Indicators of Endocrine Disruption in the Chesapeake Bay Drainage

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Endocrine Systems

- Series of organs or tissues that regulate, through hormones (receptors), everything from growth to reproduction to behavior
- Reproductive
  - HPG (hypothalamic, pituitary, gonad)
- Thyroid
- Adrenal - glucocorticoid – stress hormones
- Pancreas
- Neuroendocrine

Endocrine Disruptors

- EDCs can <u>mimic</u> normal hormone function by "fooling" the body and causing an inappropriate response.
- EDCs can <u>block</u> the effects of hormones, thereby interfering with normal function.



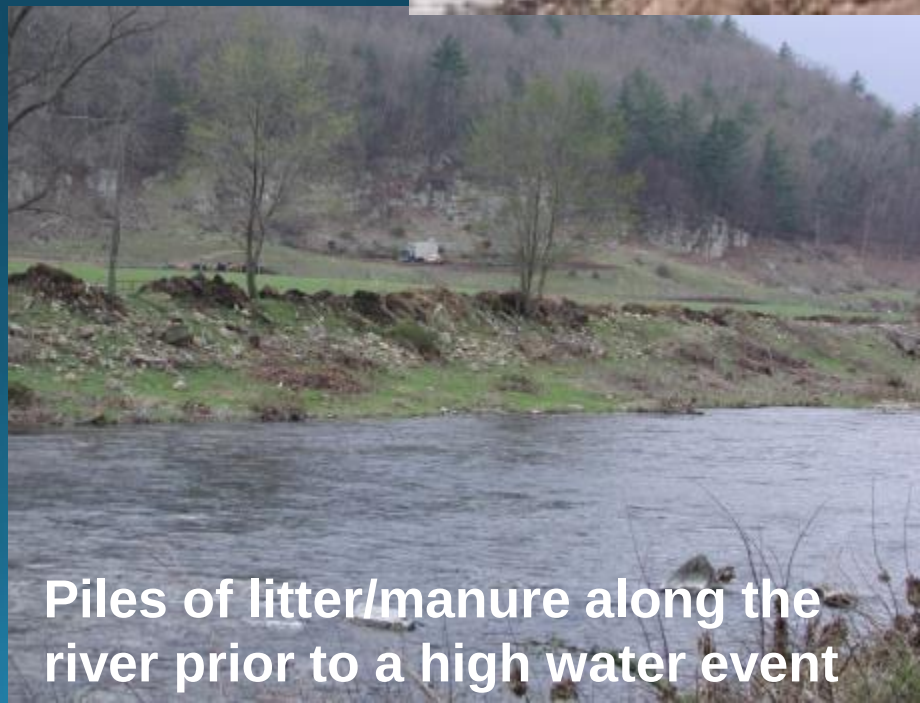


USGS

Sources

- Human development
  - WWTP effluent
  - Stormwater runoff
  - Construction sites
  - Residential runoff
- Various Industries
- Agriculture

Cattle with free access to the river



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

Contaminant Groups and CB Strategies

Widespread Severity and Occurrence

PCBs → **Policy/Prevention Strategy**

Mercury → **Next policy strategy?**

Local Effects

Dioxin, Petroleum, Insecticides, Metals PAHs → **Local impairments**

More information needed

Pesticides
Herbicides
Pharmaceuticals
HshId/Personal Care
Flame Retardants
Biogenic Hormones

→ **Research strategy: Effects, Occurrence, Sources**

Induction of Testicular Oocytes Experimentally

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

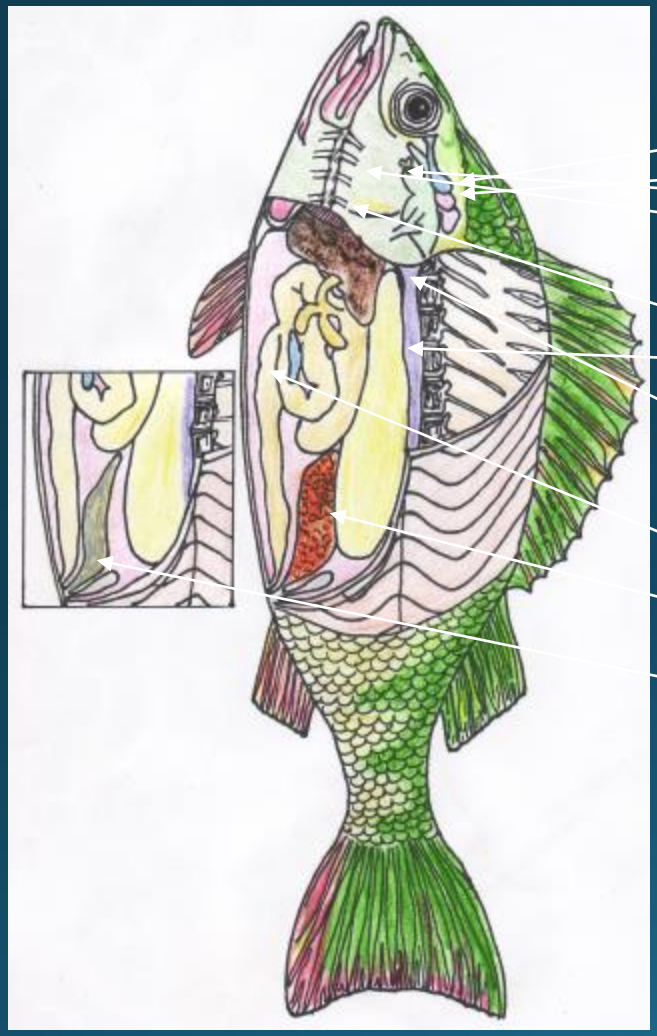
USGS Chesapeake Science Strategy Themes

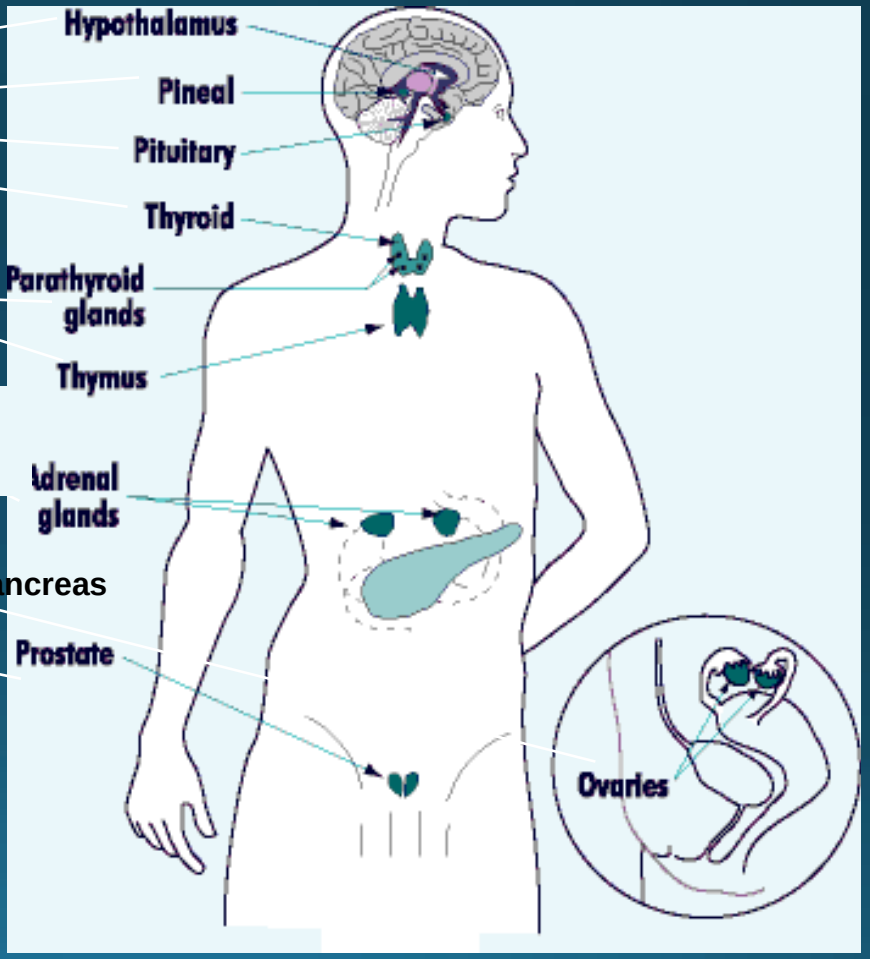
- Fish, wildlife, and habitats
- Water quality and links to ecologic responses
- Climate and land change
- Synthesize and provide science for ecosystem management
- Integration questions

Fish, Wildlife, and Habitat

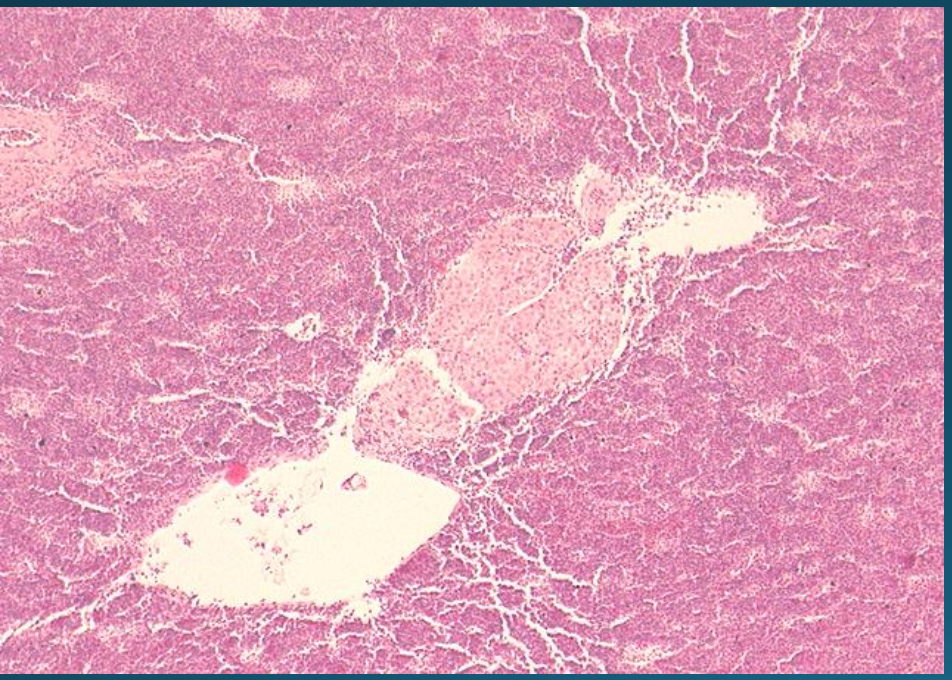
- Objectives
  - Freshwater fish and habitats
  - Toxic contaminants and stressors
  - Wetland function and waterbirds
- Integration questions
  - How do land use and climate changes affect fisheries
  - What are the relationships among fish health, land use, contaminants, and water quality?
  - How are coastal wetlands and carrying capacity for waterbirds affected by land use and climate

Fish Endocrine Glands

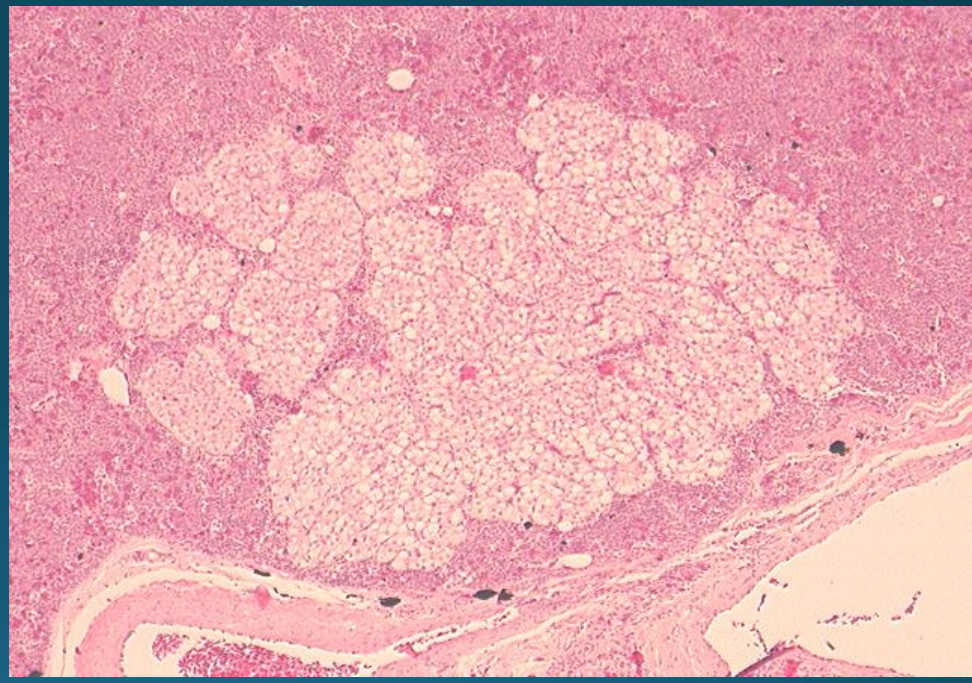




Endocrine Changes
Interrenal Tissue - Adrenal Cortex



Normal



Hyperplasia and hypertrophy

Fish as Integrators for Aquatic System Health

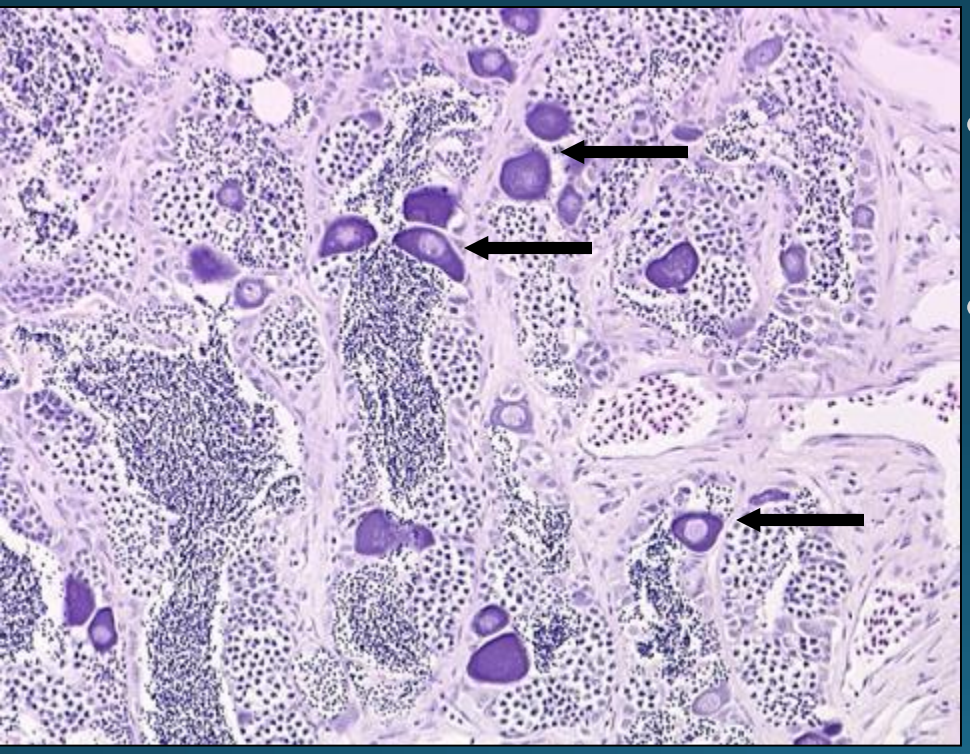
- Constantly exposed to the multitude of stressors in the water, sediment and their food source
- Stressors include complex mixtures of chemicals, climatic effects and many others
- Contaminants – many act synergistically, lack of classic dose-response, methods to measure at concentrations they cause effects, vary greatly seasonally/daily
- Integrate effects over time

Adult Fish in the Potomac - 2003

- 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

Intersex in Gonochorist Fishes

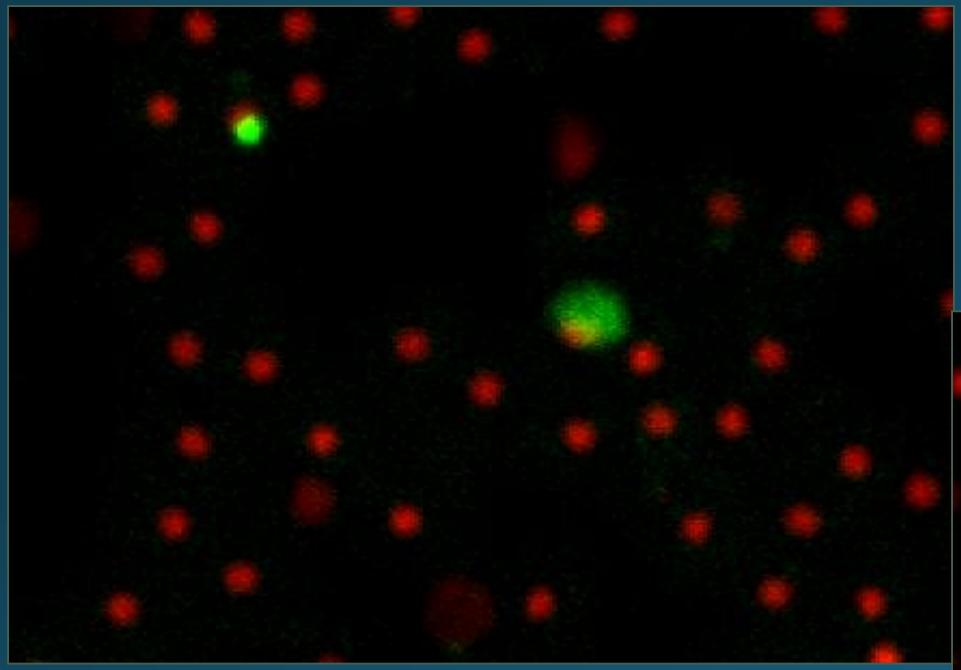


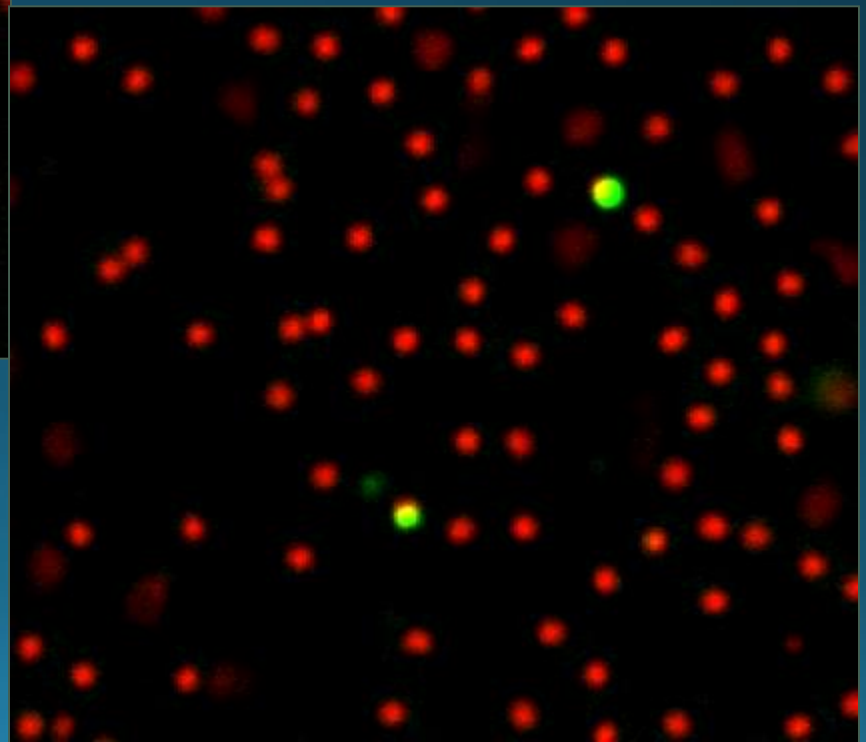
- Suggested as a marker of endocrine disruption
- Most often associated with exposure to estrogenic compounds

Susquehanna Drainage - 2005

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

Estrogen Receptor α
Fish Leukocytes





Effects of Estrogen on Fish Leukocytes

- **Estrogen exposure reduces phagocytic index** – ability of phagocytic cells, such as macrophages and neutrophils, to engulf infectious agents

- **Estrogen influences bactericidal activity** – ability of these cells to destroy engulfed agents by influencing at least two killing mechanisms:
  - **nitric oxide production**
  - **respiratory burst activity**

Complexities of EDCs 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)
- Short term exposure at sensitive life stages can have long term effects
- Additive, synergistic

Approach

- Effects-based monitoring combined with chemical analyses
- Biological effects often occur when no one chemical indicator is above “threshold benchmarks”
- Fish health is a good integrator of cumulative effects of aquatic environmental stressors – integrate over time as well as mixture effects
- In vitro bioassays to assess total estrogenicity, androgenicity, glucocorticoid, thyroid hormone activity

Methods
Chemical Sampling Water

- Discrete (grab) water samples
- Time integrated samples – passive samplers that are deployed for 30-45 days

Grab water sample

OASIS HLB

Integrative Passive Samplers

- Semi Permeable Membrane Devices (SPMDs)

  accumulate hydrophobic compounds

- Polar Organic Compound Integrative Samplers (POCIS)

  accumulate hydrophilic compounds

Plus – tend to find higher number of detects, integrates storm events
Con – hard to get an actual concentration, may equilibrate

Suite of Fish Biological Indicators

- **Morphometric and necropsy-based**
  - Comparisons based on sex, age,
  - Identify visible abnormalities
  - Provides condition factor/relative weight
- **Plasma**
  - Hormones – estrogen, testosterone, cortisol, thyroid
  - Vitellogenin
- **Histopathological**
  - Diagnose causes of gross observations, identify emerging pathogens, identify specific effects of contaminants, with image analyses quantify parasites, macrophage aggregates etc.
- **Molecular**
  - mRNA for reproductively related genes (vitellogenin, estrogen receptors), immune system indicators (TGF-β, hepcidin), contaminant-related (CYP1A, oxidative stress), stress (glucocorticoid receptors)

Biomarkers of Exposure Estrogenic Contaminants

- Intersex
  - most likely induced very early
  - exposure during sexual differentiation increases sensitivity later in life
- Vitellogenin
  - Plasma Vtg - indicative of recent exposure (days to months)
  - Vtg gene transcripts – hours to days

Findings
Biological Effects and Sources

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

Correlations with Landuse and Chemicals PA Drainages

Study done in late summer, 16 sites in three river drainages – Ohio, Susquehanna, Delaware

Discrete water samples at time of fish sampling

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		

Potomac Spawning Study

- 7 sites in Potomac/Shenandoah
- Conducted at spawning sites
  - Intersex prevalence only significantly correlated with % ag and animal density in watershed above the site; intersex severity correlated with % ag, animal density, # of AFO, # poultry houses and WWTP flow
  - No estrogen hormones were detected in discrete water samples; atrazine and metolachlor conc. correlated with prevalence and severity; total biogenic hormones and plants sterols in sediment correlated with prevalence and severity

Shenandoah Small 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

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

What We Learned from the "Historic Summary"

- Bass are very sensitive to estrogenic endocrine disruption in agricultural watersheds
- Did not see a relationship with disease or indicators of estrogenic exposure (intersex, vitellogenin) with wastewater treatment effluent
- Did see relationships with % agricultural landuse, confined animal feeding operation and agricultural herbicides/pesticides
- See considerable spatial variation as well as temporal variation monthly and yearly in total estrogenicity as well as fish health indicators
- Storms are important sources of these contaminants

Factors to Consider

- Temporal nature of chemical exposures – climatic and other factors that influence exposure
- Exposure pathways during critical life stage

Agricultural EDC Integrator Sites

- Represent a range of agricultural landuse types and density, as well as compare the two major river drainages
  - Potomac
    - Antietam Creek – MD
    - South Branch Potomac - WV
  - Susquehanna
    - Pine Creek – PA
    - Chillisquaque Creek – PA
    - West Branch Mahantango Creek – PA
    - Wyalusing Creek - PA

Monitoring at Integrator Sites

- Began wild fish sampling in Spring 2013 – present
- Intensive chemical monitoring began in Fall of 2014 at these sites
- Currently have 2 years (Nov 2014-Oct 2015; Nov 2015 – present) for which water samples were collected monthly (twice a month in the spring) plus storms

Important Exposure Periods

- Young
  - Maternal sources via the egg
  - Exposure of fry through water and sediment – during sexual differentiation and immune system development

- Adults
  - Exposure during recrudescence –
    - Effects on germ cells during development
    - Incorporation of contaminants into the yolk

Herbicide Concentrations (ng/L)

Dates	Atrazine Monocacy	Atrazine Chilli	Metolachlor Monocacy	Metolachlor Chilli	Simazine Monocacy	Simazine Chilli
May 15, 2013	364		228		343	
June 8, 2013	75		81		58	
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	43	13	83	-	24
April 30, 2014	3268		703		2,416	
May 12, 2014	201	22	93	23	112	
May 16, 2014	5,399	13,667	4,643	3,148	76	9,213
May 30, 2014	2,872	1,949	1,612	1,717	1,976	

Are Those Concentrations Significant?

- Monocacy – 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)

Molecular/Gene Expression

- **Liver – approximately 50 genes**
  - **Chose 288 samples – 1 year of integrator site comparison plus annual and seasonal comparisons at South Branch site**

- **Testes – Sequencing testes from normal and intersex males as well as immature females**

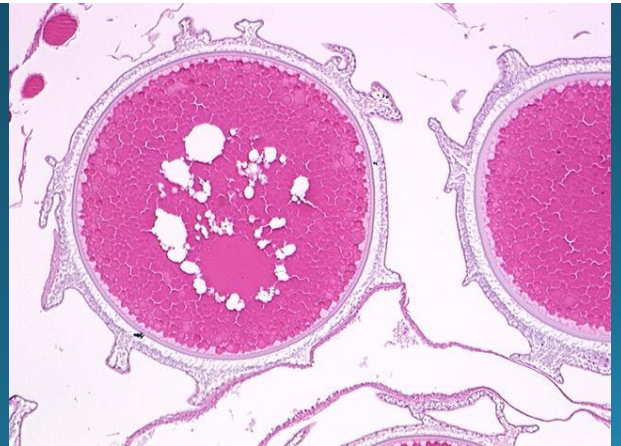
- **Anterior kidney/immune system transcriptome**
  - **Isolated anterior kidney cells from wild smallmouth bass were exposed in vitro to a variety of chemicals including known stimulants, herbicides, neonicotinoids, hormones, microcystin**

Yellow Perch

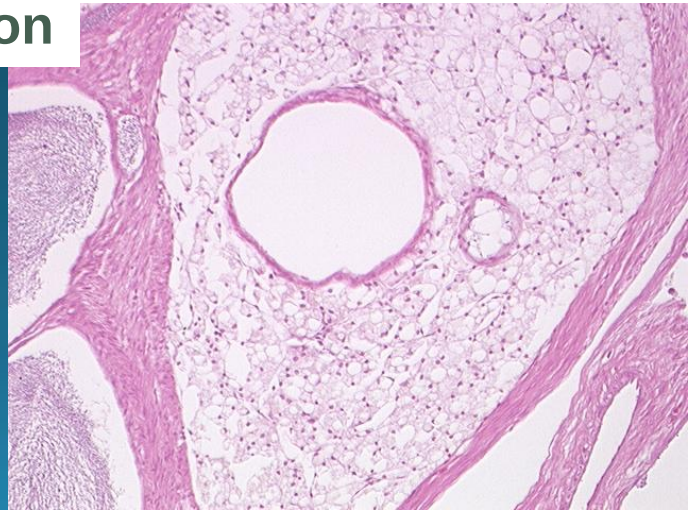
- Histological findings
  - Lack of final oocyte maturation,
  - Abnormal zona pellucida and
  - Leydig cell proliferation/Leydig cell tumors
- Both responses are regulated by dopamine
  - Raises question of exposure to dopamine agonists used to treat Alzheimer's, restless leg syndrome etc.

Normal egg

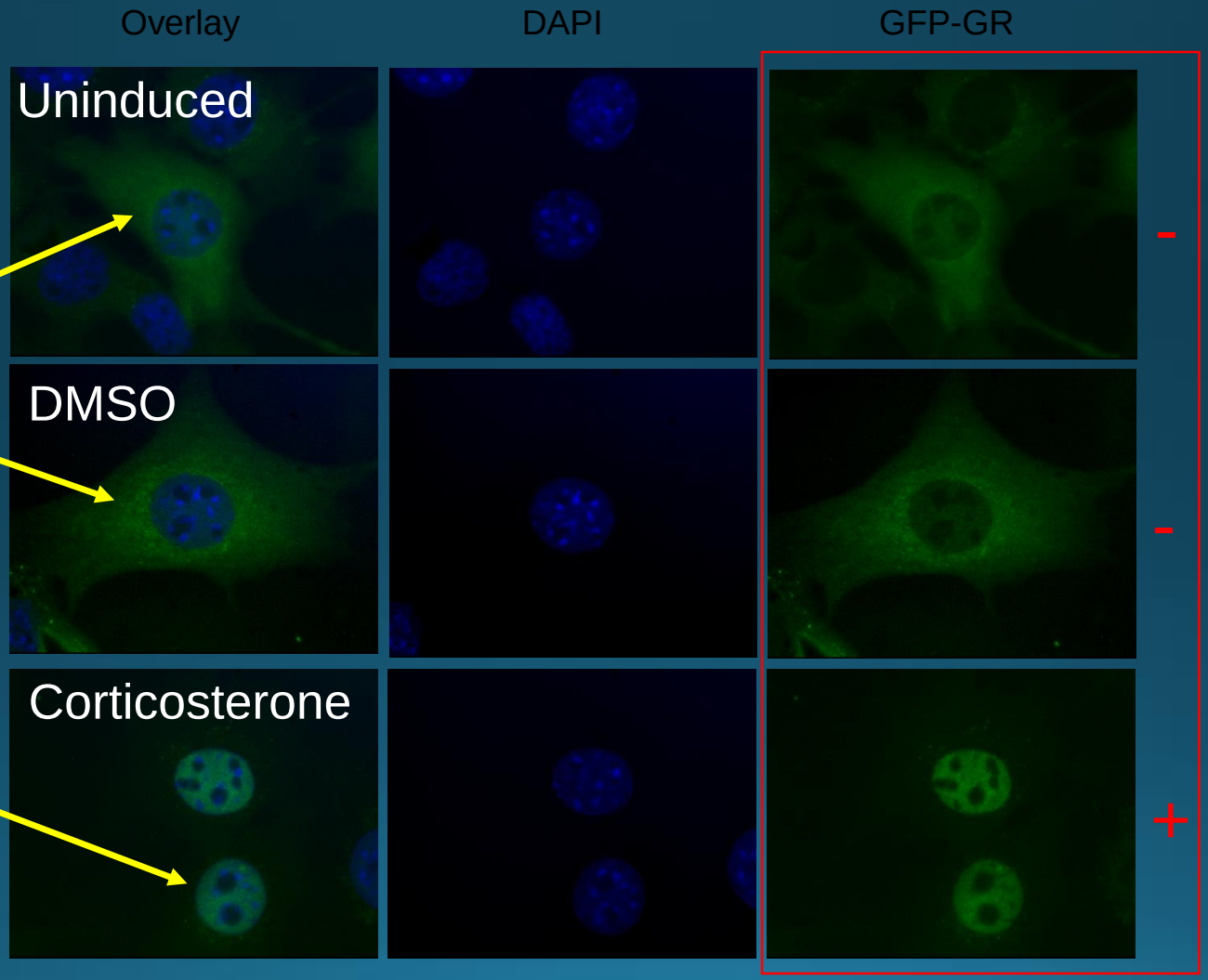
Abnormal yolk and chorion



Leydig cell proliferation



Nuclear Translocation Assays
National Cancer Institute

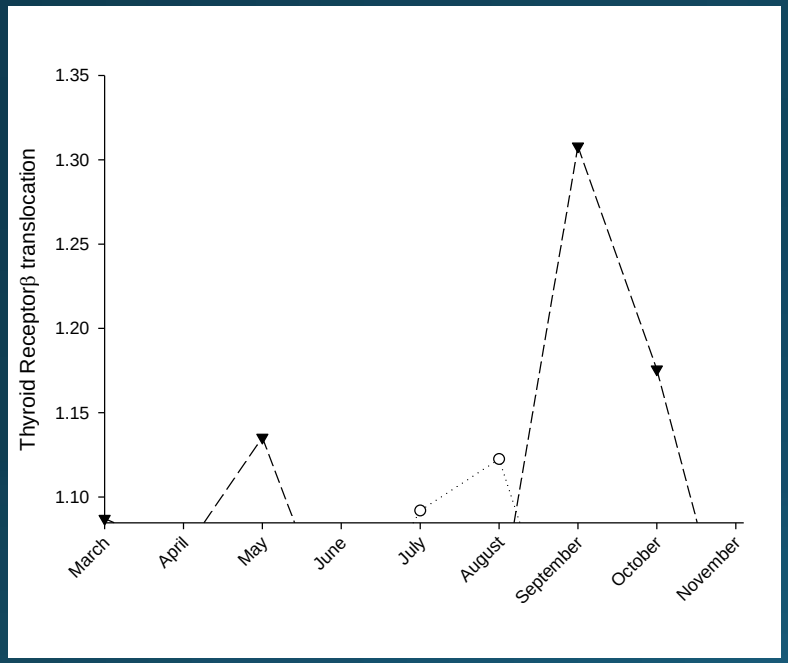


Unliganded GFP-GR resides uniquely in the cytoplasm

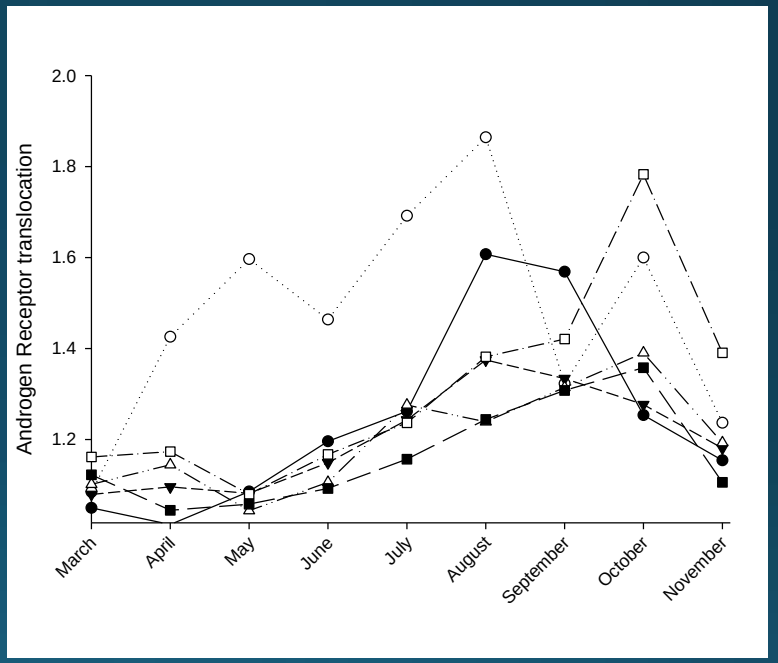
This shows GFP GR translocated to the nucleus by the water sample

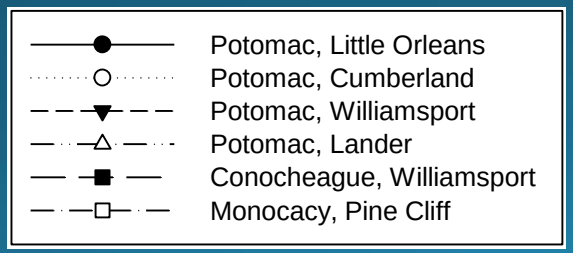
Other Hormone Activity

Thyroid



Androgen





Acknowledgements

USGS – many Programs/Centers

USFWS, Chesapeake Bay Field Office

WV Division of Natural Resources

WV Department of Environmental Protection

VA Division of Game and Inland Fisheries

MD Department of Natural Resources

PA Fish and Boat Commission

PA DEP

Potomac/ Shenandoah River Keeper

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