# The Statewide Energy Efficiency and Renewables Administration and The Public Service Commission of Wisconsin

# **Environmental and Economic Research and Development Program**

Final Report

October 2007

# Mercury in Selected Fish Species over Time

# Prepared by:

Candy S. Schrank, Environmental Toxicologist, Paul W. Rasmussen, Research Scientist, and Patrick A. Campfield, Fisheries Biologist

Department of Natural Resources, Bureau of Fisheries Management

This report in whole is the property of the State of Wisconsin and was funded through the FOCUS ON ENERGY program.



# **Mercury in Selected Fish Species Over Time**

Final Report November 2007

#### **EXECUTIVE SUMMARY**

Date of Report: November 19, 2007

**Title of Project:** Mercury in Selected Fish Species Over Time

Investigators: Candy S. Schrank (Environmental Toxicologist), Paul W. Rasmussen (Research

Scientist), and Patrick A. Campfield (Fisheries Biologist)

Institution: Wisconsin Department of Natural Resources, Bureau of Fisheries Management, 101

S. Webster Street, Madison, Wisconsin 53707

Research Category: Measurement of mercury in Wisconsin environment

Project Period: November 1, 2002 to June 30, 2007

**Object of Research:** The goal of this project was to develop and implement a monitoring design to assess changes in mercury (Hg) concentrations in selected fish species over time. Measuring temporal trends of Hg in fish is important to better understand the sources and fate of Hg released to the environment including that from generation of electricity and other sources.

#### Summary of Results and Accomplishments:

Monitoring of Hg concentrations in walleye skin-on fillets is a useful tool for detecting trends of Hg in Wisconsin's lakes if known factors that affect Hg availability and bioaccumulation are incorporated into appropriate statistical analyses. Measuring temporal trends of Hg in Wisconsin walleye should allow for a better understanding of the sources and fate of Hg released to the environment.

We explored the Wisconsin Department of Natural Resources' (WI DNR) fish contaminant database to determine the availability and utility of historical (1970 - 2002) Hg records for different species and to identify lakes with historical Hg data. Walleye skin-on fillets were selected for further examination. This project supported processing and characterization of 310 additional walleyes from 36 lakes that were collected over the years 2003 to 2006. With these data, we created a dataset of 3,024 individual walleye records from 421 lakes spanning 1982 to 2005. We evaluated temporal trends over all lakes represented in this dataset using several different mixed effects models. We explored the relationships between Hg concentrations and a suite of lake chemistry, morphometry, and other variables.

Our analyses suggest that temporal trends in walleye Hg concentrations varied latitudinally within Wisconsin. Northern lakes exhibited slight average decreases (– 0.5% per year), central lakes showed no change, and southern lakes showed modest average increases in Hg concentration (+ 0.8% per year) over the period from 1982 to 2005. Individual lakes deviate from these population averages. Our finding that walleye Hg concentrations decreased in northern Wisconsin is consistent with other studies. While there are a number of possible explanations for our finding of increased walleye Hg concentrations in southern lakes, this finding warrants further study to verify the trend and to investigate possible mechanisms that would cause Hg to increase in southern Wisconsin waters.

Walleye Hg concentrations and the Hg-fish length relationship vary greatly among lakes. Lake latitude, lake area, and alkalinity explained some of the differences in Hg concentrations, but none accounted for differences in the Hg-length relationships. We also found that Hg concentrations vary by gender and season of collection. Walleye Hg was lower in females than in males of equal size. Mercury concentrations were highest in walleye captured in the spring and lowest in the fall.

Our analysis of walleye Hg records from non-riverine Wisconsin lakes suggests that the historical data is valuable and the current sampling strategy is appropriate for detecting changes in Hg concentrations over time. Variability in fish Hg concentrations within and among lakes and with increasing fish lengths can make data interpretation challenging, but continued sampling using the current design is sufficiently robust and capable of detecting temporal trends.

This project also supported the collection, processing, and analysis of young-of-year (YOY) and yearling yellow perch. Young-of-year yellow perch (n = 66 composites) were collected in 2003 and 2006 at seven lakes where samples had been collected in the past (1992 to 2000) to create a dataset of 121 composite YOY records. In addition, yearling and YOY yellow perch (n = 477 individual fish) were collected at 24 lakes over the four sampling seasons from 2003 to 2006.

In contrast to our conclusion that monitoring of Hg concentrations in walleye skin-on fillets is a useful tool, we are unable to draw a strong conclusion about the utility of young yellow perch for detecting Hg trends in Wisconsin's lakes. Our analysis of YOY from the seven northern Wisconsin study lakes found that the average length of the all YOY sampled from the lake (grand lake-mean length) best described Hg concentrations. This finding suggests that Hg concentrations in YOY yellow perch are primarily related to factors that differ between lakes, e.g. productivity, food availability, or other factors affecting YOY growth rates. The second best model suggests that Hg concentrations in YOY yellow perch decreased from 1992 to 2006 in the range of – 0.69% per year, a result consistent with the northern Wisconsin walleye trends. These differing results do not lead to a strong conclusion about temporal trends in Hg concentrations in YOY from the seven northern Wisconsin study lakes over the 15-year study period (1992 to 2006).

The inconclusive YOY findings may be affected by the limited number of lakes with historical (1992-2000) YOY Hg data. Obtaining samples from additional lakes and years may allow for better estimates of YOY Hg trends. After we completed an interim analysis of YOY Hg data, we recommended that additional lakes be included in this study. In addition, due to the short and dynamic life history characteristics of YOY, we recommended sampling of yearling (age 1) yellow perch to determine if yearlings would be better indicators than YOY.

As follow-up to the above recommendation, individual yearling yellow perch were collected from 24 Wisconsin lakes. The limited yearling yellow perch collected to date suggest that Hq concentrations are as variable in yearling as they are in YOY. At this time, our datasets are too limited to determine if Hg concentrations changed over time in YOY or yearling yellow perch from Wisconsin lakes. Recently, Harris et al. (2007) demonstrated that Hg concentrations in YOY yellow perch responded to controlled artificial Hq spikes and that most of the increase was from Hg deposited directly to the lake but that the YOY increase was not proportional to the loading increase. These authors predict, that while biota will respond quickly to reductions, response rates will vary among lakes. Hrabik and Watras (2002) attributed reduced Hg concentrations in yellow perch from one northern Wisconsin lake to decreases in atmospheric deposition of H<sub>2</sub>SO<sub>4</sub> and Hg from 1994 to 2000. Rodger et al. (2006) found no statistically significant changes in Hg deposition measured from 1998 to 2005 at a limited number of monitoring stations. If deposition to the yellow perch study lakes did not change, then we would expect no change in yellow perch Hg concentrations. Based on monitoring conducted to date and these key studies, additional yearling yellow perch should be collected and analyzed before conclusions are made regarding the utility of yearling yellow perch to indicate temporal trends of Hg in Wisconsin lakes.

# **TABLE OF CONTENTS**

EXECUTIVE SUMMARY	i
TABLE OF CONTENTS	iii
INTRODUCTION	1
A. Purpose and objectives	2
B. Project activities	3
METHODS, RESULTS, AND DISCUSSION	5
A. Trends of mercury in walleyes 1982-2005	
Methods Results Discussion Walleye Tables and Figures	5 6 8 10
B. Trends of mercury in young-of-year yellow perch 1992-20	06
Methods Results Discussion Young-of-Year Yellow Perch Tables and Figures	15 16 17 19
C. Use of yearling yellow perch – preliminary dataset	
Methods Results Discussion Yearling Yellow Perch Tables and Figures	23 23 25 25
CONCLUSIONS	29
LITERATURE	31

#### INTRODUCTION

Mercury (Hg) concentrations in a variety of ecosystems have increased in the range of two to tenfold over pre-industrial levels. In addition to direct monitoring of emissions and deposition, other media have been examined to estimate trends in Hg deposition over time (Swain and Helwig, 1989; Engstrom and Swain, 1997; Monteiro and Furness, 1997; Braune et al., 2001; Schuster et al., 2002; Slemr et al., 2003; Frederick et al., 2004). These studies which examined Hg concentrations in ice cores, sediment cores, precipitation, the atmosphere, and the feathers and eggs of birds, indicate that Hg deposition increased through the industrial era but decreased since the late 1980s. Estimated worldwide atmospheric Hg levels peaked in the late 1980s, decreased from the 1980s to 1996, and have varied little since 1996 (Slemr et al., 2003). Recent declines may be due to limitations placed on some sources of Hg to the environment. Future trends will likely be affected by future Hg emissions.

Widespread Hg contamination and accumulation in fish poses health risks to people who consume fish and may impair wildlife health. Most of the Hg that accumulates in fish is from anthropogenic sources and enters water via atmospheric deposition (Glass and Sorensen, 1999; Wiener et al., 2006). Scientists have concluded that fish Hg concentrations will respond to changes in Hg loading rates. However, few reports demonstrate fish responses to the control of Hg emissions and Hg inputs to surface waters from atmospheric loading. In addition, the magnitude and timing of the response of fish Hg to changes in atmospheric loading will vary due to a variety of factors influencing individual ecosystems (Munthe et al., 2007).

Few studies have examined temporal trends in fish Hg from Hg-sensitive waters like the lakes typical of northern Wisconsin. Suns and Hitchin (1990) examined yearling yellow perch from 16 Canadian lakes and found no temporal trends in Hg concentrations over a 9-year period (1978-1987). The authors concluded that Hg inputs and conversion to bioavailable forms were also constant during this time. Hrabik and Watras (2002) found decreases in Hg deposition and water column concentrations in one northern Wisconsin lake from 1994 to 2000. Mercury concentrations in adult yellow perch from this lake also declined by 5% per year suggesting that Hg concentrations in adult fish decrease in response to reductions in Hg deposition. However, Hg trends in fish may not directly reflect changes in Hg emissions. Fish are exposed over a number of years and factors that affect bioaccumulation may vary spatially and temporally (Wiener et al., 2007).

Wisconsin DNR has monitored Hq in fish since the early 1970s with the main purpose of determining appropriate advice for human consumption of fish. Monitoring designs evolved over time as more was learned about environmental contaminants and risks to human health from consumption of contaminated fish. Monitoring began in the 1970s to evaluate Hg concentrations in fish from rivers receiving treated wastewater from facilities that used Hg-based slimicides. In the 1980s, atmospheric deposition, methylation, and bioaccumulation of Hg in the food chain became a concern. When Wiener (1983) reported elevated Hg concentrations in sport fish from northern Wisconsin, the focus of monitoring shifted to lakes in that area. As a result, statewide Ha monitoring in Wisconsin fish was generally not conducted under a single study design that controlled spatial and temporal sampling patterns. Sampling for fish consumption advisories focuses on popular fishing lakes. However, the data also include fish collected for other purposes, including special studies of individual lakes, and general sport fish surveys. Each sample is affected by many uncontrolled factors, including lake characteristics such as water chemistry, underlying geology, watershed position, local climate, and local landscape cover, as well as temporal and spatial patterns of sampling. Because of the large number of fish sampled and the 29-year time span, these data provide a valuable resource for examining spatial and temporal patterns of Hg contamination in Wisconsin fish.

#### A. Purpose and objectives

The goal of this project was to develop and implement a monitoring design to assess changes in Hg concentrations in selected fish species over time. More specifically, the objectives were to: 1) implement a statistically valid trend monitoring program, 2) obtain data during three 5-year phases of the project sampling 10 lakes each year, 3) analyze data for trends, 4) evaluate data and lake characteristics to identify factors affecting bioaccumulation and responses to Hg inputs, and 5) identify issues and questions for future efforts examining Hg trends. This study was funded for approximately 4.5 years encompassing four sampling seasons.

Measuring temporal trends of Hg in fish is important to better understand the sources and fate of Hg released to the environment including that released by electrical generation and other sources. Mercury moves through the environment and deposits in terrestrial and aquatic ecosystems. Bacterial action in lakes and waterways converts Hg to a form that bioaccumulates in fish. Accumulation of Hg in fish is suspected to reflect recent atmospheric deposition. Fish have been recommended as one of the important tools for assessing the long-term trends of Hg releases to our environment (Munthe et al., 2007).

One task was to evaluate temporal trends of Hg in walleye while accounting for factors not controlled or randomized during sampling. Walleye are an important game fish highly sought by anglers, represent the largest number of observations in Wisconsin's fish contaminant database, and are a top-level piscivorous species that accumulate high concentrations of Hg. Prior to initiating this project, we examined walleye Hg data and predicted that a design consisting of 30 lakes sampled every 5 years with at least 5 fish collected each time would be likely to detect an overall trend of 1% change in concentration per year over a 15 year period.

We also wanted to characterize Wisconsin's walleye Hg concentrations in relation to fish length and lake attributes that have been shown to be important to Hg accumulation including lake morphometry, watershed, and chemistry characteristics. Fish Hg concentrations increase with fish length, although the relationship varies among lakes (Lathrop et al., 1991; Somers and Jackson, 1993). Some differences in Hg accumulation among lakes may be due to differences in fish growth rates (Simoneau et al., 2005). Studies have established the importance of lake morphometry and watershed characteristics in determining fish Hg levels (Bodaly et al., 1993; Rudd, 1995; St. Louis et al., 1996). Lake chemistry, especially pH, alkalinity, and dissolved organic carbon, also affect Hg levels in fish (Wiener et al., 1990; Driscoll et al., 1995; Watras et al., 1998).

A second task was to supplement an existing YOY yellow perch Hg dataset, determine if Hg concentrations in seven study lakes changed over time, and evaluate the monitoring design to determine if changes are warranted. In the late 1980s WI DNR initiated a monitoring program to examine trends in Hg concentrations in young-of-the-year (YOY, Age 0) yellow perch as a biological indicator. Rationale for the monitoring design included: 1) YOY yellow perch 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 may be a more sensitive indicator of changes in Hg over time compared to other species and older fish (WI DNR internal correspondence 1989). Young-of-year yellow perch were purported to be a sensitive indicator of annual changes in deposition and availability of Hg because they are fast growing and are only exposed to one growing season and its recent Hg inputs, whereas Hg concentrations in older fish reflect multiple years of exposure.

Lastly, another task was to examine Hg concentrations in yearling yellow perch to evaluate their potential as Hg trend indicators, possibly in place of or to complement monitoring using YOY perch. This was effort was initiated after preliminary analysis of the YOY dataset suggested that Hg concentrations in YOY primarily depends on factors affecting the lake mean length of the YOY and recommended that the yearling life stage be examined to assess temporal trends in Hg concentrations.

#### B. Project activities

This section summarizes the particular activities that were completed. Some of these activities were directly funded by the grant while others were supported by Department of Natural Resources programs.

- Exploration of Existing Data DNR's database was explored to examine the availability and
  utility of historical (1970 2002) Hg concentration data available for different species and to
  determine the lakes with historical Hg data. This information identified lakes with valuable
  existing Hg data and also identified limitations of the existing data (limited sample numbers
  within individual lakes and unbalanced collections across fish lengths, seasons, or years).
- Walleye Characterization and Processing Determined length, weight, and gender for 310 individual walleyes from 36 lakes over the four field sampling periods (2003-2006). Extracted scale samples and determined ages of 263 walleyes. (Field collection of walleyes was primarily supported by other funds.) Filleted fish and homogenized skin-on fillet samples.
- Walleye Hg Analysis Funded analysis of Hg concentrations in skin-on fillet samples from 281 walleyes (Hg determination of other walleyes supported by other funds).
- Lake Data compilation Data sources were examined and physical, chemical, and hydrologic characteristics of 421 lakes were compiled and summarized for inclusion in statistical analyses with walleye Hg data.
- Walleye Data Analysis and Publication of Peer-reviewed Manuscript A variety of statistical analyses were conducted and final results are documented in a peer-reviewed manuscript. Statistical expertise was provided in part by other Department programs. The walleye trends manuscript was drafted, distributed for friendly review by Hg experts, and revised. The revised manuscript was submitted to the Journal of Ecotoxicology and approved for publication on 6/26/2007 with minor edits. The original publication is available at <a href="https://www.springerlink.com/content/w67723u094000164/fulltext.pdf">www.springerlink.com/content/w67723u094000164/fulltext.pdf</a>. Final results from this task are also incorporated in this final grant report.
- Young-of-Year (YOY) and Yearling Yellow Perch Sample Collection Yellow perch samples
  were collected primarily by beach seining. Under the grant, YOY were collected in 2003 and
  2006 at seven lakes where YOY were collected from 1982 to 2000 and yearling and YOY
  were collected at a total of 24 lakes over the four sampling seasons 2003 to 2006.
- YOY and Yearling Yellow Perch Characterization, Processing and Hg Determination Overall this grant supported collection, processing, and quantifying Hg concentrations of 66 composite YOY samples collected in 2003 and 2006 (resulting in 121 composite samples from 1992 to 2006) and 477 individual yearling or YOY yellow perch. Processing consisted of weighing and measuring the length of each individual fish, homogenizing whole fish samples, and determining Hg concentrations. Scales were taken from a subset of the yellow perch and age was determined by scale examination.
- YOY Yellow Perch Data Analysis and Report An interim report was completed in 2003 summarizing the data available to that point in time. A poster exhibiting preliminary YOY yellow perch Hg results was presented at the 2006 "International Conference on Mercury as a Global Pollutant" and at Wisconsin DNR's 2006 statewide fisheries meeting. Further analyses were conducted after 2006 data were obtained and a final technical report also was written describing yellow perch sampling and analyses. Again, statistical expertise was provided in part by other Department programs and a variety of statistical analyses were conducted. A YOY trends report was drafted, sent for friendly review by Hg experts, and

revised. Final results are documented in a separate report but are also incorporated in this final grant report.

 Yearling/YOY Yellow Perch Preliminary Data Description and Report – A report compiling data associated with these samples was completed in a separate report but are also incorporated into this final grant report.

#### METHODS, RESULTS, AND DISCUSSION

The detailed methods, results, and discussion of results of the three tasks of this project (walleye Hg trends, YOY yellow perch Hg trends, and yearling yellow perch Hg) are described in the following sections.

# A. Trends of mercury in walleyes 1982-2005

#### Methods

Description of datasets – WI DNR's fish contaminant database contains Hg results for 4,961 skin-on fillets from walleye collected over the years 1977 to 2005 from 607 locations. Following the recommendations of Wiener et al. (2007), we selected walleye skin-on fillets for our analysis because they are an important game fish highly sought by anglers, represent the largest number of observations in Wisconsin's fish contaminant database, and are a top-level piscivorous species that accumulate high concentrations of Hg. A dataset was selected for trend analysis by removing records from riverine-dominated flowages which may obscure responses to changes in atmospheric Hg loading (Wiener et al., 2007). WI DNR staff collected most walleye samples using typical fisheries survey methods, including electrofishing and netting (Fisheries Management Handbook 3605.9, Contaminant Fish Collection Procedures, and the WIDNR Field Procedures Manual Version IV). After collection and labeling, samples were frozen and later transported to a processing facility where they were measured, weighed, and filleted. Gender was determined for most mature individuals by gross examination of the gonads. Fish age was estimated for a subset of samples using scales and spines. Skin-on fillets were homogenized and sub-samples placed in glass jars. Samples were analyzed for total Hg content according to the methods described in Sullivan and Delfino (1982). Total Hg in fish is comprised almost entirely of methylmercury (Weiner et al., 2007). Results were reported to two significant digits and the level of detection was 0.03 μg/g for samples analyzed prior to June 1994 and 0.004 μg/g after that date.

We compiled data on lake morphometry, hydrology, and water chemistry variables previously shown to affect Hg accumulation in fish from WI DNR databases for the lakes included in the walleye Hg trend dataset (n=421). Water chemistry values were measured using conventional laboratory procedures (Wisconsin State Laboratory of Hygiene, 2005). We calculated lake-specific water chemistry values for statistical comparisons and modeling as the geometric mean of values from multiple years for each season, and then calculated a grand mean for each individual lake. Only results from surface samples were included in the calculations in order to standardize the data.

**Statistical analyses** - All statistical analyses were performed using SAS Version 9.1 (SAS Institute, Cary NC, USA). We used log-transformed Hg concentrations (log<sub>10</sub>Hg) as the response variable for analyses because the variance of residuals was more homogeneous on this scale. We converted year of collection to a value equal to the number of years since 1980. For example, a collection year of 1992 was given a value of 12 (1992-1980). Not all predictor variables were available for all lakes. Therefore the number of lakes included in the analyses varied by the selected model. We log-transformed waterbody area and adjoining wetland area to linearize the relationship between log<sub>10</sub>(Hg) and those predictors. We approximately centered fish length and latitude predictor variables by subtracting overall mean length (45 cm) and latitude (45 °), respectively, from each observation. Centering makes model intercepts more easily interpretable.

We analyzed log-transformed Hg concentrations using mixed effects models (Littell et al., 1996). Because this was an observational study, with sampling units selected by means outside of our

control (Johnson, 2002), and with many possible predictor variables, extra care was necessary in selection of predictor variables and specification of a set of candidate models to avoid overfitting and finding of spurious relationships (Burnham and Anderson, 1998). Predictor variables were included in our models if we were specifically interested in estimating their effects, if we thought it was essential to adjust for their effects to obtain unbiased estimates of other parameters, or if they were shown to be important to Hg bioaccumulation. We included lake latitude, season in which each fish was collected, and fish length (cm) in our models to account for possible changes in the spatial distribution of lakes sampled, the seasonal timing of walleye sampling, and the size of walleye targeted for sampling. Lake latitude is also correlated with important gradients in pH and alkalinity, lake productivity, and fish growth (Colby and Nepszy, 1981; Lillie and Mason, 1983; Lathrop et al., 1989; Quist et al., 2003; Nate 2004). Fish length is known to be an important determinant of walleye Hg concentrations and must be accounted for in comparing lakes or estimating trends (Wiener et al., 1990; Lathrop et al., 1991). Water chemistry data were available for only a subset of the lakes sampled for walleye Hg. We included total alkalinity in some of our models because it was available more often than other lake chemistry variables, is strongly correlated with other variables such as lake pH, and has been used in other analyses of Wisconsin walleye Hq data (Lathrop et al., 1991). We also examined the effects of some lake morphometry and watershed variables, including maximum depth, lake area, adjoining wetland area, and wetland to lake area ratio.

Both individual fish and whole lake predictor variables were included as fixed effects in our models. All models also included random effects to describe the variance of parameters among individual lakes. The random effects allow individual lakes to deviate from the population model determined by the fixed effects. The simplest model of this form allows lakes to deviate from the overall population intercept but not from the single population value for each of the coefficients (i.e., slopes) of the fixed factors. More complicated models for the random effects allow individual lakes to deviate from the population values of the coefficients for one or more of the fixed factors. We included random effects for the intercept, for fish length, and for year when selecting among models with different fixed effects structures (year was included as a random effect only for models in which it was also included as a fixed effect).

We fit models in two stages. We first fit a set of models that allowed us to estimate trends in walleye Hg, while accounting for factors that might bias trend estimates. We included models without year as a factor (i.e., a trend of zero) to provide a direct comparison for the same models with year. We ranked models on the basis of the Akaike Information Criterion (AIC) and examined models within 2 AIC units of the best-fitting model in greater detail, evaluating the random effects structure, and assessing model assumptions. We then fit additional models building on the best model from stage 1 by including factors related to lake morphometry, watershed characteristics, and gender. Model comparisons using AIC were based on maximum likelihood (ML) estimation, while final parameter estimates were obtained using restricted maximum likelihood (REML), as recommended by Verbeke and Molenberghs (2000).

#### Results

**Data Summary** - Selection of walleye, skin-on fillet records from inland, non-riverine lakes produced a dataset of 3,024 samples collected over the years 1982 - 2005 from 421 lakes. Individual samples represent a range of walleye size, age, and Hg concentrations (Table 1). The bulk of the samples were collected between 1985 - 1994 (77% of records) with 2% from before 1985, and 21% from 1995 - 2005. Records were assigned to a season based on the month of sampling. The majority (73.7%) of the samples were collected in the spring (April to June) with 15.6% collected in summer (July to September) and 10.7% collected in fall (October to December). Gender was available for a subset of walleye samples (N=1953). Of these 43.7% were females and 56.3% were males. The remaining were of uncertain gender either because the fish were not sexually mature or gender was not recorded.

The 421 lakes included in the dataset represent a range of hydrologic, morphometric, and water chemistry conditions (Table 2). The lakes are distributed in 49 of Wisconsin's 72 counties. A majority of the lakes (82%) are within the 20 most northern counties, approximating the distribution of inland lakes in Wisconsin. Hydrologic conditions include: drainage (58%) lakes that have both surface water inflow and outflow and stream drainage is the primary water source; seepage (30%) lakes that have no surface water inflow or outflow and precipitation or runoff is the primary water source; spring (8%) lakes that have no inlet and groundwater is the primary water source; and drained (3%) lakes that only have a surface water outflow and precipitation or runoff is the primary water source.

We evaluated the characteristics of the walleye records for all 421 lakes in the dataset over all years sampled (Table 3). Lake mean concentrations of Hg ranged 0.063 - 2.2 and the overall average was 0.483 µg/g. Lake mean fish length ranged from 31 - 69 cm. The years and number of years sampled varies by lake and the number of samples per lake ranged 1 - 36.

Models describing Hg bioaccumulation and trends - Mercury concentrations increased with walleye length, but the relationship varied among lakes (Figure 1). Lake differences accounted for 54.7% of the total variation in walleye Hg values, while fish length explained 52.9% of the within lake variation in walleye Hg. Of the models without lake morphometry and watershed variables, the best fitting model included fish length, season, year, latitude, and the latitude by year interaction (Model 1, Table 4). Including lake mean values for length of fish or year sampled improved the fit of the model slightly (~1 AIC unit), but did not change parameter estimates for the within-lake effects of length and year (i.e., there was no evidence for bias due to factors such as sampling changes related to fish length or year). This model included random effects for the intercept, fish length, and year; removing any one of these random effects from the model substantially reduced the fit of the model (AIC increased by 100 or more). These random effects allowed individual lakes to deviate from the overall population values for the intercept and for the slopes with respect to fish length and year sampled. The variability introduced by the random effects for the intercept and fish length is illustrated by plotting predicted Hg for all lakes in a given year (Figure 2; predictions are for 1995). The variance estimates for the random effects of fish length and year sampled were small (Table 6), but their inclusion greatly improved the fit of the model.

The parameter estimates for the fixed effects model provide the overall population values for these parameters (Table 6). Because of random effects in the model, individual lakes deviated from population values for the intercept and the effects of fish length and year, but not for the effects of season, latitude, and the latitude by year interaction (latitude has only one value for each lake in any case). Although Hg was log-transformed prior to model fitting, parameter estimates are more interpretable when converted back to the original scale. Linear relationships on the log scale become exponential relationships on the original scale (Figure 2); thus, the linear increase in log<sub>10</sub>(Hg) of 0.0181 per cm of fish length corresponds to a constant percent increase in Hg of 4.3% per cm ( $(10^{0.0181} - 1) \cdot 100 = 4.3\%$ ). Because fish length was centered at 45 cm and latitude was centered at 45 degrees, with year measured as years since 1980, the intercept of - 0.5277 corresponds to a Hg concentration of  $0.30 \mu g/g$  Hg ( $10^{-0.5277}$ ) for a 45 cm fish from a lake at 45 degrees north latitude caught in the summer of 1980. Seasonal effects were measured as deviations from summer values, with fish caught in the spring being 12.8% ((10<sup>0.0597</sup> – 1)•100) higher in Hg concentration than those caught in the summer, and fish caught in the fall being -1.5% ((10<sup>-0.0064</sup> – 1)•100) lower than summer-caught fish. Hg increased with latitude, but this relationship varied among years, as indicated by the interaction between latitude and years (Figure 3a). The rate of increase in Hg with respect to latitude was greater early in the sampling period than during recent years. Equivalently, Hg decreased in northern Wisconsin lakes during the sampling period, while Hg increased in southern lakes. Because of the exponential increase of Hg with fish length, these relationships appear stronger for larger fish (Figure 3a). We confirmed these relationships by estimating time trends separately for southern, central, and northern Wisconsin lakes – trends in Hg concentrations (µg/g) averaged +0.8% per year (linear trend = 0.0034, SE = 0.0037) for southern lakes, were approximately zero (linear trend = -0.0002,

SE = 0.0019) for central lakes, and averaged -0.5% per year (linear trend = -0.002, SE = 0.0028) for northern lakes.

Figure 3b shows the variability of estimated walleye Hg temporal trends for individual lakes based on estimates of a random effect (deviation for each lake from the overall mean for all lakes) and the latitude by year interaction. While the Hg trends for an individual lake cannot be estimated precisely due to data limitations for individual lakes, the distribution of trends for a group of lakes is more reliably estimated.

Models including lake morphometry and watershed variables - Adding log of lake area or log of adjoining wetland area to Model 1 improved the fit; in particular, the model with log of lake area fit far better than any other model (Model 11, Table 5). Log of Hg concentration decreased as the log of lake area increased and the variance among lake intercepts was reduced from its value in the simpler model because this model accounted for more of the lake differences (Table 5). Because both Hg concentration and lake area were log-transformed, the slope of -0.1487 indicates that if lake size is doubled, Hg concentration decreases by 9.8% (Figure 3c, predictions are for 2005).

Models including lake alkalinity and gender of fish - Because alkalinity was not available for all lakes and gender not known for all fish, we used subsets of the data to examine models including these predictor variables. Gender was known for 1,953 fish from 318 lakes. Alkalinity was available for 204 lakes, with 1,642 fish sampled. For each of these two subsets, we first fit the best model identified for all lakes (Model 11 with predictor variables fish length, season collected, year collected, lake latitude, year by latitude interaction, and log of lake area). Adding gender as a predictor variable significantly improved the fit of the model (AIC decreased by 68.8). The parameter estimate for gender in this model was -0.0690 (SE=0.0081, p<0.001). Walleye Hg was 14.7% lower ( $(10^{-0.069} - 1) \cdot 100$ ) for females than for males of equal size. Adding log of alkalinity as a predictor variable significantly improved the fit of the model (AIC decreased by 23.8). The parameter estimate for alkalinity in this model was -0.2236 (SE=0.0419, p<0.001). Walleye Hg decreased by 14.4% as alkalinity doubled. The model including fish length, season collected, year collected, latitude, the latitude by year interaction, log of lake area, and the log of alkalinity fit approximately as well as a slightly simpler model that did not include latitude or the latitude by year interaction, but included instead the interaction between year and log of alkalinity (AIC for the latter model was 0.5 smaller). This suggests that the geographical pattern in walleye Hg time trends may be related to water chemistry differences between northern and southern lakes. In fact, in our dataset latitude and alkalinity were strongly negatively correlated (r = -0.82. P < 0.001).

#### **Discussion**

Our analyses suggest that temporal trends in walleye Hg concentrations vary latitudinally within Wisconsin, with northern lakes exhibiting slight average decreases (-0.5% per year) and southern lakes modest average increases in Hg concentration (0.8% per year) over the time period from 1982 to 2005. The random effects associated with time trends indicate that individual lakes may deviate from these population averages. For the subset of lakes on which alkalinity was measured, we found that models in which time trend differences were related to alkalinity or to latitude fit equally well. Because northern Wisconsin lakes are generally lower in alkalinity and southern lakes generally higher, it is difficult to separate the effects of alkalinity and latitude in our observational study. Furthermore, other factors, including lake chemistry, atmospheric deposition, lake productivity, and fish growth rates, are also associated with latitude and alkalinity, so that several explanations for patterns in time trends are possible. Regional and local differences in walleye Hg trends are likely because of the complex interplay between atmospheric deposition of Hg, lake alkalinity and pH, and factors that affect microbial community composition and activity, such as the nutrient loading from runoff, or climate change (Watras et al., 2006).

Our finding that walleye Hg concentrations decreased in northern Wisconsin is consistent with other studies. Mercury concentrations in northern pike and walleye from Minnesota declined by an average of 11% between 1990 and 2000, or approximately 1% per year (MPCA, 2007). Madsen and Stern (2007) found walleyes (skin-on and skin-off fillets) from northern Wisconsin decreased 0.6%/year over 1982-2005 using hierarchical Bayesian methods. In a study of common loons in northern Wisconsin, Fevold et al. (2003) estimated that adult loon blood Hg levels decreased by 4.2% per year, while loon chick blood Hg decreased by 4.9% per year. Engstrom and Swain (1997) found sediment cores showed evidence of recent declines in Hg deposition to lakes in northeastern Minnesota after reaching a peak in the 1960s-1970s. Hrabik and Watras (2002) attributed the decreases in yellow perch Hg concentration to decreases in atmospheric deposition of H<sub>2</sub>SO<sub>4</sub> and Hg that occurred in northern Wisconsin after 1988 (Watras et al., 2000). Sulfate deposition and frequency of acidic precipitation has decreased throughout the Midwest (Lehman et al., 2007). In the poorly buffered lakes typical of northern Wisconsin, decreases in sulfate deposition may change lake chemistry in ways that reduce the efficiency of Hg methylation (MPCA, 2007; Munthe et al., 2007).

Our analyses also suggest slight increases in walleye Hg concentrations in southern Wisconsin. Mercury deposition tends to be greater in southeastern Wisconsin and these lakes may be receiving more Hg than northern lakes. Recent Hg deposition estimates (1998-2005) are available for seven Wisconsin sites but have not exhibited statistically significant temporal trends (Rodger et al., 2006). Engstrom and Swain (1997) found no evidence of decreased Hg deposition in sediment core samples from west-central Minnesota lakes. Also, the well buffered lakes of southern Wisconsin are much less likely to show changes in lake chemistry due to regional decrease in sulfate deposition than are the poorly buffered lakes of northern Wisconsin. However, our finding of increased walleye Hg in southern lakes warrants further study. There are fewer lakes in southern Wisconsin and our dataset includes fewer fish from southern lakes (573 fish from 62 lakes south of 45° latitude).

Researchers have long recognized that mean walleye Hg concentrations vary greatly among lakes, and have sought to find factors to explain this (e.g., Wiener et al., 1990; Lathrop et al., 1991; Wren et al., 1991). In our analyses, lake differences were expressed as differences in both the intercept and slope of the log<sub>10</sub>(Hg) – walleye length relationship. Although our study was not directed primarily at examining factors that account for lake differences in walleye Hg concentration, we found that lake latitude, area, and alkalinity explained some of the differences in intercepts among lakes, but that none accounted for slope differences. Neither lake latitude nor alkalinity are likely to be direct causes of differences in walleve Hg concentrations, but both are probably correlated with other causative factors. We included latitude in our models, not only to account for spatial sampling patterns in our observational study, but also because it is generally associated with gradients in pH and alkalinity, lake productivity, and fish growth, all factors suspected or demonstrated to be important to Hg bioaccumulation. In Wisconsin, pH and alkalinity, lake productivity, and fish growth rates all tend to decrease with increasing latitude (Lillie and Mason, 1983; Nate, 2004). Alkalinity and pH themselves are probably correlated with causative factors such as the activity of sulfate-reducing bacteria leading to increased rates of methylmercury production (Watras et al., 2006). Increased lake productivity and warmer water temperatures in southern Wisconsin lead to faster fish growth rates, which Simoneau et al. (2005) have found to be associated with lower Hg concentrations in fish of the same length. High lake productivity is also associated with higher densities of phytoplankton and zooplankton and reduced rates of Hg trophic transfer (Chen and Folt, 2005). Both Bodaly et al. (1993) and Greenfield et al. (2001) found that lake size and fish Hg are negatively related, just as we did. They suggested that this was due either to higher temperatures in smaller lakes, leading to higher rates of methylation, or to proportional increases in organic matter input in smaller lakes, which tends to reduce oxygen levels and increase rates of methylation by sulfate-reducing bacteria.

Differences in walleye Hg among fish within lakes were related to fish length, gender, and the season of collection. The increase in Hg concentration with fish length is well known (MacCrimmon et al., 1983; Wiener et al., 1990; Wiener and Spry, 1996) and has been modeled

using mechanistic models that account for bioenergetic and physiological parameters (Trudel and Rasmussen, 2001). The higher concentration of Hg in males than females at the same length is probably due to the higher growth rate of females; males take longer to reach the same size, and consume more Hg during that time (Henderson et al., 2003). We found that for walleye of the same length, Hg concentrations were highest in fish collected in spring; this was also the season in which most of our samples were collected. Post et al. (1996) suggested that seasonal changes in Hg concentrations in fish are caused by seasonally varying diet and growth efficiency, although they found highest Hg concentrations in late summer for age-0 yellow perch. Fish Hg may also reflect seasonally varying methylmercury water column concentrations (Monson and Brezonik, 1998; Eckley and Hintelmann, 2006).

Our findings suggest that Hg in walleye from Wisconsin lakes changed in the range of -0.5 to 0.8% per year depending on geographical position in the state during the period of 1982 to 2005. These trends may reflect geographically differing temporal trends in the amount of Hg deposited to Wisconsin lakes. However, long-term changes in other factors, such as water chemistry, fish growth rates, and lake levels, known to impact Hg bioavailability and accumulation may also be important. These findings should be re-evaluated in future years.

#### **Walleye Tables and Figures**

**Table 1.** Descriptive statistics of walleye (skin-on fillets) records included in mercury trend analysis (WIDNR 1982-2005).

Variable	N	Mean	Std Dev	C. V.	Minimum	Maximum
Fish Length (cm)	3024	45.0	8.5	19	25.4	76.7
Fish Weight (kg)	2986	0.94	0.64	68	0.1	5.46
Age (year)	160	5.5	2.1	39	2	12
Total Mercury Concentration (µg/g)	3024	0.474	0.326	69	0.030	3.1

**Table 2.** Descriptive statistics of study lakes with selected walleye (skin-on fillet) mercury records.

Variable	N	Mean	Std Dev	C.V	Minimum	Maximum
Max Depth (meters)	421	11.8	7.6	64	1.8	72.0
Drainage Area (sq miles)	189	14	46	337	1	491
Watershed Area (sq miles)	189	75	319	426	1	3929
Waterbody Size (acres)	421	1045	6818	652	17	137708
Wetland Adjoin Area (acres)	421	541	3932	727	0	78060
Wetland-Lk Area Ratio	421	0.91	5.39	591	0	87.89
pH (geo. mean, standard units)	137	7.9	0.6	8	5.7	9.5
Alkalinity (geo. mean, mg/L Total CaCO <sub>3</sub> )	204	63.7	54.4	85	2.8	213
Color (geo. mean, standard units)	207	27	28	105	5	150
Latitude (degrees)	421	45.5196	0.8286	2	42.5916	46.7951
Longitude (degrees)	421	-90.0611	1.1787	1	-92.711	-87.1542

**Table 3.** Descriptive statistics of lake means of walleye (skin-on fillet) records included in this study.

Variable	N	Mean	Std Dev	C.V.	Minimum	Maximum
Lake Mean Hg Concentration (µg/g)	421	0.483	0.306	63	0.063	2.2
Lake Mean Fish Length (cm)	421	46.00	5.89	13	30.73	69.34
Lake Mean Year Sampled	421	1990.5	4.8	0.2	1983.7	2004.0
Lake Mean Number of Years Sampled	421	1.5	0.9	57	1.0	7.0
Lake Mean Number of Mercury Results	421	7	6	80	1	36

**Table 4.** Models fit by maximum likelihood (ML) predicting walleye mercury concentrations (skinof fillets), listed in order with best fit first.  $\triangle$ AIC is the difference in AIC from best-fitting model (model with minimum AIC). All models include an intercept. Abbreviations used for factors in models are: L – fish length, lat – latitude, S – season, Y – year sampled, lat\*Y – latitude by year interaction.

Model		
Number	Fixed effects	ΔΑΙC
Model 1	L Y S lat lat*Y	0
Model 2	L Y S lat	4.6
Model 3	LYS	21.3
Model 4	L Y lat lat*Y	25.6
Model 5	L Y lat	31.4
Model 6	LY	53.2
Model 7	L S lat	110.7
Model 8	LS	125.5
Model 9	L lat	177.7
Model 10	L	198.4

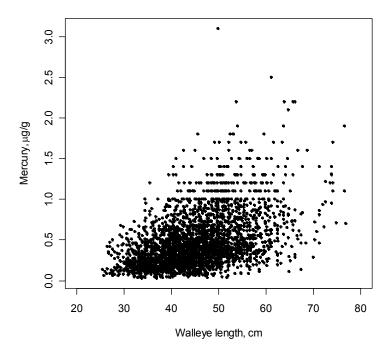
**Table 5.** Models predicting walleye mercury concentrations (skin-on fillets) based on best model from Table 4, but also including lake morphometry and watershed data. Other variables included are: logArea – log of waterbody area; logWetland – log of adjoining wetland area; depth – waterbody maximum depth; ratio – ratio of adjoining wetland area to waterbody area.

Model		
Number	Fixed effects	ΔΑΙC
Model 11	L Y S lat lat*Y logArea	0
Model 12	L Y S lat lat*Y logWetland	38.1
Model 1	L Y S lat lat*Y	42.0
Model 13	L Y S lat lat*Y ratio	43.6
Model 14	L Y S lat lat*Y depth	43.8

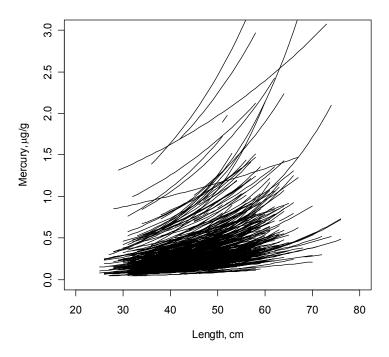
**Table 6.** Parameter estimates from best models (model 1 and 11) predicting walleye mercury concentrations (skin-on fillets) fit by restricted maximum likelihood (REML). SE = standard errors.

	Model 1		Model 11	
Parameter	Estimate	SE	Estimate	SE
Intercept	-0.5277	0.0244	-0.1426	0.0618
Fish length	0.0181	0.0005	0.0180	0.0005
Latitude	0.1072	0.0227	0.0950	0.0220
Year	0.0008	0.0016	0.0010	0.0015
Latitude*Year	-0.0038	0.0015	-0.0038	0.0014
Log waterbody area	-	-	-0.1487	0.0220
Spring - Summer	0.0597	0.0136	0.0620	0.0135
Fall - Summer	-0.0064	0.0204	-0.0051	0.0202
Variance components (exp	ressed as standard	I deviations)		
Intercept	0.2693		0.2574	
Fish length	0.0063		0.0063	
Year	0.0130		0.0127	
Within lake (residual)	0.1339		0.1340	

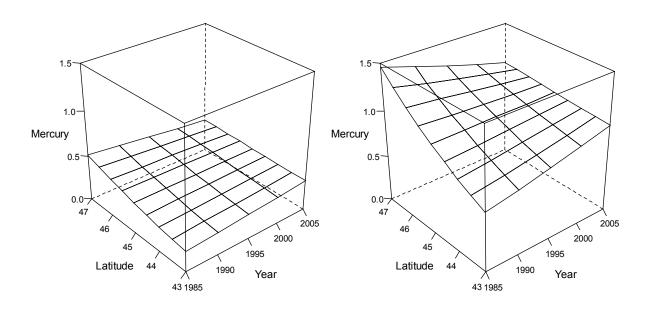
**Figure 1**. Observed total mercury concentration ( $\mu$ g/g) for 3,024 walleye skin-on fillet samples from 421 Wisconsin lakes.



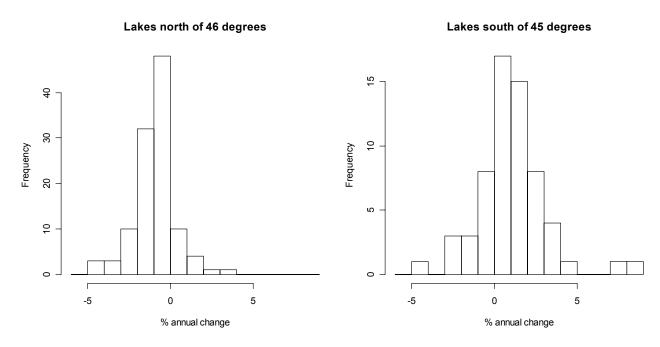
**Figure 2**. Predicted total mercury concentration in walleyes (skin-on fillets) for the year 1995 from best fitting model (Model 1) without variables on lake morphometry or watershed data. Each line represents predictions for fish from one lake, over the size range of fish observed for that lake.



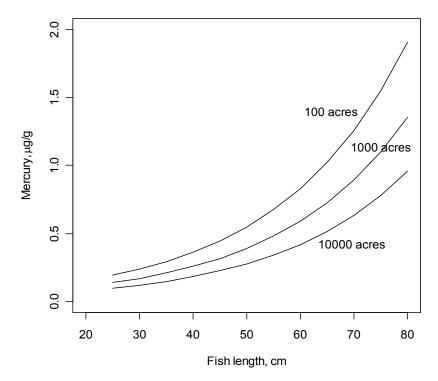
**Figure 3a**. Predicted mercury ( $\mu$ g/g) in skin-on fillets of a 45 cm walleye (top panel) and a 70 cm walleye (bottom panel) by year sampled and lake latitude (decimal degrees).



**Figure 3b**. Distribution of estimated mercury ( $\mu g/g$ ) concentration trends for study lakes north and south of 46° latitude.



**Figure 3c**. Predicted total mercury concentration in walleyes (skin-on fillets) for the year 2005 using Model 11 showing the effect of lake area. Each line represents predictions for lakes with surface areas of 100, 1000, and 10000 acres.



## B. Trends of mercury in young-of-year yellow perch 1992-2006

#### Methods

**Study Sites** - Wisconsin lakes vary considerably in appearance, size, and in chemical and physical characteristics (Lillie and Mason 1983). Young-of-the-year yellow perch were collected from seven lakes located in northern Wisconsin, USA (Oneida, Lincoln, Langlade, and Vilas Counties; Figure 4), an area rich in lake types that favor Hg bioaccumulation (Lathrop, et al. 1989). The seven lakes represent a continuum of several chemical and morphometric characteristics (Table 7), spanning a broad range in lake area (61-1128 ha) with watersheds, drainage, and adjoining wetland areas that also vary widely. 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 have broad ranges in clarity (0.5 - 3.4 m Secchi depths), alkalinity (3 - 79 mg/l CaCO<sub>3</sub> equivalents), and pH (6.6 - 8.4, slightly acidic to alkaline).

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 early spring (February-March) and late fall (October-November) and high water levels occur during 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 the 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.

**Fish Collection and Sample Preparation** – Under this project YOY yellow perch were collected in mid- to late July in 2003 and 2006 to supplement sample collected in early to mid-August 1992 and July 1996 and 2000. The sampling goal of 30 individual YOY yellow perch was increased to 50 individuals per lake in 2003 and 2006 in order to increase the sample size per lake per year from three to five composites. Overall, collections were usually within 1 to 4 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.

Young-of-the-year yellow perch were collected by shoreline seine (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 samples of ten fish of similar length. In 2003 and 2006, the age of two individuals from each composite was determined by scale examination of annuli to confirm that collected individuals were age 0.

*Hg Analysis* – Composite YOY samples were homogenized in a blender. Mercury 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  $\mu$ g/g wet weight.

**Statistical Analysis** – All statistical comparisons were performed with SAS Version 9.1 (SAS Institute, Cary, NC, USA). We log<sub>10</sub>-transformed Hg concentrations because the variance among observations was more homogeneous on the log scale. We conducted analyses of 1) individual lake trends and 2) overall trends using data combined from all study lakes.

Individual Lake Trends - We examined relationships between Hg concentrations, sample year, 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  $\log_{10}$  of Hg concentration and predictor variables were composite sample fish length, year sampled, and their interaction. If the

interaction term was not significant, we refit the model without it. Fish length was the average of lengths of individual YOY fish in each composite sample.

Overall Trends - We used several mixed effects models to analyze data from all lakes simultaneously. Mercury concentrations were log<sub>10</sub>-transformed before analysis. We used Akaike's Information Criterion (AIC) to compare models and likelihood ratio tests to examine the significance of individual factors within models. Models included random effects for lakes and fixed effects for time trends and the effects of fish length. We included both between-lake (average of samples from the lake) and within-lake effects of fish length (average of individual fish lengths contained in each composite sample) in models. Because Hg concentration generally increases in fish as they grow older and increase in length, 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.

#### Results

**Data Summary** - A total of 121 composite samples were collected from the seven study lakes over the years 1992, 1996, 2000, 2003, and 2006 (Table 8). Most of the composite samples contained 10 individuals per composite. Only seven of the 121 composite samples contained more or less than 10 individuals because the targeted number of YOYs were not obtained in some years at some sites. At least three composite samples were collected for each lake in most years. Only one composite sample was obtained for three of the five years for North Nokomis, for one of the five years for Lake Minocqua, and for two of the five years for Spirit River Flowage.

Mercury concentrations were at detectable levels in all samples. The overall mean Hg concentration for all YOY samples was 0.059  $\mu$ g/g (se = 0.004) and concentrations ranged from 0.008 to 0.220  $\mu$ g/g. The overall mean length of all samples was 45 mm (se = 0.76) and the mean lengths from individual composite samples ranged from 28 to 74 mm.

**Individual Lake Trends** – We found Hg concentrations varied significantly with year and YOY length in some lakes (Table 9). There were no significant interactions between length and year except in Rolling Stone Lake (p = 0.0021) where it appears that both Hg concentrations and YOY length increased from 1992 to 2000, and then decreased in more recent years (Figure 5).

Of the remaining lakes, the Spirit River Flowage (+7.5%/yr<sup>-</sup>) and Tug Lake (+4.5%/yr) exhibited increasing Hg concentrations over time, while Chain (-3.8%/yr<sup>-</sup>) and North Nokomis (-13.7%/yr) Lakes had decreasing Hg concentrations during the sampling period. Mercury concentrations decreased significantly with respect to length in two of the study lakes (North Nokomis and Tug).

**Overall Trends** – Seven different models used to analyze Hg trends across all lakes provided similar estimates of the effects of within and between lake factors and year on Hg concentrations (Table 10). The list of predictors shows the fixed effects included in the different models and includes year, fish length, and lake mean fish length for the lake for all years. The difference in Akaike's Information Criterion ( $\triangle$ AIC) scores is computed as the difference from the model with the lowest value of AIC among all models fit (i.e.  $\triangle$ AIC for that model is 0). The three best scoring models (models 1 to 3) scored similarly (within 2 units).

The models indicate that the between-lake effect of fish length was positive and the within-lake effect of fish length was negative but minor. The best-fitting mixed effects model (model 1) and the top scoring models all included lake mean fish length as a fixed effect. In other words, lakes with larger YOY had higher Hg concentrations. Between lakes, Hg concentrations increased 11% with each mm of lake mean fish length based on the estimate of model 1 ((10 0.04603-1)\*100). In comparison, Hg concentration decreased only 0.09% per mm of fish length of composite samples based on model 3.

The estimated change in Hg concentration per year was relatively small, ranging from -0.69% to -0.76% per year based on the four models that included year as a predictor. The second best model (model 2) included the lake mean fish length and year as predictors. This model suggests that Hg concentration in YOY yellow perch may have decreased slightly over the period 1992 to 2006 in this set of lakes.

#### Discussion

Individual Lake Trends - It is difficult to draw strong conclusions about changes in Hg concentrations over time in the seven study lakes by examination of each lake individually. Lake-by-lake analysis certainly 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.

**Overall Trends** - Analysis of data from all study lakes simultaneously using mixed effects models suggests that Hg concentrations in YOY yellow perch are primarily determined by factors that differ between lakes, e.g. productivity. 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, Littell 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 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 the opposite in mixed effects models. Within a lake, fish of longer lengths had lower Hg concentrations than samples of shorter fish. The effect of average fish length between lakes was positive. Across all years of the study, Hg concentrations in fish were higher in lakes where average YOY length was longer.

The best model of YOY Hg concentrations did not include year as a predictor. These results do not allow a strong conclusion about temporal trends in Hg concentrations in these study lakes during the 15-year study period (1992 to 2006). However, the second best scoring model suggests that Hg concentration in YOY yellow perch may have decreased slightly over the period 1992 to 2006 in this set of lakes in the range of – 0.69% per year. This finding is consistent with other estimates of temporal trends of Hg in fish from northern Wisconsin lakes. Rasmussen et al. 2007 estimated that walleye Hg concentrations in walleyes from northern Wisconsin lakes decreased slightly on average (– 0.5% per year) over the time period from 1982 to 2005. Mercury concentrations in northern pike and walleye from Minnesota declined approximately 1% per year (MPCA, 2007). Madsen and Stern (2007) found walleyes from northern Wisconsin decreased 0.6% per year over 1982-2005.

**Young-of-Year** - 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). YOY length at time of collection represent differences in growth rates within summer growing seasons from time of hatch to time of collection. Since time of hatch is unknown, our collection times were not standardized to time since hatch across years. 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 YOY. Mercury concentrations may not be strongly related to length within individual year classes, over short time frames, or within limited size ranges. Grieb 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 2 yellow perch from 10 Wisconsin seepage lakes and found no association with length or weight.

Our 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 bioavailability. 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 the first growing season. After hatching, perch larvae move from shallow water to the limnetic zone where they first begin exogenous feeding when water temperature reaches 15°C. Young-of-year prey includes zooplankton and aquatic insect larvae in this habitat. After about 30 days in the limnetic zone, or when they transform to the juvenile form at approximately 25 mm, perch return to the littoral zone and shift toward larger, benthic prey organisms such as amphipods and aquatic insects. Within a lake, YOY yellow perch Hg body burden may change over time due to the shift in diet. This shift constitutes a change in trophic feeding status of YOY during their first growing season 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, duration of incubation, and time from hatch to collection likely affect Hg accumulation in YOY. Yellow perch spawn over several weeks just after ice-out and the time until hatching can range from 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) reported that Lake Winnebago 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. Transformation to the juvenile stage is completed at about 40 days in age. Afterwards, most energy intake is directed toward increasing body weight rather than length. Therefore, body weight or condition factor may be a better measure of growth than length for juvenile yellow perch.

Study Lakes – Other possible reasons for not finding temporal changes in YOY Hg concentrations is that some of our study lakes may not be sensitive to changes in Hg deposition or that Hg deposition to the study lakes did not significantly change over the study period. The study included only two lakes with relatively low pH and four with low alkalinity, based on a limited water chemistry dataset. Inter-lake differences in fish Hg accumulation are primarily associated with 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 of 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 Hg concentrations (Hrabik and Watras 2002), drainage area, lake area (Suns and Hitchin 1990), and Hg in surficial sediments (Cope et al. 1990).

Our study lakes differ slightly in water chemistry, drainage area-to-lake area ratio, and adjoining wetland area. These differences may explain differences in YOY Hg concentrations among lakes. However, it is unlikely that these variables changed significantly during the study period. 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.

**Recommendations** - The current monitoring design does not allow assignment of cause for any observed trends or lack of trends. Wisconsin air deposition monitoring has not been sufficient to

use in determining 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 Wisconsin YOY yellow perch are sensitive to changes in deposition. Based on Hg deposition monitoring at seven stations located in Wisconsin (1998 – 2005), Hg deposition is related to amounts of wet deposition but statistically significant temporal trends have not been detected (Rodger et al., 2006).

Since this monitoring effort began, our understanding of Hg bioaccumulation has grown. Wiener et al. (2007) recently reviewed new findings and recommended assessing trends in methylmercury bioaccumulation by including 1-year-old (yearling) prey fish in monitoring, in addition to predator game fish, to indicate annual changes in the supply of methylmercury to aquatic systems.

We recommend that this monitoring effort be modified. Additional lakes known to be sensitive to Hg accumulation should be monitored. These lakes should be selected to minimize Hg inputs other than atmospheric deposition and minimize flow regimes that can alter Hg loading and methylation. Yearling yellow perch Hg should be examined to determine if yearlings would be better indicators than YOY. Future collections should be restricted to a more consistent time of year (late July) and specimens from each lake should be aged. Lastly, samples should be based on individual specimens instead of composite samples.

Our study found that Hg concentrations declined over time in some lakes, increased in some, and showed no significant change over time in other lakes. We hypothesize that YOY undergo substantial changes in food sources and growth in the first season, which is represented by length at time of capture. Our models suggest that Hg concentrations are primarily affected by factors that influence differences in YOY sizes among lakes. This dataset does not allow a strong conclusion about temporal trends in Hg concentrations in these study lakes during the 15-year study period (1992 to 2006). We suggest that future trend monitoring investigate use of yearling yellow perch in place of the young-of-year life stage. Also, the sample size should be increased by including additional lakes.

#### **Young-of-Year Tables and Figures**

**Table 7.** Characteristics and water chemistry of young-of-year yellow perch study lakes.

Name (Town Range Section)	County	Lake Type	рН	Total alkalinity (mg/l CaCO3 eq)	Secchi Disc (m)	Max. Depth (m)	Lake Area (acres)	Watershed Area (sq mi)	Drainage Area (sq mi)
Chain Lake (39 09E 28)	Oneida	Deep, lowland drainage	6.6	21*	1.3	5.8	201	32	9
Minocqua Lake (39 06E 15)	Oneida	Deep, lowland drainage	8.5	46	3 - 5	18.3	1339	80	8
North Nokomis (39 08E 26)	Oneida	Deep, headwater drainage	7.5	48	2.4	22.3	470	3	2
North Twin Lake (41 11E 18)	Vilas	Deep, lowland drainage	8.4	44	3.4	18.3	2871	17	6
Rolling Stone (34 12E 13)	Langlade	Shallow, lowland drainage	8.1	83	1.6	3.7	682	10	2
Spirit River Flowage (34 06E 16)	Lincoln	Deep, lowland drainage	7.6	24	0.5	6.7	1148	174	80
Tug Lake (33 06E 35)	Lincoln	Deep, lowland drainage	6 - 7	3 - 10	0.6	6.4	151	10	10

<sup>\*</sup> This value is methyl purple alkalinity

Sources: Wisconsin DNR Registry of Waters, water chemisty data, Surface Water Inventory File, and Lilie and Mason 1983.

Table 8. Sample sizes and descriptive statistics for young-of-year yellow perch, 1992-2006.

			Length	n (mm)		Mercury (μg/g, wet weight)				
Name	n	Mean	Minimum	Maximum	s.e.	Mean	Minimum	Maximum	s.e.	
Chain	19	47	36	57	1	0.068	0.045	0.130	0.005	
Minocqua	18	43	33	56	1	0.025	0.019	0.037	0.001	
North Nokomis	11	52	38	74	4	0.038	0.021	0.085	0.006	
North Twin	21	37	28	52	1	0.014	0.008	0.026	0.001	
Rolling Stone	20	46	35	56	1	0.046	0.018	0.110	0.006	
Spirit River Flowage	11	53	43	64	2	0.151	0.058	0.220	0.020	
Tug	21	45	38	53	1	0.097	0.056	0.140	0.005	

n = number of composite samples, s.e. = standard error.

**Table 9.** Linear regressions of  $log_{10}$ mercury on young-of-year yellow perch length (mm) and year by individual lake. %  $\Delta$  is the calculated change in mercury per year and length based on the slope estimate.

			Year co	llected			Fish lengt	th (mm)	
Name	df	slope	% Δ/yr	s.e.	р	slope	% Δ/mm	s.e.	р
Chain	18	-0.017	-3.8	0.004	<0.001 *	-0.005	-1.1	0.003	0.107
Minocqua	17	-0.0004	-0.1	0.005	0.941	-0.001	-0.2	0.004	0.743
North Nokomis	10	-0.064	-13.7	0.015	0.003 *	-0.014	-3.2	0.006	0.041 *
North Twin	20	-0.004	-0.9	0.007	0.592	-0.011	-2.5	0.006	0.073
Rolling Stone 1	19	-0.004	-0.9	0.015	0.805	0.011	2.6	0.012	0.384
Spirit River	10	0.031	7.4	0.007	0.002 *	-0.012	-2.7	0.005	0.053
Tug	20	0.019	4.5	0.003	<0.001 *	-0.007	-1.6	0.003	0.027 *

year\*length significant for Rolling Stone Lake (p = 0.0021)

df based on the number of composite samples

s.e. standard error

**Table 10.** Parameter estimates from mixed effects models of YOY yellow perch mercury concentration listed in order of Akaike Information Criterion (AIC) score. \*  $\Delta$  AIC is the difference in AIC score compared to the score of the best fitting model (model 1).

						% Δ Hg
Model	AIC*	Predictors	Slope	s.e.	p-value	per Year
1	0	Lake Mean Length	0.04603	0.01714	0.0083	
2	1.1	Lake Mean Length	0.0459	0.0171	0.0083	
		Year#	-0.0030	0.0031	0.331	-0.69
3	2	Length	-0.0004	0.0022	0.861	
		Lake Mean Length	0.0464	0.0173	0.0083	
4	2.9	Length	-0.0009	0.0022	0.673	
		Lake Mean Length	0.0468	0.0172	0.0076	
		Year #	-0.0033	0.0032	0.296	-0.76
5	4	Year #	-0.0030	0.0031	0.3321	-0.69
6	5	Length	0.0000	0.0022	0.9937	
7	6	Length	-0.0006	0.0022	0.8023	
		Year#	-0.0032	0.0032	0.3166	-0.73

<sup>\*</sup> AIC values from SAS Version 9.1

Figure 4. Location of Langlade, Lincoln, Oneida, and Vilas Counties, Wisconsin, USA.

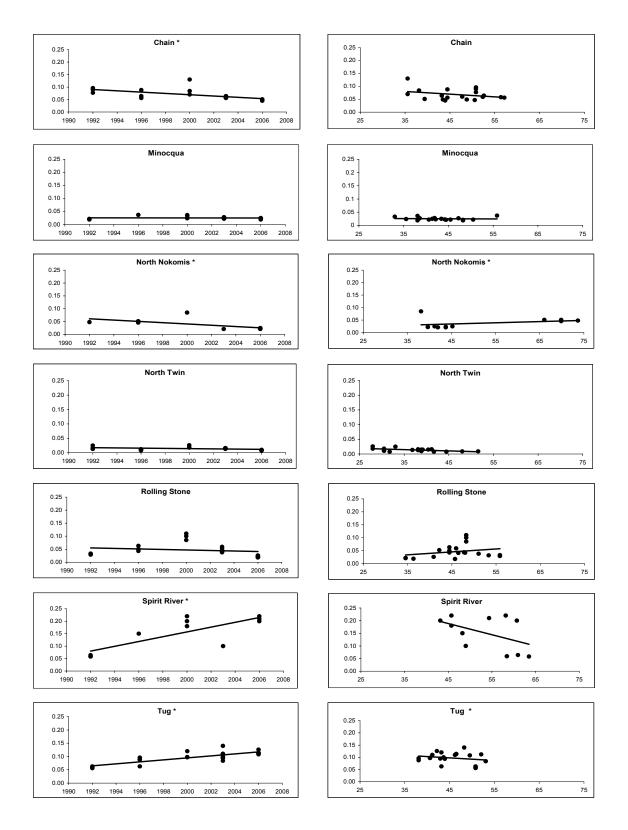


s.e. = standard error, p = level of significance at 0.05

**Figure 5.** Mercury concentrations in YOY yellow perch versus year and length by lake. \* indicates significant regression at p < 0.01.

# Mercury (µg/g) VS Year

# Mercury (µg/g) VS Length (mm)



## C. Use of yearling yellow perch – preliminary dataset

#### Methods

**Study Sites** – The goal of the sampling design was to collect yearling yellow perch in the seven lakes with historical YOY data and lakes where walleye were targeted for collection and Hg trend analysis. Lakes sampled totaled 24 and were located in 13 counties of the state of Wisconsin (Figure 6). While most of the lakes sampled are located in northern Wisconsin, four lakes are in southern and central Wisconsin. Lakes sampled included 13 drainage, 7 seepage, 3 spring and 1 drained lake. Several of the lakes have water control structures and two are considered riverine impoundments. Other physical and chemical characteristics vary among the 24 study lakes (Table 11).

Fish Collection and Sample Preparation – The goal was to collect 10 individual yearlings but additional individuals were collected, including YOY from some lakes, to ensure that yearlings were obtained by the sampling effort and to investigate differences between YOY and yearling Hg concentrations. Samples were collected in 2003 to 2006. Fish were collected using shoreline seine hauls (50-ft seine with 1/8" mesh). At some locations, mini-fyke nets were deployed to capture young perch more efficiently. Both gear types targeted moderate- to heavily-vegetated littoral areas where young perch life stages are known to be abundant. Once collected, fish were placed in a labeled plastic bag and held in a cooler. Samples were frozen until processing. Prior to homogenizing the whole body tissue for Hg analysis, length and total body weight were measured and scale samples were taken from a subset of individuals from each lake to verify age.

Mercury Analysis – Individual, whole fish were homogenized using a mortar and pestle. Mercury concentrations in individual fish 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 μg/g wet weight.

**Statistical Analysis** – Analysis of variance and basic statistics were conducted using SAS version 9.1 (SAS Institute, Cary, NC, USA). Mercury concentrations were log10-transformed because the variance among observations was more homogeneous on the log scale. Classification factors examined were lake (name), year of collection, and age (0 or 1). Box plots showing the percentile of overall lake mean Hg concentration by these factors were used to explore the variation in Hg concentrations by year and age. Scatter plots were used to examine the relationship between yearling and YOY Hg concentrations. Paired two way analysis of variance was conducted using SigmaStat version 3.1 to examine subsets of the dataset to determine: 1. differences in Hg concentrations in yearling and YOY lifestages collected in the same year and lake; 2. the variation of Hg concentrations in yearlings collected from the same lake but in different years; and 3. change in Hg concentration in a cohort or year class (YOY and yearling collected in consecutive years).

#### Results

**Data Description** - Sampling success varied in part due to the level of sampling effort available and ease of obtaining yellow perch within a lake. Yearlings were obtained from 24 lakes in 2003 to 2006, 12 of which were sampled in two different years. YOY were obtained from 15 lakes of the 24 lakes. Sampling in most lakes occurred in late July and late August of each year (29 and 59% of individual samples, respectively). Five of the study lakes were sampled in other months (May, September or October) during one collection year.

**Fish Samples** - A total of 200 YOY from 15 lakes and 277 yearlings from 24 lakes were collected over the years 2003-2006. See Table 12 for a summary of the length, weight, and Hg concentrations of individual fish by age class collected across all lakes and years. Table 13

shows the grand mean characteristics of the individual sampling means (lake-year means) of vellow perch length, weight and Hg concentrations.

<u>Variation in Hg Concentrations between lakes, collection year, and age</u> - Mercury concentrations differed among some combinations of lakes, age class, and year of collection (lake name\*year\*age, p=0.0378). Statistical analysis is limited because both YOY and yearlings were not collected in all years in all lakes and some lakes were sampled only one year. Subsets of the available data were examined further with the caveat that as additional data becomes available, these findings may change. Overall lake means by sampling year and age shows that there is high variability in Hg concentrations (Figure 7) among lakes.

Examining variation of Hg concentrations within the same age, concentrations within the same age class varied between years in some lakes but not all lakes (lake name\*year interaction, p<0.0001 for both YOY and yearling).

Both YOY and yearlings were collected in five lakes in 2005 and 15 lakes in 2006. In each of these years, Hg concentrations varied between age (YOY and yearling) depending on the lake (name\*age interaction, p=0.0316 for 2005 and p=0.0004 for 2006). That is, Hg concentrations differed between age 0 and 1 in some lakes but not in all lakes.

Fish Hg concentrations poorly or did not significantly correlate with fish length or weight of the yellow perch when examined both for individual fish and for lake means within each age class. Lake mean Hg and lake mean yellow perch length correlation coefficients were -0.145 (p=0.607) for age 0 and -0.274 (p=0.195) for age 1 fish. Additional statistical tests may prove that length or size of the yearlings is important to describing variability in Hg concentrations because length was found to be an important predictor of fish Hg concentration for both walleye and yellow perch YOY datasets.

<u>Variation in Hg Concentrations in Yearlings by Collection Year</u> - Insufficient data exists at this time to make definitive conclusions about temporal trends in Hg concentrations based on this data. However, yearlings were collected in several lakes (12 lakes) in two different years, useful to examine between year variation in Hg concentrations in yearlings. Two-way analysis of variance and multiple pair wise comparisons (Holm-Sidak method) found that Hg concentrations differed between the first and second years in four out of the eight lakes. Figure 8 shows the box plot percentiles for eight of those lakes where at least three yearlings were collected in each year. This figure shows that Hg concentrations can vary between years suggesting that larger sample sizes may be necessary to quantify temporal Hg trends.

Comparison of Yearling with YOY within the same lake and collection year - Figure 9 is a scatter plot of mean Hg concentrations for each sampling event (lake-year) for yearling versus YOY where both ages were collected in the same year and lake (n = 20). In general, Hg concentrations were higher in the yearlings compared to YOY. The average ratio of yearling to YOY Hg concentration was 1.6 (1.0 - 2.8).

Mercury concentrations in both YOY and yearling appear to vary similarly within lakes by sampling year. That is, the standard deviation of lake mean Hg concentrations for YOY averaged 0.021  $\mu$ g/g (0.002 - 0.024) and for yearling averaged 0.027  $\mu$ g/g (0.002 - 0.032). Coefficients of variations (CV) were high for both ages (Table 12 and 13). This suggests that variability may not be lessened by utilizing yearling yellow perch compared to YOY to examine temporal trends of Hg concentrations. This again suggests that larger sample sizes maybe necessary to quantify temporal Hg trends.

Hg Increases from YOY to Yearling – Examining 2005 YOY and 2006 yearling (n=6 lakes) using two-way analysis of variance again showed that interaction factors were significant (lake name\*age, p<0.001). That is, Hg concentrations differed between the two age groups in some of

the six lakes but not all. Figure 10 shows the range in lake mean Hg concentrations for 2006 yearling compared to that for 2005 YOY.

#### **Discussion**

The yearling Hg data collected to date are limited to one or two years of collection. Additional collections over the future years will allow further examination of the variation of Hg concentrations between lakes and over time. Data collected to date suggest that Hg concentrations are as variable in yearling as they are in YOY. Therefore, use of yellow perch yearling to monitor trend of Hg over time may require larger sample sizes per lake unless additional sources of variability are incorporated into the analysis. A worst-case power analysis using the observed yearling standard deviation in Hg concentrations suggests 60 individuals should be collected to detect differences between two years within one lake. For the yellow perch YOY, mixed effects models were used to analyze data from all sampled lakes. This method allowed several different models that incorporated different sources of variability to be examined. This approach may be useful for analyzing the yearling Hg data after additional samples are collected.

At this time, it is unclear if trends in Hg concentrations in Wisconsin lakes can be detected using yearling yellow perch. We found that Hg concentrations in YOY and yearling are highly variable. However, Harris et al. (2007) recently found that Hg concentrations in YOY yellow perch responded to controlled artificial Hg spikes and that most of the increase in the YOY was from Hg deposited directly to the lake but that the increase was not proportional to the loading increase. These authors predict, that while biota will respond quickly to reductions, response rates will vary among lakes.

Additional monitoring using should be conducted to further investigate the utility of young yellow perch to detect trends of Hg in Wisconsin lakes. Based on the existing data and monitoring designs conducted to date, yearling yellow perch should be collected every three years at least two more times before additional statistical analyses is conducted and this program is extended to other lakes. At that time, the data should be examined to quantify differences exhibited among lakes, temporal trends, and the importance of size of the yearling at the time of collection. The season or time of collection should be controlled to the extent feasible because Hg concentrations in some species vary by season of collection (Rasmussen et al. 2007).

# **Yearling Yellow Perch Tables and Figures**

Table 11. General characteristics of 24 study lakes.

	Maximum Depth (meters)	Waterbody Size (acres)	Drainage Area (sq mi)	Watershed Area (sq mi)	Gmean pH	Gmean ALK	Gmean COLOR	Latitude (decimal degrees)
Count	24	24	19	19	13	20	19	24
Average	13.1	670.0	5	23	7.7	66.2	27	45.5007
Minimum	3.4	46.8	1	1	6.3	5.0	5	43.0065
Maximum	27.7	3461.7	25	98	8.7	205.3	120	46.2806

Lake characteristic data compiled from DNR lake and water chemistry databases.

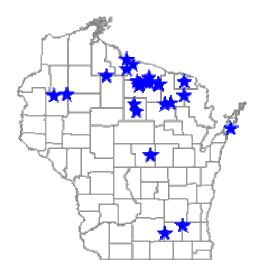
**Table 12.** General characteristics of all individual yellow perch from all lakes and years.

	Length (mm)	YOY Body weight	Mercury (ug/g)	Length (mm)	Yearling Body weight	Mercury (ug/g)
N	200	200	200	277	240	277
Mean	58.9	1.979	0.037	94.30	9.525	0.051
Min	33.0	0.300	0.010	66.80	2.600	0.017
Max	83.8	5.500	0.120	139.70	26.300	0.196
Std Dev	10.2	1.078	0.023	13.73	4.648	0.027
CV	17	55	63	15	49	52

**Table 13.** General characteristics of yellow perch by sampling event (grand mean of lake-year means). Some lakes were sampled in only 1 year and some in two separate years.

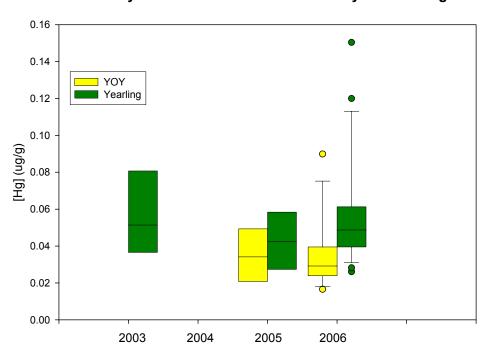
	<b>YOY</b> Body				<b>Yearling</b> Body		
	Length	weight	Mercury	Length	weight	Mercury	
	(mm)	(grams)	(ug/g)	(mm)	(grams)	(ug/g)	
N	22	22	22	36	36	36	
Mean	58.7	1.981	0.037	94.39	9.274	0.055	
Min	41.9	0.710	0.017	73.66	4.300	0.026	
Max	73.9	4.000	0.095	116.84	19.000	0.150	
Std Dev	9.3	0.981	0.021	11.37	3.623	0.027	
CV	16	50	57	12	2 39	49	

Figure 6. Location of 24 yearling yellow perch study lakes in Wisconsin, USA.



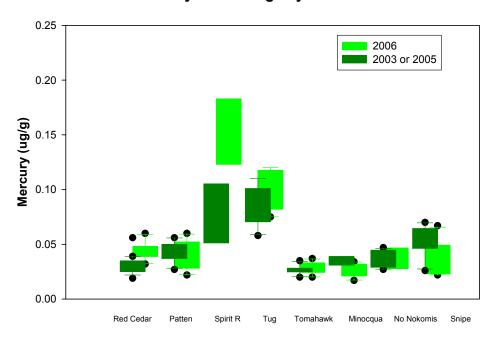
**Figure 7.** Box plot showing median, 25th and 75th percentiles (box), 10th and 90th percentile (whiskers), and outliers of lake means of mercury concentrations by collection year and age.

#### Mercury Concentrations - Lake Means by Year and Age

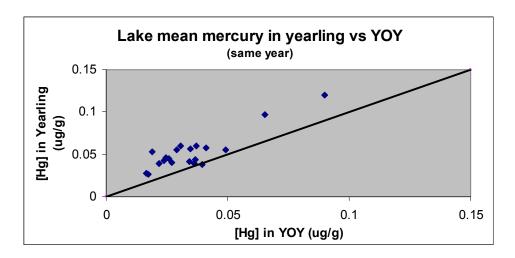


**Figure 8.** Box plot showing median, 25th and 75th percentiles (box), 10th and 90th percentile (whiskers), and outliers of lake mean yearling mercury concentrations for 8 lakes sampled 2 years (n>3 per year).

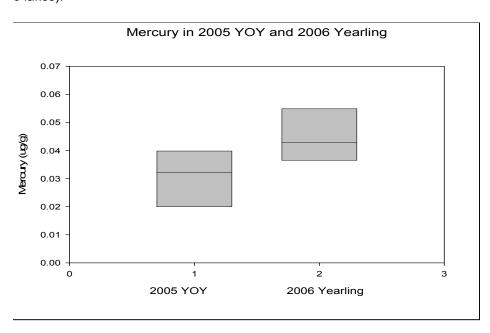
# Mercury in Yearlings by Lake and Year



**Figure 9.** Lake-year mean mercury concentrations in yearling yellow perch versus YOY collected within same lake and year (n=20).



**Figure 10.** Box plot showing median, 25th and 75th percentiles (box), 10th and 90th percentile (whiskers), and outliers of lake mean mercury concentrations in 2005 YOY and 2006 yearling (n = 6 lakes).



#### **CONCLUSIONS**

#### **Walleye**

Monitoring of Hg concentrations in walleye skin-on fillets is a useful tool for detecting trends of Hg in Wisconsin's lakes if known factors that affect Hg availability and bioaccumulation are incorporated into appropriate statistical analyses. Measuring temporal trends of Hg in Wisconsin walleye should for allow a better understanding of the sources and fate of Hg released to the environment including that from electrical generation and other sources.

While trends in individual lakes are of interest, limitations of historical data (1970 -2003) prevent estimates of temporal trends of walleye Hg concentrations within most lakes. Also, lake-by-lake analysis does not provide a straightforward method to synthesize findings and draw conclusions for the whole state. We evaluated temporal trends over all lakes using several different mixed effects models. We explored relationships between Hg concentrations and a suite of lake chemistry, morphometry, and other variables.

Our analyses suggest that temporal trends in walleye Hg concentrations varied latitudinally within Wisconsin. Northern lakes exhibited slight average decreases (– 0.5% per year), central lakes showed no change, and southern lakes showed modest average increases in Hg concentration (+ 0.8% per year) over the period from 1982 to 2005. Individual lakes deviate from these population averages. Our finding that walleye Hg concentrations decreased in northern Wisconsin is consistent with other studies. While there are several possible explanations for our finding of increased walleye Hg concentrations in southern lakes, this finding warrants further study to verify the trend and to investigate possible mechanisms that would cause Hg to increase in southern Wisconsin waters.

Walleye Hg concentrations and the Hg-fish length relationship vary greatly among lakes. We also found that lake latitude, lake area, and alkalinity explained some of the differences in Hg concentrations, but that none accounted for differences in the Hg-length relationships. Neither lake latitude nor alkalinity are likely to be direct causes of differences in walleye Hg concentrations, but both are correlated with factors like pH, lake productivity and fish growth, all factors suspected or demonstrated to be important to Hg bioaccumulation that may allow more precise estimates of Hg concentrations. In addition, we found that Hg concentrations vary by gender and season of collection. Walleye Hg concentration was lower in females than in males of equal size. Mercury concentrations were highest in walleye captured in the spring and lowest in the fall.

We recommend that walleye Hg monitoring continue. Based on our existing dataset and results in this report, we suggest that a specific monitoring design component for monitoring temporal trends be added to monitoring for fish consumption advisory purposes. Lakes (n = 30 to 60) should be selected according to a spatially balanced design accounting for lake size and alkalinity as well as geographic location. If possible, lakes with past information on walleye Hg should be included. Walleye (n = 5 to 7) should be collected every 3-5 years for Hg analysis with consideration of the collecting season. This design should allow for a more precise estimation of trends and of lake characteristics that affect trends.

#### Perch

In contrast to our conclusions that monitoring of Hg concentrations in walleye skin-on fillets is a useful tool, we are unable to draw a strong conclusion about the utility of young yellow perch for detecting trends of Hg in Wisconsin's lakes. As with walleye, lake-by-lake analysis of Hg trends suffers from the limited number of historical observations from the study lakes (1992-2000) and provides confounding results that are difficult to synthesize. We evaluated overall temporal

trends of Hg in young-of-year (YOY) yellow perch from the seven study lakes using several different mixed effects models. We explored relationships between Hg concentrations and YOY length at time of collection.

Our analysis of YOY from the seven northern Wisconsin study lakes found that Hg concentrations are best described by the average length of all YOY samples from the lake (grand lake-mean length). This suggests that Hg concentrations in YOY yellow perch are primarily related to factors that differ between lakes, e.g. productivity, food availability, or other factors that affect YOY growth rates. The second best model estimates that Hg concentration in YOY yellow perch decreased slightly over the period 1992 to 2006 in this set of lakes in the range of – 0.69% per year. While this finding is consistent with other estimates of temporal trends of Hg in fish from northern Wisconsin lakes, including our estimate based on the walleye fillet dataset, these ambiguous results do not allow a strong conclusion about Hg temporal trends in these study lakes during the 15-year study period (1992-2006).

It is possible that our findings are affected by the small number of lakes with historical (1992-2000) Hg data and that additional lakes and years would allow for better estimates of YOY Hg trends. After we completed an interim analysis of the YOY Hg dataset, we recommended that additional lakes be included in this study. Due to the short exposure time and dynamic life characteristics of YOY, we also completed preliminary examination of yearling (age 1) yellow perch to determine if yearlings would be better indicators than YOY. The limited yearling yellow perch Hg data collected to date suggest that Hg concentrations are as variable in yearling as they are in YOY.

At this time, our dataset is too limited to determine if Hg concentrations changed over time in YOY or yearling yellow perch from Wisconsin lakes. More recently, Harris et al. (2007) demonstrated that Hg concentrations in YOY yellow perch responded to controlled artificial Hg spikes and that most of the increase in the YOY was from Hg deposited directly to the lake but that the increase was not proportional to the loading increase. These authors predict, that while biota will respond quickly to reductions, response rates will vary among lakes. Hrabik and Watras (2002) attributed reduced Hg concentrations in yellow perch from one northern Wisconsin lake to decreases in atmospheric deposition of  $\rm H_2SO_4$  and Hg from 1994 to 2000. Rodger et al. (2006) found no statistically significant changes in Hg deposition measured from 1998 to 2005 at a limited number of monitoring stations. If deposition to the yellow perch study lakes did not change, then we would expect no change in yellow perch Hg concentrations. Based on monitoring conducted to date and these key studies, additional yearling yellow perch should be collected and analyzed before conclusions are made regarding the utility of yearling yellow perch to indicate temporal trends of Hg in Wisconsin lakes.

#### **LITERATURE**

Amrhein, J.F. and Geis, S.W. 2001. Mercury concentrations in yellow perch (Perca flavescens) from Vilas County, Wisconsin: 1920s versus 1980s. Bulletin of Environmental Contamination & Toxicology. 66(5):638-645.

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press. Madison, WI.

Bodaly, R.A., Rudd, J.W.M., Fudge, R.J.P. and Kelly, C.A. 1993. Mercury concentrations in fish related to the size of remote Canadian Shield Lakes. Canadian Journal of Fisheries & Aquatic Sciences. 50, 980-987.

Braune, B.M., Donaldson, G.M., and Hobson, K.A. 2001. Contaminant residues in seabird eggs from the Canadian Artic. Part I. Temporal trends 1975-1998. Environmental Pollution. 114(1): 39-54.

Burgess, N.M. and Hobson, K.A. 2005. Bioaccumulation of mercury in yellow perch and common loons in relation to lake chemistry in Atlantic Canada. Hydrobiogia in press.

Burnham, K. P. and Anderson, D.R. 1998. Model Selection and Inference: A Practical Information Theoretic Approach. Springer-Verlag, New York, USA.

Chen, C.Y. and Folt, C.L. 2005. High plankton densities reduce mercury biomagnification. Environmental Science and Technology. 39, 115-121.

Colby, P.J. and Nepszy, S.J. 1981. Variation among stocks of walleye (Stizostedion vitreum vitreum): Management implications. Canadian Journal of Fisheries and Aquatic Sciences. 38, 1814-1831.

Cope, G.W., Wiener, J.G., and Rada, R.G. 1990. Mercury accumulation in yellow perch in Wisconsin seepage lakes: relation to lake characteristics. Env. Tox. and Chem. 9: 931-940.

Diggle, P.J., Liang, K-Y., and Zeger, S.L. 1994. Analysis of longitudinal data. Oxford University Press. Oxford, Great Britain

Driscoll, C.T., Blette, V., Yan, C., Schoefield, C.L., Munson, R. and Holsapple, J. 1995. The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. Water, Air, & Soil Pollution. 80, 499-508

Eckley, C.S. and Hintelmann, H. 2006. Determination of mercury methylation potentials in the water column of lakes across Canada. Science of the Total Environment. 368(1), 111-125.

Engstrom, D.R. and Swain, E.B. 1997. Recent declines in atmospheric mercury deposition in the upper midwest. Environmental Science and Technology. 31(4), 960-967.

Fevold, B.M., Meyer, M.W., Rasmussen, P.W. and Temple, S.A. 2003. Bioaccumulation patterns and temporal trends of mercury exposure in Wisconsin Common loons. Ecotoxicology. 12, 83-93.

Frederick, P.C., Hylton, B., Heath, J.A., and Spalding, M.G.. 2004. A historical record of mercury contamination in southern Florida (USA) as inferred from avian feather tissue. Env. Tox. and Chemistry. 23 (6): 1474-1478.

Glass, G.E. and Sorensen, J.A. 1999. Six-year trend (1990-1995) of wet mercury deposition in the Upper Midwest, U.S.A. Environmental Science & Technology. 33, 3303-3312.

Greenfield, B.K., Hrabik, T.T., Harvey, C.J. and Carpenter. S.R. 2001. Predicting mercury levels in yellow perch: use of water chemistry, trophic ecology, and spatial traits. Canadian Journal of Fisheries and Aquatic Sciences. 58[7], 1419-1429.

Grieb, T.M., Driscoll, C.T, Gloss, S.P., Schofield, C.L., Bowie, G.L., and Porcella, D.B. 1990. Factors affecting mercury accumulation in fish in the upper Michigan Peninsula. Environmental Toxicology and Chemistry. 9(7):919-930.

Harris, R.C., Rudd, J.W.M., Amyot, M. Babiarz, C.L., Beaty, K.G., Blanchfield, P.F., Bodaly, R.A., Branfireun, B.A., Gilmour, C.C., Graydon, J.A., Heyes, A., Hintelmann, H., Hurley, J.P, Kelly, C.A., Krabbenhoft, D.P, Lindberg, S.E., Mason, R.P, Paterson, J.J., Podemski, C.L., Robinson, A., Sandilands, K.A., Southworth, G.R., St. Louis, V.L., and Tate, M.T. 2007. Whole –ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proceedings of the National Academy of Sciences. Early Edition. www.pnas.org/cgi/doi/10.1073/pnas.0704186104

Henderson, B.A., Collins, N., Morgan, G.E. and Vaillancourt, A. 2003. Sexual size dimorphism of walleye (Stizostedion vitreum vitreum). Canadian Journal of Fisheries & Aquatic Sciences. 60(11), 1345-1352.

Hrabik, T.R. and Watras, C.J. 2002. Recent declines in mercury concentrations in a freshwater fishery: isolating the effects of de-acidification and decreased atmospheric mercury deposition in Little Rock Lake. The Science of the Total Environment. 297, 229-237.

Johnson, D.H. 2002. The importance of replication in wildlife research. Journal of Wildlife Management. 66, 919-932.

Lathrop, R.C., Noonan, K.C., Guenther, P.M., Brasino, T.L. and Rasmussen, P.W. 1989. Mercury Levels in Walleyes from Wisconsin Lakes of Different Water and Sediment Chemistry Characteristics. Technical Bulletin No. 163. Department of Natural Resources. Madison, WI.

Lathrop, R.C., Rasmussen, P.W. and Knauer, D.R. 1991. Mercury concentrations in walleyes from Wisconsin (USA) lakes. Water, Air, and Soil Pollution. 56, 295-307.

Lehmann, C.M.B., Bowersox, V.C., Larson, R.S. and Larson, S.M. 2007. Monitoring long-term trends in sulfate and ammonium in US precipitation: results from the national atmospheric deposition program/national trends network. Water, Air, Soil Pollution: Focus 7(1-3), 59-66.

Lillie, R. and Mason, J. 1983. Limnological Characteristics of Wisconsin Lakes. Technical Bulletin No. 138. Department of Natural Resources. Madison. WI.

Littell, R.C., Milliken, G.A., Stroup, W.W. and Wolfinger, R.D. 1996. SAS System for Mixed Models. SAS Institute, Inc., Cary, NC. USA.

MacCrimmon, H.R., Wren, C.D. and Gots, B.L. 1983. Mercury uptake by lake trout, Salvelinus namaycush, relative to age, growth, and diet in Tadenac Lake with comparative data from other Precambrian shield lakes. Canadian Journal of Fisheries & Aquatic Sciences. 40(2), 114-120.

Madsen, E.R. and Stern, H.S. 2007. Time trends of methylmercury in walleye in northern Wisconsin: A hierarchical Bayesian analysis. Environmental Science & Technology. 41(13), 4568-4573.

Monson, B.A. and Brezonik, P.L. 1998. Seasonal patterns of mercury species in water and plankton from softwater lakes in Northeastern Minnesota. Biogeochemistry. 40(2-3), 147-162.

Monteiro, L.R. and Furness, W. 1997. Accelerated increase in mercury contamination in North Atlantic mesopelagic food chains as indicated by time series of seabird feathers. Environmental Toxicology and Chemistry. 16: 2489-2493.

MPCA (Minnesota Pollution Control Agency). 2007. Minnesota Statewide Mercury Total Maximum Daily Load, Final March 27, 2007. (http://www.pca.state.mn.us/publications/wq-iw4-01b.pdf).

Munthe, R.A., Bodaly, R.A., Branfireun, B.A., Driscoll, C.T., Gilmour, C.C., Harris, R., Horvat, M., Lucotte, M. and Malm, O. 2007. Recovery of mercury-contaminated fisheries. Ambio. 36(1), 33-44.

Nate, N.A. 2004. Variability in Fish Growth Rates in Relation to Physical and Landscape Characteristics of Michigan and Wisconsin Inland Lakes. Doctoral Dissertation. Michigan State University, Lansing, Michigan. USA.

Post, J.R., Vandenbos, R. and McQueen, D.J. 1996. Uptake rates of food-chain and waterborne mercury by fish: Field measurements, a mechanistic model, and an assessment of uncertainties. Canadian Journal of Fisheries & Aquatic Sciences. 53(2), 395-407.

Quist, M.C., Guy, C.S., Schultz, R.D. and Stephan, J.L. 2003. Latitudinal comparisons of walleye growth in north America and factors influencing growth of walleyes in Kansas reservoirs. North American Journal of Fisheries Management. 23, 677-792.

Rada, R.G., Wiener, J.G., Winfrey, M.R., and Powell, D.E. 1989. Recent increases in atmospheric deposition of mercury to north-central Wisconsin lakes inferred from sediment analysis. Arch. Environ. Contam. Toxicol. 18: 175-181.

Rasmussen, P.W., Schrank, C.S., and Campfield, P.A. 2007. Temporal Trends of Mercury Concentrations in Wisconsin Walleye (Sander vitreus), 1982-2005. Ecotoxicology. DOI 10.1007/s10646-007-0160-2. <a href="https://www.springerlink.com/content/w67723u094000164/fulltext.pdf">www.springerlink.com/content/w67723u094000164/fulltext.pdf</a>

Rodger, B., Gay, D.A., Prestbo, E.M. 2006. Wisconsin mercury deposition network (MDN): Findings and trends. Poster. Eighth International Conference on Mercury as a Global Pollutant. August 6-11, 2006. Madison, WI, USA.

Rudd, J.W.M. 1995. Sources of methyl mercury to freshwater ecosystems: A review. Water, Air, Soil Pollution. 80, 697-713.

Schuster, P.F., Krabbenhoft, D.P., Naftz, D.L., Cecil, L.D., Olson, M.L., Dewild, J.F., Susong, D.D., Green, J.R. and Abbott, M.L. (2002). Atmospheric Mercury Deposition during the last 270 years: A glacial ice core record of natural and anthrophogenic sources. Environmental Science and Technology. 36, 2303-2310.

Simoneau, M., Lucotte, M., Garceau, S. and Laliberté, D. 2005) Fish growth rates modulate mercury concentrations in walleye (Sander vitreus) from eastern Canadian lakes. Environmental Research. 98, 73-82.

- Slemr, F., Brunke, E., Ebinghaus, R., Temme, C., Munthe, J., Wangberg, I., Schroeder, W., Steffen, A. and Berg, T. (2003). Worldwide trend of atmospheric mercury since 1977. Geophysical Research Letters. 30(10), 1516.
- Somers, K.M. and Jackson, D.A. 1993. Adjusting mercury concentration for fish-size covariation: A multivariate alternative to bivariate regression. Canadian Journal of Fisheries & Aquatic Sciences. 50(11), 2388-2396.
- St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Beaty, K.G., Flett, R.J. and Roulet, N.T. 1996. Production and loss of methylmercury and loss of total mercury from boreal forest catchments containing different types of wetlands. Environmental Science & Technology. 30, 2719-2729.
- Sullivan, J.R. and Delfino, J.J. 1982. The Determination of Mercury in Fish. Journal of Environmental Science and Health. A17(2), 265-275.
- Suns, K. and Hitchin, G. 1990. Interrelationships between mercury levels in yearling yellow perch, fish condition, and water quality. Water, Air, Soil, Pollution. 650, 255-265.
- Swain, E.B. and D. D. Helwig. 1989. Mercury in fish from northeastern Minnesota Lakes: Historical trends, environmental correlates, and potential sources. Jo. of the Minnesota Academy of Science. 55(1):103-109.
- Trudel, M. and Rasmussen, J.B. 2001. Predicting mercury concentration in fish using mass balance models. Ecological Applications. 11(2), 517-529.
- Verbeke, G. and Molenberghs, G. 2000. Linear Mixed Models for Longitudinal Data. Springer, New York, USA.
- Watras, C.J., Back, R.C., Halvorsen S., Hudson, R.J.M., Morrison K.A. and Wente, S.P. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. The Science of the Total Environment. 219, 183-208.
- Watras, C.J., Morrison, K.A., Hudson, R.J.M., Frost, T.M. and Kratz, T.K. 2000. Decreasing mercury in northern Wisconsin: temporal patterns in bulk precipitation and a precipitation-dominated lake. Environmental Science and Technology. 34, 4051-4057.
- Watras, C.J., Morrison, K.A., Regnell, O. and Kratz, T.K. 2006. The methylmercury cycle in Little Rock Lake during experimental acidification and recovery. Limnology and Oceanography. 51, 257-270.
- Whiteside, M.C., Swindoll, C.M., and Doolittle, W.L. 1985. Factors affecting the early life history of yellow perch, Perca flavescens. Environmental Biology of Fishes. 12(1): 47-56.
- Wiener, J.G. 1983. Comparative analyses of fish populations in naturally acidic and circumneutral lakes in northern Wisconsin. U.S. Fish and Wildlife Service Report FWS/OBS-80/40.16: 107p.
- Wiener J.G., Bodaly, R.A., Brown, S.S., Lucotte, M., Newman, M.C., Porcella, D.B., Reash, R.J. and Swain, E.B. 2007. Monitoring and evaluating trends in methylmercury accumulation in aquatic biota. Chapter 4 in R.C. Harris, D.P. Krabbenhoft, R.P. Mason, M.W. Murray, R.J. Reash and T. Saltman (editors), Ecosystem Responses to Mercury Contamination: Indicators of Change, pp. 87-122. Taylor and Francis, Boca Raton, Florida. USA.
- Wiener, J.G., Knights, B.C., Sandheinrich, M.B., Jeremiason, J.D., Brigham, M.E., Engstrom, D.R., Woodruff, L.G., Cannon, W.F. and Balogh, S.J. 2006. Mercury in soils, lakes, and fish in Voyageurs National Park (Minnesota): importance of atmospheric deposition and ecosystem factors. Environmental Science & Technology. 40(20), 6261-6268.
- Wiener, J.G., Martini, R.E., Sheffy, T.B. and Glass, G.E. 1990. Factors influencing mercury concentrations in walleyes in northern Wisconsin lakes. Transactions of the American Fisheries Society. 119, 862-870.
- Wiener, J.G. and Spry, D.J. 1996. Toxicological significance of mercury in freshwater fish. In W.N. Beyer, G.H. Heinz, A.W. Redmon (editors) Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations, pp 297-339. CRC, Boca Raton, Florida, USA.
- Wisconsin State Laboratory of Hygiene. September 2005. Quality Assurance Procedures and Policies. Revision 4.0. Madison, Wisconsin. USA.
- Wren, C.D., Scheider, W.A., Wales, D.L., Muncaster, B.W. and Gray, I.M. 1991. Relation between mercury concentrations in walleye (Stizostedion vitreum vitreum) and northern pike (Esox lucius) in Ontario lakes and influence of environmental factors. Canadian Journal of Fisheries and Aquatic Sciences. 48, 132-139.