

# Measuring Mercury Trends in Freshwater Fish, 2016 Sampling Results



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## Environmental Assessment Program

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## Abstract

This report presents results from the twelfth year of a long-term monitoring program by the Washington State Department of Ecology (Ecology) to assess mercury trends in resident freshwater fish tissue.

In 2016, Ecology analyzed mercury in fish collected from Mason Lake, Lake Meridian, Moses Lake, Newman Lake, Offutt Lake, and Lake Sammamish. Results were compared to mercury concentrations previously measured in fish collected in 2006 and 2011 (and 2002 for Newman Lake). Species collected for individual analysis included largemouth bass, smallmouth bass (Moses Lake), and yellow perch (Mason Lake). Mercury was also analyzed in six composite samples of other freshwater fish species from three of the lakes for a secondary goal of supporting fish consumption advisories.

Mercury was present in all fish tissue samples. A total of 59 fish fillets were analyzed as individuals. Mercury concentrations in individual samples ranged from 22.3 to 592 ppb, with a median of 93.8 ppb.

Statistical tests revealed a significantly lower fish mercury level in the most-recent sampling year (2016) compared to one or more previous sampling years for the Newman Lake, Offutt Lake, and Lake Sammamish datasets. No significant changes among the sampling years were observed for Mason Lake, Lake Meridian, or Moses Lake.

Estimated mercury levels in 2016 Newman Lake largemouth bass were 39% lower than bass collected in 2002. The 2006 and 2011 sampling years showed a general decline in mercury levels in the lake, but changes were not significant. Offutt Lake 2016 largemouth bass estimated mercury levels were 51% and 42% lower than previous sampling in 2006 and 2011, respectively. In Lake Sammamish, estimated mercury levels were the highest in 2011 and then decreased to the lowest level in 2016. Estimated mercury in 2016 Lake Sammamish bass were 37% and 53% lower than the 2006 and 2011 bass, respectively.

# Background

The Washington State Departments of Ecology (Ecology) and Health (DOH) developed a chemical action plan (CAP) for mercury in 2003 to address the public health threat of mercury in the state and recommend actions for mercury reduction (Peele et al., 2003). Human activity has greatly increased the release of mercury into the environment and consequences of that include increased health risks to humans and wildlife due to the persistent, bioaccumulative, and toxic nature of mercury. In humans, mercury can affect the nervous system, with children and developing fetuses being most at risk (EPA, 2000). Because eating fish is the greatest source of mercury exposure for most people, Washington’s mercury CAP recommended actions to prevent the entry of mercury into the environment.

Ecology began a monitoring program to assess mercury levels in edible freshwater fish tissue in 2005. The primary goal of this

program is to characterize temporal trends in fish fillet mercury levels in Washington State. Each year, the monitoring program collects ten largemouth or smallmouth bass (or another species if no bass are present) for analysis of total mercury in individual skin-on fillets from six lakes. Ecology returns to each set of six lakes every five years to determine if mercury levels are changing. If encountered, additional species are also collected from the lakes and analyzed as composites for a secondary goal of providing data to DOH to support fish consumption advisories.

The monitoring program completed the third round of sampling from the following lakes in 2016: Mason Lake, Lake Meridian, Moses Lake, Newman Lake, Offutt Lake, and Lake Sammamish (Figure 1). Individual bass fillets were analyzed for total mercury to compare to previous collections in 2006 and 2011 (and 2002 for Newman Lake). Individual yellow perch were collected from Mason Lake instead of bass to match previous collections.

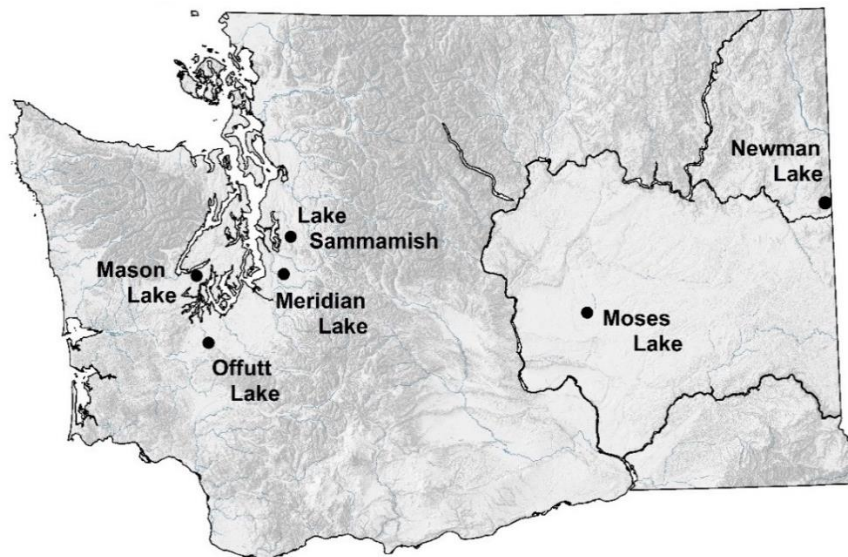


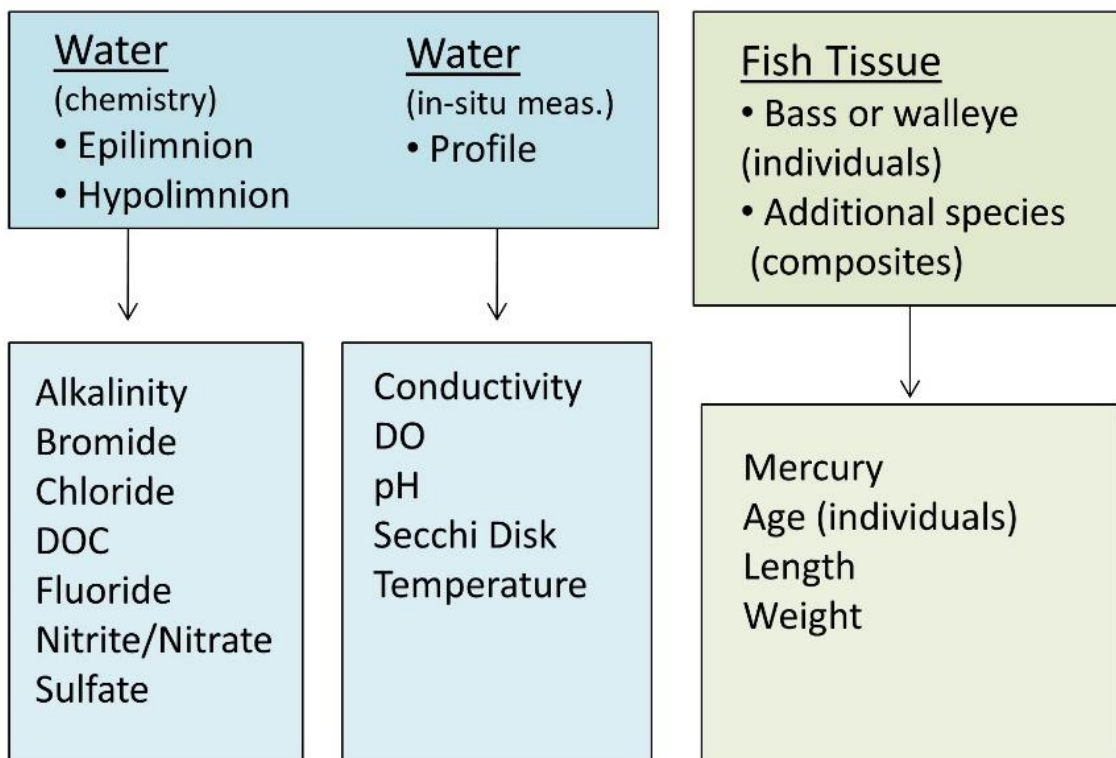
Figure 1. Locations of 2016 sampling sites.

# Methods

Figure 2 outlines the data measured or collected by this monitoring program each year. At each of the six sites, Ecology collects water chemistry and in-situ water measurements during summer stratification to better understand patterns, dynamics, and changes in mercury accumulation in fish. Water data collected in 2016 are briefly described in the Results section of this report; however, a more detailed analysis of how water chemistry influences fish mercury levels is provided every five years in a larger statewide trends report. The next

five-year report will be written in 2019 after all sites have been sampled three times.

Fish are collected in the fall to match historical sampling events and are analyzed individually for total mercury to achieve statistical power in detecting trends. Composite samples of additional species help inform DOH’s fish consumption advisories. All sampling conducted in 2016 followed the Quality Assurance Project Plan (QAPP) (Seiders, 2006) and QAPP addendum (Meredith and Furl, 2010). Ecology collected all fish via electrofishing methods and processed samples following Ecology standard operating procedures (Sandvik, 2018a and 2018b).



**Figure 2. Analytes and measurements recorded at each waterbody.**

DOC = dissolved organic carbon  
DO = dissolved oxygen

Table 1 describes the collection goals met for 2016. In September and October, Ecology collected a total of 59 fish from six waterbodies for analysis of mercury as individuals. Species and size classes of fish collected for individual analysis were targeted to match past sampling events and included largemouth bass, smallmouth bass (Moses Lake), and yellow perch (Mason Lake). For analysis of composite samples, a total of 19 fish from three of the waterbodies (Moses Lake, Offutt Lake, and Lake Sammamish) were collected and included brown bullhead, common carp, and yellow perch. Species collected for composite analysis were combined into a total of six composite samples consisting of 3-5 fish per sample.

Washington State Department of Fish and Wildlife (WDFW) biologists determined

the ages of fish analyzed as individuals. Manchester Environmental Laboratory (MEL) analyzed water and fish tissue samples following methods outlined in QAPP. Mercury data presented in this report are expressed on a  $\mu\text{g}/\text{kg}$  (ppb) wet weight basis and referred to as “ppb” throughout.

Laboratory data generally met measurement quality objectives (MQOs) outlined in the QAPP and QAPP addendum. Two matrix spike recoveries for the fish mercury analysis were just outside of MQOs (78% and 114%), but within acceptance limits of the method. All data were deemed usable as qualified. Laboratory case narratives are available upon request.

**Table 1. Summary of sampling conducted in 2016.**

Collection Goal	Mason Lake	Lake Meridian	Moses Lake	Newman Lake	Offutt Lake	Lake Sammamish
<b>10 individual bass</b>	+ <sup>1</sup>	+ <sup>2</sup>	+	+	+	+
<b>Species collected for composites</b>	---	---	CCP	---	YP	BBH
<b>2 water samples</b>	+	+	+	+	+	+
<b>Hydrolab profile</b>	+	+	+	+	+	+

<sup>1</sup>individual yellow perch obtained from Mason Lake

<sup>2</sup>only 9 individual bass obtained from Lake Meridian  
“+” = goal achieved

YP = yellow perch  
CCP = common carp  
BBH = brown bullhead

# 2016 Water Sample Results

Temperature profiles showed all six lakes were stratified at the time of water sampling in July. Dissolved oxygen (DO) levels were between 9 and 12 mg/L at the surface of the waterbodies and fell to anoxic or hypoxic conditions at the bottom of the profiles.

Figure 3 displays results of the water samples analyzed for alkalinity, dissolved

organic carbon (DOC), DO, and sulfate. Bromide, fluoride, and nitrate/nitrite were also analyzed, but not shown here because most of the samples were non-detects. Moses Lake had the highest alkalinity and sulfate concentrations of the six study sites. Newman Lake had the lowest alkalinity and chloride levels, and the highest DOC values. All values were within the range of, and similar to, water samples collected in previous sampling years at each site.

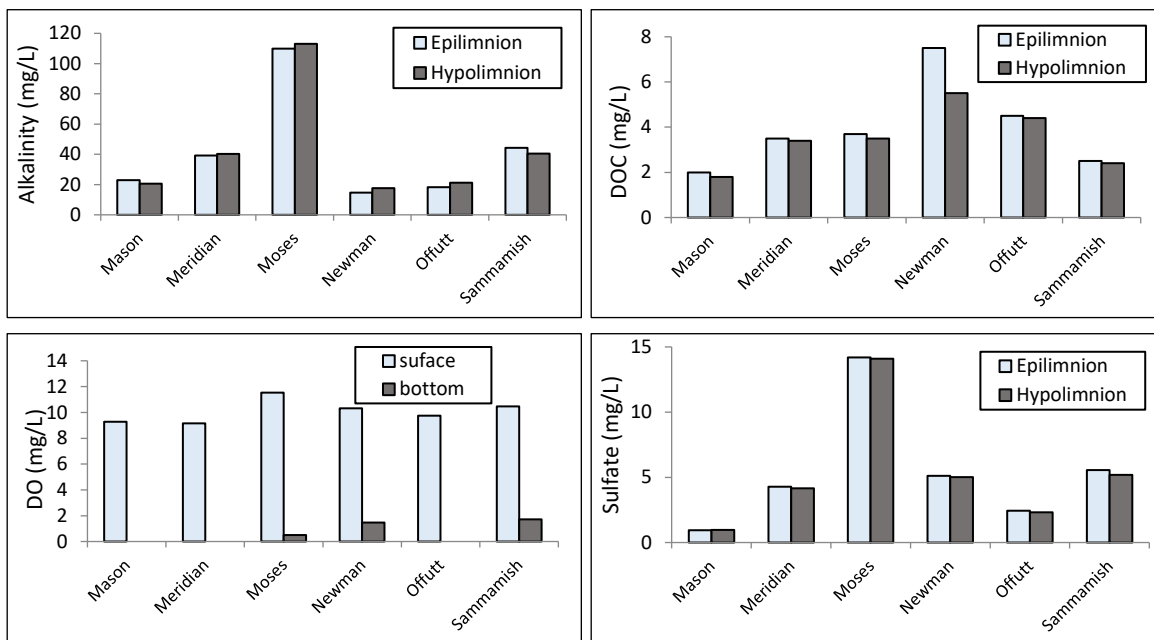


Figure 3. Alkalinity, dissolved organic carbon (DOC), dissolved oxygen (DO), and sulfate concentrations in 2016 water samples.

# 2016 Mercury Results

## Individual Fillet Data

Results of 2016 fish tissue and water chemistry sampling are available for download on Ecology’s EIM webpage<sup>1</sup> by searching Study ID: HgFish16. Mercury concentrations and ancillary data for the 2016 individual fish are summarized in Table 2.

Mercury was present in all individual fish tissue samples, with concentrations ranging from 22.3 to 592 ppb. Most of the individual fish analyzed in 2016 had mercury concentrations between 80 and 120 ppb. Exceptions to this included much lower mercury concentrations in the Moses Lake samples and three higher concentrations from Newman Lake.

Bass collected from Moses Lake have consistently contained some of the lowest

<sup>1</sup> <https://ecology.wa.gov/Research-Data/Data-resources/Environmental-Information-Management-database>

mercury concentrations measured for this monitoring program. High alkalinity in the lake and continued flushing of the lake by water from the Columbia River (via the Columbia Basin Project) are suggested drivers of the low mercury levels (Fischnaller et al., 2003; Mathieu et al., 2013). The inverse relationship between alkalinity and fish mercury levels has been explained as a result of enhanced microbial production in acidic waters and/or increased bioavailability of mercury in low-pH lakes (Xun et al., 1987; Wiener et al., 1990).

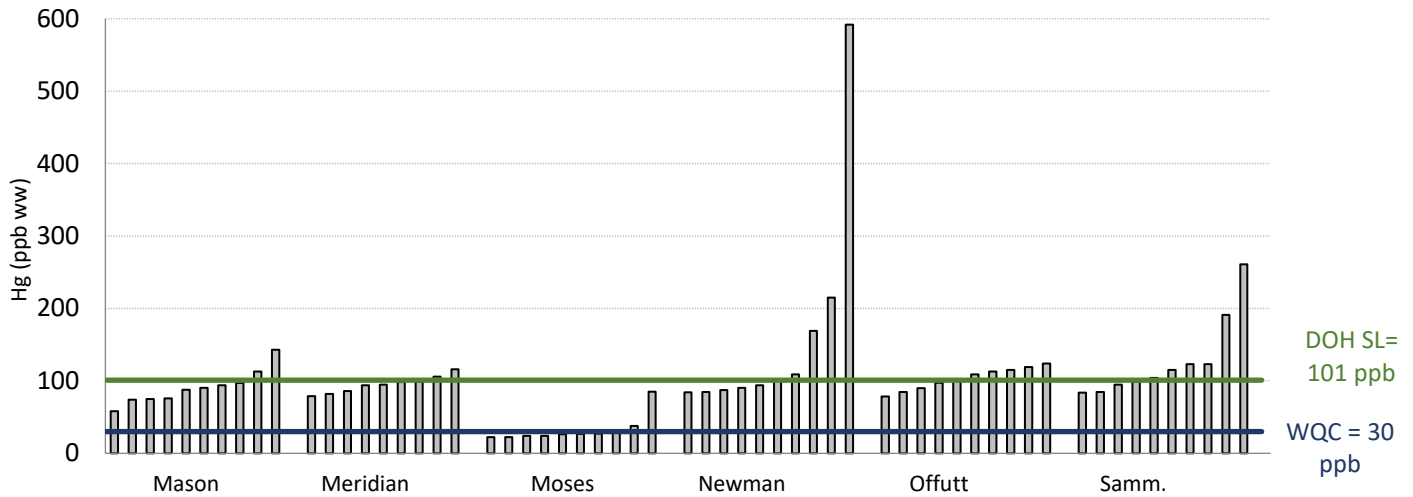
The highest levels in Newman Lake fillets came from fish that were older than other fish in the 2016 dataset, likely explaining the higher mercury concentrations as older fish have more time to accumulate mercury. The three samples came from fish that were 6, 7, and 22 years old. Newman Lake also had the lowest alkalinity and highest DOC levels of the waterbodies. DOC can influence mercury levels in a lake by facilitating transport of mercury to the lake surface water and stimulating in-lake methylation (Driscoll et al., 2007).

**Table 2. Summary statistics of individual fish lengths, weights, ages, and mercury concentrations.**

Waterbody	Species	n	Length (TL mm)		Weight (g)		Age (yr)		Hg (ppb ww)		
			Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Median
<b>Mason</b>	YP	10	210–260	227 (14.3)	103–183	136 (24)	2–3	2.2 (0.4)	58.3–143	90.8 (23.8)	89.0
<b>Meridian</b>	LMB	9	219–311	254 (33.7)	144–453	254 (116)	1–2	1.2 (0.4)	78.9–116	95.0 (11.7)	94.7
<b>Moses</b>	SMB	10	225–433	317 (65.8)	174–1477	559 (416)	1–8	2.6 (2.1)	22.3–85.3	32.5 (19.1)	26.6
<b>Newman</b>	LMB	10	299–475	357 (51.5)	342–1916	704 (460)	4–22	6.3 (5.6)	84.3–592	163 (157)	97.4
<b>Offutt</b>	LMB	10	243–305	264 (17.8)	175–407	240 (65.9)	2	2 (0)	78.4–124	103 (15.3)	105
<b>Sammamish</b>	LMB	10	260–429	337 (58.4)	238–1405	655 (397)	2–4	2.5 (0.7)	83.6–261	128 (56.0)	110
<b>All Sites</b>	—	59	210–475	293 (64.1)	103–1916	427 (370)	1–22	3 (2.9)	22.3–592	102 (78)	93.8

TL = total length  
SD = standard deviation  
YP = yellow perch

LMB = largemouth bass  
SMB = smallmouth bass



**Figure 3. Mercury concentrations of individual fish collected in 2016.**

DOH SL = Washington State Department of Health Screening Level  
 WQC = Water Quality Criterion

YP = yellow perch  
 LMB = largemouth bass  
 SMB = smallmouth bass

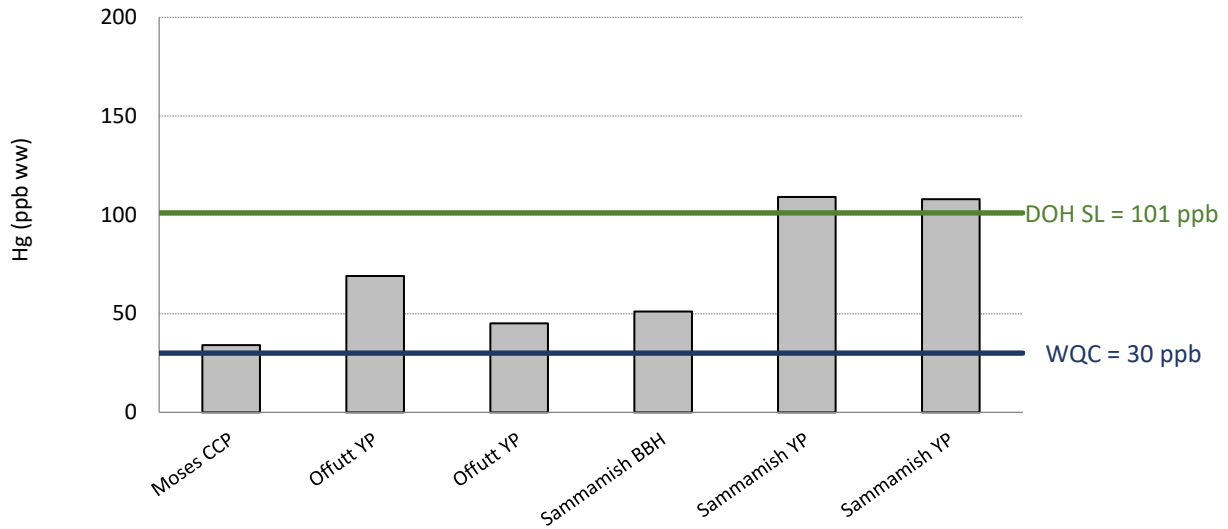
Two thresholds – the state water quality criterion (WQC) and DOH screening level (SL) – are described in Appendix A and referred to here to provide context for the mercury concentrations found in the waterbodies. Fifty-one out of fifty-nine individual samples (86%) had mercury concentrations above the Washington State WQC (Figure 5). All samples analyzed from Mason Lake, Lake Meridian, Newman Lake, Offutt Lake, and Lake Sammamish were above the WQC. The only samples with mercury concentrations below the WQC were smallmouth bass fillets collected from Moses Lake.

Nineteen individual samples (32%) had mercury concentrations above the DOH SL of 101 ppb. All sites except Moses Lake had at least two samples with concentrations above the DOH SL; however, only Offutt Lake and Lake Sammamish had median mercury

concentrations above the DOH SL.

### Ancillary Species

A total of six composite samples collected from three of the sites were analyzed for mercury in 2016 (Figure 5). Common carp was collected from Moses Lake, yellow perch was collected from Offutt Lake and Lake Sammamish, and brown bullhead was also collected from Lake Sammamish. No additional species were obtained from Mason, Meridian, or Newman Lakes. Mercury concentrations of composite samples ranged from 34 ppb in the Moses Lake carp to 109 ppb in Lake Sammamish yellow perch. All composite sample concentrations were above the WQC of 30 ppb. Only the two yellow perch samples collected from Lake Sammamish had concentrations above the DOH SL of 101 ppb.



**Figure 4. Mercury concentrations of composite fish samples.**

DOH SL = WA Department of Health Screening Level  
 WQC = Water Quality Criterion  
 CCP = common carp  
 YP = yellow perch  
 BBH = brown bullhead

## Fish Tissue Mercury Relationships

Coefficients of determination from linear regressions between  $\log_{10}$  fish size and age are presented in Table 3. To improve normality and meet other assumptions necessary for the tests, data were  $\log_{10}$ -normalized prior to running linear regressions. Relationships were considered

significant at a p-value of less than 0.05. Mercury concentrations in bass significantly increased with fish size and age for the Mason Lake, Moses Lake, Newman Lake, and Lake Sammamish datasets. No relationships with mercury were significant for Lake Meridian, where eight of the ten largemouth bass collected were the same age (1 year old). Largemouth bass collected from Offutt Lake were all 2 years old, and no mercury relationships were found.

**Table 3. Linear regression coefficients of determination for  $\log_{10}$  mercury: size-age relationships.**

Lake	$r^2$		
	Length	Weight	Age
Mason	<b>0.50</b>	<b>0.39</b>	<b>0.54</b>
Meridian	0.00	0.00	n/a
Moses	<b>0.43</b>	<b>0.44</b>	<b>0.60</b>
Newman	<b>0.48</b>	<b>0.35</b>	<b>0.80</b>
Offutt	0.00	0.06	n/a
Sammamish	<b>0.35</b>	0.31	<b>0.50</b>

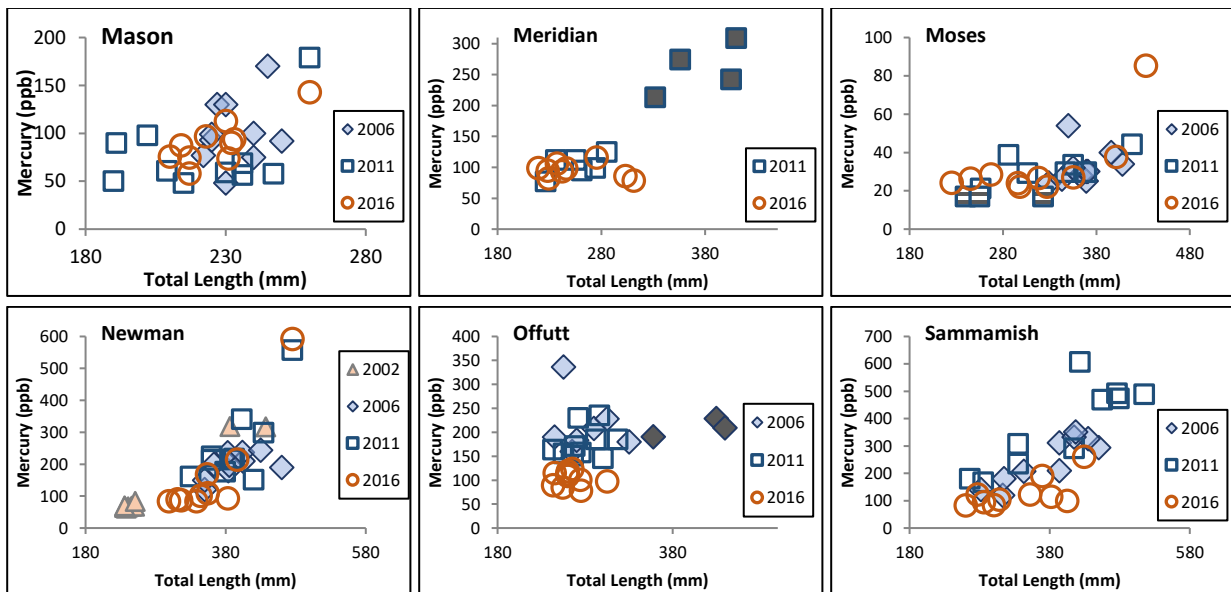
**Bolded** values indicate statistical significance ( $p < 0.05$ ).



# Temporal Trends

This monitoring program has collected individual fish for analysis of mercury from the six sites every five years starting in 2006. The 2006 results were reported by Furl (2007) and the 2011 results were reported by Mathieu and Friese (2012). Additional data was available from 2002 for Newman Lake (Fischnaller et al., 2003). Figure 6 displays the mercury and fish length data from all sampling years.

Temporal trends in fish mercury levels were assessed using analysis of covariance (ANCOVA) or ANOVA (when no covariate is appropriate) on log<sub>10</sub>-transformed data with Bonferroni post-hoc tests. Least squares means for each sampling year were back-transformed and bias-corrected; these values are referred to as “estimated mercury levels” in this report. Magnitude of change in mercury levels was calculated as the percent change between estimated mercury levels. Detailed information on the statistical analysis is provided in Appendix B.



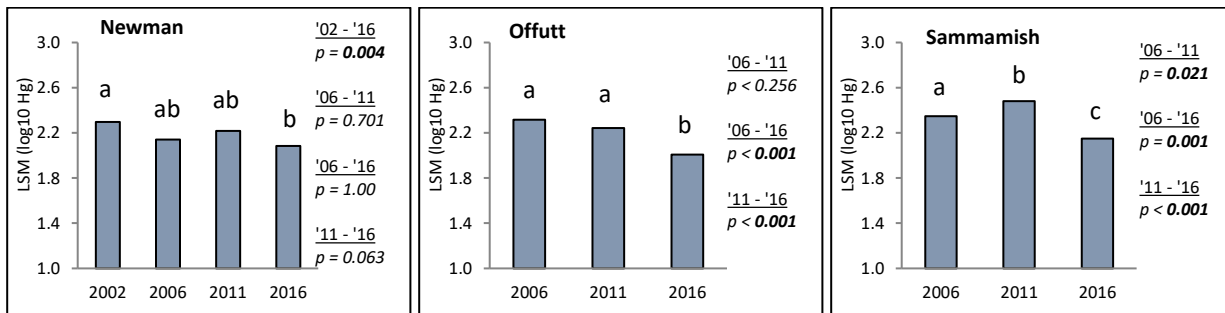
**Figure 6. Mercury concentrations and fish length data for all collection years.**

Shapes filled in with horizontal stripe pattern = non-detect value  
 Shapes filled in with dark grey = datapoints excluded from analysis

## Statistical Results

A significant difference in least squares means was found for at least one of the collection years in the Newman Lake, Offutt Lake, and Lake Sammamish datasets (Figure 7). Estimated mercury levels in Newman Lake largemouth bass collected in 2016 were 39% lower than bass collected in 2002, but not significantly different from the 2006 and 2011 sampling years. Offutt

Lake largemouth bass collected in 2016 had estimated mercury levels that were 51% and 42% lower than previous sampling in 2006 and 2011, respectively. Estimated mercury levels in Lake Sammamish largemouth bass over the three sampling years were highest in 2011, and lowest in 2016, with 2016 bass having 37% and 53% lower estimated mercury levels compared to 2006 and 2011, respectively.



**Figure 7. Bonferroni post-hoc test results.**

A difference in letters indicates a significant difference between collection years.  
LSM = least squares means

## Discussion

Mercury accumulation in fish is determined by a complex set of factors, including the amount of mercury loading to the waterbody, the availability of that mercury to the trophic system (i.e., methylation), and food web dynamics. Temporal trends in fish tissue mercury levels can also be affected by climatological fluctuations (Kolka et al., 2019; Thomas et al., 2018). The model used to determine differences between sampling years cannot attribute causes to the difference in mercury levels across years. The following is a limited discussion to examine possible factors that may influence fish mercury levels among sampling events.

Variables that were examined qualitatively for this report included watershed land use changes, precipitation and temperature patterns (PRISM Climate Group, accessed on 12/18/2018), water samples collected in each sampling year, and fish growth characteristics (length, weight, and age relationships). Unless noted in the following paragraphs, these factors did not reveal any apparent supporting explanation for differences observed in the fish mercury levels.

## Newman Lake

Mercury levels in Newman Lake largemouth bass were highest in 2002 and lowest in 2016. Water quality in the lake has improved since the early 2000s because of restoration efforts to increase oxygen levels and reduce nutrient loading (Moore et al., 2009; Moore et al., 2012). A hypolimnetic oxygenator with an alum injection system began operating at full capacity starting in 2001. Nurnberg anoxic factors calculated by Moore (2012) were dramatically lower after 2001 compared to the 1980s and 1990s, indicating fewer days of anoxia in the hypolimnion. Other changes such as oxidation of organic matter at the sediment water interface and decreased phytoplankton mass were also observed during the post-2001 time period (Moore et al., 2012). Hypolimnetic oxygenation efforts may suppress methylmercury in hypolimnion waters through binding and sedimentation (Dent et al., 2014), but it is unclear if the resultant lower mercury concentrations in bottom waters affect fish mercury levels (McCord et al., 2016).

Newman Lake water samples and DO profiles measured by this monitoring program did not show the same pattern. DO

profiles collected in July 2011 and August 2016 both reached anoxic conditions in the hypolimnion. However, our sampling was limited to one profile per sampling year and timing was targeted for peak stratification. Other water samples collected from Newman Lake remained similar across sampling years, with the exception of slightly higher DOC and sulfate concentrations in 2016 compared to the other sampling years.

### Lake Sammamish

Among the three collection years at Lake Sammamish, the highest mercury levels were seen in 2011. Several bass collected in 2011 were older (8–9 years old) compared to the other two sampling events (1–4 years old). This may help explain the higher mercury levels seen in the 2011 samples, as older fish have more time to accumulate mercury. However, a separate ANCOVA using fish age as covariate also resulted in a significant difference between the collection years. Using fish length or age as a covariate should control for the effect of the older age of fish collected in 2011. The ANCOVA model may be too simplistic to account for the older fish in this dataset. It may also omit other explanatory factors not examined here like changes in fish diet or fish community structure, which may have influenced the higher fish mercury levels in 2011.

### Offutt Lake

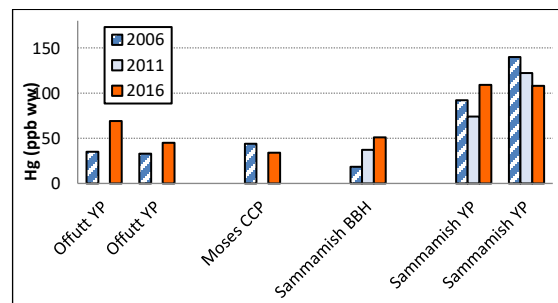
In Offutt Lake, sediment core records showed that sediment mercury concentrations and fluxes declined between the late 1990s and 2006 (Furl, 2009). The lower level of bass mercury in 2016 may be a reflection of the decrease in mercury loading to the lake, though the core record only covered up to the beginning of the fish monitoring period. All other variables that

were qualitatively assessed appeared to be similar among sampling years.

## Composite Fish Samples

Additional species were analyzed as composite samples to support DOH fish consumption advisories, not to detect trends. Figure 8 displays the mercury concentrations of similar-sized (<15% relative percent difference in fish length) composite samples collected over the three sampling periods. The following paragraph provides a qualitative discussion on the mercury levels seen throughout the sampling years.

Mercury concentrations in composite samples show no consistent pattern. Yellow perch collected from Offutt Lake in 2016 had higher concentrations of mercury in two composite samples compared to 2006, though the difference was slight in one pair. This is in contrast to the lower levels seen in the 2016 largemouth bass in Offutt Lake. Moses Lake common carp mercury levels were slightly lower in 2016 compared to 2006. Changes in Lake Sammamish mercury concentrations differed between collection years and species, with no consistent pattern.



**Figure 5. Mercury concentrations in composite samples for all collection years.**

YP = yellow perch  
 CCP = common carp  
 BBH = brown bullhead

# Conclusions and Recommendations

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Ecology analyzed mercury in freshwater fish samples collected from Mason Lake, Lake Meridian, Moses Lake, Newman Lake, Offutt Lake, and Lake Sammamish in the fall of 2016 as part of a long-term monitoring study. Results were compared to mercury concentrations previously measured from these same waterbodies in 2006 and 2011 (and 2002 for Newman Lake) to assess trends over time. Results of this study include the following:

- ◆ Mercury was present in all fish fillet samples analyzed. Mercury concentrations in individual samples ranged from 22.3 to 592 ppb, with a median of 93.8 ppb. Species collected for individual analysis included largemouth bass, smallmouth bass (Moses Lake), and yellow perch (Mason Lake).
- ◆ A total of six composite samples collected from three of the sites (Moses Lake, Offutt Lake, and Lake Sammamish) consisting of common carp, yellow perch, and brown bullhead were also analyzed for mercury. Mercury concentrations of the composite samples ranged from 34 ppb to 109 ppb.
- ◆ Statistical tests revealed a significantly lower fish mercury level in the most recent sampling year (2016) compared to one or more previous sampling years for Newman Lake, Offutt Lake, and Lake Sammamish.
  - Estimated mercury levels in Newman Lake largemouth bass collected in 2016 were 39% lower than bass collected in 2002. The 2006 and 2011 sampling years showed an overall general decline in bass mercury from the lake, but changes were not statistically significant.
  - Offutt Lake largemouth bass collected in 2016 had estimated mercury levels that were 51% and 42% lower than previous sampling in 2006 and 2011, respectively.
  - Estimated mercury levels in Lake Sammamish largemouth bass over the three sampling years were highest in 2011, and at lowest in 2016. In bass collected in 2016, estimated mercury levels were 37% and 53% lower compared to 2006 and 2011, respectively.
- ◆ No significant differences were observed among years for Mason Lake, Lake Meridian, or Moses Lake fish.

Recommendations of this study include the following:

- ◆ DOH should review the data presented in this report when updating fish consumption advisories.
- ◆ Ecology should include the data presented in this report in the next Water Quality Assessment cycle.
- ◆ This monitoring program should continue assessing fish tissue mercury levels at study sites on the planned rotation schedule through 2019. At that point, each of the 30 sites in the monitoring program will have been visited three times over a fifteen year period and a summary report should be written to assess trends observed statewide, and goals of the program should be re-evaluated.

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

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To put the mercury results into context, data in this report are compared to two methylmercury thresholds: Washington State's Water Quality criterion (WQC) for human health (40 CFR 131.45) that went into effect in 2016, and DOH's Screening Level (DOH 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). This study analyzes total mercury as a surrogate for methylmercury, as methylmercury makes up the majority of total mercury in fish tissue (Bloom, 1995; Driscoll et al., 1994).

Washington State's methylmercury WQC of 30 ppb is a tissue-based human health criterion based on a fish consumption rate of 175 g/day over a 70 year lifespan. This rate is representative of 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 of time that the assessment cycle is addressing, using median concentrations of fish tissue composite samples (Ecology, 2018).

The DOH SL is a threshold DOH uses when developing fish consumption advisories, in addition to other factors. The DOH SL of 101 ppb 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 provide advice to fish consumers in Washington, while the WQC is used to set National Pollutant Discharge Elimination System (NPDES) permit limits and assess waters, and represents full protection of the designated use of harvest.

Data exceeding these thresholds do not necessarily represent an impaired use or a fish consumption advisory. State agencies use 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.

## References:

Bloom, N. 1995. Considerations in the Analysis of Water and Fish for Mercury. In National Forum on Mercury in Fish: Proceedings. U.S. Environmental Protection Agency Office of Water, Washington D.C. EPA Publication No. 823-R95-002.

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# Appendix B. Statistical Analysis

Analysis of covariance (ANCOVA) was used to determine differences in fish mercury concentrations among sampling years. To control for the effect of fish size on mercury accumulation, fish length was used as a covariate for the Moses, Newman, and Sammamish datasets. No covariate was used for the Mason, Meridian, or Offutt datasets due to a lack of significant mercury-to-size relationship for one or more of the collection years, and thus an analysis of variance (ANOVA) was conducted for those datasets. Though there was no significant relationship at these sites, data were constrained to include only fish of overlapping size ranges. The 2006 Lake Meridian dataset was excluded from analysis because fish lengths were much larger than the 2011 and 2016 datasets and there was no significant mercury-size relationship to control for this difference.

Assumptions of normality, equality of variances, and homogeneity of slopes were met for all log<sub>10</sub> datasets prior to ANCOVA or ANOVA. Mercury was not detected at a reporting limit of 17 ppb in three samples from the 2011 Moses Lake dataset. A substitution of one half the reporting limit was used for the analysis. Least squares means from ANCOVA or ANOVA results were back-transformed and corrected for transformation bias with Duan’s Smearing estimator (Duan, 1983; Helsel and Hirsch, 2002) and these values are referred to throughout the report as “estimated mercury levels.” Magnitude of change between years where there was a significant difference was calculated as the percent change in estimated mercury levels.

Table B-1 presents the ANCOVA and ANOVA results and estimated mercury levels for each of the datasets.

**Table B-1. Results of ANCOVA/ANOVA comparing mercury levels in fish between 2006, 2011, and 2016 collections.**

Waterbody	Species	Co- variate	Sum of Squares	df	Mean Squares	F-Ratio	p-Value	2002 Hg <sub>bass</sub>	2006 Hg <sub>bass</sub>	2011 Hg <sub>bass</sub>	2016 Hg <sub>bass</sub>	Mean Fish Length* (mm)
Mason	YP	none	0.099	2	0.049	2.293	0.120	---	102	74	93	227
Meridian	LMB	none	0.005	1	0.005	1.305	0.274	---	---	104	95	255
Moses	SMB	length	0.109	2	0.055	2.004	0.155	---	28	25	35	332
Newman	LMB	length	0.199	3	0.066	5.899	<b>0.002</b>	203	142	169	124	355
Offutt	LMB	none	0.465	2	0.233	35.331	<b>&lt; 0.001</b>	---	210	178	104	273
Sammamish	LMB	length	0.494	2	0.247	24.885	<b>&lt; 0.001</b>	---	228	309	144	370

All variables were log<sub>10</sub> transformed prior to ANCOVA.

Hgbass = back-transformed least squares means with Duan’s Smearing estimator applied to correct for back-transformation bias (Duan, 1983; Helsel and Hirsch, 2002)

df = degrees of freedom

SMB = smallmouth bass

LMB = largemouth bass

\* = mean fish total length from all years sampled at each site.

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## **References:**

Duan, N. 1983. Smearing Estimate: A nonparametric retransformation method. *Journal of American Statistical Association*, Vol. 78: 605-610.

Helsel, D.R. and R.M. Hirsch. 2002. *Statistical Methods in Water Resources*, Techniques of Water Resources Investigations, Book 4, chapter A3. U.S. Geological Survey. 522 p.

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