

Smallmouth Bass Health Issues - Findings and Future Directions

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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

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
-  Myxozoans



Impaired Ecosystem - Immunosuppression

Biological Indicators in Adult Bass

Organism-level

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

Tissue/cellular level

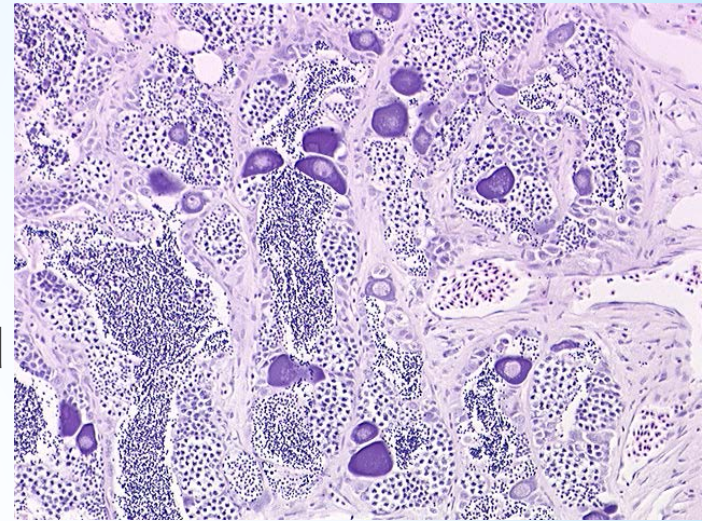
- * Microscopic pathology, identification of tissue responses, pathogens/parasites
- * Gonad stage
- * Immune function (started in 2016)

Subcellular/molecular

- * Plasma vitellogenin
- * Molecular/gene expression - liver, skin, testes, anterior kidney

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



- 🐟 **Plasma Vitellogenin**
 - 🐟 Yolk precursor
 - 🐟 Exposure to estrogenic compounds turns on the gene in males
 - 🐟 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

2003 – 2010 Projects

Lessons Learned

 **All fish are not created equal**

Differ in their response to environmental factors, chemical contaminants and pathogens/parasites

Smallmouth Bass appear to be especially sensitive to chemicals in agricultural landuse

 **Aquatic organisms are exposed to complex mixtures of chemicals in addition to other environmental stressors (nutrients, temperature etc.)**

Exposure regimes vary spatially and temporally

Sampling water once or twice a year does not provide a good indication of exposure

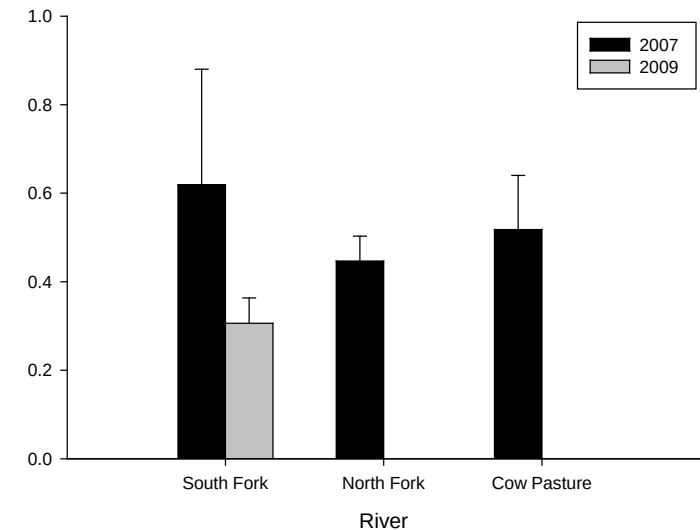
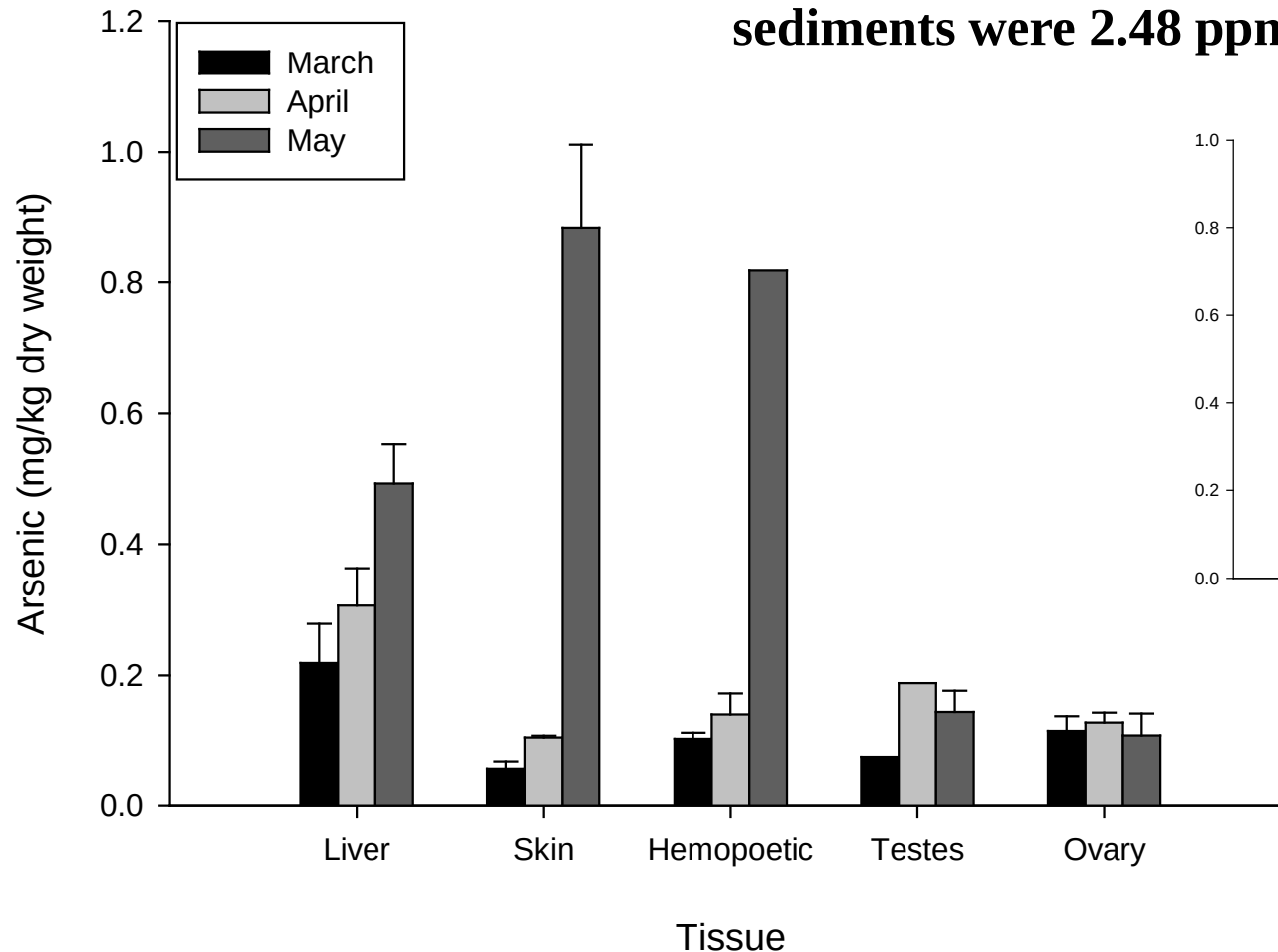
 **Multiple chemicals can have similar effects**

 **Many effects, such as intersex, may be induced early in life and not be evident until maturity and so identifying causes when looking at adults is difficult**

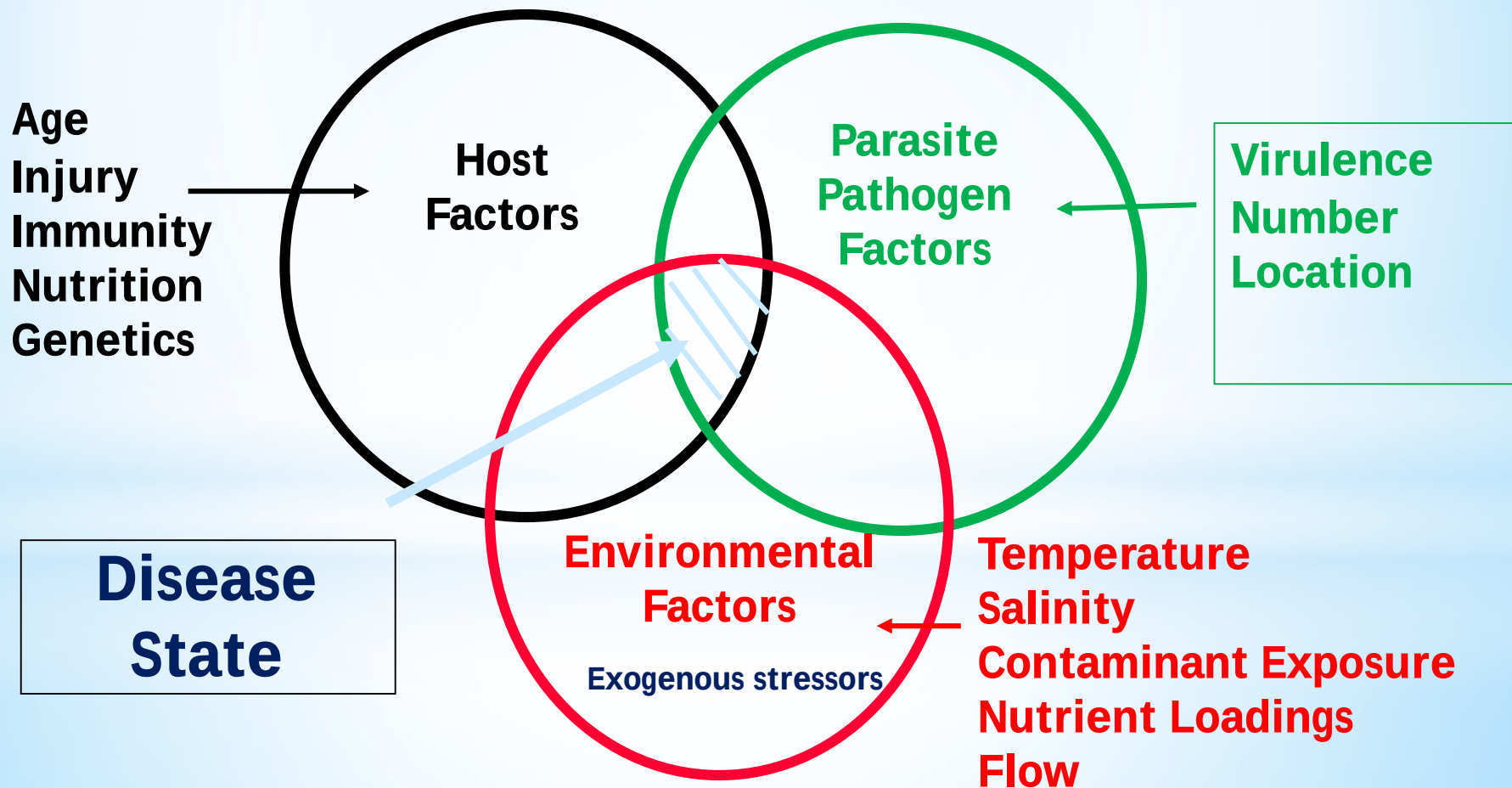
Arsenic Tissue Contaminants

Smallmouth Bass

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



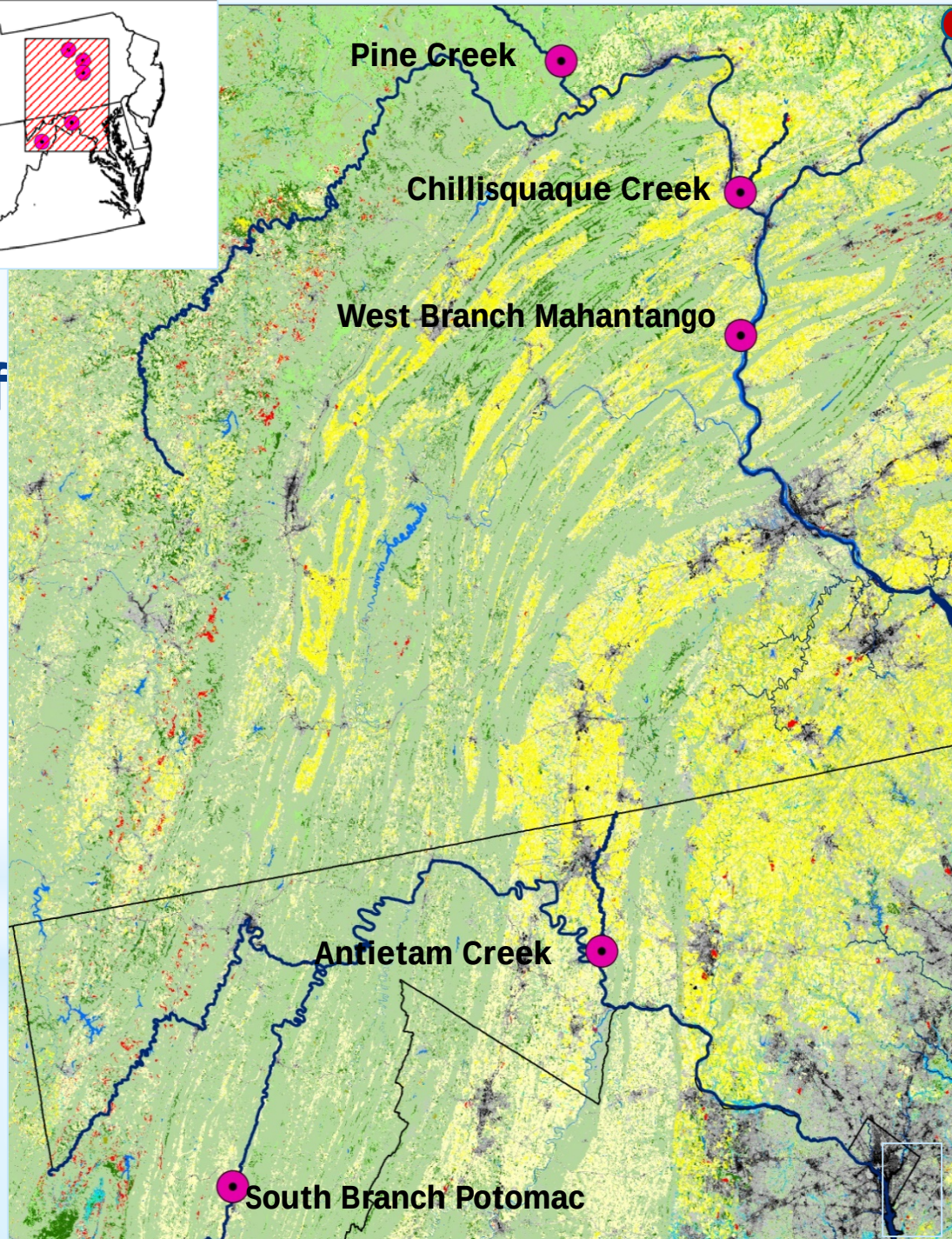
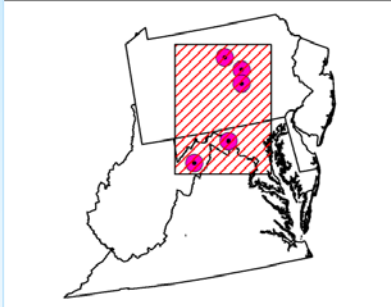
A Paradigm For Fish Health



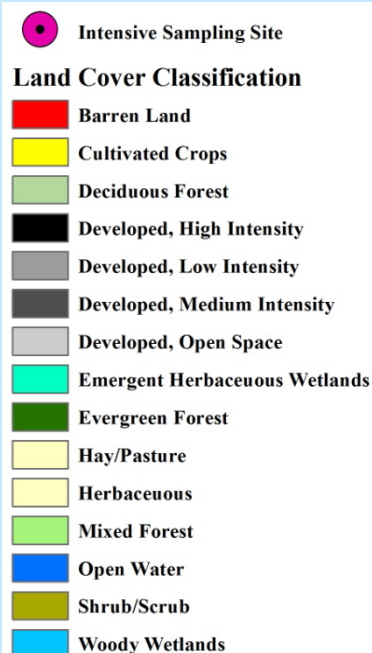
USGS Chesapeake Bay Endocrine Disruption Study Agricultural Integrator Sites

-  **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 (2013-2018) 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 Agricultural



20 hormones



130 Pesticides/Herbicides/degradates



15 phytoestrogens/plant sterols

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

Key Exposure Periods

Nest/eggs – sediment/water



Water, maternal, sediment

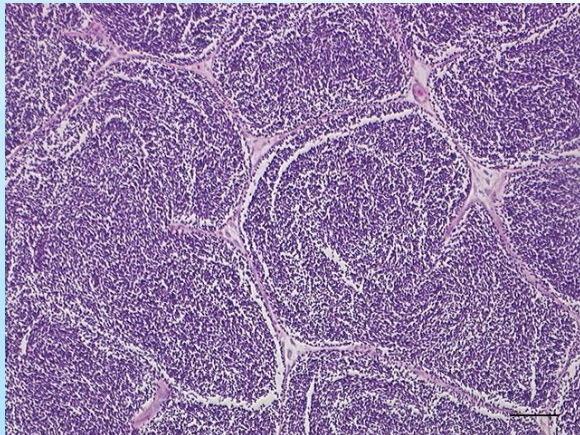


Water, food, sediment



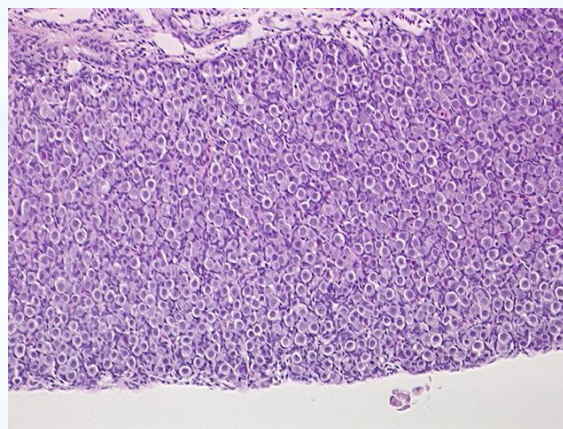
Final maturation,
sperm quality

April - May



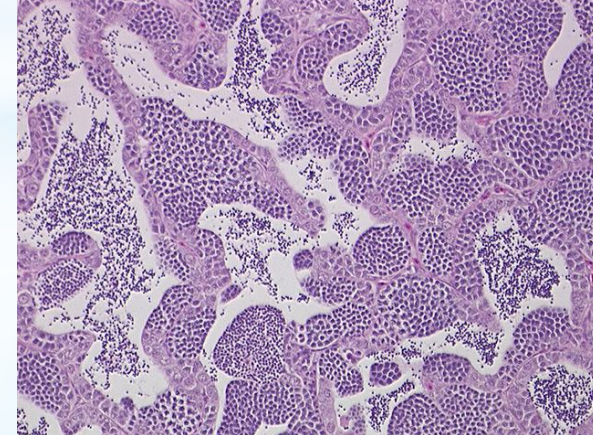
YOY – sexual differentiation
and organ development

June - July



Adults - recrudescence

August - Dec - March



Integrator Site Water Samples

- * **Analyzed for 130 pesticides or degradates (GC)**
 - * **13 including neonics (LC)**
 - * **Detected 30 at least once**
 - * **Most only occasionally detected**
- * **Atrazine, simazine, metolachlor - most common; fipronil, metalaxyl next most common**

Pesticides Detected

← **Herbicides** → Insecticide Fungicide

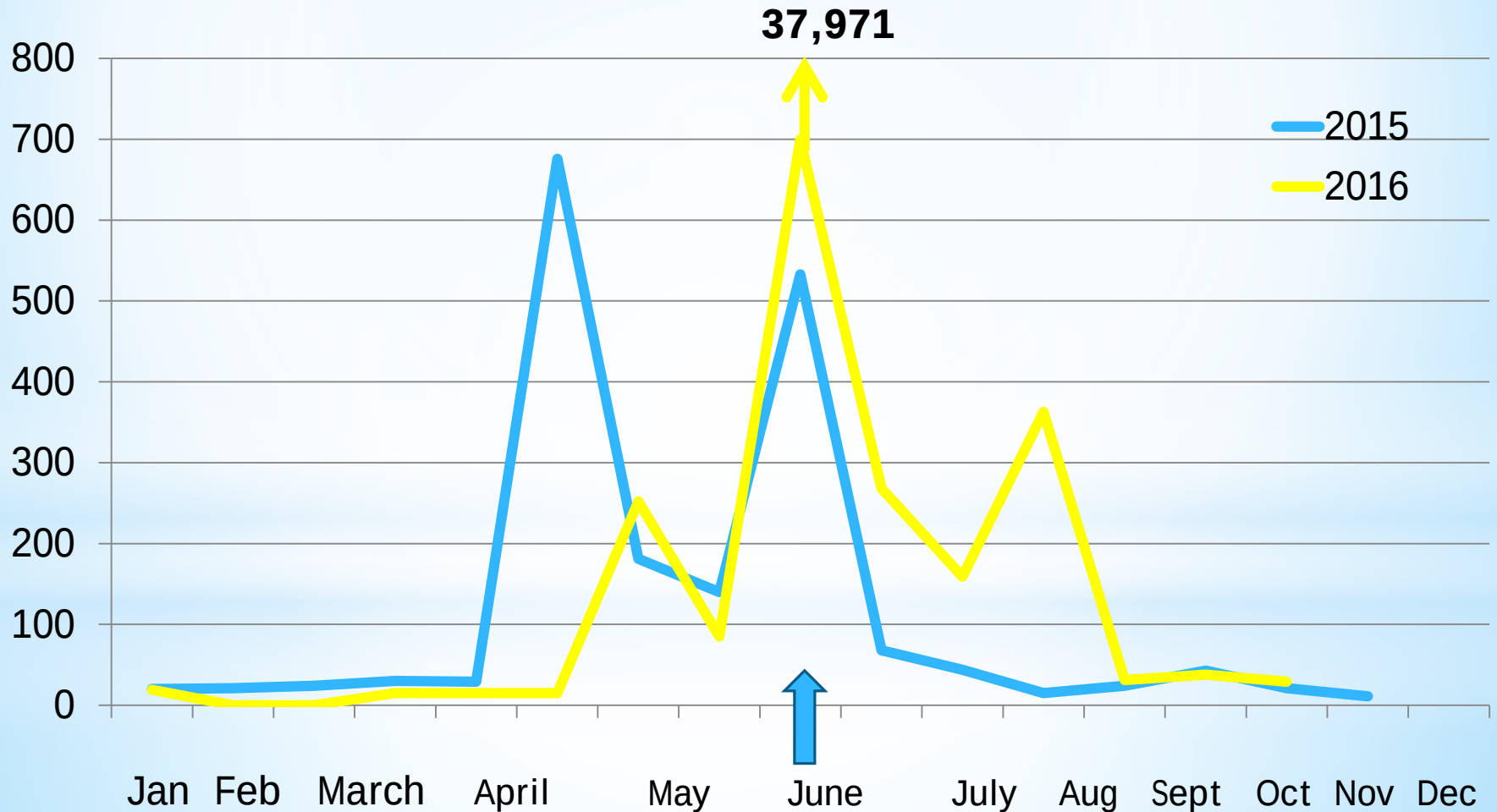
Location	# of samples	Atrazine	Simazine	Metolachlor	Fipronil	Metalaxyl
Pine	43 ^a	19 ^b (1 ^c)	1 (0)	12 (1)	0 (0)	1 (0)
WB Mahantango	47	41 (17)	12 (1)	34 (9)	2 (0)	2 (0)
Chillisquaque	36	34 (9)	14 (3)	32 (9)	0 (0)	7 (0)
Antietam	42	41 (17)	36 (3)	39 (5)	11 (0)	0 (0)
SB Potomac	41	18 (0)	3 (0)	10 (1)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

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

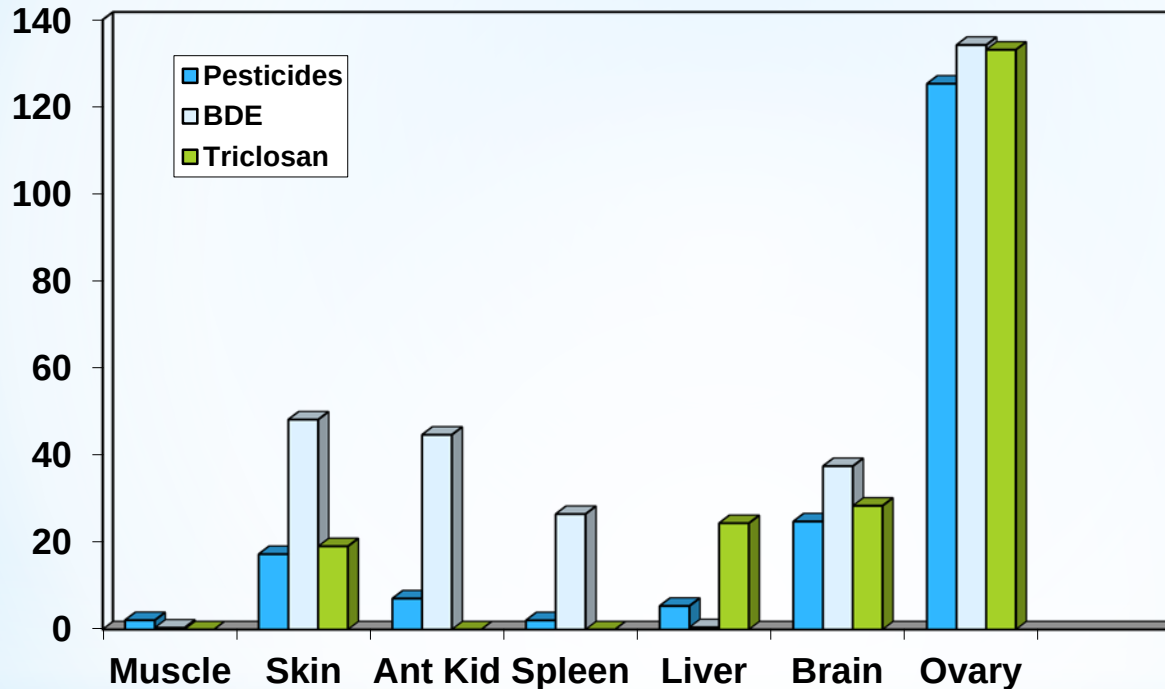
^bTotal detects

^cDetects above the 100 ng/L assessment threshold

Chillisquaque Atrazine Concentrations (ng/L)



Tissue Contaminants



🐟 Exposure of adult females - incorporation into the egg - to the young of year in the yolk sac

🐟 Collected ovaries pre-spawn and then young of year

Young of Year Smallmouth Bass Tissue Analyses

-  Compared whole body contaminants
 -  10 fish from LSC ponds and 16 fish from two sites on the Susquehanna
 -  Suite of organic contaminants including pesticides (legacy and current-use), PCBs, BDE, triclosan
-  LSC ponds – mean # of detects was 4 (range 2-5)
 -  BDE 99, BDE 200, PCB 180, hexachlorobenzene
-  Susquehanna – mean # of detect was 19 (range 14-28)
 -  BDE 47, 7 PCBs, dieldrin, chlorpyrifos, nonachlors, chlordanes, pentachloroanisole, triclosan, pyraclostrobin

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 (insecticide) detected only once on 10/29/15 at Chilli**

 **Pendimethalin (herbicide) 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**

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

Date 8/14/14	Bifenthrin	Metolachlor	p,p'DDD	p,p'-DDE	Pendimethalin
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

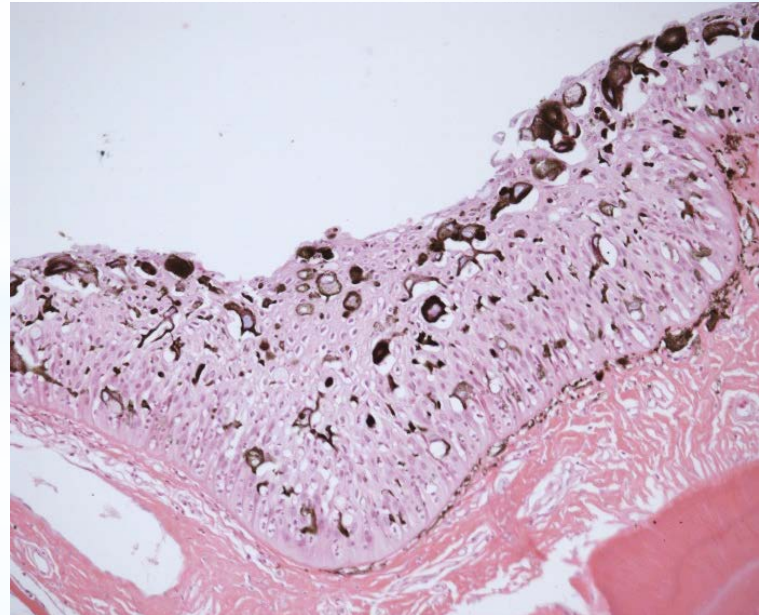
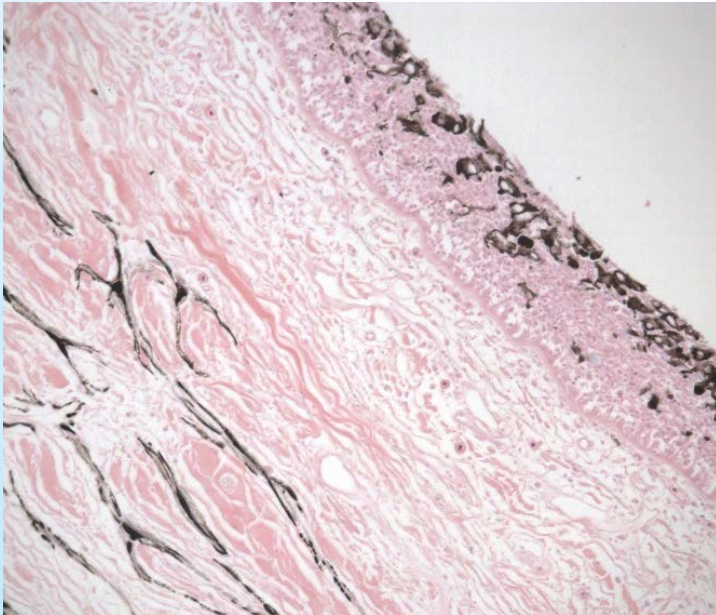
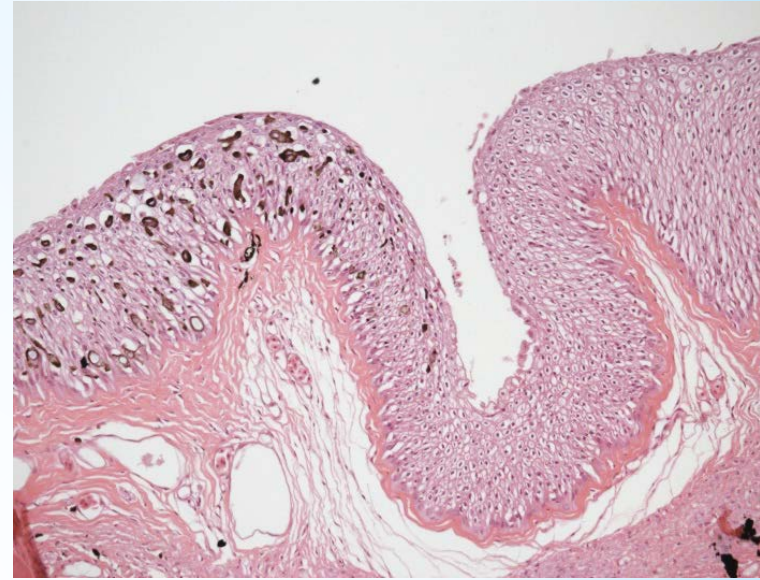
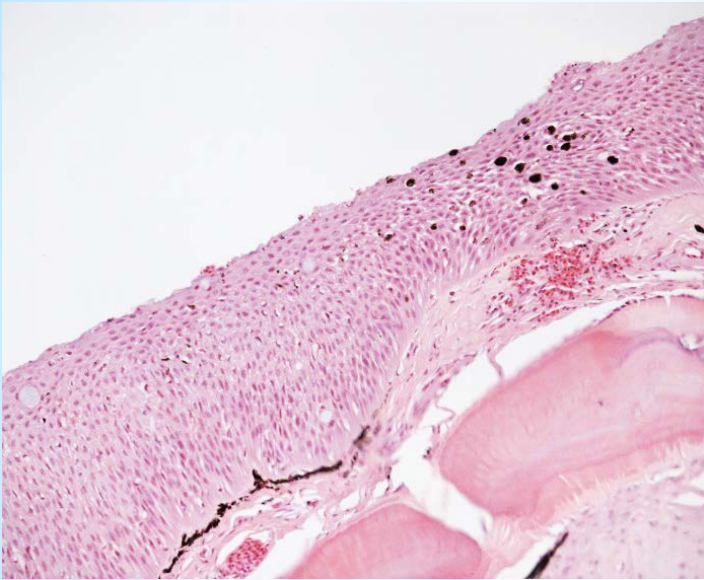
Melanistic Adult Bass

- 🐟 Irregular areas of black, hyperpigmented areas on the body surface, fins, mouth
- 🐟 Termed “blotchy bass” syndrome in the popular press and social media



Microscopic Pathology

Melanistic Lesions

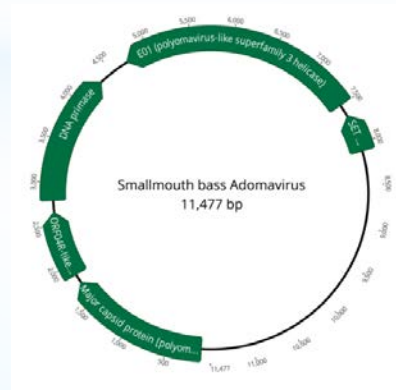


Molecular Analyses

-  Sequenced normal and melanistic skin
-  55 genes associated with melanogenesis, contaminant exposure, neoplasia and pathogen immune responses were evaluated
-  11 were significantly differentially expressed between normal and melanistic
-  2 genes were downregulated in the melanistic lesions
-  9 genes were upregulated in the melanistic lesions

E1 Helicase

- 🐟 E1 helicase, a viral-associated protein, was one of only three unique transcripts in melanistic lesions - other two were unidentified proteins
- 🐟 Further sequencing identified an adenovirus
- 🐟 A new species of virus in a recently discovered group of viruses
- 🐟 These have been associated with skin lesions in other fish species – although not with melanistic lesions



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**
-  **Phosphenolpyruvate 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)**

Pharmaceuticals Observed Chesapeake Bay Sites

-  Metformin was the only pharmaceutical found at all sites
-  Antidepressants only observed at Antietam except for two found once at Chillisquaque
-  Opioids only observed at Antietam

New Direction?

-  **Environmental Health funded Chesapeake Bay Endocrine Disruption project is ending**
-  **Chesapeake Bay Ecosystems (Environments) science plan is “evolving” and is focused on the 2014-2025 Chesapeake Bay Watershed Agreement and DOI restoration efforts**
-  **Some of the questions:**
 - *How do land and climate changes affect fish communities, health and habitats?**
 - *What are the relations among land use, water quality, contaminants and health of fish and wildlife?**
 - *What are the some opportunities for management actions to benefit multiple restoration outcomes**

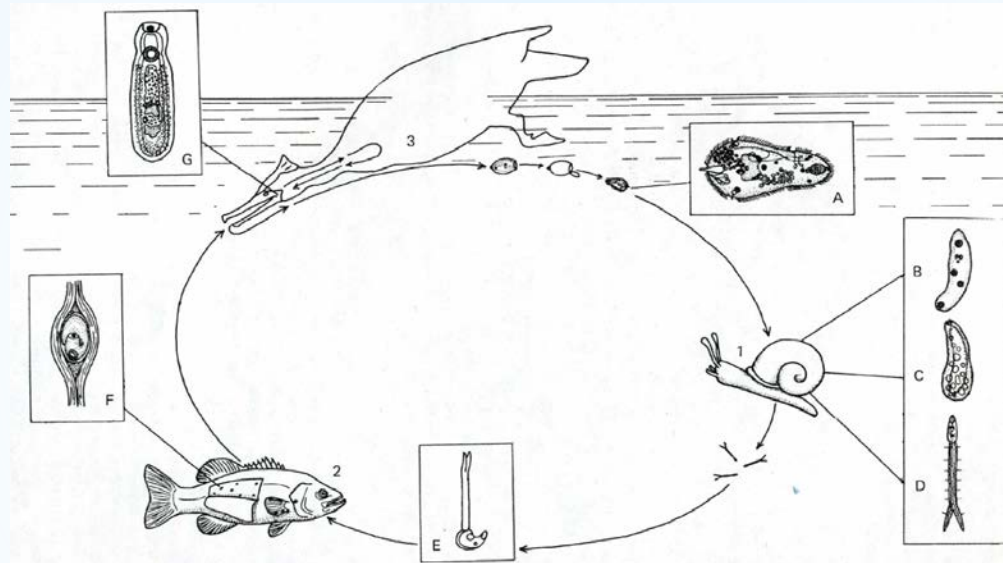
Where are We Now?

-  **Completing analyses of all collections 2013 – 2018**
 -  **Chemical and biological data**
-  **Analyze trends over time and associations with land use, climatic factors, water quality (nutrients, PA Water Quality Index), state population (adult and YOY abundance)**
-  **Assess BMP (Best Management Practices) in relation to our chemical and biological data – are BMPs directed at reducing nutrients affecting toxic contaminants and/or fish health**
-  **Utilize this data to choose “focal sites” for more indepth study**

Focal Studies

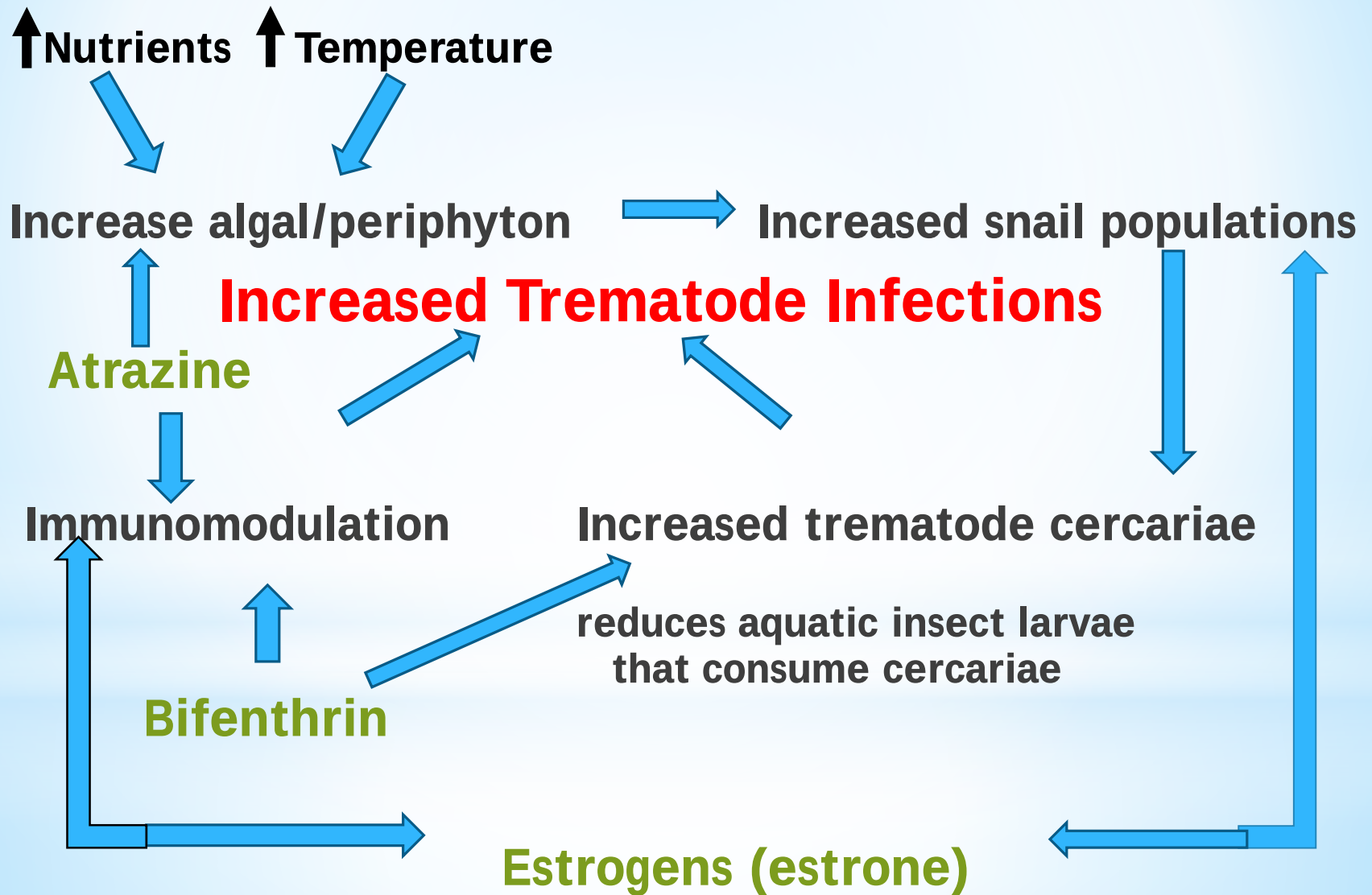
-  **Need a better understanding of food web and parasite/pathogen webs (intermediate and final hosts)**
-  **Better integration of the comprehensive fish health assessments with:**
 - * water quality – nutrient, sediment**
 - * invasive species/invasive and emerging pathogens**
 - * algal toxins**
 - * benthic macroinvertebrate data (assuming the right organisms are assessed)**
 - * population trends**
 - * fish community assessments**
 - * land use change/BMPs**

Trematode Parasites

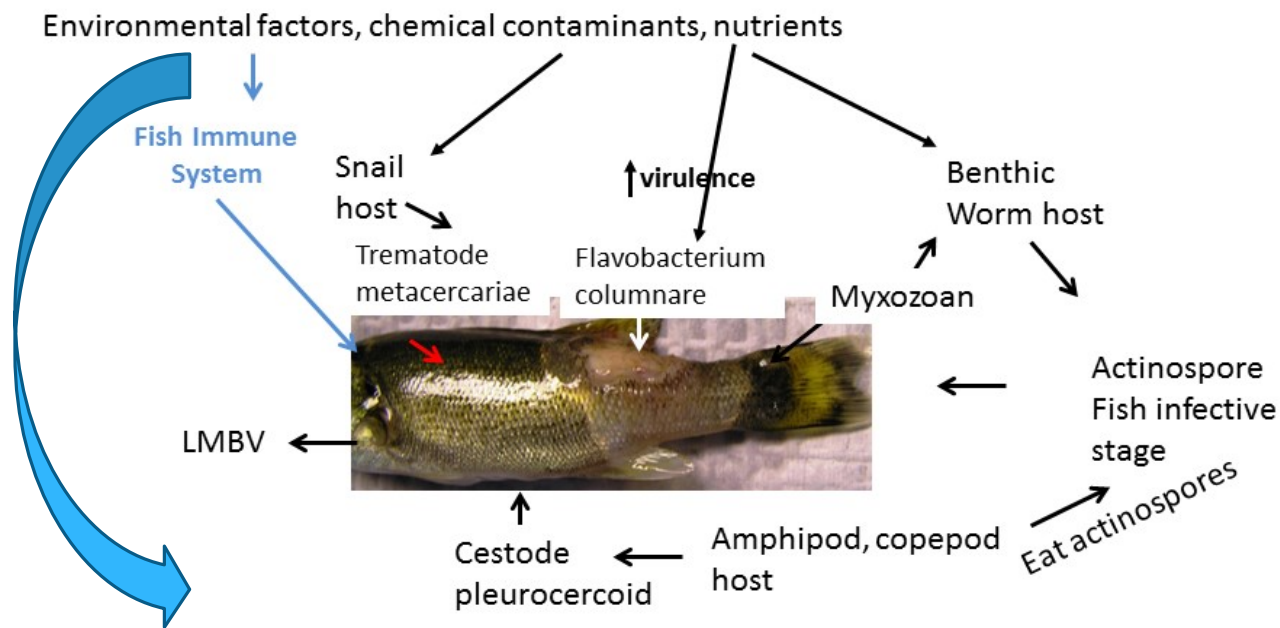


- 🐟 Atrazine augmented the richness and abundance of snails
- 🐟 Atrazine and other herbicides decrease phytoplankton which tend to increase periphytic algae
- 🐟 Increased the abundance of the cercariae
- 🐟 Increased susceptibility to cercariae
- 🐟 Low doses of atrazine have been shown to be immunosuppressive in frogs, fish and salamanders
- 🐟 Exposure increased susceptibility to *A. hydrophila* infections in fish and viral infections in salamanders

Example of Complex Interactions of Risk Factors



Young of Year Smallmouth Bass Co-infections



Focal Sites - Where?

 **Fish health studies rely on the states – in kind support, collaborative projects, other data sets**