Mercury Testing of Sea Lamprey (*Petromyzon marinus*) Captured in Tributaries to Lake Superior during 2013-2014

By
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Introduction

Three life stages (adult, larva, eggs) of sea lamprey (Petromyzon marinus) were captured in Michigan and Wisconsin tributaries to Lake Superior during 2013 and 2014 and tested for mercury. The study design, results, and interpretation are detailed in the following four attachments:

1. Moses SK, Polkinghorne CP, Mattes WP, Beesley KM. 2018. Spatial and ontogenetic variation in mercury in Lake Superior sea lamprey (*Pertromyzon marinus*). Bulletin of Environmental Contamination and Toxicology. In Press.
2. Polkinghorne CN, Beesley KM, Markee TP. Total Mercury Concentrations in Sea Lamprey Collected from Rivers in Wisconsin and Michigan During Spring 2013. Analytical Report of the Lake Superior Research Institute, University of Wisconsin Superior (Superior, WI). October 25, 2013.
3. Polkinghorne CN, Beesley KM, Markee TP. Total Mercury Concentrations in Sea Lamprey Transformers Collected from Rivers in Wisconsin and Michigan During Fall 2013. Analytical Report of the Lake Superior Research Institute, University of Wisconsin Superior (Superior, WI). January 14, 2014.
4. Polkinghorne CN, Beesley KM, Markee TP. Total Mercury Concentrations in Eggs of Female Sea Lamprey Collected from Rivers in Wisconsin and Michigan During Summer 2014. Analytical Report of the Lake Superior Research Institute, University of Wisconsin Superior (Superior, WI). December 8, 2014.

Abstract

[*Excerpted from*: Moses SK, Polkinghorne CP, Mattes WP, Beesley KM. 2018. Spatial and ontogenetic variation in mercury in Lake Superior sea lamprey (Pertromyzon marinus). Bulletin of Environmental Contamination and Toxicology. In Press.]

Mercury concentrations were measured in eggs, larvae, and adult spawning-phase sea lampreys (*Petromyzon marinus*) collected in tributaries of Lake Superior to investigate spatial and ontogenetic variation. There were significant differences in mercury concentrations between all three life stages, with levels highest in adults (mean = 3.01 μg/g), followed by eggs (mean = 0.942 μg/g), and lowest in larvae (mean = 0.455 μg/g). There were no significant differences in mercury concentrations by location for any life stage or by sex in adults. Mercury was not correlated with adult or larval lamprey length or mass. Mercury levels in adult lampreys exceeded U.S. and Canadian federal guidelines for human consumption. Mercury concentrations in all life stages exceeded criteria for the protection of piscivorous wildlife, posing a threat to local fish, birds, and mammals. High mercury levels in adult lampreys combined with their semelparous life history make them a potential source of lake-derived mercury to spawning streams.

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Spatial and Ontogenetic Variation in Mercury in Lake Superior Basin Sea Lamprey (*Petromyzon marinus*)

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Abstract
Mercury concentrations were measured in eggs, larvae, and adult spawning-phase sea lampreys (*Petromyzon marinus*) collected in tributaries of Lake Superior to investigate spatial and ontogenetic variation. There were significant differences in mercury concentrations between all three life stages, with levels highest in adults (mean = 3.01 µg/g), followed by eggs (mean = 0.942 µg/g), and lowest in larvae (mean = 0.455 µg/g). There were no significant differences in mercury concentrations by location for any life stage or by sex in adults. Mercury was not correlated with adult or larval lamprey length or mass. Mercury levels in adult lampreys exceeded U.S. and Canadian federal guidelines for human consumption. Mercury concentrations in all life stages exceeded criteria for the protection of piscivorous wildlife, posing a threat to local fish, birds, and mammals. High mercury levels in adult lampreys combined with their semelparous life history make them a potential source of lake-derived mercury to spawning streams.

Keywords Sea lamprey · Mercury · Lake Superior · Ontogenetics

The sea lamprey (*Petromyzon marinus*) is an agnathan fish native to the Atlantic Ocean but invasive in the Great Lakes (Hubbs and Potter 1971). By the late 1940s, after entering through man-made locks and canals, sea lamprey spread throughout the Great Lakes, causing a series of changes in the existing fish community. Dramatic decreases in lake trout, burbot, whitefish, rainbow trout, and catostomid populations coincided with increasing densities of lamprey (Smith 1971), impacting commercial, subsistence, and recreational fisheries.

In contrast to teleost fish, the sea lamprey has three distinct life stages. Larval lamprey hatch in spawning streams where they burrow into a sandy or silty bottom and filter-feed on detritus for 3–7 years prior to undergoing transformation to the juvenile stage. These juvenile lampreys then leave their natal streams for a lake or ocean. They attach to a host fish, and by creating a hole with their rasping teeth, feed on bodily fluids, primarily blood. After 12–20 months of parasitic behavior, sea lampreys become sexually mature and migrate back to streams where they spawn and die (Hardisty and Potter 1971).

Lake trout are the preferred host species for sea lampreys in Lake Superior (Harvey et al. 2008; Bence et al. 2003). They will selectively parasitize the largest lake trout available (Swink 1991; Schneider et al. 1996). In teleost species such as lake trout, mercury increases with length and age and is especially high in blood relative to other tissues (Vander Zanden and Rasmussen 1996; Giblin and Massaro 1973). Lampreys therefore have a high potential to biomagnify and bioaccumulate mercury, but these processes have mainly been studied in teleost fishes. This, in combination with their semelparous life history, may make lampreys a significant source of lake-derived mercury to the streams in which they spawn and die.

Data on mercury dynamics in Lake Superior lampreys may lead to a better understanding of the ecology of this species, supporting the development of population control measures in the Great Lakes. Further, such information has implications for human and wildlife consumers of lamprey. Population declines in their native range led to an interest in exporting sea lampreys to Europe, where they are considered a delicacy. In addition, eggs and larvae are eaten by a variety

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of fish species, while adults are fed upon by aquatic birds and mammals (Maitland et al. 2015).

This study measured mercury concentrations in adult and larval sea lampreys and lamprey eggs in the Lake Superior Basin. Our objectives were to: (1) examine ontogenetic variability in mercury concentrations, (2) characterize spatial variation in lamprey mercury concentrations, (3) compare mercury levels in lampreys to established health protection guidelines for human and wildlife consumers, and (4) explore the potential for spawning-phase lampreys to act as a source of mercury to tributaries. To our knowledge, this is the first publication to report on ontogenetic variation in the sea lamprey from the Great Lakes or on mercury levels in lamprey eggs or larvae from Lake Superior. It also provides the first estimate of how much mercury lampreys can move between the lake and streams within the Lake Superior Basin ecosystem.

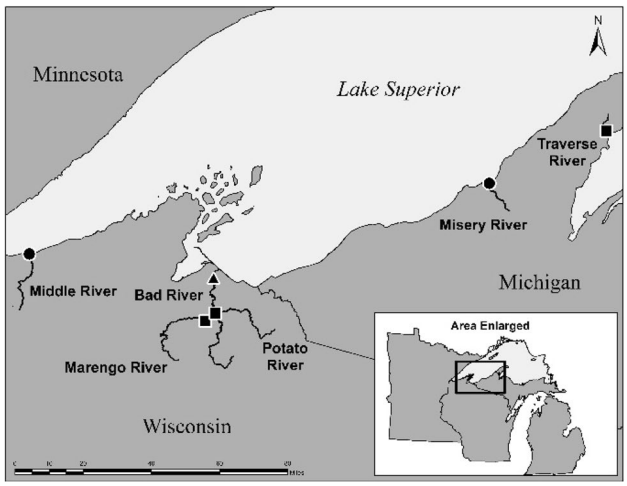

Fig. 1 Sampling locations of sea lampreys from Lake Superior tributaries (filled triangle indicates eggs, larvae, and adults; filled square indicates larvae only; black circle indicates eggs and adults only)

Materials and Methods

Adult and larval sea lampreys (*Petromyzon marinus*) were captured from Michigan and Wisconsin tributaries to Lake Superior. Collections were carried out collaboratively by the Great Lakes Indian Fish & Wildlife Commission (GLIFWC), the Bad River Band of Lake Superior Chippewa Natural Resources Department, and the U.S. Fish and Wildlife Service Sea Lamprey Control Program under the auspices of the Great Lakes Fishery Commission. Individuals were frozen within 4 h of collection and stored at − 20°C until processing. Length and mass of each lamprey were recorded in the lab.

Spawning-phase adult sea lampreys (n = 26) were collected during their spawning migration on June 6 (Middle and Bad Rivers) and July 1, 2013 (Misery River) (Fig. 1). Portable assessment traps were placed against man-made barriers to migration on the Misery and Middle Rivers and against a natural rock shelf transecting the Bad River. Sex was determined by egg expression in females or presence of a dorsal ridge in males.

Larval lampreys (n = 24) were captured between October 24 and November 8, 2013 from the Bad River system (Bad, Marengo, and Potato Rivers) and Traverse River (Fig. 1). Fyke nets used for capture were set in the lower portion of each river with cod ends facing downstream.

Eggs were recovered from spawning-phase adult female lampreys (n = 24) captured on June 12 (Misery River), June 16 (Bad River), and June 18 (Middle River), 2014 (Fig. 1). Adult lampreys were captured as described above. Frozen female lampreys were later thawed and eggs harvested through an incision made along the ventral surface from the gill slits to anus.

Prior to processing samples and between samples, all lab equipment and glassware were critically cleaned with 0.1 M hydrochloric acid and rinsed with deionized water. Whole adult and larval lampreys were partially thawed, cut into small pieces, frozen with liquid nitrogen, and ground to a fine powder with a commercial blender. Eggs were frozen with liquid nitrogen and ground in a blender. A portion of homogenized tissue was frozen in a certified clean glass vial and stored at − 20°C until analysis.

A 0.2–0.3 g portion of ground tissue was digested in 1 mL trace metal grade nitric acid plus 4 mL of trace metal grade sulfuric acid in a HotBlock™ (Environmental Express, Charleston, SC, USA) at 90 ± 5°C for 15 min. Mercury compounds were oxidized overnight with potassium permanganate and potassium persulfate. Mercuric ions in the digested samples were reduced with stannous chloride to elemental mercury and measured using a flow-injection technique (Lobring and Potter 1991) on a PerkinElmer FIMS-100 Mercury Analzyer (Waltham, MA, USA).

Blanks, duplicates, spikes, and a certified reference standard (DORM-4) from the National Research Council of Canada (Ottawa, ON, CA) were employed during analysis to ensure accurate and unbiased measurements. The detection limit for total mercury was 0.007 µg/g tissue. All mercury concentrations are reported on a wet weight basis. Duplicates, spikes, and reference materials were each analyzed nine times throughout the sample analysis and are reported here as mean ± 1 standard deviation. Relative percent difference for duplicates was 7.7% ± 8.2%. Spike recoveries were 87.8% ± 11.8%. DORM-4 results were 87.5% ± 5.5% certified reference concentrations.

ANOVA was used to test for effects of life stage and location (within each life stage) on mercury concentration. A

student's *t* test was used to compare mercury concentrations between male and female adult lampreys. Statistical outliers were identified by generating box-and-whisker plots of mercury concentrations for each life stage. Two outliers were identified, one adult (8.18 µg/g) and one larva (0.734 µg/g). The outliers were removed from the data sets when performing statistical tests for comparing group means (i.e., ANOVA and student's *t* tests) but were included where descriptive statistics are provided (e.g., Table 1; Fig. 2). Mercury concentrations were log transformed to improve normality prior to analysis. Because no significant differences between sex or location were found, data were combined for all locations and sexes when comparing life stages. All analyses were carried out using the Analysis ToolPak in Excel 2016 (Microsoft, Redmond, WA, USA) with a Type I error (α) of 0.05.

Results and Discussion

Total mercury concentrations in sea lampreys collected from Lake Superior tributaries (Table 1; Fig. 2) did not differ significantly by location for any of the three life stages studied. Adult lampreys may integrate contaminant concentrations over a large area or even the entire lake because they are highly mobile and can travel great distances either free-swimming or attached to a host. Great Lakes lampreys have been tagged and recaptured over 400 km from their natal streams (Smith and Elliott 1953; MacEachen et al. 2000; Hansen et al. 2016). Mercury concentrations in eggs, which correlate to maternal lamprey mercury levels (Drevnick et al. 2006), would therefore also be expected to show little variation relative to collection location.

Larval lamprey mercury concentrations were expected to be more variable with respect to sampling location because larvae are sedentary, living within the sediments of their nursery stream for 3–7 years and rarely leaving their burrows (Hansen et al. 2016; Quintella et al. 2003; Hardisty and Potter 1971). Drevnick et al. (2006) demonstrated a positive correlation between larval lamprey mercury concentration and mercury levels in the sediment of the stream from which they were sampled in Atlantic sea lampreys, as did Linley et al. (2016) in Pacific lampreys from the Columbia River Basin. The uniformity in mercury concentrations in larval lampreys across locations in this study may be a result of similar levels of mercury in the tributaries from which they were collected. Three of the four sampling locations (Bad, Marengo, and Potato Rivers) are part of the Bad River system. Larval lampreys from the fourth sampling location, the Traverse River, had mercury levels higher than those from the Bad River system to a degree that approached statistical significance (α = 0.07).

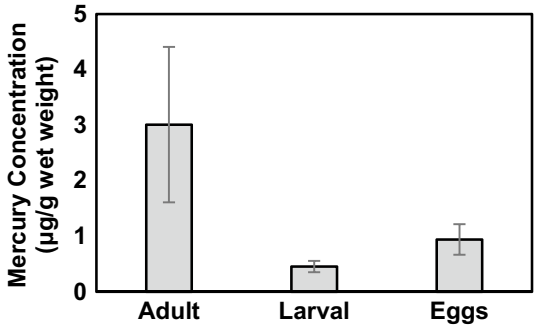

Fig. 2 Mercury concentration ± 1 standard deviation in adult spawning-phase lampreys, larval lampreys, and lamprey eggs from Lake Superior tributaries

Total mercury concentrations in sea lampreys were significantly different among the three life stages, with adults > eggs > larvae (Fig. 2). As anticipated, mercury was particularly high in adults, ranging from 1.35 to 8.18 µg/g (mean = 3.01 µg/g). Parasitic adults feed on the blood and body fluids of predatory fish for 1–2 years prior to their spawning migration (Hansen et al. 2016). Lake trout, top predators in the Great Lakes food web, are the preferred host species for lampreys in Lake Superior (Harvey et al. 2008; Bence et al. 2003). Lampreys parasitize the largest lake trout available (Swink 1991; Schneider et al. 1996). In addition to increasing with fish size, age, and trophic level (Vander Zanden and Rasmussen 1996), mercury levels in the blood of trout are higher than in other tissues (Giblin and Massaro 1973).

Mercury in adult spawning-phase sea lampreys was approximately tenfold higher than lake trout from Lake Superior, which are typically in the range of 0.1–1.0 µg/g (Zanaski et al. 2011; Bhavsar et al. 2010). This is similar to the findings of MacEachen et al. (2000) who measured lamprey mercury levels ten times that of lake trout from the same lake across the Great Lakes, although Madenjian

Table 1 Length, mass, and mercury concentrations for three life stages of sea lampreys captured in Wisconsin and Michigan tributaries to Lake Superior

Life stage	n	Length (cm)		Mass (g)		Mercury (µg/g wet weight)	
		Range	Mean ± 1 SD	Range	Mean ± 1 SD	Range	Mean ± 1 SD
Adult	26	34.0–50.5	42.4 ± 4.0	118–327	204 ± 51	1.35–8.18	3.01 ± 1.40
Larval	24	13.2–17.5	15.2 ± 1.1	3.24–8.62	5.61 ± 1.29	0.284–0.734	0.455 ± 0.102
Eggs[a]	24	35.1–47.5	41.0 ± 3.4	114–241	185 ± 41	0.490–1.55	0.942 ± 0.275

[a]Length and mass for eggs are those of the female from which the eggs were harvested

et al. (2014) saw only a threefold difference from lake trout to adult lampreys in Lake Huron.

Mercury concentrations in Lake Superior lake trout are known to be greater than in those from the other Great Lakes (Zanaski et al. 2011; Bhavsar et al. 2010). This is likely the result of numerous factors including the lake's long residence time, large surface area for atmospheric deposition, and underlying geochemistry. Similarly, mercury levels in adult lampreys from the Great Lakes have been shown to be highest in Lake Superior (MacEachen et al. 2000). Although lampreys from other Great Lakes were not measured in this study, our results support the conclusion that mercury levels are particularly high in Lake Superior. Adults from Lake Superior tributaries had significantly higher mercury levels than those measured by Madenjian et al. (2014) in Lake Huron. The mean for adults from this study (3.01 µg/g) was very similar to that measured by MacEachen et al. (2000) in Lake Superior (2.28 µg/g). To our knowledge, published mercury levels in lamprey larvae or eggs from the Great Lakes are not available for comparison to the current study.

There was no significant difference found between mercury concentrations in male (n = 13; mean = 2.79 µg/g) and female (n = 13; mean = 3.23 µg/g) adult lampreys. This is in contrast to Madenjian et al. (2014, 2016), who found concentrations in males higher than females from Lake Huron tributaries, attributing the difference to higher activity and metabolic rates, and thus dietary intake, in males.

Larval lamprey mercury concentrations were lower than adults, but still relatively high, ranging from 0.284 to 0.734 µg/g (mean = 0.455 µg/g). No other studies have measured and reported mercury in larval lampreys from the Great Lakes, but similar levels were found in Atlantic sea lampreys from the Connecticut River (mean: 0.492 µg/g) (Drevnick et al. 2006). It is unknown why larvae are as high in mercury as was observed. They are sedentary, living burrowed in stream sediments and eating a diet of algae, detritus, seston and diatoms (Sutton and Bowen 1994), which should be food sources with relatively low mercury levels. This suggests that larval lampreys may be accumulating some mercury from the surrounding environment, which is supported by the finding that they tend to have mercury levels correlating to the levels in the sediments of their nursery stream (Drevnick et al. 2006).

Although mercury levels were higher in eggs than larvae, they are not high enough to contribute a significant proportion of the mercury in larval lampreys once the large growth dilution factor from eggs to larvae is taken into account. Mercury body burdens in larval lamprey are not coming from maternal transfer alone. Further, the body burden of mercury in larvae (1.06–4.10 µg) was two orders of magnitude less than in adults (218–1615 µg), suggesting the vast majority of mercury in adult lamprey is acquired during the parasitic-phase. A similar difference in mercury body burdens between larval and adult lamprey was observed in Atlantic sea lampreys (Drevnick et al. 2006).

Unlike the typical pattern observed in teleost fishes, there was no correlation between lamprey mercury concentration and either size or mass for adult or larval lampreys. Drevnick et al. (2006) saw the same lack of correlation in adults from the Atlantic coast, but saw an increase in mercury concentration with increasing size in larvae. It is possible the correlation was not evident in larvae in the current study because the larval size range (3–9 g) was narrower than that in the Atlantic study (0.1–10 g).

Mercury concentrations in eggs were intermediate to concentrations in larvae and adults (larvae < eggs < adults). This was a different ontogenetic pattern than reported by Drevnick et al. (2006) who found eggs had the lowest concentration of the three life stages and were correlated to maternal mercury levels. Mercury was higher in adult lampreys from the current study, thus maternal transfer of mercury to the eggs was likely also higher. In addition, the relative mercury concentration of eggs versus adult female lampreys was higher in this study (30%) than the Drevnick et al. (2006) study (20%). In either case the degree of maternal transfer is much greater than for teleosts, in which the mercury concentrations in eggs are generally less than 3% of adults (Niimi 1983).

Mercury in 100% of the adult lampreys measured exceeded the U.S. FDA's action limit of 1.0 µg/g, U.S. EPA's fish tissue criterion of 0.3 µg/g, and the Health Canada protection guideline of 0.5 µg/g. Human consumption of sea lampreys is rare in the Great Lakes region, although native lampreys are consumed by Europeans and by Native American tribes in the Pacific Northwest. Mercury levels in Lake Superior sea lampreys are high enough to eliminate the possibility of commercial export to these regions, where native lampreys are endangered.

Within the Great Lakes region, a more critical concern is the consumption of mercury contaminated lampreys by wildlife. Eggs and larvae are consumed by a variety of fish species, while adults are fed upon by a number of birds and mammals, such as herons, ducks, seagulls, raptors, and otters (Maitland et al. 2015). The U.S. EPA has reported mercury criteria in fish for the protection of piscivorous wildlife ranging from 77 to 330 ng/g, depending on species and environmental factors (USEPA 1997). The lower threshold was exceeded in 100% of eggs, larva, and adult lampreys in this study. All eggs, all adult lampreys, and 21 out of 24 larval lampreys exceeded the upper threshold for protection. Therefore, lampreys may pose a significant threat to piscivorous wildlife in the Lake Superior Basin.

There is little information available on the role of anadromous fish, especially non-salmonids, in mercury transport across ecosystems. The exceptionally high mercury levels in adult lampreys combined with their semelparous life history

potentially make them a considerable source of lake-derived mercury back to the spawning tributaries. Recent population estimates of spawning-phase adult lampreys in Lake Superior are ~80,000 (Adair and Sullivan 2015). Using the mean lamprey mass and mercury concentrations from the current study, the spawning migration and subsequent death of these adult lampreys represents the movement of 49.1 g of lake-derived mercury back into Lake Superior streams annually. This mercury is expected to be virtually all in the form of methylmercury (Bloom 1992; Whittle 2000). Barbiarz et al. (2012) estimated that overall annual tributary loadings of total and methylmercury into Lake Superior are 227 and 3.4 kg, respectively, which is comparable to atmospheric deposition of mercury to the lake (Cohen et al. 2004). Thus, 1.40% of the methylmercury, and 0.02% of the total mercury, that tributaries carry into Lake Superior is returned to the streams by lampreys during spawning. The contribution to the mercury budget of an individual tributary would be much greater because lampreys only return to spawn in a small percentage of Lake Superior's tributaries (Hansen et al. 2016). Lake Superior's estimated 2.7 million larval lampreys (Hansen et al. 2016; Heinrich et al. 2003) would return ~6.9 g of this mercury back to the lake when outmigrating just prior to their parasitic phase. Thus lampreys, especially adults returning to tributaries to spawn and die, are capable of moving considerable quantities of mercury between the lacustrine and riverine systems within the Lake Superior Basin.

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Declarations Supporting data for this manuscript can be found at the website of the Great Lakes Indian Fish & Wildlife Commission (http://www.glifwc.org).

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Total Mercury Concentrations in Sea Lamprey Collected from Rivers in Wisconsin and Michigan During Spring 2013

by

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Introduction

Sea lamprey (*Petromyzon marinus*) captured during the spring of 2013 from rivers in Wisconsin and Michigan 1842 ceded territory waters were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Twenty-six sea lamprey were collected from three rivers: Middle River, Bad River (Wisconsin), and Misery River (Michigan), and delivered to LSRI and analyzed.

Methods

Adult, parasitic phase lamprey (approximately 5-10 years old) returning to spawning tributaries of Lake Superior were captured by trained GLIFWC staff using portable assessment traps. At the time of capture the sex of each lamprey was determined (egg expression in females or presence of swollen dorsal ridge in males) and its total length measured. Lamprey were assigned a unique number (i.e. a fish identification number) and placed in a Ziploc freezer bag labeled with the fish identification number. The samples were frozen within 4 hours of capture. Whole body lamprey samples and associated chain-of-custody forms were transferred to the GLIFWC laboratory freezers until they were delivered to LSRI on June 14, 2013 and July 2, 2013.

Processing and analysis of whole body lampreys was not covered under any existing Quality Assurance Project Plan (QAPP), but proceeded with only minor modifications of the QAPP entitled *Great Lakes Indian Fish and Wildlife Commission Mercury Testing and Updating Tribal-Walleye Consumption Advice* approved in June 2011. Deviations to the protocols outlined in the QAPP were implementation of an alternate grinding procedure (SOP SA/38 v.2) due to the fact that the entire lamprey was homogenized (rather than just a fillet) and the fact that a LSRI QA audit was not performed. A LSRI QA audit was recently performed during the testing of the GLIFWC walleye samples on June 20, 24, and 25, 2013. That QA report was provided to GLIFWC as an appendix to the report entitled 'Total Mercury Concentrations in Muscle Tissue from Walleye, Northern Pike, and Muskellunge Collected from Inland Lakes and the Kakagon River during Spring 2013' and dated October 17, 2013.

Before processing the whole body sea lamprey samples, all glassware, utensils, and blenders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.7). The lamprey to be processed were removed from the freezer and allowed to warm to a flexible, but stiff, consistency. The samples were cut into small pieces (about ¼ inch slices) and frozen with liquid nitrogen (LSRI SOP SA/38 v.2). The frozen sample was then placed into the blender a few pieces at a time and was ground into a fine powder. Once the entire specimen was frozen and ground, the processed tissue was combined into a bowl and mixed with a spatula. A sub-sample of the processed tissue was placed into a certified clean glass vial and stored in a freezer until mercury analysis was conducted. After each lamprey sample was processed, the blender was disassembled and washed according to the blender cleaning procedure (SOP SA/8 v.7).

Commercial canned tuna fish (*Thunnus sp.*) was used as a procedural blank for this project. This procedural blank consisted of one aliquot from a can of tuna that was transferred into a sample bottle after the packing liquid was removed and the tuna was mixed thoroughly to produce a homogeneous sample. The second portion was ground in the same manner as the sea lamprey

samples. This check was made to ensure that no contamination or loss of mercury was occurring during the processing. Two procedural blanks were prepared during this project. They were prepared on the first and the last day that the sea lamprey were processed.

Lamprey tissues were weighed for mercury analysis following standard laboratory procedure (SOP SA/11 v.6). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following the procedures in SOP SA/42 v.2. Selected samples were spiked with 1 mL of 500 µg/L mercury sub-stock solution. This spiking level is higher than the standard spiking value to compensate for the high concentrations of mercury in the lamprey samples. Mercury analyses were performed using cold vapor mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/49 v.2). All lamprey samples were diluted 1 part sample to 4 parts blank to ensure that the absorbance values would fall within the standard curve. 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 digested sample were analyzed in an attempt to obtain an RSD of less than 5%. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37 v.1. The biota method detection limit was 0.007 µg Hg/g for an average sample mass of 0.21 g (Appendix A). This limit of detection was determined using a ground tuna sample (9-19-12) containing a low concentration of mercury (SOP SA/35 v.1).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP SA/51 v.4). A portion (1 to 5 g) of ground tissue was placed into a dried and 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 approximately 23-92 hours. Six of the lamprey samples analyzed for mercury had moisture content determined, with one of these samples being analyzed in duplicate.

Data Quality Assessment

Data quality was assessed using four data quality indicators: 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 (*Squalus acanthias*) from the Canadian government (certified reference material from National Research Council Canada, Ottawa, Ontario, Canada) that has a certified concentration of mercury to measure analytical accuracy; duplicate analysis of fish tissue from the same fillet to measure analytical precision; and analysis of tissue with known additions of mercury to determine spike recovery and possible analytical interferences. Two sets of analytical standards with known amounts of mercury were analyzed with each group (maximum of 40 samples plus QA samples) of tissue samples. The concentrations of the mercury standards analyzed with each set of samples were 0, 100, 500, 1000, 5000, and 10,000 ng Hg/L. Standards 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 A).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the limits established in the Quality Assurance Project

Plan (QAPP) approved in June 2011. Results for the procedural blanks were considered acceptable when the relative percent difference was < 50%. Duplicate agreement values were acceptable when having a relative percent difference < 25%. The acceptable range for the daily mean value for the DORM standard reference material was 75 to 125% of certified value. Prior to digestion, tissues from ten percent of the lamprey 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 the calculated daily mean recovery was 70 to 130% of the spike.

Results of Fish Tissue Analyses

Quality Assurance – Two tuna procedural blanks were processed coincident with the processing of sea lamprey collected for the project. Both procedural blanks were digested with the set of mercury samples resulting in a mean of 56.1 ± 38.8 relative percent difference (Table 1). The relative percent difference values ranged from 28.6 to 83.5%. Both of the procedural blanks had values at or above the detection limit but below the limit of quantitation. This is likely to have resulted in the higher percent difference value found for one of those samples.

Analysis of dogfish shark tissue DORM-4 was conducted concurrently with lamprey tissue analysis (Table 2). The certified mercury concentration for the dogfish tissue was 0.410 ± 0.053 µg Hg/g. The individual recovery values ranged from 85.7 to 95.7% with the mean and standard deviation of the recoveries being 90.1 ± 5.1 percent of the certified value. The DORM-4 reference sample daily mean value was within the acceptance range.

Sea lamprey were analyzed for mercury in duplicate three times. Two portions of the same tissue were digested and analyzed independently. The relative percent difference between duplicate analyses of the same tissue ranged from 4.6 to 13.0% with the average and standard deviation of the differences being 9.9 ± 4.6% (Table 3).

Samples of tissue were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the three spiked samples was 97.8 ± 14.4 percent with the reported individual average recovery values ranging from 85.8 to 109.5% (Table 4).

Mercury Analysis – Samples from homogenized whole bodies of 26 sea lamprey collected from a total of three rivers in Wisconsin and Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 1.35 to 8.18 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in six of the 26 sea lamprey. Moisture analysis took place immediately following processing. The data obtained through drying and weighing the samples twice indicates that drying for 17 hours was sufficient to remove the moisture from the samples used for moisture determination. Lamprey samples had a mean moisture value of 75.6 ± 1.3 percent (Table 6). Of the six tissues analyzed for moisture, one was analyzed in duplicate, yielding a relative percent difference of 0.0 percent. Two samples were dried a minimum of an additional 24 hours and reweighed to ensure dryness, both yielding relative percent differences of 0.0 percent.

Table 1. Relative Percent Difference of Total Mercury for Procedural Blank Samples (Before and After Grinding). Data quality indicator for laboratory bias is <50% relative percent difference.

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Difference
10/10/2013	7/25/2013	0.007[Q]	0.018[Q]	0.013[Q]	83.5
10/10/2013	8/1/2013	0.012[Q]	0.016[Q]	0.014[Q]	28.6
			Mean ± Std. Dev.		**56.1 ± 38.8**

[Q] Concentration below the limit of quantitation (0.025 µg Hg/g tissue).

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-4) Analyzed during Sea Lamprey Analysis. The Standard Reference Material has a Certified Mercury Concentration of 0.410 ± 0.053 µg Hg/g Tissue. Data quality indicator for accuracy is 75.0 to 125% agreement between the certified concentration and the daily mean value for the reference standard.

	DORM 4-1		DORM 4-2		DORM 4-3		
Date of Analysis	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	Mean
10/10/2013	0.352	85.7	0.392	95.7	0.365	89.0	90.1
					Mean ± Std. Dev.		**90.1 ± 5.1**

Table 3. Relative Percent Difference for Duplicate Analysis of Total Mercury Content in Sea Lamprey. Data quality indicator for precision is <25% relative percent difference.

Date of Analysis	Sample Location and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
10/10/2013	Bad River Falls BRF-8	2.660	2.785	2.723	4.6
10/10/2013	Middle River MR-6	1.645	1.855	1.750	12.0
10/10/2013	Misery River MR-06	2.420	2.125	2.273	13.0
			Mean ± Std. Dev.		**9.9 ± 4.6**

Table 4. Percent of Mercury Recovered from Sea Lamprey Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy is a mean spike recovery of 70 to 130%.

Date of Analysis	Sample Location and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
10/10/2013	Bad River Falls BRF-8	122.3	96.7	109.5	18.1
10/10/2013	Middle River MR-6	98.8	97.3	98.0	1.04
10/10/2013	Misery River MR-06	94.5	77.1	85.8	12.3
		Mean ± Std. Dev.		**97.8 ± 14.4**	

Table 5. Total Mercury Concentration (Wet Weight) of Sea Lamprey Captured during the Spring of 2013.

Analysis Date	Sample Location	Tag Number	County	Fresh Length (mm)	Sex	µg Hg/g
10/10/2013	Bad River Falls	BRF-1	Ashland	505	M	1.35
10/10/2013	Bad River Falls	BRF-2	Ashland	401	M	2.07
10/10/2013	Bad River Falls	BRF-4	Ashland	420	F	2.70
10/10/2013	Bad River Falls	BRF-5	Ashland	426	F	3.43
10/10/2013	Bad River Falls	BRF-6	Ashland	432	M	2.31
10/10/2013	Bad River Falls	BRF-8	Ashland	438	F	2.72
10/10/2013	Bad River Falls	BRF-9	Ashland	434	M	3.71
10/10/2013	Bad River Falls	BRF-11	Ashland	427	F	8.18
10/10/2013	Bad River Falls	BRF-12	Ashland	397	F	2.24
10/10/2013	Middle River	MR-1	Douglas	480	M	4.41
10/10/2013	Middle River	MR-3	Douglas	467	M	3.33
10/10/2013	Middle River	MR-4	Douglas	390	F	1.58
10/10/2013	Middle River	MR-5	Douglas	430	F	4.77
10/10/2013	Middle River	MR-6	Douglas	410	F	1.75
10/10/2013	Middle River	MR-7	Douglas	422	M	2.95
10/10/2013	Middle River	MR-8	Douglas	430	M	3.58
10/10/2013	Middle River	MR-9	Douglas	500	F	4.94
10/10/2013	Middle River	MR-10	Douglas	378	M	2.32
10/10/2013	Misery River	MR-01	Ontonagon	459	M	1.95
10/10/2013	Misery River	MR-02	Ontonagon	389	M	2.89
10/10/2013	Misery River	MR-03	Ontonagon	403	M	2.84
10/10/2013	Misery River	MR-04	Ontonagon	439	M	2.50
10/10/2013	Misery River	MR-06	Ontonagon	449	F	2.27
10/10/2013	Misery River	MR-07	Ontonagon	416	F	2.87
10/10/2013	Misery River	MR-08	Ontonagon	394	F	2.74
10/10/2013	Misery River	MR-09	Ontonagon	342	F	1.85

Table 6. Percent Moisture in Sea Lamprey (Measured Immediately after Grinding).

Date	Sample Location	Tag Number	Species	Pan ID		Percent Moisture	Relative Percent Difference
7/25/2013	Bad River Falls	6	Lamprey	78		76.6	
7/25/2013	Bad River Falls	12	Lamprey	79		75.1	
7/25/2013	Middle River	5	Lamprey	80		74.0	
7/25/2013	Middle River	5	Lamprey	81	DUP	74.1	0.0
7/25/2013	Middle River	1	Lamprey	82		76.8	
8/1/2013	Misery River	3	Lamprey	83		77.4	
8/1/2013	Misery River	1	Lamprey	84		75.4	
				Mean and Std. Dev.		**75.6 ± 1.3**	

Appendix A

Determination of 2013 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using a ground tuna sample from September 19, 2012

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
Tuna 19 Sept 2012 -1	ground tuna	135.9	6.80	0.213	0.032
Tuna 19 Sept 2012 -2	ground tuna	139.3	6.96	0.204	0.034
Tuna 19 Sept 2012 -3	ground tuna	152.7	7.63	0.207	0.037
Tuna 19 Sept 2012 -4	ground tuna	162.7	8.14	0.214	0.038
Tuna 19 Sept 2012 -5	ground tuna	162.7	8.14	0.207	0.039
Tuna 19 Sept 2012 -6	ground tuna	149.3	7.47	0.210	0.036
Tuna 19 Sept 2012 -7	ground tuna	142.6	7.13	0.213	0.033
Tuna 19 Sept 2012 -8	ground tuna	146.0	7.30	0.211	0.035
				Mean	0.0355
				Std. Dev.	0.00245

2013 LOD = Std. Dev. x t = 0.00245 x 2.998 = 0.0073
2013 LOQ = 10/3 x LOD = 0.0245

May 1, 2013	**Hg LOD= .0073 µg/g LOQ= 0.0245 µg/g**
May 31, 2012	**Hg LOD = 0.0030 µg/g LOQ = 0.0099 µg/g**
2011	**Hg LOD=0.0017µg/g LOQ=0.0057µg/g**
2010	**Hg LOD = 0.00459 µg/g LOQ = 0.0153 µg/g**
2009	**Hg LOD = 0.00660 µg/g LOQ = 0.0220 µg/g**
2008	**Hg LOD = 0.0126 µg/g LOQ = 0.0421 µg/g**
2007	**Hg LOD = 0.0047 µg/g LOQ = 0.0157 µg/g**
2006	**Hg LOD = 0.0042 µg/g LOQ = 0.0141 µg/g**
2005	**Hg LOD = 0.0113 µg/g LOQ = 0.0368 µg/g**
2004	**Hg LOD = 0.0013 µg/g LOQ = 0.0042 µg/g**

Appendix B

Calibration Curve Data Generated during the Analysis of GLIFWC's 2013 Sea Lamprey. Indicators for Calibration Curves include a Slope of 2.0-3.0x$10^{-5}$and a Coefficient of Determination of >0.995.

Analysis Date	Standard Conc. ng Hg/L	Blank Corr. Abs. 1	Blank Corr. Abs. 2	Blank Corr. Mean	Standard Deviation	Slope	Y-Intercept	Correlation
10/10/2013	0	0.0002	0.0000	0.0000	0.0000	2.8878 E-05	0.001971	0.9996
10/10/2013	100	0.0033	0.0034	0.0034	0.0001			
10/10/2013	500	0.0156	0.0171	0.0164	0.0011			
10/10/2013	1000	0.0310	0.0317	0.0314	0.0005			
10/10/2013	5000	0.1519	0.1531	0.1525	0.0008			
10/10/2013	10,000	0.2892	0.2861	0.2877	0.0022			

Total Mercury Concentrations in Sea Lamprey Transformers Collected from Rivers in Wisconsin and Michigan During Fall 2013

by

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Introduction

Sea lamprey transformers (*Petromyzon marinus*) captured during the fall of 2013 from rivers in Wisconsin and Michigan ceded territory waters were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Twenty-four transformers were collected from four rivers: Marengo River, Traverse River, Potato River, and Bad River, and delivered to LSRI for mercury analysis.

Methods

Transformer phase sea lamprey were captured by trained GLIFWC staff using fyke nets. Specimens were placed in a Ziploc freezer bag and frozen within 8 hours of capture. Later, the transformers were assigned a unique identification number, thawed briefly to measure total length, and immediately refrozen in a Ziploc freezer bag labeled with the unique identification number. Whole body lamprey transformer samples and associated chain-of-custody forms were transferred to the GLIFWC laboratory freezers until they were delivered to LSRI on December 5, 2013.

Processing and analysis of whole body sea lamprey transformers was not covered under any existing Quality Assurance Project Plan (QAPP), but proceeded with only minor modifications of the QAPP entitled *Great Lakes Indian Fish and Wildlife Commission Mercury Testing and Updating Tribal-Walleye Consumption Advice* approved in June 2011. Deviations to the protocols outlined in the QAPP were implementation of an alternate grinding procedure (SOP SA/38 v.2) due to the fact that the entire lamprey was homogenized (rather than just a fillet) and the fact that a LSRI QA audit was not performed. A LSRI QA audit was recently performed during the testing of the GLIFWC walleye samples on June 20, 24, and 25, 2013. That QA report was provided to GLIFWC as an appendix to the report entitled 'Total Mercury Concentrations in Muscle Tissue from Walleye, Northern Pike, and Muskellunge Collected from Inland Lakes and the Kakagon River during Spring 2013' and dated October 17, 2013.

Before processing the whole body sea lamprey transformer samples, all glassware, utensils, and blenders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.7). The lamprey to be processed were removed from the freezer and allowed to warm to a flexible, but stiff, consistency. The samples were weighed prior to processing. The samples were frozen with liquid nitrogen (LSRI SOP SA/38 v.2). The frozen sample was then placed into the blender and was ground into a fine powder. A sub-sample of the processed tissue was placed into a certified clean glass vial and stored in a freezer until mercury analysis was conducted. After each lamprey sample was processed, the blender cup was disassembled and washed according to the blender cleaning procedure (SOP SA/8 v.7).

Commercial canned tuna fish (*Thunnus sp.*) was used as a procedural blank for this project. This procedural blank consisted of one aliquot from a can of tuna that was transferred into a sample bottle after the packing liquid was removed and the tuna was mixed thoroughly to produce a homogeneous sample. The second portion was ground in the same manner as the sea lamprey transformer samples. This check was made to ensure that no contamination or loss of mercury was occurring during the processing. One procedural blank was prepared during this project. It

was prepared on the last day that the lamprey transformers were processed.

Lamprey tissues were weighed for mercury analysis following standard laboratory procedure (SOP SA/11 v.6). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following the procedures in SOP SA/42 v.2. Selected samples were spiked with 500 µL of 500 µg/L mercury sub-stock solution. Mercury analyses were performed using cold vapor mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/49 v.2). 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 digested sample were analyzed in an attempt to obtain an RSD of less than 5%. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37 v.1. The biota method detection limit was 0.007 µg Hg/g for an average sample mass of 0.21 g (Appendix A). This limit of detection was determined using a ground tuna sample (9-19-12) containing a low concentration of mercury (SOP SA/35 v.1).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP SA/51 v.4). A portion (<1g, due to small sample size) of ground tissue was placed into a dried and 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 approximately 23-45 hours. Six of the lamprey samples analyzed for mercury had moisture content determined. There were no samples that were analyzed in duplicate due to small sample size and sample recovery.

Data Quality Assessment

Data quality was assessed using four data quality indicators: 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 (*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 lamprey tissue from the same individual to measure analytical precision; and analysis of tissue with known additions of mercury to determine spike recovery and possible analytical interferences. Two sets of analytical standards with known amounts of mercury were analyzed with the group of transformer lamprey samples. The concentrations of the mercury standards analyzed with each set of samples were 0, 100, 500, 1000, 5000, and 10,000 ng Hg/L. Standards were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). A summary table of the mercury calibration curve data is provided (Appendix A).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the limits established in the Quality Assurance Project Plan (QAPP) approved in June 2011. Results for the procedural blanks were considered acceptable when the relative percent difference was < 50%. Duplicate agreement values were acceptable when having a relative percent difference < 25%. The acceptable range for the daily mean value for the DORM standard reference material was 75 to 125% of certified value. Prior to digestion, tissues from ten percent of the lamprey 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 the calculated daily mean recovery was 70 to 130% of the spike.

Results of Fish Tissue Analyses

Quality Assurance – One tuna procedural blank was processed coincident with the processing of lamprey transformers collected for the project. The procedural blank was digested with the set of mercury samples resulting in a 10.5 percent difference between the ground and unground portions of tuna (Table 1).

Analysis of dogfish shark tissue DORM-4 was conducted concurrently with lamprey tissue analysis (Table 2). The certified mercury concentration for the dogfish tissue was 0.410 ± 0.053 µg Hg/g. The individual recovery values ranged from 82.5 to 89.5% with the mean and standard deviation of the recoveries being 85.0 ± 3.9 percent of the certified value. The DORM-4 reference sample daily mean value was within the acceptance range.

Lamprey transformers were analyzed for mercury in duplicate three times. Two portions of the same tissue were digested and analyzed independently. The relative percent difference between duplicate analyses of the same tissue ranged from 3.8 to 17.1% with the average and standard deviation of the differences being 8.6 ± 7.4% (Table 3).

Samples of tissue were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the three spiked samples was 83.0 ± 12.6 percent with the reported individual average recovery values ranging from 62.4 to 99.3% (Table 4).

Mercury Analysis – Samples from homogenized whole bodies of 24 lamprey transformers collected from a total of four rivers in Wisconsin and Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.284 to 0.734 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in six of the 24 lamprey transformers. Moisture analysis took place immediately following processing. The data obtained through drying and weighing the samples twice indicates that drying for 17 hours was sufficient to remove the moisture from the samples used for moisture determination. Lamprey samples had a mean moisture value of 75.2 ± 1.7 percent (Table 6). One sample was dried a minimum of an additional 24 hours and reweighed to ensure dryness, yielding a relative percent difference of 0.0 percent.

Table 1. Relative Percent Difference of Total Mercury for Procedural Blank Sample (Before and After Grinding). Data quality indicator for laboratory bias is <50% relative percent difference.

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Agreement	Relative Percent Difference
12/19/2013	12/18/2013	0.040	0.036	0.038	89.5	10.5
			Mean		**89.5**	**10.5**

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-4) Analyzed during Lamprey Transformer Analysis. The Standard Reference Material has a Certified Mercury Concentration of 0.410 ± 0.053 µg Hg/g Tissue. Data quality indicator for accuracy is 75.0 to 125% agreement between the certified concentration and the daily mean value for the reference standard.

	DORM 4-1		DORM 4-2		DORM 4-3		
Date of Analysis	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	Mean
12/19/2013	0.340	83.0	0.338	82.5	0.367	89.5	85.0
					Mean ± Std. Dev.		**85.0 ± 3.9**

Table 3. Relative Percent Difference for Duplicate Analysis of Total Mercury Content in Lamprey Transformers. Data quality indicator for precision is <25% relative percent difference.

Date of Analysis	Sample Location and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
12/19/2013	Traverse River 1158	0.720	0.748	0.734	3.8
12/19/2013	Potato River 1150	0.497	0.522	0.510	4.9
12/19/2013	Potato River 1168	0.456	0.384	0.420	17.1
			Mean ± Std. Dev.		**8.6 ± 7.4**

Table 4. Percent of Mercury Recovered from Lamprey Transformers Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy is a mean spike recovery of 70 to 130%.

Date of Analysis	Sample Location and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
12/19/2013	Traverse River 1158	62.4	80.5	71.5	12.8
12/19/2013	Potato River 1150	79.7	84.0	81.9	3.1
12/19/2013	Potato River 1168	92.0	99.3	95.6	5.1
		Mean ± Std. Dev.		**83.0 ± 12.6**	

Table 5. Total Mercury Concentration (Wet Weight) of Lamprey Transformers Captured during the Fall of 2013.

Analysis Date	Sample Location	Tag Number	County	Frozen (Thawed) Length (in)	Weight (g)	µg Hg/g
12/19/2013	Marengo River	1053	Ashland	6.1	6.240	0.432
12/19/2013	Marengo River	1054	Ashland	6.6	7.579	0.504
12/19/2013	Marengo River	1055	Ashland	6.9	8.615	0.423
12/19/2013	Traverse River	1156	Keweenaw	6.2	6.621	0.619
12/19/2013	Traverse River	1157	Keweenaw	6.1	7.066	0.465
12/19/2013	Traverse River	1158	Keweenaw	5.6	3.929	0.734
12/19/2013	Potato River	1139	Ashland	5.5	4.363	0.518
12/19/2013	Potato River	1140	Ashland	5.7	4.407	0.323
12/19/2013	Potato River	1141	Ashland	6.4	6.099	0.474
12/19/2013	Potato River	1142	Ashland	5.8	6.021	0.416
12/19/2013	Potato River	1144	Ashland	5.9	5.245	0.374
12/19/2013	Potato River	1145	Ashland	5.8	5.397	0.359
12/19/2013	Potato River	1149	Ashland	5.7	4.811	0.494
12/19/2013	Potato River	1150	Ashland	6.3	6.490	0.510
12/19/2013	Potato River	1151	Ashland	5.8	5.035	0.284
12/19/2013	Bad River	1159	Ashland	6.4	6.457	0.370
12/19/2013	Bad River	1160	Ashland	6.0	5.420	0.487
12/19/2013	Bad River	1162	Ashland	6.2	6.284	0.499
12/19/2013	Bad River	1163	Ashland	5.9	5.224	0.428
12/19/2013	Bad River	1164	Ashland	5.5	4.434	0.459
12/19/2013	Bad River	1166	Ashland	5.2	3.512	0.301
12/19/2013	Bad River	1167	Ashland	5.3	3.239	0.601
12/19/2013	Bad River	1168	Ashland	6.0	5.576	0.420
12/19/2013	Bad River	1173	Ashland	6.5	6.582	0.433

Table 6. Percent Moisture in Lamprey Transformers (Measured Immediately after Grinding).

Date	Sample Location	Tag Number	Percent Moisture
12/17/2013	Potato River	1144	75.7
12/17/2013	Potato River	1145	75.5
12/17/2013	Traverse River	1157	76.0
12/18/2013	Marengo River	1053	72.1
12/18/2013	Bad River	1159	77.0
12/18/2013	Bad River	1173	75.2
		Mean and Std. Dev.	75.2 ± 1.7

Appendix A

Determination of 2013 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using a ground tuna sample from September 19, 2012

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
Tuna 19 Sept 2012 -1	ground tuna	135.9	6.80	0.213	0.032
Tuna 19 Sept 2012 -2	ground tuna	139.3	6.96	0.204	0.034
Tuna 19 Sept 2012 -3	ground tuna	152.7	7.63	0.207	0.037
Tuna 19 Sept 2012 -4	ground tuna	162.7	8.14	0.214	0.038
Tuna 19 Sept 2012 -5	ground tuna	162.7	8.14	0.207	0.039
Tuna 19 Sept 2012 -6	ground tuna	149.3	7.47	0.210	0.036
Tuna 19 Sept 2012 -7	ground tuna	142.6	7.13	0.213	0.033
Tuna 19 Sept 2012 -8	ground tuna	146.0	7.30	0.211	0.035
				Mean	0.0355
				Std. Dev.	0.00245

2013 LOD = Std. Dev. x t = 0.00245 x 2.998 = 0.0073
2013 LOQ = 10/3 x LOD = 0.0245

May 1, 2013	**Hg LOD= .0073 µg/g LOQ= 0.0245 µg/g**
May 31, 2012	**Hg LOD = 0.0030 µg/g LOQ = 0.0099 µg/g**
2011	**Hg LOD=0.0017µg/g LOQ=0.0057µg/g**
2010	**Hg LOD = 0.00459 µg/g LOQ = 0.0153 µg/g**
2009	**Hg LOD = 0.00660 µg/g LOQ = 0.0220 µg/g**
2008	**Hg LOD = 0.0126 µg/g LOQ = 0.0421 µg/g**
2007	**Hg LOD = 0.0047 µg/g LOQ = 0.0157 µg/g**
2006	**Hg LOD = 0.0042 µg/g LOQ = 0.0141 µg/g**
2005	**Hg LOD = 0.0113 µg/g LOQ = 0.0368 µg/g**
2004	**Hg LOD = 0.0013 µg/g LOQ = 0.0042 µg/g**

Appendix B

Calibration Curve Data Generated during the Analysis of GLIFWC's 2013 Lamprey Transformers. Indicators for Calibration Curves include a Slope of 2.0-3.0x$10^{-5}$and a Coefficient of Determination of >0.995.

Analysis Date	Standard Conc. ng Hg/L	Blank Corr. Abs. 1	Blank Corr. Abs. 2	Blank Corr. Mean	Std. Dev.	Slope	Y-Intercept	Corr.
12/19/2013	0	0.0001	0.0001	0.0000	0.0000	2.8108 E-05	0.000834	1.0000
12/19/2013	100	0.0032	0.0031	0.0032	0.0001			
12/19/2013	500	0.0146	0.0153	0.0150	0.0005			
12/19/2013	1000	0.0288	0.0297	0.0293	0.0006			
12/19/2013	5000	0.1381	0.1486	0.1434	0.0074			
12/19/2013	10,000	0.2722	0.2896	0.2809	0.0123			

Total Mercury Concentrations in Eggs of Female Sea Lamprey Collected from Rivers in Wisconsin and Michigan During Summer 2014

by

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Introduction

Eggs from female sea lamprey (*Petromyzon marinus*) captured during the summer of 2014 from rivers in the 1842 ceded territory of Wisconsin and Michigan were analyzed for total mercury (Hg) content at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI). Twenty-four egg samples were collected from female sea lamprey, which were collected from three rivers: Misery River, Bad River, and Middle River, and delivered to LSRI for mercury analysis.

Methods

Adult, parasitic phase sea lamprey (approximately 5-10 years old) returning to spawning tributaries of Lake Superior were captured by trained GLIFWC staff using portable assessment traps. At the time of capture the sex of each lamprey was determined (egg expression in females or presence of swollen dorsal ridge in males) and its total length measured. Lamprey were assigned a unique number (i.e. a fish identification number) and placed in a Ziploc freezer bag labeled with the fish identification number. The samples were frozen within 4 hours of capture. At the GLIFWC laboratory, females were thawed and eggs were harvested through an incision made along the ventral surface from the gill slits to the anus. The egg samples were labeled with the same unique identification number as the adult they were harvested from and were sealed in Ziploc snack bags within a second Ziploc bag. Sea lamprey egg samples and associated chain-of-custody forms were transferred to the GLIFWC laboratory freezers until they were delivered to LSRI on September 4, 2014.

Processing and analysis of sea lamprey eggs was not covered under any existing Quality Assurance Project Plan (QAPP), but proceeded with only minor modifications of the QAPP entitled *Great Lakes Indian Fish and Wildlife Commission Mercury Testing and Updating Tribal-Walleye Consumption Advice* approved in June 2011. Deviations to the protocols outlined in the QAPP were implementation of an alternate grinding procedure (SOP SA/38 v.2) due to the fact that the egg sample was homogenized (rather than a fillet) and the fact that an LSRI QA audit was not performed. An LSRI QA audit was recently performed during the testing of the GLIFWC walleye, northern pike, and musky samples on August 11 and 12, 2014. That QA report was provided to GLIFWC as an appendix to the report entitled 'Total Mercury Concentrations in Muscle Tissue from Walleye, Northern Pike, and Muskellunge Collected from Inland Lakes during Spring 2014' and dated October 31, 2014.

Before processing the sea lamprey egg samples, all glassware, utensils, and blenders were cleaned according to the appropriate methods (LSRI SOP SA/8 v.7). The egg samples to be processed were removed from the freezer just prior to processing so that they remained frozen. The samples were further frozen with liquid nitrogen (LSRI SOP SA/38 v.2). The sample was then placed into the blender and was ground into a fine powder. A sub-sample of the processed tissue was placed into a certified clean glass vial and stored in a freezer until mercury analysis was conducted. After each lamprey egg sample was processed, the blender cup was disassembled and washed according to the blender cleaning procedure (SOP SA/8 v.7).

Commercial canned tuna fish (*Thunnus sp.*) was used as a procedural blank for this project. This

procedural blank consisted of one aliquot from a can of tuna that was transferred into a sample bottle after the packing liquid was removed and the tuna was mixed thoroughly to produce a homogeneous sample. The second portion was ground in the same manner as the lamprey egg samples. This check was made to ensure that no contamination or loss of mercury was occurring during the processing. One procedural blank was prepared during this project. It was prepared on the same day that the lamprey eggs were processed.

Lamprey eggs were weighed for mercury analysis following standard laboratory procedure (SOP SA/11 v.7). Mercury solutions for making tissue spikes and preparing analytical standards were prepared following the procedures in SOP SA/42 v.2. Selected samples were spiked with 500 µL of 500 µg/L mercury sub-stock solution. Mercury analyses were performed using cold vapor mercury analysis techniques on a Perkin Elmer FIMS 100 mercury analysis system (SOP SA/49 v.3 draft). 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 digested sample were analyzed in an attempt to obtain an RSD of less than 5%. Mercury concentrations and quality assurance calculations were done in Microsoft Excel according to SOP SA/37 v.1. The biota method detection limit was 0.0027 µg Hg/g for an average sample mass of 0.23 g (Appendix A). This limit of detection was determined using a ground tuna sample (8-2-13) containing a low concentration of mercury (SOP SA/35 v.1).

Moisture content of tissue was calculated using the wet and dried tissue weights (SOP SA/51 v.4). A portion (1-5 grams) of ground tissue was placed into a dried and 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 approximately 24 hours. Nine of the lamprey egg samples analyzed for mercury had moisture content determined. One of these samples was analyzed in duplicate.

Data Quality Assessment

Data quality was assessed using four data quality indicators: 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 (*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 lamprey eggs from the same individual to measure analytical precision; and analysis of eggs with known additions of mercury to determine spike recovery and possible analytical interferences. Two sets of analytical standards with known amounts of mercury were analyzed with the group of lamprey egg samples. The concentrations of the mercury standards analyzed with each set of samples were 0, 100, 500, 1000, 5000, and 10,000 ng Hg/L. Standards were prepared from a purchased 1000 ± 10 ppm mercury (prepared from mercuric nitrate) reference standard solution (Fisher Scientific, Pittsburgh, PA). A summary table of the mercury calibration curve data is provided (Appendix A).

Results for the quality assurance samples were considered acceptable when the value determined for a quality assurance sample fell within the limits established in the Quality Assurance Project Plan (QAPP) approved in June 2011. Results for the procedural blanks were considered

acceptable when the relative percent difference was < 50%. Duplicate agreement values were acceptable when having a relative percent difference < 25%. The acceptable range for the daily mean value for the DORM standard reference material was 75 to 125% of certified value. Prior to digestion, tissues from 12.5 percent of the lamprey egg 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 the calculated daily mean recovery was 70 to 130% of the spike.

Results of Lamprey Egg Analyses

Quality Assurance – One tuna procedural blank was processed coincident with the processing of lamprey eggs collected for the project. The procedural blank was digested with the set of mercury samples resulting in a 20.5 percent difference between the ground and unground portions of tuna (Table 1).

Analysis of dogfish shark tissue DORM-4 was conducted concurrently with lamprey egg analysis (Table 2). The certified mercury concentration for the dogfish tissue was 0.410 ± 0.053 µg Hg/g. The individual recovery values ranged from 78.7 to 92.8% with the mean and standard deviation of the recoveries being 87.5± 7.7 percent of the certified value. The DORM-4 reference sample daily mean value was within the acceptance range.

Lamprey eggs were analyzed for mercury in duplicate three times. Two portions of the same tissue were digested and analyzed independently. The relative percent difference between duplicate analyses of the same tissue ranged from 0.7 to 8.2% with the average and standard deviation of the differences being 4.8 ± 3.8% (Table 3).

Samples of eggs were spiked in duplicate with known concentrations of mercury prior to digestion. Mean recovery for the three spiked samples was 82.7 ± 10.1 percent with the reported individual average recovery values ranging from 72.4 to 98.0% (Table 4).

Mercury Analysis – Samples from homogenized eggs of 24 sea lamprey collected from a total of three rivers in Wisconsin and Michigan were analyzed for total mercury concentration. Total mercury concentrations on a wet weight basis (Table 5) ranged from 0.486 to 1.55 µg Hg/g (parts per million).

Tissue Moisture Analysis – Percent moisture was measured in nine of the 24 lamprey egg samples. Moisture analysis took place immediately following processing. The data obtained through drying and weighing the samples twice indicates that drying for 24 hours was sufficient to remove the moisture from the samples used for moisture determination. Lamprey egg samples had a mean moisture value of 61.7 ± 5.8 percent (Table 6). Two samples were dried a minimum of an additional 24 hours and reweighed to ensure dryness, yielding an average relative percent difference of 0.0 percent.

Table 1. Relative Percent Difference of Total Mercury for Procedural Blank Sample (Before and After Grinding). Data quality indicator for laboratory bias is <50% relative percent difference.

Analysis Date	Grinding Date	Before Grinding µg Hg/g	After Grinding µg Hg/g	Mean µg Hg/g	Relative Percent Difference
11/20/2014	11/18/2014	0.057	0.070	0.064	20.5
			Mean ± Std. Dev.		**20.5**

Table 2. Mercury Concentrations of Dogfish Shark Tissue (Standard Reference Material DORM-4) Analyzed during Lamprey Egg Analysis. The Standard Reference Material has a Certified Mercury Concentration of 0.410 ± 0.053 µg Hg/g Tissue. Data quality indicator for accuracy is 75.0 to 125% agreement between the certified concentration and the daily mean value for the reference standard.

Date of Analysis	DORM 4-1		DORM 4-2		DORM 4-3		Mean
	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	µg Hg/g	% of Certified Value	
11/20/2014	0.373	90.9	0.381	92.8	0.323	78.7	87.5
					Mean ± Std. Dev.		**87.5 ± 7.7**

Table 3. Relative Percent Difference for Duplicate Analysis of Total Mercury Content in Lamprey Eggs. Data quality indicator for precision is <25% relative percent difference.

Date of Analysis	Sample Location and Tag Number	µg Hg/g	Duplicate µg Hg/g	Mean µg Hg/g	Relative Percent Difference
11/20/2014	Misery River MIS-07	0.914	0.965	0.940	5.4
11/20/2014	Bad River BAD-06	1.510	1.520	1.515	0.7
11/20/2014	Middle River MID-07	1.010	0.930	0.970	8.2
			Mean ± Std. Dev.		**4.8 ± 3.8**

Table 4. Percent of Mercury Recovered from Lamprey Eggs Spiked with a Known Concentration of Mercury. Data quality indicator for accuracy is a mean spike recovery of 70 to 130%.

Date of Analysis	Sample Location and Tag Number	Spike #1	Spike #2	Mean Spike Recovery	Std. Dev.
11/20/2014	Misery River MIS-07	91.5	72.4	81.9	13.5
11/20/2014	Bad River BAD-06	98.0	81.9	90.0	11.4
11/20/2014	Middle River MID-07	74.7	77.8	76.3	2.2
		Mean ± Std. Dev.		**82.7 ± 10.1**	

Table 5. Total Mercury Concentration (Wet Weight) of Eggs from Lamprey Captured during the Summer of 2014.

Analysis Date	Sample Location	Tag Number	County	Fresh Length (in)	Female Mass (g)	Egg Mass (g)	µg Hg/g
11/20/2014	Misery River	MIS-02	Ontonagon	40.0	167.2	35.5	0.953
11/20/2014	Misery River	MIS-03	Ontonagon	40.0	173.3	50.7	0.781
11/20/2014	Misery River	MIS-04	Ontonagon	30.7	128.3	17.8	1.00
11/20/2014	Misery River	MIS-05	Ontonagon	42.0	236.3	54.9	0.763
11/20/2014	Misery River	MIS-06	Ontonagon	42.3	194.3	46.4	1.04
11/20/2014	Misery River	MIS-07	Ontonagon	45.5	234.5	55.6	0.940
11/20/2014	Misery River	MIS-09	Ontonagon	39.3	153.7	50.9	0.697
11/20/2014	Misery River	MIS-10	Ontonagon	38.3	159.9	34.3	0.748
11/20/2014	Bad River	BAD-01	Ashland	41.0	175.0	44.3	1.34
11/20/2014	Bad River	BAD-02	Ashland	39.2	130.0	21.9	0.967
11/20/2014	Bad River	BAD-03	Ashland	47.5	260.0	53.2	0.486
11/20/2014	Bad River	BAD-04	Ashland	45.0	193.0	43.1	0.700
11/20/2014	Bad River	BAD-05	Ashland	42.6	210.0	54.9	0.983
11/20/2014	Bad River	BAD-06	Ashland	42.0	199.0	51.1	1.52
11/20/2014	Bad River	BAD-07	Ashland	47.5	260.0	55.7	1.00
11/20/2014	Bad River	BAD-09	Ashland	42.5	180.0	31.9	1.11
11/20/2014	Middle River	MID-01	Douglas	38.0	157.8	29.5	0.532
11/20/2014	Middle River	MID-02	Douglas	36.5	146.8	39.9	0.883
11/20/2014	Middle River	MID-03	Douglas	35.0	114.0	39.6	0.744
11/20/2014	Middle River	MID-04	Douglas	37.0	157.7	40.4	0.745

11/20/2014	Middle River	MID-05	Douglas	39.0	178.0	38.1	1.55
11/20/2014	Middle River	MID-06	Douglas	41.5	213.8	43.9	1.33
11/20/2014	Middle River	MID-07	Douglas	40.0	184.8	39.3	0.970
11/20/2014	Middle River	MID-08	Douglas	45.0	240.7	48.2	0.830

Table 6. Percent Moisture in Lamprey Eggs (Measured Immediately after Grinding).

Sample Location	Species	Tag Number		Percent Moisture	Relative Percent Difference
Misery River	Lamprey (Eggs)	MIS-02		56.3	
Misery River	Lamprey (Eggs)	MIS-03		57.7	
Misery River	Lamprey (Eggs)	MIS-05		68.8	
Bad River	Lamprey (Eggs)	BAD-01		62.4	
Bad River	Lamprey (Eggs)	BAD-01	DUP	63.7	2.0
Bad River	Lamprey (Eggs)	BAD-02		55.9	
Bad River	Lamprey (Eggs)	BAD-03		56.6	
Middle River	Lamprey (Eggs)	MID-01		72.3	
Middle River	Lamprey (Eggs)	MID-02		58.0	
Middle River	Lamprey (Eggs)	MID-03		65.8	
		Mean ± Std. Dev.		**61.7 ± 5.8**	

Appendix A

Determination of 2014 Limit of Detection (LOD) and Limit of Quantitation (LOQ) using a ground tuna sample from August 2, 2013

Sample	Tissue Type	ng/L	ng Hg	g sample	ug Hg/g
Tuna 2 August 2013 -1	ground tuna	44.3	2.22	0.233	0.010
Tuna 2 August 2013 -2	ground tuna	44.3	2.22	0.240	0.009
Tuna 2 August 2013 -3	ground tuna	37.3	1.86	0.231	0.008
Tuna 2 August 2013 -4	ground tuna	40.8	2.04	0.241	0.009
Tuna 2 August 2013 -5	ground tuna	40.8	2.04	0.225	0.009
Tuna 2 August 2013 -6	ground tuna	40.8	2.04	0.238	0.009
Tuna 2 August 2013 -7	ground tuna	33.8	1.69	0.247	0.007
Tuna 2 August 2013 -8	ground tuna	33.8	1.69	0.219	0.008
				Mean	0.0086
				Std. Dev.	0.00092

2014 LOD = Std. Dev. x t = 0.00092 x 2.998 = 0.0028

2014 LOQ = 10/3 x LOD = 0.0093

LOD= 0.00275

LOQ= 0.00916

June 11, 2014	**Hg LOD =**	**0.0028 µg/g**	**LOQ =**	**0.0093 µg/g**
May 1, 2013	**Hg LOD =**	**0.0073 µg/g**	**LOQ =**	**0.0245 µg/g**
May 31, 2012	**Hg LOD =**	**0.0030 µg/g**	**LOQ =**	**0.0099 µg/g**
2011	**Hg LOD =**	**0.0017µg/g**	**LOQ =**	**0.0057µg/g**
2010	**Hg LOD =**	**0.0046 µg/g**	**LOQ =**	**0.0153 µg/g**
2009	**Hg LOD =**	**0.0066 µg/g**	**LOQ =**	**0.0220 µg/g**
2008	**Hg LOD =**	**0.0126 µg/g**	**LOQ =**	**0.0421 µg/g**
2007	**Hg LOD =**	**0.0047 µg/g**	**LOQ =**	**0.0157 µg/g**
2006	**Hg LOD =**	**0.0042 µg/g**	**LOQ =**	**0.0141 µg/g**
2005	**Hg LOD =**	**0.0113 µg/g**	**LOQ =**	**0.0368 µg/g**
2004	**Hg LOD =**	**0.0013 µg/g**	**LOQ =**	**0.0042 µg/g**

Appendix B

Calibration Curve Data Generated during the Analysis of GLIFWC's 2014 Lamprey Eggs. Indicators for Calibration Curves include a Slope of 2.0-3.0x$10^{-5}$and a Coefficient of Determination of >0.995.

Analysis Date	Standard Conc. ng Hg/L	Blank Corrected Abs. 1	Blank Corrected Abs. 2	Blank Corrected Mean	Standard Deviation	Slope	Y-Intercept	Corr.
11/20/2014	0	0.0001	0.0001	0.0000	0.0000	2.8677 E-05	0.001493	0.9999
11/20/2014	100	0.0028	0.0034	0.0031	0.0004			
11/20/2014	500	0.0159	0.0155	0.0157	0.0003			
11/20/2014	1000	0.0352	0.0311	0.0332	0.0029			
11/20/2014	5000	0.1536	0.1369	0.1453	0.0118			
11/20/2014	10,000	0.2844	0.2912	0.2878	0.0048			