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This interim report is preliminary to completion of the final research project titled *Mercury in Selected Fish Species over Time*, anticipated in 2007.

Use of yellow perch to determine mercury trends in lakes – 1992-2003 Interim Report

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Introduction

Mercury (Hg) releases to the atmosphere, global cycling, and deposition to surface waters have become an issue of great scientific and public interest. In addition to direct monitoring of emissions and deposition, other media have been examined to retrospectively estimate trends in deposition over time. Schuster, et al. (2002) examined ice cores from the Fremont glacier in western Wyoming USA and found trends in Hg deposition that reflect Hg usage and cycling. The ice cores indicate that Hg deposition increased through the industrial era but decreased since the late 1980s likely due to limitations placed on some sources of Hg to the environment. These findings are consistent with temporal patterns observed in sediment cores, precipitation, the atmosphere, and other studies. A recent study by scientists from Germany, South Africa, and Canada estimated that overall atmospheric Hg levels peaked in the late 1980s, decreased from the 1980s to 1996, but have varied little since 1996 (Slemr et al. 2003). Frederick et al. (2004) investigated concentrations of methylmercury (MeHg) in feathers of some bird species spanning collection years of 1910 to 1980 and found evidence of increased Hg deposition in the 1970s. Monteiro and Furness (1997) found increases in Hg levels in ocean food chains ranging from 1.1 to 4.8% per year based on seabird feathers from the 1990s compared to feathers from before 1931. Braune et al. (2001) found that eggs of 2 out of 3 species of seabirds from the Canadian artic showed increased Hg concentrations between 1975 and 1998 not attributable to shifts in trophic position. Other researchers have looked at Hg deposition recorded in sediment cores from lakes (Swain and Helwig, 1989; Engstrom and Swain, 1997) and as reflected in fish tissue concentrations (Hrabik and Watras 2002).

In the late 1980's the Wisconsin Department of Natural Resources (WI DNR) initiated a monitoring program to examine trends in Hg concentrations in young-of-year (YOY) yellow perch as a biological indicator. At that time, the rationale for the design included: 1.) YOY yellow perch were thought to bioaccumulate Hg from food and water; 2.) Yellow perch are widely distributed in lakes across the state; 3.) YOY yellow perch are easy to identify, collect and age; and 4.) YOY yellow perch would be a more sensitive indicator of changes in Hg over time compared to other species and older fish (WI DNR Internal correspondence 1989). YOY yellow perch were purported to be a sensitive indicator of annual changes in deposition and availability of Hg because they are fast growing and only exposed to one growing season whereas Hg concentrations in older fish reflect multiple years of exposure.

There are no other published reports of changes in Hg concentrations over time in YOY yellow perch. Suns and Hitchin (1990) examined yearling yellow perch from 16 Canadian lakes and found no temporal trends in Hg residues over a 9-year period. The authors concluded that Hg inputs and conversion to bioavailable forms were constant so this finding was reasonable. Amrhein et al. (2001) found that Hg in museum-preserved yellow perch from the 1920s varied by lake when compared to yellow perch collected in the 1980s from the same northern Wisconsin lakes. Hg trends from the museum specimens showed increasing Hg in fish tissue in two lakes, decreasing Hg in one lake, and no significant change in Hg concentrations in yellow perch from two other lakes. Hrabik and Watras (2002) found decreases in Hg deposition and Hg in the water column of one northern Wisconsin lake. This decrease in deposition was accompanied by an average -5% change per year in adult yellow perch Hg concentrations between 1994 and 2000 suggesting that fish Hg can respond to changes in Hg deposition over a relatively short amount of time.

The objective of this report is to determine if YOY yellow perch Hg concentrations in seven study lakes changed over the time-period 1992-2003. In addition, the YOY yellow perch trend monitoring design is evaluated to determine if changes are warranted. Continued and improved monitoring of Hg will provide better information to evaluate trends of Hg emissions and deposition in the future.

Study Sites

Wisconsin lakes are quite variable in appearance, size, chemical and physical characteristics (Lillie and Mason 1983). YOY yellow perch were collected from seven lakes located in northern Wisconsin, USA in Oneida, Lincoln, Langlade, and Vilas Counties (Figure 1), an area rich with lake types that favor Hg bioaccumulation (Lathrop, et al. 1989). The seven lakes represent a continuum in several characteristics (Table 1). The lakes vary in size from small to large (61 - 1128 ha) with watersheds, drainage, and adjoining wetland areas that also widely vary in size. Five of the lakes are classified as deep, lowland drainage lakes; one is a deep, headwater drainage lake; and one is a shallow, lowland drainage lake. The lakes range in clarity with Secchi depths from 0.5 to 3.4 m, alkalinity from low or soft to high or hard (3 - 79 mg/l $CaCO_3$ equivalents), and pH from low or slightly acidic to high or alkaline (6.6 - 8.4).

Five of the lakes have water control structures originally installed in the early 1900s and now operated by the Wisconsin Valley Improvement Company (WVIC). Lake levels in those five lakes are controlled so that low water occurs in the early spring (February-March) and late fall (October-November) and high water levels in the summer (Sam Morgan, WVIC, personal communication, May 18, 2005). Maximum annual water level fluctuation in lakes with control structures are: Chain (5.5 feet), Minocqua (2.3 feet), North Nokomis (13.25 feet), North Twin (2 feet), and Spirit River Flowage (17 feet). Spirit River Flowage is an impoundment on Spirit River that flows into the Wisconsin River near Tomahawk, Wisconsin. North Nokomis is an impoundment that is part of the Rice River Flowage system.

Methods

Fish Collection and Sample Preparation – YOY yellow perch were collected in mid- to late July in 1996, 2000, and 2003 and in early to mid-August in 1992. The sampling goal was 30 individual YOY yellow perch which was increased to 50 individuals per lake in 2003. Each year, collections from the lakes were usually within 1 to 2 consecutive days unless repeated trips were necessary to obtain the target sample size. In 1992 and 2003, collections from some lakes were up to 18 days after initial collections for that year.

The YOY were collected by shoreline seining (1/8 inch mesh). Several seine hauls were necessary to obtain the target sample size. Once collected, fish were placed in a labeled plastic bag, held in the field on ice, and then frozen until processing. Individual YOY yellow perch were combined into composite groups of ten fish of similar length. In 2003, WI DNR staff determined the age of two individuals of each composite by scale examination for annuli to confirm the assumption that collected individuals were age 0.

Hg Analysis – Composited samples were homogenized. Hg concentrations were determined by Cold Vapor Atomic Absorption Spectrophotometry (USEPA Method 1631) by the State Laboratory of Hygiene, Madison, Wisconsin USA. The level of detection was 0.004 ug/g, wet weight.

Statistical Analysis Methods – All statistical comparisons were performed with SAS Versions 7, 8, or 9.1 (SAS Institute, Cary, NC USA). We log transformed Hg concentrations since the variance among observations was more homogeneous on the log scale. We conducted two types of analysis.

Individual Lake Trends **-** We examined relationships between Hg concentrations, year sampled, and mean length of composite fish samples for each lake separately. We used linear regression to examine trends in Hg concentration in YOY yellow perch over time in individual lakes and used $\alpha = 0.05$ to judge significance. The response variable was the log of Hg concentration and predictor variables were fish length, year sampled, and their interaction. If the interaction term was not significant, we refit the model without it. Fish lengths were averages of the YOY fish in each composite sample.

Overall Trends - We used several mixed effects models to analyze data from all lakes simultaneously. Hg concentrations were log transformed before analysis. We used Akaike's Information Criterion (AIC) to score the models and likelihood ratio tests to examine the significance of individual factors within the models. Models included random effects for lakes and fixed effects for time trends and effects of fish length. We included both between lake (average fish length for all samples from the lake) and within lake effects of fish length (average length of composite samples) in our models.

Results

Table 2 shows a summary of YOY sample characteristics including the total number of composite samples (n) collected from each lake over all years (1992, 1996, 2000, and 2003). Most of the 88 composite samples included in the dataset contain 10 individuals per composite. Only six of the 88 composite samples contain more or less than 10 individuals because the targeted number of YOYs was not obtained in some years at some sites. Five of the seven lakes had at least three composite YOY Hg results for each of the four collection years but North Nokomis had three results for only one of the four collection years and Spirit River Flowage had three results for two of the four collection years.

During these years, Hg concentrations were at detectable levels in all samples. The lowest concentration was 0.008 ug/g and the highest was 0.220 ug/g. The overall mean Hg concentration for all YOY yellow perch over this period was 0.060 ug/g (se = 0.005).

Individual Lake Trends – Table 3 shows the results of the linear regression by lake and Figure 2 shows the relationship between Hg concentration and year sampled and average length of composite fish for each lake separately. There were no significant interactions between length and year except for North Nokomis Lake (p = 0.0477) from which limited samples were obtained in three of the four sampling years.

Only Tug Lake fish showed a significant change in Hg concentration over time and Spirit River Flowage showed a decrease in Hg with increasing YOY length. Tug Lake's slope estimate (p=0.0003) for the change in log Hg concentration was an increase of 0.02 per year (5% increase in Hg concentration per year). Spirit River Flowage's slope estimate represents a decrease in Hg concentration of 8.2% per mm (p=0.0063).

Overall Trends – The seven different models used to analyze all lakes simultaneously provided slightly different estimates of the effects of within and between lake factors and year on Hg concentrations (Table 4). The list of predictors shows the fixed effects included in the different models and includes year, fish length, and mean fish length for the lake (noted as Lake Fish-Length). Akaike's Information Criterion (AIC) indicates the models with the best scores and takes into account both within- and between-lake factors.

The best fitting mixed effects model (model 4) included year, fish length, and mean fish length for the lake as fixed effects in the model. Although the temporal effect (year) was not significant (by a likelihood ratio test) in this model, the estimate of the year effect corresponded to an increase in Hg concentration of 1.34% per year. The second best model included only the two length factors, and did not include a temporal effect, suggesting that the change in Hg concentration over the period 1992 to 2003 in this set of lakes was small and that both within-lake and between-lake factors affect Hg concentrations of YOY yellow perch significantly. The temporal effect was included in five of the seven models evaluated. The estimated change per year for those models ranged from –1.19% to +2.03 % per year.

The models indicate that the within lake effect of fish length was negative and the between lake effect of fish length was positive. In other words, the Hg concentration in YOY yellow perch decreased with size of the fish within each of the lakes while but lakes with larger YOY fish tended to have greater mean Hg concentrations in YOY yellow perch.

Discussion

Our results suggest that mid- to late-summer Hg concentrations in YOY yellow perch changed little over the 12-year study period. One of the seven study lakes had significantly increasing YOY Hg concentrations over time (5% per year) based on linear regression for each lake. The mixed effect model used to analyze the data from all the study lakes simultaneously suggests that Hg concentrations in YOY yellow perch from the study lakes remained the same or increased slightly over the period 1992 to 2003. The estimate of annual change in Hg concentration from the best fitting model was 1.34% per year. Even small changes of this magnitude if continued into the future would eventually result in a substantial cumulative change.

Lake-by-lake analysis provides useful information on trends within lakes and is valid because we know that Hg bioaccumulation varies between lakes (Wiener and Spry 1996) and therefore trends over time may vary between lakes (Amrhein et al. 2001). Within-lake trends are of interest to examine factors that affect Hg accumulation. However, analysis of Hg trends on an individual lake basis suffers from several shortcomings. The statistical power to detect trends is limited because of the relatively small number of observations from each lake. Lake-by-lake analysis does not provide a straightforward method to combine information and draw conclusions across lakes.

The mixed effects models include fixed effects that describe the general response of the population and random effects that account for characteristics of individual subjects or groups (lakes in this case). Such models can handle multiple levels of variability (within and between lakes) as well as serial correlation (Diggle et al. 1994, Littel et al. 1996). The variability in Hg concentrations of YOY perch may be explained in part by factors associated with individual fish in a lake, such as fish length or age, and in part by factors associated with all fish in a lake, such as lake chemistry or forage base. We used fish length in two ways in our models. The mean length of fish in individual composite samples was used for within lake effects of fish growth and annual conditions. The average length of fish over all samples from a lake was used for lake effects that may reflect overall early growth conditions in each lake.

We found that incorporating length into statistical analyses assisted in explaining the variability of Hg concentrations in fish between lakes and over time. The within and between lake effects of fish length were opposite in the mixed effects models. The effect of fish length within lakes was negative meaning that within a lake, Hg in fish samples with longer average length had lower Hg concentrations than samples of shorter fish. The effect of average fish length between lakes was positive indicating that Hg concentrations were higher in samples from lakes with YOY that averaged longer across all years of the study. That means YOY Hg concentrations were higher in lakes with larger YOY while within lakes, Hg concentrations of YOY generally decreased with increasing length.

It is generally accepted that Hg concentrations increase with age of fish and total length for most species, and that the rate of increase varies between lakes and species (Wiener and Spry 1996). Our finding of a decrease in Hg concentration with length of YOY within a lake may be a result of the limited size range of the YOY. Hg concentrations may not be strongly related to length within individual year class, over short time frames, or within limited size ranges. Greib et al. (1990) sampled 547 yellow perch from 27 lakes in the Upper Peninsula of Michigan in the United States and found that mean Hg in yellow perch was relatively constant over age classes 1 to 6 but increased in age classes 7 to 10+. Cope et al. (1990) examined Hg accumulation in age two yellow perch from 10 Wisconsin seepage lakes and found no association with length or weight. Yellow perch YOY from Rib Lake (Taylor County, Wisconsin, USA) analyzed individually as whole fish for Hg, showed no significant relation between Hg concentrations (log 10) and length of the YOY ($p>0.05$). The Rib Lake YOY log(Hg) decreased at a rate of –0.005 per mm of length (SE = 0.003, $p=0.18$) corresponding to a decrease of 1% per mm (Wisconsin Department of Natural Resources, unpublished 1999 data).

This monitoring design, conceived in the late-1980s, was based on the premise that YOY would be a sensitive indicator of changes in Hg deposition and availability. However, YOY may exhibit changing Hg accumulation rates throughout the first growing season as they develop. Whiteside et al. (1985) studied the first 70 days of yellow perch in Lake Itasca, Minnesota, and found that perch undergo changes in their habitat, diet, and growth rates in their first growing season. After hatching, perch larvae move from shallow water to the limnetic zone where they begin first exogenous feeding when water temperature reaches 15^0C, and prey includes zooplankton and aquatic insect larvae. After about 30 days of pelagic habit (or when they reach about 25 mm), the perch return to the littoral zone and shift towards larger substrate-dwelling organisms such as amphipods and aquatic insects. Within a lake, YOY yellow perch Hg body burden may change over time due to these diet shifts. These shifts constitute changes in trophic feeding status during the YOY life stage and may confound interpretation of Hg concentration trend data.

Several factors likely affect the between lake and within lake variability of Hg residues in YOY yellow perch. Time of spawning, incubation period, and time from hatch to collection likely affects Hg accumulation in YOY. Yellow perch may spawn over one to several weeks just after ice-out and hatch can range 10 to 30 days after spawning (Becker, 1983; Whiteside et al 1985). Accounting for year-to-year differences in ice-off as an estimate of spawning time and growing degree-days may be beneficial in examining trends of YOY Hg concentrations over time. However, length or condition factors should indirectly reflect both growing time and conditions (including temperature and food conditions) that affect Hg bioaccumulation.

Becker (1983) reports, based on Lake Winnebago data from 1975, perch attain 50% of their first year's growth by mid-July and reach 102 mm (4 inches) by the end of the first growing season. Development into the juvenile stage is completed at about 40 days in age and most of the energy intake is directed towards increasing body weight rather than length. Therefore body mass or condition factor may be a better measure of growth of larval and juvenile yellow perch than length. Future monitoring should include measurement of weight in addition to length of YOY.

In addition, some of our study lakes may not be sensitive to changes in Hg deposition. The study included only two lakes with lower pH and four with lower alkalinity based on limited water chemistry data. Inter-lake differences in Hg accumulation in fish are primarily related to lake pH or related indicators of acidity (Wiener and Spry 1996). Burgess and Hobson (2005) surmised that low pH is associated with increased Hg methylation, lower productivity, and decreased perch growth rates all which are factors associated with increased fish Hg levels. Greenfield et al. (2001) found that lake pH explained the majority of variability in Hg concentrations in yellow perch from 43 lakes from northern Wisconsin but that fish length or body condition explained additional variability. Other factors are also important to some degree including Hg deposition, water column concentrations (Hrabik and Watras 2002), drainage area/lake volume ratios (Suns and Hitchin 1990), and Hg in surficial sediments (Copes et al. 1990).

The study lakes differ somewhat in water chemistry, drainage-to-lake and adjoining wetland area. These differences may explain the concentrations of Hg in the YOY. However, it is unlikely that these variables changed significantly during the study period but they may make YOY more or less sensitive to changes in Hg deposition. In neutral pH lakes, Hg bioaccumulation may be limited in YOY and changes over time may be more difficult to discern.

The current monitoring design does not allow assignment of cause for any observed trends or lack of trend. Wisconsin air deposition monitoring has not been sufficient to determine Hg deposition to specific lakes although sediment core studies have been conducted on lakes in the region (Engstrom and Swain 1997, Rada et al. 1989). Monitoring Hg deposition and other factors affecting Hg accumulation in a subset of intensively monitored lakes would provide valuable information to verify that YOY yellow perch are sensitive to changes in deposition. Based on the broader scale atmospheric monitoring at three stations located in northern Wisconsin, Hg deposition is variable regionally and temporally and is related to the amount of wet deposition (WI DNR 1999). Depending on the deposition and sources of Hg to the seven study lakes, the findings of this study covering the period 1992 to 2003 are reasonable.

Since this monitoring began, our understanding of Hg bioaccumulation has grown. Wiener et al. (In Press) has recently reviewed this new information and recommends that biotic monitoring for assessing trends of MeHg bioaccumulation include 1-year-old prey fish, in addition to predator game fish, to indicate the annual changes in the supply of MeHg. In addition, seepage lakes are recommended for trend monitoring, as they should be more sensitive to Hg deposition because inflow of surface or ground water would be minimal.

We recommend continuation of this monitoring effort. Additional lakes should be monitored that are known to be sensitive to Hg accumulation. These lakes should be selected to minimize Hg input other than atmospheric deposition and minimize flow regimes that may alter Hg residence and methylation. Changes in Hg in young yellow perch should be examined to determine if young yellow perch would be better indicators than the YOY life stage. Future collections should be restricted to a more consistent time and specimens from each lake should be aged. Lastly, body weight and length should be measured on individual specimens.

While Hg trends in fish may differ between lakes, the interim findings of this study suggest that Hg in YOY yellow perch from these seven northern Wisconsin lakes changed at a rate of –1 to +2% per year during the period of 1992 to 2003. Variability of Hg in fish between lakes complicates analysis of time trends and requires either that trends be evaluated on a lake-by-lake basis or that appropriate statistical methods be used that can account for differences among lakes. These findings will be re-evaluated after a longer period of record is available.

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Table 1. Characteristics and water chemistry data for 7 study lakes (Wisconsin DNR 1992-2003 data unless otherwise noted).

WBIC*	Name (Town Range Section)*	County*	Lake Type***	pH	Total alkalinity (mg/l $CaCO_3$ eq)	Secchi Disc (m)	Max. Depth (m)*	Lake Area (ha)*	Watershed Area (ha)*	Drainage Area (ha)*	Drainage-Lake Area ratio	Adjoining Wetland (ha)*
1598000	Chain Lake (39 09E 28)	Oneida	Deep, lowland drainage	6.6***	21***	1.3	5.8	87	8287	2331	27	139
1542400	Minocqua Lake (39 06E 15)	Oneida	Deep, lowland drainage	7.9	46	3 - 5	18.3	550	20720	2072	4	397
1595800	North Nokomis (39 08E 26)	Oneida	Deep, headwater drainage	7.5	23	2.4	22.3	189	777	518	3	89
1623800	North Twin Lake (41 11E 18)	Vilas	Deep, lowland drainage	8.4	46	3.4	18.3	1128	4403	1554	1	364
389300	Rolling Stone (34 12E 13)	Langlade	Shallow, lowland drainage	8.1	79	1.6**	3.7	272	2590	518	2	553
1506800	Spirit River Flowage (34 06E 16)	Lincoln	Deep, lowland drainage	7.6	24	0.5	6.7	633	45066	20720	32	47
1482400	Tug Lake (33 06E 35)	Lincoln	Deep, lowland drainage	6 - 7	3 - 10	0.6	6.4	61	2590	2590	42	59

* Registry of Waters, Wisconsin DNR

** Lillie and Mason. 1983.

*** DNR Surface Water Inventory File (methyl purple alkalinity)

Figure 1. Location of Langlade, Lincoln, Oneida, and Vilas Counties, Wisconsin, USA.

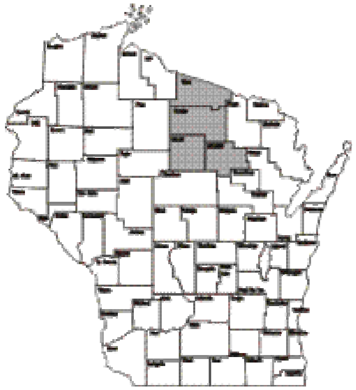

Table 2. Number of composite samples (n), length and mercury concentrations (s.e.= standard error) in YOY YP from 1992 to 2003.

		Length (mm)				Mercury (ug/g, wet weight)			
Name	**n**	**Mean**	**Minimum**	**Maximum**	**s.e.**	**Mean**	**Minimum**	**Maximum**	**s.e.**
Chain Lake	14	47	36	57	2	0.075	0.056	0.130	0.006
Minocqua Lake	13	43	33	56	2	0.026	0.019	0.037	0.002
North Nokomis	6	60	38	74	6	0.050	0.021	0.085	0.008
North Twin Lake	16	35	28	41	5	0.016	0.008	0.026	0.001
Rolling Stone Lake	15	48	42	56	4	0.055	0.029	0.110	0.006
Spirit River Flowage	8	52	43	64	8	0.130	0.058	0.220	0.024
Tug Lake	16	45	38	53	1	0.092	0.056	0.140	0.006

Table 3. Results of linear regression of log mercury on fish length (mm) and year by lake.

		Year collected			Fish length (mm)		
Name	**df**	**slope**	**s.e.**	**p**	**slope**	**s.e.**	**p**
Chain Lake	13	-0.01	0.006	0.1036	-0.007	0.004	0.0787
Minocqua Lake	12	0.007	0.007	0.3336	<0.001	0.005	0.9879
North Nokomis	5	-0.082	0.043	0.1516	-0.018	0.011	0.1993
North Twin Lake	15	0.003	0.008	0.748	-0.005	0.008	0.5038
Rolling Stone	14	0.007	0.012	0.5812	-0.016	0.023	0.2513
Spirit River Flowage	7	-0.014	0.144	0.3705	-0.037	0.008	**0.0063***
Tug Lake	15	0.02	0.004	**0.0003***	-0.006	0.004	0.1004

The interaction of year and length was significant for North Nokomis (p = 0.0477). Note that because YOY perch were composited before the Hg determination, the degrees of freedom for each lake is based on the number of composite samples analyzed not the number of individual fish included in the composites.
s.e. = standard error, p = level of significance set at 0.05.

Figure 2. Average mercury concentration in YOY yellow perch versus year and length by lake.

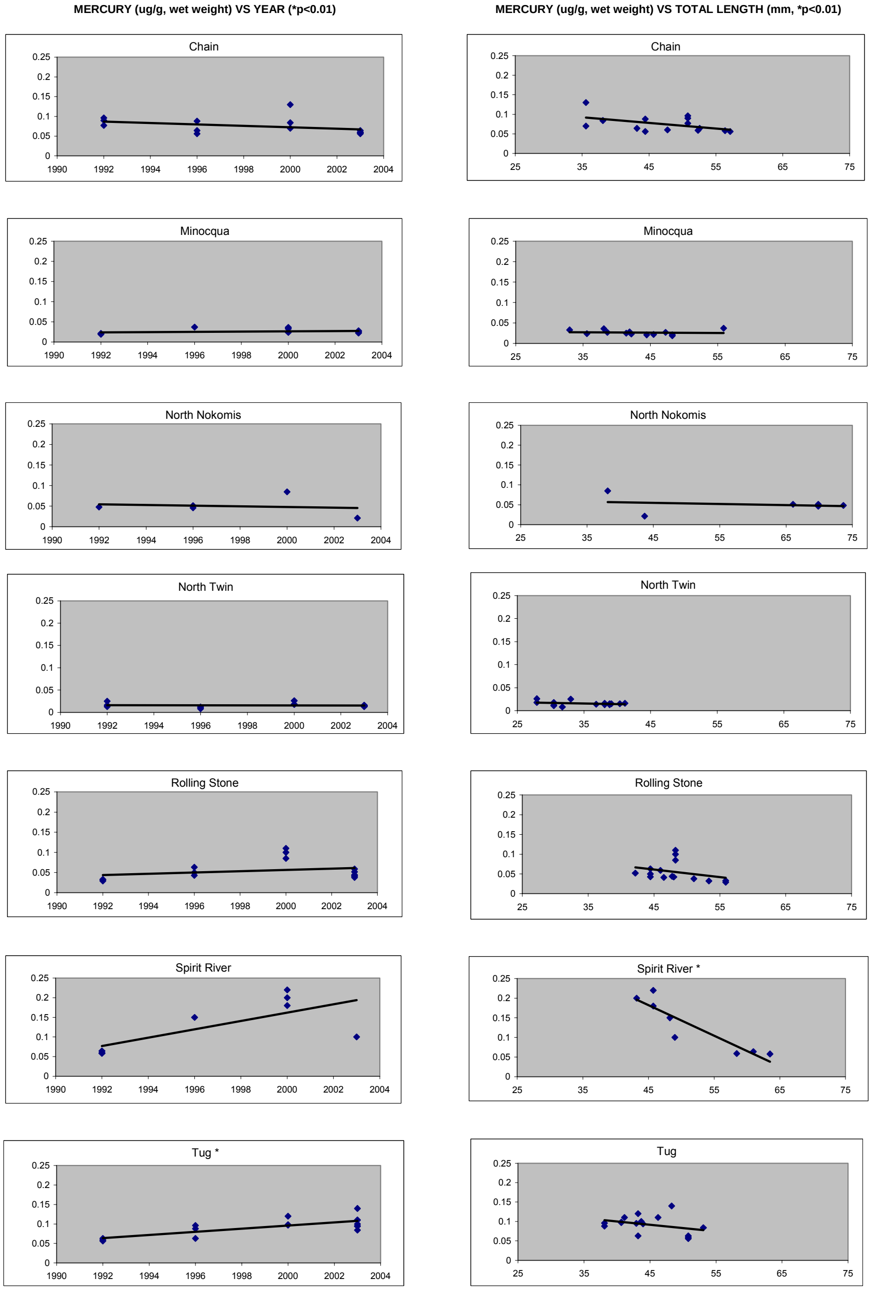

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Table 4. Parameter estimated from mixed effects models with AIC score and calculated estimate of percent change in mercury concentration per year.

Model	AIC*	Predictors	Slope	s.e.	p-value	% Change per Year
4	-65.4	Fish Length (mm)	-0.0066	0.0023	0.0060	
		Lake Fish-Length	0.0300	0.0127	0.0201	
		Year #	0.0058	0.0036	0.1071	1.34%
5	-64.8	Fish Length (mm)	-0.0077	0.0023	0.0011	
		Lake Fish-Length	0.0305	0.0126	0.0179	
2	-63.2	Fish Length (mm)	-0.0061	0.0023	0.0109	
		Year #	0.0059	0.0036	0.0996	1.36%
3	-62.5	Fish Length (mm)	-0.0072	0.0023	0.0022	
1	-61.5	Fish Length (mm)	-0.0084	0.0053	0.1183	
		Year #	-0.0052	0.0232	0.8242	-1.19%
		Fish Length*year#	0.0002	0.0005	0.6291	
7	-59.8	Lake Fish-Length	0.0237	0.0125	0.0607	
		Year #	0.0087	0.0036	0.0174	2.03%
6	-58.9	Year #	0.0086	0.0036	0.0189	2.00%

* AIC values from SAS Version 9.1

s.e. = standard error, p = level of significance at 0.05