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Water-Resources Investigations Report 00–4202

U.S. Department of the Interior
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By Kathy E. Lee[1] Vicki S. Blazer[1], Nancy D. Denslow[2], Robert M. Goldstein[1], and Philip J. Talmage[3]

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CONVERSION FACTORS AND ABBREVIATIONS

Multiply metric unit	By	To obtain inch-pound unit
micrometers (μm)	0.0000393	inch (in.)
centimeter (cm)	0.393	inch (in.)
millimeters(mm)	0.0393	inch (in.)
meter (m)	3.281	foot (ft)
gram (g)	0.035	ounce avoirdupois (oz)
liter (L)	0.264	gallon (gal)
square kilometer (km^2)	0.3861	square mile (mi^2)
degrees Celsius (°C)	1.8 (Temp. ^oC) + 32	degrees Fahrenheit (°F)
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
cubic meter per second (m^3/s)	22.82	million gallons per day(Mgal/day)

Concentrations of substances in fish plasma are given milligrams per milliliter (mg/mL), micrograms per milliliter (μg/mL), nanograms per milliliter (ng/mL), or picograms per milliliter (pg/mL). A milligram is one thousandth of a gram, a microgram is one millionth of a gram, a nanogram is one billionth of a gram, a picogram is one trillionth of a gram, and a milliliter is one thousandth of a liter.

Use of Biological Characteristics of Common Carp (*Cyprinus carpio*) to Indicate Exposure to Hormonally Active Agents in Selected Minnesota Streams, 1999

By Kathy E. Lee, Vicki S. Blazer, Nancy D. Denslow, Robert M. Goldstein, and Philip J. Talmage

ABSTRACT

The presence of hormonally active agents (HAAs) was determined in selected Minnesota streams using biological characteristics (measures of endocrine disruption) of common carp (*Cyprinus carpio*) exposed to wastewater treatment plant (WWTP) effluent and runoff from agricultural and forested land. Four biological characteristics of common carp were used as indicators of HAAs in the streams selected for this study: (1) high concentrations of vitellogenin in male fish and low concentrations in female fish, (2) high or low plasma concentrations of the sex steroid hormones (17β-estradiol and 11-ketotestosterone), (3) low gonado-somatic index (GSI) (gonad weight divided by total body weight multiplied by 100) values, and (4) abnormal gonad histopathology (high percent of atretic oocytes in female ovaries and high percent ceroid/lipofuscin tissue in male or female gonads). The study design was a paired site approach targeting sites downstream and upstream of WWTP discharges on different streams. Male (221 individuals) and female (201 individuals) common carp were collected using electrofishing techniques from seven streams with sites at two locations (upstream and downstream of WWTPs), and eight sites located downstream of WWTPs with no upstream-paired sites. Samples were collected between August 3 and September 13, 1999.

The presence of HAAs in selected Minnesota streams was indicated by biological characteristics in common carp. Biological characteristics used in this study identified WWTP effluent as a potential source of HAAs. Additionally, fish located at sites upstream of WWTP effluent primarily draining agricultural land show indications of HAAs, which may be the result of agricultural runoff or other sources of HAAs. There was variability among all sites and among sites within each site group. Differences among sites may be due to differences in water chemistry or fish exposure time. Natural variation in the biological characteristics may account for some of the differences observed in this study. This study and others indicate the presence of HAAs in surface water and the potential signs of endocrine disruption in resident fish populations. Detailed controlled studies could confirm the effects of particular chemicals such as pesticides or components of WWTPs on fish reproduction and population structure.

INTRODUCTION

Concern about the effects of chemicals in the environment that act as hormonally active agents (HAAs) is widespread (Colburn and Clement, 1992; Guillette and others, 1995; Goodbred and others, 1997). HAAs may interfere with natural regulation of the endocrine system of animals by either mimicking or blocking the function of natural hormones (U.S. Environmental Protection Agency, 1997; Kime, 1998). A variety of anthroprogenic chemicals (xenobiotics) including organochlorine compounds, surfactants (alkylphenols), plasticizers, coatings, detergent breakdown products (nonylphenols (NP)), human excreted estrogens (17β-estradiol and 17α-ethynylestradiol), agricultural pesticides, and some trace elements may act as HAAs and interfere with normal endocrine function in animals including humans (U.S. Environmental Protection Agency, 1997). It has also been suggested that HAAs may be the cause of an increased incidence of breast and ovarian cancer, and endometriosis in females (Davis and others, 1993), cryptorchidism, testicular and prostate cancer, and diminished sperm counts in males (Sharpe and Skakkebaek, 1993) although these hypotheses have not been substantiated (National Research Council, 1999).

Two potential sources of HAAs to streams in Minnesota are treated sewage (domestic and industrial) and runoff from agricultural or forested land. Domestic and industrial sewage may contain a variety of chemicals including natural and synthetic estrogens (Tabak and others 1981; Desbrow and others, 1998), alkylphenolic compounds (Ahel and others, 1994a and 1994b; Barber and others, 2000), and bisphenol-A that are known to interact with the estrogen receptors in fish (Routledge and Sumpter, 1996; Nagel

and others, 1997; Desbrow and others, 1998). Runoff from agricultural land and atmospheric deposition of pesticides has resulted in the presence of pesticides in streams. Herbicides including atrazine, cyanazine, alachlor and metolachlor are commonly detected in streams draining agricultural areas in Minnesota (Minnesota Department of Agriculture, 1996; Fallon and others, 1997).

Fish are important organisms for indicating the extent of HAAs in surface water because they are directly exposed to contaminants, and their endocrine systems have many physiological similarities to mammals, including humans. Evidence of endocrine disruption in fish was found in response to a variety of potential HAAs including wastewater treatment discharge (Purdom and others, 1994; Sumpter and Jobling, 1995; and Folmar and others, 1996), pulp and paper mill discharges (Van der Kraak, 1992), and agricultural chemicals (Goodbred and others, 1997; Grady and others, 1998; Kime, 1998).

Potential effects of HAAs on fish include disruption of the endocrine system, specifically reproductive function. Effects of HAAs on fish reproductive systems include changes in sex steroid hormone synthesis, degenerative changes in the ovaries and testes including intersex conditions (oocytes in testes tissue), abnormal oogenesis and spermatogenesis, reduced sperm viability and motility, reduced gonad size, reduced fertility, reduced viability of young, and changes in the timing of sexual reproduction (U.S. Environmental Protection Agency, 1997; and Kime, 1998).

Endocrine disruption in common carp (*Cyprinus carpio*) and walleye (*Stizostedion vitreum*) was documented in Minnesota during 1995–96. Several male carp collected in the effluent channel from the Minneapolis and St. Paul Metropolitan Wastewater Treatment Plant effluent channel had elevated concentrations of vitellogenin and were considered to be estrogenized by the effluent (Folmar and others, 1996).

Although evidence of HAAs has been documented in Minnesota by the presence of endocrine disruption in fish within a wastewater treatment plant (WWTP) channel consisting of 100 percent effluent from a major metropolitan area, little is known about the presence of HAAs in streams receiving less WWTP effluent or from streams that drain agricultural and forested land. A study was conducted to determine the presence of HAAs in selected Minnesota streams using biological characteristics (measures of endocrine disruption) of common carp exposed to WWTP effluent and runoff from agricultural and forested land. The study was conducted cooperatively among the U.S Geological Survey (USGS), the Minnesota Pollution Control Agency (MPCA), the Minnesota Department of Natural Resources (MDNR), and the Legislative Commission on Minnesota Resources (LCMR).

The purpose of this report is to present the results of this study. Specifically, this report describes: (1) the influence of gonad maturation and age on biological characteristics (2) biological characteristics as indicators of HAAs, and (3) relations of biological characteristics to WWTP discharges and land use. Results presented in this report were based on common carp collected at 22 sites from 15 streams in Minnesota during August and September 1999.

Brief Description of Fish Endocrine Systems

Fish reproduction involves a complex chain of hormonal and developmental events. Gonadal recrudescence is the growth of the gonad from the regressed to the fully mature state (Matty, 1985; Kime, 1998). After reaching the fully mature state, spawning occurs and recrudescence begins again. Common carp spawning activity is the greatest during May and June when water temperatures are between 16 and 23.9^{o}C (Becker, 1983; Billard, 1999). Common carp begin recrudescence immediately after spawning and by autumn they are ready for spawning the next spring when conditions are optimal (Down and others, 1990). External signals such as photoperiod and/or water temperature initiate gonadal recrudescence, which is modulated by the endocrine system. These external signals stimulate the hypothalamus gland to produce gonadatrophin-releasing hormone (GnRH) (fig. 1). GnRH stimulates the pituitary gland to produce gonadatrophins (GtH), which stimulate the synthesis of sex steroid hormones in the ovaries and testes. Testosterone is present in both male and female fish (Matty, 1985; Chang and Chen, 1990; Kime, 1998;). Testosterone is converted to 17β-estradiol through aromatase action or to 11-ketotestosterone via 11β-hydroxytestosterone (Kime, 1998). The function of 11-ketotestosterone in male fish is for gonadal maturation and for the development of secondary sex characteristics (Matty, 1985, Mylonas and others, 1997; Cavaco and others; 1998, Kime, 1998; Todo and others, 1999), while its function in female fish is not clear. In females, 17β-estradiol serves as a major hormone responsible for oocyte development (Matty, 1985). A major role of 17β-estradiol in females is to stimulate the liver to produce vitellogenin. Vitellogenin is a protein that is the precursor to egg yolk proteins and is responsible for increased gonad weight in females during gonadal recrudescence (Specker and Sullivan, 1994). In male fish, vitellogenesis is stimulated in the liver cells (hepatocytes) when they are exposed to various natural and synthetic estrogens and xenobiotics such as NP (Jobling and Sumpter, 1993). Concentrations of measurable amounts of plasma vitellogenin in male fish are usually very low (ng/mL) or undetectable, thus making the presence of vitellogenin in male fish an indicator of the presence of estrogen or estrogenic chemicals in the environment (Purdom and others, 1994, Sumpter and Jobling, 1995, Folmar and others, 1996). Although the presence of vitellogenin concentrations in male common carp under

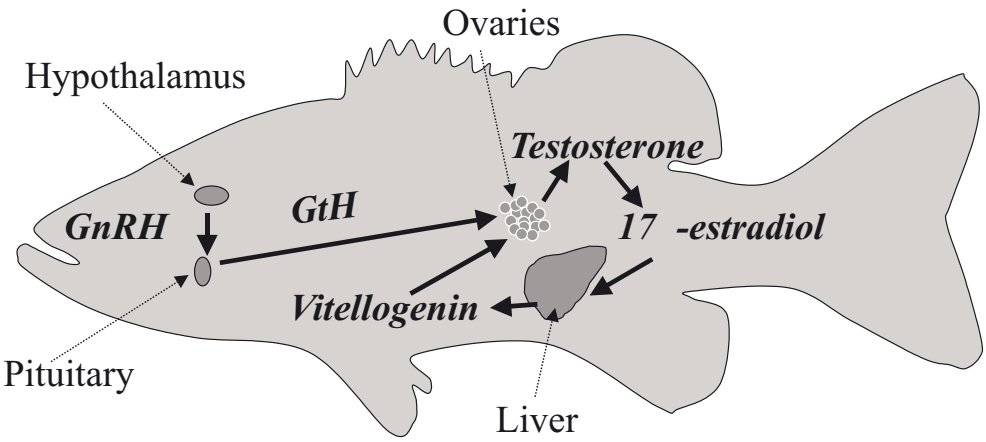

Figure 1. Endocrine regulation of selected sex steroid hormones and vitellogenin synthesis in female fish (modified from Kime, 1998; GnRH, gonadotrophin-releasing hormone; GtH, gonadotrophin).

natural conditions is not considered normal, Jobling and others (1998) measured concentrations of 0.0001 mg/mL, and Sumpter and Jobling (1995) measured 0.01 mg/mL for male fish at control sites. This suggests that some low concentrations of vitellogenin may be present in male fish. The effect of vitellogenin in male fish is largely unknown; however, elevated vitellogenin concentrations in flounder (Herman and Kincaid, 1988), and in trout (Hammer and others, in press) caused renal failure and subsequent death. Normal ranges in vitellogenin, 17β-estradiol, and 11-ketotestosterone concentrations in female and male common carp have not been established. Means or ranges of 17β-estradiol, 11-ketotestosterone, and testosterone concentrations reported in the literature are listed in table 1.

Acknowledgments

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APPROACH AND METHODS

Four biological characteristics of common carp used as indicators of HAAs in the streams selected for this study are: (1) High concentrations of plasma vitellogenin in male fish and low concentrations in female fish, (2) high or low plasma concentrations of the sex steroid hormones (17β-estradiol and 11-ketotestosterone), (3) low gonado-somatic index (GSI) (gonad weight divided by total body weight, multiplied by 100) values, and (4) abnormal gonad histopathology (high percent of atretic oocytes in female ovaries and high percent ceroid/lipofuscin tissue in male or female gonads).

These selected biological characteristics are commonly used in other studies and are a subset of the measures used by the USGS Biomonitoring of Environmental Status and Trends Program (Schmitt and Dethloff, 2000) to identify the influence of contaminants on fish throughout the United States. Features of each biological characteristic as described by Schmitt and Dethloff (2000) and Kime (1998) are listed in table 2. The selected biological characteristic can be influenced by age and gonad maturation; therefore, the influence of these factors on each biological characteristic was determined.

Study Design and Site Selection

The study design was a paired site approach targeting sites downstream and upstream of WWTP discharges on selected streams. Sites downstream of WWTPs are influenced primarily by effluent discharge and to a lesser extent by runoff from local urban and upstream land. Sites upstream of WWTP effluent were primarily influenced by runoff from agricultural land. The criteria for site selection included streams that receive a continuous effluent discharge from a WWTP and had a dam (fish barrier) upstream of that discharge. Additionally, streams with no additional WWTPs upstream of the selected site and those with the greatest amount of WWTP discharge per streamflow during summer low-flow conditions had top priority for selection. Given these constraints, 22 sites

Table 1. Concentrations of vitellogenin, 17β-estradiol, 11-ketotestosterone, and testosterone in female and male common carp plasma reported in other studies

[mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; na, not available; ^{o}C, degrees centigrade; >, greater than]

Sex	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Testosterone (pg/mL)	Number of samples	Study	Geographic location	Dates sampled	Comments
Female	[1]28.4 ± 1.80	[1]2110 ± 150	[1]1074 ± 110	[1]1116 ± 117	69	Goodbred and others, 1997	Northeastern United States	September through November 1994	Wild fish collected from streams
	[1]26.5 ± 1.5	[1]1260 ± 68	[1]707 ± 42	[1]1008 ± 65	97	Goodbred and others, 1997	Mississippi River Basin, United States	August through September 1994	Wild fish collected from streams
	[1]28.3 ± 1.3	[1]2463 ± 231	[1]617 ± 75	[1]862 ± 110	72	Goodbred and others, 1997	Northern- midcontinental United States	September 1994	Wild fish collected from streams
	[1]10.8 ± 1.1	[1]1045 ± 283	[1]282 ± 64	[1]369 ± 75	10	Goodbred and others, 1997	Southern-midcontinental United States	December 1994	Wild fish collected from streams
	[1]27.9 ± 1.5	[1]1767 ± 158	[1]1359 ± 158	[1]1288 ± 148	53	Goodbred and others, 1997	Western United States	October 1994	Wild fish collected from streams
	[1]1.7 ± 0.9	[1]216.7 ± 51.5	na	[1]720.8 ± 100	7	Folmar and others, 1996	Minneapolis and St. Paul Metropolitan Wastewater Treatment Plant effluent channel	August 1995	Wild fish collected from streams
	na	[1]750.5 ± 89.6	na	[1]1250 ± 100	10	Folmar and others, 1996	Mississippi River near St. Paul. Minnesota	September-October 1995	Wild fish collected from streams
	na	[2]490	[2]180	[2]800	5	Down and others, 1990	Indoor flow-through aquaria under a simulated natural photoperiod	Spring prespawning and active spawning period lasting into June	Wild fish collected from the Great Lakes
	na	[1]140	[1]140	[1]250	9	Down and others, 1990	Indoor flow-through aquaria under a simulated natural photoperiod	Summer postspawning period (water temperatures >15 ^{o}C)	Wild fish collected from the Great Lakes
	na	[1]140	[1]130	[1]250	10	Down and others, 1990	Indoor flow-through aquaria under a simulated natural photoperiodd	Fall period (late September through November)	Wild fish collected from the Great Lakes

Table 1. Concentrations of vitellogenin, 17β-estradiol, 11-ketotestosterone, and testosterone in female and male common carp plasma reported in other studies --Continued

[mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; na, not available; °C, degrees centigrade; >, greater than]

Sex	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Testosterone (pg/mL)	Number of samples	Study	Geographic location	Dates sampled	Comments
Male	[1]0	[1]480 ± 37	[1]1439 ± 111	[1]1917 ± 159	79	Goodbred and others, 1997	Northeastern United States	September - November, 1994	Wild fish collected from streams
	[1]0 + 0.1	[1]311 ± 25	[1]1148 ± 92	[1]1488 ± 110	77	Goodbred and others, 1997	Mississippi River Basin, United States	August - September, 1994	Wild fish collected from streams
	[1]0	[1]716 ± 49	[1]805 ± 69	[1]1084 ± 111	66	Goodbred and others, 1997	Northern- midcontinental United States	September 1994	Wild fish collected from streams
	[1]0	[1]258 ± 130	[1]627 ± 106	[1]838 ± 154	2	Goodbred and others, 1997	Southern-midcontinental United States	December 1994	Wild fish collected from streams
	[1]0 + 0.1	[1]598 ± 77	[1]1561 ± 158	[1]2141 ± 351	53	Goodbred and others, 1997	Western United States	October 1994	Wild fish collected from streams
	[1]1.13 ± 0.9	[1]125.4 ± 19.2	na	[1]2970 ± 1500	10	Folmar and others, 1996	Minneapolis and St. Paul Metropolitan Wastewater Treatment Plant effluent channel	August 1996	Wild fish collected from WWTP effluent channel
	na	[1]125.9 ± 7.3	na	[1]4160 ± 800	10	Folmar and others, 1996	Mississippi River near St. Paul, Minnesota	September - October 1996	Wild fish collected from streams
	na	[1]147.3 ± 14.3	na	[1]5130 ± 700	11	Folmar and others, 1996	Mississippi River near Hastings, Minnesota	September - October 1996	Wild fish collected from streams
	na	[1]133.6 ± 13.7	na	[1]2920 ± 400	10	Folmar and others, 1996	Minnesota River in Minnesota	September - October 1996	Wild fish collected from streams
	na	[1]105.4 ± 22.0	na	[1]6410 ± 200	5	Folmar and others, 1996	St. Croix River upstream of St. Croix Falls, Wisconsin	September - October 1996	Wild fish collected from streams
	na	[2]60	[2]300	[2]900	5	Down and others, 1990	Indoor flow-through aquaria under a simulated natural photoperiod	Spring prespawning and active spawning period lasting into June	Wild fish collected from the Great Lakes
	na	[2]50	[2]150	[2]200	9	Down and others, 1990	Indoor flow-through aquaria under a simulated natural photoperiod	Summer postspawning period (water temperatures >15 °C)	Wild fish collected from the Great Lakes
	na	[2]70	[2]300	[2]1200	9	Down and others, 1990	Indoor flow-through aquaria under a simulated natural photoperiod	Fall period (late September - November)	Wild fish collected from the Great Lakes
	na	[3]100 - 1300	na	[3]1000 - 3500	50-100	Chang and Chen, 1990	Laboratory aquaria	na	Common carp from 3 to 13 months old
	na	[3]100 - 2000	na	[3]1000 - 3300	8	Chang and Chen, 1990	Laboratory aquaria	November 1987 through March 1989	Blood samples collected from adult males every two weeks

[1] mean, plus or minus one standard error of the mean

[2] estimated mean based on evaluation of graphs

[3] estimated range based on evaluation of graphs

Table 2. Description of the biological characteristics used in this study
[modified from Schmitt and Dethloff (2000) and Kime, (1998)].

Biological characteristic	Description	Use	Precautions
Vitellogenin	A precursor of egg yolk proteins, synthesized in the liver of female fish upon stimulation by 17β-estradiol	Primarily controlled by estrogen so is a valuable indicator of exogenous estrogens and estrogen mimics. Because it is not normally present in male fish plasma, it is a good indicator of hormonally active agents that act as estrogenic substances.	Can vary with sex, age, stage of gonad maturation, species, and season. High variability among fish at one location. These factors can confound comparisons.
Sex steroid hormones (17β-estradiol and 11-ketotestosterone)	Chemical messengers that stimulate secondary sex characteristics, development of gametes, and spawning.	Indicators of reproductive health and status.	Can vary with sex, age, stage of gonad maturation, species, water temperature, and season. High variability among fish at one location. These factors can confound comparisons.
Gonado-somatic index	Gonad weight divided by total body weight, multiplied by 100.	Indicator of reproductive status and chemical exposure. Indicator is related to reproductive success.	Changes with season, sex, stage of gonad maturation, and species. Gonad size may not be associated with high sperm quality.
Gonad histopathology	Microscopic examination for the presence of abnormalities (ceroid/lipofuscin deposits in males and females, and atretic eggs in females).	Cellular level abnormalities often occur prior to macroscopic abnormalities. Good early warning system of sublethal health affects.	Standardization of methodologies is critical for comparisons among fish.

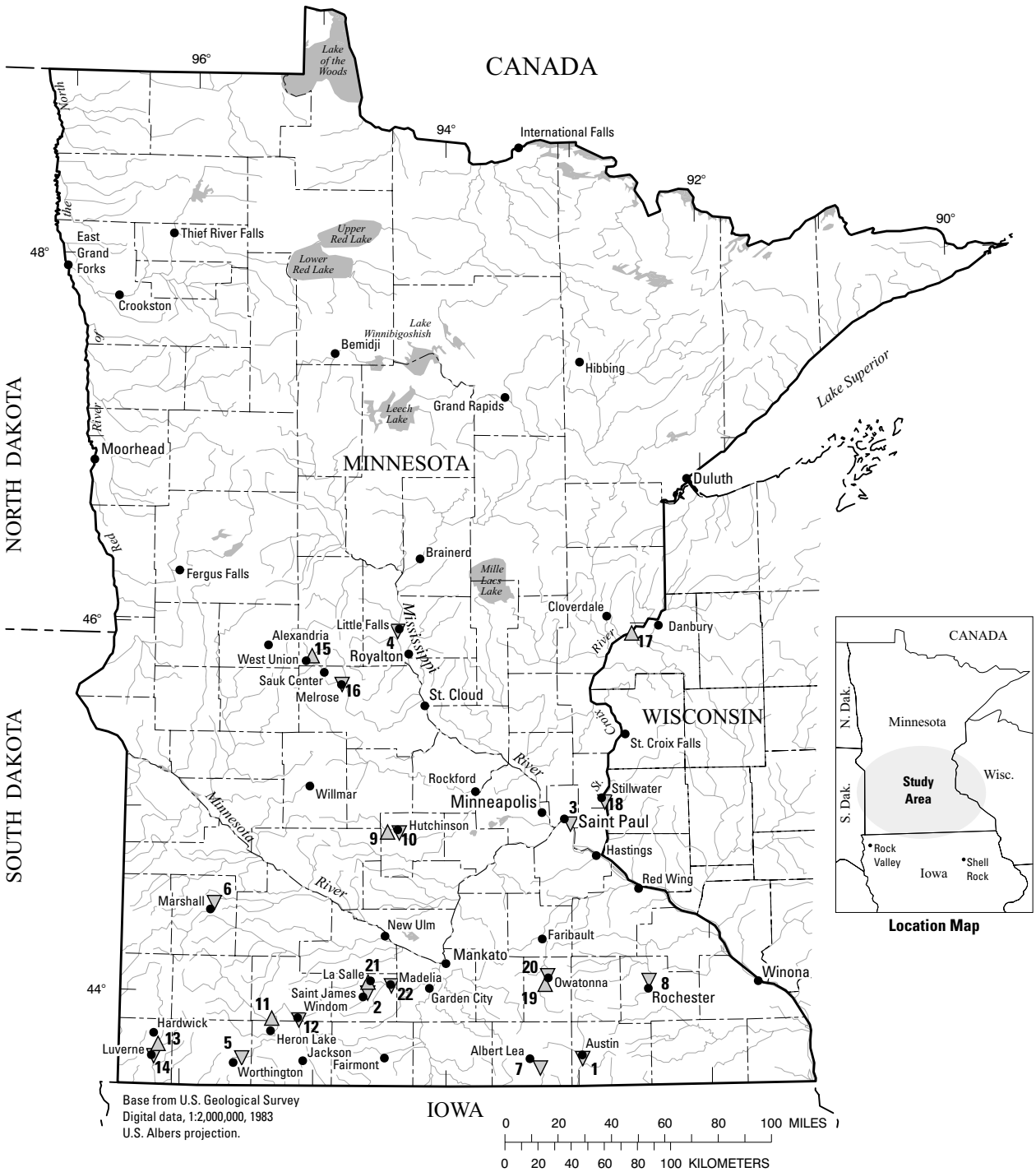

Figure 2. Location of stream sites sampled in Minnesota during August-September 1999.

Table 3. Study sites and basin land-use characteristics

[D, site downstream of wastewater treatment plant; U site located upstream of wastewater treatment plant; shaded cells are downstream sites; na, not available; km^2, square kilometers].

Site location number (fig. 2)	Sampling date	Site name	Latitude	Longitude	Site group	Drainage area (km^2)	Drainage basin land use (percent of basin)					
							Urban	Agricultural	Wetland	Forest	Water	Other
1	8/31/99	Cedar River downstream of Austin. Minn.	43°38′58″	92°58′24″	D	633	4.0	89.1	2.7	4.07	0.2	0.03
2	8/6/99	St. James Creek downstream of St. James, Minn.	44°00′20″	94°35′21″	D	118	5.9	85.8	3.0	2.4	2.9	0.00
3	8/13/99	Minneapolis and St. Paul Metropolitan Wastewater Treatment Plant effluent channel	44°55′08″	93°02′50″	D	na	100	0.00	0.00	0.00	0.00	0.00
4	8/24/99	Mississippi River downstream of Little Falls, Minn.	45°58′00″	94°22′30″	D	29,505	1.0	22.1	25.1	40.8	9.9	1.1
5	8/4/99	Okabena Creek near Worthington, Minn.	43°38′51″	95°31′57″	D	18	36.6	59.1	0.8	0.9	2.6	0.00
6	8/3/99	Redwood River near Marshall, Minn.	44°29′12″	95°45′56″	D	694	2.5	87.1	4.8	2.8	2.8	0.09
7	8/18/99	Shell Rock River near Albert Lea, Minn.	43°36′02″	93°17′30″	D	388	7.6	81.7	2.6	3.5	4.5	0.1
8	8/30/99	Zumbro River downstream of Rochester, Minn.	44°03′55″	92°27′57″	D	785	8.0	79.4	3.5	8.7	0.2	0.2
9	8/16/99	South Fork of the Crow River upstream of Hutchinson, Minn.	44°52′20″	94°21′20″	U	1,059	1.3	79.6	8.7	3.5	6.8	0.1
10	8/9/99	South Fork of the Crow River downstream of Hutchinson, Minn.	44°52′39″	94°26′45″	D	1,171	2.1	79.4	8.5	3.5	6.4	0.1
11	8/5/99	Des Moines River upstream of Windom, Minn.	43°51′54″	95°18′47″	U	1,432	1.1	89.5	3.9	2.3	3.2	0.0
12	8/5/99	Des Moines River downstream of Windom, Minn.	43°51′27″	95°06′28″	D	2,944	1.7	90.4	3.3	1.8	2.8	0.0
13	8/17/99	Rock River upstream of Luverne, Minn.	43°43′04″	96°09′49″	U	792	1.3	94.9	2.2	1.5	0.1	0.0
14	8/17/99	Rock River downstream of Luverne, Minn.	43°39′02″	96°11′47″	D	1,092	1.5	94.9	2.0	1.4	0.2	0.0
15	8/23/99	Sauk River upstream of Sauk Center, Minn.	45°49′51″	95°02′16″	U	416	1.0	67.2	14.5	8.6	8.7	0.0
16	8/19/99	Sauk River downstream of Melrose, Minn.	45°40′37″	94°48′13″	D	1,110	1.5	71.8	12.6	8.5	5.6	0.0
17	9/13/99	St. Croix near Cloverdale, Minn.	45°57′05″	92°33′20″	U	7,119	0.4	13.5	10.2	69.8	4.6	1.5
18	8/26/99	St. Croix downstream of Stillwater, Minn.	45°02′10″	92°46′50″	D	18,351	0.8	28.9	16.8	48.7	3.9	0.9
19	9/1/99	Straight River upstream of Owatona, Minn.	44°03′10″	93°15′03″	U	505	1.8	90.2	2.9	4.6	0.5	0.0
20	9/1/99	Straight River downstream of Owatona, Minn.	44°05′55″	93°13′47″	D	629	3.4	87.4	3.4	5.3	0.5	0.00
21	8/11/99	Watonwan River upstream of Madelia, Minn.	44°03′53″	94°35′22″	U	529	0.9	93.2	2.8	2.1	1.0	0.0
22	8/11/99	Watonwan River downstream of Madelia, Minn.	44°02′41″	94°24′44″	D	1,660	1.8	91.7	2.3	2.6	1.6	0.0

on 15 streams in Minnesota were selected for study (fig. 2 and table 3). Although paired sites on each stream were preferred, this was not always possible due to the inability to collect desired fish species. Seven streams had sites at two locations (upstream and downstream of WWTPs), and eight sites were downstream of WWTPs with no upstream-paired sample.

The study area includes streams draining land in Minnesota and Wisconsin. Sites are located primarily in the southern part of Minnesota in basins that drain agricultural land. Exceptions are the St. Croix River (sites 17 and 18) and the Mississippi River downstream of Little Falls, Minnesota (site 4), that drain primarily forested land. Environmental factors such as land use, hydrologic conditions, and WWTP characteristics were described for each site. The data set used to characterize land use in the study basins was produced by a cooperative effort of the USGS and the U.S. Environmental Protection Agency (USEPA) (U.S. Geological Survey, 2000). The classified data are based on 30-m-resolution Landsat satellite data. Land use for the drainage basin upstream of each site was determined with geographic-informations-system (GIS) analyses of these data.

Fish Collection and Sample Processing

Each fish species may have unique responses to HAAs because of differences in reproductive strategies. For example, the reproductive strategy of common carp includes the release of numerous gametes during several spawning periods. In contrast, smallmouth bass (*Micropterus dolomieui*) produce few gametes during a more discrete spawning period. Common carp was the only species selected for collection among all sites because of the potential confounding factors associated with the comparison among different fish species. Common carp were also selected because they are common species in Minnesota streams and have been studied previously in Minnesota (Folmar and others, 1996, Goodbred and others, 1997; and Schmitt and Dethloff, 2000). Male and female common carp were collected using electrofishing techniques with pulsed DC current. Fish were sampled between August 3 and September 13, 1999. This time period was selected because it was reported to be the post-spawn period when gonadal recrudescence occurs in carp (Down and others, 1990; Becker, 1983). At this time sex steroid hormones should be relatively stable. Paired sites were sampled within a 1-week period, to reduce potential variability in biological characteristics due to differences in hydrologic and chemical conditions, with the exception of sites 17 and 18, which were sampled within a 30-day period. The downstream sample was collected within an area of the stream where the effluent was well mixed (within 400 m downstream of the discharge). Fish were kept alive in a holding container with native water and processed within a few hours of capture. Each fish was weighed, length was measured, and scales were collected for age determination. At least 3 scales from the same area of each fish were examined under a microscope and scale annuli counted to determine fish age.

Approximately 3–5 mL of blood was drawn with a syringe from the caudal vein and transferred to a 5-mL heparinized vacutainer. The vacutainer was stored on wet ice prior to being centrifuged for 10 minutes at 3,500 revolutions per minute to separate plasma. Plasma from each fish was transferred to two cryovials and stored on dry ice or liquid nitrogen prior to transfer to laboratories. One plasma sample was sent to the University of Florida's Molecular Biomarkers Core Facility in Gainesville, Florida, for analysis of vitellogenin, and the remaining plasma sample was sent to the USGS laboratory in Gainesville, Florida, for 17β-estradiol and 11-ketotestosterone analyses. All plasma samples were stored at – 80°C until analyzed. Gonads were removed and weighed to the nearest gram for GSI calculation. Subsamples of gonad, spleen, and liver tissue were fixed in 10 percent formalin and sent to the USGS laboratory in Leetown, West Virginia, for histopathological examination.

Laboratory Analyses and Quality Control

Plasma samples from common carp were analyzed for vitellogenin, 17β-estradiol, and 11-ketotestosterone concentrations. Vitellogenin concentrations in plasma were analyzed using a direct Enzyme-Linked Immunosorbent Assay (ELISA) as previously described (Denslow and others, 2000; Folmar and others, 1996; Goodbred and others,1997; and Schmitt and Dethloff, 2000). A monoclonal antibody, HL 1638 (2D3), specifically raised against common carp vitellogenin, was used for these assays. This antibody reacts with high affinity and specificity with vitellogenin and not with other plasma proteins. The ELISA used in this study can detect 10–100 ng of vitellogenin per sample, resulting in a sensitivity of 0.001 mg/mL, as plasma must be diluted one-hundred times to eliminate interferences. Each ELISA plate had a positive control (plasma with a known vitellogenin concentration) to test for interassay and intra-assay variation. The assay was performed in triplicate and if the coefficient of variation exceeded 10 percent, the samples were rerun. Correlations between standards and field samples yielded Pearson correlation coefficients between 0.95 and 0.99.

Radioimmunoassay procedures were used for 17β-estradiol and 11-ketotestosterone determination as previously described (Goodbred and others, 1997; and Schmitt and Dethloff 2000). Each sample was analyzed in duplicate. Extraction efficiencies of 92 ± 2.8 percent and 86 ± 3.3 percent were observed for 17β-estradiol and 11-ketotestosterone, respectively. The minimum concentration detectable was 6.4 pg/mL for 17β-estradiol and 8.1 pg/mL for 11-ketotestosterone. The assays are subject to cross reac-

tivity with other steroids. Cross reactivity of 17β-estradiol with other steroids was: 11.2 percent for estrone, 1.7 percent for estriol, and less than 1.0 percent for 17α-estradiol and androstendione. The cross reactivity of 11-ketotestosterone with other steroids was 9.7 percent for testosterone, 3.7 percent for α-dihydrotestosterone, and less than 1 percent for androstendione.

Samples of female and male gonad tissues were classified into stages of sexual maturation and examined for histopathological abnormalities. Methods used for laboratory preparations and characterizations are described in Goodbred and others (1997), and Schmitt and Dethloff (2000), and briefly here. Gonad tissue was processed for light microscopy by dehydration and embedding in paraffin. Blocks of paraffin were cut at 6-µm thickness and stained with hematoxylin and eosin.

Ovaries and testes were examined for stage of sexual maturation and gonadal abnormalities (percent atretic oocytes in females, and presence of ceroid/lipofuscin in both females and males). Determination of gonad maturation stage was based on the maturity of the predominant stage of oogenesis and spermatogenesis for female and male gonads, respectively (table 4). Stage- 0, -1,and -2 females were observed in this study and are shown in figures 3a, 3b, and 3c. Although stage-3 is typical of females approaching spawning, stage-1, -2, and -3 are all sexually mature fish. Stages 1–3 are characteristic of sexually mature male fish. Stages 2 and 3 were common among male common carp in this study and are shown in figures 3d and 3e.

Atresia in the female gonadal tissue is presented as the percent of oocytes that are atretic (fig. 4). When possible, 100 oocytes were counted. A semiquantitative method (Reimschuessel and others, 1992) was used to classify ceroid/lipofuscin tissue in female and male gonads (figs 5a and 5b). The presence of ceroid/lipofuscin tissue in the female and male gonads was ranked on a scale of 0–4, with 0 being none observed, 1 rarely observed, 2 mild, 3 moderate, and 4 severe.

Data Analyses

Because the selected biological characteristics can be influenced by physiological factors such as gonad maturation and fish age, a one-way analysis of variance (ANOVA) was used to determine if any biological characteristic was different among gonad maturation stages or fish ages. Comparisons of biological characteristics between site groups (7 upstream and 15 downstream sites) were accomplished with a two-sided student's t-test for unequal sample sizes. Comparisons for the paired sites (7 upstream and downstream pairs) were accomplished with a paired t-test. This test reduces the variability between streams so that differences between groups can be detected (Helsel and Hirsch, 1992). ANOVA was used to make comparisons of biological characteristics among all sites. All variables were tested for normality through visual inspection of normal probability plots prior to analyses and transformed when necessary to achieve normality. A significance level of 0.05 was used as the criterion for statistical significance. The statistical-test result is represented by p-values, the attained level of significance.

Because baseline values for biological characteristics have not been established, the values for each characteristic were ranked relative to one another. Mean values of the selected

Table 4. Histological characteristics used to classify gonad maturation stages for female and male common carp in this study (modified from Schmitt and Dethloff, 2000; and Goodbred and others, 1997)

Gonad maturation stage	Female ovary characteristics	Male testes characteristics
0	Undeveloped (immature): Predominantly contained early perinucleolar oocytes that are previtellogenic and no developing ova.	Undeveloped (immature): Testicular tissue contained exclusively immature stages of spermatogenesis (spermatocytes) with no spermatozoa observed.
1	Previtellogenic: Ovaries contained greater than 90 percent previtellogenic oocytes, and few cortical alveoli.Ova are slightly to moderately enlarged and contain vacuoles or lipid droplets but few vitelline granules.	Early spermatogenic: Testicular tissues predominantly contained immature stages (spermatocytes to spermatids), with some spermatozoa observed.
2	Early vitellogenic: Ovaries contained a majority of oocytes that were early to mid-vitellogenic with few to moderate numbers of vitelline granules and very few fully developed oocytes.	Mid-spermatogenic: Testicular tissue contained a mix of spermatocytes, spermatids and spermatozoa in roughly equal proportions.
3	Late vitellogenic: Ovaries contained a majority of oocytes that were late vitellogenic with eosinophillic yolk globules distributed throughout the cytoplasm.	Late-spermatogenic: Testicular tissues contained mature sperm.

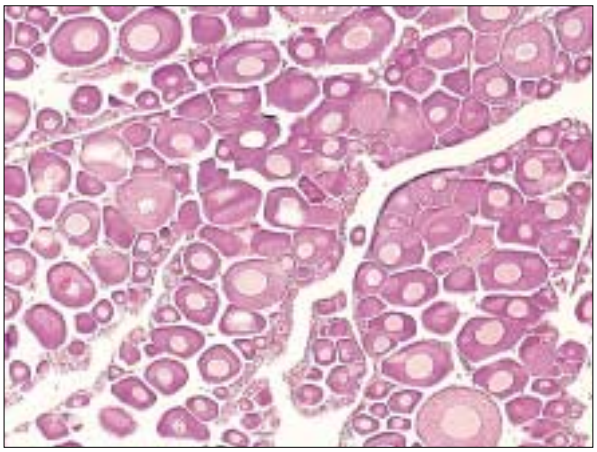

Figure 3a. Female common carp ovary containing perinucleolar oocytes; classified as stage-0, undeveloped and immature (Magnification 365x).

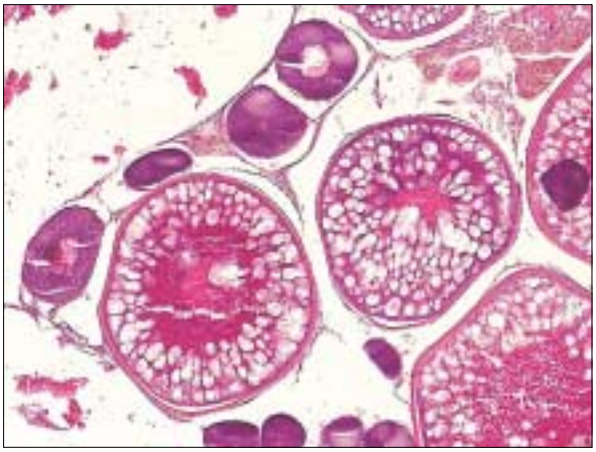

Figure 3b. Female common carp ovary containing a mixture of perinucleolar and cortical alveoli oocytes; classified as stage-1, previtellogenic (Magnification 365x).

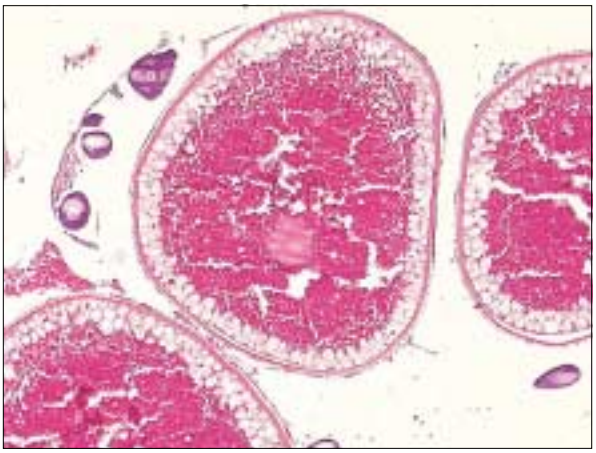

Figure 3c. Female common carp ovary containing some vitellogenic oocytes with moderate numbers of vitelline granules and a few perinucleolar and cortical alveoli oocytes; classified as stage-2, early vitellogenic (Magnification 365x).

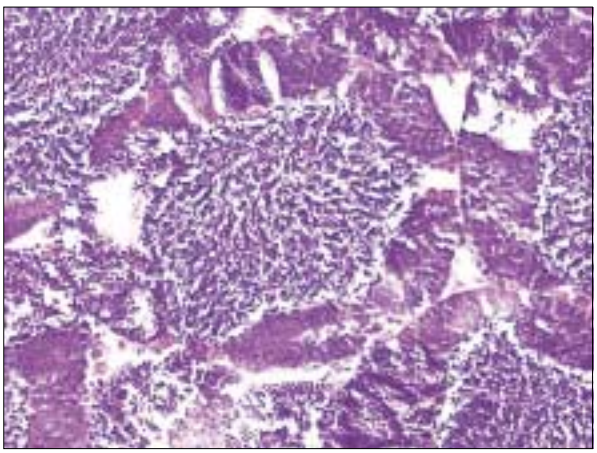

Figure 3d. Male common carp testes containing a mix of spermatocytes, spermatids, and spermatozoa in approximately equal portions; classified as stage-2, mid spermatogenic (Magnification 412x).

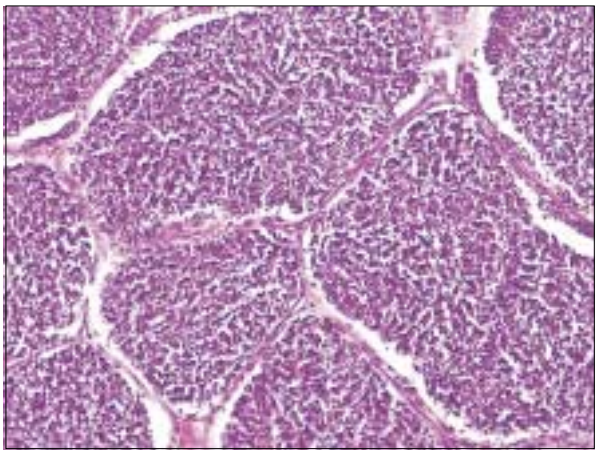

Figure 3e. Male common carp testes containing a thin germinal epithelium with scattered spermatogenic activity characteristic of full-grown testes; classified as stage-3, late spermatogenic, sexually mature (Magnification 412x).

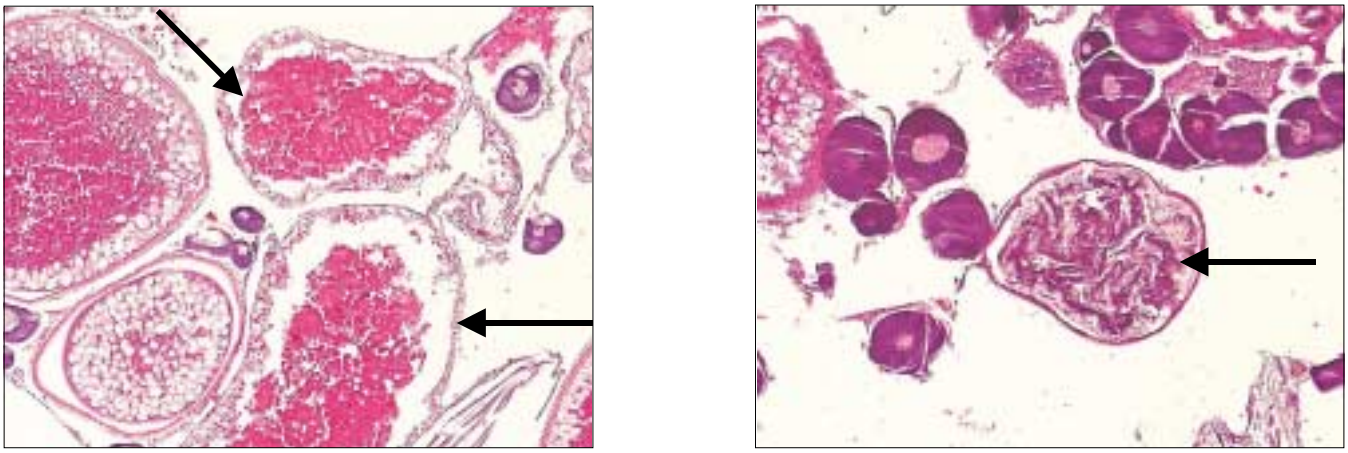

Figure 4. Examples of atretic oocytes in female common carp (Magnification 412x).

biological characteristics at each site were ranked and classified into one of three categories: high, medium, or low. The high class consisted of sites with mean values of a biological characteristic greater than or equal to the 75th percentile (the highest 25 percent). The medium class consisted of sites with mean values of a biological characteristic between the 25th and 75th percentiles, and the low class consisted of sites with mean values of a biological characteristic less than or equal to the 25th percentile (the lowest 25 percent). Samples in the high and low classes were of the greatest interest because fish in these two classes represent the samples that are distinct among all sites and thus may indicate potential HAA exposure and endocrine disruption.

SELECTED FACTORS AFFECTING BIOLOGICAL CHARACTERISTICS

Gonad Maturation and Fish Age

The selected biological characteristics can be influenced by physiological factors such as gonad maturation and fish age (Goodbred and others, 1997). Both gonad maturation stage and fish age varied among sites. Among all fish collected (201 females and 221 males), female common carp age ranged from 1 to 6 years with gonad maturation stages from 0 to 3 (table 15, at the back of the report). Male common carp age ranged from 1 to 5 years with gonad maturation stages from 1 to 4 (table 16, at the back of the report). There were small sample sizes in select gonad stages (one female in gonad stage-3, and two males each in gonad stages-1 and 4) that did not allow for analyses of the influence of those gonad stages on biological characteristics; therefore, these five samples were eliminated from further analyses.

Selected biological characteristics were different among gonad maturation stages and fish ages for both female and male fish (tables 5 and 6, respectively). In female fish, vitellogenin, 11-ketotestosterone, GSI val-

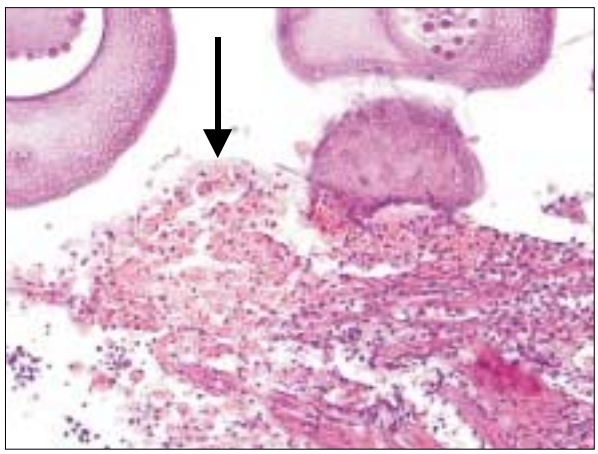

Figure 5a. Ceroid/lipofuscin tissue in female common carp gonads (Magnification 824x).

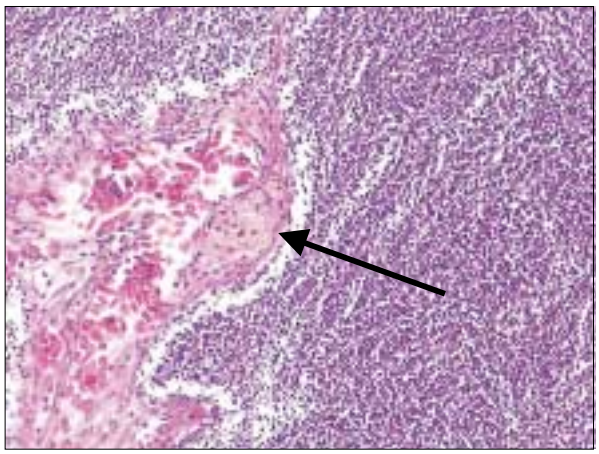

Figure 5b. Ceroid/lipofuscin tissue in male common carp gonads (Magnification 412x).

Table 5. Comparisons of biological characteristics between gonad stages, and between fish ages for female common carp[1]

[SE, one standard error; p-values shown in bold indicate statistically significant differences; n, number of samples; GSI, gonado-somatic index]

Biological characteristic	Gonad stage				Fish age						
	Gonad stage-0 Mean ± SE	Gonad stage-1 Mean ± SE	Gonad stage-2 Mean ± SE	p-value among gonad stages	1 year Mean ± SE	2 years Mean ± SE	3 years Mean ± SE	4 years Mean ± SE	5 years Mean ± SE	6 years Mean ± SE	p-value among ages
	n= 21	n=49	n=130		n=16	n=71	n=65	n=33	n=12	n=3	
Vitellogenin (mg/mL)	0.217 ± 0.048	3.58 ± 0.67	3.44 ± 0.30	**<0.001**	3.65 ± 1.25	2.85 ± 0.48	3.34 ± 0.49	2.88 ± 0.44	3.63 ± 0.52	3.19 ± 0.41	0.6
17β-estradiol (pg/mL)	428 ± 40	548 ± 46	560 ± 28	0.3	486 ± 70	544 ± 37	544 ± 37	598 ± 56	503 ± 111	358 ± 164	0.6
11-ketotestosterone (pg/mL)	299 ± 38	263 ± 28	361 ± 21	**0.02**	271 ± 48	332 ± 23	278 ± 26	352 ± 35	523 ± 118	732 ± 107	**0.001**
GSI (percent)	1.1 ± 0.1	4.2 ± 0.4	9.9 ± 0.3	**<0.001**	3.41 ± 0.68	7.0± 0.6	7.9 ± 0.5	9.1 ± 0.9	8.8 ± 1.2	3.6 ± 6.5	**<0.001**
Atretic oocytes (percent)	2.5 ± 1.2	11.4 ± 1.6	10.2± 0.7	**<0.001**	4.4 ± 1.6	8.6 ± 0.9	10.6 ± 1.3	12.0 ± 1.7	12.3 ± 2.0	4 ± 1.2	**0.003**
Ceroid/lipofuscin ranking	0.57 ± 0.16	1.1 ± 0.11	1.0 ± 0.04	**0.001**	0.69 ± 0.12	0.76 ± 0.06	1.1± 0.08	1.1 ± 0.08	1.4 ± 0.15	1.7 ± 0.67	**<0.001**

[1] Female common carp in gonad stages-0 through -2 and ranging in age from 1- to 6- years old were used in these analyses.

Table 6. Comparisons of biological characteristics between gonad stages, and between fish ages for male common carp[1]

[SE, standard error; p-values shown in bold indicate statistically significant differences; n, number of samples; GSI, gonado-somatic index]

Biological characteristic	Gonad stage			Fish age					
	Gonad stage-2 Mean ± SE	Gonad stage-3 Mean ± SE	p-value among gonad stages	1 year Mean ± SE	2 years Mean ± SE	3 years Mean ± SE	4 years Mean ± SE	5 years Mean ± SE	p-value among ages
	n = 35	n = 182		n = 14	n = 75	n = 79	n = 31	n = 18	
Vitellogenin (mg/mL)	0.02 ± 0.009	0.01 ± 0.001	**0.02**	0.009 ± 0.0002	0.018 ± 0.002	0.01 ± 0.001	0.02 ± 0.01	0.002 ± 0.001	**0.006**
17β-estradiol (pg/mL)	256 ± 22	264 ± 10	0.8	157 ± 20	263 ± 17	283 ± 13	264 ± 30	239 ± 31	**0.002**
11-ketotestosterone (pg/mL)	774 ± 70	620 ± 32	**0.04**	900 ± 159	522 ± 42	671 ± 50	804 ± 87	631.28 ± 72	**0.003**
GSI (percent)	4.5 ± 0.4	5.3 ± 0.2	**0.05**	3.5 ± 0.4	4.3 ± 0.24	5.1 0.25	6.6 ± 0.4	8.2 ± 0.6	**<0.001**
Ceroid/lipofuscin ranking	0.89 ± 0.11	1.01 ± 0.05	0.2	0.78 ± 0.15	0.56 ± 0.06	1.1 ± 0.07	1.3 ± 0.11	1.3 ± 0.11	**0.001**

[1] Male common carp in gonad stages-2 and -3 and ranging in age from 1- to 5- years old were used in these analyses.

ues, percent atretic oocytes, and ceroid/lipofuscin rankings were significantly different among gonad maturation stages (table 5). Stage-0 females had lower mean vitellogenin concentrations, GSI values, percent atretic oocytes, and ceroid/lipofuscin rankings than stage-1 and -2 females. The 11-ketotestosterone concentrations, GSI values, percent atretic oocytes, and ceroid/lipofuscin rankings were significantly different among females of different ages (table 5). The 11-ketotestosterone concentrations were greatest in 6-year-old females. GSI values and percent atretic oocytes were lowest in 1- and 6-year-old females.

In male fish, vitellogenin, 11-ketotestosterone, and GSI values differed significantly by gonad maturation stage (table 6). Vitellogenin and 11-ketotestosterone concentrations were greater in stage-2 than in stage-3 males. GSI values were greater in stage-3 than in stage-2 males. All biological characteristics in male fish differed significantly by age (table 6). Vitellogenin concentrations were lowest in 1- and 5-year old males. One-year-old males had lower concentrations of 17β-estradiol, greater concentrations of 11-ketotestosterone and lower GSI values than older males (table 6).

To account for age and gonad maturation stage as potential confounding factors, analyses of biological characteristics were limited to a subset of the original data set. Analyses of female characteristics were limited to 181 females in gonad maturation stages-0, -1, and -2 and ranging from 2- to 5-years old (table 15). Analyses of male characteristics were limited to 185 males in gonad maturation stages-2 and -3, and ranging from 2- to 4-years old (table 16). Analyses were completed for each gonad stage individually (stage-2 males were only compared to other stage-2 males). Even after removal of 1- and 5-year-old male fish, GSI values increased with increasing fish age within each stage of maturation. The GSI should normalize the relation of gonad weight to body weight. However, this was not observed for male common carp in this study. This relation should not affect the comparison between the upstream and downstream group because the percents of 2-, 3-, and 4-year old fish were similar within each site group. Likewise, fish age was similar between the paired sites. Comparisons of GSI values for male fish among sites may be biased because female and male ages were not the same at each site (tables 7 and 8, respectively). For example, the mean age for gonad stage-3 males at sites 4 and 17 was 4 years old. In contrast, gonad stage-3 males were on average 2–3 years old among other sites.

Streamflow and Wastewater Discharge

Physiological responses of fish to contaminants are often dose dependent (the response increases as the concentration of a contaminant increases). Therefore, fish in streams with more concentrated WWTP effluent may show a greater response than fish in streams with less concentrated WWTP effluent.

The flow of the receiving stream and the amount of WWTP effluent determine the dilution of the effluent in the stream. Streamflows on the day of sampling ranged from 0.10 to 248 m^3/s among all sites. Streamflows the three months prior to and during sampling in August 1999 were not all at low-flow conditions that would have been ideal to assure that the WWTP effluent was maximally concentrated. USGS streamflow data from gaging stations located near sampling locations were used to estimate streamflow conditions. Antecedent streamflows generally were greater than normal during May, June, and July (table 9), but returned to approximately normal flow during August. Exceptions to these observations were the Cedar River near Austin, Minnesota (site 1), the Mississippi River near Royalton, Minnesota (site 4), the South Fork of the Zumbro River at Rochester, Minnesota (site 8), and the St. Croix River near St. Croix Falls, Wisconsin (site 18). The streamflow at these four sites was greater than average during August by a factor of two or more.

There was also variability in the amount of discharge that each WWTP contributed to the receiving stream (table 10). To account for differences in WWTP dilution among sites, the percent of streamflow consisting of wastewater (sum of discharges from all WWTPs upstream of the sampling site, divided by streamflow at the sampling site, multiplied by 100) was estimated for each site. The discharge data for each WWTP was obtained from the Permit Compliance System (Linda Brooks, Minnesota Pollution Control Agency, written commun., 1999). The average daily WWTP effluent discharges for August 1998 (August 1999 data were not readily available) were used to represent the discharge data from each WWTP. This value is considered an estimate because of the potential temporal variability in WWTP effluent amounts.

The characteristics of the influent and waste processing at each WWTP also affect the chemistry of the effluent. While the primary influent to each plant was domestic waste, there were likely variable amounts and types of industrial waste influent. The amount and types of industrial waste entering each WWTP was not quantified in this report.

To determine if the percent of streamflow consisting of wastewater influenced the biological characteristics, Spearman rank correlations were determined for each biological characteristic and the percent of streamflow consisting of wastewater (table 11). Most Spearman correlation coefficients were low (< 0.5) between biological characteristics and the percent of streamflow consisting of wastewater. Vitellogenin concentrations in female and male fish of selected gonad stages were related to the percent of streamflow consisting of wastewater. Vitellogenin concentrations in stage-0 and-1 females were negatively correlated with the percent of streamflow consisting of wastewater (-0.41 and -0.30, respectively).

Table 7. Mean and range of female fish age by gonad stage of maturation

[shaded cells are downstream sites]

	Site location number (fig. 2)	Gonad stage–0			Gonad stage–1			Gonad stage–2		
		Number of fish	Age (years)		Number of fish	Age (years)		Number of fish	Age (years)	
			Mean	Range		Mean	Range		Mean	Range
Downstream	1							10	3.9	3 - 5
	2							6	2	2 - 2
	3	2	3.5	3 - 4	5	3.2	3 - 4	7	3.2	3 - 4
	4				3	5	5	1	5	5
	5							5	2.8	2 - 4
	6							1	3	3
	7				2	3	3 - 3	8	2.6	2 - 3
	8							4	3.5	2 - 5
Paired upstream-downstream	9				2	3.5	3 - 4	6	3.1	2 - 4
	10				2	3.5	3 - 4	10	3	2 - 4
	11	2	2.5	2 - 3				9	2.4	2 - 3
	12	1	3	3	1	2	2	7	2	2 - 2
	13				4	2.2	2 - 3	7	2.7	2 - 4
	14				6	2.6	2 - 3	5	2.6	2 - 4
	15	7	2.7	2 - 4						
	16	1	2	2	4	2.25	2 - 3			
	17							5	5	5 - 5
	18				2	4.5	4 - 5	8	3.25	2 - 4
	19							5	3.4	2 - 4
	20	1	2	2	3	3	2 - 4	4	3.7	3 - 4
	21				2	2	2 - 2	5	2	2
	22				11	2.1	2 - 3	7	2.4	2 - 3
	Summary	14			47			120		

Table 8. Mean and range of male fish age by gonad stage of maturation

[shaded cells are downstream sites]

	Site location number (fig. 2)	Gonad stage-2			Gonad stage-3		
		Number of fish	Age (years)		Number of fish	Age (years)	
			Mean	Range		Mean	Range
Downstream	1	2	3	3 - 3	9	3.6	3 - 4
	2				12	2.1	2 - 3
	3	5	3.2	3 - 4	1	2	2
	4				3	4	4 - 4
	5	1	2	2	1	3	3
	6				11	2.8	2 - 3
	7	5	2.4	2 - 3	7	3	2 - 4
	8				2	3	2 - 4
Paired upstream-downstream	9				10	3.1	3 - 4
	10				11	2.8	2 - 4
	11				8	2.2	2 - 3
	12	1	2	2	7	2.1	2 - 3
	13				13	2.2	2 - 3
	14	1	3	3	12	2.3	2 - 3
	15	3	2.3	2 - 3	1	2	2
	16	3	2.3	2 - 3	9	2.5	2 - 4
	17	2	4	4 - 4	4	4	4
	18	3	3.3	3 - 4	6	3.5	3 - 4
	19				7	3.1	2 - 4
	20				12	3.0	2 - 4
	21	1	3	3 - 3	6	2.1	2 - 3
	22				6	2.5	2 - 3
	Summary	27			158		

Table 9. Hydrologic conditions at streamflow-gaging stations located near sampling sites during May-August 1999.

U.S. Geological Survey site identifier	Corresponding site location number (fig. 2)	U.S. Geological Survey station name (period of record)	Percent difference between 1999 mean monthly streamflow and the mean monthly streamflow for the period of record[1]			
			May	June	July	August
05457000	1	Cedar River near Austin, Minn. (1909-99)	336	85	238	107
05267000	4	Mississippi River near Royalton, Minn. (1924-99)	89	42	77	135
05315000	6	Redwood River near Marshall, Minn. (1940-99)	62	55	59	-43
05462000	7	Shell Rock River at Shell Rock, Iowa (1953-99)	225	130	293	63
05372995	8	South Fork of the Zumbro River at Rochester, Minn. (1981-99)	116	1	42	102
05280000	9 and 10	Crow River at Rockford, Minn. (1906-99)	56	43	0	-26
05476000	11 and 12	Des Moines River at Jackson, Minn. (1930-99)	92	14	8	-63
06483500	13 and 14	Rock River near Rock Valley, Iowa (1948-99)	66	18	8	-41
05270500	15 and 16	Sauk River near St. Cloud, Minn. (1909-99)	105	71	-21	-6
05333500	17	St. Croix River near Danbury, Wisc. (1914-99)	-26	-6	-63	-1
05340500	18	St. Croix River near St. Croix Falls, Wisc. (1902-99)	-6	-18	20	146
05353800	19 and 20	Straight River near Faribault, Minn. (1966-99)	192	74	93	35
05319500	21 and 22	Watonwan River near Garden City, Minn. (1940-99)	38	-24	84	-54

[1]Negative numbers indicate that the 1999 stream flow was less than the mean streamflow for the period of record.

Table 10. Discharges from wastewater treatment plants upstream of sampling sites, streamflow, and percent of streamflow consisting of wastewater treatment plant discharges

[na, not available; WWTP, wastewater treatment plant; m^3/sec, cubic meters per second; shaded cells are downstream sites].

	Site location number (fig. 2)	Average discharge from WWTP (m^3/sec)	Streamflow (m^3/sec)	Percent of streamflow consisting of WWTP discharge
Downstream	1	0.242	3.85	6
	2	0.048	0.10	48
	3	11.0	11.0	100
	4	0.765	229	0.3
	5	0.093	0.14	66
	6	0.121	1.43	8
	7	0.198	2.41	8
	8	0.599	7.92	8
Paired upstream-downstream	9	0	2.08	0
	10	0.111	3.34	3.3
	11	0	1.14	0
	12	0.039	3.82	1
	13	0.002	0.71	0.3
	14	0.034	1.05	3.2
	15	0	na	0
	16	0.105	4.56	2.3
	17	0	na	0
	18	0.280	248	0.1
	19	0	2.29	0
	20	0.153	3.05	5
	21	0.001	0.68	0.1
	22	0.031	2.02	1.5

[1]Sum of the average daily discharges for August 1998 from all wastewater treatment plants upstream of the sampling site.

Table 11. Spearman correlation coefficients between biological characteristics and the percent of streamflow consisting of wastewater for female and male common carp

[mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; na, not applicable].

Biological characteristic	Spearman correlation coefficient				
	Female common carp			Male common carp	
	Gonad stage-0	Gonad stage-1	Gonad stage-2	Gonad stage-2	Gonad stage-3
Vitellogenin (mg/mL)	-0.41	-0.30	0.014	0.53	-0.003
17β-estradiol (pg/mL)	-0.35	-0.54	-0.14	0.20	0.004
11-ketotestosterone (pg/mL)	-0.71	-0.22	-0.19	-0.23	0.11
Gonado-somatic index (percent)	0.34	-0.19	0.03	-0.12	-0.20
Atretic oocytes (percent)	0.31	-0.16	0.04	na	na

Vitellogenin concentrations in stage-2 males were positively correlated with percent of streamflow consisting of wastewater (Spearman correlation coefficient = 0.53). In female fish, there were low to moderate negative correlations between 17β-estradiol and 11-ketotestosterone concentrations and the percent of streamflow consisting of wastewater. In male fish, correlations with both 17β-estradiol and 11-ketotestosterone concentrations and the percent of streamflow consisting of wastewater were low. GSI values for females and males were weakly related to the percent of streamflow that wastewater contributed.

BIOLOGICAL CHARACTERISTICS AS INDICATORS OF HORMONALLY ACTIVE AGENTS

Concentrations of vitellogenin and sex steroid hormones, GSI values, and gonad histopathology were used as biological indicators of HAAs for purposes of this report. These characteristics are described for both female and male common carp.

Vitellogenin Concentrations in Common Carp Plasma

Mean vitellogenin concentrations in females were approximately 300 times greater than those in males (fig. 6a, 6b, and 7; tables 12 and 13). A comparison of vitellogenin concentrations in female fish between the site groups (7 upstream and 15 downstream sites) revealed no significant differences. Additionally, there were no differences between the paired sites (7 upstream–downstream pairs) for female fish.

Vitellogenin concentrations in females were significantly different among all sites ($p < 0.001$) (figs. 6a and 6b). Mean vitellogenin concentrations were low (less than the 25th percentile) for females in at least one gonad stage at downstream sites 2, 3, 12, 16, and 20 and upstream sites 11, 13, and 21 (figs. 6a and 6b, and table 14).

In male fish, vitellogenin concentrations were observed at all sites. Because vitellogenin has been observed in male fish under controlled conditions at concentrations of 0.0001 mg/mL (Jobling and others, 1998), and 0.01 mg/mL (Sumpter and Jobling, 1995), concentrations of vitellogenin less than 0.01 mg/mL are considered as background concentrations for this study. Vitellogenin concentrations above background concentrations were detected in 0 to 92 percent (mean = 36 percent) of the males at each site. Vitellogenin concentrations above background were observed in males at five downstream sites (3, 5, 6, 7 and 16) and four upstream sites (9, 13, 15, and 21).Vitellogenin concentrations in stage-3 males were significantly greater in the upstream than in the downstream site group (table 13). At paired sites, vitellogenin concentrations in males were greater at upstream than downstream sites ($p = 0.01$). Vitellogenin concentrations in males were significantly different among all sites ($p < 0.001$) (fig. 7). Vitellogenin concentrations in males in at least one gonad stage were greatest (greater than or equal to the 75th percentile) at downstream sites 1, 3, and 7; and upstream sites 9, 13, 15, 21 (table 14).

Sex Steroid Hormone Concentrations in Common Carp Plasma

The 17β-estradiol concentrations in females were on average 2.7 times greater than 11-ketotestosterone concentrations. In contrast, the 11-ketotestosterone concentrations in male fish were on average 2.3 times greater than 17β-estradiol concentrations. Normal ranges of 17β-estradiol and 11-ketotestosterone in female and

male fish have not been established. However, Down and others (1990) reported that 17β-estradiol concentrations were approximately two times greater than 11-ketotestosterone concentrations in female common carp and that 11-ketotestosterone concentrations were approximately four times greater than 17β-estradiol concentrations in male common carp**.** Goodbred and others (1997) observed that 17β-estradiol concentrations were on average 2.5 times greater than 11-ketotestosterone concentrations in female common carp and that 11-ketotestosterone concentrations were approximately 2.5 times greater than 17β-estradiol concentrations in male common carp. This reoccurring pattern suggests that although both female and male fish have both 17β-estradiol and 11-ketotestosterone concentrations in their plasma, 17β-estradiol concentrations generally are expected to be greater than 11-ketotestosterone concentrations in females. Conversely, 11-ketotestosterone concentrations are expected to be greater than 17β-estradiol concentrations in males.

Steroid hormone concentrations in female fish tended to be greater in the upstream than in the downstream site group although there were some gonad maturation stages for which there were no significant differences (fig. 6a, 6b, and 7; tables 12 and 13).

At the paired sites, 17β-estradiol concentrations tended to be greater at upstream than downstream sites. However there were no statistically significant differences between upstream and downstream sites (table 13). The paired sites 17–18 have a large influence on this result. Site 17 is unusual because the 17β-estradiol concentrations in female fish were low and 11-ketotestosterone concentrations were high (in the range of male concentrations). Concentrations of 11-ketotestosterone in females were greater at upstream than downstream paired sites (p = 0.04).

Both 17β-estradiol and 11-ketotestosterone concentrations in females differed among sites (p <0.001) (figs. 6a and 6b). Mean concentrations of 17β-estradiol in female fish were low in at least one gonad stage at downstream sites 2, 4, 5, 6, 10, 12, 16, and 20; and upstream sites 17 and 21 (table 14). Mean concentrations of 11-ketotestosterone in female fish of at least one gonad stage were high at downstream sites 1, 8, 12, 20, and 22; and upstream sites 11, 15, 17, 19, and 21 (table 14). Mean concentrations of 11-ketotestosterone were greater than 17β-estradiol concentrations in female fish within at least one gonad stage at downstream sites 2, 12, and 20; and upstream site 17 (table 14).

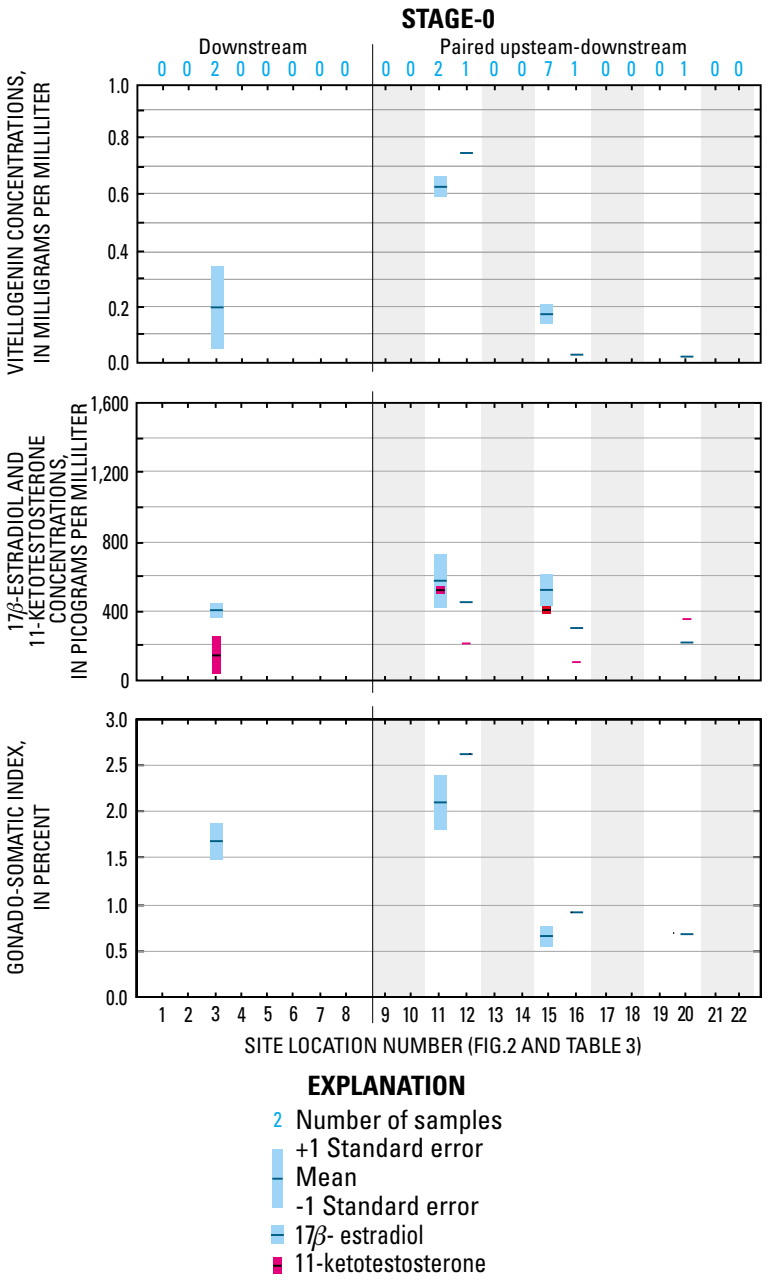

Figure 6a. Concentrations of vitellogenin, 17β-estradiol, and 11-ketotestosterone in plasma and gonado-somatic index values of stage-0 female common carp collected during 1999.

In male fish, there were some significant differences in 11-ketotestosterone, but not 17β-estradiol concentrations between the upstream and downstream site groups (table 13). In stage-2 males, 11-ketotestosterone concentrations were greater at the upstream than at the downstream site groups (p = 0.02); however, concentrations in stage-3 males were not different between site groups.

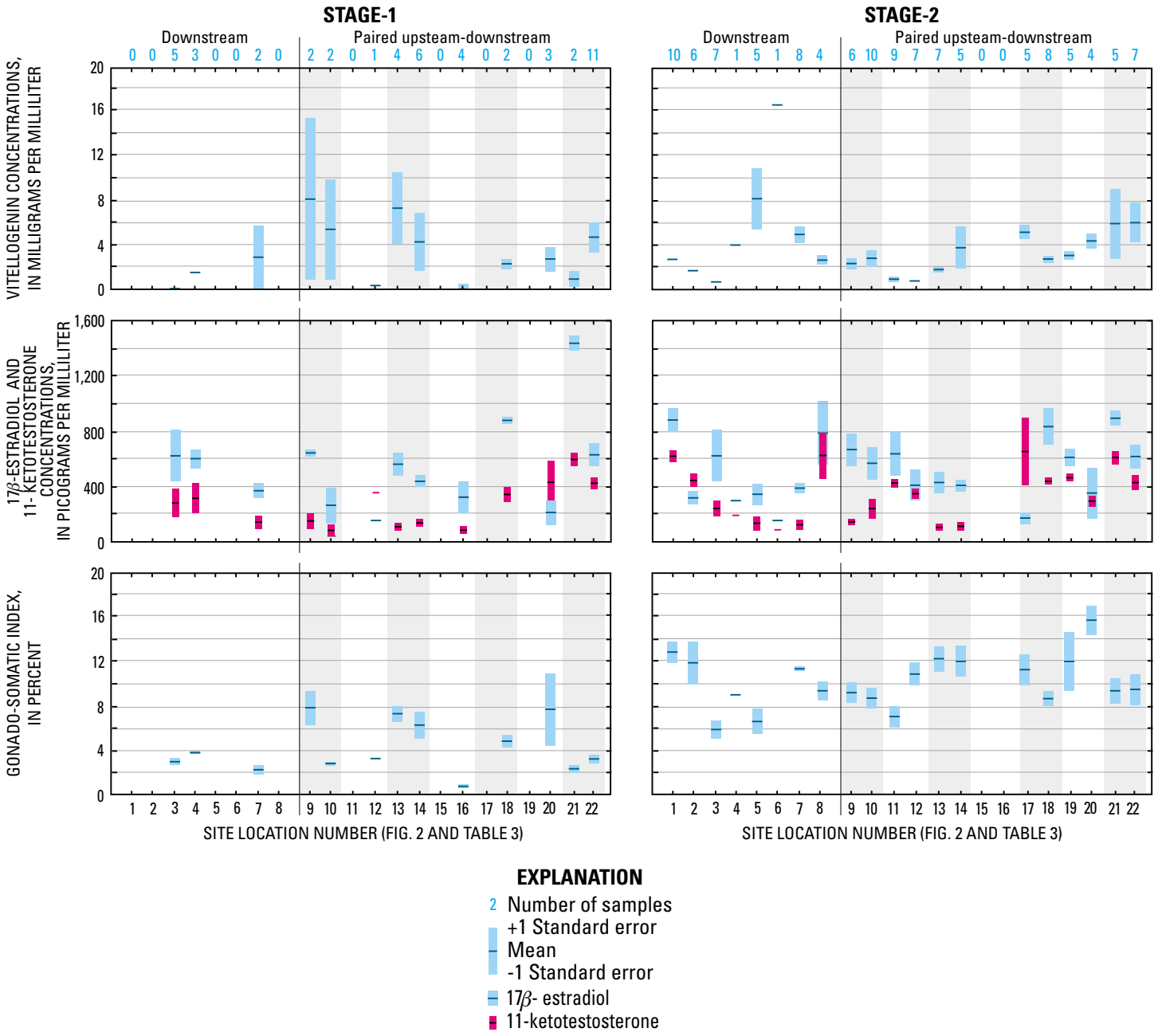

Figure 6b. Concentrations of vitellogenin, 17β-estradiol, and 11-ketotestosterone in plasma and gonado-somatic index values of stage-1 and-2 female common carp collected during 1999.

At the paired sites, there were no significant differences in 11-ketotestosterone or 17β-estradiol concentrations in male fish between upstream and downstream. Although there were no significant differences at the 0.05 significance level, 11-ketotestosterone concentrations tended to be greater at upstream than downstream sites ($p = 0.07$) at most of the paired sites.

Both 11-ketotestosterone and 17β-estradiol concentrations in male fish were significantly different among sites ($p < 0.001$). Mean concentrations of 17β-estradiol in male fish were high in at least one gonad stage at downstream sites 2, 3, 4, 5, 6, 14, and 18; and upstream sites 13 and 19 (fig. 7, table 14). Mean concentrations of 11-ketotestosterone in male fish were low in at least one gonad stage at downstream sites 5, 12, 14, and 16, and upstream sites 9, and 13. Additionally, 17β-estradiol concentrations were greater than 11-ketotestosterone concentrations at upstream site 13 and downstream site 14.

Gonado-Somatic Index Values for Common Carp

The gonads of female and male fish are considered to be fully sexually mature when GSI values reach approximately 20–30 percent of female body weight, and 5–10 percent

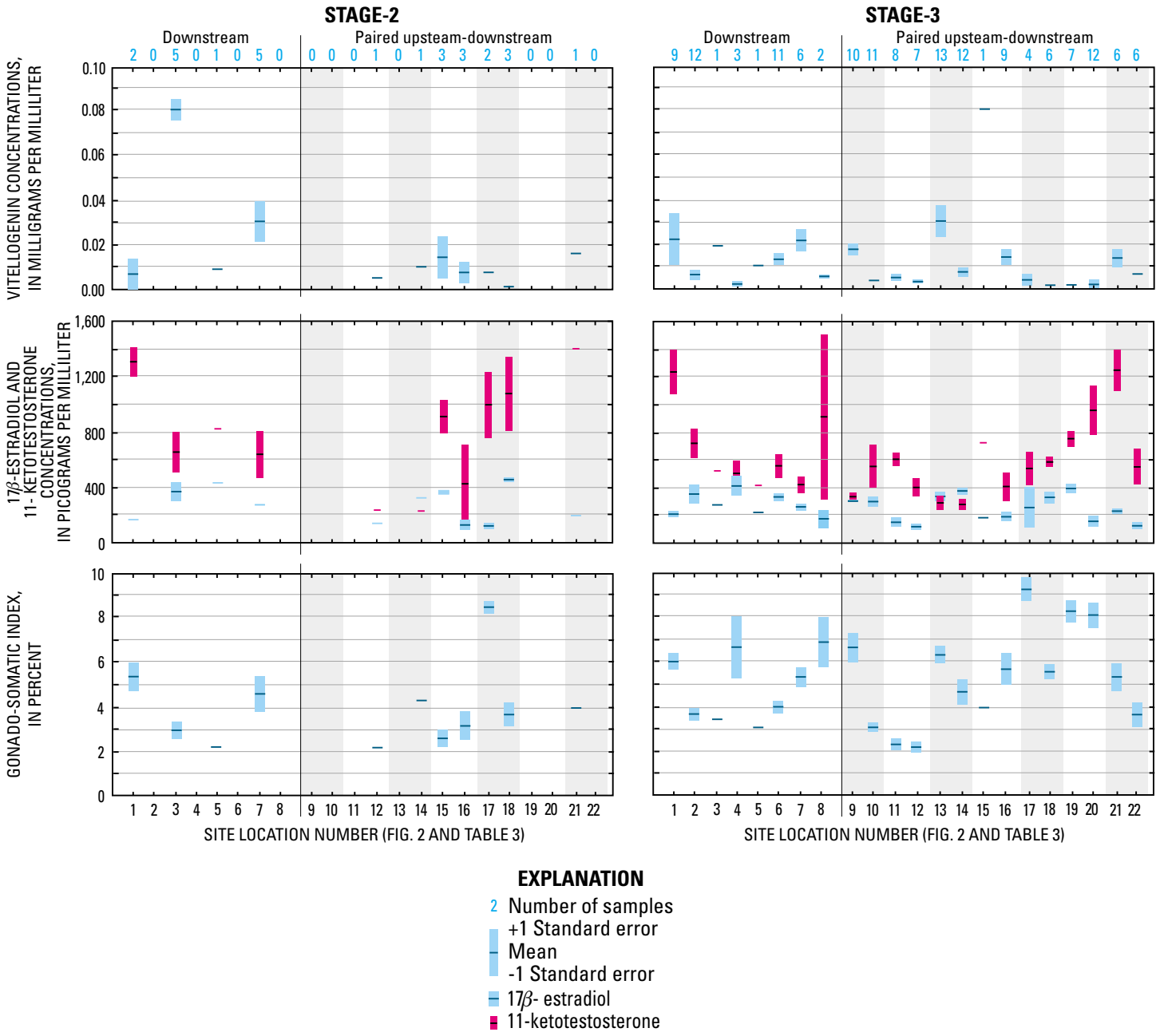

Figure 7. Concentrations of vitellogenin, 17β-estradiol, and 11-ketotestosterone in plasma and gonado-somatic index values of stage-2 and-3 male common carp collected during 1999.

of male body weight (Kime, 1998). Contaminants can result in reduced gonad weights and reduced reproductive success (Kime, 1998).

There were some differences in female GSI values between upstream and downstream site groups. GSI values in stage-1 females were greater in the upstream than the downstream site group ($p = 0.03$) (table 12). At the paired sites, there were no significant differences in GSI values for females between upstream and downstream.

GSI values in female fish were significantly different among all sites ($p < 0.001$) (figs. 6a and 6b). Mean GSI values were low for female fish in at least one gonad stage at downstream sites 3, 5, 7, 10, 16, 18, and 20; and upstream sites 11, 15, and 21 (table 14).

Male GSI values in stage-3 fish were greater in the upstream than the downstream site group ($p = 0.009$) (table 13). At the paired sites, GSI values were significantly greater in males from upstream than downstream sites ($p = 0.04$).

Male GSI values were significantly different among all sites ($p < 0.001$) (fig. 7). Mean GSI values were low in male fish in at least one gonad stage at downstream sites 3, 5,

Table 12. Comparisons of biological characteristics for female fish[1] between upstream and downstream site groups for each gonad maturation stage, and between upstream and downstream paired sites

[SE, standard error; n, number of fish sampled; p-values shown in bold indicate statistically significant differences]

Biological characteristic	Comparison of upstream and downstream site groups									Comparison of paired sites		
	Gonad stage–0			Gonad stage–1			Gonad stage–2					
	Upstream Mean ± SE	Downstream Mean ± SE	p-value [2]	Upstream Mean ± SE	Downstream Mean ± SE	p-value [2]	Upstream Mean ± SE	Downstream Mean ± SE	p-value [2]	Upstream Mean ± SE	Downstream Mean ± SE	p-value[3]
	n = 9	n = 5		n = 8	n = 39		n = 37	n = 83		n = 7	n = 7	
Vitellogenin (mg/mL)	0.27 ± 0.07	0.24 ± 0.14	0.6	6.03 ± 2.29	2.89 ± 0.63	0.2	2.87 ± 0.52	3.46 ± 0.37	0.26	3.34 ± 8.45	3.24 ± 4.14	0.45
17β-estradiol (pg/mL)	531 ± 75	357 ± 42	0.08	802 ± 147	499 ± 46	**0.04**	567 ± 49	559 ± 36	0.84	653 ± 54	474 ± 41	0.15
11-ketotestosterone (pg/mL)	428 ± 27	191 ± 53	**0.02**	242 ± 78	277 ± 31	0.63	379 ± 46	337 ±24	0.71	378 ± 71	255 ± 63	**0.04**
GSI (percent)	0.9 ± 0.2	1.5 ± 0.3	0.18	6.3 ± 0.9	3.8 ± 0.4	**0.03**	9.9 ± 0.6	10.1 ± 0.4	0.69	7.3 ± 16.3	7.3 ± 21.0	0.33
Atretic oocytes (percent)	3.2 ± 2.3	5.0 ± 5.4	0.68	15.0 ± 4.0	10.8 ± 1.8	0.09	10.9 ± 0.9	9.5 ± 1.3	0.93	10.3 ± 1.2	10.5 ± 0.9	0.13
Ceroid/lipofuscin ranking	0.82 ± 0.68	0.75 ± 0.48	0.33	0.92 ± 0.22	1.15 ± 0.48	0.94	1.06 ± 0.06	1.03 ± 0.10	**0.05**	0.93 ± 0.11	0.88 ± 0.13	0.26

[1] Female common carp in gonad stages-0 through -2, and ages ranging from 2 to 5 years old were used in these analyses
[2] Two sided t-test results
[3] Paired t-test result

Table 13. Comparisons of biological characteristics for male fish[1] between upstream and downstream site groups for each gonad maturation stage, and between upstream and downstream paired sites

[SE, standard error; n, number of fish sampled; p-values shown in bold indicate statistically significant differences]

Biological Characteristic	Comparison of upstream and downstream site groups						Comparison of paired sites		
	Gonad Stage–2			Gonad Stage–3					
	Upstream Mean ± SE	Downstream Mean ± SE	p-value [2]	Upstream Mean ± SE	Downstream Mean ± SE	p-value [2]	Upstream Mean ± SE	Downstream Mean ± SE	p-value [3]
	n = 6	n =21		n = 49	n =109		n = 7	n = 7	
Vitellogenin (mg/mL)	0.01 ± 0.0045	0.02 ± 0.01	0.44	0.016 ± 0.0028	0.0089 ± 0.0012	**0.01**	0.018 ± 0.024	0.005 ± 0.003	**0.01**
17β-estradiol (pg/mL)	254 ± 54	292 ± 30	0.57	287 ± 19	264 ± 14	0.28	260 ± 99	241 ± 126	0.35
11-ketotestosterone (pg/mL)	1022 ± 115	705 ± 96	**0.02**	561 ± 51	620 ± 42	0.44	709 ± 312	580 ± 264	0.07
GSI (percent)	4.7 ± 1.2	3.7 ± 0.3	0.49	6.0 ± 0.3	4.9 ± 0.2	**0.009**	5.8 ± 2.5	4.0 ± 1.8	**0.04**
Ceroid/lipofuscin ranking	0.83 ± 0.44	0.88 ± 0.12	0.43	0.90 ± 0.24	1.06 ± 0.08	0.21	0.86 ± 0.22	1.07 ± 0.10	0.13

[1] Male common carp in gonad stages-2 and -3, and ages ranging from 2 to 4 years old were used in these analyses
[2] Two sided t-test results
[3] Paired t-test result

Table 14. Summary of biological indicators of hormonally active agents for stage-0, -1 and -2 female, and stage-2 and 3 male common carp at individual sites collected during 1999 from Minnesota streams

[Low, lowest 25th percentile within each gonad stage; High, 75th percentile (highest 25th percentile); pg/mL, picogram per milliliter; mg/mL, milligrams per milliliter; ≤, less than or equal to; ≥, greater than or equal to; shading shows downstream sites; X indicates that the mean value for at least one gonad stage met the criteria listed in the biological indicators column]

	Biological indicators	Site location number (shown on fig. 2)																					
		Downstream sites								Paired upstream-downstream sites													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Female common carp	Low vitellogenin concentrations (≤ 0.07, ≤ 0.89 and ≤ 2.22 mg/mL for stage-0, -1 and -2, respectively)		X	X								X	X	X			X				X	X	
	Low 17β-estradiol concentrations (≤ 326, ≤ 316 and ≤ 350 pg/mL for stage-0, -1 and -2, respectively)		X		X	X	X				X		X				X	X			X	X	
	High 11-ketotestosterone (≥ 390, ≥356 and ≥ 445 pg/mL for stage -0, -1, and -2, respectively)	X							X			X	X			X		X		X	X	X	X
	11-ketotestosterone > 17β-estradiol		X										X					X			X		
	Low gonado-somatic index values (≤ 0.7, ≤ 2.9 and ≤ 8.8 percent for stage-0, -1 and -2, respectively)			X		X		X			X	X				X	X		X		X	X	
	High percent atretic oocytes (≥ 10.0, ≥ 15.3 and ≥ 13.8 for stage-0, -1 and-2, respectively)			X	X				X	X		X		X	X				X				
	High ceroid/lipofuscin ranking (≥ 1.4, ≥ 1.6 and ≥ 1.1 for stage-0, -1 and -2, respectively)			X	X				X			X		X	X			X			X		
Male common carp	High vitellogenin concentrations (≥ 0.015 and ≥ 0.016 mg/mL for stage-2 and -3, respectively)	X		X				X		X				X		X						X	
	High 17β-estradiol concentrations (≥ 366 and ≥ 339 pg/mL for stage-2 and 3, respectively)		X	X	X	X	X							X	X				X	X			
	Low 11-ketotestosterone (≤ 529 and ≤ 422 pg/mL for stage-2 and -3, respectively)					X				X			X	X	X		X						
	17β-estradiol ≥ 11-ketotestosterone													X	X								
	Low gonado-somatic index values (≤ 2.8 and ≤ 3.7 percent for stage-2 and 3, respectively)			X		X					X	X	X			X							X
	High ceroid/lipfuscin ranking (≥ 1.2 and ≥ 1.3 for stage-2 and-3, respectively)	X		X					X		X		X					X	X	X			
	Total number of biological indicators	3	4	8	4	5	2	2	4	3	4	6	7	7	5	4	4	5	4	3	6	5	2

10, 12, and 22, and upstream sites 11 and 15 (table 14).

Water temperature is a stimulus for spawning and recrudescence and can influence GSI values (Kime, 1998). Water temperatures ranged from 16 to 29 ^{o}C among all sites. Female GSI values were not consistently related to water temperature (Spearman rank correlation coefficients of 0.08, -0.01, -0.34, for stage-0, -1, and -2, respectively). In contrast, GSI values in male fish declined as water temperature increased (Spearman rank correlation coefficients of -0.52 for males in gonad stages-2 and -3). Male fish at three of the sites (5, 10, and 11) with the greatest water temperatures had low GSI values; however, it is not possible with data collected during this study to determine if lower GSI values at sites 5, 10, and 11 are due to greater temperatures or other factors.

Gonad Histopathology of Common Carp

An average of 86 percent of the female fish per site had atretic oocytes. The amount of atretic oocytes per female ranged from 0 to 57 percent (mean = 10.25 percent). There was no difference in percent atretic oocytes between site groups (table 12). At the paired sites, there were no significant differences in percent atretic oocytes between upstream and downstream.

Percent atretic oocytes in females were significantly different between sites ($p < 0.001$) (figs. 6a and 6b). The mean percent atretic oocytes in female fish within at least one gonad stage were high at downstream sites 3, 4, 8, 14, and 18; and upstream sites 9, 11, and 13) (table 14).

Ceroid/lipofuscin tissue rankings in female gonad tissue ranged from 0 to 3 among all females (table 15). Most females were ranked 1 in both upstream and downstream site groups (75 and 66 percent respectively). Ceroid/lipofuscin tissue rankings in females were not different between site groups for stage-0 and -1 fish; however, ceroid/lipofuscin rankings were greater in the upstream than downstream site group for stage-2 fish ($p = 0.05$) (table 12). Mean ceroid/lipofuscin rankings in female fish varied among sites ($p < 0.001$). Mean ceroid/lipofuscin tissue rankings were high for females in at least one gonad stage at downstream sites 3, 4, 8, 14 and 20 and at upstream sites 11, 13 and 17 (table 14).

Ceroid/lipofuscin tissue rankings ranged from 0 to 3 among all male fish (table 16). On average, 82 percent of males at each site had ceroid/lipofuscin tissue. There was no difference in ceroid/lipofuscin rankings between the upstream and downstream site groups or for the upstream–downstream paired sites. Ceroid/lipofuscin rankings varied among sites ($p = 0.006$). Ceroid/lipofuscin rankings were high for male fish in at least one gonad stage at sites 1, 3, 8, 10, 12, and 18; and upstream sites 17 and 19 (table 14).

RELATIONS OF BIOLOGICAL CHARACTERISTICS TO WASTEWATER TREATMENT PLANT EFFLUENT AND LAND USE

The presence of HAAs in selected Minnesota streams was indicated by biological characteristics in common carp. Biological characteristics used in this study identified WWTP effluent as a potential source of HAAs. Fish located at sites upstream of WWTP effluent draining agricultural and forested land also show indications of HAAs.

The paired sites offered the best opportunity to compare biological characteristics between sites upstream and downstream of WWTPs. Because the dominant chemicals at upstream sites likely influence downstream sites, the effect at downstream sites was expected to be greater due to the combination of dominant upstream factors and WWTP effluent. This pattern was not observed for vitellogenin concentrations in male fish.Vitellogenin concentrations in male fish tended to be greater in upstream than downstream paired sites. Two of the upstream sites where males had elevated vitellogenin concentrations (sites 13 and 21) had minimal percents of streamflow that consisted of WWTP discharge (0.3 and 0.1 percent, respectively). It is possible that males at these two sites were exposed to effluent, which resulted in elevated vitellogenin concentrations. However, males with elevated vitellogenin concentrations were also observed at upstream sites with no known continuous-flow WWTPs upstream (sites 9 and 15). This may suggest that HAAs that are not associated with WWTP effluent are present at upstream sites.

Sex steroid concentrations and GSI values at the paired sites indicated a greater effect downstream than upstream of WWTPs. The 17β-estradiol concentrations in females and 11-ketotestosterone concentrations in males tended to be lower at sites downstream of WWTPs. Similarly, Folmar and others (1996) found decreases in testosterone in male common carp collected from WWTP effluent. GSI values in male fish were lower at downstream than upstream sites. A similar result was reported by Jobling and others (1996), for rainbow trout exposed to NP and 17α-ethynylestradiol, which are compounds observed in WWTP effluent.

Although there were some patterns in biological characteristics between upstream and downstream sites, there was a great amount of variability among sites within each group. For example, high vitellogenin concentrations in males were observed at three downstream sites (1, 3, and 7) and four upstream sites (9, 13, 15, 21). Female fish had low vitellogenin concentrations at five downstream and three upstream sites. The presence of vitellogenin in male fish and low concentrations in females may be due to a variety of chemicals, including agricultural pesticides and chemicals in WWTP effluent, that are reported to induce vitellogenin production in male fish. Vitellogenin induction has been observed in male

fish from streams draining agricultural areas. Grady and others (1998) observed that male largemouth bass treated with 100 µg/L of atrazine for 20 days had increased vitellogenin concentrations. Atrazine at that high concentration is not typically observed in larger streams. However, it may be an environmentally relevant concentration for some periods of the year, especially for common carp, because they move upstream into very small tributaries where the concentrations tend to be greater than in larger streams.

Vitellogenin induction has also been observed in male fish exposed to WWTP effluent. Purdom and others (1994) measured vitellogenin concentrations of 0.00002–0.015 mg/mL in caged common carp after a 3-week exposure to WWTP effluent. Similarly, Folmar and others (1996) reported the presence of vitellogenin in male common carp downstream of the Minneapolis and St. Paul Metropolitan WWTP effluent channel (site 3). NP concentrations of 2.1 to 2.3 µg/L during the fall of 1997 and spring of 1998, measured by Barber and others (2000) in the Minneapolis and St. Paul Metropolitan WWTP effluent channel (site 3), are below those concentrations of NP (10–20 µg/L) reported by Sumpter and Jobling (1995) and Hammer and others (in press) to induce vitellogenin in male fish. The Minneapolis and St. Paul Metropolitan WWTP effluent channel contained 43–150 µg/L of total combined alkylphenols (AP), alkylphenolpolyethoxylates (APEO), and alkylphenolethoxycarboxylates (APEC), which in combination may have resulted in vitellogenin induction (Barber and others, 2000).

Both 17β-estradiol and 11 ketotestosterone concentrations were variable among sites. Most of the sites where female fish had low 17β-estradiol concentrations, and had 11-ketotestosterone concentrations that were greater than 17β-estradiol concentrations were located downstream of WWTPs. In male fish, 11-ketotestosterone concentrations were low at more sites located downstream than upstream of WWTPs. The tendency for 17β-estradiol concentrations to be low in female fish, and 11-ketotestosterone concentrations to be low in male fish and at sites downstream of WWTPs could be due to water chemistry. Folmar and others (1996) found reduced testosterone concentrations in male common carp exposed to WWTP effluent. HAAs associated with WWTP effluent may inhibit testosterone production and its conversion to both 17β-estradiol and 11-ketotestosterone.

GSI values were low for female and male fish at sites located upstream and downstream of WWTPs. Fish with low GSI values may have limited reproduction potential (Kime, 1998). Low GSI values for both male and female fish were observed at sites 3 and 5. Reduced GSI values have been observed by others in male fish below kraft mills (McMaster and others, 1992) and in male fish exposed to pesticides (Arora and Kulshrestha, 1984).

The percent atretic oocytes and ceroid/lipofuscin tissue rankings in females and males were not clearly related to just one site group, but were predominant at specific sites and may indicate that some contaminant or environmental factor has reduced the potential for spawning or has induced egg reabsorption. Kumar and Pant (1998) and Shukla and others (1984) found that atresia was increased with pesticide exposures.

The number of biological characteristics that indicated HAAs varied among sites. All sites had at least one biological characteristic that indicated the presence of HAAs in female and male fish (table 14). Downstream sites 3, 12, and 20 and upstream sites 11 and 13 had the greatest number of HAA indications while downstream sites 1, 6, 7, and 22 and upstream sites 9 and 19 had few indications of HAAs.

At downstream sites, the number of indications of HAAs does not seem to be strongly related to the dilution of the effluent in the receiving stream. The downstream site with the greatest number of HAA indicators was site 3, which is a 100 percent effluent stream from the Minneapolis and St. Paul Metropolitan WWTP. Sites 12 and 20 also had a high number of biological characteristics that indicate HAAs, yet had lower estimated percentages of wastewater (1 and 5 percent, respectively). In addition, sites 2 and 5 have relatively higher estimated percentages of wastewater (48 and 66 percent respectively) and have relatively fewer indications of HAAs. Differences in the types and numbers of biological characteristics that indicate HAAs among downstream sites may be due to differences in WWTP effluent chemistry rather than the percent streamflow consisting of WWTP discharge.

At the upstream sites, there is not a clear factor that explains the lack of a consistent number of biological indicators. Upstream sites 11 and 13 had the greatest number of indications of HAAs, while sites 9 and 19 had few indications of HAAs. Potential reasons may include differences in the types of pesticides used and amount of pesticide runoff into streams, unknown discharges of WWTP effluent, and runoff from animal feedlots that may contain HAAs. The lack of consistency at upstream and downstream sites may also be due to differences in the exposure time of fish sampled or natural variability in the biological characteristics.

The presence of biological indicators of HAAs at sites may be due to a variety of chemicals including agricultural pesticides and chemicals in WWTP effluent. Although water chemistry at each sampling site is unknown, observations from other water quality monitoring studies in Minnesota provide some evidence of chemicals that may be present at sites in this study that may act as HAAs. While this ancillary information does not provide the type of detailed chemical information needed to establish cause and effect relations, it can provide insight into the presence of potential HAAs in streams sampled during this study.

Determination of evidence of HAAs is complicated by the presence

of natural variability in biological characteristics. Natural variation in the biological characteristics may account for some of the differences observed in this study. Analyses were completed on separate gonad stages to remove potential natural variability in reproductive stage; however, there is likely still some variability in the biological characteristics due to natural reproductive status.

This study and others indicate the presence of HAAs in surface water and the potential signs of endocrine disruption in resident fish populations in Minnesota streams. Detailed controlled studies could confirm that endocrine disruption has occurred and establish the effects of particular chemicals such as pesticides or components of WWTPs on fish reproduction and population structure.

SUMMARY

Concern about the effects of chemicals in the environment that act as hormonally active agents (HAAs) is widespread. HAAs may interfere with natural regulation of the endocrine system of animals by either mimicking or blocking the function of natural hormones. Potential effects of HAAs on fish include disruption of the endocrine system, specifically reproductive function. Potential sources of HAAs to streams in Minnesota are treated sewage (domestic and industrial) and runoff from agricultural or forested land. Fish are important organisms for indicating the extent of HAAs in surface water because they are directly exposed to contaminants and their endocrine systems have many physiological similarities to mammals, including humans.

Male (221 individuals) and female (201 individuals) common carp were collected using electrofishing techniques from seven streams with sites at two locations (upstream and downstream of WWTPs), and eight sites located downstream of WWTPs with no upstream–paired sites. Samples were collected between August 3 and September 13, 1999. Four biological characteristics of common carp used as indicators of HAAs in the streams selected for this study are: (1) high concentrations of vitellogenin in male fish and low concentrations in female fish, (2) high or low plasma concentrations of the sex steroid hormones (17β-estradiol and 11-ketotestosterone), (3) low gonado-somatic index (GSI) (gonad weight divided by total body, weight multiplied by 100) values, and (4) abnormal gonad histopathology (high percent of atretic oocytes in female ovaries and high percent ceroid/lipofuscin tissue in male or female gonads).

The presence of HAAs in select Minnesota streams was indicated by biological characteristics in common carp. Biological characteristics used in this study identified WWTP effluent as a potential source of HAAs. Additionally, fish located at sites upstream of WWTP effluent draining agricultural and forested land show indications of HAAs.

Because the dominant chemicals at upstream sites likely influence downstream sites, the effect at downstream sites was expected to be greater due to the combination of dominant upstream factors and WWTP effluent. This pattern was not observed for vitellogenin concentrations in male fish.Vitellogenin concentrations in male fish tended to be greater in upstream than downstream paired sites. Sex steroid concentrations and GSI values at the paired sites indicated a greater effect downstream than upstream of WWTPs. The 17β-estradiol concentrations in females and 11-ketotestosterone concentrations in males were lower at sites downstream of WWTPs. GSI values in males were also lower at sites downstream of WWTPs.

Although there were some patterns in biological characteristics between upstream and downstream paired sites, there was a great amount of variability among all sites. All sites had at least one biological characteristic that indicated the presence of HAAs in female and male fish. Downstream sites 3, 12, and 20 and upstream sites 11 and 13 had the greatest number of HAA indications while downstream sites 1, 6, 7, and 22 and upstream sites 9 and 19 had few indications of HAAs. At downstream sites, the number of indications of HAAs does not seem to be strongly related to the dilution of the effluent in the receiving stream.

The presence of biological indicators of HAAs located at all sites may be due to a variety of chemicals including agricultural pesticides and chemicals in WWTP effluent. Determination of evidence of HAAs is complicated by the presence of natural variability in biological characteristics. Natural variation in the biological characteristics may account for some of the differences observed in this study.

This study and others indicate the presence of HAAs in surface water and the potential signs of endocrine disruption in resident fish populations in Minnesota streams. Detailed controlled studies could confirm that endocrine disruption has occurred and establish the effects of particular chemicals such as pesticides or components of WWTPs on fish reproduction and population structure.

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SUPPLEMENTAL INFORMATION

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
1	D	416	998	2	3	19	3.028	1,341	701	16.3	5	1	Y
1	D	416	1089	2	3	19	3.111	1,078	731	15.9	7	1	Y
1	D	485	1588	2	4	19	2.007	866	366	7.3	17	1	Y
1	D	428	1315	2	4	19	3.036	941	533	10.7	28	1	Y
1	D	454	1406	2	4	19	2.164	432	488	9.7	11	1	Y
1	D	533	2359	2	4	19	2.349	497	537	15.1	15	2	Y
1	D	410	862	2	4	19	3.402	747	623	13.3	12	1	Y
1	D	450	1452	2	4	19	3.158	972	831	14.1	15	1	Y
1	D	465	1361	2	4	19	1.785	801	595	10.9	5	1	Y
1	D	456	1452	2	5	19	2.692	1,134	760	14.3	18	1	Y
1	D	552	2132	2	6	19	2.415	383	698	6.5	2	3	N
2	D	474	1225	2	2	23	1.974	305	618	6.5	2	0	Y
2	D	477	1542	2	2	23	1.86	307	349	17.9	12	1	Y
2	D	490	1769	2	2	23	1.868	502	551	8.5	4	1	Y
2	D	483	1678	2	2	23	1.585	379	416	15.1	8	1	Y
2	D	442	1315	2	2	23	1.15	303	316	8.8	0	1	Y
2	D	481	1542	2	2	23	1.537	118	353	14.2	8	0	Y
3	D	551	2087	0	3	22	0.053	437	238	1.9	6	2	Y
3	D	571	2087	0	4	22	0.34	364	46	1.5	14	2	Y
3	D	520	2041	1	3	22	0.097	620	558	3.9	16	2	Y
3	D	511	1905	1	3	22	0.165	303	54	1.9	15	1	Y
3	D	517	1633	1	3	22	0.089	1,326	507	3.2	14	2	Y
3	D	522	1950	1	3	22	0.021	420	136	3.	6	2	Y
3	D	555	2268	1	4	22	0.446	453	125	3.4	24	2	Y
3	D	515	1996	2	3	22	0.015	343	502	6.5	1	1	Y
3	D	514	2177	2	3	22	1.342	747	284	8.4	5	1	Y
3	D	532	1814	2	3	22	0.078	283	43	4.0	4	2	Y
3	D	512	2087	2	3	22	0.75	1,035	258	3.1	7	1	Y
3	D	500	1633	2	3	22	0.252	678	237	5.5	8	1	Y
3	D	570	2812	2	4	22	1.003	247	256	9.3	0	1	Y
3	D	554	2495	2	4	22	1.329	884	111	5.1	11	1	Y
4	D	710	4536	1	5	--	1.337	716	533	4.0	12	2	Y

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
4	D	685	3856	1	5	--	1.72	619	186	3.9	26	2	Y
4	D	690	4672	1	5	--	1.68	477	202	3.6	10	2	Y
4	D	638	4763	2	5	--	4.104	296	193	8.9	15	2	Y
5	D	319	499	0	1	28	0.138	297	63	1.0	0	1	N
5	D	281	318	0	1	28	0.006	386	103	0.9	0	1	N
5	D	321	499	0	1	28	0.364	168	558	0.8	0	1	N
5	D	318	454	0	1	28	0.261	375	88	1.5	0	0	N
5	D	331	544	1	1	28	13.047	442	75	2.6	3	0	N
5	D	403	1497	2	2	28	11.597	402	113	3.5	5	0	Y
5	D	367	771	2	2	28	17.029	337	74	4.7	15	1	Y
5	D	505	1864	2	3	28	1.282	105	36	8.3	12	1	Y
5	D	425	1315	2	3	28	6.847	321	113	9.7	22	1	Y
5	D	493	1724	2	4	28	4.744	557	313	6.6	0	1	Y
6	D	429	1089	2	3	25	16.815	158	87	--	3	1	Y
7	D	415	907	1	1	23	0.989	532	58	2.5	10	1	N
7	D	418	998	1	3	23	5.909	319	97	2.6	2	1	Y
7	D	418	1043	1	3	23	0.047	419	184	1.8	3	0	Y
7	D	470	1406	2	2	23	5.752	374	106	11.0	9	0	Y
7	D	424	1089	2	2	23	1.791	342	127	12.0	9	1	Y
7	D	491	1678	2	2	23	6.454	385	123	10.3	20	2	Y
7	D	519	1860	2	3	23	5.553	243	91	11.3	5	2	Y
7	D	465	1542	2	3	23	3.146	365	52	11.9	4	1	Y
7	D	452	1225	2	3	23	4.834	343	201	11.5	18	0	Y
7	D	444	1361	2	3	23	8.415	449	129	11.6	12	1	Y
7	D	414	1043	2	3	23	4.255	556	154	10.7	18	1	Y
8	D	365	771	2	1	--	1.441	133	112	6.5	18	1	N
8	D	430	1043	2	2	--	1.781	114	141	8.8	8	1	Y
8	D	500	1905	2	3	--	1.733	955	947	11.8	29	2	Y
8	D	623	3402	2	4	--	3.309	1,014	647	7.5	15	2	Y
8	D	640	3901	2	5	--	2.832	1,120	880	9.2	12	1	Y
						--							
9	U	570	2132	1	3	24	15.395	639	191	9.3	23	1	Y

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
9	U	560	2404	1	4	24	0.861	667	107	6.3	11	1	Y
9	U	500	1678	2	2	24	2.596	783	145	12.1	3	1	Y
9	U	550	1905	2	3	24	0.686	653	152	8.1	21	1	Y
9	U	538	2087	2	3	24	4.061	259	177	10.5	19	1	Y
9	U	587	2586	2	3	24	3.251	396	107	10.4	4	1	Y
9	U	602	2631	2	4	24	1.768	1,090	139	7.8	4	1	Y
9	U	579	2313	2	4	24	1.888	791	140	6.4	12	1	Y
10	D	511	1860	1	3	26	0.759	394	53	2.7	5	1	Y
10	D	501	1814	1	4	26	10.024	38	135	3.0	12	1	Y
10	D	485	1452	2	2	26	0.461	319	191	5.2	11	1	Y
10	D	465	1361	2	3	26	2.223	235	84	7.2	5	1	Y
10	D	441	1270	2	3	26	4.959	1184	401	11.3	12	1	Y
10	D	490	1588	2	3	26	1.206	301	39	9.7	4	1	Y
10	D	525	1950	2	3	26	5.41	578	94	7.3	5	0	Y
10	D	505	1814	2	3	26	6.495	1,107	46	8.9	2	1	Y
10	D	442	1270	2	3	26	3.035	864	37	10.0	6	0	Y
10	D	499	1814	2	3	26	0.424	228	324	13.5	9	0	Y
10	D	500	1588	2	3	26	3.103	528	650	3.8	10	1	Y
10	D	515	1633	2	4	26	0.662	290	544	9.9	5	1	Y
11	U	392	998	0	2	29	0.59	722	489	1.8	20	1	Y
11	U	465	1452	0	3	29	0.664	416	552	2.4	9	2	Y
11	U	389	998	2	2	29	2.557	301	376	4.0	6	1	Y
11	U	388	1089	2	2	29	0.295	797	348	4.6	12	1	Y
11	U	421	1315	2	2	29	1.512	634	487	6.5	2	1	Y
11	U	379	998	2	2	29	0.173	401	415	4.4	18	1	Y
11	U	381	998	2	2	29	0.599	923	373	11.0	2	1	Y
11	U	548	1905	2	3	29	0.458	433	401	12.4	0	0	Y
11	U	520	2041	2	3	29	0.35	677	542	7.3	2	1	Y
11	U	403	1134	2	3	29	0.715	766	504	5.1	3	1	Y
11	U	520	1996	2	3	29	1.102	801	427	7.1	31	1	Y
12	D	462	1225	0	3	24	0.749	454	205	2.6	--	1	Y
12	D	407	680	1	2	24	0.408	161	356	3.2	--	1	Y
12	D	365	680	2	1	24	2.473	762	429	6.8	--	1	N

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
12	D	389	816	2	2	24	0.467	399	141	8.9	--	0	Y
12	D	380	816	2	2	24	1.139	833	341	14.3	--	1	Y
12	D	442	953	2	2	24	0.799	602	243	6.6	--	1	Y
12	D	375	771	2	2	24	0.131	122	517	11.8	--	0	Y
12	D	350	590	2	2	24	1.425	511	550	9.8	--	1	Y
12	D	441	1089	2	2	24	0.681	210	242	13.4	--	1	Y
12	D	404	862	2	2	24	0.496	274	390	11.0	--	0	Y
13	U	455	1089	1	2	27	12.883	638	96	5.7	13	1	Y
13	U	496	1361	1	2	27	13.066	697	121	8.8	18	2	Y
13	U	460	1315	1	2	27	2.385	588	151	6.8	19	1	Y
13	U	487	1542	1	3	27	1.878	301	84	8.4	7	1	Y
13	U	445	1270	2	2	27	1.766	333	120	13.4	15	1	Y
13	U	452	1134	2	2	27	1.987	582	98	15.0	9	1	Y
13	U	492	1406	2	2	27	2.126	828	155	13.7	17	1	Y
13	U	552	2631	2	3	27	2.094	246	100	14.7	16	1	Y
13	U	510	1814	2	3	27	0.149	368	127	11.4	8	2	Y
13	U	488	1497	2	3	27	2.116	287	61	10.8	27	1	Y
13	U	616	2812	2	4	27	2.252	301	41	6.6	8	2	Y
14	D	447	1089	1	2	22	1.066	397	100	2.9	4	1	Y
14	D	464	1270	1	2	22	1.301	449	99	2.9	20	1	Y
14	D	485	1406	1	3	22	2.232	335	203	8.8	13	2	Y
14	D	535	1860	1	3	22	2.34	365	107	9.8	9	2	Y
14	D	555	2268	1	3	22	16.996	628	159	6.7	57	3	Y
14	D	515	1814	1	3	22	2.335	433	89	6.6	9	1	Y
14	D	420	907	2	2	22	0.751	397	85	12.8	27	1	Y
14	D	405	862	2	2	22	2.499	311	86	13.0	19	1	Y
14	D	435	1179	2	2	22	2.121	561	104	10.9	7	1	Y
14	D	488	1497	2	3	22	2.007	359	147	7.6	4	1	Y
14	D	564	2495	2	4	22	11.683	381	108	15.6	19	1	Y
15	U	330	816	0	1	24	0.067	542	442	0.2	0	0	N
15	U	404	1179	0	2	24	0.201	911	355	0.68	0	0	Y
15	U	378	907	0	2	24	0.108	653	408	0.66	0	1	Y
15	U	420	1270	0	2	24	0.176	328	425	0.24	0	0	Y

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
15	U	388	1043	0	2	24	0.076	702	531	0.38	0	0	Y
15	U	420	1315	0	3	24	0.068	228	374	0.84	0	0	Y
15	U	450	1406	0	4	24	0.305	432	305	1.0	0	0	Y
15	U	455	1588	0	4	24	0.288	385	412	0.82	0	0	Y
16	D	360	816	0	1	22	0.033	331	128	1.1	0	0	N
16	D	323	635	0	1	22	0.022	327	87	0.95	0	0	N
16	D	415	1089	0	2	22	0.032	302	112	0.92	0	0	Y
16	D	424	1225	1	2	22	0.008	110	49	1.3	0	0	Y
16	D	446	1497	1	2	22	0.043	133	92	0.9	0	0	Y
16	D	410	1270	1	2	22	0.015	609	151	0.78	0	0	Y
16	D	448	14062	1	3	22	0.758	413	33	0.16	0	0	Y
17	U	770	7348	2	5	16	7.013	306	363	9.4	4	1	Y
17	U	698	4853	2	5	16	5.015	69	1594	14.4	20	1	Y
17	U	732	5670	2	5	16	6.139	199	546	11.5	6	1	Y
17	U	751	7484	2	5	16	3.953	105	220	13.6	12	2	Y
17	U	745	5715	2	5	16	4.411	132	515	6.9	2	1	Y
17	U	750	6396	2	6	16	3.326	61	565	12.8	4	1	N
17	U	785	6804	2	6	16	3.83	629	932	12.7	6	1	N
17	U	734	5216	3	5	16	0.094	158	445	9.6		2	N
18	D	610	3221	1	4	23	1.831	911	399	4.3	18	1	Y
18	D	602	3039	1	5	23	2.7	857	288	5.4	11	1	Y
18	D	485	1814	2	2	23	2.583	453	356	10.0	7	1	Y
18	D	542	2223	2	3	23	2.916	1223	515	9.3	23	1	Y
18	D	515	2041	2	3	23	2.759	394	507	7.3	6	1	Y
18	D	530	2223	2	3	23	3.449	823	420	8.3	8	1	Y
18	D	536	2268	2	3	23	2.226	923	400	12.1	4	1	Y
18	D	555	2404	2	4	23	1.606	1483	446	7.5	19	1	Y
18	D	605	3402	2	4	23	2.153	741	534	6.1	19	1	Y
18	D	614	3402	2	4	23	3.712	648	334	8.1	50	1	Y
19	U	407	907	2	2	21	2.275	381	385	4.1	0	1	Y
19	U	531	2132	2	3	21	3.715	738	497	8.0	9	1	Y
19	U	551	2767	2	4	21	2.168	685	427	13.6	1	1	Y

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
19	U	670	4309	2	4	21	3.951	591	557	15.1	8	1	Y
19	U	662	5534	2	4	21	3.262	629	445	18.7	6	1	Y
20	D	367	726	0	2	19	0.027	228	356	0.68	0	0	Y
20	D	426	771	1	2	19	1.188	129	737	3.2	0	1	Y
20	D	500	1860	1	3	19	3.323	105	251	14.0	44	3	Y
20	D	519	1542	1	4	19	3.726	386	308	6.0	1	1	Y
20	D	505	1542	2	3	19	3.533	886	428	12.2	1	1	Y
20	D	550	2359	2	4	19	2.898	287	252	18.2	13	1	Y
20	D	578	2449	2	4	19	4.485	122	183	15.3	13	1	Y
20	D	518	1905	2	4	19	6.518	113	331	17.0	11	1	Y
21	U	411	816	1	2	22	0.207	1505	645	2.0	13	0	Y
21	U	435	907	1	2	22	1.582	1382	541	2.8	16	1	Y
21	U	375	635	2	1	22	8.451	1303	503	6.0	2	1	N
21	U	350	544	2	1	22	9.35	433	489	7.7	2	1	N
21	U	394	771	2	1	22	14.563	482	383	3.1	4	1	N
21	U	354	544	2	1	22	5.703	472	382	6.1	9	1	N
21	U	380	499	2	1	22	1.507	791	431	6.8	18	1	N
21	U	425	771	2	2	22	0.069	1185	636	6.0	12	1	Y
21	U	378	726	2	2	22	17.297	1275	689	9.1	12	1	Y
21	U	369	635	2	2	22	1.696	571	689	12.6	3	1	Y
21	U	440	1179	2	2	22	0.838	902	595	8.1	7	1	Y
21	U	395	862	2	2	22	9.956	556	435	10.7	9	1	Y
22	D	402	907	1	2	24	13.358	763	722	2.4	3	1	Y
22	D	405	1043	1	2	24	8.231	334	126	4.0	18	1	Y
22	D	434	1225	1	2	24	5.775	391	562	5.5	2	1	Y
22	D	410	862	1	2	24	1.364	733	352	2.4	20	1	Y
22	D	394	816	1	2	24	0.075	431	315	1.8	1	0	Y
22	D	358	635	1	2	24	4.335	422	331	2.4	1	0	Y
22	D	402	953	1	2	24	0.692	1244	513	1.6	4	1	Y
22	D	428	998	1	2	24	--	691	415	4.7	7	1	Y
22	D	394	862	1	2	24	4.155	851	282	2.7	18	0	Y
22	D	472	1225	1	3	24	0.635	501	459	3.0	3	2	Y
22	D	444	1179	1	3	24	8.71	575	536	5.3	4	1	Y

Table 15. Water temperature and values for length, weight, age, and biological characteristics of individual female fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream; Y, yes, N, no]

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Atretic oocytes (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses [1]
22	D	411	953	2	2	24	1.352	741	345	7.0	9	1	Y
22	D	405	953	2	2	24	6.858	410	361	7.1	5	1	Y
22	D	375	816	2	2	24	1.396	691	315	14.2	11	1	Y
22	D	431	1043	2	2	24	3.146	993	662	9.7	20	1	Y
22	D	468	1497	2	3	24	5.58	442	342	4.3	6	1	Y
22	D	472	1588	2	3	24	11.295	622	382	11.3	11	1	Y
22	D	452	1270	2	3	24	13.216	401	544	12.7	17	1	Y

[1]Female common carp in gonad stages-0, 1, and 2 and ages ranging from 1 to 6 (200 samples) were used in the inital analyses to determine the influence of gonad maturation stage and age on biological characteristics. Because sample size for females in gonad stage-3 (1 sample) was too small for further analyses, this sample was removed from further analyses. Some samples (females 1- and 6-years old) were excluded from further analyses because biological characteristics were found to vary with age. Samples marked with a N were excluded from further analyses. Samples marked with a Y were used in subsequent analyses of biological characteristics between site groups, among paired sites, and among all sites.

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
1	D	394	726	2	3	19	0.0011	158	1,421	5.9	0	Y
1	D	394	726	2	3	19	0.013	172	1,189	4.7	1	Y
1	D	550	2132	2	5	19	0.024	206	1,362	4.5	1	N
1	D	518	2132	3	3	19	0.006	212	1,111	6.9	2	Y
1	D	381	816	3	3	19	0.007	319	1,740	5.1	2	Y
1	D	395	1134	3	3	19	0.007	165	1,507	7.5	1	Y
1	D	516	1860	3	4	19	<0.001	178	787	5.2	2	Y
1	D	436	1043	3	4	19	0.05	169	1,187	7.4	0	Y
1	D	526	1950	3	4	19	0.009	334	2,211	5.9	1	Y
1	D	547	2177	3	4	19	0.006	247	914	6.2	2	Y
1	D	396	1361	3	4	19	0.106	90	823	4.3	1	Y
1	D	419	953	3	4	19	0.005	185	862	5.3	1	Y
1	D	529	1996	3	5	19	0.005	226	779	6.5	2	N
2	D	336	771	3	1	23	0.02	181	514	3.1	1	N
2	D	442	1134	3	2	23	0.001	456	671	3.6	1	Y
2	D	404	907	3	2	23	0.002	361	592	2.2	1	Y
2	D	440	1225	3	2	23	0.003	478	642	4.3	1	Y
2	D	443	1179	3	2	23	0.013	223	476	4.2	0	Y
2	D	439	1270	3	2	23	0.03	222	550	5.5	1	Y
2	D	480	1542	3	2	23	0.003	298	431	3.4	0	Y
2	D	488	1633	3	2	23	<0.001	965	1,709	5.8	1	Y
2	D	442	1179	3	2	23	<0.001	438	1,006	3.1	1	Y
2	D	436	1179	3	2	23	<0.001	131	889	3.0	0	Y
2	D	445	1089	3	2	23	0.008	264	602	2.9	0	Y
2	D	482	1406	3	3	23	0.009	125	385	2.8	1	Y
2	D	276	1452	3	3	23	<0.001	297	679	3.5	1	Y
3	D	465	1361	1	3	22	0.014	252	712	1.5	1	N
3	D	505	1814	2	3	22	0.028	527	892	3.9	2	Y
3	D	532	2087	2	3	22	0.005	309	590	3.1	1	Y
3	D	500	1724	2	3	22	0.026	320	811	1.6	1	Y
3	D	480	1633	2	3	22	0.03	256	851	3.1	1	Y
3	D	550	2087	2	4	22	0.32	424	109	3.2	1	Y
3	D	460	1270	3	2	22	0.019	277	522	3.5	1	Y

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
3	D	510	1225	4	3	22	0.016	171	752	0.74	1	N
4	D	614	2994	3	4	--	<0.001	594	650	5.3	1	Y
4	D	625	3629	3	4	--	0.004	281	329	5.1	1	Y
4	D	580	3357	3	4	--	0.004	421	529	9.4	0	Y
4	D	627	4264	3	5	--	0.00073	374	901	6.0	2	N
4	D	733	5035	3	5	--	<0.001	383	618	8.3	1	N
4	D	675	4400	3	5	--	<0.001	421	435	6.3	1	N
5	D	374	771	2	2	28	0.009	434	821	2.2	1	Y
5	D	485	1406	3	3	28	0.01	224	422	3.1	1	Y
6	D	388	1089	3	1	25	0.007	214	561	3.9	0	N
6	D	378	771	3	1	25	0.021	321	844	3.6	0	N
6	D	467	1195	3	2	25	0.004	267	350	5.1	0	Y
6	D	412	1134	3	2	25	0.029	409	533	2.5	1	Y
6	D	609	2631	3	3	25	0.00012	336	464	5.1	2	Y
6	D	531	2359	3	3	25	0.016	254	389	3.5	2	Y
6	D	541	1901	3	3	25	0.015	265	281	4.3	1	Y
6	D	551	2299	3	3	25	0.012	208	822	4.7	1	Y
6	D	482	1678	3	3	25	0.021	368	502	2.9	1	Y
6	D	502	2223	3	3	25	0.009	553	1,221	3.0	2	Y
6	D	546	2586	3	3	25	0.008	346	311	4.6	0	Y
6	D	465	1452	3	3	25	0.006	245	832	4.3	1	Y
6	D	527	2132	3	3	25	0.02	424	437	3.9	1	Y
6	D	629	3447	3	5	25	<0.001	248	259	7.9	1	N
7	D	365	635	1	2	23	0.042	132	598	1.1	1	N
7	D	463	1270	2	2	23	0.026	147	155	4.4	0	Y
7	D	383	726	2	2	23	0.067	185	995	3.4	0	Y
7	D	408	907	2	2	23	0.013	386	712	4.5	1	Y
7	D	394	816	2	3	23	0.019	283	322	2.8	0	Y
7	D	440	1225	2	3	23	0.027	347	988	7.4	1	Y
7	D	427	1089	3	2	23	0.017	185	177	6.4	1	Y
7	D	495	1497	3	3	23	0.013	238	581	3.6	1	Y
7	D	482	1633	3	3	23	0.019	298	481	6.2	1	Y

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
7	D	483	1588	3	3	23	0.006	287	322	6.1	0	Y
7	D	392	907	3	3	23	0.037	224	447	4.6	1	Y
7	D	468	1315	3	3	23	0.042	339	529	5.9	1	Y
7	D	574	2313	3	4	23	0.015	--	--	4.1	1	Y
8	D	464	1361	3	2	--	0.005	102	306	6.0	1	Y
8	D	575	2586	3	4	--	0.003	230	1,498	8.1	2	Y
9	U	394	953	3	3	24	0.019	326	333	3.6	0	Y
9	U	524	1905	3	3	24	0.021	281	270	4.2	2	Y
9	U	522	1678	3	3	24	0.008	339	234	4.2	1	Y
9	U	545	2041	3	3	24	0.02	408	228	7.6	0	Y
9	U	515	1905	3	3	24	0.023	276	197	6.7	1	Y
9	U	554	1905	3	3	24	0.017	204	229	8.1	0	Y
9	U	489	1497	3	3	24	0.011	246	369	7.9	0	Y
9	U	546	2087	3	3	24	0.012	298	385	6.3	1	Y
9	U	562	2404	3	3	24	0.007	342	423	7.7	0	Y
9	U	557	2404	3	4	24	0.035	378	562	9.9	1	Y
10	D	488	1452	3	2	26	0.005	399	162	3.0	1	Y
10	D	469	1406	3	2	26	0.009	268	534	3.1	1	Y
10	D	468	1588	3	2	26	0.004	81	34	2.5	1	Y
10	D	501	1860	3	2	26	0.002	291	607	3.6	2	Y
10	D	480	1497	3	3	26	0.004	533	408	3.9	2	Y
10	D	490	1542	3	3	26	0.002	483	867	3.2	3	Y
10	D	501	1542	3	3	26	0.003	302	265	2.6	1	Y
10	D	501	1542	3	3	26	<0.001	281	125		2	Y
10	D	476	1542	3	3	26	0.003	321	415	3.9	1	Y
10	D	489	1542	3	4	26	0.002	188	752	3.	1	Y
10	D	496	1588	3	4	26	0.003	129	1,869	2.0	1	Y
10	D	529	2041	3	5	26	0.005	299	583	4.2	1	N
11	U	378	953	3	1	29	0.007	58	396	2.3	1	N
11	U	365	862	3	1	29	0	105	571	1.4	1	N
11	U	383	953	3	2	29	<0.001	108	404	1.2	1	Y
11	U	382	998	3	2	29	0.012	104	524	2.4	1	Y

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
11	U	382	1043	3	2	29	0.003	120	708	2.9	0	Y
11	U	370	907	3	2	29	0.005	232	739	3.4	0	Y
11	U	400	1134	3	2	29	0.009	298	752	1.7	0	Y
11	U	372	953	3	2	29	0.001	111	456	2.2	0	Y
11	U	564	1406	3	3	29	0.005	161	641	2.7	1	Y
11	U	515	1996	3	3	29	<0.001	33	625	2.2	1	Y
12	D	385	680	2	2	24	0.005	132	238	2.2	1	Y
12	D	367	635	3	1	24	<0.001	106	178	2.7	0	N
12	D	373	590	3	1	24	0.006	65	849	3.6	2	N
12	D	355	635	3	1	24	0.011	143	413	2.2	1	N
12	D	409	771	3	2	24	0.006	205	186	2.1	1	Y
12	D	373	635	3	2	24	0.003	102	535	1.6	1	Y
12	D	417	680	3	2	24	0.002	106	125	1.6	1	Y
12	D	385	680	3	2	24	0.006	55	412	2.9	2	Y
12	D	426	771	3	2	24	0.002	95	534	2.7	1	Y
12	D	402	862	3	2	24	<0.001	167	588	3.0	1	Y
12	D	445	1089	3	3	24	<0.001	112	420	1.6	2	Y
12	D	415	816	4	2	24	0.002	109	351	1.1	2	N
13	U	430	1043	3	2	27	0.023	185	154	4.8	1	Y
13	U	450	1134	3	2	27	0.012	256	94	5.6	1	Y
13	U	434	1089	3	2	27	0.009	298	551	5.1	1	Y
13	U	409	816	3	2	27	0.064	636	358	6.9	1	Y
13	U	460	1270	3	2	27	0.012	395	313	6.3	1	Y
13	U	445	1043	3	2	27	0.029	299	441	7.6	1	Y
13	U	455	1134	3	2	27	0.043	391	367	7.6	2	Y
13	U	397	816	3	2	27	0.02	338	197	8.5	1	Y
13	U	433	1043	3	2	27	0.04	190	82	6.9	1	Y
13	U	453	1225	3	2	27	0.094	316	148	6.9	1	Y
13	U	467	1270	3	3	27	0.024	391	227	4.3	1	Y
13	U	495	1542	3	3	27	0.007	396	482	6.3	1	Y
13	U	507	1633	3	3	27	0.015	348	308	5.0	1	Y
14	D	530	1814	2	3	22	0.01	319	222	4.3	1	Y
14	D	442	1089	3	2	22	0.02	362	137	6.0	1	Y

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
14	D	440	953	3	2	22	0.008	477	206	4.9	1	Y
14	D	480	1270	3	2	22	<0.001	301	115		1	Y
14	D	533	1361	3	2	22	0.004	335	201	2.6	2	Y
14	D	455	998	3	2	22	0.007	407	187	4.6	2	Y
14	D	591	2223	3	2	22	0.005	421	381	6.5	1	Y
14	D	405	726	3	2	22	0.023	246	279	6.3	0	Y
14	D	455	1043	3	2	22	<0.001	488	558	4.1	0	Y
14	D	535	1724	3	3	22	0.006	332	209	3.7	1	Y
14	D	523	1588	3	3	22	0.005	324	363	5.6	1	Y
14	D	561	2087	3	3	22	0.005	320	292	5.3	2	Y
14	D	505	1588	3	3	22	<0.001	525	451	6.4	1	Y
15	U	362	907	2	1	24	0.01	61	519	1.3	0	N
15	U	417	1406	2	2	24	0.002	421	759	3.3	0	Y
15	U	386	1089	2	2	24	0.008	292	814	2.0	0	Y
15	U	417	1179	2	3	24	0.033	383	1,158	2.5	0	Y
15	U	404	1270	3	2	24	0.08	182	729	4.0	0	Y
15	U	590	2948	3	5	24	<0.001	244	626	7.1	1	N
15	U	588	2994	3	5	24	<0.001	493	1,152	7.8	1	N
16	D	397	1134	2	2	22	0.017	95	992	3.9	0	Y
16	D	398	1089	2	2	22	0.003	113	102	1.9	0	Y
16	D	596	2177	2	3	22	0.003	153	178	3.5	2	Y
16	D	430	1134	3	2	22	0.039	99	178	5.8	1	Y
16	D	421	1089	3	2	22	0.008	127	469	4.6	1	Y
16	D	424	1315	3	2	22	0.006	174	175	4.9	0	Y
16	D	401	953	3	2	22	0.003	117	1,135	2.5	0	Y
16	D	461	1542	3	2	22	0.013	349	352	10.0	0	Y
16	D	437	1179	3	2	22	0.015	132	271	4.5	1	Y
16	D	552	2268	3	3	22	0.02	301	148	5.7	1	Y
16	D	542	1678	3	4	22	0.005	158	368	5.2	1	Y
16	D	507	1996	3	4	22	0.015	234	598	7.4	0	Y
17	U	619	3493	2	4	16	0.007	111	1,231	8.7	1	Y
17	U	655	4082	2	4	16	0.008	121	755	8.1	2	Y
17	U	675	4536	2	5	16	<0.001	139	826	7.1	2	N

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
17	U	670	4309	2	5	16	<0.001	175	261	9.8	1	N
17	U	700	4717	2	5	16	0.005	271	401	7.1	1	N
17	U	700	5126	2	5	16	<0.001	78	636	10.6	2	N
17	U	680	4264	3	4	16	<0.001	165	791	9.2	2	Y
17	U	624	3674	3	4	16	<0.001	679	536	8.2	2	Y
17	U	627	3810	3	4	16	0.002	104	223	8.7	2	Y
17	U	667	4309	3	4	16	0.011	75	613	10.5	2	Y
17	U	610	3357	3	5	16	<0.001	131	758	8.0	1	N
17	U	665	4672	3	5	16	<0.001	363	268	11.4	1	N
17	U	760	5987	3	5	16	<0.001	113	574	10.1	1	N
17	U	728	5625	3	5	16	<0.001	102	303	13.8	2	N
17	U	660	4445	3	5	16	0.009	31	621	10.6	1	N
18	D	530	1769	2	3	23	<0.001	518	924	2.9	2	Y
18	D	515	1905	2	3	23	0.003	482	1,596	4.7	1	Y
18	D	566	2676	2	4	23	<0.001	372	699	3.4	1	Y
18	D	500	1678	3	3	23	<0.001	287	618	6.2	1	Y
18	D	486	1633	3	3	23	0.00039	203	473	5.8	1	Y
18	D	480	1724	3	3	23	0.003	367	739	5.5	1	Y
18	D	595	3130	3	4	23	0.003	344	605	4.2	2	Y
18	D	604	3039	3	4	23	07	496	564	4.9	1	Y
18	D	554	2449	3	4	23	<0.001	285	501	6.5	2	Y
19	U	449	1089	3	2	21	<0.001	388	579	10.4	1	Y
19	U	580	2404	3	3	21	0.006	370	585	9.2	2	Y
19	U	621	3629	3	3	21	0.003	487	761	8.3	1	Y
19	U	594	2767	3	3	21	<0.001	236	950	7.8	1	Y
19	U	577	2812	3	3	21	<0.001	344	662	8.0	1	Y
19	U	718	4717	3	4	21	<0.001	389	850	6.4	1	Y
19	U	628	3130	3	4	21	<0.001	556	899	7.2	2	Y
20	D	423	907	3	2	19	<0.001	181	582	5.4	1	Y
20	D	516	1814	3	3	19	<0.001	421	1,080	8.6	1	Y
20	D	511	1724	3	3	19	0.003	101	5,13	8.3	0	Y
20	D	438	907	3	3	19	<0.001	20	624	7.0	1	Y
20	D	502	1406	3	3	19	0.002	95	1,897	10.0	1	Y

Table 16. Water temperature and values for length, weight, age, and biological characteristics of individual male fish collected during 1999

[--, no data; mm, millimeter; °C, degrees in centigrade; mg/mL, milligrams per milliliter; pg/mL, picograms per milliliter; D, downstream; U, upstream;Y, yes, N, no]..

Site location number	Site group	Length (mm)	Weight (grams)	Gonad stage	Age (years)	Water temperature (°C)	Vitellogenin (mg/mL)	17β-estradiol (pg/mL)	11-ketotestosterone (pg/mL)	Gonad-somatic index (percent)	Ceroid/ lipfuscin tissue ranking	Used in final analyses[1]
20	D	463	1452	3	3	19	0.005	211	1,846	11.6	1	Y
20	D	502	1678	3	3	19	<0.001	167	1,195	8.8	1	Y
20	D	536	1860	3	3	19	0.004	229	1,826	7.1	2	Y
20	D	429	953	3	3	19	<0.001	77	531	4.8	1	Y
20	D	441	998	3	3	19	<0.001	172	546	7.0	1	Y
20	D	567	2132	3	4	19	0.005	107	221	9.0	1	Y
20	D	557	2268	3	4	19	0.01	125	622	8.8	1	Y
21	U	383	726	2	1	22	0.021	238	1,301	4.3	1	N
21	U	343	499	2	1	22	0.009	131	844	6.2	1	N
21	U	446	1179	2	3	22	0.016	193	1,413	3.9	1	Y
21	U	374	726	3	1	22	<0.001	221	1,867	5.8	1	N
21	U	364	680	3	1	22	0.011	178	2,065	4.3	1	N
21	U	350	680	3	1	22	0.013	177	1,679	3.7	1	N
21	U	398	726	3	2	22	0.021	226	951	5.4	0	Y
21	U	393	862	3	2	22	0.01	320	1,162	7.7	1	Y
21	U	392	816	3	2	22	0.007	185	901	5.8	1	Y
21	U	420	1043	3	2	22	0.02	249	1,834	3.8	1	Y
21	U	442	1225	3	2	22	0.018	197	1,537	5.6	1	Y
21	U	447	1270	3	3	22	0.012	212	1,100	3.6	1	Y
22	D	427	998	3	2	24	0.017	107	444	4.6	1	Y
22	D	447	998	3	2	24	0.013	122	505	4.7	1	Y
22	D	322	680	3	2	24	0.006	217	252	3.8	1	Y
22	D	522	1633	3	3	24	0.032	149	491	2.0	0	Y
22	D	484	1270	3	3	24	0.002	58	419	2.1	1	Y
22	D	397	953	3	3	24	0.01	106	1,187	4.6	2	Y

[1] Male common carp in gonad stages 2 and 3 and ages ranging from 1- to 5- years old(217 samples) were used in the inital analyses to determine the influence of gonad maturation stage and age on biological characteristics. Because sample size for males in gonad stage-1 (2 samples) and gonad stage-2 (2 samples) were too small for further analyses, these samples were removed from further analyses. Some samples (males 1- and 5-years old) were excluded from further analyses because biological characteristics were found to vary with age. Samples marked with a N were excluded from further analyses. Samples marked with a Y were used in subsequent analyses of biological characteristics between site groups, among paired sites, and among all sites.