Seasonal and Sexual Differences in Liver Histomorphology Of Fish Exposed to Hg and Se

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Introduction

Mercury (Hg) is a metal contaminant that is found globally throughout our environment. When deposited in aquatic systems, Hg methylation is facilitated primarily by sulfate–reducing bacteria under anoxic conditions, warm water temperatures, and low pH (Fuhrmann et al., 2021). The methylated form of Hg is the toxic form to both fish and those consuming them.

An essential nutrient, Selenium (Se), plays a role in redox reactions and physiological functions within the body and may relate to the effects of Hg on aquatic species. The role of Se in protecting against Hg toxicity is under debate. Knott et al., 2022 reported changes in liver size of largemouth bass in relation to Hg and Se concentrations in fillets were indicative of Hg toxicity and differed by sex and season.

This study will document potential effects that Hg and Se have on different aspects of the fish systemically. Data will support the use of hepatosomatic index (HSI) as a biomarker of hepatotoxic effects and identify confounding factors that should be considered when interpreting relationship between contaminant concentrations and HSI.

Hypothesis

Males: HSI is related to Hg toxicity as indicated by a greater incidence and severity of histomorphologic changes than females and depleted glycogen as indicated by Periodic Acid-Schiff (PAS).

- ➢ Indicators of inflammation and oxidative stress will be positively related to HSI and reduced Se concentration, while indicators of necrosis and reduced glycogen content will be negatively correlated to the Se:Hg molar ratio. These changes will be independent of season.

Females: HSI is related to reproductive status as indicated by vitellogenin content, but unrelated to metal concentration.

- ➢ Histomorphologic changes will be less prominent than compared to males, and HSI will be positively related to vitellogenin during spring. These changes will be independent of metal concentration.

References

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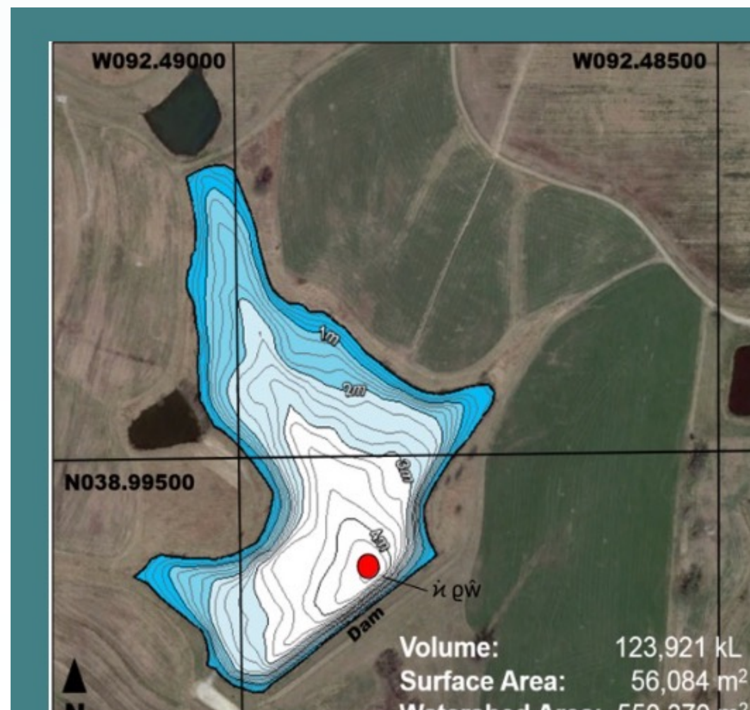
Methodology

Livers of largemouth bass used in this study were from the same fish collected from Dairy Farm Lake #1 and examined for Hg and Se in Knott et al., 2022. Only livers from LMB collected from spring and summer were used for histomorphologic assessment. Briefly, fish were collected by electroshocking. The mass and length of fish were recorded. Sex was identified and the liver mass was recorded. Fish were filleted for Hg and Se concentration and otoliths removed for aging. The HSI was calculated by (liver mass / body mass) * 100. A subsample of liver was taken for histology.

A total of 31 livers were fixed, embedded in paraffin, and mounted onto 2 separate slides. Sections were stained with H&E and PAS. Slides were randomized, and the reader was blinded to fish ID during histomorphologic assessments. Incidence and severity grading of changes were recorded.

Examined Changes	
• Small Vacuolization • Large Vacuolization • Necrosis • Inflammation	• Fibrosis • Necrosis • Parasites • Hepatocyte Density (Higher count per field)

Grading System Based Off Total Tissue Section	
• Small Vacuolization • Large Vacuolization • Necrosis • Inflammation • Fibrosis • Necrosis • Parasites • Hepatocyte Density	0 = none seen 1 = up to 25% affected 2 = 25 – 50% affected 3 = > 50% affected
• Parasites	Count < 4 Count > 4



Bathymetry of Dairy Farm Lake #1, Boone County, Missouri where largemouth bass samples were collected.

Results

Incidence and Severity of Changes by Season and Sex
Data shown as number of fish affected and severity grading 1-3 in parentheses

	Spring Female (n = 7)	Summer Female (n = 13)	Spring Male (n = 6)	Summer Male (n = 5)
Small Vacuolization	4 (1-2)	12 (1-3)	6 (1-3)	5 (1-2)
Large Vacuolization	2 (1-2)	8 (1-3)	2 (2-3)	4 (2-3)
Necrosis	2 (All 1)	12 (1-3)	4 (1-2)	5 (1-3)
Inflammation	1 (All 1)	12 (1-2)	4 (1-2)	4 (1-2)
Fibrosis	1 (All 1)	11 (1-2)	5 (1-2)	5 (1-2)
Parasites	5 (Count < 4) 2 (Count > 4)	9 (Count < 4) 4 (Count > 4)	5 (Count < 4) 1 (Count > 4)	1 (Count < 4) 4 (Count > 4)
Hepatocyte Density	0	0	0	2 present

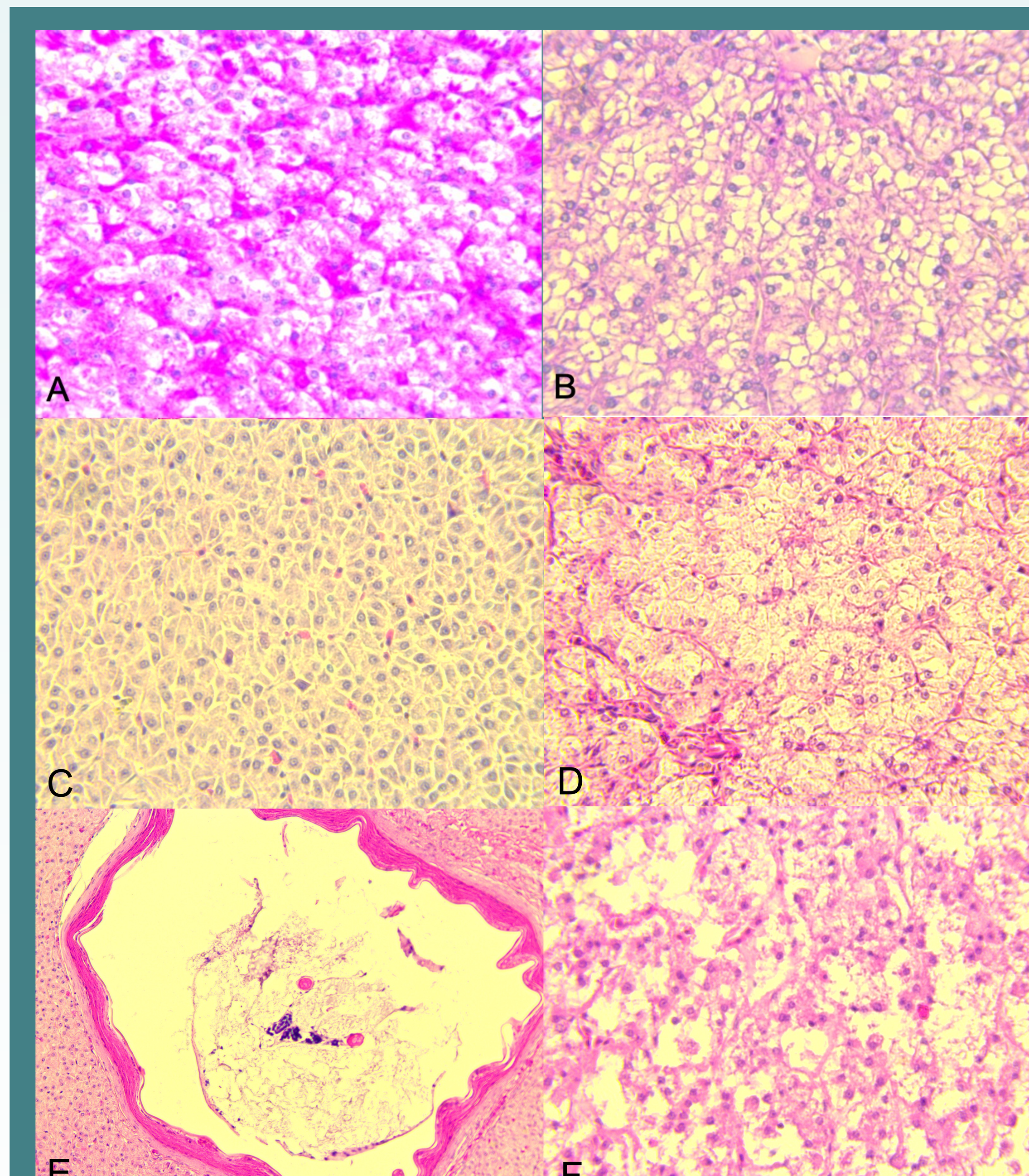


Figure 1. Histologic slides of largemouth bass livers at 40x. A. PAS (+) stained liver from summer male B. PAS (+) stained liver from summer female C. Increased hepatocyte density, note decreased cytoplasm size and increased hepatocytes per field D. larger hepatocytes, note increased cytoplasm size and small/large vacuolization E. parasite infiltrate surrounded by necrosis F. area of necrosis unassociated with parasite infiltrate, large clear vacuolization

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Conclusion

- This is our initial review of the data and we plan to further define our description and classifications of histomorphologic changes.
- This preliminary assessment suggests that:
  - ➢ Summer fish had a greater incidence of changes than spring fish regardless of sex.
  - ➢ The fewest anomalies occurred for spring females.
  - ➢ Only a few individuals exhibited necrosis, inflammation, and fibrosis that was not apparently associated with parasites.
  - ➢ Males may have darker PAS staining than females indicating greater glycogen content in hepatocytes (slides still in review).
- After we are confident of our estimates of incidence and grade, we will examine whether changes related to metals and HSI by using a quantitative health assessment index (Adams et al., 1993) and regression tree analyses.
- Further evaluation of slides will include an evaluation of vitellogenin content by immunohistochemistry to determine reproductive status.