

Smallmouth Bass Intersex and Disease Issues in the Susquehanna River

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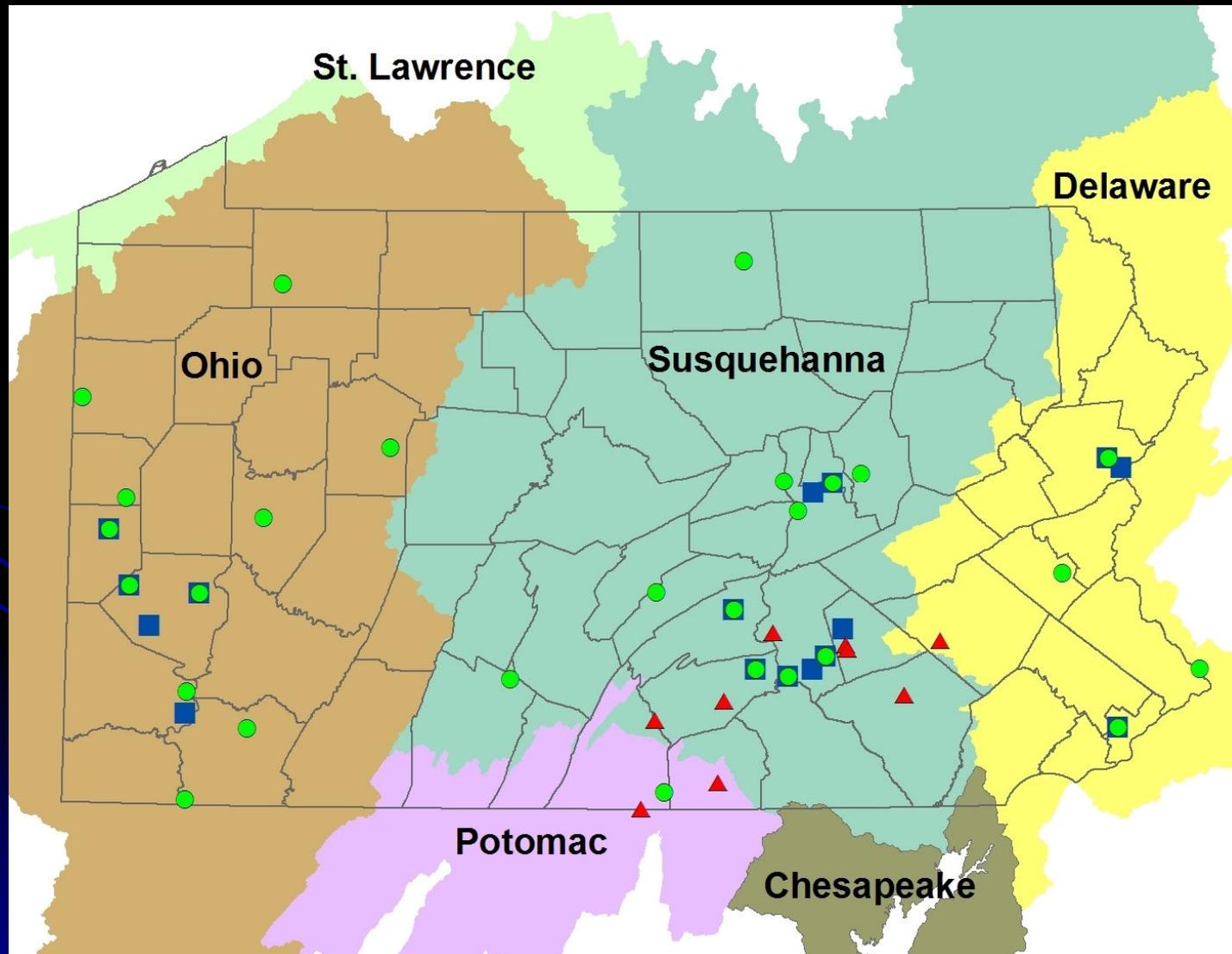
Studies/Projects

-  **2007-2008 Emerging Contaminants Project, some YOY for histopathology**
-  **2009 Adult and YOY health assessments, tissue for contaminant analyses**
-  **2010 Adult and YOY health assessments, water contaminant analyses – passive samplers and grab water samples**

Collaborators/Cooperators/Funding

-  **PA Fish and Boat Commission and PA Department of Environmental Protection**
-  **Fish and Wildlife Service Lamar Fish Health Unit – Wild Fish Survey sampling**
-  **USGS PA Water Center, Chesapeake Bay Priority Ecosystem program and the Toxic Substance Hydrology program**

PA Emerging Contaminants Project



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. in the Ohio drainage

 Included sites upstream and downstream of WWTP effluents

Fish Health Assessments

Morphometric

 Length, weight, condition factor, age, sex

Gross lesion documentation

Histopathology of major organs

 Gills, liver, spleen, anterior kidney, posterior kidney, gonad, lesions/abnormalities

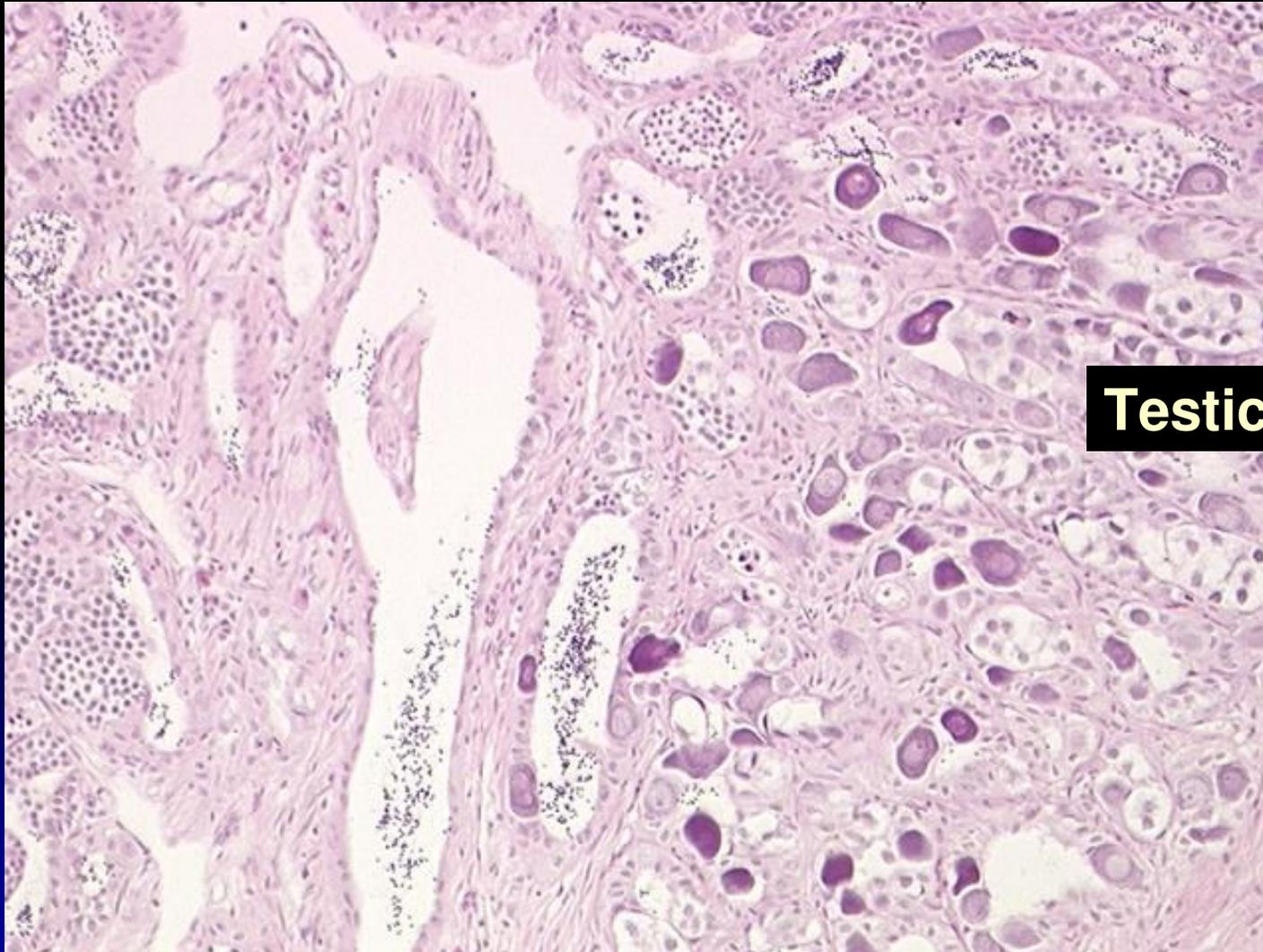
Culture for common bacteria and viruses

 FWS Lamar Fish Health Unit

Plasma analyses

 Vitellogenin (SMB, WHS)

Smallmouth Bass Susquehanna River



Testicular oocytes

Upstream/Downstream of WWTP

Site	No.	Intersex Prevalence	Intersex Severity	Vitell. In males
Susq-U	7 F	93% (13/14)	1.4	21% (3/14)
	14 M			
Susq-D	12 F	89% (8/9)	1.7	11% (1/9)
	9 M			
Swatara -U	4 F	75% (3/4)	0.6	0%
	4 M			
Swatara -M	8 F	67% (4/6)	1.0	50% (3/6)
	6 M			
Swatara-D	6 F	100% (6/6)	2.0	0%
	6 M			

Smallmouth Bass Sites

Site	Sample Size	Intersex Prevalence	Intersex Severity
Schuylkill	8	50%	0.6
Juniata	7	100%	2.6
Ohio	9	11%	0.1
Monogohela	5	20%	0.1
Mono @ Monessen	10	10%	0.1
Allegheny @ Kittering	6	33%	0.1
Allegheny @ Holten	10	10%	0.1

Lamar LMBV Isolations

Adult SMB, 20 fish/site

Negative Sites

2007

Juniata River, Newport
Yellow Breeches Creek
Swatara Creek, downstream
Schuylkill River
Susquehanna R., Shikellamy

2008

Allegheny R, Harmarville
Monongahela, Monesson
Monongahela, Braddock
Ohio R, Sewickley

Positive Sites

2007

Susquehanna R., Danville
Susquehanna, West Branch
Susquehanna R., Sunbury
Swatara Creek, upstream
Swatara Creek, park

2008

Allegheny R, Kittanning

Young-of-Year Smallmouth

 **July and August 2007 – fixed tissue from Ken Stark**

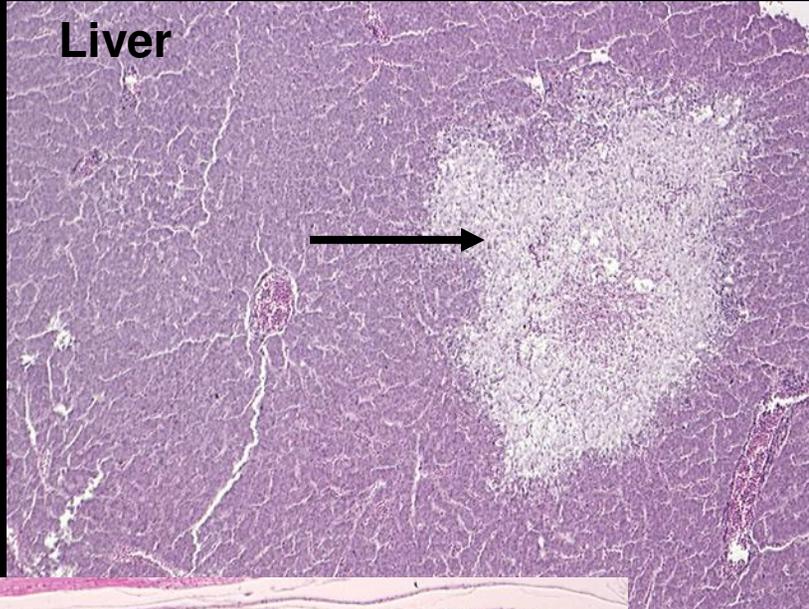
 **Susquehanna – Shady Nook, Danville boat launch, Montgomery access**

 **Juniata River Granville Access**

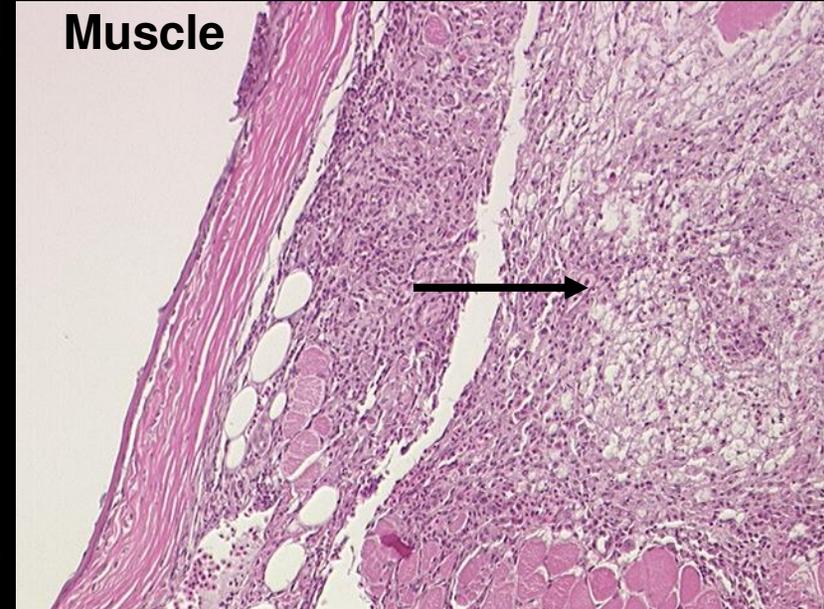


Systemic Diffuse Granulomatous Lesions

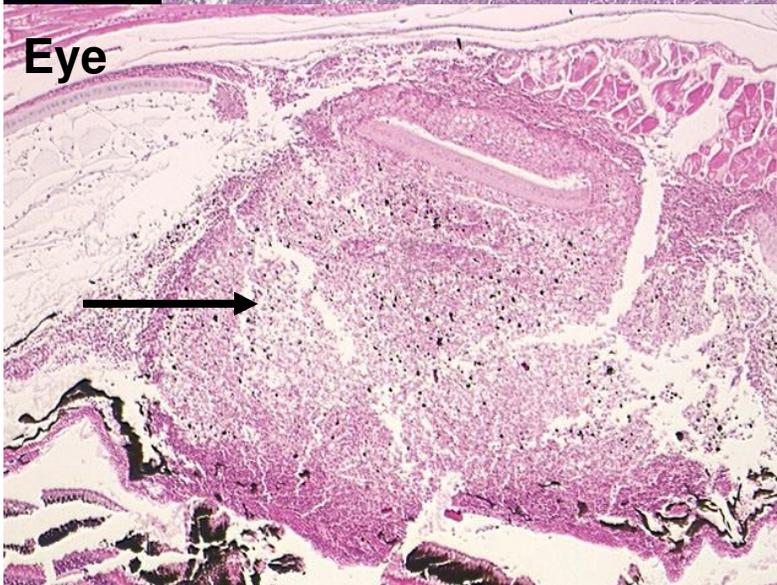
Liver



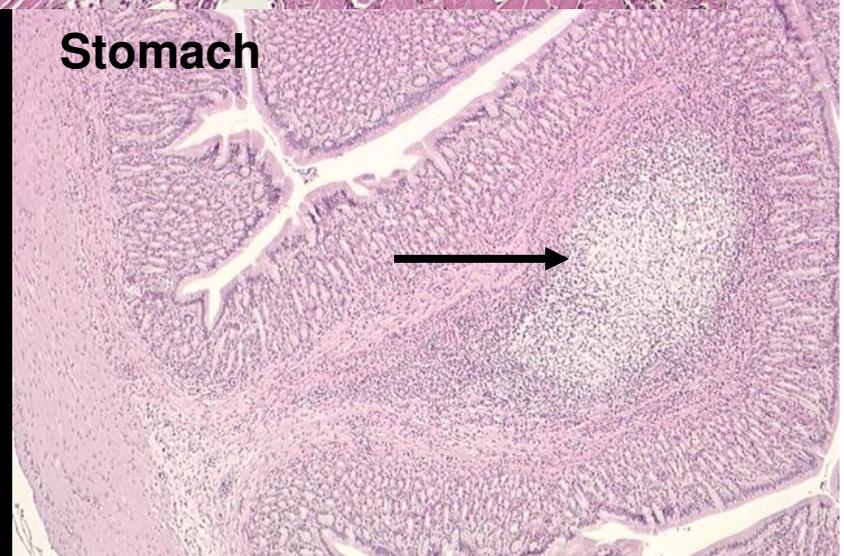
Muscle



Eye

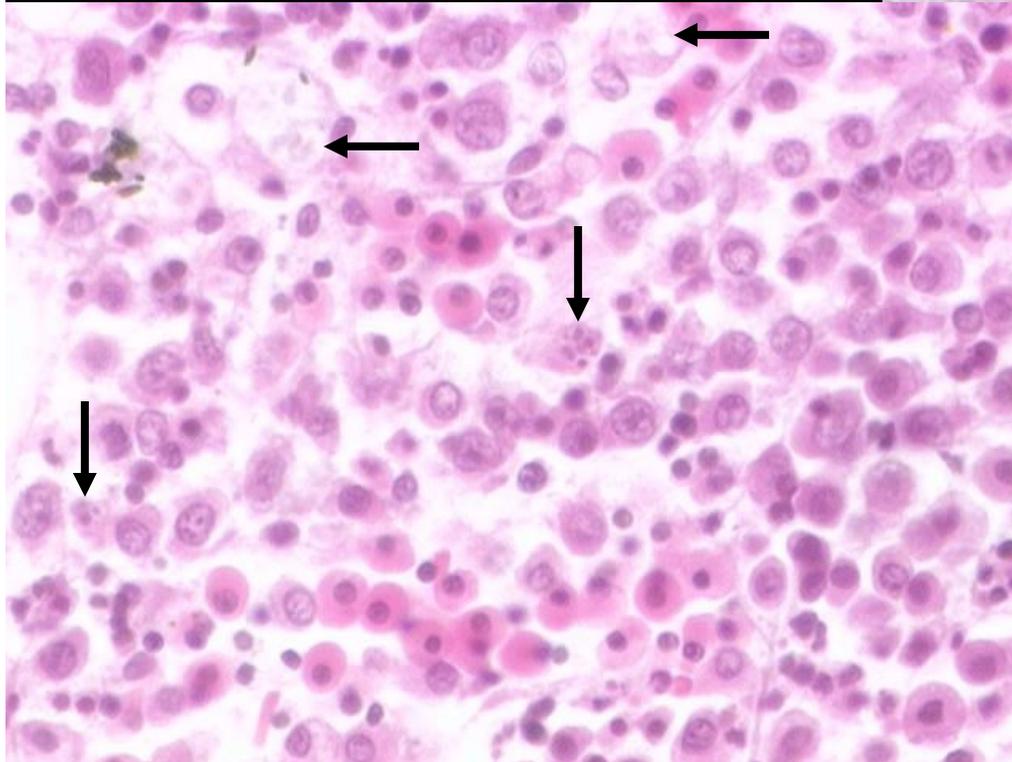
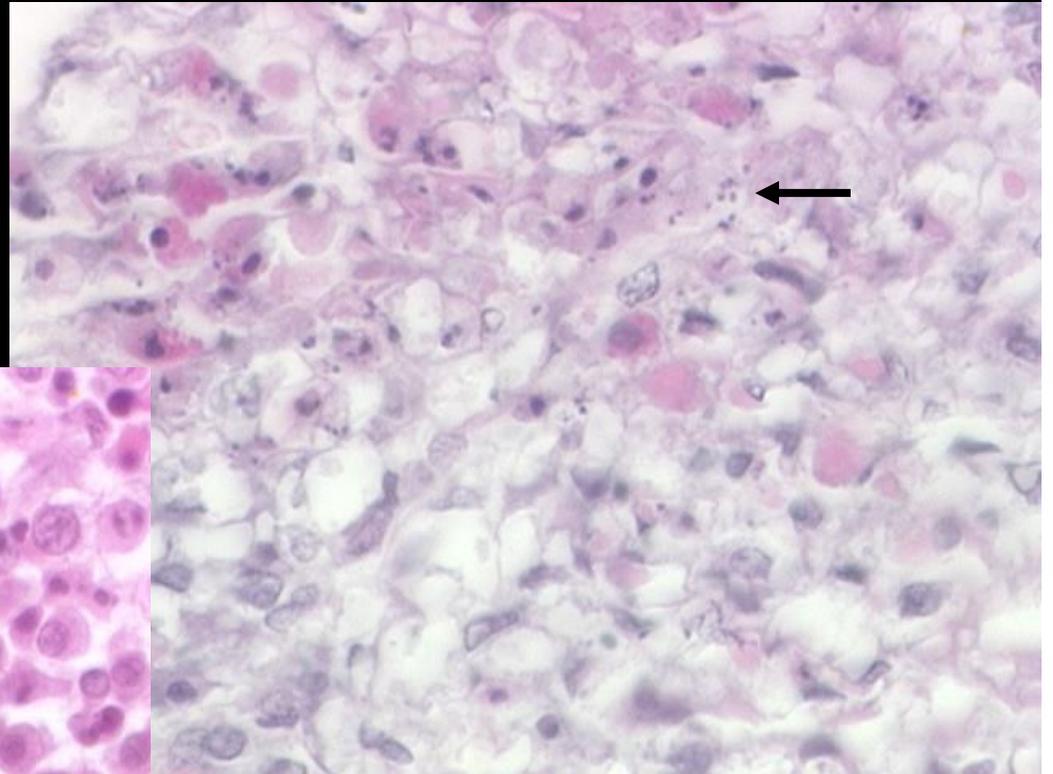


Stomach



Systemic, Diffuse Granulomatous Lesions

Anterior kidney



Liver

Pathogens

-  ***Flavobacterium columnare***
-  ***Aeromonas hydrophila* and other motile Aeromads**
-  **Numerous parasites observed**

Numerous opportunistic pathogens and high parasite loads suggests environmental stressors are leading to immunosuppression

Evidence for Exposure to Contaminants of Emerging Concern

Estrogenic Compounds

Intersex

 Moderate to high prevalence in smallmouth bass at sites where kills have occurred

 Low to moderate prevalence at sites where kills have not occurred

 High prevalence and severity in SMB from the Susquehanna; low prevalence and severity in SMB from the Allegheny

Vitellogenin

 Measurable levels in male smallmouth and white sucker

 Occasionally as high as that seen in females

Contaminants of Emerging Concern

 Include pharmaceuticals, compounds in personal care products, plastics, flame retardants, pesticides/herbicides, natural and synthetic hormones from humans and animals

 Many cause adverse effects, particularly sublethal effects, at very low concentrations

Complexities in Wild Fishes

-  **Endocrine/Immune systems - chemical communication and feedback mechanisms**
-  **Lack of classic dose response curve – hormesis**
-  **Newly recognized effects of legacy compounds**
-  **Multiple contaminant exposure routes - water, sediment, food (including yolk sac)**
-  **Complex mixtures – additive, synergistic, antagonistic effects**

Studies in 2009-2011

-  **More comprehensive attempts to culture pathogens and identify parasites**
-  **Collection of tissues for contaminant analyses**
-  **Deployment of passive samplers to attempt to identify candidate compounds of concern and use of in vitro cell-based assays to measure total estrogenicity**

YOY Cultured 2010

July - August

<i>Site</i>	<i>Total Collected</i>	<i>Total with Lesions</i>
Juniata	8	5
Danville	18	15
Laceyville	16	1
Liverpool	7	4
Clemson Island	8	6
Total	57	31

Gross Lesions of YOY SMB Susquehanna River



Media for Bacterial Isolations

General Media

Tryptic Soy

For Francisella or Rickettsia-like and others:

Blood agar base + L-cysteine + glucose

Thayer-Martin

Selective cystine heart agar + bovine hemoglobin

Non-selective cystine heart agar + bovine hemoglobin

Selective cystine heart agar + rabbit blood

Non-selective cystine heart agar + rabbit blood

Selective cystine heart agar + sheep blood

Non-selective cystine heart agar + sheep blood

For Flavobacterium columnare

Selective “#2”

Selective cytophaga agar

Culture Methods

-  **External lesions or mucus from non-lesioned fish**
-  **Internal organs (spleen, kidney, swimbladder) were homogenized**
-  **Homogenates were plated onto bacterial media and tissue culture media for viruses**

YOY Cultured 2010

<i>Site</i>	<i>Lesions Aero</i>	<i>Lesions Flavo</i>	<i>Internal Aero</i>	<i>Internal Flavo</i>
Juniata	5/5	5/5	1/8	1/8
Danville	12/15	15/15	8/18	2/18
Laceyville	1/1	0/1	4/16	0/16
<i>mucus</i>	3/9	0/9		
Liverpool	3/4	4/4	1/7	0/7
Clemson Island	6/6	4/6	1/8	0/8
Total	27/31	28/31	15/57	3/57

Largemouth Bass Virus

-  In adults, LMBV isolated at sites where fish kills have occurred and also where fish appear healthy
-  In YOY LMBV was isolated at all sites but not from all fish
-  Some indication that both environmental stressors (handling, water quality) influence the response of LMB to the virus
-  Suggested that virus-infected LMB are more susceptible to opportunistic bacterial infections
-  Unfortunately there are no specific signs of infection
-  No information on the effects of this virus on SMB

Motile Aeromonads Cultured



A. veronii-sobria biovar



A. hydrophila

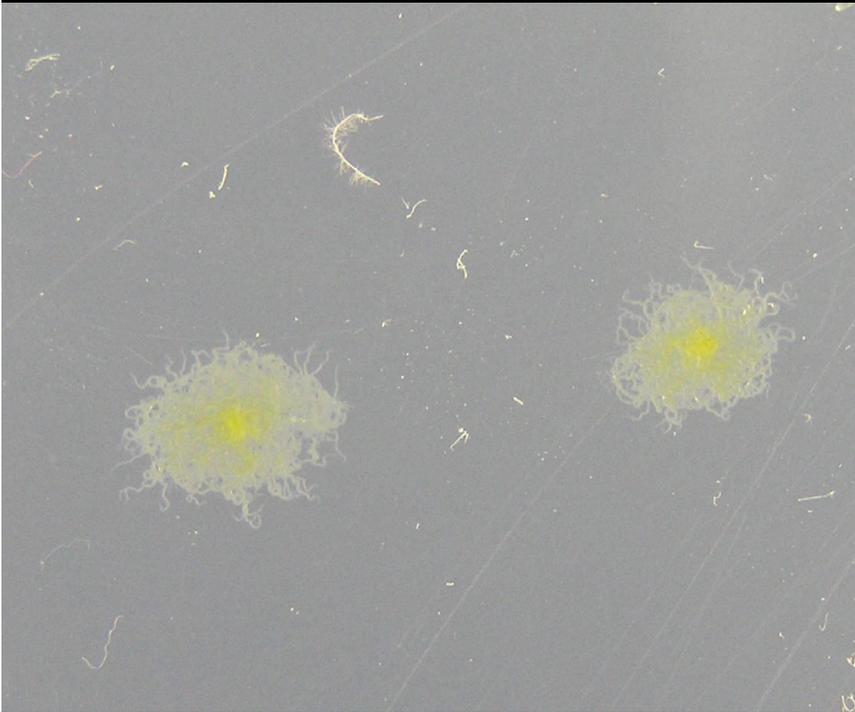


A. popoffii

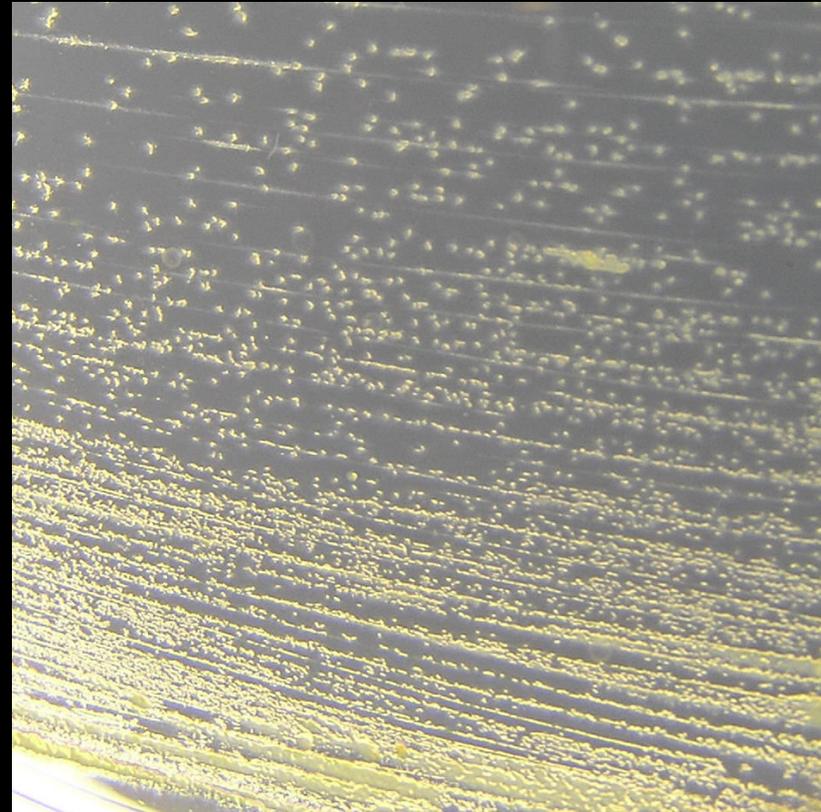


A. allosaccarophila

Flavobacterium columnare Growth Pattern

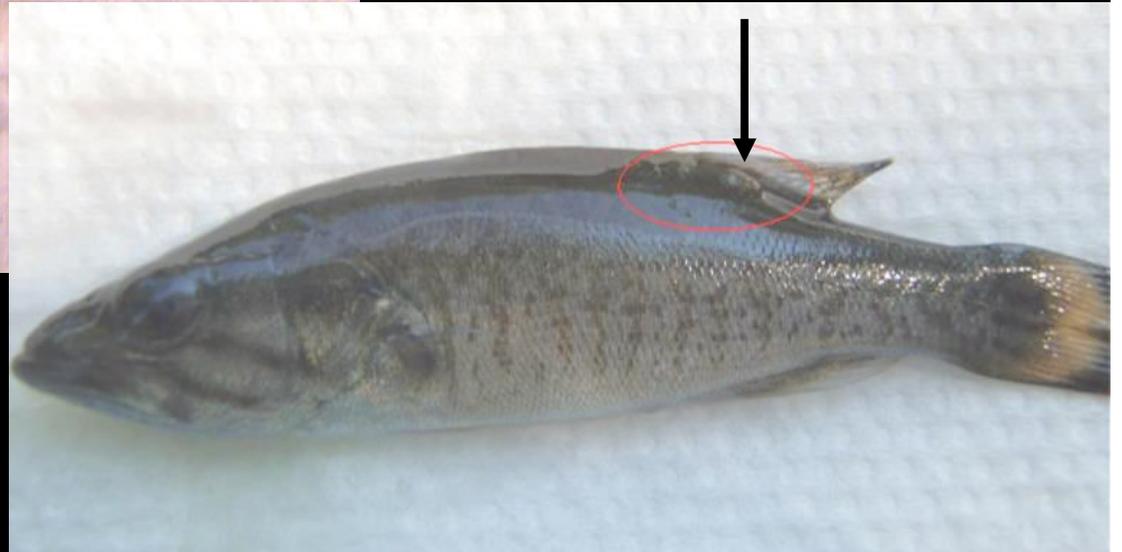


Typical *F. columnare* growth

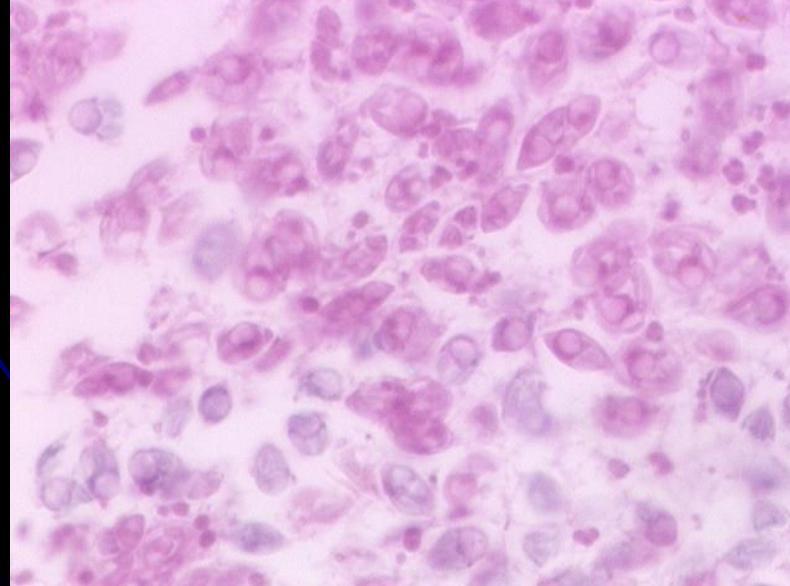
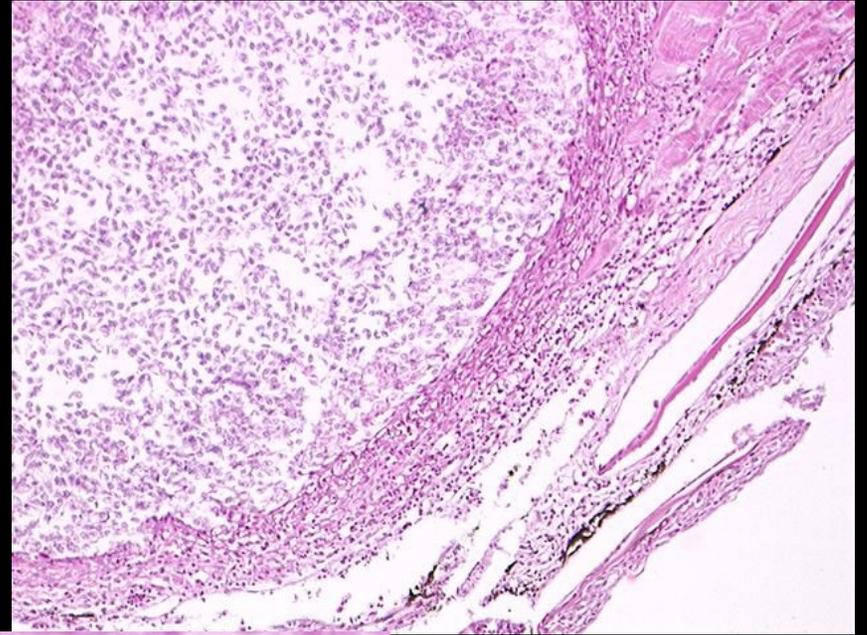


Growth of *F. columnare* from YOY SMB from
Susquehanna River
Heavier growth, smaller, less spreading
colonies

Gross Lesions - Myxozoan

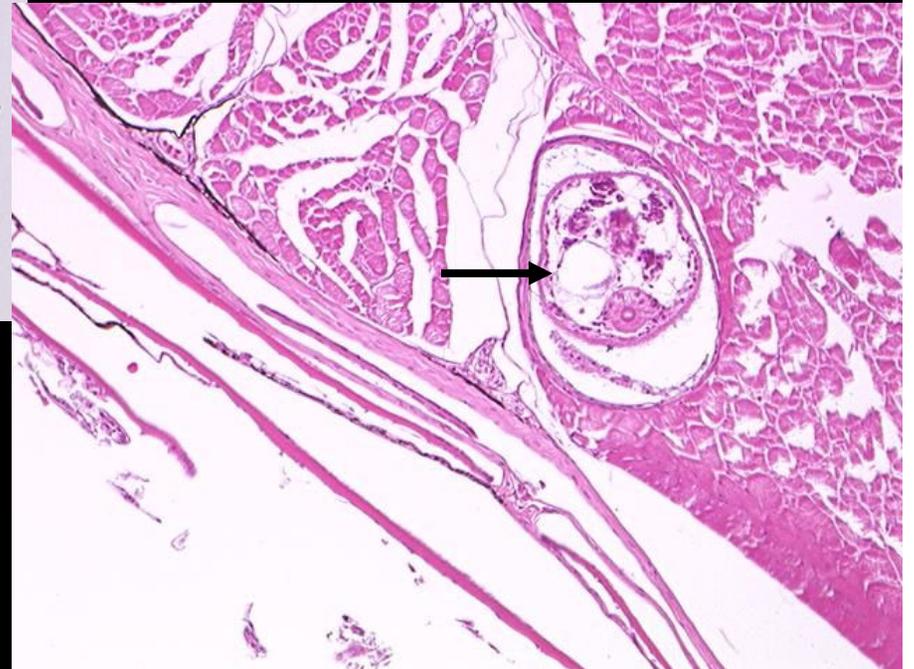


YOY SMB Parasites - Myxozoans

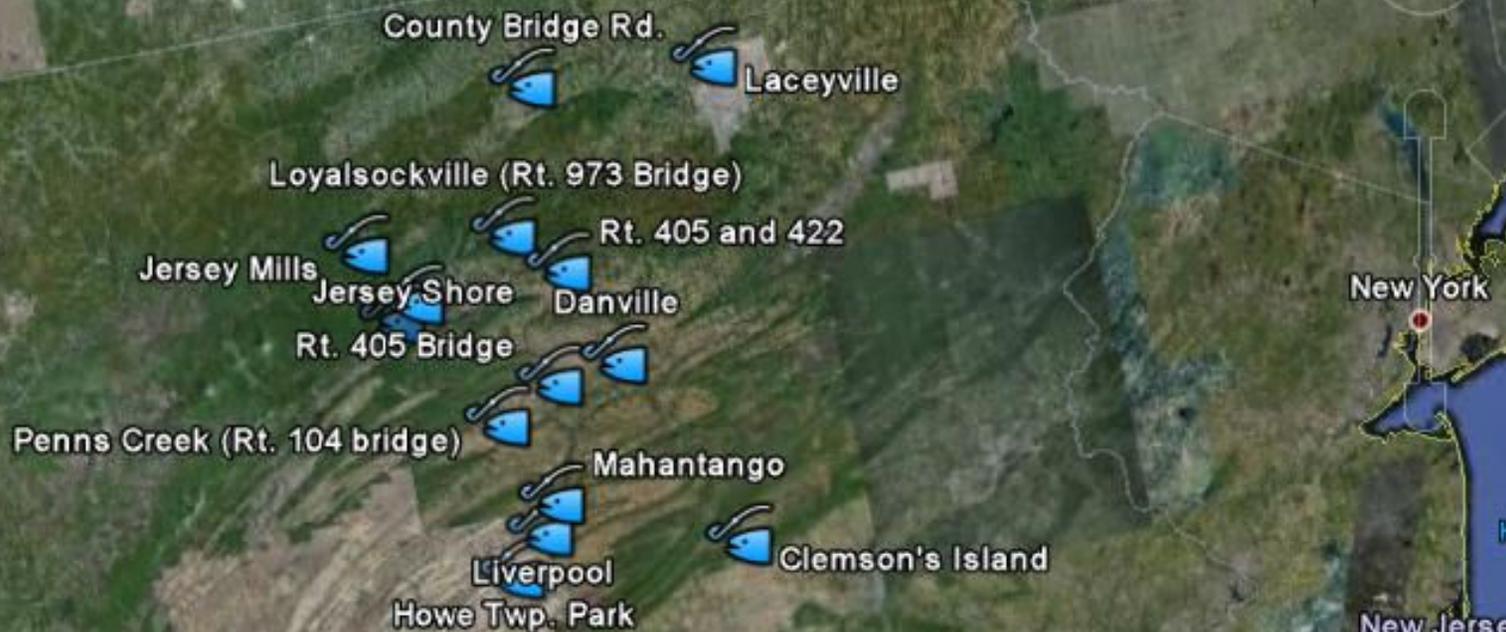


New species

YOY SMB Parasites - Trematodes



Sites at which YOY SMB were collected for histopathology and parasitology



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Image USDA Farm Service Agency
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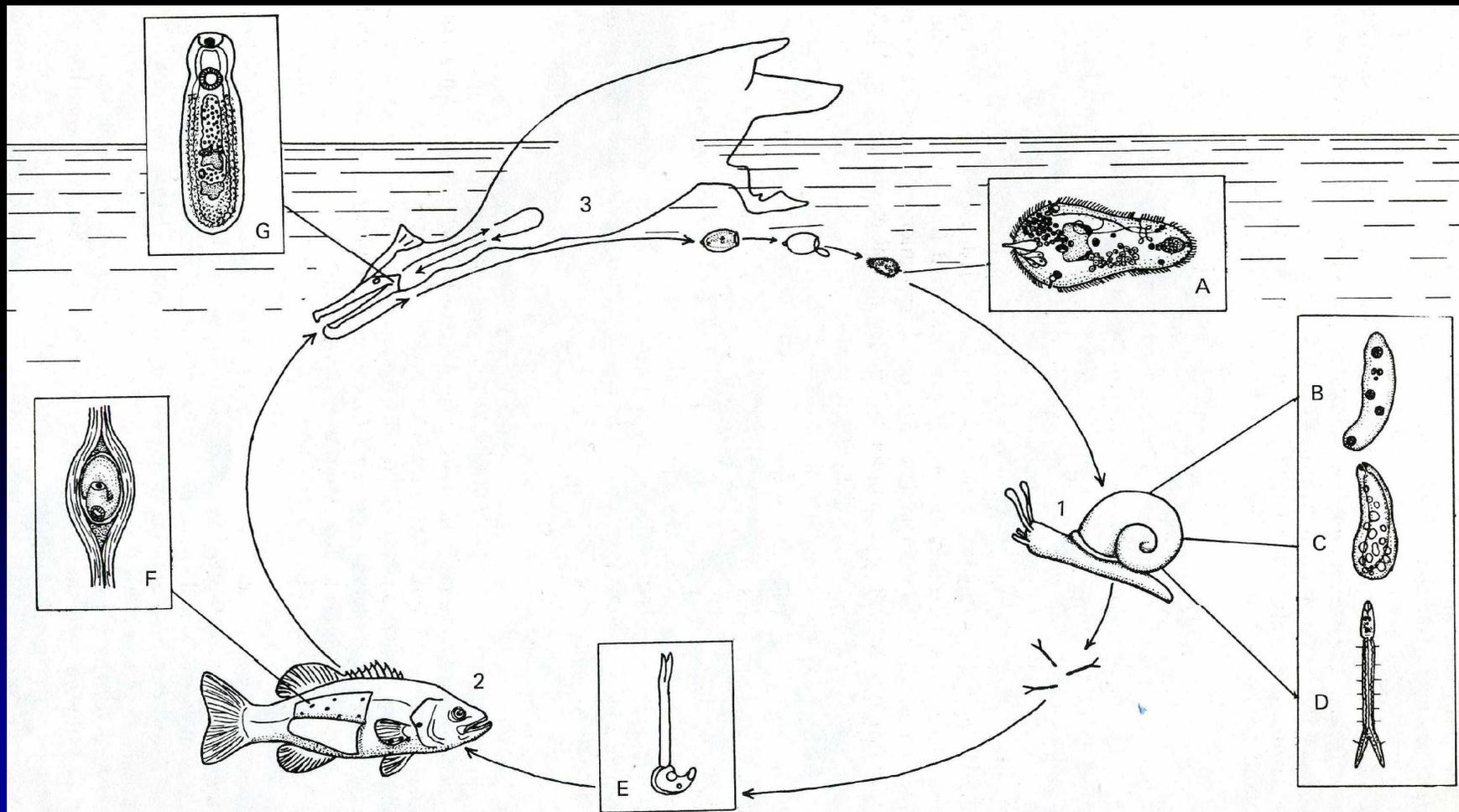
Image PA Department of Conservation and Natural Resources-PAMAP/USGS

Google

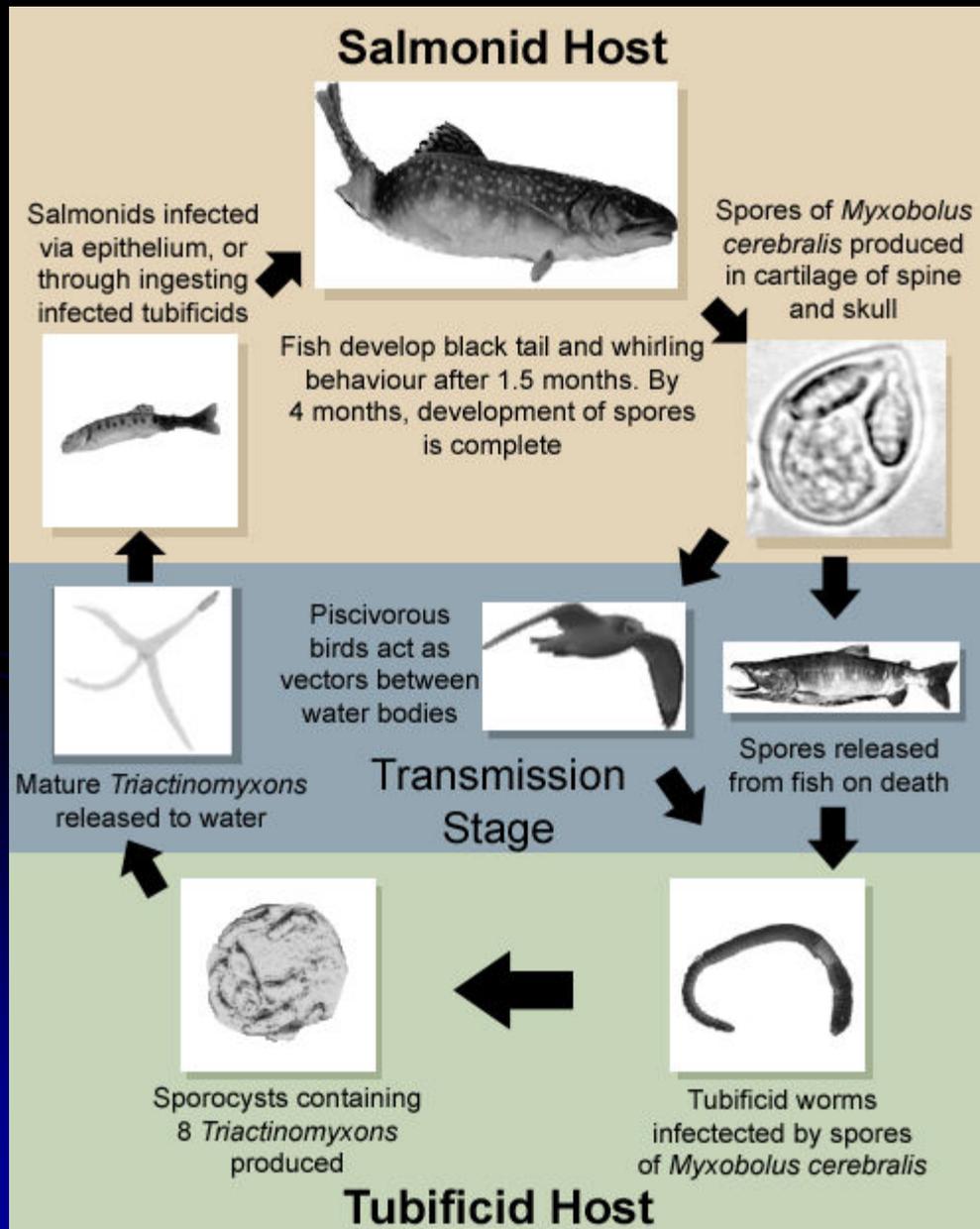
Findings to Date

-  **Multiple bacterial and viral pathogens**
-  **Multiple parasites**
-  **Environmental stressors, chemical exposures and immunosuppression**

Trematode Life Cycle

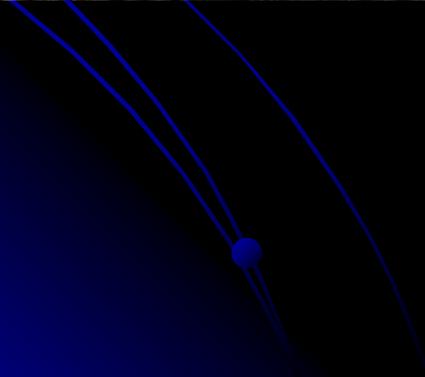


Typical Myxozoan Life Cycle



Bryozoans
Polychaetes
Oligocheates

Intermediate Hosts?



Atrazine and Trematode Infections

- 18 wetlands and 240 possible predictors of trematode infections
- Atrazine was the best predictor
- Atrazine augmented the richness and abundance of gastropods
 - Atrazine and other herbicides decrease phytoplankton which tend to increase periphytic algae
- Increased the abundance of the cercariae
- Increased susceptibility to cercariae
 - Low doses of atrazine have been shown to be immunosuppressive in frogs, fish and salamanders
 - Exposure increased susceptibility to *A. hydrophila* infections in fish and viral infections in salamanders

Water Concentrations Passive Samplers



**Semi permeable Membrane Devices (SPMDs)-
accumulate hydrophobic
compounds**

**Polar Organic Compound
Integrative Samplers
(POCIS)—accumulate
hydrophilic compounds**

Dave Alvarez, Columbia Lab

Ed Furlong, Steve Zaugg and others, Denver Water Quality Lab

Issue of Complex Mixtures

- 🐟 Screening either grab water extracts or the extracts from the passive samplers using in vitro 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

Passive Sampler Deployments

-  **Two in the Susquehanna, one in the Allegheny, one in the Delaware prespawn and postspawn**
-  **Six in various tributaries postspawn**
-  **Deployed for approximately 4-5 weeks**
-  **Grab water samples were taken at deployment and at retrieval**

Passive Samplers

-  Used Chesapeake Bay Priority Ecosystems funding for purchase of samplers
-  Chesapeake EDC supplemental funding for extraction, specific hormone analyses and EEQ for the mainstem samplers
-  F&B will fund chemical analyses for tributary samplers

No data yet

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	

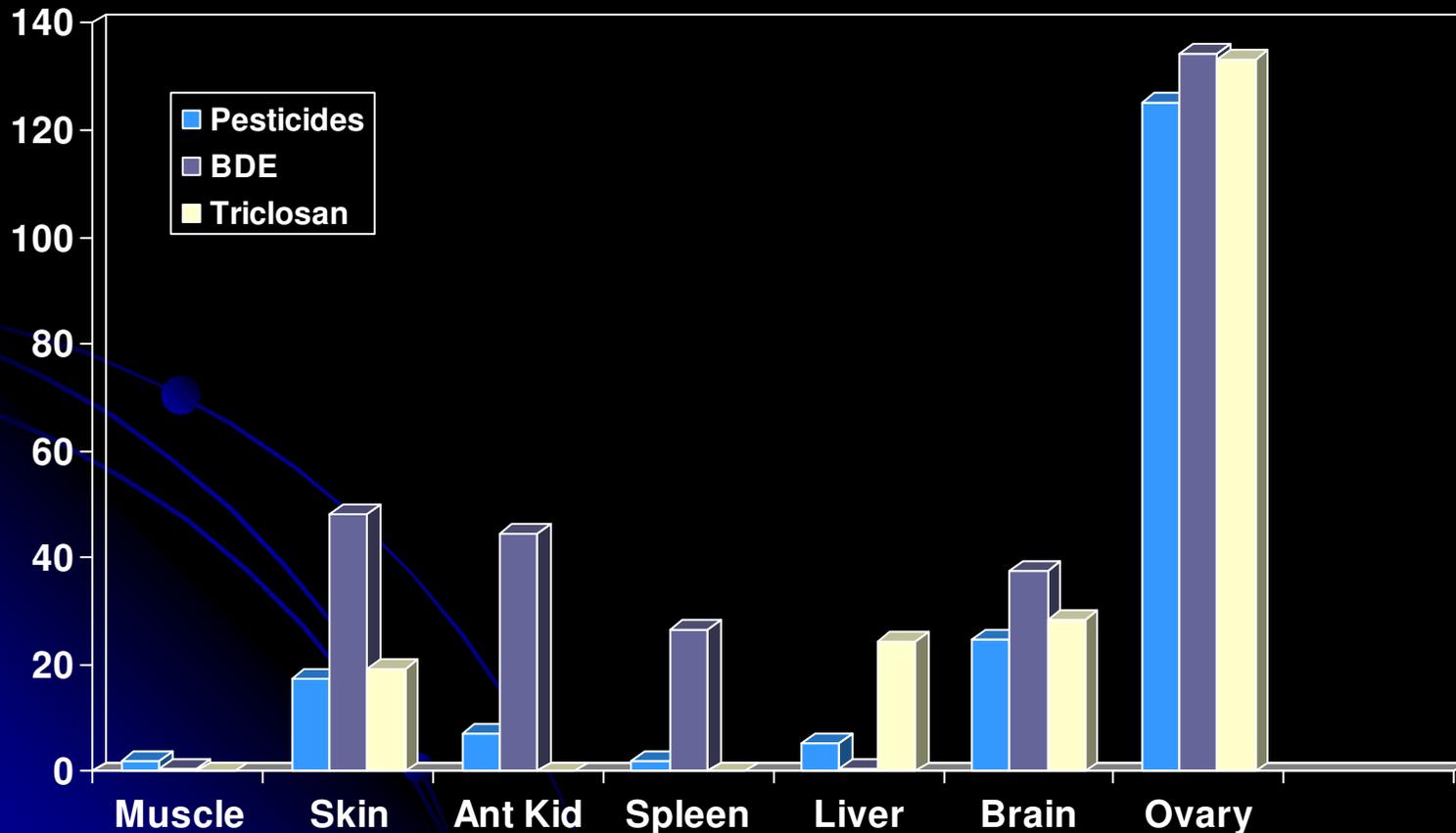
Dana Kolpin - Toxic Substance Hydrology

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



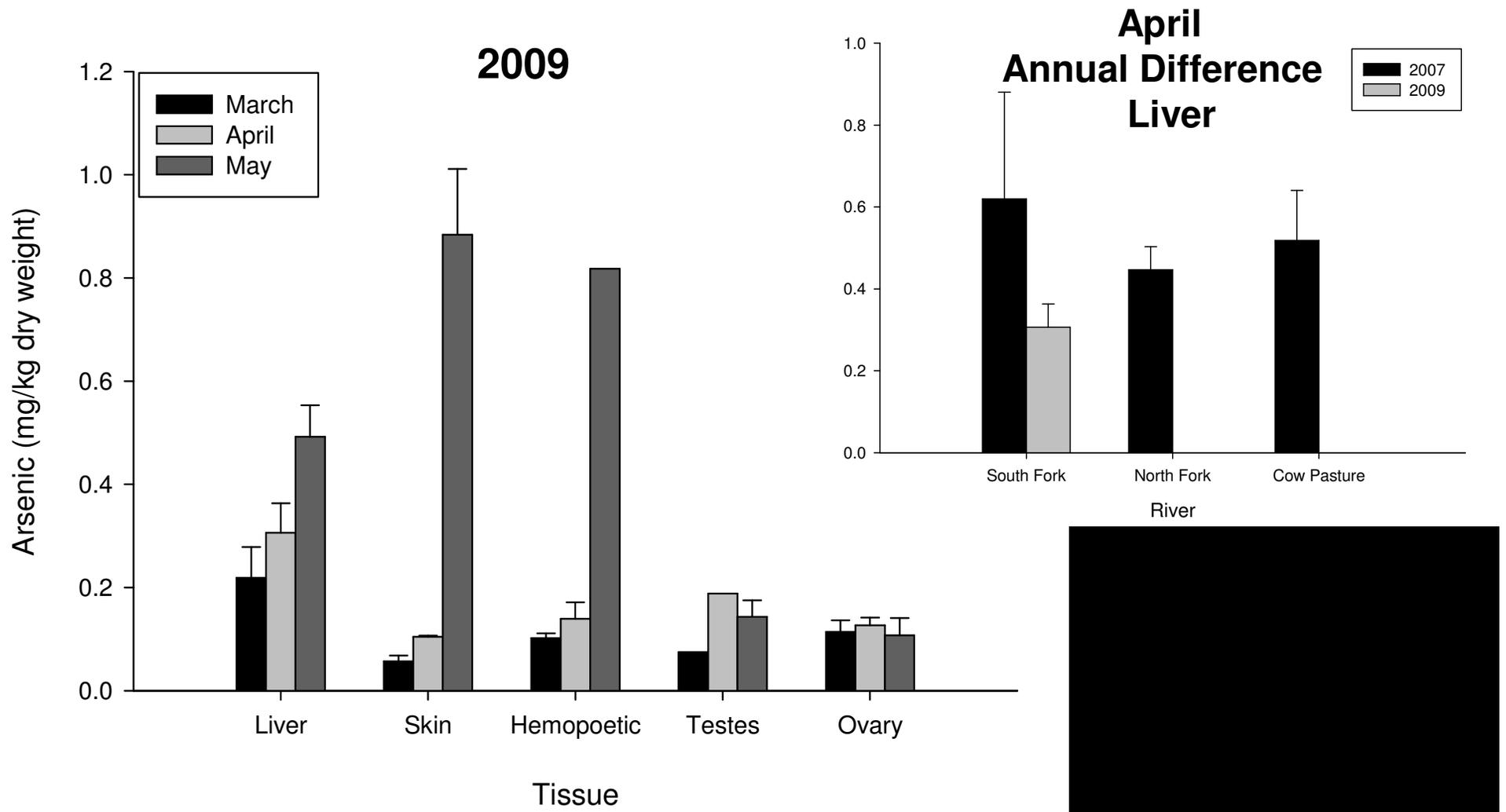
Tissue Contaminants

-  **Relevance of whole body/fillet results in terms of fish health**
-  **Most sensitive stage for induction of intersex and probably development of the immune system is first few weeks after hatch – absorbing yolk and exposure to potentially higher water concentrations of many chemicals**

Ongoing/Future Studies

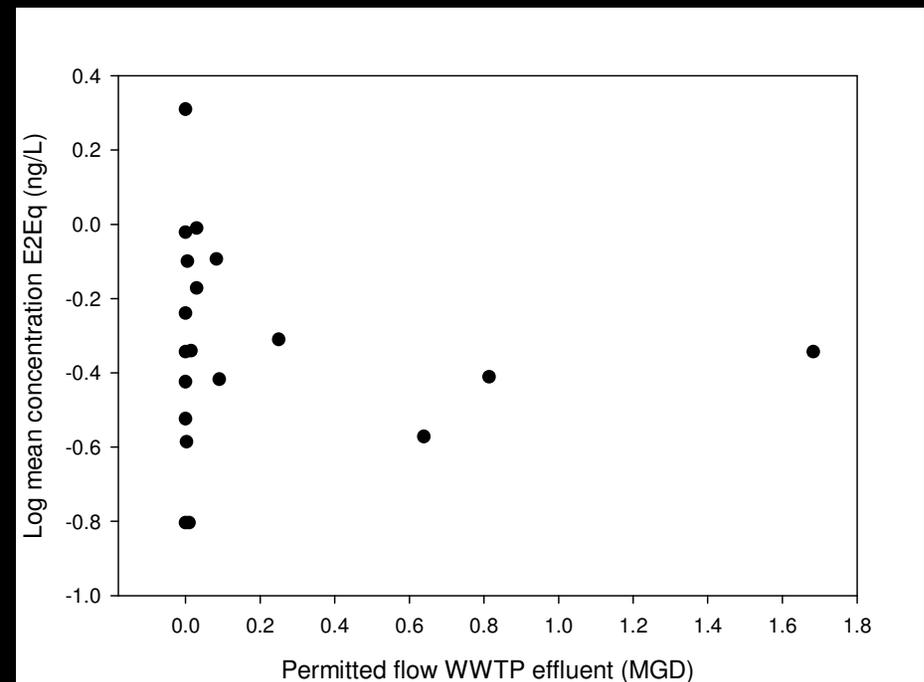
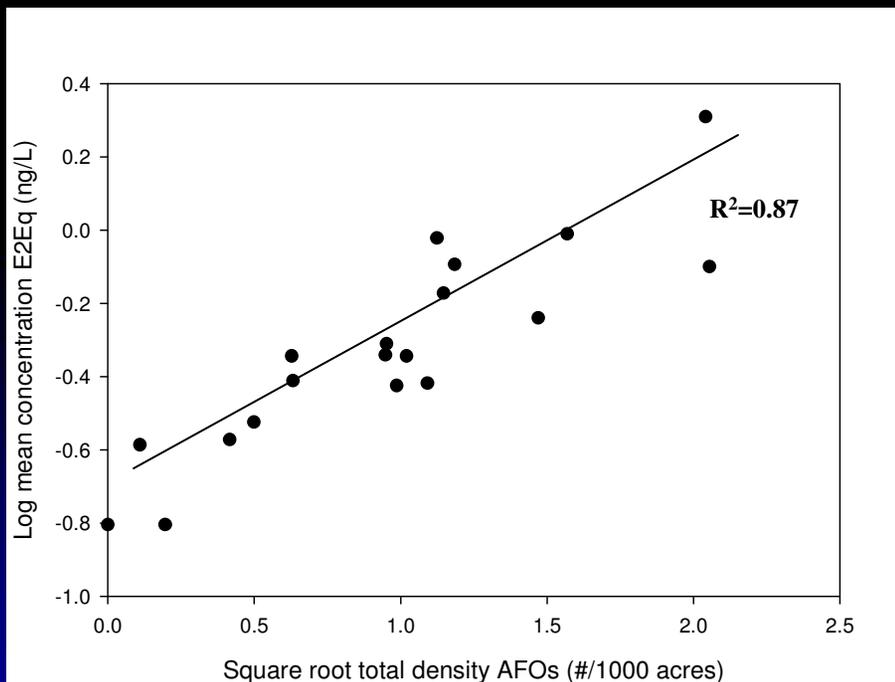
-  **Chemical analyses of passive sampler extracts and identification of priority contaminants**
-  **Determination of intermediate hosts of parasites and environmental factors that may lead to their proliferation**
-  **Determination of virulence factors in the bacterial pathogens and environmental factors that may influence them**
-  **Assessment of disease resistance of the YOY from fish kill sites and reference sites and association with chemical exposure both aqueous and through yolk/food**
-  **Evaluate the role of water quality – particularly nutrients and water temperature**
-  **Assessing temporal changes in contaminant concentrations (water and tissue) and possible associations with landuse**

Arsenic Tissue Contaminants Smallmouth Bass



Total Estrogenic Equivalents of Water Extracts

Smaller Tribs of Shenandoah Drainage



Serena Ciparis, Reese Voshell – VT Tech

Agricultural Pesticides Fall 2005 (Spring 2006)

Chemical Estimated ng/L	Con Up	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 certain amphibians

Also shown to affect disease resistance

Spring Study 2006-2007

Sites	Landuse Characteristics
Gauley	Human population density
South Branch Petersburg	# WWTP
South Branch Moorefield	WWTP flow
South Branch Springfield	% Agriculture
Shenandoah NF	# AFO – total and poultry
Shenandoah MS	# of animals
Shenandoah SF	
Conococheague	

Regression Analysis with intersex prevalence and intersex severity

WWTP flow ($R^2=0.62$, $p=0.02$)

% agriculture, ($R^2=0.50$, $p=0.05$)

poultry AFO ($R^2=0.50$, $p=0.05$)

Correlation with intersex severity