# Monitoring for the occurrence and effects of endocrine disrupting chemicals in fish


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**U.S. Geological Survey**

# Overview

- Biomonitoring Environmental Status and Trends – Large Rivers Monitoring Network
  - Endpoints
  - Summary findings
  - Evaluation of endocrine metrics
- Lessons learned
- Recommendations for biological monitoring

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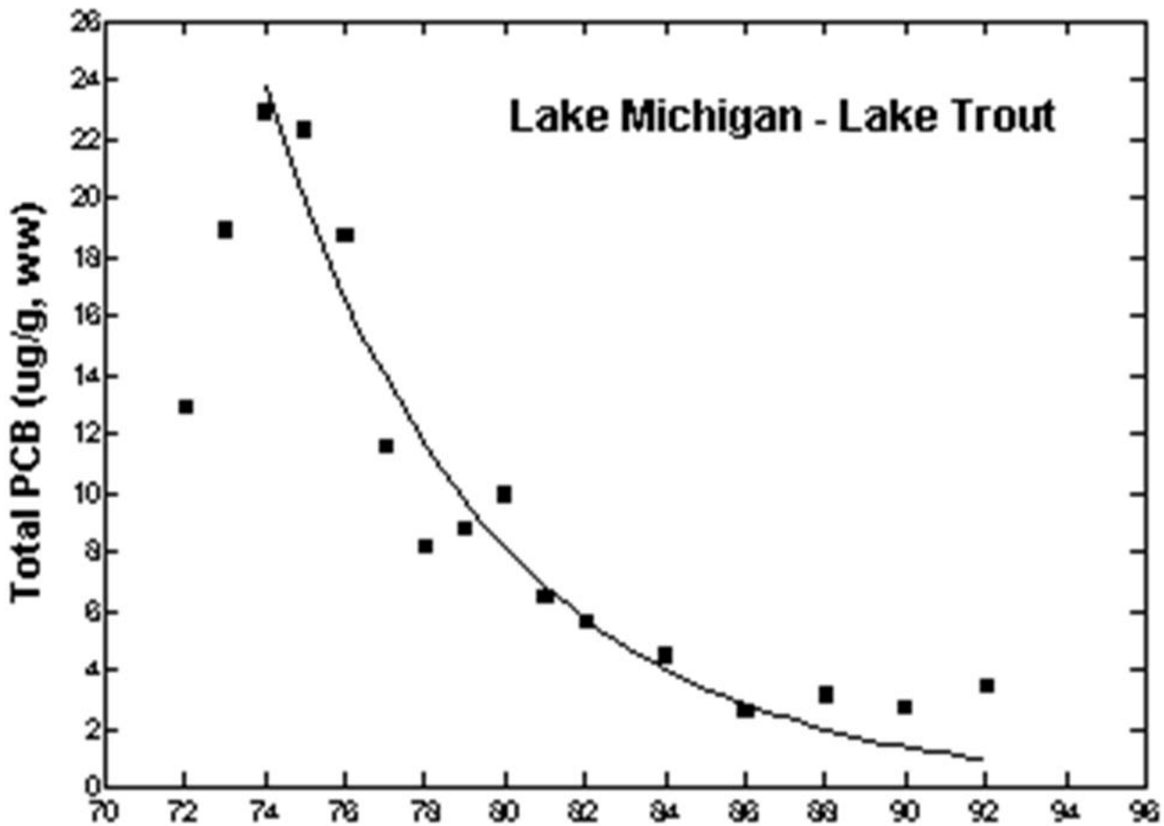
# National Contaminants Biomonitoring Program (NCBP)

**USFWS monitoring program 1970’s-1992**




De Vault et al. 1996 JGLR 22:884

# Effects Biomonitoring: How we got here.

- 1940-50's New Age Pesticides
  - Organochlorine pesticides
- 1960-70's Contaminant monitoring
  - NCBP, NOAA Status & Trends, EPA
- 1970-80's Ban of Certain Pesticides and PCBs
  - DDT, Toxaphene, Dieldrin, PCBs
- 1980-present New Generation Pesticides
  - Organophosphates, Carbamates, Triazenes………
  - Shortened half-life, little or no accumulation in biota
- 1990's-present Pharmaceuticals, Veterinary products, Antibiotics, Personal Care Products
- Are effects occurring even without residual chemicals being present?

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# Common wastewater constituents

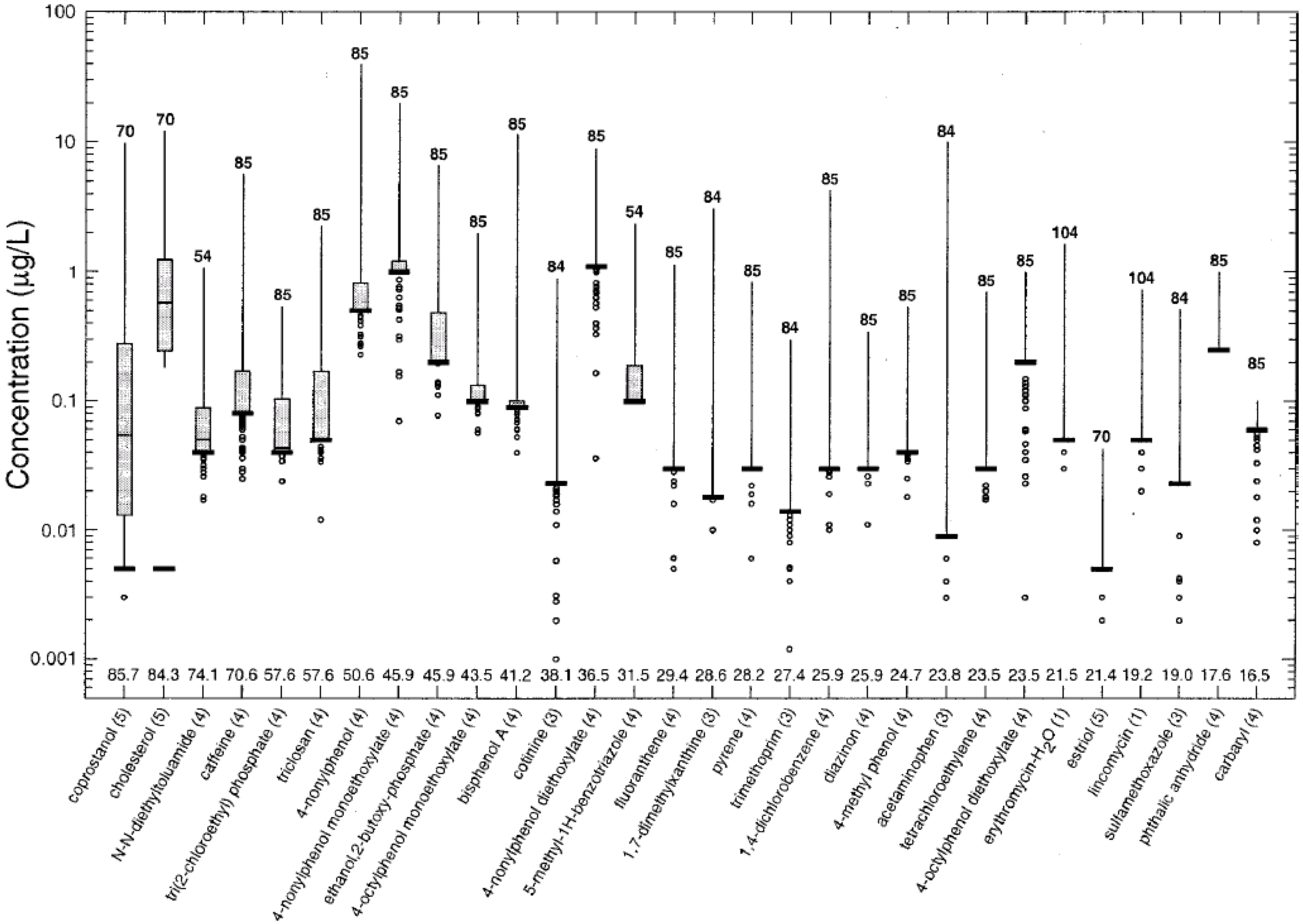


FIGURE 2. Measured concentrations for the 30 most frequently detected organic wastewater contaminants. Boxplots show concentration distribution truncated at the reporting level. Estimated values below the reporting level are shown. Estimated maximum values for coprostanol and cholesterol obtained from Method 5 (Table 1) are not shown. The analytical method number is provided (in parentheses) at the end of each compound name. An explanation of a boxplot is provided in Figure 3.

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**Kolpin et al. 2002 ES&T 36(6):1202-1211**

# Organic wastewater contaminants

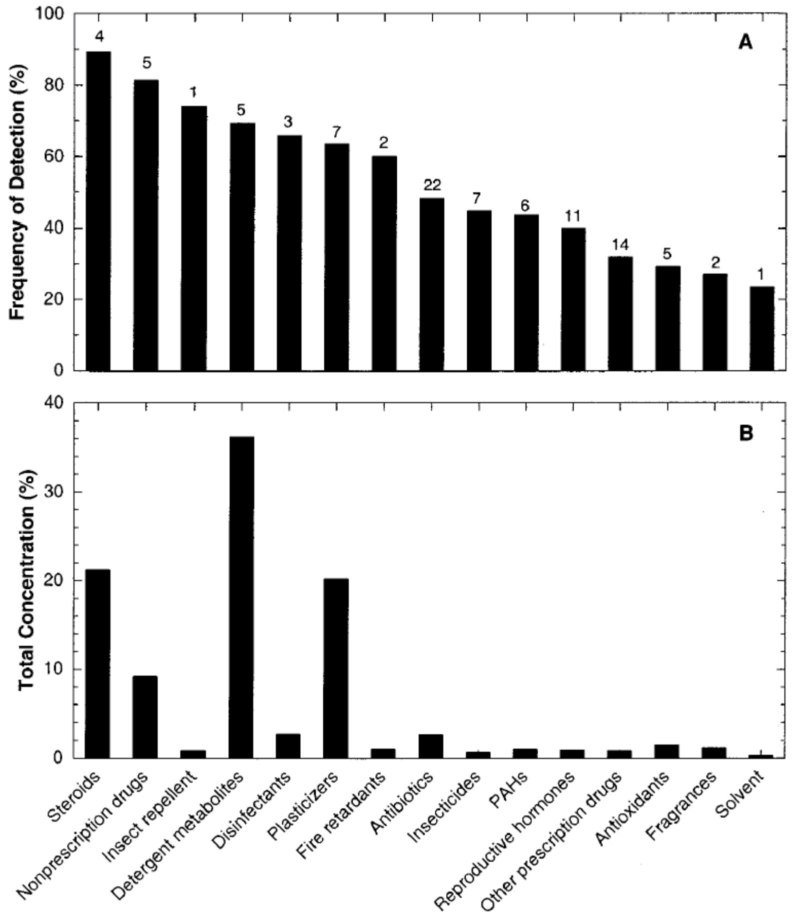


FIGURE 4. Frequency of detection of organic wastewater contaminants by general use category (4A), and percent of total measured concentration of organic wastewater contaminants by general use category (4B). Number of compounds in each category shown above bar.

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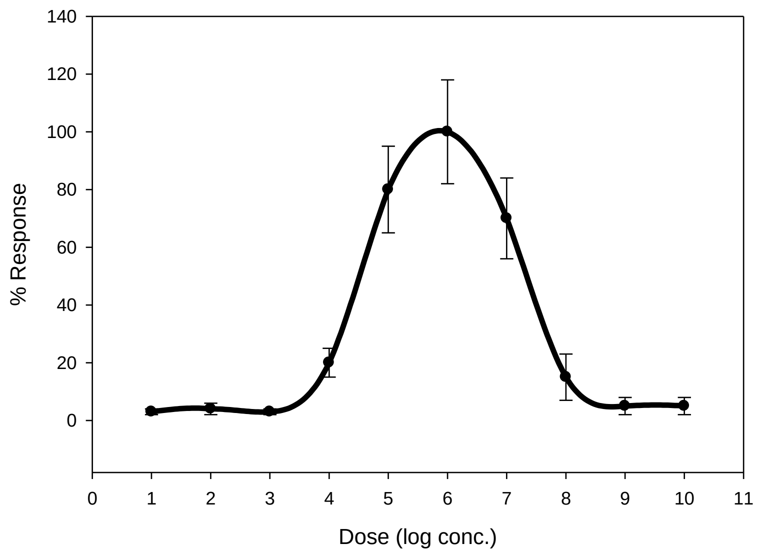
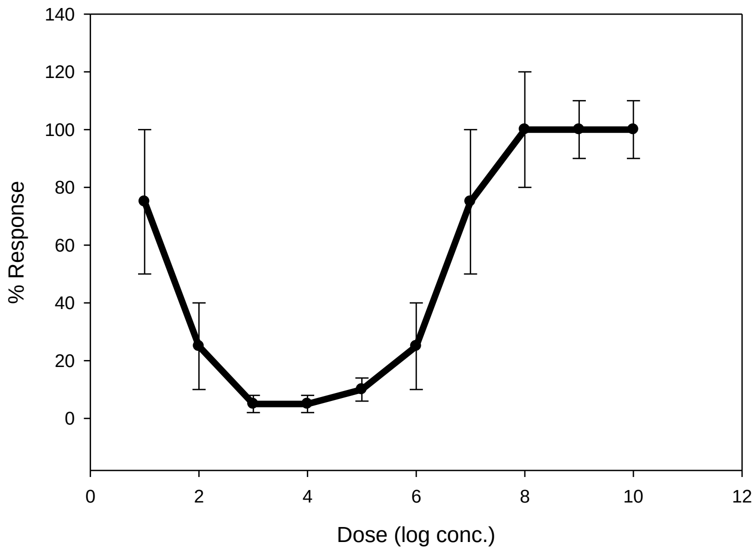
**Kolpin et al. 2002 ES&T 36(6):1202-1211**

# Non-monotonic dose-response relationships with EDCs

- **Non-linear dose-response relationships are common**
- **Hormesis**
- **Predictive models must incorporate**




- **Shapes of response relationships vary**

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# Paradigm shift for environmental chemical monitoring

OC pesticides and industrial chemicals (POPs)

- High Kow
- Low metabolism
- WS <<< LOD
- TRV <<< WS

➢ Tissue burdens critical

- Spatial distribution
- Temporal distribution
- Toxicity evaluation

New generation pesticides, veterinary and health care products, and industrial chemicals

- Lower Kow (70% < 3.0)
- Greater metabolism
- WS >> LOD
- TRV ???

➢ Tissue analysis useless

➢ Water analysis ???

➢ Effects biomonitoring

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# Biomonitoring of Environmental Status and Trends (BEST) Program: Large River Monitoring Network (LRMN)

**Objective:** Identify, monitor, and assess environmental contaminants and their effects in fish

**Endpoints**

- Contaminant concentrations ***(PCBs, organochlorine pesticides, metals, H4IIE bioassay)***
- Fish health indicators ***(somatic indices, health assessment)***
- Histopathology ***(general health, gonad)***
- Reproductive biomarkers ***(vitellogenin, steroid hormones)***
- Hepatic ethoxyresorufin *O*-deethylase (EROD) activity

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# Endpoints used by LRMN

| Factors to consider | Rating |
|---|---|
| Cost | |
| Historical data | |
| Collection method | |
| Analytical method | |
| Interpretation | |

- Field, Laboratory
- Temporal, Spatial, Species
- Time, Training, Preservation
- Expertise, Equipment, Time
- Expertise, Influencing factors

**Green = good/easy/useful**
**Red = bad/difficult/challenging**

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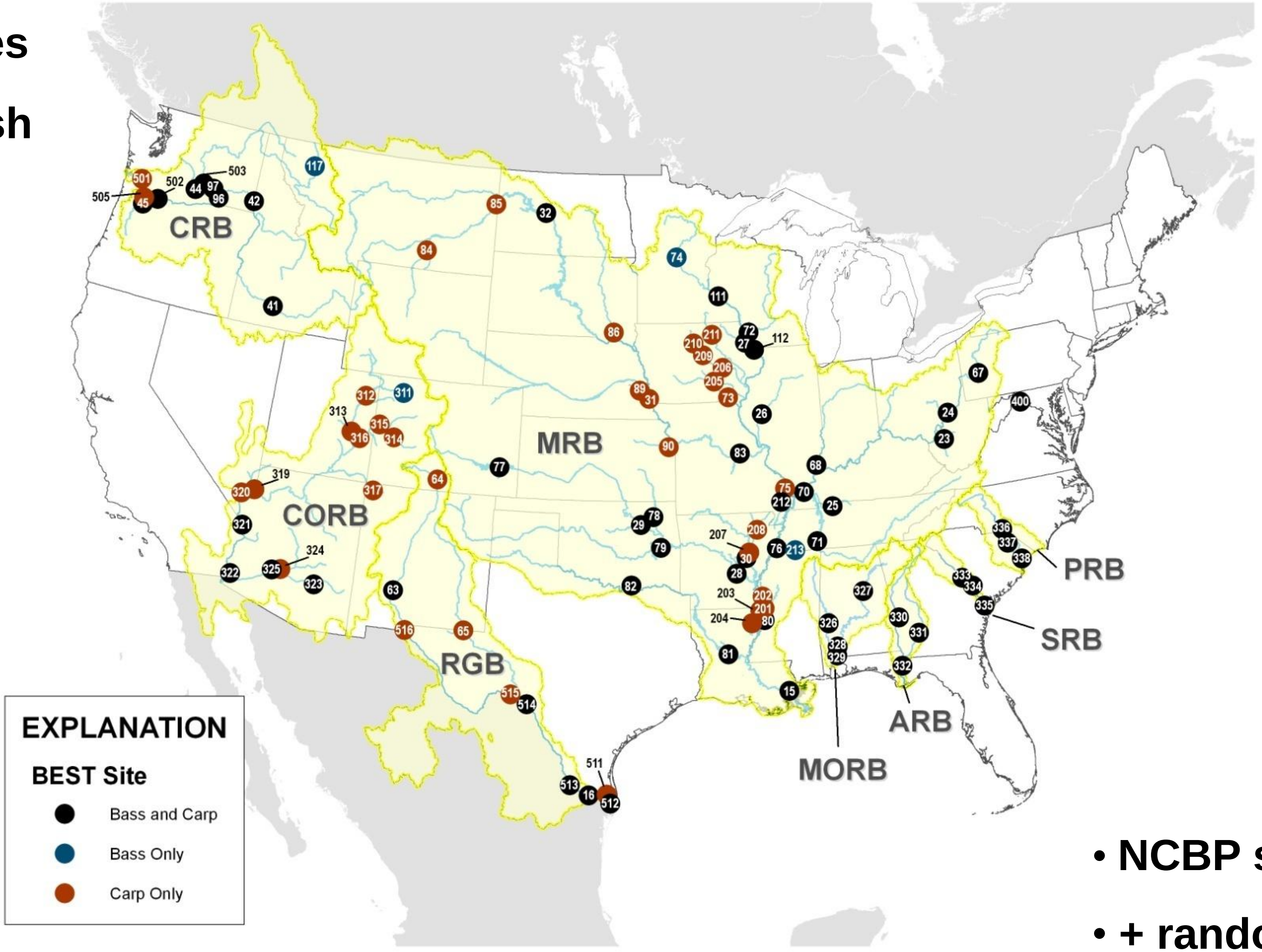
# LRMN Sampling Sites

- > 100 sites
- > 3200 fish




- NCBP sites
- + random sites

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# BEST-LRMN Program

Many endpoint responses are species specific; therefore the program targets certain fish species

**Predator: Largemouth Bass**


**Benthivore: Common carp**


**Endpoint data may be limited for certain species**

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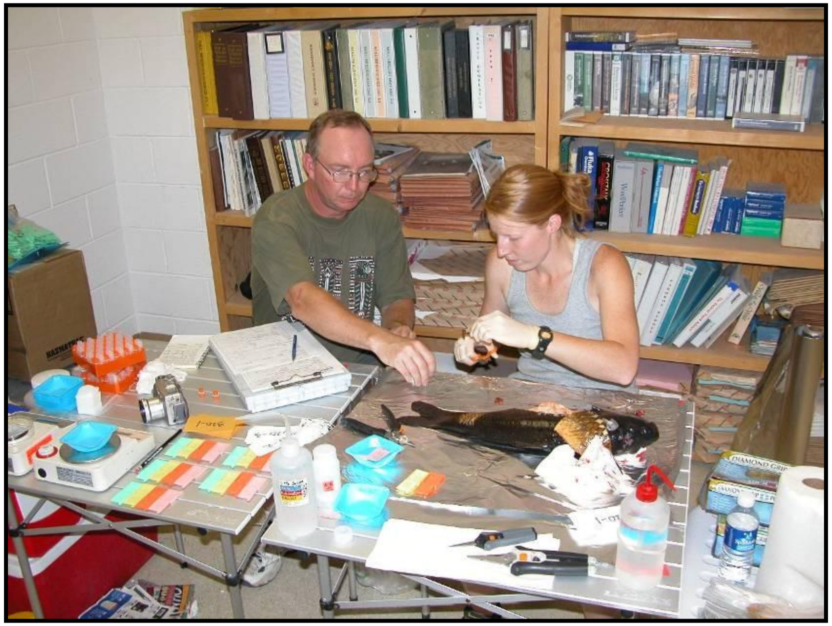
# Collection logistics of LRMN

Live fish
Equipment
Min. 2 person crew



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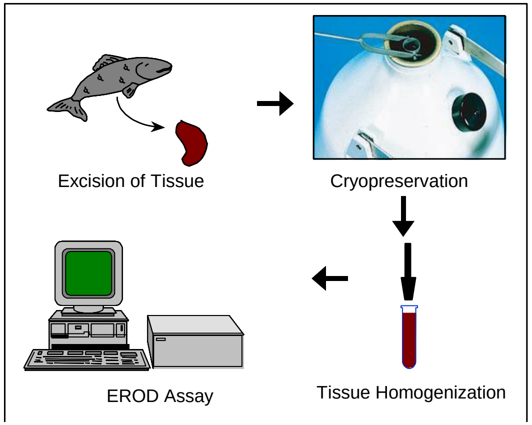
# Hepatic microsomal ethoxyresorufin *O*-deethylase (EROD)




| Factors to consider | Rating |
|---|---|
| Cost | |
| Historical data | |
| Collection method | |
| Analytical method | |
| Interpretation | |

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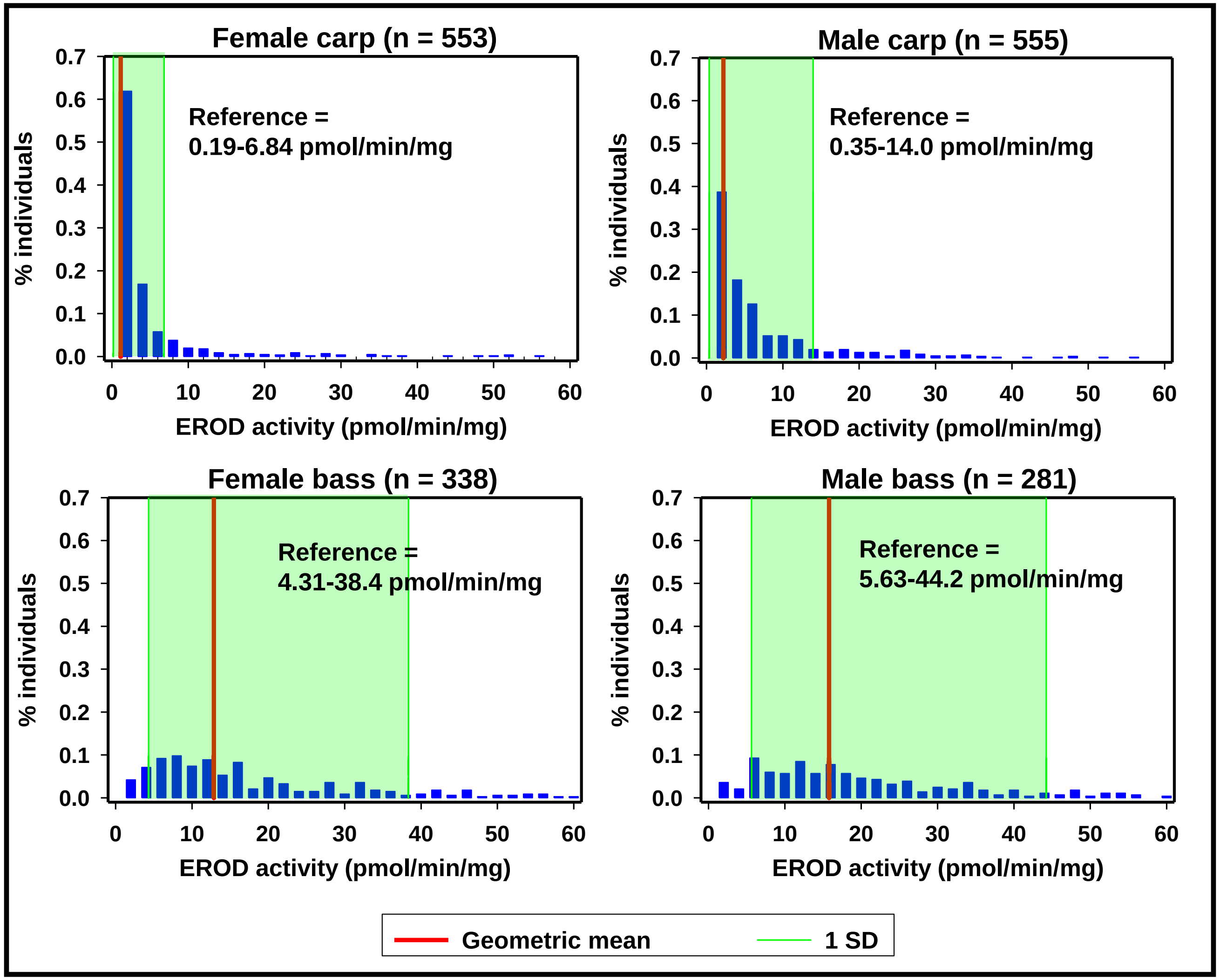
# Frequency distribution of EROD

EROD activity in green area are reference or background

Influencing factors:
- Species
- Gender
- Reproductive stage

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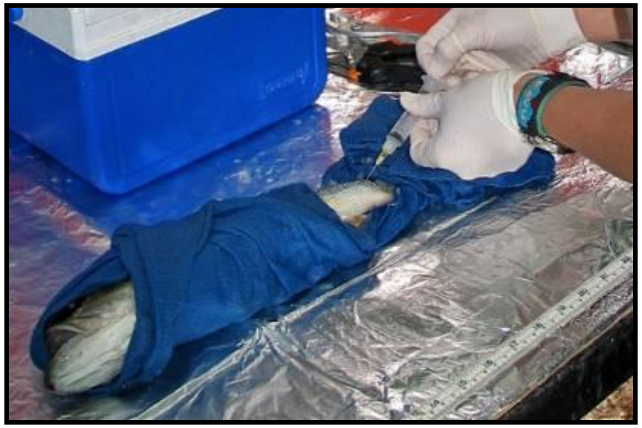
# Plasma vitellogenin and steroid hormones



| Factors to consider | Rating |
|---|---|
| Cost | |
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| Interpretation | |

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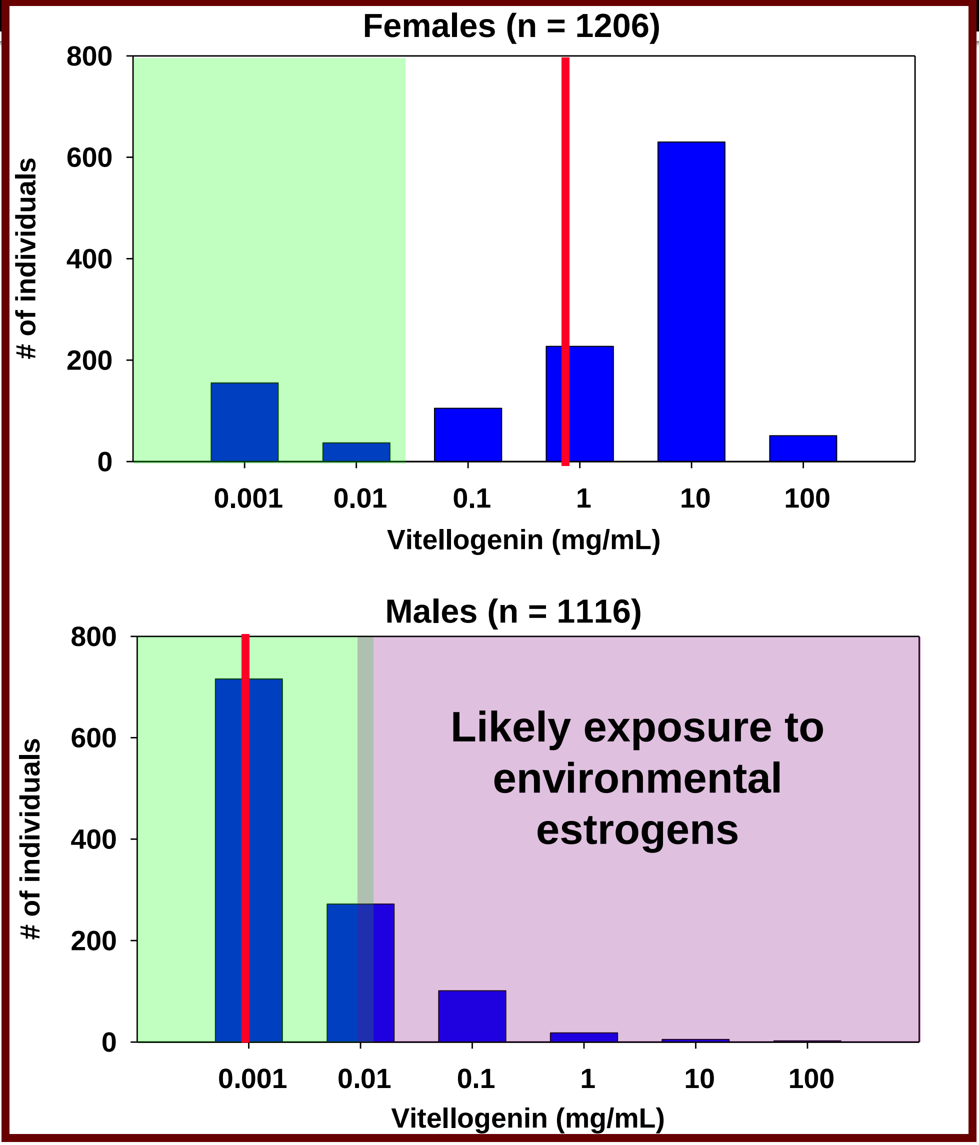
# Frequency distribution of Vtg concentrations

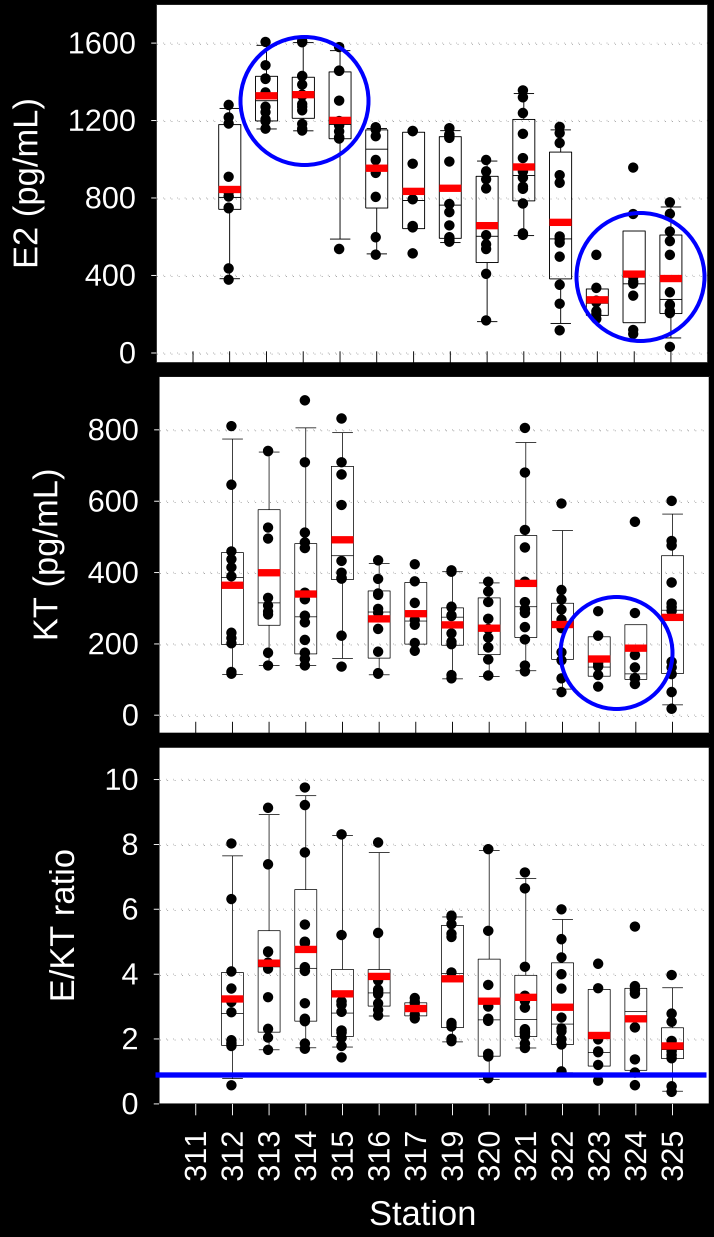
**Conc. < detection limit:**
- **13% of females**
- **87% of males**

**Conc. >0.01 mg/mL in males is anomalous**



# Steroid hormones in female carp

- **Reference condition difficult to determine**
- **Samples collected Aug-Oct to minimize stage effects**
- **17β –estradiol conc. differed among sites – delayed maturation (as determined by histopathology) at 323, 324, and 325**
- **11-ketotestosterone conc. also relatively low at 323 and 324**
- **Compare hormone ratios**

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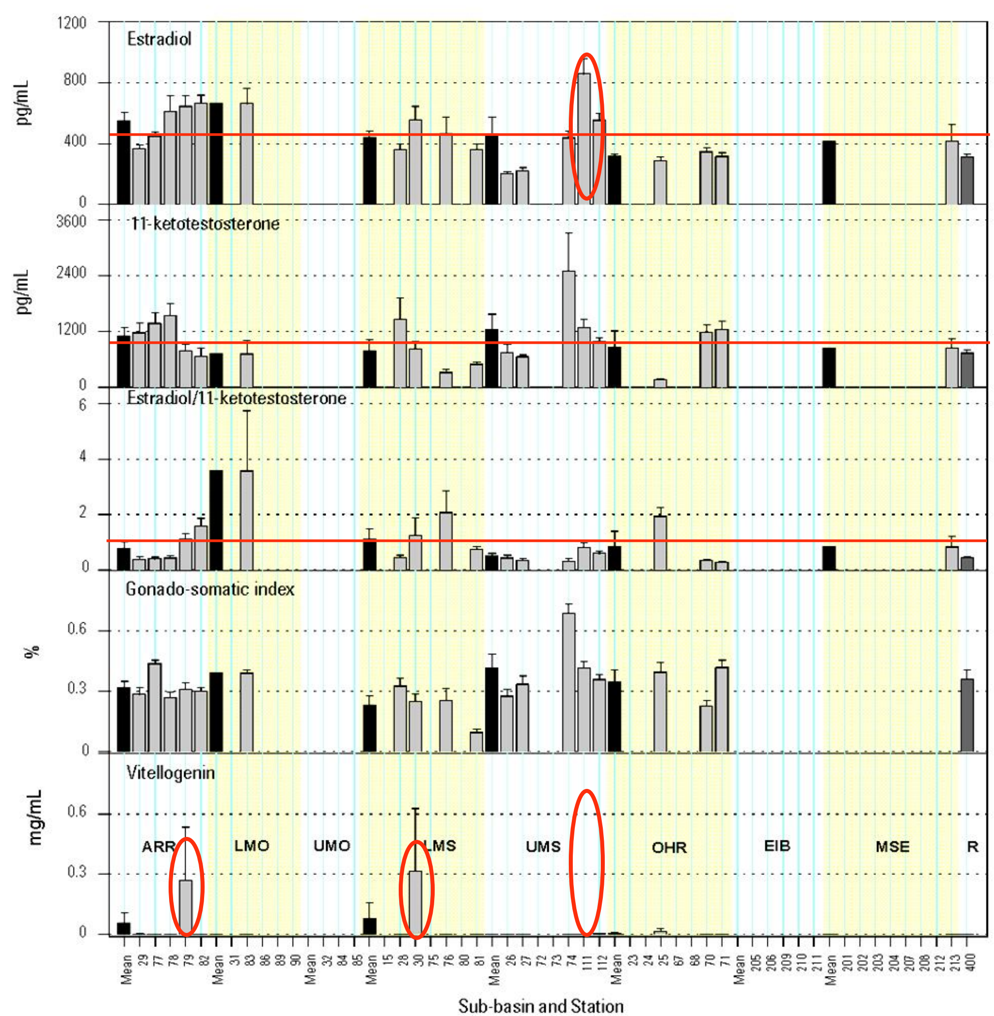
# Reproductive biomarkers in male LMB collected in the Mississippi River Basin - LRMN

- Identify average values for steroid hormones
- Identify abnormal values for each biomarker
- Inter-comparisons among reproductive biomarkers

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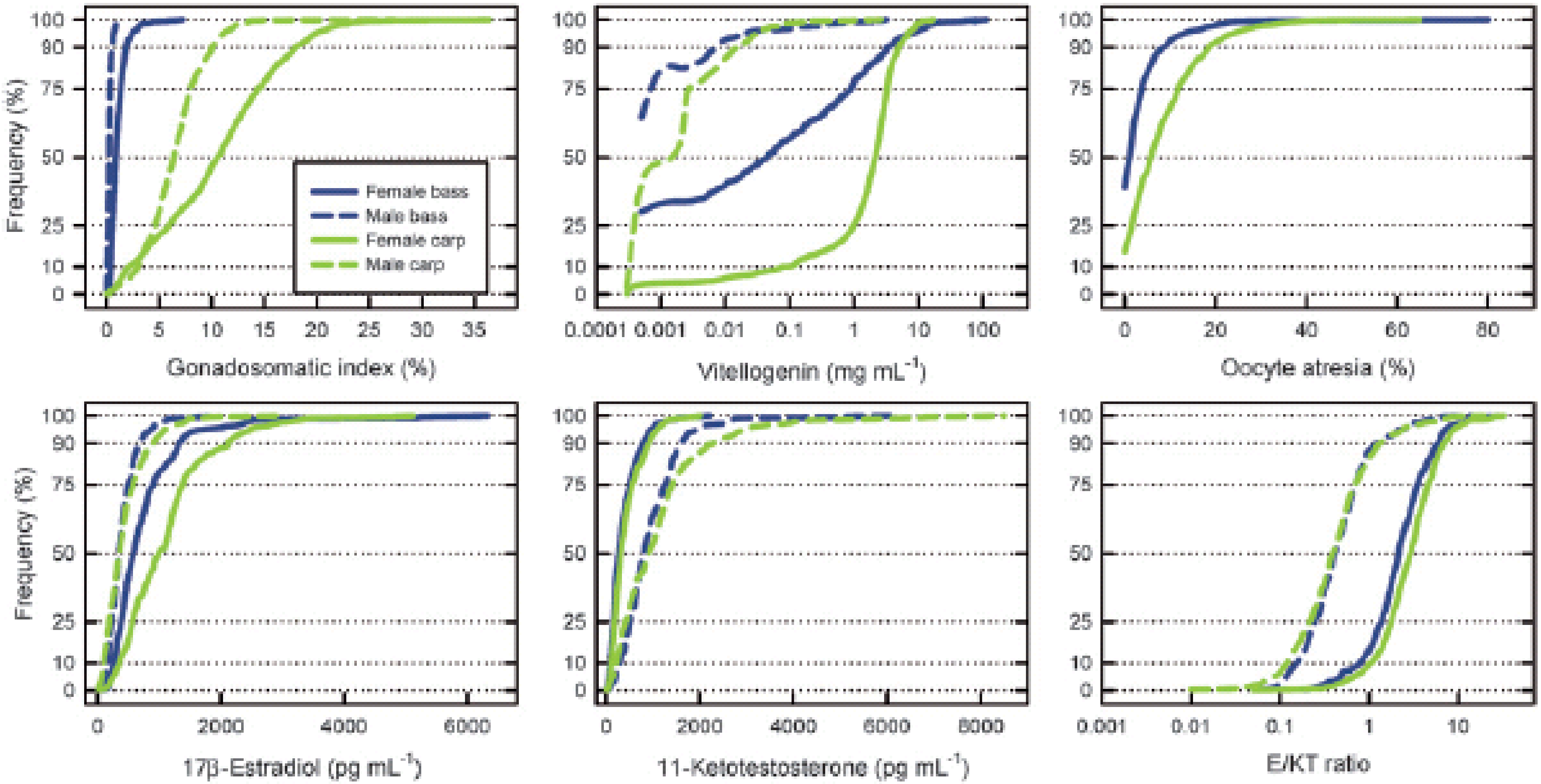
# Cumulative frequency distributions of reproductive biomarkers in fish from the BEST-LRMN

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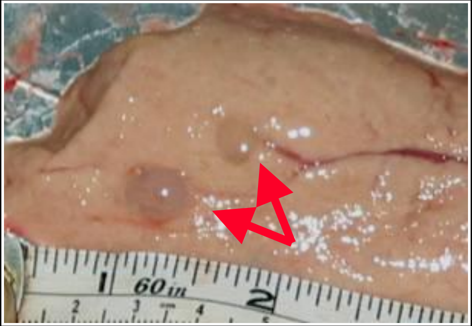
# Fish Health Assessment Index (HAI)

**External anomalies**

**Internal anomalies**



| Factors to consider | Rating |
|---|---|
| Cost | |
| Historical data | |
| Collection method | |
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| Interpretation | |

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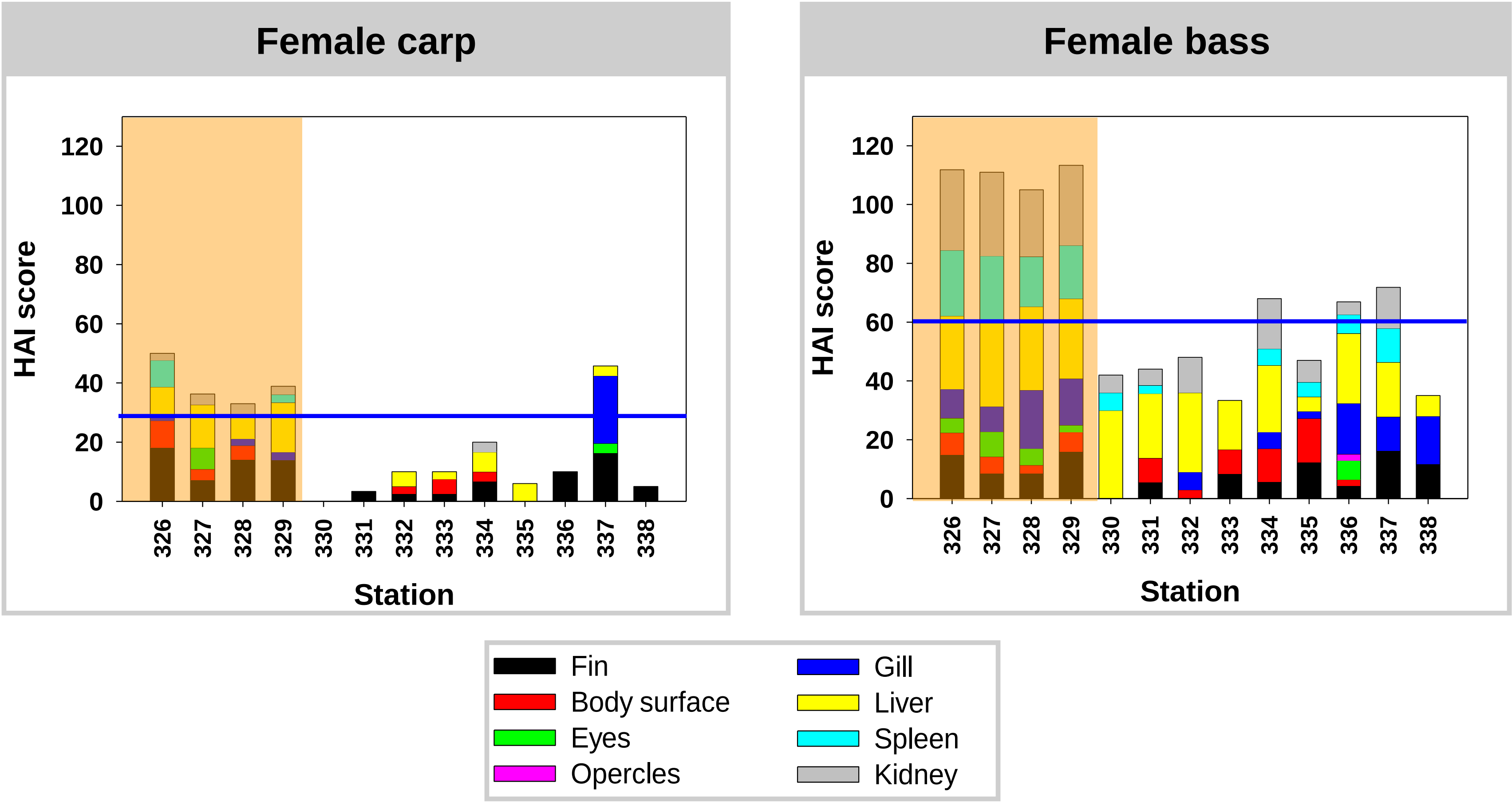
# HAI scores and species differences



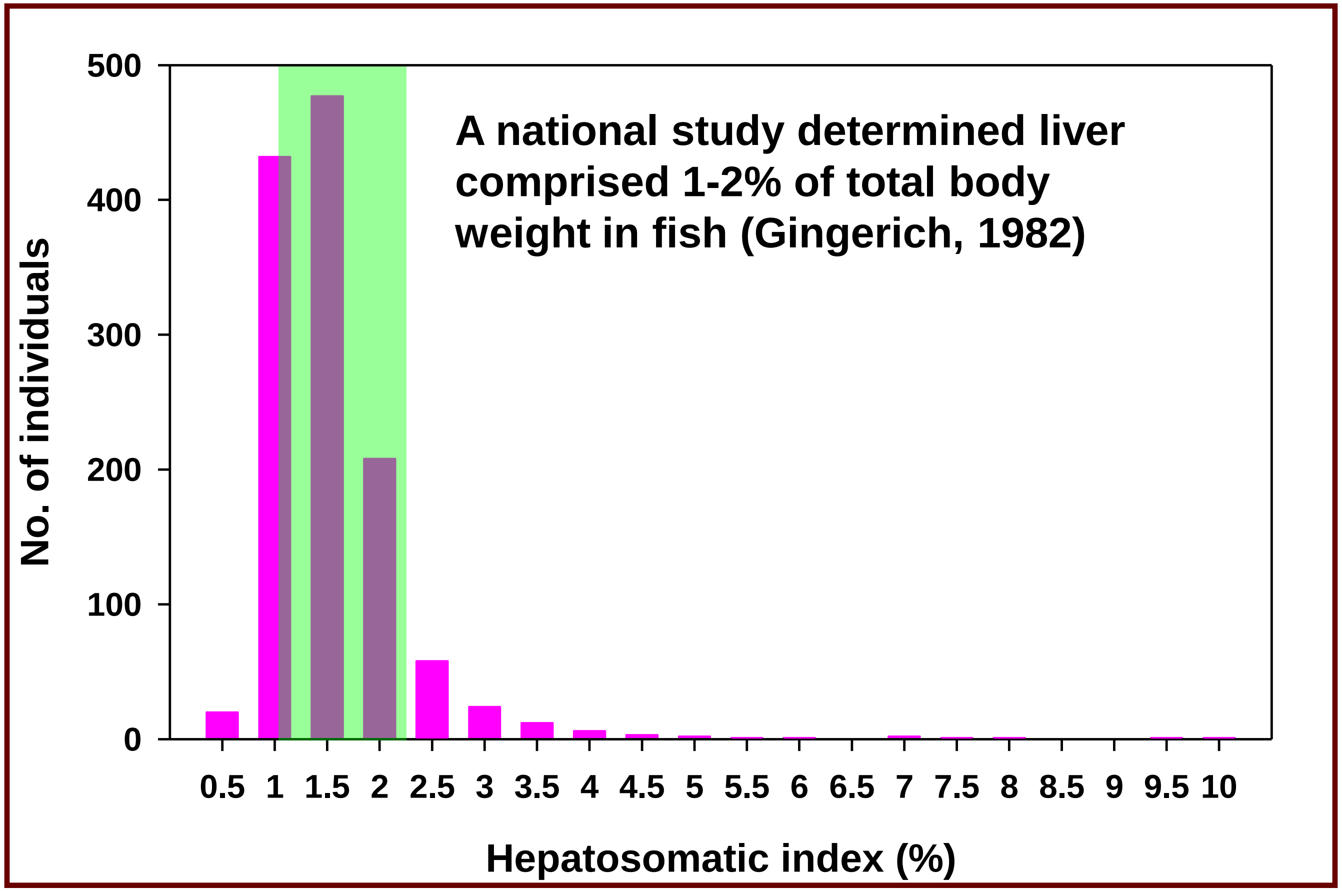
# Age, length, weight, somatic indices

Otoliths


Enlarged spleen

| Factors to consider | Rating |
|---|---|
| Cost | |
| Historical data | |
| Collection method | |
| Analytical method | |
| Interpretation | |

# Frequency distribution of hepatosomatic index in all LRMN fish




HSI = liver weight/(total body weight – gonad weight)*100

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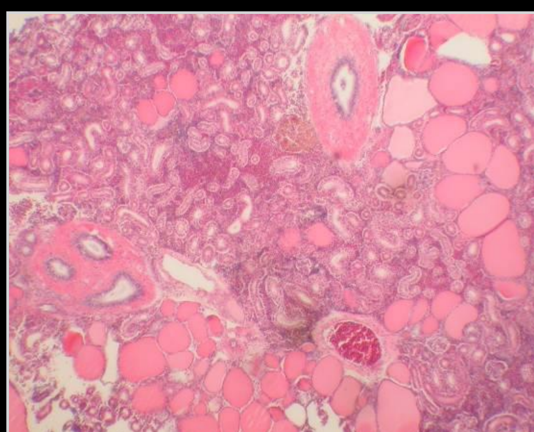
# Histopathology

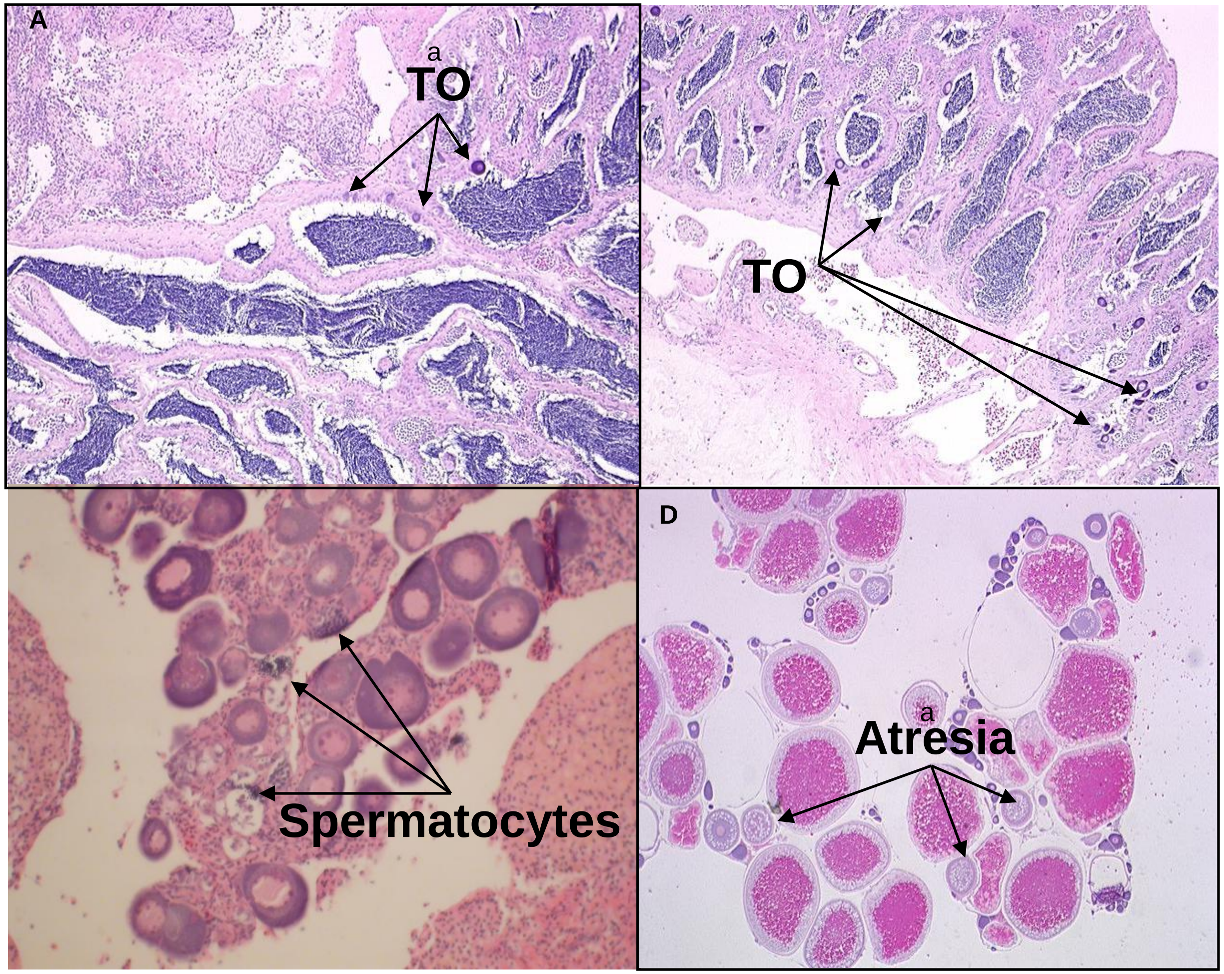
Testes with granulomas

Anterior kidney with thyroid follicles



| Factors to consider | Rating |
|---|---|
| Cost | |
| Historical data | |
| Collection method | |
| Analytical method | |
| Interpretation | |

# Gonad Histopathology

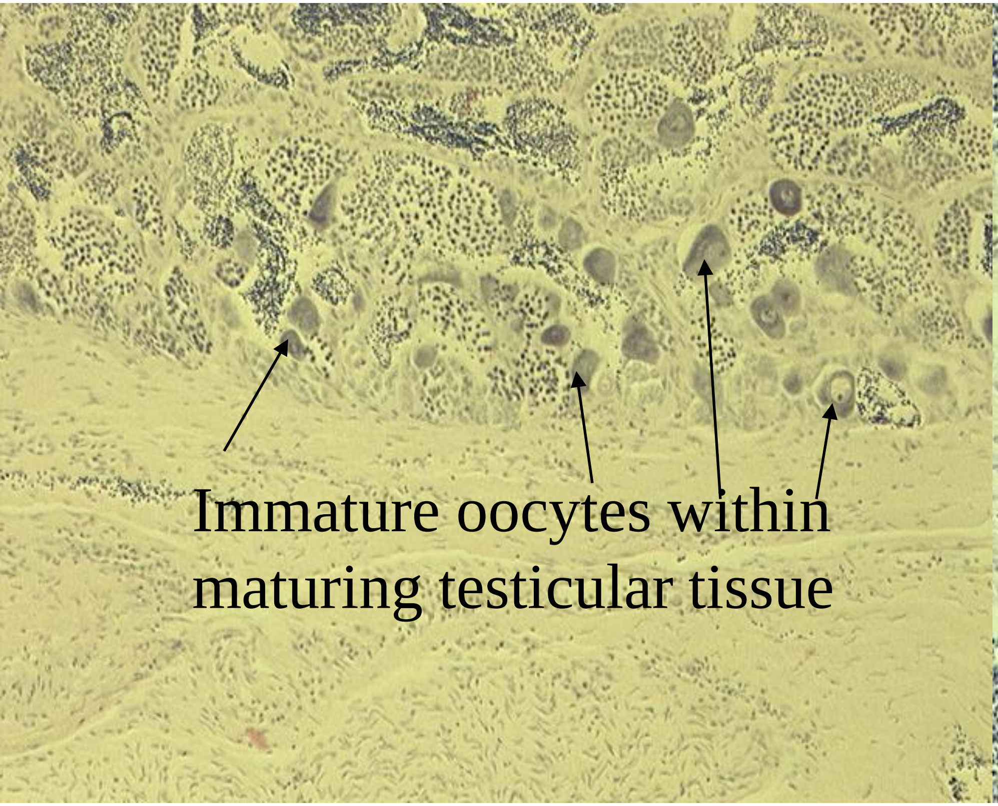


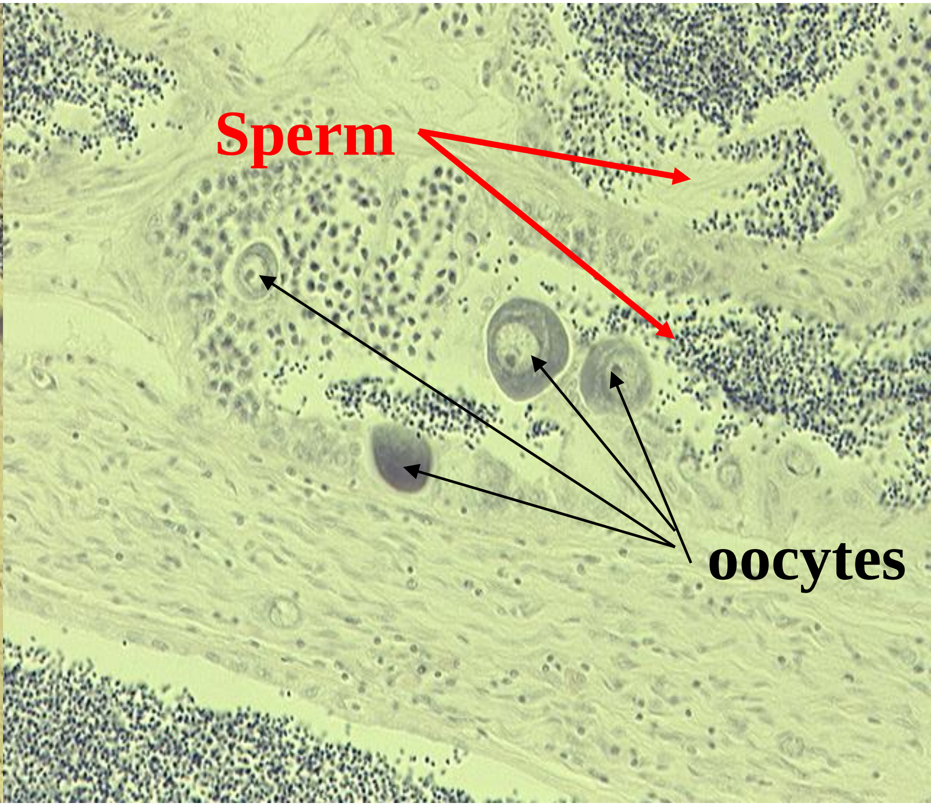

Endocrine - sensitive tissues

Abnormalities associated with reproductive function

Variety of AOPs (Adverse Outcome Pathways)

# Histologic Appearance of Intersex Fish




**Low magnification showing extent of oocyte development within testicular tissue**

**Higher magnification**

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# Definition of Intersex

- An organism possessing both testicular and ovarian tissue simultaneously or sequentially (hermaphrodite)
- Or can be an organism possessing only male or female gonadal tissue, **but** also having secondary sexual characteristics, behavior, physiological characteristics, or sex chromosomes of the opposing sex

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# Normally Hermaphroditic Fishes:

- Aulopiformes – grinners
- Atheriniformes – silversides
- Cyprinodontiformes – egg-laying tooth carps
- Myctophiformes –blackchins & lanternfishes
- Perciformes – perch-like fishes
- Scorpaeniformes – scorpionfishes & flatheads
- Stomiiformes – lightfishes & dragonfishes
- Synbranchiformes – spiny- & swamp-eels

# Historical Accounts of Abnormal Hermaphroditism:

- Acipenseriformes – sturgeons
- Clupeiformes – herring
- Cypriniformes – minnows
- Cyprinodontiformes – tooth carps
- Gadiformes – cods
- Gasterosteiformes - sticklebacks
- Perciformes - perches
- Pleuronectiformes – flatfishes
- Salmoniformes – trouts and salmons
- Siluriformes - catfishes

# Intersex in feral fish reported in the literature

| Species, location | Gender | No. fish with intersex/n | Comments | Reference |
|---|---|---|---|---|
| White sucker | | | | |
| Athabasca R., Alberta | Male | 1/Uknown | Herm | Sikstrom et al., 1975 |
| Boulder Creek, Colorado | Female | 4/39 | Ovaries with testicular tissue | Woodling et al., 2006 |
| South Platte R., Colorado | Male | 4/20 | TO | Woodling et al., 2006 |
| Boulder Creek, Colorado | M/F | 11/57 | TO/herm | Vajda et al., 2008 |
| Largemouth bass | | | | |
| Ridge Lake, Illinois | Herm | 1/Unknown | Herm | James, 1946 |
| Fort Lake, Illinois | Herm | Several | TO | James, 1946 |
| Hudson R., New York | Male | 4/15 | TO | Baldigo et al., 2006 |
| Smallmouth bass | | | | |
| Kalamazoo River, Michigan | Male | 15/15 | TO | Anderson et al., 2003 |
| Hudson R., New York | Male | 12/33 | TO | Baldigo et al., 2006 |
| Potomac River drainages VA/WV | Male | 146/241 | TO | Blazer et al., 2007 |
| Common carp | | | | |
| Ebro R., Spain | Male | 1/6 | TO | Lavado et al., 2004 |
| Hudson R., New York | Male | 1/9 | TO | Baldigo et al., 2006 |
| Northern pike | | | | |
| Lake Oahe, South Dakota | Female | 2/1936 | Herm | June, 1977 |
| Various rivers, United Kingdom | Male | 1/54 | TO | Vine et al., 2005 |
| Various rivers, United Kingdom | Female | 15/58 | Male germ cells in ovary | Vine et al., 2005 |
| Striped bass | | | | |
| Coos Bay, Oregon | Herm | 11/42 | Herm | Moser et al., 1983 |
| Brown trout | | | | |
| Unknown, Ireland | Herm | 1/Unkown | Herm | O'Ferrall and Peirce, 1989 |
| Three rivers in Switzerland | Female | 27/121 | Spermatogenic nests in oocytes | Korner et al., 2005 |

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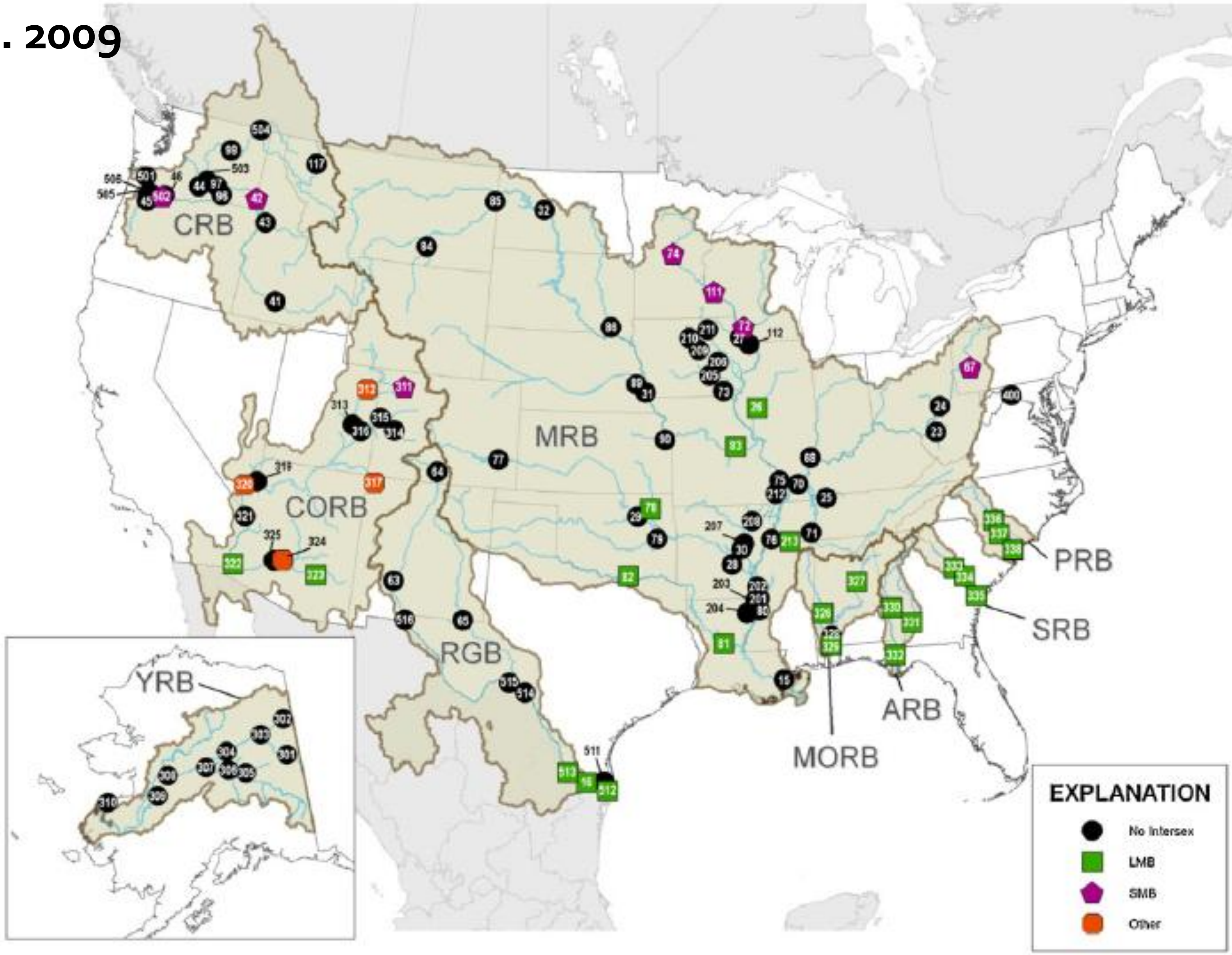
# National Distribution of Intersex Fish

Hinck et al. 2009

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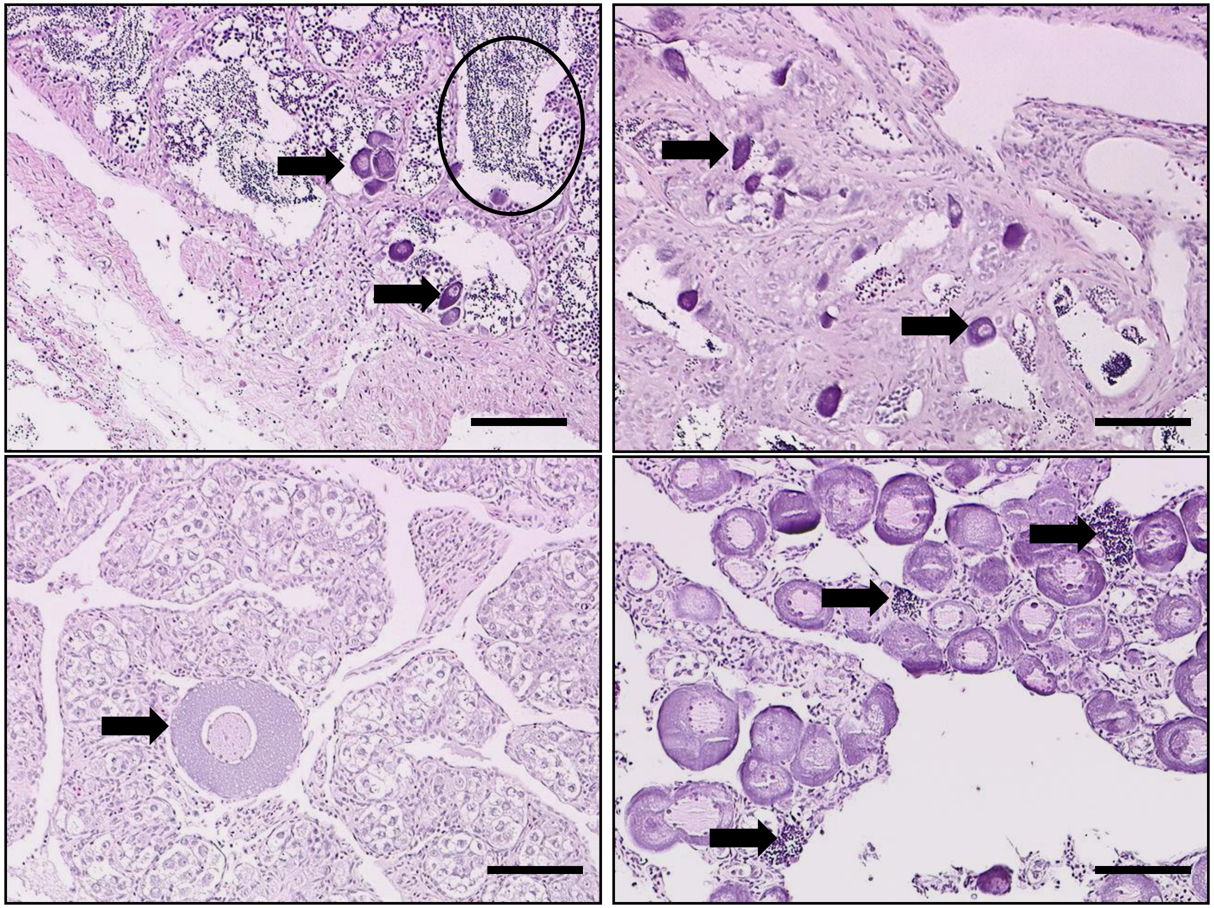
# Intersex examples from Biomonitoring Environmental Status and Trends



Hinck et al. 2009

# Occurrence of intersex in fish from the BEST-LRMN

| Species | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| | #/n | (%) | Sites | (%) | #/n | (%) | Sites | (%) |
| **LM Bass** | 0/426 | 0 | 0/55 | 0 | 70/390 | 18 | 23/52 | 44 |
| **SM Bass** | 0/90 | 0 | 0/15 | 0 | 23/70 | 33 | 7/16 | 44 |
| **C. Carp** | 1/798 | 0.1 | 1/89 | 1 | 0/774 | 0 | 0/89 | 0 |
| **C. Catfish** | 0/44 | 0 | 0/6 | 0 | 3/42 | 7 | 3/6 | 50 |

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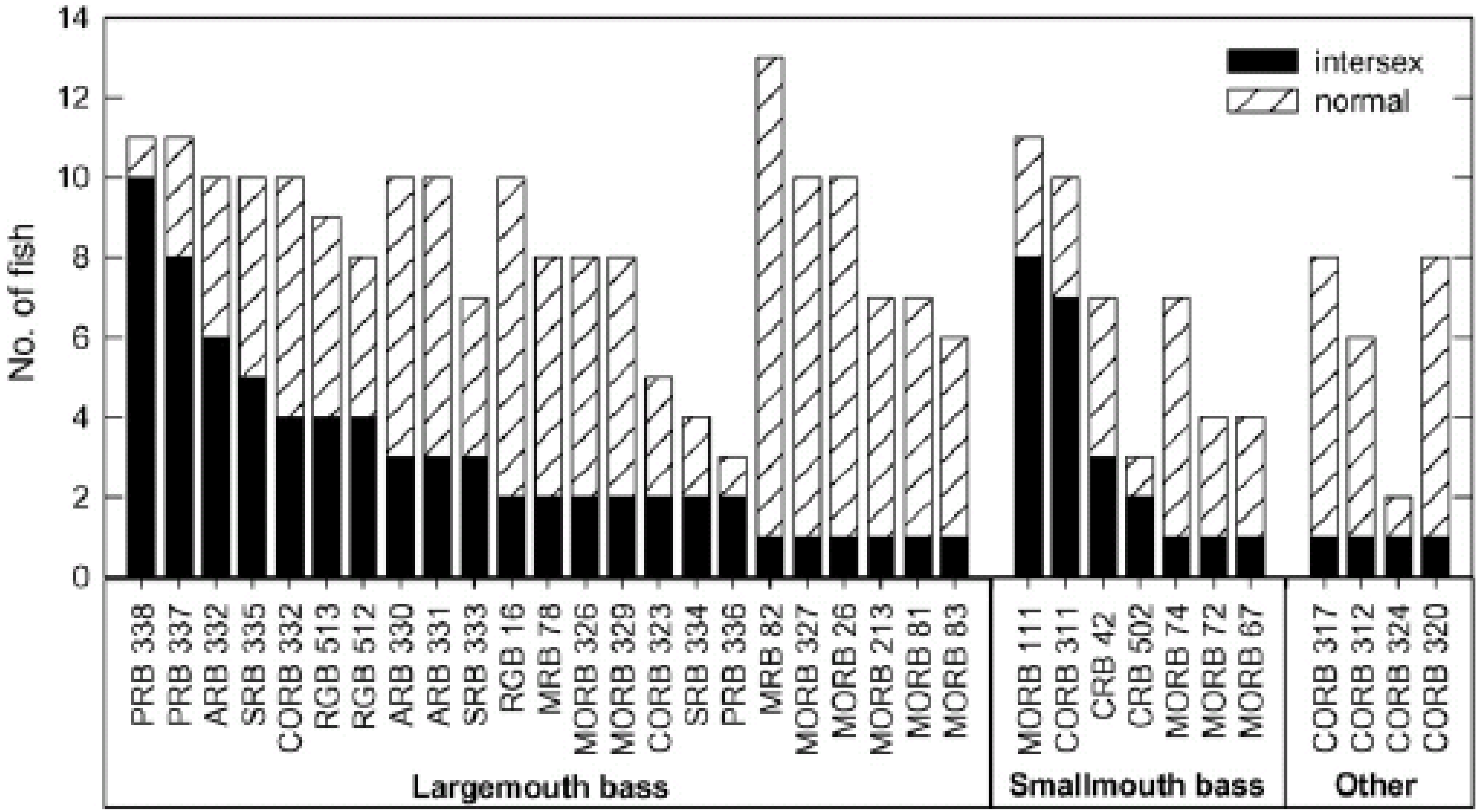
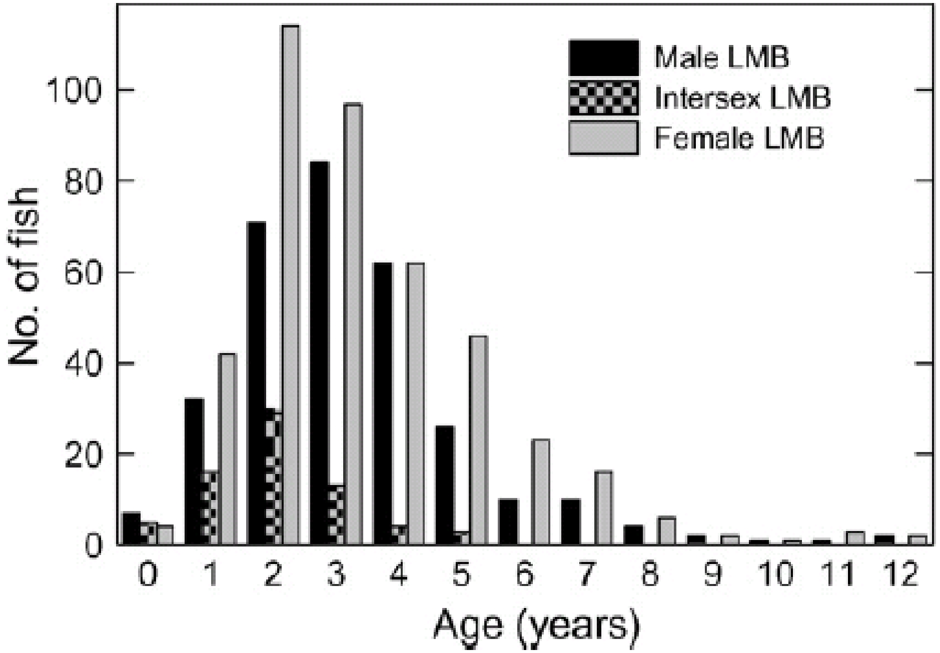
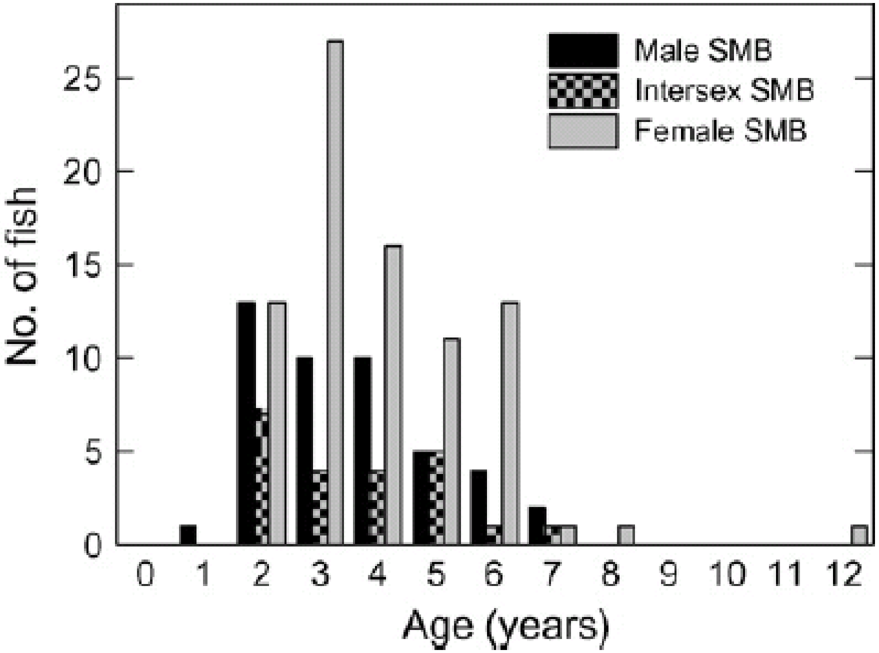
# Intersex in male fish by site



# Intersex condition by age



# Intersex occurrence by river basin and species with related associations

| Basin-species | # Fish | % | # Sites | % | Associations |
|---|---|---|---|---|---|
| MRB-LMB | 174 | 4 | 22 | 27 | (-) Length, (-) MA |
| MRB-SMB | 27 | 41 | 5 | 80 | (+) 17β-E2, (+) MA |
| Columbia RB-LMB | 34 | 0 | 7 | 0 | |
| Columbia RB-SMB | 24 | 21 | 8 | 25 | (-) HAI, (+) MA |
| RGB-LMB | 29 | 35 | 4 | 75 | None |
| RGB-SMB | 1 | 0 | 1 | 0 | |
| CO RB-LMB | 42 | 14 | 6 | 3 | (-) EROD |
| CO RB-SMB | 18 | 39 | 2 | 50 | (-) EROD, (+) Age/HAI/SSI/HSI |
| Apalachicola RB-LMB | 30 | 67 | 3 | 100 | None |
| Mobile RB-LMB | 36 | 14 | 4 | 75 | (+) HSI |
| Pee Dee RB-LMB | 25 | 80 | 3 | 100 | (-) Age, (-) Stage |
| Savannah RB-LMB | 21 | 48 | 3 | 100 | None |

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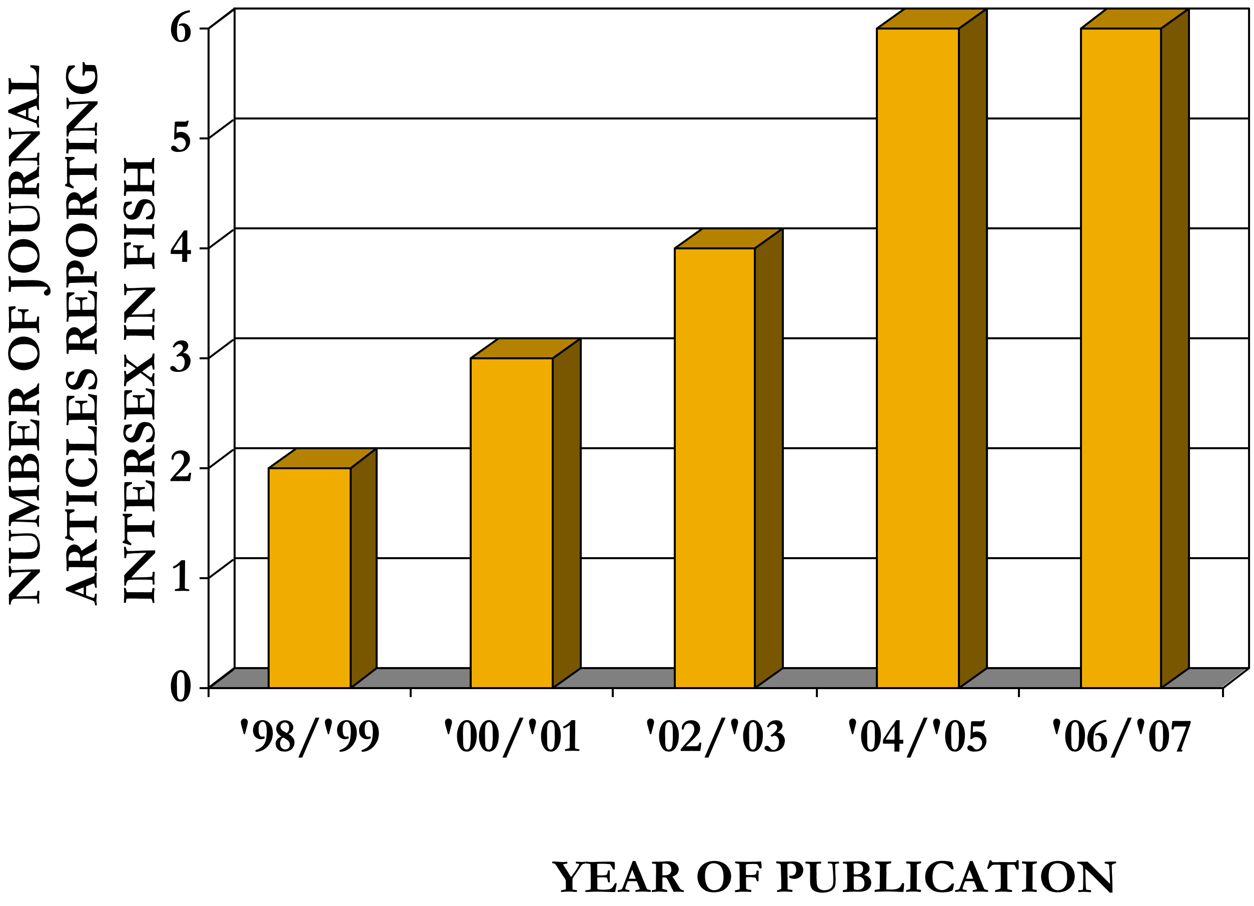
# Observations of Fish with Intersex Gonads Increasing

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# Occurrence and Severity of Intersex Gonads in SNS

- Missouri River research on sturgeon
- Monitor reproductive status of large numbers of shovelnose sturgeon
- Observations of intersex in different parts of the river
- Comparison with historical

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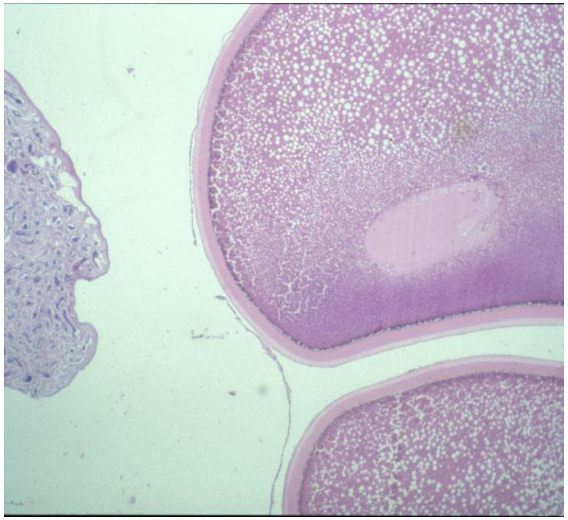
# Example: Shovelnose Sturgeon (SNS), Missouri River

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# EDCs induce intersex in fish

- **Controlled chemical exposures**
  - Ethinylestradiol (EE2)
  - Methyl testosterone (MT)
  - Trenbolone acetate
  - Nonylphenol
  - WWTP effluent
  - Pulp & Paper Mill effluents

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# EDC action

- Dose
  - Can be extremely low and have effects.
- Timing
  - Critical  in terms of development.
  - Critical in terms of reproductive cycle.

# Non-chemical factors in gonad development and differentiation

- Age
- Genetic abnormalities
- Hybridization
- Radiation
- Diet
- Temperature
- Hypoxia

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# Intersex in fish

- Testicular oocyte are most common (feminized males)
- Wide geographic occurrence
- Apparent regional differences in severity (?)
- Multiple species of fish express intersex condition
- Temporal increases in incidence in sturgeon
  - Other species?

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# Summary of endpoint use in fish health assessment

| Endpoint | Method | Interpretation | Overall Use |
|---|---|---|---|
| Age, length, weight, somatic indices | | | |
| Health Assessment Index | | | |
| EROD | | | |
| Vitellogenin | | | |
| Steroid hormones | | | |
| Pesticides, Inorganic contaminants | | | |
| Histopathology | | | |

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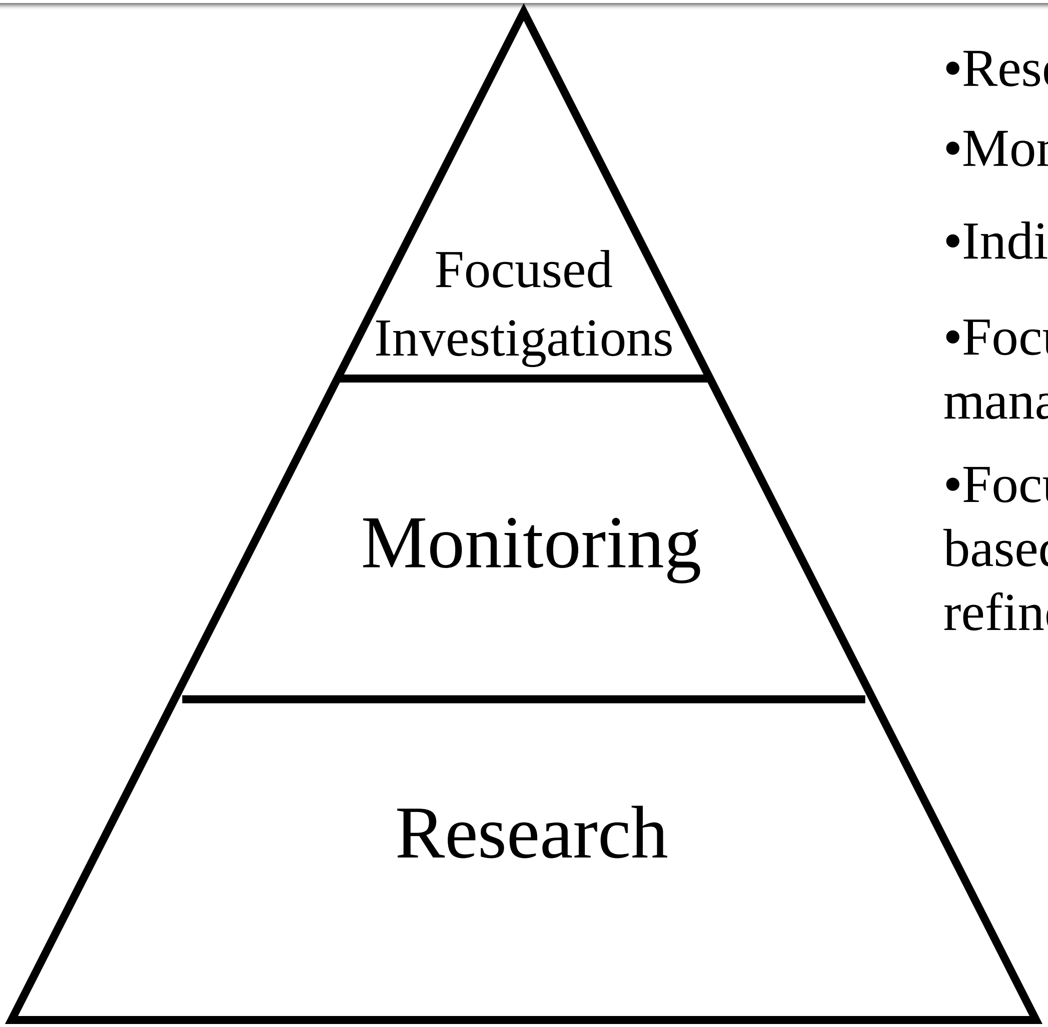
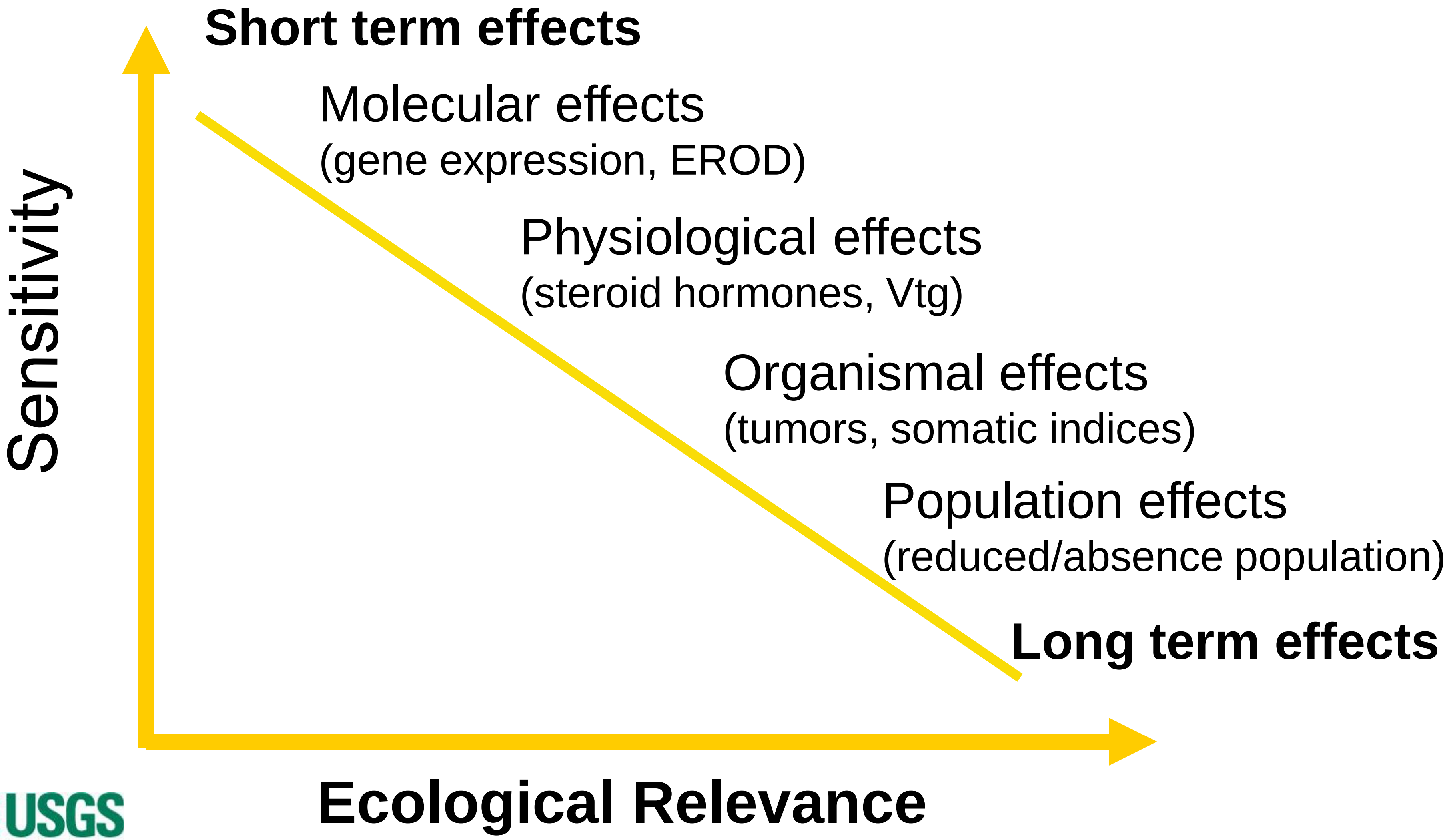
# Biomonitoring Paradigm

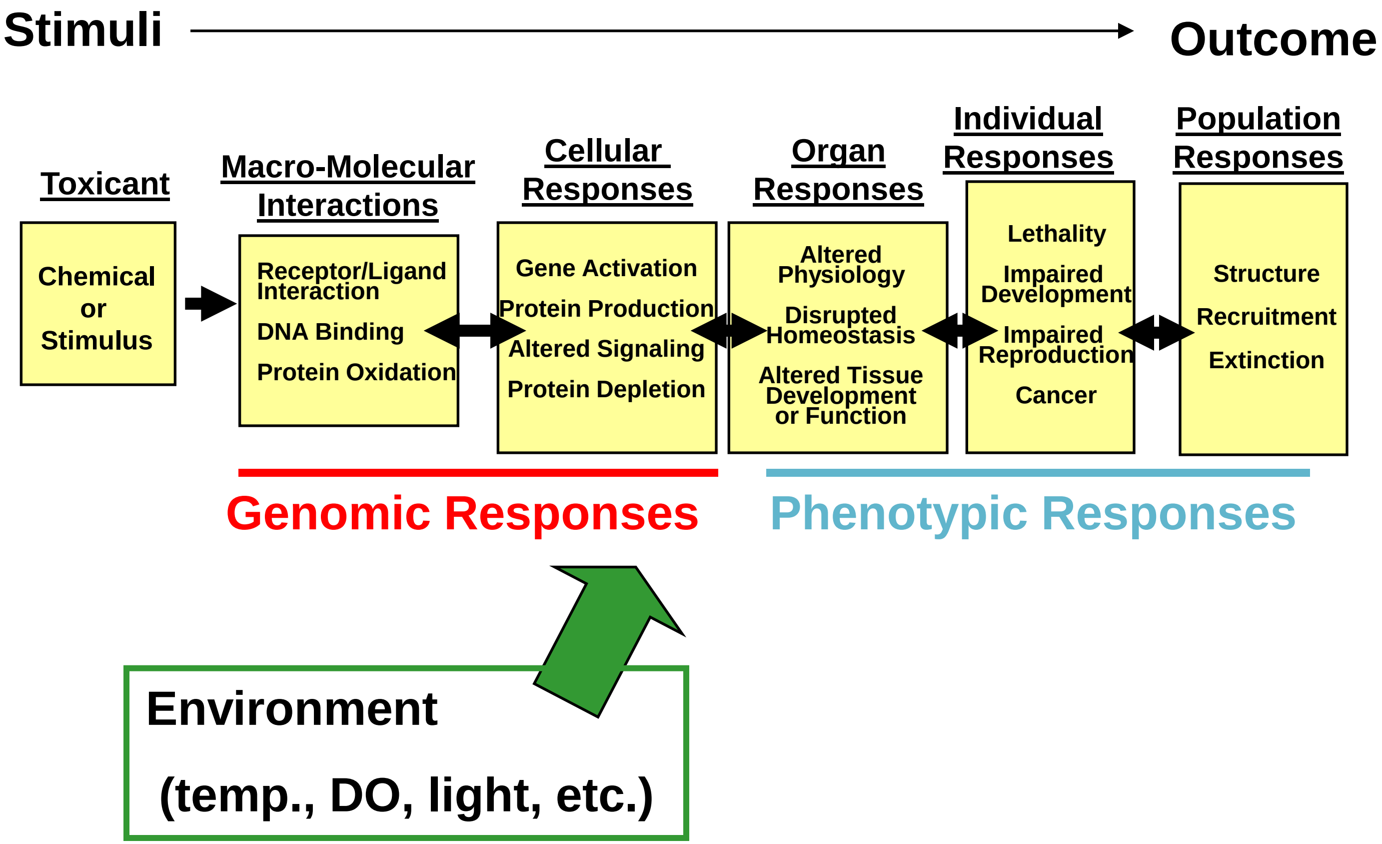



- Research provides foundation
- Monitoring - endpoint evaluation
- Indicators provide an indication
- Focused investigation direct management actions
- Focused investigations (field-lab based studies) serve to validate and refine models

# Monitoring endpoints in fish health and ecosystem assessment



# Understanding Toxicity Pathways and Complex Biological Processes



# Summary/Conclusions

- Standard ecological risk approaches will not suffice for PCPPs
  - Lacking TRVs and non-linear dose-responses
- Biomonitoring for endocrine active substances requires biochemical, histological, and organism level responses
- Current reproductive biomarkers appear to be useful
  - "normal" or baseline description is required
- Development of "omics" will aide forensic/diagnostic sciences
- Integrated lab-field studies help define utility of reproductive biomarkers

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# Acknowledgements

USGS Columbia Environmental Research Center:
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University of Florida: N. Denslow

USFWS Environmental Contaminants Program

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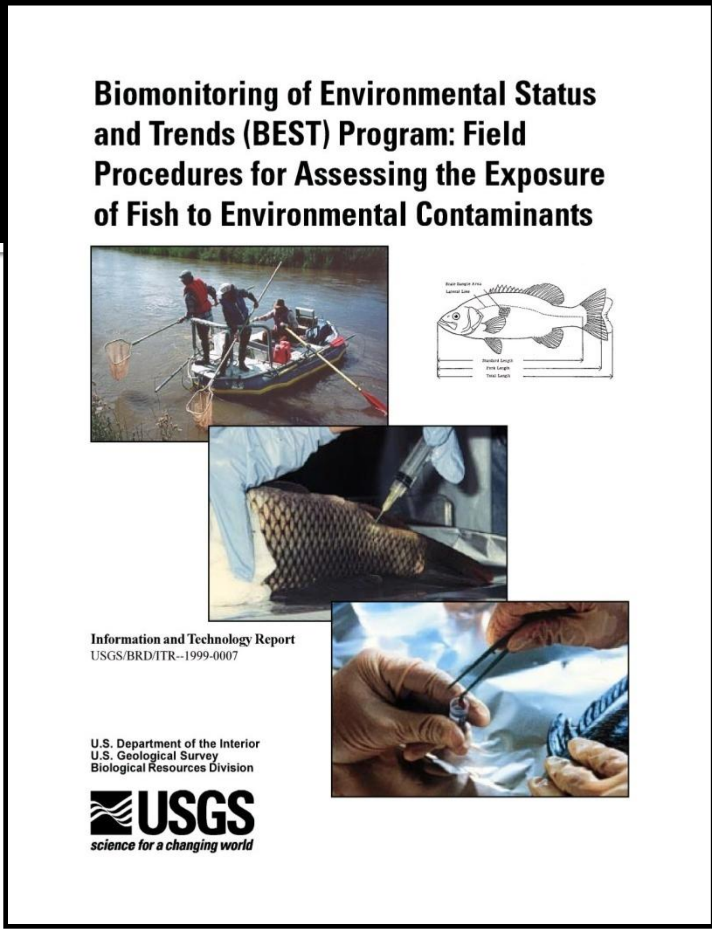

**Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants**

Information and Technology Report
USGS/BRD/ITR--1999-0007

U.S. Department of the Interior
U.S. Geological Survey
Biological Resources Division

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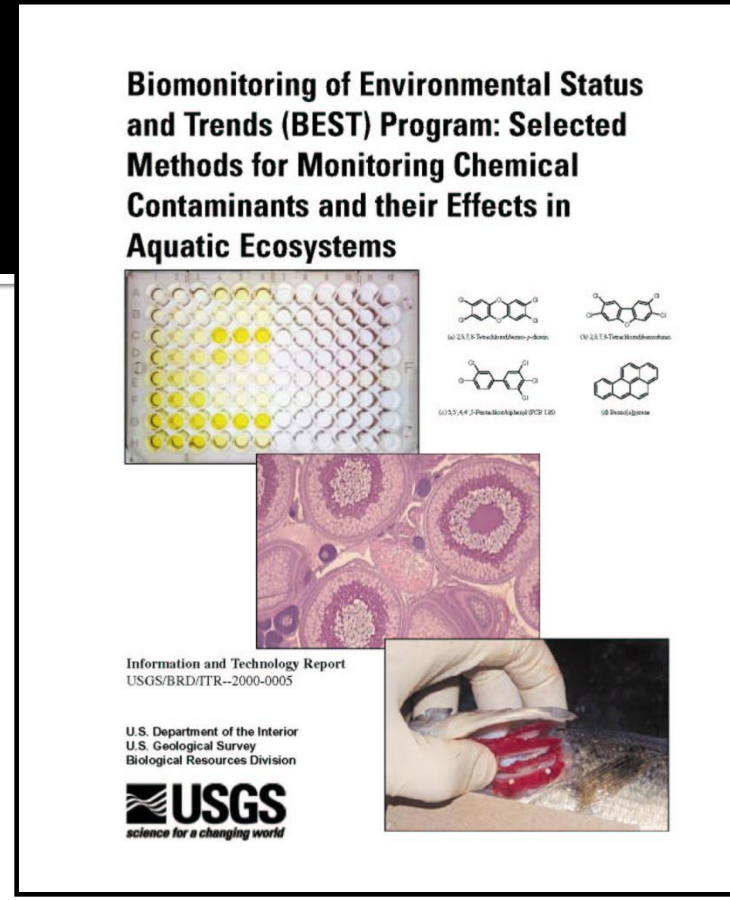

**Biomonitoring of Environmental Status and Trends (BEST) Program: Selected Methods for Monitoring Chemical Contaminants and their Effects in Aquatic Ecosystems**

Information and Technology Report
USGS/BRD/ITR--2000-0005

U.S. Department of the Interior
U.S. Geological Survey
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***<http://www.cerc.usgs.gov/pubs/pubs.htm>***

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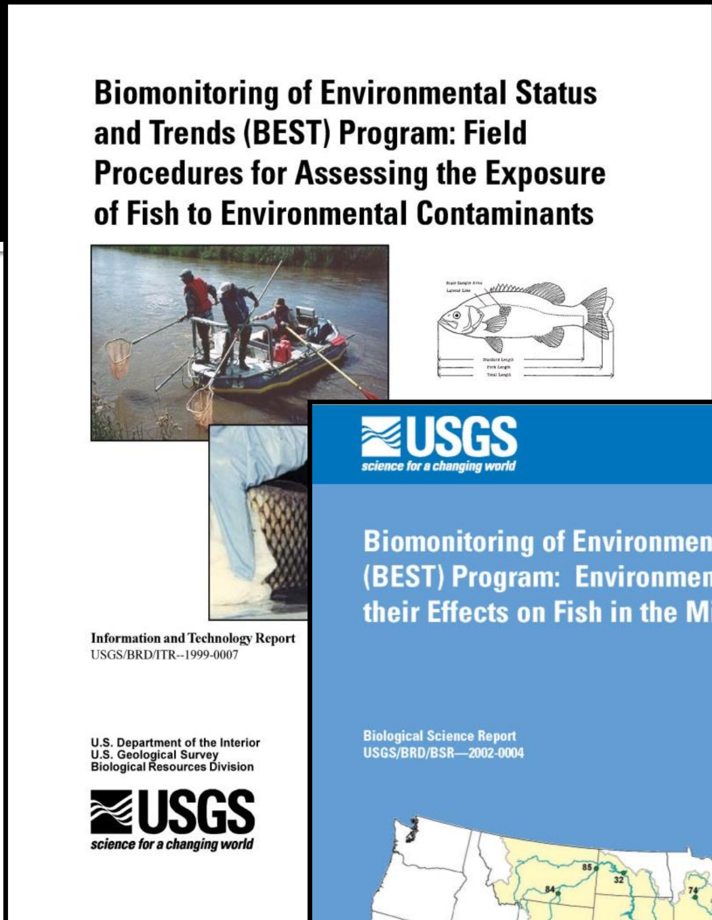
Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants

Information and Technology Report
USGS/BRD/ITR--1999-0007

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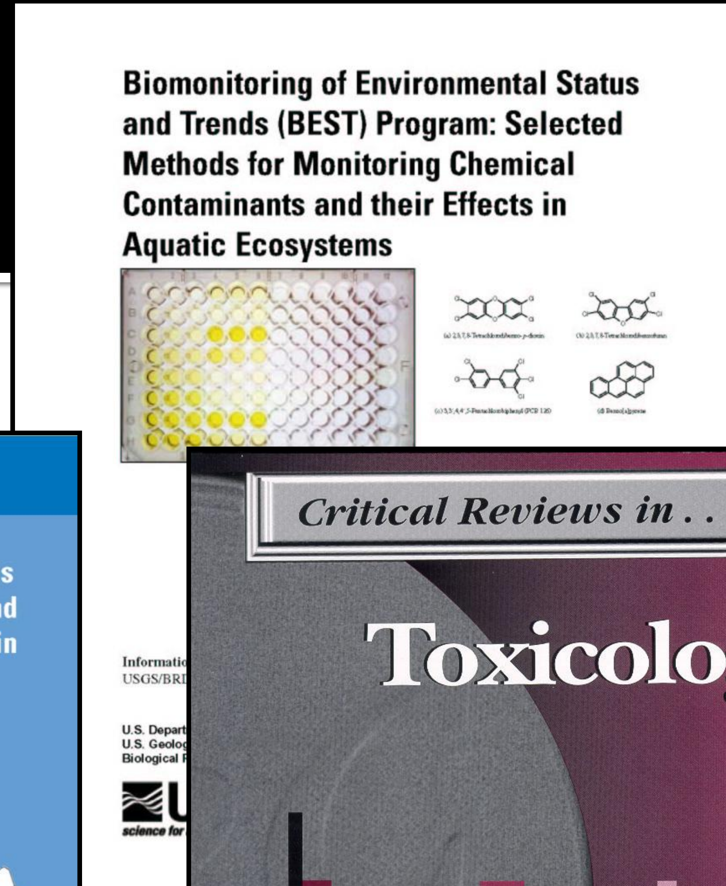
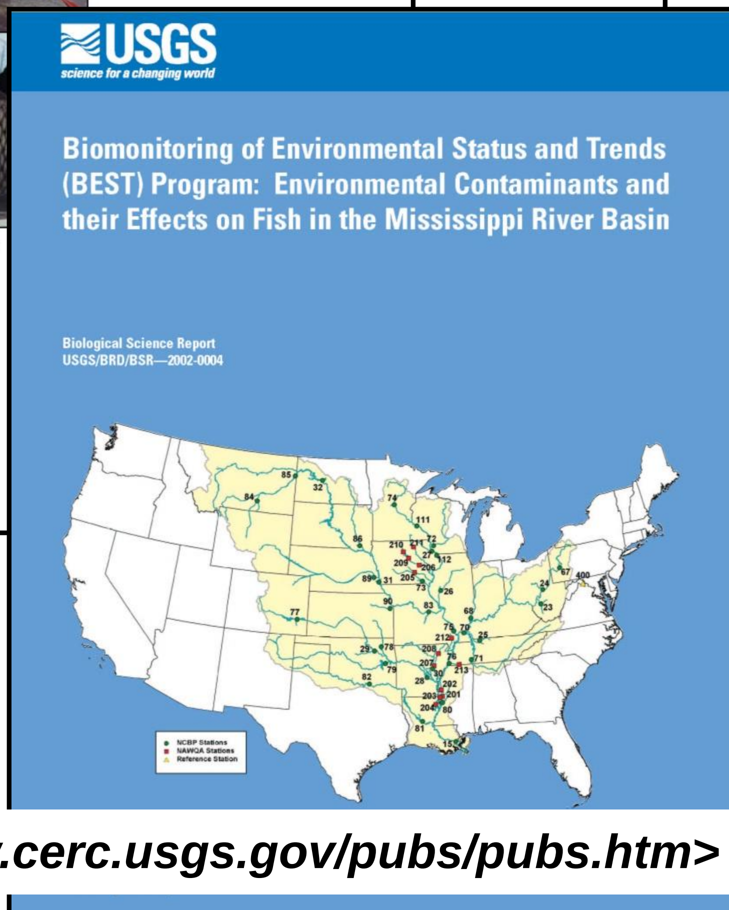

Biomonitoring of Environmental Status and Trends (BEST) Program: Selected Methods for Monitoring Chemical Contaminants and their Effects in Aquatic Ecosystems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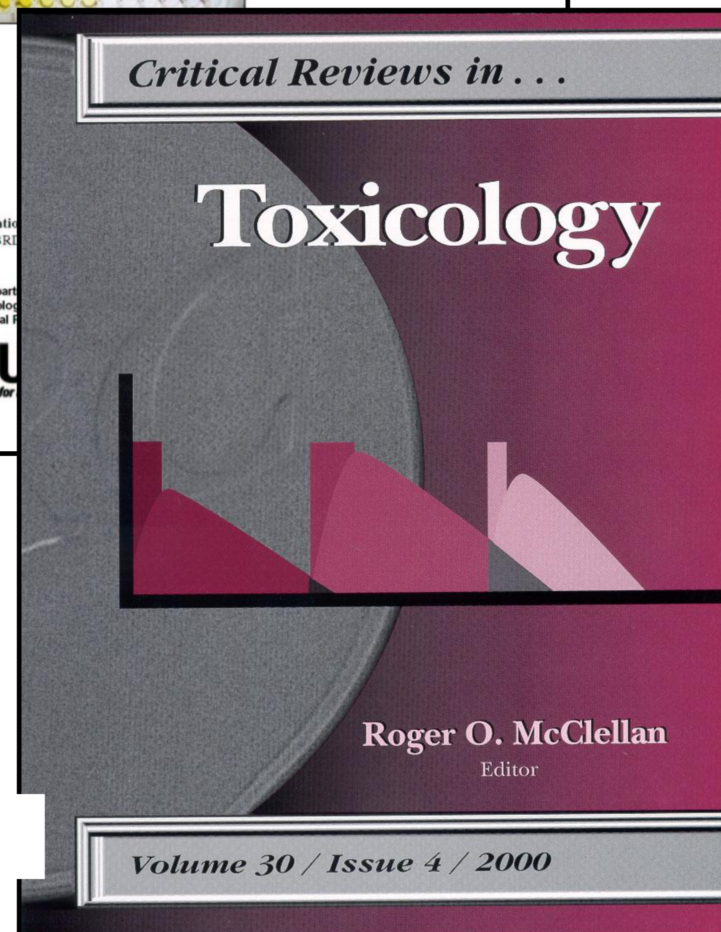
Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants and their Effects on Fish in the Mississippi River Basin

Biological Science Report
USGS/BRD/BSR—2002-0004




Critical Reviews in . . .

Toxicology

Roger O. McClellan
Editor

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