Evidence for Estrogenic Endocrine Disruption in Bass Throughout the Northeast

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Background

- Beginning in 2003 major fish kills of adult bass, suckers and other species were observed on the South Branch Potomac, Shenandoah River (2004-2005) and Monocacy River (2009)
- Lower level chronic skin lesions and mortality, and sporadic fish kills have occurred since then

Skin Lesions and Mortalities Adult Fishes in the Potomac Drainage

Adult Fish in the Potomac - Findings

- **Multiple bacterial pathogens, but no consistent findings**
  - ***Aeromonas hydrophila* and other motile Aeromonads**
  - ***Aeromonas salmonicida***
  - ***Flavobacterium columnare***
- **Multiple, often heavy parasite infestations**
  - **Leeches, trematodes, myxozoans, cestodes**
- **Opportunistic fungal infections**
- **Skin papillomas**
- **Largemouth Bass Virus**
- **High prevalence of testicular oocytes and vitellogenin in male fishes**

Impaired Ecosystem - Immunosuppression

Chemicals of "Emerged Concern"

- Defined as synthetic or naturally occurring chemicals that are not commonly monitored in the environment, are generally not regulated, but have the potential to enter the environment and cause adverse effects
- Newly recognized effects such as endocrine disruption of exposure to low concentrations of legacy contaminants as such PCBs, mercury, arsenic

Chemicals of Emerging Concern Sources

- Wastewater Treatment Plants
- Industrial effluent
- Stormwater runoff
- Agriculture
- Landfill leachate
- Gas extraction/Marcellus shale

Inputs and Management

Cattle with free access to the river

Piles of litter/manure along the river prior to a high water event

Chemicals of Emerging Concern WWTP and Stormwater-Related

- Pharmaceuticals
  - Synthetic Hormones – birth control, hormone replacement therapy
  - Antibiotics
  - Viagra to Prozac

- Personal care products
  - Antimicrobials – soap, detergent, toothpaste
  - Fragrances
  - Organic UV filters
  - DEET

  - BDE – brominated diphenyl ethers (flame retardants)
  - Bisphenol A – plasticizers, epoxy resins

Agricultural Sources
Residential Sources

- Animal manure and litter
  - Natural and synthetic hormones
  - Phytoestrogens
  - Antibiotics/antimicrobials
- Pesticides and herbicides
- Human biosolids used for fertilizer

Complexities of CECs 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, maternal (yolk sac)
- Short term exposure at sensitive life stages can have long term effects
- Complex mixtures – additive, synergistic or antagonistic interactions

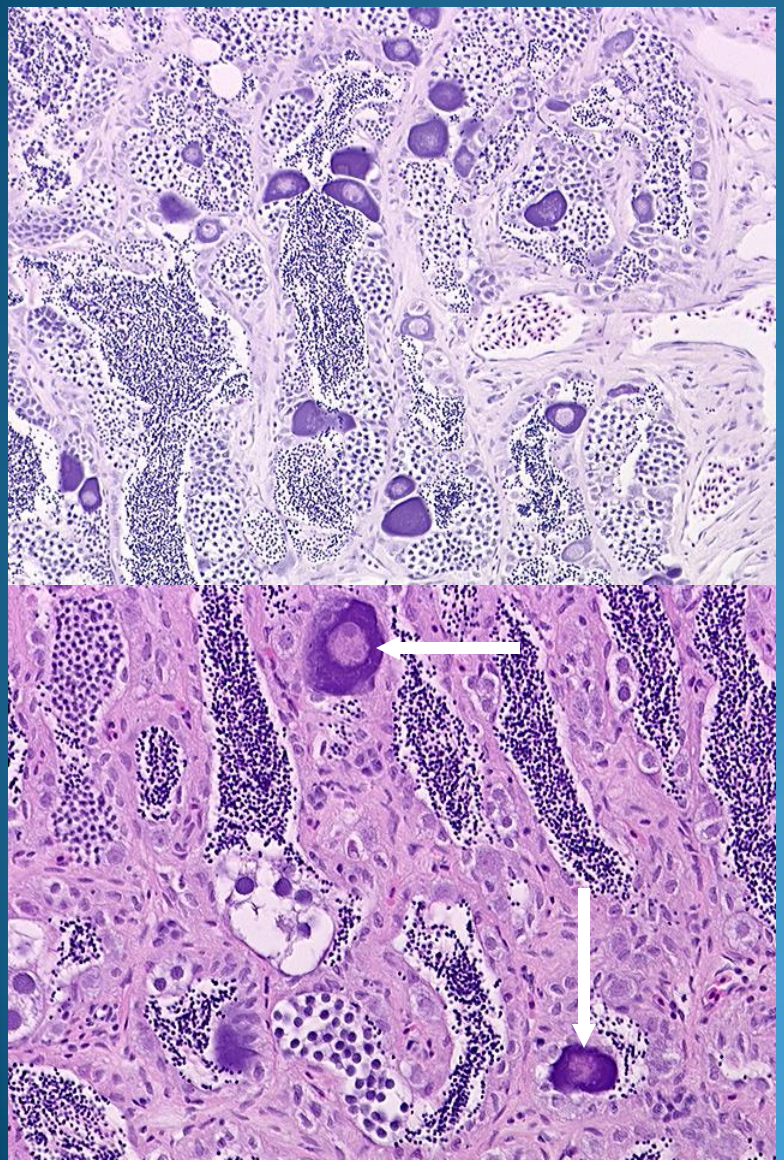
Fish as Integrators for Aquatic System Health

- Constantly exposed to the multitude of stressors in the water, sediment and their food source
- Integrate effects over time
- Stressors include complex mixtures of chemicals, climatic effects, infectious agents and many others
- Contaminants – many act synergistically, complex interactions with parasites/ pathogens
- Hence, adverse effects monitoring can identify environmental problems

Indicators of Exposure to Estrogenic Chemicals

- Intersex – testicular oocytes
  - Most likely induced early in life (fertilized egg to first few weeks)
  - Severity may increase with increased exposure throughout life
- Vitellogenin
  - Yolk precursor
  - Exposure turns the gene on in males
  - Plasma vitellogenin – days to months
  - Vitellogenin mRNA – hours to days

Testicular Oocytes in Bass



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

Another Complexity

- All fish are not created equal
- Different sensitivities to a variety of contaminants
- Species choice and choice of the biomarker are very important
- Using bass because they have been shown to be very sensitive to estrogenic endocrine disruption
- Multiple contaminants can have the same effect and so “the cause” in one environmental setting may not be “the cause” in another setting/landuse

Collaborative Approach

- Chemical Monitoring
  - Water (discrete and time-integrated)
  - Sediment
  - Fish tissue
- Biological Effects Monitoring
  - Resident fish species or other aquatic organisms
  - In vitro cell-based assays
- Landuse/Landcover Analyses

Bioactivity of chemical extracts from water samples

Grab water sample

OASIS HLB

Elute

MeOH
MeOH: DCM

DMSO

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Total Estrogenicity

- In vitro assay using genetically engineered yeast cells – bioluminescent (BLYES)
- Extracts of discrete or passive water samples
- Measures the total estrogenicity in relation to a standard curve with estradiol
- Benefit – provides an indication of the activity of complex mixtures
- Doesn't indicate what those chemicals are

Multiple Projects Addressing Adverse Effect Related to Emerging Contaminants

- Chesapeake Bay watershed
  - Potomac River – WWTP, agriculture and other sources
  - Susquehanna – WWTP, agriculture and other sources
  - EDC Integrator sites
- Out of basin Site Comparisons
  - Ohio, Delaware and Lake Erie drainages
- Great Lakes
  - Areas of Concern on lakes Ontario, Erie, Michigan and Superior

National Wildlife Refuge Project

- Collaboration with FWS
- 19 refuges – sites on and around refuges
- Sampled in the Fall
- Biological endpoints were testicular oocytes (TO), plasma vitellogenin (Vtg) and total estrogenicity of water samples

Overall Results

- Testicular oocytes
- Smallmouth Bass – 60 – 100% (mean of 85%)
- Largemouth bass – 0 – 100% (mean of 27%)
  - "Reference sites" for SMB 10-14% with severity 0.2 or less
  - For LMB – many sites with 0 %

- Estrogenicity – many had some activity
- Only 6 refuges had > 1 EEQ (ng/L)
  - No effects level ranges from 0.73 to 10

Delaware Drainage Results

- Walkill – 5 male SMB
  - no hormone activity measured in water,
  - no vitellogenin in male fish
  - 100% of males had TO but low severity (<1.0)
- Great Swamp – 16 male LMB
  - no hormone activity in water
  - no vitellogenin in male fish
  - 8% of males had TO, low severity (<1.0)
- Cherry Valley (2 sites) - 10, 11 male SMB
  - estrogenicity in water
  - Vitellogenin in male fish – actually similar to females
  - 80-90% TO (severity of 1.0)

Refuge Project

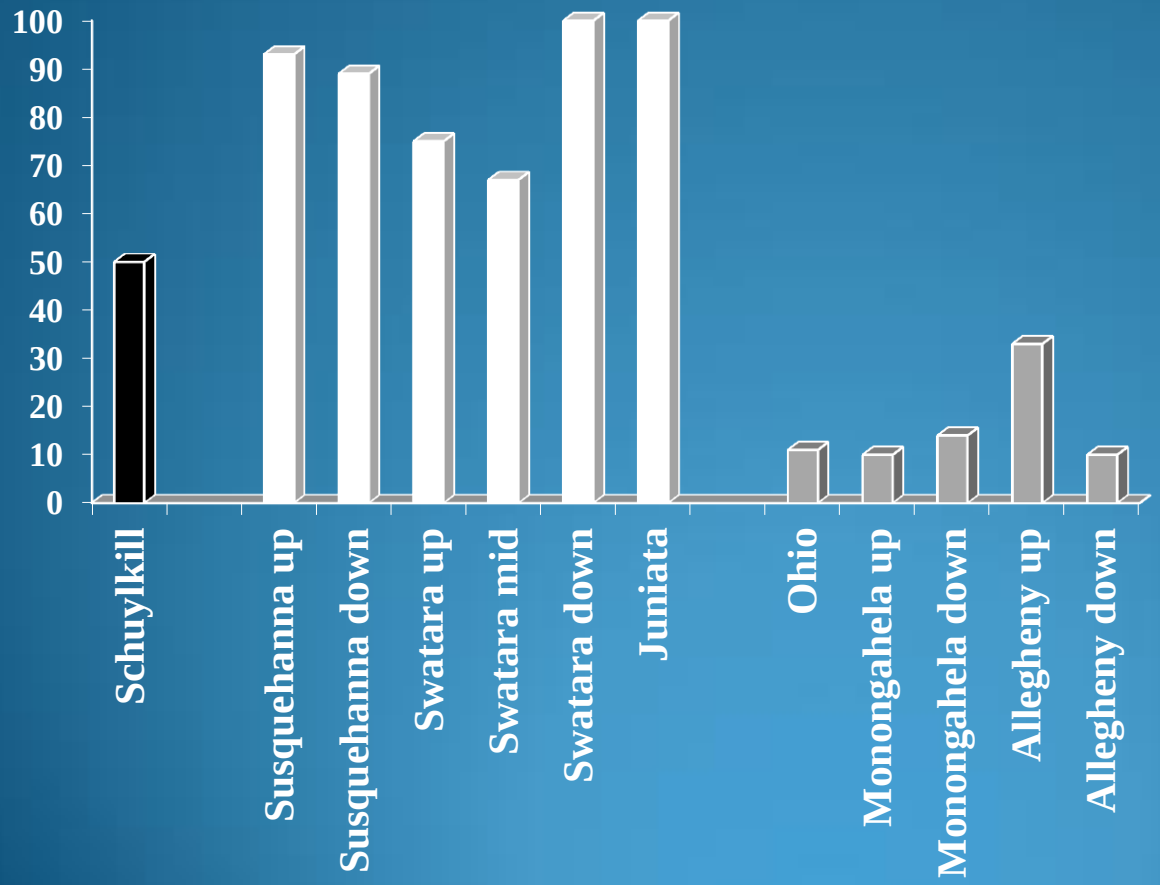
- A reconnaissance to identify potential adverse effects
- No water/sediment/fish tissue contaminants were measured
- Identify sites for further study

Chesapeake Projects

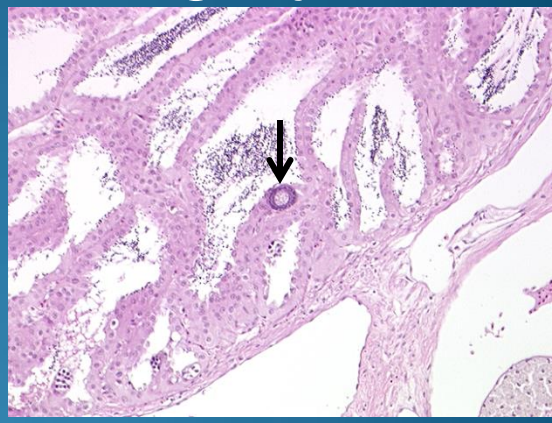
- Do have chemical analyses for many of the projects
- Allows us to look for correlations
  - Although not cause and effect
  - Provides weight-of-evidence for chemical groups and sources that may be contributing to adverse effects

- Overall observation
  - For bass, agricultural inputs rather than WWTP seem to be major contributing factor
  - Do not consistently see increased prevalence of TO downstream of WWTP – often higher upstream

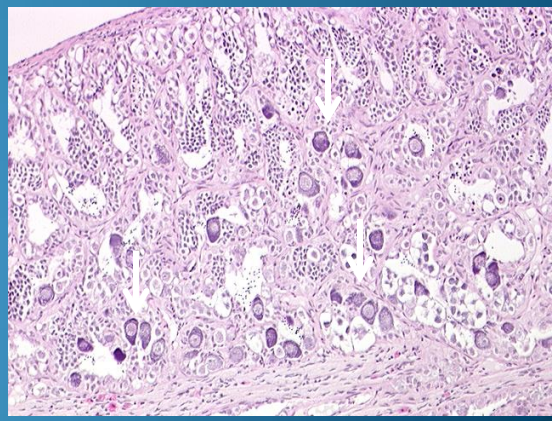
Intersex in SMB from PA River Drainages



Allegheny River



Susquehanna River



Correlations with Landuse and Chemicals PA Drainages

Chemical Contaminants or Landuse	Intersex Prevalence *rho*	*p*	Intersex Severity *rho*	*p*
Estrone (water)	0.6530	0.0238	0.7609	0.0055
Agricultural landuse	0.6843	0.0170	0.7044	0.0129
WWTP/sewage facilities	-0.5298	0.0794	-0.8441	0.0936
	Prevalence of males with vitellogenin			
Estrone (water)	0.7914	0.0033		

Spawning Study

- Spring spawning study in Potomac
  - No estrogen hormones were detected in discrete water samples
  - Atrazine and metalochlor conc. correlated with prevalence and severity of intersex
  - Total biogenic hormones and plants sterols in sediment correlated with prevalence and severity

Spawning Study
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

Shenandoah Tributary Study

Land-use Characteristics	Estrogenicity	
	rho	*p*
% Forest	-0.654	0.008
% Pasture/ Hay	0.629	0.012
% Crop	0.586	0.021
% Developed	0.453	0.086
Poultry Density	0.696	0.004
Beef Density	0.530	0.041
Dairy Density	0.360	0.180
WWTP (MGD)	-0.006	0.974

No fish data

Total estrogenicity based on the estrogen equivalents using the BLYES

POCIS pesticides (26 total)	Estrogenicity	
	rho	*p*
Desethylatrazine	0.670	0.006
Metolachlor	0.631	0.011
Atrazine	0.582	0.022
Simazine	0.541	0.037

Herbicides

- Atrazine and simazine – triazine herbicides
  - Groundwater concerns – banned in Germany because groundwater exceeded 100 ng/L
- Metolochlor – chloroacetanilide herbicide
  - Health advisory at 525 ng/L

Phytoestrogens

- Induction of intersex and other reproductive endpoints
- Influences on immune response and disease resistance

Correlations of Herbicides with Intersex and Estrogenicity

- Atrazine has been associated with intersex in frogs – controversial
- Atrazine is not thought to bind to the estrogen receptor
- Has been shown to alter phytoplankton and algal populations, increase trematode infections and cause immunosuppression
- Could algal/cyanobacteria and their toxins be contributing to endocrine disruption and fish health issues

Herbicide Concentrations (ng/L) Monocacy – Big Pipe Creek

	Dates	Atrazine	Metolachlor	Simazine
	May 15, 2013	364	228	343
	June 8, 2013	75	81	58
Storm	June 11, 2013	227	215	215
	July 11, 2013	45	61	20
	Aug 28, 2013	37	34	13
	April 4, 2014	26	30	12
	April 14, 2014	31	47	15
	April 25, 2014	16	13	-
Storm	April 30, 2014	3268	703	2416
	May 12, 2014	201	93	112
Storm	May 16, 2014	5399	4643	76
	May 30, 2014	2872	1612	1976

Herbicide Concentrations (ng/L) Chillisquaque Creek

Dates	Atrazine	Metolachlor	Simazine
April 25, 2014	43	83	24
April 30, 2014			
May 12, 2014	22	23	
May 16, 2014	13,667	3,148	9,213
May 30, 2014	1,949	1,717	

Are Those Concentrations Significant?

- Big Pipe Creek – 4.8 to 5.7 ppb late April to late May – spawning period
- Chillisquaque Creek – 1.9 to 22.9 ppb

- 2.5 ppb induced complete feminization in frogs when exposed from hatching through metamorphosis (Hayes et al. 2010)
- 0.1 ppb induced intersex in frogs exposed during larval period (Hayes et al. 2003)
- Atrazine has been shown to increase susceptibility of fish to *Aeromonas hydrophila* and reduce immune responses (most studies in the ppm; one study 42 ppb)
- Increased trematode infections in amphibians (3 to 200 ppm)

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