

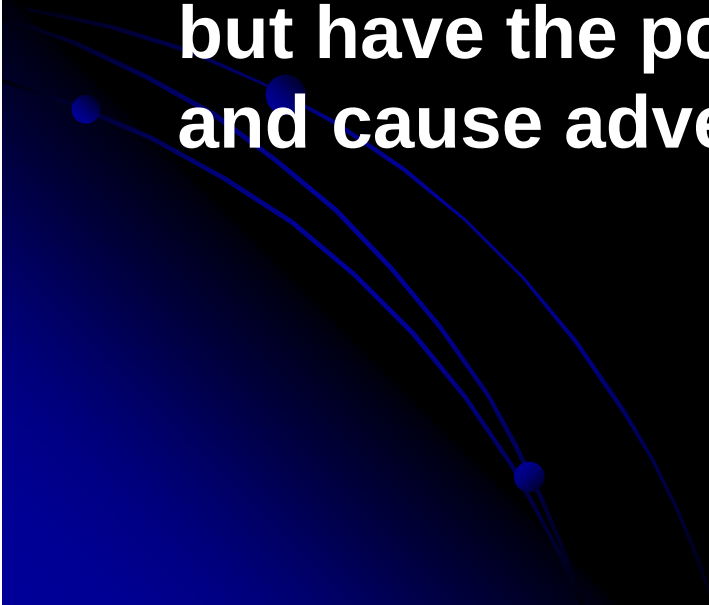
Contaminants of Emerging Concern in Rivers and Streams and Effects on Fish Health

**Vicki Blazer
U.S. Geological Survey
National Fish Health Research Laboratory
Kearneysville, WV**



Chemicals of “Emerging (Emerged) Concern”

 Defined as synthetic or naturally occurring chemicals that are not commonly monitored in the environment, are generally not regulated, but have the potential to enter the environment and cause adverse effects



Chemicals of Emerging Concern Sources

 **Wastewater Treatment Plants**

 **Industrial effluent**

 **Stormwater runoff**

 **Agriculture**

 **Landfill leachate**

“Emerging Contaminants” WWTP-Related

 **Pharmaceuticals – Human and Animal**

 **Synthetic Hormones – birth control,
hormone replacement therapy**

 **Antibiotics**

 **Viagra to Prozac**

 **Personal care products**

 **Antimicrobials – soap, detergent, toothpaste**

 **Fragrances**

 **Organic UV filters**

 **DEET**

“Emerging Contaminants”

 **Industrial effluents, urban/suburban stormwater runoff, landfill leachate**

 **Current-use and legacy pesticides**

 **Brominated flame retardants**

 **Bisphenol A, other plastic-derived compounds**

 **Nanoparticles**

 **Phytoestrogens**

Agricultural Sources

-  **Animal manure and litter**
-  **Natural and synthetic hormones**
-  **Antibiotics/antimicrobials**
-  **Feed additives**
-  **Pesticides and herbicides**
-  **Human Biosolids**

Effects

Chemical of Emerging Concern

-  Endocrine disruption (EDC)
-  Immune system/disease resistance
-  Cancer/Neoplasia - promoters
-  Numerous physiological and pathological effects – secondary sex characteristics, oxidative damage
-  Behavior

Factors To Consider

Many are chemicals that are produced to have a biological effect

May have biological effects on nontarget organisms at very low (ppb-ppt) levels

Endocrine/immune systems are regulated by soluble factors and feedback mechanisms – not classic dose responses

Interactive effects of the complex mixtures

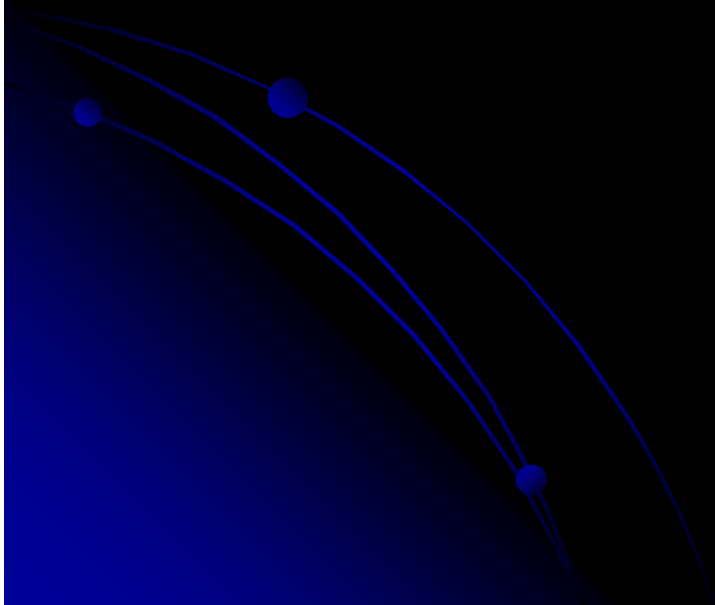
In vitro and laboratory studies suggest many of these compounds are additive or even synergistic in estrogenicity, immunotoxicity and other effects

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).

Questionable Statements

- “no trace elements or pesticide contaminants have been found at concentrations sufficient to stress fish and thereby be factors in fish kills”



Factors to Consider

-  Most criteria are based on acute toxicity or gross effects such as growth
-  In most cases there are no criteria for sublethal effects such as immune modulation or endocrine disruption
-  Detection limits (MDL) for the methods used may be well above the concentrations known to cause effects

Estrogens and Intersex

Natural estrogen

-  Based on studies in >25 fish species 1 ng/L has been suggested as the “no effects levels”
-  1.3 ng/L MDL for the Potomac/Shenandoah passive sampler studies

Ethynylestradiol

-  0.3 ng/l exposure of roach embryo caused feminization
-  0.35 ng/l recently set as the aquatic “no effects level”
-  0.66 ng/L MDL for the Potomac/Shenandoah studies

Passive Samplers



- Semi permeable Membrane Devices (SPMDs)- accumulate hydrophobic compounds
- Polar Organic Compound Integrative Samplers (POCIS)—accumulate hydrophilic compounds

**Dave Alvarez, Columbia Environmental Research Laboratory
WRD Chemists**

Issue of Complex Mixtures

 Screening either grab water extracts or the extracts from the passive samplers using in vitro reporter cell assays

- YES, BLYES, breast cancer cells – total estrogenicity – estrogen equivalents
- Total androgenicity

 Passive sampler hormone results – no hormones above method quantification levels (N. Fork Shen)

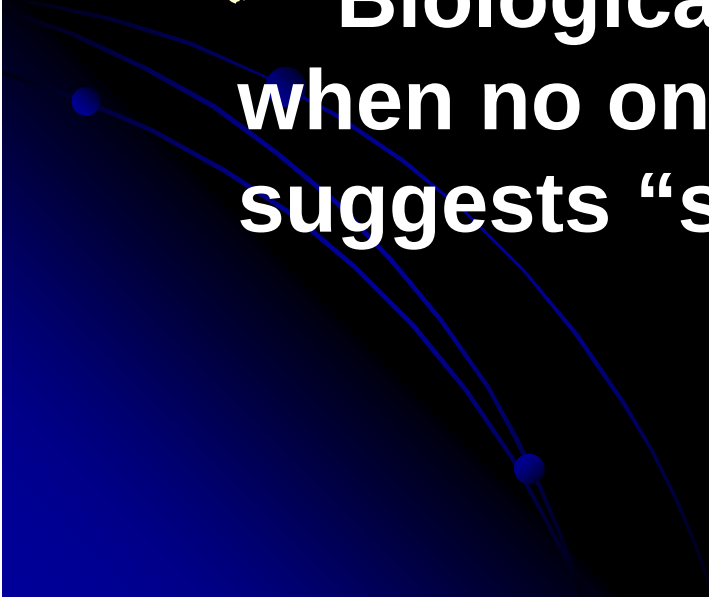
- 17β estradiol, 17α -ethynylestradiol, estrone, estriol

 Estrogen equivalents ranged from 14-79 ng estradiol/sample depending on the site

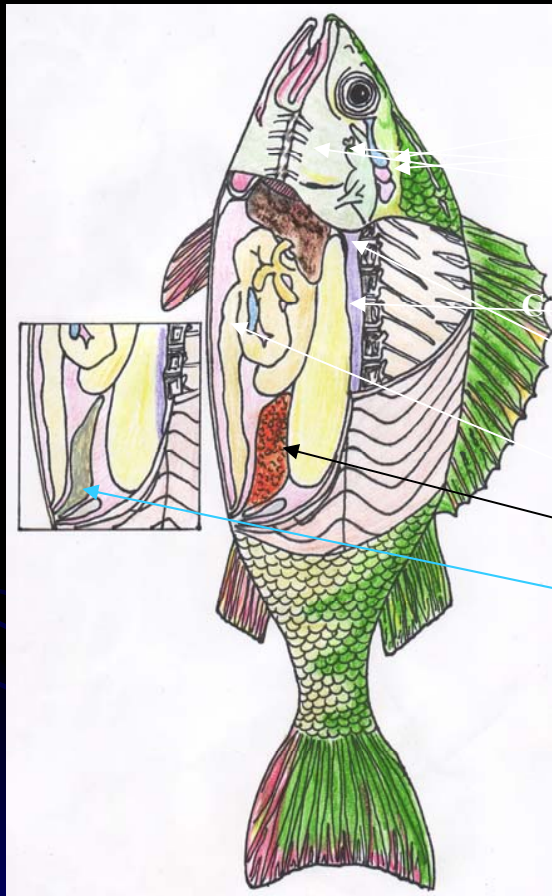
Fish As Indicators of Environmental or Ecosystem Health

 Fish health is a good integrator of cumulative effects of environmental stressor

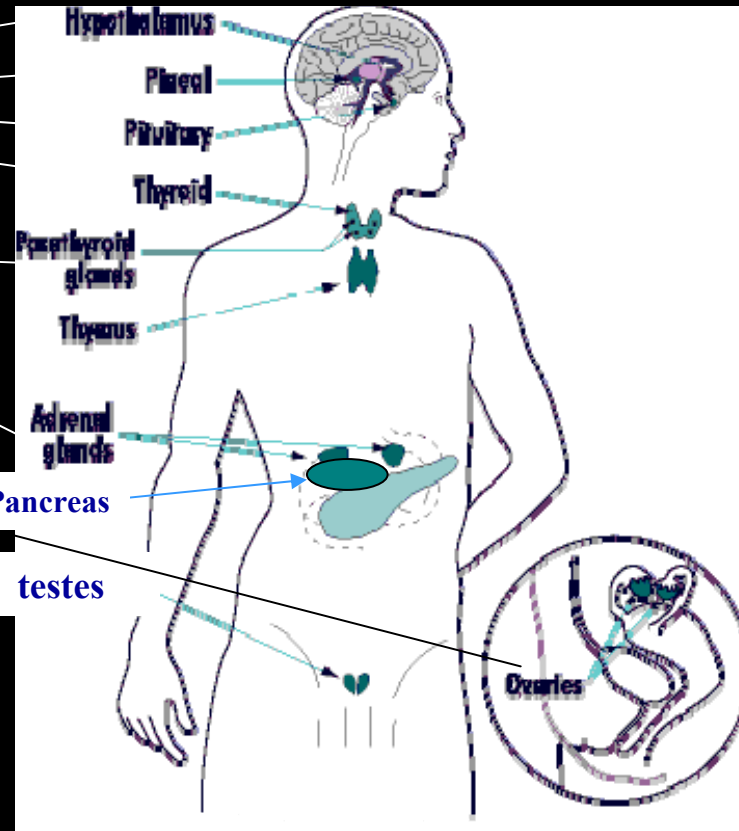
 Biological effects often occur when no one chemical indicator suggests “stress”



Endocrine Organs



Fish



Human

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

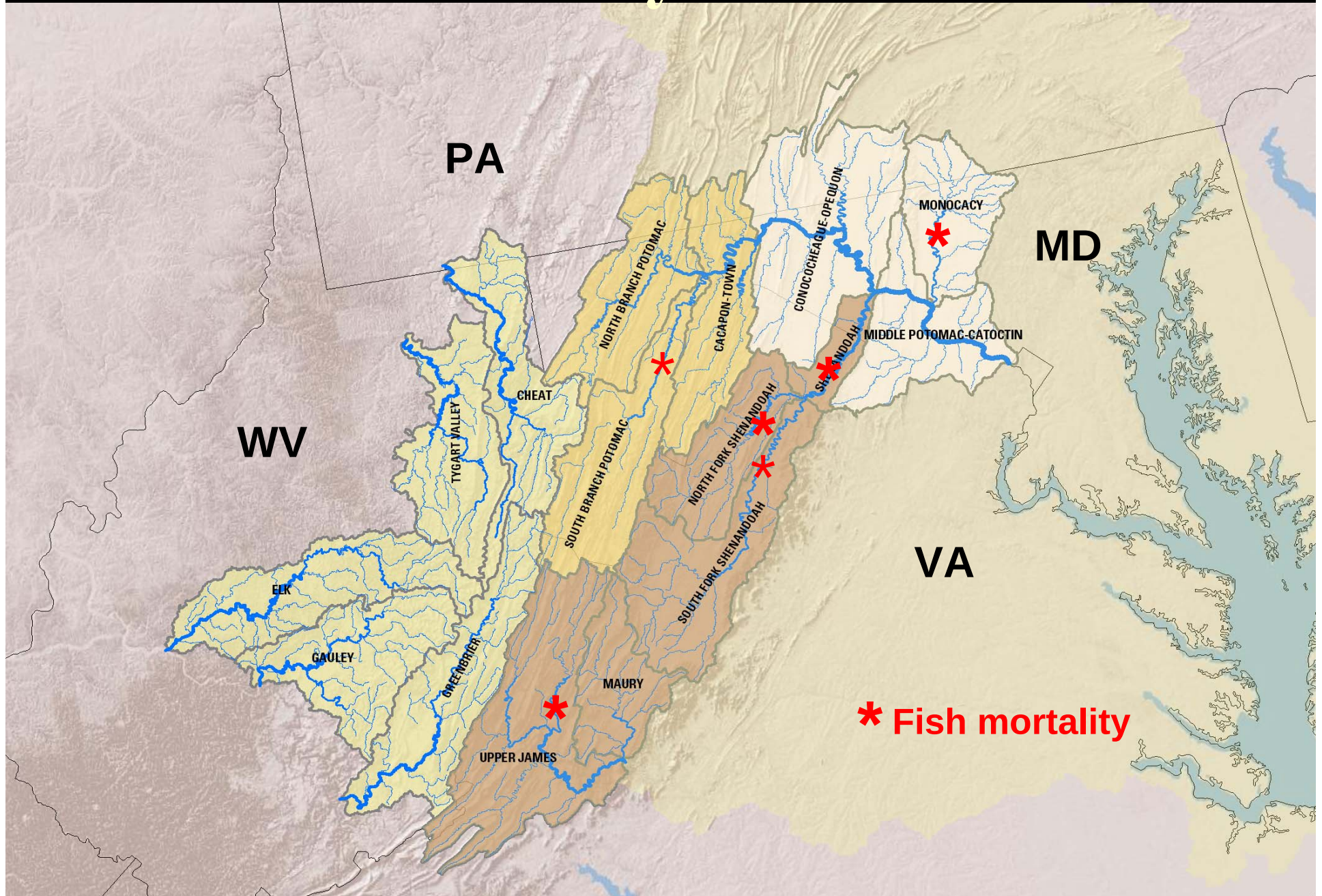
Histopathological

- Diagnose causes of gross observations, identify emerging pathogens, identify specific effects of contaminants, with image analyses quantify parasites, macrophage aggregates etc.

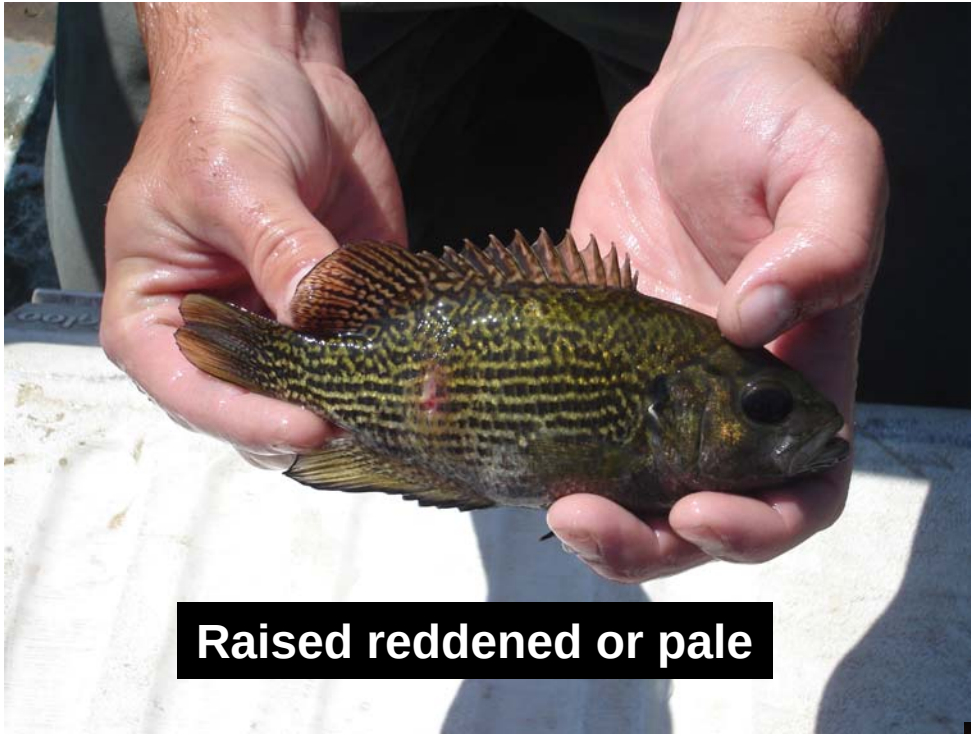
Molecular

- 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

Study Areas



Variety of Lesions



Raised reddened or pale

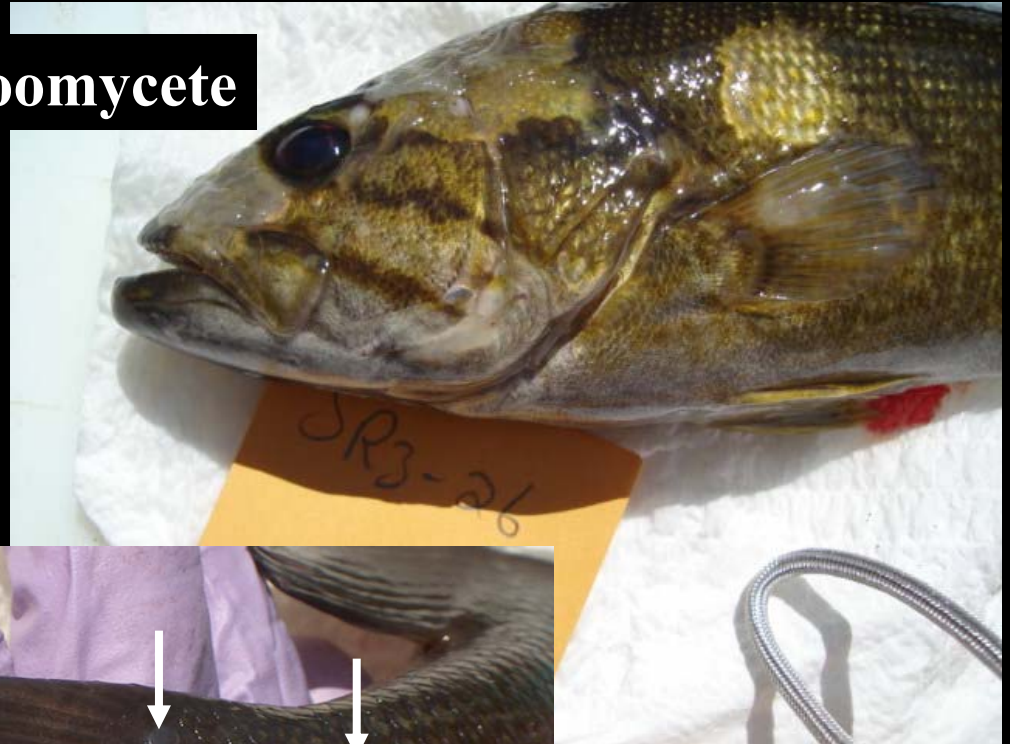
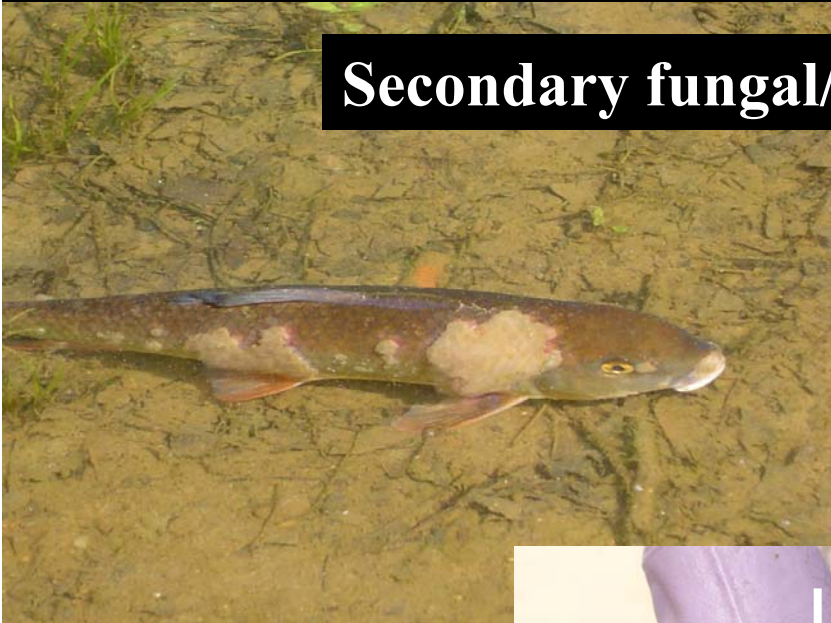


Erosion of epidermis and dermis

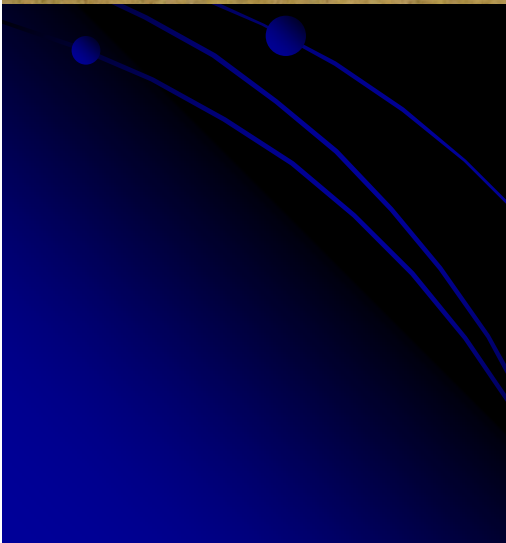


Variety of Skin Lesions

Secondary fungal/oomycete



Raised clear, mucoid



Variety of Pathogens Cultured

Bacteria

- *Flavobacterium columnare*
- *Aeromonas hydrophila* – and other motile Aeromonads
- *Aeromonas salmonicida*

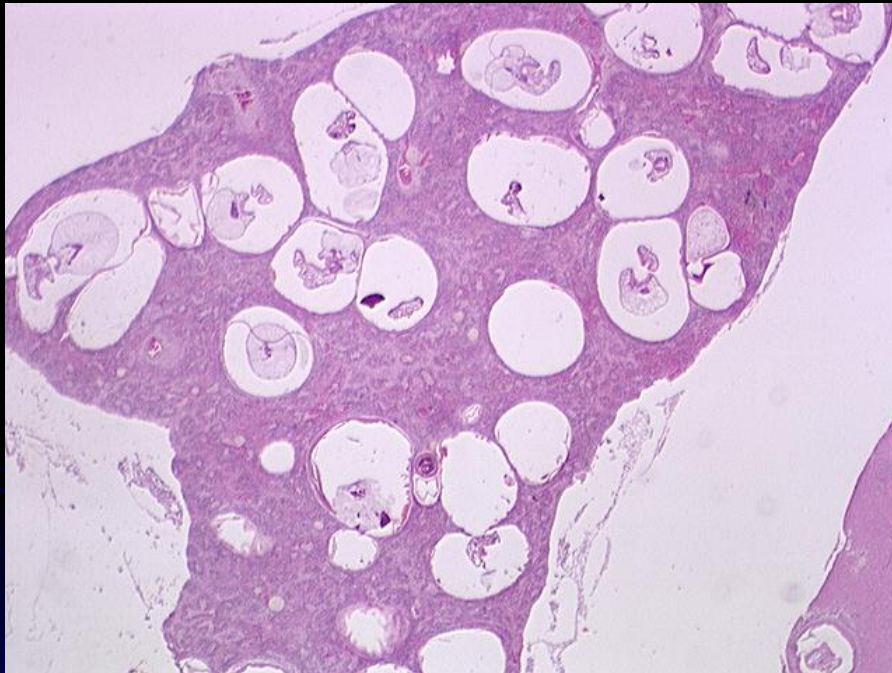
Virus

 Largemouth Bass Virus

Fungi

No consistent findings

High Parasite Loads



Trematodes



Myxozoans

Both groups have complex life cycles that include benthic invertebrates – snails, bryozoans, worms – polychaetes and oligochaetes (*Tubifex*)

Potomac Fish Kills Findings Suggest:

 There are stressed populations of sensitive species and at some point the “perfect storm” of conditions overwhelm the fish and mortalities occur

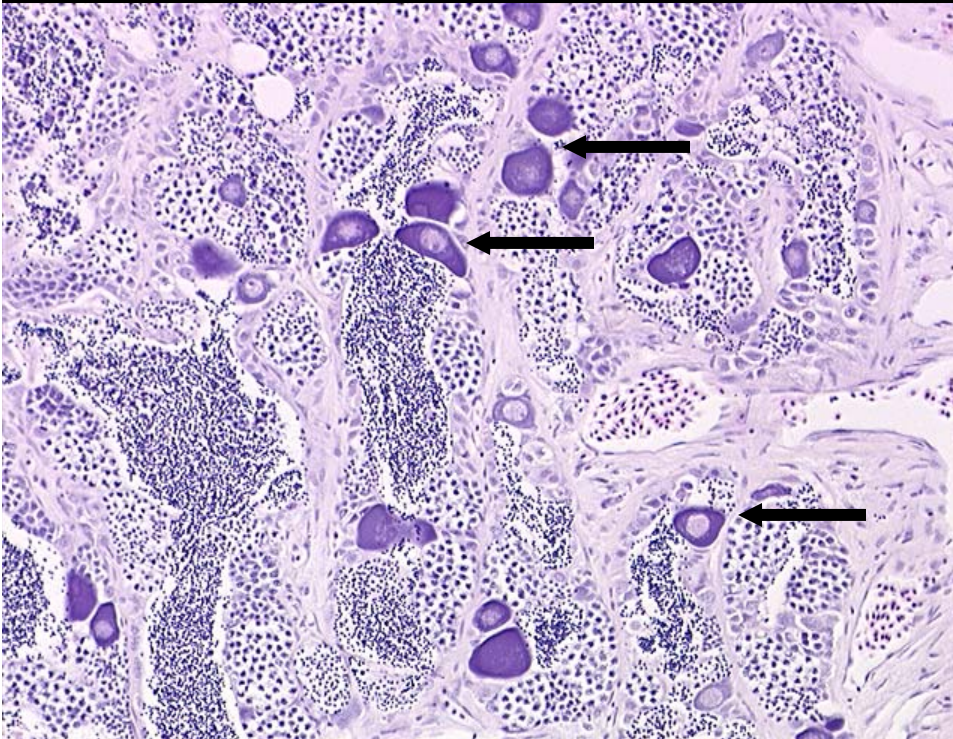
 No one infectious agent responsible – variety of “opportunistic” infections

 Environmental stressors:

 Water quality issues – high pH, ammonia, increased water temperatures, nutrients, low DO

 Chemicals that cause immunosuppression leading to a variety of infections

Intersex in Normally Gonochorist Fishes



- Suggested as a marker of endocrine disruption
- Most often associated with exposure to estrogenic compounds
- Probably induced early in life, but may occur due to exposure later in life

Vitellogenin in Male/Immature Fish

-  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

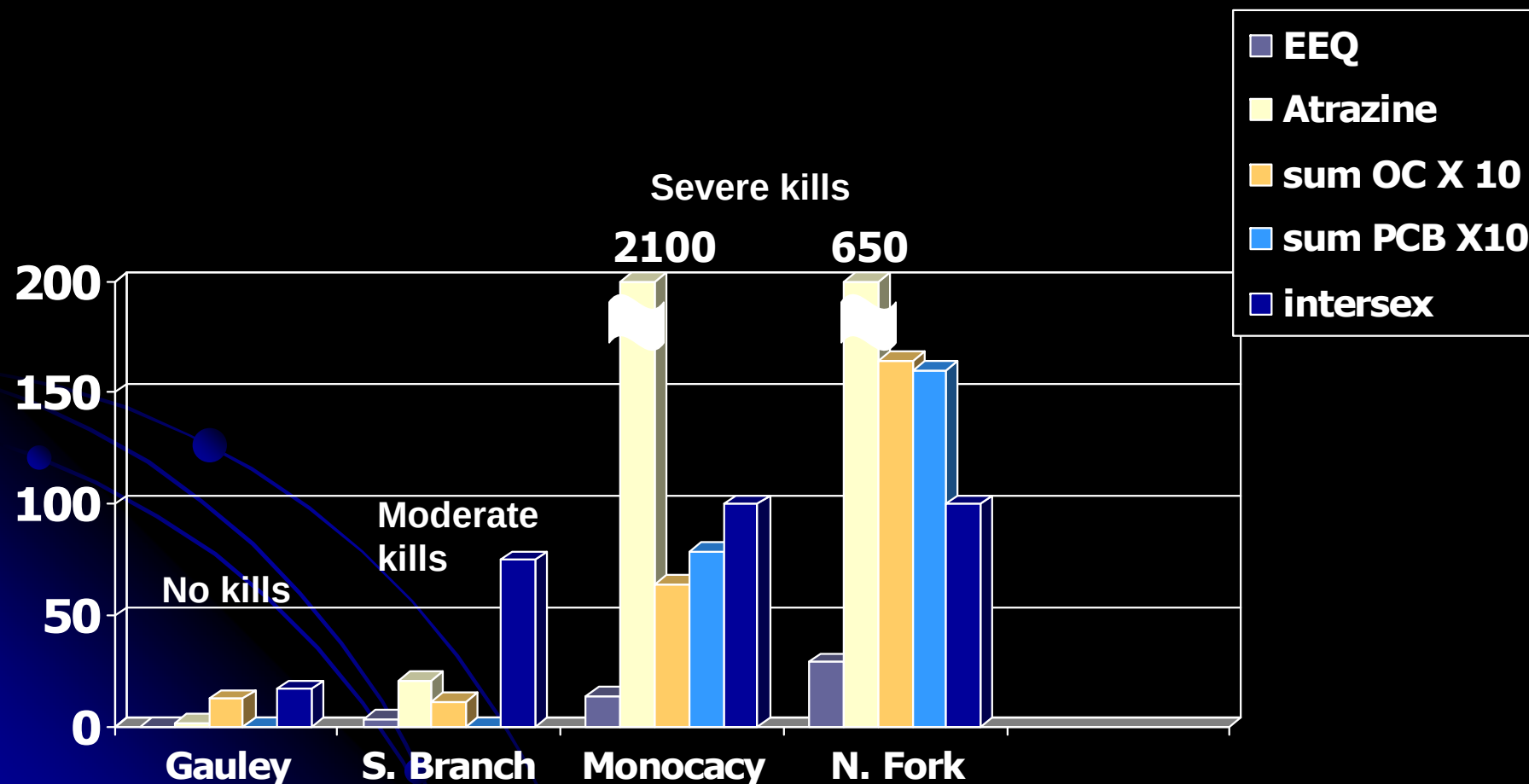
Fish Kill Issues Related to Intersex and Other Reproductive Findings?

 **Estrogens and estrogenic chemicals 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**

Gradient Spring 2006-2007

Passive Sampler Results/Intersex



WWTP Study

Smallmouth Bass

Site	Testicular Oocytes	Male Vitellogenin	Female Vitellogenin
Conococheague Upstream	100%	60%	80%
Conococheague Downstream	90%	90%	80%
Monocacy Upstream	82%	45%	45%
Monocacy Downstream	100%	33%	77%

WWTP Susquehanna Drainage

Site	No.	Intersex Prevalence	Intersex Severity	Vitell. In males
Susq - U	7 F 14 M	93%	1.4	21%
Susq - D	12 F 9 M	89%	1.7	11%
Swatara - U	4 F 4 M	75%	0.6	0%
Swatara - M	8 F 6 M	67%	1.0	50%
Swatara - D	6 F 6 M	100%	2.0	0%

Agricultural Inputs

Cattle with free access to the river



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

Agricultural Pesticides

Fall 2005 (Spring 2006)

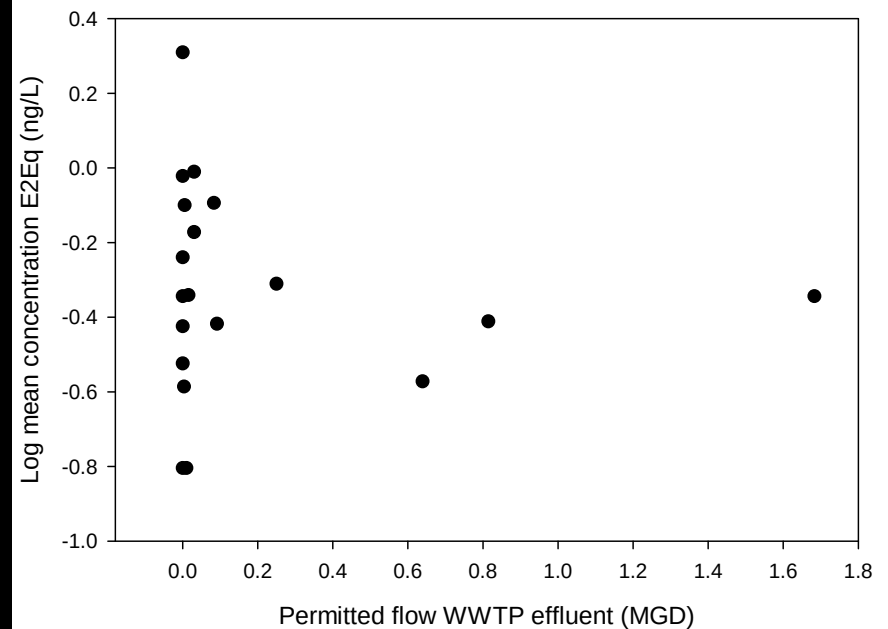
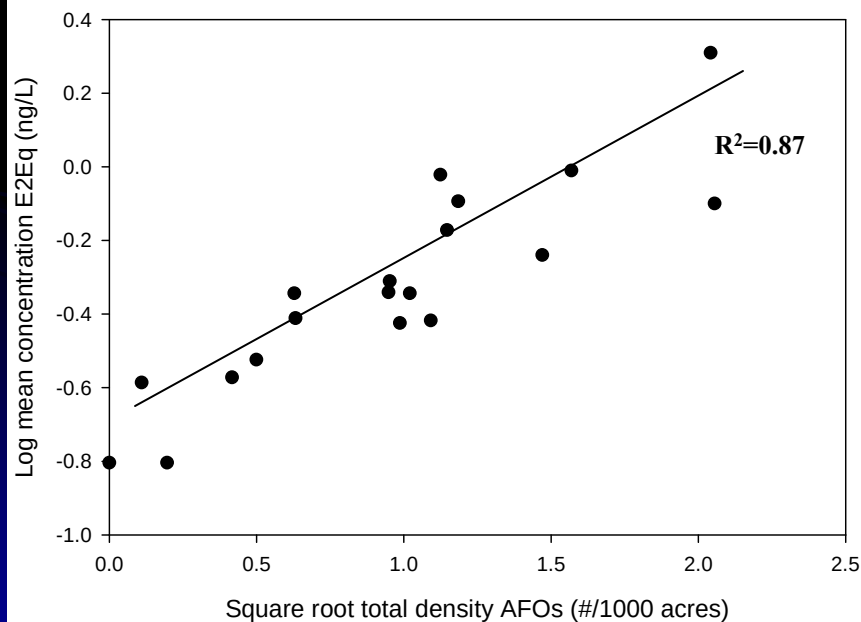
Chemical	Con	Con	Mon	Mon
Estimated ng/L	Up	Down	Up	Down
Metolachlor	0.73 (7.5)	1.1 (9.0)	12.0	10.8 (97)
Atrazine	47 (380)	110 (430)	92	2 (2100)
Prometon	1.1 (1.2)	3.2 (1.4)	2.1	1.4 (1.8)

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

Total Estrogenic Equivalents of Water Extracts

Smaller Tribs of Shenandoah Drainage



Serena Ciparis, Reese Voshell – VT Tech

Chemical Compounds in Tissue

PCBs		Flame Retardants	
PCB101	PCB174	BDE47	BDE100
PCB110	PCB177	BDE66	BDE138
PCB118	PCB180	BDE71	BDE153
PCB138	PCB183	BDE85	BDE154
PCB146	PCB187	BDE99	BDE183
PCB149	PCB 194	Fire Master	
PCB151	PCB206	Dechlorane Plus	
PCB170		Pentabromotoluene	
Personal Care Products			
Triclosan		Methoxytriclosan	

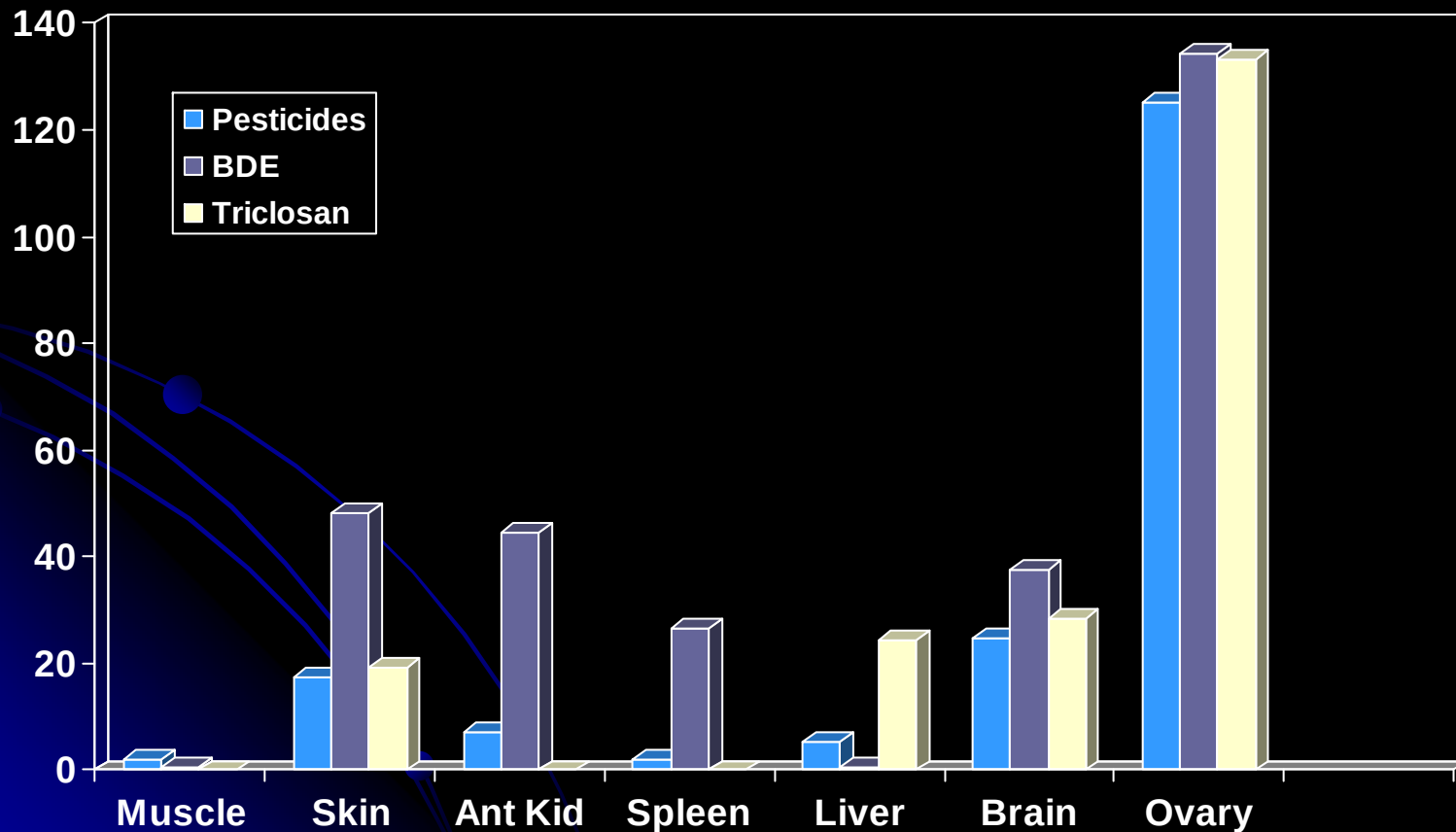
Dana Kolpin - Toxic Substance Hydrology

Mark Burkhardt and Steve Zaugg – Denver USGS Lab

Chemical Compounds in Tissue

Organochlorine Pesticides		Pesticides
Hexachlorobenzene	trans-Nonachlor	Cyfluthrin
Pentachloroanisole	cis-Nonachlor	Tefluthrin
Pentachloronitrobenzene	Tetradifon	Cyhalothrin
Dimethyltetrachlorophthalate	Dieldrin	Chlorpyrifos
Chlordane, Oxychlordane	Trifluralin	Fipronil
cis and trans-Chlordane	Benfluralin	Fipronilsulfide
p,p-DDT, DDD, DDE		Desulfnylfipronil
Oxychlorostyrene		DCPA(Dacthal)
		Oxyfluorfen

Comparison of Tissue Contaminant Concentrations Female Bass South Fork Shenandoah



Antidepressant Study Iowa and Colorado

-  7 different antidepressants upstream and downstream of WWTP
-  Analyzed in water, sediment and fish brains
 - Were not present upstream, but were downstream
 - Levels decreased in water further downstream
 - Levels increased in sediment at downstream sites
 - Were measurable in fish brains
 - Different profile was observed in brain tissue than that observed in water

Tissue Levels?



Understanding what effects the individual compounds and complex mixtures have in the individual tissues in which they accumulate

- **Spleen, anterior kidney – influence disease resistance**
- **Brain – effects on reproduction, behavior etc.**
 - **Oocyte maturation depends the pituitary gonadotropin, luteinizing hormone stimulating the production of maturation-inducing hormone.**
- **Egg**
 - **Effect vitellogenin incorporation, final maturation etc.**
 - **Fish hatch as sac fry – for the first 2-3 weeks of life live by absorbing the yolk.**
 - **How do chemicals present in the egg (and water and sediment) affect the fish during this critical stage of sexual differentiation, immune system development etc.**

Human Effects

-  **Cancer – particularly breast cancer, testicular cancer**
-  **Infertility**
-  **Disorders of sex development (intersex)**
-  **Asthma and other immune related syndromes**
-  **Autism, ADHD, Learning/behavioral disorders**
-  **Diabetes**
-  **Thyroid disorders**

Critical Windows of Exposure – Fetal/Newborn Exposure

Testicular Dysgenesis Syndrome in Humans

-  Increase in reproductive system problems
 -  Declining sperm counts
 -  Reduced semen quality
 -  Genital abnormalities - cryptorchidism
 -  Reduction in testicle size
 -  Increased prevalence of testicular cancer

Suggested this syndrome is result of disruption of fetal programming and gonadal development during fetal life and is related to adverse environmental influences/contaminant exposures or other factors affecting hormone levels.

Human Testicular Cancer

-  Rates of testicular cancer have increased sharply in the past three decades in many countries (US, Denmark, Norway, Canada)
-  Particularly those affecting younger men - the seminomas
-  Recent study compared EDCs in breast milk – dioxins, PCBs and some organochlorine pesticides correlated with high incidence

Testicular Tumors (Seminoma) Lake Michigan Yellow Perch

Sample Time

Prevalence

Winter

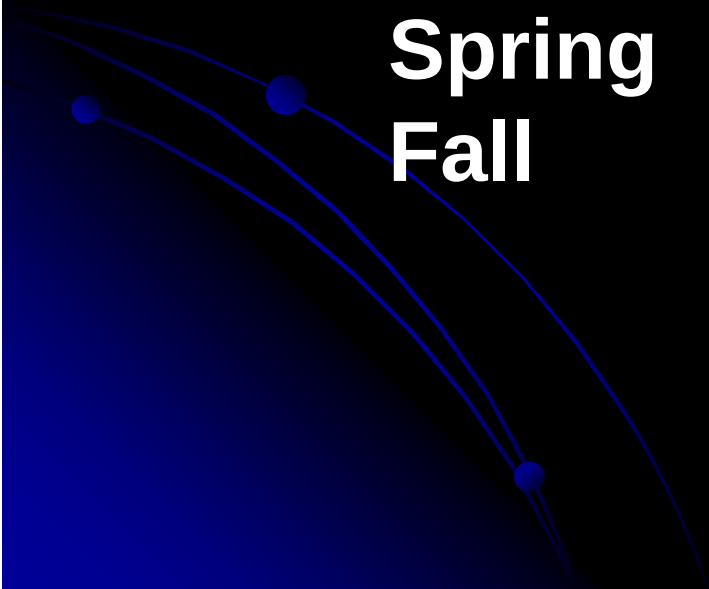
31%

Spring

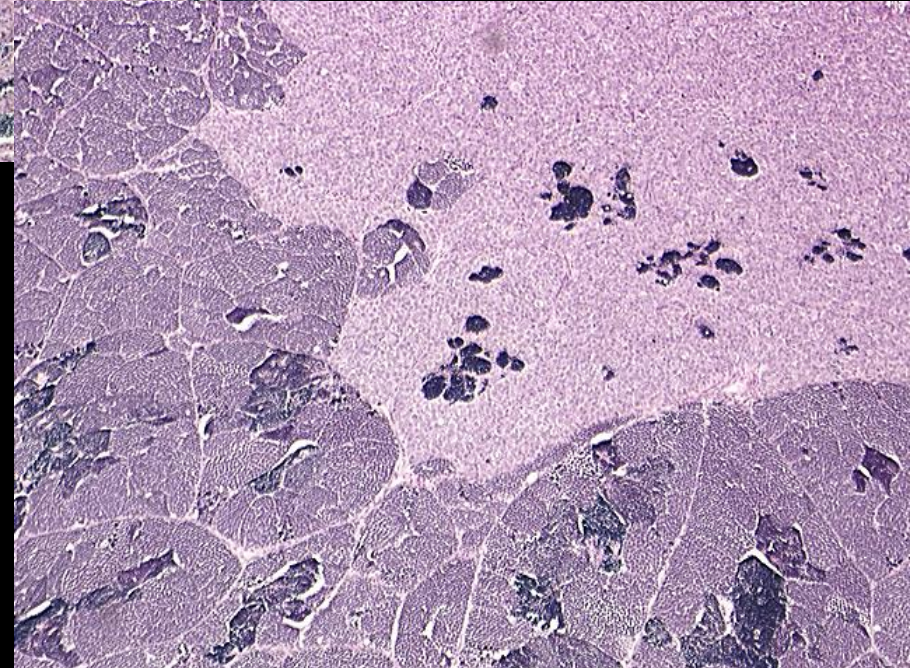
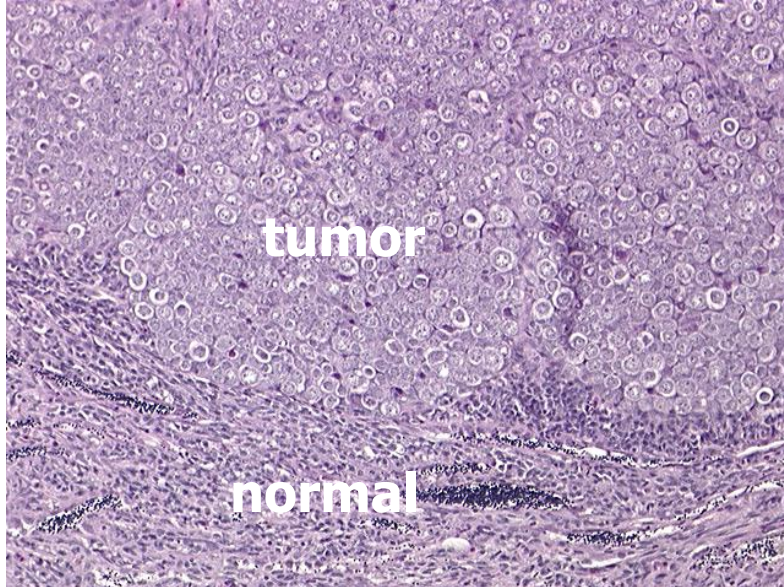
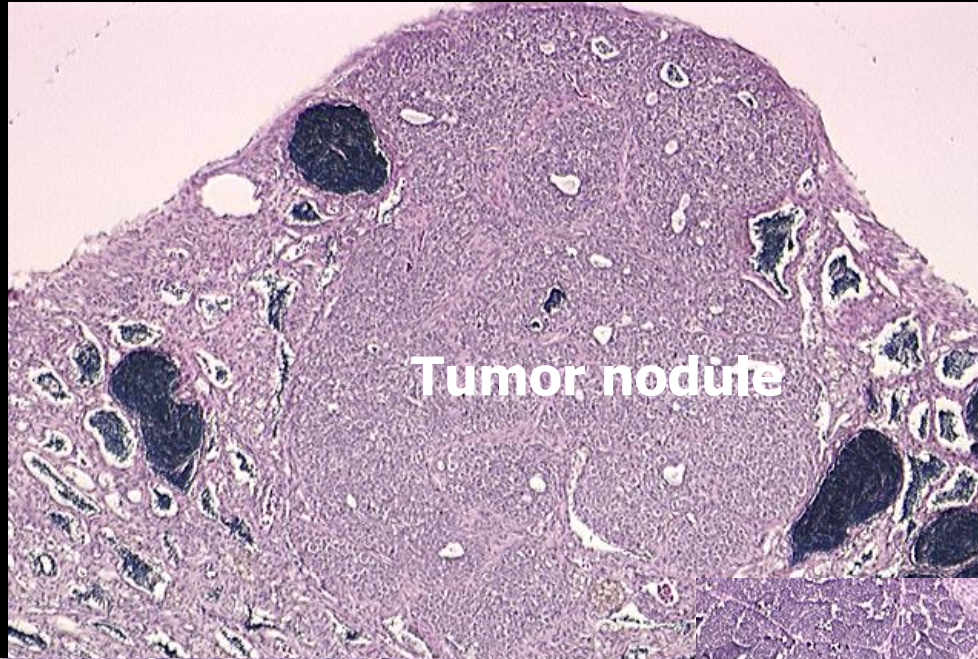
27%

Fall

25%



Perch Testicular Neoplasia



Results for Male Perch

Testicular dysgenesis syndrome in Lake Michigan yellow perch?

Lake Michigan compared to Lake Mendota

-  **Smaller testes – smaller GSI**
-  **Presence of a variety of gonadal abnormalities including intersex**
-  **High prevalence of testicular tumors**
-  **Higher concentrations of PCBs, organochlorine pesticides**

Pharmaceutical Formulation Facilities

Compound	Class
Butalbital	Barbiturate
Carisoprodol	Muscle relaxant
Diazepam	Tranquilizer
Metaxalone	Muscle relaxant
Methadone	Opioid
Oxycodone	Opioid
Phendimetrazine	Amphetamine

Pharmaceutical Formulation Facilities

National Survey of 23 municipal WWTP

-  5 of 7 were detected in at least one
-  Butalbital (83%) and oxycodone (56%) most common
-  Concentrations up to 0.73 µg/L

Effluent of 2 WWTP receiving flows from pharmaceutical formulation facilities

-  Concentrations of oxycodone and metaxalone up to 1700 and 3800 µg/L (1.7 and 3.8 mg/L)
-  Oxycodone human dosage ranges from 2.2 to 9.0 depending on strength – 2 liters of water would have an effective dose

Acknowledgements

- ❖ Virginia DGIF and DEQ
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- ❖ Maryland DNR
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- ❖ Priority Ecosystem
- ❖ US EPA
- ❖ US Fish Wildlife Service
- ❖ NOAA

