

Fish Health Concerns in Agricultural Settings

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Observed Fish Health Issues Chesapeake Bay Watershed

-  Major fish kills
-  Chronic low level mortality
-  Skin lesions
-  Reproductive endocrine disruption

Adult Fishes in the Potomac

Major fish kills of centrarchids, suckers and other species

2003-recent South Branch Potomac

2004-2007 – Shenandoah

2009 Monocacy

Multiple bacterial pathogens, but no consistent findings

Aeromonas hydrophila and other motile Aeromonads

Aeromonas salmonicida

Flavobacterium columnare

Multiple, often heavy parasite infestations

Leeches, trematodes, myxozoans, cestodes

Opportunistic fungal infections

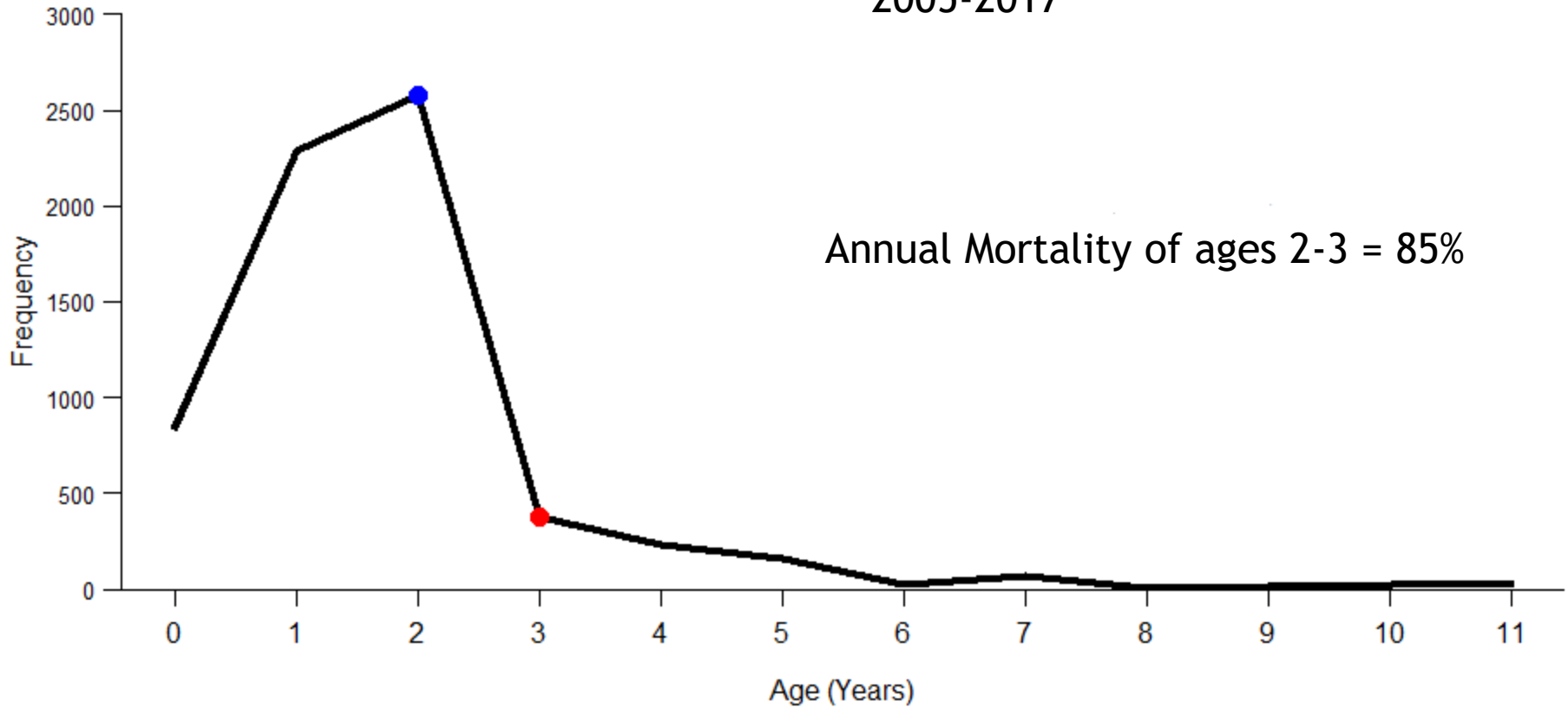
Largemouth Bass Virus



Impaired Ecosystem, Immunosuppression, Perfect storm

WV DNR Population Assessments

Catch-Curve for South Branch Smallmouth Bass
2005-2017









Adult Bass in Susquehanna

- 🐟 No major kills of adult bass
- 🐟 High prevalence of various skin lesions



Young of Year Smallmouth Mortality Susquehanna

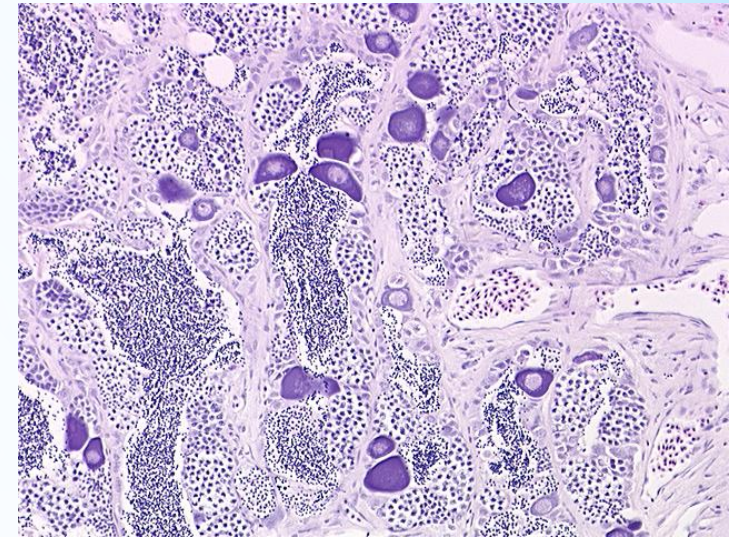
-  *Aeromonas hydrophila* and other motile Aeromonads
-  *Flavobacterium columnare*
-  Largemouth Bass Virus
-  Trematodes
-  Cestodes
-  Myxozoan parasites



Impaired Ecosystem - Immunosuppression

Indicators of Exposure to Estrogenic Chemicals

- 🐟 Intersex - testicular oocytes
 - 🐟 Immature oocytes in the testes of male fish
 - 🐟 Most likely induced early in life (fertilized egg to first few weeks)
 - 🐟 Severity may increase with increased exposure throughout life



- 🐟 Vitellogenin
 - 🐟 Yolk precursor
 - 🐟 Exposure to estrogenic compounds turns on the gene in males
 - 🐟 Can measure in plasma
 - 🐟 Indicative of recent exposure - weeks to months
- 🐟 Can also measure the gene transcripts for vitellogenin in the liver - indicate very recent exposure hours to days

Spawning Study Potomac Intersex and Land-use

Conducted in the spring, 5 sites in the Potomac, 1 out of basin

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

Intersex and Chemical Correlations

Discrete water samples collected in spring at Potomac sites
No estrogens were present at levels above the detection limit

Chemical Contaminants	Intersex Prevalence		Intersex Severity	
	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>
Atrazine	0.93	0.003	0.88	0.009
Deethylatrazine	0.78	0.039	0.68	0.090
Acetochlor	0.65	0.116	0.79	0.036
Metolachlor	0.87	0.011	0.81	0.028

Correlations with Landuse and Chemicals PA Drainages

Study done in late summer
Discrete water samples at time of fish sampling
11 sites in the Susquehanna, 5 out of basin

Chemical Contaminants or Landuse	Intersex Prevalence		Intersex Severity	
	<i>rho</i>	<i>p</i>	<i>rho</i>	<i>p</i>
Estrone (water)	0.6530	0.0238	0.7609	0.0055
Agricultural landuse	0.6843	0.0170	0.7044	0.0129
WWTP/sewage facilities	-0.5298	0.0794	-0.8441	0.0936
	Prevalence of males with vitellogenin			
Estrone (water)	0.7914	0.0033		

Key Exposure Periods

Nest/eggs - sediment/water



Water, maternal, sediment

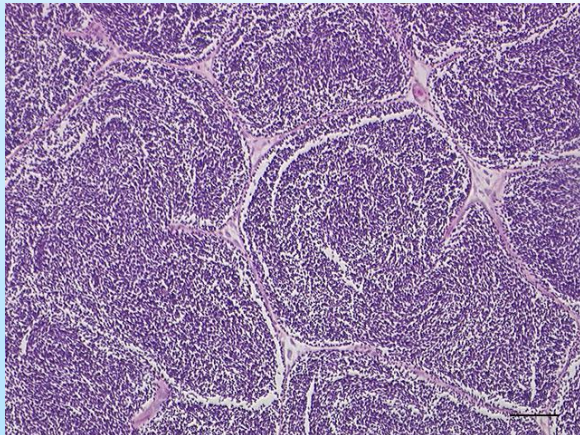


Water, food, sediment



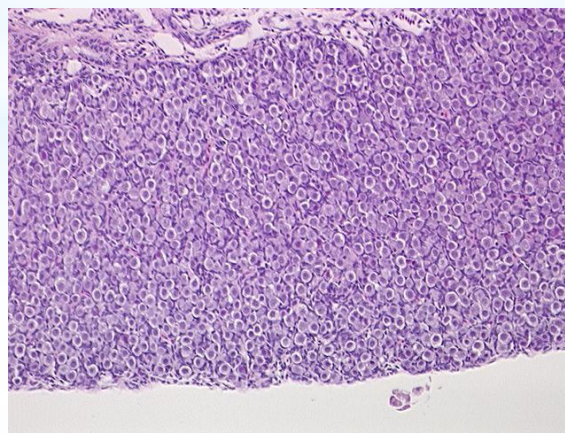
Final maturation,
sperm quality

April - May



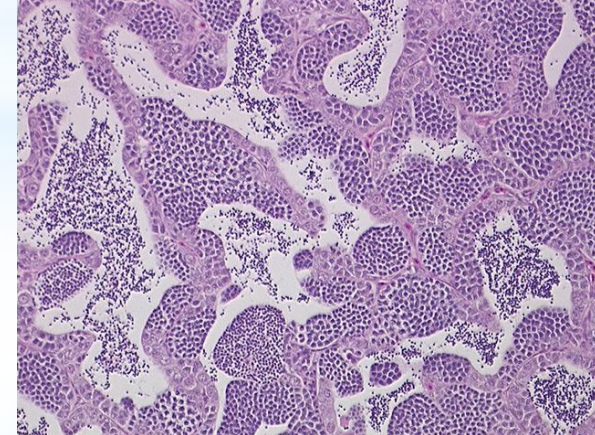
YOY - sexual differentiation
and organ development

June - July








Adults - recrudescence

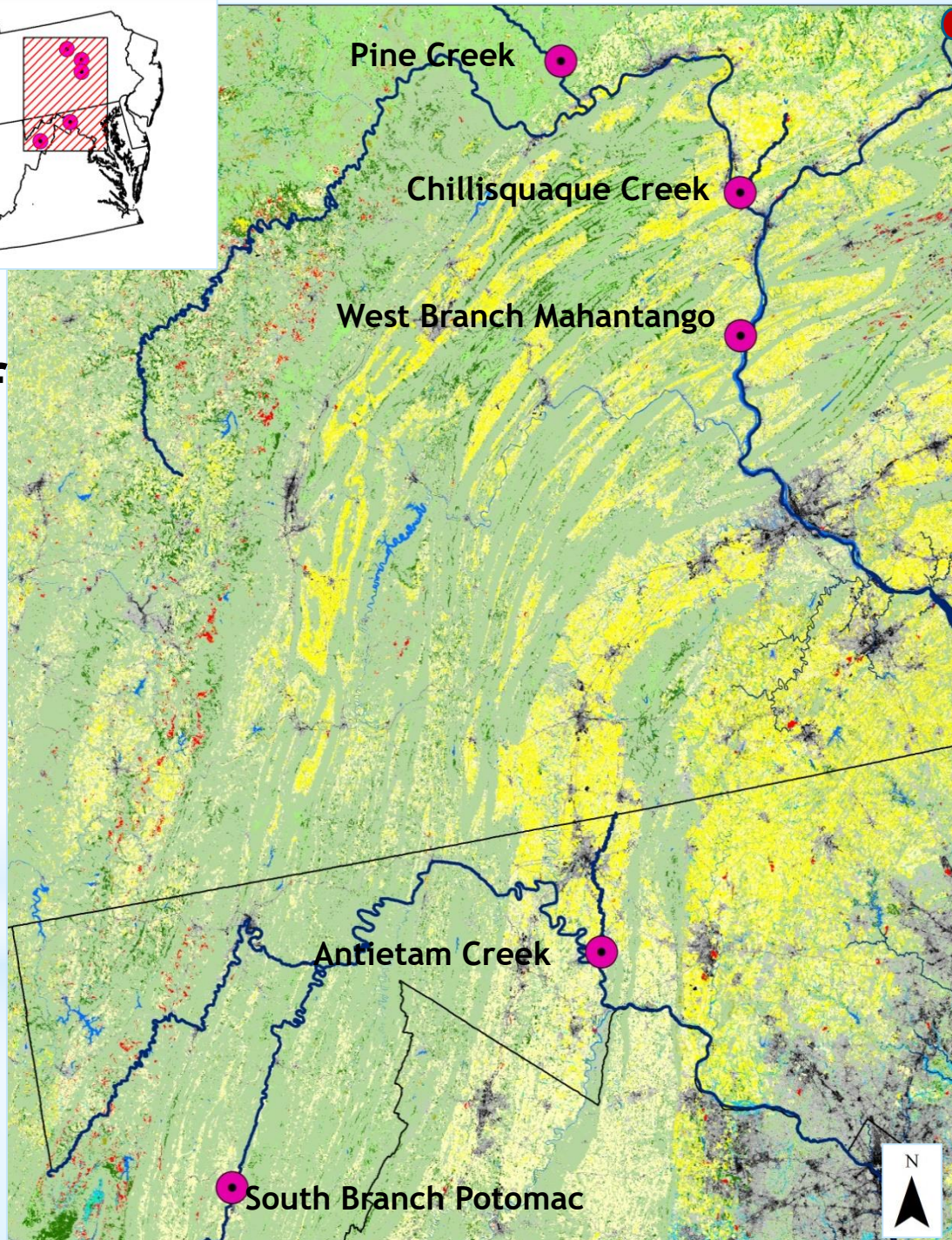
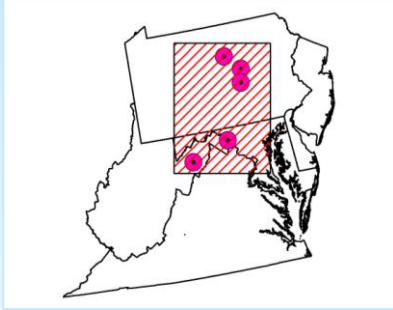
August - Dec - March



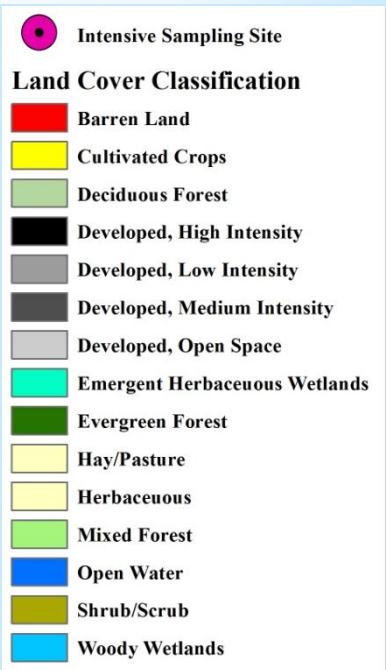
USGS Chesapeake Bay Endocrine Disruption Study Agricultural Integrator Sites

-  4 - 6 sites where monthly to biweekly water samples, plus storm events, are collected from Nov. 2014 to present
-  Sediment samples in spring and fall each year
-  Adult fish are collected in the spring prespawn (2013-2019) and at some sites in the summer and fall
-  YOY (1-3 months of age) are collected in early summer (2013-2018)
-  Tissue samples for contaminant analyses




Location of Integrator Sites



Wyalusing Creek



Integrator Site Water Samples

-  20 hormones
-  130 Pesticides/Herbicides/degradates
-  15 phytoestrogens/plant sterols

Pesticides Detected

← Herbicides → Insecticide Fungicide

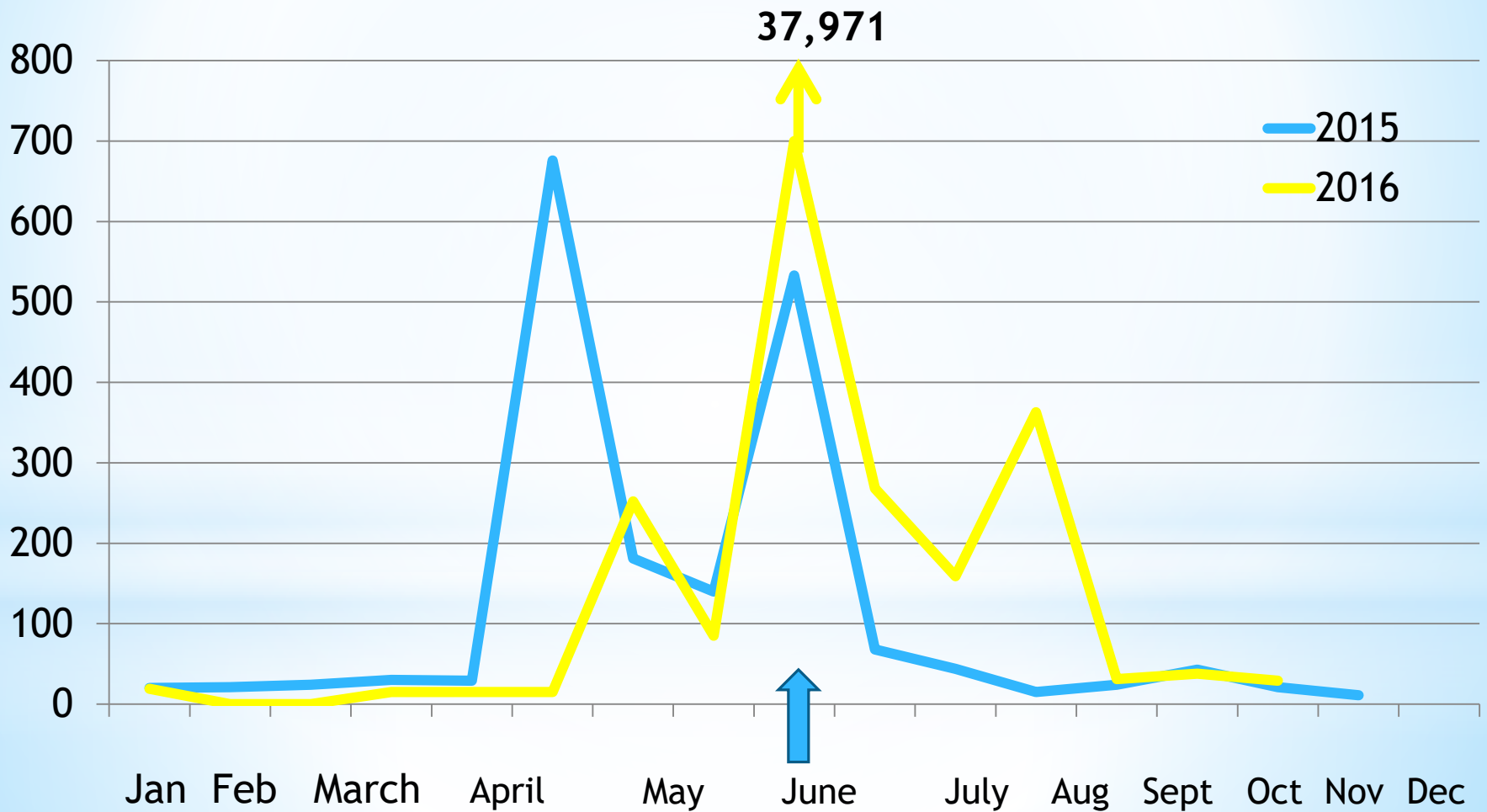
		Atrazine	Simazine	Metolachlor	Fipronil	Metalaxyl
Pine	(43 ^a)	19 ^b (1 ^c)	1 (0)	12 (1)	0 (0)	1 (0)
WB Mahantango	(47)	41 (17)	12 (1)	34 (9)	2 (0)	2 (0)
Chillisquaque	(36)	34 (9)	14 (3)	32 (9)	0 (0)	7 (0)
Antietam	(42)	41 (17)	36 (3)	39 (5)	11 (0)	0 (0)
SB Potomac	(41)	18 (0)	3 (0)	10 (1)	0 (0)	1 (0)

^aTotal number of water samples from 11/2014 through 7/2017






^bTotal detects

^cDetects above the 100 ng/L assessment threshold

Chillisquaque Atrazine Concentrations (ng/L)









Long-term Analyses

-  Ability to look at year classes and compare the exposure regimes and biological responses
 -  Fish spawned in 2015 would be 2 years of age in 2017, 3 years of age in 2018
 -  We have exposure of adults through recrudescence, spawning, young of year, as they go through sexual maturity
-  Identify key compounds and environmentally relevant concentrations for lab exposures
-  Identify times when management actions would be most important for fish health





YOY Whole Body Pesticides (ng/g; ppb) Chillisquaque

Date	Bifenthrin	Metolachlor	p,p'DDD	p,p'-DDE	Pendimethalin
8/14/14					
1				6.3	7.3
2	0.7		1.0	3.4	11.6
3	0.3	0.5	1.0	2.4	4.4
4	0.5	0.6	1.1	2.4	5.2
5	0.9			3.7	9.4
6					20.1
7	0.6	1.0		3.5	7.6
8					14.5
9	1.6	1.4	1.5	3.4	14.1

Water Versus YOY Tissue

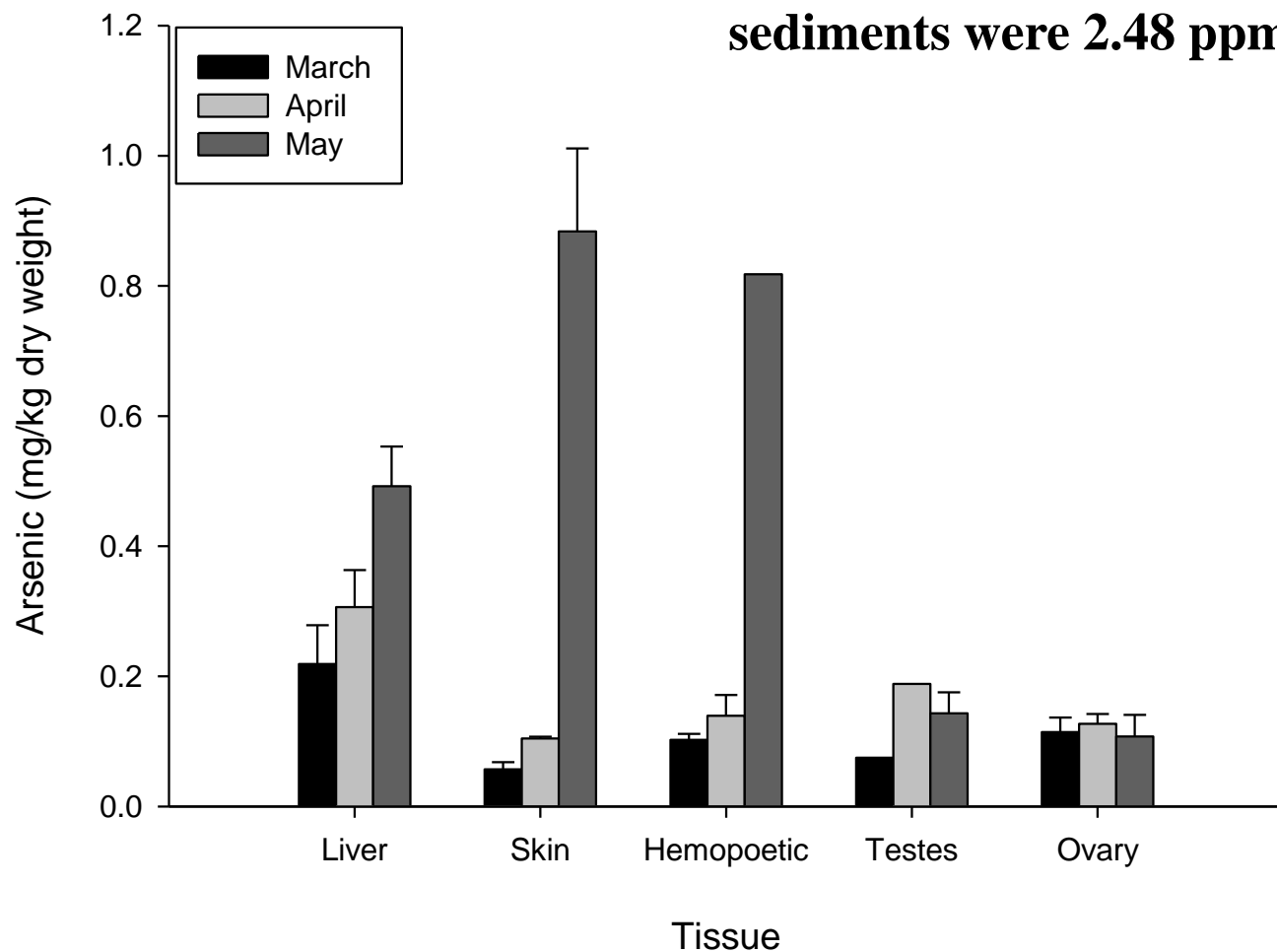
-  Young of year whole body concentrations analyzed at the USGS lab in California
-  In the water:
 -  Bifenthrin (4) and pendimethalin (6) only occasionally detected
 -  Bifenthrin detected only once on 10/29/15 at Chilli
 -  Pendimethalin not detected at all in water samples in 2015
-  Both compounds have been shown to modulate the immune response and bifenthrin is toxic to aquatic insect larvae which can have food-web consequences

Young of Year Tissue

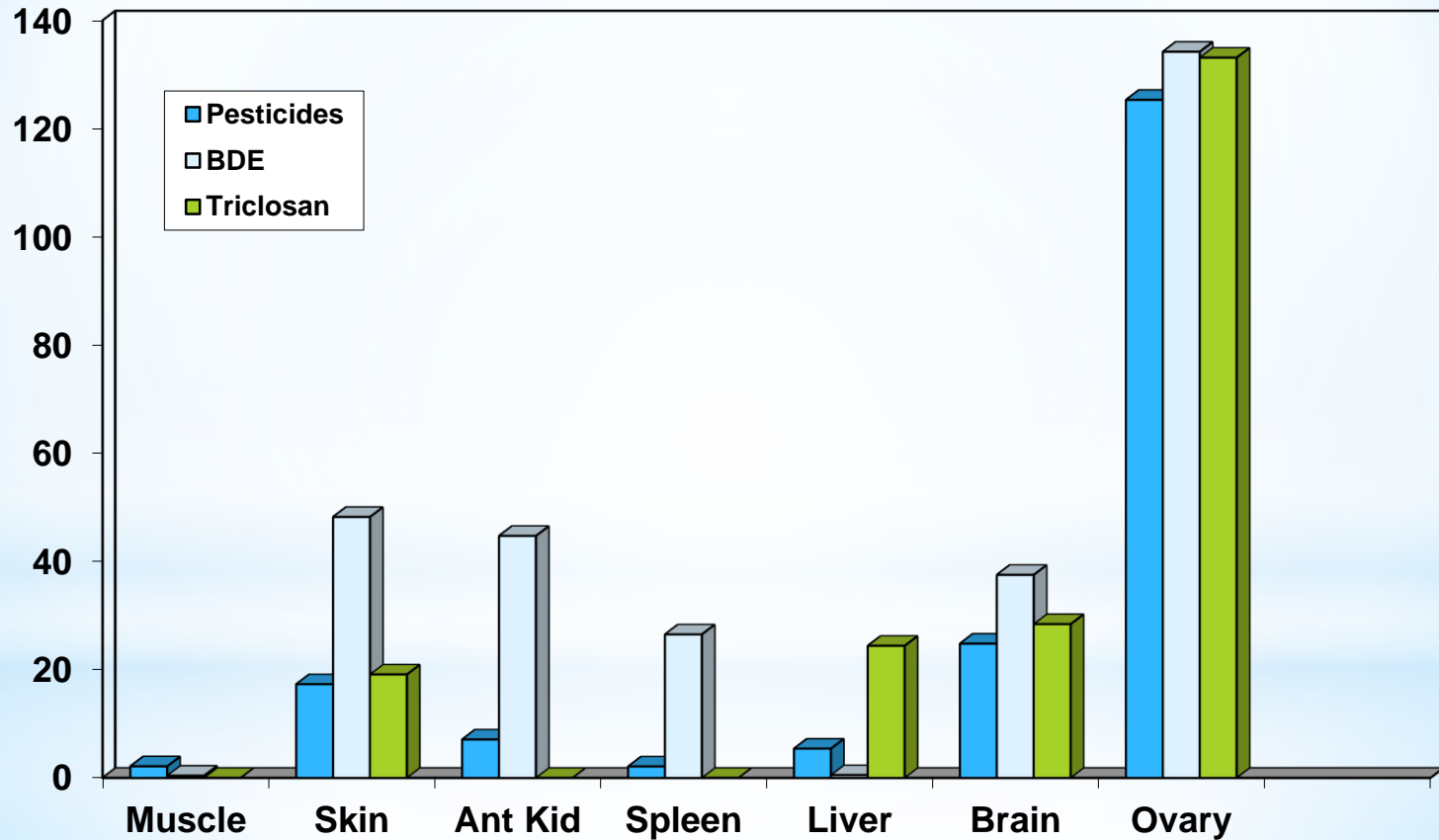
-  Additional samples from WB Mahantango, South Branch Potomac and Pine
-  Bifenthrin - only pesticide found in YOY from all three sites
-  Pendimethalin and metolachlor found at WB Mahantango and South Branch
-  Atrazine and p,p-DDE at WB Mahantango

Arsenic Tissue Contaminants Smallmouth Bass

Water concentrations even during storm runoff were 0.78 ppb, however, levels in the suspended sediments were 2.48 ppm



Comparison of Adult Tissue Contaminant Concentrations



Biological Indicators in Adult Bass

Organism-level

- * Length, weight, age, year class
- * Visible abnormalities
- * Condition factor, hepatosomatic index, gonadosomatic index

Tissue/cellular level

- * Microscopic pathology, identification of tissue responses, pathogens/parasites
- * Gonad stage
- * Immune function (started in 2016)





Subcellular/molecular


- * Plasma vitellogenin
- * Molecular/gene expression - transcriptome analyses of liver and anterior kidney - identified 50 gene transcripts to be quantified
- * Testes transcriptome - identified 40 genes to quantify

Great Lakes Emerging Contaminants Study





Parameter	Parameter	Spearman rho	p value
Male			
Bass (both species) testicular oocyte severity	Plasma vitellogenin	0.3397	0.0011
	Hepatic vitellogenin transcripts	0.2462	0.0193
	Estrogen receptor α	0.3866	0.0002
	Estrogen receptor $\beta 2$	0.3416	0.0010
	Androgen receptor β	-0.3256	0.0017
	Phosphoenolpyruvate carboxykinase	-0.2370	0.0245

Metformin

-  Metformin one of the most commonly prescribed drugs
 - diabetes, polycystic ovary
-  Phosphoenolpyruvate carboxykinase is downregulated by metformin
-  Recently found to induce intersex in laboratory exposures at environmentally relevant concentrations
-  Studies done in response to findings in the Milwaukee River (R. Klaper and associates)

-  Metformin was the only pharmaceutical measured often at all of the integrator sites

Conclusions

-  Fish are being exposed to complex mixtures of both legacy and “emerging” chemicals
-  Multiple stressor approach - including not only mixtures of chemicals but other stressors
-  Many of these chemicals influence the immune response, disease resistance and the endocrine system
-  Effects on food web and pathogen webs (sources, intermediate host effects, effects on virulence factors) need to be better understood

Acknowledgements

**USGS - Chesapeake Bay Ecosystems's
Environments and Environmental Health
programs**

WV Division of Natural Resources

WV Dept. of Environmental Protection

MD Department of Natural Resources

PA Fish and Boat Commission

PA Dept of Environmental Protection

VA Dept Game and Inland Fisheries

U.S. Fish and Wildlife Service

West Virginia University

Penn State University