Chemicals in the Aquatic Environment What are Fish Telling Us?

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science for a changing world

Fish

- Aquatic vertebrates that have most of the same organs (tissues) systems as humans
- Live and reproduce in the habitats humans recreate, from which many get their drinking water and may be used as food (particularly subsistence living)
- Raises concerns when fish that look like this are caught

Complexities in Determining Risk of Contaminant Exposures

- Thousands on chemicals in complex mixtures
- Often a disconnect in timing of sensitive exposure periods versus water/sediment sampling
- Effects of early life stage exposure that may not be evident until adult
- Effects on disease resistance that require understanding the fish immune response and influences on the pathogens/parasites/ intermediate hosts

Issues with Addressing "Toxic" Chemicals in the Aquatic Environment

- Monitoring concentrations in water/sediment provide only a snapshot in time
- Most monitoring of fish tissue chemical contaminants is whole body or fillet
  - Evidence for differential tissue accumulation
- See serious biological effects when no one chemical is above "threshold benchmarks"
  - Complex mixtures – additive, synergistic, antagonistic

Fish as Integrators for Aquatic System Health

- Constantly exposed to the multitude of stressors in the water, sediment and their food source
- Stressors include complex mixtures of chemicals, climatic effects and many others
- Integrate effects over time
- Emerging contaminants – many act synergistically, lack of classic dose-response, methods to measure at concentrations they cause effects, vary greatly seasonally/daily
- Adverse effects monitoring - indicators of effects (adverse outcome pathways) at various levels of organization

Suite of Fish Biological Indicators

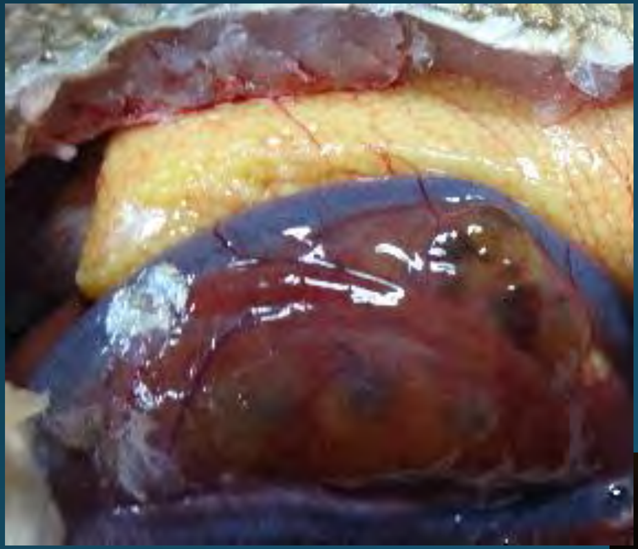
- Morphometric and necropsy-based
  - Comparisons based on sex, age,
  - Identify visible abnormalities
  - Provides condition factor/relative weight
- Plasma
  - Hormones – estrogen, testosterone, cortisol, thyroid
  - Vitellogenin
- Histopathological
  - Diagnose causes of gross observations, identify 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-β, hepcidin), contaminant-related (CYP1A, oxidative stress), stress (glucocorticoid receptors)

Lessons Learned

- All fish are not created equal
- Different species sensitivities to a variety of contaminants
- Species choice and choice of the biomarkers are very important
- Multiple contaminants can have the same effect and so “the cause” in one environmental setting may not be “the cause” in another setting/landuse

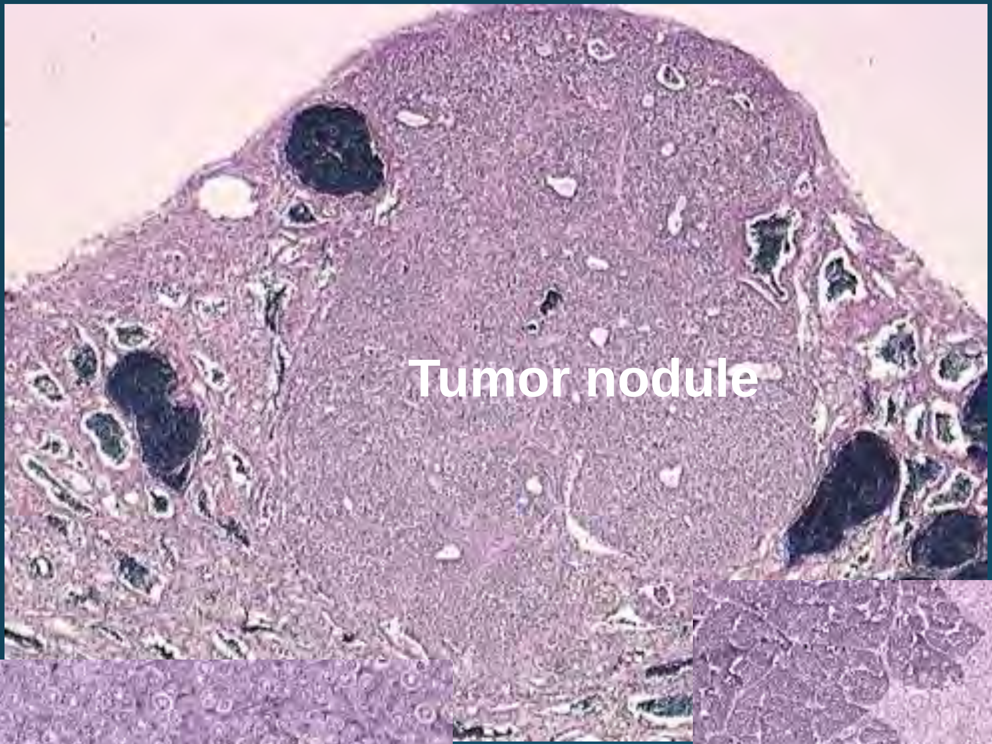
Examples of Fish Health Issues

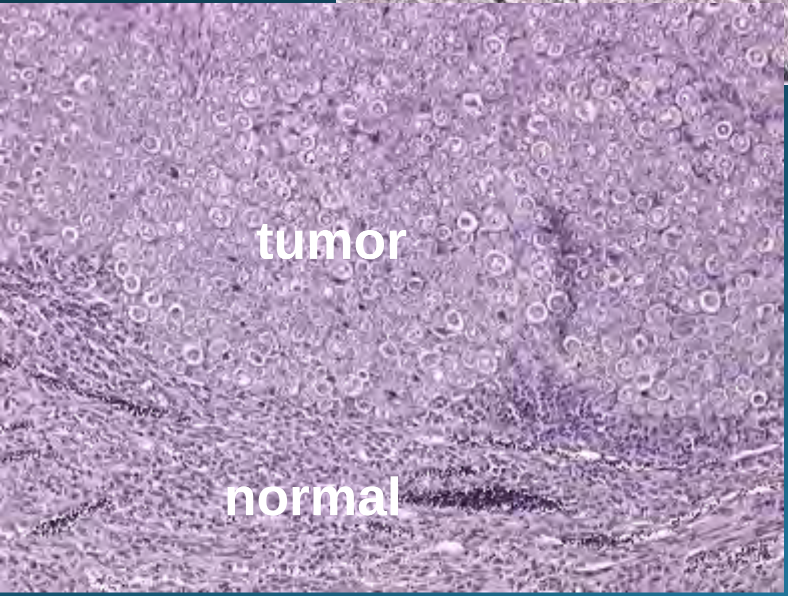
- Skin and liver tumors of brown bullhead and white sucker – indicator at Great Lakes Areas of Concern – PAHs, PCBs

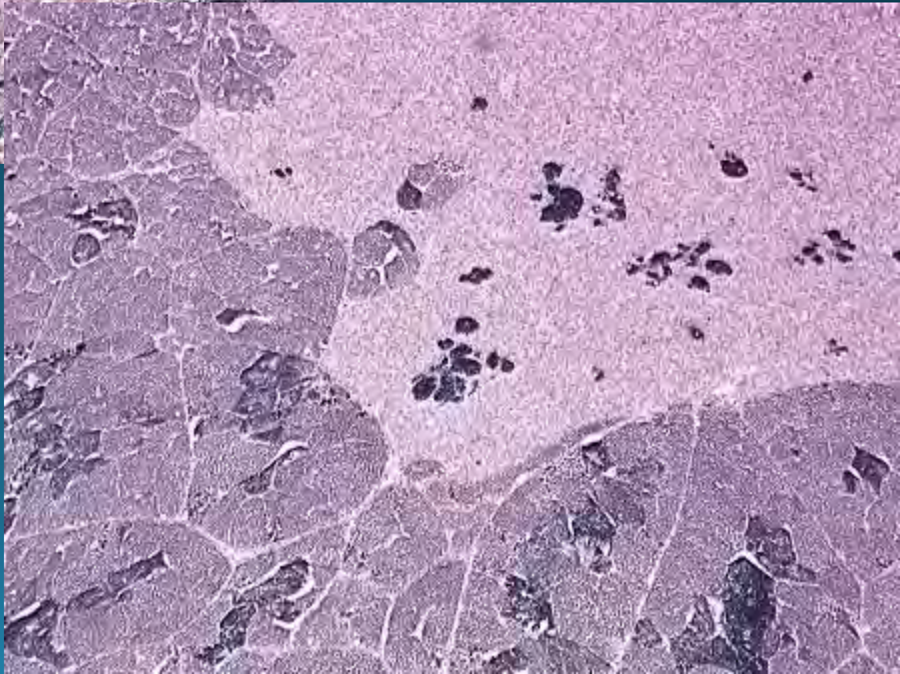


Yellow Perch Reproductive Declines/Testicular tumors

Perch Testicular Neoplasia







Testicular Tumors (Seminoma) Lake Michigan Yellow Perch

Sample Time	Prevalence
Winter	31%
Spring	27%
Fall	25%

Testicular Dysgenesis Syndrome in Humans

- Increase in male reproductive system problems
  - Declining sperm counts
  - Reduced semen quality
  - Genital abnormalities - cryptorchidism
  - Reduction in testicle size
  - Increased prevalence of testicular cancer - seminoma

Suggestion this syndrome is result of disruption of fetal programming and gonadal development during fetal life and is related to adverse environmental influences/contaminant exposures or other factors affecting hormone levels.

Results for Male Perch

Testicular dysgenesis syndrome in Lake Michigan yellow perch?

Lake Michigan compared to Lake Mendota

- Smaller testes – smaller GSI
- Presence of a variety of gonadal abnormalities
- High prevalence of testicular tumors

Smallmouth Bass Fish Kills
Endocrine Disruption

- In 2003 at the request of WV DNR we began sampling adult smallmouth bass in the South Branch Potomac because of spring fish kills and skin lesions
- In 2004 at the request of VA DGIF we began sampling smallmouth in the Shenandoah River because of adult fish kills and skin lesions
- In 2005 PA F & B sent samples of diseased YOY smallmouth bass
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Adult Fish in the Potomac

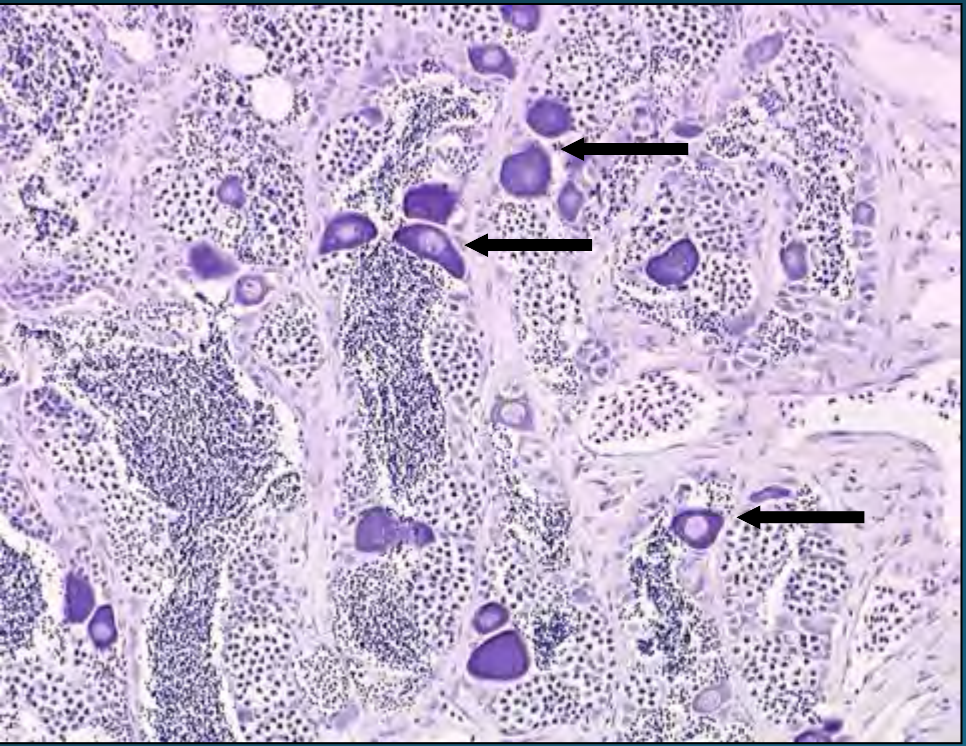
- Multiple bacterial pathogens, but no consistent findings
  - *Aeromonas hydrophila* and other motile Aeromonads
  - *Aeromonas salmonicida*
  - *Flavobacterium columnare*
- Multiple, often heavy parasite infestations
  - Leeches, trematodes, myxozoans, cestodes
- Opportunistic fungal infections
- Largemouth Bass Virus
- *High prevalence of intersex, vitellogenin in male fishes*

Impaired Ecosystem
Immunosuppression

Susquehanna Drainage - YOY

- *Aeromonas hydrophila* and other motile Aeromonads
- *Flavobacterium columnare*
- Largemouth Bass Virus
- Trematodes, cestode
- Myxozoan parasite

Intersex in Gonochorist Fishes



- Suggested as a marker of endocrine disruption
- Most often associated with exposure to estrogenic compounds

Biomarkers of Exposure Estrogenic Contaminants

- Intersex
  - most likely induced very early
  - exposure during sexual differentiation increases sensitivity later in life
- Vitellogenin in males
  - Plasma Vtg - indicative of recent exposure (days to months)
  - Vtg gene transcripts – hours to days

Effects of Estrogen on Fish Leukocytes and Disease Resistance

- **Estrogen exposure reduces phagocytic index** – ability of phagocytic cells, such as macrophages and neutrophils, to engulf infectious agents

- **Estrogen influences bactericidal activity** – ability of these cells to destroy engulfed agents by influencing at least two killing mechanisms:
  - **nitric oxide production**
  - **respiratory burst activity**

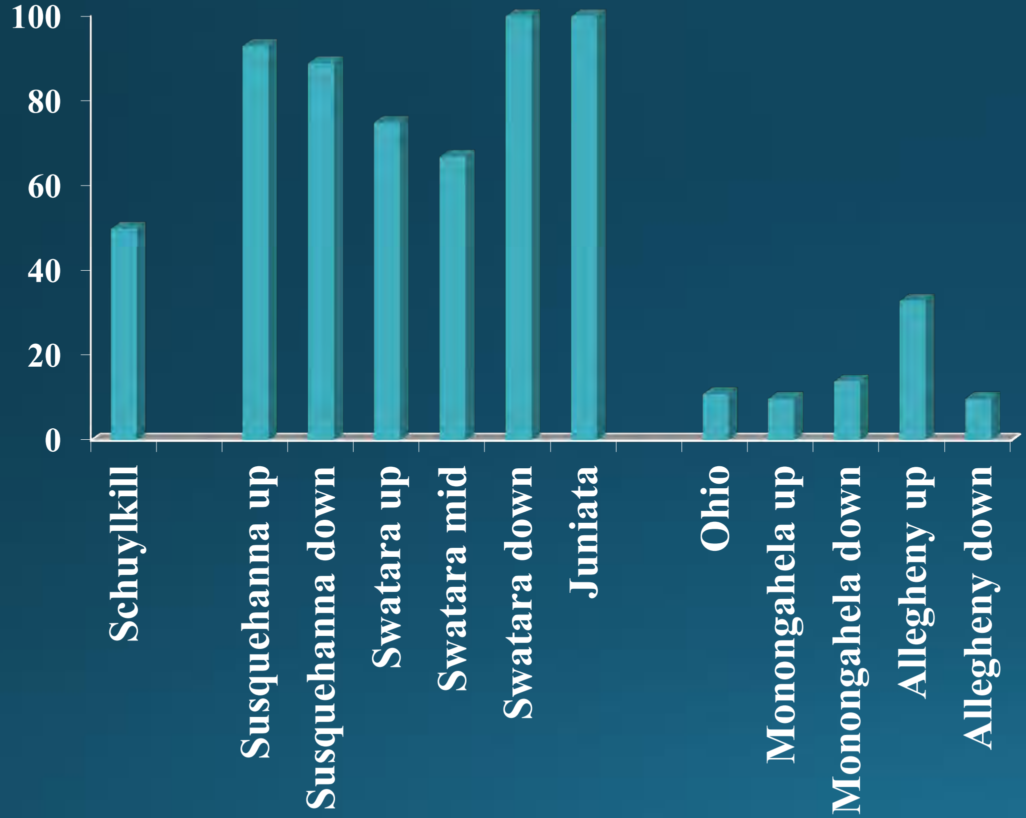
Findings
Biological Effects and Sources

- A number of our studies have evaluated fish upstream and downstream of WWTP
  - Do not see higher prevalence of intersex or skin lesions at downstream sites, however, in both Potomac and Susquehanna studies intersex severity was slightly higher at most downstream sites
- Do consistently find an association with agricultural landuse

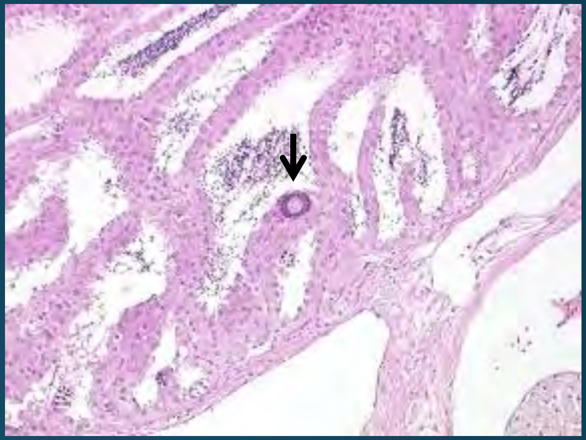
Associations with Contaminants

- PA study, low flow, summer – correlations with estrone
- Potomac study, spring, prespawn
  - atrazine and metalochlor conc. correlated with prevalence and severity of intersex
  - Total biogenic hormones and plants sterols in sediment correlated with prevalence and severity

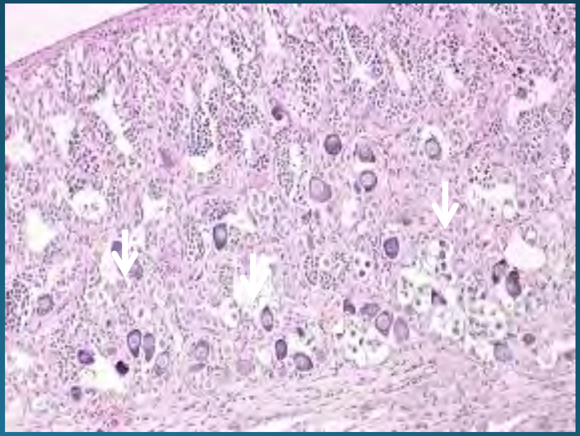
Intersex in SMB from PA River Drainages



Ohio Drainage



Susquehanna Drainage



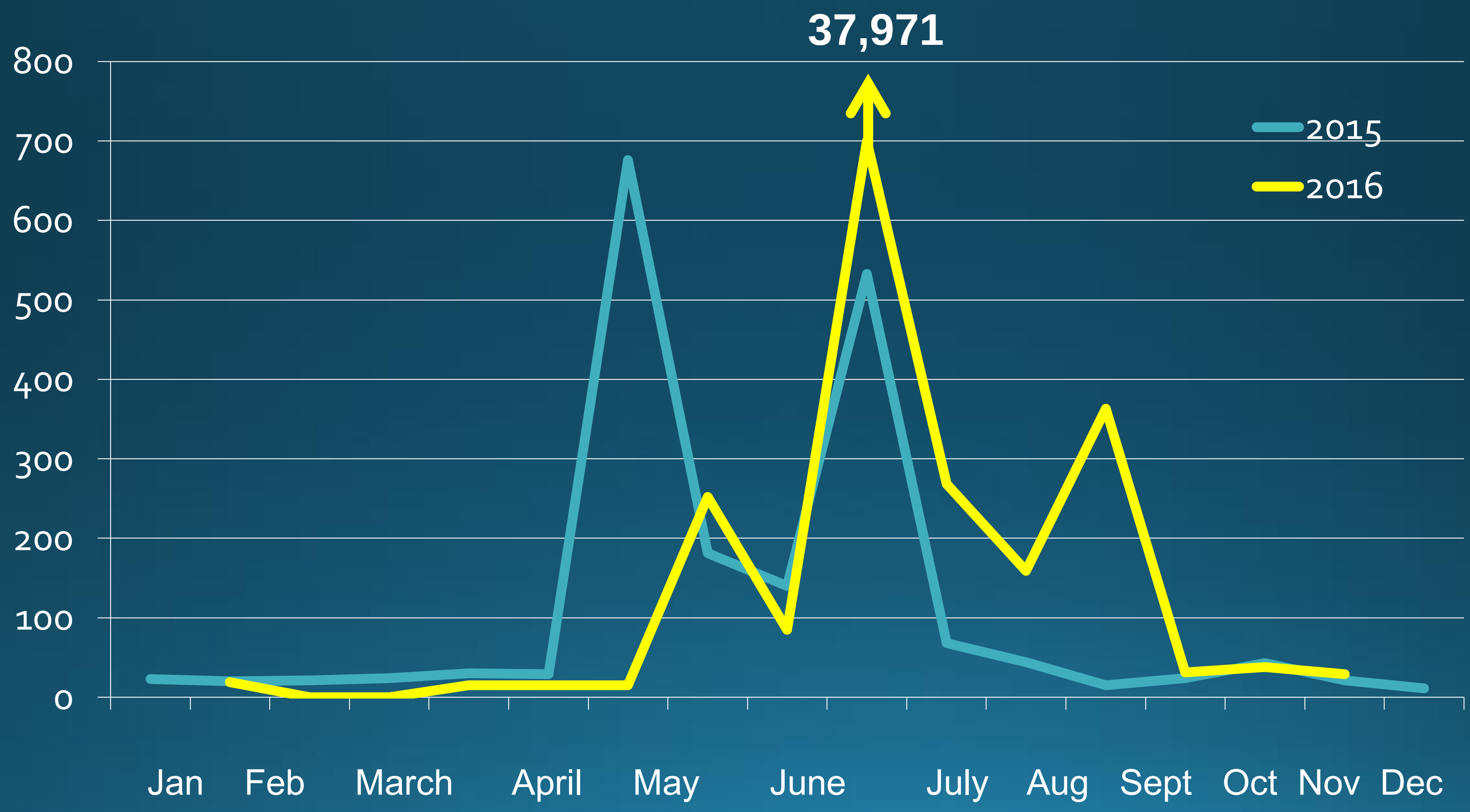
Factors to Consider

- Temporal nature of chemical exposures – climatic and other factors that influence exposure
- Exposure pathways during critical life stage

Chesapeake Bay Endocrine Disruption Study Agricultural Integrator Sites

- Long term monitoring – allow us to look at year classes
- 4 - 6 sites where monthly to biweekly water samples, plus storm events, are collected and analyzed for hormones, pesticides, phytoestrogens and total estrogenicity from Nov. 2014 to present
- Sediment samples in spring and fall
- Tissue samples for contaminant analyses
- Adult fish are collected in the spring prespawn (2013-2017) and at some sites in the summer and fall
- YOY (1-3 months of age) are collected in early summer (2013-2017)

Chillisquaque Atrazine Concentrations (ng/L)



Approach

- Effects-based monitoring of wild fishes
- Chemical analyses (water, sediment, tissue)
- In vitro bioassays of water samples to assess total estrogenicity, androgenicity, glucocorticoid, thyroid hormone activity

- Risk assessment/population modeling
- Geospatial/landuse/land use change (BMPs; restoration)

Acknowledgements

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