DEPARTMENT OF ECOLOGY
State of Washington

Methylmercury in Freshwater Fish, 2020 Comparison Study

July 2023

Publication 23-03-011

Publication Information

This report is available on the Department of Ecology's website at:
https://apps.ecology.wa.gov/publications/SummaryPages/2303011.html.

Data for this project are available in Ecology's EIM Database. Study ID: HgFish20

The Activity Tracker Code for this study is 06-501

Suggested Citation
Foster, K., and C. Mathieu. 2023. Methylmercury in Freshwater Fish, 2020 Comparison Study. Publication 23-03-011. Washington State Department of Ecology, Olympia. https://apps.ecology.wa.gov/publications/SummaryPages/2303011.html.

Water Resource Inventory Area (WRIA) and 8-digit Hydrologic Unit Code (HUC) numbers for the study area:

- WRIAs: 26, 54, 57, 59
- HUC Numbers: 17080005, 17010305, 17010307, 17020003

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Methylmercury in Freshwater Fish, 2020 Comparison Study

by

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Abstract

In 2020, the Washington State Department of Ecology (Ecology) analyzed total mercury in 70 fish tissue samples collected from five waterbodies throughout Washington State. This is part of an ongoing monitoring study for mercury in freshwater fish.

A 40-sample subset from four lakes was analyzed for methylmercury to characterize the percent methylmercury content. Species collected for analysis included largemouth bass (Liberty Lake, Loon Lake, Silver Lake) and smallmouth bass (Lake Spokane). Methylmercury results were compared to total mercury concentrations measured in the paired samples, and relationships with fish growth characteristics were evaluated.

Total mercury and methylmercury were present in all tissue samples. The average percent methylmercury level was 66% (±16%), which is lower than the 95% conservative estimate referenced by the U.S. Environmental Protection Agency (EPA) in their guidance for state fish contaminant monitoring programs. Overall, percent methylmercury, total mercury concentrations, and methylmercury concentrations varied widely across bass size, age, and location.

Statistical analysis revealed that each mercury fraction (total mercury, methylmercury, or percent methylmercury) had a significant, positive relationship with one or more growth variables, showing increased concentration with bass length. When tested for differences by location, location and length explained the most variability in bass percent methylmercury, total mercury, and methylmercury concentrations. Statistical differences by lake varied with each mercury fraction.

While percent methylmercury varied widely, most samples (93%) would have exceeded state thresholds using the total mercury or methylmercury concentration. Ecology plans to continue measuring total mercury in fish tissue for its long-term monitoring programs. Data users should be aware of the range in percent methylmercury demonstrated by this report in largemouth and smallmouth bass.

Introduction

Mercury in the Environment

Mercury is a naturally occurring element that is present in the environment as elemental, inorganic, and organic forms. As a byproduct of industrialization and commercial production, these forms have become widespread in the environment at concentrations far exceeding natural levels (Barrett 2010). The common sources are from mining, smelting, chemical production, fossil fuel combustion, and waste incineration (ATSDR 2022). All forms of mercury are toxic, and the severity of the effects changes with the mercury form and level of exposure. Mercury exposure is dangerous for human and ecosystem health, especially the organic, neurotoxic form methylmercury (MeHg) (Barrett 2010).

The general global cycle of human caused mercury release is emission into the atmosphere (often as the elemental gaseous form Hg^0), deposition onto lands and waterbodies, and partial re-emission from natural processes back into the atmosphere (ATSDR 2022). Mercury that doesn't return to the atmosphere stays in water or binds to soil and sediment as inorganic mercury (Hg^2). In water, naturally occurring microorganisms in sediments convert a fraction of inorganic mercury into methylmercury. This organic form can accumulate and magnify more efficiently in the tissues of organisms than inorganic forms (Lavoie et al. 2013). This bioavailability makes methylmercury especially hazardous because of its neurotoxicity. Its bioaccumulation in fish makes it accessible to the humans and wildlife as a shared food resource.

Over time, inorganic and organic mercury increases in concentration up the food web with each trophic level. This biomagnification leads to higher mercury concentrations in consumer species and often ends up in apex predators at harmful levels. In fish, an indicator of bioaccumulation and biomagnification is high tissue concentrations of total and methylmercury in older and larger individuals and species that feed at higher trophic levels (Gilmour & Riedel 2000; Cizdziel et al. 2002; Yamashita et al. 2005; Mason et al. 2006; Polak-Juszczak 2018).

In freshwater systems, walleye, pike, and largemouth bass feed on other fish and occupy upper trophic levels. These fish tend to have higher tissue mercury concentrations and are frequently fished for recreation. Where bass differs from walleye and pike is in their ontogenetic changes, or life stage shifts, in their feeding habits. As upper-level trophic feeders, largemouth and smallmouth bass at larger sizes can consume other fish but also consume benthic organisms, crayfish, and detritus (Olson 1996; Vander Zanden et al. 1997).

Monitoring Mercury Trends

Since 2005, the Washington State Department of Ecology (Ecology) has been engaged in a long-term monitoring study of mercury levels in freshwater fish to support Washington's mercury chemical action plan (CAP). The Mercury Trends Study monitors the extent of mercury contamination in fish by characterizing concentrations in fish tissue and tracking the spatial and temporal trends in 30 waterbodies across Washington State (Mathieu and Bednarek 2020).

Annually, Ecology collects the target fish species, largemouth and smallmouth bass, from six waterbodies across Washington State. Regional sample sites are resampled every five years. Total mercury is analyzed in the collected fish muscle tissue as processed fillets or non-lethal fillet tissue plugs. The data is used to inform the extent and trends in mercury contamination in different regions of the state.

It is common practice for mercury monitoring studies to use total mercury (inorganic and organic forms combined) as an acceptable and cost-effective surrogate for methylmercury. This decision was based on early studies by Bloom (1992, 1994) that estimated about 95% or more of total mercury in fish tissue was methylmercury. The United States Environmental Protection Agency (EPA) acknowledges additional studies that report a wide range of percent methylmercury values in fish but recommends that state monitoring programs use this estimate and assume that total mercury measurements are equivalent to methylmercury. EPA (2000) states this is the "most conservative assumption . . . as to be most protective of human health," and Ecology adheres to this guidance.

More inclusive studies that cover species at multiple trophic levels found that methylmercury does not consistently make up 95% of total mercury and that this percentage varies across species, size, and age in addition to trophic level and habitat (Jewett et al. 2003; Yamashita et al. 2005; Arcagni et al. 2018; Lescord et al. 2018; Polak-Juszczak 2018). In bass specifically, size, habitat type and location, and trophic level were linked to differences in methylmercury concentrations and percent methylmercury levels (Sveinsdottir & Mason 2005; Mason et al. 2006; Bowling et al. 2011).

Most notably, these studies found the following:

- Smaller-sized bass tended to have lower methylmercury concentrations.
- Bass methylmercury concentrations had intra- and inter-region variations with the overall trend of different tissue concentrations between reservoirs.
- Growth rates were not equal across all bass, which could have additional implications for mercury tissue dilution or concentration during growth or starvation events.
- Bass consuming crayfish with preexisting mercury exposure had the highest methylmercury tissue concentrations over uncontaminated or artificial food sources.

When we consider the conditions listed above, we would expect their combination would create conditions where methylmercury and total mercury accumulation would change throughout the lifetime of a bass.

This comparison study will examine the percent methylmercury levels in a species-specific, regional sample set and compare the relationship between percent and measured methylmercury and total mercury concentrations. To accomplish this, we evaluated total mercury, methylmercury, and percent methylmercury in a subset of large and smallmouth bass tissue samples from four lakes in Washington State. Figure 1 displays the locations where tissue samples were collected.

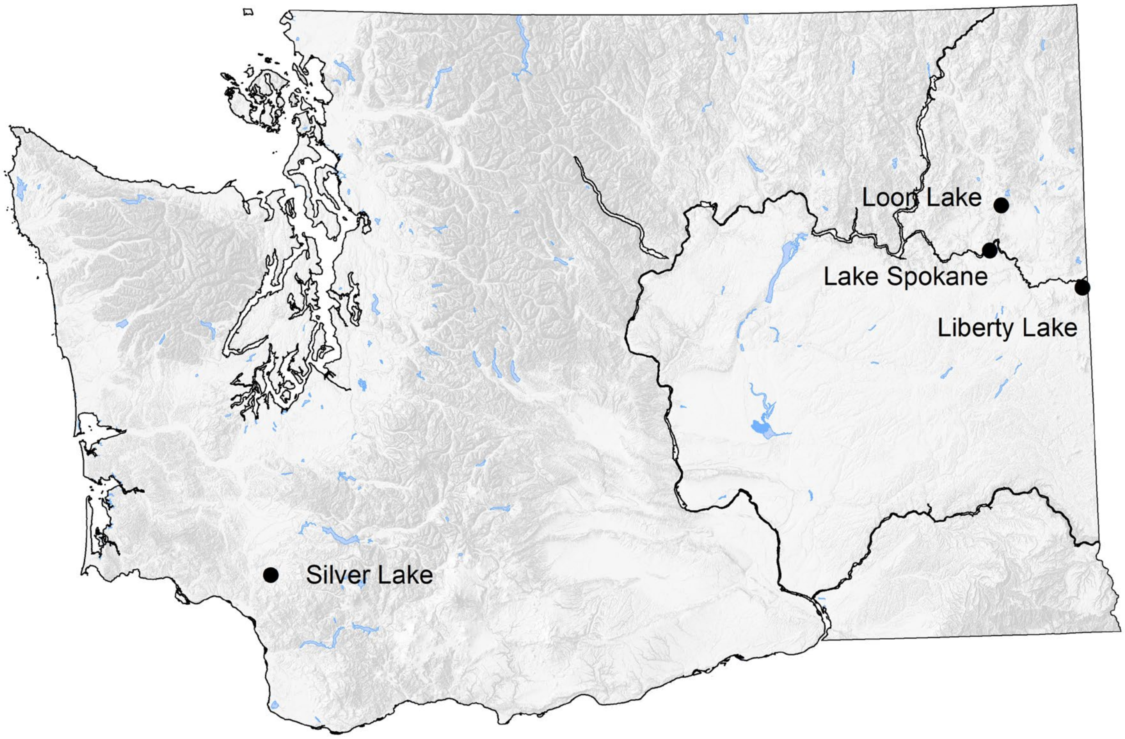

Figure 1. Locations of Methylmercury Sample Collection Sites, 2020.

Methods

Study Design

In October 2020, Ecology collected 70 bass, either largemouth or smallmouth, from five lakes across Washington State. Liberty Lake, Loon Lake, Silver Lake, Potholes Reservoir, and Lake Spokane are five of six waterbodies routinely sampled every five years for the mercury trends study (Mathieu and Bednarek 2020). The Yakima River is the sixth waterbody and was excluded from the 2020 fish collections to comply with Covid health and safety restrictions at the time of sampling.

This comparison study analyzed 40 of the 70 bass collected for the 2020 collection year for methylmercury, as outlined in the QAPP addendum (Mathieu and Bednarek 2021). The cost of methylmercury analysis is significantly more than total mercury, and budget constraints limited the size of our sample subset. A total of 30 largemouth and 10 smallmouth bass were selected for methylmercury analysis: 10 largemouth bass (LMB) each from Liberty Lake, Loon Lake, and Silver Lake, and 10 smallmouth bass (SMB) from Lake Spokane (Figure 1).

This comparison study was completed alongside Ecology's ongoing monitoring study for mercury trends in freshwater fish. The 2020 collection year included a pilot sampling method for non-lethal tissue biopsy. Biopsy plugs from the left fillet side of each bass were collected for a side-by-side comparison of total mercury concentrations with the whole right fillet side for each bass. For this reason, only the right fillet from each bass was homogenized, then divided, creating a set of two samples per individual bass. Of the two samples, one was submitted for total mercury analysis, and the other was archived pending sample selection for methylmercury analysis. Two of these sample sets, LIBLMB10 and SILLMB10, included both fillet sides from the individual bass. This adjustment is allowed when there is not enough tissue from one fillet to meet the tissue weight requirements for the lab analysis. It is not expected to impact mercury concentration results (Mathieu and Bednarek 2020).

During fish collection and processing, we collected data for length, weight, and sex and collected scales and otoliths for fish aging. These fish aging structures were sent to the Washington Department of Fish and Wildlife (WDFW) to estimate the age of each bass. All fish samples were collected and processed following Ecology's Environmental Assessment Program's (EAP's) standard operating procedures (SOPs) (Sandvik 2018 2020).

Laboratory Analysis

We used two laboratories to complete the mercury analyses.

- Ecology's Manchester Environmental Laboratory (MEL) is accredited for the total mercury analysis (EPA method 245.6) and has been routinely used for the mercury in freshwater fish project. MEL is not accredited for methylmercury analysis (EPA method 1630).
- For methylmercury, we contracted with Brooks Applied Labs (BAL), a third-party lab that met project method requirements detailed in the 2020 QAPP addendum (Mathieu and Bednarek 2021). BAL adapted EPA method 1630 for tissue samples and can provide documentation and details upon request.

Total mercury (THg) and methylmercury (MeHg) concentrations were reported as milligrams per kilogram (mg/kg) or nanograms per gram (ng/g) wet weight (ww) by the laboratory. They were converted to parts per billion (ppb) for comparison purposes for this study. Percent methylmercury (% MeHg) was calculated from the measured concentrations of total mercury and methylmercury in mg/kg using the following equation:

$$\% \text{ MeHg} = (\text{MeHg concentration}/\ \text{THg concentration}) \times 100.$$

Only results from the 40-sample subset are included in this report. An upcoming report will summarize total mercury concentrations and trends for all 70 bass samples.

Quality Assurance and Quality Control

Results were reviewed against the measurement quality objectives (MQOs) outlined in the QAPP (Mathieu and Bednarek 2020) and the QAPP addendum (Mathieu and Bednarek 2021) for data reliability and accuracy. The lab-provided case narratives were reviewed for data quality. Copies of analysis-specific MQOs and lab narratives can be provided on request. Each analysis has several MQOs, including matrix spikes, matrix spike duplicates, laboratory control samples, laboratory control sample duplicates, and method blanks for acceptance criteria and data reliability. These results are highlighted with bolded text in Table 1.

Laboratory case narratives include a summary of sample handling and receipt and lab analysis quality assurance and control (QAQC) measures that could affect data quality. Lab comments in the case narratives from MEL and BAL on sample hold times, chain-of-custody, sample temperature, sample preparation, and data qualifiers were all satisfactory and without issue for this comparison study. Because BAL is a third-party lab, their data and lab report was reviewed and approved through an EPA Stage 3 data validation completed by MEL's Quality Assurance Coordinator. All lab QAQC acceptance criteria were met except for one lab duplicate. The only qualifier reported for the data sets was a "U" (non-detectable at or above the reported result) for the total mercury and methylmercury method blanks.

One lab duplicate from BAL returned 31% Relative Percent Difference (RPD) which exceeded our QAPP MQO of 20% (Mathieu and Bednarek 2020). However, this return fell within BAL's lab acceptance criteria for duplicate samples of 35%. All other duplicate samples fell within the 20% MQO limit (3% – 16% RPD). The data validator chose not to qualify results based on this exception. For the total mercury analyses performed by MEL, a matrix spike duplicate (MSD) was analyzed instead of a lab duplicate per MEL SOP 720027 v2.3. All MSDs were within the MQO of <20% RPD (0.6% – 6%).

Table 1 summarizes our data review and verification for the project MQOs. Project MQOs were met for total mercury and methylmercury as outlined in the QAPP (Mathieu and Bednarek 2020) and QAPP addendum (Mathieu and Bednarek 2021).

Table 1. Measurement Quality Objectives Met for 2020 Total Mercury and Methylmercury Results.

MQO Type	QAPP MQOs	MEL MQO Met? (Yes/No)	QAPP Addendum MQOs	BAL MQO Met? (Yes/No)
Parameter	Mercury, Total	Yes	Mercury, Methyl	Yes
Matrix	Tissue	Yes – Fillets	Tissue	Yes – Fillets
Reporting Limit (mg/kg, ww)	0.017	Yes	0.017	NA
Matrix Spike (% recovery)	75–125	**Yes**	75–125	**Yes**
Matrix Spike Duplicate (RPD)	<20	**Yes**	<20	**Yes**
Lab Control Sample (% recovery)	85–115	**Yes**	17–125[1a]	**Yes**
Laboratory Control Sample Duplicate (RPD)	<20	**Yes**	<20[1b]	**Yes**
Standard Reference Material (% recovery)	75–125	Yes	75–125	NA
Lab Duplicate Sample (RPD)	NA	NA	<20	Partial[2]
Method Blank	<MDL mg/kg	**Yes**	≤5 ng/L	**Yes**

Note. **Bold** text indicates MQOs used for lab acceptance and project data reliability.
[1a and 1b] BAL used a Certified Reference Material instead of Laboratory Control Sample to meet the project MQOs.
[2] One of four duplicates exceeded QAPP MQO of 20% but was within BAL acceptance limits.
BAL = Brooks Applied Labs, MDL = method detection limit, MEL = Manchester Environmental Laboratory.
MRL = method reporting limit, NA = not listed in the QAPP as an MQO, RPD = relative percent difference.

All samples were analyzed within holding times. However, the total mercury analysis was performed in January 2021, and the methylmercury analysis was performed in May 2021. We do not expect the additional holding time of the methylmercury samples to affect results, but it may introduce variability that we cannot account for. Another limitation of comparing the two analyses is the use of two separate labs for analyses. Since MEL does not conduct methylmercury analyses, we used a contract lab. The same lab should be used for total mercury and methylmercury analyses in future comparisons.

Statistical Analysis

Methylmercury and total mercury results are presented and analyzed as described in the QAPP (Mathieu and Bednarek 2020) and QAPP addendum (Mathieu and Bednarek 2021). This report includes a summary of results, summary statistics, and any additional length, weight, and age relationships that help meet the goal of this comparison study. Data was analyzed using paired t-tests, multiple linear regression, and ANCOVA (Helsel et al. 2020). Measured data were tested for normality using the Shapiro-Wilks test (Table 2), and the significance of linear model relationships was assessed using Bonferroni post hoc testing. Total mercury, methylmercury, length, weight, and age data were log 10 transformed when appropriate for analysis and reported as back-transformed results. Non-parametric equivalent tests such as the Wilcoxon rank sum and Kruskal-Wallis tests were used for non-normally distributed data. All statistical tests were completed using R v4.2.2, Systat v13.2, and Microsoft Excel.

Results – Mercury in Fish Tissue

Percent Methylmercury Levels

Overall, percent methylmercury in bass for all locations, sizes, and ages ranged from 39% – 124% (Table 2), with a mean of 66% ±16% and a 95% C.I. of 5%. The lowest percent methylmercury level was found in LIBLMB9, a 4-year-old largemouth bass from Liberty Lake that weighed 293 grams (g) at 293 millimeters (mm). The highest percent methylmercury level was found in SPOKSMB1, a 7-year-old smallmouth bass from Lake Spokane that weighed 1,652 g at 480 mm. Table 2 summarizes the minimum and maximum values for total mercury, methylmercury, percent methylmercury, and growth variables for all bass by location. A detailed table of these same variables for individual bass is included in the appendix of this report (Table A1). Summary statistics for percent methylmercury levels are listed in Table 3.

Table 2. Mercury Concentrations and Growth Variables by Location for all Bass.

Site	Species	Count	Weight (g)	Length (mm)	Age (years)	THg (ppb)	MeHg (ppb)	% MeHg
All Lakes	LMB, SMB	30, 10	197–1652	243–480	2–14	18.8–337	12.3–220	39–124
Liberty	LMB	10	201–1458	262–459	3–13	92.7–363	36.4–266	39–73
Loon	LMB	10	364–1452	295–444	3–14	58.4–318	30.6–220	46–85
Silver	LMB	10	197–1137	243–436	2–5	18.8–163	12.3–100	61–94
Spokane	SMB	10	288–1652	284–480	3–7	46.8–156	29.1–119	58–124
Shapiro-Wilks Normality Test[1]			0.010	**0.072**	4.01×10^{-5}	0.001	9.15×10^{-5}	0.005

Note. **Bold** text indicates a normal distribution.
[1]P-value of test results for the variable across all lakes
THg = Total mercury, MeHg = methylmercury.

The boxplot for percent methylmercury (Figure 2) shows that our one outlying value was also our highest at 124%. Methylmercury concentrations are not expected to exceed 100% because the total mercury analysis results should include all organic and inorganic fractions. After reviewing our field notes and the lab analytical reports, we could not find an explanation for the outlying value, so it was included in the analysis. Similar studies, like Lescord et al. (2018), handled analysis of percent methylmercury values >100% similarly and recognized that the outliers were likely due to "small but cumulative errors in the analytical process" (Lescord et al. 2018). The Shapiro-Wilks normality tests on the residuals for percent methylmercury regression and ANCOVA relationships passed when 124% was included in the analysis.

A comparison of our bass percent methylmercury levels with Bloom's (1992) conservative 95% estimate shows that all but one fish was below the EPA monitoring guidance level (Figure 3). Figure 3 has percent methylmercury levels arranged from smallest to largest fish by length, and there is noticeable variability in the values as fish increase in size. Because of the difference between the conservative estimate and the percent methylmercury levels in this dataset, we assessed possible explanatory relationships between percent methylmercury levels and multiple growth characteristics. These include age, length, weight, and sample location.

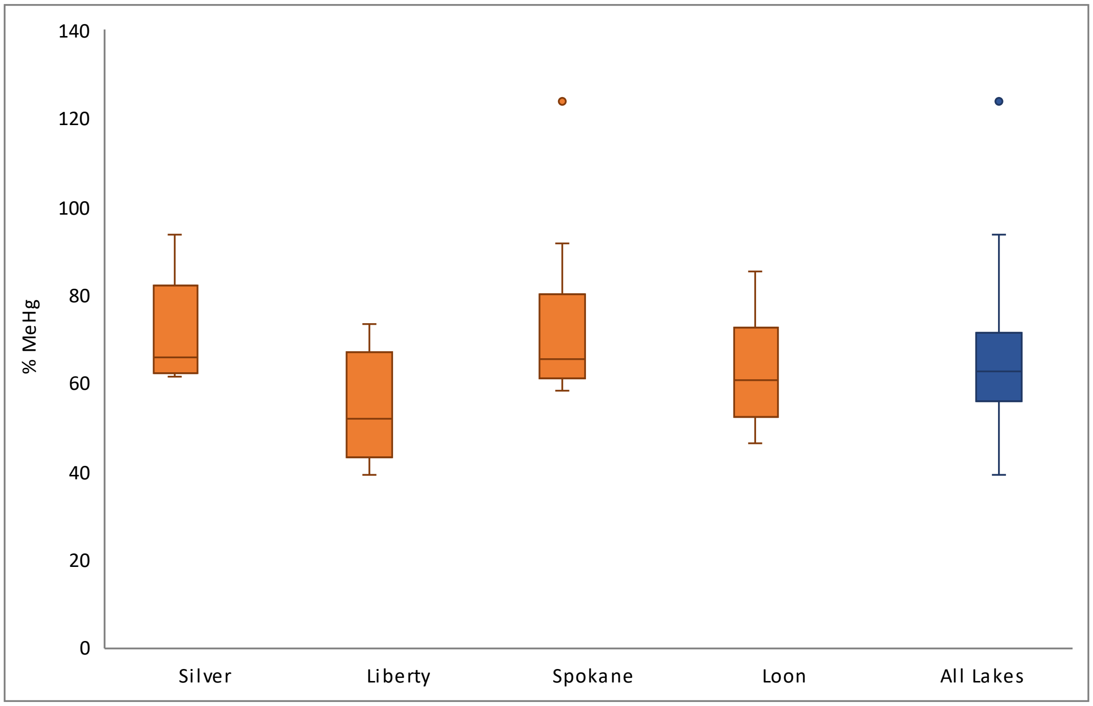

Figure 2. Boxplot of Percent Methylmercury (% MeHg) in Bass by Location and All Locations Combined.

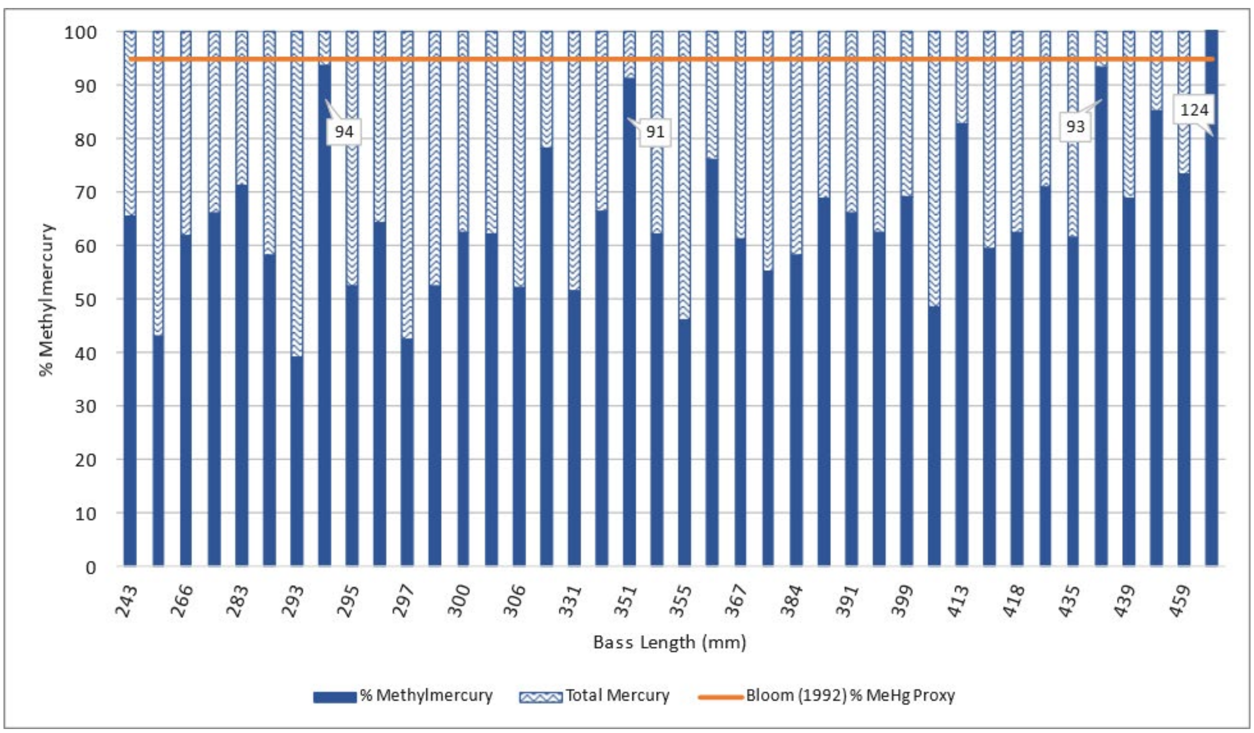

Figure 3. Mercury Fractions in Bass Tissue from the Smallest to Largest Fish.

Table 3. Boxplot Summary Statistics for Bass Percent Methylmercury Values in Total and by Location.

Type	Total	Silver Lake	Liberty Lake	Lake Spokane	Loon Lake
Count	40	10	10	10	10
Minimum	39.0	61.0	39.0	58.0	46.0
Median	63.0	65.5	52.0	65.5	60.5
Mean	65.7	71.4	54.8	73.5	63.2
Interquartile Range	14.5	16.0	23.0	14.0	17.0
Maximum	124	94.0	73.0	124	85.0
Standard Deviation	16.2	12.8	12.2	20.2	13.2
Standard Error	2.56	4.03	3.84	6.40	4.18
Lower 95%	60.5	62.3	46.1	59.0	53.7
Upper 95%	70.9	80.5	63.5	88.0	72.7

Percent Methylmercury and Growth Characteristics

The bass included in this sample set followed a positive allometric growth pattern ($b = 3.2$), where an increase in bass weight correlated with increased length (Figure 4), demonstrated by a strong, positive linear relationship ($p < 0.05$, $R^2 = 0.98$; Figure 5). In Figure 4, when percent methylmercury levels are plotted over this length-weight relationship, it varies as the size of the fish increases. However, the individual linear trends with these growth variables show increased percent methylmercury levels with size (Figure 6). In the scatterplots in Figure 6, percent methylmercury (shown as solid, dark blue squares) increases with length and weight. Pearson's correlation for both relationships was significant ($p < 0.05$) and indicates a similar strength between percent methylmercury and length or weight, where the $R^2 = 0.44$ and $R^2 = 0.41$, respectively (Figure 5).

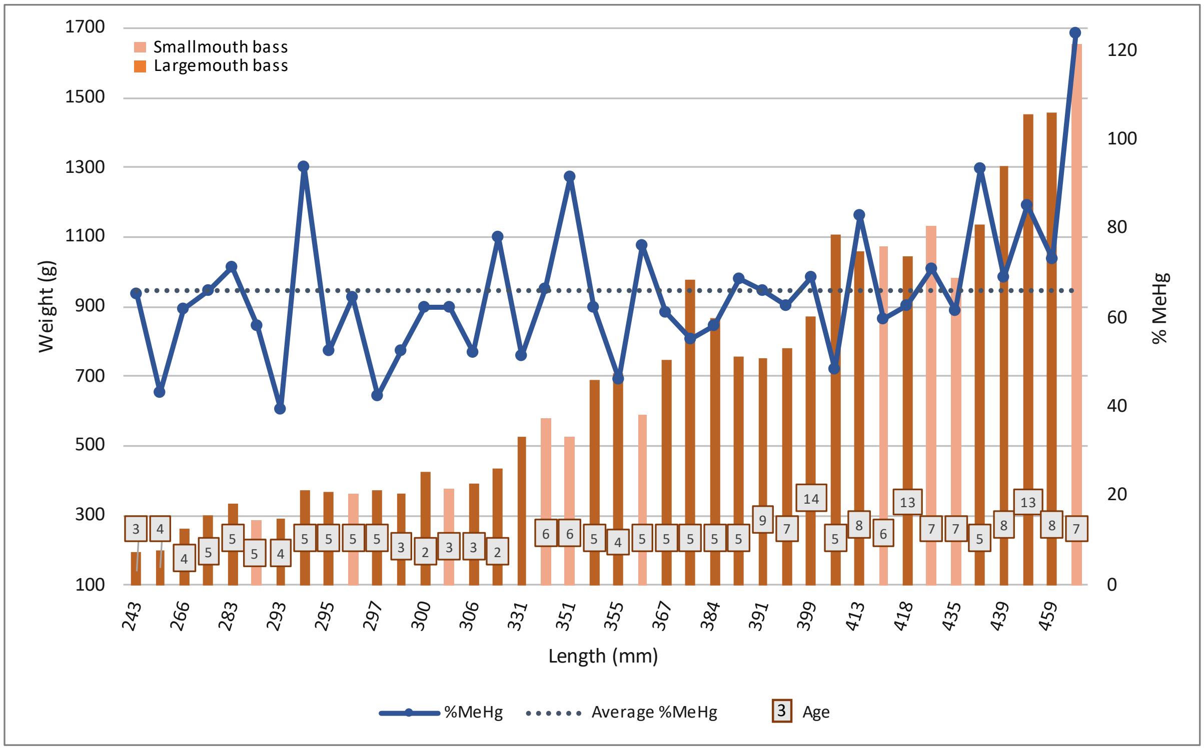

Figure 4. Bass Percent Methylmercury (% MeHg) Levels and Weight with Increasing Length.

Pearson's Correlation Coeff.

-1.00	-0.50	0.00	0.50	1.00

Variables	Weight	L10 Weight	Length	L10 Length	% MeHg	THg	L10 THg	MeHg	L10 MeHg
Weight	1								
L10 Weight	**0.97**	1							
Length	**0.97**	**0.98**	1						
L10 Length	**0.95**	**0.99**	**0.99**	1					
% MeHg	**0.47**	**0.41**	**0.44**	**0.41**	1				
THg	**0.55**	**0.59**	**0.60**	**0.61**	0.017	1			
L10 THg	**0.54**	**0.62**	**0.62**	**0.65**	-0.040	**0.93**	1		
MeHg	**0.64**	**0.65**	**0.68**	**0.68**	0.28	**0.96**	**0.87**	1	
L10 MeHg	**0.66**	**0.72**	**0.73**	**0.75**	0.28	**0.91**	**0.95**	**0.93**	1

Figure 5. Pearson's Correlation Matrix for Different Mercury Fractions and Growth Variables of Bass.

All bass and locations combined. **Bolded** values are significant correlations (p-value < 0.05).
Darker cells represent stronger correlations. THg = total mercury, MeHg = methylmercury, and L10 = Log 10.

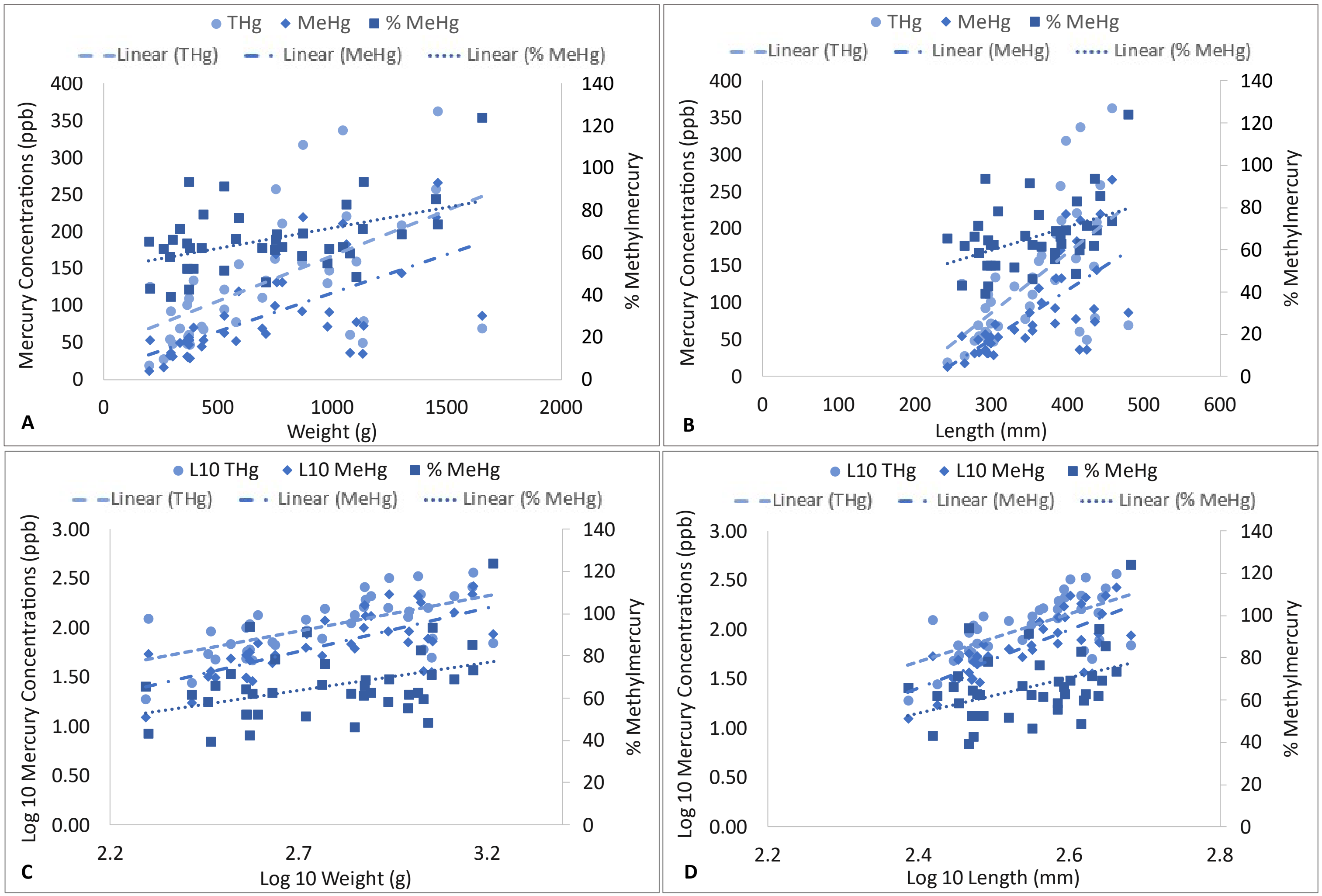

Figure 6. Methylmercury Concentration Relationships with Growth Variables for Bass.
Light blue circles with dashed lines represent total mercury concentrations and their log-transformed values, and the medium blue diamonds with dash and dot lines represent methylmercury concentrations and their log 10 transformed values. Individual linear trends for total mercury (THg), methylmercury (MeHg), and percent methylmercury (% MeHg) increase with length and weight of Bass regardless of log 10 transformation. The letter of each graph corresponds to a different variable on the x-axis. L10 = Log 10, MeHg = methylmercury, and THg = total mercury.

We tested the percent methylmercury relationships with the growth variables in two ways; a median assessment of values as length or age categories using the Kruskal-Wallis test and a multiple linear regression with length and weight. When we bin percent methylmercury values into length groups, boxplots show that the variability of percent methylmercury levels is greatest in the smallest fish (<300 mm, Figure 7). The median percent methylmercury level for each group increases with size, with the largest change occurring between fish in the 350 – 399 mm group and fish greater than 400 mm.

Despite the increase in the medians with length, a Kruskal-Wallis test of this relationship showed no significant difference in percent methylmercury among size groups (Table A8). When testing the linear length and weight relationships with percent methylmercury using a multiple linear regression, length and weight had similar results. Length, weight, and the interaction between length and weight were not considered significant for percent methylmercury levels ($p > 0.05$, $R^2 = 0.26$, Table A2).

Like length, a Kruskal-Wallace test showed no significant difference in median percent methylmercury levels for fish ages. The age for each fish is shown in Figure 4 in a box centered over the weight-by-length columns.

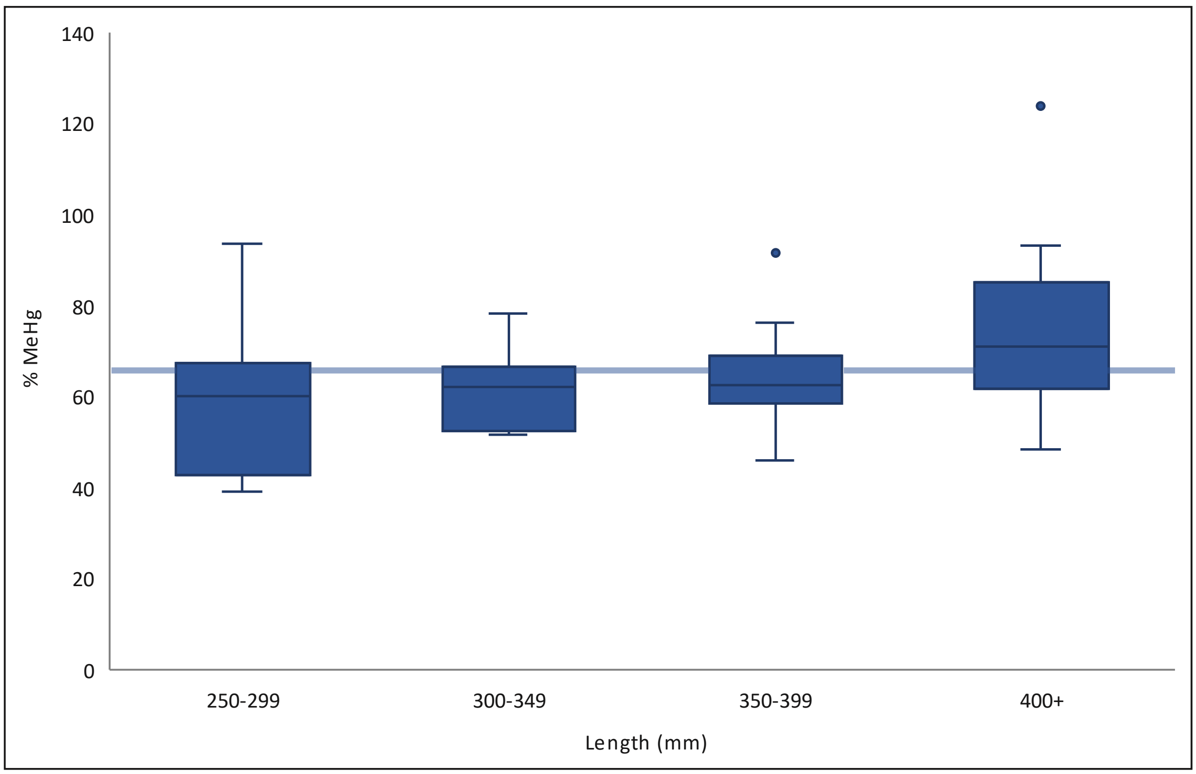

Figure 7. Distribution of Percent Methylmercury in Bass by Length Groups.
Bass are grouped in 50 mm increments: 250 – 299 (n = 10), 300 – 399 (n = 7), 350 – 399 (n = 11), and 400+ (n = 11). The 400+ group also includes percent methylmercury (% MeHg) data for the two fish with lengths greater than 450 mm. The light blue line shows the overall average % MeHg level of 66%.

Percent Methylmercury at Different Lakes

In Figure 2, the distribution of percent methylmercury levels by location is shown as four solid-orange boxplots on the left side of the graph. The median distribution of percent methylmercury varies by lake, indicating that location could contribute to the variability in percent methylmercury in bass. Scatter plots in Figure A1 show differences in percent methylmercury trends with each growth characteristic when grouped by location, some of which have stronger correlation relationships depending on the lake. Pearson's correlation values for percent methylmercury vary between the sampling locations for length, weight, and age ranging from 0.21 – 0.82 (Figure 8).

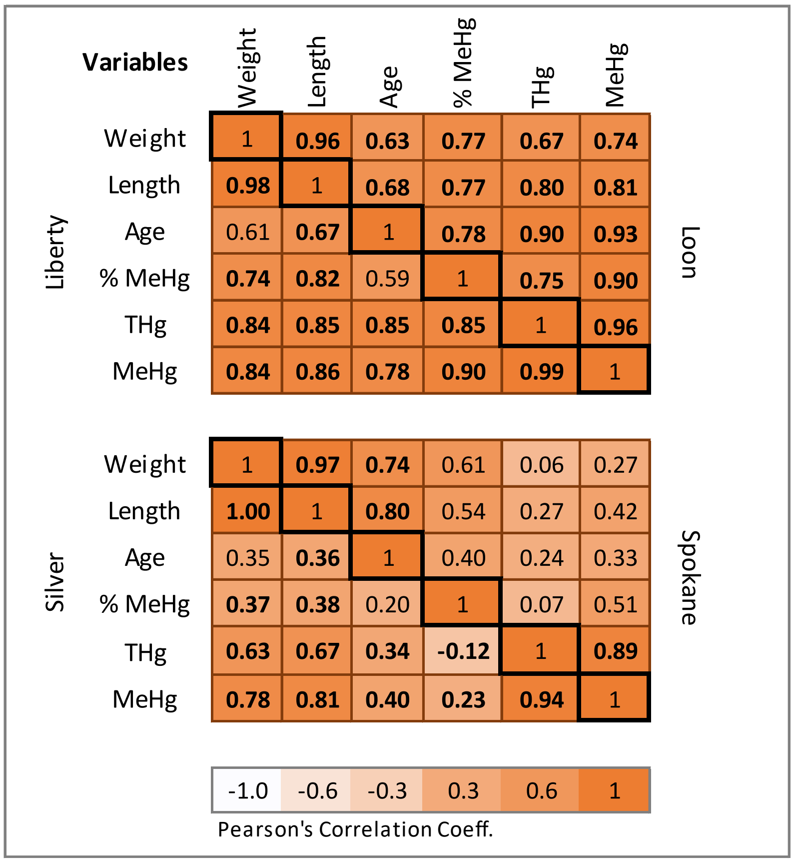

Figure 8. Pearson's Correlation Matrix for Mercury Fractions and Growth Variables for Bass Grouped by Lake.

Bolded values indicate significant correlations (p-value <0.05). THg = total mercury, and MeHg = methylmercury. Darker cells represent stronger correlations.

An ANCOVA that looked at location and controlled for length offers the best explanation for percent methylmercury variability in our bass samples (Table A5). Location and length ($p < 0.05$) explain about 50% of percent methylmercury values ($R^2 = 0.499$). A Bonferroni post hoc test found significant differences between percent methylmercury values from Lake Spokane and Liberty Lake ($p = 0.030$), Silver Lake and Liberty Lake ($p = 0.001$), and Silver Lake and Loon Lake ($p = 0.022$) (Table A6).

Total Mercury and Methylmercury Concentrations

Overall, total mercury and methylmercury concentrations ranged from 18.8 – 363 ppb and 12.3 – 266 ppb, respectively (Table 2). The lowest total and methylmercury concentrations were found in an individual fish from our sample set. SILLMB10 was a 3-year-old male largemouth bass from Silver Lake that weighed 197 g at 243 mm, with the lowest total mercury and methylmercury concentrations of 18.8 ppb and 12.3 ppb, respectively. The highest total and methylmercury concentrations were also found in the one fish. LIBLMB1 was an 8-year-old female largemouth bass from Liberty Lake that weighed 1,458 g at 459 mm, with the highest total and methylmercury concentrations of 363 ppb and 266 ppb, respectively. For all bass, the mean total mercury concentration was 130 ppb (±86 ppb), and the mean methylmercury concentration was 86 ppb (±63 ppb).

A Wilcoxon signed-rank test for total mercury and methylmercury concentrations shows that the means were significantly different across all bass ($p < 0.05$, $z = -5.39$, Table A9). When total mercury and methylmercury concentrations are plotted against each other (Figure 9), they have a strong, positive linear relationship, where methylmercury concentrations correlate strongly with total mercury concentrations ($r = 0.96$; $p < 0.05$).

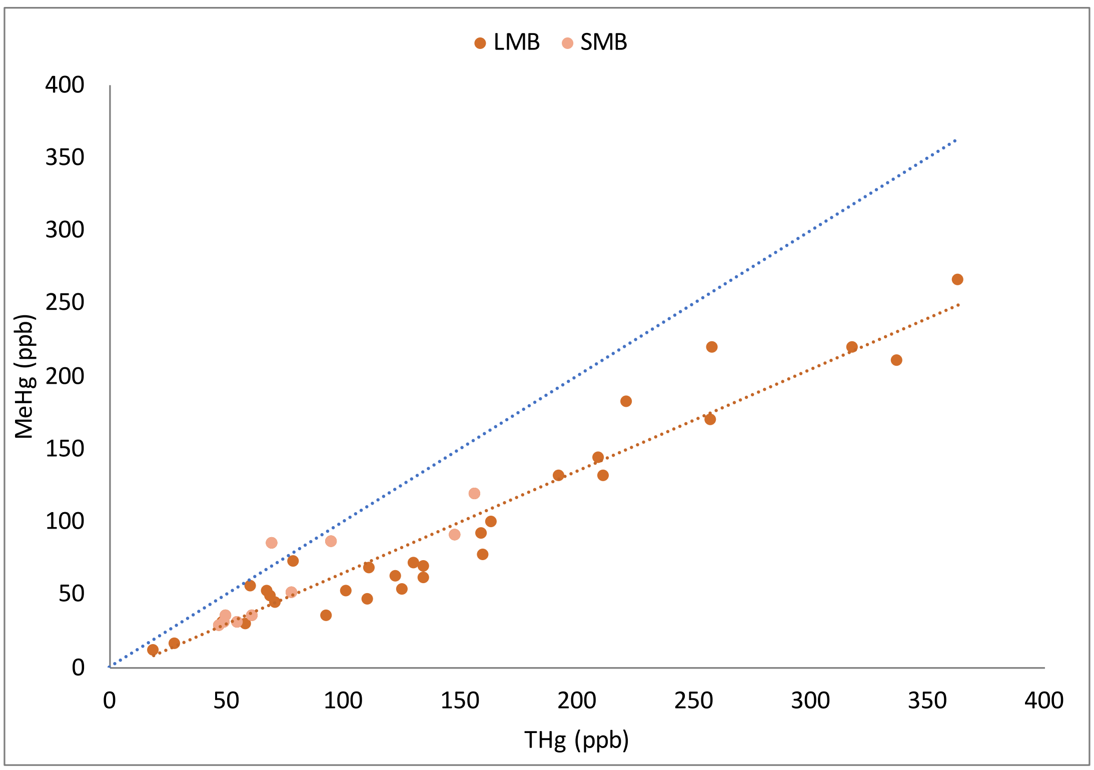

Figure 9. Scatterplot of Total Mercury and Methylmercury in Largemouth and Smallmouth Bass.

The dotted orange line shows the positive linear relationship between THg and MeHg concentrations. The dotted blue line represents a 1:1 relationship. MeHg = methylmercury, THg = total mercury, LMB = largemouth bass, and SMB = smallmouth bass.

Mercury Concentrations and Growth Variables

In Figure 6C and 6D, the distance between the methylmercury and total mercury trendlines decrease as weight and length increase. The Pearson's correlation coefficients (Figure 5) for the log10 transformed concentration-growth variable relationships follow the same pattern as the measured data. All four variations have strong correlations, but log10 length and methylmercury have the highest correlation ($R = 0.75$), and log10 weight and total mercury have the lowest ($R = 0.62$). Methylmercury concentrations correlated the best with both growth variables regardless of data transformation.

As with percent methylmercury, we tested the total mercury and methylmercury relationships with growth characteristics using several methods, including; a Kruskal-Wallis test, a Wilcoxon signed rank test, and a multiple linear regression. We binned total mercury and methylmercury values into the same length groups used in Figure 7. We compared their median concentrations between both fractions for a given size and among the length groups (Figure 10). The boxplots in Figure 10 show that the variability in total mercury and methylmercury is greatest in larger fish (>400 mm). The median concentrations for total mercury and methylmercury increase with size, with the largest change among the groups occurring between fish at 300 – 349 mm and 350 – 399 mm. The median methylmercury concentrations decreased for larger fish (>400 mm).

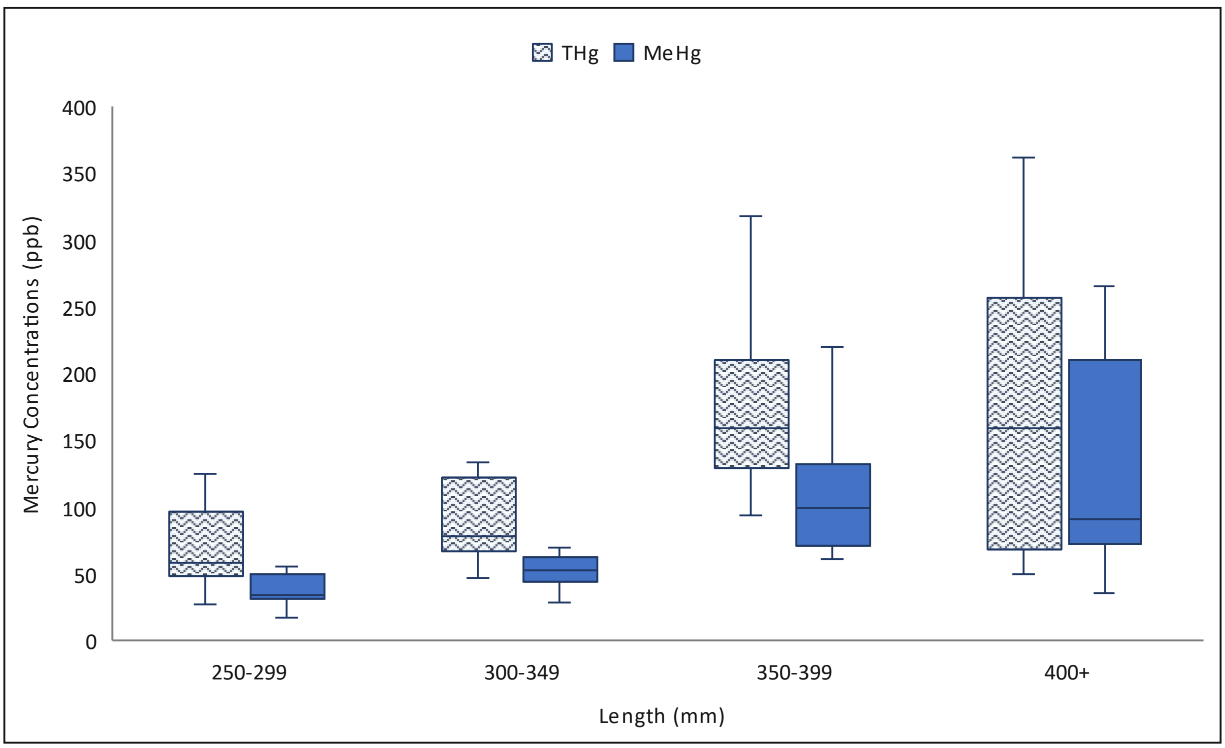

Figure 10. Distribution of Total Mercury and Methylmercury in Bass When Grouped by Length.

The Kruskal-Wallis test showed that median total mercury and methylmercury concentrations significantly differed across length groups ($p < 0.05$, Table A8). When we look at differences between both fractions within these groups, Wilcoxon signed rank tests show that total mercury concentrations were significantly different from methylmercury concentrations for all length categories (Table A9). Both fractions had similar results when testing the linear relationships of total mercury and methylmercury with length and weight (Table A3 and Table A4). A multiple linear regression showed that length and the interaction between length and weight were significant ($p > 0.05$), accounting for 44% of the variability in total mercury (R2 = 0.44) and 60% of the variability in methylmercury (R2 = 0.59). For this regression test, the relationship between weight and changes in total mercury and methylmercury concentrations was not significant ($p > 0.05$).

Mercury Concentrations at Different Lakes

The boxplots in Figure 11 show the distribution of total mercury and methylmercury concentrations by location and for all lakes combined. The median distribution for both fractions varies by lake, indicating that location could contribute to mercury concentrations in bass. When grouped by location, we also see differences in linear trends for total mercury and methylmercury for each growth factor (Figure A1). The Pearson's correlation coefficients for total mercury and methylmercury vary between the sampling locations for length, weight, and age ranging from 0.06 – 0.90 and 0.27 – 0.93, respectively (Figure 8).

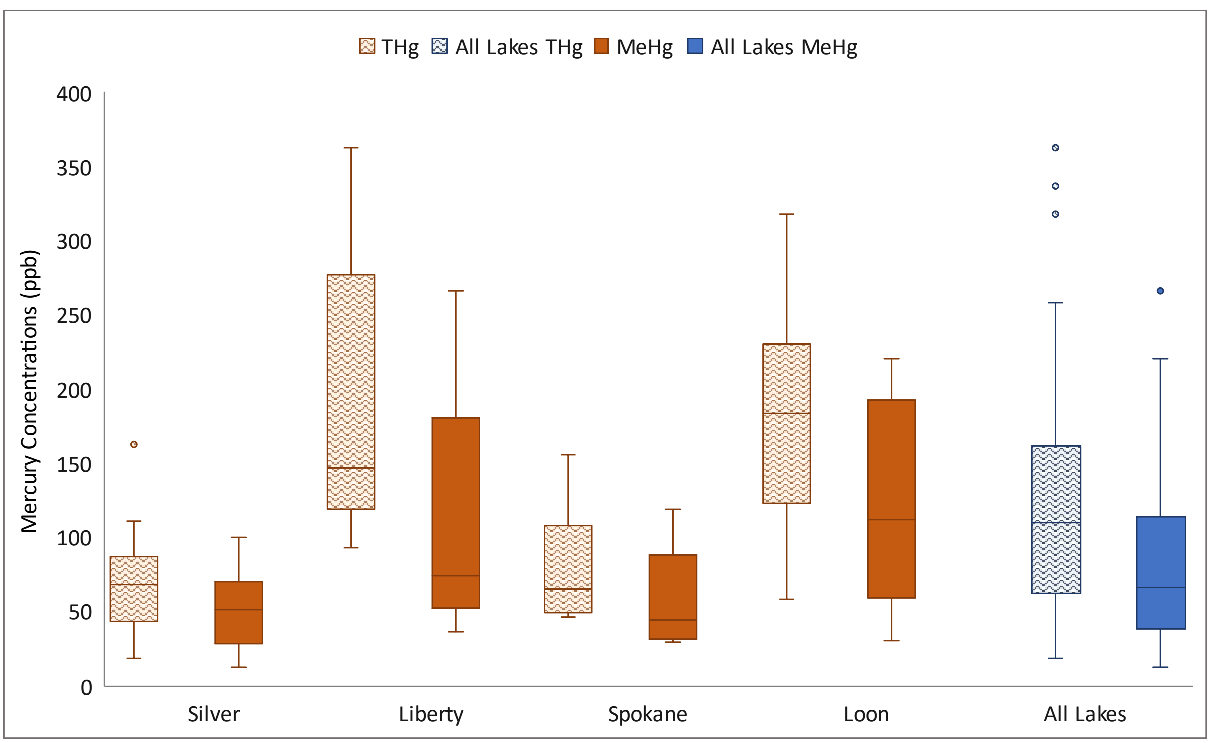

Figure 11. Distribution of Total Mercury and Methylmercury in Bass by Location.
MeHg = methylmercury, and THg = total mercury.

Using an ANCOVA, we tested whether location contributed to the variability in total mercury and methylmercury concentrations while controlling for length. Both variables were significant ($p < 0.05$) for total mercury and methylmercury, explaining about 70% of the variability in both ($R2 = 0.71$, $R2 = 0.68$). Tables A10 – A15 (Appendix A) summarize the results of ANCOVA and post-hoc testing.

A Bonferroni post-hoc test for total mercury showed that Lake Spokane mean concentrations differed from Liberty and Loon Lake, and Liberty Lake differed from Silver Lake ($p < 0.05$) (Table A11). A Bonferroni post-hoc test for methylmercury showed differences in the mean methylmercury concentrations for Lake Spokane with Liberty and Loon Lake ($p < 0.05$) (Table A14).

Mercury Thresholds in Washington State

Figure 12 shows total mercury and methylmercury concentrations for every fish in this study relative to Washington State's methylmercury water quality criterion (WQC) for human health (40 CFR 131.45; Ecology 2018) and the Washington State Department of Health (DOH) methylmercury screening levels (DOH SL) for fish consumption advisories. These thresholds are set at 30 ppb and 101 ppb, respectively. Although the WQC and DOH SL are specific to methylmercury, we chose to include total mercury concentrations in this comparison because it is generally accepted to represent the methylmercury fraction in fish and is the EPA (2000) recommended analyte for "state fish contaminate monitoring programs" (p. 4 – 20). An explanation of how we use these thresholds is provided in Appendix B. Note that any exceedance of a threshold discussed in this section does not necessarily mean non-compliance but is included for context.

For this sample set, 93% (37 bass) had methylmercury tissue concentrations above the WQC, and 95 % (38 bass) had total mercury concentrations at or above the WQC. Two fish, SILLMB9 and SILLMB10, had both mercury fractions below the mercury WQC. One fish (3% of the sample set), SPOKSMB9, had total mercury above 30 ppb and methylmercury below 30 ppb (Table A1). Despite this discrepancy, we still accurately capture about 97% of methylmercury contamination as above or below WQC threshold values when we measure total mercury.

Overall, 25% (10 bass) of the methylmercury tissue concentrations were above the DOH SL compared to 55% (22 bass) of the total mercury concentration at or above the DOH SL. There are 12 fish (30% of the sample set) with total mercury concentrations above 101 ppb and methylmercury concentrations below 101 ppb. This demonstrates that we can only accurately capture approximately 70% of methylmercury contamination as above or below DOH SL threshold values by measuring total mercury as a proxy for methylmercury. This suggests that relying on THg concentration would overestimate the methylmercury concentrations in reference to the DOH SL.

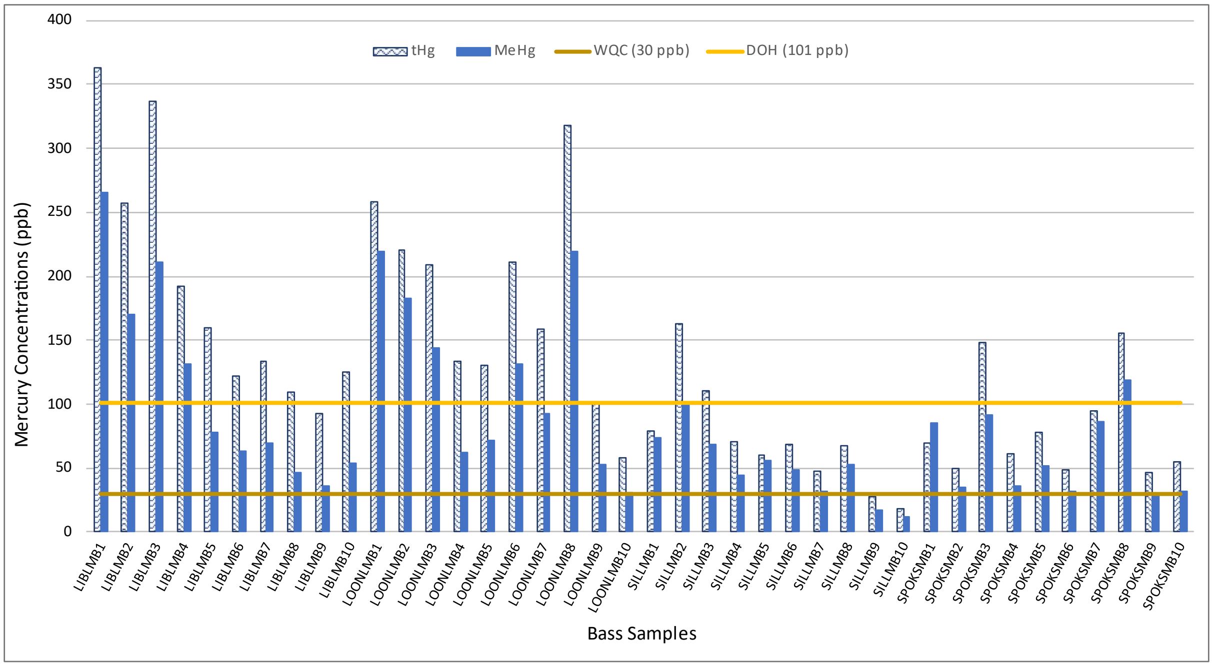

Figure 12. Mercury Concentration in Bass Tissue Relative to State WQC and DOH SL Thresholds for Methylmercury.

WQC = Washington State Water Quality Criterion, DOH SL = Washington State Department of Health Screening Level, THg = total mercury, and MeHg = methylmercury.

Discussion – Mercury in Bass

Mercury accumulation in fish is complex; multiple variables interact across individuals and populations. This can create a lot of variability in organic and inorganic tissue concentrations and ultimately affect the ratio of mercury fractions in fish. Some of these variables include tissue accumulation through growth, feeding habits and diet, and location or habitat (Jewett et al. 2003; Sveinsdottir & Mason 2005; Yamashita et al. 2005; Mason et al. 2006; Bowling et al. 2011; Arcagni et al. 2018; Lescord et al. 2018; Polak-Juszczak 2018). This study focused on total mercury, methylmercury, and percent methylmercury and explored relationships with fish growth characteristics.

In our study, methylmercury made up an average of 66% of total mercury concentrations. Our result contrasts with Bloom's (1992) early findings, which suggested that the methylmercury fraction of total mercury is 95%. However, studies by Yamashita et al. (2005), Mason et al. (2006), Lescord et al. (2018), and Polak-Juszczak (2018) all report a range of values for percent methylmercury in fish tissue that fall anywhere from 28% – 100% or more. Our results are similar to the mean percent methylmercury levels that Mason et al. (2006) measured in striped bass from Maryland reservoirs.

Changes With Growth

Percent Methylmercury

We routinely collect length, weight, and age data as part of our study design, and bass are collected from different lakes across Washington state. When we looked at the relationships between these variables and percent methylmercury, we noted they varied widely across bass size, age, and location. Smaller fish (<299 mm) tended to have greater variability in percent methylmercury levels than larger fish (>400 mm).

In the scatterplots from Figure 6, the increase in percent methylmercury trendlines with growth variables show that methylmercury concentrations are increasing in tissues relative to total mercury and size. However, the gradual slope in the lines suggests that the percent change is minimal. Specifically with length, statistical tests showed no overall difference in median percent methylmercury levels between smaller and larger fish when we compared length groups, despite the positive trends in the boxplots (Figure 7) and scatterplots (Figure 6). Linear relationships with weight and median change with age were also not significant for percent methylmercury levels. Our results differ from Lescord et al. (2018), who found significant differences in percent methylmercury by weight for multiple species.

There are additional accumulation variables discussed by Schultz & Newman (1997), Sveinsdottir & Mason (2005), and Lavoie et al. (2013) that could cause loss or dilution of methylmercury in tissues. Conditions where inorganic and organic fractions accumulate in tissues, fish excreting methylmercury, or growth-induced methylmercury tissue dilution, could all result in variable percent values as methylmercury and total mercury concentrations change.

Total Mercury and Methylmercury

Unlike percent methylmercury levels, we found statically significant relationships between the growth variables and total mercury and methylmercury concentrations. The strong correlation between total mercury and methylmercury concentrations in Figure 9 shows that both are changing relative to each other and are most likely responding similarly to one or more of the variables that control mercury accumulation in fish tissues.

The linear trends in Figures 6C and 6D show accumulation with size for both fractions. However, methylmercury is the most affected overall, especially in longer bass, where the correlation coefficients were strongest for methylmercury and length. The regression tests show us that length and the interaction between length and weight best estimated total and methylmercury concentrations in bass tissue. Of the two fractions, more of the variability was explained in methylmercury than in total mercury. Weight was not considered significant for either fraction.

We could interpret these results as bass in our sample set continue accumulating inorganic mercury and methylmercury with growth, but the greatest change is in the organic fraction with length.

Changes With Location

Percent Methylmercury

When we tested differences in mean percent methylmercury values while accounting for length, these two variables combined explain 50% of the variability (Table A5). This tells us that there are differences related to size and location, something noted by Mason et al. (2006) for total mercury concentrations in largemouth bass between habitat types (estuarine vs. fresh) and by Sveinsdottir and Mason (2005) for largemouth bass methylmercury concentrations across Maryland. Mean percent methylmercury concentrations were significantly different between Lake Spokane and Liberty Lake and between Loon and Liberty Lakes with Silver Lake. Lakes Spokane, Liberty, and Loon are in eastern WA, and Silver Lake is in western WA (Figure 1).

Statistically different percent methylmercury levels between some eastern and western WA lakes demonstrate the potential of spatial influence on percent methylmercury levels in bass. There are intraregional differences between eastern and western WA lakes and interregional differences between two lakes (Lake Spokane and Liberty Lake). Although this study was not designed to evaluate what could cause regional variability (e.g., background mercury levels, proximity to sources, and lake productivity), variability exists and indicates that percent methylmercury levels are inconsistent across the state.

Total Mercury and Methylmercury

We found similar spatial variability in total mercury and methylmercury concentrations by lake. When we accounted for length, location and length explained approximately 70% of the variability in total mercury and methylmercury concentrations (Tables A10 – A15). Concentrations of both fractions were significantly different between Lake Spokane and both Liberty Lake and Loon Lake. Total mercury concentrations were also significantly different between Liberty Lake and Silver Lake. Our results are similar to Sveinsdottir and Mason (2005), who found regional differences in largemouth bass methylmercury concentrations from coastal, central, and western reservoirs.

State Water Quality Criterion and Health Screening Levels

Only 1 sample out of 40 analyzed had a total mercury concentration at or above the state WQC for human health when the methylmercury concentration was below the WQC. This sample had tissue concentrations of 46.8 ppb total mercury compared to 29.1 ppb methylmercury, demonstrating a situation where using total mercury overestimates the risk when we reference the WQC. This happens more frequently with the DOH SL.

Thirty percent of the samples (12 fish) had total mercury concentrations at or above the DOH SL when the methylmercury concentrations were below this threshold (Table A1). Because the DOH SL is set at a higher concentration than the state WQC, we only accurately capture 70% of methylmercury contamination above or below this threshold when we measure total mercury.

One of the most important takeaways from this comparison study is that designing monitoring studies around a conservative estimate (95% of total mercury is methylmercury) has not led to underestimating the overall risk of methylmercury to human health.

Overestimating Methylmercury in Bass

Using total mercury concentrations to represent methylmercury concentrations in fish tissue overestimates the toxic organic fraction in most of our sample set. Because of this, using total mercury as a surrogate for methylmercury should not underestimate the more hazardous fraction of mercury. Of the 40 samples collected, 37 (92.5%) had methylmercury and total mercury at or above the WQC, and 10 (25%) had methylmercury and total mercury above the DOH SL.

Conclusions

During 2020, we studied percent methylmercury levels, total mercury concentrations, and methylmercury concentrations in bass from four lakes in Washington state and evaluated the effects of fish growth characteristics. Results from our analysis were compared to state mercury thresholds for human health as context for how data from these monitoring studies are used.

Following are our key findings:

- The average percent methylmercury content in bass tissue was lower than the conservative estimate of 95% used by EPA in their guidance to states for fish contaminant monitoring programs.
  - Mean percent methylmercury level in bass was 66% (±16%) (median = 63%)
- Percent methylmercury levels, total mercury concentrations, and methylmercury concentrations for all bass covered a wide range of values and varied by location, size, and age. Each mercury fraction increased with one or more of the growth characteristics.
- All fish tissue contained methylmercury and total mercury. Percent methylmercury ranged from 39% – 124%, total mercury concentrations ranged from 18.8 – 363 ppb, and methylmercury concentrations ranged from 12.3 – 266 ppb.
- Length and location explained percent methylmercury variability in bass tissue better than other variables.
  - Statistical tests showed that percent methylmercury was best explained by location when we accounted for length. Combined, these two factors accounted for 50% of change.
- Length and location explained mercury concentrations in bass tissue overall but differed by lake for total mercury and methylmercury.
- Assuming total mercury consists almost entirely of methylmercury in bass tissue overestimates the methylmercury fraction, making it a conservative measure for human health. Of the samples analyzed in this study, 92.5% exceeded the state WQC for human health, using either total mercury or methylmercury concentrations. Only 25% of the samples would exceed the DOH SL using either value.

Recommendations

The following are recommendations from this 2020 study in Washington State:

- The Mercury Trends project should continue to monitor total mercury in fish tissue following EPA's guidance for contaminant monitoring studies. We will continue to use total mercury concentrations as (1) a conservative estimate for methylmercury to protect human health, (2) for continuity of sampling methods over a long-term monitoring program, and (3) to continue testing fish at the sample size allowed by the cost of total mercury.
- Ecology's fish monitoring programs should consider additional testing for methylmercury and total mercury to expand the dataset in the state and to either confirm or reduce the variability in methylmercury concentrations found in this study. Future studies should use the same laboratory for total mercury and methylmercury analyses and consider adding triplicate analyses to assess within-sample variability.
- Users of these data should be aware that the methylmercury fraction in largemouth and smallmouth bass caught in the state varies, and EPA's 95% estimate is not necessarily representative of these species. In this study, most methylmercury concentrations in fish tissue made up 50% – 82% of total mercury.

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Acronyms and Abbreviations

Acronyms and Abbreviations

BAL	Brooks Applied Labs
DOH SL	Washington State Department of Health Screening Level
Ecology	Washington State Department of Ecology
EPA	U.S. Environmental Protection Agency
MEL	Manchester Environmental Laboratory
MeHg	methylmercury
MDL	method detection limit
MQO	measurement quality objectives
MRL	method reporting limit
PBT	persistent, bioaccumulative, and toxic substance
RPD	relative percent difference
SOP	standard operating procedures
THg	total mercury
WQC	water quality criterion
% MeHg	percent Methylmercury

Units of Measurement

g	gram, a unit of mass
kg	kilograms, a unit of mass equal to 1,000 grams
mg	milligram
mg/kg	milligrams per kilogram (parts per million)
mm	millimeters
ng/g	nanograms per gram
µg/kg	micrograms per kilogram (parts per billion)
ww	wet weight

Appendices

Appendix A. Supplemental Tables and Graphs

Table A1. Mercury Fractions and Growth Variables for Each Bass by Location.

Lake Location	Species	Station ID	Weight (g)	Length (mm)	Sex	Age	THg (ug/kg)	MeHg (ug/kg)	% MeHg
Liberty	LMB	LIBLMB1	1458	459	F	8	363	266	73
Liberty	LMB	LIBLMB2	752	391	M	9	257	170	66
Liberty	LMB	LIBLMB3	1043	418	M	13	337	211	63
Liberty	LMB	LIBLMB4	756	385	M	5	192	132	69
Liberty	LMB	LIBLMB5	1105	412	F	5	160	77.6	49
Liberty	LMB	LIBLMB6	525	331	F	-	122	63.0	52
Liberty	LMB	LIBLMB7	392	306	F	3	134	70.1	52
Liberty	LMB	LIBLMB8	373	297	F	5	110	46.7	42
Liberty	LMB	LIBLMB9	293	293	F	4	92.7	36.4	39
Liberty	LMB	LIBLMB10	201	262	F	4	125	53.9	43
Loon	LMB	LOONLMB1	1452	444	M	13	258	220	85
Loon	LMB	LOONLMB2	1060	413	F	8	221	183	83
Loon	LMB	LOONLMB3	1302	439	M	8	209	144	69
Loon	LMB	LOONLMB4	709	355	M	4	134	61.9	46
Loon	LMB	LOONLMB5	978	384	M	5	130	71.8	55
Loon	LMB	LOONLMB6	781	392	F	7	211	132	63
Loon	LMB	LOONLMB7	867	384	F	5	159	92.8	58
Loon	LMB	LOONLMB8	872	399	M	14	318	220	69
Loon	LMB	LOONLMB9	364	300	M	3	101	53.0	52
Loon	LMB	LOONLMB10	367	295	F	5	58.4	30.6	52
Silver	LMB	SILLMB1	1137	436	F	5	78.6	73.4	93
Silver	LMB	SILLMB2	747	367	M	5	163	100	61
Silver	LMB	SILLMB3	691	354	F	5	111	69.0	62
Silver	LMB	SILLMB4	427	300	F	2	71.1	44.4	62
Silver	LMB	SILLMB5	372	293	F	5	60.3	56.5	94
Silver	LMB	SILLMB6	332	283	M	5	68.8	49.1	71
Silver	LMB	SILLMB7	301	279	F	5	48.0	31.7	66
Silver	LMB	SILLMB8	435	310	M	2	67.5	52.8	78
Silver	LMB	SILLMB9	262	266	F	4	27.8	17.2	62
Silver	LMB	SILLMB10	197	243	M	3	18.8	12.3	65
Spokane	SMB	SPOKSMB1	1652	480	F	7	69.3	85.9	124
Spokane	SMB	SPOKSMB2	1130	426	M	7	49.8	35.4	71
Spokane	SMB	SPOKSMB3	984	435	F	7	148	91.3	62
Spokane	SMB	SPOKSMB4	1075	416	M	6	60.8	36.2	60
Spokane	SMB	SPOKSMB5	578	345	F	6	78.1	52.0	67
Spokane	SMB	SPOKSMB6	365	296	M	5	48.9	31.5	64
Spokane	SMB	SPOKSMB7	528	351	M	6	94.7	86.6	91
Spokane	SMB	SPOKSMB8	588	363	M	5	156	119	76
Spokane	SMB	SPOKSMB9	378	303	M	3	46.8	29.1	62
Spokane	SMB	SPOKSMB10	288	284	M	5	54.8	31.9	58

Note. LMB = largemouth bass, SMB = smallmouth bass, THg = total mercury, and MeHg = methylmercury.

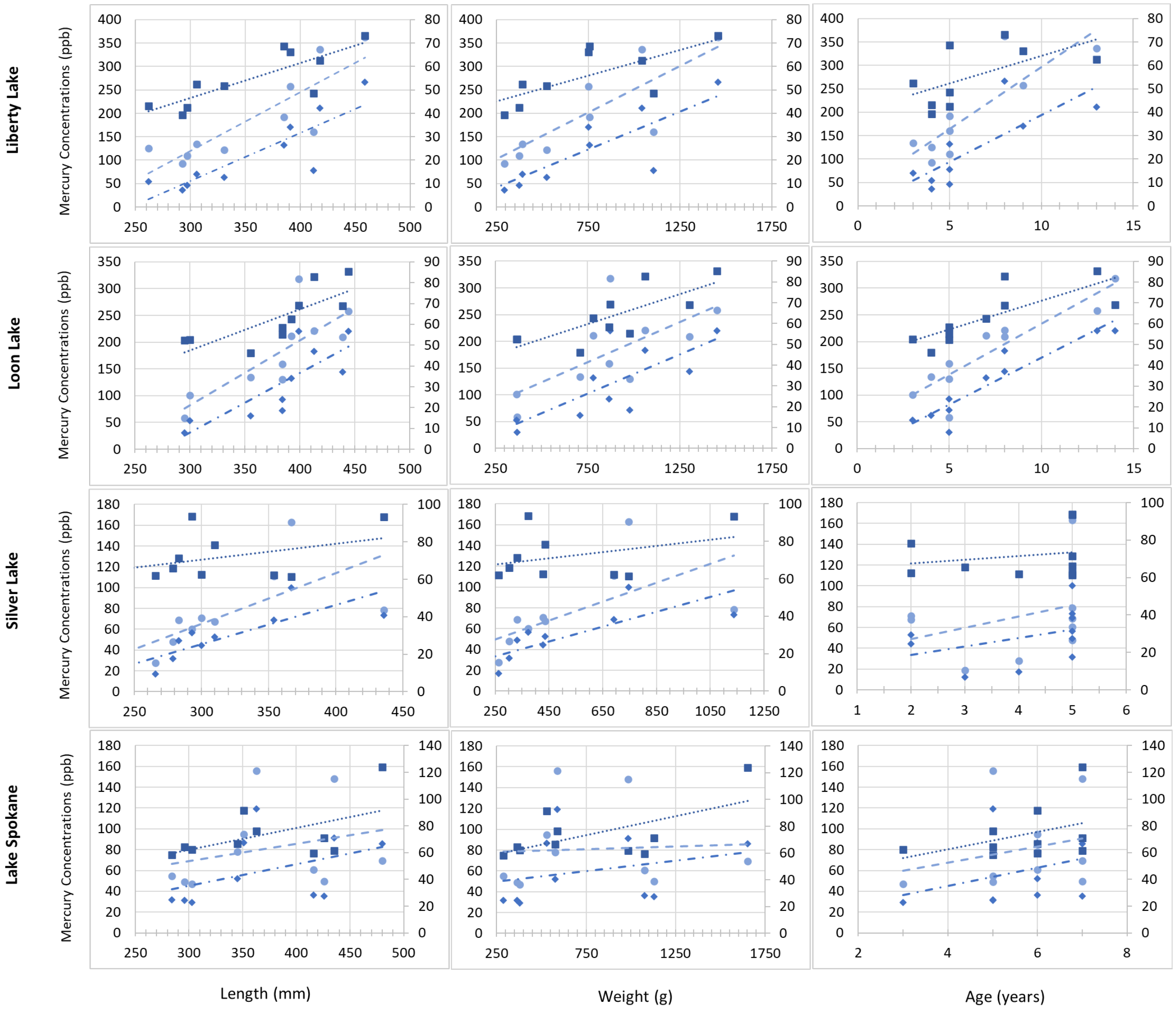

Figure A1. Scatterplots for Mercury Fractions and Growth Variables by Location.
Light blue circles with dashed lines represent total mercury concentrations, methylmercury concentrations are represented by medium blue diamonds with dash and dot lines, and the dark blue squares with dotted lines represent percent methylmercury values. Percent methylmercury values are plotted on the secondary y-axis.

Table A2. Regression Results for % Methylmercury and Growth Variables.

Variable	B	SE	β	Sig.	R^2
Constant	314	158	1.98	0.055	0.260
Length	-0.725	0.648	-1.12	0.271	0.260
L10 Weight	-104	64.0	-1.63	0.113	0.260
Length*L10 Weight	0.298	0.183	1.63	0.113	0.260

Note. **α** (alpha level) = 0.05.
B = intercept or coefficient, SE = standard error, β = beta coefficient, and Sig. = p-value.

Table A3. Regression Results for Log 10 Total Mercury and Growth Variables.

Variable	B	SE	β	Sig.	R^2
Constant	-4.95	2.41	-2.05	0.0473	0.442
Length	0.0267	0.00987	2.71	**0.0103**	0.442
L10 Weight	1.75	0.973	1.79	0.0814	0.442
Length*L10 Weight	-0.00734	0.00278	-2.64	**0.0122**	0.442

Note. Bold Sig. values indicate significant correlations. **α** (alpha level) = 0.05.
B = intercept or coefficient, SE = standard error, β = beta coefficient, and Sig. = p-value.

Table A4. Regression Results for Log 10 Methylmercury and Growth Variables.

Variable	B	SE	β	Sig.	R^2
Constant	-4.03	2.24	-1.80	0.0798	0.597
Length	0.0231	0.00915	2.53	**0.0160**	0.597
L10 Weight	1.27	0.903	1.41	0.167	0.597
Length*L10 Weight	-0.00588	0.00258	-2.28	**0.0288**	0.597

Note. Bold Sig. values indicate significant correlations. **α** (alpha level) = 0.05.
B = intercept or coefficient, SE = standard error, β = beta coefficient, and Sig. = p-value.

Table A5. ANCOVA for Percent Methylmercury by Location Controlled for Length.

Variable	Type III SS	df	Mean Sq.	F	Sig.
Location	3.1×10^3	3.0	1.0×10^3	7.2	**0.0010**
Length	2.9×10^3	1.0	2.9×10^3	20	**<0.05**
Error	5.1×10^3	35	-	-	-

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05, RSE of 12.1, and $r^2 = 0.50$.
df = degrees of freedom, F = F-value, and Sig. = p-value.

Table A6. Bonferroni's Post Hoc Test for ANCOVA for Percent Methylmercury by Location Controlled for Length.

Location 1	Location 2	Difference	Sig.	Lower 95% CI	Upper 95% CI
Spokane	Liberty	16.60	**0.026**	5.56	27.6
Spokane	Loon	11.80	0.221	0.76	22.80
Spokane	Silver	-6.47	1.000	-18.10	5.170
Liberty	Loon	-4.81	1.000	-15.9	6.3
Liberty	Silver	-23.10	**0.001**	-34.40	-11.7
Loon	Silver	-18.20	**0.022**	-30.10	-6.3500

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05. Sig. = p-value and CI = confidence interval.

Table A7. Adjusted Means for ANCOVA for Percent Methylmercury by Location Controlled for Length.

Location	Est. Mean	SE	df	Lower 95% CI	Upper 95% CI
Spokane	71.3	3.86	35	63.5	79.1
Liberty	54.7	3.83	35	46.9	62.5
Loon	59.5	3.92	35	51.6	67.5
Silver	77.8	4.07	35	69.5	86.00

Note. SE = standard error, df = degrees of freedom, and CI = confidence interval.

Table A8. Kruskal-Wallis Results for Mercury Fractions Between Length Groups.

Length (mm)*	% MeHg *Sig.*	THg *Sig.*	MeHg *Sig.*
Total	0.14	**0.0010**	**<0.0010**
250 & 300	0.0030	0.86	0.76
250 & 350	0.52	**<0.0010**	**<0.0010**
250 & 400	0.11	**0.0010**	**<0.0010**
300 & 350	<0.0010	**<0.0010**	**<0.0010**
300 & 400	<0.0010	**<0.0010**	**<0.0010**
350 & 400	0.57	0.74	0.66

Note. Bold Sig. values indicate a significant relationship.
α (alpha level) = 0.05 and df=3.
*Dwass-Steel-Chritchlow-Fligner for pairwise comparisons
Sig. = p-value.

Table A9. Wilcoxon Signed-Rank Test: Total Mercury and Methylmercury Concentrations Within Length Groups.

Length (mm)	Z-Score	Sig.	Count
Total	-5.4	**<0.0010**	40
250+	-2.8	**0.0050**	10
300+	-2.4	**0.018**	7
350+	-2.9	**0.0030**	11
400+	-2.7	**0.0080**	11

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05.
Sig. = p-value.

Table A10. ANCOVA for L10 Total Mercury by Location Controlled for Length.

Variable	Type III SS	df	Mean Sq.	F	Sig.
Location	1.12	3	0.372	27.8	**0.00**
L10 Length	0.83	1	0.827	13.3	**0.00**
Error	0.98	35	-	29.59	-

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05, RSE of 0.1672, and r^2 = 0.71.
df = degrees of freedom, F = F-value, and Sig. = p-value.

Table A11. Bonferroni's Post Hoc Test for ANCOVA for L10 Total Mercury by Location Controlled for Length.

Location 1	Location 2	Difference	Sig.	Lower 95% CI	Upper 95% CI
Spokane	Liberty	-0.401	**0.000**	-0.554	-0.249
Spokane	Loon	-0.32	**0.001**	-0.472	-0.168
Spokane	Silver	-0.062	1.000	-0.223	0.099
Liberty	Loon	0.082	1.000	-0.072	0.235
Liberty	Silver	0.339	**0.001**	0.182	0.496
Loon	Silver	0.258	0.018	0.093	0.422

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05
Sig. = p-value and CI = confidence interval.

Table A12. Adjusted Means for ANCOVA for L10 Total Mercury by Location Controlled for Length.

Location	Est. Mean	SE	df	Lower 95% CI	Upper 95% CI
Spokane	67.6	0.053	35	52.5	87.1
Liberty	170	0.053	35	132	219
Loon	141	0.054	35	110	182
Silver	77.6	0.056	35	60.3	100

Note. Table A12 estimated means and associated CI are back-transformed.
SE = standard error, df = degrees of freedom, and CI = confidence interval.

Table A13. ANCOVA for L10 Methylmercury by Location Controlled for Length.

Variable	Type III SS	df	Mean Sq.	F	Sig.
Location	0.53	3	0.175	5.12	**0.005**
L10 Length	1.56	1	1.56	45.54	**0.000**
Error	1.20	35	-	-	-

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05, RSE of 0.185, and R^2=0.68
df = degrees of freedom, F = F-value, and Sig. = p-value.

Table A14. Bonferroni's Post Hoc Test for ANCOVA for L10 Methylmercury by Location Controlled for Length.

Location 1	Location 2	Difference	Sig	Lower 95% CI	Upper 95% CI
Spokane	Liberty	-0.290	0.008	-0.458	-0.121
Spokane	Loon	-0.249	0.030	-0.417	-0.081
Spokane	Silver	-0.110	1.000	-0.288	0.068
Liberty	Loon	0.041	1.000	-0.129	0.211
Liberty	Silver	0.180	0.256	0.006	0.353
Loon	Silver	0.139	0.777	-0.043	0.321

Note. Bold Sig. values indicate a significant relationship. **α** (alpha level) = 0.05
Sig. = p-value and CI = confidence interval.

Table A15. Adjusted Means for ANCOVA for L10 Methylmercury by Location Controlled for Length.

Location	Est. Mean	SE	df	Lower 95% CI	Upper 95% CI
Spokane	46.8	0.059	35	35.5	61.7
Liberty	91.2	0.059	35	69.2	120
Loon	83.2	0.060	35	61.7	110
Silver	60.3	0.062	35	44.7	79.4

Note. Table A15 estimated means and associated CI are back transformed.
SE = standard error, df = degrees of freedom, and CI = confidence interval.

Appendix B. Numerical Thresholds for the Protection of Human Health from Mercury in Fish Tissue

To provide context for the mercury results in this 2020 study, we compared data to two methylmercury thresholds:

- Washington State's Water Quality Criterion (WQC) for human health (40 CFR 131.45) that went into effect in 2016.
- DOH's screening level (SL) for fish consumption advisories.

Both thresholds are based on the toxicological effects of methylmercury, the bioaccumulative and toxic form of mercury in fish tissue, while values in this report reflect total mercury. EPA guidance for fish contaminant monitoring programs recommends analyzing mercury as a surrogate for methylmercury as a conservative approach to be most protective of human health (EPA 2000).

Washington State's methylmercury WQC of 30 ppb (tissue) is a human health criterion based on a fish consumption rate of 175 g/day over a 70-year lifespan. This rate represents the average consumption of all fish and shellfish (including salmon and fish/shellfish eaten at restaurants, locally caught, imported, or obtained from other sources) for highly exposed populations that consume both fish and shellfish from Puget Sound waters. Washington State assesses waterbodies for impairment using all data collected from a waterbody over the period that the assessment cycle is addressing, using median concentrations of fish tissue composite samples (Ecology 2018).

The DOH SL is a threshold DOH toxicologists use when developing fish consumption advisories, in addition to other factors. The DOH SL of 101 ppb (tissue) is based on a general population consumption rate of 59.7 g/day, which the American Heart Association recommends for a healthy diet (two 8 oz fish meals per week). DOH uses the SL to advise fish consumers in Washington. In contrast, the WQC is used to set the National Pollutant Discharge Elimination System (NPDES) permit limits, assess waters, and represents full protection of the designated use of harvest.

Data exceeding these two thresholds do not necessarily represent an impaired use or a fish consumption advisory. State agencies use fish tissue mercury data, including data provided in this report, as part of an overall assessment of a waterbody, using an approach to address average exposures over a period of time.