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U.S. FISH AND WILDLIFE SERVICE  
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ENVIRONMENTAL CONTAMINANTS PROGRAM  
ON-REFUGE INVESTIGATIONS  
FINAL REPORT

**Endocrine Disruption in Razorback Sucker and Common Carp on  
National Wildlife Refuges along the Lower Colorado River**

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## ABSTRACT

To provide the entire Lower Colorado River with the first endocrine disruption reconnaissance study on native and non-native fishes (razorback suckers and common carp), the U.S. Fish and Wildlife Service (FWS) chose three sampling locations, Lake Mohave, Havasu National Wildlife Refuge, and Cibola National Wildlife Refuge, below Lake Mead, in 2002, to collect steroid hormones, vitellogenin, thyronine, thyroxine, sperm viability, histology, and environmental contaminants concentrations in whole body tissue and sediments. Tissue concentrations of total p,p'-DDT homologs, total brominated diphenyl ethers (BDEs), total chlordanes, and total polychlorinated biphenyls (PCBs) were all relatively low (parts per billion (ppb) range). Total p,p'-DDT homologs were detected at the highest concentrations of any environmental contaminant in this study. There were significant differences between environmental contaminants and sites. For example, while total DDT-homologs were highest at Cibola and lowest at Havasu, total PCBs and total BDEs were greatest at Mohave and lowest at Cibola. Endocrine disruption in endangered razorback suckers was not found although data indicates slightly elevated concentrations of 17 $\beta$ -estradiol in female razorback suckers. A few indications of endocrine disruption in carp were found. Male carp vitellogenin concentrations (0.18-0.38 mg/mL) were elevated compared to normal male vitellogenin concentrations around the country and previous Lake Mead studies (0.01-0.1 mg/mL). Other carp data, such as elevated 17 $\beta$ -estradiol in females and lowered 11-ketotestosterone in females, may reflect exposure to a variety of environmental stressors or intrinsic differences between the Lower Colorado River (LCR) and other sites, but do not indicate endocrine disruption. Differences in biomarker results between LCR sampling sites were also apparent. In most cases, Mohave had significantly different hormone concentrations or condition indices than Havasu or Cibola. For example, razorback sucker E/T ratios were significantly higher in both males and females at Mohave than the other sites, although male E/T ratios never exceeded one, an indicator of endocrine disruption. Also, sperm viability in Mohave carp was significantly lower (78.5%) compared with Havasu carp (95%). Regressions between environmental contaminants and hormones or condition indices yielded significant results, but no consistent relationships were found. Thus, while environmental contaminants may be affecting the health of fish in the Lower Colorado River, clear cause-and-effect relationships could not be determined at this time. Other factors in the Lower Colorado River that may influence hormone concentrations or condition indices and should be taken into consideration include trophic dynamics of each sampling site, nutrient status of fishes, and other stressors besides environmental contaminants (season, photoperiod, and hydrology of each site).

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### KEYWORDS:

endocrine disruption, razorback sucker, *Xyrauchen texanus*, carp, *Cyprinus carpio*, hormones, tissue concentrations, Havasu National Wildlife Refuge, Cibola National Wildlife Refuge, FFS# 1261-2N47, DEC ID# 200020005, Congressional District 2, and Congressional District 7

### LIST OF ACRONYMS/ABBREVIATIONS

11-KT	11-Ketotestosterone		
ANOVA	Analysis of Variance	USGS	United States Geological Survey
AZFRO	Arizona Fisheries Resources Office		
BC	British Columbia	Vtg	Vitellogenin
BDEs	Brominated diphenyl ethers	WW	Wet Weight
BEST	Biomonitoring Environmental Status and Trends		
BHA	3-tert-Butyl-4-hydroxyanisole		Total p,p'-DDT homologs = p,p'-DDT + p,p'-DDD + p,p'-DDE
BRD	Biological Resources Division		
DCPA	Dacthal		
DDD	Dichlorodiphenyldichloroethane		Total PCBs = PCB 70 + ... + PCB 206 as listed in Table 1.
DDE	Dichlorodiphenyldichloroethylene		
DDT	Dichlorodiphenyltrichloroethane		
E2	17 $\beta$ -estradiol		Total chlordanes = cis-chlordane + trans-nonachlor + trans-chlordane + cis-nonachlor + heptachlor epoxide + oxychlordane
EDC	Endocrine Disrupting Chemicals		
EI	Electronic ionization		
EPA	Environmental Protection Agency		
E/T ratio	Estradiol:11-Ketotestosterone ratio		
ELISA	Enzyme-linked immunosorbent assay		Total BDEs = BDE 47 + BDE 99 + BDE 100 + BDE 153 + BDE 154
FWS	Fish and Wildlife Service		
GSI	Gonadal somatic indices		
HBSS	Hank's Balanced Salt Solution		
HCB	Hexachlorobenzene		
HCHs	Hexachlorocyclohexanes		
LCR	Lower Colorado River		
MA	Macrophage aggregates		
NCI	Negative chemical ionization		
NCBP	National Contaminant Biomonitoring Program		
ND	Non-detect		
NFH	National Fish Hatchery		
NPS	National Park Service		
NWQL	National Water Quality Laboratory		
NWR	National Wildlife Refuge		
PAHs	Polynuclear aromatic hydrocarbons		
PCA	Pentachloraniline		
PCBs	Polychlorinated biphenyls		
PCNB	Pentachloronitrobenzene		
PIT	Passive inductive transponder		
PPB	Parts per billion		
PPM	Parts per million		
PPT	Parts per trillion or Parts per thousand		
RIA	Radioimmunoassay		
SPMDs	Semi Permeable Membrane Devices		
T	Testosterone		
T <sub>3</sub>	Tri-iodothyronine		
T <sub>4</sub>	Tetra-iodothyroxine		
USA	United States of America		

## INTRODUCTION

Non-point sources of agricultural chemicals and point sources of industrial pollution and wastewater treatment plants have been implicated in endocrine disruption in fishes in Lake Mead and present a concern to the health of FWS resources in the Lower Colorado River (Bevans et al. 1996; Jobling et al. 1998). The water in the Lower Colorado River flows from Lake Mead, formed by Hoover Dam, toward four Arizona National Wildlife Refuges (NWR): the Havasu NWR, Bill Williams River NWR, Cibola NWR, and Imperial NWR along the Lower Colorado River (Figures 1 and 2). These NWRs are located downstream from municipalities with wastewater treatment plants and agricultural return flows. Evidence of reproductive effects (endocrine disruption) has been documented in fish from Las Vegas Wash and Las Vegas Bay of Lake Mead (Bevans et al. 1996; Goodbred et al. 1997, Tuttle and Orsak 2002, Patiño et al. 2003). Several endocrine-active agents, including pesticides, industrial compounds, pharmaceuticals, and personal care products, have been identified in water, sediment, and fish from Las Vegas Wash and Las Vegas Bay. Concentrations of hormones responsible for spermatogenesis were substantially lower in male razorback suckers (*Xyrauchen texanus*), sport-fish, and common carp (*Cyprinus carpio*) from Las Vegas Bay. Ratios of estrogens and androgens were also skewed. Sperm motility, sperm viability, and gonad weight were depressed in carp from Las Vegas Bay (Bevans et al. 1996; NPS 2001).

Low levels of some endocrine disrupting compounds were found in American coots (*Fulica americana*) on Havasu NWR (Andrews et al. 1997). Another concern is perchlorate surface and groundwater contamination in Lake Mead from an ammonium perchlorate manufacturer in Henderson, Nevada. Perchlorate is a threat to fishes in the Lower Colorado River because of its ability to disrupt the normal function of the thyroid. These concerns threaten the Arizona refuges which provide critical refugia to endangered big river fishes such as the razorback sucker and the bonytail chub (*Gila elegans*). Although dam construction and flow modification on the Lower Colorado River was one of the greatest reasons for decline of the razorback sucker and bonytail chub, recruitment of these species under current conditions has not been sufficient to sustain viable populations.

Cibola NWR is the only refuge with razorback suckers and bonytail chub; however, other refuges are in different stages in developing grow-out ponds for these species. Most of these growout ponds use water from the Colorado River. Similarly, the Arizona Fishery Resources Office (AZFRO) regularly stocks razorback suckers and bonytail chub along the Lower Colorado River. Growth, development, and metabolism could also be affected if these fishes are exposed to perchlorate. Habitat necessary for endangered birds such as the southwestern willow flycatcher (*Empidonax traillii extimus*) and Yuma clapper rail (*Rallus longirostris yumanensis*) is present at the NWRs along the Lower Colorado River. The Lower Colorado River also provides important habitat for migrating and wintering waterfowl and shorebirds throughout the arid southwest. Egrets, herons, grebes, cormorants, and ibis feed and nest in the backwaters of the Lower Colorado River.

Widespread concern about endocrine disruption escalated in the 1990s when researchers began recording hormone imbalances in fish and wildlife inhabiting contaminated environments. Many chemicals are now suspected of playing a role in this increasingly common phenomenon. Industrial chemicals, organochlorines, polycyclic aromatic hydrocarbons, phthalates, phenols,

pharmaceuticals and personal care products have been implicated (Thomas 1988; Colburn et al. 1993). Although the mode of action that produces the hormone imbalance is unknown, the effects of the exposure can be estrogenic, anti-estrogenic, androgenic, or anti-androgenic. Fish exposed to chemicals that are estrogen agonists or androgen antagonists may exhibit increased estrogen production and/or decreased androgen synthesis in males and females, respectively. Fish exposed to chemicals that are estrogen antagonists may have decreased levels of estrogen and fish exposed to chemicals that are androgen agonists may exhibit increased circulating levels of testosterone. In addition to abnormal hormone levels, hormone ratios are another tool used to indicate the potential for reproductive dysfunction after exposure to endocrine disrupting compounds. Vitellogenin, a female egg yolk precursor protein and a biomarker of a physiological response, can also be used to indicate a potential adverse impact. Vitellogenin production is the result of the expression of a gene found only in non-eutherian mammals, amniotes, and vertebrates with megalecithal eggs. It is expressed in females under the influence of estrogen and/or the absence of testosterone. Male carp often have induced vitellogenin concentrations if contaminated sites are significantly elevated above the reference site.

Results from investigations of potential endocrine disrupting chemicals (EDCs) in Lake Mead revealed several chemicals in the water, sediment, and common carp (Bevans et al. 1996, Tuttle and Orsak 2002). The source of contamination includes the influx of household chemicals, pesticides, personal care products (including musk derivatives) and pharmaceuticals including ethinyl estradiol in treated wastewater into Las Vegas Wash (Bevans et al. 1996; Goodbred et al. 1997). Musk derivatives are used as fragrances in personal care products and include both natural and synthetic compounds. Other potential endocrine disrupting chemicals, including industrial chemicals and pesticides such as nonylphenol, PCBs, hexachlorobenzene, DCPA, chlordane, dieldrin, DDT and its metabolites, dioxins, and furans, were detected by USGS in Lake Mead using semipermeable membrane devices (SPMDs) (Bevans et al. 1996; Goodbred et al. 1997, Tuttle and Orsak 2002).

Perchlorate is also a potential problem in Lake Mead and the Colorado River at low levels (4 to 16 parts per billion (ppb)). Ammonium perchlorate was released by the Kerr-McGee Chemical Company in Henderson, Nevada into the groundwater and subsequently moved into Lake Mead (NDEP 2007). Perchlorate competitively inhibits the uptake of iodide anion by the thyroid, thus disrupting thyroid function. Iodine is necessary for the conversion of tri-iodothyronine ( $T_3$ ) to tetra-iodothyroxine ( $T_4$ ). Additional functions affected by perchlorate exposure include growth, development, and metabolism. Compromising thyroid function could alter the ability of the animal to handle environmental and behavioral stressors because the thyroid also regulates immune function (Capps et al. 2004, Brown et al. 2004).

Havasu NWR, Bill Williams River NWR, Cibola NWR, and Imperial NWR are dependent upon the quantity and quality of Colorado River water to manage lands and wildlife, including several endangered species. All of these refuges are adjacent to the river and three are bisected by the river with refuge lands on both the California and Arizona side. River water or agricultural irrigation water is used to restore and maintain much of the native vegetation on these refuges. The Lower Colorado River historically had elevated selenium levels in the water, sediment, and biota as a result of seleniferous inputs from the upper Colorado River basin (Martinez 1994; Marr and Velasco 2005). Upstream from these refuges are the wastewater treatment plants for the following municipalities: Bullhead City/Laughlin, Lake Havasu City, and Blythe. The

Colorado River NWRs also receive agricultural return flows from California and Arizona. There is a concern that the Las Vegas Water Authority would choose an alternative to pipe wastewater to an outfall downstream of Hoover Dam, thus directly discharging wastewater that has been shown to negatively affect carp and razorback suckers into the Lower Colorado River (Patiño et al. 2003, Tuttle and Orsak 2002). Given the evidence of endocrine disruption upstream from nearly half of Arizona's nine National Wildlife Refuges, the amount of water (industrial and agricultural) that returns to the Colorado River as effluent, and the need to obtain data for wastewater discharge decision-making, indicators of endocrine disruption in common carp and razorback suckers were measured downstream of the Hoover Dam. Benthivorous fish species such as the razorback sucker, which is an endangered species, and the common carp, which uses a similar foraging strategy, were included for tissue collection and determination of whole-body concentrations of contaminants. Endocrine disruption has been documented in common carp, protocols for endocrine disruption biomarkers are well established (Goodbred et al. 1997, Patiño et al. 2003, Snyder et al. 2004), and data on razorbacks above and just below Hoover Dam are being compiled (Erik Orsak, pers. comm., FWS contaminants specialist, Las Vegas, NV).

Hormone levels in male and female fish and vitellogenin production in males were measured. Reproductive dysfunction in endangered species resulting from unbalanced hormone levels or abnormal female protein production could decrease the probability of successful recovery. Also examined as a higher level effects in razorback suckers and carp, respectively, was sperm viability and gonad histology. For carp, fecundity as determined by gonad histology was also examined.

### **Scientific Objectives**

1. Investigate evidence for endocrine disruption in carp, a bottom feeding, non-native fish and the razorback sucker, an endangered native species.
2. Measure levels of tri-iodothyronine (T<sub>3</sub>) and tetra-iodothyroxine (T<sub>4</sub>) in carp of the Lower Colorado.
3. Evaluate reproductive condition of fish using gonadal somatic indices (GSI) and histology.
4. Provide information for Willow Beach NFH or AZFRO to prioritize stocking sites if effects are seen in razorback suckers.
5. Obtain data that could be used in an Environmental Impact Statement or Biological Opinion should the Las Vegas Water Authority choose to discharge treated wastewater south of Hoover dam.

### **METHODS**

#### **Data Collection and Analysis**

Selection of sampling sites was based upon a documentation of EDCs in the vicinity, proximity to wastewater treatment plants, and proximity to other point sources that could contribute EDCs. An electrofishing boat and trammel nets were used to collect male and female carp from Cibola

NWR and Havasu NWR (Figures 1 and 2). Trammel nets were used to collect male and female razorback suckers only from High Levee Pond<sup>1</sup> at Cibola NWR and Lake Mohave (Mohave); razorback suckers occur in Lake Havasu, but they are not localized in specific areas. Therefore, no razorback suckers were collected from Havasu NWR. Razorback suckers and carp collected at Lake Mohave were used as reference for fish collected at the two refuge locations. Common carp were collected beginning in January 2002 at Cibola NWR (Cibola-January) from Walter's Camp and Three Fingers Lake and then in March 2002 at Lake Mohave from Cottonwood Cove south towards the main body of the lake, Havasu NWR (Havasu) from Needles to the Topock Gorge, and Cibola NWR (Cibola-March) from Mitchell's Camp and Pretty Water and High Levee Pond (Figures 1 and 2). Samples were processed according to Goodbred et al. (1997). Fish were successfully collected in January 2002 at Cibola (carp = 18; razorbacks = 18), but the rest of the collections were delayed because January water temperatures were cold and fish were not spawning. Field collections resumed at the end of March 2002. Beginning at Lake Mohave, 27 razorback sucker and 19 carp were collected and processed. Twenty carp were collected at Havasu NWR and in March, 13 carp and 15 razorback suckers were processed from Cibola NWR. All efforts were taken to collect and process fish in the same age class based on field measurements of length and weight, the same reproductive stage based on visual observations of gonad maturation stage, and the same ratio of male to female fishes. Fish were collected during spawning in order to minimize the number of times wild razorback suckers would be handled. Biologists monitor razorback sucker populations yearly on the Lower Colorado River. This study was conducted at the same time to take advantage of population counts and larval fish collections that were already scheduled.

Carp samples were necropsied and the following were collected: scales, gonads, blood, sperm, and the whole carcass. GSIs were also measured as an indicator of fish health (Schmitt et al. 1999). Razorbacks collected from High Levee Pond were examined for passive inductive transponder (PIT) tags to determine their origin, and then processed non-lethally for blood. Blood from each fish was collected and stored in heparinized vacutainers, kept on wet ice and centrifuged as close to collection time as possible. Plasma was sent to the USGS-Florida Integrated Science Center and University of Florida Ecotoxicology Program for sex steroid, vitellogenin, and thyroid hormone analyses. Testes were collected from male carp and analyzed by flow cytometry for sperm viability and motility by USGS-BRD at the National Wetlands Research Center. Gonad samples were sent to the USGS-Texas Cooperative Fish and Wildlife Research Unit for histology. Carp were held no longer than 24 hours in live cars (in-situ netted cages) in the river prior to tissue collection and razorback suckers were held up to 1-2 days in live cars in the river in order to keep the number of capture events on wild razorback suckers to a minimum.

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<sup>1</sup>High Levee Pond is an isolated 6 acre pond, formed by the old Colorado River channel and man-made levees on two sides. The pond holds two endangered big river fishes, the razorback sucker and bonytail. Razorback suckers were sampled twice in 2002 in High Levee Pond, once in January and once in March.

Sediment was sampled in 2003 at High Levee Pond and Pretty Water on Cibola NWR, at Topock Marina and below the Needles wastewater treatment plant outfall on Havasu NWR, and at Yuma Cove and North Cove in Lake Mohave (Figures 1 and 2). Grab samples were collected with a ponar dredge, placed in a glass jar, kept on ice in a cooler in the field, and refrigerated until shipment to the analytical laboratory. Sediment was analyzed for a variety of polynuclear aromatic hydrocarbons (PAHs), organochlorine pesticides, PCBs, and BDEs.



Figure 1. Map of the northern sampling sites along the Lower Colorado River, Arizona in 2002 and 2003.



Figure 2. Map of the southern sampling sites along the Lower Colorado River, Arizona in 2002 and 2003.

### Determination of Sex Steroids

Plasma samples from common carp and razorback suckers were analyzed by the USGS-Florida Integrated Science Center and University of Florida Ecotoxicology Program using radioimmunoassay (RIA) procedures for sex steroid hormones, 17 $\beta$ -estradiol (E2), testosterone (T) and 11-ketotestosterone (11-KT). The methods developed for this reconnaissance study are described in detail in Goodbred et al. (1997), Schmitt and Dethloff (2000), Smith et al. (2002) and Gross et al. (2002). Results are expressed as concentrations (picograms per mL - pg/mL) of E2, T, 11-KT or a ratio of the two (E2:11-KT); as potential indicators of gender and contaminant exposure in both species. All plasma samples were assayed in duplicate, and values are reported as pg/mL of plasma. Plasma samples (50  $\mu$ L) were extracted twice with 5 mL diethyl ether prior to RIA analysis (Scintillation Counter Packard Tricarb, Model 1600). Plasma samples were corrected for extraction efficiencies of  $87 \pm 3.5\%$ ,  $82 \pm 4.1\%$ , and  $83 \pm 2.8\%$  for E2, T, and 11-KT. Standard curves (1, 5, 10, 25, 50, 100, 250, 500 and 1000 pg) were prepared in buffer with known amounts of radioinert E2, T (ICN Biomedicals, Costa Mesa, CA) and 11-KT (Sigma Chemical, St. Louis, MO). Detection limits were 5.1 pg/mL for E2; 12.8 pg/mL for T; and 9.3 pg/mL for 11-KT. Specific antibodies against sex steroids were purchased from either ICN Biomedicals (E2 and T) or Helix Biotech, Richmond, BC, Canada (11-KT). Cross-reactivities of the E2 antiserum with other steroids were: 11.2% for estrone; 1.7% for estriol; < 1.0% for 17-estradiol and androstenedione; and < 0.1% for all other steroids examined. Cross-reactivities of the T antiserum with other steroids were: 18.75% for 5-dihydrotestosterone; 3.0% for 5-androstenediol, < 1.0% for androstenedione, and < 0.1% for all other steroids examined. Cross-reactivities of the 11-KT antiserum with other steroids were: 9.65% for T; 3.7% for -dihydrotestosterone, < 1.0% for androstenedione, and < 0.1% for all other steroids examined.

### Determination of Vitellogenin

Vitellogenin (Vtg) concentrations in carp plasma were assayed and quantified by Direct Enzyme-Linked Immunosorbent Assay (ELISA) at the Biotechnology Laboratory, University of Florida as described by Denslow et al. (1996), Goodbred et al. (1997), Schmitt and Dethloff (2000), and Gross et al. (2002). The detection limit of Vtg was 0.005 pg/mL. Vitellogenin was first purified by anion exchange chromatography, and its protein concentration determined by the Bradford method (Bradford 1976) for use as a standard. Standard curves were constructed by adding serial dilutions of purified Vtg (0 mg/mL to 0.001 mg/mL) to male control plasma and also assayed and quantified. The intensity of yellow color that developed was quantified at 405 nm with an automated ELISA reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA). Standard curves fitted by quadratic regression were used to calculate Vtg concentrations, with R<sup>2</sup> values usually between 0.95 and 0.99. In order to test for interassay and intra-assay variation, each assay was run with a positive control that had a known Vtg concentration. Samples were rerun if the coefficient of variation between triplicates exceeded 10%. The minimum concentration detectable in this assay was 0.001 mg/mL. Vitellogenin values were reported as mg/mL of plasma.

### Determination of Thyroid Hormones

Plasma samples from carp were analyzed by the USGS-Florida Integrated Science Center and University of Florida Ecotoxicology Program using RIA procedures hormones tri-iodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>). All plasma samples were assayed in duplicate by direct radioimmunoassay, and values are reported as pg/mL of plasma. Direct RIA procedures for T<sub>3</sub> and T<sub>4</sub> utilized kit procedures from ICN Biomedicals, Costa Mesa, CA. The minimum concentration distinguishable from zero was 63 pg/mL for T<sub>3</sub> and 92 pg/mL for T<sub>4</sub>. Specific antibodies against T<sub>3</sub> and T<sub>4</sub> were purchased from ICN Biomedicals. For carp plasma: interassay and intrassay coefficients of variation were 8.4 and 9.2%, respectively for T<sub>3</sub>; 9.2 and 7.1% for T<sub>4</sub>. Thyroid hormone values were reported as ng/mL of plasma (Gross 2005).

### Sperm Cell Viability

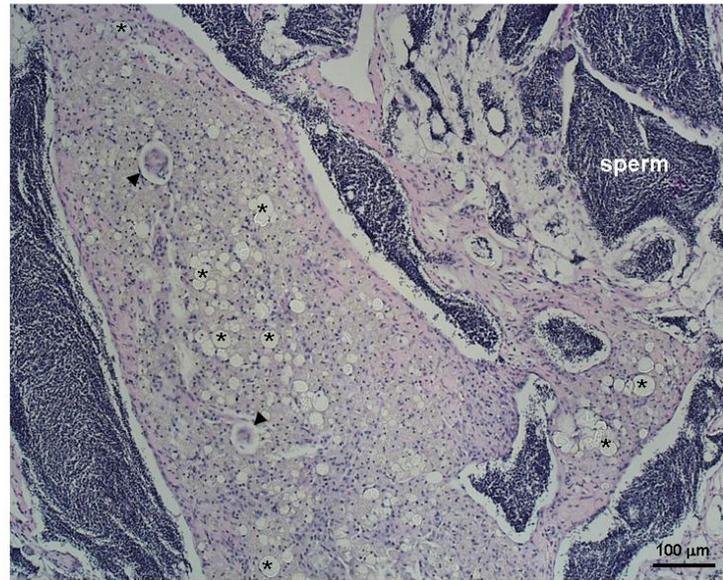
Sperm cell viability was examined in carp collected during the March sampling trips. Originally, a different method that required on-site viability analysis of sperm cells was going to be used; this method was tested at Cibola in January. When January water temperatures were too cold for fish spawning, the sampling schedule was moved to March. On-site viability analysis of sperm cells could not be performed in March, so methods were changed and Dr. Jill Jenkins, USGS-BRD at the National Wetlands Research Center began performing sperm viability analysis. For sperm viability and motility analyses, testes were shipped overnight to the National Wetlands Research Center (in calcium-free Hank's balanced salt solution (HBSS) with 10% v/v streptomycin/penicillin). A portion of cells were removed from the posterior section for viability analyses. Flow cytometry using a dual-staining technique with SYBR 14 and propidium iodide was used with 10,000 cells per sample analyzed in triplicate. Dissection methods, buffer choice, and sperm cell preparation methods were optimized (Jenkins 2005). Validation procedures are found in Jenkins and Goodbred (2006).

Sperm suspensions were filtered with 30  $\mu\text{m}$  nylon mesh (Small Parts, Miami Lakes, FL) prior to dilution ( $1 \times 10^6$  /mL). Cells were stained using a live/dead sperm viability kit (Molecular Probes) using a starting stock solution of 1:100 SYBR-14 into 250  $\mu\text{l}$  aliquots of cell suspensions (Segovia et al. 2000). The analytical instrumentation used to study the sperm was a flow cytometer (FACScan, Becton Dickinson Immunocytometry System, San Jose, CA). This instrument provides multiparameter analyses for assessing nuclear and cellular components of interest in freely flowing cells. Individual cells or nuclei of any tissue from any species can be probed by a laser light and emitted light signals are transferred quickly into electronic measures, so that typical sample sizes approximate 10K – 50K events and data are collected within about 1 minute per sample. For the verification of cell condition and probes employed, epifluorescence microscopy (Leitz Diaplan) was used in conjunction with flow cytometry.

In membrane integrity staining, SYBR-14 stains the nuclei of living sperm, and the counterstain propidium iodide stains dead or moribund sperm.

### Gonad Histology

Carp gonads were preserved in 10% buffered formalin and sent to USGS-Texas Cooperative Fish and Wildlife Research Unit (Dr. Reynaldo Patiño) for documentation of stage of maturation, analysis for anomalies, macrophage aggregates (MA) in testes, and fecundity in ovaries. Macrophage aggregates were analyzed according to previously published methods (Capps et al. 2004). Stage of maturation of testicular tissue, ovarian follicle diameter and fecundity estimates were analyzed according to Patiño et al. (2003). Relative incidences of testicular MA histopathologies were measured by a histopathologist. The relative incidences of specific histopathologies associated with MAs, such as the presence of sporozoan-like parasites, vacuolization of MAs (Figure 3), and focal granulomas (Figure 4), was accomplished by counting the number of crosshairs on an ocular grid that fell on the specific histopathologies. The percent area of testes covered by specific histopathologies was estimated by dividing the number of crosshairs falling on specific histopathologies by the corrected total crosshair count on the grid (procedure was similar to that described for kidneys in Capps et al. 2004).



Arrowheads: sporozoan-like structures  
Asterisks: vacuoles

Figure 3. Sporozoan-like structures and vacuolization of macrophage aggregates found in carp testes on the Lower Colorado River, Arizona in 2002. Hematoxylin and eosin stain.



Arrows: focal granulomas

Figure 4. Focal granulomas associated in carp testes from the Lower Colorado River, Arizona in 2002. Hematoxylin and eosin stain.

## Analytical Chemistry

Whole body carp from the all sites were sent to the National Water Quality Laboratory (NWQL, Dr. Tom Leiker, USGS) for organic chemical analysis. Whole body fish were analyzed for 54 organochlorine contaminants, fungicides, and other endocrine-disrupting chemicals (Table 1). The basic methods for analysis of tissues, sediments, and plasma used at NWQL included whole-body homogenization (tissue only), freeze-drying, accelerated-solvent extraction, and analysis by dual capillary-column gas chromatography with mass spectrometry. Electron ionization (EI) was used in mass spectrometry to lower the detection limits for PAHs and electron-capture negative ion (negative chemical ionization; NCI) was used in mass spectrometry to lower the detection limits for halogenated compounds (USGS 2007). Specific details for NWQL analytical methods were described in Leiker et al. (1995), USGS (2006), and USGS (2007). Data reporting limits for most compounds were 1 µg/kg wet weight (ww) for most samples except for 5 p,p'-DDT homologs, which were 50 µg/kg ww. p,p'-DDT had a reporting limit of 1 µg/kg ww. Triclosan degradates, toxaphene, and chlorothalonil were not detected nor listed in Table 1. Some tissue data values were reported below the method reporting limit and as a result, were noted as estimates. These values were treated the same as data without qualifiers. Laboratory reagent spikes and laboratory blanks were analyzed with each set of 10-15 samples. Surrogate compounds were added to samples prior to extraction to monitor method performance. Percent mean recovery of spiked samples was within normal recovery limits. Recoveries for surrogates were not adjusted based on quality control data.

Sediment was collected in 2003 and analyzed for 32 PAHs; analysis was conducted according to the sample extraction and analysis methods described above. Data reporting limits for compounds ranged from 25 - 250 ng/g for EI compounds and 2 – 80 ng/g for NCI compounds. Some sediment data values were reported below the method reporting limit and as a result, were noted as estimates. These values were treated the same as data without qualifiers. Laboratory spikes, blanks, and surrogates were performed as described above. Additional plasma was analyzed for pharmaceuticals and musk derivatives and was also analyzed according to the same procedures as tissue and sediment (USGS 2006, 2007). Data reporting limits for compounds ranged from 12.5 ng/mL for acetophenone, 2-methylnaphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, and diethyl phthalate; 25 ng/mL for BDE 47 and diethylhexyl phthalate; 125 ng/mL for 3-tert-Butyl-4-hydroxyanisole (BHA), and 1,250 ng/mL for cholesterol for EI compounds and 2 – 80 ng/mL for NCI compounds.

Table 1. Organochlorine pesticides, fungicides, and other endocrine disrupting chemicals analyzed in fish tissue samples.

Trifluralin	HCB	Heptachlor Epoxide	cis-chlordane	p,p'-DDT	Endrin aldehyde	PCB 118	PCB 174	PCB 206
Benfluralin	PCA	Oxychlordane	trans-nonachlor	o,p'-DDT	Endrin Ketone	PCB 138	PCB 177	BDE 47
alpha-HCH	Aldrin	Fipronil	trans-chlordane	p,p'-DDD	Mirex	PCB 146	PCB 180	BDE 99
beta-HCH	Chlorpyrifos	Endosulfan I	cis-nonachlor	o,p'-DDD	PCB 70	PCB 149	PCB 183	BDE 100
gamma-HCH	DCPA	Endosulfan II	Dieldrin	p,p'-DDE	PCB 101	PCB 151	PCB 187	BDE 153
delta-HCH	Octachlorostyrene	Endosulfan Sulfate	Endrin	o,p'-DDE	PCB 110	PCB 170	PCB 194	BDE 154

DDT = dichlorodiphenyltrichloroethane; DDD= dichlorodiphenyldichloroethane; DDE=dichlorodiphenyldichloroethylene; HCHs = hexachlorocyclohexanes; PCA = pentachloraniline. DCPA = dacthal; HCB =hexachlorobenzene.

The following abbreviations will be used throughout the text:

Total p,p'-DDT homologs = p,p'-DDT + p,p'-DDD + p,p'-DDE

Total PCBs = PCB 70 + ... + PCB 206 as listed in Table 1.

Total chlordanes = cis-chlordane + trans-nonachlor + trans-chlordane + cis-nonachlor + heptachlor epoxide\* + oxychlordane

Total BDEs = BDE 47 + BDE 99 + BDE 100 + BDE 153 + BDE 154

\* Heptachlor was not analyzed by itself.

## Statistics

Mean hormone levels, Vtg, GSI, hormone ratios, and contaminant levels in fish were compared between species and among areas using one-way analysis of variance (ANOVA, Neter and Wasserman 1974). Where significant differences were found, Bonferroni's test to compare group means was used (Sokal and Rohlf 1995). A 95% confidence level was used for all tests ( $\alpha=0.05$ ). Kruskal-Wallis nonparametric statistics was used when homogeneity of variance was not achieved (Sokal and Rohlf 1995) and the Kruskal-Wallis Multiple-Comparison Z-Value Test to determine if median concentrations between sampling sites were statistically different. All statistics were conducted using SAS (SAS 2001).

Because there were so many site-specific differences between hormone concentrations and environmental contaminants in fish tissue (see below), site-specific regressions were analyzed. Also, sexes were separated for regression purposes since many hormones were significantly different between the males and females. When site-specific regressions yielded no significant relationships, hormone/contaminant relationships were analyzed with data pooled between sites. Forward step-wise regressions were used with  $\alpha=0.05$ . R-squared and p-values are presented with the data.

## RESULTS AND DISCUSSION

### Endocrine Disrupting Compounds in Whole Body Tissue

Whole body carp from all sites and a few whole body razorback suckers from Mohave were analyzed for organochlorine contaminants, fungicides, and other endocrine-disrupting chemicals (Table 2). Total p,p'-homologs of DDT were greatest at Cibola-January (gmean = 55.4 µg/kg) and lowest at Havasu (gmean = 10.3 µg/kg). Dieldrin was not detected in any samples. Total PCBs were greatest at Mohave (gmean = 0.92 µg/kg) and lowest at Cibola-March (gmean = 0.52 µg/kg). Total chlordanes were greatest at Cibola-January (gmean = 1.02 µg/kg) and lowest at Cibola-March (gmean = 0.57 µg/kg), although no chlordanes were detected in Mohave razorback suckers. Total BDEs were greatest in razorback suckers from Mohave (gmean = 1.39 µg/kg) and lowest at Cibola-March (gmean = 0.59 µg/kg).

Table 2. Arithmetic mean, weighted geometric mean, minimum, maximum concentrations ( $\mu\text{g}/\text{kg}$  (ppb), ww) of organic contaminants in whole body carp from the Lower Colorado River, Arizona in 2002.

Station		Total p,p'- homologs of DDT	Dieldrin	Total PCBs	Total Chlordane	Total BDEs
Mohave (n=19)	Mean	79.8	ND	19.9	4.65	15.3
	Gmean	22.0	ND	0.92	0.65	1.19
	Min	ND <sup>1</sup>	ND	ND	ND	ND
	Max	164	ND	46.84	12.98	32.51
Havasu (n=20)	Mean	57.2	ND	12.6	4.51	10.8
	Gmean	10.3	ND	0.66	0.62	0.93
	Min	ND	ND	ND	ND	ND
	Max	100.6	ND	35.09	9.27	29.91
Cibola January (n=18)	Mean	383.3	ND	14.5	8.24	5.07
	Gmean	55.4	ND	0.71	1.02	0.69
	Min	ND	ND	8.51	ND	ND
	Max	626	ND	67.02	26.63	23.58
Cibola March (n=13)	Mean	172	ND	8.57	3.04	3.48
	Gmean	36.2	ND	0.52	0.51	0.59
	Min	ND	ND	ND	ND	ND
	Max	464	ND	12.03	3.55	8.79
Mohave Razorbacks (n=2)	Mean	62.1	ND	16.6	ND	17.6
	Gmean	15.7	ND	0.76	ND	1.39
	Min	ND	ND	10.38	ND	14.16
	Max	73.6	ND	22.91	ND	20.98

<sup>1</sup> ND=Non-detect; Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means.

Other endocrine disrupting chemicals including aldrin, fipronil, endrin, oxychlordane, heptachlor epoxide, toxaphene, mirex, total HCHs, and endosulfan were analyzed in whole body fish (Table 3). None of these contaminants were detected in Lower Colorado River carp, razorback suckers, or bonytail chub. Octachlorostyrene was detected in three samples from Mohave and Havasu, with the greatest concentration found in a male carp from Mohave at  $1.28 \mu\text{g}/\text{kg}$  (Table 4). The herbicides benfluralin, trifluralin, dacthal (=DCPA) were also measured (Table 4). Benfluralin was not detected in any samples, although trifluralin was detected at Havasu (range = ND-0.63  $\mu\text{g}/\text{kg}$ ), Cibola-January (range = ND-22.9  $\mu\text{g}/\text{kg}$ ), and Cibola-March (range = ND-17.7  $\mu\text{g}/\text{kg}$ ). Trifluralin was not detected in any Mohave samples. DCPA was detected in Mohave carp from ND-2.02  $\mu\text{g}/\text{kg}$ , in Havasu carp from ND-1.74  $\mu\text{g}/\text{kg}$ , in Cibola-January from ND-0.53  $\mu\text{g}/\text{kg}$ , and in Cibola-March carp from ND-2.68  $\mu\text{g}/\text{kg}$ . DCPA was also found from 0.43-3.0  $\mu\text{g}/\text{kg}$  in bonytail chub from Cibola in January (High Levee Pond).

The fungicide chlorothalonil was not detected in any samples, but the fungicide PCNB's primary metabolite, PCA, was detected at Havasu from ND-1.82 µg/kg. The fungicide HCB was detected in carp at Mohave from ND-2.05 µg/kg, Havasu from ND-1.68 µg/kg, Cibola-January from ND-2.7 µg/kg, and Cibola-March from ND-1.11 µg/kg. HCB was also found in razorback suckers at Mohave from 0.96-1.16 µg/kg and in a razorback sucker from Cibola at 0.79 µg/kg.

Table 3. Other endocrine disrupting chemicals analyzed for in whole body fish from the Lower Colorado River, Arizona in 2002 (µg/kg (ppb), ww).

	Sampling Sites	<i>n</i> <sup>1</sup>	Aldrin	Fipronil	Endrin	Endrin aldehyde	Endrin Ketone	Mirex	Total HCHs	Endosulfan	Chlorpyrifos
Carp	Mohave	19	ND <sup>2</sup>	ND	ND	ND	ND	ND	ND	ND	ND
	Havasu	20	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Cibola-Jan	18	ND	ND	ND	ND	ND	ND	ND	ND	ND-10.5
	Cibola-Mar	13	ND	ND	ND	ND	ND	ND	ND	ND	ND
Razorback Suckers	Mohave	2	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Cibola-Jan	1	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Cibola-Mar	1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bonytail Chub	Cibola-Jan	2	ND	ND	ND	ND	ND	ND	ND	ND	ND

<sup>1</sup>*n* = sample size; <sup>2</sup>ND = non-detect. The limit of detection for all of the chemicals was 1 µg/kg ww. A range is reported when one occurred (min-max). Endosulfan concentrations reported here were the same as endosulfan I and II. Oxychlorodane and heptachlor epoxide were not reported because they were not detected and because these chemicals were also a component of the total chlordanes calculation. Toxaphene, chlorothalonil, and triclosan degradates were not detected or reported.

Table 4. Continued, other endocrine disrupting chemicals analyzed for in whole body fish from the Lower Colorado River, Arizona in 2002 (µg/kg (ppb), ww).

	Sampling Sites	<i>n</i> <sup>1</sup>	Benfluralin	Trifluralin	DCPA	Octachlorostyrene	PCA	HCB
Carp	Mohave	19	ND <sup>2</sup>	ND	ND-2.02	ND-1.28	ND	ND-2.05
	Havasu	20	ND	ND-0.63e <sup>3</sup>	ND-1.74	ND-0.28e	ND-1.82	ND-1.68
	Cibola-Jan	18	ND	ND-22.9	ND-0.53e	ND	ND	ND-2.7
	Cibola-Mar	13	ND	ND-17.7	ND-2.68	ND	ND	ND-1.11
Razorback Suckers	Mohave	2	ND	ND	ND	ND	ND	0.96e-1.16
	Cibola-Jan	1	ND	ND	ND	ND	ND	ND
	Cibola-Mar	1	ND	ND	ND	ND	ND	0.79e
Bonytail Chub	Cibola-Jan	2	ND	ND	0.43-3.0	ND	ND	ND

<sup>1</sup>*n* = sample size; <sup>2</sup>ND = non-detect. <sup>3</sup>e = data flagged because concentration was estimated during analysis. The limit of detection for all of the chemicals was 1 µg/kg ww. A range is reported when one occurred (min-max).

Statistical analysis of total p,p'-DDT homologs, total PCBs, total chlordanes, and total BDEs between sites was also performed. The greatest p,p'-homolog concentration was at Cibola in January and the lowest was at Havasu ( $P < 0.0001$ ; Figure 5). Total PCB concentrations in carp

were greatest at Mohave and lowest Cibola in March ( $P=0.02$ ; Figure 6). Total chlordanes were greatest at Cibola in January ( $P=0.0007$ ; Figure 7). Total BDEs were greatest in Mohave carp and lowest in Cibola carp in March ( $P=0.0001$ ; Figure 8).

In summary, Mohave had the highest concentrations of total PCBs and total BDEs and Cibola in January had the highest concentrations of total p,p'-DDT homologs and total chlordanes. Total p,p'-DDT homologs ranged from 0.26-0.37 ppm ww (geometric means) in a nationwide survey of organochlorine concentrations in different fishes from 1976-1984 (National Contaminant Biomonitoring Program (NCBP); Schmitt et al. 1990). Geometric means of p,p'-DDE in carp from the Yuma Valley, Arizona ranged from 0.055 – 0.691 ppm ww in 1995 (Tadayon et al. 1997). The mean concentration of p,p'-DDT homologs in benthivores from the Colorado River Basin was 0.06 ppm ww and ranged from 0.002 ppm in the Yukon River system to 0.220 ppm in the Columbia River Basin (Hinck et al. 2006). Compared with other studies around the country, geometric means of total p,p'-DDT concentrations in this study (10.3-55.4 ppb ww) were very similar to the geometric means reported elsewhere in the United States.

The NCBP reported geometric means of total chlordanes that ranged from 0.12-0.2 ppm ww from 1976-1984 (Schmitt et al. 1990). Chlordane was only reported in a few carp from the Yuma Valley in 1995 and residue concentrations ranged from ND-0.01 ppm ww (Tadayon et al. 1997). The mean concentration of total chlordanes in benthivores in the Colorado River basin was 0.012 ppm ww and ranged from 0.003 ppm ww in benthivores from the Yukon River system to 0.054 ppm ww in the Mississippi River Basin (Hinck et al. 2006). Compared with other studies around the country, total chlordanes in carp from this study (0.57-1.02 ppb ww) were at least 5 times lower than reported elsewhere in the United States.

Geometric means of total PCBs ranged from 0.39-0.89 ppm ww in the NCBP study from 1976-1984 (Schmitt et al. 1990). In carp in the Yuma valley, total PCBs were only detected in three carp, from 0.05-0.07 ppm ww (Tadayon et al. 1997). Hinck et al. (2006) reported a geometric mean of total PCBs in benthivores from the Colorado River basin of 0.066 ppm ww; they also reported geometric means for total PCBs ranging from 0.037 ppm ww in the Yukon River system to 0.129 ppm ww in the Mississippi River Basin. Compared with the geometric means in carp reported in this study (0.52-0.92 ppb ww), total PCBs in fish from around the United States were at least 2 orders of magnitude greater than total PCBs reported in this study.

Concentrations of total BDEs in LCR fish were below all reported concentrations in freshwater fishes in a worldwide review paper (range = 26-4,600 ng/g lipid weight; de Wit 2002) and below carp in the United States (13-22 ng/g, ww; Loganathan et al. 1995). Mean concentrations of total BDEs in carp from the Des Plaines River and the Detroit River ranged from 3.96-6.38 ppb ww (Rice et al. 2002). Mean concentrations of total BDEs in trout (*Salvelinus namaycush*) from the Great Lakes ranged from 5.35 ppb ww in Lake Erie to 20.65 ppb in Lake Ontario (Luross et al. 2002). Mean concentrations of total BDEs in smelt (*Osmerus mordax*) from the Great Lakes ranged from 1.47 ppb ww in Lake Superior to 22.4 ppb ww in Lake Michigan (Chernyak et al. 2005). Compared with other studies around the country, total BDEs in carp from this study (mean = 3.48-15.3 ppb ww) were very similar to concentrations reported elsewhere in the United States. Although the science on the toxicity of BDEs continues to grow, in general, the acute toxicity of BDEs is low, while sublethal effects to endocrine systems are the greatest threat to

biota exposed to BDEs (Law et al. 2002). Hardy (2002) also reported bioaccumulation of BDEs 47, 99, and 100 in fish tissues without acute or chronic toxicity.

Across all contaminant categories, concentrations decreased at Cibola from January to March. It is unknown whether this was strictly a seasonal response but it is possible that it was in response to either increased agriculture return flows at Cibola in March, increased chemical use in Cibola in January, or increased flows on the LCR due to Bureau of Reclamation's water release schedule to meet increased demand in the summer.

Fish from Mohave had several general indices that were lower when compared to other sites:  $T_3$ , male and female GSIs, and sperm viability. Fish from Mohave also had the greatest amount of macrophage aggregates and other histopathologies as well as an increased percentage of interstitium in testes. Mohave was also the only site to have a significant relationship between female GSIs and DDT tissue concentrations. While there were no significant relationships between Mohave carp reproductive indices and PCBs or BDEs, studies have shown that increased human PCB concentrations resulted in lower sperm quality and quantity (Rozati et al. 2000). Others found that the stress response in fishes exposed to PCBs was potentiated by poor nutritional status (Quabius et al. 2000).

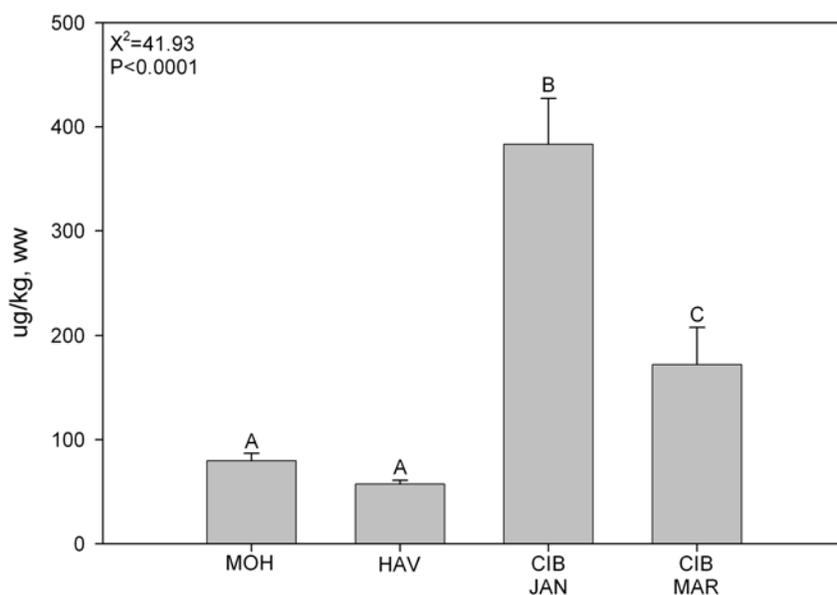


Figure 5. Total p,p'-homologs (DDT, DDD, and DDE) in carp ( $\mu\text{g}/\text{kg}$  (ppb), ww) from Lower Colorado River, Arizona sites in 2002. Stations are ordered from upstream to downstream and then by chronologically by sampling date. Data are presented as arithmetic means and standard errors. Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means. Different letters represent statistical differences between sampling locations as determined by a Kruskal-Wallis Test and a Bonferroni separation of means at  $\alpha=0.05$ .

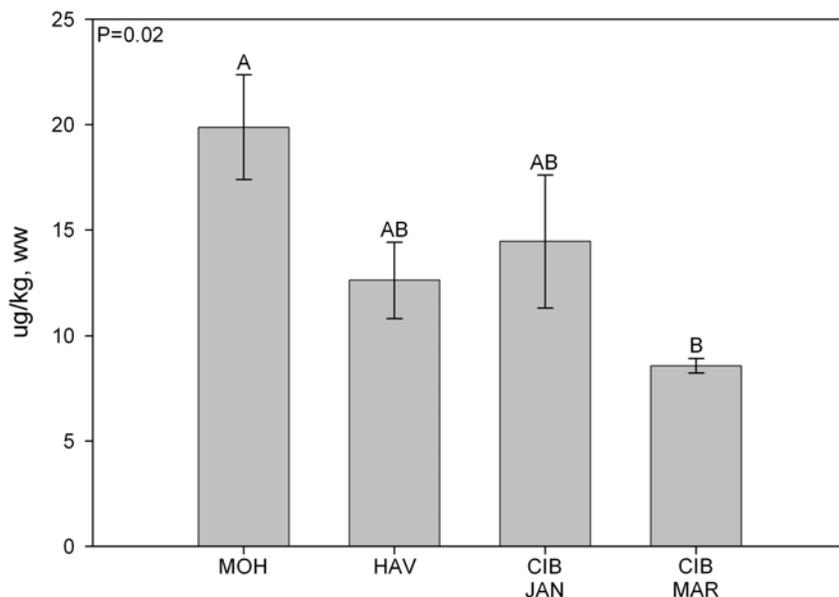


Figure 6. Total PCBs in carp ( $\mu\text{g}/\text{kg}$  (ppb), ww) along the Lower Colorado River, Arizona in 2002. Stations are ordered from upstream to downstream and then by chronologically by sampling date. Data are presented as arithmetic means and standard errors. Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ .

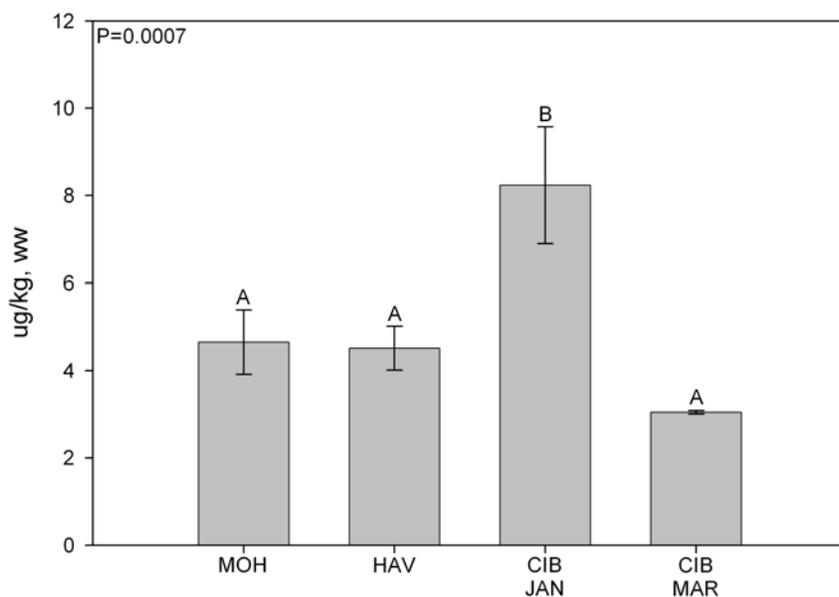


Figure 7. Total chlordanes in carp ( $\mu\text{g}/\text{kg}$  (ppb), ww) from the Lower Colorado River, Arizona in 2002. Stations are ordered from upstream to downstream and then by chronologically by sampling date. Data are presented as arithmetic means and standard errors. Censored values

were set to  $\frac{1}{2}$  the limit of detection for calculation of means. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ .

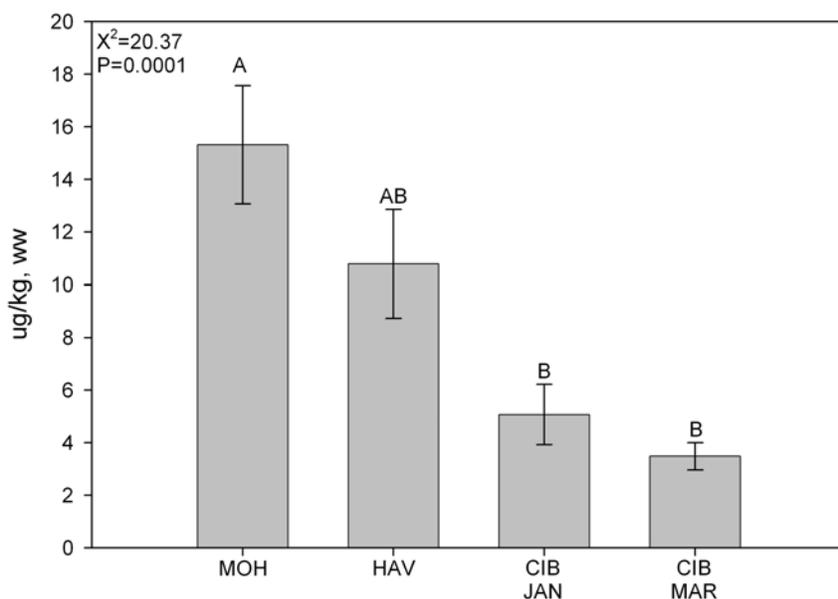


Figure 8. Total BDEs in carp ( $\mu\text{g}/\text{kg}$  (ppb), ww) from the Lower Colorado River, Arizona in 2002. Stations are ordered from upstream to downstream and then by chronologically by sampling date. Data are presented as arithmetic means and standard errors. Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means. Different letters represent statistical differences between sampling locations as determined by a Kruskal-Wallis Test and a Bonferroni separation of means at  $\alpha=0.05$ .

## Reproductive Hormones and Vitellogenin

### Razorback Sucker

#### *Estradiol*

17 $\beta$ -estradiol concentrations (estradiol; E2) were measured in razorback suckers in the LCR. Concentrations of E2 in male and female razorback suckers were significantly different between sexes ( $P<0.0001$ ). Therefore, analysis between sexes was not pooled. Concentrations of E2 in males were all lower than females (Figure 9). Mohave males had the lowest E2 concentrations ( $P=0.0051$ ) and Mohave females had the greatest E2 concentrations ( $P<0.0001$ ). Razorback suckers at Cibola-January had greater E2 concentrations (males) and lower E2 concentrations (females) than at Cibola-March. This could be due to colder water temperatures in January at the outset of razorback suckers reproductive cycle, limnological differences between sampling locations, or nutritional differences between sampling locations.

Median concentrations of E2 in male razorback suckers in the LCR were  $\leq 500$  pg/mL, which are similar to those reported in razorback suckers in Lake Mead (200-375 pg/mL) at Echo Bay (reference) and Las Vegas Bay (Tuttle and Orsak 2002). Therefore, male razorback suckers in the LCR, regardless of sampling site, have similar E2 concentrations.

Median E2 concentrations in female razorback suckers in the LCR ranged from 602-1,720 (total range = 121-2,343) pg/mL. Other studies in Lake Mead reported median E2 concentrations in female razorback suckers from 1,250 pg/mL (744-1,777 pg/mL) (Tuttle and Orsak 2002).

Median E2 concentrations in female razorback suckers at Mohave were elevated compared to other LCR data, since the median E2 concentration in female razorback suckers from Mohave was 1,720 pg/mL and the median E2 concentration in female carp in the reference site at Lake Mead was 1,250 pg/mL (Tuttle and Orsak 2002).

### *11-Ketotestosterone*

The male hormone, 11-ketotestosterone (11-KT), was measured in razorback suckers on the LCR. 11-KT ratios in male and female razorback suckers were significantly different between sexes ( $P < 0.0001$ ). Therefore, analysis between sexes was not pooled. Males had greater 11-KT concentrations than females, as expected (Figure 9). Concentrations of 11-KT in Mohave males were statistically greater than the Cibola sampling sites ( $P < 0.0001$ ). Site characteristics (lake vs. pond), nutritional differences between sites, water dynamics between sites, and temperature differences between sites could explain the differences in male 11-KT concentrations between sampling sites. Females had similar 11-KT concentrations at Mohave and Cibola when sampled in March. The January sampling at Cibola resulted in lower 11-KT concentrations in females ( $P = 0.0008$ ), but this could be attributed to lower, wintertime water temperatures.

Median concentrations of 11-KT in razorback sucker males ranged from 668-1,377 pg/mL in the LCR (Mohave, Cibola-January, Cibola-March). Tuttle and Orsak (2002) found that the median concentration of 11-KT in reference males was 1,000 pg/mL (411-3,587 pg/mL) for razorback suckers although they collected their samples post-spawn, whereas our samples were collected before or during spawning. Concentrations of 11-KT in male razorback suckers from the LCR were within a similar range reported previously for razorback suckers.

Median concentrations of 11-KT in female razorback suckers ranged from 182 pg/mL at Cibola in January to 448 pg/mL at Mohave in March. Female razorback suckers from Lake Mead had a median concentration of 11-KT around 400 pg/mL (299-524 pg/mL) at Echo Bay (Tuttle and Orsak 2002). Overall, concentrations of 11-KT in females in this study were most similar to reference sites from a Lake Mead study.

### *E/T Ratios*

The ratio of estradiol:11-ketotestosterone (E/T ratio) was analyzed as an indicator of endocrine disruption. In typical males and female fishes, E/T should be less than one and greater than one, respectively.

E/T ratios in male and female razorback suckers were significantly different between sexes ( $P < 0.0001$ ). Therefore, analysis between sexes was not pooled. All E/T ratios in male razorback

suckers were below one and all E/T ratios in female razorback sucker were above one. There was a significant difference among sites in male razorback suckers (Figure 9). Male razorback suckers at Mohave had a significantly lower mean E/T ratio than both sampling events at Cibola ( $P < 0.0001$ ), but since there were no E/T ratios that exceeded one, this could be attributed to site differences (lake vs. pond), nutritional quality of foods between sites, water dynamics between sites, and temperature differences between sites. All male razorback suckers at a reference site in Lake Mead had E/T ratios below one (0.09-0.71) (Tuttle and Orsak 2002). Based on this biomarker alone and given sampling time differences between studies, no evidence of the occurrence of endocrine disruption in male razorback suckers at the LCR sites in 2002 was found.

Median E/T ratios in female razorback suckers on the LCR were similar ( $P = 0.145$ ) and they ranged from 3.16 at Cibola-March to 3.89 at Mohave. Female razorback suckers in Lake Mead had a median E/T ratio slightly greater than 3.0 (1.8-5.9) (Tuttle and Orsak 2002). Given that the medians and ranges of female E/T ratios in the LCR were similar to those reported in Tuttle and Orsak (2002) and all the female ratios were greater than one, although sampling times differed between studies, there was no evidence of endocrine disruption in female razorback suckers in this study.

## Carp

### *Estradiol*

Concentrations of E2 in male and female carp were significantly different between sexes ( $P < 0.0001$ ). Therefore, analysis between sexes was not pooled.

E2 concentrations in male carp were not significantly different between sites on the LCR (Figure 10). Median concentrations ranged from 210-349 pg/mL. Median concentrations of E2 in male carp from Lake Mead were between 200-600 pg/mL (excluding Las Vegas Wash, where concentrations were skewed) (Bevans et al. 1996). Male carp collected from several western sampling sites had a mean E2 concentration of  $598 \pm 77$  SE pg/mL (Goodbred et al. 1997). Hinck et al. (2007) found that male carp from the upper portion of Lake Mead had the lowest mean E2 concentrations compared to the other carp collected in the Colorado River basin ( $29 \pm 4$  pg/mL). Studies outside of the LCR reported mean E2 concentrations in male carp from  $175.7 \pm 46.14$  to  $1,208 \pm 255.8$  pg/mL in the Mississippi River Basin (Schmitt 2002) and  $632 \pm 212$  pg/mL in male longnose suckers (*Catostomus catostomus*) in the Yukon River Basin (Hinck et al. 2004b). Although all of the studies used for comparison sampled after spawning occurred, which differs from our study, where fish were collected prior to or during spawning, no evidence of endocrine disruption was found in E2 in male carp from the LCR.

E2 concentrations in female carp were significantly different between sites on the LCR (Figure 10). Mohave and Havasu female carp had the greatest E2 concentrations ( $P < 0.0001$ ). Median E2 concentrations were 1,627, 1,431, and 813 pg/mL for Mohave, Havasu, and Cibola-March, respectively. Bevans et al. (1996) found that median E2 concentrations in female carp were 900 pg/mL at Callville Bay, 800 pg/mL at Las Vegas Bay, and 1,250 pg/mL at Las Vegas Wash in Lake Mead. Goodbred et al. (1997) found that the mean E2 concentration in female carp for the

west was  $1,767 \pm 158$  SE pg/mL. Hinck et al. (2006) reported E2 concentrations in female carp ranging from 657-961 pg/mL at Willow Beach, Lake Mohave, AZ to Imperial Dam, AZ. Since Havasu and Mohave are directly downstream of Lake Mead, continued monitoring of E2 concentrations in females should occur. Studies outside of the LCR reported mean E2 concentrations in female carp from  $357.4 \pm 45.64$  to  $2,409 \pm 264.3$  pg/mL in the Mississippi River Basin (Schmitt 2002) and  $1,402 \pm 106$  pg/mL in female longnose suckers in the Yukon River Basin (Hinck et al. 2004b). Therefore, E2 concentrations in female carp in the LCR were higher than other values reported for the Colorado River, but not higher than E2 concentrations from the Mississippi River Basin, although the fish from these comparison studies were collected post-spawn.

Hormone concentrations may be influenced by the reproductive stage of fish and environmental contaminant concentrations. The reproductive stage of carp from the LCR was measured; these findings are discussed with the results of the histology analysis. Whole body carp were analyzed for environmental contaminant concentrations such as total PCBs, total chlordanes, total BDEs, and total homologs of DDT. One significant site-specific regression in female carp was detected for E2. Female carp at Cibola-January had E2 concentrations with significant negative relationships with total BDEs and total PCB concentrations in tissue ( $R^2 = 0.96$ ,  $P = 0.0376$ ). For females at Cibola-January, 96% of the variation in E2 was explained by total BDE and total PCB concentrations in tissue. As concentrations of BDEs and PCBs increased, E2 concentrations in female carp at Cibola-January decreased. Yano and Matsuyama (1986) found that carp exposed to PCBs metabolized E2 and testosterone faster, reducing circulating levels of E2 and testosterone in the blood. While female carp at Cibola-January had the lowest E2 concentration between sites, it is unlikely that it was due to the relative fecundity of females at Cibola-January because Cibola-January relative fecundity was statistically similar to the relative fecundity of females from the other sampling sites and times (Figure 23). Although it is possible that total PCBs or BDEs affected E2 concentrations in female carp at Cibola-January, other evidence (E2 concentrations in females increased at Cibola in March and E2 concentrations in males were similar between sampling dates) suggests contaminants did not contribute to differing E2 concentrations.

The only significant relationship between E2 concentrations and tissue BDEs was negative for Cibola-January females ( $R^2=0.90$ ,  $P=0.01$ ). The strong associations between E2 concentrations and environmental contaminants in tissue show that adverse effects may be occurring in female carp on the LCR. While the evidence that BDE negatively affected E2 concentrations in female carp at Cibola in January was not strong (it did not continue to occur at Cibola in March, BDEs have been found to be estrogenic in in-vitro human cell lines (Meerts et al. 2001).

### *11-Ketotestosterone*

11-KT concentrations in male and female carp were significantly different between sexes ( $P<0.0001$ ). Therefore, analysis between sexes was not pooled.

Concentrations of 11-KT in male carp were significantly different between sites ( $P<0.0001$ ) (Figure 10). Mohave and Havasu males had the greatest median concentrations, 1,424 and 1,453 pg/mL, respectively. Cibola-March males had the next greatest median concentration of 11-KT

at 773 pg/mL. Cibola-January males had a median concentration of 11-KT at 497 pg/mL, but it was probably much lower due to lower water temperatures in January. Male carp at Callville Bay and Las Vegas Bay had median concentrations of 11-KT of 1,850 pg/mL and 500 pg/mL, respectively (Bevans et al. 1996). The mean concentration of 11-KT in male carp from western sampling sites was  $1,561 \pm 158$  SE pg/mL (Goodbred et al. 1997). Hinck et al. (2007) reported lower mean 11-KT concentrations ( $121 \pm 4$  pg/mL) in male carp from the upper portion of Lake Mead compared to other male fish in the study. Studies outside of the LCR reported mean 11-KT concentrations in male carp from  $215.1 \pm 17.4$  to  $3,663 \pm 1,023$  pg/mL in the Mississippi River Basin (Schmitt 2002) and  $755 \pm 148$  pg/mL in male longnose suckers in the Yukon River Basin (Hinck et al. 2004b). However, all of the studies used for comparison sampled after spawning occurred, which differs from our study, where fish were collected prior to or during spawning. Although the 11-KT concentrations at Mohave and Havasu seem high, they were lower than those reported in the Mississippi River Basin.

Concentrations of 11-KT in female carp were not significantly different between sites (Figure 10). Median concentrations of 11-KT in female carp ranged from 234-407 pg/mL from all sites. At Callville Bay and Las Vegas Wash, median concentrations of 11-KT in female carp were 650 pg/mL and 500 pg/mL (Bevans et al. 1996). The mean concentration of 11-KT in female carp from the west was  $1,359 \pm 158$  SE pg/mL (Goodbred et al. 1997). Hinck et al. (2006) reported 11-KT concentrations in female carp ranging from 244-369 pg/mL at Willow Beach, AZ to Imperial Dam, AZ. Studies outside of the LCR reported mean 11-KT concentrations in female carp from  $109.2 \pm 16.42$  to  $987.1 \pm 62.81$  pg/mL in the Mississippi River Basin (Schmitt 2002) and  $361 \pm 31$  pg/mL in female longnose suckers in the Yukon River Basin (Hinck et al. 2004b). 11-KT concentrations in female carp from the LCR were similar to concentrations in female carp reported in other studies around the United States.

Regressions of 11-KT in male and female carp with whole body contaminant concentrations resulted in only one site-specific significant relationship. At Cibola-January, 11-KT in males was negatively associated with total p,p'-DDT homologs in fish tissue ( $R^2=0.56$ ,  $P=0.0032$ ). Therefore, 56% of the variation in 11-KT concentrations in males at Cibola-January can be explained by total DDT homolog concentrations in carp tissue. As DDT concentrations increased in male carp, 11-KT concentrations decreased. A similar result was found when sites were pooled. Concentrations of 11-KT in males were significantly associated with all of the environmental contaminants that were analyzed ( $R^2=0.76$ ,  $P<0.0001$ ). Concentrations of 11-KT in male carp had positive relationships with BDEs and PCBs and negative relationships with DDT and chlordanes. Seventy-six percent of the variation in 11-KT pooled between sites was explained by environmental contaminant concentrations in male carp. Exposure to complex mixtures of chemicals in the LCR could be causing adverse effects to male carp. Scientists have found that p,p DDE is anti-androgenic (Kurihara 2000; Kelce et al. 1995). However, our findings do not agree with the relationship between PCBs and testosterone reported by Yano and Matsuyama (1986) since the relationship between 11-KT in males at all sites and PCBs was positive, but relationships between hormones and environmental contaminants highlight how environmental contaminants may adversely affect hormone concentrations.

### *E/T Ratios*

E/T ratios in male and female carp were significantly different between sexes ( $P < 0.0001$ ). Therefore, analysis between sexes was not pooled.

E/T ratios in male carp were significantly different between sites ( $P = 0.0002$ ), but they were all below one (Figure 10). Median E/T ratios in male carp ranged from 0.16-0.61 at all LCR sampling sites. Tuttle and Orsak (2002) reported that median E/T ratios in male carp in Lake Mead, Willow Beach, and Lake Mohave were below one. Goodbred et al. (1997) found that the mean E/T ratio in male carp was  $0.5 \pm 0.0$  SE. Hinck et al. (2006) reported E/T ratios in male carp ranging from 0.3-0.5 at Willow Beach, AZ to Imperial Dam, AZ. Studies outside of the LCR reported mean E/T ratios in male carp from approximately 0.2 to  $2.78 \pm 0.26$  in the Mississippi River Basin (Schmitt 2002) and  $1.51 \pm 0.56$  in male longnose suckers in the Yukon River Basin (Hinck et al. 2004b). Although the studies used for comparison sampled after spawning occurred, E/T ratios in male carp in this study were all comparable to previous studies conducted around the country and do not indicate that endocrine disruption is occurring in male carp in the LCR.

E/T ratios in female carp were significantly different between sites ( $P = 0.0016$ ), although none of the ratios were below one. Mohave and Havasu had the greatest median E/T ratios, at 4.07 and 3.72, respectively. Tuttle and Orsak (2002) reported median E/T ratios in females from 2 (1.3-2.5) at Willow Beach to 4 (1.3-16) at Lake Mead. Goodbred et al. (1997) reported a mean E/T ratio in western female carp of  $2.0 \pm 0.2$  SE. Hinck et al. (2006) reported E/T ratios in female carp ranging from 3.0-3.3 at Willow Beach, AZ to Imperial Dam, AZ. Studies outside of the LCR reported mean E/T ratios in female carp from approximately 1.5 to 12.9 in the Mississippi River Basin (Schmitt 2002) and  $5.27 \pm 0.77$  in female longnose suckers in the Yukon River Basin (Hinck et al. 2004b). E/T ratios for female carp in the LCR were very similar to those reported in other studies. Therefore, there was no evidence of endocrine disruption because E/T ratios in female carp in the LCR were above one.

Site-specific regression of E/T ratios and environmental contaminants in razorback suckers did not result in any significant associations. However, a multiple regression of E/T ratios and environmental contaminants in male carp resulted in a significant association between E/T ratios and total p,p'-DDT homologs, total chlordanes, and total BDEs ( $R^2 = 0.51$ ,  $P < 0.0001$ ). Therefore, 51% of the variation in E/T ratios in male carp on the LCR was explained by DDT, chlordanes, and BDE concentrations. The relationships with male E/T ratios in this multiple regression were positive for DDT and chlordanes, but negative for BDEs. A significant relationship between E/T ratios and environmental contaminants (DDT, BDEs, and PCBs) was also detected in female carp ( $R^2 = 0.46$ ,  $P = 0.0002$ ). Therefore, 46% of the variation in female carp E/T ratios was explained by DDT, BDE, and PCB concentrations in fish tissue. The relationships with female E/T ratios in this multiple regression were positive for BDEs, but negative for DDT and PCBs. Although there was no consistency between the types of relationships between the sexes, the strong relationships found in these regressions indicate that exposure to DDT, chlordanes, PCBs, and BDEs in the LCR could affect carp endocrine systems. PCBs have been found to be anti-estrogenic in in-vitro carp hepatocyte studies (Letcher et al. 2002).

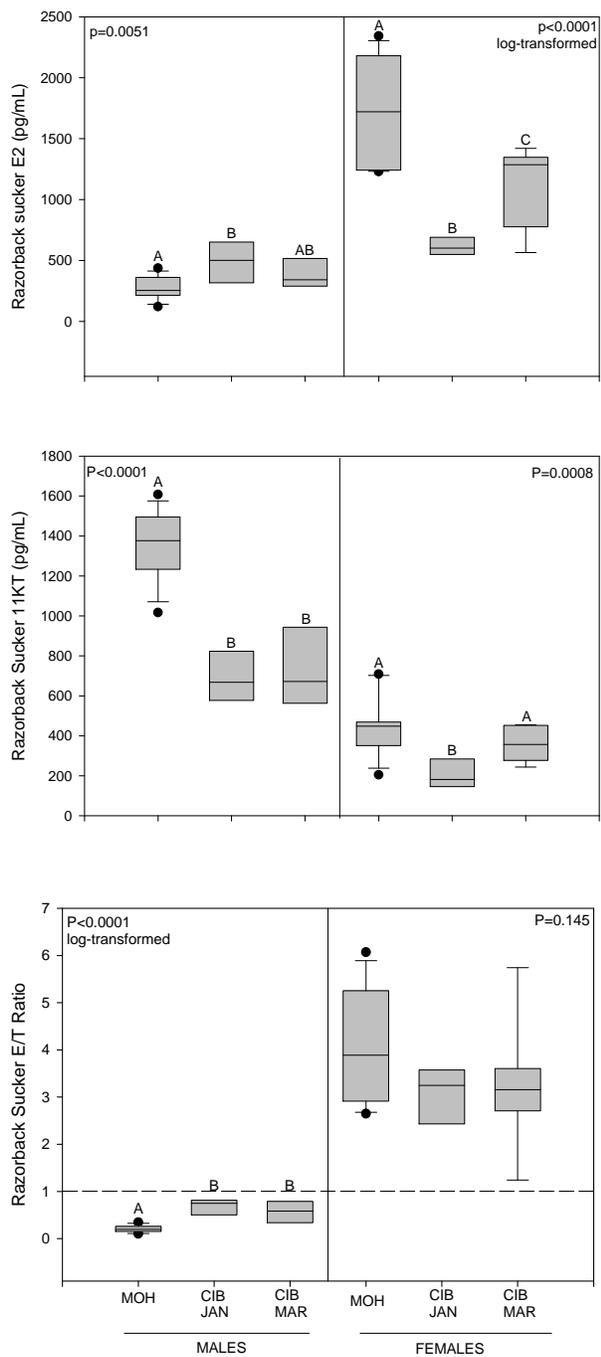


Figure 9. Concentrations (pg/mL or ppt) of E2, 11-KT, and E/T ratios (E2/11-KT) in razorback suckers from Mohave, Cibola-January, and Cibola-March sampling sites from the Lower Colorado River, Arizona in 2002. Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ .

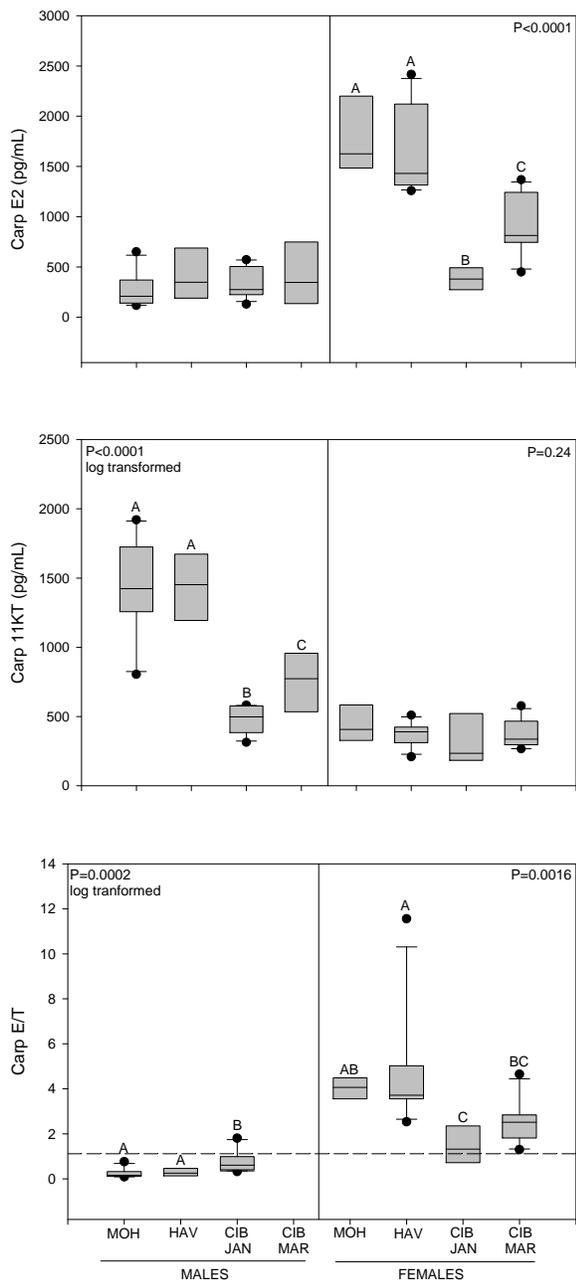


Figure 10. Concentrations (pg/mL or ppt) of E2, 11-KT, and E/T ratios (E2/11-KT) in carp from Mohave, Havasu, Cibola-January, and Cibola-March sampling sites from the Lower Colorado River, Arizona, 2002. Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Since only two carp were collected at Cibola in March, a box plot could not be drawn for this sampling location and date. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ .

### *Vitellogenin*

Vitellogenin is a biomarker indicative of endocrine disruption because under normal circumstances, it is not present in males. Vtg was analyzed in carp. Females had significantly greater concentrations of Vtg ( $P < 0.00001$ ) than males (Figure 11). The arithmetic mean concentration of Vtg among all sites on the Lower Colorado River was 4.67 mg/mL. Differences in Vtg between males and females were also analyzed between sites. There was no statistical difference between all sites with the sexes combined ( $P = 0.16$ ) or between sites in males only ( $P = 0.21$ ), but there was a statistical difference between females between sites ( $P < 0.0001$ ) (Figure 12).

Males had median concentrations of Vtg from 0.18 mg/mL (Mohave) to 0.38 mg/mL (Cibola-January). Ten males (91%) from Mohave had a Vtg concentration greater than 0.1 mg/mL which may indicate a potential estrogenic response in these fish (Hinck et al. 2006). Eight males from Havasu (100%) and 10 males from Cibola-January (77%) had a Vtg concentration greater than 0.1 mg/mL. Only two male carp were collected at Cibola-March, so no statistics were conducted with these data. The male Vtg response was lower in Cibola-March (0.087-0.092 mg/mL) compared to Cibola-January (median  $\pm$  SE =  $0.38 \pm 0.03$  mg/mL), indicating that water temperatures influenced Vtg production in males. No Vtg was detected in males at a reference site for a Lake Mead endocrine disruption study, but the median male Vtg from Las Vegas Bay was 4 mg/mL (Bevans et al. 1996). Another study found no Vtg in males from Lake Mead and very low concentrations in males from Willow Beach and Lake Mohave, although they do not state specifically what these concentrations were (Tuttle and Orsak 2002). The mean male Vtg concentration was  $0.0 \pm 0.1$  mg/mL in the western portion of a nationwide endocrine disruption reconnaissance study (Goodbred et al. 1997). Studies outside of the LCR reported mean Vtg concentrations in male carp at  $0.002 \pm 0.001$  mg/mL in the Columbia River Basin (Hinck et al. 2004a), 0.129 mg/mL in the Rio Grande Basin (Schmitt et al. 2004), and  $0.018 \pm 0.004$  mg/mL in male longnose suckers in the Yukon River Basin (Hinck et al. 2004b). However, all of the comparison studies sampled after spawning occurred, which differs from our study, where fish were collected prior to or during spawning.

The median female Vtg concentration on the LCR ranged from 3.02 mg/mL at Cibola-March to 13.71 mg/mL at Havasu. There were statistical differences between Mohave/Havasu and Cibola ( $P < 0.0001$ ). Median female Vtg concentrations were also slightly higher than those reported in Tuttle and Orsak (2002;  $< 1-3$  mg/mL). When compared to other data from Lake Mead, Vtg concentrations at our sampling sites in this study were more similar to those from the reference site (4 mg/mL) than one of the contaminated sites (54 mg/mL) (Bevans et al. 1996). The mean female Vtg concentration was  $27.9 \pm 1.5$  mg/mL for western sites in a national reconnaissance study (Goodbred et al. 1997). Hinck et al. (2006) reported vtg in female carp ranging from 3.46-5.95 at Willow Beach, AZ to Imperial Dam, AZ. Studies outside of the LCR reported mean Vtg concentrations in female carp at  $1.57 \pm 0.15$  mg/mL in the Columbia River Basin (Hinck et al. 2004a), 1.66 mg/mL in the Rio Grande Basin (Schmitt et al. 2004), from  $0.1 \pm 0.036$  to  $6.30 \pm 1.25$  mg/mL in the Mississippi River Basin (Schmitt 2002), and  $3.86 \pm 0.61$  mg/mL in female longnose suckers in the Yukon River Basin (Hinck et al. 2004b).

Overall, it appears Vtg concentrations from LCR sites were elevated in males and slightly elevated in females when compared with other Colorado River data and other Biomonitoring Environmental Status and Trends (BEST) studies around the country. Given that endocrine disruption has been documented in Las Vegas Wash and Bay, the elevated Vtg concentrations in LCR males should be monitored closely.

Since none of the site specific multiple regressions of Vtg and environmental contaminants in fish tissue were significant, sites were pooled. The only significant relationship found in female carp was Vtg and DDT, BDE, and chlordanes ( $R^2=0.53$ ,  $P<0.0001$ ). Therefore, 53% of the variation in female carp Vtg was explained by tissue concentrations of DDT, BDE and chlordanes. The relationship between Vtg and BDEs (and chlordanes) was positive, while it was negative for Vtg versus DDT. Again, there has been no clear pattern of the effect of contaminant concentrations on hormones or Vtg in blood in female carp, but the strong relationship found in this regression indicates possible adverse effects on the endocrine system in the LCR due to accumulation of environmental contaminants.

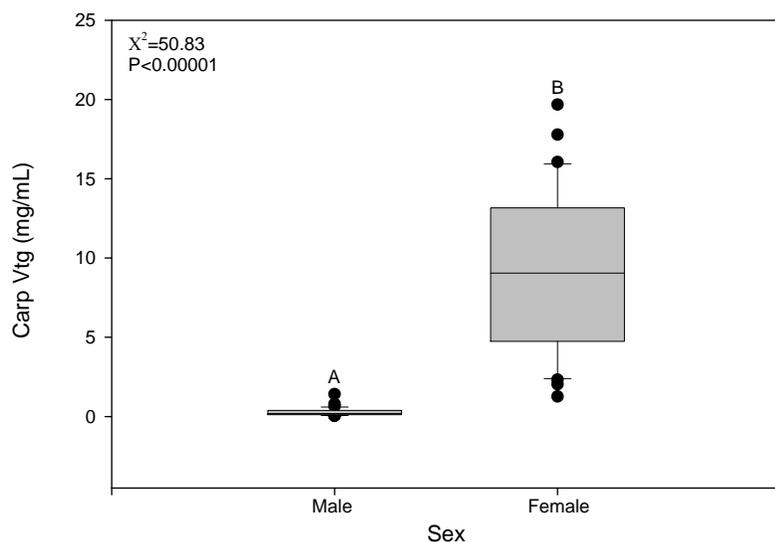


Figure 11. Vitellogenin concentrations (mg/mL or ppt) in male and female carp from the Lower Colorado River, Arizona in 2002. Different letters represent statistical differences between sampling locations as determined by a Kruskal-Wallis Test at  $\alpha=0.05$ . Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers).

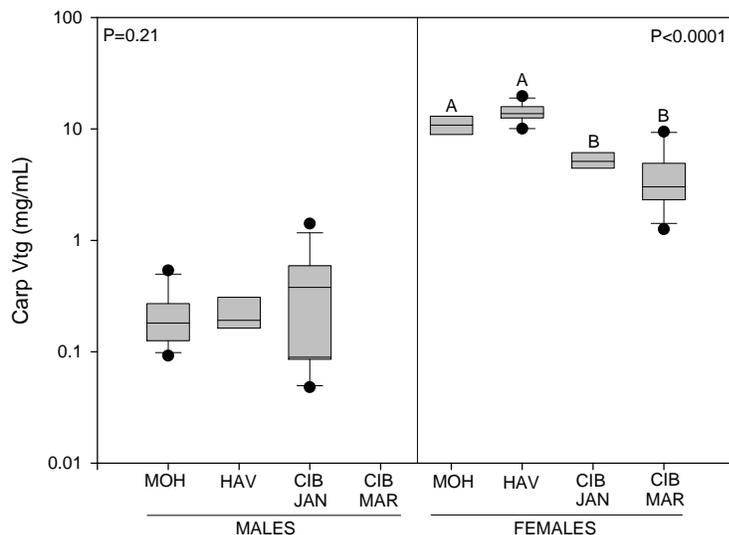


Figure 12. Vitellogenin concentrations (mg/mL or ppt) in male and female carp between the Mohave, Havasu, Cibola-January, and Cibola-March sampling sites from the Lower Colorado River, Arizona in 2002. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Since only two carp were collected at Cibola in March, a box plot could not be drawn for this sampling location and date.

### Thyroid Hormones

Thyroid hormones  $T_3$  and  $T_4$  were analyzed in carp at Lake Mohave, Havasu, and Cibola. To our knowledge, this is the first analysis of thyroid hormones in fishes in the Lower Colorado River. There were no significant differences in  $T_3$  and  $T_4$  concentrations between sexes (for  $T_3$   $P=0.30$ ;  $T_4$   $P=0.12$ ); therefore, sexes were pooled to analyze for differences between sites. Mean concentrations of  $T_3$  ranged from 1.76 ng/mL at Mohave and 3.67 ng/mL at Cibola in January (next greatest was Cibola in March at 2.86 ng/mL). Concentrations of  $T_3$  did vary significantly among sites ( $P<0.0001$ ), with the greatest concentrations at Cibola in January and the lowest concentrations at Mohave in March (Figure 13). This suggests that water temperature plays a role in regulating  $T_3$  concentrations. Stryjek-Kaminska et al. (1988) found that seasonal variations concentrations of  $T_3$  in serum from carp could be regulated by water temperature, food intake, or daylight.  $T_3$  concentrations in this study were at least 750-times lower than those reported by Stryjek-Kaminska et al. (1988) in carp in outdoor ponds in Germany. Since there are so few literature references for  $T_3$  concentrations in carp in the environment, this is discrepancy between carp  $T_3$  concentrations could be due to longitudinal differences between sampling sites or due to the difference in  $T_3$  analysis in plasma versus serum.  $T_3$  concentrations were slightly

lower than  $T_3$  concentrations in Lake Mead fishes from 2006 (4-8 ng/mL; Dr. Reynaldo Patiño, pers. comm., USGS Texas Cooperative Fish and Wildlife Research Unit, 4/19/07). Mean concentrations of  $T_4$  in our study ranged from 24.11 ng/mL at Mohave to 27.79 ng/mL at Havasu. The variances of concentrations of  $T_4$  were also significantly different among sites ( $P=0.034$ ), but the means were not significantly different after a Bonferroni multiple comparison test at  $\alpha=0.05$  (Figure 14). Mukhi et al. (2005) exposed zebrafish to perchlorate and measured  $T_4$  concentrations during and after exposure. The  $T_4$  concentrations were 1.37 ng/g (=1.37 ng/mL using the specific gravity of water ( $1 \text{ g/cm}^3 = 1 \text{ g/mL}$ )) after 12 weeks of exposure and 1.73 ng/g (=1.73 ng/mL) after 12 weeks of recovery.  $T_4$  concentrations in this study are 13.9-times greater than the post-recovery concentrations in zebrafish.  $T_4$  concentrations were within the same range as fishes from Lake Mead in 2006 (5-30 ng/mL; Dr. Reynaldo Patiño, pers. comm., USGS Texas Cooperative Fish and Wildlife Research Unit, 4/19/07).

The only significant site specific regression for thyroid hormones found in this study was between  $T_4$  and BDEs and chlordanes at Cibola-March ( $T_4$  and BDEs/chlordanes;  $R^2=0.48$ ,  $P=0.0367$ ). This was a positive relationship for both BDEs and chlordanes, where 48% of the variation in female carp  $T_4$  concentrations was explained by tissue BDE concentrations. When all sites were pooled, no relationships between  $T_4$  concentrations and environmental contaminants were found, but the multiple regression between  $T_3$  and DDT, PCBs, and chlordanes was significant ( $R^2=0.28$ ,  $P<0.0001$ ). The relationship between  $T_3$  and DDT (and chlordanes) was positive, but it was negative for  $T_3$  and PCBs. A negative relationship between  $T_4$  and BDEs was reported in rats exposed to BDEs in utero (Zhou et al. 2002). Although the exact effects of DDT, chlordanes, and PCBs on thyroid hormones are unknown, environmental contaminants in carp tissue could have adversely affected thyroid hormone concentrations in carp in the LCR.

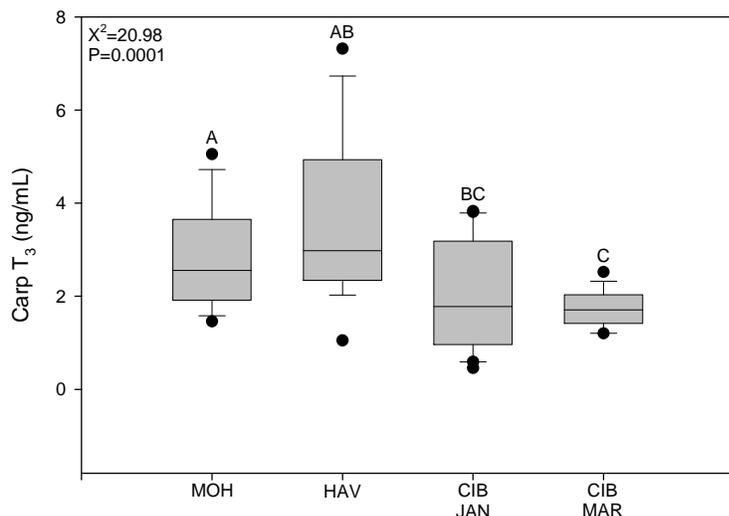


Figure 13. Thyronine ( $T_3$ ) concentrations in carp (ng/mL or ppb) between the Mohave, Havasu, Cibola-January, and Cibola-March sampling sites on the Lower Colorado River, Arizona in 2002. Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Different letters represent statistical differences between sampling locations as determined by a Kruskal-Wallis Test and a Bonferroni separation of means at  $\alpha=0.05$ .

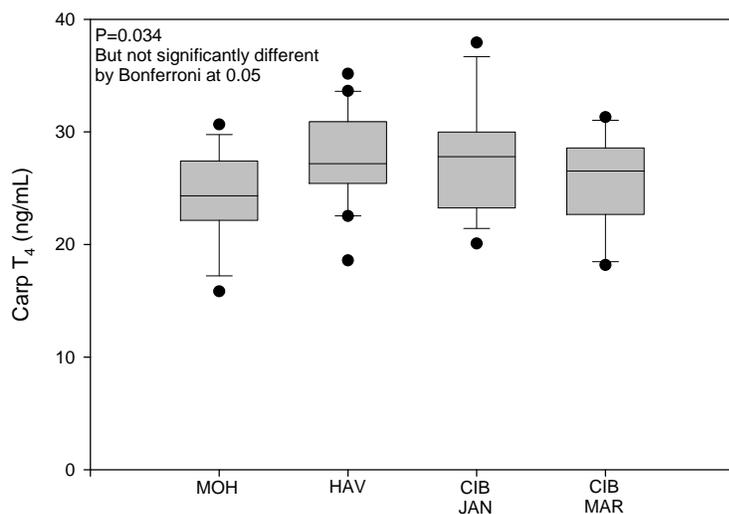


Figure 14. Thyroxine ( $T_4$ ) concentrations in carp (ng/mL or ppb) among sites on the Lower Colorado River, Arizona in 2002. Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date.

## Gonadal Somatic Indices, Histology in Males, and Sperm Viability in Males and Fecundity Analysis in Females

### Gonadal Somatic Indices

There were no significant differences in carp lengths between males and females ( $P=0.06$ ), so data between sexes was pooled for analysis among sites. There was no significant difference in carp length between sites although Mohave had the smallest carp (Figure 15). Only two males were collected from Cibola-March, so statistical comparison was not possible. Male and female carp weights were significantly different ( $P=0.0066$ ), so data between the sexes were not pooled. There were no significant differences in carp weights between sites in males or females (Figure 16). Again, Mohave carp weighed the least compared to the other sites, but this difference was not significant.

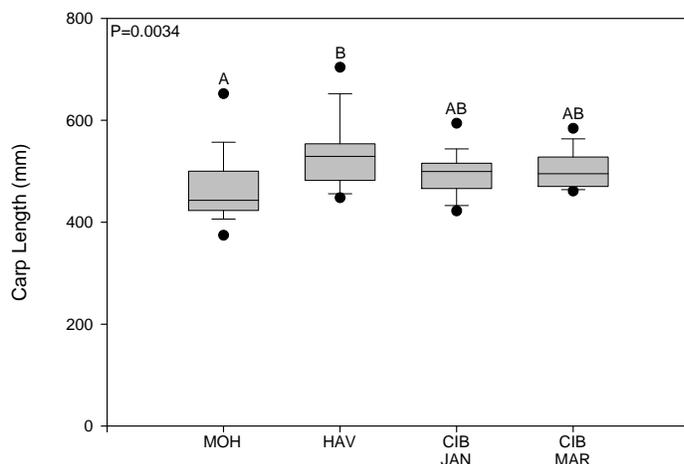


Figure 15. Carp lengths between sites on the Lower Colorado River, Arizona 2002. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Since only two carp were collected at Cibola in March, a box plot could not be drawn for this sampling location and date.

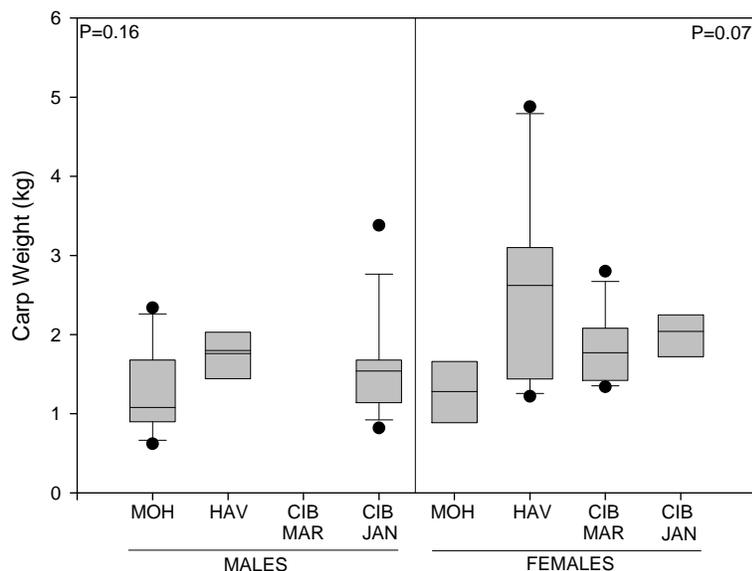


Figure 16. Carp weights between sites on the Lower Colorado River, Arizona 2002. Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Since only two males were collected at Cibola in March, a box plot could not be drawn for this sampling location and date.

GSI measures structural changes related to reproductive status, seasonal dynamics, or environmental stressors (Schmitt and Dethloff 2000). Gonadal somatic indices are calculated as the percentage of gonadal weight per total body weight. Although follicular development was examined in female carp, gonadal stage was not measured. There were significant differences among sites in GSIs in male and female carp (Figures 17 and 18). Male GSIs were lowest at Mohave (median GSI = 5%) and highest at Havasu (median GSI = 7%) ( $P=0.0007$ ). There were not enough male samples from Cibola-March for statistical analysis. Tuttle and Orsak (2002) reported male GSIs from 3.5% at Willow Beach to 6% at Lake Mohave. Male carp from a year-long study in Overton Arm (reference site) and Las Vegas Bay (contaminated site), Lake Mead had mean GSIs from 5-8% at Overton Arm to 6-19% at Las Vegas Bay (Patiño et al. 2003). Male GSIs from the LCR were similar to those found at other non-contaminated sites on the Colorado River.

Female GSIs were lowest at Mohave (median GSI = 11%) and highest at Havasu and Cibola-March (median GSIs = 21%) ( $P=0.0017$ ). Tuttle and Orsak (2002) reported female GSIs from 10% at Lake Mohave to 16% in Lake Mead. Female carp from Lake Mead had mean GSIs from 5-18% at Overton Arm to 6-18% at Las Vegas Bay (Patiño et al. 2003). Female GSIs from the LCR were similar to those found at other non-contaminated sites on the Colorado River.

Even though the Mohave carp did not have significantly smaller carp when compared to the other sites, their smaller sizes could have affected the GSI results. Smaller fishes and lower GSIs could result as a function of different environmental variables, although age did not appear to be the cause because fish from Mohave were not the youngest fish collected (Carrie Marr, unpublished data). Seasonal differences in water temperature can also affect reproductive condition and potentially GSIs as seen in Figure 18. Female carp GSIs at Cibola-January are lower than in Cibola-March and were statistically similar to those values from Mohave in March. Female carp from January to March would have had an increase in ovary size and weight, which caused the increase in GSI between the two Cibola sampling events. Yet Mohave carp had lower GSIs in March. Smaller body size and weight would have contributed to this.

Other environmental factors that could lead to smaller body size, weight, and gonadal weight include nutritional status of the fish as well as exposure to environmental chemicals. Lake Mohave is more oligotrophic than the other two sampling locations, suggesting that limnological differences caused the lower GSIs and sperm viability rather than exposure to xenobiotics (Paul Marsh, pers. comm., Arizona State University, 2003). Patiño et al. (2003) found that most of Lake Mead is mildly mesotrophic and does not support high levels of productivity. Priscu (1978) noted that primary productivity and limnological characteristics in Lake Mohave reflected its receiving waters from Lake Mead. Any nutrient gains that Lake Mohave made over a year were lost due to its high outflow and, therefore, low residence time (Priscu 1978). Another study on the LCR found that the agricultural drains on the LCR were constant sources of particulate organic matter and nutrients into the main channel of the river (Lieberman and Burke 1991). These additional sources of nutrients into the LCR are further down the main channel at Cibola NWR. Although chlorophyll and phosphorus concentrations increased from upstream to downstream (indicating higher productivity downstream), Minckley (1979) did not think this was a pattern of downstream eutrophication. Instead, he hypothesized that these characteristics reflected the displacement of organic materials downstream concurrently with degradation, storage, and utilization within the LCR (Minckley 1979). However, his study did not include Lake Mohave; Davis Dam was his northernmost sampling site. Differences in productivity (including trophic status and nutrient levels) between sites could have influenced fish health indices in this study. Inter-site variability was reduced by sampling in similar habitats. However, razorback suckers sampled at High Levee Pond were in a more lentic system than the main channel, although High Levee Pond has a high water turnover since it is directly connected to the old river channel. A few carp were collected at Cibola-January at Three Fingers Lake and most carp at Cibola-March were collected at Pretty Water, which are both lentic systems. Pretty Water is directly downstream from High Levee Pond, is hydrologically connected to both High Levee Pond and the old river channel, and also has a low residence time similar to High Levee Pond. All of the other fishes collected for this study were collected from riverine portions of the sampling sites.

Regressions were also conducted to determine the influence of environmental contaminants on GSIs. The only significant site specific regression occurred in Mohave female carp where GSIs were negatively associated with DDT concentrations ( $R^2=0.50$ ,  $P=0.049$ ). Therefore, 50% of the variation of female GSIs was due to DDT concentrations in tissue. As DDT concentrations in whole body female carp increased, GSIs decreased.

When sites were pooled, a negative relationship between male GSIs and PCBs was found ( $R^2=0.22$ ,  $P=0.005$ ). In females, GSIs had a significant relationship with PCBs, BDEs, and chlordanes ( $R^2=0.28$ ,  $P=0.0152$ ). The relationship between GSIs and BDEs (and chlordanes) was positive, although it was negative with PCBs. The same negative relationship between PCBs and GSIs was seen in females as in males. Although the influence of environmental contaminants on GSIs may not be considered endocrine disruption, the strong negative relationship between Mohave female GSIs and DDT concentrations could account for the smaller GSIs compared to other sites. Exposure to environmental contaminants has resulted in reduced GSIs in previous studies (Schmitt and Dethloff 2000, Patiño et al. 2003, Lavado et al. 2004, Diniz et al. 2005).

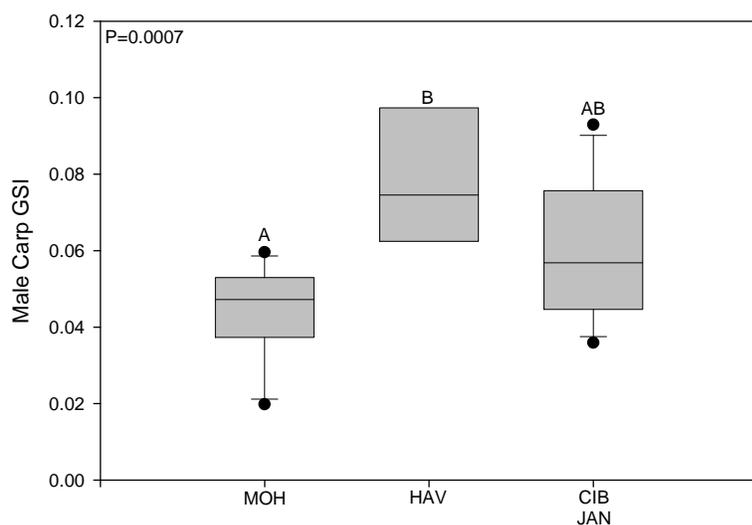


Figure 17. Male GSIs for carp in the Lower Colorado River, Arizona in 2002. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date. Since only two carp were collected at Cibola in March, a box plot could not be drawn for this sampling location and date.

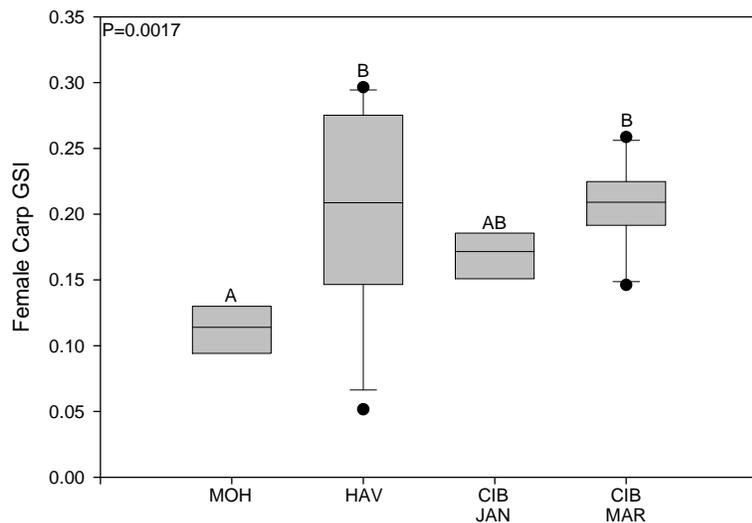


Figure 18. Female GSIs for carp in the Lower Colorado River, Arizona in 2002. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date.

### Sperm Viability

Sperm viability assays assess the functional capacity of sperm in an objective manner. This is opposed to the subjective measure of motility by microscopy. Use of this technique has been successful for several mammalian (Garner et al. 1997) as well as non-mammalian species (Segovia et al. 2000; Salinas-Flores et al. 2005). Sperm viability is influenced by a variety of environmental factors, such as nutritional and reproductive status and exposure to endocrine disrupting chemicals. Sperm viability was exclusively studied in carp during the March sampling trips.

Sperm viability was significantly lower in Mohave males compared to males at Havasu using non-parametric statistics ( $P=0.0064$ ) (Figure 19). Too few data were available for statistical analysis at Cibola since only two males were collected. Since GSIs in male and female carp from Mohave were lower, it was not surprising to find decreased sperm viability at Mohave also (Jenkins 2005).

There were no significant relationships between sperm viability and whole body environmental contaminant concentrations. Based on GSI, interstitial space in testes, and sperm viability, Mohave males may have inferior or reduced testicular development compared to the other LCR sites.

Sperm counts were not determined in this study, but in the future, using sperm counts in addition to sperm viability can help determine the stage of the reproductive process during which impacts occur. Perreault (1998) found that sperm numbers were normal even though sperm viability was impaired when damage to sperm occurred at later stages of spermiogenesis (Jenkins and Goodbred 2006). Future studies should also assess the nutritional status of the fish via isotope analysis as well as determine the trophic status of the water body since these factors can influence sperm quality.

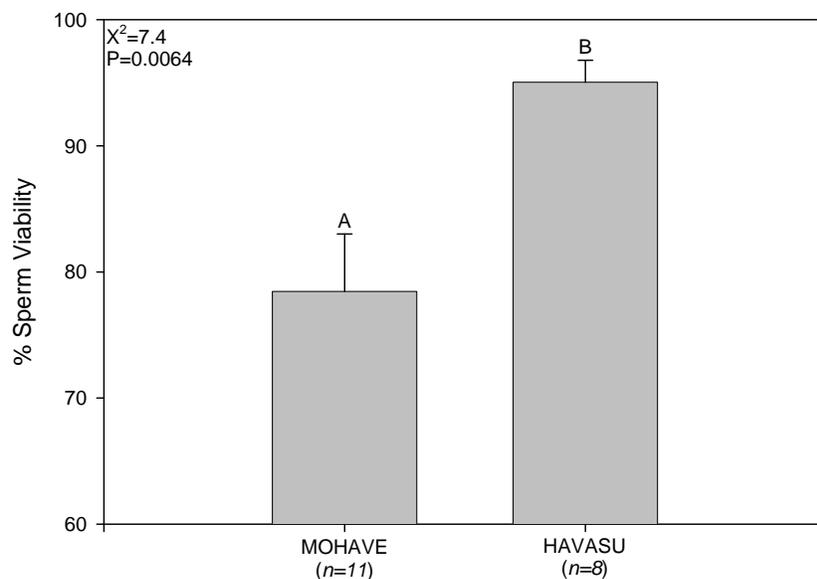


Figure 19. Sperm viability (arithmetic mean + SE) in carp from the Lower Colorado River, Arizona in 2002. The sample size from Cibola was too low to be included in this analysis (n=2). Different letters represent statistical differences between sampling locations as determined by a Kruskal-Wallis Test and a Bonferroni separation of means at  $\alpha=0.05$ .

### Histology

Quantitative MA analysis, qualitative histopathology, and average stage were analyzed in male carp testes from the Lower Colorado River. Macrophage aggregates are generally considered to be indicative of either disease or exposure to environmental contaminants (Patiño 2002a), and histological preparations indicated that testicular MAs were present in all fish. Mohave males showed higher incidences of testicular MAs (Figure 20), although this difference was not statistically significant ( $P=0.12$ ). The qualitative histopathology revealed the incidences of specific histopathologies associated with MAs, such as the presence of sporozoan-like parasites, vacuolization of MAs, and focal granulomas (Figure 21). No statistics were conducted between sites for the incidences of sporozoan-like structures and focal granulomas because none were found at Havasu or Cibola. Sporozoan-like parasites were found in about 27% of males from Mohave. Focal granulomas were not found in any of the preparations from Havasu or Cibola, but they were present in about 4% of the males from Mohave (Patiño 2002a). There was a

significant difference between the incidence of vacuole-like structures ( $P=0.0006$ ).

Vacuolization of MAs was observed in a small percentage of males from Havasu and Cibola (15% and 10%, respectively), but more than 70% of the males from Mohave had these vacuoles. The results of average stage analysis of testes development indicated a trend for Mohave males to have slightly less sperm per unit area and slightly more non-germinal tissue than males from Havasu and Cibola in March ( $P=0.026$  for Percent Interstitium only) (Figure 22). These quantitative and qualitative analyses indicated that the testes of males from Mohave were less 'healthy' than those from Havasu and Cibola (Patiño 2002a). Mohave males had higher incidences of MAs and other histopathologies associated with disease. Mohave males also had the lowest GSI when compared to the other sites. The greater incidence of testicular MAs at Mohave may be related to their reduced GSI and increased testicular interstitial space as a result of greater exposure to environmental contaminants, different water quality conditions, different nutritional/trophic status, or different immunological strengths of fishes at the LCR sampling sites. Increased concentrations of MAs in ovaries, spleen, liver, and kidney have been positively correlated with concentrations of environmental contaminants (Johnson et al. 1988; Wolke 1992; Couillard and Hodson 1996) although infectious diseases can also contribute to increased MA concentrations in fishes (Wolke 1992). Hinck et al. (2007) found large macrophage aggregates ( $>15,000 \mu\text{m}^2$ ) in carp as well as pigmented cell accumulations, similar to MAs, in the gonadal tissue of several male and female carp near Lake Mead. Patiño et al. (2003) found significantly lower GSIs associated with increased MAs in testes from the same site. Wolke (1992) found that infectious disease contributed to increased MA formation.

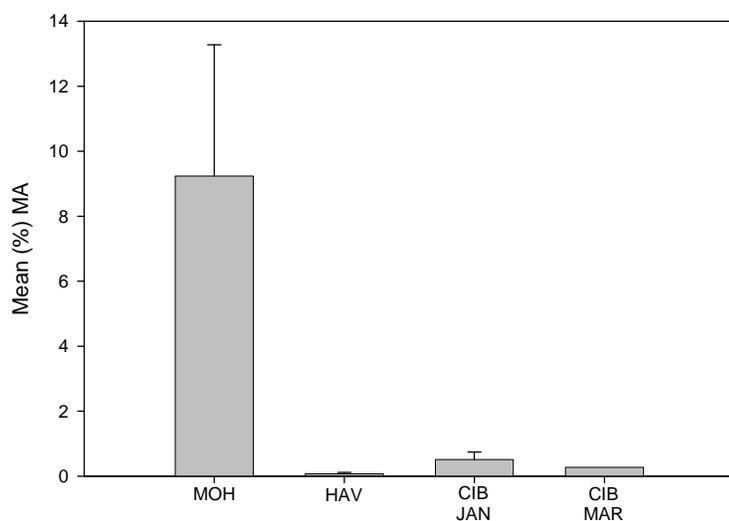


Figure 20. Quantitative MA analysis of carp testes in the Lower Colorado River, Arizona in 2002. Shown for each group are the arithmetic mean + SE (error bars). Stations are ordered from upstream to downstream and then by chronologically by sampling date.

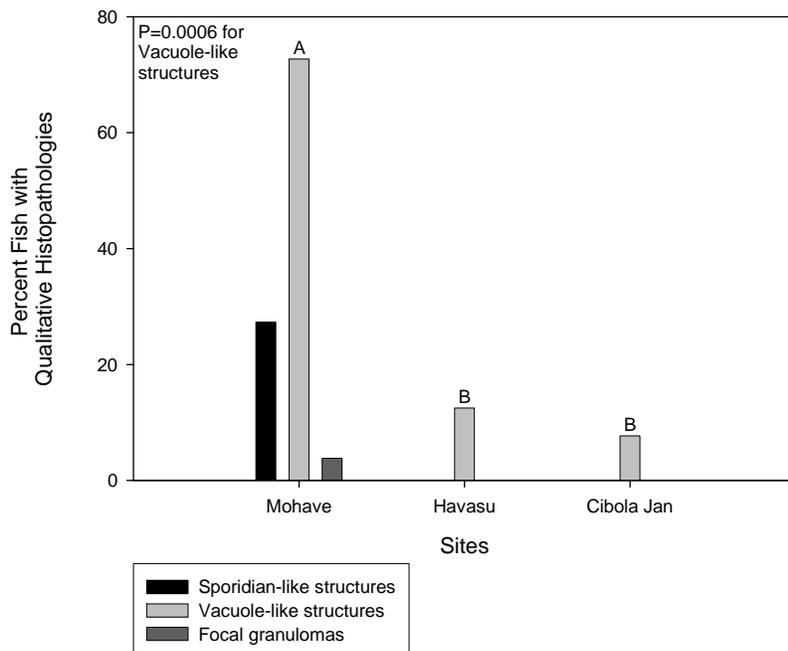


Figure 21. Qualitative histopathologies in male carp testes on the Lower Colorado River, Arizona in 2002. Macrophage aggregates were found in the two males from Cibola-March but no sporidian-like structures, vacuole-like structures, or focal granulomas were found. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are the total percent of fish with each histopathology. Since only two male carp were collected at Cibola in March, a box plot could not be drawn for this sampling location and date.

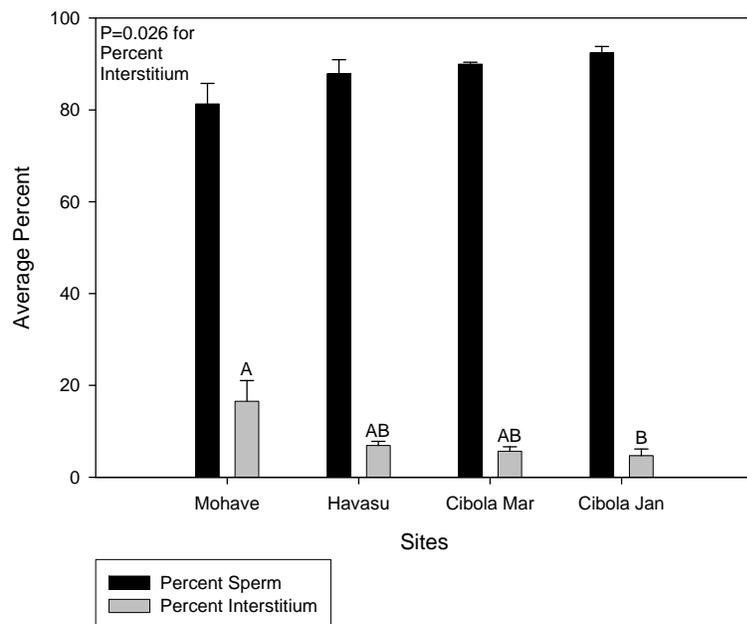


Figure 22. Average stage of development of testes in carp from the Lower Colorado River, Arizona in 2002. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are the arithmetic mean + SE (error bars). Stations are ordered from upstream to downstream and then by chronologically by sampling date.

### Fecundity Analysis in Females

Fecundity, like sperm viability, can be affected by a number of environmental factors, such as nutrient intake levels and environmental contaminants. Fecundity in female carp was examined between the LCR sites. Fecundity was defined as the number of eggs per body weight of the adult female. Cibola-March statistically had the greatest fecundity and lowest fecundity was at Mohave ( $P<0.0013$ ) (Figure 23).

Follicle frequency distributions in carp ovaries were also examined between sites. Although no statistics were performed, both Havasu and Cibola-March had peak follicle sizes of 1.4 mm. Mohave lagged behind with a peak follicle size at 1.3 mm. A smaller peak follicle size at Mohave could mean that follicle development at Mohave lagged behind development at Havasu and Cibola and would have caught up to the other sites if sampling occurred again at a later date (Patiño 2002b). This is possible given that surface water temperatures in Mohave were around 16.0 °C and 15.6 °C at Havasu but were warmer at 17.2°C at Cibola-March during fish collections. Another explanation is that follicle sizes at Mohave are inherently smaller than at Havasu or Cibola (Patiño 2002b). If follicle sizes are naturally smaller at Mohave, it could indicate a problem, because egg size has been correlated with larval survival in fishes (Palace and Werner 2005). A third explanation could be smaller fish size (length) at Mohave, at least in

comparison to Lake Havasu (Figure 15). Weights were also smaller at Mohave although they were not statistically significant.

Additionally, fish were collected prior to spawning, because after the onset of spawning, a second distinct peak around 0.6 mm forms (Patiño 2002b). A second peak would have helped determine if follicle sizes were typically smaller at Mohave than the other LCR sites. This has broader implications for the reproductive success of female carp at Mohave, since their fecundity was also significantly lower than the other sites (Patiño 2002b).

Given that Mohave female carp had reduced fecundity (Figure 23) and had the lowest GSI between sites, it was not surprising to find that they also had delayed follicular development (Figure 24).

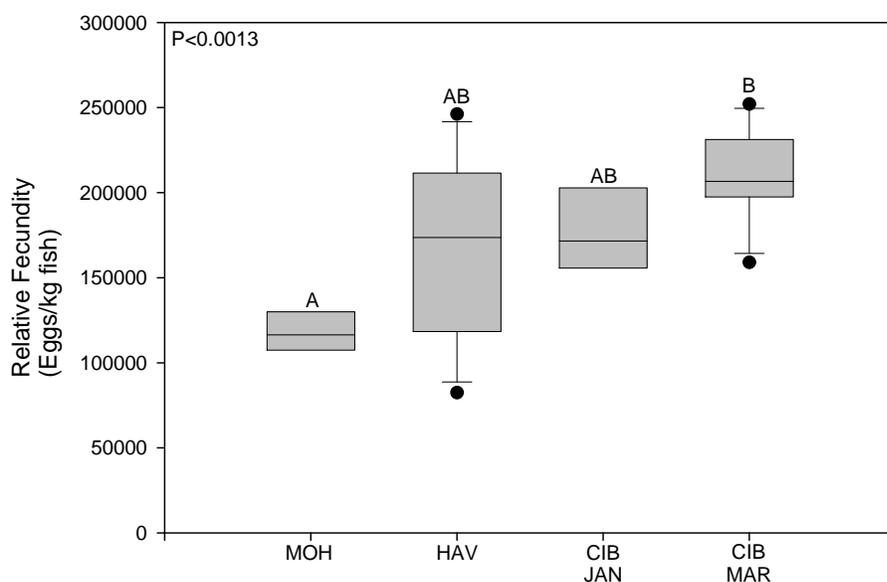


Figure 23. Fecundity of female carp from the Lower Colorado River, Arizona in 2002. Different letters represent statistical differences between sampling locations as determined by a Bonferroni t Test at  $\alpha=0.05$ . Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Stations are ordered from upstream to downstream and then by chronologically by sampling date.

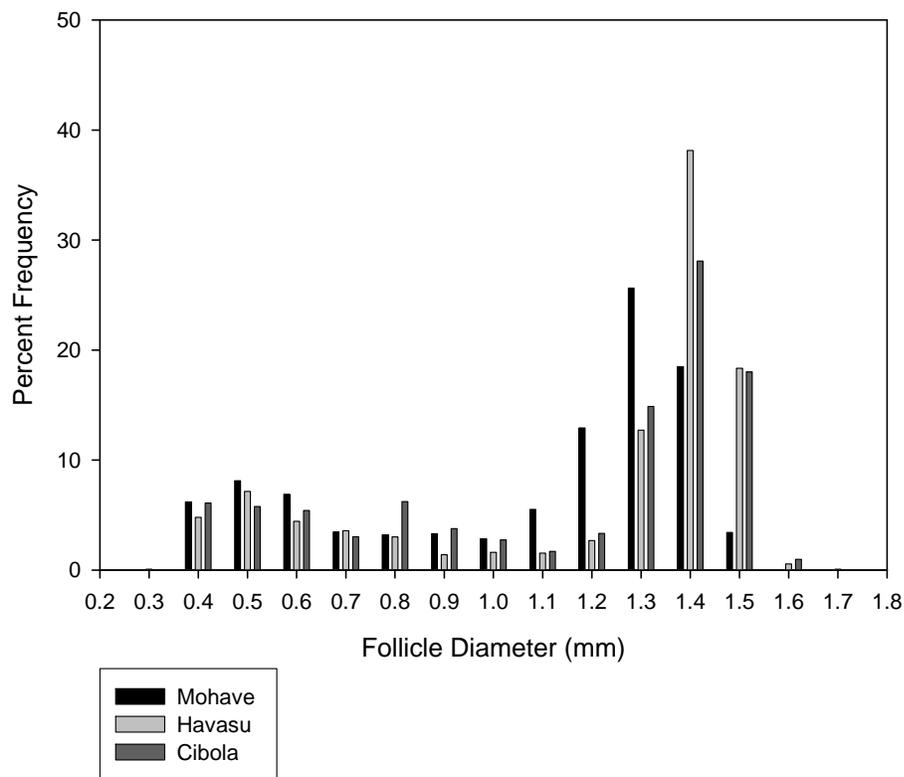


Figure 24. Carp ovarian follicle frequency distribution among Lower Colorado River, Arizona sites in 2002. Follicle frequency is presented as a percent, averaged for each site.

## PAHs and Organics in Sediment

Table 5. Detected concentrations (ng/g (ppb), ww) of PAHs and BDEs found in Lower Colorado River, Arizona sediments in 2002. Numbers in bold indicate values greater than the detection limit.

Sediment ID	Havasu	Havasu	Cibola		Mohave	Mohave	Mohave
	Topock Marsh	Needles WWTP	High Levee Pond	Cibola Pretty Water	Cottonwood Cove	Yuma Cove	Yuma Cove DUP
<i>n</i>	1	1	1	1	1	1	1
phenol	<25	<25	<b>122.0</b>	<b>110.0</b>	<b>132.0</b>	<25	<25
acetophenone	<25	<25	<b>34.8</b>	<b>68</b>	<25	<25	<25
diethyl phtalate	<b>228</b>	<b>189</b>	<b>289</b>	<b>339</b>	<b>298</b>	<b>331</b>	<b>302</b>
diethylhexyl phtalate	<b>293</b>	<b>148</b>	<b>183</b>	<b>417</b>	<b>308</b>	<b>313</b>	<b>353</b>
BDE 47	<2	<2	<b>5.84</b>	<2	<2	<2	<2
BDE 99	<4	<4	<b>8.69</b>	<4	<4	<4	<4
BDE 100	<4	<4	<b>4.14</b>	<4	<4	<4	<4

*n* = sample size. No benzo(a)pyrene, naphthalene, or anthracene were detected.

No organochlorine pesticides were detected in sediments. No halogenated compounds were detected except for brominated diphenyl ethers. Brominated diphenyl ethers were detected in one sediment sample, Cibola's High Levee Pond.

Percent recovery of spiked samples ranged from 48.16 to 117.3. Detection of these compounds in blanks ranged from ND to 392 ng/g.

Few PAHs and BDEs were detected in sediment samples (Table 5). No organochlorine pesticides or PCBs were detected in sediments. Of all the compounds detected, Cibola High Levee Pond was the only site to have all of these compounds. Both Cibola sites are entrained water bodies that receive water inputs from the old Colorado River channel and are near an agricultural drain. It is not clear why High Levee Pond would be the only site to have detectable BDEs, however. It is possible that all other sites receive greater flow volumes and/or flow rates than the Cibola sites. Whether or not smaller flow rates of water through these water bodies contributed to increased deposition of PAHs and BDEs is not known. An agricultural drain empties into the Lower Colorado River downstream of Cibola's Pretty Water (which is downstream of High Levee Pond). Therefore, the agricultural drain could not be the source of PAHs and BDEs at High Levee Pond and Pretty Water. Concentrations of BDEs in biosolids ranged from 51.5-192 ng/g ww for BDE 47 in Maine, 129 ng/g ww in the Gulf to 640.3 ng/g for BDE 99 in Maine, and 28 ng/g ww in the Gulf to 116 ng/g ww in Maine for BDE 100 (assuming 75% moisture content; desJardins Anderson and MacRae 2006). Although some BDEs were detected at one Cibola site and not another, the detected concentrations appear more similar to the limit of detection than the concentrations of BDEs reported in biosolids around the country.

## Contaminants in Plasma

Few NCI and EI target compounds were detected in carp and razorbacks from the Lower Colorado River, although most fish were analyzed for these compounds. Five NCI compounds were detected in individual fish plasma (both carp and razorback sucker): trifluralin, BDEs 47 and 99, and PCBs 170 and 180 (Figure 25 and Appendix 4). The rationale for measuring these compounds in plasma was to find an alternative method to measuring the compounds in whole body fish, which requires the fish to be sacrificed. Concentrations of NCI compounds ranged from non-detect (all compounds) to 24.6 ng/mL (BDE 47 in a Mohave razorback sucker). Ninety-three percent of the BDE 47 detected was in two Mohave razorback suckers. The frequency of detection of NCI compounds was 64% in carp (all three sites) and 36% in razorbacks (at Mohave only). Nine EI compounds were detected in fish plasma (Figures 26 and 27), ranging from non-detect (all compounds) to 1,495 ng/mL (diethyl phthalate in a Mohave razorback sucker) and 24,941 ng/mL (cholesterol in a Havasu carp). Sixty-seven percent of the diethyl phthalate detected was in the Cibola and Mohave razorback suckers. When diethyl phthalate concentrations were compared in razorback suckers only, 41% of the diethyl phthalate was in Cibola razorback suckers and 59% in Mohave razorback suckers. The frequency of detection of EI compounds was 64% in carp (all three sites) and 36% in razorbacks (Cibola and Mohave). Two of the greatest concentrations of cholesterol detected were in Havasu carp (carp# 13 and 19, 0.026 mg/mL and 0.012 mg/mL cholesterol, respectively;  $P=0.0018$ ) (Figure 27). In the literature, cholesterol concentrations for teleosts ranged from 150-360 mg/mL (Folmar 1993). The average concentration of plasma cholesterol from common carp at a reference site in Spain was 173 mg/mL (Carballo et al. 2005) and the average serum cholesterol concentration in the control group of common carp in a gallium exposure study was 0.137 mg/mL [reported as 137 mg/dL] (Yang and Chen 2003). Therefore, the cholesterol concentrations from the LCR are much lower (four orders of magnitude) compared to the first two studies, but only a little lower than the control carp in the exposure study (one order of magnitude). Yang and Chen (2003) found increased cholesterol concentrations due to gallium exposure which could have been associated with increased stress, but Carballo et al. (2005) reported decreased cholesterol concentrations at treatment sites due to either environmental variation or pollution. It is unknown whether lower cholesterol concentrations found on the LCR are due to environmental factors or contaminant concentrations compared to literature values. The cause of the variation of cholesterol between sites is also unknown. Some of the Havasu carp were collected near the Topock Marina, where they may have eaten food provided by humans.

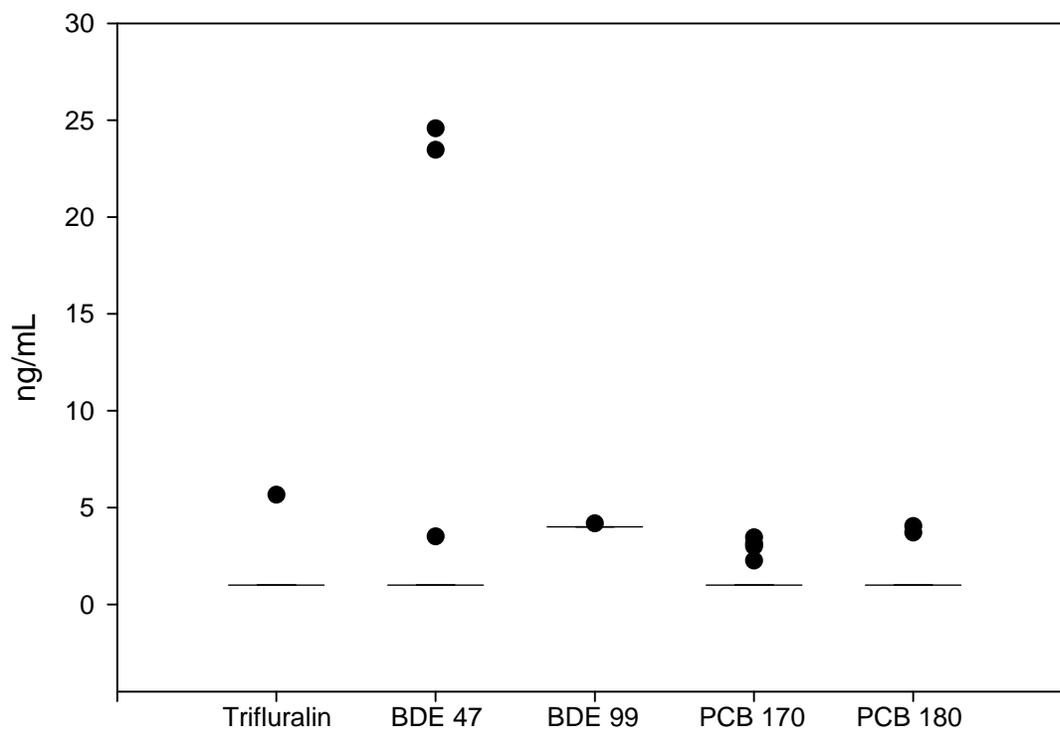


Figure 25. Halogenated (NCI) compounds in Lower Colorado River, Arizona fish plasma (ng/mL or ppb) in 2002. These contaminants were measured in 94 fish plasma samples, including 31 razorback suckers from Mohave. Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means. The box plots do not show up due to the high number of non-detect data and the resultant low variation created. Shown for each group are points representing the median (black horizontal line) and outliers (dots).

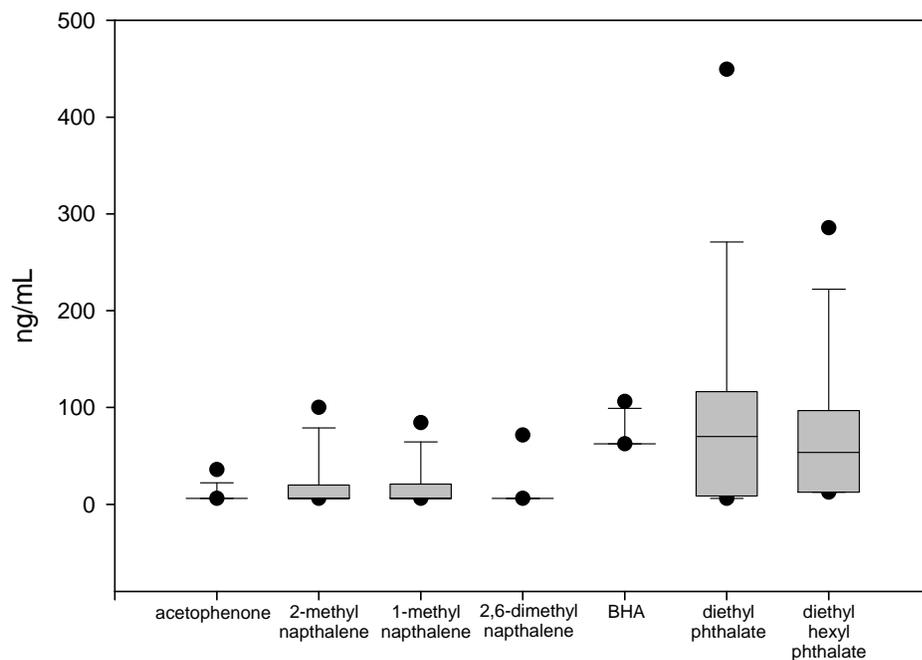


Figure 26. EI compounds in Lower Colorado River, Arizona fish plasma (ng/mL or ppb) in 2002. These contaminants in plasma were measured in 85 fish, including 28 razorback suckers. Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means. Shown for each group are points representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles, median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Some of the box plots do not show up due to the high number of non-detect data and the resultant low variation created.

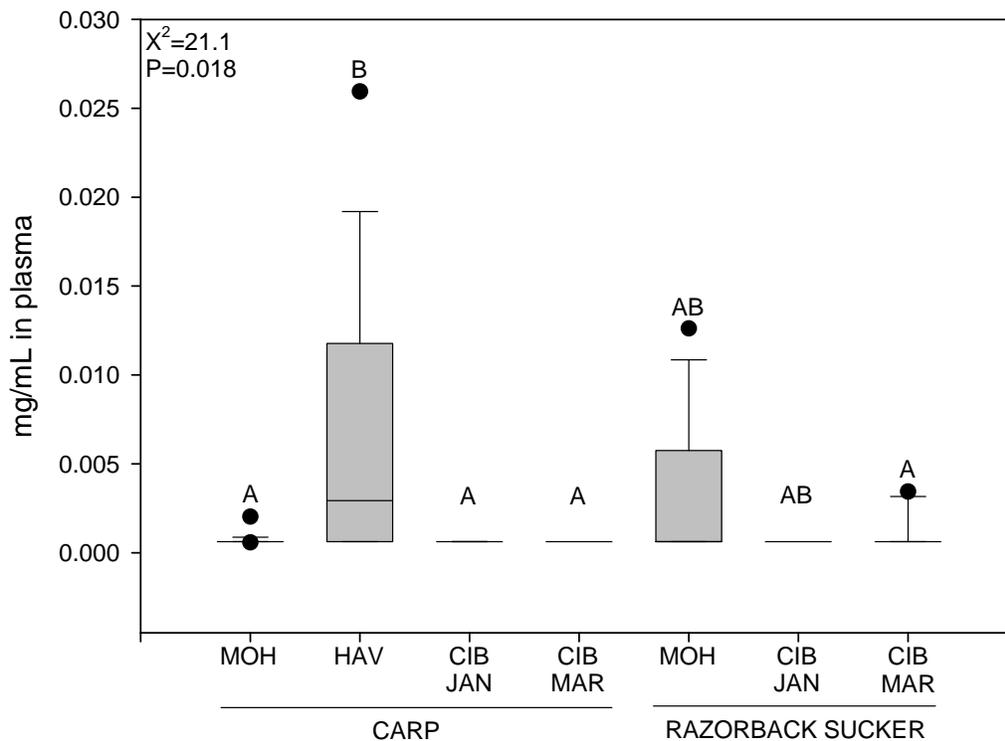


Figure 27. Cholesterol, also an EI compound, in Lower Colorado River, Arizona fish plasma (ng/mL or ppb) in 2002. Cholesterol in plasma was measured in 85 fish, including 28 razorback suckers. Censored values were set to  $\frac{1}{2}$  the limit of detection for calculation of means. Shown for each group are points representing individual fish (outliers), median (black horizontal line), interquartile range (box), and the 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers). Some of the box plots do not show up due to the high number of non-detect data and the resultant low variation created. Different letters represent statistical differences between sampling locations as determined by a Kruskal-Wallis Test and a Bonferroni separation of means at  $\alpha=0.05$ .

## SUMMARY

While no consistent evidence of endocrine disruption in razorback suckers or carp was found in the Lower Colorado River, several hormones were found in greater concentrations than expected. For example, female carp had elevated E2 and Vtg when compared to other studies. Male carp also had elevated Vtg concentrations. Thyroid concentrations were skewed low ( $T_3$ ) or high ( $T_4$ ) compared to other studies. There were also significant differences between sampling sites. Despite its location downstream of Lake Mead and the endocrine disruption reported in Las Vegas Wash, Mohave was chosen as the reference site because of the lack of industrial and agricultural inputs into it. However, according to most of the hormone data and condition indices, carp from Mohave were significantly different than those from Havasu or Cibola. For

instance, male carp from Mohave had reduced GSIs, increased incidence of MAs, increased incidence of histopathologies, reduced quantity of space for spermatocyte development, and decreased sperm viability. Mohave females had lowered fecundity and were delayed in follicle development behind the other sites. Mohave was not different than the other sampling sites due to environmental contaminants because the site specific regressions between hormones or condition indices and environmental contaminants did not consistently indicate significant relationships at Mohave. There were also differences between the quantities of environmental contaminants found between sites. Mohave did have the greatest concentrations of total BDEs and total PCBs, but not total p,p'-DDT homologs or total chlordanes. While this was not an exhaustive examination of potential contaminants in the environment, other factors could contribute to site differences. For example, Mohave could be limnologically different than Havasu or Cibola. Additional nutrients from agricultural drains enter the river at Cibola which adds confounding variables to this analysis. Also, some fish were collected from more lotic systems and others from lentic systems. Some of these additional variables were not measured so inter-site differences cannot be analyzed.

## MANAGEMENT ACTIONS

Endocrine disruption was not found in LCR razorback suckers. At this time, it is unnecessary to determine prioritized release sites for future razorback sucker stockings. However, Mohave carp seemed to have reduced reproductive fitness. It is unknown if it could be due to the endocrine disruption reported in Las Vegas Wash or Lake Mead. Since reproductive data was not collected from razorback suckers, it is not known how Mohave razorback sucker reproductive fitness compares to Cibola. This should be a research priority for the future, especially since the FWS manages Willow Beach National Fish Hatchery below Hoover Dam on Lake Mohave.

Reproductive effects were seen in carp. Only one slight indication of endocrine disruption was detected in slightly elevated male Vtg concentrations, while elevated concentrations of E2 and Vtg in female carp were also found. These results warrant further investigation. Future studies should also monitor thyroid concentrations in fishes since the results were bimodal (v. low T<sub>3</sub> and v. high T<sub>4</sub>). Since perchlorate is a concern on the LCR, continued monitoring of thyroid concentrations on the LCR is a priority.

Although significant relationships between hormones and environmental contaminants were found, no specific causative agents were identified. Future studies must eliminate confounding variables such as nutritional or trophic differences between sites, especially considering Mohave.

Data collected in this study can be used in an Environmental Impact Statement or Biological Opinion should the Las Vegas Water Authority choose to discharge treated wastewater south of Hoover dam. It can also be used as baseline data, even if wastewater inputs do not increase below Hoover Dam. Alternative wastewater disposal options are being considered in Lake Mead, but these could also affect downstream resources. Basin-wide monitoring on a fixed schedule, such as USGS's BEST Program, should be able to continue tracking the occurrence of endocrine disruption in the LCR. In the event the BEST Program ceases sampling on the LCR,

then the FWS or EPA in Las Vegas should monitor endocrine disruption in the LCR every 10-15 years.

Direct management is also possible through cooperation with the participating refuges and the Arizona Fisheries Resources Office at Parker. These offices work together in regular monitoring of native fishes. Hormone analysis is inexpensive and if blood from native fishes during routine monitoring is collected periodically, it could be another alternative for continued endocrine disruption monitoring.

This report will also be provided to the FWS Southern Nevada Field Office for use in discussions with the Las Vegas Water Authority to help with their management decisions regarding discharge of treated wastewater into Lake Mead.

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## Appendices

## Appendix 1. Summary statistics for steroid hormone concentrations from carp and razorback suckers on the Lower Colorado River in 2002.

Fish Species and Sampling Site	<i>n</i>	E2 (pg/mL)					11-KT (pg/mL)				
		Mean	Median	Min	Max	SE	Mean	Median	Min	Max	SE
<b>Male Carp</b>											
Mohave	11	273	210	117	652	15	1,428	1,424	804	1,920	32
Havasu	8	425	349	146	891	34	1,435	1,453	1,111	1,751	32
Cibola-Jan	13	368	298	131	749	14	511	524	313	957	13
Cibola-Mar	2	242	242	137	347	74	654	654	534	773	84
<b>Female Carp</b>											
Mohave	8	1,778	1,627	1,314	2,262	47	465	407	298	787	21
Havasu	11	1,619	1,431	1,259	2,415	38	374	389	209	510	7
Cibola-Jan	5	383	380	241	504	22	329	234	181	538	36
Cibola-Mar	11	916	813	449	1,368	28	372	336	266	577	9
<b>Male Razorback Suckers</b>											
Mohave	13	271	254	121	438	7	1,360	1,377	1,017	1,608	13
Cibola-Jan	7	478	501	205	678	24	672	668	407	834	22
Cibola-Mar	5	391	342	285	559	24	737	672	488	974	40
<b>Female Razorback Suckers</b>											
Mohave	12	1,727	1,721	1,229	2,343	41	445	448	205	709	12
Cibola-Jan	7	607	602	474	743	13	212	182	136	351	11
Cibola-Mar	9	1,107	1,286	565	1,421	35	357	357	244	456	9

Appendix 2. Summary statistics for E2/11-KT ratios and vitellogenin concentrations from carp and razorback suckers on the Lower Colorado River, Arizona in 2002.

Fish Species and Sampling Site	n	E2/11-KT					Vtg (mg/mL)				
		Mean	Median	Min	Max	SE	Mean	Median	Min	Max	SE
Male Carp											
Mohave	11	0.20	0.15	0.08	0.53	0.01	0.23	0.18	0.09	0.54	0.01
Havasu	8	0.30	0.25	0.10	0.55	0.02	0.24	0.19	0.12	0.49	0.02
Cibola-Jan	13	0.77	0.61	0.32	1.81	0.04	0.41	0.38	0.05	1.42	0.03
Cibola-Mar	2	0.35	0.35	0.26	0.45	0.07	0.09	0.09	0.09	0.09	0.00
Female Carp											
Mohave	8	4.02	4.07	2.82	5.30	0.09	11.25	10.83	6.96	17.78	0.43
Havasu	11	4.69	3.72	2.53	11.56	0.22	14.11	13.71	10.05	19.68	0.25
Cibola-Jan	5	1.49	1.32	0.45	2.60	0.17	5.25	5.12	4.05	7.10	0.23
Cibola-Mar	11	2.56	2.51	1.30	4.65	0.09	4.14	3.02	1.26	9.46	0.24
Male Razorback Suckers											
Mohave	13	0.20	0.19	0.10	0.35	0.01					
Cibola-Jan	7	0.70	0.75	0.47	0.93	0.02					
Cibola-Mar	5	0.57	0.58	0.30	0.83	0.05					
Female Razorback Suckers											
Mohave	12	4.07	3.89	2.65	6.07	0.10					
Cibola-Jan	7	3.13	3.25	1.56	4.43	0.13					
Cibola-Mar	9	3.25	3.16	1.24	5.74	0.13					

Appendix 3. Summary statistics for T<sub>3</sub> and T<sub>4</sub> in carp on the Lower Colorado River, Arizona in 2002.

Fish Species and Sampling Site	n	T <sub>3</sub> (ng/mL)					T <sub>4</sub> (ng/mL)				
		Mean	Median	Min	Max	SE	Mean	Median	Min	Max	SE
Male Carp											
Mohave	11	1.87	1.99	1.21	2.52	0.04	25.44	24.32	19.81	30.66	0.31
Havasu	8	1.15	0.89	0.46	2.84	0.10	28.69	28.38	26.01	33.63	0.32
Cibola-Jan	13	3.41	2.66	1.05	7.32	0.14	27.83	27.71	20.09	37.93	0.40
Cibola-Mar	2	2.67	2.67	2.25	3.09	0.30	28.92	28.92	26.52	31.31	1.69
Female Carp											
Mohave	8	1.59	1.52	1.20	2.15	0.04	22.28	24.27	15.83	27.41	0.58
Havasu	11	2.55	2.49	1.30	3.83	0.09	27.53	26.55	18.59	35.16	0.47
Cibola-Jan	5	4.33	3.90	2.79	6.24	0.31	27.65	27.88	21.57	34.20	1.04
Cibola-Mar	11	2.90	2.56	1.46	5.05	0.11	25.15	26.23	18.18	30.63	0.37

Appendix 4. Concentrations of NCI compounds (ng/mL; ppb) in fish plasma from the Lower Colorado River, Arizona in 2002. Numbers in bold are greater than the detection limit.

ng/mL	<b>Trifluralin</b>	<b>BDE 47</b>	<b>BDE 99</b>	<b>PCB 170</b>	<b>PCB 180</b>
LOD	2	2	8	2	2
MOHCARP3	<2	<2	<8	<b>2.25</b>	<2
MOHCARP4	<2	<2	<8	<b>3.13</b>	<2
MOHCARP7	<2	<2	<8	<b>3.46</b>	<2
MOHCARP11	<2	<2	<8	<b>2.99</b>	<2
MOHRZB8	<2	<b>23.47</b>	<8	<2	<b>3.70</b>
MOHRZB10	<2	<b>24.57</b>	<8	<2	<b>4.04</b>
CIBCARMAR2	<b>5.66</b>	<2	<8	<2	<2
HAVCARP14	<2	<b>3.51</b>	<b>4.18</b>	<2	<2