

Fish Health and Reproductive Issues in the Potomac Drainage

South Branch Potomac

- ◆ **Spring –Summer 2002 – Fish kills and fish lesions**
- ◆ **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 With WV DNR

- ◆ More comprehensive fish health assessments of bass from 8 sites
 - 5 in the South Branch
 - 1 each in Cacapon, Lost and North Rivers
- ◆ Assessments (10 male, 10 female bass) include:
 - Necropsy-based – gross lesions, length, weight, condition factor, age, gonadosomatic index
 - Blood/plasma collection – vitellogenin, reproductive hormones, thyroid hormones, cortisol
 - Histologic (microscopic) analysis of all organs – gill, liver, spleen, kidney, gonads, skin lesions or other abnormalities





South Branch Potomac 2003

- ◆ Findings included:
 - High parasite (helminth and protozoan) loads at some sites
 - Signs of oxidative damage in a number of organs
 - Intersex




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)

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

Endocrine Disruption/Modulation

-  Fairly recently been recognized that small amounts of certain chemicals can have very significant effects on the endocrine systems of vertebrates
-  Due to human activities many of these chemicals end up in the aquatic environment
-  However, effects have been documented in alligators, turtles, birds and mammals, as well as invertebrates

Chemicals

‘Emerging Contaminants’

 **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**

Mechanisms of Hormone Disruption

1. **Interaction with hormone receptors**
 - ❖ **Bind and activate receptor**
 - (“mimic” or agonist)
 - ❖ **Bind receptor without activation**
 - Antagonist
2. **Alter hormone synthesis and metabolism**
 - ❖ **Modulates hormone synthesis and clearance**
3. **Alteration of receptor expression**
 - ❖ **Increase of decrease available receptors for hormone binding**

Indicators of Endocrine Modulation in Fishes

Morphological changes

Secondary sex characteristics, altered growth, altered organosomatic indices, deformities

Circulating hormone levels




Testosterone, estrogen, thyroid (T_3 , T_4), cortisol

Molecular changes – mRNA, estrogen receptors



Vitellogenin in male fish

Microscopic (histologic) changes

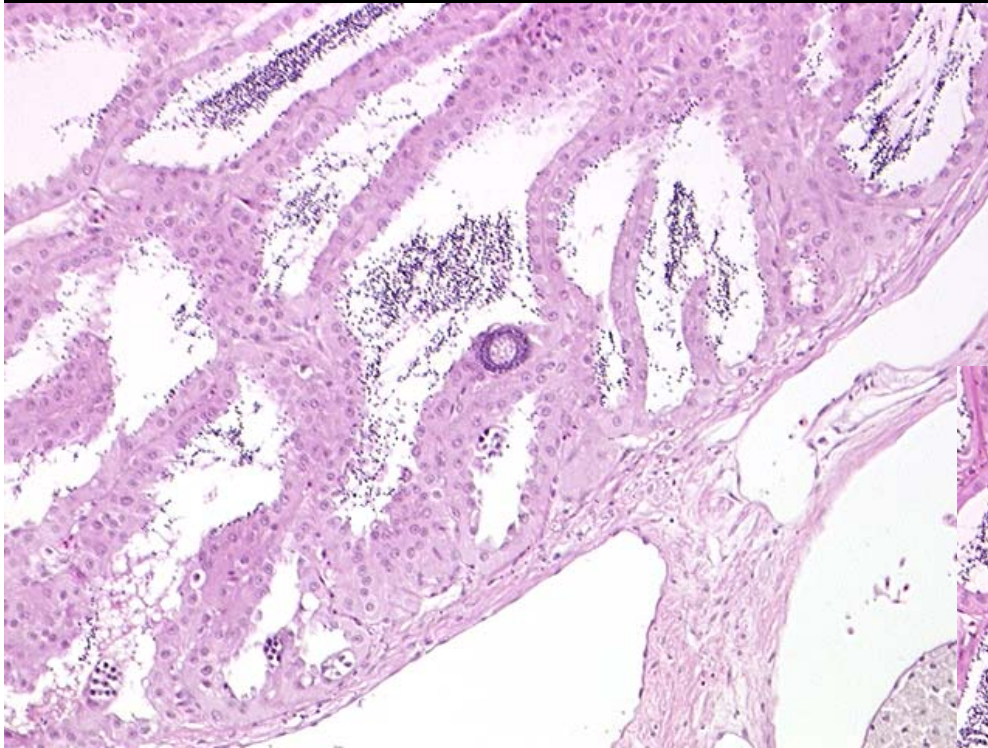
Vitellogenin

-  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
-  Estrogen stimulates the liver to produce vitellogenin which travels to the ovaries via bloodstream and is sequestered by developing oocytes

Vitellogenin in Male Fish

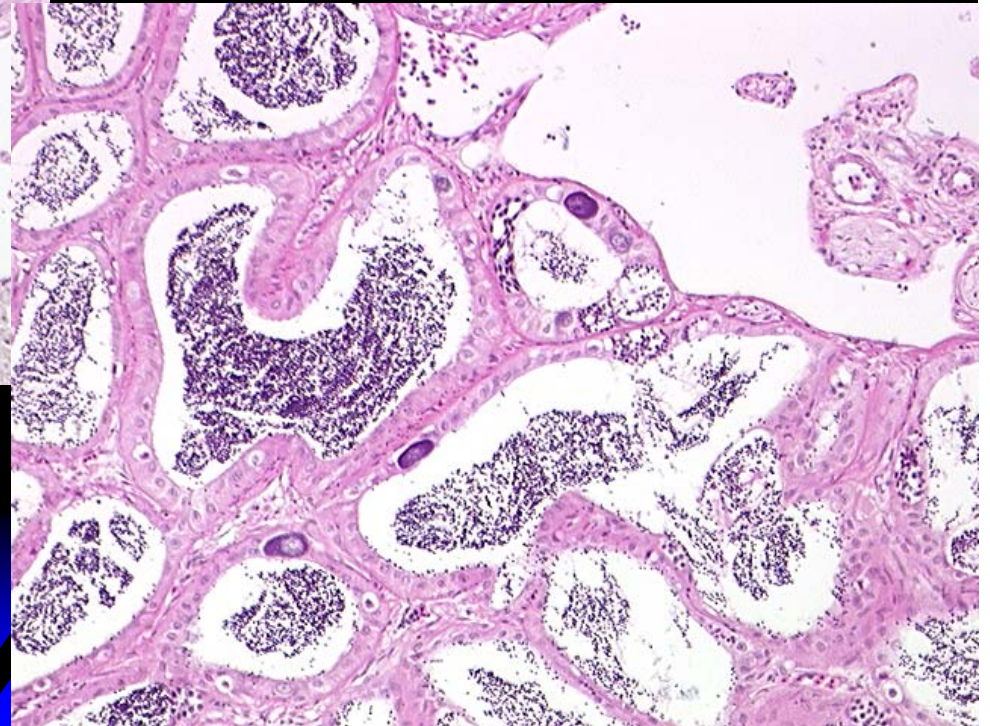
-  Males have the gene to produce vitellogenin - usually not turned on
-  Exposure to natural estrogens (phytoestrogens), synthetic or natural estrogens from human and agricultural sources, and estrogen mimics such as toxaphene, dieldrin, endosulfan, PCBs, alkylphenols and other chemicals may stimulate vitellogenin production by males

Intersex Severity Index



Score 1
Single oocyte

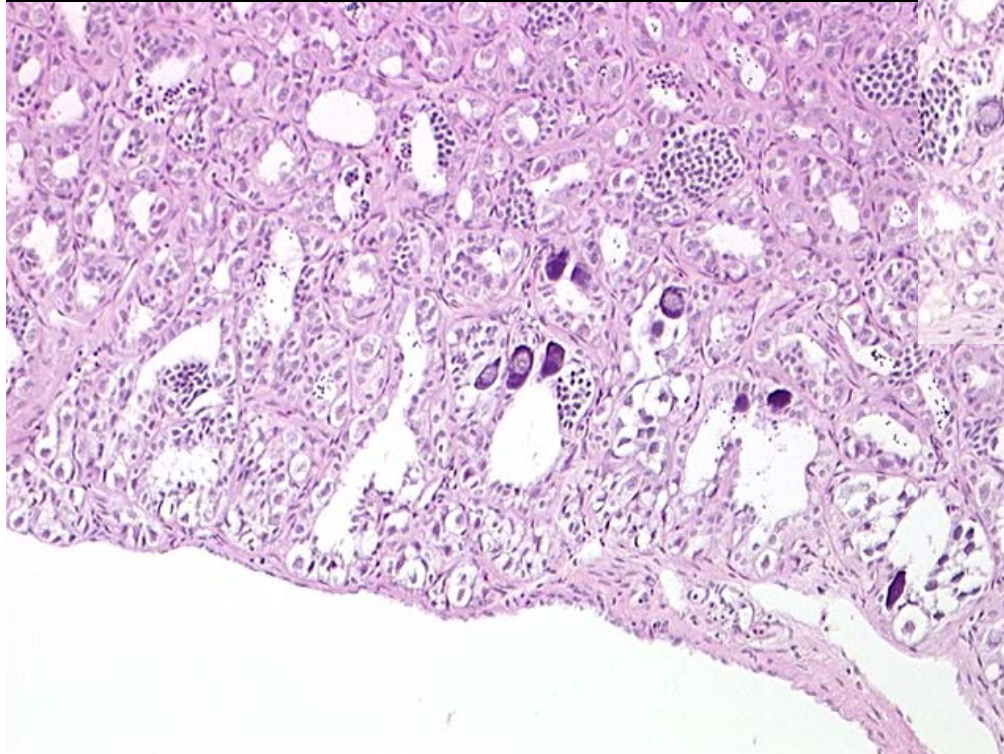
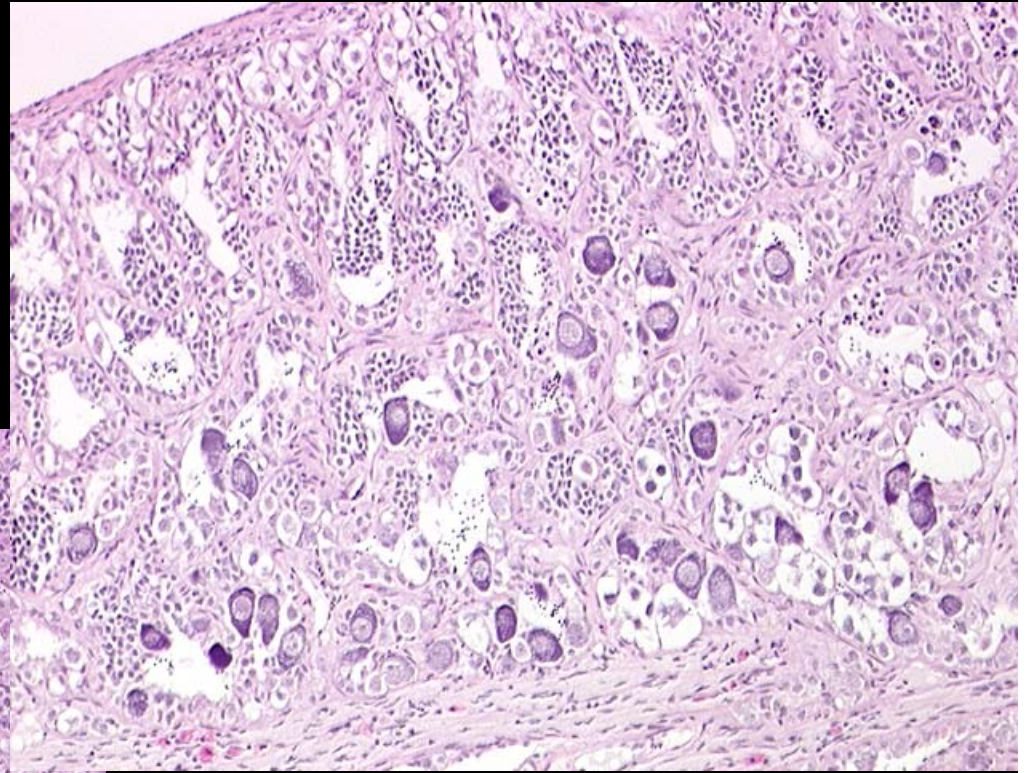
Score 2
More than 1 oocyte
Not associated - multifocal



Intersex Severity Index

Score 3 Cluster

>1 but <5 associated oocytes



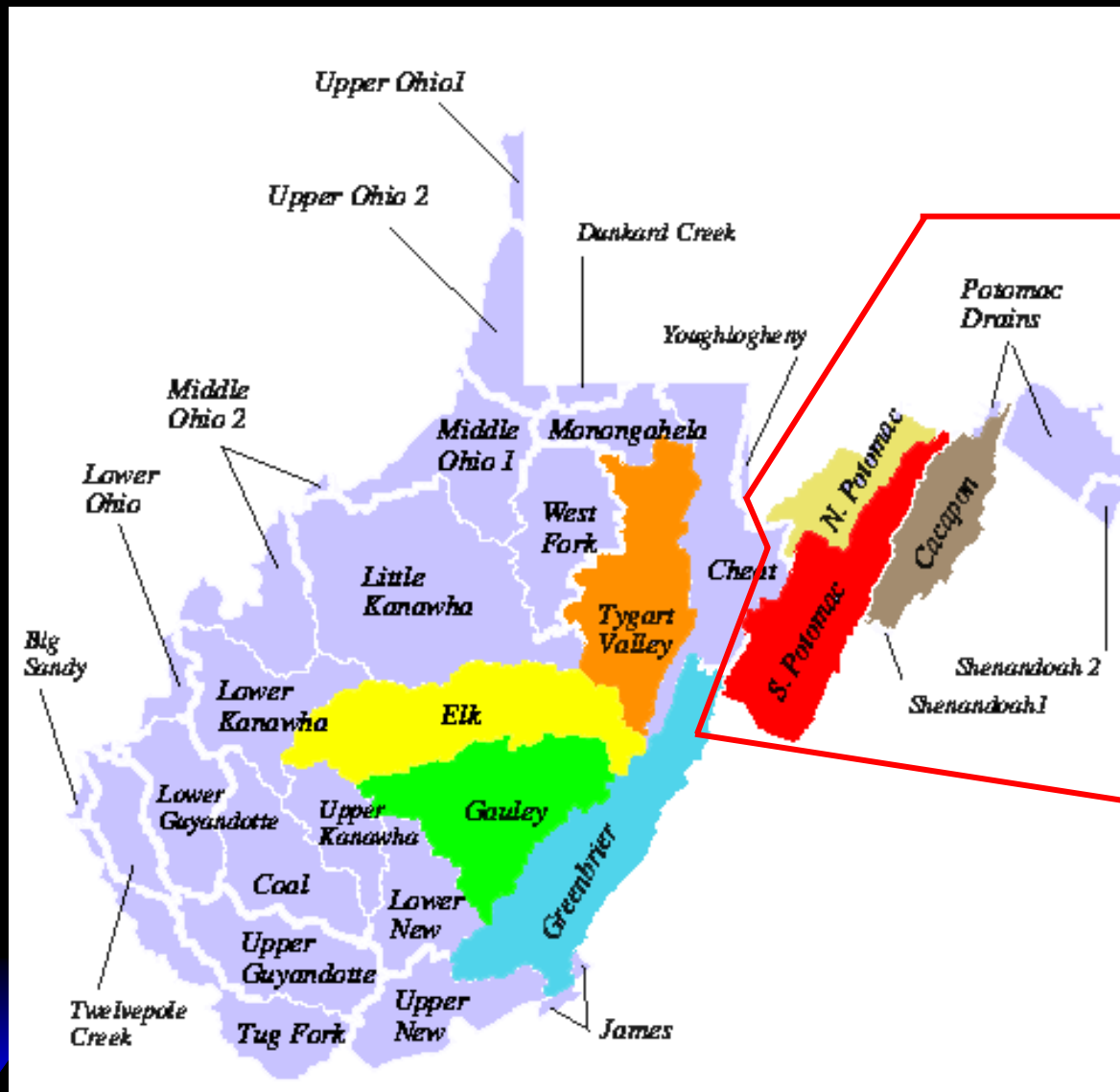
Score 4 Zonal

**multiple clusters or
>5 associated oocytes**

2005 South Branch

- ◆ Resampled smallmouth at selected sites within South Branch
- ◆ Sampled largemouth from a number of areas in WV for comparison
- ◆ Sampled smallmouth at “out of basin” sites
 - WV DNR and the Army Corp of Engineers

West Virginia Watersheds



South Branch – Summary

- ◆ **Overall, in 2003-2005 incidence of intersex in male SMB throughout the South Branch is approximately 58%**
- ◆ **Incidence at out of basin sites is lower – 14-25%**
- ◆ **Incidence does vary from site to site, seasonally**
- ◆ **Male smallmouth from the some of the same sites as those with intersex also have circulating vitellogenin**
- ◆ **Largemouth bass from some areas in the Potomac drainage also have intersex, although at a lower prevalence, 0-30%, depending on site**
- ◆ **Suckers collected at some of the same sites did not have intersex**

2005 Maryland Portion of the Potomac

- ◆ Project with FWS and MD DNR, funded by FWS Off Refuge Contaminant program
- ◆ Targeted wastewater treatment
 - **Smallmouth were collected upstream and downstream of wastewater treatment plants on the Monacacy and Concocheague**
 - **Largemouth collected around the Blue Plains treatment plant for Washington, DC**

Effects Other Than Reproductive



Immunomodulation

- 2002 – fish kills and fish lesions in the South Branch
- 2003-2004 – fish kills in the North Fork, Shenandoah
- 2005 – fish kills in South Fork Shenandoah, and the Susquehanna

2005-2006 Virginia Studies

- ◆ **Fall 2005 – 3 sites in the Shenandoah and 1 “reference”**
 - South Fork, North Fork, Mainstem
 - Cowpasture
- ◆ **Spring, summer and fall 2006**
 - Same sites
 - Routine comprehensive fish health assessment
 - Immune function assays
 - Samples collected for bacteriology and virology (Lamar FWS Unit)

Virginia Sampling

- ◆ Found intersex in the Shenandoah
- ◆ Found similar high parasite loads at some sites
- ◆ Observed differences in the immune response among sites

Effects of Intersex

Individual and Population Levels


- ◆ **Studies with wild roach (*Rutilus rutilus*)**
- ◆ **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

Reproductive Effects on Smallmouth Bass

- ◆ Cooperative Fish and Wildlife Unit at WVU – Holly Henderson and Pat Mazik
 - Attempted to compare sperm quantity and quality at 3 sites in South Branch and an out of basin site

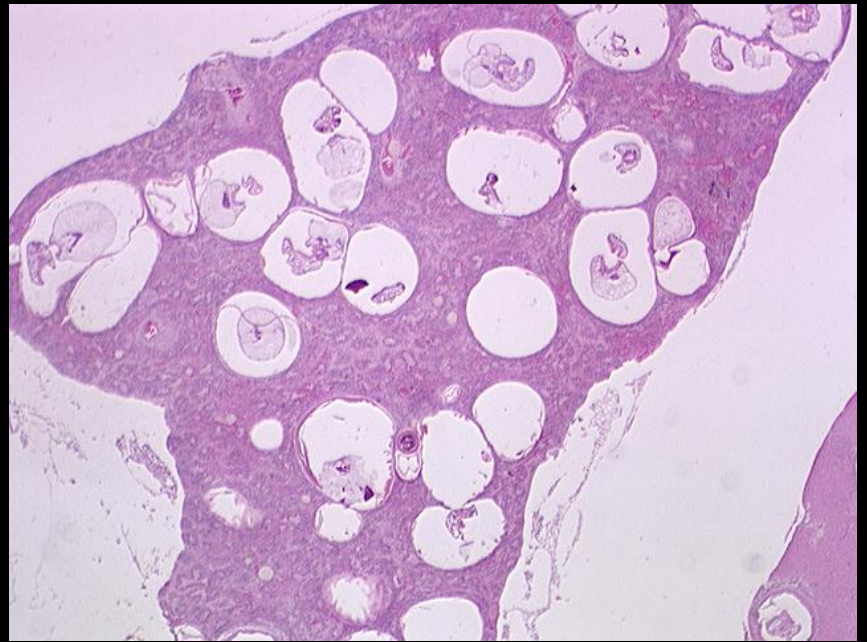
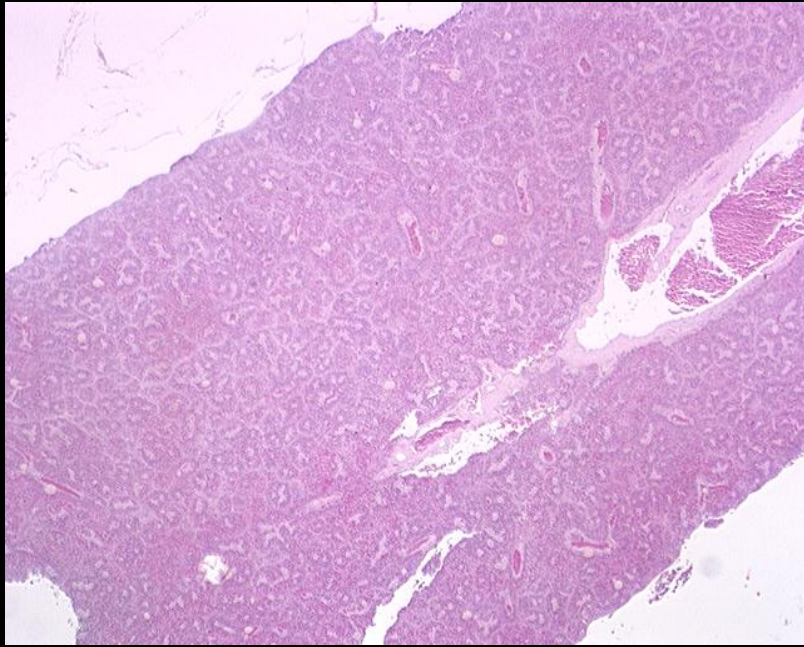
Exposure Issues

 Interactive effects of the complex mixtures
In vitro studies and studies with rats 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).

In Vitro Assays

- ◆ Fish hepatocytes and macrophages
 - Luke Iwanowicz
- ◆ Human cell lines
 - Mike Miller and Rosana Schafer - WVU



Fish Kills 2006

- ◆ South Branch, WV – May 21
 - Worked up 30 moribund/abnormally-behaving fish collected by WV DNR
 - ❖ 25 golden redhorse suckers
 - ❖ 4 fall fish
 - ❖ 1 rock bass
- ◆ Shenandoah – May 30
 - VA DGIF delivered 12 fish to Leetown
 - ❖ 8 golden redhorse suckers
 - ❖ 3 bluegill
 - ❖ 1 rock bass

Culture Findings

- ◆ No viruses isolated
- ◆ Bacteriology:
- ◆ Shenandoah – 2/10 fish with skin lesions were positive for *Flexibacter columnaris*, no internal positives. One skin lesion was positive for *Aeromonas salmonicida*.
- ◆ Potomac – 6/10 fish with skin lesions were positive for *Flexibacter columnaris*, 2 had systemic infections. One skin lesion was positive for *Aeromonas salmonicida*.

Histology

- ◆ Gill – signs of both environmental problems and infectious diseases including protozoan and helminth parasites and bacteria

Liver Pathology

- ◆ Signs of environmental problems and infectious disease
 - High density of macrophage aggregates
 - Signs of oxidative damage
 - Myxosporidian parasite

Findings Suggest:

- ◆ Environmental stressors:
 - Water quality problems – pH (affect O₂ binding to hemoglobin), ammonia, nutrients (lead to proliferation of bacteria and possibly intermediate hosts of parasites)
 - Chemicals that
 - ❖ cause immunosuppression
 - ❖ affect other physiological functions (oxidative damage)

Suggestions for Further Research

- ◆ During Fall collection – collect suckers to compare with findings observed during the fish kills

Further Fish Health Work

- ◆ Analyze the various samples already collected and compile and analyze data
- ◆ Identify parasites, potential intermediate hosts and the environmental factors that influence them
- ◆ Further investigate possible other infectious intracellular agents – viruses, microsporidian, rickettsia – molecular technique