Non-Steady State Mercury Bioaccumulation and Dynamics

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Non-steady state mercury bioaccumulation and dynamics

By

Rachel Abma

A Thesis
Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research (GLIER)
in Partial Fulfillment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada
2014

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Non-steady state mercury bioaccumulation and dynamics

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September 8, 2014
DECLARATION OF PREVIOUS PUBLICATION

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ABSTRACT

This study explored Hg bioaccumulation with age in three Lake Huron lake trout populations, considering effects of growth and trophic dynamics. Hg concentrations and stable isotopes were measured in trout, smelt, round goby, zooplankton and zebra mussels. Trout populations demonstrated exponentially increasing Hg concentrations with age and revealed basin-specific accumulation patterns. High biomagnification Factor (BMF) correlated with low prey densities suggest that physiological and ecological factors regulating fish growth rates such as foraging efficiencies are important in regulating Hg bioaccumulation.

Physiological processes affect Hg bioaccumulation, specifically elimination dynamics. Hg in liver, gonads, dorsal muscle, and remaining carcass in pre-spawn, spawning, and post-spawn yellow perch populations were investigated. Ratio of Hg in each tissue to whole-body Hg were different between male and female perch, as well as among pre-, during-, and post-spawning perch. Thus, changes in Hg tissue concentrations during spawning could result in high variability of Hg elimination rates.
DEDICATION

This thesis is dedicated to the members of my ever-loving, ever-supportive family:

John, Mary, Bryan and Evan.
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There are many people who deserve special mention for their help on my project. First and foremost I would like to thank my supervisor, Dr. Douglas Haffner, for his support—both financial and academic—along the way. This thesis would not have been possible without you. I also couldn’t have done this without the endless help of Dr. Gordon Paterson. Your comments and hard work were invaluable. Next I must acknowledge all of the government employees who let me tag along on their fishing and science vessels. Specifically, thank-you to Mike Keir, Mandy Clark, John Brookham, Darrell Wilson, Terry Walmsley, the staff of the MNR-UGLMU and the Canada Coast Guard. Thank-you also to JC Barrette and Anna Hussey who helped me analyze my samples, to my fellow graduate students, Anne McLeod, Mark Ryder, Yanna Altshuler, Brennen Coristine and Bill Glass, who helped me with field collections, and to the undergraduates who helped me collect data—Lauren DiPierdomenico, Michael Dufour, Alexander McIsaac, Jule Ridel, and Brittany Hedges. Finally, I would like to thank my family and friends for their constant support through this process.
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of variance with interaction, p<0.05), and the (i) indicate significant differences in ratios (ANCOVA, p<0.05)).
LIST OF ABBREVIATIONS/SYMBOLS

Hg- Mercury
GLIER- Great Lakes Institute for Environmental Research
OMNR- Ontario Ministry of Natural Resources
UGLMU- Upper Great Lakes Management Unit
EPA- Environmental Protection Agency
W-CntVG- Wet Control
BT-Cnt2M-Spk- Biological Tissue Control M Spiked
BT-Dorm-3- Biological Tissue Control
BT-Dolt 4- Biological Tissue Control
ANCOVA- Analysis of covariance
k- Growth rate coefficient
$L_{\text{inf}}$- asymptotic length
LT- total length
$\delta^{13}C$- Carbon isotopic signature
$\delta^{15}N$- Nitrogen isotopic signature
$R_x$ - Ratio of heavy to light isotope
NOMENCLATURE

*Salvelinus namaycush*- Lake Trout

*Osmerus mordax*- Rainbow Smelt

*Neogobius melanostromus*- Round Goby

*Dreissena polymorpha*- Zebra Mussel

*Perca Flavescens*- Yellow Perch
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Appendix A: John Wiley and Sons License Terms and Conditions
CHAPTER 1
General Introduction

Mercury (Hg) has been recognized as a toxin for centuries, but the level of its risk to human health was not realized globally until the 1800s (Stein 1996). The dangers of exposure to Hg through food became a relevant, global issue when it was discovered as the cause of Minamata disease in the 1950s, and fish consumption was shown to be the main route of exposure to humans (Harada 1995). Hg is a highly toxic and ubiquitous metal that is emitted into the atmosphere by both natural and anthropogenic activity (UNEP 2008). Atmospheric Hg is present mainly in three forms: elemental Hg, divalent Hg, and particulate Hg. In aquatic systems, however, Hg is converted to monomethylmercury (MeHg), the most toxic and bioaccumulative species of Hg (Bloom 1992, Ullrich 2001), and dimethylmercury (Me2Hg). MeHg accounts for approximately 90% of the total Hg in fish tissues (Jernelov 1979). Although Hg dynamics in trophic interactions have been extensively studied, the mechanisms that drive these processes remain poorly understood.

The Laurentian Great Lakes contain 21% of the world’s fresh water and the Great Lakes fishery is highly productive, supporting more than 30 million people (Hagreen 2004). The discovery of high Hg levels in fish was the primary cause of fishery closures in Lake St. Clair in 1970, and since then government initiatives have been put in place to clean up existing pollution and prevent further contamination (MNR-MOE 1993, Zananski 2011). After the initiation of government programs, Hg levels in water and sediment have been declining throughout the Great Lakes, and water and sediment
mercury concentrations have been reported to be homogeneous across much of the Great Lakes with very few exceptions (SOLEC 2011). Despite lowered background levels of Hg, however, Hg levels in fish tissues are still a concern today and fish consumption advisories have been established to prevent mercury poisoning in humans (Evers 2011). Fish consumption advisories are published bi-yearly by the Ontario Ministry of the Environment (OMOE 2013). All guidelines are based on length and species of fish, and muscle concentrations of Hg are used to measure guideline levels to determine which fish are safe to eat. According to the most recent consumption guideline, average Hg concentrations in largemouth bass, walleye lake trout, smallmouth bass, and common carp exceed consumption thresholds in multiple locations in the Great Lakes and their surrounding small inland lakes (OMOE 2013).

Hg bioaccumulation is complex, and there are a number of factors that regulate variability in muscle concentrations. Background Hg, trophic structure, and variable growth rates contribute to the uptake coefficient of Hg in bioaccumulation models. Physiological processes, including reproduction, can contribute to the elimination parameter. This thesis investigates Hg bioaccumulation in populations of lake trout in Lake Huron, testing the null hypothesis ($H_0$) that Hg bioaccumulation is a steady-state process that is constant both spatially and temporally in a large body of water. The first study assesses Hg bioaccumulation as a non-steady state process by comparing whole-body Hg concentrations among lake trout from three basins in Lake Huron. The study investigates Hg bioaccumulation in the contexts of growth, trophic ecology, and background Hg. Growth, trophic ecology, and background Hg are all parameters
expected to contribute to the bioaccumulation of Hg in predatory fish. These parameters primarily affect the uptake of Hg in the bioaccumulation process (Trudel 2000).

The second study in this thesis investigates tissue distribution of Hg and temporal differences in Hg tissue concentration during different times in a reproductive cycle by measuring Hg concentrations in liver, gonad, muscle and carcass tissue in yellow perch from Lake Erie, another large body of water in the Great Lakes. Tissue concentrations of Hg before, during, and after spawning provide insight into factors regulating the elimination coefficient of Hg bioaccumulation models, specifically elimination during reproduction (Trudel 2001).
REFERENCES


CHAPTER 2

Bioaccumulation of Mercury: Non-steady state dynamics

Introduction

A thorough understanding of the processes regulating Hg bioaccumulation dynamics in fish remains a challenge (Morel 1998, Sackett 2013). Hg contamination is a concern to both human and environmental health such that size dependant fish consumption guidelines have been established (OMOE 2013). Although there are a number of studies quantifying Hg accumulation as a function of size and trophic structure, only a few studies have addressed year-to-year changes in Hg contamination of tissues in predatory fish such as walleye (Evers 2011, Zananski 2011) and lake trout (Bhavsar 2010, Borgmann and Whittle 1991). Risk models of Hg exposures in aquatic ecosystems have utilized different uptake and elimination rates due to factors including growth, trophic ecology, and reproduction. Current models are often species-specific but not basin-specific within large systems such as Lake Huron. Furthermore, some models have assumed elimination rates in older fish that are similar to uptake rates (Trudel 2001).

Biomagnification factors (BMFs) are used to quantify bioaccumulation in this study. BMFs quantify the increase in Hg between trophic levels in a given food web. BMFs in the literature range anywhere between 0.5- and 10- fold increase in Hg between
trophic levels in different ecosystems (Baeyens 2003, Rolfhus 2011), but differences in BMFs among basins in a large water body have not been extensively studied.

Hg concentration in the sediments of the Great Lakes have decreased significantly between 1970 (0.5-1.7mg/kg) and 2002 (<0.17mg/kg), and are now relatively low throughout the basin (Forsythe 2009, SOLEC 2012). Average Hg concentrations in water have also decreased since 1970 (from 1.7-3.8 to <0.06-0.11mg/L). Although background Hg levels in the sediment and water of the Great Lakes region are generally lower than the guideline level of 1.0ug/L (SOLEC 2012), concentrations of Hg in predatory fish tissues continue to remain a concern (Dove 2011). Hg concentrations in predatory fish of the Great Lakes decreased between 1970 and 1990 but then began to show slight increases especially in Lakes Erie and Huron after 1990 (Bhavsar 2010).

As background levels of Hg in the water and sediment in the Lake Huron are relatively homogenous (Dove 2011) it would be predicted that top predators at common trophic levels would accumulate Hg at similar rates. Therefore it is predicted that predators sharing the same trophic level would have similar contaminant burdens across basins and predator–prey relationships would reflect similar BMFs. Recently it has been observed that factors regulating predator growth rates can also modify the bioaccumulation dynamics of Hg, such that bioenergetics and ecological processes need to be considered.

Lake trout have been used as a contaminant biomonitor in the Great Lakes since the 1970s (Borgmann and Whittle 1991). These populations are also monitored by the Ontario Ministry of Natural Resources for growth rates and population trends, thus producing one of the better-integrated Hg monitoring programs in the world (OMNR-
UGLMU 2012). This study builds on their Lake Huron data set, with the goal of developing a better understanding of factors regulating Hg bioaccumulation in upper trophic levels, specifically in response to the collapse of the Lake Huron food web (Bunnell 2014). Lake trout are primarily a cold-water fish and are found in the North Channel, Georgian Bay, and the Main Basin of Lake Huron (Zananski 2011). As a top predator in Lake Huron, they prey on a variety of forage fish including rainbow smelt (*Osmerus mordax*) (Borgmann and Whittle 1991). With the recent crash of the forage fish base in Lake Huron (Riley 2008) it is surmised that predator–prey interactions have changed considerably over the past decade. This in turn impacts the growth rates of lake trout, which can alter Hg bioaccumulation dynamics in fish.

According to gut content analysis, performed by the OMNR-UGLMU as part of their lake trout rehabilitation project, lake trout have eating Round Goby (*Neogobius melanostomus*) in the Main Basin of Lake Huron (OMNR-UGLMU 2012). Since 2003, gobies were shown to occupy approximately 10% of the lake trout diet in the Main Basin, so gobies were included as a potential prey item for Lake Huron lake trout as well.

The aim of this research was to determine whether or not Hg bioaccumulation in lake trout with age is the same across basins in Lake Huron. The null hypothesis ($H_0$) is that Hg bioaccumulation trends are the same across basins in the context of trophic ecology, growth, and background Hg levels. Long lived lake trout were chosen as the test species in that they would have sufficient time to show clear bioaccumulation patterns.

**Materials and methods**
**Fish Collection and Processing**

Lake trout and forage fish were collected from sites on Lake Huron in the three Canadian basins: North Channel, Georgian Bay, and Main Basin (Fig. 1). Fish species collected, processed and analyzed for this study include lake trout (*Salvelinus namayacush*), rainbow smelt (*Osmerus mordax*), and round goby (*Neogobius melanostomus*). Gill nets were set overnight by the Upper Great Lakes Unit of the Ontario Ministry of Natural Resources to capture lake trout and forage fish from all sites. Gill nets were set as part of the lake trout rehabilitation project (OMNR UGLMU 2012) on the OMNR vessel, *Huron Explorer*. A total of 18 nets were set per sampling site. Each net consisted of a 15m panel of 32mm mesh and a 25 m panel of 38 mm mesh followed by 50m panels of 51, 64, 76, 89, 102, 114 and 127mm meshes. Rainbow smelt, round goby and lake trout were caught in the same way from all sites.

Whole individuals of lake trout and forage fish and composite samples of zooplankton and zebra mussels (*Dreissena polymorpha*) were transported back to the laboratory at the Great Lakes Institute for Environmental Research (GLIER) on ice and stored there at -25°C. Forage fish were thawed for processing. Total length and body mass were measured. Forage fish were homogenized using a high-powered kitchen blender. 5g subsamples of homogenate were kept from each fish for Hg analysis and 1-2g subsamples of homogenate were kept for stable isotope analysis. Lake trout were homogenized using a Hobart meat grinder. 10g subsamples of homogenate were set aside from each fish for Hg analysis and 1-2g subsamples of homogenate were kept for stable isotope analysis. Lake trout otoliths were collected and analyzed according to the
protocol from the Otolith Research Laboratory (Bedford Institute of Oceanography, Dartmouth, Nova Scotia). Other methods of ageing used for lake trout in this study included fin clip data (OMNR-UGLMU 2012) and coded wire tags.

Zebra mussels were collected using bottom trawls, then put on ice and brought back to the laboratory. There they were thawed, shucked and combined to make a total of ten samples, each weighing between 1-2g. Each sample was analyzed for Hg and stable isotopes. Zooplankton samples were collected using a 10m long, 1m diameter conical plankton net with 62µm mesh size. Composites were put on ice and brought back to the lab. They were then thawed and dried for three days in a fume hood before analysis.

**Chemical Analysis**

Hg concentrations were determined using Direct Mercury Analyzer (DMA-80, SOP 1-007, Great Lakes Institute for Environmental Research) according to EPA method 7473 (USEPA 2007). Forage fish and lake trout samples were subsampled and 0.2g of homogenate was placed in routine-washed (soap and water) nickel boats and analyzed 32 at a time. The automatic steps of the DMA-80 are described in detail in Haynes et al, 2006. DMA-80 was calibrated prior to analysis using in house quality controls, including liquid control (W-CntVG, 0.1mg/L), solid wet biological tissue control (BT-Cnt2M-Spk, 0.1mg/kg) and solid dry biological tissue controls (BT-Dorm-3, 0.4mg/kg; BT-Dolt 4, 2.58mg/kg). The same quality controls were used during each run of 32 samples. Two blind burns (nickel boat alone) were conducted per 32 samples to establish the low Hg background level. All sample Hg concentrations were calculated in mg/kg wet weight. Detection limit of the DMA-80 is 0.0002mg/kg.
Stable isotope analyses were conducted in order to determine trophic level and feeding strategies of lake trout, forage fish, zooplankton and zebra mussels. For stable isotope analysis, homogenates were first freeze-dried for 24 hours (Labconco co., Kansas City, Missouri). Freeze-dried samples were then homogenized using mortar and pestle and lipid extracted using Chloroform-Methanol. After lipid extraction, 400-600ug of each sample was wrapped in a tin cup (3.0 x 5.5mm) and placed into a 96-well plate for analysis. Determination of isotope signature was conducted in the Chemical Tracers laboratory at GLIER. Samples were analyzed for stable isotope signatures using a continuous-flow isotope ratio mass spectrometer (Finnigan MAT Deltaplus; Thermo Finnigan, San Jose, California). Samples were quantified against two reference standards (Bovine NIST 8414 and tilapia internal fish standard). Isotope results were calculated using the formula

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where R is the ratio of heavy to light isotope (e.g. $\delta^{15}N/\delta^{14}N$) relative to the standard.

Carbon and Nitrogen stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$ respectively) were used to determine basin specific feeding strategies and trophic structures. Populations from the three Canadian basins of Lake Huron were compared and contrasted with one another.

BMFs were calculated in the following way:

\[
\text{BMF} = \frac{\text{Conc}_{\text{Hg, predator}}}{\text{Conc}_{\text{Hg, prey}}}
\]
To investigate growth as a driver of Hg bioaccumulation, a von Bertalanffy (VBL) growth curves were generated for each basin using the formula

\[ LT = L_{inf}(1-e^{-k(t-t_0)}) \]

where \( LT \) is total length of fish (cm), \( L_{inf} \) is the asymptotic length (cm), \( k \) is the growth rate coefficient (yr\(^{-1}\)), and \( t \) is fish age in years (Ricker 1984).

Volumetric prey densities were calculated as an estimate of prey abundance in each basin. For each basin, prey fish densities (kg/km\(^3\)) were estimated from prey abundance (kg/ha) data reported by Schaeffer et al. (2012) and available basin-specific morphometric information. North Channel and Georgian Bay basin volumes were estimated using mean depth and surface area information provided by Ridgway et al. (2006). Main Basin morphometric information, provided by the National Oceanic and Atmospheric Administrations’ Great Lakes Environmental Research Laboratory website (was used to estimate Main Basin volume.

**Data Analyses**

Data were tested for normality using the Shapiro-Wilks test. Non-normal data (i.e. Hg concentration) were ln transformed, and then ANOVAs with interaction, ANCOVAs, and linear regressions were performed on the normal data sets. Data were subsequently un-transformed and graphed.
Results

In total, 104 trout were captured in the North Channel, ranging in length from 18-68 cm and in mass from 37-3650 g. In Georgian Bay, despite a similar collection effort, only 22 lake trout were collected, ranging in length from 27-74 cm and in mass from 170.6-5750 g, and 69 trout were collected in the Main Basin, ranging in length from 26-78 cm and in mass from 160-5500 g. Lake trout ranged in age from 1-13 years old. Von Bertalanffy growth analysis revealed that growth rates in the colder North Channel trout population were slower and plateaued earlier resulting in smaller fish than those caught in the other two basins (Fig. 2). Generally, specific growth rates were very slow for fish greater than five years old in all three basins.

Mean Hg concentrations ± standard error (SE), mean $\delta^{13}$C and $\delta^{15}$N signatures ± SE, mass ranges, and numbers of lake trout caught at each site in each year are summarized in Table 1. Mean Hg concentrations in trout of all ages were significantly lower in the North Channel than the in other two basins (0.07±0.001 0.15±0.02, and 0.19±0.01 mg/kg in the North Channel, Georgian Bay, and Main Basin, respectively; ANOVA p<0.01). $\delta^{13}$C signatures were significantly more littoral in North Channel trout (-21.3±0.1%) than in Georgian Bay and Main Basin trout (-23.0±0.2% and -23.3±0.1%, respectively; ANOVA p<0.01). Finally, $\delta^{15}$N signatures were significantly higher in the Georgian Bay and Main Basin (12.7±0.2% and 12.7±0.1%, respectively), than the North Channel trout (12.3±0.1%; ANOVA p<0.01), although the difference did not result in a change in trophic level designation.
Concentrations of Hg for rainbow smelt and round goby populations are summarized in Table 2, and were significantly higher in Main Basin populations. Significant differences were observed between North Channel and Georgian Bay smelt (0.04±0.03, 0.02±0.001, and 0.05±0.001 mg/kg in North Channel, Georgian Bay, and Main Basin, respectively, ANOVA p<0.05). $\delta^{13}$C isotope signatures were significantly higher for the North Channel populations, however, no significant difference existed between Main Basin and Georgian Bay populations (-21.6±0.2%, -23.5±0.2%, -24.4±0.3% in the North Channel, Georgian Bay, and Main Basin, respectively, ANOVA p<0.05). $\delta^{15}$N isotope signatures were also significantly lower in the North Channel population (10.3±0.2%, 9.6±0.1%, 9.1±0.2%, in the North Channel, Georgian Bay, and Main Basin, respectively, ANOVA p<0.05).

As summarized in Table 2, significant differences were found among concentrations of Hg in each basin for round goby populations (0.03±0.001, 0.03±0.001, 0.02±0.001, in the North Channel, Georgian Bay, and Main Basin, respectively). $\delta^{13}$C isotope signatures for round goby populations were significantly different between the North Channel and Main Basin populations (-17.4±0.0%, -20.0±0.9%, -21.7±0.6%, in the North Channel, Georgian Bay, and Main Basin, respectively). Similarly, $\delta^{15}$N isotope signatures for round goby populations were only significantly different between the North Channel populations and the Main basin populations (9.28±0.08%, 8.31±0.30%, and 7.55±0.17%, in the North Channel, Georgian Bay, and Main Basin, respectively). Isotope signatures for zooplankton also did not differ
significantly among basins (δ¹³C: \(-27.18\pm0.5\%\), \(-27.80\pm0.7\%\), \(-27.9\pm1.4\%\) in the North Channel, Georgian Bay, and Main Basin, respectively. δ¹⁵N: 3.2\pm0.2\%, 4.1\pm0.3\%, 3.7\pm0.5\% in the North Channel, Georgian Bay, and Main Basin, respectively.). As only one composite zebra mussel sample was analyzed per basin, no statistical comparison was available. Background levels of Hg were not observed to be different according to the mussel data seen in Table 3 (0.01\pm0.001\%, 0.01 \pm0.001\%, and 0.02 \pm0.001\% in the North Channel, Georgian Bay, and Main Basin, respectively). Food web isotope data for lake trout, forage fish, zooplankton and zebra mussels of Lake Huron are summarized in Fig. 2.3.

Whole-body Hg concentrations increased exponentially with age in lake trout from all three basins of Lake Huron (ANOVA on ln normalized data, \(p<0.05\)) (Fig. 4). Hg accumulation rates are reflected in the exponential equations of the best-fit curve for each population. The equations of the curves in North Channel, Georgian Bay and Main Basin populations were \(0.044e^{0.113x}\), \(0.047e^{0.164x}\), and \(0.044e^{0.173x}\), respectively. North Channel revealed a statistically lower accumulation curve than the Georgian Bay or Main Basin populations (ANOVA with interaction on ln normalized data \(p<0.05\)).

The relationship between δ¹⁵N signatures and whole-body Hg concentrations in the three lake trout populations were significant (linear regression on lnHg, \(p<0.05\)). Rate of accumulation of Hg related to δ¹⁵N signatures, however, did not differ among basins (ANOVA with interaction, \(p>0.05\)). The equations of the curves in North Channel, Georgian Bay, and Main Basin populations were \((2.61x10^{-4})e^{0.452x}\), \((3.61x10^{-4})e^{0.476x}\), and \((1.36x10^{-4})e^{0.711x}\), respectively (Fig. 2.5).
Average BMF was significantly lower in the North Channel than in the other two basins (5.1-, 4.1-, and 1.9-fold increases for Georgian Bay, Main Basin, and North Channel, respectively. Student’s T-test p<0.05). Figure 2.6 summarizes BMF for all lake trout as a function of volumetric prey densities, an estimate of the abundance of available prey items in each basin. Low volumetric prey densities in Georgian Bay were associated with higher BMFs, and high volumetric prey densities in the North Channel and Main Basin were associated with lower BMFs.

Discussion

The growth and Hg accumulation data suggest that the lake trout from the three Canadian basins of Lake Huron are relatively distinct populations, especially the North Channel population. Basin-specific Hg accumulation as a function of age, stable isotope signatures and BMFs, supports the conclusion that trout do not frequently move from basin to basin. These independent populations, occupying three distinct ecosystems within the same lake, provide an excellent study system to test the relative importance of chemical, physiological and ecological factors regulating Hg accumulation in lake trout.

Lake trout populations in the three basins of Lake Huron revealed similar age-specific Hg accumulation patterns, and indicated that long-lived trout accumulate Hg exponentially with age, although the rates of accumulation were observed to be basin specific. There were no significant differences among the basins in zooplankton Hg concentrations or in stable isotope signatures. Although the zebra mussel data were not replicated within the basins, they do provide further support that the lower trophic levels
of the food web of each basin are similar. Combined with the literature suggesting that Hg concentrations in sediment and water of Lake Huron are relatively homogenous, the basin specific accumulation dynamics observed in this study indicate that ecological factors are relatively important in understanding Hg bioaccumulation dynamics in aquatic food webs.

Signatures of δ¹⁵N indicated that the lake trout in the three basins occupied similar trophic levels. Although significantly lower δ¹⁵N values were observed in the North Channel, the difference represents less than 10% of a trophic level. Therefore it is concluded that trophic level alone is not sufficient to explain the basin dependent Hg accumulation dynamics observed in Lake Huron. Furthermore, the relationship between Hg and δ¹⁵N is similar among all three basins. These data support the conclusion that basin-dependent processes regulate Hg bioaccumulation and biomagnification dynamics.

The three populations of lake trout, as well as smelt and gobies, in this study showed significantly different mean Hg, δ¹⁵N and δ¹³C signatures in the three basins despite similar background levels reported in the literature and confirmed by the zooplankton and zebra mussel data presented here. Sediment and water Hg values from previous studies in Lake Huron (Forsythe 2009, SOLEC 2012) suggest that background Hg contamination in the three basins cannot account for the basin specific patterns in Hg bioaccumulation in lake trout.

Previous studies have shown that differences in growth rates can have an effect on Hg bioaccumulation (Simoneau 2005, MacCrimmon 1983, Swanson 2003). If differences in growth rate among the North Channel, Georgian Bay and Main Basin lake trout populations accounted for the differences, then it would be expected that Georgian Bay
and Main Basin trout would have similar Hg bioaccumulation rates. The bioaccumulation trend according to BMF, however, is significantly different between Main Basin and Georgian Bay trout populations, providing evidence that growth rates alone cannot explain age variation in Hg bioaccumulation dynamics with age. Furthermore, Hg levels are the lowest in the North Channel, where growth dilution would have had the least effect (Simoneau 2005, MacCrimmon 1983).

Basin-to-basin differences in Hg exposure dynamics and bioaccumulation suggest that ecological processes are important in regulating Hg concentrations and levels in aquatic food webs. Declining prey fish populations have been reported in Lake Huron, especially since the introduction of Driessenid mussels and the round goby (Riley 2008). Declines in forage fish will force trout to change foraging strategies which might result in declines in foraging efficiency. Changes in feeding strategies have been observed in Lake Ontario, where lake trout were observed to be moving to shallower, more littoral zones (Rush 2012). There is little evidence of this in Lake Huron, where smelt have remained the main prey item of trout. However, OMNR-UGLMU gut content analysis has shown that since 2003, approximately 10% of lake trout diet consists of round goby, suggesting that lake trout are moving to littoral areas to find food. If lake trout were expending more energy to find food, then Hg bioaccumulation would reflect such energy acquisition strategies, as Hg uptake is primarily a function of food uptake and utilization. The relationship of low BMFs associated with higher prey density in conjunction with the lower growth rates of lake trout in the North Channel observed in this study indicates that trout in the North Channel are feeding sufficiently to maintain body mass but growth efficiency in these trout is much lower than for trout in Georgian Bay. Thus,
bioenergetics and metabolic processes such as growth efficiency must be taken into consideration in Hg bioaccumulation modelling.

The results of this study clearly point to the complex nature of Hg bioaccumulation. Bioaccumulation trends were different among basins in Lake Huron when considering year-to-year changes, trophic dynamics, and growth dynamics.
Table 2.1: Sample size, mass ranges, average Hg, $\delta^{15}$N, $\delta^{13}$C, and Hg in lake trout caught in 2011 and 2012 in North Channel, Main Basin, and Georgian Bay

<table>
<thead>
<tr>
<th>Species</th>
<th>Basin</th>
<th>n</th>
<th>mass range</th>
<th>mean $\delta^{13}$C $\pm$ SE</th>
<th>mean $\delta^{15}$N $\pm$ SE</th>
<th>mean Hg (mg/kg) $\pm$ SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvelinus namaycush</td>
<td>North Channel</td>
<td>104</td>
<td>37.5-3650.0</td>
<td>-21.48 $\pm$ 0.12</td>
<td>12.53 $\pm$ 0.04</td>
<td>0.073 $\pm$ 0.003</td>
</tr>
<tr>
<td>Salvelinus namaycush</td>
<td>Georgian Bay</td>
<td>23</td>
<td>170.6-5750.0</td>
<td>-22.79 $\pm$ 0.19</td>
<td>12.87 $\pm$ 0.13</td>
<td>0.111 $\pm$ 0.021</td>
</tr>
<tr>
<td>Salvelinus namaycush</td>
<td>Main Basin</td>
<td>75</td>
<td>160.4-6600.0</td>
<td>-23.50 $\pm$ 0.12</td>
<td>13.01 $\pm$ 0.08</td>
<td>0.157 $\pm$ 0.007</td>
</tr>
</tbody>
</table>
Table 2.2: Sample size, mass range, average Hg, $\delta^{15}$N, $\delta^{13}$C in rainbow smelt and round goby from North Channel, Main Basin, and Georgian Bay

<table>
<thead>
<tr>
<th>Basin</th>
<th>Species</th>
<th>n</th>
<th>Mass range (g)</th>
<th>mean $\delta^{13}$C ±SE</th>
<th>mean $\delta^{15}$N ±SE</th>
<th>mean Hg (mg/kg) ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Channel</td>
<td>Osmerus mordax</td>
<td>28</td>
<td>6.7-45</td>
<td>-21.64 ± 0.23</td>
<td>10.28 ± 0.15</td>
<td>0.037 ± 0.003</td>
</tr>
<tr>
<td>North Channel</td>
<td>Neogobius Melanostomus</td>
<td>2</td>
<td>18.2-21.2</td>
<td>-17.43 ± 0.03</td>
<td>9.28 ± 0.08</td>
<td>0.028 ± 0.020</td>
</tr>
<tr>
<td>Georgian Bay</td>
<td>Osmerus mordax</td>
<td>13</td>
<td>6.3-22.2</td>
<td>-20.37 ± 0.19</td>
<td>8.24 ± 0.08</td>
<td>0.032 ± 0.003</td>
</tr>
<tr>
<td>Georgian Bay</td>
<td>Neogobius Melanostomus</td>
<td>8</td>
<td>31.3-44.7</td>
<td>-23.49 ± 1.25</td>
<td>9.59 ± 0.47</td>
<td>0.026 ± 0.010</td>
</tr>
<tr>
<td>Main Basin</td>
<td>Osmerus mordax</td>
<td>16</td>
<td>8.8-48.3</td>
<td>-23.62 ± 0.26</td>
<td>8.64 ± 0.17</td>
<td>0.052 ± 0.004</td>
</tr>
<tr>
<td>Main Basin</td>
<td>Neogobius Melanostomus</td>
<td>4</td>
<td>10.3-25.1</td>
<td>-21.13 ± 0.57</td>
<td>8.15 ± 0.17</td>
<td>0.019 ± 0.010</td>
</tr>
</tbody>
</table>
Figure 2.1: Study basins in Lake Huron: North Channel, Georgian Bay, and Main Basin
Figure 2.2: Von Bertalanffy growth curves for lake trout populations in the three study basins.
Figure 2.3: Carbon vs. Nitrogen stable isotope signatures for Lake Huron lake trout, forage fish, zooplankton and zebra mussels.

Stable isotope signatures for Lake Huron lake trout, forage fish, zooplankton and zebra mussels

- North Channel Lake Trout
- Georgian Bay Lake Trout
- Main Basin Lake Trout
- North Channel Smelt
- Georgian Bay Smelt
- Main Basin Smelt
- North Channel Goby
- Georgian Bay Goby
- Main Basin Goby
- North Channel Zebra Mussels
- Georgian Bay Zebra Mussels
- Main Basin Zebra Mussels
- North Channel Zooplankton
- Georgian Bay Zooplankton
- Main Basin Zooplankton
Figure 2.4: Whole-body Hg concentration vs. age in lake trout populations in the three study basins
Figure 2.5: Whole-body Hg concentration vs. $\delta^{15}$N signature in lake trout population from the three study basins.
Figure 2.6: BMF vs. volumetric prey density in lake trout populations from the three study basins

BMF vs. Volumetric Prey Density in Lake Huron lake trout

Volumetric Prey Density (kg/km$^3$)
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USEPA. METHOD 7473, Revision 0. Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry. **2007**.


CHAPTER 3

Tissue distribution of Hg in pre-spawn, spawning, and post-spawn yellow perch (*Perca flavescens*)

**Introduction**

Many people in the Great Lakes region participate in recreational and/or subsistence fishing. The Great Lakes fishery supports approximately 30 million people (Hagreen 2004), and the most important route of Hg exposure to humans is through fish consumption, so Hg in fish tissue is a concern for humans (Harada 1995). In order to educate the public on safe fish consumption, the Ontario Ministry of the Environment publishes a fish consumption guide every two years. These fish consumption advisories take into consideration length and species of fish, and Hg concentrations are measured only in muscle tissue (Ministry of the Environment, 2013).

Previous studies have investigated Hg uptake and elimination in order to provide more insight into the bioaccumulation patterns of Hg in fish muscle (Trudel 1997, VanWallegham 2007, VanWallegham 2013). These studies have concluded that the bioaccumulation of Hg is a complex process, particularly the elimination of Hg. The elimination of the Hg is a first order process (Li et al, 2014), and exists in different states. Elimination rates are highly variable based on the form of Hg and the tissue where it is stored (Trudel 1997). Rates of Hg elimination vary according to the time of the year (VanWallegham 2007), and the elimination of Hg in larger fish eventually becomes so low that growth dilution, rather than elimination, becomes the main driver of changing Hg concentrations in muscle (VanWallegham 2013).
When Hg is eliminated from muscle tissue, it is not directly eliminated from the body and the redistribution of Hg among tissues has been observed by Oliviera Ribeiro (1999), who observed low levels of MeHg transfer in and out of the liver, presumably mobilized in the blood. The true nature of Hg redistribution among fish organs has since not been extensively studied. In our study, we test the hypothesis that tissue distribution of Hg remains constant throughout the reproductive cycle and that Hg is not redistributed among tissues and that elimination through reproduction is negligible. We tested this hypothesis by analyzing Hg in muscle, liver, gonad, and carcass tissue in male and female yellow perch collected from Lake Erie pre-spawn, during spawning, and post-spawn.

**Materials and Methods**

Yellow perch of varying sizes (9.0-29.0cm in length) were purchased from a commercial supplier (Presteve Foods, Wheatley, ON) on Lake Erie between April 2012 and April 2013. Fish were collected in March (pre-spawn), May (during spawning), and October (post-spawn). Total length, fork length, and body mass were measured for each individual. Liver, gonads and dorsal muscle were excised, weighed and collected for Hg analysis. Remaining fish carcasses were weighed and homogenized for Hg analysis. All Hg analyses were completed using a Milestone Direct Mercury Analyzer (DMA-80) as described in USEPA method 7473 (USEPA 2007). A summary of the automatic steps of the DMA-80 can be found in Haynes (2006).
Hg concentrations were compared among tissues in pre-spawn, spawning, and post-spawn male and female yellow perch. Ratios between liver, gonad, muscle or carcass Hg concentration and total Hg concentration were determined and proportions were compared between sexes and among pre-spawn, spawning, and post-spawn perch. Shapiro-Wilks test for normality of residuals and Levene’s test of homogeneity of variances were performed. ANOVAs with mass as the interacting term and ANCOVAs with mass as the covariate were performed where applicable.

Results

Significant differences in ratio of Hg concentration in muscle, gonad, liver, and carcass tissue to whole-body Hg were observed between male and female perch before and during spawning. Females were found to have significantly higher proportions of Hg in muscle tissues, and lower proportions of Hg in gonads than males prior to spawning (ANCOVA p<0.05, Figure 3.1a). During spawning, small vs. large females had greater differences in Hg concentration in all tissues than small vs. large males. Large males and large females had lower proportions in liver and carcass than small males and females, but the proportion of Hg in muscle tissue increased with mass for both sexes during spawning (ANOVA, p<0.05, Figure 1b). Small females had higher proportions of Hg in their gonads than large females, while small males, on the other hand, had lower proportions of Hg in their gonads than large ones during spawning (ANOVA p<0.05, Figure 3.1b). No significant differences in the ratio of Hg in any organ and whole-body
Hg were observed between post-spawn male and female perch (ANCOVA p>0.05, Figure 3.1c).

Female perch were found to have a significantly higher proportion of Hg in their muscle tissues pre-spawn than either spawning or post-spawn female perch (ANCOVA, p<0.05, Figure 3.2a). Pre-spawn male perch, however, showed significantly lower proportions of Hg in their muscle tissues than spawning males (ANCOVA, p<0.05, Figure 2b). Female perch were found to have significantly lower proportions of Hg in gonad tissues pre-spawn than during or after spawning (ANCOVA p<0.05), whereas male perch showed no significant difference in proportion of Hg in gonad tissues pre-, or post-spawn (ANCOVA, p= >0.05). However, male perch during spawning showed a significantly different relationship between proportion of Hg in gonads and mass than pre- or post-spawn males: larger males were shown to have a higher proportion of Hg in their gonads than smaller males during spawning (ANOVA, p<0.05, Figure 3.2b). Spawning female perch had a significantly different relationship between proportion of Hg in carcass and mass than pre- or post-spawn females, with smaller females having higher proportions of Hg in carcass tissue than larger females (ANOVA, p<0.05, Figure 3.2c).

No significant differences in liver or muscle mass were seen among pre-, during, and post-spawn perch. Gonad mass, however, was significantly larger during spawning in both male and female perch. MANCOVA analysis was performed to assess the interactions of organ mass and proportion of Hg in the different tissues. Although organ mass was significantly larger, and Hg concentration was significantly lower in the gonads
of spawning perch, there were no significant three-way interactions among gonad mass, gonad Hg, and any other variable (time of year, sex, or fish total mass; p>0.05).

Discussion

Analysis of the proportion of Hg in different organs at different times during a reproductive cycle in both male and female perch revealed the redistribution of Hg among tissues. These observations are similar to conclusions of Dutton and Fisher (2013) that Hg is redistributed among fish tissues after it is taken up into the body. Redistribution of Hg in fish has been shown in previous studies to take place when Hg forms metal-protein complexes and moves through the bloodstream (Branco 2012).

The higher proportion of Hg in the muscle tissue and lower proportion of Hg in the gonad tissue of females before spawning suggests that Hg has mobilized out of the gonads and into the muscles in females. Although the lower Hg concentrations in gonad tissue seen during spawning can be attributed to gonad growth, statistical analysis showed that three-way interactions among gonad size, Hg, and time of year were not significant, so the increase in gonad mass cannot fully explain this phenomenon. This observation is further supported by the decrease in muscle Hg during spawning despite no change in muscle mass. Further research is required to determine the mechanism for the mobilization of Hg before spawning, although it is presumed that Hg mobilization takes place in the bloodstream as a metal-protein complex (Khan, 2009; Mottet, 1997).

This particular redistribution of Hg was only apparent in females. In fact, Hg was distributed from muscle tissue in pre-spawn males. Large males were shown to have significantly higher proportions of Hg in their gonad tissue than small males during
spawning, which suggests that with increasing sexual maturity, more Hg is distributed into the gonads during spawning. This effect is magnified when large increases in gonad mass during spawning are taken into consideration, since the increase in gonad mass during spawning are expected to dilute Hg.

Maternal transfer of Hg has been investigated previously. Niimi et al (1983) concluded that only small concentrations of Hg were transferred into fish eggs. Johnson et al (1990) came to similar conclusions. A more recent study found that maternal transfer of Hg into eggs was 4-6 times higher than previously reported values in fish with high body burdens of Hg. Fish with low body burdens of Hg, however, showed lower Hg concentrations in eggs (Sackett et al 2013). The results of this study conclude that although the eggs do contain Hg during spawning, Hg proportion is actually lower than in the gonads during the rest of the year. Although maternal transfer of Hg is occurring, the transfer of Hg from the gonads and into the muscle is also occurring at the same time.

This research reveals that mercury is mobilized throughout the year in fish and moves among tissues in the body. The changes seen in Hg tissue distribution before, during, and after spawning are likely associated with the physiological and dietary changes that occur during these times within the fish life cycle. During reproduction, this mobilization could be an explanation for highly variable elimination rates of Hg observed in fish. Mobilization of Hg depends on sex and time of year, thus both tissue concentrations and elimination rates are highly variable. To our knowledge, no studies have documented sexual differences in tissue distribution during spawning. A few studies, however, have demonstrated sexual differences in Hg concentration in muscle tissue, concluding that males have higher Hg concentrations than females (Montiero and
Lopes 1990, Walker 1972), whereas some have found females to have higher concentrations of Hg in muscle tissue than males (Nicoletto and Hendricks1988, Renzoni 1981). The variability observed in these studies are possibly explained by tissue redistribution of Hg as observed in our study.

Difference in gross growth efficiency is a plausible explanation for sexual differences in Hg, but the results of this study suggest that spawning is a major contributor as well. Hg is redistributed differently among organs in larger, more sexually mature, fish than in smaller, less sexually mature fish. All significant differences in the relationship between proportion of Hg in organs and mass mainly occurred during spawning in both males and females. Therefore, sexual maturity plays a role in the tissue redistribution of Hg.

The findings of this study have major implications for fish consumption advisories based on Hg concentration in muscle. Fish consumption advisories are based on length and species of fish, and not on reproductive status (Ministry of the Environment, 2013). While muscle Hg levels are of main concern to the general human population, some human subpopulations, such as Inuit populations, consume more than just muscle tissue (Johansen 2004). The redistribution of Hg among fish organs must be considered for these populations. Due to the significant differences between males and females in proportion of Hg in different organs, fish sex should also be considered for the setting of consumption advisories. The differences between males and females were especially marked during spawning, which means that time of sampling has to be considered when using consumption guidelines to protect human health.
The results of this study conclude that muscle concentration in fish is highly variable during different times of the year. This conclusion points to the potential for inaccuracy of long-term monitoring data based solely on Hg concentration in fish muscle. Fish collections for long-term studies do not always take place at the same time of year, and fish collected at different times of year (Gandhi 2014). When this is the case, muscle Hg concentrations are not necessarily representative of whole-body Hg concentrations due to tissue redistribution at different times of the year. Long-term monitoring data should be based on whole-body concentrations of Hg rather than on muscle concentrations so that fish from each year can be accurately compared with one another.
Figure 3.1a, b and c: Relationship between the ratio of Hg in the different organs (liver, muscle, gonads, carcass) in males and females for the whole body Hg content of yellow perch in the April (pre-spawn (a)), May (during spawning (b)), and October (post-spawn (c)). The (*) indicate significant differences between slopes (analysis of variance with interaction, p<0.05), and the (ǂ) indicate significant differences in ratios (ANCOVA, p<0.05).
Figure 3.2a, b and c: Relationship between the ratio of Hg in the different organs (liver, muscle, gonads, carcass) and the whole body Hg content of yellow perch in April (pre-spawn), May (during spawning), and October (post-spawn) for females (a) and males (b). The (*) indicate significant differences between slopes (analysis of variance with interaction, $p<0.05$), and the (ǂ) indicate significant differences in ratios (ANCOVA, $p<0.05$).
REFERENCES


CHAPTER 4

General Discussion

This thesis examines factors regulating Hg accumulation in feral fish populations of the Great Lakes. Factors regulating both uptake and elimination of Hg were examined. Hg is bioaccumulated exponentially in trout populations across Lake Huron and Hg accumulation dynamics are different among populations despite background Hg concentrations being homogenous across all basins. Bioaccumulation trends, growth curves, and BMFs indicated that bioaccumulation of Hg cannot be explained only by tropho-dynamics and growth dilution. The relationship between high BMFs and low prey density as well as growth trends in the three study populations of lake trout suggest that growth efficiency and foraging efficiency also play key roles in the bioaccumulation of Hg.

The complex nature of Hg bioaccumulation is further supported by the analysis of the proportion of Hg in different organs at different times during a reproductive cycle in both male and female perch. This study provided insight into Hg elimination during reproduction, which is often considered in Hg bioaccumulation models to be negligible (Trudel and Rasmussen 2006). Hg was shown to be redistributed among tissues before, during, and after spawning. The higher proportion of Hg in the muscle tissue and lower proportion of Hg in the gonad tissue of females before spawning suggests that in preparation for reproduction, Hg is distributed out of the gonads and into the muscles in female perch, whereas Hg is distributed away from muscle tissue in pre-spawning males.

The results of this thesis warrant the recommendation that caution be taken when assessing muscle Hg with respect to risk modeling. A wide variety of size or age classes
should be collected when studying Hg contamination in muscle tissue. Although Hg models can predict Hg concentrations in large fish based on Hg concentrations in smaller fish at the whole-fish level, Hg concentrations in large fish are highly variable, so it is far more accurate to use whole fish homogenates. Males and females should also be assessed separately. Although differences were not staggering, the results of this thesis suggest that sex and reproductive status can contribute to the regulation Hg concentrations in muscle tissues, and should be considered in Hg bioaccumulation models.

The high variability in Hg concentrations, accumulation rates, and tissue distribution observed in these studies calls for further research in a number of areas including effects of environmental changes on Hg bioaccumulation, effects of bioenergetic processes on Hg bioaccumulation, and elimination of Hg during reproduction. Further research in all of these fields will be instrumental in the development of accurate predictive models of Hg exposure.

This thesis clearly observed the complex nature of Hg bioaccumulation in predatory fish. Non-steady state dynamics must be taken into consideration when developing predictive Hg bioaccumulation models and when conducting risk assessments dealing with Hg toxicity in fish. Sampling design must be carefully planned considering reproduction of fish when investigating Hg bioaccumulation. Date of collection and type of sample are important to consider in addition to size and species of fish. This thesis provides strong evidence for non-steady state Hg accumulation, and highlights the importance of further study on Hg bioaccumulation dynamics and the bioenergetics and reproductive processes that regulate Hg bioaccumulation in fish.
REFERENCES


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