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Organic Chemical Concentrations and Reproductive Biomarkers in Common Carp (*Cyprinus carpio*) Collected from Two Areas in Lake Mead, Nevada, May 1999–May 2000

Data Series 286

U.S. Department of the Interior
U.S. Geological Survey

Cover: Mosaic photograph of Lake Mead taken from Boulder Basin looking north showing drop in water level since 2000. (Photograph taken by Michael R. Rosen, U.S. Geological Survey, 2006)
Inset 1: Photograph of drawing blood from common carp (Photograph taken by E. Orsak, U.S. Fish and Wildlife Service, 2006)
Inset 2: Photograph of electrofishing boat used to sample fish in Lake Mead (Photograph taken by E. Orsak, U.S. Fish and Wildlife Service, 2006)

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U.S. Department of the Interior
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U.S. Geological Survey
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U.S. Geological Survey, Reston, Virginia: 2007

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Suggested citation:
Goodbred, S.L., Leiker, T.J., Patiño, R., Jenkins, J.A., Denslow, N.D., Orsak, E.., and Rosen, M.R., 2007, Organic chemical concentrations and reproductive biomarkers in common carp (*Cyprinus carpio*) collected from two areas in Lake Mead, Nevada, May 1999–May 2000: U.S. Geological Survey Data Series 286, 18 p.

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Conversion Factors

Multiply	By	To obtain
centimeter (cm)	0.3937	inch (in.)
cubic meter (m^3)	264.2	gallon (gal)
cubic meter per day (m^3/d)	264.2	gallon per day (gal/d)
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
liter (L)	1.057	quart (qt)
liter (L)	61.02	cubic inch (in^3)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
square centimeter (cm^2)	0.1550	square inch (ft^2)

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

$$°F=(1.8\times°C)+32.$$

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

$$°C=(°F-32)/1.8.$$

Water-Quality Units

Water-Quality Units	Definition
cm/s	centimeter per second
g	gram
mg	milligram
mg/mL	milligram per milliliter
mL/min	milliliter per minute
mm^2	square millimeter
mM	milli Molar
ng	nanogram
ng/µL	nanogram per microliter
nm	nanometer
pg/µL	picogram per microliter
µg	microgram
µg/kg	microgram per kilogram
µg/L	microgram per liter
µg/mL	microgram per milliliter
µL	microliter
µm	micrometer
µM	micro Molar

Abbreviations and Acronyms

Abbreviations and Acronyms	Meaning
BDE	brominated diphenyl ethers
Dalton	unified atomic mass
eV	electron volt, one electron volt equals $1.60217646 \times 10^{-19}$ joules
ECNI	electron-capture negative ion
EI	electron ionization
FSCC	fused-silica capillary column
GPC	gel permeation chromatographic
GSI	gonadosomatic index
HBSS	Hank’s balanced salt solution
kV	killovolts
KIU/mL	Kallikrein inhibitory units per milliliter
m/z	mass of a molecule to its electric charge ratio
PAH	poly aromatic hydrocarbons
PCB	polychlorinated biphenyls
torr	millimeters of mercury
µA	microampere
Vtg	vitellogenin
v/v	volume-to-volume dilution

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Organic Chemical Concentrations and Reproductive Biomarkers in Common Carp (*Cyprinus carpio*) Collected from Two Areas in Lake Mead, Nevada, May 1999–May 2000

By Steven L. Goodbred[1], Thomas J. Leiker[1], Reynaldo Patiño[1], Jill A. Jenkins[1], Nancy D. Denslow[2], Erik Orsak[3], and Michael R. Rosen[1]

Abstract

The U.S. Geological Survey, in cooperation with the U.S. Fish and Wildlife Service, National Park Service, Bureau of Reclamation, and Nevada Department of Wildlife, collected and assessed data to determine the general health and reproductive status of common carp (*Cyprinus carpio*) at two study areas in Lake Mead, Nevada, during May 1999–May 2000. These data will form the basis of interpretations and provide a comparison for continuing studies on the health of the ecosystem in Lake Mead. One study area, Las Vegas Bay, is in the western part of Lake Mead. Las Vegas Bay receives inflows from Las Vegas Wash, which is predominantly tertiary-treated wastewater effluent, and to a lesser extent stormwater runoff from Las Vegas, Henderson, and other nearby communities, and from ground water underlying Las Vegas Valley. The other study area, Overton Arm, is in the northern extent of Lake Mead. Overton Arm receives inflow from the Virgin and Muddy Rivers, which historically are not influenced by wastewater effluent. Both sexes of common carp were collected bimonthly for 12 months using boat-mounted electrofishing gear (a direct electric current is used to temporarily immobilize fish for capture) to determine their health and reproductive status and any relation between these factors and environmental contaminants.

This report presents fish tissue chemistry, organic chemical compound concentrations, and biomarker data for 83 male common carp collected from Las Vegas Bay, similar organic chemistry results for 15 male common carp, and similar biomarker measures for 80 male common carp collected from Overton Arm. Tissue chemistry results also are presented for 16 female common carp and biomarker measures for 79 female common carp collected from Las Vegas Bay, and tissue chemistry results for 15 female common carp and biomarker measures for 81 female common carp collected from Overton Arm.

Thirty-three organic chemical compounds plus total concentrations for four groups of compounds (chlordanes, polychlorinated biphenyls [PCBs], brominated diphenyl ethers [BDEs], and triclosans) were analyzed from extracts of whole-body tissue using gas chromatography/mass spectrometry in male common carp from Las Vegas Bay during May 1999 through May 2000. All 33 compounds were detected in at least one sample of whole-body tissue from male common carp collected in Las Vegas Bay. In Overton Arm, 37 organic compounds plus total concentrations of three groups of compounds (PCBs, BDEs, and triclosans) were analyzed in male common carp where 20 (54 percent) of the compounds were detected. Sixteen of the 33 compounds detected in male common carp from Las Vegas Bay and 10 compounds detected in males from Overton Arm have the potential to disrupt the endocrine system in fish in Lake Mead. During May and June 1999, the mean concentration of all organic compounds detected in male common carp was 670 micrograms per kilogram from Las Vegas Bay and 109 micrograms per kilogram from Overton Arm.

Twenty-seven organic compounds plus total PCBs were analyzed from extracts of whole-body tissue in female common carp collected in Las Vegas Bay and Overton Arm during May 1999. Twenty-four (86 percent) of these compounds were detected in at least one sample of whole-body tissue from female common carp collected from Las Vegas Bay while 10 (36 percent) chemical compounds were detected in female common carp from Overton Arm during that same period. Median concentrations of all chemical compounds were higher in female common carp from Las Vegas Bay compared to those collected from Overton Arm except Dacthal (DCPA), which was similar between sites.

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Biomarker measures obtained for male and female common carp include gonadosomatic index (percentage of gonad weight to total body weight), plasma vitellogenin (a phospholipid protein normally produced by female common carp and other oviparous fish), and condition factor [body weight/(fork length)^3]. Biomarker measures for male common carp include five indicators of sperm quality: viability, motility, mitochondrial function, distribution of germ cell stages, and concentration of sperm. Biomarker measures for female common carp include total fecundity (estimate of total number of follicles per fish available for spawning), normalized fecundity (number of follicles per kilogram of body weight) during January and March 2000 only, and follicle-size frequency distribution.

Monthly medians for reproductive biomarkers were variable between sites and among months. Median concentrations of vitellogenin in blood plasma from male common carp collected from Las Vegas Bay ranged from not detected (detection limit 0.005 milligram per milliliter) to 0.631 milligram per milliliter, whereas monthly median concentrations in male common carp from Overton Arm ranged from not detected to a high of 0.100 milligram per milliliter. Vitellogenin concentrations in individual male common carp collected from Las Vegas Bay were as high as 68.1 milligrams per milliliter. Monthly median sperm counts also varied between sites and among months; values ranged from 1.20×10^{10} per milligram of testis to a high of 6.42×10^{10} per milligram of testis in male common carp from Las Vegas Bay and from 1.52×10^{10} per milligram to 7.41×10^{10} per milligram of testis in male common carp from Overton Arm. Monthly medians for another sperm quality biomarker, viability, ranged from 79.5 percent to a high of 98.2 percent in male common carp from Las Vegas Bay, and from 90.0 percent to 97.3 percent in male common carp from Overton Arm.

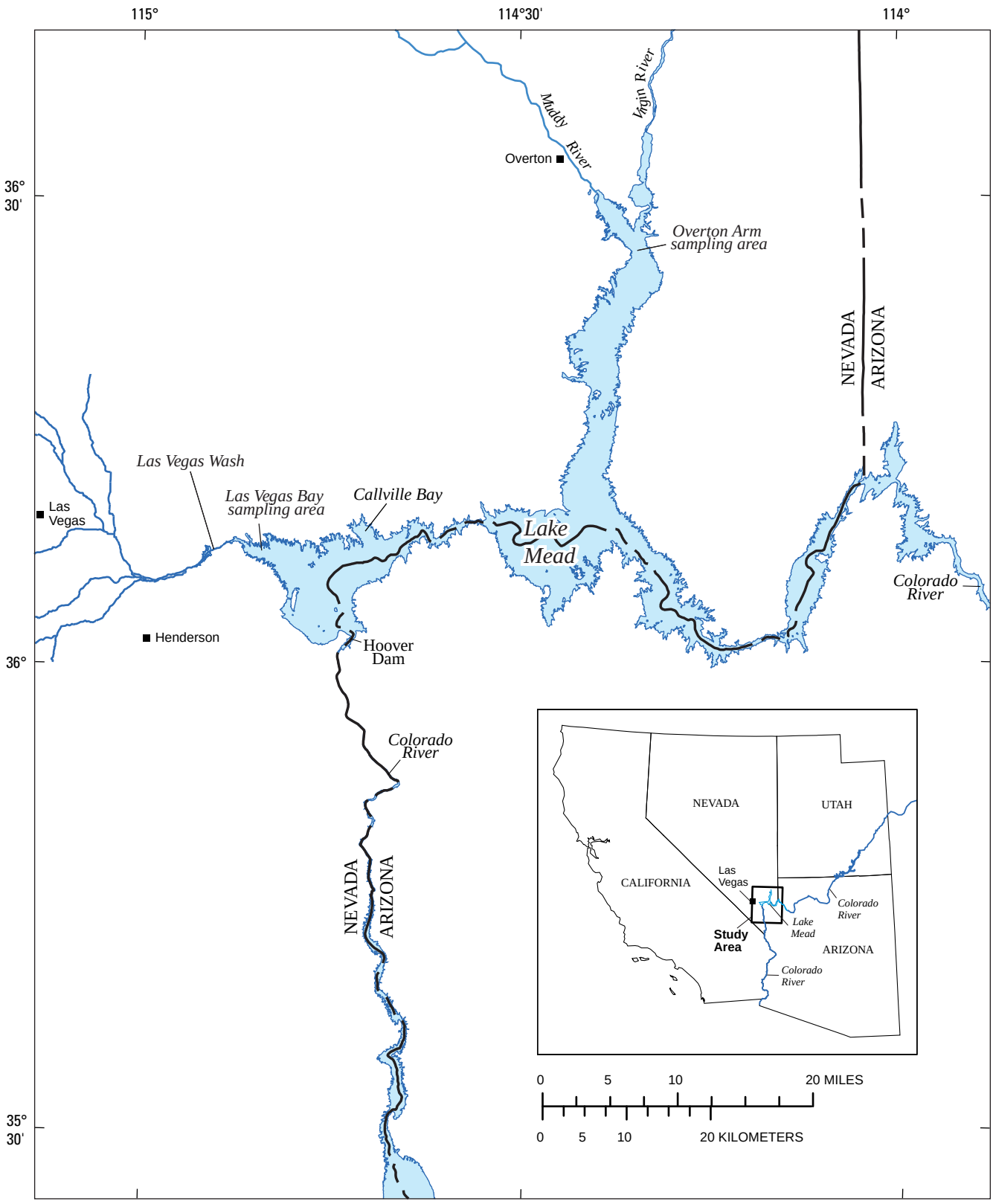
Monthly median concentrations of vitellogenin in blood plasma ranged from 0.863 to 9.368 milligrams per milliliter in female common carp collected from Las Vegas Bay and from 0.785 to 11.126 milligrams per milliliter in female common carp collected from Overton Arm. Vitellogenin concentrations generally increased in late autumn through winter and reached a maximum just before spawning in March. Normalized fecundity was more variable during the pre-spawning months of January and March 2000 in female common carp from Las Vegas Bay (medians of 190,883 and 231,232 follicles per kilogram, respectively) but was fairly consistent in female common carp from Overton Arm (medians of 164,054 and 162,409 follicles per kilogram, respectively).

Introduction

Hoover Dam was built between 1931 and 1936 on the Colorado River in Black Canyon along the Nevada-Arizona border. Lake Mead, the reservoir created behind the 221.4-m high Hoover Dam (fig. 1), is the largest manmade reservoir by volume in the United States (Covay and Beck, 2001). Hoover Dam created a new water supply, which is used for hydroelectric power, irrigation, and municipal and industrial use. Lake Mead stores Colorado River water for municipal-water supply to the Las Vegas Valley area and is used to store and regulate flows for downstream users along the Colorado River. Lake Mead also is well known for its year-round recreational activities, including sport fishing, boating, swimming, and scuba diving. Several species of fish are present in Lake Mead including striped bass (*Morone saxatalis*), channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*), rainbow trout (*Oncorhynchus mykiss*), and the endangered razorback sucker (*Xyrauchen texanus*). Lake Mead National Recreation Area was established in 1964 and is managed by the U.S. Department of the Interior through a cooperative agreement between the National Park Service and Bureau of Reclamation.

Major inflows to Lake Mead are from the Colorado, Virgin, and Muddy Rivers, and Las Vegas Wash. Las Vegas Wash is perennial because of inflows from municipal wastewater-treatment plants (Covay and Leiker, 1998) and excess irrigation water from urban landscapes, which flows into the wash through surface- and ground-water discharge. Population within the Las Vegas Valley has significantly increased since the 1940s and the volume of treated effluent, which in 1993 constituted about 96 percent of the annual discharge of Las Vegas Wash, has increased concurrently (Bevans and others, 1996). The mean flow of Las Vegas Wash into Las Vegas Bay from 1992 to 1998 was about 490,000 m^3/d (Preissler and others, 1999); in 2001, mean flow increased to 606,000 m^3/d (Bureau of Reclamation, 2001). Other sources of environmental contaminants like perchlorate, a rocket fuel component, have been seeping into Las Vegas Wash from former manufacturing sites in Henderson, Nev. (National Park Service, 2006).

In 1995, the U.S. Geological Survey (USGS) in cooperation with other Federal agencies including the U.S. Fish and Wildlife Service, the National Park Service, the Bureau of Reclamation, and the Nevada Department of Wildlife, began a study to determine the potential for endocrine disruption in common carp (*Cyprinus carpio*) from Lake Mead. In that study, male and female common carp were collected from Las Vegas Wash, Las Vegas Bay, and Callville Bay (fig. 1) during the spawning season and analyzed for several reproductive biomarkers. The results showed that common carp from Las Vegas Wash and Las Vegas Bay exhibited evidence of endocrine disruption (Bevans and others, 1996). Significantly lower concentrations of the sex steroid hormone 11-ketotestosterone, the major androgen responsible for spermatogenisis (Kime, 1999) were found in male common carp from Las Vegas Wash and Bay than in reference male common carp from Callville Bay.



Base from U. S. Geological Survey digital data 1:100,000. scale 1987:
Universal Transverse Mercator Projection, Zone 11

Figure 1. Study area and sampling areas in Lake Mead, Nevada, May 1999–May 2000.

Low concentrations of sex steroid hormones can affect reproductive development and cause reduced gonad size and altered secondary sex characteristics (Munkittrick and others, 1992). Another indication of endocrine disruption was the induction of vitellogenin (Vtg) in male common carp, which was present in high concentrations in fish collected at Las Vegas Wash and Las Vegas Bay (Bevans and others, 1996). Vitellogenin, a phospholipoprotein produced in the liver under control of 17β-estradiol, is the precursor of egg yolk normally only detected in female oviparous vertebrates. However, male fish do have estrogen receptors in the liver and can produce Vtg when exposed to estrogenic compounds (Purdom and others, 1994). Some researchers have proposed that low concentrations of Vtg are normally present in males in some fish species (Sepulveda and others, 2004).

Lake Mead is mildly mesotrophic (Carlson, 1977) with generally good water quality. However, the water quality at Las Vegas Bay has significantly higher numbers and higher concentrations of environmental contaminants (Bevans and others, 1996) and higher nutrient concentrations (LaBounty and Horn, 1997) than other parts of Lake Mead. More organochlorine pesticides, polyaromatic hydrocarbons (PAHs), furans, phthalates, phenols, and polychlorinated biphenyls (PCBs) were detected in bed sediment, fish tissue, and passive sampler extracts from Las Vegas Bay compared to Callville Bay, which has no point source discharges (Bevans and others, 1996). Sediment from the bottom-sediment core-sampling study done in 1998 by Covay and Beck (2001) showed twice as many organic contaminants detected from Las Vegas Bay compared to the sediment from Overton Arm where maximum concentrations of pesticides and PCBs were one order of magnitude higher in Las Vegas Bay compared to sediment in Overton Arm. Covay and Beck (2001) also detected several dioxins and furans in sediment from Las Vegas Bay, but no dioxins and furans were detected in sediment from Overton Arm.

Recent studies have documented the presence of emerging chemical contaminants and endocrine disrupting compounds in Lake Mead subsequent to the evidence of endocrine disruption in fish reported by Bevans and others (1996). Water samples collected from Las Vegas Bay in April 1997 contained four xenoestrogens: octylphenol at 0.027 µg/L; nonlyphenol at 0.750 µg/L, polyethoxylates at 4.85 µg/L; 17*a*-ethinyl estradiol at 0.0005 µg/L; and one natural estrogen, 17β-estradiol at 0.0022 µg/L (Snyder and others, 1999).

Another study by Snyder and others (2001) determined that the compounds with the highest estrogenic activity at Las Vegas Bay were two steroid hormones, 17β-estradiol, a naturally occurring estrogen, and 17*a*-ethinyl estradiol, a synthetic estrogen used in oral contraception. Fish exposed to ethinyl estradiol in concentrations as low as 0.0005 µg/L have shown evidence of endocrine disruption (Purdom and others, 1994; Routledge and others, 1998; Bayley and others, 1999; Doyle and Lim, 2002; Thorpe and others, 2003). Boyd and Furlong (2002) detected 13 human-health pharmaceutical compounds in surface water in Las Vegas Wash between October 2000 and August 2001. Caffeine, carbamazepine (used to treat epilepsy), cotinine (a metabolite of nicotine), and dehydronifedipine (a metabolite of the antianginal Procardia) were the most frequently detected compounds of the 33 they targeted for analysis. The effects of pharmaceuticals and personal care products on aquatic biota are largely unknown, but several examples indicate effects have been detected at low concentrations (Daughton and Ternes, 1999).

Purpose and Scope

Concern about initial 1995 study results from Lake Mead prompted a more thorough and comprehensive study which began in 1999 by the U.S. Department of the Interior. This study, led by USGS, included the U.S. Fish and Wildlife Service; National Park Service; Bureau of Reclamation; University of Nevada, Las Vegas; University of Florida, Gainesville; Texas Tech University, and the Nevada Department of Wildlife. The purpose of this study, partially reported here, was to determine if the evidence of endocrine disruption observed in 1995 could be confirmed during a 1-year common carp reproductive cycle from May 1999 through May 2000. Results from this study can serve as a baseline for the reproductive health of Lake Mead fish and a comparison for other ongoing research and monitoring of fish reproductive studies.

The scope of work was to characterize the environmental contaminants in Lake Mead including estrogenic and other emerging compounds in pharmaceuticals and personnel care products, and examine possible correlations with changes in endocrine and reproductive biomarkers. Specific results for the current 1999–2000 study pertaining to morphometric and histopathological biomarkers were reported by Patiño and others (2003). This part of the study compared male and female common carp from Las Vegas Bay with those from Overton Arm. The Las Vegas Bay male common carp had lower gonadosomatic indices (a measure of testicular development) throughout the study, a higher proportion of spermatocytes (a less mature stage of sperm) for one sampling date, and higher incidence of gonadal macrophages aggregates (biomarkers of contaminant exposure). Differences in biomarkers from female common carp between the two sampling areas are most likely strongly influenced by differences in water temperature and could not be attributed to environmental contaminants.

This report presents organic chemical compound concentrations in fish tissue and biomarker data for 83 male common carp collected from Las Vegas Bay, similar organic chemistry results for 15 male common carp, and similar biomarker measures for 80 male common carp collected from Overton Arm. Tissue chemistry results also are presented for 16 female common carp and biomarker measures for 79 female common carp collected from Las Vegas Bay, and tissue chemistry results for 15 female common carp and biomarker measures for 81 female common carp collected from Overton Arm.

Study Methods

Fish Sampling and Field Processing

Adult common carp (greater than 300 mm total length) were collected seven times from May 1999 through May 2000 using boat-mounted, electrofishing gear (Model SR-16, Smith-Root, Inc.) at Las Vegas Bay and Overton Arm in Lake Mead (fig. 1). Samples were collected in coves, bays, and along the shoreline in the two sampling areas usually at depths of less than 3 m. Due to equipment failure of the electroshocking boat during July 1999, fish were collected using a gill net (mesh size, 8 cm), which was set in the early morning and retrieved 4 hours later. All fish were held alive in an aerated live well or a netted cage submerged on site for less than 2 hours between the time of capture and time of processing. Nine to 16 male and female common carp were selected at each site for processing. Following blunt force trauma to the head, both total length and fork length were measured to the nearest millimeter and body weighed to the nearest decagram. A 5-mL syringe with a 20-gage needle was used to collect blood from the caudal vein for Vtg analysis. Blood was transferred to heparinized Vaccu-tubes and centrifuged at approximately 1,000 × gravity for 10–15 minutes to separate the plasma. Plasma then was placed into cryovials, which were immediately placed on dry ice and stored at -80°C until the plasma was ready for analysis. Gonads were carefully dissected and weighed to the nearest 0.1 g. A small section (about 5 g) from one testis or ovary was dissected from a point one-third of its length starting from the anterior end, fixed in NoTox fixative (Earth Safe Industries, Inc., Belle Mead, N.J.), and sent to the USGS Texas Cooperative Fish and Wildlife Research Unit in Lubbock, Tex. for further processing and histological evaluation. After several weeks in NoTox fixative, tissues were transferred to 70 percent ethanol for further processing.

The other testis was removed intact, rinsed with calcium-free Hank's balanced salt solution (HBSS) containing 10 percent streptomycin/penicillin by volume (HBSS-SP), and stored in 500-mL sterile containers filled with HBSS-SP. These containers were packed in a cooler with blue ice and shipped overnight at 4°C to the USGS National Wetlands Research Center in Lafayette, La. for sperm-quality analysis. The remaining fish carcass was wrapped in aluminum foil, double bagged, and frozen on dry ice and then sent to the USGS National Water Quality Laboratory in Lakewood, Colo. for organic chemical analysis. The gonadosomatic index (GSI) and condition factor (K) were determined according to the formulas 100 × (gonad weight/body weight) (Schmitt and Dethloff, 2000) and body weight/(fork length)^3 (Carlander, 1969), respectively.

Analytical Chemistry

Individual whole body common carp, minus one gonad in the case of males, were thawed at room temperature and then thoroughly homogenized with a Hobart meat grinder (Model No. 4146) at the USGS National Water Quality Laboratory in Lakewood, Colo. Samples were prepared for analysis using a method described by Leiker and others (1995) and were treated as follows: A 10-g aliquot of homogenate was added to 100 g of anhydrous sodium sulfate. The sodium sulfate and tissue mixture was frozen at –20°C. While frozen, the mixture was homogenized into a free-flowing powder using a blender and then quantitatively transferred into a fritted disk glass Soxhlet extraction thimble. Surrogate standards (1 ng each) consisting of decafluorobiphenyl; α-HCH-d6: *p,p*'-DDT-d8; and nonachlorobiphenyl were added to the sample prior to extraction. The tissue/sodium sulfate mixture was extracted overnight with a Soxhlet apparatus using dichloromethane. The extract was concentrated in a Kuderna-Danish (K–D) apparatus to a volume of 5.0 mL. A 1-mL aliquot was removed for lipid determination. Two Waters Envirogel (Milford, MA) gel permeation chromatographic (GPC) preparative columns (19 × 150 mm and 19 × 300 mm) were used to remove lipid material from a 2-mL aliquot of the extract. The mobile phase was dichloromethane and the flow rate was 5 mL/min. The fraction from 14 to 30 minutes was collected for analysis in a clean K-D unit and concentrated to 5 mL. The sample then was exchanged into hexane, and the extract was re-concentrated to about 1 mL.

The extract was purified further and divided into two fractions by passing it through a column dry packed (from top to bottom) with 5 g of 8.5 percent water-deactivated neutral alumina, 3 g of 2 percent water-deactivated silica, and 0.5 cm of granular sodium sulfate. The column was pre-rinsed with 50 mL of hexane and the rinse discarded. The sample extract was applied to the column and eluted with 30 mL of hexane (fraction 1). The column then was eluted with 25 mL of 50 percent (v/v) acetone in hexane (fraction 2). Each fraction was collected in a 35-mL K-D receiver, concentrated to a volume of 5 mL, and then concentrated further under a gentle stream of filtered ultrapure nitrogen at ambient temperature to a volume of 0.5 mL. The extract was transferred quantitatively,

with hexane rinses, to a 1-mL gas chromatography (GC) vial. The volume of the extract was adjusted to 0.5 mL. The GC vial was sealed with a Teflon®-lined cap and stored at –20°C until analysis. Just prior to analysis, injection standards consisting of 4,4´-dibromooctafluorobiphenyl (25 ng, resulting in a concentration of 50 pg/µL in the final extract) and naphthalene-d_8 (1 µg, resulting in a concentration of 2 ng/µL in the final extract) were added to each sample as injection standards.

Authentic standards of methyl triclosan and its higher chlorinated forms were not available. A commercial triclosan standard was derivatized with ethereal diazomethane according to the procedure of Fales and others (1973). Ethereal diazomethane produced from N-methyl-N'-nitrosoguanidine (97 percent) was allowed to react with triclosan overnight at an ambient temperature in a sealed conical-shaped reaction vial. Based on gas chromatography/mass spectrometry (GC/MS) analysis, the purity of the derivatized triclosan is estimated to be greater than 95 percent. The molecular structure of the derivatized product was established by capillary gas chromatography under low- and high-resolution electron ionization (EI) mass spectrometry. Chromatographic and mass spectral conditions for confirming the structure of the derivative methyl triclosan and methyl triclosan in the sample extract were identical. The response of 4,4´-dibromooctafluorobiphenyl was used to estimate the concentration of the higher chlorinated analogues of methyl triclosan during the electron-capture negative ion (ECNI) analysis and derivatized triclosan was used to estimate the concentration of methyl triclosan during the EI analysis. Reporting limits for compounds detected in common carp from Lake Mead are given in table 1. Reported values less than these concentrations are estimated and based on the lowest point of the calibration curve of the compound.

Quality assurance of analytical methods was further validated by analyzing certified chlorinated organic compounds in cod liver oil from the National Institute of Standards and Technology (SRM 1588) (table 2).

Gas Chromatograph-Mass Spectrometry Analyses

The capillary gas chromatographic low-resolution electron-capture negative ion mass spectral (GC/ECNIMS) analyses were carried out on a Hewlett Packard 5890 gas chromatograph interfaced to Hewlett Packard 5989A mass spectrometer at the USGS National Water Quality Laboratory in Lakewood, Colo. Chromatographic separations were achieved on a 60 m × 0.25 mm inside diameter, 0.25 µm film thickness, 5 percent phenyl-95 percent dimethylpolysiloxane fused-silica capillary column (FSCC) using the following temperature program: initial oven temperature was held at 50°C for 3 minutes, oven temperature was ramped to 150°C at 20°C per minute, from 150°C to 275°C at 2°C per minute, from 275°C to 300°C at 20°C per minute and held for 20 min at 300°C. Splitless injections of 2 µL of each sample extract were made. The carrier gas was helium with a linear velocity of 25 cm/s. The injection port temperature was 250°C, and the transfer line temperature was 275°C. The GC/ECNIMS analyses were conducted under the following conditions: modifying gas, methane; source temperature, 125°C; quadrupole temperature, 110°C. Instrument was repetitively scanned from 150 to 600 Daltons with a cycle time of 1.29 seconds per scan; emission current, 300 µA; and electron energy, 200 eV. Perfluorotributyl amine (FC-43) was used for mass axis calibration and tuning. Source pressure (2×10^{-4} torr) was adjusted to maximize m/z 633 while minimizing m/z 452 with methane (modifying gas), and then tuned to maximize m/z 452.

Low-resolution and high-resolution capillary gas chromatographic electron ionization mass spectral (GC/EIMS) analyses were conducted using a Varian 3400 gas chromatograph interfaced to a MAT-95 high-resolution mass spectrometer. Chromatographic separations for both low- and high-resolution mass spectral analyses were described above. Low-resolution EI analysis was made under the following conditions: resolution, 1,000; electron energy, 70 eV; emission current, 100 µA; high voltage, 5 kV; and source temperature, 200°C. The instrument was scanned repetitively from 50 to 700 Daltons with a scan-rate of 1-second per decade. High-resolution EI analysis was conducted under the following conditions: resolution, 7,500 (set statically); electron energy, 70 eV; emission current, 100 µA; high voltage, 5 kV; and source temperature, 200°C. The instrument was repetitively scanned from 50 to 600 Daltons with a scan-rate of 3 seconds per decade. Perfluorokerosene was used for low- and high-resolution mass axis calibration and tuning for all EI analysis.

Vitellogenin

Vtg concentration in common carp blood plasma was determined at the University of Florida, Gainesville, with a direct enzyme-linked immunosorbent assay (ELISA). Plasma samples were diluted 1:100, 1:10,000, 1:100,000 and 1:1,000,000 with 10 mM phosphate, 150 mM NaCl, 0.02 percent azide, 10 KIU/mL aprotinin, pH 7.6 (PBSZ-AP). Carp Vtg standards (0, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 µg/mL) containing 1:200, 1:10,000, 1:100,000, and 1:1,000,000 male plasma (in PBSZ-AP) were added to account for matrix effect (Denslow and others, 1999). Samples and standards were loaded onto a 96-well ELISA plate in triplicate and stored overnight at 4ºC in a humidified container. The following day the plates were washed four times with PBSZ and then blocked with 1percent bovine serum albumin (BSA) in 10 mM tris, 150 mM NaCl, 0.05 percent tween, 0.02 percent azide, 10 KIU/mL Aprotinin, pH 7.6 (1 percent BSA/TBSTZ-AP) for 2 hours at room temperature. The plates were rewashed with PBSZ

Table 1. Organic chemical compounds analyzed in common carp (*Cyprinus carpio*) from Las Vegas Bay and Overton Arm, Lake Mead, Nevada, May 1990–May 2000.

[Concentrations reported in this study that are less than the reporting limit are estimated. **Reporting limit**: Reporting limit is the concentration above which values are reliably quantified. **Abbreviations:** CAS, Chemistry Abstracts Service Registry; µg, microgram; kg, kilogram; g/mole, grams per mole; Log K_{ow}, log octanol-water partition coefficient; NA, not available. **Symbols:** α, alpha; β, beta; δ, delta; γ, gamma; *o*, ortho, *p*, para]

Compound name	CAS No.	Class/Use	Reporting limit (µg/kg)	Molecular weight (g/mole)	Log K_{ow}
2,2´,4,4´-tetrabromodiphenyl ether (BDE 47)	40088-47-9	Fire retardant	5.0	485.80	6.05
2,2´,4,4´,6-pentabromodiphenyl ether (BDE 100)	189084-64-8	Fire retardant	5.0	564.80	7.24
2,2´,4,4´,5,6´-hexabromodiphenyl ether (BDE 154)	207122-15-4	Fire retardant	5.0	643.60	7.82
Acetyl-hexamethyl-tetrahydro-naphthalene (AHTN)	21145-77-7	Fragrance	25.0	258.40	5.70
α-HCH	319-84-6	Organochlorine insecticide	5.0	290.83	3.80
Arochlor 1248	12672-29-6	Polychlorinated biphenyl	5.0	288.00	6.34
Arochlor 1254	11097-69-1	Polychlorinated biphenyl	5.0	327.00	6.98
Arochlor 1260	11096-82-5	Polychlorinated biphenyl	5.0	372.00	8.27
ß-HCH	319-85-7	Organochlorine insecticide	5.0	290.83	3.80
Chlorothalonil	1897-45-6	Chloronitril fungicide	5.0	265.92	2.88
Chlorpyrifos	2921-88-2	Organophosphate insecticide	5.0	350.59	4.96
cis-Chlordane	5103-71-9	Organochlorine insecticide	5.0	409.78	5.38
cis-Nonachlor	5103-73-1	Organochlorine insecticide	5.0	444.23	6.20
Dacthal (DCPA)	1861-32-1	Chlorobenzoic acid ester herbicide	5.0	331.96	4.28
δ-HCH	319-86-8	Organochlorine insecticide	5.0	290.83	4.10
Dieldrin	60-57-1	Organochlorine insecticide	5.0	380.91	5.40
Endosulfan I	000959-98-8	Organochlorine insecticide	5.0	406.93	3.83
Endosulfan II	033213-65-9	Organochlorine insecticide	5.0	406.93	3.83
Endosulfan sulfate	103-07-8	Organochlorine / degradate	5.0	442.95	3.66
γ-HCH	58-89-9	Organochlorine insecticide	5.0	290.83	3.72
Heptachlor epoxide	1024-57-3	Organochlorine insecticide	5.0	389.32	4.98
Hexachlorobenzene (HCB)	118-74-1	Organochlorine insecticide	5.0	284.78	5.73
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	1222-05-5	Fragrance	25.0	258.40	5.90
methoxy 3-Chlorotriclosan	63709579	Antimicrobial / degradate	25.0	335.93	NA
methoxy 5-Chlorotriclosan	3380-44-7	Antimicrobial / degradate	25.0	335.93	NA
methoxy 3,5-Dichlorotriclosan	53555-01-4	Antimicrobial / degradate	25.0	369.99	NA
methyl Triclosan	4640-01-1	Antimicrobial / degradate	500	166.15	5.00
(*o*,*p*´-DDD) 1-(2-Chlorophenyl)-1(4-chlorophenyl)-2,2-dichloroethane	53-19-0	Organochlorine / degradate	5.0	320.05	6.02
(*o*,*p*´-DDE) Benzene-1-chloro2[2,2-dichloro-1(4-chlorophenyl)ethenyl]	3424-82-6	Organochlorine / degradate	5.0	318.03	6.00
Octachlorostyrene	29082-74-4	Organochlorine / industrial by product	5.0	379.10	7.46
Oxychlordane	27304-13-8	Organochlorine insecticide	5.0	423.77	5.48
(*p*,*p*´-DDD) 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane	72-54-8	Organochlorine / degradate	5.0	320.05	6.02
(*p*,*p*´-DDE) 1,1-dichloro2,2bis(p-chlorophenyl)ethylene	72-55-9	Organochlorine / degradate	5.0	318.03	6.51
Pentachloroanisole (PCA)	1825-21-4	Organochlorine / degradate	5.0	280.37	5.45
trans-Chlordane	005103-74-2	Organochlorine insecticide	5.0	409.78	6.10
trans-Nonachlor	39765-80-5	Organochlorine insecticide	5.0	444.23	6.08
Trifluralin	1582-09-8	Dinitroaniline herbicide	5.0	335.29	5.34

Table 2. Results for organochlorine compounds in standard reference material and percent recovery of spiked surrogate compounds, May and June 1999.

[All values are in micrograms per kilograms unless otherwise noted. Standard reference material analyses for each batch of field samples. **Abbreviations**: ng/g, nanogram per gram; ND, not detected above reporting limit; <5.0, compound not present above the reporting level; E, estimated concentration; <, less than]

Compound	Standard reference material analyses							Average	Expected concentration	
	May 1999				June 1999				(ng/g)	Range
alpha-HCH	[1]<5.0	87	58	87	37	74	88	62	86	67–105
HCB	[1]160	120	70	130	E57	E130	120	112	148	127–169
trans-chlordane	[1]<5.0	30	22	34	E14	37	46	26	50	37–63
cis-chlordane	[1]110	140	97	160	54	130	140	119	158	150–166
trans-nonachlor	[1]130	182	E136	200	71	E171	188	154	209	198–220
p,p'-DDE	[1]380	450	240	420	214	418	E420	363	641	579–703
Dieldrin	[1]<5.0	140	100	150	72	150	180	113	150	138–162
p,p'-DDD	[1]<5.0	<5.0	<5.0	180	83	180	180	89	277	262–292
o,p'-DDT	[1]<5.0	<5.0	<5.0	<5.0	48	88	<5.0	19	156	151–161
p,p'-DDT	[1]<5.0	410	300	530	170	420	E540	339	529	484–574
	Surrogate recovery (percent recovery)									
Decafluorobiphenyl	65	61	[2]103	83	33	90	71	67		
alpha-HCH-d6	89	71	[2]142	86	38	79	97	77		
Nonachlorobiphenyl	75	83	[2]132	86	39	82	86	75		
p,p'-DDT-d8	ND	58	[2]131	90	42	91	105	77		

[1]GPC fraction problems with standard reference material.

[2]Suspect samples double surrogated.

(four times) and the monoclonal antibody (mAb) was loaded into wells on each plate. The lowest dilution (1:100) was probed with 1 µg/mL of the mAb and dilutions of 1:10,000 and higher with 0.1 µg/mL. After the addition mAb, the plates were stored at 4ºC overnight in the humidified container. The following day the plates were washed and the biotinylated secondary antibody (goat anti mouse IgG-biotin) was added to each well at 1:1000 dilution in 1 percent BSA/TBSTZ-AP and incubated at room temperature for 2 hours. The plates were washed, and strepavidin-alkaline phosphatase was added at 1:1,000 dilution in 1 percent BSA/TBSTZ-AP and incubated for 2 hours at room temperature. After a final wash of the plates, the color was developed by adding 1 mg/mL p-nitrophenyl phosphate in carbonate buffer (0.03M carbonate, 2 mM $MgCl_2$, pH 9.6) and the color was measured using an ELISA plate reader (SpectraMax Plus384, Applied Biosystems) at 405 nm. Concentrations of the unknowns were determined from the standard curves.

The limit of detection for carp Vtg direct ELISA was 0.005 mg/mL. All assays were performed in triplicate and reported as the mean of the three measurements. The coefficient of variation was less than 10 percent for all samples analyzed. Inter- and intra-assay variability was routinely measured by analyzing controls on several plates and different runs and was determined to be less than 10 percent, and less than 5 percent, respectively.

Histology

Gonadal tissue processing was done at the USGS Texas Cooperative Fish and Wildlife Research Unit in Lubbock, Tex., according to Patiño and others (2003). Standard paraffin sections of 7 µm of ovarian and testicular tissue were prepared and stained with Weigert's hematoxylin and eosin (Luna, 1992). Testicular sections also were stained using the Sigma HT20 iron stain kit (Sigma Chemical Co., St. Louis, Mo.) for demonstration of hemosiderin using bright field microscopy and ceroid-lipofuscin using fluorescence microscopy and with periodic acid-Schiff's reagent to visualize polysaccharide-rich tissue elements (Sigma 395-B). The NoTox solution did not adequately fix yolky ovarian follicles, so the exposed paraffin block tissue face (0.5-1 cm^2) was soaked from overnight to several days in equal parts of glycerol and water to improve the quality of the sections. The presence of any postovulatory and atretic follicles in each ovarian section was determined by visual examination under 100× magnification. The percentage of distribution of germ cell stages was determined for each testicular section according to the general method described by Jobling and others (1996). Testicular sections were randomly placed under a 0.7-mm^2 ocular grid at a total magnification of 400 ×, and the stage of the germ cell over each crosshair was recorded. For each sample, the total counts per stage were expressed as a percentage of the total crosshairs in the

grid, including the corners. The following categories were recorded: spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, sperm, and interstitial (interlobular) tissue (Patiño and Redding, 2000).

Fecundity and Follicle Size

Fecundity and follicle size-frequency distributions were determined according to Patiño and others (2003) at the Texas Cooperative Fish and Wildlife Research Unit in Lubbock, Tex. A small fragment of ovarian tissue containing about 50–100 follicles was randomly dissected off the fixed tissue and the diameters of all vitellogenic and fully grown follicles were measured under a stereoscope at the Texas Cooperative Fish and Wildlife Research Unit. The follicle size-frequency distribution was determined for all female fish collected. Early vitellogenic follicles were identified by their light yellow-brown color as opposed to the bright white appearance of the small previtellogenic follicles. Most follicles had a slight ovoid shape so their diameter was expressed as the average of the long and short diameter. Fecundity estimates were determined for female common carp collected in January and March 2000 prior to spawning. A larger ovarian section weighing 400–500 mg was dissected from the fixed ovarian tissue (stored in 70 percent ethanol), briefly blotted, weighed, rehydrated in water for 2 hours, and briefly blotted and reweighed. All follicles with yolk were then counted and the rehydrated weight was used to express follicle counts per gram of ovary. The total number of follicles with yolk in each female was then estimated by multiplying the number of follicles per gram of ovary by the total weight of both ovaries and expressed both as total fecundity (follicles per fish) and normalized fecundity (follicles per kilogram of fish).

Sperm Quality

Upon arrival at the USGS National Wetland Research Center in Lafayette, La., the condition of each testis was noted, and milt was removed from the collecting duct at the posterior end of the testis. Sperm motilities in undiluted milt were estimated by thorough mixing of 0.5 µL milt with 20 µL distilled water and by viewing under darkfield microscopy at 100× magnification (Jenkins and Tiersch, 1997). Triplicate readings were made per investigator, two investigators examined each sample, and the average percent (to the nearest 5 percent) of total motile cells was recorded. Milt was diluted to 1×10^6 cells/mL in HBSS in 250 µL aliquots and viability and mitochondrial function analyses were performed according to modifications of Garner and others (1994) and Segovia and others (2000) with Live/Dead Sperm Viability Kit and rhodamine 123 (Molecular Probes, Eugene, OR).

The viability assay is based on the simultaneous determination of live and dead cells using the membrane-permeant nucleic acid stain SYBR-14 (5 µL of 1 µM stock), and the conventional dead-cell stain propidium iodide (PI; 2.5 µL of 2.4 mM stock) to 250 µL sample. Ten minutes following the addition of SYBR-14 and incubation at 24°C in the dark, PI was added and tubes incubated 10 min more prior to flow cytometry (FCM). The mitochondrial function assay is based on mitochondrial membrane potential, whereby 2.5 µL of rhodamine (0.13 µM) was added to 250 µL sample and incubated similarly with PI as in the viability assay.

Quantitative assessments of fluorescent-stained sperm were made using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems [BDIS], San Jose, CA) equipped with an argon laser emitting at 488 nm. The instrument had been calibrated with AUTOComp software (BDIS) and Calibrite beads (BDIS) prior to each session. Fluorescence ranges included FL1 (525 nm band pass filter) for green fluorescence, FL2 (575 nm band pass filter) for red fluorescence, and FL3 (635 nm band pass filter) for far red fluorescence. For the three assays, data from approximately 10^4 cells or nuclei per sample in triplicate were acquired using LYSIS II software (BDIS). For viability analyses, instrument settings were forward scatter threshold at 48, scale at E01, side scatter at 483, FL1 at 412, FL2 at 376; and FL3 at 521. Density plots of FL1 (green fluorescence; live spermatozoa) and red fluorescence (FL3; dead spermatozoa) were generated. For mitochondrial function, instrument settings were forward scatter threshold at 52, scale at E01, side scatter at 414, FL1 at 712, FL2 at 505, and FL3 at 549. Density plots of FL1 (green fluorescence; functional mitochondrial with high membrane potential) and red fluorescence (FL2; dead spermatozoa) were generated.

For sperm counts, a Makler counting chamber (MidAtlantic Diagnostics, Mt. Laurel, NJ) was used by placing a 25 uL drop of a 1:400 cell dilution, and 10 squares were counted. Two samples per fish were counted in triplicate. The count formula is cells/mL= count × 400 × 1 × 10^6.

Organic Chemical Concentrations and Reproductive Biomarkers

Concentrations of organic chemical compounds in whole body tissue, reproductive biomarker measures, and physical characteristics for individual common carp collected at Las Vegas Bay and Overton Arm in Lake Mead during May 1999 through May 2000 are presented in eight attached Microsoft® Excel spreadsheets. Reproductive biomarkers for common carp are presented as follows: Las Vegas Bay females (appendix A), Overton Arm females (appendix B), Las Vegas Bay males (appendix C), and Overton Arm males (appendix D). Organic chemical concentrations are presented as follows: Las Vegas Bay females (appendix E), Overton Arm females (appendix F), Las Vegas Bay males (appendix G), and Overton Arm males (appendix H). Each spreadsheet contains all data for each month of sampling as well as monthly medians. Relevant quality assurance and quality control

Table 3. Summary of monthly median concentrations of organic chemical compounds in common carp (*Cyprinus carpio*) from Las Vegas Bay and Overton Arm, Lake Mead, Nevada, May 1999–May 2000.

[Units for all analytes in microgram per kilogram of fish tissue, wet weight. **Abbreviations:** HCB, hexachlorobenzene; HCH, hexaclorocyclohexine; *o,p´*-DDE, dichlorodiphenyldicloroethene; *o,p´*-DDD, dichlorodiphenyldicloroethane; PCA, pentachloroanisole; PCB, polychlorinated biphenyl; *p,p´*-DDD, 2,2-bis(*p*-chlorophenyl)-1, 1-dichloroethane; *p,p´*-DDE, 1.1-dichloro-2,2bis(*p*-chlorophenyl) ethylene; ND, not detected above reporting limit. **Symbols**: α, alpha; β, beta; δ, delta; γ, gamma; *o*, ortho, *p*, para]

Sex	Site	Sample size	Percent lipid	Trifluralin	α-HCH	HCB	PCA	β-HCH	γ-HCH	δ-HCH	Chlorpyrifos	Dacthal	Octachlorostyrene	Heptachlor epoxide
							May 1999							
Male	Las Vegas Bay	(14)	8.5	ND	1.55	5.00	3.05	ND	3.55	5.75	0.55	3.75	1.70	0.30
Male	Overton Arm	(15)	7.0	ND	ND	.01	ND	ND	ND	ND	ND	3.00	ND	ND
Female	Las Vegas Bay	(16)	8.3	ND	2.15	3.95	2.15	6.35	2.90	7.15	ND	3.40	1.15	ND
Female	Overton Arm	(15)	5.4	ND	ND	.29	ND	ND	ND	ND	ND	3.40	ND	ND
							June 1999							
Male	Las Vegas Bay	(15)	5.5	ND	0.68	0.42	ND	3.80	ND	ND	1.60	2.70	ND	0.62
Male	Overton Arm	(14)	6.0	ND	ND	.73	ND	ND	ND	ND	1.45	3.45	ND	ND
							September 1999							
Male	Las Vegas Bay	(10)	8.1	0.46	ND	2.64	0.32	ND	ND	ND	0.66	1.56	3.00	ND
							November 1999							
Male	Las Vegas Bay	(10)	6.7	0.67	1.07	3.90	0.45	1.54	0.97	1.96	0.95	1.34	5.33	ND
							January 2000							
Male	Las Vegas Bay	(9)	12.7	ND	1.15	2.00	0.81	3.24	1.84	1.87	0.95	1.68	1.81	ND
							March 2000							
Male	Las Vegas Bay	(10)	11.5	ND	6.95	8.95	3.80	7.00	9.90	4.55	8.65	5.95	14.50	ND
							May 2000							
Male	Las Vegas Bay	(13)	10.2	ND	8.50	7.10	1.60	ND	6.00	ND	5.50	5.00	12.00	ND

Sex	Site	Sample size	Oxychlordane	*trans*-chlordane	*o,p'*-DDE	*cis*-chlordane	*trans*-nonachlor	*p,p'*-DDE	Dieldrin	*o,p'*-DDD	*p,p'*-DDD	*cis*-nonachlor	Total PCB
							May 1999						
Male	Las Vegas Bay	(14)	1.25	5.00	23.20	7.50	6.45	115.50	3.90	19.50	13.00	1.45	2,920.0
Male	Overton Arm	(15)	ND	.05	ND	.37	1.00	12.00	ND	ND	ND	.40	26.0
Female	Las Vegas Bay	(16)	.93	3.55	16.00	5.65	5.60	88.50	ND	14.50	13.00	2.15	187.5
Female	Overton Arm	(15)	ND	ND	ND	ND	ND	4.80	ND	ND	ND	ND	9.5
							June 1999						
Male	Las Vegas Bay	(15)	1.00	1.10	0.96	1.70	2.20	50.00	1.40	7.90	2.20	1.10	156.0
Male	Overton Arm	(14)	.99	.16	ND	.49	1.50	14.50	1.20	ND	ND	.19	23.1
							September 1999						
Male	Las Vegas Bay	(10)	0.10	0.97	8.08	1.36	3.22	87.95	1.25	2.57	5.27	0.71	176.5
							November 1999						
Male	Las Vegas Bay	(10)	ND	2.20	30.90	2.85	4.41	162.50	0.97	7.79	14.18	1.05	288.0
							January 2000						
Male	Las Vegas Bay	(9)	ND	0.66	5.30	0.97	2.28	103.00	0.96	1.54	5.19	0.51	290.0
							March 2000						
Male	Las Vegas Bay	(10)	ND	7.85	62.50	11.50	13.50	380.00	2.05	38.00	ND	4.80	480.5
							May 2000						
Male	Las Vegas Bay	(13)	ND	6.60	32.00	13.00	14.00	410.00	ND	ND	ND	4.40	392.0

Table 4. Summary of monthly median biomarker and physical characteristic data for female common carp (*Cyprinus carpio*) from Las Vegas Bay and Overton Arm, Lake Mead, Nevada, May 1999–May 2000.

[**Abbreviations:** TL, total length; W, weight; GSI, Gonadosomatic Index; Vtg, Vitellogenin; mm, millimeter; kg, kilogram; mg/mL, milligram per milliliter; follicles/kg fish, follicles per kilogram of fish; NA, not available]

Site	Sample size	TL (mm)	W (kg)	GSI	Vtg (mg/mL)	Normalized fecundity (follicles/kg fish)
May 1999						
Las Vegas Bay	(16)	508	1.81	17.52	2.337	NA
Overton Arm	(15)	456	1.36	18.15	1.974	NA
June 1999						
Las Vegas Bay	(15)	488	1.52	4.28	0.863	NA
Overton Arm	(15)	450	1.26	16.40	.785	NA
September 1999						
Las Vegas Bay	(10)	500	1.63	6.00	1.433	NA
Overton Arm	(10)	473	1.18	4.07	1.125	NA
November 1999						
Las Vegas Bay	(10)	463	1.27	7.99	2.792	NA
Overton Arm	(10)	440	1.13	12.34	2.822	NA
January 2000						
Las Vegas Bay	(10)	529	2.20	14.53	3.003	190,883
Overton Arm	(10)	460	1.36	12.36	2.386	164,054
March 2000						
Las Vegas Bay	(10)	485	1.72	18.44	4.961	231,232
Overton Arm	(11)	465	1.30	14.57	4.661	162,409
May 2000						
Las Vegas Bay	(8)	504	1.77	10.30	9.368	NA
Overton Arm	(10)	452	1.29	19.29	11.126	NA

information also is included. Monthly median summaries for selected biomarkers and analytical chemistry for common carp in Lake Mead are presented in the following tables: table 3, analytical chemistry for both males and females; table 4, biomarkers for females; and table 5, biomarkers for males.

Chemistry

Thirty-three organic chemical compounds plus total concentrations for four groups of compounds (chlordanes, PCBs, BDEs, and triclosans) were analyzed in extracts of whole body tissue by GC/MS with electron ionization and electron capture negative ion detection in male common carp from Las Vegas Bay and Overton Arm during May 1999 through May 2000. All 33 compounds analyzed were detected in at least one sample of whole body tissue from male common carp collected in Las Vegas Bay. In Overton Arm, 37 organic compounds plus total concentrations of 3 groups of compounds (PCBs, BDEs, and triclosans) were analyzed in male common carp where 20 (54 percent) of the compounds were detected. Sixteen of the compounds detected in male common carp from Las Vegas Bay and 10 compounds detected in male common carp from Overton Arm are suspected of being able to disrupt the endocrine system in fish (Keith, 1997). During May and June 1999, the mean concentration of all organic compounds detected in male common carp was 670 µg/kg at Las Vegas Bay and 109 µg/kg at Overton Arm (fig. 2).

Twenty-seven organic compounds plus total PCBs were analyzed from extracts of whole body tissue in female common carp collected in both Las Vegas Bay and Overton Arm during May 1999. Twenty-four (86 percent) of these compounds were detected in at least one sample of whole body tissue from female common carp collected from Las Vegas Bay while 10 (36 percent) chemical compounds were detected in female common carp from Overton Arm during that same period. Median concentrations of all chemical compounds were higher in female common carp from Las Vegas Bay compared to those collected from Overton Arm except Dacthal (DCPA) which was similar between sites (table 3).

Biomarkers

Biomarker measures obtained for male and female common carp include gonadosomatic index (percentage of gonad weight to total body weight), plasma vitellogenin (a phospholipid protein normally produced by female common carp and other oviparous fish), and condition factor [body weight/(fork length)^3]. Biomarker measures for male common carp include five indicators of sperm quality: viability, motility, mitochondrial function, distribution of germ-cell stages, and concentration of sperm. Biomarker measures for female common carp include total fecundity (estimate of total number of follicles available for spawning), normalized fecundity (number of follicles per kilogram of body weight) during January and March 2000 only, and follicle-frequency size distribution.

Monthly medians for reproductive biomarkers were variable between sites and among months. Median concentrations of Vtg in blood plasma from male common carp collected from Las Vegas Bay ranged from not detected (detection limit 0.005 mg/mL) to 0.631 mg/mL, whereas median concentrations in male common carp from Overton Arm ranged from not detected to a high of 0.100 mg/mL. Vitellogenin concentrations in individual male common carp collected from Las Vegas Bay were as high as 68.1 mg/mL.

Table 5. Summary of monthly median biomarker and physical characteristic data for male common carp (*Cyprinus carpio*) from Las Vegas Bay and Overton Arm, Lake Mead, Nevada, May 1999–May 2000.

[**Abbreviations:** TL, total length, W, weight; GSI, Gonadosomatic Index; Vtg, Vitellogenin; mm, millimeter; kg, kilogram; mg/mL, milligram per milliliter; NA, data not available]

Site	Sample size	TL (mm)	W (kg)	GSI	Vtg (mg/mL)	Sperm quality				
						Sperm count/ mg testis	Viability (percent)	Mitochondrial function (percent)	Motility (percent)	Germ cell stage (percent sperm)
May 1999										
Las Vegas Bay	(14)	504	1.58	6.30	0.034	1.20×10^{10}	79.6	63.8	NA	77.3
Overton Arm	(15)	435	1.10	8.02	.024	1.52×10^{10}	90.0	82.1	NA	90.6
June 1999										
Las Vegas Bay	(15)	452	1.38	4.56	0.056	5.53×10^{10}	97.4	96.7	NA	81.3
Overton Arm	(15)	449	1.14	6.92	.030	7.41×10^{10}	97.1	96.5	NA	84.4
September 1999										
Las Vegas Bay	(12)	479	1.41	4.17	0.105	6.08×10^{10}	97.4	97.4	NA	49.2
Overton Arm	(12)	459	1.07	6.15	.043	6.15×10^{10}	95.7	95.4	NA	64.8
November 1999										
Las Vegas Bay	(10)	460	1.30	6.25	0.631	6.42×10^{10}	97.8	97.6	76.7	76.6
Overton Arm	(9)	432	.94	7.73	.058	7.31×10^{10}	96.0	95.7	79.2	85.9
January 2000										
Las Vegas Bay	(10)	469	1.35	5.50	0.015	5.52×10^{10}	98.2	97.8	62.5	81.3
Overton Arm	(10)	442	1.06	7.70	.019	4.71×10^{10}	95.2	94.5	83.3	93.0
March 2000										
Las Vegas Bay	(10)	468	1.52	7.32	0.028	4.06×10^{10}	97.3	95.8	75.0	83.6
Overton Arm	(10)	447	.98	8.62	.026	4.32×10^{10}	96.5	96.1	75.8	82.0
May 2000										
Las Vegas Bay	(12)	474	1.30	5.40	0.014	3.12×10^{10}	96.4	96.2	79.4	93.8
Overton Arm	(10)	433	1.02	6.68	.022	2.49×10^{10}	97.3	96.9	95.0	93.8

Monthly median sperm counts also varied between sites and among months; values in male common carp ranged from 1.20×10^{10} per milligram of testis to a high of 6.42×10^{10} per milligram of testis at Las Vegas Bay, and from 1.52×10^{10} per milligram to 7.41×10^{10} per milligram of testis at Overton Arm. Another sperm quality biomarker, viability had monthly medians from 79.6 percent to a high of 98.2 percent in male common carp from Las Vegas Bay and from 90.0 percent to 97.3 percent in male common carp from Overton Arm. Monthly median Vtg concentrations in blood plasma ranged from 0.863 to 9.368 mg/mL in female common carp from Las Vegas Bay and from 0.785 to 11.126 mg/mL in female common carp from Overton Arm (fig. 3). Vitellogenin generally increased in late autumn through winter reaching a maximum just before spawning in March. Total fecundity (number of follicles per kilogram of body weight) was more variable during the pre-spawning months of January and March 2000 in female common carp from Las Vegas Bay (ranges from 190,883 to 231,232 follicles per kilogram of fish, respectively) but was fairly consistent in female common carp from Overton Arm (medians of 164,054 and 162,409 follicles per kilogram of fish, respectively).

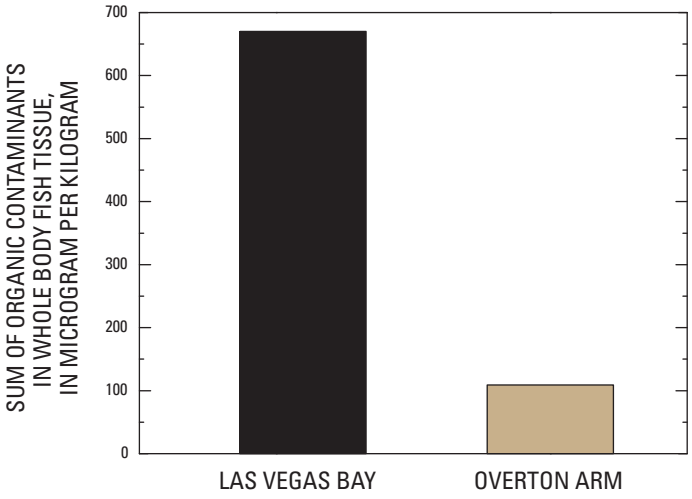


Figure 2. Sum of total contaminants in whole body male common carp collected from Las Vegas Bay and Overton Arm sampling areas, Lake Mead, Nevada, May and June 1999.

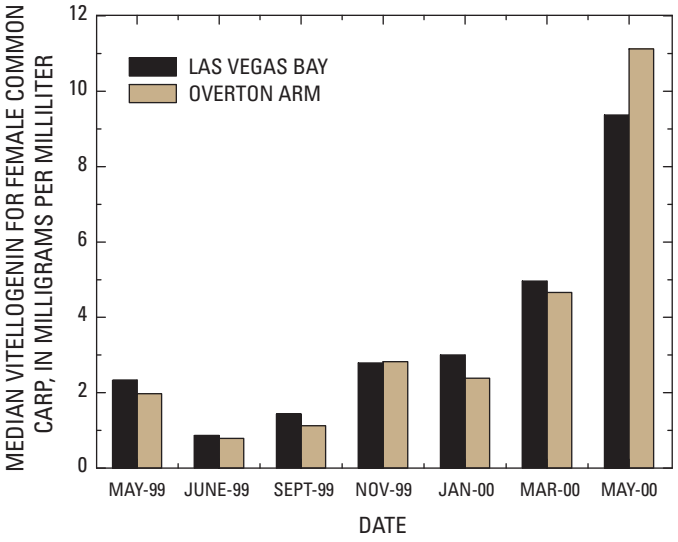


Figure 3. Seasonal change in monthly median vitellogenin (Vtg) concentrations for female common carp from Las Vegas Bay and Overton Arm sampling areas, Lake Mead, Nevada, May 1999–May 2000.

Acknowledgments

This study was funded by the U.S. Geological Survey and supported with in-kind services from the National Park Service, Bureau of Reclamation, U.S. Fish and Wildlife Service and the Nevada Department of Wildlife. The USGS Texas Cooperative Fish and Wildlife Research Unit is jointly supported by the U.S. Geological Survey, Texas Tech University, Texas Parks and Wildlife, U.S. Fish and Wildlife Service, and the Wildlife Management Institute. Specific assistance for electroshocking fish was provided by Jim Heinrich of Nevada Department of Wildlife. Tim Gross and technicians Carla Weiser, Shane Ruessler, and John Wiebe from the U.S. Geological Survey, Florida Integrated Science Center in Gainesville, Fla., provided substantial help planning the study, organizing trips, purchasing equipment and field assistance. Bill Burke and Bryan Moore of the National Park Service helped with field sampling, permits, and reconnaissance. Ken Covay of the U.S. Geological Survey, provided excellent assistance in all field sampling and shipping samples. Many helped analyzing samples in the laboratory including Melody Wainscott and Catherine Barry for fecundity, histology, and follicular diameter; Sonja Abney for analytical chemistry; Lita Hazlett for sperm quality; and Kevin Kroll for vitellogenin. Finally, we owe a debt of gratitude to Rachael Muir for her far sighted thinking to have the U.S. Geological Survey become involved in assessing endocrine disruption in aquatic resources in streams and rivers of the United States including Lake Mead.

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Appendixes

Concentrations of organic chemical compounds in whole body tissue, reproductive biomarker measures, and physical characteristics for individual common carp collected at Las Vegas Bay and Overton Arm in Lake Mead during May 1999 through May 2000 are presented in eight Microsoft® Excel spreadsheets. The appendixes can be accessed and downloaded at URL http://pubs.water.usgs.gov/ds286/.

Appendix A. Biomarker data and physical characteristics for female common carp (*Cyprinus carpio*) collected from Las Vegas Bay, Lake Mead, Nevada, May 1999–May 2000. (Excel, 55 KB)

Appendix B. Biomarker data and physical characteristics for female common carp (*Cyprinus carpio*) collected from Overton Arm, Lake Mead, Nevada, May 1999–May 2000. (Excel, 55 KB)

Appendix C. Biomarker data and physical characteristics for male common carp (*Cyprinus carpio*) collected from Las Vegas Bay, Lake Mead, Nevada, May 1999–May 2000. (Excel, 47 KB)

Appendix D. Biomarker data and physical characteristics for male common carp (*Cyprinus carpio*) collected from Overton Arm, Lake Mead, Nevada, May 1999–May 2000. (Excel, 91 KB)

Appendix E. Analytical chemical results for female common carp (*Cyprinus carpio*) collected from Las Vegas Bay, Lake Mead, Nevada, May and June1999. (Excel, 25 KB)

Appendix F. Analytical chemical results for female common carp (*Cyprinus carpio*) collected from Overton Arm, Lake Mead, Nevada, May 1999. (Excel, 24 KB)

Appendix G. Analytical chemical results for male common carp (*Cyprinus carpio*) collected from Las Vegas Bay, Lake Mead, Nevada, May 1999–May 2000. (Excel, 108 KB)

Appendix H. Analytical chemical results for male common carp (*Cyprinus carpio*) collected from Overton Arm, Lake Mead, Nevada, May and June 1999. (Excel, 32 KB)

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