2007 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 2007). 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 2007. 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 made available to tribal members at offices where permits for off-reservation spearing are issued and recently, 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, personal communication). 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 2007. Funding for the collection and analysis of these samples came from carry-over of United States Environmental Protection Agency (EPA) Supplemental Funds, received to test for mercury levels in walleye from 25 lakes in each of three years (2004-2006).

METHODS

Collection of Samples

Walleye from inland lakes were collected during spring from tribal spearers and netters and by GLIFWC fishery assessment crews. 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).

Upon collection, walleye 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 to the Lake Superior Research Institute (LSRI) in Superior (Appendix 2).

Processing

Walleye were processed into skin-off fillets at GLIFWC using stainless steel knives and cutting surfaces. 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.

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: 2007
Lake Name: Sherman Lake (Vilas)	Sample Processing: Hg
Tissue type: Fillet	Processor: LSRI

Total Mercury Analyses

Walleye fillets 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 audits 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 2004-2006 studies (mean ± 2 times the standard deviation of all DORM-2 analyses). The calculated acceptable range was 3.36 to 5.28 µg Hg/g.

DORM-2 was analyzed in duplicate with each of the 11 sets of 20 samples. The recovery values ranged from 81.3 to 106% with the grand mean and standard deviation of the recoveries being 94.8 ± 7.7 percent of the certified value. All results were within the acceptable range of 72.4 to 114% of the certified value.

Spikes

A total of 45 spike samples were analyzed (11 percent of total samples). Spike recovery was considered acceptable when it was in the range of 61.9 to 120 percent 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 2004-2006 sample analysis. Mean recovery for the 45 spiked samples was 87.0 ± 11.3 percent with the values ranging from 56.6 to 105%. Two spike recovery values (Pine-12966 and Squirrel-12551) were outside the acceptance range (61.9 to 120 %). Both values outside the acceptance range had low recoveries. These samples were reanalyzed on August 9, 2007 and were found to have acceptable recoveries on that date. All other spike recovery values were within the acceptance range.

Duplicates

Fish tissues were analyzed for mercury in duplicate 45 times (11 percent of total samples). Two portions of the same tissue were digested and analyzed independently on the same date. Duplicate agreement values were acceptable when having a relative percent agreement > 80.5%. The acceptable value was calculated as the mean ± 2 times the standard deviations of all duplicate analyses conducted from Spring Walleye 2004-2006 sample analysis at the LSRI laboratory. Relative percent agreement between the duplicate analyses of the same tissue ranged from 87.1 to 100% with the average and standard deviation of the agreements being 94.8 ± 3.1 percent. All relative percent agreement values were above the acceptance range of > 80.5%.

In addition, 10 tissue samples digested and analyzed on two different occasions. The relative percent agreements for the samples analyzed on two dates ranged from 71.4 to 100% with a mean of 87.2 ± 8.3%. Eight of the ten samples reanalyzed had relative percent agreements above the minimum acceptance criteria used for duplicates digested and analyzed on the same date.

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 2004-2006 projects. Four tuna procedural blanks were processed coincident with the grinding of walleye collected for the GLIFWC EPA Mercury/Mapping Grant. One of the four procedural blanks was analyzed with each set of mercury samples for a total of eleven analyses resulting in a mean of 92.6 ± 5.8 relative percent agreement (Table 1). The relative percent agreement values ranged from 77.8 to 100% which were all within the acceptable range of > 69.7%. The procedural blank percent agreement analyses suggest that processing did not change the mercury content of the samples.

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 131 quality control samples measured. Four samples, or 3 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 2007, skinless fillets of 395 walleye from 37 lakes in Wisconsin were analyzed for total mercury concentration (Appendix 3). Overall, total mercury concentrations on a wet weight basis ranged from 0.059 to 1.86 µg Hg/g. Walleye lengths ranged from 11.9 to 29.2 inches. Walleye length and mercury data are summarized for each lake in Table 1.

Table 1. Summary statistics for mercury concentration (ug Hg/g fish tissue) and fresh length (inches) for walleye collected from Wisconsin lakes during spring 2007.

COUNTY	LAKE	# Fish	Mean Conc	St.Dev. Conc.	Median Conc.	Max Conc.	Min Conc.	Mean Length	St.Dev. Length
Vilas	Annabelle	3	0.634	0.207	0.516	0.873	0.514	13.0	1.0
Washburn	Bass-Patterson	12	0.439	0.316	0.265	0.952	0.152	18.8	5.3
Vilas	Big Arbor Vitae	12	0.174	0.079	0.161	0.385	0.072	18.1	4.6
Vilas	Boulder	8	0.449	0.150	0.447	0.643	0.266	16.7	2.6
Forest	Butternut	12	0.233	0.134	0.207	0.450	0.097	18.8	4.4
Oneida	Carrol	8	0.239	0.194	0.152	0.645	0.108	17.5	4.9
Sawyer	Chetac	12	0.164	0.102	0.143	0.392	0.059	19.0	4.9
Sawyer	Chippewa	12	0.458	0.326	0.337	1.37	0.165	18.0	5.2
Vilas	Eagle	12	0.373	0.191	0.321	0.762	0.159	17.9	3.6
Vilas	Forest	12	0.588	0.428	0.502	1.58	0.135	18.2	3.9
Sawyer	Grindstone	12	0.389	0.286	0.298	0.893	0.094	18.5	4.7
Oneida	Island	7	0.668	0.307	0.648	1.06	0.350	17.2	5.1
Forest	Jungle	12	0.566	0.253	0.533	1.04	0.245	16.7	2.2
Vilas	Kentuck	13	0.255	0.066	0.264	0.379	0.167	16.0	2.6
Burnett	Lipsett	8	0.296	0.096	0.293	0.448	0.135	18.0	2.7
Vilas	Little Arbor Vitae	12	0.136	0.040	0.132	0.226	0.088	18.1	3.7
Sawyer	Little Round	11	0.358	0.246	0.272	0.944	0.108	19.1	3.2
Washburn	Long	12	0.189	0.101	0.179	0.418	0.072	16.9	2.3
Sawyer	Lost Land	10	0.211	0.045	0.221	0.279	0.147	17.6	3.2
Douglas	Lower Eau Claire	12	0.289	0.170	0.210	0.703	0.128	18.2	3.4
Forest	Metonga	12	0.276	0.202	0.205	0.737	0.097	18.6	4.1
Oneida	Minocqua	12	0.246	0.195	0.180	0.682	0.118	18.6	4.5
Bayfield	Namekagon	12	0.320	0.103	0.314	0.511	0.184	18.4	4.6
Washburn	Nancy	10	0.528	0.249	0.432	1.05	0.264	19.3	3.4
Iron	Pine	5	0.560	0.140	0.605	0.748	0.404	14.9	2.1
Oneida	Planting Ground	12	0.736	0.247	0.716	1.24	0.344	18.8	4.1
Oneida	Rainbow Flowage	11	0.580	0.348	0.491	1.04	0.187	17.9	4.1
Burnett	Rooney	6	0.598	0.664	0.334	1.86	0.132	19.3	5.5
Vilas	Sherman	12	0.367	0.181	0.297	0.786	0.197	18.0*	5.4
Bayfield	Siskiwit	9	0.734	0.260	0.652	1.18	0.437	16.4	1.8
Oneida	Squirrel	12	0.323	0.186	0.273	0.667	0.124	18.1*	4.0
Sawyer	Teal	12	0.357	0.162	0.323	0.695	0.147	18.6	4.1
Vilas	Trout	12	0.324	0.254	0.208	0.935	0.120	18.9	4.8
Iron	Turtle-Flambeau Flowage	12	0.569	0.234	0.527	1.04	0.209	18.5	3.7
Vilas	Twin Lake Chain	10	0.288	0.148	0.277	0.659	0.143	17.6	4.4
Oneida	Two Sisters	12	0.352	0.134	0.330	0.629	0.135	18.2	3.0
Oneida	Willow Flowage	12	0.674	0.394	0.601	1.30	0.219	18.5	3.6

* Reported mean includes one or more fish measured as "frozen length" at GLIFWC laboratory.

Percent Moisture

Percent moisture was measured in 128 of the 395 walleye tissues. Walleye muscle tissue had a mean moisture value of 79.3 ± 1.5 percent (Appendix 3). Of the 128 tissues analyzed for moisture, 16 were analyzed in duplicate, all yielding relative percent agreements of 99.1 percent or greater. Fifteen 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 2007 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

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 2007 in Wisconsin Ceded Territory Waters

Total Mercury Concentrations in Muscle Tissue from Walleye Captured during the Spring 2007 in Wisconsin Ceded Territory Waters

by

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Introduction

Skinless fillet samples from walleye (*Sander vitreus*) captured during the spring of 2007 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). Three hundred ninety five skinless walleye fillets from thirty-seven lakes in Wisconsin collected by tribal spearers and GLIFWC Inland Fisheries assessment crews were analyzed as part of the EPA Mercury/Mapping Grant Number GL-96540801.

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 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 liquid was squeezed out of the can. 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. Four procedural blanks were prepared during this project. The initial procedural blank was prepared on the second day fish were ground for the project and the last procedural blank was generated on the last day fish were processed. The other two 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 by 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/13). 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 0.0047 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 30 percent of the walleye analyzed for mercury had moisture content determined. In general, 3 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 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 2004 through 2006 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 > 80.5%. The calculated acceptable range for the DORM-2 standard reference material was 3.36-5.28 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 61.9 to 120 percent of the expected value.

Eleven walleye tissue samples had an initial RSD of >5% for the triplicate measurements made on the digested sample. The digestate of ten of those samples was reanalyzed on the same date and each resulted in an RSD of <5%. One sample was digested and analyzed on a second date because it had failed the required 5%RSD limit on the initial analysis date and was mistakenly overlooked for reanalysis on that date. A number of QA/QC samples also failed the 5% RSD check for their initial analysis. Most of these samples were reanalyzed and met the RSD requirement. Calibration blanks and the two lowest concentration mercury standards were normally not reanalyzed if they failed the RSD requirement because they have low absorbance values and thus are likely to fail the RSD limit.

Several tissue samples were reanalyzed on a second analysis date to determine the reproducibility of results from samples analyzed on two different days. The results are provided in Table 5.

A quality assurance audit was conducted by the LSRI quality assurance officer during the Spring Walleye 2007 project. That report is provided in Appendix C.

Results from Fish Tissues Analyzed for GLIFWC's EPA Mercury/Mapping Grant (Number 96540801)

Quality Assurance – Four tuna procedural blanks were processed coincident with the grinding of walleye collected for GLIFWC's EPA Mercury/Mapping Grant. One of the four procedural blanks was analyzed with each set of mercury samples for a total of eleven analyses resulting in a mean of 92.6 ± 5.8 relative percent agreement (Table 1). The relative percent agreement values ranged from 77.8 to 100% which were all within the acceptable range of > 69.7%.

Analysis of the dogfish shark tissue (DORM-2) standard reference material was conducted in duplicate with all 11 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 81.3 to 106% with the grand mean and standard deviation of the recoveries being 94.8 ± 7.7 percent of the certified value. All results were within the acceptable range of 72.4 - 114%.

Fish tissues were analyzed for mercury in duplicate 45 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 87.1 to 100% with the average and standard deviation of the agreements being 94.8 ± 3.1 percent (Table 3). All relative percent agreement values were above the acceptance range of > 80.5%.

Samples of tissue were spiked with known concentrations of mercury prior to digestion. Mean recovery for the 45 spiked samples was 87.0 ± 11.3 percent with the individual

values ranging from 56.6 to 105% (Table 4). Two spike recovery values (Pine-12966 and Squirrel-12551) were outside the acceptance range (61.9-120 %). Both values outside the acceptance range had low recoveries. These samples were reanalyzed on August 9, 2007 and were found to have acceptable recoveries on that date.

Table 5 provides the results of the tissue samples digested and analyzed on two occasions. The relative percent agreements for the samples analyzed on two dates ranged from 71.4 to 100% with a mean of 87.2 ± 8.3%. Eight of the ten samples reanalyzed had relative percent agreements above the minimum acceptance criteria used for duplicates digested and analyzed on the same date.

Mercury Analysis – Skinless fillets of 395 walleye from 37 lakes in Wisconsin were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 6) ranged from 0.059 to 1.86 µg Hg/g (parts per million). The mercury concentrations reported in Table 6 for samples (Pine-12966 and Squirrel-12551) that originally failed our acceptance criteria for spike recoveries are the values determined on August 9, 2007.

Tissue Moisture Analysis – Percent moisture was measured in 128 of the 395 walleye tissues. Moisture analysis took place immediately following grinding of the fillets. Walleye muscle tissue had a mean moisture value of 79.3 ± 1.5 percent (Table 7). Of the 128 tissues analyzed for moisture, sixteen were analyzed in duplicate, all yielding relative percent agreements of 99.1 percent or greater. Fifteen 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*
7/6/2007	6/13/2007	0.072	0.070	0.071	97.2
7/11/2007	6/28/2007	0.055	0.055	0.055	100
7/19/2007	5/31/2007	0.099	0.094	0.097	94.8
7/24/2007	7/17/2007	0.058	0.054	0.056	92.9
7/25/2007	6/28/2007	0.069	0.076	0.073	90.3
7/27/2007	7/17/2007	0.046	0.051	0.049	89.7
7/31/2007	6/13/2007	0.053	0.051	0.052	96.2
8/1/2007	6/28/2007	0.049	0.051	0.050	96.0
8/7/2007	7/17/2007	0.040	0.050	0.045	77.8
8/8/2007	5/31/2007	0.097	0.089	0.093	91.4
8/9/2007	6/13/2007	0.061	0.066	0.064	92.1
			Mean ± Std. Dev.		92.6 ± 5.8

* 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 µg Hg/g	Percent of Expected Dorm 2-1	Dorm 2-2 µg Hg/g	Percent of Expected Dorm 2-2
7/6/2007	4.62	99.6	4.69	101
7/11/2007	4.66	101	4.52	97.5
7/19/2007	4.33	93.4	4.61	99.4
7/24/2007	3.77	81.3	4.75	102
7/25/2007	4.93	106	4.73	102
7/27/2007	4.37	94.2	4.19	90.4
7/31/2007	4.85	105	3.97	85.5
8/1/2007	4.69	101	4.02	86.7
8/7/2007	4.54	97.9	3.79	81.6
8/8/2007	4.55	98.0	4.21	90.8
8/9/2007	4.02	86.6	3.88	83.7
	Mean ± Std. Dev.		4.40 ± 0.36	94.8 ± 7.7

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

Date of Analysis	Lake	Tag number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Agreement
7/6/2007	Bass-Patterson	12534	0.848	0.905	0.877	93.5
7/6/2007	Bass-Patterson	12544	0.152	0.153	0.153	99.3
7/6/2007	Big Arbor Vitae	12718	0.169	0.174	0.172	97.1
7/6/2007	Butternut	12675	0.106	0.111	0.109	95.4
7/11/2007	Eagle	12809	0.159	0.155	0.157	97.5
7/11/2007	Grindstone	12697	0.893	0.910	0.902	98.1
7/11/2007	Grindstone	12710	0.128	0.133	0.131	96.2
7/11/2007	Jungle	12842	1.04	1.08	1.06	96.2
7/19/2007	Chetac	12606	0.157	0.165	0.161	95.0
7/19/2007	Chetac	12618	0.129	0.123	0.126	95.2
7/19/2007	Kentuck	12422	0.167	0.190	0.179	87.1
7/19/2007	Little Arbor Vitae	12779	0.089	0.083	0.086	93.0
7/24/2007	Lower Eau Claire	12749	0.132	0.124	0.128	93.8
7/24/2007	Minocqua	12682	0.172	0.171	0.172	99.4
7/24/2007	Minocqua	12691	0.187	0.195	0.191	95.8
7/24/2007	Namekagon	12588	0.249	0.232	0.241	92.9
7/25/2007	Pine	12966	0.657	0.616	0.637	93.6
7/25/2007	Sherman	12553	0.306	0.319	0.313	95.8
7/25/2007	Siskiwit	12432	0.652	0.621	0.637	95.1
7/25/2007	Squirrel	12551	0.633	0.644	0.639	98.3
7/27/2007	Turtle-Flambeau Fl.	12506	0.498	0.465	0.482	93.1
7/27/2007	Two Sisters	12817	0.300	0.333	0.317	89.6
7/27/2007	Two Sisters	12827	0.423	0.421	0.422	99.5
7/27/2007	Willow Flowage	12603	1.20	1.20	1.20	100
7/31/2007	Boulder	12791	0.643	0.674	0.659	95.3
7/31/2007	Carrol	12912	0.143	0.138	0.141	96.4
7/31/2007	Chippewa	12521	0.165	0.161	0.163	97.5
7/31/2007	Forest	12852	0.246	0.268	0.257	91.4
8/1/2007	Lipsett	12963	0.255	0.244	0.250	95.6
8/1/2007	Little Round	12928	0.243	0.221	0.232	90.5
8/1/2007	Long	5581	0.201	0.208	0.205	96.6
8/1/2007	Long	5592	0.101	0.091	0.096	89.6
8/7/2007	Island	12986	1.06	1.02	1.04	96.2
8/7/2007	Lost Land	5544	0.174	0.176	0.175	98.9
8/7/2007	Metonga	5553	0.201	0.182	0.192	90.1
8/7/2007	Nancy	12865	0.339	0.313	0.326	92.0
8/8/2007	Rooney	12950	0.302	0.279	0.291	92.1
8/8/2007	Teal	12629	0.302	0.276	0.289	91.0
8/8/2007	Trout	5567	0.199	0.191	0.195	95.9
8/8/2007	Twin Lake Chain	12659	0.291	0.314	0.303	92.4

8/9/2007	Pine	12966	0.748	0.766	0.757	97.6
8/9/2007	Planting Ground	12733	0.970	0.943	0.957	97.2
8/9/2007	Rainbow Flowage	12757	0.280	0.299	0.290	93.4
8/9/2007	Rainbow Flowage	12767	0.747	0.676	0.712	90.0
8/9/2007	Squirrel	12551	0.667	0.694	0.681	96.0
				Mean ± Std. Dev.		94.8 ± 3.1

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.
7/6/2007	Bass-Patterson	12534	66.7	69.2	67.9	1.8
7/6/2007	Bass-Patterson	12544	97.0	93.5	95.2	2.5
7/6/2007	Big Arbor Vitae	12718	94.3	91.6	93.0	1.9
7/6/2007	Butternut	12675	95.7	98.6	97.1	2.1
7/11/2007	Eagle	12809	97.6	93.2	95.4	3.1
7/11/2007	Grindstone	12697	79.6	77.2	78.4	1.7
7/11/2007	Grindstone	12710	98.4	100	99.2	1.1
7/11/2007	Jungle	12842	95.8	75.4	85.6	14.4
7/19/2007	Chetac	12606	93.0	95.5	94.2	1.8
7/19/2007	Chetac	12618	93.9	93.6	93.7	0.3
7/19/2007	Kentuck	12422	91.8	92.5	92.1	0.6
7/19/2007	Little Arbor Vitae	12779	101	99.7	100	0.9
7/24/2007	Lower Eau Claire	12749	91.7	88.7	90.2	2.1
7/24/2007	Minocqua	12682	93.5	84.8	89.1	6.1
7/24/2007	Minocqua	12691	76.0	90.2	83.1	10.0
7/24/2007	Namekagon	12588	111	85.2	98.1	18.2
7/25/2007	Pine	12966	58.8	59.2	59.0	0.3
7/25/2007	Sherman	12553	95.6	93.0	94.3	1.9
7/25/2007	Siskiwit	12432	89.6	68.8	79.2	14.7
7/25/2007	Squirrel	12551	57.6	55.5	56.6	1.5
7/27/2007	Turtle-Flambeau Fl.	12506	84.0	93.7	88.8	6.9
7/27/2007	Two Sisters	12817	91.2	89.6	90.4	1.1
7/27/2007	Two Sisters	12827	81.2	81.5	81.4	0.2
7/27/2007	Willow Flowage	12603	81.3	65.3	73.3	11.3
7/31/2007	Boulder	12791	91.5	85.2	88.4	4.5
7/31/2007	Carrol	12912	102	107	105	3.5

7/31/2007	Chippewa	12521	94.4	90.8	92.6	2.6
7/31/2007	Forest	12852	87.1	90.5	88.8	2.4
8/1/2007	Lipsett	12963	103	96.4	99.7	4.7
8/1/2007	Little Round	12928	96.6	92.2	94.4	3.1
8/1/2007	Long	5581	92.3	89.5	90.9	2.0
8/1/2007	Long	5592	90.8	89.4	90.1	1.0
8/7/2007	Island	12986	69.9	68.9	69.4	0.7
8/7/2007	Lost Land	5544	97.5	94.6	96.0	2.1
8/7/2007	Metonga	5553	87.7	91.0	89.3	2.4
8/7/2007	Nancy	12865	76.0	74.3	75.1	1.2
8/8/2007	Rooney	12950	90.2	90.5	90.3	0.2
8/8/2007	Teal	12629	94.3	94.7	94.5	0.3
8/8/2007	Trout	5567	96.0	91.4	93.7	3.2
8/8/2007	Twin Lake Chain	12659	93.6	92.6	93.1	0.7
8/9/2007	Pine	12966	89.8	83.8	86.8	4.2
8/9/2007	Planting Ground	12733	63.5	77.9	70.7	10.2
8/9/2007	Rainbow Flowage	12757	91.6	92.1	91.8	0.4
8/9/2007	Rainbow Flowage	12767	63.5	68.5	66.0	3.5
8/9/2007	Squirrel	12551	69.2	76.2	72.7	5.0
				Mean ± Std. Dev.		87.0 ± 11.3

Table 5. Relative Percent Agreement of Samples that Were Digested and Analyzed on Two Analysis Dates.

Analysis Date	Lake	Tag number	µg Hg/g	Mean	Relative Percent Agreement
7/25/2007	Pine	12966	0.657	0.703	87.0
8/9/2007	Pine	12966	0.748		
7/25/2007	Pine	12967	0.438	0.463	89.2
8/1/2007	Pine	12967	0.488		
7/25/2007	Pine	12980	0.607	0.640	89.8
8/1/2007	Pine	12980	0.672		
7/25/2007	Sherman	12547	0.227	0.208	81.2
8/1/2007	Sherman	12547	0.188		
7/25/2007	Sherman	12553	0.306	0.277	78.7
8/1/2007	Sherman	12553	0.247		
7/25/2007	Sherman	12554	0.264	0.248	87.1
8/1/2007	Sherman	12554	0.232		

7/25/2007	Squirrel	12551	0.633	0.650	94.8
8/9/2007	Squirrel	12551	0.667		
7/27/2007	Two Sisters	12823	0.347	0.405	71.4
8/9/2007	Two Sisters	12823	0.463		
7/27/2007	Two Sisters	12828	0.154	0.154	100
8/1/2007	Two Sisters	12828	0.154		
7/27/2007	Willow Flowage	12591	0.763	0.791	93.0
8/1/2007	Willow Flowage	12591	0.818		
			Mean ± Std. Dev.		87.2 ± 8.3

Table 6. Total Mercury Concentration (Wet Weight) in Walleye Fillets from Fish Captured during the Spring of 2007.

Analysis Date	Lake	Tag Number	Fresh Length (in)	Sex	µg Hg/g tissue
7/6/2007	Annabelle	12403	13.1	M	0.514
7/6/2007	Annabelle	12404	12.0	M	0.516
7/6/2007	Annabelle	12405	13.9	M	0.873
7/6/2007	Bass-Patterson	12531	14.0	M	0.159
7/6/2007	Bass-Patterson	12532	26.9	F	0.880
7/6/2007	Bass-Patterson	12534	26.1	F	0.848
7/6/2007	Bass-Patterson	12535	18.5	M	0.558
7/6/2007	Bass-Patterson	12536	27.3	F	0.952
7/6/2007	Bass-Patterson	12538	18.5	F	0.243
7/6/2007	Bass-Patterson	12540	15.2	M	0.171
7/6/2007	Bass-Patterson	12541	21.0	F	0.643
7/6/2007	Bass-Patterson	12542	15.5	M	0.286
7/6/2007	Bass-Patterson	12543	15.4	M	0.204
7/6/2007	Bass-Patterson	12544	13.7	M	0.152
7/6/2007	Bass-Patterson	12545	13.1	M	0.176
7/6/2007	Big Arbor Vitae	12711	25.4	F	0.385
7/6/2007	Big Arbor Vitae	12712	16.6	M	0.195
7/6/2007	Big Arbor Vitae	12713	12.7	M	0.093
7/6/2007	Big Arbor Vitae	12714	11.9	M	0.072
7/6/2007	Big Arbor Vitae	12715	14.2	M	0.143
7/6/2007	Big Arbor Vitae	12716	15.1	M	0.152
7/6/2007	Big Arbor Vitae	12717	16.3	M	0.190
7/6/2007	Big Arbor Vitae	12718	23.2	F	0.169
7/6/2007	Big Arbor Vitae	12719	25.7	F	0.231
7/6/2007	Big Arbor Vitae	12720	18.2	M	0.167
7/6/2007	Big Arbor Vitae	12721	18.4	M	0.154
7/6/2007	Big Arbor Vitae	12722	18.9	F	0.140
7/31/2007	Boulder	12786	17.3	M	0.582

7/31/2007	Boulder	12787	15.0	M	0.357
7/31/2007	Boulder	12788	15.2	M	0.321
7/31/2007	Boulder	12789	14.4	M	0.266
7/31/2007	Boulder	12790	13.8	M	0.307
7/31/2007	Boulder	12791	20.4	F	0.643
7/31/2007	Boulder	12799	20.6	F	0.537
7/31/2007	Boulder	12800	16.5	M	0.578
7/6/2007	Butternut	12669	16.5	M	0.117
7/6/2007	Butternut	12670	16.2	M	0.153
7/6/2007	Butternut	12671	15.5	M	0.100
7/6/2007	Butternut	12672	14.1	M	0.105
7/6/2007	Butternut	12673	13.5	M	0.097
7/6/2007	Butternut	12674	20.1	M	0.276
7/6/2007	Butternut	12675	14.5	M	0.106
7/6/2007	Butternut	12676	19.0	M	0.368
7/6/2007	Butternut	12677	21.3	M	0.383
7/6/2007	Butternut	12678	23.4	F	0.374
7/6/2007	Butternut	12679	26.2	F	0.450
7/6/2007	Butternut	12680	25.3	F	0.261
7/31/2007	Carrol	12907	23.8	F	0.430
7/31/2007	Carrol	12908	26.3	F	0.645
7/31/2007	Carrol	12909	14.2	M	0.108
7/31/2007	Carrol	12910	16.2	M	0.176
7/31/2007	Carrol	12911	16.6	M	0.134
7/31/2007	Carrol	12912	15.4	M	0.143
7/31/2007	Carrol	12913	12.3	M	0.160
7/31/2007	Carrol	12914	14.9	M	0.115
7/19/2007	Chetac	12606	26.5	F	0.157
7/19/2007	Chetac	12607	19.9	M	0.289
7/19/2007	Chetac	12608	19.0	M	0.221
7/19/2007	Chetac	12610	26.9	F	0.392
7/19/2007	Chetac	12611	25.8	F	0.214
7/19/2007	Chetac	12614	18.5	M	0.178
7/19/2007	Chetac	12615	16.2	M	0.097
7/19/2007	Chetac	12616	14.1	M	0.059
7/19/2007	Chetac	12617	16.9	M	0.105
7/19/2007	Chetac	12618	16.8	M	0.129
7/19/2007	Chetac	12619	13.9	M	0.061
7/19/2007	Chetac	12620	13.5	M	0.062
7/31/2007	Chippewa	12516	12.3	M	0.358
7/31/2007	Chippewa	12517	13.3	M	0.269
7/31/2007	Chippewa	12518	15.2	M	0.296
7/31/2007	Chippewa	12519	22.7	F	0.640
7/31/2007	Chippewa	12520	13.7	M	0.297
7/31/2007	Chippewa	12521	15.7	M	0.165

7/31/2007	Chippewa	12522	24.3	F	0.698
7/31/2007	Chippewa	12523	13.3	M	0.268
7/31/2007	Chippewa	12525	29.2	F	1.37
7/31/2007	Chippewa	12526	18.8	M	0.422
7/31/2007	Chippewa	12528	18.9	F	0.315
7/31/2007	Chippewa	12530	18.9	M	0.403
7/11/2007	Eagle	12801	23.7	F	0.762
7/11/2007	Eagle	12802	22.9	F	0.481
7/11/2007	Eagle	12803	18.2	M	0.666
7/11/2007	Eagle	12804	22.8	F	0.468
7/11/2007	Eagle	12807	18.0	F	0.209
7/11/2007	Eagle	12809	17.0	F	0.159
7/11/2007	Eagle	12810	15.8	M	0.269
7/11/2007	Eagle	12811	13.5	M	0.266
7/11/2007	Eagle	12812	14.3	M	0.186
7/11/2007	Eagle	12813	16.6	M	0.377
7/11/2007	Eagle	12814	18.9	F	0.254
7/11/2007	Eagle	12815	13.5	M	0.373
7/31/2007	Forest	12846	15.1	M	0.256
7/31/2007	Forest	12847	17.7	M	0.497
7/31/2007	Forest	12848	18.2	M	0.519
7/31/2007	Forest	12849	19.1	M	0.928
7/31/2007	Forest	12850	18.0	M	0.507
7/31/2007	Forest	12851	25.0	F	1.58
7/31/2007	Forest	12852	15.0	M	0.246
7/31/2007	Forest	12853	12.2	M	0.135
7/31/2007	Forest	12854	23.0	F	0.946
7/31/2007	Forest	12857	23.5	F	0.927
7/31/2007	Forest	12858	14.7	M	0.234
7/31/2007	Forest	12860	16.4	M	0.279
7/11/2007	Grindstone	12696	19.4	M	0.460
7/11/2007	Grindstone	12697	26.4	F	0.893
7/11/2007	Grindstone	12698	18.3	M	0.316
7/11/2007	Grindstone	12699	13.2	M	0.120
7/11/2007	Grindstone	12700	20.0	M	0.435
7/11/2007	Grindstone	12701	22.8	M	0.664
7/11/2007	Grindstone	12703	26.2	F	0.876
7/11/2007	Grindstone	12705	17.1	M	0.267
7/11/2007	Grindstone	12707	12.9	M	0.094
7/11/2007	Grindstone	12708	13.8	M	0.133
7/11/2007	Grindstone	12709	17.3	M	0.280
7/11/2007	Grindstone	12710	15.1	M	0.128
8/7/2007	Island	12981	13.2	M	0.648
8/7/2007	Island	12982	16.4	M	0.885
8/7/2007	Island	12983	12.4	U	0.405

8/7/2007	Island	12984	26.0	F	0.975
8/7/2007	Island	12985	12.7	M	0.355
8/7/2007	Island	12986	21.3	F	1.06
8/7/2007	Island	12987	18.1	M	0.350
7/11/2007	Jungle	12831	17.5	M	0.820
7/11/2007	Jungle	12832	18.1	M	0.588
7/11/2007	Jungle	12833	19.5	F	0.639
7/11/2007	Jungle	12835	13.0	M	0.245
7/11/2007	Jungle	12836	13.7	M	0.308
7/11/2007	Jungle	12837	16.1	M	0.403
7/11/2007	Jungle	12838	17.0	M	0.477
7/11/2007	Jungle	12840	19.6	F	0.708
7/11/2007	Jungle	12842	18.1	M	1.04
7/11/2007	Jungle	12843	18.2	M	0.859
7/11/2007	Jungle	12844	14.5	M	0.308
7/11/2007	Jungle	12845	15.0	M	0.393
7/19/2007	Kentuck	12417	12.5	M	0.208
7/19/2007	Kentuck	12419	17.6	M	0.305
7/19/2007	Kentuck	12420	12.8	M	0.168
7/19/2007	Kentuck	12421	14.0	M	0.181
7/19/2007	Kentuck	12422	13.9	M	0.167
7/19/2007	Kentuck	12423	18.9	F	0.265
7/19/2007	Kentuck	12425	18.9	M	0.307
7/19/2007	Kentuck	12426	18.4	F	0.278
7/19/2007	Kentuck	12427	15.7	M	0.379
7/19/2007	Kentuck	12428	13.2	M	0.204
7/19/2007	Kentuck	12429	15.6	M	0.327
7/19/2007	Kentuck	12430	16.6	M	0.264
7/19/2007	Kentuck	12418	20.4	F	0.256
8/1/2007	Lipsett	12958	23.1	M	0.376
8/1/2007	Lipsett	12959	19.6	M	0.329
8/1/2007	Lipsett	12960	18.2	M	0.256
8/1/2007	Lipsett	12961	13.8	M	0.135
8/1/2007	Lipsett	12962	18.8	M	0.448
8/1/2007	Lipsett	12963	17.0	M	0.255
8/1/2007	Lipsett	12964	17.2	M	0.331
8/1/2007	Lipsett	12965	16.6	M	0.238
7/19/2007	Little Arbor Vitae	12771	18.2	M	0.141
7/19/2007	Little Arbor Vitae	12772	17.8	M	0.168
7/19/2007	Little Arbor Vitae	12773	15.4	M	0.094
7/19/2007	Little Arbor Vitae	12774	17.9	M	0.136
7/19/2007	Little Arbor Vitae	12775	19.2	F	0.126
7/19/2007	Little Arbor Vitae	12776	14.0	M	0.118
7/19/2007	Little Arbor Vitae	12777	22.2	F	0.127
7/19/2007	Little Arbor Vitae	12778	18.8	M	0.140

7/19/2007	Little Arbor Vitae	12779	13.0	M	0.089
7/19/2007	Little Arbor Vitae	12780	13.1	M	0.088
7/19/2007	Little Arbor Vitae	12781	23.7	F	0.175
7/19/2007	Little Arbor Vitae	12782	23.4	F	0.226
8/1/2007	Little Round	12921	22.6	M	0.569
8/1/2007	Little Round	12923	17.8	M	0.180
8/1/2007	Little Round	12924	16.9	F	0.134
8/1/2007	Little Round	12926	19.8	M	0.466
8/1/2007	Little Round	12927	18.2	M	0.189
8/1/2007	Little Round	12928	17.3	F	0.243
8/1/2007	Little Round	12930	12.5	M	0.108
8/1/2007	Little Round	12931	19.0	M	0.272
8/1/2007	Little Round	12932	19.0	M	0.465
8/1/2007	Little Round	12933	23.0	F	0.372
8/1/2007	Little Round	12934	23.8	F	0.944
8/1/2007	Long	5578	19.5	M	0.418
8/1/2007	Long	5579	18.9	M	0.249
8/1/2007	Long	5580	18.6	M	0.322
8/1/2007	Long	5581	15.5	M	0.201
8/1/2007	Long	5582	18.1	M	0.178
8/1/2007	Long	5583	17.8	M	0.200
8/1/2007	Long	5584	18.0	M	0.136
8/1/2007	Long	5585	17.2	M	0.116
8/1/2007	Long	5586	17.6	M	0.097
8/1/2007	Long	5587	16.6	M	0.179
8/1/2007	Long	5591	12.1	M	0.072
8/1/2007	Long	5592	12.9	M	0.101
8/7/2007	Lost Land	5533	21.8	F	0.279
8/7/2007	Lost Land	5534	14.3	M	0.227
8/7/2007	Lost Land	5540	14.7	M	0.241
8/7/2007	Lost Land	5541	19.0	M	0.264
8/7/2007	Lost Land	5542	19.5	F	0.218
8/7/2007	Lost Land	5543	23.5	F	0.224
8/7/2007	Lost Land	5544	15.3	M	0.174
8/7/2007	Lost Land	5545	15.8	M	0.191
8/7/2007	Lost Land	5546	17.5	M	0.147
8/7/2007	Lost Land	5547	14.8	M	0.149
7/24/2007	Lower Eau Claire	12741	23.4	F	0.703
7/24/2007	Lower Eau Claire	12742	14.4	M	0.215
7/24/2007	Lower Eau Claire	12743	23.7	F	0.417
7/24/2007	Lower Eau Claire	12745	22.4	F	0.427
7/24/2007	Lower Eau Claire	12748	18.7	M	0.361
7/24/2007	Lower Eau Claire	12749	14.6	M	0.132
7/24/2007	Lower Eau Claire	12750	14.3	M	0.128
7/24/2007	Lower Eau Claire	12751	18.0	F	0.204

7/24/2007	Lower Eau Claire	12752	19.2	M	0.358
7/24/2007	Lower Eau Claire	12753	17.9	F	0.173
7/24/2007	Lower Eau Claire	12754	15.9	M	0.185
7/24/2007	Lower Eau Claire	12755	15.9	M	0.167
8/7/2007	Metonga	5548	18.7	F	0.193
8/7/2007	Metonga	5549	16.7	M	0.219
8/7/2007	Metonga	5550	15.3	F	0.147
8/7/2007	Metonga	5551	13.8	M	0.097
8/7/2007	Metonga	5552	18.0	F	0.210
8/7/2007	Metonga	5553	17.9	F	0.201
8/7/2007	Metonga	5554	14.5	M	0.102
8/7/2007	Metonga	5555	14.4	M	0.161
8/7/2007	Metonga	5556	25.2	F	0.497
8/7/2007	Metonga	5557	24.6	F	0.541
8/7/2007	Metonga	5558	24.5	F	0.737
8/7/2007	Metonga	5559	19.9	F	0.209
7/24/2007	Minocqua	12681	15.5	M	0.226
7/24/2007	Minocqua	12682	18.3	F	0.172
7/24/2007	Minocqua	12683	14.8	M	0.148
7/24/2007	Minocqua	12684	14.1	M	0.122
7/24/2007	Minocqua	12685	16.6	F	0.137
7/24/2007	Minocqua	12686	18.0	F	0.189
7/24/2007	Minocqua	12687	17.3	F	0.197
7/24/2007	Minocqua	12688	18.0	M	0.144
7/24/2007	Minocqua	12689	14.2	M	0.118
7/24/2007	Minocqua	12690	25.4	F	0.682
7/24/2007	Minocqua	12691	22.2	F	0.187
7/24/2007	Minocqua	12692	28.4	F	0.633
7/24/2007	Namekagon	12576	24.0	F	0.386
7/24/2007	Namekagon	12577	26.3	F	0.464
7/24/2007	Namekagon	12578	24.0	F	0.337
7/24/2007	Namekagon	12579	15.0	M	0.333
7/24/2007	Namekagon	12580	18.6	M	0.511
7/24/2007	Namekagon	12581	21.7	F	0.379
7/24/2007	Namekagon	12582	12.5	M	0.199
7/24/2007	Namekagon	12583	13.5	M	0.184
7/24/2007	Namekagon	12587	14.5	M	0.293
7/24/2007	Namekagon	12588	16.5	M	0.249
7/24/2007	Namekagon	12589	15.2	M	0.211
7/24/2007	Namekagon	12590	18.6	F	0.294
8/7/2007	Nancy	12861	19.3	M	0.353
8/7/2007	Nancy	12862	16.3	M	0.378
8/7/2007	Nancy	12863	19.8	M	0.791
8/7/2007	Nancy	12864	17.9	M	0.429
8/7/2007	Nancy	12865	18.3	M	0.339

8/7/2007	Nancy	12866	17.8	M	0.435
8/7/2007	Nancy	12867	24.8	F	0.512
8/7/2007	Nancy	12868	22.2	F	1.05
8/7/2007	Nancy	12869	13.4	M	0.264
8/7/2007	Nancy	12870	23.1	F	0.731
8/9/2007	Pine	12966	18.4	M	0.748
7/25/2007	Pine	12967	15.0	M	0.438
7/25/2007	Pine	12978	13.0	M	0.404
7/25/2007	Pine	12979	13.9	M	0.605
7/25/2007	Pine	12980	14.0	M	0.607
8/9/2007	Planting Ground	12726	26.3	F	1.24
8/9/2007	Planting Ground	12729	14.4	M	0.690
8/9/2007	Planting Ground	12730	15.7	M	0.406
8/9/2007	Planting Ground	12731	19.0	F	0.826
8/9/2007	Planting Ground	12732	16.9	F	0.682
8/9/2007	Planting Ground	12733	18.5	F	0.970
8/9/2007	Planting Ground	12734	21.0	F	0.599
8/9/2007	Planting Ground	12735	25.2	F	0.764
8/9/2007	Planting Ground	12737	14.1	M	0.742
8/9/2007	Planting Ground	12738	15.6	F	0.962
8/9/2007	Planting Ground	12739	22.2	F	0.344
8/9/2007	Planting Ground	12740	16.3	M	0.601
8/9/2007	Rainbow Flowage	12756	12.8	M	0.187
8/9/2007	Rainbow Flowage	12757	14.8	M	0.280
8/9/2007	Rainbow Flowage	12758	25.8	F	0.980
8/9/2007	Rainbow Flowage	12759	15.8	F	0.274
8/9/2007	Rainbow Flowage	12760	16.0	M	0.491
8/9/2007	Rainbow Flowage	12761	15.9	M	0.253
8/9/2007	Rainbow Flowage	12762	18.4	M	1.04
8/9/2007	Rainbow Flowage	12764	21.7	M	0.853
8/9/2007	Rainbow Flowage	12765	23.4	F	1.01
8/9/2007	Rainbow Flowage	12766	13.9	M	0.265
8/9/2007	Rainbow Flowage	12767	18.2	M	0.747
8/8/2007	Rooney	12936	26.0	F	1.86
8/8/2007	Rooney	12937	13.8	M	0.132
8/8/2007	Rooney	12938	23.9	F	0.792
8/8/2007	Rooney	12939	12.6	M	0.134
8/8/2007	Rooney	12940	17.6	M	0.366
8/8/2007	Rooney	12950	21.6	F	0.302
7/25/2007	Sherman	12546	12.5	M	0.288
7/25/2007	Sherman	12547	12.7	M	0.227
7/25/2007	Sherman	12548	12.1	M	0.197
7/25/2007	Sherman	12549	20.2	M	0.368
7/25/2007	Sherman	12552	17.8*	M	0.413
7/25/2007	Sherman	12553	17.8*	F	0.306

7/25/2007	Sherman	12554	16.4*	F	0.264
7/25/2007	Sherman	12555	14.6*	M	0.247
7/25/2007	Sherman	12556	16.5*	M	0.257
7/25/2007	Sherman	12557	23.3	F	0.661
7/25/2007	Sherman	12646	23.4	F	0.786
7/25/2007	Sherman	12647	22.1	F	0.393
7/25/2007	Siskiwit	12431	18.8	F	1.04
7/25/2007	Siskiwit	12432	15.1	M	0.652
7/25/2007	Siskiwit	12433	15.2	F	0.437
7/25/2007	Siskiwit	12434	14.7	F	0.600
7/25/2007	Siskiwit	12435	14.9	M	0.501
7/25/2007	Siskiwit	12436	14.8	F	0.516
7/25/2007	Siskiwit	12437	17.5	F	0.758
7/25/2007	Siskiwit	12438	18.2	M	0.921
7/25/2007	Siskiwit	12439	18.4	F	1.18
7/25/2007	Squirrel	12550	22.6	F	0.466
8/9/2007	Squirrel	12551	23.7*	F	0.667
7/25/2007	Squirrel	12636	25.3	F	0.623
7/25/2007	Squirrel	12637	19.0	F	0.251
7/25/2007	Squirrel	12638	19.6	F	0.346
7/25/2007	Squirrel	12639	22.0	F	0.295
7/25/2007	Squirrel	12640	16.6	M	0.213
7/25/2007	Squirrel	12641	13.4	M	0.157
7/25/2007	Squirrel	12642	16.0	M	0.424
7/25/2007	Squirrel	12643	14.7	M	0.124
7/25/2007	Squirrel	12644	16.7	M	0.164
7/25/2007	Squirrel	12645	13.2	M	0.147
8/8/2007	Teal	12621	23.5	F	0.568
8/8/2007	Teal	12622	23.4	F	0.451
8/8/2007	Teal	12623	19.2	M	0.432
8/8/2007	Teal	12624	18.9	M	0.410
8/8/2007	Teal	12625	16.3	M	0.274
8/8/2007	Teal	12627	17.5	M	0.344
8/8/2007	Teal	12628	21.3	F	0.272
8/8/2007	Teal	12629	17.9	M	0.302
8/8/2007	Teal	12630	25.1	F	0.695
8/8/2007	Teal	12631	13.8	M	0.147
8/8/2007	Teal	12632	13.5	M	0.189
8/8/2007	Teal	12635	13.2	M	0.205
8/8/2007	Trout	5563	16.8	F	0.120
8/8/2007	Trout	5564	15.8	F	0.195
8/8/2007	Trout	5565	19.9	F	0.198
8/8/2007	Trout	5566	20.0	F	0.237
8/8/2007	Trout	5567	16.5	F	0.199
8/8/2007	Trout	5568	13.3	M	0.216

8/8/2007	Trout	5569	18.8	F	0.248
8/8/2007	Trout	5570	26.4	F	0.654
8/8/2007	Trout	5571	14.7	M	0.156
8/8/2007	Trout	5572	22.7	M	0.568
8/8/2007	Trout	5573	28.2	F	0.935
8/8/2007	Trout	5575	14.2	M	0.159
7/27/2007	Turtle-Flambeau Flowage	12501	14.5	M	0.312
7/27/2007	Turtle-Flambeau Flowage	12502	18.5	M	0.594
7/27/2007	Turtle-Flambeau Flowage	12503	14.9	M	0.209
7/27/2007	Turtle-Flambeau Flowage	12504	16.6	M	0.543
7/27/2007	Turtle-Flambeau Flowage	12505	14.9	M	0.511
7/27/2007	Turtle-Flambeau Flowage	12506	16.3	M	0.498
7/27/2007	Turtle-Flambeau Flowage	12507	15.4	M	0.420
7/27/2007	Turtle-Flambeau Flowage	12508	19.2	M	0.434
7/27/2007	Turtle-Flambeau Flowage	12509	19.9	F	0.610
7/27/2007	Turtle-Flambeau Flowage	12510	24.2	F	0.827
7/27/2007	Turtle-Flambeau Flowage	12511	24.6	F	0.831
7/27/2007	Turtle-Flambeau Flowage	12515	22.5	F	1.04
8/8/2007	Twin Lake Chain	12655	20.6	F	0.339
8/8/2007	Twin Lake Chain	12656	18.7	F	0.263
8/8/2007	Twin Lake Chain	12658	14.1	F	0.143
8/8/2007	Twin Lake Chain	12659	16.9	F	0.291
8/8/2007	Twin Lake Chain	12660	15.2	M	0.304
8/8/2007	Twin Lake Chain	12661	26.1	F	0.659
8/8/2007	Twin Lake Chain	12662	15.1	M	0.250
8/8/2007	Twin Lake Chain	12663	14.0	M	0.155
8/8/2007	Twin Lake Chain	12664	12.5	M	0.161
8/8/2007	Twin Lake Chain	12665	22.7	F	0.314
7/27/2007	Two Sisters	12816	19.1	M	0.409
7/27/2007	Two Sisters	12817	16.8	M	0.300
7/27/2007	Two Sisters	12818	18.6	M	0.450
7/27/2007	Two Sisters	12819	17.4	M	0.449
7/27/2007	Two Sisters	12820	16.3	M	0.311
7/27/2007	Two Sisters	12821	19.3	M	0.305
7/27/2007	Two Sisters	12822	14.7	M	0.135
7/27/2007	Two Sisters	12823	22.6	F	0.347
7/27/2007	Two Sisters	12825	14.8	M	0.313
7/27/2007	Two Sisters	12826	22.6	M	0.629
7/27/2007	Two Sisters	12827	22.2	F	0.423
7/27/2007	Two Sisters	12828	14.5	M	0.154
7/27/2007	Willow Flowage	12591	19.8	M	0.763
7/27/2007	Willow Flowage	12594	19.7	F	0.326
7/27/2007	Willow Flowage	12595	24.1	F	1.30
7/27/2007	Willow Flowage	12597	22.5	F	0.906
7/27/2007	Willow Flowage	12598	16.0	M	0.546

7/27/2007	Willow Flowage	12599	14.5	M	0.373
7/27/2007	Willow Flowage	12600	23.6	F	1.19
7/27/2007	Willow Flowage	12601	17.4	F	0.219
7/27/2007	Willow Flowage	12602	17.0	M	0.655
7/27/2007	Willow Flowage	12603	19.1	M	1.20
7/27/2007	Willow Flowage	12604	13.5	M	0.269
7/27/2007	Willow Flowage	12605	14.2	M	0.336

* Frozen length. Fresh length lost with datasheets.

Table 7. Percent Moisture in Walleye Fillets (Measured Immediately after Grinding).

Lake	Tag Number	Percent Moisture	Relative Percent Agreement
Annabelle	12403*	79.9	
Annabelle	12404*	80.4	
Annabelle	12405*	81.4	
Bass-Patterson	12535	78.6	
Bass-Patterson	12535 Dup	78.7	99.9
Bass-Patterson	12540	78.2	
Bass-Patterson	12542	79.2	
Big Arbor Vitae	12717	78.3	
Big Arbor Vitae	12715	79.9	
Big Arbor Vitae	12712	78.1	
Boulder	12789	79.0	
Boulder	12790	79.8	
Boulder	12787	80.2	
Boulder	12787 Dup	79.5	99.1
Butternut	12672	80.6	
Butternut	12675	80.4	
Butternut	12675 Dup	80.1	99.6
Butternut	12673	81.9	
Carrol	12909	78.9	
Carrol	12910	78.5	
Carrol	12913	78.6	
Eagle	12812	79.6	
Eagle	12813	79.6	
Eagle	12811	79.9	
Forest	12858	79.4	
Forest	12846	79.3	
Forest	12852	79.0	
Grindstone	12707*	80.8	

Grindstone	12699*	79.9	
Grindstone	12710*	78.2	
Grindstone	12710 Dup*	78.6	99.5
Island	12983	79.3	
Island	12985	79.0	
Island	12981	78.7	
Jungle	12835	79.9	
Jungle	12831	81.4	
Jungle	12831 Dup	81.6	99.8
Jungle	12832	80.4	
Kentuck	12421	79.0	
Kentuck	12422	78.9	
Kentuck	12422 Dup	78.9	100
Kentuck	12428	80.5	
Kentuck	12419	66.6	
Chetac	12619	79.2	
Chetac	12616	77.9	
Chetac	12617	78.6	
Chippewa	12517	79.9	
Chippewa	12520	80.0	
Chippewa	?	79.2	
Lipsett	12961*	78.1	
Lipsett	12963*	79.4	
Lipsett	12964*	78.8	
Little Arbor Vitae	12779	78.1	
Little Arbor Vitae	12780	78.8	
Little Arbor Vitae	12776	78.3	
Little Round	12923	78.8	
Little Round	12923 Dup	78.5	99.6
Little Round	12924	79.6	
Little Round	12928	79.6	
Long	5592	77.9	
Long	5583	77.1	
Long	5583 Dup	77.3	99.7
Long	5591	77.8	
Lost Land	5540	79.8	
Lost Land	5540 Dup	79.8	100
Lost Land	5534	79.7	
Lost Land	5544	79.7	
Lower Eau Claire	12750*	79.1	
Lower Eau Claire	12749*	79.0	

Lower Eau Claire	12755*	79.1	
Metonga	5550	79.7	
Metonga	5551	80.0	
Metonga	5554	79.4	
Minocqua	12681	78.7	
Minocqua	12681 Dup	78.9	99.7
Minocqua	12689	79.5	
Minocqua	12682	79.3	
Namekagon	12583	79.8	
Namekagon	12587	78.9	
Namekagon	12589	78.8	
Nancy	12869	78.4	
Nancy	12862	78.5	
Nancy	12861	79.3	
Pine	12967	80.8	
Pine	12979	79.8	
Pine	12979 Dup	79.8	100
Pine	12980	81.7	
Planting Ground	12729	79.3	
Planting Ground	12737	82.5	
Planting Ground	12730	77.9	
Planting Ground	12730 Dup	78.4	99.4
Rainbow Flowage	12760	79.9	
Rainbow Flowage	12761	79.9	
Rainbow Flowage	12762	79.9	
Rooney	12939	79.9	
Rooney	12937	78.7	
Rooney	12940	78.2	
Sherman	12555	80.0	
Sherman	12554	79.2	
Sherman	12552	79.3	
Siskiwit	12436*	79.6	
Siskiwit	12434*	80.5	
Siskiwit	12431*	80.3	
Siskiwit	12431 Dup*	80.0	99.6
Squirrel	12641	79.2	
Squirrel	12642	79.3	
Squirrel	12642 Dup	79.2	99.9
Squirrel	12643	78.3	
Teal	12631	80.1	
Teal	12632	80.1	

Teal	12635	80.5	
Trout	5563	77.5	
Trout	5568	78.8	
Trout	5571	78.6	
Turtle-Flambeau Flowage	12507	78.5	
Turtle-Flambeau Flowage	12501	78.1	
Turtle-Flambeau Flowage	12502	78.9	
Turtle-Flambeau Flowage	12502 Dup	78.9	99.9
Twin Lake Chain	12658	81.4	
Twin Lake Chain	12662	79.9	
Twin Lake Chain	12663	80.6	
Two Sisters	12825	81.8	
Two Sisters	12828	78.3	
Two Sisters	12817	78.2	
Willow Flowage	12604	79.0	
Willow Flowage	12599	78.7	
Willow Flowage	12599 Dup	79.0	99.6
Willow Flowage	12602	79.1	
	Mean ± Std. Dev.	79.3 ± 1.5	

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

Appendix A

Determination of 2007 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
GP-RT-HRC-3 #1	whole fish composite	170.46	8.523	0.211	0.0404
GP-RT-HRC-3 #2	whole fish composite	186.58	9.329	0.226	0.0413
GP-RT-HRC-3 #3	whole fish composite	214.78	10.74	0.255	0.0421
GP-RT-HRC-3 #4	whole fish composite	210.75	10.54	0.267	0.0395
GP-RT-HRC-3 #5	whole fish composite	190.61	9.530	0.222	0.0429
GP-RT-HRC-3 #6	whole fish composite	214.78	10.74	0.241	0.0446
GP-RT-HRC-3 #7	whole fish composite	178.52	8.926	0.212	0.0421
GP-RT-HRC-3 #8	whole fish composite	202.69	10.13	0.248	0.0409
				Mean	0.0417
				Std. Dev.	0.00158

LOD = Std. Dev. x t = 0.00158 x 2.998 = 0.0047

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 Walleye from GLIFWC's EPA Mercury/Mapping Grant.

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs 1	Blank Corrected Abs 2	Blank Corrected Mean	Std. Dev.	Slope	Y-Intercept	Correlation
07/06/07	0	0.0010	0.0011	0.0000	0.0001			
07/06/07	50	0.0011	0.0012	0.0012	0.0001			
07/06/07	100	0.0023	0.0024	0.0024	0.0001			
07/06/07	500	0.0113	0.0118	0.0116	0.0004			
07/06/07	1000	0.0233	0.0233	0.0233	0.0000			
07/06/07	6000	0.1353	0.1402	0.1378	0.0035	2.295E-05	8.809E-05	1.00000
07/11/07	0	0.0013	0.0012	0.0000	0.0001			
07/11/07	50	0.0011	0.0011	0.0011	0.0000			
07/11/07	100	0.0023	0.0023	0.0023	0.0000			
07/11/07	500	0.0121	0.0125	0.0123	0.0003			
07/11/07	1000	0.0237	0.0234	0.0236	0.0002			
07/11/07	6000	0.1397	0.1354	0.1376	0.0030	2.290E-05	2.731E-04	0.99997
07/19/07	0	0.0013	0.0012	0.0000	0.0001			
07/19/07	50	0.0011	0.0013	0.0012	0.0001			
07/19/07	100	0.0024	0.0027	0.0026	0.0002			
07/19/07	500	0.0122	0.0124	0.0123	0.0001			
07/19/07	1000	0.0240	0.0249	0.0245	0.0006			
07/19/07	6000	0.1413	0.1438	0.1426	0.0018	2.373E-05	2.506E-04	0.99999
07/24/07	0	0.0017	0.0013	0.0000	0.0003			
07/24/07	50	0.0015	0.0023	0.0019	0.0006			
07/24/07	100	0.0030	0.0032	0.0031	0.0001			
07/24/07	500	0.0167	0.0118	0.0143	0.0035			
07/24/07	1000	0.0319	0.0270	0.0295	0.0035			
07/24/07	6000	0.1828	0.1511	0.1670	0.0224	2.776E-05	5.438E-04	0.99996
07/25/07	0	0.0013	0.0013	0.0000	0.0000			
07/25/07	50	0.0014	0.0014	0.0014	0.0000			
07/25/07	100	0.0027	0.0024	0.0026	0.0002			
07/25/07	500	0.0140	0.0113	0.0127	0.0019			
07/25/07	1000	0.0274	0.0211	0.0243	0.0045			
07/25/07	6000	0.1629	0.1196	0.1413	0.0306	2.349E-05	3.983E-04	0.99998
07/27/07	0	0.0014	0.0016	0.0000	0.0001			
07/27/07	50	0.0016	0.0014	0.0015	0.0001			
07/27/07	100	0.0030	0.0025	0.0028	0.0004			
07/27/07	500	0.0140	0.0122	0.0131	0.0013			
07/27/07	1000	0.0279	0.0252	0.0266	0.0019			
07/27/07	6000	0.1613	0.1532	0.1573	0.0057	2.619E-05	1.314E-04	1.00000
07/31/07	0	0.0014	0.0013	0.0000	0.0001			
07/31/07	50	0.0015	0.0014	0.0015	0.0001			
07/31/07	100	0.0025	0.0025	0.0025	0.0000			
07/31/07	500	0.0125	0.0119	0.0122	0.0004			
07/31/07	1000	0.0245	0.0219	0.0232	0.0018			

07/31/07	6000	0.1448	0.1226	0.1337	0.0157	2.222E-05	5.140E-04	0.99997
08/01/07	0	0.0013	0.0010	0.0000	0.0002			
08/01/07	50	0.0010	0.0010	0.0010	0.0000			
08/01/07	100	0.0022	0.0017	0.0020	0.0004			
08/01/07	500	0.0111	0.0098	0.0105	0.0009			
08/01/07	1000	0.0220	0.0179	0.0200	0.0029			
08/01/07	6000	0.1248	0.1045	0.1147	0.0144	1.907E-05	3.511E-04	0.99995
08/07/07	0	0.0015	0.0011	0.0000	0.0003			
08/07/07	50	0.0006	0.0009	0.0008	0.0002			
08/07/07	100	0.0017	0.0017	0.0017	0.0000			
08/07/07	500	0.0096	0.0084	0.0090	0.0008			
08/07/07	1000	0.0199	0.0162	0.0181	0.0026			
08/07/07	6000	0.1184	0.0926	0.1055	0.0182	1.758E-05	8.417E-05	0.99999
08/08/07	0	0.0009	0.0009	0.0000	0.0000			
08/08/07	50	0.0008	0.0011	0.0010	0.0002			
08/08/07	100	0.0019	0.0018	0.0019	0.0001			
08/08/07	500	0.0095	0.0091	0.0093	0.0003			
08/08/07	1000	0.0188	0.0175	0.0182	0.0009			
08/08/07	6000	0.1103	0.1011	0.1057	0.0065	1.759E-05	2.303E-04	0.99998
08/09/07	0	0.0013	0.0014	0.0000	0.0001			
08/09/07	50	0.0014	0.0012	0.0013	0.0001			
08/09/07	100	0.0024	0.0023	0.0024	0.0001			
08/09/07	500	0.0131	0.0109	0.0120	0.0016			
08/09/07	1000	0.0264	0.0251	0.0258	0.0009			
08/09/07	6000	0.1530	0.1448	0.1489	0.0058	2.482E-05	6.883E-05	0.99997

* Absorbance values for 0 ng/L standards are actual absorbances measured. All standards including the 0 ng/L are corrected for the mean absorbance of the blank (0 ng/L) in calculating the standard curve.

Appendix C

Quality Assurance Audit Report on the Spring 2007 Walleye Project

Audit Date: June 2007
Report Date: September 24, 2007

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 contaminant analysis procedures; data recording, entry, and reduction; and QA/QC training exercises. The sample preparation for analysis procedures for the Spring 2007 Walleye Project (date of contract = May 1 - October 31, 2007) were observed by the LSRI QA Manager. 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),* three-ring notebook containing bench sheets for *GLIFWC Spring Walleye 2007,* and the sample analysis preparation procedures which occurred on August 6, 2007. One of the project chemists, along with a student assistant prepared the frozen tissue samples for digestion on August 6 following the procedures as outlined in SOPs: GLM/16, SA/11, SA/13, and SA/42. The findings are listed under the subheadings.

2. Major Findings

Spring 2007 Walleye Project [Project Notebook *Vol. 06-07-10-CNP (GLIFWC)*]

On August 1, 2007, 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 complete. The Table of Contents had been filled out, researchers names and initials were recorded on the front inside cover, project/subcontract labels with the project ID number and year were affixed to each page of the notebook, and a copy of the subcontract 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. Although the notebook contained multiple sampling years' information, each year was clearly delineated by using plastic tab dividers.
- The transfer chain-of-custody forms contained blank sections where no information had been recorded. These sections should either have a line drawn through them or the code "NA" written in the blanks to show that no further

samples were collected rather than the information being misinterpreted as not recorded.

- On page 15 versus page 19 of the project notebook, only one of the lakes sampled listed on the transfer chain-of-custody also listed the county of sample collection. The transfer chain-of-custody form on page 19 listed all the counties that the samples were collected from. Because some of the lake names are common and could be the same for different counties, the county of sample collection should also be listed (*NOTE: This suggested change would need to be decided by the sponsor, since the chain-of-custody forms originate with them*).
- In the daily diary information for grinding and processing the fish tissue, the calibration of the balance using the Class 1 weights was recorded in the notebook *Volume 05-9-26-BAL* for the entry dated 5/30/07. Some of the succeeding diary entries did not contain which notebook the calibration information was recorded in. Because there are at least three separate balance calibration notebooks located in the chemistry laboratories, it is important to delineate which balance calibration notebook was used. Rather than writing the title of the calibration notebook in the daily diary, it is recommended that the staff use labels containing the appropriate information and affix those to the daily diary entries. The recording of specific SOPs used in the analysis process was also listed in the daily diary entry. Labels listing the SOP version and number could be used in this same manner.
- All project notebook entries were dated and initialed. Errors were lined-through, initialed, and contained an explanation.
- A daily diary entry on 6/11/07 recorded a QA/QC exercise to determine if the sample dry weights changed over time. The tissue sample dry weights from three Siskiwit (plus one duplicate) and three Lower Eau Claire lake fish were measured twice, approximately 24 hours apart to measure variance due to length of drying time. It might be useful to compile this information into a spreadsheet and monitor this activity over time.
- A daily diary entry on 5/31/07 documented a mismatched fish sample tag versus the bag label. The explanation of corrective action was well detailed and resulted in appropriate resolution.
- The type of procedural blank used for sample analysis was listed in the daily diary entry of 5/31/07, but not for 6/28/07. It may be useful to list the brand of water-packed tuna purchased for use as procedural blanks.
- The sample ID number for Chippewa Lake on page 25 was missing.
- The students had formal project SOP training, as summarized in the following spreadsheet:

SOP Category	SOP Title:	SOP Number:	Version Number and/or Revision Date:	Training Year	Student Name
Personnel	*Personnel Clothing*	PER/2	No revisions	2007	Graumann, Amanda; Herstad, Hannah
Recordkeeping	*Preparing Laboratory Notebooks*	REC/9	August 23, 2005	2007	Graumann, Amanda; Herstad, Hannah
Recordkeeping	*Xeroxing Contents of Study Notebooks*	REC/12	May 31, 2006	2007	Graumann, Amanda; Herstad, Hannah

General Lab Maintenance	*Procedures for Calibrating Laboratory Balances*	GLM/12	August 31, 2005	2007	Graumann, Amanda; Herstad, Hannah
General Lab Maintenance	*Procedures for Taring and Weighing Samples Using Laboratory Balances*	GLM/16	June 7, 2006	2007	Herstad, Hannah
Neurobehavioral Toxicology	*Procedures for Determining Percent Moisture in Tissue Samples*	NT/15	October 19, 2005	2007	Graumann, Amanda; Herstad, Hannah
Sample Analysis	*Routine Labware Cleaning for Metals Analysis*	SA/8	October 25, 2005	2006/2007	Graumann, Amanda; Herstad, Hannah
Sample Analysis	*Sample Grinding for Metals Analysis*	SA/10	October 25, 2005	2006/2007	Graumann, Amanda; Herstad, Hannah
Sample Analysis	*Sample Weighing for Metals Analysis*	SA/11	October 19, 2005	2007	Graumann, Amanda; Herstad, Hannah
Sample Analysis	*Cold Vapor Mercury Determination in Biota*	SA/13	July 2, 2002	2007	Graumann, Amanda; Herstad, Hannah

- The staff members and students had received training in Quality Systems and Good Laboratory Practices. Training certificates were on file for the staff members and students.
- The laboratory SOP notebook located in Barstow Room 9 contained older versions of the project SOPs. The LSRI QA Manager replaced the older versions of SOPs with new versions within a few days of completing this audit.

Spring 2007 Walleye Project - Bench Sheets for Analysis of Samples Completed in July 2007

Reviewed the three-ring binder entitled *GLIFWC Spring Walleye 2007.*

- The study ID number appeared on all output sheets. 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 should also be written into the project notebook *Vol. 06-07-10-CNP (GLIFWC).* The bench sheets were transferred to a larger 3-ring notebook, which will need a label with the project title, notebook custodian, sponsor, and dates of the study.
- The beginning of the notebook contained a hardcopy of the color-coded labels for all the project samples. It would be helpful to have a descriptive title on this page, such as: "GLIFWC 2007 Walleye Samples Processed for Mercury Content", along with a version date. It would also be useful to have a brief summary at the end of the label hardcopy listing each lake and the number of samples processed per lake. If possible, a column could be added for when the tissue was ground for sample analysis.
- 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.

- The project chemists changed the format of the data analysis bench sheets to include a spreadsheet table listing the QC criteria (i.e., detection limits and acceptance ranges). This change began with the dataset for 7/6/07 and is very useful for accessing outlier data upon initial review of the summarized information. For the dataset that was analyzed on 7/6/07, the header on the Hg Analysis Master summarizing the QA/QC data should have the date listed.
- In analyzing the samples for tissue moisture content, approximately 32% (128/395 samples) were chosen for this parameter. The contract stated that up to 119 fillets would be tested for percent moisture, so the researchers analyzed nine more samples for this parameter. Of the 128 samples, 12.5% (n = 16 samples) were analyzed in duplicate and checked for relative percent agreement. The percent duplicate agreement for tissue moisture ranged from 99.1 -100%. Of the 128 samples, 13.3% (n = 17 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. This was recorded on preprinted bench sheets for the analyses dates of: 7/6/07, 7/11/07, 7/19/07, 7/24/07, 7/25/07, 7/27/07, 7/31/07, 8/1/07, 8/7/07, 8/8/07, and 8/9/07. Approximately 730 samples, standards, and QA/QC samples were analyzed for mercury content, which included QA/QC samples and samples that had to be rerun because they did not meet the quality assurance criteria.
- The lowest values recorded for the QA/QC analytical parameters were: percent recovery for the dorm samples – 81.3 %; relative percent agreement between duplicates - 87.1%; mean percent spike recovery – 55.2% (two sets of spike recovery samples were redigested and reanalyzed for the 7/25/07 analyses date) and the relative percent agreement for procedural blanks – 77.8 %. The minimum acceptance values established for the QA/QC analytical parameters for 2007 were: percent recovery for the dorm samples – 72.5%; relative percent agreement between duplicates – 80.5%; and mean percent spike recovery – 61.9%.
- The highest values recorded for the QA/QC analytical parameters were: percent recovery for the dorm samples – 106 %; relative percent agreement between duplicates - 100%; mean percent spike recovery – 105 % and the relative percent agreement for procedural blanks - 100%. The maximum acceptance values established for the QA/QC analytical parameters for 2007 were: percent recovery for the dorm samples – 114%; relative percent agreement between duplicates – 100%; and mean percent spike recovery – 120%.
- For some samples the %RSD (percent relative standard deviation) was greater than 5.0%, for the triplicate measurements made on the digested sample. The following samples, standards and QA/QC samples were reanalyzed for that reason:

GLIFWCSW 2007 Lake Samples Reanalyzed		
Sample ID	**% RSD per Triplicate Analyses**	**Analyses Date**
bav-714	27.71	7/6/2007
lower eau claire 12748	7.89	7/24/2007
minoqua 12682	5.44	7/24/2007
namekagon 12576	9.11	7/24/2007
namekagon 12576rerun	11.01	7/24/2007
namekagon12577	14.32	7/24/2007
turtle flam fl-12510	23.21	7/27/2007
two sisters 12823	12.79	7/27/2007
willow fl 12598	5.36	7/27/2007
caroll 12907	5.15	7/31/2007
caroll 12907rerun	7.72	7/31/2007
long-5591	6.66	8/1/2007
tsi-12828	8.94	8/1/2007
Average %RSD =	**11.18**	
Standard Deviation %RSD =	**6.99**	
13/395 = 3.29% of samples did not meet the QA/QC criteria and had to be reanalyzed.		

GLIFWCSW 2007 Standards and QA/QC Samples Reanalyzed		
Sample ID	**% RSD per Triplicate Analyses**	**Analyses Date**
tb-6-13-07	107.31	7/6/2007
tb-6-13-07	45.46	7/6/2007
ta-6-13-07	56.76	7/6/2007
6000 ng/L	20.95	7/6/2007
tb-6-13-07	37.01	7/6/2007
dorm 2-2#2	21.59	7/6/2007
calib blank	7.88	7/24/2007
50	5.79	7/24/2007
100 ng/L	7.59	7/24/2007
calib blank	94.6	7/24/2007
calib blank	12.24	7/24/2007
50	12.12	7/24/2007
dorm 2-1-1 rerun	5.71	7/24/2007
dorm 2-2-2	7.58	7/24/2007
dorm 2-2-2rerun	132.26	7/24/2007
tb-28-07	13.67	7/25/2007
blank-1	152.89	7/31/2007
50-1	8.56	7/31/2007
100-1	8.36	7/31/2007
1000-1	81.76	7/31/2007
calib blank	7.04	8/1/2007
50	11.43	8/1/2007
100 ng/L	5.31	8/1/2007
blank-1	362.36	8/1/2007
50-1	10.46	8/1/2007
calib blank	87.33	8/7/2007
Sample	10.39	8/7/2007
calib blank	11.68	8/7/2007
calib blank	11.47	8/7/2007
50	17.06	8/7/2007
100 ng/L	8.46	8/7/2007
calib blank	12.22	8/7/2007
50	14.6	8/7/2007
100 ng/L	6.32	8/7/2007

100 ng/L	6.71	8/7/2007
calib blank	6.81	8/9/2007
50	7.08	8/9/2007
pg-12733spk 1(1:2)	8.83	8/9/2007
dorm 2-2#1	6.82	8/9/2007
Average %RSD =	**37.24**	
Standard Deviation %RSD =	**65.5**	
39/330 = 11.82% of samples did not meet the QA/QC criteria and had to be reanalyzed.		
Typical daily set of standards and QA samples analyzed = 12 standards, 4 dorms, 4 duplicates, 8 spikes and 2 tuna samples.		

- On some of the mercury analyzer print-outs, data were crossed-out with no explanation, researcher initials or date of error. When a rerun of a sample was warranted, there were not always explanations as to why. Due to the limited space on the print-outs, it may be useful to write a brief note/date on the print-out and write an explanation in the project notebook when the sample measurements do not meet QA/QC criteria. This would allow the researcher to write a lengthier explanation if needed. The units of measurement for some of the calibration standards were missing on the print-outs.

Spring 2007 Walleye Project – August 6, 2007 Sample Analyses Preparation Procedures

- The project chemist and student assistant wore lab coats, safety glasses, and gloves.
- Sample vials were removed from the freezer one hour prior to processing. The vials had ID labels attached to the sides and on the caps.
- The student assistant calibrated Balance PB 303-S using Class I weights prior to weighing the tissue samples. The assistant used SOP GLM/16 to prepare the balance and weigh the samples, however, SOP GLM/12 should also be available as it contains further information on balance calibrations. Calibration weights were properly recorded in the three-ring notebook *05-9-26-BAL*.
- Assistant was careful to remove 0.2 – 0.3 g of tissue from the labeled vials for transfer into the certified clean digestion tubes. On one observed occasion, the assistant measured more that 0.3 g, and had to repeat the process. The assistant labeled a new digestion tube and removed the necessary tissue for analysis from the designated vial. All the digestion tubes had been labeled prior to their being filled with tissue samples. The assistant was careful to zero the balance with the empty digestion tube in place before adding the tissue sample.
- The tissue sample in the vials was stirred with a spatula according to SOP SA/11 to insure homogeneity. In between samples, the assistant rinsed the spatula (in use) with deionized water, then wiped it with a Kimwipe before transferring it to a beaker containing 10% nitric acid. This beaker contained three other spatulas that were rotated through when removing tissue from the vials. The spatulas were again rinsed with deionized water following the acid soaking. It would be useful to change the Kimwipe more often between samples. The assistant continually checked the balance to insure that no tissue had dropped onto the weighing pan.
- The project chemist prepared the standards according to SOP SA/42. Only the

project chemists prepare the dorm samples and standards. The volumetric flasks used for the standard preparations had been soaked in a 10% nitric acid solution. Observed that the mercury reference standard that had been received on 5/30/07 had an expiration date of 10/2008. The project chemist used an adjustable micropipette, Class A pipettes, and Nalgene squirt bottles containing deionized water to carefully prepare the standards. When filling the volumetric flasks to the bottom of the meniscus, pipettes were used to deliver the deionzed water a few drops at a time. Once the standards were prepared, the project chemist inverted the bottles ten times to insure adequate mixing.

- When preparing the dorm samples, the project chemist weighed the digestion tubes, then added 0.1 g of the powdered dorm sample.
- The SOP SA/13 should be revised to add a section about the apparatus that was developed to minimize the amount of gases released when the digestion tubes are placed in the Hot Block.

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. The *SOP NT/15 Procedures for Determining Percent Moisture in Tissue Samples* should be amended to reflect the recording of time/date for drying aluminum pans. The *SOP SA/8 Routine Labware Cleaning for Metals Analysis* should be amended to include a statement regarding the frequency of changing the Liquinox® detergent water (according to the LSRI staff member, the cleaning water is changed every fourth time when cleaning the grinding equipment). Not all of the grinding equipment pieces fit into the 0.1 M HCl solution bath, necessitating the rotation of the stainless steel bowls so they can be acid rinsed. Perhaps a larger 0.1 M HCl solution bath could be used to fully submerge in equipment in the acid solution. If this change is made, it would also need to be reflected in *SOP SA/8 Routine Labware Cleaning for Metals Analysis*. The *SOP SA/8 Routine Labware Cleaning for Metals Analysis* may need to be amended to include wording that the 0.1 M HCl solution should be remade prior to grinding the fish tissue samples. The SOPs GLM/16, SA/11, SA/13, and SA/42 should be reviewed and revised. Some of the SOPs contain the same basic information and might cause confusion when preparing the samples for analyses. The percent moisture spreadsheet could be amended to include the "n" values for total number of samples, number of samples reweighed for the drying exercise, and number of samples analyzed twice for relative percent agreement. The samples that were analyzed multiple times because of percent RSD being greater than 5% for the first analysis isn't included on the percent moisture spreadsheet.

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., Mettlers 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.

COLD VAPOR MERCURY DETERMINATION IN BIOTA

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 LIST

- Stannous Chloride, Analytical Reagent
- Magnesium Perchlorate, Anhydrous for Elemental Analysis
- 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
- Mercury Cold Vapor Analyzer
- Hollow Cathode Mercury Lamp
- Variable Autotransformer
- Neptune Dyna-Pump Model 4K
- Hot Block (Environmental Express)
- Varian SpectrAA 200 Spectrophotometer
- FIMS-100 (Perkin Elmer) Mercury Analyzer
- Labindustries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Polypropylene Digestion Cups and Covers
- Pipets/Pipettors
- Beakers
- Spatulas
- 5% (w/v) Potassium Permanganate
- 5% (w/v) Potassium Persulfate
- 10% (w/v) Hydroxylamine Hydrochloride-10%(w/v) Sodium Chloride
- 10% (w/v) Stannous Chloride-0.5M Sulfuric Acid for Spectra AA Analysis
- 0.05M Potassium Permanganate-5% (v/v) Sulfuric Acid
- 1000 ug/mL Mercuric Nitrate Stock
- 5 ug/mL Mercuric Nitrate Substock for Spectra AA Analysis
- 50 ng/mL Mercuric Nitrate Substock for Spectra AA Analysis
- 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 in Teflon Squirt Bottle

PROCEDURE

Digestion

1. 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 110°C and allow to digest for approximately 15 minutes or until all the fish tissue is dissolved.

3. Turn off the Hot Block and allow the digestion cups to cool to room temperature.

4. Add 5.0 mL of 5% potassium permanganate to each bottle in 1.0 mL increments swirling the digestion cups after each addition.

5. Add 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 cup 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 Using Varian SpectraAA 200

Instrument Conditions

Current = 3.0 mA	Wavelength = 253.7 nm
Atomic Absorption Mode (AA)	Double Beam Mode (DB)
Statistics = 99	Integration = 1.0 seconds

D_2 Background Correction with diffraction grating filter
Circulating Pump autotransformer = 70% power

Instrument Conditions for Varian SpectrAA 200

Sampling Mode = AutoMix	Wavelength = 253.7 nm
Calibration Mode = Scale Expansion	Slit Width = 1.0 nm
Measurement Mode = Integrate	Lamp Current = 3.0 mA
Replicates Standard = 20	Background Correction = BC on
Replicates Sample = 20	Cal. Zero Rate = 0
Expansion Factor 1.0	Measurement Time = 4.5 s
Minimum Reading = Disabled	Pre-Read Delay = 0 s
Smoothing = 9 pt	Vapor Type = Cold Vapor
Conc. Units = ng	Burner Height = 16.0 mm
Conc. Decimal places = 2	

1. Set the AA to the instrument conditions listed above and allow instrument warm-up time. Prepare the 10% stannous chloride/0.5 M sulfuric acid solution and the magnesium perchlorate drying tube. Attach the drying tube in the cold vapor mercury analyzer.
2. Autozero the AA by aerating deionized water through the cold vapor mercury analyzer.
3. Transfer the sample from the digestion cup to a glass bottle. Add 10 mL of hydroxylamine hydrochloride/10% sodium chloride to the digestion cup, then transfer to the glass bottle with the sample. Swirl sample until no purple or brown color remains. Rinse the digestion cup with three portions of deionized water, adding the rinse to the sample in the glass bottle each time. Be careful not to end up with the bottle more than two-thirds full.
4. Add 5.0 mL of 10% stannous chloride/0.5 M sulfuric acid to a sample and immediately attach to the mercury analyzer.
5. Measure the absorbance of the sample until the maximum absorbance is reached and begins to decline and record the maximum absorbance as the response.
6. Change the valves of the mercury analyzer to draw the mercury into a 0.05 M potassium permanganate/5% sulfuric acid trap. Purge the mercury analyzer of mercury until the absorbance reaches a minimum similar to the background absorbance.
7. Return the valves to the "analyze" position and rinse the aerator with deionized water before analyzing the next sample. Dispose of the analyzed and purged sample into an Acid Waste container.
8. Alternate analyzing the samples, standards and blanks by use of steps 3-7.
9. Neutralize the "Acid Waste" in a fume hood with ammonium hydroxide until the pH is between 6 and 10. Pour the neutralized waste down the drain with running cold water. Record the volume of waste neutralized in the Acid/Base Waste Log.
10. Collect the exhausted stocks and standards in a glass bottle identified as "Hazardous Waste - Mercuric Nitrate in % acid solutions. Corrosive Toxic." Note the start date. Each waste bottle will require an analysis before it will be accepted for disposal.

Sample Analysis Using Perkin Elmer FIMS-100 Flow Injection Mercury Analysis System

1. Prepare the following:
   Carrier Solution (3% HCl)
   Reductant Solution (5% $SnCl_2$, 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.
2. Turn on computer and printer.
3. Turn on Nitrogen (400 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 and enter a new data set name (DateProject). Be sure that the save data and print log boxes are both checked.
13. Turn clamps on the peristaltic pump rollers in order to allow pump to work.
14. Check filter compartment cover to see that it has been tightened.
15. Attach tubing from filter compartment to 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.
19. Rinse the collection tube with deionized water and place in the blank solution. Click on "analyze blank" and allow instrument time to complete triplicate analysis.

Rinse the collection 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 that 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.

1. Rinse the collection tube with deionized water and place in appropriate sample. Enter sample ID code into the appropriate field. Rinse the collection tube with DI water and place in appropriate sample. Click on "analyze sample" and allow 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.
2. 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) (~13 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:

3. Place sample collection tube, and lines from reductant and carrier solutions into beaker of deionized water.
4. Flush/clean tubing with deionized water by running FIAS two times.
5. 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.
6. Raise waste lines out of liquid in waste container so liquid does not back up.
7. Release the peristaltic pump rollers so that tubing is not compressed.
8. Detach line from cell.
9. Unscrew the filter compartment cover and, using forceps to handle filter, dry filter with a Kimwipe.
10. 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.
11. Save Excel file to floppy disk.
12. Turn off FIMS instrument, computer, nitrogen, gas and printer.
13. Record the date, project, analyst, number of injections, and time run in FIMS-100 usage record book located on top of instrument.

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.

Appendix 4

Quality Assurance Report: 2007 Field Data Collection for EPA Grant # 96540801-0

Quality Assurance Report: 2007 Field data collection for EPA Grant # 96540801-0

By:

Matt Hudson
Environmental Biologist
Great Lakes Indian Fish and Wildlife Commission
Field Manager, EPA Grant # 96540801-0

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".

Quality Assurance Summary

1. System and Performance Audits - Results from the field audit, which included an audit of field walleye collections and an audit of GLIFWC laboratory tissue processing and data collection, are described in Appendix 4A. In general, protocols for data collection and sample handling were followed well by staff observed during the audits. Minor comments were made on improving the completion of chain of custody forms, but no major problems or deviations were noted.

2. Completeness and Quality of Field Sampling Process and Data - Carry-over funds were available to analyze 300 walleye for mercury from 25 lakes in 2007 under EPA Grant # 96540801-0. 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). Because twelve fish are not typically collected from all lakes, additional lakes were selected to reach the goal of 300 fish. A total of 41 lakes were selected for sampling and a total of 395 walleye samples from 37 lakes were collected (Table 1). In addition, 15 samples of fish compost, soil and corn from a garden plot used to test the growth of plants with fish compost from the walleye mercury project were analyzed, bringing the total number of mercury analyses conducted in 2007 to 410. Results from the compost pile samples will be submitted in a separate administrative report.

Overall, sample collection and analysis exceeded project goals. 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 2007 walleye mercury project.

4. Significant Quality Assurance Problems and Recommended Solutions - No significant quality assurance problems were noted during the 2007 field sample and data collection process.

Table 1. Summary of completeness of mercury walleye collections during spring 2007 as part of EPA Grant # 96540801-0.

			Size Group					
State	County	Lake Name	12.0 to 14.9	15.0 to 17.9	18.0 to 22.0	> 22.0	Total Collected	% of Goal
WI	VILAS	ANNABELLE L	3	0	0	0	3	25%
WI	WASHBURN	BASS-PATTERSON L	3	3	3	3	12	100%
WI	VILAS	BIG ARBOR VITAE L	3	3	3	3	12	100%
WI	WASHBURN	BIRCH L	0	0	0	0	0	0%
WI	ONEIDA	BLUE L	0	0	0	0	0	0%
MI	ONTONAGON	BOND FALLS FL	0	0	0	0	0	0%
WI	VILAS	BOULDER L	2	4	2	0	8	67%
WI	FOREST	BUTTERNUT L	3	3	3	3	12	100%
WI	ONEIDA	CARROL L	3	3	0	2	8	67%
WI	VILAS	EAGLE L	3	3	3	3	12	100%
WI	VILAS	FOREST L	2	4	3	3	12	100%
WI	IRON	GILE FL	0	0	0	0	0	0%
WI	SAWYER	GRINDSTONE L	3	3	3	3	12	100%
WI	ONEIDA	ISLAND L	3	1	2	1	7	58%
WI	FOREST	JUNGLE L	3	3	3	3	12	100%
WI	VILAS	KENTUCK L	5	4	4	0	13	108%
WI	SAWYER	L CHETAC	3	3	3	3	12	100%
WI	SAWYER	L CHIPPEWA	4	2	3	3	12	100%
WI	FOREST	L METONGA	3	3	3	3	12	100%
WI	WASHBURN	L NANCY	1	3	3	3	10	83%
WI	BURNETT	LIPSETT L	1	3	3	1	8	67%
WI	VILAS	LITTLE ARBOR VITAE L	3	3	3	3	12	100%
WI	SAWYER	LITTLE ROUND L	1	3	4	3	11	92%
WI	WASHBURN	LONG L	2	5	5	0	12	100%
WI	SAWYER	LOST LAND L	3	3	3	1	10	83%
WI	DOUGLAS	LOWER EAU CLAIRE L	3	3	3	3	12	100%
WI	ONEIDA	MINOCQUA L	3	3	3	3	12	100%
WI	BAYFIELD	NAMEKAGON L	3	3	3	3	12	100%
WI	IRON	PINE L	3	1	1	0	5	42%
WI	ONEIDA	PLANTING GROUND L	2	4	3	3	12	100%
WI	ONEIDA	RAINBOW FL	3	3	3	2	11	92%
WI	BURNETT	ROONEY L	2	1	1	2	6	50%
WI	VILAS	SHERMAN L	0	0	0	0	12	100%
WI	BAYFIELD	SISKIWIT L	3	3	3	0	9	75%
WI	ONEIDA	SQUIRREL L	0	0	0	0	12	100%
WI	SAWYER	TEAL L	3	3	3	3	12	100%
WI	VILAS	TROUT L	3	3	3	3	12	100%
WI	IRON	TURTLE-FLAMBEAU FL	3	3	3	3	12	100%
WI	VILAS	TWIN L CHAIN	3	3	2	2	10	83%
WI	ONEIDA	TWO SISTERS L	3	3	3	3	12	100%
WI	ONEIDA	WILLOW FL	3	3	3	3	12	100%
		TOTAL COLLECTED	**97**	**101**	**96**	**77**	**395**	
		% OF REQUESTED	**77.0%**	**80.2%**	**76.2%**	**61.1%**	**78.4%**	

Appendix 4A

Field audits of walleye collection and tissue processing data collection for EPA Grant # 96540801-0

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.

Field Audit Form

Audit of Lab Processing of Walleye
by Shane Crumb, Environmental Biologist Aide

GLIFWC Procedure No. AD.005
Revision No. 1
Revision Date. 6/4/2004
Initial Date. 8/3/2001

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
Whole fish wt	+		4/30/07
Fillet wt	+	Reminded him to tare scale before fillet weight or account for Ziplock bag weight if subtracting	4/30/07
Frozen length	+		4/30/07
Age[b] Pine Gill	+		4/30/07

[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: Same processor as previous 4 years. Has organized system for processing fillets. Reminded him to calibrate digital scale each day & write calibration data on one of the data sheets for that day.

Section 2: Tissue Collection

Data Type	(+/-)[a]	Comments	Date Observed
fillet removal	+		4/30/07

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

General Comments:

Section 3: Sample Packaging

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

fillet	+	Very good method	4/30/06
spine	+		

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

General Comments: LSKI lab appreciates the well-packaged fillets from Shane. follows orderly & neat process

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
freezer	+	< -10°C		throughout spring sample season

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

General Comments: I check this often & told Shane to let me know if he notices something out of the ordinary on the temperature monitor

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

Data Type	(+/-)[a]	Comments	Date Observed
Data entry			
to Computer			

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

General Comments:

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
Thawing of	+		4/20/07
fish for processing			

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

General Comments:

Auditor Name: Matt Hudson

Auditor Signature: Matt Hudson Date Signed: 5/1/07

Audit of Warden Purchase
of walleye for Mercury Analysis

GLIFWC Procedure No. AD.005
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.

Field Audit Form

Section 1: Data Collection

Data Type	(+/-)[a]	Comments	Date Observed
sex	+		4/16/07
Fish Length	+		4/16/07
Tag ID	+		4/16/07
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: Make suggestion that fish purchaser make sure mercury fish tag number be written next to the appropriate fish on the catch report form so I can track fish that have been collected from the office

Section 2: Tissue Collection

Data Type	(+/-)[a]	Comments	Date Observed
Fish tissue data collection	+		4/30/07
Spine Collection	+		4/30/07

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

General Comments: Processor has organized system of collecting fillet tissue and fillet data. See Lab audit form

Section 3: Sample Packaging

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

Packaging Fillets	+		4/30/07

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

General Comments: CSRT was commented on how good a job he does at packaging the fillets because it makes them easier to process in the lab. He takes pride in how well he packages the fillets & it shows.

Section 4: Storage

Data Type	(+/-)[a]	Temp (°C)[b]	Comments	Date Observed
Freezer Storage at Minisinakwa	+	< -10°C	See below	4/23/07

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

General Comments: Assessment crew fish were stored in a freezer @ the hotel. The freezer was kept outside. It had a lock, but the freezer was unlocked. The temperature monitor showed the freezer temp. never got warmer than -10°C. But next year the freezer needs to be stored in a more secure location.

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

Data Type	(+/-)[a]	Comments	Date Observed
CoC form	+		4/16/07

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

General Comments:

Section 6: Transport

Data Type	(+/-)[a]	Comments	Date Observed
In Cooler on ice	+	Fish purchased put fish on ice immediately after purchase. Time put into freezer was also marked on COC.	4/16/07

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

General Comments:

Auditor Name: Matt Hudson

Auditor Signature: Matt Hudson Date Signed: 4/16/07

Field

Lab 5/1/07

See lab audit form