Emerging Contaminants: Biological Effects Monitoring in Rivers of Pennsylvania

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Contaminants of Emerging Concern

- Include trace organic compounds such as pharmaceuticals, personal care products, pesticides and hormones
- Defined as synthetic or naturally occurring chemicals (or microorganisms) that are not commonly monitored in the environment, are generally not regulated, but have the potential to enter the environment and cause adverse effects

Contaminants of Emerging Concern

- Many are endocrine modulators working through hormone receptors
- Recently recognized low-level effects of legacy contaminants – endocrine disruption and immune suppression
- Many of these chemicals (hormones and pharmaceuticals) are produced to have biological effects and hence may have effects on nontarget organisms at very low (ppb-ppt) levels

Emerging Contaminant Issues Aquatic Environment

- Minimum detection limits are often higher than effective doses
- Interactive effects of the complex mixtures
  - In vitro and laboratory studies suggest many of these compounds are additive in effects
- Lack of classical dose response curves
- What ends up in the sediment and individual tissues?

Levels of estrogenic activity have been found to be much higher in sediments than in the overlying water

Methods to Address These Issues

- Use of integrative water samplers
  - Accumulate chemicals over time (4-6 weeks)
  - Extracts of the membranes (or of grab water samples) can be used for chemical analyses, in vitro testing for total estrogenicity, androgenicity, etc.
- Biological effects monitoring

Effects-Based Monitoring

- Biological effects often occur when no one chemical indicator is above “threshold benchmarks”
- Fish health is a good integrator of cumulative effects of environmental stressors

Biological Indicators

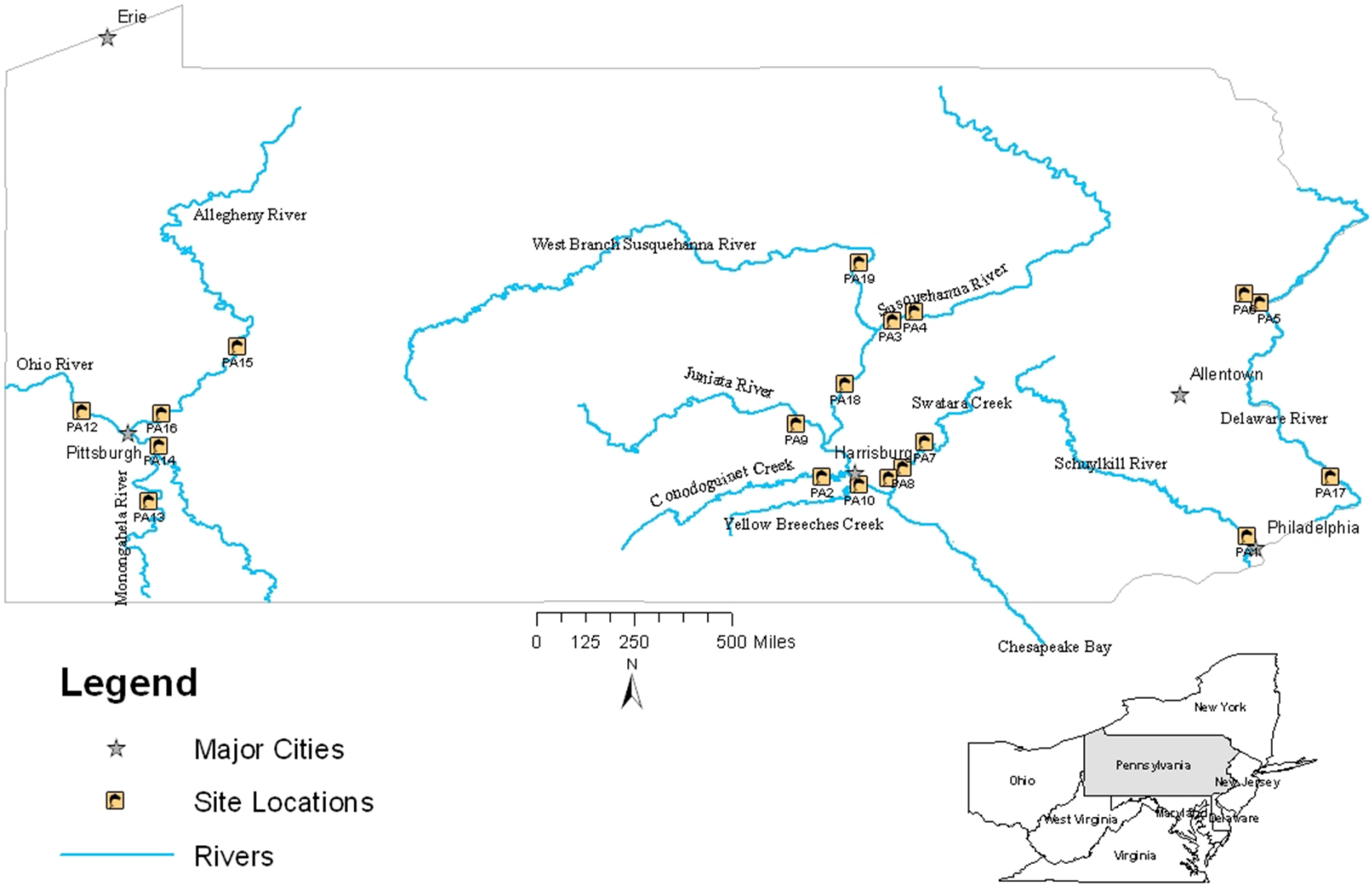
- Morphological – condition factor, gonadosomatic and hepatosomatic indices
- Gross observations – lesions, parasites, abnormalities such as skeletal deformities, eye abnormalities
- Plasma vitellogenin
- Histopathological changes - intersex, ceroid/lipofusin accumulations, macrophage aggregates, ovarian atresia, gill lesions, liver lesions, pathogens/parasites, etc.

Study Design

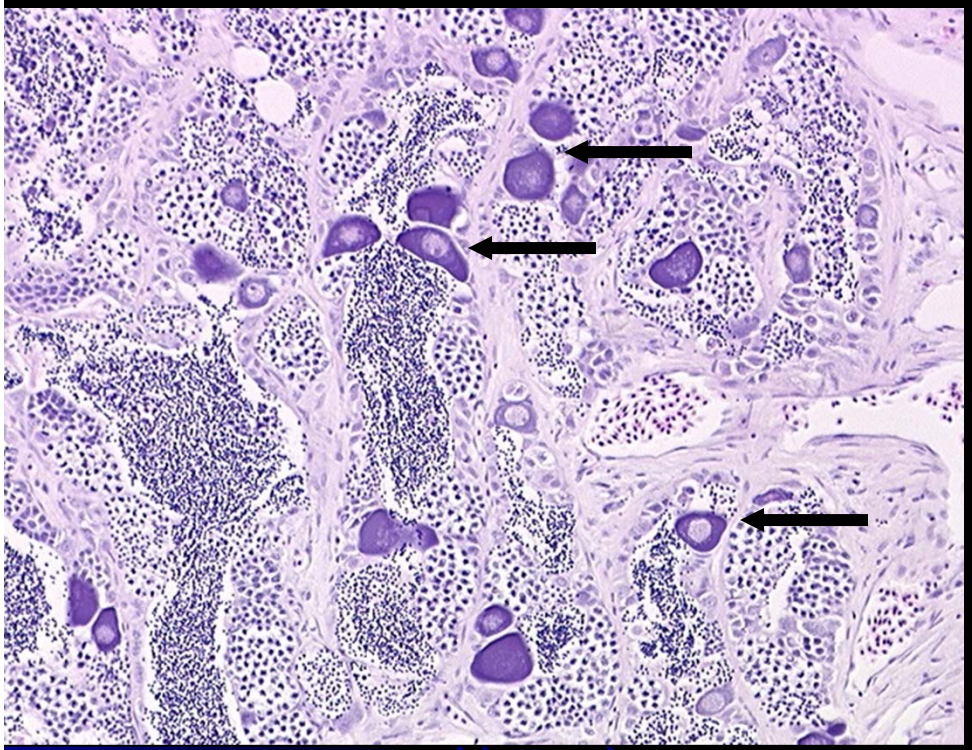
- Species comparisons
  - 2007 – smallmouth bass, white suckers and/or rock bass at 11 sites in the Susquehanna and Delaware drainages
  - 2008 – smallmouth bass and redhorse sucker sp. at 5 sites in the Ohio drainage

- Included sites upstream and downstream of WWTP effluents

Fish Collection Sites in Pennsylvania



Intersex in Normally Gonochorist Fishes



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

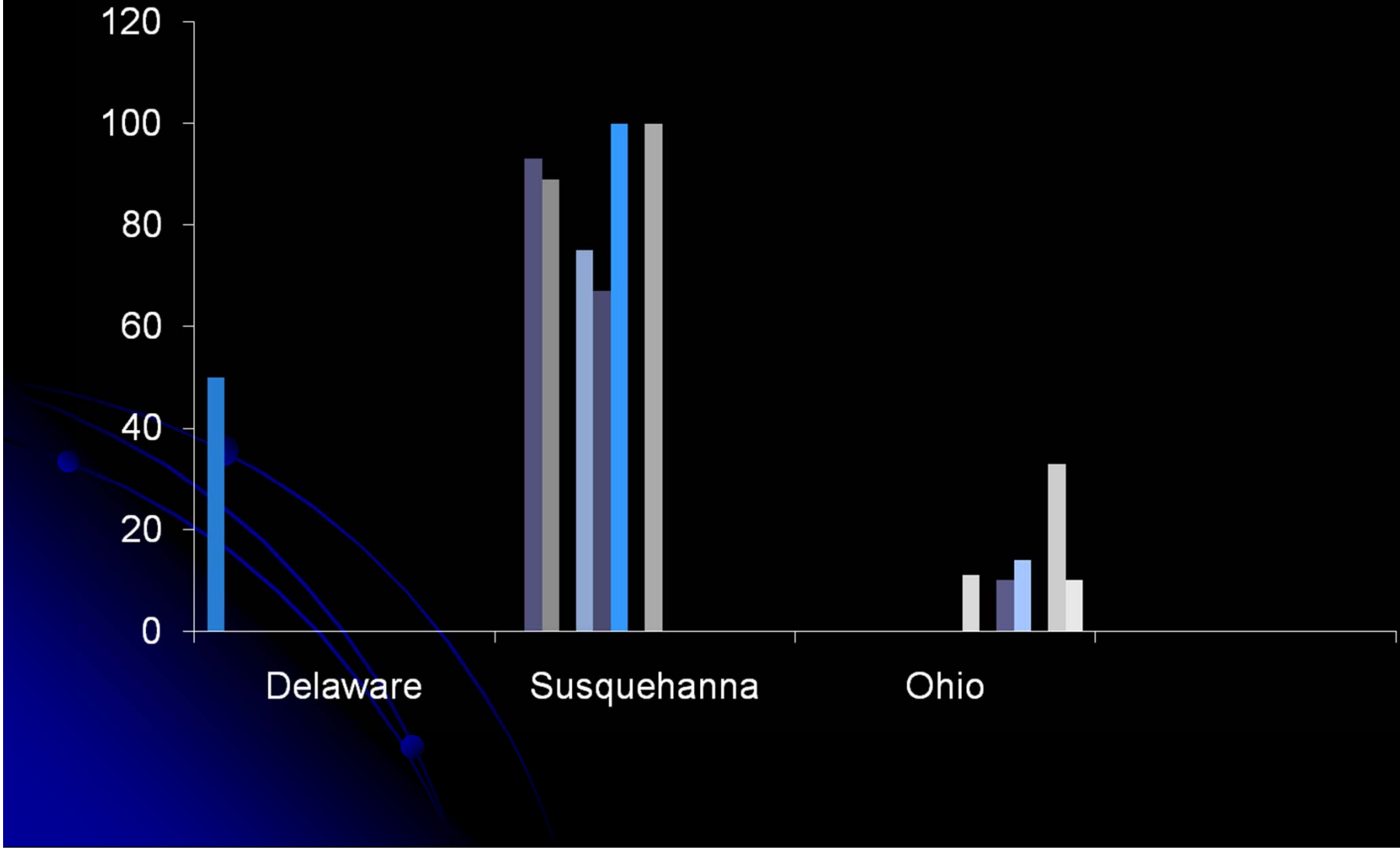
Vitellogenin

- Vitellogenin is the serum/plasma phospholipoglycoprotein precursor to egg yolk
- Normally found in measurable amounts only in the blood of sexually mature egg-laying vertebrates (females)
- Estrogen stimulates the liver to produce vitellogenin which travels to the ovaries via bloodstream and is sequestered by developing oocytes
- Males have the gene to produce vitellogenin - usually not turned on, however exposure to estrogenic compounds turns on the gene

Biomarkers

- Intersex
  - most likely induced very early
  - exposure during sexual differentiation increases sensitivity later in life
- Vitellogenin
  - more indicative of recent exposure (days to months)

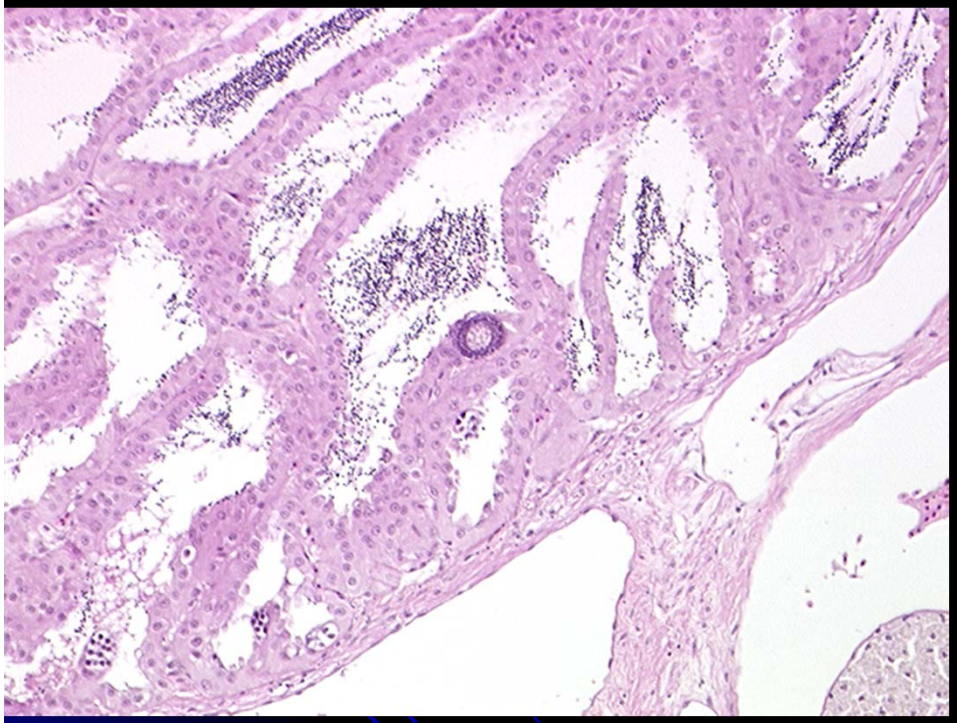
Intersex Prevalence Smallmouth Bass



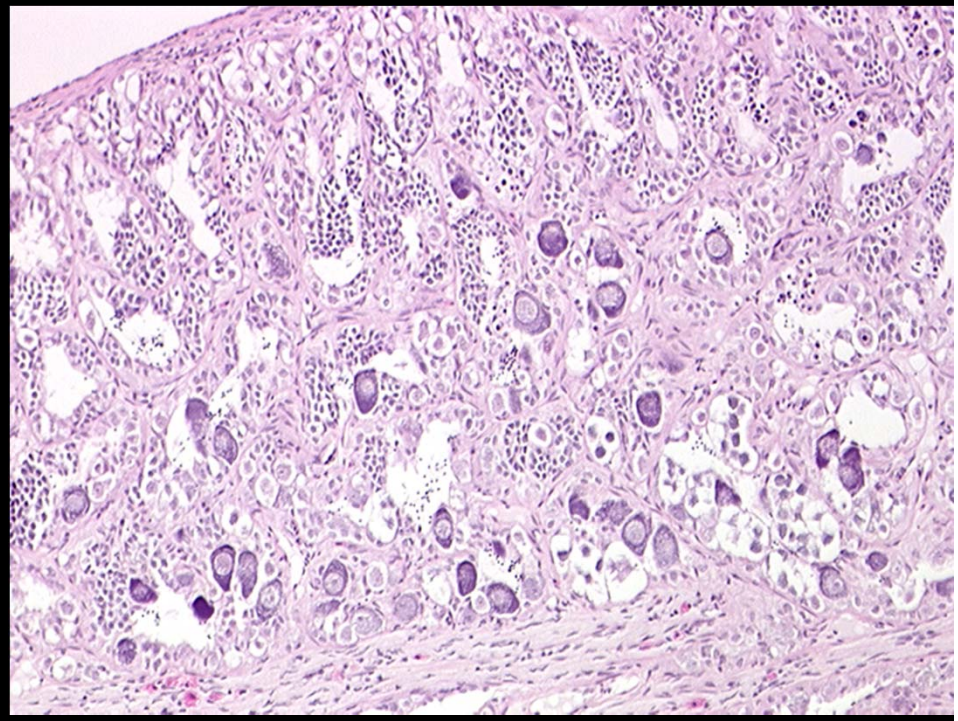
Upstream/Downstream of WWTP

Site	Sample No.	Intersex Prevalence	Intersex Severity	Vitell. In males
Susq-U	14 M	93%	1.4	21%
Susq-D	9 M	89%	1.7	11%
Swatara -U	4 M	75%	0.6	0%
Swatara -M	6 M	67%	1.0	50%
Swatara-D	6 M	100%	2.0	0%
Allegheny-U	6 M	33%	0.2	17%
Allegheny-D	10 M	10%	0.1	20%
Monogahela-U	10 M	10%	0.1	10%
Monogahela-D	7 M	14%	0.1	20%

Smallmouth Bass



Allegheny River

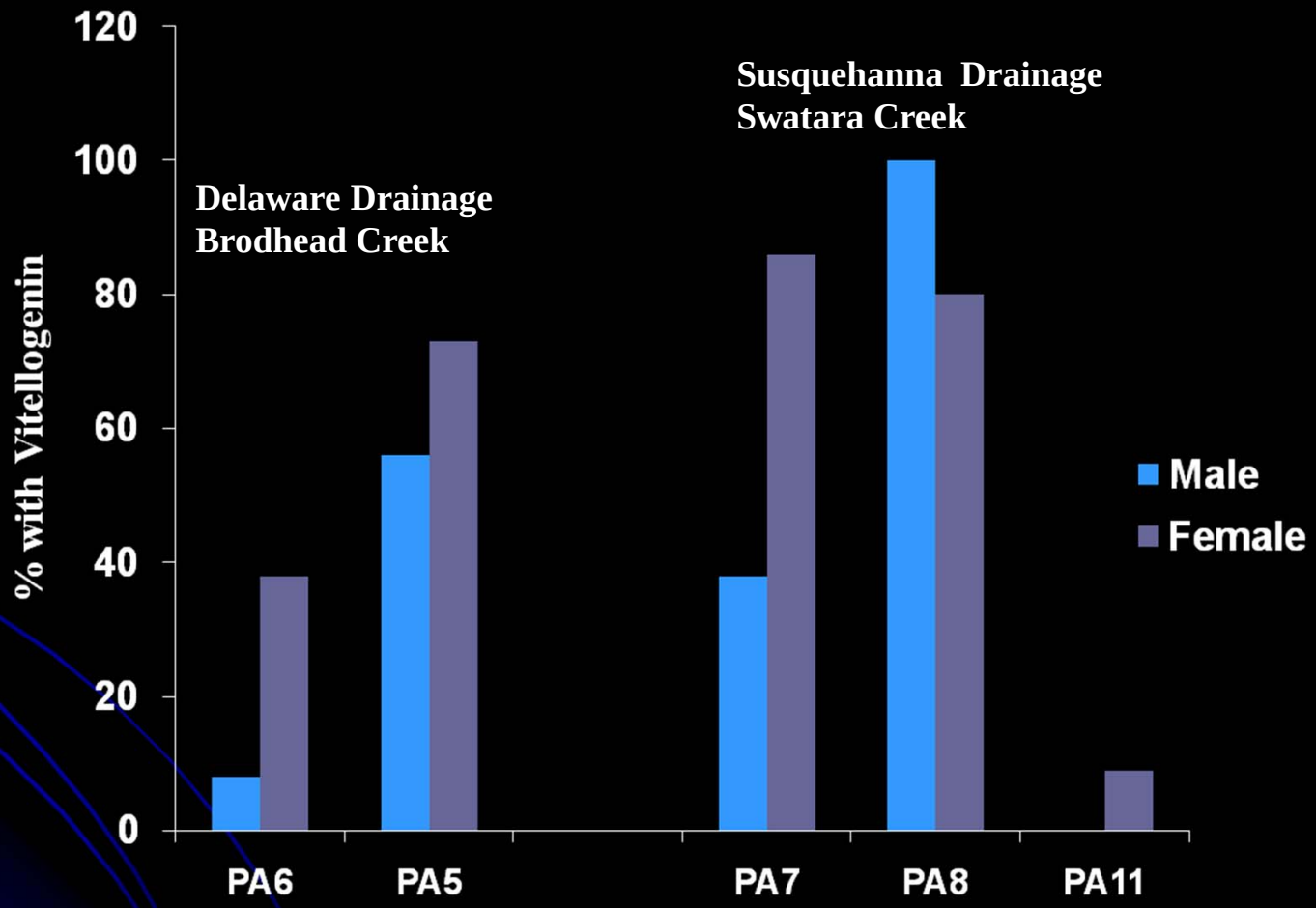


Susquehanna River

Overview of the Intersex Results

- Ohio drainage
  - Low prevalence and severity of intersex in bass;
  - No intersex in redhorse suckers
- Delaware drainage
  - Moderate intersex prevalence and severity in bass
  - No intersex in white sucker or rock bass
- Susquehanna drainage
  - High prevalence and severity of intersex in bass
  - No intersex in white suckers or rock bass

White Sucker Vitellogenin



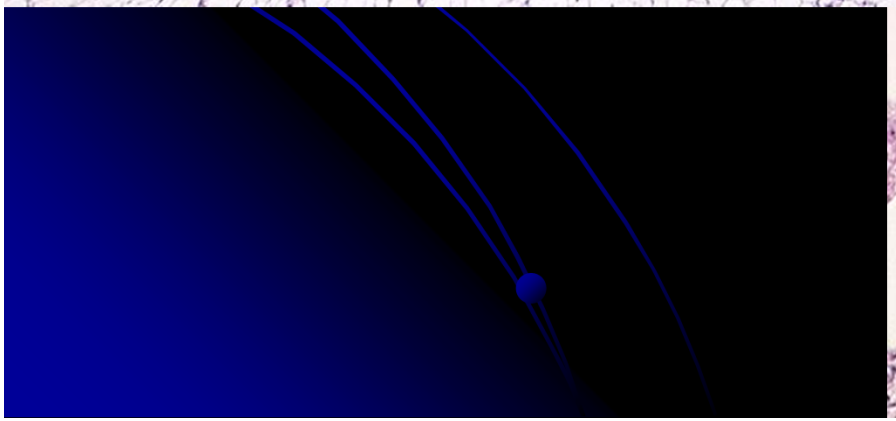
Reports of “Genderless” Fish

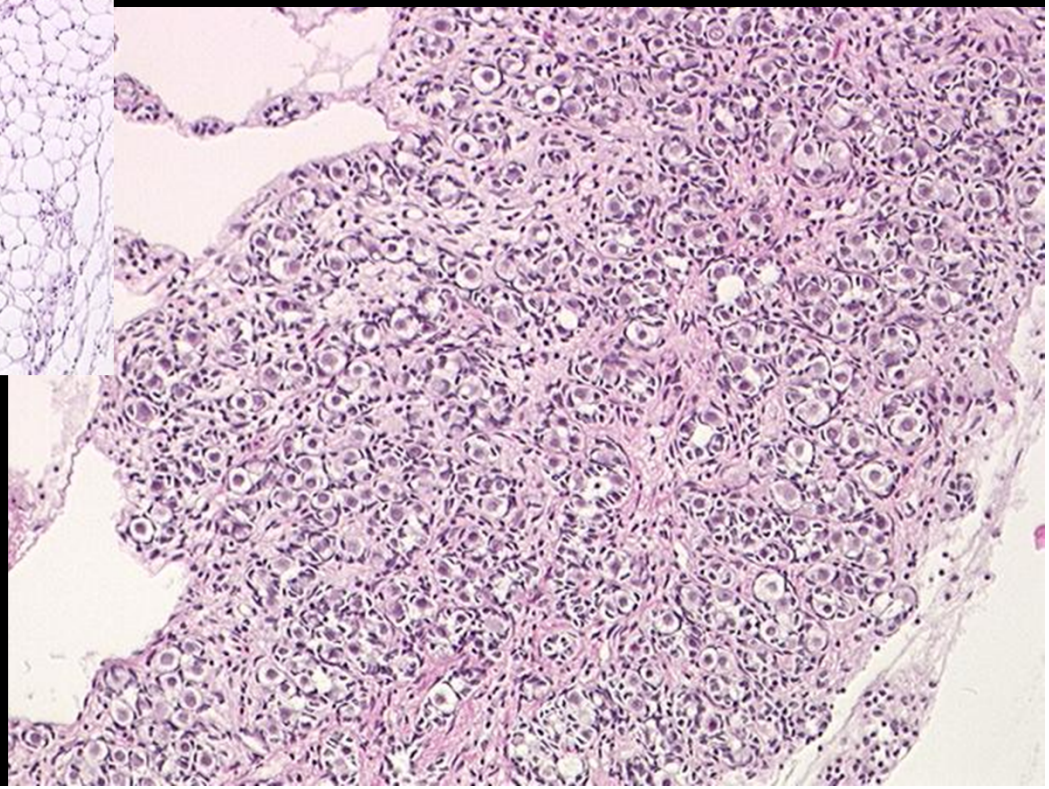
- Pittsburgh area
- Newspaper articles stating 85% of the fish examined were genderless
- “cells don’t look like they are male or female”
- Reported to be associated with estrogen in the river

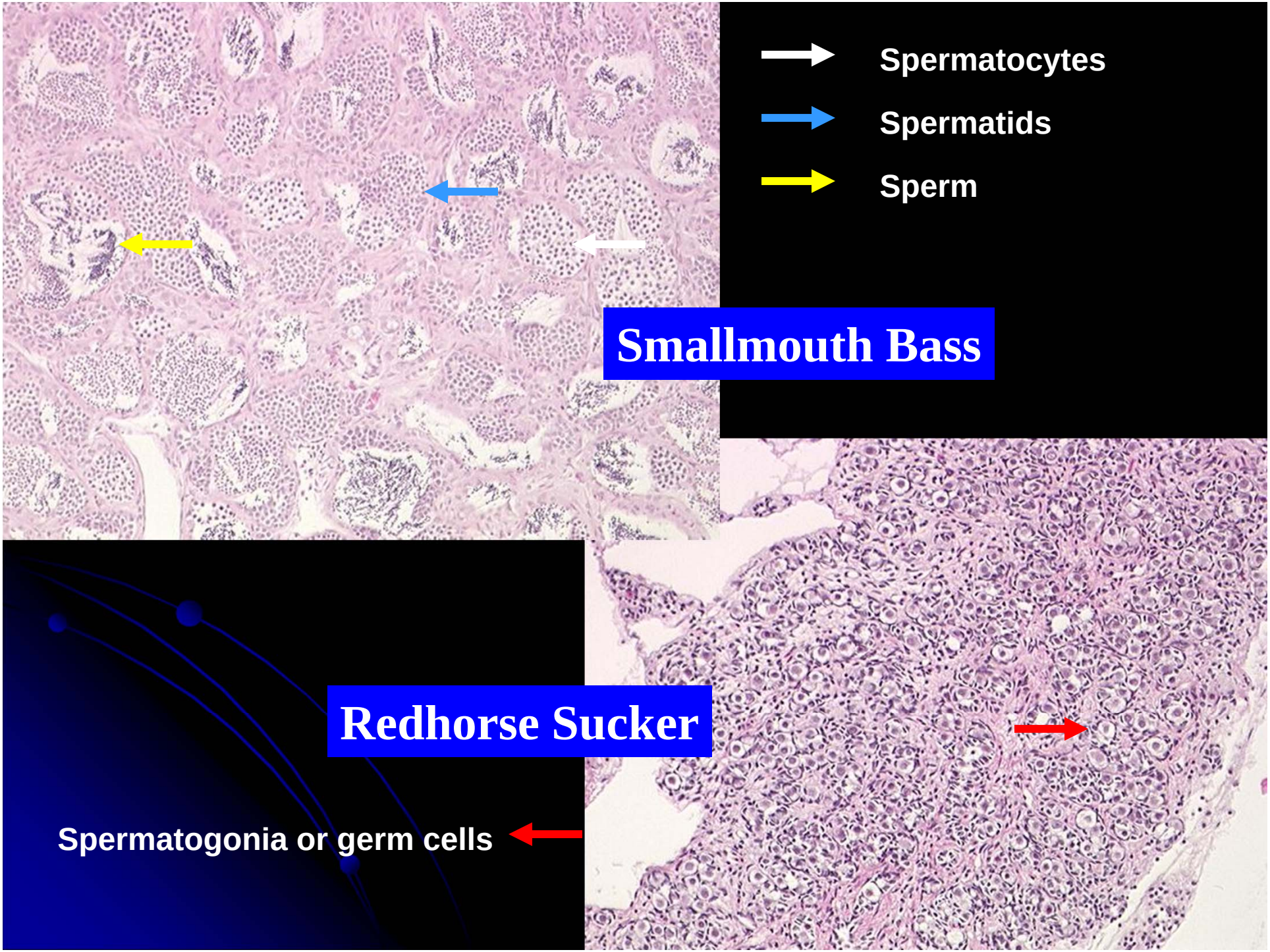
PA Emerging Contaminant Project 2008 - Results

- All the fish had a gender – histologically males and females were easily distinguished
- Redhorse sucker males were immature and gonads were very small and undeveloped
  - Ranged in total length from 260 to >400 mm

Redhorse Suckers






Spermatocytes
Spermatids
Sperm
Smallmouth Bass
Redhorse Sucker
Spermatogonia or germ cells

Summary

- High prevalence and severity of intersex in smallmouth bass in the Susquehanna drainage
- Do not see intersex in rock bass or white suckers – see other potential contaminant effects
- Low prevalence and severity of intersex in smallmouth bass from the Ohio drainage
- Possibly delayed/lack of maturation in the male redhorse sucker species
- There was no consistent association of these effects with WWTP proximity

Chemicals of Emerging Concern Sources

- Wastewater Treatment Plants
- Industrial effluent
- Stormwater runoff
- Agriculture sources
- Landfill leachate
- Atmospheric deposition

Induction of Testicular Oocytes Experimentally

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

Types of Chemicals

- Estrogens
- Anti-androgens

- Androgens
- Anti-estrogens

Inputs and Manangement?

Human development
- WWTP effluent
- Stormwater runoff
- Construction sites

Industry

Agriculture

Cattle with free access to the river

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

Intersex and Landuse
Potomac River Drainage 7 Sites

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**

Association with Fish Lesions/Kills

- High prevalence and severity of intersex in same areas as fish lesions/kills
- Similarities to Potomac findings
  - Multiple bacterial pathogens, often co-infections
  - High parasite loads – trematodes and myxozoans
  - Largemouth bass virus
- Increasing evidence that many chemicals of emerging concern influence disease resistance and immune system
  - Estrogens, atrazine, DDE, triclosan, arsenic

Acknowledgements

- Collaborators/Cooperators
  - PA DEP
  - PA Fish and Boat Commission
  - USGS PA Water Center