Reconnaissance of 17β-Estradiol, 11-Ketotestosterone, Vitellogenin, and Gonad Histopathology in Common Carp of United States Streams: Potential for Contaminant-Induced Endocrine Disruption

By Steven L. Goodbred, Robert J. Gilliom, Timothy S. Gross, Nancy P. Denslow, Wade L. Bryant, *and* Trenton R. Schoeb

---

U.S. GEOLOGICAL SURVEY

Open-File Report 96-627

8056-46

Sacramento, California
1997

U.S. DEPARTMENT OF THE INTERIOR
BRUCE BABBITT, Secretary

U.S. GEOLOGICAL SURVEY
Gordon P. Eaton, Director

For additional information write to:

District Chief
U.S. Geological Survey
Water Resources Division
Placer Hall
6000 J Street
Sacramento, CA 95819-6129

Copies of this report can be purchased from:

U.S. Geological Survey
Information Services
Box 25286
Federal Center
Denver, CO 80225

CONTENTS

FIGURES

TABLES

CONVERSION FACTORS, ABBREVIATIONS, AND ACRONYMS

Multiply	By	To Obtain
centimeter	0.3937	inch
gram	0.03527	ounce, avoirdupois
kilogram	2.205	pound, avoirdupois
square kilometer	0.3861	square mile
liter	33.82	ounce, fluid
milliliter	0.061024	cubic inches
millimeter	0.03937	inch

Temperature is given in degrees Celsius (°C), which can be converted to degrees Fahrenheit (°F) by the following equation: $°F = 1.8(°C) + 32$.

Abbreviations

cm, centimeter
dw, dry weight
g, gram
in., inch
kg, kilogram
km^2, square kilometer
L, liter
mg, milligram
mg/mL, milligram per milliliter
mL, milliliter
mm, millimeter
ng, nanogram
nm, nanometer
pg, picogram
pg/mL, picogram per milliliter
ww, wet weight
µg, microgram
µg/kg, microgram per kilogram
µg/L, microgram per liter
µg/mL, microgram per milliliter
µg/well, microgram per well
µL, microliter
µm, micrometer

Acronyms

ANCOVA, analysis of covariance
ANOVA, analysis of variance
E_2/11-KT, ratio of 17β-estradiol to 11-ketotestosterone
ELISA, Enzyme-Linked Immunosorbent Assay
GIRAS, Geographic Information Retrieval and Analysis System
HSD, Tukey's studentized range test
NAWQA, National Water Quality Assessment
NWQL, National Water Quality Laboratory
PAHs, polycyclic aromatic hydrocarbons
PCBs, polychlorinated biphenyls
RIA, radioimmunoassay
STP, sewage treatment plants
TBST, tris-buffered saline Tween

CONTRIBUTING U.S. GEOLOGICAL SURVEY STAFF

TECHNICAL SUPPORT

Larry R. Brown, Biologist (Fisheries)
James F. Coles, Ecologist
Rod DeWeese, Ecologist
Steven A. Frenzel, Hydrologist (Fisheries)
Jeffery W. Frey, Hydrologist
Robert M. Goldstein, Biologist (Fisheries)
Michael J. Lydy, Ecologist
Bruce J. Moring, Biologist
Thomas A. Muir, Ecologist (Project Manager)
Karen R. Murray, Ecologist
Barbara C. Scudder, Hydrologist/Biologist
Stephen Smith, Ecologist
Stephen K. Sorenson, Biologist
Daniel J. Sullivan, Hydrologist
Cathy M. Tate, Biologist
Ian R. Waite, Biologist
Humbert Zappia, Biologist

TECHNICAL REVIEWERS

Reynaldo Patiño, Assistant Cooperative Leader (Fisheries)
Christopher J. Schmitt, Biologist (Fisheries)

EDITORIAL, GRAPHICS, AND PRODUCTION TEAM

James B. Baker, Editor
Susan G. Davis, Production Editor
Yvonne M. Gobert, Scientific Illustrator
Glenn R. Schwegmann, Technical Editor
Thomas A. Sklarsky, Technical Editor

Reconnaissance of 17β-Estradiol, 11-Ketotestosterone, Vitellogenin, and Gonad Histopathology in Common Carp of United States Streams: Potential for Contaminant-Induced Endocrine Disruption

By Steven L. Goodbred, Robert J. Gilliom, Timothy S. Gross, Nancy P. Denslow, Wade L. Bryant, *and* Trenton R. Schoeb

Abstract

A reconnaissance of sex steroid hormones and other biomarkers in common carp was used to assess whether endocrine disruption may be occurring in fish in United States streams, to evaluate relations between endocrine disruption and contaminant levels, and to determine requirements for further studies. 17β-estradiol, 11-ketotestosterone, vitellogenin, and gonadal histopathology were measured in adult carp (usually 10–15 for each sex) at 25 sites (647 fish), representing a wide range of environmental settings typical of major regions of the nation. Fish were collected during August–December 1994, a period of gonadal maturation after spawning. Contaminants evaluated were organochlorine pesticides and polychlorinated biphenyls in tissue; phthalates, phenols, and polycyclic aromatic hydrocarbons in bed sediment; and dissolved pesticides in water. Mean site concentrations of steroid hormones spanned two orders of magnitude for both sexes. No significant regional differences in steroid hormones were detected for males, but females from the Northern and Southern Midcontinent were significantly different from other regions of the country in one or both hormones. Within all regions there were significant differences between sites in one or both hormones for both sexes. Most correlation coefficients between biomarkers and contaminants were negative. Contaminants that had significant (α=0.05) correlations with biomarkers were organochlorine pesticides, phenols, and dissolved pesticides. The strongest pattern common to both males and females was a negative correlation between the hormone ratio (E_2/11-KT) and dissolved pesticides. The significant site-to-site differences in biomarkers, and the presence of significant correlations between biomarkers and contaminants, are evidence that fish in some streams may be experiencing endocrine disruption. Improved information is needed to evaluate whether endocrine disruption is actually occurring and if there are reproductive effects on individual or populations of carp or other species. Future studies should shift to more intensive study of fewer sites, including reference and contaminated sites, in order to address these additional questions.

INTRODUCTION

Interest in endocrine disruption—the effects of environmental contaminants on the endocrine system of animals—has increased markedly over the past 20 years. McLachlan (1980) initiated a debate on the effects of environmental contaminants on the endocrine system. Based on a recent symposium on endocrine disruption, Colborn and Clement (1992) concluded that "a large number of man-made chemicals that have been released into the environment * * * have the potential to disrupt the endocrine systems of animals including humans." At least 45 chemicals have been identified as potential endocrine-disrupting contaminants (Colborn and others, 1993),

including industrial contaminants (such as dioxins and polychlorinated biphenyls [PCBs]), insecticides (such as DDT and carbaryl), and herbicides (such as dichlorophenoxy acetic acid [2,4-D] and atrazine).

Injury to endocrine function by environmental contaminants is potentially debilitating to a variety of physiological systems. The endocrine system in animals consists of glands that produce hormones that enter the bloodstream to maintain physiological homeostasis. This is accomplished through regulation of immune, metabolic, morphogenic, neural, and reproductive functions. Previous studies have found correlations between specific impairments of reproductive activity and elevated tissue concentrations of xenobiotic agents (Hose and others, 1989 and Tillitt and others, 1992). The reproductive injuries reported to date include reduced fertility, hatchability, and viability of offspring; impaired reproductive hormone activity; and altered sexual development and behavior. There are also reports of slow growth, atrophy, and lower rates of metabolic activity (Colborn and Clement, 1992). Abnormalities of these types may be caused by disruption of normal endocrine function either before or after hormone interactions with specific cellular receptors. Though some cause and effect relations are known, the underlying mechanisms of endocrine disruption are poorly understood (Colborn and Clement, 1992; Bern, 1992; McLachlan and others, 1992). Reproductive impairment often is correlated with altered or decreased circulating concentrations of sex steroid hormones or other critical reproductive factors (Colborn and Clement, 1992; Mayer and others, 1992). The cause-effect linkage between xenobiotic compounds and observed effects is, however, clouded by limited knowledge of the biochemical effects of these compounds in tissues, limited data on exposure to contaminants, and inconsistent data for both contaminants and their effects in terms of analytical methodologies, sampling media, species, age, and sex (Swain and others, 1992).

Studies of aquatic wildlife that are directly exposed to contaminants in our waterways, and thus act as sentinels, often provide early indications of potential environmental problems. Recent studies have found evidence of endocrine disruption in fish from a variety of contaminated ecosystems. Fitzsimmons (1990) reported depressed steroid hormone concentrations in male lake trout (*Salvelinus namaycush*) from Lake Ontario as compared to those from less polluted sites. Systemic concentrations of 11-ketotestosterone, a sex steroid hormone, were inversely proportional to contaminant body burdens. A similar relation between 11-ketotestosterone and contaminant body burden also was reported for coho salmon (*Oncorhynchus kisutch*) by Leatherland (1992). In addition, Munkittrick and others (1992) reported depressed gonadal sex steroids, as well as delayed sexual maturity, reduced gonad size and altered secondary sex characteristics in white suckers (*Catostomus commersoni*) and lake whitefish (*Coregonus clupeaformis*) from Lake Superior in the vicinity of effluents from a bleach kraft pulp mill. Kraft mill effluents also have been reported in association with masculinization of female Poeciliid fish (Davis and Bortone, 1992).

These studies have used sex steroid concentrations as biomarkers of contaminant exposure. Monitoring of sex steroid hormones, such as 17β-estradiol and 11-ketotestosterone in fish, may be useful for the assessment of biological effects and exposure to environmental contamination (Mayer and others, 1992). In addition to sex steroid hormones, vitellogenin, an estrogen-inducible phosphoprotein, also can be used as a biomarker of contaminant exposure in fish and in other oviparous vertebrates. Vitellogenin is normally synthesized by the liver of female oviparous vertebrates during oogenesis (Specker and Sullivan, 1994) and is a precursor of egg yolk. Recent reports have documented vitellogenin synthesis in both male common carp (*Cyprinus carpio*) and male rainbow trout (*Oncorhynchus mykiss*) in streams impacted by sewage effluent (Purdom and others, 1994; Folmar and others, 1996). The use and development of these and other biomarkers of potential endocrine disruption in fish are important for detecting and monitoring potential adverse effects of environmental contaminants on aquatic organisms.

The purpose of this paper is to present results of a reconnaissance of selected biomarkers of potential endocrine disruption in fish from streams throughout the United States. The primary objectives of the study were: (1) to determine if endocrine disruption is potentially widespread in fish of United States streams; (2) to evaluate potential relations between endocrine disruption and contaminant levels; and (3) to aid in determining whether further study of this issue is needed and what type of investigations are likely to be most useful. The study was designed to sample and analyze common carp from streams with a wide range of environmental settings and contaminant levels throughout the United States. Most of the 23 streams and 2 impoundments sampled are established water-quality sites of the National Water Quality Assessment (NAWQA) Program (Gilliom and others, 1995), where levels of a variety of contaminants have been characterized. The study was a collaboration of the

National Biological Service (now the Biological Resources Division of the U.S. Geological Survey), the U.S. Geological Survey, and the University of Florida's Biotechnologies for the Ecological, Evolutionary, and Conservation Sciences Program.

METHODS

The study focuses on measurements of biomarkers for male and female adults of common carp (*Cyprinus carpio*), hereafter referred to as carp, an omnivore that is widely distributed and has been used in other endocrine-disruption studies. Site selection was based on availability of carp and on attaining broad geographic coverage and a representative range of land use and contaminant conditions. Six hundred and forty-seven carp were sampled during 1994 from 25 sites that represent varying degrees of contamination within 11 NAWQA study units (fig. 1). The sampling sites span a wide range of environmental and land-use settings (table 1) that occur in substantial portions of the nation. Land-use calculations were derived from land use- and land-cover digital data in the Geographic Information Retrieval and Analysis System (GIRAS), which are organized by 1:250,000 or 1:100,000-scale quadrangle maps (U.S. Geological Survey, 1990).

Fish Collection, Plasma Sampling, and Age Determination

All fish were sampled with pulsed DC electroshocking between August 29 and December 14, 1994, which is a postspawn period when gonadal recrudescence occurs (Down and others, 1990). Table 2 summarizes the characteristics of fish sampled. Fish were kept alive in a holding net or a live car until processed, which was usually less than 2 hours. The objective was to sample 10–15 fish of each sex at each site, but this was not always obtained. To ensure that adult fish were sampled, the target minimum total lengths were set at 300 mm for males and 375 mm for females (Panek, 1987), and almost all fish sampled met these criteria. Fish were weighed to the nearest 0.1 g and

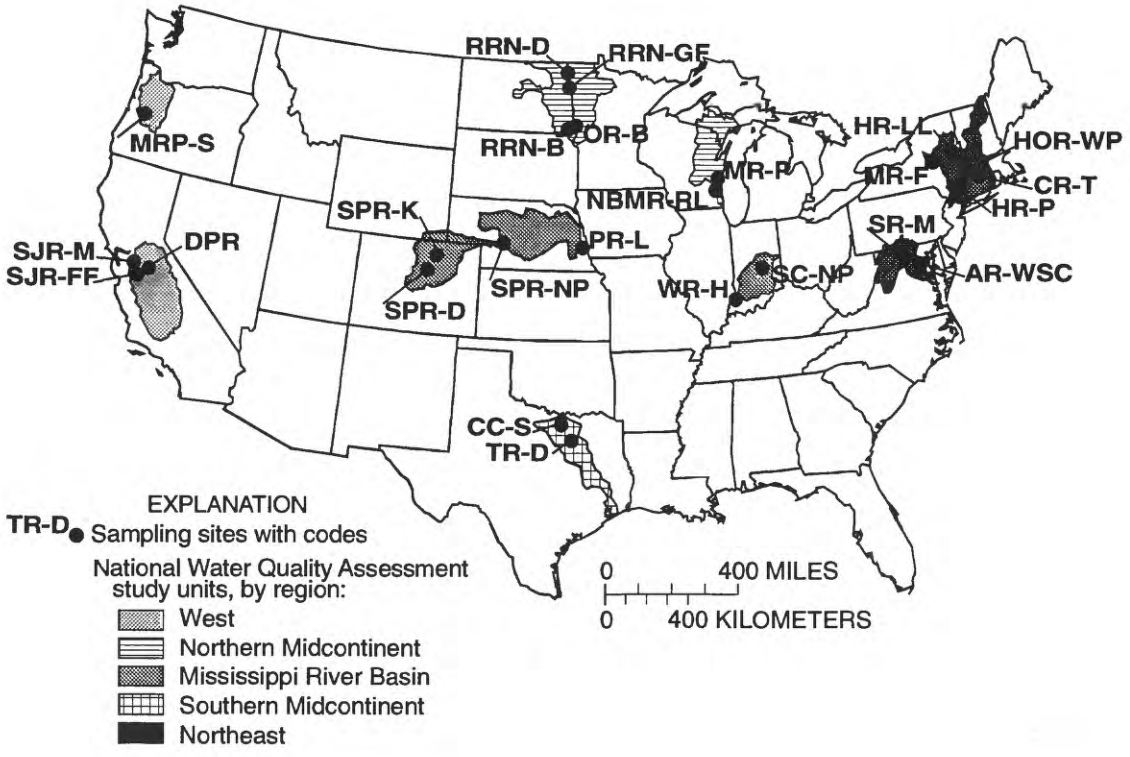

Figure 1. Locations of sites sampled in relation to National Water Quality Assessment study units and regions used for data analysis. Site codes are described in table 1.

Table 1. Study sites and basin land-use characteristics

[* surface inflow to Mill Race Pond is diverted from the Middle Fork Willamette River, which has a drainage area of 3,530 square kilometers (km^2) above the diversion point; land use is 95 percent forested, 1 percent agricultural, and 1 percent urban. The drainage area contributing direct surface runoff to the pond is 13.4 km^2; land use is 13 percent forested, 16 percent agricultural, and 67 percent urban (much of the latter industrial)]

Site name and location	Site code	Drainage basin area (km^2)	Drainage basin land use (percentage of basin)				
			Forest	Range	Cropland and pasture	Orchards and vineyards	Urban
Northeast							
Connecticut River at Thompsonville, Connecticut	CR-T	25,042	80	0	11	0	4
Housatonic River at Woods Pond, Massachusetts	HOR-WP	438	66	0	11	0	17
Hudson River, south of Lake Luzerne, New York	HR-LL	7,028	91	0	1	0	1
Hudson River near Poughkeepsie, New York	HR-P	30,890	63	0	25	0	6
Mohawk River at Frankfort, New York	MR-F	1,620	40	0	44	0	11
Anacostia River at Washington Ship Channel, District of Columbia.	AR-WSC	410	21	0	10	0	66
Shenandoah River at Millville, West Virginia	SR-M	7,853	51	0	40	1	7
Mississippi River Basin							
Platte River at Louisville, Nebraska	PR-L	78,149	0	43	55	0	1
South Platte River at Denver, Colorado	SPR-D	10,009	48	35	6	0	5
South Platte River near Kersey, Colorado	SPR-K	25,144	38	29	21	0	6
South Platte River at North Platte, Nebraska	SPR-NP	62,486	16	41	37	0	3
Sugar Creek at New Palestine, Indiana	SC-NP	246	1	0	95	0	3
White River at Hazleton, Indiana	WR-H	29,290	22	0	69	0	7
Northern Midcontinent							
Mullet River near Plymouth, Wisconsin	MR-P	117	15	0	72	0	3
North Branch Milwaukee River near Random Lake, Wisconsin.	NBMR-RL	138	6	0	87	0	1
Otter Tail River above Breckenridge, Minnesota	OR-B	5,091	20	0	60	0	1
Red River of the North near Breckenridge, Minnesota	RRN-B	10,192	10	0	78	0	1
Red River of the North at Drayton, North Dakota	RRN-D	78,148	8	2	80	0	1
Red River of the North at Grand Forks, North Dakota	RRN-GF	66,501	9	3	78	0	1
Southern Midcontinent							
Clear Creek near Sanger, Texas	CC-S	773	8	38	53	0	0
Trinity River below Dallas, Texas	TR-D	9,372	8	13	62	0	14
West							
Don Pedro Reservoir, California (Tuolumne River)	DPR	3,970	78	12	0	0	2
San Joaquin River at Fremont Ford, California	SJR-FF	1,224	0	2	78	1	1
San Joaquin River at Mossdale, California	SJR-M	18,939	48	20	18	5	2
Mill Race Pond at Springfield, Oregon	MRP-S	*	*	*	*	*	*

measured to the nearest millimeter. Fish scales were collected and external anomalies were noted (Meador and others, 1993). Blood was collected from the caudal vein using a 3 or 5 cc syringe and 20-gauge 1.5 in. (3.8 cm) needle. The sample then was transferred to a heparinized 5 mL vacutainer, chilled on wet ice, and centrifuged in the field for 10 minutes at $1000 \times g$ (where the constant g is acceleration due to gravity). Plasma was pipetted into 2 mL cryotubes, immediately frozen on dry ice, and shipped on dry ice using overnight mail to the laboratory at the University of Florida's Biotechnologies for the Ecological, Evolutionary, and Conservation Sciences Program. All samples were stored at –80°C until analyzed.

Ages were determined from scales taken above the lateral line and slightly anterior to the middle of the fish using methods described in Jearld (1983). The ages of some fish could not be determined because regeneration caused scales to be unreadable. Scales from 10 percent of fish at each site were later reaged to assess variability, with at least 90 percent agreement between measurements.

Table 2. Characteristics of female and male adult carp sampled

[Site codes, with corresponding site names and locations, are listed in table 1. Standard error is the standard deviation divided by $n^{1/2}$. mm, millimeter; g, gram. —, no data]

Site code	Sampling date (month/day/year)	Number of fish sampled	Characteristics of fish [Mean ± standard error (Range)]		
			Total length (mm)	Weight (g)	Age (years)
Northeast					
CR-T	9/13/1994	Females: 9	729±19 (618–810)	6,613±581 (2,423–8,286)	6.3±0.5 (5–8)
		Males: 12	543±42 (339–690)	3,249±640 (601–6,356	4.6±0.4 (2–7)
HOR-WP	9/13/1994	Females: 2	586 (565–606)	2724 (2,270–3,178)	5.5 (5–6)
		Males: 0	—	—	—
HR-LL	9/27/1994	Females: 7	556±32 (487–727)	3,023±592 (1,569–6,200)	4.7±1.2 (2–10)
		Males: 19	557±14 (475–674)	2,628±251 (1,356–5,500)	4.2±0.6 (1–9)
HR-P	9/29/1994	Females: 11	635±35 (513–920)	4,113±757 (2,175–10,100)	5.4±0.7 (3–9)
		Males:18	607±11 (508–673)	3,461±201 (1,946–5,352)	5.1±0.3 (3–7)
MR-F	9/28/1994	Females: 16	561±9 (486–614)	2,774±129 (1,813–3,516)	6.1±0.9 (1–11)
		Males: 24	539±5 (466–576)	2,212±67 (1,582–3,037)	5.7±0.6 (1–9)
AR-WSC	11/18/1994	Females: 10	591±23 (496–692)	3,493±373 2,032–5,477	7.8±1.1 (3–13)
		Males: 3	473±37 (401–521)	1,630±306 (1,073–2,126)	8.0±1.2 (6–10)
SR-M	11/16/1994	Females: 14	629±14 (518–726)	3,740±220 (1,834–5,335)	4.2±0.6 (1–8)
		Males: 3	582±2 (577–585)	2,981±105 (2,777–3,126)	3.0±0 (3–3)
Mississippi River Basin					
PR-L	9/1–2/1994	Females: 13	560±20 (368–662)	2,629±236 (672–3,702)	8.9±0.7 (5–13)
		Males: 13	489±23 (317–586)	1,524±174 (464–2,456)	8.3±0.6 (4–13)
SPR-D	9/12/1994	Females: 21	586±15 (441–675)	3,083±232 (1,200–4,588)	8.1±0.3 (5–10)
		Males: 18	569±11 (496–633)	2,602±141 (1,700–3,700)	8.5±0.7 (2–13)
SPR-K	9/13/1994	Females: 25	533±8 (480–620)	2214±96 (1,559–3,275)	12.6±0.2 (11–14)
		Males: 15	516±11 (440–585)	1922±150 (1,077–3,107)	12.5±0.4 (10–14)
SPR-NP	9/14/1994	Females: 12	521±12 (435–573)	1,872±171 (910–2,709)	9.1±0.6 (7–13)
		Males: 5	500±41 (390–615)	1,609±277 (838–2,152)	10.0±2.0 (8–12)
SC-NP	8/29/1994	Females: 9	627±23 (524–742)	3,582±448 (1,775–5,600)	4.3±0.6 (2–7)
		Males: 7	586±14 (551–665)	2,347±146 (1,797–3,020)	4.2±0.8 (1–6)
WR-H	9/1/1994	Females: 17	569±14 (446–668)	2,722±202 (1,314–4,530)	4.8±0.5 (2–8)
		Males: 19	533±11 (457–610)	1,970±80 (1,389–2,756)	4.3±0.4 (1–7)

Table 2. Characteristics of female and male adult carp sampled—Continued

Site code	Sampling date (month/day/year)	Number of fish sampled	Characteristics of fish [Mean ± standard error (Range)]		
			Total length (mm)	Weight (g)	Age (years)
Northern Midcontinent					
MR-P	9/20/1994	Females: 10	441±10 (398–522)	1,242±135 (926–2,405)	6.6±0.6 (3–9)
		Males: 10	433±8 (405–490)	980±62 (712–1,430)	5.9±0.5 (3–8)
NBMR-RL	9/21/1994	Females: 17	511±15 (402–630)	1,809±161 (892–2,994)	6.8±0.5 (3–10)
		Males: 22	486±9 (414–560)	1,384±75 (868–2,154)	5.8±0.4 (3–10)
OR-B	9/12/1994	Females: 10	521±4 (495–530)	1,819±53 (1,520–2,020)	8.1±0.3 (6–9)
		Males: 10	494±7 (460–530)	1,457±57 (1,253–1,763)	8.5±0.7 (6–12)
RRN-B	9/12/1994	Females: 14	533±9 (485–610)	2,031±112 (1,467–3,164)	9.8±0.5 (7–12)
		Males: 10	505±15 (450–620)	1,604±170 (998–2,918)	8.7±0.3 (7–10)
RRN-D	9/14/1994	Females: 11	714±25 (518–831)	4,686±424 (1,644–6,100)	10.6±0.7 (6–13)
		Males: 10	589±17 (520–675)	2,352±203 (1,645–3,470)	9.5±0.6 (7–14)
RRN-GF	9/15/1994	Females: 10	488±14 (424–534)	1,575±104 (1,182–2,063)	—
		Males: 4	471±15 (436–510)	1,252±83 (1,079–1,470)	—
Southern Midcontinent					
CC-S	12/13/1994	Females: 5	534±18 (472–575)	2,110±153 (1,589–2,423)	7.8±0.2 (7–8)
		Males: 0	—	—	—
TR-D	12/14/1994	Females: 5	626±23 (559–690)	4,116±601 (2,690–5,997)	7.0±1.2 (3–10)
		Males: 2	618 (580–655)	3,148 (3,140–3,155)	8.5 (8–9)
West					
DPR	10/6/1994	Females: 9	490±36 (415–766)	1,714±437 (1,023–5,176)	4.2±0.9 (1–9)
		Males: 12	445±9 (416–526)	1,233±66 (1,009–1,853)	3.3±0.6 (1–7)
SJR-FF	10/5/1994	Females: 17	489±24 (323–625)	1,668±217 (460–3,295	6.2±0.6 (3–13)
		Males: 16	442±28 (302–640)	1,237±201 (334–2,665)	5.8±0.6 (2–10)
SJR-M	10/4/1994	Females: 18	567±25 (429–795)	3,032±425 (1,043–7,355)	4.6±0.5 (2–8)
		Males: 10	542±23 (440–660)	2,437±320 (1,221–3,806)	4.2±0.4 (2–6)
MRP-S	10/20/1994	Females: 9	414±9 (390–482)	934±78 (681–1,476)	2.8±0.2 (2–4)
		Males: 15	380±5 (339–411)	704±27 (454–908)	2.6±0.2 (1–4)

Biomarkers

Analysis of Sex Steroid Hormones

Plasma samples from carp were analyzed for 17β-estradiol, 11-ketotestosterone, and testosterone using radioimmunoassay (RIA) procedures. Methods are described below for 17β-estradiol and 11-ketotestosterone, which were used in data analysis. The method for testosterone is similar. Testosterone was not used in data analysis because it was closely correlated with 11-ketotestosterone (R^2=0.90 for males and 0.81 for females), which is more important for spermatogenesis in males. Samples (50 µL) were extracted twice with 5 mL diethyl ether prior to RIA analysis. Each sample was analyzed in duplicate for both 17β-estradiol and 11-ketotestosterone and corrected for extraction efficiencies of 92 ± 2.8 percent and 86 ± 3.3 percent, respectively. Standard curves were prepared in buffer with known amounts of radioinert 17β-estradiol or 11-ketotestosterone (1, 5, 10, 25, 50, 100, 250, 500, and 1,000 pg). The minimum concentration detectable was 6.4 pg/mL for 17β-estradiol and 8.1 pg/mL for 11-ketotestosterone.

Cross-reactivities of 17β-estradiol antiserum (produced and characterized by Dr. T.S. Gross, University of Florida) with other steroids were as follows: 11.2 percent for estrone, 1.7 percent for estriol, less than 1.0 percent for 17α-estradiol and androstenedione, and less than 0.1 percent for all other steroids examined. Cross-reactivities of the 11-ketotestosterone antiserum (also produced and characterized by Dr. Gross) with other steroids were, 9.7 percent for testosterone, 3.7 percent for α-dihydrotestosterone, less than 1.0 percent for androstenedione, and less than 0.1 percent for all other steroids examined. A pooled sample (approximately 275 pg of 17β-estradiol/mL and 220 pg 11-ketotestosterone/mL) was 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, and the tests for homogeneity of regression indicated 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 17β-estradiol or 11-ketotestosterone in 50 µL of charcoal-stripped plasma. Values of R^2 for correlations between actual and measured amounts were 0.93 for 17β-estradiol and 0.88 for 11-ketotestosterone. Interassay and intra-assay coefficients of variation were 7.3 and 9.5 percent, respectively, for plasma 17β-estradiol; and 9.1 and 8.7 percent, respectively, for plasma 11-ketotestosterone.

Analysis of Vitellogenin

Vitellogenin concentrations in plasma of carp were assayed and quantified by capture ELISA (Enzyme-Linked Immunosorbent Assay) as previously described (Folmar and others, 1996) and summarized below. Initially, vitellogenin from carp was purified by chromatography, its protein concentration was determined by the Bradford assay (Peterson, 1993), and it was used as a standard. The monoclonal antibody, Mab HL 1147 2D3-3A9 (produced by Hybridoma facility, University of Florida and characterized by Dr. N.S. Denslow) was used in the ELISA assay. This antibody reacts specifically, and with high affinity, to carp vitellogenin and not with other plasma proteins.

Purified antibody was diluted to 10 µg/mL in phosphate-buffer saline and coated onto 96-well microtitre plates (50 µg/well), and stored overnight at 4°C. Plates then were washed with tris-buffered saline Tween (TBST), blocked with 360 µL per well of 0.1 percent bovine serum albumin in TBST for 2 hours at room temperature, and thoroughly washed again three times with TBST. Plasma samples were diluted from 1:500 to 1:5,000 in 0.1 percent bovine serum albumin in TBST and 50 µL was added in duplicate to microtitre plate wells and incubated overnight.

Standard curves were constructed by adding serial dilutions of purified carp vitellogenin (0.0001 mg/mL to 0.002 mg/mL) to male control plasma and processed the same way as samples. Male control plasma was made from a pool of plasma from fish collected at an uncontaminated site, which was shown by Western Blot assay to have no vitellogenin. The next day plates were washed with TBST, incubated with 50 µL per well rabbit anti-vitellogenin polyclonal antibody OF114 (produced and characterized by Dr. N.S. Denslow, University of Florida), diluted to 1:500, and incubated for 2 hours at room temperature. This discloses the vitellogenin captured by the monoclonal antibody in the first step. The polyclonal antibody was in turn disclosed by a goat antirabbit immunoglobulin class G, which was diluted 1:1,000, linked to alkaline phosphatase, and incubated for 2 hours at room temperature.

After a final series of washes with TBST, 100 µL of *p*-nitro phenyl phosphate in carbonate buffer (pH 9.6) was added to each well and incubated for 30 minutes. The intensity of yellow color that developed was quantified at 405 nm with an automated ELISA reader. Vitellogenin concentrations were calculated from standard curves after subtracting the small value (around 0.2 $A_{405\ nm}$) of a nonspecific color reaction with male control plasma.

The ELISA assay used in this study can detect between 10 and 100 ng of vitellogenin per well, resulting in a sensitivity of about 0.001 mg/mL. Each ELISA assay included a positive control, which was plasma with a known vitellogenin concentration, to test for interassay and intra-assay variation. The coefficient of variation was calculated for each duplicate sample and, if it exceeded 10 percent, samples were rerun. Standard curves fit by linear regression were used to calculate vitellogenin concentration, with R^2 values usually between 0.95 and 0.99.

Histopathology

Samples of male and female gonads were taken after blood had been sampled, fixed in the field with Bouins' Solution, and transferred to 100-percent ethanol in the laboratory prior to processing. Testes were cut longitudinally and ovaries were cut transversely. Samples were embedded in paraffin, sectioned to 5 µm, and stained with hematoxylin and eosin for histological evaluation. All tissue slides were evaluated by a histopathologist for anomalies.

Gonads of female fish were classified according to four stages of sexual maturation, based on evaluation of histological slides (fig. 2*A,B,C,D*). Ovaries containing mostly perinucleolar oocytes at various stages of previtellogenic growth were classified as undeveloped (stage 0). Ovaries showing a mixture of both perinucleolar and cortical alveoli oocytes were classified as previtellogenic (stage 1). Ovaries classified as early vitellogenic (stage 2) had some vitellogenic oocytes of various sizes and development, with few to moderate numbers of vitelline granules, and no (or only a few) fully developed oocytes. The latest stage of sexual development for females, classified as late vitellogenic (stage 3), had ovaries in which most oocytes were at or near maximum size and contained numerous, densely packed vitelline granules.

Male gonads were classified according to three stages of sexual maturation (fig. 2*E,F,G*). Testes that were classified as early spermatogenic (stage 1) had thick germinal epithelium, with diffuse pronounced proliteration and maturation of spermatozoa. Midspermatogenic (stage 2) testes had germinal epithelium of moderate thickness, with diffuse moderate proliferation and maturation of sperm. Testes classified as late spermatogenic (stage 3) had mostly thin germinal epithelium, with only scattered spermatogenic activity characteristic of full-grown testes and the latest stage of maturity.

Analysis of Hormone and Vitellogenin Data

Values for 17β-estradiol, 11-ketotestosterone, and 17β-estradiol/11-ketotestosterone (E_2/11-KT) in males and females, and for vitellogenin in females, were $\log_{10}$ transformed prior to analysis. Significant differences between biomarkers within and between regions were tested using analysis of covariance (ANCOVA), accounting for age as a potential concomitant variable. When significant differences were found, Tukey's studentized range test (HSD) was used to compare group means. Vitellogenin values in males were ranked, and an analysis of variance (ANOVA) was performed. Tukey's HSD test then was used to compare group means of ranks. The 95 percent confidence level (α=0.05) was used in all tests of significance.

Potential complications to the large-scale reconnaissance approach taken in this study include geographic variation in biomarkers that are associated with stages of sexual maturation and variation because of environmental factors, such as photoperiod and water temperature. To be conservative in defining significant differences in biomarkers between sites, the biomarkers were evaluated and controlled for variation from stages of sexual maturation, geographical region, and age.

Excluding vitellogenin in males, significant differences were detected in all biomarkers measured in both males and females using stage of sexual maturation as the classification variable. Fish with unknown stage of maturation (because gonads were not available) were treated as a distinct category. Age was not a significant factor, and the interaction between age and stage of maturation was not significant for any biomarker tested. The results of Tukey's HSD test for female carp showed no differences between stages 0 and 1 or between stages 2 and 3 and the unknowns, but these two groups were significantly different from each other. Therefore, female carp with ovaries in stages 2 and 3 (vitellogenic) and unknowns were combined for all subsequent analyses. Only 20 female carp in sexual maturation stages 0 and 1 were eliminated. Similar analyses for males showed no significant differences between stages 1 and 2 (early and midspermatogenic) and unknowns, but this group was significantly different from stage 3. Male carp with stages 1, 2, or unknown were combined for further analyses. Forty-nine male carp in the late spermatogenic stage were eliminated.

The potential influence of regionally varying conditions, such as temperature and photoperiod, was

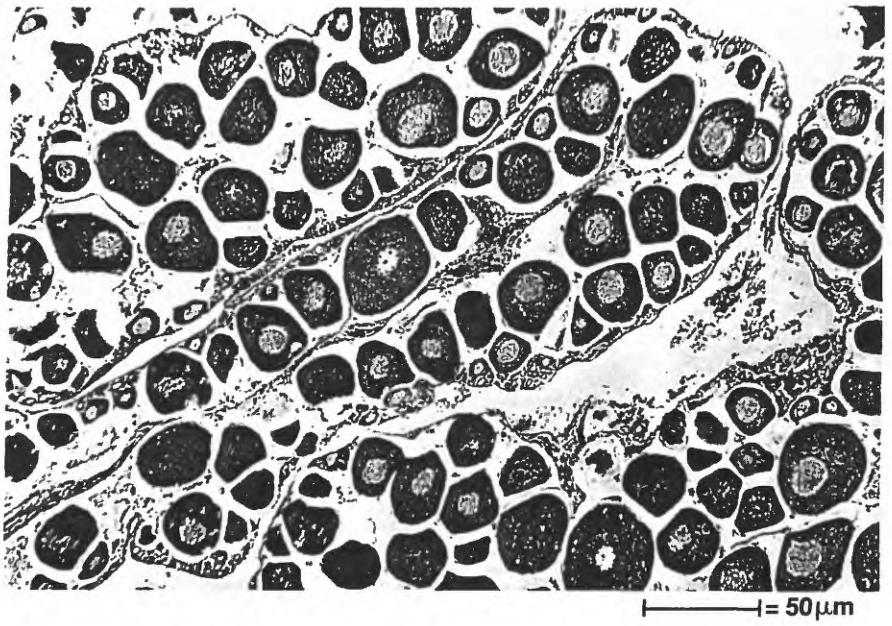

Figure 2*A*. Female adult carp ovary from Platte River at Louisville, Nebraska, with perinucleolar oocytes at various stages of previtellogenic growth. Some larger oocytes show indications of cortical alveoli stage; classified as stage 0, undeveloped. The carp was 8 years old, 368 mm in length, and weighed 672 g.

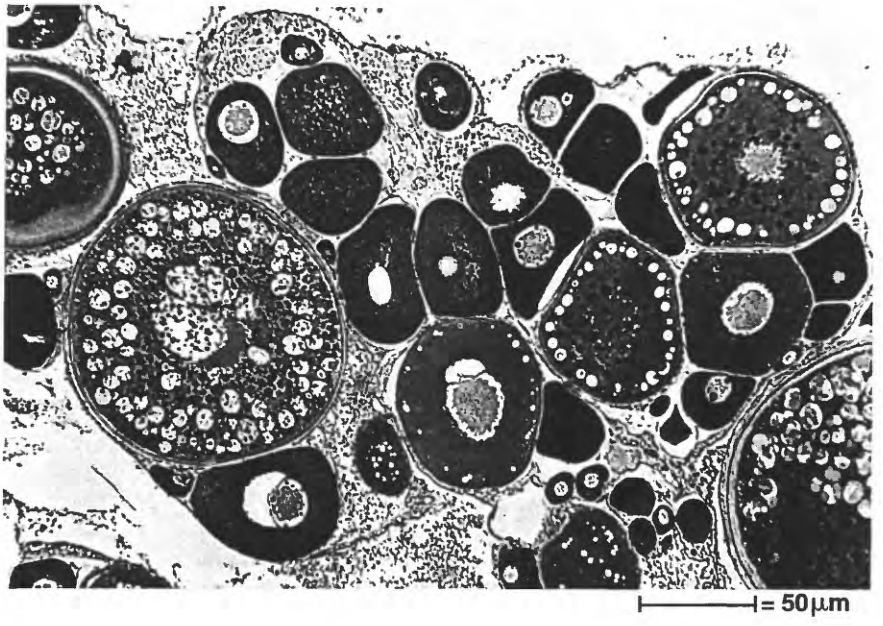

Figure 2*B*. Female adult carp ovary from South Platte River at Denver, Colorado, showing mixture of perinucleolar and cortical alveoli oocytes. Larger oocytes are early vitellogenic; classified as stage 1, previtellogenic. The carp was 7 years old, 604 mm in length, and weighed 2,900 g.

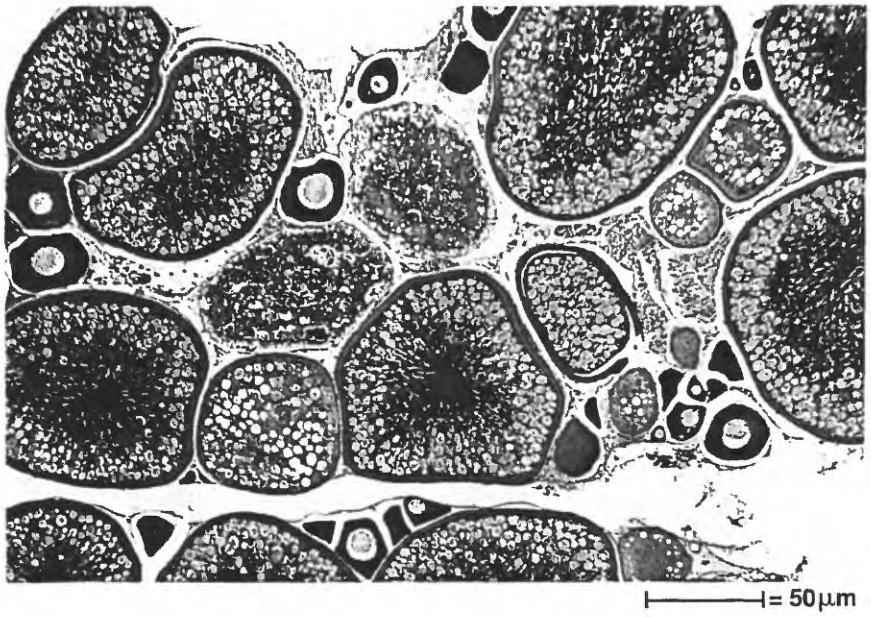

Figure 2*C*. Female adult carp ovary from Platte River at Louisville, Nebraska, containing some vitellogenic oocytes with moderate numbers of vitelline granules and a few perinucleolar and cortical alveoli oocytes. Two atretic oocytes (left and upper center) show granuosa cell hypertrophy; classified as stage 2, early vitellogenic. The carp was an undetermined age, 592 mm in length, and weighed 2,948 g.

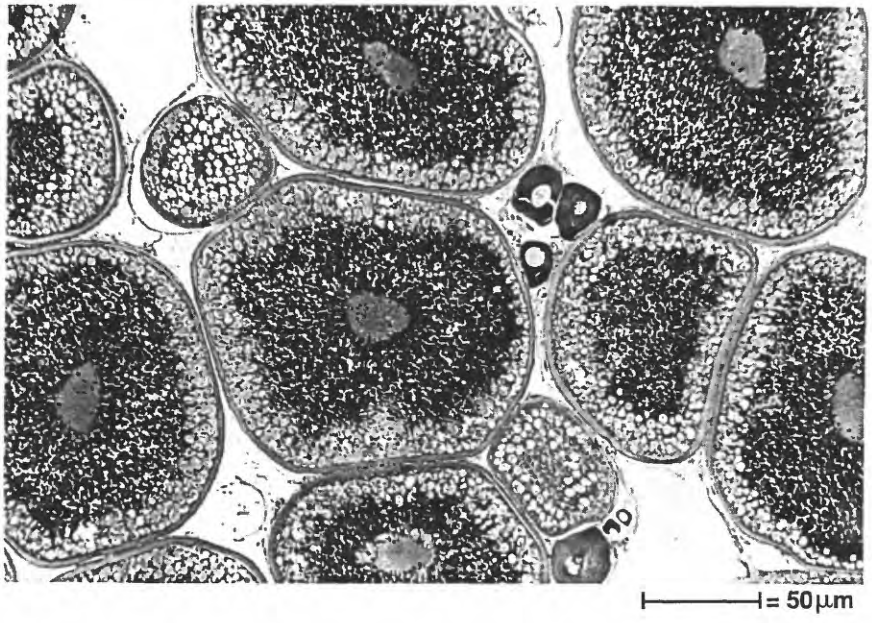

Figure 2*D*. Female adult carp ovary from Hudson River, south of Lake Luzerne, New York, showing fully developed oocytes with numerous vitelline granules along with a few oocytes in earlier stages of development; classified as stage 3, late vitellogenic. The carp was 8 years old, 727 mm in length, and weighed 6,200 g.

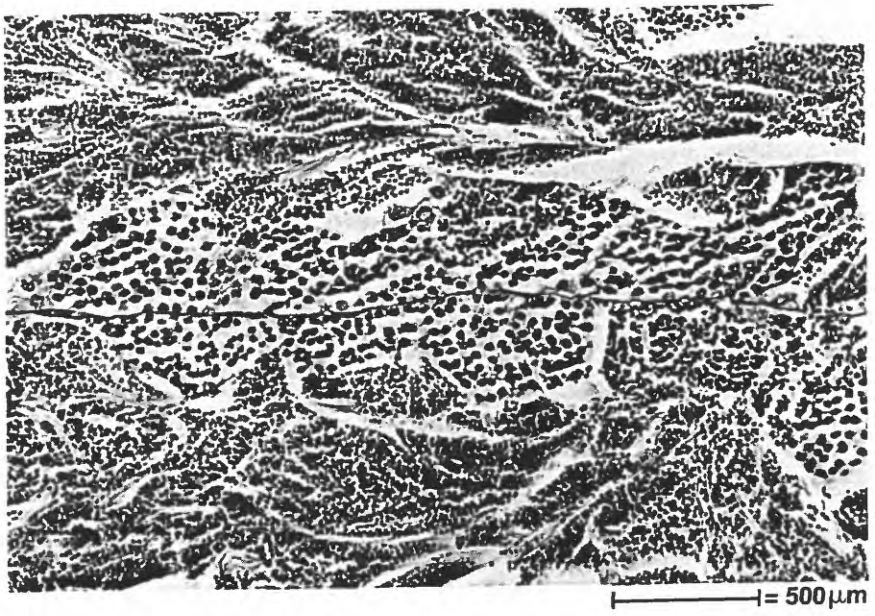

Figure 2*E*. Male adult carp testis from Platte River at Louisville, Nebraska, showing thick germinal epithelium with diffuse pronounced proliferation and maturation of spermatozoa; classified as stage 1, early spermatogenic. The carp was 8 years old, 475 mm in length, and weighed 1,454 g.

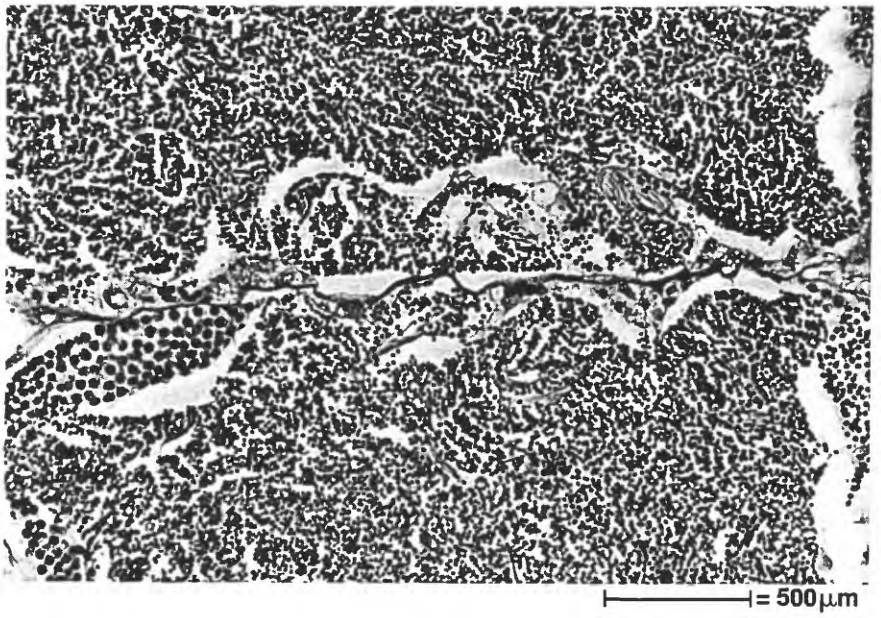

Figure 2*F*. Male adult carp testis from Hudson River, south of Lake Luzerne, New York, showing moderately thick germinal epithelium with diffuse moderate proliferation and maturation of spermatozoa; classified as stage 2, mid-spermatogenic. The carp was 2 years old, 518 mm in length, and weighed 2,334 g.

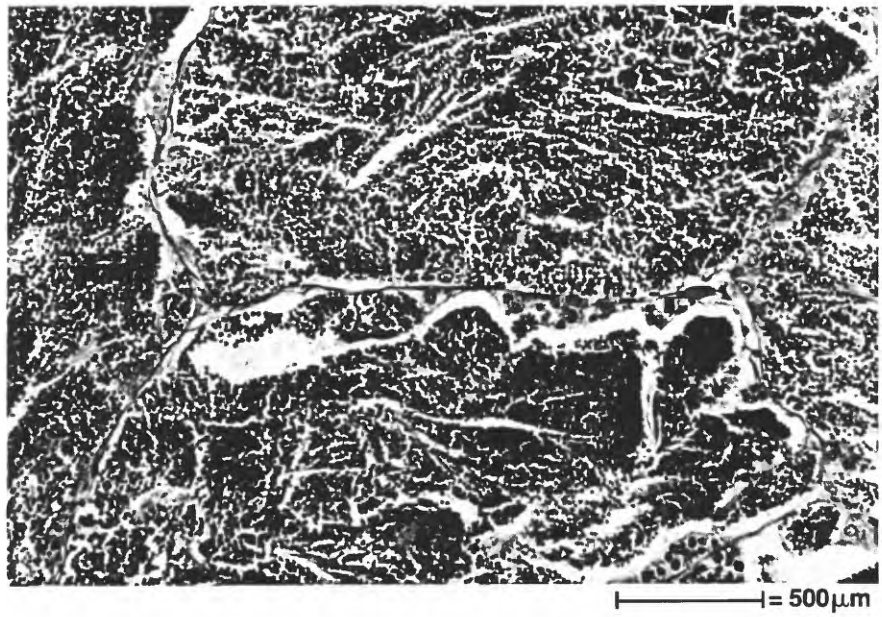

Figure 2G. Male adult carp testis from Anacostia River at Washington Shipping Channel, District of Columbia, where germinal epithelium is mostly thin with only scattered spermatogenic activity characteristic of full-grown testes; classified as state 3, late spermatogenic. The carp was 5 years old, 530 mm in length, and weighed 2,286 g.

investigated in an aggregated manner by grouping sites into major regions (fig. 1) and testing for significant differences in biomarkers between regions. Analysis of covariance showed significant differences between regions by some measures, but not others. There were no consistent patterns in differences of biomarkers among regions. For males, the only significant differences were for E_2/11-KT ratio, with the Northern Midcontinent found different (higher) than the Northeast and Mississippi River Basin regions. For females, excluding the Southern Midcontinent (with only two sites), there were significant differences between regions in 11-ketotestosterone (less in the Northern Midcontinent than in the Mississippi River Basin or the West) and in E_2/11-KT ratio (less in the Mississippi River Basin and West than in the Northeast or the Northern Midcontinent). Because differences were apparent in some of the biomarkers between some regions, and because of the potential for natural regional differences in biomarkers, site-to-site differences were tested only within each region.

Contaminants

The optimal characteristics of contaminant data for an exposure assessment, such as sampling media, timing, frequency, and specific constituents, are difficult to determine and are frequently too expensive to obtain. This is particularly true for a reconnaissance, such as the one described in this report, which needs to cover highly variable site conditions in a large geographic area and characterize exposure to a wide range of contaminants. To meet these objectives, sites were chosen where several aspects of contaminant exposure had already been assessed either at or near the same location as part of National Water Quality Assessment. Contaminant levels were characterized using tissue, bed sediment, and water data collected within 1 to 2 years of the time that fish were sampled for biomarker analysis. This approach results in an approximate and relative indication of recent exposure of fish to environmental contaminants at each site.

Specifically, three primary types of contaminant data were used to characterize exposure: (1) analyses of organochlorine pesticides and PCBs in tissue; (2) analyses of polycyclic aromatic hydrocarbons (PAHs), phenols, and phthalates in bed sediment; and (3) analyses of dissolved pesticides in water. Not all types of data were available for all sites. Though each type of contaminant data was managed somewhat differently before analysis, as described below, the general approach was to attain a balance between logical, relatively homogeneous groupings of contaminants and a small enough number of contaminant parameters to evaluate the 25-site data set. Tables 3, 4, and 5 summarize the individual compounds included in each contaminant group. Generally, there was a high degree of intercorrelation among the most detected individual contaminants within each group.

Table 3. Organochlorine pesticides analyzed in fish tissue samples

[Data reporting limits for most compounds were 5–10 micrograms per kilogram (µg/kg) wet weight for most samples, except for toxaphene, which was 200 µg/kg]

Aldrin	*p,p'*-DDT	Hexachlorobenzene
alpha-Chlordane	Dieldrin	*o,p'*-Methoxychlor
gamma-Chlordane	Endrin	*p,p'*-Methoxychlor
Dacthal (DCPA)	*alpha*-HCH	Mirex
o,p'-DDD	*beta*-HCH	*cis*-Nonachlor
p,p'-DDD	*delta*-HCH	*trans*-Nonachlor
o,p'-DDE	*gamma*-HCH (Lindane)	Oxychlordane
p,p'-DDE	Heptachlor	Pentachloroanisole
o,p'-DDT	Heptachlor epoxide	Toxaphene

This intercorrelation, combined with the small data set and the reconnaissance-level nature of the study design, supported a grouped analysis rather than an approach based on individual contaminants.

Organochlorine Pesticides and PCBs in Tissues

Fish for tissue analysis were collected at 21 sites, and a freshwater clam *(Corbicula fluminea)* was collected at 4 sites. Collections were made during 1992–1995, with most sites sampled during summer or autumn. Methods used for collecting both fish and clams are described by Crawford and Luoma (1993). The five species of fish collected were carp at 15 sites, white sucker (*Catostomus commersoni*) at 3 sites, black redhorse (*Moxostoma duquesnei*) at 1 site, channel catfish (*Ictalurus punctatus*) at 1 site, and largemouth bass (*Micropterus salmoides*) at 1 site. All tissue samples were whole-body composites, with fish having 5 to 10 individuals and clams 50 to 100 individuals. Samples were wrapped in aluminum foil and frozen with dry ice in the field until analysis.

Twenty-seven organochlorine pesticides (table 3), total PCBs, and lipid content were analyzed in 20 tissue samples by the U.S. Geological Survey's National Water Quality Laboratory (NWQL), with five tissue samples analyzed by the Mississippi State Chemical Laboratory, under contract with the U.S. Fish and Wildlife Service. The basic methods used at the NWQL included Soxhlet extraction, gel permetation and adsorption chromatographic fractionation, and analysis by dual capillary-column gas chromatography with electron capture detection. Detailed analytical methods for NWQL tissue analysis are described by Leiker and others (1995).

Methods for analyzing organochlorine pesticides and PCBs in tissue at the Mississippi State Chemical Laboratory included soxhlet extract with hexane for 7 hours, concentration by rotary evaporation dissolution in petroleum ether, and extraction four times with acetonitrile. Residues were partitioned into petroleum ether, washed, concentrated, and transferred to a glass chromatographic column with florisil. The column was eluted with 5 percent diethyl ether and 94 percent petroleum ether into Fraction I, and with 15 percent diethyl ether and 85 percent petroleum ether into Fraction II. Fraction II is concentrated by packed or capillary column and quantified by electron-capture gas chromatography. Fraction I is concentrated and transferred to a silicic and acid chromatographic column to separate PCBs into three fractions and quantified by electron-capture gas chromatography.

Total organochlorine pesticides in tissue from each site were calculated by summing concentrations of all individual analytes listed in table 3, with zero concentration assigned to all nondetections. This approach results in a comparatively low approximation of the actual concentration. To minimize effects of different species, total organochlorine pesticide and PCB values were normalized by dividing the values by the lipid concentration for each composite sample. Both total organochlorine pesticides and PCBs were significantly (α=0.05) correlated with lipid concentration for the complete, multispecies national data set. Except for carp, data on individual species are too limited to individually evaluate correlations with lipid concentration. By dividing each composite sample value by its respective lipid concentration, all species were treated similarly, and the resulting values showed no remaining correlation with lipid concentration. In addition, analysis of correlations between contaminants and biomarkers showed no major differences (based on p values and direction of correlation) between results for lipid-normalized and non-normalized data.

At sites for which tissues were analyzed, but no PCBs or organochlorine pesticides detected, one-half of the lowest detected lipid-normalized value for the respective total was used for further data analyses. This procedure ensured that the few sites with no detections had the lowest lipid-normalized concentrations compared to the other study sites. All values were then $\log_{10}$ transformed for correlation analysis.

Table 4. PAHs, phenols, and phthalates analyzed in bed sediment samples

[The data reporting limit was 50 micrograms per kilogram (µg/kg) dry weight for most compounds in most samples. Compounds for which values were corrected for median concentrations in blanks of 28–35 µg/kg are shown by *]

	PAHs	
Acenaphthene	Chrysene	Isoquinoline
Acenaphthylene	Dibenzo(*ah*)anthracene	2-Methylanthracene
Acridine	Dibenzothiophene	4,5-Methylenephenanthrene
Anthracene	1,2-Dichlorobenzene	1-Methyl-9H-fluorene
Anthraquinone	1,3-Dichlorobenzene	1-Methylphenanthrene
Azobenzene	1,4-Dichlorobenzene	1-Methylpyrene
Benzo(*a*)anthracene	1,2-Dimethylnaphthalene	Naphthalene
Benzo(*c*)cinnoline	1,6-Dimethylnaphthalene	Nitrobenzene
Benzo(*b*)fluoranthene	2,6-Dimethylnaphthalene	N-Nitrosodi-*n*-propylamine
Benzo(*k*)fluoranthene	2,4-Dinitrotoluene	N-Nitrosodiphenylamine
Benzo(*ghi*)perylene	2,6-Dinitrotoluene	Pentachloronitrobenzene
Benzo(*a*)pyrene	2-Ethylnaphthalene	Phenanthrene
2,2'-Biquinoline	Fluoranthene	Phenanthridine
4-Bromophenyl-phenylether	9H-Fluorene	Pyrene
9H-Carbazole	Hexachlorobenzene	Quinoline
bis(2-Chloroethoxy)methane	Indeno(1,2,3-*cd*)-pyrene	1,2,4-Trichlorobenzene
2-Chloronaphthalene	Isophorone	2,3,6-Trimethylnaphthalene
4-Chlorophenyl-phenylether		
	Phenols	
C_8-Alkylphenol	2-Chlorophenol	3,5-Dimethylphenol
4-Chloro-3-methylphenol	*p*-Cresol	Phenol
	Phthalates	
Butylbenzylphthalate*	Dimethylphthalate	D-*n*-butylphthalate*
Diethylphthalate	Di-*n*-octylphthalate	*bis*(2-Ethylthexyl)-phthalate*

Two weaknesses of the tissue contaminant data are that analyses were not made on the same individual fish that were analyzed for biomarkers, and that the samples for contaminant and biomarker analyses were collected during different years for most sites. The relative stability of these contaminants over time, the reduced variability caused by summing of contaminants, and the averaging accomplished by compositing should, however, result in a robust indication of overall site conditions from the available data. Data from 34 river sites of the National Biocontaminant Monitoring Program (U.S. Fish and Wildlife Service, 1992) for 1984 and 1986 indicate that analysis of composite carp samples at a site had an average difference of about 64 percent between years for organochlorine pesticides and about 47 percent for PCBs (for organochlorine pesticides, five sites with extreme percentage changes resulting from a very low value in 1 of the 2 years were eliminated). These levels of interannual variability are relatively low compared to the order of magnitude of differences in contaminant levels among sites, but such levels may be an important source of unexplained variation in data analysis, particularly when comparing sites with similar contaminant concentrations.

PAHs, Phenols, and Phthalates in Bed Sediment

Composite bed sediment samples were collected at 22 sites. Collections were made during 1992–1995, with most sites sampled during summer or autumn. At each site, bed sediment was collected from depositional zones in the stream channel, where recently deposited, fine-grain material accumulates. A Teflon sampler or spoon was used to collect bed sediment from the upper 2 cm in 5 to 10 deposition zones and composited into a glass container. Sediment was sieved through a 2.0 mm stainless-steel sieve, decanted, and frozen prior to analysis. Further details of bed sediment sampling are available in Shelton and Capel (1994).

Fifty-two PAHs, 6 phenols, 6 phthalates, and organic-carbon content were analyzed by the NWQL. PAH, phenol, and phthalate compounds were extracted from the sediment with dichloromethane, followed by partial isolation using high-performance gel

Table 5. Dissolved pesticides analyzed in water samples

[Method detection limits were less than 0.01 micrograms per liter (µg/L) for all compounds except disulfoton, prometon, propargite, and terbufos, which were between 0.01 and 0.02 µg/L]

Amides:			
Alachlor	Napropamide	Propachlor	
Metolachlor	Pronamide	Propanil	
Carbamates:			
Aldicarb	Carbaryl	Methiocarb	Thiobencarb
Aldicarb sulfone	Carbofuran	Pebulate	Triallate
Aldicarb sulfoxide	Carbofuran, 3-Hydroxy	Propham	
Butylate	EPTC	Propoxur	
Dinitroanilines:			
Benfluralin	Oryzalin	Trifluralin	
Ethafluralin	Pendimethalin		
Organochlorines:			
p,p'-DDE	Dieldrin	*alpha*-HCH	*gamma*-HCH
Organophosphates:			
Azinphos-methyl	Dimethoate	Fonofos	Parathion
Chlorpyrifos	Disulfoton	Malathion	Phorate
Diazinon	Ethoprop	Methyl parathion	Terbufos
Pyrethroids:			
Dimethoate	*cis*-Permethrin		
Triazine herbicides:			
Atrazine	Cyanazine	Prometon	
Atrazine, desethyl	Metribuzin	Simazine	
Uracils:			
Terbacil			
Miscellaneous:			
2,6-Diethylaniline	Propargite		

permeation chromatography and elution with dichloromethane. Compounds were then identified and quantified using dual capillary-column gas chromatography with electron-capture detection. Details on methods are reported in Furlong and others (1996).

Total concentrations of PAHs, phenols, and phthalates for each site were calculated by summing concentrations of all individual analytes listed for each group in table 4, with zero concentration assigned to all nondetections. This approach results in a comparatively low approximation of the actual concentration. Total concentration values were normalized by dividing the values by the organic carbon content of the sample to minimize affects of different site sediment characteristics in the results. Concentrations of PAHs, phenols, and phthalates in bed sediment were not significantly correlated with organic carbon concentrations for the complete national data set (21 sites), but p values for the three regressions ranged from 0.15 to 0.26, with positive slope coefficients of 0.14 to 0.17. Removal of two sites (NBMR-RL in the Northern Midcontinent and MRP-S in the West) with particularly high organic carbon levels resulted in a significant correlation between PAHs and organic carbon. Organic carbon normalization of bed-sediment data, similar to lipid normalization, probably results in the most comparable contaminant data possible for all sites. Analysis of relations between contaminants and biomarkers, however, showed no major differences (based on p values and directions of correlation) in results for carbon normalized and non-normalized data.

For any site where bed sediments were analyzed, but where no PAHs, phenols, or phthalates were detected, one half of the lowest detected organic carbon-normalized value for the respective group total was assigned for further data analysis. This procedure ensured that the sites with no detections had the lowest organic-carbon normalized concentrations compared to the other study sites. All values were $\log_{10}$ transformed for correlation analysis.

A potential weakness of the bed sediment contaminant data, as with tissue data, is that most samples were collected during a different year than that of the biomarkers. For reconnaissance purposes, however, the relative stability of these contaminants over time, the summation of contaminants by major groups, and the averaging accomplished by compositing, should result in a robust indication of overall site conditions. Nevertheless, the differences in timing of sample collection may be an important source of unexplained variation in data analysis.

Dissolved Pesticides

At 11 of the study sites, 7 to 34 filtered water samples were collected during 1993–1994, at least monthly from March through September at most sites. Depth-integrated, discharge-weighted water samples were collected and prepared for laboratory analysis of dissolved pesticides as described by Shelton (1994). Fifty-two pesticides (table 5) were analyzed by extracting compounds from filtered water (0.7 µm glass-fiber filter) using a C-18 solid-phase extraction cartridge, eluting the cartridge with hexaneisopropanol, and analyzing by capillary-column gas chromatography with spectrometric detection in the "selected-ion" monitoring mode (Zaugg and others, 1995).

For each site, a time-weighted annual mean concentration of dissolved pesticides was computed by summing all detected pesticides from each sample, weighting each sample total by the number of days it represents within the year, summing the time-weighted concentrations over the year, and dividing by 365 days. Nondetections were treated as zero concentrations, resulting in a comparatively low estimate of the total pesticide concentration. Annual medians, which were considered but not used in final analyses, yielded the same overall findings as time-weighted means. Time-weighted means were $\log_{10}$ transformed for correlation analysis.

The most relevant measure of exposure of fish to dissolved pesticides for assessing potential endocrine disruption is difficult to determine. Dissolved pesticides vary over time within and between seasons in different ways for different chemicals and in different regions. The time-weighted annual mean was chosen as a relatively stable measure of average exposure. One potential weakness of the dissolved pesticide values is that pesticide sampling was done in a different year than when fish were sampled for biomarker analysis. However, year-to-year seasonal patterns and overall levels tend to repeat annually at a site, as shown by data in Richards and Baker (1993) and Coupe and others (1995). Nevertheless, important potential sources of unexplained variation in data analysis are temporal variability and, in particular, the uncertainty in which temporal measure of pesticide concentrations (for example seasonal mean or annual mean) is most relevant to endocrine effects.

RESULTS

Sex Steroid Hormones and Vitellogenin

Mean concentrations and ranges of plasma 17β-estradiol, 11-ketotestosterone, and vitellogenin for male and female carp, are summarized by site and region in table 6. Complete data for all 578 individual fish used in data analysis are listed in Appendixes A and B (back of this report) for males and females, respectively, including testosterone. Considerable variation and ranges are apparent from the summaries in table 6. Nationally, site means of both steroid hormones for male and female fish span more than 2 orders of magnitude. The lowest mean concentration of plasma 17β-estradiol for male carp was 70 pg/mL at the Platte River at Louisville, Nebraska (PR-L), and the lowest concentration for females was 382 pg/mL at Clear Creek near Sanger, Texas (CC-S). The highest mean concentrations of 2,086 and 4,175 pg/mL for male and female, respectively, were both from Don Pedro Reservoir, California (DPR). The lowest mean concentration of 11-ketotestosterone for male carp was 411 pg/mL from the Shenandoah River at Millville, West Virginia (SR-M), and the lowest concentration for females was 119 pg/mL at Clear Creek near Sanger, Texas (CC-S). The highest mean 11-ketotestosterone concentrations of 4,289 and 2,180 pg/mL for male and female, respectively, were both found at Don Pedro Reservoir, California (DPR).

Table 6. Summary of sex steroid hormone and vitellogenin levels in blood plasma of male and female adult carp

[Site codes, with corresponding site names and locations, are listed in table 1. Site HOR-WP is not included because there were no male fish and only two females. Results of Tukey's studentized range test (HSD) are summarized by letter codes, with X, Y, and Z used to show groupings among regions, and A, B, C, and D used to show groupings among sites within each region. pg/mL, picogram per milliliter; mg/mL, milligram per milliliter. NS, not significant; —, no data]

Site code	17β-Estradiol (in pg/mL)		11-Ketotestosterone (in pg/mL)		E_2/11-KT		Vitellogenin (in mg/mL)	
	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups
MALES								
Northeast								
CR-T	738±93 (114–1,227)	A	1,393±166 (442–2,161)	AB	0.6±0.1 (0.2-1.3)	A	0.0±0.0 (0.0-0.1)	B
HR-LL	506±81 (72–1,183)	AB	857±92 (254–1,638)	ABC	0.6±0.1 (0.1-1.1)	A	0.1±0.0 (0.0-0.6)	A
HR-P	216±46 (29–857)	B	1,762±143 (565–3,012)	A	0.1±0.0 (0.0-0.4)	B	0.0±0.0 (0.0-0.0)	B
MR-F	190±30 (30–509)	B	837±114 (144–2,186)	BC	0.3±0.1 (0.0-1.2)	AB	0.0±0.0 (0.0-0.2)	B
AR-WSC	378±115 (218–601)	AB	737±322 (341–1,375)	BC	0.6±0.1 (0.4-0.6)	A	0.0±0.0 (0.0-0.0)	B
SR-M	341±156 (111–479)	AB	411±77 (323–565)	C	0.8±0.3 (0.3-1.3)	A	0.0±0.0 (0.0-0.0)	B
Regional Summary	480±37 (29–1,227)	NS	1,439±111 (144–3,012)	NS	0.6±0.1 (0.0-1.3)	Y	0.0±0.0 (0.0-0.6)	NS
Mississippi River Basin								
PR-L	70±27 (17–380)	D	605±162 (76–2,007)	B	0.3±0.1 (0.0-1.0)	B	0.0±0.0 (0.0-0.6)	NS
SPR-D	245±39 (20–591)	BC	790±83 (221–1,528)	AB	0.4±0.1 (0.0-1.3)	B	0.0±0.0 (0.0-0.0)	NS
SPR-K	118±15 (31–227)	CD	675±114 (152–1,733)	AB	0.3±0.0 (0.0-0.7)	B	0.0±0.0 (0.0-0.0)	NS
SPR-NP	526±56 (396–691)	AB	514±82 (254–723)	B	1.1±0.2 (0.6-1.6)	A	0.0±0.0 (0.0-0.0)	NS
SC-NP	804±141 (436–1,491)	A	1,234±126 (726–1,619)	A	0.7±0.1 (0.3-1.0)	AB	0.0±0.0 (0.0-0.0)	NS
WR-H	726±85 (176–1,728)	A	1821±147 (755–2,604)	A	0.4±0.1 (0.2-1.0)	AB	0.1±0.0 (0.0-0.8)	NS
Regional Summary	311±25 (17–1,728)	NS	1,148±92 (76–2,604)	NS	0.4±0.0 (0.0-1.6)	Y	0.0+0.1 (0.0-0.8)	NS
Northern Midcontinent								
MR-P	656±51 (365–859)	NS	707±100 (253–1,306)	NS	1.1±0.2 (0.4-2.8)	AB	0.0±0.0 (0.0-0.0)	NS
NBMR-RL	775±65 (214–1,340)	NS	621±55 (108–1,118)	NS	1.5±0.2 (0.5-4.3)	A	0.0±0.0 (0.0-0.0)	NS
OR-B	580±127 (57–1,105)	NS	1,127±307 (77–2,935)	NS	0.6±0.1 (0.3-1.1)	BC	0.0±0.0 (0.0-0.1)	NS
RRN-B	759±118 (267–1,573)	NS	822±258 (105–2,261)	NS	1.8±0.6 (0.3-6.6)	A	0.0±0.0 (0.0-0.0)	NS
RRN-D	444±95 (100–901)	NS	965±129 (508–1,947)	NS	0.5±0.1 (0.1-1.1)	C	0.1±0.1 (0.0-0.5)	NS
RRN-GF	1431±348 (465–2,104)	NS	857±238 (387–1,507)	NS	2.1±0.8 (0.5-3.8)	—	0.0±0.0 (0.0-0.1)	NS
Regional Summary	716±49 (57–2,104)	NS	805±69 (77–2,935)	NS	1.3±0.1 (0.1-6.6)	X	0.0±0.0 (0.0-0.5)	NS

Table 6. Summary of sex steroid hormone and vitellogenin levels in blood plasma of male and female adult carp—Continued

Site code	17β-Estradiol (in pg/mL)		11-Ketotestosterone (in pg/mL)		E_2/11-KT		Vitellogenin (in mg/mL)	
	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups
Southern Midcontinent								
CC-S	—	—	—	—	—	—	—	—
TR-D	258±130 (128–388)	—	627±106 (521–732)	—	0.4±0.1 (0.2-0.5)	—	0.0±0.0 (0.0-0.0)	—
Regional Summary	258±130 (128–388)	—	627±106 (521–732)	—	0.4±0.1 (0.2-0.5)	—	0.0±0.0 (0.0-0.0)	—
West								
DPR	2,086±108 (1,338–2,542)	A	4,289±136 (3,589–5,009)	A	0.5±0.0 (0.3-0.7)	B	0.0±0.0 (0.0-0.1)	NS
SJR-FF	207±26 (90–454)	C	1,021±145 (287–2040)	B	0.3±0.1 (0.1-1.3)	B	0.0±0.0 (0.0-0.0)	NS
SJR-M	536±63 (242–871)	B	695±140 (149–1,511)	C	1.1±0.2 (0.3-2.9)	A	0.0±0.0 (0.0-0.0)	NS
MRP-S	126±18 (23–227)	D	642±81 (84–1,218)	C	0.3±0.1 (0.0-1.6)	B	0.1±0.0 (0.0-0.6)	NS
Regional Summary	598±77 (23–2,542)	NS	1,561±158 (84–5,009)	NS	0.5±0.0 (0.0-2.9)	XY	0.0+0.1 (0.0-0.6)	NS
FEMALES								
Northeast								
CR-T	3,343±644 (1,646–7,703)	AB	1,568±191 (769–2,740)	A	2.2±0.3 (1.4-4.6)	B	32.7±2.5 (21.3-38.3)	A
HR-LL	2,694±660 (1,002–5,780)	AB	336±101 (113–920)	BC	10.6±3.0 (3.7-22.1)	A	20.4±2.5 (8.0-29.0)	AB
HR-P	3,329±389 (435–4,499)	AB	1,102±146 (304–1,702)	AB	3.3±0.5 (1.4-7.6)	AB	34.6±2.5 (19.0-50.2)	A
MR-F	1,746±342 (5,624,741)	BC	586±101 (115–1,508)	ABC	5.5±1.7 (0.8-21.8)	AB	13.7±1.4 (6.4-26.6)	B
AR-WSC	787±124 (323–1765)	C	552±129 (66–1,125)	ABC	3.2±1.2 (0.6-11.8)	B	25.5±3.4 (6.6-39.6)	AB
SR-M	1,159±126 (315–1,760)	BC	278±53 (13–700)	C	8.8±2.5 (1.2-28.2)	AB	14.9±1.1 (5.8-20.4)	B
Regional Summary	2110±150 (315–7,703)	X	1,074±110 (13–2,740)	XY	4.5±0.6 (0.6-28.2)	X	28.4±1.8 (5.8-50.2)	X
Mississippi River Basin								
PR-L	476±89 (40–1,104)	C	812±221 (87–2,332)	NS	1.2±0.5 (0.2-6.3)	D	25.3±4.8 (0.0-72.5)	AB
SPR-D	1,758±144 (791–3,249)	A	809±79 (182–1,500)	NS	2.5±0.2 (1.1-5.2)	AB	20.4±3.1 (0.0-46.5)	B
SPR-K	1,519±112 (559–2,320)	AB	586±68 (185–1,250)	NS	3.0±0.2 (1.6-5.7)	A	29.5±1.4 (14.7-45.9)	AB
SPR-NP	1,169±136 (561–1,889)	AB	334±26 (180–433)	NS	3.7±0.5 (1.7-7.8)	A	24.2±2.7 (1.8-37.4)	AB
SC-NP	809±110 (504–1,423)	B	810±98 (383–1,257)	NS	1.1±0.1 (0.5-1.6)	CD	24.8±3.6 (0.0-33.4)	AB
WR-H	1,722±133 (884–2,799)	A	1,177±117 (325–1,969)	NS	1.8±0.3 (0.8-4.7)	BC	30.4±1.2 (21.4-41.1)	A
Regional Summary	1,260±68 (40–3,249)	XY	707±42 (87–2,332)	X	2.3±0.2 (0.2-7.8)	Y	26.5±1.5 (0.0-72.5)	X
Northern Midcontinent								
MR-P	2,370±261 (1,276–3,830)	NS	684±118 (72–1503)	NS	5.3±1.5 (1.5-17.7)	NS	27.6±1.5 (20.9-36.2)	NS
NBMR-RL	2,584±405 (354–6,147)	NS	633±130 (84–2020)	NS	8.8±2.6 (1.1-40.6)	NS	25.3±2.3 (16.0-47.5)	NS

Table 6. Summary of sex steroid hormone and vitellogenin levels in blood plasma of male and female adult carp—Continued

Site code	17-β Estradiol (in pg/mL)		11-Ketotestosterone (in pg/mL)		E_2/11-KT		Vitellogenin (in mg/mL)	
	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups	Mean ± one standard error (Range)	HSD groups
OR-B	3,134±923 (421–9,942)	NS	1013±356 (51–3641)	NS	4.4±0.8 (1.7-8.3)	NS	31.1±0.8 (25.8-34.4)	NS
RRN-B	1,968±425 (462–6,852)	NS	284±44 (63–540)	NS	10.6±2.8 (1.8-38.5)	NS	32.6±1.3 (23.4-41.0)	NS
RRN-D	1,400±289 (533–3,530)	NS	342±78 (123–832)	NS	5.4±1.2 (1.6-11.4)	NS	26.7±3.3 (0.4-44.1)	NS
RRN–GF	3,543±891 (443-8,075)	NS	895±250 (57–2639)	NS	5.8±1.3 (0.7-13.0)	NS	27.2±7.2 (7.8-87.8)	NS
Regional Summary	2,463±231 (354–9,942)	X	617±75 (51–3641)	Y	7.1±0.9 (0.7-40.6)	X	28.3±1.3 (0.4-87.8)	X
Southern Midcontinent								
CC-S	382±77 (110–558)	B	119±31 (57–237)	B	3.4±0.6 (1.9-5.1)	NS	11.7±1.8 (7.0-16.5)	NS
TR-D	1,708±367 (947–2,922)	A	445±63 (265–654)	A	3.8±0.4 (2.3-4.5)	NS	9.9±1.2 (7.0-12.8)	NS
Regional Summary	1,045±283 (110-2,922)	Y	282±64 (57–654)	Z	3.6±0.4 (1.9-5.1)	X	10.8±1.1 (7.0-16.5)	Y
West								
DPR	4,175±418 (2,929–6,398)	A	2,180±177 (1,416–2,868)	A	2.0±0.2 (1.1-3.2)	AB	31.7±1.9 (22.9-41.3)	A
SJR-FF	993±163 (204–2,299)	BC	692±79 (177–1,172)	B	1.4±0.2 (0.4-3.0)	B	31.4±2.3 (2.0-42.6)	A
SJR-M	1,591±235 (135–3,196)	B	570±70 (121–1,144)	B	2.7±0.2 (1.1-5.4)	A	37.8±1.5 (25.7-47.9)	A
MRP-S	723±167 (67–1,522)	C	692±171 (22–1,738)	B	1.4±0.3 (0.4-3.0)	B	8.1±3.0 (0.0-23.5)	B
Regional Summary	1,767±158 (67–6,398)	XY	1,359±158 (22–2,868)	X	2.0±0.2 (0.4-5.4)	Y	27.9±1.5 (0.0-47.9)	X

Variations in mean hormone levels for sites within geographic regions are generally much less than the national variation (usually less than 1 order of magnitude). Nevertheless, within most regions, there were significant differences in plasma hormone concentrations between some sites. In all regions except the Northern Midcontinent and the Mississippi River Basin, there were significant differences in 17β-estradiol and 11-ketotestosterone levels for males and females. In the Mississippi River Basin, there were differences between sites in both hormones for males and in 17β-estradiol levels for females. In the Northern Midcontinent, where variability was high at each site, there were no significant differences for either hormone for either males or females.

Another way to evaluate sex steroid hormones is to examine their ratios. In this study, all mean ratios of 17β-estradiol to 11-ketotestosterone (E_2/11-KT) for female carp were above 1.0, whereas mean ratios for male carp were generally below 1.0. Four sites in the Northern Midcontinent had mean E_2/11-KT ratios above 1.0 for males. Significant differences in E_2/11-KT ratios were found between sites in all regions for males, whereas for females, only the Northern Midcontinent and the Southern Midcontinent had no significant site-to-site differences.

Mean plasma vitellogenin in female carp ranged from 8.1 mg/mL for the Mill Race Pond at Springfield, Oregon (MRP-S) to 37.8 mg/mL for the San Joaquin River at Mossdale, California (SJR-M). The highest individual plasma vitellogenin for a female was 87.8 mg/mL from Red River of the North at Grand Forks, North Dakota (RRN-GF). A few females at several sites had no detectable vitellogenin. Male carp had detectable vitellogenin in one or more individuals at 13 of the 25 sites, including at least one site in every region except the Southern Midcontinent. Mean values were much lower in males than females, with the highest being 0.1 mg/mL (found at four sites). The highest individual male plasma vitellogenin concentration was only 0.8 mg/mL, two orders of

magnitude lower than the highest female concentration. The only significant difference between sites evident from the sparse detections of vitellogenin in males was between the Hudson River south of Lake Luzerne, New York (HR-LL) and all other sites in the Northeast. For females, there were significant differences in vitellogenin levels between sites within the Northeast, Mississippi River Basin, and the West.

Gonad Histopathology

Histopathological analyses of carp testes showed that all 216 males evaluated had at least some spermatogenic activity. Most fish had moderate or high spermatogenic activity, indicating a recrudescent reproductive period. However, 86 percent of fish from the Shenandoah and Anacostia Rivers had low spermatogenic activity, which is associated with a late stage of sexual maturation and may be related to the late November sampling dates. All fish with low spermatogenic activity were not used in any statistical data analyses. One male from the Shenandoah River had a few large basophilic cells resembling perinucleolar oocytes (fig. 3), which may be attributed to contaminant exposure and endocrine disruption. All other male gonadal tissue examined in this study was normal.

Ovaries in all but twenty of the female fish were vitellogenic and had varying stages of developing ova, again indicating recrudescence. Abnormalities, such as multinuclear ova, sometimes associated with endocrine disruption, were not seen in any female carp from this study. The general gonadal condition for both male and female carp was similar for all sites and regions and indicated a similar stage of sexual maturation.

Contaminant Distribution

Contaminant levels are summarized in table 7 by group and sample matrix for the 25 study sites. Total concentrations for all contaminant groups measured in tissues and bed sediments spanned at least 2 orders of magnitude among sites. Dissolved pesticides varied within a factor of 10.

Organochlorine pesticide concentrations in tissues were detected at all but two sites, with total concentrations ranging from 5 to 1,310 µg/kg wet weight (ww). The South Platte River near Kersey, Colorado (SPR-K), and Hudson River near Poughkeepsie, New York (HR-P), had total tissue organochlorine pesticide concentrations above 1,000 µg/kg ww. Don Pedro Reservoir, California (DPR) and Clear Creek near Sanger, Texas (CC-S) had no detectable

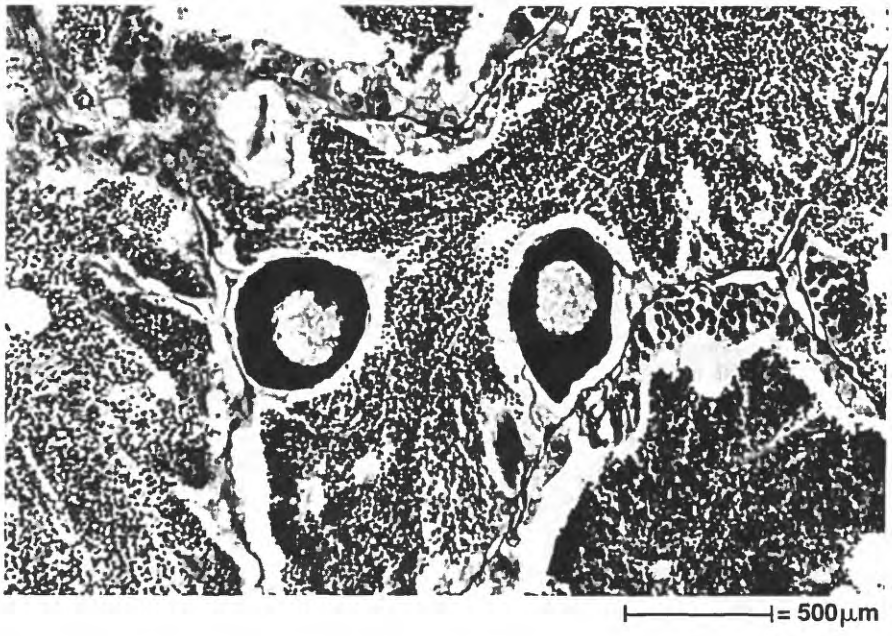

Figure 3. Male adult carp gonad from Shenandoah River near Millville, West Virginia showing intratubular basophylic cells resembling perinucleolar oocytes among maturing spermatozoa, indicating a possible hermaphrodite. The carp was 3 years old, 577 mm in length, and weighed 3,041 g.

Table 7. Contaminant levels at study sites

[Site codes, with corresponding site names and locations, are listed in table 1. Most contaminant data were collected at or near biomarker study sites. For site RRN-D, the bed sediment sample was collected at the Red River of the North at Pembina, North Dakota, about 30 miles downstream and below the inflow of the Pembina River. For site DPR, bed sediment and tissue samples were from the Tuolumne River downstream of the reservoir. For site SJR-FF, bed sediment, tissue, and water samples were from Salt Slough near Stevinson, which usually accounts for most of the flow at SJR-FF. Errors introduced by these site differences are believed to be negligible in comparison to variability between sites and considering the mobility of fish and the reconnaissance nature of the study. Constituents summed for each category are listed in tables 3, 4, and 5. PCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons. nd, none detected; —, no data. µg/kg, microgram per kilogram; µg/L, microgram per liter]

Site code	Tissues					Bed Sediments					Water
	Date of sample	Species	Lipids (percent)	Sum of organo-chlorine pesticides (µg/kg wet weight)	Total PCBs (µg/kg wet weight)	Date of sample	Organic carbon (percent)	Sum of phenols (µg/kg dry weight)	Sum of phthalates (µg/kg dry weight)	Sum of PAHs (µg/kg dry weight)	Annual mean sum of dissolved pesticides (µg/L)
Northeast											
CR-T	7/14/1993	White sucker	10.9	321	1,400	7/14/1993	2.2	26	431	5,560	—
HOR-WP	9/13/1994	White sucker	2.5	260	72,000	—	—	—	—	—	—
HR-LL	5/23/1995	Common carp	4.7	71	109	8/25/1993	1.6	48	20	568	—
HR-P	5/19/1995	Common carp	14.0	1,006	9,200	8/31/1993	2.0	61	499	4,502	—
MR-F	9/28/1994	Common carp	9.6	287	6,700	8/23/1993	2.2	104	134	13,827	—
AR-WSC	11/18/1994	Largemouth bass	3.3	509	1,800	—	—	—	—	—	—
SR-M	9/15/1992	Asiatic clam	0.4	5	140	8/21/1992	2.7	632	223	5,080	0.33
Mississippi River Basin											
PR-L	9/1/1994	Common carp	5.2	29	nd	3/17/1995	0.9	34	245	378	2.90
SPR-D	8/17/1993	Common carp	7.8	267	1,100	8/23/1993	0.8	99	2,310	13,302	0.84
SPR-K	9/18/1992	Common carp	6.0	1,310	410	10/6/1992	0.3	250	285	646	0.97
SPR-NP	9/30/1992	Common carp	4.1	83	140	9/30/1992	0.3	117	104	297	—
SC-NP	10/29/1992	Black redhorse	7.1	97	nd	10/9/1992	1.7	100	6	1,115	1.24
WR-H	6/1/1995	Common carp	9.3	235	420	10/6/1992	0.9	20	1	123	2.55
Northern Midcontinent											
MR-P	9/20/1994	Common carp	2.8	17	51	—	—	—	—	—	—
NBMR-RL	8/2/1995	White sucker	1.2	6	nd	8/17/1995	4.6	nd	105	377	0.34
OR-B	8/20/1992	Common carp	6.4	12	nd	8/20/1992	1.8	60	85	1,032	—
RRN-B	9/12/1994	Common carp	10.8	13	nd	8/18/1992	1.5	72	168	1,004	—
RRN-D	8/19/1992	Common carp	3.8	63	70	8/11/1992	1.2	31	57	333	0.22
RRN-GF	9/15/1994	Common carp	6.8	98	62	9/2/1992	1.3	62	344	1,807	—
Southern Midcontinent											
CC-S	10/13/1992	Asiatic clam	0.9	nd	nd	10/2/1994	0.3	50	42	nd	—
TR-D	8/29/1995	Common carp	4.2	483	580	8/29/1995	8.0	nd	206	802	0.69
West											
DPR	10/14/1992	Asiatic clam	1.0	nd	nd	10/14/1992	1.2	nd	6	28	—
SJR-FF	10/15/1992	Channel catfish	3.3	348	nd	10/15/1992	0.7	9	10	39	0.81
SJR-M	10/14/1992	Asiatic clam	1.9	328	nd	10/14/1992	0.5	18	nd	27	0.38
MRP-S	10/20/1994	Common carp	2.7	139	nd	7/17/1995	6.3	1,019	368	623	—

Table 8. Pearson product-moment correlation matrix of contaminant groups

[All tissue and bed sediment concentrations were lipid or carbon standardized, respectively, and all data were log transformed for correlation analysis. PCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons. Correlations that are significant at α=0.05 are shown by *]

	Organochlorine pesticides	PCBs	Phenols	Phthalates	PAHs	Dissolved pesticides
Organochlorine pesticides	1.00					
PCBs	*0.52	1.00				
Phenols	0.14	0.15	1.00			
Pthlalates	0.00	0.35	*0.50	1.00		
PAHs	0.29	*0.66	0.37	*0.61	1.00	
Dissolved pesticides	-0.05	-0.11	0.09	0.04	0.11	1.00

organochlorine pesticides. Total PCBs in tissue follow a regional pattern, with the four highest concentrations occurring in the Northeast. The South Platte River at Denver, Colorado (SPR-D), a site affected by urban development, was the only site outside of the Northeast with a concentration greater than 1,000 µg/kg ww. Ten sites had no detectable PCBs in tissue.

Phenol concentrations in bed sediment were usually less than 100 µg/kg dry weight (dw), with only the Mill Race Pond at Springfield, Oregon (MRP-S), exceeding 1,000 µg/kg dw. Only three sites had no detectable phenols. Phthalates in bed sediment were frequently over 10 µg/kg dw, but only the South Platte at Denver, Colorado (SPR-D), with 2,300 µg/kg dw, had a concentration greater than 1,000 µg/kg dw. Concentrations of PAHs generally were higher than phenols and phthalates. Nine sites had PAH concentrations higher than 1,000 µg/kg dw, with the highest of 13,300 µg/kg dw found in the South Platte River at Denver, Colorado (SPR-D). PAHs were found at all sites except Clear Creek near Sanger, Texas (CC-S).

Different organic contaminants are frequently found together in the environment and this co-occurrence is important to consider when evaluating possible biological effects. The Pearson product-moment correlation matrix of log-transformed contaminant data shows significant (α=0.05) correlations between PCBs and organochlorine pesticides, PCBs and PAHs, PAHs and phthalates, and phthalates and phenols (table 8). There were no significant correlations between dissolved pesticides in water and any contaminant group in tissue or bed sediment. Principal component analysis based on the correlation matrix for log-transformed data was used to examine patterns in bed sediment and tissue contaminants among sites (table 9). The first principal component accounts for 50 percent of the total variance and is composed of an almost equally weighted sum of all contaminant groups. The second component accounts for 24 percent of the variance and has high positive loadings for organochlorine pesticides (0.66) and PCBs (0.39), which are correlated with each other, and negative loadings for phenols (–0.45) and phthalates (–0.46). The third component accounts for 15 percent of the variance and has high positive loadings for phenols (0.69) and organochlorine pesticides (0.54). The second and third components indicate that the relative proportions of phenols, phthalates, and organochlorine pesticides account for most of the total variability in contaminants after variations in total contaminant levels are accounted for.

Relations Between Contaminants and Biomarkers

One of the primary objectives of this reconnaissance study was to investigate potential relations between contaminant levels and the biomarkers of potential endocrine disruption: 17β-estradiol, 11-ketotestosterone, and vitellogenin. To investigate these relations, all sites from all regions were combined. Potential interactions between contaminant influences on biomarkers and inherent regional differences were tested, but found to be insignificant. Regional influences were also investigated graphically, but the small and variable numbers of sites in each region prevented conclusive analysis. Results for male and female carp are reported separately below and then comparatively evaluated in the "Discussion" section of this report.

Male Carp

Table 10 is the Pearson product-moment correlation matrix for $\log_{10}$-transformed values for contaminant groups and sex steroid hormones. The detection frequencies of vitellogenin were not log transformed. Nonparametric rank correlation analysis

Table 9. Principal component analysis of tissue and bed sediment contaminants (22 sites)

[PCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons]

	Eigenvectors and variable loadings		
	V1	V2	V3
Organochlorine pesticides	-0.29	0.66	0.53
PCBs	-0.49	0.39	-0.33
Phenols	-0.37	-0.45	0.69
Phthalates	-0.47	-0.46	-0.19
PAHs	-0.56	0.01	-0.29
Percentage of variance accounted for	50	24	15

Table 10. Pearson product-moment correlations between biomarkers and contaminant groups for male adult carp

[All data are log-transformed concentrations, except for vitellogenin, which is frequency of detection and not transformed. Correlations that are significant at α=0.05 are shown by *. PCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons]

	17β-Estradiol	11-Ketotestosterone	E_2/11-KT	Vitellogenin (frequency)
11-ketotestosterone	* 0.48	1.00		
E_2/11-KT	* 0.81	-0.10	1.00	
Vitellogenin (frequency)	0.11	0.15	0.02	1.00
Organochlorine pesticides	*-0.46	-0.15	-0.34	-0.36
PCBs	-0.23	-0.04	-0.26	-0.14
Phenols	-0.33	*-0.45	-0.10	-0.23
Phthalates	-0.39	-0.34	-0.25	-0.11
PAHs	-0.21	-0.23	-0.14	-0.04
Dissolved pesticides	-0.41	0.18	*-0.63	0.04

to test robustness of relations showed essentially the same results as the log transformed analysis and is not presented.

Among the biomarkers, there is a significant correlation (α=0.05) between the two major sex steroid hormones, 17β-estradiol and 11-ketotestosterone, but variation in the E_2/11-KT ratio is mainly determined by 17β-estradiol. The detection frequency of vitellogenin in the male carp was not correlated significantly with either of the two steroid hormones or E_2/11-KT.

Correlation coefficients between 17β-estradiol in male carp and all contaminant groups are negative, but the only significant correlation is with organochlorine pesticides (fig. 4*A*). With removal of one extreme outlier (PR-L), the proportion of variance in 17β-estradiol that is explained by organochlorine pesticides in tissue increases from 21 to 48 percent. Multiple regression relations were examined by stepwise addition to the correlation with organochlorine pesticides, but there were no significant additions.

Correlation coefficients between 11-ketotestosterone and contaminant groups are either negative or close to zero, with a significant negative correlation with phenols. Phenols in bed sediments explain 20 percent of the variance in median 11-ketotestosterone among sites (fig. 4*B*). No other contaminant groups were found to be significant additions to the phenol relation in stepwise multiple regression analysis.

Correlation coefficients between the E_2/11-KT ratio and all contaminant groups also are negative, reflecting the dominant influence of negative correlations with levels of 17β-estradiol. There is a significant negative correlation with dissolved pesticides. The time-weighted mean dissolved pesticide concentration explained 40 percent of the variance in the E_2/11-KT ratio for the 11 sites with dissolved pesticides data (fig. 4*C*). Examination of multiple regression relations showed that phthalates were a significant addition to the regression relation for dissolved pesticides, also with a negative coefficient. The addition of phthalates increased the variance explained from 40 to 65 percent for the 11 sites. Although the data set for this multiple regression relation is small and does not support conclusive analysis, the significant relation is consistent with the

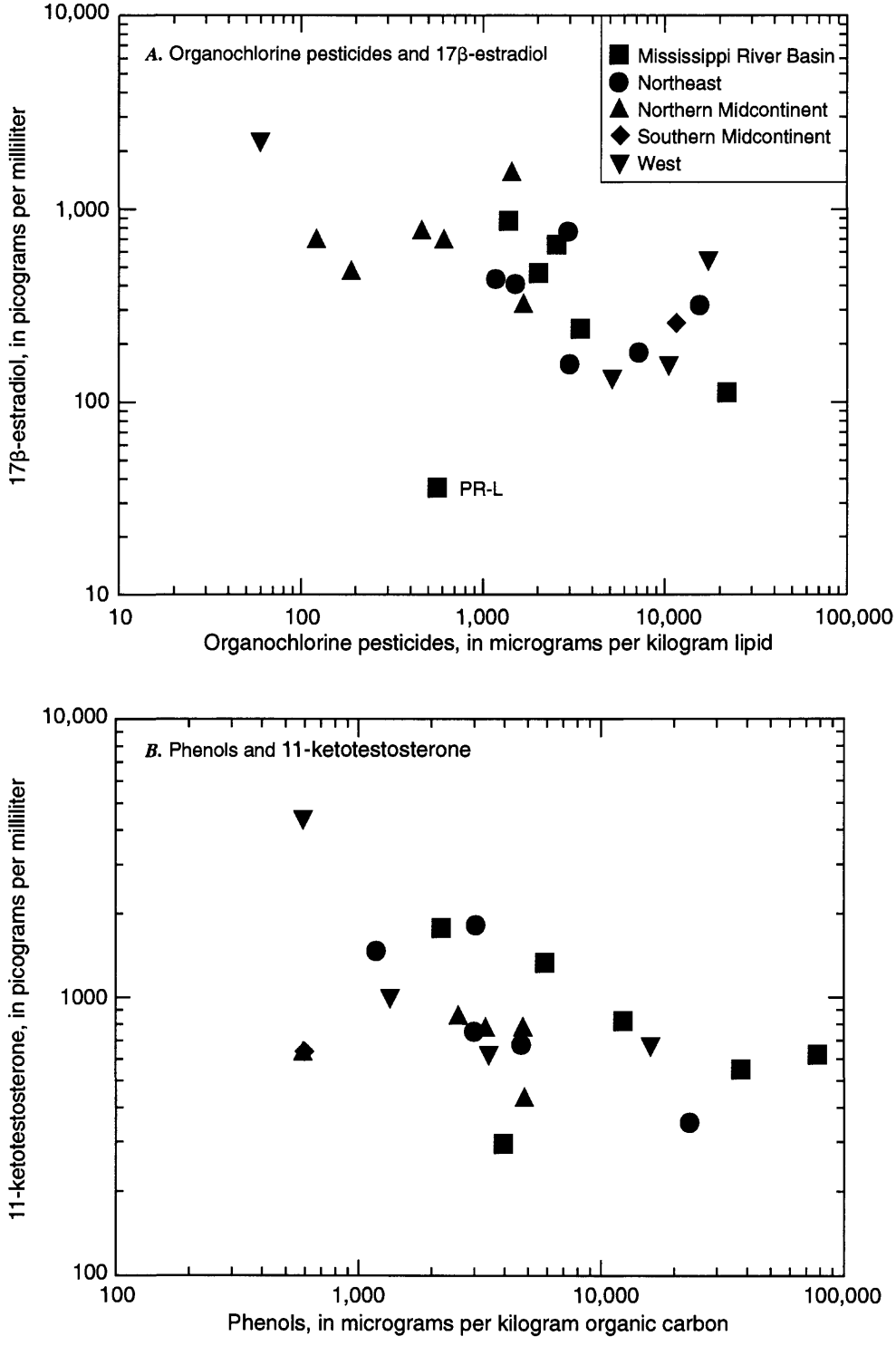

Figure 4. Significant correlations between biomarkers and contaminants for male adult carp. *A*, organochlorine pesticides and 17β-estradiol (R^2=0.21); *B*, phenols and 11-ketotestosterone (R^2=0.20); and *C*, dissolved pesticides and E_2/11-KT (R^2=0.40). PR-L denotes Platte River at Louisville, Nebraska.

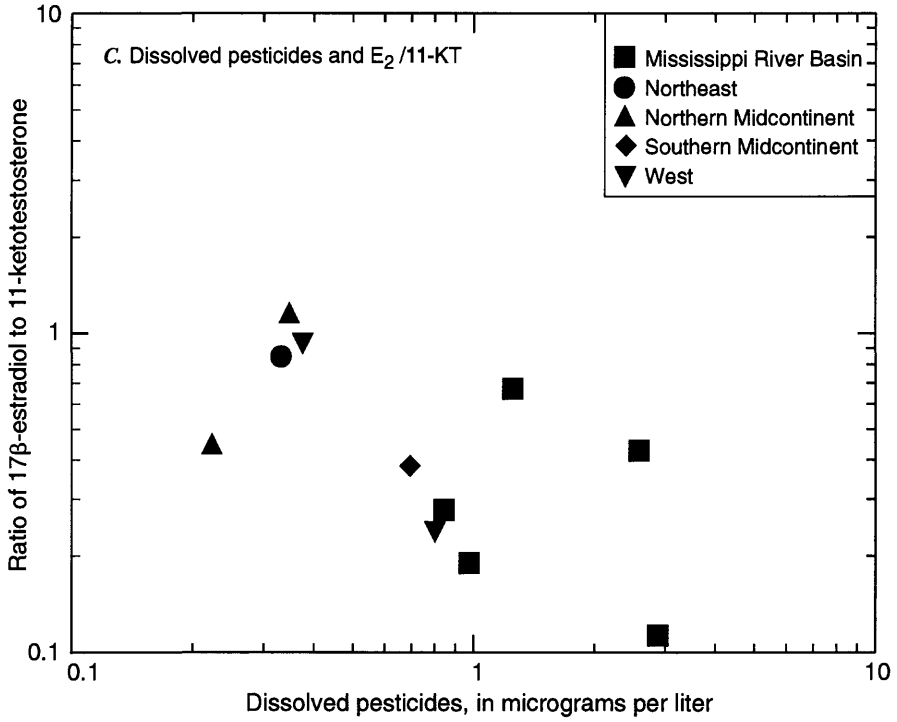

Figure 4. Continued.

occurrence of simultaneous influences of multiple contaminants. The detection frequency of vitellogenin in male carp was not significantly correlated with any of the contaminant groups.

Figure 4 shows varying degrees of consistency between small regional subsets of data and the national data for the statistically significant correlations between biomarkers and contaminants for male carp. Generally, within-region correlations are insignificant or are controlled by one or two observations. The small number of sites per region, the uncontrolled variability, and the possibility that other contaminant factors not measured in this study may be the dominant influence at a particular site, combine to make detailed interpretation of individual relations within each region inappropriate from this reconnaissance data.

Female Carp

Table 11 is the Pearson product-moment correlation matrix for $\log_{10}$-transformed values for contaminant groups and sex steroid hormones plus vitellogenin for female carp. As for males, 17β-estradiol and 11-ketotestosterone are significantly correlated with each other in female carp, but the two hormones have a more balanced influence on variations in the E_2/11-KT ratio for females compared to males.

Correlation coefficients between 17β-estradiol and contaminant groups vary in both direction and magnitude. There are no significant correlations with contaminant groups, although p=0.06 for the correlation between 17β-estradiol and phenols.

Correlation coefficients between 11-ketotestosterone in female carp and contaminant groups also vary in both direction and magnitude. There is one significant positive correlation with dissolved pesticides (fig. 5*A*). No other contaminant groups were found to be significant additions to this relation in stepwise multiple regression analysis.

Correlation coefficients between the E_2/11-KT ratio and contaminant groups are mostly low, but there is a significant negative correlation with dissolved pesticides that explains 71 percent of the variance in E_2/11-KT (fig. 5*B*). Examination of multiple regression relations showed that PCBs are a significant addition to the dissolved pesticide relation with a positive coefficient, and increased the variance explained to 92 percent (HOR-WP is not included because there are no dissolved pesticide data for that site). Vitellogenin in female carp is not significantly correlated with any of the contaminant groups.

As for males, figure 5 shows varying degrees of consistency between small regional subsets of data and the national data for the two statistically

Table 11. Pearson product-moment correlations between biomarkers and contaminant groups for female adult carp

[All data are log-transformed concentrations. Correlations that are significant at α=0.05 are shown by *. PAHs, polycyclic aromatic hydrocarbons. Results for 17β-estradiol and polychlorinated biphenyls (PCBs), and E_2/11-KT and PCBs, exclude an extreme outlier, site HR-WP; results for vitellogenin and phenols exclude an extreme outlier, site MRP-S]

	17β-Estradiol	11-Ketotestosterone	E_2/11-KT	Vitellogenin
11-ketotestosterone	*0.47	1.00		
E_2/11-KT	*0.50	*-0.46	1.00	
Vitellogenin	0.34	0.27	0.05	1.00
Organochlorine pesticides	0.04	0.22	-0.13	-0.02
PCBs	0.20	0.12	0.10	0.05
Phenols	-0.41	-0.39	0.11	-0.15
Phthalates	-0.03	-0.17	0.16	-0.18
PAHs	0.25	0.14	0.11	0.07
Dissolved pesticides	-0.44	*0.74	*-0.84	0.14

significant correlations between biomarkers and contaminants for female carp. Particularly for dissolved pesticides, the number of sites in each region is small (five in the Mississippi River Basin and two or less in others). Generally, within-region correlations are insignificant or are governed by one or two observations. As stated for males, the small number of sites per region, the uncontrolled variability in the data, and the possibility that other contaminant factors may be the dominant influence at a particular site, make detailed interpretation of individual relations within each region inappropriate.

DISCUSSION

Use of Biomarkers as Indicators of Potential Endocrine Disruption

An important issue with regard to use of sex steroid hormones as biomarkers of potential endocrine disruption is the natural temporal variability of hormones in fish. This is particularly true for this reconnaissance in which one-time samples were collected from a wide geographic area. The sampling period of this study (late August to early December) is after carp spawn (March to mid August [Panek, 1987]), during a time of gonadal recrudescence, and before water temperatures go below 15°C (Down and others, 1990). Samples were collected during this part of the reproductive cycle to minimize hormone variance, which is much greater in the spring through summer spawning period (Barry and others, 1990; Chang and Chen, 1990; and Down and others, 1990). Even within this part of the reproductive cycle, sex steroid hormones among individual fish at a site can vary up to 30-fold (Chang and Chen, 1990; Down and others, 1990; and Folmar and others, 1996). In this study, however, the 10th and 90th percentiles of 17β-estradiol and 11-ketotestosterone in individual males and females were within a factor of 5 of each other for most sites. Nevertheless, detecting possible endocrine disruption through differences in sex steroid hormones, even within the same period of the reproductive cycle, is difficult because of natural variability. Generally, subtle effects of potential endocrine disrupters are unlikely to be detected, and only the strongest influences of contaminants are likely to be evident.

In addition to the individual sex steroid hormones—17β-estradiol and 11-ketotestosterone—their ratio was used in this study as an indicator of possible endocrine disruption. Folmar and others (1996) concluded that the ratio of 17β-estradiol to testosterone appears to be a sensitive marker of abnormal sex steroid concentrations in carp, but has little functional significance because normal ranges have not been established. However, Hileman (1994) concluded that a specific ratio of estrogen to testosterone is necessary for sexual differentiation in developing animals and that alteration of the ratio can result in incomplete or improper gonadal development. Additionally, the balance between these two hormones determines a fish's phenotype, which includes sex characteristics, differentiation of the brain and behavior, and development of other reproductive organs (Lehninger, 1982; and Hunter and Donaldson, 1983). There may be an acceptable range of proportions of female to male sex steroid hormones at various stages in a fishes life cycle, and the range may be most critical in immature and developing fish. In this study, values of the E_2/11-KT ratio were mostly below 1.0 for males, and

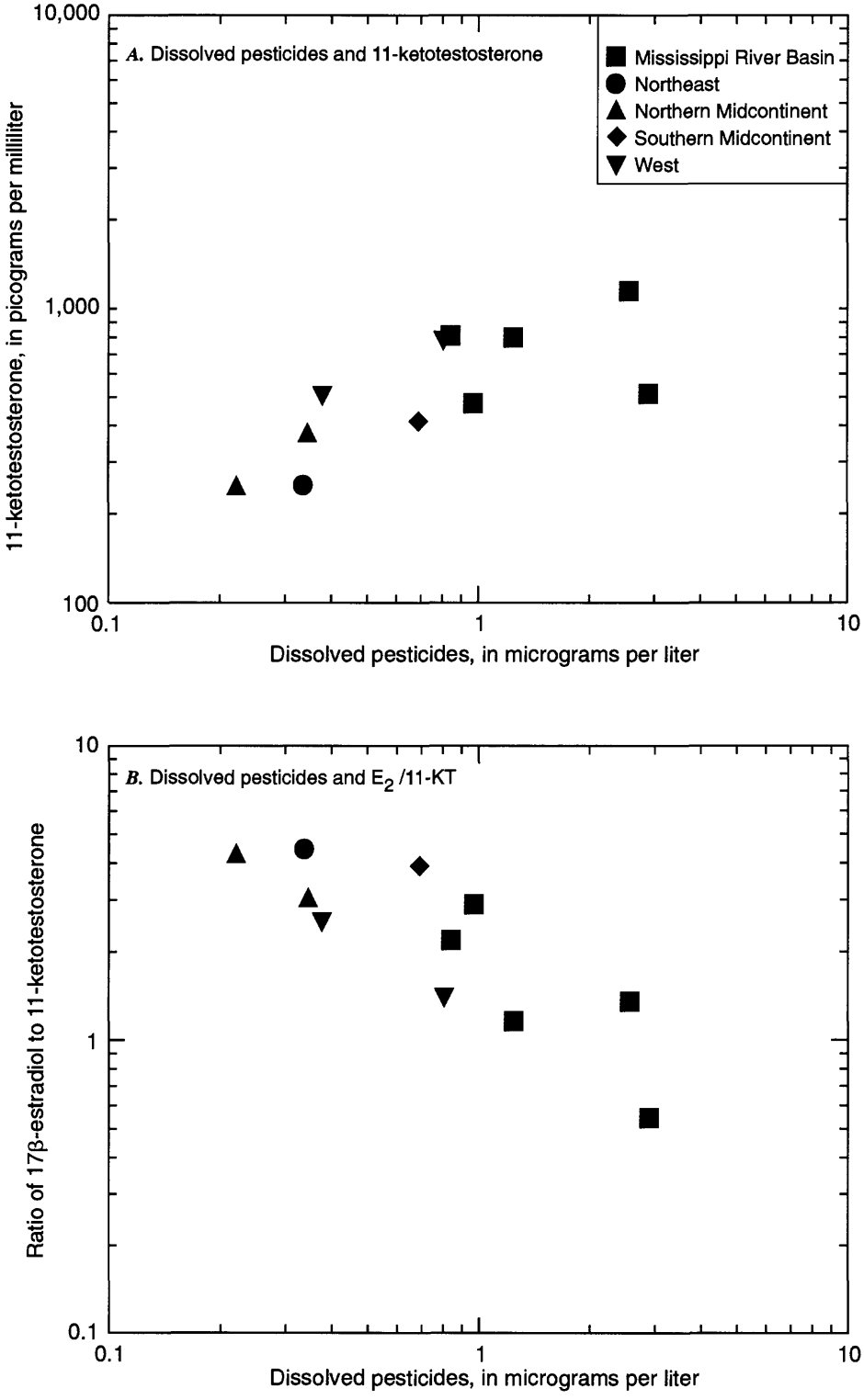

Figure 5. Significant correlations between biomarkers and contaminants for female adult carp. *A*, dissolved pesticides and 11-ketotestosterone (R^2=0.55); *B*, dissolved pesticides and E_2/11-KT (R^2=0.71).

mostly above 1.0 for females. Although ranges have not been established for classifying fish as normal or not, extreme values of the ratio compared to other fish, or correlations between the ratio and contaminant levels, are useful indicators of potential endocrine disruption.

Vitellogenin induction in male fish has also been presented as evidence of endocrine disruption (Purdom and others, 1994 and Folmar and others, 1996), and vitellogenin was detected in one or more male fish at over half the study sites. However, the level of vitellogenin was never over 1 mg/mL in any individual, far below the normal range found in females. Purdom and others (1994) and Folmar and others (1996) found vitellogenin induction in male carp below sewage treatment plants (STP) and their results suggested that exposure to estrogenic compounds in the effluent, such as alkylphenol-ethoyxlates could be the cause. Folmar and others (1996) also showed that an increase in 17β-estradiol was not responsible for this vitellogenin induction, but a reduction in testosterone lowered the ratios of 17β-estradiol/testosterone in males at the STP impacted site. Some of our sites where vitellogenin was found in male carp may have been influenced by sewage effluent, and substances in the effluent may be partly responsible for the induction. However, vitellogenin also was detected in males at several minimally contaminated sites with no sewage effluent, such as Don Pedro Reservoir in California (DPR), which indicates that some male fish have low background vitellogenin during some portion of the reproductive cycle. Male fish have a vitellogenin gene that is not usually expressed, but male fish of several species not exposed to endocrine disrupting compounds have been documented to have low concentrations of vitellogenin present (Copeland and Thomas, 1988 and Goodwin and others, 1992).

To supplement the hormone and vitellogenin biomarkers, gonads also were evaluated for abnormalities. Evidence of endocrine disruption in mammals and reptiles has been documented through gonadal histopathology, such as multinuclear eggs, too many eggs in the ovary, and dark bar-shaped structures in tubules of testes (McLachlan and Arnold, 1996). However, this study found only one possible gonadal abnormality (a few basophyllic cells in a testes, which are probably primary ova) out of 438 gonads examined. Other possible endocrine-disruption effects that have been observed in fish, such as reduced ovary size, lower egg viability, and delayed sexual maturity (McMaster and others, 1991; Munkittrick and others, 1992; and Hontela and others, 1995), were not assessed in this study. Thus, the evidence for potential endocrine disruption that is indicated by differences in hormones and vitellogenin among sites, and significant correlations between hormone levels and contaminants, is not confirmed by gonad abnormalities that were evaluated in this study.

Contaminants and Biomarkers

An important objective of this reconnaissance study was to evaluate whether differences in biomarkers among sites may be related to environmental contaminants. There is strong evidence that xenobiotics can induce toxicity and changes in endocrine systems (Atterwill and Flack, 1992), and field studies have found correlations between the levels of endocrine disruption in fish and the types and degree of contaminant exposure (Folmar, 1993). Most of the evidence in teleost fish shows contaminants reducing levels of circulating sex steroid hormones and vitellogenin (Folmar, 1993), although some contaminants, such as cadmium (Sangalang and Freeman, 1974), DDT (Denison and others, 1981), β-hexachlorocyclohexane (β-HCH [Webster and others, 1985]), nonylphenol (Waldock and others, 1994) and PAHs (Janssen and others, 1995), may increase steroid hormones or vitellogenin.

Table 12. Summary of analyses of correlations between biomarkers and contaminants for male and female adult carp

[The plus and minus symbols indicate the direction of correlation: 3 symbols signifies statistical significance with α=0.05. For characterizing patterns in the direction of correlations that did not meet the 0.05 significance levels, 2 symbols signifies $0.05 < p < 0.10$, and 1 symbol signifies $0.10 < p < 0.25$. PCBs, polychlorinated biphenyls; PAHs, polycyclic aromatic hydrocarbons]

	17β-Estradiol		11-Ketotestosterone		E_2/11-KT ratio		Vitellogenin	
	Males	Females	Males	Females	Males	Females	Males	Females
Organochlorine pesticides	− − −				−		− −	
PCBs					−			
Phenols	−	− −	− − −	− −				
Phthalates	− −		−					
PAHs								
Dissolved pesticides	−	−		+ + +	− − −	− − −		

Table 12 summarizes the results of the pairwise correlation analyses of contaminant-biomarker relations for both males and females. There are five statistically significant (α=0.05) correlations between biomarkers and contaminant groups with four negative and one positive. Of 15 correlations with p values less than 0.25, 14 were negative. The strongest patterns common to both males and females are: (1) negative correlations between the E_2/11-KT ratio and dissolved pesticides, and (2) negative correlations between phenols and both 17β-estradiol and 11-ketotestosterone. Notable differences between males and females are: (1) the significant negative correlation between 17β-estradiol and organochlorine pesticides shown in males and not females, and (2) the significant positive correlation between 11-ketotestosterone and dissolved pesticides shown in females and not males.

The significant correlations between dissolved pesticides in water and E_2/11-KT for both males and females are shown in figure 6. Best-fit regression lines for males and females follow the same general pattern, with the greatest reduction in E_2/11-KT occurring from 0.2 to 1 µg/L dissolved pesticides. The lowest E_2/11-KT ratios in both male and female carp were found in the Platte River at Louisville (PR-L) in Nebraska, which had the highest total concentration of dissolved pesticides in water. Studies have shown that some water soluble pesticides, such as atrazine (Babic-Gojmerac and others, 1989 and Simic and others, 1991), alachlor (U.S. Environmental Protection Agency, 1984 and Amdur and others, 1991) and carbaryl (Amdur and others, 1991), all of which were detected at one or more sites in this study, affect endocrine systems. However, the available

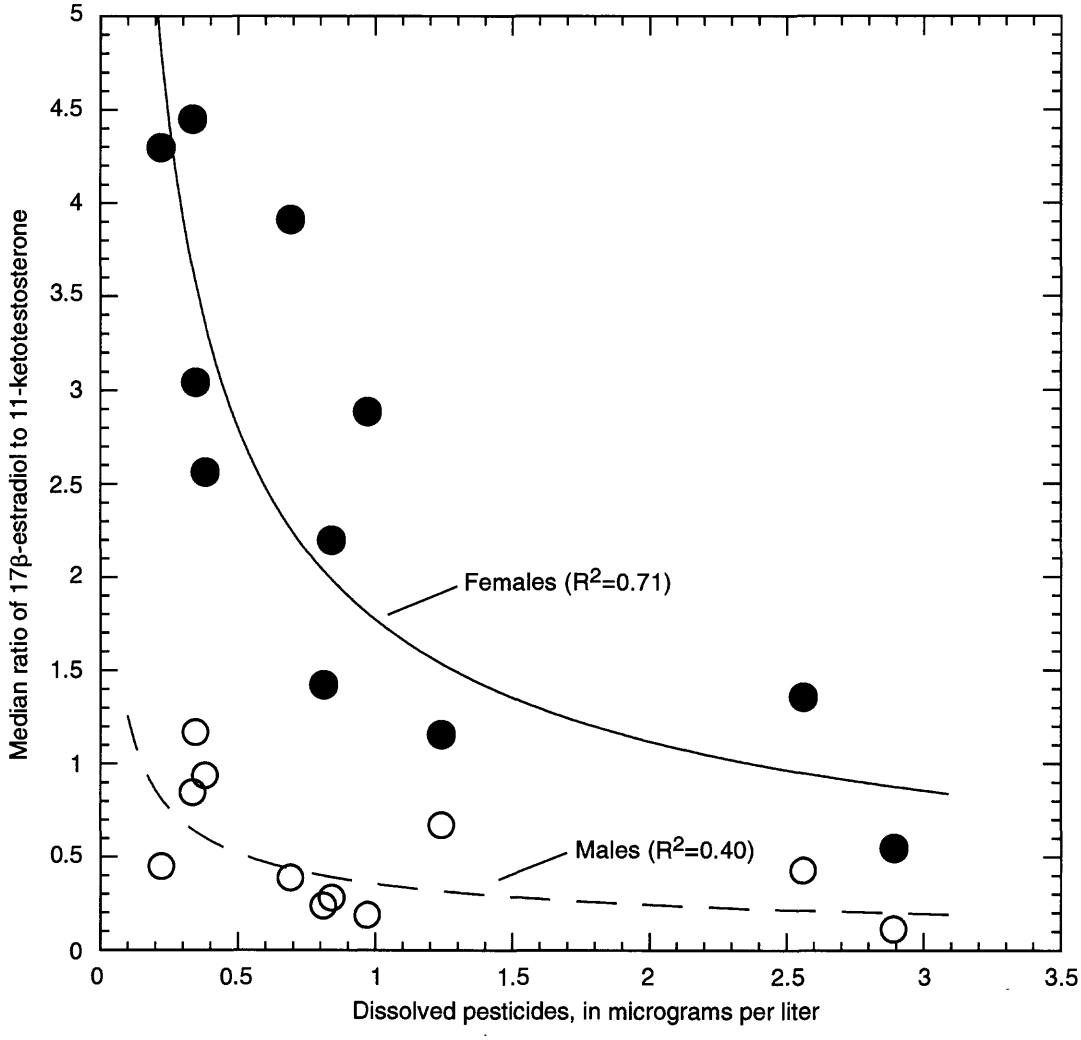

Figure 6. Correlations of the 17β-estradiol/11-ketotestosterone (E_2/11-KT) ratio with dissolved pesticide concentrations for male (○) and female (●) adult carp.

contaminant data are not sufficient to determine which specific pesticides or groups of pesticides could be responsible for the reduction in E_2/11-KT ratios.

The contaminant group having the most consistent direction of correlation with biomarkers is the phenols, which have negative correlation coefficients with 17β-estradiol and 11-ketotestosterone in male and female fish (although most are not significant at the 0.05 level). Only the correlation with 11-ketotestosterone in males was statistically significant. Within the phenol chemical group, the alkylated phenols have been shown to bind to the estrogen receptor, displaying estrogenic effects, such as vitellogenin induction in male fish (Waldock and others, 1994 and White and others, 1994).

Organochlorine pesticides showed a significant negative correlation with male 17β-estradiol. A similar response was seen in largemouth bass from a site in Florida that is contaminated with organochlorine pesticides (Gross and others, 1995). Results from another study (Singh and Singh, 1987) also showed that organochlorine pesticides (lindane and γ-benzene hexachloride [γ–BHC]) reduced estradiol in fish.

Significant pair-wise correlations with biomarkers were not found for PCBs, phthalates, or PAHs. Others, however, have reported reduced estradiol in fish exposed to PCBs. PCB concentration was found in this study to explain a significant portion of the variance in E_2/11-KT in female carp in a multiple regression with dissolved pesticides. Phthalates, widely used chemicals in plastics, are routinely found in sampling of aquatic environments and have been shown by others to affect endocrine systems (Wams, 1987; Treinen and others, 1990; and Laskey and Berman, 1993). Phthalate concentration was found in this study to explain a significant portion of the variance in E_2/11-KT in male carp in a multiple regression with dissolved pesticides. Available literature on fish mostly show that PAHs reduce sex steroid hormones and vitellogenin (Johnson and others, 1988; Thomas 1988; Singh, 1989; and Sol and others, 1995), although one study showed that PAHs in contaminated sediment increased steroid hormones and vitellogenin (Janssen and others, 1995). The absence of strong findings that these contaminant groups are correlated with biomarkers at the reconnaissance study sites may be due to one or a combination of several reasons: there are no effects at the levels of exposure studied; effects are subtle in relation to uncontrolled sources of variability; or that various contaminants have effects in opposite directions that confound simple correlation analysis.

Direction of Future Studies

An important objective of this reconnaissance study was to determine the need and priorities for further endocrine disruption studies. Improved information is needed in several areas to evaluate whether endocrine disruption in fish is actually occurring in some streams and, if so, to determine its causes and its effects on fish populations.

Improved knowledge of the occurrence of endocrine disruption in fish will require more detailed and integrated assessment of the exposure of fish to contaminants over time in relation to reproductive cycles and variability in endocrine systems. Major elements of study that should be included are:

- Assessment of biological effects on both individual fish and populations, and how these effects correlate with the degrees of endocrine disruption indicted by biomarkers and other measures.
- Inclusion of fish species that are more sensitive to endocrine disruption than carp.
- Contemporaneous temporal characterization of contaminant exposure and endocrine status over the complete annual reproductive cycle.
- Broader characterization of contaminant exposure (including background levels), such as trace elements, additional pesticides, and nitrates.
- Improved pairing of reference sites with contaminated sites. Such site pairs need to be as similar as possible in all respects, except contaminant levels.

Investigation of biological effects associated with altered endocrine systems is probably the most difficult task. Detailed discussion of methods to assess individual and population-level effects is beyond the scope of this paper. Initially, however, reproductive impairment of individuals should be assessed using a range of techniques on selected fish species, including gonadal somatic index, fecundity, egg size and hatchability, and sperm quantities and qualities. These indicators should be combined with basic characterization of fish populations, such as sex and age distribution, condition, health, and growth. Multiple lines of evidence in assessing reproductive impairment are critical because of the high degree of inherent variability.

Achieving the types of improvements in information on biological effects and contaminant influences outlined above will require a distinct shift in study design from the "one sample from many sites" approach of the reconnaissance, to the "intensive study of a few sites" approach for more detailed study. A paired-site approach is suggested, with one "contaminated" site and one "reference" site located in each of the three regions with the clearest evidence of endocrine disruption: the Northeast, the Mississippi

River Basin, and the West. Criteria for selection of contaminated sites should include representation of the regions' streams, historical availability of contaminant and fish population data, availability of comparable species with sufficient numbers to sample, and availability of a comparable reference site. Potential "contaminated" sites for further study include the Shenandoah River at Millville, West Virginia (SR-M) or the Mohawk River at Frankfort, New York (MR-F) in the Northeast; the Platte River at Louisville, Nebraska (PR-L) or the White River at Hazleton, Indiana (WR-H) in the Mississippi River Basin; and the San Joaquin River at Fremont Ford, California (SJR-FF) in the West.

SUMMARY

Reconnaissance assessment of sex steroid hormones in carp from United States streams indicates that fish in some streams within all regions studied may be experiencing some degree of endocrine disruption. Primary lines of evidence for the occurrence of endocrine disruption are the significant site-to-site differences in biomarkers within regions and the presence of significant correlations between biomarkers and contaminants. Gonad histopathology, however, showed that only one fish out of 438 had an abnormality that could be associated with sex-steroid hormone levels. Specific findings are summarized below:

- For 23 streams and 2 impoundments studied in five major regions of the United States, mean levels of 17β-estradiol and 11-ketotestosterone in common carp spanned 2 orders of magnitude for each sex during recrudescence.
- There were no significant (α=0.05) differences between regions in sex steroid hormones for males, but females from the Northern Midcontinent and the Southern Midcontinent were significantly different from other regions in one or both hormones.
- Within most regions, there were significant differences between sites in one or both sex steroid hormones for both sexes.
- The ratio of 17β-estradiol to 11-ketotestosterone (E_2/11-KT) also had significant differences between sites in most regions for both male and female carp.
- Vitellogenin showed some differences between sites for females in the Northeast, the Mississippi River Basin, and the West.
- The gonad conditions of most males and females were similar for all sites and regions, indicated similar stages of sexual maturation, and showed only one abnormality in 438 fish.
- Total organochlorine pesticides and PCBs in tissues (lipid normalized) and phenols, PAHs, and phthalates in bed sediment (organic carbon normalized) each spanned at least two orders of magnitude among sites. Mean total dissolved pesticides in water varied within a factor of 10. There were significant correlations between PCBs and organochlorine pesticides, PCBs and PAHs, PAHs and phthalates, and phthalates and phenols.
- Of 15 correlations between biomarkers and contaminants that had p values less than 0.25 (males and females), 14 were negative.
- For male carp: (1) 17β-estradiol had a significant (α=0.05) negative correlation with total organochlorine pesticides; (2) 11-ketotestosterone had a significant negative correlation with phenols; and (3) E_2/11-KT ratio had a significant negative correlation with total dissolved pesticides.
- For female carp: (1) 11-ketotestosterone had a significant positive correlation with dissolved pesticides; and (2) E_2/11-KT ratio had a significant negative correlation with dissolved pesticides.
- The strongest patterns common to both males and females are: (1) negative correlations between the E_2/11-KT ratio and dissolved pesticides, and (2) negative correlations between phenols and both 17β-estradiol and 11-ketotestosterone.
- Notable differences between males and females are: (1) the significant negative correlation between 17β-estradiol and organochlorine pesticides shown in males and not in females, and (2) the significant positive correlation between 11-ketotestosterone and dissolved pesticides shown in females and not in males.

More information is needed in several areas to evaluate whether endocrine disruption is actually occurring in fish of some streams, what may be its cause, and whether there are biological effects on individuals or populations. A distinct shift in study design will be required from the "one sample from many sites" approach of the reconnaissance, to the "intensive study of a few sites" approach for more detailed study. A paired site approach is suggested for further investigation, with one "contaminated" site and one "reference site" located in the regions with the clearest evidence of endocrine disruption.

REFERENCES

Amdur, M.O., Doull J., and Klaassen C.D., eds., 1991, Casarett and Doull's toxicology, the basic science of poisons (4th ed.): New York, Pergamon Press, 1,033 p.

Atterwill, C.K., and Flack, J.O., 1992, Endocrine toxicology: Cambridge, England, Cambridge University Press, 475 p.

Babic-Gojmerac, T., Kniewald, Z., and Kniewald, J., 1989, Testosterone metabolism in neuroendocrine organs in male rats under atrazine and deethylatrazine influence: Journal of Steroid Biochemistry, v. 33, p. 141-146.

Barry, T.P., Santos, A.J.G., Furukawa, K., Aida, K., and Hanyu, I., 1990, Steroid profiles during spawning in male common carp: General and Comparative Endocrinology, v. 80, p. 223.

Bern, H.A., 1992, The fragile fetus, *in* Colborn Theo, and Clement, Coralie, eds., Chemically-induced alterations in sexual and functional development: The wildlife/human connection: Princeton New Jersey, Princeton Scientific Publishing Co., Advances in Modern Environmental Toxicology, v. 21, p. 9-16.

Chang, C.F., and Chen, M.R., 1990, Fluctuation in sex steroids and sex-binding protein during the development and annual cycle of the male common carp, (*Cyprinus carpio*): Comparative Biochemistry and Physiology, v. 97A, no. 4, p. 565-568.

Colborn, Theo, and Clement, Coralie, eds., 1992, Chemically induced alterations in sexual and functional development: the wildlife/human connection: Princeton New Jersey, Princeton Scientific Publishing Co., Advances in Modern Environmental Toxicology, v. 21, p. 403 p.

Colborn, Theo, Vom Saal, F.S., and Soto, A.M., 1993, Developmental effects of endocrine disrupting chemicals in wildlife and humans: Environmental Health Perspectives, v. 101, p. 378-384.

Copeland, P.A., and Thomas, P., 1988, The measurement of plasma vitellogenin levels in a marine teleost, the spotted seatrout (*Cynoscion nebulosus*) by homologous radioimmunoassay: Comparative and Biochemistry Physiology, v. 91B, p. 17-23.

Coupe, R.H., Goolsby, D.A., Iverson, J.L., Markovchick, D.J., and Zaugg, S.D., 1995, Pesticide, nutrient, water-discharge and physical-property data for the Mississippi River and some of its tributaries: April 1991-September 1992: U.S. Geological Survey Open-File Report 93-657, 116 p.

Crawford J.K., and Luoma, S.N., 1993, Guidelines for studies of contaminants in biological tissues for the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 92-494, 69 p.

Davis, W.P., and Bortone, S.A., 1992, Effects of kraft mill effluent on the sexuality of fishes: An environmental early warning?, *in* Colborn, Theo, and Clement, Coralie, eds., Chemically induced alterations in sexual and functional development: The wildlife/human connection: Princeton New Jersey, Princeton Scientific Publishing Co., Advances in Modern Environmental Toxicology, v. 21, p. 113-128.

Denison, M.S., Chambers, J.E., and Yarbrough, J.D., 1981, Persistent vitellogenin-like protein and binding of DDT in the serum of insecticide-resistant mosquito fish (*Gambusia affinis*): Comparative Biochemistry and Physiology, v. 69, no. 1, p. 109-112.

Down, N.E., Peter, R.E., and Leatherland, J.F., 1990, Seasonal changes in serum gonadotropin, testosterone, 11-ketotestosterone, and estradiol-17β levels and their relation to tumor burden in gonadal tumor-bearing carp x goldfish hybrids in the Great Lakes: General Comparative Endocrinology, v. 77, p. 192-201.

Fitzsimmons, J., 1990, Steroid hormones in male lake trout. Proceeding, round table on contaminant and reproductive problems in Salmonids: Winsor, Ontario, Canada (April 24-25, 1990), p. 29-35.

Folmar, L.C., 1993, Effects of chemical contaminants on blood chemistry of teleost fish: A bibliography and synopsis of selected effects: Environmental Toxicology and Chemistry, v. 12, no. 2, p. 337-375.

Folmar, L.C., Penslow, N.D., Rao, V., Chow, M., Crain, P.A., Enblom, J., Marcino, J., and Guillette, L.J., Jr., 1996, Vitellogenin induction and reduced serum testerone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant: Environmental Health Perspectives, v. 104, p. 1096-1101.

Furlong, E.T., Vaught, D.G., Merten, L.M., Foreman, W.T., and Gates, P.M., 1996, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of semivolatile organic compounds in bottom sediment by solvent extraction, gel permeation chromatographic fractionation, and capillary-column gas chromatography/mass spectrometry: U.S. Geological Survey Open-File Report 95-719, 67 p.

Gilliom, R.J., Alley, W.M. and Gurtz, M.E. 1995, Design of the National Water-Quality Assessment Program: Occurrence and distribution of water-quality conditions: U.S. Geological Survey Circular 1112, 33 p.

Goodwin, A.E., Grizzle, J.M., Bradley, J.T., and Estridge, B.H., 1992, Monoclonal antibody-based immunoassay of vitellogenin in the blood of male channel catfish (*Ictalurus punctatus*): Comparative Biochemistry and Physiology, v. 101B, no. 3, p. 441-446.

Gross, D.A., Gross, T.S., Johnson, B., and Folmar, L., 1995, Characterization of endocrine-disruption and clinical manifestations in large-mouth bass from Florida lakes, *in* SETAC World Congress, abstract book, Second SETAC World Congress, 16th Annual Meeting, Global environmental protection–science, politics, and common sense, 5–9 November, 1995, Vancouver, British Columbia, Canada: Pensacola, Florida, SETAC Press, p. 185.

Hileman, B., 1994, Environmental estrogens linked to reproductive abnormalities, cancer: Chemical and Engineering News, January 31, p. 19-23.

Hontela A., Dumont, P., Duclos, D., and Fortin, R., 1995, Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River: Environmental Toxicology and Chemistry, v. 14, no. 4. p. 725-731.

Hose, J.E., Cross, J.N., Smith, S.G., and Diehl, D., 1989, Reproductive impairment in a fish inhabiting a contaminated coastal environment off of Southern California: Environmental Pollution, v. 57, p. 139-148.

Hunter, G.A., and Donaldson, E.M., 1983, Hormonal sex control and its application to fish culture. *In* Hoar, W.S., Randall, D.J., and Donaldson, E.M., eds., Fish Physiology, v. 1X Reproduction: Orlando, Florida, Academic Press, p. 223-303.

Janssen, P.A.H, Lambert, J.G.D., Goos, J.J. Th., van Wezel, A.P., and Opperhuizen, A., 1995, Polluted harbor sediment and the annual reproductive cycle of the female flounder *Platichthys fiesus (L.)*, *in* SETAC World Congress, abstract book, Second SETAC World Congress, 16th Annual Meeting, Global environmental protection–science, politics, and common sense, 5–9 November, 1995, Vancouver, British Columbia, Canada: Pensacola, Florida, SETAC Press, p. 60.

Jearld, A., Jr., 1983, Age determination *in* Nielsen, L.A., Johnson, D.L., and Lampton, S.S., eds., Fisheries Techniques.: Bethesda, Maryland, American Fisheries Society, p. 301-324.

Johnson, L.L., Casillas, E., Collier, T.K., McCain, B.B., and Varanasi, U., 1988, Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington: Canadian Journal of Fisheries and Aquatic Science, v. 45, p. 2133-2146.

Laskey, J.W., and Berman, E., 1993, Steroidogenic assessment using ovary culture in cycling rats: Effects of bis(2-diethylhexyl) phthalate on ovarian steroid production: Reproductive Toxicology, v. 7, no. 1, p. 25-34.

Leatherland, J.F., 1992, Endocrine and reproductive function in Great Lakes salmon, *in* Colborn, Theo, and Clement, Coralie, eds., Chemically induced alterations in sexual and functional development: the wildlife/human connection: Princeton New Jersey, Princeton Scientific Publishing Co., Advances in Modern Environmental Toxicology, v. 21, p. 129-146.

Lehninger, A.L. 1982, Principles of biochemistry: New York, Worth Publishers, 1011 p.

Leiker, T.J., Madsen, J.E., Deacon, J.R., and Foreman, W.T., 1995, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of chlorinated pesticides in aquatic tissue by capillary-column gas chromatography with electron-capture detection: U.S. Geological Survey Open-File Report 94-710, 42 p.

Mayer, F.L., Versteeg, D.J., McKee, M.J., Folmar, L.C,. Graney, R.L., McClume, D.C., and Ruttner, B.A., 1992, Physiological and nonspecific biomarkers, *In* Huggett, R.J., Kimerle, R.A., Merrle, P.M., Jr., and Bergman, H.A., eds., Biomarkers, Biochemical Physiological, and Histological Markers of Anthropogenic Stress: Chelsea, Michigan, Lewis Publishers, p. 5-85.

McLachlan, J.A., ed., 1980, Estrogens in the Environment: New York, Elsevier Science, 427 p.

McLachlan, J.A., Newbold, P. R., Teng, C.T., and Korach, K.S., 1992, Environmental estrogens: Orphan receptors and genetic imprinting, *in* Colborn, Theo, and Clement, Coralie, eds., Chemically-induced alterations in sexual and functional development: The wildlife/human connection: Princeton New Jersey, Princeton Scientific Publishing Co., Advances in Modern Environmental Toxicology, v. 21, p. 107-112.

McLachlan, J.A. and S.F. Arnold, 1996, Environmental estrogens—Chemicals in the environment can mimic the effects of biological signaling molecules: American Scientist, v. 84, no. 5, p. 452-461.

McMaster, M.E., VanDerKraak, G.J., Portt, C.B., Munkittrick, K.R., Sibley, P.K., Smith, I.R., and Dixon, D.G., 1991, Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent: Aquatic Toxicology, v. 21, nos. 3-4, p. 199-218.

Meador, M.R., Cuffney, T.F., and Gurtz, M.E., 1993, Methods for sampling fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey, Open-File Report 93-104, 40 p.

Munkittrick, K.R., VanDerKraak, G.J., McMaster, M.E., and Portt, C.B., 1992, Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdown: Environmental Toxicology and Chemistry. v. 11, no. 10, p. 1427-1439.

Panek, F.M., 1987, Biology and ecology of carp. *In* Cooper, E.L., ed., Carp in North America: Bethesda, Maryland, American Fisheries Society, p. 1-13.

Peterson, G.L., 1993, Determination of total protein: Methods of Enzymology, v. 91, p. 95-121.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., and Sumpter, J.P., 1994, Estrogenic effects of effluents from sewage treatment works: Chemistry and Ecology, v. 8, no. 4, p. 275-285.

Richards, P.R., and Baker, D.B., 1993, Pesticide concentration patterns in agricultural drainage networks in the Lake Erie Basin: Environmental Toxicology and Chemistry, v. 12, no. 1, p. 13-26.

Sangalang, B., and Freeman, H.C., 1974, Effect of sublethal cadmium on maturation and testosterone, and 11-ketotestosterone production in vivo in brook trout (*Salvelinus fontinalis*): Biology of Reproduction, v. 11. p. 429-435.

Shelton, L.R., 1994, Field guide for collecting and processing stream-water samples for the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 94-455, 42 p.

Shelton, L.R., and Capel, P.D., 1994, Guidelines for collecting and processing samples of stream bed sediment for analysis of trace elements and organic contaminants for the National Water Quality Assessment Program: U.S. Geological Survey Open-File Report 94-458, 20 p.

Simic, B., Kniewald, Z., Davies, J.E., and Kniewald, J., 1991, Reversibility of the inhibitory effect of atrazine and lindane on cytosol 15 alpha-dihydrotestosterone-receptor complex formation in rat prostate: Bulletin of Environmental Contamination and Toxicology, v. 46, no 1, p. 92-100.

Singh, H., 1989, Interaction of xenobiotics with reproductive endocrine functions in a protogynous teleost (*Monopterus albus*): Response of Marine Organisms to Pollutants, v. 28, no. 1-4, p. 285-289.

Singh, S., and Singh, T.P., 1987, Evaluation of toxicity limits and sex hormone production in response to cythion and BHC in the vitellogenic catfish *Clarias batrachus*: Environmental Research, v. 42. p. 482–488.

Sol, S.Y, Lomas, D.P., Jacobson, J.C., Sommers, F.C., Anulacion, B.F., and Johnson, L.L., 1995, Effects of chronic contaminant exposure in reproductive development of English sole (*Pleuronectes vetulus*), *in* SETAC World Congress, abstract book, Second SETAC World Congress, 16th Annual Meeting, Global environmental protection–science, politics, and common sense, 5–9 November, 1995, Vancouver, British Columbia, Canada: Pensacola, Florida, SETAC Press, p. 60.

Specker, J., and Sullivan, C.V., 1994, Vitellogenesis in fishes: status and perspectives. *In* Davey, K.G., Peter, R.G., and Tobe, S.S., eds.: Ottawa, Canada, National Research Counsil of Endocrinology, p. 304-315.

Swain, W., Colborn, T., Bason, C., Howarth, R., Larney, L., Palmer, B., and Swackhamer, D., 1992, Exposure and effects of airborne contaminants: Draft report to Great Lakes Water Program, U.S. Environmental Protection Agency.

Thomas, P., 1988, Reproductive endocrine function in female atlantic croaker exposed to pollutants: Marine Environmental Research, v. 24, p. 179-183.

Tillitt, D.E., Ankley, G.T., Giesy, J.P., Ludwig, J.P., Kurita-Matsuba, H., Wesehloh, D.V., Ross, P.S., Bishop, C.A., Sileo, L., Stromborg, K.L., Larson, J., and Kubiak, T.J., 1992, Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes: Environmental Toxicology and Chemistry, v. 11, no. 9, p. 1281-1288.

Treinen, K.A., Dodson, W.C., Heindel, J.J., 1990, Inhibition of FSH-stimulated cAMP accumulation and progesterone production by mono (2-ethylyhexyl) phthalate in rat granulosa cell cultures: Toxicology of Applied Pharmacology, v. 106, p. 334-340.

U.S. Environmental Protection Agency, 1984, Guidance for the reregistration of pesticide products containing as the active ingredient Alachlor (090501): Washington, D.C.: U.S. Environmental Protection Agency, Office of Pesticide Programs, 104 p.

U.S. Fish and Wildlife Service, 1992, U.S. Fish and Wildlife Service National Contaminant Biomonitoring Program fish data file, data for 1969-1986 [3 ASCII database files]: Columbia, Missouri, U.S. Fish and Wildlife Service National Fisheries Contaminant Research Center (now the Midwest Science Center, Biological Resources Division, U.S. Geological Survey).

U.S. Geological Survey, 1986 [1990], Land use and land cover digital data from 1:250,000- and 1:100,000-scale maps, Data user guide 4: Reston, Virginia., U.S. Geological Survey, 25 p.

Waldock, M.J., Blackburn, M., Sheahan, D.A., Osborne, J.A., Sumpter, J.P., Jobling, S., and Routledge, E.J., 1994, Concentrations of alkylphenols in UK. sewage effluents, and the oestrogenic effects of trace concentrations in nonylphenol on male rainbow trout: Essex, England, Ministry of Agriculture, Fisheries and Food, project summary, 1 p.

Wams, T.J., 1987, Diethylhexylphthalate as an environmental contaminant—a review: Science of the Total Environment, v. 66, p. 1-16.

Webster, P.W., Canton, J.H., and Bisschop, A., 1985, Histopathological study of (*Poecilia reticulate*) (guppy) after long term beta hexachlorocyclohexane exposure: Aquatic Toxicology, v. 6, no. 4, p. 271-296.

White, R., Jobling, S., Hoare, S.A., Sumpter, J.P., and Parker, M.G., 1994, Environmentally persistent alkylphenolic compounds are estrogenic: Endocrinology, v. 135, no. 1, p. 175-182.

Zaugg, S.D., Sandstrom, M.K., Smith, S.G., and Fehlberg, K.M., 1995, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Pesticides in water by C-18 solid-phase extraction and capillary-column gas chromatography/mass spectrometry with selected-ion monitoring: U.S. Geological Survey Open-File Report 95-181, 49 p.

Appendix A. Characteristics and biomarker values for individual male adult carp

[pg/mL, picogram per milliliter; mg/mL, milligram per milliliter. —. no data]

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
Northeast									
Connecticut River at Thompsonville, Connecticut.	CR-T	9/13/1994	5	—	556	3,169	1,043	0.53	0.00
			5	1	610	4,328	1,937	0.31	0.00
			4	—	114	136	629	0.18	0.00
			5	1	1,120	6,276	2,109	0.53	0.00
			7	1	995	3,435	1,271	0.78	0.00
			4	1	1,227	2,811	1,394	0.88	0.00
			3	1	327	307	803	0.41	0.00
			7	1	923	2,615	2,161	0.43	0.00
			4	1	857	2,077	1,593	0.54	0.00
			4	1	869	968	1,462	0.59	0.00
			5	—	586	675	442	1.33	0.00
			2	—	673	6,427	1,876	0.36	0.06
Housatonic River at Woods Pond, Massachusetts.	HOR-WP	9/13/1994	—	—	—	—	—	—	—
Hudson River south of Lake Luzerne, New York.	HR-LL	9/27/1994	6	1	654	1,140	729	0.90	0.00
			5	1	1,070	2,229	1,426	0.75	0.02
			9	1	889	1,428	913	0.97	0.00
			1	1	1,183	1,690	1,081	1.09	0.08
			8	1	1,103	2,559	1,638	0.67	0.00
			—	1	742	1,844	1,180	0.63	0.00
			6	2	468	1,893	1,211	0.39	0.24
			2	2	72	2,203	1,410	0.05	0.09
			4	1	93	1,757	1,125	0.08	0.57
			3	1	339	1,018	651	0.52	0.15
			4	1	76	405	319	0.24	0.00
			2	1	405	734	528	0.77	0.00
			7	1	182	378	254	0.72	0.16
			4	2	253	574	458	0.55	0.38
			2	1	277	772	659	0.42	0.50
			5	1	446	685	510	0.87	0.00
			2	2	623	1,174	995	0.63	0.18
			1	1	323	781	626	0.52	0.00
			5	1	409	600	578	0.71	0.00
Hudson River near Poughkeepsie, New York.	HR-P	9/29/1994	7	1	183	1,884	1,145	0.16	0.00
			5	1	857	3,253	1,994	0.43	0.00
			6	1	503	4,889	3,012	0.17	0.00
			6	1	105	3,252	1,996	0.05	0.00
			7	1	105	2,949	1,806	0.06	0.00
			4	1	160	4,183	2,572	0.06	0.00
			3	1	304	4,111	2,528	0.12	0.00
			5	1	158	2,351	1,435	0.11	0.00
			4	1	178	2,954	1,809	0.10	0.00
			5	1	161	1,844	1,120	0.14	0.00
			6	1	186	3,493	2,144	0.09	0.00
			3	1	213	3,468	2,128	0.10	0.00
			5	1	262	2,865	1,754	0.15	0.00
			7	—	195	2,264	1,381	0.14	0.04
			5	1	59	1,729	1,049	0.06	0.00
			4	—	186	2,731	1,671	0.11	0.00
			3	1	37	2,621	1,602	0.02	0.00
			6	1	29	947	565	0.05	0.02

Appendix A. Characteristics and biomarker values for individual male adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
Mohawk River at Frankfort, New York.	MR-F	9/28/1994	6	1	149	2,593	1,653	0.09	0.00
			9	2	39	2,643	1,685	0.02	0.00
			1	2	77	1,971	1,259	0.06	0.17
			6	2	116	1,010	639	0.18	0.00
			8	2	99	887	558	0.18	0.00
			8	2	166	1,789	1,143	0.15	0.00
			1	1	198	2,503	1,904	0.10	0.00
			3	2	114	1,070	679	0.17	0.00
			9	1	189	3,440	2,186	0.09	0.00
			9	1	69	528	319	0.22	0.00
			1	1	89	1,404	895	0.10	0.00
			2	1	290	1,532	978	0.30	0.10
			4	2	91	1,147	728	0.13	0.00
			6	1	508	1,334	850	0.60	0.00
			6	1	172	462	275	0.63	0.01
			8	1	218	694	431	0.51	0.00
			6	—	47	308	167	0.28	0.18
			8	—	30	81	144	0.21	0.00
			9	—	59	595	365	0.16	0.05
			2	—	216	1,677	1,071	0.20	0.00
			2	—	377	721	638	0.59	0.00
			9	—	292	1,065	610	0.48	0.06
			6	—	509	930	526	0.97	0.00
			7	—	449	691	377	1.19	0.00
Anacostia River at Washington Ship Channel, District of Columbia.	AR-WSC	11/18/1994	8	1	317	774	495	0.64	0.00
			6	2	601	2,149	1,375	0.44	0.00
			10	2	218	549	341	0.64	0.00
Shenandoah River at Millville, West Virginia.	SR-M	11/16/1994	3	2	433	536	323	1.34	0.00
			3	2	111	568	344	0.32	0.00
			3	2	479	918	565	0.85	0.00
Mississippi River Basin									
Platte River at Louisville, Nebraska.	PR-L	9/1–2/1994	4	1	79	163	99	0.80	0.00
			9	1	59	1,733	1,109	0.05	0.00
			13	1	22	840	615	0.04	0.00
			9	1	53	2,137	2,007	0.03	0.00
			7	1	19	199	166	0.11	0.00
			8	—	380	1,255	1,032	0.37	0.60
			—	2	21	1,202	987	0.02	0.00
			8	1	17	338	262	0.06	0.00
			8	—	19	926	968	0.02	0.00
			7	—	36	145	132	0.27	0.00
			9	1	19	149	76	0.25	0.00
			9	1	127	321	123	1.03	0.01
			9	—	61	357	289	0.21	0.00

Appendix A. Characteristics and biomarker values for individual male adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
South Platte River at Denver, Colorado.	SPR-D	9/12/1994	7	—	235	1,394	818	0.29	0.00
			8	—	448	2,081	1,249	0.36	0.00
			—	—	419	2,522	1,528	0.27	0.00
			8	—	306	1,372	804	0.38	0.00
			7	1	118	1,515	894	0.13	0.00
			9	—	231	2,223	1,339	0.17	0.00
			11	—	57	1,107	639	0.09	0.00
			7	—	243	1,160	672	0.36	0.00
			—	—	42	1,546	913	0.05	0.00
			2	—	368	962	550	0.67	0.03
			10	—	20	1,373	805	0.02	0.00
			12	1	189	1,833	1,093	0.17	0.00
			13	—	25	869	494	0.05	0.00
			11	—	102	713	399	0.26	0.02
			7	—	362	1,018	585	0.62	0.00
			9	—	413	576	316	1.31	0.00
			—	—	247	419	221	1.12	0.00
			7	—	591	1,018	902	0.66	0.00
South Platte River near Kersey, Colorado.	SPR-K	9/13/1994	—	—	112	709	454	0.25	0.00
			14	—	75	957	612	0.12	0.00
			—	—	103	960	614	0.17	0.00
			13	—	131	286	183	0.72	0.00
			11	—	149	443	284	0.52	0.00
			12	—	51	237	152	0.34	0.00
			—	—	118	951	609	0.19	0.00
			13	—	85	704	450	0.19	0.00
			—	—	196	509	326	0.60	0.00
			12	—	91	1,096	701	0.13	0.00
			13	—	211	2,707	1,733	0.12	0.00
			10	—	66	2,114	1,353	0.05	0.00
			13	—	129	1,229	788	0.16	0.00
			12	—	31	1,129	723	0.04	0.00
			14	—	227	1,775	1,136	0.20	0.00
South Platte River at North Platte, Nebraska.	SPR-NP	9/14/1994	8	—	451	837	538	0.84	0.00
			—	—	466	906	723	0.64	0.00
			12	—	396	396	254	1.56	0.00
			—	—	627	949	629	1.00	0.00
			—	—	691	636	424	1.63	0.00
Sugar Creek at New Palestine, Indiana.	SC-NP	8/29/1994	4	—	871	2,397	1,524	0.57	0.00
			5	—	436	2,319	1,474	0.30	0.00
			6	—	502	2,061	1,306	0.38	0.00
			—	—	891	1,598	1,004	0.89	0.00
			1	—	488	1,174	726	0.67	0.00
			6	—	946	1,090	986	0.96	0.00
			3	—	1,491	2,544	1,619	0.92	0.00

Appendix A. Characteristics and biomarker values for individual male adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
White River at Hazleton, Indiana.	WR-H	9/1/1994	7	—	733	3,929	2,514	0.29	0.00
			3	—	659	3,578	2,288	0.29	0.00
			4	—	598	1,429	836	0.72	0.00
			6	—	396	1,754	755	0.52	0.00
			4	—	1,049	1,694	1,067	0.98	0.00
			5	—	546	2,384	1,515	0.36	0.83
			3	—	693	2,027	1,284	0.54	0.05
			4	—	1,324	2,736	1,743	0.76	0.00
			6	—	737	1,823	2,446	0.30	0.00
			4	—	1,728	3,248	2,075	0.83	0.00
			7	—	655	2,232	1,417	0.46	0.01
			1	—	652	4,030	2,580	0.25	0.02
			6	—	430	3,814	2,441	0.18	0.00
			7	—	1,145	4,068	2,604	0.44	0.00
			1	—	564	2,073	1,314	0.43	0.00
			5	—	443	1,775	2,416	0.18	0.00
			3	—	389	1,825	2,448	0.16	0.00
			4	—	869	2,699	1,720	0.51	0.06
			2	—	176	1,349	1,140	0.15	0.00
Northern Midcontinent									
Mullet River near Plymouth, Wisconsin.	MR-P	9/20/1994	6	—	735	923	882	0.83	0.00
			3	—	701	1,494	1,306	0.54	0.00
			6	—	704	701	634	1.11	0.00
			4	—	365	400	829	0.44	0.00
			6	—	399	396	255	1.56	0.02
			7	1	638	483	549	1.16	0.00
			7	—	859	687	726	1.18	0.02
			8	—	715	782	253	2.83	0.00
			6	1	820	1,691	940	0.87	0.00
			6	—	620	533	691	0.90	—
North Branch Milwaukee River near Random Lake, Wisconsin.	NBMR-RL	9/21/1994	6	1	759	803	732	1.04	0.00
			5	1	708	854	779	0.91	0.00
			7	1	483	457	422	1.14	0.00
			10	1	822	1,021	985	0.83	0.00
			6	1	821	912	842	0.98	0.02
			8	1	690	746	609	1.13	0.00
			3	1	899	916	792	1.14	0.00
			4	1	811	600	484	1.68	0.06
			7	1	571	504	267	2.14	0.00
			8	1	302	981	518	0.58	0.00
			3	1	751	1,043	628	1.20	0.01
			5	—	862	971	668	1.29	0.00
			5	1	828	680	619	1.34	0.00
			6	1	464	411	108	4.30	0.00
			7	1	214	158	262	0.82	0.00
			6	1	1,032	702	699	1.48	0.00
			4	2	1,296	1,178	449	2.89	0.00
			6	1	951	659	225	4.23	0.00
			7	1	222	534	423	0.52	0.03
			4	—	653	1,149	942	0.69	0.00
			7	—	1,285	434	736	1.75	0.00
			4	—	1,340	1,293	1,118	1.20	0.00

Appendix A. Characteristics and biomarker values for individual male adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
Otter Tail River above Breckenridge, Minnesota.	OR-B	9/12/1994	—	—	682	3,125	1,988	0.34	0.00
			6	1	1,097	1,628	1,017	1.08	0.00
			7	1	1,096	3,767	2,404	0.46	0.05
			7	1	1,105	4,585	2,935	0.38	0.09
			10	1	57	123	77	0.74	0.00
			—	1	123	651	383	0.32	0.00
			9	1	534	1,080	697	0.77	0.00
			10	1	376	433	329	1.14	0.00
			12	1	308	984	599	0.51	0.00
			7	—	426	1,320	837	0.51	0.00
Red River of the North near Breckenridge, Minnesota.	RRN-B	9/12/1994	8	1	694	431	105	6.61	0.00
			8	1	1,112	685	466	2.39	0.00
			10	1	778	529	404	1.93	0.00
			8	1	714	242	303	2.36	0.00
			8	1	489	586	427	1.15	0.00
			9	1	1,573	1,580	1,340	1.17	0.05
			10	1	925	3,540	2,221	0.42	0.00
			7	1	267	336	264	1.01	0.00
			10	1	463	410	431	1.07	0.00
			9	1	579	2,613	2,261	0.26	0.00
Red River of the North at Drayton, North Dakota.	RRN-D	9/14/1994	9	1	812	3,074	868	0.94	0.40
			8	2	750	1,986	1,947	0.39	0.00
			10	1	233	1,679	1,245	0.19	0.06
			9	1	100	369	1,048	0.10	0.00
			11	2	901	1,075	826	1.09	0.00
			7	2	342	289	658	0.52	0.00
			9	2	308	1,250	508	0.61	0.50
			8	2	156	1,723	771	0.20	0.00
			10	1	199	1,149	1,077	0.18	0.00
			14	1	641	191	706	0.91	0.00
Red River of the North at Grand Forks, North Dakota.	RRN-GF	9/15/1994	—	—	1,457	928	387	3.76	0.00
			—	—	1,696	2,586	1,507	1.13	0.00
			—	—	2,104	979	665	3.16	0.07
			—	—	465	542	868	0.54	0.00
Southern Midcontinent									
Trinity River below Dallas, Texas.	TR-D	12/14/1994	8	2	388	991	732	0.53	0.00
			9	2	128	684	521	0.25	0.00
West									
Don Pedro Reservoir, California (Tuolumne River).	DPR	10/6/1994	4	1	1,338	5,626	3,589	0.37	0.00
			3	1	2,123	6,454	4,121	0.52	0.00
			2	1	2,219	7,787	4,978	0.45	0.00
			3	1	2,319	7,009	4,478	0.52	0.00
			7	1	1,376	6,181	3,945	0.35	0.00
			7	1	2,220	7,389	4,722	0.47	0.00
			5	1	2,218	5,802	3,702	0.60	0.12
			2	1	2,412	6,920	4,421	0.55	0.00
			1	1	2,542	6,082	3,882	0.65	0.01
			2	1	2,253	6,484	4,141	0.54	0.00
			1	1	1,950	7,005	4,476	0.44	0.00
			2	1	2,066	7,835	5,009	0.41	0.00

Appendix A. Characteristics and biomarker values for individual male adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
San Joaquin River at Fremont Ford, California.	SJR-FF	10/5/1994	10	1	279	3,287	2,040	0.14	0.00
			6	1	189	3,236	2,008	0.09	0.00
			8	1	151	3,239	2,010	0.08	0.00
			6	1	122	1,680	1,035	0.12	0.00
			6	2	336	1,281	787	0.43	0.00
			7	1	90	1,596	983	0.09	0.00
			8	2	136	2,346	1,451	0.09	0.00
			4	1	309	1,784	1,100	0.28	0.00
			8	2	129	1,592	981	0.13	0.00
			4	1	295	1,121	687	0.43	0.00
			2	1	454	588	355	1.28	—
			8	1	126	848	517	0.24	0.00
			6	1	157	1,105	677	0.23	0.00
			2	1	139	759	462	0.30	0.00
			3	1	130	479	287	0.45	0.00
			5	1	272	1,544	951	0.29	0.00
San Joaquin River at Mossdale, California.	SJR-M	10/4/1994	6	1	596	1,606	1,511	0.39	0.00
			4	1	804	1,528	1,354	0.59	0.00
			3	1	307	716	458	0.67	0.00
			2	1	431	232	149	2.89	0.00
			4	1	434	636	408	1.06	0.00
			5	2	871	809	790	1.10	0.00
			3	2	242	765	762	0.32	0.00
			6	2	514	476	305	1.69	0.00
			4	1	563	740	473	1.19	0.00
			5	1	601	757	739	0.81	0.00
Mill Race Pond at Springfield, Oregon.	MRP-S	10/20/1994	3	—	227	646	414	0.55	0.57
			3	—	135	502	321	0.42	0.39
			1	—	23	1,131	723	0.03	0.00
			3	1	124	1,224	783	0.16	0.00
			3	—	174	1,754	1,122	0.16	0.00
			2	—	185	1,904	1,218	0.15	0.00
			2	1	135	1,014	649	0.21	0.16
			3	1	124	1,358	869	0.14	0.00
			3	1	132	131	84	1.57	0.00
			4	1	30	596	382	0.08	0.00
			3	2	46	872	558	0.08	0.00
			3	2	47	435	279	0.17	0.00
			1	1	79	1,047	751	0.11	0.00
			3	2	217	1,385	590	0.37	0.00
			2	1	213	1,417	886	0.24	0.00

Appendix B. Characteristics and biomarker values for individual female adult carp

[pg/mL, picogram(s) per milliliter; mg/mL, milligram(s) per milliliter. —, no data]

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
Northeast									
Connecticut River at Thompsonville, Connecticut.	CR-T	9/13/1994	—	—	4605	3,486	2,740	1.68	36.00
			5	3	2,729	2,173	1,962	1.39	25.60
			7	2	4,358	2,705	1,621	2.69	38.00
			8	2	2,564	2,090	1,532	1.67	38.00
			5	3	1,996	1,420	924	2.16	38.30
			8	2	7,703	5,418	1,663	4.63	26.60
			5	3	2,505	2,332	1,537	1.63	37.70
			6	3	1,982	1,663	1,368	1.45	21.30
			6	3	1,646	1,106	769	2.14	
Housatonic River at Woods Pond, Massachusetts.	HOR-WP	9/13/1994	5	3	4,195	784	621	6.76	60.00
			6	3	5,447	851	722	7.54	12.90
Hudson River south of Lake Luzerne, New York.	HR-LL	9/27/1994	2	3	2,216	177	113	19.61	17.00
			8	3	3,964	1,524	920	4.31	20.00
			3	3	3,244	344	220	14.75	8.00
			2	3	1,281	542	346	3.70	22.00
			4	3	5,780	409	261	22.15	29.00
			4	3	1,372	298	272	5.04	24.00
			10	3	1,002	231	217	4.62	23.00
Hudson River near Poughkeepsie, New York.	HR-P	9/29/1994	9	3	4,426	2,608	1,594	2.78	26.00
			7	3	2,429	545	318	7.64	35.00
			5	3	4,141	1,401	846	4.90	31.00
			8	3	3,696	2,793	1,702	2.17	19.00
			3	3	3,364	1,921	1,168	2.88	35.60
			4	3	3,626	2,281	1,391	2.61	39.60
			8	3	1,756	1,514	916	1.92	43.50
			6	3	4,476	2,682	1,640	2.73	50.20
			3	3	4,499	2,023	1,231	3.66	35.80
			3	3	3,773	1,661	1,007	3.75	33.90
			3	3	435	523	304	1.43	31.00
Mohawk River at Frankfort, New York.	MR-F	9/28/1994	9	3	594	1,212	771	0.77	13.10
			8	3	1,279	677	419	3.05	19.30
			8	3	730	962	608	1.20	17.40
			7	3	656	1,263	804	0.82	11.60
			1	3	562	699	435	1.29	19.90
			8	3	1,753	1,597	1,130	1.55	11.50
			10	3	4,741	2,363	1,508	3.14	9.50
			4	3	625	136	153	4.09	10.80
			8	3	1,544	710	441	3.50	7.70
			3	3	1,062	528	320	3.32	12.20
			2	3	1,425	1,518	969	1.47	15.60
			11	3	834	1,523	972	0.86	9.20
			1	3	4,347	371	213	20.41	6.40
			11	3	1,559	155	145	10.75	9.40
			5	3	3,713	614	377	9.85	19.60
			1	—	2,512	260	115	21.84	26.60

Appendix B. Characteristics and biomarker values for individual female adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
Anacostia River at Washington Ship Channel, District of Columbia.	AR-WSC	11/18/1994	5	3	631	1,759	1,125	0.56	32.20
			11	3	877	946	605	1.45	39.60
			13	3	782	301	66	11.85	24.30
			7	3	1,765	1,503	1,089	1.62	17.20
			3	3	323	528	338	0.96	6.64
			12	2	633	561	359	1.76	17.71
			5	3	696	140	90	7.73	34.29
			4	3	1,018	966	746	1.37	38.42
			9	3	605	248	159	3.81	16.30
			9	3	540	1,475	944	0.57	28.22
Shenandoah River at Millville, West Virginia.	SR-M	11/16/1994	6	3	1,760	705	430	4.09	19.30
			2	3	1,275	411	245	5.20	5.80
			—	3	536	419	250	2.14	20.40
			3	3	1,440	397	237	6.08	18.50
			5	3	1,366	632	385	3.55	18.30
			6	3	1,639	846	520	3.15	14.80
			3	3	1,448	1,130	700	2.07	14.30
			8	3	1,045	76	37	28.24	8.90
			1	3	880	312	183	4.81	15.55
			6	2	1,298	156	86	15.09	11.80
			3	3	348	21	13	26.77	16.40
			2	3	1,294	730	443	2.92	17.97
			7	3	315	494	271	1.16	13.60
			3	3	1,580	155	85	18.59	13.10
Mississippi River Basin									
Platte River at Louisville, Nebraska.	PR-L	9/01/1994	7	3	1,104	614	515	2.14	20.00
			5	3	862	3,066	1,789	0.48	28.00
			8	—	901	3,027	2,173	0.42	26.50
			9	3	538	984	986	0.55	28.00
			10	—	448	2,532	2,332	0.19	31.50
			—	—	550	144	87	6.32	24.50
			12	3	505	269	200	2.53	0.00
			13	—	40	135	180	0.22	0.00
			—	2	243	564	536	0.45	72.50
			7	3	129	174	152	0.85	30.00
			8	3	321	401	323	0.99	18.50
			11	3	385	1,880	992	0.39	23.00
			8	3	166	407	295	0.56	26.00

Appendix B. Characteristics and biomarker values for individual female adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
South Platte River at Denver, Colorado.	SPR-D	9/12/1994	9	—	1,312	991	1,192	1.10	0.00
			8	3	3,249	2,113	1,270	2.56	33.50
			9	3	1,787	1,189	694	2.58	34.00
			10	3	2,851	2,477	1,500	1.90	16.00
			8	—	1,249	782	440	2.84	0.00
			9	3	2,643	2,069	1,243	2.13	0.00
			8	3	2,863	1,884	1,127	2.54	16.00
			6	3	1,477	1,412	829	1.78	26.00
			8	3	791	651	360	2.20	30.70
			10	3	1,666	1,377	808	2.06	15.80
			9	3	1,943	1,635	970	2.00	14.70
			8	3	1,863	1,160	674	2.76	41.50
			8	3	1,523	1,218	708	2.15	15.20
			8	3	1,416	908	517	2.74	12.70
			—	3	1,829	1,833	1092	1.68	14.60
			—	3	1,724	1,059	610	2.83	28.00
			7	3	952	356	182	5.23	39.50
			7	—	1,983	1,749	1,040	1.91	0.00
			8	3	971	776	436	2.23	12.23
			5	3	1,536	546	296	5.19	46.50
			8	3	1,297	866	992	1.32	31.50
South Platte River near Kersey, Colorado.	SPR-K	9/13/1994	—	3	2,320	1,871	1,198	1.94	37.40
			—	3	2,038	1,953	1,250	1.63	27.00
			—	3	2,152	747	478	4.50	34.00
			—	3	2,288	1,736	1,111	2.06	29.10
			12	3	1,329	1,169	748	1.78	27.70
			12	3	2,195	1,422	910	2.41	32.70
			—	3	2,163	1,218	780	2.77	21.40
			12	3	1,891	1,399	895	2.11	21.60
			13	3	1,464	1,367	875	1.67	23.80
				3	1,349	1,221	782	1.73	33.80
			12	3	2,150	1,499	958	2.24	32.50
			—	3	1,976	1,014	649	3.05	32.70
			11	—	1,546	556	536	2.88	14.70
			14	3	1,438	598	383	3.76	21.00
			12	—	762	395	253	3.01	45.90
			14	3	851	309	198	4.30	25.80
			—	3	1,094	472	302	3.62	25.10
			14	3	1,177	409	261	4.51	25.70
			—	3	1,885	519	332	5.68	25.30
			—	3	819	381	244	3.36	39.90
			13	3	633	289	185	3.42	25.20
			13	—	559	321	205	2.73	38.80
			—	—	1,428	566	363	3.93	29.30
			12	—	951	569	365	2.61	38.00
			13	—	1,518	615	394	3.85	28.70

Appendix B. Characteristics and biomarker values for individual female adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
South Platte River at North Platte, Nebraska.	SPR-NP	9/14/1994	—	3	1,873	655	421	4.45	28.60
			—	3	1,005	597	383	2.62	19.20
			—	3	1,356	527	338	4.01	23.60
			—	3	1,074	535	343	3.13	30.10
			—	3	838	665	427	1.96	29.60
			—	3	719	675	433	1.66	24.00
			—	—	745	434	278	2.68	1.76
			—	3	1,682	338	217	7.75	14.00
			—	3	813	354	227	3.58	37.40
			—	2	1,469	660	409	3.59	23.30
			—	3	561	261	180	3.12	30.97
			—	3	1,889	979	352	5.37	27.56
Sugar Creek at New Palestine, Indiana.	SC-NP	8/29/1994	4	3	1,423	1,477	925	1.54	28.40
			3	3	924	815	1,146	0.81	29.20
			—	3	504	739	436	1.16	24.10
			5	—	656	985	1,257	0.52	0.00
			2	3	577	502	942	0.61	27.10
			6	3	827	665	670	1.23	31.60
			7	3	525	417	737	0.71	33.40
			5	3	1,245	819	797	1.56	33.30
			2	3	601	218	383	1.57	16.90
White River at Hazleton, Indiana.	WR-H	9/01/1994	8	3	1,939	1,251	1,429	1.36	23.80
			3	3	1,130	268	440	2.57	33.90
			4	3	1,559	810	791	1.97	34.70
			6	3	2,087	1,825	1,152	1.81	24.40
			8	3	2,125	1,321	1,474	1.44	32.90
			3	3	2,485	1,785	1,126	2.21	28.90
			7	3	2,799	1,277	794	3.53	32.50
			4	3	1,283	640	681	1.88	26.10
			2	3	1,521	580	325	4.68	30.80
			8	3	1,957	1,949	1,881	1.04	21.40
			2	3	884	1,228	862	1.03	27.00
			4	3	1,328	1,614	1665	0.80	31.00
			4	3	1,774	1,164	1,373	1.29	29.70
			3	3	1,890	1,545	1,621	1.17	31.50
			5	3	1,248	1,118	1,091	1.14	37.20
			4	3	2,313	1,084	1,969	1.18	29.20
			7	3	948	1,132	1,342	0.71	41.10
Northern Midcontinent									
Mullet River near Plymouth, Wisconsin.	MR-P	9/20/1994	7	2	2,976	86	590	5.04	29.60
			9	3	3,830	111	503	7.61	26.20
			9	3	2,398	430	686	3.50	24.60
			9	—	1276	99	72	17.72	28.60
			3	3	3245	244	532	6.10	20.90
			8	3	2,419	779	441	5.49	30.80
			6	3	1,725	948	782	2.21	36.20
			5	3	2,580	1,108	1,503	1.72	22.40
			5	3	1,837	182	779	2.36	32.30
			5	3	1,410	646	948	1.49	24.60

Appendix B. Characteristics and biomarker values for individual female adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
North Branch Milwaukee River near Random Lake, Wisconsin.	NBMR-RL	9/21/1994	8	3	1,178	942	731	1.61	16.00
			4	3	2,253	1,274	1,062	2.12	29.50
			8	3	354	321	223	1.59	22.50
			10	3	6,147	1,842	2,020	3.04	30.50
			9	2	2,233	1,109	985	2.27	43.00
			7	3	876	417	84	10.43	47.50
			8	3	2,742	2,187	1,400	1.96	32.00
			8	3	4,466	809	323	13.83	29.50
			6	3	1926	154	373	5.16	22.00
			3	3	1,117	881	984	1.14	19.50
			6	3	1,254	148	564	2.22	20.50
			3	3	1,853	1,092	945	1.96	31.50
			10	3	2,877	219	114	25.24	19.00
			7	3	5,719	397	141	40.56	16.00
			8	3	4,128	301	254	16.25	16.00
			4	3	3,094	568	192	16.12	16.50
			7	3	1,707	351	364	4.69	18.70
Otter Tail River above Breckenridge, Minnesota.	OR-B	9/12/1994	6	3	5,114	2,932	1,863	2.75	30.90
			8	3	5,162	2,851	1,810	2.85	29.60
			8	3	9,942	5,675	3,641	2.73	32.10
			9	3	3,213	1,568	978	3.29	30.00
			8	3	2,143	466	262	8.18	25.80
			9	3	2,154	786	471	4.57	32.40
			9	3	1,086	635	372	2.92	33.20
			8	3	1,288	349	187	6.89	34.10
			8	3	421	94	51	8.26	28.70
			8	—	816	817	490	1.67	34.40
Red River of the North near Breckenridge, Minnesota.	RRN-B	9/12/1994	11	3	2,291	779	240	9.55	31.90
			12	3	1,475	143	117	12.61	31.00
			7	3	808	719	443	1.82	23.40
			8	3	1,368	175	63	21.71	33.50
			12	3	1,154	282	337	3.42	35.10
			—	3	462	404	143	3.23	29.90
			8	3	1,090	354	540	2.02	28.80
			10	3	1,728	781	190	9.10	28.60
			11	2	1,208	470	467	2.59	35.30
			9	3	6,852	650	178	38.49	36.60
			8	3	2,953	243	208	14.20	38.50
			9	3	2,609	536	117	22.30	27.80
			12	3	2,534	830	481	5.27	35.00
			11	3	1,013	725	445	2.28	41.00
Red River of the North at Drayton, North Dakota.	RRN-D	9/14/1994	12	3	3,530	1,333	311	11.35	23.50
			10	3	1,290	1,401	825	1.56	21.20
			10	3	1,212	383	206	5.88	22.00
			12	3	2,740	384	248	11.05	24.80
			12	3	670	61	231	2.90	31.20
			10	3	533	495	124	4.30	30.20
			12	3	1,822	1,690	832	2.19	35.00
			—	3	723	841	410	1.76	31.40
			13	2	552	561	127	4.35	0.43
			5	3	970	121	327	2.97	29.40
			9	3	1,352	487	123	10.99	44.10

Appendix B. Characteristics and biomarker values for individual female adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
Red River of the North at Grand Forks, North Dakota.	RRN-GF	9/15/1994	—	—	8,075	2,111	1,656	4.88	30.00
			—	—	4,249	491	1,329	3.20	19.00
			—	—	673	657	57	11.81	23.50
			—	—	4,811	1,302	563	8.55	8.50
			—	—	4,697	535	806	5.83	28.00
			—	—	1,774	292	307	5.78	26.00
			—	—	443	843	149	2.97	87.80
			—	—	1,720	4,169	2,639	0.65	16.00
			—	—	7,763	2,138	596	13.03	7.80
			—	—	1,226	870	848	1.45	25.00
Southern Midcontinent									
Clear Creek near Sanger, Texas.	CC-S	12/13/1994	7	3	491	370	237	2.07	12.80
			8	3	337	189	79	4.27	7.00
			8	3	558	183	109	5.12	16.50
			8	3	415	113	115	3.61	14.00
			8	3	110	69	57	1.93	8.00
Trinity River below Dallas, Texas.	TR-D	12/14/1994	6	3	1,527	513	413	3.70	7.10
			8	3	947	553	412	2.30	12.80
			8	3	2,922	813	654	4.47	10.50
			10	3	1,036	340	265	3.91	12.30
			3	3	2,110	545	480	4.40	7.00
West									
Don Pedro Reservoir, California (Tuolumne River).	DPR	10/06/1994	6	3	2,929	1,414	1,416	2.07	26.00
			7	3	3,121	2,241	2,168	1.44	38.00
			9	3	3,156	4,504	2,868	1.10	30.00
			5	3	5,922	2,925	1,854	3.19	30.40
			3	3	4,553	3,996	2,542	1.79	41.30
			3	3	3,532	2,670	1,691	2.09	22.90
			1	3	4,344	3,884	2,470	1.76	31.00
			2	3	6,398	4,478	2,851	2.24	34.90
			2	3	3,623	2,774	1,757	2.06	30.70
San Joaquin River at Fremont Ford, California.	SJR-FF	10/05/1994	5	3	287	683	414	0.69	38.50
			10	3	1,996	1,898	1,172	1.70	42.60
			3	3	1,390	1,401	861	1.61	31.30
			13	3	1,400	1,391	855	1.64	21.90
			8	3	1,379	1,576	970	1.42	33.80
			3	3	2,299	1,229	778	2.96	38.00
			7	2	875	645	391	2.24	39.60
			5	3	896	1,405	864	1.04	31.20
			9	3	333	1,253	769	0.43	34.50
			5	3	456	1,392	856	0.53	34.80
			6	3	1,451	1,036	634	2.29	30.80
			6	3	1,006	1,307	1,085	0.93	36.00
			6	3	411	726	441	0.93	35.60
			5	3	1,847	1,750	1,079	1.71	31.20
			4	3	392	322	189	2.07	29.80
			7	3	253	377	224	1.13	22.90
			4	—	204	301	177	1.15	2.00

Appendix B. Characteristics and biomarker values for individual female adult carp—Continued

Site name and location	Site code	Sampling date	Age (years)	Sexual maturation	17β-Estradiol (pg/mL)	Testosterone (pg/mL)	11-Ketotestosterone (pg/mL)	E_2/11-KT	Vitellogenin (mg/mL)
San Joaquin River at Mossdale, California.	SJR-M	10/04/1994	5	3	2,126	851	834	2.55	44.70
			6	3	2,298	803	514	4.47	42.60
			5	3	2,503	819	808	3.10	42.20
			3	3	808	536	354	2.28	34.30
			6	3	2,735	1,016	994	2.75	—
			6	3	3,196	1,316	1,144	2.79	37.40
			2	3	2,384	1,001	971	2.46	34.80
			7	3	2,778	823	807	3.44	37.90
			5	3	1,445	777	497	2.91	40.20
			2	3	2,784	813	520	5.35	32.50
			8	3	1,159	658	659	1.76	40.70
			4	2	135	191	121	1.12	47.90
			7	—	1,032	626	401	2.57	46.40
			4	—	377	363	232	1.63	39.60
			2	3	658	371	238	2.77	36.30
			3	3	939	687	439	2.14	28.60
			4	2	500	636	407	1.23	25.70
			—	—	784	508	325	2.41	31.40
Mill Race Pone at Springfield, Oregon.	MRP-S	10/20/1994	2	3	1,044	609	1,030	1.01	20.40
			4	—	860	943	603	1.43	3.60
			3	—	67	10	22	3.05	2.30
			2	2	1,522	1,175	752	2.02	1.50
			3	2	215	486	311	0.69	0.15
			3	—	1,232	1,048	670	1.84	0.00
			3	—	875	2,715	1,738	0.50	23.49
			2	3	358	404	907	0.40	15.00
			3	3	335	499	191	1.75	6.90