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LOUISIANA

Health Assessment and Mercury Analyses of Fishes of the Atchafalaya National Wildlife Refuge

Study Identifiers:

1997-4F54 — 4N54

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Jill A. Jenkins

for

The Lafayette, Louisiana, Field Office

JULY 2002

U.S. FISH AND WILDLIFE SERVICE/SOUTHEAST REGION/ATLANTA, GEORGIA

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¹U.S. Geological Survey, National Wetlands Research Center, Lafayette, Louisiana 70508

**REGION 4
LAFAYETTE, LOUISIANA**

**HEALTH ASSESSMENT AND MERCURY ANALYSES OF FISHES
OF THE ATCHAFALAYA NATIONAL WILDLIFE REFUGE**

by

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Lafayette, LA**

July 2002

**National Wetlands Research Center
Final Laboratory Report (project number 536.01)
30 July 2002**

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PREFACE

This report documents the 1996-97 mercury and fish health monitoring performed by USGS and USFWS personnel in the Sherburne Wildlife Management Area of the Atchafalaya National Wildlife Refuge. The study was conducted as a pre-diversion monitoring study by the Lafayette USFWS Field Office, and was designed to investigate water management and water quality within the Big Alabama Bayou hydrologic unit. Over the last 3 years, plans have not been imminent for diverting water from the Atchafalaya River into Big Alabama Bayou, once a distributary of the River. Thus, the future dates for post-diversion monitoring are in question.

This investigation was a cooperative effort between the National Wetlands Research Center, Biological Resources Discipline, U.S. Geological Survey (USGS), and the Lafayette, Louisiana Ecological Services Field Office of the U.S. Fish and Wildlife Service (USFWS). The overall study was designed by the USFWS, samples collected by USFWS Fisheries Resource personnel, and NWRC conducted analytical testing, interpreted the results, and prepared this report.

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ABSTRACT

Mercury and poor water quality in the Atchafalaya River Basin have been documented by the Louisiana Department of Environmental Quality's Mercury Monitoring Program. The Atchafalaya River, a distributary of the Mississippi River in the Lower Mississippi River ecosystem, receives most of its volume by way of the U.S. Army Corps of Engineer's Old River Control Structure. This study assessed potential biological and ecotoxicological impacts of mercury contamination on resources in the Atchafalaya National Wildlife Refuge (NWR). Forty largemouth bass (*Micropterus salmoides*) and common carp (*Cyprinus carpio*) were collected by electroshocking in December 1996 and October 1997. Samples were analyzed for tissue mercury levels, liver enzyme activity (EROD), DNA integrity in blood cells, reproductive hormone levels, vitellogenin (Vg) levels, percentage of white blood cells, percentage of phagocytes, macrophage aggregates (MA) and parasites in spleen tissue, and health assessments as per the Biomonitoring and Environmental Status and Trends Program. Wet weight mercury levels ranged from 0.04 to 0.52 ppm. Largemouth bass had elevated EROD activity (decontamination enzymes) in both males and females, but carp did not have elevated EROD activity in either gender. Mercury level was correlated with increased EROD ($p=0.0001$). Aneuploidy was demonstrated with blood from one carp, where tissue mercury levels were significantly, positively correlated with an increase in DNA coefficient of variation (CV) ($p=0.0012$). Spleen weights were positively correlated with increased DNA CV ($p=0.0236$). Vg (egg protein precursor) was found in one male carp. Splenic parasites were prevalent in two fish. Bass spleens had significantly fewer MA than carp spleens ($p=0.0247$), but significantly more area of tissue was involved ($p=0.0003$). Tissue mercury levels were significantly, negatively correlated with overall gonadosomatic index for both species ($p<0.0001$), and when separated by sex (male, $p=0.0002$ and female, $p=0.0001$). For carp, there was a significant negative correlation for the percent of phagocytes ($p=0.0492$), indicating possible immunosuppression. The study of biomarker responses of two fish species with different life histories provided a thorough assessment of an ecosystem experiencing low dissolved oxygen and anthropogenic inputs.

INTRODUCTION

The Atchafalaya River Basin (ARB), being the largest remaining hardwood swamp in the United States, is a highly productive river-floodplain system (Rutherford et al. 2001). The dominant feature of the ARB is the Atchafalaya River, a distributary formed from 30% of the combined river flows of the Mississippi and Red rivers that is regulated by the U.S. Army Corps of Engineers. The ARB contains diverse aquatic habitats, including the distributaries from the Atchafalaya River, shallow headwater and backwater lakes, a network of bayous and canals (dredged oil and gas channels), and seasonally flooded swamps (Rutherford et al. 2001) (see map, p. 8).

In a 1981 study of the ARB (Winger et al. 1985), mercury, cadmium, and lead concentrations in both fish and sediment samples exceeded the 85th percentile of the National Pesticides Monitoring Program for these metals. Since 1986, the Louisiana Department of Environmental Quality (LDEQ) has conducted analyses to detect mercury contamination of fish from several water bodies throughout the state (LDEQ, 1995; Cormier, 1995). As part of their statewide monitoring program for mercury, reports have documented that levels of mercury in the ARB were elevated above background levels (LDEQ 1995; LDEQ 1996). In contrast, fish collected by LDEQ from the Mississippi River since 1990 did not have elevated mercury levels (Henrich, M.D. et al. 1995).

Mercury in natural waters exists in multiple forms, where most water bodies in the Northern Hemisphere are probably contaminated because of long-range transport and deposition of mercury from anthropogenic sources (Wiener and Spry 1996). The methylation of mercury to the toxic methyl mercury is primarily a microbial process. Some environmental conditions often associated with high mercury levels include newly flooded reservoirs, low acid-neutralizing capacity waters, high humic content, shallow lakes with high littoral/pelagic ratios, gold mining, industrial discharges, agricultural use of alkylmercury fungicides, disturbed wetlands (USDI 1998). The diet is the primary route of methylmercury uptake by fish in natural waters, where accumulation seems to be most rapid in summer months (Wiener and Spry 1996).

The regulatory and scientific focus on mercury in aquatic ecosystems has been motivated largely by the health risks of consuming contaminated fish, because exposure to methylmercury is almost wholly due to consumption of fish. The U.S. Environmental Protection Agency (EPA) aquatic life criterion for mercury (EPA 1980) was based upon the hazard to humans rather than hazards to fish, where the criterion regulated that the concentration in water which can result in 1 mg/kg (ppm) wet weight (ww) in fish. Louisiana has adopted a more restrictive human health advisory standard of 0.5 mg/kg, consistent with the National Academy of Sciences recommendations (NAS) 1978.

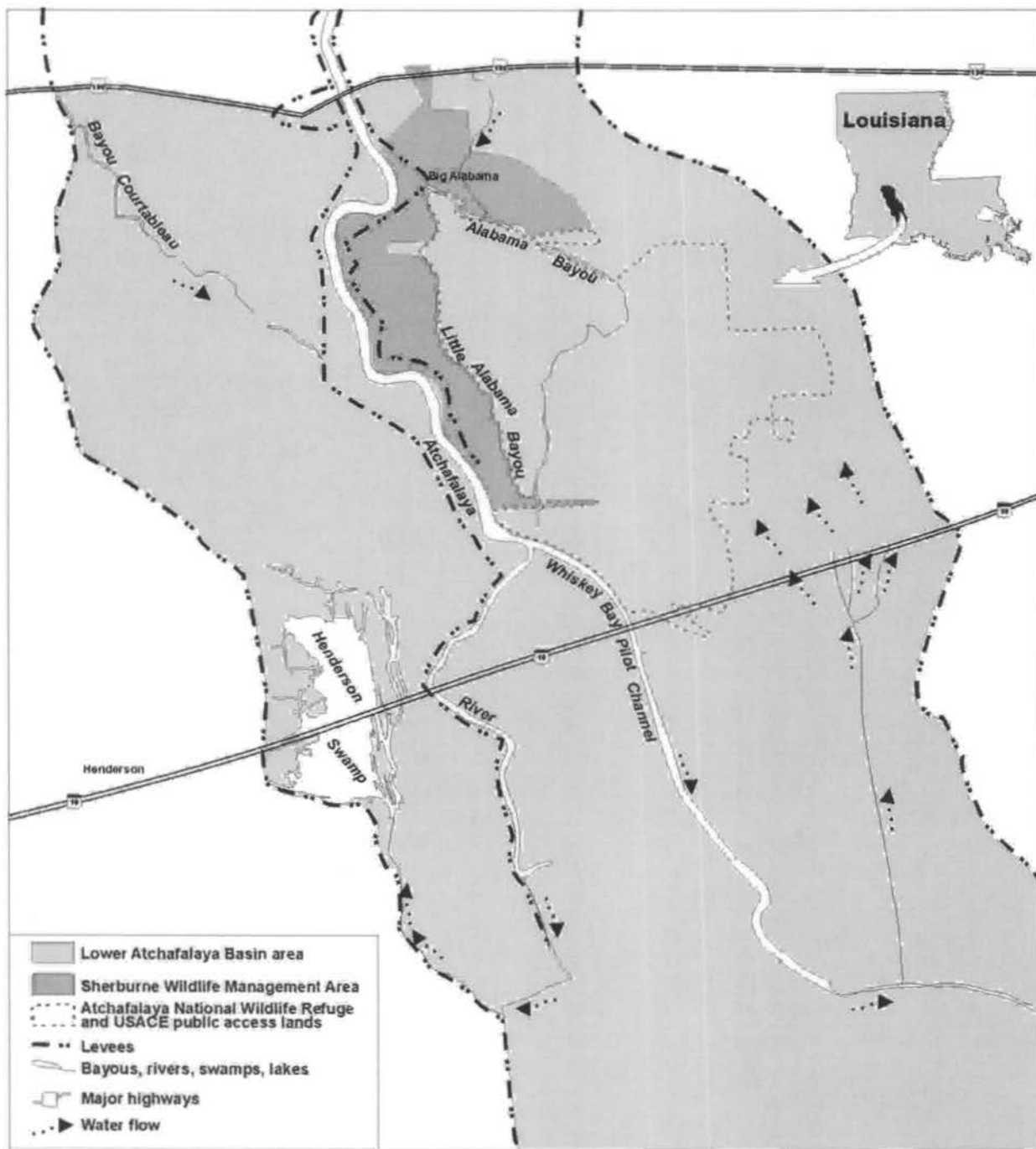
Monitoring programs for mercury typically employ fillet or whole body samples. Muscle tissue is the only tissue for which data have been obtained on methylmercury-intoxicated fish in both laboratory and field studies. The range for lab studies is similar (Wiener and Spry 1996). Background levels at 0.08 mg/kg ww have been seen in redbreast sunfish (*Lepomis auritus*) and 0.11 mg/kg wet weight in bluegill (*Lepomis machrochirus*), whereas rainbow trout (*Oncorhynchus mykiss*) exhibited chronic effects at 1 to 5 mg/kg wet weight whole body.

The objective of this study was to examine fish from an ecosystem with historically high mercury (Winger and Andreasen 1985) and low dissolved oxygen (DO)

levels. Data were collected prior to a scheduled increase in freshwater flow through the watershed by way of the Old River Control Structure. Water management regimes may change in the future, as which time a parallel post-diversion study would provide information on how the environmental conditions are influencing the health of the fish. In light of this study not addressing both pre- and post-diversion biological data, the revised objective was to of performing an interspecies comparison of bioindicator data. Compound bioavailability and metabolism can vary tremendously among chemicals and across species (Immunotoxicology Technical Committee 2001).

Biomarkers are physical changes reflective of exposure to contaminants. The magnitude of change is measurable and often related to the severity of the stressor. Genomic DNA alterations or fragmentations are widely used in physiological, genetic and toxicological studies. In addition to increased DNA coefficient of variation (CV), deviations from normal diploid histograms can be noted in cells from animals exposed to contaminants. Macrophage aggregates (MA) are accumulations of macrophages found in organs that have been correlated to low DO and xenobiotic exposure. The mixed-function oxygenase enzyme system, playing roles in detoxification and molecular breakdown, is induced by contaminants. Hepatic vitellogenin in males has been used as a biomarker for environmental estrogens. Deviant reproductive hormone ratios have been used to indicate endocrine abnormalities.

Sublethal stress is generally first manifested at the sub-organismal level, where effects can be measured via cellular components such as enzymes or functions, such as the immune system. Depending on its severity, sublethal stress may limit physiological systems, reduce growth, and impair reproduction. Animal health and biological integrity of ecosystems may be impacted. In this study, largemouth bass, highly popular for sportfishing, and common carp, ubiquitous in U.S. watersheds, were chosen as for study.



MATERIALS AND METHODS

Animals

Fish were collected by electrofishing in December 1996 and October 1997. Twenty largemouth bass (*Micropterus salmoides*)(LMB) (10 males and 10 females), and twenty common carp (*Cyprinus carpio*) (10 males and 10 females) were collected, held in live wells, and processed immediately at WMA headquarters building. Average water quality parameters for the time periods at the collection location were dissolved oxygen at 2.5 mg/L and 18.7°C average temperature. Health assessments were recorded as per the USGS Biomonitoring of Environmental Status and Trends (BEST) protocol (Schmitt et al. 1995). Observations were made on length, weight, internal and external body appearance, parasite incidence, and organ color and condition.

Mercury Concentrations

The sample matrix for mercury determinations was whole fish bodies. Data were obtained by Geochemical and Environmental Research Group, Texas A & M, College Station, Texas, subcontracted laboratory through Patuxent, USGS, Laurel, MD. Mercury was determined by EPA method 245.5 with minor revisions. Tissue samples were homogenized prior to subsampling. After preparation and digestion, samples were reduced to elemental mercury, aerated, and measured by atomic absorption spectrophotometry.

Reproductive Effects

Typical biomarkers used to assess possible reproductive abnormalities have included the vitellogenin, plasma sex hormones (17 β -estradiol and 11-ketotestosterone), and organ histopathology. Gonadosomatic indices (GSI) were calculated according to (gonad weight/total weight) x 100.

Blood serum was stored in cryovials in liquid nitrogen containers until analysis for Vg. Egg protein, or vitellogenin, concentrations were determined by Dr. Nancy Denslow of the University of Florida, Gainesville.

Blood serum stored in liquid nitrogen was sent for analysis of estradiol and testosterone. Equitech laboratories provided those data.

Liver enzymes

Ethoxyresorufin-O-deethylase activity (EROD assay) were determined by Dr. Don Tillett, Columbia Environmental Research Center, BRD, USGS, Columbia, MO.

Age Estimates

Otoliths from largemouth bass were processed and analyzed by FWS personnel, Baton Rouge, LA, to estimate ages of individuals.

Spleen Tissues

Splenic indices were calculated according to spleen weight/body weight = spleen index.

Spleens were stored in buffered formalin, and histologically processed and stained by the Louisiana State University Veterinary School Pathology Laboratory. Sections were stained using periodic acid schiff protocol for optimal visualization of macrophage aggregates. Macrophage aggregates were scored at NWRC using brightfield microscopy and image analysis for frequency, area, and percent total area.

The presence of splenic parasites were noted during examination of histological sections.

Differential Blood Cell Count

Whole blood was collected into sodium heparin anticoagulant for smearing and fixation. Duplicate slides per Wright Geimsa (Fisher Scientific,) and Sudan Black B (Ellsaesser et al. 1984). For total white blood cell (WBC) counts, leukocytes and erythrocytes were counted to total between 300 and 500 on Wright Giemsa-stained slides. For percent phagocytes, at least 100 WBC were categorized as either lymphocyte (thrombocyte, lymphocyte, and others) or phagocytes (neutrophil or monocyte) per Sudan Black B slides.

Blood DNA Integrity

Whole blood was collected into acid citrate dextrose anticoagulant for analysis of nuclear DNA integrity. Cells at one million nuclei per mL were stained using a standard propidium iodide nucleic acid staining dye, and measurements made by flow cytometry with a FACScan instrument (Becton Dickinson Immunocytometry Systems, San Jose, CA)(BDIS). Data were collected in one parameter histograms, and analyzed using CellQuest software (BDIS) primary peak coefficient of variation (CV).

Statistical Analyses

Data were analyzed using SAS by Rassa Dale, NWRC statistician. Models for detecting differences between and among species and sexes, as well as for detecting correlations and covariance were used. Data were transformed as necessary.

RESULTS

Please see Tables and Figures for data displays. Results from statistical analyses are bulleted below:

1. Average concentrations of mercury, coefficients of variation of blood cell DNA, length to weight ratios (L/Wt), estradiol to testosterone levels, and EROD levels, and spleen to body weight ratios were lower in carp than in largemouth bass.
2. Significant differences between species were noted in mercury concentration, DNA CV, L/Wt, EROD.
3. Mercury (ppm): At average concentrations of 0.5 ppm, LA DEQ issues fish consumption advisories. Overall, tissue Hg levels were significantly, positively correlated with increased DNA CV, L/Wt, and EROD.
4. Blood from one male carp (fish #14) showed aneuploidy.
5. LMB DNA CVs were significantly wider than those from carp blood.
6. Although 5 individuals exhibited high E/T ratios, no significant differences or correlations were found.
7. One male carp (fish 17) displayed vitellogenin at (0.003ug/mL).
8. LMB ages ranged between 1 and 3 years.
9. Hepatic EROD rates in LMB were elevated 3-10 fold greater than what has been previously reported for LMB species from reference locations (D. Tillett, personal communication).
10. Hepatic EROD rates in carp were similar to those in carp collected from a reference site in another study (D. Tillett, personal communication).
11. As per L/W ratio analyses, estimated total fish weights were similar to actual weights.
12. In carp, there were significantly higher numbers of spleen macrophage aggregates than in LMB, but they were smaller and occupied significantly less area/spleen than those from LMB.
13. Spleen Wt/Body Wt was significantly, positively correlated with DNA CV.
14. Splenic parasites were noted only in male LMB: fish 2 (4 parasites), fish 6 (38 parasites), fish 22 (39 parasites.)

15. Tissue mercury levels were significantly, negatively correlated with overall GSI ($p < 0.0001$) and when separated by sex (female $p < 0.0001$, and male $p < 0.0002$).
16. The only significant correlation of tissue mercury levels with microscopic blood parameters was a negative correlation with the percent phagocytes for carp ($p < 0.0492$).

Table 1. SUMMARY OF CORRELATIONS: '+' indicates a significant positive correlation, '-' indicates a significant negative correlation, and 'NS' indicates no correlation (non-significant).

MERCURY

<u>VARIABLE</u>	<u>Overall</u>	<u>Female</u>	<u>Male</u>
DNA-CV	+	+	+
WEIGHT (Wt)	NS	NS	-
LENGTH	-	NS	-
L/ Wt	+	NS	+
E/T	NS	NS	NS
EROD	+	+	+

SPLEEN WT/WT

dna-cv	+	+	ns
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Table 2. SUMMARY STATISTICS for Macrophage Aggregate (MA) analyses.

SPECIES

<u>VARIABLE</u>	<u>CARP</u>	<u>LMB</u>
Avg. Frequency/View	12.7	7.9
Avg. Total MA Area/View	23001 μ	45096 μ
Avg. Size MA	2177 μ	6317 μ
Avg. Area/Spleen	1.6%	3.2%

Table 3. Carp E/T ratio, Vitellogenin, and Mercury Concentrations at Atchafalaya NWR

Sample I. D.	Species	Sample Matrix	Collection Date	Sex by Necropsy	E/T ratio	Sex by E/T ratio	Vitello-genin	Sex by Vitellogenin	Hg Conc. ww (ppm)
BA-14	Com. carp	Wholebody	12/ 4-5 /96	Male	0.016	Male	0.000	Male	0.09
BA-17	Com. carp	Wholebody	12/ 4-5 /96	Male	0.016	Male	0.003	Male	0.14
BA-30	Com. carp	Wholebody	10/21-22/97	Male	0.083	Male	0.000	Male	0.07
BA-33	Com. carp	Wholebody	10/21-22/97	Male	0.009	Male	0.000	Male	0.14
BA-35	Com. carp	Wholebody	10/21-22/97	Male	0.014	Male	0.000	Male	0.08
BA-36	Com. carp	Wholebody	10/21-22/97	Male	0.015	Male	0.000	Male	0.06
BA-37	Com. carp	Wholebody	10/21-22/97	Male	0.020	Male	0.000	Male	0.15
BA-38	Com. carp	Wholebody	10/21-22/97	Male	0.010	Male	0.000	Male	0.19
BA-39	Com. carp	Wholebody	10/21-22/97	Male	0.008	Male	0.000	Male	0.14
BA-40	Com. carp	Wholebody	10/21-22/97	Male	0.012	Male	0.000	Male	0.11
				GEO. MEAN	0.015		0.000		0.11
BA-7	Com. carp	Wholebody	12/ 4-5 /96	Female	0.397	?Female?	1.327	Female	0.10
BA-8	Com. carp	Wholebody	12/ 4-5 /96	Female	0.495	Female	0.632	Female	0.12
BA-16	Com. carp	Wholebody	12/ 4-5 /96	Female	1.243	Female	0.107	Female	0.08
BA-18	Com. carp	Wholebody	12/ 4-5 /96	Female	0.659	Female	0.165	Female	0.09
BA-19	Com. carp	Wholebody	12/ 4-5 /96	Female	0.606	Female	0.144	Female	0.04
BA-24	Com. carp	Wholebody	10/21-22/97	Female	0.548	Female	0.130	Female	0.16
BA-25	Com. carp	Wholebody	10/21-22/97	Female	0.494	Female	2.210	Female	0.13
BA-28	Com. carp	Wholebody	10/21-22/97	Female	0.600	Female	4.203	Female	0.14
BA-29	Com. carp	Wholebody	10/21-22/97	Female	0.715	Female	0.854	Female	0.09
BA-31	Com. carp	Wholebody	10/21-22/97	Female	0.604	Female	3.232	Female	0.17
				GEO. MEAN	0.6073 4		0.6092		0.10

**Table 4. Largemouth Bass E/T ratio, Vitellogenin, and Mercury Concentrations
Atchafalaya NWR**

Sample I. D.	Species	Sample Matrix	Collection Date	Sex by Necropsy	E/T ratio	Sex by E/T ratio	Vitello- genin	Sex by Vitellogenin	Hg Conc. ww (ppm)
BA-1	LMB	Whole	12/ 4-5 /96	Male	0.007	Male	0.000	Male	0.17
BA-2	LMB	Whole	12/ 4-5 /96	Male	0.013	Male	0.000	Male	0.19
BA-3	LMB	Whole	12/ 4-5 /96	Male	0.032	Male	0.000	Male	0.22
BA-5	LMB	Whole	12/ 4-5 /96	Male	0.104	Male	0.000	Male	0.41
BA-6	LMB	Whole	12/ 4-5 /96	Male	0.066	Male	0.000	Male	0.45
BA-9	LMB	Whole	12/ 4-5 /96	Male	0.007	Male	0.000	Male	0.44
BA-12	LMB	Whole	12/ 4-5 /96	Male	0.017	Male	0.000	Male	0.42
BA-21	LMB	Whole	10/21-22/97	Male	0.064	Male	0.000	Male	0.36
BA-22	LMB	Whole	10/21-22/97	Male	0.130	Male	0.000	Male	0.21
BA-23	LMB	Whole	10/21-22/97	Male	0.775	?Female?	0.000	Male	0.28
				GEO. MEAN	0.042		0.000		0.29
BA-4	LMB	Whole	12/ 4-5 /96	Female	0.562	Female	0.334	Female	0.14
BA-10	LMB	Whole	12/ 4-5 /96	Female	0.183	?????	0.491	Female	0.25
BA-11	LMB	Whole	12/ 4-5 /96	Female	1.276	Female	0.913	Female	0.52
BA-13	LMB	Whole	12/ 4-5 /96	Female	1.333	Female	0.534	Female	0.20
BA-15	LMB	Whole	12/ 4-5 /96	Female	1.302	Female	0.277	Female	0.39
BA-20	LMB	Whole	10/21-22/97	Female	0.163	?????	0.000	?Male?	0.35
BA-26	LMB	Whole	10/21-22/97	Female	1.895	Female	0.134	Female	0.41
BA-27	LMB	Whole	10/21-22/97	Female	0.732	Female	0.008	?Male?	0.20
BA-32	LMB	Whole	10/21-22/97	Female	0.694	Female	0.000	?Male?	0.36
BA-34	LMB	Whole	10/21-22/97	Female	0.790	Female	0.000	?Male?	0.42
				GEO. MEAN	0.700		0.269		0.30

**Table 5. Percent Coefficient of Variation for Whole
Blood DNA and Mercury Concentration per Fish**

<u>Fish ID #</u>	<u>DNA CV</u>	<u>Hg (ppm)</u>	<u>Species</u>
1	2.74	0.17	LMB
2	2.01	0.19	LMB
3	2.27	0.22	LMB
4	2.23	0.14	LMB
5	3.16	0.41	LMB
6	2.26	0.45	LMB
7	1.39	0.1	CARP
8	1.65	0.12	CARP
9	2.08	0.44	LMB
10	3.32	0.25	LMB
11	2.76	0.52	LMB
12	2.1	0.42	LMB
13	2.89	0.2	LMB
14	1.96	0.09	CARP
15	1.96	0.39	LMB
16	1.08	0.08	CARP
17	1.68	0.14	CARP
18	2.34	0.09	CARP
19	1.27	0.04	CARP
20	2.07	0.35	LMB
21	2	0.36	LMB
22	3.25	0.21	LMB
23	2.13	0.28	LMB
24	1.24	0.16	CARP
25	1.48	0.13	CARP
26	2.08	0.41	LMB
27	2.38	0.2	LMB
28	2.18	0.14	CARP
29	1.54	0.09	CARP
30	1.82	0.07	CARP
31	1.48	0.17	CARP
32	2.02	0.36	LMB
33	1.5	0.14	CARP
34	2.34	0.42	LMB
35	1.65	0.08	CARP
36	1.54	0.06	CARP
37	1.46	0.15	CARP
38	1.32	0.19	CARP
39	1.47	0.14	CARP
40	1.42	0.11	CARP

**Table 6. Percent White Blood Cells per Fish
and Percent Phagocytes of Total WBC**

<u>Fish ID #</u>	<u>%WBC</u>	<u>%Phagocytes</u>
1	5.48%	49.00%
2	16.41%	48.00%
3	6.64%	48.00%
4	1.02%	70.45%
5	10.05%	56.00%
6	14.16%	24.00%
7	3.81%	54.55%
8	5.49%	69.23%
9	4.46%	57.69%
10	4.85%	66.15%
11	5.41%	75.47%
12	3.16%	43.14%
13	5.78%	74.14%
14	3.37%	67.27%
15	4.03%	49.15%
16	2.92%	70.59%
17	4.69%	61.76%
18	6.88%	76.47%
19	6.84%	70.69%
20	7.01%	73.68%
21	3.46%	56.14%
22	1.01%	26.42%
23	7.89%	34.48%
24	11.50%	20.37%
25	11.20%	41.00%
26	8.07%	66.67%
27	4.00%	33.33%
28	5.80%	50.00%
29	9.90%	47.17%
30	4.50%	67.31%
31	6.96%	55.74%
32	7.33%	69.09%
33	4.82%	72.73%
34	5.69%	73.53%
35	3.20%	40.98%
36	6.20%	51.79%
37	4.89%	40.74%
38	3.48%	42.86%
39	5.92%	51.79%
40	1.48%	n.d.

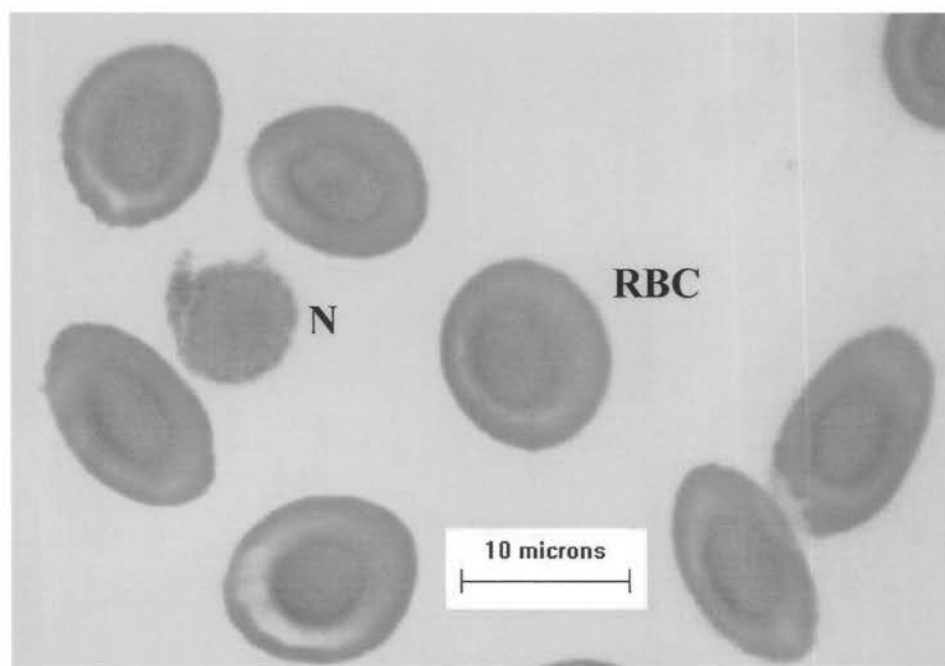
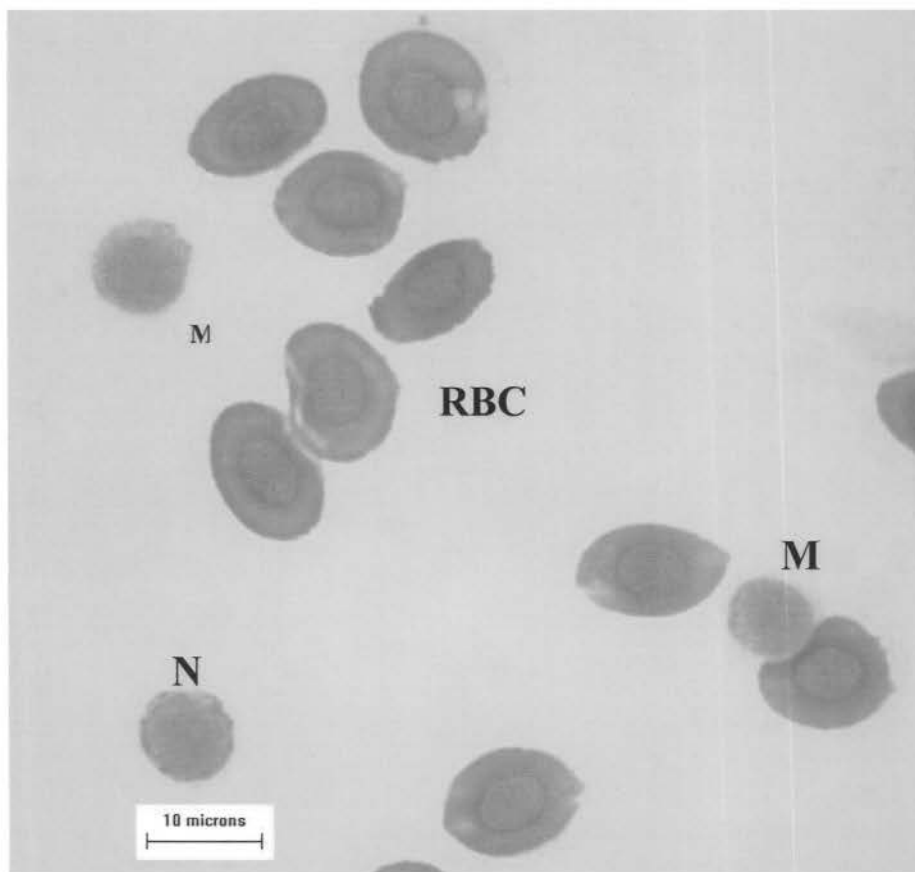


Figure 1. Carp (Fish #7) whole blood stained with Wright Giemsa. Red blood cells (RBC), neutrophils (N), and monocytes (M) are labeled. Less mature RBC are round rather than oval.

Weight(Log10) vs Estimated Weight(Log10)

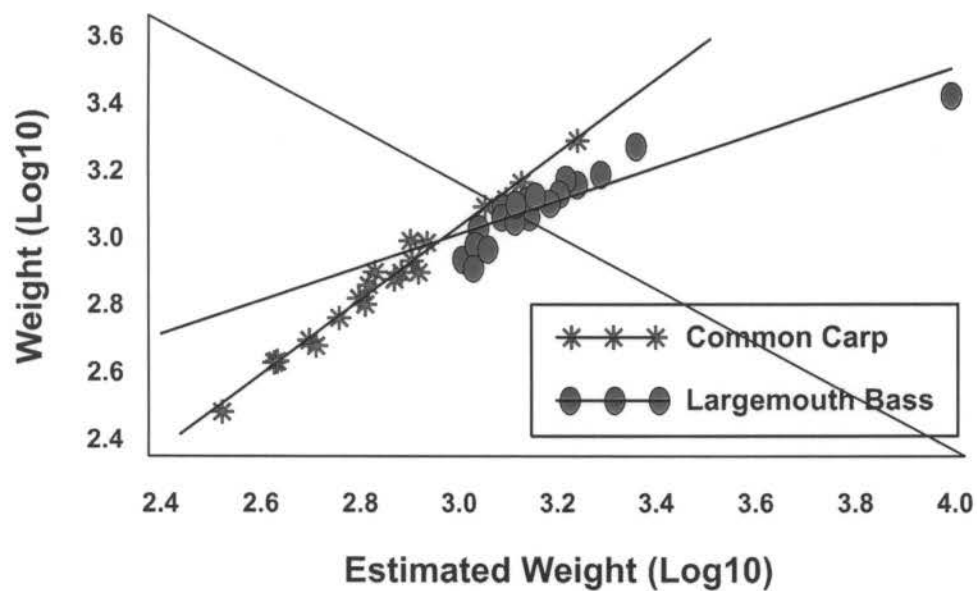


Figure 2. Weight of Carp and LMB (log 10) versus Estimated Weight (log 10)

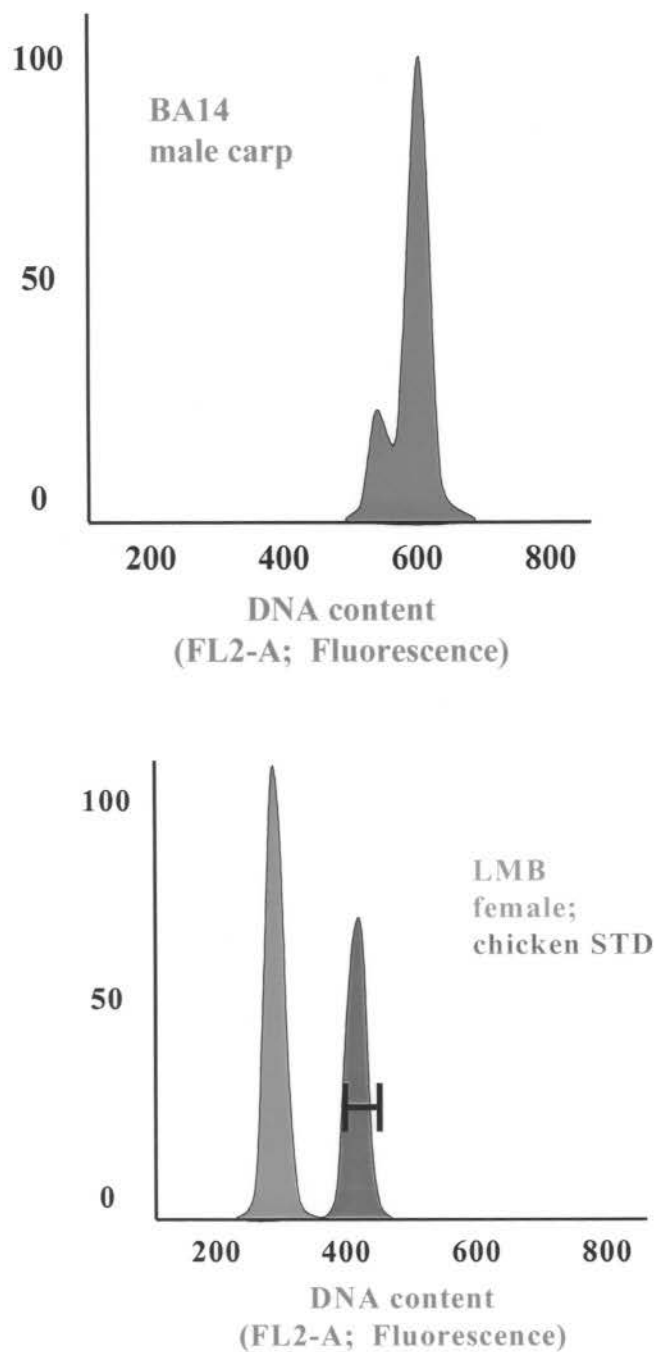


Figure 3. The top graphic shows a hypodiploid peak of whole blood DNA stained with propidium iodide. The bottom graphic is scanned from a flow cytometric analysis of normal largemouth bass blood compared with an internal chicken blood standard. Coefficients of variation were calculated at half maximum peak height and width, as shown with brackets on the chicken blood peak.

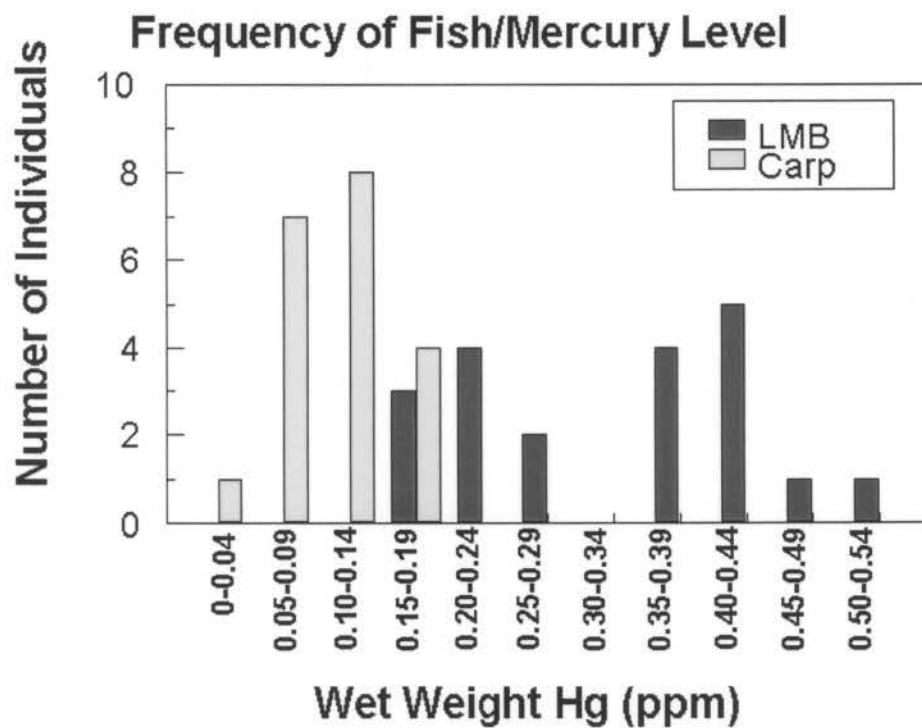


Figure 4. Number of fish per mercury concentration level.

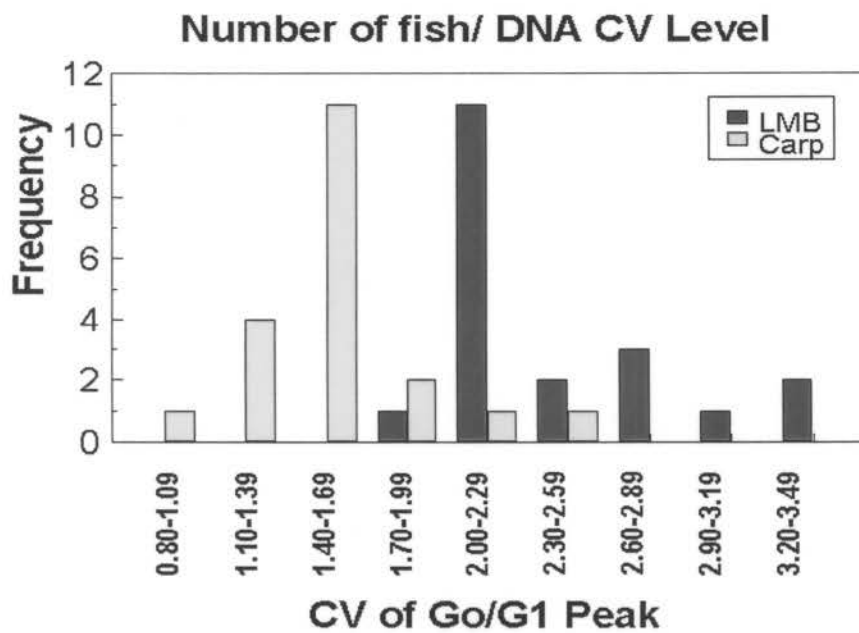


Figure 5. Number of fish per DNA CV level.

Mercury vs DNA_CV

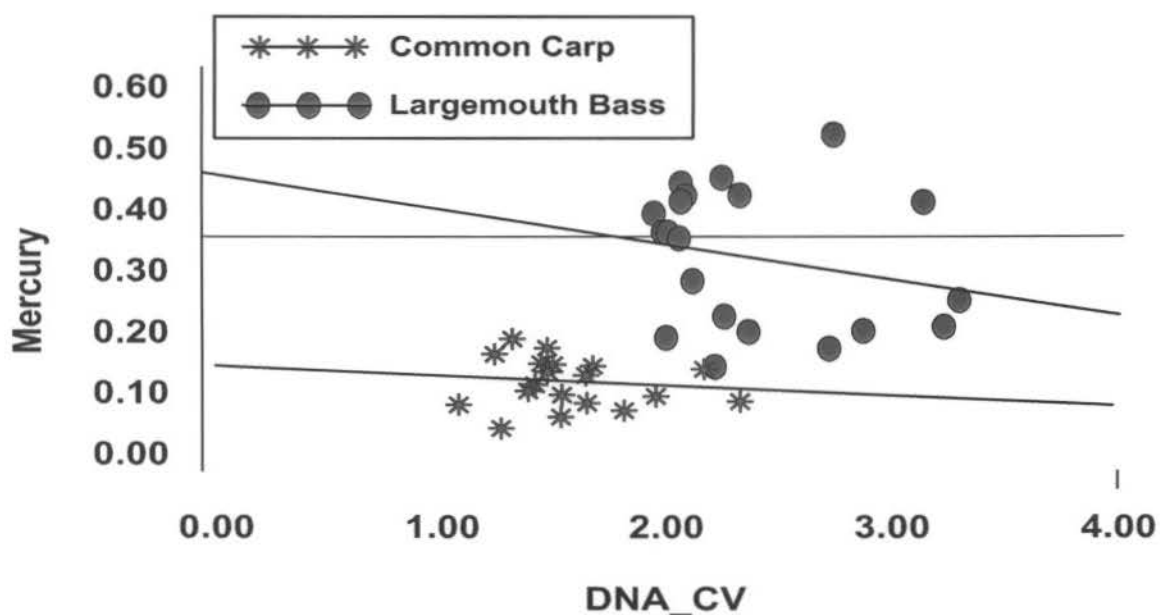


Figure 6. Mercury versus DNA CV per fish.

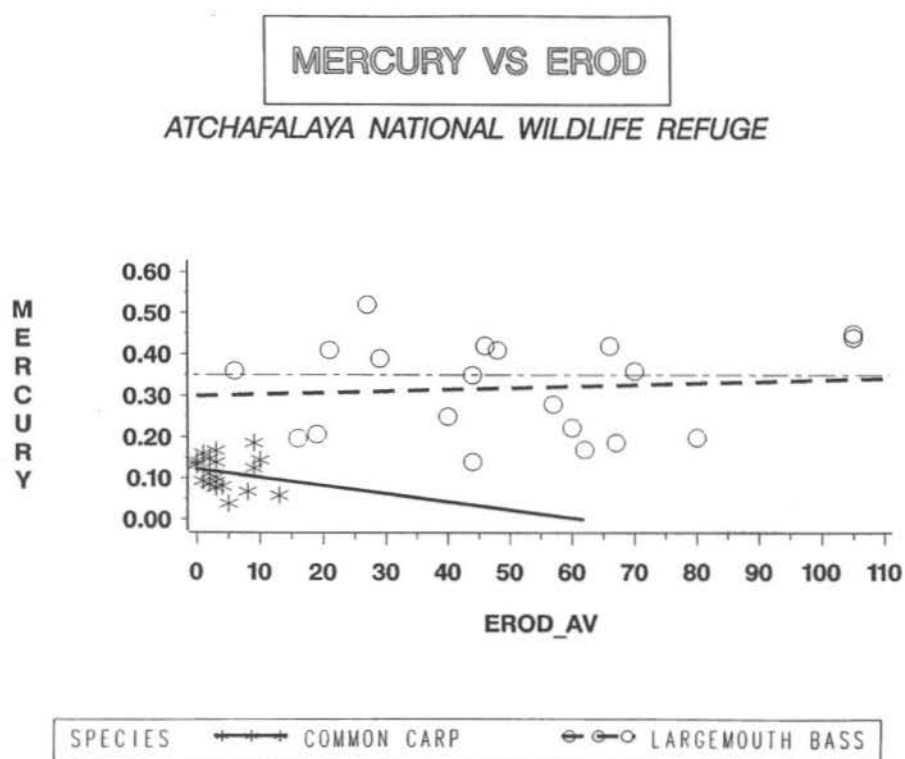


Figure 7. Mercury versus average EROD level.

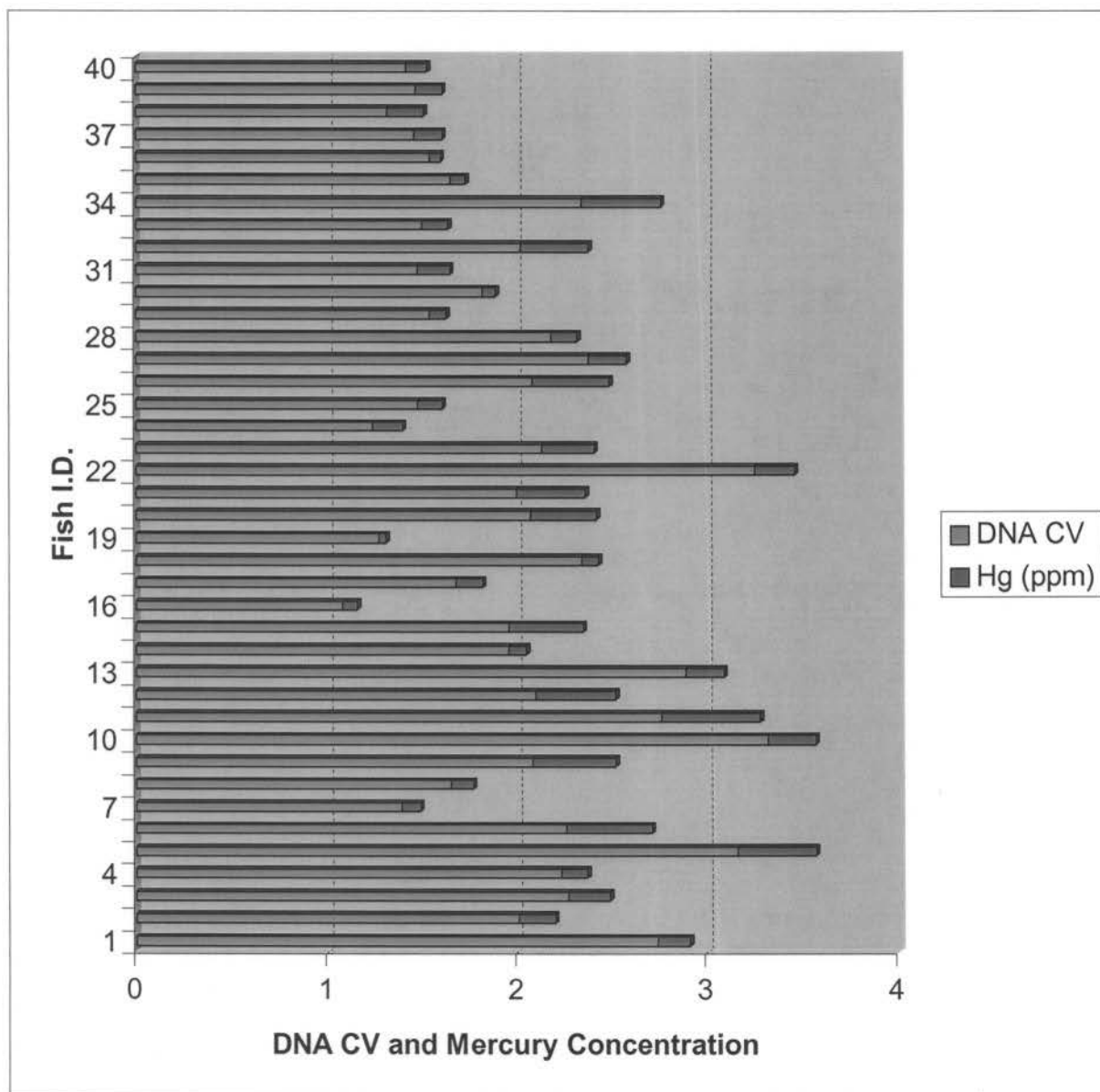


Figure 8. Mercury concentrations and DNA CV of G_0 peak per fish I.D.. Carp are denoted with green and largemouth bass are denoted with blue bars.

DISCUSSION

Prior to disturbances being realized at the ecosystem level, changes are noted in lower levels of biological organization, such as the tissue, cell, and molecular levels. In the case of potential negative influences of nonpoint source mercury, measuring contaminants levels in fish tissues, biomarkers are useful for assessing impacts. As in this study, the efficient use of financial resources available for thorough biological monitoring should take advantage of interspecies differences in responses. The original intent of this project was to study animals from the site in pre- and post-diversion samplings, therefore no reference sites, having known low levels of environmental mercury, were designated. Therefore, some comparisons were made from other studies, from scientific literature, having employed carp and LMB. In essence, a robust interspecies comparison was performed at a site with historically high mercury levels (Cormier, 1995; LDEQ, 1995; LDEQ, 1996; Winger and Andreasen 1985).

Heavy metals, pesticides, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) are considered persistent pollutants (Weiner and Spry 1996). Because analytical costs for contaminants is not inexpensive, and high mercury levels occur in many aquatic ecosystems worldwide due to human consumption of fishes, mercury is often the sole analyte. By supplementing such studies with biomarker data, which may be obtained non-destructively as in the case of bioindicators using blood, a more thorough assessment of environmental condition may be derived. For instance, increases in splenic MA's parameters indicates that some organic contaminants are present, as high MA are coincident with organic contaminants and low DO (Fournie et al. 2000). The macrophage aggregate analyses indicate that each species has a different mode of macrophage aggregate formation, and that baseline and experimental values are valid per species and not between species.

Only 1 of 40 animals displayed mercury concentrations over 0.5 ppm. Although tissue mercury levels were within legal limits, these results show that both the carp and LMP populations experienced bioaccumulation and biological responses. Water quality conditions at the site were less than optimum throughout the study. Because mercury concentrations can fluctuate seasonally, times for sampling should be consistent, as in this study, over the course of a single year or over multiple years.

Biaccumulation can be effected by factors specific to the fish species, such as diet and reproductive status (Foster et al. 2000). Fish at lower trophic levels tend to have lower concentrations of mercury than fish at higher trophic levels (Potter et al. 1975), as also seen with this study. Diet is the primary route of methylmercury uptake by fish in natural waters (Weiner and Spry 1996).

Hematology, a useful diagnostic tool for animal health, has been employed in establishing a blood profile for LMB (Clark et al. 1979). Hematological effects can be detected by examination of the relative proportions can be detected by examination of the relative proportions of different blood cell types (Blaxhall 1972; Llorrente et al. 2002). In this study of blood cells identified by differential blood cell staining, the results showed that phagocyte number was elevated in carp. In response to stressors in the aquatic environment, an overall drop in WBC would indicate immunosuppression. This drop would be mediated by corticosteroids and is a fundamental mechanism in the increased susceptibility of fish to disease organisms. Phagocyte (neutrophil) numbers, as

in this study, increase in response to stressors or an increase in cortisol. Following exogenous cortisol administration or exposing fish to stressful conditions, readily observed effects were changes in the total number of circulating leukocytes, and quantitative shifts with the differential pattern (Wojtazek et al. 2002). Age influences the distribution of leukocytes among tissue compartments (Maule and Schreck 1990). In this study, the range of fish ages was not large. Interspecific differences have been seen in circulating cortisol levels (Pottinger and Brierley 1999).

Chronic stress due may result in reproductive abnormalities and loss of fish numbers and diversity. The metrics for measuring reproductive effects for this study included vitellogenin, estradiol, testosterone, and GSI. Tissue mercury levels were significantly, negatively correlated with GSI, and vitellogenin in one male carp. Mercury is an organometallic contaminant, and its tissue distribution does not correlate with tissue lipid content (Foster et al. 2000). However, total mercury in liver and gonads has been correlated with gonad mercury, thereby indicating that reproductive status and/or season may be a factor to be considered. Seasonal differences in mercury concentration in LMB were noted in ovaries and liver, whereas no seasonal differences were noted for muscle tissues.

DNA measurements are widely used in physiological, genetic and toxicological studies. Many waterborne pollutants have cytogenetic properties which in fish cause enhanced frequency of chromosomal aberrations or the alteration of the structure of DNA. Induced changes can be heritable. Use of flow cytometry allows the determination of DNA changes in the circulating blood population. Altered synthesis of DNA content in blood from non-tumor-bearing fish from polluted areas would be consistent with the presence of promoting influences acting upon initiated cells, where cell proliferation could be detected. In this study, the integrity of DNA was measured, where compromised DNA can be seen by additional peaks other than the G_0 diploid peak, or by wide G_0 diploid peaks (Dallas et al. 1998; Easton et al. 1997) as measured by its coefficient of variation (Figures 3). Increased CVs have indicated chronic exposure to genotoxins (Dallas et al. 1998; Easton et al. 1997). Comparing mean DNA CVs between groups is helpful when sample sizes are large and the level of genetic damage is high. However, when sample sizes are small and there are subtle influences, frequencies (Figure 5) are useful to show non-normal distributions of individual CVs. Even with a sample size of 40 animals, one fish (male carp #14) displayed a hypodiploid peak. Significant differences in DNA CV's were noted between species, where CVs were significantly correlated with mercury levels.

The differences in species in cytogenetic effects, phagocyte proportions, spleen histology results, indicate differences in how the animals respond to chronic exposure to an ecosystem with historically high mercury levels. Spleens, the site of hematopoietic activity, were significantly positively correlated with DNA CV. This relationship might be further explored in bioindicator studies.

Biomarkers that directly indicate injurious effects on animal reproduction contribute valuable information on mechanisms underlying effects of xenobiotic agents, and in identifying problems earlier than typical surveys used to assess population declines. In the present study, a suite of measured biomarkers at different levels of biological organization was analyzed. Differences and correlations were analyzed

between species, sites, sexes, and parameters. Such studies allow for understanding the links between water regulation and animal responses due to changes in water regimes.

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