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SPATIAL AND TEMPORAL VARIABILITY OF MERCURY IN UPPER AND LOWER RED LAKE WALLEYE

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Mercury is a global pollutant that is released into our environment by natural and anthropogenic processes resulting in extensive studies conducted on mercury cycling in aquatic ecosystems which has led to the issuance of human-health-based fish-consumption advisories. We examined total mercury concentrations in Walleye *Sander vitreus* from Upper and Lower Red Lakes, located in north central Minnesota, between 2019 and 2020. Upper and Lower Red Lake form a contiguous water body consisting of two large (483.1 and 665.6 km^2, respectively) basins that are naturally connected by a 1.4 km-wide strait. Both basins are important fisheries for recreation, subsistence, and commercial fishing for the Red Lake Band of Chippewa Indians. The eastern half of Upper Red Lake is also an important sport fishery for non-Tribal anglers in Minnesota. Sampled Walleye ($n = 265$) ranged from 158 to 610 mm in total length from an age range of 0 to 16 years. Mercury concentrations within the Red Lakes' Walleye ranged from 0.030 mg/kg to 0.564 mg/kg ($\bar{x} = 0.179 \pm 0.105$ mg/kg; $\bar{x}$ = mean ± sd, all fish-mercury concentrations expressed on wet-weight basis). The best supported model for predicting mercury concentrations in Red Lake Walleye included the independent variables: length, age, sex, and lake basin. This model indicated that basin was an important predictor variable for estimating Hg in Walleye from Upper and Lower Red Lake. This model also suggests that individuals who rely on fish for subsistence should target Walleye that are ≤ 400 mm from Lower Red Lake. With no physical barriers between the Lakes to prohibit migration, observed differences in mercury concentrations could be linked to the differences in wetland area, fish growth rates, and physicochemical parameters between the two basins. Spatial variability of mercury showed that Upper and Lower Red Lake exhibit fish-mercury concentrations comparable to other large lakes within the region after adjusting for length as the covariate. Given that basin was an important predictor variable for estimating fish-Hg concentrations, future pollutant monitoring efforts should treat Upper and Lower Red Lake as separate lakes and not assume that data from one basin can apply to the other. This will be important over a longer time scale as ecosystems respond to changes in mercury emissions and other environmental changes.

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ABSTRACT

Mercury is a global pollutant that is released into our environment by natural and anthropogenic processes resulting in extensive studies conducted on mercury cycling in aquatic ecosystems which has led to the issuance of human-health-based fish-consumption advisories. We examined total mercury concentrations in Walleye *Sander vitreus* from Upper and Lower Red Lakes, located in north central Minnesota, between 2019 and 2020. Upper and Lower Red Lake form a contiguous water body consisting of two large (483.1 and 665.6 km^2, respectively) basins that are naturally connected by a 1.4 km-wide strait. Both basins are important fisheries for recreation, subsistence, and commercial fishing for the Red Lake Band of Chippewa Indians. The eastern half of Upper Red Lake is also an important sport fishery for non-Tribal anglers in Minnesota. Sampled Walleye (n = 265) ranged from 158 to 610 mm in total length from an age range of 0 to 16 years. Mercury concentrations within the Red Lakes' Walleye ranged from 0.030 mg/kg to 0.564 mg/kg ($\bar{x}$ = 0.179 ± 0.105 mg/kg; $\bar{x}$ = mean ± sd, all fish-mercury concentrations are expressed on a wet-weight basis). The best supported model for predicting mercury concentrations in Red Lake Walleye included the independent variables: length, age, sex, and lake basin. This model indicated basin was an important predictor variable for estimating Hg in Walleye from Upper and Lower Red Lake. This model also suggests that individuals who rely on fish for subsistence should target Walleye that are ≤ 400 mm from Lower Red Lake. With no physical barriers between the Lakes to prohibit migration, observed differences in mercury concentrations could be linked to the differences in wetland area, fish growth rates, and physicochemical parameters between the two basins. Spatial variability of mercury showed that Upper and Lower Red Lake exhibit fish-mercury concentrations comparable to other large lakes within the region after adjusting for length as the covariate. Given that basin was an important predictor variable for estimating fish-Hg concentrations, future pollutant monitoring efforts should treat Upper and Lower Red Lake as separate lakes and not

assume that data from one basin can apply to the other. This will be important over a longer time scale as ecosystems respond to changes in mercury emissions and other environmental changes.

INTRODUCTION

Mercury (Hg) is a global pollutant that is released into our environment by natural and anthropogenic processes which has resulted in extensive studies on Hg cycling in aquatic ecosystems which has led to the issuance of human-health-based fish-consumption advisories (Brigham et al., 2009, 2003; Brumbaugh et al., 2001). Mercury is commonly found in three forms (elemental Hg^0, inorganic Hg^{+2}, and methylmercury MeHg), each displaying different physical and chemical properties, economic uses, and human-health risks (Park and Zheng, 2012; US EPA, 1997). Since 1990 through the Clean Air Act, the United States has been regulating Hg compound uses and emissions (Rustagi and Singh, 2010; US EPA, 2015); however, MeHg can still be found in the most remote locations. Through co-metabolic processes in microbes, Hg^{+2} ions inadvertently undergo a methylation process that converts the inorganic Hg^{+2} into bioavailable MeHg (Gilmour et al., 1992; Myrbo et al., 2017; Shao et al., 2012). Due to MeHg's ability to biomagnify in food chains, MeHg poses the greatest health concerns for wildlife and humans (Fitzgerald and Clarkson, 1991). Human-health risks of chronic and/or high-dose acute exposures to MeHg can result in neurological implications; especially for individuals in their early developmental stages of life (Bernhoft, 2012; Minai, 2016; Myers and Davidson, 1998). Because of these implications and extensive epidemiology studies on Hg, the United States Environmental Protection Agency (EPA) established a reference dose (RfD) of 0.1 µg of Hg per kg of body weight per day (µg/kg/day) that should be considered for consumption advisories for sensitive populations (ATSDR, 1999; Goldman et al., 2001; US EPA, 1997). Sensitive populations consist of women of child-bearing age, breast-feeding women, and children under 15 years of age (US EPA, 1997). In a nationwide study by Xue et al. (2015), MeHg exposure of tribal populations from fish were 3 to 10 times higher than the United States general population. This elevated mercury exposure poses potential health risks to Red Lake Tribal members who rely on fish for subsistence.

The origin of mercury inputs to our waters are well documented (Brigham et al., 2003; Krabbenhoft et al., 1998; Lamborg et al., 2002; Scudder et al., 2009). A review of past and present research has indicated that there are known environmental conditions that promote the production of MeHg which include wetland acreage, dissolved organic carbon (DOC), pH, dissolved oxygen (DO), acid neutralizing capacity (ANC or alkalinity), acid-volatile sulfides (AVS) in wetland sediment, and land use and land cover (LULC) (Gilmour et al., 1992; Rypel, 2010; Scudder et al., 2009). Many of these variables are associated with wetland properties which, in general, exhibit high DOC and AVS sediments and low pH, DO, and ANC levels that are resultant of anaerobic conditions necessary for the reduction of sulfate. Sulfur, in general, enters aquatic ecosystems by rock erosion, atmospheric deposition, or human influences and can be readily oxidized to sulfate (SO_4^{-2}) which is essential for plant growth (Clayden et al., 2017). Although essential for plant growth, excess loading of SO_4^{-2} to aquatic ecosystems has been shown to harm some sensitive plant species (e.g. wild rice), and exacerbate MeHg production from sulfate-reducing bacteria (Myrbo et al., 2017). Sulfate-reducing bacteria are typically found in low pH (<6) environments in the transitional zone of aquatic ecosystems, and will incorporate available metal cations, such as Hg^{+2}, during their co-metabolic processes resulting in the inadvertent production of organically available MeHg. The abundance of wetlands located around the Red Lake Indian Reservation in northcentral Minnesota would indicate that there is a potential for higher MeHg concentrations in fish than other locations in northern Minnesota. More specifically, Upper Red Lake is influenced by a large expanse of wetlands to the north. Most of these wetlands are connected to the Tamarac River that discharges into the northeastern portion of Upper Red Lake (Appendix A) leading to the possibility of an influx of MeHg during snowmelt and flood-like conditions.

The ubiquity of Hg in our environment has led scientists performing decades of regional, national, and global scale studies on Hg (Chalmers et al., 2011; Engstrom et al., 2007; Krabbenhoft et al., 1998). These studies aided regulatory agencies to establish fish-mercury monitoring programs to inform health-based fish-consumption advisories. The Red Lakes, consisting of the connected Upper and Lower Red Lakes (referred to as Lakes and/or basins throughout the remaining text), is the largest freshwater lake ecosystem contained entirely within Minnesota. The Walleye fishery within the Red Lakes is both a culturally and economically important subsistence resource for the Red Lake Nation, whose lands surround much of the Red Lakes. Additionally, the eastern half of Upper Red Lake is an important sport fishery and recreational resource for the state of Minnesota. Although some fish-mercury monitoring has been done in the Red Lakes, existing data are too limited, hindering the assessment of Hg concentrations with respect to: fish size (length), time, or space. In particular, existing data are inadequate to determine if the Red Lakes can be treated as a single ecosystem, or if fish-mercury concentrations differ between the Upper and Lower basins.

Currently, the Red Lake Indian Reservation has a Hg dataset that dates to 2002. This 20-year-old dataset is what the Tribe uses to inform its members about Hg levels in fish caught from Tribal waters of Upper and Lower Red Lake. Additionally, the Minnesota Department of Natural Resources (MN DNR), in conjunction with the Minnesota Pollution Control Agency (MPCA) and Minnesota Department of Health (MDH), have been monitoring the state waters since 1987 with the last assessment made in 2012. However, this dataset is too small to accurately determine Hg concentration in fish populations due to small sample sizes ranging from 3 to 24 fish per sampling event. With inconsistent and generally small sample sizes per event, determining statistical differences in fish populations is challenging. Collecting consistent data is paramount for sound science and the results of this study will provide Tribal and State agencies with models needed to estimate Hg concentrations in Walleye *Sander vitreus*. The objectives of this research are to (1) develop models for Walleye to determine Hg concentrations by length, age, sex, and lake basin; (2) statistically determine temporal variability; and (3) determine how Hg concentrations from the Red Lakes compare to similar large-lake systems within Minnesota. Focusing our efforts on Walleye will give us an accurate representation of how much Hg is in the ecosystem and the potential MeHg concentrations being consumed by humans from the Red Lakes. Achieving these objectives should provide Tribal and State agencies the necessary tools for long-term monitoring of Hg along with the appropriate sample size to produce statistically significant results.

METHODS

Study area

Located in north central Minnesota, USA, Upper and Lower Red Lakes are primarily within the Red Lake Indian Reservation. The Red Lake Indian Reservation is one of two “closed Reservations” in the United States, meaning that there is no outside law enforcement and the Red Lake Tribal Council governs over its members including their natural resources. The Reservation’s total land holdings are in excess of 3300 km^2 consisting of approximately 2190 km^2 of mixed forests and wetlands; 975 km^2 of lakes (Upper and Lower Red Lake encompassing 963 km^2 within the Reservation boundary); and over 597 km of rivers and streams. Situated within the Red Lakes Watershed which has an area of 5000 km^2 (Appendix A), Upper and Lower Red Lake form a contiguous water body consisting of two large basins (483.1 and 665.6 km^2, respectively) that are naturally connected by a 1.4 km-wide strait. The watersheds’ land use and land cover consists primarily of open water/wetlands (mostly peatlands) and forested land, 79% and 13% respectively.

There are 12 major streams, along with numerous ephemeral streams or ditches, that flow into Upper and Lower Red Lake. The Red Lakes form the headwaters of the Red Lake River which is controlled by a dam that is owned by the United States Army Corp of Engineers located at the outlet of Lower Red Lake. The Red Lake River is the only outlet of the two lakes and flows west for approximately 310 km before discharging into the Red River of the North in East Grand Forks, Minnesota. Upper and Lower Red Lake are classified as eutrophic lakes that rarely stratify; driven by prevailing west winds. The maximum depths for Upper and Lower Red Lake are 5.5 and 9.1 meters, respectively. A summary of mean water quality parameters for Upper and Lower Red Lake are found in Table 1. The two lakes exceed Minnesota’s Clean Water standards for phosphorus which is set at 30 µg/L, however they are not listed as impaired waters. Both the State and Tribe recognized the need for site specific standards after a 2016 winter sediment-coring experiment performed by the St. Croix Watershed Research Station indicated that the phosphorus levels are consistent with historic diatom inferred phosphorus levels for this system (Burge, 2021). In the mid-1990s, the Tribe voluntarily stopped Walleye harvest efforts after the Red Lakes experienced a crash in the Walleye population; by 1997 Walleye harvesting was prohibited. However, through cooperative management and recovery plans by fry-stocking, the fishery recovered by 2006. Today, the Red Lakes, especially Upper Red Lake, is considered one of the most productive Walleye fisheries within the United States.

Sample Size Determination

Previous Walleye samples (n = 62) collected from Upper Red Lake by the MN DNR from 1987 to 2012 were used to generate linear regression models in R software (R Core Team, 2021). These models were used to determine a mean maximum effect of varying samples sizes (n = 3 - 200). The mean maximum effect was determined from the maximum confidence interval width from 1,000 simulations at each sample size. Plotting the confidence interval width at each sample size gave us an inflection point at 60 Walleye samples (Appendix B). Based off these calculations to efficiently and accurately measure Hg concentrations in the Red Lakes, a minimum sample size of 60 Walleye from Upper Red Lake and 60 Walleye from Lower Red Lake were needed to perform our statistical analysis and produce Hg models for the Lakes.

Walleye Sampling

Walleye samples were collected through a collaborative effort working in conjunction with the MN DNR and Red Lake Department of Natural Resources (RL DNR) fisheries crews during the fall 2019 and 2020 experimental gill netting population assessments. These nets consist of five different mesh sizes (19, 25, 32, 38, and 51 mm) measuring 15.2 m each for a total span of 76.2 m. The RL DNR set four nets (paired net sets) per location each day from 3 September to 27 September 2019 and 1 September to 25 September 2020 (Appendix A); two nets oriented in deep water and two nets oriented in shallow water for a total of 48 net sets (12 locations). The MN DNR used a grid pattern for their net sets for a total of 20 sets or 20 locations during the same sampling period as the RL DNR (Appendix A). These net sets were fished for approximately 24 hours before assessment at the DNR headquarters. A total of 265 Walleye samples, 131 Walleye (66 male and 65 female) from Upper Red Lake and 134 Walleye (62 male and 72 female) from Lower Red Lake were collected for Hg analysis (Table 3). When possible, a minimum of three male and three female Walleye from 50 mm size classes were collected (Table 3).

Sample Collection

Sample collection followed procedures similar to the US EPA (2000) and US Geological Survey (Scudder et al., 2008). Briefly, work surfaces were covered with a new plastic sheet or bag for each Walleye sample taken. All field personnel participating in processing samples wore Nitrile gloves. All total lengths (± 1 mm), weights (± 1.0 grams), sexual identification, and aging structures (fin ray, scales, and otoliths) were taken before collecting a ~12.9 cm^2 tissue sample. Skin-off tissue samples were taken on the left side anterior to the dorsal fin using a clean stainless-steel fillet knife. Tissue samples were rinsed with deionized water, weighed (wet weight) to the nearest 0.01 gram, and placed in a clean sterile Whirl-Pak® plastic bag with the respective serial numbers from the netting assessment. Tissue samples were transferred to wet ice in an insulated cooler before being transferred to a laboratory freezer (-20°C) to be stored until the lyophilization and homogenization process.

Sample Preparation

Lyophilization occurred through the use of a Harvest Right® stainless-steel freeze dryer; approximately 28 hours of run time from frozen to a freeze dried sample. Ten percent of the samples were lyophilized a second time to determine equipment efficiency. Each sample was homogenized using porcelain mortar and pestles, weighed (± 0.0001 g) for wet vs. dry weight conversions, and placed in 40 mL borosilicate scintillation vials for dry storage.

Sample Analysis/QAQC

Samples were analyzed in Red Lake, Minnesota at the RL DNR office using a Milestone TriCell Dual Beam Direct Mercury Analyzer (DMA-80evo) following EPA 7473 (US EPA, 2007). Briefly, samples were introduced into the DMA-80evo quartz decomposition tube by a nickel sample boat. The decomposition tube is heated by two programmable furnaces to dry and thermally decompose the sample to release mercury vapors in an oxygenated environment. The mercury vapors are transported over the amalgamator that traps the mercury. Once all the mercury vapors are trapped, the amalgamator is rapidly heated to release the vapors in order to pass through the three absorbance cuvettes and spectrometer. Detection is then sent to the desktop controller. The instrument's detection limit (0.0003 ng Hg) was 100 times lower than EPA 7473 requirement. A new calibration curve (created using stock 1000 mg/L Hg to gravimetrically dilute to 1.0, 0.1, 0.01 mg/L Hg) was created to span the width of all three cuvette cells at the beginning of the project. Two calibration standard samples were analyzed daily before the start of the sample run at 0.5 ng and 50 ng of Hg. Calibration standards were made daily or weekly depending on the change in percent absorbance; new check standards were made when percent absorbance exceeded 10% from the calibration curve. Certified reference material, DORM-4 (dried fish protein homogenate), was purchased from National Research Council Canada and used to verify EPA method 7473. Matrix spikes and matrix spike duplicates (MS/MSD) at an average spike of 30.834 ng Hg of the original sample were analyzed to test the DMA-80evo percent recovery of Hg (105.7-120.5%, $\bar{x}$ = 111.7% from 26 samples). The method detection limit was 0.745 ng Hg and was estimated by analyzing 15 replicates of known Hg additions (50 ng Hg) to sterile sample boats and multiplying the standard deviation among replicates by 2.624, the single-tailed t value for a 99% confidence interval. A sample run consisted of 2 calibration check samples, 20 Walleye tissue samples, 3 equipment blanks, 2 DORM-4 samples, and 1 MS/MSD paired sample. All sample concentrations were converted back to wet weight. Sample boats were brushed clean of ash and ran back through the DMA-80evo for sterilization after each sample run and stored in a new zip-sealed bag.

DATA ANALYSIS

All statistical analyses were performed in R (R version 4.1.1; R Core Team, 2021) and significance was evaluated at $\alpha = 0.05$. First, an exhaustive model selection procedure was used to evaluate a suite of variables for predicting mercury concentrations in Walleye from Upper and Lower Red Lake. These linear models (Table 2) were used to determine the significance of Hg concentration as a response to length, weight, age, sex, and basin. The best fit model for predicting Hg concentrations was based on the lowest Akaike information criterion (AIC). By theoretical definition, as a model becomes more complex, the AIC score will penalize those models (Rossi et al., 2020). Diagnostic plots were used to check for heteroscedasticity and verify the best fit model. Observed versus predicted plots with 95% confidence intervals were used to illustrate the data.

To compare mean length by age of Walleye between basins, we modeled the relationship between fish total length and fish age using the von Bertalanffy growth rate model (Olge, 2016) in R using packages FSA, FSAdata, plotrix, and dplyr (Lemon, 2006; Olge et al., 2021; Olge, 2019; Wickham et al., 2021). A t-test was used to determine differences in mean length per age by basin.

Analysis of variance (Type III ANOVA; car package (Fox and Weisberg, 2019)) was used to evaluate the interactive effect of length (continuous variable) and years sampled to determine temporal differences in Hg concentrations within the Red Lakes. We evaluated the interactive effect of length (continuous variable) and Minnesota's top ten largest bodies of water (discrete variable) on fish Hg concentrations using two-way ANOVA (Type III ANOVA; car package (Fox and Weisberg, 2019)). The significant interaction between length and body of water was evaluated with post-hoc comparisons using ANCOVA (emmeans package (Lenth, 2021)) to estimate the mean Hg concentrations with a confidence level of 0.95. Finally, a Tukey pairwise comparison of estimated marginal mean Hg concentrations was used to determine significant differences between lakes.

RESULTS

A total of 265 Walleye ranging from 158 to 610 mm were collected from Upper and Lower Red Lake (Table 3; Figure 1) during the 2019 and 2020 study. Total mercury concentrations varied between 0.030 mg/kg to 0.564 mg/kg ($\bar{x}$ = 0.179 ± 0.105 mg/kg; $\bar{x}$ = mean ± sd, all fish-mercury concentrations expressed on wet-weight basis) with the highest Hg concentration found in a 10 year old, 517 mm female Walleye from Upper Red Lake. Total mercury concentration in Walleye were higher in Upper versus Lower Red Lake (Figure 2; $\bar{x}$ = 0.215 ± 0.117 and 0.144 ± 0.077 mg/kg, respectively). Mercury concentrations in Red Lake Walleye illustrated a positive linear relationship with length (Figure 1 & 2) and age (Figure 2 & 3). Age of Walleye ranged from young-of-the year (0) to 16 with a mean ± sd of 4.3 ± 3.3. Walleye at age-1, 2, and 6 through 10 from Lower Red Lake exhibited significantly faster growth rates than Walleye from Upper Red Lake (Figure 4). Lastly, Hg concentrations between male and female walleye in Upper and Lower Red Lake were similar ($\bar{x} = 0.176 \pm 0.103$ and $\bar{x} = 0.181 \pm 0.107$ mg/kg, respectively; Figure 5).

An interactive model with the main effects of length, age, sex, and basin produced the best AIC score and explained 80% of the Hg concentration variability in Walleye (Table 2, Figure 6A). A simplified interactive model, which could be used by the general public to estimate Hg concentrations in Walleye, contained the main effects of length and basin. This model explains 53% of the Hg variability found in Walleye from the Red Lakes (Table 2, Figure 6B). The assumption would be made that the general public cannot accurately estimate fish age and/or sex a Walleye from the Red Lakes due to fast growth rates and an abundance of visceral fat.

Individual Walleye samples for Hg analysis have been collected from 1997 to 2020. Sampling events have been inconsistent ranging from 1 to 7 years and sample sizes ranging from 8 to 224 individuals. Trends in Hg concentrations over time were not noticeable, and after adjusting for length (394 mm to 460 mm) to fit years sampled, there were no significant differences temporally (Figure 7).

Spatial comparisons of Hg concentrations in Walleye from Minnesota's ten largest lakes in relation to the Red Lakes showed significant differences among lakes (Table 4, Figure 8). After adjusting for length (325 to 610 mm) to fit all water bodies, Lower Red Lake's Hg concentrations were significantly lower than Otter Tail and Rainy Lake ($\bar{x} = 0.140 \pm 0.080$ mg/kg; $\bar{x} = 0.460 \pm 0.236$ mg/kg; $\bar{x} = 0.477 \pm 0.203$ mg/kg; P-value < 0.05, respectively), while Upper Red Lake's Hg concentrations were lower than Rainy Lake ($\bar{x} = 0.260 \pm 0.118$ mg/kg; $\bar{x} = 0.477 \pm 0.203$ mg/kg; P-values < 0.05, respectively).

DISCUSSION

The Red Lakes in north central Minnesota are considered one of the most important fisheries within the state. Both basins are important fisheries for recreation, subsistence, and commercial fishing for the Red Lake Band of Chippewa Indians. The eastern half of Upper Red Lake is also an important sport fishery and recreational resource for non-Tribal anglers in Minnesota. Even with its popularity, scientific advancements in regards to pollutant monitoring has been limited, in part, due to cooperative management efforts. The robust dataset collected for this study on the Red Lakes allowed us to determine important factors influencing Hg concentrations observed in the Walleye population. Previous Hg studies conducted on the Red Lakes' Walleye focused primarily on a certain size range near 400 mm. These individuals are known to be the most targeted or harvestable fish from anglers which coincides with a protected slot limit between 432 mm to 660 mm; current regulations for non-Tribal members allows anglers to harvest one Walleye over 432 mm. However, with data gaps from both smaller and larger individuals and virtually zero individuals collected from Lower Red Lake, making accurate assumptions about Hg concentrations in Walleye is challenging; this study provided those missing components. All size classes were represented from both Upper and Lower Red Lake in our dataset from young-of-the year (< 254 mm) to 16 year old (> 600 mm) individuals. Collecting lengths, weights, age, sex, and location (basin) provided us the ability to perform model predictions about the Hg concentrations found in Walleye. Based on AIC scores, the best predictive model incorporates length, sex, age, and basin; these factors are interactive and intuitive explaining 80% of the Hg variability we observed in the Red Lake Walleye. Previous studies have shown that as fish grow, in length and age, Hg concentrations generally increase (Depew et al., 2013; Eagles-Smith et al., 2008; Mathers and Johansen, 1985). However, explaining Hg differences in male and female walleye is complex due to sexual dimorphism or growth dilution, energy and reproductive requirements. Studies on smallmouth bass (Murphy et al., 2007) and on Walleye (Henderson et al., 2003) both illustrated differences in Hg concentrations between sexes; however, this was only after sexual maturity where sexual dimorphism is most noticeable. In this study, sexual differences in mercury concentrations was an important predictor variable for the model and is likely linked to the differences in energy requirements and growth dilution (Madenjian et al., 2016).

Observing a significant difference in Hg concentrations between Upper and Lower Red Lake Walleye was an important finding from a management and recreational standpoint (Figure 2). Historically, samples for Hg analysis were collected primarily from the easternmost portion of Upper Red Lake. These samples were then used to infer Hg levels throughout the entire Red Lake ecosystem. Observing differences in Hg concentrations could be explained by a couple of factors, even though the two basins lack any barrier to fish movement where they are connected. The first factor being the limnology of the two basins. Both basins are shallow, windswept basins with Lower Red Lake being twice the depth of Upper at ~10 m. Lower Red Lake provides more fish habitat to promote faster growth rates while Upper Red Lake is primarily sand, small cobble, and soft sediment. Second, wetlands are a dominant land cover type within the Red Lake watershed which are suitable sites for certain bacteria species (e.g. sulfate reducing bacteria) to methylate Hg (Hall et al., 2008; Jeremiason et al., 2006). Upper Red Lake is situated down gradient to a large wetland expanse, primarily peatland, to the north and east and has one major tributary, the Tamarac River to the northeast. There are also numerous perennial and semi-perennial streams and ditches along the north shore of Upper Red Lake that may facilitate the transport of MeHg from sites of methylation at the peatland-upland interface (Mitchell et al., 2008a, 2008b; Wang et al., 2021). In contrast, Lower Red Lake has six major tributaries located along the south and east shores which are influenced more by upland forest and agriculture. Studies have shown that wetlands contribute to increased bioavailable Hg to aquatic systems (Hall et al., 2008; Louis et al., 1994; Rypel, 2010), whereas upland forests generalize in accumulation and retention of total Hg (Demers et al., 2013). Due to the abundance of potential wetlands influencing Upper Red Lake, there is evidence to suggest that wetlands provide a disproportionate Hg load that impacts Upper Red Lake Walleye. Even though direct Hg deposition to surface water is an important source for MeHg concentrations found in fish (Harris et al., 2007), direct Hg inputs to the Red Lakes does not explain the Hg differences between the basins. The third factor that alludes to the Hg difference we found in this study are the growth rates in Walleye between the two basins. Walleye from age-classes 1 through 10 exhibited faster growth rates on average in Lower Red Lake than Upper Red Lake. Before sexual maturity, age-0 through 4 Walleye from Lower Red Lake, on average, were 12 mm longer than Upper Red Lake Walleye. After sexual maturity (age-5 through 10), Lower Red Lake Walleye exhibit an average growth rate of 53 mm of increased length per age than Upper Red Lake Walleye. Although growth rates were not statistically different in all age-classes (age-classes 0 and 3-5), the observed average growth differences from age-classes 1, 2, and 6 through 10 could contribute to the Hg differences we observed between the two basins. Simoneau et al. (2005)

concluded that slower growing fish from Québec experienced increased Hg levels compared to faster-growing fish. Because basin was an important predictor variable for estimating Hg concentrations in Walleye and the observed growth rate differences by age, this dataset suggests that the Walleye populations do not frequently mix between the two basins. Observing these Hg trends in Walleye between the two basins warrants standardize sampling throughout Upper and Lower Red Lake.

Since the early 1990's, the US EPA has been regulating Hg uses and emissions. The Toxic Release Inventory Program has shown steady declines in Hg emissions from ~57,000 kg in 2007 to ~14,000 kg in 2019 (US EPA, 2019). However, the temporal variability of Hg within the Red Lake Walleye has been inconsistent with data showing no significant trends from 1997-2020 which is linked to limited data. What our data suggests is the need for frequent sampling of a specific number of individuals from a certain size class to determine temporal changes. Secondly, the Red Lakes are situated within a large complex of wetlands to the north and east; previous studies have shown that wetlands can act as a massive storage system for Hg and also increase the methylation rates of elemental Hg (Gabriel et al., 2009; Mitchell et al., 2009; Rypel, 2010; Snodgrass et al., 2000). Due to wetlands' ability to store and release MeHg into surface water, observing a significant increase or decrease in Hg levels found in fish could take decades.

Monson et al. (2011) compared Hg levels across all of Minnesota and found evidence to suggest that Hg increases from south to north and west to east. However, based on this study, Lower Red Lake Walleye have one of the lowest mean Hg concentrations than other large lakes (> 52 km^2) in Minnesota. Also, two distinctly different lakes, Otter Tail Lake and Rainy Lake, exhibit some of the highest Hg levels found in Walleye. Otter Tail Lake is located in west central Minnesota and is part of a chain of lakes. The shorelines are well established by residential and commercial uses and the surrounding watershed land cover is primarily agriculture, forest, and water. Studies have indicated that agricultural and forested land covers can contribute to high total Hg levels due to the retention of Hg in foliage (Brumbaugh et al., 2001; Krabbenhoft et al., 1998). Rainy Lake, in contrast, is located in north eastern Minnesota and is primarily undeveloped. The lake is situated within a boreal forest that exhibits shallow soils, bedrock, and peat bogs. Due to minimal anthropogenic processes, elevated Hg levels in Rainy Lake likely occur from atmospheric deposition, leaching geologic formation, and suitable physical and chemical water quality parameters for Hg methylation. In contrast to Monson et al. (2011) above, Simoneau et al. (2005) and Strandberg et al. (2018) illustrated that spatially comparing lakes for Hg is difficult due to different food availability and growth rates, water chemistry, watershed influences, and anthropogenic processes which is also what we experienced in this study. When adjusting length as a covariate between other large lakes in Minnesota, Lower Red Lake Walleye exhibit significantly lower Hg concentrations than Otter Tail and Rainy Lake, whereas Upper Red Lake Walleye only exhibit significantly lower Hg concentrations than Rainy Lake.

Within Minnesota, the MPCA and MN DNR collect fish samples for Hg analysis every five years on average. The last known study conducted within Tribal waters' was in 2002 resulting in the tribal community relying on data collected from State waters for fish consumption guidelines. The State waters account for approximately 25% of the entire Red Lake ecosystem and with inconsistency between sample sizes and time between collection events, there was a need to produce a robust Hg dataset that encompasses all of Upper and Lower Red Lake. This dataset provided the necessary information to create an updated Red Lake-specific Walleye consumption advisory and the coding required to produce an interactive web-based application for predicting Hg concentrations within an individual Walleye. Anticipated informational graphics will mirror those in Figure 2. The current fish consumption guidelines that the state of Minnesota implements is an effective guideline to use for the Red Lakes. The Minnesota fish consumption guideline has minor differences from the EPA recommendations which are broken down into four categories for sensitive populations: no restrictions (≤ 0.05 mg/kg), one meal per week (0.05-0.2 mg/kg), one meal per month (0.2-1.0 mg/kg), and no consumption (≥ 1.0 mg/kg) (MDH, 2004); whereas EPA's guidelines are weekly fish servings: three 4 ounce servings per week (≤ 0.15 mg/kg), two 4 ounce servings per week (0.15-0.23 mg/kg), one 4 ounce serving per week (0.23-0.46 mg/kg), and zero servings per week (> 0.46 mg/kg) (US EPA, 2001). After standardizing the EPA consumption guidelines to mimic Minnesota's guidelines, the advisories are similar with Minnesota's guideline slightly more restrictive for sensitive populations. The Hg data collected from this study suggests that all Walleye sampled between 2019 and 2020 are within safe consumption levels, however moderation is still recommended to meet the consumption guidelines. For people who wish to consume Walleye frequently, one meal per week (MN guidelines) or one to three servings per week (EPA guidelines), should target individuals that are ≤ 400 mm (Figure 2).

In conclusion, collecting Walleye from a wide size distribution was beneficial for this project by providing insights on how mercury can accumulate within a species (e.g. growth, trophic status, energy acquisitions, etc.). The most important finding in this study suggests that management and pollutant monitoring (e.g. mercury) should be sampled throughout the entire Red Lake ecosystem. This study also allowed us to fill in the mercury-data gaps when making comparisons between Upper and Lower Red Lake Walleye. The development of models to estimate mercury levels by length, age, sex, and lake basin will help inform the general public about up-to-date Hg levels in Walleye. Lastly, this study provides the foundation for future research within the Red Lakes from trophic level comparisons through carbon and nitrogen isotope analysis to determining mercury loads from wetlands.

REFERENCES

Agency for Toxic Substance and Disease Registry (ATSDR), 1999. Toxicological profile for mercury. US Department of Health and Human Services, Public Health Service, Atlanta, GA. https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=115&tid=24 (accessed 3.24.2019).

Bernhoft, R.A., 2012. Mercury toxicity and treatment: A review of the literature. J. Environ. Public Health 2012, 1-10. https://doi.org/10.1155/2012/460508 (accessed 4.21.2019).

Brigham, M.E., Krabbenhoft, D.P., Hamilton, P.A., 2003. Mercury in stream ecosystems-New studies initiated by the U.S. Geological Survey, 1-4. https://pubs.usgs.gov/fs/fs-016-03/ (accessed 12.14.2018).

Brigham, M.E., Wentz, D.A., Aiken, G.R., Krabbenhoft, D.P., 2009. Mercury cycling in stream ecosystems. 1. Water column chemistry and transport. Environ. Sci.

Technol., Environmental Science and Technology 43, 1-6. https://doi.org/10.1021/es802694n (accessed 9.18.2018).

Brumbaugh, W.G., Krabbenhoft, D.P., Helsel, D.R., Wiener, J.G., Echols, K.R., 2001. A national pilot study of mercury contamination of aquatic ecosystems along multiple gradients: Bioaccumulation in fish. Biological Science No. 2001– 0009. USGS. http://pubs.er.usgs.gov/publication/bsr20010009 (accessed 9.9.2018).

Burge, D.R.L., 2021. Chapter 2: Managing the Red Lake Nation's and Minnesota's largest lake: Monitoring and paleolimnology support a site-specific standard for Upper and Lower Red Lakes (Red Lake Nation and Minnesota, USA). Modern Limnological and Paleolimnological Applications of Diatoms in Minnesota Lakes (Doctoral dissertation, University of Minnesota). 18-45.

Chalmers, A.T., Argue, D.M., Gay, D.A., Brigham, M.E., Schmitt, C.J., Lorenz, D.L., 2011. Mercury trends in fish from rivers and lakes in the United States, 1969–2005. Environ. Monit. Assess. 175, 175–191. https://doi.org/10.1007/s10661-010-1504-6 (accessed 10.20.2018).

Clayden, M.G., Lescord, G.L., Kidd, K.A., Wang, X., Muir, D.C.G., O'Driscoll, N.J., 2017. Using sulfur stable isotopes to assess mercury bioaccumulation and biomagnification in temperate lake food webs: Sulfur isotopes and mercury. Environ. Toxicol. Chem. 36, 661–670. https://doi.org/10.1002/etc.3615 (accessed 9.18.2018).

Demers, J.D., Blum, J.D., Zak, D.R., 2013. Mercury isotopes in a forested ecosystem: Implications for air-surface exchange dynamics and the global mercury cycle. Global Biogeochem. Cycles 27, 222–238. https://doi.org/10.1002/gbc.20021 (accessed 11.14.2018).

Depew, D., Burgess, N., Anderson, M., Baker, R., Bhavsar, S., Bodaly, R.A., Eckley, C., Evans, M., Gantner, N., Graydon, J., Jacobs, K., LeBlanc, J., St.Louis, V., Campbell, L., 2013. An overview of Hg concentrations in freshwater fish species: A national fish mercury (Hg) dataset for Canada. Can. J. Fish. Aquat. Sci. 70, 1–16. https://doi.org/10.1139/cjfas-2012-0338 (accessed 10.13.2021).

Eagles-Smith, C.A., Suchanek, T.H., Colwell, A.E., Anderson, N.L., 2008. Mercury trophic transfer in a eutrophic lake: The importance of habitat-specific foraging.

Ecol. Appl. 18, A196–A212. https://doi.org/10.1890/06-1476.1 (accessed 10.25.2018).

Engstrom, D.R., Balogh, S.J., Swain, E.B., 2007. History of mercury inputs to Minnesota lakes: influences of watershed disturbance and localized atmospheric deposition. Limnol. Oceanogr. 52, 2467–2483. https://doi.org/10.2307/4502395 (accessed 12.14.2018).

Fitzgerald, W.F., Clarkson, T.W., 1991. Mercury and monomethylmercury: present and future concerns. Environ. Health Perspect. 96, 159–166. https://doi.org/10.1289/ehp.9196159 (accessed 3.19.2019).

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, 3rd ed. SAGE Publications, Inc., Thousand Oaks, CA. https://us.sagepub.com/en-us/nam/an-r-companion-to-applied-regression/book246125 (accessed 8.31.2021).

Gabriel, M.C., Kolka, R., Wickman, T., Nater, E., Woodruff, L., 2009. Evaluating the spatial variation of total mercury in young-of-year yellow perch (*Perca flavescens*), surface water and upland soil for watershed–lake systems within the southern Boreal Shield. Sci. Total Environ. 407, 4117–4126. https://doi.org/10.1016/j.scitotenv.2009.03.019 (accessed 11.13.2020).

Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26, 2281–2287. https://doi.org/10.1021/es00035a029 (accessed 10.25.2018).

Goldman, L.R., Shannon, M.W., Committee on Environmental Health, 2001. Technical Report: Mercury in the environment: Implications for pediatricians. Pediatrics 108, 197–205. https://doi.org/10.1542/peds.108.1.197 (accessed 3.21.2019).

Hall, B.D., Aiken, G.R., Krabbenhoft, D.P., Marvin-DiPasquale, M., Swarzenski, C.M., 2008. Wetlands as principal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region. Environ. Pollut. 154, 124–134. https://doi.org/10.1016/j.envpol.2007.12.017 (accessed 11.4.2021).

Harris, H.C., Rudd, J.W.M., Amyot, M., Babiarz, C.L., Beaty, K.G., Blanchfield, P.J., 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, M.J., Podemski, C.L., Robinson, A., Sandilands, K.A., Southworth, G.R., St. Louis, V.L., Tate, M.T., 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. PNAS. 104, 16586-16591. https://doi.org/10.1073/pnas.0704186104 (accessed 1.29.2022).

Henderson, B., Collins, N., Morgan, G., Vaillancourt, A., 2003. Sexual size dimorphism of walleye (*Stizostedion vitreum vitreum*). Can. J. Fish. Aquat. Sci. 60, 1345–1352. https://doi.org/10.1139/f03-115 (accessed 10.13.2021).

Jeremiason, J.D., Engstrom, D.R., Swain, E.B., Nater, E.A., Johnson, B.M., Almendinger, J.E., Monson, B.A., Kolka, R.K., 2006. Sulfate addition increases methylmercury production in an experimental wetland. Environ. Sci. Technol. 40, 3800–3806. https://doi.org/10.1021/es0524144 (accessed 11.13.2020).

Krabbenhoft, D.P., Wiener, J.G., Brumbaugh, W.G., Olson, M.L., DeWild, J.F., Sabin, T.J., 1998. A national pilot study of mercury contamination of aquatic ecosystems along multiple gradients. U.S. Geological Survey Toxic Substance Hydrology Program-Proceedings of the technical meeting, Charleston, S.C., March 8-12, 1999. USGS Water-Resources Investigations Report 99-4018B, Vol 2, 147-160. https://pdfs.semanticscholar.org/8780/28e45ab5027ed861928eb0d2addca43fb387.pdf (accessed 12.14.2018).

Lamborg, C.H., Fitzgerald, W.F., Damman, A.W.H., Benoit, J.M., Balcom, P.H., Engstrom, D.R., 2002. Modern and historic atmospheric mercury fluxes in both hemispheres: Global and regional mercury cycling implications. Global Biogeochem. Cycles 16, 51-1-51–11. https://doi.org/10.1029/2001GB001847 (accessed 10.20.2018).

Lemon, J., 2006. Plotrix: A package in the red light district of R. R-News 6(4), 8-12.

Lenth, R.V., 2021. emmeans: Estimated marginal means, aka least-squared means. R package version 1.6.2-1. https://cran.r-project.org/web/packages/emmeans/emmeans.pdf (accessed 8.31.2021).

Louis, S., Rudd, J., Kelly, C., Beaty, K., Bloom, N., Flett, R., 1994. Importance of wetlands as sources of methyl mercury to boreal forest ecosystems. Can. J. Fish. Aquat. Sci. 51, 1-12. https://doi.org/10.1139/f94-106 (accessed 11.4.2021).

Madenjian, C.P., Rediske, R.R., Krabbenhoft, D.P., Stapanian, M.A., Chernyak, S.M., O'Keefe, J.P., 2016. Sex differences in contaminant concentrations of fish: a synthesis. Biol. Sex Differ. 7, 1-16. https://doi.org/10.1186/s13293-016-0090-x (accessed 11.4.2021).

Mathers, R., Johansen, P., 1985. The effects of feeding ecology on mercury accumulation in walleye (*Stizostedion vitreum*) and pike (*Esox lucius*) in Lake Simcoe. Can. J. Zoology 63, 2006–2012. https://doi.org/10.1139/z85-295 (accessed 10.13.2021).

Minai, M., 2016. Methylmercury and human embryonic development. Embryo Proj. Encycl. 1-9. http://embryo.asu.edu/handle/10776/11335 (accessed 3.21.2019).

Minnesota Department of Health (MDH), 2004. Eat Fish Often? A Minnesota Guide to Eating Fish. http://www.health.state.mn.us/divs/eh/fish/index.html (accessed 10.13.2021).

Mitchell, C.P.J., Branfireun, B.A., Kolka, R.K., 2008a. Assessing sulfate and carbon controls on net methylmercury production in peatlands: An in situ mesocosm approach. App. Geochem. 23, 503-518. https://doi.org/10.1016/j.apgeochem. 2007.12.020 (accessed 1.29.2022).

Mitchell, C.P.J., Branfireun, B.A., Kolka, R.K., 2008b. Spatial characteristics of net methylmercury production hot spots in peatlands. Environ. Sci. Technol. 42, 1010-1016. https://doi.org/10.1021/es0704986 (accessed 1.29.2022).

Mitchell, C.P.J., Branfireun, B.A., Kolka, R.K., 2009. Methylmercury dynamics at the upland-peatland interface: Topographic and hydrogeochemical controls: Methylmercury at the upland-peatland interface. Water Resour. Res. 45, 1-12. https://doi.org/10.1029/2008WR006832 (accessed 11.13.2020).

Monson, B.A., Staples, D.F., Bhavsar, S.P., Holsen, T.M., Schrank, C.S., Moses, S.K., McGoldrick, D.J., Backus, S.M., Williams, K.A., 2011. Spatiotemporal trends of mercury in walleye and largemouth bass from the Laurentian Great Lakes Region. Ecotoxicol. 20, 1555–1567. https://doi.org/10.1007/s10646-011-0715-0 (accessed 7.26.2021).

Murphy, G.W., Newcomb, T.J., Orth, D.J., 2007. Sexual and seasonal variations of mercury in smallmouth bass. J. Freshw. Ecol. 22, 135–143. https://doi.org/ 10.1080/02705060.2007.9664153 (accessed 10.13.2021).

Myers, G.J., Davidson, P.W., 1998. Prenatal methylmercury exposure and children: neurologic, developmental, and behavioral research. Environ. Health Perspect. 106, 841–847. https://doi.org/10.1289/ehp.98106841 (accessed 3.21.2019).

Myrbo, A., Swain, E.B., Johnson, N.W., Engstrom, D.R., Pastor, J., Dewey, B., Monson, P., Brenner, J., Shore, M.D., Peters, E.B., 2017. Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms. J. of Geophys. Res.: Biogeosciences 122, 2769–2785. https://doi.org/10.1002/2017JG003788 (accessed 9.19.2018).

Ogle, D.H., 2019. FSAdata: Fisheries stock analysis, datasets. R package version 0.3.8.

Ogle, D.H., 2016. Introductory Fisheries Analyses with R, 1st Ed., Chapman and Hall/CRC. 337. https://doi.org/10.1201/9781315371986 (accessed 3.15.2019).

Ogle, D.H., Doll, J.C., Wheeler, P., Dinno, A., 2021. FSA: Fisheries stock analysis. R package version 0.9.1. https://github.com/droglenc/FSA (accessed 10.13.2021).

Park, J.-D., Zheng, W., 2012. Human exposure and health effects of inorganic and elemental mercury. J. Prev. Med. Public Health 45, 344–352. https://doi.org/10.3961/jpmph.2012.45.6.344 (accessed 3.18.2019).

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (accessed 3.18.2021).

Rossi, R., Murari, A., Gaudio, P., Gelfusa, M., 2020. Upgrading model selection criteria with goodness of fit tests for practical applications. Entropy 22, 447. https://doi.org/10.3390/e22040447 (accessed 10.13.2021).

Rustagi, N., Singh, R., 2010. Mercury and health care. Indian J. Occup. Environ. Med. 14, 45–48. https://doi.org/10.4103/0019-5278.72240 (accessed 3.18.2019).

Rypel, A.L., 2010. Mercury concentrations in lentic fish populations related to ecosystem and watershed characteristics. Ambio 39, 14–19. https://doi.org/10.1007/s13280-009-0001-z (accessed 10.20.2018).

Scudder, B.C., Chasar, L.C., DeWeese, R.L., Brigham, M.E., Wentz, D.A., Brumbaugh, W.G., 2008. Procedures for collecting and processing aquatic invertebrates and fish for analysis of mercury as part of the National Water-Quality Assessment

Program. USGS Open-File Report No. 2008–1208, 1-46. https://pubs.usgs.gov /of/2008/1208/pdf/ofr20081208.pdf (accessed 3.25.2019).

Scudder, B.C., Chasar, L.C., Wentz, D.A., Bauch, N.J., Brigham, M.E., Moran, P.W., Krabbenhoft, D.P., 2009. Mercury in fish, bed sediment, and water from streams across the United States, 1998–2005. USGS, Scientific Investigation Report 2009-5109, 1-74. https://pubs.usgs.gov/sir/2009/5109/pdf/sir20095109.pdf (accessed 9.9.2018).

Shao, D., Kang, Y., Wu, S., Wong, M.H., 2012. Effects of sulfate reducing bacteria and sulfate concentrations on mercury methylation in freshwater sediments. Sci. Total Environ. 424, 331–336. https://doi.org/10.1016/j.scitotenv.2011.09.042 (accessed 3.18.2019).

Simoneau, M., Lucotte, M., Garceau, S., Laliberté, D., 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. Environ. Res. 98, 73–82. https://doi.org/10.1016/j.envres.2004.08.002 (accessed 10.20.2018).

Snodgrass, J.W., Jagoe, C.H., Bryan, Jr., A.L., Brant, H.A., Burger, J., 2000. Effects of trophic status and wetland morphology, hydroperiod, and water chemistry on mercury concentrations in fish. Can. J. Fish. Aquat. Sci. 57, 171–180. https://doi.org/10.1139/cjfas-57-1-171 (accessed 3.29.2019).

Strandberg, U., Bhavsar, S., Parmar, T.P., Arts, M., 2018. Spatial and length-dependent variation of the risks and benefits of consuming Walleye (*Sander vitreus*). Environ. Int. 112, 251–260. https://doi.org/10.1016/j.envint.2017.12.029

United States Environmental Protection Agency (US EPA), 2019. 2019 Toxics Release Inventory National Analysis. US EPA. https://www.epa.gov/trinationalanalysis /introduction-2019-tri-national-analysis (accessed 10.13.2021).

United States Environmental Protection Agency (US EPA), 2015. Clean Air Act Text. US EPA. https://www.epa.gov/clean-air-act-overview/clean-air-act-text (accessed 3.18.19).

United States Environmental Protection Agency (US EPA), 2007. Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. Method 7472, US EPA. https://www.epa.gov/sites/production/files/2015-07/documents/epa-7473.pdf (accessed 3.25.2019).

United States Environmental Protection Agency (US EPA), 2001. Water Quality Criterion for the Protection of Human Health: Methylmercury. Office of Science and Technology, Washington, D.C. EPA-823-R-01-001. http://www.epa.gov/waterscience/criteria/methylmercury/ (accessed 10.13.2021).

United States Environmental Protection Agency (US EPA), 2000. Quality assurance project plan for analytical control and assessment activities in the national study of chemical residues in lake fish tissue. Report No. EPA-823-R-02-006.

United States Environmental Protection Agency (US EPA), 1997. Mercury study report to congress (Executive Summary No. EPA-452/R-97-003). US EPA. https://www3.epa.gov/airtoxics/112nmerc/volume1.pdf (accessed 3.21.2019).

Wang, B., Zhong, S., Bishop, K., Nilsson, M.B., Hu, H., Eklof, K., Bravo, A.G., Akerblom, S., Bjorn, E., Skyllberg, U., 2021. Biogeochemical influences on net methylmercury formation proxies along a peatland chronosequence. Geochimica et Cosmochimica Acta. 308, 188-203. https://doi.org/10.1016/j.gca.2021.06.010 (accessed 1.29.2021).

Wickham, H., François, R., Henry, L., Müller, K., 2021. dplyr: A grammar of data manipulation. R package version 1.0.7. https://CRAN.R-project.org/package=dplyr.

Xue, J., Zartarian, V., Mintz, B., Weber, M., Bailey, K., Geller, A., 2015. Modeling tribal exposures to methyl mercury from fish consumption. Sci. Total Environ. 533, 102–109. https://doi.org/10.1016/j.scitotenv.2015.06.070 (accessed 8.3.2021).

TABLES

Table 1: General mean water quality parameters for Upper and Lower Red Lake based on a 2013 10-year water quality assessment conducted by the Red Lake DNR Water Resources Program.

Parameter	Units	Upper Red Lake	Lower Red Lake
Alkalinity	mg/L	127.9	140.4
Area	km^2	482.8	665.9
Chlorophyll α, corrected for pheophytin	µg/L	15.2	12.1
Conductivity	µS/cm	259.5	286.2
Depth, Secchi	m	0.8	1.2
Depth, Water	m	5.5	9.1
Dissolved Oxygen	mg/L	10.3	9.7
pH	pH	8.4	8.3
Phosphorus	mg/L	0.04	0.03
Temperature, Water	°C	15.5	15.2
Turbidity	NTU	7.4	5.0
Orthophosphate	mg/L	0.00	0.00
Kjeldahl Nitrogen	mg/L	1.2	0.8
Nitrogen	mg/L	1.2	0.8
Inorganic Nitrogen (NO_3/NO_2)	mg/L	0.01	0.01
Ammonia-Nitrogen	mg/L	0.03	0.03

Table 2: Model used to predict Hg concentrations in Walleye from Upper and Lower Red Lake from study years of 2019 and 2020.

Function	AIC	Δ AIC	R^2
Hg~Length*Sex*Age*Basin	-852.5	0.0	0.80
Hg~Length*Age*Basin	-825.2	-27.2	0.77
Hg~Length+Sex+Age+Basin	-773.5	-79.0	0.72
Hg~Length+Age+Basin	-773.2	-79.2	0.72
Hg~Length*Age	-716.4	-136.1	0.65
Hg~Length+Age	-704.9	-147.5	0.63
Hg~Age	-686.9	-165.6	0.61
Hg~Length*Basin	-637.6	-214.8	0.53
Hg~Length*Sex*Basin	-636.9	-215.6	0.54
Hg~Length+Sex+Basin	-628.6	-223.9	0.51
Hg~Length+Sex*Basin	-627.0	-225.5	0.51
Hg~Length+Sex	-625.4	-227.1	0.35
Hg~Length*Sex	-550.3	-302.1	0.35
Hg~Length+Weight	-548.5	-304.0	0.34
Hg~Length	-548.4	-304.1	0.34
Hg~Weight	-529.0	-323.4	0.29
Hg~Basin	-471.1	-381.4	0.11
Hg~Length+Basin	-459.2	-393.2	0.51
Hg~Sex	-438.7	-413.8	0.00

Table 3: Size class distribution of Walleye sampled from Upper and Lower Red Lake during 2019 and 2020. Concentrations converted to wet weight.

	Size Class (mm)							
	< 254	**254-304**	**305-355**	**356-406**	**407-457**	**458-508**	**509-559**	**560-610**
n	31	38	40	39	41	40	28	8
Min mg/kg Hg	0.030	0.065	0.061	0.059	0.060	0.085	0.112	0.186
Max mg/kg Hg	0.176	0.187	0.279	0.407	0.530	0.502	0.564	0.416
Mean mg/kg Hg	0.096	0.114	0.123	0.163	0.231	0.240	0.264	0.282
SD mg/kg Hg	0.036	0.028	0.041	0.080	0.125	0.093	0.124	0.078
Mean Age	0.9	1.2	2.2	3.7	5.5	7.3	8.6	10.4
				Male Walleye				
n	18	21	22	21	23	19	5	
Min mg/kg Hg	0.033	0.074	0.061	0.095	0.077	0.125	0.168	
Max mg/kg Hg	0.152	0.187	0.279	0.407	0.530	0.413	0.496	
Mean mg/kg Hg	0.094	0.111	0.131	0.175	0.244	0.261	0.300	
SD mg/kg Hg	0.031	0.026	0.047	0.092	0.122	0.077	0.119	
Mean Age	0.8	1.2	2.2	4.1	6.1	8.9	11.0	
				Female Walleye				
n	13	17	18	18	18	21	23	8
Min mg/kg Hg	0.030	0.065	0.065	0.059	0.060	0.085	0.112	0.186
Max mg/kg Hg	0.176	0.173	0.179	0.325	0.441	0.502	0.564	0.416
Mean mg/kg Hg	0.099	0.117	0.114	0.149	0.215	0.221	0.256	0.282
SD mg/kg Hg	0.041	0.030	0.031	0.060	0.126	0.101	0.124	0.078
Mean Age	1.0	1.1	2.1	3.2	4.8	5.8	8.1	10.4

Table 4: Estimated marginal means and 95% CIs for Hg concentrations in Walleye between 12 large lakes in Minnesota. Analysis of covariance was used to compare the Hg concentrations between lakes by total length as the covariate. Groups with similar letters indicates no significant difference ($p > 0.05$) when comparing Upper and Lower Red Lake to other large lakes in MN.

Lake Names	Estimated Marginal Hg Means (mg/kg)	SE	df	Lower CI	Upper CI	Groups
Cass	0.33	0.04	485	0.25	0.41	a,b
Kabetogama	0.24	0.04	485	0.16	0.32	a,b
Lake of the Woods	0.31	0.04	485	0.22	0.39	a,b
Leech	0.26	0.04	485	0.18	0.34	a,b
Lower Red	0.16	0.04	485	0.09	0.24	a,b
Mille Lacs	0.25	0.04	485	0.17	0.32	a,b
Minnetonka	0.25	0.04	485	0.16	0.34	a,b
Otter Tail	0.46	0.05	485	0.36	0.57	c
Rainy	0.48	0.03	485	0.42	0.53	d
Upper Red	0.26	0.03	485	0.21	0.32	a,b,c
Vermilion	0.27	0.04	485	0.20	0.34	a,b
Winnibigoshish	0.21	0.04	485	0.12	0.29	a,b

FIGURES

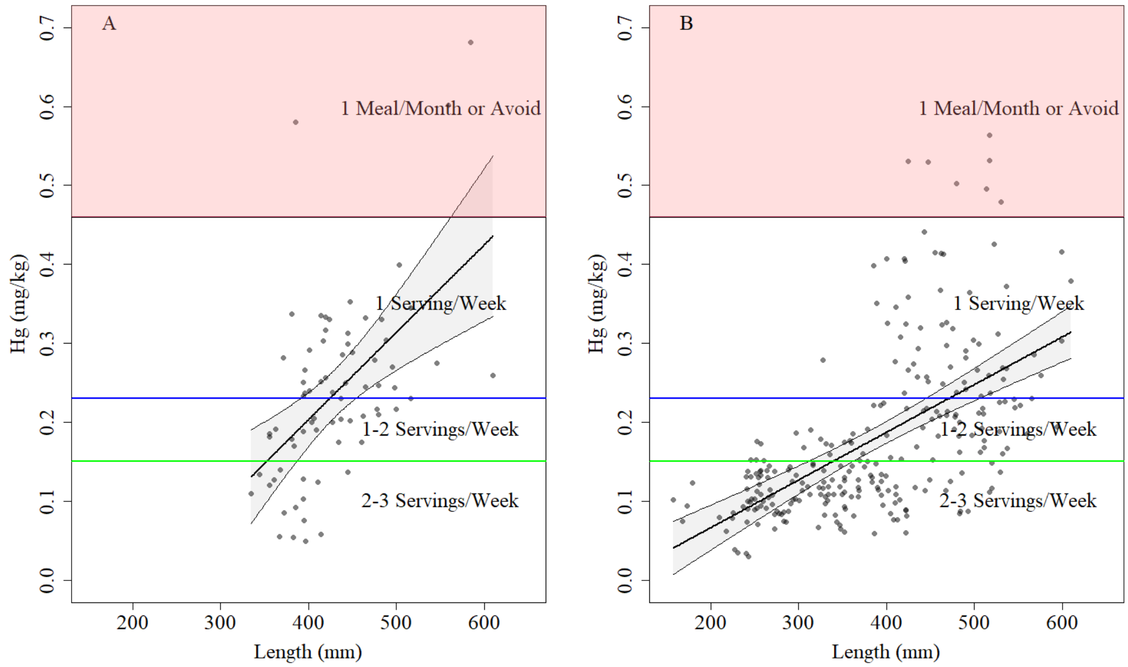


Figure 1: Mercury concentrations as a response to length. (A) Data that was collected from Upper Red Lake by MNDNR from 1987 to 2012. (B) Current data that was collected during the 2019 and 2020 experimental gill netting to assess population densities by the Red Lake DNR and MNDNR. Green horizontal lines indicate unrestricted consumption of 0.15 mg/kg Hg in raw fish fillet; blue horizontal lines indicate a consumption of two meal/week at levels of ≤ 0.23 mg/kg Hg in raw fish fillet; below the red polygon indicates a consumption of one meal/week at levels ≤ 0.46 mg/kg Hg in raw fish fillet; the area in red indicates a consumption of one meal/month at levels ≥ 0.46 mg/kg Hg in raw fish fillet.

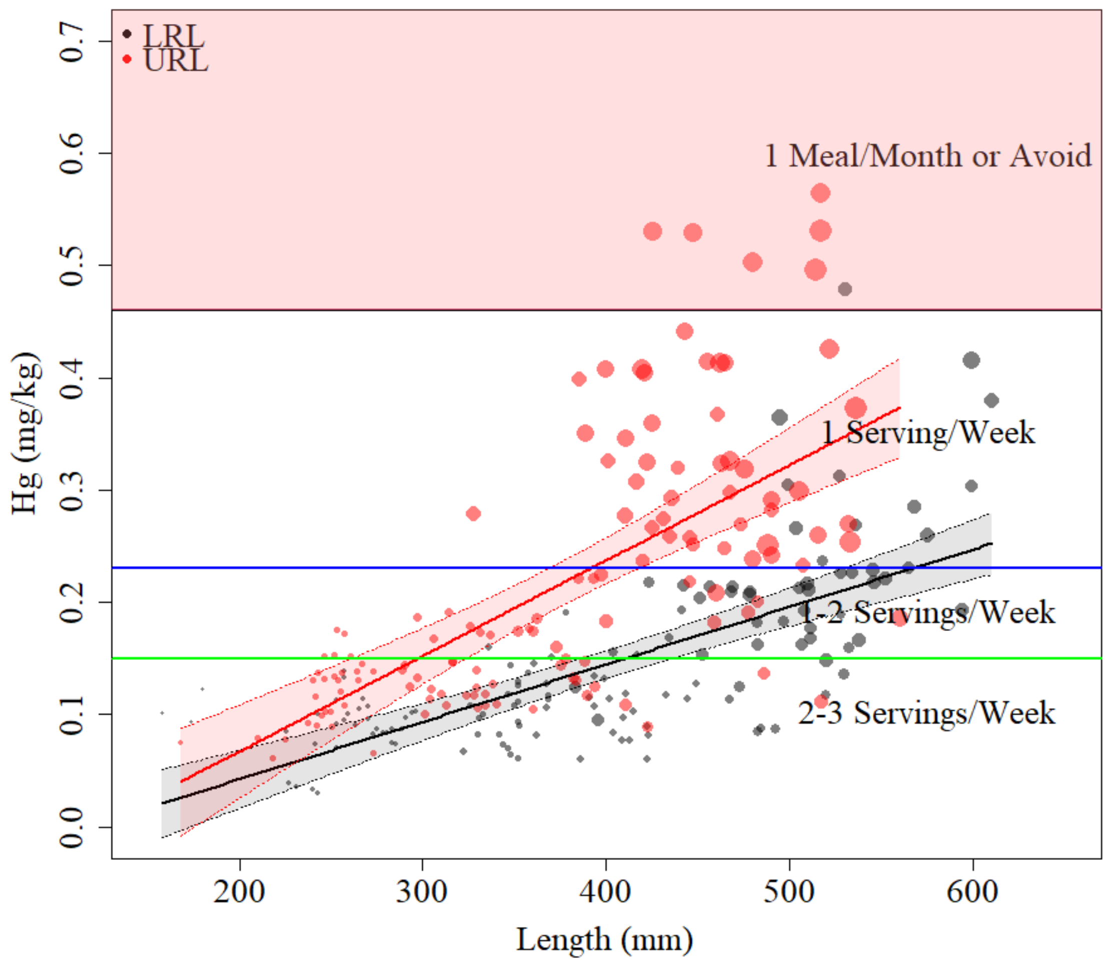


Figure 2: Mercury concentrations by length from Upper (red) and Lower (black) Red Lake collected by the Red Lake DNR and MNDNR during the 2019 and 2020 study. Point sizes are weighted based on the age of individual Walleye; larger points are older individuals. Age zero Walleye received a 0.5 age in order to illustrate the point indication. The 99% confidence interval indicates that the linear regression will fall inside the interval bounds. Green horizontal lines indicate unrestricted consumption of 0.15 mg/kg Hg in raw fish fillet; blue horizontal lines indicate a consumption of two meal/week at levels of ≤ 0.23 mg/kg Hg in raw fish fillet; below the red polygon indicates a consumption of one meal/week at levels ≤ 0.46 mg/kg Hg in raw fish fillet; the area in red indicates a consumption of one meal/month at levels ≥ 0.46 mg/kg Hg in raw fish fillet.

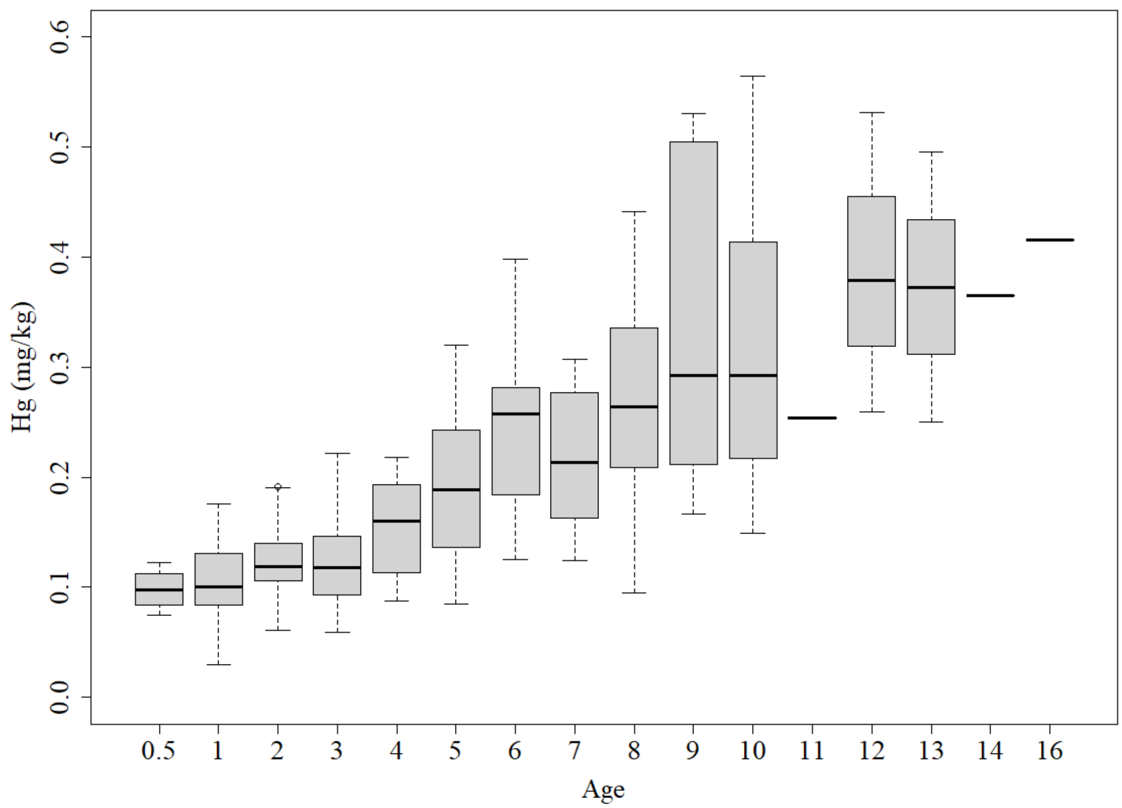


Figure 3: Mercury concentrations as a response to age from the 2019 and 2020 Upper and Lower Red Lake study. The line within the box represents the median mercury concentrations in wet weight (mg/kg). Dimensions of the box represent the 25^{th} and 75^{th} percentiles with the dark bar representing the median (50^{th} percentile). Error bars represent the minimum and maximum outliers within the data.

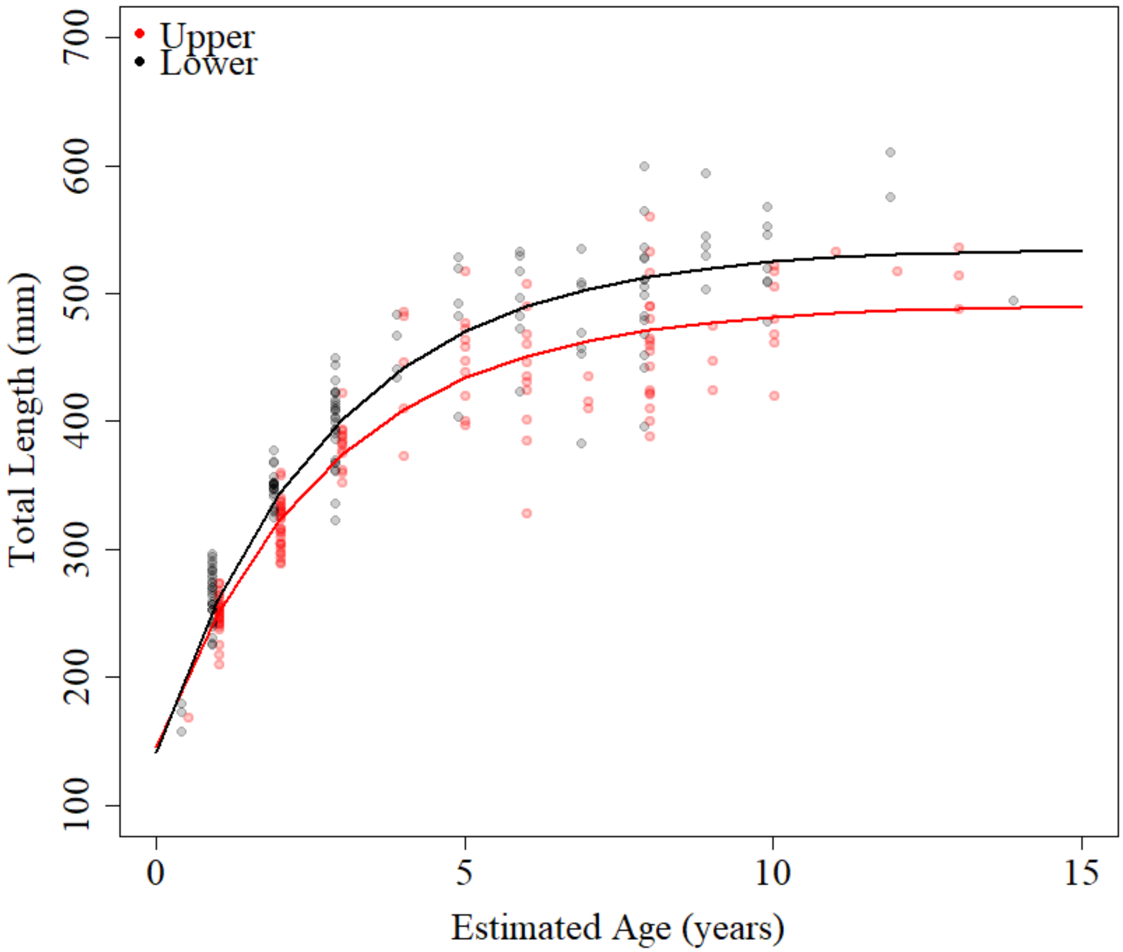


Figure 4: Comparison of mean length by age of Red Lake Walleye based on 265 examined dorsal fin rays and otoliths for age determination using von Bertalanffy method from the 2019 and 2020 fall experimental population netting assessment.

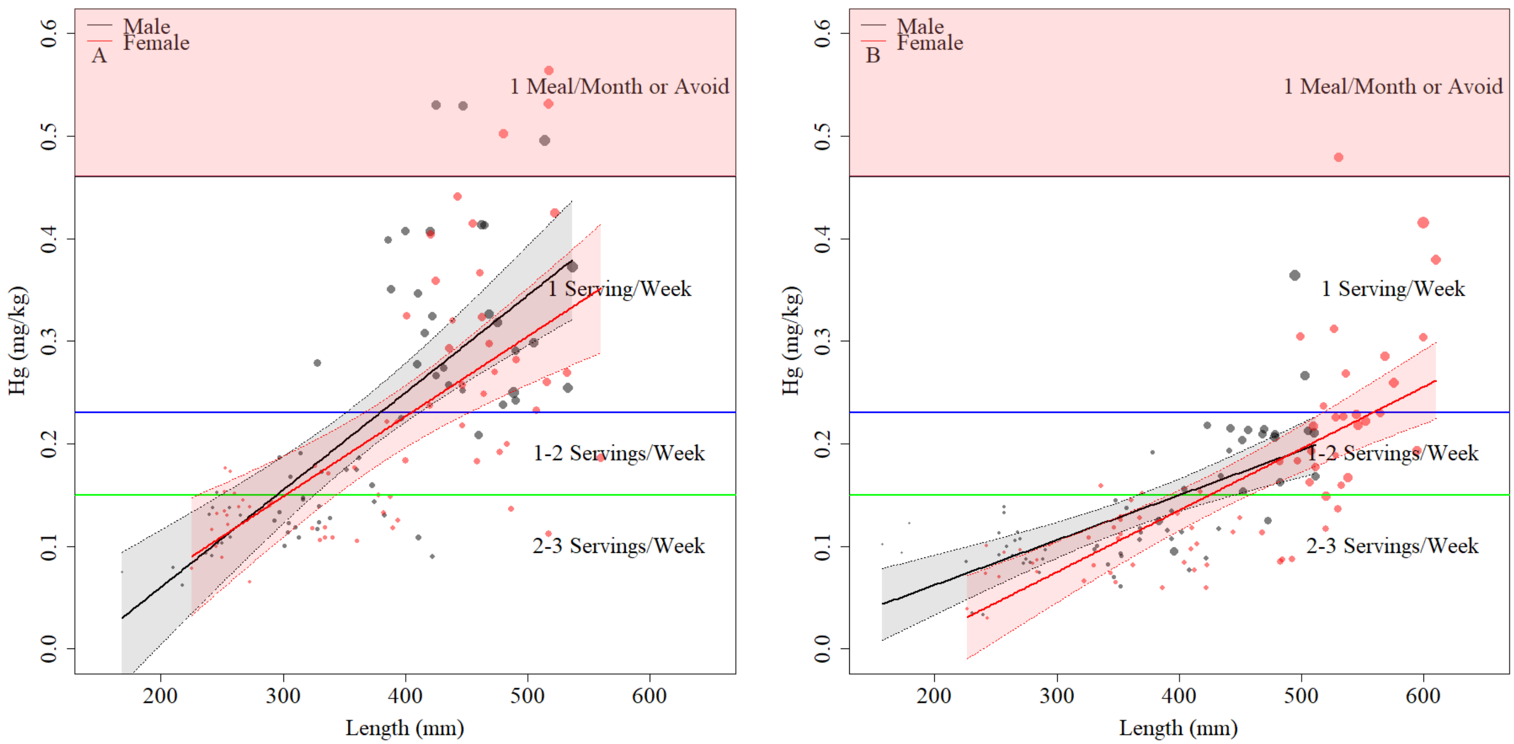


Figure 5: Mercury concentrations as a response to sex from the 2019 and 2020 study on Upper and Lower Red Lake. Plot A is Hg concentration in male and female Walleye from Upper Red Lake and plot B is Lower Red Lake. Size of data points are weighted based on age of male and female Walleye. Green horizontal lines indicate unrestricted consumption of 0.15 mg/kg Hg in raw fish fillet; blue horizontal lines indicate a consumption of two meal/week at levels of ≤ 0.23 mg/kg Hg in raw fish fillet; below the red polygon indicates a consumption of one meal/week at levels ≤ 0.46 mg/kg Hg in raw fish fillet; the area in red indicates a consumption of one meal/month at levels ≥ 0.46 mg/kg Hg in raw fish fillet.

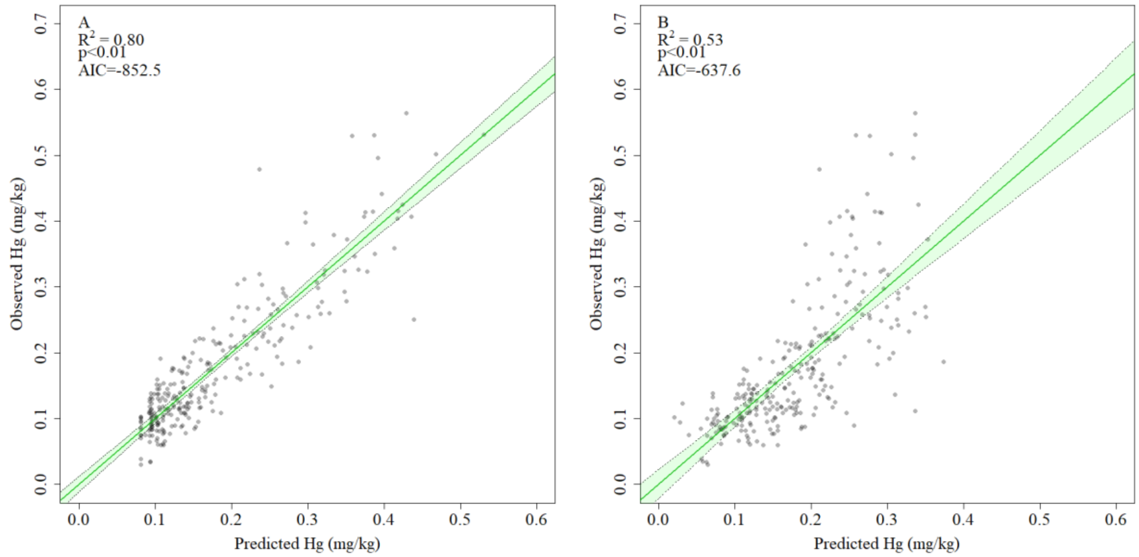


Figure 6: Predictive models to estimate Hg in Walleye from Upper and Lower Red Lake. Plot A is an interactive function with four different variables (length, age, sex, and basin) to estimate Hg concentrations in Walleye from Upper and Lower Red Lake (AIC=-852.5). Plot B is also an interactive function with two variables (length and basin) to estimate Hg concentrations in Walleye from Upper and Lower Red Lake (AIC=-637.6). Plot A is the best fit model for the data with a linear regression line that will fall within the confidence interval, in green, 95% of the time: F-statistic: 71.39 on 15 and 249 DF, p-value: < 0.01.

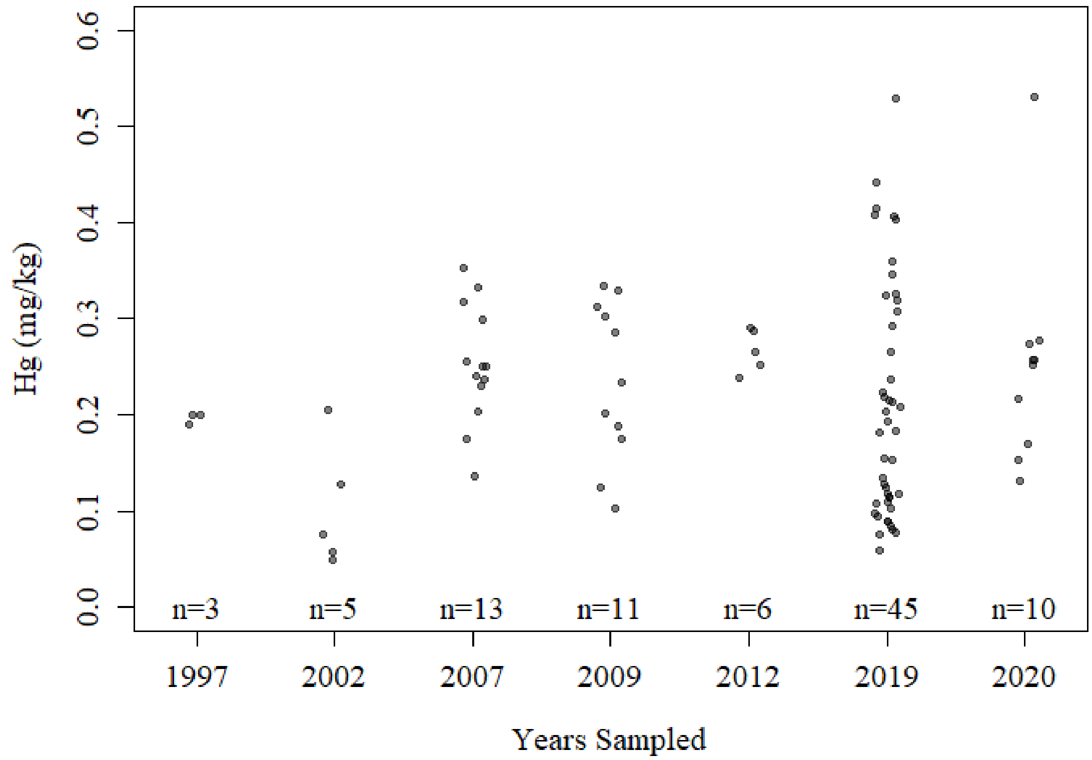


Figure 7: Scatter plot of Walleye mercury concentrations (mg/kg) by years sampled in Upper and Lower Red Lake adjusted for length as the covariate. A two-way ANOVA (Type III ANOVA) was used to evaluate the interactive effect of length (continuous variable) and years sampled to determine temporal differences in Hg concentrations within the Red Lakes. The ANOVA suggests that there are no significant differences in Hg concentrations between years sampled.

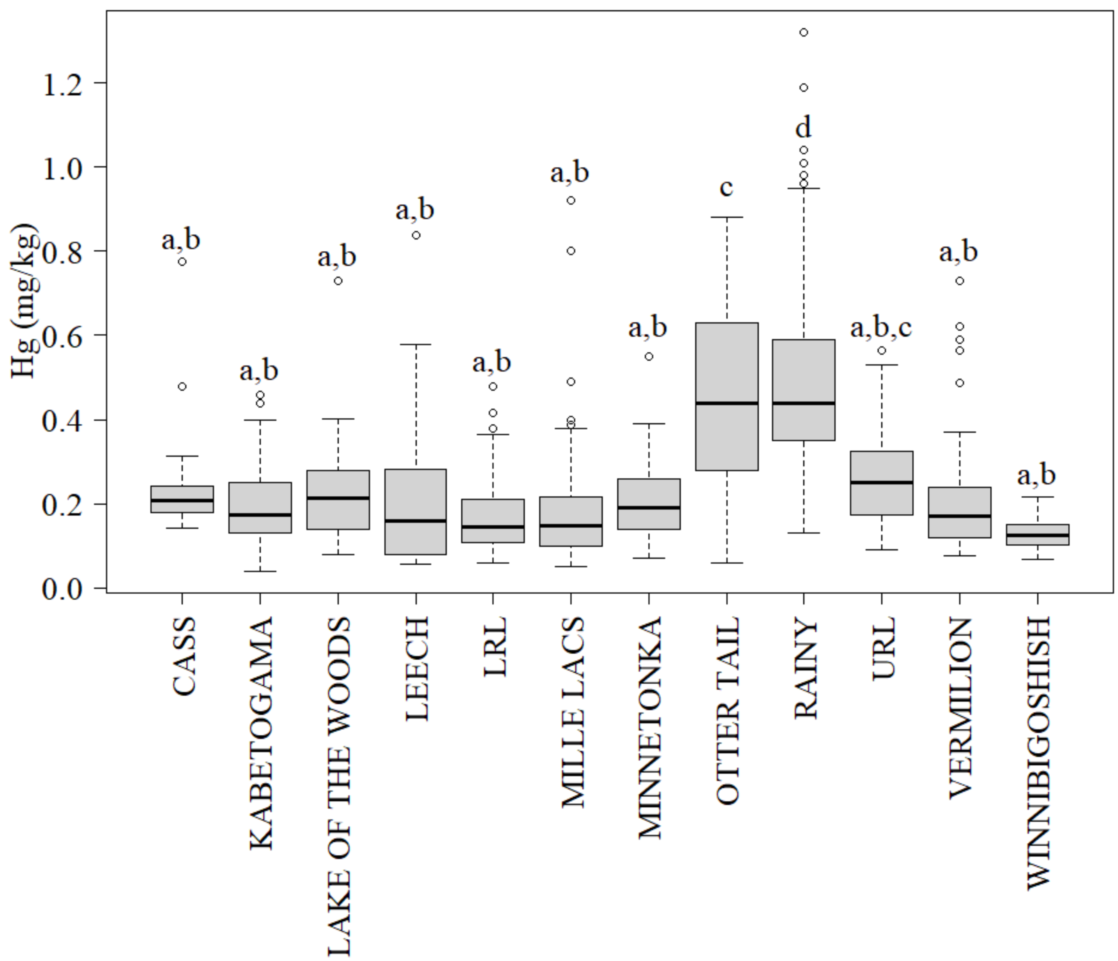
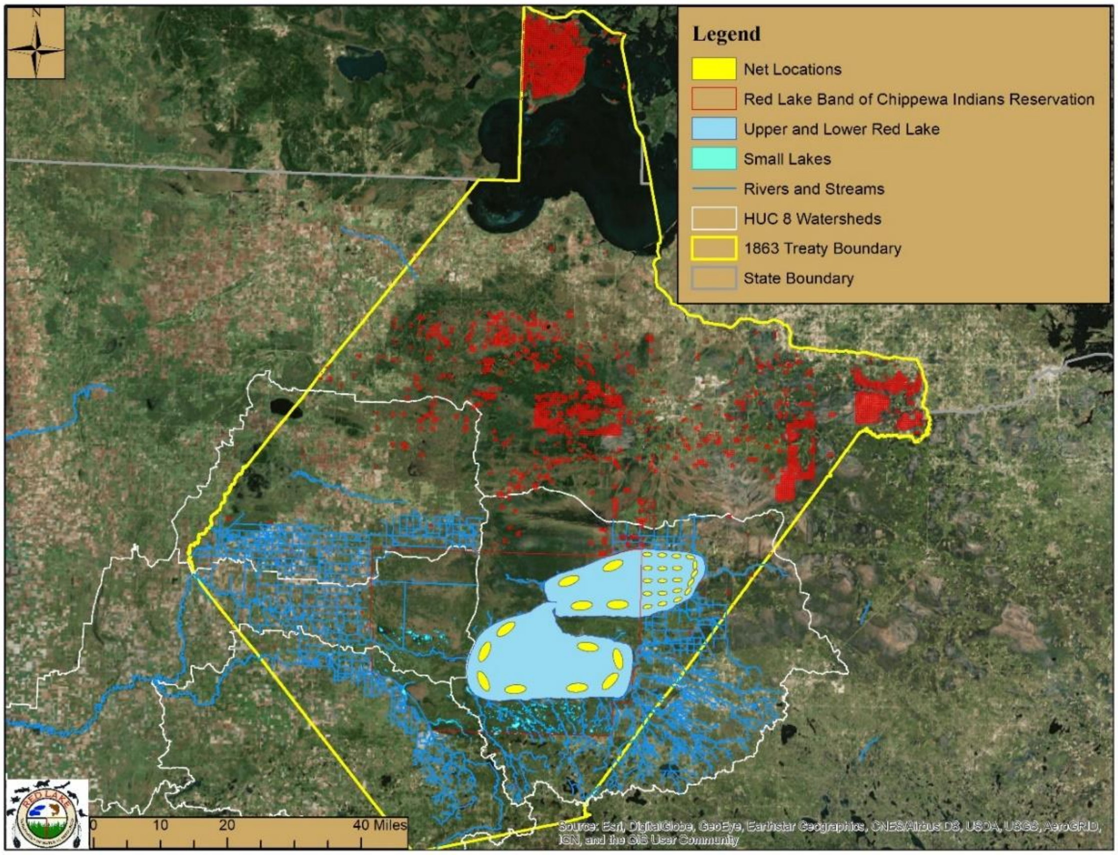
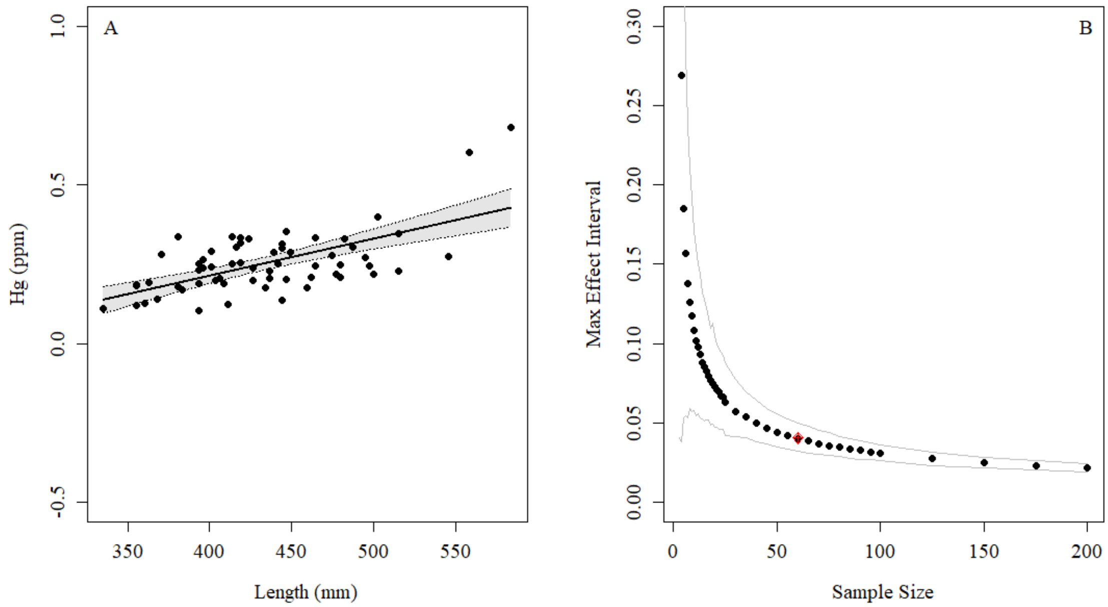


Figure 8: Distribution of Hg concentrations (mg/kg) in Walleye from Minnesota's 10 largest lakes in comparison in the Red Lakes (denoted as LRL for Lower Red Lake and URL for Upper Red Lake). The line within the box represents the median value. Dimensions of the box represent the 25th and 75th percentiles with the dark bar representing the median (50th percentile). Error bars represent the minimum and maximum outliers within the data. Individual points are extreme outliers. Boxes sharing a common letter are not significantly different ($p > 0.05$), analyzed using ANCOVA and Tukey method for multiple comparisons.

APPENDIX



Appendix A: Location of sampling sites on the Red Lakes and the watersheds that influence the system.



Appendix B: (A) Current MNDNR Hg concentrations by length from 1987-2012 with a 95% confidence interval polygon of the regression line. (B) Sample size determination based on the simulated effect interval from (A). The red point indicates 60 samples needed to achieve a maximum mean effect of 0.04 mg/kg of Hg.