Monitoring Endocrine Disruptors and Other Chemicals of Emerging Concern (CECs) in the Aquatic Environment

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Chemicals of Emerging Concern

- Defined as synthetic or naturally occurring chemicals that are not commonly monitored in the environment, but have the potential to enter the environment and cause adverse effects.
- Many are chemicals that are produced to have biological effects at low levels – hormones, pharmaceuticals
- Many end up in human sewage, wastewater, runoff and ultimately the aquatic environment, so aquatic organisms may be constantly exposed

- Current treatment technologies are not designed to remove CECs.

Chemicals of Emerging Concern Human Wastewater

- Pharmaceuticals
  - Synthetic Hormones – birth control, hormone replacement therapy
  - Antibiotics
  - Viagra to Prozac
- Personal care products
  - Antimicrobials – soap, detergent, toothpaste
  - Fragrances
  - Organic UV filters
  - DEET

Agricultural Sources
Residential Sources

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

Endocrine Disrupting Chemicals

- 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

Other Effects of CECs

- Immunosuppression
- Indirect Effects:
  - Pathogen and parasite populations
  - Atrazine example
    - Effects phytoplankton populations leading to increased periphyton
    - Increased periphyton leads to increased snails
    - Increased trematode parasites

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 (yolk sac)
- Short term exposure at sensitive life stages can have long term effects
- Additive, synergistic

Approaches and Considerations

- Chemical Monitoring
  - Water, sediment, fish tissue
- Biological Monitoring
  - Variety of aquatic organisms that vary in exposure (habitats) and sensitivity
- Both approaches have pros and cons and is best to combine the two whenever possible

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
Con – hard to get an actual water concentration

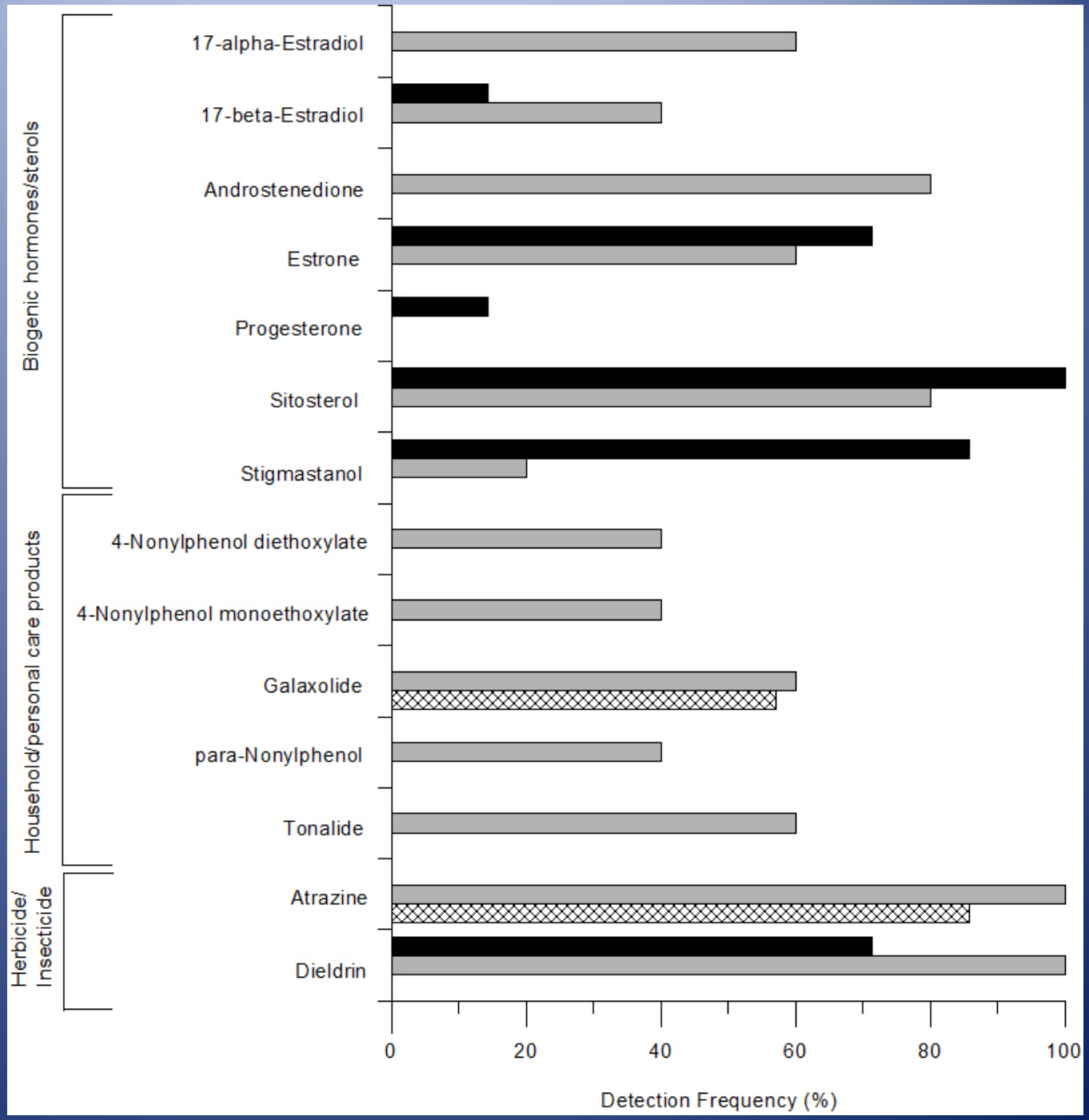
Water Chemical Monitoring

- Discrete water sample – one snapshot in time
- Integrated samplers – longer time period that may integrate different flow regimes, storm events etc.

- Seasonal
  - Climatic factors, landuse characteristics, hydrological – runoff versus groundwater sources

Endocrine disruptors (EDs) measured in all sample types

Discrete water samples underestimate ED exposure



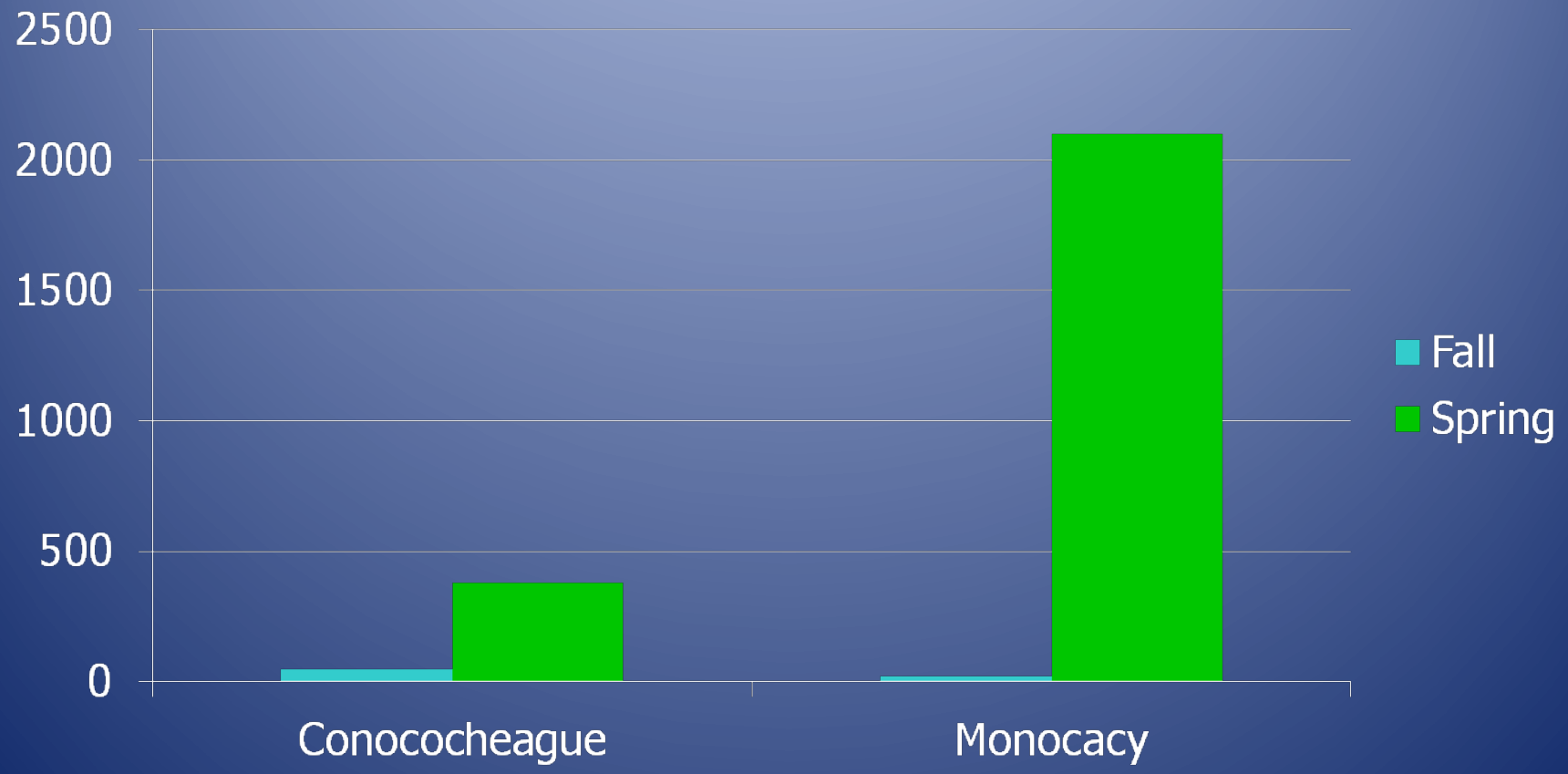
Agricultural Pesticides
Fall (Spring)

Chemical Estimated ng/L	Con Upstream	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)

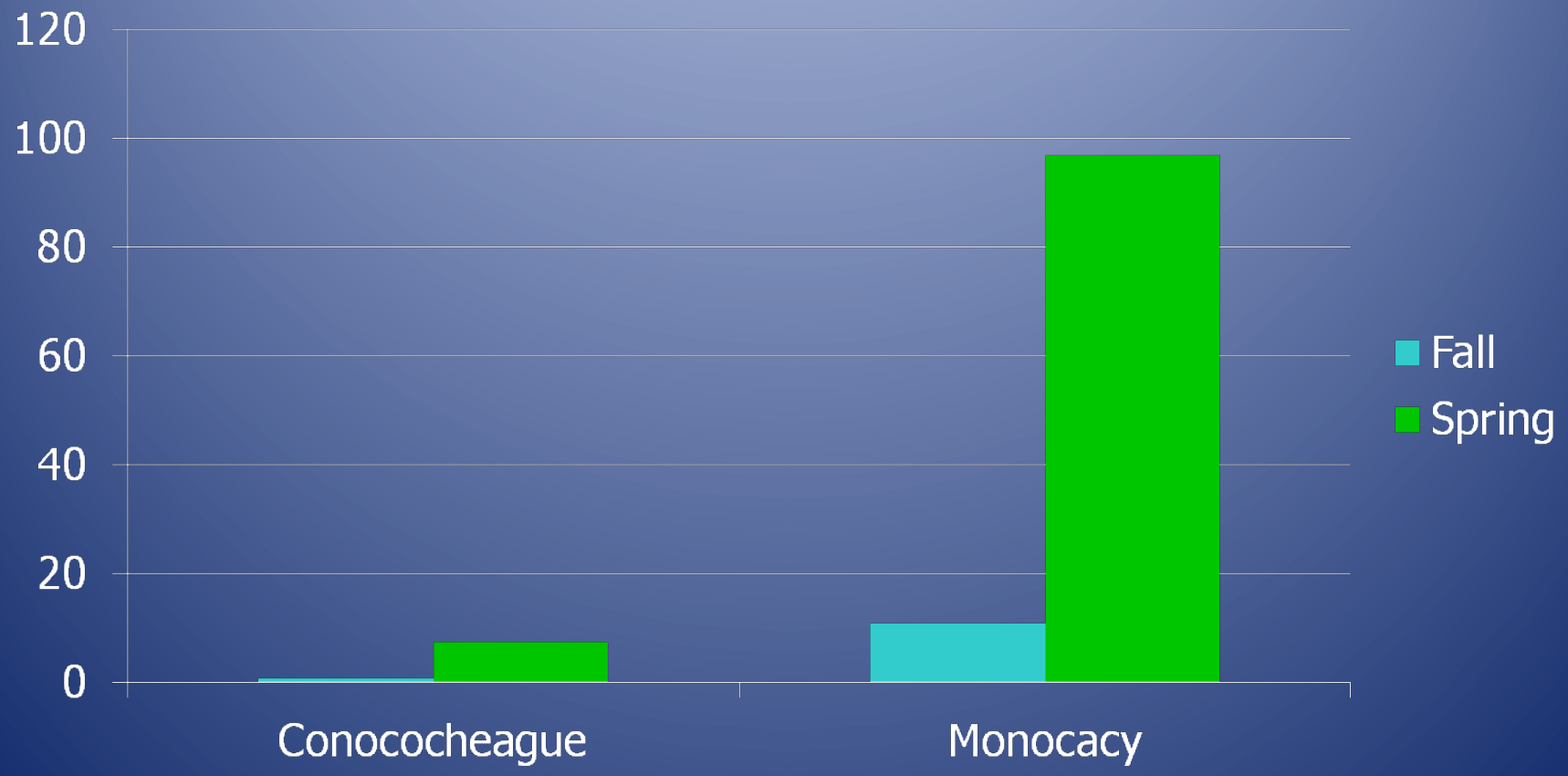
2.5 ppb recently shown to induce complete feminization and chemical castration in frogs - Hayes et al. (March 2010)

Earlier work found 0.1 ppb induced intersex in frogs

Seasonal Differences in Atrazine in Tributaries of the Potomac River, Concentrations (ng/L)



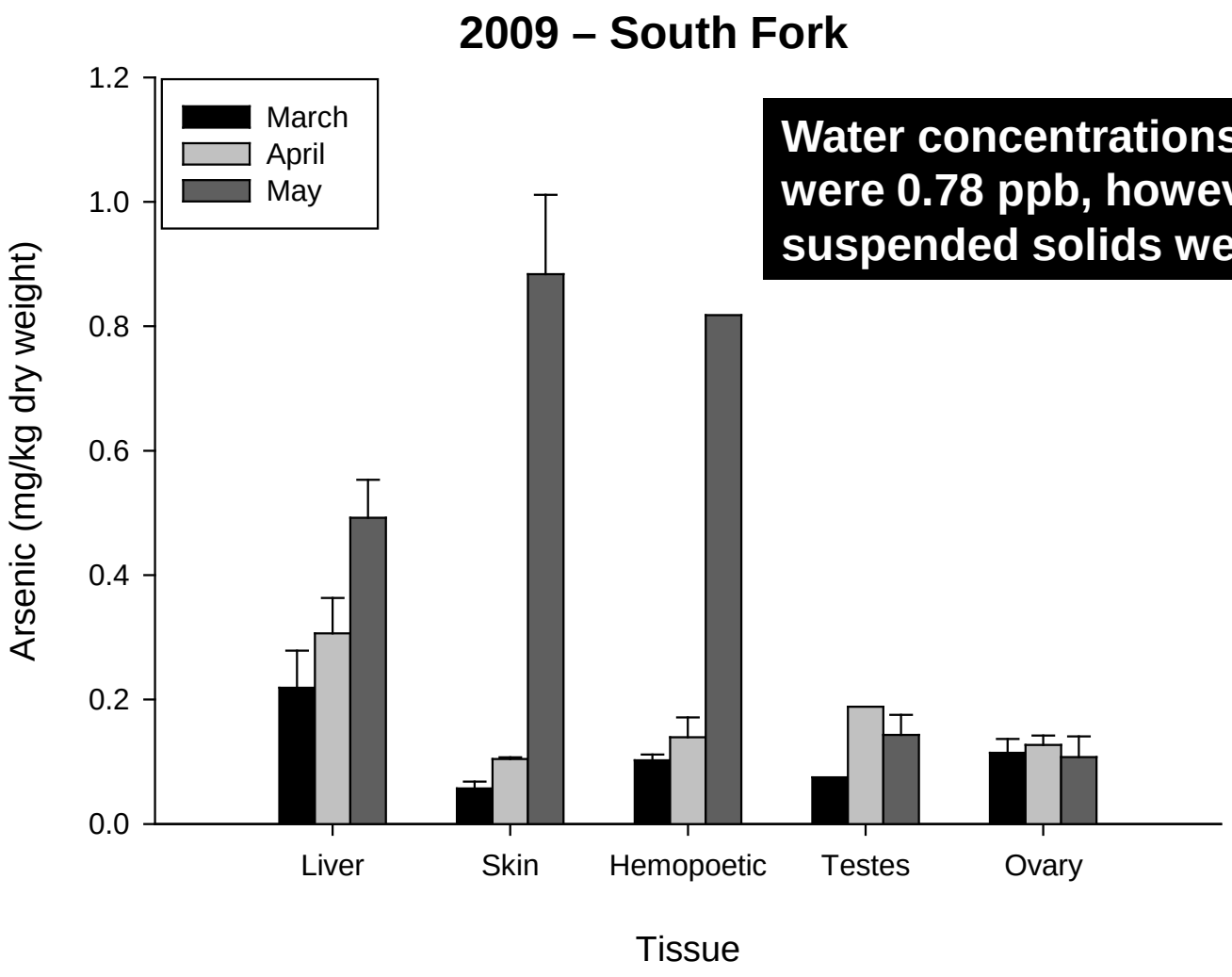
Seasonal Differences in Metalochlor Concentrations (ng/L)



Tissue Contaminants

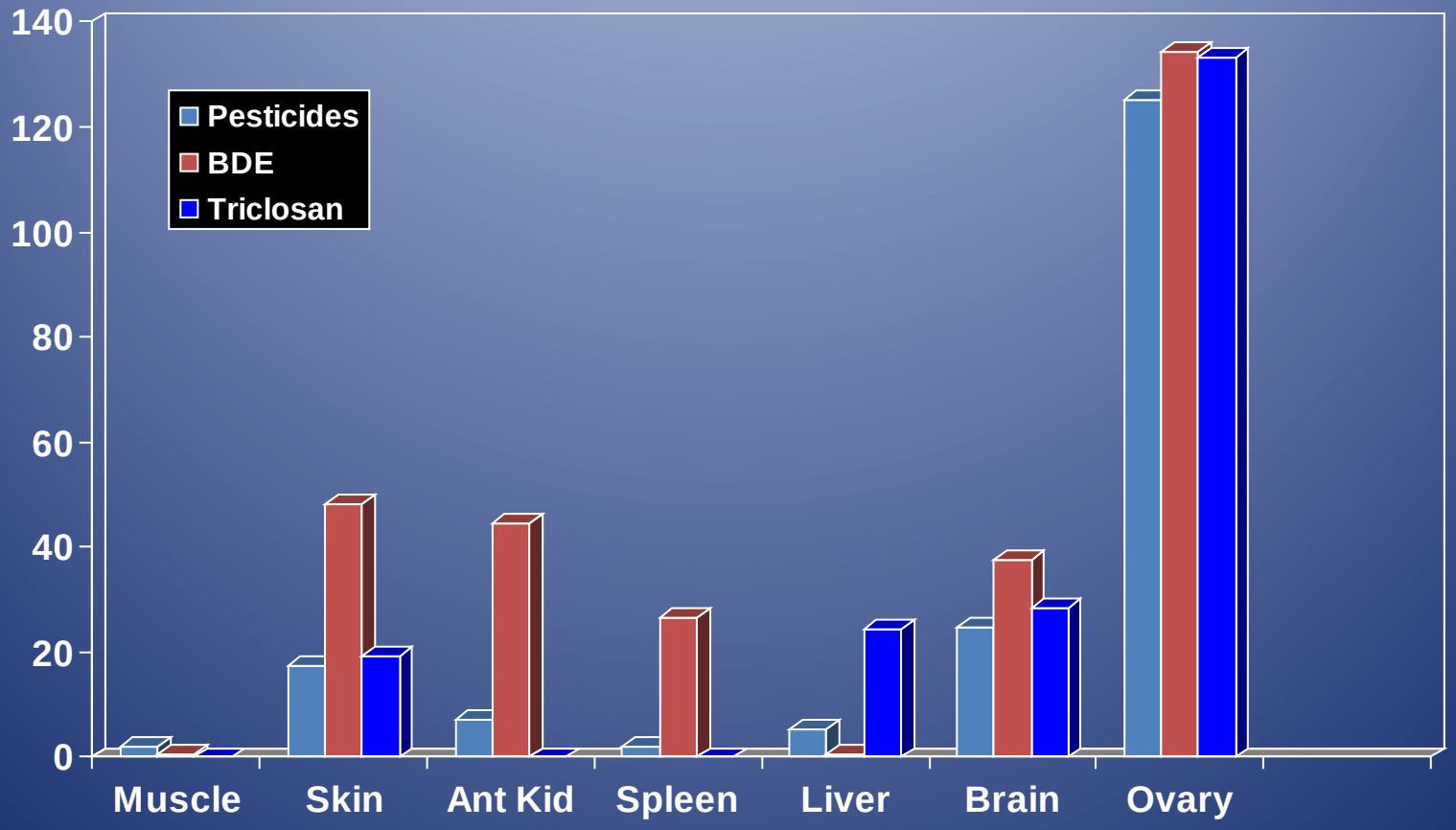
- Classically, whole body or filets
  - Filets are important for human consumption concerns
  - May not be most useful for understanding aquatic animal health issues
- Legacy contaminants that accumulate
- Method development for CECs

Arsenic Tissue Contaminants Smallmouth Bass



Water concentrations even during storm runoff were 0.78 ppb, however, levels in the suspended solids were 2.48 ppm

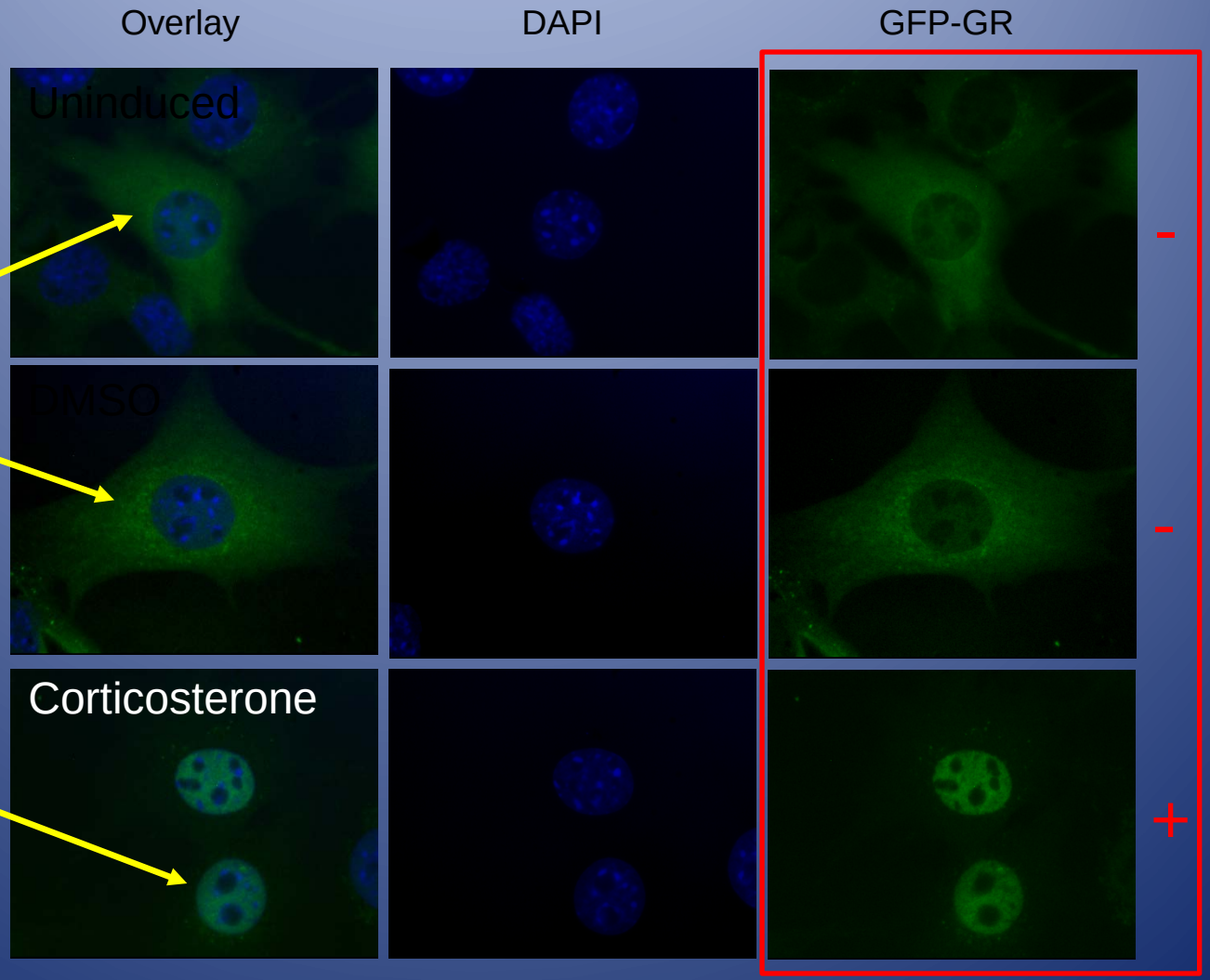
Comparison of Tissue Contaminant Concentrations Female Bass South Fork Shenandoah



Biological Effects Monitoring

- Organism (fish) in the environment
  - Species differences in response
  - Exposure to young – effects in mature adults
  - Important to use multiple endpoints
- In vitro cell/organism reporter assays
  - BLYES – bioluminescent yeast estrogen screen – total estrogenicity
  - BLYAS – androgenicity
  - Nuclear translocation assays – androgenicity, glucocorticoid receptors

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

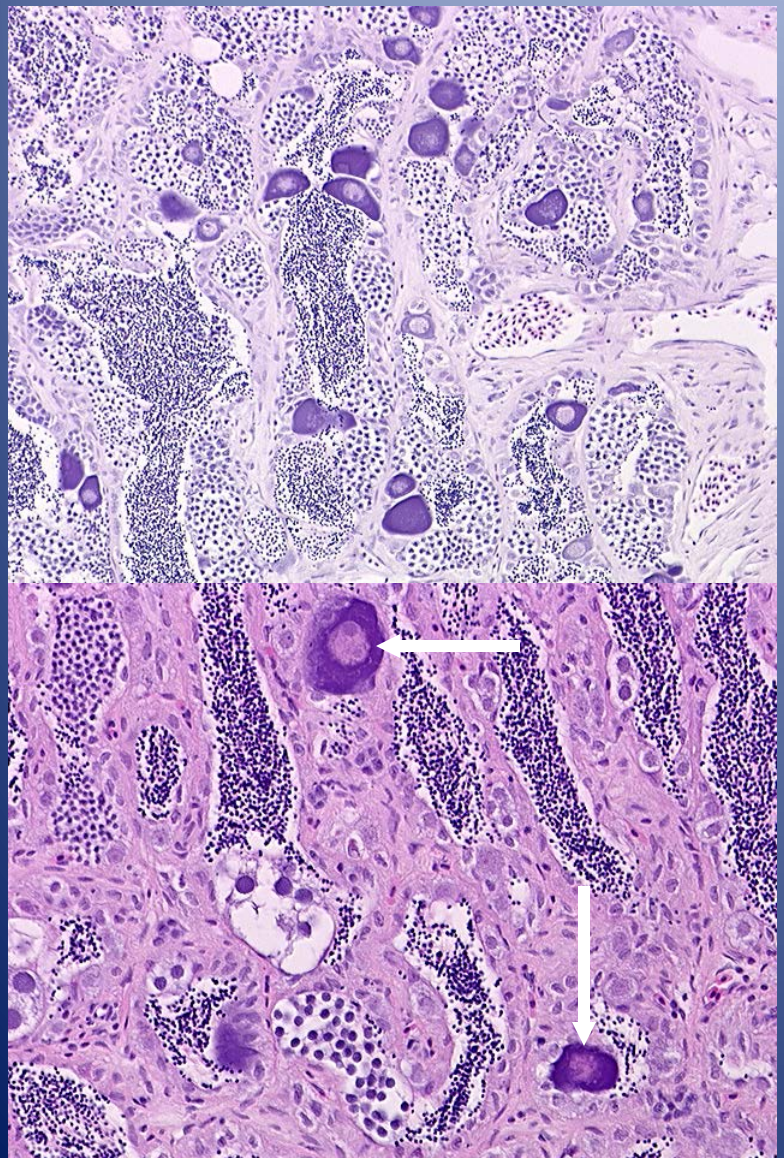
Fish as Integrators for Aquatic System Health

- 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

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)
  - Mechanisms
  - Identification of parasites/pathogens

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

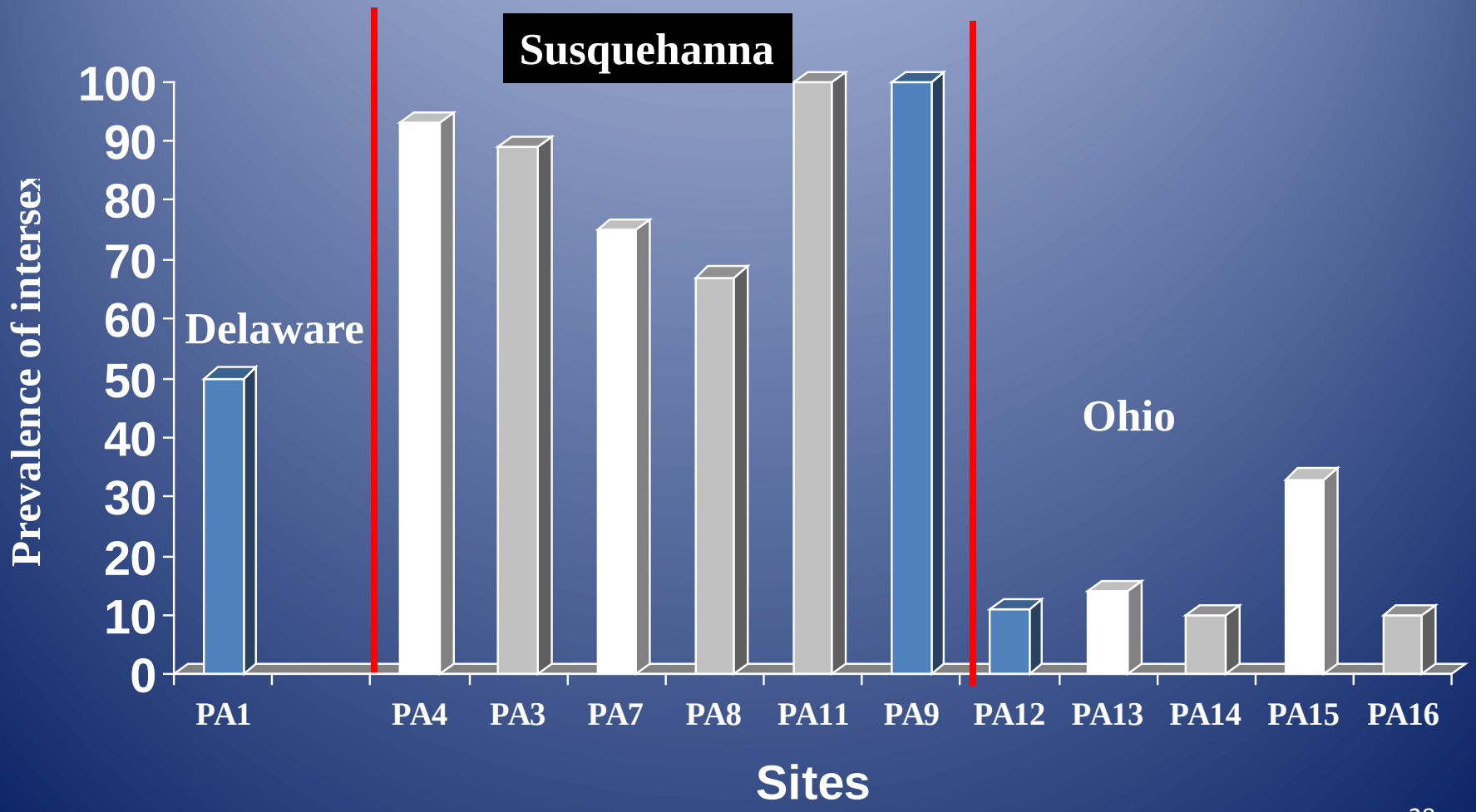
Vitellogenin

- Vitellogenin (Vtg) is the serum/plasma phospholipoglycoprotein precursor to egg yolk
- Normally found in measurable amounts only in the blood of sexually mature female egg-laying vertebrates
- Estrogen stimulates the liver to produce vitellogenin which travels to the ovaries via bloodstream and is sequestered by developing oocytes
- Presence in plasma of male fish indicators of exposure to estrogenic chemicals – more recent

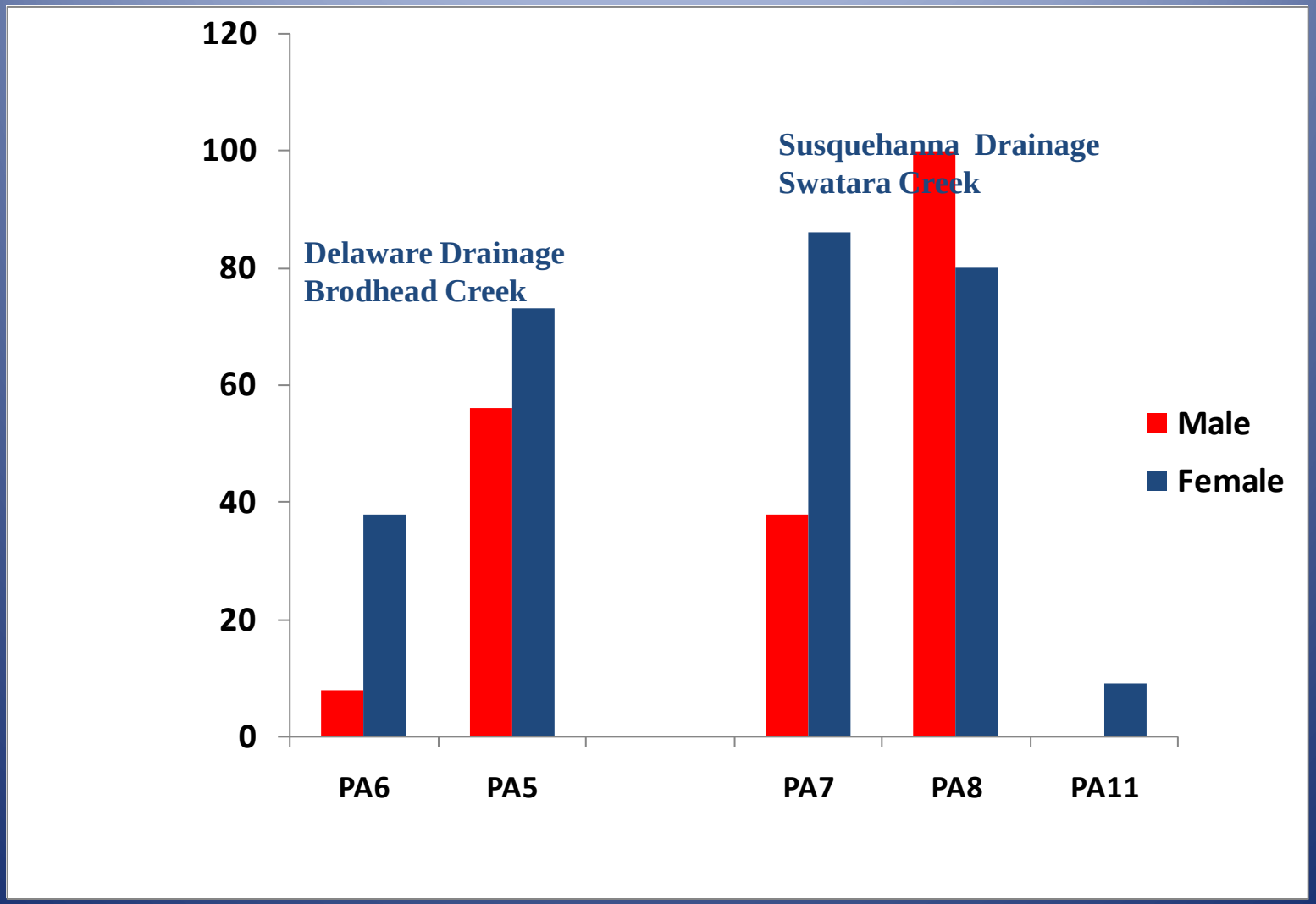
Upstream (U) / Downstream (D) of WWTP

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%

Intersex Prevalence Smallmouth Bass

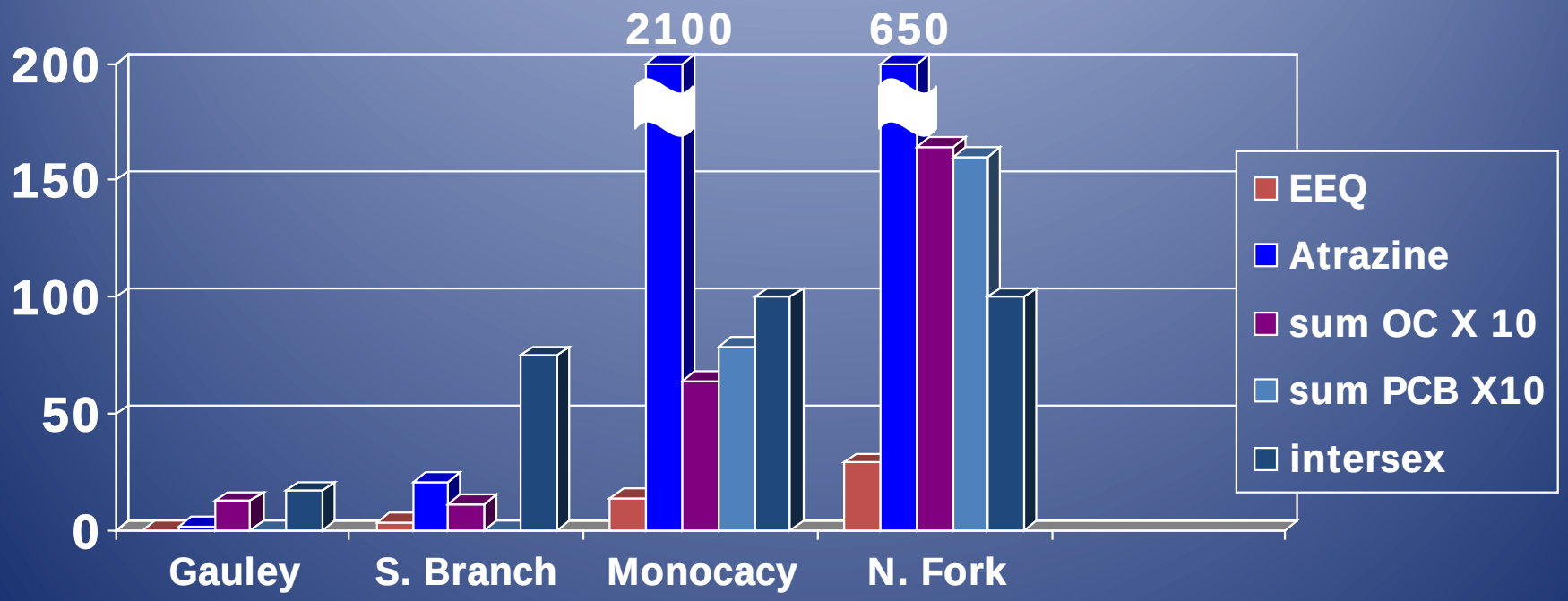


White Sucker Vitellogenin



PA6 & PA7 = upstream

Gradient Spring 2006-2007
Passive Sampler Results/Intersex

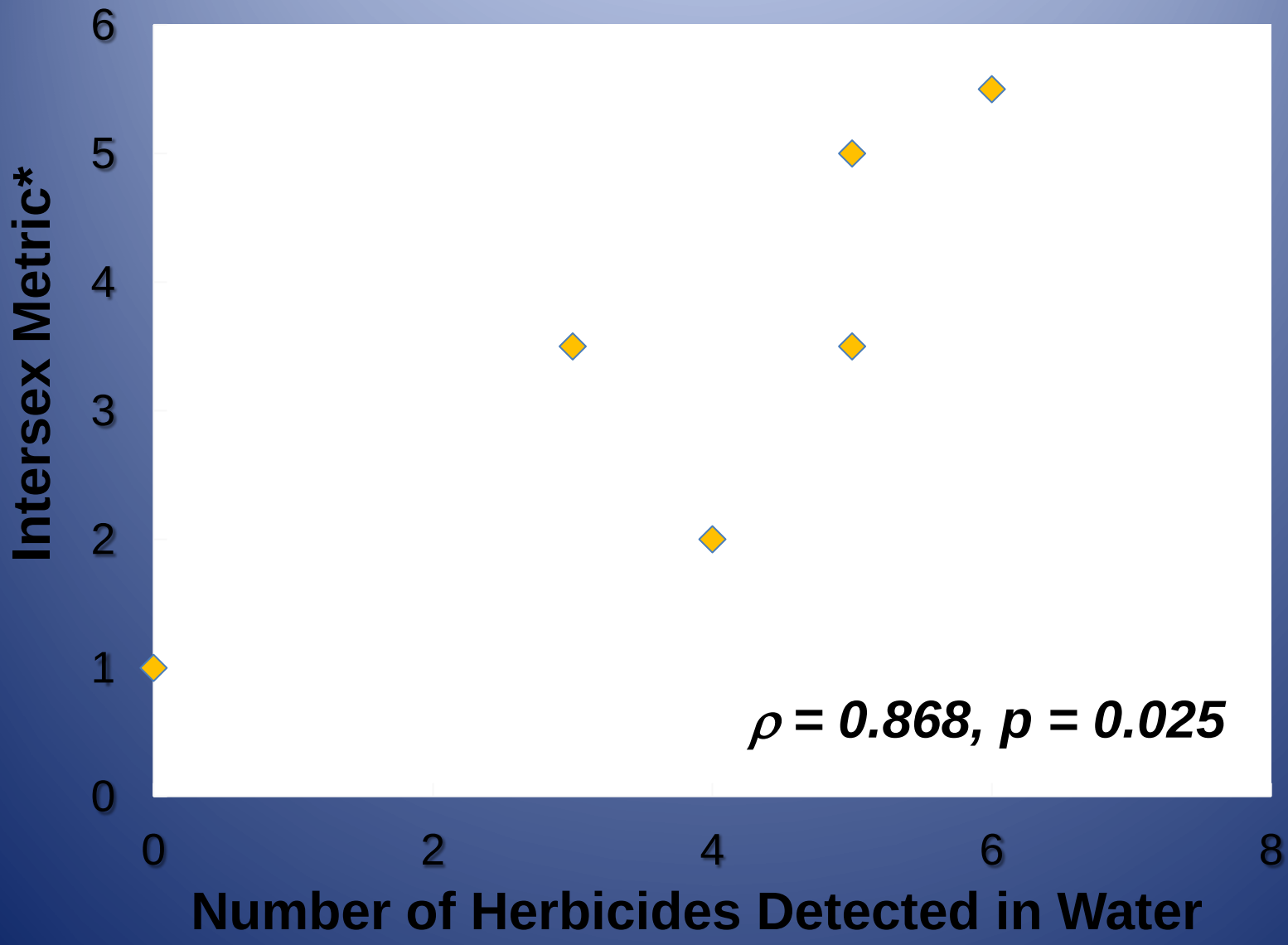


Intersex and Land-use

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. 2012. 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

Water Results – Chemicals and TO*



***Intersex metric is a nonparametric indicator of prevalence and severity**

*TO = (*Testicular Oocytes*)

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. 2013. Chemical Contaminants in Water and Sediment near fish nesting sites in the Potomac River Basin: Determining Potential Exposures to Smallmouth Bass (*Micropterus dolomieu*). Sci. Total Environ.

Shenandoah Tributary Study

Land-use Characteristics	Estrogenicity	
	r^2	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

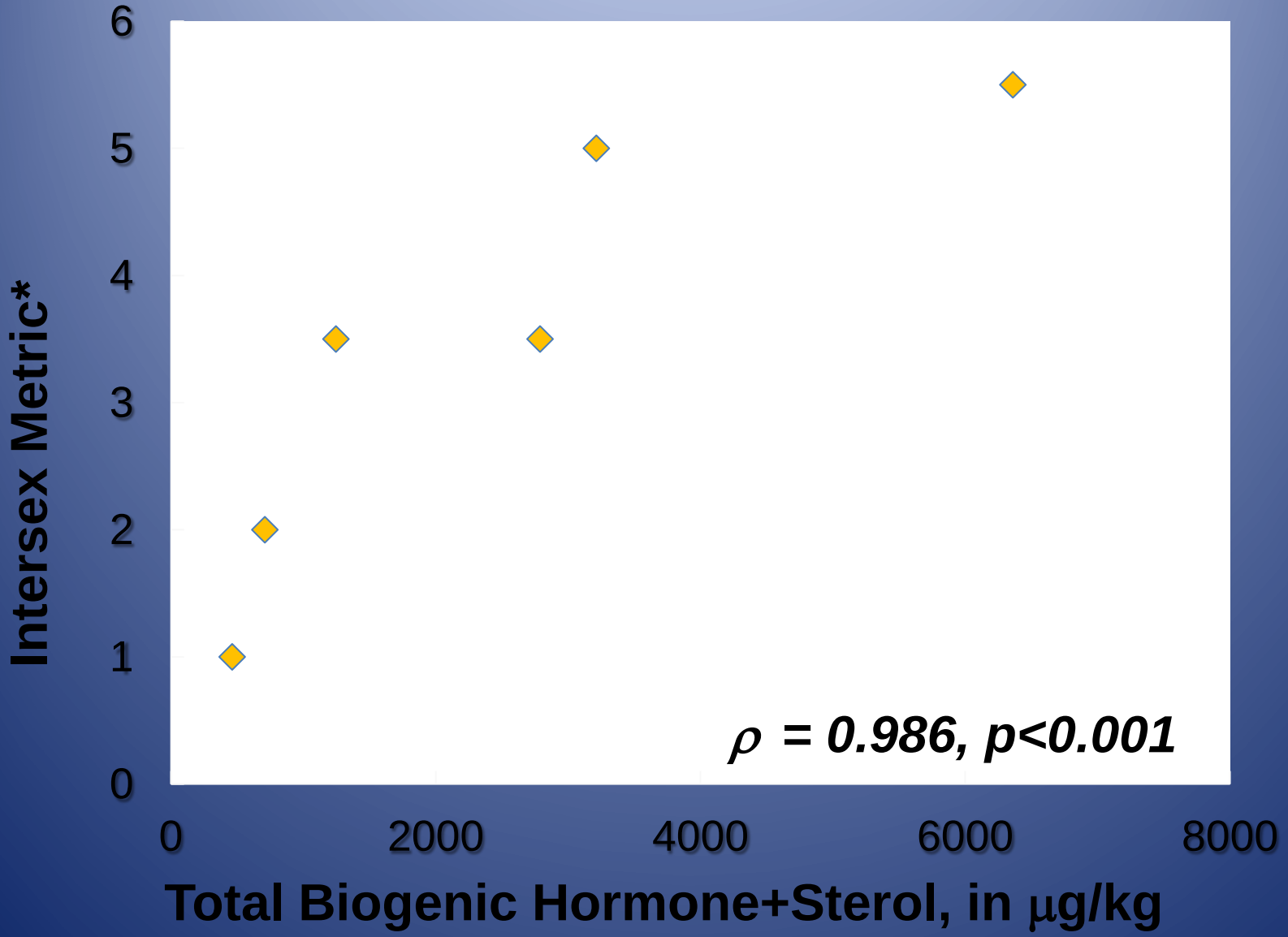
Shenandoah Tributary Study

POCIS pesticides (26 total)	Estrogenicity	
	r^2	*p*
Deethylatrazine	0.670	0.006
Metolachlor	0.631	0.011
Atrazine	0.582	0.022
Simazine	0.541	0.037

Atrazine and Estrogenicity

- Atrazine does not bind to the estrogen receptor
- Is there some type of synergy with the herbicides and co-occurring contaminants?
- Does the use of herbicides lead to high levels of phytoestrogens in the water/sediment?

Sediment Results – Chemicals and TO



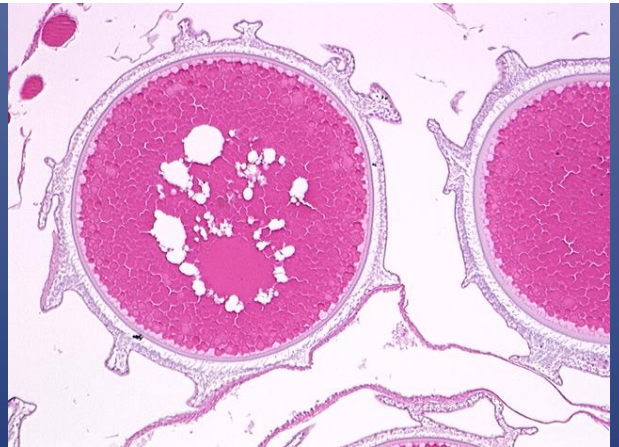
*Intersex metric is a nonparametric indicator of prevalence and severity

Yellow Perch

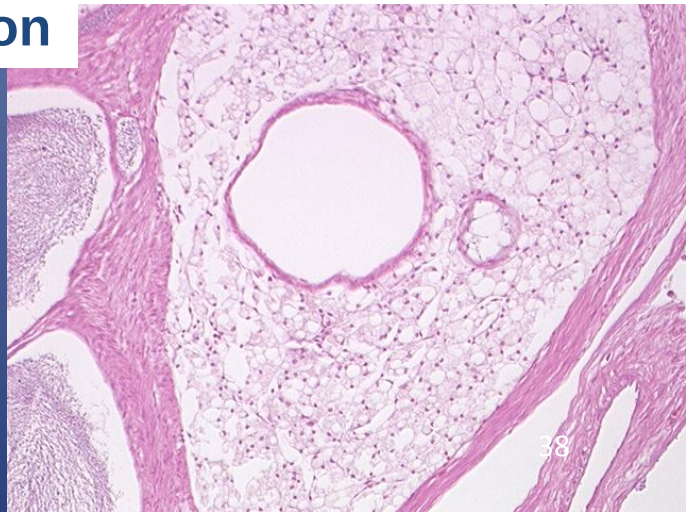
- Histological findings
  - Lack of final oocyte maturation,
  - Abnormal zona pellucida and
  - Leydig cell proliferation/Leydig cell tumors
- Both responses are regulated by dopamine
  - Raises question of exposure to dopamine agonists used to treat Alzheimer's, restless leg syndrome etc.

Normal egg

Abnormal yolk and chorion

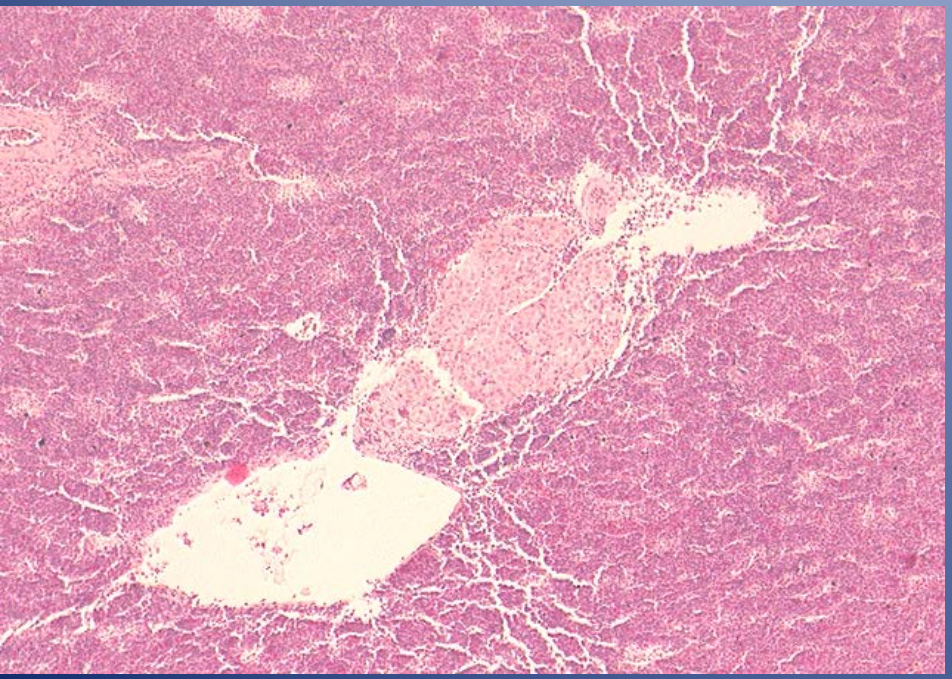


Leydig cell proliferation



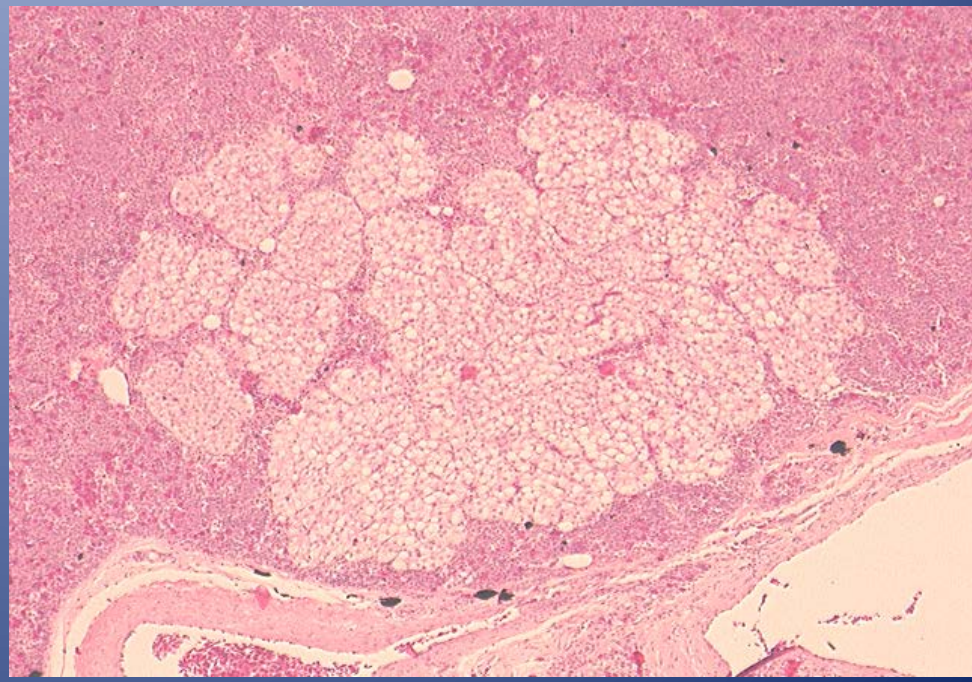
Endocrine Changes
Interrenal Tissue - Adrenal Cortex

Reference site



Normal

Contaminated site



Hyperplasia and hypertrophy

Skin Lesions and Mortalities

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