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Investigations of Endocrine Disruption in Aquatic Systems Associated with the National Water Quality Assessment (NAWQA) Program

Collecting a blood sample from a largemouth bass to analyze for bioindicators of endocrine disruption.

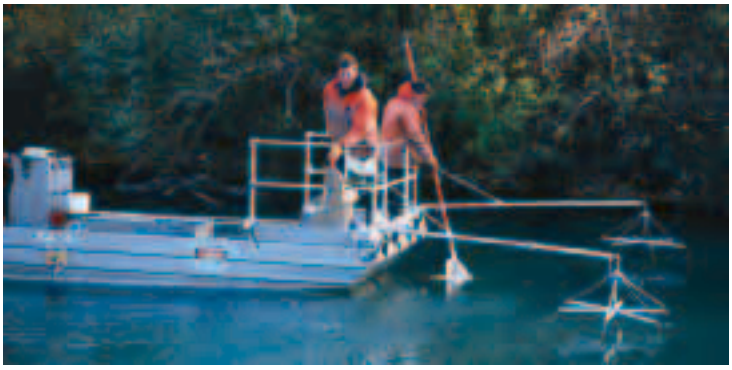
Does Endocrine Disruption Occur in U.S. Waters?

The U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA) program recently found evidence of endocrine disruption in common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) collected from waterways that contain synthetic organic compounds. Evidence indicates concentrations of sex steroid hormones (estrogen and testosterone) and vitellogenin (egg protein produced by females) were different in fish from contaminated and reference sites.

Endocrine disruption has been the focus of an increasing number of scientific investigations in recent years. At least 45 synthetic chemicals from several chemical groups have been identified as potentially having endocrine disrupting effects (Table 1). Some of these chemicals have the potential to cause reproductive impairment in animals, including both terrestrial and aquatic organisms. Alteration in blood concentrations of sex steroid hormones and vitellogenin may be associated with reproductive impairment and other critical reproductive factors (Colborn and Clement, 1992). These include: reduced penis size in juvenile male otters of the Columbia River (Henny and others, 1996), feminized behavior in male Western gulls of southern California (Fry and Toone, 1981), non-descended testicles in male Florida panthers (Facemire and others, 1995), nonfunctional testes in male American alligators in Florida (Guillette and others, 1994), and masculinized female mosquito fish (Davis and Bortone, 1992).

Table 1. Synthetic chemicals that may cause endocrine disruption in wildlife.

POTENTIAL ENDOCRINE DISRUPTERS	
CHEMICAL GROUPS	**SPECIFIC CHEMICALS**
• Herbicides	atrazine, 2,4-D
• Fungicides	vinclozolin
• Insecticides	DDTs, carbaryl
• Nematocides	aldicaryl
• Industrial Chemicals	phenols, PCBs, phthalates



Sampling for live carp and bass using electrofishing boat.

Highlights

- The NAWQA program of the USGS is conducting several investigations related to endocrine disruption of fish from contaminants.
- A national reconnaissance investigation found that the potential for endocrine disruption in common carp was widespread and appeared to be related to waterborne pesticides.
- An investigation of common carp and largemouth bass in rivers in the Northeast indicates disruption in their endocrine system that is associated with elevated levels of polychlorinated biphenyls (PCBs) in sediments and fish tissue residue.
- High levels of vitellogenin (an egg protein normally found in females) were detected in male common carp in areas of Lake Mead that receive industrial and sewage effluent from the Las Vegas, Nevada area.
- Additional investigations are planned by USGS to establish baseline conditions and to understand the potential impacts of endocrine disruption to fish populations.

U.S. Department of the Interior
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What is an Endocrine Disrupter?

An environmental endocrine disrupter is defined as an external compound that interferes with or mimics natural hormones in the body that are responsible for the maintenance, reproduction, development, and/or behavior of an organism (U.S. Environmental Protection Agency, 1997). Hypotheses about which chemicals may be endocrine disrupters, about the mechanisms through which they operate, and about which animals may be affected have been discussed in numerous publications (cited earlier); however, few regional or national studies related to fish populations have been conducted to test these hypotheses.

The effort to investigate endocrine disrupting chemicals in surface waters and their potential effects on fish began as a cooperative effort between the Biological Resources Division (BRD) and the Water Resources Division (WRD) of the USGS and the University of Florida, Gainesville, Fla. The NAWQA program provided background information on contaminant levels at many of the study sites and cooperated in both the field collection and synthesis of the data. NAWQA studies of endocrine disruption have focused on three goals:

1) To determine if endocrine disruption is occurring in fish and, if so, is the phenomenon widespread in the Nation's rivers and streams;

2) To evaluate the relation between endocrine disruption and concentrations of chemical contaminants in water and sediments; and

3) To provide information that may guide future monitoring and research related to endocrine disruption in aquatic biota.

What Evidence Has Been Found?

Examination of common carp and largemouth bass in 11 river basins around the country indicates the potential for endocrine disruption is most prevalent in industrialized, agricultural or urbanized areas. The primary forms of endocrine disruption in male carp were reductions in the levels of estrogen and testosterone, which has also been reported by Folmar (1993).

These results do not establish a direct causal connection between endocrine disruption and exposure to waterborne and sediment-borne chemicals, but they verify that differences in hormonal patterns are indeed occurring in fish and that they are correlated with the presence of contaminants in aquatic habitats. Population level effects were not among the study objectives. These findings demonstrate a need for further investigation into the mechanisms through which endocrine disruption occurs and into the prevalence of endocrine disruption in animal populations. Although these studies examined only fish, other investigations (Henny and others, 1996; Fry and Toone, 1981; and Guillette and others, 1994) suggest the possibility of similar effects in the endocrine systems of other vertebrates.

Results of Specific NAWQA Studies

National Reconnaissance Investigation

In 1994, the NAWQA Program and the BRD collaborated to examine carp and bass at 26 stream sites in 11 river basins (fig. 1) to determine if endocrine disruption is widespread across the United States. The sampling design permitted comparison of results among agricultural, urban, and forested areas that have different chemicals in their surface waters. If a relation between land use and endocrine disruption could be established, then scientists might be better able to anticipate locations where similar effects are likely to occur and to target additional studies aimed at assessing endocrine disruption (Goodbred and others, 1996).

Analysis of estrogen and testosterone in the blood of carp showed a relation between concentrations of waterborne pesticides and levels of sex steroid hormones. Male and female adult carp and bass, where available, were sampled at each site and analyzed by the University of Florida for estrogen, testosterone, vitellogenin, and maturity of sex organs. Alterations in sex steroid hormones and vitellogenin appear to be related to certain chemical groups. These chemical groups include: organochlorine compounds in tissue (DDT, chlordane, and PCBs); phenols, polyaromatic hydrocarbons, and phthalates (cresol and phenol) in bed sediments; and dissolved pesticides (atrazine) in water (Table 2).

Northeastern United States

Carp and bass were collected from industrial and reference sites during the nonreproductive period (Fall 1994) and the reproductive period (Spring 1995). Evidence of endocrine disruption at some sites appears related to higher concentrations of contaminants in fish tissue and sediments. These collections at sites with known contaminants were made in cooperation with the NAWQA Program in the Connecticut River, Conn., Housatonic River, Mass., Hudson River Basin, N.Y., and the Potomac River Basin, W. Va. and D.C.

As concentrations of PCBs increased, estrogen and testosterone concentrations in the blood of both carp and bass were altered. The presence of vitellogenin in the plasma of male fish is an indication of exposure to an estrogenic stimulus (N. D. Denslow, Univ. of Florida, oral comm., 1998). Male fish with vitellogenin in their plasma from the Hudson River at Lake Luzerne, N.Y., and the Mohawk River at Frankfort, N.Y., indicate they may have been

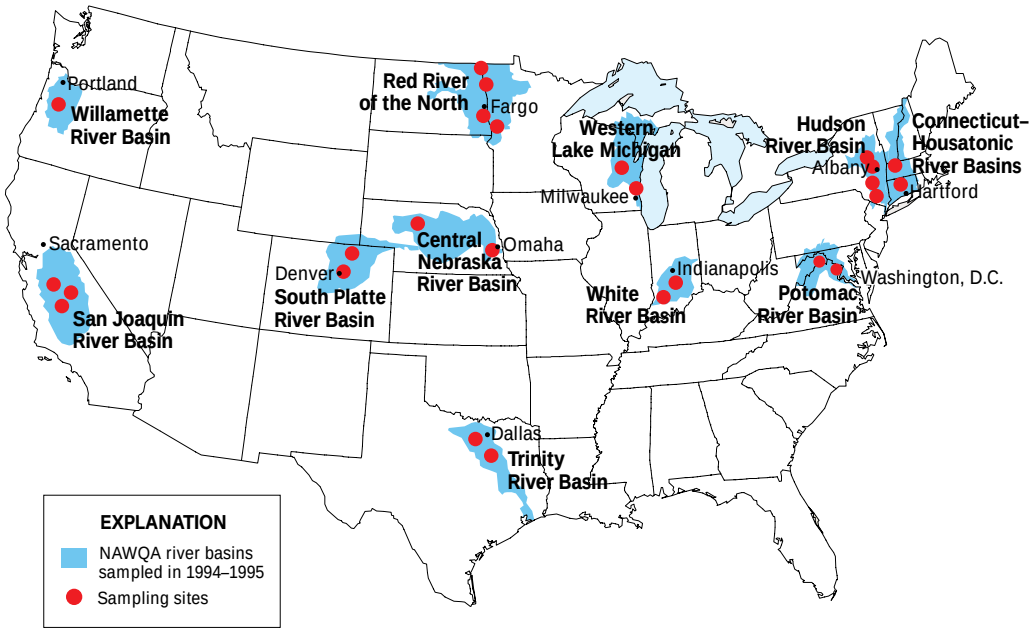

Figure 1. Sampling site locations in basins for national reconnaissance investigation, 1994. Major cities within basins are identified for reference.

Table 2. Positive (+) and negative (-) correlations between biomarkers and contaminants for female and male adult carp.

BIOMARKERS vs CONTAMINANTS

	ESTROGEN (E)	TESTOSTERONE (T)	E/T RATIO	VITELLOGENIN
*OCs[1]	-			- -
*PCBs			-	
Phenols	- - -	- - -		
Phthalates	- -	-		
*PAHs				
*Diss Pest	- - -	+ + +	- - -	

EXPLANATION
- correlative
- highly correlative
- very highly correlative
- female
- male

*OCs-organochlorine pesticides, PCBs-polychlorinated biphenyls, PAHs-polyaromatic hydrocarbons, Diss Pest-dissolved pesticides.

[1]Correlations for OCs are modified from Goodbred and others (1996) based on updated data from Mill Race Pond, Oregon.

exposed to an estrogenic compound (fig. 2). Lake Luzerne, a site previously selected as a reference site because of low levels of contaminants in the sediments, contained a large number of male carp (53%) with vitellogenin. A point source of minimally treated sewage or other unidentified sources might be the origin of estrogenic exposure for these fish (Smith and others, 1997). Lake Luzerne demonstrates that traditional chemical characterization of a stream does not insure reference conditions.

At some sites, female fish had lower than expected vitellogenin concentrations, suggesting interference with the production of egg yolk protein in the fish. Documenting the rise and fall of vitellogenin concentrations in females during the reproductive season will necessitate measurements during the same time periods (and water temperatures) at both the contaminated and reference sites.

Lake Mead, Nevada

An interagency study initiated by the USGS, National Park Service, and U.S. Fish and Wildlife Service (USFWS) was conducted in 1995. Investigations were conducted to determine potential effects of contaminants from Las Vegas Wash on water quality and the endocrine system of fish in Las Vegas Bay, a popular recreation site in Lake Mead National Recreation Area. Two contaminated sites (Las Vegas Wash and Las Vegas Bay) and a reference site (Callville Bay) were assessed for contaminant levels in water, bed sediments and fish tissue, and to determine if endocrine disruption was occurring in carp. High concentrations of organochlorine compounds, dioxins, and furans were found in the water and sediments from the two contaminated sites (Bevans and others, 1996).

Evidence of potential endocrine disruption was shown by reduced sex steroid hormone levels in male carp from contaminated sites, compared to Callville Bay (fig. 3). The most compelling evidence was vitellogenin production in males from

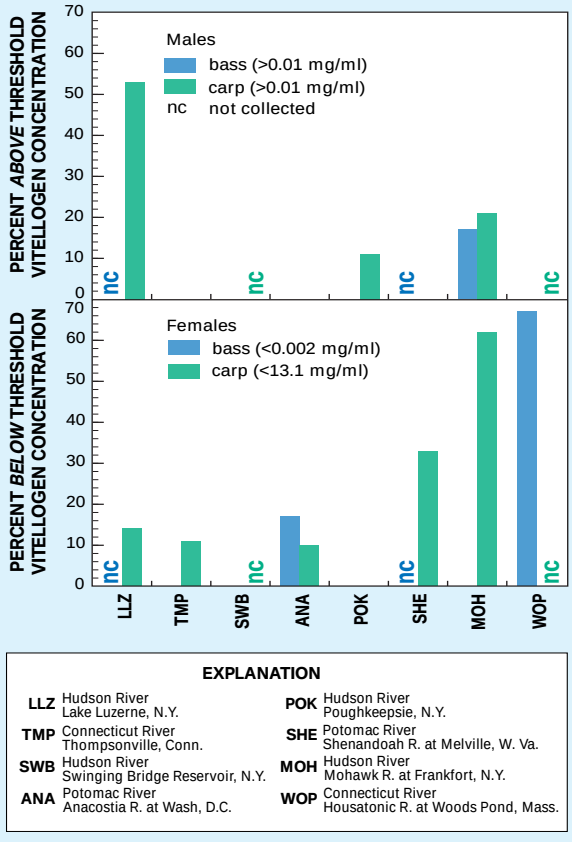

Figure 2. Percentages of male (top) and female (bottom) bass and carp (fall 1994 collections) with vitellogenin concentrations above or below potential statistical threshold levels.

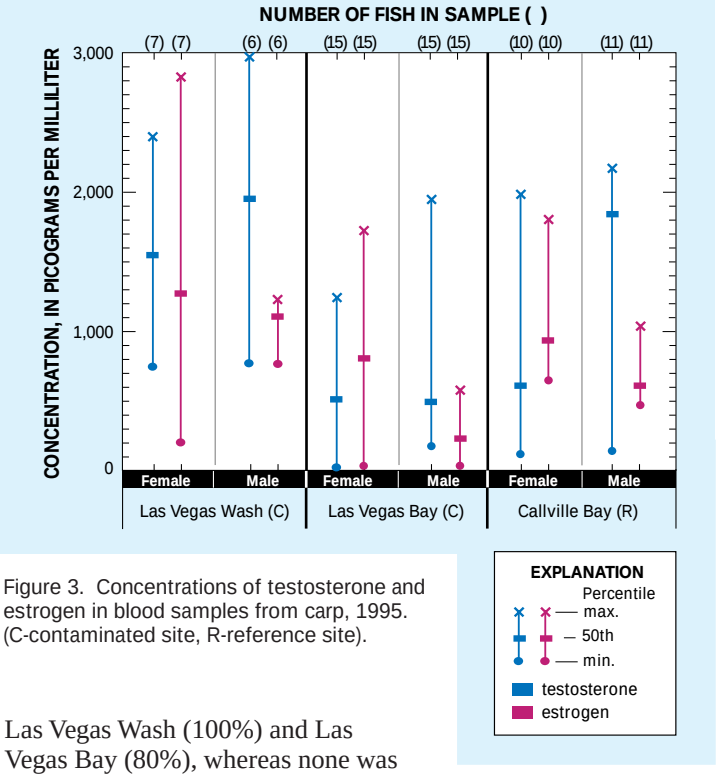

Figure 3. Concentrations of testosterone and estrogen in blood samples from carp, 1995. (C-contaminated site, R-reference site).

Las Vegas Wash (100%) and Las Vegas Bay (80%), whereas none was detected in any male carp from the reference site. Some vitellogenin concentrations in males from the contaminated sites were comparable to those in females sampled at the same site, up to 60 mg/mL (fig. 4). High vitellogenin concentrations in males has been associated with a decrease in testes weight and changes in the development of sperm (Jobling and others, 1996).

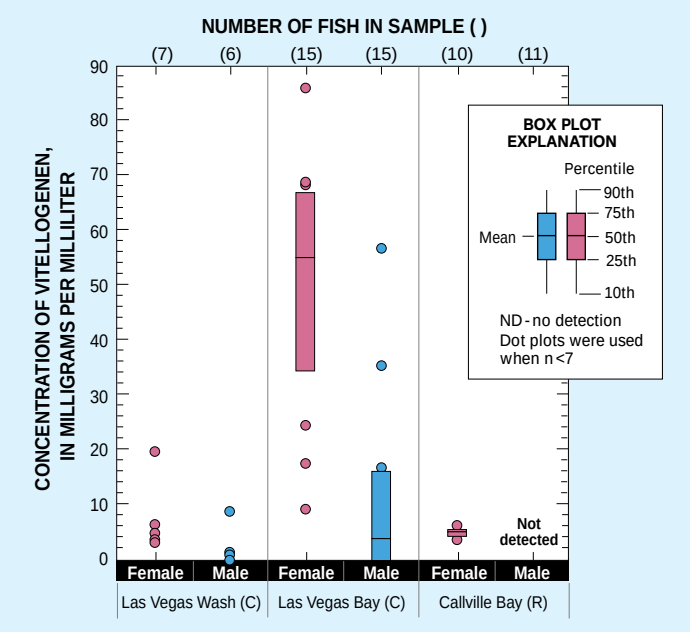

Figure 4. Vitellogenin concentrations in blood samples from carp sampled in Lake Mead, Nevada, 1995 (C-contaminated site, R-reference site).

Current and Future Research

As part of the BRD Biomonitoring of Environmental Status and Trends (BEST) program, scientists from the Environmental and Contaminants Research Center, Columbia, Mo., in collaboration with USFWS and the NAWQA program, conducted an investigation of contaminants and the potential effects on carp and bass throughout the Mississippi River basin. Among the suite of biomarkers analyzed during the study were estrogen and testosterone, vitellogenin, and sex organ maturity and condition. The sites in the current Mississippi River Basin study had been previously sampled for the National Contaminant Biomonitoring Program in the mid-1980's (Schmitt and others, 1990). These biomarker and tissue residue data are currently being analyzed and will be compared to similar endocrine biomarkers from the national reconnaissance investigation discribed above.

Large carp collected on the Hudson River near Poughkeepsie, N.Y.

Follow-up research planned or on-going by the USGS include documenting natural fluctuations in sex steroid hormone and vitellogenin concentrations and laboratory experiments to establish their baseline concentrations. The measurement of specific variables to test the ecological implications of observed hormone alterations, such as reproductive failure, sperm mobility, egg quality, induction of biomarker specific messenger-RNA, and fertilization and hatching success may be included in future studies.

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