

# Health of Fishes and Association with Complex Chemical Mixtures

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## Recent Fish Health Issues Chesapeake Drainage

 Endocrine disruption, emerging contaminants and reproductive health of fishes

 Intersex and other reproductive abnormalities in smallmouth bass

 Lack of recruitment of yellow perch in selected tributaries

 Skin lesions and kills of smallmouth bass, sunfish and selected sucker species

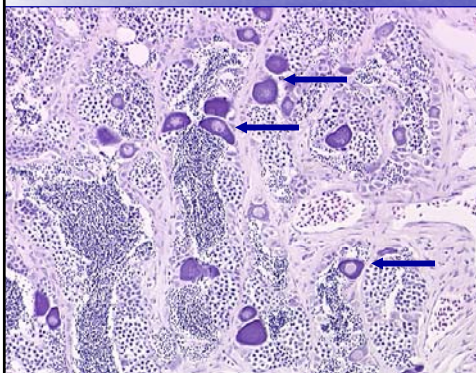
 Spring kills of mature adults – Potomac, James

 Summer kills of young of the year smallmouth  
- Susquehanna

## Overall Findings/Conclusions/ Progress – Fish Kills

- 🐟 Sensitive species, stressed populations close to a threshold between healthy and sick (dead)
- 🐟 Variety of stressors leading to immunosuppression
- 🐟 Numerous pathogens contributing to the skin lesions and eventual death – no consistent findings
  - 🐟 Bacteria – *Aeromonas hydrophila*, *A. salmonicida*, *Flavobacterium columnare*
  - 🐟 Virus – largemouth bass virus
  - 🐟 Numerous parasites
  - 🐟 Possible rickettsia in YOY bass in Susquehanna
- 🐟 Prevalence/severity of intersex appears to be related to fish lesions/kills – predictive?
  - 🐟 James/Cowpasture
  - 🐟 Monocacy

## 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 later

## Induction of Testicular Oocytes Experimentally

- 🐟 Estradiol
- 🐟 Ethinyl estradiol – synthetic estrogen
- 🐟 Bisphenol A
- 🐟 Isoflavones – equol, genistein
- 🐟 Nonylphenol
- 🐟 4-tert-pentylphenol and octylphenol – surfactants in pesticides
- 🐟 DDT
- 🐟 Atrazine - amphibians

Many pesticides may add to the “estradiol equivalents”

## Induction of Intersex

- 🐟 Ethynylestradiol
  - 🐟 0.3 ng/l exposure of roach embryo caused feminization
  - 🐟 0.35 ng/l recently set as the aquatic “no effects level”

## Suite of Biological Indicators

- 🐟 **Morphometric and necropsy-based**
  - Comparisons based on sex, age,
  - identifies 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- $\beta$ , hepcidin), contaminant-related (CYP1A, oxidative stress), stress (glucocorticoid receptors)
  - Mechanisms
  - Identification of parasites/pathogens

## Benefits of the Variety of Endpoints

- 🐟 **Tease out sources/inputs of important contaminants for better management**
- 🐟 **Identify previously unidentified problems**
- 🐟 **Better understand the range of effects – reproductive, immune system and others**

For instance

- 🐟 **Testicular oocytes have been induced with both estrogenic and anti-androgenic chemicals while vitellogenin in male fish is strictly a response to estrogenic exposure**

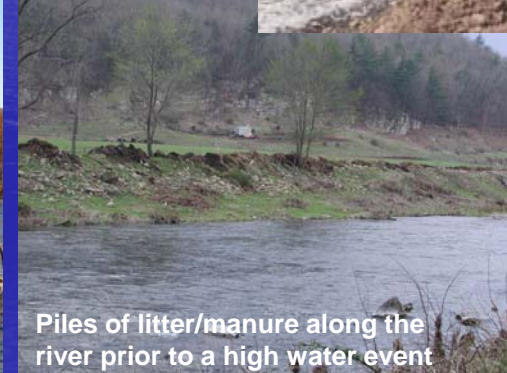
# Inputs and Management?



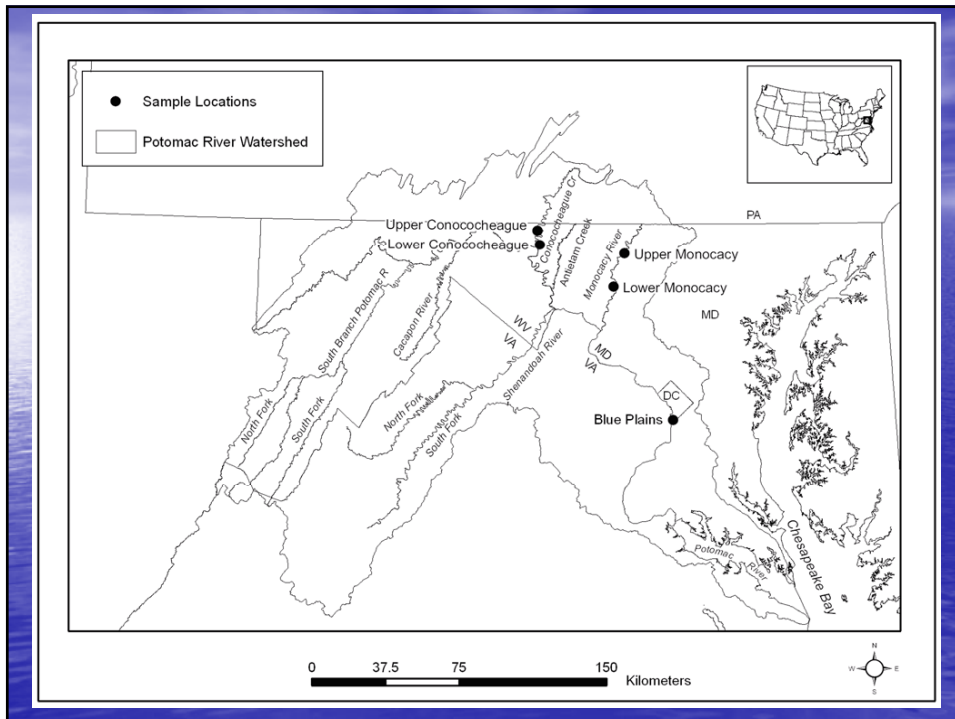
Human development  
 WWTP effluent  
 Stormwater runoff  
 Construction sites  
 Industry  
 Agriculture



Cattle with free access to the river



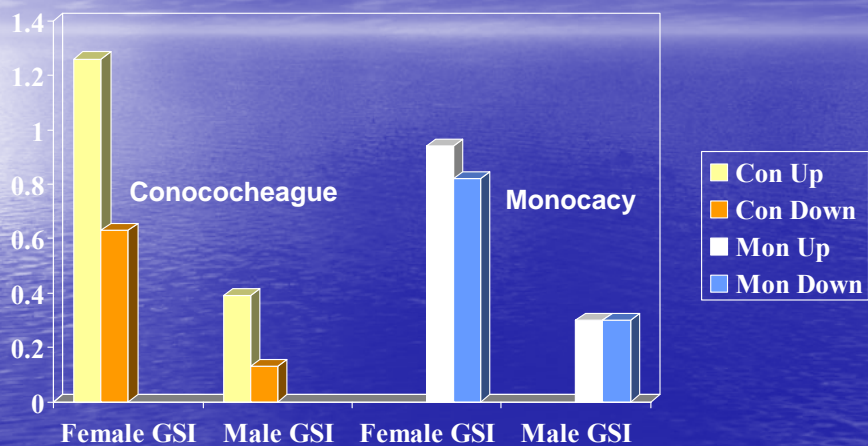
Piles of litter/manure along the river prior to a high water event



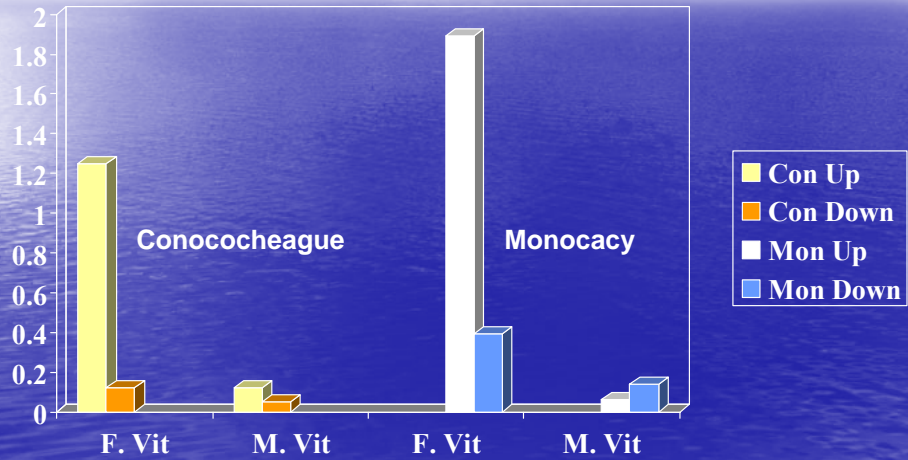
## WWTP Study – MD Tributaries

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 Study – MD Tributaries Fall 2005



## MD WWTP Study Fall 2005 Plasma Vitellogenin (mg/ml)



## 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%

## Chemical Data Passive Samplers



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

D. Alvarez, Columbia Environmental Research Lab, USGS

### Wastewater-related Chemicals

Presented as ng/POCIS

Chemical	Con Up	Con Down	Mon Up	Mon Down	Blue Plains
<b>Fragrances</b>					
Celestolide	<MDL <sup>a</sup>	130	<MDL	<MDL	130
Tonalide	<MDL	120	<MDL	<MDL	515
Galaxolide	<MDL	335	<MDL	210	955
<b>Flame Retardant</b>					
Tri(2-chloroethyl) phosphate	<MQL	165	<MQL	100	360

## Organochlorines and PCBs

Chemical Estimated pg/L	Con Up	Con Down	Mon Up	Mon Down	Blue Plains
Total PCBs	66	215	<MQL	410	2,550
Total PAHs	4,136	4,114	3,921	16,790	21,825
Total DDTs	94	144	<MQL	655	355
Hexachlorobenzene <sup>1</sup>	83	58	<MQL	<MQL	59
Pentachloranisole <sup>2</sup>	56	225	110	190	310

<sup>1</sup>Fungicide

<sup>2</sup>Rodent toxin

## Insecticides

Chemical Estimated pg/L	Con Up	Con Down	Mon Up	Mon Down	Blue Plains
Lindane	<MQL	620	<MQL	550	<MQL
Total Benzenehexachloride	89	404	<MQL	93	218
Chlorpyrifos	<MQL	115	<MQL	48	475
Total Chlordane	43	136	35	95	620
Dieldrin	175	295	104	195	550
Heptachlor epoxide	<MQL	165	<MQL	150	405
Endosulfans	630	1,470	830	1,330	6,050

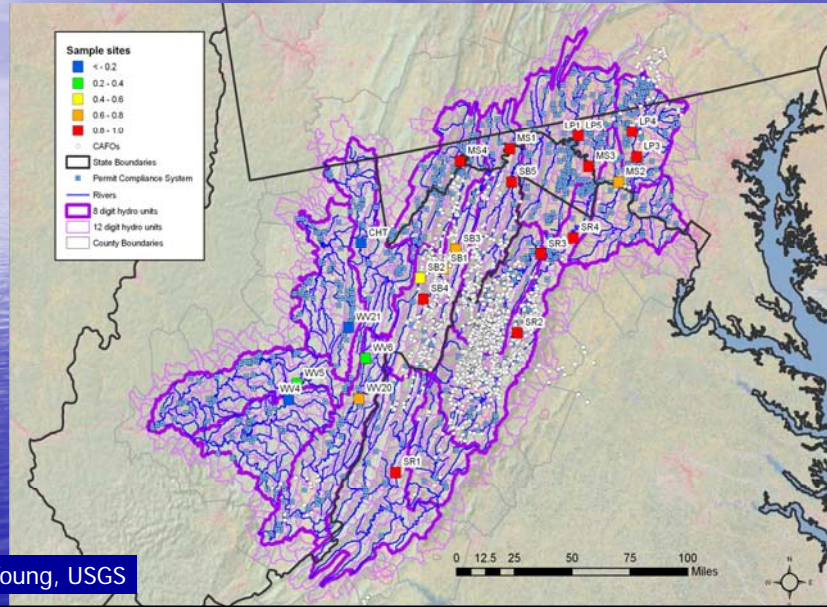
## Agricultural Pesticides Fall 2005 (Spring 2006)

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)

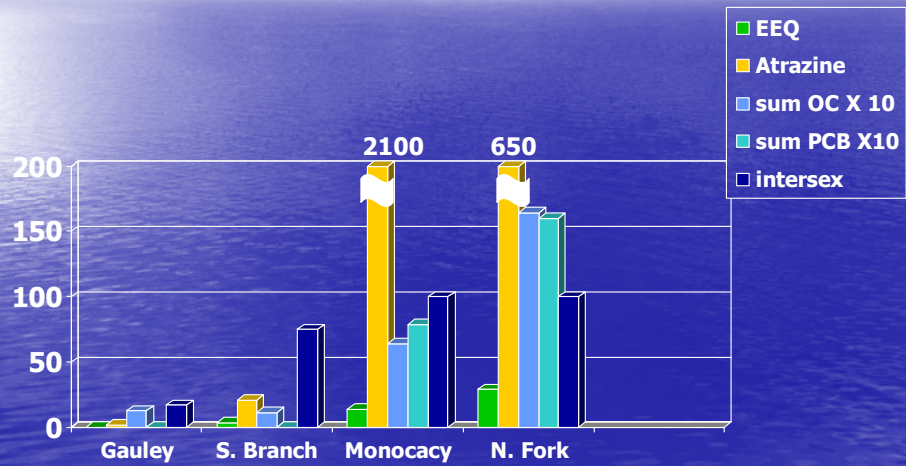
## Issue of Complex Mixtures

- Screening either grab water extracts or the extracts from the passive samplers using in vito 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)
  - 17 $\beta$  estradiol, 17 $\alpha$ -ethynylestradiol, estrone, estriol
- Estrogen equivalents ranged from 14-79 ng estradiol/sample depending on the site

# Intersex Severity and Selected Landuse Parameters



# Gradient Spring 2006-2007 Passive Sampler Results/Intersex



## 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

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## 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

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## Chemicals Measured In Gonads and Liver of SMB

- HCB (fungicide)
  - PCA (rodent toxin)
  - Fipronil & metabolites
  - Chlorpyrifos
  - Chlordane & metabolite
  - Triclosan & metabolites
  - Cis-Nonachlor
  - Pentabromotoluene
  - Dieldrin
  - Cyfluthrin
  - Numerous PCBs
  - p,p'-DDE
  - Numerous BDEs
- Brominated flame retardants

Mark Burkhardt, Steve Zaugg and Dana Kolpin

## Ovary Contaminants Spring Pre-Spawn Smallmouth Bass

Compound µg/kg	Cheat	South Branch	South Fork Shenandoah
PCA	ND	0.48	0.39
dieldrin	ND	ND	2.41
pesticides	1.4	16.9	20.2
BDEs	3.7	119.6	76.9
DDx	1.9	13.6	67.0
PCBs	14.3	13.1	49.6

## Contaminants Measured in Tissues Yellow Perch Choptank

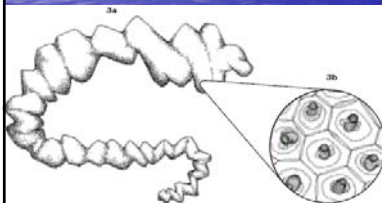
Chemical ( $\mu\text{g}/\text{kg}$ )	Brain	Ovary	Liver	Muscle
<b>HCB</b>	<b>ND</b>	<b>ND</b>	<b>2.47</b>	<b>5.90</b>
<b>PCA</b>	<b>ND</b>	<b>ND</b>	<b>1.31</b>	<b>4.04</b>
<b>DCPA</b>	<b>0.22</b>	<b>0.25</b>	<b>ND</b>	<b>ND</b>
<b>Fipronil compounds</b>	<b>0.21</b>	<b>0.09</b>	<b>ND</b>	<b>ND</b>
<b>Chlordane compounds</b>	<b>4.30</b>	<b>5.41</b>	<b>3.28</b>	<b>0.49</b>
<b>Nonachlor compounds</b>	<b>3.36</b>	<b>3.51</b>	<b>3.18</b>	<b>0.20</b>
<b>Dieldrin</b>	<b>0.64</b>	<b>0.82</b>	<b>0.60</b>	<b>0.10</b>
<b>DD<sub>x</sub></b>	<b>20.50</b>	<b>19.30</b>	<b>4.95</b>	<b>1.30</b>
$\Sigma$ PCBs	<b>61.34</b>	<b>39.30</b>	<b>41.37</b>	<b>2.81</b>
$\Sigma$ BDEs	<b>7.41</b>	<b>0.75</b>	<b>1.03</b>	<b>0.59</b>

## Yellow Perch Pre-Spawn

Egg Mass Counts (#/50 meter reach)

Broadcast or pelagic spawners

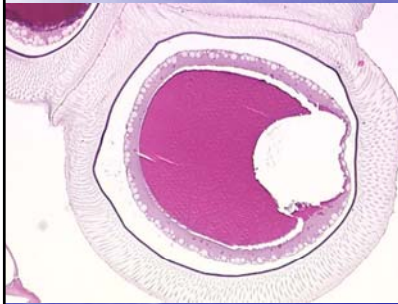
Females release Jellylike ribbons of eggs



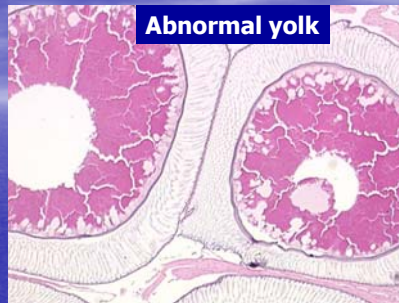
River	2005	2006	2007
<b>Choptank</b>	<b>&gt;300</b>	<b>&gt;200</b>	<b>&gt;300</b>
<b>Allen's Fresh</b>	<b>75</b>	<b>350</b>	<b>&gt;200</b>
<b>Matta-woman</b>	<b>&gt;100</b>	<b>280</b>	<b>&gt;100</b>
<b>South</b>	<b>NS</b>	<b>75</b>	<b>50</b>
<b>Severn</b>	<b>20</b>	<b>7</b>	<b>NS</b>

## Egg Abnormalities Observed

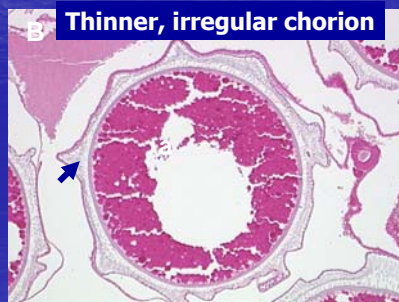
Yellow perch



Choptank –  
Most appeared normal  
Thick, intact, regular chorion  
Hydrated yolk



Abnormal yolk



Thinner, irregular chorion

## 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.

# Acknowledgements

- 🐟 US Fish Wildlife Service
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