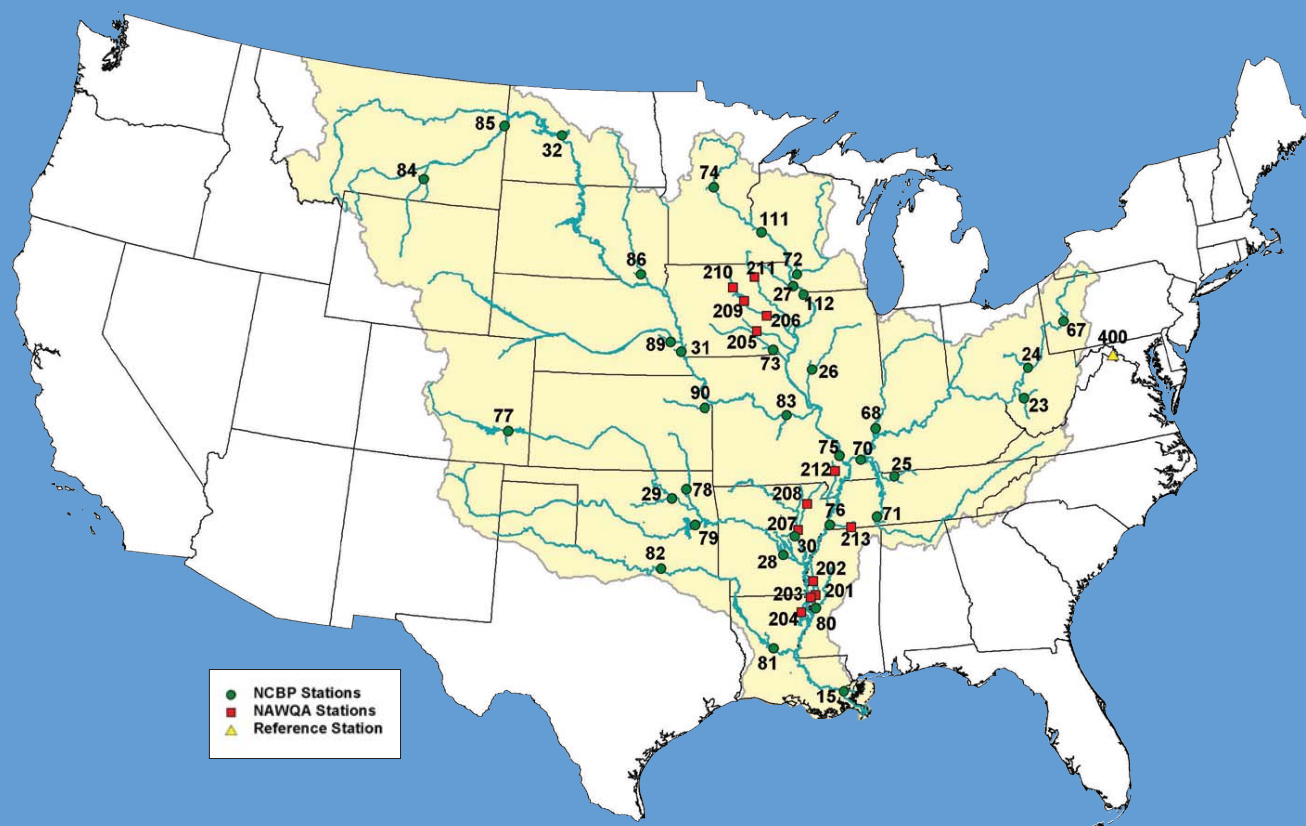


Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants and their Effects on Fish in the Mississippi River Basin

Biological Science Report
USGS/BRD/BSR—2002-0004



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Front cover: U.S. map showing locations sampled in 1995 (1996 for Station 400).

Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants and their Effects on Fish in the Mississippi River Basin

Biological Science Report
USGS/BRD/BSR—2002-0004
June 2002

edited by

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BIOMONITORING OF ENVIRONMENTAL STATUS AND TRENDS (BEST) PROGRAM: ENVIRONMENTAL CONTAMINANTS AND THEIR EFFECTS ON FISH IN THE MISSISSIPPI RIVER BASIN

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Abstract: We collected, examined, and analyzed 1378 fish of 22 species from 47 sites in the Mississippi River basin (MRB) during 1995 and from a reference site in 1996. The sampling sites in the MRB represented National Contaminant Biomonitoring Program (NCBP) stations situated at key points on major rivers and National Water-Quality Assessment Program (NAWQA) stations located on lower-order rivers and streams in the Eastern Iowa Basins (EIB) and Mississippi Embayment (MSE) Study Units. The reference site was the water supply system of the USGS-Leetown Science Center in rural Jefferson County, WV. Common carp (*Cyprinus carpio*; carp) and black basses (*Micropterus* spp.; bass), the targeted species, together represented 82% of the fish collected. Each fish was examined in the field for externally and internally visible gross lesions, selected organs were weighed to compute various ponderal and organo-somatic indices, and selected tissues and fluids were obtained and preserved for analysis of biomarkers. Fish health indicators included splenic macrophage aggregates, lysozyme activity, and histopathological analysis of liver, kidney, and other tissues. Reproductive biomarkers included analysis of plasma concentrations of vitellogenin (vtg) and the sex steroid hormones 17 β -estradiol (E2) and 11-ketotestosterone (11-kt); and the histological determination of percent oocyte atresia (in female fish) and gonadal stage. Hepatic ethoxyresorufin *O*-deethylase (EROD) activity was also measured. Composite samples of whole fish from each station were grouped by species and gender and analyzed for persistent organochlorine and elemental contaminants and for dioxin-like activity (TCDD-EQ) using the H4IIE rat hepatoma cell bioassay.

Organochlorine and inorganic contaminant concentrations in fish were generally low relative to historical levels at most sites, but remained present at concentrations representing threats to piscivorous wildlife in some locations. Toxaphene and DDT (mostly as *p,p'*-DDE) concentrations remained elevated in fish from the cotton-growing regions of the lower Mississippi valley, and were generally greater in the smaller streams draining agricultural areas (that is, in the MSE Study Unit) than at large river sites. Cyclodiene pesticide concentrations were also greatest in the EIB Study Unit and elsewhere in the corn-growing regions of the mid-MRB. Former point-sources of organochlorine pesticides also remained evident, especially in the Mississippi River near Memphis, TN. Consistent with previous findings, total PCB concentrations tended to be greatest (1-3 $\mu\text{g/g}$) in the industrialized and urbanized Ohio River and Upper Mississippi sub-basins and at Memphis, TN, and were generally correlated with TCDD-EQ and EROD activity. Conversely, PCB concentrations were low (<0.1 $\mu\text{g/g}$) in the more agricultural parts of the MRB. Concentrations of inorganic contaminants were also relatively low and stable or declining relative to past levels at most sites. Exceptions were Hg and Se; Hg concentrations were slightly elevated (>0.3 $\mu\text{g/g}$) in bass from the Mississippi River at Memphis and several other sites and in carp from one MSE site. Concentrations of Se were also great enough to constitute a hazard to piscivorous wildlife (>0.6 $\mu\text{g/g}$) at several MRB sites in the western parts of the MRB and were especially high (4-5 $\mu\text{g/g}$) in fish from John Martin Reservoir, CO, where elevated concentrations were reported previously.

Biomarker results indicated that fish from many stations had been exposed to contaminants, but at no sites did findings indicate exposure to high concentrations of toxic chemicals. Noteworthy among biomarker findings was that 73% of the male smallmouth bass (*Micropterus dolomieu*) from the Mississippi River at Lake City, MN (Lake Pepin) were intersex as indicated by the histological detection of ovotestes; and the combined EROD and H4IIE results indicated that fish from several rural sites in the Lower Mississippi valley contained a dioxin-like contaminant. EROD results also indicated that fish from some sites had been exposed to oil or other non-accumulative organic contaminants. Individual male carp from two sites and male bass from two other sites contained vtg concentrations >1.0 mg/mL, levels typical of female fish in early- to mid-vitellogenesis. Overall, most of methods

selected for evaluation in this investigation proved logistically feasible to implement and were recommended for inclusion in subsequent investigations, subject to the caveats and recommendations presented in this report.

Keywords: Contaminants, biomarkers, fish health, organochlorines, DDT, toxaphene, PCBs, chlordane, dieldrin, aromatic hydrocarbons, elemental contaminants, lead, zinc, cadmium, arsenic, selenium, copper, heavy metals, histopathology, atresia, sex steroid hormones, 17 β -estradiol, 11-ketotestosterone, vitellogenin, GSI, HIS, SSI, HAI, EROD, H4IIE, condition factor, lysozyme, macrophage aggregates.

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PREFACE

The study described in this report was developed and implemented to test biological and chemical methods for use in the U.S. Geological Survey (USGS) Biomonitoring of Environmental Status and Trends (BEST) Program (BEST, 1996; Schmitt and Dethloff 2000). In this study, fish were collected in late 1995 from National Contaminant Biomonitoring Program (NCBP—Schmitt and others, 1999b) and National Water Quality Assessment Program (NAWQA—Hirsch and others, 1988) sites located at key points in the rivers and streams Mississippi River Basin (MRB). Fish were also collected from a reference site located in West Virginia. The fish were analyzed for concentrations of accumulative environmental contaminants and for a suite of biological measures (biomarkers) indicative of contaminant exposure and their effects (Schmitt and Dethloff 2000). The study was conducted by the USGS-BEST Program and by three USGS research centers: the Columbia Environmental Research Center, in Columbia, MO; the Florida-Caribbean Science Center in Gainesville, FL; and the Leetown Science Center in Kearneysville, WV. Cooperating scientists at the University of Florida, Gainesville, FL also participated. Field aspects of the study were coordinated by the U.S. Fish and Wildlife Service (FWS) through its Environmental Contaminants program. Field work was conducted by FWS and USGS personnel stationed throughout the MRB.

The 1995 project had two primary objectives: (1) providing contemporary information on the distribution, abundance, and ecological risk of organochlorine and elemental contaminants in rivers of the MRB; and (2) providing a platform for field testing some of the biological methods nominated for inclusion in the BEST program (BEST, 1996). The study described here, along with similar investigations conducted in 1997 in the Columbia River and Rio Grande basins (Bartish and others, 1997), also represents part of a pilot, national-scale USGS monitoring program for large rivers. This report describes the MRB project and presents its findings.

Content and Organization of This Report

Chapter 1 of this report describes the objectives and general approach used to conduct the MRB study, the study area and sampling sites, the statistical methods used to analyze the data, and the field methods employed to capture and process the fish. Chapter 1 also summarizes the demographics (size, age, etc.) of

the fishes collected in the study. Documenting the distribution, abundance, size, and age of fish in the rivers and streams of the MRB was not an objective of our study. Nevertheless, these factors are important in the interpretation of the chemical and biological end-points we measured (Schmitt and Dethloff 2000). Appendix A of this report therefore contains more detailed information on the species, numbers, size, ages, and sex ratios of the fish collected at the sites. Chapter 2 describes the laboratory methods used to analyze the fish samples for organochlorine and elemental contaminants, dioxin-equivalents using the H4IIE rat hepatoma cell bioassay, and ethoxyresorufin *O*-deethylase (EROD) activity, and presents the results of these analyses. Chapter 3 describes the methods used to assess fish health and summarizes the results of these analyses, and Chapter 4 summarizes the reproductive biomarkers. Chapter 5 summarizes Chapters 1-4 and discusses the findings in terms of environmental significance and future monitoring. Future reports will present more in-depth statistical analyses and interpretations of the results presented here, and incorporate and evaluate additional results from the NAWQA study units. Evaluation and optimization of methods were also objectives of the 1995 study, as was an evaluation of logistic and administrative feasibility. These objectives will be addressed following completion of the 1997 studies in the Columbia River and Rio Grande basins. Data for the MRB project may be obtained on the World-wide Web at <http://www.cerc.usgs.gov/data/data.htm>.

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Chapter 1. Project Overview

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BACKGROUND AND INTRODUCTION

The BEST Program

The BEST program was initiated in the late 1980s by the U.S. Fish and Wildlife Service (FWS) as a revision and expansion of the National Contaminant Biomonitoring Program (NCBP). The NCBP, which originated in 1967 as the FWS component of the National Pesticide Monitoring Program (NPMP), documented temporal and geographic trends in concentrations of accumulative environmental contaminants through periodic collections and chemi-

cal analyses of freshwater fish, European starlings (*Sturnus vulgaris*), and duck wings (Johnson and others, 1967; Schmitt and Bunck, 1995). Although accumulative contaminants were still perceived to be important, by the 1980's FWS concerns had shifted to newer pesticides (that is, herbicides and short-lived insecticides), oil and petrochemicals, and other chemicals not amenable to monitoring through chemical analyses of animal carcasses. In addition, the NCBP lacked a biological focus; because the program was designed to monitor chemicals, it was difficult to extrapolate findings to resources (lands and species) of direct concern to FWS (Schmitt, 2000). Consequently, the FWS began planning for an expansion of the NCBP that would be more focused on agency concerns by incorporating biological indicators

that respond to a wider variety of chemicals and which would sample more species and habitats. The BEST program was transferred to the National Biological Service (NBS) in 1993. The NBS became part of USGS in 1996.

The BEST program documents spatial and temporal trends in the exposure of organisms and ecosystems to contaminants, and assesses the effects of contaminant exposure on selected organisms (Biomonitoring of Environmental Status and Trends Program, 1996; Schmitt, 2000). This is accomplished through the periodic application of chemical and biological assessment methods and through the incorporation of information from other programs and sources. Monitoring methods include chemical analyses of selected media (biota, sediments, etc.) for contaminants and biological indicators of chemical exposure and effects. For monitoring aquatic ecosystems, the BEST program relies on chemical analyses of water, sediment, and the tissues of organisms (primarily fishes and birds); biochemical indicators of chemical exposure (biomarkers) in indicator species; and toxicity tests and bioassays of sediment pore-waters and animal tissue extracts (BEST, 1996). The suite of methods, including the subset evaluated in this project, was selected to maximize sensitivity, cost-effectiveness, and the variety of contaminants and their effects that could be detected (BEST, 1996; Schmitt, 2000).

Although environmental concentrations of many persistent chemicals have declined compared to historic levels (Schmitt and Bunck, 1995; Schmitt and others, 1999b), the analysis of fish for accumulative contaminants is an important part of the BEST program and the 1995 project. Accumulative contaminants in fish has also been proposed as an indicator of sustainable economic development (Council on Environmental Quality (CEQ), 1997), and remains an integral part of many environmental monitoring programs (BEST, 1996; Hirsch and others, 1988; Messer and others, 1991). Despite declining concentrations, there is a substantial body of information indicating that concentrations of accumulative contaminants in fish may remain sufficiently elevated to harm fish and wildlife in some locales (for example, Gooch and Matsamura, 1987; Colborn, 1991; Tillitt and others, 1992; Schmitt and others, 1999b). In addition, no large-scale monitoring program, including the NCBP and NAWQA, has incorporated assessments of the polychlorinated dibenzo-*p*-dioxins or -furans because of the expense involved in the analysis of these compounds. Certain of these and other highly toxic and accumulative polyhalogenated hydrocarbons (PHHs), as well as other structurally and toxicologically similar compounds, are present in many U.S. waters at concentrations harmful to pisciv-

orous organisms (Kubiak and others, 1989; Mac and Edsall, 1991; Colborn, 1991; U.S. Environmental Protection Agency (USEPA), 1992; Tillitt and others, 1992). In the Northeast, concentrations of mercury in fish from many lakes are sufficiently high to represent a threat to fish-eating wildlife (Yearley and others, 1998) and may be increasing in some remote areas (Monteiro and Furness, 1998), largely as a result of distribution by atmospheric and sediment transport processes. Finally, continuing reports of reproductive impairment, immune system dysfunction, and other health problems in wildlife has renewed interest in organochlorine chemicals, mercury, and other substances believed to interfere with the endocrine and immune systems (for example, Matthews and others, 1990; Colborn and others, 1993; Hutchinson and Simmonds, 1994).

OBJECTIVES AND DESIGN OF THE MISSISSIPPI RIVER BASIN STUDY

Objectives

The specific objectives of the 1995 study were to: (1) document the geographic distribution of selected chemical contaminants and their effects on fish in the large rivers of the Mississippi River basin (MRB), and compare the concentrations to historic and contemporaneous findings (for example, Goodbred and others, 1994; Tate and Heiny, 1996; Heiny and Tate, 1997; Goodbred and others, 1997; Schmitt and others, 1999b); (2) evaluate the performance of a subset of the biological and chemical indicators selected for the BEST program (BEST, 1996); and (3) evaluate the technical and logistic feasibility of implementing a large-scale monitoring program through partnerships with USGS research centers and programs, cooperative research units, universities, and other Department of Interior (DOI) agencies.

General Approach

Fish were collected from selected sites in the MRB (Fig. 1-1, Table 1-1) during the fall and early winter of 1995 and from a reference site in West Virginia in October 1996. Composite samples of whole fish were analyzed for organochlorine and elemental contaminants. Individual fish were analyzed for a suite of biomarkers (Table 1-2). The biomarkers included indicators of immune and endocrine system function, general fish health and condition, and biochemical responses to several classes of chemicals

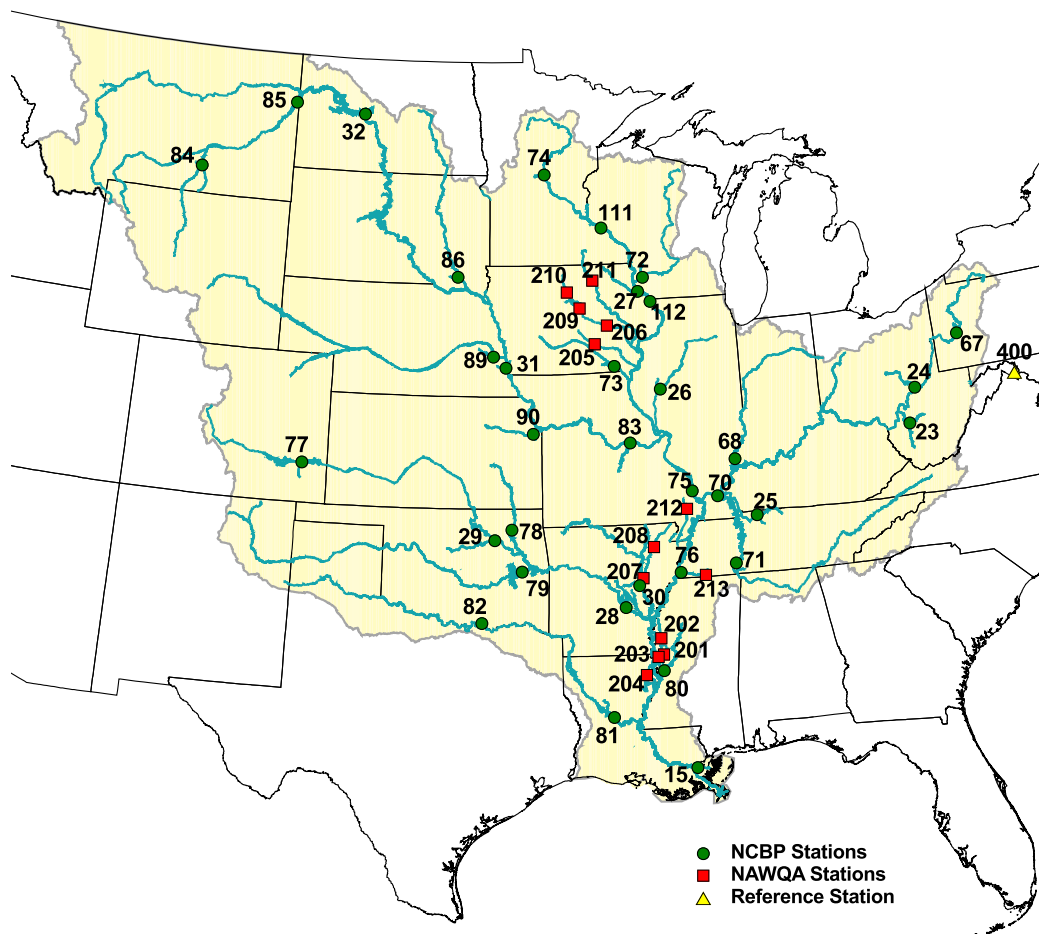


Figure 1-1. Locations sampled in 1995 (1996 for Station 400). See Table 1-1 for more information.

(BEST, 1996; Schmitt and Dethloff, 2000). For geographic and temporal comparisons, historic NCBP sites situated on the largest rivers of the MRB were sampled. Sites on lower-order streams in two NAWQA Study Units, each of which comprised watersheds lying wholly within the MRB, were also sampled to examine issues of spatial scale with respect to contaminants and their effects on fish. The NAWQA sites were also included to allow the evaluation of the broader suite of methods employed by both programs.

Study Area

The MRB is the largest river basin in North America. It drains all or parts of 32 states (about 41% of the conterminous U.S.) and two Canadian provinces (Fig. 1-1), and has a human population of more than 72

million. Agricultural development in the basin is extensive; the MRB accounts for >50% of U.S. corn, wheat, soybean, cattle, and hog production (Goolsby, 1996). Many programs and studies (for example, Thurman and others, 1991; Goolsby and others, 1993; Meade, 1995; Ellis and others, 1995; Goodbred and others, 1997; Schmitt and others, 1999b; Wong and others, 2000) have documented the presence and widespread distribution of numerous contaminants in the Mississippi River and its tributaries. Organochlorine pesticides, including DDT, chlordane, heptachlor, and aldrin, were used heavily in both agricultural and urban areas, and also originate from well-known point-sources (Biglane and others 1964; Yurawecz and Roach, 1978; O'Shea and others, 1980; Leppanen and others 1998; Schmitt and others, 1999b). Some of these compounds were also used against termites in much of the basin (Arruda and others, 1987). In the 1970s and 80s, concentrations of

Table 1-1. Stations sampled in 1995 (1996 for reference site), collection dates (first-last), and collection organization (FWS, U.S. Fish and Wildlife Service; USGS, U.S. Geological Survey).

Program, sub-basin, and station number	River	Nearest city or feature	Collection date(s)	Collection organization	Latitude (dd-mm-ss)	Longitude (dd-mm-ss)
NCBP						
Arkansas-Red River (ARR)						
29	Arkansas	Keystone Res., OK	12/4-12/5	FWS, Tulsa, OK	36-07-54	96-20-47
77	Arkansas	John Martin Res., CO	10/11-10/12	FWS, Columbia, MO & Manhattan, KS	38-03-55	102-56-02
78	Verdigris	Oologah, OK	12/6-12/7	FWS, Tulsa, OK	36-31-16	95-33-37
79	Canadian	Eufaula, OK	11/28-11/29	FWS, Tulsa, OK	35-16-43	95-34-39
82	Red	Lake Texoma, TX/OK	11/28-12/7	USGS, Austin, TX	33-52-08	96-47-04
Lower Missouri River (LMO)						
31	Missouri	Nebraska City, NE	10/19-10/20	FWS, Columbia, MO & Manhattan, KS	40-40-15.9	95-49-44.58
83	Missouri	Hermann, MO	10/24-10/25	FWS, Columbia, MO & Manhattan, KS	38-42-24.06	91-26-17.52
86	James	Olivet, SD	9/5-9/7	FWS, Pierre, SD	43-13-45	97-41-05
89	Platte	Louisville, NE	10/17-10/18	FWS, Columbia, MO & Manhattan, KS	40-59-33.06	96-12-30.9
90	Kansas	Bonner Springs, KS	11/7	FWS, Columbia, MO & Manhattan, KS	39-02-47	94-47-05
Upper Missouri River (UMO)						
32	Missouri	Garrison Dam, ND	9/5-9/7	FWS, Bismarck, ND	47-28-27.3	101-26-15.5
84	Big Horn	Hardin, MT	10/2-10/3T	FWS, Helena, MT	45-52-12.2	107-34-34
85	Yellowstone	Sidney, MT	9/18-9/19	FWS, Bismarck, ND	47-34-46.8	104-13-10.7
Lower Mississippi River (LMS)						
15	Mississippi	Luling, LA	11/6-11/8	FWS, Vicksburg, MS & Lafayette, LA	29-59-53.2	90-25-31.1
28	Arkansas	Pine Bluff, AR	10/11-10/12	FWS, Vicksburg, MS & Lafayette, LA	34-16-27	94-57-12
30	White	Devall's Bluff, AR	9/19	USGS, Jackson, MS	34-47-01	91-26-28
75	Mississippi	Cape Girardeau, MO	10/11-10/12	FWS, Rock Island, IL	37-18-36	89-31-1.2
76	Mississippi	Memphis, TN	10/31-11/1	FWS Vicksburg, MS & Lafayette, LA	38-08-30.3	90-03-36.6
80	Yazoo	Redwood, MS	8/23	USGS, Jackson, MS	32-24-36	90-55-27
81	Red	Alexandria, LA	10/3-10/5	FWS Vicksburg, MS & Lafayette, LA	31-20-48	92-27-37
Upper Mississippi River (UMR)						
26	Illinois	Beardstown, IL	9/26-9/28	USGS, Urbana, IL	40-07-50.64	90-20-45.6
27	Mississippi	Guttenburg, IA	10/8	FWS, Rock Island, IL	42-43-37.2	91-01-30
72	Wisconsin	Woodman, WI	9/5-9/7	FWS, Green Bay, WI	43-05-42	90-48-57.6
73	Des Moines	Keosauqua, IA	10/24-10/25	FWS, Rock Island, IL	40-44-52.8	91-59-38.4
74	Mississippi	Little Falls, MN	9/18-9/26	FWS, Twin Cities, MN	45-58-48	94-22-00
111	Mississippi	Lake City, MN	9/11-9/13	FWS, Twin Cities, MN	44-22-49.8	92-07-33
112	Mississippi	Dubuque, IA	10/4	FWS, Rock Island, IL	42-26-27.6	90-35-06
Ohio River (OHR)						
23	Kanawha	Winfield, WV	10/16-10/17	FWS, Elkins, WV	38-29-06	81-48-57.6
24	Ohio	Marietta, OH	9/25-10/31	FWS, Reynoldsburg, OH	39-24-36.8	81-26-26.3
25	Cumberland	Clarksville, TN	10/17-10/19	FWS, Vicksburg, MS & Lafayette, LA	36-32-28.6	87-22-04.7
67	Allegheny	Natrona, PA	10/30-10/31	FWS, Elkins, WV	40-39-54	79-41-24
68	Wabash	New Harmony, IN	8/30-11/28	FWS, Bloomington, IN	38-11-58.37	87-58-35.98
70	Ohio	Metropolis, IL	10/10	FWS, Rock Island, IL	37-07-40.8	88-39-25.2
71	Tennessee	Savannah, TN	10/25-10/26	FWS, Vicksburg, MS & Lafayette, LA	35-12-52	88-18-36

Table 1-1. Stations sampled in 1995 (1996 for reference site), collection dates (first-last), and collection organization (FWS, U.S. Fish and Wildlife Service; USGS, U.S. Geological Survey) --Continued.

Program, sub-basin, and station number	River	Nearest city or feature	Collection date(s)	Collection organization	Latitude (dd-mm-ss)	Longitude (dd-mm-ss)
NAWQA						
Eastern Iowa Basins (EIB)						
205	S. Skunk	Oskaloosa, IA	9/19	USGS, Urbana, IL	41-21-19	92-39-31
206	Iowa	Morengo, IA	9/20	USGS, Urbana, IL	41-50-23	92-11-54
209	S. Fork Iowa	New Providence, IA	9/21	USGS, Urbana, IL	42-19-26	93-10-10
210	Iowa	Rowan, IA	9/22	USGS, Urbana, IL	42-45-36	93-37-23
211	Cedar	St. Charles City, IA	9/23	USGS, Urbana, IL	43-03-45	92-40-23
Mississippi Embayment (MSE)						
201	Big Sunflower	Anguilla, MS	8/21	USGS, Jackson, MS	32-58-18	90-46-40
202	Bogue Phalia	Leland, MS	8/22	USGS, Jackson, MS	33-24-22	90-50-26
203	Steele Bayou	Rolling Fork, MS	8/24	USGS, Jackson, MS	32-54-71	90-57-10
204	Tensas	Tendal, LA	8/25	USGS, Jackson, MS	32-25-56	91-21-57
207	Cache	Corton Plant, AR	9/20	USGS, Jackson, MS	35-02-32	91-19-12
208	Cache	Egypt, AR	9/21	USGS, Jackson, MS	35-51-23	90-56-15
212	Little River Ditch	Moorehouse, MO	10/18	USGS, Jackson, MS	36-50-03	89-43-48
213	Wolf	LaGrange, TN	10/19	USGS, Jackson, MS	35-01-57	89-14-48
Reference site	Leetown Res.	Kearneysville, WV	10/24-10/31	USGS, Kearneysville, WV	39-21-2.15	77-55-32.69

organochlorine insecticides and PCBs were elevated in eggs and chicks of great blue heron (*Ardea herodias*) inhabiting the Upper Mississippi River basin (Ohlendorf and others, 1979; Nosek and Faber, 1984). Great blue heron eggs remained contaminated with PCBs and cyclodiene pesticides in 1993, and biomarker results (hepatic EROD activity, brain asymmetry, eggshell thinning, and erythrocyte aneuploidy) were consistent with exposure to organochlorine chemicals (Custer and others, 1997). Contemporary-use agricultural chemicals, most notably atrazine and other herbicides, are commonly detected in waters from much of the MRB (Thurman and others, 1991; Goolsby and others, 1993; Meade, 1995). Somewhat elevated concentrations of PCBs have also been reported in fish from the Ohio R. near Marietta and Cincinnati, OH, and farther downstream, at Metropolis, IL; in the Mississippi R. below the Twin Cities (MN) and Quad-Cities (IA/IL); and in the Kanawha R., WV and Wabash R., IN (Lee and Anderson, 1998; Schmitt and others, 1999b; Zajicek and others, 2000). Petrochemicals are present in the heavily industrialized lower Mississippi River below Baton Rouge, LA, and in the Kanawha R., WV. Sources of chlorinated dioxins and related compounds are located near the Mississippi R. in Arkansas (Johnson and others, 1996) and on the Kanawha R. (Schmitt and others, 1999b).

Metals and other elemental contaminants from mining, smelting, and other industrial activities are also common in the MRB. These pollutants emanate from point-sources located on the Mississippi, Missouri, Verdigris, and Ohio Rivers. In addition, non-point sources in the form of wastes from historic and active mining and urban runoff are present in many parts of the basin (May and McKinney, 1981; Schmitt, 1999). Mercury from historic chlor-alkali and paper production facilities originated from sites on the Kentucky and Wisconsin Rivers (May and McKinney, 1981; Rada and others, 1986).

The continuing widespread occurrence of bio-accumulable contaminants in fish has caused human fish consumption advisories to remain in effect for cyclodiene pesticides, PCBs, chlorodioxins, mercury, and lead in parts of the basin (USEPA, 1998). In addition, seleniferous geologic formations underlie western parts of the MRB (Lakin, 1973; May and McKinney, 1981), where selenium-associated wildlife poisoning (Lemly and others, 1993) and elevated concentrations in fish (Schmitt and others, 1999b) have been reported. And finally, recent studies (Folmar and others, 1996; Goodbred and others, 1997; Harshbarger and others, 1999) have identified sites and regions within the MRB where endocrine disruption in fish caused by chemical contaminants may be occurring.

Table 1-2. Methods incorporated into the MRB project.

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 et al. (1992); Hinton (1993); Goodbred et al. (1997)
Ethoxyresorufin-O-deethylase (EROD) activity	Enzyme induction by planar hydrocarbons	Liver	PCBs, PAHs, dioxins, and furans	Pohl and Fouts (1980); Kennedy and Jones (1994)
Lysozyme activity	A disease resistance factor that can be suppressed in the presence of contaminants	Blood plasma	Overall organism health	Blazer et al. (1994)
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, hemopoetic kidney, and liver	Multiple contaminants including PAHs and metals	Blazer et al. (1994); Blazer (1997)
H4IIE bioassay	A screening tool to determine the presence of certain classes of planar halogenated compounds	Whole fish (composites)	PCBs, dioxins and furans; and PAHs	Tillitt et al. (1991)
Vitellogenin	A precursor of egg yolk, normally synthesized in the liver of female fish	Blood plasma	Endocrine modulating compounds	Folmar et al. (1996)
Sex Steroids (estradiol and testosterone)	Determine reproductive health and status	Blood plasma	Endocrine modulating substances	Guillette et al. (1994); Goodbred et al. (1997)
Chemical analyses	Organochlorine chemical residues and elemental contaminants	Whole fish (composites)	Specific analytes	Schmitt et al. (1999)
Somatic indices	The relative mass of some organs is often indicative of chemical exposure	Gonads, spleen, liver	Overall organism health	Grady et al. (1992)
Stable N isotopes (^{14}N and ^{15}N)	The ratio of ^{15}N to ^{14}N ($\delta^{15}\text{N}$) increases with trophic position and sewage pollution	Whole fish (composites)	Trophic position, nitrogen sources	Cabana and Rassmussen (1996)
Necropsy-based fish health assessment	Visual assessment of external/internal anomalies (e.g., lesions, parasites, tumors), which may indicate contaminant-related stress	All	Overall organism health	Goede (1988, 1996); Adams et al. (1993); Adams (1990)

In addition to the wide variety of contaminants present in the MRB, the basin was selected because the information on organochlorine chemicals and metals was in need of revision. Substantial quantities of these contaminants were re-distributed by severe flooding in 1993 and early 1995, and large quantities of bio-accumulables were trans-

ported out of the MRB (Rostad, 1997). Nevertheless, concentrations in some waters increased following the 1993 flood (Petty and others, 1995; Petty and others, 1998), ostensibly because contaminated soils and sediments were washed from watersheds and re-deposited in the floodplain. The MRB was also chosen because the fishes in which the biological indicators were best

validated are widely distributed in the basin and were historically collected at the NCBP sites (Schmitt and others, 1999b).

Collection Sites

The 38 NCBP stations in the MRB, which were last sampled in 1984-88 (Schmitt and others, 1999b; Zajicek and others, 2000), were the initial focus of the 1995 study. For financial reasons, the scope was reduced to 35 NCBP stations; we eliminated from consideration NCBP Stations 87 (S. Platte R. at L. McConaughy, NE) and 88 (S. Platte R. at Brule, NE) because contaminants in fish from the Platte R. system had been studied recently (Goodbred and others, 1994; Tate and Heiny, 1996; Heiny and Tate, 1997; 1997); and Station 33 (Missouri R. at Great Falls, MT), which historically yielded fishes that were not targeted by the 1995 study (Schmitt and others, 1999b). Collection locations are listed in Table 1-1 and are shown on Fig. 1-1.

As part of the original NPMP-NCBP monitoring networks, the NCBP sites in the MRB were originally selected because they represent key points (that is, confluences of major tributaries, impoundments) in some of the largest U.S. rivers (Johnson and others, 1967; Schmitt and Bunck, 1995). Among the assumption implicit in this design is that concentrations of bio-accumulable contaminants in long-lived organisms inhabiting such sites integrate large expanses of space and time with respect to the contaminants and their sources. To evaluate this assumption for both chemical and biological endpoints, sites in lower-order streams were also sampled. In addition to the NCBP sites, we sampled 13 NAWQA sites—nine in the Mississippi Embayment (MSE) Study Unit (Mallory, 1994) and four in the Eastern Iowa Basins (EIB) Study Unit (Kalkhoff and others, 1994; Fig. 1-1, Table 1-1). Specifically, these sites were included to evaluate how well contaminant conditions documented in the large rivers reflected conditions in lower-order streams, and *vice-versa*. The sub-basins comprised by the NAWQA Study Units are intensively farmed for pesticide-intensive crops (that is, corn, cotton, rice, soybeans, and wheat). Fish from NCBP sites within or affected by the sub-basins historically contained elevated concentrations of organochlorine pesticide residues (Schmitt and others, 1999b). In addition, NAWQA collects information on water quality, aquatic community composition (algae, benthic macroinvertebrates, and fish), and land use and cover. Similar information was recommended for inclusion in the BEST program (BEST, 1996) as corollary data for analyzing and interpreting monitoring results. The NAWQA program and the MSE and EIB Study Units are described by Hirsch and others

(1988), Mallory (1994), and Kalkhoff and others (1994), respectively.

The nominal station locations at which we collected fish were either the site of the last NCBP collection (for most, 1986-87—Schmitt and others, 1999b) or the NAWQA fixed site location (Fig. 1-1; Table 1-1); however, the actual collection locations and spatial extents (that is, stream area sampled) varied. Collectors were instructed to seek local expertise, as needed, to obtain the sought-after numbers of the preferred species as near to the nominal station location as possible. The actual collection locations were documented with a geographic position system (GPS) receiver.

Species Selection and Sampling Strategy

Historically, the NCBP was based on the analysis of composite samples of whole, adult fish, with all five individuals being of the same species. A total of three samples—two of a representative bottom-dwelling species and one of a predatory species were collected, with each sample comprising five individuals of similar size (Schmitt and others, 1999b). Cooperators could select from a list of preferred taxa stratified by habitat type (that is, cold-, cool-, or warm-water). Through the 1980s, common carp (*Cyprinus carpio*, hereafter carp) and largemouth bass (*Micropterus salmoides*) were the most commonly collected species at NCBP sites in the MRB (Schmitt and others, 1999b). These are also the taxa targeted by NAWQA for monitoring contaminants in fish (Crawford and Luoma, 1993; Goodbred and others, 1994; Tate and Heiny, 1996; Heiny and Tate 1997; Goodbred and others, 1997).

The 1995 study was designed to accommodate biomarkers, many of which are gender-specific and require live or freshly killed individual fish, while retaining comparability with the historic NCBP data base and contemporaneous studies of contaminants in fish based on composite samples (for example, Goodbred and others, 1997). Accordingly, the collection target at each NCBP site was ten each of male and female carp and largemouth bass (total of 40 fish per site), to be collected by electrofishing. At the NAWQA sites, only one species (carp at 12 sites, largemouth bass at one) was targeted. Although the preferred species are widely distributed, they are not ubiquitous in the MRB. Alternate species were therefore permitted, as was also true for the NCBP (Schmitt and others, 1999b) and NAWQA (Crawford and Luoma, 1993). Preferred alternates for largemouth bass included other black basses (*Micropterus* spp.), which we collectively refer to as bass; other centrarchids (*Pomoxis* spp., *Lepomis* spp.); northern pike (*Esox lucius*); and certain percichthyids

(*Morone* spp.), and percids (*Stizostedion* spp.). Preferred alternates for carp included certain catfishes (Ictaluridae) and suckers (Catostomidae). Fall was selected to maintain continuity with historic NCBP protocol (Schmitt and others, 1999b) and the previous study of endocrine disruption in the MRB (Goodbred and others, 1997). Fall is the reproductively quiescent season for most fishes (Down and others, 1990) during which we expected temporal and spatial variability of the chemical and biological endpoints (including the reproductive biomarkers) to be lowest (Goodbred and others, 1997).

Chemical and Biological Indicators

The 1995 study was designed to link the BEST program with the historic fish data of the NCBP. It therefore comprises the analysis of fish carcasses for bioaccumulative contaminants following NCBP protocol (Schmitt and others, 1999b) and the biological indicators recommended for use in the BEST program (BEST, 1996) that could be performed on fish collected from large rivers. The study also incorporated the suite of reproductive biomarkers that had been tested in selected NAWQA Study Units during 1994 (Goodbred and others, 1994; 1997—Table 1-2). To facilitate comparisons with historic NCBP findings, the fish carcass samples were composited by species and gender and analyzed for elemental and organic chemical contaminants by instrumental analyses (gas chromatography with electron capture detection [GC-ECD]; inductively coupled plasma emission spectroscopy; atomic absorption spectroscopy). The composite fish samples were also analyzed for stable isotopes of nitrogen ($\delta^{15}\text{N}$), a potential indicator of trophic position and nitrogen source (Cabana and Rasmussen, 1996; Kendall, 1998).

As noted previously, the PHHs are among the most persistent and toxic of environmental contaminants. The PHHs comprise the halogenated biphenyls, dioxins and dibenzofurans, as well as other less familiar groups of chemicals. These compounds, along with the polycyclic aromatic hydrocarbons (PAHs), are ubiquitous environmental pollutants released from a variety of industrial, petroleum, and combustion sources (Schmitt and others, 1999b). The most toxic constituents of these classes share a similar planar chemical structure, and their toxicity is mediated by the aryl hydrocarbon hydroxylase (AHH) receptor (Whyte and others, 2000). Routine monitoring of the PHHs and PAHs is precluded by the relatively great expense of the high-resolution methods necessary for their measurement at environmental concentrations. We evaluated a combination of biological and chemical methods for evaluating these classes of compounds without high-resolution instru-

mental analyses. Total PCBs were among the analytes included in the GC-ECD analysis of composited fish carcasses (Table 1-2). Extracts from the composites were then screened for the presence of AHH-active compounds by the H4IIE rat hepatoma cell bioassay, an *in vitro* method for documenting the cumulative concentrations of planar PHHs (Tillitt and others, 1991; 1992). Liver samples from individual fish were analyzed for ethoxyresorufin *O*-deethylase (EROD) activity (Table 1-2). EROD is a cytochrome P450-dependent mono-oxygenase enzyme that is induced by several classes of planar aromatic compounds (Whyte and others, 2000). This strategy is presented in more detail in Chapter 2.

In addition to hepatic EROD activity, fish were analyzed for the following biomarkers (Table 1-2): Each specimen was examined and scored in the field using a quantitative health assessment (Schmitt and others, 1999b) based on the methods of Goede (1989; 1996), Adams (1990), and Adams and others (1993) that included determinations of relative organ sizes (liver, spleen, and gonad). For the targeted species (carp and bass), selected tissues and organs were also examined histopathologically for evidence of parasites, tumors, and other lesions potentially indicative of chemical exposure (Hinton and others, 1992), and additional reproductive biomarkers were determined. These included histological examination of the gonads to assess developmental stage and pathological conditions such as the occurrence of atretic eggs in females and ovotestes in males; and the reproductive biomarkers used in the previous NAWQA investigation (Goodbred and others, 1994; 1997; McDonald and others, 2000). The latter included plasma concentrations of reproductive hormones (Guillette and others, 1994) and the protein vitellogenin, an egg-yolk precursor synthesized by the fish in response to endogenous or exogenous estrogens (Folmar and others, 1996). Many contaminants are also known or suspected to suppress immune system function in animals (for example, Matthews and others, 1990; Hutchinson and Simmonds, 1994). We therefore included two immune system indicators—splenic macrophage aggregates, which are determined histopathologically; and plasma lysozyme activity (Blazer and others, 1994). Field procedures are described later in this chapter; laboratory methods are presented in subsequent chapters. Further information on the biological methods, including the rationale for their inclusion in this study, is presented by Schmitt and Dethloff (2000).

CONDUCT OF THE MRB STUDY

Project oversight was maintained by the USGS-Columbia Environmental Research Center (CERC), Columbia, MO. CERC also had the lead responsibility for data management, statistical analysis, and interpretation. Field portions of the study were supervised and implemented by cooperating FWS and USGS personnel and were implemented at the NCBP and NAWQA sites from late August to early December 1995 (Table 1-1). Fish were collected and processed by 17 field teams, each of which comprised 4-7 biologists and other personnel. Team leaders had participated in a 3-d training session to gain familiarity with collection, dissection, storage, handling, and recording protocols; some teams also included other trained individuals. Teams typically spent 1-4 d at each station, but three sites had to be sampled more than once to obtain the necessary fish: Station 24 was sampled three times over a 4-wk period; Station 68 was revisited after 3 months; and Station 82 was revisited after 1 wk (Table 1-1). The West Virginia reference site (Station 400) was sampled by USGS personnel in October 1996 (Table 1-1). Frozen or preserved samples were shipped to cooperating laboratories for analysis, as follows: Carcass samples were prepared and analyzed for organochlorine chemicals and elemental contaminants by contract laboratories managed by the FWS Patuxent Analytical Control Facility in Laurel, MD. Laboratory analyses for fish health indicators (histopathology, plasma lysozyme activity, and splenic macrophage aggregates) were conducted by the USGS National Fish Health Laboratory in Kearneysville, WV. Reproductive biomarkers in plasma (hormones and vitellogenin) were analyzed by cooperating scientists at the University of Florida, Gainesville, FL. The H4IIE bioassays and analyses of hepatic EROD activity were conducted by the CERC.

FIELD METHODS

Fish Collection

At most sites fish were collected by DC boat electrofishing. At Station 77 fish were gill-netted, but such injurious sampling methods were generally avoided. Fish were generally collected along shorelines or from backwater areas of the rivers and reservoirs sampled. Although electrofishing tends to be somewhat biased toward larger fish (Reynolds, 1983), all individuals of the target species were collected, irrespective of size. The collection of more than one species of bass at several sites facilitated biomarker

comparisons among species. At some sites more than 10 fish of a given species and gender were collected. GPS coordinates were obtained at the geographic extremes of each area sampled.

Following capture, fish were held in on-board live wells and transported to on-shore processing sites, where they were usually processed within a few hours of collection. At several stations, fish were held alive overnight in net pens or in tanks containing ambient water because all fish could not be processed on the day of collection.

Fish Processing (Tissue and Fluid Collection Procedures and General Observations of Fish Health and Condition)

The tissues and fluids collected, along with the endpoints associated with them, are summarized in Table 1-3. The general sequence of fish processing was as follows: A live fish was removed from the holding tank and identified to species. Blood was collected from the posterior caudal artery and vein with a heparinized needle and syringe; from this sample, plasma was later obtained for determination of reproductive hormones, vitellogenin, and lysozyme activity. The fish was weighed and measured, then subdued with a sharp blow to the head. Observations of external features were recorded, and any grossly visible anomalies were removed and placed in fixative for later histological examination. The abdominal cavity of the fish was cut open from the vent forward to the pectoral girdle. The liver (in species with a discrete liver—see next section), spleen, and gonads were removed and weighed for later computation of condition factor and organo-somatic indices. The gender of the fish was determined by gonadal observation and recorded. Pieces of liver for EROD analysis were collected and frozen immediately in dry ice-ethanol slush. Additional pieces of liver, as well as samples of gonad, kidney, and spleen, were collected and preserved for histopathological examination, gender confirmation (gonad) and macrophage aggregate analysis (spleen). Prior to excision of the pieces, the liver, gall bladder, posterior and anterior kidneys, gonads, mesenteric fat (in certain species), and spleen were visually observed as part of the overall fish assessments. Upon completion of the internal examination, scales (or spines from ictalurids) were collected for age determination. Remaining tissues (those not frozen or fixed) were returned to the body cavity and the entire fish was wrapped in the aluminum foil upon which it was processed. The wrapped carcass was placed in a plastic bag with other carcasses of the same species and gender, chilled, and later frozen; they were used for chemical, H4IIE, and $\delta^{15}\text{N}$ analyses. The entire procedure typically took 15-20 min

Table 1-3. Measured indicators, associated tissues, and field preservation techniques for assessment of fish from the Mississippi River basin.

Analysis	Tissue or fluid	Preservation technique
EROD	Liver	Quick frozen in field, stored at -80°C
Reproductive hormones	Plasma	Quick frozen in field, stored at -80°C
Vitellogenin	Plasma	Quick frozen in field, stored at -80°C
Lysozyme activity	Plasma	Quick frozen in field, stored at -80°C
Macrophage aggregates	Spleen	NOTOXhisto™ preservative
Histopathology	Liver, spleen, kidney, gonads, grossly visible lesions	NOTOXhisto™ preservative
Chemical analysis (organochlorine scan w/ total PCBs, metal scan, Pb)	Carcass (whole body minus pieces used in other tests)	Placed on wet ice in field, stored frozen at 0°C
H4IIE	Carcass (whole body minus pieces used in other tests)	Placed on wet ice in field, stored frozen at 0°C
Age	Scales or spines	Scale envelope
Fish health assessments	Visual observations of body surface, eyes, gills, opercula, fins, liver, gall bladder, spleen, kidneys, gonads, mesenteric fat	NOTOXhisto™ preservative (anomalies matched with normal tissue)
Weight	Whole body	Not applicable
Length	Whole body	Not applicable
Somatic indices (relative weights of tissues)	Gonads, spleen, liver (some species)	Not applicable

(per fish), and tissue samples, especially plasma and liver for EROD analysis, were collected and frozen as rapidly as possible to avoid clotting, enzyme breakdown, and tissue necrosis. Blood samples were centrifuged after all or several fish were processed, and the plasma was aspirated and frozen in the dry ice-ethanol bath. Frozen plasma and liver samples were shipped in dry ice to the laboratories, where they were stored at -80 °C.

A synopsis of the specific field procedures for each endpoint (for example, reproductive hormones) is provided in the following paragraphs. The complete field procedures are described in Schmitt and others (1995), an updated version of which was published by Schmitt and others (1999a) and which can be viewed online at

<<http://www.cerc.usgs.gov/pubs/pubs.htm>>.

Fish Size: Large fish (>2 kg) were weighed (g) with a hanging spring balance; smaller fish were weighed with an electronic pan balance calibrated daily before and after sampling. Maximum total length (TL, mm) was measured with a measuring board from the anterior of the fish to the tip of the caudal fin rays, with the

lobes of the caudal fin compressed dorso-ventrally (Anderson and Gutreuter, 1983).

Fish Health Assessments: Both the external and internal features of each fish were observed for grossly visible abnormalities based on the criteria of Goede (1989).

External: The body surface of each fish was examined for deformities, tumors, lesions, parasites, and scale loss; the presence of such conditions was recorded. Suspected anomalies were excised and preserved in NoToXhisto™ (Earthsafe Technologies), an ethanol-based histological fixative. A sample of normal tissue was also collected if possible. Fins were examined and recorded as normal, frayed, eroded (mild or severe), embolic, or hemorrhagic. The condition of the eyes was recorded as normal, exophthalmic, hemorrhagic, opaque, embolic, or missing. Opercula were observed for degree of shortening (normal, slight, severe). The gill lamellae were rated as normal, frayed, clubbed, marginate, or pale. In salmonids, the pseudobranchs were scored as normal, swollen, lithic, or hemorrhagic.

Internal: The color of the intact liver was noted (dark to light red, general discoloration) and was examined for the presence of nodules, focal discoloration, or lesions. The gall bladder was observed for fullness (empty, partially full, full and distended) and bile color (yellow, light to grass green, dark green to blue-green). The spleen was examined for granulations, nodules, enlargement, and color (red to black). The gonads were examined to determine gender and gonadal condition (ripe, spent, or intermediate). For fishes with prominent pyloric caeca (for example, bass, salmonids), the extent of mesenteric fat (none, slight, 50%, >50%, completely covered) was determined. The posterior and anterior kidneys were observed for swelling, mottling, granulations, and urolithiasis. Samples of kidney, spleen, liver, gonad, and gill were collected for histopathological examination from all specimens (Table 1-3). In addition, all grossly visible abnormalities (internal or external) were excised and preserved for subsequent histological evaluation. When possible, a sample of normal tissue was also collected for comparison.

Organ Weights: The weights of the excised gonads (to 0.1 g), spleen (to 0.01 g), and liver (to 0.1 g, in species with a discrete liver) of each fish were determined with electronic pan balances that were calibrated daily before and after sampling. Livers were weighed with the gall bladder intact to avoid bile contamination of the sample collected for EROD analysis. Livers of carp, which are dispersed, were not weighed.

Histopathology (Including Macrophage Aggregates): Kidney, spleen, liver, gill, and gonadal tissues were preserved in 125-mL polyethylene bottles (one per fish) containing approximately 85 mL of NoToXhisto™ preservative (Table 1-3). At least five 1-cm³ pieces of liver were collected from distinct hepatic nodules (species with a dispersed liver) or from separate regions of the liver (species with a discrete liver). Five 1-cm³ pieces of gonad were collected and preserved from the anterior, middle, and posterior areas. One piece of posterior kidney (1-cm³), as much of the anterior kidney as possible, and the entire spleen (or a 1-cm³ piece if the spleen was larger than 0.8 g) were also preserved. In addition, and as described above, any grossly visible external and internal abnormalities (for example, lesions, parasites, nodules) were excised and placed in preservative along with a corresponding sample of normal tissue for comparison. Upon completion of sampling, the bottles were completely filled with preservative and shipped to the cooperating laboratory for histopathological processing and analysis. Kidney, spleen, liver, and gonad samples were used in histological examinations of fish health. Spleen samples were also used for macrophage aggregate

quantification. Laboratory methods for these endpoints are described in Chapter 3 of this report.

Reproductive Hormones, Vitellogenin, and Lysozyme

Activity: Plasma samples were analyzed for concentrations of 17 β -estradiol, testosterone, 11-ketotestosterone, and vitellogenin, and for lysozyme activity (Table 1-3). In the field, 3-5 mL of blood were obtained from the posterior caudal vessels of each fish using a heparinized (100 IU/mL) 5-mL syringe equipped with a 20-gauge needle. After removing the needle, the blood was transferred to a chilled, heparinized Vacutainer® and stored on wet ice. When either several or all fish from a station had been processed, blood samples were centrifuged at 3000 rpm (Centrif Model 228, Fisher-Scientific) for 10 min and the plasma was aspirated with a disposable pipette. At least 0.75 mL of plasma from each fish was transferred into each of two 2.0-mL Cryovials®, which were then quick-frozen in a previously prepared dry ice-ethanol slush. Typically, blood samples were centrifuged and plasma aspirated within 1 h of blood collection. Once frozen, the plasma samples were transferred from the dry ice-ethanol slush to a cooler containing dry ice. Upon completion of sampling, plasma samples were shipped (overnight mail, on dry ice) to the cooperating laboratories and stored at -80 °C. Laboratory methods used to conduct the reproductive hormone and vitellogenin analyses are presented in Chapter 4. Lysozyme methods are described in Chapter 3.

EROD Activity: Pieces (1 cm³) of liver from each fish were placed in two 1.2-mL Cryovials®, each of which was filled to approximately 0.6 mL. Liver samples were immediately frozen in the previously prepared dry ice-ethanol slush (Table 1-3). At the end of the day, the samples were transferred to a cooler containing dry ice. Following the completion of sampling, samples were shipped (overnight mail, on dry ice) to the cooperating laboratory for EROD analysis, where they were stored at -80 °C. Further details on the processing and analysis of liver samples for EROD activity are provided in Chapter 2.

Chemical Analysis and H4IIE: Following the health assessment and the collection of tissues and fluids for biomarker analyses, all remaining parts of the fish were wrapped in the foil on which the fish was processed and chilled on wet ice (Table 1-3). After all fish had been collected at a station and the species and gender of all specimens were confirmed (and gonadal tissue collected for histological verification), fish were composited by species and gender for chemical analysis and the H4IIE bioassay, double-bagged in polyethylene, and chilled. Individuals of unknown

gender were composited separately from others of the same species until gender was confirmed histologically. Upon return from the field, the chilled composite carcass samples were stored frozen (-20°C) and shipped frozen to the lead analytical laboratory (organic analysis) for processing and analysis. Results of residue chemistry and H4IIE analyses on carcass samples approximated whole fish concentrations minus approximately 5 mL of blood, 5-8 1-cm³ pieces of liver, five 1-cm³ gonad pieces, the entire spleen, and a 1-cm³ piece of both the posterior and anterior kidneys. Although many of these tissue pieces were not weighed, we estimate that the total mass of tissues not included in the carcass analyses constituted <1% of the mass of the fish. Analyses conducted on the composite carcass samples are described in Chapter 2.

Age: Scales were collected from the left side of the fish, from the area of the appressed pectoral fin of spiny-rayed fish (for example, bass) and from beneath the anterior portion of the dorsal fin, above the lateral line, of soft-rayed fish (carp, suckers, etc., Jearld, 1983). For channel catfish (*Ictalurus punctatus*), the entire disarticulated pectoral spine was collected. Scales or spines were stored in Whirlpak[®] bags or scale envelopes for later examination. Fish age was estimated from scales and spines by the field cooperators. Although exact methods varied, they generally followed the procedures described by Tesch (1968), which included cleaning the structures and observing the number of completed annuli under magnification (that is, dissecting microscope or equivalent). For some sites, acetate scale impressions made with a scale press were read.

Scales from carp collected in the EIB Study Unit (Stations 205, 206, 210, and 211) were not available to read. Ages of these fish were estimated using regression analysis based on the ages and lengths of different carp collected concurrently by NAWQA personnel at Stations 206, 210 and 211, which were aged using the distance-annuli method (Tesch, 1968). The relationships between age and TL were established separately for male ($n=11$) and female ($n=17$) fish from these three stations using the Forecast function of Microsoft Excel. The age of each un-aged fish was then estimated from its TL using the appropriate function. Estimated ages were rounded down to the nearest year for comparison; they were not included in the computation of means, however.

STATISTICAL ANALYSES

Because of the large area sampled and the temporal variability inherent in many of the biological

variables, it was necessary to adjust or otherwise account for spatio-temporal bias in comparisons among or between stations. Differences in taxa, sex, gonadal stage, age, and size of fish, as well as spatio-temporal differences, can potentially confound station comparisons. For variables measured on individual fish, the primary question we attempted to answer was whether biomarker levels differed between sampling locations (that is, stations). Descriptive statistics were computed and presented graphically for all variables and all data. We attempted to account for these sources of variation both statistically, by testing and adjusting for significant factors and covariates; and non-statistically, by restricting comparisons to smaller groups of samples in situations where confounding effects were evident. Rigorous statistical testing was only conducted for carp and bass. To partly control for bias associated with the reproductive cycle, comparisons were further restricted by eliminating immature fish and those in advanced stages of gonadal development from the statistical analysis when indicated by preliminary analysis.

To further account for spatio-temporal variability, we grouped the stations into geographic "regions" (that is, sub-basins), as recommended by Goodbred and others (1997), and by program of origin (Table 1-1). NCBP stations were grouped as follows: Arkansas-Red River (ARR) sub-basin (Stations 29, 77, 78, 79, and 82); Lower Missouri River (LMR) sub-basin (Stations 31, 83, 86, 89, and 90); Upper Missouri River (UMO) sub-basin (Stations 32, 84, and 85); Lower Mississippi River (LMS) sub-basin (Stations 15, 28, 30, 75, 76, 80, and 81); and Ohio River (OHR) sub-basin (Stations 23, 24, 25, 67, 68, 70, and 71). NAWQA sites were assigned numbers from 201-213 and grouped by Study Unit, as follows: Eastern Iowa Basins (EIB, Stations 205, 206, 209, 210, and 211), which is wholly contained within the UMS sub-basin; and Mississippi Embayment (MSE, Stations 201, 202, 203, 204, 207, 208, 212, and 213), which lies entirely within the LMS sub-basin. NCBP and NAWQA stations in their respective sub-basins could then be compared to each other and to the reference site (Station 400), and higher-level comparisons (that is, between and among sub-basins and programs) could be made without bias attributable to the presence or absence of NAWQA sites within a sub-basin. Given the designs of the NCBP and NAWQA programs, NCBP vs. NAWQA contrasts are effectively comparisons based on stream order and basin size.

Depending on the nature of the response variable (that is, biomarker), we used several statistical methods to compare stations and groups of stations. For measured and computed variables with continuous distributions (EROD activity, vitellogenin, relative organ weights, etc.) we used a nested ANOVA

(Keuhl, 1994) to compare responses among stations within regions, among regions within programs, and between programs; station and region were both considered fixed effects in these analyses. The significance of additional explanatory variables (for example, age, weight, and histologically determined gonadal stage) was also investigated using regression and correlation analyses. The actual terms included in each model for testing depended on the biomarker being analyzed. Lacking a more complete dataset, modeling of these additional explanatory terms and their interactions could only be carried out on subsets of the data. It is important to note that by using this approach we implicitly assumed that what was found for the subsets also held for the entire dataset. Compliance with distributional and other assumptions was evaluated by examining residual plots and with formal tests when possible [for example, Levene's test for homogeneity of variance in one-way ANOVA models (Ramsey and Shafer, 1997)]. Data were log (base e)- or rank-transformed (Conover, 1999) as necessary when standard ANOVA assumptions were not met. Significant ANOVA F -tests were followed by the Tukey-Kramer multiple comparison procedure (Ramsey and Shafer, 1997).

Unlike most of the biomarkers we evaluated, "normal" values are known (or at least presumed) for the ratio of the plasma concentrations of the reproductive hormones [that is, 17β -estradiol to 11-ketotestosterone (E2/KT)]; E2/KT in females is typically >1.0 , whereas in males it is <1.0 (Goodbred and others, 1997). Accordingly, log-transformed E2/KT values in the reduced-rank data sets were analyzed using one-sample, one-tailed t -tests in which the null hypothesis that each station mean E2/KT = 1.0 was evaluated against the alternative hypotheses of E2/KT >1.0 for males and <1.0 for females.

Some biomarkers (for example, the presence or absence of external lesions; vitellogenin in males) had to be analyzed as binary variables. For these, we compared the proportions of fish with and without the feature being analyzed among stations and regions using Fisher's Exact Test. This test is typically used to determine whether the row and column variables of 2-by-2 contingency tables are independent; however, it is also appropriate in some situations (for example, with product binomial sampling) for a test of equal population proportions (Ramsey and Shafer, 1997). All P -values were adjusted for multiple comparisons using a Bonferroni procedure (Keuhl, 1994).

For variables measured on composite samples (that is, concentrations of organochlorine and elemental contaminants and H4IIE bioassay results) we used an approach similar to the one used for individual fish; however, due to the small sample sizes and large numbers of censored values for many analytes, we did not test statistically for differences

among stations. Instead, we tested for differences among sub-basins and between programs using appropriately transformed variables where there were sufficient uncensored data. For small numbers of censored values ($<15\%$), we substituted half the nominal detection limit. No formal statistical tests of significance were conducted for some highly censored variables (that is, most of the organochlorine chemical residues). Where possible, we also compared concentrations of organochlorine chemicals and elemental contaminants at NCBP sites to historic values (Schmitt and others, 1999b).

Deducing the cause or causes of observed biological responses was not an objective of this study. We did, however, conduct preliminary exploratory statistical analyses to identify simple relations between pairs of variables by computing and examining rank correlation coefficients to test for monotonic relationships between concentrations of selected contaminants measured in composite samples and biomarkers determined for individual fish. Spearman's rho (as opposed to Pearson's r) was computed because the data for many variables contained censored values, outliers, or both; failed to meet normality assumptions; or possessed combinations of these traits. For the correlation analyses, concentration values for composite samples were paired with biomarker medians representing the fish comprised by the corresponding composite sample. Concentrations of cyclodiene pesticides (dieldrin, endrin, heptachlor epoxide, and chlordane constituents) were summed because many of the individual concentrations were below detection limits. In these analyses E/KT was treated as a binary response (1 for males >1.0 and females <1.0 ; 0 for males <1.0 and females >1.0). Because of the differences among species and genders noted for many biomarkers, correlation analyses were performed separately for male and female carp and bass; eight mixed-gender composites were excluded from these analyses to avoid bias. Geometric mean concentrations were then computed for each station and taxon (that is, mean concentrations in males and females of each species at each station) and paired with the corresponding biomarker medians. These statistics were chosen because they were the most universally representative indicators of central tendency for their respective groups; biomarkers were inconsistent with respect to distributional assumptions whereas geometric means are typically used to characterize carcass concentrations (Schmitt and others, 1999b). The means and medians for male and female carp and bass were then averaged to obtain a single value for each species at each station, and another set of Spearman correlation coefficients were computed for all carp and all bass. The mixed-gender composites were included in these station-level analyses, which were not performed for reproductive biomarkers.

because they respond differently in each sex.

DATASET COMPOSITION

Although documenting the distribution, abundance, size, and other attributes of fishes in the rivers and streams of the MRB was not an objective of the 1995 study, these factors must be considered in the interpretation of the chemical and biological endpoints measured (Schmitt and Dethloff, 2000). Accordingly, we present an overview of the distribution of the fishes collected, and summarize the sizes ages of carp and bass. A more detailed presentation of this data, including information on other taxa, is contained in Appendix A of this report.

Geographic Distribution of the Fishes Collected

A total of 1378 fish representing 22 species was collected from the 48 stations sampled (34 NCBP sites, 13 NAWQA sites, and Station 400, the reference site; Table 1-4). Although selected for sampling, no fish were collected from NCBP Station 69 (Ohio R. at Cincinnati, OH). Together, the two primary target species (carp and largemouth bass) accounted for 82%

of the total (1130 individuals), and bass (all *Micropterus* spp.) and carp together (1224 fish) represented 89% (Table 1-4). Each of the other 19 species collected represented <2.5% of the total (Table 1-4).

Carp were collected at 46 of the 48 stations (96%; Fig. 1-2, Table 1-4); they were not collected at Station 74 (Mississippi R. at Little Falls, MN), and only largemouth bass were targeted by NAWQA at Station 213. At sites where carp were collected, both males and females were caught at all stations except Station 23, which was represented by only a single male (Table 1-5). Largemouth bass were collected at 25 sites (52%—Stations 15, 23-30, 32, 68, 70-71, 76-83, 112, 212-213, and 400; Fig. 1-3). The lower number for this species resulted partly from the targeted collection of only carp at 11 of the 13 NAWQA sites and partly because largemouth bass are not distributed throughout the MRB. At sites that yielded largemouth bass, both males and females were collected at all but Stations 15, 23, and 32, from which only females were collected (Table 1-5). Smallmouth bass (*Micropterus dolomieu*) of both sexes were caught at five sites (10%—Stations 24, 67, 72, 74, and 111; Fig. 1-2, Table 1-5). At Stations 67, 72, 74, and 111 smallmouth bass were the only black bass captured whereas at Station 24 largemouth bass were also collected (Fig. 1-3, Table 1-5). If the NAWQA sites at which only one species was targeted

Table 1-4. Fishes collected in the Mississippi River basin and at the reference site.

Species	Number collected	Number of stations ¹	Taxon grouping
Black crappie	1	1	Sunfish
Brown trout	10	1	Trout
Burbot	1	1	Burbot
Channel catfish	6	2	Catfish
Common carp	777	46	Carp
Goldeye	33	2	Goldeye
Largemouth bass	353	25	Bass
Largemouth X spotted bass	1	1	Bass
Northern pike	5	1	Pike
Quillback carpsucker	1	1	Sucker
Rainbow trout	7	1	Trout
River redhorse	2	2	Sucker
Sauger	16	3	Percid
Smallmouth bass	72	5	Bass
Smallmouth buffalo	15	2	Sucker
Spotted bass	21	6	Bass
Striped bass	4	1	<i>Morone</i>
Walleye	4	1	Percid
Striped X white bass	3	1	<i>Morone</i>
White bass	31	3	<i>Morone</i>
White crappie	2	1	Sunfish

¹48 total stations including the reference site

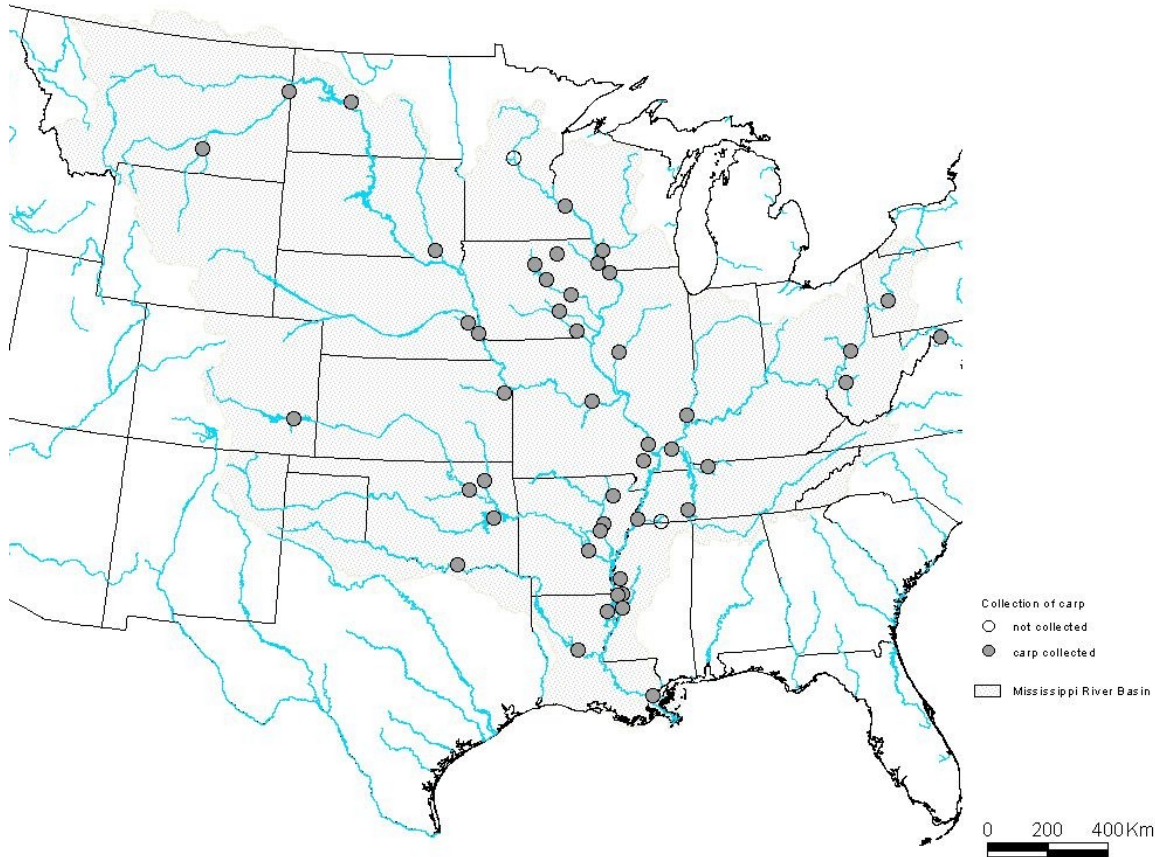


Figure 1-2. Stations from which carp (*Cyprinus carpio*) were collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations.

(all except Station 212) are eliminated from the total, carp were collected at 35 of 36 stations (97%), largemouth bass at 24 of 36 (67%), and smallmouth bass at five of 36 (14%).

Among the other species collected in substantial numbers (>10 individuals), spotted bass (*Micropterus punctulatus*) were collected at six sites (Stations 23, 24, 25, 68, 78, and 83), but at no site were they the only black bass collected (Fig. 1-2, Table 1-5). Male and female spotted bass were collected at all of these except Station 78 (one male). Other predator species represented by 10 or more fish included white bass (*Morone chrysops*), which were collected at Stations 15 (both males and females), 68 (females only), and 75 (males and females); goldeye (*Hiodon alosoides*), of which both sexes were collected at Stations 85 and 86; and sauger (*Stizostedion canadense*), collected at Stations 73 (both sexes), 85 (males and females) and 84 (one male). Benthivorous fishes represented by 10 or more individuals included white sucker (*Catostomus commersoni*), which were collected exclusively at Station

74 (both males and females), and smallmouth buffalo (*Ictiobus bubalus*), which were captured at Stations 23 and 68 (both sexes). For the species represented by ten or fewer individuals, we noted a few groupings of interest: Brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), and burbot (*Lota lota*) were collected only at Station 84; white crappie (*Poxomis annularis*) and black crappie (*P. nigromaculatus*) were collected together at Station 68; and walleye (*Stizostedion vitreum*) and northern pike were collected together at Station 32.

When grouped by higher-level taxon (Table 1-5), the following distributions were noted: Carp, northern pike, goldeye, burbot, catfishes (Ictaluridae), trouts (Salmonidae), and sunfishes (Centrarchidae other than *Micropterus*) were not changed from the distribution of their composite species because the first five taxa each comprise only one species and the last two each comprise two species that were collected together (Table 1-5). White basses (*Morone* spp.) were collected only at Stations 15, 68, and 75 (corresponding to the distribution of the most widespread

Table 1-5. Numbers of each species collected in 1995, by station, species, and gender.

Sub-basin and station number	Species	Females	Males	Species total	Station total
Arkansas-Red River (ARR)					
29					35
	Carp	11	9	20	
	Largemouth bass	7	8	15	
77					36
	Carp	11	7	18	
	Largemouth bass	8	10	18	
78					38
	Carp	10	9	19	
	Largemouth bass	9	8	17	
	Largemouth X spotted bass	1	0	1	
	Spotted bass	0	1	1	
79					42
	Carp	10	10	20	
	Largemouth bass	12	10	22	
82					50
	Carp	13	11	24	
	Largemouth bass	13	13	26	
Lower Missouri R. (LMO)					
31	Carp	12	11	23	23
83					32
	Carp	6	9	15	
	Largemouth bass	7	6	13	
	Spotted bass	1	3	4	
86					40
	Carp	10	10	20	
	Goldeye	10	10	20	
89	Carp	2	7	9	9
90	Carp	10	10	20	20
Upper Missouri R. (UMO)					
32					30
	Carp	9	11	20	
	Largemouth bass	1	0	1	
	Northern pike	3	2	5	
	Walleye	2	2	4	
84					41
	Brown trout	6	4	10	
	Burbot	0	1	1	
	Carp	12	8	20	
	Channel catfish	0	2	2	
	Rainbow trout	7	0	7	
	Sauger	0	1	1	
85					40
	Carp	12	8	20	
	Channel catfish	2	2	4	
	Goldeye	7	6	13	
	Sauger	1	2	3	
Lower Mississippi R. (LMS)					
15					22
	Carp	8	2	10	
	Largemouth bass	4	0	4	
	Striped bass	1	0	1	
	White bass	5	2	7	

Table 1-5. Numbers of each species collected in 1995, by station, species, and gender--Continued.

Sub-basin and station number	Species	Females	Males	Species total	Station total
28					39
	Carp	9	10	19	
	Largemouth bass	10	10	20	
30					36
	Carp	7	10	17	
	Largemouth bass	10	9	19	
75					41
	Carp	10	10	20	
	White bass X Striped bass	3	0	3	
	White bass	8	10	18	
76					35
	Carp	9	8	17	
	Largemouth bass	10	8	18	
80					15
	Carp	5	7	12	
	Largemouth bass	2	1	3	
81					36
	Carp	8	4	12	
	Largemouth bass	17	7	24	
Upper Mississippi R. (UMR)					40
26					
	Carp	10	10	20	
	Largemouth bass	10	10	20	
27					40
	Carp	10	10	20	
	Largemouth bass	10	10	20	
72					38
	Carp	10	12	22	
	Smallmouth bass	12	4	16	
73					32
	Carp	10	10	20	
	Sauger	10	2	12	
74					34 ¹
	River redhorse	0	1	1	
	Smallmouth bass	10	7	17	
	White sucker	9	6	16 ¹	
111					42
	Carp	10	10	20	
	Smallmouth bass	11	11	22	
112					40
	Carp	10	10	20	
	Largemouth bass	10	10	20	
Ohio R. (OHR)					
23					19
	Carp	0	1	1	
	Largemouth bass	1	0	1	
	Smallmouth buffalo	6	5	13 ¹	
	Spotted bass	3	1	4	
24					17
	Carp	2	3	5	
	Largemouth bass	2	2	4	
	Quillback carpsucker	0	1	1	
	River redhorse	0	1	1	
	Smallmouth bass	3	1	4	

Table 1-5. Numbers of each species collected in 1995, by station, species, and gender--Continued.

Sub-basin and station number	Species	Females	Males	Species total	Station total
25	Spotted bass	1	1	2	21
	Carp	2	2	4	
	Largemouth bass	4	5	9	
	Spotted bass	5	3	8	
67	Carp	6	5	11	24
	Largemouth bass	9	4	13	
68	Black crappie	0	1	1	39
	Carp	9	8	17	
	Largemouth bass	4	5	9	
	Smallmouth buffalo	1	1	2	
	Spotted bass	1	1	2	
	White bass	6	0	6	
	White crappie	1	1	2	
70	Carp	5	6	11	35 ¹
	Largemouth bass	9	14	24 ¹	
71	Carp	5	10	15	27
	Largemouth bass	3	9	12	
Eastern Iowa Basins (EIB)					
205	Carp	10	10	20	20
206	Carp	10	10	20	20
209	Carp	3	5	8	8
210	Carp	10	10	20	20
211	Carp	10	10	20	20
Mississippi Embayment (MSE)					
201	Carp	9	8	17	17
202	Carp	10	10	20	20
203	Carp	8	10	18	18
204	Carp	5	10	15	15
207	Carp	8	10	18	18
208	Carp	10	10	20	20
212					23
213	Carp	10	10	20	11
	Largemouth bass	1	2	3	
	Largemouth bass	4	7	11	
Reference Site					
400					39
	Carp	11	8	19	
	Largemouth bass	10	10	20	

¹Total includes individual of unknown gender.

member, white bass); both sexes were collected at Stations 15 and 75 but only females were obtained at Station 68. Suckers were collected at Stations 23, 24, 68 and 74; both males and females were captured at all stations except Station 24 (males only). *Stizostedion* spp. were obtained from Stations 32, 73,

and 85 (males and females) and at Station 84 (one male). Bass were collected from 29 sites (60%—Stations 15, 23-30, 32, 67, 68, 70-72, 74, 76-83, 111-112, 212-213, and 400; Fig. 1-3). Both male and female bass were collected from all Stations except 15 and 32, from which only females were obtained.

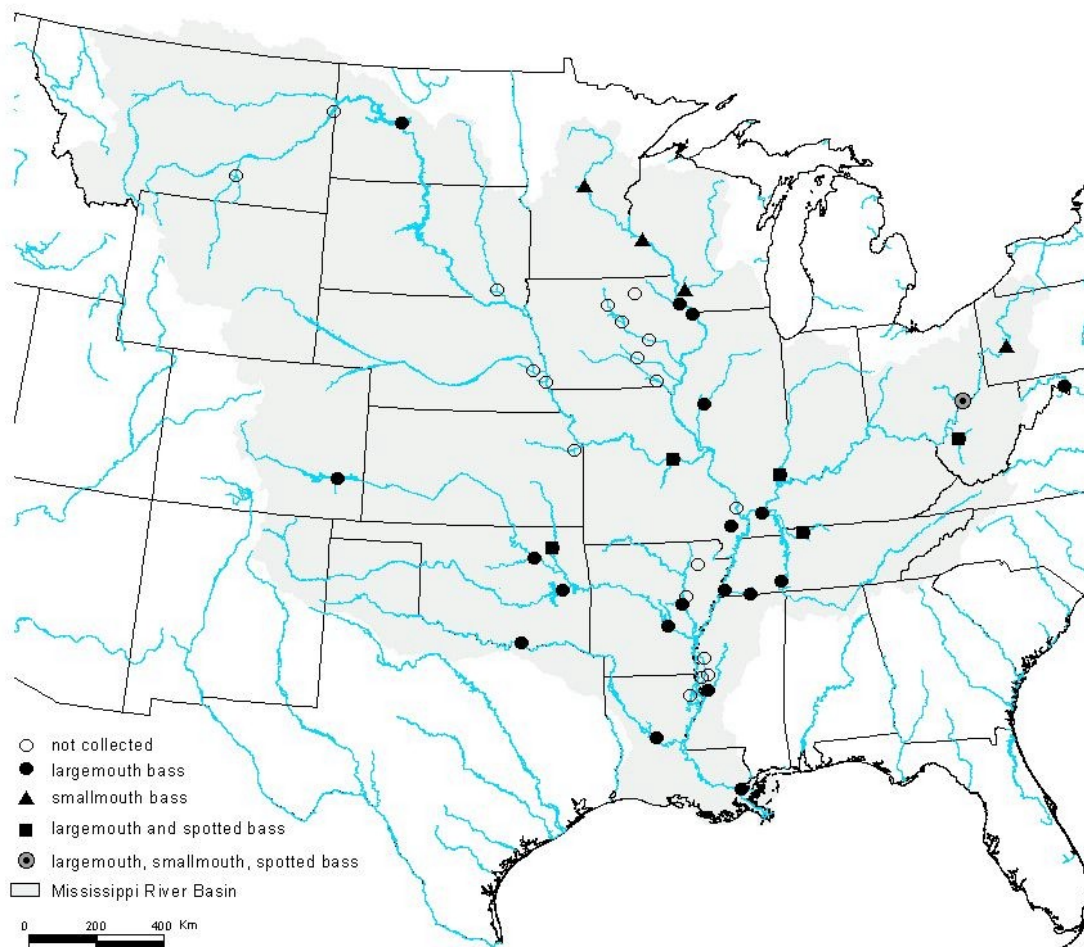


Figure 1-3. Stations from which bass (*Micropterus* spp.) were collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations.

The 1995 species makeup was identical to past collections at most of the 34 NCBP stations sampled in the MRB (Stations 25, 27-31, 67, 70-73, 79, 82, 86, 89, 90, and 112; Appendix A). Differences were as follows: Carp were collected at Stations 26, 76, 77, 80, 81, 83 and 111 both historically and in 1995, but the 1995 predator species differed. At Stations 15, 68, 75, 78, and 84, two or three species collected historically were also collected in 1995, but additional species accounted for a small percentage of captured individuals. At Station 23, where carp and largemouth bass had previously been collected, most of the 1995 fish were smallmouth buffalo and spotted bass; only one largemouth bass and one carp were collected in 1995. At Station 74, white suckers were collected exclusively in the past, but in 1995 smallmouth bass were also collected. At Station 85, carp

and sauger were collected in the past. Although three saugers (and three channel catfish) were also collected in 1995, most of the 1995 fish from Station 85 were carp and goldeye. Finally, although largemouth bass and carp were collected at Station 24 in past collections and in 1995, these two species accounted for only small percentage of the 1995 fish collected at this station. Overall, the differences were minor; the composition of the 1995 collection is sufficiently consistent relative to past collections to allow for temporal comparisons of chemical concentrations on a species-by-species basis at many sites, as recommended by Schmitt and others (1999b).

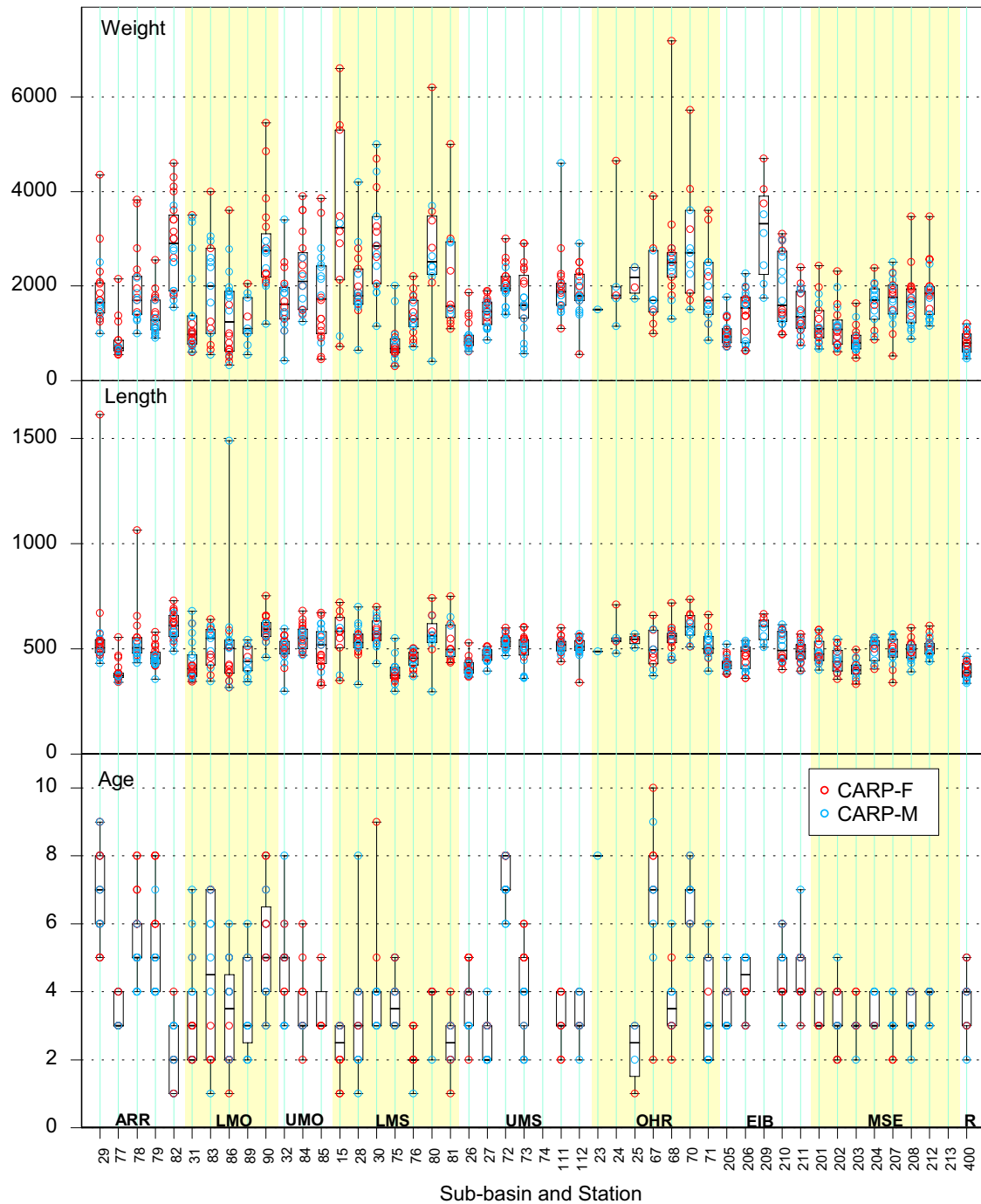


Figure 1-4. Weight, total length, and age of male (M) and female (F) carp collected in 1995 (1996 for Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station and sub-basin locations.

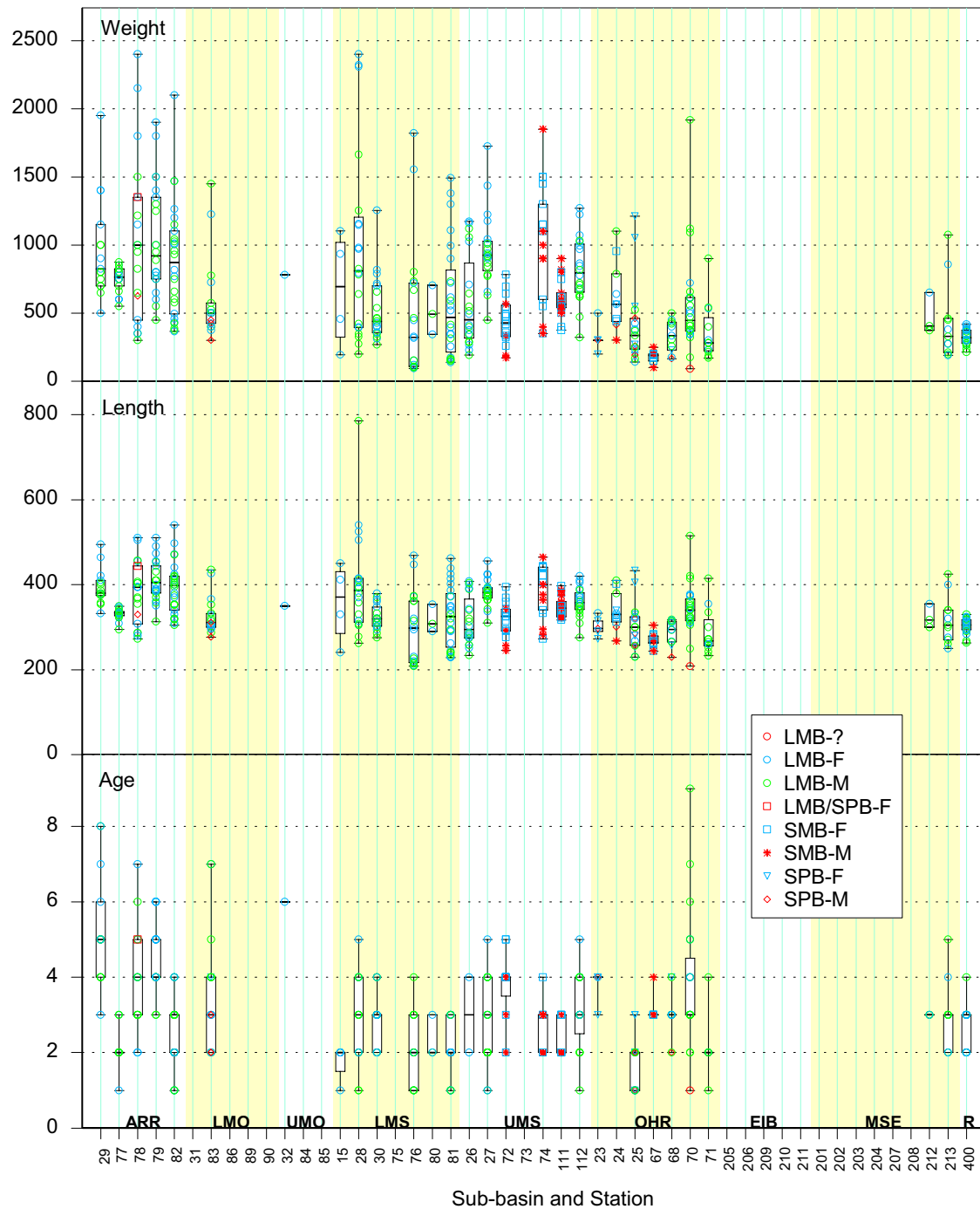


Figure 1-5. Weight, total length, and age of male (M) and female (F) bass (*Micropeterus* spp.) collected in 1995/1996 for Station 400). LMB, largemouth bass (*M. salmoides*); SMB, smallmouth bass (*M. dolomieu*); SPB, spotted bass (*M. punctulatus*). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Table 1-6. Lengths, weights, and ages of common carp, by sub-basin.

Sub-basin	Gender	Number of stations	Total Length (mm)			Weight (g)			Age (y)	
			Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)
Arkansas-Red R. (ARR)	All	5	515	390-608	342-1614	1773	825-2871	550-4600	4.7	2.1-7.0
	F	5	551	404-635	342-1614	2047	928-3292	550-4600	5.0	2.2-7.0
	M	5	472	369-576	347-670	1453	663-2373	600-3700	4.3	2.1-6.9
Lower Missouri R. (LMO)	All	5	500	439-596	316-1489	1761	1267-2838	325-5450	4.0	3.2-5.4
	F	5	486	409-619	316-752	1779	1077-3238	400-5450	4.0	2.5-5.8
	M	5	518	429-594	319-1489	1797	1143-2438	325-3450	4.2	3.4-5.0
Upper Missouri R. (UMO)	All	3	520	496-555	299-681	1915	1690-2272	420-3900	3.9	3.5-4.7
	F	3	519	479-572	328-681	1970	1538-2603	450-3900	4.0	3.5-4.6
	M	3'	523	489-551	299-619	1850	1625-2150	420-3400	3.9	3.0-4.8
Lower Mississippi R. (LMS)	All	7	516	388-580	297-750	2219	790-3394	304-6602	3.1	2.1-4.2
	F	7	524	375-609	344-750	2404	663-3710	304-6602	3.3	2.1-5.3
	M	7	504	401-566	297-700	1944	918-2773	336-4996	3.0	1.8-3.7
Upper Mississippi R. (UMS)	All	6	491	413-534	340-605	1661	930-2016	555-4600	4.0	2.5-7.2
	F	6	506	421-552	340-605	1834	1053-2255	555-3000	4.2	2.6-7.6
	M	6	476	405-519	360-578	1495	806-1975	566-4600	3.8	2.5-6.9
Ohio R. (OHR)	All	7'	538	488-606	373-736	2192	1500-2893	850-7181	5.2	2.3-8.0
	F	6'	572	519-640	429-736	2686	1975-3304	1000-7181	4.8	2.0-7.0
	M	7'	520	472-578	373-620	1947	1500-2550	850-3600	5.2	2.5-8.0
Eastern Iowa Basins (EIB)	All	5'	496	427-594	360-665	1790	1005-3170	629-4700	4.2	3.5-4.6
	F	5'	503	422-645	360-665	1990	968-4166	629-4700	4.2	3.7-4.6
	M	5'	493	432-563	383-615	1671	1042-2572	650-3512	4.2	3.3-4.6
Mississippi Embayment (MSE)	All	7	475	403-510	332-610	1446	872-1870	479-3474	3.3	3.0-3.8
	F	7	478	405-528	332-610	1544	942-2150	479-3474	3.2	2.7-3.9
	M	7	470	401-509	362-580	1337	816-1768	649-2505	3.3	2.9-3.7

Lengths, Weights, and Ages of Carp and Bass

Length, weight and age data for each species were examined for extremes in variation and overall consistency across stations. Observational comparisons were not made for species that were found at only one station or had less than three stations with more than two individuals (for example, white sucker, small-mouth buffalo, sauger, goldeye). Here we describe the size and age of carp and bass. A more detailed examination of the size and age data for all taxa is presented as Appendix A.

Most carp were 300-750 mm long, with four individuals >1000 mm (Fig. 1-4, Table 1-6). Examination of the scatter of points revealed no notable differences among stations. Weight was more variable than length for carp, but most fish weighed 500-5000 g; ten individuals weighed 300-500 g; and eight were 5000-7500 g. Variability was noticeably greater than most for Station 15; conversely, variation was low at Station 25, but no other stations were noteworthy (Fig. 1-4). Carp from most stations were 2-6 or 7 y old; ages were most variable at Stations 67 and 83.

Most largemouth bass were 200-600 mm long; only two were 600-800 mm (Fig. 1-5, Table 1-7). No station had inordinately high or low variation in TL. As noted for carp, weights were more variable than lengths; most were 100-1500 g. The greatest variation in largemouth bass weight was at Stations 28 and 78. Variation in age was relatively consistent, with most largemouth bass 2-6 y old (Fig. 1-7). Stations 77 and 212 had comparatively low variation, but only three bass were collected at Station 212 (Table 1-7). For smallmouth bass, which were typically the smallest of the black basses, only the weights for Station 74 seemed to vary more than average, as did weights of spotted bass from Station 25 (Fig. 1-7). The latter was due to two fish that weighed more than 1000 g; all other spotted bass weighed less than 650 g.

The reference site (Station 400) yielded largemouth bass and carp that were, on average, smaller than those from most MRB stations (Figs. 1-4, 1-5 Tables 1-6, 1-7). However, the mean ages of both species were similar to the respective MRB-wide means. Stations 24 (Ohio R. at Marietta, OH) and 67 (Allegheny R. at Natrona, PA) were the nearest (geographically) locations to Station 400 from which more than one largemouth bass or carp was collected. Carp from both stations equaled or exceeded the MRB-wide mean for both TL and weight. Largemouth bass from Station 24 also exceeded the MRB-wide means. These observations suggest that growth is not slower in this geographic area compared to the rest of the MRB. A mean age was only available for carp from Station 67; the average age for these fish was 6.9

Table 1-6. Lengths, weights, and ages of common carp, by sub-basin—Continued.

Sub-basin	Gender	Number of stations	Total Length (mm)			Weight (g)			Age (y)		
			Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)	Range (individual fish)
Reference	All	1	399		337-464	824		463-1205	3.8		2-5
	F	1	406		362-442	875		584-1205	4.0		3-5
	M	1	389		337-464	753		463-1130	3.4		2-5

Mean age calculated with one less station.

Table 1-7. Lengths, weights, and ages of bass (*Micropterus* spp.), by sub-basin.

Sub-basin	Gender	Number of stations	Total Length (mm)			Weight (g)			Age (y)		
			Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)	Range (individual fish)
Arkansas-Red R. (ARR)	All	5	381	331-408	272-540	935	751-1043	300-2400	3.7	2.1-5.2	1-8
	F	5	393	332-419	280-540	1043	741-1240	350-2400	3.8	2.0-5.4	1-8
	M	5	369	331-394	272-470	824	759-944	300-1500	3.5	2.2-5.0	1-8
Lower Missouri R. (LMO)	All	1	327		277-435	591		300-1450	3.6		2-7
	F	1	329		296-426	581		375-1225	3.5		2-7
	M	1	325		277-435	600		300-1450	3.7		2-7
Upper Missouri R. (UMO)	All	1	350			780			6.0		
	F	1	350			780			6.0		
Lower Mississippi R. (LMS)	All	6	337	297-400	208-785	627	489-980	94-2400	2.4	1.8-3.0	1-5
	F	6	347	308-419	210-540	723	524-1286	94-2400	2.4	1.8-3.4	1-5
	M	5	315	285-381	208-785	469	361-673	100-1662	2.4	1.8-3.1	1-4
Upper Mississippi R. (UMS)	All	6	352	315-386	234-465	735	439-1009	172-1850	2.9	2.4-3.8	1-5
	F	6	362	319-398	251-456	801	480-1102	228-1726	3.2	2.5-3.9	1-5
	M	6'	336	284-369	234-465	651	315-929	172-1850	2.6	2.1-3.3	1-4
Ohio R. (OHR)	All	7'	305	269-348	229-515	401	180-622	100-1918	2.9	1.6-3.9	1-9
	F	7'	307	267-341	231-433	394	176-603	142-1212	2.9	1.7-3.8	1-5
	M	7'	303	274-352	229-515	400	190-652	100-1918	3.0	1.6-4.1	1-9
Eastern Iowa Basins (EIB)	All	2	320	317-324	250-425	452	428-477	191-1075	3.0	3.0-3.0	2-5
	F	2	333	311-355	250-400	538	426-651	191-859	2.9	2.8-3.0	
	M	2	314	309-320	260-425	409	390-429	198-1075	3.1	3.0-3.1	2-5
Mississippi Embayment (MSE)	All	1	304		263-330	323		214-418	2.8		2-4
	F	1	308		284-325	352		278-418	2.7		2-3
	M	1	300		263-330	294		214-378	2.8		2-4
Reference	All	1	304		263-330	323		214-418	2.8		2-4

Table 1-7. Lengths, weights, and ages of bass (*Micropterus* spp.), by subbasin—Continued.

Sub-basin	Gender	Number of stations	Total Length (mm)			Weight (g)			Age (y)		
			Mean	Range (station means)	Range (individual fish)	Mean	Range (station means)	Range (individual fish)	Mean (station means)	Range (station means)	Range (individual fish)
	F	1	308		284-325	352		278-418	2.7		2-3
	M	1	300		263-330	294		214-378	2.8		2-4

¹Mean age calculated with one less station.

years. Their size was not substantially different relative to carp from other stations with similar-aged fish. Overall, largemouth bass and carp from the reservoir at Kearneysville, WV were younger, on average, than other fish collected in the area and appeared to be growing more slowly compared to fish of similar ages in MRB rivers. The efficacy of the reference site is addressed further in subsequent chapters of this report.

Certain trends in the sub-basin means for TL, weight, and age of carp and bass (all species, male, and female) were also evident. Carp from the OHR sub-basin were either greatest or second-greatest relative to other sub-basins in terms of mean TL, weight, and age (Tables 1-6 and 1-7). Carp from the MSE Study Unit, on the other hand, were among the smallest and youngest. Carp from the LMS sub-basin, in which the MSE Study Unit is contained, were relatively large on average, but were young (lowest or second lowest mean age). Both carp and largemouth bass from the reference site were, on average, the smallest fish, and were relatively young (Figs. 1-4, 1-5; Tables 1-6, 1-7). The magnitude of the sub-basin means for the length and weight of bass were similarly ordered for the combined sexes, females, and males: ARR > UMS > LMS or LMO (one station) > MSE (two stations) > OHR or reference. The regional means for age were not as consistent although the ARR and LMO sub-basins were always high and the LMS was the lowest for all bass, females, and males. Both bass and carp were largest, but youngest, at the NCBP sites in the LMS sub-basin. This could be related to high growth rates in a warmer section of the main basin; carp from the MSE Study Unit seemed to grow slower, however, suggesting that other factors were also involved. Although carp from the OHR sub-basin were comparatively large and old, the sub-basin means for bass indicated smaller fish (due in part to proportionately large representation of smallmouth and spotted bass). The ARR sub-basin was notable for its comparatively large, old largemouth bass (Table 1-7). The ARR means for female carp TL and weight were also in the upper third among sub-basins, but the means for TL and weight were not particularly high for male carp or all carp (Table 1-6). However, the ARR mean for carp age was highest or second highest. At the program level, carp and bass from the NCBP sites were, on average, longer, heavier, and older than those from NAWQA sites. Carp and bass from NCBP sites also had greater ranges of size and age.

General Observations

The fish collected in 1995 were relatively consistent among stations with respect to species, size, and age. Although we noted differences among stations in the

sizes and ages for each species, TL, weight, and age within species were generally within well-defined ranges. Fish size and age varied more at some stations than at others, but not at any one station for more than one species. At certain stations, a few relatively large or small individuals contributed to this result. Also, the number of individuals of each species collected at each station was not constant, so some degree of difference in variation among stations was expected.

Females were, on average, longer and heavier than males of all species for which 15 or more individuals were collected. With the lone exception of male sauger, which were typically older, females were also older than males. Generally, mean age and size did not correspond well across stations for the species collected in the 1995 study. This finding suggests that ages determined from scales or other structural components are more reliable than ages estimated from fish size, particularly for fish collections spanning large areas where conditions affecting growth can differ substantially.

Three or more largemouth bass and carp were collected together at 22 stations (Table 1-5). The ordering of those stations in terms of average size of largemouth bass or carp differed. The four stations at which both smallmouth bass and carp were collected also differed with respect to size trends for each species, as did the largemouth bass, spotted bass, and carp collected together at two stations. The size trends for white bass and carp across three stations also were not similar. Therefore, no station at which multiple species were collected stood out as having consistently small or large fish. These endpoints alone do not suggest further investigation of natural or xenobiotic factors at any station; however, together with other endpoints, this information could suggest or explain potential problems at certain stations (that is, impaired fish health).

In contrast with size, there were parallels in the ordering of stations in terms of mean ages among species. Stations with older largemouth bass often had older carp (relative to the MRB-wide mean for the species), and those with younger largemouth bass frequently had a lower mean age for carp. Similar age trends were found for carp and smallmouth bass, carp and white bass, and carp, spotted bass and largemouth bass. This suggests that the relative age at a station is somewhat consistent across species when compared to other stations in the MRB. Such a situation could make it easier to interpret physiological endpoints across stations for which age is a confounding factor.

Overall, the 1995 collection was more homogeneous (that is, more taxa in common to more stations) than in previous NCBP collections in the MRB, and the range of fish sizes and ages was acceptable given the spatial extent of the study. By permitting

other black basses as alternates to largemouth bass, it was possible to collect bass over a greater number of sites (60% vs. 52%). The three black bass species collected were not the same size, but the ages were similar. Size differences can be factored into interpretation of the endpoints, as needed, to determine whether these species yielded equivalent results. If the results of this study show that the collection of different species in this genus is acceptable for the endpoints being monitored, it will allow for a greater number of locations to be sampled.

Chapter 2. Accumulative Contaminants, H4IIE Bioassay-Derived Dioxin-Equivalents, and Ethoxyresorufin *O*-Deethylase (EROD) Activity

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INTRODUCTION

Environmental concentrations of mercury (Hg), lead (Pb), organochlorine pesticides, polychlorinated biphenyls (PCBs), and other persistent contaminants have declined compared to historic concentrations throughout most of the conterminous U.S. (Schmitt and others, 1999b). Nevertheless, and as documented in Chapter 1 (this report), concentrations of accumulative contaminants remain sufficiently high in some parts of the Mississippi River basin (MRB) to constitute a hazard to piscivorous wildlife. The analysis of fish tissues for accumulative contaminants consequently remains an important component of BEST and other monitoring programs.

The approach selected for monitoring the exposure of organisms to bioaccumulable contaminants had three objectives: (1) Maintain continuity with the historic NCBP data base on organochlorine chemical and elemental contaminant concentrations in samples of whole fish composited by species at each station. This objective was necessary for the documentation of temporal and geograph-

ic trends in the concentrations of accumulative contaminants, the first objective of the study. (2) Ensure that the suite of biological and chemical methods employed would detect exposure to the widest variety of organic and inorganic contaminants possible without redundancy and at lowest possible cost, which is an overarching objective of the BEST program (BEST, 1996). And (3) accommodate a variety of biological measurements and analyses (biomarkers) to gauge and evaluate the exposure of the fish to contaminants (including those that do not accumulate) and other environmental stressors without compromising objectives (1) and (2). Many biomarkers are species- and gender-specific, and are performed on individual fish. Therefore, biomarker analyses (field and laboratory) were performed on individual fish, which were then composited by station, species and gender for chemical analyses (organochlorine chemical residues and elemental contaminants). By averaging the male and female samples of each species at a station, comparisons with historic NCBP data, which were composited only by species, were facilitated. The organochlorine chemical and elemental contaminants analyzed in the composite samples and reported in this chapter are identified in Table 2-1.

Table 2-1. Organochlorine chemical and elemental contaminants measured in composite fish samples.

Contaminant class and analyte	Chemical name(s) or atomic symbol	Principal uses and sources to aquatic ecosystems
Organochlorine chemicals		
<i>p,p'</i> -DDE	2,2-bis (<i>p</i> -chlorophenyl)-1,1-dichloroethylene	DDT-metabolite
<i>p,p'</i> -DDD (TDE)	2,2-bis (<i>p</i> -chlorophenyl)-1,1-dichloroethane	Insecticide; DDT-metabolite
<i>p,p'</i> -DDT	2,2-bis (<i>p</i> -chlorophenyl)-1,1,1-trichloroethane	Insecticide
<i>o,p'</i> -DDE	2-(<i>o</i> -chlorophenyl)-2-(<i>p</i> -chlorophenyl)-1,1-dichloroethylene	<i>o,p'</i> -DDT metabolite
<i>o,p'</i> -DDD (TDE)	2-(<i>o</i> -chlorophenyl)-2-(<i>p</i> -chlorophenyl)-1,1-dichloroethane	<i>o,p'</i> -DDT metabolite
<i>o,p'</i> -DDT	2-(<i>o</i> -chlorophenyl)-2-(<i>p</i> -chlorophenyl)-1,1,1-trichloroethane	<i>p,p'</i> -DDT impurity
Total polychlorinated biphenyls (PCBs)	Mixture containing as many as 209 mono-through octa-chloro-substituted biphenyl congeners.	Dielectric, hydraulic, and transformer fluids; lubricants; extenders; de-dusting agents; carbonless copy paper
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,8,8a-hexahydro-1,4-endomno-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-octahydro-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
<i>cis</i> -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
<i>trans</i> -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
<i>cis</i> -Nonachlor	1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-	Technical chlordane constituent

Table 2-1. Organochlorine chemical and elemental contaminants measured in composite fish samples--Continued.

Contaminant class and analyte	Chemical name(s) or atomic symbol	Principal uses and sources to aquatic ecosystems
<i>trans</i> -Nonachlor	hexahydro-4,7-methano-1H-indene (1- α ,2- α ,3- α ,3a- α ,4- β ,7- β ,7a- α) 1,2,3,4,5,6,7,8-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)	<i>cis</i> -Chlordane metabolite
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 know 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
γ -HCH (Lindane)	1,2,3,4,5,6-hexachlorocyclohexane	Insecticide; technical HCH (BHC) constituent
Hexachlorobenzene (HCB)	Perchlorobenzene	Fungicide; industrial intermediate
Mirex	1,1a,2,2,3,3a,4,5,5a,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
Copper	Cu	Mining, smelting and other industrial sources
Lead	Pb	Mining, smelting and other industrial sources; urban runoff; atmospheric pollution; fishing sinkers; lead shot

Table 2-1. Organochlorine chemical and elemental contaminants measured in composite fish samples—Continued.

Contaminant class and analyte	Chemical name(s) or atomic symbol	Principal uses and sources to aquatic ecosystems
Mercury	Hg	Herbicides; pulp, paper, and textile effluents; open-cycle chloralkali cells; landfills; mining; atmospheric pollution
Selenium	Se	Coal-fired powerplants; irrigation return flows
Zinc	Zn	Mining, smelting and other industrial sources; urban runoff

As noted in Chapter 1, we evaluated a combination of biological and chemical methods for evaluating PHHs without using high-resolution instrumental analyses such as gas chromatography/mass spectrometry (GC/MS). Total PCBs were among the analytes included in the analysis of composite fish carcasses (Table 2-1). Solvent extracts from the composite samples were then screened for the presence of AHH-active compounds by the H4IIE rat hepatoma cell bioassay, an *in vitro* method for documenting the cumulative concentrations of planar PHHs (Tillitt and others, 1991; 1992). In the H4IIE assay, cultured H4IIE cells are exposed to sample extracts, and the activity of ethoxyresorufin *O*-deethylase (EROD), a cytochrome P450 dependent monooxygenase enzyme, is measured and compared to the EROD activity induced by a 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD) standard. Results are reported as dioxin-equivalents (TCDD-EQ), in pg/g wet-weight. EROD activity in H4IIE cells also responds to AHH-active PAHs. To remove the PAHs and other labile compounds, the extracts were subjected to a reactive, sulfuric acid cleanup (Schwartz and Lehmann, 1982) that does not affect PHHs and other recalcitrant compounds. In this manner, the H4IIE assay provides semi-quantitative information on the cumulative concentration of AHH-active PCBs, chlorodioxins, chlorodibenzofurans, and related compounds of concern (Table 2-2), and augments the information on total PCBs provided by the instrumental analysis of the fish carcasses. In addition to the instrumental analysis and H4IIE analyses of composite sample extracts, EROD activity was also measured in samples of liver from individual fish. Hepatic EROD in the fish can be induced by PHHs as well as by PAHs and other compounds removed from the composite sample extracts by the reactive cleanup (Pohl and Fouts, 1980; Whyte and others, 2000). Consequently, hepatic EROD documents the cumulative exposure of the fish to all AHH-active compounds, including the PAHs and other labile compounds that would not otherwise be accounted for by instrumental analysis or the H4IIE assay with reactive cleanup. By comparing EROD results with those from the instrumental analysis and the H4IIE assay, information on the classes of chemicals to which the fish were exposed can be obtained (Table 2-2). In addition, the cytochrome P450-dependent monooxygenase enzymes of fish respond to fewer PHHs than mammalian enzymes; in particular, some highly toxic mono-*ortho* substituted PCBs and their analogs from other chemical classes present in the carcass extracts may be detected by the H4IIE cells that would not induce hepatic EROD in the fish themselves (Whyte and others, 2000) and might thereby go undetected. With this three-component approach, a degree of causative assessment for planar aromatic contaminants

Table 2-2. Monitoring and assessment strategy for polycyclic aromatic and polyhalogenated hydrocarbons (PAHs and PHHs).

Endpoint	Contaminants		
	PCBs	PCDDs & PCDFs	PAHs ⁺
GC-ECD ¹ (carcass)	+	-	-
EROD ² activity (liver)	*	*	*
H4IIE assay (carcass) ³	*	*	-

¹Total PCBs by gas chromatography with electron-capture detection²7-ethoxysorufin *O*-deethylase³After reactive cleanup to remove AhR-active PAHs

*AhR-active isomers and congeners only

+And other planar organic compounds

(halogenated or not) was obtained even though no high-resolution instrumental analyses were performed.

METHODS AND MATERIALS

Field Procedures

Fish (nominally 40 per station) were captured by electrofishing and held alive until needed for processing (generally <4 h). Common carp (*Cyprinus carpio*, hereafter “carp”) and black basses (*Micropterus* spp., “bass”) were the preferred taxa, with alternate taxa permitted (see Chapter 1). In the field, each fish was processed as described in Chapter 1; they were identified to species, measured, weighed, and examined for grossly visible external lesions and pathologies. A blood sample (ca. 5 mL) was collected by caudal veinipuncture. The abdominal cavity was opened with a mid-ventral incision, and the internal organs were dissected from the fish for examination. The sex of the fish was determined by observation, and the internal organs were quickly examined for grossly visible lesions. The liver of fishes with a discrete liver (all but carp) was dissected from the remaining viscera and weighed. The liver (all fishes) was then cut into ca. 1-cm cubes from which two 1.2-mL Cryovials® were filled and flash-frozen in a dry-ice/ethanol bath, then stored in dry ice for shipment to the laboratory. These cryogenically preserved samples were analyzed for EROD activity. Five additional liver pieces were preserved for histopathological analysis. The gonads and spleen were dissected free of the viscera and weighed. Samples of gonad, kidney, and gill; the

entire spleen; and all grossly visible lesions were preserved for histopathological analysis (see Chapter 1). All remaining tissues and fluids were returned to the carcass, which was wrapped in foil, labeled for chemical analysis, and chilled. Individual fish were then composited by station, species, and gender; frozen; and shipped to the lead analytical laboratory (Lab 1).

Laboratory Analyses

Analyses of composite fish samples for organochlorine chemical residues and elemental contaminants (Table 2-1) were performed by contract laboratories (Labs 1 and 2) managed by the Patuxent Analytical Control Facility (PACF) of the U.S. Fish and Wildlife Service (FWS). Quality assurance (QA) oversight was provided by the PACF. Round-robin tests among PACF and contract analytical labs were also part of the quality control. Based on the QA program, PACF determined that the results of the contract laboratory analyses were acceptable. In keeping with past NCBP practice (Schmitt, 1999), results were not adjusted to reflect spike recoveries or moisture loss during storage. EROD assays and analyses of composites fish extracts for TCDD-EQ with the H4IIE rat hepatoma cell bioassay (Tillitt and others, 1989) were performed at the USGS Columbia Environmental Research Center (CERC), Columbia, MO. Details of the laboratory methods are presented in the following sections.

Composite Sample Preparation: Carcass samples were shipped to and stored frozen at Lab 1 until prepared for analysis. Individual fish carcasses were composited and homogenized (generally, by station, species,

and gender) by first band-sawing each fish into pieces, then grinding the pieces of all the fish together three times using a commercial meat grinder. Three sub-samples of each composite sample were prepared. Sub-sample 1 (100 g) was re-frozen and shipped frozen to the inorganic laboratory (Lab 2) for analysis of moisture content and elemental contaminants. Sub-sample 2 (10 g) was extracted with methylene chloride, subjected to the reactive cleanup procedure described below, amputated, and shipped to the CERC for use in the H4IIE bioassay. Sub-sample 3 (10 g) was retained by Lab 1 for analysis of organochlorine chemical residues by gas chromatography with electron capture detection (GC-ECD) and gravimetric determination of lipid content.

Elemental Contaminants and Moisture Content: At Lab 2, the 100-g sub-samples (sub-sample 1) were re-homogenized using a food processor, freeze-dried using a Virtis UniTrap® Model 10-100V lyophilizer, and ground to 100-mesh with a cutter-hammer mill that was rinsed with HNO₃ between samples. Moisture content was determined by weight loss during lyophilization. Concentrations of total arsenic (As), mercury (Hg), lead (Pb), and selenium (Se) were determined by atomic absorption spectroscopy (AA). Concentrations of aluminum (Al), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), silver (Ag), strontium (Sr), thallium (Th), vanadium (Va), and zinc (Zn) were determined by inductively coupled plasma emission spectroscopy (ICPES); no pre-concentration was used. Digestions for graphite furnace (GF) and cold vapor (CV) AA analyses were conducted in a microwave oven. A freeze-dried sample (0.25-0.50 g) was heated in a capped 120-mL Teflon® vessel in the presence of 5 mL of Baker Instra-Analyzed nitric acid for 3 min at 120 w, 3 min at 300 w, and 15 min at 450 w. The residue was then diluted to 50 mL with laboratory-pure water. Hg was measured by CVAA with a Leeman PS200 Hg Analyzer using SnCl₄ as the reducing agent. The GFAA measurements were made using a Perkin-Elmer Zeeman 3030 or 4100ZL atomic absorption spectrometer. ICPES measurements were made using a Leeman Plasma Spec I sequential or ES2000 simultaneous spectrometer. QA measures included analyses of blanks, fortified samples, duplicates and standard reference materials. Limits of detection (LODs) were determined individually for each analyte in each sample, but were nominally 0.15 µg/g dry-weight for Be, Cd, and Hg; 0.3 µg/g for Pb and Sr; 0.5 µg/g for Al; 0.6 µg/g for Mo; 0.7 µg/g for As, Ba, Cr, Cu, Mo, Ni, Se, and V; 2.5 µg/g for Zn; and 15 µg/g for Fe and Mn. In keeping with past NCBP reports (Schmitt and

others, 1999b), these values, as well as the analytical results, were converted to wet-weight concentrations for statistical analysis and reporting.

Organochlorine Chemical Residues and Lipid Content:

At Lab 1, one 10-g sub-sample (sub-sample 3) of each ground composite sample was thoroughly mixed with anhydrous sodium sulfate and soxhlet-extracted with hexane for 7 h. The extracts were then concentrated by rotary evaporation; transferred to tared test tubes and further concentrated to dryness; and weighed for gravimetric determination of lipid content. After weighing, the lipid samples were re-dissolved in petroleum ether and extracted four times with acetonitrile saturated with petroleum ether. Residues were partitioned into petroleum ether, then washed, concentrated, and transferred to a glass chromatographic column containing 20 g of Florisil®. The column was eluted with 200 mL of 6% diethyl ether/94% petroleum ether (Fraction I) followed by 200 mL of 15% diethyl ether/85% petroleum ether (Fraction II). Fraction II, which contained relatively polar organochlorine insecticides, was concentrated to appropriate volume for quantification of residues by dual megabore (DB-608 and DB-5)-column GC-ECD. To separate PCBs from other organochlorine chemical residues, Fraction I was concentrated and transferred to a silicic acid chromatographic column for additional fractionation and cleanup. Three fractions were eluted from this column: The first (20 mL of petroleum ether) contained hexachlorobenzene (HCB) and mirex; the second (150 mL of petroleum ether) contained PCBs; and the third contained organochlorine pesticides. Each of these fractions was concentrated to appropriate volume for quantification of residues by megabore-column GC-ECD. The nominal LODs for individual compounds was 0.01 µg/g wet-weight; for multi-component residues (that is, toxaphene and PCBs) the detection limit was 0.05 µg/g wet-weight.

Precision and accuracy of laboratory results were confirmed through analyses of procedural blanks, duplicates, fortified samples, and reference materials. Duplicate analyses ($n=9$) typically differed by 3-5% except for total PCBs (9%). Mean recovery efficiency of fortified samples ($n=9$) was 92-104% except for dieldrin (88%) and HCB (70%). The identities of residues were confirmed by GC/MS in about 10% of the samples with a Varian Saturn 2000 ion-trap MS, positive EI mode, on a 30-M X 0.25-mm (id) DB-5 capillary column and a Model 1078 injector (14 psi head pressure, trap 235 °C, manifold 50 °C, transfer line 285 °C). Injector temperature was 300 °C. Column temperatures were 40 °C for 2 min; increase 25 °C/min to 150 °C; increase 4 °C/min to 290 °C; hold 3.6 min. Round-robin tests among PACF and contract laboratories were also concluded.

H4IIE Rat Hepatoma Cell Bioassay: The other ground, re-frozen 10-g sub-samples (sub-sample 3) were kept frozen at Lab 1 until the initiation of sample processing for H4IIE analysis. Each sample was then thawed at room temperature and homogenized in a blender with four times its weight of anhydrous sodium sulfate, packed in an extraction column, and extracted with methylene chloride. Percent lipid was determined on a 1% portion of the extract. The remainder was concentrated by roto-evaporation and taken through two stages of reactive column clean-up: first by chromatography using a sulfuric acid-silica gel/potassium silicate (SASG/KS) column; and second using a sulfuric acid-silica gel/silica gel (SASG/SG) column (Schwartz and Lehmann, 1982; Tillitt and others, 1991). Extracts were then evaporated to near-dryness, re-dissolved with 150 μ L of isooctane, ampulated, and shipped to the CERC for analysis. Matrix quality control samples (blanks and spikes) prepared at Lab 1 and at the CERC included ground tissues from laboratory-raised bluegill (*Lepomis macrochirus*) and samples of a CERC standard positive control tissue (carp from Saginaw Bay, MI). The quality control samples, along with procedural blanks, were processed concurrently with the 1995 samples. No internal recovery surrogates were used to monitor recovery efficiencies out of concerns that any added chemicals might alter the response of the cells. Extraction efficiencies for these procedures are typically greater than 80%, however (Schwartz and Lehmann, 1982; Peterman and others, 1996).

At CERC, the H4IIE bioassay was performed on extracts of composite fish samples (prepared as described above) according to the method of Tillitt and others (1991) as modified for 96-well microtiter plates (Tysklind and others, 1994). The H4IIE cells were seeded at 7000 cells/well in 300 μ L of D-MEM culture media (Tillitt and others, 1991). After a 24-h incubation, the cells were dosed with sample extracts or standards in 5 μ L of isooctane. The cells were exposed to eight different concentrations (doses) of the samples in a 25% dilution series, with four replicates at each dose. The samples were calibrated against TCDD for the determination of TCDD-EQ in the samples. TCDD standards were dosed at eight concentrations (0, 0.125, 0.25, 0.5, 1.25, 2.5, 5.0, 12.5, and 50 pg/well) with each dose replicated four times. At least three TCDD curves were analyzed on the respective day.

A 72-h incubation followed dosing of the cells, after which the plates were washed twice with ultra-pure water and the cells allowed to lyse. The following reagents were added to each well: 20 μ L of PBS buffer with dicumarol (20- μ M final concentration) and 20 μ L of 5- μ M 7-ethoxyresorufin (1.25- μ M final concentration). The reactions were initiated with 20 μ L of 5-mM NADPH (1.25-mM) and allowed to

proceed for 10 min in the fluorometric plate reader (Cytofluor 2300, Millipore Corp.). Resorufin production was measured once per minute kinetically with an excitation filter wavelength centered at 530 nm and an emission filter wavelength centered at 595 nm. The relative fluorescence intensity of the samples was then compared to a quadratic fit of an eight point resorufin standard curve (six replicates per concentration) and the relative intensity units were converted to pmoles of resorufin. Resorufin in each well was plotted against time to observe any deviations from linearity of the reaction. A linear regression was then performed on the data from each well to determine an EROD rate (pmol/min) from the slope of the linear regression line along with its associated estimates of variance. The amount of protein in each well was determined by the fluorescamine assay (Lorenzen and Kennedy, 1993) and the values used to normalize dose to each well and EROD activity. The doses of each sample (g-equivalents/mg cellular protein) or TCDD standards (pg TCDD/mg cellular protein) were plotted against EROD activity (pmol/min/mg cellular protein) to develop dose-response curves. The linear portions of these curves were used to compare the relative potencies of the samples with that of the TCDD standard. Determination of TCDD-EQ was by slope ratio assay (Finney, 1964) as described by Ankley and others (1991). Variance estimates were based on an additive model (Finney, 1964) and were computed as previously described (Ankley and others, 1991; Tillitt and others, 1991).

The quantitative QA objective of the H4IIE bioassay was a coefficient of variation (CV) of $\leq 25\%$ based on replicate analyses for the potency estimates of the environmental extracts and TCDD standard. Method LODs and limits of quantitation (LOQs) were computed as recommended by Keith and others (1983). Accuracy of the bioassay results was based on TCDD standard curves and replicate analyses. The acceptance criterion for TCDD potency estimates was < 2.5 standard deviations from the mean value as determined from previous values. Replications and QA checks were performed at many stages of the H4IIE assays. A composite TCDD dose-response curve was generated from the mean of four independent determinations for each composite sample. Four percent of the tissue extracts samples were assayed in triplicate. Eight-point resorufin standard curves and seven-point BSA standard curves were prepared at six replicates per concentration and were analyzed concurrently with the TCDD standards and samples. Scatter plots for the resorufin and bovine serum albumin (BSA) standard curves were prepared and examined to monitor the temporal consistency of the fluorescence response over the course of sample evaluation. And finally, a positive control fish extract (carp from Saginaw Bay, MI) was included with the

samples on each assay date. Based on this QA program, we determined that the results of the H4IIE bioassays accurately reflect the dioxin-like potency of the extracts.

Microsomal Preparation and Microsomal Assay for Ethoxyresorufin O-deethylase (EROD) Activity: Liver samples were shipped to CERC in dry ice and stored frozen in Cryovials® at -80 °C until needed for the preparation of microsomal fractions for analysis. Microsomes were prepared following ECRC SOP B5.264, "Microsomal Preparation from Liver Tissues." Briefly, approximately 0.5 g of liver was weighed directly into labeled 12- × 75-mm centrifuge tubes and 1.5 mL of phosphate buffer (pH 8) was added to each tube. The samples were twice homogenized for 20 sec with an Omni Hand-Held Tissuemizer® and centrifuged at 9,000 g for 25 min at 4 °C. The supernatants (S9 portions) were transferred to Ultrafuge® tubes and centrifuged for 50 min at 30,000 rpm, at 4 °C. The supernatants from the second centrifugation were discarded and the pellets were re-suspended in 0.5-1.0 mL of pH-8 phosphate buffer.

The kinetic microsomal assays were conducted in 96-well microtiter plates. Microsomal preparations were used the day they were prepared. Triplicate determinations (three wells per plate) of EROD activity were performed on 5-μL portions of each microsomal preparation; mean EROD activity was reported. Protein content was determined using the fluorescamine protein assay (Lorenzen and Kennedy, 1993) on the same samples and in the same 96-well microtiter plate as the EROD analyses. The positive control material for the EROD assay was liver microsomal preparation from channel catfish (*Ictalurus punctatus*) injected with 10 mg/kg of benzo(a)pyrene; an additional reference material for the EROD assay was liver microsomes of flathead catfish (*Pylodictis olivaris*) obtained from the Missouri River near Easley, MO. Excess microsomal fractions not used on the day of preparation were re-frozen and stored in a -80 °C freezer.

The EROD assay was performed by first adding the following reagents to each well: 50 μL of 10-μM 7-ethoxyresorufin at 25 °C (3.84 μM/well final concentration); 50 μL of 4.3-mM NADPH at 25 °C (1.54 mM/well final concentration); and 50 μL of phosphate buffer (pH 8) at 25 °C. The sample plate was incubated at 25 °C for 10 min. Each 96-well microtiter plate was scanned once per minute for 20 min of kinetic analysis in a fluorometric plate reader (Cytofluor® 2300, Perspective Bioscience). Resorufin and BSA protein standards were analyzed on separate plates. The excitation and emission filter wavelengths and the sensitivity were centered at 530 nm, 595 nm, and 4, respectively, for the EROD assay; and 400 nm,

460 nm, and 3 for the fluorescamine protein assay. The relative fluorescence intensity of the samples was then compared to a 7-point resorufin standard curve (six replicates per concentration) and the relative intensity units were converted to pmol resorufin. Resorufin in each well was plotted against time to observe any deviations from linearity of the reaction. A linear regression was then performed on the data from each well to determine an EROD rate (pmol/min) from the slope of the linear regression line along with its associated estimates of variance. The amount of protein was used to normalize EROD activity in each well.

The EROD assays were subjected to a rigorous QA program. Replication and performance checks were performed at many stages of the microsomal assay procedure. LODs and LOQs were computed according to Keith and others (1983). A composite EROD slope/activity value was determined from the mean of three independent determinations for each microsome sample. Five percent of the liver samples were split into triplicates and processed independently. Seven-point resorufin standard and six-point BSA standard curves were prepared at six replicates per concentrations and were analyzed concurrently with the microsomal samples. Scatter plots for the resorufin and BSA standard curves were prepared and examined to monitor the temporal consistency of the fluorescence response over the course of sample evaluation. The concentrations of the resorufin, ethoxyresorufin, and NADPH reagents were verified spectrophotometrically on each assay date and deemed acceptable if the measured values were within 10% of the nominal concentrations. Samples of both reference materials (channel and flathead catfish) were included with each batch of samples analyzed. Based on this QA program, we determined that the results obtained accurately reflect the hepatic EROD rates of the fish liver samples analyzed.

Data Set Composition and Statistical Analyses

A total of 163 composite samples from 47 of the 48 stations (including the reference site) sampled were analyzed instrumentally and by H4IIE bioassay [carrass samples from Station 209, in the Eastern Iowa Basins (EIB) Study Unit, were lost in shipment]. Of those analyzed, 89 (54%) from 45 stations (96%) were carp and 58 (35%) from 30 stations (64%) were bass. The remaining 17 samples (11%) comprised white suckers (*Catostomus commersoni*, two samples from one station), white bass (*Morone chrysops*; four samples, two stations), sauger (*Stizostedion canadense*; three samples, two stations), brown trout (*Salmo trutta*, two samples, one station), goldeye

(*Hiodon alosoides*, two samples, one station), small-mouth buffalo (*Ictiobus bubalus*; two samples, one station), and northern pike (*Esox lucius*, one sample, one station). A total of 1316 individual fish from the 48 stations, which collectively represented 22 species, were analyzed for EROD activity. Carp and bass comprised 90% of the fish analyzed. As noted in Chapter 1, there was at least one species common to both the 1986 and 1995 collections at most NCBP stations for the examination of within-taxon temporal trends in composite samples. Chapter 1 and Appendix A contain additional information on the species composition of the 1995 and 1986 data sets and summary statistics for the data reported here. Raw data may be obtained at <http://www.cerc.usgs.gov/data/data.htm>.

All results for composite samples (that is, elemental and organochlorine chemical contaminant concentrations and TCDD-EQ) were converted to, statistically analyzed, and reported as wet-weight concentrations to maintain continuity with previous NCBP reports. Concentrations of some organic contaminants were low or non-detected in many samples, which precluded rigorous statistical analysis for these analytes. As in the past (Schmitt and others, 1999b), a value of one-half the detection limit was substituted for censored values in the computation of unweighted species, sex, and station means, which were compared graphically for most analytes due to the large number of censored values. Concentration data were transformed (log) as necessary to meet distributional and other assumptions.

Temporal and geographic comparisons are readily confounded by differences among taxa, especially for elemental contaminants (Lowe and others, 1985; Schmitt and Brumbaugh, 1990; Schmitt and others, 1999b). Accordingly, within-taxon comparisons were made where possible. For geographic comparisons, there were too few uncensored observations for statistical testing at the station level. Instead, stations were compared graphically, then aggregated by sub-basin and program (NCBP vs. NAWQA—see Chapter 1) for statistical testing of the four analytes with sufficient numbers of uncensored observations in both carp and bass—*p,p'*-DDE, Hg, Se, and TCDD-EQ. The Mississippi Embayment (MSE) and EIB NAWQA Study Units are wholly contained within the Lower Mississippi (LMS) and Upper Mississippi (UMS) sub-basins, respectively. Therefore, comparisons of these sub-basins represent regional contrasts of large-river stations against those on lower-order rivers and streams. In these analyses, we tested log-transformed concentrations of those contaminants with sufficient numbers of uncensored observations (DDT, TCDD-EQ, Pb, Hg, and Se) in carp and bass using a nested linear model that included terms for sub-basin, program, and sex. This analysis revealed small but statistically significant

($P < 0.05$) differences between genders for DDE, total PCBs, Hg, and Se in carp, but none in bass. Consequently, four stations (23, 29, 76, and 77) were eliminated from the in-depth statistical analysis of these four analytes in carp due to either mixed-gender compositing (caused by fish that either could not be identified to sex or were misidentified in the field) or the presence of only one gender at the station. Temporal comparisons for NCBP sites were based on unweighted species and station-species (or higher-order taxon) means, which were compared graphically with findings from the most recent NCBP collection (1986) for all but Station 90, which was last sampled in 1984 (Schmitt and others, 1990; Schmitt and Brumbaugh, 1990; Schmitt and others, 1999b).

Hepatic EROD activity was measured on individual fish and was analyzed using the same statistical models used for other individual fish variables (see Chapter 1). In addition, and as noted in Chapter 1 of this report, preliminary analyses sought to determine whether hepatic EROD activity varied with age or histopathologically determined gonadal stage (McDonald and others, 2000) within each species and taxon. There were insufficient numbers of observations for a full evaluation of the effects of these variables in combination at each station. Instead, exploratory linear ANOVA models were developed to test for the effect of stage on EROD activity within each species/gender combination. These analyses suggested that there was no effect of stage on EROD activity in either male or female carp or in male bass. However, in female bass, the preliminary analyses suggested an effect of stage, which is consistent with the known effects of estradiol on EROD activity in female fish (Whyte and others, 2000). Further analyses were therefore conducted using three separate linear models. Two models that included “stage” and “station” main effects and an interaction term were fit separately to the data grouped by available stages (1-2 and 2-3). In these models only the interaction term was significant ($P = 0.0444$), which suggested that the evidence for a stage effect on EROD was weak. The final ANOVA model combined data across all stages (0-4) and no stage effect was observed. These results suggested that no further adjustments were necessary, and that log-transformed EROD activity could be compared at the station and sub-basin level within each taxon-sex category. For correlation and regression analyses and other comparisons with measurements made on the composite samples we used the geometric mean EROD activity of the individual fish in each composite sample.

RESULTS AND DISCUSSION

In this section we summarize findings for 1995 and, where appropriate, compare them to previous NCBP findings and other studies. Chemical results are also compared with extant information on ecological risk, most of which is based on the risks posed by the accumulated contaminants to piscivorous wildlife rather than to the fish.

Elemental Contaminants

Lead: Environmental releases of Pb in North America have been reduced over the last two decades. Nevertheless, substantial large quantities are still emitted by mining, smelting, and other activities (Table 2-1), and there is atmospheric transport from elsewhere. In addition, much remains from past emissions. Consequently, Pb was detected ($\text{LOD} > 0.006$ – > 0.032 $\mu\text{g/g}$) in 87% of the samples and in least one sample from all 1995 stations (Table 2-3). At the reference site, Pb was detected in both carp samples but not in either largemouth bass sample; concentrations were uniformly low (< 0.03 $\mu\text{g/g}$ in largemouth bass, 0.09 – 0.12 $\mu\text{g/g}$ in carp). The greatest concentration (0.69 $\mu\text{g/g}$) was in male carp from Station 111 (Mississippi R. at Lake City, MN; Fig. 2-1); concentrations in the other samples from this site were low (< 0.04 – 0.10 $\mu\text{g/g}$), however. Pb concentrations were also relatively high (> 0.2 $\mu\text{g/g}$ in one or both samples) in carp from NCBP Stations 67 (Allegheny R.), 85 (James R. at Olivet, SD), 24 (Ohio R. at Marietta, OH), 25 (Tennessee R. at Savannah, TN), 28 (Arkansas R. at Pine Bluff, AR), 78 (Verdigris R. at Oolagah, OK), 79 (Canadian R. at Eufaula, OK), 73 (Des Moines R. at Keosauqua, IA), and at NAWQA Station 204 (Tensas R. at Tenda, LA); in smallmouth buffalo from Station 23; and in largemouth bass (*Micropterus salmoides*) from Stations 28 and 70 (Ohio R. at Metropolis, IL; Fig. 2-1). Geometric station means (Fig. 2-2) were greatest (> 0.1 $\mu\text{g/g}$) at NCBP Stations 70, 24, 67, and 90, and at NAWQA Stations 204, 203 (Steele Bayou at Rolling Fork, LA), 208 (Cache R. at Egypt, AR), and 201 (Big Sunflower R. at Anguilla, MS), all in the MSE Study Unit and all represented by only carp in the 1995 collection. In 1986, greatest Pb concentrations (individual samples, station means, or both) occurred at several sites in the MRB at which concentrations were also relatively high in 1995: Stations 78 (Verdigris R.), 79 (Canadian R. at Eufaula, OK), 89 (Platte R. at Louisville, NE), 73 (Des Moines R. at Keosauqua, IA) and 69 (Ohio R. at Cincinnati, which was not sampled in 1995). In 1986, concentrations were also elevated at Stations 76 (Mississippi R. at Memphis, TN) and 83 (Missouri R.

at Hermann, MO) in 1986 (Schmitt and others, 1999b). Relative to 1986 (1984 for Station 90), geometric mean Pb concentrations decreased at many NCBP stations where concentrations were high (Stations 76, 79, 85, and 73, and 89), but also increased markedly at Stations 67 and 70 (Fig. 2-3). Within-taxa, these changes were clearly evident as increases in carp at Station 67 and in bass at Station 70, but not vice-versa (Fig. 2-4).

Concentrations of Pb in carp (2% censored) did not differ significantly among sub-basins (Table 2-4) or programs (Table 2-5), nor did either group of stations (NCBP or NAWQA) differ significantly from the reference site. In bass (24% censored), however, Pb concentrations differed significantly among sub-basins, with greatest levels occurring in the Ohio River (OHR) sub-basin and lowest in the MSE Study Unit (Table 2-4). None of the sub-basins differed significantly from the reference site, however. At the program level, Pb concentrations in bass from the NCBP stations were significantly greater than levels at the NAWQA sites, but neither group differed from the reference site (Table 2-5).

In terms of ecological risk, Pb is readily accumulated by fish from food and water (for example, Farag and others, 1994) but does not biomagnify (Settle and Patterson, 1980); there appears to be little risk to piscivorous wildlife from the Pb incorporated into, sorbed onto, or ingested by fish (Henny and others, 1994). In fish, effects on heme synthesis have been detected at carcass concentrations exceeding about 1.0 $\mu\text{g/g}$, depending also on Zn burden (Schmitt and others, 1984; 1993). The greatest 1995 concentrations (ca. 0.5 – 0.7 $\mu\text{g/g}$) were therefore about half the values associated with impaired heme synthesis in fish. Higher-level effects specifically associated with Pb at environmental concentrations have not been reported; however, in combination with other elemental contaminants, effects on individual fish and fish populations have been documented (Farag and others, 1994; 1995; Woodward and others, 1997; Wildhaber and others, 2000).

Cadmium: Cadmium is present in many materials and is released to the environment from mining, smelting, and a variety of other sources (Table 2-1). In 1995, Cd was detected (LOD ca. 0.05 $\mu\text{g/g}$) in 49% of the samples from 91% of the NCBP, NAWQA, and reference stations sampled (Table 2-3). Concentrations ranged from < 0.05 $\mu\text{g/g}$ to about 0.5 $\mu\text{g/g}$, the latter in carp from Station 67 (Allegheny R.; Fig. 2-5). Comparatively high Cd concentrations (> 0.15 $\mu\text{g/g}$) were also present, as individual samples (Fig. 2-5), station means (Fig. 2-6), or both at NCBP Stations 24 (Ohio R. at Marietta, OH), 25 (Cumberland R. at Clarksville, TN), 90 (Kansas R. at

Table 2-3. Occurrence (percentages of samples and stations, including the reference site), limits of detection (LOD), and maximum concentrations of analytes in composite carcass samples (all concentrations in wet weight). Also shown are the station, sex, and species associated with the maximum concentration.

Analyte(s)	Samples (% of 163)	Stations (% of 47)	LOD range (µg/g) ¹	Maximum 1995 concentration			Species
				Conc. (µg/g)	Station	Sex	
<i>p,p'</i> -DDT	8	15	0.01	0.14	24	F	Carp
<i>p,p'</i> -DDD	58	74	0.01	2.80	201	M	Carp
<i>p,p'</i> -DDE	91	100	0.01	8.30	201	M	Carp
Total <i>p,p'</i> -homologs	91	100	0.01	11.10	201	M	Carp
<i>o,p'</i> -DDT	3	4	0.01	0.24	24	F	Carp
<i>o,p'</i> -DDD	12	24	0.01	0.34	24	F	Carp
<i>o,p'</i> -DDE	1	2	0.01	0.02	24	F	Carp
Dieldrin	42	57	0.01	0.25	76	F	Carp
Endrin	2	2	0.01	0.70	76	F	Carp
<i>cis</i> -Chlordane	37	48	0.01	0.12	76	F	Carp
<i>trans</i> -Chlordane	9	30	0.01	0.35	76	F	Carp
<i>cis</i> -Nonachlor	21	35	0.01	0.05	23	F	Smallmouth buffalo
<i>trans</i> -Nonachlor	51	70	0.01	0.31	23	M	Spotted bass
Oxychlordane	6	9	0.01	0.03	76	F	Carp
Heptachlor epoxide	9	15	0.01	0.08	206	M	Carp
Total chlordane-related residues	51	70	0.01	0.54	76	F	Carp
Toxaphene	7	11	0.05	8.3	201	M	Carp
Mirex	4	4	0.01	0.08	204	M	Carp
HCB	2	7	0.01	0.07	24	M	Carp
Total PCBs	21	35	0.05	3.3	24	M	Carp
TCDD-EQ ²	87	100	<0.05-0.40 ²	68 ²	208	F	Carp
Arsenic	28	48	0.11-0.51	0.56	78	M	Largemouth bass
Cadmium	49	91	0.02-0.10	0.51	67	M	Carp
Copper	100	100	0.11-0.51	3.8	15	F	White bass
Lead	87	100	0.01-0.04	0.69	111	M	Carp
Mercury	97	100	0.02-0.10	0.45	76	F	Largemouth bass
Selenium	100	99	0.11-0.51	4.7	77	M	Carp
Zinc	100	100	0.23-1.66	150	79	M	Carp

¹ uniform censoring levels for organochlorine residues² estimated, pg/g

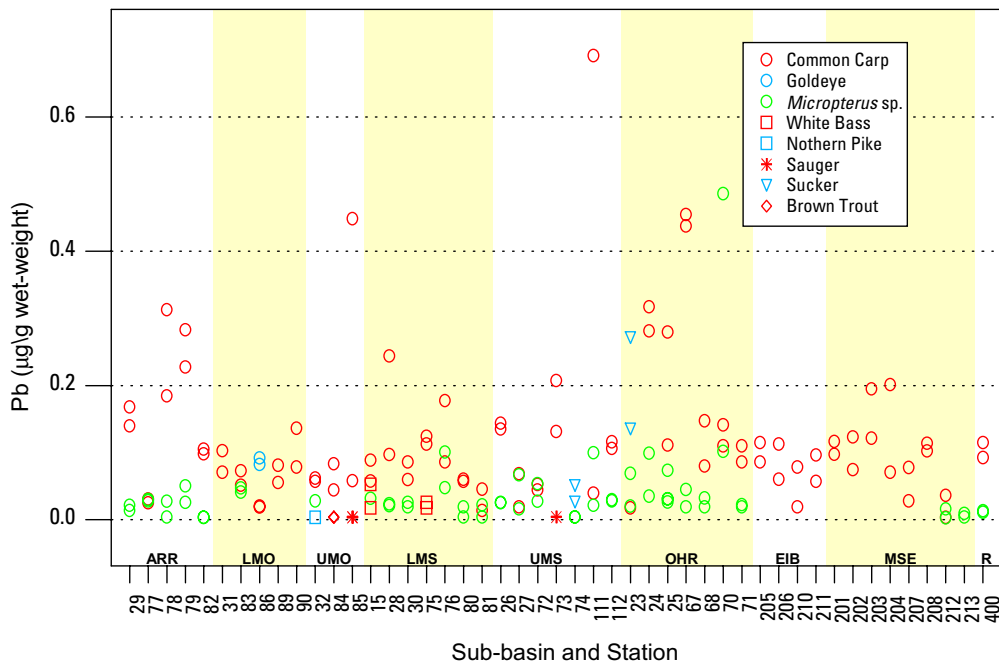


Figure 2-1. Concentrations of Pb in composite fish samples, by sub-basin, station, and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

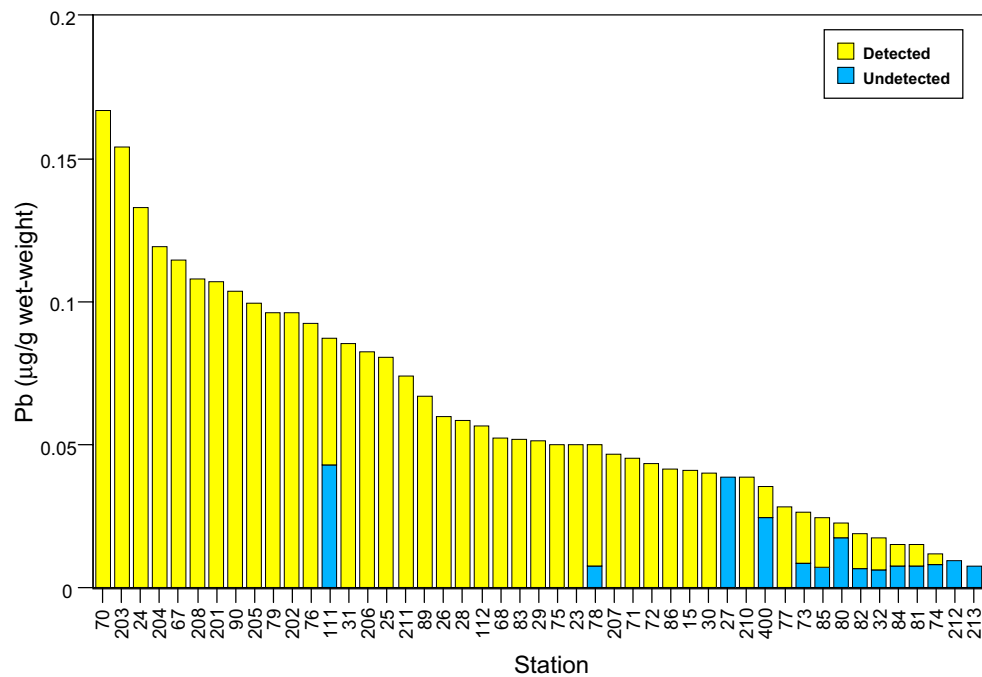


Figure 2-2. Ranked geometric mean concentrations of Pb in composite fish samples, by station. Shaded areas are means of censored observations (represented by 50% of LOD) used to compute the respective station means. See Table 1-1 for station descriptions.

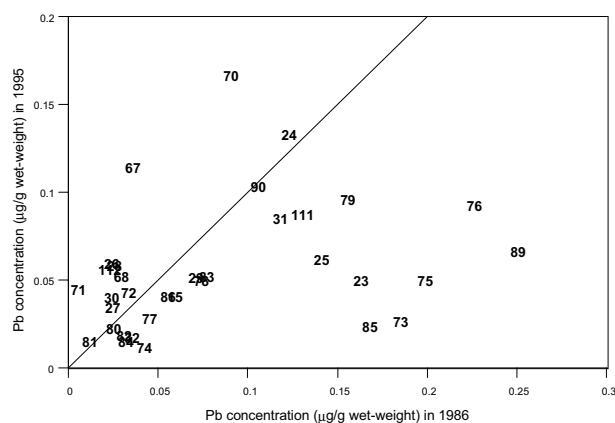


Figure 2-3. Geometric mean Pb concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

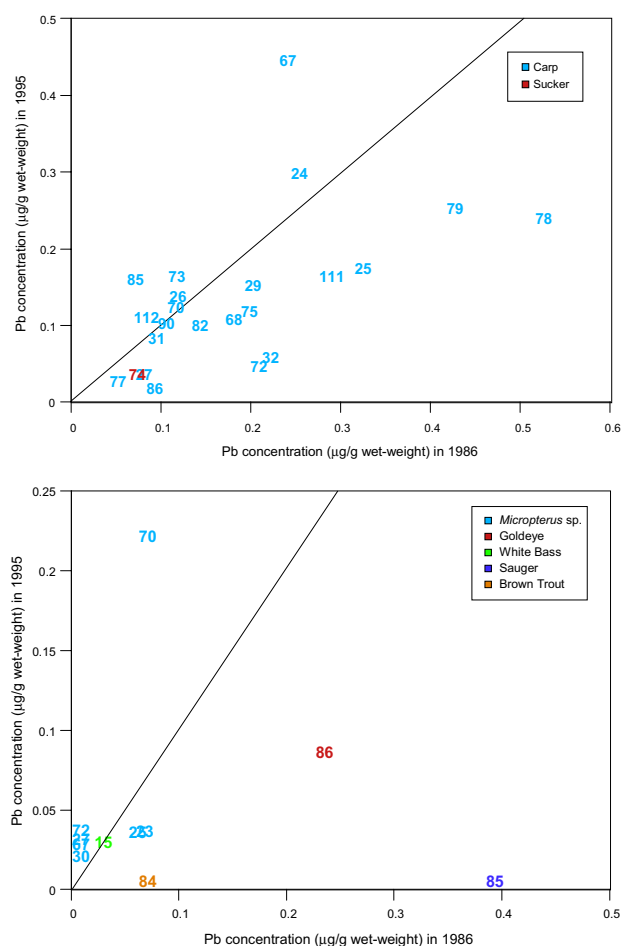


Figure 2-4. Geometric mean Pb concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

Bonner Springs, KS), 78 (Verdigris R. at Oologah, OK), 73 (Des Moines R. at Keosauqua, IA), and 30 (White R. at DeVall's Bluff, AR). At all of these sites except Station 30 the elevated concentrations occurred in carp (Fig. 2-5); at Station 30, one relatively high value also occurred in male largemouth bass. Among these sites, Stations 67, 78, and 24 have been identified as among the uppermost for Cd in previous NCBP collections (May and McKinney, 1981; Lowe and others, 1985; Schmitt and Brumbaugh, 1990; Schmitt and others, 1999b). Station 78 has a long history of contamination by metals; the Verdigris R. upstream of the collection site drains part of the Tri-State Mining District where Zn and other metals were mined for many years and where there are abandoned mines and ore-processing facilities (Pita and Hyne, 1975; May and McKinney, 1981). Cd concentrations at the NAWQA sites were generally lower than at the NCBP sites; the greatest values at the former (about 0.1 µg/g) were in carp from Stations 201 and 203, in the MSE Study Unit (Figs. 2-5 and 2-6). At the reference site (Station 400), Cd concentrations did not exceed detection limits (0.02-0.03 µg/g) in any sample (Fig. 2-6). Relative to previous NCBP collections, geometric mean Cd concentrations were generally lower or did not change (Fig. 2-7); however, geometric mean concentrations increased at Stations 30, 78, 90 (Kansas R.; Fig. 2-7).

Schmitt and others (1999b) noted that Cd concentrations tended to be greater in carp than in other taxa, a trend that held through 1995 (Fig. 2-5). In temporal comparisons within taxa (Fig. 2-8), Cd concentrations in carp increased relative to 1986 at Stations 67 and 90 (1984) and decreased at Stations 32 (Missouri R. at Garrison Dam, ND) and 79 (Canadian R. at Oologah, OK). Concentrations also increased in largemouth bass at Station 30, white bass at Stations 15 and 25, and in other taxa at stations in the Missouri River system—goldeye at Station 86 (James R. at Olivet, SD), sauger at Station 85 (Yellowstone R. at Sidney, MT), and brown trout at Station 84 (Big Horn R. at Hardin, MT—Fig. 2-8). Carp were collected at Stations 85 and 86 in both 1986 and 1995, but Cd concentrations in that species remained low (Fig. 2-8).

According to Eisler (1985), whole-organism Cd concentrations of 2 µg/g are indicative of contamination, levels of 5 µg/g are hazardous to the organism, and dietary levels of 13-15 µg/g represent a hazard to higher trophic levels. Even the greatest 1995 concentrations were 4-5 fold below the lowest of these toxicity thresholds.

Mercury: The discharge of Hg to North American waters has been greatly reduced over the last two decades. Nevertheless, Hg is present in many effluents and is released to the atmosphere via fossil fuel

Table 2-4. Geometric mean concentrations (all $\mu\text{g/g}$ wet-weight except TCDD-EQ, in pg/g) and results of analysis-of-variance for indicated analytes in carp and bass (arithmetic means for Hg and Se in carp) and EROD activities (arithmetic means, $\text{pmol/min/mg protein}$) for each sub-basin¹ and for the reference site in West Virginia. Within each row, means containing the same subscript are not significantly different (ANOVA, $P > 0.05$). EROD means were not tested at the species level (combined genders). See Table 2-5 for ANOVA F -values.

Taxon and analyte	Sub-basin ¹							Reference
	ARR	LMO	UMO	LMS	UMS	OHR	EIB	MSE
Carp								
<i>p,p'</i> -DDE	0.11 ² _{ac}	0.02 _b	0.01 _b	0.15 ³ _a	0.04 _c	0.07 ⁴ _{ac}	0.09 _c	1.00 _d
Hg	0.09 ² _a	0.09 _a	0.11 _a	0.09 ³ _a	0.09 _a	0.10 ⁴ _a	0.10 _a	0.17 _b
Se	0.82 ² _{abcd}	0.73 _{abcd}	0.89 _{ad}	0.57 ³ _c	0.59 _c	0.59 ⁴ _c	0.69 _{bcd}	0.76 _{bcd}
Pb	0.12 _a	0.06 _a	0.08 _a	0.08 _a	0.10 _a	0.14 _a	0.07 _a	0.07 _a
TCDD-EQ	1.21 _a	1.05 _a	1.49 _{ac}	6.25 _{bc}	6.79 _{bc}	8.25 _b	4.33 _{ab}	7.37 _b
EROD (all)	2.1	3.9	1.6	8.0	4.6	5.4	1.1	12.6
Males	2.6 _{ab}	4.0 _a	2.3 _{ac}	10.3 _{bcd}	6.4 _{ac}	7.3 _{bcd}	2.0 _e	14.7 _d
Females	1.6 _{ab}	3.7 _{ace}	0.8 _b	5.7 _{ce}	2.7 _{ac}	3.5 _{ace}	0.2 _d	10.5 _e
Bass								
<i>p,p'</i> -DDE	0.04 _a	0.01 _b	0.01 _b	0.12 _c	0.02 _{bd}	0.04 _{ad}	* ⁵	0.04 _{ad}
Hg	0.15 _a	0.33 _{bc}	0.16 _{ac}	0.23 _{bc}	0.14 _a	0.14 _a	* ⁵	0.27 _{bc}
Se	0.75 _a	0.59 _{ac}	0.98 _a	0.36 _b	0.40 _b	0.40 _b	* ⁵	0.40 _{bc}
Pb	0.01 _a	0.04 _{ac}	0.03 _{ac}	0.02 _{ac}	0.02 _{ac}	0.04 _{bc}	* ⁵	0.01 _{abc}
TCDD-EQ	4.02 _a	1.10 _a	6.00 _a	5.35 _a	8.77 _a	7.12 _a	* ⁵	2.91 _a
EROD (all)	14.0	26.5	* ⁶	22.3	22.3	22.5	* ⁷	* ⁷
Males	15.0	30.0 _b	* ⁶	21.8 _a	22.3 _a	22.4 _a	* ⁵	* ⁸
Females	13.0	23.0 _b	2.0 _a	22.7 _a	22.6 _a	20.3 _a	* ⁵	* ⁸

¹ As defined in Table 1-1.

² Does not include Stations 29 or 77.

³ Does not include Station 76.

⁴ Does not include Station 23.

⁵ Only carp were collected in the EIB Study Unit.

⁶ Only females were collected (from one site) in the UMO sub-basin.

⁷ Only carp from the MSE Study Unit were analyzed for EROD activity.

⁸ EROD activity was not analyzed in fish from the reference site.

Table 2-5. Mean concentrations and results of analysis-of-variance (as ANOVA *F*-values: * $P < 0.05$; ** $P < 0.01$) for NCBP and NAWQA sites and for the reference site in West Virginia. All are geometric means except for Hg and Se in carp and all EROD means; see text for explanation of transformations used. Within each group of three analyte-taxon means, those containing the same subscript are not significantly different (ANOVA, $P > 0.05$). EROD means were not tested at the species level (i.e., with genders combined). Units for all analytes are $\mu\text{g/g}$ wet-weight except TCDD-EQ ($\mu\text{g/g}$) and EROD activity (pmol/min/mg protein).

Analyte	Carp				Bass			
	NCBP	NAWQA	Reference	<i>F</i> -value	NCBP	NAWQA	Reference	<i>F</i> -value
<i>p,p'</i> -DDE	0.05 ¹ _a	0.29 _b	0.27 _b	35.05**	0.03 _a	0.04 _{ab}	0.06 _b	18.82**
Hg	0.10 ¹ _a	0.14 _b	0.04 _c	16.68**	0.18 _a	0.27 _b	0.22 _{ab}	11.68
Se	0.70 ¹ _a	0.73 _a	0.18 _b	11.39**	0.54 _a	0.39 _b	0.29 _b	21.49**
Pb	0.09 _a	0.07 _a	0.11 _a	3.00**	0.03 _a	0.01 _b	0.01 _{ab}	3.52**
TCDD-EQ	2.96 _a	5.64 _a	2.45 _a	3.00**	4.55 _a	2.91 _a	1.48 _a	0.28
EROD (all)	4.2	6.9	*	*	21.3	*	*	*
EROD (males)	5.5 _a	8.4 _a	* ²	4.80**	22.3	* ²	* ²	6.92**
EROD (females)	3.0 _a	5.4 _b	* ²	6.17**	17.2	* ²	* ²	10.03**

¹ Does not include Stations 23, 29, 76, or 77

² EROD activity was not analyzed in any fish from the reference site or in bass from the NAWQA Study Units.

combustion and the incineration of Hg-containing materials (Table 2-1). In addition, residual Hg remains from historic discharges from chlor-alkali production and gold silver mining. In 1995, Hg was detected ($>0.05 \mu\text{g/g}$) in 97% of the samples and at 100% of the NCBP and NAWQA stations sampled (Table 2-3). Concentrations in fish ranged from barely detectable ($\leq 0.05 \mu\text{g/g}$) to $0.45 \mu\text{g/g}$ (Table 2-3, Fig. 2-9), the latter in largemouth bass from Station 76 (Mississippi R. at Memphis, TN). In addition to Station 76, relatively high concentrations (that is, $>0.25 \mu\text{g/g}$) were present in at least one sample of largemouth bass from NCBP Stations 81 (Red R. at Alexandria, LA), 70 (Ohio R. at Metropolis, IL), 30 (White R. at DeVall's Bluff, AR), 83 (Missouri R. at Hermann, MO), 79 (Canadian R. at Eufaula, OK), 80 (Yazoo R. at Redwood, MS), and 82 (Red R. at L. Texoma, TX); and at NAWQA Stations 212 (Little River Ditch at Moorehouse, MO) and 213 (Wolf R. at LaGrange, TN), both in the MSE Study Unit (Figs. 2-9, 2-10). Hg also exceeded $0.25 \mu\text{g/g}$ in smallmouth bass (*Micropterus dolomieu*) from NCBP Station 74 (Mississippi R. at Little Falls, MN), white bass from NCBP Station 75 (Mississippi R. at Cape Girardeau, MO), and carp from NAWQA Station 207 (Cache R. at Cotton Plant, AR), the latter in the ME Study Unit (Fig. 2-9). Hg Concentrations were 0.20 - $0.25 \mu\text{g/g}$ in largemouth bass, but only 0.03 - 0.04 in carp, from the reference site (Station 400; Fig. 2-9).

Geometric mean Hg concentrations at the NCBP stations sampled in 1995 were generally slightly higher than when these sites were last sampled in the mid-1980s (Figs. 2-10, 2-11). At Station 76, neither largemouth bass nor carp were collected in 1986; however, carp were collected there in 1984, when the Hg concentration in this species was 0.06 - $0.07 \mu\text{g/g}$ (Schmitt and Brumbaugh, 1990) vs. $<0.05 \mu\text{g/g}$ in 1995. Largemouth bass had not been collected at this site previously, so no comparison with 1995 data is possible. The greatest Hg increases were at Stations 81 and 82, both on the Red R. (Fig. 2-11); however, at these sites temporal differences are confounded by a change in species from 1986 to 1995. Largemouth bass and carp were collected in 1995 whereas other species [channel catfish, bigmouth buffalo (*Ictalurus cyprinellus*), white crappie (*Pomoxis annularis*), etc.] were collected in previous years. However, largemouth bass were collected from Lake Texoma (Station 82) prior to 1986, and concentrations were lower— $0.06 \mu\text{g/g}$ in 1981 (Lowe and others, 1985) and $0.03 \mu\text{g/g}$ in 1984 (Schmitt and Brumbaugh, 1990). It is important to note also that the largemouth bass analyzed in 1981 and 1984 were considerably smaller (mean length 249-287 mm, mean wt 181-318 g) than those collected in 1995 (males 381 mm, 796 g; females 403 mm, 961 g). Because Hg accumulates with size and age in predatory fishes to a greater

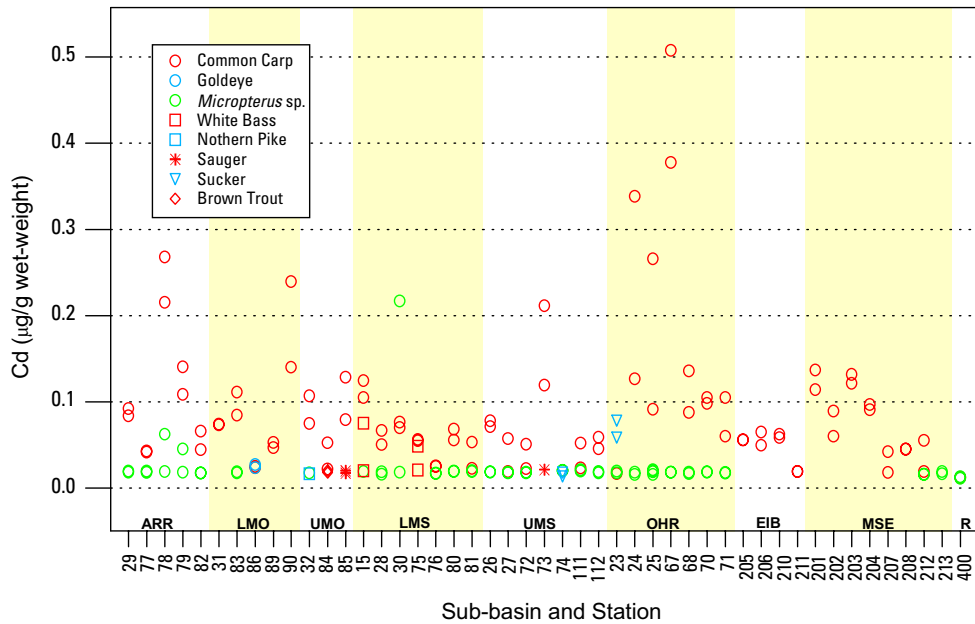


Figure 2-5. Concentrations of Cd in composite fish samples, by sub-basin, station, and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

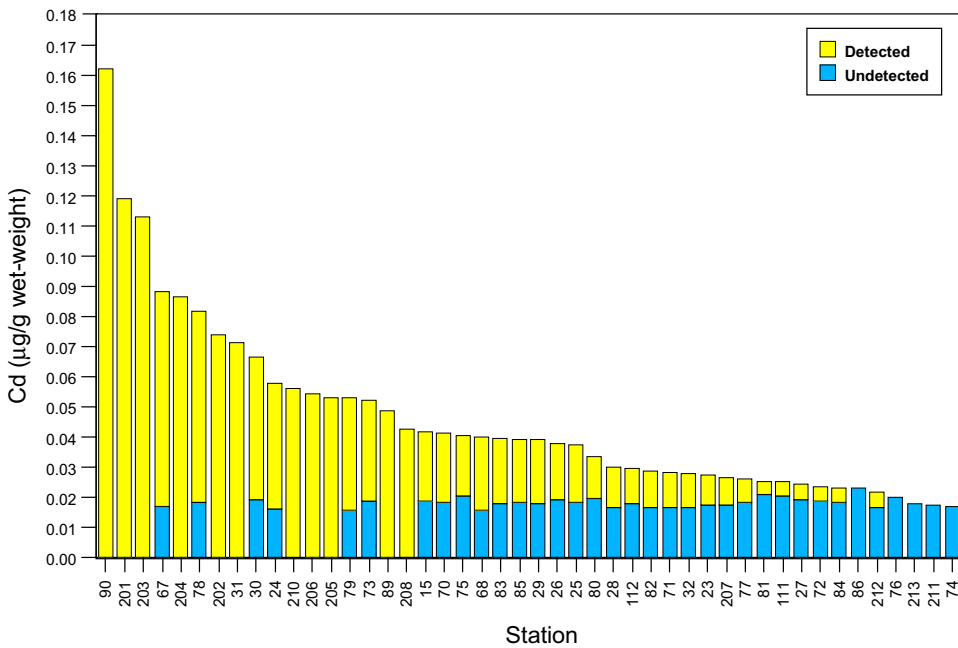


Figure 2-6. Ranked geometric mean concentrations of Cd in composite fish samples, by station. Shaded areas are means of censored observations (represented by 50% of LOD) used to compute the respective station means. See Table 1-1 for station descriptions.

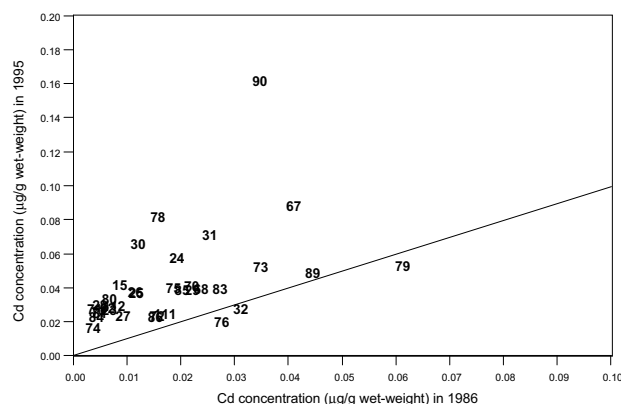


Figure 2-7. Geometric mean Cd concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

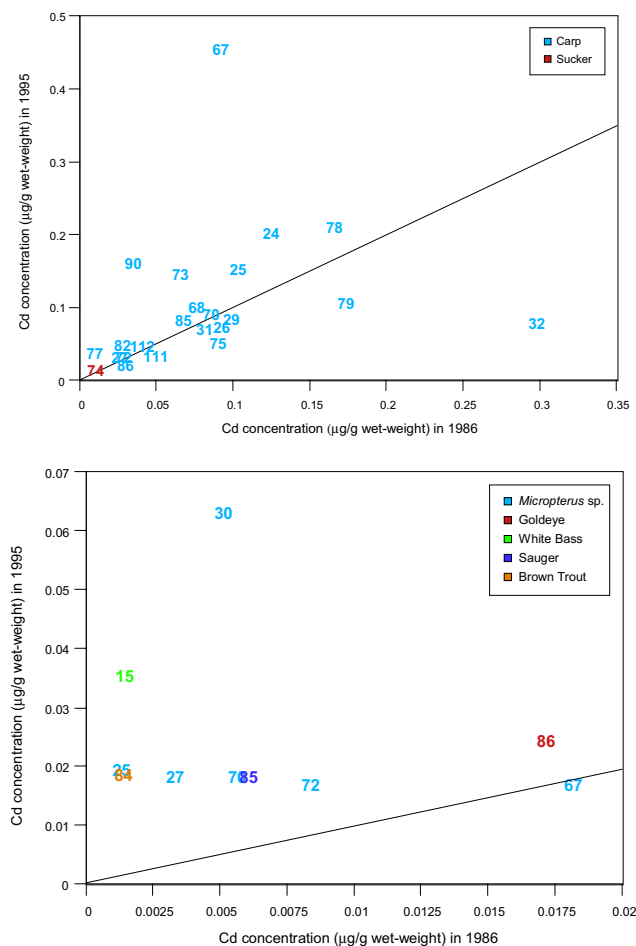


Figure 2-8. Geometric mean Cd concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

extent than most other contaminants (Wiener and Spry, 1996), at least some of the observed increase at Station 82 can be attributed to the larger size of the fish collected in 1995. Mercury accumulation in aquatic ecosystems is also highly dependent on ecosystem structure and trophic dynamics (Cabana and others, 1994). Consequently, temporal and geographic concentration differences may reflect ecosystem changes in addition to (or in lieu of) changes in Hg flux. We hope to evaluate this aspect in the future using information on nitrogen isotopic ratios.

Geometric mean Hg concentrations in fish from Stations 30, 31, and 85 declined slightly from 1986 to 1995 (Figs. 2-10, 2-11). For within-species comparisons, concentrations increased at some of these stations and decreased at others (Fig. 2-12). Hg in carp increased from 1986 to 1995 at Stations 67 (Allegheny R.), 112 (Mississippi R. at Dubuque, IA), 90 (Kansas R.), and 86 (James R.); and declined at Stations 25 (Cumberland R. at Clarksville, TN), 29 (Arkansas R. at Keystone Bluff, OK), 72 (Wisconsin R. at Woodman, WI), 78 (Verdigris R.), and 85 (Yellowstone R.). Hg concentrations increased from 0.072 µg/g to 0.14-0.20 µg/g in smallmouth bass at Station 72; from 0.18 µg/g to 0.24-0.39 µg/g in largemouth bass at Station 70 (Ohio R. at Metropolis, IL); and from 0.029 µg/g to 0.10-0.11 µg/g in spotted bass (*Micropterus punctulatus*) at Station 25 (Fig. 2-12). At Station 30 (White R.), Hg in largemouth bass increased steadily from 0.16 µg/g in 1979 (Lowe and others, 1985) to 0.23 µg/g in 1986 (Schmitt and others, 1999b) and 0.27-0.35 µg/g in 1995 (Fig. 2-12). Moreover, the largemouth bass collected at this site in 1995 were similar in size or smaller (mean for males 300 mm, 428 g; females 330 mm, 618 g) than those collected in 1986 (302 mm, 417 g) and 1979 (384 mm, 999 g), so the upward trend is not confounded with a decrease in fish size. Similarly, the largemouth bass collected from Station 70 in 1995 were slightly smaller (mean for males 352 mm TL, 637 g; females 341 mm, 488 g) than those collected in 1986 (358 mm, 699 g). Therefore, the observed increases at these stations are also not confounded by a change in fish size (in fact, the concentration increases may be underestimated). At Station 72, the smallmouth bass collected in 1995 (mean for males 260 mm, 200 g; females 320 mm, 450 g) were somewhat larger than those collected in 1986 (232 mm, 194 g), as were the spotted bass from Station 25 (males 280 mm, 220 g; females 320 mm, 500 g in 1995 vs. 218 mm, 73 g in 1986), so some of the increase noted at this station may also have been related to an increase in the size of the fish analyzed, as also noted for Station 82. Ecosystem changes may also have occurred.

Nationally, the concentration of Hg (as determined by NCBP fish collections) did not change from 1976 to 1986 after a period of decline from 1972 to

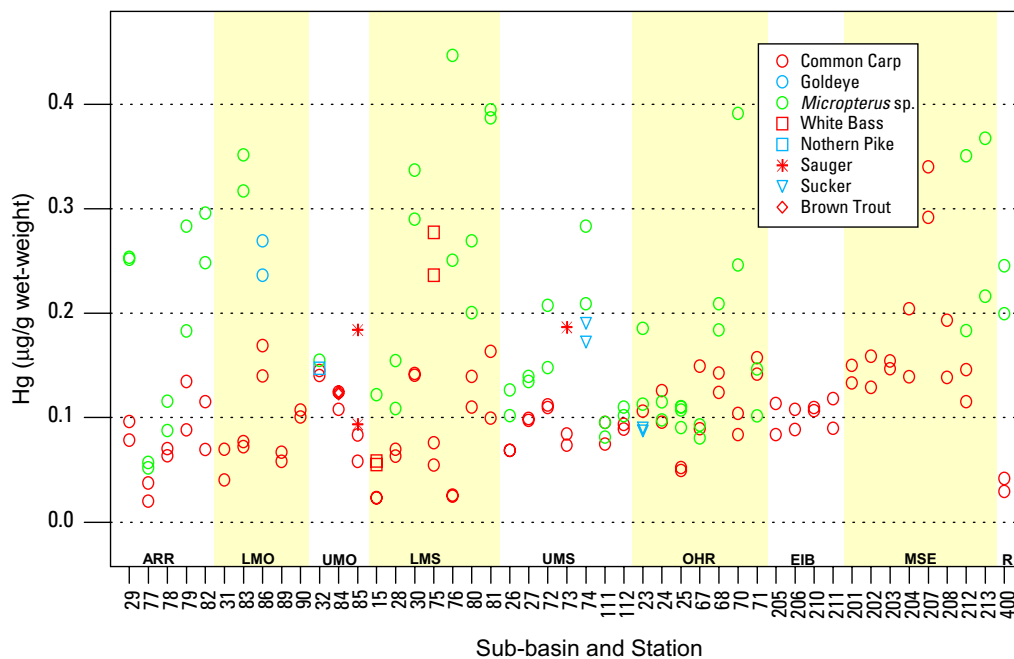


Figure 2-9. Concentrations of Hg in composite fish samples, by sub-basin, station, and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

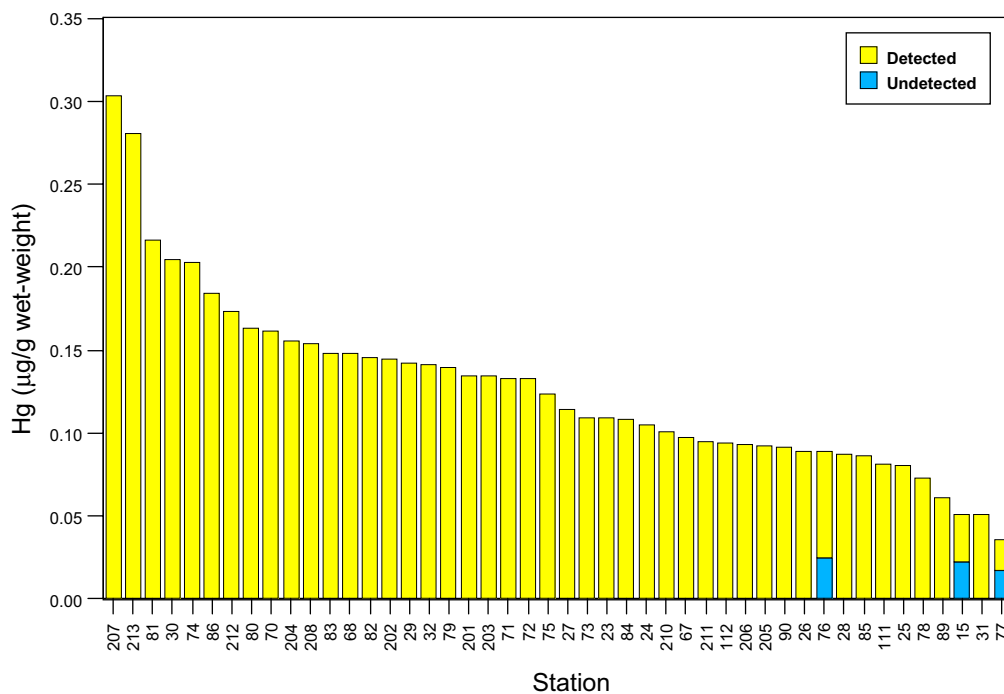


Figure 2-10. Ranked geometric mean concentrations of Hg in composite fish samples, by station. Shaded areas are means of censored observations (represented by 50% of LOD) used to compute the respective station means. See Table 1-1 for station descriptions.

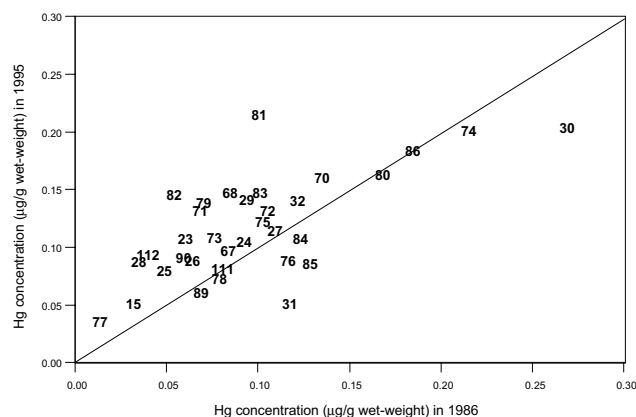


Figure 2-11. Geometric mean Hg concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right line indicate decreases between the two years. See Table 1-1 for station descriptions.

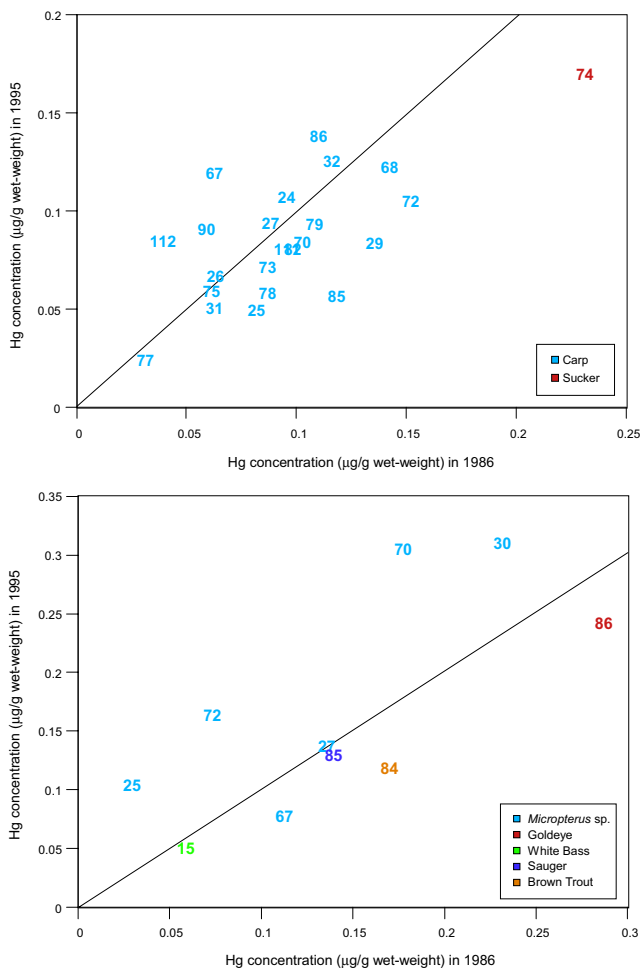


Figure 2-12. Geometric mean Hg concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

1976 (May and McKinney, 1981). From 1976 to 1986, Hg concentrations in fish declined significantly at 11 NCBP stations. Among these were several in the MRB with historically elevated Hg concentrations attributed to point-sources (May and McKinney, 1981; Schmitt and Brumbaugh, 1990). These included Station 71 (Tennessee R. at Savannah, TN), where concentrations fell steadily through 1984-86 and remained comparatively low in 1995 (Figs. 2-9—2-12). Conversely, Hg concentrations increased significantly from 1976 to 1986 at eight NCBP stations, including Station 30 (discussed above) and 76 (Mississippi R. at Memphis, TN), where levels had increased significantly from 0.06-0.16 µg/g in 1984 to 0.07-0.44 µg/g in 1986 (Schmitt and others, 1999b) and remained relatively high through 1995 (Figs. 2-9—2-12). Hg concentrations at Station 74 (Mississippi R. at Little Falls, MN) were also higher than most in 1986 (Schmitt and others, 1999b), but did not change appreciably from 1986 to 1995 (Fig. 2-11 and 2-12).

There were comparatively few censored Hg concentrations in carp (6%) or bass (none). Hg concentrations differed significantly among sub-basins and between programs in both taxa. In carp, mean concentrations were significantly greater ($P<0.01$) in the MSE Study Unit (due largely to Station 207) than in all other sub-basins, including the Lower Mississippi (LMS; Table 2-4). Conversely, concentrations at the reference site were significantly lower than in all sub-basins (Table 2-4). Overall, Hg concentrations in carp were significantly greater at NAWQA than at NCBP sites (Table 2-5), and were greater for both programs overall than at the reference site ($P<0.01$). Hg concentrations also differed significantly among sub-basins in bass ($P<0.01$), but no sub-basins differed significantly from the reference site (Table 2-4). In bass, the greatest Hg concentrations were in the Lower Missouri (LMO) sub-basin; however, this sub-basin included only Station 83 for bass. Nevertheless, Hg concentrations in the LMO sub-basin were significantly greater ($P<0.01$) than in the Arkansas-Red River (ARR), Upper Mississippi (UMS), and OHR sub-basins, in which concentrations were generally low. Hg concentrations in bass from the MSE Study Unit (two stations) did not differ significantly from the NCBP sites in the LMS sub-basin. As a group, Hg concentrations in bass from NCBP sites were significantly ($P<0.01$) lower than at NAWQA sites ($n=2$), but overall neither program differed significantly from the reference site (Table 2-5).

Most (>90%) of the Hg in whole fish occurs as the highly toxic methylmercury [MeHg—(Bloom, 1992; Southworth and others, 1995)]. Based on an extensive review of the literature, Wiener and Spry (1996) concluded that the threshold whole-fish con-

centration for adverse effects of Me-Hg on fish is in the range 0.7-5.3 µg/g, varying with taxon and endpoint. The maximum concentrations detected in the 1995 samples were all below these levels, albeit by a factor of less than two for the most contaminated samples (0.4 µg/g). All 1995 concentrations were also below the thresholds for adverse effects on piscivorous non-marine wildlife of 2-6 µg/g (wet-weight) for mammals and 3 µg/g dry weight (about 0.6 µg/g wet-weight) for birds proposed by Thompson (1996); however, the margin of safety for some of the most contaminated samples is less than two-fold. In addition, threshold 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), values exceeded by 1995 concentrations in bass from many stations (including the reference site).

Arsenic: Arsenic is released to the environment from industrial sources and from the use of arsenical pesticides and defoliants (Table 2-1). In 1995, As was detected (0.2-0.3 µg/g) in only 28% of the samples from 48% of the stations sampled (Table 2-3). Greatest concentrations (0.30-0.56 µg/g in one or more samples) were found at NCBP Stations 78 (Verdigris R. at Oologah, OK), 79 (Canadian R. at Eufaula, OK), 29 (Arkansas R. at Keystone Bluff, OK), 15 (Mississippi R. at Luling, LA), 80 (Yazoo R.), 26 (Illinois R. at Hardin, IL), 76 (Mississippi R. at Memphis, TN), and 75 (Mississippi R. at Cape Girardeau, MO; Fig. 2-13 and 2-14). Except for one sample of carp from Station 76, these greatest values all occurred in largemouth bass (Fig. 2-13). Concentrations were below detection limits (ca. 0.12 µg/g) in all samples from the NAWQA sites, from most of which only carp were collected (Fig. 2-13); and in all samples from Station 400 (reference site).

Concentrations of As in NCBP fish have historically been greatest at stations outside the Mississippi basin [that is, in the Southwest and Great Lakes—(Schmitt and Brumbaugh, 1990; Schmitt and others, 1999b)], largely because of its tendency to accumulate in planktivorous fishes not collected in the MRB. The lone exception has been Station 69 (Ohio R. at Cincinnati), where As concentrations were 0.07-0.11 µg/g in 1984 and increased to 0.06-0.78 µg/g in 1986. This increase was attributed to a change in species, however; one smallmouth buffalo containing 0.78 µg/g was collected in 1986 whereas carp had been collected previously (Schmitt and others, 1999b). Unfortunately, Station 69 was not sampled in 1995. Relative to 1986, geometric mean concentrations of As were slightly higher at most NCBP sites in 1995, the exceptions being Stations 70 (Ohio R. at Metropolis, IL) and 32 (Missouri R. at Nebraska City, NE), where concentrations increased slightly (Fig. 2-

15.). In comparisons within taxa, As concentrations decreased from 1986 to 1995 in carp and largemouth bass at Station 70, but increased in brown trout at Station 84 (Big Horn R.), goldeye at Station 85 (Yellowstone R.), and white sucker at Station 74 (Mississippi R. at Little Falls, MN—Fig. 2-16). Elsewhere concentrations did not change.

As noted, planktivorous fishes (and also sculpins, *Cottus* spp.) tend to accumulate As to a greater degree than other fishes (Wageman and others, 1978; Hunter and others, 1981; Schmitt and Brumbaugh, 1990), and can be further accumulated by piscivores that prey upon them (Hunter and others, 1981). Hence, the occurrence of planktivorous taxa at some sites and the dynamics of the ecosystems in which they occur may confound the interpretation of temporal trends for As, as noted for Hg. The comparatively high concentrations of As in largemouth bass in the southern parts of the MRB, especially in the impoundments, may therefore be as much or more a function of the occurrence of planktivorous clupeids (that is, *Dorosoma* spp.) as a reflection of environmental concentrations. However, it should also be noted that large amounts of arsenical compounds (herbicides and defoliants) have been used in cotton farming and other applications.

Concentrations of As in freshwater fish (ca. 0.5-1 µg/g) are low relative to naturally occurring concentrations in marine fishes and invertebrates, and are therefore not perceived to constitute a hazard to either the fish or to higher trophic level organisms that might consume them (USEPA, 1984). In addition, As is largely accumulated by fish as arsenobetaine, which is relatively non-toxic (Law, 1996).

Selenium: Selenium is released to the environment through the combustion of fossil fuels and can be leached from seleniferous soils (Table 2-1). In 1995, Se was detected (>0.02 µg/g) in all but two samples (female white sucker from Station 74, male carp from Station 400). Except for Station 77 (Arkansas R. at John Martin Reservoir, CO), concentrations were relatively low; individual sample concentrations ranged from barely detectable (≤0.20 µg/g) to 1.40 µg/g; Fig. 2-17), and geometric station means were 0.2-1.2 µg/g (Fig. 2-18). In contrast, concentrations were 3.5-4.7 µg/g in all samples (carp and largemouth bass) from Station 77, with a geometric station mean of about 4.0 µg/g (Fig. 2-18). The maximum 1986 concentration (3.4 µg/g) was also in carp from Station 77 (Table 2-3), with a geometric station mean of about 2.0 µg/g (Fig. 2-18). Concentrations at Station 77 increased from 1976-77 through 1986 (May and McKinney, 1981; Lowe and others, 1985; Schmitt and Brumbaugh, 1990; Schmitt and others, 1999b), a trend that seemed to continue into 1995 (Figs. 2-19 and 2-20). In 1995, Se concentrations exceeded 1.0 µg/g in

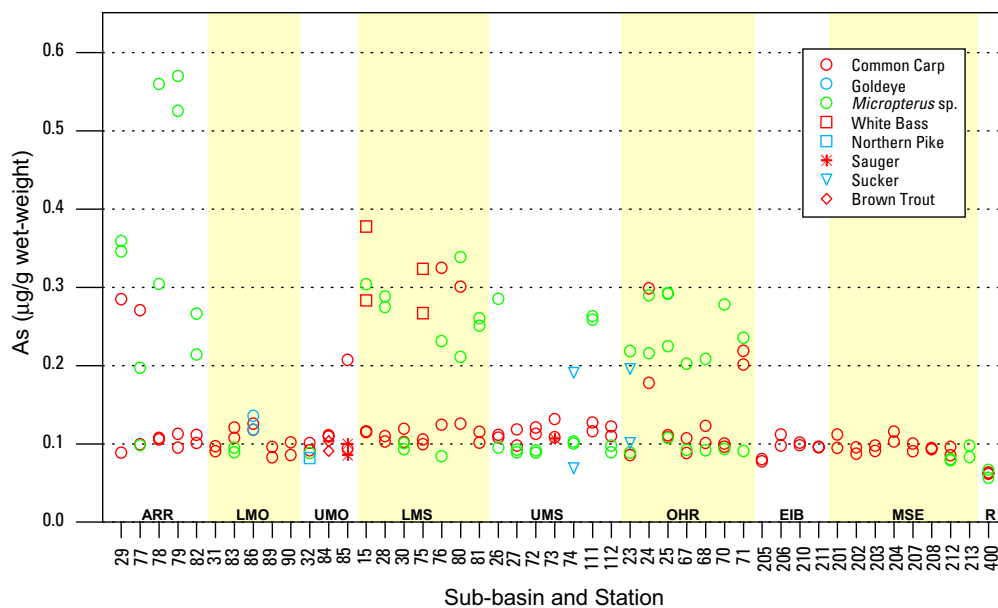


Figure 2-13. Concentrations of As in composite fish samples, by sub-basin, station and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

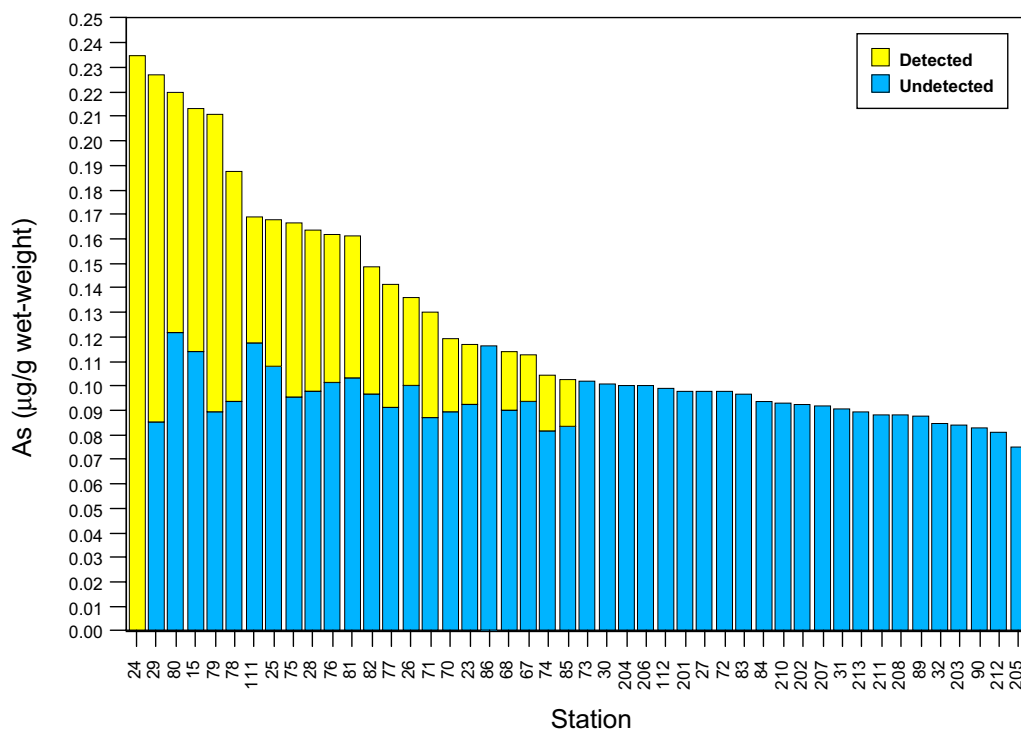


Figure 2-14. Ranked geometric mean concentrations of As in composite fish samples, by station. Shaded areas are means of censored observations (represented by 50% of LOD) used to compute the respective station means. See Table 1-1 for station descriptions.

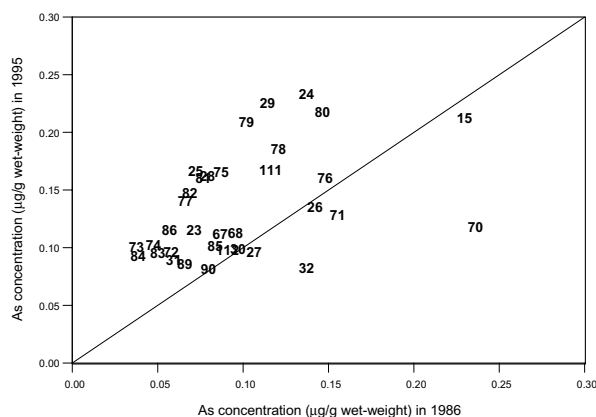


Figure 2-15. Geometric mean As concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

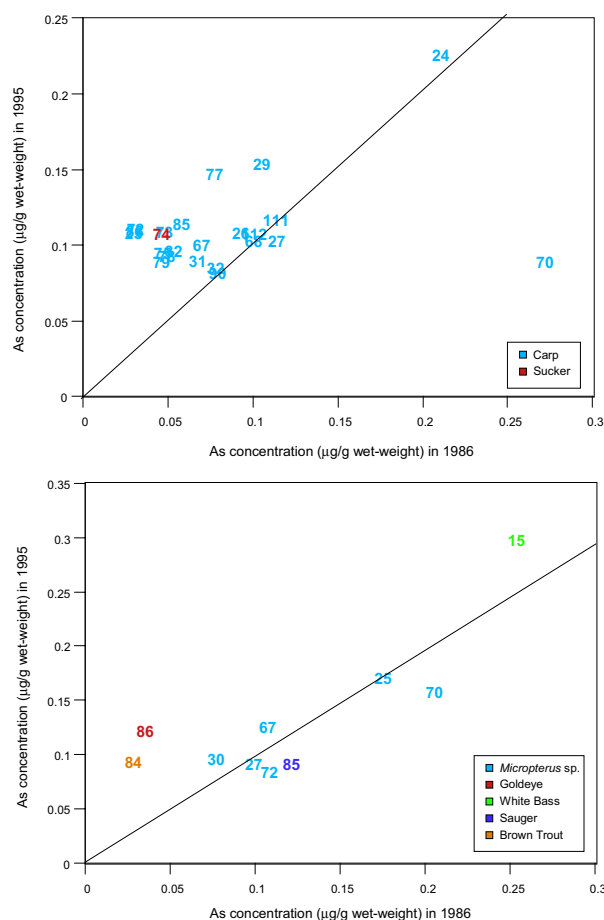


Figure 2-16. Geometric mean As concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

samples from only two other stations: NCBP Station 84 (Big Horn R. at Hardin, MT), where concentrations were 1.0-1.4 $\mu\text{g/g}$ in all samples (carp and brown trout), as they were in 1986 (Schmitt and others, 1999b; Fig. 2-20); and NCBP Station 78 (Verdigris R.), where concentrations were about 1.2 $\mu\text{g/g}$ in both 1995 samples of carp but were lower (0.7 $\mu\text{g/g}$) in largemouth bass (Fig. 2-20). The 1995 concentrations in carp were two-fold greater than the 1986 concentrations, but largemouth bass were not collected there in 1986 (Fig. 2-20; Schmitt and others, 1999b). Concentrations of Se also increased slightly in sauger at Station 85 (Yellowstone R.), but declined in white sucker at Station 74 (Mississippi R. at Little Falls, MN—Fig. 2-20). Concentrations in fish from Station 400 were low (<0.12-0.29 $\mu\text{g/g}$ in carp, 0.27-0.30 $\mu\text{g/g}$ in largemouth bass; Fig. 2-17).

There were no censored values for Se in bass, and only one value was below detection limits in carp. Se concentrations in both carp and bass differed significantly among sub-basins ($P<0.01$). Concentrations in carp from NCBP sub-basins with the greatest concentrations (ARR, UMO, and LMO) were significantly greater than those from the other sub-basins and from the reference site; and concentrations at the reference site were significantly lower than in all sub-basins (Table 2-4). Concentrations in both NAWQA Study Units (MSE and EIB) were significantly ($P<0.01$) greater than in their respective NCBP sub-basin (UMS and LMS). Overall, Se concentrations in carp did not differ significantly between NCBP and NAWQA stations, but concentrations at both were significantly greater than at the reference site ($P<0.01$; Table 2-5). It is important to note that Station 77, where Se concentrations were 5- to 10-fold greater than at most other stations, was deleted from the statistical analyses because of mixed-gender compositing of carp samples; had Station 77 been included, differences among sub-basins and between programs would have been more evident. For bass, no stations were deleted. Se concentrations in the UMO, LMO, and ARR sub-basins were significantly ($P<0.01$) greater than in all others and at the reference site, but the three greatest did not differ among themselves (Table 2-4). As a group, Se concentrations in bass from the NCBP stations were significantly greater than at the reference site and the NAWQA sites (only two stations from the MSE Study Unit), but the NAWQA sites did not differ from the reference site (Table 2-5).

Nationally, the geometric mean concentration of Se in fish declined slightly from 1978-81 to 1984 (Schmitt and Brumbaugh, 1990), but increased slightly in 1986 (Schmitt and others, 1999b). Elevated Se in fish partly reflects high soil concentrations in some areas of the west and the Great Plains. The bioaccumulation of Se can become a problem for fish and

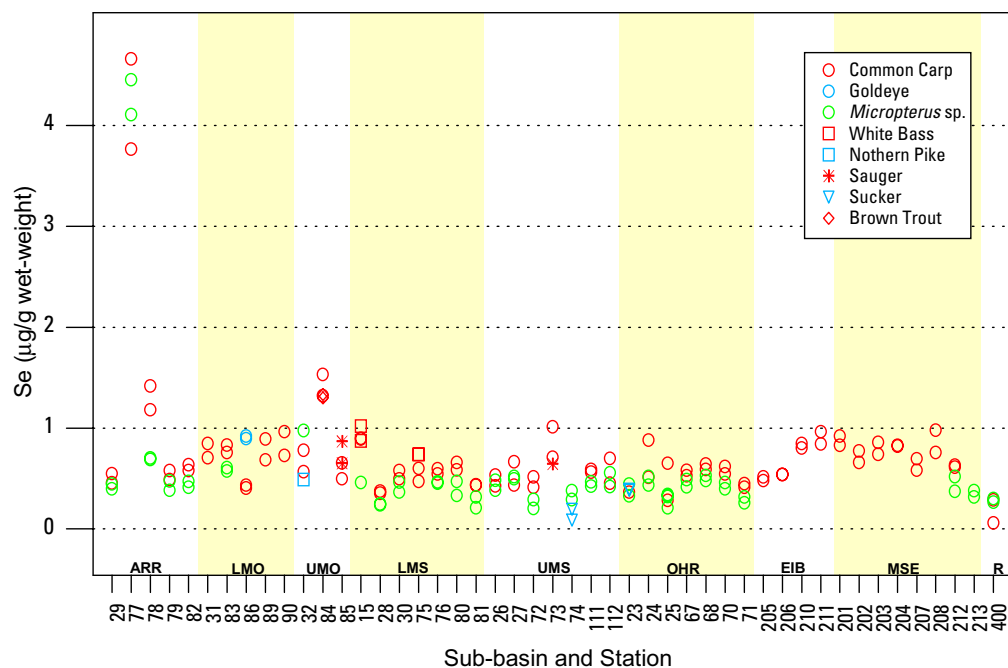


Figure 2-17. Concentrations of Se in composite fish samples, by sub-basin, station and taxon. Censored values (Station 74 only) are plotted as 50% of LOD. See Table 1-1 for station descriptions.

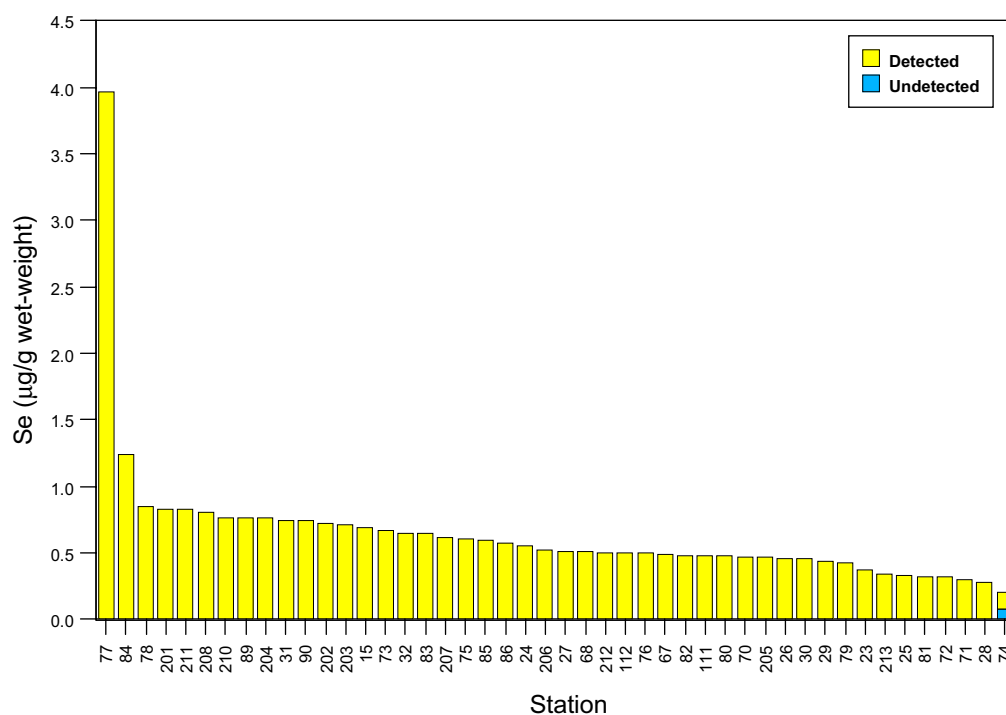


Figure 2-18. Ranked geometric mean concentrations of Se in composite fish samples, by station. Shaded area for Station 74 is the mean of censored observations (represented by 50% of LOD) used to compute the station mean. See Table 1-1 for station descriptions.

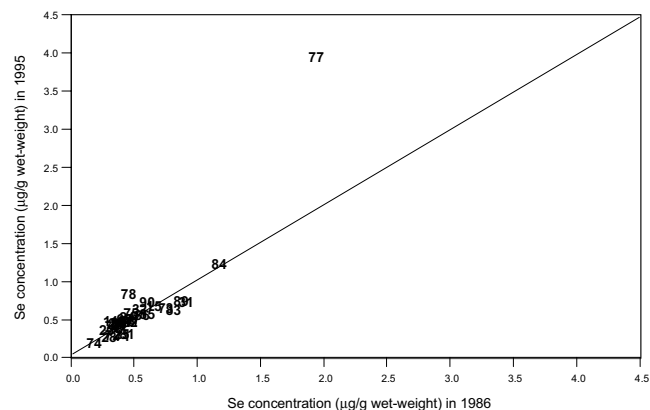


Figure 2-19. Geometric mean Se concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right line indicate decreases between the two years. See Table 1-1 for station descriptions.

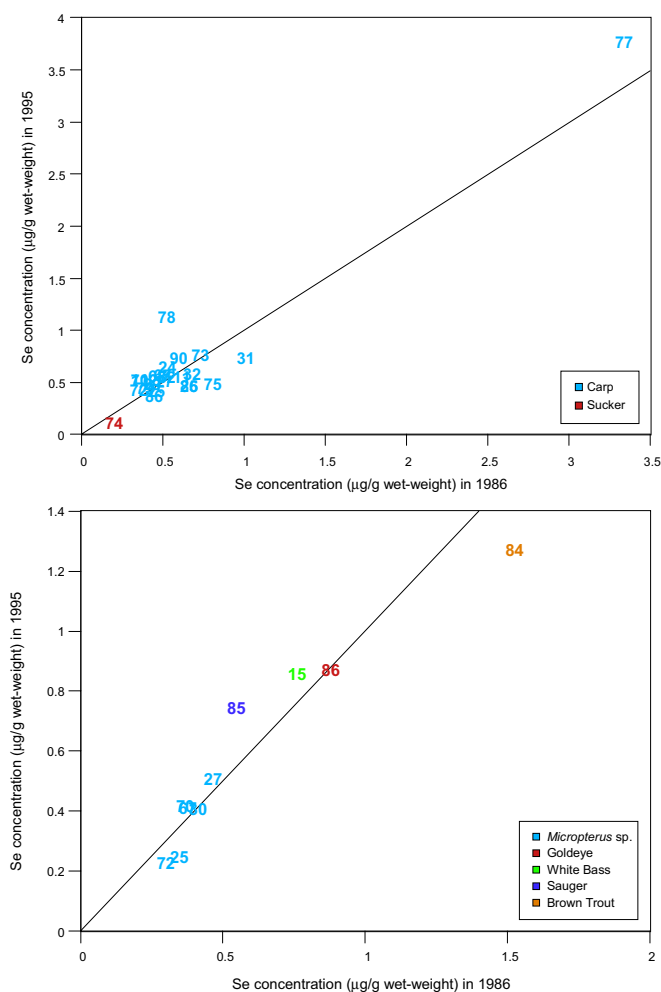


Figure 2-20. Geometric mean Se concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

wildlife when arid, seleniferous soils are leached by irrigation, as they have been in agricultural areas of the West. Selenium bioaccumulation to problematic levels has also been noted in cooling reservoirs associated with coal-fired powerplants (Baumann and Gillespie, 1986). According to Lemly (1996), the whole-fish threshold for Se toxicity (to the fish) is about 4 µg/g dry weight (0.8 µg/g wet-weight), and for the protection of piscivorous wildlife it is about 3 µg/g dry weight (0.6 µg/g wet-weight). The carp from Stations 77, 78, and 84 exceeded both values in 1995. So also did the largemouth bass from Station 77 and one sample of carp from each of NCBP Stations 32 (Missouri R. at Garrison Dam, MT), 89 (Platte R. at Louisville, NE), 90 (Kansas R. at Bonner Springs, KS), 85 (Yellowstone R. at Sidney, MT); both samples of carp from each of NCBP Stations 15 (Mississippi R. at Luling, LA) and 86 (James R. at Olivet, SD); and one carp sample from NAWQA Stations 201 (Big Sunflower R., Anguilla, MS), 211 (Cedar R. at St. Charles City, IA), and 208 (Cache R. at Egypt, AR).

Other Elements: The ICPES elemental scan yielded data for some analytes of marginal environmental interest and, without pre-concentration, lacks sufficient sensitivity for some elements of concern. Although all can be toxic to fish under certain conditions, many are also essential trace elements that do not accumulate in fish to concentrations that represent a threat to either the fish or to higher-level consumers, even in heavily contaminated areas. Many elements accumulate preferentially in specific organs and tissues (for example, May and McKinney, 1981; Harrison and Klaverkamp, 1990; Farag and others, 1995; Goldstein and others, 1996; Goldstein and DeWeese, 1999; Taylor and others, 2000) such as liver (Zn, Cu, As, etc.), kidney (Cd), bone (Pb), gill (Cu), or muscle (Hg). The accumulation of these elements, especially Zn, also differs greatly among taxa; for example, carp seem to accumulate Zn to a greater extent than other fishes (Schmitt and Brumbaugh, 1990; Schmitt and others, 1999b), and white perch (*Morone americana*) accumulate Cu in their livers in a condition analogous to Wilson's disease in humans (Bunton and others, 1987). In addition, the elements Al, Fe, and Mn are major constituents of sediments, and their concentrations in fish carcasses may reflect the accumulation of particulate material in or on the fish (Brumbaugh and Kane, 1985). The concentrations of other elements in whole fish may similarly include ingested or sorbed sediments.

Concentrations of the more abundant crustal elements may also be used as covariates to account for otherwise unexplained variation in the concentrations of other analytes, however (for example, Schmitt and Finger, 1987; Sutherland and others, 2000). In keep-

ing with past NCBP reports (for example, Schmitt and others, 1999b) only results for Cu and Zn are presented here. Data for the other elements can be obtained at <http://www.cerc.usgs.gov/data/data.htm>.

Zinc is released to the environment from mining, smelting, and a variety of other activities and sources (Table 2-1). In 1995, Zn concentrations in carp ranged from about 40 µg/g to 150 µg/g (Table 2-3); in all other taxa, the range was 15-30 µg/g (Fig. 2-21). Greatest concentrations (>90 µg/g, all in carp) occurred at NCBP Stations 79 (Canadian R. at Eufaula, OK) and 84 (Big Horn R. at Hardin, MT); at two OHR stations—67 (Allegheny R. at Natrona, PA) and 24 (Ohio R. at Marietta); and at five NCBP stations in the Upper Mississippi River system—26 (Illinois R. at Beardstown, IL), 112 (Mississippi R. at Dubuque, IA), 27 (Mississippi R. at Guttenburg, IA), 73 (Des Moines R. at Keosauqua, IA), and 111 (Mississippi R. at Lake City, MN); and at one EIB NAWQA site—Station 210 (Iowa R. at Rowan, IA). At the reference site (Station 400), Zn concentrations in carp were low (0.25 µg/g) in both samples. Because of the preferential accumulation of Zn by carp, geometric station means reflect the collection of carp at the sites (Fig. 2-22).

Geometric mean Zn concentrations increased from 1986 to 1995 at many NCBP stations (Fig. 2-23; however, at some of these sites the increases reflect the more widespread distribution of carp in the 1995 collection. In carp, Zn concentrations increased from 1986 to 1995 at Stations 79, 26, 112, 73, 27 and 67, and decreased at Stations 75, 85, 68, 86, and 32 (Fig. 2-24). Concentrations also increased in goldeye at Station 86 (James R.), white bass at Station 25 (Cumberland R.), sauger at Station 85 (Yellowstone R.), and white suckers at Station 74 (Mississippi R. at Little Falls, MN—Fig. 2-24).

Like Zn, Cu is an essential element that is also released from a variety of sources (Table 2-1). Although Cu was detected by ICPES in all samples (Table 2-3), the data in Figs. 2-27 and 2-28 indicate a lack of sensitivity near the LOD relative to concentrations in 1986, when Cu was measured by AA, and temporal trends at low concentrations cannot be evaluated. Nevertheless, at higher concentrations and in contrast to Zn, there were fewer clearly evident trends in the 1995 results for Cu. Measured Cu concentrations ranged from about 0.4 to 3.8 µg/g, with greatest concentrations in white bass from NCBP Station 15 (Mississippi R. at Luling, LA; Fig. 2-25, Table 2-3). Concentrations were also higher than most (>1.5 µg/g) in white bass from Station 75 (Mississippi R. at Cape Girardeau, MO); and in carp from Stations 30 (White R. at DeVall's Bluff, AR), 89 (Platte R. at Louisville, NE), 67 (Allegheny R.) and 24 (Ohio R. at Marietta). Copper concentrations at

the reference site (Station 400) were also about 1.0 µg/g in carp; in largemouth bass they were 0.35-0.66 µg/g. Geometric station means reflect these general patterns (Fig. 2-26). Although high in comparison to other species, the 1995 concentrations in white bass from Station 15 are nevertheless about 10-fold lower than those typical of the congeneric white perch from Atlantic coastal rivers and estuaries (Bunton and others, 1987; Schmitt and Brumbaugh, 1990). Relative to 1986, geometric mean concentrations increased at Stations 89, 90 (Kansas R. at Bonner Springs, KS), and 24; and decreased at Stations 75, 15, and 31 (Missouri R. at Nebraska City, NE; Fig. 2-27). Concentrations in white bass were slightly higher at Station 15 in 1986 than in 1995 (Fig. 2-28). In carp, Cu concentrations increased from 1986 to 1995 at Stations 67 (Allegheny R.) and 24 (Ohio R.), and decreased at Station 72 (Wisconsin R.; Fig. 2-28). Concentrations also declined in largemouth and smallmouth bass at Stations 72 (Wisconsin R.), 27 (Mississippi R. at Guttenburg, IA), 67 (Allegheny R.), and 70 (Ohio R. at Metropolis, IL); in sauger at Station 85 (Yellowstone R.); in goldeye at Station 86 (James R.); in white bass at Station 15 (Mississippi R. at Luling, LA); in white sucker at Station 74 (Mississippi R. at Little Falls, MN); and in brown trout at Station 84 (Big Horn R., MT; Fig. 2-28). On a dry-weight basis, the 1995 Cu concentrations in the two samples (1.83-2.52 µg/g) from Station 84 were within the range of values reported for this species at reference sites in Montana, but were 2- to 3-fold lower than concentrations associated with adverse effects on organism health and physiology in brown trout from mining-contaminated sites on the Clark Fork River (Farag and others, 1995).

For the other elements detected in all or most of the samples (that is, Al, Ba, Cr, Fe, Mg, Mn, Ni; and Sr), concentrations were elevated in several samples relative to the bulk of the collection, but there were few clearly evident geographic or other trends. Vanadium was detected by ICPES in only 12% of the samples, Mo in four samples, Be in one, and Bo was not detected in any sample.

Organochlorine Chemical Residues

DDT and its Primary Metabolites: Prior to its ban in 1972 the insecticide DDT was used to control many pests throughout the U.S. Environmental residues of DDT and its degradation products (Table 2-1) persist in many areas from historic use, especially in cotton-growing regions (Schmitt, 1999). Residues also remain evident near sites of former DDT production and synthesis and as a result of atmospheric transport from parts of the world where DDT is still used.

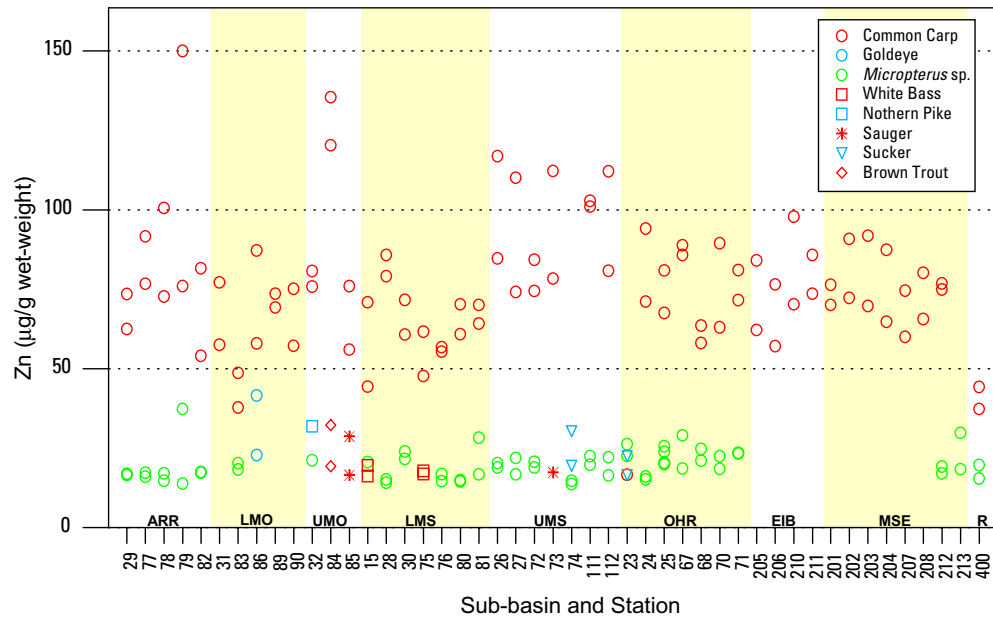


Figure 2-21. Concentrations of Zn in composite fish samples, by sub-basin, station, and taxon. See Table 1-1 for station descriptions.

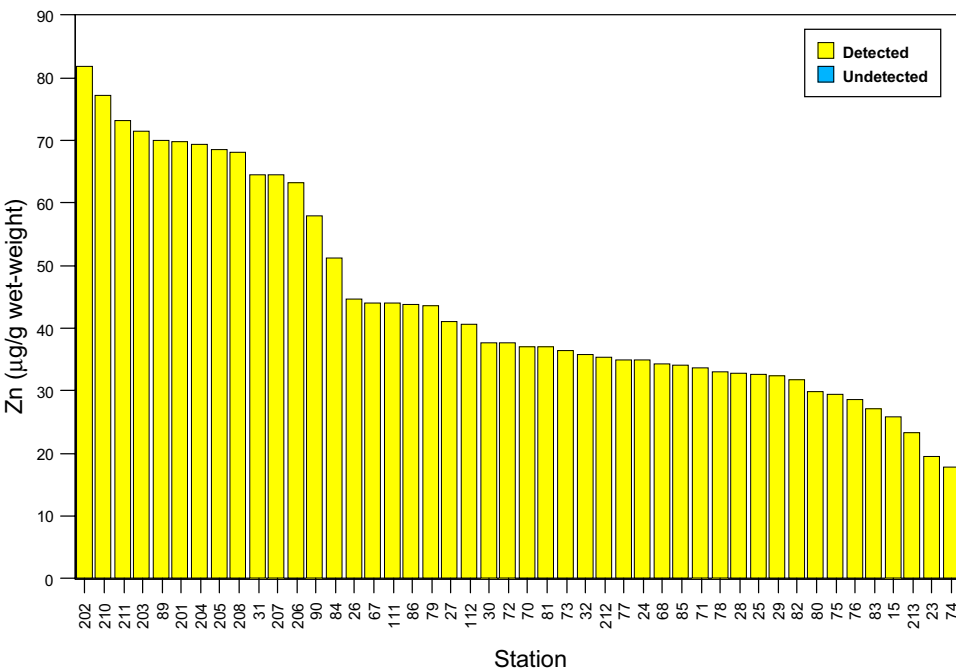


Figure 2-22. Ranked geometric mean concentrations of Zn in composite fish samples, by station. See Table 1-1 for station descriptions.

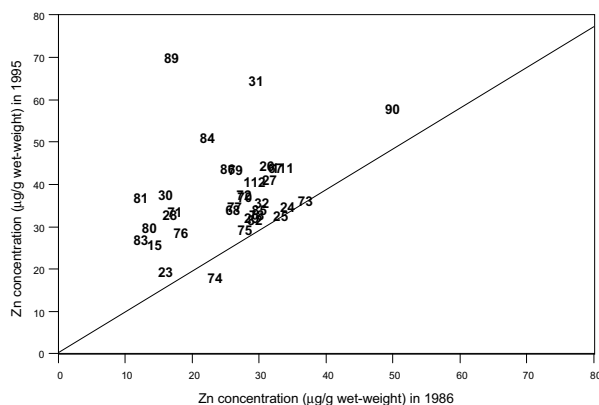


Figure 2-23. Geometric mean Zn concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

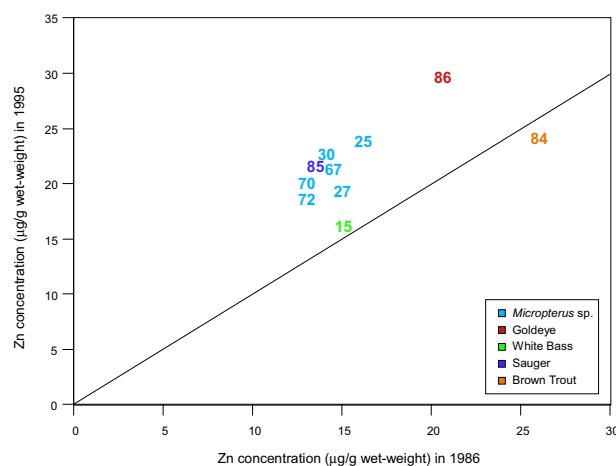
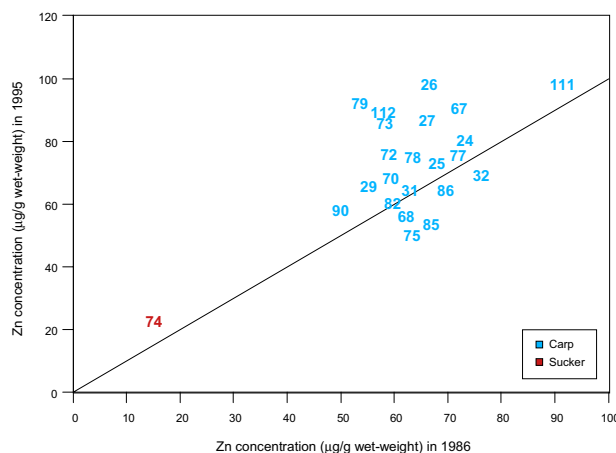


Figure 2-24. Geometric mean Zn concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

Although long-lived, *p,p'*-DDT (the active insecticide) is metabolized by vertebrates to a number of other residues, the most stable and toxic of which is *p,p'*-DDE. Although banned in the U.S. in 1972, DDT residues (as *p,p'*-DDE) were detected in 91% of the samples and at all stations sampled in 1995 (Table 2-3), including Station 400 (the reference site). Total DDT concentrations in individual samples ranged from non-detectable (<0.01 µg/g) to 11 µg/g (Table 2-3). All of the greatest DDT concentrations (that is, ≥1.0 µg/g) occurred in the southernmost part of the MRB (Figs. 2-29 and 2-30), at Stations 201, 202, 203, and 204 (in the MSE Study Unit) and at NCBP Station 80 (Yazoo R. at Redwood, MS). Total DDT concentrations in fish from Station 400 were 0.3-0.4 µg/g (mostly as DDE) in carp, and <0.1 µg/g in largemouth bass (Fig. 2-29).

The predominant homolog in all 1995 samples with detectable residues was *p,p'*-DDE (Fig. 2-29). Next most abundant was *p,p'*-DDD, an anaerobic metabolite of *p,p'*-DDT that was also used historically as an insecticide. Traces (<0.02 µg/g) of *p,p'*-DDT, the parent insecticide, were found in only seven samples—two from each of Stations 80, 24 (Ohio R. at Marietta, OH), and 28 (Arkansas R. at Pine Bluff, AR); and one from Station 67 (Allegheny R. at Natrona, PA). Station 80 has a long history of contamination by DDT and other organochlorine pesticides from intensive cotton farming in the Yazoo River basin, and Station 28 is influenced by a point-source (Pine Bluff Arsenal) at which DDT was synthesized for military use. There was no detectable *p,p'*-DDT in any sample from the NAWQA sites, including those in the Yazoo basin, despite 5-fold greater total DDT concentrations in all samples from Stations 201-204 than from Station 80 (Fig. 2-29). Traces of *o,p'*-DDT homologs (mostly as the degradation product *o,p'*-DDD), which occur as impurities in insecticidal DDT, were present in fish from the reference site (Station 400) and at Stations 24 (Ohio R. at Marietta, OH), 28 (Arkansas R.), 67 (Allegheny R.), 68 (Wabash R. at New Harmony, IN), 80, and 81; and Stations 201-204, in the Mississippi Embayment NAWQA Study Unit; and Station 211 (Cedar R. at St. Charles City, IA).

Among NCBP sites in the MRB, total DDT concentrations were greatest at Stations 80 and 81 in 1995, as they were in 1986 (Schmitt and others, 1999b). The mean 1995 total DDT concentration at Station 80 was 1.2 µg/g, mostly as *p,p'*-DDE (Fig. 2-31), down from 2.5 µg/g in 1986 (Fig. 2-32). The 1986 total included about 0.3 µg/g (12%) of *p,p'*-DDT (Schmitt and others, 1999b). Total DDT concentrations declined less at NCBP Station 81 (Red R. at Alexandria, LA), from a mean of 0.5 µg/g in 1986 to about 0.3 µg/g in 1995 (Figs. 2-31 and 2-32).

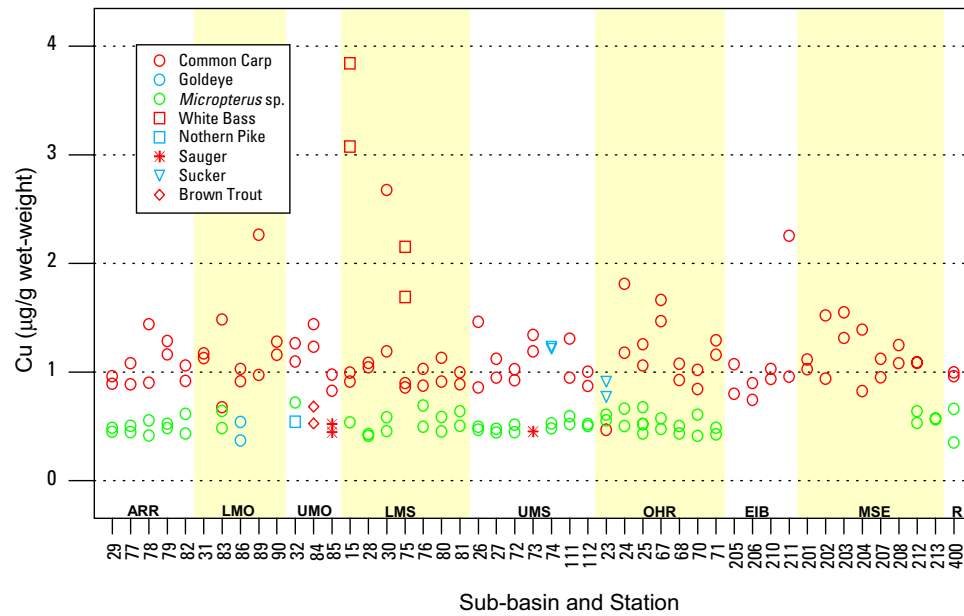


Figure 2-25. Concentrations of Cu in composite fish samples, by sub-basin, station, and taxon. See Table 1-1 for station descriptions.



Figure 2-26. Ranked geometric mean concentrations of Cu in composite fish samples, by station. See Table 1-1 for station descriptions.

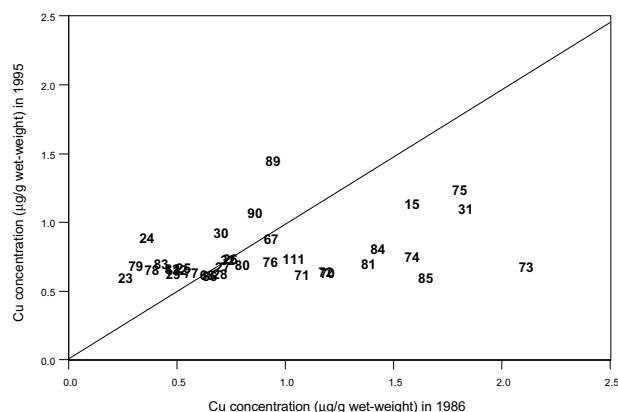


Figure 2-27. Geometric mean Cu concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

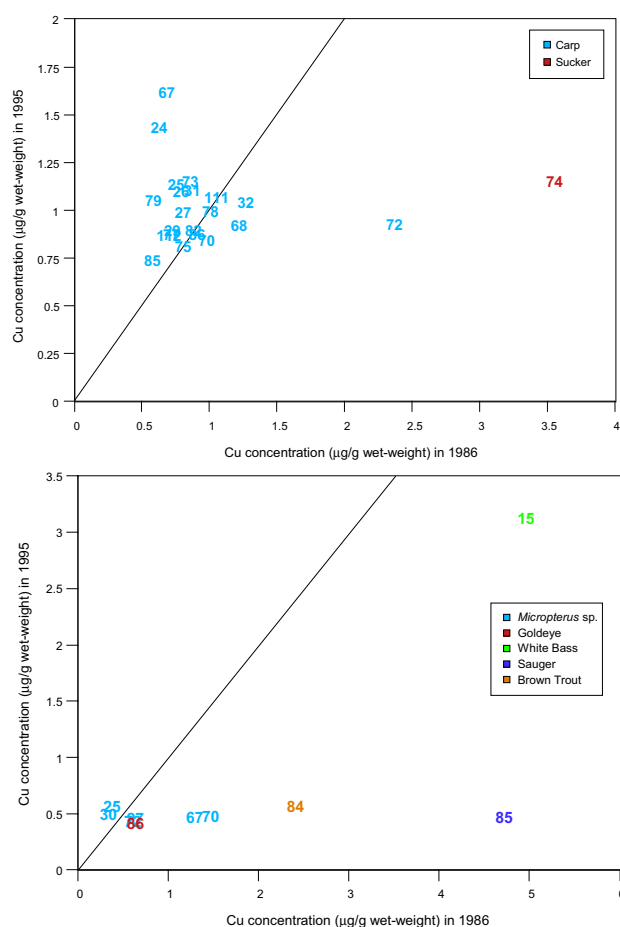


Figure 2-28. Geometric mean Cu concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

However, it should be noted that there were no taxa in common to both the 1986 and 1995 collections at either of these stations (see Appendix A). Nationally, Station 80 historically yielded fish with the greatest concentrations of total DDT; concentrations were 10-30 µg/g in the early 1970s (Schmitt and others, 1981) and declined steadily to 2-6 µg/g in 1986 (Schmitt and others, 1999b) and <3 µg/g in 1995 (Figs. 2-31 and 2-32). In comparison, 1995 total DDT concentrations in fish from NAWQA Stations 201-204 (4-11 µg/g) were comparable to Station 80 concentrations of the late 1970s and early 1980s (Schmitt and others, 1983; 1985). Total DDT concentrations declined at most of the stations where within-taxon comparisons could be made (Fig. 2-32). The exceptions were total DDT in carp and largemouth bass from Station 67 (Allegheny R.) and in carp from Station 82 (L. Texoma), where concentrations increased (Fig. 2-32).

Residues of DDT (as *p,p'*-DDE, the predominant homolog) were detected in about 95% of the carp and bass samples analyzed. Concentrations differed significantly among sub-basins and between NAWQA and NCBP stations (Tables 2-4 and 2-5). In both carp and bass, most sub-basins differed significantly ($P<0.01$) from each other and from the reference site; however, most mean concentrations were low and the differences were small. The exception was the MSE Study Unit, where the greatest mean concentrations in carp occurred. Concentrations in MSE carp were significantly ($P<0.01$) greater than in other sub-basins, including the LMS (in which the MSE Study Unit is contained; Table 2-4). In contrast to the MSE, DDE concentrations in EIB carp were significantly lower than in carp from the UMS sub-basin; however, the concentrations were low in both and the differences were small. As a group, DDE concentrations were significantly greater in carp from the NAWQA sites than from the NCBP sites due to the very high levels in fish from the MSE Study Unit; however, only the mean for NCBP sites differed from the concentrations at the reference site, which in carp were very low (Table 2-5). Concentrations in bass did not differ between NCBP and NAWQA sites (two stations); however, concentrations at the NCBP stations, but not the NAWQA sites, were significantly lower than at the reference site (Table 2-5).

Following the 1972 U.S. ban on DDT use the proportional composition of the DDT mixture present in U.S. freshwater fish gradually changed. From 1970 to 1980-81, *p,p'*-DDE accounted for about 70% of total *p,p'*-DDT homologs (Schmitt and others, 1981; 1983; 1985), then increased to 73% in 1984 (Schmitt and others, 1990) and 74% in 1986 (Schmitt and others, 1999b), reflecting the reduced influx and continuing weathering of *p,p'*-DDT in the environment. In 1995, the average among stations with detectable DDT residues was still about 75%. Nationwide, the

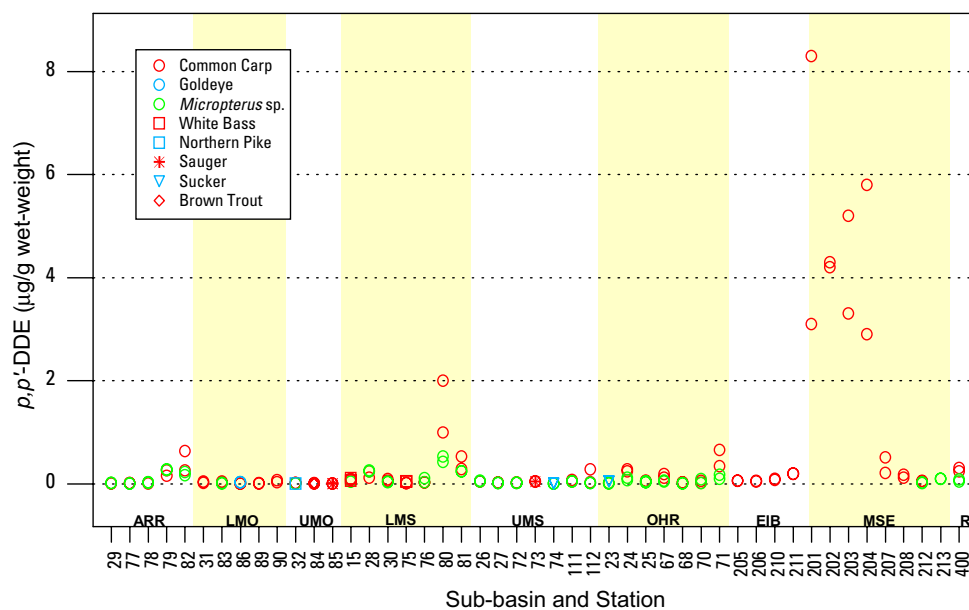


Figure 2-29. Concentrations of p,p' -DDE in composite fish samples, by sub-basin, station, and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

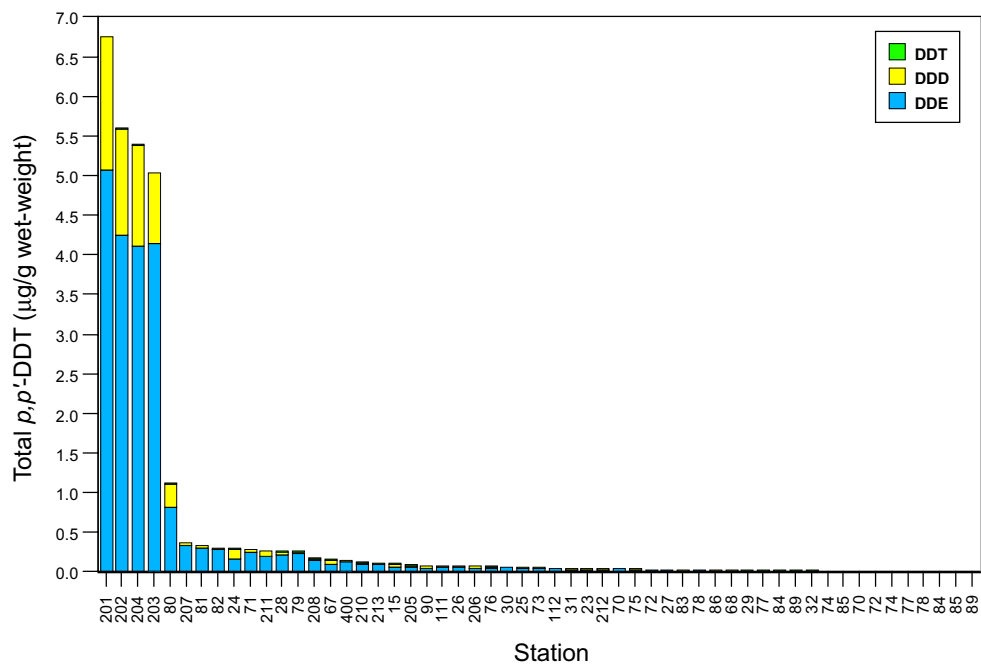


Figure 2-30. Ranked geometric mean concentrations of p,p' -DDT homologs in composite fish samples, by station. (Note: Censored values are represented by 50% of LOD in the totals and means but are not shown). See Table 1-1 for station descriptions.

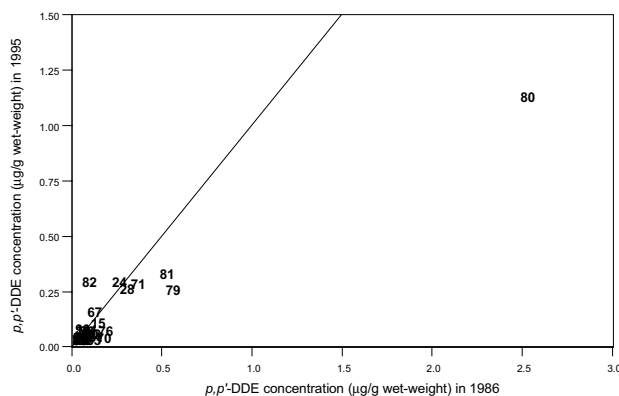


Figure 2-31. Geometric mean p,p' -DDE concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

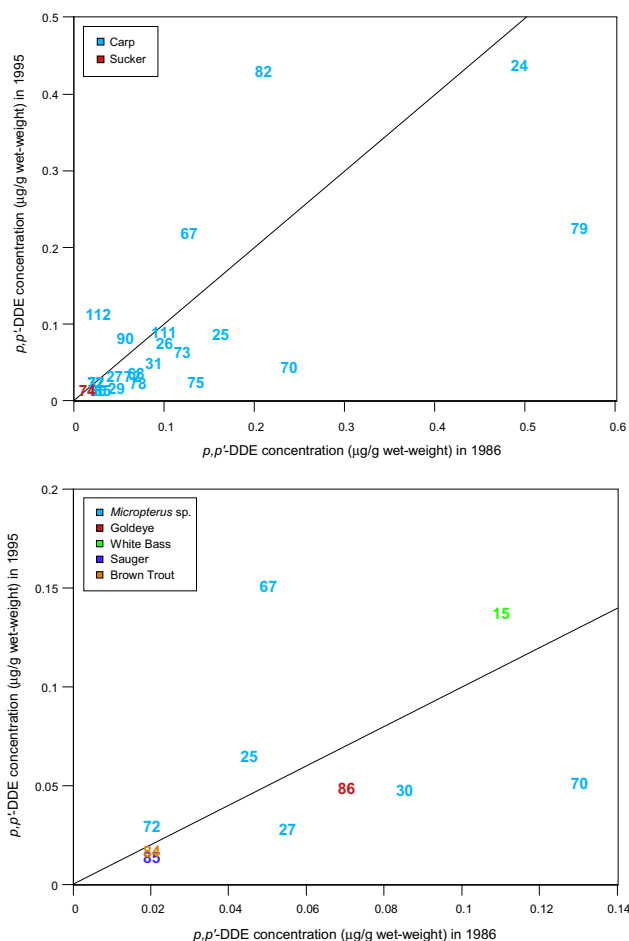


Figure 2-32. Geometric mean p,p' -DDE concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

NAWQA program reported a higher percentage of p,p' -DDE (86%) in fish collected from 234 sites in 1991-1995 (Wong and others, 2000). The occurrence of proportionally high concentrations of p,p' -DDT may signify recent inputs of unweathered insecticide (Aguillar, 1984), which was not indicated for any station sampled in 1995. DDT-derived residues have been nearly ubiquitous since the 1960s; detectable residues of p,p' -DDE, the most persistent metabolite of DDT, have been present at all or nearly all NCBP stations in every collection since 1970 (Schmitt and others, 1985; 1990; 1999b).

In terms of ecological risk, the USEPA ambient water quality criterion for DDT (USEPA, 1980) is based on a value of 0.15 µg/g (total DDT) in fish for the protection of reproduction in the brown pelican (*Pelicanus occidentalis*), the most sensitive species evaluated (Anderson and others, 1975); for other avian species, the range is 1-3 µg/g (Blus, 1996). However, the Canadian Council of Ministers of the Environment [Environment Canada (EC), 1999] guideline is 0.14 µg/g and the New York State Department of Environmental Quality (NYSDEC) guideline is 0.2 µg/g (Newell and others, 1987). Relative to the higher values of Blus (1996), the 1995 DDT residues in fish from NCBP and NAWQA sites in the lower MRB represent a hazard to most fish-eating birds. Based on the lower values (USEPA, 1980; Newell and others, 1987; EC, 1999), sensitive species may also represent a hazard to sensitive wildlife species at several stations in the ARR and OHR sub-basins and at the reference site (Figs. 2-29, 2-32). Fitzsimons (1995) concluded that 4 µg/g was the threshold for early life stage effects in salmonids, a concentration also exceeded by some 1995 samples from the lower MRB. In laboratory-exposed freshwater fish, toxic effects have been observed at whole-body total DDT concentrations <0.5 µg/g in some studies (Jarvinen and Ankley, 1999), but there is great variation among species and exposure regimes.

Toxaphene: Following the 1972 U.S. ban on DDT use, toxaphene became the insecticide most heavily used on cotton until it also was banned in the early 1980s (USEPA, 1982). The geographic distribution of toxaphene residues among the 1995 samples reflects this historic use pattern; toxaphene was detected in only 12 samples, all of which came from five sites in the lower MRB—NCBP Station 80 (Yazoo R.) and NAWQA Stations 201-204, the latter all in the MSE Study Unit (Figs. 2-33 and 2-34). These represent only 7% of the samples and 11% of the stations sampled (Table 2-3), and are the same sites at which total DDT concentrations were greatest (Figs. 2-29 and 2-30). Toxaphene concentrations in fish from NCBP Station 80 were 0.8-2.5 µg/g in carp and 0.5-0.7 µg/g in largemouth bass (mean about 1 µg/g), but

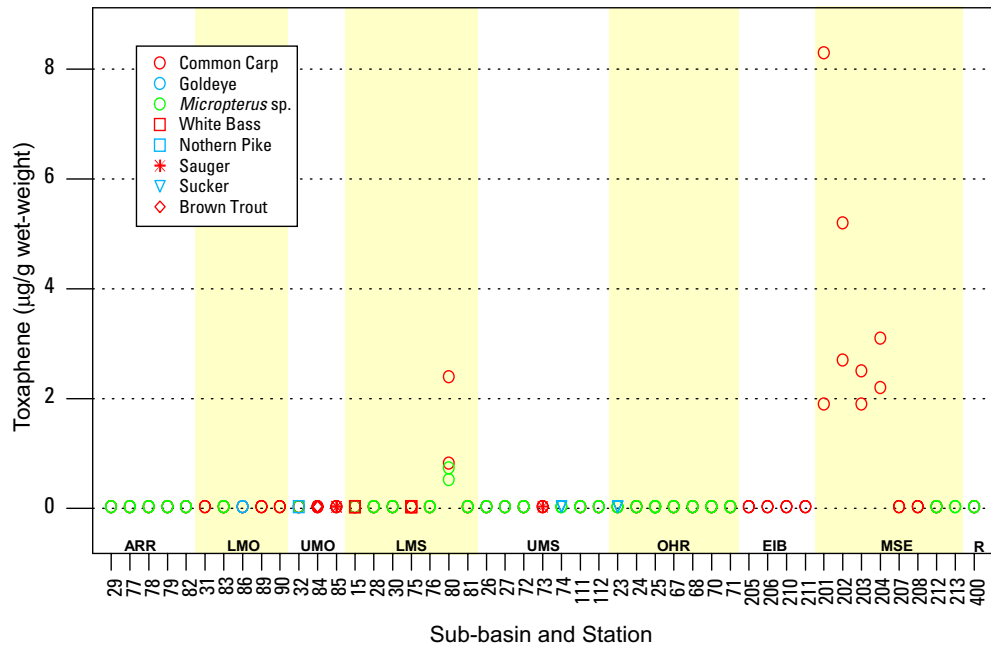


Figure 2-33. Concentrations of toxaphene in composite fish samples, by sub-basin, station and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

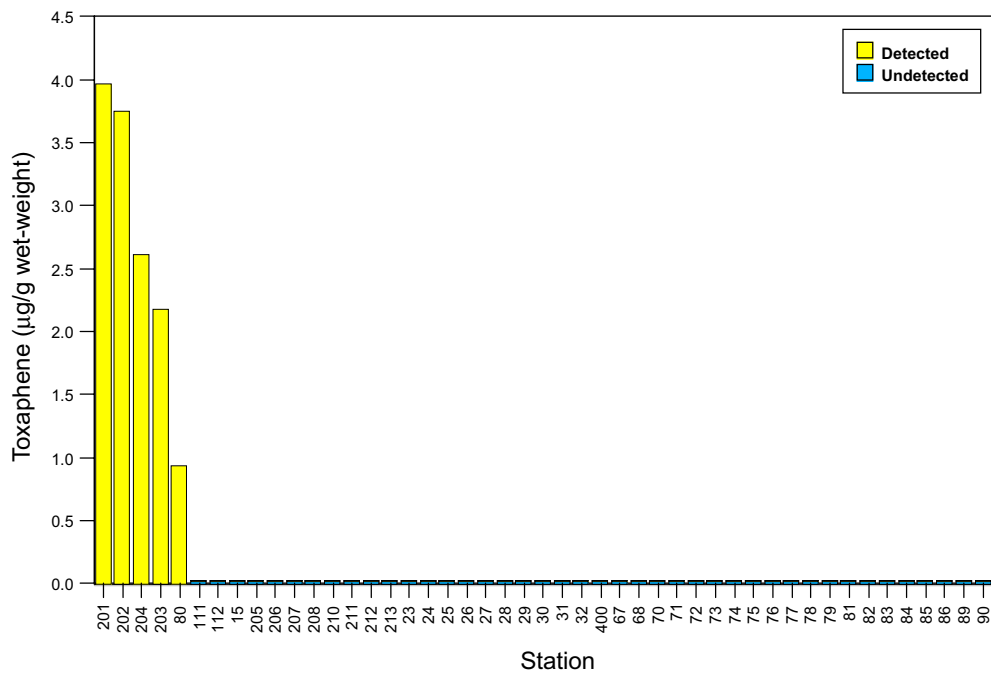


Figure 2-34. Ranked geometric mean concentrations of toxaphene in composite fish samples, by station. Shaded portions of bars are means of censored observations (represented by 50% of LOD) used to compute the respective station mean. See Table 1-1 for station descriptions.

were generally higher (means 2.0-4.0 $\mu\text{g/g}$, maxima 2.0->8.0 $\mu\text{g/g}$) in carp from Stations 201-204 (Figs. 2-33 and 2-34; Table 2-3). Although elevated relative to other 1995 sites, levels at Station 80 have declined substantially over the last two decades; concentrations were 0.4-2.4 $\mu\text{g/g}$ in 1986 (Schmitt and others, 1999b) and 10-20 $\mu\text{g/g}$ in the late 1970s and early 1980s (Schmitt and others, 1983; 1985). Similar to the pattern observed for DDT, toxaphene concentrations in fish from NAWQA Stations 201-204 remained about at the levels observed at Station 80 in the early 1980s (Schmitt and others, 1983; 1985). In contrast to DDT, which declined by about 50% from 1986 to 1995, the geometric mean concentration of toxaphene at Station 80 was about the same in 1995 as in 1986 (Fig. 2-35); however, as noted for DDT, there were no taxa common to both collections at Station 80, so this comparison must be interpreted with caution. At those NCBP stations where there were species common to both collections, the trends were uniformly downward. In the 1980s, toxaphene was more widely distributed among NCBP sites in the MRB; in addition to Station 80, >0.2 $\mu\text{g/g}$ was present in at least one 1986 sample from Station 81 (Red R.) and all three Ohio R. stations—24 (Marietta, OH), 69 (Cincinnati, OH; not sampled in 1995), and 70 (Metropolis, IL).

Toxaphene is a complex mixture of chlorinated camphenes that is difficult to analyze by GC-ECD because of interferences from co-eluting PCB congeners and other compounds. Consequently, negative chemical ionization GC/MS (GC/MS-NCI) is the preferred analytical method (Ribick and others, 1982; Muir and de Boer, 1993). Nevertheless, reasonably good concentration estimates can be achieved by capillary-column GC-ECD if toxaphene is fractionated away from PCBs (for example, Krock and others, 1997), as was done with the 1995 samples; and concentrations of PCBs and chlordane are low relative to those of toxaphene, as was true in the 12 toxaphene-containing samples MSE Study Unit and Station 80 (see discussion following). Nevertheless, the toxaphene concentrations reported here and in the past should be considered estimates.

Toxaphene is highly toxic to fish (Johnson and Finley, 1980). In laboratory studies with technical toxaphene, adverse effects on freshwater fish have been associated with whole-body residues $\geq 1.0 \mu\text{g/g}$ (Jarvinen and Ankley, 1999), a concentration exceeded in some 1995 samples. However, all the samples from Station 80 and the MSE NAWQA sites exceeded 0.0063 $\mu\text{g/g}$, the Canadian guideline (EC, 1999). Relatively few components of technical-grade toxaphene are toxic; however, the most toxic constituents are also among the most persistent (Harder and others, 1983; Gooch and Matsamura, 1987). Because the composition of the weathered toxaphene present in the 1995 samples cannot be determined

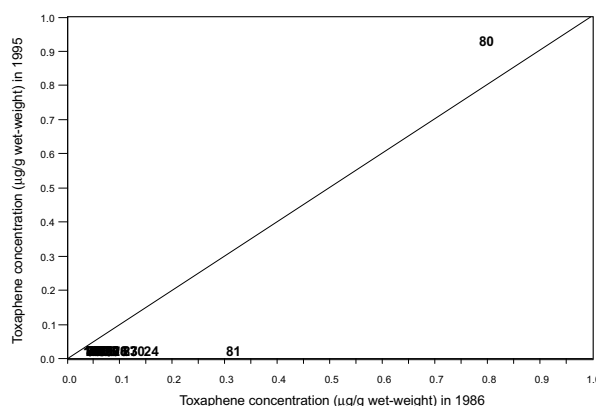


Figure 2-35. Geometric mean toxaphene concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

from the low-resolution analytical methods employed, it is impossible to evaluate the ecological risk of the residues (Bidleman and others, 1993).

Cyclodiene Insecticides: The insecticides dieldrin, aldrin (which is metabolized to dieldrin), chlordane, and heptachlor (metabolized to heptachlor epoxide) were used against a variety of soil-dwelling insects, including corn rootworms (*Diabrotica* spp.) and termites (Table 2-1). Endrin was used extensively on cotton and, to a lesser extent, against army cutworms (*Euoxoa axilliaris*) infesting wheat in the Great Plains, and to protect orchards from rodents (Schmitt and others, 1990). No cyclodiene pesticides are currently used in North America, but they are synthesized domestically for export. These compounds bind tightly to soil particles and persist in many areas from historic uses (Schnoor, 1981; Rostad, 1997).

Dieldrin: Historically, the greatest concentrations of dieldrin were found in fish from the Corn Belt and the Great Lakes (Schmitt and others, 1981; 1983; 1985; 1990; 1999b). For the MRB, this pattern persisted into 1995; dieldrin residues were present in 42% of the samples from 57% of the stations sampled (Table 2-3), at concentrations ranging from barely detectable (ca. 0.01 $\mu\text{g/g}$) to 0.25 $\mu\text{g/g}$ (Figs. 2-36 and 2-37). Greatest concentrations occurred in the central and southern parts of the basin, as they have historically. Geometric station means were $\geq 0.025 \mu\text{g/g}$ at NCBP Stations 76 (Mississippi R. at Memphis, TN), 26 (Illinois R. at Hardin, IL), 68 (Wabash R. at New Harmony, IN), 73 (Des Moines R. at Keosauqua, IA), 75 (Mississippi R. at Cape Girardeau, MO), 31 (White R. at DeVall's Bluff, AR), 83 (Missouri R. at Hermann, MO), 80 (Yazoo R.), 90 (Kansas R. at

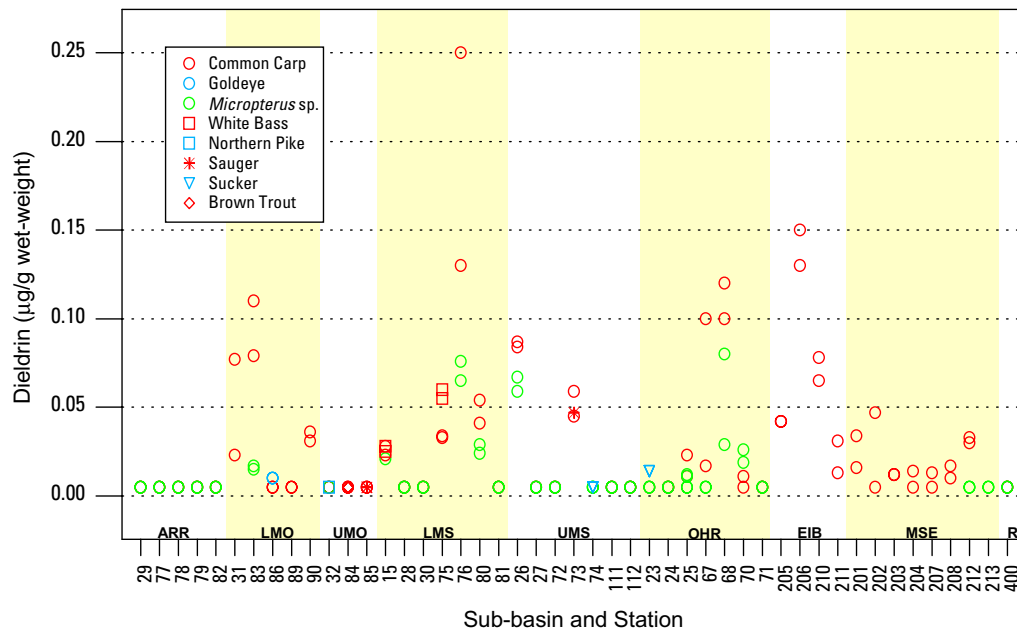


Figure 2-36. Concentrations of dieldrin in composite fish samples, by sub-basin, station and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

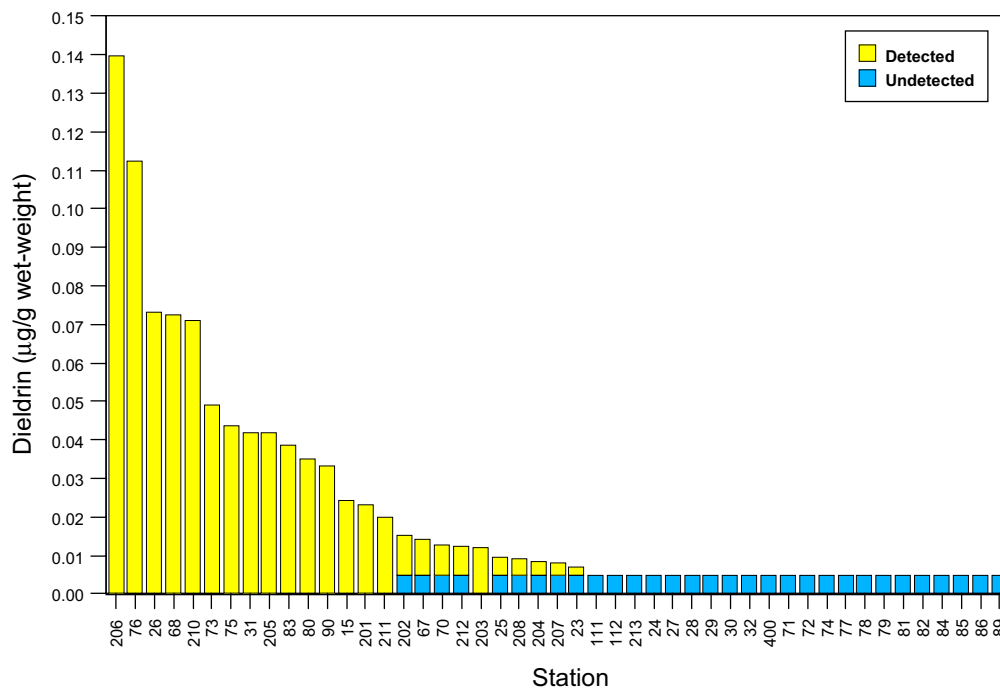


Figure 2-37. Ranked geometric mean concentrations of dieldrin in composite fish samples, by station. Shaded areas represent means of censored observations (represented by 50% of LOD) used to compute the respective station mean. See Table 1-1 for station descriptions.

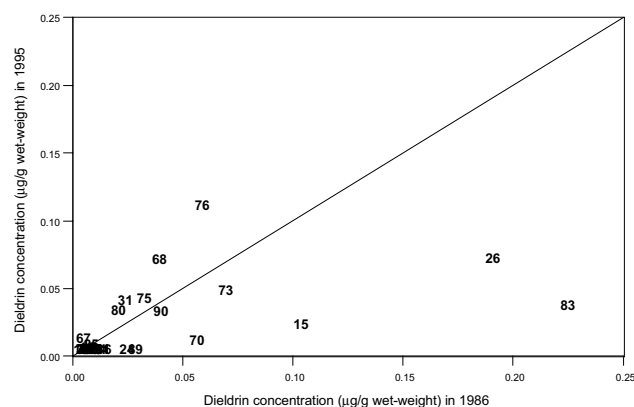


Figure 2-38. Geometric mean dieldrin concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

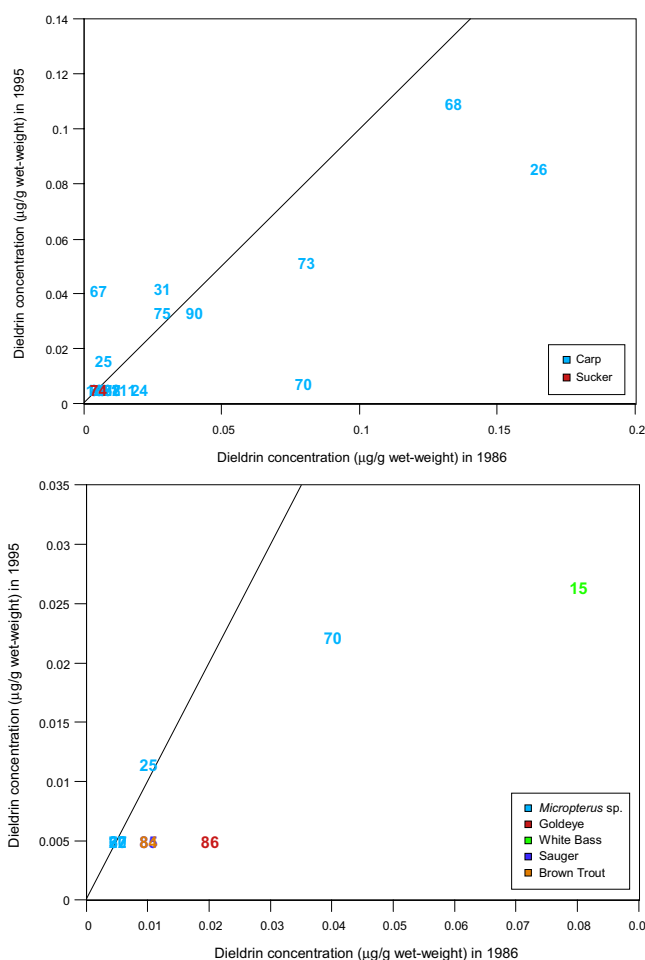


Figure 2-39. Geometric mean dieldrin concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

Bonner Springs, KS); and at NAWQA Stations 205, 206 and 210, in the EIB Study Unit (Fig. 2-37). The greatest individual sample concentrations (0.10-0.25 µg/g) were from Stations 76, 206, 83, 68, and 67 (Allegheny R. at Natrona, PA). There were no detectable dieldrin residues in fish from Station 400, the reference site.

From 1986 to 1995, dieldrin concentrations declined by 50% or more at most of the NCBP stations historically evidencing the greatest geometric mean concentrations (Fig. 2-38; Schmitt and others, 1999b). This finding supports other research showing that significant amounts of this and other soil-associated compounds were transported out of the MRB by the floods of 1993 and 1995 (Rostad, 1997). Dieldrin concentrations were also relatively high in the past at NCBP Station 90 (Kansas R.), which was not sampled in 1986; however, compared to 1984, concentrations there also declined, albeit not to the extent that it did at other stations. Station 76 was an exception, however; at that site, the mean concentration of dieldrin in fish was two-fold higher in 1995 than in 1986 (Fig. 2-38). Some of this increase may reflect taxonomic differences, however; freshwater drum (*Aplodinotus grunniens*) and bluegill were collected in 1986, whereas carp and largemouth bass were collected in 1995. Carp were collected in 1984, however, when concentrations were 0.08-0.11 µg/g (Schmitt and others, 1990)—ca. 50% lower than the 0.13-0.25 µg/g present in this species in 1995. This site has historically evidenced high concentrations of cyclodiene insecticides and related chemicals emanating from a manufacturing source near Memphis (Schmitt and others, 1981; 1983; 1985; 1990; 1999b) and from a municipal/industrial landfill known to contain and leach pesticide manufacturing wastes (Leppanen and others, 1998). In the past, chemical spills at the manufacturing site caused massive fish kills (Biglane and others, 1964), and fish from the Mississippi River also contained residues of cyclodiene insecticide precursors (Yurawecz and Roach, 1978). Among stations with taxa in common to the 1986 and 1995 collections, dieldrin concentrations declined or changed only slightly at most (Fig. 2-39). The exception was dieldrin in carp at Station 67 (Allegheny R.), which increased slightly.

In laboratory studies with freshwater fish, adverse effects have been observed at whole-body dieldrin concentrations ≥ 1.2 -1.4 µg/g (Jarvinen and Ankley, 1999), which is about five-fold greater than the highest 1995 concentration (ca. 0.25 µg/g at Station 76). This concentration is also more than 10-fold lower than dietary concentrations associated with adverse effects in wildlife (see review by Peakall, 1996). However, it is two-fold greater than the NYSDEC wildlife guideline of 0.12 µg/g (Newell and others, 1987). Based on this lower value, dieldrin in

fish from Station 206 may also represent a hazard to piscivorous wildlife. Evidence cited by Schmitt and others (1985; 1990) suggested that dieldrin was still being carried into receiving waters from fields in the Midwest, despite the fact that no aldrin (the source of most environmental dieldrin residues) had been used in agriculture since 1974 (Schnoor, 1981). The more recent data of Rostad (1997) and the 50% lower concentrations present in fish in 1995 relative to 1986 indicate that these compounds are still present, but that amounts are generally declining.

Endrin: Low concentrations of endrin (ca. 0.2 µg/g) were historically present in fish from NCBP sites in the Cotton Belt and the Great Plains (Schmitt and others, 1981; 1983; 1985; 1990; 1999b). In 1995, endrin was present in only the four samples from NCBP Station 76 (Mississippi R. at Memphis, TN; Table 2-3), which has historically produced the highest concentrations among NCBP stations owing to the point-source described previously. The 1995 concentrations at Station 76 were 0.22 µg/g in largemouth bass (both samples) and 0.40-0.71 µg/g in carp (geometric station mean 0.34 µg/g), which is about 5-fold greater than the 1986 mean for bluegill and freshwater drum at that site (Schmitt and others, 1999b). In 1984, the concentrations in carp were 0.01-0.22 µg/g (Schmitt and others, 1990). As noted earlier, cyclodiene insecticides were manufactured near Memphis; there were extensive chemical spills and fish kills (Biglane and others, 1964), and fish from the Mississippi River historically contained residues of cyclodiene insecticides and precursors (Yurawecz and Roach, 1978). Consequently, endrin concentrations were also elevated at Station 15, farther downstream on the Mississippi R. (Schmitt and others, 1981; 1983; 1985). By 1984-86, however, fish from Station 15 contained only 0.01 µg/g of endrin (Schmitt and others, 1990; 1999b), and in 1995 none was detected. Although high concentrations of endrin were once found at many NCBP stations in the Cotton Belt, levels at all were <0.05 µg/g by 1980-81 (Schmitt and others, 1985) and declined to ≤0.03 µg/g in 1984 (Schmitt and others, 1990) and ≤0.02 µg/g in 1986 (Schmitt and others, 1999b) everywhere except Station 76. Elsewhere, concentrations remained uniformly low at NCBP stations having taxa common to the 1986 and 1995 collections.

Endrin is among the most toxic organochlorine insecticides to fish (Grant and Mehrle, 1970; 1973; Grant, 1976; Johnson and Finley, 1980); in laboratory studies with freshwater fish, adverse effects have been observed at whole-body concentrations as low as 0.01 µg/g, the nominal 1995 detection limit that was exceeded only by the 1995 samples from Station 76. In contrast to the safety margin observed for dieldrin, the 1995 endrin

concentrations (0.2-0.7 µg/g) in carp from Station 76 are only about 2- to 4-fold lower than the lowest dietary levels known to be toxic to avian wildlife (see review by Peakall, 1996). In addition, these concentrations are 10-fold greater than the NYSDEC guideline for endrin (Newell and others, 1987).

Chlordane and Heptachlor: Residues of chlordane components, heptachlor (as heptachlor epoxide), and their metabolites were among the most widely distributed organochlorine compounds detected in the 1995 samples. Residues of at least one of the measured chlordane-related compounds (that is, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxy-chlordane, heptachlor epoxide) were present in 51% of the samples from 70% of the stations sampled (Table 2-3). None were found in any of the samples from Station 400 (reference site; Figs. 2-40, 2-41). The geographic distribution of these compounds closely resembled that of dieldrin. The greatest individual and mean total concentrations (0.07-0.25 µg/g) of chlordane-related compounds occurred in the central part of the MRB—at NCBP Stations 76 (Mississippi R. at Memphis, TN), 68 (Wabash R. at New Harmony, IN), 23 (Kanawha R. at Nitro, WV), 24 (Ohio R. at Marietta, OH), 67 (Allegheny R. at Natrona, PA), 73 (Des Moines R. at Keosauqua, IA), 90 (Kansas R. at Bonner Springs, KS), and 26 (Illinois R. at Hardin, IL); and at NAWQA Stations 206 (Iowa R. at Morengo R., IA), 205 (S. Skunk R. at Oskaloosa, IA), and 201 (Big Sunflower R. at Anguilla, MS; Figs. 2-40 and 2-41). The individual samples containing the highest concentrations (0.25-0.55 µg/g of total chlordane-related compounds) were the two carp samples from Station 76, one carp sample from Station 206, and one each of bass and sucker from Station 23 (Figs. 2-40 and 2-41). In 1984, the most recent NCBP collection in which carp were collected from that site, concentrations were about the same as in 1995—0.21-0.59 µg/g (Schmitt and others, 1999b).

Heptachlor, aldrin, dieldrin, and chlordane were all used against ants, termites, corn rootworms, and other soil-dwelling insects. Residues of the individual compounds and mixtures and their metabolites therefore, tend to co-occur (Schmitt and others, 1990). Heptachlor also occurs as a minor component (≤10%) of technical chlordane [National Research Council of Canada (NRCC), 1974], and small amounts of *cis*- and *trans*-chlordane are present in technical heptachlor (Eisler, 1990; Wiemeyer, 1996). It is therefore difficult to differentiate the source(s) of environmental heptachlor- and chlordane-derived residues (Schmitt and others, 1985). Heptachlor is rapidly converted to heptachlor epoxide and other metabolites by many organisms, and the use of this compound was phased out by the early 1980s (Wiemeyer, 1996). Consequently, little or no unmetabolized heptachlor

has been detected in NCBP fish samples since 1976-77. Both the occurrence and the concentrations of heptachlor epoxide were declining through the mid-1980s; in 1986, concentrations were $\geq 0.04 \mu\text{g/g}$ in one or more samples from only NCBP Stations 75 (Mississippi R. at Cape Girardeau, MO) 26 (Illinois R. at Hardin, IL), and 83 [Missouri R. at Hermann, MO—(Schmitt and others, 1999b)]. In 1995, residues of heptachlor epoxide were present in 14 samples (9%) from seven stations (15%), mostly as trace concentrations ($<0.02 \mu\text{g/g}$). Greater-than-trace concentrations ($0.03\text{--}0.05 \mu\text{g/g}$) were present only in carp from NAWQA Station 206 (Iowa R. at Morengo, IA—Fig. 2-40).

As noted for dieldrin and endrin, the 1995 concentrations of chlordane-related compounds were lower at most NCBP sites than they were in 1986 (Schmitt and others, 1999b). Nationally, chlordane concentrations declined steadily from 1976 to 1981 (Schmitt and others, 1983; 1985), but then changed little from 1980-81 to 1986 (Schmitt and others, 1990; 1999b). As described for other cyclodiene insecticides, Station 76 was an exception; concentrations were higher in 1995 than in 1986 (Fig. 2-42), with the same caveat that different taxa were collected in 1995. Chlordane concentrations generally declined or changed little from 1986 to 1995 at stations with taxa in common to both collections (Fig. 2-43).

The incidence of the most abundant and persistent chlordane constituents has been declining since the early 1980s. In 1980-81, *cis*-chlordane was detected at 74% of the NCBP stations sampled, and *trans*-nonachlor at 85%, having declined from 93% in 1978-79 (Schmitt and others, 1983; 1985). By 1984-86, residues of *cis*-chlordane and *trans*-nonachlor were present at only 70% and 74%, respectively, of the NCBP stations sampled (Schmitt and others, 1990; 1999b). In 1995, *trans*-nonachlor was again the most frequently encountered residue; it was present 51% of the samples from 70% of the stations (Table 2-3). Residues of *cis*-chlordane were next in concentration and abundance; they were detected in 21% of the samples from 48% of the stations (Table 2-3). At Station 76, however, *trans*-chlordane was the most abundant component (Fig. 2-41), probably reflecting the influence of the previously noted point-source and landfill in Memphis. In 1986, the maximum chlordane concentration ($0.78 \mu\text{g/g}$) occurred at NCBP Station 69 (Ohio R. at Cincinnati, OH), which was not sampled in 1995, and relatively high concentrations of one or more chlordane-related compounds (*cis*- or *trans*-chlordane or nonachlor; oxychlordane; heptachlor epoxide) were also present in fish from Stations 70 (Ohio R. at Metropolis, IL), 83 (Missouri R. at Hermann, MO), and 67 (Allegheny R.) Oxychlordane, a highly toxic metabolic of *cis*-chlordane, and heptachlor epoxide were also present at

most of these sites in 1986, but at lower concentrations than the other chlordane components. As noted for dieldrin, chlordane concentrations were also relatively high in the past at NCBP Station 90 (Kansas R.), which was not sampled in 1986; however, 1995 dieldrin concentrations at this site were about the same as they were in 1984 (geom. mean $0.03 \mu\text{g/g}$). In general, the compositional change in the chlordane mixture present in fish collected in 1995 compared to 1986 reflects the continued weathering of these compounds, and the decline in concentrations at most NCBP sites from 1986 to 1995 further supports the hypothesis that large amounts of cyclodiene insecticides were removed from the MRB by the floods of 1993 and 1995 (Rostad, 1997).

In terms of ecological risk, a total fish concentration of $0.3 \mu\text{g/g}$ for *cis*-chlordane, *trans*-chlordane, and oxychlordane was proposed as a temporary guideline for vertebrate wildlife protection (Eisler, 1990). This level was exceeded by some of the most heavily contaminated 1995 samples (Fig. 2-40), but not by any geometric station means (Fig. 2-41). However, only one sample from Station 76 exceeded $0.5 \mu\text{g/g}$, the NYSDEC wildlife guideline for chlordanes (Newell and others, 1987). In laboratory exposures of freshwater fish, the lowest heptachlor and heptachlor epoxide residue concentrations associated with adverse effects are several orders of magnitude greater than levels present in the 1995 samples (Jarvinen and Ankley, 1999), but there are no data for chlordane. Chlordane residues in paddlefish (*Polyodon spathula*) eggs from the Ohio River collected in 1997 contained $0.35 \mu\text{g/g}$ of chlordane and $0.74 \mu\text{g/g}$ of PCBs (Gundersen and others, 2000), similar to levels observed in our 1995 fish from the OHR sub-basin; however, testes of male paddlefish contained 4-fold greater concentrations. Hatching success of the Ohio River paddlefish eggs was not affected, but possible contaminant-related effects on the parent fish were noted (Gundersen and others, 2000).

Hexachlorocyclohexane (HCH): HCH (also known as benzene hexachloride, BHC) is a mixture of five isomers formerly used extensively on cotton and other crops (Table 2-1). Technical HCH use in the U.S. was curtailed in the late 1970s but it remained in use elsewhere into the 1990s (Li and others, 1996). The purified γ -isomer (lindane), which also contains small amounts of the other isomers, is still used in North America for a few agricultural and domestic applications (Poissant and Koprivnjak, 1996; Li and others, 1996). Compared to some organochlorine insecticides HCH isomers are volatile and short-lived, and they can be difficult to quantify. Consequently, these compounds occurred infrequently and at low concentrations in NCBP fish samples, and both inci-

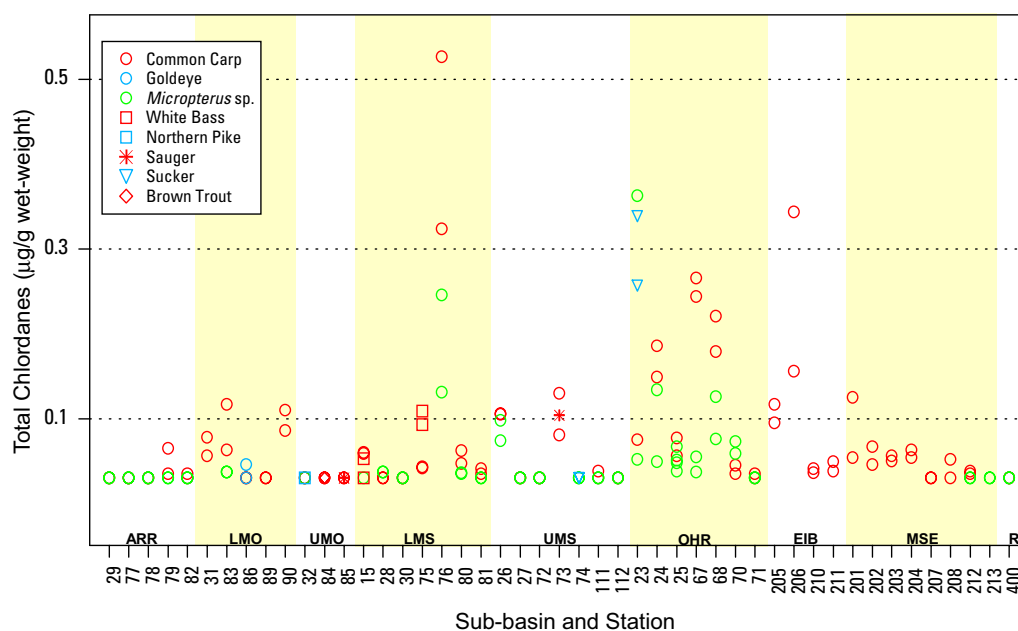


Figure 2-40. Concentrations of chlordane-related compounds (sum of *cis* and *trans* chlordanes and nonachlors; oxychlordane; and heptachlor epoxide) in composite fish samples, by sub-basin, station, and taxon. Censored values were represented in totals by 50% of LOD. See Table 1-1 for station descriptions.

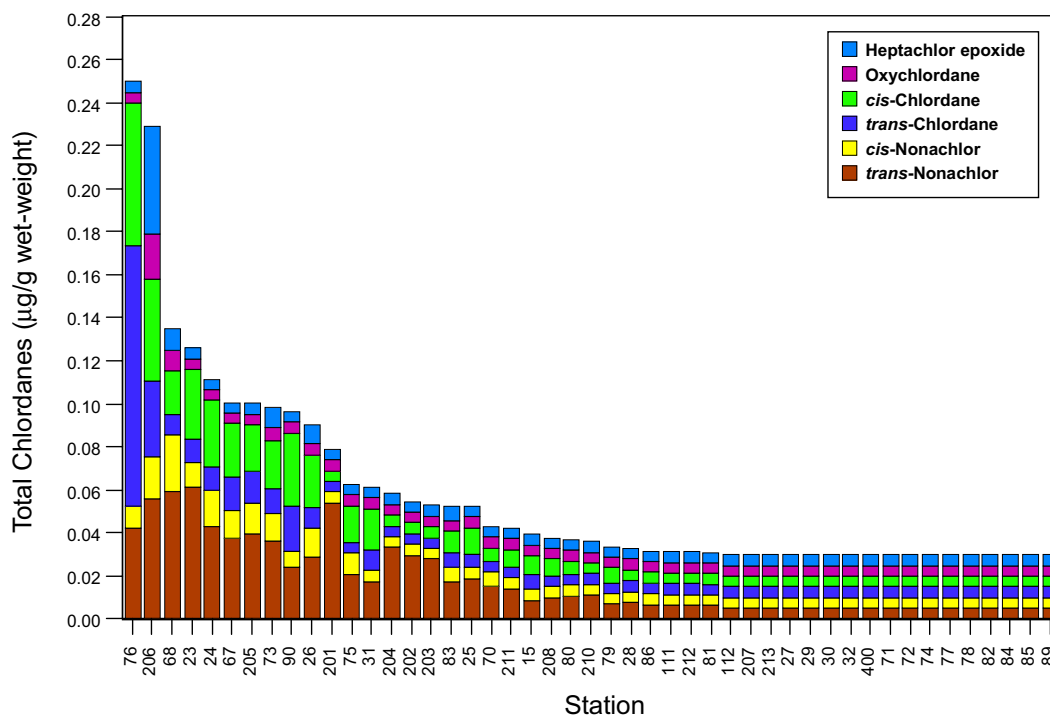


Figure 2-41. Ranked geometric mean concentrations of chlordane-related compounds (sum of *cis* and *trans* chlordanes and nonachlors; oxychlordane; and heptachlor epoxide) in composite fish samples, by station. (Note: Censored values are represented by 50% of LOD in the totals and means but are not shown). See Table 1-1 for station descriptions.

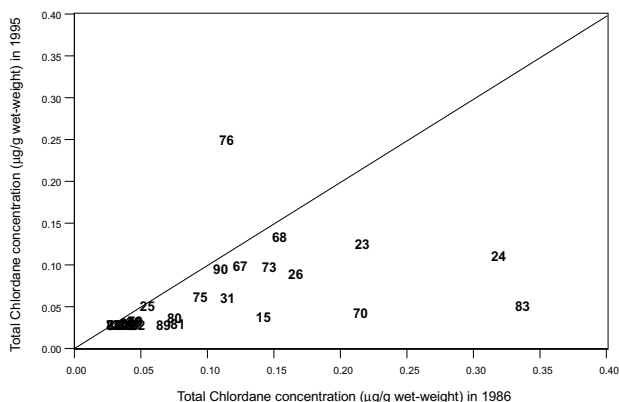


Figure 2-42. Geometric mean concentrations of chlordane-related compounds (sum of *cis* and *trans* chlordanes and nonachlors; oxychlordane; and heptachlor epoxide), by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

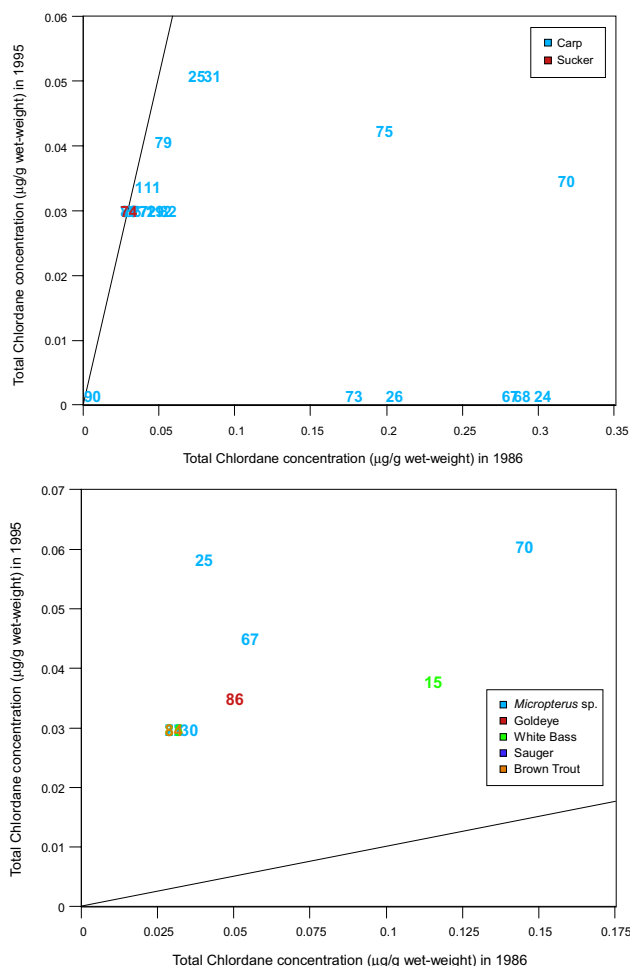


Figure 2-43. Geometric mean concentrations of chlordane-related compounds (sum of *cis* and *trans* chlordanes and nonachlors; oxychlordane; and heptachlor epoxide), by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

dence and concentrations of the two isomers measured (α and γ) were declining through the mid-1980s (Schmitt and others, 1999b). The 1995 samples were analyzed for four HCH isomers (α , β , γ , and δ); no samples contained detectable concentrations (≥ 0.01 $\mu\text{g/g}$) of any isomer.

Mirex: This highly recalcitrant compound was used historically as a fire retardant and as an insecticide (Table 2-1) to combat red imported fire ants (*Solenopsis invicta*) in the South (Kaiser, 1987). The historic distribution of mirex in NCBP samples reflected those patterns; residues at greater than trace concentrations were found only at sites in the South, from insecticidal use of mirex, and in Lake Ontario and the St. Lawrence River, reflecting two sources of mirex synthesis on Lake Ontario tributaries (Kaiser, 1987; Schmitt and others, 1990; 1999b).

Concentrations in fish from both areas had been declining through 1986 (Schmitt and others, 1999b). In 1995, mirex was detected (≥ 0.01 $\mu\text{g/g}$) in only four samples (4%) from two sites (4%, Table 2-3) in Louisiana—NCBP Station 81 (Red R. at Alexandria) and NAWQA Station 204 (Tensas R. at Tenda), the latter in the MSE Study Unit. Concentrations at these sites ranged from about 0.02 $\mu\text{g/g}$ to 0.075 $\mu\text{g/g}$ (data not shown). In 1986, traces of mirex were also present in fish from NCBP Stations 25 (Tennessee R. at Clarksville, TN) and 69 (Ohio R. at Cincinnati); the latter was not sampled in 1995. Concentrations at Station 81 were 0.02-0.04 $\mu\text{g/g}$ in 1995, slightly higher than they were in 1986 (0.01-0.02 $\mu\text{g/g}$); however, different species were collected in 1995 (carp and largemouth bass) than in 1986 (channel catfish and white bass). At stations with taxa common to both the 1986 and 1995 collections, mirex concentrations changed little (data not shown). Eisler (1985) stated that sensitive wildlife species are affected at dietary mirex levels of 0.1 $\mu\text{g/g}$, a level that was approached but not exceeded by any sample collected in 1995. However, the NYSDEC wildlife guideline for mirex is 0.33 $\mu\text{g/g}$ (Newell and others, 1987), suggesting that residues in fish from Stations 81 and 204 represent a risk to piscivorous wildlife. In laboratory-exposed freshwater fish, toxic effects have been observed at concentrations ≥ 0.35 $\mu\text{g/g}$ (Jarvinen and Ankley, 1999), 10-fold greater than the highest 1995 concentrations.

Hexachlorobenzene: Residues of hexachlorobenzene (HCB), which are virtually ubiquitous (Zell and Ballschmiter, 1980), occur in the environment as a result of this compound's use as a fungicide (Vizethum and Goerz, 1979) and because it is a by-product of the production of other chlorinated hydrocarbons (Villanueva and others, 1974; Table 2-1). In fish, HCB is shorter lived (Villanueva and others,

1974) and much less toxic (Jarvinen and Ankley, 1999) than DDT and most other persistent organochlorine compounds; however, commercial formulations once contained toxic impurities, including polychlorinated dibenzo-*p*-dioxins (PCDDs) and -dibenzofurans (PCDFs) (Villanueva and others, 1974). In addition, HCB has a low level of dioxin-like activity [ca. 0.0001-0.001 relative to 2,3,7,8-TCDD—(Hahn and others, 1996; Sinclair and others, 1997)], and may therefore contribute to toxicity in combination with other polyhalogenated hydrocarbons (PHH). In 1995, trace HCB residues were detected (LOD=0.01 µg/g) in only four samples (2%) from three sites (7%, Table 2-3): NCBP Stations 24 (Ohio R. at Marietta, OH), 76 (Mississippi R. at Memphis), and 23 (Kanawha R. at Nitro, WV). Concentrations in these samples were 0.020-0.075 µg/g (data not shown), which are at least 10-fold lower than the Canadian wildlife guideline (EC, 1999). In 1986, traces (<0.02 µg/g) of HCB were also present in fish from NCBP Stations 15 (Mississippi R. at Luling, LA), 81 (Red R. at Alexandria, LA) and 69 (Ohio R. at Cincinnati, which was not sampled in 1995), but not at the three sites at which it was present in 1995.

Polychlorinated Biphenyls (PCBs), H4IIE Bioassay-Derived Dioxin Equivalents (TCDD-EQ), and Ethoxyresorufin *O*-Deethylase (EROD) Activity

PCBs, chlorodioxins, and aromatic hydrocarbons emanate from a variety of urban and industrial sources and are ubiquitous environmental pollutants. Although their production has been banned in the U.S., large quantities of PCBs were historically manufactured and distributed for many industrial and consumer products such as lubricants, dielectric fluids, and carbonless copy papers (Table 2-1). Chlorodioxins and related compounds occur as impurities in and byproducts of the manufacture of many products, including PCBs and many pesticides, and they are also released from combustion sources. Polycyclic aromatic hydrocarbons (PAHs) are present in oil and petroleum products and are also released from combustion sources. These three broad classes of compounds, which are present in the sediments of most industrialized waterways, share many chemical and toxicological properties. The toxic effects of these structurally similar classes of planar aromatic compounds are believed to be mediated in part through the cellular aryl-hydrocarbon receptor, (AhR) which leads to the induction of proteins that can disrupt cellular homeostasis (De Vito and Birnbaum, 1994). Because they derive from similar sources,

these compounds also tend to co-occur in the environment. They continue to enter U.S. waters from landfills, urban runoff, oil spills, and the atmosphere. Their chemical analysis by instrumental methods requires extensive and expensive fractionation and cleanup. As noted in Chapter 1, the exposure of fish to these classes of chemicals was assessed by the three endpoints described here.

Total Polychlorinated Biphenyls: PCBs continued a downward trend in concentration and occurrence that has been in evidence since the early 1980s. In 1995 PCB residues were detected (≥ 0.05 µg/g) in only 21% of the samples from 35% of the stations sampled (Table 2-3), and none were detected in any samples from Station 400 (reference site; Fig. 2-44). Nationwide, PCBs were detected at 65% of the NCBP stations sampled in 1986; in the MRB, they were detected in 1986 at 25 of 34 NCBP stations sampled in 1995 (73%). PCBs were also present in 1984 at Station 90, which was not sampled in 1986 (Schmitt and others, 1990; 1999). In 1986, no PCBs were present in any samples from Stations 30 (White R.), 32 (Missouri R. at Garrison Dam), 74 (Mississippi R. at Little Falls, MN), 77 (Arkansas R. at John Martin Res.), 78 (Verdigris R. at Oologah, OK), 84 (Big Horn R. at Hardin, MT), 85 (Yellowstone R. at Sidney, MT), 86 (James R. at Olivet, SD), or 88 (S. Platte R. at L. McConaughy, NE, which was not sampled in 1995) nor were any detected at most of these stations in 1995 (traces were present in samples from Stations 30 and 86). From 1976-1984, PCBs were present at about 91% of the NCBP stations nationwide (Schmitt and others, 1990). It should be noted that the declining incidence of PCBs in the MRB from 1984-86 to 1995 occurred despite the lower LOD for total PCBs in 1995 (0.05 µg/g) than in 1984-86 (0.1 µg/g); however, GC-ECD-based analyses of weathered PCBs based on Aroclor mixtures can vary considerably (Schwartz and others, 1987; Eganhouse and Gossett, 1991), and the quantitation method used for the 1995 samples differed slightly from that used in 1986 (Schmitt and others, 1990).

Within the MRB, greatest PCB concentrations historically occurred at stations in the industrialized OHR and UMS sub-basins, a geographic trend that persisted into 1995. Concentrations of 1.0-3.2 µg/g in individual 1995 samples and station means >0.3 µg/g occurred at NCBP Stations 24 (Ohio R. at Marietta, OH), 23 (Kanawha R. at Nitro, WV), 67 (Allegheny R.), 76 (Mississippi R. at Memphis), 111 (Mississippi R. at Lake City, MN), 26 (Illinois R.), 28 (Arkansas R.), 70 (Ohio R. at Metropolis, IL), 27 (Mississippi R. at Guttenburg, IA), 112 (Mississippi R. at Dubuque, IA), 25 (Cumberland R. at Clarksville, TN), and 15 (Mississippi R. at Luling, LA; Fig. 2-45). At most of these sites, mean

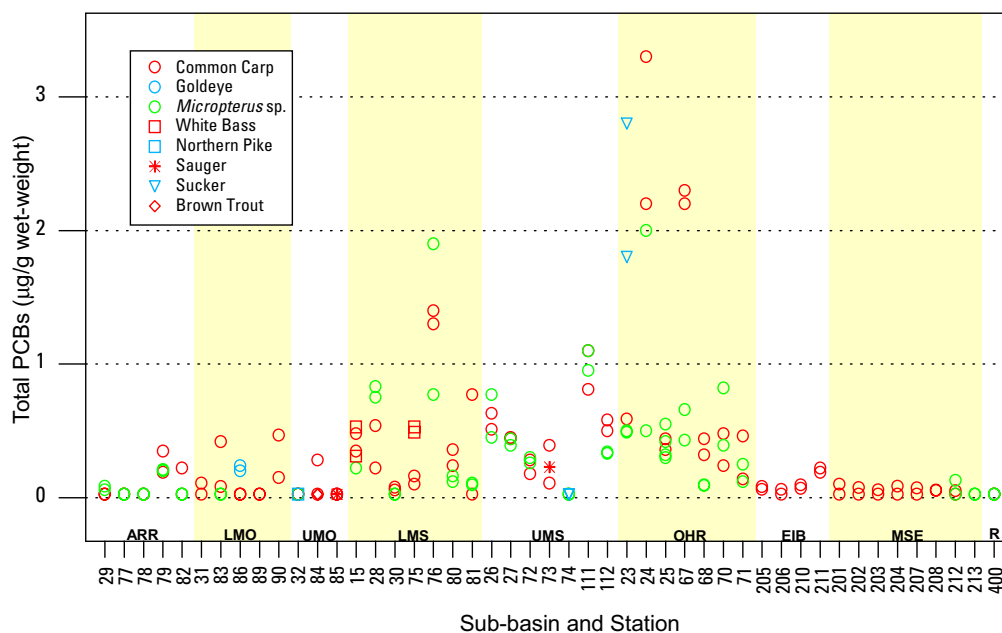


Figure 2-44. Concentrations of total PCBs in composite fish samples, by sub-basin, station, and taxon. Censored values are plotted as 50% of LOD. See Table 1-1 for station descriptions.

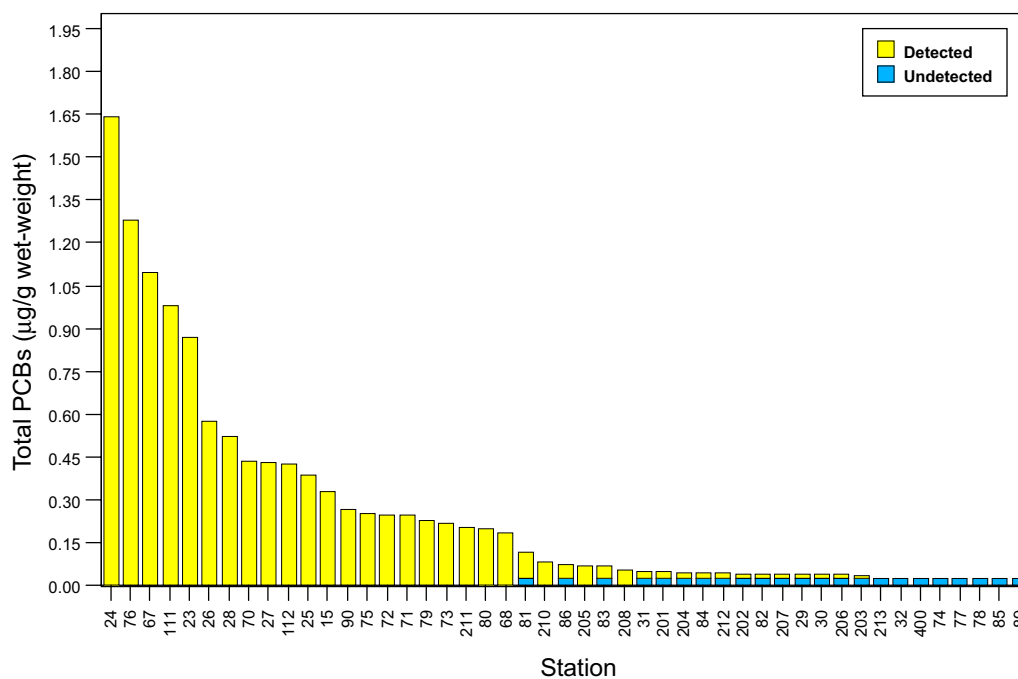


Figure 2-45. Ranked geometric mean concentrations of total PCBs in composite fish samples, by station. Shaded areas represent means of censored observations (represented by 50% of LOD) used to compute the respective station means. See Table 1-1 for station descriptions.

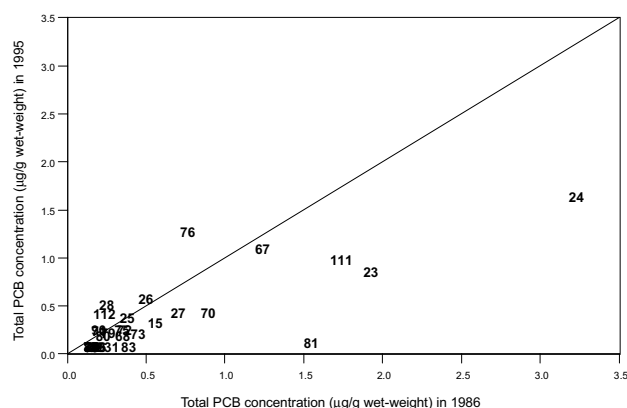


Figure 2-46. Geometric mean total PCB concentrations, by station, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

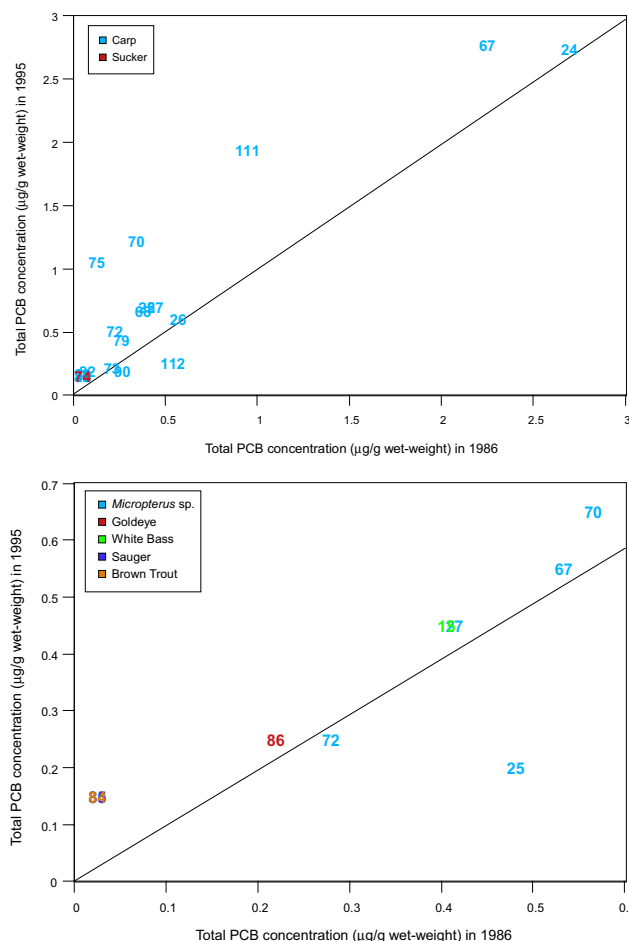


Figure 2-47. Geometric mean total PCB concentrations, by station and taxon, in composite fish samples collected from NCBP stations in 1986 (1984 for Station 90) and 1995. Upper panel, benthivores; lower panel, piscivores. Diagonal line: 1995=1986; points to the left of the line indicate increases and points to the right indicate decreases between the two years. See Table 1-1 for station descriptions.

concentrations either declined substantially (Stations 23, 81, 111, and 24) or did not increase appreciably (Fig. 2-46) since the mid-1980s (Schmitt, 1999; Zajicek and others, 2000). Lee and Anderson (1998) also reported declining PCB concentrations in carp and walleye from urbanized Minnesota areas of the MRB during 1975-1995. Total PCB concentrations increased slightly at Stations 112, 28, and 76, however (Fig. 2-46). Although there was a change in the species collected at some of these sites from 1986 to 1995 [especially at Station 28, where channel catfish and white crappie were replaced by carp and largemouth bass, and at Station 76, as noted earlier], at the other sites at least one species was common to both collections. Total PCB concentrations in carp increased at Stations 75 (Mississippi R. at Cape Girardeau, MO), 70 (Ohio R. at Metropolis, IL), 111 (Mississippi R. at Lake City, MN), and 67 (Allegheny R. at Natrona PA—Fig. 2-47). Concentrations declined in carp at Station 112 (Mississippi R. at Dubuque, IA) and in spotted bass at Station 25 (Cumberland R. at Clarksville, TN—Fig. 2-47).

The NYSDEC wildlife guideline for total PCBs is 0.11 µg/g (Newell and others, 1987), a concentration exceeded by at least one sample of fish from Stations 76, 23, 24, and 67 (Fig. 2-44). However, it is important to note that 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). Moreover, the congener composition of weathered PCBs varies greatly among NCBP locations and taxa (Zajicek and others, 2000). Consequently, we prefer not to speculate on the ecological risk represented by the PCB residues in the 1995 fish samples based solely on their total PCB concentrations. The toxic actions of PCBs and other dioxin-like compounds occur through multiple mechanisms, both AhR and non-AhR mediated. The biomarkers we used (H4IIE and EROD) are based on aseptic AhR-mediated event; it is therefore important to note that other potential modes of toxicity are not addressed. In addition, there are profound differences among taxa with respect to the uptake and metabolism of PCB congeners. Carp (especially) accumulate lower-chlorinated congeners that are not AhR-active but which may be neurotoxic or induce thyroid- or endocrine-mediated toxicity (Gerstenberger and others, 1997).

H4IIE Bioassay-Derived Dioxin Equivalents (TCDD-EQ)

H4IIE bioassay-derived dioxin equivalents are a measure of the total amount of AhR agonists present in the fish at the point of collection. The processing of the fish extracts was designed to remove the more labile

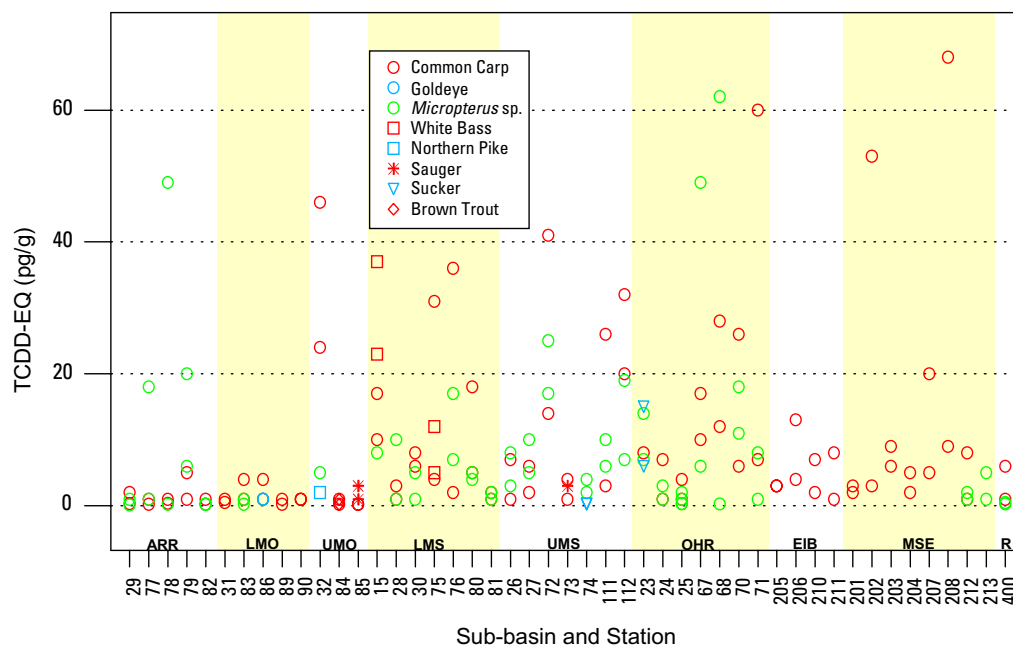


Figure 2-48. Concentrations of H4IIE bioassay-derived TCDD-EQ in composite fish samples, by sub-basin, station and taxon. See Table 1-1 for station descriptions.

compounds, such as PAHs. As such the bioassay results are largely a measure of PCBs, PCDDs and PCDFs present in the fish. Extracts of each composite fish sample were analyzed in triplicate and reported as the arithmetic mean of the three assays, in units of pg/g 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ), wet-weight, rounded to the nearest whole number. Samples were run in blocks of about 40, with limits-of-detection (LOD) and -quantitation (LOQ) computed separately for each block. LODs were <0.05-0.51 pg/g, and LOQs were 0.43-2.0 pg/g. Values <LOQ were replaced with one-half ($\frac{1}{2}$) the LOQ in all computations.

TCDD-EQ in Bass: The range of H4IIE bioassay-derived TCDD-EQ in composite samples of bass was <LOD-62 pg/g (Fig. 2-48; Table 2-3). Approximately 16% of the 57 bass samples analyzed were below the LOQ (<~0.5 pg/g). The four greatest values were all measured in females, whereas TCDD-EQ in male bass did not exceed 20 pg/g (Fig. 2-48). Examination of the frequency distributions (Fig. 2-49) revealed that in male bass the modal concentration was ≤ 1 pg/g, whereas in female bass it was 2 pg/g. These are essentially background concentrations owing to the ubiquity of polyhalogenated hydrocarbon contaminants. TCDD-EQ was ≤ 5 pg/g in 48% of the

male bass composites and 51% of the females, and was ≤ 10 pg/g in 68% of the males and 78% of the females (Fig. 2-49, 2-50). In bass, the greatest TCDD-EQ concentration was 62 pg/g in female largemouth bass from Station 68 (Wabash R. at New Harmony, IN); however, the sample of male bass from that site contained <0.5 pg/g (Fig. 2-50). At the station level, mean TCDD-EQ in bass (sexes combined) was ≥ 10 pg/g at nine sites (of 29; 31%) and ≥ 5 pg/g at 15 sites (52%; Fig. 2-51). The greatest mean TCDD-EQ concentration in bass was at Station 68, where the mean for the composite of seven female largemouth bass was 31 pg/g (Fig. 2-51). The other locations at which mean TCDD-EQ concentrations in bass exceeded 10 pg/g were, in descending order, Stations 67 (Allegheny R. at Natrona, PA), 78 (Verdigris R. at Oolaga, OK), 72 (Wisconsin R. at Woodman, WI), 70 (Ohio R. at Metropolis, IL), 79 (Canadian R. at Eufala, OK), 112 (Mississippi R. at Dubuque, IA), 76 (Mississippi R. at Memphis, TN), and 23 (Kanawha R. at Winfield, WV; Fig. 2-51). Total PCB concentrations were ≥ 0.2 μ g/g in bass from all of these sites except Station 78 (Verdigris R.), where PCBs were not detected (<0.05 μ g/g) in either sample of largemouth bass (Fig. 2-44). At the reference site (Station 400) there was no detectable TCDD-EQ in either sample of largemouth bass (female ≤ 0.96 pg/g,

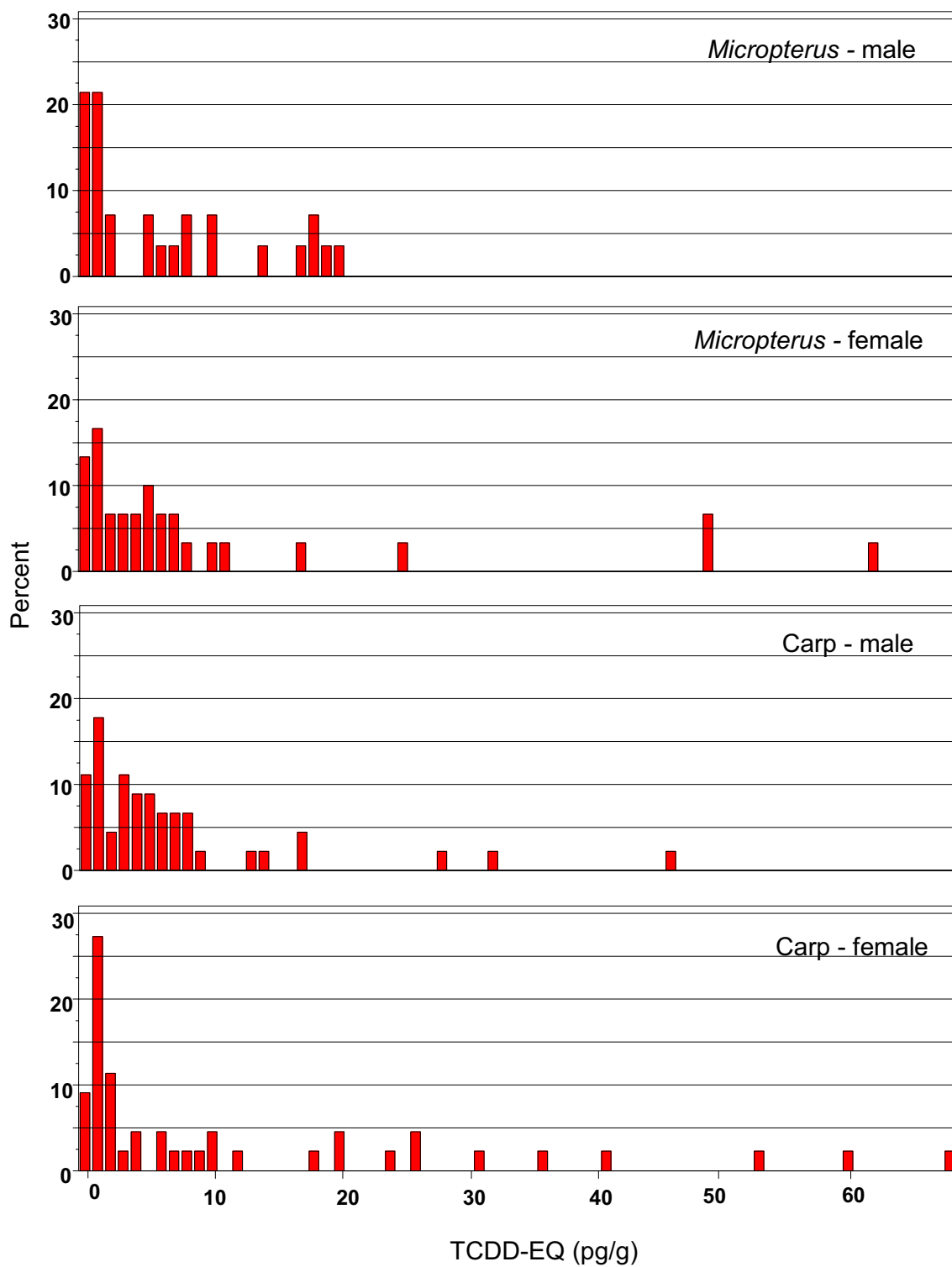


Figure 2-49. Frequency distributions of H4IIE bioassay-derived TCDD-EQ in composite samples of male and female carp and bass. See Table 1-1 for station descriptions.

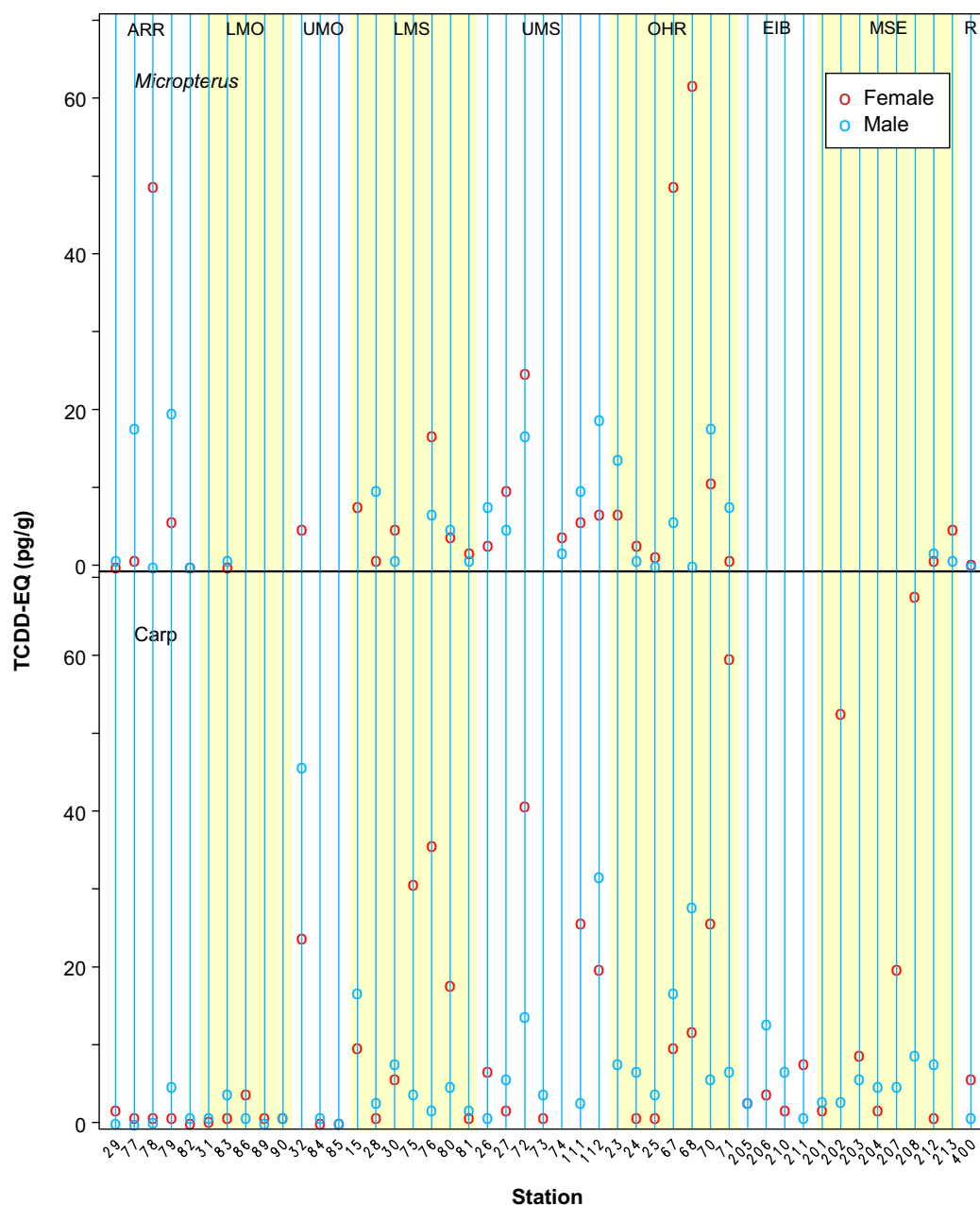


Figure 2-50. Concentrations of H4IIE bioassay-derived TCDD-EQ (pg/g) in composite samples of bass (upper panel) and carp (lower panel), by sub-basin, gender, and station. See Table 1-1 for station descriptions.

male ≤ 0.51 pg/g; Figs. 2-48, 2-50, 2-51); that is, composite samples of bass contained no extractable dioxin-like compounds that could be detected in the H4IIE cells. Each of the extracts was analyzed in triplicate and all of the values were less than the LOQ.

Dioxin-like contamination was present in bass from all sub-basins; sub-basin means for TCDD-EQ in bass ranged from 1.1 (LMO) to 8.8 (UMS) pg/g

(Table 2-4). As noted, the composite sample of bass from the reference site contained <1 pg/g of TCDD-EQ. Nevertheless, TCDD-EQ in bass did not differ significantly among sub-basins ($P=0.16$) or programs ($P=0.32$), and no sub-basin or program means differed significantly from the reference site (Tables 2-4 and 2-5).

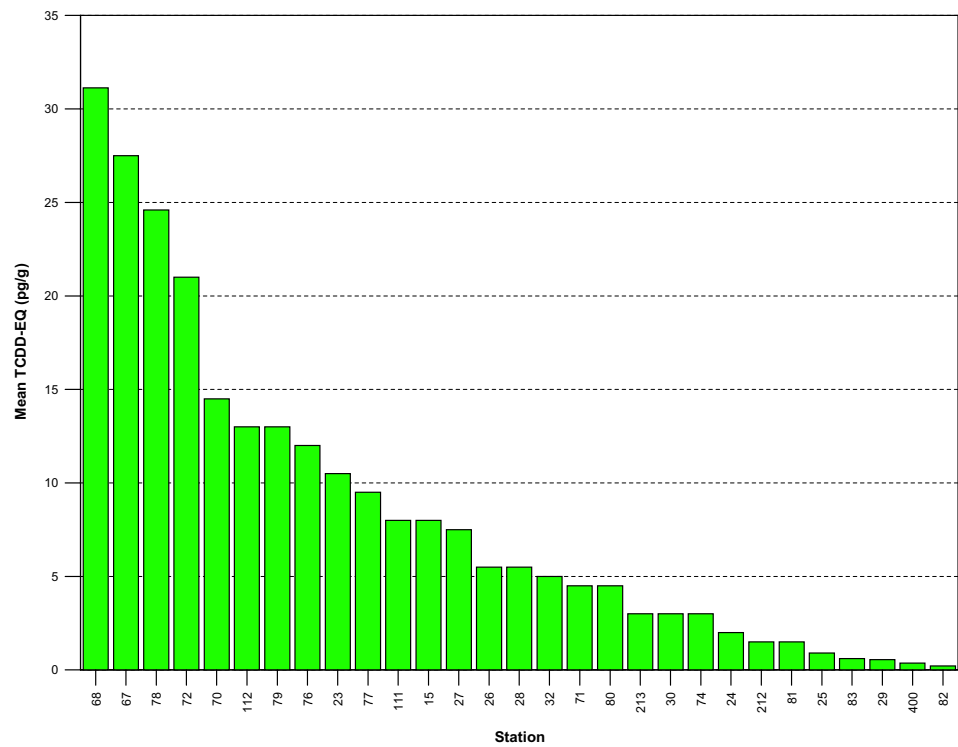


Figure 2-51. Ranked geometric mean concentrations of H4IIE bioassay-derived TCDD-EQ (pg/g) in composite samples of bass, by station. See Table 1-1 for station descriptions.

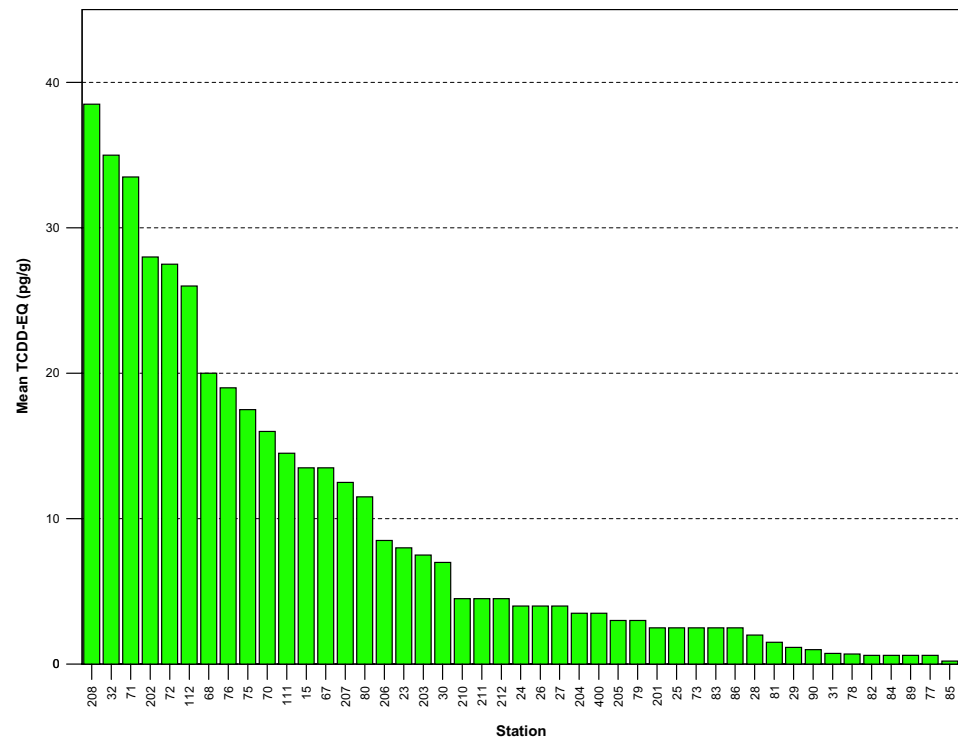


Figure 2-52. Ranked geometric mean concentrations of H4IIE bioassay-derived TCDD-EQ (pg/g) in composite samples of carp, by station. See Table 1-1 for station descriptions.

TCDD-EQ in Carp: H4IIE bioassay-derived TCDD-EQ in composite samples of carp ranged from <LOD to 68 pg/g (Figs. 2-48 and 2-50). Approximately 10% of the 89 carp composite samples analyzed were below the LOQ (Fig. 2-49). Concentrations were generally greater in females than in males (Fig. 2-50). However, in contrast to male bass, there were composites of male carp in which TCDD-EQ was >20 pg/g (Fig. 2-50). The mode for TCDD-EQ in both male and female carp was 2 pg/g (Fig. 2-49). These are essentially background concentrations owing to the ubiquity of the PHHs. In general, TCDD-EQ concentrations in carp were greater than in bass; nevertheless, concentrations were ≤ 5 pg/g in 53% of male composites and 55% of the females (Fig. 2-49). The station means for TCDD-EQ in carp were ≥ 30 pg/g at three sites (three of 45, 7%), ≥ 20 pg/g at six sites (13%), ≥ 10 pg/g at 15 sites (33%), and ≥ 5 pg/g at 19 sites (42%; Fig. 2-52). The greatest TCDD-EQ concentration in carp was 68 pg/g in a female from Station 208 (Cache R. at Egypt, AR; Fig. 2-50, Table 2-3), where the station mean for carp was also the greatest (39 pg/g; Fig. 2-52). The other sites at which mean TCDD-EQ concentrations in carp was >10 pg/g were, in descending order, Stations 32 (Missouri R. at Garrison Dam, ND), 71 (Tennessee R. at Savannah, TN), 202 (Bogue Phalia at Leland, MS), 72 (Wisconsin R. at Woodman, WI), 112 (Mississippi R. at Dubuque, IA), 68 (Wabash R. at New Harmony, IN), 76 (Mississippi R. at Memphis), 75 (Mississippi R. at Cape Girardeau, MO), 70 (Ohio R. at Metropolis, IL), 111 (Mississippi R. at Lake City, MN), 15 (Mississippi R. at Luling, MS), 67 (Allegheny R.), 207 (Cache R. at Cotton Plant, AR), and 80 (Yazoo R. at Redwood, MS; Fig. 2-52). As was true for bass, total PCB concentrations in carp from most of these sites were ≥ 0.2 $\mu\text{g/g}$ (Fig. 2-44). However, at Stations 32, 202, 207, and 208, which are located in relatively rural areas, total PCB concentrations in carp were low (<0.05-0.08 $\mu\text{g/g}$; Fig. 2-44).

In contrast to the largemouth bass from the reference site, which contained no detectable TCDD-EQ, both samples of carp from Station 400 had measurable but low concentrations of TCDD-EQ—1 pg/g in males and 6 pg/g in females (Figs. 2-48 and 2-50), with a geometric station mean of 2.4 pg/g (Fig. 2-52). Also in contrast to bass, TCDD-EQ concentrations in carp differed significantly ($P < 0.0001$) among sub-basins, with sub-basin means ranging from 1.1 to 8.3 pg/g (Table 2-4). The ARR, UMR, and LMR sub-basins and the reference site had comparatively low (<2.5 pg/g) TCDD-EQ means that did not differ significantly from each other ($P > 0.05$). In contrast, the means for the UMR, LMR, and OHR sub-basins and the MSE Study Unit all exceeded 6.3 pg/g (Table 2-4). These means differed significantly ($P < 0.05$)

from the three sub-basins with low TCDD-EQ means and the reference site, but also did not differ from each other (Table 2-4). The mean for the EIB Study Unit (4.33 pg/g) was intermediate relative to the two groups and did not differ significantly from any other sub-basin mean or the reference site (Table 2-4). As was also true for bass, TCDD-EQ in carp did not differ significantly between programs, and neither program-level mean differed significantly from the reference site ($P = 0.07$; Table 2-5).

TCDD-EQ in Other Taxa: Most of the H4IIE-derived TCDD-EQ concentrations in taxa other than carp and bass were <10 pg/g (Fig. 2-48). The only exceptions were white bass from Stations 15 (Mississippi R. at Luling, LA), which contained 23-37 pg/g, 75 (Mississippi R. at Cape Girardeau, MO), which had 5-12 pg/g; and smallmouth buffalo from Station 23 (Kanawha R. at Nitro, WV), which contained 6-15 pg/g. Total PCB concentrations were about 0.5 $\mu\text{g/g}$ in all the white bass samples from Stations 15 and 75, and were 1.8-2.9 $\mu\text{g/g}$ in the smallmouth buffalo from Station 23 (Fig. 2-44).

Ecological Risk of TCDD-EQ: In laboratory studies with mink (*Mustela vison*) fed diets containing varying proportions of PCB-contaminated carp from Saginaw Bay, MI, significant effects on liver pathology and reproduction occurred at 19.4 pg of TCDD-EQ per g of diet (as measured by the H4IIE bioassay), the lowest nominal dose (the Lowest Observed Adverse Effects Level, or LOAEL) (Heaton and others, 1995; Tillitt and others, 1996). In these studies the calculated threshold dietary concentration for reproductive effects was 4.4 pg/g. The dietary threshold for reproductive effects in birds is also about 5 pg/g. (Nosek and others, 1992). Although sensitivity varies among taxa, fish are generally less sensitive than birds or mammals; the threshold for effects on hatching success in Chinook salmon (*Oncorhynchus tshawytscha*) is about 100 pg TCDD-EQ/g egg (Ankley and others, 1991). Walker and others (1996) suggested a NOEL for the most sensitive salmonids (lake trout, *Salvelinus namaycush*) to be approximately 30 pg TCDD/g egg. This threshold, however, is not based on whole body measurements of TCDD, but rather egg concentrations. If one assumes the lipid content is similar between eggs and whole bodies of fish, then the 30 pg TCDD/g threshold would be applicable to whole body estimates of dioxin-like potency. None of the 1995 samples exceeded 100 pg/g (Ankley and others, 1991). The 30 pg/g threshold was exceeded by one sample of carp from each of Stations 32 (Missouri R. at Garrison Dam, ND), 75 (Mississippi R. at Cape Girardeau, MO), 76 (Mississippi R. at Memphis, TN), 72 (Wisconsin R. at Woodman, WI), 112 (Mississippi R. at Dubuque, IA), 71 (Tennessee R. at Savannah,

TN), 202 (Bogue Phalia at Leland, MS), and 208 (Cache R. at Egypt, AR), one sample of white bass from Station 75, and one sample of bass from each of Stations 67 (Allegheny R. at Natrona, PA), 68 (Wabash R. at New Harmony, IN) and 78 (Verdigris R. at Oologah, OK; Figs. 2-48 and 2-50). One or both samples of carp from Stations 68 (Wabash R. at New Harmony, IN), 75, 111 (Mississippi R. at Lake City, MN), and 207 (Cache R. at Cotton Plant, AR); one sample of bass from Station 72; and both samples of white bass from Station 15 (Mississippi R. at Luling, LA) also exceeded 19.4 pg/g, the LOAEL for reproductive effects in mink [(Heaton and others, 1995; Tillitt and others, 1996); Figs. 2-48, 2-50]. In addition, fish from many more stations contained >5 pg/g of TCDD-EQ (Figs. 2-48-2-50), the dietary threshold for reproductive effects in wildlife (Nosek and others, 1992; Tillitt and others, 1996).

Correlations between TCDD-EQ and Other

Contaminants: We used TCDD-EQ as a surrogate for the congener-specific instrumental analysis of the samples for a suite of dioxin-like PHHs that should rise and fall together in each sample irrespective of the taxon and gender of the fish. Accordingly, we examined correlations between TCDD-EQ and other contaminants only for all composite samples ($N=163$). As expected, TCDD-EQ was positively correlated with total PCBs ($r=0.37$, $P<0.001$). TCDD-EQ was also correlated to a lesser extent with p,p' -DDE ($r=0.19$, $P<0.05$) and total cyclodiene pesticides (sum of dieldrin, *cis*- and *trans*- chlordanes and nonachlors, oxychlordane, heptachlor epoxide, and endrin; $r=0.19$, $P<0.05$). Relations among TCDD, EROD, and PCBs are discussed in greater detail later in this section.

Hepatic Ethoxyresorufin O-Deethylase (EROD) Activity

Hepatic EROD activity is a catalytic measure of the microsomal de-alkylase activity of mono-oxygenase enzymes of the liver. These are heme-containing, detoxification enzymes that are induced by certain chemicals. Mono-oxygenase enzymes oxidize chemicals, making the chemicals more hydrophilic and readily available for elimination from the body. As noted earlier in this chapter, an increase in mono-oxygenase enzymes and a corresponding increase in catalytic activity, as measured by EROD, are symptomatic of exposure to chemicals with a dioxin-like structure. The enzymatic activity toward EROD is a measure of the catalytic activity of the mono-oxygenase system of the liver and state of exposure of the organism with respect to AhR-agonists.

Basal EROD Activity

A determination of basal EROD activity is required to

place the EROD dataset into context. Basal EROD is the enzyme activity expected in an unexposed organism or in an organism exposed to chemicals below some threshold amount. Basal EROD activities or rates are specific to each species and gender (Whyte and others, 2000). Females typically have greater estradiol concentrations, especially during reproductively active times of the year, which can reduce the inductive EROD response to chemicals. The mechanism or mechanisms of EROD suppression by estrogens and estrogenic compounds is not fully understood at this time, but the phenomenon causes females to have reduced EROD activities during periods of elevated estrogen compared with males of the same species and exposure. Therefore, it is necessary to develop gender-specific assessments of basal EROD rates. We estimated basal rates through observation of the frequency distributions and associated measures of median values and variances of our own data (Figs. 2-53, 2-54). These estimated basal rates were verified by comparisons with basal EROD rates from the published scientific literature (see review by Whyte and others, 2000). These taxon- and gender-specific estimates were used to help define the basal activity of EROD in the taxa we analyzed.

Unfortunately, the samples of largemouth bass and carp liver from the reference site were compromised during transport, so it is impossible to make comparisons with these samples. However, the other quality control measures for evaluation of data quality indicated that the EROD data we report accurately reflect the catalytic activity of this enzyme in the samples.

Basal EROD Activity in Bass: The modal hepatic EROD rate in male bass was 10-12 pmol/min/mg resorufin/minute/mg protein (pmol/min/mg), and approximately 72% of the EROD rates in male bass were <22 pmol/min/mg (Fig. 2-53). In female bass the modal EROD rate was 4-8 pmol/min/mg, and 73% of the EROD rates of female bass were <20 pmol/min/mg (Fig. 2-53). Based on these data, we considered hepatic EROD activity in bass to be within the normal range and not elevated at rates of 0-16 pmol/min/mg in females and 0-22 pmol/min/mg in males. Only at EROD rates greater than these activities did we consider enzyme activity induced above background. A search of the published literature (Whyte and others, 2000) revealed three references for basal EROD rates in black basses, all dealing with largemouth bass. Adams and others (1994) reported hepatic EROD rates of approximately 5 pmol/min/mg in females and 17 pmol/min/mg in males from a reference area in South Carolina. Schlenk and others (1996a, 1996b) reported baseline rates of hepatic EROD of 0-10 pmol/min/mg in male and female

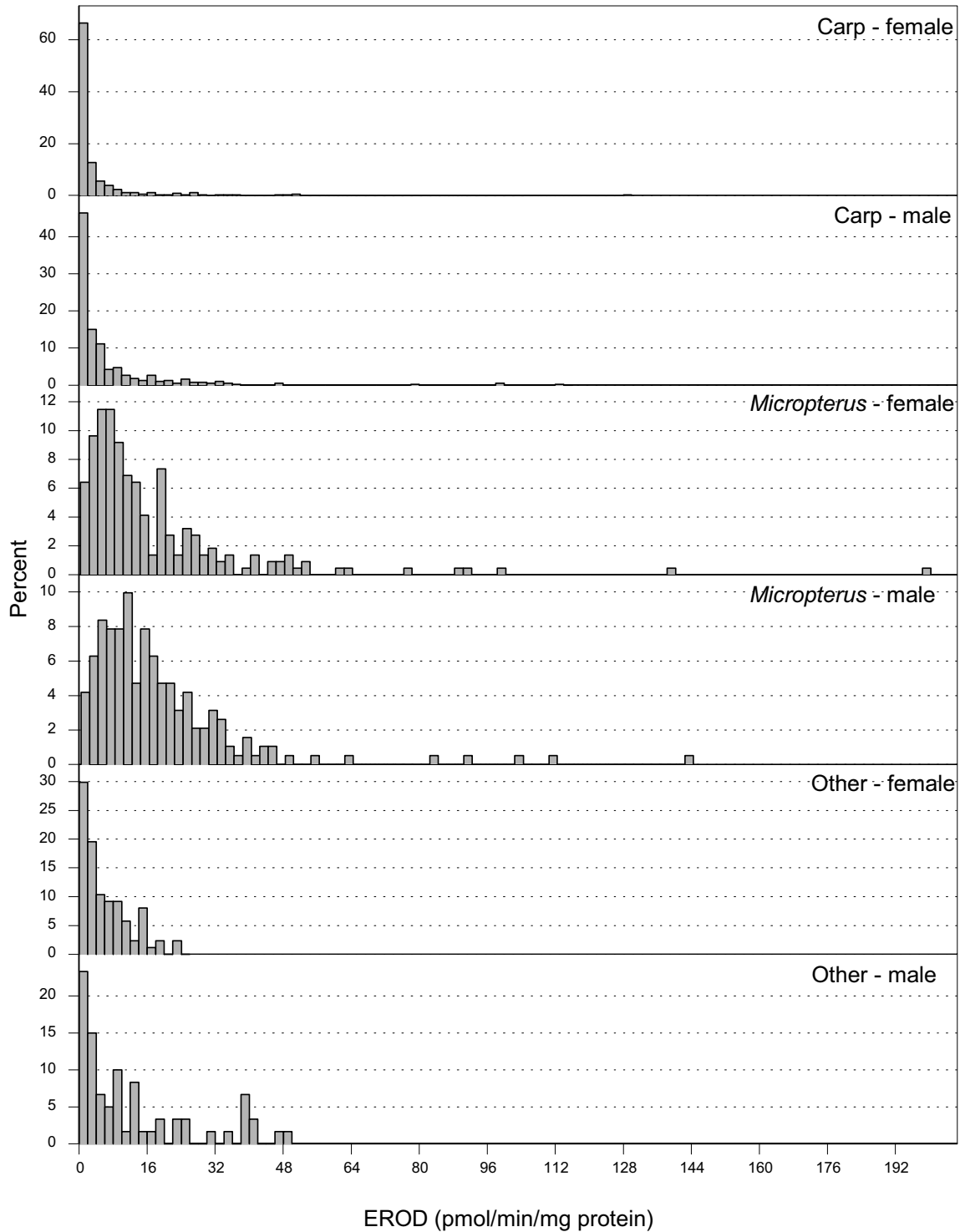


Figure 2-53. Frequency distributions of EROD activity in individual fish, by taxon and gender. Censored values are represented by 50% of LOD.

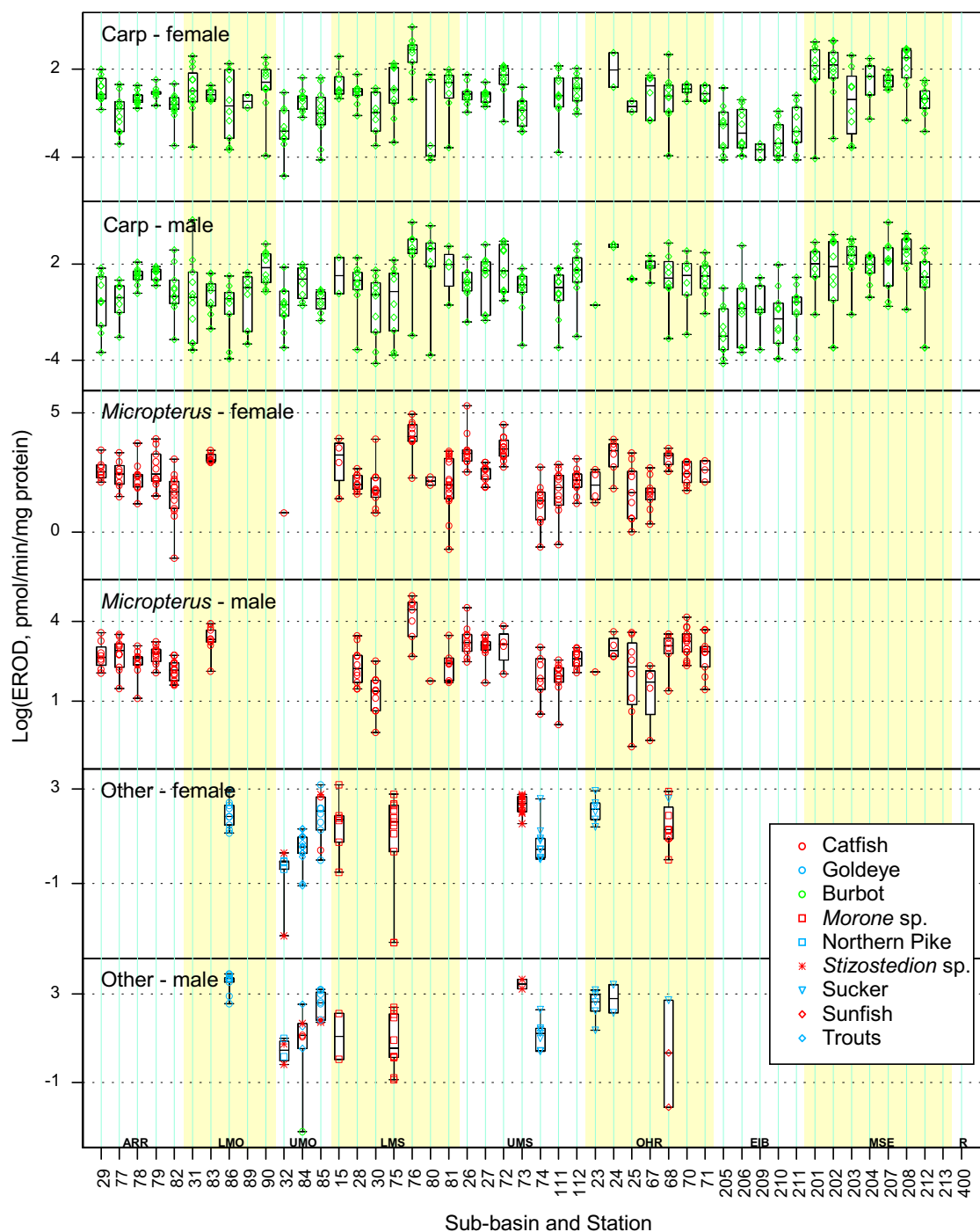


Figure 2-54. EROD activity (log) in individual fish, by taxon, gender, sub-basin, and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). Censored values are represented by 50% of LOD. See Table 1-1 and Figure 1-1 for station locations.

largemouth bass from Bayou Bartholomew, AR. Thus, our data and its interpretation, as outlined above, appear to be consistent with the literature on basal hepatic EROD rates in bass. When differences in assay conditions are included in the comparison, our estimate of basal or “background activity” appears to be consistent with the literature. As such, we consider hepatic EROD rates in female bass >16 pmol/min/mg and >22 pmol/min/mg in male bass to be induced or indicative of elevated exposure to Ah-R agonists. It is important to note that there are no published studies of EROD rates in smallmouth or spotted bass; however, our examination of EROD data from sites at which these species were collected together with largemouth bass (Fig. 2-54) suggests that EROD rates in smallmouth and spotted bass do not differ from those of largemouth bass.

Basal EROD Activity in Carp: EROD rates in carp are generally lower than in bass (Whyte and others, 2000). In carp, modal rates of hepatic EROD activity in our study were ≤ 1 -2 pmol/min/mg or less in both males and females (Fig. 2-53). The hepatic EROD activity in 82% of the male carp was ≤ 6 pmol/min/mg whereas the frequency distribution in females was slightly skewed to lower values, as would be expected and similar to our findings for bass (Fig. 2-53). In female carp, 84% of the hepatic EROD rates were ≤ 4 pmol/min/mg. Thus, hepatic EROD rates in carp of 0-4 pmol/min/mg in females and 0-6 pmol/min/mg in males appear to normal, based on the population of samples collected and analyzed in our study. As in bass, these results were consistent with the literature; Schlenk and others (1996a; 1996b) reported EROD rates of 0-5 pmol/min/mg in carp from a reference site in Bayou Bartholomew, AR. For comparative purposes we therefore considered hepatic EROD rates >6 pmol/min/mg in male carp and >4 pmol/min/mg in female carp as induced (that is, elevated above background).

Hepatic EROD Activity in Bass: Hepatic EROD rates in bass were 0-200 pmol/min/mg (Fig. 2-53). The greatest EROD rate in female bass (200 pmol/min/mg) was found at Station 26 (Illinois R. at Beardstown, IL) whereas in males the greatest value was 139 pmol/min/mg at Station 76 (Mississippi R. at Memphis, TN; Fig. 2-54). Station means of the EROD rates for male and female bass are presented in Fig. 2-55. The greatest mean EROD activity in males was 77 pmol/min/mg at Station 76 (Mississippi R. at Memphis). Other locations at which male bass had elevated mean EROD rates, in descending order, were Stations 26 (Illinois R. at Beardstown, IL), 83 (Missouri R. at Hermann, MO), 70 (Ohio R. at Metropolis, IL), 72 (Wisconsin R. at Woodman, WI),

24 (Ohio R. at Marietta, OH), and 27 (Mississippi R. at Guttenburg, IA; Fig. 2-55). Mean hepatic EROD activity in male bass was elevated at 15 of the 24 sites from which they were collected (Fig. 2-55) based our criterion of ≥ 22 pmol/min/mg. Thus, 29% of the sites had mean values indicating elevated EROD activity (Fig. 2-55), and 63% of the sites had at least one male bass that exceeded our criterion (Fig. 2-54).

Mean hepatic EROD in female bass rates exceeded our designated threshold (16 pmol/min/mg) at 8 of 26 sites (31%), slightly more than in males (Fig. 2-55). In addition, at least one individual female bass from 20 of the 26 sites (77%) had hepatic EROD rates that exceeded this threshold (Fig. 2-54). EROD activities in female bass were elevated at many of the sites at which the males were also induced; for example, female bass from Station 76 (Mississippi R. at Memphis) had the greatest mean EROD rate (Fig. 2-55). The other sites with elevated mean rates of hepatic EROD activities were, in descending order, Stations 26 (Illinois R.), 72 (Wisconsin R.), 24 (Ohio R. at Marietta, OH), 15 (Mississippi R. at Luling, LA), 68 (Wabash R. at New Harmony, IN), 83 (Missouri R. at Hermann, MO), and 79 (Canadian R. at Eufala, OK; Fig. 2-55).

Mean hepatic EROD rates in both male and female bass indicated exposure to Ah-R agonists at five sites (Stations 76, 26, 72, 83, and 24). That is, 19% (five of 26) of the stations sampled had both male and female bass with mean values over the designated EROD threshold for their respective gender. Only female liver samples were available from Station 15, so it is impossible to compare the response of the genders in the same fashion. At four other stations (68, 79, 70, and 27) mean hepatic EROD activities were above the gender-specific thresholds in only one of the sexes.

Hepatic EROD activities in bass differed significantly among sub-basins (Table 2-4). Both male and female bass from the LMO sub-basin had gender-specific mean rates of hepatic EROD that differed from all of the other sub-basins. However, it must be noted that bass were only found at one station in this sub-basin. Nevertheless, mean hepatic EROD rates were elevated in both males and female largemouth bass from Station 83 (Missouri R. at Hermann, MO). Mean hepatic EROD rates were also elevated in both male and female largemouth bass from the LMS, UMS, and OHR sub-basins. In contrast, mean hepatic EROD activities in both males and female largemouth bass from the ARR and UMO sub-basins were basal or baseline. Additionally, no individual stations in these two sub-basins had elevated rates of hepatic EROD activity. Bass from the MSE NAWQA Study Unit were not analyzed for EROD activity.

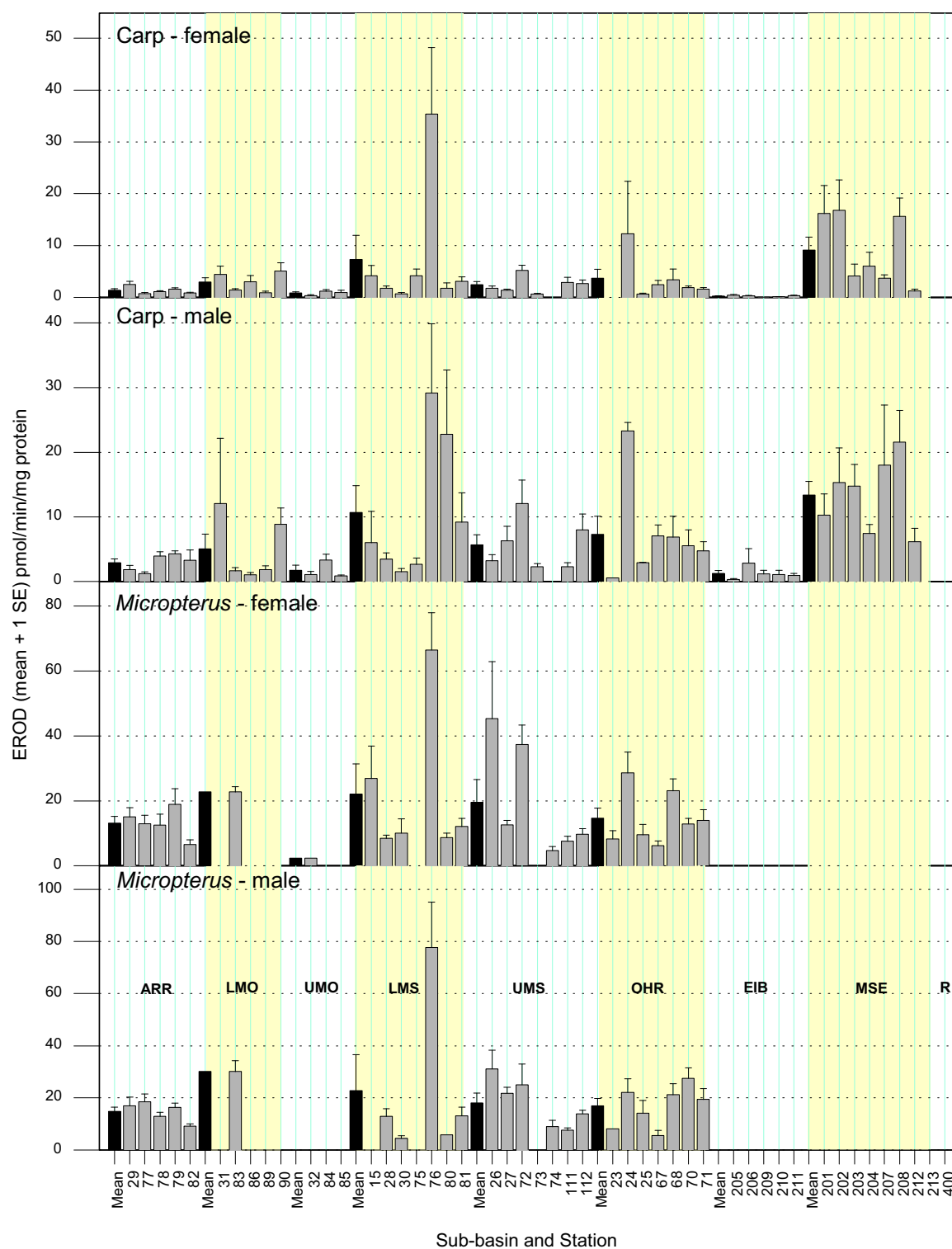


Figure 2-55. EROD activity in carp and bass, by taxon, gender, sub-basin (black bars), and station (gray bars, $n > 1$). Shown are arithmetic means + 1 SE. Sub-basin estimates were based on station means rather than individual fish. Censored values are represented by 50% of LOD. See Table 1-1 and Figure 1-1 for station locations.

Hepatic EROD Activity in Carp: Hepatic EROD activity in carp was 0-129 pmol/min/mg (Fig. 2-53). The greatest individual EROD rates (129 pmol/min/mg in females, 100 pmol/min/mg in males) were both from Station 76 (Mississippi R. at Memphis; Fig. 2-54). Consequently, the greatest mean EROD value in male carp (29 pmol/min/mg) was also at Station 76 (Fig. 2-55). Other sites with elevated mean rates of hepatic EROD activity in male carp were, in descending order, Stations 24 (Ohio R. at Marietta, OH), 80 (Yazoo R.), 208 (Cache R. at Egypt, AR), 207 (Cache R. at Cotton Plant, AR), 202 (Bogue Phalia at Leleand, MS), 203 (Steele Bayou at Rolling Fork, MS), 31 (Missouri R. at Nebraska City, NE), 72 (Wisconsin R.), 201 (Big Sunflower R. at Anguilla, MS), 81 (Red R. at Alexandria, LA), 90 (Kansas R. at Bonner Springs, KS), 112 (Mississippi R. at Dubuque, IA), 204 (Tensas R. at Tendal, LA), 67 (Allegheny R.), 68 (Wabash R.), 27 (Mississippi R. at Guttenburg, IA), 212 (Little River Ditch at Moorehouse, MO), and 15 (Mississippi R. at Luling, LA; Fig. 2-55). Based on our criterion of ≥ 6 pmol/min/mg, mean EROD activity was elevated at 19 of the 45 sites from which male carp were collected and analyzed (42%; Fig. 2-55) and 67% of the sites had at least one male carp with EROD > 6 pmol/min/mg (Fig. 2-54).

Mean hepatic EROD rates in female carp exceeded our threshold (≥ 4 pmol/min/mg) at 12 of 44 sites (27%), slightly fewer than the number and percentage at which male carp exceeded their threshold (Fig. 2-55). At 25 sites (57%) at least one female carp had a hepatic EROD rate ≥ 4 pmol/min/mg (Fig. 2-54). In female carp, as with male carp, the greatest mean EROD rate was at Station 76 (Mississippi R. at Memphis, TN). The other stations with elevated mean hepatic EROD rates in female carp also included many of the same sites identified for males. They were, in descending order, Stations 202 (Bogue Phalia), 201 (Big Sunflower R.), 208 (Cache R. at Egypt, AR), 24 (Ohio R. at Marietta, OH), 204 (Tensas R.), 72 (Wisconsin R.), 90 (Kansas R.), 83 (Missouri R. at Hermann, MO), 15 (Mississippi R. at Luling, LA), 75 (Mississippi R. at Cape Girardeau, MO), and 203 (Steele Bayou at Rolling Fork, MS; Fig. 2-55).

Mean hepatic EROD rates at 11 sites (Stations 31, 90, 15, 76, 72, 24, 201, 202, 203, 204, and 208) indicated elevated exposure to Ah-R agonists in both male and female carp (Fig. 2-55). That is, at 25% (11 of 44) of the stations sampled the mean for both male and female carp exceeded the designated EROD threshold for their respective gender. At Station 75 only liver samples from female carp were obtained, and at eight other sites (Stations 80, 81, 27, 112, 67, 68, 207, and 212) mean hepatic EROD activities exceeded the gender-specific threshold in only

male carp. At no site was the criterion for EROD activity exceeded only in female carp.

Mean hepatic EROD rates in carp (both males and females) differed significantly among sub-basins (Table 2-4). The sub-basins with elevated mean hepatic EROD rates were the LMS (both males and females), UMS (males only), and OHR (males only); and the MSE-Study Unit (both males and females). Means for carp from the ARR (both males and females), LMO (both males and females), the UMO (both males and females), UMS (females only), and OHR (females only) sub-basins; and from the EIB Study Unit (both males and females) were not elevated in hepatic EROD rates with respect to the gender-specific, estimated basal activity for carp (≥ 4 pmol/min/mg for females, ≥ 6 for males). Hepatic EROD rates were significantly greater in female carp from NAWQA sites than in carp from NCBP sites, but male carp did not differ at the program-level (Table 2-5).

Hepatic EROD Activity in Other Fishes: In addition to carp and bass, hepatic EROD activity was measured in a number of other taxa. Unfortunately, EROD activity varies greatly among fishes, and there relatively few published reports of basal activity against which to judge the relative induction associated with many of these results (Whyte and others, 2000). Those for which such comparisons can be made are summarized below. For the other taxa, we present and discuss the data in Fig. 2-54 primarily for future reference.

Northern pike were collected only at Station 32 (Missouri R. at Garrison Dam, ND), where EROD activity was 0.6-0.9 pmol/min/mg in females and 1.1-2.7 pmol/min/mg in males (Fig. 2-54). These values are ≥ 10 -fold lower than previously reported basal activities for this species (Förlin and Celander, 1993; Williams and others, 1997). Walleye (*Stizostedion vitreum*) were also collected exclusively at Station 32, where hepatic EROD activity was < 0.8 -1.3 pmol/min/mg in females and 0.8-2.1 pmol/min/mg in males (Fig. 2-54). These levels were also 10-fold lower than the basal activities for this species reported by Williams and others (1997). White suckers were collected only at Station 74 (Mississippi R. at Little Falls, MN), where EROD activity was 1.0-13.1 pmol/min/mg in females (most 1-3) and 1.4-3.4 pmol/min/mg in males (Fig. 2-54). These are all within previously reported values for basal EROD activity in fall-caught white sucker (Servos and others, 1992; Munkittrick and others, 1994; Gagnon and others, 1994). Channel catfish (females only, $n=2$) were collected exclusively at Station 85 (Yellowstone R. at Sydney, MT), where all EROD values were within the basal ranges in the studies reviewed by Whyte and others (2000). The only burbot (*Lota lota*) collected

in 1995 was a male from Station 84 (Big Horn R. at Hardin, MT). The EROD activity in this fish was <0.08 pmol/min/mg (Fig. 2-54). This value is lower than all previously reported values for this species (Lockhart and Metner, 1992; Kloepper-Sams and Benton, 1994; Williams and others, 1997), and therefore suggests basal activity in this species at this location. Similarly, rainbow trout (*Oncorhynchus mykiss*) were collected exclusively at Station 84 (one female); its EROD activity was very low (<0.08 pmol/min/mg; Fig. 2-54), even relative to basal activities in laboratory studies (many, reviewed by Whyte and others 2000). Brown trout were also collected exclusively at Station 84, where hepatic EROD activity was 0.3-2.6 pmol/min/mg in females and 1.6-12.3 (only one fish >10) pmol/min/mg in males (Fig. 2-54). These values are similar to previously reported basal EROD activities in reproductively active brown trout (Nakari, 1997). In contrast to most of the fishes collected in 1995, brown trout spawn in the fall. Results of the reproductive biomarkers indicated that most of these fish were approaching spawning condition at the time they were collected.

There are no previously published reports of EROD activity in white bass or white bass-striped bass (*Morone saxatilis*) hybrids (Whyte and others, 2000), but a laboratory study with striped bass (Washburn and others, 1996) indicated a comparatively low level of basal activity (mean 1.2 pmol/min/mg) in this species. Most of the white and hybrid bass we collected at Stations 15 (Mississippi R. at Luling, LA), 68 (Wabash R. at New Harmony, IN), and 75 (Mississippi R. at Cape Girardeau, MO) had relatively low EROD rates (most 2-8 pmol/min/mg; Fig. 2-54). Nevertheless, a few values in white bass from each of these sites were elevated relative to other 1995 values and to basal activities in striped bass (Washburn and others, 1996), suggesting exposure of these fish to exogenous AhR agonists. Similarly, there are no previously published reports of EROD activity in sauger (Whyte and others, 2000). However, relative to basal activity in the congeneric walleye (Williams and others, 1997), most of the values for sauger [from Stations 73 (Des Moines R. at Keosauqua, IA), 84, and 85] were within the range of basal activity (Fig. 2-54). The EROD activities in all of the sauger collected from these stations were greater than that of the walleyes collected from Station 32 (Fig. 2-54), however. In addition, EROD activity in both of the male sauger from Station 73 was somewhat elevated relative to the other saugers collected in 1995, suggesting that these fish had also been exposed to exogenous AhR agonists.

Correlations Among TCDD-EQ, Hepatic EROD Activity, and Instrumentally Determined Contaminant

Concentrations: In contrast to TCDD-EQ, we explored relations among hepatic EROD activity, TCDD-EQ, and instrumentally determined contaminant concentrations separately for male and female carp and bass because of the well-documented species- and gender-specific nature of the EROD response to AhR agonists (Whyte and others, 2000). We also analyzed for correlations at the taxon level (that is, with the sexes combined). Generally, correlations were stronger and there were more of them in carp than in bass. Our analyses revealed statistically significant ($P<0.1$) weak to moderate correlations between geometric mean hepatic EROD activity and contaminant concentrations in composite carcass samples. EROD was positively correlated with Cd, Hg, total PCBs, and DDE in male carp ($r=0.25$, $P=0.12$ in female carp); TCDD-EQ in female carp ($r=0.21$, $P=0.20$ in male carp); Hg, total PCBs, DDE, and TCDD-EQ in the combined-sex analysis of carp; and with total PCBs in the combined-sex analysis of bass (Table 2-6). EROD activity was also positively correlated with Pb in male and female carp and bass and in all bass, but not in all carp (Table 2-6). EROD activity was negatively correlated with Cd in male bass, but there were no other significant negative correlations (Table 2-6). Relations between hepatic EROD activity, total PCBs, and TCDD-EQ were evaluated further using multiple linear regression. The results of these analyses are presented in the next section.

An important point to remember with respect to these correlations is that they quantify only the degree of association between pairs of variables, not cause-effect relationships. As such, correlations may be artifacts of contaminant co-occurrence and other sources of variation not accounted for in the analyses. Contaminants tend to co-occur at most of the sites we investigated; consequently, many of the statistically significant correlations between EROD activity and measured contaminants may not represent causal relationships. For example, heavy metals are generally regarded as AhR antagonists, but may also enhance enzyme activity through non-AhR-mediated mechanisms (Whyte and others, 2000). So, the fact that we observed both positive and negative associations between EROD activity and metals concentrations is not surprising. In addition, metals such as Pb, Hg, and Cd tend to co-occur with PCBs at sites affected by urban and industrial activities (Schmitt, 1999). Consequently, positive correlations between EROD activity and metals may reflect correlations with PCBs and other industrial pollutants. More rigorous statistical analyses are required to better characterize these relationships. Regardless, even the best statistical

Table 2-6. Statistically significant¹ rank correlation coefficients describing relationships between concentrations of indicated contaminants in whole fish and hepatic EROD activity in carp and bass of the indicated genders.

Contaminant	Carp			Bass		
	Male	Female	All	Male	Female	All
Cd	0.27*	ns	ns	-0.51**	ns	ns
Hg	0.36**	ns	0.34**	ns	ns	ns
Pb	0.27*	0.31**	ns	0.46**	0.48**	0.49**
<i>p,p'</i> -DDE	0.56***	ns	0.51***	ns	ns	ns
Total PCBs	0.27*	ns	0.32**	ns	ns	ns
TCDD-EQ	ns	0.39**	0.39**	ns	ns	0.35*

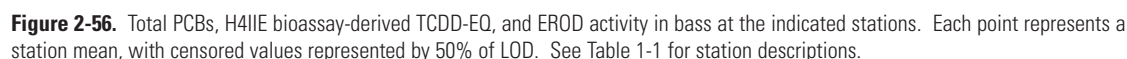
¹ ns, $P > 0.1$; * $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$.

analyses can only suggest relationships, not causes; the latter can only be investigated in the laboratory and in semi-controlled field studies conducted on contaminant gradients. Moreover, and in spite of the fact that hepatic EROD activity in fish is among the most often employed biomarkers in aquatic toxicology, surprisingly few contaminants have been tested under controlled laboratory conditions, especially in commonly collected North American fishes (Whyte and others, 2000). Laboratory investigators have tended to further refine existing mechanisms of AhR-mediated toxicity with known ligands in laboratory organisms rather than to broaden the base of knowledge with respect to chemicals, fish species, and factors other than contaminants that may influence the EROD response. Even fewer contaminants have been tested in combination. Consequently, the correlations reported here may in fact represent real, but as-yet undocumented relationships between contaminants and EROD activity. We therefore feel that these correlations are worth reporting, if for no other reason than to stimulate discussion and further study.

Total PCBs, TCDD-EQ, and Hepatic EROD Activity: Our monitoring and assessment strategy allowed us to evaluate exposure of the fish to PAHs, PCBs, and PCDDs and PCDFs even though most of these compounds were not analyzed (Table 2-2). PAHs were not analyzed because they are readily metabolized and the results of chemical analysis does not reflect exposure in fish. Instrumental analyses of individual PCDD, PCDF, and AhR-active PCB congeners were not included due to high cost. Instead, the combination of total PCB analysis, hepatic EROD analysis, and H4IIE bioassay-derived TCDD-EQ are used together, as noted earlier. Chemical analysis of total PCBs provides general information on exposure to PCBs, but not for specific AhR-active congeners; hepatic EROD activity is indicative of exposure to PAHs, PCBs, and PCDDs/PCDFs; and the H4IIE bioassay was performed on acidified extracts and thereby provides

exposure information on dioxin-like chemicals (PCDDs/PCDFs, etc.) exclusive of PAHs (Table 2-2). The acidic silica gel columns through which the fish sample extracts used in the H4IIE bioassay were processed effectively remove most labile compounds, including PAHs (Schwartz and Lehmann, 1982). Because of the differences in the processing methods, the combination of measures for AhR agonists (total PCBs, EROD, and H4IIE) taken from the same samples can be used to give inference about PCDD, PCDF, and PAH exposure in these fish. For example, if H4IIE bioassay-derived TCDD-EQ is elevated in a sample with elevated hepatic EROD and low total PCB concentration, then other AhR agonists are probably present. Likely candidates to explain such a situation are PCDDs, PCDFs, and similar recalcitrant PHHs capable of surviving the reactive cleanup (Table 2-2). Alternatively, if total PCB and TCDD-EQ concentrations were low, elevated EROD activity would suggest exposure to PAHs or other labile compounds that did not survive the reactive cleanup (Table 2-2). When used together in this manner, these comparatively inexpensive assays may provide information beyond their own routine measures.

In bass, hepatic EROD activities, concentrations of total PCBs, and H4IIE-derived TCDD-EQ suggested that concentrations of total PCBs in fish were the most important component of AhR-related toxicity, but the relationships among the three variables were weakly defined (Fig. 2-56). TCDD-EQ generally increased with concentrations of total PCBs in bass at most stations at which all three measurements were made (note: EROD activity was not measured in bass from Stations 212 and 213 in the MSE NAWQA Study Unit), and hepatic EROD activity was elevated relative to basal activity at most of the sites with the greatest PCB concentrations in bass (Fig. 2-56). Nevertheless, and as noted previously, hepatic EROD activity was only marginally correlated with total PCBs ($r=0.35$, $P<0.01$) in the combined-sex analysis of bass and was not significantly correlated



total PCB concentrations and hepatic EROD activity.

Bass from Station 83 (Missouri R. at Hermann, MO) were particularly noteworthy in terms of deviation from the PCB-TCDD-EQ-EROD axis (Fig. 2-56). Bass from this site contained very low total PCB and TCDD-EQ concentrations (Figs. 2-44, 2-48, and 2-50), yet had comparatively high hepatic EROD rates (Figs. 2-54, 2-55, and 2-56). Elevated EROD activity in the absence of PCBs and TCDD-EQ suggest that the fish were exposed to PAHs, which is consistent with the recent environmental history of this site. In contrast to the carp from Station 83, which were collected from the mainstem of the river, field records revealed that the bass were actually collected from the lower reaches of the Gasconade River, a tributary draining rural areas that joins the Missouri River about 2 km upstream of Hermann. On December 24, 1988, a pipeline rupture caused the largest known inland oil spill in U.S. history; some 3.3

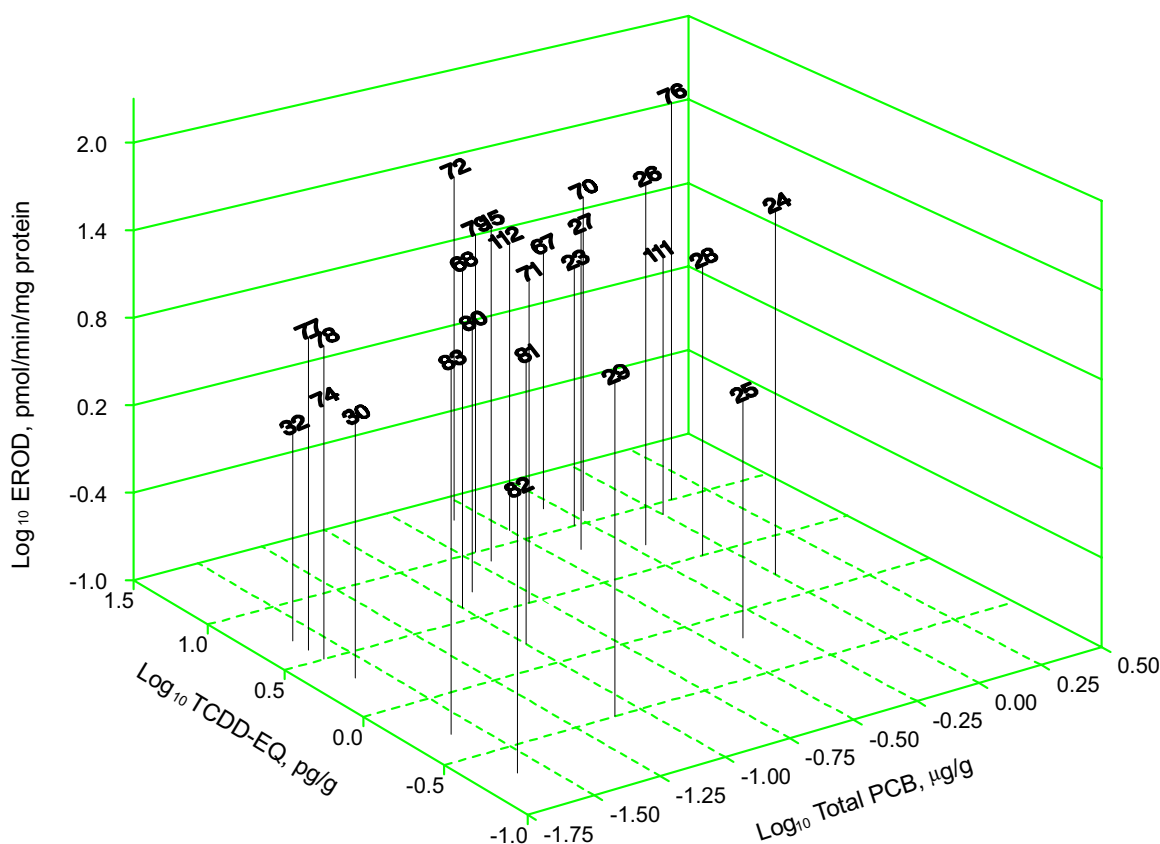


Figure 2-57. Total PCBs, H4IIE bioassay-derived TCDD-EQ, and EROD activity in bass at indicated NCBP stations. Each point represents a station mean. Censored values are represented by 50% of LOD. See Table 1-1 for station descriptions.

million L of crude oil entered the Gasconade 40 km upstream of its confluence with the Missouri (Poulton and others, 1997). Our finding of elevated EROD activity in bass, but not carp, from Station 83 suggests that oil from this spill remained present in the lower reaches of the Gasconade River through 1995. This finding also supports the use of the 3-pronged approach for documenting the exposure of fish to planar organic contaminants and differentiating the class or classes of compounds present.

The relationships among hepatic EROD activities, total PCB concentrations, and TCDD-EQ in carp indicated a much stronger dependence of EROD and TCDD-EQ on PCBs (Fig. 2-57) than in bass, which is consistent with the greater sensitivity of EROD in carp. This also suggests that PCBs were an important component of observed AhR-related toxicity in these taxa. Hepatic EROD activity in carp was correlated with both TCDD-EQ ($r=0.35$, $P<0.01$) and

total PCBs ($r=0.32$, $P<0.05$; both with sexes combined). The only samples that did not follow this general pattern of increasing EROD and TCDD-EQ with total PCBs were those from the MSE NAQWA Study Unit. In particular, carp from Stations 201, 202, 203, 204, 207, and 208 had elevated TCDD-EQ and EROD that were not accounted for by total PCB concentrations, which were uniformly low (Fig. 2-57). The absence of PCBs is not surprising in that these sites are all located in rural areas of the Mississippi Delta. Subsequent high-resolution GC-MS analysis of these samples revealed very low concentrations (<9 pg/g) of PCDD and PCDF congeners. When multiplied by their respective toxic equivalency factors (TEFs) relative to TCDD (Whyte and others, 1998) and summed, the totals were sufficient to account for most of the elevated EROD and TCDD-EQ activity in carp from some, but not all, of the MSE sites (USGS, Columbia Environmental Research Center,

Table 2-7. Results of regression analyses in which the linear model $\log(\text{EROD}) = b_0 (\text{intercept}) + b_1 [\text{Gender (coded male=1, female=0)}] + b_2 \log(\text{Total PCB}) + b_3 \log(\text{TCDD-EQ}) + b_4 [\log(\text{Total PCB}) * \log(\text{TCDD-EQ})]$ was fit to the data for carp and bass from the NCBP sites and for carp from NAWQA sites in the MSE Study Unit. For individual model terms the Type-III (order-independent) parameter estimates and their associated standard errors, F -values, and P -values are shown. In these analyses, Total PCB and TCDD-EQ were as measured in composite samples (by station, taxon, and gender) and EROD was represented by the geometric mean of the individual fish comprised by each respective composite sample. df , degrees-of-freedom.

Taxon, stations	Total df	R^2	MS_{Error}	Source	df	F	P	Parameter estimate	SE
Carp, MSE sites	13	0.35	0.130	Model	4	1.19	>0.05	--	--
				b_0	--	--	--	2.206	1.440
				b_1	1	0.19	>0.05	-0.182	0.416
				b_2	1	1.55	>0.05	-0.756	0.608
				b_3	1	1.41	>0.05	1.094	0.922
Carp, NCBP sites	64	0.51	0.106	b_4	1	1.54	>0.05	-0.564	0.454
				Model	4	15.42	<0.001	--	--
				b_0	--	--	--	4.327	1.301
				b_1	1	1.42	>0.05	0.100	0.084
				b_2	1	8.97	<0.01	2.412	0.801
Bass, NCBP sites	51	0.08	0.116	b_3	1	10.84	<0.01	8.248	2.505
				b_4	1	9.75	<0.01	4.878	1.562
				Model	4	1.06	>0.05	--	--
				b_0	--	--	--	2.621	3.865
				b_1	1	0.19	>0.05	0.042	0.097
				b_2	1	0.14	>0.05	0.919	2.419
				b_3	1	0.22	>0.05	2.137	4.538
				b_4	1	0.19	>0.05	1.246	2.834

unpublished data). These latter finding suggested the presence of one or more additional PHHs in samples from several of these sites.

We used multiple linear regression to further investigate relations between hepatic EROD activity, total PCBs, and TCDD-EQ (Figs. 2-56, 2-57). As noted in the preceding paragraph, visual inspection of the data indicated that EROD in carp and bass from the NCBP sites in the MRB and in carp from the NAWQA sites in the MSE and EIB Study Units were responding differently. We therefore analyzed carp from the NCBP and MSE sites and bass from the NCBP sites with the following linear model: $\log(\text{EROD}) = b_0 (\text{Intercept}) + b_1 [\text{Gender (coded male=1, female=0)}] + b_2 \log(\text{Total PCB}) + b_3 \log(\text{TCDD-EQ}) + b_4 [\log(\text{Total PCB}) * \log(\text{TCDD-EQ})]$. In these analyses, as in the correlation analyses described previously, EROD was represented by the geometric mean of the individual fish in each composite sample. Fish gender was included as a dummy variable (1, 0) to account for among-sex differences. Small sample numbers precluded regression analyses of carp from the EIB Study Unit and bass from the MSE Study Unit. The regression model accounted for 51% of the observed variability in EROD activity in carp from NCBP sites and was highly significant ($P < 0.001$; Table 2-7). This is a surprisingly large percentage considering the wide range of PCB concentrations, sources, and congener mixtures represented by the samples from these geographically dispersed sites. The Type-III parameter estimates for b_2 , b_3 , and b_4 , but not b_1 , were also highly significant ($P < 0.01$; Table 2-7). It is important to note that the Type-III estimates are very conservative because they measure the reduction in the unexplained sum-of-squares accounted for by each term after all other terms in the model have been fit (that is, independent of the order in which the model is specified). The Type-I parameter estimates (fit in the order indicated in the model identified above; data not shown) for carp from NCBP sites indicated a significant gender effect (b_1 , $P < 0.01$), but the linear term for Total PCBs (b_2) was not significant ($P > 0.05$). For carp from the MSE NAWQA sites the model also accounted for 35% of the observed variability in EROD activity, but neither it nor any of the Type-I or -III parameter estimates differed significantly from zero ($P > 0.05$, Table 2-6). Although this finding suggests that EROD in these fish was responding to factors other than fish gender, total PCB, and TCDD, it should be noted that the sample size was small ($n=14$; 9 error DF).

In contrast to carp from the NCBP sites, the regression model for bass (52 samples) accounted for only 8% of the variability in EROD activity, and neither the overall model nor any of the individual Type-

III parameter estimates were significant ($P > 0.05$, Table 2-6). However, the Type-I estimate for b_3 was marginally significant ($P = 0.086$), suggesting a weak effect attributable to TCDD-EQ. Collectively, the results of these analyses support the interpretations we presented. Hepatic EROD in carp is more sensitive to the effects of PHH and PAH than in bass. Consequently, PCB and TCDD-EQ concentrations at the NCBP sites are above the threshold for EROD induction in carp, but not bass, and these contaminant classes accounted for about half of the observed variability in EROD. The other half was presumably attributable to PAH and other factors not included in the model. At the MSE NAWQA sites it appears that other factors, including other contaminants, were involved. However, it should be noted that only 14 samples from seven sites were included in the MSE model, with only nine error degrees-of-freedom. In addition, factors such as reproductive stage and related variables (for example, estradiol levels) were not included in any of the models.

Among the factors not accounted for in any of our analyses is temperature. The 1995 MRB fish were collected at temperatures ranging from $>30^\circ\text{C}$ (measured) at several MSE sites during late August (Stations 201-204) to $<15^\circ\text{C}$ (estimated) at Stations 68 (sampled several times, as late as November 28) and 29, 78, 79, and 82 (sampled November 28-December 6—Table 1-1). In marine fishes, temperature compensation (greater rates of hepatic EROD activity at lower temperatures) has been reported (Sleiderink and others, 1995; Lange and others, 1998; reviewed by Whyte and others, 2000) over the range of $10\text{--}20^\circ\text{C}$. In freshwater fishes, no such effect has been reported; enzyme activity generally seems to increase with temperature (Whyte and others, 2000). Consequently, some of the EROD variability and differences among sites we noted may reflect temperature effects.

Carp appear to be among the most sensitive fish species towards the effects of dioxin-like chemicals, especially compared to largemouth bass (Kleeman and others, 1988). The greater sensitivity of carp compared to largemouth bass may therefore explain the generally more consistent association between total PCBs and hepatic EROD activity in carp (Fig. 2-57). Total PCB concentrations in our carp samples (Fig. 2-44) lie within the response range for EROD induction and these variables are correlated with each other. This correlation was more evident when the samples from the sites in the MSE Study Unit, in which TCDD-EQ and hepatic EROD activity appear to be responding to AhR agonists other than PCBs, were excluded from the computations; a statistical model that included Total PCB and TCDD-EQ terms accounted for more than 50% of EROD

variability in carp from NCBP sites (Table 2-7)

SUMMARY AND CONCLUSIONS

With few exceptions, 1995 concentrations of most contaminants measured in fish were low. Although DDT-derived residues (mostly as *p,p'*-DDE) were detected at all of the stations sampled, potentially toxic (to fish-eating wildlife) concentrations (>1.0 $\mu\text{g/g}$) occurred only in fish from sites in the MSE NAWQA Study Unit (Stations 201-204) and at NCBP Station 80 (Yazoo R., MS). These sites are all in the Lower Mississippi valley and drain watersheds farmed extensively for cotton. However, even at these sites little or no *p,p'*-DDT was detected, indicating the continued weathering of residual DDT rather than the input of new material. Concentrations of DDT were also low at NCBP sites historically influenced by point-sources of contamination (Stations 28 and 71). Similar to DDT, toxaphene was present in more than trace quantities only at sites in the Lower Mississippi region (Stations 201-204 and 80). Because of the historically heavy use of DDT and toxaphene on cotton, average concentrations were greatest in the LMS sub-basin and MSE Study Unit. Mirex was also used extensively only in the South, against red imported fire ants. Consequently, mirex residues were detected (traces) at only two sites in Louisiana (Stations 81 and 204); at Station 81, concentrations were higher in 1995 than in 1986.

Cyclodiene insecticides were present at fewer stations (70%) than DDT, but relatively high concentrations were more widely distributed. Although lower than levels reported in the past, elevated concentrations of one or more cyclodiene insecticide residues (dieldrin, endrin, and chlordane-heptachlor) were present at sites in all sub-basins except the ARR and UMO. Concentrations were generally highest in the EIB Study Unit (Stations 205, 210, and 211) and at most of the NCBP sites draining the cotton and corn-producing regions of the central part of the MRB, which encompasses parts of the UMR, LMR, OHR, and LMO sub-basins. Cyclodiene pesticide concentrations were especially high at NCBP Station 76, where there are point-sources. Concentrations of all other organochlorine pesticides were very low.

Total PCB concentrations were also generally low. Although detected at 35% of the stations sampled, levels exceeding 1.0 $\mu\text{g/g}$ occurred only in fish from three sites in the OHR sub-basins (Stations 23, 24, and 67) and from one each in the UMR (Station 111) and LMS (Station 76) sub-basins; and average concentrations were correspondingly greatest in these three sub-basins. PCB concentrations were generally lowest in the NAWQA Study Units (EIB and

MSE), which drain primarily agricultural areas, than in the larger rivers represented by the NCBP sites. Similarly, HCB was detected in trace quantities only at two sites in the industrialized OHR sub-basin (Stations 24 and 25) and at Station 76 (Mississippi R. at Memphis, TN). Dioxin-like contaminants, as indicated by the H4IIE rat hepatoma cell bioassay, were detected in all sub-basins except the LMO. Dioxin-like activity was present at greater than background levels in one or more samples from Stations 77-79, in the ARR sub-basin; Station 32, in the UMO sub-basin; Stations 15, 75, 76, and 80, in the LMS sub-basin; Stations 72, 111, and 112, in the UMS sub-basin; Stations 67, 68, 70, and 71, in the OHR sub-basin; Station 206, in the EIB Study Unit; and Stations 202 and 207, and 208, in the MSE Study Unit. At the sub-basin level, TCDD-EQ concentrations were greatest in the OHR, LMS, UMR, and UMO sub-basins and in the MSE Study Unit. At most sites with elevated TCDD-EQ, the dioxin-like activity was reasonably well correlated with total PCB concentrations. At the MSE sites they were not, however, suggesting the presence of one or more other dioxin-like compounds.

Results of the EROD assays confirmed the PCB and H4IIE findings; hepatic EROD rates tended to rise with total PCB and TCDD-EQ levels in both carp and bass. Greatest rates of hepatic EROD activity tended to occur at some of the sites with the highest concentrations of PCBs, TCDD-EQ, or both (that is, Stations 24 and 76). Correlations among EROD activity, TCDD-EQ, and total PCB concentrations were generally better for carp than for bass. A plausible explanation for the stronger correlation in carp is the greater sensitivity of carp than bass to PHH toxicity coupled with the generally low PCB and TCDD-EQ concentrations in the fish, which may not have been sufficient to induce EROD activity in bass at many sites. Hepatic EROD activity was not uniformly correlated with TCDD-EQ and total PCBs, however, suggesting that at some sites elevated rates of hepatic EROD were caused by exposure of the fish to labile contaminants that did not survive the reactive cleanup used to process the samples for the H4IIE bioassay. These include PAHs, exposure to which was indicated at Station 83 (Missouri R. at Hermann, MO), where an oil spill had occurred. In addition, elevated TCDD-EQ and hepatic EROD activity in the near complete absence of PCBs suggested the presence of dioxin-like contaminants in fish from several sites in the MSE Study Unit. Subsequent analyses ruled out PCDDs and PCDFs at most sites, suggesting that other structurally and toxicologically similar, but as yet unidentified, contaminants may be present. There were no other sites at which the combined results of the H4IIE bioassay and EROD analyses suggested the presence of dioxins or related compounds.

Elemental contaminant concentrations were also relatively low. The most notable exception was Se, concentrations of which were high (ca. 5 µg/g) in all samples from Station 77. These samples heavily influenced the mean concentrations for bass in the ARR sub-basin (carp were excluded because of mixed-gender compositing). Slightly elevated concentrations were also present in fish from several sites in the UMO sub-basin. Concentrations of Hg were generally higher in predatory fishes (mostly bass) than in bottom-feeders; levels exceeded 0.3 µg/g in predators from sites in the LMO (Station 83) and LMS (Stations 30, 76, and 81) sub-basins and the MSE Study Unit (Stations 212, and 213). It should be noted, however, that no predators were collected at most NAWQA sites; even so, concentrations were relatively high (>0.3 µg/g) in carp from MSE Station 207. At the sub-basin level, Hg concentrations were greatest in the LMO and LMS sub-basins and MSE Study Unit (bass only); and for carp from the MSE (due to Station 207, as noted). Concentrations of Cd were generally greater in carp than in bass, exceeding 0.2 µg/g only in carp from one site in each of the ARR (Station 78), LMO (Station 90), LMS (Station 30), and UMS (Station 73) sub-basins, and Stations 67 and 70 in the OHR sub-basin. At the sub-basin level, Cd concentrations were correspondingly greatest in the OHR sub-basin (carp only). Pb concentrations were also greatest in carp, but were generally low; they were <0.2 µg/g in carp from all sites except Stations 78 and 79, in the ARR sub-basin; 89, in the LMO; 85, in the UMO sub-basin; 28, in the LMS sub-basin; 73 and 111, in the UMS sub-basin; 24, 25, 67, 68, and 70, in the OHR sub-basin; and 204, in the MSE Study Unit. Mean concentrations were also greatest in the OHR sub-basin.

Comparisons of 1995 concentrations with 1984-86 data revealed that most temporal trends for organochlorine and elemental contaminants at NCBP sites were downward, continuing two-decade trends for accumulative contaminants at these sites. There were several noteworthy increases, however. Overall, Hg concentrations tended to be slightly higher than when these sites were last sampled in 1986; increases in the station means were evident at both Red River stations (81, Alexandria, LA; and 82, Lake Texoma), in carp at Stations 67 (Allegheny R.) and 86 (James R.), and in bass at Stations 25 (Tennessee R.), 30 (White R.), 70 (Ohio R.), and 72 (Wisconsin R.). Concentrations of Cd also increased at Stations 30 (bass) and 67 (carp), and Pb increased at Stations 67 and 70 (carp and bass). Se concentrations at Station 77 (Arkansas R. at John Martin Reservoir, CO) increased slightly, continuing a previously noted trend.

Chapter 3. Fish Health Indicators

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INTRODUCTION

The approach selected for assessing the health of fish species collected for the Mississippi River Basin (MRB) BEST project was to choose indicators on various levels of biological organization—organism, tissue or cellular, and sub-cellular. Fish health information can be placed in four categories: 1) observations of gross (visible to the naked eye) lesions or abnormalities; 2) condition and organosomatic indices; 3) lesions or changes at the cellular (microscopic or histologic) level; and 4) subcellular or soluble disease resistance factors. The biomarkers or methods evaluated in this project represented different levels of complexity and specificity. Observations of gross external abnormalities and condition indices based on body and organ weights are inexpensive, rapid measurements that can be performed in the field by personnel with minimal equipment and training. These indicate responses to chemicals or other stressors at the organ or organism level and often represent an advanced condition. For example, a high incidence of severe gross abnormalities may indicate that a significant impact on the population has already occurred. However, changes at this level can also result from numerous factors other than contaminants, such as

food availability, meteorological or hydrological events, anthropogenic nutrient inputs, and infectious agents. Changes at the cellular or subcellular level are more difficult to evaluate; special handling of tissues, specialized equipment, and personnel with generally advanced training are required, the techniques are not performed in the field so transport of tissue/cells is an issue, and results take longer to generate. Nevertheless, cellular or sub-cellular changes often occur soon after exposure and are subtle, and may therefore precede significant higher-level effects. They may also be diagnostic for grossly observable changes.

Evaluating the prevalence of fish with external gross lesions is the most simplistic necropsy-based assessment. It has been used in a number of large-scale monitoring programs such as the Environmental Monitoring and Assessment (EMAP)-Estuaries Program of the USEPA (Fournie and others, 1996) and the Status and Trends Program of the National Oceanographic and Atmospheric Administration (Long and Morgan, 1990; O'Connor and Ehler, 1991), as well as in many smaller-scale state and regional studies. In most of these studies a higher prevalence of external lesions was found in fish from contaminated sites when compared to less-impacted areas. Most of these programs have concentrated on marine or estuarine environments, however, and few large-scale

studies in freshwater environments have included a standardized assessment of external lesions. A number of smaller-scale studies, especially in tributaries of the Great Lakes, have reported a higher incidence of lip papillomas and hepatic tumors at heavily impacted freshwater sites (reviewed by, Baumann and others, 1991). Assessment of external abnormalities is also part of the index of biotic integrity (IBI) that has been used extensively in freshwater systems (Karr, 1981; Leonard and Orth, 1986).

The necropsy-based procedure used in this study (Schmitt and others, 1999a; Blazer, 2000) was modified from a fish health/condition system developed for use by fisheries personnel at the field level (Goede, 1989; Goede and Barton, 1990). In this procedure both internal and external grossly visible changes are observed, and the results obtained can be used to compute a variety of indices with which to compare populations either spatially or temporally. Among these is the health assessment index (HAI), a modification of the Goede (1989) system developed to provide a more quantitative basis for comparing populations. To compute the HAI, numerical ratings are assigned to the gross observations (Adams and others, 1992; 1993; Blazer, 2000). The necropsy-based methods were developed to provide condition profiles of populations and are best suited to documenting changes over time in the health of a population.

Indices such as condition factor (CF), hepatosomatic index (HSI) and splenosomatic index (SSI) are commonly used as indicators of growth and health by fishery biologists (Dethloff and Schmitt, 2000). These indices are based on body length and weight and organ weight and are amenable to field use. A decline in CF (weight/length^3) is generally interpreted as depletion of energy stores that may reflect differences in feeding behavior or increased metabolism due to stress (Goede and Barton, 1990). Condition factor can fluctuate seasonally (Adams and others, 1982) and throughout physiological development and sexual maturation (Denton and Yousef, 1976), however, and may vary among locations within a species (Doyon and others, 1988). Relative liver size, that is, hepatosomatic index (HSI), has also been reported to decline in response to reduced food intake or starvation (Adams and others, 1982), season (Delahunty and de Vlaming, 1980) and, like CF, physiological development and sexual maturation (Bulow and others, 1978). A reduction in HSI has been reported in fish stressed by handling, altered water flow, acidity, and exposure to certain chemicals (reviewed by Goede and Barton, 1990). In contrast, increased HSI may result from exposure to toxicants that cause hypertrophy or hyperplasia of hepatocytes (Slooff and others, 1983; Everaarts and others, 1993).

Immunotoxicology is a relatively new and

emerging branch of environmental toxicology. In the early 1970s it became apparent that chemicals known to be present in the environment could compromise immunity in animals. In the 1980s it was confirmed that a variety of environmental contaminants such as toxaphene (Allen and others, 1983), lead and polychlorinated biphenyls (Koller and others, 1983), and pentachlorophenol (Kerkvliet and others, 1982) produced immunosuppression at dosages lower than those that altered other known and commonly used toxicological endpoints (Koller, 1996). Hence, it was recognized that the immune system is very sensitive to chemical exposure and may be affected well before other functions. The immune system of fishes has been shown to be as sensitive as that of homeotherms to a variety of environmental contaminants (Weeks and others, 1992; Zelikoff, 1994; Wester and others, 1994). For this reason there is strong interest in incorporating measures of immunity and disease resistance for fish into the overall organism health assessments conducted as part of the BEST program. It is important to note, however, that this represents an emerging area of aquatic toxicology, and both the methods and the significance that can be attached to their results are developing rapidly.

The most relevant endpoint for immune system dysfunction is altered host resistance, which can lead to infectious (bacterial, viral, fungal, parasitic) or noninfectious (neoplasia) diseases. For this reason signs of infectious disease, either gross or histological, are noted in internal and external observations and during histological examinations. Generally, assessment of immune system function requires harvesting live cells (macrophages or lymphocytes), maintaining them at optimal conditions in sterile, liquid media, and performing functional assays within a short period of time. Most of the functional assays require sterile techniques, sophisticated equipment, and specially trained personnel, and often utilize radiolabeled substrates. These types of assays have been used in field studies of limited scope; however, they are not logistically feasible in large-scale endeavors such as the BEST program. Weeks and others (1992) suggested a tiered approach for use in screening or comprehensive analysis of immunomodulatory effects of chemicals on aquatic organisms. Organosomatic indices, particularly splenosomatic index (SSI), histology of spleen and lymphoid tissue, lysozyme activity and macrophage aggregate analyses were all included in tier 1. These tier-1 methods were chosen for the BEST program (Schmitt and Dethloff, 2000) because they can be performed in the field (SSI) or on cryogenically frozen plasma or serum (lysozyme) or on preserved tissue (histology of lymphoid tissue; macrophage aggregate parameters).

Pigment-bearing macrophages are a

prominent feature in fish spleen, kidney, and sometimes liver (Agius, 1980). In advanced teleosts they form discrete aggregations called macrophage aggregates (MA) or melanomacrophage centers. Macrophage aggregates are believed to be functional equivalents of germinal centers, active in centralization of foreign material and cellular debris for destruction, detoxification or reuse, storage of exogenous and endogenous waste products, the immune response, and iron storage and recycling (Ferguson, 1976; Ellis and others, 1976). Occurrence of MA in selected tissues may vary depending on the size, nutritional status, or health of a particular fish (Agius, 1979; 1980; Agius and Roberts, 1981; Wolke and others, 1985). In addition, the number, size or both of MA increase with age, at least in some fish (Brown and George, 1985; Blazer and others, 1987). In spite of the variety of factors known to influence MA parameters (Blazer and Dethloff, 2000) this histologic as well as potential immune system biomarker, although quite general, has been shown through both field and laboratory studies to respond to exposure of fish to a variety of contaminants (reviewed by Wolke, 1992; and Blazer and others, 1997). In addition, these measurements are logistically reasonable to accommodate in a suite of health assessment indicators because preserved tissues are collected as part of the overall procedure (Schmitt and others, 1999a).

Lysozyme is an enzyme believed to be an important component of the nonspecific humoral disease resistance mechanisms of fish (Yano, 1996). It is a disease resistance factor that has been studied for many years, particularly in relation to infectious diseases and vaccination of cultured fishes (Blazer and Dethloff, 2000). Seasonal, sexual, species, strain, and age-dependent variations in plasma lysozyme activity have been reported (Fletcher and White, 1976; Fletcher and others, 1977; Studnicka and others, 1986; Lie and others, 1989; Røed and others, 1993). More recently there has been some interest in using this assay as an indicator of environmental stress, but its use in either field studies or contaminant-related laboratory studies has been limited. General stress, such as that induced by handling and transport, can affect lysozyme activity (Möck and Peters, 1990; Fevolden and others, 1994), but the effects appear to depend on the type or duration of the stress (Möck and Peters, 1990; Røed and others, 1993; Hutchinson and Manning, 1996). In a laboratory study, serum lysozyme activity of dab (*Limanda limanda*) decreased after exposure to oil-contaminated sediments from drilling sites (Tahir and others, 1993). Lysozyme activity also decreased in common carp (*Cyprinus carpio*, hereafter carp) following exposure to the organophosphate insecticide trichlorphon (Siwicki and others, 1990), but was not affected significantly in dab exposed to sewage sludge for 12

weeks (Secombes and others, 1991).

In the following sections of this chapter we describe the methods used to assess the health of fish from the Mississippi River Basin (MRB) and present the results of the fish health indicators described in the preceding paragraphs. Each biomarker is presented and compared at the station, sub-basin and program level. We thus compare fish health at the sites and in the sub-basins, document potential contaminant effects, and evaluate the performance of individual, as well as the suite, of fish health biomarkers. Information about the sites, including the sub-basins and programs of origin, is presented in Chapter 1 of this report. Chapter 1 also contains a detailed description of the field procedures and the statistical methods used to compare and evaluate the results. Further information on the species composition, sizes, and ages of the fish collected at each site is also presented in Chapter 1 and in Appendix A. Raw data for the entire project can be found on the World-wide-web at <http://www.cerc.usgs.gov/data/best/index.htm>.

METHODS AND MATERIALS

Field Procedures

As described in Chapter 1, fish were collected by electrofishing and held alive until they were processed using a procedure similar to the one described by Schmitt and others (1999a). Carp and black bass (*Micropterus* spp., hereafter bass) were the preferred species, with others permitted as necessary (see Chapter 1). Fish were identified to species, measured, weighed, bled by caudal venipuncture, and examined for grossly visible external abnormalities. The abdominal cavity was opened and the internal organs were examined for grossly visible lesions or abnormalities. Lesions or abnormalities were classified and entered onto a data sheet (Schmitt and others, 1999a). The liver of non-cyprinid fishes (that is, all species with discrete livers) and the spleen and gonads of all fishes were removed and weighed. Pieces of liver, spleen, gonad, posterior and anterior kidney, and any grossly visible lesions were placed in plastic containers containing NoToX[®] solution for fixation.

Blood tubes were centrifuged for 10 min @ 3500 rpm. The resulting plasma was aspirated with a sterile transfer pipette into a cryogenic vial and quick-frozen on dry ice. The samples were stored and shipped frozen on dry ice to the USGS-National Fish Health Research Laboratory of the Leetown Science Center (LSC) and stored at -80 °C until assayed.

Laboratory Analyses

External Gross Abnormalities

The information collected in the field was used for two types of necropsy-based assessments: 1) the prevalence of gross external pathological disorders; and 2) a more comprehensive necropsy-based fish health assessment incorporating both internal and external observations. Ratings of present (1) or not present (0) were assigned to each fish based on the occurrence of abnormalities and the proportions were analyzed statistically to compare species, stations, sub-basins and programs for prevalence of external disorders. For consistency with other monitoring programs that have used this type of assessment (for example, Fournie and others, 1996) only certain

observations were included (Table 3-1). These included any visible disorders of the eye (exophthalmia, hemorrhage, opacity, emboli, missing), opercles (shortening, deformities, parasites) and body surface (ulcers, parasites, discolored or raised areas). Severe erosion, emboli, parasites, and deformities of the fins and skeleton were also included.

Fish Health Assessment Index (HAI)

Numerical values were assigned to abnormalities to facilitate comparisons of species, stations, sub-basins, and programs using the more comprehensive necropsy-based fish HAI (Table 3-2). Each fish was assigned a health index by summing values for all organs examined. An index value was only computed for a fish if there were observations for all

Table 3-1. Classification of external lesions.

Organ, condition	Field observation	Classification
Eyes		
Normal	No visible abnormalities	0
Exophthalmic	Protruding or "pop-eye"	1
Hemorrhagic	Reddened within or around the eye	1
Opaque	Cloudiness of the eye; cataracts	1
Emboli	Gas bubbles visible within the eye	1
Missing	Eye appears to be gone; may be healed over	1
Body Surface		
Normal	No visible abnormalities, possibly missing scales, pinpoint reddened areas	0
Tumors	Raised and/or discolored areas on the body surface	1
Lesions	Large reddened areas, ulcerations, or erosions	1
Parasites	Visible parasites	1
Opercles		
Normal	No visible abnormalities	0
Slight shortening	Opercle is slightly shortened; a small area of the gill may be visible	1
Severe shortening	Shortening is severe; a large area of the gill may be exposed	1
Other	Deformity or visible parasite attached	1
Fins		
Normal	No visible abnormalities	0
Mild erosion	Some erosion but no evidence of bleeding or secondary infection	0
Severe erosion	Active erosion with hemorrhage, evidence of secondary infection, or both	1
Frayed	Margins of fins are ragged or torn	0
Hemorrhagic	Reddened, bloody areas within fin	0
Emboli	Gas bubbles visible within fin	1
Other	Deformities, parasites	1

Table 3-2. Necropsy observations and their substituted health assessment index (HAI) values.

Organ	Field observation	Value
Body surface	Normal; no aberrations	0
	Lesions; tumors; parasites; other	30
Fins	Normal	0
	Mild erosion, frayed, hemorrhagic, emboli	10
	Other	10
	Severe active erosion	30
Eyes	No aberration; eyes clear	0
	Opaque, exophthalmic, hemorrhagic, missing	30
	Other; deviation not fitting any above	30
Opercles	Normal	0
	Slight shortening	10
	Severe shortening	30
Gills	Normal; no apparent aberrations	0
	Frayed; tips eroded, ragged	30
	Clubbed; tips swollen	30
	Marginate; distal portion light-colored	30
	Pale; whole filament very light-colored	30
	Other	30
Spleen	Normal; black, very dark red, or red	0
	Nodular; containing fistulas or nodules	30
	Enlarged	30
	Other; aberration not fitting any above	30
Kidney	Normal; firm, dark, flat	0
	Swollen; enlarged or distended	30
	Mottled; gray discoloration	30
	Granular in appearance and texture	30
	Urolithiasis or nephrocalcinosis	30
	Other; aberration not fitting any above	30
Liver	Normal; uniform red or light red color	0
	Fatty liver; “coffee with cream” color	30
	Nodules or cysts in liver	30
	Focal discoloration	30
	General discoloration	30
	Other; deviation not fitting any above	30

components or tissues.

Condition and Organosomatic Indices

Condition and organosomatic indices were computed from the body and organ weights obtained in the field. Condition factor was calculated as $K = \text{body weight} / \text{total length}^3$. Hepatosomatic index or $\text{HSI} = \text{liver weight} / (\text{total body weight} - \text{gonad weight})$

$\times 100$. Splenosomatic index or $\text{SSI} = \text{spleen weight} / (\text{total body weight} - \text{gonad weight}) \times 100$. These “gonad-free” indices were computed to minimize the spatio-temporal variability associated with the reproductive cycle (Dethloff and Schmitt, 2000).

Cellular or Histopathological Analyses - General:

Pieces of tissue fixed in the field were shipped to the

LSC where they were processed for routine histopathological slide preparations (Luna, 1992). Each piece was trimmed into smaller pieces, placed in labeled cassettes, dehydrated through a series of alcohols followed by an organic solvent, and infiltrated with paraffin. Blocks of paraffin containing the tissues were allowed to harden and then cut into sections of approximately 6 μm . Sections were placed on glass slides, allowed to dry, deparaffinized with organic solvent, and stained with hematoxylin and eosin (H&E) for routine evaluation. Sections of liver, spleen, kidney, and gonad, if collected by field personnel, of all the carp and bass were processed and evaluated histologically. In addition, grossly abnormal tissue collected in the field for any fish species was examined. The majority of observations made histologically were rated for extent and severity on a scale of 0 (not present) to 4 (severe).

Macrophage Aggregates

A special staining procedure (Perl's method, Luna, 1992) was used to increase the ease in visualizing MAs and all the pigments within the MAs. With this stain, melanin, the melanosome pigment derived from tyrosine metabolism, is black; hemosiderin, a protein-bound iron pigment, is blue; and ceroid/lipofuscin, lipogenic pigments arising from the oxidation of unsaturated lipids, are yellow-tan. The following macrophage aggregate parameters were measured using a computer-based image analysis system: the number of aggregates in 2 mm^2 of tissue and their mean size. From this information the percentage of tissue occupied by MAs was computed.

Subcellular or Plasma Lysozyme Activity

Measurement of lysozyme activity is based on the lysis of suspensions of the bacteria *Micrococcus lysodeikticus* by serum or plasma. We used the microplate method developed by Tahir and others (1993) as modified by Blazer and others (1996) to analyze plasma. A 0.075% suspension of dried *M. lysodeikticus* was prepared in the appropriate buffer. A 25- μL aliquot of plasma was added (triplicate determinations for each fish) to wells of a 96-well, flat-bottomed microtiter plate. A 175- μL aliquot of the *M. lysodeikticus* suspension was then added and the plate was immediately shaken and read on a kinetic microplate reader at 450 nm every 15 seconds for 5 min. Activity was reported as mOD/min (that is the change in optical density per minute). Preliminary studies with largemouth bass (*Micropterus salmoides*) from the reference site (Station 400) indicated that the pH optimum differed among fish species. We determined that the optimal buffer pH was 5.5 for carp and 6.5 for largemouth bass, which was used for all bass species (*M. salmoides*, *M. dolomieu*, and *M.*

punctatus).

Statistical Analyses

For all biomarkers except external abnormalities, only data for carp and bass were analyzed and reported. Biomarker data for the other species, as well as that for other components of this study, can be obtained from the World-wide Web at <http://www.cerc.usgs.gov/data/data.htm>. All biomarkers reported here were performed on individual fish and were analyzed using statistical methods described in Chapter 1. As noted therein, a nested ANOVA (Keuhl, 1994) was used to compare responses among stations (including the reference site, Station 400); among sub-basins and the reference; and among combined NCBP sites, combined NAWQA sites, and the reference site for measured or computed variables with continuous distributions (CF, HSI, SSI, MA parameters, lysozyme). The HAI, an ordered categorical variable (that is, measured on an ordinal scale) was also analyzed in a nested ANOVA. The presence or absence of external lesions at the fish level is a Bernoulli random variable that becomes a binomial random variable when summed for fish at a station. Fisher's Exact Test was used to compare stations for this endpoint because this test is appropriate for any sample size. Transformations necessary to meet the assumptions of the statistical methods were determined independently for each variable, as described in Chapter 1. A significance level of $\alpha=0.05$ was used for all statistical tests unless otherwise indicated.

The significance of additional explanatory variables was investigated in preliminary regression and correlation analyses; the additional variables included taxon (bass vs. carp) for external abnormalities, gender for all but MA parameters and HSI (genders were separated for HSI investigations without preliminary analysis due the liver's role in vitellogenesis), age for MA parameters, and reproductive stage for CF, HSI, SSI, and lysozyme. There were insufficient numbers of observations for a full evaluation of the effects of these additional variables in combination at each station, with station considered a fixed effect. Exploratory linear ANOVA models tested for the effects of additional variables on endpoints within subsets of data for each species or species/gender combination. Preliminary and final models were tested for carp and bass at stations where $n>1$ for all endpoints except external abnormalities. Station, sub-basin, and program-level differences were evaluated using analysis of covariance (ANCOVA) when preliminary analyses indicated statistically significant relationships with explanatory variable. Final models for CF, HSI, SSI, and lysozyme were restricted to female and male carp and male bass in gonadal stages >0 and female bass in stages 1-3.

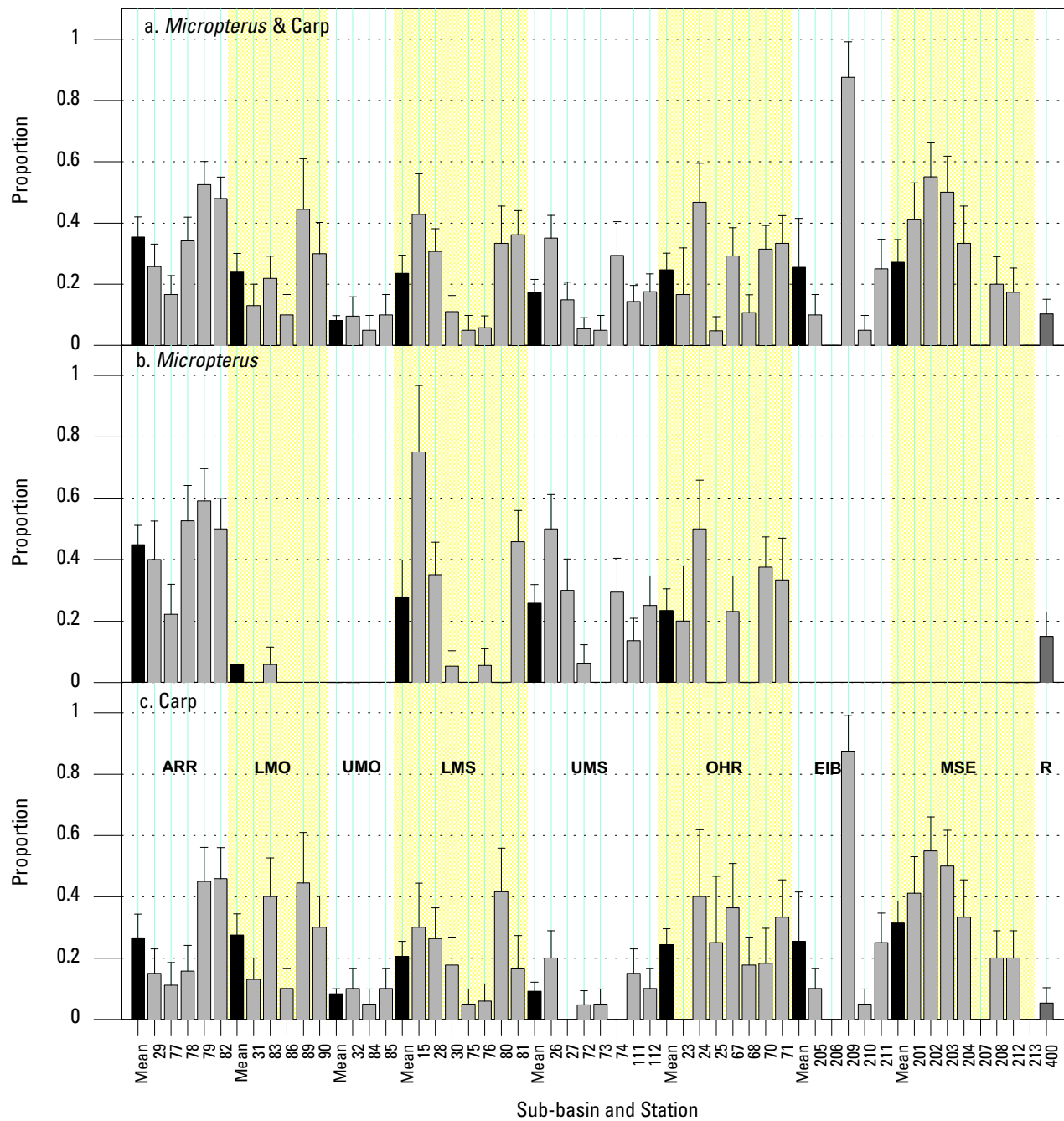


Figure 3-1. Proportion of external lesions in (a) bass and carp, (b) bass only, and (c) carp only, by sub-basin (black bars) and station (gray bars, $n > 1$). Shown are arithmetic means + 1 SE. Sub-basin estimates are based on the station proportions rather than individual fish across the sub-basin. See Table 1-1 and Figure 1-1 for station locations.

Graphics include box plots of individual data points that illustrate the 25th and 75th percentile (box) and the median. Whiskers on these plots extend to the minimum and maximum values. Bar graphs illustrate arithmetic means and standard errors (SE's) except for external lesions where the proportions of fish with lesions, and their respective SE's are represented. Sub-basin means are unweighted and were computed using the means of their respective stations within each sub-basin.

RESULTS

Organism-Level Indicators

Gross Lesions and Abnormalities

External Abnormalities

External Lesions, All Fish: All fish collected (1376 from 48 stations) were included in an initial comparison of external abnormalities (Table 3-3). The proportion of fish with external abnormalities ranged from 0 (Stations 206, 207, 213) to 0.875 (Station 209). At 12 stations (25, 32, 68, 72, 73, 75, 76, 84, 206, 207, 210, 213) <10% of the fish had external lesions, and at another 12 stations (23, 27, 30, 31, 77, 85, 86, 111, 112, 205, 212, 400) external lesions were present on 10-19%. External lesions were observed on 20-29% of the fish from five stations (29, 67, 83, 208, 211); on 30-39% from 10 stations (15, 26, 28, 70, 71, 78, 80, 81, 90, 204); and on 40-49% from four stations (24, 82, 89, 201). At five stations (74, 79, 202, 203, 209) external lesions were present on ≥50% of the fish examined.

Table 3-3 also presents the types of lesions in a sub-basin comparison for all fish. The most commonly observed lesions (17.4% of fish) were on the body surface, most of which were external parasites and reddened or ulcerated areas. A total of 14 fish were rated as having tumors; however, it must be recognized that tumors cannot be diagnosed by the naked eye. Moreover, for most of these (six of nine bass and four of four carp) the tumors were not described, no pieces of tissue collected by field personnel for histopathology, and no diagnosis could be made. Among the tumors that could be diagnosed was a snout tumor on a white sucker (*Catostomus commersoni*) from Station 74 (Mississippi R. at Little Falls, MN; fish 74-31) that was microscopically determined to be a papilloma. The other "tumors" examined histologically were determined to be parasite-induced.

External lesions of all other tissues were observed on less than 10% of the fish. The second

most common observation was abnormalities of the fins (6.2% of fish). Only 2.1% of the fish had any abnormality of the eyes and only 1.2% had opercular lesions (Table 3-3). Opercular abnormalities were observed only at stations in the ARR, LMO, LMS, and UMS sub-basins and at the reference site. Although eye lesions were also relatively rare, only in the two NAWQA Study Units (EIB and MSE) were all eye observations normal (Table 3-3).

For consistency with other sections of this report, statistical analyses were conducted only on the carp and bass data. However, a station summary of external lesions on other species is provided in Table 3-4.

External Lesions on Bass and Carp (Combined): There was no evidence that males and females differed with respect to the proportion of lesions after accounting for differences among stations (carp: $P=0.39$; bass: $P=0.22$, from signed rank test). Furthermore, there was no evidence that carp and bass differed with respect to the proportion of lesions after adjusting for station ($P=0.22$ from signed rank test). For this reason, comparisons were made for carp and bass together in addition to each taxon individually. Of the 1222 carp and bass from 48 stations included in the comparisons, 283 (23.2%) had external abnormalities.

The proportion of carp and bass with external abnormalities ranged from 0 at Stations 206, 207, 213 to 0.875 at Station 209 (Fig. 3-1a). External lesions were present on 1-10% of the carp and bass at 12 stations (25, 32, 72, 73, 75, 76, 84, 85, 86, 205, 210, 400), on 11-20% at 10 stations (23, 27, 30, 31, 68, 77, 111, 208, 112, 212) and on 21-30% at six stations (29, 67, 74, 83, 90, 211). Higher proportions were present at fewer stations; 31-40% at eight stations (26, 28, 70, 71, 78, 80, 81, 204), 41-50% at six stations (15, 24, 82, 89, 201, 203), and >50% at three stations (79, 202, 209). Based on Fisher's Exact Tests with Bonferroni-adjusted P -values, Station 209 was significantly greater than Stations 25, 27, 30, 32, 68, 72, 73, 75, 76, 84, 111, 206, 207, 210, 213 and 400, and Stations 206 and 207 were significantly lower than Stations 79, 82, 202 and 209.

Carp and bass data were available for all sub-basins (Fig. 3-1a). The lowest arithmetic means were those for the reference site and the UMO sub-basin whereas the highest were for the ARR and MSE sub-basins. However, the sub-basins did not differ significantly (ANOVA F -tests of rank-transformed data). In the ARR, LMO, UMO, LMS, UMS, and OHR sub-basins, stations were not significantly different from each other. Within the EIB Study Unit, Station 209 was significantly greater than 206 and 210, and in the MSE Study Unit, Station 202 exceeded Station 207. Although the arithmetic mean for the

Table 3-3. External lesions for all fish, by station and sub-basin. See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Sub-basin and station	n	Lesion location				Total no. w/ lesions	Proportion
		Body	Eyes	Opercles	Fins		
Arkansas-Red R. (ARR)	201	66	4	4	13	74	0.368
29	35	6	1	1	3	9	0.257
77	36	4	0	0	2	6	0.167
78	38	11	0	2	3	13	0.342
79	42	22	0	0	0	22	0.524
82	50	23	3	1	5	24	0.480
Sub-basin proportion	--	0.328	0.020	0.020	0.065	--	0.368
Lower Missouri R. (LMO)	124	12	2	2	13	25	0.202
31	23	0	1	0	3	3	0.130
83	32	5	0	0	2	7	0.219
86	40	2	0	2	1	5	0.125
89	9	1	0	0	4	4	0.444
90	20	4	1	0	3	6	0.300
Sub-basin Proportion	--	0.097	0.016	0.016	0.105	--	0.202
Upper Missouri R. (UMO)	111	9	1	0	0	10	0.090
32	30	2	0	0	0	2	0.067
84	41	2	1	0	0	3	0.073
85	40	5	0	0	0	5	0.125
Sub-basin proportion	--	0.081	0.009	0.000	0.000	--	0.090
Lower Mississippi R. (LMS)	224	35	5	2	14	46	0.200
15	22	8	1	0	4	8	0.364
28	39	8	1	2	1	12	0.308
30	36	1	0	0	3	4	0.111
75	41	2	0	0	0	2	0.049
76	35	1	0	0	2	2	0.057
80	15	5	0	0	0	5	0.333
81	36	10	3	0	4	13	0.361
Sub-basin proportion	--	0.156	0.022	0.009	0.063		0.200
Upper Mississippi R. (UMS)	265	28	12	8	18	54	0.204
26	40	6	2	0	9	14	0.350
27	40	2	2	0	2	6	0.150
72	37	0	1	0	1	2	0.054
73	32	0	0	0	1	1	0.031
74	34	13	6	4	1	18	0.529
111	42	5	0	1	3	6	0.143
112	40	2	1	3	1	7	0.175
Sub-basin proportion	--	0.106	0.045	0.03	0.068	--	0.204

Table 3-3. External lesions for all fish, by station and sub-basin. See Table 1-1 and Figure 1-1 for station and sub-basin locations--Continued.

Sub-basin and station	<i>n</i>	Lesion location				Total no. w/ lesions	Proportion
		Body	Eyes	Opercles	Fins		
Ohio River (OHR)	182	34	5	0	11	42	0.231
23	19	1	0	0	2	3	0.158
24	17	5	3	0	1	8	0.471
25	21	1	0	0	0	1	0.048
67	24	4	1	0	5	7	0.292
68	39	3	1	0	2	3	0.077
70	35	11	0	0	1	11	0.314
71	27	9	0	0	0	9	0.333
Sub-basin proportion	--	0.187	0.027	0.000	0.060	--	0.231
Eastern Iowa Basins (EIB)	88	14	0	0	1	15	0.170
205	20	1	0	0	1	2	0.100
206	20	0	0	0	0	0	0.000
209	8	7	0	0	0	7	0.875
210	20	1	0	0	0	1	0.050
211	20	5	0	0	0	5	0.250
Sub-basin proportion	--	0.160	0.000	0.000	0.023	--	0.170
Mississippi Embayment (MSE)	142	36	0	0	7	40	0.282
201	17	7	0	0	0	7	0.412
202	20	11	0	0	2	11	0.550
203	18	9	0	0	0	9	0.500
204	15	5	0	0	0	5	0.333
207	18	0	0	0	0	0	0.000
208	20	3	0	0	2	4	0.200
212	23	1	0	0	3	4	0.174
213	11	0	0	0	0	0	0.000
Sub-basin proportion	--	0.254	0.000	0.000	0.049	--	0.282
Reference (Station 400)	39	2	1	1	0	4	0.103
Proportion	--	0.051	0.026	0.026	0.000		0.103
Overall	1376	238	29	16	85	310	0.225
Proportion	--	0.174	0.021	0.012	0.062	--	0.225

reference site was only 0.103 compared to 0.243 for NCBP sites and 0.311 for NAWQA sites, these differences were not statistically significant.

External Lesions on Bass: A total of 447 bass from 29 stations were included in the comparisons. Of these 125, or 28%, had some type of external lesion. At the individual station level, the proportion of bass with external lesions ranged from 0 at Stations 25, 32, 68, 80, 212 and 213 to 0.750 at Station 15 (Fig. 3-1b). External lesions were present on 1-10% of the bass at four stations (30, 72, 76, 83), 11-20% at three stations (23, 111, 400), and 21-30% at five stations (27, 67, 74, 77, 112). External lesions were present on 31-40% of the bass from four stations (28, 29, 70, 71), and 41-50% at three stations (24, 26, 81, 82). At two stations (78 and 79) 51-60% of the bass had external lesions, and external lesions were present on three of the four bass collected (75%) at Station 15. Despite the wide range in the proportion of bass with external lesions, there were few statistically significant differences among stations. Station 25, where there were no external lesions in bass, was significantly lower than Stations 78, 79 and 82. In contrast, although no bass from Stations 32, 68, 80, 212 or 213 had external lesions, these stations were not significantly lower than any others nor was Station 15, with the highest proportion, significantly greater than any others.

Bass from seven sub-basins were evaluated for external lesions. However, the LMO and UMO sub-basins contained only one station from which bass were collected, and in the UMO sub-basin only one fish was evaluated. Sub-basin means ranged from 0 in UMO and MSE to 0.448 in ARR (Fig. 3-1b). Despite this range, the differences among sub-basins were not statistically significant. Within sub-basins containing more than one station, no significant differences were found between stations. Program means ranged from 0 for the NAWQA sites (two stations) to 0.213 for NCBP sites, but the differences between these means and with the reference site were not statistically significant.

External Lesions on Carp: Data for external lesions were available for 775 carp from 46 stations. Of these, 158 fish (20.4%) had some type of external lesion. At the station level, the proportion of carp having any external abnormalities ranged from 0 at Stations 23 (only one carp collected), 27, 206, and 207 to 0.875 at Station 209 (Fig. 3-1c). At 12 stations (32, 72, 73, 75, 76, 84, 85, 86, 112, 205, 210, 400) 5-10% of the carp had external lesions, whereas at another 12 stations (26, 29, 30, 31, 68, 70, 77, 78, 81, 111, 208, 212) 11-20% had external lesions. Five stations (15, 25, 28, 90, 211) had 21-30% and another 5 (24, 67, 71, 83, 204) had 31-40% of the carp with external

lesions. There were six stations (79, 80, 82, 89, 201, 203) at which 41-50% of the carp had external lesions and another two (202, 209) with >50%. Station 23, from which only one carp was collected, did not differ significantly from any other station. Stations 27, 206, and 207 were also stations at which no external lesions were noted in carp; for these the proportions were only significantly lower than Stations 209 and 202. Station 209, with the highest proportion (0.875), was significantly greater than 10 stations (27, 72, 73, 75, 76, 84, 206, 210, 207, 400).

Carp data were available from all eight sub-basins and the reference site. Means for the proportion of external lesions ranged from 0.053 at the reference site to 0.398 in the MSE sub-basin. The reference site and UMO sub-basin had the lowest arithmetic means whereas the MSE sub-basin had the highest (Fig. 3-1c); however, there was inconclusive evidence of a difference among sub-basins ($P=0.06$, ANOVA F -test). Within the EIB Study Unit, Station 209 was significantly greater than either 206 or 210, whereas in the MSE Study Unit, Station 202 was greater than 207. No significant differences were found among stations in the ARR, LMO, UMO, LMS, UMS, or OHR sub-basins. Although means for the proportion of external lesions ranged from 0.053 for the reference site to 0.281 for the combined NAWQA sites, differences among reference, NCBP, and NAWQA sites were not statistically significant.

Health Assessment Index (HAI)

HAI in Bass: Based on preliminary statistical analyses there was no evidence that mean HAI differed between sexes, so data were analyzed for all bass (434) at 28 stations. The HAI was found to be non-normal with heterogeneous variance. The data were therefore rank-transformed and analyzed in a nested ANOVA.

Of the 434 bass included in these comparisons, 81 (18.7%) were judged normal—that is, no abnormal organ ratings were recorded. A total of 108 bass (24.9%) received ratings indicating only one abnormal observation (10 or 30) and the same number received ratings indicating two abnormalities (40 or 60); 71 (16.4%) received ratings of 70, 80, or 90, which indicate three or four abnormal observations; and 53 (12.2%) received ratings of 100, 110 or 120 (four or five abnormalities). Only 13 bass (3.0%) received ratings of 130 or higher, indicating ≥ 5 abnormalities (Table 3-5).

At seven stations (23, 72, 74, 77, 111, 212, 213) most fish had one or less abnormal rating. In contrast, at Station 15 three of the four bass examined received ratings of 150, indicating five abnormal organ ratings of 30 (Fig. 3-2a; Table 3-5). Overall, station means for bass HAI ranged from 14 at Station

Table 3-4. External lesions in species other than carp and bass, sorted by station and incidence. See Table 1-1 and Figure 1-1 for station locations.

Station	Taxon	No. lesions	No. fish	Proportion
Sorted by Station				
15	<i>Morone</i>	2	8	0.250
23	Sucker	2	13	0.154
24	Sucker	1	2	0.500
32	Northern pike	0	5	0.000
32	<i>Stizostedion</i>	0	4	0.000
68	<i>Morone</i>	0	6	0.000
68	Sucker	0	2	0.000
68	Sunfish	0	3	0.000
73	<i>Stizostedion</i>	0	12	0.000
74	Sucker	13	17	0.764
75	<i>Morone</i>	1	21	0.048
84	Catfish	0	2	0.000
84	<i>Lota</i>	0	1	0.000
84	<i>Stizostedion</i>	0	1	0.000
84	Trout	2	17	0.118
85	Catfish	3	4	0.750
85	<i>Hiodon</i>	0	13	0.000
85	<i>Stizostedion</i>	0	3	0.000
86	<i>Hiodon</i>	3	20	0.150
Sorted by Increasing Incidence				
32	Northern pike, <i>Stizostedion</i>	0	9	0.000
68	Sucker, sunfish	0	11	0.000
73	<i>Stizostedion</i>	0	12	0.000
75	<i>Morone</i>	1	21	0.048
84	Catfish, <i>Lota</i> , <i>Stizostedion</i> , trout	2	21	0.105
23	Sucker	2	17	0.118
85	Catfish, <i>Hiodon</i> <i>Stizostedion</i> ,	3	20	0.150
86	<i>Hiodon</i>	3	20	0.150
15	<i>Morone</i>	2	8	0.250
24	Sucker	1	2	0.500
74	Sucker	13	17	0.764
All	All	27	154	0.175

Table 3-5. Distribution of bass among HAI scores, by station. See Table 1-1 and Figure 1-1 for station locations.

Station	HAI score																Total bass
	0	10	30	40	60	70	80	90	100	110	120	130	150	160	200		
15	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	4	
23	3	0	1	0	1	0	0	0	0	0	0	0	0	0	0	5	
24	3	0	1	0	2	0	0	0	0	0	3	0	0	0	0	9	
25	6	2	2	4	0	2	0	1	0	0	0	0	0	0	0	17	
26	0	0	0	0	7	2	0	2	6	0	2	1	0	0	0	20	
27	1	0	1	1	3	1	0	8	2	0	2	1	0	0	0	20	
28	1	2	1	8	1	4	0	0	1	1	1	0	0	0	0	20	
29	3	3	4	3	0	0	0	1	0	0	1	0	0	0	0	15	
30	1	0	13	1	4	0	0	0	0	0	0	0	0	0	0	19	
67	5	0	6	1	0	0	0	0	1	0	0	0	0	0	0	13	
68	3	0	1	0	6	0	0	1	0	0	0	0	0	0	0	11	
70	0	0	1	0	9	3	0	7	1	0	2	1	0	0	0	24	
71	0	0	4	2	3	1	0	1	1	0	0	0	0	0	0	12	
72	9	1	4	1	1	0	0	0	0	0	0	0	0	0	0	16	
74	8	0	7	0	1	0	0	1	0	0	0	0	0	0	0	17	
76	4	6	3	2	2	0	0	0	1	0	0	0	0	0	0	18	
77	10	3	1	3	0	1	0	0	0	0	0	0	0	0	0	18	
78	4	0	5	3	4	1	1	1	0	0	0	0	0	0	0	19	
79	0	2	7	1	6	3	0	0	2	0	0	1	0	0	0	22	
80	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2	
81	2	0	4	4	2	1	0	1	7	0	0	1	0	1	0	23	
82	1	0	3	1	4	2	0	7	1	0	4	2	0	0	1	26	
83	0	0	1	0	4	0	0	7	4	0	1	0	0	0	0	17	
111	12	1	5	0	1	1	0	0	0	0	0	0	0	0	0	20	
112	1	0	1	0	6	1	0	2	5	1	2	1	0	0	0	20	
212	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	
213	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	5	
400	1	0	7	2	3	4	0	3	0	0	0	0	0	0	0	20	
Total	81	20	88	38	70	27	1	43	33	2	18	8	3	1	1	434	

77 to 130 at Station 15 (Fig. 3-3a). At the seven stations mentioned above, many of the fish received no abnormal ratings (HAI=0) and, at only three of these stations did any fish have ratings >60 (one each at Stations 74, 77, and 111). At eight stations (15, 26, 27, 70, 81, 82, 83) the mean HAI values were >60, indicating that many bass at these stations received two or more abnormal ratings, and one bass at each of Stations 81 and 82 had HAI ratings >150.

Only one bass was collected in the UMO sub-basin so it was not considered in sub-basin comparisons. Sub-basin means for HAI ranged from 16.5 (MSE) to 83.5 (LMO) (Fig. 3-3a). The LMO sub-basin included only one station at which bass

were collected and the MSE included only two. Statistical testing indicated that the LMO was significantly greater than all other sub-basins. The MSE sub-basin was also significantly lower than the LMS sub-basin and the reference site, but the ARR, LMS, UMS, and OHR sub-basins did not differ significantly nor did any of them differ from the reference site.

The two stations at which bass were collected in the MSE sub-basin did not differ significantly for HAI. Within the ARR sub-basin, mean bass HAI at Station 82 was significantly greater than at Stations 29, 77, and 78 but not at Station 79. Station 77 was significantly lower than Station 79. In the LMS sub-

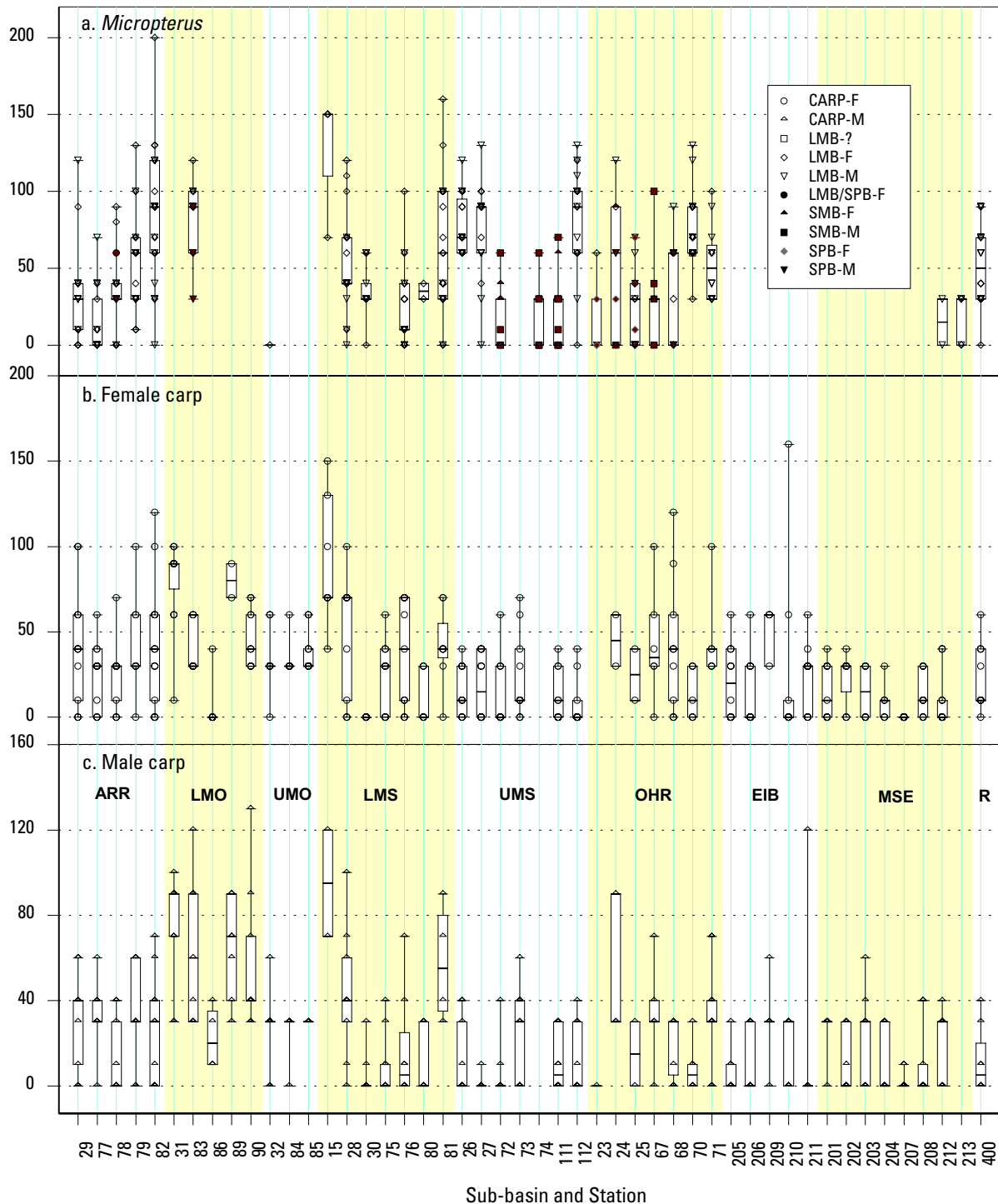


Figure 3-2. Health assessment index (HAI) in (a) bass, (b) female carp, and (c) male carp, by sub-basin and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and the interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

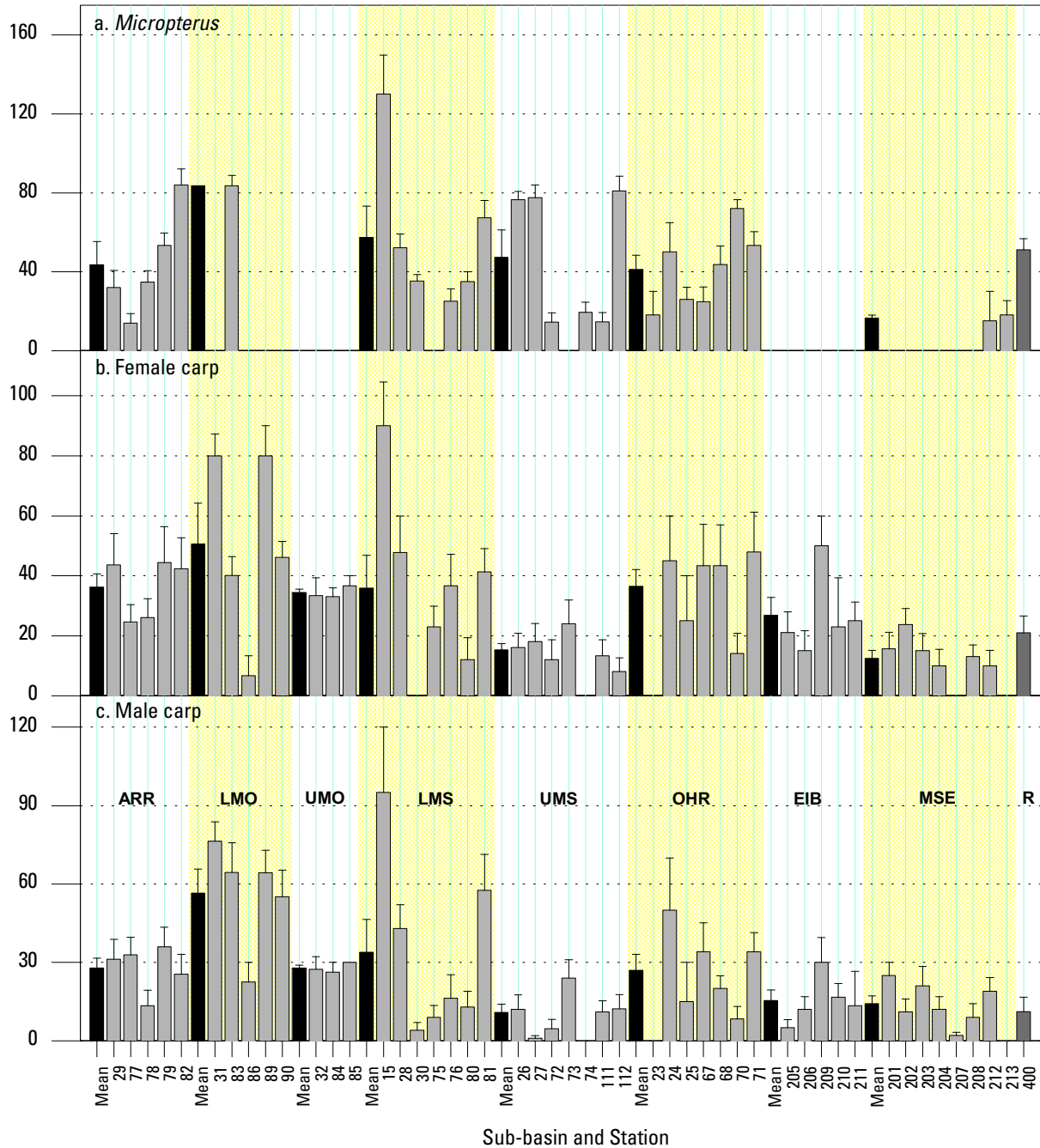


Figure 3-3. Health assessment index (HAI) scores in (a) bass, (b) female carp, and (c) male carp, by sub-basin (black bars) and station (gray bars, $n \geq 1$). Shown are arithmetic means + 1 SE. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

basin, mean bass HAI at Station 15 was significantly greater than at Stations 30 and 76. Station 76 was also significantly lower than Station 81. Bass were also collected at six stations in the UMS sub-basin. Of these, Stations 72, 74 and 111 clustered together and did not differ significantly. Stations 26, 27 and 112 also clustered and were not significantly different from each other; however, HAI at these three were significantly greater than at the other three stations. In the OHR sub-basin, Station 70 had the highest mean HAI and was significantly different from Stations 23, 25 and 67. At the program level bass HAI at the NAWQA sites (MSE only) was significantly lower than either the combined NCBP sites or the reference site, which were not significantly different from each other.

HAI in Carp: A total of 748 carp were examined from 45 stations. Of these, 271 (36.2%) were rated as normal (0 HAI) and 273 (36.5%) received scores of 10-30, indicating one abnormal observation. Of the remainder, 130 (17.4%) received scores of 40-60, 53 (7.1%) received scores of 70-90, and 17 (2.3%) received scores of 100-120. Only four carp (0.4%) received scores above 120. There was strong evidence that HAI in carp differed between the sexes, so males and females were analyzed separately.

HAI in Female Carp: Female carp were collected from 45 stations. The HAI data for female carp were non-normal with heterogeneous variance. Therefore, the data were rank-transformed and analyzed in a nested ANOVA. Of the 374 female carp collected, 119 were rated as normal (HAI=0), 138 (36.9%) received ratings indicating only one abnormality (10 or 30), 76 (20.3%) received ratings indicating two abnormalities (40 or 60), 26 (7%) had ratings of 70-90, 12 (3.2%) had ratings of 100-120, and only three (0.8%) had ratings of 130 or above (Table 3-6). HAI for female carp ranged from 0 at all but 11 stations to 160 at Station 209 (Fig. 3-2b).

Station means for female carp HAI ranged from 0 at Stations 30 and 207 to 90 at Station 15 (Fig. 3-3b). At 16 stations (26, 27, 30, 70, 72, 80, 86, 111, 112, 201, 203, 204, 206, 207, 208, 212) mean HAI was <20, indicating that most female carp at these sites received one or less abnormal rating (Table 3-6). Only two of these stations (72 and 206) contained any fish with ratings >40; one fish from each of these sites had a 60 rating. Only four stations (15, 31, 89, 209) had a mean HAI >60, indicating that most fish at these stations had two or more abnormal ratings. At Station 89 only two female carp were examined and at 209 only three; HAI was >30 for all. At Station 15, all seven fish had ratings of ≥40 and at Station 31, 11 of 12 fish were >40.

At the sub-basin level, mean HAI in female carp ranged from a low of 12.5 (MSE Study Unit) to 50.5 (LMO sub-basin; Fig. 3-3b). At the reference site and in the MSE Study Unit and UMS sub-basin mean HAI was <21, but were not significantly different from each other. The EIB NAWQA sites had a mean HAI of 26.8, which was also not significantly different from the reference site, MSE Study Unit, or UMS. The UMO, LMS, ARR and OHR sub-basins all had mean HAI scores of 30-40 and did not differ significantly from each other. These sub-basins also did not differ from the LMO, where the mean was 50.5. The LMO sub-basin was significantly greater than the UMS sub-basin and the MSE and EIB Study Units. Differences between stations within sub-basins were noted in the LMO sub-basin, where Station 31 was significantly greater than Station 86; and in the LMS sub-basin, where Station 15 was significantly greater than Stations 30 and 80, and where Stations 28 and 81 were significantly greater than Station 30. Mean HAI for female carp at the reference site was not significantly different from either the NAWQA or NCBP sites. Although the mean for NCBP stations was somewhat greater than that for the NAWQA sites, they did not differ significantly.

HAI in Male Carp: A total of 375 male carp was collected at 45 stations; however, because only one male carp was collected at Station 23 this station was not included in the comparisons. Of the 374 male carp included, 152 (40.6%) were rated as normal (HAI=0), 135 (36.1%) had ratings of 10-30, 54 (14.4%) had ratings of 40-60, 27 (7.2%) received ratings of 70-90, and five (1.3%) received ratings of 100-120. Only one male carp received a rating >120 (Table 3-7). Overall, HAI values for male carp ranged from 0 at all but nine stations to 130 at Station 90 (Fig. 3-2c).

Station means for male carp HAI ranged from 1.0 (Station 27) to 95.0 (Station 15) (Fig. 3-3c). At 20 stations (25, 26, 27, 30, 68, 70, 72, 75, 76, 78, 80, 111, 112, 202, 204, 205, 206, 207, 208, 210, 211, 212, 400) the mean HAI score was ≤20, indicating that most male carp at these stations were normal or had only one abnormality. Indeed, at only eight of the 20 stations (26, 72, 75, 76, 78, 112, 208, 212, 400) were there individuals with ratings >30, and all of these were ratings of 40 with the exception of one fish at Station 76 that received a score of 70. Four stations (15, 31, 83, 89) had mean HAI scores >60. Only two male carp were collected at Station 15 and both received scores of 70 or greater. At Station 31, 10 of the 11 male carp collected received scores of 70 or greater. At the remaining 18 stations (24, 28, 29, 32, 67, 71, 73, 77, 79, 81, 82, 84, 85, 86, 90, 201, 203, 209) the mean HAI was in the intermediate ranges of

Table 3-6. Distribution of female carp among HAI scores, by station. See Table 1-1 and Figure 1-1 for station locations.

Station	HAI score												Total female carp
	0	10	30	40	60	70	90	100	120	130	150	160	
15	0	0	0	1	0	3	0	1	0	1	1	0	7
24	0	0	1	0	1	0	0	0	0	0	0	0	2
25	0	1	0	1	0	0	0	0	0	0	0	0	2
26	3	3	3	1	0	0	0	0	0	0	0	0	10
27	5	0	2	3	0	0	0	0	0	0	0	0	10
28	2	1	0	1	0	4	0	1	0	0	0	0	9
29	2	1	1	3	2	0	0	2	0	0	0	0	11
30	7	0	0	0	0	0	0	0	0	0	0	0	7
31	0	1	0	0	2	0	7	2	0	0	0	0	12
32	1	0	6	0	2	0	0	0	0	0	0	0	9
67	1	0	2	1	1	0	0	1	0	0	0	0	6
68	2	1	1	2	1	0	1	0	1	0	0	0	9
70	2	1	2	0	0	0	0	0	0	0	0	0	5
71	0	0	2	2	0	0	0	1	0	0	0	0	5
72	7	0	2	0	1	0	0	0	0	0	0	0	10
73	2	4	1	1	1	1	0	0	0	0	0	0	10
75	4	0	3	2	1	0	0	0	0	0	0	0	10
76	2	2	0	1	1	3	0	0	0	0	0	0	9
77	3	1	4	2	1	0	0	0	0	0	0	0	11
78	2	1	6	0	0	1	0	0	0	0	0	0	10
79	1	0	3	0	2	0	0	1	0	0	0	0	7
80	3	0	2	0	0	0	0	0	0	0	0	0	5
81	1	0	1	4	0	2	0	0	0	0	0	0	8
82	3	1	2	2	3	0	0	1	1	0	0	0	13
83	0	0	4	0	2	0	0	0	0	0	0	0	6
84	0	0	9	0	1	0	0	0	0	0	0	0	10
85	0	0	8	2	2	0	0	0	0	0	0	0	12
86	5	0	0	1	0	0	0	0	0	0	0	0	6
89	0	0	0	0	0	1	1	0	0	0	0	0	2
90	0	0	4	2	2	2	0	0	0	0	0	0	10
111	4	2	2	1	0	0	0	0	0	0	0	0	9
112	7	1	1	1	0	0	0	0	0	0	0	0	10
201	4	1	3	1	0	0	0	0	0	0	0	0	9
202	2	0	5	1	0	0	0	0	0	0	0	0	8
203	4	0	4	0	0	0	0	0	0	0	0	0	8
204	2	2	1	0	0	0	0	0	0	0	0	0	5
205	4	1	2	2	1	0	0	0	0	0	0	0	10
206	6	0	3	0	1	0	0	0	0	0	0	0	10
207	8	0	0	0	0	0	0	0	0	0	0	0	8
208	3	4	3	0	0	0	0	0	0	0	0	0	10
209	0	0	1	0	2	0	0	0	0	0	0	0	3
210	7	1	0	0	1	0	0	0	0	0	0	1	10
211	3	0	5	1	1	0	0	0	0	0	0	0	10
212	6	2	0	2	0	0	0	0	0	0	0	0	10
400	1	6	1	2	1	0	0	0	0	0	0	0	11
Total	119	38	100	43	33	17	9	10	2	1	1	1	374

Table 3-7. Distribution of male carp among HAI scores, by station. See Table 1-1 and Figure 1-1 for station locations.

Station	HAI score										Total male carp
	0	10	30	40	60	70	90	100	120	130	
15	0	0	0	0	0	1	0	0	1	0	2
24	0	0	2	0	0	0	1	0	0	0	3
25	1	0	1	0	0	0	0	0	0	0	2
26	6	1	1	2	0	0	0	0	0	0	10
27	9	1	0	0	0	0	0	0	0	0	10
28	1	1	1	4	1	1	0	1	0	0	10
29	2	1	1	3	2	0	0	0	0	0	9
30	8	1	1	0	0	0	0	0	0	0	10
31	0	0	2	0	0	2	6	1	0	0	11
32	2	0	8	0	1	0	0	0	0	0	11
67	1	0	2	1	0	1	0	0	0	0	5
68	2	1	5	0	0	0	0	0	0	0	8
70	3	2	1	0	0	0	0	0	0	0	6
71	2	0	4	2	0	2	0	0	0	0	10
72	9	1	0	1	0	0	0	0	0	0	11
73	4	0	2	3	1	0	0	0	0	0	10
75	6	2	1	1	0	0	0	0	0	0	10
76	4	2	0	1	0	1	0	0	0	0	8
77	1	0	3	2	1	0	0	0	0	0	7
78	5	1	1	2	0	0	0	0	0	0	9
79	2	0	4	0	4	0	0	0	0	0	10
80	4	0	3	0	0	0	0	0	0	0	7
81	0	0	1	1	0	1	1	0	0	0	4
82	4	1	2	2	1	1	0	0	0	0	11
83	0	0	3	1	1	0	3	0	1	0	9
84	1	0	7	0	0	0	0	0	0	0	8
85	0	0	8	0	0	0	0	0	0	0	8
86	0	2	1	1	0	0	0	0	0	0	4
89	0	0	1	1	1	2	2	0	0	0	7
90	0	0	2	5	0	1	1	0	0	1	10
111	5	2	3	0	0	0	0	0	0	0	10
112	5	1	2	1	0	0	0	0	0	0	9
201	1	0	5	0	0	0	0	0	0	0	6
202	5	1	3	0	0	0	0	0	0	0	9
203	4	0	3	1	1	0	0	0	0	0	9
204	6	0	4	0	0	0	0	0	0	0	10
205	7	2	1	0	0	0	0	0	0	0	10
206	6	0	4	0	0	0	0	0	0	0	10
207	8	2	0	0	0	0	0	0	0	0	10
208	7	1	0	2	0	0	0	0	0	0	10
209	1	0	3	0	1	0	0	0	0	0	5
210	4	0	5	0	0	0	0	0	0	0	9
211	8	0	0	0	0	0	0	0	1	0	9
212	4	0	5	1	0	0	0	0	0	0	10
400	4	2	1	1	0	0	0	0	0	0	8
Total	152	28	107	39	15	13	14	2	3	1	374

21-58 (Table 3-7).

Sub-basin means for HAI in male carp ranged from 10.8 in the UMS sub-basin to 56.5 in the LMO (Fig. 3-3c). Sub-basin means for the reference site, UMS sub-basin, and EIB and MSE Study Units were all ≤ 20 and did not differ significantly. The LMO sub-basin had the highest mean HAI for male carp (56.5) and was significantly greater than all other sub-basins and the reference site. The LMS sub-basin had the second highest mean HAI at 33.9; the LMS exceeded significantly the UMS sub-basin and the EIB and MSE Study Units, but did not differ significantly from the ARR, UMO, or OHR sub-basins or the reference site. Only within the LMS sub-basin did stations differ significantly from each other; HAI at Station 30 was significantly lower than at Stations 15, 28 and 81. At the program level, the reference site and NAWQA (MSE and EIB) means were both < 20 and not statistically different from each other; however, the mean for the NCBP sites (30.6) was significantly greater than the NAWQA mean.

Condition and Organosomatic Indices

Condition Factor (CF)

CF in Bass: There was moderate statistical evidence that CF differed between male and female bass. Consequently, CF was analyzed separately for the two genders.

CF in Female Bass: CF values for female bass were available for 25 stations. Preliminary statistical results indicated that analysis of differences in means required adjustment for gonadal stage. No data were available from Stations 212 and 213 and female bass from Stations 32 and 80 did not meet the criteria for inclusion ($n > 1$).

Across stations, individual CF values ranged from 0.80 at Station 67 to 2.35 at Station 77 (Fig. 3-4a). Ten female bass had CF values < 1.0 ; five of these were from Station 67, two were from Station 23, and one was from each of Stations 68, 72, and 82. Six female bass had CF values > 2.0 ; of these, four were collected at Station 77 and one each at Stations 76 and 78. For female bass, 92% of the CF values were between 1.0 and 2.0. All values at Stations 15, 23, 67, 68 (without Stage 0), 70, 72, and 400 were < 1.5 whereas all values at Stations 27, 77, 78, and 112 were > 1.5 . Female bass excluded from the statistical analysis but present in the box plot were Stage-0 fish from Stations 68, 74, 76, and 81; Stage-4 fish from Stations 32, 67, and 74; and fish for which no stage information was available from Stations 79, 111, and 112. A Stage-1 female from Station 80 was excluded.

Arithmetic station means for CF in female bass ranged from 0.92 at Station 67 to 2.00 at Station 77 (Fig. 3-5a). Stations 68 and 71 had relatively large standard errors. The statistical comparisons indicated the following significant differences among stage-adjusted station means: Station 77 was greater than all other stations whereas Station 67 was exceeded by all stations except 23 and 68. Stations 15, 76, 79, 82, and 111 were all lower than Stations 26, 27, 78 (except 15), and 112; Stations 26, 27, 28, 78, 83, and 112 all exceeded Stations 25, 68, 70, 72, and 400. The mean for Station 23 was lower than that at Stations 26, 27, 28, 29, 74, 78, 83, and 112; and, the mean for Station 30 was lower than that for Station 27.

The reference site mean for female bass CF (1.19) was exceeded by all sub-basin means (largest mean = 1.58 for the ARR sub-basin; Fig. 3-5a). Statistical comparisons indicated that the OHR sub-basin and the reference site were both significantly lower than all other sub-basins, but did not differ from each other. Also, the LMS sub-basin was significantly lower than the ARR sub-basin. The five lowest CF station means were for sites in the OHR sub-basin and at the reference site, whereas the six greatest were in the UMS and ARR sub-basins.

Differences between stations in the same sub-basin were found in only two sub-basins (Fig. 3-5a). Within the UMS sub-basin, Stations 72 and 111 were significantly lower than Stations 26, 27 and 112. Also in the UMS sub-basin, Station 74 (smallmouth bass only) was greater than Stations 72 and 111 but lower than Stations 26, 27, and 112. In the OHR sub-basin, Station 67 was significantly lower than Stations 24, 25, 70, and 71. Note that like Station 74, only smallmouth bass were collected at Stations 67, 72, and 111. So, within these two sub-basins (UMS and ARR), the stations with the lowest mean CF are those where only smallmouth bass were collected. At the program level, data for CF in female bass were only available from the reference site (mean = 1.19) and NCBP sites (mean = 1.45), which differed significantly.

CF in Male Bass: In contrast to CF in females, no gonadal stage effect was detected by preliminary statistical analysis, so no further adjustment was necessary. Values of CF for male bass were available for 23 stations. No data were available from Stations 15, 32, 80, 212, and 213 and Station 23 did not meet the criteria for inclusion ($n > 1$).

Across stations, individual CF values for male bass ranged from 0.34 at Station 28 to 2.47 at Station 79 (Fig. 3-4b). Four fish had CF values < 1.0 ; these were found at Stations 28, 67, and 400. Nine males had CF values > 2.0 ; one was from Station 79 and eight were from Station 77. For male bass, 93% of the CF values were between 1.0 and 2.0. All values

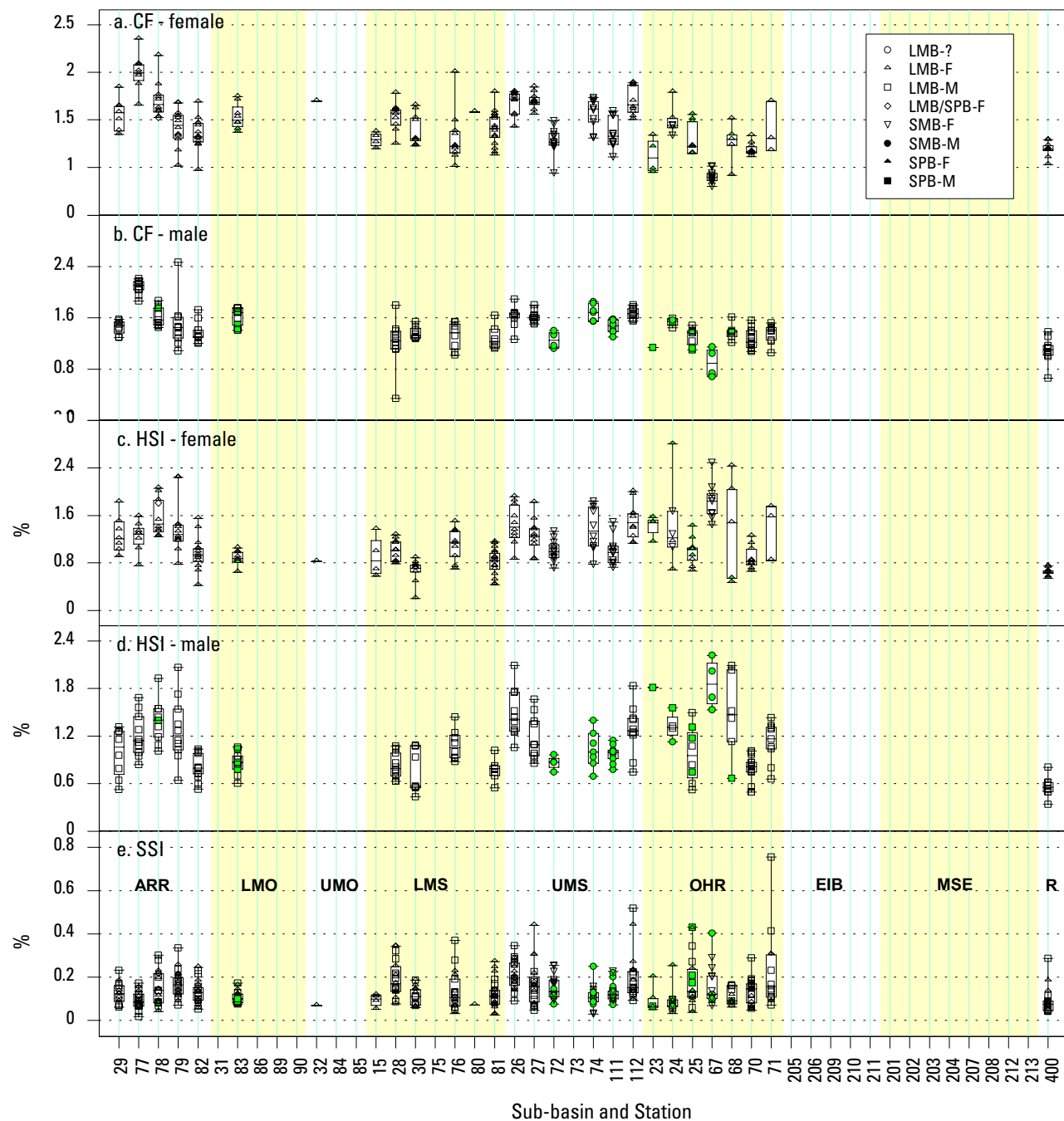


Figure 3-4. Condition factor (CF) in female bass (a), CF in male bass (b), hepato-somatic index (HSI) in female bass (c), HSI in male bass (d), and spleno-somatic (SSI) in bass (e), by sub-basin and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and the interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

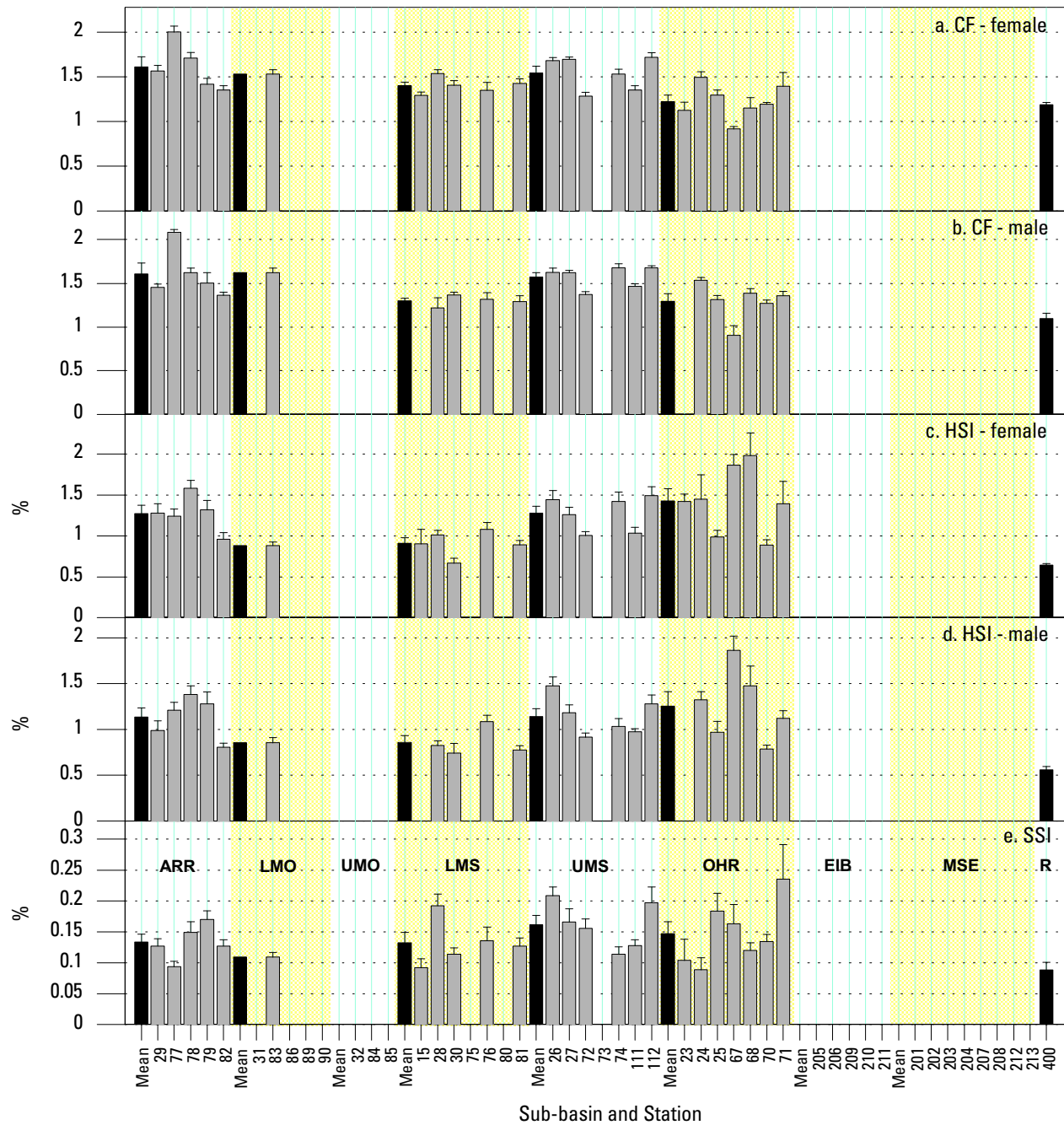


Figure 3-5. Condition factor (CF) in female bass, (b) CF in male bass, (c) hepato-somatic index (HSI) in female bass, (d) HSI in male bass, and (e) spleno-somatic index in bass, by sub-basin (black bars) and station (gray bars, $n > 1$). Shown are arithmetic means + 1 SE. Fish in gonadal stages 0 or 4 were not included in the computations. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

at Station 77 were >1.65 , with eight >1.95 . All CF values at Stations 27, 74, and 112 were also >1.5 . Stations where all values were <1.5 included 25, 67, 72, and 400. Male bass excluded from analysis but present in the box plot were Stage-0 fish from Station 72 and fish without stage information from Station 83. A 4-y-old male from Station 23 was also excluded.

Arithmetic station means ranged from 0.9 at Station 67 to 2.1 at Station 77 (Fig. 3-5b). At Stations 28, 67 and 79 the standard errors were relatively large, which, for Stations 28 and 79, appeared to be caused by single outliers. The statistical comparison of the transformed data from each station indicated the following significant differences: Station 77 was greater than all others except 24, 26, 27, 74, 78, 83 and 112; Stations 26, 27, 74, 78, 83 and 112 were greater than Stations 25, 28, 30, 67, 68 (Stations 74 and 112 only), 70, 71, 76, 79 (Station 112 only), 81, 82, and 400; and Stations 67 and 400 were less than Stations 24, 29, 79, and 111.

The mean for male bass CF at the reference site (1.1) was significantly lower than that for any sub-basin (largest mean=1.6 in the LMO; Fig. 3-5b). In addition, the OHR and LMS sub-basins were each significantly less than the LMO, UMS, and ARR sub-basins. The seven smallest station means (excluding the reference site) occurred in the OHR and LMS sub-basins whereas the seven greatest were in the UMS, ARR, and LMO sub-basins.

Within sub-basins, significant differences among CF station means were noted only in the ARR and OHR (Fig. 3-5b): In the ARR sub-basin Station 77 was significantly greater than Stations 29, 79, and 82; and Station 78 was greater than Station 82. In the OHR sub-basin, Station 24 was significantly greater than Station 67 in the OHR sub-basin. As noted for female bass, CF station means for male bass from Station 67 in the OHR sub-basin and Stations 72 and 111 in the UMS sub-basin were low relative to other stations in their respective sub-basins. Along with Station 74, only smallmouth bass were collected at these sites. Nevertheless, Station 74 had the greatest mean CF in the UMS sub-basin and was the second-greatest overall. At the program level, data on CF for male bass were only available for the reference site (mean=1.1) and NCBP stations (mean=1.5); this difference was significant.

CF in Carp: Gender was not significant in the preliminary model for carp CF so the data for males and females were combined. Only the fixed Station effect remained in the model after fitting, so no further adjustment was necessary. Carp data from 43 stations were included in the analyses investigating possible differences in CF. Data were not available from Stations 201 and 202 (the fish were not weighed) and

Station 23 did not meet the criteria for inclusion ($n>1$).

Across all stations 96% of the carp examined had CF values between 1.0 and 1.7 (Fig. 3-6a). Of these, 22 CF values <1.0 occurred at 15 stations. Comparatively high values were calculated for individuals at Stations 67, 68, 79, and 111. The highest value was recorded at Station 67 (3.2) whereas values <0.5 were recorded at Stations 29, 70, 78, and 86. Male and female carp excluded from the analyses but included in the box plot were Stage 0 fish from Stations 29, 32, 68, 76, 79, 86, and 89, and fish without stage information from Stations 15, 28, 30, 68, 71, 82, 86, 111, 112, 203, 205, 209, and 210.

Arithmetic station means for CF in carp ranged from 1.1 at Station 24 to 1.5 at Station 67 (Fig. 3-7a). The standard errors differed among stations; nine stations (25, 29, 67, 68, 70, 79, 86, 89, 111) had relatively high variance. Station 67 had the largest standard error due to the presence of two very large values, including the maximum value. Results of statistical analyses on the rank-transformed data indicated the following significant differences: Station 82 was less than Stations 15, 30, 31, 68, 76, 80, 111, 208, 209, and 210; Station 70 was less than Stations 15, 31, 68, 76, and 80. Station 31 also exceeded Stations 24, 26, 28, 71, 72, 78, 79, 81, 84, 85, 86, 204, 205, 211, and 400 and Station 30 exceeded Stations 24, 26, 28, 84, 205, 211, and 400. Station 80 exceeded Stations 24, 26, 28, 71, 72, 79, 81, 84, 85, 204, 205, 211, and 400; Station 15 was also greater than Stations 24, 26, 27, 28, 29, 71, 72, 73, 75, 78, 79, 81, 84, 85, 86, 90, 204, 205, 206, 211, 212, and 400. Station 76 exceeded the same stations as Station 15 as well as Stations 32, 112, 203 and 207. Station 68 was greater than Stations 24, 26, 28, 72, 79, 84, 85, 205, 211, and 400 as well; Station 111 was greater than Stations 28, 205, and 211; Station 209 was greater than Stations 24, 26, 28, 205, 211 and Station 211 was less than Stations 77, 208, and 210.

The mean for carp CF at the reference site (1.16) was lower than that for any sub-basin (largest mean=1.30 in the LMS sub-basin; Fig. 3-7a). Statistical analysis of the ranked data indicated that the LMS sub-basin was significantly greater than the reference site and all other sub-basins except the LMO, which exceeded the ARR, UMO, and OHR sub-basins and the reference site. In no sub-basin were all station means in the upper or lower 50% of means. The highest and lowest 10 station means were scattered across six and seven sub-basins, respectively.

In general, mean CF for stations within sub-basins were evenly scattered (Fig. 3-7a). Multiple comparisons of the ranked data indicated that the following differences were significant: In the LMO sub-basin, Station 31 exceeded Station 86; in the OHR

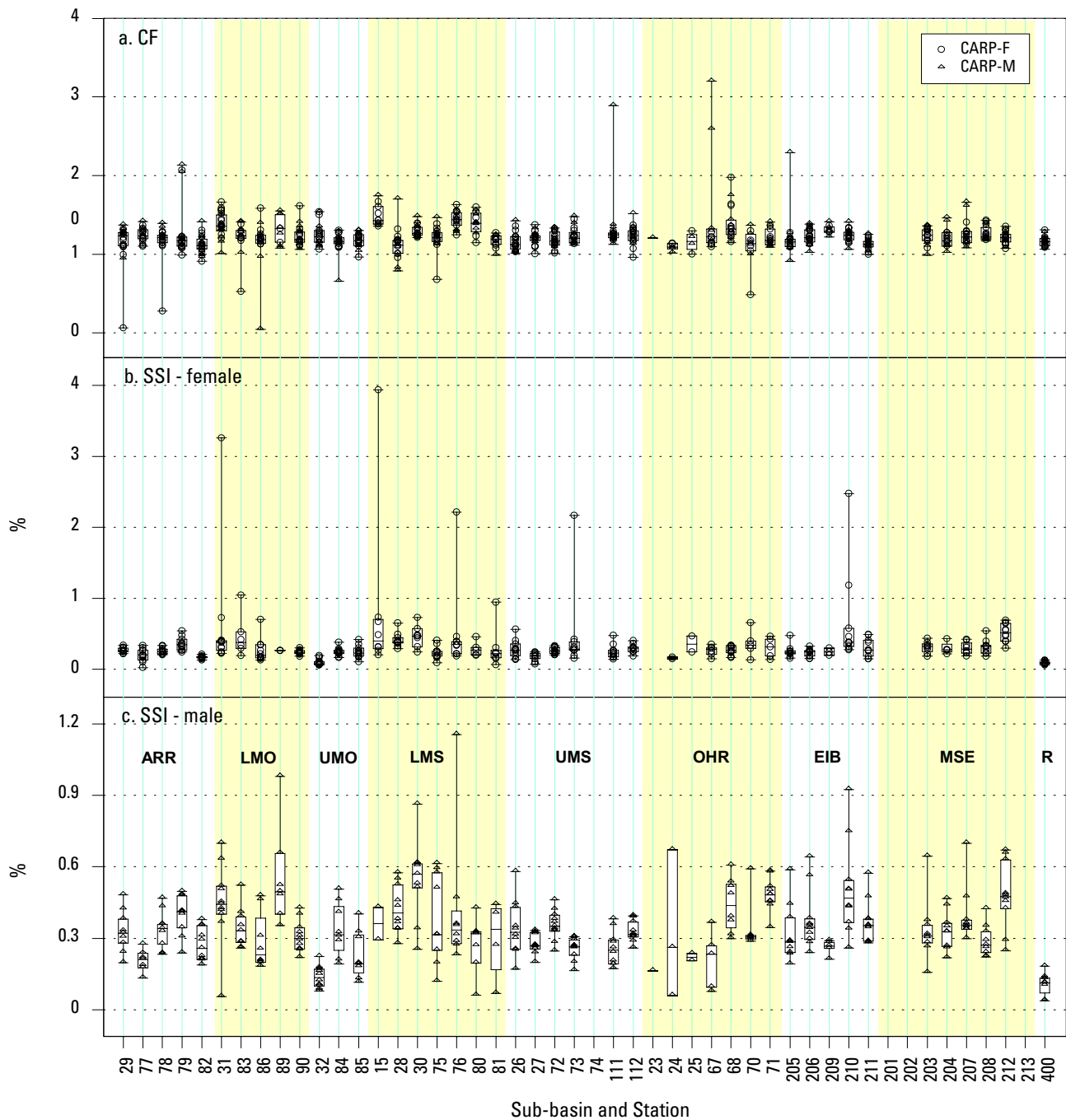


Figure 3-6. (a) Condition factor (CF) in carp, (b) spleno-somatic index (SSI) in female carp, and (c) SSI in male carp, by sub-basin and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and the interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

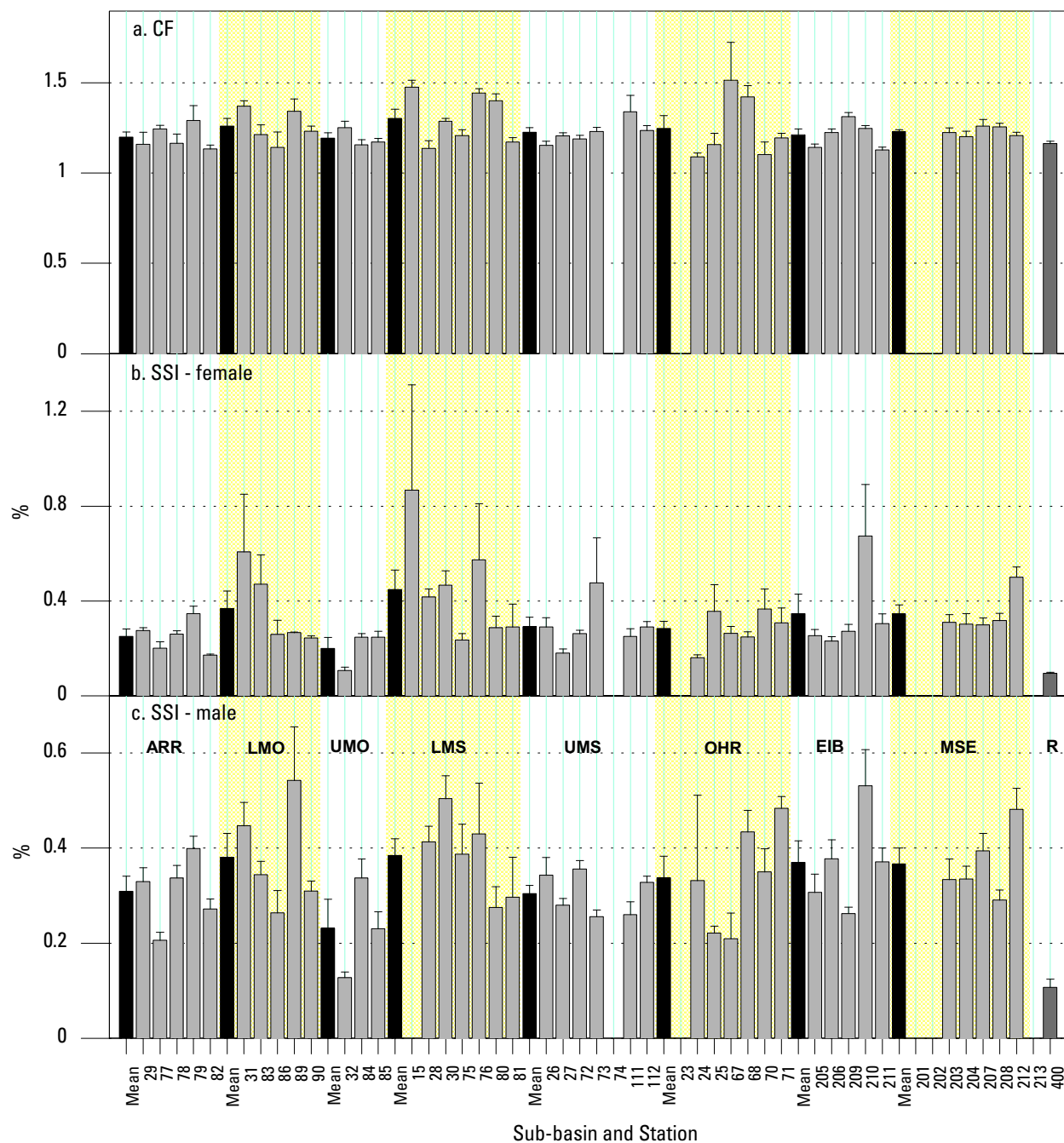


Figure 3-7. Condition factor (CF) in carp, (b) spleno-somatic index (SSI) in female carp, and (c) SSI in male carp, by sub-basin (black bars) and station (gray bars, $n > 1$). Shown are arithmetic means + 1 SE. Fish in reproductive stage 0 were not included in the computations. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

sub-basin, Station 68 was greater than Stations 24 and 70; in the EIB Study Unit, Station 209 exceeded Stations 205 and 211, and Station 210 was greater than Station 211. In the LMS sub-basin, Station 15 exceeded Stations 28, 75 and 81, Station 76 was greater than 28, 75, and 81, Station 80 exceeded Stations 28 and 81, and Station 30 exceeded 28. At the program level, carp CF at the NAWQA sites (mean=1.22) and NCBP sites (mean=1.24) were not significantly different, but both were significantly greater than the reference site.

Hepatosomatic Index (HSI)

The diffuse nature of carp liver did not allow accurate liver weights to be obtained. For this reason only bass HSI values were calculated. Due to the role of the liver in vitellogenesis, genders were not combined for the evaluation of HSI.

HSI in Female Bass: After initial model fitting, only the fixed station effect remained in the model. Female bass from 25 stations were included in the analysis of differences in HSI. Data were not available from Stations 80, 212, and 213, and Station 32 did not meet the criteria for inclusion ($n > 1$).

Most of the values (94%) for HSI in female bass fell within the range of 0.5–2.0%, and 82% were in the range of 0.5–1.5% (Fig. 3-4c). One HSI value at Station 30 was noticeably low (0.2%). Individual values $\geq 2.0\%$ occurred at Stations 24, 67, 68, 78, 79, and 112. At Stations 67 and 112 all values except one were $> 1.5\%$ and 1.0% , respectively, whereas all measurements at Stations 30 and 400 were $< 1.0\%$. Females excluded from statistical analyses but presented in the box plot were stage-4 females from Station 32, 67, and 74, stage-0 females from Station 68, 74, 76, and 81, and females without stage data from Stations 79, 111, and 112. With these data points excluded, HSI values for female bass from Station 68 were uniformly $\geq 1.5\%$.

Station means for HSI ranged from 0.6% at Station 400 to 2.0% at Station 68 (Fig. 3-5c). Means were between 0.6% and 1.6% for all but two stations (67 and 68). Variability was relatively high at Stations 24, 68, and 71. Mean separation tests on the log-transformed data indicated the following significant differences: Stations 82 and 83 were both less than Stations 26, 67, 68, 78, and 112; Station 30 was exceeded by all stations except 15, 70, 81, 82 (approached significance), 83, and 400; Station 15 was less than 67, 68, and 78; Stations 81 and 70 were both less than 26, 67, 68, 74, 78, and 112; and, Station 400 was exceeded by all stations except 15, 25 (approached significance), 30, 70, 81, 82, and 83. Along with the previously noted differences, Station 78 was significantly greater than 25, 28, 72, and 111.

Stations 67 and 68 were also significantly greater than 25, 28, 72, 76 (67 only), and 111.

Mean HSI for female bass from the reference site (0.6%) was less than that for all sub-basins (largest mean=1.4% in the OHR sub-basin; Fig. 3-5c). Excluding Station 83 (the only LMO station) and the reference site, the five stations in the LMS sub-basin were all among the 10 with the lowest mean HSI. Of the other five, two were in the OHR sub-basin, two were in the UMS, and one was in the ARR. Mean HSI for female bass from the LMO sub-basin was significantly less than means for all but the LMS sub-basin and the reference site, which was in turn less than all but the LMO sub-basin. In comparisons of stations within sub-basins, differences between Stations 82 and 78 in the ARR sub-basin, and between Station 30 and Stations 28 and 76 in the LMS sub-basin were statistically significant. So also were differences in the OHR sub-basin between Stations 67 and 68 and Stations 25 and 70. At the program level data for HSI were only available from the reference site (mean=0.6%) and NCBP sites (mean=1.2%), which differed significantly.

HSI in Male Bass: After initial model fitting, only the fixed station effect remained in the model. Male bass from 23 stations were included in the analysis of differences in HSI. Data were not available from Stations 15, 80, 212, and 213, and Station 23 did not meet the criteria for inclusion ($n > 1$).

As noted for female bass, most (94%) of the HSI values for male bass were between 0.5% and 2.0%; 85% were between 0.5% and 1.5% (Fig. 3-4d). The lowest HSI value (0.3%) occurred at Station 400, the reference site. The only other male bass $< 0.5\%$ was from Station 30. Values at or above 2.0% were measured in male bass from Stations 26, 67, 68, and 79. Only at Station 67 were all HSI values $> 1.5\%$. Values at Stations 24, 26, 68 (one exception), and 78 were all $\geq 1.0\%$. Stations at which all values were $\leq 1.0\%$ included 70, 72, 81, 82, 83 (one exception) and 400. Male bass excluded from the calculation of descriptive statistics and from the ANOVA but included in the box plot were two stage-0 males from Station 72 and one fish without a stage designation from Station 83.

Arithmetic mean HSI values for male bass ranged from 0.6% at Station 400 to 1.9% at Station 67 (Fig. 3-5d). Means were between 0.6% and 1.5% for all but one station (68), where variability was also relatively high. Significant differences indicated by mean separation tests on the log-transformed data were as follows: Stations 30 and 70 were exceeded by Stations 24, 26, 27, 67, 68, 77, 78, 79, and 112; Stations 28, 81, and 82 were less than 26, 67, 68, 77 (Station 82 only), 78, 79, and 112; Stations 25 and 83

were less than 26, 67, and 78 (Station 83 only); and, Station 67 was also greater than 29, 74, and 111. Finally, Station 400 was exceeded by Stations 24, 25, 26, 27, 29, 67, 68, 71, 74, 76, 77, 78, 79, 111, and 112.

Again as noted for female bass, mean HSI for males was lowest at the reference site (0.6%) and highest in the OHR sub-basin (1.3%; Fig. 3-5d). The stations with the five greatest means were all located in the OHR (three), UMS (one) and ARR (one) sub-basins. Three of the five stations with the lowest means were in the LMS sub-basin, and one was in each of the OHR and ARR sub-basins. Mean HSI in male bass from the reference site was significantly lower than in all sub-basins. In addition, HSI in the LMS sub-basin was exceeded significantly by all sub-basins except the LMO, which was in turn exceeded by the OHR. In analyses of stations within sub-basins, significant differences were noted between Station 82 (low) and Stations 77, 78 and 79 in the ARR sub-basin; and, in the OHR sub-basin, between Station 70 (low) and Stations 24, 67, and 68 and between Stations 25 (low) and 67. At the program-level, HSI data were not available from the NAWQA sites; however, NCBP sites (mean=1.1%) significantly exceeded the reference site.

Splenosomatic Index (SSI)

SSI in Bass: Because gender was not significant in the exploratory model, male and female bass were combined for analysis. Only the fixed station effect remained in the model. Data from 25 stations were included in the analysis of SSI in bass. Data were not available from Stations 212 and 213, and Stations 32 and 80 did not meet the criteria for inclusion ($n > 1$).

Across all stations, 91% of the SSI values for bass were between 0.05% and 0.3% (Fig. 3-4e). Values of SSI $< 0.05\%$ were calculated for fish from Stations 24, 25, 27, 70, 74, 76, 77, 78, 81, and 400. Calculated SSI values exceeded 0.3% for fish from Stations 25, 26, 27, 28, 67, 71, 76, 78, 79, and 112. The highest SSI value was recorded for an individual at Station 71. There were a number of low values, none of which appeared to be obvious outliers. Male and female bass excluded from the analysis but presented in the box plot included Stage-0 fish from Stations 68, 72, 74, 76, and 81; Stage-4 fish from Stations 32, 67, and 74; fish without stage information from Stations 79, 83, 111, and 112; and one male from Station 23.

In terms of descriptive statistics, the mean SSI across the 25 stations ranged from 0.09% at Stations 15, 24, 77, and 400 to 0.24% at Station 71 (Fig. 3-5e). Standard errors across stations were within a factor of three except for Station 71 (six-fold greater than the lowest). Results of the analyses on

the log-transformed data were as follows: Station 77 was exceeded significantly by Stations 26, 28, 71, 79, and 112; Stations 30, 74, and 83 were all exceeded by Station 26; Station 24 was significantly less than Stations 26, 28, 71, 79, and 112; and, Station 400 was exceeded by Stations 25, 26, 27, 28, 71, 72, 79, and 112.

At the sub-basin level, means for bass SSI ranged from 0.11% in the LMO sub-basin to 0.16% in the UMS. The mean for the reference site was 0.09% (Fig. 3-5e), which was significantly lower than all but the LMO sub-basin (Station 83 only). Within sub-basins, most station means were clustered. The OHR sub-basin included the station with the highest mean (Station 71) as well as the second lowest (Station 24). Statistical analysis of the log-transformed data indicated the following differences between stations in the same sub-basin: in the ARR, Station 79 exceeded Station 77; in the UMS, Station 26 exceeded Station 74; and in the OHR Station 71 exceeded Station 24. At the program level, the mean for bass SSI from the NCBP sites was 0.14%, which was significantly greater than the reference site. No data from the NAWQA sites were available.

SSI in Carp: The third-order interaction of station, gonadal stage, and gender was significant in the preliminary statistical model. Because of this, SSI in carp was analyzed separately for each gender.

SSI in Female Carp: Only the fixed station effect remained in the model for SSI in female carp, for which data from 43 stations were analyzed. No data were available from Stations 201 and 202 and no females were collected at Station 23.

Across all stations the range of SSI values for female carp was 0.03%–3.9%; 93% of the values were between 0.06% and 0.5% and 88% were between 0.1% and 0.5% (Fig. 3-6b). The 25 values $> 0.5\%$ occurred at 16 stations. The lowest 26 SSI values ($< 0.13\%$) occurred primarily at three stations: Station 77 (three of 11 females), 32 (seven of nine females), and 400 (all 11 females). The smallest individual SSI value for female carp was calculated for a fish from Station 77 whereas the greatest from Station 15. Female carp excluded from analyses but presented in the box plot were Stage-0 fish from Stations 29, 68, 76, and 79 and fish without stage information from Stations 68, 82, 86, 112, 203, and 209.

The arithmetic station means for SSI in female carp ranged from 0.09% at Station 400 to 0.87% at Station 15 (Fig. 3-7b). The standard errors were similar to each other at most stations. Notably large standard errors characterized stations with one or more outlying values; these included Stations 15, 210, 31, 76, 73, 83 and 81. Results of statistical analyses

of the log-transformed data were as follows: SSI in female carp from Station 400 was significantly less than at all stations except 24, 25, 27, 32, 77, 82, 89, and 209; Stations 27, 77 and 82 were all exceeded by Stations 15, 28, 30, 31, 76, 210, and 212; and SSI at Station 32 was less than all stations except 24, 25, 27, 75, 81, 89, and 209. SSI at Station 15 was also significantly greater than Stations 75 and 206, and, Station 210 exceeded Stations 75, 85, 86, and 206.

At the sub-basin level, SSI in female carp from the reference site (0.09%) was exceeded significantly by SSI in all sub-basins (largest mean=0.45%, LMS sub-basin; Fig. 3-7b). The UMO sub-basin was significantly lower than all sub-basins except the OHR and ARR; the ARR was exceeded by the MSE and LMS; and the UMS sub-basin was exceeded by the LMS, in which four of the 10 stations with the largest means were located. Of the remaining six, two were in the LMO sub-basin and the rest were in the OHR sub-basin, the UMS sub-basin, and the MSE and EIB Study Units. For SSI in female carp all stations in the MSE Study Unit were in the upper half of the ranked station means. In contrast, all the UMO sub-basin stations were in the lower half. Statistical analysis of the log-transformed data indicated that the following differences among stations within sub-basins were significant: In the UMO sub-basin, SSI in female carp from Station 32 was exceeded by Stations 84 and 85, in the LMS, Station 15 exceeded Station 75, and in the EIB, Station 210 exceeded Station 206.

Data were available from both the NCBP sites (mean=0.31%) and the NAWQA sites (mean=0.35%) for program-level comparisons of female carp SSI. The combined NCBP sites were exceeded significantly by the combined NAWQA sites, and combined sites from both programs were significantly greater than the reference site.

SSI in Male Carp: Final model fitting left only the fixed station effect for SSI in male carp, for which data from 42 stations were analyzed. No data was available from Stations 201 and 202, and Stations 15 and 23 did not meet the criteria for inclusion (gonadal stage >0, $n > 1$).

The range of SSI values for male carp across all stations was 0.04%–1.2%. Of these, 87% were between 0.06% and 0.5%, and 85% were between 0.1% and 0.5% (Fig. 3-6c). Only one value exceeded 1.0%, but 43 exceeded 0.5%. The lowest 25 SSI values (<0.16%) occurred primarily at two stations, 32 (seven of nine males) and 400 (seven of eight males). The male carp with the lowest SSI value was from Station 400 whereas the highest was from Station 76. Male carp excluded from statistical analyses but presented in the box plot were stage-0 fish from Stations 15, 28, 68, 71, 86, 111, 205, and 210, fish without

stage information from Stations 86 and 89, and Stations 15 and 23, from which only one male carp of stage >0 was collected.

The arithmetic station means for SSI in male carp ranged from 0.11% at Station 400 to 0.54% at Station 89 (Fig. 3-7c). Most of the stations had similar standard errors; however, notably large standard errors resulted at stations with outlying values. These included Stations 76, 89, and 210. Station 24 also had a relatively large standard error because its three SSI values were scattered. There were fewer high, outlying values for males than for females. Results of statistical analyses of the rank-transformed data indicated that the following among-station differences were significant: Stations 400 and 32 were both exceeded by Stations 26, 28, 29, 30, 31, 68, 71, 72, 75, 76, 78, 79, 83, 84, 89, 112, 203, 204, 206, 207, 210, 211, and 212; Station 77 was less than Stations 28, 30, 31, 68, 71, 72, 79, 89, 207, 210, 211, and 212; Station 82 was less than Stations 30, 31, 71, 210, and 212; Station 86 was less than Stations 30, 71, and 212; Stations 85 and 73 were both significantly exceeded by Stations 28, 30, 31, 71, 89, 210, and 212; and Stations 27, 111, and 67 were all significantly less than Stations 30, 31, 71, 89 (67 only), 210 (111 only), and 212. In addition, Station 71 was greater than Stations 205, 208, and 209.

The mean for male carp SSI from the reference site (0.11%) was lower than the means of all sub-basins (largest mean=0.38% for the LMO and LMS sub-basins; Fig. 3-7c). These differences were statistically significant for all except the UMO sub-basin. The UMO sub-basin was in turn significantly lower than the LMO, LMS, OHR, EIB and MSE, and the UMS was exceeded by the LMS. Station means were relatively evenly distributed within each sub-basin and were more evenly distributed than for females. Multiple comparisons of the ranked data indicated the following significant differences between stations in three sub-basins: In the ARR sub-basin SSI at Station 79 was significantly greater than at Station 77; in the UMO sub-basin Station 84 exceeded Station 32; and in the OHR sub-basin Station 71 was significantly greater than Station 67. At the program level, data for SSI in male carp were available from both the NCBP sites (mean=0.32%) and the NAWQA sites (mean=0.37%); these differences were statistically significant and mean SSI in male carp from both NAWQA and NCBP sites were significantly greater than at the reference site.

Cellular or Histopathological Indicators

As stated in the methods, pieces of liver, spleen, kidney and gonad were collected, processed and

evaluated histologically. Most of the observations were rated, based on severity and extent, on a scale of 0 (not present) to 4 (severe). These observations included hepatocyte vacuolization, presence of macrophage aggregates, parasites, bile duct proliferation, altered foci, neoplasia, inflammation, and necrosis (liver); developing tubules, tubular or glomerular lesions, macrophage aggregates, thyroid follicles, parasites, necrosis, and inflammation (kidney); parasites and thyroid follicles (spleen); stage, atretic eggs, ceroid deposits, parasites, inflammation, intersex, and neoplasia (gonad). The splenic macrophage aggregates were quantified using image analysis, and these results are presented in the following section. The gonad histology results are presented in Chapter 4 of this report. The remaining data are not presented for a number of reasons. First, the use of NoToX[®] and the number of groups/individuals involved in field collections resulted in some quality control issues. One of the problems encountered with the use of multiple field teams was inconsistency in the size and location of tissue samples removed and fixed for histology. Nevertheless, some observations strongly indicated that fish from certain sites had experienced environmental stress. For example, the high incidence of intersex male smallmouth bass detected at Station 111 (see Chapter 4) seems significant. However, it is not known whether the foci of immature oocytes in intersex smallmouth bass males is randomly distributed throughout the gonad or are there areas, if cut, are more likely to demonstrate this change. Similarly, we noted an increased size and number of thyroid follicles in kidney of carp from sites in the MSE Study Unit (especially Stations 208 and 212). Thyroid proliferation in Great Lakes salmonids has been attributed to a number of causes, including iodine deficiency and exposure to environmental contaminants (for example, Noltie and others, 1988; Leatherland, 1992). In laboratory studies varying degrees of thyroid hyperplasia have been induced in a number of fishes by exposures to many widespread environmental contaminants including DDT (Shukla and Pandey, 1986), β -HCH (Wester and Canton, 1986), carbofuran (Ram, 1988), thiocyanate (Lanno and Dixon, 1994; 1996), cadmium (Pandey and Shrivastava, 1986), and ammonium sulfate fertilizer (Ram and Sathyanesan, 1987). As noted in Chapter 2, concentrations of DDT and other organochlorine pesticides in fish from the MSE sites were comparatively high. Additional sampling should therefore be conducted at these sites (MSE stations and Station 111) to determine whether these were "real" responses or sampling bias introduced by the tissue sectioned. The second reason for not presenting the histopathological results is that a large amount of data was generated, most of which was unremarkable. As noted previously, most of the tumors identified by gross observations were parasite-induced, and we did

not find high incidences of preneoplastic or neoplastic changes or other lesions of the types that have previously been associated with chemical exposure (Baumann and others, 1991). Hence, for this report we concentrated on the gonad results (see Chapter 4) and on quantification of macrophage aggregates.

Macrophage Aggregates

Macrophage aggregate measurements were completed for 432 bass from 28 stations and for 618 carp from 37 stations. As noted previously, three MA parameters—density (the number of aggregates per mm², MAMM), mean size of aggregates in μm^2 (MEANAREA), and percent of tissue occupied by MA (TISOCC), were analyzed for bass and carp.

Macrophage Aggregate Density (MAMM)

MAMM in Bass: Arithmetic station means for MA density (MAMM) ranged from 2.2 MA/mm² at Station 77 to 11.2 MA/mm² at Station 15 (Table 3-8). Preliminary statistical analysis indicated that age significantly influenced MA numbers in bass; we estimated that an increase in age of one year was associated with a multiplicative change of 1.17 in median MAMM, that is median MAMM increased by 9-26% for each year increase in age. Accordingly, age was included as a factor in the statistical analyses. However, age data were not available for all fish and statistical comparisons were restricted to 385 bass from 27 stations.

Bass spleens from most stations contained <20 MA/mm². Only at Stations 68, 74 and 112 was there at least one fish with ≥ 20 MA/mm² (Fig. 3-8a). Station 71 had the lowest age-adjusted median density; it was significantly lower than Stations 15, 25, 27, 30, 72, 79, 81, 82, 111, 112, and 400. The highest adjusted median densities were observed at Stations 15 (four fish) and 30. Stations 15 and 30 were significantly greater than Stations 67, 71, 74, 77, 78 (Station 30 only), and 213.

Linear regressions of MAMM vs. age in bass for each station indicated that the slopes for bass at various stations were similar, but not identical, across stations. Consequently, the stations could not be combined and age adjustment by ANCOVA was performed independently for each station. Stations 15, 30 and 111 had the highest age-adjusted means whereas Stations 67, 71, 74, 77 and 213 had the lowest. The ANCOVA suggests that starting at an early age, MA numbers in bass at some stations begin increasing (or decreasing), a trend that continues throughout their lifetimes.

Data for MAMM in bass were available for at least one station in each of six sub-basins (ARR, LMO, LMS, UMS, OHR, MSE) and the reference site (Table 3-8). In bass, MAMM differed significantly

Table 3-8. Arithmetic station and sub-basin means, numbers of samples, and standard errors for macrophage aggregate density (no./mm²) in bass. See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Station or sub-basin	<i>n</i>	Mean	SE
Stations			
15	4	11.2	1.8
23	4	5.9	2.1
24	10	5.4	1.0
25	17	6.2	0.5
26	20	5.0	0.6
27	20	7.4	0.7
28	20	5.5	0.6
29	15	5.7	0.4
30	12	10.7	0.7
67	13	4.0	1.0
68	11	9.8	2.6
70	23	7.3	0.9
71	11	3.7	0.9
72	16	9.4	1.5
74	17	4.2	1.3
76	18	5.9	0.9
77	18	2.2	0.5
78	19	5.3	0.6
79	20	9.1	0.7
80	3	5.7	1.0
81	23	7.4	1.0
82	26	7.6	0.6
83	17	5.6	1.0
111	22	8.5	0.7
112	20	7.8	1.0
212	3	7.1	0.3
213	11	3.8	1.0
400	19	6.2	0.7
Sub-basins			
Arkansas-Red R. (ARR)	5	6.0	1.2
Lower Missouri R. (LMO)	1	5.6	--
Lower Mississippi R. (LMS).	6	7.7	1.1
Upper Mississippi R. (UMS)	6	7.1	0.9
Ohio R. (OHR)	6	6.0	0.8
Mississippi Embayment (MSE)	7	5.5	1.7

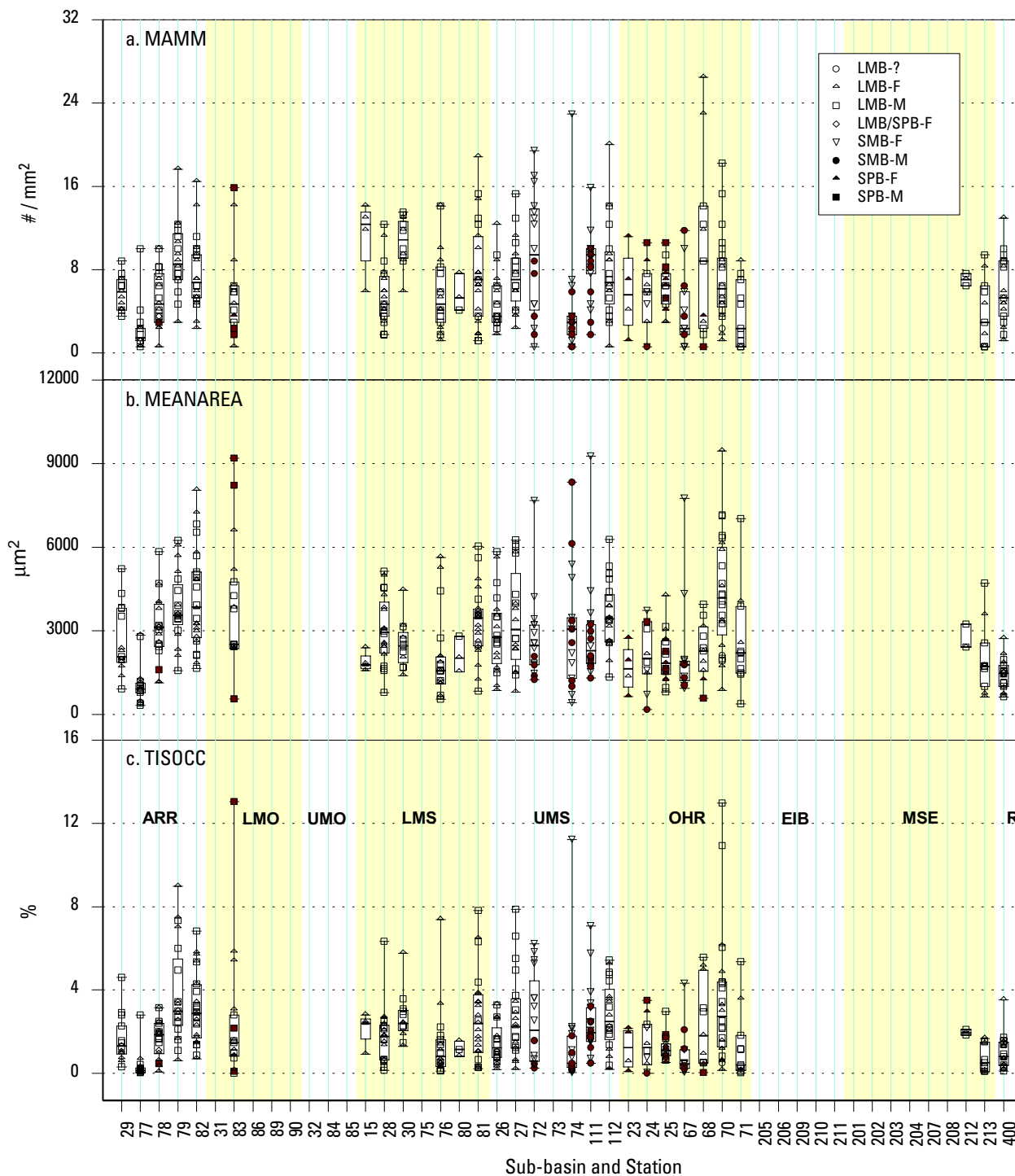


Figure 3-8. (a) Macrophage aggregate density (in MA/mm²), (b) mean area of MA (MEANAREA, in µm²), and (c) percent of tissue occupied by macrophage aggregates (TISOCC) for bass, by sub-basin and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

among sub-basins; age-adjusted MAMM for the ARR and OHR sub-basins were significantly lower than for the LMS. In addition, some stations in the ARR, UMS and OHR sub-basins differed significantly from each other. In the ARR sub-basin, MAMM in bass was less at Station 77 than at either Station 79 or 82; in the UMS sub-basin, MAMM in bass from Station 74 was significantly less than at Stations 27 and 111; and in the OHR sub-basin, MA density in bass from Station 25 was significantly greater than at Stations 67 and 71. At the program level MAMM in bass did not differ significantly between the NCBP and NAWQA sites, nor did either group differ from the reference site.

MAMM in Carp: Arithmetic station means for MAMM ranged from 5.1 MA/mm² in carp from the reference site (Station 400) to 18.3 MA/mm² at Station 26 (Table 3-9). In the initial statistical model there was a moderately significant effect of age on MAMM in carp; however, the effect of age was not consistent—at some sites MAMM increased with age whereas at others it decreased. Age was therefore excluded from the model.

Most individual values for MAMM in carp were <20 MA/mm², as was also true for bass (Fig. 3-9a). However, MA density was ≥20 MA/mm² in at least one fish from each of Stations 26, 70, 72, 73, 75, 78, 81, 82, 201, 203, 205, 206, 207, and 208, and at Stations 26, 203, and 208 MAMM in at least one fish was >30 MA/mm². Carp from the reference station had the lowest MAMM and were significantly lower than 23 of the 36 other stations. The only stations where MAMM in carp was not significantly greater than at Station 400 were Stations 15, 24, 25, 30, 31, 67, 75, 76, 77, 81, 209, 210, and 211. The greatest density of MA occurred at Station 26, which differed significantly from Stations 15, 30, 31, 67, 68, 75, 76, 77, 81, 202, 209, 210, 211, 212 and 400.

Carp from all eight sub-basins were collected and analyzed for MA (Table 3-9). At the reference site MAMM in carp was significantly lower than in all sub-basins except the LMO; the difference between the LMO and the reference site was not significant, however. The LMO was significantly lower than all other sub-basins except the LMS, however. Carp MAMM in the UMS sub-basin was significantly greater than in all other sub-basins. Within sub-basin comparisons of MAMM in carp indicated significant differences only among stations of the ARR sub-basin and EIB Study Unit. In the ARR sub-basin the mean for Station 77 was significantly less than at Station 82. In the EIB Study Unit, Station 205 had the highest MAMM in carp and was significantly greater than Stations 210 or 211 whereas the lowest MAMM was at Station 211, which was significantly lower than Stations 206 and 205. Program-level comparisons of

MAMM in carp indicated that mean MAMM was lowest at the reference site, intermediate at the NCBP sites, and highest at the NAWQA sites; these differences were all statistically significant.

Macrophage Aggregate Size (MEANAREA)

MEANAREA in Bass: Arithmetic station means for the area occupied by MA ranged from 1048.9 μm² at Station 77 to 4439.9 μm² at Station 70 (Table 3-10). Age had a significant effect on MA size; we estimated that an increase in age of one year was associated with a multiplicative change of 1.09 μm² in the median mean area, that is median mean area increased by 4-15% for each year's increase in age. Hence, statistical comparisons included age as a factor. Due to missing age data, only 385 bass from 27 stations were compared statistically.

The size of most MA in individual bass across all stations ranged from 1000 to 6000 μm² (Fig. 3-8b). At nine stations (27, 67, 70, 71, 72, 74, 82, 83, 111) MEANAREA in at least one individual fish exceeded 6000 μm². Based on age-adjusted data, Stations 400 and 77 had the smallest MA. These two stations were not significantly different from each other, but MA in bass from Station 77 were significantly smaller than at 16 other stations (25, 26, 27, 28, 30, 68, 70, 72, 74, 78, 79, 81, 82, 83, 111, 112) whereas MA at Station 400 were significantly smaller than at 11 other stations (27, 28, 70, 74, 78, 79, 81, 82, 83, 111, 112). Although Stations 26 and 70 had the largest median MEANAREA, the median MEANAREA for bass from Station 26 was only significantly greater than that of Station 77. In contrast, Station 70 exceeded Stations 29, 67, 76, 77, 213 and 400.

The reference site MEANAREA for bass (1416.0 μm²) was significantly lower than in any sub-basin (largest mean=3858.4 μm² for the LMO sub-basin; Table 3-10) except the MSE Study Unit. The only other significant difference between sub-basins was for the OHR and UMS; MA in bass from the UMS were significantly larger than in the OHR. Only within the ARR, LMS, and OHR sub-basins did mean MA size in bass differ significantly among stations. In the ARR sub-basin, MEANAREA in bass from Station 77 was significantly smaller than that from Stations 78, 79, and 82, and Station 82 was also significantly larger than Station 29. In the LMS sub-basin the only significant among-station difference was between Stations 81 and 76; MEANAREA in bass from Station 76 was smaller. Stations in the OHR sub-basin were clustered, with only two stations differing significantly; MA's in bass from Station 67 were smaller than those from Station 70.

Bass MA data were available from two NAWQA sites (both in the MSE Study Unit), the NCBP sites, and the reference site. Mean MA size in

Table 3-9. Arithmetic station and sub-basin means, numbers of samples, and standard errors for macrophage aggregate density (no./mm²) in carp. See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Station or sub-basin	<i>n</i>	Mean	SE
Stations			
15	10	8.4	1.7
24	5	11.1	1.9
25	4	13.2	1.1
26	19	18.3	1.4
27	20	14.3	1.1
28	18	11.9	1.1
29	20	12.5	0.9
30	17	9.3	0.7
31	23	7.3	0.7
32	20	12.6	0.9
67	11	9.6	0.6
68	17	11.2	0.8
70	11	14.3	1.5
71	14	11.5	1.3
72	21	15.7	0.8
73	20	16.3	0.9
75	19	10.3	1.4
76	17	9.2	1.1
77	18	9.0	1.2
78	19	12.9	0.8
79	20	12.1	0.8
80	12	12.0	1.6
81	11	11.1	2.1
82	24	15.3	0.9
201	16	12.4	1.5
202	20	11.8	1.0
203	18	17.8	1.2
204	15	13.1	1.1
205	18	17.5	1.1
206	19	16.0	1.1
207	18	15.9	1.2
208	19	15.9	1.9
209	8	10.7	1.0
210	18	10.6	0.9
211	20	7.0	0.5
212	20	10.9	0.7
400	19	5.1	0.4
Sub-basins			
Arkansas-Red R. (ARR)	5	12.3	1.0
Lower Missouri R. (LMO)	1	7.3	--
Upper Missouri R. (UMO)	1	12.6	--
Lower Mississippi R. (LMS)	7	10.3	0.5
Upper Mississippi R. (UMS)	4	16.2	0.8
Ohio R. (OHR)	6	11.8	0.7
Eastern Iowa Basins (EIB)	5	12.3	1.9
Mississippi Embayment (MSE)	7	14.0	1.0

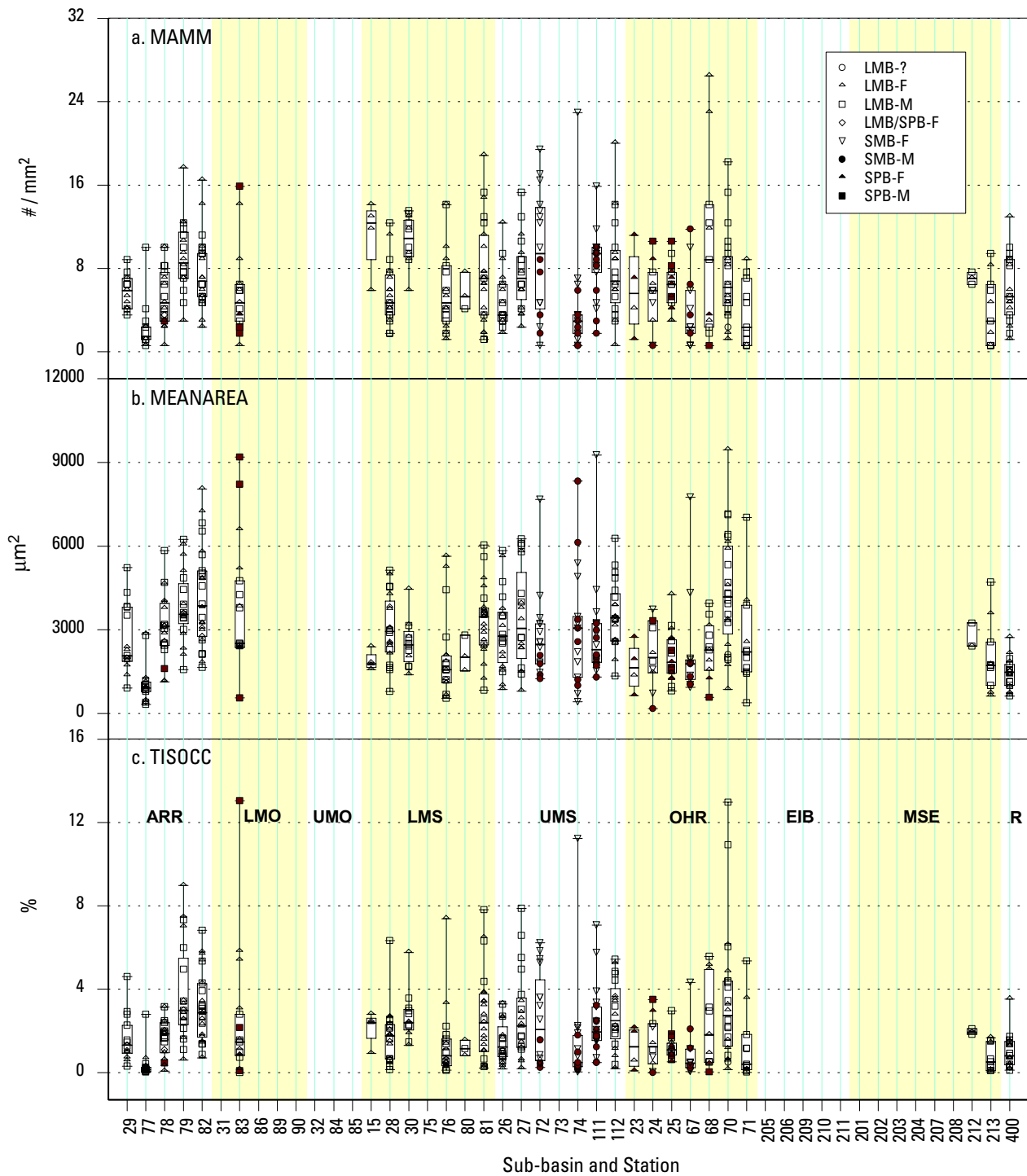


Figure 3-9. (a) Macrophage aggregate density (in MA/mm²), (b) mean area of MA (MEANAREA, in μm²), and (c) percent of tissue occupied by macrophage aggregates (TISOCC) for carp, by sub-basin and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

Table 3-10. Arithmetic station and sub-basin means, numbers of samples, and standard errors for macrophage aggregate size (μm^2) in bass. See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Station or sub-basin	<i>n</i>	Mean	SE
Stations			
15	4	1874.5	177.6
23	4	1660.2	445.3
25	17	2064.2	219.9
26	2	4363.8	1243.4
27	19	3537.3	406.9
28	18	3016.8	299.9
29	15	2607.3	317.3
30	12	2511.1	240.9
67	13	2172.4	523.9
68	11	2325.6	303.2
70	23	4439.9	432.4
71	8	2414.7	700.5
72	16	2733.5	390.1
74	14	3248.4	595.7
76	15	1600.6	252.1
77	17	1048.9	175.4
78	18	3192.9	278.6
79	20	3820.9	281.0
80	3	2113.0	373.5
81	21	3433.2	258.0
82	25	4072.9	341.6
83	17	3858.4	560.6
111	20	2750.7	387.2
112	20	3570.4	269.7
212	3	2689.8	276.9
213	11	1978.9	369.0
400	19	1416.0	123.0
Sub-basins			
Arkansas-Red R. (ARR)	5	2948.6	539.0
Lower Missouri R. (LMO)	1	3858.4	--
Lower Mississippi R. (LMS)	6	2424.9	286.0
Upper Mississippi R. (UMS)	6	3367.4	248.9
Ohio R. (OHR)	6	2512.8	400.1
Mississippi Embayment (MSE)	2	2334.4	355.4

bass from the NCBP sites was significantly larger than from the reference site, and the NAWQA sites were intermediate and not significantly different from either the NCBP sites or the reference site.

MEANAREA in Carp: Arithmetic station means for MEANAREA in carp ranged from 1668.6 μm^2 at Station 205 to 4684.0 μm^2 at Station 67 (Table 3-11).

Although age was not a significant factor for MA density in carp, it was found to have a significant effect on MA size. It was estimated that an increase in age of one year was associated with a multiplicative change of 1.05 in the median mean area of MAs. In other words median MA size increased 2–9% each year. Age was therefore retained in the final model; however, due to a lack of age data, only 511 carp from

Table 3-11. Arithmetic station and sub-basin means, numbers of samples, and standard errors for macrophage aggregate size (μm^2) in carp. See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Station or sub-basin	<i>n</i>	Mean	SE
Stations			
15	10	1826.5	277.9
25	4	2066.7	586.9
26	18	3341.5	339.3
27	15	2189.2	173.5
28	12	2908.3	305.8
29	20	2991.6	333.6
30	11	2608.7	190.8
31	21	1668.6	121.7
32	19	2964.5	206.7
67	11	4684.0	508.9
68	10	2213.8	327.0
70	10	4449.9	531.0
71	10	2743.5	328.3
72	21	3293.2	254.8
73	18	3751.6	358.5
75	19	2325.0	377.2
76	11	2005.1	318.4
77	13	2365.0	265.2
78	18	3251.9	259.7
79	19	3289.1	288.0
80	6	3281.7	766.7
81	9	2001.0	182.2
82	14	2715.8	220.9
201	10	2627.5	273.6
202	20	2778.1	253.0
203	14	2371.7	274.0
204	11	2638.9	328.9
205	18	1668.6	204.1
206	19	2146.6	304.3
207	15	2514.6	278.6
208	16	2910.2	418.2
210	18	2373.0	330.0
211	20	4405.5	500.8
212	18	2148.0	185.5
400	13	2328.2	266.2
Sub-basins			
Arkansas-Red R. (ARR)	5	2922.7	173.4
Lower Missouri R. (LMO)	1	1668.6	--
Upper Missouri R. (UMO)	1	2964.5	--
Lower Mississippi R. (LMS)	7	2422.3	202.7
Upper Mississippi R. (UMS)	4	3143.9	334.4
Ohio R. (OHR)	5	3231.6	557.9
Eastern Iowa Basins (EIB)	4	2648.5	603.8
Mississippi Embayment (MSE)	7	2569.9	96.1

35 stations were included in the statistical analysis.

Most carp had a MEANAREA between 1000 and 6000 μm^2 ; however, at 12 of the stations with age data (26, 29, 67, 70, 72, 73, 74, 79, 206, 208, 210, 211) at least one fish had a MEANAREA greater than 6000 μm^2 and at two stations (208, 211) values were $>8000 \mu\text{m}^2$ (Fig. 3-9b). Age-adjusted median MEANAREA were largest in carp from Stations 67, 70 and 211. Station 211 had the largest age-adjusted MA, which were significantly larger than those at Stations 15, 31, 68, 75, 76, 205, 206, 210, 212 and 400. Stations 67 and 70 were only significantly greater than Stations 31, 205 and 206, however. The smallest median age-adjusted MA in carp were from Stations 205 and 31; MA were significantly smaller at Station 205 than at Stations 26, 28, 32, 67, 70, 73, 78, 79, 82, 202, 208 and 211. Station 31 MA were also significantly smaller than those at Stations 26, 67, 70, 73, 79 and 211.

Sub-basin means for MEANAREA in carp ranged from 1668.6 μm^2 in the LMO sub-basin to 3231.6 μm^2 in the OHR (Table 3-11). Age-adjusted MEANAREA medians were largest in the UMS and OHR sub-basins and smallest in the LMO; the LMO was significantly less than the ARR, UMO, UMS, and OHR sub-basins and the MSE Study Unit. The only other significant sub-basin difference detected was between the EIB Study Unit and UMS sub-basin; MEANAREA in carp from the UMS exceeded that in the EIB. Only one sub-basin contained stations that differed significantly for age-adjusted MA size in carp; in the EIB Study Unit Station 211 was greater than the other three stations (205, 206, 210). Program-level comparisons indicated that MA size at the reference site and at the NCBP and NAWQA sites did not differ significantly.

Tissue Occupied by Macrophage Aggregates (TISSOC)

TISSOC in Bass: Arithmetic station means for TISSOC in bass ranged from 0.34% at Station 77 to 3.77% at Station 79 (Table 3-12). The percent of tissue occupied by MA utilizes both density and size data. Preliminary statistical analysis indicated that both of these parameters were significantly affected by age in bass. Therefore, age was included as a covariate in statistical comparisons.

Most of bass collected in the MRB had $<5\%$ of splenic tissue occupied by MAs (Fig. 3-8c). However, at 15 stations (27, 28, 30, 68, 70, 71, 72, 74, 76, 79, 81, 82, 83, 111, 112) there was at least one fish (often more than one) in which TISSOC was $>4\%$, and, for four bass from three stations (70, 74, 83), TISSOC was $>10\%$. Bass from Station 77 had the lowest age-adjusted median TISSOC, which was significantly less than all other sites sampled except Stations 23, 26, 29, 67, 71, 74, 80, 212 and 213.

Stations 15, 30 and 82 had the highest age-adjusted median TISSOC for bass and did not differ significantly from each other. Station 82, with the highest age-adjusted median TISSOC, significantly exceeded Stations 67, 71, 74, 76, 77, 83, 213, and 400, however.

Bass from six sub-basins were compared for MA TISSOC. Sub-basin means ranged from 1.34% in the MSE Study Unit to 2.45% in the LMO sub-basin (Table 3-12). The reference site mean (0.99%) was the lowest mean age-adjusted median TISSOC; however, it was similar to and not significantly different from the ARR, LMO, and OHR sub-basins and the MSE Study Unit. The only statistically significant differences were between OHR and the two sub-basins with the highest median values, the LMS and UMS; both were significantly larger than OHR but did not themselves differ. Differences among stations within sub-basins occurred in only two sub-basins. In the ARR sub-basin, Station 77 was significantly lower than all other stations (78, 79, 82) except Station 29; and in the OHR sub-basin, Station 70 exceeded Stations 67 and 71. The age-adjusted median for bass TISSOC at the reference site was slightly lower than that for the NAWQA sites whereas the age-adjusted median for the NCBP sites was slightly higher than the other two; however, these differences were not statistically significant.

TISSOC in Carp: Arithmetic mean TISSOC ranged from 1.17% at Station 400 to 6.36% at Station 70 (Table 3-13). Although there were some significant interactions between age and TISSOC, as described previously for MAMM in carp, they were not consistent and age was therefore not included as a covariate in the statistical comparisons.

All station means were $<7\%$ for carp TISSOC. Nevertheless, at most stations MAs occupied $>5\%$ of splenic tissue in at least one fish. The exceptions were Stations 15, 25, 30, 76, 205, 209, 210, 212 and 400. At 10 stations (26, 31, 70, 72, 73, 80, 82, 203, 204, 208) TISSOC of one or more carp was $>10\%$ (Fig. 3-9c). The reference station had the lowest TISSOC and was significantly lower than 23 of the remaining 36 stations whereas Stations 15, 25, 30, 31, 71, 75, 76, 77, 81, 205, 209, 210, and 212 did not differ significantly from the reference site. Station 70 had the highest TISSOC in carp and differed significantly from Stations 15, 30, 31, 75, 76, 77, 81, 205, 209, 210, 212 and 400.

Mean TISSOC in carp from the reference site (1.17) was lower than that for all sub-basins (largest mean=5.23 in the UMS sub-basin; Table 3-13). The reference site was significantly lower than all sub-basins except LMO, and the LMO was significantly lower than all other sub-basins. The UMS sub-basin

Table 3-12. Arithmetic station and sub-basin means, numbers of samples, and standard errors for the percentage of splenic tissue occupied by macrophage aggregates in bass. See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Station or sub-basin	<i>n</i>	Mean	S.E.
Stations			
15	4	2.13	0.41
23	4	1.18	0.51
25	17	1.22	0.15
26	2	2.11	1.19
27	19	2.82	0.49
28	18	1.74	0.33
29	15	1.60	0.29
30	12	2.68	0.34
67	13	0.88	0.33
68	11	2.36	0.63
70	23	3.59	0.66
71	8	0.97	0.64
72	16	2.63	0.54
74	14	1.51	0.77
76	15	0.90	0.17
77	17	0.34	0.16
78	18	1.73	0.20
79	20	3.77	0.53
80	3	1.17	0.21
81	21	2.57	0.43
82	25	2.96	0.30
83	17	2.45	0.78
111	20	2.36	0.37
112	20	2.77	0.36
212	3	1.96	0.07
213	11	0.72	0.20
400	19	0.99	0.19
Sub-basins			
Arkansas-Red R. (ARR)	5	2.08	0.59
Lower Missouri R. (LMO)	1	2.45	--
Lower Mississippi R. (LMS)	6	1.86	0.30
Upper Mississippi R. (UMS)	6	2.37	0.20
Ohio R. (OHR)	6	1.70	0.44
Mississippi Embayment (MSE)	2	1.34	0.62

Table 3-13. Arithmetic station and sub-basin means, numbers of samples, and standard errors for percentage of tissue occupied by macrophage aggregates in carp. See Table 1-1 and Figure 1-1 for station locations.

Station or sub-basin	<i>n</i>	Mean	SE
Stations			
15	10	1.39	0.29
24	5	5.13	1.05
25	4	2.54	0.47
26	19	6.02	0.57
27	20	3.48	0.44
28	18	3.47	0.47
29	20	3.58	0.42
30	17	2.78	0.27
31	23	1.79	0.52
32	20	3.77	0.31
67	11	4.48	0.53
68	17	3.38	0.48
70	11	6.36	0.98
71	14	2.90	0.49
72	21	5.08	0.47
73	20	6.33	0.62
75	19	2.51	0.56
76	17	1.76	0.34
77	18	2.78	0.55
78	19	4.24	0.43
79	20	3.53	0.33
80	12	5.14	0.87
81	11	2.20	0.45
82	24	5.28	0.40
201	16	3.45	0.50
202	20	3.11	0.36
203	18	4.82	0.54
204	15	4.27	0.76
205	18	2.80	0.32
206	19	3.25	0.42
207	18	3.67	0.27
208	19	4.85	0.61
209	8	2.38	0.20
210	18	2.23	0.21
211	20	3.00	0.34
212	20	2.63	0.28
400	19	1.17	0.17
Sub-basins			
Arkansas-Red R. (ARR)	5	3.88	0.42
Lower Missouri R. (LMO)	1	1.79	--
Upper Missouri (UMO)	1	3.77	--
Lower Mississippi R. (LMS)	7	2.75	0.47
Upper Mississippi R. (UMS)	4	5.23	0.64
Ohio R. (OHR)	6	4.13	0.60
Eastern Iowa Basins (EIB)	5	2.73	0.19
Mississippi Embayment (MSE)	7	3.83	0.32

had the highest mean, which was significantly higher than all sub-basins except the UMO and OHR. The UMO sub-basin was only greater than LMO, LMS and the reference, whereas the OHR was greater than the LMO and LMS sub-basin, the EIB Study Unit, and the reference site. The LMS and EIB had similar, intermediate means that did not differ significantly; however, the LMS differed significantly from all other sub-basins except the EIB Study Unit. In comparisons of stations within sub-basins, Station 77 was significantly lower than Station 82 in the ARR sub-basin and, in the LMS sub-basin, Station 80 was statistically greater than Stations 15, 75, and 76. Both NCBP and NAWQA stations were represented for TISSOC in carp. The means for the combined NCBP and NAWQA sites did not differ significantly, but both differed from the reference site.

Subcellular-Level Indicators

Soluble Disease Resistance Factors

Lysozyme Activity

Lysozyme Activity in Bass: Because gender was not significant in the preliminary model, lysozyme activity in male and female bass was analyzed and reported together. After model fitting, only the fixed station effect was significant. Bass from 24 stations were included in the analysis. Data were not available from Stations 212, 213, and 400 whereas Stations 32 and 80 did not meet the criteria for inclusion (gonadal stage 1-3 for females, $n > 1$).

Lysozyme activity in 97% of the bass analyzed was between 10 and 200 mOD/min. In 92%, levels were between 10 and 150 mOD/min, with 72% falling between 50 and 150 mOD/min (Fig. 3-10a). Three fish from Station 28 had lysozyme levels > 200 mOD/min including the highest measured level, 273 mOD/min. In contrast, one bass from Station 28 had a plasma lysozyme level of 12.3 mOD/min. Other values > 200 mOD/min were measured at Stations 30, 72, 80, and 81. Five bass from Station 77 had lysozyme levels of < 20 mOD/min, as did five from Station 111. Other stations where single-digit values were measured included Stations 68 and 112. Females and males excluded from the analysis but presented in the box plot included fish at Stations 83 and 112 without stage information, Stage 4 females from Stations 32, 67, and 74, stage-0 fish from Stations 68, 72, 74, 80, and 81, and the one female that met the stage criteria at Station 80.

The mean lysozyme value across the 24 stations ranged from 47.5 mOD/min at Station 68 to 126.8 mOD/min at Station 71 (Fig. 3-11a). Most sta-

tions (18) had mean values of 59.3-99.5 mOD/min. Standard errors across stations were relatively constant. Bass from the reference site were not analyzed for lysozyme activity. Results of the statistical analyses on the rank-transformed data indicated that differences between Station 68 and five stations (28, 76, 81, 72, and 71) and between Station 71 and seven stations (29, 79, 83, 111, 112, 67, and 68) were significant.

Sub-basin means for lysozyme activity in bass lysozyme ranged from 59.3 mOD/min for the LMO (Station 83 only) to 107.7 mOD/min for the LMS (Fig. 3-11a). The LMS sub-basin was significantly greater than all other sub-basins except the OHR. The LMS sub-basin consisted of stations that had the 2nd, 3rd, 5th, 6th and 8th highest arithmetic means and included five of the seven fish with measurements over 200 mOD/min. Stations in the OHR sub-basin rounded out the 10 stations with the greatest mean lysozyme levels except for Station 72, in the UMS sub-basin. In examining differences between stations within a sub-basin, analysis of the rank-transformed data for lysozyme activity in bass indicated that only within the OHR sub-basin were stations significantly different from one another; Station 71 exceeded Stations 67 and 68.

Lysozyme Activity in Carp: Preliminary statistical analysis indicated that lysozyme activity in carp did not differ among genders so results for males and females were combined. After model fitting, only the fixed station effect remained. Carp from 37 stations were included in the analysis of differences in plasma lysozyme levels. Data were not available from Stations 201-204, 207, 208, 212, and 400 and Station 23 did not meet the criteria for inclusion ($n > 1$).

Plasma lysozyme levels in carp had a narrower range than in bass. Values ranged from 1.1 to 12.0 mOD/min (Fig. 3-10b). Lysozyme activity was 1.1-8.0 mOD/min for 97% of the carp and 2.0-8.0 mOD/min for 88%. Only four carp from NCBP sites were 1.1-1.9 mOD/min; the other 45 in this range were from NAWQA sites in the EIB Study Unit. Comparatively high values (≥ 9.0 mOD/min) were detected in carp from Stations 28, 67, 83, 84, 90, and 112. Females and males presented in Fig. 3-10b but not included in the analysis included stage 0 fish at Stations 29, 32, 68, 79, 86, and 89, fish without stage data at Stations 28, 30, 68, 82, 86, 111, and 209, and males at Stations 15 and 23.

Mean lysozyme levels across the 37 stations ranged from 1.6 mOD/min at Station 209 to 6.4 mOD/min at Station 111 (Fig. 3-11b). The five lowest arithmetic means were for the EIB NAWQA sites. For NCBP sites the range of means was 3.6 mOD/min (Station 32) to 6.4 mOD/min (Station 111). Standard

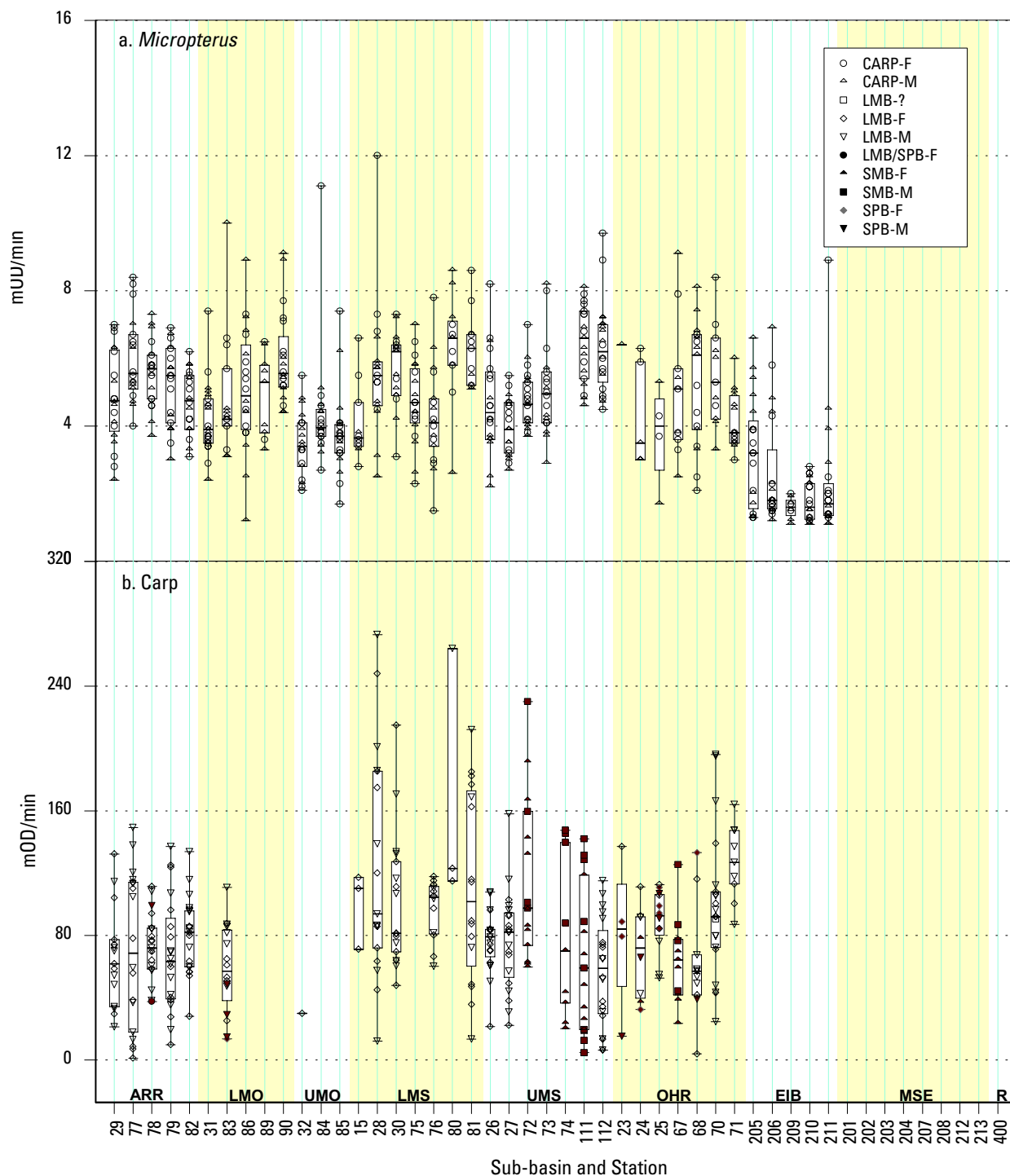


Figure 3-10. Lysozyme activity in (a) bass and (b) carp, by sub-basin and station. Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

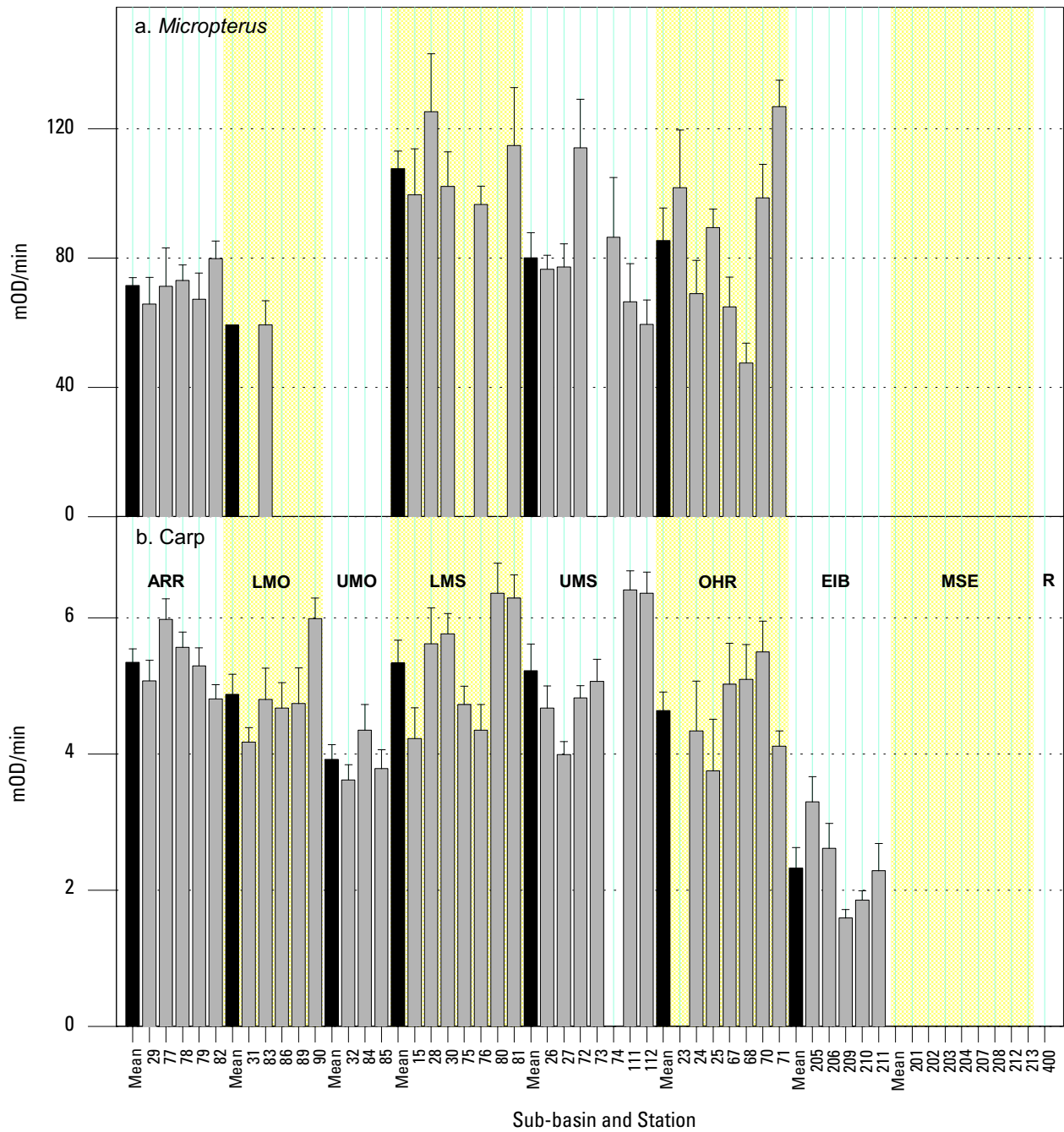


Figure 3-11. Lysozyme activity in (a) bass and (b) carp, by sub-basin (black bars) and station (gray bars, $n \geq 1$). Shown are arithmetic means + 1 SE. Female bass in gonadal stage 0 or 4 and male bass, male carp, and female carp in stage 0 were not included in the computations. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

errors were relatively uniform across stations. No comparison between basin stations and the reference station could be made because lysozyme levels were not available from Station 400. Many NCBP and NAWQA sites differed significantly, however. Mean lysozyme activity in carp from Station 31 was significantly less than at Stations 77, 80, 81, 90, 111, and 112, whereas the means for Stations 32 and 85 were less than those for Stations 28, 30, 77, 78, 80, 81, 90, 111, and 112. Station 84 was significantly less than Stations 80, 111, and 112. Carp from Stations 27 and 71 had comparatively low mean lysozyme that differed significantly from Stations 77 (Station 27 only), 80, 81, 90, 111, and 112. Stations 111 and 112 were also significantly greater than Stations 15 and 76. Carp from Stations 205, 206, 209, 210, and 211 had relatively low lysozyme activity that was exceeded significantly by Stations 28, 29, 30, 70, 73, 77, 78, 79, 80, 81, 90, 111, and 112. Stations 26, 67, 68, 72, 75, 82, 83, 84, and 86 had significantly greater mean lysozyme than Stations 206, 209, 210, and 211 whereas mean lysozyme at Stations 27, 31, 76, and 89 was greater than at Stations 209-211. Finally, Station 71 exceeded Stations 209 and 210, and Stations 15 and 85 were greater than Station 210.

Sub-basin means for lysozyme activity in carp ranged from 2.3 mOD/min in the EIB Study Unit to 5.2 mOD/min in the ARR and LMS sub-basins (Fig. 3-11b). Statistical comparisons indicated that the UMO sub-basin was significantly lower than the ARR, LMO, LMS and UMS sub-basins, and that the EIB Study Unit was significantly lower than all other (NCBP) sub-basins. In comparing mean lysozyme activity for stations within sub-basins, activity at Station 31 was significantly less than at Station 90 within the LMO sub-basin; levels at Stations 111 and 112 were significantly greater than at Station 27 in the UMS sub-basin. Stations 111 and 112 had the two greatest mean lysozyme levels for carp in the MRB.

No data for lysozyme activity in carp were available from the reference site. Program means for NCBP (4.89 mOD/min) and NAWQA 2.33 (mOD/min) sites differed. As noted above, 45 of the 49 carp with lysozyme levels <2.0 mOD/min were collected at the EIB NAWQA sites and the NAWQA sites had the lowest mean values. Of the 84 fish included in the analysis for NAWQA sites, only six had lysozyme levels ≥ 5.0 mOD/min whereas at the NCBP sites 46% of carp had lysozyme levels of ≥ 5.0 mOD/min.

DISCUSSION

Many of the fish health metrics measured in this study have not been used previously in large-scale, regional or large river, basin-wide evaluations of freshwater systems. Hence, an important objective of the study was to accumulate information on the species of choice for comparison with other drainages and regions. However, because these indicators have not been used routinely in freshwater systems, we currently lack an understanding of how factors other than contaminants such as age, sex, reproductive status, season, geographic location, and innate species differences influence many of the indicators/markers. For most, we lack knowledge of "normal" ranges, even in carp and bass. In an attempt to eliminate as many confounding factors as possible, fish were all collected in late summer to fall, they were sexed and aged, and most species (or sometimes genera) were analyzed separately. Potential effects of sex and age on comparisons among stations or sub-basins were evaluated statistically. Analysis of the data indicated no effect of sex on external lesions, MA parameters or lysozyme activity in either carp or bass, HAI or SSI in bass, or condition factor in carp. Of the remaining indicators, HAI and SSI in carp and condition factor in bass differed between sexes. For these, the sexes were evaluated separately. Bass HSI was analyzed by gender due to the role of the liver in the female reproductive cycle. Age is known to significantly influence MA density, at least in some fishes (Brown and George, 1985) including largemouth bass (Blazer and others, 1987). For this reason age was considered in the statistical model for station and sub-basin comparisons of these parameters.

Bass and carp differed with respect to lysozyme activity. Plasma lysozyme activity in carp was consistently lower than in bass, often by more than 10-fold, which appears to be a species difference rather than an effect of environmental factors. Significant species differences have been reported previously, with activity levels ranging from 40 units/g in kidney tissue in tusk (*Bromse bromse*) to 33,500 units/g in rainbow trout (*Oncorhynchus mykiss*; Lie and others, 1989; Achazi and Leydecker, 1992). Although we could find no studies in which lysozyme activity was measured in largemouth bass, other researchers have reported low lysozyme values for carp. Some difficulties occur when comparing results due to differing methodologies and units of measurement, however. In one study, lysozyme values in control carp ranged from means of 0.607 $\mu\text{g/mL}$ for 1-year-old fish to 0.829 $\mu\text{g/mL}$ for 5-8-year old fish (Studnicka and others, 1986). Other studies demonstrated an increase in serum/plasma lysozyme activity

when carp were exposed to bacteria (Siwicki and others, 1990; Studnicka and Siwicki, 1990) or parasites (Studnicka and others, 1986; Studnicka and Siwicki, 1990). Conversely, stressful conditions such as low dissolved oxygen, starvation, and high salinity caused a decrease in lysozyme activity whereas transportation stress caused an increase (Hajji and others, 1990). Carp exposed to trichlorophen and sewage sludge had decreased lysozyme activity (Siwicki and others, 1990; Dunier and others, 1991). In a variety of studies involving other fish species, exposures to metals most often caused an increase (Sanchez-Dardon and others, 1999) or no change (Low and Sin, 1996; Sanchez-Dardon and others, 1999). An exception to this was plaice (*Pleuronectes platessa*) exposed to mercury (Fletcher and White, 1986) in which a decrease was noted. Conversely, exposure of various fish species to pesticides (Dunier and others, 1995), organic contaminants such as oil (Tahir and others, 1993; Tahir and Secombes, 1995) and creosote (Karrow and others, 1999), and sewage sludge (McVicar and others, 1988; Secombes and others, 1991; 1995; Price and others, 1997) have caused decreased levels, while no change was observed after exposure to PCBs (Hutchinson and others, 1999).

Little is known about the "normal" ranges of lysozyme activity in wild carp and *Micropterus* spp. In the MRB, comparatively high mean lysozyme activity (>120 mOD/min) in bass was recorded at Stations 71 (in the OHR sub-basin) and 28 (in the LMS), with Station 68 (OHR) having the lowest mean (<50 mOD/min). The highest means in carp (>6 mOD/min), which were much lower than in bass, occurred at Stations 80 and 81 (LMS) and 111 and 112 (UMS). Low lysozyme activity in carp (<2 mOD/min) was noted at stations in the EIB. The highest lysozyme activity in individual carp (12 mOD/min) and bass (>240 mOD/min) was at Station 28, which is also in the LMS sub-basin. Based on examination of the means, there were no stations at which lysozyme activity was consistently low or high in both species, but only carp were collected at many of our sites (for example, Station 80 and most NAWQA sites).

Laboratory investigations and field studies in which fish were collected from specific contaminated sites have generally indicated increases in MA parameters relative to reference sites or groups (reviewed by Wolke, 1992; Blazer and others, 1994; Blazer and others, 1997). As noted in the introduction, the USEPA's EMAP-Estuarines program (Summers and others, 1993; Fournie and others, 2001) and NOAA's Status and Trends programs (Chang and others, 1998) have both used MA as bioindicators. Some studies have used splenic MA's whereas others have utilized hepatic MA's; and most of the studies have been done with marine or estuarine fishes. In addition, most previous

studies have only evaluated MA density. Even for MA density, however, no regional baseline information exists to establish a "normal" value for any species. To our knowledge, there have been no studies evaluating carp or bass MA and potential effects of contaminant exposure; however, one study reported an increase in MA in largemouth bass exposed to thermal effluent from a nuclear power plant (Blazer and others, 1987).

Station means for MA density in the MRB were 2.2-11.2 MA/mm² in largemouth bass and slightly higher, ranging from 5.1 to 18.3 MA/mm², in carp. Statistical analysis of the carp data also suggested that unlike many fish species (see Agius, 1981), there is no consistent relationship between age and MA density in carp. Utilizing data collected in the EMAP-Estuarines program for a variety of estuarine fishes and irrespective of age, Fournie and others (2001) suggested that splenic MA densities of >40 MA/mm² in at least one fish from a site were correlated with hypoxic stress or high levels of sediment contamination. There is insufficient data to know if this is a reasonable reference number for freshwater fishes or how MA may be correlated with body burdens of various contaminants. However, none of the MRB fish exceeded 40 MA/mm². In fact, only three stations had individual carp with more than 30 MA/mm² (Stations 26, in the UMS sub-basin, and Stations 203 and 208, in the MSE Study Unit); and individual bass exceeded 20 MA/mm² at only three stations (74 and 112, in the UMS sub-basin) and 68 (in the OHR).

Condition factor (CF) may indicate changes at the organism level. Condition factor is affected most directly by nutrition (Tyler and Dunn, 1976), but factors such as season, sexual maturation, and disease can also affect it (Denton and Yousef, 1976; Adams and others, 1982; Möller, 1985). Exposure to contaminants such as pulp mill effluent has been linked to elevated CF (McMaster and others, 1991; Adams and others, 1992), whereas diminished CF has been observed after exposure to contaminants such as metals and petroleum (Kiceniuk and Khan, 1987; Munkittrick and Dixon, 1988; Miller and others, 1992). Condition factor can also vary among locations within a species (Doyon and others, 1988; Fisher and others, 1996). A survey of carp in the U.S. found mean CF ranging from 1.2 to >2.0 (Carlander, 1969). A similar survey of largemouth and smallmouth bass found mean CF of 1.1-1.9 and 1.2-1.9, respectively (Carlander, 1977). The range of CF station means for carp in the MRB was relatively narrow (1.1 to 1.5), but individual values ≥ 2.5 were computed for carp from Stations 67 and 111 (in the UMS sub-basin). Individual values <1.0 occurred at 15 stations, but no single station was notable for low CF in carp. Mean CF for bass had greater ranges (0.9-2.1 for males and 0.8-2.4 for females). Most of the bass with high CF

were from Station 77 whereas most of the low (<1.0) values were from Station 67.

The HSI may vary with season (Delahunty and de Vlaming, 1980; Beamish and others, 1996) and nutrition (Daniels and Robinson, 1986; Foster and others, 1993) as well as with gender and changes in gonadal status (Fabacher and Baumann, 1985; Förlin and Haux, 1990; Grady and others, 1992). It is also the organosomatic index for which changes are most often associated with contaminant exposure (Adams and McLean, 1985). Increased HSI has been reported with exposure of fish in the wild to organic contaminants, most often PAHs and PCBs whereas laboratory exposures of fish to metals, crude oil, certain pesticides, and bleached kraft mill effluent have resulted in HSI decreases (Dethloff and Schmitt, 2000). A comparative range for normal liver weight in fish is 1-3% of body weight, with relative weights greater than 2% being uncommon (Gingerich, 1982). In the MRB, only bass were included in HSI calculations due to the diffuse nature of carp liver. For male and female bass combined, 94% of HSI values were between 0.5% and 2.0%. All values at the reference site were <1.0%, which supports the conclusion from all of the data that low HSIs of 0.5-1.0% are not abnormal relative liver weights for bass. In contrast, relatively high mean HSIs (>1.8%) characterized female bass from Stations 67 and 68, both in the OHR sub-basin. Individual values >2.0% were also found in females from Stations 24 (also in the OHR) and 78 (in the ARR sub-basin) and in males from Stations 26 (in the UMS sub-basin), 67, and 68.

The SSI is measured to determine changes in the relative size of the spleen, a primary hematopoietic organ in fish. The SSI can differ among species, genders, and locations and can change over age and season (Krykhtin, 1976; Ruklov, 1979; White and Fletcher, 1985). Research has also documented changes in relative spleen size with exposure to chemical contaminants. Decreased SSI has been noted in fish exposed to organic contaminants alone or in combination with metals but increased SSI has rarely been seen with contaminant exposure (Dethloff and Schmitt, 2000). An increase in SSI is considered indicative of disease or immune system problems (Goede and Barton, 1990).

Because data on "normal" ranges for SSI of bass and carp are not available, we note stations with high and low SSI relative to others in the MRB. For bass, the lowest mean was calculated at four stations: 15 (LMS sub-basin); 24 (OHR); 77 (ARR); and 400, the reference site. There were multiple bass with low SSI values at a number of stations. Station 71 (OHR) had the highest mean and the highest individual SSI value. Station 112 (UMS) also had a high mean (> 0.2% of the body weight) and two individual values >0.4% of body weight. Station 26 (UMS) had a high

mean due to consistently high SSI. Considering all carp data, SSI covered a much wider range than SSI in bass. In carp, 88% of females and 85% of males were in a narrow range (0.1-0.5%) compared to the range for 91% of bass (0.05-0.3% of body weight). Low mean SSI and low individual SSI values occurred in both male and female carp from Stations 32 (UMO sub-basin) and 400. Relatively high station means for females (>0.5%) were generally influenced by one or two unusually large values (> 1.0%); these high individual values were found at Stations 15 and 76, in the LMS sub-basin; 31 and 83, in the LMO; 73, in the UMS; and 210, in the EIB Study Unit. High means for males (> 0.5%) at Stations 89 (LMO sub-basin) and 210 were also influenced by a few high individual values. Values for both carp and bass were all relatively low at Station 400. In contrast, a relatively high station mean (0.48%) was computed for male carp as well as all bass at Station 71 (OHR sub-basin). A low mean for bass at Station 15 contrasted with a high mean for female carp; however, the carp value was largely driven by one observation.

Most of the fish health variables presented here were limited to results for bass and carp collected at the various sites to remain consistent with other parts of the study. The exception was the proportion of fish with external lesions. The proportion of fish with disease or anomalies, irrespective of species, is used as a health metric in the IBI (Karr, 1981; Leonard and Orth, 1986) and the estuarine biotic integrity index (EIB—Deegan and others, 1997). In addition, a number of recent studies have compared sites using external anomalies of all fish species collected in estuarine and freshwater systems (Fournie and others, 1996; Sanderson and van den Berg, 1999). A high prevalence of external anomalies has been correlated with exposure to anthropogenic stressors in numerous studies (Sindermann, 1979; McCain and others, 1992; Fournie and others, 1996). Fin erosion (Murchelano and Ziskowski, 1982; Cross, 1985; Reash and Berra, 1989; Lindesjoo and Thulin, 1990), skin and liver tumors (Malins and others, 1988; Vogelbein and others, 1990; Baumann and others, 1991), and skeletal deformities (Bengtsson, 1979; Mehrle and others, 1982; Bengtsson, and others 1985) are the anomalies most commonly associated with degraded environments. However, other lesions such as skin ulcerations and eye abnormalities have also been suggested to be a result of anthropogenic stress (Hargis and Zwerner, 1988).

The overall proportion of fish with external abnormalities in the MRB was 0.225 (of a total of 1376 examined). In a study evaluating the use of the IBI in small coolwater streams, Leonard and Orth (1986) found that the proportion of fish with abnormalities was 0.080-0.344 in "degraded" streams, versus 0-0.01 for streams only mildly affected. In a study

of streams in Ohio, proportions ranged from 0.004 to 0.081 for DELT (deformities, erosions, lesions, tumors) anomalies (Sanderson and van den Berg, 1999). Background prevalences of gross abnormalities in estuarine fishes were estimated to be 0.5% in the mid-Atlantic and 0.7% in Gulf Coast estuaries, whereas weighted-average prevalences ranging from 0.45% in the mid-Atlantic to 0.88% in the Louisiana Providence (Fournie and others, 1996). Care must be taken in comparing our results with those of other studies and even in comparing stations within this study for a number of reasons. First, it is widely recognized that errors in proportion of anomalous fish can result from biased or differential examination of fish, species composition, habitat, and other factors unrelated to environmental degradation (Leonard and Orth, 1986). A higher incidence of anomalies was noted the second year versus the first in the study of small, coolwater streams. Since this was consistent throughout the study area, the authors suggested that it represented systematic error caused by increased familiarity and efficiency in identifying anomalies (Leonard and Orth, 1986). In our study this error could be compounded by the fact that many individuals from several offices and organizations were involved in assessing external lesions. A second confounding factor in comparing the results of various studies is the different anomalies that are considered. In the fish health metric of the IBI all external signs of disease, parasites, or anomalies are considered (Karr, 1981). In contrast, Fournie and others (1996) examined only the eyes, body surface, fins and branchial chamber, noting discolorations, raised scales, exophthalmia, white or black spots, ulcers, fin erosion, visible tumors and parasites. Sanders and others (1999) noted only deformities of the fins, head, vertebrae, barbels, and opercles; erosion of the fins, opercles, or barbells; and lesions (open sores, ulcerations); and tumors, but not external parasites. We evaluated abnormalities of the body surface, eyes, opercles, and fins, including deformities and parasites.

We found external lesions on 28% of the 447 bass examined from 29 stations and on 20.4% of 775 carp from 46 stations. At 16 of the 27 stations where both species were collected, a greater proportion of bass than of carp had external lesions. Conversely, Fournie and others (1996) reported a higher prevalence of external lesions on demersal than on pelagic fishes from the Gulf of Mexico. Our findings also differ from the findings of Sanders and others (1999), who used external anomalies to characterize biological integrity in seven Ohio streams. Of the 2624 carp they collected, 28.5% had external anomalies versus only 2.9% of 5037 bass (largemouth, spotted, and smallmouth). In the MRB, high proportions of bass with external lesions (>40%) were collected at Stations 15 and 81, in the LMS sub basin; Station 24,

in the OHR; Station 26, in the UMS; and at Stations 78 79, and 82, in the ARR. At Stations 15 and 24 fewer than 10 bass were collected, however. Overall, a lower percentage of carp than bass had external lesions, but at eight stations [79, 80 (LMS), 82, 89 (LMO), 201, 202 and 203 (MSE), and 209 (EIB)] >40% of carp had lesions. Again, at two of these stations (89 and 209) the sample size was small (<10). High percentages of both carp and bass had external lesions at Stations 79 and 82 (both in the ARR sub-basin). For both carp and bass, histopathological examination indicated that most external lesions were parasites or acute inflammation, the latter probably from bacterial infections.

The HAI, which is also an assessment of grossly visible lesions or changes, is more comprehensive than the incidence of external lesions because it accounts for both external and internal abnormalities. To our knowledge this methodology has not previously been used with carp, thus precluding comparisons with other studies. However, the HAI has been used to assess largemouth bass populations, particularly in Tennessee Valley Authority (TVA) reservoirs in the Southeast. In a survey of 28 reservoirs, the mean HAI for all TVA reservoirs was 62, the "healthiest" reservoir averaged 17, and the worst had a mean of 79 (Adams and others, 1993). The HAI of largemouth bass from Hartwell Reservoir, which is contaminated with PCBs, ranged from 42 at the reference site to 64 at an intermediate site and 74 at the most contaminated site. Mean PCB concentrations (wet weight basis) of bass filets from these sites were 0.3, 2.0 and 21 µg/g, respectively (Adams and others, 1993). In the Catawba River system, which originates in the Appalachian Mountains and ultimately becomes part of the Santee-Cooper basin in South Carolina, largemouth bass were collected and assessed from 27 stations representing habitats ranging from minimally impacted to areas influenced by industrial and sewage effluents and combinations of stressors (Coughlan and others, 1996). In this study, station mean HAI scores averaged 42 and ranged from 18 at minimally impacted sites to 94 at sites with combined stressors. A positive linear relation between average fish weight and HAI score and between age and HAI score was also noted. For this reason Coughlan and others (1996) suggested that only bass between 250 and 450 mm (TL) be included. In our study, individual bass from a number of stations in the MRB exceed 450 mm. However, if we assume from previous studies that a mean bass HAI for an un-impacted or minimally impacted site is ≤20, seven stations (23, in the OHR sub-basin; 77, in the ARR; 72, 74, and 111, in the UMS; and 212 and 213, both in the MSE Study Unit) fit this category. Carp of both sexes from Stations 80, 26, 27, 72, 111, 112, 70, 203, 204, 206, 208, 212 and 400 also had mean HAI values of <20 as did female

carp from Stations 86 and 201 and male carp from Stations 78, 30, 75, 76, 25, 68, 205, 210, 211, 202. Bass from four stations (82, 83, 15, and 112) exceeded the "worst" HAI station mean of 79 in the TVA study (Adams and others, 1993), whereas only one (Station 15) exceeded the highest HAI station mean of 94 in the Catawba study (Coughlan and others, 1996). Carp of both sexes from Stations 15 (in the LMS sub-basin) and 31 (in the LMO) exceeded the TVA station mean of 79, whereas only female carp exceeded this level at Station 89 (also in the LMO; no bass collected at Station 31 or 89).

A combination of the fish health indicators in both species indicated potential contaminant stress at certain stations. Station 67 had low CF for both bass and carp, high HSI in bass, low SSI in male carp and large carp MA. Station 68 had low CF and high HSI for both male and female bass, low lysozyme and a high density of splenic MA in bass; however, a low prevalence of external lesions in bass and high CF in carp were also found at this station. Station 70 also had low CF for bass and carp, high HSI in bass, as well as large MAs in both species. All of these stations were in the OHR sub-basin. As noted elsewhere in this report, fish from Station 67 contained somewhat elevated concentrations of many contaminants including DDT, dieldrin, PCBs, TCDD-EQ, Cd and Pb. Fish from Station 68 had elevated levels of chlor-dane, dieldrin, PCBs and TCDD-EQ, and at Station 70 concentrations of DDT and dieldrin were slightly elevated, along with somewhat elevated PCB, TCDD-EQ, Hg and Pb. Station 79, in the ARR sub-basin, had high prevalence of external lesions in both bass and carp, low bass SSI and a high percent of bass splenic tissue occupied by MAs. Station 82, also in the ARR sub-basin, also had a high prevalence of external lesions in bass and carp as well as a high mean HAI for bass. Low levels of DDT contamination in carp and DDT and Hg in bass were detected at Stations 79 and 82 as well as high dioxin-like activity in one bass at Station 79. Concentrations of Pb, Zn and As were also slightly elevated at Station 79. Although only four bass were collected at Station 15 (in the LMS sub-basin), it was notable for the high incidence of external lesions, the highest mean HAI score (and the only mean to exceed the highest station mean in the Catawba study; Coughlan and others, 1996), low lysozyme activity and low SSI in bass. Supporting the bass data, the mean HAI for male carp (only two individuals) and female carp (seven individuals) at Station 15 exceeded the HAI mean for the "worst" station in the TVA study (Adams and others, 1993). At Station 15, carp contained small amounts of dieldrin and moderately high concentrations of PCBs.

In summary, with the exception of the few sites noted above, most of the carp and bass examined in this study appeared relatively healthy. At no sta-

tions did we observe high incidences of fish health problems indicative of contaminant exposure, such as skeletal deformities, fin erosion, skin ulcers, papillomas, or liver neoplasms. The biomarker differences noted between stations and sub-basins in the MRB were subtle and probably indicate sublethal stress that may have been caused by many factors including, but not necessarily or exclusively, exposure to chemicals.

Chapter 4. Reproductive Biomarkers

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INTRODUCTION

The 1995 Mississippi River Basin (MRB) study was implemented to field test a suite of methods for evaluating exposure to and effects of contaminants in aquatic environments (BEST, 1996; McDonald and others, 2000; Chapter 1, this report). Among the methods under consideration was a group of biomarkers for assessing the reproductive health of individual fish in a given population. Reproductive success is often difficult to evaluate, especially in aquatic environments where fish and other wildlife are not easily captured or contained for convenient monitoring. Consequently, the development of techniques for measuring reproductive indicators, such as sex steroid hormones, vitellogenin (vtg), gonadosomatic indices (GSI), and gonadal histopathology, has aided researchers in assessing the reproductive health of many fish species.

The primary objective of this chapter is to evaluate the performance of the reproductive biomarkers incorporated into the study (Chapter 1, this

report) and, in doing so, examine the reproductive health of fish inhabiting the MRB. The reproductive biomarkers used in this study and nominated for incorporation into future BEST program projects include: sex steroid hormones, 17 β -estradiol (E2) and 11-ketotestosterone (11-KT); vtg; GSI; and gonadal histopathology. The latter was used for the analysis of sex, stage, oocyte atresia, and other gonadal anomalies including ovotestes. With regard to the monitoring effort, this study sought to establish biomarker ranges for the species sampled and identify specific stations where contaminant exposure is occurring or has occurred and where reproductive/endocrine function may be abnormal.

A number of environmental factors are known to influence endocrine and reproductive activity in fish, including sex, age, species, reproductive stage, season, photoperiod, and water temperature. In addition, a growing body of evidence implicates anthropogenic contaminants as a source of many adverse reproductive alterations (Atterwill and Flack, 1992). Exposure to both natural and synthetic chemicals has been linked to reduced fertility, hatchability of eggs, and survival of offspring; impaired hormone

production or activity; and modified adult sexual behavior (Colborn and Clement, 1992). Over the last few decades, sex steroid hormones have evolved as convenient biomarkers for detecting contaminant-induced biochemical alterations. Studies have documented alterations in reproductive hormone concentrations of fish exposed to bleached kraft mill effluent (Munkittrick and others, 1991; McMaster and others, 1991; Munkittrick and others, 1992; Munkittrick and van der Kraak, 1994), agricultural pesticides (Singh and Singh, 1987; Singh and Singh, 1991; Singh and others, 1994; Gross and others, 1997; Goodbred and others 1997), industrial chemicals (Sivarajah and others, 1978; Spies and others, 1996), and heavy metals (Thomas, 1988; Allen-Gil and others, 1993).

Vtg, a yolk protein precursor secreted by the liver of non-mammalian female vertebrates in response to estrogen stimulation, provides an additional biomarker for assessing reproductive health and predicting the estrogenicity of various compounds. Early studies suggested that healthy males do not produce vtg, while males treated with estrogen produced significant concentrations (Bromage and Cumaratunga, 1988; Purdom and others, 1994). Therefore, it was believed that the detection of plasma vtg in males would serve as a reliable biomarker of exposure to environmental estrogens (Sumpter and Jobling, 1995; Heppell and others, 1995; Denslow and others, 1996; Palmer and Selcer, 1996; Denslow and others 1997; Sherry and others 1999).

GSI and gonadal histopathology fall into a category of indicators that provide both structural and functional information about maturational stage. There is substantial evidence that most animal species undergo reproductive cycling, and frequently dramatic variation in gonadal size is observed throughout the cycle (de Vlaming and others, 1981). Therefore, GSI measurements have routinely been used to determine reproductive maturity, as well as assess gonadal changes in response to environmental dynamics (for example, seasonal changes) or exogenous stresses (for example, contaminant exposure). A reduction in GSI and impaired gonadal development has been reported in response to environmentally relevant doses of dietary mercury in juvenile walleye (*Stizostedion vitreum*) (Friedmann and others, 1996), organophosphate insecticides in female Asian redtailed catfish (*Mystus vittatus*) (Choudhury and others, 1993), and Metacid-50 and carbaryl in climbing perch (*Anabas testudineus*) (Haider and Upadhyaya, 1985).

Gonadal histopathology has been used to confirm gonadal phenotype, determine the stage of sexual development, and investigate reproductive impairment. In field studies, this technique allows investigators to rapidly examine many potential sites

of injury under *in vivo* conditions while conserving the structural integrity of the cells and organelles from the isolated organ or tissue (Wester and Canton, 1986). Although histopathology is routinely used to detect higher level responses expressed as morphological abnormalities, such as the presence of ovotestes and oocyte atresia, this method is capable of providing information at all levels of biological organization (that is, distribution of molecules; distribution, number, volume, morphology of organelles, cells, and organs). Observed alterations in cells and tissues are often subsequent to, and reflective of, previous biochemical and physiological modifications.

In a field study of fathead minnows (*Pimephales promelas*) subjected to acidic water conditions, histological examination revealed an association between oocyte atresia and reproductive success (McCormick and others, 1989). The ability to detect increased degeneration or necrosis of developing oocytes by histological examination has inspired the use of oocyte atresia as a biomarker of reproductive impairment.

Each of the biomarkers examined in this study has proven to be a valuable tool for measuring reproductive activity in a variety of laboratory and field studies. However, with the exception of the 1994 reconnaissance study conducted by the USGS National Water Quality Assessment (NAWQA) program (Goodbred and others, 1997), information regarding the ability of these techniques to produce interpretable information when applied to large-scale (for example, regional or national) studies of fish in their natural habitats is limited. Results from Goodbred and others (1997) suggested that fish from various streams were experiencing endocrine disruption and, in some areas, the alterations were correlated with contaminant exposure. Goodbred and others (1997) identified the MRB as a region demonstrating some of the strongest evidence of potential endocrine disruption. As noted in Chapter 1 (this report), these findings were among the reasons this study focused on the MRB.

METHODS AND MATERIALS

Laboratory Procedures

The following section describes the laboratory procedures used in this study. Methods and materials for fish collection and processing are described in Chapter 1.

Sex Steroid Hormones

Concentrations of E2 and 11-KT in plasma samples collected from common carp (*Cyprinus carpio*,

hereafter carp), black bass (*Micropterus* spp.—bass), and other fishes (as described in Chapter 1 of this report) were measured by radioimmunoassay (RIA). Blood was collected in the field, transferred to a heparinized 5-mL vacutainer, chilled on wet ice, and centrifuged for 10 minutes at 1000 x g. Plasma was pipetted into 2-mL cryotubes, immediately frozen on dry ice and shipped on dry ice from field sites to the Florida Caribbean Science Center (FCSC) and stored at -80°C . 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 min, 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 11-KT (1, 5, 10, 25, 50, 100, 250, 500 and 1000 pg) and a constant concentration of radiolabeled hormone. Cross-reactivities of the E2 antiserum with other steroids were: 11.2% for estrone; 1.7% for estriol; $<1.0\%$ for 17α -estradiol and androstenedione; and $<0.1\%$ for all other steroids examined. Cross-reactivities of the 11-KT antiserum with other steroids were: 9.65% for testosterone; 3.7% for dihydrotestosterone; $<1.0\%$ for androstenedione; and $<0.1\%$ for all other steroids examined. 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 1000 x g for 10 min. Supernatant aliquots (0.4 mL) containing bound radiolabeled hormone were carefully removed so as not to disturb the charcoal pellet 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.

Hormone concentrations in plasma samples from common carp were corrected for an extraction efficiency of $92 \pm 2.8\%$ for E2 and $86 \pm 3.3\%$ for 11-KT. The minimum concentration distinguishable from zero was 6.4 pg/mL for E2 and 8.1 pg/mL for 11-KT. Pooled samples (approximately 275 pg E2/mL and 220 pg 11-KT/mL) 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 stan-

dard curve, with the tests for homogeneity of regression indicating that the curves did not differ. Further characterization of the assays involved measurement of known amounts (1, 2, 5, 10, 25, 50, 100, 250 and 500 pg) of E2 or 11-KT in 50 μL charcoal-stripped plasma. Results of these analyses were $Y=11.36 + 0.97X$, $r^2=0.9271$ for E2; and $Y=22.3 + 0.94X$, $r^2=0.8767$ for 11-KT, where Y = the amount of E2 or 11-KT measured (pg) and X = the amount of E2 or 11-KT added (pg). Inter- and intra-assay coefficients of variation were 7.3% and 9.5% respectively for plasma E2 and 9.1% and 8.7% respectively for plasma 11-KT.

Plasma samples from bass were analyzed similarly. They were corrected for an extraction efficiency of $87 \pm 3.5\%$ for E2 and $83 \pm 2.8\%$ for 11-KT. The minimum concentration distinguishable from zero was 5.1 pg/mL for E2 and 9.3 pg/mL for 11-KT. Pooled samples (approximately 310 pg E2/mL and 275 pg 11-KT/mL) 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 measurement of known amounts (1, 2, 5, 10, 25, 50, 100, 250 and 500 pg) of E2 or 11-KT in 50 μL charcoal-stripped plasma. Results of these analyses were $Y=-9.63 + 1.12X$, $r^2=0.9081$ for E2; $Y=21.4 + 0.97X$; and $Y=13.8 + 0.93X$, $r^2=0.8767$ for 11-KT, where X and Y are as described above. Inter- and intra-assay coefficients of variation were 5.8% and 7.9% respectively for plasma E2 and 6.4% and 8.4% respectively for plasma 11-KT.

The ratio of E2 to 11-KT (E/KT) is an additional variable used to analyze sex steroids (Folmar and others, 1996; Hileman 1994). Typically, female fish will have an E/KT ratio greater than 1.0, and male fish will have an E/KT ratio below 1.0, although exact ranges of normality and seasonal fluctuations in this variable have not been established.

Vitellogenin: Vtg concentrations in plasma samples from male and female carp and bass were quantified using an Enzyme-Linked Immunosorbent Assay (ELISA) as described by Folmar and others (1996). Microtitre plates (96-well) were coated with purified anti-carp vtg monoclonal antibody and incubated overnight. The next day, plates were washed with Tris Buffered Saline/Tween 20 (TBST) solution, and incubated with bovine serum albumin (BSA) to block non-specific antibody binding. After thoroughly washing with TBST, plasma samples (diluted from 1:500 to 1:5000) were added in duplicate to the plates and incubated overnight. Standard curves were constructed by adding serial dilutions of purified vtg to male control plasma (from male control fish) and processed

according to the same method. The following day, the plates were washed with TBST and incubated with a rabbit anti-vtg polyclonal antibody for 2 h. The rabbit antibody binds to the vtg captured by the monoclonal antibody in the first step. The polyclonal antibody was in turn bound by a goat anti-rabbit IgG conjugated to alkaline phosphatase, which was applied to the wells and allowed to incubate for 2 h. After a final series of washes with TBST, *p*-nitrophenyl phosphate in carbonate buffer was added to each well and incubated for 30 min. The *p*-nitrophenyl phosphate served as a substrate for the alkaline phosphatase; this reaction generated a yellow color that was quantified by measuring the absorbance at 405 nm using an automated ELISA reader. Vtg concentrations were determined by subtracting values obtained from male control plasma and comparing to standard curves.

Gonado-somatic Index and Gonadal Histopathology:

Body and organ weights of individual fish were determined in the field, as described in Chapter 1. GSI was computed as gonad weight (W_g) expressed as a percentage of body weight (W_b): $GSI=100(W_g / W_b)$.

For gonadal histology, one-third to one-half of a gonad was removed and immediately fixed in NoToX® solution (Earth Safe Industries) in the field. Transverse sections were routinely processed for light microscopy, embedded in paraffin, sectioned at 5 μ m and stained with hematoxylin and eosin (H & E). Developmental stages (designated 0-5) were used to classify each section (Treasurer and Holliday, 1981; Nagahama, 1983; Rodriguez and others, 1995; McDonald and others, 2000). In carp and bass it is not unusual to observe a number of oocyte stages in one ovary. Consequently, ovaries were classified on the basis of the most prevalent stage. Stage 0 was assigned to samples with undeveloped, previtellogenic oocytes. These oocytes, representative of chromatin-nucleolus and early perinucleolus stages, generally measured less than 250 μ m in diameter and contained cytoplasm that stained basophilic with H & E. Stage 1 was assigned to samples in early development where most of the oocytes (>90%) were previtellogenic; the rest were early to mid-vitellogenic. There were no late-vitellogenic or post-ovulatory follicles observed in samples assigned to stage 1. Stage 2 was defined as mid-development and contained follicles that were primarily early and mid-vitellogenic. Early vitellogenic oocytes are defined as oocytes with few to moderate numbers of vitelline granules measuring up to 300 μ m in diameter. Mid-vitellogenic oocytes were larger (approximately 300-600 μ m in diameter), contained yolk vesicles at the periphery of the cytoplasm that stained eosinophilic with H & E stain, and chorion tissue was uniformly observed. Oocytes in stage 3, the late development stage, contained mostly late-

vitellogenic follicles. These oocytes measured 600-1000 μ m in diameter, contained yolk globules throughout the cytoplasm, and the chorion increased in thickness. Stage-4 oocytes were also in late development; however, the developing follicles were much larger (>1000 μ m in diameter) than stage-3 oocytes. Stage-4 oocytes were only detected in female bass. Although post-ovulatory follicles did not appear often in any tissue sample, these oocytes were designated stage 5. For the assessment of oocyte atresia, ovarian tissue samples were prepared according to the protocol above and evaluated by light microscopy. One hundred oocytes were counted when possible, and those showing morphological evidence of resorption or necrosis were quantified and the percentage of atretic oocytes was calculated.

Analogous to the procedure used to stage ovaries, male gonadal tissue was classified into five developmental stages (0-4) according to the maturity of the predominant stage of spermatogenesis of each tissue sample (Nagahama, 1983). Immature, undeveloped testes undergoing the earliest stages of spermatogenesis were assigned to Stage 0. The tissue samples classified as stage 0 contained primarily spermatocytes, with no apparent spermatozoa. Stage 1, the early spermatogenic stage, was assigned to tissue largely containing spermatocytes and spermatids, with some spermatozoa present. A combination of cell types, including spermatocytes, spermatids, and spermatozoa, were present in roughly equal amounts in stage 2 (mid-spermatogenic) tissue. In stage 3, the late spermatogenic stage, mature spermatozoa predominated although all stages of development are present, and in stage 4 the testes are spent.

RESULTS

Preliminary Analysis and Presentation of Raw Data

In addition to carp and bass, 154 fish of mixed sex and age representing 17 other species were collected from 11 stations, as described in Chapter 1 and Appendix A of this report. The gonadal stage of most of these additional fish were not determined. Preliminary analyses of data for carp and bass indicated that the reproductive biomarkers varied considerably between species and between sexes. This necessitated the separate assessment of each sex/taxon group and precluded statistical comparisons of species. Many of the reproductive indicators were also correlated with reproductive stage. Consequently, none of the other taxa were sufficiently represented to

make comparisons or statistically test for differences among stations or sub-basins. Therefore, reproductive indicators were assessed and reported by station for carp and bass, the predominant taxa. For these species, the biomarkers were also evaluated separately by gender. Results for other taxa are presented graphically in Appendix B of this report (Figs. B-1, B-2).

For carp and bass, statistical analysis began with descriptive and graphical displays of the complete data set and a preliminary examination to detect relationships among the variables (biomarkers), identify outliers, and determine the most appropriate statistical methods for analyzing each biomarker. These preliminary observations were also used to select an appropriate trimmed data set for complete statistical analysis. Possible confounding effects included temporal and geographical influences, age, and reproductive stage, each of which was assessed in the preliminary analyses.

Examination of reproductive biomarker results from stations where more than one *Microperus* sp. (largemouth bass, *Micropterus salmoides*; smallmouth bass, *M. dolomieu*; and spotted bass, *M. punctulatus*) were collected indicated that these taxa could be combined for analysis. In general, males and females of both carp and bass differed with respect to each of the reproductive indicators. E2 was typically higher in females, whereas 11-KT was higher in males. As a result, E/KT ratios were normally >1 for females and <1 for males. GSI was higher in female carp than male carp, and reproductive status was more advanced; female carp were primarily in stage 3, whereas male carp were usually in stages 1 to 2 (Fig. 4-1). Female bass were primarily in stages 2 to 3, males were most often in stages 1 to 2 (Fig. 4-2), and GSI was higher in the females. Although vtg was detected in a small percentage of males (approximately 16%), the concentrations were rarely as high as they were in females.

The following is a descriptive summary of results from the analysis of the entire data set. Fish were collected from 48 stations, each assigned a number between 15 and 400. Of these stations, numbers 15 through 112 were National Contaminant Biomonitoring Program (NCBP) stations and were located along large rivers; 201 through 213 were National Water Quality Assessment program (NAWQA) fixed sites and were located along smaller streams or reservoirs; and Station 400, which is located on the water supply reservoirs of the Leetown Science Center in Kearneysville, WV. Station 400 was included as a potential reference site. Station locations are presented in Chapter 1 (Fig. 1-1 and Table 1-1).

Female Carp

E2 concentrations in 387 female carp from 45 stations were analyzed (Fig. 4-3). E2 was only weakly correlated with stage. Concentrations reported in individual fish ranged from 23 pg/mL at Station 201 (stage 3) to 5126 pg/mL at Station 207 (stage 3). The highest E2 concentrations (>3000 pg/mL) were reported at Stations 27, 28, 72, 84, 85, 112, 205, 206, 207, 210, and 211. Conversely, E2 concentrations were uniformly low (<1000 pg/mL) at Stations 15, 24, 25, 31, 32, 68, 70, 75, and 89, although all or most of these females were in stage 2 or 3. E2 concentrations reported for females (all stage 3) at the reference site (Station 400) were between 300-1250 pg/mL.

11-KT concentrations in 375 female carp from 45 stations were analyzed (Fig. 4-3). 11-KT was not correlated with stage. Individual fish reported concentrations ranging from 31 pg/mL at Station 77 to 2008 pg/mL at Station 212. Stations 30, 31, 67, 78, 84, 86, 208 and 212 each contained one female with relatively high 11-KT concentrations (>1400 pg/mL). Conversely, Stations 24, 25, 32, and 68 reported females with uniformly low 11-KT concentrations (all fish below 250 pg/mL). Concentrations reported for females at the reference site (Station 400) were between 103-481 pg/mL.

E/KT ratios were determined for 375 female carp from 45 stations (Fig. 4-3). Of the 375 individuals, 49 (13%) had ratios <1.0. Ratios below 1.0 were observed in all stages of gonadal maturation examined (0-3) and were abundant or predominant at Stations 26, 31, 70, 80, 86, 89, and 90. All females at the reference site (Station 400) were in stage 3 and reported E/KT ratios >1.0.

GSI was measured in 367 female carp from 43 stations (Fig. 4-3). GSI, which was strongly correlated with stage, ranged from 0.162% at Station 76 (stage 0) to 25.9% at Station 83 (stage 3). The high variability may have resulted in part from fractional spawning, which is common in this species (Carlander, 1969). However, there were several stations that reported less variability. For instance, GSI was uniformly medium to high (10-20% of body weight) at Stations 25, 26, 27, 81, 84, 89, and 212, whereas at Stations 32, 67, 75, 76, and 111 GSI was uniformly medium to low (2-10% of body weight). Values for females (all stage 3) at the reference site (Station 400) ranged from 4% to 13%.

Vtg concentrations in 384 female carp from 45 stations were analyzed (Fig. 4-3). Vtg was correlated with stage. Vtg concentrations >5 mg/mL occurred in primarily stage-3 females at Stations 26, 27, 32, 68, 77, 84, 86, 111, 112, 210, 211, and 212; the highest vtg concentration (16,2010 mg/mL) was detected in a stage-3 female at Station 27. Conversely, there was at least one female with undetectable concentrations of vtg at Stations 29, 30, 31,

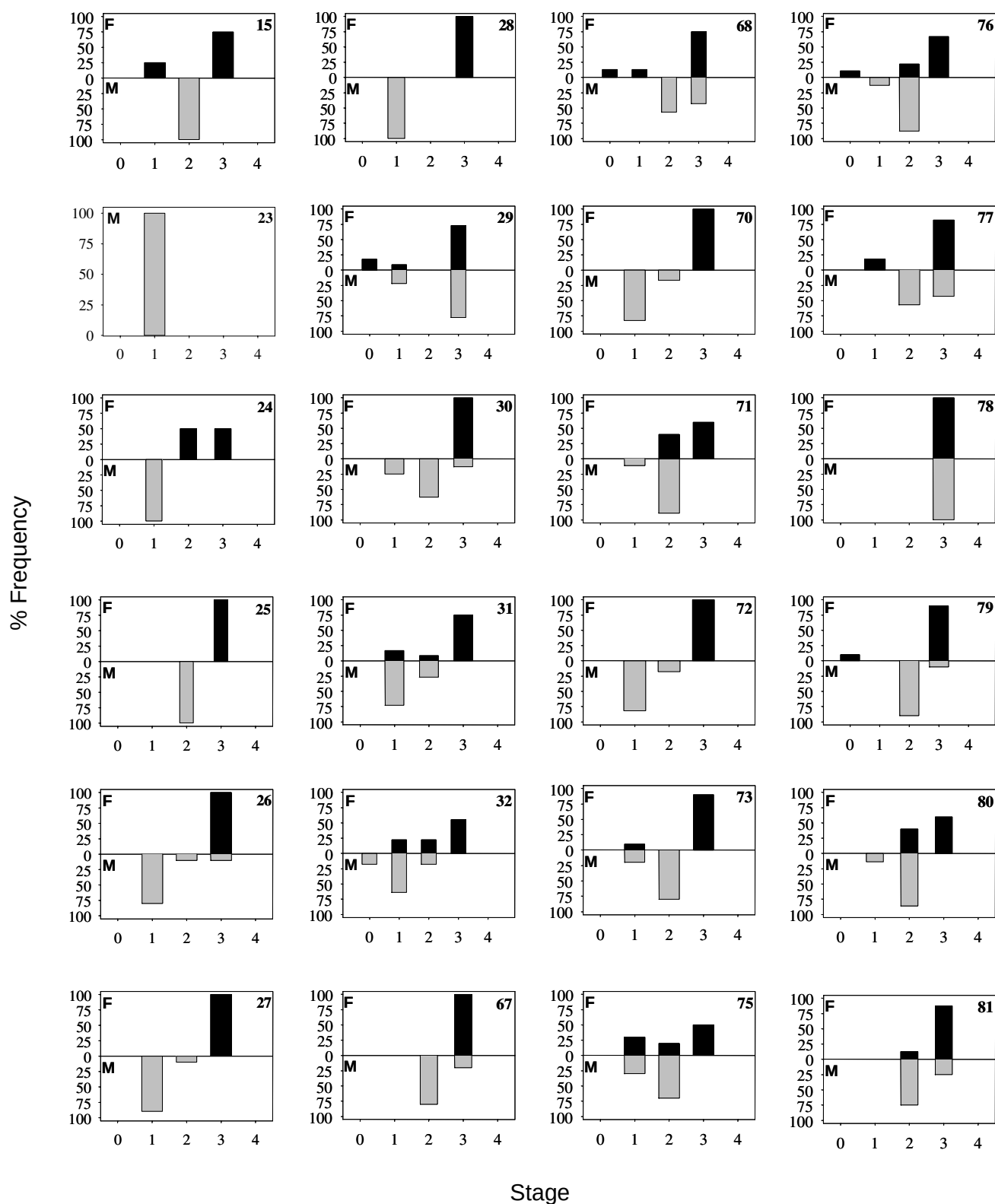


Figure 4-1. Proportional distribution of reproductive stage in carp (all data), by station, for the Mississippi River basin and the reference site (Station 400). See Table 1-1 and Figure 1-1 for station locations.

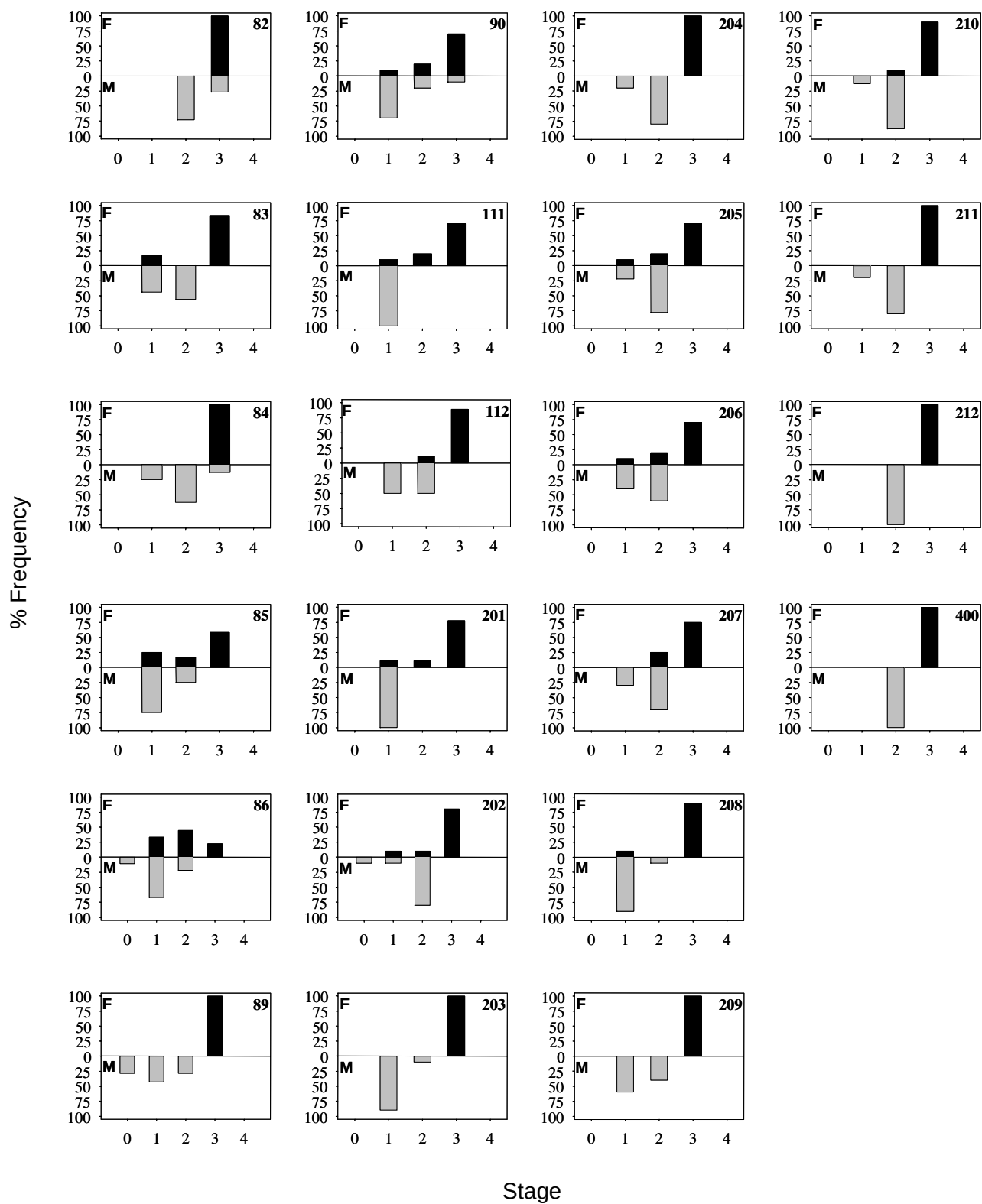


Figure 4-1. Proportional distribution of reproductive stage in carp (all data), by station, for the Mississippi River basin and the reference site (Station 400). See Table 1-1 and Figure 1-1 for station locations--Continued.

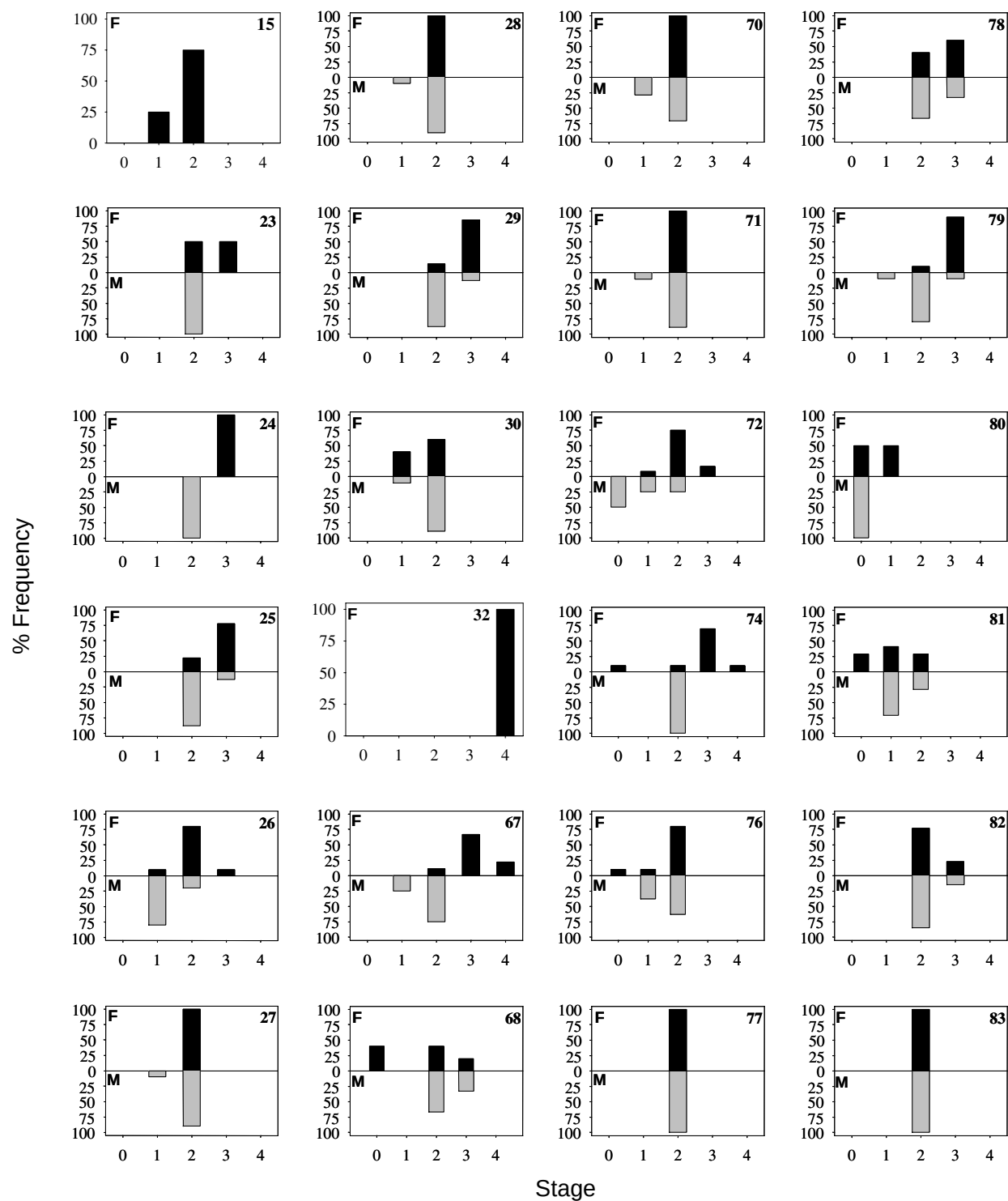


Figure 4-2. Proportional distribution of reproductive stage in bass (all data), by station, for the Mississippi River basin and the reference site (Station 400). See Table 1-1 and Figure 1-1 for station locations.

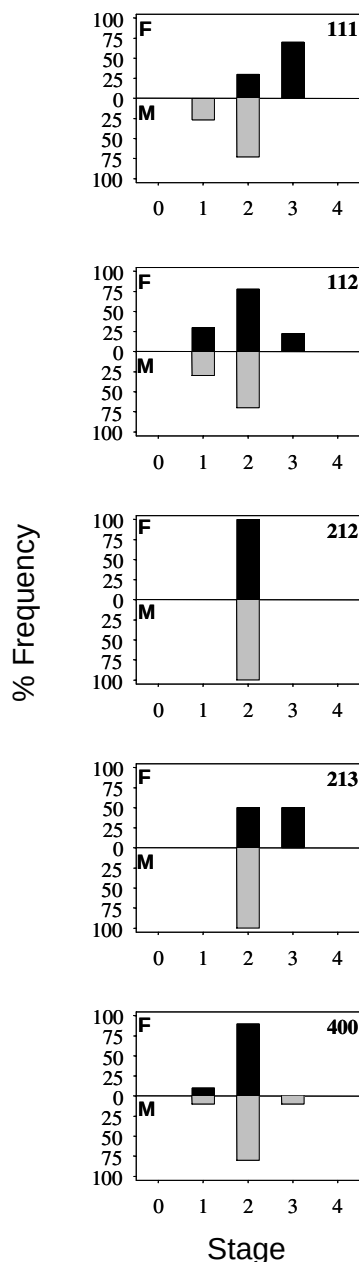


Figure 4-2. Proportional distribution of reproductive stage in bass (all data), by station, for the Mississippi River basin and the reference site (Station 400). See Table 1-1 and Figure 1-1 for station locations--Continued.

71, 77, 80, 84, 85, 90, 112, 201, 210, and 400. The collection dates ranged from late August to early November at stations with fish having both high and low vtg; therefore, time of collection did not appear to

influence vtg trends for the stations identified above. Furthermore, all of the females reporting no detectable vtg were at least 2 years of age, and most were older. Uniformly low vtg concentrations characterized fish from Stations 71, 80, 89, and 201, although most females at those sites were in stages 2 and 3, and none were estimated to be younger than 3 years of age. Vtg concentrations in females (all stage 3) at the reference site (Station 400) were also somewhat low, ranging from non-detectable to 2.8870 mg/mL.

Percentages of atretic oocytes in 380 female carp from 45 stations were determined (Fig. 4-3). Percent atresia was not correlated with stage. Individual fish percentages ranged from 0% (at least one female at approximately half of the stations) to 65% at Station 204. In addition to Station 204, one or more females with high percentages ($\geq 25\%$) were identified at Stations 26, 29, 32, 70, 71, 75, 82, 112, 201, 202, 205, 207, 210, and 212. Atresia in females from the reference site (Station 400) was 0-18%; however, atresia was $>10\%$ in only one female from this station.

Male Carp

E2 concentrations in 388 male carp from 45 stations were analyzed (Fig. 4-4). E2 was not correlated with stage. Concentrations in individual fish ranged from 17 pg/mL at Station 212 to 2918 pg/mL at Station 85. At no stations were concentrations uniformly high or low. At Stations 72, 79, 83, 84, 85, 90, 112, 206, 207, 209, and 212 there was at least one male with an E2 concentration above 1400 pg/mL. Conversely, one or more males at Stations 28, 202, 203, 204, 205, 207, 209, 210, and 212 had an extremely low E2 concentration (<100 pg/mL). Other than Station 28, all of the low-E2 stations were NAWQA sites. E2 concentrations in males at the reference site (Station 400) were between 135-419 pg/mL.

11-KT concentrations in 388 male carp from 45 stations were analyzed (Fig. 4-4). Males from stations with elevated 11-KT concentrations were primarily in later gonadal stages (2 and 3); nevertheless, 11-KT was not strongly correlated with stage. Concentrations in individual fish ranged from 58 pg/mL at Station 204 (stage 2) to 8492 pg/mL at Station 28 (stage 1). Greatest concentrations and variability occurred at Stations 24, 26, 27, 28, 32, 70, 73, and 75. Although not among stations with the highest concentrations, 11-KT concentrations at Stations 67, 73, and 85 were uniformly high, ranging from approximately 1200 to 4300 pg/mL. Conversely, 11-KT concentrations at Stations 15, 80, 201, 204, and 207 were uniformly low (<750 pg/mL). Otherwise, there was substantial variability within stations. It should also be noted that stations with the lowest concentrations were all located in the lower Mississippi region, and concentrations were lower

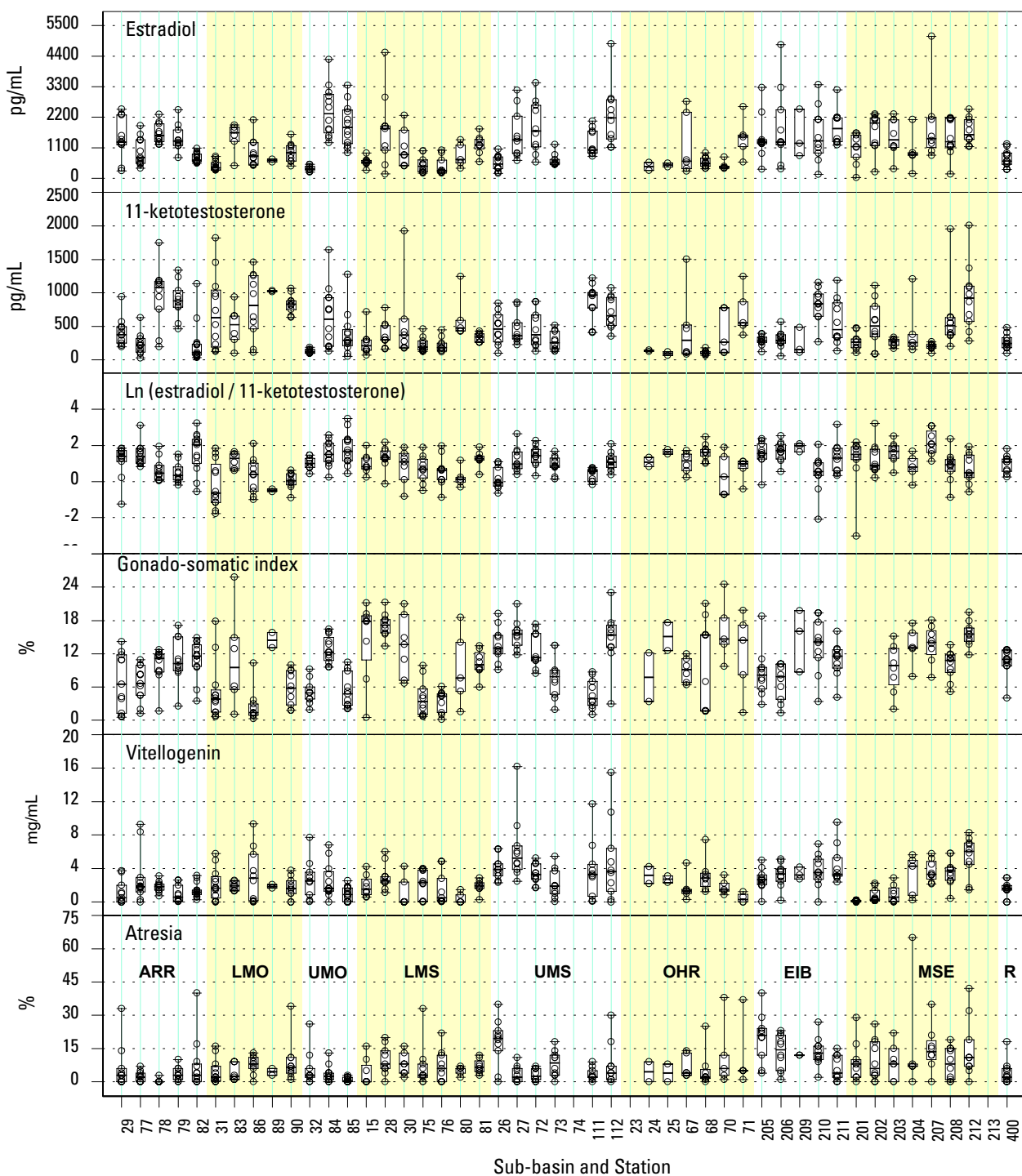


Figure 4-3. Reproductive biomarkers in female carp (all data), by sub-basin and station, for the Mississippi River basin and the reference site (Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

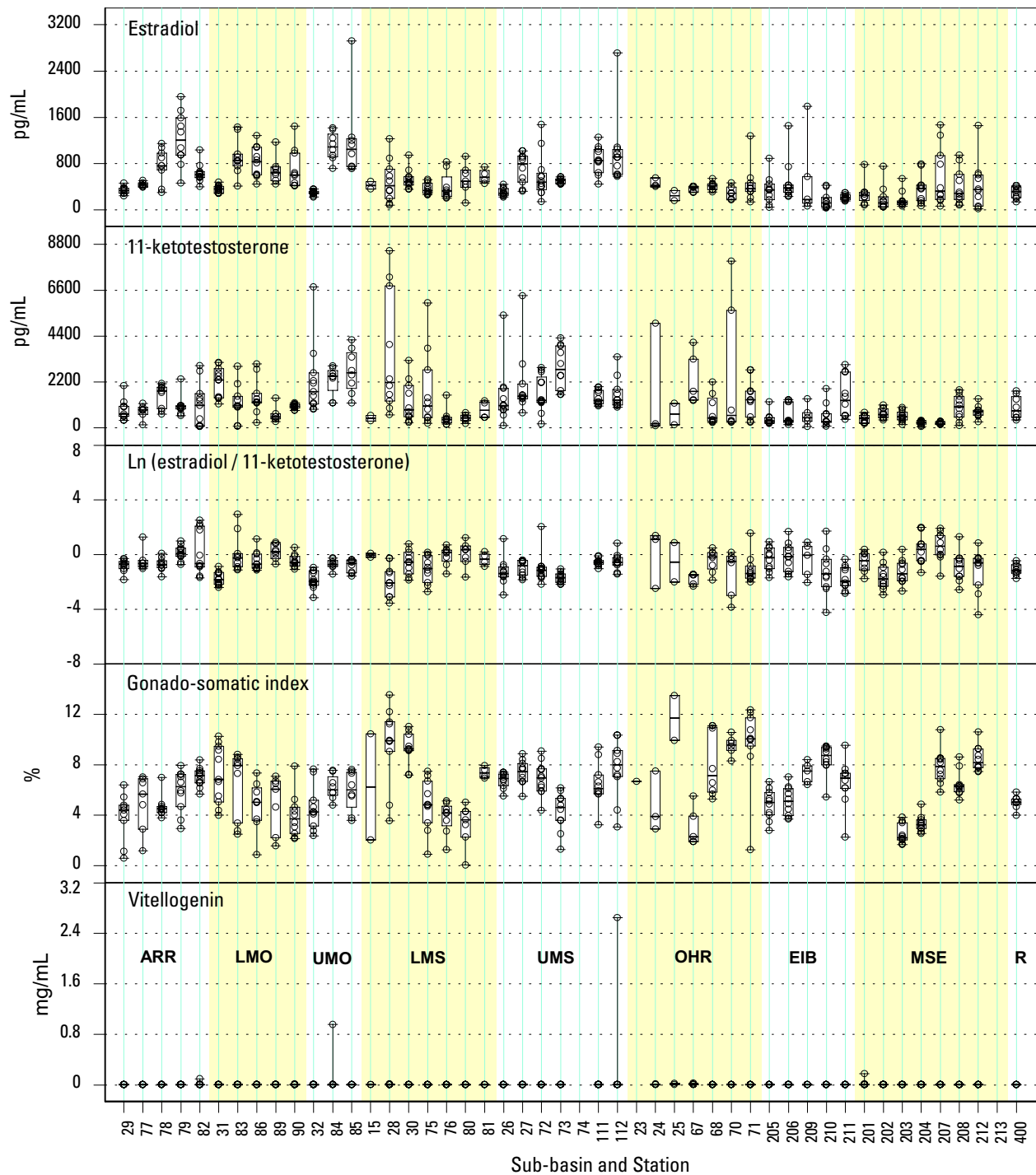


Figure 4-4. Reproductive biomarkers in male carp (all data), by sub-basin and station, for stations in the Mississippi River basin and the reference site (Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

within each stage at all of the NAWQA sites (201-212) except 211 compared with other stations in the MRB. Concentrations in males (all stage 3) at the reference site (Station 400) were between 371 and 1764 pg/mL.

E/KT ratios were >1.0 in 82 (21%) of the 388 male carp from the 45 stations examined (Fig. 4-4). The highest individual fish ratios (one or more fish with E/KT of 5.0-15.0) were at Stations 72, 82, 83, 204, 206, 207, and 210. Males with E/KT >1.0 were present at (Stations 15, 24, 25, 30, 68, 76, 79, 80, 82, 83, 86, 201, 204, 205, 206, 207, and 209). One or more male carp with E/KT ratios >5.0 , well into the female range, were present at Stations 24, 30, 68, 76, and 83. One male carp from Station 83 had an E/KT of almost 20. E/KT ratios of all male carp (stage 3) at the reference site (Station 400) were <1.0 .

GSI was evaluated in 365 male carp from 44 stations (Fig. 4-4). GSI in individual male carp, which was not correlated with stage, ranged from 0.06% at Station 80 to 13.6% at Station 28. Although GSI was typically 1-10% of body weight for most male carp, at a given station and within similar stages, GSI varied considerably. At Station 28, GSI in two fish was approximately 30% of body weight. Values for males at the reference site (Station 400) ranged from 4.0% to 5.8%.

In general Vtg was detected (>0.001 mg/mL) in 28 of the 384 male carp from the 45 stations analyzed (Fig. 4-4). Trace amounts (0.001-0.01 mg/mL) were present in at least one male from Stations 24, 25, 28, 32, 67, 72, 76, 78, 82, 84, 86, 89, 90, 111, and 201. Greater concentrations (0.01-0.3 mg/mL) were present in one male from each of Stations 82 and 201. However, vtg concentrations commonly found in females were present in only one male from Station 84 (0.958 mg/mL) and one male from Station 112 (2.645 mg/mL). Vtg was not detected (>0.001 mg/L) in males from the reference site (Station 400).

Female Bass

E2 concentrations in 229 female bass from 28 stations were analyzed (Fig. 4-5). E2 was only weakly correlated with stage. E2 concentrations in individual fish ranged from 102 pg/mL at Station 26 (stage 2) to 6330 pg/mL at Station 213 (stage 2). One stage-1 female bass from Station 30 had an E2 concentration >5000 pg/mL, whereas E2 concentrations in three of four females (stages 2-3) from Station 213 were >5000 pg/mL. Conversely, E2 concentrations at Stations 15, 24, 25, 26, 32 ($n=1$), 67, 76, 83, and 212 ($n=1$) were uniformly low (<800 pg/mL), as compared to the majority of sites examined. Gonadal stages of these females ranged from 0 to 4. E2 concentrations in female bass (mostly stage 2) from the reference site (Station 400) were 300-1400 pg/mL.

11-KT concentrations in 229 female bass

from 28 stations were analyzed (Fig. 4-5). 11-KT was not correlated with stage. Concentrations in individual fish ranged from 23 pg/mL at Station 25 to 2203 pg/mL at Station 78. Stations 30, 78, 79, and 213 each had one female in stage 2 or 3 with relatively high 11-KT concentrations (>1500 pg/mL).

Conversely, concentrations in female bass from Stations 15, 24, 25, 28, 32 ($n=1$), 68, and 76 were uniformly low (all <500 pg/mL), as compared to the majority of sites examined. Female bass from these stations were in gonadal stages 0 to 4. Concentrations in females (mostly stage 2) at the reference site (Station 400) were 100-600 pg/mL.

E/KT ratios were determined for 229 female bass from 28 stations (Fig. 4-5). Of these, 37 (16%) had ratios <1.0 . These females were present at Stations 26, 28, 67, 70, 74, 76, 78, 79, 81, 82, 83, 112, 212, and 213. More than half the fish from Stations 26 and 70 had E/KT ratios <1.0 . At Station 30, one female had an E/KT ratio of >20 due to a low 11-KT concentration, and one female from Station 213 had a ratio >20 due to a combination of low 11-KT and high E2 concentrations. Below average E/KT ratios (<1.0) were identified in females in all reproductive stages except stage 4. E/KT ratios of females (mostly stage 2) at the reference site (Station 400) ranged from approximately 2 to 4.

GSI in 229 female bass from 26 stations was computed (Fig. 4-5). GSI, which was strongly correlated with stage, ranged from 0.033% at Station 68 (stage 0) to 6.8% at station 32 (stage 4). With the exception of the one female from Station 32, GSI in all individuals ranged from $<1\%$ to about 3% of body weight. For the female at station 32, GSI was 6.8% of the body weight. Values for females (mostly stage 2) at the reference site (Station 400) ranged from approximately 0.3% to 1.7%.

Vtg concentrations in 210 female bass from 26 stations were analyzed (Fig. 4-5). Vtg was correlated with stage. The highest concentration (77.918 mg/mL) was detected in a stage-3 female at Station 213. Conversely, individual fish with non-detectable concentrations of vtg (<0.001 mg/L) were present at $>50\%$ of the stations sampled. Most females with non-detectable vtg were in early gonadal stages (0-2); however, Stations 25, 29, 79, and 111 had stage-3 fish with no detectable vtg. No vtg was detected in any of the stage-0 fish, and only a few of the stage-1 fish had detectable concentrations (at Stations 15 and 30). At Stations 15, 24, 25, 26, 28, 70, 71, 76, 81, and 400 vtg concentrations were uniformly low (<1.0 mg/mL), whereas concentrations in 3 of 4 fish from Station 213 were >50 mg/mL. The other fish from Station 213 had no detectable vtg, even though all four fish from this station were in similar stages (2 or 3). The only other female bass with unusually high vtg (48.675 mg/mL) was a stage-4 female from station 74. As

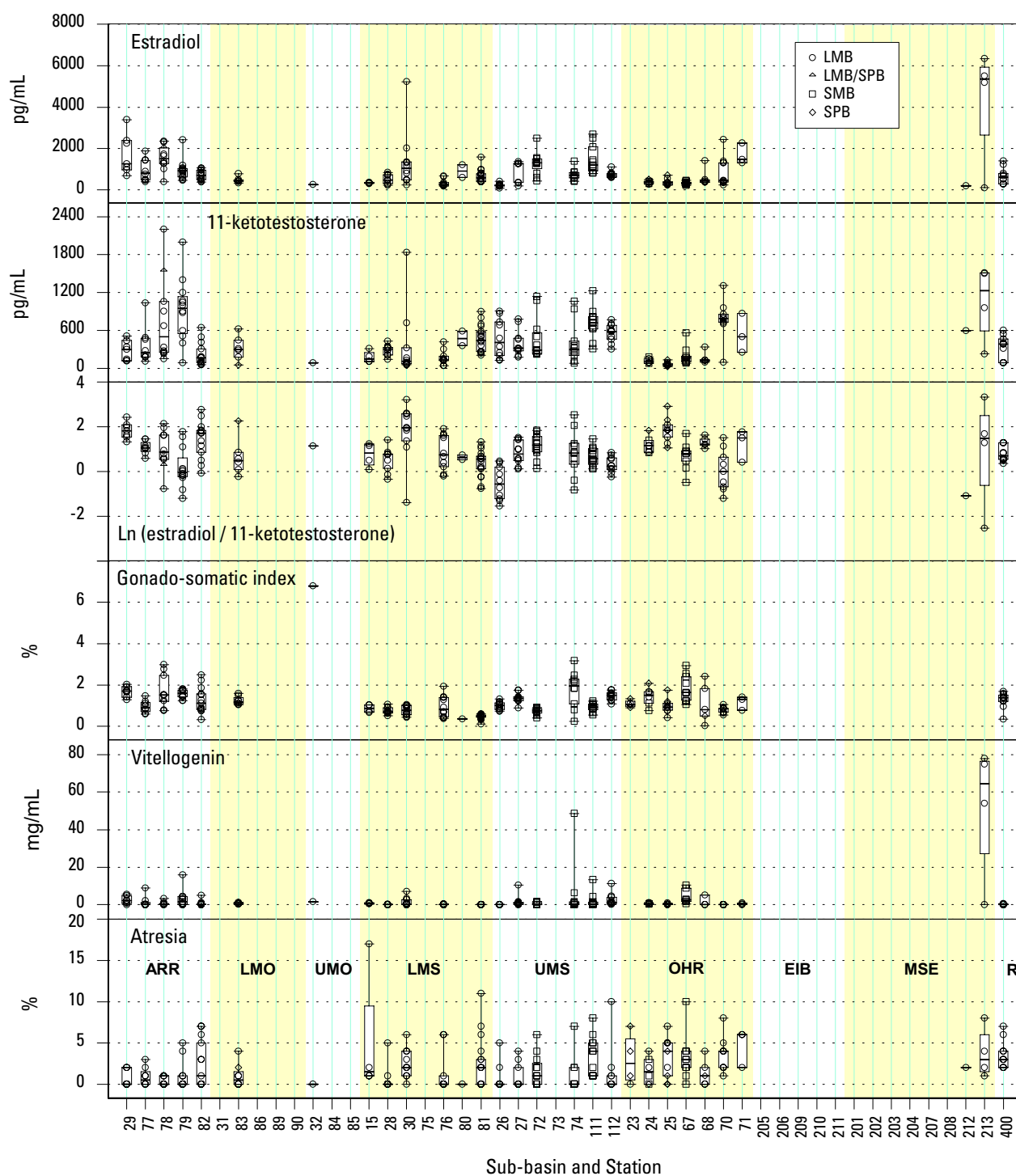


Figure 4-5. Reproductive biomarkers in female bass (all data), by sub-basin and station, for stations in the Mississippi River basin and the reference site (Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and the interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

with female carp, collection date did not appear to influence vtg concentrations, since stations with uniformly low vtg were sampled within the same timeframe as Stations 213 and 74 (late September to late October). Conversely, female bass from several stations with low vtg were somewhat younger (1-year old fish were collected at four stations) than females from station 213 (ages 2-4). Vtg concentrations in female bass (mostly stage 2) from the reference site (Station 400) were somewhat low, ranging from non-detectable to 0.512 mg/mL.

The percentage of atretic oocytes was determined for 230 female bass from 29 stations (Fig. 4-5). Per cent atresia was not correlated with stage. Percentages of atretic oocytes in individual fish ranged from 0% (at least one female with no discernable atresia at most stations) to 17% at Station 15. In addition to station 15, female bass with high percentages (>10%) of atretic eggs were present at Stations 67, 81, and 112. Atresia in females (mostly stage 2) at the reference site (Station 400) ranged from approximately 2% to 7%.

Male Bass

E2 concentrations in 208 male bass from 26 stations were analyzed. E2 was only weakly correlated with stage. Concentrations in individual fish ranged from 99 pg/mL at Station 27 (stage 2) to 1886 pg/mL at Station 212 (stage 2). One or two fish with relatively high concentrations (>1000 pg/mL) were observed at Stations 30, 68, 78, 79, 83, 111, and 212. Whereas most of the male bass from these stations were in gonadal stages 2 or 3, three stage-1 fish from Station 111 had unusually high E2 concentrations (900-1200 pg/mL). Conversely, E2 concentrations were uniformly low (<300 pg/mL) in male bass from Stations 26 and 27 (all stages 1 and 2). E2 concentrations in male bass (mostly stage 2) from the reference site (Station 400) were 200-400 pg/mL.

11-KT concentrations in 208 male bass from 26 stations were analyzed (Fig. 4-6). 11-KT was not correlated with stage. Concentrations of individual fish ranged from 41 pg/mL at Station 83 to 6040 pg/mL at Station 74. Stations 28 and 74 had males with unusually high 11-KT concentrations (>5000 pg/mL), whereas 11-KT concentrations in males from Stations 24, 25, and 80 ($n=1$) were uniformly low (<500 pg/mL). Male bass with elevated 11-KT concentrations were almost exclusively in stage 2, while those from stations with the lowest concentrations were in stages 0, 2, and 3. Concentrations in males (mostly stage 2) from the reference site (Station 400) were 400-1100 pg/mL.

E/KT ratios were determined for 208 male bass from 26 stations (Fig. 4-6). Of these, 44 (21%) had ratios >1.0. Proportionately large numbers of males with E/KT >1.0 were present at Stations 24, 25,

68, 76, 79, 82, and 83. One or more male bass with E/KT ratios >5.0, well into the female range, were present at Stations 24, 30, 68, 76, and 83; one male at Station 83 had an E/KT >16.0. Males with high ratios (>1.0) occurred at all stages of gonadal maturation (0-3). E/KT ratios of male bass (mostly stage 2) from the reference site (Station 400) were all <1.0.

GSI in 199 male bass from 24 stations was determined (Fig. 4-6). GSI, which was correlated with stage, ranged from 0.047% at Station 27 (stage 1) to 0.900% at Station 74 (stage 2). Although GSI was generally <1% of body weight irrespective of stage, GSI in male bass collected from the same station and within similar stages displayed a large degree of variability. There were only two stage-0 males (from Station 72) for which GSI was determined; they were among the lowest GSIs. The converse was not true; greatest GSI values were generally found in stage-2 smallmouth bass, not in stage-3 fish. This may be due at least partly to the fact that GSI was among the few biomarkers for which preliminary analyses suggested differences among the *Micropterus* spp. collected (due to architectural differences). GSI values for male bass (mostly stage 2) at the reference site (Station 400) ranged from approximately 0.1-0.6%.

Vtg concentrations were analyzed in 198 male bass from 25 stations (Fig. 4-6). Of these, vtg was present at detectable concentrations (>0.001 mg/mL) in 14 fish (approximately 9%). At least one male contained detectable vtg at 11 of the 25 stations (44%) analyzed. Trace amounts (0.001-0.01 mg/mL) were present in at least one male bass from Stations 25, 27, 29, 30, 68, 70, 71, and 213. Greater concentrations (0.01-0.3 mg/mL) were present in one male from each of Stations 25, 112, and 400. Only at Stations 30 and 79 were there males (one from each station) with concentrations >2 mg/mL. Both of those fish were also highly estrogenic. There was only one vitellogenic male (0.114 mg/mL) at the reference site (Station 400).

Summary

High variability in biomarker responses, even within the same sex/taxon group, made it difficult to determine statistically valid responses with the raw data set. This raw data set contains several stations for each sex/taxon from which low numbers (<5) of fish were collected and/or fish spanned a wide range of gonadal stages. Although most fish collected were in similar reproductive stages, there were outliers in each sex/taxon group (Figs. 4-1, 4-2). Reproductive biomarkers are known to vary over the course of the reproductive cycle; therefore, the high variability within the same sex/taxon likely resulted from the analysis of fish from a wide range of reproductive stages. These and other factors, including spatio-temporal influences, were considered when preparing the

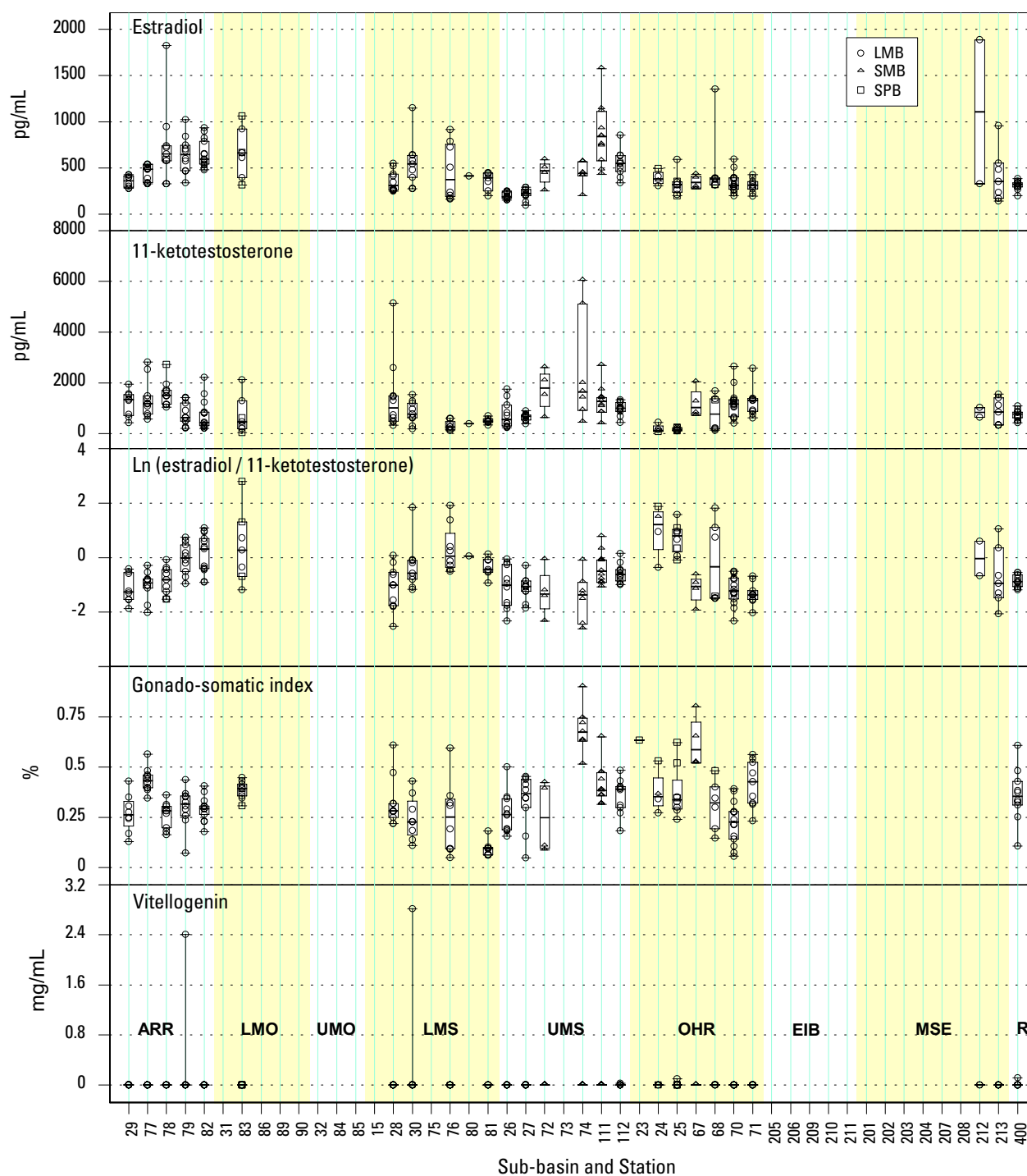


Figure 4-6. Reproductive biomarkers in male bass (all data), by sub-basin and station, for stations in the Mississippi River basin and the reference site (Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and the interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

trimmed data set for statistical analysis (see next section).

Analysis and Presentation of the Reduced-Rank Data Set

Prior to a thorough statistical examination of the reproductive biomarkers, preliminary analyses were performed to test the hypothesis that the biomarkers in this study were influenced by differences in reproductive stage. These procedures are described in detail in Chapter 1 of this report. As expected, many biomarker responses were correlated with gonadal stage and differed significantly ($P < 0.05$) among stages in at least one sex/taxon group, and stage was a significant ($P < 0.05$) covariate for many biomarkers. Consequently, we decided that all further statistical analyses should be restricted to fish in a narrow range of gonadal stages. Although the distribution of stages did not differ significantly between stations for all sex-taxon combinations investigated (see the following sections), examination of the data (Figs. 4-1, 4-2) indicated that most stations yielded fish in more than one gonadal stage. Nevertheless, and despite differences in sampling time and location, >80% of carp and bass in each sex/taxon group were in similar stages of their reproductive cycles, making it relatively easy to narrow the dataset to one or, at the most, two stages. Stage was also treated as a response variable (much like the other reproductive biomarkers) to determine whether the distribution differed between stations. Results of the stage analyses are reported according to sex and taxon.

In the following sections the first paragraph under each of the biomarker results sections presents the range of station means ($\pm$ one standard error) and identifies specific stations where means are unusually high or low compared with other stations within the MRB. Although normal differences between stations are to be expected, the fact that the MRB is the largest river basin in the United States, spanning 17 states, cannot be ignored and therefore may be expected to exhibit high variability. The reproductive indicators analyzed in this chapter are likely sensitive to seasonal and/or geographical differences, meaning that variation in climate, environment, and land use may be responsible for some of the differences observed between distant sites. Furthermore, this study included both the NCBP and NAWQA stations, which represent rivers of vastly different sizes. Characteristics of the different types of waterways along which these stations are located may influence the reproductive physiology of the fish at these sites.

To partly address the spatio-temporal issues, and as recommended by Goodbred and others (1997),

stations were grouped by sub-basin (based on location within the MRB) and by program of origin, as noted in Chapter 1 of this report. The NCBP stations were divided into six sub-basins: Arkansas-Red River (ARR), Lower Missouri River (LMO), Upper Missouri River (UMO), Lower Mississippi River (LMS), Upper Mississippi River (UMS), and Ohio River (OHR). The NAWQA stations were grouped according to their respective Study Units: Eastern Iowa Basins (EIB) and Mississippi Embayment (MSE). The EIB is wholly contained within the Upper Mississippi sub-basin, and the MSE is similarly contained within the Lower Mississippi sub-basin. Nevertheless, the NCBP and NAWQA sites were treated separately for the purpose of computing and comparing sub-basin means, due to the differences between the NCBP and NAWQA stations (See Chapter 1).

The range of NCBP sub-basin means and standard errors are presented for each biomarker, and the statistically significant differences between and within sub-basins are summarized. The NAWQA Study Units are not included in the general sub-basin comparisons; instead, we compare the EIB with only UMS and the MSE with the LMS. As noted, the reproductive indicators may be influenced by the physical and hydrologic properties (basin size, flow, temperature etc.) of the waterways from which fish were collected. Within-sub-basin comparisons were made because a station appearing to be an outlier when compared with all other stations in the MRB may be quite normal when compared with the other stations in its sub-basin. Conversely, if a station differs significantly from the other stations in the sub-basin, this may be more meaningful for identifying potential sites of endocrine disruption. Stations were also grouped and compared at the program level (that is, NCBP vs. NAWQA). The reference site (Station 400), was compared at the program level was compared with all station, sub-basin, and program means.

To provide the most accurate and informative results, the final dataset used for this report was selected according to several specific terms. As mentioned, the results section below is restricted to carp and bass of both sexes, and each sex/taxon group is analyzed independently. Although the numbers of fish collected differed among stations, no station with less than five individuals was included in the final analyses; and all biomarkers were restricted to the analysis of fish within similar stages (to eliminate variance due to differences in reproductive cycles). For simplicity, the comparisons are reported in terms of statistical differences. It should be noted, however, that these differences only refer to differences in means for untransformed data. If the data were log-transformed, then differences refer to medians; if rank-transformed,

tion was required, then differences refer to distributions. A significance level of $P < 0.05$ was used in all statistical tests. Details of the statistical procedures are presented in Chapter 1 of this report.

Reproductive Biomarkers in Carp

A total of 777 common carp were collected from 46 stations. Of these, 387 females and 388 males were analyzed; two samples were not analyzed.

Histological examination detected no intersex male or female carp. Further details of fish collection and information on the number, location, gender, size, and age are presented in Chapter 1 and Appendix A of this report.

Female Carp

Stage

Of the 380 female carp ovaries examined histologically, the majority (83%) were in stage 3 (late vitellogenic), 7% were in stage 1, and 9% were in stage 2 (Fig. 4-1). There were no stage-4 or -5 female carp, but five females were in stage 0 (1%). Preliminary statistical analysis indicated that differences in the proportional distribution of the fish among stages did not differ significantly ($P > 0.05$) among stations, however. Based on this information, rigorous statistical testing of reproductive biomarkers in female carp was restricted to Stage 3, and stations were eliminated from the trimmed data set analyzed statistically as follows: At Stations 75 and 86, $\geq 50\%$ of the females analyzed were in early stages (1 and 2); and Stations 24, 25, 71, 80, 86, 89, and 209 there were < 5 females. The actual number of fish analyzed for each biomarker differed slightly, but in general 38 stations met the criteria (stage 3, $n > 4$) for statistical analysis of reproductive biomarkers in females except GSI, which was analyzed at 36 stations.

Estradiol (E2)

Station analysis: A wide range of E2 concentrations, reported in pg/mL, was observed in female carp. Station means ranged from 2409.75 ± 264.29 at Station 84 to 357.40 ± 45.64 at Station 32, and the mean E2 at the reference site (Station 400) was 715.55 ± 92.81 (Fig. 4-7). Mean E2 concentrations at Stations 15, 26, 31, 32, 68, 70, 75, and 76 were all < 600 pg/mL, whereas E2 means at Stations 84, 85, 112, and 207 were > 2000 pg/mL.

Between sub-basins: E2 means for all sub-basins and the NAWQA Study Units were > 715.55 pg/mL, the value reported for the reference site (Station 400; Fig. 4-7). NCBP sub-basin means ranged from 1662.15 ± 654.69 for the Upper Missouri sub-basin to $739.56 \pm$

216.36 for the OHR. The LMS and OHR E2 means were the lowest. Both sub-basins differed significantly (lower) from the ARR, UMO, and UMS, but not from the LMO, Station 400, or each other. E2 means of the two NAWQA Study Units (EIB and MSE) were high compared with most of the NCBP sub-basins. Despite the geographical proximity of their stations, the MSE differed significantly ($P < 0.05$) from the LMS but there was no difference between the EIB and the UMS.

Within sub-basins: No significant differences ($P > 0.05$) were detected among stations within the ARR or OHR sub-basins or in either NAWQA Study Unit for E2 in female carp (Fig. 4-7). In several sub-basins, only one station differed significantly from the others. For instance, in the LMO sub-basin, Station 31 was significantly ($P < 0.05$) lower than Station 83, and in the UMO, Station 32 was lower than Stations 84 and 85. In the LMS sub-basin, E2 means for female carp from Stations 15, 75, and 76 ranged from 479.17 (76) to 585.00 (15), whereas means for Stations 28, 30, and 81 ranged from 1104.57 (30) - 1754.67 (28); however, only Stations 28 and 76 differed statistically ($P < 0.05$). In the UMS sub-basin, Station 26 was significantly lower ($P < 0.05$) than Stations 27, 112, and 72; and Station 112 was significantly greater than Station 73. E2 means for Stations 26 and 73 were < 700 , whereas the means for Stations 111, 112, 27, and 72 were all > 1300 (Station 112 had the highest E2 mean of all stations).

NCBP vs. NAWQA sites: The E2 means calculated for the NCBP and NAWQA sites (as a group) were 1179.18 ± 135.85 and 1622.32 ± 106.08 , respectively. The NAWQA sites differed significantly from the NCBP stations and the reference site (Station 400, mean = 715.55 pg/mL), but the NCBP stations were not statistically different from the reference site.

11-Ketotestosterone (11-KT)

Station analysis: Mean concentrations of 11-KT (in pg/mL) ranged from 987.14 ± 62.81 at Station 111 to 109.17 ± 16.42 at Station 68 (Fig. 4-7). The mean 11-KT concentration at the reference site (Station 400) was 261.82 ± 33.28 . In addition to Station 111, 11-KT means were highest at Stations 78, 79, 90, and 212 (all > 880 pg/mL). Conversely, mean 11-KT concentrations at Stations 32, 76, and 207 (and 68, as noted above) were all < 200 pg/mL.

Between sub-basins: Mean 11-KT concentrations ranged from 311.20 ± 60.34 for the LMS sub-basin to 702.92 ± 96.21 for the LMO (Fig. 4-7). The LMS differed significantly ($P < 0.05$) from the ARR, LMO, and UMO sub-basins, and the LMO differed from the

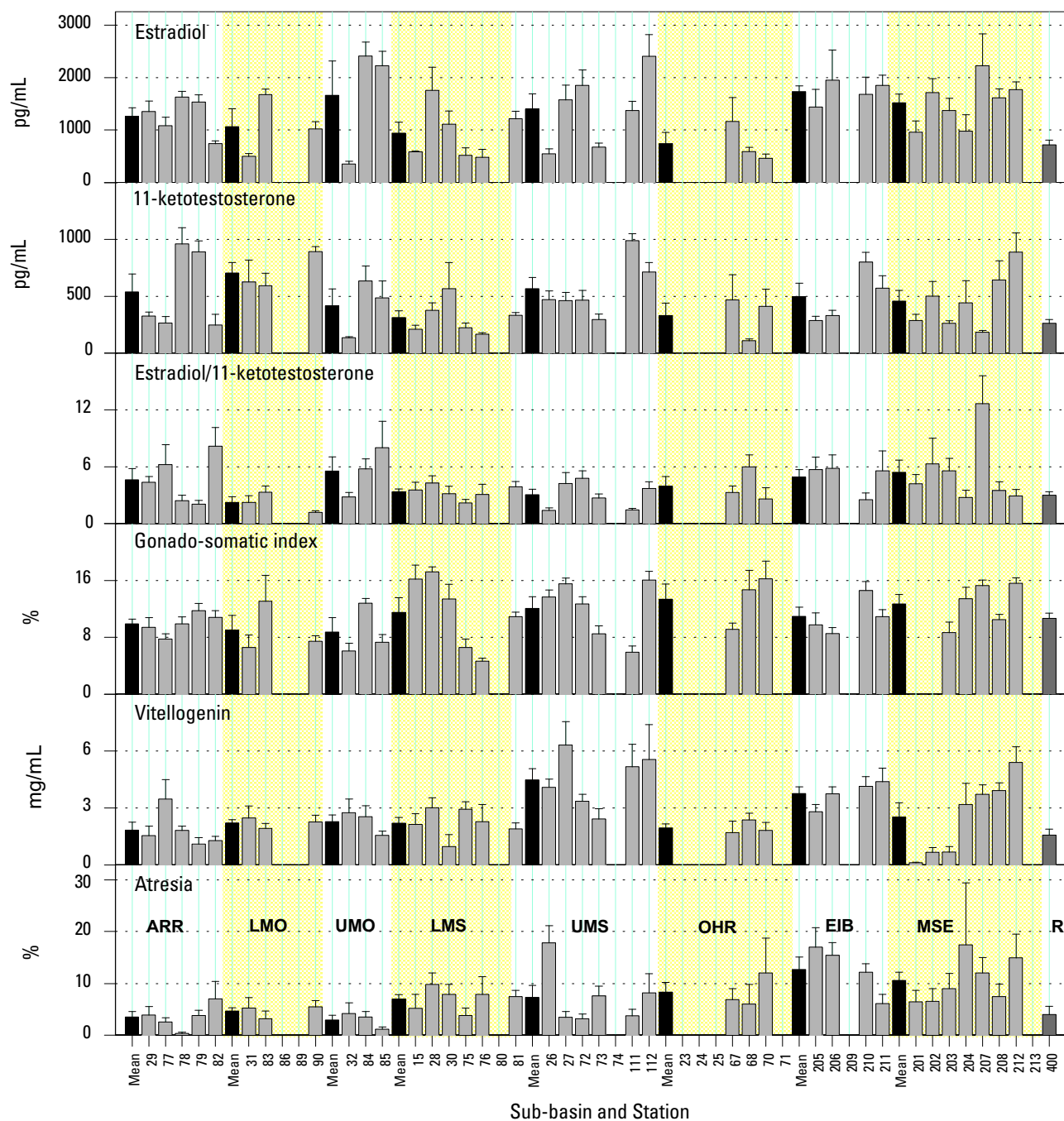


Figure 4-7. Reproductive biomarkers in female carp (reduced-rank dataset), by sub-basin (black bars) and station (grey bars, $n > 1$), for stations in the Mississippi River basin and the reference site (Station 400). Shown are arithmetic means + 1 SE. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

LMS and OHR sub-basins and the reference site (Station 400). Although the 11-KT mean for the reference site (261.81 pg/mL) was lower than all other sub-basins means, the reference site only differed statistically ($P < 0.05$) from the LMO sub-basin. 11-KT means for female carp from the NAWQA Study Units were 496.61 ± 118.98 (EIB) and 457.27 ± 93.44 (MSE). The EIB did not differ statistically from the UMS sub-basin nor did the MSE differ from the LMS.

Within sub-basins: Only the ARR and UMS sub-basin and MSE Study Unit were there stations at which 11-KT in female carp differed significantly (Fig. 4-7). Within the ARR sub-basin, Stations 78 and 79 (with two of the highest station means) differed significantly ($P < 0.05$) from Stations 29, 77, and 82. Within the UMS, Station 111 (with the highest station mean) differed statistically from Station 73, and within the MSE Study Unit Station 212 differed from Stations 203 and 207 (two of the lowest station means).

NCBP vs. NAWQA sites: There was no significant difference ($P > 0.05$) between 11-KT in female carp from the NCBP and NAWQA sites, and neither of these groups differed significantly from the reference site (Station 400). The mean 11-KT concentrations measured for the NCBP and NAWQA sites were 476.85 ± 62.08 and 476.94 ± 19.67 , respectively.

E/KT Ratio

There were no stations in the reduced-rank data set analyzed statistically (38 stations including the reference site, stage=3, $n > 4$) for which the mean E/KT in female carp was significantly < 1.0 ($P > 0.05$).

Gonado-somatic Index (GSI)

Station analysis: The greatest GSI mean in female carp was at Station 28 ($17.2 \pm 0.8\%$), and the lowest was at Station 76 ($4.7 \pm 0.4\%$; Fig. 4-7). At the reference site (Station 400), mean GSI in female carp was $10.7 \pm 0.7\%$. At Stations 31, 32, 75, and 76, the mean GSIs were in the lower range ($< 7\%$), whereas at Stations 15, 27, 28, 70, 112, 207, and 212, they were among the highest ($> 15\%$).

Between sub-basins: The GSI means ranged from $8.7 \pm 2.1\%$ in the UMO sub-basin to $13.3 \pm 2.2\%$ in the OHR (Fig. 4-7). The UMO and LMO (also one of the lowest mean GSIs) differed significantly ($P < 0.05$) from the UMS. The LMO sub-basin also differed significantly from the OHR. Differences between the EIB Study Unit ($10.9 \pm 1.3\%$) and the UMS sub-basin (12.1 ± 1.7) and between the MSE ($12.7 \pm 1.4\%$) and the LMS (11.5 ± 2.1) were not significant ($P > 0.05$).

Within sub-basins: There were no significant differences

($P > 0.05$) for GSI in female carp between stations in the ARR, LMO, UMO, or OHR sub-basins or in the EIB or MSE Study Units (Fig. 4-7). In the LMS sub-basin, however, Station 76 was significantly ($P < 0.05$) lower than all other stations in the sub-basin except Station 75; and Station 28 differed significantly from Stations 75 and 76. Stations 75 and 76 had the lowest mean GSIs (< 7), whereas all other station means within the sub-basin were $\geq 10\%$. In the UMS sub-basin, Station 111 (with a low mean GSI) differed significantly from all others except Station 73.

NCBP vs. NAWQA sites: The GSI means for the NCBP and NAWQA sites were $10.8 \pm 0.8\%$ and $11.8 \pm 0.9\%$, respectively. These groups of sites differed significantly ($P < 0.05$) from one another, but neither differed from the reference site (Station 400).

Vitellogenin (vtg)

Station analysis: The mean vtg concentrations (mg/mL) in female carp ranged from 0.100 ± 0.036 at Station 201 to 6.299 ± 1.248 at Station 27 (Fig. 4-7). Mean vtg in female carp from the reference site (Station 400) was 1.556 ± 0.309 . At Stations 30, 79, 202, and 203 vtg means were in the lower range (< 1.100), whereas means at Stations 27, 111, 112 and 212 were among the highest (> 5.000).

Between sub-basins: The vtg sub-basin means ranged from 1.827 ± 0.429 in the ARR to 4.473 ± 0.596 in UMS (Fig. 4-7). The vtg means for the ARR, LMO, UMO, LMS, and OHR were similar and did not differ significantly ($P > 0.05$), whereas the UMS mean was significantly ($P < 0.05$) higher than all other NCBP sub-basins and the reference site (Station 400). However, the UMS did not differ significantly from the EIB Study Unit (mean= 3.762 ± 0.349) nor did the LMS sub-basin (mean= 2.191 ± 0.307) differ significantly from the MSE Study Unit (mean= 2.510 ± 0.769). The vtg mean for the reference site (Station 400) was lower than all sub-basin and Study Unit means.

Within sub-basins: Only the MSE contained stations from which vtg in female carp differed significantly (Fig. 4-7); Stations 201, 202, and 203 (with the lowest vtg means in the basin) differed significantly ($P < 0.05$) from Stations 207, 208, and 212 (one of the highest station means) but Station 204 did not differ from any other station within the MSE.

NCBP vs NAWQA sites: Vtg concentrations in female carp from the NAWQA sites as a group (mean= 3.136 ± 0.626) were only slightly higher than at the NCBP sites (mean= 2.486 ± 0.404); nevertheless, these differed statistically. Only the NAWQA sites differed

significantly from the reference site (Station 400), however.

Atresia

Station analysis: The highest mean atresia in female carp was at Station 26 ($17.80 \pm 3.34\%$), and the lowest was at Station 78 ($0.30 \pm 0.30\%$; Fig. 4-7). Mean atresia at the reference site (Station 400) was $4.00 \pm 1.58\%$. In addition to Station 26, Stations 70, 204, 205, 206, 207, 210, and 212 all had means $\geq 12.00\%$, whereas the lowest means ($<3.00\%$) were at Stations 77, 78 (the lowest in the MRB).

Between sub-basins: NCBP sub-basin means for atresia ranged from $2.95 \pm 0.92\%$ for the UMO to $8.28 \pm 1.88\%$ for the OHR (Fig. 4-7); however, neither of these sub-basins differed significantly ($P>0.05$) from the other NCBP sub-basins. The ARR mean was the second lowest mean ($3.50 \pm 1.09\%$) and differed significantly ($P<0.05$) from the LMS and UMS sub-basins, both of which had means in the higher range. Atresia in female carp from the NAWQA Study Units ($12.66 \pm 2.41\%$ for EIB, $10.52 \pm 1.64\%$ for MSE) was greater than any of the NCBP sub-basins. The MSE Study Unit did not differ significantly from the LMS sub-basin, but the EIB and the UMS ($P<0.05$).

Within sub-basins: Although atresia varied considerably among the sub-basins, there were no significant differences ($P>0.05$) between the stations within any sub-basin (Fig. 4-7).

NCBP vs. NAWQA sites: Atresia in female carp from the NAWQA sites as a group (mean= $11.59 \pm 1.07\%$) was substantially and significantly ($P<0.05$) higher than at the NCBP sites (mean= $5.60 \pm 0.90\%$) and the reference site (mean= 4.00%).

Male Carp (Bar graphs of reproductive biomarkers in male carp are displayed in Figure 4-8.)

Gonadal Stage

Of the 374 male carp examined, the vast majority (86%) were in stages 1, 1.5, and 2 (early to mid-spermatogenic; Fig. 4-1). Only 12% were in stages 2.5 - 3 (mid- to late spermatogenic), and six (2%) were in stage 0 - 0.5 (immature or early spermatogenic). Of the latter, only three were truly immature (Stage 0) and there were no stage 4 carp. Preliminary statistical analysis revealed that, in contrast to female carp, the proportional distribution of stages differed significantly ($P<0.05$) among stations for males. Accordingly, further statistical analysis of reproductive biomarkers in male carp was restricted to stages 1 and 2 (intermediate stage values were rounded down for analysis), and stations were eliminated

from further testing as follows: Stations 15, 23, 24, 25, 29, 67, 68, 77, 78, and 81 had <5 males in the appropriate stages (1 and 2) for statistical analysis. Although the actual number of fish analyzed for each biomarker differed slightly, 36 stations met the criteria (stage=1 or 2, $n>4$) for statistical analysis of reproductive biomarkers in male carp; for GSI, data from 34 stations were analyzed.

Estradiol (E2)

Station analysis: Mean E2 concentrations (in pg/mL) in male carp varied substantially among stations (Fig. 4-8). The highest E2 mean was at Station 85 (1208.75 ± 255.83), and the lowest was at Station 203 (175.70 ± 46.14). The mean E2 for male carp from the reference site (Station 400) was 300.88 ± 37.93 . Mean E2 concentrations at stations 79, 84, 85, and 112 were the highest in the MRB (>1000 pg/mL), whereas concentrations at Stations 202, 203, and 210 were among the lowest (<200 pg/mL).

Between sub-basins: Concentrations of E2 in male carp from the ARR, LMO, and UMO sub-basins differed significantly ($P<0.05$) from concentrations in males from the LMO and OHR sub-basins and the reference site (Station 400; Fig. 4-8). The ARR, LMO, and UMO means were similar, ranging from 707.15 ± 96.38 to 907.78 ± 279.90 , whereas the LMO and OHR sub-basin means were lower (357.35 ± 72.71 to 481.15 ± 37.80). The UMS mean was in between (mean= 647.85 ± 106.70) and did not differ significantly from those in any other NCBP sub-basin. However, the UMS differed significantly from the EIB even though the stations of the UMS sub-basin and EIB Study Unit are located in the same geographic area. In contrast, E2 in male carp from the LMS sub-basin did not differ significantly from the MSE Study Unit ($P>0.05$). Overall, E2 concentrations in male carp from the EIB (mean= 357.35 ± 72.71) and MSE (mean= 358.40 ± 52.88) Study Units were low compared with all NCBP sub-basins. The E2 mean for the reference site (mean= 300.88 ± 37.93) was lower than all NCBP and NAWQA sub-basin means; however, the reference site only differed significantly ($P<0.05$) from the ARR, LMO, and UMO sub-basins.

Within sub-basins: E2 in male carp only the UMO and UMS sub-basins contained stations that differed statistically ($P<0.05$) from each other (Fig. 4-8). In the UMO, Station 32 differed from Stations 84 and 85. The E2 mean at Station 32 (mean= 293.67 ± 14.50) was considerably lower than the means at Stations 84 (mean= 1118.86 ± 96.77) and 85 (mean= 1208.75 ± 255.83), both of which had two of the highest E2 means in the entire MRB. In the UMS sub-basin,

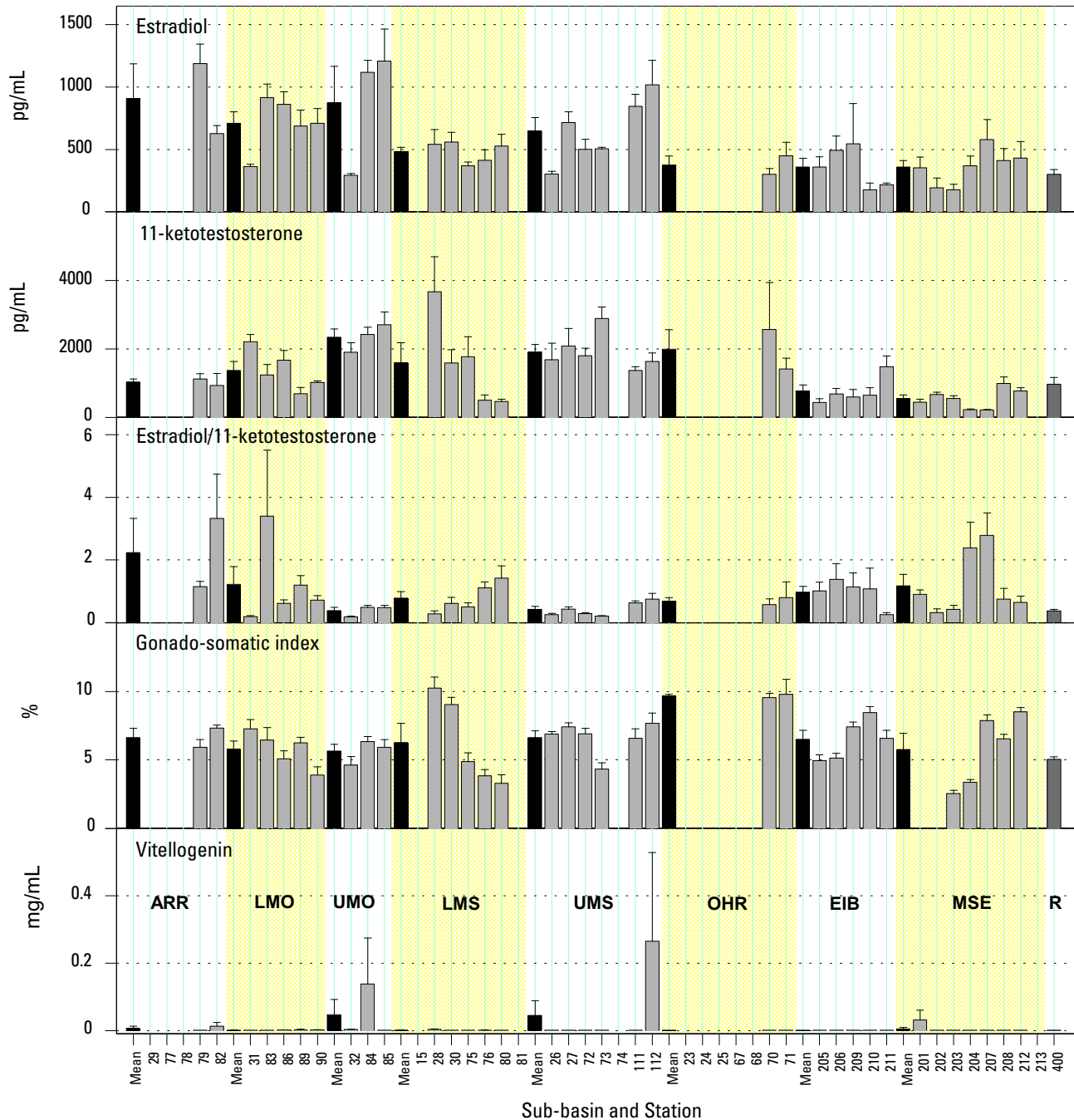


Figure 4-8. Reproductive biomarkers in male carp (reduced-rank dataset), by sub-basin (black bars) and station (grey bars, $n > 1$), for stations in the Mississippi River basin and the reference site (Station 400). Shown are arithmetic means + 1 SE. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

only Stations 26 and 112 differed significantly from one another. Station 26 (mean=303 $\pm$ 23.90) had one of the lower E2 means in the basin, whereas Station 112 (mean=1018.60 $\pm$ 197.20) had one of the highest. *NCBP vs NAWQA sites:* E2 concentrations in male carp from the NCBP sites as a group (mean=665.16 $\pm$ 86.31) were significantly greater ($P<0.05$) than concentrations for the NAWQA sites (mean=357.88 $\pm$ 0.52). Only the NCBP stations differed statistically from the reference site (mean=300.88), however.

11-Ketotestosterone (11-KT)

Station analysis: Station means (in pg/mL) for 11-KT ranged from 3663.44 $\pm$ 1022.50 at Station 28 to 215.10 $\pm$ 17.40 at Station 207 (Fig. 4-8). The 11-KT mean for male carp at the reference site (Station 400) was 971.25 pg/mL $\pm$ 196.47. In addition to Station 28, the mean 11-KT concentrations at Stations 27, 31, 70, 73, 84, and 85 were uniformly >2000 pg/mL, whereas the means for Stations 76, 80, 201, 204, 205, and 207 (the lowest in the basin) were <500 pg/mL.

Between sub-basins: 11-KT concentrations in male carp also differed considerably among sub-basins (Fig. 4-8). Means ranged from 1989.14 pg/mL $\pm$ 575.03 in the OHR sub-basin to 2343.43 pg/mL $\pm$ 236.90 in the UMO. Concentrations in the UMO differed significantly ($P<0.05$) from those at the reference site (Station 400) and all other sub-basins except the UMS. The UMS differed from the ARR and LMS. Only for the EIB and MSE Study Units were the means <1000 pg/mL. Moreover, both Study Units differed significantly ($P<0.05$) from their respective NCBP sub-basins (MSE vs. LMS, EIB vs UMS).

Within sub-basins: Despite the variation between sub-basins, there was only moderate variability among stations within sub-basins for 11-KT in male carp (Fig. 4-8). There were no significant differences ($P>0.05$) among stations in the UMO, UMS, and OHR sub-basins or in the EIB Study Unit. In the LMO sub-basin only Stations 31 (at which the 11-KT mean was comparatively high) and 89 differed significantly ($P<0.05$); in the LMS, Station 28 (with the highest mean in the MRB) differed from Stations 76 and 80 (with two of the lowest means); and in the MSE Study Unit, Stations 207 (with the lowest 11-KT station mean) and 208 were the only stations that differed significantly.

NCBP vs NAWQA sites: 11-KT concentrations in male carp differed significantly (and substantially) between the NCBP (mean=1704.65 $\pm$ 192.84) and NAWQA (mean=659.55 $\pm$ 108.31) stations, but neither group differed significantly from the reference site (Station 400).

E/KT Ratio

For male carp analyzed statistically (36 stations including the reference site, stages 1 and 2, $n>4$) only Station 207 had a mean E/KT ratio (mean=2.778 $\pm$ 0.257) significantly ($P<0.05$) >1.0.

Gonado-somatic Index (GSI)

Station analysis: The GSI station means for male carp ranged from 2.53 $\pm$ 0.25% at Station 203 to 10.23 $\pm$ 0.82% at Station 28 (Fig. 4-8). The mean GSI at the reference site (Station 400) was 5.03 $\pm$ 0.19%. In addition to Station 203, Stations 76, 80, 90, and 204 had mean GSIs in the lower range (<4%), whereas Stations 28 (the highest), 30, 70, 71, 210, and 212 were among the highest (>8%).

Between sub-basins: GSI means for male carp from five of the six NCBP sub-basins were remarkably similar, ranging from 5.63 $\pm$ 0.51% in the UMO sub-basin to 6.62 $\pm$ 0.49% in the UMS (Fig. 4-8). The OHR sub-basin, however, differed significantly ($P<0.05$) from all others; the mean GSI for the OHR sub-basin was 9.65 $\pm$ 0.12%, considerably higher than the others. GSI means for all sub-basins were higher than that of the reference site; however, only the OHR differed significantly from Station 400. The LMS sub-basin and the MSE Study Unit did not differ significantly ($P>0.05$), nor did the EIB from the UMS.

Within sub-basins: Although variability among sub-basins was low for GSI in male carp, there were notable differences among stations within several sub-basins (Fig. 4-8). In the LMO sub-basin, Stations 90 (somewhat low compared with other stations) and 31 differed statistically ($P<0.05$). In the UMS sub-basin, Station 73 (slightly low) differed from Stations 27 and 112; and in the EIB Study Unit, Station 210 (with one of the higher station means) differed from Stations 205 and 206. Stations in the LMS sub-basin and MSE Study Unit differed the most. In the LMS, GSI in male carp from Stations 28 and 30 were similar (two of the highest in the basin), but differed significantly ($P<0.05$) from all other stations in the sub-basin (75, 76, and 80). In the MSE, Stations 203 and 204 were similar, yet differed significantly from the other MSE stations (207, 208, 212). There were no significant differences ($P>0.05$) between stations in the ARR, UMO, and OHR sub-basins.

NCBP vs NAWQA sites: For GSI in male carp the NCBP stations (mean=6.75 $\pm$ 0.60%) as a group differed significantly from the NAWQA sites (mean=6.12 $\pm$ 0.37%) with respect to GSI in male carp. Although this difference was statistically significant, these means were biologically very similar. Furthermore, the NCBP mean may be somewhat skewed by the

high mean for the OHR sub-basin. Only the NCBP stations differed significantly from the reference station.

Vitellogenin (vtg)

Most (93%) of the vtg values (in mg/mL) in male carp were censored (<0.001 mg/mL; Fig. 4-8), which precluded rigorous statistical analyses of the concentrations. Instead, vtg in males was analyzed statistically as a binary variable (that is, detected at 0.001 mg/mL or not). After trimming the data set to the 36 stations meeting the criteria for analysis (stage=1 or 2, $n>4$), there was no evidence that the proportion of male fish with detectable vtg differed by stage or among stations.

At the 36 stations remaining in the reduced-rank data set, vtg was detected in 17 of 312 male carp (5.4%) and in at least one fish at the following 11 stations: 28 (2 of 9), 32 (3 of 9), 72 (1 of 11), 76 (1 of 8), 82 (2 of 8), 84 (2 of 7), 86 (1 of 8), 89 (1 of 5), 90 (1 of 9), 112 (1 of 10), and 201 (2 of 6; Fig. 4-8). As noted previously, only the two fish from Stations 84 and 112 had vtg concentrations in the range of vtg station means for female carp in this study (0.100 – 6.299 mg/mL). There were no male carp (out of 8) with detectable concentrations of vtg at the reference site (Station 400).

Reproductive Biomarkers in Bass

Three species of black bass (largemouth, smallmouth, and spotted) were collected in the MRB (see Chapter 1 and Appendix A). Most of these were largemouth bass ($n=353$), which were collected at 25 stations (15, 23, 24, 25, 26, 27, 28, 29, 30, 32, 68, 70, 71, 76, 77, 78, 79, 80, 81, 82, 83, 112, 212, 213, and 400). Smallmouth bass ($n=72$) were collected at five stations (24, 67, 72, 74, and 111), and spotted bass ($n=21$) were collected at six sites (23, 24, 25, 68, 78, and 83). For the reproductive biomarkers evaluated in this study, the only discernable differences between the three species appeared to be anatomical. For instance, GSI was generally greater in male smallmouth and spotted bass than in male largemouth bass (Fig. 4-6). This is not surprising since largemouth bass grow considerably larger than the other *Micropterus* spp. GSI in females, however, did not differ among species (Fig. 4-5). For the other biomarkers, there were no significant differences among species, and all bass were pooled for statistical analyses. Additional information on the species, number, location, gender, size, and age of the fish are presented in Chapter 1 and Appendix A.

Histological examination of 2-3 gonad slices was used to confirm the sex of fish that were initially

classified in the field. In the process, foci of ovarian tissue were discovered in the gonads of 8.6% of the male bass examined. Consequently, the following male bass were identified as abnormal and potentially intersex: Largemouth bass from Stations 26 (1 of 7), 78 (2 of 7), 81 (1 of 8), 82 (1 of 13), 83 (1 of 6), and 213 (1 of 7); and smallmouth bass from Stations 67 (1 of 5), 72 (1 of 4), 74 (1 of 7), and 111 (8 of 11). No intersex spotted bass were found. With the exception of two 1-year old males (81-29 and 82-6), all of the intersex individuals were ≥ 2 y old, and all but two (81-29 and 72-30, both stage 1) were in stage 2 or 3. Of the 18 intersex male bass, only three had E/KT ratios >1.0 (fish 81-29, E/KT=1.15; fish 83-20, E/KT=1.32; fish 111-10, E/KT=2.17). The high E/KT values resulted from low 11-KT concentrations, rather than elevated E2 concentrations, in all three fish. Two intersex fish had abnormally high E2 concentrations (fish 78-38, E2=1823 pg/mL; fish 111-33, E2=1574 pg/mL); however, these fish also had high 11-KT concentrations (11-KT=1951 and 1713 pg/mL, respectively) and their E/KT ratios were within the normal limits for males (0.93 and 0.92, respectively). Although these males showed evidence of ovarian tissue, none expressed detectable concentrations of vtg. However, vtg analysis was not performed for the following intersex fish: 83-20, 74-2, 111-10, and 111-19.

Female Bass (Bar graphs of reproductive biomarkers in female bass are displayed in Figure 4-9).

Gonadal Stage

Of the 231 female bass examined histologically, the majority (58%) were in stage 2, 4% were in stage 0, 7% were in stage 1, 29% were in stage 3, and 2% were in stage 4 (Fig. 4-2). At Stations 80 and 81, 75% or more of the fish analyzed were in early stages of maturation (stages 0 and 1); however, it should be noted that only two fish from Station 80 were staged. Statistically significant differences ($P<0.05$) were found between several stations from which fish in drastically different stages were collected. However, only Stations 79 and 81 differed significantly ($P<0.05$) from more than three other stations with respect to stage. Within sub-basins only Stations 27 and 74 (in the UMS sub-basin) differed significantly. Stations 15, 23, 32, 68, 71, 80, 212, and 213 had <5 females in stages 2 and 3 combined and, therefore, were excluded from the statistical analyses of the other biomarkers. Although the number of fish differed for each biomarker, 21 stations met the criteria (stage=2 or 3, $n>4$) for statistical analysis of the reproductive biomarkers in female bass.

Estradiol (E2)

Station analysis: Mean E2 concentrations (in pg/mL) in

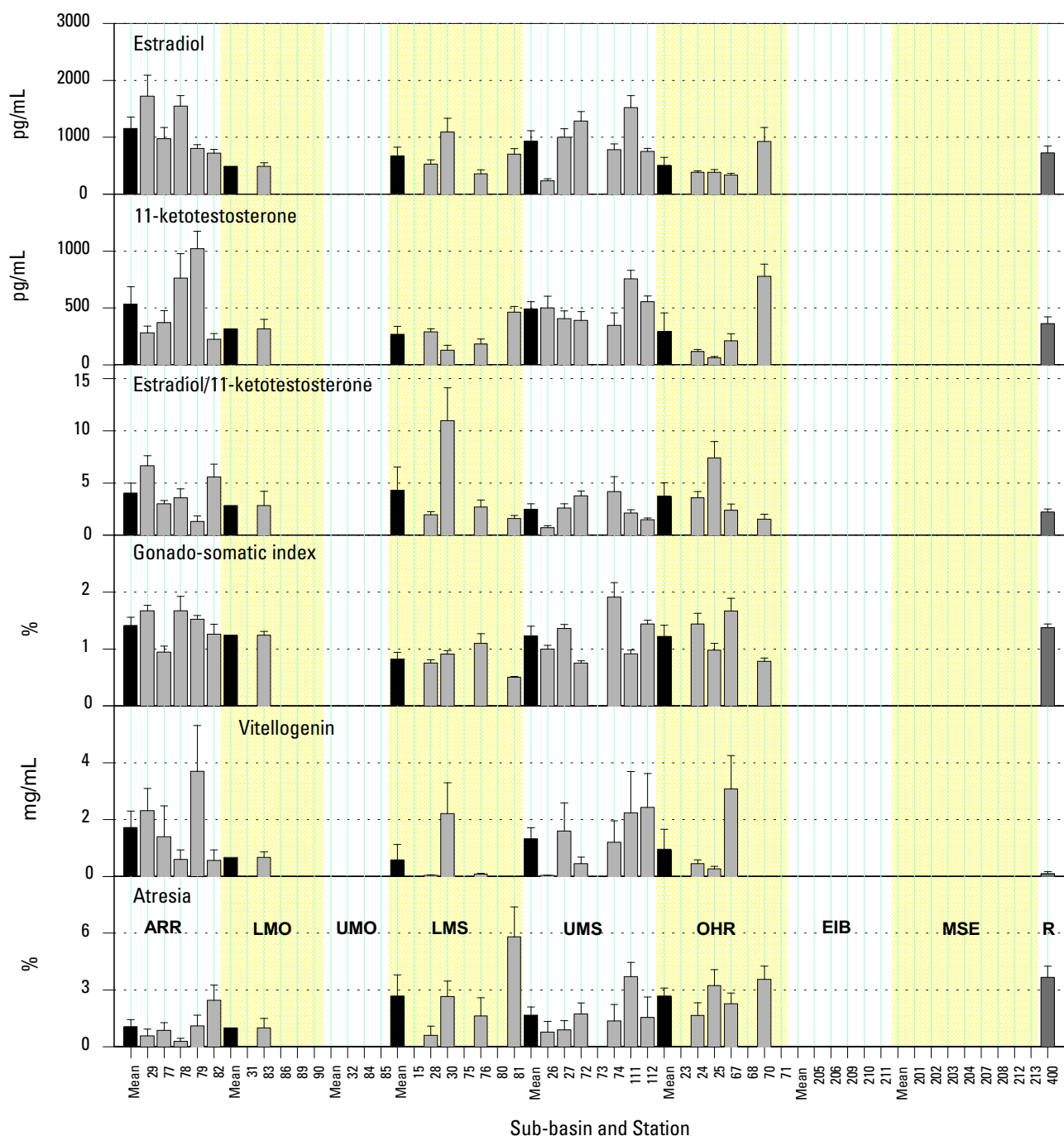


Figure 4-9. Reproductive biomarkers in female bass (reduced-rank dataset), by sub-basin (black bars) and station (grey bars, $n > 1$), for stations in the Mississippi River basin and the reference site (Station 400). Shown are arithmetic means + 1 SE. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

female bass ranged from 237.89 ± 29.74 at Station 26 to 1725.57 ± 368.15 at Station 29 (Fig. 4-9). Other than Station 26, only Stations 78 and 111 were >1500 pg/mL. Whereas none of the station means were unusually high, E2 means at Stations 24, 25, 67, and 76 were all <400 pg/mL. The mean E2 concentration for female bass at the reference site (Station 400) was 724.67 ± 126.87 .

Between sub-basins: Five sub-basins (ARR, LMO, LMS, UMS, and OHR) contained stations that met the criteria for inclusion, but there were no NAWQA sites with sufficient numbers of female bass. The E2 sub-basin means ranged from 1154.29 ± 202.83 in the ARR to 489.83 in the LMO (Fig. 4-9); however, it should be noted that there was only one station (83) in the LMO, so the E2 mean for this sub-basin is actually the station mean. The OHR sub-basin, which also had a comparatively low E2 mean (506.68 ± 140.34), differed significantly ($P<0.05$) from the ARR, LMS, and UMS sub-basins. The only other significant difference was between the ARR and LMS sub-basins.

Within sub-basins: For E2 in female bass there were no significant differences ($P>0.05$) among stations in the ARR sub-basin (Fig. 4-9). However, within the LMS sub-basin, Stations 30 and 76 differed significantly ($P<0.05$), as did Stations 67 and 70 within the OHR sub-basin. Stations 76 (in the LMS) and 67 (in the OHR) had two of the lowest station means in the MRB. Within the UMS, Station 26 differed significantly ($P<0.05$) from the other five stations in the sub-basin. Station 26 was also the site with the lowest E2 mean in the basin.

NCBP vs NAWQA sites: Overall, E2 in female bass from the NCBP sites (mean= 750.34 ± 128.35) did not differ significantly ($P>0.05$) from the reference site (mean= 724.67). Female bass from the reference site (mean= 724.67). Female bass from the NAWQA sites (Stations 212 and 213) did not meet the criteria for inclusion in the analysis.

11-Ketotestosterone (11-KT)

Station analysis: Mean 11-KT concentrations (in pg/mL) in female bass ranged from 62.44 ± 10.70 at Station 25 to 1018.80 ± 157.09 at Station 79 (Fig. 4-9). Although concentrations were not excessively high, Stations 70, 78, and 111 had 11-KT means >750 pg/mL. Conversely, means at Stations 24, 30, and 76 were <200 pg/mL. The mean 11-KT concentration in female bass at the reference site (Station 400) was 362.44 ± 60.11 .

Between sub-basins: The sub-basin means for 11-KT ranged from 265.14 ± 73.00 (LMS) to $530.72 \pm$

154.07 (ARR; Fig. 4-9). The ARR and UMS sub-basins, which had two of the highest 11-KT means, differed significantly ($P<0.05$) from the LMS and OHR sub-basins, which had two of the lowest. None of the sub-basins differed significantly ($P>0.05$) from the reference site (Station 400).

Within sub-basins: Stations within the ARR, LMS, and OHR sub-basins differed significantly ($P<0.05$) with respect to 11-KT in female bass (Fig. 4-9). In the ARR sub-basin, Station 82 differed from Stations 78 and 79; Station 79 also differed from Station 29. Stations 78 and 79 had two of the highest 11-KT means in the sub-basin, whereas Stations 29 and 82 were slightly low. In the LMS sub-basin, Station 30 (with one of the lowest 11-KT means in the sub-basin) differed significantly ($P<0.05$) from Station 81; in the OHR sub-basin, Station 70 differed from the other three stations in the sub-basin, all of which had comparatively low mean 11-KT concentrations.

NCBP vs NAWQA sites: The NCBP stations as a group (mean= 378.91 ± 54.94) did not differ significantly ($P>0.05$) from the reference site (Station 400, mean= 362.44). Female bass from the NAWQA sites were not included in the analysis.

E/KT Ratio

For female bass analyzed statistically (21 stations including the reference site, stage=2 or 3, $n>4$), only Station 26 had an E/KT ratio (mean= 0.564) significantly ($P<0.05$) <1.0 .

Gonadosomatic Index (GSI)

Station analysis: GSI in female bass varied considerably between stations, with the lowest mean at Station 81 ($0.50 \pm 0.02\%$) and the highest at Station 74 ($1.91 \pm 0.26\%$). Although significantly ($P<0.05$) greater than Station 81, the GSI Station means for Stations 28, 70, and 72 (ranging from 0.75 to 0.78%) were in the lower range, whereas means at Stations 29, 67, and 78 were among the highest ($>1.60\%$). The mean GSI of female bass from the reference site (Station 400) was $1.37 \pm 0.07\%$.

Between sub-basins: Mean GSIs for the sub-basins ranged from $0.82 \pm 0.13\%$ for the LMS to $1.42 \pm 0.14\%$ for the ARR. Only the LMS had a mean GSI $<0.12\%$. The LMS differed significantly ($P<0.05$) from the ARR, LMO, and UMS sub-basins, but not from the OHR sub-basin. The OHR sub-basin, however, differed from the ARR and LMO sub-basins. [Note: the Lower Missouri comprised only one station (83) in the trimmed data set.] The mean GSI for the reference site (Station 400), which differed significantly ($P<0.05$) from the LMS, UMS, and OHR

sub-basins, was higher than all sub-basin means except the ARR.

Within sub-basins: There were no statistically significant ($P<0.05$) differences among stations in the ARR and OHR sub-basins. In the LMS sub-basin Stations 76 and 81 (with the lowest mean GSI in the MRB) differed significantly ($P<0.05$) from one another. Greater variability was observed among stations within the UMS sub-basin, where GSI station means ranged from 0.75% to 1.91%. Within the UMS sub-basin, Station 111 differed significantly (lower, $P<0.05$) from Stations 112, 27, and 74; and Station 72 (with one of the lower GSI means) differed from Stations 27, 74, and 112.

NCBP vs NAWQA sites: As a group the NCBP stations (mean = $1.18 \pm 0.10\%$) differed significantly ($P<0.05$) from the reference site (mean = 1.37%) with respect to GSI in female bass. The NAWQA sites were not included in the analysis.

Vitellogenin (vtg)

Station analysis: The vtg station means (in mg/mL) for female bass ranged from 0.0005 at Stations 70 and 81 to 3.687 ± 1.621 at Station 79. [Note: the value 0.0005 is half the level of detection (0.001), which was assigned to those fish with non-detectable quantities of vtg for computational purposes.] Other than Station 79, only the vtg mean at Station 67 was >3.000 . Conversely, vtg means at several stations were at the lower extreme; these included Stations 26, 28, and 76, all of which had means <0.080 . The mean vtg for female bass at the reference site (Station 400) was also one of the lowest in the MRB (0.098 ± 0.073).

Between sub-basins: Sub-basin means for vtg in female bass ranged from 0.578 ± 0.542 in the LMS sub-basin to 1.705 ± 0.590 in the ARR. The LMS sub-basin differed significantly ($P<0.05$) from the LMO and OHR sub-basins and the reference site (Station 400); however, the LMO sub-basin comprised only one station (83). There were no other significant differences ($P>0.05$) among sub-basins.

Within sub-basins: Although little variability was observed among sub-basins for vtg in female bass, stations within most sub-basins differed considerably. In the LMS sub-basin, Station 30 differed significantly ($P<0.05$; higher) from Stations 28 and 81 (two of the lowest station means). In the UMS sub-basin, Station 112 differed significantly ($P<0.05$) from Stations 26 (one of the lowest station means) and 72; Station 26 also differed from Station 27. In the OHR sub-basin, Station 67 (one of the highest station means) differed

significantly ($P<0.05$) from Station 70 (one of the lowest station means). There were no significant differences among the stations in the ARR sub-basin, and the LMO had only one station (83) in the trimmed data set.

NCBP vs NAWQA sites: Overall, vtg concentrations of female bass from the NCBP stations as a group (mean = 1.043 ± 0.210) were significantly ($P<0.05$) greater than at the reference site (mean = 0.098 ± 0.073). Female bass from the NAWQA sites were not included in the analysis.

Atresia

Station analysis: Mean atresia in female bass was greatest at Station 81 ($5.8 \pm 1.59\%$) and lowest at Station 78 ($0.30 \pm 0.15\%$; Fig. 4-9). The mean at the reference site (Station 400) was $3.67 \pm 0.60\%$. In addition to Station 81 and the reference site, mean atresia was $>3.0\%$ at Stations 25, 70, and 111. In contrast, mean atresia was $<1.0\%$ at Stations 26, 27, 28, 29, 77, and 78 (the lowest in the basin).

Between sub-basins: Atresia means for the sub-basins ranged from 1.00% in the LMO to $2.68 \pm 0.43\%$ in the OHR (Fig. 4-9); however, the LMO sub-basin comprised only one station (83) in the reduced-rank data set. Overall, the sub-basin means were similar; only the ARR (with the second lowest sub-basin mean) differed significantly ($P<0.05$) from LMS and OHR sub-basins. Atresia at the reference site (Station 400) was greater than in all sub-basins, but differed statistically from only the ARR sub-basin.

Within sub-basins: The LMS was the only sub-basin in which mean atresia in female bass differed significantly among stations (Fig. 4-9). Within this sub-basin, Station 81 (which had the highest station mean in the MRB) differed ($P<0.05$) from Station 28 (among the lowest station means).

NCBP vs NAWQA sites: Overall, atresia in female bass from the reference site was significantly greater ($p>0.05$) than at the NCBP stations (mean = $1.82 \pm 0.37\%$). Female bass from the NAWQA sites were not included in the analysis.

Male Bass (Bar graphs of reproductive biomarkers in male carp are displayed in Fig. 4-10).

Gonadal Stage

Of the 210 male bass examined histologically, most (77%) were in stage 2, 16% were in stage 1, and 5% were in stage 3 (Fig. 4-2). Only three male bass (1%) were in stage 0 and none were in stage 4. Two of the four male bass from station 72 were in stage 0. In

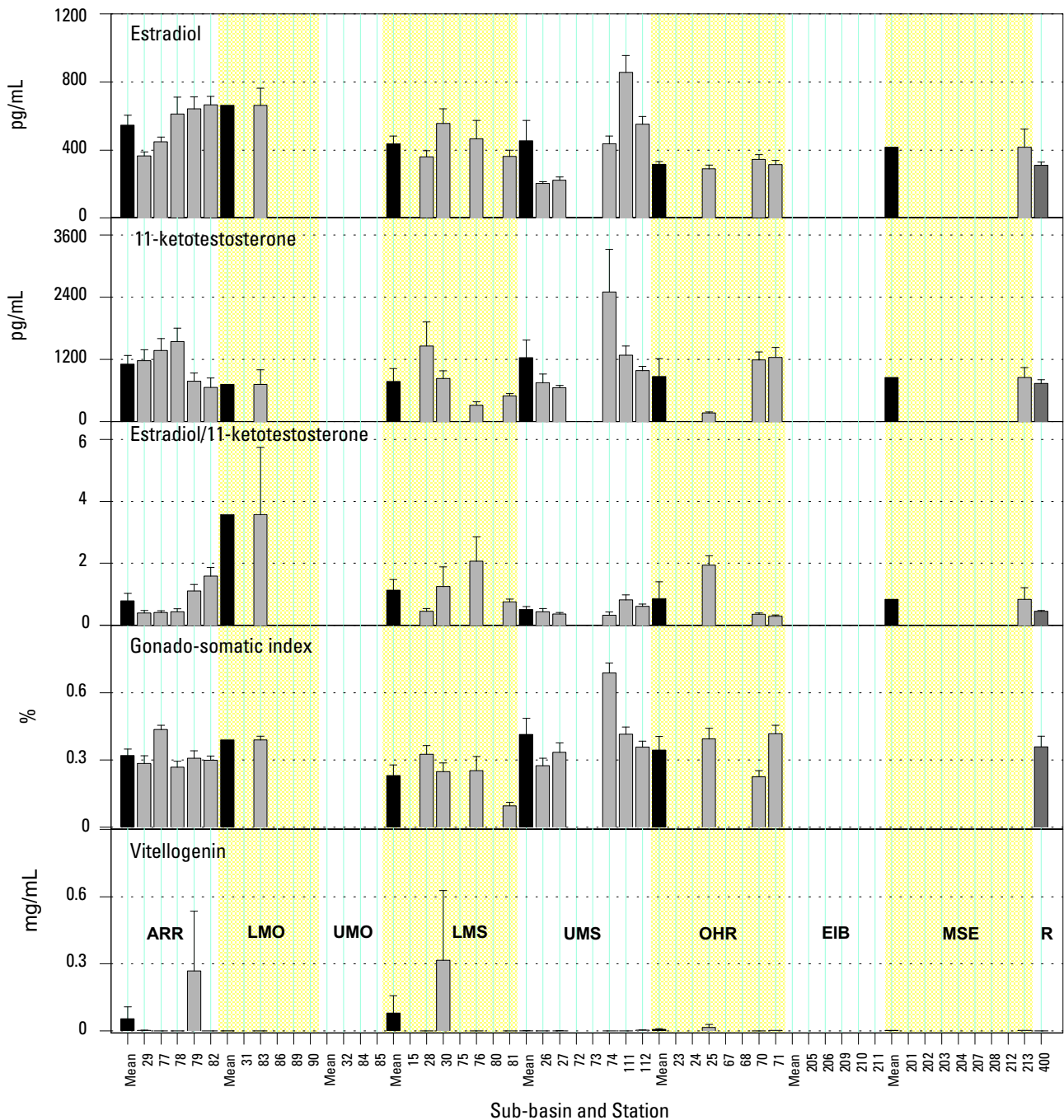


Figure 4-10. Reproductive biomarkers in male bass (reduced-rank dataset), by sub-basin (black bars) and station (grey bars, $n > 1$), for stations in the Mississippi River basin and the reference site (Station 400). Shown are arithmetic means + 1 SE. Sub-basin estimates were based on station means rather than individual fish. See Table 1-1 and Figure 1-1 for station locations.

terms of stage distributions among stations, the only substantial differences were between Stations 82 (with fish in stages 2 and 3) and 26 (with fish in stages 1 and 2). Stations 23, 24, 67, 68, 72, 80, and 212 had <5 males in stages 1 and 2 combined and were therefore excluded from the statistical analyses and comparisons. Although the number of fish varied among biomarkers, 20 stations met the criteria (stage=1 or 2, $n>4$) for statistical analysis of reproductive biomarkers in male bass. In addition, five NCBP sub-basins (ARR, LMO, LMS, UMS, and OHR) and the MSE Study Unit (Station 213 only) met the criteria for statistical analysis. However, GSI data are not presented for Station 213 because no gonadal weights were obtained.

Estradiol (E2)

Station analysis: E2 station means (in pg/mL) for male bass ranged from 201.30 ± 11.78 at Station 26 to 855.00 ± 99.70 at Station 111 (Fig. 4-10). Stations 25 and 27 had the lowest E2 means (<300 pg/mL), whereas Station 111 was the only station with an E2 mean >670 pg/mL. The mean E2 for male bass at the reference site (Station 400) was 310.00 ± 18.06 .

Between sub-basins: Sub-basin means for E2 in male bass ranged from 315.19 ± 16.12 in the OHR sub-basin to 662.71 in the LMO (Fig. 4-10); however, as reported for female bass, there was only one station (83) with male bass in LMO sub-basin. Nevertheless, the LMO differed significantly ($P<0.05$) from all other sub-basins except the ARR. Similarly, the ARR sub-basin (with the second highest sub-basin mean) differed from all sub-basins except the LMO. The LMS sub-basin and the MSE Study Unit did not differ significantly, however ($P>0.05$). Although the E2 mean at the reference site (Station 400) was lower than all sub-basin means (including the MSE), the reference site only differed significantly from the ARR and LMO sub-basin.

Within sub-basins: There were no significant differences ($P>0.05$) between stations of the LMS and OHR sub-basins for E2 in male bass. In the ARR sub-basin, only Stations 29 and 82 differed significantly ($P<0.05$; Fig 4-10). Greater variability was observed among stations of the UMS sub-basin, where Station 111 (with the highest station mean in the MRB) differed significantly ($P<0.05$) from Stations 26 and 27 (with two of the lowest E2 means in the MRB). Stations 26 and 27 also differed significantly from Stations 74 and 112.

NCBP vs NAWQA sites: Because only one NAWQA station (213) was included in the analysis of E2 in male bass, the NAWQA mean is actually the mean for

Station 213. Nevertheless, the NCBP stations (mean= 482.18 ± 58.15) and NAWQA stations (mean= 414.57) were not significantly different. However, the NCBP stations differed significantly ($P<0.05$) from the reference site (Station 400).

11-Ketotestosterone (11-KT)

Station analysis: 11-KT station means (in pg/mL) for male bass ranged from 166.71 ± 21.13 at Station 25 to 2502.29 ± 820.12 at Station 74 (Fig. 4-10). Most station means ranged from 600-1400 pg/mL; only Stations 28 and 78 were slightly higher and Stations 76 and 81 were lower. The mean 11-KT concentration for male bass from the reference site (Station 400) was 737.67 ± 71.40 .

Between sub-basins: Overall, there were few statistically significant differences between sub-basins for 11-KT in male bass. The means ranged from 718.71 in the LMO sub-basin (containing only station 83) to 1234.04 ± 335.11 in the UMS sub-basin (Fig. 4-10). The LMS (with the second lowest 11-KT mean) differed significantly ($P<0.05$) from the ARR and UMS sub-basins (with the highest 11-KT mean). The LMS sub-basin and the MSE Study Unit (containing only Station 213) were not significantly ($P<0.05$) different.

Within sub-basins: Station means for 11-KT in male bass differed significantly ($P<0.05$) within all sub-basins except the UMS (only one station within the LMO and MSE, however). Within the ARR, Station 78 differed significantly (higher) from Station 82, and within the LMS sub-basin, Station 28 differed (higher) from Station 76. Stations 78 and 28 (Fig. 4-10) had mean 11-KT concentrations in the upper range, whereas Stations 82 and 76 were slightly low. Within the OHR sub-basin, Station 25 (the lowest 11-KT mean in the MRB) differed significantly ($P<0.05$) from the other two stations (70 and 71).

NCBP vs NAWQA sites: For 11-KT in male bass the NCBP stations as a group (mean= 940.18 ± 98.74) and NAWQA station (that is, Station 213; mean= 846.86) did not differ significantly ($P>0.05$), nor did either differ from the reference site (mean= 737.67).

E/KT Ratio

For male bass analyzed statistically (21 stations including the reference site, stage 1 and 2, $n>4$), only Stations 25 (mean= 1.936 ± 0.309) and 82 (mean= 1.585 ± 0.276) had E/KT ratios significantly ($P<0.05$) >1.0.

Gonado-somatic Index (GSI)

Station analysis: GSI station means for male bass ranged from $0.23 \pm 0.04\%$ at Station 30 to $0.69 \pm$

0.05% at Station 74 (Fig. 4-10). GSI means were 0.25% and 0.45% for all other stations. Only Station 74, which differed significantly ($P < 0.05$) from all other stations, seemed to be somewhat unusual (greater GSI, as noted above). The mean GSI for male bass from the reference site (Station 400) was $0.37 \pm 0.05\%$.

Between sub-basins: The only significant differences among sub-basins for GSI in male bass involved the UMS, which differed (higher) significantly ($P < 0.05$) from the ARR, LMS, and OHR sub-basins (Fig. 4-10). The Upper Mississippi contained Station 74, which may have shifted the sub-basin mean upward slightly.

Within sub-basins: The UMS was the only sub-basin in which GSI in male differed significantly among stations (Fig. 4-10); Station 74 was significantly ($P < 0.05$) greater than all other stations in the sub-basin (27, 111, and 112). Station 74 was also the only station to differ significantly from the reference site (Station 400).

NCBP vs NAWQA sites: GSI in male bass did not differ significantly ($P > 0.05$) between the NCBP stations as a group (mean = $0.37 \pm 0.03\%$) and the reference site. No GSI data were available for male bass from the NAWQA stations.

Vitellogenin (vtg)

The reference site, 18 NCBP stations, and NAWQA station 213 met the criteria for vtg analysis (reported in mg/mL). Due to the high degree of censoring (undetectable vtg), the response was converted to a binary variable (proportion) for analysis; fish with non-detectable concentrations of vtg (< 0.001 mg/mL) were assigned a value of zero and fish with detectable vtg (> 0.001 mg/mL) were assigned a value of 1.0. In this analysis, there was no evidence that the proportion of fish with detectable vtg differed by stage or among stations.

Of the 20 stations remaining in the reduced-rank data set, vtg was detected in 14 of 174 (8%) stage-1 and -2 male bass from the following nine sites (45%): NCBP Stations 25 (two of seven), 27 (one of 10), 29 (two of seven), 30 (two of nine), 70 (one of 14), 71 (one of nine), 79 (one of nine), 112 (one of 10), and at NAWQA Station 213 (three of seven; Fig. 4-10). Overall, male bass with detectable vtg were present at nine of 20 stations (45%), and vtg was detected in 14 (8%) of the 174 male bass analyzed. Except for two stations at which vtg was non-detectable in all fish, the vtg station means for female bass in this study ranged from 0.026 to 3.687 mg/mL. Of the male bass with detectable concentrations of vtg, only four had concentrations within the range of

vtg detected in the female bass—0.102 mg/mL (Station 25), 2.817 mg/mL (Station 30), 2.408 mg/mL (Station 79), and 0.026 mg/mL (Station 112). There were no vitellogenic male bass (out of nine analyzed) at the reference site (Station 400).

DISCUSSION

Objectives of the Study

One of the overall objectives of the 1995 MRB study was to evaluate the performance of a suite of biomarkers designed to determine reproductive status and potentially predict contaminant exposure or effect. The primary purpose of this chapter was to document and compare reproductive biomarker responses from several species of fish distributed throughout the basin. This study sought to establish normal biomarker ranges and identify specific sites where reproductive activity may be affected by chemical exposure. With the contaminant data presented in Chapter 2, the results from this chapter form a preliminary database that would assist in evaluating effects of contaminants on reproductive function and future changes.

Reproductive Biomarkers

The biomarkers used in this study—sex steroid hormones (E2 and 11-KT), vtg, GSI, and gonadal histopathology (used for the analysis of sex, stage, and oocytic atresia)—are the best techniques available for measuring reproductive function as well as the effects of contaminants, whether endocrine disrupting or otherwise, on reproductive health. However, these factors are influenced by photoperiod, water temperature, age, species, as well as other biotic and abiotic factors and undergo great fluctuations during the reproductive cycle. A well designed study controlling for these factors can provide valuable data and give important insights into reproductive health. These biomarkers have proven to be valuable measures of reproductive activity and dysfunction in a variety of laboratory studies, as well as several field studies (including this one) designed to monitor the effects of environmental contaminants on the reproductive activity of fish in nearby streams. The use of this suite of reproductive biomarkers is necessary to evaluate the effects of contaminants on reproductive health and should be incorporated into future BEST program projects.

Sex Steroids: Measuring sex steroid hormones in plasma is a technique that has been used to study gonadal development and reproductive cycles of healthy individuals (Johnson and others, 1991; Freund and others, 1995), as well as the endocrine-disrupting effects of various contaminants. The literature has shown

that although males and females produce both estrogens and androgens, males will typically produce considerably higher concentrations of 11-KT than estradiol, and females will produce substantially higher concentrations of estradiol than 11-KT. Often the ratio of the two hormones (E/KT) is used, rather than actual concentrations, to evaluate reproductive health. An E/KT >1.0 for females and <1.0 for males is generally considered normal (Hileman, 1994; Folmar and others, 1996). Overall, the reproductive status and health of males and females throughout the MRB appeared to be normal, although the ranges of hormone concentrations were large (Figs. 4-3—4-6). This was to be expected, however, since these hormones are influenced by a number of intrinsic and extrinsic factors, including sex, age, species, reproductive stage, geographical location, season, and exposure to various nutrients and chemicals.

This study was designed to control for some of these factors by analyzing each sex/taxon group separately and restricting hormone analyses to fish in the same (or similar) reproductive stage, as determined by gonadal histopathology. In order to minimize temporal or seasonal variability, as well as maintain consistency with earlier studies (Goodbred and others, 1997; Schmitt and others, 1999a), all fish were collected between August and December (1995 for NCBP and NAWQA stations 1996 for the reference site), which is the reproductively quiescent period for most non-salmonid North American fishes (Down and others, 1990). Other studies demonstrating that the reproductive cycle for certain fishes (for example, largemouth bass in Florida) begins in the fall would suggest that some of the variation observed in this study may be due to differences in reproductive stages (Gross and others, in press). Nonetheless, there did not appear to be substantial differences in reproductive stage nor the biomarkers altered as a function of the collection date. Despite these measures, a large degree of variability in sex steroid concentrations was observed between stations. For instance, E2 station means for female carp (Fig. 4-7), female bass (Fig. 4-9), and male carp (Fig. 4-8) varied approximately 7-fold, and the variability in E2 concentrations for male bass (Fig. 4-10) was approximately 4-fold. 11-KT station means varied 15-fold for male bass (Fig. 4-10), 17-fold for male carp (Fig. 4-8), 9-fold for female carp (Fig. 4-7), and 16-fold for female bass (Fig. 4-9). This is not surprising, since several reports in the literature have documented extreme variability (up to 30-fold) in sex steroid concentrations from individual fish at the same site (Down and others, 1990; Chang and Chen, 1990; Folmar and others, 1996). Although the ranges were large, sex steroid concentrations at most stations were in the middle of the two extremes. By comparing sex steroid means across the MRB, stations

with extreme concentrations (high and low) were identified for further examination and possibly future monitoring (see Results section for details). Overall, extreme variability made it difficult to draw meaningful conclusions regarding station differences.

To control for spatio-temporal effects, stations were categorized and evaluated on a regional (sub-basin) basis, as recommended by Goodbred and others (1997). Substantial variability was also demonstrated between and within sub-basins, although station means in the same sub-basin were often less variable. For instance, in male carp, mean 11-KT concentrations at two of the MSE NAWQA stations (204 and 207) were low compared with other stations in the basin (Fig. 4-8); however, these stations were not substantially lower than the other stations within their MSE Study Unit. Lower 11-KT concentrations may be typical of fish in the lower-order streams sampled by the NAWQA program as evidenced by the fact that mean 11-KT concentrations at most of the EIB NAWQA sites were also low. However, there were also NCBP stations in the same vicinity (76 and 80 in the LMS sub-basin) with low 11-KT means. The NAWQA sites and Station 80 were sampled early (mid-August to mid-September), which may account for lower hormone concentrations; however, this does not explain the low concentrations at Station 76, which was sampled in early November. Regardless of collection date, most male carp collected from Stations 76, 80, and the NAWQA sites were in stage 2; only at Station 201 were all the fish in gonadal stage 1. Another observation is that E2 concentrations in female carp from the MSE Study Unit (Stations 201-204, 207, 208, and 212) were average to high (Fig. 4-23), which suggests that steroidogenesis was in progress, at least for females, despite early sampling. Therefore, if endocrine disruption is occurring, it appears to be selectively affecting androgens.

As noted in Chapter 2 of this report, concentrations of one or more organochlorine pesticides (DDE, toxaphene, dieldrin, endrin, chlordane, heptachlor, and mirex) were relatively high at stations in the LMS sub-basin and in both the EIB and MSE Study Units. Preliminary statistical analyses indicated a negative correlation between 11-KT and Hg, cyclo-dienes, and DDE in male carp (see Chapter 5 of this report). Therefore, it is possible that similar contaminant exposure was responsible for the comparable hormone responses observed at the above stations, all of which were located in the lower Mississippi River valley. As noted above, however, the contaminants measured in this study represent only a small fraction of the agricultural (and other) chemicals to which fish in these areas are exposed. All of the factors mentioned previously need to be explored further to determine the extent, the etiology, and the implications of the

endocrine alterations occurring in the lower Mississippi region.

Grouping stations by sub-basins was also useful for identifying single stations, rather than entire sub-basins, where endocrine disruption may be occurring. For example, the E2 and 11-KT means for female carp at Station 32 were drastically lower than those of the other two UMO stations (84 and 85) (Fig. 4-7). Because these stations were in close geographic proximity and were sampled at similar times, the low sex steroid concentrations at Station 32 may be related to conditions at that particular site. A preliminary assessment of the contaminant data from Station 32 (Chapter 2) did not reveal unusually high levels of any of the chemicals or elements examined during this monitoring effort.

Measuring sex steroids is a rapid and inexpensive method for obtaining valuable information at the biochemical or molecular level. Biomarkers that detect alterations at this level are highly sensitive and are often the first detectable responses to an environmental change or stressor (McCarthy and Shugart, 1990). Consequently, they have the potential to provide early warning signals of endocrine disruption and may be predictive of perturbations at higher levels of organization. Furthermore, sex steroid concentrations can be determined from blood, urine, and fecal samples, all of which are typically easy to obtain in most species and do not require sacrificing the animal. This is advantageous for studying reproductive toxicity in endangered species, allows for serial sampling throughout lifecycle or trans-generational studies, and serves the interests of wildlife conservation.

Considering the advantages, sex steroids have become popular biomarkers for determining reproductive status and detecting endocrine alterations; however, there are a number of caveats or limitations to using this technique. The most obvious involves the large degree of natural variability that occurs between individuals, even those of the same sex, species, and gonadal stage. The data from this study clearly show a wide range of concentrations, even when comparing neighboring stations or fish from the same station. Although endocrine disruption due to contaminants or other environmental stressors probably accounts for some of the variability, fish at the same stations should be exposed to similar conditions, yet they often differ with respect to hormone concentrations. This has made it difficult to define normal ranges, especially because these ranges are typically species-specific and vary throughout the reproductive season. As mentioned earlier, sex steroid concentrations are also influenced by many environmental factors. Some of these can be controlled in laboratory and field experiments; however, there is evidence that stress from collecting, holding, relocating, and obtaining blood may also influence sex

steroid levels (van der Kraak and others, 1992; McMaster and others, 1994; van den Heuvel and others, 1995). Because of the large number of teams involved in this study these stressors may not have been well controlled. When possible, in future studies, sampling should be conducted by as few teams as possible in order to minimize variations due to collecting techniques.

Sex steroids have been used successfully to document exposure to certain endocrine-disrupting chemicals; however, they are rarely able to provide information concerning mechanism of endocrine disruption or ultimate biological consequences. This is partially due to the complexity of the endocrine system and the fact that chemicals have been shown to alter sex steroid levels by interfering at multiple sites along the hypothalamo-pituitary-gonadal axis. Furthermore, it is possible that multiple endocrine pathways are affected simultaneously (Singh and others, 1994), especially considering the combinations of chemicals that wildlife populations are exposed to in their natural habitats. Despite several limitations, sex steroid assays can provide valuable information in both field and laboratory studies, and they are most reliable when field studies are validated with laboratory experiments.

Vitellogenin: Vitellogenin (vtg) has gained popularity over the last decade as a biomarker for characterizing gonadal stage, assessing reproductive health, and predicting the estrogenicity of various compounds. The structural diversity of many environmental estrogens has made it necessary to establish a bioassay that tests for estrogen action, independent of a chemical's structural properties. The fact that vtg synthesis is primarily regulated by circulating estrogens (Emmersen and Petersen, 1976; Sundararaj and others, 1982; Ueda and others, 1984) made vtg an attractive candidate for the development of a universal assay to test for estrogenicity (Heppell and others, 1995; Palmer and Palmer, 1995; Palmer and Selcer, 1996). Early studies indicated that healthy males do not synthesize vtg under normal circumstances (Sumpter and Jobling, 1995; Denslow and others, 1996) but that exposing males to estrogens stimulated significant vtg production. Consequently, it was believed that vtg could provide an effective instrument for identifying males that had been exposed to environmental estrogens.

Recent studies that localized vtg receptors to the testes, muscle, and spermatocytes (Bidwell and Carlson, 1995; Tao and others, 1996), as well as the 1995 reconnaissance study by Goodbred and others (1997), which reported detectable vtg concentrations in untreated males from the reference site, raised the possibility that vtg may occur at low levels in a percentage of healthy males. Vitellogenin was measured in only one male (bass) from the reference site in this

study; the protein appeared in 6% of the males (carp and bass) from other stations. At no station were there more than 3 fish with detectable concentrations (most stations had a total of 0 or 1 male fish with vtg), and stations with vitellogenic fish were distributed throughout the basin; no sub-basin trends were observed. In most cases, vtg concentrations were low and did not correlate with elevated estradiol in males, although this does not preclude the possibility that external estrogens stimulated vtg production. The exceptions were the few males (2 carp, 4 bass) with high vtg concentrations (0.026-2.817 mg/mL, similar to female concentrations) that also had high E2 concentrations of 958-2713 pg/mL. Furthermore, the six males with the highest E2 also had the highest vtg concentrations.

PCBs were present at several of the stations where the six highly vitellogenic males were collected, and preliminary statistical analyses suggested a positive correlation between PCBs and vtg in male carp (see Chapter 5 of this report). If these contaminants, or others not tested for in this study, were contributing to elevated vtg in males, the fact that some males responded by producing vtg while others (at the same station) did not is puzzling. However, this may be the nature of the vitellogenic response in males, given the fact that diverse responses to estrogenic substances have been observed, even in studies where exposures were constant and carefully monitored. Laboratory experiments with sheepshead minnows (*Cyprinodon variagatus*) suggest that a threshold between 20 and 100 ng/L of exogenous estradiol is required before vtg is produced in measurable quantities (Folmar and others, 2000; Hemmer and others, 2001). In the wild, concentrations and durations of actual exposure are impossible to measure, especially given the natural variability in diet and the fact that fish are free ranging and may move in and out of contaminated areas.

Although variables related to exposure have complicated the interpretation of vtg data from males collected in field studies (as in this study), vtg has proven to be a valuable biomarker in carefully controlled laboratory experiments, where concentrations can be measured before and after estrogen administration or exposure, and in field studies where fish reside in extremely estrogenic environments (for example, downstream of sewage treatment facilities), can be compared to those in unpolluted waters (Purdom and others, 1994; Sumpter and Jobling, 1995; Bevans and others, 1996). However, the successful application of vtg as a biomarker for male fish in large-scale field studies and laboratory studies is contingent on a thorough understanding of the species specific pattern of vtg production and the documentation of baseline concentrations and variance. Similar to the studies with sheepshead minnows, species-specific experi-

ments which seek to determine exposure requirements and magnitude of response are needed in order to accurately assess the condition of males in their natural habitats. Because vtg assays performed by different laboratories are not routinely cross validated and detection limits may vary between laboratories it is difficult to define normal ranges. Additionally, it is important to understand the biological implications, if any, that vtg (at a range of concentrations) may have on fish. Although vtg at abnormally high concentrations can lead to abnormal pathologies (that is renal damage) following experimental induction in the laboratory (Folmar and others, 2001), effects of vtg at concentrations within the normal range for male or female fish have not been shown to induce any abnormal pathologies in male fish. Indeed, the comprehensive biomarker results from this study suggested that males with detectable or abnormal vtg concentrations were otherwise normal. It is possible that the concentrations measured have no biological implications and, indeed, baseline concentrations <0.01 mg/mL are likely normal. These results indicate that the presence of a detectable concentration of vtg in male fish is not in itself a biomarker of estrogenic exposure. Although concentrations significantly higher than the species-specific baseline would suggest exposure to an estrogenic contaminant and/or other hormonally active agent in male fish, vtg is primarily utilized as a biomarker of reproductive function and status in female fish.

Although vtg is a highly reliable biomarker of reproductive function and status in female fish, it has rarely been utilized or proposed as an indicator of potential contaminant effects/exposure in females. In male fish, vtg has no known reproductive or physiological function, whereas in females, it is a critical component of the developing oocyte. Therefore, identifying females with abnormally low vtg concentrations, as compared to other female fish during similar seasonal periods, may indicate potential contaminant effects. Indeed, the identification of abnormal vtg concentrations in female fish will likely have more relevance to subsequent reproductive function and the detection of potential adverse effects than does the detection of low or abnormal concentrations in male fish. All female oviparous fish produce vitellogenin, and in the prespawning period, a lack of vtg may indicate serious reproductive problems. Within the MRB, female bass from Stations 70 (stage-2) and 81 (stage-0, -1, and -2) had no detectable vtg, and female bass at Stations 28 (stage-2), 76 (stage-0, -1, and -2), 26 (stage-1, -2, and -3), and the reference site (stage-1 and -2) had extremely low concentrations (Fig. 4-5). Although the stations with low vtg concentrations also had low mean E2 concentrations, the two stations without vtg showed relatively average E2 means. This suggests that endocrine disruption

may be occurring at a point in the biochemical pathway following estrogen production. Although PCBs or other organochlorines were detected at each of the stations where the vtg concentrations were extremely low, preliminary statistical analyses did not find a correlation between these contaminants and vtg concentrations (see Chapter 5 of this report).

Seasonality and gonadal stage, and the relationship between these factors, are other important issues to consider when analyzing vtg data in females. For female bass in this study, vtg concentrations were correlated with stage and differed significantly among stages. Indeed, stage assessments are based in part on the deposition of vitelline granules in the developing oocyte; therefore, it is not surprising that stage and vtg in females are correlated. Although fish were collected in their non-reproductive season, sampling took four months to complete, so it is reasonable that females sampled months apart could be in different stages of their respective reproductive cycle. Most female bass were in stages 2 and 3 and, therefore, statistical analysis was restricted to females in these two stages; however, even slight differences between stages 2 and 3 may have accounted for the wide range of vtg concentrations observed. Low or non-detectable concentrations of vtg at Stations 26 and 81 may have been related to early sampling (late September). At both stations, the females analyzed were almost exclusively in stage 2. On the other hand, Stations 28, 71, 76, and 400 were sampled in October and November, yet females were also stage 2 (or lower) and vtg concentrations were uniformly low. At many of the stations with higher vtg means, such as Stations 79 and 67, fish were predominantly in stage 3. Both of these stations were also sampled late in the fall; however, Station 111 reported predominantly stage 3 fish and was sampled in early to mid-September. Thus, while sampling over a four-month period is not ideal for assessing any of the reproductive biomarkers, it did not appear to be the reason for slight differences in stage, although geographical location is another potentially complicating factor. Regardless of the time of collection, stage differences (although often slight) did appear to influence vtg concentrations. In addition, immaturity may have contributed to lower concentrations observed at Stations 15, 25, 76 and 81, since some of the female bass collected at these sites were determined to be only 1-year old. Although some of these females were stage-0 (one fish from Station 15 and five from Station 81), most were in stages 1-3 and should have been vitellogenic.

All female carp in this study had detectable concentrations of vtg, although the means were low at several stations. Unlike female bass that were distributed across five reproductive stages with a substantial

number of individuals in stages 2 and 3, female carp were predominantly stage 3 (83%). Even so, vtg concentrations of stage 3 females ranged from 0.1 to 6.3 mg/mL. Although the stations with the lowest vtg means (30, 201, 202, and 203) were also among the earliest sites sampled (late August – mid-September), most female carp (and the only individuals contributing to the station means) were in stage 3. Thus, early collection, immature gonads, or both are not reasonable explanations for the variability observed. Age did not appear to be a factor either, as none of the stage 0 fish were age 1, and the youngest female at any of the stations with low vtg was age-3.

E2 concentrations in female carp did not always correlate with vtg concentrations. For instance, Stations 84 and 85 had two of the highest E2 means (Fig. 4.23); yet, vtg concentrations were low to average. Conversely, the E2 mean for Station 26 was extremely low, but the vtg mean at this site was among the highest in the MRB. These are not terribly surprising observations, as other studies have demonstrated that increases in estradiol are not necessarily paralleled by increases in vtg. Furthermore, it appears that vtg production can be stimulated by relatively small increases in estradiol concentrations. The fact that vtg is constantly being absorbed from the blood by the maturing oocyte may mean that vtg levels in the blood are somewhat transient. Measuring vtg during the reproductive season, when concentrations are much higher, may consequently prove to be valuable in future studies.

Vtg concentrations are affected by many of the same factors that influence sex steroids, including sex, species, reproductive stage, season, temperature, and chemical composition of the environment; therefore, a number of factors need to be considered when interpreting vtg data. Furthermore, there is evidence for the regulation of vtg synthesis by circadian rhythms in catfish (*Heteropneustes fossilis*) (Lamba and others, 1983), photoperiod in rainbow trout (Bromage and others, 1982), and winter sea temperatures in small-spotted cat shark (*Scyliorhinus caniculus*) (Craik, 1978). In addition to measuring plasma vtg, the appearance of vitelline granules, which are storage compartments within oocytes composed of lipid-bound vtg fragments, aids histopathologists in the evaluation of gonadal development and seasonal activity changes in females.

Vtg assays are sensitive and reasonably inexpensive; however, a better understanding of the natural variability and the factors that influence variability is needed to improve the effectiveness and reliability of vtg as a biomarker of reproductive health or chemical exposure. Also, the sequence variability of vtg, even among closely related species (Campbell and Idler, 1980; So and others, 1985; Benfey and others, 1989;

Lee and others, 1992), has complicated the development of a universal assay for detecting the protein by immunological methods. Heppell and coworkers recently generated a polyclonal vtg antibody that cross-reacts with fish from diverse families (Heppell and others, 1995), and several monoclonal antibodies with wide cross reactivities are now available (Denslow and others, 1997). To date, a truly universal assay has not been developed, and it is unlikely that traditional techniques will produce an antiserum with such broad taxonomic applicability.

Gonadosomatic Index (GSI) and Gonadal

Histopathology: Considerable variation in gonad size is observed throughout the reproductive cycle of many animal species (de Vlaming and others, 1981). Therefore, GSI is often used to evaluate reproductive status and health. As with the appraisal of sex steroid hormones and vitellogenin, interpretation of GSI measurements relies on a thorough understanding of the natural variability between fish of the same age, sex, and species, as well as the environmental influences and behavioral patterns that may complicate or confound data. In addition, evaluating GSI data obtained from a particular species requires knowledge of the specific reproductive strategy practiced by that species. Over the years, the accuracy and reliability of this method have been scrutinized by some, due to the variability observed between relative ovarian weights (de Vlaming and others, 1981), and the inability of some field studies to correlate tissue levels of contaminants or plasma sex steroid levels with GSI (Monosson and others, 1994; Johnson and Landahl, 1994; Sepúlveda and others, in press).

Large variability in GSI was also observed in the current study, despite the fact that fish from each sex/taxon group were in the same (or similar) reproductive stages (see Figs. 4-3—4-6). However, GSI was often consistent with sex steroid and vtg concentrations, especially at those stations with extremely high or low measurements. For example, Station 74 had the highest 11-KT mean as well as the highest GSI mean for male bass (Fig. 4-10), whereas Station 76 documented the lowest GSI mean and one of the lowest E2 means for female carp (Fig. 4-7). There were exceptions, however. At most of the NAWQA stations male carp showed normal GSI means, although they also had the lowest 11-KT means in the MRB (Fig. 4-8). In such instances the mechanisms underlying the reproductive alterations may be more complicated than reduced steroidogenesis due to immature gonads, and more thorough investigations at these stations may be required to identify the underlying cause or causes.

Gonadal histopathology was used in this

study to confirm sex, assign reproductive stage, and detect anatomical abnormalities such as the presence of ovotestes and excessive oocyte atresia. In general, fish of the same sex/taxon were in similar stages of gonadal maturity, despite differences in sampling times and locations. Males were predominantly in stages 1 and 2 and females in stages 2 and 3. Fish falling outside of these ranges were excluded from the final statistical analysis because the reproductive biomarkers used in this study are known to vary over the course of the reproductive cycle. Therefore, for the purposes of this study, gonadal stage was treated primarily as a covariate. However, those stations at which most fish were outside of the normal range of maturation were identified in the results section, and future efforts should be made to reveal the reason or reasons for either advanced or delayed gonadal development.

Oocyte atresia, as defined by an involution or resorption of vitellogenic oocytes by the ovaries, has been sufficiently validated as a histological biomarker; that is, lesions in laboratory studies have been correlated with chemical exposure, and these same lesions detected in fish from contaminated sites. Although oocyte atresia is a normal physiological event in all fish, it can become a pathological condition following exposure to certain environmental contaminants (Kirubakaran and Joy, 1988; Cross and Hose, 1988; Johnson and others, 1988; Cross and Hose, 1989). In the MRB, there was a large degree of variability between stations for atresia, especially in female carp (Figs. 4-3, 4-7). At several stations mean atresia in female carp was high compared with the reference site and other stations; however, without a more thorough understanding of normal percentages in healthy individuals it is difficult to reach a conclusion regarding the degree of oocyte atresia that affects reproduction. Furthermore, the other biomarkers appeared to be normal in female carp from the stations at which mean atresia was high. There was, however, a positive correlation between atresia and several contaminants, including Hg, cyclodienes insecticides, and DDE (see Chapter 5). Female bass had less oocyte atresia (Figs. 4-5, 4-9), in general, than female carp (Figs. 4-3, 4-7) and at only one station (81) was the mean for female bass unusually high compared with the reference site and other stations. Female bass from Station 81 also had a particularly low GSI, extremely low mean vtg, and relatively low E2 mean. Multiple reproductive anomalies and the presence of the organochlorine insecticide mirex and other contaminants make Station 81 an appropriate candidate for future investigations.

Eighteen male bass (7 largemouth, 11 smallmouth) from 11 stations distributed throughout the MRB were identified as histologically abnormal and potentially intersex; that is, they were male fish with

foci of ovarian tissue. The significance of this is unclear because most of these males had normal sex steroid concentrations, E/KT ratios, and GSIs; and none had detectable vtg concentrations. Furthermore, the natural incidence of intersex gonadal tissue in various species is unknown. These intersex fish were not functional hermaphrodites. The testes appeared normal, and the oocytes found were perinucleolar and not previtellogenic. Interestingly, eight of 11 male small-mouth bass from station 111 were classified as intersex, although only one had an E2 concentration in the range of females and one other had an unusually high E/KT ratio. At all other stations except Station 78, where there were two intersex male bass, there was only more than one. The histological examination involved the analysis of two to three slices (5 μ m) taken from the distal portion of the gonads. Therefore, when foci of ovarian tissue were found, the males were positively identified as intersex; however, no evidence of ovarian tissue within the few slices examined does not preclude the possibility of intersex in other specimens. Additional slices would need to be examined before concluding with confidence that no ovarian tissue was present in a single gonad sample.

Histological examination and GSI are often most informative when used concurrently to examine gonadal composition and verify reproductive stage. GSI is easy and inexpensive to measure, although the gonads must be weighed on freshly killed specimens to avoid post-mortem weight fluctuations. Accurate analysis of histological data is more complex and relies strongly on the interpreter's knowledge of the seasonal reproductive cycle and normal variations in the anatomy of the species under investigation, as well as differences that may occur due to gender, age, and environmental conditions. In addition, the investigator must be able to differentiate between lesions of different origins, including exposure to toxic chemicals, infectious diseases, congenital anomalies, and stress from handling. Histopathology can often provide information regarding the magnitude, or occasionally the source, of toxic impairment and this method may be used to study alterations in animals too small for standard biochemical analyses. Using control animals is crucial for distinguishing between normal morphology and lesions derived from experimental treatments or environmental exposures. Although subjectivity of the examiner can also lead to variance, microcomputers and associated software programs now exist that allow for the quantitative analysis of morphological data.

Using a Suite of Biomarkers

Currently, the best approach towards studying reproductive health involves using a repertoire of

biomarkers to gain information at different levels of organization. Each of the indicators used in this study has advantages and disadvantages, and analyzing them together may help to overcome the individual weaknesses of any one test. Individually, the reproductive biomarkers can provide only limited information regarding the effects of environmental stressors.

At the biochemical level, the sex steroids and vitellogenin respond quickly to both intrinsic (physiological) and extrinsic (environmental) stimuli, and they are rapidly and easily measured. The high sensitivity of these indicators can be a disadvantage when considering the number of factors (for example, temperature, photoperiod, handling and drawing blood) that can affect circulating levels. These and many other contributing factors can make it difficult to discern whether the observed response is normal for the particular sex and species or due to an assortment of environmental stimuli. Although vitellogenesis is affected by a variety of environmental factors, a greater problem is the lack of knowledge regarding the normal patterns of vtg synthesis in males and females of different species, as well as baseline concentrations of vtg for specific species and seasonal fluctuations and patterns. GSI and histological examination provide structural information concerning the gonads and appear to be advantageous methods for identifying effects of long-term contaminant exposure. GSI is easy and inexpensive to determine, but it is definitely crude compared with the more informative, yet more labor-intensive, histological exam. Both techniques require knowledge of the variations due to age, species, and season, and histology requires extensive knowledge of the gross and microscopic anatomy of the specimen under investigation.

When used collectively, the biomarkers can provide a more holistic account of an animal's reproductive health. Although each of the biomarkers is a valuable indicator of a potential toxic response, none are indicative of either reproductive success or dysfunction. Furthermore, they are not capable of distinguishing between natural variability and endocrine disruption due to environmental factors, and they provide little information regarding the underlying mechanisms and causal relationships between reproductive impairment and environmental stressors. However, they are currently the most inexpensive and time efficient methods for assessing reproductive status and identifying abnormal populations in large-scale studies. Once such populations are identified, more thorough investigations may be conducted using reproductive endpoints such as fertility, fecundity, hatchability, and sexual behavior. These, along with mechanistic studies, are necessary for a complete understanding of the reproductive mechanisms in healthy and compromised wildlife populations.

Suggesting mechanisms for reproductive alterations is complicated and, certainly, beyond the scope of this study. However, analyzing the biomarkers collectively may help to design or prioritize mechanistic studies, since the integrated information may suggest that disruption is occurring prior to or subsequent to a specific reproductive function (for example, steroidogenesis, vtg synthesis). Currently, there is evidence that environmental contaminants can cause endocrine or reproductive alterations by 1) affecting the synthesis and/or elimination of sex steroids, 2) interfering with interactions between sex hormones and sex hormone binding globulins (SHBG), 3) disrupting the signaling processes from the hypothalamus and pituitary, 4) interfering with the binding of sex steroids to their receptors, and 4) affecting transcription of hormone receptors through mechanisms involving the AhR receptor.

SUMMARY AND FUTURE DIRECTIONS

In general, the MRB pilot project was successful in evaluating the potentials and limitations of select reproductive biomarkers. The methodologies themselves are confined to the current limits of detection; however, data interpretation was more often impeded by the study design. For instance, the success of the reproductive biomarkers relies strongly on the ability to compare control and experimental groups. Just as proper controls are required in the laboratory, field studies are most effective when experimental stations are carefully matched with reference sites. Although the reference site was established, the few fish at this site served as control animals for the entire basin. Pertinent information regarding base-line values and normal ranges for the biomarkers could be gained by analyzing larger sample numbers from additional reference stations. However, finding truly uncontaminated waters to serve as reference sites poses a challenge, and monitoring efforts should not be curtailed as a result of this limitation. Sampling more fish from the monitoring sites would also facilitate data interpretation. Grouping individuals based on sex, species, and stage was necessary for data analysis, however, often reduced the sample sizes to less than ideal numbers (although no station was analyzed with less than 5 individuals).

This study was also successful in identifying stations at which extreme responses (both high and low) for one or more biomarker occurred; however, and as expected in a study not conducted at pre-selected contaminated sites, most stations appeared to fall within expected ranges, and few sub-basin differences were detected. Contaminants as a possible cause of

the endocrine alterations were discussed earlier in this chapter; however, except for preliminary analyses, documenting correlations between contaminants and biomarker responses was beyond the scope of this report. An in-depth statistical evaluation of the combined data set, which is planned for the future, may further define the extent to which chemical exposure was involved in the differences observed among stations in the MRB. This information will also help to prioritize locations for future studies. Furthermore, continued validation of the reproductive biomarkers is necessary for the effective application of these tools, especially in field studies. Increasing sample numbers, establishing additional reference sites, and focusing on smaller geographic regions or sub-basins, may all increase the amount of information gained from future studies.

Chapter 5. Project Summary, Discussion, and Recommendations

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As described in Chapter 1 and Appendix A of this report, 1378 fish of 22 species were collected, examined, and analyzed from 46 sites in the Mississippi River basin (MRB) during 1995 and from a reference site during 1996. The 1995 sites represented National Contaminant Biomonitoring Program (NCBP) stations situated at key points on major rivers in the MRB, and National Water-Quality Assessment Program (NAWQA) stations located on lower-order rivers and streams in the Eastern Iowa Basins (EIB) and Mississippi Embayment (MSE) Study Units (see Table 1-1 and Fig. 1-1 of this report). Contaminants in fish had been studied at these sites in the past. Common carp (*Cyprinus carpio*; carp) and black basses (*Micropterus* spp.; bass) were the targeted species; together, they represented 82% of the fish collected.

Each fish was subjected to a field examination for externally and internally visible gross lesions, and selected tissues and fluids were obtained for analysis of fish health and reproductive biomarkers. Composite samples of whole fish grouped by species and gender were analyzed for organic and inorganic bioaccumulative contaminants and for dioxin-like activity (TCDD-EQ) using the H4IIE rat hepatoma cell bioassay (Table 1-2). The results of these analyses are presented and discussed in Chapters 2-4 of this report.

In this chapter we describe the combined results of the study and suggest further analyses and research. The findings of Chapters 2-4 are summarized and presented geographically (by station and sub-basin), and correlations between biomarkers and

bioaccumulative contaminant concentrations are described and discussed. We then discuss the limitations of the study, and make some recommendations regarding future use of the approach and methods in the BEST program and elsewhere.

GEOGRAPHIC SUMMARIES

Figures 5-1—5-9 and Table 5-1 present the pertinent findings described in Chapters 2-4 of this report for each station. The highlighted findings indicate either that known threshold were exceeded, or where no thresholds exists, that the indicated results were anomalous (that is, high or low) relative to other locations in this study. For the reproductive and fish health biomarkers the colors are relative and indicate the number, magnitude, or both of the anomalies (including the number of sex-taxon categories in which they occurred) at a station. They are intended only to draw attention to particular stations highlighted in the text, possibly for further investigation, and not to categorize the sites. It is important to recognize that increased frequencies of external lesions or elevated health assessment index (HAI) scores, which represent the cumulative total number of grossly visible internal and external lesions, do not necessarily indicate direct contaminant effects. Many factors, including contaminants, can indirectly influence these markers. Factors such as increased nutrients or organic matter, particularly when coupled with increased water temperature can lead to the proliferation of opportunistic bacterial and fungal pathogens. In addition, contaminants may have a negative effect on the intermediate hosts of many parasites, which could result in fewer external lesions or lower HAI scores. Similarly, and as noted in Chapter 4, many factors other than chemicals can influence the reproductive biomarkers. And finally, it is important to note that considerably more is known about risk to fish and piscivorous wildlife associated with bioaccumulative contaminants and ethoxyresorufin *O*-deethylase (EROD) than about long- and short-term risks represented by the other biomarkers. Accordingly, in Table 5-1, Figures 5-1—5-9 and the discussions following, greater relative risk has been associated with elevated contaminant concentrations and EROD rates than with anomalous fish health or reproductive biomarkers.

“Reference” Site: The reference site (Station 400) represents the water supply system of the USGS-Leetown Science Center in rural Jefferson County, WV. The watershed contains farms and a relatively sparse, but growing, human population. Both male and female carp and bass (but no other species) were collected at

this site, but the liver samples were lost in transit and the complete suite of analyses was not performed. The fish were collected in late October 1996.

As expected, concentrations of most contaminants in carp and bass from Station 400 were fairly low, but there were exceptions (Table 5-1). Surprisingly high (for a reference site) concentrations of *p,p'*-DDE were present in both carp (0.3-0.4 µg/g) and bass (0.1 µg/g; Fig. 5-2). These levels are about 10-fold greater than background (Schmitt and others, 1999b; Wong and others, 2000). As noted in Chapter 2, concentrations of this magnitude may represent a risk to the most sensitive piscivorous avian species. A low level of dioxin-like activity (TCDD-EQ, as determined by H4IIE bioassay) was also detected in one carp sample (Fig. 5-4), but no EROD values were available with which to further compare and evaluate these data. All other organochlorine chemical residue concentrations were below detection limits; however, the largemouth bass contained moderate concentrations (0.2-0.3 µg/g) of Hg (Fig. 5-1).

Both male and female carp and largemouth bass from Station 400 were generally smaller (shorter and lighter) and younger than those from most NCBP and NAWQA stations. Condition factors in both species were also relatively low, but nevertheless similar to those of fish from many NCBP and NAWQA stations. Both carp and bass had comparatively small spleens, but otherwise seemed healthy (Table 5-1; Figs. 5-7—5-9).

Collectively, our findings indicate that fish from the reference site are exposed to concentrations of bioaccumulative contaminants similar to those at many MRB stations. Although smaller than those from many MRB sites, carp and bass from Station 400 appeared to be healthier. The slow growth at Station 400 appears to be site-specific rather than regional; that is, it reflects productivity and nutritional differences between the sites, including the fact that the reference site represents a small impoundment whereas the MRB sites all were situated on rivers or much larger impoundments.

Arkansas-Red River (ARR) Sub-Basin: This sub-basin is influenced by a variety of contaminants from mining and agriculture, including Se from the leaching of seleniferous soils by irrigated agriculture in the upper Arkansas River watershed (May and McKinney, 1981; Schmitt and others, 1999b). Consequently, stations in this sub-basin differ greatly in their contaminant profiles and pollution histories. In addition, and in contrast to all other sub-basins, all five ARR stations represent relatively large impoundments with permanent pools rather than flowing-water reaches of their respective rivers. Both male and female carp and bass, but no other species, were collected from all



Figure 5-1. Maximum concentrations (µg/g, wet-weight) of Hg (upper panel) and Se (lower panel) in composite samples of whole fish from the indicated stations. See Chapter 1, Table 1-1 for station locations and Chapter 2 for explanation of thresholds.

Table 5-1. Summary of chemical and biological indicator results, by sub-basin and station. Within each column, colors indicate the severity, incidence, or both of the indicated condition or conditions at each station (green<yellow<orange<red). These designations are relative; see text for explanations. Male and female bass¹ and carp¹ were collected from all sites unless otherwise indicated. See footnote¹ for abbreviations and Chapter 1 of this report (Table 1-1, Fig. 1-1) for station and sub-basin locations.

Program, sub-basin, and station	Contaminants and EROD	Fish health indicators	Reproductive biomarkers
Reference Site			
400	DDE (b, c), TCDD-EQ (c), Hg (b)	SSI (b-, c-)	Atresia (fb).
NCBP Stations			
Arkansas-Red River (ARR)			
29	As (b), Hg (b)	Ext. lesions (b), lys (c)	
77	Se (b, c), TCDD-EQ (b)	Lys (c), SSI (b-)	GSI
78	As (b), Cd (c), Pb (c), TCDD-EQ (b)	Ext. lesions (b), HSI (b), lys (c)	Ovt (mb)
79	As (c), Hg (b) DDE (b, c), PCBs (c, b), TCDD-EQ (b), EROD (b)	Ext. lesions (b, c), MA (b), HSI (b), SSI (b, c), lys (c)	E/KT (mc, mb, fb), vtg (mb), ovt (mb)
82	Hg (b), DDE (b), PCBs (c)	Ext. lesions (b, c), HAI (b), MA (b, c)	E/KT (mb), ovt (mb), atresia (fc)
Lower Missouri River (LMO)			
31 (carp only)	CI (c), PCBs (c), EROD (c)	HAI (c), SSI (c)	E/KT (fc)
83	Hg (b), CI, PCBs (c), EROD (b)	Ext. lesions (c), HAI (b)	E/KT (b), ovt (b)
86 (no bass)	Hg (g), PCBs (g)		
89 (no bass)	Pb (c)	Ext. lesions (af), HAI (af), SSI (c)	E/KT (mc)
90 (no bass)	CI (c), Cd (c), PCBs (c), EROD (c)	Ext. lesions (af), HAI (af), MA (c), lys (c)	E/KT (fc)
Upper Missouri River (UMO)			
32 (one f bass)	TCDD-EQ (c)	SSI (c-)	E2 (fc); 11KT (fc)
84 (no bass)			Vtg (mc)
85 (no bass)			
Lower Mississippi River (LMS)			
15 (no m bass)	CI (c, wb), PCB (c, wb), TCDD-EQ (c, wb), EROD (c, b, wb), As (wb), Cu (wb)	Ext. lesions (c, wb), HAI (c, wb), lys (b), SSI (b-)	E2 (fc)
28	DDE (c, b), PCB (c, b), TCDD-EQ (b), Pb (c, b)	Ext. lesions (b), SSI (c, b), lys (c, b)	11-KT (mc), vtg (mc)
30	Hg (b), Cd (b), TCDD-EQ (c, b)	SSI (c), MA (b), lys (c, b)	Vtg (mb)
75 (no bass)	CI (c, wb), PCB (c, wb), TCDD-EQ (c), Hg (wb), As (c, wb), EROD (c)	SSI (c), lys. (c, b)	E2 (fc)
76	CI (c, b), PCBs (c, b), TCDD-EQ (c, b), EROD (c, b), Hg (c, b)	SSI (c)	11-KT (mc, mb), GSI (mc, fc), E/KT (mc), E2 (fc)
80	DDE (c, b), CI (c, b), tox (c, b), PCBs (c), TCDD-EQ (fc), EROD (mc)	Ext. lesions (c), MA (c, b), lys (c)	11-KT (mc), GSI (mc), E/KT (mc, mb)
81	DDE (b, c), PCB (b, c), Hg (b), mirex (c, b), EROD (c)	Ext. lesions (b), HAI (b), MA (b), lys (c, b)	Ovt (mb), vtg (fb), atresia (fb)

¹DDE, *p,p'*-DDE; tox, toxaphene; CI, cyclodiene insecticides (dieldrin, chlordane, endrin, heptachlor epoxide); PCBs, polychlorinated biphenyls; TCDD-EQ, dioxin-like activity as determined by H4IIE bioassay; As, arsenic; Cd, cadmium; Cu, copper; Hg, mercury; Pb, lead; Zn, zinc; EROD, ethoxyresorufin *O*-deethylase; CF, condition factor; lys, lysozyme; vtg, vitellogenin; E2, 17 β -estradiol; 11-KT, 11-ketotestosterone; E/KT, E2/11-KT; HAI, health assessment index; SSI, spleno-somatic index; HSI, hepato-somatic index; GSI, gonado-somatic index; ovt, ovotestis; MA, macrophage aggregates (one or more parameters); thy, thyroid proliferation; c, carp (*Cyprinus carpio*); b, bass (*Micropterus* spp.); wb, white bass (*Morone chrysops*); g, goldeye (*Hyodon alosoides*); m, male; f, female. For SSI, - indicates smaller; all others larger.

Table 5-1. Summary of chemical and biological indicator results, by sub-basin and station. Within each column, colors indicate the severity, incidence, or both of the indicated condition or conditions at each station (green<yellow<orange<red). These designations are relative; see text for explanations. Male and female bass¹ and carp¹ were collected from all sites unless otherwise indicated. See footnote¹ for abbreviations and Chapter 1 of this report (Table 1-1, Fig. 1-1) for station and sub-basin locations—Continued.

Program, sub-basin, and station	Contaminants and EROD	Fish health indicators	Reproductive biomarkers
Upper Mississippi River (UMS)			
26	CI (b, c), PCBs (c, b), TCDD-EQ (c, b), EROD (b), As (c), Zn (c)	Ext. lesions (b), HAI (b) HSI (b), SSI (b), MA (c, b),	E/KT (fc), atresia (fc)
27	PCBs (c, b), TCDD-EQ (c, b), EROD (c)	MA (c, b)	
72	TCDD-EQ (c, b), EROD (c, b), PCBs (c, b)	MA (c, b), lys (c)	Ovt (mb), E2 (fc)
73 (no bass)	CI (c), PCBs (c), As (c), Pb (c)	MA (c), lys (c)	E2 (fc)
74 (no carp)	Hg (b)		11-KT (mb), ovt (mb)
111	PCBs (c, b), TCDD-EQ (c), Zn (c)	MA (c, b), lys (c)	Ovt (mb)
112	PCBs (c, b), TCDD-EQ (c, b), EROD (c), Zn (c)	MA (b), lys (c), HAI (b)	E2 (fc), vtg (mc)
Ohio River (OHR)			
23 (only one carp)	CI (c, b), PCBs (c, b), TCDD-EQ (c, b), Pb (c)	Lys (b)	
24	CI (c, b), PCBs (c, b), TCDD-EQ (c), DDE (c, b), Pb (c), Cd (c), EROD (c, b)	Ext. lesions (c, b), CF (c), SSI (b-)	
25	PCBs (c, b)	CF (c), lys (b), MA (b)	E/KT (mb)
67	CI (c, b), DDE (c, b), PCBs (c, b), TCDD-EQ (c, b), Cd (c), Pb (c), EROD (c)	Ext. lesions (c), CF (b), HSI (b), MA (c)	Ovt (mb)
68	CI (c, b), PCBs (c, b), TCDD-EQ (c, b), EROD (c)	HSI (b), SSI (c), lys (c), MA (b)	E2 (fb)
70	CI (c, b), DDE (c, b), PCBs (c, b), TCDD-EQ (c, b), EROD (b), Hg (b), Pb (c)	Ext. lesions (c), CF (c), HAI (b), lys (c, b), MA (b, c)	Atresia (fc), E2 (fb), E/KT (fb), GSI (fb), vtg (fb)
71	DDE (c, b), PCBs (c, b), TCDD-EQ (c)	Ext. lesions (c), lys (b, c)	
NAWQA Stations			
Eastern Iowa Basins (EIB) Study Unit			
205 (carp only)	CI	CF, MA	E/KT (m), atresia (f)
206 (carp only)	CI, TCDD-EQ	MA	E/KT (m), atresia (f)
209 (carp only)	No samples analyzed	Ext. lesions	E/KT (m)
210 (carp only)	CI, Zn, TCDD-EQ	SSI	E/KT (m), atresia (f)
211 (carp only)	CI, DDE, PCBs, TCDD-EQ	CF, MA	
Mississippi Embayment (MSE) Study Unit			
201 (carp only)	DDE, tox, CI, EROD	Ext. lesions	
202 (carp only)	DDE, tox, TCDD-EQ, EROD	Ext. lesions	Vtg (f)
203 (carp only)	DDE, tox, CI, TCDD-EQ, EROD	Ext. lesions, MA	GSI (m), vtg (f)
204 (carp only)	DDE, tox, As, Pb, TCDD-EQ, EROD, mirex	Ext. lesions, MA	11-KT (m), GSI (m), atresia (f)
207 (carp only)	TCDD-EQ, EROD, CI, Hg	MA, SSI	11-KT (m), E/KT (m)
208 (carp only)	DDE, TCDD-EQ, EROD	MA, thy	
212 (carp and bass)	EROD (b, c), CI (c), PCBs (b)	MA (b, c), SSI (c); thy (c)	E/KT (mb, fb), atresia (fc)
213 (bass only; no EROD, HSI, or GSI)	Hg	MA	Ovt (m), vtg (m), E/KT (f)

¹DDE, *p,p'*-DDE; tox, toxaphene; CI, cyclodiene insecticides (dieldrin, chlordane, endrin, heptachlor epoxide); PCBs, polychlorinated biphenyls; TCDD-EQ, dioxin-like activity as determined by H4IIE bioassay; As, arsenic; Cd, cadmium; Cu, copper; Hg, mercury; Pb, lead; Zn, zinc; EROD, ethoxyresorufin *O*-deethylase; CF, condition factor; lys, lysozyme; vtg, vitellogenin; E2, 17 β -estradiol; 11-KT, 11-ketotestosterone; E/KT, E2/11-KT; HAI, health assessment index; SSI, spleno-somatic index; HSI, hepato-somatic index; GSI, gonado-somatic index; ovt, ovotestis; MA, macrophage aggregates (one or more parameters); thy, thyroid proliferation; c, carp (*Cyprinus carpio*); b, bass (*Micropterus* spp.); wb, white bass (*Morone chrysops*); g, goldeye (*Hiodon alosoides*); m, male; f, female. For SSI, - indicates smaller; all others larger.

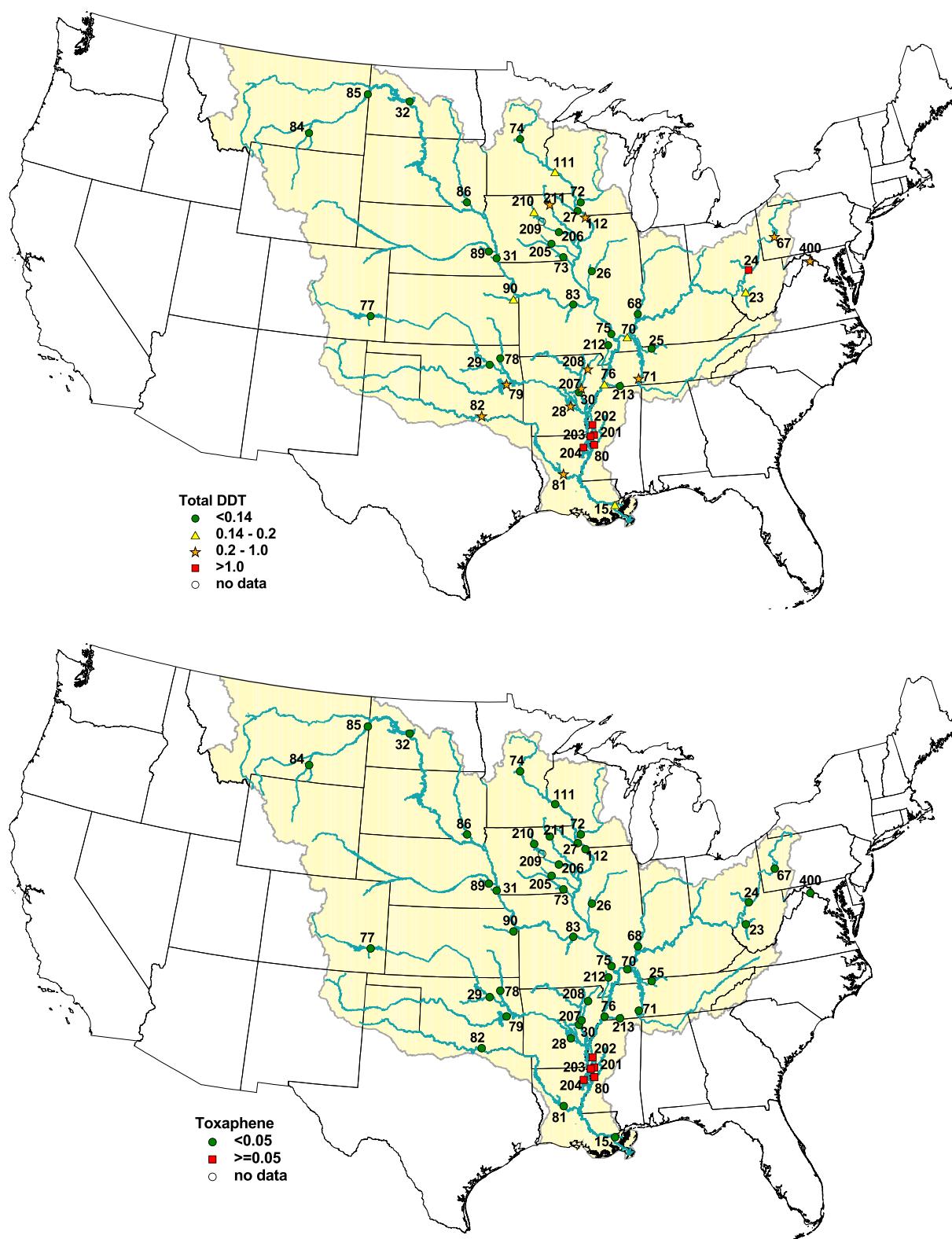


Figure 5-2. Maximum concentrations ($\mu\text{g/g}$, wet-weight) of total DDT (sum of p,p' homologs, upper panel) and toxaphene (lower panel) in composite samples of whole fish from the indicated stations. See Chapter 1, Table 1-1 for station locations and Chapter 2 for explanation of thresholds.

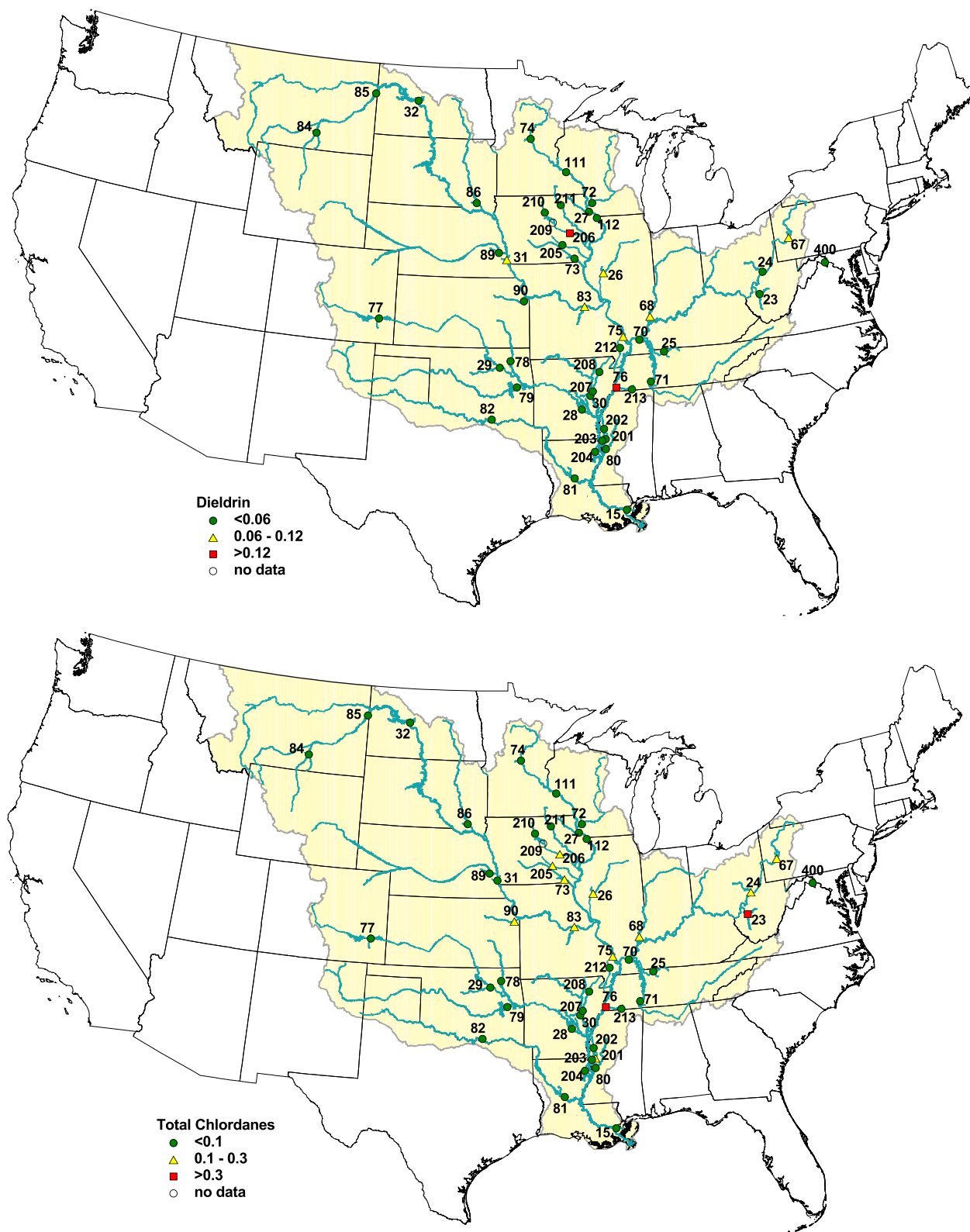


Figure 5-3. Maximum concentrations ($\mu\text{g/g}$, wet-weight) of cyclodiene pesticides in composite samples of whole fish from the indicated stations. Upper panel: dieldrin. Lower panel: sum of chlordane-related compounds (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, and heptachlor epoxide). Not shown are concentrations of endrin, which were detected ($>0.01 \mu\text{g/g}$) only at Station 76. See Chapter 1, Table 1-1 for station locations and Chapter 2 for explanation of thresholds.

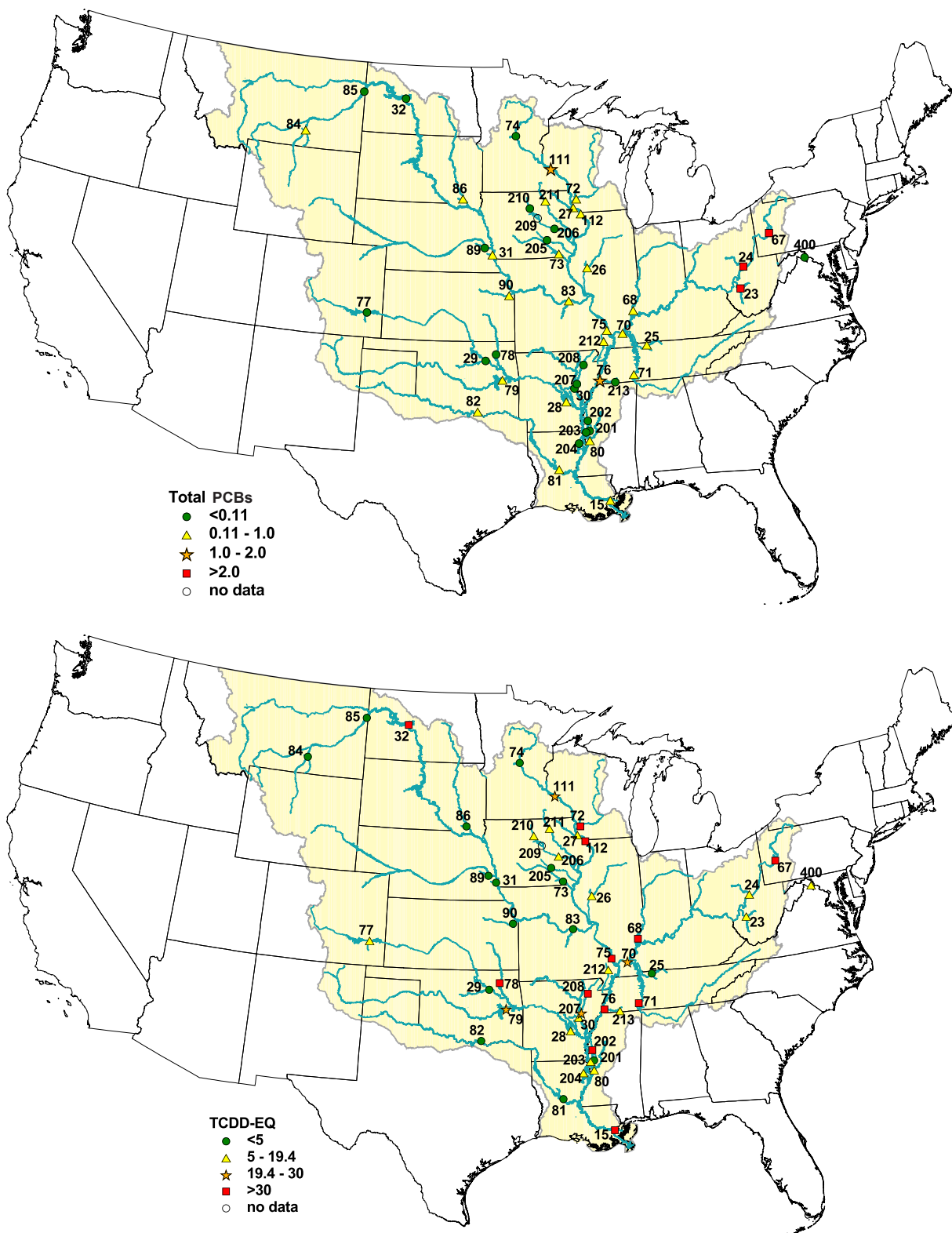


Figure 5-4. Maximum concentrations of total PCBs (upper panel, $\mu\text{g/g}$ wet-weight) and TCDD-EQ (lower panel; pg/g , based on H4IIE bioassay) in composite samples of whole fish from the indicated stations. See Chapter 1, Table 1-1 for station locations and Chapter 2 for explanation of thresholds.

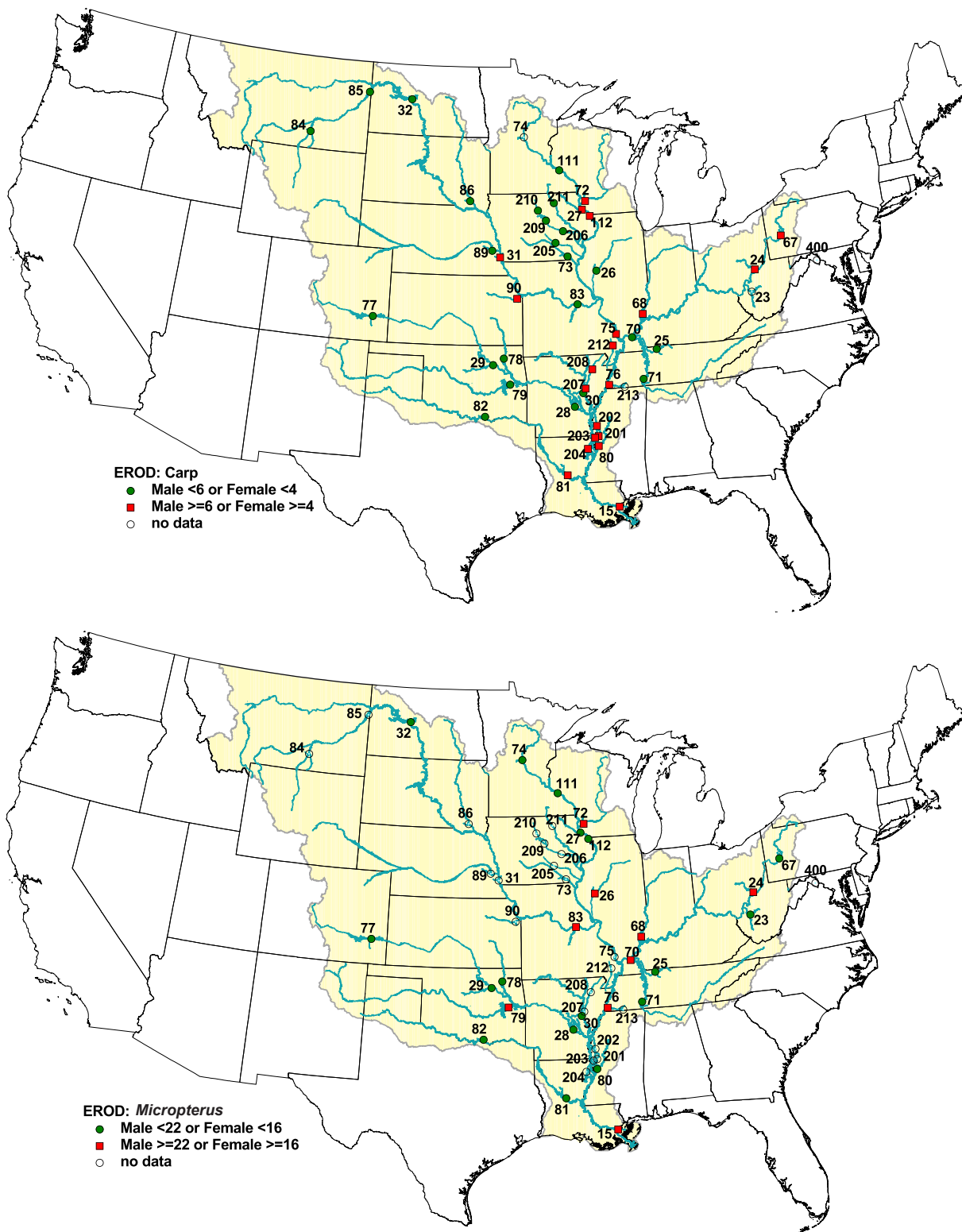


Figure 5-5. Mean ethoxyresorufin *O*-deethylase (EROD) activity (pmol/min/mg protein) in male or female carp (upper panel) and bass (lower panel). The thresholds are levels of EROD activity indicative of exposure of the fish to exogenous Ah-R agonists, as described in Chapter 2.

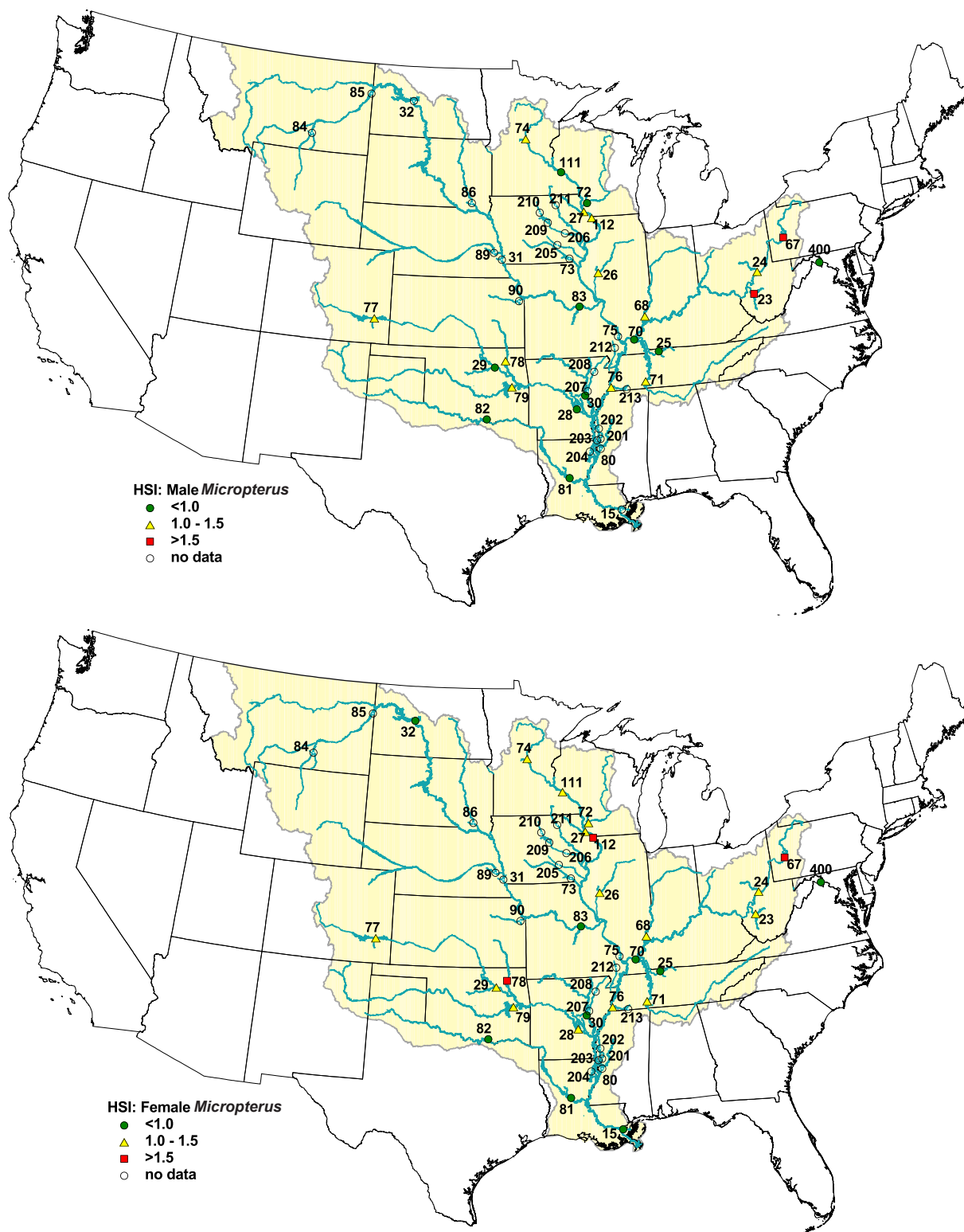


Figure 5-6. Mean hepatosomatic index (HSI) values in male and female bass from the indicated stations. See Chapter 1, Table 1-1 for station locations.

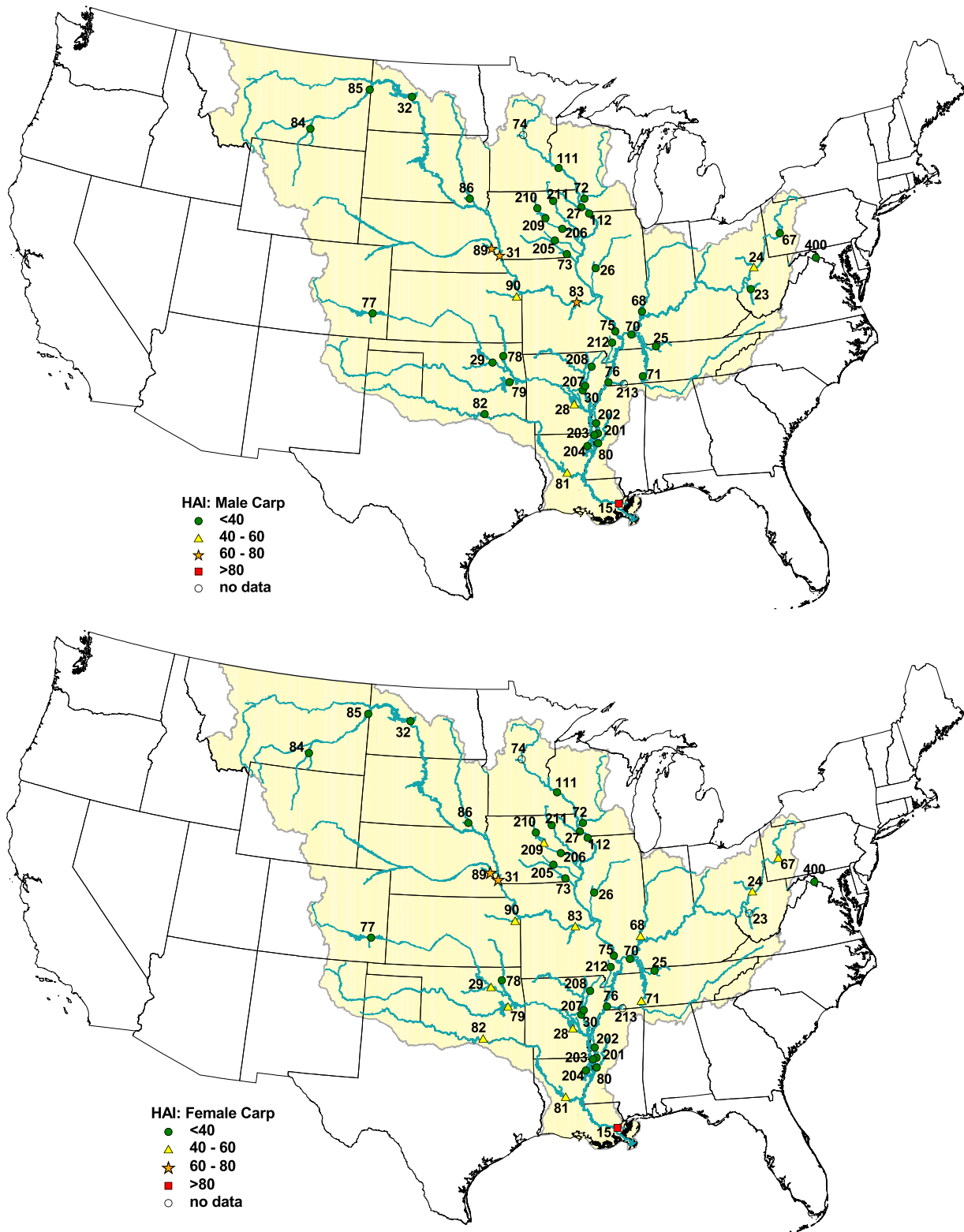


Figure 5-7. Mean health assessment index (HAI) values in male and female carp from the indicated stations. See Chapter 1, Table 1-1 for station locations.

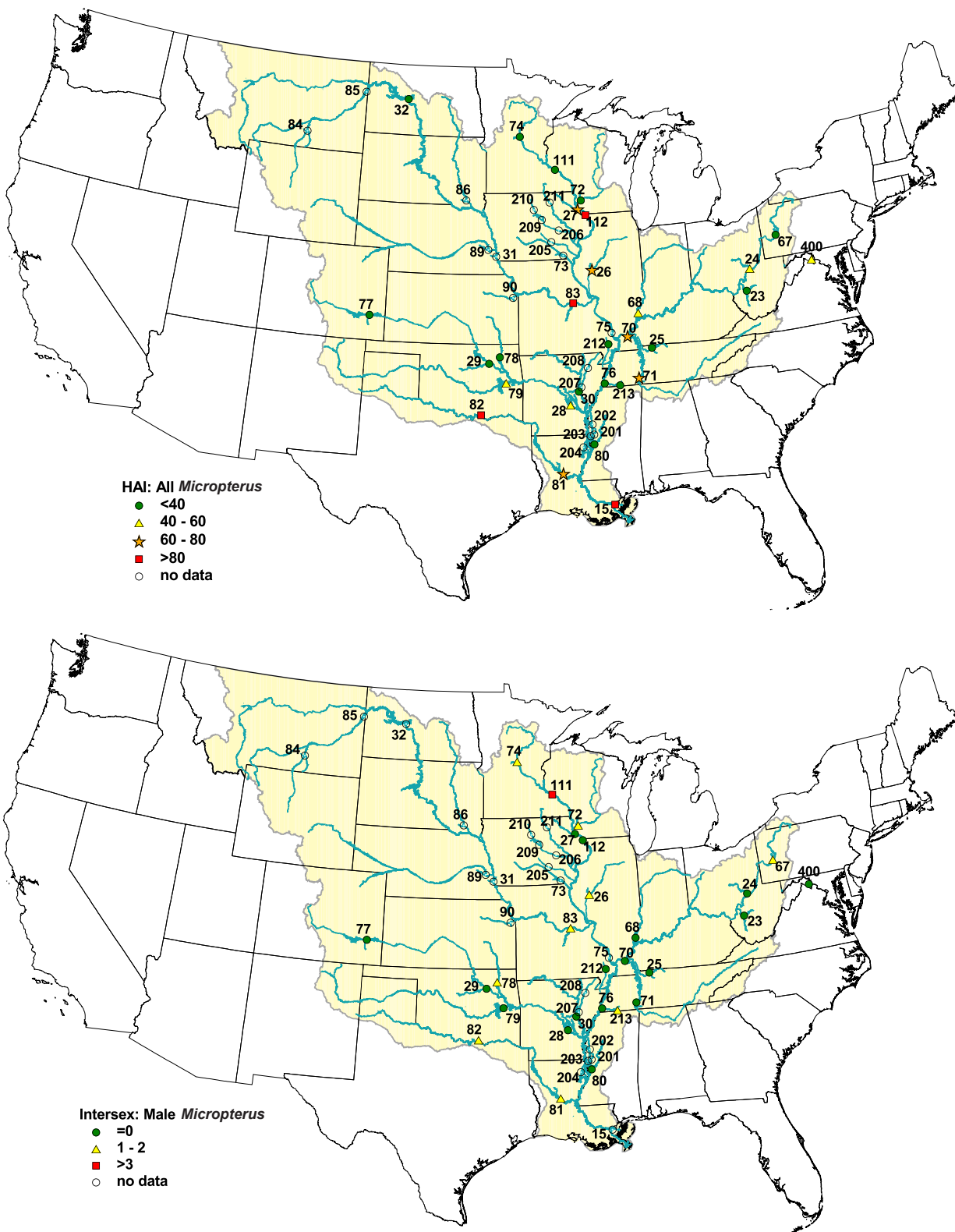


Figure 5-8. Mean health assessment index (HAI) values in bass (upper panel, males and females combined) and numbers of male bass with ovotestes detected through histopathological examination of the gonads (lower panel) from the indicated stations. No ovotestes were detected in male carp. See Chapter 1, Table 1-1 for station locations.

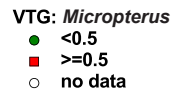


Figure 5-9. Stations at which concentrations of vitellogenin (vtg) exceeded 0.5 mg/mL in male carp (upper panel) and bass (lower panel). See Chapter 1, Table 1-1 for station locations.

ARR stations. The ARR stations were sampled from mid-October to early December 1995.

Concentrations of organochlorine chemical residues in fish from Stations 29 (Arkansas R. at Keystone Res., OK), 77 (Arkansas R. at John Martin Res., CO), and 78 (Verdigris R. at Oologah, OK—Oologah Lake) were low and EROD rates did not indicate exposure to exogenous AhR agonists at these sites; however, there was some dioxin-like activity in bass from stations 77 and 78 (Table 5-1; Figs. 5-2—5-5). Inorganic contaminants were more evident than organic compounds in fish from Stations 77 and 78. At Station 77, Se concentrations (ca. 5 µg/g in both carp and largemouth bass) were by far the highest in the MRB and exceeded known thresholds for toxicity to both fish and piscivorous wildlife; a low level of Hg contamination (0.2-0.3 µg/g) was evident in largemouth bass from Station 29; and elevated Cd (0.2-0.3 µg/g) and Pb (>0.2 µg/g) concentrations were present in carp from Station 78, which is downstream from a mining area (Table 5-1; Fig. 5-1).

Concentrations of As in largemouth bass were also slightly elevated (>0.3 µg/g) at Stations 29 and 78.

Organochlorine chemical residues were present at Stations 79 (Canadian R. at Eufaula, OK—L. Eufaula) and 82 (Red R. at L. Texoma, Table 5-1). Slightly elevated levels (ca. 0.5 µg/g) of *p,p'*-DDE were detected in carp from Station 79 and both *p,p'*-DDE and Hg (0.2-0.3 µg/g) were present in largemouth bass from Stations 79 and 82 (Figs. 5-1, 5-2). In addition, EROD rates indicated that female bass from Station 79 had been exposed to exogenous AhR ligands (Table 5-1; Fig. 5-5). Concentrations of Pb, Zn, and As were also elevated slightly in carp from Station 79. Polychlorinated biphenyl (PCB) concentrations at all ARR stations were low (<0.5 µg/g) compared to other areas of the MRB but TCDD-EQ levels were moderately high in one sample of bass from each of Stations 77, 78, and 79 (Table 5-1; Fig. 5-4). The latter indicate that dioxin-like contaminants were present at these sites.

Overall, fish from the ARR sub-basin had the largest percentage of external lesions in the MRB. Proportionately large numbers of bass from Stations 29, 78, 79, and 82 had external lesions, as did carp from Stations 79 and 82 (Table 5-1). Health assessment index (HAI) scores, which represent cumulative numbers of certain grossly visible internal and external anomalies (greater scores associated with more anomalies), were among the highest in the MRB for bass from Station 82 (Fig. 5-8). These elevated HAI scores were primarily due to parasite infestations. The livers of female bass and the spleens of carp from Station 79 were relatively large, but the spleens of bass from Station 77 were comparatively small (Table 5-1; Fig. 5-6). In addition, bass from Stations 79 and 82 and carp from Station 82 had high MA scores, and

lysozyme activity in carp from Stations 77, 78, 79, and 82 was comparatively high.

Reproductive biomarkers potentially indicative of chemical exposure were noted at all ARR sites, especially in male fish (Table 5-1). At Station 79 the concentrations of 17β-estradiol (E2) in male carp were unusually high and 11-ketotestosterone (11-KT) levels were comparatively low, as were 11-KT concentrations at Station 82. Consequently, more than 50% of the male carp from both stations had E/KT ratios >1.0 (Table 5-1). E/KT was also significantly ($P<0.05$) >1.0 in Stage-2 male bass from Station 82. The vtg concentration was also >2 mg/mL (well into the range of females in early- to mid-vitellogenesis) in one male bass from Station 79 (Fig. 5-9). Histological evaluation revealed that two of seven male largemouth bass from Station 78 were intersex (that is, ovotestes were detected), as was one of 13 male bass from Station 82 (Fig. 5-8). In contrast to the males, reproductive biomarkers in female carp and bass from the ARR sub-basin were generally unremarkable. The exception was Station 79, where the mean 11-KT concentration in female largemouth bass was comparatively high and the E/KT ratio was <1.0 in six of 12 fish (Table 5-1).

In summary, fish from all five ARR stations were noteworthy in at least one respect. At Station 77, Se concentrations in fish were high enough to constitute a hazard to fish and wildlife, but with the exception of SSI, biomarker responses were unremarkable. In contrast, fish from Station 79 contained *p,p'*-DDE and other organochlorine pesticides, elevated TCDD-EQ in one sample of bass, and comparatively high concentrations of As, Pb, and Zn. Bass from Station 82 had relatively high HAI scores, and a large percentage of the fish from all stations except 77 had grossly visible lesions. Collectively, the fish health indicators for Stations 78, 79, and 82 suggest the presence of pathogens or parasites whereas the small spleens of bass from Station 77 are more consistent with contaminant exposure. At several ARR sites the reproductive biomarkers were also consistent with exposure to endocrine-modulating chemicals, although other factors may have also been involved. The relatively high concentrations of As, Hg, and other accumulative contaminants in fish may also reflect the dynamics of the reservoir ecosystems represented by the ARR stations.

Lower Missouri (LMO) Sub-Basin: Stations in this sub-basin are influenced by agriculture and by urban-industrial pollutants from the Kansas City and Omaha metropolitan areas, the latter including defunct oil refineries and Pb smelters. There is a history of cyclodiene pesticide contamination from past agricultural uses and termite control in the lower parts of the sub-basin, and several major oil spills have occurred.

Male and female carp were collected from all LMO sites; however, bass were only obtained at Station 83 (Missouri R. at Hermann, MO). Most fish were collected between mid-October and early November 1995, but Station 86 (James R. at Olivet, SD) was sampled in early September.

Relative to other sub-basins and the reference site, concentrations of most contaminants in carp and bass were low (Table 5-1). The exception was cyclodiene insecticides; as in the past (Schmitt and others, 1999), concentrations were relatively high in fish from Stations 31 (Missouri R. at Nebraska City, NE), 83, and 90 (Kansas R. at Bonner Springs, KS; Fig. 5-3). Concentrations of Cd in carp from Station 90 and of Pb in carp from Station 89 were also slightly elevated relative to other stations, and fish from several stations contained moderately elevated concentrations of PCBs (Table 5-1; Fig. 5-4). Bass from Station 83 also contained elevated concentrations of Hg (Fig. 5-1), as did goldeye (*Hiodon alosoides*) from Station 86. Hepatic EROD rates were elevated only in bass from Station 83 (Fig. 5-5), where PCB and TCDD-EQ concentrations were low (Fig. 5-4), indicating that these fish had been exposed to non-accumulative exogenous AhR ligands such as polycyclic aromatic hydrocarbons (PAHs).

A comparatively large percentage of fish from Stations 83, 89 (Platte R. at Louisville, NE), and 90 had high incidences of external lesions and correspondingly large HAI scores (Table 5-1; Figs. 5-7, 5-8). Carp from Stations 31 and 89 also had relatively large spleens, and HAI scores were comparatively high in carp from Station 31 (Fig. 5-7). Macrophage aggregate values were elevated only in carp from Station 90, however. Four of seven male bass from Station 83 had E/KT ratios >1.0 , one of which was the largest value for male bass in the MRB. Another male bass from Station 83 was intersex (that is, ovotestes were detected by histopathology), but none of the male fish from this site (six bass, nine carp) analyzed contained detectable concentrations of vtg (that is, ≥ 0.001 mg/mL; Figs. 5-8, 5-9). At Station 89 more than 50% of the male carp had E/KT ratios >1.0 . At Station 31 the mean E2 concentration was comparatively low in female carp, and seven of 12 had E/KT ratios <1.0 . Five of 10 female carp from Station 90 also had E/KT ratios <1.0 , but these were due to slightly elevated 11-KT concentrations.

In summary, Station 86 (James R.) was the only LMO station at which the concentrations of most measured accumulative contaminants were relatively low and none of the biomarkers were noteworthy. Even at Station 86, Hg was evident in goldeye (the piscivorous species collected), however. Further downstream, cyclodiene pesticides remained evident in fish from the Missouri and Kansas rivers, and some of the fish health and reproductive biomarkers were

consistent with the exposure of the fish to chemicals. At Station 83 (Missouri R. at Hermann, MO), all samples contained measurable concentrations of organochlorine pesticides. Concentrations of Hg in bass from this site were elevated and their EROD rates exceeded basal activities. The latter suggests that the fish were exposed to PAHs, possibly residual material from a large oil spill that occurred in 1988 (Poulton and others, 1997). In addition, the E/KT ratios of some male bass from this site were among the highest in the MRB; many were well into the range typical of female fish (that is, >1.0).

Upper Missouri (UMO) Sub-Basin: Contaminants in the UMO sub-basin derive primarily from agriculture (including irrigation), mining, and energy extraction. Part of the sub-basin also drains areas containing seleniferous rocks and soils, and fish from some sites historically contained slightly elevated Se concentrations along with some Hg. Both male and female carp were collected at all three UMO stations; however, only one bass was obtained (a female) from Station 32 (Missouri R. at Garrison Dam, ND). All fish were collected between early September and early October 1995.

Contaminant concentrations in fish from the three UMO stations were generally low. The exception was TCDD-EQ, which was inexplicably elevated in both male and female carp from Station 32; however, PCB concentrations there were low as was EROD activity (Table 5-1; Figs. 5-4, 5-5). Because few bass were collected, many of the health indicators and reproductive biomarkers were not analyzed at all UMO sites. Nevertheless, most of the fish collected and analyzed appeared healthy and the fish health and reproductive biomarkers were largely unremarkable. The only exceptions were comparatively small spleen size in carp from Station 32, and a high vtg concentration (in the range of females in early- to mid-vitellogenesis) in one male carp from Station 84 (Fig. 5-9).

In general, and relative to the other sub-basins, fish from the UMO stations contained low concentrations of bioaccumulative contaminants. Biomarker results also indicated that the fish were healthy, subject to the limited number of stations and species examined. The exceptions were the TCDD-EQ and small spleen size in carp from Station 32 and the elevated vtg concentration in one male carp from Station 84; the latter may reflect exposure to exogenous estrogens.

Lower Mississippi (LMS) Sub-Basin: Stations in this part of the MRB are affected by chemically intensive agriculture (cotton, corn, soybeans, rice, etc.) and receive urban and industrial pollutants from the St. Louis, MO and Memphis, TN metropolitan areas. The latter include point-sources where pesticides were

manufactured and formulated. Consequently, contaminant burdens in fish have historically been comparatively high. In addition, mirex was used extensively in this sub-basin to control red imported fire ants (*Solenopsis invicta*). Male and female carp were collected from all LMS sites. Male and female bass (all largemouth) were obtained everywhere except at Stations 15 (Mississippi R. at Luling, LA), where only females were collected; and Station 75 (Mississippi R. at Cape Girardeau, MO), where no bass (*Micropterus* spp.) were collected. Sampling in the LMS sub-basin began in late August 1995 at Station 80 (Yazoo R. at Redwood, MS) but was not completed until early November (at Station 15); most stations were sampled in October (see Chapter 1, Table 1-1).

As in the past, fish from the LMS sub-basin contained comparatively high concentrations of bioaccumulative contaminants. Carp and white bass (*Morone chrysops*) from Station 15 contained small amounts of dieldrin, moderately high concentrations of PCBs, and corresponding TCDD-EQ (Table 5-1; Fig. 5-4). The white bass also contained slightly elevated concentrations of As and Cu, and EROD rates in both carp and largemouth bass indicated exposure to exogenous AhR agonists (Fig. 5-5). At Station 28 (Arkansas R. at Pine Bluff, AR), which is influenced by a former DDT point-source, fish were slightly contaminated with *p,p'*-DDE, PCBs, TCDD-EQ, and Pb, but EROD rates were not elevated (Table 5-1; Figs. 5-2, 5-4, 5-5). At Station 30 (White R. at DeVall's Bluff, AR), largemouth bass contained moderately high concentrations of Hg (Fig. 5-1) and, in one sample, Cd (the only bass sample from the MRB containing substantial Cd). TCDD-EQ was also present in both carp and bass (Table 5-1). Carp and white bass from Station 75 contained dieldrin, PCBs, TCDD-EQ (carp only), Hg (white bass only), and slightly elevated As; and EROD rates in carp indicated exposure to AhR agonists (Figs. 5-1, 5-3, 5-4, 5-5). Carp and largemouth bass from Station 76 (Mississippi R. at Memphis), which is heavily influenced by point- and non-point- sources of pesticides and other contaminants, contained very high (by 1995 standards) concentrations of cyclodiene insecticides (including endrin in all four samples); substantial PCBs, TCDD-EQ, and Hg; traces of HCB; and slightly elevated As concentrations (Table 5-1; Figs. 5-1, 5-3, 5-4). EROD rates at Station 76 were also very high in all species and genders, indicating exposure to exogenous AhR agonists (Fig. 5-5). Fish from Station 80 contained relatively high concentrations of chemicals used historically on cotton, including *p,p'*-DDE, toxaphene, and dieldrin (Figs. 5-2, 5-3). The fish also contained slightly elevated PCB levels and, in female carp, correspondingly high TCDD-EQ (Figs. 5-4, 5-5). Conversely, EROD rates indicated that male carp from Station 80 (Yazoo R.) had been exposed to exogenous

AhR agonists, but not female carp or largemouth bass (Table 5-1; Fig. 5-5). Carp from Station 80 also contained slightly elevated concentrations of As. Here and elsewhere in the LMS sub-basin some Hg and As may derive from the historical and, for As, continuing use of mercurial and arsenical pesticides. Fish from Station 81 (Red R. at Alexandria, LA) contained slightly elevated concentrations of DDT and total PCBs, relatively high concentrations of Hg (in bass), and traces of mirex (Figs. 5-1—5-4). EROD rates in male carp from Station 81 were also elevated slightly (Table 5-1).

Both carp and largemouth bass from the LMS tended to be large for their age, indicating rapid growth, but condition factors were not exceptionally large or small. Nevertheless, many of the other health indicators suggested that fish from stations in this sub-basin were in comparatively poor health (Table 5-1). Large numbers of external lesions were present on fish from Stations 15, 28, 80, and 81, but the species involved (carp, largemouth bass, or both) varied among sites. Most of these lesions were frayed and hemorrhagic fins and frayed gills. HAI scores were correspondingly high for both carp and bass from Station 15 and for bass at Station 81 (Figs. 5-7, 5-8). Carp from Station 28, 30, 75, and 76 also had relatively large spleens, as did bass from Station 28. Conversely, the spleens of largemouth bass from Station 15 were comparatively small. The other immune system indicators also suggested that fish from stations in this sub-basin were in comparatively poor health; high levels of lysozyme activity, high macrophage aggregate scores, or both conditions were present in carp, largemouth bass, or both at all stations in the sub-basin except Station 76 (Table 5-1). Elevated SSI in several carp was the only noteworthy fish health indicator at Station 76, where fish contained the some of highest bioaccumulative contaminant burdens in the MRB.

Potentially anomalous reproductive biomarkers were detected at most LMS stations (Table 5-1). Histological examination revealed that one (of eight) male bass from Station 81 was intersex (that is, ovotestis detected; Fig. 5-8). E/KT ratios were >1.0 in 50% of the male carp from Stations 76 and 80, but vtg was not present at high concentrations in male fish from any LMS station (Table 5-1; Fig. 5-9). 11-KT concentrations were relatively low in male bass from Station 76, and the E/KT ratio of four of eight fish was >1.0. Sex steroid concentrations in female bass were also uniformly low at Station 76, but the E/KT ratios were unremarkable. At Station 30 the vtg concentration in one of nine male largemouth bass was >2 mg/mL, which is in the range typical of females in early- to mid-vitellogenesis (Fig. 5-9). Conversely, vtg concentrations were comparatively low in female largemouth bass from Stations 28 and 76, and no vtg

was detected (<0.001 mg/mL) in any of the 14 female bass from Station 81 analyzed. Although three of the female bass from Station 81 were immature (gonadal stage 0), seven were in gonadal stage 1, and four were in stage 2. Stages 1 and 2 are defined in part by the presence of vtg in the developing oocytes (McDonald and others, 2000). The percentage of atretic oocytes (% atresia) was also comparatively high in female largemouth bass from Station 81.

Collectively, the chemical and biological indicators suggest that fish in the LMS sub-basin are exposed to comparatively high concentrations of organic and inorganic contaminants. Fish from all the stations sampled contained relatively high concentrations of bioaccumulable contaminants; concentrations at Station 76 were especially high. The fish health biomarkers indicated that fish were in sub-optimal condition at most sites. In addition, the reproductive biomarkers for most of the LMS sites were consistent with exposure of the fish to endocrine-modulating contaminants.

Mississippi Embayment (MSE) Study Unit: The MSE Study Unit is wholly contained within the LMS sub-basin, and the two areas are affected by many of the same contaminants (including mirex); however, and in contrast to the large rivers represented by the LMS sites, the MSE sites were located exclusively on lower-order rivers and streams in rural areas. As such, they are primarily affected by farming for the same pesticide-intensive crops (for example, corn, cotton, rice, soybeans) as the NCBP stations in the LMS sub-basin, but the NAWQA sites are less affected by point-sources. Both male and female carp were obtained at all MSE sites except Station 213 (Wolf R. at LaGrange, TN), from which only bass (male and female) were collected. A few bass were also obtained from Station 212 (Little R. Ditch at Moorehouse, MO). Not all analyses were completed at all sites, and some of the stations were sampled earlier than the rest of the MRB. As in the LMS sub-basin, sampling began in the MSE sub-basin during late August 1995 (Stations 201-204, 207, and 208), but Stations 212 and 213 were not sampled until early October.

Overall, concentrations of *p,p'*-DDE, toxaphene, and Hg in carp from MSE sites were high, even compared to nearby NCBP stations in the LMS sub-basin (Table 5-1; Figs. 5-1, 5-2). Carp from Stations 201 (Big Sunflower R. at Anguilla, MS), 202 (Bogue Phalia at Leland, MS), 203 (Steele Bayou at Rolling Fork, MS), and 204 (Tensas R. at Tenda, LA) contained very high (by 1995 standards) concentrations of *p,p'*-DDE (5-10 $\mu\text{g/g}$; Fig. 5-2) and toxaphene (1-4 $\mu\text{g/g}$). Carp from Stations 201 and 203 also contained slightly elevated concentrations of dieldrin (both stations) and chlordane (Station 201

only; Fig. 5-3); those from Station 204 contained slightly elevated As and Pb; and carp from Stations 202, 207 (Cache R. at Cotton Plant, AR), and 208 (Cache R. at Egypt, AR) had elevated TCDD-EQ (Fig. 5-4). Traces of mirex were also present in both carp samples from Station 204. EROD rates were indicative of exposure to exogenous AhR agonists in carp (males, females, or both) at all sites where they were collected (Fig. 5-5). In carp from Stations 202, 207, and 208, EROD was correlated with TCDD-EQ, but not with PCBs, which were uniformly low at all MSE stations. At Stations 207 (Cache R. at Cotton Plant, AR) and 208 (Cache R. at Egypt, AR), *p,p'*-DDE concentrations in carp were also slightly elevated (0.1-0.5 $\mu\text{g/g}$), but there was no detectable toxaphene (Fig. 5-2). However, at Station 207 dieldrin concentrations were slightly elevated and the Hg concentration was the maximum encountered in MRB carp (>0.3 $\mu\text{g/g}$; Figs. 2-1, 2-1). At Stations 212 and 213, Hg concentrations in bass were also slightly elevated (ca. 0.3 $\mu\text{g/g}$), and carp from Station 212 contained slightly elevated concentrations of dieldrin. As noted for the LMS sub-basin, some of the Hg and As in MSE fish may derive from pesticides and defoliants.

In terms of fish health, MSE carp and bass tended to be smaller (both length and weight) and younger than fish from most NCBP sub-basins. In the LMS sub-basin (in which the MSE is contained), both carp and bass tended to be especially large, but young, indicating rapid growth and accentuating the smaller fish size at the MSE sites. Condition factors for carp and bass (two sites) were normal at all sites, but other biomarkers indicated sub-optimal health at many stations (Table 5-1). The incidence of external lesions on carp from Stations 201, 202, 203, and 204 was high, but these were not reflected in large HAI scores (Fig. 5-7). High MA scores (one or more measures) were also documented in carp from Stations 203, 204, 207, 208, and 212 and in bass from Stations 212 and 213. In addition, carp from Stations 207 and 212 had relatively large spleens (Table 5-1); and histological examination indicated the presence of an increased number and size of thyroid follicles in the kidney of carp from Stations 208 and 212. As noted in Chapter 3, this condition has been documented in fish exposed to a number of contaminants in laboratory studies and in Great Lakes salmonids. Nevertheless, there are several potential underlying causes (including sampling bias introduced by the way the kidneys were sampled), and further investigation would be necessary to document the condition and determine its cause or causes.

Reproductive biomarker responses consistent with exposure to endocrine-modulating contaminants were detected at many MSE sites (Table 5-1). Of the seven male bass collected from Station 213, two had E/KT ratios >1.0 , as did one of two male bass from

Station 212; the other male from Station 212 was intersex (as indicated by ovotestes). The E/KT ratio of the one female bass from Station 212 and one of four from Station 213 was <1.0 . In male carp the mean 11-KT concentrations at Stations 204 and 207 were among the lowest in the MRB, and more than 50% of the male carp from these stations had E/KT ratios >1.0 . In addition, E/KT averaged significantly >1.0 in stage-1 and -2 male carp from Station 207—the only one of 36 stations analyzed statistically in which this condition was found (see Chapter 4 of this report). Male carp from Stations 203 and 204 had unusually low mean GSIs, and % atresia in female carp from Stations 204 and 212 was exceptionally high.

Collectively, the results indicate that MSE fish were exposed to relatively high concentrations of contaminants, as they were also in the LMS sub-basin. MSE fish contained the greatest concentrations of toxaphene and DDT (as *p,p'*-DDE) in the MRB, the latter great enough to represent a risk to piscivorous wildlife at most stations. In addition, the fish health and reproductive biomarkers, albeit limited in that carp and bass were not collected and analyzed from all sites and all biomarkers were not analyzed, indicated that the health of the fish was less than optimal at most stations and consistent with exposure to endocrine-modulating and other contaminants. Hydrologic and ecological differences between the NAWQA and NCBP sites were also reflected in fish size differences.

Upper Mississippi (UMS) Sub-Basin: Stations in the UMS sub-basin are affected by a variety of agricultural, industrial, and urban contaminant sources. The exception is Station 74 (Mississippi R. at Little Falls, MN), which is located in a relatively rural area not substantially affected by urban or agricultural activities. Fish from the UMS sites have historically been contaminated to greatly differing degrees by a variety of pollutants. Male and female carp and bass were collected at all UMS sites except Stations 73 (Des Moines R. at Keosauqua, IA; no bass) and 74 (no carp). All stations were sampled between mid-September and late October 1995.

The primary contaminants in UMS fish were cyclodiene insecticides (chlordane and dieldrin) and PCBs (Table 5-1; Figs. 5-3, 5-4). Chlordane and dieldrin were evident in carp and bass from Station 26 (Illinois R. at Beardstown, IL) and in carp from Station 73 (Fig. 5-3). Fish from Station 26 also contained moderately elevated total PCB concentrations (0.5-1.0 $\mu\text{g/g}$), but not correspondingly elevated TCDD-EQ (Fig. 5-4), and slightly elevated As and Zn (only in carp). Carp from Station 73 also contained slightly elevated PCB concentrations (0.2-0.5 $\mu\text{g/g}$) as well as Pb, and As. Carp and bass from Station 27

(Mississippi R. at Guttenburg, IA) contained elevated PCB concentrations and corresponding TCDD-EQ whereas those from Station 72 (Wisconsin R. at Woodman, WI) contained TCDD-EQ, but PCB concentrations were low (Fig. 5-4). Consistent with previous findings (Schmitt and others, 1999), smallmouth bass from Station 74 contained slightly elevated Hg concentrations (0.2-0.3). At Station 111 (Mississippi R. at Lake City, MN—L. Pepin), PCB concentrations were somewhat elevated and there was corresponding TCDD-EQ in carp, but not in bass (Fig. 5-4). Concentrations of Zn in carp were also slightly elevated at Station 111, as they also were in carp from Station 112 (Mississippi R. at Dubuque, IA). In addition, PCB concentrations in both carp and bass were slightly elevated and there was corresponding TCDD-EQ activity in fish from Station 112 (Fig. 5-4). EROD rates indicated exposure to exogenous AhR agonists in one or more taxa at all UMS stations except 74 and 111 (Fig. 5-5), the latter despite the presence of PCBs and TCDD-EQ (Fig. 5-4).

Carp from the UMS sub-basin were of average size and age, but bass tended to be larger and older compared to other sub-basins. This is somewhat surprising considering that only smallmouth bass (*Micropterus dolomieu*), which tend to be the smallest of the black basses, were collected at Stations 72 (Wisconsin R. at Woodman, WI), 74, and 111. A large percentage of bass from Station 26 had external lesions and the mean HAI for bass from this site was correspondingly high as it also was at Stations 27 and 112 (Table 5-1; Fig. 5-8). Both male and female bass from Station 26 also had enlarged livers and spleens (Fig. 5-6) and had high MA scores (one or more variables). Carp from Station 26 also had high MA scores and elevated lysozyme activity, but were normal in other respects. One or more MA variables were also elevated in carp, bass, or both from Stations 27, 72, 73, 111, and 112 (Table 5-1). In addition, enlarged spleens were present in bass from Station 112, as was elevated lysozyme activity in carp from Stations 72, 73, 111, and 112. Collectively, the health indicators suggested that the fish were in a diseased condition at many UMR sites.

Most reproductive biomarkers in UMS carp appeared normal (Table 5-1). An exception was one (of 10) male carp from Station 112 with very a very high vtg concentration (level typical of females in early to mid-vitellogenesis; Fig. 5-9). In addition, intersex male smallmouth bass (that is, ovotestis detected) were identified at three stations: 72 (one of four fish), 74 (one of seven), and 111 (eight of 11; Fig. 5-8). Only one of 10 male bass from each of Stations 27 and 112 had detectable concentrations of vtg, however, and in none of these fish were the vtg concentrations particularly high. In female fish reproductive biomarker anomalies were detected only

at Station 26, where E/KT was <1.0 in more than 50% of the female carp and mean % atresia was the highest in the MRB (Table 5-1). Female bass from Station 26 also had comparatively low E2 and vtg concentrations, and more than 50% had E/KT ratios <1.0 .

In general, fish from the UMR sub-basin were moderately contaminated. Smallmouth bass from Station 74 contained slightly elevated concentrations of Hg, as they have in the past. However, concentrations of all other measured contaminants were low at this site and the fish appeared to be healthy. In contrast, fish from all of the other stations in this sub-basin were contaminated with bioaccumulative organic compounds to some degree, and the health indicators and reproductive biomarkers were consistent with the exposure of the fish to contaminants.

Eastern Iowa Basins (EIB) Study Unit: The EIB Study Unit is wholly contained within the UMS sub-basin and is consequently affected by similar contaminant sources. EIB stations are influenced primarily by agricultural activities, and several sites also receive industrial and urban pollutants from cities such as Waterloo and Cedar Rapids, IA. Exclusively carp (both male and female) were collected from all of the EIB sites; however, the carcass samples from Station 209 (S. Fork Iowa R. at New Providence, IA) were lost in transit. All EIB stations were sampled in mid-September 1995.

Cyclodiene pesticides were the most noteworthy contaminants in EIB fish; concentrations were comparatively high at all sites (Table 5-1; Fig. 5-3). In contrast, concentrations of PCBs and TCDD-EQ were comparatively low at all EIB sites, and EROD rates were generally not indicative of exposure to exogenous AhR agonists (Table 5-1; Figs. 5-4, 5-5). At Station 205 (S. Skunk R. at Oskaloosa, IA), concentrations of all contaminants except chlordane, which was present at slightly elevated levels, were low. At Station 206 (Iowa R. at Morengo, IA), elevated concentrations of chlordane and dieldrin were evident, and the proportional contribution of heptachlor epoxide was high. Dieldrin was also present at slightly elevated concentrations at Stations 210 (Iowa R. at Rowan, IA) and 211 (Cedar R. at St. Charles City, IA). In addition, carp from Stations 210 and 211 contained slightly elevated concentrations of DDT (as *p,p'*-DDE; Fig. 2-2) and PCBs (0.2-0.5 $\mu\text{g/g}$; Fig. 2-4), and fish from Station 210 contained slightly elevated Zn concentrations.

Carp from the EIB Study Unit were about the same size and age as those from the UMS sub-basin, and condition factors were generally within normal ranges except at Stations 205 and 211, where they were lower than most (Table 5-1). More than 50% of the carp from Station 209 had grossly visible lesions,

and the HAI values for this site were correspondingly high (Fig. 2-7). Fish from Stations 205, 206, and 211 had high MA scores, and average relative spleen size was large at Station 210. In contrast, lysozyme activity was not elevated at any EIB sites.

Station 211 was the only EIB site at which E/KT ratios for all male carp were normal (that is, <1.0 ; Table 5-1), generally because mean concentrations of 11-KT were low at all sites. In contrast, most reproductive biomarker responses in female carp were unremarkable. The exceptions were a few fish with E/KT ratios <1.0 at several sites and mean % atresia, which was unusually high in fish from Stations 205, 206, and 210 (Table 5-1).

Collectively, the chemical and biological indicators suggest that carp from all the EIB sites were exposed to and affected by chemicals to some degree. At Station 206, concentrations of cyclodiene pesticides were comparatively high.

Ohio River (OHR) Sub-Basin: Stations in the OHR sub-basin are influenced by industrial, agricultural, and urban pollution sources. Fish from this sub-basin historically contained relatively high concentrations of PCBs and cyclodiene pesticides (Schmitt and others, 1999b). Male and female carp were obtained from all OHR sites except Station 23 (Kanawha R. at Nitro, WV), where bass were obtained but only one male carp was collected. Sampling in the OHR sub-basin began in late August 1995 at Station 68 (Wabash R. at New Harmony, IN) but was not completed until late November (also Station 68) because several sites had to be visited more than once to obtain the necessary complement of fish.

In keeping with past findings, fish from all OHR stations contained a wide variety of contaminants (Table 5-1). Fish from Station 23 contained elevated concentrations of chlordane, moderately elevated PCB concentrations, and, in carp, slightly elevated Pb (Figs. 5-3, 5-4). Traces of HCB were also present, but neither TCDD-EQ nor EROD rates were elevated. Fish from Station 24 (Ohio R. at Marietta, OH) also contained traces of HCB along with elevated levels of chlordane; relatively high PCB concentrations ($>1.0 \mu\text{g/g}$); and slightly elevated concentrations of *p,p'*-DDE (0.1-0.5 $\mu\text{g/g}$), Cd ($>0.3 \mu\text{g/g}$ in carp); and Pb (Table 5-1; Figs. 5-2, 5-3, 5-4). TCDD-EQ concentrations were low, but EROD rates indicated exposure to AhR agonists in both carp and bass from Station 24 (Figs. 5-4, 5-5). Fish from Station 25 (Cumberland R. at Clarksville, TN) contained slightly elevated concentrations of dieldrin, PCBs, Cd, and Pb, and traces of HCB (Table 5-1; Fig. 5-4). Like Stations 23 and 24, there was no evidence of elevated TCDD-EQ in fish from Station 25 despite the presence of PCBs and HCB, and hepatic EROD rates were low in both carp and bass (Figs. 5-4, 5-5).

In contrast, fish from Station 67 (Allegheny R. at Natrona, PA) contained at least somewhat elevated concentrations of many contaminants including *p,p'*-DDE, dieldrin, PCBs ($>1.0 \mu\text{g/g}$), TCDD-EQ (in both carp and bass), Cd ($>0.5 \mu\text{g/g}$ in carp), and Pb, but no detectable HCB (Table 5-1; Figs. 5-1—5-4). In addition, EROD rates in male carp from Station 67 indicated exposure to exogenous AhR agonists. Fish from Station 68 (Wabash R. at New Harmony, IN) also contained slightly elevated concentrations of chlordane, dieldrin, PCBs, and TCDD-EQ; and EROD was elevated in male carp (Figs. 5-3, 5-4). At Station 70 (Ohio R. at Metropolis, IL) fish contained slightly elevated concentrations of *p,p'*-DDE and dieldrin, PCBs, TCDD-EQ, Hg ($>0.3 \mu\text{g/g}$), and Pb (Table 5-1; Figs. 5-1—5-4). In addition, hepatic EROD rates in male bass from Station 70 indicated exposure to exogenous AhR agonists (Fig. 5-5). This site is immediately downstream from a uranium processing facility with a history of contaminant releases. Fish from Station 71 (Tennessee R. at Savannah, TN) contained slightly elevated concentrations of DDE and PCBs (Figs. 5-2, 5-4). Carp from Station 71 also contained TCDD-EQ, and EROD rates indicated exposure to exogenous AhR ligands; however, TCDD-EQ levels and EROD rates in bass from this site were low (Figs. 5-4, 5-5).

Carp from the OHR sub-basin were, on average, the largest (length and weight) and oldest of any MRB sub-basin, but OHR bass were comparatively small. Many of the fish health indicators suggested that fish from OHR sites were in less than optimal health (Table 5-1). In bass, condition factors at Station 67 were lower than most in both males and females. In carp, condition factors were relatively low at Stations 24, 25, and 70. The livers of male and female bass from Stations 67 and 68 were relatively large (Fig. 5-6), a condition indicative of chronic chemical exposure. The spleens of carp from Station 68 were also enlarged, a condition often associated with disease. Conversely, the spleens of bass from Station 24 were comparatively small, which is more indicative of contaminant exposure. Large percentages of carp, bass, or both from Stations 24, 67, 70, and 71 also had grossly visible external lesions, and HAI values were comparatively high in bass from Station 70 (Fig. 5-8). Lysozyme activity was elevated in bass from Stations 23, 25, 70, and 71, and in carp from Stations 68, 70, and 71. One or more MA parameters were also elevated in bass from Stations 25, 68, and 70 and in carp from Stations 67 and 70.

The reproductive biomarkers were also consistent with contaminant exposure at several OHR stations (Table 5-1). Sex steroid concentrations in male largemouth bass from Station 25 were comparatively low, and seven of eight had E/KT ratios >1.0 . One of

five male smallmouth bass from Station 67 was inter-sex (as indicated by ovotestes; Fig. 5-8), but none had exceptionally high vtg concentrations. At Station 70 mean % atresia in female largemouth bass was the highest in the MRB, and most female bass had E/KT ratios <1.0 . Mean GSI in female bass (mostly gonadal stage 2, mid-development) from Station 70 was also comparatively low and no vtg was detected ($<0.001 \text{ mg/mL}$) in any of the nine female bass analyzed, but traces of vtg were detected in one male. Female bass from Station 68 also had slightly elevated % atresia (Table 5-1). The mean E2 concentrations in female bass from Stations 24, 25, and 67 were unusually low, but 11-KT concentrations at these stations were also low and E/KT ratios were generally unremarkable (>1.0).

In summary, fish from all OHR stations contained comparatively high concentrations of bioaccumulative contaminants, and the health indicators suggested that some fish were in sub-optimal condition. The reproductive biomarkers were also consistent with the exposure of the fish to contaminants at several stations. Stations 67 (Allegheny R.) and 70 (Ohio R.) were particularly noteworthy in that concentrations of several contaminants were elevated in both carp and bass, and there were multiple biomarker anomalies.

Program-Level Comparisons

One objective of this study was to compare contaminants and biomarkers in fish from large rivers, as represented by the NCBP sites, with those in the smaller rivers and streams represented by the NAWQA sites. Accordingly, we examined for differences at the program level (that is, between all NAWQA and NCBP sites). For most chemical and biological indicators there were few clearly evident trends because mean concentrations varied greatly among stations and sub-basins. In carp, concentrations of Cd and Pb were greater overall at NCBP than at NAWQA sites, whereas concentrations of Hg and DDT (as *p,p'*-DDE) were greater at the NAWQA sites; however, the Hg results were heavily influenced by high concentrations at one NAWQA site (Station 207), and Station 77 was excluded from the analysis of program- and sub-basin trends because of mixed-gender compositing. Nevertheless, the comparatively high concentrations of certain bioaccumulative contaminants in fish from the NAWQA sites relative to nearby NCBP sites were particularly evident. In bass, mean Hg concentrations were greater at the two NAWQA sites from which bass were collected than the overall mean for NCBP sites. For Se, concentrations in bass were greater at NCBP sites (Station 77 was included for bass). When the NAWQA Study Units were compared with their corre-

sponding NCBP sub-basins, differences were more apparent; these were noted in the preceding sections.

In general, carp and bass from the NCBP stations were larger (TL and weight) and older than at the NAWQA sites; however, bass were obtained from only two NAWQA sites (212 and 213), and three species of black bass (*Micropterus* spp.) were collected at the NCBP sites. Despite these size differences, most of the fish health indicators did not differ significantly at the program level. Although statistically significant overall differences between NCBP and NAWQA sites were noted for several biomarkers in carp, most of these were subtle. The exception was % atresia in female carp from the NAWQA sites, which was substantially greater than at the NCBP sites. Many factors, including (but not limited to) chemical exposure, may have caused this condition.

Correlations Between Biomarkers and Bioaccumulative Contaminants

Correlations between biomarkers and contaminants were examined using Spearman rank correlations. For most variables, separate analyses were conducted for each gender and for all fish of a species; reproductive biomarkers were analyzed separately. TCDD-EQ was considered both as both a biomarker and a contaminant and analyzed only for all fish combined. Many of the biomarkers were correlated with one or more contaminants (Table 5-2). Some of the responses were consistent (that is, were present in more than one species, gender, or both and, for non-reproductive biomarkers, in the combined-sex analyses), whereas others were not. Statistically significant ($P \leq 0.1$) and borderline ($0.1 < P < 0.15$) correlations for each group of variables are presented in the following sections and in Table 5-2. Statistical methods are described in Chapter 1 of this report.

Fish Size and Age: Sizes and ages of carp and bass differed between genders, so no combined-sex correlations were investigated for these variables. Within the genders, the concentrations of some contaminants in composite samples were correlated with fish size, age, or both (Table 5-2). Cd increased with age in female carp, as did Hg in male bass. In contrast, DDE decreased with age in male carp ($r = -0.25$, $P = 0.11$ in female carp). Cd was also positively correlated with both total length (TL) and weight in male carp and female bass (Table 5-2). Total PCBs also increased with TL in male and female carp, but TL was negatively correlated with total cyclodiene pesticides in both male and female bass and with weight in female bass. Cd increased with weight in male carp and female bass, as did Se in both female and male bass

and total PCBs in male and female carp (Table 5-2).

EROD and TCDD-EQ: As noted in Chapter 2 of this report, EROD rates generally increased with the concentrations of many contaminants, but the strengths of the relationships varied. In male carp EROD was positively correlated with Cd, Hg, PCBs, and DDE, but the correlation with TCDD-EQ was surprisingly weak ($r = 0.21$, $P = 0.20$). In female carp EROD was significantly correlated with TCDD-EQ and with Hg, PCBs, and DDE. EROD was also correlated with TCDD-EQ in the combined-sex analysis of carp (all carp) and with PCBs in the combined-sex analysis of bass (all bass). Among the elemental contaminants, EROD was positively correlated with Pb in all sex-taxon combinations and in all bass, but not in all carp, and EROD was negatively correlated with Cd in male bass (Table 5-2). TCDD-EQ was positively correlated with total PCBs, p,p' -DDE, and total cyclodiene pesticides in the all-fish analysis. More information on relations between EROD and contaminants is presented in Chapter 2.

Organo-Somatic and Ponderal Indices: Some of the organo-somatic and ponderal indices were correlated with measured contaminant concentrations (Table 5-2). The spleno-somatic index (SSI) increased with Se in male carp, total cyclodiene pesticides in female carp, and TCDD-EQ in all bass, but there were no significant correlations between SSI and any contaminants in male or female bass considered separately or in all carp. The hepato-somatic index (HSI, bass only) increased with Cd and Se in males, but not in females; and with TCDD-EQ in all bass (Table 5-2). In contrast, HSI decreased with p,p' -DDE in male bass and with Hg in female bass and all bass. Condition factor (CF) increased with TCDD-EQ in female carp and in all carp, but not in male carp. Condition factor also increased with Cd in female bass and with Se in male carp, female bass, male bass, and all bass. In contrast, CF decreased with p,p' -DDE in male bass and with Pb in female carp (Table 5-2).

Immune System Indicators: The macrophage aggregate (MA) variables were positively correlated with concentrations of several contaminants. The only significant correlation for MA density (MAMM) was an increase with Hg in female carp ($r = 0.25$, $P = 0.13$ for males) and in all carp; there were no significant negative correlations (Table 5-2). Mean MA area (MEANAREA) increased with Cd and Pb in male carp and in all carp, with Hg in male bass and in all carp, and with total PCBs in both female and male carp (Table 5-2). There were also no significant correlations between MEANAREA and any contaminants in all bass; however, MEANAREA decreased with

Table 5.2. Statistically significant correlations¹ between biomarkers and contaminants. See text for biomarker definitions.

Biomarker	Contaminant							
	<i>p,p'</i> -DDE	Cyclo. Pestic. ⁵	PCBs	TCDD-EQ ³	Cd	Hg	Pb	Se
Total Length ²		mb, FB	mc, fc		mc, fb			
Weight ²		FB	mc, fc		mc, fb			MB, fb
Age ²	mc, fc				fc	mb		
EROD	MC, AC, ab		mc, ac	fc, AC	mb, mc	mc, ac	mc, fc, mb, fb, AB	
TCDD-EQ ³	af	af	AF	--				
SSI		fc		ab				mc
HSI ⁴	mb			AB	mb	mb, ab		mb
CF	mb			fc, ac	fb		fc	mc, mb, fb, AB
MAMM						FC, ac		
MA-AREA		fb	MC, fc		mc, ac	mb, ac	MC, ac	
MA-TISSOC		fb	mc, fc		mc, fc, ac	fc	mc, ac	
Lysozyme	fb, mb, AB	fc			mb			FB, AB
HAI	ab			fc, AC	mc, ac	FC, ac	fc	mc
Ext. Lesions	MC, fc, AC, fb, ab				MC, AC		ac	MC
E2	mc	mc, fc, mb, FB	fc, mb			fc		mb
11-KT	MC	mc, mb		mc		mc		
E/KT	mc	mc			mc	fb		
GSI					mb			mc, fb
% Atresia ⁵	fc	fc			fb	FC		fb
Stage	mc					fb	fb	
Vtg	mb		mc		fc, mc		mc	fb

¹Blue text, positive correlations; Red text, negative correlations; plain text, $P < 0.10$; bold italics, $P < 0.05$; BOLD SMALL CAPS, $P < 0.01$;

BOLD ALL CAPS, $P < 0.001$; mb, male bass; fb, female bass; ab, all bass; mc, male carp; fc, female carp; ac, all carp; af, all fish.

²Because fish size and age differed between genders; "all fish" (af) correlations were not computed for these variables.

³TCDD-EQ was considered as both a biomarker and a contaminant in this analysis and was only evaluated for all fish combined.

⁴HSI was evaluated in bass only

⁵% Atresia in females only.

⁶Total cyclodiene pesticides (sum of *cis*- and *trans*-chlordanes and nonachlors, oxychlordane, heptachlor epoxide, dieldrin, and endrin).

increasing cyclodiene pesticide concentrations in female bass. MA % tissue occupied (TISSOC) also increased with Cd in female and male carp and in all carp, with Pb in male carp and in all carp, with Hg in female carp, and with total PCBs in male carp ($r = 0.26$, $P = 0.1$ for female carp). Similar to MAMM and MEANAREA, TISSOC was negatively correlated with cyclodiene pesticide concentrations in female bass but was not significantly correlated with any contaminants in all bass.

In contrast to the MA parameters, lysozyme activity was correlated with concentrations of relatively few contaminants; however, some of the correlations were comparatively strong (Table 5-2). Lysozyme increased with DDE in female and male bass and in all bass, but decreased with Cd in male bass and with Se in female bass and all bass (Table 5-2). Lysozyme activity was also negatively correlated (weakly) with cyclodiene pesticides in female carp, albeit weakly ($r = -0.29$, $P < 0.1$). There were no significant correlations between lysozyme activity and any contaminant concentrations in either male carp or in all carp.

Gross Lesions and HAI: The health assessment index (HAI; greater scores indicate poorer health) was positively correlated with concentrations of many contaminants (Table 5-2). HAI increased with Cd in

male carp and in all carp, Se in male carp, Pb in female carp, and DDE in all bass. HAI was negatively correlated with TCDD-EQ and Hg in both female carp and all carp (Table 5-2). The proportion of fish with externally visible lesions also increased with Cd in male carp and all carp, and with Pb in male carp and *p,p'*-DDE in all analyses except male bass (Table 5-2). There were no significant negative correlations between external lesions and any contaminants in carp or bass.

Reproductive Biomarkers: Many of the reproductive biomarkers, which were only evaluated separately for male and female carp and bass, were correlated with contaminants (Table 5-2). E2 concentrations increased with Hg in female carp and with Se in male bass, but E2 was negatively correlated with many organochlorine chemical residues. These included cyclodiene pesticides in both female and male carp and bass, total PCBs in female carp and male bass ($r = -0.30$, $P = 0.12$ in female bass); and *p,p'*-DDE in male carp (Table 5-2). 11-KT concentrations also decreased with Hg and total cyclodiene pesticides in male carp ($r = -0.20$, $P = 0.21$ in female carp) and male bass ($r = -0.30$, $P = 0.12$ in female bass) and with *p,p'*-DDE and TCDD-EQ in male carp (Table 5-2). There were no significant positive correlations between 11-KT and any contaminants in carp or bass of either sex.

E/KT ratios increased with Cd, cyclodiene pesticides, and *p,p'*-DDE in male carp and with Hg in female bass. Conversely, E/KT was not significantly correlated with any contaminants in male bass.

Compared to the hormone-based variables there were fewer statistically significant correlations between the other reproductive biomarkers and contaminant concentrations. GSI increased with Cd in male bass and decreased with Se in male carp and female bass, but was not correlated with any contaminants in female carp (Table 5-2). In bass % atresia (females only) was not positively correlated with any contaminants, but it decreased with Cd and Se (Table 5-2). In carp the correlations were opposite of those in bass; there were no significant negative correlations with any contaminants, but % atresia increased with Hg, total cyclodiene pesticides, and DDE (Table 5-2). Gonadal stage decreased with Hg and Pb in female bass, but it increased with *p,p'*-DDE in male carp. Stage was not correlated with any contaminants in male bass or female carp, however. Vtg decreased with Cd in female carp and with *p,p'*-DDE in male bass, but it increased with Cd, Pb, and PCBs in male carp and with Se in female bass.

Summary: Some biomarkers were significantly correlated with measured concentrations of bioaccumulative contaminants, but in general none of the correlations were particularly strong; that is, no single contaminant or group of contaminants (cyclodiene pesticides, TCDD-EQ) investigated explained more than about 50% of any biomarker response in either male or female carp or bass or in the combined-sex analysis of either species. Nevertheless, some of the relationships were consistent across species or genders (for example, EROD and Pb; external lesions and *p,p'*-DDE; E2 and cyclodiene pesticides), implying that these conditions at least tended to occur together. Some of the relationships were the opposite of expectations, however; for example, EROD activity was negatively correlated with Cd in male bass whereas the statistically significant correlations with Cd were positive in other taxon-sex groups. Cd is a known EROD-inducer (Whyte and others, 2000), and our expectation was a positive correlation. Such findings are also not surprising, however. The simple coefficients are based on all the data (regardless of stage, age, fish size, etc.), so the effects of many variables are confounded (see discussion following). It is likely that more in-depth statistical analyses using more sophisticated techniques would increase the amount of variation explained. In addition, the rank correlations of medians presented here are extremely conservative. These correlations nevertheless indicate that some of the biomarker responses resulted at least partly from exposure of the fish to chemicals, and that further sta-

tistical analyses may yield better resolution. In addition, the correlations between contaminant concentrations and fish size and age indicate that these variables should continue to be controlled or otherwise accounted for in future studies.

Discussion

Statistically, the 1995 data set was challenging; few variables met the distributional and homogeneity-of-variance assumptions necessary for parametric tests, and there were many missing and censored values. Our strategy was to apply the minimal transformation necessary (generally log) to meet these assumptions and to analyze only those variables with at least 85% uncensored observations. If no transformation succeeded in rendering the data suitable for parametric analysis, we ranked the data and used non-parametric methods. This was an iterative and time-consuming process, but a necessary and expected exercise in a pilot project. It was also somewhat difficult to interpret and present the results because different transformations were sometimes indicated for closely related variables and even for the same variable in different taxon-sex categories. Nevertheless, having completed this exercise, the intermediate steps are probably unnecessary in future projects; because so many variables ultimately had to be ranked, we recommend that future analyses use the rank transformation exclusively for biomarkers, which should greatly expedite data processing and statistical analysis. We recognize at least two shortcomings of this approach, however. One is the statistical power loss often associated with non-parametric tests; the other is that complex statistical models, which may be necessary for controlling spatio-temporal variability, may not be well suited to ranked data.

Limitations of Correlation Analyses: Studies such as ours, which span broad geographic areas, are exclusively exploratory, not explanatory. Correlations quantify associations between measured variables, regardless of the number of variables and statistical tools available. Consequently, determining the causes of the biological findings was not an objective of the study. Rather, carefully planned and controlled field and laboratory research is required to document cause-effect relationships. The foundation of biomarker-based monitoring is the understanding of the factors that influence the biomarkers based on such research; that is, interpretation of biomarker findings is based more on knowledge of the biomarkers than on empirical correlations. Such correlations typically generate more questions than answers, but may suggest testable hypotheses to be evaluated through subsequent laboratory research and more focused field studies. Such

studies have already begun; based on the results reported here, selected 1995 fish samples from MSE and LMS sites were analyzed for chlorinated dioxins and dibenzofurans (unpublished data, USGS-Columbia Environmental Research Center), the occurrence of intersex fish near sewage treatment outfalls was investigated further in Minnesota (Lee and others, 1999), and the normal cycle of reproductive biomarkers in several fish taxa is being investigated at USGS laboratories (see list of projects at http://www.cerc.usgs.gov/Other_Webs/endocrine/summary.htm).

Simple correlations are inherently deceptive because many variables are inter-correlated and cannot be controlled or otherwise accounted for. In addition, the notion of biological responses rising or falling monotonically with the concentrations of one or more contaminants in fish collected over broad expanses of time and space is grossly simplistic. Curvilinear and asymptotic relationships on environmental gradients are common, and many variables are closely inter-related. Nevertheless, exploratory data analyses typically begin with the examination of correlations between pairs of variables to determine whether any basic relationships are present, as we did here. These should be followed by more carefully structured tests involving greater numbers of variables and fewer observations (that is, subsets of the data partitioned by sub-basins, reproductive stages, etc.), as recommended by Goodbred and others (1997). Ultimately, associations between and among groups of chemical and biological indicators should be explored using multiple regression and multivariate statistical methods to reduce the complexity of the data set (Chen and others, 1998). Such methods may be useful for expressing groups of related variables as smaller numbers of metrics, exploring relations between groups of variables, or characterizing samples or sites in multi-dimensional space. Correlated variables also contain the same information, and further analyses may reveal that closely related biomarkers are redundant and subject to elimination from future studies.

Need for More Quantitative Methods: We analyzed many biological variables as either categorical (for example, gonadal stage) or binary (vtg in males) responses, both of which are limiting. For variables such as stage, it would be advantageous to have the either the original data (that is, cell diameters) or the distributions of cell numbers in each stage rather than the fully classified observations. Our analyses showed graduated responses across stages for many reproductive biomarkers; although the responses differed among stages, there was considerable overlap that would be better addressed on a continuous scale. Other binary variables (for example, presence/absence of lesions) would also benefit from further quantifica-

tion (numbers of lesions, area, etc.); macrophage aggregates, for example, are quantified three ways (number, area, and percentage of tissue occupied). Such comparisons are inherently more powerful than categorical comparisons or, in the extreme, binary responses, but are also more costly and time consuming. Subsequent investigations should therefore determine, on a smaller scale with well-defined sites and an appropriate sampling design, whether the additional information gained through quantification justifies the additional effort and expense.

Design and Implementation Issues

Fixed vs. Probability Sampling: As noted in Chapter 1, the present study evolved from the NCBP (Schmitt and others, 1999b) and earlier NAWQA studies (Goodbred and others, 1997). Consequently, the collection sites were at least nominally “fixed”; they were historical NCBP collection stations and NAWQA program fixed sites, which were selected to be hydrologically rather than statistically representative. Our cooperators collected fish where they could be obtained in the vicinity of the nominal station location; considering this and the unknown degree to which the fish may have moved prior to their capture, the degree to which the locations are “fixed” is questionable. Nevertheless, the locations were clearly not selected probabilistically, which therefore implies an element of spatial bias that limits inferences beyond the sites actually sampled (Olsen and others, 1999). A design with less inherent spatial bias will be required to characterize and monitor conditions in large rivers rather than at selected sites. Methods for implementing spatially unbiased sampling without compromising historical fixed-station databases such as the NCBP are available (Urquhart and others, 1998).

Quality Control and Consistency Among Field Teams: The protocol developed for this project required the internal and external examination of the fish at the time of capture for grossly visible lesions. Various internal organs and external structures were examined and categorized in terms of color and other somewhat subjective criteria. Fish were collected by 18 teams led by biologists who had been trained in the conduct of the protocol but who varied greatly in experience and expertise relative to fish anatomy and health. It was the overall consensus of all study participants that greater consistency would be achieved if smaller numbers of more experienced personnel led the field teams, a recommendation incorporated into subsequent studies (Bartish and others, 1997).

Other Data and Information: Monitoring contaminants

in aquatic ecosystems and their effects on living resources requires considerably more data and information than the bioindicators and contaminant burdens we measured. Accordingly, the methods evaluated in this study represent only a subset of the suite of BEST program indicators for monitoring in aquatic habitats (BEST, 1996; Schmitt, 2000). By design, the BEST program shares many methods with the NAWQA program (BEST, 1996). A secondary objective of this study was therefore to provide a database for evaluating the compatibility of the BEST aquatic methods with those of the NAWQA program, as a template for monitoring rivers in concert with other USGS water monitoring programs. Accordingly, 13 fixed monitoring stations in two NAWQA Study Units were sampled in 1995. Subsequent studies should evaluate these more complete data sets for the MSE and EIB Study Units.

Controlling for Spatio-Temporal Variability: Many of the biological endpoints we evaluated cycle seasonally, and tend to vary as a group. Consequently, many are correlated. Our analyses sought to untangle these inter-correlations, and to present and test for spatial differences free of biases associated with the reproductive cycle. Future analyses of these data will necessitate the use of spatio-temporally adjusted values for biomarkers. This adjustment could be done statistically, or by restricting comparisons to geographic or stage-based subsets (or both), as we did in the presentations of the univariate biomarker findings in Chapters 2-4.

Comparing locations and, ultimately, time periods (that is, monitoring) will require accounting for cyclic variability, as we attempted to do for some biomarkers. In this regard several alternatives have been suggested and some are currently being pursued. Goodbred and others (1997) suggested sampling a series of regional “reference sites”; that is, ecologically representative, but uncontaminated, sites in close proximity to the study areas at which fish would presumably be in the same or similar reproductive stages as those at the sites under investigation in the same region. The study sites would then presumably be compared to the reference condition for the appropriate region. Unfortunately, and as documented elsewhere in this report, this is an unrealistic expectation because all large U.S. rivers have been modified by human activities (including pollution) to some extent. Detectable concentrations of *p,p'*-DDE, Hg, Pb, and other substances capable of inducing biomarker responses at low concentrations have become ubiquitous (Zell and Ballshmiter, 1980; Settle and Patterson, 1980; Bidleman and others, 1993; Kidd and others, 1995; Yeardley and others, 1998; Monteiro and Furness, 1998; Muir and others, 1999; Schmitt

and others, 1999b). In addition, many of the biological variables we measured can be influenced by temperature and other environmental factors (Schmitt and Dethloff, 2000). Consequently, and as we showed through comparisons between the MSE sites with NCBP sites in the LMS sub-basin, even fish collected at about the same time of year and in relatively close geographic proximity may grow at different rates and be in different reproductive stages due to natural environmental factors such as temperature, water level, and productivity.

Not surprisingly, biomarker-based studies completed to date that have attempted to incorporate regional reference sites have yielded equivocal results; that is, biomarker anomalies consistent with chemical exposure have been reported at putatively uncontaminated “reference” sites (for example, Goodbred and others, 1997; Lee and others, 1999). There are several plausible explanations for these findings. First, and as noted in the preceding paragraph, some endocrine-modulating contaminants (*p,p'*-DDE, PCBs, cyclodiene insecticides, Pb, Hg), are ubiquitous; note that we found slightly elevated concentrations of *p,p'*-DDE and Hg, as well as some TCDD-EQ, in fish from Station 400, which represents a water supply system in a relatively rural area. Anomalous biomarker results may therefore represent responses to low or undetected concentrations of known or unknown contaminants, either singly or in combination. Alternatively, equivocal biomarker findings at reference sites may also reflect how little is known about the “normal” range of many biomarkers. It is impossible to quantify all the natural and anthropogenic substances that may influence biomarker results. Consequently, because the true extent of contamination and the normal range of many responses are unknown, positive findings are possible even at putatively “clean” sites.

In addition to analyzing regional subsets, we attempted to control for spatio-temporal bias by comparing biomarker results in fish of similar gonadal stages as determined by histopathology; that is, by restricting comparisons to subsets of the data defined by gonadal stage (McDonald and others, 2000), and eliminating fish outside the desired reproductive stages. Generally, we selected mature fish early in the reproductive cycle, when biomarker differences among individual fish were expected to be smallest and the rate-of-change in the seasonally varying parameters is slowest. Because most fish were collected in the fall, relatively few individuals fell outside the desired range of stages. Although expedient, there are several drawbacks to this approach, the most important of which is that gonadal stage may itself be influenced by contaminants. (This is one reason we also treated stage as a dependent variable). Seasonal

development of the gonads is controlled by hormones, the concentrations of which can be affected by chemicals (see Chapter 4 of this report and McDonald and others, 2000). In addition, many chemicals, including some that bioaccumulate (and which were present in the fish we analyzed), may act as hormone mimics or antagonists. Consequently, adjusting for stage in the manner we did may have inadvertently masked differences among locations. As an alternative, we might attempt to better characterize the "normal" range of the biomarkers through the reproductive cycle, and then seek to identify groups of fish that lie outside this normal range. As noted earlier, the high degree of inter-correlation among the reproductive biomarkers will probably necessitate the use of multivariate statistical procedures, as recommended by Adams and others (1994) for biomarkers in general, to characterize the "normal" condition and identify deviations from normal. A second drawback of eliminating individual fish (for any reason) from the data set is that it reduces the correspondence between chemical analyses based on composite samples and biomarkers determined for individual fish. As noted, the chemical concentrations may be influenced by gonadal stage and development, so eliminating fish in advanced (or retarded) stages could affect these relationships. Accounting for stage differences through the application of multivariate statistical approaches and reducing the need to eliminate fish from considerations would also mitigate this problem.

Spatial vs. Temporal Comparisons

Our analyses have thus far focused on controlling for spatio-temporal bias in the biomarkers, to be as certain as possible that our observations reflect differences attributable to environmental degradation and not seasonal or other factors. To that end, we documented and tested for differences among locations, on the grounds that the same level of understanding will be necessary to document temporal trends. As this study was a pilot for a monitoring program, the assumption was and remains that temporal trends are important, and that the methods we employed for testing and controlling spatio-temporal bias in this study will be applicable in future analyses. Nevertheless, and as noted, we would prefer to know the normal ranges for more biomarkers, and to thereby evaluate the biological rather than purely statistical significance of the findings. We note that similar complaints were historically lodged against the measurement of chemical contaminants, but that continuing ecotoxicological research has enabled their evaluation to a far greater extent than in the past. Biomarker research continues at a rapid rate and it, along with information synthesis activities (for example, Schmitt and Dethloff, 2000; Whyte and others, 2000) will facilitate future evalua-

tions.

Three-Pronged Approach for Planar Organic Compounds

At most of the MRB sites sampled, EROD and TCDD-EQ were correlated with PCBs. This was not the case in the MSE Study Unit, where comparatively high EROD rates and TCDD-EQ were measured in carp in the near absence of measurable PCB residues. Although it is possible that low concentrations of very potent PCB congeners were present, this would be unprecedented and highly unlikely in such a predominantly agricultural area. More likely causes for the EROD activity and TCDD-EQ are low levels of chlorinated dibenzodioxins, which emanate from a variety of combustion sources. They were present as impurities in early formulations of chlorophenolic herbicides such as 2,4,5-trichlorophenol (Cleveland and others, 1982), which were heavily used in the past. Analysis of samples from the LMS sub-basin and MSE Study Unit for chlorinated dibenzodioxins and dibenzofurans by high-resolution analytical methods failed to detect sufficient concentrations of these compounds to account for the TCDD-EQ in all samples (USGS, Columbia Environmental Research Center, unpublished data), and ongoing research seeks to determine whether other dioxin-like contaminants are present in these samples. Regardless, this screening approach was highly successful and cost-effective; we identified a few sites for further, more detailed studies without having had to incur the larger expense of analyzing samples from all sites for individual polyhalogenated hydrocarbons by instrumental methods.

SUMMARY AND CONCLUSIONS

Our study, as a pilot for a contaminants monitoring program, was designed to characterize specific sites in the MRB with respect to contaminants and their potential effects on fish; it was not designed to find or characterize contaminant or fish health problem spots, nor was it intended to provide an unbiased characterization of the MRB. We suspected based on recent studies (Goodbred and others, 1997; Schmitt and others, 1999b) that concentrations of bioaccumulative contaminants would be low, and that biomarker results indicative of exposure to high concentrations of contaminants were not likely to be widespread. Our expectation was that we would find low contaminant concentrations at most sites and correspondingly few sites where biomarker results would indicate problematic exposure to chemicals (that is, anomalous biomarker results). In general, these expectations were met; nevertheless, we also found that

contaminants and their effects on fish remain evident at some sites and in some parts of the MRB. Although organochlorine and inorganic contaminant concentrations in fish were generally low relative to historical levels at most sites, these chemicals remained present at concentrations representing threats to piscivorous wildlife in some locations. In particular, toxaphene and DDT concentrations remained elevated in fish from the cotton-growing regions of the lower Mississippi River valley, and were generally greater in the smaller streams draining agricultural areas than at most of the large river sites. The same was generally true for cyclodiene pesticides in the mid-MRB. Although not designed to find or characterize "hot spots", former point-sources of organochlorine pesticides remained evident, most notably in the Mississippi River near Memphis, TN. PCB concentrations tended to be greatest where they were historically, in the industrialized and urbanized OHR and UMS sub-basins, and were generally correlated with TCDD-EQ and EROD activity and with several other biomarkers. Conversely, PCB concentrations were very low in the more agricultural sub-basins.

Except for Hg and Se, concentrations of inorganic contaminants were relatively low and stable or declining relative to past levels at most sites. Concentrations of Hg were slightly elevated in bass from the Mississippi River at Memphis and several other sites and in carp from one MSE site. Concentrations of Se in fish also remained great enough to constitute a hazard to piscivorous wildlife at several stations in the western parts of the MRB. Levels were especially high at John Martin Reservoir, CO, where elevated concentrations had been reported previously.

In general, and as indicated by Table 5-1, the concentrations of bioaccumulative contaminants were a greater risk to higher trophic level organisms (that is, fish-eating wildlife) than to the fish themselves. With the exception of TCDD-EQ and Se, which exceeded thresholds for early life-stage mortality in fish at several stations, no individual contaminants exceeded known levels associated with impaired health in fish. In contrast, thresholds for dietary exposure of wildlife were approached or exceeded at a number of sites, especially in the LMS sub-basin and MSE Study Unit. The chemical findings were supported by the biomarker results, which indicated that most fish from the MRB were generally healthy; that is, we found no evidence of tumors or other conditions indicating that the fish had been exposed to high concentrations of chemical contaminants. Nevertheless, fish from many sites had external lesions of various types, and at sites where the contaminant burdens of the fish were high, one or more biomarkers were consistent with chemical exposure.

Such findings at sites where accumulative contaminant burdens were low suggest that the fish were exposed to PAHs, hydrophylic pesticides such as herbicides and organo-phosphate and carbamate insecticides, sewage, or other substances not measured.

As monitoring methods, the utility of several of the biomarkers we evaluated are unclear. The causes and significance of vtg in male fish have been questioned, and the apparent need for reference sites and high degree of variability inherent in hormone concentrations and ratios suggest caution in the interpretation of these biomarkers when applied in a program such as BEST. Some of the biomarkers may be better indicators of reproductive health and condition during the spawning season, which is generally the time shunned for the measurement of chemical concentrations and organo-somatic and ponderal indices because of variability introduced by the maturation of the gonads and movement of the fish. Alternatively, and as recommended by Goodbred and others (1997), measurement of the biomarkers over the entire reproductive cycle at a site may be necessary for their full interpretation.

Despite the problems outlined in this chapter and elsewhere in this report, some of the geographic differences and correlations noted for the biomarkers support the contention that MRB fish were exposed to contaminants. Although other factors may have been involved, the enlarged spleens and prevalence of external lesions at some sites suggest that the fish were diseased, possibly as a consequence of immune suppression caused by chemical exposure (Anderson and others, 1989; Hutchinson and Manning, 1996). However, at no sites were MA parameters elevated to the extent previously associated with contaminated sites in marine fish (Fournie and others, 1996). Conversely, the relatively small spleens of fish from several sites is a condition that has been associated with exposure to a number of different chemicals (Schmitt and Dethloff, 2000). A few male bass with ovotestes were found at many sites, but intersex bass were prevalent at only one site (Mississippi R. at Lake Pepin). A 2001 follow-up study at this site confirmed that the prevalence of intersex male bass persisted (U.S. Geological Survey, Columbia Environmental Research Center, unpublished data). Although many factors may be involved, similar ovotestes have been induced in males of other species exposed to organochlorine pesticides, natural and synthetic estrogens, and sewage in controlled laboratory and field studies (Wester and Canton, 1986; Purdom and others, 1994; Jobling and others, 1995; van Aerle and others, 2001). Moreover, we may have underestimated the incidence of ovotestes by examining only a small proportion of the gonad of each fish. Male bass and carp containing low concentrations of vtg were relatively common, but males with comparatively high vtg concentrations

(that is, levels typical of early- to mid-vitellogenic females) were also collected from several sites. Conversely, at two sites female bass in gonadal stage 2 contained no detectable vtg. Male and female carp and bass with seemingly anomalous hormone ratios ($E/T > 1.0$ in males, < 1.0 in females) were also present at many sites, and at some sites all fish of a given gender were anomalous in this respect. The ovaries of female carp, bass, or both from some sites contained large percentages of atretic eggs, and at a few sites enlarged livers were found in bass. Although it is important to remember that wide variety factors can cause these conditions, all can nevertheless be induced in fish by exposure to contaminants (see reviews in Schmitt and Dethloff, 2000).

Most biomarkers are non-specific with respect to contaminants, so identifying the chemical or chemicals responsible for these conditions is not possible without more focused follow-up studies. Nevertheless, many of the biomarker anomalies we report are consistent with contaminant exposure as demonstrated in either controlled laboratory studies or in field studies at known contaminated sites, which is the accepted definition of a "valid" ecotoxicological method (USDOJ, 1987). Moreover, some biomarkers were correlated with the measured concentrations of one or more contaminants in the fish. Collectively, our findings indicate that fish at some sites in the MRB are exposed to contaminants sufficient to induce biological responses in the fish or in fish-eating wildlife. At some sites, concentrations of contaminants no longer in use increased since last measured in the mid-1980s, indicating that continued monitoring is warranted. Except as noted elsewhere in this report for lysozyme activity and some of the reproductive biomarkers, the suite of methods was satisfactory for documenting exposure of fish to contaminants and highlighting some potentially adverse biological effects of exposure.

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Appendix A. Fishes Collected and Demographics

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INTRODUCTION

Although documenting the distribution, abundance, size, and other attributes of fishes in the rivers and streams of the Mississippi River Basin (MRB) was not an objective of the 1995 study, these factors must be considered in the interpretation of the chemical and biological endpoints measured (Schmitt and Dethloff, 2000). Accordingly, Chapter 1 of this report contains an overview of the fishes collected and summarizes demographic information on the two primary taxa, common carp (carp, *Cyprinus carpio*) and black basses (bass, *Micropterus* spp.). Here, we present more detailed information on the ages, sizes, and sex ratios of the fish, focusing on carp and bass. We also present data for the species not originally targeted for collection; however, data for species that numbered ten or fewer individuals are not presented unless they are

included in discussions of taxon groupings. We present and summarize results by collection location, species, gender, taxon grouping, sub-basin (as described in Chapter 1), and program [National Contaminant Biomonitoring Program (NCBP) or National Water-Quality Assessment Program (NAWQA)]. Raw data can be obtained on the World-wide Web at
<<http://www.cerc.usgs.gov/data/data.htm>>

Collection Strategy

As described in Chapter 1, we sought to collect the same species at each site. In all previous NCBP collections (Schmitt and others, 1999b), the most prevalent bottom-dwelling species at NCBP sites in the MRB was carp, and the most prevalent predator

species was the largemouth bass (*Micropterus salmoides*). In addition, these were the species targeted by NAWQA in its studies of contaminants in fish (Crawford and Luoma, 1993) and of reproductive biomarkers (Goodbred and others, 1997). Consequently, these were the targeted species at NCBP sites (Stations 15-112) and at the reference site (Station 400). If common carp and largemouth bass could not be collected, preferred alternate species were collected [for example, suckers (*Catostomidae*) as bottom-dwellers and another black bass (*Micropterus* sp.) or other species as a predator]. Collection goals at each site were 10 individual fish of each sex of each species (one predator and one bottom-dweller, total $n=40$). More than two species were collected at many NCBP stations due to incomplete quotas for the target predator or bottom-dwelling species. Carp were the only species targeted at the NAWQA sites in the Mississippi Embayment (MSE) and Eastern Iowa Basins (EIB) Study Units (Stations 201-213) except at Stations 212 (carp and largemouth bass) and 213 (largemouth bass only). Alternate species were not collected at NAWQA sites.

Fish Collection and Processing

Sampling at NCBP and NAWQA sites commenced in late August and was completed in December 1995. The reference site (Station 400) was sampled in October 1996. Fish were collected and processed by 17 field teams, which spent 1-4 d at each station. Three sites had to be sampled more than once to obtain the necessary fish: Station 24 was sampled three times over a 4-wk period; Station 68 was revisited after 3 months; and Station 82 was revisited after 1 wk.

At most sites fish were collected by DC boat electrofishing. At Station 77 fish had to be gill-netted due to high conductivity, but such injurious sampling methods were generally avoided. Fish were held in on-board live wells and transported to on-shore processing sites, where they were usually processed within a few hours of collection. At several stations, fish were held alive overnight in net pens or in tanks containing ambient water because all fish could not be processed on the day of collection. Fish processing procedures are described in Chapter 1.

Statistical Analyses

Descriptive statistics were computed for TL, weight, and age for important species and taxon groupings; for all relevant sub-basins (see Chapter 1, Table 1-1 for sub-basin descriptions); and for NCBP and NAWQA stations collectively. Species were grouped taxonomically for analysis (see Table 1-4). Age data were not available for Station 24. Fish for which only regenerated scales were obtained (100 total: 84 carp, 12

largemouth bass, three smallmouth bass, one spotted bass) were not included in analyses of age data. The main comparisons of interest were between genders within a species, among stations within a species, and among stations for a given gender within a species. For comparisons among stations, a station was only included if three or more members of the designated species were collected there. For comparisons among stations for a given gender, a station was only included if two or more members of the designated gender and taxon were collected there. In situations where these minimal numbers were not met, the data were presented for the station or the station and gender, but direct comparisons were not made. All comparisons were of magnitudes of means. Combination vertical box-scatter plots (S-Plus 2000, MathSoft Inc, Seattle, WA) illustrating the minimum, maximum, median, and 25th and 75th percentile and individual data points were also prepared and examined. For each taxon grouping, the primary comparisons of interest were among-species within the grouping and within each gender. Descriptive statistics for sub-basins and programs (NCBP, NAWQA) were computed only for carp and bass. We computed un-weighted sub-basin and program-level statistics (that is, means of station means based on all stations with carp or bass regardless of the number of individuals) to eliminate bias associated with the variable number of fish at each station. Statistical analyses are described in more detail in Chapter 1.

SIZES, AGES, AND GEOGRAPHIC DISTRIBUTIONS OF FISHES COLLECTED

We collected 1378 fish of 22 species from 48 stations (including Station 400, the reference site; Table 1-4). Of these, the two species targeted at NCBP sites (carp and largemouth bass) accounted for 82% (1130 individuals). With smallmouth bass (the first alternate predator species) included, the three species together accounted for 87% (1202 individuals) of the fish collected. Of the remaining 19 species, each represented <2.5% of the total. Bass (*Micropterus* spp.) and carp together (1224 fish) comprised 89% of the total. Species totals by station and station totals are presented in Table 1-5.

Geographic Distributions of the Collected Species

As noted above and in Chapter 1, the species collected at the greatest number of stations were carp and largemouth bass (Fig. 1-2; Tables 1-4, 1-5). Carp were collected at 46 of the 48 stations (96%); they

were not collected at Station 74, and only largemouth bass were targeted by NAWQA at Station 213. At sites where carp were collected, both males and females were caught at all stations except Station 23, which was represented by only a single male (Table 1-5). Largemouth bass were collected at 25 sites (52%—Stations 15, 23-30, 32, 68, 70-71, 76-83, 112, 212-213, and 400; Fig. 1-3; Table 1-5). The lower number for this targeted predator species resulted partly from the targeted collection of exclusively carp at 11 of the 13 NAWQA sites and partly because largemouth bass are not present at all the MRB sites we sampled. Both male and female largemouth bass were collected at all but three Stations (15, 23, and 32); at these, only females were collected (Table 1-5). Smallmouth bass of both sexes were caught at five sites (10%—Stations 24, 67, 72, 74, and 111; Fig. 1-3; Table 1-5). If the NAWQA sites at which only one species was targeted are eliminated from the total, carp were collected at 35 of 36 stations (97%), largemouth bass at 24 of 36 (67%), and smallmouth bass at five of 36 (14%).

Among the other alternate taxa, spotted bass were collected at six sites (Stations 23, 24, 25, 68, 78, and 83; Fig. 1-3; Table 1-5). Males and females were collected at all of these except Station 78 (one male). White bass were found at Stations 15 (males and females), 68 (females only), and 75 (males and females; Table 1-5). Both male and female white suckers were collected at Station 74 (Table 1-5). Male and female smallmouth buffalo were found at Stations 23 and 68, and goldeye of both sexes were collected at Stations 85 and 86 (Table 1-5). Saugers were collected at Stations 73, 85 (males and females) and 84 (one male). For the species represented by ten or fewer individuals, a few groupings of interest were noted. Brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), and burbot were collected only at Station 84. White crappie (*Poxomis annularis*) and black crappie (*P. nigromaculatus*) were collected together at Station 68, and walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*) were collected together at Station 32.

When grouped by higher-level taxon (Table 1-4), the following basin distributions were found: Carp, northern pike, burbot, catfishes (Ictaluridae), trouts (Salmonidae), and sunfishes (Centrarchidae other than *Micropterus*) were not changed from the distribution of their composite species because the first five contain only one species and the last two each comprise two species that were found together. White basses were collected only at Stations 15, 68, and 75 (corresponding to the distribution of the most widespread member, white bass); both sexes were collected at Stations 15 and 75 but only females were found at Station 68. Suckers were collected at

Stations 23, 24, 68 and 74; both males and females were captured at all stations except Station 24 (males only). *Stizostedion* spp. were obtained from Stations 32, 73, and 85 (males and females) and at Station 84 (one male). Bass were collected from 29 sites (60%—Stations 15, 23-30, 32, 67, 68, 70-72, 74, 76-83, 111-112, 212-213, and 400; Fig. 1-3). Both male and female bass were collected from all Stations except 15 and 32, from which only females were obtained.

Lengths, Weights, and Ages of Carp and Bass

Carp: Carp averaged 499 mm TL (range 297-1614) and 1771 g (range 304-7181). The mean age for all carp was 4.0 y (range 1-10). Females were substantially heavier (mean_F=1911 g; mean_M=1630 g) and slightly longer (mean_F=507 mm; mean_M=491 mm) than males but of similar age (mean_F=4.1 y; mean_M=3.9 y).

We compared station means for carp size and age to each other (Table A-1) and the to the MRB-wide mean (Station 23 was not compared). Mean lengths exceeded the MRB-wide mean at 26 stations; 19 stations, including the reference site, were below (Table 2-4). Mean weights followed a similar pattern—23 stations exceeded the MRB-wide mean and 22 were below (Table 2-4). If the mean weight exceeded the MRB-wide mean for a station, the mean TL also either equaled or exceeded the MRB-wide mean. At the six stations (including the reference site) where carp were smallest (TL) they were also lightest (that is, lowest weight). At Stations 86 and 29, mean weights for carp were low relative to mean TL: At all stations with a mean TL >550 mm mean weights were >2200 g except Station 29 (570 mm, 1861 g); at all stations with a mean TL >525 mm mean weights were >2000 g except Station 85 (543 mm, 1783 g); and at all stations with a mean TL >500 mm mean weights were >1850 g except Station 86 (511 mm, 1374 g). The average age equaled or exceeded the MRB-wide mean at 11 stations and was below the MRB-wide mean at 28 (no data for Stations 24 and 209; calculated ages for Stations 205, 206, 210, and 211 not included—Table 2-4). The six stations with the greatest average ages also exceeded the basin means for TL and weight. Carp from Stations 15 and 82 were much younger, on average, than expected given their relatively large size. In addition to being small, the mean age of carp at Station 400 was slightly below the MRB-wide mean. In terms of rank, mean ages did not generally correspond with mean lengths and weights (Table 2-4).

At the sub-basin level, mean carp TL was greatest in the Ohio River (OHR) sub-basin (538 mm) and least in the MSE Study Unit (475 mm; Table 2-5).

Table A-1. Lengths, weights, and ages of common carp collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (years)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
Arkansas-Red R. 29	F	11	632	473-1614	11	2093	1250-4350	11	7.0	5-9
	M	9	494	432-575	9	1578	1000-2500	9	6.9	5-9
77	F	11	404	342-555	11	928	550-2150	10	3.4	3-4
	M	7	369	347-382	7	663	600-720	7	3.2	3-4
78	F	10	593	470-1064	10	2318	1400-3825	10	6.3	5-8
	M	9	485	433-530	9	1500	1000-2175	9	4.8	4-6
79	F	10	490	420-580	10	1603	1000-2550	10	6.0	4-8
	M	10	435	356-470	10	1153	900-1700	10	4.7	4-7
82	F	14	632	535-730	14	3243	1800-4600	14	2.1	1-4
	M	11	576	490-670	11	2373	1550-3700	11	2.1	1-3
Lower Missouri R. 31	F	12	409	345-620	12	1123	600-3500	12	2.8	2-5
	M	11	472	352-680	11	1655	600-3450	11	3.9	2-7
83	F	6	494	375-640	6	1758	650-4000	6	3.8	2-7
	M	9	524	346-610	9	2078	550-3050	9	4.8	1-7
86	F	10	420	316-588	10	1078	400-3600	10	2.5	1-5
	M	10	594	319-1489	10	1670	325-2775	10	4.1	2-6
89	F	2	487	462-512	2	1700	1350-2050	2	5.0	5-5
	M	7	429	343-543	7	1143	550-1850	7	3.4	2-6
90	F	10	619	533-752	10	3238	2075-5450	10	5.8	3-8
	M	10	573	460-621	10	2438	1200-2950	10	5.0	3-8

Table A-1. Lengths, weights, and ages of common carp collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations--Continued.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (years)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
Upper Missouri R.	F	9	505	409-565	9	1770	1050-2500	9	4.6	3-6
	M	11	489	299-595	11	1625	420-3400	11	4.8	3-8
	F	12	572	474-681	12	2603	1400-3900	12	3.9	2-6
	M	8	529	470-585	8	1775	1250-2600	8	3.0	3-3
	F	12	479	328-672	12	1538	450-3850	4	3.5	3-5
	M	8	551	390-619	8	2150	800-2800	1	*	
Lower Mississippi R.	F	8	578	350-720	8	3710	720-6602	8	2.1	1-3
	M	2	488	375-600	2	2132	938-3325	2	3.0	3-3
	F	9	525	473-570	9	2026	1480-2792	8	3.2	2-4
	M	10	545	330-700	10	2016	643-4200	10	3.4	1-8
	F	7	599	540-700	7	3241	2052-4690	4	5.3	3-9
	M	10	566	430-674	10	2773	1147-4996	7	3.6	3-4
	F	10	375	344-415	10	663	304-990	10	3.7	3-5
	M	10	401	300-550	10	918	339-2009	10	3.7	3-5
	F	9	451	379-504	9	1454	799-2200	9	2.3	2-3
	M	8	445	370-498	8	1292	711-1778	8	1.8	1-2
	F	5	609	497-742	5	3605	2071-6200	3	4.0	4-4
	M	7	527	297-657	7	2289	407-3693	3	3.3	2-4
	F	8	531	435-750	8	2129	1097-5000	8	2.7	1-4
	M	4	554	473-651	4	2192	1410-2950	4	2.3	2-3

Table A-1. Lengths, weights, and ages of common carp collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations--Continued.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (years)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
Upper Mississippi R. 26	F	10	421	366-529	10	1053	616-1864	10	4.0	2-5
	M	10	405	376-441	10	806	629-961	10	3.5	3-4
27	F	10	483	441-513	10	1595	1230-1895	10	2.6	2-3
	M	10	458	395-496	10	1277	862-1672	10	2.5	2-4
72	F	10	552	508-600	10	2255	1900-3000	10	7.6	7-8
	M	12	519	468-578	12	1817	1400-2400	11	6.9	6-8
73	F	10	538	462-605	10	2164	1310-2900	10	4.6	2-6
	M	10	460	360-528	10	1269	566-1760	10	3.2	2-4
111	F	10	531	439-600	10	1975	1100-2800	10	3.1	2-4
	M	10	504	470-536	10	1975	1450-4600	10	3.5	3-4
112	F	10	510	340-560	10	1964	555-2500	10	3.4	3-4
	M	10	510	470-570	10	1827	1396-2900	10	3.1	2-4
Ohio R. 23	F	0			0			0		
	M	1	488		1	1500		1	8.0	
24	F	2	623	535-710	2	3225	1800-4650	0		
	M	3	523	479-552	3	1624	1150-1981	0		
25	F	2	545	545-545	2	2184	1968-2400	2	2.0	1-3
	M	2	538	505-570	2	2065	1729-2400	2	2.5	2-3
67	F	6	519	429-660	6	1975	1000-3900	6	7.0	2-10
	M	5	472	373-594	5	1870	1500-2750	5	6.8	5-9

Table A-1. Lengths, weights, and ages of common carp collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations--Continued.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (years)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
68	F	9	562	447-718	9	3008	1800-7181	9	3.5	2-5
	M	8	536	450-592	8	2275	1300-2800	8	3.8	3-6
70	F	5	640	510-736	5	3304	1700-5720	4	6.8	6-8
	M	6	578	515-620	6	2550	1500-3600	6	6.7	5-8
71	F	5	543	455-662	5	2420	1200-3600	5	4.7	4-5
	M	10	505	395-570	10	1745	850-2500	10	3.1	2-6
Eastern Iowa Basin										
205	F	10	422	381-482	10	968	711-1355	10	3.7**	3-4
	M	10	432	385-523	10	1042	717-1768	10	3.3**	3-5
206	F	10	461	360-530	10	1353	629-1970	10	4.2**	3-5
	M	10	470	383-540	10	1412	650-2265	10	4.3**	3-5
209	F	3	645	624-665	3	4166	3751-4700	0		
	M	5	563	508-615	5	2572	1744-3512	0		
210	F	10	500	402-595	10	1928	970-3102	10	4.6**	4-6
	M	10	520	440-615	10	1951	1190-2971	10	4.6**	3-6
211	F	10	490	400-570	10	1534	811-2398	10	4.4**	3-5
	M	10	480	395-550	10	1377	740-1965	10	4.4**	3-7
Mississippi Embayment										
201	F	9	501	430-590	9	1342	792-2432	6	3.2	3-4
	M	8	479	400-580	8	1098	671-1829	6	3.4	3-4

Table A-1. Lengths, weights, and ages of common carp collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations--Continued.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (years)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
202	F	10	445	356-528	10	1157	613-2316	10	3.1	2-4
	M	10	444	383-546	10	1119	685-1977	9	3.2	2-5
203	F	8	405	332-495	8	942	479-1638	5	3.4	3-4
	M	9	401	362-460	10	816	649-1347	9	2.9	2-3
204	F	5	479	404-554	5	1588	868-2380	3	3.0	3-3
	M	10	509	421-555	10	1652	942-2065	8	3.4	3-4
207	F	8	473	340-550	8	1610	523-2278	6	2.7	2-3
	M	10	504	426-570	10	1768	1189-2505	9	3.2	3-4
208	F	10	516	465-600	10	2022	1358-3472	8	3.5	3-4
	M	9	465	391-519	9	1317	882-1758	9	3.1	2-4
212	F	10	528	480-610	10	2150	1519-3474	8	3.9	3-4
	M	10	491	440-560	10	1590	1158-2467	10	3.7	3-4
Reference 400	F	11	406	362-442	11	875	584-1205	11	4.0	3-5
	M	8	389	337-464	8	753	463-1130	8	3.4	2-5

* regenerated scales

** calculated ages

Carp from the reference site (Station 400) were shorter (TL mean=399 mm) than those from the MSE Study Unit, and were also very light (mean weight=824 g). Mean carp weight was greatest in the Lower Mississippi (LMS) sub-basin (2219 g) and least (excluding the reference site) in the MSE Study Unit (1446 g; Table 2-5). Despite the wide size disparity between carp from the MSE Study Unit and LMS sub-basin, fish from both of these areas were comparatively young (MSE mean age=3.3 y, LMS mean=3.1 y). In contrast, carp from the EIB Study Unit were about the same size (mean TL=496 mm, mean weight=1790 g) as those from the Upper Mississippi (UMS) sub-basin (491 mm, 1661 g); however, only estimated ages were available for EIB carp. Mean age at the reference site (3.8 y) was greater than that of the LMS sub-basin and MSE Study Unit but less than the mean age for all other sub-basins; mean age was greatest in the OHR sub-basin (5.2 y). Overall, the mean length of carp from the NCBP sites was 513 mm (range 297-1614), the mean weight was 1920 g (range 304-7181), and the mean age was 4.1 y (range 1-10). Carp from the NAWQA sites were generally somewhat smaller (mean length=486 mm, range 332-665; mean weight=1618 g, range 479-4700) and younger (mean age=3.7 y, range 2-7).

Because many biomarkers are gender-specific, the sizes and ages of male and female carp were evaluated separately for each station (Table A-1). As expected, stations for which the overall means were greatest were generally, but not always, the ones from which the longest and heaviest males and females were captured (Fig. 1-4). Carp were generally long at Station 86 (Table 2-4), but this was due to males; the females were relatively short compared to the males and to females at most other stations (Table A-1; Fig. 1-4). At Stations 73 and 208 the females were relatively heavy whereas the males were lighter than expected given the ranking of the overall station mean compared to the MRB-wide mean whereas at Stations 31, 85, and 86 males were heavier and females lighter than expected (Fig. 1-4; Tables A-1, A-2). At several stations, the mean ages of male and female carp differed by >1 y; females were older than males at Stations 30, 71, and 73 whereas males were older at Stations 31, 83, and 86 (Fig. 1-4; Tables A-1). Carp of both sexes from Stations 15 and 82 were relatively young.

At the sub-basin level, most of the trends evident for carp in general also pertained for each gender. Female carp were largest in the OHR sub-basin (mean TL=572 mm, mean weight=2686 g) and smallest in the MSE Study Unit (478 mm, 1544 g; Table 1-4); they were smaller still at the reference site (406 mm, 875 g). Females from the MSE Study Unit were substantially smaller than females from the LMS NCBP

sites (mean TL=524 mm, mean weight=2404 g), which were among the largest but relatively young (mean age=3.3 y). On average, female carp from the MSE Study Unit were also the youngest (mean age=3.2 y), whereas those from the Arkansas-Red River (ARR) sub-basin were the oldest (mean=5.0 y). Female carp from the EIB Study Unit were about the same size (mean TL=503 mm, mean weight=1990 g) as those from the UMS sub-basin (1834 g); only estimated ages were available for EIB carp, however. The mean age of female carp at the reference site (4.0 y) was greater than that of the LMS sub-basin and the MSE Study Unit, but about the same as that of the Upper Missouri (UMO) and Lower Missouri (LMO) sub-basins. Overall, female carp from NCBP sites were larger (mean length=526 mm, range 316-1614; mean weight=2120 g, range 304-7181 g) and older (mean age=4.2 y, range 1-10) than females collected from the MSE and EIB NAWQA Study Units combined (mean length=491 mm, range 332-365; mean weight=1764 g, range 479-4700; mean age=3.7 y, range 2-6).

For male carp, the trends differed slightly. Mean TL in male carp was greatest in the UMO sub-basin (523 mm) but, like the females, was least in the MSE Study Unit (470 mm; Table 1-4). Also like the females, male carp from the OHR sub-basin were heaviest (mean weight=1947 g), and those from the MSE Study Unit lightest (1337 g; Table 2-5). Males from the MSE Study Unit were smaller than those from NCBP sites in the LMS sub-basin (mean TL=504 mm, mean weight=1944 g). Male carp from the reference site were also small (mean TL=389 mm, mean weight=753 g). In contrast to the female carp, EIB males were slightly larger (mean TL=493 mm, mean weight=1671 g) than those from NCBP sites in the UMS sub-basin (476 mm, 1495 g). Male carp from the OHR sub-basin were the oldest (mean age=5.2 y) whereas those from the LMS sub-basin were the youngest (mean age=3.0 y; Table 1-4). Male carp from the reference site (mean age=3.4 y) were older than those from the LMS sub-basin and the MSE Study Unit but younger than those of any other sub-basin. Like the females, male carp from the NCBP sites were longer (mean TL=502 mm, range 297-489), heavier (mean weight=1748 g, range 325-4996) and older (mean age=4.1 y, range 1-9) than those from the NAWQA sites (mean TL=482 mm, range 362-615; mean weight=1504 g, range 649-3512; mean age=3.7 y, range 2-7).

Black Basses (*Micropterus* spp.): Largemouth bass were characteristically the largest of the *Micropterus* spp. collected. Overall, the mean TL of largemouth bass was 346 mm (range 208-785), the mean weight was 692 g (range 92-2400), and the mean age was 3.1

Table A-2. Ranking of stations by descending mean length, weight, and age of carp. For each variable, means in bold were greater than the MRB-wide mean, those in plain text less were less than the MRB-wide mean, and means separated by hyphens were equal. See Fig. 1-1 and Table 1-1 for station locations.

Length

82, 70, 90, 209, 30, 29, 24, 80, 15, 84, 68, 78, 25, 81, 28, 72, 71-111, 83, 86, 112-210-212, 85, 73-204, 67, 32, 208, 207-201, 211, 27, 206, 79, 76, 202, 89, 31, 205, 26, 203, 400, 77, 75

Weight

15, 209, 30, 70, 82, 90, 80, 68, 84, 24, 81, 25, 28, 72, 111, 71, 83, 210, 78, 67, 112, 212, 29, 85, 73, 207, 32, 208, 204. 211, 27, 206, 76-79, 31, 86, 89, 201, 202, 205, 26, 203, 77, 400, 75

Age

72, 29, 67, 70, 78, 90, 79, 32, 83, 30, 73, 212-400, 26-68-75-80, 89-84, 71-85, 28-77-86-208-201-204-112, 31-111-202, 203, 207, 81-27, 15-25, 82-76

y (range 1-9). Females were, on average, longer (356 mm vs. 337 mm) and heavier (776 g vs. 609 g) but not older (3.1 y vs. 3.0 y) than males. The 1-y-old fish of indeterminate gender (208 mm, 92 g) was the smallest largemouth bass collected. Stations were again compared to each other and the MRB-wide mean (Stations 23 and 32 were not included; stations listed in descending size order). Mean TL exceeded the MRB-wide mean at nine stations (79, 28, 29, 82, 27, 78, 24, 112, and 15) and was less than the MRB-wide mean at thirteen stations (70, 83, 77, 81, 212, 30, 213, 80, 26, 68, 76, 71, and 25) and the reference site (Station 400). Mean weights followed a similar pattern. Largemouth bass from Station 15 averaged slightly longer but were lighter than the MRB-wide mean whereas those from Station 77 were shorter but heavier. For all other stations, largemouth bass that exceeded the MRB-wide mean for TL also exceeded the MRB-wide mean for weight. Mean weight exceeded the MRB-wide mean at nine stations (79, 28, 29, 82, 27, 78, 24, 112, and 15) and was less than the MRB-wide mean at 14 (70, 83, 77, 81, 212, 30, 213, 80, 26, 400, 68, 76, 71, and 25). The mean age for 11 stations (29, 79, 78, 70, 83, 112, 28, 26, 68, 212, and 213) equaled or exceeded the MRB-wide mean; the rest, including Station 400 (mean=2.8 y), were below. In terms of rank, mean age correspond well to mean length and weight at only nine stations (25, 29, 30, 71, 76, 78, 79, 80, and 112).

Largemouth bass were examined by gender at each station (Table A-3). As expected, stations with the longer and heavier female or male largemouth bass were generally, but not exclusively, the 11 stations from which larger fish were collected (Fig. 1-7). For females, the two exceptions were stations with few individuals, as follows: All the females from Station 24 were smaller than the MRB-wide means for TL and weight; the relatively high overall mean TL and

weight of largemouth bass from Station 24 were due to unusually large males (greatest mean TL and weight in the MRB). Conversely, the single female collected at Station 212 was about the same length as the MRB-wide mean; the two males were much smaller and decreased the overall station mean. Males from Stations 70 and 83 were longer and heavier than expected given the overall station means and the MRB-wide mean for males. Age data for males and females followed the pattern for all largemouth bass (Fig. 1-7) with one notable exception: Males from Station 112 were younger than expected given the station rank; they averaged more than a year younger than females from this station. Mean ages of males and females from Station 79 also differed by more than 1 y (Table A-3).

Smallmouth bass were generally the smallest and youngest of the *Micropterus* spp. (Fig. 1-7); they averaged 335 mm (range 244-465), 583 g (range 100-1850), and 2.9 y (range 2-5). Mean TL and weight of females and males were similar (337 mm vs. 331 mm; 580 g vs. 589 g), but females were older (mean age=3.1 y for females, 2.6 y for males). Smallmouth bass were largest at Station 74 (mean TL=386 mm, mean weight=1009 g) followed by Stations 111 (348 mm, 605 g), 24 (326 mm, 538 g), 72 (319 mm, 439 g), and 67 (269 mm, 180 g). Smallmouth bass were oldest at Station 72 (mean age=3.8 y) followed by Stations 67 (3.1 y), 74 (2.6 y), and 111 (2.4 y).

The patterns of mean sizes and ages of female smallmouth bass across stations were similar to that of all smallmouth bass (Fig. 1-7). Females were generally largest at Station 74 (mean TL=398 mm, range=272-444; mean weight=1065 g, range=350-1500) followed by Stations 24 (mean TL=346 mm, range 312-402; mean weight=617 g, range 440-954), 111 (mean TL=345 mm, range 317-397; mean weight=567 g, range 375-800), 72 (mean

Table A-3. Lengths, weights, and ages of largemouth bass collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (y)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
Arkansas-Red R. 29	F	7	411	332-495	7	1161	500-1950	7	5.4	3-8
	M	8	379	354-405	8	800	650-1000	8	5.0	4-8
77	F	8	332	309-348	8	741	600-850	8	2.0	1-3
	M	10	331	295-349	10	759	550-875	10	2.2	2-3
78	F	9	393	280-510	9	1228	350-2400	9	4.0	2-7
	M	8	365	272-457	8	849	300-1500	8	3.9	3-6
79	F	12	419	351-510	12	1110	600-1900	12	5.0	4-6
	M	10	394	313-453	10	944	450-1500	10	3.9	3-5
82	F	13	403	309-540	13	961	366-2100	13	2.5	1-4
	M	13	381	305-470	13	796	373-1467	12	2.5	1-4
Lower Missouri R. 83	F	7	329	296-426	7	593	375-1225	7	3.4	2-7
	M	6	340	287-435	6	704	400-1450	6	4.3	2-7
Upper Missouri R. 32	F	1	350		1	780		1	6.0	
Lower Mississippi R. 15	F	4	358	241-450	4	672	194-1103	4	1.8	1-2
28	F	10	419	305-540	10	1286	391-2400	10	3.4	2-5
	M	10	381	262-785	10	673	200-1662	10	2.9	1-4
30	F	10	333	294-379	10	618	313-1255	10	2.7	2-4
	M	9	313	275-370	9	428	269-655	9	3.1	2-4
76	F	10	308	210-469	10	566	94-1822	10	2.0	1-3

Table A-3. Lengths, weights, and ages of largemouth bass collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations--Continued.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (y)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
80	M	8	285	208-373	8	392	100-802	8	2.3	1-4
	F	2	322	290-354	2	524	344-704	2	2.5	2-3
	M	1	308		1	493		1	2.0	
81	F	17	341	228-462	17	670	140-1493	17	2.1	1-3
	M	7	290	235-372	7	361	149-733	7	1.9	1-3
Upper Mississippi R.										
26	F	10	319	251-408	10	618	228-1173	10	3.0	2-4
	M	10	310	234-404	10	539	192-1119	10	3.3	1-5
27	F	10	398	337-456	10	1102	656-1726	10	3.0	1-5
	M	10	367	310-393	10	817	449-1008	10	2.6	1-4
112	F	10	382	355-420	10	972	682-1272	10	3.7	3-5
	M	10	337	275-387	10	661	323-1030	10	2.6	1-4
Ohio R.										
23	F	1	333		1	500		1	4.0	
24	F	2	330	328-332	2	602	564-640	0		
	M	2	395	379-410	2	945	789-1100	0		
25	F	4	286	231-325	4	295	142-425	4	1.0	1-1
	M	5	284	230-335	5	330	167-525	5	1.6	1-2
68	F	4	297	266-316	4	340	176-450	4	3.0	
	M	5	301	265-318	5	385	258-500	5	3.0	2-4
70	F	9	341	310-377	9	488	343-721	9	3.7	3-5
	M	14	352	249-515	14	637	176-1918	14	4.1	2-9

Table A-3. Lengths, weights, and ages of largemouth bass collected in 1995 (1996 for Station 400). See Fig. 1-1 and Table 1-1 for station locations--Continued.

Sub-basin and Station	Gender	Length (mm)			Weight (g)			Age (y)		
		Number of observations	Mean	Range	Number of observations	Mean	Range	Number of observations	Mean	Range
71	?	1	208		1	92		1	1.0	
	F	3	292	260-355	3	355	232-534	3	2.0	
	M	9	287	233-415	9	356	170-900	9	2.1	1-4
Mississippi Embayment 212	F	1	355		1	651		1	3.0	
	M	2	309	300-317	2	390	372-407	2	3.0	3-3
213	F	4	311	250-400	4	426	191-859	4	2.8	2-4
	M	7	320	260-425	7	429	198-1075	7	3.1	2-5
Reference 400	F	10	308	284-325	10	352	278-418	10	2.7	2-3
	M	10	300	263-330	10	294	214-378	10	2.8	2-4

TL=330 mm, range 276-395; mean weight=480 g, range 259-784), and 67 (mean TL=267 mm, range 244-284; 176 g, range 150-200). The average age was greatest at Station 72 (3.9 y, range 2-5) followed by Stations 67 (all 3.0 y), 74 (2.9 y, range 2-4), and 111 (2.5 y, range 2-3). Male smallmouth bass were also largest at Station 74 (mean TL=369 mm, range 282-465; mean weight=929 g, range 350-1850) followed by Stations 111 (mean TL=350 mm, range 322-387; mean weight=644 g, range 500-900), 72 (mean TL=284 mm, range 245-343; mean weight=315 g, range 172-568), and 67 (mean TL=274 mm, range 244-305; mean weight=190 g, range 100-250; Fig. 2-4). [Note: The single male collected at Station 24 (268 mm, 303 g) was not included in comparisons of males across stations]. Males from Stations 67 (mean age=3.3 y, range 3-4) and 72 (mean age=3.3 years, range 2-4) were older than those from Stations 74 (mean age=2.4 y, range 2-3) and 111 (mean=2.3 y, range=2-3; Fig. 1-7). Overall, greater mean age was not reflected in greater mean size at a station for either male or female smallmouth bass.

Spotted bass were generally intermediate in size and age relative to largemouth and smallmouth bass. They averaged 229-433 mm in TL (overall mean=305 mm), weighed 166-1212 g (mean=426 g), and ranged in age from 1-4 y (mean=2.7 y). Females were, on average, longer (318 mm vs. 290 mm), heavier (488 g vs. 359 g) and older (3.0 y vs. 2.3 y) than males. Spotted bass from Station 23 were shorter than those from Stations 25 and 83 (mean₂₃=294 mm, mean₂₅=320 mm, mean₈₃=302 mm). The pattern for mean weights was the same (mean₂₃=275 g, mean₂₅=523 g, mean₈₃=419 g), but the order of the age means was reversed (mean₂₃=3.8 y, mean₂₅=2.0 y, mean₈₃=2.8 y). Only two spotted bass were collected at Stations 24 (mean TL=320 mm, mean weight=489 g, no age data) and 68 (mean TL=243 mm, mean weight=198 g, mean age=3.0 y). The one spotted bass (male) collected at Station 78 was 329 mm long and weighed 625 g; all collected scales were regenerated. Following the overall pattern for spotted bass, females at Station 23 were shorter (mean TL=292 mm, range 272-315) and lighter (mean=267 g, range 200-300) but older (mean age=3.7 y, range 3-4) than females at Station 25 (mean TL=40 mm, range 253-433; mean weight=654 g, range 188-1212; mean age=2.2 y, range 1-3 y; Fig. 2-4). In contrast, males from Stations 25 and 83 did not follow the size pattern for all spotted bass (Fig. 2-4). Males at Stations 25 and 83 were similar in length (mean₂₅=287 mm, range 257-320; mean₈₃=293 mm, range 277-311) but, on average, those from Station 25 were lighter (mean₂₅=304 g, range 192-462; mean₈₃=392 g, range 300-450). Males from Station 25 were also younger

(mean age=1.7 y, range 1-2) than males from Station 83 (mean=2.3 y, range=2-3; Fig. 2-4).

With all *Micropterus* spp. considered together, bass averaged 343 mm TL, weighed 663 g and were 3.0 y old. Largemouth bass and spotted bass were collected together at Stations 23 (one largemouth bass), 25, 68, 78 (one spotted bass), and 83 (Table 1-5, Fig. 1-7). At Station 83, the largemouth bass were, on average, longer, heavier, and older than the spotted bass. At Station 68, largemouth bass were longer and heavier, but they were the same age. Only at Station 25 were the spotted bass larger and older than the largemouth bass collected. A largemouth bass-spotted bass hybrid, collected at Station 78, was larger and older than representatives of both parent species at the station. Male largemouth bass from Station 83 were larger and older than male spotted bass (only one female spotted bass was collected). At Station 25, both genders followed the overall trends in size and age except for weights; male largemouth bass were heavier than male spotted bass. At Station 68 only one spotted bass of each gender was collected. Largemouth, smallmouth and spotted bass were captured at Station 24, where largemouth bass were, on average, larger than smallmouth bass and smallmouth bass were larger than spotted bass. This size relationship also held for comparisons of males and females across species. Females of the three species were, on average, about the same age (no data from Station 24); for males, largemouth bass were slightly older than smallmouth bass, and smallmouth bass were older than spotted bass.

On a sub-basin basis, bass were typically largest and heaviest in the ARR sub-basin (all largemouth; mean TL=381 mm, mean weight=935 g) and smallest in the OHR sub-basin (mixed species; mean TL=305 mm, mean weight=401 g; Table 1-7) owing at least partly to the proportionately large representation of smallmouth and spotted bass in the latter (Fig. 2-2; Table 2-3). Largemouth bass from the MSE NAWQA sites were also small (see below), as were those from the reference site, which were about the same size as the overall means for bass (mixed species) from the OHR sub basin (mean TL=304 mm, mean weight=323 g). Average age was greatest in the UMO sub-basin (6.0 y; one fish from one station) and least in the LMS sub-basin (2.4 y; Table 2-8). Mean age at the reference site (2.8 y) was greater than that of the LMS sub-basin but lower than the mean age for all other sub-basins. Bass from NCBP sites were typically longer (mean TL=342 mm, range 208-785), heavier (mean weight=678 g, range 94-2400), and older (mean age=3.6 y, range 1-9) than those from NAWQA sites (Stations 212 and 213) in the MSE Study Unit (mean TL=320 mm, range 250-425; mean weight=452 g, range 191-1075; mean age=3.0 y, range

2-5).

The size patterns were generally the same for each gender as for bass as a group. Female bass (all largemouth) from the ARR sub-basin were the largest (mean TL=393 mm, mean weight=1043 g) and those from the OHR sub-basin (mixed species; mean TL=307 mm, mean weight=394 g) and the reference site (largemouth, mean TL=308 mm, mean weight=352 g; Table 2-8) were the smallest. The mean age of female bass was greatest in the UMO sub-basin (6.0 y, one fish from one station) and lowest in the LMS sub-basin (2.9 y; Table 1-7). Mean age at the reference site was 2.7 y. Overall, female bass from NCBP stations were longer (mean TL=348 mm, range=210-540), heavier (mean weight=720 g, range 94-2400), and older (mean age=3.6 y, range 1-8) than female bass from the MSE Study Unit (mean TL=333 mm, range 250-400; mean weight=538 g, range 191-859; mean age=2.9 y, range 2-4).

Male bass reflected the same general trends. Male bass from the ARR sub-basin (all largemouth) were largest (mean TL=369 mm, mean weight=824 g) and those from the OHR sub-basin (mixed species; mean TL=303 mm, mean weight=400 g) and at the reference site (all largemouth; mean TL=300 mm, mean weight=294 g) were the smallest (Table 1-7). Also like the females, the mean age of males was greatest in the UMO sub-basin (3.7 y) and lowest in the LMS sub-basin (2.4 y; Table 1-7). Mean age at the reference site (2.8 y) was greater than that of the LMS and UMS sub-basins but lower than that of any other sub-basin. Also like the females, male bass from the NCBP sites were generally longer (mean TL=330 mm, range=208-785) heavier (mean weight=589 g, range=100-1918), and older (mean age=3.0 y, range=1-9) than male bass from NAWQA Stations 212 and 213 (mean TL=314 mm, range=260-425; mean weight=409 g, range=198-1075; mean age=3.1 y, range=2-5).

Lengths, Weights, and Ages of Other Fishes

White Basses (*Morone* spp.): White bass ranged from 235 to 430 mm (mean=322 mm) in TL, from 159 to 1044 g (mean=474 g) in weight, and were 1-6 y old (mean=3.2 y; Fig. A-1). Females, on average, were heavier (mean weight=522 g) and longer (mean TL=330 mm) than males (mean weight=400 g, mean TL=311 mm). Males and females did not differ in mean age (3.2 y), however. White bass from Stations 15, 68, and 75 were of similar mean TL (328 mm, 316 mm, 323 mm, respectively), but those from Station 15 were typically heavier (mean weight=508 g) than those from Stations 68 (438 g) and 75 (470 g). Despite their large size, white bass from Station 15 were younger (mean age=2.1 y) than those from

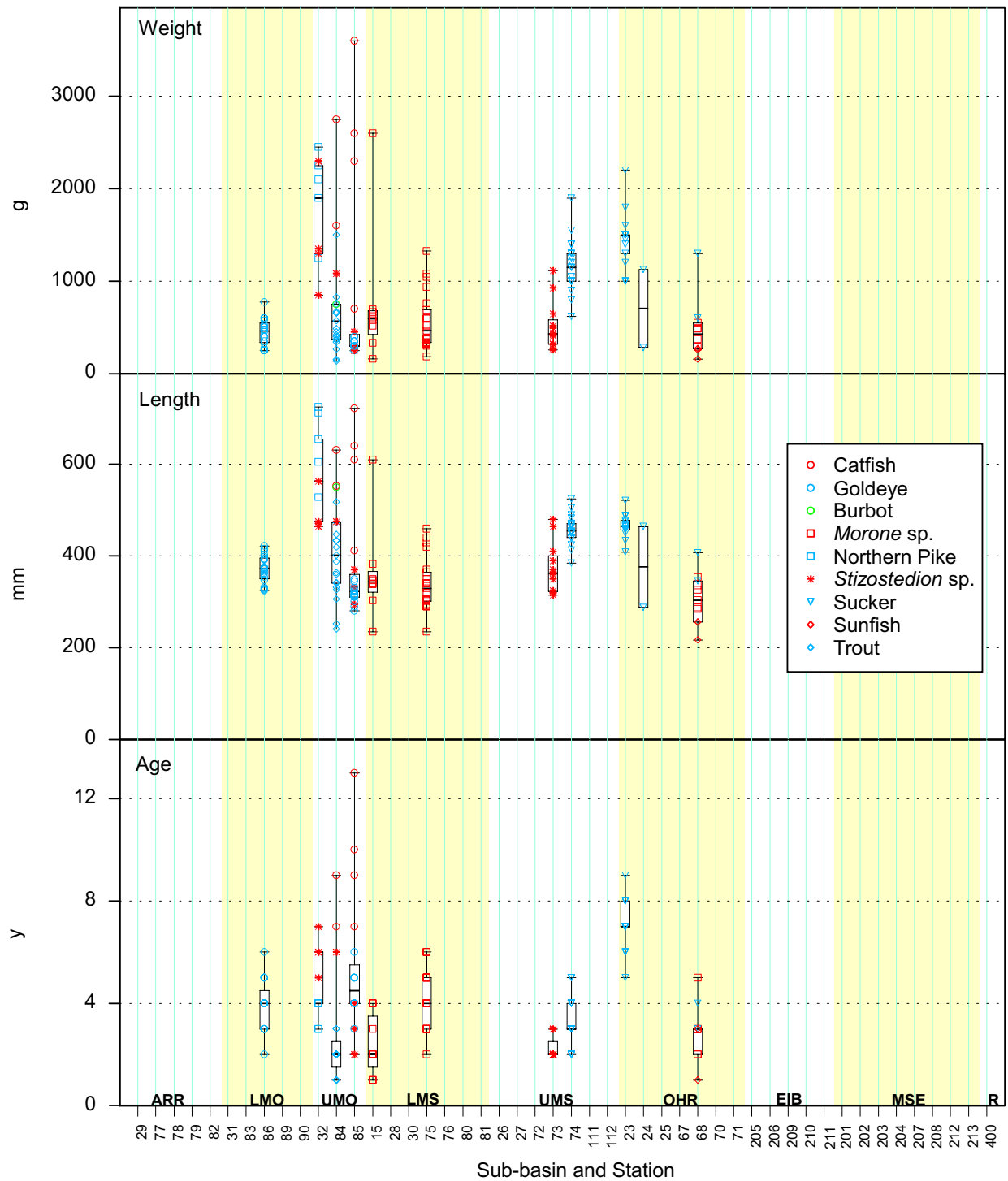


Figure A-1. Weight, total length, and age of fish other than carp and bass collected in 1995 (1996 for Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station and sub-basin locations.

Stations 68 (3.0 y) and 75 (3.7 y). Across stations, the mean TL of females was greatest at Station 15 (342 mm), whereas males were longer at Station 75 (mean=314 mm); no males were collected at Station 68. Females from Station 68 were, on average, lighter (mean weight=438 g, range 293-549) than those from Stations 15 (mean=558 g, range=334-695) and 75 (mean=553 g, range 184-1044). Males from Stations 15 (mean=384 g, range=159-609) and 75 (mean=404 g, range 301-691) were more similar in weight than females. Males from Station 75 were typically younger than females (mean_M=3.5 y, mean_F=3.9 y) but both of these mean ages were greater than those of females from Stations 68 (3.0 y) and 15 (2.4 y), and of males from Station 15 (1.5 y). As with the *Micropterus* spp., a few stations yielded larger, younger fish (Fig. A-1).

Collectively, the *Morone* spp. averaged 341 mm TL, 593 g, and 3.4 y old. The mean length, weight, and age of three female striped bass (*Morone saxatilis*) X white bass hybrids collected at Station 75 were greater than the mean length, weight, and age of all white bass (440 mm vs. 322 mm; 1115 g vs. 474 g; 5.3 y vs. 3.2 y). Female white bass also were smaller (330 mm vs. 440 mm), lighter (522 g vs. 1115 g) and younger (3.2 y vs. 5.3 y) than female hybrids, whereas the single female striped bass collected at Station 15 was larger (610 mm, 2600 g), but not older (4 y). This size distribution is typical of these fishes.

Suckers (Catostomidae): White suckers were collected only at Station 74 (Table 1-5; Fig. A-1). They weighed, on average, 1130 g (range 620-1550), measured 454 mm (range 385-505), and had a mean age of 3.4 y (range 2-5). Females exceeded males in mean TL (457 mm vs. 449 mm) and weight (1175 g vs. 1075 g), but not age (3.2 y vs. 3.5 y). The gender of one white sucker that was not identified in the field could not be determined because no gonadal tissue was obtained for histological analyses. This individual measured 455 mm, weighed 1050 g, and was 4 y old.

Smallmouth buffalo, which were collected only at Stations 23 and 68 (Table 1-5; Fig. A-1), averaged 454 mm in TL (range 346-521), 1390 g (range 600-2200), and 6.6 y old (range 3-9). Mean TL of females, males, and two individuals of unknown gender (again, no gonadal tissue collected for confirmation) were almost identical (mean_F=456 mm, mean_M=450 mm, mean_{UN}=455 mm), but the mean weight of females (1521 g) was greater than that of males (1333 g) and of the unknowns (1100 g); and females were, on average, older (7 y) than males (6.2 y) and unknowns (6.5 y). Of the 15 smallmouth buffalo collected MRB-wide, 13 were captured at Station

23 (Table 1-5; Fig. A-1). The mean TL of Station 23 smallmouth buffalo was 466 mm, the mean weight was 1458 g, and the mean age was 7.1 y. At Station 68, smallmouth buffalo were, on average, 377 mm, 950 g, and 3.5 y old. Males and females were compared across stations (Fig. A-1); the unidentified individuals were not included in these comparisons. Females from Station 23 were, on average, 475 mm long (range 434-521, 1675 g (range 1450-2200), and 7.5 y old (range 6-9). The single female from Station 68 was 346 mm, 600 g, and 4 y old. Males from Station 23 averaged 459 mm TL (range=409-488), weighed 1340 g (range=1000-1500), and were 6.8 y old (range=5-8). The single male from Station 68 was 407 mm long, 1300 g, and 3 y old (Fig. 2-5).

Collectively, the suckers (white sucker, smallmouth buffalo, river redhorse, quillback carpsucker) averaged 451 mm, 1242 g, and 4.9 y. Mean TL of smallmouth buffalo and white sucker was identical (454 mm), whereas river redhorse averaged longer (mean TL=495 mm). The mean weight of river redhorse (two males) was 1513 g, higher than the mean weight of either white sucker (1130 g) or smallmouth buffalo (1390 g). White suckers were, on average, younger (3.4 y) than smallmouth buffalo (6.6 y). Only one river redhorse was aged (5 y, from Station 74); the second (from Station 24) was not aged. The single male quillback carpsucker collected at Station 24 was 288 mm and 283 g. Smallmouth buffalo females were about the same length (mean TL=456 mm) as white sucker females (mean TL=457 mm) but heavier (1175 g vs. 1075 g) and older (7.0 y vs. 3.2 y). Male smallmouth buffalo were also about the same length as white sucker males (buffalo mean=450 mm, white sucker mean=449 mm) but heavier (1333 g vs. 1075 g) and older (6.2 y vs. 3.5 y).

Stizostedion spp.: Sauger from Stations 73, 84, and 85 (Table 1-5, Fig. A-1) weighed 250-1116 g (mean=514 g), measured 295-480 mm (mean=371 mm), and were 2-6 y old (mean=2.6 y). Females and males were of similar length (mean_F=375 mm; mean_M=363 mm), but females were heavier (mean_F=527 g; mean_M=485 g) and males were older (mean_M=3.4 y; mean_F=2.3 y). Sauger from Station 73 were, on average, longer (373 mm vs. 332 mm) and heavier (511 g vs. 333 g) but younger (2.3 y vs. 3.0 y) than those from Station 85. Descriptive statistics were computed separately for male and female sauger from Stations 73 and 85 (Fig. A-1). Most of the sauger (10 of 16) from Station 73 were females; they averaged 383 mm (range 320-480), 555 g (range 256-1116), and 2.3 y old (range 2-3). Males from Stations 73 and 85 averaged 320 mm (range 315-325) and 351 mm (range 331-370), respectively and had mean weights of 294 g (range 272-316) and 375 g (range 300-450), and mean age ages of 2.0

y (all) and 3.5 y (range 3-4). The sauger from Station 84 was a 6-y-old male that was 475 mm long and weighed 1085 g. This was the oldest sauger collected and, with the exception of a female at Station 73, the longest and heaviest. The single female captured at Station 85 was the smallest sauger (295 mm; 250 g; 2 y old; Fig. A-1).

Collectively, *Stizostedion* spp. averaged 396 mm, 701 g, and 3.3 y. As expected, the four walleye collected (all from Station 32) were longer (mean_w=494 mm vs. mean_s=371 mm), heavier (mean_w=1450 g vs. mean_s=514 g) and older (mean_w=6 y vs. mean_s=2.6 y) than sauger (Fig. A-1). This trend held for both sexes; female walleye were, on average, longer (517 mm vs. 375 mm), heavier (1800 g vs. 527 g), and older (6.5 years vs. 2.3 years) than female sauger as were males (470 mm vs. 363 mm; 1100 g vs. 485 g; 5.5 y vs. 3.4 y).

Goldeye: Goldeye weighed, on average, 396 g (range 250-775), measured 351 mm (range 281-422), and were 2-6 y old (mean=4.2 y). Females were typically heavier (451 g vs. 338 g), longer (361 mm vs. 340 mm), and older (4.3 y vs. 4.0 y) than males. Goldeye from Station 85 were, on average, lighter and shorter than those from Station 86 (315 g vs. 449 g; 316 mm vs. 371 mm); however, they were slightly older (4.6 y vs. 4.0 y; Fig. A-1). Males from Station 85 were shorter (mean TL=314 mm, range 281-345) and lighter (mean weight=317 g, range 250-400) than males from Station 86 (mean TL=354 mm, range 325-412; mean weight=350 g, range 250-525). They were, however older (mean₈₅=4.5 y, range 3-6; mean₈₆=3.7 y, range 2-5; Fig. A-1). Females followed a similar pattern (mean₈₅=318 mm, range₈₅ 301-350 vs. mean₈₆=392 mm, range₈₆ 371-422; mean₈₅=314 g, range₈₅ 300-400 vs. mean₈₆=548 g, range₈₆ 400-775, mean₈₅=4.4 y, range₈₅ 4-5 vs. mean₈₆=4.2 y, range₈₆ 3-6; Fig. A-1). As we found for other species, a greater mean age at a station did not necessarily correspond to a greater mean size.

General Observations on Variability in Length, Weight, and Age

Length, weight and age data for each species were examined for extremes in variation and overall consistency across stations. Observational comparisons were not made for species that were found at only one station or had less than three stations with more than two individuals (for example white sucker, smallmouth buffalo, sauger, goldeye). Most common carp were 300-750 mm long, with four individuals >1000 mm (Fig. 1-4). When the scatter of points among stations was compared, no notable differences were found. Weight data were more variable than length

data for carp [possibly due to variation in moisture on external surfaces of the fish (Anderson and Gutreuter 1983)], but most fish weighed 500-5000 g; ten individuals weighed 300-500 g; and eight were 5000-7500 g. The variance was noticeably greater for Station 15; conversely, Station 25 had low variation, but no other stations were noteworthy (Fig. 1-4). Carp from most stations were 2-6 or 7 y old; ages were most variable at Stations 67 and 83. Most of the largemouth bass were 200-600 mm long; only two largemouth bass were 600-800 mm (Fig. 1-5). No station had an inordinately high or low variation in TL. Weights were also more variable than lengths for largemouth bass. Most largemouth bass were 100-1500 g, with the greatest variation in weight at Stations 28 and 78. Most largemouth bass were 2-6 y old. Variation in the age data was relatively consistent. Variation was low at Stations 77 and 212, but only three bass were collected at Station 212 (Table 1-5). For smallmouth bass, only the weights for Station 74 seemed to vary more than average, as did weights of spotted bass from Station 25 (Fig. 1-5). The latter was due to two fish that weighed more than 1000 g; all other spotted bass weighed <650 g. For white bass, no outliers or stations with high or low variance were noted (Fig. A-1).

DISCUSSION

A few general observations can be made regarding the demographic data on fish collected from the MRB in 1995. Although we noted differences among stations in the measured morphologic and demographic parameters of each species, TL, weight, and age for each species across stations were generally within well-defined ranges. Fish size and age varied more at some stations than at others, but not at any one station for more than one species. At certain stations, a few relatively large or small individuals contributed to this result. Also, the number of individuals of each species collected at each station was not constant, so some degree of difference in variation among stations was expected.

Females were, on average, longer and heavier than males of all species for which 15 or more individuals were collected. Except for sauger, females were also older; male sauger were, on average, older than females. Generally, mean age and size did not corresponded well across stations for the species we collected in this study. This finding suggests that scales or other appropriate structural components should be collected for aging rather than estimating

ages from fish size, particularly for fish collections covering large areas where conditions affecting growth can differ substantially.

Three or more largemouth bass and carp were collected together at 22 stations (Table 2-3). The ordering of those stations in terms of average size of largemouth bass or carp differed. The four stations at which both smallmouth bass and carp were collected also differed with respect to size trends for each species, as did the largemouth bass, spotted bass, and carp collected together at two stations. The size trends for white bass and carp across three stations also were not similar. Therefore, no station at which multiple species were collected stood out as having consistently small or large fish. These endpoints alone do not suggest further investigation of natural or xenobiotic factors at any station; however, together with other endpoints, this information could suggest or explain potential problems at certain stations (that is, impaired fish health).

In contrast with size, there were parallels in the ordering of stations in terms of mean ages among species. Stations with older largemouth bass often had older carp (relative to the MRB-wide mean for the species), and those with younger largemouth bass frequently had a lower mean age for carp. Similar age trends were found for carp and smallmouth bass, carp and white bass, and carp, spotted bass and largemouth bass. This suggests that the relative age at a station is somewhat consistent across species when compared to other stations in the basin. Such a situation could make it easier to interpret physiological endpoints across stations for which age is a confounding factor.

The reference site (Station 400) yielded largemouth bass and carp that were, on average, smaller than those from most MRB stations sampled. However, the mean ages of both species were similar to the respective MRB-wide means. Stations 24 and 67 were the nearest (geographically) locations to Station 400 from which more than one largemouth bass or carp was collected. Carp from both stations equaled or exceeded the MRB-wide mean for TL and weight. Largemouth bass from Station 24 also exceeded the MRB-wide means. These observations suggest that growth is not slower in this geographic area compared to the rest of the MRB. A mean age was only available for carp from Station 67; the average age for these fish was 6.9 years. Their size was not substantially different relative to carp from other stations with similar-aged fish. Overall, largemouth bass and carp from the reservoir at Kearneysville, WV were younger, on average, than other fish collected in the area and appeared to be growing more slowly compared to fish of similar ages in MRB rivers. The efficacy of the reference site is addressed further in subsequent chapters of this report.

Certain trends in the sub-basin means for TL, weight, and age of carp and bass (all species, male, and female) were notable. Carp from the OHR sub-basin were either greatest or second-greatest relative to other sub-basins in terms of mean TL, weight, and age. Carp from the MSE Study Unit, on the other hand, were among the smallest and youngest; however, carp from the LMS sub-basin, in which the MSE Study Unit is contained, were relatively large on average, but were young (lowest or second lowest mean age). Both carp and largemouth bass from the reference site were, on average, the smallest fish, and were relatively young. The magnitude of the sub-basin means for the length and weight of bass were similarly ordered for the combined sexes, females, and males: ARR > UMS > LMS or LMO (one station) > MSE (two stations) > OHR or reference. The sub-basin means for age were not as consistent although the ARR and LMO sub-basins were always high and the LMS was the lowest for all bass, females, and males. A noticeable trend for both bass and carp was the larger, younger fish at the NCBP sites in the LMS sub-basin. This could be related to high growth rates in warmer parts of the MRB; carp from the MSE Study Unit seemed to grow more slowly, however. Although carp from the OHR sub-basin were comparatively large and old, the sub-basin means for *Micropterus* indicated smaller fish due in part to proportionately large representation of smallmouth and spotted bass. The ARR sub-basin was notable for its comparatively large, old largemouth bass. The ARR means for female carp TL and weight were also in the upper third among sub-basins, but the means for TL and weight were not high when considering male carp or all carp. However, the ARR sub-basin mean for carp age was highest or second highest. Carp and bass from NCBP sites were, on average, longer, heavier, and older than those from NAWQA sites. NCBP sites also had a greater range of data for all measurements.

By collecting other *Micropterus* spp. as alternates to largemouth bass, it was possible to collect this genus at a greater number of sites (60% vs. 52%). The three black basses were not necessarily the same size, but the ages were similar. Size differences can be factored into interpretation of the endpoints, as needed, to determine whether these species yielded equivalent results. If the results of this study show that the collection of different species in this genus is acceptable for the endpoints being monitored, it will allow for a greater number of locations to be sampled.

Relative to the past collections, the 1995 species makeup was identical at most of the 34 NCBP stations sampled (Stations 25, 27-31, 67, 70-73, 79, 82, 86, 89, 90, and 112; Table A-4). Differences were as follows: Carp were collected at Stations 26, 76, 77,

Table A-4. Fishes collected historically at NCBP stations in the MRB. See Fig. 1-1 and Table 1-1 for station locations.

Station Number	Species	<i>n</i> ¹	Year (begin-end)
15	Common carp	6	1969-1979
	Largemouth bass	2	1979-1981
	White bass	2	1984-1986
23	Common carp	7	1969-1976
	Largemouth bass	1	1986
24	Common carp	10	1969-1986
	Largemouth bass	2	1969-1971
25	Common carp	11	1969-1986
	Largemouth bass	7	1969-1980
	Spotted bass	2	1984-1986
26	Common carp	10	1969-1986
27	Common carp	11	1969-1986
	Largemouth bass	10	1969-1986
28	Common carp	10	1969-1984
	Largemouth bass	2	1981-1984
29	Common carp	11	1969-1986
	Largemouth bass	7	1969-1984
30	Common carp	6	1969-1981
	Largemouth bass	2	1979-1986
31	Common carp	11	1969-1986
32	Common carp	5	1969-1986
	Northern pike	1	1979
67	Common carp	6	1970-1986
	Smallmouth bass	5	1972-1986
68	Common carp	10	1970-1986
	Largemouth bass	3	1976-1980
70	Common carp	10	1970-1986
	Largemouth bass	4	1976-1986
71	Common carp	8	1970-1984
	Largemouth bass	6	1970-1980
72	Common carp	9	1970-1986
	Smallmouth bass	8	1970-1986
73	Common carp	10	1970-1986
	Sauger	2	1971-1978

Table A-4. Fishes collected historically at NCBP stations in the MRB. See Fig. 1-1 and Table 1-1 for station locations--Continued.

Station Number	Species	<i>n</i> ¹	Year (begin-end)
74	White sucker	8	1970-1986
75	Common carp White bass	10 1	1970-1986 1980
76	Common carp	5	1970-1984
77	Common carp	6	1970-1986
78	Common carp Largemouth bass	9 5	1970-1986 1970-1984
79	Common carp Largemouth bass	7 5	1970-1986 1970-1984
80	Common carp	6	1970-1979
81	Common carp	2	1973-1974
82	Common carp Largemouth bass	7 7	1970-1986 1970-1984
83	Common carp	5	1970-1974
84	Common carp Brown trout	5 3	1970-1979 1981-1986
85	Common carp Sauger	5 7	1973-1986 1972-1986
86	Common carp Goldeye	8 10	1970-1986 1970-1986
89	Common carp	5	1970-1974
90	Common carp	9	1970-1984
111	Common carp	3	1974-1986
112	Common carp Largemouth	5 2	1976-1986 1976-1986

*n*¹=number of years for which data exists for a species at a designated station.

80, 81, 83 and 111 both historically and in 1995, but the 1995 predator species differed. At Stations 15, 68, 75, 78, and 84, two or three species collected historically were also collected in 1995, but additional species accounted for a small percentage of captured individuals. At Station 23, where carp and largemouth bass had been collected previously, most of the 1995 fish were smallmouth buffalo and spotted bass; only one largemouth bass and one carp were collected. At Station 74, white suckers were collected exclusively in the past, but in 1995 smallmouth bass were also collected. At Station 85, carp and sauger were collected in the past. Although three saugers (and

three channel catfish) were also collected in 1995, most of the 1995 fish from Station 85 were carp and goldeye. Finally, although largemouth bass and carp were collected at Station 24 in past collections and in 1995, these two species accounted for only small percentage of the 1995 fish collected at this station. Overall, these differences are minor; the composition of the 1995 collection is sufficiently consistent relative to past collections to allow for temporal comparisons of chemical concentrations on a species-by-species basis at many sites, as recommended by Schmitt and others (1999b).

Appendix B. Reproductive Biomarkers in Fishes other than Carp and Bass

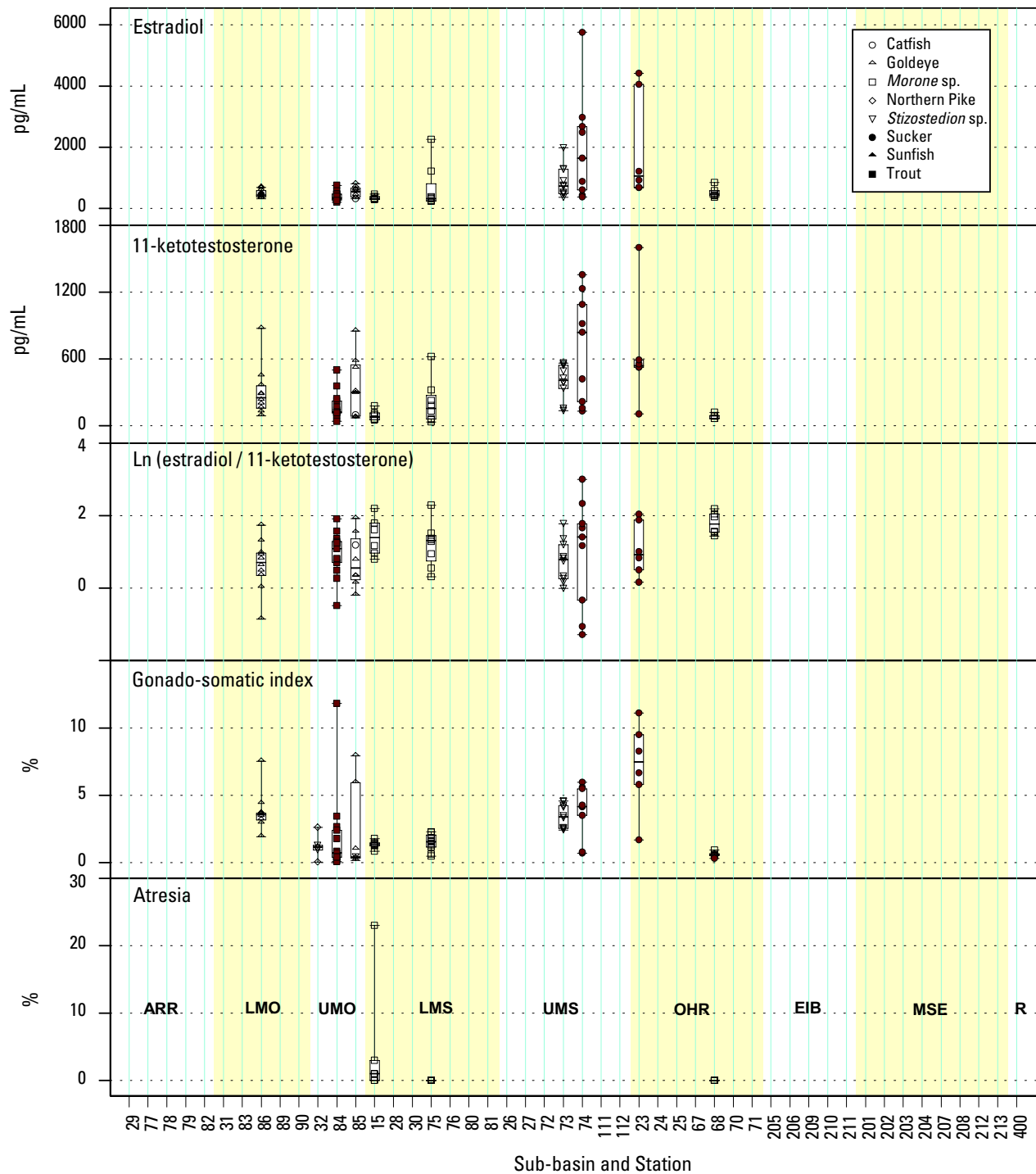


Figure B-1. Reproductive biomarkers in females of species other than bass and carp (all data) by sub-basin and station, for the Mississippi River basin and the reference site (Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

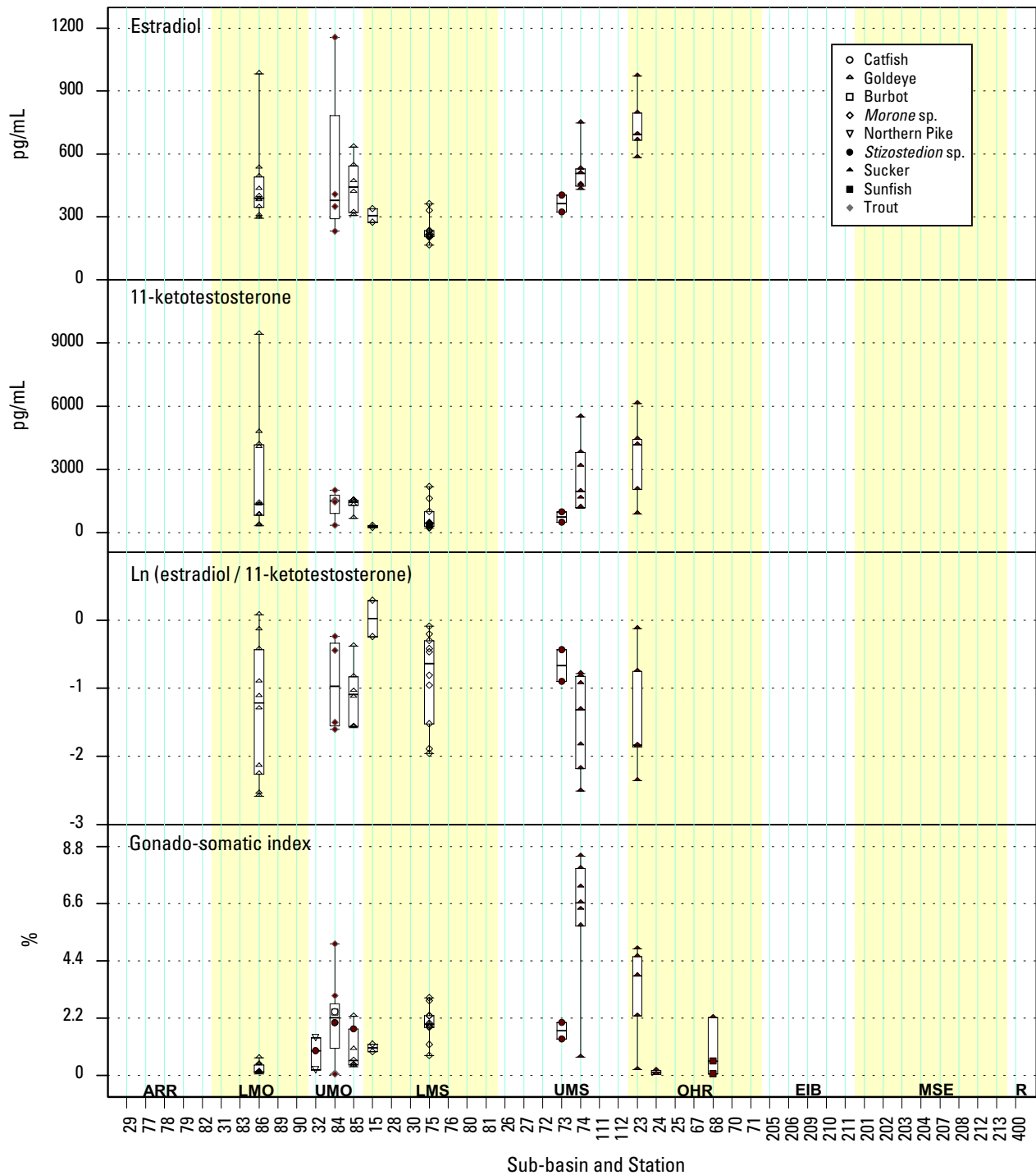


Figure B-2. Reproductive biomarkers in males of species other than bass and carp (all data) by sub-basin and station, for the Mississippi River basin and the reference site (Station 400). Shown for each station are points representing individual fish and the range (vertical line), median (horizontal bar), and interquartile range (box). See Table 1-1 and Figure 1-1 for station locations.

NOTE: The mention of trade names does not constitute endorsement or recommendation for use by the Federal Government.

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13. ABSTRACT (<i>Maximum 200 words</i>) A total of 1378 fish representing 22 species were collected from 47 sites in the Mississippi River basin (MRB) during 1995 and from a reference site in 1996. Common carp (<i>Cyprinus carpio</i>) and black basses (<i>Micropterus</i> spp.) together represented 82% of the fish collected. Each fish was examined in the field for externally and internally visible gross lesions, selected organs were weighed to compute various ponderal and organo-somatic indices, and selected tissues and fluids were obtained and preserved for biomarker analyses. Fish health indicators included splenic macrophage aggregates, lysozyme activity, and histopathological analysis of liver, kidney, and other tissues. Reproductive biomarkers included plasma concentrations of vitellogenin and sex steroid hormones (17 β -estradiol and 11-ketotestosterone) and the histological determination of percent oocyte atresia and gonadal stage. Hepatic ethoxresorufin O-deethylase (EROD) activity was also measured. Composite samples of whole fish from each station were analyzed for organochlorine and elemental contaminants and for dioxin-like activity using the H4IIE rat hepatoma cell bioassay. Organochlorine and inorganic contaminant concentrations in fish were generally low relative to historical levels at most sites, but remained present at concentrations representing threats to piscivorous wildlife in some agricultural areas and near point-sources. Biomarker results indicated that fish from many stations had been exposed to contaminants, but exposure to high concentrations of toxic chemicals was not indicated.			
14. SUBJECT TERMS Contaminants, biomarkers, fish health, organochlorines, DDT, toxaphene, PCBs, chlordane, dieldrin, aromatic hydrocarbons, elemental contaminants, lead, zinc, cadmium, arsenic, selenium, copper, heavy metals, histopathology, atresia, sex steroid hormones, 17 β -estradiol, 11-ketotestosterone, vitellogenin, GSI, HIS, SSI, HAI, EROD, H4IIE, condition factor, lysozyme, macrophage aggregates.		15. NUMBER OF PAGES 217 pages +appendices	
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U.S. Department of the Interior
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As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This responsibility includes fostering the sound use of our lands and water resource; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities.

