GREAT LAKES INDIAN FISH & WILDLIFE COMMISSION
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• MEMBER TRIBES •

MICHIGAN	WISCONSIN		MINNESOTA
Bay Mills Community	Bad River Band	Red Cliff Band	Fond du Lac Band
Keweenaw Bay Community	Lac Courte Oreilles Band	St. Croix Chippewa	Mille Lacs Band
Lac Vieux Desert Band	Lac du Flambeau Band	Sokaogon Chippewa	

To: Neil Kmiecik, Biological Services Director
John Coleman, Environmental Section Leader

From: Sara Moses, Environmental Biologist

Date: December 2, 2010

Subject: Review of Existing Muskellunge Mercury Data

The attached report is a review of existing GLIFWC muskellunge harvest and mercury data. Included in the report are summaries of:

1. Open water spearing statistics for GLIFWC member tribes in Wisconsin (1995-present)
2. Mercury levels in muskellunge collected in WI, MN and MI (1976-present)
3. Uniformity of mercury within muskellunge fillets
4. Review of literature investigating seasonal differences in mercury levels in predatory fish
5. Summary of important points for developing a GLIFWC muskellunge mercury sampling plan

The report is intended to assist in developing a sampling scheme for future mercury testing of muskellunge by GLIFWC within the ceded territory waters.

Review of GLIFWC Muskellunge Harvest and Mercury Data
By Sara K. Moses
December 2, 2010

Introduction

Muskellunge (*Esox masquinongy*) are often harvested by GLIFWC member tribes within the ceded territories. Between 1995 and 2009, tribal members harvested 207-333 fish annually during the spring spearing season. Muskellunge are the largest predatory subsistence or game fish in Wisconsin, capable of attaining an “ultimate length” of 56 inches and potentially surpassing 50 pounds (WDNR, 2008). The Wisconsin Department of Natural Resources (WDNR) currently manages muskellunge as a trophy fish by establishing high minimum length requirements and low daily bag limits for fishers, promoting the increased presence of larger individuals within the population. These fish may live to the age of 20 years or more. They are highly piscivorous, residing at the top of the aquatic food web. As a result of their trophic status, longevity and size, muskellunge have an increased potential to bioaccumulate environmental contaminants, including mercury.

There is a scarcity of published literature documenting mercury levels in muskellunge. A search of peer reviewed literature identified only three studies that included such information. None of these studies directly tested mercury in muskellunge, but rather relied on information available in existing state and federal databases (i.e., WDNR and U.S. EPA). Flaherty et al. (2003), in an assessment of mercury exposure through fish consumption by Wisconsin ice anglers, cited a mean muskellunge mercury concentration of 0.28µg/g. The data was supplied by WDNR, but there is no information regarding the number of samples, tissue type, fish size, harvest location, or other summary statistics (e.g. range or standard error of mercury concentrations). Kamman et al. (2003) reported a mean muskellunge mercury concentration of 0.98µg/g for fish averaging 79.4cm (31.3 inches). This value represented the mean of 18 fillets (skin on versus off not specified) and was the highest level reported for the 13 freshwater species included in the study. The mercury data was collected from various state and provincial governments in northeastern North America, encompassing a region that extended east to, but did not include, the Great Lakes Region. Finally, a recent study (Rypel, 2010) compiled mercury data for fish in Wisconsin Lakes from the U.S. EPA national mercury database (http://www.epa.gov/waterscience/fish/mercurydata.html). The mean muskellunge mercury concentration was 0.845µg/g (range: 0.041-2.200µg/g), which encompassed data from 32 lakes and represented fish with a mean length of 85.4cm.

GLIFWC and its member tribes are interested in expanding the GLIFWC mercury database to include additional muskellunge records due to this species’ potential for accumulating significant concentrations of mercury, its tribal importance and the scarcity of existing muskellunge mercury data both within the existing database and within the published scientific literature. This report summarize past muskellunge harvest by GLIFWC member tribes and reviews existing GLIFWC and WDNR mercury data for this species. In addition, topics relevant to the future development of a muskellunge sampling protocol (e.g. uniformity of mercury within muskellunge fillets, seasonality of mercury levels in piscivorous lake fish) are reviewed. Highlight of the main points that should be considered when developing a GLIFWC muskellunge mercury sampling plan are included in the last section of this report.

Open Water Spearing of Muskellunge by GLIFWC Member Tribes in Northern Wisconsin

[Adapted in part from GLIFWC Administrative Report 2010-03 (Krueger, 2010)]

Between 1995 and 2009, GLIFWC member tribes harvested 207-333 muskellunge annually from northern Wisconsin lakes during the spring spearing season (Figure 1).

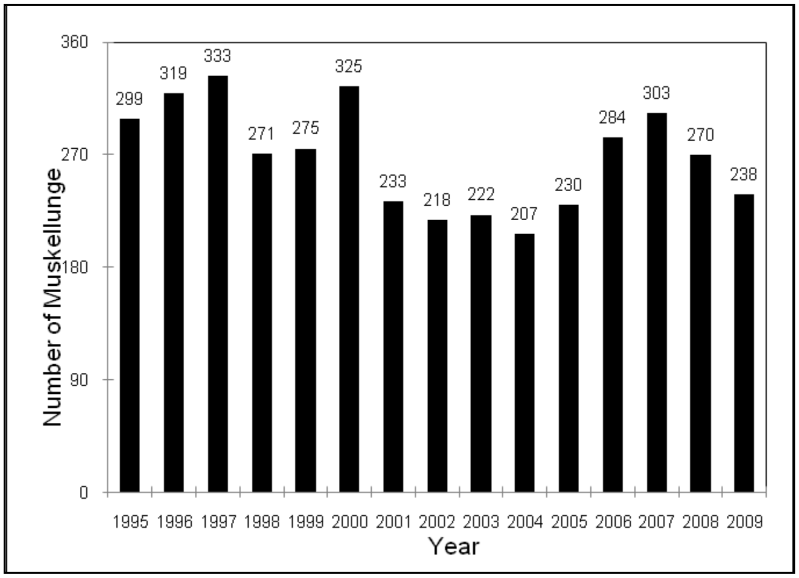

Figure 1. Number of muskellunge harvested from northern Wisconsin lakes each year from 1995-2009 by GLIFWC member tribes during the spring spearing season

Muskellunge were harvested during spring spearing from 52-73 lakes each year from 1995-2009. The annual spring harvest represented only 11.6-23.1% of the tribal muskellunge quota (Table I).

Table I. Number of lakes with tribal muskellunge quota, harvest, and percent of overall quota harvested from 1995-2009

Year	Tribal Muskellunge Quota (*n*)	Number of Lakes with Quotas	Number of Lakes with Harvest	Number of Muskellunge Harvested	Percent (%) of Quota Harvested
1995	1364	153	59	299	21.9
1996	1555	171	68	319	20.5
1997	1489	169	66	333	22.3
1998	1381	166	60	271	19.6
1999	1431	169	63	275	19.2
2000	1410	169	59	325	23.1
2001	1580	198	61	233	14.7
2002	1555	206	52	218	14.0
2003	1522	203	65	222	14.6
2004	1509	206	69	207	13.7
2005	1733	209	65	230	13.3
2006	1655	203	69	284	17.2
2007	1703	201	73	303	17.8
2008	2080	249	62	270	13.0
2009	2051	249	60	238	11.6

In 2009, the number of muskellunge harvested ranged from 1 to 19 fish per lake with harvest occurring in 60 lakes. The harvest per lake for 2009 is shown in Table II.

Table II. Muskellunge harvest by lake in 2009 with lakes ranked by number of fish harvested[1]

Harvest Ranking	County	Lake	Muskellunge Harvested (*n*)		Harvest Ranking	County	Lake	Muskellunge Harvested (*n*)
1	**Oneida**	**Pelican**	**19**		31	Oneida	Fifth	3
2	Vilas	Little Arbor Vitae	16		32	Oneida	Kawaguesaga	3
3	**Washburn**	**Shell**	**15**		33	Oneida	Sand	3
4	**Vilas**	**Big Arbor Vitae**	**12**		**34**	**Vilas**	**Big (Boulder Jct)**	**3**
5	**Oneida**	**Tomahawk**	**8**		35	Vilas	Lac Vieux Desert	3
6	**Sawyer**	**Sissabagama**	**7**		36	Bayfield	Twin Bear	2
7	Vilas	Kentuck	7		**37**	**Burnett**	**Big McKenzie**	**2**
8	Iron	Turtle Flambeau Fl	6		38	Oneida	Katherine	2
9	Oneida	Bearskin	6		39	Oneida	Sevenmile	2
10	**Sawyer**	**Lac Courte Oreilles**	**6**		40	Oneida	Squirrel	2
11	Sawyer	Whitefish	6		**41**	**Sawyer**	**Chippewa**	**2**
12	Vilas	Clear	6		**42**	**Sawyer**	**Grindstone**	**2**
13	Vilas	Upper Buckatabon	6		43	Sawyer	Spider	2
14	Barron	Sand	5		44	Vilas	Big Sand	2
15	Lincoln	Nokomis	5		45	Vilas	High	2
16	**Oneida**	**Minocqua**	**5**		46	Vilas	Papoose	2
17	Vilas	Cranberry	5		47	Washburn	Nancy	2
18	Vilas	Laura	5		**48**	**Bayfield**	**Namekagon**	**1**
19	Vilas	Lower Buckatabon	5		49	Burnett	Twenty-six	1
20	Bayfield	Middle Eau Claire	4		50	Oneida	Buckskin	1
21	Oneida	Dam	4		51	Oneida	Laurel	1
22	**Sawyer**	**Round**	**4**		52	Oneida	Muskellunge	1
23	Vilas	Ballard	4		**53**	**Sawyer**	**Lost Land**	**1**
24	**Vilas**	**Big St Germain**	**4**		**54**	**Sawyer**	**Sand**	**1**
25	**Vilas**	**N Twin**	**4**		55	Sawyer	Teal	1
26	**Vilas**	**Plum**	**4**		56	Vilas	Big (MI Border)	1
27	Vilas	White Sand	4		57	Vilas	Big Muskellunge	1
28	**Burnett**	**Yellow**	**3**		58	Vilas	Catfish	1
29	Lincoln	Alice	3		59	Vilas	Harris	1
30	Oneida	Clear	3		60	Vilas	Squaw	1

[1]Shaded entries represent lakes for which one or more muskellunge mercury records exist. See **Table IV**.

Fish length has been recorded for 5675 of the 5725 muskellunge speared between 1985 and 2009. The mean length was 37.6 inches for this entire time period. Annual averages have been similar, ranging from 35.4-39.0 inches. In 2009, 238 of the 249 muskellunge harvested were measured. Lengths ranged from 21.9-54.4 inches for individual years, with a mean annual average length of 38.8 inches. Length-frequency for 2009 as well as for the entire period from 1985-2009 is shown in Figure 2. Approximately 25% of the records (1985-2009) fell into each of the following length categories (in inches): <34, 34-37, 38-40, and ≥41.

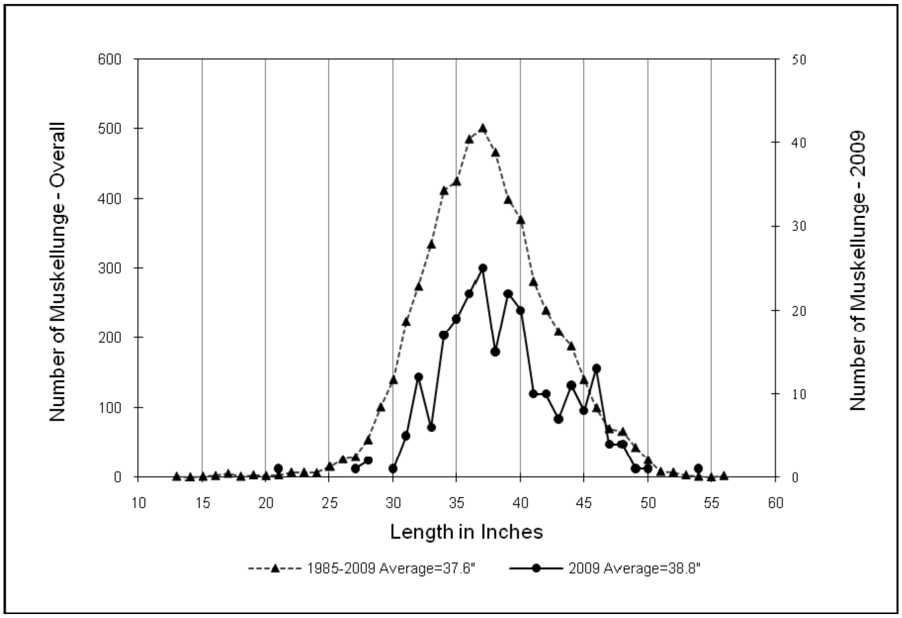

Figure 2. Length-frequency of muskellunge speared during spring 2009 and during the twenty-five year period from 1985-2009.

Existing GLIFWC and WDNR Mercury Data on Muskellunge

The number of existing muskellunge mercury records are summarized in Table III. There are currently a total of 359 records (1976-2007), with 292 records (1982-2007) within the ceded territories. Records include both skin-on and skin-off fillets.

Table III. Number of existing muskellunge mercury records sorted by presence within ceded territory, agency and state.

All Areas					**Ceded Territories Only**			
State	Total Records	GLIFWC Records	DNR Records		State	Total Records	GLIFWC Records	DNR Records
WI, MN, MI	359	99[1]	260		WI, MN, MI	292	98[1]	194
WI	297	99[1]	198		WI	280	98[1]	182
MN	44	0	44		MN	5	0	5
MI	18	0	18		MI	7	0	7

[1] Includes 4 records collected by Lac du Flambeau.

Wisconsin lakes within the ceded territories that are represented in the database (n=85) are shown in Table IV. Number of muskellunge harvested in 2009 in each of those lakes is also included for comparison. Only 19 of the 60 lakes from which muskellunge were harvested in 2009 have one or more muskellunge mercury records in the database (also see Table II).

Table IV. Number of muskellunge mercury records for Wisconsin lakes within the ceded territories, most recent record year, and number of muskellunge harvested in each lake during 2009 spring spearing (Lakes ranked by number of existing mercury records)

Lake	County	Most Recent Record Year	Hg Records (*n*)	Musky Harvest in 2009 (*n*)	Lake	County	Most Recent Record Year	Hg Records (*n*)	Musky Harvest in 2009 (*n*)
Deer L	Polk	2004	23	0	L Wausau	Marathon	1986	2	0
L Winter	Sawyer	2001	15	0	L Wissota	Chippewa	2005	2	0
Bone L	Polk	1996	13	0	Loretta L	Sawyer	1987	2	0
Shell L	Washburn	2003	11	15	Lyman L	Douglas	1987	2	0
Big McKenzie L	Burnett	2002	9	2	Pixley Fl	Price	1990	2	0
L Chippewa	Sawyer	2003	8	2	Solberg L	Price	1986	2	0
N Twin L	Vilas	2003	8	4	Wilson L	Price	1993	2	0
Potter L	Ashland	1994	8	0	Yellow L	Burnett	2003	2	3
Round L	Sawyer	2003	8	4	Amacoy L	Rusk	1986	1	0
Grindstone L	Sawyer	2001	7	2	Apple R Fl	Polk	1987	1	0
Lac Courte Oreilles	Sawyer	2001	7	6	Augustine L	Ashland	1985	1	0
Big Arbor Vitae L	Vilas	2001	6	12	Bass L	Price	1985	1	0
Big St Germain L	Vilas	2003	6	4	Big Carr L	Oneida	1982	1	0
Sissabagama L	Sawyer	2002	6	7	Big Rib R	Marathon	1989	1	0
Trout L	Vilas	2001	6	0	Caldron Falls Reservoir	Marinette	2006	1	0
Sand L	Sawyer	2001	5	1	Crowley Fl	Price	1989	1	0
Spider L	Ashland	1996	5	0	Evergreen L	Sawyer	1986	1	0
Tomahawk L	Oneida	2000	5	8	Fence L	Vilas	2001	1	0
Callahan L	Sawyer	1985	4	0	Grandfather Fl	Lincoln	1989	1	0
Namekagon L	Bayfield	2003	4	1	Greater Bass L	Langlade	1985	1	0
Pelican L	Oneida	2001	4	19	Hayward L	Sawyer	1986	1	0
Spillerberg L	Ashland	1993	4	0	Ike Walton L	Vilas	2001	1	0
Tiger Cat Fl	Sawyer	1986	4	0	Jag L	Vilas	1982	1	0
Big L (Boulder Jct)	Vilas	2002	3	3	L of the Pines	Sawyer	1988	1	0
Black L	Sawyer	1985	3	0	L Superior	Douglas	1987	1	0
Day L	Ashland	1985	3	0	Lac Sault Dore	Price	1986	1	0
Elk L	Price	1985	3	0	Little Trout L	Vilas	2001	1	0
English	Ashland	1993	3	0	Long L	Iron	1990	1	0
Little St Germaine L	Vilas	2002	3	0	Mason L	Sawyer	1986	1	0
Long L	Price	1986	3	0	Mondeaux Fl	Taylor	1985	1	0
Lost Land L	Sawyer	2003	3	1	N Spirit L	Price	1988	1	0
Mead L	Clark	1985	3	0	Pike L	Price	1989	1	0
Mineral L	Ashland	2007	3	0	Pike Chain L	Bayfield	1986	1	0
Minocqua L	Oneida	2003	3	5	Plum L	Vilas	1996	1	4
Musser L	Price	1987	3	0	Rib L	Taylor	1988	1	0
S Harper L	Taylor	1989	3	0	Round L	Price	1986	1	0

St Louis R	Douglas	1999	3	0	Spider L	Sawyer	1993	1	2
Big Muskellunge L	Vilas	1989	2	1	Spirit L	Price	1988	1	0
Butternut L	Price	2003	2	0	Squirrel L	Oneida	2002	1	2
Deerskin L	Vilas	1993	2	0	Teal L	Sawyer	2004	1	1
Duroy L	Price	1993	2	0	White Sand L	Vilas	2001	1	4
Flambeau R	Price	1990	2	0	Whitefish L	Sawyer	2003	1	6
L Hallie	Chippewa	1986	2	0					

All but one of the 359 muskellunge records in the mercury database had associated fish length information recorded. Table V contains summary statistics on length and mercury content for all records in the database as well as for the subset (n=292) collected within the ceded territories. All values over 3µg/g (n=4) occurred in either Shoepack Lake (MN) or Lake St. Clair (MI), which both lie outside the ceded territories, and were collected prior to 2000. The fish lengths represented in the database are generally less than for muskellunge typically harvested by GLIFWC member tribes in the spring.

Table V. Summary statistics on muskellunge length and mercury content for records existing in the mercury database.

Region	Statistic	Length (inches)	Mercury (µg/g)
All	Mean (±1 SD)	34.0 (±6.7)	0.78 (±0.61)
	Median	34.0	0.60
	Range	13.1-54	0.063-3.7
Ceded Territories	Mean (±1 SD)	34.7 (±6.2)	0.67 (±0.42)
	Median	34.6	0.56
	Range	18.2-54	0.086-2.9

Histograms of muskellunge length and mercury data (Table VI, Figures 3 and 4) reveal that length was relatively normally distributed among the data records, but mercury content was skewed toward the lower concentrations.

Table VI. Data percentiles for muskellunge length and fillet mercury concentration for all database records collected within the ceded territories.

Percentile	Length (Inches)	Mercury (µg/g)
100% (Max)	54	2.9
99%	50.75	1.9
95%	45.5	1.5
90%	42.75	1.2
75%	37.8	0.92
50%	34.6	0.56
25%	31	0.35
10%	26.75	0.255
5%	23.8	0.18
1%	20.8	0.1
0% (Min)	18.2	0.086

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             Histogram                        #              Boxplot
 55+*                                         1                 0
   .
   .**                                        4                 0
   .**                                        3                 0
   .***                                       6                 |
   .****                                      8                 |
   .********                                 15                 |
   .*******                                  13                 |
   .***********                              22                 |
 37+**************************               52              +-----+
   .********************                     39              *--+--*
   .*******************                      38              |     |
   .*******************                      37              +-----+
   .********                                 15                 |
   .*******                                  13                 |
   .*****                                     9                 |
   .****                                      8                 |
   .****                                      7                 0
 19+*                                         1                 0
    ----+----+----+----+----+-
    * may represent up to 2 counts
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Figure 3. Distribution of muskellunge lengths (inches) for all database records collected within the ceded territories.

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                    Histogram                              #             Boxplot
 2.9+*                                                     1                *
    .
    .
    .*                                                     1                0
    .
    .**                                                    4                0
    .****                                                  7                |
 1.5+*****                                                 9                |
    .*******                                              14                |
    .*********                                            18                |
    .*********************                                41             +-----+
    .********************                                 40             |  +  |
    .*********************************                    65             *-----*
    .*************************************                73             +-----+
 0.1+*********                                            18                |
     ----+----+----+----+----+----+----+--
     * may represent up to 2 counts
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Figure 4. Distribution of muskellunge fillet mercury concentration (µg/g) for all database records collected within the ceded territories.

Mercury-length correlations were analyzed among all records from within the ceded territories, as shown in Figure 5. Although there is a considerable amount of scatter in the data (r^2=0.143), the regression showed a highly significant relationship between muskellunge length and mercury content (F-test, p<0.0001).

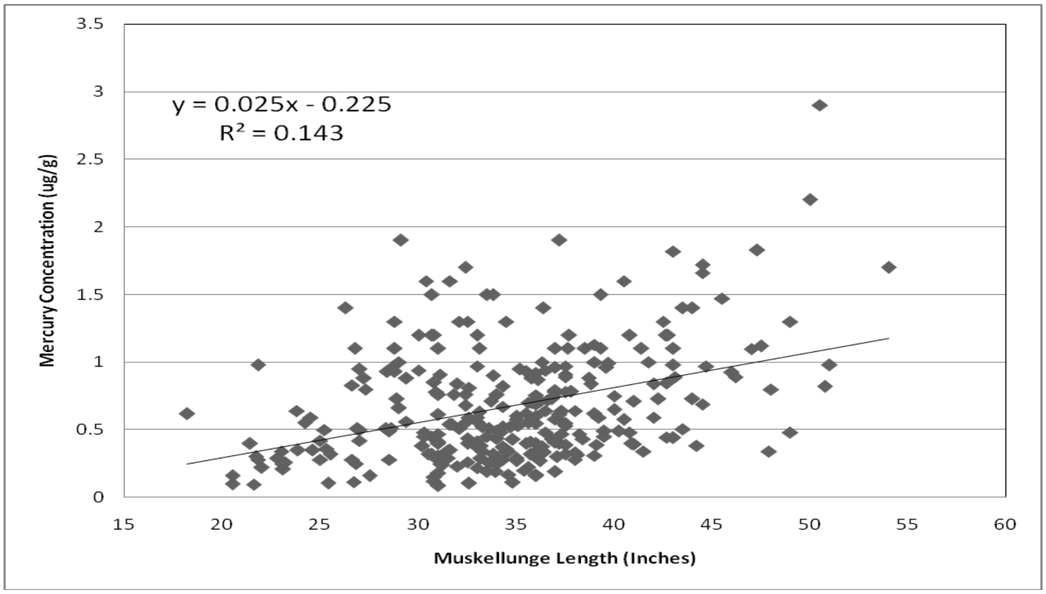

Figure 5. Muskellunge mercury content versus length for all database records collected within the ceded territories.

Uniformity of Mercury within Fish Muscle

Typically, GLIFWC provides whole fish fillets for mercury testing. These fillets are ground and homogenized prior to mercury analysis to account for any uniformity issues present. It is unlikely that spring spearers would be willing to provide whole muskellunge fillet samples to GLIFWC for contaminant testing. But, it is likely possible that a small portion of muscle can be obtained. Therefore, it is necessary to gather information on the uniformity of mercury within fish muscle fillets to estimate the sample size required to provide a mercury concentration comparable to that which would be obtained from a homogenized whole fillet.

During 1999 and 2000, fillets from 9 muskellunge were subdivided into 8 equal sections [dorsal-anterior (A), dorsal-anterior medial (B) dorsal-posterior medial (C), dorsal-posterior (D) ventral-anterior (E), ventral-anterior medial (F), ventral-posterior medial (G), ventral-posterior (H)]. The mercury concentration in each section and in a composite sample representing the entire homogenized fillet was measured (Table VII). Comparison of the mean concentrations for the eight sections with that for the composite (whole fillet) resulted in an average percent difference of 8.0%. It does not appear that any given sub-sampling location within the fillet results in consistently higher or lower concentration values. A Kruskal-Wallis one-way analysis of variance (non-parametric) revealed no significant differences were found tissues ($p = 0.904$), indicating that the mercury was uniformly distributed throughout a skin-off muskellunge fillet. The uniform mercury distribution suggests that sub-sampling a fillet for the purpose of mercury analysis would yield similar results to analyzing homogenized skin-off whole fillet samples.

Table VII. Mercury concentration (µg/g) in muskellunge whole fillets and 1/8-fillet subsections

Fish ID	Mercury Concentration (µg/g)										
	Section of Fillet									Mean (±SD) of Sections	Difference (%) Between Section Mean and Whole Filet
	A	B	C	D	E	F	G	H	Whole Fillet		
1069	0.679	0.566	0.575	0.591	0.608	-	0.707	0.540	0.491	0.609 (±0.061)	21.5
1070	0.597	0.638	0.644	0.526	0.399	0.628	0.589	0.555	0.636	0.572 (±0.081)	10.6
1084	0.616	0.515	0.534	0.555	0.618	0.579	0.471	0.520	0.552	0.551 (±0.051)	0.2
11074	0.421	0.511	0.489	0.513	0.442	0.459	0.441	0.413	0.438	0.461 (±0.039)	5.1
2176	0.362	0.395	0.315	0.357	0.375	0.373	0.326	0.630	0.397	0.358 (±0.026)	10.3
2177	0.48	0.518	0.445	0.418	0.382	0.433	0.415	0.508	0.389	0.450 (±0.048)	14.5
2179	0.44	0.418	0.457	0.416	0.433	0.354	0.344	0.368	0.388	0.404 (±0.043)	4.0
2452	0.34	0.383	0.389	0.375	0.364	0.310	0.321	0.308	0.347	0.349 (±0.033)	0.6
2453	0.410	0.366	0.392	0.358	0.297	0.378	0.323	0.344	0.376	0.358 (±0.037)	4.9
										Mean	**8.0**

Similar results were obtained for walleye (data not shown). During 1997 and 1999, fillets from 10 walleye were each divided into 4 equal sections (dorsal-anterior (A), dorsal-posterior (B), ventral-anterior (C), ventral-posterior (D)). The mercury concentration in each section was compared to each other and to the mercury concentration in the remaining homogenized fillet tissue using a one-way analysis of variance. No significant differences were found between tissues (p = 0.989).

As a follow-up to the 1997 and 1999 work which indicated that the mercury concentration was similar throughout a single walleye fillet, a smaller sub-sampling technique was tested during 2000. A “fillet plug” (~0.2 g of fillet tissue) was sub-sampled from each of 23 skin-off walleye fillets and analyzed for mercury. Subsamples were taken using a biopsy punch (5 mm diameter x 7 mm depth) prior to grinding. Three plugs were necessary for each subsample to achieve the mass needed for analysis. The mercury-plug concentration was compared to mercury concentration in the remaining fillet tissue from which the plug was collected to determine if it is necessary to use the whole filet to accurately determine the mercury concentration (Table IIX). The measured values in the biopsy plug samples were lower than the whole filet value in 16 of 20 samples. The mean percent difference between the plug and whole fillet values was 14.4%. A paired t-test revealed that the mean mercury concentration determined for the plug samples was not significantly different (p = 0.173) from the mercury concentration in the paired whole fillet samples.

Table IIX. Mercury concentration (µg/g) in walleye "fillet plug" versus whole fillet (Note: Multiple plugs were analyzed from some fish).

Fish ID	Fillet Hg Concentration (µg/g)	Plug Hg Concentration (µg/g)	Percent Difference Between Fillet and Plug
6466	0.434	0.407	6.4
6467	0.659	0.721	9.0
		0.703	6.5
		0.548	18.4
6468	1.70	1.12	41.1
2330	0.312	0.252	21.3
2331	0.405	0.378	6.9
		0.365	10.4
		0.351	14.3
2332	0.737	0.651	11.5
5048	0.256	0.218	12.0
5049	0.253	0.227	14.4
		0.233	16.7
		0.235	16.5
5050	0.354	0.254	13.8
322	0.900	0.868	12.9
		0.926	13.6
		0.700	14.0
2419	0.354	0.338	13.9
2426	1.10	1.49	14.2
		Mean	**14.4**
		Standard Deviation	**7.4**

A literature search located four published manuscripts and one state agency report describing the uniformity of mercury within fish muscle and the implications of using biopsy sized samples versus whole homogenized fillets for mercury analysis. The first study (Baker et al., 2004) evaluated mercury concentrations in whole fillets of lake whitefish (n=5) and northern pike (n=10), versus two non-lethal sampling methods: a 14-gauge biopsy needle (mean sample weight: 47mg, composite of 2 biopsies) and a 4mm dermal punch (mean sample weight: 126mg, composite of 2 punches). Both techniques provided accurate and precise measurements of mercury concentration relative to the benchmark value obtained from whole fillets, although samples <80mg did not provide reliable results.

The second study (Pearson, 2000) evaluated mercury concentrations in biopsies (anterior, dorsal, and posterior) relative to concentrations in the whole fillets of 11 walleye and 18 northern pike. Pearson concluded that mercury concentrations in biopsies and fillets from the same fish were not significantly different and that dorsal muscle biopsies were slightly more accurate predictors of fillet mercury concentrations than biopsies from the anterior or posterior areas of the fillet. Unfortunately, a copy of this report from the North Dakota Department of Health could not be obtained online. Therefore, additional information, such as biopsy size, is not available.

Cizdziel et al. (2002) had very similar conclusions to Pearson regarding the utility of muscle biopsies for mercury testing and the ideal biopsy location within the fillet. Using a 5mm biopsy punch, which provided approximately 150mg of muscle tissue, the researchers sampled individual fish of 5 different species (rainbow trout, striped bass, largemouth bass, bluegill, blue tilapia) at 27 different body locations. The results indicated a relatively uniform distribution of mercury within the dorsal muscle, with mean relative standard deviation (RSD) between 4-10%. Muscle mercury did not vary by sampling depth, with dorsal muscle plugs collected 0-3 versus 3-6mm below the skin showing no statistical difference. In addition, plugs removed with a 2mm biopsy punch (9-17mg) were compared to those taken with the 5mm punch (95-115mg). There was no statistical difference between the mean (n=3 per punch size) mercury concentrations, although the lower sample size showed greater variability. The authors suggest the mid-dorsal area approximately 1-2mm from the dorsal fin as the preferred biopsy site because this region appeared to be particularly homogenous with respect to mercury and gave mercury concentrations close to the mean value for all plugs. Single 5mm plugs from this region were compared to homogenized whole fillets in six fish. The mean relative percent difference between results was low (5.4%).

Schmitt and Brumbaugh (2007) evaluated two alternatives to fillet sampling for determining mercury concentrations in smallmouth bass (n=59). Muscle was collected with a biopsy needle (10mm x 14 gauge) and a biopsy punch (7mm diameter x 5mm depth). The biopsy needle was used three times per fish to collect approximately 50-100mg muscle. In addition, one muscle plug, approximately 150-250mg, was taken from each fish using the biopsy punch. Samples were collected in the area beneath the dorsal fin. Samples were stored in 1.8mL polyethylene cryogenic vials. Mercury concentrations in both plug and needle samples were "nearly identical" to those of the fillet from the same fish (note: authors did not test the statistical significance of the concentration differences among sample types). Plug and needle mercury values in all fish differed from the fillet values by ≤4.1%. Unlike previous studies, the researchers did not find an increased coefficient of variation for the biopsy samples relative to the plug or fillet.

Peterson et al. (2005) tested 210 fillet biopsies from 13 species for mercury. These values were compared to whole body mercury from the same fish, rather than to fillets. Although this study does not provide information on the relative mercury concentrations in the biopsies relative to whole fillets, it does provide useful information on the storage of muscle biopsy samples. As sample size decreases, sample desiccation becomes an increasing concern, drawing into question maximum sample holding times in the freezer and appropriate sample storage containers. Moisture loss from a sample could potentially affect the mercury concentration obtained upon analysis. Peterson placed biopsy samples (6mm diameter, 8mm depth) from a northern pike minnow into each of three storage containers: 1) Packard LSC 20-mL polyethylene scintillation vials, 2) I-Chem brown borosilicate 40-mL vials, and 3) I-Chem clear borosilicate 40-mL vials. Biopsies from each container type were removed at various time intervals up to 100 days and tested for mercury. There were no significant container dependent differences among the three container types within this time period.

Seasonal Variation in the Mercury Content of Fish Muscle: Literature

GLIFWC member tribes harvest muskellunge in both the winter and the spring. It is possible that mercury concentrations in fish may vary seasonally in response to factors such as changes in diet, growth efficiency, reproductive cycle, body condition or mercury methylation rate. Relevant literature was reviewed to gain insight into whether seasonal differences in mercury concentrations may exist between spring and winter harvested muskellunge. Since no information exists specifically for muskellunge, literature for other predatory freshwater fish from the U.S. is summarized.

A review of Wisconsin Department of Natural Resources (WDNR) walleye mercury data from 1982-2005 (n=3024) found a significant effect of season on mercury content in skin-on fillets (Rasmussen et al., 2007). Fish caught in the spring had 12.8% higher mercury than those caught in the summer. Fall-caught fish were slightly lower (1.5%) than summer caught fish. Ward and Neumann (1999) found similar results in largemouth bass from two lakes in Connecticut. Mean mercury concentrations in skin-off fillets adjusted for fish length were significantly (26-43%) higher during spring than summer and fall in both lakes. Fowlie et al. (2008) also observed that in the period from May-September, yellow perch from the

St. Lawrence River had the highest muscle mercury concentrations during the period from June-August. Mercury concentrations were lower in May and September. Unfortunately, winter-caught fish were not included in any of these studies.

Weis and Ashley (2007) monitored mercury in white perch (n=168) from New Jersey between February and December and observed higher mercury levels in skin-off fillets during warmer summer months (June-July). The authors hypothesize that since mercury depuration continues during winter months when food is scarce, this pattern would result in the lowest tissue mercury concentrations occurring during the winter.

The Massachusetts Department of Environmental Protection (2006) saw different seasonal mercury patterns in muscle of yellow perch (30 in each of 7 lakes) and largemouth bass (12-15 in each of 7 lakes). Mercury concentrations were generally highest in the spring and lowest in the summer and fall, with winter being intermediate.

One study (Foster et al., 2000) found no seasonal effect on mercury concentration in fish muscle. 53 largemouth bass were collected in the spring, summer and fall from an Oregon reservoir. Mercury concentrations in liver and gonad were found to vary with season in a pattern similar to that determined for muscle in the other studies.

Seasonal Variation in the Mercury Content of Muskellunge: GLIFWC Mercury Database

Since no published information is available on seasonal mercury variation in muskellunge, the existing records in the GLIFWC mercury database were examined. The number of records available for each month is shown in Table IX. Virtually no winter records exist. The mean mercury concentration and fish length for each month are shown.

Table IX. Number of muskellunge records, mean mercury concentration (± 1 SD) and mean length (± 1 SD) in the GLIFWC database by month

Month	Number of Records	Mean (±SD) Hg (µg/g)	Mean (±SD) Length (in.)
January	1	0.66	29
February	0	-----	-----
March	0	-----	-----
April	126	0.69 (±0.39)	36.4 (±4.7)
May	77	0.59 (±0.42)	36.3 (±5.7)
June	18	0.48 (±0.31)	28.3 (±5.3)
July	19	0.53 (±0.31)	29.6 (±8.7)
August	5	0.48 (±0.19)	27.5 (±4.5)
September	17	0.85 (±0.71)	33.2 (±7.8)
October	21	1.03 (±0.35)	30.7 (±5.2)
November	8	0.72 (±0.38)	37.5 (±3.5)
December	0	-----	-----

Muskellunge mercury concentrations were plotted by day of the year (Figure 6). A positive linear correlation of fish length with tissue mercury concentrations exists, as described above. In order to adjust for the effects of this covariate prior to examining mercury concentration trends over time, individual fish mercury concentrations were adjusted to the concentration of a standard-sized fish, defined as the mean fish length over all fish sampled. For the existing data set, the standard sized muskellunge is 34.7 inches. Fish-length adjusted mercury concentrations were then plotted over time to see if seasonal trends exist.

Unfortunately, only one winter sample was collected. Fish-length adjusted mercury concentrations by month are shown in Figure 7.

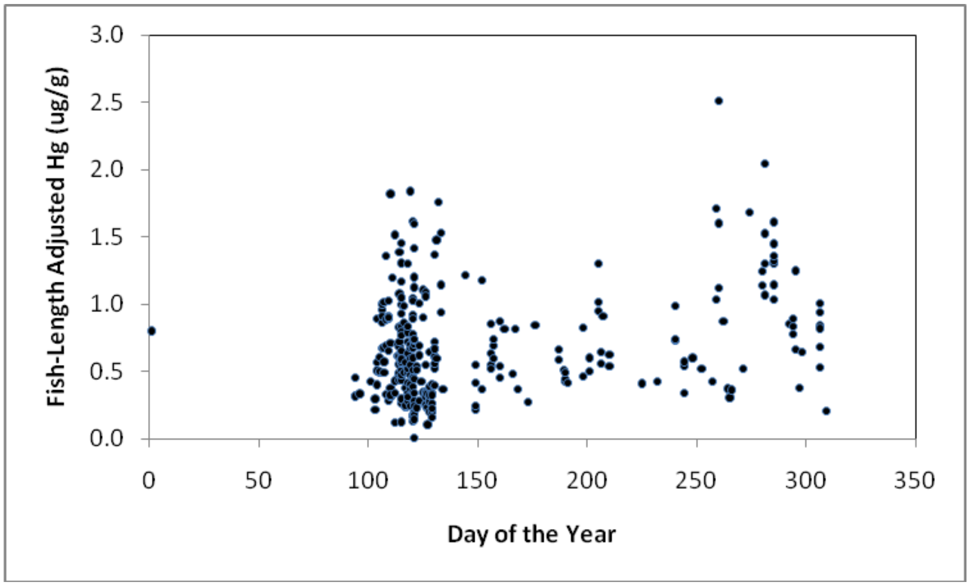

Figure 6. Muskellunge length adjusted mercury concentration in muscle versus day of the year the fish was harvested. All database recordscollected within the ceded territories (n=292) are included.

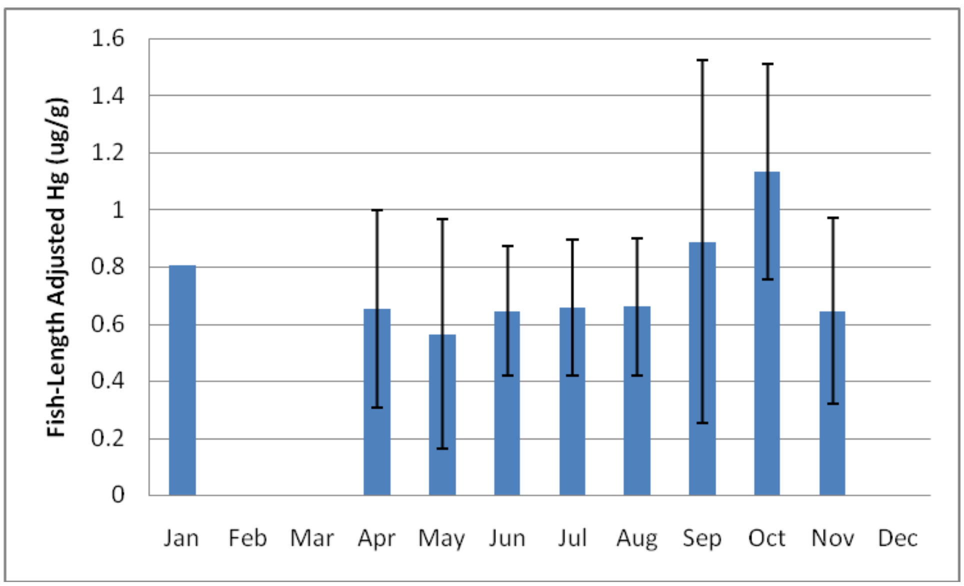

Figure 7. Muskellunge mean (±SD) length adjusted mercury concentration in muscle versus month of harvest. All database recordscollected within the ceded territories (n=292) are included.

The large variance among the available samples prevented the clear detection of seasonal trends in muskellunge mercury concentration. Figures 6 and 7 suggest that mercury may be higher in the fall than during other times of the year. Analysis of variance (ANOVA, Tukey test) of fish-length adjusted mercury concentration by month revealed that length-adjusted muskellunge mercury concentrations were greater in October than in April, May June, July or November. In addition, mercury concentration was significantly greater in September than in May. Unfortunately, the lack of winter data makes it difficult to speculate on whether seasonal variation in mercury concentrations in muskellunge would impact the interpretation of data collected during winter versus spring spearing.

Highlights for Consideration when Developing a GLIFWC Muskellunge Sampling Plan

- The muskellunge harvest represents an important tribal resource, yet little data from within the ceded territories (n=292 since 1982) exists within the GLIFWC mercury database. The data that does exist was collected by WDNR only, not from tribal harvesters, and therefore is not necessarily representative of the tribal harvest (i.e., harvest seasons, fish size, harvest lake, etc.).
- If it is possible to collect a sufficient number of samples to allow for the targeting of specific lakes, there are a number of important harvest lakes for which little or no muskellunge mercury data exists. For example, only 7 of the 19 lakes in which ≥5 muskellunge were harvested in 2009 are represented in the mercury database.
- Mercury concentration and fish length are significantly positively correlated for muskellunge records in the GLIFWC mercury database. If it is possible to collect a sufficient number of samples to allow for the targeting sample representation within specified fish size classes, historical harvest data is sufficient to assist in setting appropriate size classes. If four size classes are desired, as is the practice for the collection of walleye during spring spearing, historical data might be split into quartiles resulting in the following four target size classes (in inches): <34, 34-37, 38-40, and ≥41.
- Mercury concentrations in muskellunge from within the ceded territories are relatively high (mean = 0.56µg/g, range = 0.09-2.9µg/g) and warrant further investigation so that GLIFWC can provide accurate and appropriate information to tribal fishers and their families, allowing them to make informed fishing and consumption decisions.
- A comparison of muskellunge length for tribally harvest fish versus the fish in the GLIFWC mercury database reveals that the fish represented in the database are smaller overall than those that are harvested. Since larger fish generally have a higher mercury content, it is important to take this into consideration if muskellunge consumption advice relative to mercury exposure is to be provided to tribal members.
- Previous work conducted by GLIFWC on walleye and muskellunge as well as published literature on a number of predatory species in this region suggest that mercury is relatively uniform within muscle tissue. All results suggest that sampling a small portion of fish muscle (as little as 80mg can provide mercury concentration data that is equivalent to that obtained from the analysis of an entire homogenized fillet. This will likely allow GLIFWC scientists greater access to muskellunge samples from tribal fishers who may be less willing to sacrifice an entire fillet for mercury analysis.
- Scientific literature is inconsistent on the pattern and extent of seasonal variation in mercury in fish muscle. Most published studies have found significant differences between mercury concentrations during different seasons, although some have not. The majority of the studies detecting seasonal variation have found mercury to be the highest in fish during the warm summer months, although some have also seen higher levels in the spring. The data in the GLIFWC database suggests muskellunge mercury concentrations could be highest in the fall, although the sample size is relatively small leading to a large variance within the data. Unfortunately, few data sets include data on fish harvested in the winter. The GLIFWC database has only one record collected during the months of December-March. As a result, it is unknown what effect collecting muskellunge during the winter versus the spring spearing season might have on mercury concentrations and therefore on subsequent consumption advice for this species.

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