.

Mercury Bioaccumulation and Hepatosomatic Index of Yellow Perch (*Perca flavescens*) in Drainage versus Seepage Lakes in Upper Peninsula Michigan

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Abstract

Mercury is found around the world, causing both environmental and human health impacts. Bioaccumulation of mercury in fish tissues is dependent on numerous characteristics of bodies of water such as flow of water through a system. Bioaccumulation of mercury is important to understand because of the important role fish plays in the diets of cultures around the world. Yellow Perch (*Perca flavescens*) were collected by angling from five different study locations with varying water flow characteristics and analyzed for Hepatosomatic Index (HSI) and Total Mercury (ug/g) concentration in muscle tissue. For total mercury concentration based on location, a significantly higher concentration of mercury was observed in Morris Lake compared to Tenderfoot Lake and Tenderfoot Creek. Tenderfoot Lake and the Ontonagon River were observed to have a significantly higher HSI compared to Morris Lake. Furthermore, there were no significant differences in total mercury concentration and HSI detected between Palmer Lake, the Ontonagon River, Tenderfoot Lake, and Tenderfoot Creek, which all form a system. There was no relationship found between total mercury concentration and HSI. Total mercury concentration was found to be the least in a system with flowing water compared to an isolated seepage lake. There was no evidence showing that total mercury concentration had an effect on HSI.

Key Words: Yellow Perch (*Perca flavescens*); Mercury bioaccumulation; Hepatosomatic Index; Drainage and Seepage Lake; Trophic Levels; Human Consumption; Environmental Impacts

Introduction

Mercury is known to be a harmful contaminant to both wildlife and humans, and it is found throughout the world in numerous different habitats. In the United States, 35% of all freshwater systems have restrictions on fish consumption due to the risk factors to human health

associated with mercury levels (Ward et al. 2010). There are several sources of mercury that can contribute to the bioaccumulation in aquatic predators. Deposition of atmospheric mercury, contribution of mercury from surrounding wetlands, amount of urbanization, and general productivity of an area all contribute as possible sources of mercury in aquatic systems (Chasar et al. 2009). Anthropogenic sources of mercury input from the atmosphere range from mining, coal burning power plants, and metal smelting, all of which are historically rooted in the Great Lakes region. Even after metal smelting sites were abandoned, the mass of mercury left behind still threatens surrounding habitats at concentrations of up to two orders of magnitude larger than the site had emitted into the atmosphere during the smelting process (Kerfoot et al. 2016).

Another natural source of mercury in the environment includes wetlands. Fluctuating water regimes, often characteristic of wetlands, can enhance methylation rates of mercury and facilitate the release of mercury from sediments. This can have great impacts on downstream habitats by increasing the amount of mercury available in the environment for bioaccumulation (Ackerman & Eagles-Smith 2010). In remote or pristine lakes, the aquatic mercury cycle is primarily dominated by direct inputs from the atmosphere via the surface of the water body or indirectly through runoff from the surrounding watershed. Terrestrial organic matter accumulated by terrestrial runoff is also a possible major source of mercury into aquatic systems (Watras & Morrison 2008).

Numerous environmental and ecological factors play roles in how effective mercury can be bioaccumulated. Environmental factors include mercury loading rates, pH, dissolved organic carbon, and temperature. Furthermore, ecological factors include system productivity, number of trophic levels, and trophic position (Watras et al. 1998). In aquatic systems, persistent hydrophobic chemicals and heavy metals can accumulate in several different ways: direct

diffusion across gills or skin, ingestion of suspended particles, and the consumption of contaminated prey species. Accumulation of mercury, a heavy metal, in the tissues of fish, such as Yellow Perch (*Perca flavescens*), likely result from the consumption of contaminated food sources (Van der Oost & Vermeulen 2003). Yellow Perch have a sub-terminal mouth which allows them to be efficient at benthic feeding (Guzzo et al. 2011). If contaminants are released from sediments, benthic organisms are likely to uptake the contaminant, making it available to bioaccumulate into benthic predators (Van der Oost & Vermeulen 2003).

Predator fish are located at the top of food chains. Therefore, they are exposed to mercury from their surroundings and diet throughout their entire lifespan, causing concern for toxic consequences (Has-Schön et al. 2015). At a concentration of 0.5 to 1.0 ug/g of mercury, fish can begin to experience changes in biochemical processes, reduced reproduction and fecundity, and damage to cell structure and tissues (Sheuhammer et al. 2015). The conditions of fish are dependent on several environmental factors, such as heavy metal contamination for instance. Hepatosomatic Index (HSI) is a common metric used to determine fish condition using a ratio of liver weight to body weight in order to estimate energy reserves, possible parasitism, and overall health of fish (Rajotte & Couture 2002).

Since mercury is known to bioaccumulate, top level predators are most likely affected by mercury toxicity (Scheuhammer et al. 2015). Humans along with fish are exposed to mercury primarily through diet (Bradley et al. 2017). Since fish are a widespread component of the human diet, especially in many developing countries where fish is a primary source of protein, humans are at risk for experiencing negative effects caused by mercury consumption (Marine 2011). Health effects suffered by humans from exposure to toxic levels of mercury include immune system suppression, neurodevelopmental complications in children, and cardiovascular

health and infertility issues in adults (Selin et al. 2010). As a result of both ecosystem and human threats due to mercury in the environment, whether from natural or anthropogenic sources, mercury is a major cause for concern.

In this study, we set out to look at how the type of lake and connectivity of lakes affects mercury bioaccumulation in Yellow Perch. The difference in the amount of water carrying terrestrial organic matter due to watershed extensions and water cycling through a system could have effects on mercury bioaccumulation. Lakes in northern Wisconsin and Michigan are ideal for studying mercury bioaccumulation because the areas are remote, reducing the effects of anthropogenic sources of mercury into the systems (Grieb et al. 1990). If tributary streams transport organic material and sediment containing mercury into downstream lakes, then the total mercury concentration in the muscle tissues of fish will be higher in drainage lakes compared to seepage lakes due to bioaccumulation.

Materials and Methods

Study Site

The University of Notre Dame Environmental Research Center (UNDERC) lies on the border of Wisconsin and the Upper Peninsula of Michigan in Vilas County, Wisconsin and Gogebic County, Michigan. Within the 7,500 acre research center, numerous lakes classified as both drainage and seepage lakes exist. Morris Lake, Palmer Lake, Tenderfoot Lake, the Ontonagon River, and Tenderfoot Creek were used as study locations. Morris Lake is 12 acres (4.9 ha) in size and is an isolated seepage lake. Palmer Lake is a 644 acre (260 ha) drainage lake connected to Tenderfoot Lake by the Ontonagon River. Tenderfoot Lake is 442 acres (179 ha) in size and also a drainage lake. The Ontonagon River is a navigable 1.1 mile (1.73 km) stretch of river running East-West connecting the Northern side of Palmer Lake to the South Side of

Tenderfoot Lake. Tenderfoot Creek drains the whole system out the North end of Tenderfoot Lake, and 3.0 miles (4.87 km) of Tenderfoot Creek lies on UNDERC property. In all three lakes and the Ontonagon River, both emergent and submergent vegetation were targeted to catch Yellow Perch. In Tenderfoot Creek, deep holes were targeted for Yellow Perch. The Ontonagon River was sampled halfway between Palmer Lake and Tenderfoot Lake. Tenderfoot Creek was sampled at the northern stretch on UNDERC property. These areas were chosen to reduce possible effects on mercury levels within our study locations due to migration among populations.

Techniques

All Yellow Perch specimens were collected by angling. Fishing jigs imitating minnows were used. Yellow Perch specimens were kept alive until returning to shore for processing. The Yellow Perch were measured for total length and weighed for live weight while still alive. After total length and live weight measurements were taken, each specimen was submerged in a Tricaine methanesulfonate (MS-222) solution. Tap water was used to dilute the MS-222 powder to a concentration of 2.0 g/L. The specimens were left in the solution for one minute after movement had ceased to ensure death. Each specimen was labeled and placed on ice for preservation until further processing in the lab.

In the lab, each specimen was filleted to remove muscle tissue. The muscle tissue from each specimen was packaged separately from the carcass and labeled to correlate to the appropriate specimen. After filleting a specimen, the body cavity was cut open, and the liver was completely removed. The liver weight was recorded to calculate the hepatosomatic index. Processing surface and tools were rinsed with water and scrubbed with a sponge after the

completion of each specimen to prevent cross contamination between specimens. Labeled carcasses and fillets were stored frozen.

Mercury Analysis

Frozen Yellow Perch fillets were mailed to the Bioinorganic and Environmental Analysis Facility at Florida International University. In preparation of the analysis, three 4 mm cores using a stainless steel core tube were taken from the fillets (without scales or bones). The cores were combined and weighed (homogenate) before putting them into ampules. Tissue samples between 0.3 g and 0.4 g are used for analysis. For each batch of samples, which includes 20 samples, two aliquots of 0.02 g of SRM (unbaked DORM-2) are transferred to the ampules as QA/QC control samples. These samples are then incorporated to the unknown sample ampule set to follow the analytical procedure. 1 mL of DIW and 2 mL of concentrated HNO_3 were added into each ampule. Ampules were left standing for 20 minutes. The method blanks are prepared by adding 1 mL of DIW and 2 mL of concentrated HNO_3 to dry the ampules which were incorporated at this point to the unknown sample ampule set to follow the remaining steps. Ampules were sealed using Ampulmatic (Bioscience Inc.) ampule sealer. The ampule holder was then placed in a plastic tray, water added to the fill mark, and the container was covered with an inverted plastic pan to protect autoclave in case of ampule explosion. Ampules were autoclaved for one hour at 105 °C. After autoclaving, the ampules were left standing until room temperature was reached. Next, one 50-ml polyethylene vial per ampule was put in a tray. 40 ml of 1% HCL were added to each vial. The ampules were opened using an ampule cracker (a paper towel should be used to cover the ampules before opening due to explosion risk). A volume of the digested solution (1000 μL for the unknown samples and 50 μL for the SRM sample) was pipetted to the corresponding 50-ml polyethylene vial containing 40 ml of 1% HCl solution. The total mercury

analysis was carried out by running the calibration standards, method blanks, samples, and QA/QC control samples on a cold vapor atomic fluorescence spectrometry (CVAFS) from PS Analytical (Millennium/Merlin 10.035 Model). Instrument conditions for the equipment was set as follows:

PSA Vapor Generator

Delay–20 s

Analysis–60 s

Memory–60 s

Pump 1 Speed–Full

Pump 2 Speed–Full

Mercury Fluorescence Detector:

Carrier gas (argon) flow rate–A flow of 200 mL/min is kept constant by PSA system

Sheath gas (argon) flow rate–A flow of 330 mL/min is kept constant by PSA system

Air flow rate to the Perma Pure dryer–Controlled by a flow meter at 2500 mL/min

Range–1000

Filter–64.

Statistical Analyses

The Hepatosomatic Index (HSI) is a ratio of the liver weight to body weight of the Yellow Perch. The equation for HSI is as follows:

$$HSI = [\text{liver weight (g)} / \text{total body weight (g)}] \times 100$$

The Shapiro-Wilks test was used to determine if the data for total mercury concentration and HSI per location was normally distributed. Two one-way Analysis of Variances (ANOVAs) were used to test for statistical differences in total mercury concentration between locations and HSI

between locations. The Tukey Test was used to determine if there were significant differences between each location based on total mercury concentration and HSI after significant results were determined from the one-way ANOVAs. A Pearson Correlation test was used to determine if there was a relationship between total mercury concentration and HSI.

Results

A total of 42 specimens were collected and analyzed for HSI data, with 25 of the total number of specimens being analyzed for total mercury concentrations in the muscle tissue of the Yellow Perch. Five random specimens from each location were analyzed for total mercury concentration. The data from one specimen analyzed for total mercury was discarded from Morris Lake due to it being an outlier. The 24 specimens analyzed for both HSI and total mercury concentration were used for the correlation between HSI and total mercury concentration.

Average total mercury concentration for each location are Morris Lake=0.3079, Palmer Lake=0.2134, Ontonagon River=0.2230, Tenderfoot Lake=0.1621, Tenderfoot Creek=0.1683. Average HSI for each location are Morris Lake=1.0120, Palmer Lake=1.1948, Ontonagon River=1.7698, Tenderfoot Lake=1.5079, Tenderfoot Creek=1.3543 (Table 3).

For total mercury concentration based on location, there was a significant difference found among study sites ($F_{4,19}$=3.357 p-Value=0.031; Figure 1). A post-hoc test showed that Morris Lake was significantly higher in total mercury concentration compared to Tenderfoot Lake (p-Value=0.012). Morris Lake was also statistically higher in total mercury concentration compared to Tenderfoot Creek (p-Value=0.005). Between all other study sites, no significance was detected.

For the HSI data based on location, there was a significant difference found among study sites ($F_{4,37}$=4.274 p-Value=0.006; Figure 2). A post-hoc test showed that Morris Lake had a significantly lower HSI compared to the Ontonagon River (p-Value=0.006). Morris Lake also had a significantly lower HSI compared to Tenderfoot Lake (p-Value=0.041). Between all other study sites, no significance was detected.

When comparing HSI to total mercury concentration, there was not a statistically significant relationship detected (r =0.431 p-Value=0.431 N=24; Figure 3).

Discussion

The experimental results contradicted the original hypothesis. The average mercury concentration was highest in Morris Lake, which is an isolated seepage lake. These results indicate that systems with flowing water such as Tenderfoot Lake and Tenderfoot Creek reduce the amount of mercury bioaccumulated into Yellow Perch compared to a seepage lake, such as Morris Lake, where there is minimal water exchange. There was a trend in reducing bioaccumulation of mercury from Morris Lake to Palmer Lake and the Ontonagon River to finally Tenderfoot Lake and Tenderfoot Creek (Figure 1). The non-significant result between Palmer Lake, Ontonagon River, Tenderfoot Lake, and Tenderfoot Creek indicates that within a system, bioaccumulation of mercury is generally similar throughout the system. Bioaccumulation of metals is generally lower in flowing systems, such as rivers, compared to lakes, which have minimal water flow (Has-Schön et al. 2015). The Palmer and Tenderfoot Lake system is a flowing system even though they are considered lakes. Morris Lake is a seepage lake with little exchange or flow of water, indicating it should have a higher bioaccumulation rate.

Furthermore, hepatosomatic index is a biological measure of fish health. High HSI values can indicate an unhealthy fish due to increased activity to detoxify harmful heavy metals (Kumar et al. 2017). On the other hand, a high HSI can indicate a healthy fish in a rich environment with healthy energy reserves (Environmental 2007). In our study, Morris Lake had the highest mercury bioaccumulation and the lowest average HSI. The mercury concentrations detected in Morris Lake did not reach a concentration of 0.5 to 1.0 ug/g when Yellow Perch would begin to experience changes in biological processes (Sheuhammer et al. 2015). Therefore, HSI is not likely influenced by the mercury concentrations detected in this study. Since Morris Lake is an isolated seepage lake, there is a limited inflow of nutrients into the lake to support healthy liver energy reserves in Yellow Perch from Morris Lake. The absence of a relationship between HSI and mercury concentration was supported in our research (Figure 3).

Yellow Perch are a commonly targeted panfish species for human consumption. By collecting all of the specimens by angling, the data set represents what would normally be caught by panfish anglers. Mercury also accumulates at the highest concentrations in muscle tissue of fish (Has-Schön et al. 2015). The FDA recommended mercury concentrations in fish based on best choices and meals per week to eat can be seen in Table 1 (United 2017). Total mercury concentrations in the muscle tissue of the 25 specimens we analyzed (Table 2). All of the specimens fall safely in the “Good Choices to Best Choices.” It is commonly accepted that mercury is a metal that bioaccumulates up the food chain with highest concentrations being found in top level predators. In all of our study sites, Yellow Perch are mid-level predators; therefore, other popular game fish located in higher trophic levels such as Walleye (*Sander vitreus*), Muskellunge (*Esox masquinongy*), and Northern Pike (*Esox Lucius*) will have greater concentrations of mercury in their muscle tissues.

Mercury, a heavy metal, is persistent in the environment allowing it to be bioaccumulated in fish from diet and the surrounding water (Bradley et al. 2017). Fish are also exposed through the sediments in a water body where mercury often accumulates (Has-Schön et al. 2015). Mercury from anthropogenic sources can stay in the atmosphere for long periods of time allowing for global distribution (Steffen et al. 2015). Even exposure to low concentrations of mercury from the environment for long periods of time can result in high mercury levels in the tissues of fish (Deb & Fukushima 1999). Not only is there a threat to our biodiversity due to mercury contamination and toxicity, but humans are also adversely affected by the toxic capabilities of mercury and the environmental impacts. In many developing countries, fish is the primary source of protein, and worldwide contamination by mercury could have significant impacts on the people within these already struggling countries (Marine 2011). As the world continually changes due to global climate change and human expansion, there are going to be more and more rising issues with mercury in the environment. It is imperative to understand how mercury influences the environment on which earth's biodiversity, including humans, depends on for life.

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Figures

Table 1. Screening values for fish categories. Suggested weekly servings for consumption of fish based on concentration of total mercury (ug/g) found in fish muscle tissue (United 2017).

Weekly fish servings	Screening value (µg/g)	Chart category
0	> 0.46	Choices to Avoid
1	≤ 0.46	Good Choices
2	≤ 0.23	
3	≤ 0.15	Best Choices

Table 2. Total mercury concentrations for all samples analyzed by location. Concentrations are in ug/g. Note: "*" indicates an outlier identified by statistical analysis.

	Location				
	Tenderfoot Lake	Ontonagon River	Morris Lake	Tenderfoot Creek	Palmer Lake
Total Hg (ug/g)	0.1723	0.2801	0.4058	0.2259	0.1983
	0.2016	0.1758	0.2416	0.1520	0.1683
	0.2087	0.1497	0.2149	0.0871	0.2867
	0.0665	0.2864	*0.1044	0.1645	0.2774
	0.1616	0.2230	0.3694	0.2119	0.1364

Table 3. Averages for total mercury concentration, hepatosomatic index, and length of fish for each study location. Standard Error for total mercury, HSI, and length are listed.

Location	Average Total Mercury (ug/g)	Standard Error for Total Mercury	Average Hepatosomatic Index (HSI)	Standard Error for HSI	Average Length (cm)	Standard Error for Average Length
Morris Lake	0.3079	0.1540	1.0120	0.3200	23.2	1.7248
Palmer Lake	0.2134	0.0954	1.1948	0.4878	19.8	1.9396
Otonagon River	0.2230	0.0997	1.7698	0.7915	17.5	0.9494
Tenderfoot Lake	0.1621	0.0725	1.5079	0.4768	18.1	1.2961
Tenderfoot Creek	0.1683	0.0753	1.3543	0.4083	17.2	0.8517

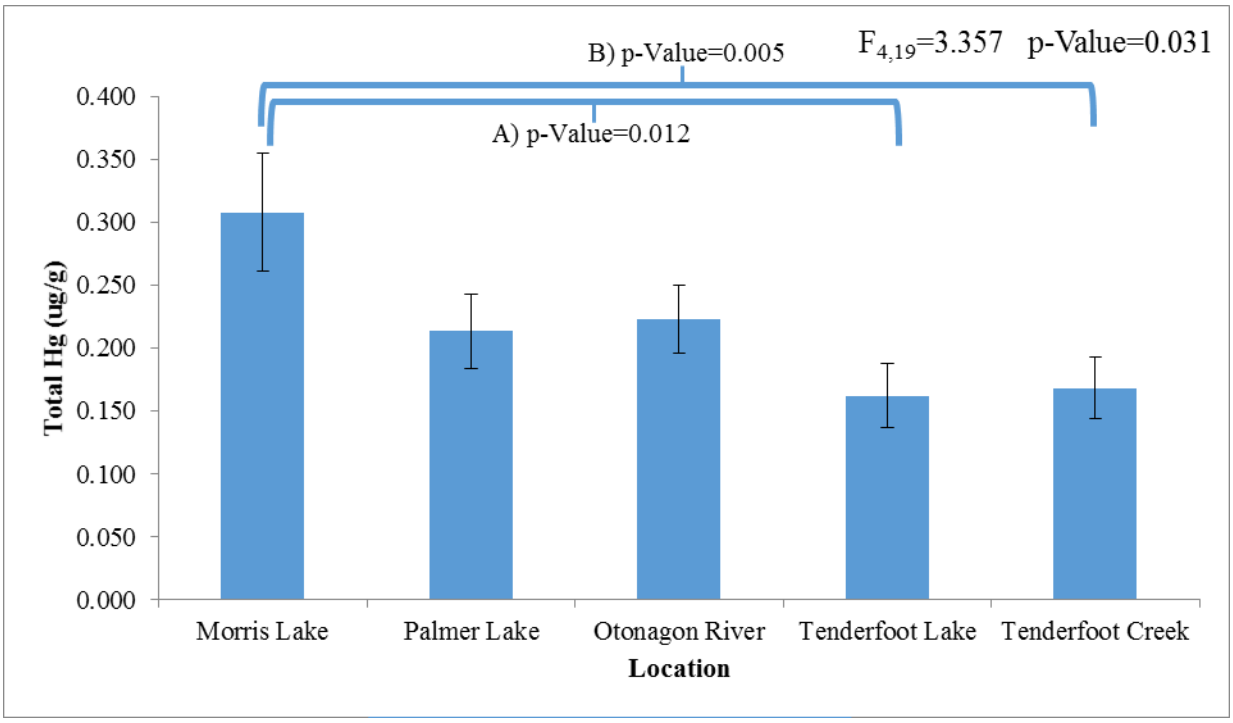

Figure 1. Total mercury concentration (ug/g) based on study location. Results of a one-way ANOVA and a Tukey's Honesty Significant Difference Test showed significance between locations connected by brackets. A) p-Value=0.012 B) p-Value=0.005. Error bars represent standard error.

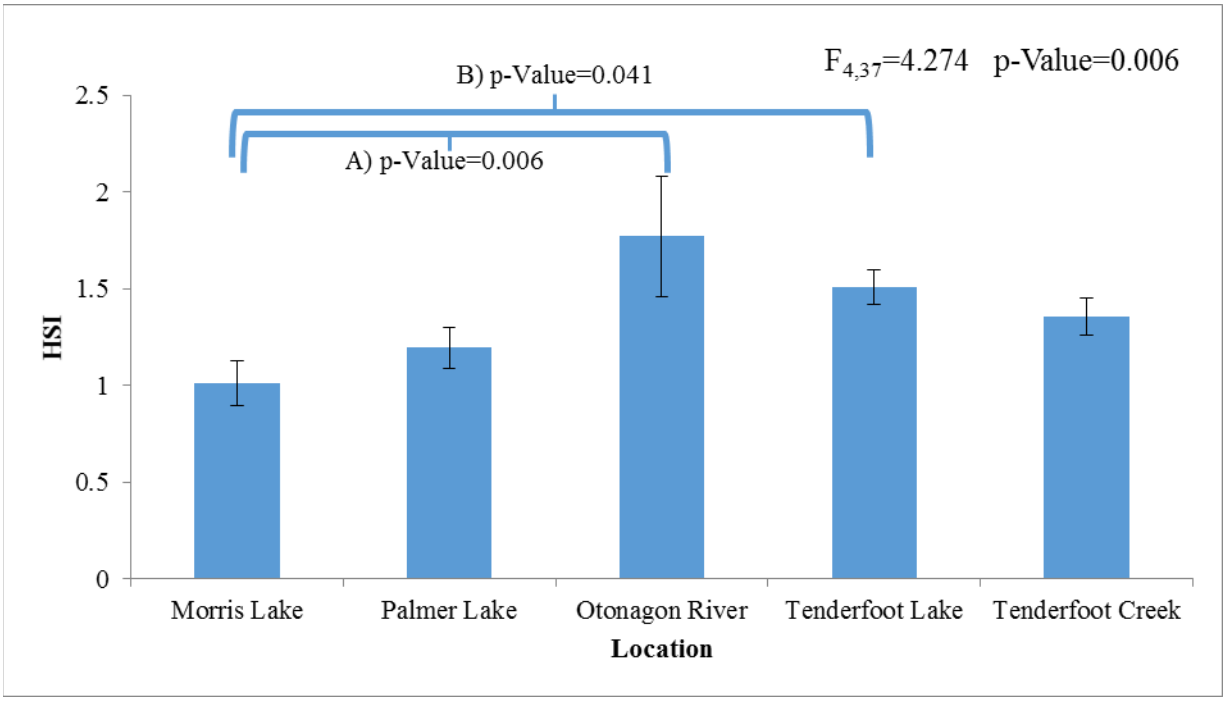

Figure 2. Hepatosomatic Index (HSI) based on study location. Results of a one-way ANOVA and a Tukey's Honesty Significant Difference Test showed significance between locations connected by brackets. A) p-Value=0.006 B) p-Value=0.041. Error bars represent standard error.

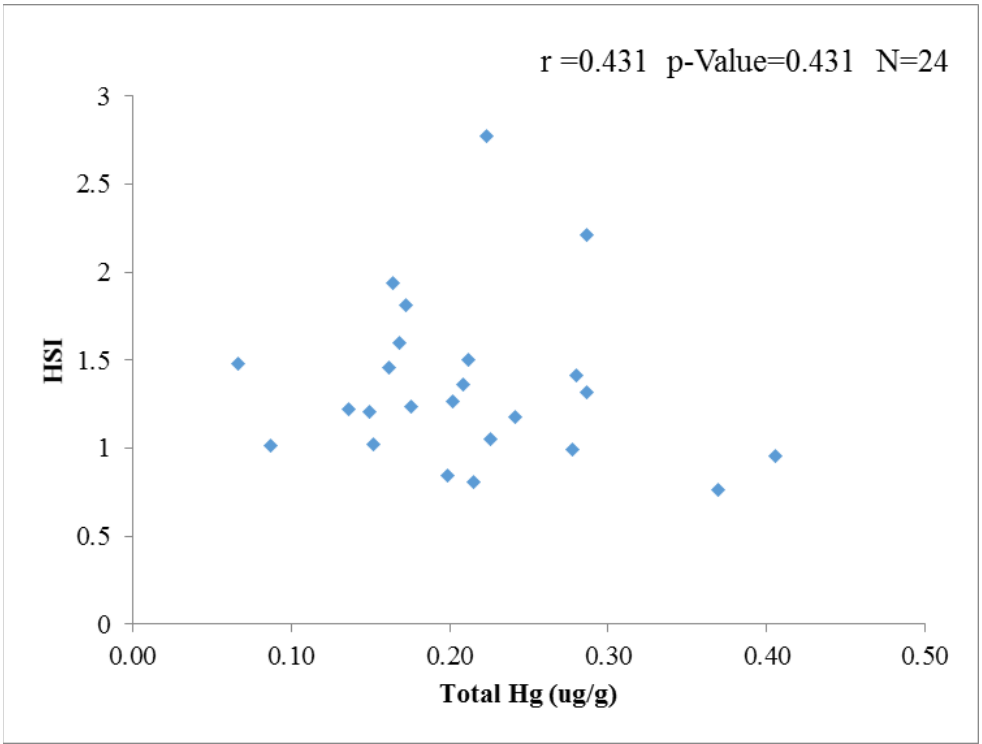

Figure 3. Hepatosomatic Index (HSI) compared to Total Mercury concentration (ug/g). A Pearson Correlation test comparing total mercury concentration to HSI reveals that there is no significant correlation between total mercury concentration and HSI within the samples.

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