Biological Effects Monitoring to Identify Consequences of Exposure to Endocrine Disruptors

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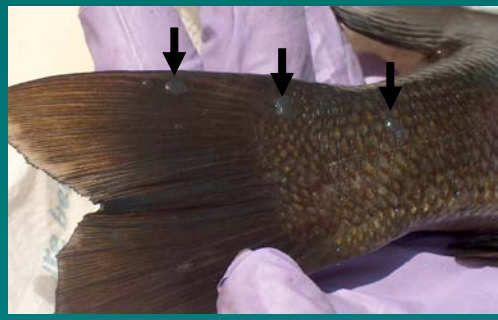
Endocrine Disruption in the Chesapeake Watershed

- First observed during assessments to determine the cause of fish kills
  - Adult fishes
    - South Branch Potomac (WV) beginning in 2003
    - Shenandoah River (VA) beginning in 2004 – 2005
    - Monocacy River (MD) – 2009
  - Young of year Smallmouth Bass
    - Susquehanna River (PA) – beginning in 2005

Adult Fish in the Potomac

- 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 intersex, vitellogenin in male fishes



Impaired Ecosystem
Immunosuppression

Young of Year in the Susquehanna

- *Aeromonas hydrophila* and other motile Aeromonads
- *Flavobacterium columnare*
- Largemouth Bass Virus
- Trematodes
- Myxozoan parasites

Importance of Wild Fish (or other Organism) Monitoring

- Integrate the various environmental stressors over time
  - Complex mixtures of chemical contaminants
  - Temporal changes – short term (weekly/monthly) and annual
  - Cumulative over the life span

Adverse Effects Monitoring

- **Sensitive species – not all fish are created equal**
  - **Species sensitivity due to genetic, physiological factors**
  - **Habitat usage, spawning habitat and timing**
- **Indicators of effects (adverse outcome pathways) at various levels of organization**

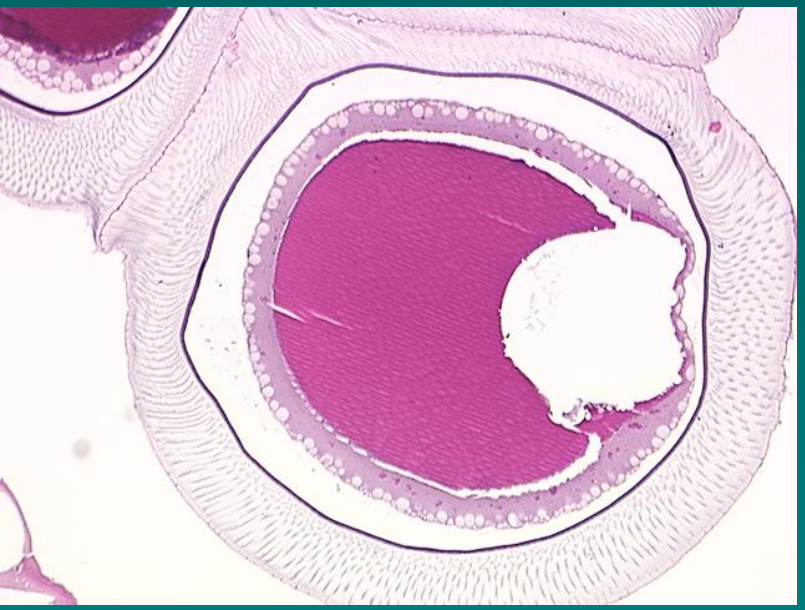
- **Understand the effects of complex mixtures of chemicals and other environmental stressors**
- **Evaluate the efficacy of restoration, remediation or other management actions**

Fish Health Issues Potentially Related to EDC

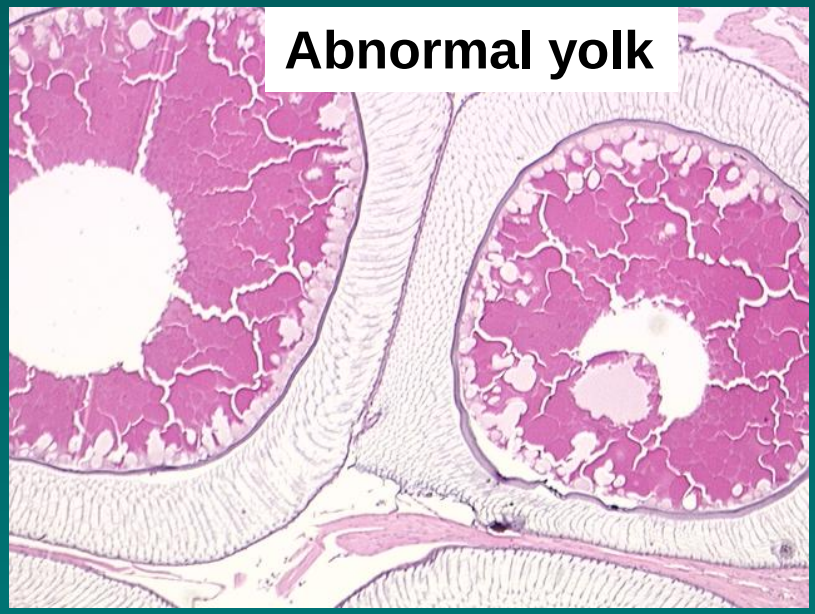
- Agricultural areas
  - Fish kills/chronic mortality/skin lesions
  - Estrogenic endocrine disruption
- Urban
  - Lack of reproductive success of yellow perch and other anadromous fishes in urban tributaries
  - Skin and liver tumors in bottom fishes such as brown bullhead

Egg Abnormalities Observed

Yellow perch



Choptank –
Most appeared normal
Thick, intact, regular chorion
Hydrated yolk



Levels of Organization

Population

Organism → **Visible lesions, condition factor, relative weight, growth**

Tissue → **Gonadosomatic index, parasite load, microscopic pathology – pathogens, tumors, inflammation**

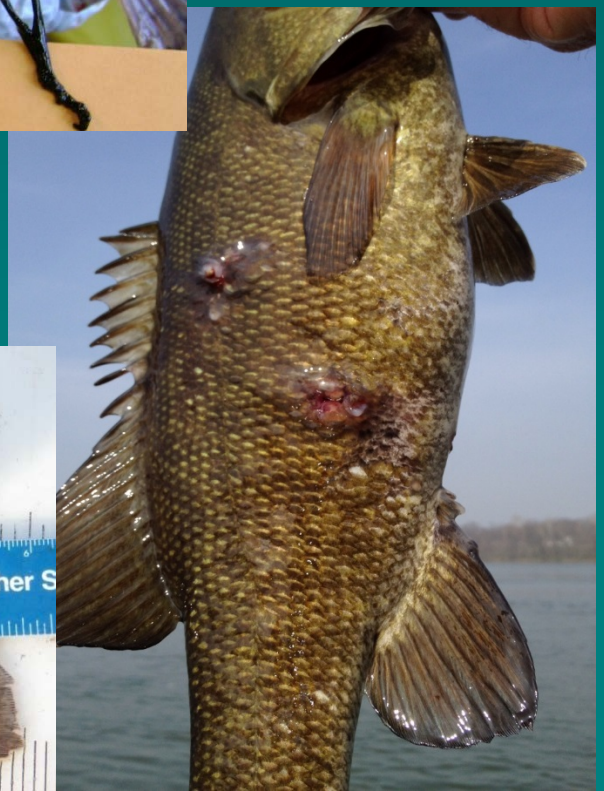
Cellular → **Histopathology – necrosis (cell death), hypertrophy, cytoplasmic vacuoles/inclusions**
Function of immune cells such as lymphocytes and macrophages

Molecular → **Expression of genes – estrogen and androgen receptors, metabolic, contaminant-responsive, immune regulatory**

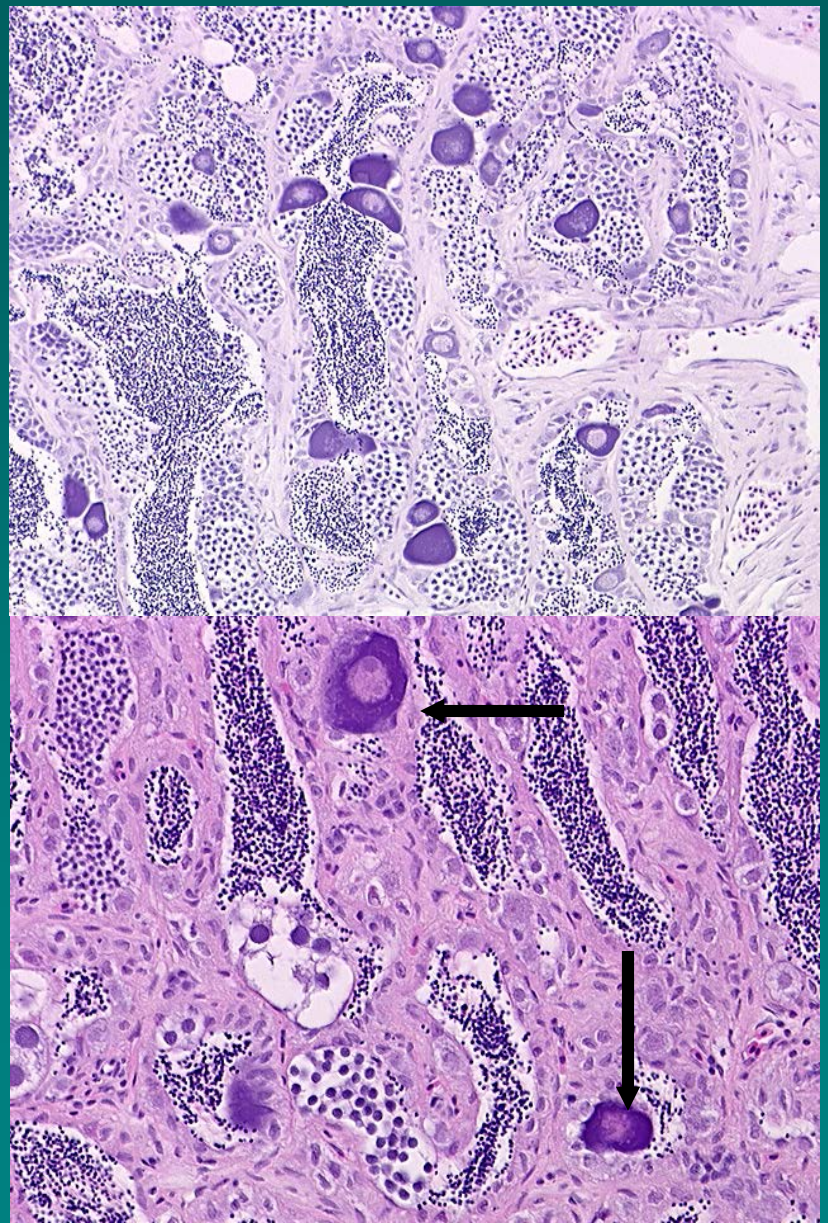
Subcellular → **Plasma vitellogenin, hormones, proteins**

Organism Level - Visible Lesions

- Anglers and the general public see and are most concerning



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

Estrogenic Effects in Male Fish

- **Intersex – most likely induced early in life, first few weeks, severity may increase with age**
- **Plasma vitellogenin – indicative of more short term exposure – days to months**
- **Vitellogenin mRNA – indicative of current conditions**

Initial Findings

- A number of our studies have evaluated fish upstream and downstream of WWTP
  - Do not see higher prevalence of intersex at downstream sites, however, in both Potomac and Susquehanna studies intersex severity was slightly higher downstream
- Did consistently find an association with agricultural landuse and effects in bass

Correlations with Landuse and Chemicals PA Drainages

Evaluated smallmouth bass and white sucker at 16 sites in three river drainages in the summer (low flow)

Bass – intersex and vitellogenin in males
White sucker – no intersex, did have vitellogenin in males

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		

Intersex and Land-use
Spring Pre-spawn Study Potomac

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

Intersex and Chemical Contaminants Spawning Study

Chemical Contaminants	Intersex Prevalence		Intersex Severity	
	rho	*p*	*rho*	*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

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

Associations of Herbicides with Intersex, Estrogenicity and Disease

- Atrazine augments the richness and abundance of gastropods
  - Intermediate hosts for trematode parasites
  - Increase abundance of the cercariae (infective stage of trematodes for fish and amphibians
- Atrazine and other herbicides decrease phytoplankton which tend to increase periphyton
- Could algal/cyanobacteria and their toxins be contributing to endocrine disruption and fish health issues

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)**

Better Understanding

- **Integration of long term biological data sets**
  - **Few sites with > 10 years of biological data**
  - **Chemical data (water, sediment, tissue)**
  - **Nutrient data**
  - **Climatic data – flow, temperature**
  - **Landscape analyses – land use, land use change, changes in agricultural practices, BMPs etc.**

Understanding of the most important risk factors for the fish health issues and identify steps that could be used to restore healthy ecosystems

Acknowledgements

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