Chemicals in the Aquatic Environment
What are Fish Telling Us?

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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

Yellow Perch Male Gonads

Abnormal testes
Lake Michigan

Normal testes
Lake Mendota
Perch Testicular Neoplasia

Tumor nodule

tumor

normal
<table>
<thead>
<tr>
<th>Sample Time</th>
<th>Prevalence</th>
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<tbody>
<tr>
<td>Winter</td>
<td>31%</td>
</tr>
<tr>
<td>Spring</td>
<td>27%</td>
</tr>
<tr>
<td>Fall</td>
<td>25%</td>
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Testicular Tumors (Seminoma) Lake Michigan Yellow Perch
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.
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

Fish

- 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)

37,971

May June July Aug Sept Oct Nov Dec

2015

2016
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/land use change (BMPs; restoration)
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