Intersex and Other Signs of Endocrine Disruption in Fishes Within the Chesapeake Watershed

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USGS
science for a changing world

Chesapeake Bay Executive Order

- Historically – primary concerns
  - Nutrients, suspended sediment
  - Legacy contaminants – whole body or fillet
    - Human consumption
- EO
  - Nutrients, suspended sediment
  - Fish and wildlife health
  - Contaminants of emerging concern
    - Endocrine disruption, intersex

"Toxic" Chemicals

- Ecosystem or Environmental Health
- See serious biological effects when no one chemical is above "threshold benchmarks"
- Classic toxicology/current regulations
  - Benchmark criteria – one contaminant at a time and one or two, often fairly unsensitive species
    - Acute toxicity - death
    - Chronic toxicity – growth
- Endocrine disruption
- Immune system/disease resistance

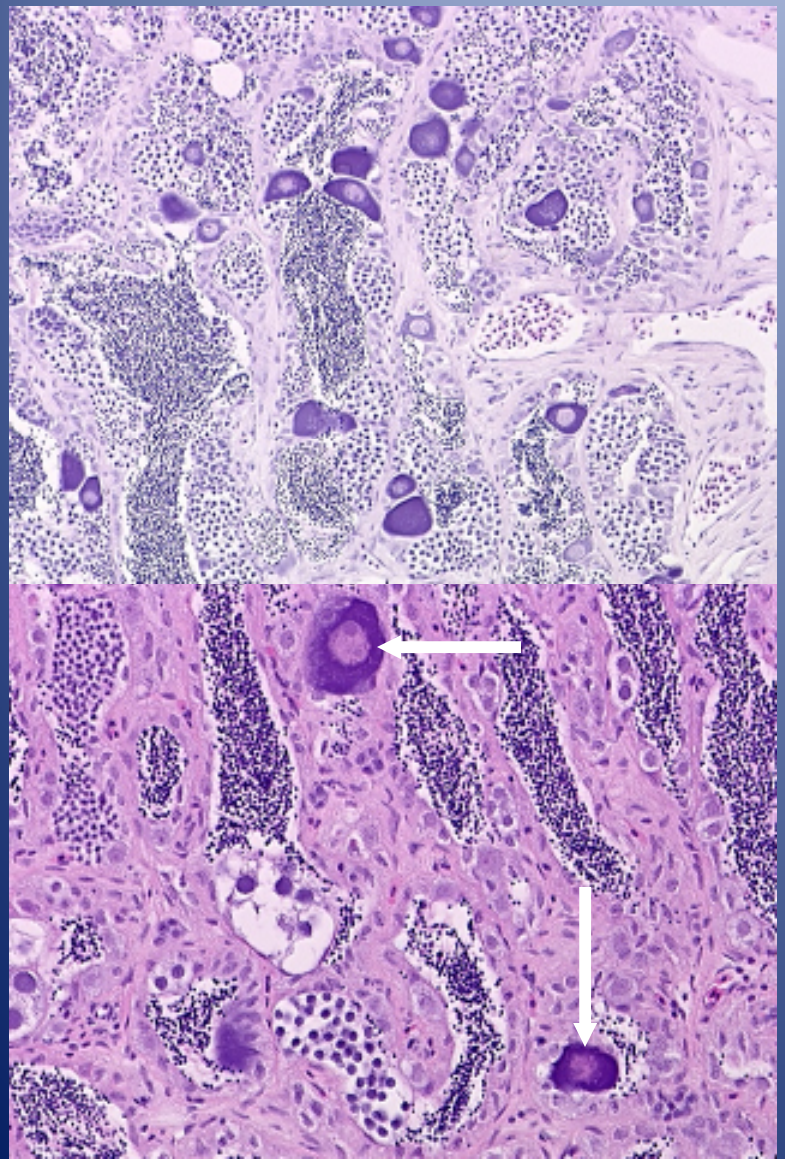
Complexities of Contaminants in Wild Populations

- Many were produced to have a biological effect and so may affect nontarget organisms at very low levels
- Endocrine/Immune systems - chemical communication and feedback mechanisms
- Lack of classic dose response curve – hormesis
- Multiple contaminant exposure routes - water, sediment, food (yolk sac)
- Short term exposure at sensitive life stages can have long term effects

Biological Effects

- Fish kills and skin lesions
  - Centrarchids and suckers in the Potomac and Susquehanna drainages
  - Striped bass in the Bay and selected tributaries
- High prevalence of intersex and other signs of reproductive endocrine disruption
- Lack of recruitment of yellow perch in certain urbanized tributaries
- Skin and liver tumors in brown bullhead

Intersex in Normally Gonochorist Fishes



- Immature oocytes within testes
- Suggested as a marker of endocrine disruption
- Used as an indicator of exposure to estrogenic compounds

Induction of Testicular Oocytes Experimentally

- Estradiol
- Ethinyl estradiol – synthetic estrogen
- Nonylphenol
- 4-tert-pentylphenol and octylphenol
- DDT
- Bisphenol A
- Phytoestrogens/Isoflavones – equol, genistein
- Atrazine - amphibians

Sensitive Life Stage Exposure

- Experimental exposures of numerous fish species have shown that the most sensitive time period for induction of intersex is the first few weeks of life (sexual differentiation).
- Exposure during that time can increase sensitivity later in life.
- Exposure throughout life can increase incidence and severity

Distribution, Prevalence, Severity Intersex in the Potomac Watershed

- Moderate (30%) to high (100%) prevalence
- Low (< 0.2) to moderate (> 2.0) mean severity
- Varied by season, site and species
- Out-of-basin sites on Gauley, Tygart, Greenbrier had significantly lower prevalence and severity
- Appeared to be associated with increased human population and increased agricultural landuse

Blazer, V.S., L.R. Iwanowicz, D.D. Iwanowicz, D.R. Smith, J.A. Young, J. D. Hedrick, S. W. Foster, S. J. Reeser. 2007. Intersex (testicular oocytes) in Smallmouth Bass *Micropterus dolomieu* from the Potomac River and selected nearby drainages. J. Aquat. Animal Health 19:242-253.

Subsequent and Ongoing Studies to Address Intersex and CEC

- Wastewater treatment plants as sources
  - Collaborative study with FWS and MD DNR
  - Upstream and downstream sites on Conococheague Creek and Monocacy River

Iwanowicz, L.R., V.S. Blazer, C.P. Guy, A.E. Pinkney, J. Mullican, D.A. Alvarez. 2009. Reproductive health of bass in the Potomac, USA, drainage: Part 1. Exploring the effects of proximity to wastewater treatment plant effluent. Environ. Toxicol. Chem. 28:1072-1083.

Alvarez, D.A., W.L. Cranor, S.D. Perkins, V. Schroeder, L.R. Iwanowicz, R.C. Clark, C.P. Guy, A.E. Pinkney and V.S. Blazer. 2009. Reproductive health of bass in the Potomac, USA, drainage: Part 2. Seasonal occurrence of persistent and emerging organic contaminants. Environ. Toxicol. Chem. 28:1084-1095

Upstream/Downstream of WWTP

Site	No. Males	Intersex Prevalence	Intersex Severity	% males w Vtg
Susquehanna – U	14	93%	1.4	21%
Susquehanna – D	9	89%	1.7	11%
Swatara – U	4	75%	0.6	0%
Swatara – M	6	67%	1.0	50%
Swatara – D	6	100%	2.0	0%
Monocacy – U	11	82%	1.2	45%
Monocacy – D	7	100%	1.8	33%
Conococheague – U	10	100%	2.2	60%
Conococheague – D	10	90%	1.8	90%

Blazer, V.S., D.D. Iwanowicz, H. Walsh, L.R. Iwanowicz, A. Sperry, D. Alvarez and G. Smith. In Prep. Indicators of reproductive endocrine disruption in Pennsylvania watersheds.

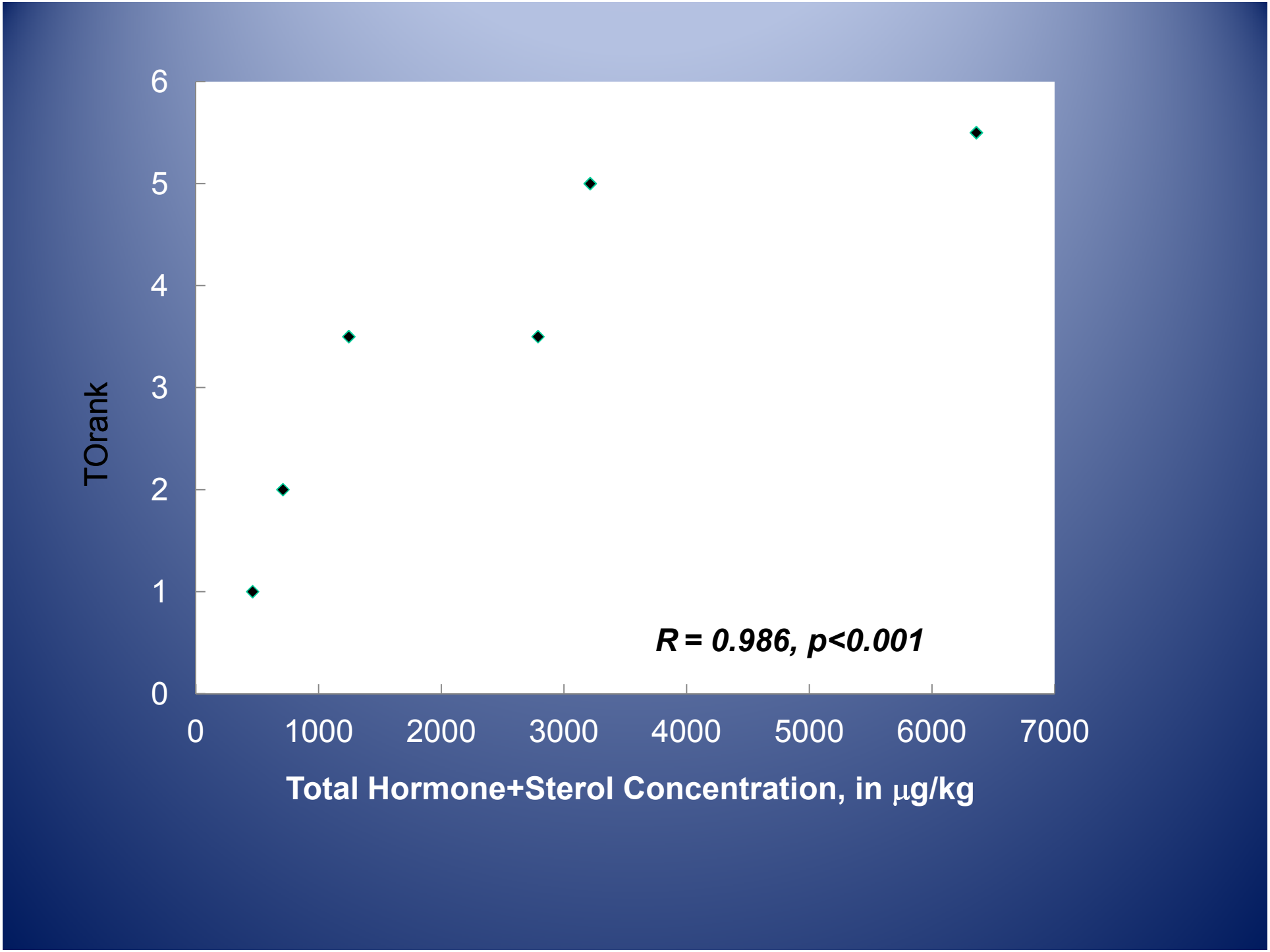
Potomac Spawning Study

- **Objectives**
  - Comprehensive assessment of chemicals in water and sediment
  - Evaluation of biological endpoints
- **Approach**
  - 7 sites, 6 in the Potomac and one reference (Gauley)
  - Represent a range of TO and landuse
  - Sampling during an important life stage
  - Multiple sample types collected

Intersex and Land-use

Landuse Characteristics	Intersex Prevalence		Intersex Severity	
	r^2	*p*	r^2	*p*
Human population	0.39	0.10	0.42	0.08
# WWTP	0.22	0.24	0.34	0.13
WWTP flow	0.32	0.15	**0.63**	**0.02**
Percent agriculture	**0.63**	**0.02**	**0.50**	**0.05**
# Animal feeding operations	0.28	0.17	**0.56**	**0.03**
Total animal numbers	0.27	0.18	0.48	0.06
Animal density	**0.49**	**0.05**	**0.58**	**0.03**
Poultry Houses	0.27	0.18	**0.50**	**0.05**

Blazer, V.S., L.R. Iwanowicz, H. Henderson, P.M. Mazik, J.A. Jenkins, D.A. Alvarez and J.A.Young. 2011. Reproductive endocrine disruption in Smallmouth Bass *(Micropterus dolomieu)* in the Potomac River Basin: Spatial and temporal Comparisons of biological effects. Environ. Monitor. Assess. DOI 10.1007/s10661-011-2266-5


TOrank
0
1
2
3
4
5
6
0
1000
2000
3000
4000
5000
6000
7000
R = 0.986, p<0.001
Total Hormone+Sterol Concentration, in μg/kg

Intersex and Chemical Correlations

Chemical Contaminants	Intersex Prevalence		Intersex Severity	
	r^2	*P*	r^2	*p*
Atrazine	**0.93**	**0.003**	**0.88**	**0.009**
Deethylatrazine	**0.78**	**0.039**	0.68	0.090
Acetochlor	0.65	0.116	**0.79**	**0.036**
Metolachlor	**0.87**	**0.011**	**0.81**	**0.028**

Kolpin, D.W., V. S. Blazer, J. L. Gray, M.J. Focazio, J. A. Young, D. A. Alvarez, L.R. Iwanowicz, W.T. Foreman, E.T. Furlong, G.K. Speiran, S.D. Zaugg, L. E. Hubbard, M.T. Meyer, M.W. Sandstrom, L.B. Barber. In Review. Chemical Contaminants in Water and Sediment near fish nesting sites in the Potomac River Basin: Determining Potential Exposures to Smallmouth Bass (*Micropterus dolomieu*).

Agricultural Pesticides
Fall (Spring)

Chemical Estimated ng/L	Con Up	Con Down	Mon Up	Mon Down
Metolachlor	0.73 (7.5)	1.1 (9.0)	12.0	10.8 (97)
Atrazine	47 (380)	110 (430)	92	2 (2100)
Prometon	1.1 (1.2)	3.2 (1.4)	2.1	1.4 (1.8)

2.5 ppb recently shown to induce complete feminization and chemical castration in frogs - Hayes et al. (March 2010)

Earlier work found 0.1 ppb induced intersex in frogs

Fish Kill Issues

Related to Intersex and Other Reproductive Findings?

- Estrogens and estrogenic chemicals (estrogen mimics) are most often associated with intersex and vitellogenin production in male fishes
- Increasing evidence that estrogenic chemicals and other endocrine-disrupting substances modulate the immune response and disease resistance
- Also other chemicals such as arsenic, atrazine, PCBs act as immunosuppressors by other mechanisms

Overall Findings/Conclusions

- **Sensitive species, stressed populations close to a threshold between healthy and sick (dead)**
- **Variety of stressors leading to immunosuppression**
- **Numerous pathogens contributing to the skin lesions and eventual death – no consistent findings**
  - **Bacteria – *Aeromonas hydrophila*, *A. salmonicida*, *Flavobacterium columnare***
  - **Virus – largemouth bass virus**
  - **Numerous parasites**
- **Prevalence/severity of intersex appears to be related to fish lesions/kills**

Blazer, V.S., L.R. Iwanowicz, C.E. Starliper, D.D. Iwanowicz, P. Barbash, J.D. Hedrick, S.J. Reeser, J.E. Mullican, S.D. Zaugg, M.R. Burkhardt and J. Kelble. 2010. Mortality of centrarchid fishes in the Potomac drainage: Survey results and overview of potential contributing factors. J. Aquat. Animal Health 22:190-218.

Populations Effects

- Increased mortality due to opportunistic infectious and parasitic disease
  - Smallmouth bass
  - Roach (Europe)
- Reduced reproductive success
  - Experimental lake work (Kidd et al. 2007)
  - Smallmouth bass – decreased sperm/motility
  - Roach (Harris et al. 2011)

Yellow Perch Study

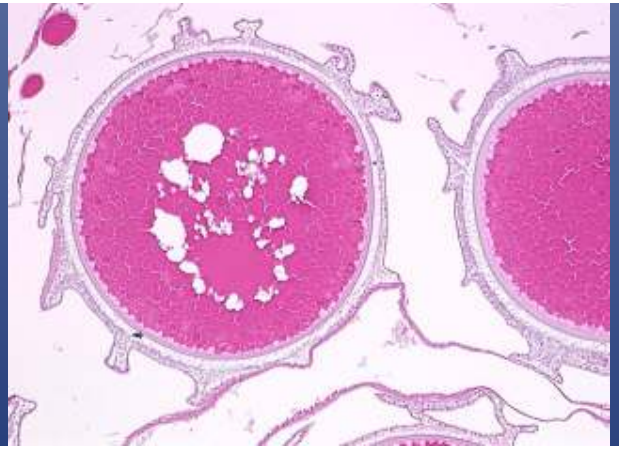
- Lack of recruitment of yellow perch in certain urban Chesapeake tributaries
- Relationship with percent impervious surface
- Five tributaries varying percent of urban landuse and impervious surface
  - South and Severn rivers – high % urban landuse and impervious surface
  - Mattawoman Creek – moderate and increasing
  - Allen's Fresh and Choptank - low

Yellow Perch

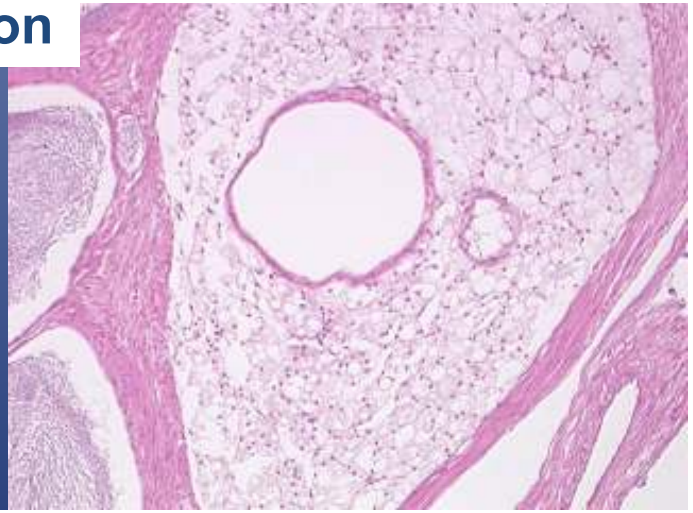
- Histological findings
  - Lack of final oocyte maturation,
  - Abnormal zona pellucida and
  - Leydig cell proliferation/Leydig cell tumors
- Both responses are regulated by dopamine
  - Raises question of exposure to dopamine agonists

Normal egg

Abnormal yolk and chorion



Leydig cell proliferation



Acknowledgements

USGS – many Programs/Centers

USFWS

NOAA

EPA

WV Division of Natural Resources

WV Department of Environmental Protection

VA Division of Game and Inland Fisheries

VA Department of Environmental Quality

MD Department of Natural Resources

University of Tennessee, Center for Environmental Biotechnology

Virginia Tech

PA Fish and Boat Commission

Potomac/ Shenandoah River Keeper

Friends of the Shenandoah