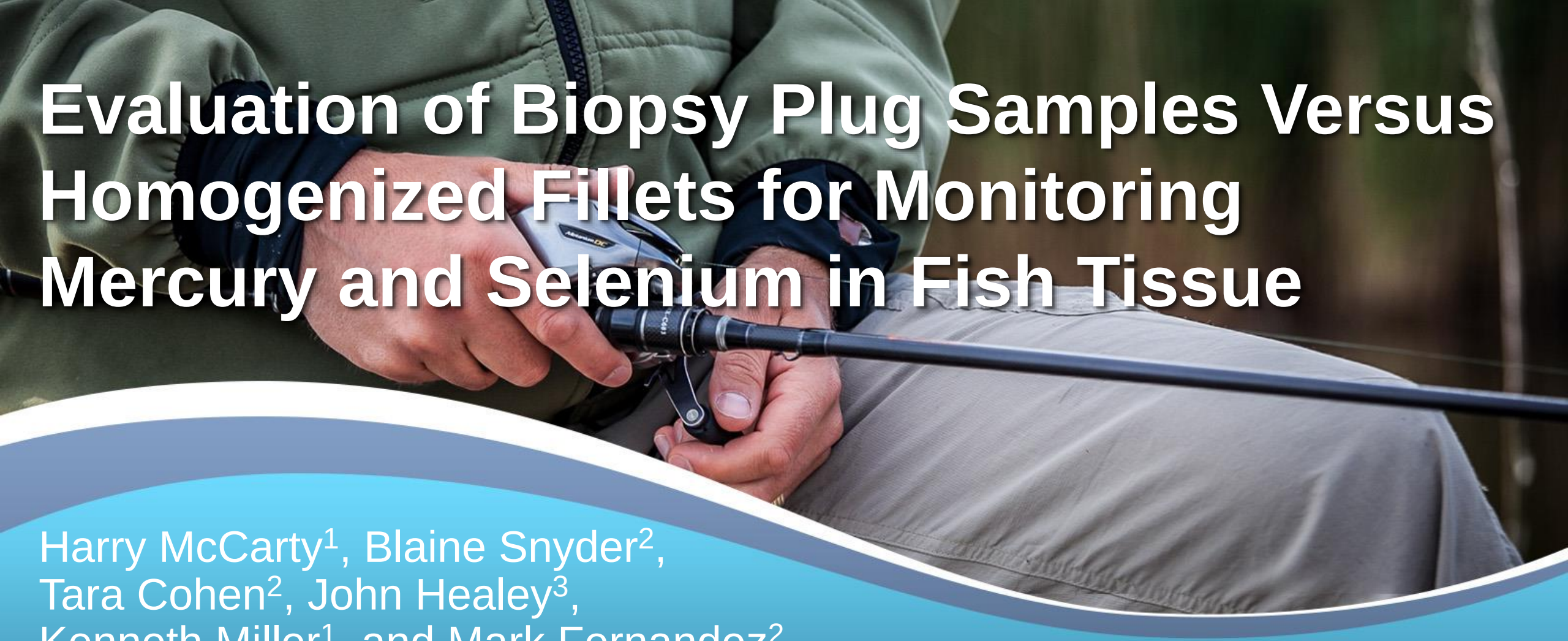

Evaluation of Biopsy Plug Samples Versus Homogenized Fillets for Monitoring Mercury and Selenium in Fish Tissue

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The presenter is *not* an EPA employee, but a contractor.

Background

Monitoring chemical contaminant levels in the aquatic environment continues to be important for the characterization of water resource conditions, identification of associated impacts, and protection of human and ecological health.

- Fish are important indicators for water quality and human health assessments
- Fish tissue contaminant studies with human health protection objectives typically focus on fish species that are important to commercial, recreational, and subsistence fisheries, and on the commonly eaten tissue fraction (i.e., fillets)
- Such contaminant studies typically require that whole fish be collected and killed to remove and homogenize the entire fillets

Challenges

In addition to sacrificing multiple fish specimens, the typical approach for contaminant studies involves shipping either the whole fish specimens, or field-prepared fillets, to a laboratory for further preparation and analysis.

- Overnight shipping costs are not trivial
- Fish are usually shipped with large quantities of dry ice, adding expense and often logistical challenges associated with dry ice availability
- Storing whole fish samples for large studies requires lots of freezer space
- Field preparation of the fish presents additional sources of contamination that would not occur in a laboratory, as well as the need for additional equipment, suitable containers, and equipment decontamination in the field

Alternative Tissue Collection Methods

Researchers have considered various alternative collection methods since the early 1970s, including, but not limited to, published reports by:

- Uthe, 1971, using a biopsy needle
- Crawford et al., 1977 and Waddell and May, 1995, using a biopsy punch to collect a “plug” of tissue
- Heltsley et al., 2005, assessing the use of adipose fins
- Rolfhus et al., 2008 and Piraino and Taylor, 2013, using fin clips

Beginning in 2002, the use of a biopsy punch became the most frequent alternative, with at least 10 published studies.

In 2013, EPA began using a biopsy punch to collect plug samples to its National Aquatic Resource Surveys for monitoring mercury in whole fish, while retaining use of homogenized fillets to allow the analysis of multiple contaminants for the human health component of the study.

Potential Advantages of Plug Sampling

- As implemented by many researchers, removing a small plug sample from fish of harvestable size is *assumed* to be non-lethal and that the fish can be returned to their environment alive.
- The equipment needed is simple to use and relatively inexpensive, such that it can be considered disposable, minimizing the risk of cross-contamination, and eliminating the need for cleaning equipment between samples.
- Shipping costs and sample storage costs are greatly reduced, although dry ice is still required.
- Sample preparation costs are reduced because the laboratory does not need to process either whole fish or fillet samples.

So, Why Not?

There are limitations to the use of biopsy plug samples:

- The punch only collects about 0.5 to 1 g of tissue, which many not be enough for analyses of some contaminants, particularly organics.
- The non-lethality claims for the technique are not well studied (only one known study that involved long-term monitoring, in a hatchery setting).
- Concerns about effects of frozen storage on the small samples (freezer burn)
- It is not clear how well the plug sample results represent the concentrations derived from homogenized fillet tissue samples.

It is this last concern that led us to conduct our study.

Fish Plug Evaluation Study Design

EPA's Standards and Health Protection Division (SHPD) in the Office of Water embarked on the Fish Plug Evaluation Study in 2017 to assess the comparability of mercury concentrations in fish fillet plugs vs. homogenized whole fillet tissue samples and to test the feasibility and applicability of fish fillet plug sampling and analysis for conducting compliance monitoring associated with EPA's fish tissue-based selenium water quality criterion. The study was conducted in two phases:

- Mercury phase, beginning in 2017, and
- Selenium phase, beginning in 2018

Fish Plug Evaluation Study Design (continued)

Design Element	Mercury Phase	Selenium Phase	Description
Waterbody Types	2	2	Great Lakes and East Coast Rivers
Sampling Sites and Fish Species Collected	6	6	Lake Erie, Walleye; Lake Michigan, Lake Trout; Lake Ontario, Chinook Salmon; Anacostia River, Blue Catfish; Potomac River, Largemouth Bass; St. Lawrence River, Smallmouth Bass
Fish Collected per Site	10	5	Each fish sample consisted of a single specimen
Fish Tissue Sample Types	2	2	Fillet plug samples (2 plugs per sample) and Homogenized fillet tissue samples
Replicates per Sample Type	5	4	Number applies to each individual fish sample
Total Plug Samples	300	120	Sampling sites (6) x Fish collected per site x Replicates per sample type
Total Homogenized Samples	300	120	Sampling sites (6) x Fish collected per site x Replicates per sample type

The Players

- **SHPD** – Leanne Stahl (now retired), who provided overall direction for planning and implementing the Fish Plug Evaluation Study, and John Healey, who is a co-author of this presentation
- **Tetra Tech** – Blaine Snyder, Tara Cohen, and Mark Fernandez, who were responsible for sampling QAPP development, fish sample collection and processing (plugs and fillets), and statistical analyses
- **CSRA (now GDIT)** – Harry McCarty and Ken Miller, who were responsible for analytical QAPP development, laboratory contracting, data review and database development, and statistical analyses
- **Labs** - Tetra Tech for fish sample preparation (filleting and homogenization), ALS (Kelso) for mercury, and Brooks Applied Laboratories for selenium

Mercury Phase Flowchart

FIELD (6 Sampling Sites)

LABORATORY

Ship to Laboratory

Fillet

Homogenize

Prepare Aliquots

5 Fillet Plug Samples
Collected and Analyzed from Each Fish

5 Homogenized Fillet Samples
Collected and Analyzed from Each Fish

Mercury Analysis
600 Sample Results

Selenium Phase Flowchart

FIELD (6 Sampling Sites)

LABORATORY

Ship to Laboratory

Fillet

Homogenize

Prepare Aliquots

4 Fillet Plug Samples
Collected and Analyzed from Each Fish

4 Homogenized Fillet Samples
Collected and Analyzed from Each Fish

Selenium Analysis
240 Sample Results

Fish Sample Processing

Analytical Methods

- Fillet plug samples and homogenized fillet samples were prepared and analyzed for mercury using Appendix to *Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation from Method 1631 Revision B and Revision E*, respectively (USEPA 2001 and 2002).
- Fillet plug samples and homogenized fillet samples were prepared and analyzed for selenium using a collision cell modification to *Method 200.8, Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry* (USEPA 1994) to achieve better sensitivity and address interferences.
- Because the water quality criterion for selenium is based on the dry-weight concentration in fish tissue, the selenium phase samples also were analyzed for total solids, using SM 2540G.

QC Operations

QC Operation	Mercury	Selenium
Bubbler blank	X	NA
Method blank	X	X
Lab Control Sample	X	X
Reference material sample	X	X
Matrix spike sample	X	X

There were no substantive QC failures for either mercury or selenium

Statistical Methods

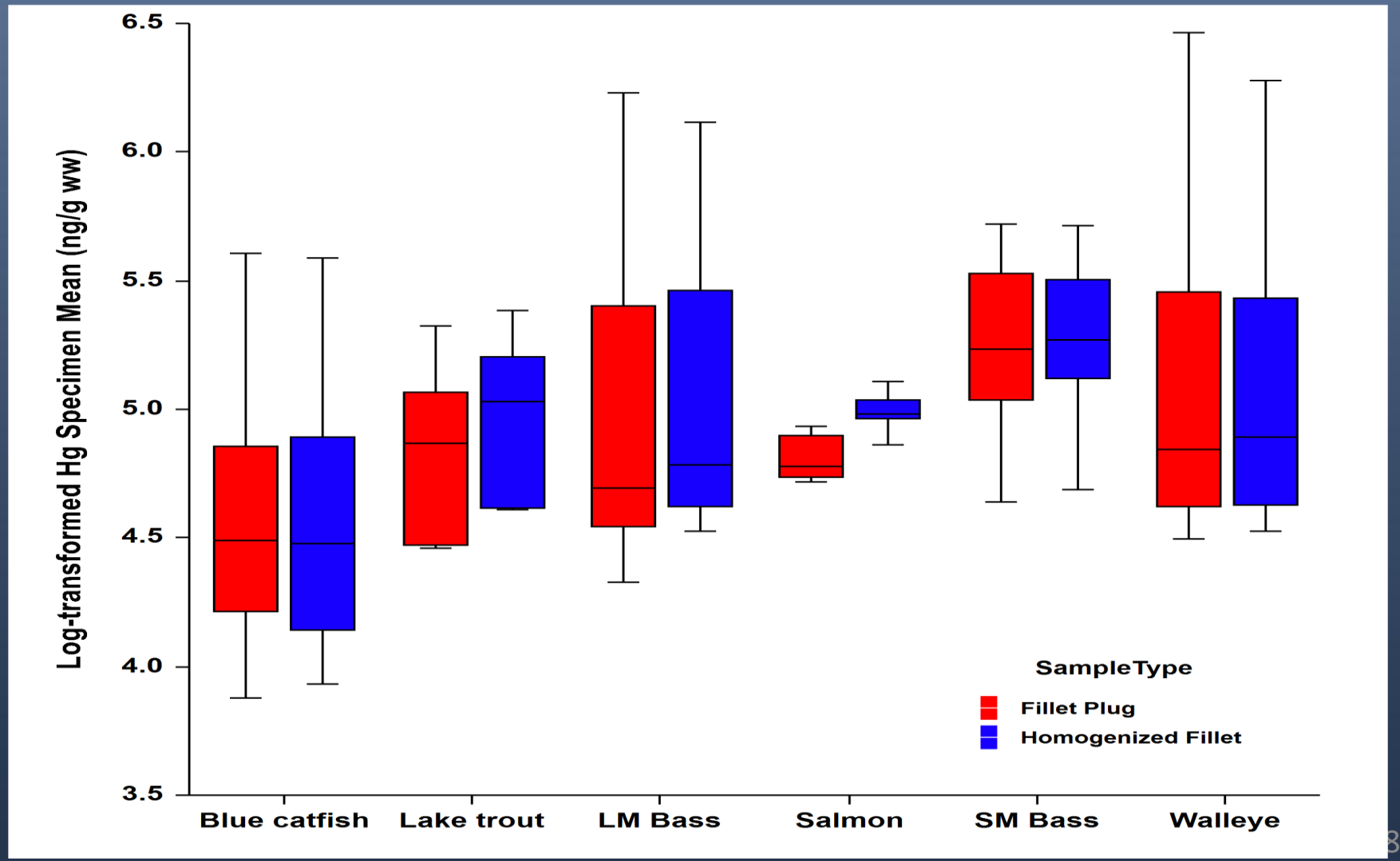
- Null hypotheses (H_0) for mercury and selenium: both methods of collecting samples (fillet plugs vs. homogenized fillets) would yield equivalent mean concentrations of mercury and of selenium, respectively, for any given specimen.
- An analysis of variance (ANOVA) model on log-transformed data averaged across specimens was used to determine whether there are any significant differences across the two sampling methods, for each analyte (mercury and selenium), and an alpha value of 0.05 was used to assess significance.
- The statistical methods evaluated the potential impact of factors that could affect results, including waterbody type (lake vs. river), specific waterbody (6 locations), and fish species (6 species).

Summary Statistics by Sample Type

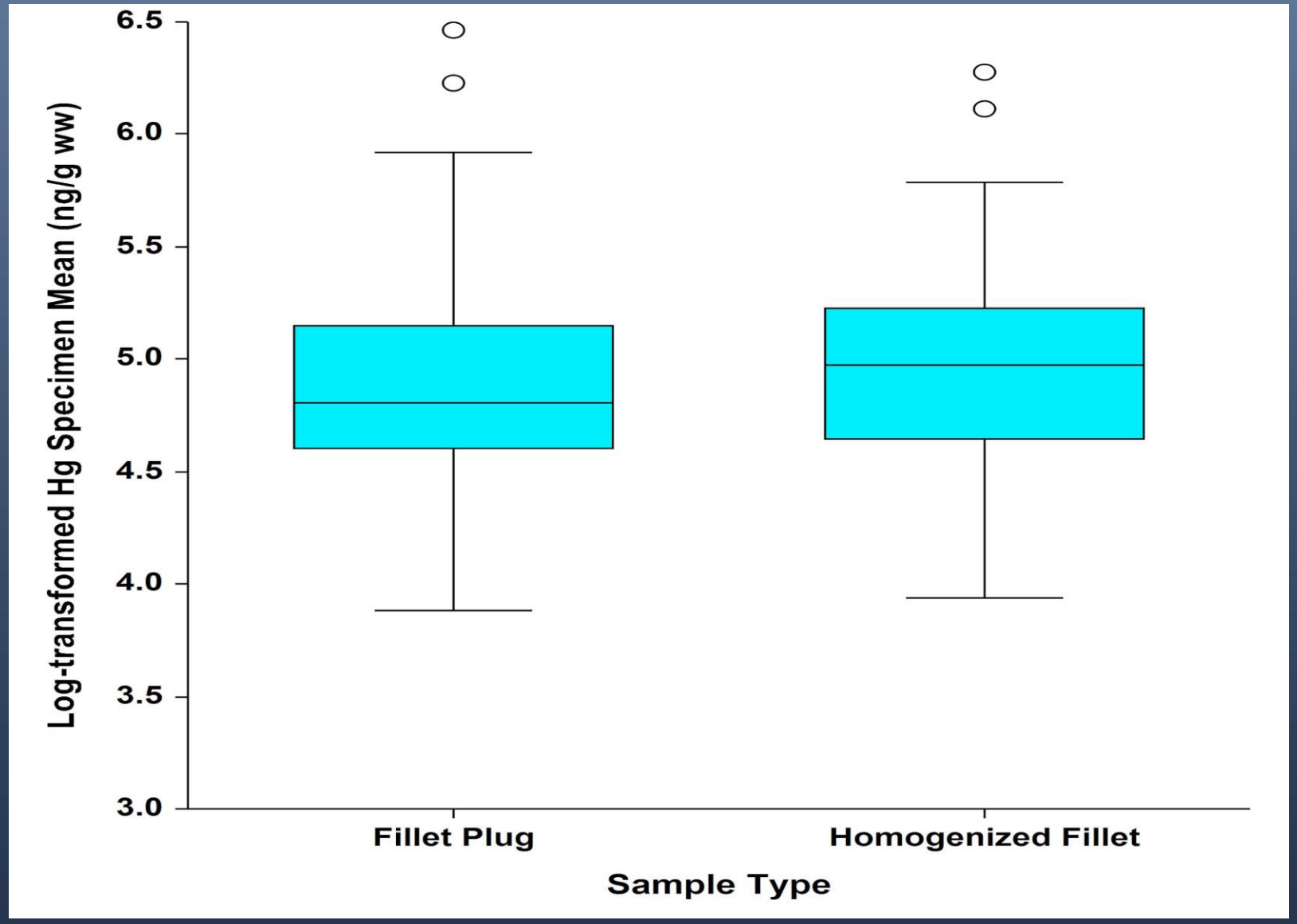
	Mercury (ng/g wet weight)		Selenium (ng/g dry weight)	
	Fillet Plug (n=300)	Homogenized Fillet (n=300)	Fillet Plug (n=120)	Homogenized Fillet (n=120)
Minimum	44.2	23.0	711.0	750.0
Median	121.0	143.0	1762.0	1781.5
Mean	155.2	161.4	1922.0	1996.0
Maximum	649.0	556.0	3814.0	4084.0
SD	101.6	86.5	810.1	936.2
RSD	65.5%	53.6%	42.2%	46.9%

For reference, the mercury human health criterion is 300 ng/g (wet weight) and the selenium water quality criterion is 11,300 ng/g (dry weight).

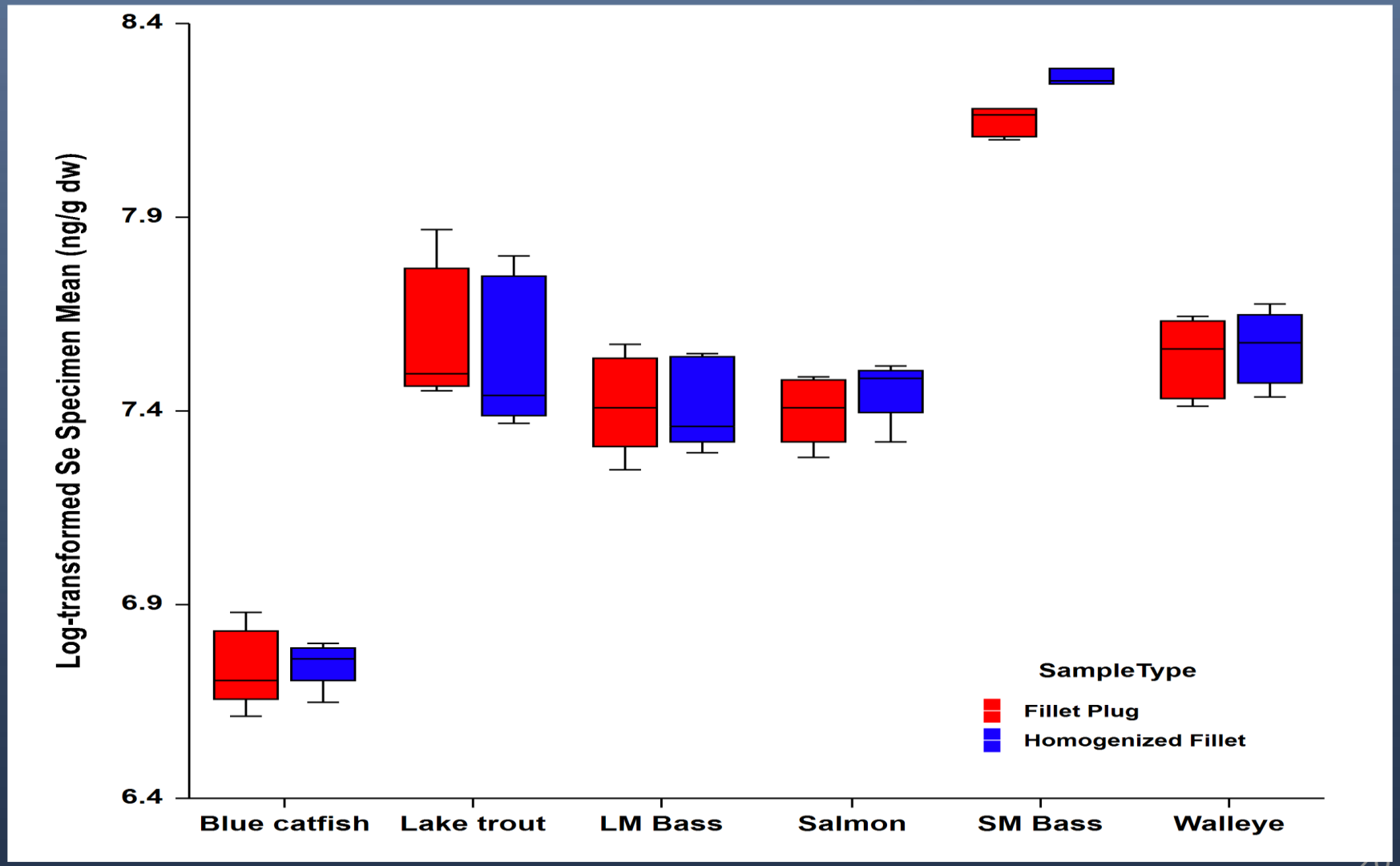
Mercury Results by Species and Sample Type



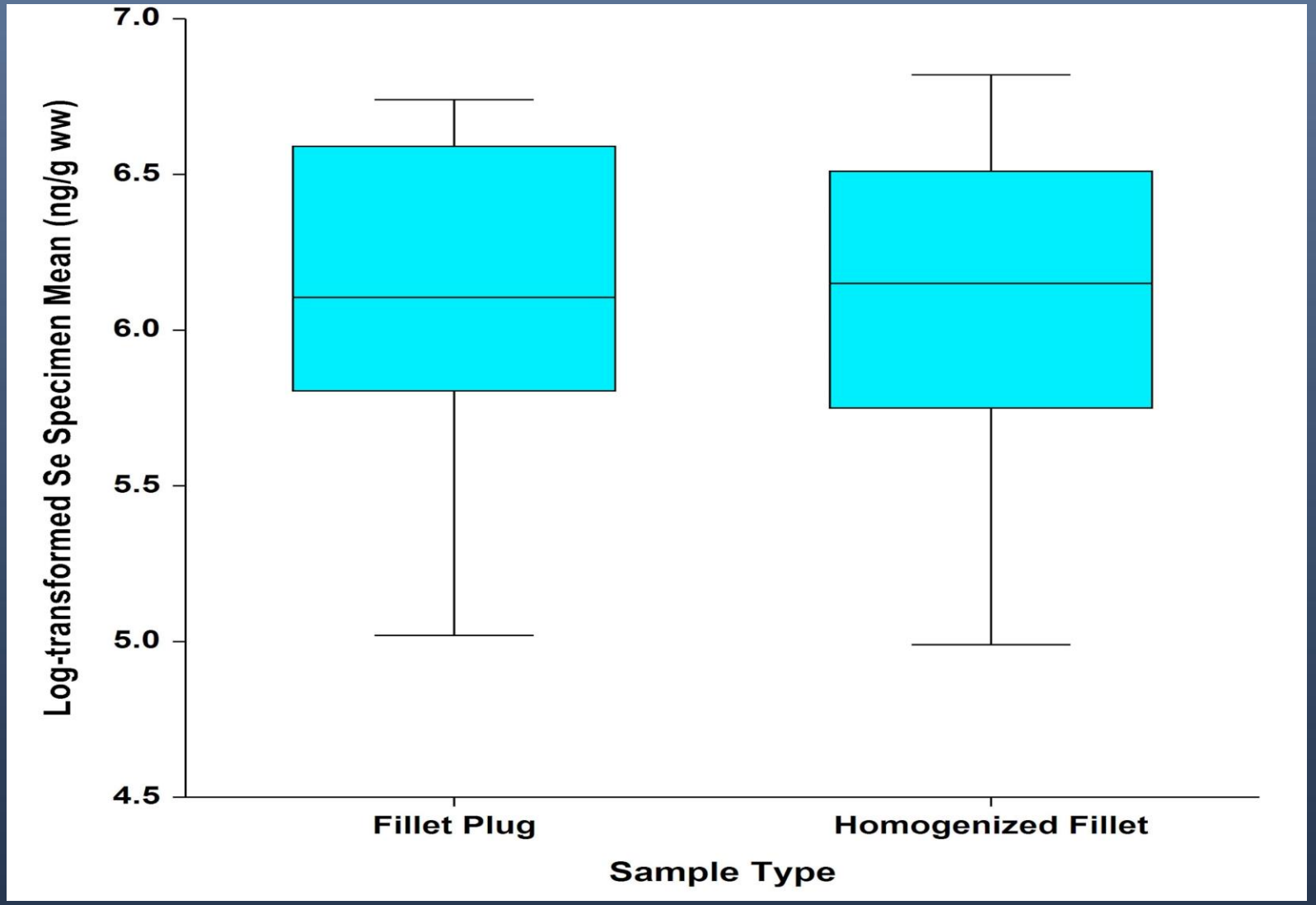
Mercury Results by Sample Type



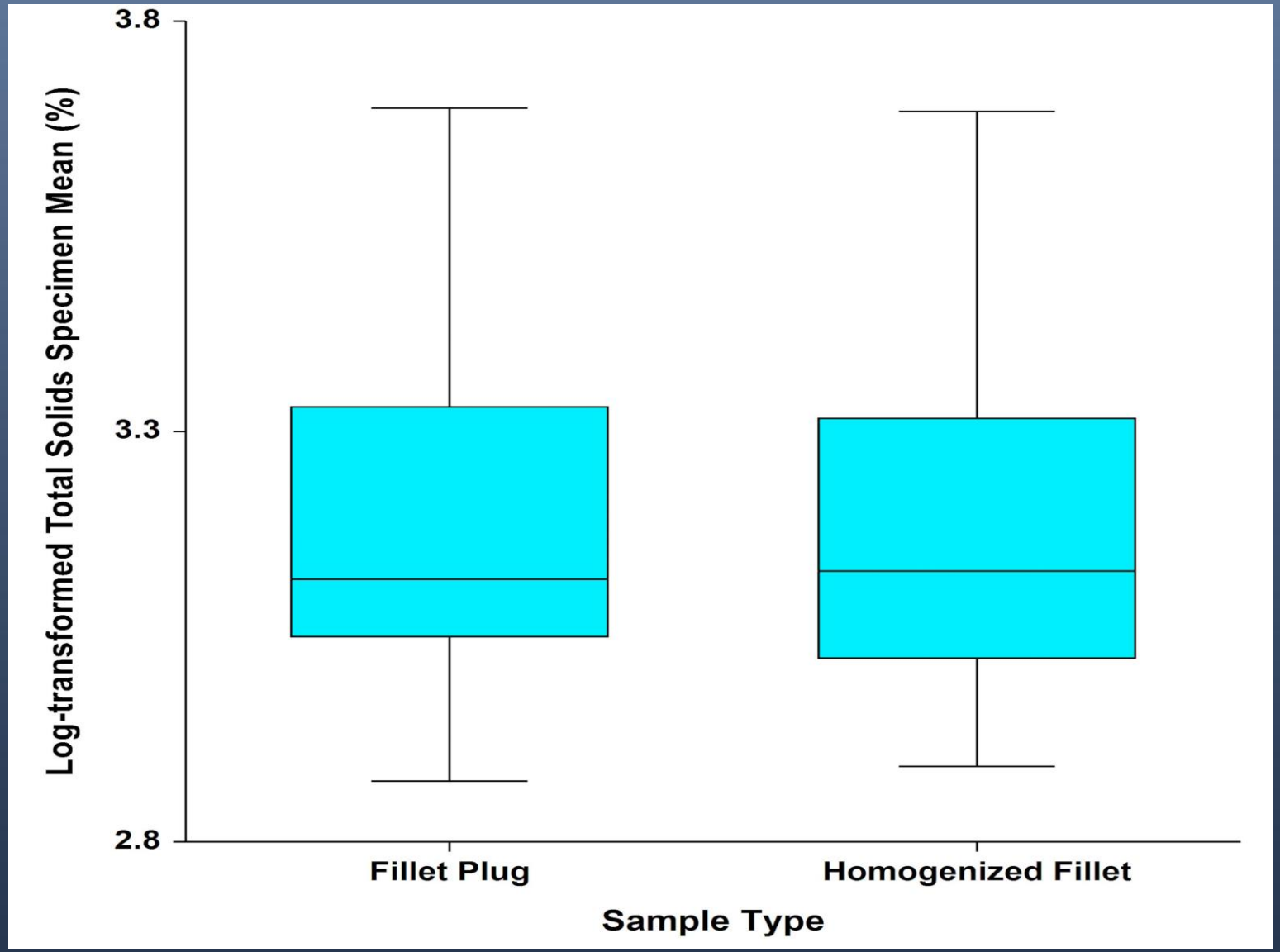
Selenium Results by Species and Sample Type



Selenium Results by Sample Type



Total Solids Results by Sample Type



Statistical Findings

- The ANOVA main effects models indicated that for mercury (p=0.4048) and for selenium (p=0.3786), ***there was no significant difference among the two fish fillet tissue sample types***.
- The mercury data and the selenium data were log-normally distributed. For each analyte, there was a large overlap across the different fillet tissue sampling methods, and large variability across waterbodies.
- Since there was a large variance across waterbodies, waterbody was included as a blocking factor in the ANOVA model equation used for each analyte.
- The interaction term (Method:Waterbody) was not significant (p=0.9728 for mercury, p=0.6740 for selenium), indicating the effect of sample types was not impacted by site- or species-specific factors.

Practical Implications for Typical Fish Monitoring Studies

- Many state and local studies of mercury in fish are carried out to assess fish mercury concentrations to the human health decision level of 300 ng/g (wet weight) and issue local consumption advisories.
- 62 mercury results (31 plug and homogenized pairs) from this study fell between 250 and 650 ng/g. 10 pairs from lake sites and 21 pairs from river sites.
- Of those 31 pairs, only 4 would have resulted in a different conclusion relative to the decision level. For 3 of the 4, the plug result was above the decision level and the homogenized fillet result was below that level (FP>HF). The remaining pair was reversed (FP<HF). All 4 pairs were for smallmouth bass samples from one river site.
- None of the selenium samples were anywhere near the water quality criterion, so no similar assessment was possible.

Conclusions

- Both phases of this study showed that there were no statistically significant differences between fillet plug and homogenized fillet results *at the population level*.
- A choice between sampling alternatives ultimately depends on study objectives, target chemicals, and tissue volumes needed for analysis.
- Homogenized fillet sampling provides sufficient mass for the analysis of multiple contaminants, but requires fish to be sacrificed for analysis, whereas plug sampling may allow fish survival following collection, but may only provides adequate tissue mass for a single analyte (e.g., mercury or selenium).
- For selenium, the plug sampling alternative must employ a sufficiently sensitive analytical method and should consider total solids if the EPA water quality criterion is to be used as the decision level.

Limitations

- This study does *not* address fish survival *nor* provide any confirmation that plug sampling is a non-lethal technique.
- Our conclusions apply to mercury and selenium only and cannot be extrapolated to other contaminants, especially lipophilic organics.
- The target species in the study are representative of freshwater sportfish species commonly caught and consumed in the U.S. Therefore, these findings *can be* extrapolated to similar freshwater species, but they *may not* apply to all fish species (e.g., estuarine or marine species were not tested).
- Studies analyzing contaminants in whole fish (rather than fillet tissue) would not be expected to yield similar plug sample comparison conclusions to those described here.

Areas for Future Study

- Can plug sampling be expanded to organic contaminants such as PFAS?
  - Sample size is limited, so PFAS methods would need to be optimized for small tissue samples and focus on the low levels of concern.
  - PFAS may not be evenly distributed among tissues in fish, so a similar side-by-side comparison study would be essential.
- Additional research on fish survival after plug removal is warranted
  - More information is needed on survival differences between species and life stages under various ambient conditions upon release.

For Further Information

Consult:

Stahl, et al., *Archives of Environmental Contamination and Toxicology* (2021) 81:236–254.

https://doi.org/10.1007/s00244-021-00872-w

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Questions?