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
National Contaminants Biomonitoring Program (NCBP)

USFWS monitoring program 1970’s-1992
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?
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.
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.
Non-monotonic dose-response relationships with EDCs

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

• Shapes of response relationships vary
**Paradigm shift for environmental chemical monitoring**

<table>
<thead>
<tr>
<th>OC pesticides and industrial chemicals (POPs)</th>
<th>New generation pesticides, veterinary and health care products, and industrial chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>• High Kow</td>
<td>• Lower Kow (70% &lt; 3.0)</td>
</tr>
<tr>
<td>• Low metabolism</td>
<td>• Greater metabolism</td>
</tr>
<tr>
<td>• WS &lt;&lt;&lt; LOD</td>
<td>• WS &gt;&gt; LOD</td>
</tr>
<tr>
<td>• TRV &lt;&lt;&lt; WS</td>
<td>• TRV ???</td>
</tr>
<tr>
<td>➢ Tissue burdens critical</td>
<td>➢ Tissue analysis useless</td>
</tr>
<tr>
<td>• Spatial distribution</td>
<td>➢ Water analysis ???</td>
</tr>
<tr>
<td>• Temporal distribution</td>
<td>➢ Effects biomonitoring</td>
</tr>
<tr>
<td>• Toxicity evaluation</td>
<td></td>
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</table>
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
Endpoints used by LRMN

<table>
<thead>
<tr>
<th>Factors to consider</th>
<th>Rating</th>
</tr>
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<tbody>
<tr>
<td>Cost</td>
<td></td>
</tr>
<tr>
<td>Historical data</td>
<td></td>
</tr>
<tr>
<td>Collection method</td>
<td></td>
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<tr>
<td>Analytical method</td>
<td></td>
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<tr>
<td>Interpretation</td>
<td></td>
</tr>
</tbody>
</table>

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

- Field
- Laboratory
- Temporal
- Spatial
- Species
- Time
- Training
- Preservation
- Expertise
- Equipment
- Time
- Expertise
- Influencing factors
LRMN Sampling Sites

- > 100 sites
- > 3200 fish

EXPLANATION
BEST Site
- Black: Bass and Carp
- Blue: Bass Only
- Orange: Carp Only

- NCBP sites
- + random sites
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
Collection logistics of LRMN

Live fish
Equipment
Min. 2 person crew
Hepatic microsomal ethoxyresorufin O-deethylase (EROD)

Factors to consider

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</tbody>
</table>

Excision of Tissue → Cryopreservation

EROD Assay → Tissue Homogenization
Frequency distribution of EROD activity in green area are reference or background

Influencing factors:
Species
Gender
Reproductive stage

EROD activity in green area are reference or background

Reference = 0.19-6.84 pmol/min/mg
Reference = 4.31-38.4 pmol/min/mg
Reference = 0.35-14.0 pmol/min/mg
Reference = 5.63-44.2 pmol/min/mg
Plasma vitellogenin and steroid hormones

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<td>Interpretation</td>
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</tbody>
</table>
Frequency distribution of Vtg concentrations

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

Conc. >0.01 mg/mL in males is anomalous

Likely exposure to environmental estrogens
Steroid hormones in female carp

- Reference condition difficult to determine
- Samples collected Aug-Oct to minimize stage effects
- $17\beta$-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
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
Cumulative frequency distributions of reproductive biomarkers in fish from the BEST-LRMN
Fish Health Assessment Index (HAI)

Factors to consider

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</table>

External anomalies

Internal anomalies
HAI scores and species differences

Female carp

Female bass

- Fin
- Body surface
- Eyes
- Opercles
- Gill
- Liver
- Spleen
- Spleen
- Kidney

USGS science for a changing world
## Age, length, weight, somatic indices

### Otoliths

![Otoliths Image]

### Enlarged spleen

![Enlarged Spleen Image]

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</tr>
<tr>
<td>Interpretation</td>
<td></td>
</tr>
</tbody>
</table>
Frequency distribution of hepatosomatic index in all LRMN fish

A national study determined liver comprised 1-2% of total body weight in fish (Gingerich, 1982)

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

#### Factors to consider

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<tr>
<td>Interpretation</td>
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</tr>
</tbody>
</table>

- **Testes with granulomas**
- **Anterior kidney with thyroid follicles**
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

Immature oocytes within maturing testicular tissue

Sperm

oocytes
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
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

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

Hinck et al. 2009
Intersex examples from Biomonitoring Environmental Status and Trends

Hinck et al. 2009
## Occurrence of intersex in fish from the BEST-LRMN

<table>
<thead>
<tr>
<th>Species</th>
<th>Female #/n (%)</th>
<th>Sites #/n (%)</th>
<th>Male #/n (%)</th>
<th>Sites #/n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM Bass</td>
<td>0/426 0%</td>
<td>0/55 0%</td>
<td>70/390 18%</td>
<td>23/52 44%</td>
</tr>
<tr>
<td>SM Bass</td>
<td>0/90 0%</td>
<td>0/15 0%</td>
<td>23/70 33%</td>
<td>7/16 44%</td>
</tr>
<tr>
<td>C. Carp</td>
<td>1/798 0.1%</td>
<td>1/89 1%</td>
<td>0/774 0%</td>
<td>0/89 0%</td>
</tr>
<tr>
<td>C. Catfish</td>
<td>0/44 0%</td>
<td>0/6 0%</td>
<td>3/42 7%</td>
<td>3/6 50%</td>
</tr>
</tbody>
</table>
Intersex in male fish by site

![Bar chart showing the number of fish with intersex traits and normal traits by site for largemouth bass, smallmouth bass, and other species.](chart.png)
Intersex condition by age

![Graphs showing the number of fish by age for different categories including Male LMB, Intersex LMB, Female LMB, Male SMB, Intersex SMB, and Female SMB.](image)
## Intersex occurrence by river basin and species with related associations

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

YEAR OF PUBLICATION

NUMBER OF JOURNAL ARTICLES REPORTING INTERSEX IN FISH

'98/'99 | '00/'01 | '02/'03 | '04/'05 | '06/'07
---|---|---|---|---
2 | 3 | 4 | 6 | 6
Occurrence and Severity of Intersex Gonads in SNS

- Missouri River research on sturgeon
- Monitor reproductive status of large numbers of shovel-nose sturgeon
- Observations of intersex in different parts of the river
- Comparison with historical
Example: Shovelnose Sturgeon (SNS), Missouri River
EDCs induce intersex in fish

- Controlled chemical exposures
  - Ethinylestradiol (EE2)
  - Methyl testosterone (MT)
  - Trenbolone acetate
  - Nonylphenol
  - WWTP effluent
  - Pulp & Paper Mill effluents
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
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?
## Summary of endpoint use in fish health assessment

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Method</th>
<th>Interpretation</th>
<th>Overall Use</th>
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<tbody>
<tr>
<td>Age, length, weight, somatic indices</td>
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<tr>
<td>Health Assessment Index</td>
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<td></td>
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<tr>
<td>EROD</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vitellogenin</td>
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<tr>
<td>Steroid hormones</td>
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<td></td>
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<tr>
<td>Pesticides, Inorganic contaminants</td>
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<td></td>
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<tr>
<td>Histopathology</td>
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</table>
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

**Short term effects**
- Molecular effects (gene expression, EROD)
- Physiological effects (steroid hormones, Vtg)

**Long term effects**
- Organismal effects (tumors, somatic indices)
- Population effects (reduced/absence population)

**Sensitivity**

**Ecological Relevance**
Understanding Toxicity Pathways and Complex Biological Processes

Stimuli → Outcome

Toxicant
- Chemical or Stimulus

Macro-Molecular Interactions
- Receptor/Ligand Interaction
- DNA Binding
- Protein Oxidation

Cellular Responses
- Gene Activation
- Protein Production
- Altered Signaling
- Protein Depletion

Organ Responses
- Altered Physiology
- Disrupted Homeostasis
- Altered Tissue Development or Function

Individual Responses
- Lethality
- Impaired Development
- Impaired Reproduction
- Cancer

Population Responses
- Structure
- Recruitment
- Extinction

Genomic Responses

Phenotypic Responses

Environment
- (temp., DO, light, etc.)
Summary/Conclusions

Standard ecological risk approaches will not suffice for PCPPs
  - Lacking TRVs and non-linear dose-responses
Biomonitoting 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
Acknowledgements


USGS Leetown Science Center: V. Blazer

USGS BEST Program: T. Bartish, J. Coyle, P. Anderson

University of Florida: N. Denslow

USFWS Environmental Contaminants Program
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/ITE--1999-0007

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

U.S. Geological Survey


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/ITE--2000-0005

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

USGS
science for a changing world
Biomonitring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants

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

Critical Reviews in... Toxicology

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Roger O. McClellan
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