2008 Walleye Total Mercury Analyses

by

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INTRODUCTION

Walleye (*Sander vitreus*) are targeted for harvest by Chippewa tribal members from many off-reservation inland lakes in Wisconsin each spring (Krueger 2008). Tribal representatives have expressed concern about the health risk that mercury in fish poses to tribal members. As a result of this concern, the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) has been collecting walleye annually since 1989 during spring from various lakes routinely harvested by tribal members. Muskellunge (*Esox masquinongy*) and northern pike (*Esox lucius*) are collected occasionally, but were not collected in 2008. Several funding sources have been used for collection and analysis of the fish for total mercury concentration. The fish were measured for total mercury as a surrogate for methylmercury because most mercury (>95%) in top predator fish is in the form of methyl mercury (Bloom 1992, Lasorsa and Allen-Gil 1995).

The walleye data are used to prepare tribal and lake specific, color-coded GIS maps that include walleye consumption advice (Appendix 1). These maps are intended to help tribal members reduce their risk to methyl mercury exposure by offering lake specific meal consumption advice and information to help them select lakes for harvest where walleye contain lower mercury concentrations. The maps have been updated every 2-3 years and have been made available to tribal members at offices where permits for off-reservation spearing are issued and at health service provider offices. Large, wall-sized maps were also posted at these offices and in various public locations such as tribal administration buildings, grocery stores, school libraries, or community centers (DeWeese *et al.* 2009). The maps for the six Wisconsin Ojibwe tribes were updated in 2005 using a methodology described in Madsen et al. (2008) and were expanded in 2006 to include walleye lakes within the 1837 ceded territory in Minnesota and select walleye lakes in the 1842 ceded territory in the Upper Peninsula of Michigan.

This report presents results of mercury testing of walleye collected from off-reservation lakes during 2008. Funding for the collection and analysis of these samples came from the United States Environmental Protection Agency (EPA) Great Lakes National Program Office (GLNPO) as part of grant #GL00E06501 and from the Bureau of Indian Affairs (BIA).

METHODS

Collection of Samples

Walleye from inland lakes were collected during spring from tribal spearers and netters and by GLIFWC or St. Croix Natural Resources Department fishery assessment crews. Plans for samples collected by GLIFWC called for twelve walleye to be collected with three fish taken from each of four size ranges (12.0 to 14.9, 15.0 to 17.9, 18.0 to 22.0, and greater than 22.0 inches). Samples collected by St. Croix were collected as part of a larger contaminant analysis project and followed procedures outlined in the Quality Assurance Project Plan (QAPP) for their project. Their plans called for collecting nine walleye from each lake between 17 and 20 inches.

Once the walleye were collected, a St. Croix Biologist contacted GLIFWC staff and four of the nine walleye from seven of the lakes sampled by St, Croix were selected for mercury testing.

Walleye collected by GLIFWC were measured for total length and sex was determined. A metal identification tag with a unique number was attached to each fish. Fish were then placed on ice in a cooler and transferred to a freezer (at temperatures at or below -10 °C) within 36 hours. A chain-of-custody form was filled out to identify fish collected from individual lakes each night (Appendix 2). The form also served as a record of who collected and transported the samples and when they were placed on ice or transferred to a freezer. A second chain-of-custody form was used when transferring fish collected by GLIFWC to the Lake Superior Research Institute (LSRI) in Superior (Appendix 2). St. Croix also used a chain-of-custody form for collection and transport of fish. GLIFWC received copies of all St. Croix chain-of-custody forms.

Processing

Walleye were processed into skin-off fillets at GLIFWC or at the St. Croix Natural Resources Department. St. Croix followed processing methods based on those used by GLIFWC. Stainless steel knives and cutting surfaces were used to fillet the walleye. All surfaces and equipment were washed with a mild dish detergent then rinsed with tap water prior to processing each fish. The following descriptive data were collected from each fish: a second length measurement (denoted as frozen length), sex, round weight, fillet weight, and the second or third dorsal spine was removed for aging. A single skin-off fillet was removed from each walleye, weighed on a digital scale, and placed into a one-gallon plastic bag with an interlocking seal. A sample label containing the name of the lake, fish identification number, year, date of filleting, analytical processing lab, species, type of sample and title of study was placed into each bag with the fillet (Figure 1). The tag identification number was recorded on the outside of each bag. All descriptive data were recorded on a laboratory data sheet. All individually bagged fillets for a given lake were placed into a single 15-gallon plastic bag, sealed, and labeled with the name of the lake. Spines were placed into small envelopes with a label, similar to the fillet labels (Figure 1), affixed to the outside of the envelope. The age of the fish was determined by counting the number of annuli (translucent zones) in the spine cross-section consistent with Schram (1989). Experienced GLIFWC Inland Fisheries technicians aged the spines. Ages were not available from walleye collected by St. Croix. All chain-of custody forms and GLIFWC laboratory data sheets were filed and kept in a three-ring binder at GLIFWC's main office.

Figure 1. Example of a sample label placed into one-gallon walleye fillet bags.

Project: Spring Mercury Walleye	Client: GLIFWC
Species: Walleye	Tag No. 0551
Month/Day Collected: 4/23	Year: 2008
Lake Name: Sherman Lake (Vilas)	Sample Processing: Hg
Tissue type: Fillet	Processor: LSRI

Total Mercury Analyses

Walleye fillets from GLIFWC and St. Croix were received by LSRI in good condition with chain-of-custody documentation. A complete description of fillet grinding, total mercury analysis and associated quality control and assurance is provided in the LSRI laboratory report (Appendix 3). Briefly, the fillets were partially thawed and ground three times with a stainless steel motorized meat grinder. An aliquot (200 mg) of the ground tissue was digested and analyzed for total mercury using a Cold Vapor Atomic Absorption Spectroscopy (Perkin Elmer FIMS-100 Flow Injection Mercury Analysis System) method based on EPA Method 245.6.

Quality Control

Quality control at LSRI was monitored using four methods: 1) the analysis of a certified reference tissue (DORM-2, *Squalus acanthias*) to determine accuracy, 2) tissue spikes to test the extraction method for efficiency and interferences, 3) duplicate analyses to determine precision, and 4) procedural blanks to determine whether sample processing changed the mercury content of the samples.

A quality assurance report from an audit of the laboratory processing and analysis is included with the LSRI laboratory report in Appendix 3. In addition, a quality assurance report for the field data collection and audit is found in Appendix 4.

RESULTS

Quality Control

Standard Reference Material

The DORM-2 reference tissue has a certified concentration of 4.64 ± 0.26 µg Hg/g tissue. An acceptable range of mercury concentrations for DORM-2 standard reference material samples was calculated for this study based upon the analyses conducted from the Spring Walleye 2005-2007 studies (mean ± 2 times the standard deviation of all DORM-2 analyses). The calculated acceptable range was 3.48 to 5.23 µg Hg/g.

DORM-2 was analyzed in duplicate with all eight sets of walleye tissues analyzed. The recovery values ranged from 80.6 to 111.2% with the grand mean and standard deviation of the recoveries being 95.3 ± 7.9 percent of the certified value. On July 1, 2008, one DORM-2 recovery was not within the acceptance range of 3.48 to 5.23 µg Hg/g and as a result, the entire set was reanalyzed on August 7, 2008. Analytical results for mercury content for that set of samples are reported based on the data obtained on August 7, 2008, which were within the acceptable DORM-2 recovery range.

Spikes

A total of 26 spike samples were analyzed (11 percent of total samples). Spike recovery was considered acceptable when it was in the range of 60.8 to 115% of the expected value. This was based upon the mean ± 2 times the standard deviation of all analyses of the spiked samples conducted from Spring Walleye 2005-2007 sample analysis. Mean recovery for the 26 spiked samples was 88.7 ± 13.7% with the values ranging from 35.9 to 106.8%. One spike recovery value (Two Sisters 12573) was below the acceptance range. This sample was reanalyzed on August 7, 2008 and was found to have an acceptable recovery on that date.

Duplicates

Fish tissues were analyzed for mercury in duplicate 33 times (14 percent of total samples). Two portions of the same tissue were digested and analyzed independently. Duplicate agreement values were acceptable when having a relative percent agreement > 85.3%. The acceptable value was calculated as the mean ± 2 times the standard deviations of all duplicate analyses conducted from Spring Walleye 2005-2007 sample analysis at the LSRI laboratory. Relative percent agreement between the duplicate analyses of the same tissue ranged from 72.1 to 99.7% with the average and standard deviation of the agreements being 92.3 ± 7.4 percent. Seven relative percent agreement values were below the acceptance range of > 85.3% and those samples were reanalyzed in duplicate on another date. The results for the reanalyzed samples fell within the acceptance range.

Procedural Blanks

Procedural tissue blanks (canned tuna, *Thunnus* sp.) were split into two aliquots on each processing day. One aliquot was processed in the same manner as the walleye fillets and the second aliquot was directly digested without processing. Results for the procedural blanks were considered acceptable when the relative percent agreement was > 69.7%. This is based on the mean ± 2 times the standard deviation of all the relative percent agreement values determined for the procedural blanks from the Spring Walleye 2005-2007 projects. Five tuna procedural blanks were processed coincident with the grinding of walleye. One of the five procedural blanks was analyzed with each set of mercury samples for a total of eight analyses resulting in a mean of 79.4 ± 10.6 relative percent agreement. The relative percent agreement values ranged from 58.9 to 92.2%, all but two were within the acceptable range of > 69.7%.

Quality Control Data Completeness

An assessment of the overall acceptability of the quality control data was made by adding up the total number of quality control samples that were outside of control limits and dividing by the total number of quality control samples. The project QAPP suggests a goal of fewer than 10 percent of the total quality control samples should exceed quality control parameters. Overall, there were a total of 83 quality control samples measured. Two samples, or 2.4 percent of the

total samples, exceeded the quality control parameters. This percentage was less than the goal of <10 percent of the quality control samples not meeting project quality control parameters. Overall, the sample data were in good agreement with the quality assurance parameters, so the data were determined to be precise and accurate.

Sample Results

During 2008, skinless fillets of 218 walleye from 26 lakes in Wisconsin and 12 walleye from one lake in Minnesota were analyzed for total mercury concentration (Appendix 3). A total of 28 of the 218 walleye samples from Wisconsin were collected by St. Croix. The remainder were collected by GLIFWC staff. Overall, total mercury concentrations on a wet weight basis ranged from 0.062 to 1.36 µg Hg/g from Wisconsin lakes and from 0.066 to 0.413 from the Minnesota lake. Walleye lengths ranged from 12.2 to 28.2 inches from Wisconsin lakes and 12.6 to 23.9 inches from the Minnesota lake. Walleye length and mercury data are summarized in Table 1.

Table 1. Summary statistics for wet weight mercury concentration (ug Hg/g fish tissue) and fresh length (inches) for walleye collected from 26 Wisconsin lakes and 1 Minnesota lake (Mille Lacs) during spring 2008.

COUNTY	LAKE	#Fish	Mean Conc.	St.Dev. Conc.	Median Conc.	Max Conc.	Min Conc.	Mean Length	St.Dev. Length
OCONTO	ARCHIBALD L	11	0.398	0.308	0.249	1.07	0.208	18.3	4.4
POLK	BALSAM L*	4	0.173	0.075	0.140	0.284	0.127	18.2	0.7
BARRON	BEAR L*	4	0.281	0.073	0.277	0.356	0.213	18.5	1.2
ONEIDA	BEARSKIN L	12	0.136	0.083	0.118	0.373	0.062	18.4	4.1
BARRON	BEAVER DAM L*	4	0.283	0.209	0.195	0.595	0.148	18.9	1.5
BURNETT	BIG MCKENZIE L*	4	0.244	0.134	0.197	0.432	0.148	18.4	1.0
POLK	BIG ROUND L*	4	0.294	0.093	0.282	0.402	0.209	18.6	0.9
VILAS	BIRCH L	8	0.619	0.267	0.500	1.13	0.374	17.3	4.1
WASHBURN	BIRCH L	11	0.287	0.155	0.271	0.687	0.134	18.2	3.7
ST CROIX	CEDAR L*	4	0.130	0.058	0.122	0.201	0.076	18.1	1.0
ONEIDA	FOUR MILE L	6	0.979	0.245	0.913	1.36	0.694	14.7	1.6
BAYFIELD	L OWEN	12	0.474	0.343	0.404	1.25	0.133	19.0	4.3
VILAS	LAC VIEUX DESERT	12	0.217	0.145	0.154	0.547	0.099	18.2	4.3
ONEIDA	LONG L	10	0.451	0.185	0.404	0.742	0.228	17.4	4.0
SAWYER	NELSON L	5	0.696	0.279	0.758	0.920	0.242	21.0	2.0
LANGLADE	OTTER L	8	0.105	0.039	0.101	0.173	0.062	17.2	2.1
FLORENCE	PATTEN L	12	0.415	0.233	0.386	0.750	0.150	18.7	4.5
ONEIDA	PELICAN L	12	0.228	0.124	0.177	0.454	0.098	18.0	3.8
LINCOLN	RICE R FL CHAIN	12	0.434	0.205	0.356	0.863	0.222	18.2	4.2
SAWYER	ROUND L	12	0.265	0.184	0.175	0.562	0.100	18.2	3.8
WASHBURN	SHELL L	9	0.350	0.228	0.266	0.712	0.133	16.6	2.9
VILAS	SQUAW L	11	0.347	0.116	0.295	0.534	0.189	16.9	3.1
SAWYER	TIGER CAT FL	6	0.369	0.137	0.388	0.532	0.122	17.7	3.4
ONEIDA	TWO SISTERS L	12	0.447	0.337	0.332	1.14	0.130	19.9	4.5
POLK	WAPOGASSET L*	4	0.239	0.043	0.242	0.288	0.185	18.3	1.1
VILAS	WILDCAT L	9	0.195	0.105	0.152	0.401	0.105	17.3	2.9
MILLE LACS	MILLE LACS L	12	0.205	0.134	0.150	0.413	0.066	18.5	3.8

* Walleye collected and processed by St. Croix Natural Resources Deparment.

Percent Moisture

Percent moisture was measured in 80 of the 230 walleye tissues. Walleye muscle tissue had a mean moisture value of 79.4 ± 0.94 percent (Appendix 3). Of the 80 tissues analyzed for moisture, 12 were analyzed in duplicate, all yielding relative percent agreements of 98.8 percent or greater. Nine samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 99 percent.

SUMMARY

Walleye total mercury results from 2008 are summarized in this report. Quality control results indicated that the measured total mercury concentrations were precise and accurate. Total mercury concentrations in walleye tended to vary within a lake by size (larger fish generally having higher mercury concentrations) and between lakes for similar size groups of fish. These data have been entered into GLIFWC's mercury database used to produce GIS-based mercury in walleye consumption advisory maps (Madsen et al. 2008).

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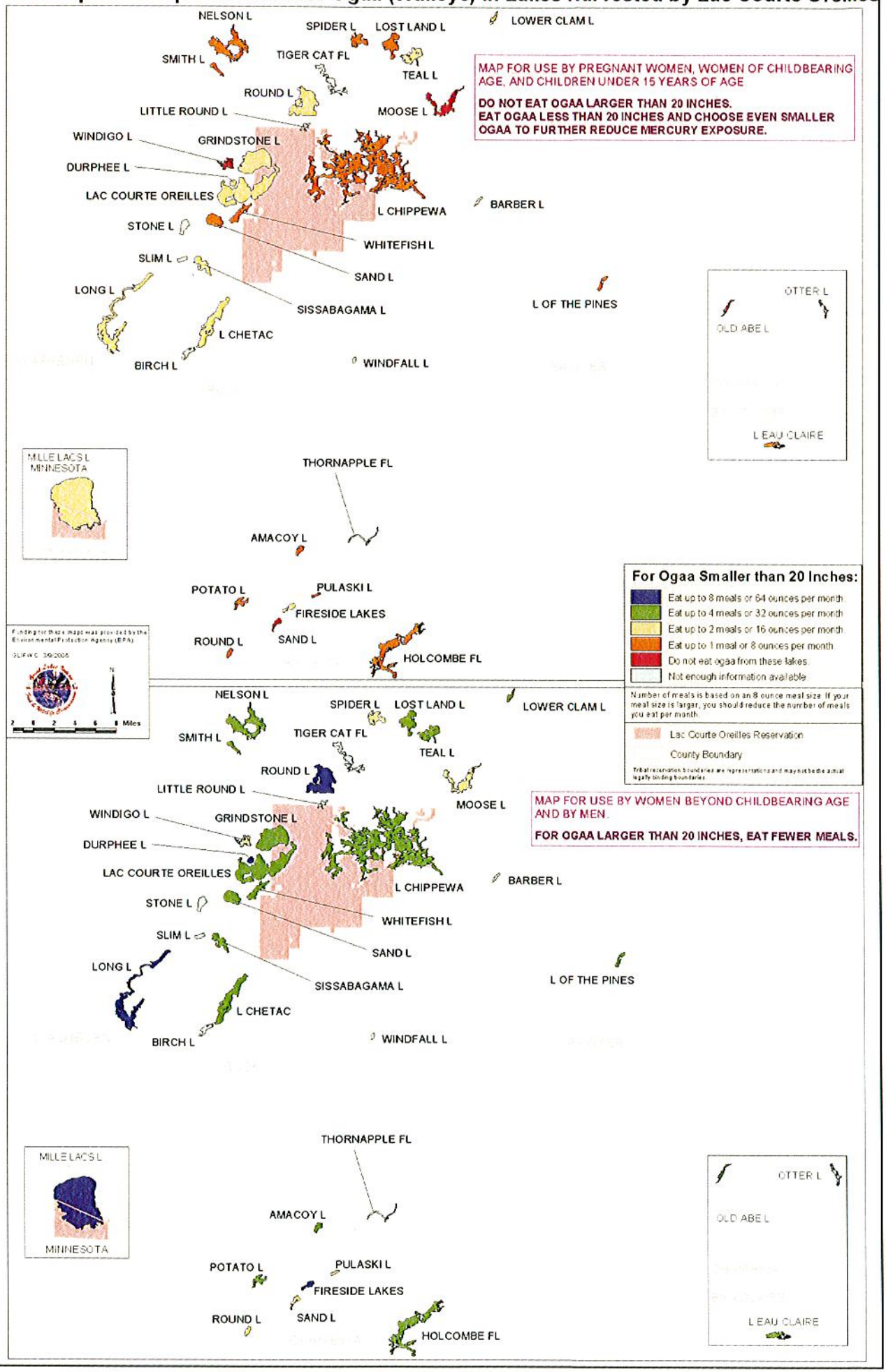
LIST OF APPENDICES

Appendix 1

Example Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Geographic Information System (GIS) - Based Mercury in Walleye Consumption Advisory Map

This Map is to Help You Find Safe Ogaa (Walleye) in Lakes Harvested by Lac Courte Oreilles



MAP FOR USE BY PREGNANT WOMEN, WOMEN OF CHILDBEARING AGE, AND CHILDREN UNDER 15 YEARS OF AGE

DO NOT EAT OGAA LARGER THAN 20 INCHES.
EAT OGAA LESS THAN 20 INCHES AND CHOOSE EVEN SMALLER OGAA TO FURTHER REDUCE MERCURY EXPOSURE.

For Ogaa Smaller than 20 Inches:

- Eat up to 8 meals or 64 ounces per month
- Eat up to 4 meals or 32 ounces per month
- Eat up to 2 meals or 16 ounces per month
- Eat up to 1 meal or 8 ounces per month
- Do not eat ogaa from these lakes
- Not enough information available

Number of meals is based on an 8 ounce meal size. If your meal size is larger, you should reduce the number of meals you eat per month.

Lac Courte Oreilles Reservation

County Boundary

Tribal reservation boundaries are representations and may not be the actual legally binding boundaries

Funding for these maps was provided by the Environmental Protection Agency (EPA)
GLIFWC 09/2005

MAP FOR USE BY WOMEN BEYOND CHILDBEARING AGE AND BY MEN.

FOR OGAA LARGER THAN 20 INCHES, EAT FEWER MEALS.

Recommended Maximum Number of Ogaa Meals per Month for Lakes Harvested by Lac Courte Oreilles

SORTING AND LABELING *OGAA* PRIOR TO FREEZING

When Cleaning *Ogaa*:

- Put *ogaa* under 20 inches in bags labeled "under 20 inches."
- Put *ogaa* over 20 inches in bags labeled "over 20 inches."
- Label bags with the lake name.
- Follow the advice below for maximum number of meals per month.

USING THIS CHART TO FIND SAFER *GIIGOONH*

MAXIMUM NUMBER OF MEALS PER MONTH

Advice is for all lakes combined. For example, if you eat four meals in a month from green lakes you should not eat any other meals of *ogaa* in that month.

MEAL SIZE

Meal size is based on 8 ounces. An average 19 inch *ogaa* will have 8 ounces of meat. If your meal size is larger you should eat fewer meals of *ogaa*. If it is smaller you can eat more meals of *ogaa*.

OTHER *GIIGOONH*

Giigoonh such as muskellunge, largemouth bass, smallmouth bass, and northern pike will have more mercury than *giigoonh* such as lake whitefish, herring, bluegill, sunfish, crappie or perch. Try to choose safer *giigoonh*.

LAKE	COUNTY	Women of childbearing age and children less than 15 Maximum number of meals per month	Women beyond childbearing years and men 15 and older Maximum number of meals per month
AMACOY L	RUSK	1	4
BARBER L	SAWYER	Not Enough Information	
BIRCH L	WASHBURN	Not Enough Information	
DURPHEE L	SAWYER	2	8
FIRESIDE LAKES	RUSK	2	8
GRINDSTONE L	SAWYER	2	4
HOLCOMBE FL	CHIPPEWA	1	4
L CHETAC	SAWYER	2	4
L CHIPPEWA	SAWYER	1	4
L EAU CLAIRE	EAU CLAIRE	1	4
L OF THE PINES	SAWYER	1	4
LAC COURTE OREILLES	SAWYER	2	4
LITTLE ROUND L	SAWYER	Not Enough Information	
LONG L	WASHBURN	2	8
LOST LAND L	SAWYER	1	4
LOWER CLAM L	SAWYER	2	4
MILLE LACS L	MILLE LACS	2	8
MOOSE L	SAWYER	0	2
NELSON L	SAWYER	1	4
OLD ABE L	CHIPPEWA	1	4
OTTER L	CHIPPEWA	Not Enough Information	
POTATO L	RUSK	1	4
PULASKI L	RUSK	0	2
ROUND L	SAWYER	2	8
ROUND L	CHIPPEWA	1	2
SAND L	RUSK	0	2
SAND L	SAWYER	1	4
SISSABAGAMA L	SAWYER	2	4
SLIM	WASHBURN	Not Enough Information	
SMITH L	SAWYER	1	4
SPIDER L	SAWYER	1	2
STONE L	WASHBURN	Not Enough Information	
TEAL L	SAWYER	2	4
THORNAPPLE FL	RUSK	Not Enough Information	
TIGER CAT FL	SAWYER	Not Enough Information	
WHITEFISH L	SAWYER	1	4
WINDFALL L	SAWYER	Not Enough Information	
WINDIGO L	SAWYER	0	2

For many native people, *giigoonh* are part of a traditional and healthy diet. If you rely on *giigoonh*, choose safer *giigoonh* with lower levels of mercury by following the advice on this map.

RISKS AND BENEFITS

Risk: Mercury can damage the nervous system, especially the brain. Fetuses and babies are the most at risk because their nervous systems are rapidly developing. Children exposed to unsafe levels while in the womb have been found to experience delayed development in walking and talking, even though the mother was not affected. Mercury cannot be removed by trimming or cooking.

Benefit: Eating even as few as two to three meals of *giigoonh* a month may reduce your risk of death due to heart disease.

If you have questions about finding safer ogaa, call GLIFWC at 1-800-250-7574.
To learn more about mercury in ogaa, visit GLIFWC's website at www.glifwc.org/bio/mercury.htm

Appendix 2

Great Lakes Indian Fish and Wildlife Commission Chain of Custody Forms for Collection and Transport of Fish for Mercury Analysis

FIELD CHAIN-OF-CUSTODY/DATA FORM

Study Title: Spring Walleye Sampling For Mercury Year:

Name of Lake:_____ County_____ Area _____

SECTION A: SAMPLE COLLECTION

COLLECT WALLEYE IN THE FOLLOWING SIZE GROUPS				
Size Ranges	12.0-14.9	15.0-17.9	18.0-22	>22
Number of Walleye	3	3	3	3

No	Fish Tag No	Length (in.)	Sex (M/F/U)	No	Fish Tag No	Length (in.)	Sex (M/F/U)
1				7			
2				8			
3				9			
4				10			
5				11			
6				12			

SECTION B: SAMPLE STORAGE AND CUSTODY

Check (X) either Cooler or Freezer(<0^{0}C)

1. Crew Leader/ Warden:_____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
2. Custody given to : _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
3. Custody given to : _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____

Comments:_____

_____

_____

_____

OFFICE USE ONLY– DO NOT WRITE BELOW THIS LINE

_____

3. 3rd Custody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
4. 4th Custody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
5. 5th Custody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
6. 6thCustody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____
7. 7thCustody: _____ Date:_____ Time:_____ Cooler on Ice _____ Freezer_____

TRANSFER CHAIN-OF-CUSTODY FORM

Study Title: Spring Walleye Sampling For Mercury **Year:**
Purpose: Transfer Filets to UW-Superior, LSRI

PAGE 1 of 2

SECTION A: SAMPLE STORAGE

Container Type Enter: 1 = Cooler + Ice 2 = Freezer (≤-10°C)		Placed INTO Container				Taken OUT of Container			
		Date	Time	Initials	°C	Date	Time	Initials	°C
A	GLIFWC	placement into the freezer is recorded on the field COC forms.							
B									
C									
D									
E									
F									

SECTION B: SAMPLE COLLECTION

The individual samples for each lake are listed on the attached sheets.
The lakes being delivered are:
WALLEYE:

1. _____ _____
2. _____ _____
3. _____ _____
4. _____ _____
5. _____ _____
6. _____ _____
7. _____ _____
8. _____ _____
9. _____ _____
10. _____ _____
11. _____ _____
12. _____ _____
13. _____ _____
14. _____ _____
15. _____ _____
16. _____ _____
17. _____ _____
18. _____ _____
19. _____ _____
20. _____ _____

SECTION C: SAMPLE CUSTODIAN

1. **Collected by:** Collection information list on Field COC at GLIFWC Office.

2. **Transferred by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

3. **Received by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

4. **Received by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

5. **Received by:**_____ **Date:**_____ **Time:**_____

   Relinquished by:_____ **Date:**_____ **Time:**_____

Appendix 3

Lake Superior Research Institute Final Report: Total Mercury Concentrations in Muscle Tissue from Walleye Captured during the Spring 2008 in Wisconsin Ceded Territory Waters

Total Mercury Concentrations in Muscle Tissue from Walleye Captured during the Spring 2008 in Wisconsin Ceded Territory Waters

by

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Introduction

Skinless fillet samples from walleye (*Sander vitreus*) captured during the spring of 2008 from waters in the 1837 and 1842 Treaty ceded territories were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Two hundred and eighteen skinless walleye fillets from twenty-six lakes in Wisconsin and 12 from one lake in Minnesota collected by tribal spearers and GLIFWC Inland Fisheries assessment crews were analyzed. Funding for the analysis of these samples was associated with GLIFWC's BIA Department 345 FY-05 funds and EPA Grant #GL96520701-1.

Methods

At the time fish were captured, a tribal warden or biologist was present to measure the total length of each fish. Fish were tagged with a unique number (i.e. a fish identification number), were immediately placed on ice and were frozen within 36 hours of capture. Whole fish with chain-of-custody forms were transferred to the Great Lake Indian Fish and Wildlife Commission (GLIFWC) laboratory. At the GLIFWC laboratory, one fillet was removed from each fish, the skin was removed from the fillet and the fillet was placed into a plastic bag along with a label containing the fish identification number. This fish processing followed SOPs developed by GLIFWC. Sex of the fish was determined during the filleting process. A dorsal fin spine was removed from each fish to determine its age. At the LSRI laboratories, the walleye were received frozen and in good condition with chain-of-custody documentation. Samples were stored in a freezer at approximately -20°C until they were removed and thawed for processing and analysis.

Before processing the fish tissues, all glassware, utensils, and grinders were cleaned according to the appropriate methods (SOP SA/8). Each day, the fish to be processed were removed from the freezer and allowed to warm to a flexible, but stiff, consistency. The skinless fillet was passed through a grinder three times. A small amount of the initial tissue that passed through the grinder was collected and discarded (SOP SA/10). A sub-sample of the ground tissue was placed into a certified clean glass vial and frozen until mercury analysis was conducted. The grinder was disassembled after each fillet was ground and the unit was washed according to the grinder cleaning procedure (SOP SA/8).

Commercial canned tuna fish (*Thunnus sp.*) were used as procedural blanks for this project. These procedural blanks consisted of one aliquot from a can of tuna that was transferred directly into a sample bottle after the packing liquid was removed from the tuna . The second portion was ground in the same manner as the walleye fillets. This check was made to ensure that no contamination or loss of mercury was occurring in the grinding process. Five procedural blanks were prepared during this project. The initial procedural blank was prepared on the first day fish were ground for the project and the last procedural blank was generated on the last day fish were processed. The other three were prepared on intermediate dates when fish were being ground.

Fish tissues were weighed for mercury analysis following standard laboratory procedure (SOP SA/11). Mercury solutions for making tissue spikes and preparing analytical standards were

prepared following the procedures in SOP SA/42. Mercury analyses were performed using cold vapor mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/49). Sample analysis yielded triplicate absorbance readings whose mean value was used to calculate the concentration of each sample. If the relative standard deviation (RSD) of the three measurements was greater than 5%, additional aliquots of the sample were analyzed in an attempt to obtain an RSD of less than 5%. If an RSD of < 5% was not able to be achieved, the sample was redigested and reanalyzed. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37. The biota method detection limit was 0.0126 µg Hg/g for a tissue mass of 0.2 g (Appendix A). This limit of detection was determined using a whole fish composite of rainbow trout containing a low concentration of mercury (SOP SA/35).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP NT/15). A portion (1 to 4 g) of ground tissue was placed into a pre-dried and pre-weighed aluminum pan immediately following tissue grinding. The pan and wet tissue were immediately weighed and placed into an oven (60°C) and dried for various time intervals. Drying times varied from 24 to 96 hours. Approximately 35 percent of the walleye analyzed for mercury had moisture content determined. In general, 4 fish per lake were randomly selected for determination of percent moisture.

Quality Assurance

Data quality was monitored by four methods: analysis of similar fish tissues (commercial canned tuna; *Thunnus* sp.) before and after the tissue grinding process (procedural blanks) to measure laboratory bias; analysis of dogfish shark (DORM-2, *Squalus acanthias*) from the Canadian government (certified reference material from National Research Council Canada, Ottawa, Ontario, Canada) that has a certified concentration of mercury to measure analytical accuracy; duplicate analysis of fish tissue from the same fillet to measure analytical precision; and analysis of tissue with known additions of mercury to determine spike recovery and possible analytical interferences. Two sets of standard solutions with known amounts of mercury (analytical standards) were analyzed with each group (maximum of 40 samples plus QA samples) of tissue samples. These analytical solutions contained 0, 50, 100, 500, 1000 and 6000 ng Hg/L. They were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). Summary tables of the mercury calibration curve data are provided (Appendix B).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the mean ± 2 times the standard deviation of the values obtained from the Spring Walleye 2005 through 2007 projects for the respective quality assurance parameter. Results for the procedural blanks were considered acceptable when the relative percent agreement was > 69.7%. Duplicate agreement values were acceptable when having a relative percent agreement > 85.3%. The calculated acceptable range for the DORM-2 standard reference material was 3.48-5.23 µg Hg/g. Prior to digestion, tissues from ten percent of the fish samples were spiked, in duplicate, with a known quantity of mercury and analyzed for recovery of the spiked mercury. Spike recovery was considered acceptable when it was in the

range of 60.8 to 115 percent of the expected value.

Five walleye tissue samples had an initial RSD of >5% for the triplicate measurements made on the digested sample. The digestate of four of those samples was reanalyzed on the same date and each resulted in an RSD of <5%. One sample, Bear 1141, had a RSD of 5.20% and was not rerun. Several QA/QC samples initially failed the RSD of >5% check. These included one spiked sample which was rerun and passed the check; two DORM samples, one which was rerun and passed and a second which was not rerun that had a RSD of 5.56%; and one tuna sample that was rerun and the rerun also failed the RSD check. The tuna sample had a low concentration making it more likely to fail the RSD check. A number of calibration blanks and the three lowest concentration mercury standards also failed the RSD of >5% check. This included eight blanks, seven 50 ng standards, three 100 ng standards, and one 500 ng standard. Standards or samples with low absorbance values are likely to fail the RSD limit of 5% and frequently are not reanalyzed. Based on the limit of detection determined for mercury with this project it was noted that the 50 ng mercury standard was below the detection limit.

A quality assurance audit was conducted by the LSRI quality assurance officer during the Spring Walleye 2008 project. That report is provided in Appendix C.

Results of Fish Tissue Analyses

Quality Assurance – Five tuna procedural blanks were processed coincident with the grinding of walleye collected for the project. One of the five procedural blanks was analyzed with each set of mercury samples for a total of eight analyses resulting in a mean of 79.4 ± 10.6 relative percent agreement (Table 1). The relative percent agreement values ranged from 58.9 to 92.2%, all but two of which were within the acceptable range of > 69.7%.

Analysis of the dogfish shark tissue (DORM-2) standard reference material was conducted in duplicate with all eight sets of walleye tissues analyzed (Table 2). The certified mercury concentration for the dogfish tissue was 4.64 ± 0.26 µg Hg/g. The recovery values ranged from 80.6 to 111.2% with the grand mean and standard deviation of the recoveries being 95.3 ± 7.9 percent of the certified value. On July 1, 2008, one DORM-2 recovery was not within the acceptance range of 75.0 to 113% and as a result, the entire set was reanalyzed on August 7, 2008. Analytical results for mercury content for that set of samples are reported based on the data obtained on August 7, 2008.

Fish tissues were analyzed for mercury in duplicate 33 times. Two portions of the same tissue were digested and analyzed independently. Relative percent agreement between the duplicate analyses of the same tissue ranged from 72.1 to 99.7% with the average and standard deviation of the agreements being 92.3 ± 7.4 percent (Table 3). Seven relative percent agreement values were below the acceptance range of > 85.3% and those samples were reanalyzed in duplicate on another date. The results for the reanalyzed samples fell within the acceptance range.

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 26 spiked samples was 88.7 ± 13.7 percent with the individual values ranging

from 35.9 to 106.8% (Table 4). One spike recovery value (Two Sisters 12573) was below the acceptance range (60.8 to 115 %). This sample was reanalyzed on August 7, 2008 and was found to have an acceptable recovery on that date.

Mercury Analysis – Skinless fillets of 218 walleye from 26 lakes in Wisconsin and 12 walleye from one lake in Minnesota were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.062 to 1.36 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in 80 of the 230 walleye tissues. Moisture analysis took place immediately following grinding of the fillets. Walleye muscle tissue had a mean moisture value of 79.4 ± 0.94 percent (Table 6). Of the 80 tissues analyzed for moisture, twelve were analyzed in duplicate, all yielding relative percent agreements of 98.8 percent or greater. Nine samples were also dried an additional 24 hours and reweighed to ensure dryness, all yielding agreements greater than 99 percent.

Table 1. Relative Percent Agreement of Total Mercury for Procedural Blank Samples (Before and After Grinding).

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
6/13/2008	5/23/2008	0.043	0.038	0.041	87.9
7/9/2008	7/8/2008	0.067	0.062	0.065	92.2
7/16/2008	7/15/2008	0.247	0.209	0.228	83.3
7/22/2008	6/30/2008	0.049	0.035	0.042	66.7
7/23/2008	6/23/2008	0.085	0.056	0.071	58.9
8/1/2008	7/15/2008	0.250	0.215	0.233	84.9
8/7/2008	6/23/2008	0.075	0.064	0.070	84.2
8/20/2008	7/15/2008	0.260	0.206	0.233	76.8
			Mean ± Std. Dev.		79.4 ± 10.6

* Relative percent agreement is calculated by the equation (1-|before – after| /mean)100

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-2) Analyzed during Fish Analysis. The Standard Reference has a Certified Mercury Concentration of 4.64 ± 0.26µg Hg/g Tissue.

Date of Analysis	DORM 2-1		DORM 2-2		DORM 2-3	
	µg Hg/g	Percent of Expected	µg Hg/g	Percent of Expected	µg Hg/g	Percent of Expected
6/13/2008	4.68	100.8	4.56	98.2		
7/9/2008	3.96	85.4	5.16	111.2		
7/16/2008	4.73	101.9	4.88	105.1		
7/22/2008	4.84	104.4	4.00	86.2		
7/23/2008	4.33	93.4	4.41	95.1		
8/1/2008	4.05	87.3	3.74	80.6		
8/7/2008	4.33	93.3	4.27	92.1	4.30	92.6
8/20/2008	4.47	96.4				
			Mean ± Std. Dev.		4.42 ± 0.37	95.3 ± 7.9

Table 3. Relative Percent Agreement for Duplicate Analysis of Total Mercury Content in Skinless Walleye Fillet Tissue.

Date of Analysis	Lake	Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
6/13/2008	Big Mckenzie	9929	0.147	0.149	0.148	98.6
6/13/2008	Big Round	8770	0.217	0.200	0.209	91.8
6/13/2008	Bear	1147	0.385	0.326	0.356	83.4
7/9/2008	Lake Owen	12371	0.137	0.130	0.134	94.8
7/9/2008	Otter	11232	0.073	0.056	0.065	73.6
7/9/2008	Round	12384	0.510	0.604	0.557	83.1
7/9/2008	Birch	11377	0.479	0.507	0.493	94.3
7/16/2008	Pelican	11118	0.297	0.319	0.308	92.9
7/16/2008	Rice River Flowage	11187	0.482	0.364	0.423	72.1
7/16/2008	Rice River Flowage	11199	0.364	0.363	0.364	99.7
7/16/2008	Two Sisters	12573	1.080	1.160	1.120	92.9
7/22/2008	Archibald	11221	0.215	0.212	0.214	98.6
7/22/2008	Four Mile	11253	0.822	0.829	0.826	99.2
7/22/2008	Long	11266	0.526	0.517	0.522	98.3
7/22/2008	Tiger Cat Flowage	12352	0.408	0.452	0.430	89.8
7/23/2008	Lac Vieux Desert	11206	0.124	0.131	0.128	94.5
7/23/2008	Squaw	11347	0.201	0.236	0.219	84.0
7/23/2008	Squaw	11359	0.270	0.276	0.273	97.8
7/23/2008	Birch	12901	0.333	0.319	0.326	95.7
8/1/2008	Shell	11312	0.134	0.131	0.133	97.7
8/1/2008	Wildcat	11166	0.126	0.131	0.129	96.1
8/7/2008	Patten	11367	0.168	0.139	0.154	81.1
8/7/2008	Mille Lacs	11320	0.105	0.103	0.104	98.1
8/7/2008	Mille Lacs	11329	0.062	0.076	0.069	79.7
8/7/2008	Bearskin	11343	0.107	0.110	0.109	97.2
8/7/2008	Bear	1147	0.276	0.295	0.286	93.3
8/7/2008	Otter	11232	0.063	0.055	0.059	86.4
8/7/2008	Round	12384	0.458	0.476	0.467	96.1
8/7/2008	Rice River Flowage	11187	0.454	0.449	0.452	98.9
8/7/2008	Squaw	11347	0.187	0.191	0.189	97.9
8/7/2008	Two Sisters	12573	1.100	1.140	1.120	96.4
8/20/2008	Mille Lacs	11329	0.073	0.070	0.070	95.8
8/20/2008	Patten	11367	0.147	0.153	0.150	96.0
				Mean ± Std. Dev.		92.3 ± 7.4

Table 4. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with a Known Concentration of Mercury.

Analysis Date	Lake	Tag Number	Spike #1	Spike #2	Mean	Std. Dev.
6/13/2008	Big Mckenzie	9929	100.8	90.8	95.8	7.0
6/13/2008	Big Round	8770	93.0	96.8	94.9	2.7
6/13/2008	Bear	1147	85.2	78.8	82.0	4.6
7/9/2008	Lake Owen	12371	103.2	101.3	102.2	1.4
7/9/2008	Otter	11232	103.6	102.4	103.0	0.8
7/9/2008	Round	12384	106.6	55.8	81.2	35.9
7/9/2008	Birch	11377	67.8	78.1	72.9	7.3
7/16/2008	Pelican	11118	85.4	85.9	85.6	0.3
7/16/2008	Rice River Flowage	11187	75.5	102.8	89.2	19.3
7/16/2008	Rice River Flowage	11199	90.9	87.4	89.2	2.5
7/16/2008	Two Sisters	12573	35.9	46.4	41.2	7.4
7/22/2008	Archibald	11221	101.5	89.8	95.7	8.3
7/22/2008	Four Mile	11253	71.9	60.3	66.1	8.2
7/22/2008	Long	11266	85.5	84.4	85.0	0.8
7/22/2008	Tiger Cat Flowage	12352	76.3	66.5	71.4	7.0
7/23/2008	Lac Vieux Desert	11206	101.9	99.2	100.5	1.9
7/23/2008	Squaw	11347	93.8	88.2	91.0	4.0
7/23/2008	Squaw	11359	91.1	94.7	92.9	2.6
7/23/2008	Birch	12901	98.4	94.7	96.6	2.6
8/1/2008	Shell	11312	92.1	110.9	101.5	13.3
8/1/2008	Wildcat	11166	106.8	106.4	106.6	0.3
8/7/2008	Patten	11367	98.4	98.7	98.5	0.2
8/7/2008	Mille Lacs	11320	97.4	96.9	97.2	0.3
8/7/2008	Mille Lacs	11329	91.8	94.5	93.2	1.9
8/7/2008	Bearskin	11343	84.2	83.9	84.0	0.2
8/7/2008	Two Sisters	12573	88.6	87.1	87.8	1.1
			Mean ± Std. Dev.		88.7 ± 13.7	

Table 5. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured during the Spring of 2008.

Analysis Date	Lake	Tag Number	County	Fresh Length (in)	Sex	µg Hg/g tissue
6/13/2008	Bear	1141	Barron	20.0	M	0.223
6/13/2008	Bear	1144	Barron	18.4	M	0.330
6/13/2008	Bear	1146	Barron	17.0	M	0.213
8/7/2008	Bear	1147	Barron	18.5	M	0.356
6/13/2008	Beaver Dam	10984	Barron	17.3	M	0.148
6/13/2008	Beaver Dam	10985	Barron	19.8	F	0.190
6/13/2008	Beaver Dam	10988	Barron	18.0	M	0.199
6/13/2008	Beaver Dam	10989	Barron	20.5	M	0.595
7/9/2008	Owen	12366	Bayfield	23.0	F	0.656
7/9/2008	Owen	12367	Bayfield	19.5	M	0.657
7/9/2008	Owen	12368	Bayfield	21.0	M	0.722
7/9/2008	Owen	12369	Bayfield	25.7	F	0.755
7/9/2008	Owen	12370	Bayfield	21.2	F	0.399
7/9/2008	Owen	12371	Bayfield	14.9	M	0.134
7/9/2008	Owen	12372	Bayfield	15.0	M	0.241
7/9/2008	Owen	12373	Bayfield	15.7	M	0.133
7/9/2008	Owen	12374	Bayfield	17.0	M	0.409
7/9/2008	Owen	12375	Bayfield	14.6	M	0.157
7/9/2008	Owen	12376	Bayfield	14.3	M	0.174
7/9/2008	Owen	12377	Bayfield	26.0	F	1.250
6/13/2008	Big Mckenzie	9927	Burnett	17.0	M	0.148
6/13/2008	Big Mckenzie	9929	Burnett	18.5	M	0.148
6/13/2008	Big Mckenzie	9931	Burnett	18.5	F	0.432
6/13/2008	Big Mckenzie	9932	Burnett	19.4	M	0.246
8/7/2008	Patten	11361	Florence	18.0	M	0.398
8/7/2008	Patten	11362	Florence	21.2	M	0.546
8/7/2008	Patten	11363	Florence	24.9	F	0.746
8/7/2008	Patten	11364	Florence	17.5	M	0.374
8/7/2008	Patten	11365	Florence	15.8	M	0.299
8/7/2008	Patten	11367	Florence	12.2	M	0.150
8/7/2008	Patten	11368	Florence	27.3	F	0.750
8/7/2008	Patten	11370	Florence	19.3	M	0.548
8/7/2008	Patten	11371	Florence	14.7	M	0.167
8/7/2008	Patten	11373	Florence	22.6	F	0.676

8/7/2008	Patten	11374	Florence	14.3	M	0.155
8/7/2008	Patten	11375	Florence	16.9	M	0.170
7/9/2008	Otter	11231	Langlade	14.6	M	0.076
8/7/2008	Otter	11232	Langlade	15.8	M	0.065
7/9/2008	Otter	11235	Langlade	20.6	F	0.146
7/9/2008	Otter	11236	Langlade	18.4	M	0.173
7/9/2008	Otter	11237	Langlade	14.5	M	0.062
7/9/2008	Otter	11242	Langlade	18.0	M	0.109
7/9/2008	Otter	11244	Langlade	17.8	M	0.115
7/9/2008	Otter	11245	Langlade	17.7	M	0.093
7/16/2008	Rice River Flowage	11186	Lincoln	18.4	F	0.272
8/7/2008	Rice River Flowage	11187	Lincoln	20.7	M	0.452
7/16/2008	Rice River Flowage	11188	Lincoln	13.3	M	0.222
7/16/2008	Rice River Flowage	11189	Lincoln	18.2	M	0.561
7/16/2008	Rice River Flowage	11190	Lincoln	13.6	M	0.273
7/16/2008	Rice River Flowage	11192	Lincoln	23.2	F	0.719
7/16/2008	Rice River Flowage	11194	Lincoln	25.1	F	0.573
7/16/2008	Rice River Flowage	11195	Lincoln	24.4	F	0.863
7/16/2008	Rice River Flowage	11197	Lincoln	14.0	M	0.249
7/16/2008	Rice River Flowage	11198	Lincoln	15.2	M	0.348
7/16/2008	Rice River Flowage	11199	Lincoln	16.6	F	0.364
7/16/2008	Rice River Flowage	11200	Lincoln	15.6	M	0.306
8/7/2008	Mille Lacs	11319	Mille Lacs	12.6	M	0.066
8/7/2008	Mille Lacs	11320	Mille Lacs	17.3	M	0.104
8/7/2008	Mille Lacs	11321	Mille Lacs	18.4	M	0.155
8/7/2008	Mille Lacs	11322	Mille Lacs	16.9	M	0.145
8/7/2008	Mille Lacs	11323	Mille Lacs	21.3	M	0.394
8/7/2008	Mille Lacs	11324	Mille Lacs	16.7	M	0.115
8/7/2008	Mille Lacs	11325	Mille Lacs	21.8	M	0.365
8/7/2008	Mille Lacs	11326	Mille Lacs	22.3	M	0.273
8/7/2008	Mille Lacs	11327	Mille Lacs	23.9	F	0.413
8/7/2008	Mille Lacs	11328	Mille Lacs	22.3	M	0.287
8/7/2008	Mille Lacs	11329	Mille Lacs	14.1	M	0.072
8/7/2008	Mille Lacs	11330	Mille Lacs	14.1	M	0.066
7/22/2008	Archibald	11216	Oconto	21.8	F	0.446
7/22/2008	Archibald	11217	Oconto	27.1	F	1.066
7/22/2008	Archibald	11218	Oconto	24.2	F	0.941
7/22/2008	Archibald	11219	Oconto	18.4	M	0.228

7/22/2008	Archibald	11220	Oconto	19.4	F	0.307
7/22/2008	Archibald	11221	Oconto	17.0	M	0.214
7/22/2008	Archibald	11222	Oconto	14.0	M	0.208
7/22/2008	Archibald	11223	Oconto	13.9	M	0.249
7/22/2008	Archibald	11224	Oconto	16.4	M	0.219
7/22/2008	Archibald	11225	Oconto	15.4	M	0.256
7/22/2008	Archibald	11227	Oconto	13.9	M	0.241
8/7/2008	Bearskin	11331	Oneida	13.0	M	0.079
8/7/2008	Bearskin	11332	Oneida	13.5	M	0.075
8/7/2008	Bearskin	11333	Oneida	24.7	F	0.373
8/7/2008	Bearskin	11336	Oneida	22.5	F	0.112
8/7/2008	Bearskin	11338	Oneida	21.5	F	0.168
8/7/2008	Bearskin	11339	Oneida	22.1	F	0.081
8/7/2008	Bearskin	11340	Oneida	16.8	M	0.125
8/7/2008	Bearskin	11341	Oneida	20.7	F	0.176
8/7/2008	Bearskin	11342	Oneida	16.7	F	0.123
8/7/2008	Bearskin	11343	Oneida	15.4	M	0.109
8/7/2008	Bearskin	11344	Oneida	13.4	M	0.062
8/7/2008	Bearskin	11345	Oneida	20.9	F	0.143
7/22/2008	Four Mile	11248	Oneida	13.2	M	0.967
7/22/2008	Four Mile	11249	Oneida	16.3	M	1.356
7/22/2008	Four Mile	11253	Oneida	13.2	M	0.826
7/22/2008	Four Mile	11254	Oneida	16.5	M	1.174
7/22/2008	Four Mile	11256	Oneida	13.5	M	0.694
7/22/2008	Four Mile	11257	Oneida	15.5	M	0.859
7/22/2008	Long	11261	Oneida	14.0	M	0.405
7/22/2008	Long	11262	Oneida	12.2	M	0.228
7/22/2008	Long	11263	Oneida	14.9	M	0.672
7/22/2008	Long	11264	Oneida	15.0	M	0.290
7/22/2008	Long	11265	Oneida	15.0	F	0.229
7/22/2008	Long	11266	Oneida	21.6	F	0.522
7/22/2008	Long	11267	Oneida	15.0	M	0.403
7/22/2008	Long	11268	Oneida	22.9	F	0.742
7/22/2008	Long	11269	Oneida	21.2	F	0.381
7/22/2008	Long	11270	Oneida	22.2	F	0.640
7/16/2008	Pelican	11111	Oneida	18.8	M	0.411
7/16/2008	Pelican	11112	Oneida	25.0	F	0.454
7/16/2008	Pelican	11115	Oneida	15.1	M	0.145

7/16/2008	Pelican	11116	Oneida	14.8	M	0.175
7/16/2008	Pelican	11117	Oneida	18.2	M	0.253
7/16/2008	Pelican	11118	Oneida	23.2	F	0.308
7/16/2008	Pelican	11120	Oneida	13.9	M	0.098
7/16/2008	Pelican	11121	Oneida	23.2	F	0.350
7/16/2008	Pelican	11122	Oneida	18.4	M	0.179
7/16/2008	Pelican	11123	Oneida	16.1	M	0.123
7/16/2008	Pelican	11124	Oneida	15.1	M	0.124
7/16/2008	Pelican	11125	Oneida	14.6	M	0.115
7/16/2008	Two Sisters	12561	Oneida	24.0	F	0.406
7/16/2008	Two Sisters	12562	Oneida	21.4	M	0.459
7/16/2008	Two Sisters	12563	Oneida	18.8	M	0.326
7/16/2008	Two Sisters	12565	Oneida	15.8	M	0.130
7/16/2008	Two Sisters	12566	Oneida	14.8	M	0.182
7/16/2008	Two Sisters	12567	Oneida	28.2	F	1.135
7/16/2008	Two Sisters	12568	Oneida	17.7	M	0.305
7/16/2008	Two Sisters	12569	Oneida	17.4	M	0.337
7/16/2008	Two Sisters	12571	Oneida	21.1	M	0.499
8/7/2008	Two Sisters	12573	Oneida	26.7	M	1.120
7/16/2008	Two Sisters	12574	Oneida	14.7	M	0.177
7/16/2008	Two Sisters	12575	Oneida	18.7	M	0.282
6/13/2008	Balsam	1336	Polk	19.1	M	0.284
6/13/2008	Balsam	174246	Polk	18.3	M	0.152
6/13/2008	Balsam	174261	Polk	17.5	M	0.128
6/13/2008	Balsam	174262	Polk	17.9	M	0.127
6/13/2008	Big Round	8770	Polk	18.2	M	0.209
6/13/2008	Big Round	8772	Polk	17.7	M	0.224
6/13/2008	Big Round	8773	Polk	18.6	M	0.339
6/13/2008	Big Round	8774	Polk	19.9	M	0.402
6/13/2008	Wapogasset	10962	Polk	17.9	M	0.288
6/13/2008	Wapogasset	10964	Polk	18.6	M	0.255
6/13/2008	Wapogasset	10966	Polk	19.6	M	0.229
6/13/2008	Wapogasset	10968	Polk	17.1	M	0.185
6/13/2008	Nelson	11107	Sawyer	18.5	M	0.920
6/13/2008	Nelson	12345	Sawyer	21.1	M	0.643
6/13/2008	Nelson	12346	Sawyer	20.6	F	0.242
6/13/2008	Nelson	12347	Sawyer	20.5	M	0.758
6/13/2008	Nelson	12348	Sawyer	24.1	U	0.918

7/9/2008	Round	12382	Sawyer	19.1	F	0.199
7/9/2008	Round	12383	Sawyer	22.5	F	0.452
8/7/2008	Round	12384	Sawyer	22.8	F	0.557
7/9/2008	Round	12385	Sawyer	23.0	F	0.454
7/9/2008	Round	12389	Sawyer	14.3	M	0.150
7/9/2008	Round	12390	Sawyer	13.8	M	0.100
7/9/2008	Round	12391	Sawyer	21.0	M	0.562
7/9/2008	Round	12392	Sawyer	12.4	M	0.129
7/9/2008	Round	12393	Sawyer	20.5	F	0.167
7/9/2008	Round	12394	Sawyer	15.8	M	0.118
7/9/2008	Round	12395	Sawyer	17.1	F	0.182
7/9/2008	Round	12396	Sawyer	15.6	M	0.106
7/22/2008	Tiger Cat Flowage	12351	Sawyer	20.8	F	0.355
7/22/2008	Tiger Cat Flowage	12352	Sawyer	21.5	F	0.430
7/22/2008	Tiger Cat Flowage	12353	Sawyer	19.7	M	0.532
7/22/2008	Tiger Cat Flowage	12354	Sawyer	15.2	M	0.358
7/22/2008	Tiger Cat Flowage	12355	Sawyer	15.9	M	0.418
7/22/2008	Tiger Cat Flowage	12356	Sawyer	13.1	M	0.122
6/13/2008	Cedar	10992	St Croix	17.6	M	0.201
6/13/2008	Cedar	10993	St Croix	17.2	F	0.076
6/13/2008	Cedar	10994	St Croix	19.4	F	0.153
6/13/2008	Cedar	10996	St Croix	18.2	F	0.090
7/9/2008	Birch	11376	Vilas	22.6	F	0.761
7/9/2008	Birch	11377	Vilas	15.2	M	0.493
7/9/2008	Birch	11378	Vilas	22.8	F	1.132
7/9/2008	Birch	11379	Vilas	15.0	F	0.507
7/9/2008	Birch	11380	Vilas	13.3	M	0.375
7/9/2008	Birch	11383	Vilas	20.9	F	0.834
7/9/2008	Birch	11389	Vilas	13.1	M	0.478
7/9/2008	Birch	11390	Vilas	15.1	M	0.374
7/23/2008	Lac Vieux Desert	11201	Vilas	18.2	M	0.130
7/23/2008	Lac Vieux Desert	11202	Vilas	16.3	M	0.137
7/23/2008	Lac Vieux Desert	11203	Vilas	14.8	M	0.118
7/23/2008	Lac Vieux Desert	11204	Vilas	13.2	M	0.108
7/23/2008	Lac Vieux Desert	11205	Vilas	13.5	M	0.099
7/23/2008	Lac Vieux Desert	11206	Vilas	15.8	M	0.128
7/23/2008	Lac Vieux Desert	11207	Vilas	15.2	M	0.171
7/23/2008	Lac Vieux Desert	11208	Vilas	18.0	F	0.171

7/23/2008	Lac Vieux Desert	11209	Vilas	24.4	F	0.342
7/23/2008	Lac Vieux Desert	11210	Vilas	23.3	F	0.431
7/23/2008	Lac Vieux Desert	11214	Vilas	26.1	F	0.547
7/23/2008	Lac Vieux Desert	11215	Vilas	19.5	M	0.219
7/23/2008	Squaw	11346	Vilas	17.7	M	0.270
8/7/2008	Squaw	11347	Vilas	12.2	M	0.189
7/23/2008	Squaw	11348	Vilas	20.4	F	0.424
7/23/2008	Squaw	11349	Vilas	17.8	F	0.492
7/23/2008	Squaw	11353	Vilas	18.0	M	0.258
7/23/2008	Squaw	11354	Vilas	22.7	F	0.534
7/23/2008	Squaw	11355	Vilas	15.3	F	0.410
7/23/2008	Squaw	11356	Vilas	17.8	M	0.236
7/23/2008	Squaw	11357	Vilas	17.2	F	0.295
7/23/2008	Squaw	11358	Vilas	14.5	F	0.439
7/23/2008	Squaw	11359	Vilas	12.8	M	0.273
8/1/2008	Wildcat	11156	Vilas	14.6	M	0.110
8/1/2008	Wildcat	11163	Vilas	16.4	M	0.160
8/1/2008	Wildcat	11164	Vilas	17.3	M	0.152
8/1/2008	Wildcat	11165	Vilas	16.5	M	0.274
8/1/2008	Wildcat	11166	Vilas	14.6	M	0.129
8/1/2008	Wildcat	11167	Vilas	14.3	M	0.105
8/1/2008	Wildcat	11168	Vilas	18.4	M	0.302
8/1/2008	Wildcat	11169	Vilas	22.2	F	0.401
8/1/2008	Wildcat	11170	Vilas	21.3	M	0.125
7/23/2008	Birch	12891	Washburn	21.3	F	0.234
7/23/2008	Birch	12892	Washburn	23.0	F	0.207
7/23/2008	Birch	12893	Washburn	19.6	M	0.292
7/23/2008	Birch	12894	Washburn	17.4	M	0.271
7/23/2008	Birch	12895	Washburn	14.0	M	0.144
7/23/2008	Birch	12896	Washburn	14.3	M	0.158
7/23/2008	Birch	12897	Washburn	13.0	M	0.134
7/23/2008	Birch	12898	Washburn	17.7	M	0.344
7/23/2008	Birch	12899	Washburn	16.0	M	0.359
7/23/2008	Birch	12900	Washburn	23.8	M	0.687
7/23/2008	Birch	12901	Washburn	19.8	F	0.326
8/1/2008	Shell	11301	Washburn	19.8	M	0.656
8/1/2008	Shell	11302	Washburn	19.8	M	0.712
8/1/2008	Shell	11309	Washburn	17.4	M	0.266

8/1/2008	Shell	11310	Washburn	12.8	M	0.199
8/1/2008	Shell	11311	Washburn	15.8	M	0.166
8/1/2008	Shell	11312	Washburn	13.1	M	0.133
8/1/2008	Shell	11313	Washburn	13.5	M	0.150
8/1/2008	Shell	11314	Washburn	17.5	F	0.318
8/1/2008	Shell	11315	Washburn	19.3	M	0.549

Table 6. Percent Moisture in Walleye Fillets (Measured Immediately after Grinding).

Lake	Tag Number		Percent Moisture	Relative Percent Agreement
Otter	11232*		77.9	
Otter	11242*		77.2	99.9
Otter	11242*	dup	77.1	
Otter	11237*		77.5	
Otter	11244*		77.8	
Tiger Cat Flowage	12356*		79.8	
Tiger Cat Flowage	12355*		79.9	
Tiger Cat Flowage	12354*		79.7	
Tiger Cat Flowage	12352*		79.7	
Four Mile	11248		79.3	
Four Mile	11249		79.7	
Four Mile	11256		79.7	
Four Mile	11527		79.4	
Long	11261		80.3	
Long	11265		80.1	
Long	11262		80.9	
Long	11266		79.7	99.7
Long	11266	dup	80.0	
Squaw	11359		81.2	100.0
Squaw	11359	dup	81.2	
Squaw	11353		78.7	
Squaw	11349		80.0	99.7
Squaw	11349	dup	79.8	
Mille Lacs	11319		81.2	
Mille Lacs	11320		79.1	99.7
Mille Lacs	11320	dup	79.3	
Mille Lacs	11321		79.6	
Mille Lacs	11322		79.7	
Squaw	11346		79.1	
Patten	11361		78.5	
Patten	11367		79.7	
Patten	11363		79.8	

Patten	11365		78.6	99.2
Patten	11365	dup	79.2	
Bear Skin	11332		81.0	
Bear Skin	11336		79.2	
Bear Skin	11344		80.4	
Bear Skin	11338		79.5	
Archibald	11223		79.3	99.9
Archibald	11223	dup	79.4	
Archibald	11225		79.2	
Archibald	11221		80.6	
Archibald	11220		80.0	
WildCat	11156		79.6	
WildCat	11163		80.5	
WildCat	11164		79.5	
WildCat	11170		79.2	
BirchLake Vilas	11389		79.4	
BirchLake Vilas	11380		79.5	
BirchLake Vilas	11390		80.0	
BirchLake Vilas	11383		80.5	100.0
BirchLake Vilas	11383	dup	80.5	
Lac Vieux Desert	11201		78.4	
Lac Vieux Desert	11204		80.1	
Lac Vieux Desert	11207		80.4	
Lac Vieux Desert	11203		79.7	
Nelson	11107		79.3	
Nelson	12345		78.8	
Nelson	12346		80.1	
Nelson	12347		79.4	
Owen	12376		78.8	98.8
Owen	12376	dup	77.9	
Owen	12374		79.8	
Owen	12373		77.4	
Owen	12375		78.5	
Round	12395		79.9	
Round	12389		78.4	
Round	12390		79.6	
Round	12394		78.5	
Pelican	11115		79.1	
Pelican	11124		79.2	99.96
Pelican	11124	dup	79.2	
Pelican	11116		79.3	
Pelican	11125		79.1	
Two Sister	12566		78.6	
Two Sister	12574		79.4	

Two Sister	12569		78.7	99.6
Two Sister	12569	dup	79.0	
Two Sister	12575		78.8	
Shell	11301		78.0	
Shell	11310		79.9	
Shell	11302		80.3	
Shell	11313		79.7	
Rice River	11200		79.2	
Rice River	11199		78.7	
Rice River	11197		81.9	
Rice River	11186		81.2	
Birch Lake Washburn	12891		78.4	
Birch Lake Washburn	12895		78.6	99.0
Birch Lake Washburn	12895	dup	77.8	
Birch Lake Washburn	12897		78.8	
Birch Lake Washburn	12892		78.2	
	Mean ± Std. Dev.		79.4 ± 0.9	

* Sample was returned to the oven and reweighed after an additional 24 hours of drying time.

Appendix A

Determination of 2008 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using GP-RT-HRC-3 sample from 2006

Sample	Tissue Type	ng/L	ng Hg	g sample	µg Hg/g
GP-RT-HRC-3 #1	whole fish composite	140.4	7.355	0.205	0.036
GP-RT-HRC-3 #2	whole fish composite	135.5	7.134	0.260	0.027
GP-RT-HRC-3 #3	whole fish composite	152.0	8.02	0.215	0.037
GP-RT-HRC-3 #4	whole fish composite	168.2	8.90	0.246	0.036
GP-RT-HRC-3 #5	whole fish composite	145.7	7.576	0.258	0.029
GP-RT-HRC-3 #6	whole fish composite	119.4	6.03	0.219	0.028
GP-RT-HRC-3 #7	whole fish composite	184.8	9.790	0.289	0.034
GP-RT-HRC-3 #8	whole fish composite	159.2	8.46	0.232	0.036
				Mean	0.0330
				Std. Dev.	0.00421

LOD = Std. Dev. x t = 0.00421 x 2.998 = 0.0126

LOQ = 10/3 x LOD = 0.0421

2008 Hg LOD = 0.0126 µg/g LOQ = 0.0421 µg/g

2007 Hg LOD = 0.0047 µg/g LOQ = 0.0157 µg/g
2006 Hg LOD = 0.0042 µg/g LOQ = 0.0141 µg/g
2005 Hg LOD = 0.0113 µg/g LOQ = 0.0368 µg/g
2004 Hg LOD = 0.0013 µg/g LOQ = 0.0042 µg/g

Appendix B

Calibration Curve Data Generated During the Analysis of GLIFWC's 2008 Walleye Fillets

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs 1	Blank Corrected Abs 2	Blank Corrected Abs 3	Blank Corrected Mean	Std. Dev.	Slope	Y-Intercept	Correlation
6/13/2008	0	0.0016	0.0016		0.0000	0.0000			
6/13/2008	50	0.0016	0.0009		0.0013	0.0005			
6/13/2008	100	0.0022	0.0020		0.0021	0.0001			
6/13/2008	500	0.0118	0.0113		0.0116	0.0004			
6/13/2008	1000	0.0230	0.0214		0.0222	0.0011			
6/13/2008	6000	0.1369	0.1250		0.1310	0.0084	2.18E-05	0.000214	0.99999
7/9/2008	0	0.0016	0.0013		0.0000	0.0002			
7/9/2008	50	0.0012	0.0012		0.0012	0.0000			
7/9/2008	100	0.0020	0.0020		0.0020	0.0000			
7/9/2008	500	0.0119	0.0110		0.0115	0.0006			
7/9/2008	1000	0.0230	0.0257		0.0244	0.0019			
7/9/2008	6000	0.1539	0.1445		0.1492	0.0066	2.49E-05	-0.00041	0.99998
7/16/2008	0	0.0012	0.0012		0.0000	0.0000			
7/16/2008	50	0.0012	0.0010		0.0011	0.0001			
7/16/2008	100	0.0021	0.0027		0.0024	0.0004			
7/16/2008	500	0.0124	0.0124		0.0124	0.0000			
7/16/2008	1000	0.0234	0.0239		0.0237	0.0004			
7/16/2008	6000	0.1515	0.1347		0.1431	0.0119	2.38E-05	4.3E-05	0.99999
7/22/2008	0	0.0022	0.0016		0.0000	0.0004			
7/22/2008	50	0.0007	0.0015		0.0011	0.0006			
7/22/2008	100	0.0024	0.0022		0.0023	0.0001			
7/22/2008	500	0.0108	0.0123		0.0116	0.0011			
7/22/2008	1000	0.0225	0.0211		0.0218	0.0010			
7/22/2008	6000	0.1476	0.1226		0.1351	0.0177	2.25E-05	-6.5E-05	0.99998
7/23/2008	0	0.0018			0.0000				
7/23/2008	50	0.0009			0.0009				
7/23/2008	100	0.0020			0.0020				
7/23/2008	500	0.0108			0.0108				
7/23/2008	1000	0.0223			0.0223				
7/23/2008	6000	0.1330			0.1330		2.22E-05	-0.00013	1.00000
7/23/2008	0	0.0024	0.0016		0.0000	0.0006			
7/23/2008	50	0.0001	0.0011		0.0006	0.0007			
7/23/2008	100	0.0012	0.0022		0.0017	0.0007			
7/23/2008	500	0.0095	0.0106		0.0101	0.0008			
7/23/2008	1000	0.0204	0.0205		0.0205	0.0001			

7/23/2008	6000	0.1265	0.1186		0.1226	0.0056	2.05E-05	-0.0002	0.99999
8/1/2008	0	0.0018	0.0016		0.0000	0.0001			
8/1/2008	50	0.0013	0.0014		0.0014	0.0001			
8/1/2008	100	0.0022	0.0028		0.0025	0.0004			
8/1/2008	500	0.0129	0.0108		0.0119	0.0015			
8/1/2008	1000	0.0227	0.0233		0.0230	0.0004			
8/1/2008	6000	0.1484	0.1231		0.1358	0.0179	2.26E-05	0.000278	0.99999
8/7/2008	0	0.0015	0.0015	0.0017	0.0000	0.0001			
8/7/2008	50	0.0011	0.0010	0.0009	0.0010	0.0001			
8/7/2008	100	0.0024	0.0022	0.0021	0.0022	0.0002			
8/7/2008	500	0.0112	0.0107	0.0101	0.0107	0.0006			
8/7/2008	1000	0.0229	0.0210	0.0208	0.0216	0.0012			
8/7/2008	6000	0.1333	0.1270	0.1247	0.1283	0.0045	2.14E-05	3.16E-05	1.00000
8/20/2008	0	0.0015			0.0000				
8/20/2008	50	0.0012			0.0012				
8/20/2008	100	0.0024			0.0024				
8/20/2008	500	0.0114			0.0114				
8/20/2008	1000	0.0224			0.0224				
8/20/2008	6000	0.133			0.1330		2.21E-05	0.000167	1.00000

Appendix C

Quality Assurance Audit Report on the Spring 2008 Walleye Project

Audit Date: September 2008
Report Date: October 13, 2008

Auditor: Dianne Brooke

1. Description and Scope of Audit

As part of a contaminant environmental monitoring study that was begun due to increased concerns about health risks and the consumption of fish, LSRI biologists and chemists are analyzing fish samples for contaminant levels. This audit report contains a review of the data recording, entry, and reduction; and QA/QC training exercises for the Spring 2008 Walleye Project (date of contract = May 1 - October 31, 2008. The primary staff members involved with the project are: Ms. Christine Polkinghorne (chemist), Ms. Heidi Saillard (chemist), and Mr. Tom Markee (chemist). Two LSRI students have assisted with the grinding, cleaning, and weighing processes this past year. This audit outlines a review of the study notebook *Vol. 06-07-10-CNP (GLIFWC),* notebook containing the project SOPs, three-ring notebook containing bench sheets for *GLIFWC Spring Walleye 2008,* and the three-ring notebook containing bench sheets for *St. Croix 2008.* The findings are listed under the subheadings.

2. Major Findings

Spring 2008 Walleye Project [Project Notebook *Vol. 06-07-10-CNP (GLIFWC)*]

On September 29, 2008, Dianne Brooke (LSRI QA Manager) reviewed the contents of the project notebook. The following observations were made and discussed with the project staff.

- The project notebook *Vol. 06-07-10-CNP (GLIFWC)* was well organized and mostly complete. The Table of Contents had been filled out, researchers names and initials were recorded on the front inside cover (with the exception of one of the student technicians working on the project), project/subcontract labels with the project ID number and year were affixed to each page of the notebook, and copies of the subcontracts outlining the project tasks had been Xeroxed and pasted into the notebook. Copies of the transfer chain-of-custody forms had also been pasted into the notebook. The notebook contained information for multiple sampling years; however, page 29 of the notebook needed a tab to indicate the start of the 2008 project.
- Information for the calibration of the balance using Class I weights was missing in the log book, in addition to the balance identification number. A note referring to the three-ring binder calibration book would suffice.
- The name/title of the SOP used for processing the fish was missing in the diary entries.
- A diary entry for 6/23/08 on page 33 needs a label for the final column of numbers.
- A diary entry for 6/27/08 was lined-through, but not dated or initialed.

- The COC form on page 37 was missing information for the date and time the samples were transferred. Recording information on this form is not a responsibility of LSRI; however, it needs to be brought to the attention of the sponsor.

Active SOPs Three-Ring Binder Notebook

- Resumes were on file for the project student technicians. The students had formal project SOP training, as summarized in the following spreadsheet, however, the certificates of completion and compliance had not been signed:

SOP Category	SOP Title:	SOP Number:	Version Number and/or Revision Date:	Training Year	Student Name
Recordkeeping	*Preparing Laboratory Notebooks*	REC/9	August 23, 2005	2008	Koirala, Damodar Atwater, Emily
Recordkeeping	*Xeroxing Contents of Study Notebooks*	REC/12	May 31, 2006	2008	Koirala, Damodar
General Lab Maintenance	*Procedures for Calibrating Laboratory Balances*	GLM/12	August 31, 2005	2008	Koirala, Damodar Atwater, Emily
General Lab Maintenance	*Procedures for Taring and Weighing Samples Using Laboratory Balances*	GLM/16	June 7, 2006	2008	Koirala, Damodar Atwater, Emily
Neurobehavioral Toxicology	*Procedures for Determining Percent Moisture in Tissue Samples*	NT/15	August 28, 2007	2008	Koirala, Damodar
Sample Analysis	*Routine Labware Cleaning for Metals Analysis*	SA/8	August 28, 2007	2008	Koirala, Damodar Atwater, Emily
Sample Analysis	*Sample Grinding for Metals Analysis*	SA/10	October 25, 2005	2008	Koirala, Damodar
Sample Analysis	*Sample Weighing for Metals Analysis*	SA/11	October 19, 2005	2008	Koirala, Damodar
Sample Analysis	*Cold Vapor Mercury Determination in Biota*	SA/13	August 27, 2007	2008	Atwater, Emily
Sample Analysis	*New Labware Cleaning for Metals Analysis*	SA/14	June 1, 2000	2008	Atwater, Emily
Sample Analysis	*Procedures for Measuring Organic Compounds Using High Performance Liquid Chromatography HPLC*	SA/15	December 3, 2007	2008	Koirala, Damodar
Sample Analysis	*Ammonia Analysis by Ion Specific Electrode*	SA/25	May 31, 2006	2008	Koirala, Damodar
Sample Analysis	*Preparation of Tissues for Analytical Determinations Using Liquid Nitrogen*	SA/38	December 22, 1999	2008	Atwater, Emily
Sample Analysis	*Processing Several Fish into Three Homogenous Composites of Different Tissue Type*	SA/45	June 13, 2005	2008	Atwater, Emily
Sample Analysis	*Processing Several Fish into One Homogenous Fish Composite*	SA/46	June 13, 2005	2008	Koirala, Damodar
Sample Analysis	*Acid Digestion of Biota Samples*	SA/48	April 25, 2006	2008	Koirala, Damodar Atwater, Emily

- The staff members and students had received training in Quality Systems and Good Laboratory Practices. One student had completed the training on March 3, 2008 and the other student had completed the training on June 16, 2008. Workshop competency

quizzes were given to both students and each one scored 100% on the questions. Training certificates were on file for the staff members and students.

- The SOP notebook located in Barstow Room 7 contained older versions of the project SOPs. The LSRI QA Manager removed the following SOPs and listed them in the LSRI Inactive Standard Operating Procedure Spreadsheet: SA/13, SA/21, SA/33, SA/39, and SA/41. Copies of the Active and Inactive Standard Operating Procedure Spreadsheets were updated and placed in the SOP notebook. The SOP Training Record and GLP Training Workshop Spreadsheets were also updated and copies were placed in the SOP notebook. Newer versions of SA/8, NT/15, and SA/49 were added to the SOP notebook. Project staff should notify the LSRI QA Manager when the SOPs need to be updated, and the LSRI QA Manager will make the changes. In the absence of the LSRI QA Manager, project staff should refer to the *LSRI Active SOP* spreadsheet to make sure that the latest SOP versions are being used.

Spring 2008 Walleye Project - Bench Sheets for Analysis of Samples Completed in July/August 2008

Reviewed the three-ring binder entitled *GLIFWC Spring Walleye 2008.*

- The study ID number appeared on all output sheets, with the exception of pages 1 and 2 for the 7/9/2008 analyses. Dates of analysis labels were affixed to the bench sheets to separate the data in the notebook. The data bench sheets also contained good descriptors of where the data is stored electronically (i.e., computer drive designation). It would be helpful to add this information to the project notebook *Vol. 06-07-10-CNP (GLIFWC)* and describe the process for how the data is backed-up on the drives and who has assess to the data entry. A summary of the data reduction process and/or referral to the appropriate SOP should also be written into the project notebook *Vol. 06-07-10-CNP (GLIFWC).*
- The data in the binder appeared to be thoroughly proofed, both for entry errors and calculation errors. The person checking the data initialed the rechecks and recorded the date when the data were proofed.
- Some of the bench sheets containing the spreadsheet table listing the QC criteria (i.e., detection limits and acceptance ranges) were for 2007, rather than 2008 and had the LOD and LOQ values reversed
- In analyzing the samples for tissue moisture content, approximately 35% (80/230 samples) were chosen for this parameter. The contract stated that up to 92 fillets would be tested for percent moisture. Of the 80 samples, 15.0% (n = 12 samples) were analyzed in duplicate and checked for relative percent agreement. The percent duplicate agreement for tissue moisture ranged from 98.8 -100%. Of the 80 samples, 11.2% (n = 9 samples) were placed back into the oven and reweighed after an additional 20 - 24 hours to ensure dryness. The QA/QC drying exercise yielded values that were above 99.0% duplicate agreement.
- Typically an analysis set consists of 40 samples being analyzed for mercury content. For each data set, the following QA/QC samples were analyzed: two dorm samples in duplicate, four duplicate agreement samples, and four spike recovery samples in

duplicate. A calibration blank and six standards were also analyzed with the data set. One set of standards was run at the beginning of the analyses and the other set interspersed throughout the analysis. The St. Croix analyses consisted of 28 samples and this analysis set was considered separate when determining the number of QA/QC samples needed (10% minimum). The QA/QC samples were as follows: analysis date (7-23-08, run 1) contained a value for only one dorm sample; the St. Croix analyses contained three duplicate agreement samples; analysis date (8-1-08) contained two duplicate agreement samples; analysis date (8-07-08) contained ten duplicate agreement samples and analysis date (8-20-08) contained two duplicate agreement samples. The spike recovery samples in duplicate were: the St. Croix analyses contained three sets of spike recovery samples; analysis date (8-1-08) contained two sets of spike recovery samples; and analysis date (8-07-08) contained five sets of spike recovery samples. All samples that failed QA/QC were reanalyzed.

- The lowest values recorded for the QA/QC analytical parameters were: percent recovery for the dorm samples – 80.6 %; relative percent agreement between duplicates – 62.5%; mean percent spike recovery – 41.2%. The minimum acceptance values established for the QA/QC analytical parameters for 2008 were: percent recovery for the dorm samples – 75.0%; relative percent agreement between duplicates – 85.3%; and mean percent spike recovery – 60.8%.
- The highest values recorded for the QA/QC analytical parameters were: percent recovery for the dorm samples – 114.6 %; relative percent agreement between duplicates – 99.7%; mean percent spike recovery – 106.6 %. The maximum acceptance values established for the QA/QC analytical parameters for 2008 were: percent recovery for the dorm samples – 113%; relative percent agreement between duplicates – 100%; and mean percent spike recovery – 115%.

Reviewed the three-ring binder entitled *St. Croix 2008.*

- The SOP SA/45 placed in the beginning of the notebook was an old version. This was updated by the QA/QC Manager. For the analysis date of 6-13-08, some of the computer-generated sample analysis sheets contained cross-outs of the data without a date, initials of the researcher, and an explanation. A brief overview of the remaining data sheets and COC forms revealed no major problems in data recording.

3. Recommendations

The overall reviews of the methodology and data recording indicate that study personnel are highly organized and intentional in their QA/QC protocols for conducting research. The time/date when the weighing pans are initially placed into the oven (and removed from the oven) should be recorded in the notebook.

PROCEDURES FOR DETERMINING PERCENT MOISTURE IN TISSUE SAMPLES

INTRODUCTION

This SOP includes general guidelines for the analysis of tissue samples for moisture content. It is a gravimetric technique requiring careful weighing techniques.

EQUIPMENT LIST

- Balance (i.e., Mettler AG245, PB303, AB204, H34, H72 and H80)
- Aluminum Weighing Pans
- Drying Oven (60° C)
- Desiccation Container
- Spatula

PROCEDURE

1. Calibrate balance using Class 1 weights. Label the aluminum weighing pans and dry at 60° C for 16 hours.
2. Place dried weighing pans in desiccator until cool.
3. Weigh the dried and cooled weighing pans on balance to the 0.001 g.
4. Weigh approximately 1.0 g of thawed tissue and place in the labeled weighing pan.
5. Weigh the pan and the tissue on balance to the nearest 0.001 g.
6. Dry pan and tissue in drying oven at 60° C for 16 hours or until constant dry weight is achieved.
7. Remove dried pans and tissue from the oven and place in desiccator until cool.
8. Weigh the pan with the tissue on balance to the nearest 0.001 g.
9. It may be necessary to dry the pan and tissue a second time when the tissue is a large mass. Desiccate and re-weigh to prove that an equilibrium dry weight has been achieved.
10. Calculations:

    Aluminum pan with wet tissue- Dry Aluminum Pan = Wet weight of tissue

    (Aluminum pan and wet tissue weight - Aluminum pan and dry tissue/Wet tissue weight) X 100 = Percent moisture of tissue

ROUTINE LABWARE CLEANING FOR METALS ANALYSIS

INTRODUCTION

This cleaning procedure is used for the routine cleaning of labware and equipment used for metals analysis. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Deionized Water
- Dish Pan
- Gloves
- Goggles
- Lab Coat
- Labware to be Washed
- Liquinox Detergent
- pH Indicator Strips
- Various Labware Washing Brushes
- Wash Bottle
- Plastic Dish Rack
- Grinder
- Plastic Tank with Cover
- Stainless Steel Bowls
- Ammonium Hydroxide, 30% (VWR Reagent)
- Fillet Knife
- Nitric Acid, Concentrated (Fisher Reagent)
- Spatula (Stainless Steel)
- Hydrochloric Acid, Concentrated (Fisher Reagent)
- Nalgene 2½ Gallon Carboy
- Sodium Bicarbonate
- Stainless Steel Bowls

PROCEDURE: CLEANING EQUIPMENT USED FOR FISH GRINDING [Grinder, Stainless Steel Bowls, Fillet Knife, Spatula]

1. Dismantle the meat grinder before washing.
2. Scrub equipment in hot water containing Liquinox detergent.
3. Rinse equipment with tap water until there is no presence of soap.
4. Rinse equipment once with deionized water.
5. Soak equipment in 0.1 M HCl for 30 seconds (be sure the equipment is completely immersed).
6. Rinse equipment three times with deionized water.
7. Upon drying, cover equipment with aluminum foil to store until used.

PROCEDURE: LABWARE CLEANING [Scintillation Vials]

1. Scrub the labware thoroughly in hot water containing Liquinox detergent.
2. Rinse the labware with hot water until there is no presence of soap.
3. Rinse the labware once with deionized water.
4. Place the labware in the plastic tank containing 10% nitric acid. Be sure the labware is completely filled with acid. Allow the labware to soak for a minimum of 60 minutes.

5. Remove the labware from the tank, emptying the acid back into the tank.
6. Rinse the labware three times with deionized water.
7. Place the clean labware in a plastic rack to air dry. When the labware is dry, cover the labware with a lid, stopper, or aluminum foil. Place the labware in a proper storage location until used.

PROCEDURE: PLASTIC TANK CONTAINING 10% (V/V) NITRIC ACID

1. Fill the tank with 14.4 liters of deionized water. Then add 1.6 liters of concentrated nitric acid and stir. The tank is now ready to be used to soak labware.
2. Every few months change the acid in the tank. Neutralize the acid with ammonium hydroxide until a pH of between 5 and 9 is achieved. Measure the pH in the tank with pH indicator strips.
3. Pour the neutralized acid down the drain with running cold water. Run the cold water for an additional 10 minutes.
4. Rinse the tank with warm tap water and then with deionized water. Fill the tank with 10% nitric acid as in step 1.

PROCEDURE: 0.1 M HYDROCHLORIC ACID

1. Fill a 2½ gallon carboy to the 10-L mark with the deionized water. Add 83 mL concentrated hydrochloric acid. Cover the solution and mix. The 0.1 M hydrochloric acid is now ready to be used to soak the labware.
2. Remake the 0.1 M hydrochloric solution once a week. Neutralize the acid with ammonium hydroxide or sodium bicarbonate until a pH of between 5 and 9 is achieved. Measure the pH in the tank with pH indicator strips.
3. Pour the neutralized acid down the drain with running cold water.

SAMPLE GRINDING FOR METALS ANALYSIS

INTRODUCTION

This procedure is for the grinding of biological tissues into homogeneous samples. The grinder and labware used to grind the tissue is cleaned by the "Routine Labware Cleaning for Metals Analysis (SA/8)" procedure. The proper safety equipment must be worn during the entire grinding procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Tissue Samples
- Fillet Knife
- Gloves
- Goggles
- Lab Coat
- Grinder
- Spatula
- Scintillation Vials or Jars
- Aluminum Foil
- Procedural Blank (i.e., Tuna Fish)
- Beaker or Stainless Steel Bowls
- Food Processor with Grinding Attachments

PROCEDURE: GRINDING TISSUE SAMPLES

1. Cut the tissue sample into small pieces that will fit through the grinder feed tube or food processor with grinding attachments.

2. Pass the tissue through the grinder or food processor, discarding the first few grams of tissue that come through. Collect the tissue in a beaker or bowl.

3. Mix the tissue with a spatula.

4. Pass the collected tissue through the grinder or food processor a second and third time and collect in the same beaker or bowl.

5. Mix the tissue with a spatula to insure homogeneity.

6. Place the tissue in a scintillation vial or jar previously washed (use procedure as described in SA/8). Seal securely with the screw top lid. Label the vial with the appropriate information and place in a freezer until analyzed.

7. Wash the grinder (or food processor) and labware by the "Routine Labware Cleaning for Metals Analysis (SA/8)" procedure before grinding the next sample.

8. Continue to grind each sample by repeating steps 1 - 7.

PROCEDURE: PREPARING THE PROCEDURAL BLANK

1. Prepare a procedural blank. When using the tuna, drain the liquid from the can. Grind half the procedural blank tissue as a procedural blank by use of steps 2-7. Label the procedural blank as "ground" and include with the analysis set.

2. The other half of the procedural blank is left unground and handled like a sample by use of steps 5 + 6. Label the procedural blank as "unground" and include with the analysis set.

SAMPLE WEIGHING FOR METALS ANALYSIS

INTRODUCTION

This procedure is for the weighing of biological tissue for metals analysis. The tissue should be ground according to the "Sample Grinding for Metals Analysis SA/10" or "Preparation of Tissues for Analytical Determinations Using Liquid Nitrogen SA/38" procedures. The labware used in this procedure should be cleaned using the "Routine Labware Cleaning for Metals Analysis (SA/8)" procedure. The proper safety equipment must be worn during this entire procedure. This includes gloves, safety glasses or goggles, and lab coat.

EQUIPMENT LIST

- Ground Samples
- Gloves
- Goggles or Safety Glasses
- Lab Coat
- Kimwipes
- Spatula
- Deionized Water
- Nitric Acid (10%)
- Balance Capable of Reading to Nearest 0.001 g
- Polypropylene Digestion Vessels (Environmental Express)

PROCEDURE

1. Remove the sample to be analyzed from the freezer and allow to thaw.

2. Check the level of the balance and adjust if necessary. Clean the top of the balance of any foreign materials with a soft brush.

3. Zero the balance with the zero adjustment to read 0.000 g. Check balance calibration, if not previously done today, following "Procedures for Calibrating Laboratory Balances (GLM/12)".

4. Place a clean sample container on the balance and tare the balance.

5. With a spatula, stir the sample to insure homogeneity. Weigh the appropriate quantity (approximately 0.2 - 0.3 g for mercury analyses and 1.0 g for other metals analyses) of tissue into the sample container.

6. Record the weight of the sample.

7. Rinse the spatula with water, 10% nitric acid and deionized water. Wipe the spatula clean with a Kimwipe.

8. Label and record each sample container and sample. Be sure that none of the tissue adheres to the side of the sample container.

PROCEDURES FOR DETERMINING DETECTION LIMITS

INTRODUCTION

Detection limits should be calculated by the following procedure for analytical methods utilizing a calibration curve. Examples of instruments that would provide data used to generate calibration curves are: gas chromatograph, organic carbon analyzer, high pressure liquid chromatograph, atomic absorption instrument, and the specific ion electrodes.

EQUIPMENT

- Standard or sample estimated to be within 5 times of the detection limit
- Calculator capable of doing standard deviations
- Student t chart

PROCEDURE

1. Select a low level standard that is estimated to be within 1-5 times the detection limit.
2. Analyze the standard a minimum of 7 times in the same manner as the samples.
3. Determine a mean and standard deviation, $SD_{(n-1)}$, for the response of the 7 replicates.
4. Calculate the instrument detection limit by multiplying the standard deviation by the student t value for the number of replicates (n-1):

$$DL = SD \times t_{(n-1)}$$

Student's t:

# Observations	$t_{(n-1)}$
7	3.143
8	2.998
9	2.896
10	2.821
11	2.764

5. Calculate the detection limit concentration using the calibration curve.
6. Compare the detection limit to the mean concentration. If the mean concentration is greater than 5-10X the calculated detection limit, repeat steps 1-7 using a lower concentration for the replicates.
7. Compare the calculated response of the detection limit concentration. During some procedures the calculated response at the detection limit will be a fictional number below the instrument's sensitivity. This may indicate that the calibration curve is not representative at that level. These procedures should be evaluated on a case-by-case basis with the project director.

PROCEDURES FOR CALCULATING MERCURY CONCENTRATIONS USING COLD VAPOR MERCURY ANALYSIS

INTRODUCTION

The following equations are used in calculating mercury concentrations.

PROCEDURE

Concentration of Mercury Stock Solution:

$$\frac{\text{mass } HgCl_2 \text{ (g)}}{271.50 \text{ g/mol } HgCl_2} \times \frac{200.59 \text{ g/mol Hg}}{100 \text{ mL}} \times \frac{\text{purity (\%)}}{100\%} \times \frac{10^6 \text{ µg}}{\text{g}} = \text{conc. Hg(µg/mL)}$$

Concentration of Mercury Sub-Stocks:

$$C_1V_1 = C_2V_2$$

where C_1 = concentration of mercury stock solution
C_2 = concentration of diluted solution
V_1 = volume of stock solution used
V_2 = volume of diluted solution

Amount of Hg in Each Standard:

ng of Hg = concentration of Hg sub-stock (ng/mL) x mL of sub-stock used

Calibration Curve:

ng of Hg (x) vs. maximum response (y)
Results in a linear regression with an intercept and slope. Using the equation for the regression:

$y = mx + b$ where m = slope and b = intercept

and inserting the response for any given sample, the concentration of Hg or y can be determined.

Calculation of µg Hg/g Tissue:

Divide the µg Hg calculated using the calibration curve by the mass of tissue analyzed.

FIMS MERCURY ANALYSIS - STOCK, STANDARD AND SPIKE PREPARATION

INTRODUCTION

This procedure is used for the preparation of the stock, analytical standards, blanks and spikes for analysis using the Perkin Elmer FIMS-100 Mercury Analyzer. The fish/tissue used for the spikes should be weighed by the use of the "Sample Weighing for Metals Analysis (SA/11)" procedure. The labware used in this procedure should be cleaned by the "Routine Labware Cleaning for Metals Analysis" (SA/8) procedure.

EQUIPMENT LIST

- Ground Tissue Samples for Spikes
- Class A Pipettes (1 mL and 3 mL)
- Deionized Water
- Pipette Bulb
- 1000 mg/L Mercuric Nitrate Stock/Reference Solution
- Concentrated Hydrochloric Acid (Trace Metal Grade)
- 5% (w/v) Potassium Permanganate ($KMnO_4$)
- Micropipettes and Tips
- Teflon Beakers for Making Substocks
- Mercury Waste Container
- 2 Volumetric Flasks (100 mL)
- Polypropylene Digestion Cups (Environmental Express)

PROCEDURE

1. Pipet 1 mL of a 1000 mg/L mercuric nitrate stock solution into a 100 mL volumetric flask containing ~60 mL of deionized water, 1 mL trace metal grade concentrated HCl, and 100 µL 5% $KMnO_4$. Dilute to 100 mL with deionized water to prepare a 10 mg/L Hg substock. Label this solution with the concentration, date and initials as it must be remade once a month.

2. Pipet 1 mL of the 10 mg/L Hg substock solution into a 100 mL volumetric flask containing ~60 mL of deionized water, 0.5 mL trace metal grade concentrated HCl, and 100 µL 5% $KMnO_4$. Dilute to 100 mL with deionzed water to prepare a 100 µg/L Hg substock. Label this solution with the concentration, date and initials as it must be remade once a week.

3. Pipet the following volumes of deionized water and 100 µg/L Hg substock into digestion cups labeled with the appropriate concentrations which are based on the final volume (50 mL) of standard at time of analysis. Use a micropipette to deliver all water volumes and stock Hg volumes less than 1 mL. Use a class A pipet to deliver 3 mL 100 µg Hg/L substock.

Concentration (ng/L)	Amount of 100 µg/L substock	Amount of DI water
Blank	0	3 mL
50	25 µL	2975 µL
100	50 µL	2950 µL
500	250 µL	2750 µL
1000	500 µL	2500 µL
6000	3 mL	0 mL

4. Each blank and standard should be prepared in duplicate.

5. A total of 10% of samples analyzed for mercury should be spiked in duplicate. Spiking is accomplished by pipetting a known volume of the 100 µg/L Hg substock into a digestion cup containing a known weight of fish tissue. A micropipette may be used to deliver two 750 µL aliquots onto pre-weighed tissue to give a total spiking volume of 1.5 mL.

6. All mercury waste from rinsing pipettes, beakers, etc. should be disposed of in mercury waste container. Volume and concentration placed in waste container should be recorded on the hazardous waste container inventory form for that bottle.

COLD VAPOR MERCURY DETERMINATION IN BIOTA USING THE FMS-100

INTRODUCTION

This procedure is used for the determination of total mercury in fish, hair and other tissue samples. Do not use this procedure for analyzing human blood.

REFERENCES

"Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry", Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, April 1991.

EQUIPMENT AND REAGENT LIST

- Stannous Chloride, Analytical Reagent
- Potassium Persulfate, Reagent Suitable for Mercury Determination
- Hydroxylamine Hydrochloride, Reagent Suitable for Mercury Determination
- Potassium Permanganate, Certified A.C. S.
- Sodium Chloride, Certified A.C. S.
- Sulfuric Acid, A.C.S. Reagent, Suitable for Mercury Determination
- Hydrochloric Acid, Trace Metals Grade
- Nitric Acid, Fisher, Trace Metals Grade
- Hot Block (Environmental Express)
- FIMS- 100 (Perkin Elmer) Mercury Analyzer
- Lab industries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Polypropylene Digestion Cups and Covers (Environmental Express)
- Pipets/Pipettors
- Beakers
- Spatulas
- Kimwipes
- 5% (w/v) Potassium Permanganate
- 5% (w/v) Potassium Persulfate
- 3% Hydrochloric Acid
- 10% (w/v) Hydroxylamine Hydrochloride- 1 O%(w/v) Sodium Chloride
- 5% Stannous Chloride in 3% Hydrochloric Acid
- 1000 ug/mL Mercuric Nitrate Stock
- 10 mg/L Mercuric Nitrate Substock for FIMS-100 Analysis
- 100 ug/L Mercuric Nitrate Substock for FIMS-100 Analysis
- Silicon Defoaming Agent (Perkin Elmer)
- Deionized Water

PROCEDURE

Digestion

1. **The addition of acids and digestion of samples must be conducted in a fume hood.** Add 4.0 mL of concentrated sulfuric acid and 1.0 mL of concentrated nitric acid to each sample, standard, spike, duplicate and blank.
2. Place the digestion cups in Hot Block at a setting of 110°C. Place the Plexiglas fume cover over the Hot Block with tubing connected to all Erlenmeyer flasks and vacuum pump. Turn on vacuum pump. Allow samples to digest for approximately 15 minutes or until all the fish tissue is dissolved.
3. Disconnect and remove Plexiglas fume cover. **Be careful in handling the fume cover as it will contain acid vapors!** Turn off the Hot Block. Remove the digestion cups from the Hot Block and allow to cool to room temperature in the fume hood.
4. Add 5.0 mL of 5% potassium permanganate to each digestion cup, swirling the digestion cups after each addition.
5. Add an additional 10.0 mL of 5% potassium permanganate to each digestion cup in 5.0 mL increments, swirling the digestion cup after each addition. Additional 5% potassium permanganate solution (maximum of 5 mL) or solid potassium permanganate should be added to the samples if necessary so that the samples remain purple in color for at least 15 minutes. If extra potassium permanganate is added to a sample, an equal amount should be added to one set of standards and a blank.
6. Add 8 mL of 5% potassium persulfate to each digestion cup, and cover and swirl.
7. Allow the digestion cups to set overnight to oxidize organic mercury compounds to inorganic mercury ions.
8. The samples will remain stable for several days before analysis.

Sample Analysis

1. Prepare the following:
   Carrier Solution (3% HCl)
   Reductant Solution (5% SnC12, 1% Silicon Defoaming Agent, in 3% HCl)
   Weigh 50g $SnCl_2$ and add to 990 mL 3% HCl.
   Add 10 mL Silicon Defoaming Agent using 5 mL micropipettor.
   Note: The Silicon Defoaming Agent is optional, needed only if the samples appear to be producing foam during analysis.
2. Turn on computer and printer.
3. Turn on Nitrogen (400 kPa or 60 psi).
4. Turn on FIMS 100 mercury analyzer and allow to warm-up for 10 minutes minimum.
5. Press Ctrl+Alt+Del (on computer).
6. Username: administrator.
7. Leave password field blank. Click on "OK
8. Open appropriate project Excel file prepared from Hg Calculations-Master and minimize the Excel window.
9. Double click on **AA** Winlab Analyst icon.
10. Choose "Use a custom designed workspace".

11. Choose "Hg.fms" > "file" > "open" > "method"> "Hg Analysis".
12. Click on "Browse" in Results Data Set window, choose drive D: AAUSER SW2007 and enter a new data set name (DateProject). Be sure that the save data box is checked.
13. Turn pump magazine pressure adjustment levers so that they fit into the notch on the back of the pump magazine.
14. Check gas/liquid separator cover to see that it has been tightened.
15. Attach tubing from gad/liquid separator to the FIMS-cell.
16. Click on Manual button (on top toolbar).
17. Click on FIAS button (on top toolbar). Run FIAS once using clean deionized water (Click on the "FIAS on/off" button). Place collection tubes into appropriate solution bottles (Red = Reductant solution, Yellow = Carrier Solution) and run FIAS two more times checking the flow of the instrument and the lines for bubbles while it is running. Remember while running a sample set to periodically check carrier and reductant volumes, so they do not deplete.
18. Just prior to analysis of all blanks, standards and samples (steps 19-22), add 10 mL of 10% (w/v) Hydroxylamine Hydrochloride - 10% (w/v) Sodium Chloride in two 5 mL aliquots, mix sample until no purple or brown color remains. Dilute to 50 mL with deionized water using the correct line on the digestion cup and mix thoroughly.
19. Rinse the sample aspiration tube with deionized water and place in the blank solution. Click on "analyze blank" and allow instrument time to complete triplicate analysis.
20. Rinse the sample aspiration tube with deionized water and place in the lowest standard. Choose appropriate standard concentration and click on "analyze standard" and allow instrument time to complete triplicate analysis. In the appropriate Excel file for the project, enter 0.000 for the blank absorbance and enter the mean Blank Corrected Signal value for the standard. Repeat this step for each of the five standards to be run in order of lowest to highest to develop the standard curve.
21. Prior to analyzing samples check the following parameters:
    a. The slope of the line should fall between 2.0×10^{-5} - 3.0×10^{-5}
    b. Review peak shape
    c. The 6000 ng/L standard should give a response between 0.12 and 0.18.
    d. If these checks do not fall in the acceptable range, check carrier and reductant flows and/or perform other maintenance as needed.
22. Rinse the collection tube with deionized water and place in appropriate sample. Enter sample ID code into the appropriate field. Rinse the sample aspiration tube with deionized water and place in appropriate sample. Click on "analyze sample" and allow the instrument time to complete triplicate analysis. Enter the mean Blank Corrected Signal value into the appropriate Excel file for that project. Repeat this step for each of the samples to be analyzed.
23. The second Blank, second set of standards, and Dorm-2 samples should be run as they were above, sometime in between samples, to check the precision of the instrument. For example, if the sample set contains 52 samples, including duplicates and spikes, run the first set of standards (~13 samples), the Blank and the lowest

standard (50 ng/L), Dorm 2-1 (1) and (2) (~1 3 samples), the next two standards (100 ng/L and 500 ng/L), Dorm 2-2 (1) (~13 samples), the last two standards (1000 ng/L and 6000 ng/L) and finally Dorm 2-2 (2). It is best to try to analyze the duplicates and spikes without interruption, so more or less than 13 samples may be analyzed between standards in order to keep the samples together and in order.

WHEN ANALYSIS OF ALL SAMPLES AND STANDARDS IS COMPLETE:

24. Place sample aspiration tube, and lines from reductant and carrier solutions into beaker of deionized water.
25. Flush/clean tubing with deionized water by running FIAS two times.
26. Lift collection tubing out of deionized water and run FIAS one more time to allow air to pass through all tubing. When FIAS is finished running, place collection tubing back into beaker of DI water for storage.
27. Raise waste lines out of liquid in waste container so liquid does not back up.
28. Release the pump magazine pressure adjustment levers so that tubing is not compressed.
29. Detach line from FIMS-cell.
30. Unscrew the gas/liquid separator cover and, using forceps to handle filter, dry filter with a Kimwipe.
31. Print report. Choose "file" > "utilities" > "reporter". "Open Design" Choose "WR01 Mussel" (double-click), then double-click on the number 1 under result name and choose the data set for that day. Click "OK" > "Print Report" and close the reporter window.
32. Save Excel file to floppy disk.
33. Turn off FIMS instrument, computer, nitrogen gas, and printer.
34. Record the date, project, analyst, number of injections, and time run in FIMS-100 usage record book located in the drawer below the instrument.

Appendix 4

Quality Assurance Report: 2008 Field Data Collection

Quality Assurance Report: 2008 Field data collection

By:

Matt Hudson
Environmental Biologist
Great Lakes Indian Fish and Wildlife Commission

Introduction

The following report satisfies quality assurance reporting requirements outlined in section 14.1 of the Quality Assurance Project Plan entitled "Great Lakes Indian Fish and Wildlife Commission (GLIFWC) Testing of Fish for Mercury Using EPA Supplemental Funds - EPA Grant # 96540801-0". Although the grant referenced in this QAPP has expired, the QAPP is still used as the guidance document for implementing the field and lab portions of GLIFWC's spring mercury project.

Quality Assurance Summary

1. System and Performance Audits - Results from an audit of field walleye collections are described in Appendix 4A. Only field walleye collections were audited in 2008. Laboratory processing of fish into fillets was not audited in 2008. The same individual who has processed walleye fillets for the past four seasons was hired again in 2008. This individual has been reliable at following QAPP procedures in the past and based on review of the completed data sheets and processed fillets, there was no indication the individual did not follow protocols in 2008.

In general, protocols for data collection and sample handling were followed well by staff observed during the field audit. The staff member collecting the walleye samples had not done so before, so the collection was as much a training session as it was an audit. The main improvement to be made would be to make sure that the individual carries the mercury packet with tags and COC forms in their work vehicle at all times and makes sure to double check their list of field collection lakes each night.

2. Completeness and Quality of Field Sampling Process and Data - BIA carry-over funds and funds from EPA grant #GL00E06501 were used to collect and analyze 230 walleye for mercury from 27 lakes in 2008. In addition, 28 of the 230 walleye were collected by the St. Croix Natural Resources Department, but were analyzed using the funding sources listed above. GLIFWC plans called for twelve walleye to be collected, with three fish taken from each of four size ranges (12.0 to 14.9, 15.0 to 17.9, 18.0 to 22.0, and greater than 22.0 inches). St. Croix collected nine walleye between 17.0 and 20.0 inches. Four of these walleye were selected per lake to be tested for mercury by GLIFWC. Because the number of walleye requested are not always able to be collected, additional lakes were selected to reach a goal of 247 fish. A total of 29 lakes were selected for sampling and a total of 230 walleye samples from 27 lakes were collected (Table 1). The total samples collected were 93% of the goal.

Observed collection of field samples and tissue processing and data collection was adequately followed according to QAPP guidelines. Therefore, no problems are seen with the quality of field data for this project.

3. Deviations - No deviations were reported during the 2008 walleye mercury project.

4. Significant Quality Assurance Problems and Recommended Solutions - No significant quality assurance problems were noted during the 2008 field sample and data collection process.

Table 1. Summary of completeness of mercury walleye collections during spring 2008. Samples collected by St. Croix Natural Resources Department are noted with *. All St. Croix walleye were between 17 and 20 inches. The "% Requested" field refers to the number of walleye that could be collected per lake (12 for GLIFWC lakes and 4 for St. Croix lakes). This is not the same as the overall collection goal (i.e. 247).

State	County	Lake Name	12.0 to 14.9	15.0 to 17.9	18.0 to 22.0	> 22.0	Total Collected	% of Requested
WI	BAYFIELD	L OWEN	3	3	3	3	12	100%
WI	SAWYER	ROUND L	3	3	3	3	12	100%
WI	WASHBURN	BIRCH L	3	3	3	2	11	92%
WI	SAWYER	TIGER CAT FL	1	2	3		6	50%
WI	LINCOLN	RICE R FL CHAIN	3	3	3	3	12	100%
WI	ONEIDA	TWO SISTERS	2	3	4	3	12	100%
WI	VILAS	SCATTERING RICE	0	0	0	0	0	0%
WI	VILAS	WILDCAT L	3	3	2	1	9	75%
MI	GOGEBIC	L GOGEBIC	0	0	0	0	0	0%
MI	ONTONAGON	BOND FALLS FL	0	0	0	0	0	0%
WI	ONEIDA	PELICAN L	3	3	3	3	12	100%
WI	VILAS	LAC VIEUX DESERT	3	3	3	3	12	100%
WI	OCONTO	ARCHIBALD L	3	3	3	2	11	92%
WI	LANGLADE	OTTER L	2	3	3	0	8	67%
WI	ONEIDA	FOUR MILE L	3	3	0	0	6	50%
WI	ONEIDA	LONG L	3	3	2	2	10	83%
WI	SAWYER	NELSON L	0	0	4	1	5	42%
WI	WASHBURN	SHELL L	3	3	3	0	9	75%
WI	ST CROIX	CEDAR L*					4	100%
WI	POLK	BIG ROUND L*					4	100%
WI	BARRON	BEAVER DAM L*					4	100%
WI	POLK	WAPOGASSET L*					4	100%
WI	BURNETT	BIG MCKENZIE*					4	100%
WI	BARRON	BEAR L*					4	100%
WI	POLK	BALSAM L*					4	100%
WI	ONEIDA	BEARSKIN L	3	3	3	3	12	100%
WI	VILAS	SQUAW L	3	5	2	1	11	92%
MN	MILLE LACS	MILLE LACS L	3	3	3	3	12	100%
WI	FLORENCE	PATTEN L	3	3	3	3	12	100%
WI	VILAS	BIRCH L	2	3	1	2	8	67%
		Total Collected	**52**	**58**	**54**	**38**	**230**	
		% of Requested	**75%**	**84%**	**78%**	**55%**	**76%**	
		% of Goal (goal 247)					**93%**	

Appendix 4A

Field audit of 2008 walleye collection

GLIFWC Procedure No. AD.001
Revision No. 1
Revision Date. 6/4/2004
Initial Date. 8/3/2001

Title: Field Audit of Biota Collection for Chemical Contaminant Samples

Introduction:
This procedure describes the auditing process for the collection of fish to be analyzed for chemical contaminants. The project manager or an appointed and properly trained GLIFWC staff member not involved in the fish collection will perform this audit.

Equipment:

Audit Form (see attachment)
Black indelible ink pen

Procedures:

1. All aspects of the biota sampling involving data collection, sample storage, sample processing, and transport should be audited.

2. At a minimum, audits will occur once during a sampling season less than or equal to 6 weeks in length. Two audits should occur for longer sampling seasons. Single audits should be conducted during the initial part or the sampling season, with second audits occurring after 6 weeks of sampling. If non-compliance to procedures is observed, further audits may be scheduled as deemed necessary by the project manager.

3. All types of field data collection should be observed such as the following possible parameters:
   a. Length
   b. Weight
   c. Sex
   d. Age

   Collection methods of this data will be according to the quality assurance protocol plan or work plan for which the data is being collected.

4. Tissue collection, packaging, storage, custody and transport procedures should be observed and documented for compliance to the quality assurance protocol plan or work plan for which the data is being collected.

5. The attached form should be completed and returned to the project manager for review and archiving.

Warden collection of walleye
Nelson Lake, Tim Stone, 5/7/08

GLIFWC Procedure No. AD.001
Revision No. 1
Revision Date. 6/4/2004
Initial Date. 8/3/2001

Field Audit Form

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
Age[b]			

[a]: + = in compliance, - = out of compliance
[b]: Age will be determined at lab and not in the field, only scales or spines will be collected.

General Comments: This was Jim Stone's 1st experience with mercury fish collection. He was not part of the training I gave the wardens (Mike S. picked up the packets) so I decided to work with him directly both to help him learn the process & because of time constraints.

Section 2: Tissue Collection

Data Type	(+/-)[a]	Comments	Date Observed
walleye	+	placed in plastic bag & in cooler	5/7/08

[a]: + = in compliance, - = out of compliance

General Comments:

Jim did not have the mercury packet with him with COC form and tags. He improvised by using evidence tags instead and I filled out fish data on a sheet of paper I had

Section 3: Sample Packaging

Data Type	(+/-)[a]	Comments	Date Observed
	+	See section 2	5/7/08

[a]: + = in compliance, - = out of compliance

General Comments:

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
	+	Ambient		5/7/08

[a]: + = in compliance, - = out of compliance
[b]: Temperature of storage container

General Comments:

Section 5: Custody (Chain-of-Custody Forms)

Data Type	(+/-)[a]	Comments	Date Observed
CoC Form	+		5/7/08

[a]: + = in compliance, - = out of compliance

General Comments: I filled this out & showed him how to do it since there was not form & he hadn't done it before.

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
	+	Coolers ice	5/7/05

[a]: + = in compliance, - = out of compliance

General Comments: I transported the fish since I was going back to the office.

Auditor Name: Matt Hudson

Auditor Signature: Matt Hudson Date Signed: 5/26/04