Multi-level Assessment of Biological Effects Associated with Mercury Concentrations in Smallmouth Bass in the Chesapeake Bay Watershed

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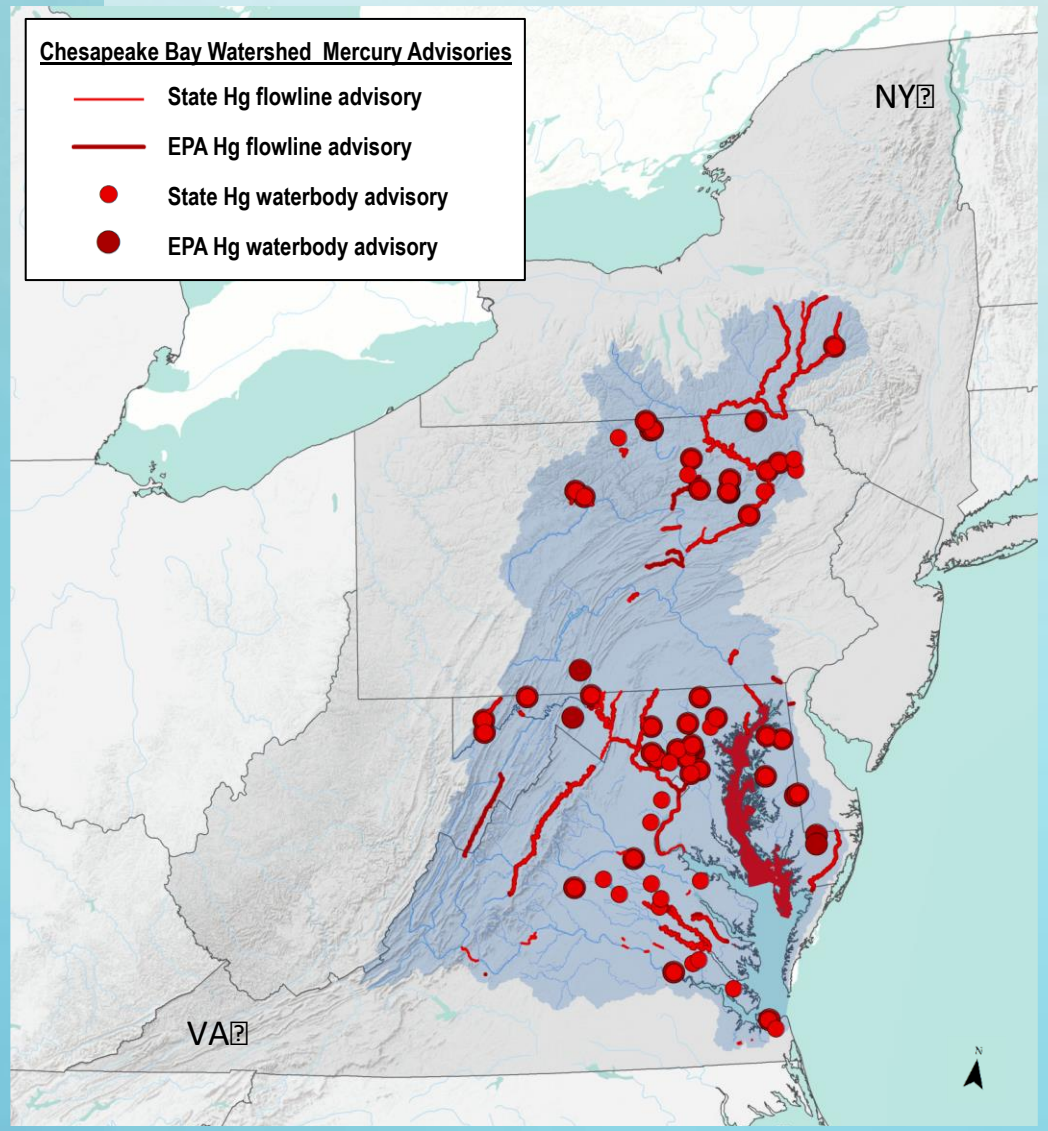
Mercury in the Chesapeake Bay Watershed



Mercury is responsible for more listed waters for human consumption advisories than any other pollutant

Mercury was identified as a priority for the Chesapeake Bay Toxic Contaminant Workgroup established in 2014

Mercury is a contaminant that has been shown to have both reproductive and health effects

Mercury in Freshwater Fishes in the Chesapeake Bay Watershed

- In 2020 Willacker et al. published a synthesis of mercury bioaccumulation in freshwater fishes
- Compiled data from state monitoring programs and research projects
- Summarized 7937 fish Hg measurements representing 61 species at 600 locations
- 32 species were well represented and geometric mean THg concentrations in fillet were highest in smallmouth bass which were also in the highest category (with striped bass, bowfin, walleye, largemouth bass, flathead catfish) when accounting for spatial and temporal variation
- Mean concentrations were highest in the Potomac and the Susquehanna

Smallmouth Bass Heath, Reproduction and Population Declines

- **Since 2002 we have monitored smallmouth bass health to determine risk factors/stressors/"causes" for observed fish kills, high occurrence of skin lesions, high prevalence of intersex (testicular oocytes) and population declines in the Susquehanna and Potomac watersheds.**
- **Signs of estrogenic endocrine disruption – intersex and vitellogenin (egg yolk precursor) in male bass**
- **Signs of immunomodulation - multiple parasites, bacteria and viruses associated with mortality and skin lesions**

Integrated Sampling

- **Between 2013 and 2021 smallmouth bass have been sampled at many sites throughout the Potomac and Susquehanna River drainages for fish health assessments**
- **2013-2019 integrated surface water and fish at 4 sites**
  - **Adult bass were sampled in spring (prespawn) and fall (recrudescence)**
  - **Young of year in early summer**
  - **Surface water was sampled monthly (bi-monthly in spring/early summer)**
  - **Focus on agricultural land use and estrogenic endocrine disruption and so routinely pesticides, hormones, phytoestrogens were analyzed with pharmaceuticals and wastewater less often**
- **Numerous other sites were sampled in conjunction with state agencies in MD, PA, and WV in response to management needs/requests**

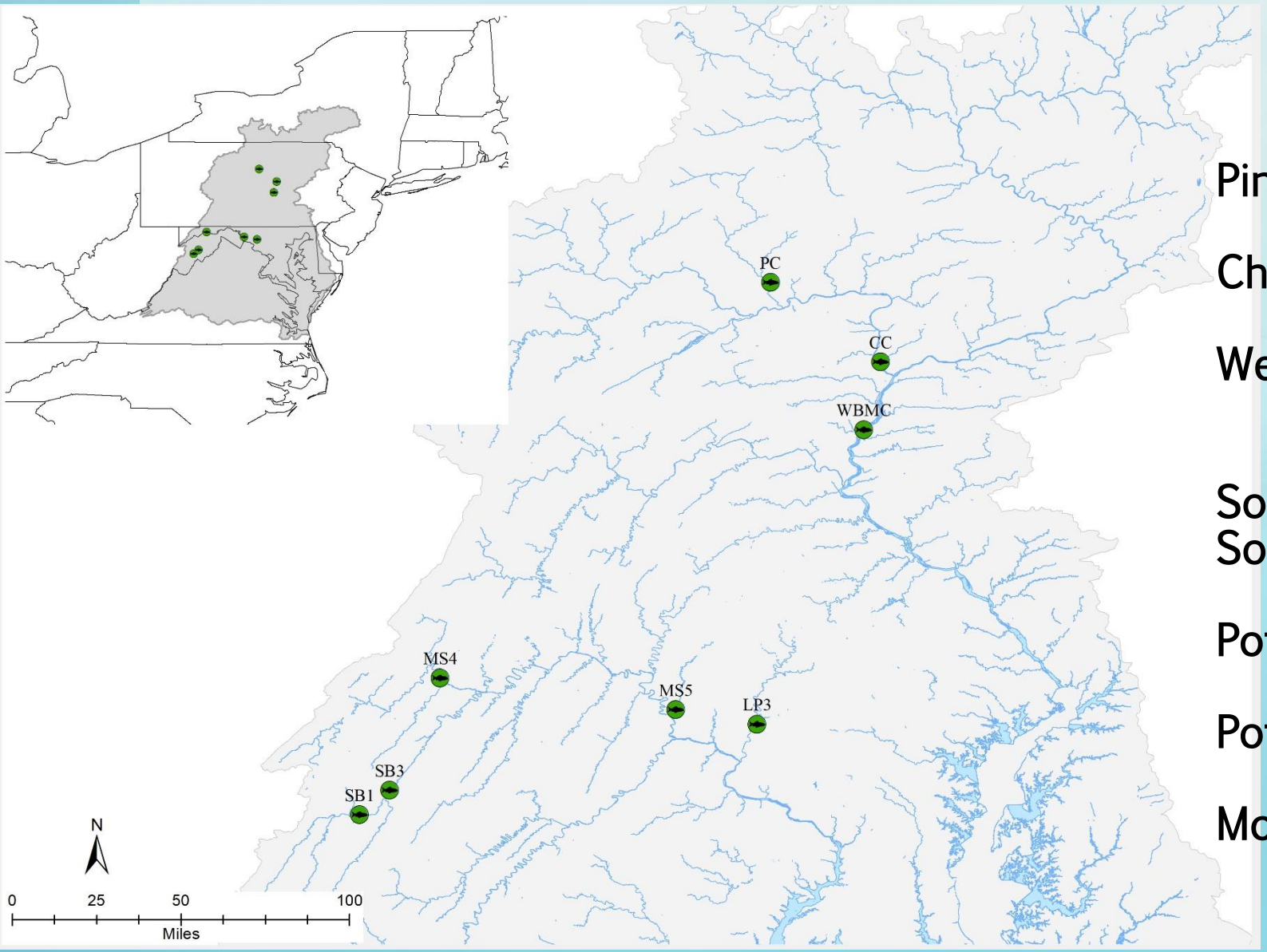
Adult Fish Sampling

- **Weigh, measure, document visible abnormalities, blood sample, preserve pieces of all organs for histopathological (cellular level) and pieces of liver, gonad, anterior kidney for gene expression (molecular level). Otoliths are collected for age analysis**
- **Multi-level biological effects monitoring – from the organism level (visible abnormalities/condition factor) to the molecular**
- **From numerous sites also archive plasma, muscle, gonad and liver for potential contaminant analyses should funding become available.**

Mercury in Smallmouth Bass

- **A total of 294 smallmouth bass samples (muscle or liver) collected at 9 sites in the Potomac and Susquehanna were analyzed for total mercury**
- **273 were collected in the spring of 2013 or 2017 at 8 sites and we focused on those to assess associations with biological endpoints**
- **For bass with only liver the muscle (fillet) total Hg was estimated based on the relationship between liver and muscle when both were analyzed and compared**

Sites – Spring Samples



Pine Creek (PC)

Chillisquaque Creek (CC)

West Branch Mahantango Creek (WBMC)

South Branch Potomac Petersburg (SB1)
South Branch Potomac Moorefield (SB3)

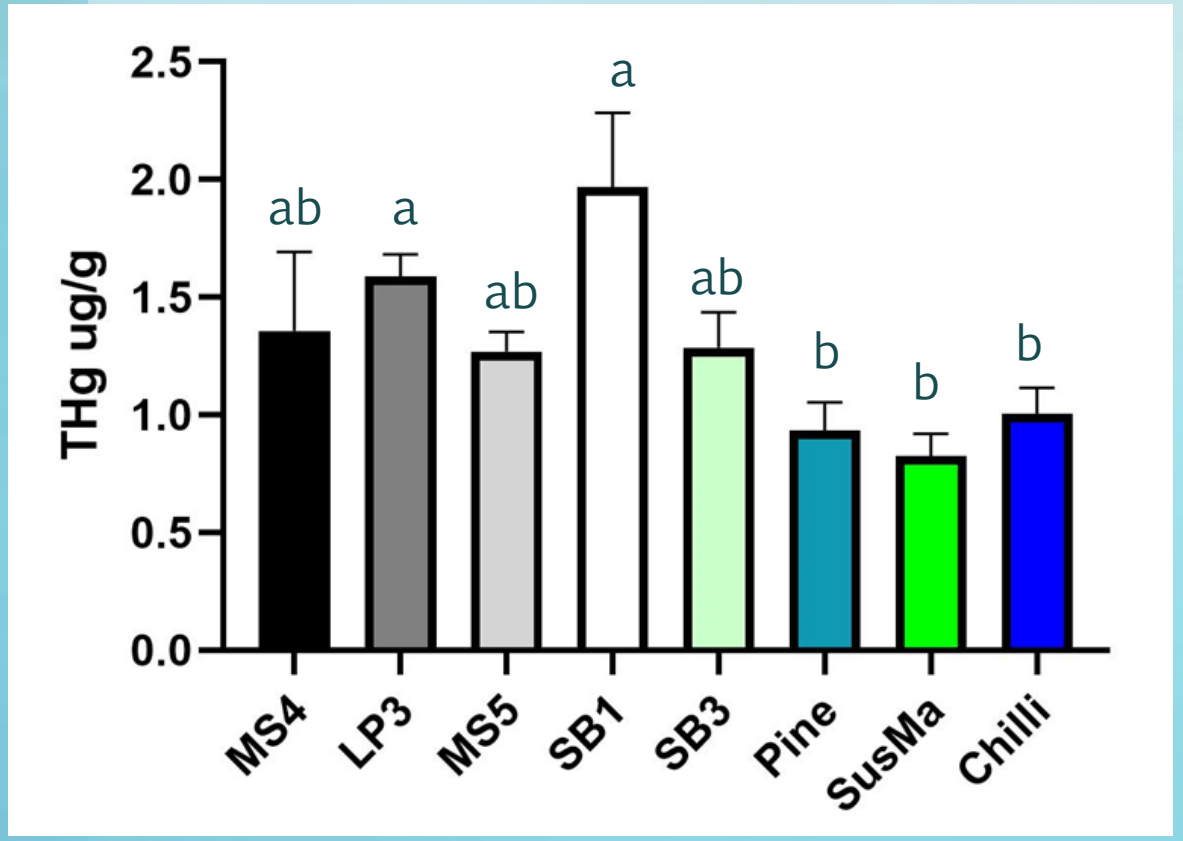
Potomac River at Cumberland (MS4)

Potomac River at Antietam Creek (MS5)

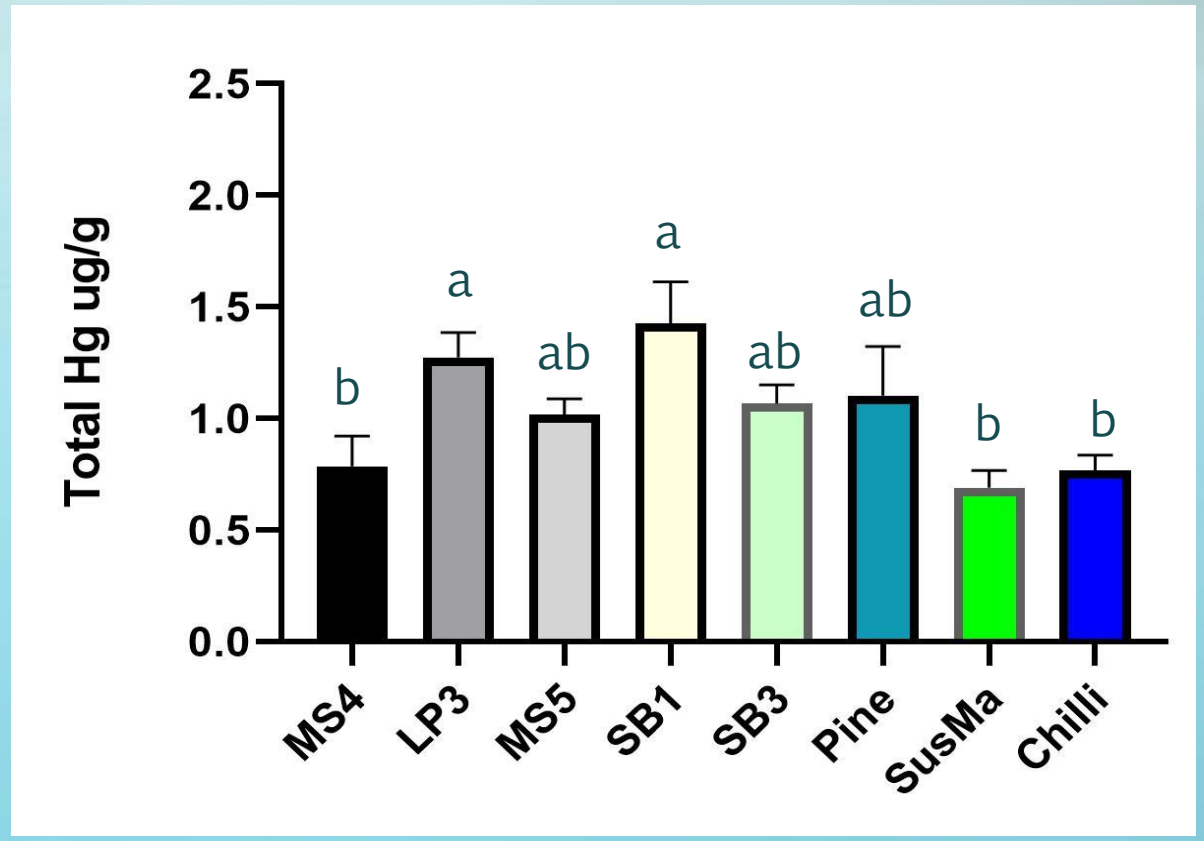
Monocacy River (LP3)

Site and Sex Differences
Mercury in Potomac and Susquehanna

Female



Male



Multi-level Biological Indicators

- **Organismal**
  - **Condition factor**
  - **Health Assessment Index**
- **Organ**
  - **Hepatosomatic index**
  - **Gonadosomatic index**
  - **Visual abnormalities**
- **Tissue/Cellular**
  - **Macrophage aggregate density**
  - **Splenic, hepatic and kidney parasite density**
  - **Testicular oocytes – prevalence, severity rating**
- **Plasma Analyses**
  - **Vitellogenin, estradiol, 11-keto testosterone, testosterone**
- **Molecular**
  - **Transcript abundance of 50 hepatic genes**

Correlation of Mercury with Morphometric and Organ Endpoints in Smallmouth Bass

	Spearman Correlation Coefficients			
Parameter	**Female**	**p value**	**Male**	**p value**
Age	**0.56**	**< 0.0001**	**0.64**	**< 0.0001**
Length	**0.23**	**0.0076**	**0.21**	**0.0111**
Weight	**0.19**	**0.0237**	**0.20**	**0.0205**
Hepatosomatic Index	**0.27**	**0.0012**	**0.26**	**0.0029**

Condition factor and gonadosomatic index were not correlated with THg

Health Assessment Index (HAI)

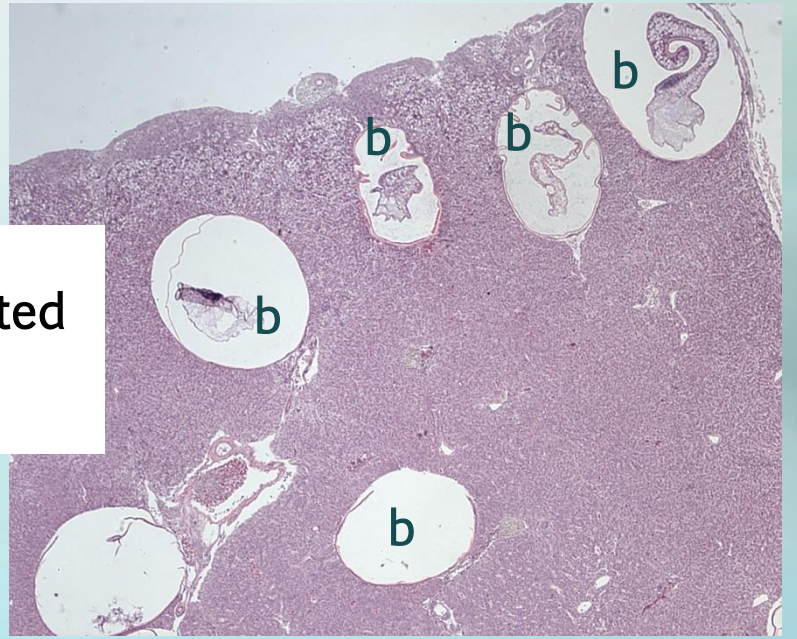
- **Based on visual observation of both external and internal organs with a numerical rating of some changes based on severity**
- **Fins and body surface, gills, eyes, liver, spleen, anterior kidney, posterior kidney, swimbladder, gonad, other**
- **Fins and body surface:**
  - **Erosions/ulcer 30**
  - **Raised/reddened 30**
  - **Melanistic areas 30**
  - **Leech 10**
  - **Black spots 10**
  - **Parasite cysts/grubs 10**

Eyes

- **Opaque 30**
- **Missing 30**
- **Exopthalmia 30**
- **Other 30**

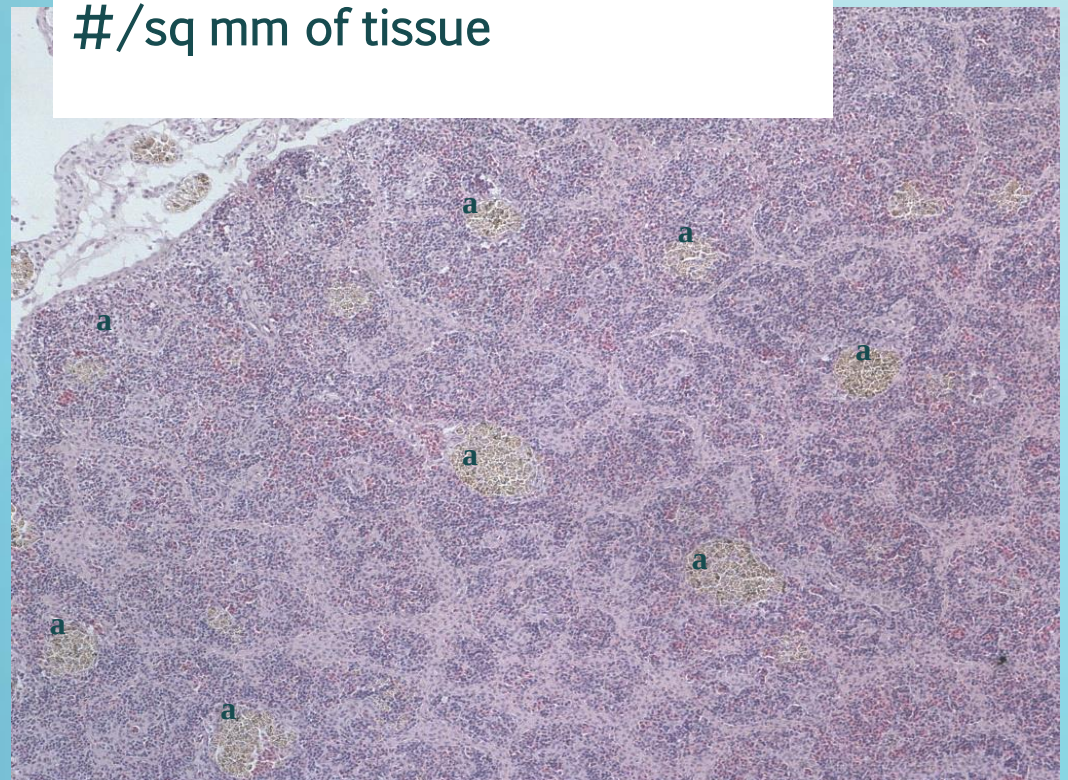
Numerical ratings for all organs are summed for index value that can be compared statistically

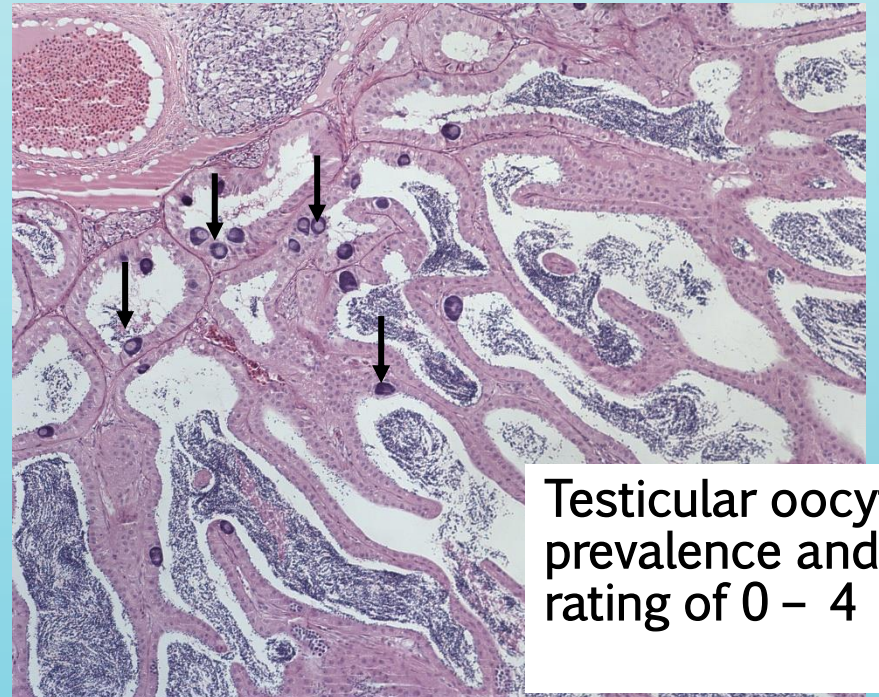
Histological (Cellular) Indicators



Parasite density – counted and calculated #/sq mm of tissue

Macrophage aggregate density – counted and calculated #/sq mm of tissue





Testicular oocytes – prevalence and severity rating of 0 – 4

Correlations with Health Endpoints and Mercury

	Spearman Correlation Coefficients			
Parameter	**Female**	**p value**	**Male**	**p value**
HAI	**0.37**	**< 0.0001**	**0.41**	**< 0.0001**
Liver parasites	**0.36**	**< 0.0001**	**0.23**	**0.0069**
Spleen parasites	**0.30**	**0.0004**	**0.27**	**0.0017**
Liver macrophage aggregates	**0.25**	**0.0033**	**0.36**	**< 0.0001**
Spleen macrophage aggregates	**ns**	**ns**	**0.34**	**0.0001**

Correlations with Liver Transcripts Associated with Immunity and Inflammation

	Spearman Correlation Coefficients			
Transcripts	Female	p value	Male	p value
Transforming growth factor β (*tgfβ*)	0.28	0.0022	ns	ns
Arginase (*arg*)	-0.30	0.0011	-0.30	0.0010
Apolipoprotein A1 (*apa1*)	ns	ns	-0.39	<0.0001
Heme oxygenase 1B (*h01b*)	ns	ns	0.23	0.0122
C-reactive-protein-like (*crp-like*)	ns	ns	0.42	<0.0001
C3 complement (*c3*)	ns	ns	0.23	0.0133

Correlations with Reproductive Endpoints

	Spearman Correlation Coefficients			
Parameter	**Female**	**p value**	**Male**	**p value**
Plasma vitellogenin	**-0.37**	**< 0.0001**	**0.47**	**< 0.0001**
Plasma estradiol	**ns**	**ns**	**0.21**	**0.0156**
***vtg* (vitellogenin)**	**-0.20**	**0.0334**	**ns**	**ns**
***chg* (choriogenin)**	**-0.23**	**0.0120**	**ns**	**ns**
***erα* (estrogen receptor α)**	**ns**	**ns**	**-0.32**	**0.0005**
***erβ1* (estrogen receptor β1)**	**ns**	**ns**	**0.31**	**0.0006**
***erβ2* (estrogen receptor β2)**	**-0.24**	**0.0097**	**-0.31**	**0.0006**
***arα* (androgen receptor α)**	**ns**	**ns**	**0.20**	**0.0336**
***arβ* (androgen receptor β)**	**ns**	**ns**	**-0.20**	**0.0299**

Correlations with Other Indicators

- No correlation between THg and testicular oocyte prevalence (high at all sites) or severity
- Correlations with thyroid transcripts and metallothionein

	Spearman Correlation Coefficients			
Parameter	**Female**	**p value**	**Male**	**p value**
thrβ **(thyroid hormone receptor)**	**-0.21**	**0.0243**	**-0.30**	**0.0011**
doi1 (**deiodinase 1)**	**-0.32**	**0.0005**	**-0.52**	**< 0.0001**
doi2 **(deiodinase 2)**	**ns**	**ns**	**-0.46**	**< 0.0001**
mt **(metallothionein)**	**0.33**	**0.0004**	**0.44**	**< 0.0001**

Summary

- **Although these are correlations and not necessarily cause and effect, the results suggest mercury may be an important factor in both the reproductive and disease issues in smallmouth bass**
- **Many of the effects have been associated with mercury exposure in other wild fish studies as well as laboratory exposures**
- **HOWEVER, these same fish have been exposed to numerous other stressors – high concentrations of pesticides and phytoestrogens in surface water in spring of certain years; PCBs, pesticides and PFAS have detected in tissues of YOY and/or adults.**
- **Cumulative effects of complex mixtures in wild populations need to be better understood**
- **Utilizing gene expression for adverse outcome pathways in conjunction with biological indicators at multiple levels will help tease out the role of various stressors in population declines**

Acknowledgements

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