

Intersex in Bass

“Emerging” Contaminant Issues

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Sampling With WV DNR

- u Spring –Summer 2002 – Fish kills and fish lesions
- u Randomly examined a variety of fish species focusing on the external lesions
 - Variety of causes – bacterial, parasitic, noninfectious
 - Suggested some environmental stressors that may cause immunosuppression and increased disease susceptibility



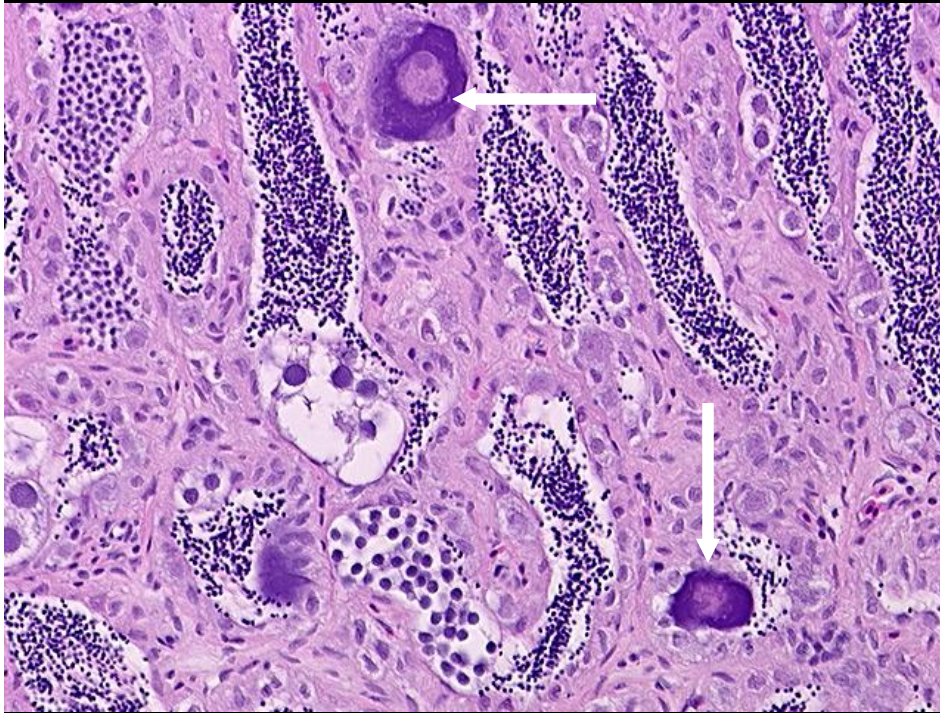
Sampling 2003

- u More comprehensive fish health assessments of bass from 7 sites
- u Necropsy-based, plasma collection, histologic (microscopic) analysis of all organ
- u Findings included:
 - High parasite loads at some sites
 - Oxidative damage in a number of organs
 - Intersex

Intersex in Fish

- Increasingly there are reports of wild and laboratory-exposed fishes with intersex
 - Intersex (ovotestis; testisova) is a general term for gonadal abnormalities most often noted microscopically, occasionally macroscopically
 - Most reports involve observation of female germ cells or immature oocytes within a predominantly male gonad
 - Includes malformed/intersex reproductive ducts; large areas of ovarian tissue; testicular tissue within ovaries

Intersex in Normally Gonochorist Fishes



- ⌋ Suggested as a marker of endocrine disruption
- ⌋ Most often associated with exposure to estrogenic compounds
- ⌋ Has been reported in a number of fish species

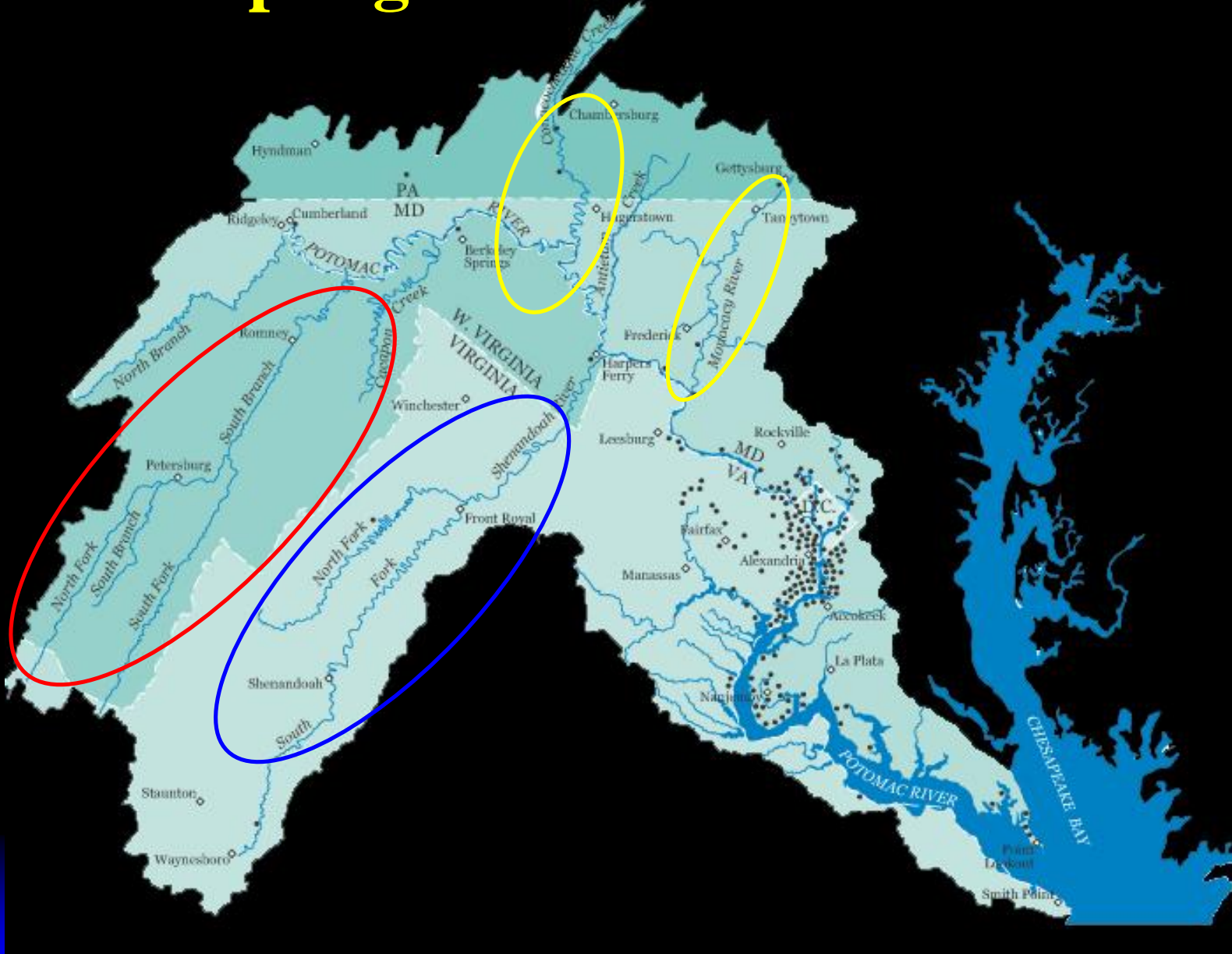
Frequency of Intersex in Smallmouth Bass

Site #	River System	Summer 2003 % Intersex	Spring 2004 % Intersex	Summer 2004 % Intersex
1	South Branch	33% (6)	100% (7)	50% (4)
2	North Fork	ND	75% (12)	25% (8)
3	South Branch	0% (4)	85% (13)	40% (10)
4	South Branch	ND	69% (13)	36% (11)
5	South Branch	60% (5)	90% (10)	67% (12)
6	North River	0% (3)	ND	33% (6)
7	Cacapon	14% (7)	70% (10)	57% (7)
8	South Branch	80% (10)	72% (11)	33% (6)

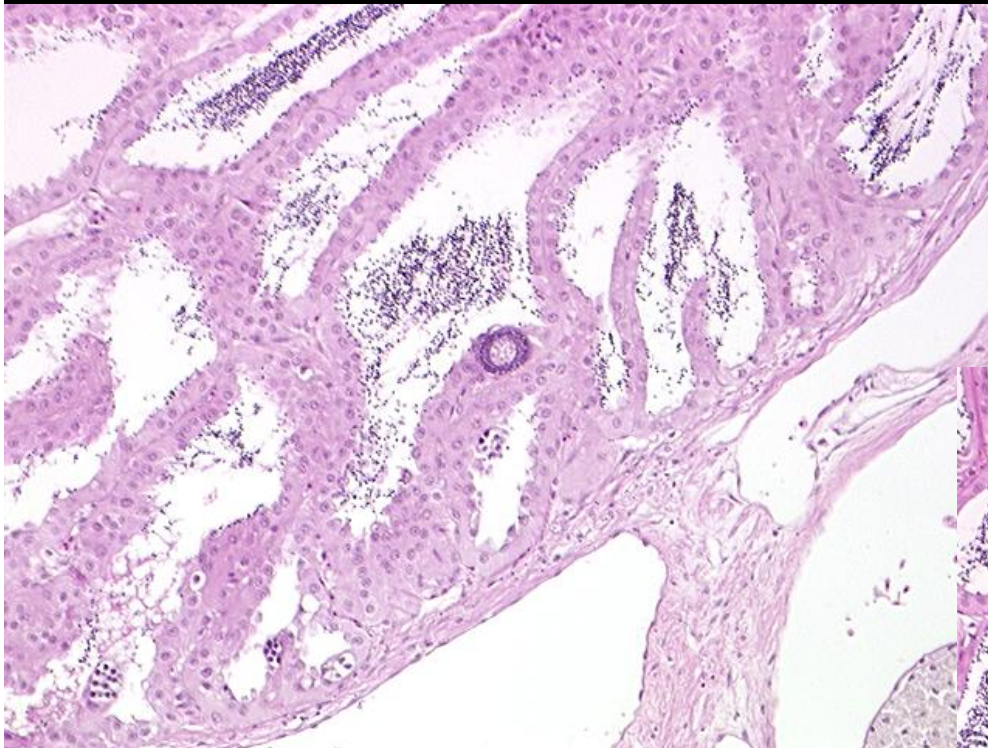
Questions

- u How widespread is it within the Potomac and ultimately Chesapeake drainage?
- u Is it somehow “normal” for smallmouth bass?
 - Or are there other indications of exposure?
- u How does the incidence in the Potomac compare to other, out of basin drainages?
- u Does it affect reproductive capacity and hence eventually population levels

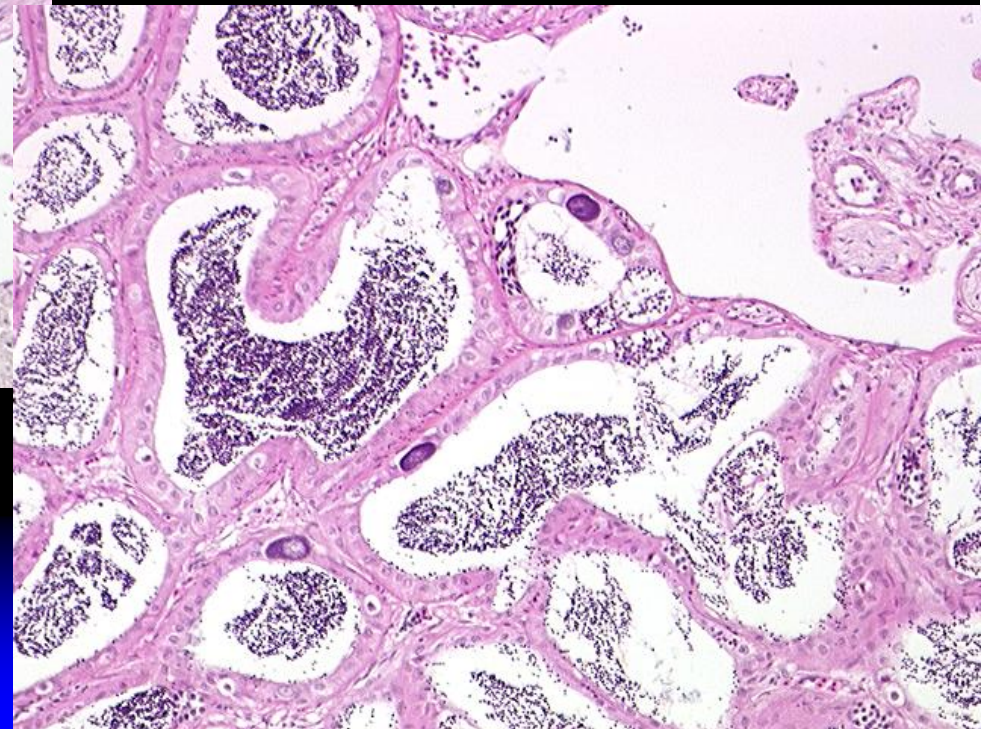
Sampling in the Potomac Watershed



Ovotestis Severity Index



Score 1
Single oocyte

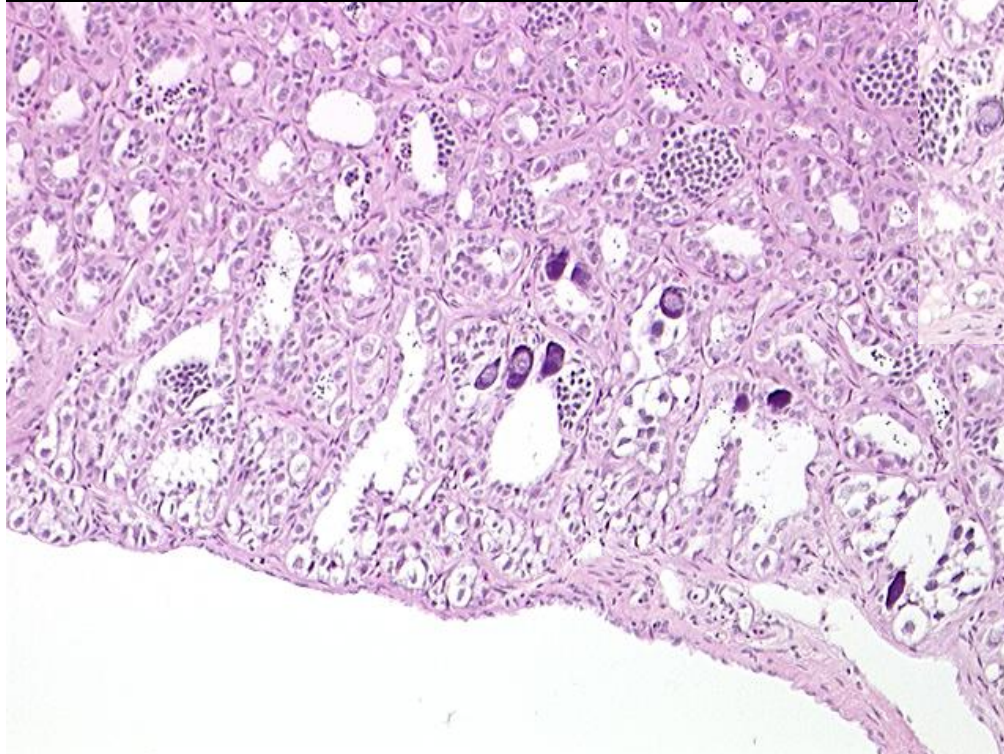
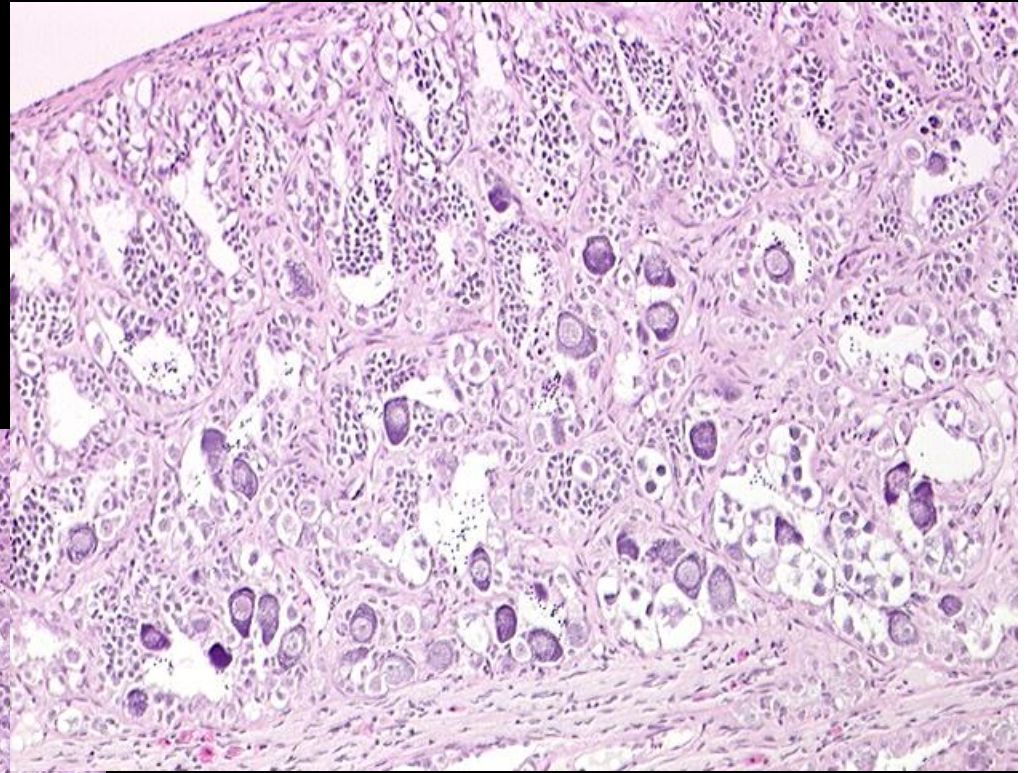


Score 2
More than 1 oocyte
Not associated - multifocal

Ovotestes Severity Index

Score 3 Cluster

>1 but <5 associated oocytes

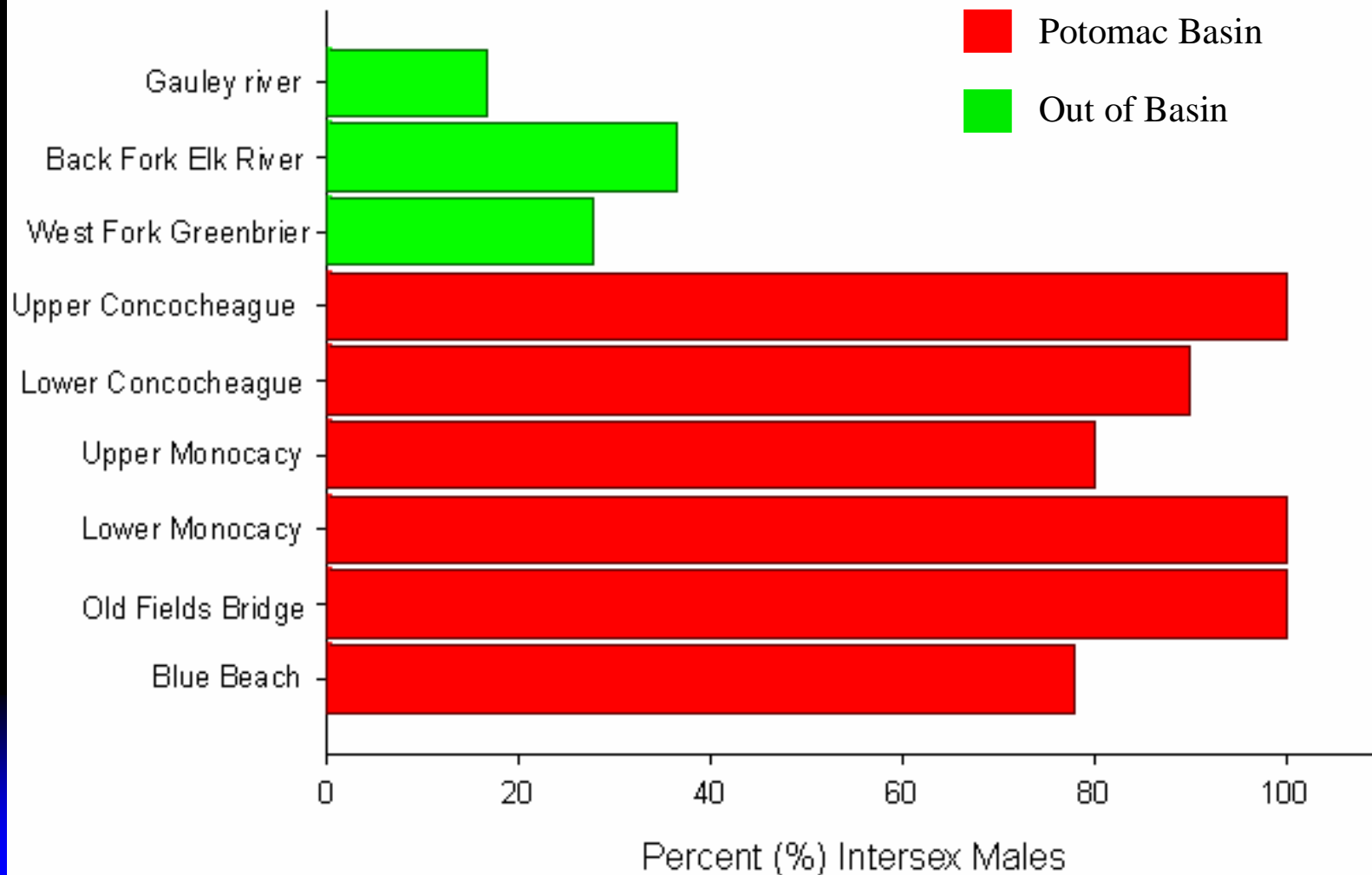


Score 4 Zonal

**multiple clusters or
>5 associated oocytes**

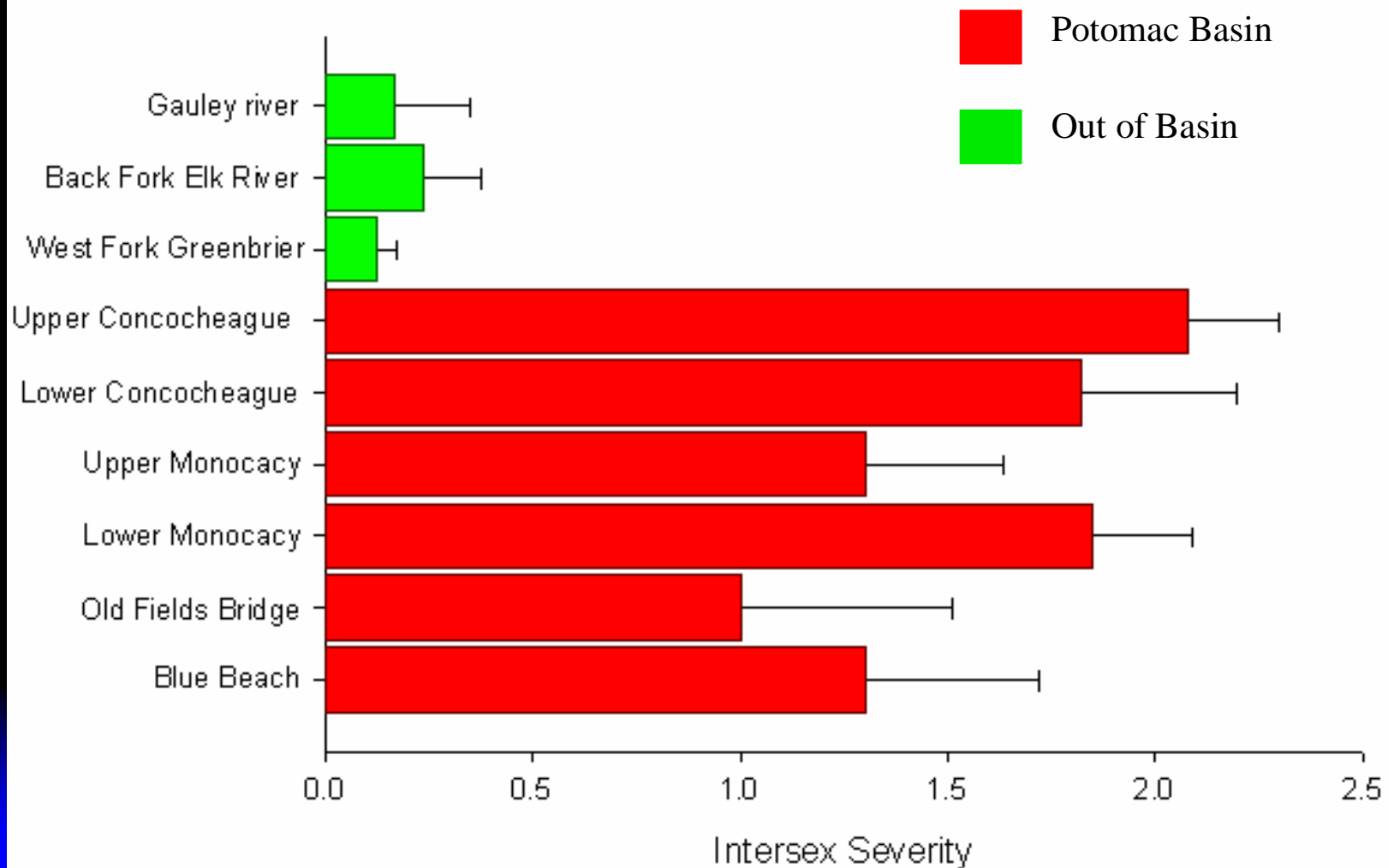
Fall 2005

Prevalence of Intersex



Fall 2005

Severity of Intersex



Other Biomarkers of Exposure

Vitellogenin

- Vitellogenin 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

Vitellogenin in Male Fish

- Males have the gene to produce vitellogenin - usually not turned on
- Exposure to natural or synthetic estrogens from human and agricultural sources, phytoestrogens and a variety of estrogen mimics can turn the genes on

Vitellogenin in Male Bass in the Potomac

Site	Incidence Intersex	Incidence Vitellogenin	Male GSI	Female GSI
Upper Conococheague	100%	50%	0.39	1.24
Lower Conococheague	90%	50%	0.13	0.62
Upper Monocacy	80%	50%	0.32	0.85
Lower Monocacy	100%	13%	0.30	0.81
Blue Plains	23%	69%		

A Problem??

- u Intersex
- u Circulating vitellogenin in male fish
- u Other histologic observations
- u Apparent immunosuppression
 - Opportunistic infections
 - Lesions
 - Fish kills

Cause(s) in the Potomac

- Estrogenic chemicals are most likely culprit
- Unlikely that there is one cause
- Mixture of contaminants from various sources
- Cause(s) may not be the same in all areas
- Human wastewater, agricultural runoff, industrial, atmospheric deposition



Chemicals

“Emerging Contaminants”

- Agricultural Sources - Animal
 - Natural and synthetic hormones
- Pesticides and herbicides
 - Agricultural and household use
- Personal care products
 - Pharmaceuticals
 - Synthetic Hormones – birth control, hormone replacement therapy
 - Antimicrobials – soap, detergent, toothpaste
 - Fragrances, organic UV filters, DEET

“Emerging Contaminants”

Pharmaceutical, Hormones, Organic Wastewater Contaminants

- Ô These are chemicals that are produced to have biological effects at low levels**
- Ô For many that end up in human sewage and ultimately the aquatic environment, aquatic organisms may be constantly exposed**
- Ô National Reconnaissance (Kolpin et al. 2002)**
 - Ô Measured 95 in water from 139 streams in 30 states**
 - Ô Most downstream of urban areas or livestock production**
 - Ô Median of 7, as many as 38, in a given sample**

Are there EDCs in the South Branch?

Contaminant

Category

Ethynyl estradiol (EE₂)

Personal Care/Contraceptive

Trifluralin

Pesticide/ Herbicide

Chlorpyrifos

Organophosphate insecticide

Triclosan

Chlorinated anti-bacterial

Lindane (γ-HCH)

Insecticide

Hexachlorobenzene

Pesticide/ Fungicide

trans-Chlordane

Pesticide

cis-Chlordane

Pesticide

trans-Nonachlor

Pesticide

cis-Nonachlor

Pesticide

BDE 47

Brominated flame retardant

BDE 99

Brominated flame retardant

BDE100

Brominated flame retardant



Questions That Need to be Addressed

- **Interactive effects of the complex mixtures**
In vitro studies and experimental exposures suggest many of these compounds are additive in estrogenicity
- **What ends up in the sediment?**
Levels of estrogenic activity have been found to be much higher (532 to 748-fold) in sediments than in the overlying water, suggesting these chemicals accumulate in the bed sediment phase (Peck et al 2004).

Effects of Intersex

Individual and Population Levels

- u Studies with wild roach (*Rutilus rutilus*)
- u Intersex males
 - Produced up to 50% less milt (per gram)
 - Reduced percent of motile sperm
 - Lowered ability of sperm to successfully fertilize eggs and produce viable offspring

Research Needs

- u Need to identify the contaminants and sources
- u Need a risk assessment of various landuse influences
- u Need to understand exposure times and routes
 - Does exposure at a young age predispose the fish for the rest of its life to lower reproductive capacity and increased disease susceptibility?
- o Need to understand that the intersex problem is just one part of a complex issue

Acknowledgements

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