MERCURY BIOMAGNIFICATION IN THE UPPER SOUTH SASKATCHEWAN RIVER BASIN

by

LARS BRINKMANN
Bachelor of Science, University of Lethbridge, 2004

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfilment of the
Requirements for the Degree

MASTER OF SCIENCE

Biological Sciences
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

Abstract

In this thesis mercury concentrations in biota of the upper South Saskatchewan River Basin are assessed in three non-industrialized systems. Mercury levels in large sport fish (northern pike, walleye, lake trout) frequently exceeded the consumption limit of 0.5ppm. Goldeye and mooneye of the Oldman River and lake whitefish of Waterton Lakes were below 0.5ppm total mercury. Agricultural and urban effluents constituted no sources of significant mercury loadings to the Oldman River. A doubling of mercury biomagnification factors between longnose dace and their food suggests bioenergetic heterogeneity of these fish along the river gradient. Basin-specific mercury levels were detected for the upper and middle basins in Waterton Lakes, and are associated with food web characteristics, and fish bioenergetics. High mercury levels in a new reservoir were in part attributed to increased loadings from flooded soils, as is commonly observed, but also to bioenergetic constraints and growth inefficiency as a result of non-piscivory of this population.

Acknowledgements

First of all I would like to thank my supervisor Joe Rasmussen for his countless suggestions on this project, support, and financial generosity.

Thank you to Alberta Conservation Association for the Grant Eligible Conservation Fund, which also contributed significant funding to this project. A great deal of thanks goes to Alberta Ingenuity Centre for Water Research for the grant to Joe Rasmussen, out of which much of this project was funded. Many thanks to NSERC for the operating grant to Joe Rasmussen.

Thank you to the other committee members Alice Hontela, Cam Goater, Peter Dibble for their time during meetings and suggestions on my work.

Thank you to my assistants during field and lab work, especially Will Warnock, April Matisz and Jillian Barnes for their help with sample preparations and sampling efforts.

Thank you to Rob Watt at Waterton Lakes National Park for his assistance with sampling a deep cold lake in nasty weather.

Last but not least I would like to thank my parents for their financial and emotional support and for letting me use their tools and floor space during countless times of mechanical distress.

Thank you again to Alice and Joe for putting up with me a number of nights, and for great meals and parties. It was fun.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Definition
Hg	Mercury
MeHg	Methylmercury
MMHg	Monomethylmercury
THg	Total mercury
NRC	National Research Council
ANCOVA	Analysis of Covariance
Hwy	Highway
SSR	South Saskatchewan River
SSRB	South Saskatchewan River Basin
DOC	Dissolved organic carbon
CVAAS	Cold-vapor atomic absorption spectrometry
FIAS	Flow-injection analysis system
WWTP	Waste-water treatment plant

Chapter I

Introduction

The incidence at Minamata, Japan in 1956 (see Kurland et al. 1960) has resulted in broad public awareness of methyl mercury in food obtained from aquatic environments (Gochfeld 2003). The fact that methyl mercury biomagnifies through the food chain as much as 6 orders of magnitude has resulted in strict legislation regarding the release of mercury into the environment as well as the monitoring of food items from systems known to be affected (Hagreen and Lourie 2004). Mercury is a persistent contaminant in the environment and spills that happened decades ago still cause severe problems in the affected ecosystems (Gochfeld 2003, also see Parks et al. 1991, Parks & Hamilton 1987, Jackson et al. 1982), hence most research has focused on systems where distinct industrial point sources are known. The Wabigoon English River system, which received an estimated 10 tonnes of elemental mercury from a chlor-alkali plant near Dryden, Ontario between 1962 and 1969, is probably the most studied contaminated system in North America, with research and monitoring continuing to the present day (Kinghorn et al. 2007). Nonetheless examples of mercury contamination do not only pertain to industrial point source loadings.

1.1 The global mercury cycle

Elemental mercury is volatile, its vapour pressure is 0.002mm Hg at 25°C. This feature makes mercury a global problem. It can readily disperse through the atmosphere to even the remotest regions, thousands of kilometers away from the source (Lindqvist et al. 1991). The global pre-industrial background level of mercury in fish was established to be around 0.15mg/kg. The current range of background levels is 0.2-1.0mg/kg (Downs et al. 1998). Background concentrations in water currently range from 1-20ng/L (Morel et al. 1998). Overall it has been estimated that the atmospheric burden of mercury in the atmosphere has tripled due to industrial emissions (Mason et al. 1994) and possibly other anthropogenic activities since the mid to late 19th century. This estimate has been corroborated by the examination of lake sediment cores, in which the distribution of mercury suggests drastic increases of atmospheric mercury loading since the mid 19th century (Swain et al. 1992). Seventy-eighty percent of the current atmospheric mercury budget is believed to be associated with anthropogenic activity (Downs et al. 1998). Sources of mercury to the atmosphere are various industries and contaminated terrestrial and aquatic sites, but primarily combustion of fossil fuels such as coal and crude oil (Hagreen and Lourie 2004). Other sources mentioned elsewhere include incineration of plant matter such as wood and peat (Lindqvist et al. 1991). The incineration of sewage sludge and other waste handling and treatment have also been identified as point sources for atmospheric mercury (Glass et al.1990, Hagreen and Lourie 2004, Lindqvist et al. 1991). Other significant sources that are more recently receiving increased attention include geological disturbances (plate tectonics, volcanoes) (Gustin et al. 1996, Gustin et al. 1997 Rasmussen 1994) and forest fires (Lindqvist et al. 1991, Rasmussen et al. 1994, Kelly et al. 2006, Sigler et al. 2003, Friedli et al. 2003, Turetsky et al. 2006, Biswas et al. 2007).

Chemical cycling of mercury in the atmosphere has been reviewed numerous times (e.g. Morel et al. 1998, Boudou & Ribeyre 1997). Elemental mercury in the air becomes eventually oxidized to mercuric ion, although this process is slow. The residence time of mercury in the atmosphere is about 1 year (Morel et al. 1998). Hg(II) readily complexes with particles like dust and water droplets. Deposition of mercury from the atmosphere occurs chiefly by wet precipitation; on land, where aerosols are abundant, dry precipitation is also significant (Morel et al. 1998). Hg(II) falling out over the ocean undergoes and number of chemical and biological transformations, is reduced to the elemental state and released into the atmosphere. Deposition in ocean sediments is minor (Morel et al. 1998). Acidification of precipitation amplifies the fallout of mercury from the atmosphere (Downs et al. 1998, Lindqvist et al. 1991). This can exacerbate contamination of remote systems. Examples mentioned in the literature include many lakes in Sweden (Lindqvist et al. 1991) and Norway (Fjeld and Rognerud 1993), and the Arctic Ocean (Wagemann et al. 1998). Inorganic mercury is converted to methylmercury by sulfate reducing bacteria (Compeau & Bartha 1985), a process that chiefly takes place in anoxic sediments. Since methylated mercury is the biomagnified species, watersheds that are dominated by wetlands (sites of standing, anoxic water to favour methylation) along their course have the potential for high levels of mercury in their fish populations. More recently Cleckner et al. (1999) describe mercury methylation in periphyton communities that support an active microbial sulfur cycle. This process could be of significance in shallow, well-oxygenated rivers in the upper SSRB.

1.2 Factors affecting mercury levels in fish

Mercury levels observed across a broad range of remote ecosystems are highly variable. This variability has been attributed to a number of physicochemical and biological factors. Acidity (pH) and dissolved organic carbon concentration ([DOC], water color) are arguably two of the most important factors identified in the literature. In most cases a negative correlation exist between pH and mercury levels in biota (e.g. Greenfield et al. 2001, Fjeld & Rognerud 1993), mainly because lower pH results in less inorganic mercury bound to ligands, hence increasing its availability for methylation (Scheuhammer & Graham 1999, Mason et al. 2000, Bloom et al. 1991). [DOC] has been implicated as a major positive correlate; it has been suggested that it may be indicative as the major transfer mechanism between the soils of the watershed and the water body (Kolka et al. 1999, Mierle & Ingram 1991). Mercury species tend to bind to DOC and are consequently flushed out with runoff, thus increasing the loading to the aquatic system (see Rennie et al. 2005, Westcott & Kalff 1996, Gorski et al. 2003). In the same respect a positive correlation often exists between mercury in biota and the drainage area of the water body (reviewed by Schindler et al. 1995 among others), but specific features such as amount and type of wetlands, which are significant methylation sites, also affect mercury loadings from a watershed and levels in biota (St. Louis et al. 1996, Schindler et al. 1995, Greenfield et al. 2001).

It has been shown that mercury levels in fish negatively correlate with nutrient concentrations in the water (Essington & Houser 2003). High nutrient loadings obviously result in increased productivity of a given system. This has a depressing effect on mercury levels in biota through two dominant mechanisms: Algal bloom dilution is the consequence of a given background mercury concentration in water being distributed among a greater amount of biomass, thus reducing per-biomass mercury levels (Chen et al. 2005). This immediately has the consequence that primary and successive consumers experience low loadings in their respective food source (Chen et al. 2005). Furthermore high availability of food in enriched systems can result in bioenergetic growth dilution in fish. Fish in high-productivity systems typically experience high food densities and thus can allocate a significant energy surplus into growth (rather than activity), thus are diluting the assimilated mercury into a greater amount of new body tissue.

The cumulative effect of all factors combined determines final mercury levels in biota. Thus individual disturbances of the same nature may produce variable results in different systems. For example, enrichment and associated alterations of food webs following a forest fire resulted in elevated mercury levels in fish in at least one case (Kelly et al. 2006), however, other authors have either shown that forest fires result in temporary decreases of mercury levels in biota as a consequence of enrichment-associated growth dilution at the base of the food web (Allen et al. 2003), or detected no difference between burned and undisturbed systems (Garcia & Carignan 2000, 1999). Altered runoff regimes as a result of removal of the forest canopy by fire seemed to have only short-term effects on chemical exports from the watershed into lakes in boreal and Canadian Shield forests (Schindler et al. 1996), but increases in water temperatures and increased sulfate reduction were detected, which may increase bioavailability of methylmercury.

Alterations of runoff regimes seem to play an important role in elevating mercury levels in biota after man-made disturbances such as logging (Lamontagne et al, 2000), and the evidence suggests that logging has greater effects on mercury loadings within the immediate watersheds than wildfires (Garcia & Carignan 2000, 1999).

1.3 The potential for mercury contamination in the upper South Saskatchewan River Basin

The upper South Saskatchewan River Basin is shown in Figure 1.1. Located in a semi-arid region, the watershed of the Oldman River is devoid of heavy forestation or wet lands, and the classical industries associated with contamination (smelters, pulp mills) are absent as well. Land use in the upper SSRB is dominated by agriculture, a significant proportion of which is irrigation-based. Newly constructed reservoirs and the resulting flooding of soil have been identified as significant sources of mercury in river systems (Tremblay et al. 1998, Hall et al. 1998, Schetagne et al. 2000). Irrigation of soils and drainage of excess water may be a significant source of mercury to the river.

Mercury is an abundant element in the earth’s crust and a trace in any rock and soil. It is therefore conceivable that any disturbance of the geosphere or lithosphere will result in the release of mercury to the atmosphere and deposition on the surrounding landscape. Exposure of the lower strata to the elements will result in weathering and erosion, releasing trace elements into the biosphere.

Examples of aforementioned disturbances pertinent to Alberta are:

- Tar sands + refining
- Coal bed methane
- Oil drilling + refining
- Gas wells + refining
- Coal mining/strip mining
- Smelting of ores in BC

- Land use: from natural to cultural
- Agriculture: tillage, irrigation
- Flooding (reservoirs)
- Wildfires along the West coast
- Logging

These potential sources in connection with the notion that methylation can occur in periphyton communities lead to the realization that this seemingly pristine river has the potential for contamination. In addition, several communities discharge their treated or untreated sanitary and storm sewage into the river, and sewage has been shown to be a source of mercury contamination in water bodies before.

1.4 Mercury in sewage

Data from independent sources on sewage derived mercury is very limited. Glass et al. (1990) identified sewage sludge incineration as the main source for increased mercury levels at their study sites. Bodaly et al. (1998) reported that there was great variation of mercury contents in raw sewage at their sampling locations (Red and Assiniboine Rivers; Winnipeg sewage). They also state that sewage treatment removed about 88% of mercury from the raw sewage, resulting in a less variable and low concentration of mercury in the effluent. During the treatment process mercury likely adsorbs to suspended organic matter and precipitates in the sludge. These authors also mention the concept of combined and separate storm and sanitary sewers and the potential for combined sewer overflows during storm events. During storm events the throughput capacity of sewage plants can easily be exceeded and untreated sewage will be discharged into the river. Bodaly et al. (1998) mention a high concentration of mercury in sewage sludge of the sewage plants under investigation. The authors also speculate that sewage lagoons may be sites of significant mercury methylation when they become anoxic. Although the authors do not present any data specifically on this, they argue that methylation in lagoons in combination with combined sewer overflows may result in significantly increased mercury and methylmercury loads in the river.

The waste water treatment facility in Lethbridge, AB features a tertiary treatment system with sludge-settling lagoons. Since the system is relatively new, sanitary and storm sewers are not combined. Storm events do not result in dramatically increased flows of effluent, and the concentration of suspended particles in the effluent is constant throughout the year (WWTP Lethbridge, personal communication). Mercury in the effluent is measured once a year and has been just above the detection limit.
Storm sewers spill into the river untreated. Whether this system is conducive to lowering mercury loads in rivers has not attracted much attention to this point. Runoff from city streets and roof tops could potentially carry an elevated mercury load, due to adsorption of mercury to dust particles. Soot particles accumulate during dry periods and get washed into the sewers during storm events. In southern Alberta, where storm events are variable (short and strong, with long intermittent dry periods), mercury loads in urban storm runoff have the potential to spike significantly during storm events.

Figure 1.1: The upper South Saskatchewan River Basin. Systems relevant to this thesis include the Oldman River, Little Bow River, Twin Valley Reservoir, and Waterton Lakes National Park.

1.5 Research presented in this thesis

The general scope of this thesis is to gather knowledge about mercury contamination in biota of the upper SSRB. It deals with three different systems:

Chapter 3 deals with the Oldman River. Sport fish of this river are analyzed for mercury to find out whether there is a mercury problem in this system. A mercury problem is identified when levels in fish muscle tissue exceed the Health Canada consumption limit of 0.5ppm. Furthermore the question is answered whether there is a gradient of increasing mercury levels found along the course of the river as evinced by a sentinel fish species (longnose dace) and invertebrates (hydropsychid caddisflies) collected at sites along the river gradient. This chapter also deals with agricultural and urban effluents as potential sources of mercury to the river. This is accomplished by comparing mercury levels in fish from irrigation drainage canals and urban sewage outfalls with reference sites along the river gradient.

Chapter 4 deals with mercury levels in biota of newly constructed Twin Valley Reservoir. The Little Bow River feeding the reservoir is an augmented river system. The question is asked whether mercury levels in sport fish from the reservoir are elevated as is observed in numerous other reservoirs of the northern hemisphere. An answer is found to the question whether the flow-augmented Little Bow River contributes to elevated mercury levels in fish, by comparison with fish from the Oldman River.

Chapter 5 deals with mercury levels in Waterton Lakes National Park, which features nearly undisturbed watersheds. The question is answered whether mercury levels in fish from the upper and middle lake exceed 0.5ppm. Moreover, the results are compared with published literature data to determine where the system may fit in terms of food web complexity and mercury levels.

Chapter II

Measurement of total mercury (THg) concentrations in animal tissues using cold-vapour atomic absorption spectrometry (CVAAS).

2.1 Introduction

The threat to human health associated with methylmercury in fish has lead to the development of sensitive analytical techniques capable of detecting trace levels of mercury (organic or inorganic) in biological tissues or the surrounding environments (e.g. Perna et al. 2005, Sanz et al. 2003, Cizdziel et al. 2002, Gelaude et al. 2002, Chou and Naleway 1984, El-Awady et al. 1976, Hawley and Ingle, 1975). Techniques in current use include derivatives of inductively-coupled-plasma mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS) or atomic absorption spectrometry (AAS). While the first two analysis methods feature detection limits suitable for ultra-trace level determination of Hg (on the order of 0.01 pg/g), the relative cost of equipment and/or ongoing operating and maintenance costs (especially with ICP-MS) render these techniques uneconomical to most research labs involved in fish research. While CVAAS technology is not suitable for ultra-trace determinations, with detection limits commonly around 0.05ppb (for conventional CVAAS, specialized techniques can achieve 0.5 to 0.9ppt (Cizdziel et al. 2002)) it is perfectly capable of delivering accurate and reproducible results at Hg concentrations commonly observed in biota (ppb-ppm levels). Reasonable operating and purchasing costs coupled with relatively simple procedures make this technology accessible to many research labs.

Objectives

This chapter summarizes the technique used to analyze total Hg in fresh fish tissue throughout this thesis. It is intended as instructional material to familiarize future employees/graduate students with CVAAS technology available in our lab and aid in the understanding of procedures and processes.

2.2 Mercury analysis with CVAAS

2.2.1 The functional principle of CVAAS

As the name implies, atomic absorption spectrometry utilizes the property of gaseous elements to absorb light at specific wave lengths. Elemental mercury absorbs light at 253.65nm. In cold-vapour setups specifically, a beam of lights passes through the quartz windows of a cell through which the sample of mercury vapour is driven, followed by detection of the exiting light intensity (Figure 2.1). The exiting light intensity depends on the concentration of mercury vapour within the sample cell. Comparison of absorbances of known concentrations of mercury standards are used to make inferences about the concentration of mercury in unknown samples.

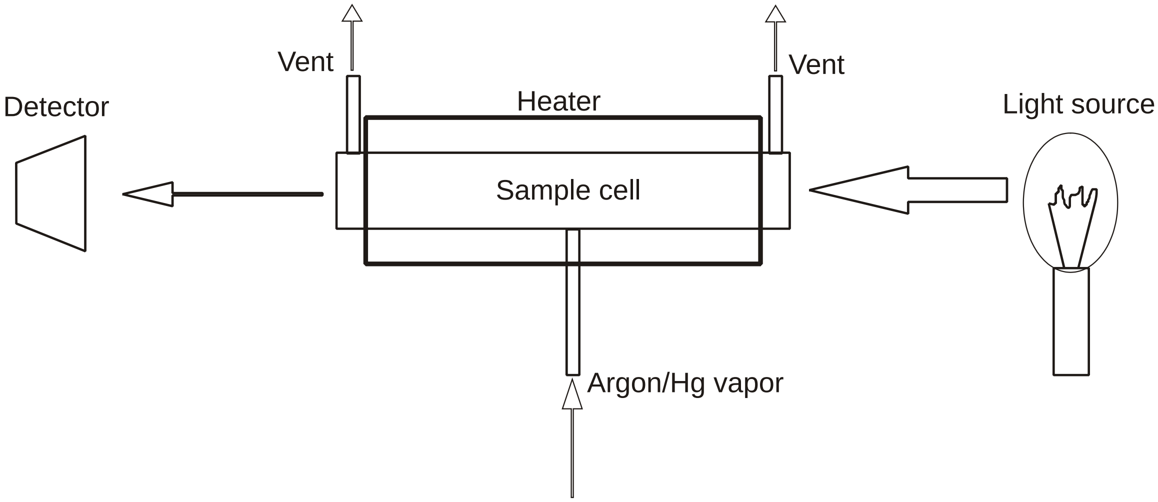

Figure 2.1: Schematic outlining the functional principle of CVAAS.

2.2.2 Mercury vapour generation and throughput automation using a flow injection analysis system (FIAS).

In biological tissues mercury exists as mercuric ion (Hg^{2+}) and organic methylmercury (CH_3Hg^+, $MeHg^+$)), which is tightly bound to protein. Mercury in a solution is detected with CVAAS as elemental, volatile mercury (Hg^0). For any accurate measurement with CVAAS mercury must exist in solution as mercuric ion, since it is stable in acidic medium, and will not volatilize prematurely.

A known amount (e.g. 500µl) of Hg^{2+}-containing solution is mixed in the chemifold of a FIAS with stannous chloride ($SnCl_2$) solution, which reduces the mercuric ion to volatile elemental mercury. The elemental mercury is stripped from the liquid in a gas-liquid separator by inert argon gas, which carries vapourized mercury through a moisture filter to the sample cell for detection. The sample cell itself is heated (100 deg.C) to prevent condensation of residual moisture. Finally the Hg vapour/argon mixture exits the sample cell through vents at both ends. The Perkin Elmer FIAS 400 coupled with the AS91 autosampler automates sample delivery and mixing of chemicals. It consists of two peristaltic pumps (each with several channels), a switching valve with sample loop (500µl), chemifold, gas-liquid separator, and argon gas flow regulator (see schematic in Figure 2.2). Pump 1 has the sole function to draw sample solution from test tubes in the autosampler tray and deliver it to the 500µl sample loop. Pump 2 constantly mixes carrier acid and stannous chloride solution in the chemifold, and withdraws waste from the bottom of the gas-liquid separator. When the sample loop is filled the valve switches it into the path of the carrier acid, which then pushes the plug of sample into the chemifold, where Hg^{2+} is reduced by stannous chloride and volatilized. A constant stream of argon gas then delivers the vapour to the sample cell.

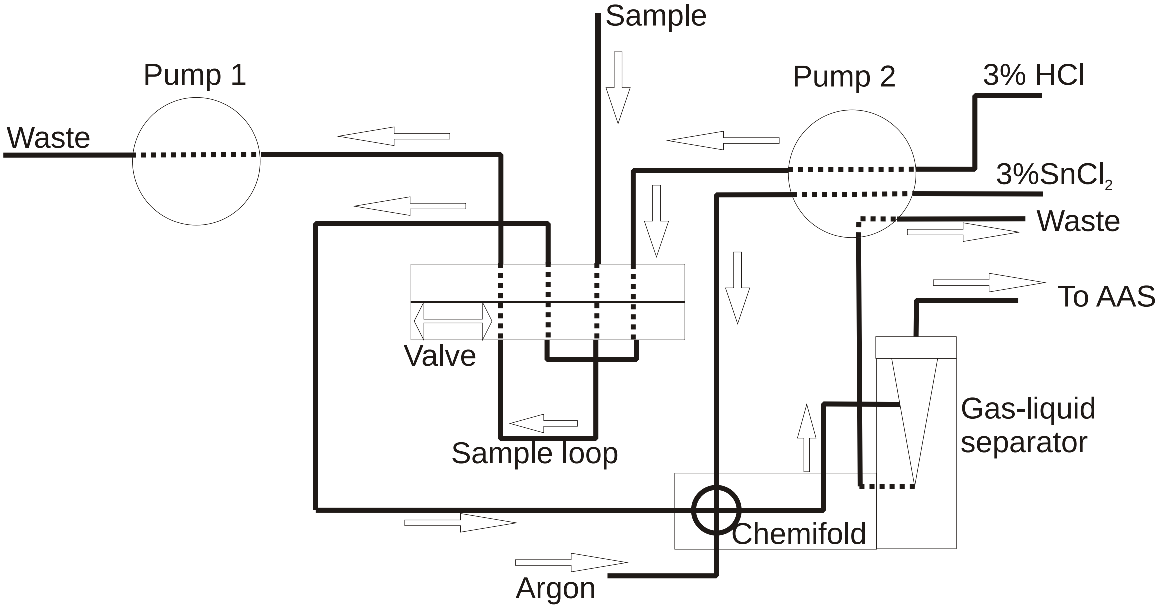

Figure 2.2: Schematic of the FIAS 400 showing key components and direction of flow. At the depicted stage Pump 1 fills the sample loop. Then the valve block will shift to the right (indicated by two-headed arrow) aligning the sample loop with the stream of HCl carrier and sending the sample to the chemifold.

2.2.3 Analysis reagents

The carrier acid used is 3% (v/v in nanopure water) HCl as recommended by PerkinElmer, but 3% (w/v in 3% HCl) $SnCl_2$ instead of the recommended 1.1%. Using 1.1% resulted in poor estimates of mercury in DORM-2 certified reference material. This can probably be explained by the fact that the acid concentration in the samples was much higher than recommended, thus resulting in premature oxidation and consumption of $SnCl_2$ and incomplete reduction of Hg^{2+}. Therefore it was necessary to increase the concentration of $SnCl_2$.

BrCl solution (5% in solution) was prepared in-house. The synthesis protocol was derived from EPA method 1631 (U.S.EPA 2002). This synthesis is carried out in a fume hood:

Potassium bromide (2.7g) was dissolved in 250mL conc. HCl in an Erlenmeyer flask by stirring over the period of one hour. Potassium bromate (3.8g) was then added slowly. Violent fizzing ensued immediately, the solution changed color from orange to red to yellow within seconds. The solution was stirred for another 20min and allowed to cool.

2.2.4 Preparation of biological tissue samples

From a variety of sample preparation protocols available in the literature (see Trudel and Rasmussen 2001, Tao et al. 1998, Clesceri et al. 1998) a protocol derived from Jewett et al. (2003) and Scott Willie (NRC Institute for National Measurement Standards, personal communication; see also U.S.EPA 2002, Method 1631) produced the best results. In principle the objective of this method is to oxidize all organic matter in strong acid with assistance of bromine monochloride (BrCl, a strong oxidizing agent) and convert all organic mercury to mercuric ion. Alterations were made to steps associated with BrCl addition (mainly due to the unpredictable reactivity of this chemical) and dilution prior to analysis. Reagents suitable for trace metal analysis were used exclusively. Each tissue sample (~1g wet weight fish muscle or homogenous whole body tissue, or ~1g pooled invertebrate bodies) was digested with 10ml of 7:3 mix nitric and sulfuric acid (*aqua regia*), aided by 1.0ml of 5% BrCl solution (in conc. HCl) at 90 degrees C on a 36-place aluminum block dry bath. Prior to addition of BrCl the samples incubated under *aqua regia* at room temperature for one hour. Premature addition of BrCl can lead to violent fizzing and consequential loss of sample and damage to equipment. BrCl addition after digestion produced poor results due to unpredictable fizzing within the tubing of the FIAS, moreover, addition of BrCl before digestion greatly improved methylmercury conversion.

Samples were placed on the dry bath overnight for 12-14hrs. After this period the samples were allowed to cool and subsequently received 0.2ml of 5% (w/v in 3% HCl) potassium dichromate solution. Potassium dichromate is supposed to aid in the retention of mercuric ion in solution, rather than forming complexes with domains on the glass walls of the test tube (Pamela Wee, PerkinElmer, personal communication). Finally the samples were diluted to 30ml (graduation mark) with nanopure water and capped with Teflon-lined caps until analyzed. Hot samples were generally clear, but occasionally developed precipitates upon cooling, depending on fish species digested. Longnose dace frequently produced lipid droplets that would not get digested, whereas goldeye and mooneye muscle occasionally produced a white, flaky substance of unknown composition. These impurities probably have no effect on mercury readings, since multiple trials of extraction with fresh acid produced negative results (data not shown). However, it is imperative that large solidified impurities be removed, since they could potentially obstruct the autosampler probe or the orifice in the chemifold, resulting in inconvenient downtime. Fatty impurities can be easily removed from the digestion test tube or the sample tube (prior to placing it on the autosampler) with a clean Teflon-coated micro-spatula.

2.2.5 Standards and quality control

Mercury standards were prepared in *aqua regia*, stabilized with potassium dichromate solution, and diluted with water in a similar manner as the samples, i.e. the acid to water ratio was 1/3 acid to 2/3 water (which approximates the mixture of the diluted samples). The final standard concentrations were blank, 1.00ppb, 5.00ppb, 10.0ppb, 50.0ppb. A new calibration was run for each sample run of 33 samples (+3 quality control samples). Calibration curves were highly accurate, with R^2 values greater than 0.99992 in all cases. Best achievements were R^2 values of 0.999998. Furthermore frequent comparison of new versus old calibration curves showed that standards stabilized with potassium dichromate and stored in borosilicate glass bottles with Teflon-lined caps had long shelf-lives. "Drift" of calibration equations was not observed.

Quality was assured by analysis of interspersed quality control samples (certified reference material DORM-2 by NRC Canada). Approximately 0.1g of reference material was digested the same way as unknown samples. Initially only three samples of DORM-2 were prepared for each sample run. Later the technique was refined by including spiked samples (spiked duplicates). For this quality control technique two DORM-2 samples (duplicates) were prepared, one of which received 90µl 1.00ppm mercury stock solution prior to digestion; the same procedure was done on one of the unknown samples. The rationale is that the unspiked sample serves as a reference with which the spiked (i.e. with additional mercury) sample can be compared. This is a way of identifying losses during the digestion in addition to reproducing referenced DORM-2 values. With this analysis protocol DORM-2 tissue concentrations of 4.50ppm (mean, S.D. ±0.48ppm, n=96; Figure 2.3) were measured, which constitutes 97% of the referenced THg concentration. This is a slight, yet statistically significant (one-tailed t-test, t= 2.367, p=0.009) underestimation. Spike recoveries (n=10) averaged around 99%.

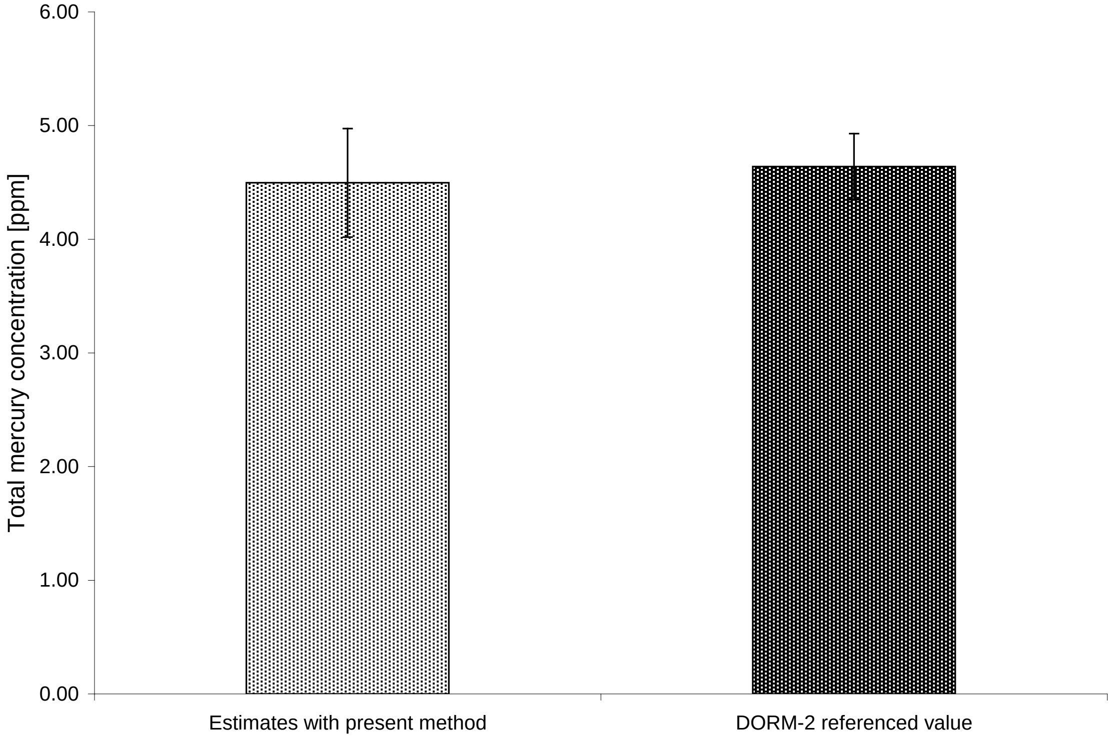

Figure 2.3: Comparison of THg concentrations in DORM-2 certified reference material determined using the method described herein.

2.3 Conclusion

The method for determination of total mercury in biological samples described in this chapter is simple and economical. A single worker can prepare many samples in one day and analyze them after digestion the next morning. The length of time required for digestion is a limiting factor to sample throughput but can be overcome by employing larger or multiple dry bath digesters. Moreover this technique produces results with acceptable accuracy and precision, as has been demonstrated by quality controls using certified reference material DORM-2 and spike recovery experiments.

Chapter III

Mercury biomagnification in the Oldman River, upper South Saskatchewan River Basin, and urban and agricultural effluents as potential sources

Abstract

Mercury levels in northern pike (*Esox lucius*), Walleye (*Stizostedion vitreum*), and Goldeye and Mooneye (*Hiodon alosoides*, *Hiodon tergisus*) from the Oldman River, upper South Saskatchewan River basin, were measured to assess the necessity for consumption advisories. Moreover, mercury levels in longnose dace (*Rhinichthys cataractae*) and net-spinning caddisfly larvae were measured to assess whether a mercury gradient exists in the biota along the Oldman River continuum. Mercury levels in longnose dace and suckers (*Catostomus catostomus*, *Catostomus commersoni*) affected by domestic sewage, urban and agricultural runoff were measured to identify potential sources of mercury to the food webs of the river system. Mercury concentrations in pike and walleye exceeding the legal minimum length exceeded the consumption limit of 0.5ppm, while goldeye/mooneye had lower mercury levels. Mercury levels in caddisfly larvae and longnose dace increased from upstream to downstream sites; however, the gradient was more than 2-fold steeper in dace relative to caddisflies, which suggests that bioenergetic factors greatly affect mercury levels in this species, although the underlying mechanism is unknown. Fish exposed to agricultural and urban effluents had significantly lower mercury levels, or showed no difference, relative to reference sites, which suggests that these effluents contribute no significant mercury loading to river food webs. Although it cannot be ruled out that these effluents contribute a net mercury loading to

the water, enrichment-derived biological processes in the food webs (biodilution) may offset the effect of increased loading.

3.1 Introduction

The atmospheric mercury burden has increased since the onset of industrialization (Fitzgerald et al. 1998). Current estimates suggest a tripling of atmospheric concentrations over pre-industrial background levels (Mason et al. 1994). Atmospheric mercury is deposited in remote landscapes through precipitation and fallout of particulate matter (Lindqvist et al. 1991, Downs et al. 1998). In undisturbed landscapes the majority of mercury is sequestered in soil and only a small fraction is transported into the associated aquatic ecosystem (Grigal 2002). Loadings of elemental or ionic mercury can become methylated in aquatic ecosystems (Compeau & Bartha 1985), yielding highly toxic methylmercury (MMHg, MeHg). Mercury poisonings, leading to neurological degeneration, are largely associated with the ingestion of tainted foods, especially fish (see review by Gochfeld 2003, also see Kurland et al. 1960, Amin-Zaki et al. 1974). Therefore strict legislation regulates emissions of mercury from industrial sources, fish stocks are monitored, and consumption advisories are posted when required.

The effect of land use practices such as cultivation and urbanization on mercury levels in fish in lakes and rivers has received little attention in the literature. Most work has focused on humid regimes in the eastern and mid-west United States. Chen et al. (2000) show that aqueous mercury levels in eutrophic lakes affected by agriculture were low relative to pristine oligotrophic lakes. Mercury levels in fish were closely correlated with mercury levels in preceding steps of the food chain (also see Cabana et al. 1994). Other authors indicate that silt loads associated with agricultural drainage are responsible for elevated aqueous levels of mercury (Balogh et al. 2005, 2000). Lyons et al. (2006) assessed mercury levels along the Scioto River, Ohio continuum during a flood event and found that mercury levels gradually increase from the headwaters affected by agriculture, towards the City of Columbus and reached the highest levels downstream of the Columbus sewage treatment facilities. The authors conclude that urban runoff contributes a greater loading relative to agricultural runoff. The contribution of urban sewage to mercury loadings in waterways is generally considered to be low, since sewage treatment removes most of the mercury burden in raw sewage (Bodaly et al. 1998, Glass et al. 1990, Balogh & Liang 1995), however, Mason & Sullivan (1998) show that export of mercury from an urban watershed is elevated. This is attributed to surface sealing and thus lack of retention of atmospheric mercury outfall in soils as is observed in undisturbed watersheds. Thus, elevated mercury loadings from urbanized regions seem to be associated with storm runoff; cities with separate domestic and storm sewer systems may contribute significant mercury loadings to the associated waterway, since storm runoff is commonly not treated before discharge into the waterway. While these studies focus solely on mercury loadings, the effects on biota along the continuum of the associated waterway are unknown. It is well understood that mercury levels in biota are highly variable and affected strongly by a variety of factors other than loadings. Enrichment-associated biodilution at the base of the food web (Chen & Folt 2005) and bioenergetic variation (Simoneau et al. 2005, Doyon et al. 1998, Trudel & Rasmussen 2001) are equally important determinants of mercury concentration throughout the food web.

The watershed of the Oldman River (upper South Saskatchewan River Basin) in semi-arid southern Alberta is largely affected by agriculture. The effects of land use practices such as irrigation-based agriculture and urbanization on mercury levels in semi-arid geographic regimes have not been studied. Since undisturbed soils are a major storage pool for atmospherically deposited mercury, anthropogenic disturbances can potentially result in the release of significant mercury loadings. Runoff events have been shown to transport silt-associated mercury from the watershed into the waterway. In semi-arid southern Alberta, where storm events are highly variable and runoff originates primarily in the mountains rather than the plains, the mercury burden in prairie soils may be high. It is therefore conceivable that irrigation-based agriculture is a significant factor in the release of mercury from the watershed. Urbanization along the Oldman River continuum is moderate with a number of small towns discharging sewage effluent into the river after secondary treatment. The City of Lethbridge is the largest urbanized centre on the river, with a population of 82,000, no heavy industry, tertiary domestic sewage treatment and a separate storm sewage system, which discharges untreated effluent.

Objectives

The objective of this study was to determine whether mercury levels in sport fish in the Oldman River are elevated to the point that consumption advisories are necessary, and whether or not there is a mercury gradient in biota along the Oldman River continuum. Moreover, the role of agricultural irrigation runoff, and urban storm and domestic sewage effluent as potential sources of mercury to the system is assessed.

3.2 Materials and methods

3.2.1 Sites and sampling

Northern pike (*Esox lucius*), Walleye (*Stizostedion vitreum*), Mooneye (*Hiodon tergisus*) and Goldeye (*Hiodon alosoides*) were collected by angling and seining in the Oldman River between Popson Park in the City of Lethbridge and Picture Butte in the months of June through September 2005).

Longnose dace (*Rhinichthys cataractae*) are abundant in every stretch of fast water throughout the river continuum analyzed in this study and were collected by electrofishing during July and August 2006. Sites ranged from Maycroft to Taber (Figure 3.1). Small suckers (*Catostomus catostomus* and *Catostomus commersoni*) and longnose dace (where available) were collected by electrofishing in irrigation drainage canals (July, August 2005) and storm outfalls (July, August 2006) that featured some form of entrainment (pool or stream) of effluent prior to discharge into the river. This was thought to eliminate dilution effects caused by mixing with river water. In the river main stem suckers were collected by seining in July and August 2005. Stomach contents were analyzed qualitatively for species composition in sport fish and longnose dace where possible. A sample of ~500 hydropsychid caddisfly larvae was collected for each site where available; they are typically found in longnose dace habitat and were hand-picked from rocks at the same time the corresponding fish sample was collected (Table 3.1).

3.2.2 Mercury analysis

Reagents suitable for trace metal analysis were used exclusively. Each tissue sample (~1g fish muscle fresh weight or ~1g pooled invertebrate bodies) was digested with 10ml of

7:3 mix nitric and sulfuric acid (*aqua regia*), aided by 1.0ml of 5% BrCl solution (in conc. HCl) at 90 degrees C on a 36-place aluminum block dry bath. Prior to addition of BrCl the samples incubated under *aqua regia* at room temperature for one hour. BrCl solution was prepared in-house according to EPA method 1631 (U.S.EPA 2002). Samples were placed on the dry bath overnight for 12-14hrs. After this period the samples were allowed to cool and subsequently received 0.2ml of 5% (w/v in 3% HCl) potassium dichromate solution. Finally the samples were diluted to 30ml (graduation mark) with nanopure water. Samples were analyzed by means of CVAAS on a PerkinElmer AAnalyst 400 spectrometer coupled to a FIAS 400 with an AS91 autosampler. The carrier acid was 3% (v/v in nanopure water) HCl and the reducing agent was 3% (w/v in 3% HCl) $SnCl_2$. Mercury vapour was carried to the sample cell by high purity argon gas.

Pooled samples of lyophilized caddisfly larvae were sent to Flett Research Ltd. (440 DeSalaberry Ave.,Winnipeg, Manitoba R2L 0Y7, Canada) for total and organic mercury analysis.

3.2.3 Standards and quality control

Mercury standards were prepared in *aqua regia*, stabilized with potassium dichromate solution, and diluted with water in the same manner as the samples. The final standard concentrations were blank, 1.00ppb, 5.00ppb, 10.0ppb, 50.0ppb. A new calibration was run for each sample run of 33 samples (+3 quality control samples, 2 of which were spiked).

Quality was assured by analysis of interspersed quality control samples (certified reference material DORM-2 by NRC Canada). Approximately 0.1g of reference material was digested the same way as unknown samples. With our analysis protocol we have been able to measure tissue concentrations of 97% (average) of the referenced THg concentration; spike recoveries averaged around 99%.

3.2.4 Statistical analysis

Statistical computations (ANCOVA, linear regression) were performed in R Version 2.2.1 (2005) and JMP IN Version 5.1 (SAS Institute, Cary, North Carolina). Total mercury data for fish species were $\log_{10}$ transformed prior to statistical analysis to normalize the data and reduce heteroscedasticity where necessary. Suckers were available for narrow length ranges and were compared across exposure and reference/gradient sites along the Oldman River by analysis of covariance with fork length as covariate followed by Tukey's or Dunnett's multiple comparisons. Samples of longnose dace incorporated broad size ranges and $\log_{10}$ transformed mercury levels were standardized to least squares means followed by Tukey contrasts as part of ANCOVA using fork length as the covariate.

Table 3.1: Summary of sampling sites in along the Oldman river continuum

site number	site name	Location	type	samples collected	site number	site name	location	type	samples collected
1	Maycroft/Hwy22	SW12-10-2-W5	gradient/reference	suckers, LNDC	9	Peenaquim Park	Lethbridge	Lethbridge sewage plume	LNDC,
2	Poulsen	NW28-8-1-W5	gradient/reference	suckers, LNDC, caddisfly larvae	10	Peenaquim Park	Lethbridge	gradient/reference	LNDC,
3	Summerview	SW13-7-29-W4	gradient/reference	LNDC, caddisfly larvae	11	Peenaquim Park	Lethbridge	storm outfall	LNDC, crayfish
4	Ft. Macleod	Town of Ft. Macleod, bridge	gradient/reference	suckers, LNDC,	12	Coalhurst sewage	SE14-9-22-W4	Coalhurst sewage input	suckers, daphnia, notonectids
5	Pearce Rd	NW9-10-24-W4	gradient/reference	suckers, LNDC, caddisfly larvae	13	Pavan	NE24-9-22-W4	gradient/reference	suckers, LNDC, caddisfly larvae
A	Monarch	NW9-10-24-W4	irrigation canal	Suckers	C	Piyami	SW23-10-21-W4	irrigation canal	LNDC, suckers
B	BASF	NW26-9-23-W4	irrigation canal	Suckers	14	Coaldale bridge	NE27-10-20-W4	gradient/reference	LNDC, suckers, caddisfly larvae
6	Popson	Lethbridge	gradient/reference	suckers, LNDC, caddisfly larvae	D	Battersea	NE35-10-20-W4	irrigation canal	Suckers
7	Paradise Canyon	Lethbridge	storm outfall	LNDC,	15	Church of Cameron	SE36-10-19-W4	gradient/reference	Caddisfly larvae
8	Ridgewood	Lethbridge	storm outfall	suckers	16	Hwy 36 north	NE35-11-16-W4	gradient/reference	LNDC, crayfish, caddisfly larvae

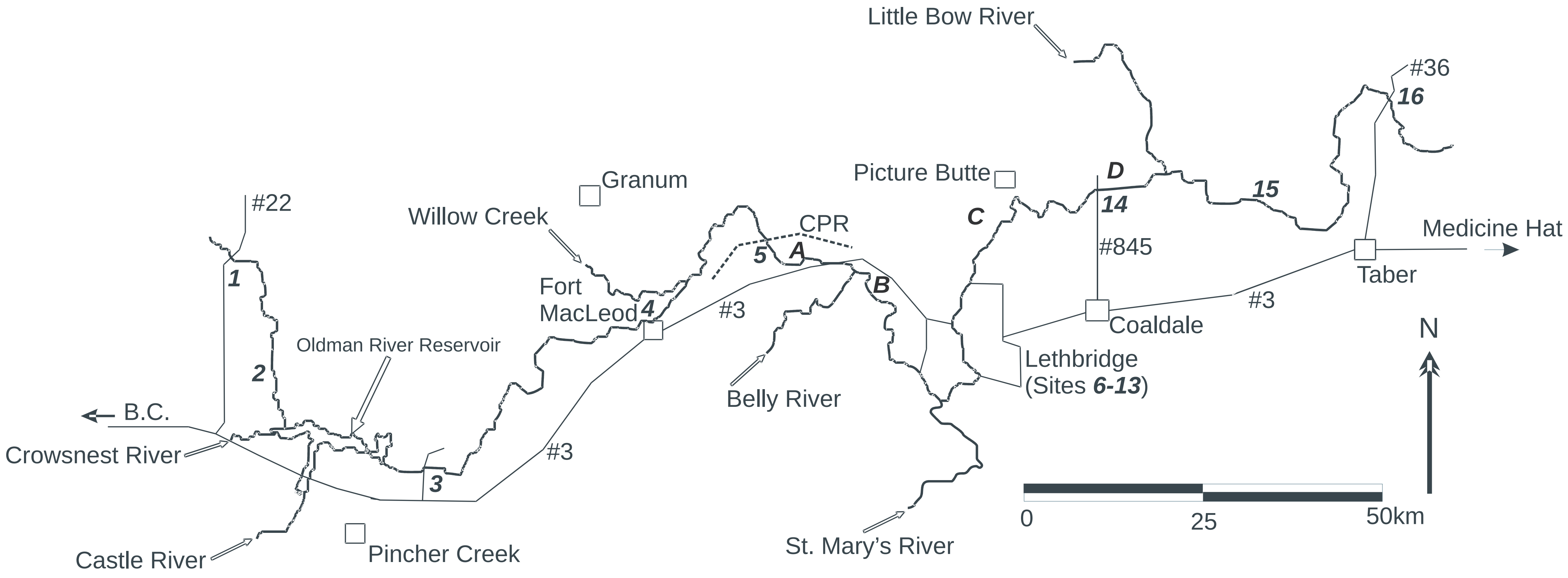

Figure 3.1: Location of sites along the Oldman River continuum. Bold italic numerals indicate sites along the river, including reference sites and sewage outfalls (see Table 3.1 for details), bold italic letters indicate irrigation canals (see Table 3.1 for details). Sites 6-13 see Appendix A.

3.3 Results

3.3.1 Mercury levels in sport fish

Mercury concentrations in walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*) above the legal minimum angling size exceeded the Health Canada consumption limit mercury in fresh muscle tissue (Figure 3.2). Mercury concentrations were highest in walleye, exceeding mercury concentrations in northern pike of comparable fork length 2-3-fold. Mercury concentrations in mooneye (*Hiodon tergisus*) and goldeye (*Hiodon alosoides*) were generally below the consumption limit, with only one fish (goldeye) in the sample exceeding the consumption guideline. The descriptive statistics of the three species are presented in Table 3.2.

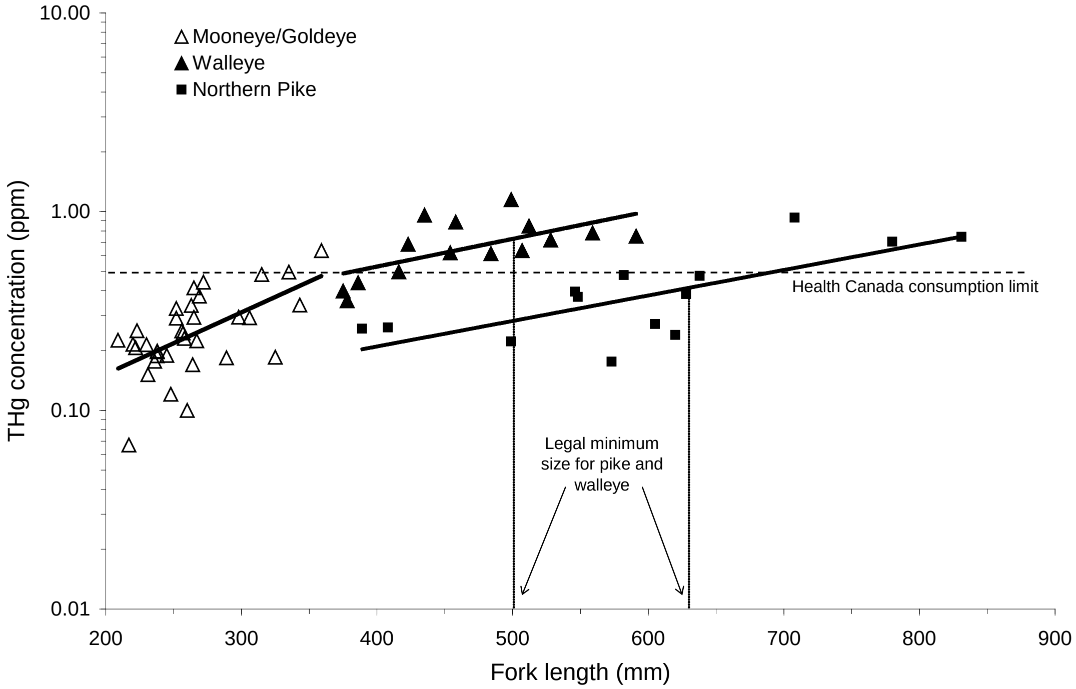

Figure 3.2: Mercury concentrations in important sport fish species in the Oldman River.

Table 3.2: Descriptive statistics of sport fish species.

Species	Mean fork length (mm)	Length range (mm)	Mean THg (ppm)	THg S.E.	n
Mooneye/Goldeye	264	209-359	0.26	0.021	34
Walleye	467	375-591	0.69	0.056	15
Northern pike	597	389-780	0.42	0.061	14

3.3.2 Mercury levels in longnose dace and hydropsychid caddisfly larvae

Mercury concentrations in size-standardized 52.52mm (global mean fork length) longnose dace from the Oldman River ranged from 0.024 to 0.104ppm (Table 3.3). Mercury concentrations generally tended to increase with decreasing elevation along the section of the river assessed herein. Human alterations such as the Oldman reservoir and inputs of sewage effluent coincided with reductions of mercury concentrations in longnose dace. Comparing least squares mean THg values for all sites by analysis of covariance (covariate fork length) followed by Tukey contrasts returned a significant interaction term, suggesting that slopes of THg versus fork length distributions were different among sites. Groups not statistically different at the global mean fork length (52.52mm) are shown on Table 3.3. Mercury levels in dace increased significantly between Maycroft and Poulsen above the Oldman reservoir (Figure 3.3, Table 3.3). Summerview was significantly lower relative to all other sites and coincided with a 3-fold reduction of mercury levels relative to Poulsen. The section of river between Summerview and Lethbridge (Popson Park) is characterized by continuously increasing mercury levels.

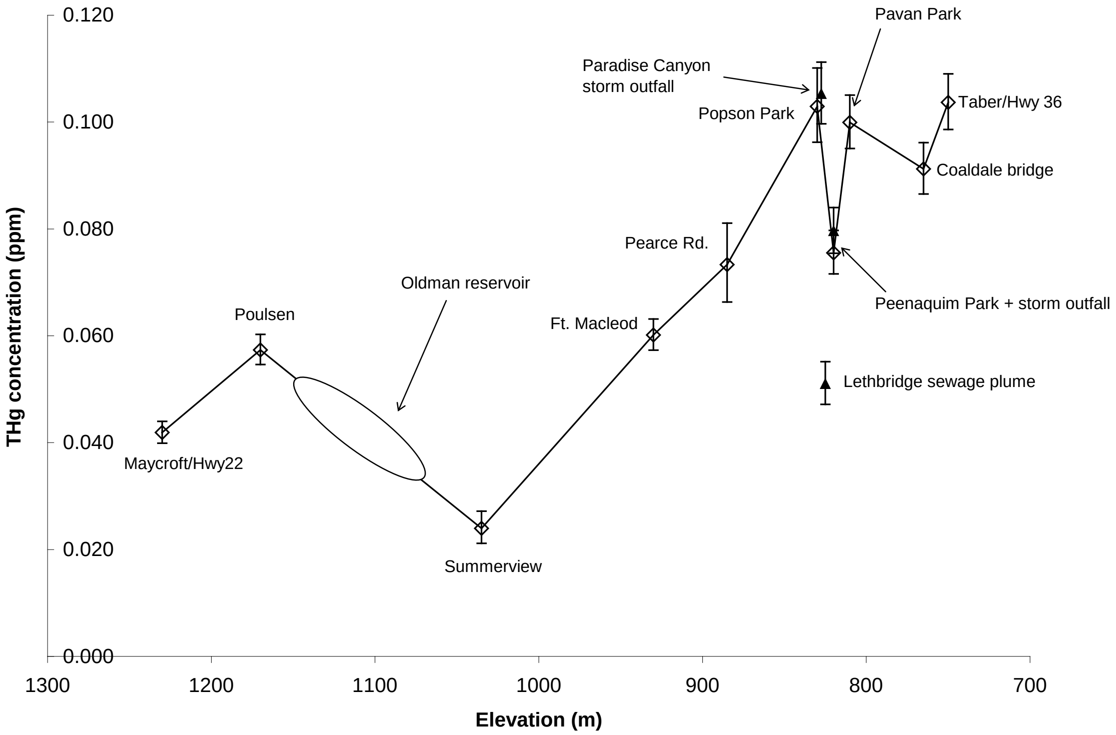

Figure 3.3: Total mercury concentration (least squares means) in longnose dace along the Oldman River continuum. Open diamonds indicate reference sites, solid triangles indicate sewage outfalls. Error bars indicate standard errors of least squares means. Sample sizes: Maycroft, Poulsen, Summerview, Pearce Rd., Peenaquim Park, Peenaquim storm outfall, Coaldale bridge, Taber/Hwy 36: n=33; Ft Macleod: n=38; Popson Park: n=22; Lethbridge sewage: n=30; Paradise Canyon storm outfall: n=32.

Linear regression of mercury concentration (least squares means) in dace versus elevation for gradient sites below the Oldman reservoir (Summerview, Ft. Macleod, Pearce Rd., Popson Park, Peenaquim Park, Pavan Park, Coaldale bridge, Taber/Hwy 36), showed an overall increase of mercury levels by a factor of 3.6 (0.030-0.11ppm, by regression model) between Summerview and Hwy 36, which constitutes a drop in elevation of approximately 285m. Mercury levels in fish from storm sewers did not differ significantly from close upstream reference sites (Table 3.3). Sewage effluent from the waste water treatment facility below Lethbridge coincided with a statistically significant depression of mercury concentrations comparable with levels above the Oldman reservoir (Table 3.3), followed by a rebound at Pavan Park. Further increases downstream of the City of Lethbridge were not detected.

Table 3.3: Statistical comparisons of mercury concentrations in longnose dace from Oldman River gradient sites and sewage outfalls. Tukey contrasts performed on least squares means, corresponding to the pooled mean fork length of 52.52mm. Sites not connected by same letter are significantly different.

Sites	Least squares means THg (ppm)	Groups alike						
Maycroft	0.042						F	
Poulsen	0.057					E		
Summerview	0.024							G
Ft. Macleod	0.060				D	E		
Pearce Rd	0.073	A	B	C	D	E		
Popson Park	0.103	A	B					
Peenaquim Park (reference)	0.076			C	D			
Pavan Park	0.100	A	B					
Coaldale bridge	0.091	A	B	C				
Hwy 36	0.104	A						
Paradise Canyon storm sewer	0.105	A						
Peenaquim Park storm sewer	0.080		B	C				
Lethbridge sewage plume	0.051					E	F	

Total mercury levels in hydropsychid caddisflies ranged between 0.013 and 0.023ppm wet weight equivalent, the mean MeHg fraction was 59.5% (Figure 3.4). Linear regression of MeHg concentration and elevation was employed to examine the increase in mercury concentration between Summerview and Taber/Hwy 36. The analysis yielded a significant increase in Hg concentration with decreasing elevation (Statistics provided in Appendix B). Mercury concentrations in hydropsychid caddisflies increase by a factor of 1.5 (0.008-0.012ppm, by regression model) between Summerview and Taber/Hwy36.

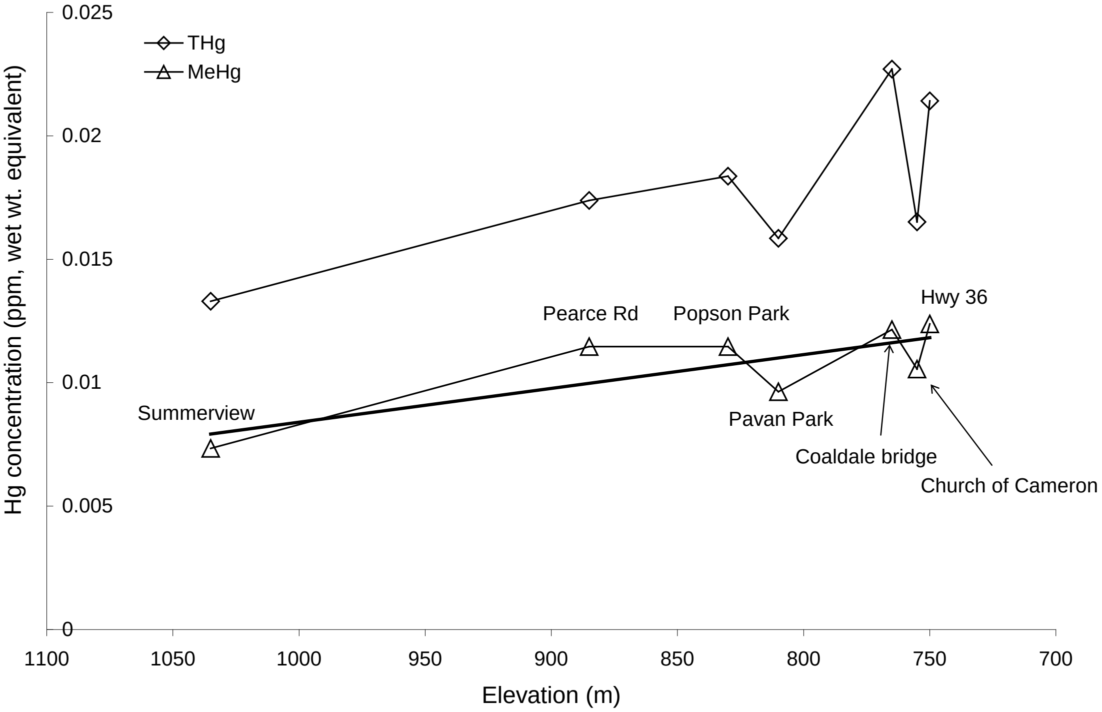

Figure 3.4: Total mercury and methylmercury concentration in hydropsychid caddisfly larvae along the Oldman River continuum.

3.3.3 Agricultural and urban effluents as potential mercury sources

To identify further inputs of sewage as potential sources of mercury to the Oldman river, longnose and white suckers (*Catostomus catostomus*, *Catostomus commersoni*) were analyzed for total mercury at sites where longnose dace were absent. Suckers from the Ridgewood storm sewer and Coalhurst sewage effluent were compared with fish of comparable size range from Popson Park. The results are summarized in Figure 3.5. Analysis of covariance followed by Dunnett's contrasts yielded significant differences only between Popson Park and Coalhurst sewage (Appendix B).

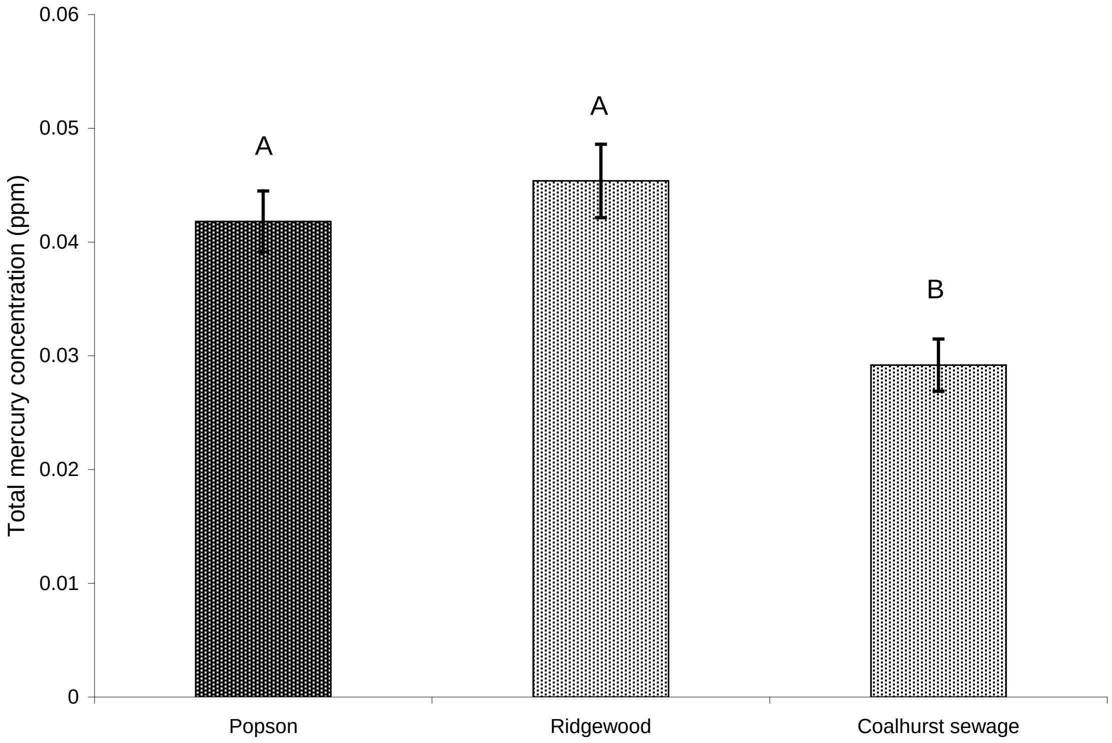

Figure 3.5: Mean total mercury concentration in small longnose and white suckers from Popson Park, Ridgewood storm outfall, and Coalhurst sewage. Letter codes identify sites that were statistically different (ANCOVA, Dunnett's contrasts). Error bars represent +- 1 standard error.

Mercury levels in suckers from irrigation drainage canals were compared with suckers from reference sites on the Oldman River upstream of the outfall into the river, in order to assess potential mercury loadings from agricultural runoff (Figure 3.6). Mean THg concentrations of four sites along the river (Ft Macleod, Pearce Rd., Popson Park, Pavan Park) were compared with mean THg concentrations of canal sites (Monarch, BASF, Pyami, Battersea) using Student's t-test (assuming unequal variances). A significant difference between river and canal THg concentrations was not detected (t = -1.584, df = 6, p = 0.1652). A dependency on fish length was ruled out (analysis of covariance, details not shown).

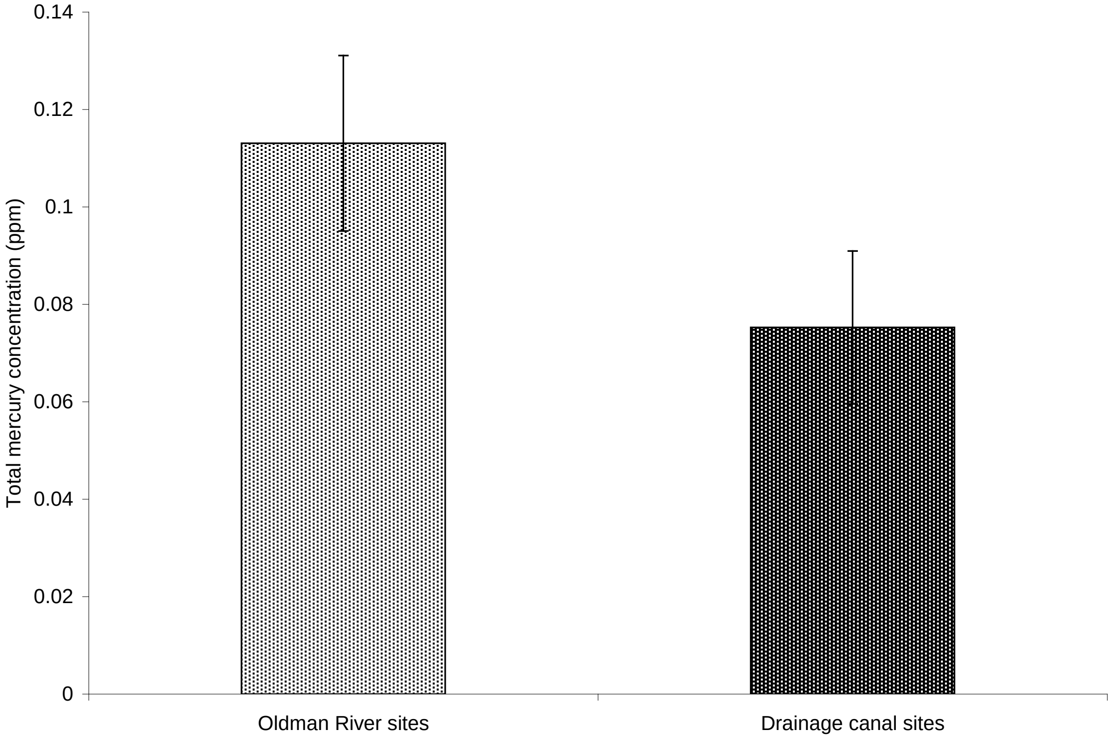

Figure 3.6: Mean total mercury concentration in small longnose and white suckers from irrigation drainage canals and sites along the river. Error bars represent +- 1 standard error.

3.4 Discussion

3.4.1 Mercury levels in sport fish

Mercury levels in northern pike and walleye exceed the Health Canada consumption limit of 0.5ppm and should therefore be of concern for fisheries managers. Mercury levels increased with fish size, as is commonly observed by others (Scott & Armstrong 1972). For these two species consumption advisories are warranted. Goldeye and mooneye are generally below the consumption limit, at least in the size ranges analyzed in this study. These fish feed predominantly on invertebrates, but are also known to be piscivores, especially when they get larger. Both species are known for a migratory lifestyle and cover vast distances within the South Saskatchewan River system between central Saskatchewan and Lethbridge. It is expected that larger individuals will most likely exceed the consumption limit and consumption advisories are therefore warranted as a precautionary measure. High mercury levels (>1.0ppm average) in Goldeye were measured in the North Saskatchewan during the mid 1970’s and could not be explained by local exposure (Munson & Daniel 1973, also see Nelson & Paetz 1992). Moreover, mercury levels in these fish exceeded those of pike and walleye and thus appeared to be inconsistent with the commonly observed positive relationship between mercury levels and trophic position. This prompted a closer study of the biology of goldeye which revealed movements as far downstream as Tobin reservoir, Saskatchewan, a section of river impacted by kraft pulp mills and chlor-alkali industry (notorious sources of mercury pollution). Following the elimination of large-scale mercury pollution from industry in the affected section of river in 1972 mercury levels in Goldeye declined to 0.65ppm in 1976 (Munson 1978). The upper South Saskatchewan population of hiodontids would not have been affected significantly by industrial pollution since the construction of Lake Diefenbaker in central Saskatchewan (1959-1967) restricted the range of migration of the upper South Saskatchewan River population to regions of lower mercury exposure.

Mercury levels in northern pike and walleye from the Oldman River are comparable with pike and walleye of similar size from uncontaminated control sites in the Wabigoon-English River system (Kinghorn et al. 2007, Parks et al. 1991, also see Bishop & Neary 1976, Parks et al. 1984). The observation that walleye have much higher mercury levels than northern pike of comparable size has been made by other authors as well (Kinghorn et al. 2007, Parks et al. 1991). Jackson (1991) notes that different mercury levels in species occupying the same trophic level can be ascribed to differences in metabolic rate, habitat preferences, and diet, among other factors. While northern pike and walleye may feed on similar prey items in lakes (Scott & Crossman 1973), physiological restrictions – especially of northern pike – may result in dissimilar primary food sources for pike and walleye in the Oldman River. The Oldman River is a typical prairie river with alternating fast-flowing riffles and boulder runs, deep channels and shallow, weedy pools. Whereas walleye prefer deeper, faster water at the tail-end of runs and riffles, northern pike are usually found in the weed beds of shallow pools. This pattern of habitat choice of both species suggests that walleye may feed almost entirely on longnose dace, whereas northern pike most likely encounter sucker species, which share the habitat preferences of northern pike. The mean mercury concentration in 90-190mm suckers is ~0.07ppm on average at Pavan Park, whereas mercury levels in 55-65mm longnose dace were approximately twice as high. Obviously, on a size-corrected scale, mercury levels in longnose dace are higher than in suckers. Assuming that walleye will preferentially consume longnose dace of 55 to 65mm (the size most commonly available downstream of Lethbridge) biomagnification factors range from 4 to 7. The corresponding biomagnification factor estimates for northern pike, assuming that their primary food source consists of suckers 9-20cm long (ranging in Hg from approximately 0.04-0.18ppm at Pavan Park), range between 4 and 5. These findings compare very well with published data on comparable trophic relationships (Vander Zanden & Rasmussen 1996, Cabana et al. 1994, Atwell et al. 1998, also see Yoshinaga et al. 1992). Mercury levels in walleye may also be exacerbated by higher activity costs associated with maintaining position in fast water; in fact activity levels of northern pike have been shown to be approximately 25% less than those of walleye of comparable size (see Rowan & Rasmussen 1996).

3.4.2 Mercury levels in longnose dace and hydropsychid caddisflies

Mercury concentrations in longnose dace increased significantly between Maycroft and Lethbridge. Downstream of Lethbridge further increases in mercury concentration could not be detected with the tests and samples used in this study. The Oldman River reservoir upstream of Summerview coincides with a strong depression of mercury levels, which is a surprising result, since reservoirs have been known to discharge high mercury loadings in their spill (Canavan et al. 2000, Schetagne et al. 2000). Although mercury burdens in reservoirs reach a maximum within two years of impoundment, the normalization periods to background levels are highly variable (Porvari 1998, Therriault & Schneider 1998, Verdon et al. 1991). The tributaries of the Oldman reservoir (Oldman River, Crowsnest River, Castle River) flow through regions with large deposits of glacial clay, which is eroded in severe runoff events and subsequently deposited in the reservoir. This factor may have aided significantly in the recovery of baseline levels in the reservoir after flooding, since clay strongly binds cations such as Hg^{2+}, eliminates mercury from the bioavailable pool, and sequesters it in sediments (Parks & Hamilton 1987). Moreover, the discharge of nutrient-rich hypolimnetic water from the reservoir results in a high-productivity ecosystem in the river downstream (Rasmussen 2006, unpublished data). Biodilution by enhanced algal growth (Chen & Folt 2005) and growth dilution of mercury signatures are likely factors contributing of the low mercury levels in longnose dace, as has been observed in other species (Simoneau et al. 2005, Doyon et al. 1998, also see Essington & Houser 2003). Similar effects of growth dilution are observed at sites of high nutrient loadings around Lethbridge (e.g. sewage treatment plant).

Although a 1.5-fold increase of mercury levels in hydropsychid caddisflies was observed between Summerview and Taber, it was not sufficient to explain the comparably steeper gradient observed in longnose dace, which constituted a 3.6-fold increase of mercury concentrations along the same river gradient. This difference in gradient suggests a doubling of mercury biomagnification factors along the river gradient, which could be explained either by a gradual increase in trophic position of dace, or bioenergetic heterogeneity associated with the river gradient. Stomach contents of longnose dace suggest that the primary food source of this species is detritus and periphyton, and thus is similar to what is likely consumed by net-spinning caddisfly larvae, although longnose dace are known to consume aquatic insects as well (Thomson et al. 2001, Culp 1989, Beers & Culp 1990, Scott & Crossman 1973). Moreover, diets did not appear to change along the river gradient, which suggests that the observed gradient difference is not due to dietary exposure, and bioenergetic factors play a more important role. Doyon et al. (1998) show that slow-growing lake whitefish bioaccumulate mercury at an increased rate relative to normal lake whitefish. In a regression of length versus THg this would translate into a greater slope for slow-growing fish relative to fast-growing fish (also see Simoneau et al. 2005, Harris & Bodaly 1998). The magnitudes of regression slopes (THg versus fork length in longnose dace for each site) versus elevation are presented in Figure 3.7. Slopes tend to increase with decreasing elevation and slopes for sewage outfalls tend to be less than nearby reference sites. The river exhibits a temperature gradient, with sites below Lethbridge frequently reaching 24 deg C, whereas temperatures around Summerview rarely reach 14 deg C (Rasmussen 2006, unpublished data). Growth rates in fish have been negatively correlated with activity levels previously (Rennie et al. 2005, Trudel & Rasmussen 2006). It is possible that longnose dace begin to spawn at a younger age in the lower reaches where temperatures are higher compared with the upper reaches. It is also known that longnose dace spawn several times per season (Roberts & Grossman 2001), and it is possible that the frequency of annual spawning events increases with stream temperatures. Thus, much of their energy budget is spent on gonadal rather than somatic growth as well as high activity levels during spawning.

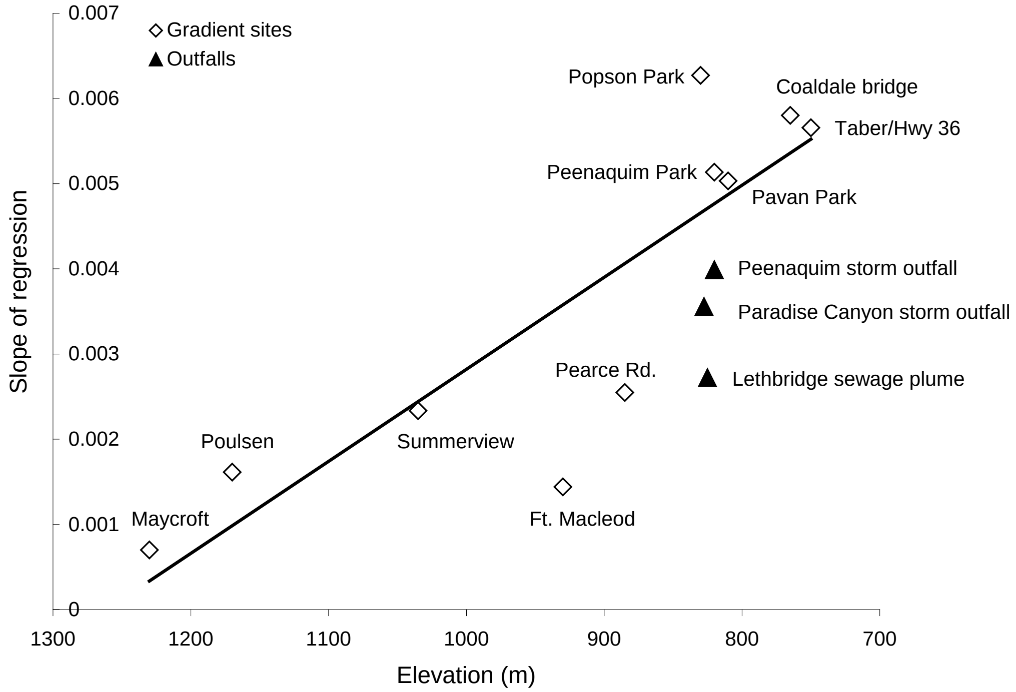

Figure 3.7: Slopes of individual regressions of fork length versus THg in longnose dace from the Oldman River. Shown trendline applies to gradient sites only (statistics and model parameters shown in Appendix B)

Methylmercury is associated with the protein fraction of the body (Harris et al. 2003) hence the export of mercury into the gonads is minimal. Also, since the ratio of mercury accumulation to body mass accumulation is greater in a slow-growing fish, mercury concentration reach much higher levels compared with fast-growing fish. Thus the mercury gradient observed in longnose dace is likely to be more influenced by the bioenergetic responses of the fish to the temperature gradient along the river rather than to exposure to the contaminant. Exposure to pesticides could also induce stress in fish, which would lead to a stress-induced increase in metabolic rate and thus exacerbate mercury accumulation in somatic tissue. Field studies have shown that toxicant stress has profound consequences for bioenergetics and is known to impair fish growth (Couture & Rajotte 2003, Sherwood et al. 2000). Jeffries (2007) has shown that sex ratios in longnose dace populations of the Oldman River become increasingly female-biased along the river gradient between Summerview and Taber. He identified a number of organic contaminants in river water among which were many endocrine disrupting compounds. Based on physiological evidence he concluded that endocrine disrupting compounds entering the river with agricultural and urban effluents adversely affect longnose dace in the Oldman River. Thus it is conceivable that the stress response of longnose dace to contaminants has the secondary effect of elevating mercury levels in these fish. The results we obtained for suckers from reference sites between Ft. Macleod and Pavan Park show very little congruence with the results for longnose dace, underscoring the interpretation that the observed pattern is more due to species-specific variability rather than exposure. In fact, it seems that hydropsychid caddisflies are the best indicator to assess actual exposure of biota to mercury, because the aquatic period of their lifecycle involves virtually only feeding and growing, thus eliminating confounding factors such as increased activity levels associated with reproduction.

Although background exposure levels at the base of the food web increase only slightly along the course of the river, the bioenergetic response to the river gradient observed in longnose dace constitutes a significant route of elevated mercury exposure to predatory fish feeding on longnose dace. Thus mercury levels in walleye, for example, are expected to be high, despite the fact that no point source loadings are identifiable and background level increases are small along the river gradient.

3.4.3 Urban and agricultural runoff as potential sources of mercury to river food webs

We could not identify any significant sources of high mercury loading to the river. Mercury levels in fish from irrigation canals or sewage effluent and storm outfall were either significantly lower or not different than reference sites within the river. A comparison of pooled means of canal sites with pooled means of river sites showed that mercury levels in canals were not significantly different than the river. It cannot be ruled out that the observations made earlier by other workers with respect to mercury export from urban and agricultural watersheds (see Balogh et al. 2005, 2000, Mason & Sullivan 1998) are not relevant here, since background mercury levels in water were not measured, however, our data suggest that other factors offset the effect of potentially high loadings in the favour of low levels in biota. Irrigation drainage, sewage effluent, and urban runoff are expected to carry high nutrient loads. As discussed earlier, high productivity in the food web results in low mercury levels in the biota as a result of biodilution, which is clearly reflected by the data presented herein. The effects of high mercury levels in longnose dace on higher trophic levels may be nonetheless profound, as the comparison of northern pike and walleye has shown.

3.5 Conclusion

Mercury concentrations in sport fish legal to be kept by anglers generally exceed the consumption limit if 0.5ppm. A mercury gradient along the Oldman River continuum could be identified in hydropsychid caddisfly larvae and longnose dace, however, the pattern observed in longnose dace suggests that bioenergetic factors exacerbate mercury contamination in this species more than loading (exposure gradient). Thus this river may also exhibit a bioenergetic gradient, which could be a direct consequence of the temperature gradient, toxicant stress or other unknown factors. This hypothesis requires further attention in the future.

Land use practices and urbanization are not significant contributors of mercury to biota in the river. Although it cannot be ruled out that land use practices contribute mercury loadings to the river, the observed low levels may be a consequence of biological processes associated with enrichment.

Chapter IV

Mercury levels in biota of a newly constructed irrigation reservoir in a flow-augmented stream system.

Abstract

Mercury data for northern pike (*Esox lucius*) from a newly constructed irrigation reservoir in southern Alberta are presented. Mercury levels (range 0.37ppm to 1.54ppm) generally exceeded the consumption limit of 0.5ppm total mercury concentration (THg) in fresh muscle tissue, were significantly higher (3.5-fold) than northern pike mercury levels in the Oldman River, and in most cases exceeded (up to 2-fold) previously published data from other reservoirs of the northern hemisphere. We tested the hypothesis that the flow–augmented stream system feeding the reservoir contributes to the mercury loading in the reservoir and found, by comparison with sites along the Oldman River, that it is unlikely that the tributary contributes to the high mercury loadings within the reservoir. Mercury levels in northern pike from this reservoir were considered extremely high given that these fish were not piscivorous. Elevated feeding rates and reduced growth rates of 3 and 4-year old fish in this new irrigation reservoir are presented as possible explanations for high mercury levels.

4.1 Introduction

The large demand for water by irrigation-based agriculture in semi-arid southern Alberta has required the construction of numerous reservoirs as storage pools. Mercury concentrations in biota of newly impounded reservoirs spike to high levels within 2-5 years from initial impoundment (Abernathy and Cumbie1977, Tremblay et al. 1998, Hall et al. 1998) and decline to background levels only slowly during periods as long as 15-30 years after impoundment (Porvari 1998, Therriault and Schneider 1998, Verdon et al. 1991). Discharge of hypolimnetic water from reservoirs has also been identified as a source of mercury contamination in the river system downstream (Canavan et al. 2000, Schetagne et al. 2000). Most work in this field has focused on reservoirs in eastern Canada and the sub-arctic, probably because of the problems associated with high mercury levels in fish traditionally harvested for sustenance by the native population (Chevalier et al. 1997). The watersheds of these reservoirs are dominated by forests whose soils and vegetation have been implicated as large storage pools of mercury (St. Louis et al. 1996). In addition surface water flows through vast wetlands, which have been identified as major methylation sites (St. Louis et al. 1998, 2004), before entering the main-stem rivers. Thus high mercury levels in fish are expected. The watersheds of most prairie rivers in semi-arid southern Alberta are characterized by grasslands and the absence of large wetlands. Yet we suspect that disturbances of the geosphere caused by human activity (i.e. agriculture) in the surrounding watersheds in conjunction with flooding of soils can lead to high mercury levels in Alberta reservoirs. Augmentation of the Little Bow River results in increased flows, which lead to erosion of the banks and high silt-loads. This in turn has been shown to increase the availability of soil-borne mercury (Balogh et al. 2005). The stream bottom is characterized by alternating clean gravel beds and mud, which may be important methylation sites, leading to increased availability of methylmercury to the biota in this system.

The impoundment of prairie rivers results in the creation of lake habitats that are fundamentally different from the original stream. Disrupted and simplified food webs can be the result of alteration of flow regimes (Wootton et al. 1996), and alter trophic relationships in freshwater communities. Trophic position is an important predictor of mercury levels in biota, and variable food web complexity can significantly affect mercury levels at higher trophic levels in different systems (Cabana et al 1994, Cabana & Rasmussen 1994). The importance of a fish-based diet for northern pike is well documented (see Bregazzi and Kennedy 1980, Diana 1979, Wolfert and Miller 1978, Lawler 1965, Seaburg and Moyle 1964), however, examples of feeding plasticity have also been shown for larger pike (Chapman et al. 1989). Seasonal feeding plasticity is observed in northern pike in at least one system where forage fish become more abundant in late summer and replace invertebrates as the primary food source (Chapman and Mackay 1990). Furthermore, opportunistic feeding habits of pike have been observed in small boreal lakes, where winterkill events can reduce forage fish stocks (Venturelli and Tonn 2006). Thus the trophic position of northern pike can be variable across systems, and mercury levels in prey species must be studied to understand mercury biomagnification in this top predator.

Objectives

The objective of this study is to present mercury levels in northern pike (*Esox lucius*) from a newly constructed irrigation reservoir as part of an augmented system in southern Alberta, and to compare the results with previously collected data for the Oldman River. Secondly, mercury levels in Twin Valley reservoir are compared with published data for other reservoirs. Finally, the question that flow augmentation increases mercury loadings in the Little Bow river is addressed by comparing longnose and white suckers (*Catostomus catostomus*, *Catostomus commersoni*) from the Little Bow River with previously analyzed samples of suckers from the Oldman River.

4.2 Materials and Methods

4.2.1 Sites and Sampling

Twin Valley reservoir is a new reservoir located approximately 45km due south east of High River, Alberta. The legal land description of the access point/boat launch is SE 8 15 25 W4, (Figure 4.1). It was constructed as storage for irrigation purposes and to regulate peak flows in the Little Bow River downstream.

The reservoir incorporates the confluence of the Little Bow River and Mosquito Creek. Both tributary streams are augmented through diversion canals from the Highwood River drainage during peak flows. Construction of the impoundment structures commenced in 2000 and flooding began in 2003. The reservoir is estimated to impound approximately 2.73×10^6 m^3 and has a surface area of approximately 835ha at FSL. Thus the mean depth is estimated to be 3.06m. The maximum depth at FSL is 21m. The water residence time so far is 2.3 years (Statistics kindly provided by Mike Bryski, Alberta Environment, personal communication). We took a sample of 28 northern pike (NRPK, *Esox lucius*) from the reservoir in August 2005. Fish were caught exclusively by angling from a boat and from shore. Fish were caught in depths of approximately 0.5 to 2m and 3 to 20m from shore. The entire perimeter of the reservoir was covered in that fashion. A sample of 15 white and longnose suckers

(*Catostomus commersoni*, *Catostomus catostomus*) was collected by electro-shocking from the Little Bow River upstream of Twin Valley reservoir at the Hwy 533 bridge. Fish were kept cool and later measured and weighed. In the lab scales, opercular bones and cleithra were taken for aging purposes and a sample of dorsal muscle tissue was frozen under nanopure water for mercury analysis. Stomach contents were collected where available, washed through a sieve with nanopure water, sorted by species and frozen under nanopure water.

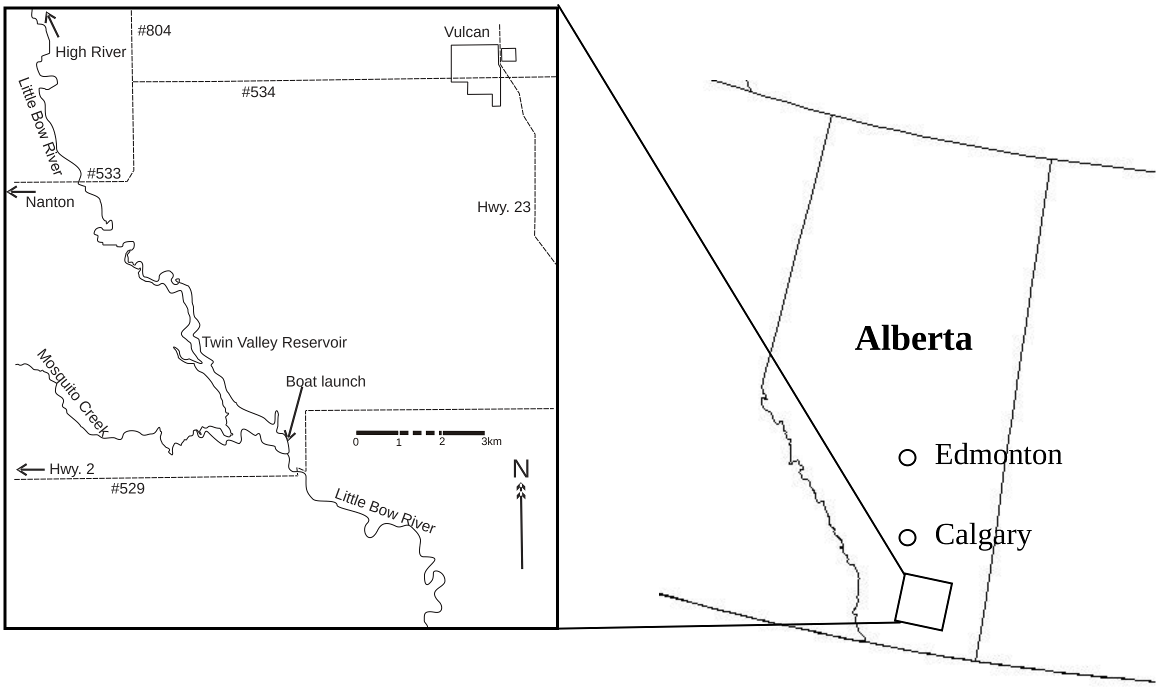

Figure 4.1: Location of Twin Valley Reservoir.

4.2.2 Mercury analysis

Reagents suitable for trace metal analysis were used exclusively.

Each tissue sample (~1g fish muscle fresh weight or ~1g pooled invertebrate bodies) was digested with 10ml of 7:3 mix nitric and sulfuric acid (*aqua regia*), aided by 1.0ml of 5% BrCl solution (in conc. HCl) at 90 degrees C on a 36-place aluminum-block-drybath. Prior to addition of BrCl the samples incubated under *aqua regia* at room temperature for one hour. Premature addition of BrCl can lead to violent fizzing and consequential loss of sample and damage to equipment. BrCl solution was prepared in-house (refer to protocol below).

Samples were placed on the drybath overnight for 12-14hrs. After this period the samples were allowed to cool and subsequently received 0.2ml of 5% (w/v in 3% HCl) potassium dichromate solution. Finally the samples were diluted to 30ml (graduation mark) with nanopure water.

Samples were analyzed by means of CVAAS on a PerkinElmer AAnalyst 400 spectrometer coupled to a FIAS 400 with an AS91 autosampler. We used 3% (v/v in nanopure water) HCl as carrier acid and 3% (w/v in 3% HCl) $SnCl_2$ as reducing agent. Mercury vapour was carried to the sample cell by high purity argon gas.

4.2.3 Standards and quality control

Mercury standards were prepared in *aqua regia*, stabilized with potassium dichromate solution, and diluted with water in the same manner as the samples. The final standard concentrations were blank, 1.00ppb, 5.00ppb, 10.0ppb, 50.0ppb. A new calibration was run for each sample run of 33 samples (+3 quality control samples, 2 of which were spiked).

Quality was assured by analysis of interspersed quality control samples (certified reference material DORM-2 by NRC Canada). Approximately 0.1g of reference material was digested the same way as unknown samples. With our analysis protocol we have been able to measure tissue concentrations of 97% (average) of the referenced THg concentration; spike recoveries averaged around 99%.

4.2.4 Statistical analysis and literature comparisons

Statistical analyses were carried out using R Version 2.2.1 (2005). Twin Valley northern pike were compared with Oldman River northern pike using analysis of covariance. Total mercury concentration (THg) data were $\log_{10}$ transformed to normalize the residuals and reduce heteroscedasticity. For literature comparisons publications were chosen that report mercury data for northern pike 2-4 years after initial impoundment. If fish sizes reported in published materials were different than sizes analyzed in this study the ANCOVA-derived model of $\log_{10}$THg versus fork length was used to extrapolate mercury levels across fish sizes not available in the sample. Statistical parameters other than means are frequently not reported in the relevant literature, so this comparison is qualitative accordingly.

4.3 Results

The plot of fork length versus total mercury concentration (THg) (Figure 4.2) shows that the majority of fish in this sample exceed the consumption limit issued by Health Canada of 0.5ppm total mercury in fresh muscle tissue. Mercury concentrations in northern pike from the Oldman River were significantly lower relative to Twin Valley reservoir (Appendix D). The compilation of previously published mercury concentrations in northern pike from other reservoirs (Table 4.1) shows that mercury levels in Twin Valley reservoir exceed those of other North American reservoirs in 12 out of 14 cases.

Table 4.1: Comparison of published mercury levels in northern pike from other reservoirs with expected mercury levels in Twin Valley reservoir. Twin Valley mercury data was estimated using the ANCOVA-derived regression model.

Source	System	Site	Years after impoundment	Mean length (mm)	THg (ppm)	Expected THg in Twin Valley (ppm)
Bodaly et al. (1984)	Southern Indian Lake, Churchill River diversion, Manitoba	The Channel	3	497	0.57	0.91
		Camp 9	3	554	0.58	1.11
		Region 4	3	528	0.49	1.01
		Region 6	2	666	0.77	1.64
			3	530	0.69	1.02
	Notigi reservoir, Churchill River diversion, Manitoba	Issett Lake	2	573	0.61	1.18
		Rat Lake	2	698	2.14	1.83
		Notigi Lake	4	788	1.95	2.51
	downstream of Notigi reservoir	Wapisu	1	672	1.08	1.67
		Footprint	2	457	0.6	0.79
		Threepoint	4	688	1.28	1.77
		Wuskwatim	3	560	0.91	1.13
		Mystery	3	462	1.13	0.80
Porvari (1998)	Western and northern Finland	Kalajärvi	3-6	505*	1.6	0.93
		Kivi-ja levalampi	3-6	505*	1.9	0.93
Verdon et al. (1991)	La Grande complex, Quebec	La Grande 2	3	700	1.31	1.84

* The publication lists mercury values for weight standardized (1kg) northern pike. Fork length was reconstructed using *length(mm) = 145.93•ln(weight(g)) - 502.77*; R^2 = 0.95 based on Twin Valley data.

Northern pike in Twin Valley reservoir fed exclusively on amphipods (*Gammarus lacustris, Hyalella azteca*). Not a single instance of piscivory was recorded in the sample. Mercury concentrations in amphipods from stomach contents ranged from 0.11ppm to 0.42ppm (mean 0.21ppm).

Mercury levels in suckers from the Little Bow River upstream of Twin Valley reservoir and sites along the Oldman River are summarized in Figure 3. The Little Bow sample was different from all sites except Pavan Park downstream of the City of Lethbridge (Appendix D).

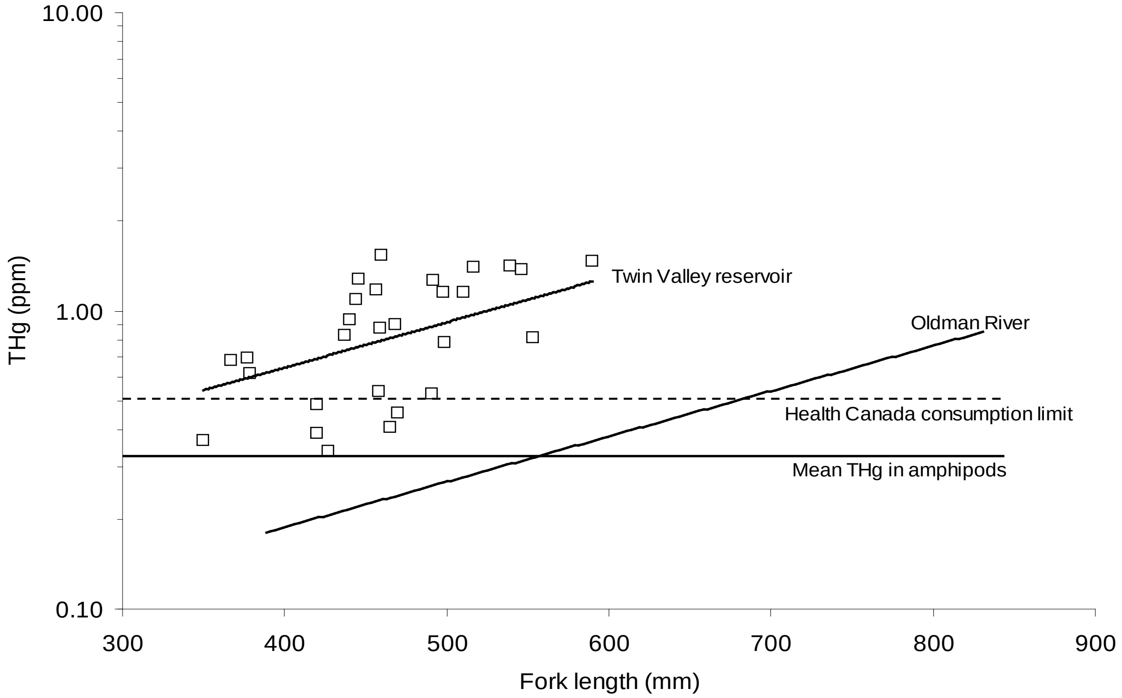

Figure 4.2: Total mercury concentration (THg) versus fork length in northern pike from Twin Valley reservoir. Mean THg in amphipods represents mercury levels in the primary food source of pike in the reservoir.

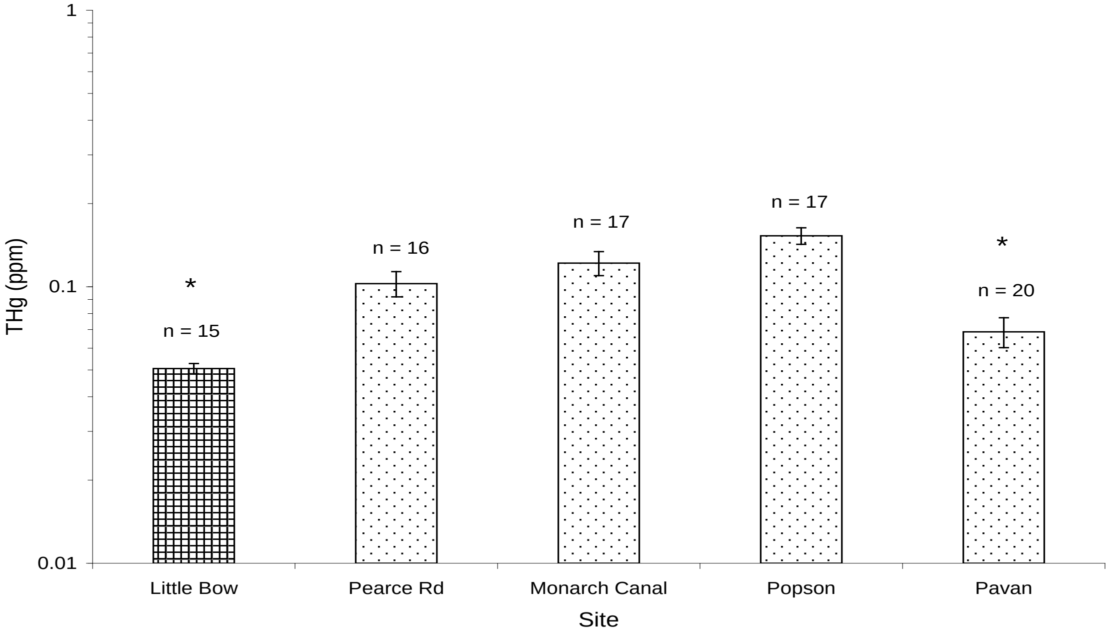

Figure 4.3: Mean total mercury (THg) in sucker species from the Little Bow River upstream of Twin Valley reservoir and various sites along the Oldman River for comparison. Little Bow: Hwy 533 bridge; Pearce Rd: Between Ft. Macleod and Monarch; Monarch Canal: Highest input of mercury from irrigation drainage; Popson: Upstream of Lethbridge; Pavan: Downstream of Lethbridge sewage treatment plant. Error bars represent ± 1 S.E. Asterisks indicate no difference.

4.4 Discussion

4.4.1 Mercury levels in Twin Valley Reservoir and the Little Bow River

Mercury levels in the majority of northern pike from this reservoir exceeded the consumption limit of 0.5ppm. Mercury levels in northern pike were significantly higher relative to the Oldman River. In Southern Indian and other lakes, mercury levels reached 2.55ppm in extreme cases, but generally ranged between 0.3 and 1.5ppm in northern pike of sizes comparable to the present sample (Bodaly et al. 1984). Mercury levels in 700mm northern pike from the La Grande complex in northern Quebec reached 1.31ppm within three years after impoundment and increased further to about 3ppm within 9 years (Verdon et al. 1991). Mercury levels in Finnish reservoirs are higher relative to Twin Valley, however, only ranges of years after impoundment are presented in the publication and the precise age is unknown. The comparison of published mean mercury levels with estimates from Twin Valley data indicates that Twin Valley values exceed those of other reservoirs in most cases, in some instances up to two-fold. This suggests that flooding of prairie soils in Alberta can result in significant mercury contamination. The comparison of fish samples from the Little Bow River and reference sites along the Oldman River shows that mercury levels in the Little Bow are significantly lower relative to all sites on the Oldman River upstream of the City of Lethbridge. Little Bow mercury levels were not significantly different than Pavan Park, a site that is affected by enrichment from urban sewage effluent downstream of the City of Lethbridge. Enrichment has been shown to cause biodilution at the base of the food web and results in lower mercury levels in zooplankton and higher trophic levels (Chen & Folt 2005). Enrichment from agricultural runoff is likely the factor responsible for low mercury levels in suckers upstream of Twin Valley reservoir, and flow augmentation of the Little Bow River has no appreciable effect on elevating mercury loadings in this system.

4.4.2 Possible factors affecting mercury levels in northern pike

The data presented here suggest that mercury loadings are restricted to the reservoir alone and are not exacerbated by flow augmentation of the tributary. Although mercury loadings from flooded soils may account for part of the observed mercury contamination, it must be considered that pike in Twin Valley are non-piscivorous and occupy the trophic position of suckers. Yet, northern pike from the Oldman River are piscivorous and have mercury levels 3.5-fold lower than Twin Valley fish. Moreover biomagnification factors between Oldman River pike and their preferred prey (suckers 9-

20cm long, ranging in Hg from approximately 0.04-0.18ppm at Pavan Park) are 4-5, whereas biomagnification factors in Twin Valley range between 5 and 7. Mercury levels in fish are a balance of feeding rates, growth rates, and mercury concentrations in the food source. Field studies have shown that high mercury biomagnification factors are associated with bioenergetic factors. For example, lake whitefish (*Coregonus clupeaformis*) of the dwarf ecotype feed predominantly on zooplankton throughout their life span, whereas normal whitefish consume zooplankton only at early life stages and switch to benthos later in life (Doyon et al. 1998, Trudel et al. 2001). In lake trout (*Salvelinus namaycush*) growth rates are influenced by food web structure within a given system (Pazzia et al. 2002). Lake trout forced to be non-piscivorous by thermal constraints during the summer have lower growth rates, mature at younger ages and are stunted, whereas their exclusively piscivorous counterparts in other systems grow normally (Pazzia et al. 2002). The authors also show that daily rations of non-piscivorous lake trout are 2-3-fold higher on a g/g/day basis, but when corrected for caloric value of the food source, the difference amounted to less than 10% (Pazzia et al. 2002). This suggests that non-piscivorous lake trout make up the lack of caloric value of their food source by consuming more of it. The resulting expenditure of energy for feeding on comparably low value food would explain the reduction in growth rates compared with piscivorous lake trout in other systems (Pazzia et al. 2002). Furthermore, the importance of diet shifting for fish growth has been shown (Sherwood et al. 2002a,b). For example, in metal-impacted lakes, in which benthos of a certain size range was absent, juvenile yellow perch (*Perca flavescens*) did not find an intermediate food source between the planktivorous and piscivorous trophic stages of development (Sherwood et al. 2002a).

Unusually high activity costs, low growth efficiency and stunted growth resulted from this energetic bottleneck, and were attributed to the greater number of activity bursts required to achieve the daily ration when feeding on small food items (Sherwood et al. 2002a).

It is conceivable that low forage fish densities within the reservoir force northern pike into perpetual non-piscivory and pike may thus allocate a greater proportion of their energy budget towards activity. Elevated energy demands may in turn result in elevated feeding rates which exacerbate mercury uptake in these fish. Thus the simplified food web observed in this reservoir ultimately translates into elevated biomagnification factors and high mercury concentrations in northern pike.

To test the possibility that low growth rates and high feeding rates affect mercury levels in this system, growth rates were compared with other system for which appropriate data are published, and feeding rates were calculated and compared with published data for other systems.

4.4.3 Northern pike growth rates and daily ration

Growth rates of northern pike from Twin Valley reservoir are generally lower relative to others systems (Figure 4.4A & B). This may suggest that northern pike in this reservoir indeed encounter a bioenergetic bottleneck as a result of low-quality food sources and higher energy demands for activity.

The mean fork length and mean total mercury (THg) concentration of each age class are shown in Figure 4.4 B) and C). Due to their relative strength in n, daily ration was estimated for age classes 3+ and 4+ (modelling approach summarized in Appendix C).

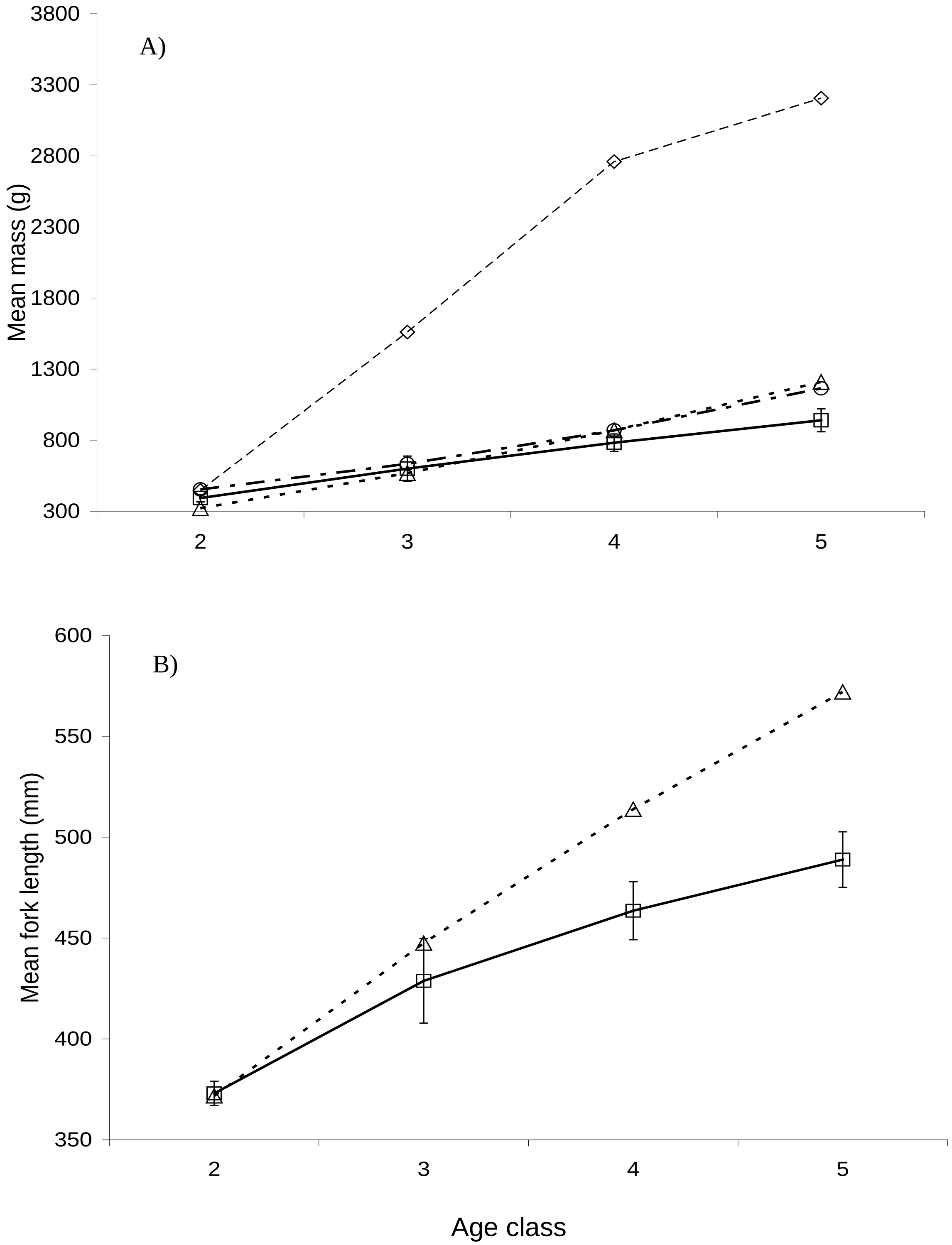

Figure 4.4: A)&B) (-□-) Data collected from Twin Valley reservoir, (-Δ-) Data reconstructed from Griffiths et al. (2000). A) Mean mass against age class of northern pike from Twin Valley reservoir, estimated fork length using von Bertalannfy function from Griffiths et al. (2000), (-◊-) Lake Simcoe, (-○-) Lac Rond-de-Poêle (Trudel et al. 2000); B) Mean fork length against age class; reconstructed data (total length) using regression model in Fig. 7 and length data in Fig.3 in Griffiths et al. (2000). Error bars in A) and B) represent 1 S.E.

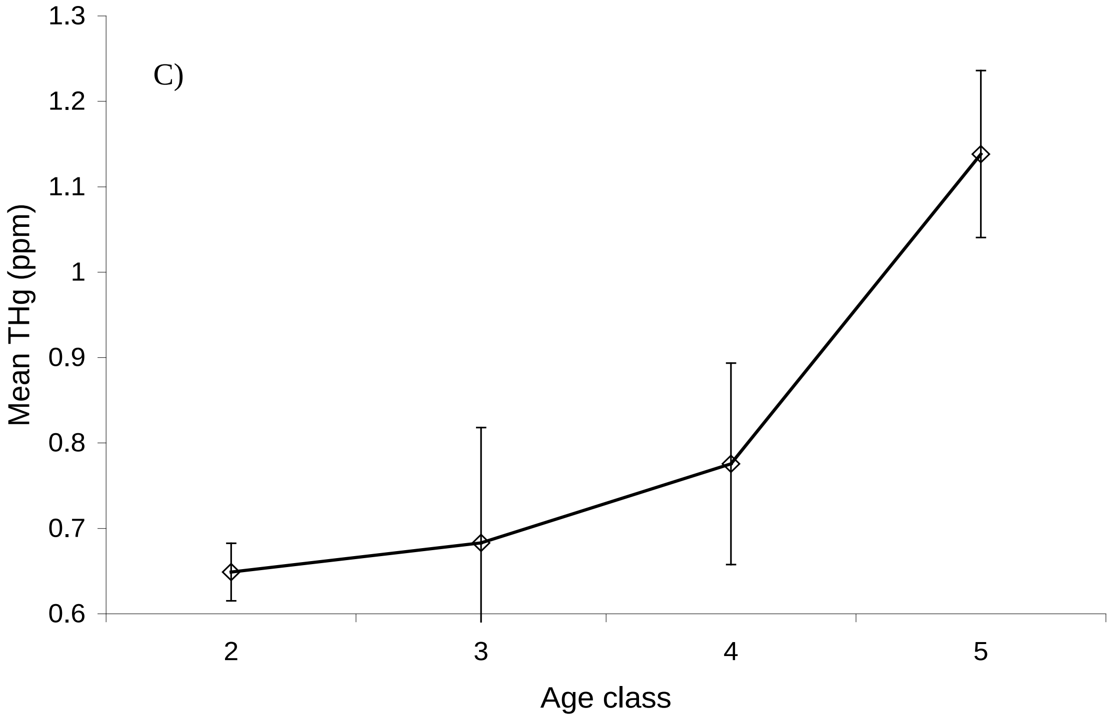

Figure 4.4 continued: **C) Mean total mercury (THg) against age class from Twin Valley. Error bars represent 1 S.E.**

For 3+ northern pike the mean daily ration estimated for August was 0.020g/g/d, reached a minimum of 0.0096g/g/d in December and ended at a maximum of 0.025g/g/d in July (Figure 4.5). Mean daily ration for 4+ northern pike behaved similarly, but started at 0.026g/g/d in Aug, was 0.015g/g/d in December, and peaked in July 0.038g/g/d (Figure 4.5). The annual averages were 0.016g/g/d for 3+ pike and 0.023g/g/d for 4+ pike.

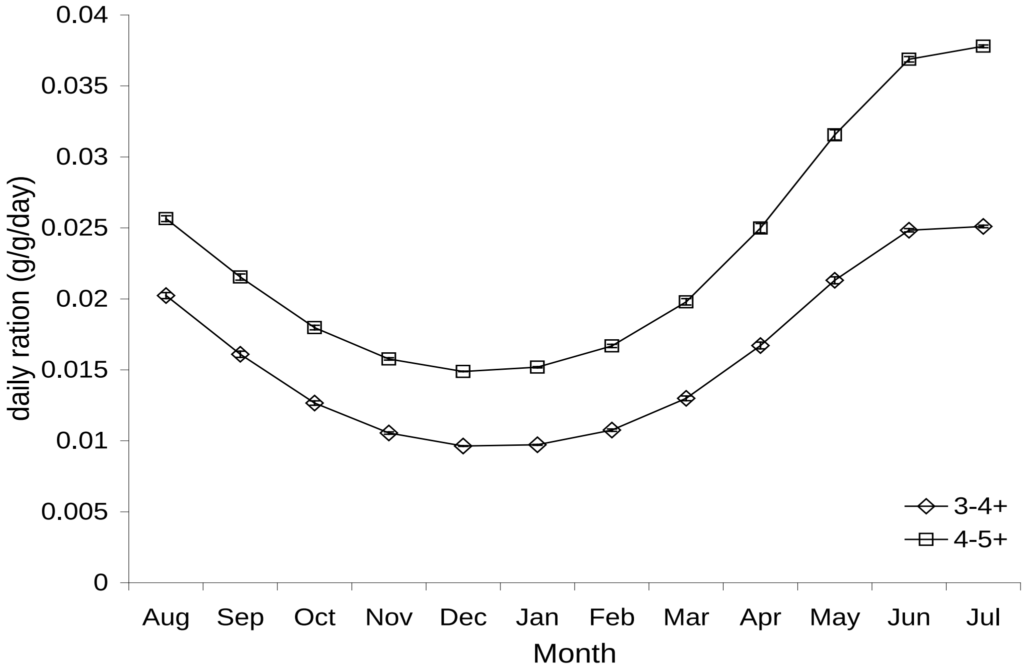

Figure 4.5: Mean monthly daily ration of benthivorous 3+ and 4+ northern pike estimated using the mercury mass balance model. Error bars represent 1 S.E.

A number of authors have addressed daily ration of northern pike, directly or indirectly, by experimentation in various systems, but a large, comprehensive data set is absent from the literature. A summary of published data used for comparisons is presented in Appendix E. Lucas et al. (1991) have used telemetry to track 2 individual pike of ~ 500mm total length, a size that is reasonably comparable with 4-5+ fish from my sample. The authors indirectly estimate (through heart rate monitoring) daily ration to be 1.6 and 1.5% B.W. for their two fish for the month of June and water temperatures around 20 deg. C. In this study pike were considered piscivorous. Although sparse, these data would suggest that feeding rates of benthivorous pike in Twin Valley reservoir are 2-fold higher. The caloric content of freshwater *Gammarus* has been estimated around 4.07kcal/g before (Hanson et al 1997, also see Jorde and Owen 1990). Based on the conversion, the estimates herein exceed those of J.S. Diana (1979) 50 to 130-fold in winter and 3 to 4-fold in Summer assuming the highest feeding rate is representative. Data presented by other authors, using the mercury mass balance approach, fall within the range of our results (Trudel et al. 2000). Feeding rates of 3 and 4-year old pike from Twin Valley are 1.5 to 2.5-fold, respectively, higher than estimates for pike from northern Quebec lakes and reservoirs (compare annual estimates, Appendix E). This would support the hypothesis that feeding rates of benthivorous pike should exceed that of purely piscivorous fish, although caution is necessary when comparing a warm lake like Twin Valley with cold lakes and reservoirs of northern Quebec (Trudel et al. 2000), since temperature is one of the main determinants of feeding rates. Rowan and Rasmussen present estimates of daily rations for pike from a number of systems based on ^{137}Cs concentrations (Rowan and Rasmussen 1996). In the Ottawa River, 4 year old pike are heavier (872.6g versus 782g in Twin Valley) and have a slightly greater feeding rate (0.0259g/g/d versus 0.0229g/g/d in Twin Valley). In Great Slave Lake (Christie Bay) feeding rates of 9-15+ year old pike are less than 3+ pike in Twin Valley (compare 0.0113g/g/d (highest in age range) versus 0.0158g/g/d in Twin Valley). Unfortunately data on age classes comparable to the present sample are not available from Great Slave Lake. Old fish as presented in Rowan and Rasmussen (1996) are expected feed at decreased rates (consuming large meals less frequently). Pike from Great Slave Lake and reservoirs in northern Quebec are expected to be entirely piscivorous early on in their life.

4.5 Conclusion

Mercury levels in northern pike from Twin Valley reservoir mostly exceed the consumption limit of 0.5ppm and exceed the levels measured in the Oldman River 3.5-fold. The figures presented here also exceed those of other reservoirs of the northern hemisphere. We have also shown that the flow-augmented Little Bow River system is probably not higher in mercury loadings than other rivers in southern Alberta, leading to the conclusion that high mercury levels in Twin Valley reservoir are solely due to impoundment and flooding.

Feeding rates of non-piscivorous northern pike are higher compared to piscivorous pike, as expected from previous examples in lake trout. A comprehensive data set of a system comparable to Twin Valley reservoir is absent from the current body of literature, and thus the question whether feeding rates of non-piscivorous and piscivorous northern pike are different must receive additional attention. There is a gap of knowledge to be filled and future work on other systems in southern Alberta is required.

Chapter V

Mercury biomagnification in Waterton Lakes National Park.

Abstract

Mercury data for biota in the upper and middle lakes in Waterton Lakes National Park, Canada were collected in order to assess the necessity for consumption advisories and compare the data with remote systems in other geographic regimes. Mercury levels in lake trout averaged 0.40ppm, and values ranged between 0.16 and 1.41. Fish from 400mm fork length and up had the potential to exceed the consumption limit of 0.5ppm. Lake whitefish were on average 0.1ppm and none exceeded 0.26ppm within a size range of 150-570mm. Analysis of stomach samples and zooplankton communities revealed that the upper lake is a class 3 lake (lake trout, forage fish and *Mysis relicta*) and the middle is a class 2 lake (lake trout, forage fish). Literature comparisons showed that lake whitefish from Waterton had lower mercury levels than whitefish from Flathead Lake, Montana; however, *Mysis relicta* from Waterton were 2.4-fold higher in mercury than Flathead Lake *Mysis*. Lake trout mercury levels were not different between the two lakes. Waterton Lakes mercury levels in lake trout were not different compared with Ontario class 2 lakes, however, no difference was detected between Waterton and class 2 and 3 lakes when only upper Waterton Lake samples were included in the analysis. Results indicate that lake trout belong to two different groups, with middle lake fish having lower mercury levels than upper lake fish for their length.

5.1 Introduction

Human exposure to monomethylmercury, one of the most toxic mercury species, has been largely associated with the ingestion of contaminated food, predominantly fish (WHO 1990, Gochfeld 2003). Methylation of inorganic mercury by sulfate-reducing bacteria (Compeau & Bartha 1985) results in highly toxic monomethyl mercury, which biomagnifies through the food web and can reach concentrations in terminal predators six orders of magnitude higher than background levels (Boudou & Ribeyre 1997). Perhaps the most famous and severe cases of mercury poisonings have been associated with direct pollution by industrial point sources (e.g. Minamata Bay, Japan, see Kurland et al. 1960). Mercury is a volatile element and circulates through the earth's atmosphere (Morel et al. 1998). Thus mercury contamination not only constitutes a problem near industrial point sources, but often also in remote regions devoid of all immediate development, where wet and dry deposition of mercury in the watersheds constitute the major source of contamination in biota (Lindqvist et al. 1991, Downs et al. 1998, Mason et al. 1994, Fitzgerald et al. 1998). Mercury released into the atmosphere is sequestered in soil and vegetation, but may become mobilized after natural or man-made disturbances such as wildfires (Kelly et al. 2006, Sigler et al. 2003, Friedli et al. 2003, Turetsky et al. 2006, Biswas et al. 2007) and logging (Garcia & Carignan 2000, 1999).

Mercury levels observed across a broad range of remote ecosystems are highly variable due to both physicochemical and biological factors. Mercury levels in biota are commonly observed to be negatively correlated with water pH (e.g. Greenfield et al. 2001, Scheuhammer & Graham 1999, Mason et al. 2000, Bloom et al. 1991), and positively correlated with [DOC] (dissolved organic carbon concentration, e.g. Rennie et al. 2005, Westcott & Kalff 1996, Gorski et al. 2003, also see Kolka et al. 1999, Mierle & Ingram 1991). Since mercury biomagnifies within the food chain, food web interactions and bioenergetic differences among fish population play significant roles in determining mercury levels at higher trophic levels. For example, Cabana et al. (1994) show that lake trout feeding on forage fish that feed on *Mysis relicta* (class 3 lakes) reach higher mercury levels than lake trout feeding only on forage fish that feed on herbivorous zooplankton (Class 2 lakes) or lake trout that feed directly on invertebrates (Class 1 lakes), often exceeding the Health Canada consumption limit of 0.5ppm. The same pattern was shown earlier for PCB levels by Rasmussen et al. (1990).

High levels of mercury had been detected in lakes and streams of Banff and Jasper National Parks and mercury advisories were posted in 2005 (Parks Canada 2005). Mercury data were not available for Waterton Lakes National Park prior to this study. Background mercury data from water samples collected in Glacier National Park by Watras et al. (1995), in immediate proximity of Waterton Lakes, indicated that background methylmercury loadings were lower in Glacier National Park than in Wisconsin lakes; however, only sparse fish data were provided. The authors suggest that low sulfate background levels result in reduced activity of sulfate-reducing bacteria, which are thought to be the primary agents in mercury methylation (Compeau & Bartha 1985).

The upper and middle lakes are closely connected via a narrow channel. Water drains directly from the upper lake into the middle lake. The Waterton Lakes system is considered a class 3 system due to the documented presence of *Mysis relicta* in the upper lake (e.g. Chess & Stanford 1998). *Mysis relicta* migrate diurnally in the upper lake, and are likely a major component of the pelagic food web, as observed in other systems (Cabana et al. 1994, Stafford et al. 2004). Yet data on the food webs in Waterton Lakes are sparse, and mercury levels are potentially high in this class 3 system.

Objectives

The objectives of this research were to assess whether mercury levels in lake trout and lake whitefish exceed the consumption limit of 0.5 ppm posted by Health Canada. This evaluation was intended to provide Parks Canada with information regarding the necessity for fish consumption advisories for lake trout and lake whitefish in the upper and middle lake in WLNP. A second objective was to compare mercury levels in lake trout and lake whitefish with published results for other class 3 systems in Montana and Ontario in order to assess potential variability among alpine and Precambrian shield systems with similar food webs. The final objective of this study was to characterize fish diets by examining stomach contents of lake trout and lake whitefish.

5.2 Materials and methods

5.2.1 Sites and sampling

We collected 29 lake trout and 89 lake whitefish for mercury analysis by gill-netting in the upper lake (Cameron Bay, 54-66m depth) and the middle lake (southern cliff face, 9-20m depth) in May and September/October 2005 (Figure 5.1). Fish were caught in 2.4m (8ft) wide experimental gill nets consisting of four panels 13.5m (45ft) long each, with

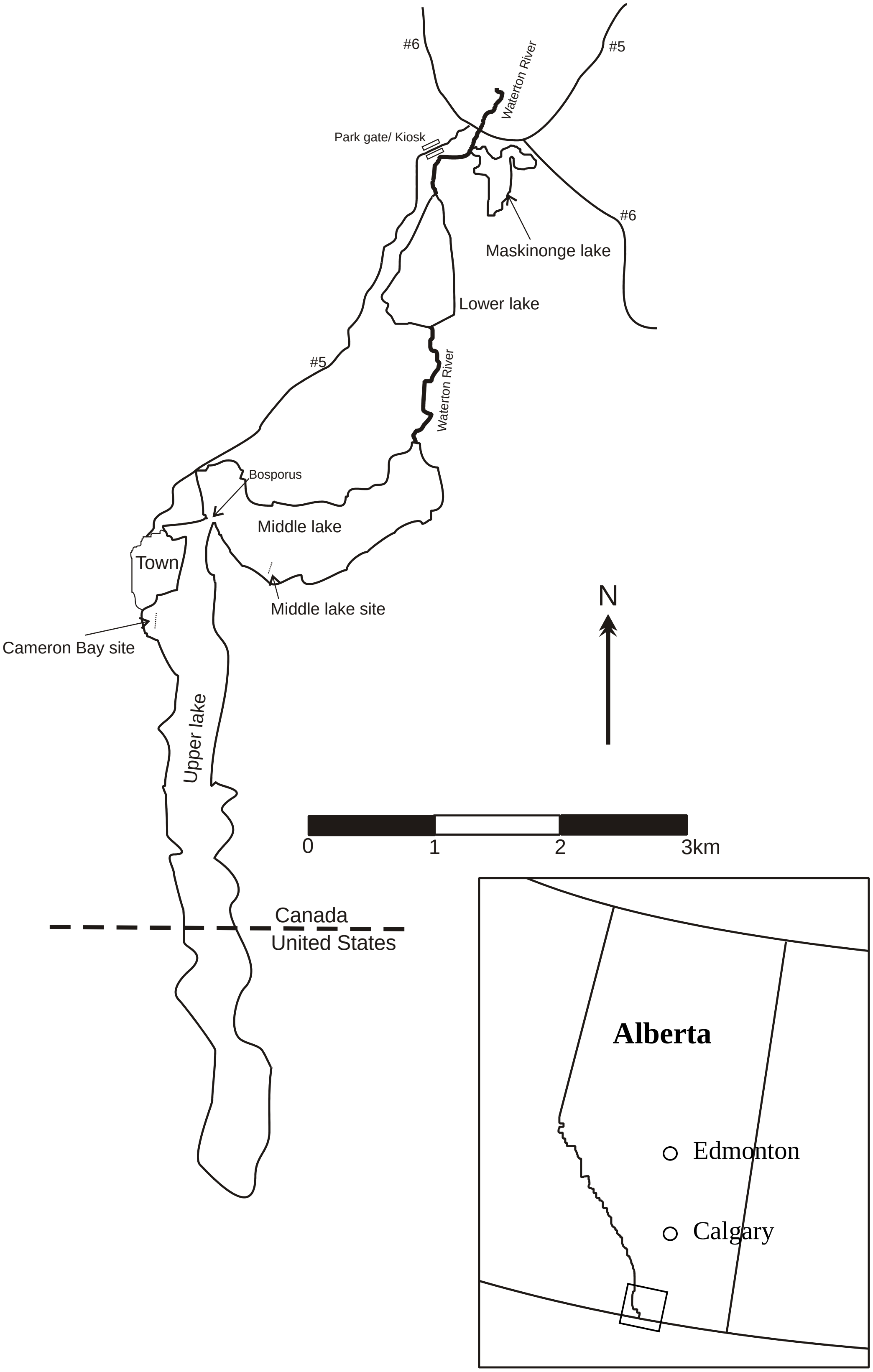

Figure 5.1: System of lakes in Waterton Lakes National Park and 2005 sampling location.

mesh sizes of 10.1, 6.4, 3.8, and 1.3cm. Fish fork lengths (+/- 1mm) and weights (+/- 1g) were recorded and scales, opercular bones and otoliths were collected from lake trout for ageing purposes. Sexes were determined for lake trout. Samples of dorsal skinless, boneless muscle tissue for mercury analysis were frozen under nanopure water. Stomach contents were analyzed qualitatively for species composition. Opossum shrimp (*Mysis relicta*) were collected in plankton trawls in summer 2006, as well as additional stomach samples from lake trout and lake whitefish in both lakes.

5.2.2 Mercury analysis

Reagents suitable for trace metal analysis were used exclusively.

Each tissue sample (~1g fish muscle fresh weight or ~1g pooled invertebrate bodies) was digested with 10ml of 7:3 mix nitric and sulfuric acid (*aqua regia*), aided by 1.0ml of 5% BrCl solution (in conc. HCl) at 90 degrees C on a 36-place aluminum-block-drybath. Prior to addition of BrCl the samples incubated under *aqua regia* at room temperature for one hour. Premature addition of BrCl can lead to violent fizzing and consequential loss of sample and damage to equipment. BrCl solution was prepared in-house.

Samples were placed on the drybath overnight for 12-14hrs. After this period the samples were allowed to cool and subsequently received 0.2ml of 5% (w/v in 3% HCl) potassium dichromate solution. Finally the samples were diluted to 30ml (graduation mark) with nanopure water. Samples were analyzed by means of CVAAS on a PerkinElmer AAnalyst 400 spectrometer coupled to a FIAS 400 with an AS91 autosampler. We used 3% (v/v in nanopure water) HCl as carrier acid and 3% (w/v in 3% HCl) $SnCl_2$ as reducing agent. Mercury vapour was carried to the sample cell by high purity argon gas.

A pooled sample of lyophilized *Mysis* was sent to Flett Research for total and organic mercury analysis.

5.2.3 Standards and quality control

Mercury standards were prepared in *aqua regia*, stabilized with potassium dichromate solution, and diluted with water in the same manner as the samples. The final standard concentrations were blank, 1.00ppb, 5.00ppb, 10.0ppb, 50.0ppb. A new calibration was run for each sample run of 33 samples (+3 quality control samples, 2 of which were spiked).

Quality was assured by analysis of interspersed quality control samples (certified reference material DORM-2 by NRC Canada). Approximately 0.1g of reference material was digested the same way as unknown samples. With our analysis protocol we have been able to measure tissue concentrations of 97% (average) of the referenced THg concentration; spike recoveries averaged around 99%.

5.2.4 Statistical analysis and literature comparisons

Statistical computations (ANCOVA, linear regression, t-tests) were performed in R Version 2.2.1 (2005). Total mercury data for lake trout and lake whitefish were $\log_{10}$ transformed prior to statistical analysis to normalize the data and reduce heteroscedasticity.

Data were compared with data provided in Stafford et al. (2004) and Cabana et al. (1994). For the comparison with Stafford et al. (2004) total mercury versus fork length distributions were reconstructed using equations provided in the publication. Regression models were adjusted from total length to fork length by multiplying the coefficient by 1.09 for lake trout, and 1.12 for lake whitefish, based on estimates from Waterton Lakes fish (Table 5.1; see also Stafford et al. 2004 and references therein). Moreover, since regression models in Stafford et al. (2004) predict $\log_{10}$THg in ng/g, the intercept was adjusted by subtracting 3 ($\log_{10}$ 1000 = 3) to convert to μg/g (ppm). Model parameters (coefficients and intercepts) were then compared with parameters from Waterton Lakes models using t-tests, assuming that standard errors of parameters from Flathead Lake were equal to standard errors from Waterton Lakes (standard errors or mean squares were not shown in Stafford et al. 2004).

Weight estimates for lake trout reported in Cabana et al. (1994) were converted to fork length (mm) based on a model generated from weight versus fork length plots using Waterton Lakes data (Table 5.1). THg data provided by Cabana et al. (1994) were $\log_{10}$ transformed and compared with Waterton Lakes data using analysis of covariance followed by Dunnett's contrasts.

Table 5.1: Models used for conversions and reconstruction of data.

Purpose	Published model	Conversion	Model used
Estimate fork lengths from weights from Cabana et al. (1994)	Weight data (from Cabana et al. 1994)	Fork length versus weight plots (Waterton Lakes samples)	Fork length = 52.697•weight$^{0.3089}$ (R^2 = 0.991)
Reconstruct $\log_{10}$THg data from Stafford et al. (2004)	lake trout: $\log_{10}$THg=0.00129•TL+1.78 lake whitefish: $\log_{10}$THg=0.00126•TL+1.60	lake trout : TL=1.09•FL lake whitefish: TL=1.12•FL (Waterton Lakes samples) 1μg/g=1000ng/g	lake trout: $\log_{10}$THg=0.00141•FL-1.22 lake whitefish: $\log_{10}$THg=0.00141•FL-1.40

5.3 Results

5.3.1 Mercury levels

Mercury levels in lake trout and lake whitefish increased with fork length (Figure 5.2A & B, for linear regression results refer to Appendix F). The average mercury concentration in lake trout was 0.40ppm (range of values between 0.16 and 1.41ppm). Lake trout around 700mm length approximated or exceeded the 0.5ppm Health Canada consumption limit for total mercury (Figure 5.2A). Mercury levels in all lake trout below 400mm length were below the consumption limit. Fish within the length range of 400 and 600mm ranged between 0.23 and 1.41ppm total mercury (Figure 5.2A). Mercury levels in lake whitefish were 0.10ppm on average (range 0.03 to 0.26ppm) and were below the Health Canada consumption limit. The concentration of total mercury in *Mysis* was 139ng/g dry weight (0.023ppm wet weight equivalent); the fraction of methylmercury was 70.5%. Thus an eight-fold increase of mercury concentration is observed between *Mysis relicta* and <350mm lake trout (the size class feeding predominantly on *Mysis*, Table 5.2) in the upper lake. Lake trout >700mm from the middle lake biomagnify mercury ten-fold over their preferred prey of 200-300mm lake whitefish.

5.3.2 Literature comparisons

Regression model parameters of fork length versus $\log_{10}$THg distributions presented in this study were compared with parameters for Flathead Lake, Montana using t-tests (Appendix F). The results indicate that lake trout from both systems were not significantly different when samples from both Waterton basins were included. Lake whitefish mercury levels were significantly higher in Flathead Lake than in Waterton

Lakes. Slopes of the regression models were not significantly different (Appendix G). The mercury concentration in *Mysis relicta* from Waterton Lakes (0.023µg/g) was about 2.4 times higher relative to Flathead Lake (9.6ng/g).

The comparison with class 1, 2, and 3 lakes in Ontario, using analysis of covariance, showed that lake trout mercury levels in Waterton Lakes do not differ from class 2 lakes, but were significantly higher compared with class 1 lakes and significantly lower compared with class 3 lakes, when data from both Waterton basins are included (Appendix F).

5.3.3 Stomach contents

The composition of stomach contents of lake trout and lake whitefish is summarized in Table 5.2. Stomach contents suggest that *Mysis relicta* is no significant food source for lake whitefish, being absent from stomach samples from all but 1 individual. Piscvory was observed in only one lake whitefish, which had consumed a deepwater sculpin (*Myoxocephalus thomsoni*) prior to capture.

Young lake trout feed predominantly on *Mysis relicta*, but shift towards more piscivory at a length of approximately 350mm.

Table 5.2: Summary of the qualitative analysis of stomach contents in lake trout and lake whitefish from Waterton Lakes National Park. Combined observations from 2005 and 2006 samples.

Basin	Fish species	Composition of stomach contents
Middle lake	**Lake trout**	
	500-700mm	Lake whitefish 100-170mm, chironomid pupae
	>700mm	Lake whitefish 200-300mm, chironomid pupae, occasionally notonectidae, small lake trout
	Lake whitefish	
	200-500mm	Trichoptera larvae, *Pisidium*, amphipods, chironomid larvae, *Limnaea*, sphaerids
Upper lake	**Lake trout**	
	<350mm	*Mysis*, occasionally fish <75mm
	350-400mm	*Mysis*, YoY sculpins, lake whitefish <100mm
	400-500mm	Lake whitefish <150mm, *Mysis*, YoY sculpins
	500-600mm	Lake whitefish 100-180mm occasionally burbot and suckers
	>600mm	Lake whitefish >150mm
	Lake whitefish	
	200-400mm	*Pisidium*, *Diporeia*, chironomid larvae

Initially prey fish are dominated by young deepwater sculpins and lake whitefish; lake trout gradually shift diets towards larger lake whitefish as they get larger. *Mysis relicta* was absent from stomachs of lake trout >500mm in either lake. Plankton trawls at night showed that *Mysis relicta* was abundant in the upper lake but absent from the middle lake. The plankton community in the middle lake at the time of sampling consisted of calanoid copepods, chironomid pupae, and larval burbot (*Lota lota*). In the upper lake the plankton community was dominated by *Mysis relicta* (both juvenile and adult), besides calanoid copepods, fish larvae (mostly sculpins but also burbot), and occasionally *Diporeia*.

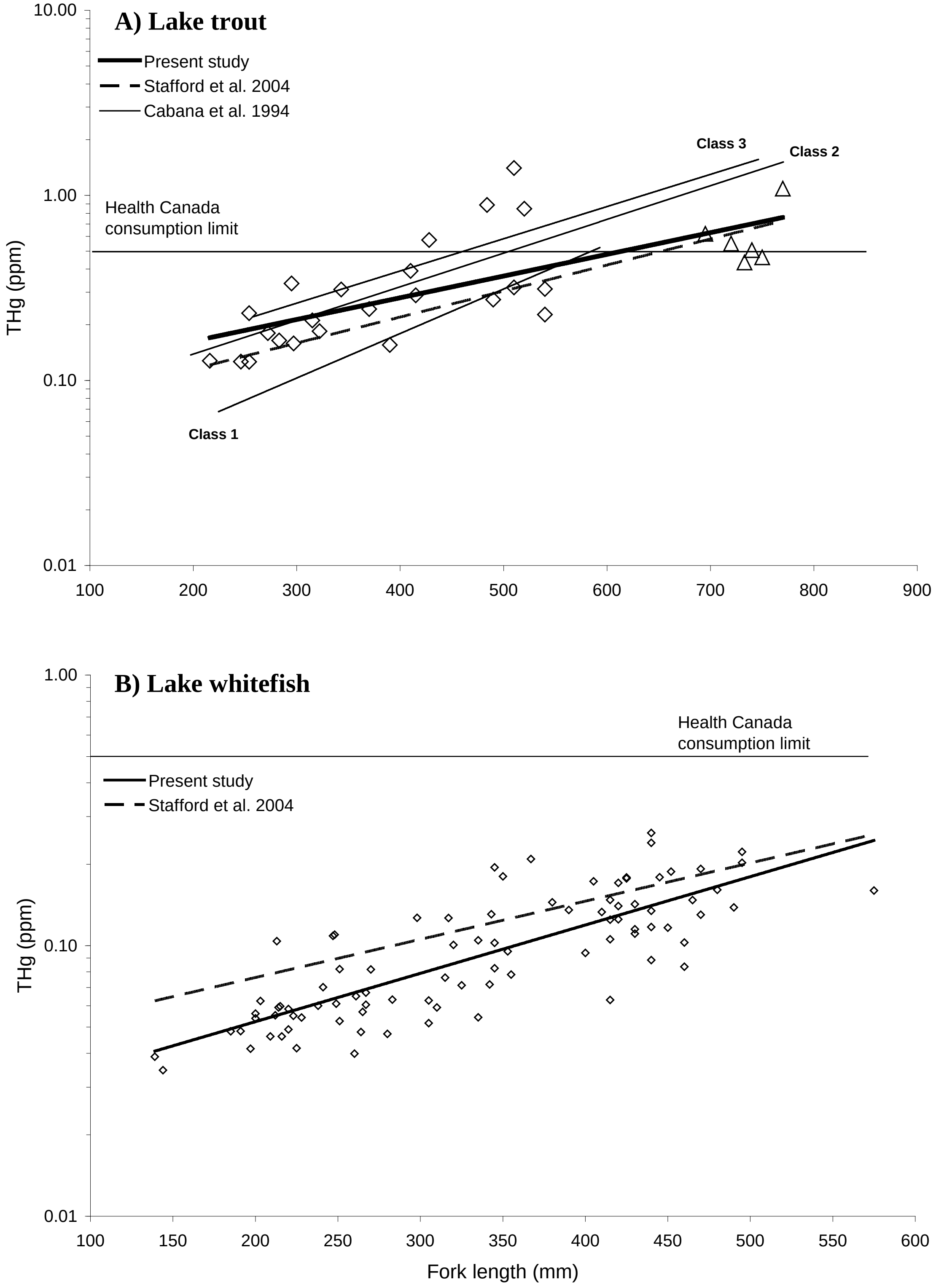

Figure 5.2: A) Total mercury concentration (THg) versus fork length of lake trout in Waterton Lakes National Park. Diamonds indicate upper lake samples, triangles indicate middle lake samples. Reproduced estimates for Flathead Lake, Montana (Stafford et al. 2004) and Ontario lakes (Class 1-3, Cabana et al. 1994) as individual trendlines for illustrative purposes (for ANCOVA-derived models see Appendix F. B) THg versus fork length in lake whitefish from Waterton Lakes National Park. Combined samples for both lakes. Reproduced estimates for Flathead Lake, Montana (Stafford et al. 2004) as individual trendline.

5.4 Discussion

5.4.1 Mercury levels

Mercury levels in lake trout approached or exceeded the Health Canada consumption limit of 0.5ppm in fresh muscle in lake trout >500mm fork length. Based on these findings Parks Canada amended their 2006 fishing regulations with a mercury advisory for Waterton Lakes National Park. All lake whitefish within the sample were well below the consumption limit and were thus no reason for concern. Since Waterton Park features pristine watersheds, minimally impacted either by human activities or by recent forest fires, the mercury loading in the lakes is most likely the result of historical deposition of atmospheric mercury (anthropogenic or natural) to the lakes and their watersheds. A growing body of evidence suggests that forest fires may release large quantities of mercury (Sigler et al. 2003, Friedli et al. 2003, Turetsky et al. 2006, Biswas et al. 2007) to the atmosphere and thus exacerbate mercury contamination in the biota of remote aquatic ecosystems through dry deposition of ashes downwind. With predominantly southwesterly air currents, it is conceivable that Alberta received elevated mercury loadings from large wildfires in the forests of British Columbia, Washington, and Oregon, prior to human intervention and fire suppression. Other geochemical processes may also contribute mercury to Waterton Lakes. Weathering of mercury-rich soil and rock (Downs et al. 1998, Rasmussen 1994), as well as volcanic activity are mentioned as important contributors (Nriagu 1979, Lindqvist et al. 1984, Lindberg et al. 1987). The Circum-Pacific belt has been implicated as one of the most important geological sources of mercury (Jonasson & Boyle 1972), and may be particularly important in northwestern North America. Most of the northwestern United States and Canada received deposits of tephra (see Beierle & Bond 2002, Beierle & Smith 1998) following the eruption of Mount St. Helens in 1980 and from earlier events, and therefore these events may have contributed to the mercury burden in Alberta's landscapes and elsewhere (see Schuster et al. 2002).

5.4.2 Basin-specific variation and fish growth

All upper lake fish were <550mm long, whereas middle lake fish were >690mm long, yet mercury concentrations of certain upper lake fish exceeded those of middle lake fish. Thus there were two groups that had mercury concentrations above the 0.5ppm consumption limit; these were the >690mm individuals from the middle lake, and, even higher in mercury, the >450mm group from the upper lake. Analysis of covariance (Appendix F) showed that lake trout from the middle basin were significantly lower in mercury than trout from the upper basin (Figure 5.3). Stafford et al. 2004 indicate that different mercury levels of lake trout from in Flathead Lake, Montana correlate with habitat choice: deep-dwelling fish have higher mercury levels than littoral-dwelling fish, likely due to their proximity to mercury-enriched hypolimnetic water (see Bloom et al. 1991, Herrin et al. 1998). Since our samples were collected at only one depth at each location, a depth dependency as observed in Flathead Lake by Stafford et al. (2004) cannot be accounted for.

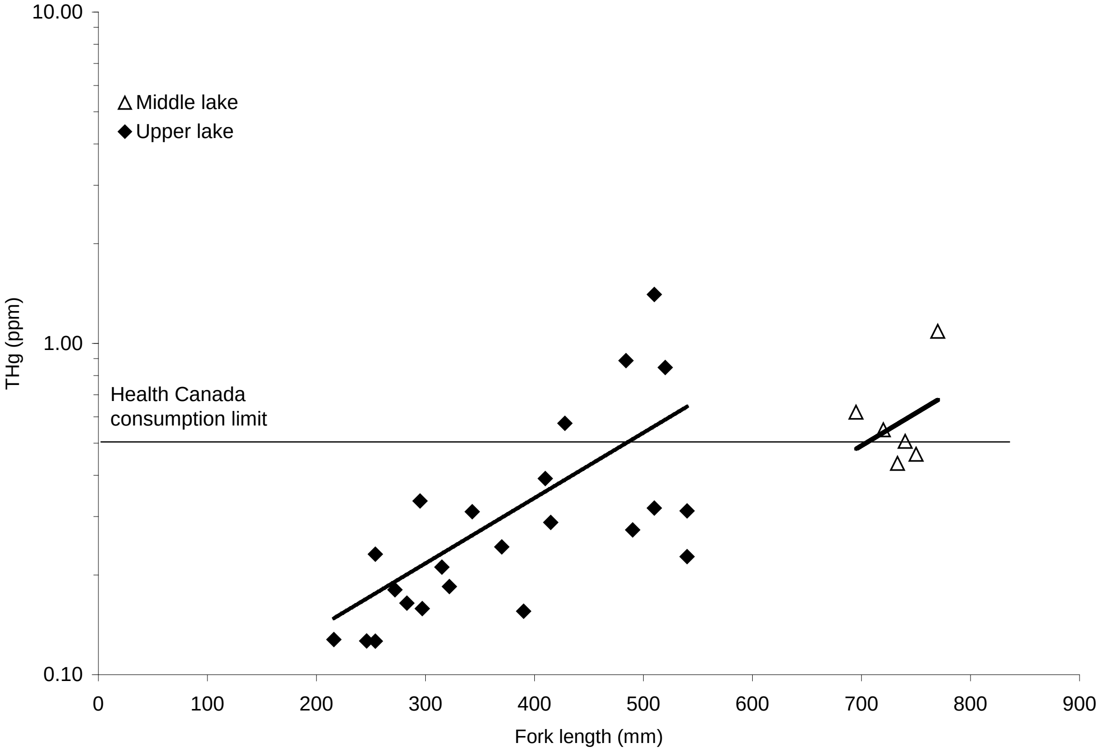

Figure 5.3: **Distribution of mercury signatures based on lake of origin.**

Plots of age versus fork length and weight (Figure 5.4A & B) showed that middle lake fish reached greater lengths and weights compared with upper lake fish of the same age (ANCOVA results are shown in Appendix F). Lake trout in the middle lake were longer and heavier relative to upper lake fish of the same age. Thus growth rates of lake trout in the middle lake are greater. Growth rates depend on the bioenergetic budget of the fish. The implications of bioenergetics on bioaccumulation of contaminants in fish are increasingly understood in the literature (Trudel et al. 2000, Trudel & Rasmussen 2001, 2006, deBruyn & Gobas 2006, Harris & Bodaly 1998, Korhonen et al. 1995, Borgmann & Whittle 1992). Field studies have shown that growth rates at least partially modulate mercury levels in fish (Simoneau et al. 2005, Essington & Houser 2003, Jackson 1991, Swanson et al. 2003, but see Stafford & Haines 2001). A more productive food web translates into lower activity costs while feeding and thus a greater proportion of the energy budget available for growth. The contaminant burden assimilated from the food source is thus diluted in a greater amount of new body tissue (growth dilution). Doyon et al. (1998) have observed that dwarf lake whitefish bioaccumulate mercury faster than normal lake whitefish, and Trudel et al. (2001) have established the link between mercury levels and bioenergetics of these fish. Riget et al. (2000) observe that overall mercury levels were 10-15-fold lower in anadromous arctic charr (*Salvelinus alpinus*) relative resident individuals. The authors suggest that feeding in low-exposure estuarine food webs results in lower mercury levels in anadromous arctic charr, yet, at the same time, observe that resident individuals were smaller at a given age relative to anadromous individuals. Thus bioenergetic variability is likely an important additional factor determining mercury levels in arctic charr as well. In Waterton Lakes elevated growth rates in middle lake fish may be caused by a greater abundance of large lake whitefish, enabling lake trout to make a diet shift. The bioenergetic advantages resulting from such diet shifts as well as the effects of food web structure on growth rates in lake trout have been shown before (Sherwood et al. 2002, Pazzia et al. 2002).

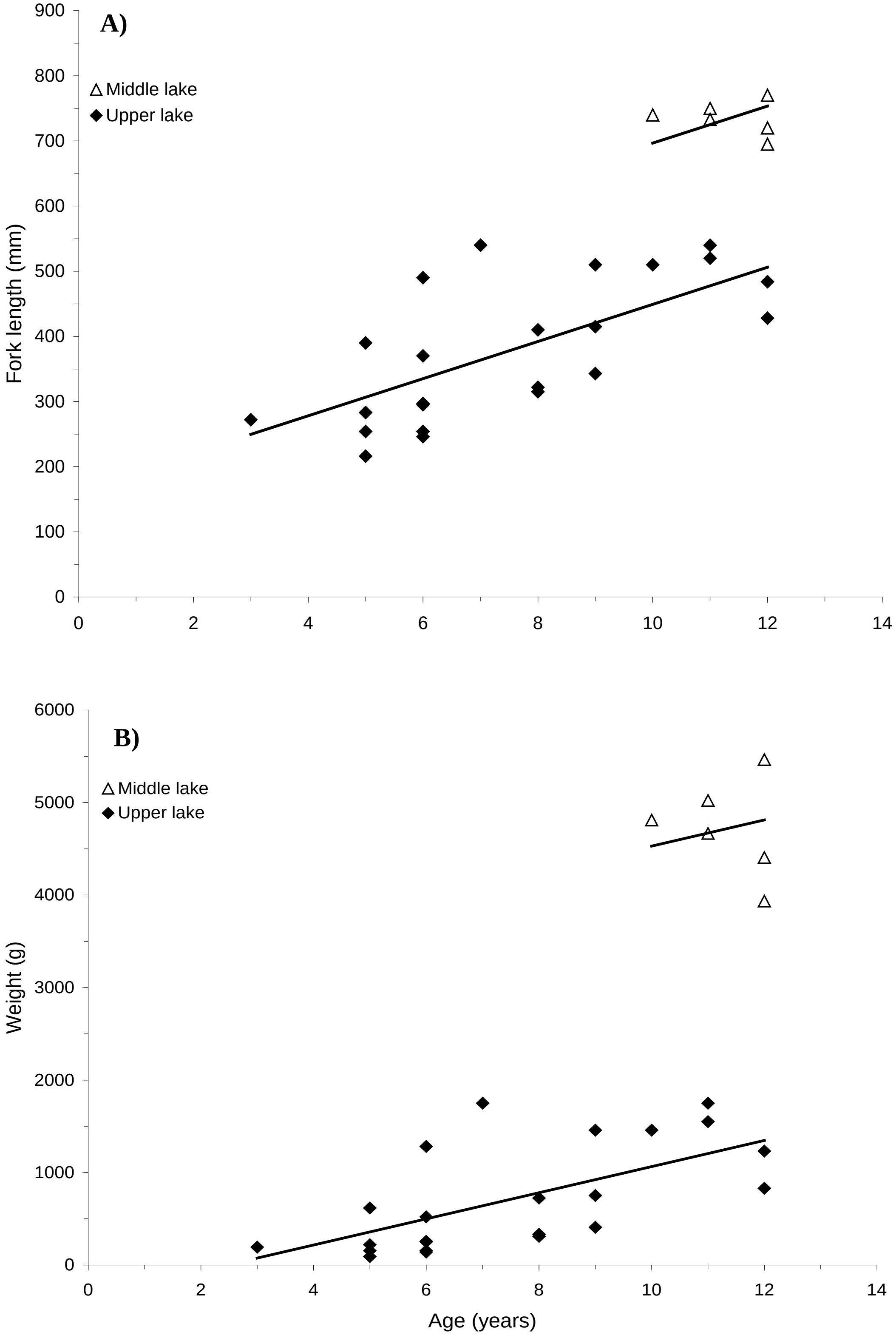

Figure 5.4: A) & B) Growth parameters of lake trout from the upper and middle lake.

5.4.3 Literature comparisons

Mercury levels in lake trout from Waterton Lakes were not different compared with Flathead Lake, Montana, however, the analysis was repeated eliminating middle lake data points (Appendix G). The overall outcome of the comparison did not change significantly and a difference between the populations in Waterton and Flathead Lakes was still not detected.

Lake whitefish from Flathead Lake were found to have approximately 1.7-fold higher mercury levels. Absence of *Mysis* from lake whitefish stomachs may in part explain the low mercury levels in Waterton lake whitefish, which may in turn be an indication that *Mysis* is not a significant part of the whitefish diet at any time. Yet, the relatively higher mercury levels in Flathead lake whitefish are hard to explain, because lake whitefish appear to have similar diets in the two lakes.

Mercury concentrations in *Mysis relicta* were more than twice as high in Waterton compared with Flathead Lake. Chess and Stanford (1998) have shown that Flathead Lake is a much more productive system for *Mysis* than Waterton Lakes. *Mysis* successfully exploited *Daphnia thorata* in Flathead Lake (*D. thorata* is not found in Waterton Lakes). Energy assimilation was efficient and resulted in a reduction in generation time to one year (two years in Waterton), greater fecundity, and elevated lipid reserves for offspring. It is conceivable that growth dilution is a significant factor resulting in low mercury levels in *Mysis*.

Analysis of covariance suggested that mercury levels in Waterton lakes differed from class 1 and 3, but were equal to class 2 lakes. This may be in part caused by lower mercury levels in lake trout from the middle basin. Analysis of covariance after removing middle lake data points from the distribution showed that the upper basin (class 3) lake trout had mercury levels not significantly different than either class 2 and 3 lakes (Appendix G). Interaction terms were not significant in any case.

This analysis supports the idea that mercury levels in lake trout are sensitive to trophic regimes, and results seem to be consistent across broad geographic regimes.

5.4.4 Food webs

Examination of stomach contents and zooplankton samples suggested that the middle lake is a class 2 lake (Rasmussen et al. 1990, Cabana et al. 1994), in which forage fish and lake trout are present and *Mysis relicta* is absent. The upper lake in contrast is a typical class 3 lake (Rasmussen et al. 1990, Cabana et al. 1994) with forage fish, lake trout and *Mysis*. Cabana et al. (1994) report that Hg levels in lake trout increase in the order of class 1, 2, and 3 lakes, respectively. A similar observation was made earlier by Rasmussen et al. (1990) with respect to PCB levels in lake trout. This explanation would assume that there are separate populations of lake trout in the two lakes, implying limited exchange across the Bosporus (the narrow channel connecting the two basins). Moreover, the model requires that forage fish in class 3 lakes are trophically elevated compared with class 2 lakes, by feeding on *Mysis*. The overall homogeneity of whitefish mercury levels and their uniform diet, which consists mainly of mollusks rather than Mysis, does not support this explanation. Yet lake trout were observed to feed on deepwater sculpins, which may result in trophic elevation of lake trout in the upper lake relative to middle lake fish. This observation should be substantiated by nitrogen stable isotope analysis, which provides a more time-integrated estimate of trophic position than stomach contents

(Vander Zanden et al. 1997). Even though lake trout in the upper basin may be trophically elevated relative to middle basin lake trout, further research has to show which link in the food chain is causing it. The role of *Mysis* in this system remains to be shown.

5.5 Conclusion

Mercury levels in lake trout larger than 400mm fork length potentially exceed the consumption limit of 0.5ppm, and fish larger than 700mm are all within close proximity or above the consumption limit. Based on these results consumption limits are warranted for lake trout. Parks Canada posted consumption advisories in the 2006 fishing regulations for Waterton Lakes National Park. The current data suggest that lake trout from the middle lake have lower mercury levels relative to upper lake individuals. Growth rates of middle basin lake trout were elevated relative to upper basin lake trout and we conclude that bioenergetic heterogeneity may play a role in modulating mercury levels in lake trout.

The presence of deepwater sculpins and *Mysis relicts* in lake trout stomachs from the upper lake suggests that lake trout in this system may be trophically elevated relative to middle lake fish. Trophic elevation of lake whitefish in the upper lake is not evident so far since feeding habits do not seem to contrast in both lakes, and disjunction of mercury signatures was not obvious. Mercury levels in lake trout compare well with other class 2 and 3 systems even across broad geographical regimes.

Chapter VI

Conclusion

6.1 Mercury biomagnification in the upper South Saskatchewan River Basin

6.1.1 Mercury levels in sport fish

One of the main objectives of this research was to collect mercury data for sport fish in order to assess the necessity for consumption advisories. The data presented herein show that mercury levels in piscivorous sport fish are high. Mercury levels in northern pike (*Esox lucius*), Walleye (*Stizostedion vitreum*) from the Oldman River, and lake trout (*Salvelinus namaycush*) from Waterton Lakes typically exceed the Health Canada consumption limit of 0.5ppm THg in fresh muscle tissue. Invertebrate-feeding sport fish, such as Goldeye and Mooneye (*Hiodon alosoides, Hiodon tergisus*) from the Oldman River, and lake whitefish (*Coregonus clupeaformis*) from Waterton Lakes are typically below 0.5ppm. Mercury levels in northern pike from Twin Valley Reservoir, a newly constructed storage reservoir exceeded 0.5 ppm and were significantly elevated (3.5-fold) compared with northern pike from the Oldman, and in most cases exceeded (up to 2-fold) previously published data from other reservoirs of the northern hemisphere.

6.1.2 Mercury sources

Mercury levels in longnose dace (*Rhinichthys cataractae*) and net-spinning caddisfly larvae were measured to assess whether a mercury gradient exists in the biota along the Oldman River continuum. Mercury levels in longnose dace and suckers (*Catostomus catostomus, Catostomus commersoni*) affected by domestic sewage, urban and agricultural runoff were measured to identify potential sources of mercury to the food webs of the river system. Mercury levels in caddisfly larvae and longnose dace increased from upstream to downstream sites; however, the gradient was more than 2-fold steeper in dace relative to caddisflies, which shows that an interpretation focused on mercury loadings is insufficient in explaining the observed mercury levels in longnose. Since the aquatic portion of the lifecycle of hydropsychid caddisflies is centered around feeding and growth, the observed gradient of mercury concentrations in these organisms is likely reflecting the actual background loading in the river. Fish exposed to agricultural and urban effluents had significantly lower mercury levels, or showed no difference, relative to reference sites, which suggests that these effluents contribute no significant mercury loading to river food webs. Although it cannot be ruled out that these effluents contribute a net mercury loading to the water, enrichment-derived biological processes in the food webs (biodilution) may offset the effect of increased loading.

With respect to the hypothesis that the flow-augmented stream system feeding Twin Valley Reservoir contributes to the mercury loading in the reservoir, it was shown that mercury levels in fish from the Little Bow River upstream of the reservoir were lower by comparison with sites along the Oldman River. Thus the tributary unlikely contributes to the high mercury loadings within the reservoir and the source for mercury contamination lies within the recently inundated soils of the basin.

6.1.3 Bioenergetic factors affect mercury levels in fish of the SSRB

Longnose dace exhibited a more pronounced mercury gradient relative to hydropsychid caddisfly larvae, which form part of the diet of dace. This suggests that mercury levels in this species are more affected by bioenergetic factors, although the underlying mechanism is unknown. It is conceivable that mercury levels correlate more closely with the temperature gradient in the river, causing dace in downstream reaches to invest more energy into gonadal growth and spawning rather than somatic growth. Furthermore toxicant induced stress could lead to elevated metabolic rates in these fish, thus slowing down their growth rate in downstream reaches. The bioenergetic gradient in longnose dace may bear significant consequences for mercury levels in sport fish, especially walleye, which presumably feed significantly on dace. Even though background mercury levels are relatively low and point sources are not found, longnose dace act as significant concentration step, thus providing walleye with an abundant high mercury food source. This may in part explain the high mercury levels observed in walleye.

Mercury levels in northern pike from Twin Valley Reservoir were considered extremely high given that these fish were not piscivorous. Mercury mass balance modeling has shown that elevated feeding rates and reduced growth rates of 3 and 4-year old fish in this new irrigation reservoir are possible explanations for high mercury levels, exacerbating the effects of increased loadings from inundated soils.

Mercury data for biota in the upper and middle lakes in Waterton Lakes National Park, Canada generally fell within the range of data published for similar systems in the northern hemisphere. More interestingly, differences were detected between the closely connected upper and middle basins of the Waterton Lakes system. Analysis of stomach samples and zooplankton communities revealed that the upper lake is a class 3 lake (lake trout, forage fish and *Mysis relicta*) and the middle is a class 2 lake (lake trout, forage fish). Results indicate that lake trout belong to two different groups, with middle lake fish having lower length-corrected mercury levels than upper lake fish. In addition, on an age-corrected scale, middle lake fish reach larger sizes compared with upper lake fish. This suggests that mercury levels in lake trout are determined significantly by bioenergetic factors.

6.1.4 Implications and future trends

Management of the mercury problem in the Oldman River will be a challenge. The effect of temperature requires additional data to be elucidated conclusively and not much is known about the interactions of toxicant stress and mercury bioaccumulation in fish. Assuming that temperature regimes will play an important role one can speculate that higher temperatures due to global warming will exacerbate the problem. Furthermore the scarcity of water in Alberta could result in further reduction of summer flows in the Oldman which will by itself result in elevated stream temperatures. Future work focusing on in-stream flow needs should include the bioenergetic gradient in longnose dace (or potentially other fish species) as another criterion to maintain ecological integrity in the river.

Mercury levels in northern pike of Twin Valley Reservoir may reach maximum levels by 2007 or 2008, since peak mercury levels in reservoirs are commonly observed within 2-5 years after impoundment, given that nothing else changes. In the case of Twin Valley Reservoir, however, it is possible that the forage fish population increases in size and northern pike are given the chance to change diets and become piscivorous. Nothing is known about mercury relationships between piscivorous and non-piscivorous northern pike. Mercury levels depend on trophic guild and one would expect non-piscivorous pike to exhibit lower mercury levels compared with piscivorous pike. On the other hand, non-piscivorous pike encounter a bioenergetic bottleneck early on in their life, which would result in elevated mercury levels. The question comes to mind whether bioenergetic constraints of non-piscivorous pike are sufficient to override the effects of trophic guild. Systems suitable for such a comparison are numerous in Southern Alberta.

Despite the otherwise pristine nature of Waterton Lakes National Park the atmospheric mercury burden constitutes a significant source of the contaminant to this and other remote regions. A combination of physico-chemical factors and biological interactions can lead to high mercury levels in attractive sport fish species such as lake trout. In Waterton Lakes mercury concentrations in lake trout can exceed levels considered by health authorities to be safe for human exposure. Since there exists no commercial fishery in this park, recreational fishing pressure is generally low, and daily catch limits (2 lake trout per day) are hardly ever filled, the risk of human exposure to high levels of the contaminant from this particular source is negligible. Yet mercury advisories should be maintained to increase the public's understanding of the risks associated with mercury and raise awareness of the problem of mercury in remote areas. The future trend that mercury levels in Waterton are going to follow is hard to estimate. It is known that wildfires release large quantities of mercury stored vegetation and forest soils into the atmosphere. With global warming inevitable, hotter and drier summers along the Pacific coast may increase the frequency and severity of wildfires. This may elevate atmospheric fallout of mercury in the Waterton Lakes watersheds and result in increasing mercury levels in the biota.

While this research was successful in showing that mercury levels in lake trout exceed the consumption guideline of 0.5ppm, it also served as a preliminary assessment of pelagic communities and trophic interactions among organisms, and as such, clearly identifies gaps in our knowledge with respect to these lakes. While the Waterton Lakes system was previously considered a class 3 system, this present research suggests that this classification is too general and only partially correct. The middle basin, although closely connected to the upper basin by a narrow channel, is likely not inhabited by *Mysis relicta*, which occurs in large numbers in the upper basin. Additional research is required to fully understand the mechanisms at work in Waterton Lakes. Further questions to be asked may include: What is the role of *Mysis relicta* in the pelagic food chain? How separate are middle basin and upper basin lake trout populations? Are there separate spawning grounds? What is the significance of bioenergetic differences? Do lake trout exhibit partially migratory life-histories and what is their range of movement? This last question is based considerably on knowledge obtained during 2006 sampling efforts, the data from which have not yet been analyzed in great detail. Yet, despite great efforts, and large sample sizes, lake trout smaller than 55cm have not been caught in the middle lake, and we know that large lake trout occur temporarily in the lower lake as well. Future work should involve growth rate measurements of forage fish and lake trout, estimates of bioenergetic budgets, and trophic position estimates using carbon and nitrogen stable isotope techniques for the upper and middle basins. It is conceivable that “lake residency” can be traced in the annuli of opercular bones using C and N stable isotopes and it may be possible to locate the age at which a shift in habitat occurred.

6.2 Future approaches to the question of mercury sources:

6.2.1 Mercury in sediments

The ultimate source (i.e. anthropogenic versus natural) of the mercury burden in the upper SSRB remains unknown. It has been argued that elevated atmospheric mercury inputs are reflected in the distribution pattern of mercury in lake sediments (Fitzgerald et al 1998, Swain et al. 1992), a perspective that has also been debated in the literature (Kemp et al. 1978). Some authors argue that the observed pattern could also be caused by post-depositional diagenetic processes that render mercury mobile within the sediments (see Kemp et al. 1978, Gobeil & Cossa 1993, Shaw et al. 1990). Evidence for this possibility has been presented (Krabbenhoft & Babiarz 1992, Matty & Long 1995), but post-depositional fluxes seem insufficient to explain observed patterns entirely (Gobeil & Cossa 1993). More recently it has been proposed that geological factors and biogeochemical cycling of mercury are the key factors causing the highly variable patterns of mercury concentrations in sediments of their study lakes, and thus are severely challenging the traditional view of human-caused atmospheric loading as the dominant factor (El Bilali et al. 2002, Gobeil et al. 1999, Rasmussen et al. 1998). Schuster et al. (2002) have shown that depth profiles of mercury concentrations in glacial ice cores exhibit remarkable resolution and clearly display changing atmospheric mercury loadings over time. The authors can clearly associate mercury concentrations in the core with historical man-made or natural events. Whereas industrial activity contributed to the bulk loading over time, volcanic events resulted in significant short term peaks in mercury concentration (Schuster et al. 2002).

6.2.2 Mercury isotope signatures

Recently the utility of mercury stable isotopes for tracing natural and anthropogenic sources has been proposed (Jackson 2001a, Jackson & Muir 2004), and vigorously debated (Hintelmann et al. 2001, Jackson TA 2001b, Ridley & Stetson 2006). Industrial processes such as smelting, mining, and burning of fossil fuels are thought to result in characteristic fractionation of mercury isotopes. Hence, recently (within the last 150-200 years of industrial activity) deposited anthropogenic mercury is believed to have different isotopic signatures than older, non-anthropogenic mercury. It has been suggested that the isotopic signatures are conserved in sediment cores from lakes (Jackson 2001a), but reliable undisputed data have not been produced until recently (Jackson & Muir 2004). With the recent developments in the field of multi-collector-ICP-MS, the tools for reliable determination of heavy isotopes like mercury are available (Foucher & Hintelmann 2006). Current research conducted on sediment cores from high arctic lakes (Jackson &Muir 2004) focused only on the upper strata (~20cm deep) of sediment. Critics say that the observed fractionation pattern of Hg stable isotopes could have been the result of *biological activity* (methylation and demethylation reactions and metabolism) rather than the alleged *industrial* processes (Hintelmann 2007, personal communication). Possible natural sources of mercury could be geological anomalies that occurred further back in time (eg. the Mazama ash found throughout southern Alberta and in lake sediment cores). Mercury data on such deposits are very limited in the literature, and it would be important to know whether these deposits still constitute, in part or entirely, the pool of active mercury in Alberta. Secondarily the hypothesis can be addressed that the Hg isotopic signature of tephra in the sediment is biologically altered, by comparing it with material from the volcano that produced the tephra, *and* with the corresponding layer of material preserved in ice cores. Mercury deposits preserved in ice cores unlikely underwent post-depositional fractionation and are thus likely to show the true isotopic composition of both man-made and natural atmospheric mercury loadings nicely separated.

References

Abernathy AR and Cumbie PM (1977). Mercury accumulation by largemouth bass (*Micropterus salmoides*) in recently impounded reservoirs. Bulletin of Environmental Contamination and Toxicology 17: 595-602.

Allen EW, Prepas EE, Gabos S, Strachan WMJ, Zhang W (2005). Methyl mercury concentrations in macroinvertebratesand fish from burned and undisturbed lakes on the boreal plain. Canadian Journal of Fisheries and Aquatic Sciences 62: 1963-1977.

Amin-Zaki L, Elhassani S, Majeed MA, Clarkson TW, Doherty RA, Greenwood M (1974). Intra-uterine methylmercury poisoning in Iraq. Pediatrics 54(5): 587-595.

Atwell L, Hobson KA, Welch HE (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis.

Bajkov AD (1935). How to estimate the daily food consumption of fish under natural conditions. Trans. Am. Fish. Soc. 65: 288-89.

Balogh S & Liang L (1995). Mercury pathways in municipal wastewater treatment plants. Water, Air, and Soil Pollution 80: 173-183.

Balogh SJ, Meyer ML, Hansen NC, Moncrief JF, Gupta SC (2000). Transport of mercury from a cultivated field during snowmelt. Journal of Environmental Quality 29 (3): 871-874.

Balogh SJ, Nollet YH, Offerman HJ (2005). A comparison of total mercury and methylmercury export from various Minnesota watersheds. Science of the Total Environment 340: 261-270.

Beers CE & Culp JM (1990). Plasticity in foraging behaviour of a lotic minnow (*Rhinichthys cataractae*) in response to different light intensities. Canadian Journal of Zoology 68: 101-105.

Beierle B, Bond J (2002). Density-induced settling of tephra through organic lake sediments. Journal of Paleolimnology 28(4): 433-440.

Beierle B, Smith DG (1998). Severe drought in the early Holocene (10000-6800 BP) interpreted from lake sediment cores, southwestern Alberta, Canada. Palaeogeography, Palaeoclimatology, Palaeoecology 140(1-4): 75-83.

Palaeoclimatology, Palaeoecology 140(1-4): 75-83.

Bishop JN & Neary BP (1976). Mercury levels in fish from Northwestern Ontario, 1970-1975. In: Report of Laboratory Services Branch, Ontario Ministry of the Environment, Toronto, Ontario, p84.

Biswas AJD, Blum JD, Klaue B, Keeler GJ (2007). Release of mercury from Rocky Mountain forest fires. Global Biogeochemical Cycles 21: GB1002.

Bloom NS, Watras CJ, Hurley JP (1991). Impact of acidification on the methymercury cycle of remote seepage lakes. Water, Air, and Soil Pollution 56: 477-491.

Bodaly R.A., Rudd J.W.M., and R.J. Flett (1998): “Effect of urban sewage treatment on total and methyl mercury concentrations in effluents”, Biogeochemistry 40, pp. 279-291

Bodaly RA and Lesack LFW (1984). Response of a boreal northern pike (*Esox lucius*) population to lake impoundment: Wupaw Bay, Southern Indian Lake, Manitoba. Can. J. Fish. Aquat. Sci. 41:706-714.

Bodaly RA, Hecky RE, Fudge RJP (1984). Increase in Fish Mercury Levels in Lakes Flooded by the Churchill River Diversion, Northern Manitoba. Can. J. Fish. Aqu. Sci. 41: 682-691.

Bodaly RA, Rudd JWM, Flett RJ (1998) Effect of urban sewage treatment on total methyl mercury concentrations in effluents. Biogeochemistry 40: 279-291.

Borgmann U & Whittle DM (1992). Bioenergetics and PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): a model based on surveillance data. Canadian Journal of Fisheries and Aquatic Sciences 49: 1086-1096.

Boudou A & Ribeyre F (1997). Mercury in the Food Web: Accumulation and Transfer Mechanisms. Metal Ions in Biological Systems 34: 289-319

Bregazzi PR and Kennedy CR (1980). The biology of pike, *Esox lucius* L., in a southern eutrophic lake. J. Fish. Biol. 17: 91-112.

Brylinski M (2000). An Evaluation of Changes in the Yellow Perch (*Perca flavescens*) Population of Garfton Lake, Kejimkujik National Park after Dam Removal. Acadia Centre for Estuarine Research Publ. No. 59.

Burgess NM & Hobson KA (2006). Bioaccumulation of mercury in yellow perch (*Perca flavescens*) and common loon in relation to lake chemistry in Atlantic Canada. Hydrobiologia 567: 275-282.

Cabana G & Rasmussen JB (1994). Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372: 255-257.

Cabana G, Tremblay A, Kalff J, Rasmussen JB (1994). Pelagic food chain structure in Ontario lakes: A determinant of mercury levels in lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 51: 381-389.

Canavan CM, Caldwell CA, Bloom NS (2000). Discharge of methylmercury-enriched hypolimnetic water from a stratified reservoir. Science of the Total Environment 260 (1-3): 159-170.

Chapman LJ and Mackay WC (1990). Ecological correlates of feeding flexibility in northern pike (*Esox lucius*). Journal of Freshwater Ecology 5 (3): 313-322.

Chapman LJ, Mackay WC, Wilkinson CW (1989). Feeding flexibility in northern pike (*Esox lucius*) – fish versus invertebrate prey. Can. J. Fish. Aqu. Sci. 46 (4): 666-669.

Chen CY & Folt CL (2005). High plankton densities reduce mercury biomagnification. Environmental Science and Technology 39: 115-121.

Chess DW & Stanford JA (1998). Comparative energetics and life cycle of the opossum shrimp (*Mysis relicta*) in native and non-native environments. Freshwater Biology 40 (4): 783-794.

Chou H.N. and C.A. Naleway (1984). Determination of Mercury by Cold Vapour Atomic Absorption Spectrometry. Analytical Chemistry 56: 1737-1738

Cizdziel JV, Hinners TA, Heithmar EM (2002). Determination of Total Mercury in Fish Tissues using Combustion Atomic Absorption Spectrometry with Gold Amalgamation. Water, Air, and Soil Pollution 135: 355-370.

Clesceri LS, Greenberg AE, Eaton AD (1998). Standard Methods for the Examination of Water and Wastewater, 20th Edition. American Public Health Association, Washington, DC.

Compeau GC & Bartha R (1985). Sulfate-reducing bacteria: principal methylaotors of mercury in anoxic estuarine sediment. Applied Environmental Microbiology 50: 498-502.

Couture P, Rajotte JW (2003). Morphometric and metabolic indicators of metal stress in wild yellow perch (Perca flaveseens) from Sudbury, Ontario: A review. Journal of Environmental Monitoring 5 (2): 216-221.

Culp JM (1989).Nocturnally constrained foraging of a lotic minnow (*Rhinichthys cataractae*). Canadian Journal of Zoology 67: 2008-2012.

deBruyn AMH & Gobas FAPC (2006). A bioenrgetic biomagnification model for the animal kingdom. Environmental Science and Techology 40: 1581-1587.

DesLandes J-C, Guenette S, Prairie Y, Roy D, Verdon R, Fortin R (1995). Changes in fish populations affected by the construction of the La Grande complex (Phase I), James Bay region, Quebec. Can. J. Zool. 73: 1860-1877.

Diana JS (1979). Feeding pattern and daily ration of a top carnivore, the northern pike (*Esox lucius*).Canadian Journal of Zoology 57 (11): 2121-2127.

Diana JS (1983). An energy budget for northern pike (*Esox lucius*). Canadian Journal of Zoology 61 (9): 1968-1975

Diana JS and Mackay WC (1979). Timing and magnitude of energy deposition and loss in the body, liver and gonads of northern pike (*Esox lucius*). J. Fish. Res. Board Can. 36 (5): 481-487.

Downs SG, Macleod CL, Lester JN (1998). Mercury in Precipitation and its Relation to Bioaccumulation in Fish: A Literature Review. Water, Air, and Soil Pollution 108: 149-187

Doyon J-F, Bernatchez L. Gendron M, Verdon A, and Fortin A (1998). Comparison of normal and dwarf populations of lake whitefish (*Coregonus clupeaformis*) with reference to hydroelectric reservoirs in northern Quebec. Arch. Hydrobiol. Spec. Issues Adv. Limnol. 50: 97-108.

Doyon J-F, Schetagne R, Verdon R (1998). Different mercury bioaccumulation rates between sympatric populations of dwarf and normal lake whitefish (*Coregonus clupeaformis*) in the La Grande complex watershed, James Bay, Quebec. Biogeochemistry 40: 203-216

El Bilali L, Rasmussen PE, Hall GEM, Fortin D (2002). Role of sediment composition in trace metal distribution in lake sediments. Applied Geochemistry 17: 1171-1181

El-Awady AA, Miller RB, Carter MJ (1976). Automated Method for the Determination of Total and Inorganic Mercury in Water and Wastewater Samples. Analytical Chemistry 48 (1):110-116

Essington TE and Houser JN (2003). The effect of whole-lake nutrient enrichment on mercury concentration in age-1 yellow perch. Transactions of the American Fisheries Society 132: 57-68.

Fitzgerald WF, Engstrom DR, Mason RP, Nater ED (1998). The case for atmospheric mercury contamination in remote areas. Environmental Science and Technology 32(1): 1-7.

Fjeld E & Rognerud S (1993). Use of Path Analysis to Investigate Mercury Accumulation in Brown Trout (*Salmo Trutta*) in Norway and the Influence of Environmental Factors. Canadian Journal of Fisheries and Aquatic Sciences 50: 1158-1167

Forseth T, Næsje TF, Jonsson B, Hårsaker K (1999). Juvenile migration in brown trout: a consequence of energetic state. Journal of Animal Ecology 68: 783-793.

Foucher D & Hintelmann H (2006). High-precision measurement of mercury isotope ratios in sediments using cold-vapour generation multi-collector inductively coupled plasma mass spectrometry. Analytical and Bioanalytical Chemistry 384: 1470-1478.

Friedli HR, Radke LF, Prescott R, Hobbs PV, Sinha P (2003). Mercury emissions from the August 2001 wildfires in Washington State and an agricultural waste fire in Oregon and atmospheric mercury budget estimates. Global Biogeochemical Cycles 17(2): 1039.

Frost WE (1954). The food of pike, *Esox-lucius* L., in Windermere. J. Anim. Ecol. 23 (2): 339-360.

Garcia E & Carignan R (1999). Impact of wildfire and clearcutting in the boreal forest on methyl mercury in zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 56: 339-345.

Garcia E & Carignan R (2000). Mercury concentrations in northern pike (Esox lucius) from boreal lakes with logged, burned, or undisturbed catchments. Canadian Journal of Fisheries and Aquatic Sciences 57 (Suppl. 2): 129-135

Glass GE, Sorensen JA, Schmidt KW, Rapp GR Jr., (1990). New Source Identification of Mercury Contamination in the Great Lakes. Environmental Science and Technology 24: 1059-1069

Gobeil C, & Cossa D. (1993). Mercury in sediments and sediment pore-water in the Laurentian trough. Canadian Journal of Fisheries and Aquatic Sciences 50: 1794-1800.

Gobeil C, Macdonald RW, Smith JN (1999). Mercury profiles in sediments of the Arctic Ocean basins. Envrionmental Science and Technology 33: 4194-4198.

Gochfeld M (2003). Cases of mercury exposure, bioavailability, and absorption. Ecotoxicology and Environmental Safety 56:174-179

Gorski PR, Cleckner LB, Hurley JB, Sierszen ME, Armstrong DE (2003). Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. The Science of the Total Environment 304: 327-348.

.

Greenfield BK, Hrabik TR, Harvey CJ, Carpenter SR (2001). Predicting mercury levels in yellow perch: use of water chemistry, trophic ecology, and spatial traits. Canadian Journal of Fisheries and Aquatic Sciences 58: 1419-1429.

Griffiths RW, Newlands NK, Noakes DLG, Beamish FWH (2004). Northern pike (*Esox lucius*) growth and mortality in a northern Ontario river compared with that in lakes: influence of flow. Ecology of Freshwater Fish 13 (2): 136-144.

Grigal DF (2002). Inputs and outputs of mercury from terrestrial watersheds: a review. Environmental Reviews 10: 1-39.

Gu B, Schelske CL, Hoyer MV (1997). Intrapopulation feeding diversity in blue tilapia: evidence from stable-isotope analyses. Ecology 78: 2263-66.

Gustin MS, Taylor GE, Maxey RA (1997). Effect of temperature and air movement on the flux of elemental mercury from substrate to the atmosphere. Journal of Geophysical Research 102(D3): 3891-3898.

Hagreen L.A. and B.A. Lourie (2004): ”Canadian mercury inventories: the missing pieces”, Environmental Research 95, pp. 272-281

Hall BD, Bodaly RA, Fudge RJP, Rudd JWM, Rosenberg DM (1997). Food as the predominant pathway of methylmercury uptake by fish. Water, Air, and Soil Pollution 100: 12-24.

Hall BD, Rosenberg DM, Wiens AP (1998). Methyl mercury in aquatic insects from an experimental reservoir. Canadian Journal of Fisheries and Aquatic Sciences 55: 2036-2047.

Hanson PC, Johnson TB, Schindler DE, Kitchell JF (1997). Fish Bioenergetics 3.0. University of Wisconsin, Sea Grant Institute, Madison.

Harris HH, Pickering IJ, George GN (2003). The chemical form of mercury in fish Science 301 (5637): 1203-1203.

Harris RC & Bodaly RA (1998). Temperature, growth and dietary effects on fish mercury dynamics in two Ontario lakes. Biogeochemistry 40: 172-187.

Herrin RT, Lathrop RC, Gorski PR, Andren AW (1998). Hypolimnetic methylmercury and its uptake by plankton during fall destratification: A key entry point of mercury into lake food chains? Limnology and Oceanography 43(7): 1476-1486.

Hickie B.E., Kingsley M.C.S., Hodson P.V., Muir D.C.G., Beland P., and D. Mackay (2000): "A modeling-based perspective on the past, present, and future polychlorinated biphenyl contamination of the St. Lawrence beluga whale (*Delphinapterus leucas*) population", Canadian Journal of Fisheries and Aquatic Sciences 57 (suppl. 1), pp. 101-112

Hintelmann H, Dillon P, Evans RD, Rudd JWM, Bodaly RA (2001). Comment: Variations in the isotope composition of mercury in a freshwater sediment sequence and food web. Canadian Journal of Fisheries and Aquatic Sciences 58: 2309-2311.

Hunt BP and Carbine WF (1951). Food of young pike *Esox lucius* L., and associated fishes in Peterson's ditches, Houghton Lake, Michigan. Trans. Am. Fish. Soc. 80: 67-83.

Jackson TA & Muir DC (2004). Historical variations in the stable isotope composition of mercury in arctic lake sediments. Environmental Science and Technology 38: 2813-2821.

Jackson TA (1991). Biological and environmental control of mercury accumulation by fish in lakes and reservoirs of northern Manitoba, Canada. Canadian Journal of Fisheries and Aquatic Sciences 48 (12): 2449-2470.

Jackson TA (2001a). Variations in the isotope composition of mercury in a freshwater sediment sequence and food web. Canadian Journal of Fisheries and Aquatic Sciences 58: 185-196.

Jackson TA (2001b). Reply: Variations in the isotope composition of mercury in a freshwater sediment sequence and food web. Canadian Journal of Fisheries and Aquatic Sciences 58: 2312-2316.

Jackson TA, Parks JW, Jones PD, Woychuk RN, Sutton JA, Hollinger JD (1982). Dissolved and suspended mercury species in the Wabigoon River (Ontario, Canada): seasonal and regional variations. Hydrobiologia 92: 473-487.

Jewett SC, Zhang X, Naidu AS, Kelley JJ, Dasher D, Duffy LK (2003). Comparison of mercury and methylmercury in northern pike and Arctic grayling from western Alaska rivers. Chemosphere 50: 383-392.

Jonasson IR & Boyle RW (1972). Geochemistry of mercury and origins of natural contamination of environment. Canadian Mining and metallurgical Bulletin 65 (717): 32&

Jorde DG and Owen RB (1990). Changes in caloric content of the amphipod *Gammarus oceanicus* along the coast of Maine. Canadian Field-Naturalist 104 (2): 303-304. Journal of Animal Ecology 23 (2): 339-360.

Kelly EN, Schindler DW, St. Louis VL, Donald DB, Vladicka KE (2006). Forest fire increases mercury accumulation by fishes via food web restructuring and increased mercury inputs. Proceedings of the National Academy of Sciences 103(51): 19380-19385.

Kemp ALW, Williams JDH, Thomas RL, Gregory ML (1978). Impact of mans activities on the chemical composition of the sediments of lakes Superior and Huron. Water, Air, and Soil Pollution 10: 381-402.

Kinghorn A, Solomon P, Chan HM (2007). Temporal and spatial trends of mercury in fish collected in the English-Wabigoon river system in Ontario, Canada. Science of the Total Environment 372: 615-623.

Kolka RK, Grigal DF, Verry ES, Nater EA (1999). Hg and organic coarbon relationships in streams draining forested upland/peatland watersheds. Journal of Environmental Quality 28: 766-775.

Korhonen P, Virtanen M, Schultz T (1995). Bioenergetic calculation of mercury accumulation in fish. Water, Air, and Soil Pollution 80: 901-904.

Kurland LT, Faro SN, Siedler H (1960). Minamata disease. World Neurology 1(4): 370-395.

Lamontagne S, Carignan R, D'Arcy P, Prairie YT, Paré D (2000). Element export in runoff from eastern Canadian Boreal Shield drainage basins following forest harvesting and wildfires. Canadian Journal of Fisheries and Aquatic Sciences 57: 118-128.

Lawler G (1965). The food of the pike, *Esox lucius*, in Heming Lake, Manitoba. J. Fish. Res. Board Can. 22: 1357-77.

Lindberg S, Stokes P, Goldberg E, Wren C (1987). Group report: Mercury. In: Hutchinson, TW & Meema, KM, ed. Lead, mercury, cadmium and arsenic in the environment, New York, Chichester, Brisbane, Toronto, John Wiley & Sons, pp. 17-34.

Lindqvist O, Jernelov A, Johansson K, Rodhe R (1984). Mercury in the Swedish environment: global and local sources, Solna, National Swedish Environment Protection Board, 105 pp (Report No. 1816).

Lindqvist O, Johansson K, Aastrup M, Andersson A, Bringmark L, Hovsenius G, Hakanson L, Iverfeldt A, Meili M, Timm B (1991). Mercury in the Swedish Environment – Recent Research on Causes, Consequences and Corrective Methods. Water, Air, and Soil Pollution 55 (xi-xiii)

Lucas MC and Armstrong JD (1991). Estimation of meal energy-intake from heart-rate records of pike, *Esox lucius* L. J. Fish. Biol 38 (2): 317-319.

Lucas MC, Priede IG, Armstrong JD, Gindy ANZ, Devera L (1991). Direct measurements of metabolism, activity and feeding-behaviour of pike, *Esox lucius* L.in the wild, by the use of heart rate telemetry. J. Fish. Biol. 39 (3): 325-345.

Lyons WB, Fitzgibbon TO, Welch KA, Carey AE (2006). Mercury geochemistry of the Scioto River, Ohio: Impact of agriculture and urbanization. Applied Geochemistry 21: 1880-1888.

Machniak K 1975. The effects of hydroelectric development on the biology of northern fishes (reproduction and population dynamics) II, Northen pike *Esox lucius* (Linnaeus). A literature review and bibliography. Fish. Mar. Serv. Res. Div. Tech. Rep. No. 528.

Mason RP & Sullivan KA (1998). Mercury and methylmercury transport through an urban watershed. Water Research 32: 321-330.

Mason RP, Fitzgerald WF, Morel FMM (1994). The biogeochemical cycling of elemental mercury – anthropogenic influences. Geochimica et Cosmochimica Acta 58(15): 3191-3198.

Mason RP, Laporte J-M, Andres S (2000). Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology 38: 283-297.

Matty JM, & Long DT (1995). Early diagenesis of mercury in the Laurentian Great Lakes. Journal of Great Lakes Research 21: 574-586.

Mierle G & Ingram R (1991). The role of humic substances in the mobilization of mercury from watersheds. Water, Air, and Soils Pollution 56: 349-357.

Morel FMM, Kraepiel AML, Amiot M (1998). The Chemical Cycle and Bioaccumulation of Mercury. Annual Review of Ecology and Systematics 29: 543-566

Munson BA & Daniel EE (1973). North Saskatchewan River Mercury Project. 1972-1973 Report. 43pp. Alberta Environment.

Munson BA (1978). The Biology of Goldeye, Hiodon alosoides, in the North Saskatchewan River with Special Reference to Mercury Contamination in this Species of Fish. Report 1978/1. Alberta Environment.

Nakashima BS and Leggett WC (1978). Daily ration of yellow perch (*Perca flavescens*) from Lake Memphremagog, Quebec-Vermont, with a comparison of methods for in situ determinations. J. Fish. Res. Board Can. 35: 1597-1603.

Nelson SJ & Paetz MJ (1992). The fishes of Alberta, 2nd edition. University of Alberta Press, Edmonton Alberta.

Nelson WR 1974. Age, growth and maturity of thirteen species of fish from Lake Oahe during early years of impoundment, 1963-1968. U.S. Fish. Wild. Serv. Tech. Pap. No 77.

Nriagu JO (1979) The biogeochemistry of mercury in the environment, Amsterdam, Oxford, New York, Elsevier Science Publishers.

Parks Canada (2005). http://www.pc.gc.ca/pn-np/ab/waterton/visit/visit14b_e.asp

Parks JW, Curry C, Romani D, Russell DD (1991). Young northern pike, yellow perch and crayfish as bioindicators in a mercury contaminated watercourse. Environmental Monitoring and Assessment 16: 39-73

Parks JW & Hamilton AL (1987). Accelerating recovery of the mercury-contaminated Wabigoon English river system. Hydrobiologia 149: 159-188.

Parks JW, Craig PJ, Neary BP, Ozburn G, Romani D (1991). Biomonitoring in the mercury-contaminated Wabigoon-English-Winnipeg River (Canada) system:

selecting the best available bioindicator. Applied Organometallic Chemistry 5: 487-495.

Parks JW, Sutton JA, Hollinger JD (1984). Mercury contamination of the Wabigoon/Emglish Winnipeg River System—causes, effects and possible remedial measures. Final Rep. of the Steering Committee. Vol.1 and 2. Published by Government of Ontario (Dep.of Environment). 570pp.

Paterson MJ, Rudd JWM, St. Louis VL (1998). Increases in Total and Methylmercury in Zooplankton following of a Peatland Reservoir. Environ. Sci. Technol. 32: 3868-3874.

Pazzia I, Trudel M, Ridgway M, Rasmussen JB (2002). Influence of food web structure on the growth and bioenergetics of lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 59: 1593-1605.

Porvari P (1998). Development of fish mercury concentrations in Finnish reservoirs from 1979 to 1994. Science of the Total Environment 213 (1-3): 279-290.

R Development Core Team (2005). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rasmussen JB, Rowan DJ, Lean DRS, Carey JH (1990). Food chain structure in Ontario lakes determines PCB levels in lake trout (Salvelinus namaycush) and other pelagic fish. Canadian Journal of Fisheries and Aquatic Sciences 47: 2030-2038.

Rasmussen PE (1994). Current methods of estimating atmospheric mercury fluxes in remote areas. Environmental Science and Technology 28(13): 2233-2241.

Rennie MD, Collins NC, Purchase CF, Tremblay A (2005). Predictive models of benthic invertebrate methylmercury in Ontario and Quebec lakes. Canadian Journal of Fisheries and Aquatic Sciences 62: 2770-2783.

Rennie MD, Collins NC, Shuter BJ, Rajotte JW, Couture P (2005). A comparison of methods for estimating activity costs of wild fish populations: more active fish observed to grow slower. Canadian Journal of Fisheries and Aquatic Sciences 62: 767-780.

Ridley WI & Stetson SJ (2006). A review of isotopic composition as an indicator of the natural and anthropogenic behavior of mercury. Applied Geochemistry 21: 1889-1899.

Riget F, Asmund G, Aastrup P (2000). Mercury in arctic char (Salvelinus alpinus) populations from Greenland. The Science of the Total Environment 245: 161-172.

Roberts JH & Grossman GD (2001). Reproductive characteristics of female longnose dace in the Coweeta Creek Drainage, North Carolina, USA. Ecology of Freshwater Fish 10 (3): 184-190.

Rowan DJ and Rasmussen JB (1996). Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (Cs-137). Can. J. Fish. Aqu. Sci. 53 (4): 734-745.

Schetagne R, Doyon J-F, Fournier J-J (2000). Export of mercury downstream from reservoirs. The Science of the Total Environment 260: 135-145.

Scheuhammer AM & Graham JE (1999). The bioaccumulation of mercury in aquatic organisms from two similar lakes with differing pH. Ecotoxicology 8: 49-56.

Schindler DW, Bayley SE, Parker BR, Beaty KG, Cruikshank DR, Fee EJ, Schindler EU, Stainton MP (1996). The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. Limnology and Oceanography 41(5): 1004-1017.

Schuster PF, Krabbenhoft DP, Naftz DL, Cecil LD, Olson ML, Dewild JF, Susong DD, Green JR, Abbott ML (2002). Atmospheric mercury deposition during the last 270 years: A glacial ice core record of natural and anthropogenic sources. Environment Science and Technology 36: 2303-2310.

Scott DP & Armstrong FAJ (1972). Mercury concentration in relation to size in several species of freshwater fishes from Manitoba and Northwestern Ontario. Journal of the Fisheries Research Board of Canada 29:1985-1690.

Seaburg KG and Moyle JB (1964). Feeding habits, digestion rates, and growth of some Minnesota warmwater fishes. Trans. Am. Fish. Soc. 93: 269-85.

Sherwood GD, Kovecses J, Hontela A, Rasmussen JB (2002). Simplified food webs lead to energetic bottlenecks in polluted lakes. Can. J. Fish. Aqu. Sci. 59 (1): 1-5.

Sherwood GD, Pazzia I, Moeser A, Hontela A, Rasmussen JB (2002). Shifting gears: enzymatic evidence for the energetic advantage of switching diet in wild-living fish. Can. J. Fish. Aqu. Sci. 59 (2): 229-241.

Sherwood GD, Rasmussen JB, Rowan DJ, Brodeur J, Hontela A (2000). Bioenergetic costs of heavy metal exposure in yellow perch (Perca flavescens): in situ estimates with a radiotracer (Cs-137) technique. Canadian Journal of Fisheries and Aquatic Sciences 57 (2): 441-450.

Sigler JM, Lee X, Munger W (2003). Emission and long-range transport of gaseous mercury from a large-sclae Canadian borel forest fire. Environmental Science and Technology 37: 4343-4347.

Simoneau M, Lucotte M, Garceau S, Laliberte D (2005) Fish growth rates modulates mercury concentration in walleye (*Sander vitreus*) from eastern Canadian lakes. Environ. Res. 98: 73-82.

Skov C, Lousdal O, Johansen PH, Berg S (2003). Piscivory of 0+pike (*Esox lucius* L.) in a small eutrophic lake and its implication for biomanipulation. Hydrobiologia 506 (1-3): 481-487.

St. Louis VL, Rudd JWM, Kelly CA, Beaty KG, Flett RJ, Roulet NT (1996). Production and Loss of Methylmercury and Loss of Total Mercury from Boreal Forest Catchments Containing Different Types of Wetlands. Environ. Sci. Technol. 30: 2719-2729.

St. Louis VL, Rudd JWM, Kelly CA, Bodaly RA, Paterson MJ, Beaty KG, Hesslein RH, Heyes A, Majewski AR (2004). The Rise and Fall of Mercury Methylation in an Experimental Reservoir. Environ. Sci. Technol. 38: 1348-1358.

Stafford CP & Haines TA (2001). Mercury contamination and growth rate in two piscivore populations. Environmental Toxicology and chemistry 20(9): 2099-2101.

Stafford CP, Hansen B, Stanford JA (2004). Mercury in fishes and their diet items from Flathead Lake, Montana. Transactions of the American Fisheries Society 133: 349-357.

Stow CA and Carpenter SR (1994). PCB accumulation in Lake Michigan coho and Chinook salmon: individual based models using allometric relationships. Environ. Sci. Technol. 28: 1543-1549.

Strange NE, Fudge RJP, Bodaly RE (1991). Post-impoundment response of a boreal northern pike (*Esox lucius*) population in Wupaw Bay, Southern Indian Lake, Manitoba 1976-88. Can. Tech. Rep. Fish. Aquat. Sci. No. 1823.

Swanson HK, Johnston TA, Leggett WC, Bodaly RA, Doucett RR, Cunjak RA (2003). Trophic positions and mercury bioaccumulation in rainbow smelt (*Osmerus mordax*) and native forage fishes in northwestern Ontario lakes. Ecosystems 6: 289-299.

Swenson WA and Smith LL (1973). Gastric digestion, food consumption, feeding periodicity, and food conversion efficiency in walleye (*Stizostedion-vitreum-vitreum*). J. Fish. Res. Board Can. 30 (9): 1327-1336.

Tao G, Willie SN, and Sturgeon RE (1998). Determination of total mercury in biological tissues by flow injection cold vapour generation atomic absorption spectrometry following tetramethylammonium hydroxide digestion. The Analyst 123: 1215-1218

Therriault TW and Schneider D (1998). Predicting change in fish mercury concentrations following reservoir impoundment. *Environmental Pollution* 101 (1): 33-42.

Thomann RV (1981) Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Canadian Journal of Fisheries and Aquatic Sciences 38:280-296.

Thomson AR, Petty JT, Grossman GD (2001). Multi-scale effects of resource patchiness on foraging behaviour and habitat use by longnose dace, *Rhinichthys cataractae*. Freshwater Biology 46 (2): 145-160.

Tremblay A, Lucotte M, Schetagne R(1998). Total mercury and methylmercury accumulation in zooplankton of hydroelectric reservoir in northern Quebec (Canada). Science of the Total Environment 213: 307-315.

Trudel M & Rasmussen JB (2001). Predicting mercury concentration in fish using mass balance models. Ecological Applications 11: 517-529.

Trudel M & Rasmussen JB (2006). Bioenergetics and mercury dynamics in fish: a modeling perspective. Canadian Journal of Fisheries and Aquatic Sciences 63: 1890-1902.

Trudel M and Rasmussen JB (1997). Modeling the elimination of mercury by fish Environmental Science & Technology 31 (6): 1716-1722.

Trudel M, Tremblay A, Schetagne R, Rasmussen JB (2000). Estimating food consumption rates of fish using a mercury mass balance model. Canadian Journal of Fisheries and Aquatic Sciences 57 (2): 414-428.

Trudel M, Tremblay A, Schetagne R, Rasmussen JB (2001). Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). Canadian Journal of Fisheries and Aquatic Sciences 58 (2): 394-405.

Turetsky MR, Harden JW, Friedli HR, Flannigan M, Payne N, Crock J, Radke L (2006). Wildfires threaten mercury stocks in northern soils. Geophysical Research Letters 33: L16403.

U.S.EPA Method 1631, Revision E (2002). Mercury in Water by Oxydation, Purge and Trap, and Cold Vapour Atomic Fluorescence Spectrometry. United States Environmental Protection Agency, Office of Water 4303.

Vander Zanden MJ & Rasmussen JB (1996). A trophic position model of pelagic food webs: Impact on contaminant bioaccumulation in lake trout. Ecological Monographs 66 (4): 451-477.

Vander Zanden MJ, Cabana G, Rasmussen JB (1997). Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54: 1142-1158.

Venturelli PA and Tonn WM (2006). Diet and growth of northern pike in the absence of prey fishes: Initial consequences for persisting in disturbance-prone lakes. Trans. Am. Fish. Soc. 135 (6): 1512-1522

Verdon R, Brouard D, Demers C, Lalumiere R, Laperle M, Schetagne R (1991). Mercury Evolution (1978-1988) in Fishes of the La-Grande Hydroelectric Complex, Quebec, Canada. *Water Air and Soil Pollution* 56: 405-417.

Wahl DH and Stein RA (1991). Food-consumption and growth of 3 esocids – field-tests of a bioenergetic model. Trans. Am. Fish. Soc. 120 (2): 230-246.

Watras CJ, Morrison KA, Bloom NS (1995). Mercury in mremote Rocky Mountain lakes of Galcier National Park, Montana, in comparison with other temperate North American regions. Canadian Journal of Fisheries and Aquatic Sciences 52: 1220-1228.

Westcott K & Kalff J (1996). Environmental factors affecting methyl mercury accumulation in zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 53: 2221-2228.

Wolfert DR and Miller TJ (1978). Age, growth, and food of northern pike in eastern Lake-Ontario. Trans. Am. Fish. Soc. 107 (5): 696-702.

Wootton JT, Parker MS, Power ME (1996). Effects of disturbance on river food webs. Science 273 (5281): 1558-1561.

World Health Organization (1990). Methylmercury. World Health Organization, Geneva, Switzerland.

Yoshinaga J, Suzuki T, Hongo T, Minegawa M, Ohtsuka R, Kawabe T, Inaoko T, Akimichi T (1992). Mercury concentration correlates with the nitrogen stable isotope ratio in the animal food of Papuans. Ecotoxicology and Environmental Safety 24: 37-45.

Appendix A

Chapter III: Supplementary maps of Oldman River sampling sites (City of Lethbridge sites 6-13).

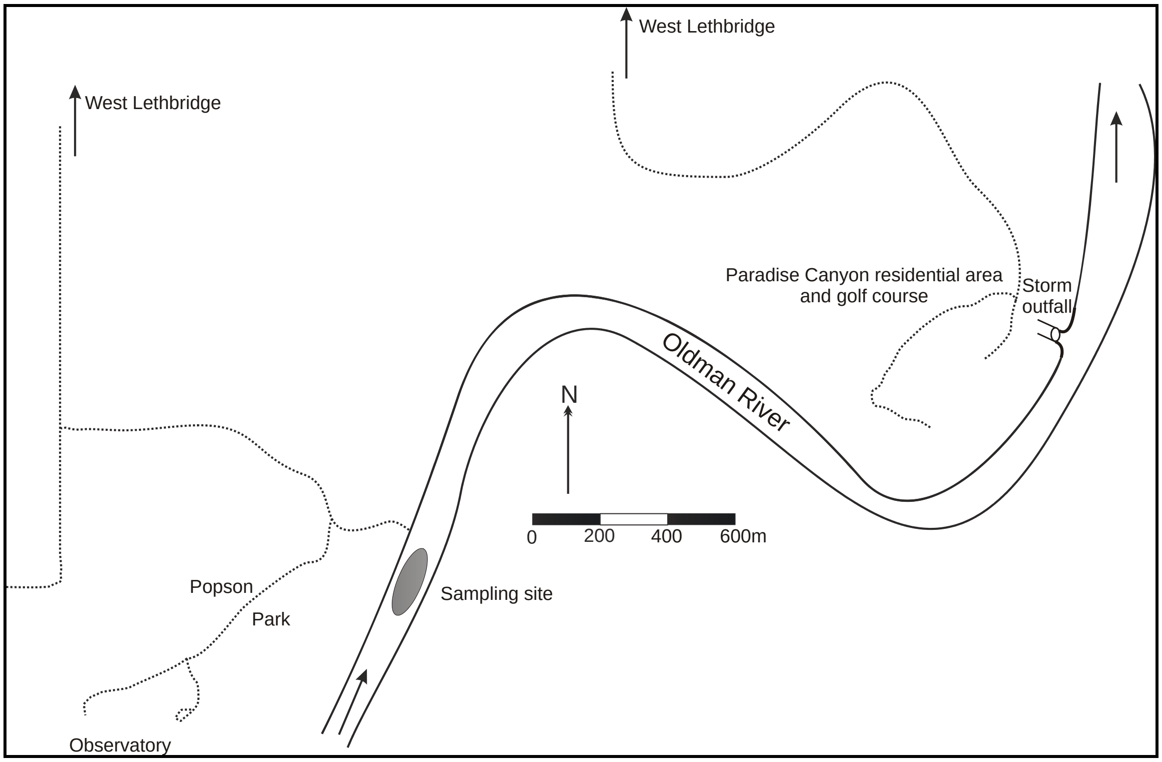

Figure A1: Popson Park and Paradise Canyon storm outfall.

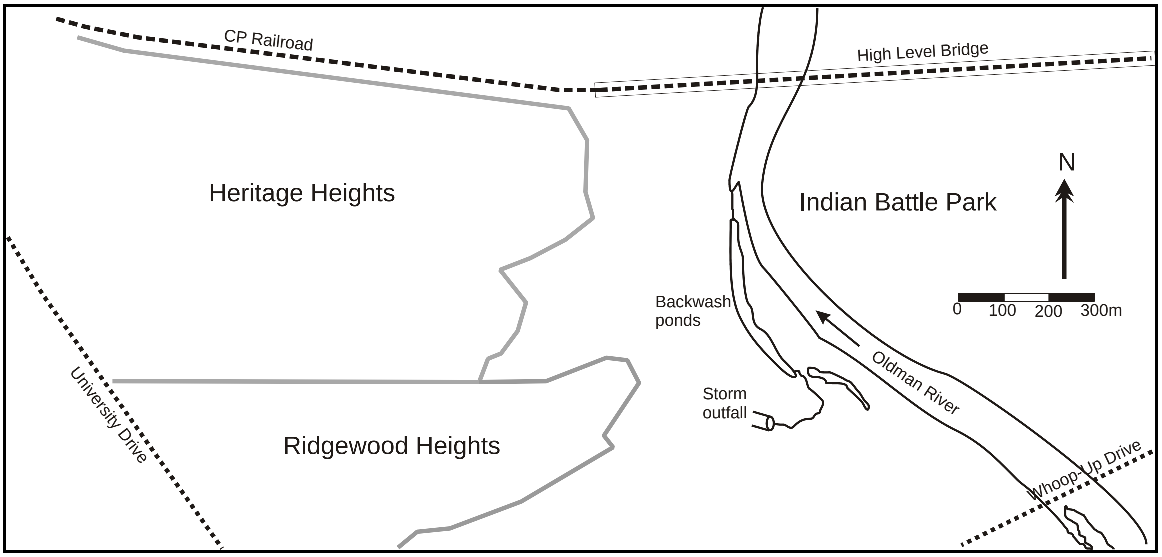

Figure A2: Ridgewood Heights storm outfall.

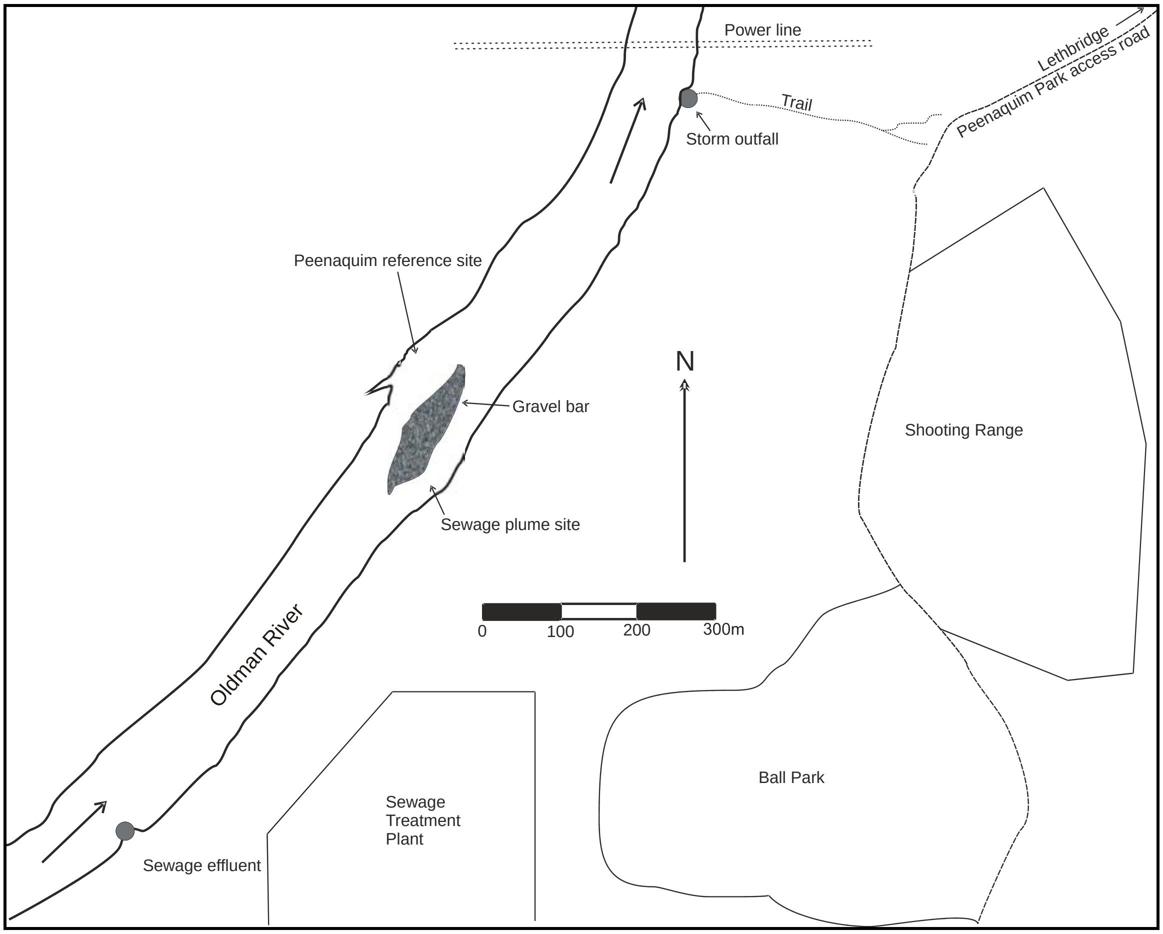

Figure A3: Lethbridge sewage effluent, Peenaquim Park reference site, storm outfall.

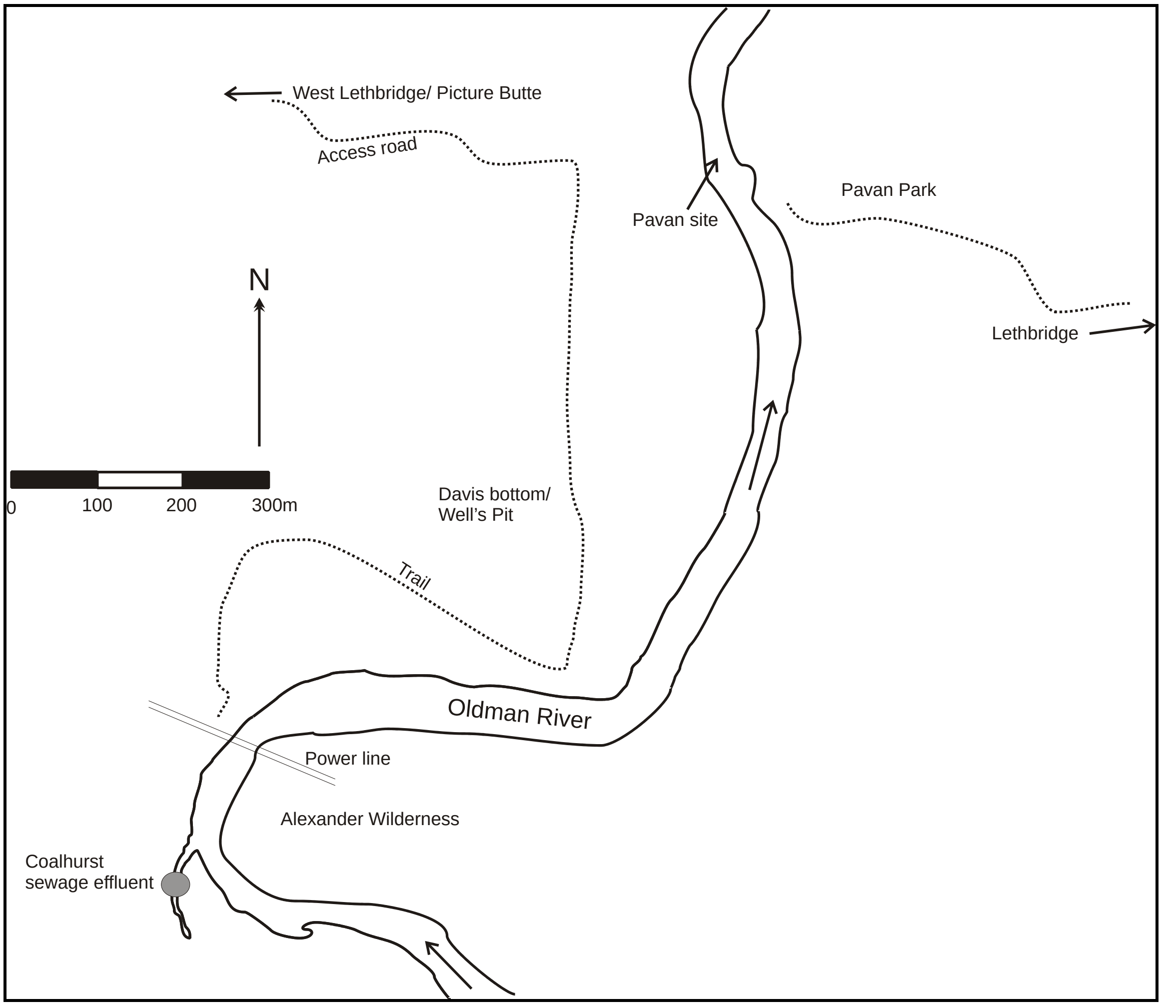

Figure A4: Coalhurst sewage outfall, Pavan Park.

Appendix B

Table B1: Chapter III statistical output tables. Computations were performed in R (2005) unless otherwise noted.

A) ANCOVA on THg in sport fish (northern pike, goldeye/mooneye, walleye). Response: $\log_{10}$THg; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	p
fork length	1	1.6396	1.6396	66.461	3.02E-11
species	2	1.15331	0.57665	23.375	3.34E-08
residuals	59	1.45553	2.47E-02		

(simplified model: interaction term was not significant at p = 0.07

Model parameters	Estimate	Std. Error	t value	p	R^2
Goldeye/Mooneye	-1.0375008	0.0795131	-13.048	< 2e-16	0.64
slope	0.0015845	0.0002837	5.585	6.25E-07	
Northern pike*	-1.37156	0.1068649	-3.126	0.00275	
Walleye*	-0.92304	0.075486	1.516	0.13477	

*Ho: no difference relative to Goldeye/Mooneye estimate.

B) ANCOVA on THg in longnose dace, gradient sites and sewage inputs included. Response: $\log_{10}$THg; covariate: length (statistical analysis carried out in JMP IN 5.1)

Predictor	Df	Sum Sq	Mean Sq	F value	p
fork length	1	9.6093455	9.6093455	645.1387	<.0001
site	12	6.6678498	0.55565415	37.3047	<.0001
interaction	12	1.3702561	0.11418801	7.6662	<.0001
residuals	393	5.853738	0.014895		

Model parameters	Estimate	Std. Error	t value	p	R^2
Lethbridge sewage	-2.11392	0.038145	-55.42	<.0001	0.79
slope	0.0181355	0.000714	25.4	<.0001	
Coaldale bridge*	0.1214588	0.022572	5.38	<.0001	
Ft Macleod*	-0.059398	0.02109	-2.82	0.0051	
Hwy 36*	0.1771483	0.021665	8.18	<.0001	
Maycroft*	-0.216523	0.021237	-10.2	<.0001	
Paradise storm*	0.1837115	0.023368	7.86	<.0001	
Pavan*	0.1610113	0.021543	7.47	<.0001	
Pearce Rd*	0.0266804	0.041026	0.65	0.5159	
Peenaquim*	0.0394976	0.023034	1.71	0.0872	
Peenaquim storm*	0.0622815	0.022987	2.71	0.007	
Popson*	0.173968	0.028155	6.18	<.0001	
Poulsen*	-0.079969	0.021343	-3.75	0.0002	
Summerview*	-0.458793	0.050685	-9.05	<.0001	
Coaldale bridge*(Length-52.5247)*	0.0037998	0.002298	1.65	0.0991	
Ft Macleod*(Length-52.5247)*	-0.007426	0.002386	-3.11	0.002	
Hwy 36*(Length-52.5247)*	0.0050856	0.002591	1.96	0.0504	
Maycroft*(Length-52.5247)*	-0.011476	0.001925	-5.96	<.0001	
Paradise storm*(Length-52.5247)*	0.000789	0.001578	0.5	0.6173	
Pavan*(Length-52.5247)*	0.0067002	0.002161	3.1	0.0021	
Pearce Rd*(Length-52.5247)*	-0.000262	0.002969	-0.09	0.9297	
Peenaquim*(Length-52.5247)*	0.0082467	0.002433	3.39	0.0008	

Peenaquim storm*(Length-52.5247)*	-0.001759	0.001795	-0.98	0.3277
Popson*(Length-52.5247)*	0.0025887	0.002463	1.05	0.2938
Poulsen*(Length-52.5247)*	-0.00904	0.001928	-4.69	<.0001
Summerview*(Length-52.5247)*	0.0019092	0.003061	0.62	0.5331

*Ho: no difference relative to Lethbridge sewage estimate.

Least Squares Means Table

Site	Least Sq Mean (log$_{10}$ THg)	Std Error	Mean
Coaldale bridge	-1.0399	0.022862	-0.9619
Ft Macleod	-1.220757	0.02112264	-1.1889
Hwy 36	-0.984211	0.02180005	-1.0261
Maycroft	-1.377882	0.02129631	-1.3829
Paradise storm	-0.977648	0.02378773	-1.1016
Pavan	-1.000348	0.02165625	-1.0474
Pearce Rd	-1.134679	0.04369949	-1.3525
Peenaquim	-1.121861	0.02339939	-1.2242
Peenaquim storm	-1.099078	0.02334538	-1.0106
Popson	-0.987391	0.02928047	-0.8788
Poulsen	-1.241328	0.02142162	-1.2285
Summerview	-1.620152	0.05437473	-1.3101
sewage	-1.292433	0.03402466	-1.4152

C) Linear regression of MeHg concentration versus elevation in hydropsychid caddisflies.

Predictor	Df	Sum Sq	Mean Sq	F value	p
elevation	1	1.16E-05	1.16E-05	8.2051	0.03522
Residuals	5	7.04E-06	1.41E-06		
Model parameters	estimate	SE	t	p	R^2
Intercept	2.21E-02	4.01E-03	5.521	0.00267	0.55
slope	-1.37E-05	4.78E-06	-2.864	0.03522	

D) Linear regression of total mercury concentration (least squares means) versus elevation in longnose dace downstream of Oldman reservoir (gradient sites only).

Predictor	Df	Sum Sq	Mean Sq	F value	p
elevation	1	0.0044798	0.0044798	36.466	0.0009325
Residuals	6	0.0007371	0.0001228		
Model parameters	estimate	SE	t	p	R^2
Intercept	3.08E-01	3.82E-02	8.071	1.94E-04	0.84
slope	-2.69E-04	4.46E-05	-6.039	0.000932	

E) ANCOVA on THg in suckers from Ridgewood, Popson, Coalhurst sewage, Response: log$_{10}$THg; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	p
length	1	2.59E-06	2.59E-06	0.0001	9.93E-01
site	2	0.5422	0.2711	8.5735	3.60E-04
Residuals	103	3.2568	0.0316		

(simplified model: interaction term was not significant at p = 0.25

Dunnett's contrasts	estimate	SE	t	p
Coalhurst sewage-Popson	-0.205	-3.491	0.059	0.001
Ridgewood sewer-Popson	0.025	-0.65	0.039	5.17E-01

F) Linear regression of regression slopes (individual regression models of fork length vs THg in longnose dace) versus elevation of sampling site

Predictor	Df	Sum Sq	Mean Sq	F value	p
elevation	1	2.9950e-05	2.9950e-05	23.083	0.001348
Residuals	8	1.0380e-05	1.2975e-06		
Model parameters	estimate	SE	t	p	R^2
Intercept	1.363e-02	2.107e-03	6.468	0.000195	0.71
slope	-1.081e-05	2.250e-06	-4.804	0.001348	

Appendix C

Chapter IV Mercury Mass Balance Model

Daily ration was estimated using a simplified version of the mercury mass balance model (MMBM) devised by Trudel et al. (2000) to estimate food consumption rates of fish:

$$(1) \qquad \frac{dC}{dt} = (\alpha \cdot C_d \cdot I) - (E + G + K) \cdot C$$

where α is the coefficient of assimilation of mercury from the food source, C_d is the mercury concentration in the food source, I is the ingestion rate or daily ration, E is the elimination rate of mercury from the fish body, G is the growth rate of fish, and K is the elimination rate of mercury from the gonads during spawning. A summary of parameters, values and units is presented in Table C1.

Table C1: Summary of parameters used in the MMBM (adapted from Trudel and Rasmussen (2000) and references therein).

Parameter	Description	Estimation	Value	Units
A	Assimilation efficiency of Hg from food	experimental	0.8	n/a
C_d	Hg concentration in fish diet	Experimental/measurement	0.2	mg/kg
I	Food consumption rates	Estimate from MMBM		per day
E	Hg elimination rate	$E = \varphi \cdot W^{\beta} \cdot e^{\gamma T}$		per day
Φ	Coefficient of mercury elimination	Empirically derived constant	0.0029	n/a
ß	Allometric exponent of mercury elimination	"	-0.20	n/a
Γ	Temperature coefficient of mercury elimination	"	0.066	n/a
W	Mass of fish in grams	Average daily increase		
T	Temperature in deg. C	Estimate mathematically		
G	Specific growth rate	$G = \frac{1}{\Delta t} \cdot \ln\left(\frac{W_{t+\Delta t}}{W_t}\right)$		

In later publications it becomes evident that the MMBM is relatively insensitive to changes in the elimination of mercury through the gonads (Trudel and Rasmussen 2001, Trudel and Rasmussen 2006). Finally this specific term K is dropped altogether (Trudel and Rasmussen 2006) based on the notion that elimination of mercury from the gonads is negligible. The authors make the argument that mercury is predominantly bound to the protein fraction within the body (also see Harris et al. 2003) and thus the sequestration of mercury into gonads is small. After performing this adjustment to equation (1), integration, and solving for *I*, the final version of the MMBM to estimate daily ration in fish is:

$$(2) \qquad I = \frac{C_{t+\Delta t} - C_t \cdot e^{-(E+G)\Delta t}}{\alpha \cdot C_d [1 - e^{-(E+G)\Delta t}]} (E + G)$$

The model was run on a daily basis estimating an individual value I_i for each day of a cycle of 365 days. Parameters used in the model were estimated accordingly as follows. Elimination of mercury from fish was estimated according to Trudel and Rasmussen (1997) on a daily basis using the equation:

(3) $E_i = \varphi \cdot W_i^{\beta} \cdot e^{\gamma T_i}$

where W_i is the fish weight and T_i is the water temperature at day i. Temperature data were generated using simple harmonic oscillation, assuming a range of +4 to 26 deg. C over a cycle of 365 days (Figure 5), setting day 1 at the beginning of August:

(4) $T_i = -11 \cdot \cos(2 \cdot \pi \cdot 1/365 \cdot i) + 15$

The specific growth rate G was estimated based on the three strongest age classes in the sample, using the equation:

(5) $G = \frac{1}{\Delta t} \cdot \ln\left(\frac{W_{t+\Delta t}}{W_t}\right)$

where Δt is 365 days and W_t and $W_{t+\Delta t}$ are fish mass of two consecutive age classes. The mean weight of fish W_i at each day was then calculated for 365 days of growth, assuming G is constant throughout a consecutive year.

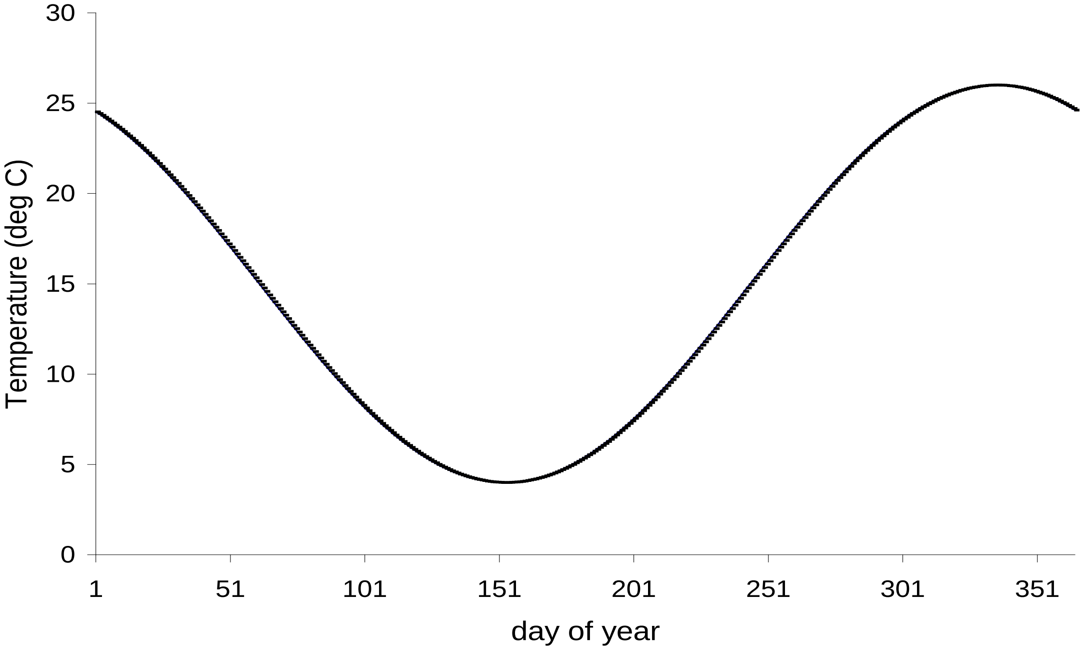

Figure C1: Simulated annual temperature cycle in Twin Valley reservoir.

The daily gain of mean THg, ΔTHg_i, was estimated by averaging the difference of two consecutive age classes over 365 days, thus assuming linear gain in THg throughout one year:

(6) $\Delta THg_i = \frac{THg_{t+\Delta t} - THg_t}{365}$

where $THg_{t+\Delta t}$ and THg_t are mean mercury concentrations in two consecutive age classes in the sample.

Finally, The data were summarized and reported as monthly a averages.

Appendix D

Table D1: Chapter IV statistical output tables. Computations were performed in R (2005) unless otherwise noted.

A) ANCOVA on THg in northern pike from Twin Valley reservoir and Oldman River. Response: $\log_{10}$THg; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	p
fork length	1	0.00135	0.00135	0.0482	0.8274
system	1	1.69399	1.69399	60.5151	1.87E-09
residuals	39	1.09172	0.02799		

(simplified model: interaction term was not significant at P = 0.26)

Model parameters	estimate	SE	t	p	R^2
intercept	-1.33374	0.191418	-6.968	2.37E-08	0.59
slope	0.001521	0.000312	4.877	1.84E-05	
Twin Valley	0.534797	0.068748	7.779	1.87E-09	

B) ANCOVA on THg in suckerts from the Little Bow River and 4 reference sites along the Oldman River. Response: $\log_{10}$THg; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	p
fork length	1	0.56403	0.56403	24.548	4.36E-06
site	4	1.94869	0.48717	21.203	9.99E-12
interaction	4	0.23601	0.059	2.568	0.0448
residuals	75	1.72322	0.02298		

Dunnett's contrasts	Estimate	t value	Std.Err	p adj
Popson-Little Bow	0.419	-6.845	0.056	<0.001
Monarch-Little Bow	0.339	-6.045	0.055	<0.001
Pearce Rd-Little Bow	0.221	-3.521	0.063	0.001
Pavan-Little Bow	0.068	-1.229	0.061	0.223

Appendix E

Table E1: Chapter IV summary of previously published data on daily rations of northern pike in various systems, including the present study.

Authors	mass (g)	length (mm)	Age class (years)	sex	season	feeding rates	units	conversion estimates (g/g/d)	method	notes
present study	600 (SEM: 88.5)	429 (SEM: 21.0)	3+	-	January	0.0094	g/g/d	-	mercury mass balance (Trudel et al. 2000)	
					June, July	0.024				
					December	0.01				
					annual mean	0.016				
	782 (SEM: 61.6)	464 (SEM: 14.4)	4+	-	January	0.014				
					July	0.034				
					December	0.017				
					annual mean	0.023				
Diana JS (1979)	-	See Diana (1979), Diana & Mackay (1979), Diana (1983)	3+	♂	Winter	0.4	kcal/kg/d	8.51x10^{-5}*	Bajkov (1935)	* assuming calorific equivalent of 4.7kcal/g dry weight (Diana 1979).
				♀		0.9		1.91x10^{-4}*		
				♂	Summer	11.4		0.0024*		
				♀		17.4		0.0037*		
				♂	June (max)	18.1		0.0039*		
				♀		30.9		0.0066*		
				♂	April (min)	0.3		6.38x10^{-5}*		
				♀		0.3		6.38x10^{-5}*		
Lucas MC, et al. (1991)	900**	530	-	-	June	1.6	%B.W.	0.016	Lucas MC & Armstrong JD (1991)	** only 2 fish tested in study
	800**	510	-	-		1.5		0.015		
Wahl DH & Stein RA (1991)	30.7 (95% CL 1.9)	179 (95% CL 2.5)	-	-	Sep 1984 (max)	0.05	g/g/d	-	Swenson WA & Smith LL (1973)	
					April 1985 (min)	0.003				
	44.6 (95% CL 2.6)	205 (95% CL 4.7)	-	-	Sep 1985 (max)	0.048				
					Jan 1986 (min)	0.006				

Table E1 continued...

Authors	mass (g)	length (mm)	age class (years)	sex	season	feeding rates	units	conversion extimates (g/g/d)	method	notes
Rowan DJ & Rasmussen JB (1996)	1250	-	9+	♀	annual mean[a]	0.0108	g/g/d	-	^{137}Cs (Rowan DJ & Rasmussen JB 1996)	[a]Christie Bay, Great Slave Lake; [b]Macleod Bay, Great Slave Lake; [c]Western Basin, Great Slave Lake; [d]Ottawa river.
	1400		10+	♀		0.0112				
	1500		10+	♂		0.0075				
	2200		12+	♂		0.008				
	4200		15+	♀		0.0113				
	1800	-	11+	♀	annual mean[b]	0.0083				
	2100		12+	♀		0.0082				
	1550	-	10+	-	annual mean[c]	0.0172				
	89.8	-	1+	-	annual mean[d]	-				
	544.3		3+	♂		0.0259				
	872.6		4+	♂		0.0259				
	1614.3		5+	♂		0.0266				
Trudel M et al. (2000)	1048.7	-	4+	♀	annual mean[e]	0.011	g/g/d	-	mercury mass balance (Trudel et al. 2000)	[e]Caniapiscau Reservoir; [f]Lac Rond-de-Poêle; [g]Lake Simcoe.
	1301.5		5+	♀		0.011				
	1373.9		5+	♂		0.009				
	1615.4		6+	♀		0.011				
	1596.3		6+	♂		0.009				
	2004.8		7+	♀		0.011				
	1854.6		7+	♂		0.007				
	2488.2		8+	♀		0.01				
	2154.8		8+	♂		0.006				
	2088.1		9+	♀		0.009				
	452.8	-	2+	-	annual mean[f]	0.008				
	633.1		3+			0.009				
	868.9		4+			0.012				
	1164.7		5+			0.014				
	1516.7		6+			0.015				
	1910.4		7+			0.016				
	2321.2		8+			0.017				
	2720.1		9+			0.017				
	446	-	2+	-	annual mean[g]	0.01				
	1561		3+			0.01				
	2760		4+			0.009				
	3206		5+			0.011				

Appendix F

Table F1: Chapter V statistical output tables. Computations were performed in R (2005) unless otherwise noted.

A) Linear regression on $\log_{10}$THg versus fork length in lake trout

	Df	Sum Sq	Mean Sq	F value	p
fork length	1	1.18354	1.18354	27.693	1.506E-05
residuals	27	1.15392	0.04274		

Model parameters	estimate	SE	t	p	R^2
Slope	0.00117	0.0002229	5.262	1.51e-05	0.49
intercept	-1.022	0.1078549	-9.480	4.41e-10	

B) Linear regression on $\log_{10}$THg versus fork length in lake whitefish

	Df	Sum Sq	Mean Sq	F value	p
fork length	1	2.83347	2.83347	162.94	<2.2e-16
residuals	87	1.51293	0.01739		

Model parameters	estimate	SE	t	p	R^2
Slope	0.00178	0.0001399	12.77	<2e-16	0.65
intercept	-1.63813	0.0488	-33.55	<2e-16	

C) ANCOVA on fork length of lake trout separated by lakes. Response: fork length; covariate: age

Predictor	Df	Sum Sq	Mean Sq	F value	p
age	1	522762	522762	96.052	3.22E-10
lake	1	195749	195749	35.967	2.48E-06
residuals	26	141504	5442		

(simplified model: interaction term was not significant at P = 0.38)

Model parameters	estimate	SE	t	p	R^2
slope	28.5	6.211	5.376	1.25E-05	0.82
intercept middle basin	411.6	76.6	4.589	9.93E-05	
intercept upper basin	164.0	41.3	-5.997	2.48E-06	

Table F1 continued...

D) ANCOVA on weight of lake trout separated by lakes. Response: weight; covariate: age

Predictor	Df	Sum Sq	Mean Sq	F value	p
age	1	40714514	40714514	181.11	3.17E-13
lake	1	38318983	38318983	170.45	6.32E-13
residuals	26	5844916	224804		

(simplified model: interaction term was not significant at P = 0.296)

Model parameters	estimate	SE	t	p	R^2
slope	141.21	39.92	3.537	0.00154	0.93
intercept middle basin	3116.7	492.09	6.334	1.05E-06	
intercept upper basin	-347.23	265.32	-13.056	6.32E-13	

E) ANCOVA on seasonal variation of lake trout from the upper lake. Response: weight; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	p
fork length	1	0.91076	0.91076	24.6326	7.48E-05
season	1	0.08789	0.08789	2.3771	0.139
interaction	1	0.12172	0.12172	3.2922	0.0846
residuals	20	0.73948	0.03697		

Model parameters	estimate	SE	t	p	R^2
slope fall	0.00319	0.00074	4.303	0.00035	0.54
intercept fall	-1.626	0.235	-6.922	1.01E-06	
slope spring	0.00139	0.00099	-1814	0.0846	
intercept spring	0.516	0.403	1.278	0.216	

Appendix G

Table G1: Chapter V summary of t-test comparisons of mercury data from Waterton Lakes and Flathead Lake (Stafford et al. 2004).

	Models compared				
A	Waterton lake trout		Flathead lake trout		
Equations	$\log_{10}$THg=0.00155•FL-1.19		$\log_{10}$THg=0.00141•FL-1.22		
parameter comparisons	slope:		intercept:		
	T	p	t	p	Df
	0.483	0.685	0.186	0.57	56
B	Waterton lake whitefish		Flathead lake whitefish		
Equations	$\log_{10}$THg=0.00179•FL-1.64		$\log_{10}$THg=0.00141•FL-1.40		
parameter comparisons	slope:		intercept:		
	T	p	t	p	Df
	1.893	0.970	-3.45	0.0004	113
C	Waterton upper basin lake trout		Flathead lake trout		
Equations	$\log_{10}$THg=0.00188•FL-1.27		$\log_{10}$THg=0.00141•FL-1.22		
parameter comparisons	slope:		intercept:		
	T	p	t	p	Df
	1.309	0.902	-0.314	0.38	56

Table G2: Summary of analysis of covariance comparisons of mercury data from Waterton Lakes and Ontario class 1-3 lakes (Cabana et al. 1994).

A) ANCOVA on all-inclusive Waterton lake trout THg and class 1-3 lakes, Ontario. Response: log10THg; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	P
fork length	1	7.9277	7.9277	103.5189	<2.2E-16
lake type	3	2.1036	0.7012	9.1564	1.67E-05
residuals	120	9.1898	0.0766		

(simplified model: interaction term was not significant at P = 0.297)

Model parameters	estimate	SE	t	p
intercept	-1.192	0.1069395	-11.145	<2E-16
slope	0.00155	0.002074	7.462	1.48E-11
class 1 intercept	-1.365	0.0703034	-2.461	0.0153
class 2 intercept	-1.088	0.0738219	1.413	0.1604
class 3 intercept	-1.003	0.0720655	2.615	0.0101

B) ANCOVA on upper Waterton lake trout THg and class 1-3 lakes Ontario. Response: log10THg; covariate: length

Predictor	Df	Sum Sq	Mean Sq	F value	P
fork length	1	8.4482	8.4482	110.4638	<2.2E-16
lake type	3	1.6769	0.559	7.3086	1.58E-04
residuals	114	8.7187	0.0765		

(simplified model: interaction term was not significant at P = 0.881

Model parameters	estimate	SE	t	p
intercept	-1.269	0.1125356	-11.281	<2E-16
slope	0.00188	0.0002554	7.358	3.11E-11
class 1 intercept	-1.498	0.0744494	-3.063	0.00274
class 2 intercept	-1.251	0.0831931	0.223	0.82418
class 3 intercept	-1.177	0.0839918	1.097	0.27507

Appendix H: Data tables

Table H1: Mercury concentrations in sport fish from the Oldman River.

Mooneye/Goldeye			Northern pike			Walleye		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
209	104	0.23	389	442	0.26	375	575	0.40
217	123	0.07	408	560	0.26	378	n/a	0.36
220	119	0.22	499	952	0.22	386	721	0.44
222	121	0.21	546	1331	0.40	416	776	0.50
223	120	0.25	548	1153	0.37	423	1679	0.68
230	145	0.21	573	n/a	0.18	435	820	0.96
231	149	0.15	582	1499	0.48	454	1057	0.62
236	159	0.18	605	1834	0.27	458	980	0.89
238	160	0.20	620	1729	0.24	484	1538	0.61
238	153	0.19	628	2113	0.38	499	1429	1.15
238	153	0.20	638	1936	0.48	507	1673	0.64
245	185	0.19	708	2600	0.93	512	1280	0.84
248	183	0.12	780	4080	0.71	528	1671	0.72
252	185	0.33	831	4300	0.75	559	521	0.78
252	193	0.29				591	n/a	0.75
256	177	0.25						
257	193	0.25						
258	195	0.23						
260	227	0.10						
263	184	0.34						
264	214	0.17						
265	223	0.29						
265	191	0.41						
267	244	0.22						
269	215	0.38						
272	230	0.44						
289	266	0.18						
298	313	0.29						
306	344	0.29						
315	337	0.48						
325	416	0.19						
335	441	0.50						
343	456	0.34						
359	521	0.64						

Table H2: Mercury levels in longnose dace from sites along the Oldman River gradient (2006 samples).

Maycroft			Poulsen			Summerview		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
24.3	n/a	0.029	37.3	0.471	0.038	54.4	1.756	0.018
30.4	n/a	0.028	41.7	0.753	0.091	58.9	2.164	0.043
34.9	0.466	0.029	42.3	0.820	0.057	58.9	2.070	0.030
36.0	0.469	0.029	44.0	0.823	0.044	59.0	2.057	0.038
41.5	0.789	0.047	44.7	0.894	0.060	60.0	2.350	0.026
41.6	0.718	0.024	44.8	0.935	0.045	62.0	2.344	0.032
42.2	0.868	0.031	44.9	0.920	0.046	62.6	2.744	0.031
43.0	0.883	0.004	45.2	0.988	0.065	64.0	2.800	0.048
43.8	0.864	0.028	45.4	0.957	0.073	64.0	2.517	0.028
44.9	0.992	0.026	45.9	0.880	0.045	64.5	3.511	0.084
46.1	1.103	0.041	45.9	0.955	0.070	65.5	2.939	0.036
46.1	1.137	0.029	46.0	0.860	0.047	65.9	3.420	0.029
46.8	1.057	0.035	47.0	0.998	0.062	66.1	3.266	0.038
50.4	1.395	0.081	47.2	0.982	0.059	66.5	3.321	0.063
52.1	1.670	0.072	48.1	1.008	0.041	66.5	3.499	0.065
53.1	1.462	0.054	48.3	1.095	0.058	66.7	3.310	0.058
54.8	1.815	0.043	49.9	1.341	0.056	67.2	3.596	0.069
55.4	1.874	0.049	51.5	1.397	0.064	67.3	2.891	0.029
55.7	1.619	0.054	55.2	1.701	0.049	68.0	3.315	0.047
56.5	2.118	0.060	56.7	2.003	0.054	69.8	4.294	0.105
57.5	2.293	0.046	56.8	1.783	0.063	70.5	4.287	0.077
57.8	2.094	0.072	57.2	1.799	0.070	70.8	3.859	0.041
59.3	2.107	0.044	57.4	1.950	0.066	71.9	3.495	0.044
60.4	2.496	0.042	58.2	2.020	0.051	72.1	4.650	0.061
60.8	2.278	0.039	59.5	2.244	0.061	72.3	4.453	0.048
61.4	2.467	0.050	60.6	2.117	0.079	72.6	4.218	0.071
62.3	2.587	0.059	63.6	2.397	0.068	72.7	4.262	0.059
62.5	2.274	0.038	65.8	2.905	0.080	72.8	4.571	0.055
62.6	2.406	0.046	66.6	3.204	0.088	73.5	4.741	0.075
62.9	2.298	0.043	71.8	3.804	0.085	73.6	4.711	0.077
65.3	2.987	0.048	73.0	4.094	0.125	77.1	5.286	0.057
67.5	3.303	0.042	73.2	3.655	0.076	77.6	5.296	0.075
68.7	3.432	0.037	84.2	6.754	0.175	88.6	8.508	0.108

Table H2 continued...

Ft Macleod			Pearce Rd			Popson Park		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
31.1	0.316	0.026	27.8	n/a	0.021	41.6	0.725	0.048
35.9	2.843	0.064	31.3	0.299	0.039	43.8	0.940	0.048
45.3	0.894	0.027	33.2	0.404	0.035	44.0	0.839	0.044
46.1	1.071	0.037	33.9	0.349	0.040	45.9	0.944	0.072
48.6	1.013	0.051	34.6	0.399	0.035	49.0	1.111	0.061
48.7	1.416	0.060	35.0	0.367	0.049	50.2	1.170	0.139
49.6	1.144	0.070	35.1	0.405	0.032	51.8	1.253	0.097
50.8	1.874	0.044	35.3	0.476	0.035	52.9	1.329	0.135
51.7	1.630	0.067	36.1	0.533	0.037	53.6	1.573	0.121
51.9	1.487	0.077	36.8	0.454	0.045	57.6	2.476	0.102
52.0	2.792	0.061	36.9	0.453	0.060	58.3	1.987	0.151
52.3	1.681	0.095	37.1	0.489	0.044	58.4	2.251	0.220
52.9	1.601	0.058	37.5	0.470	0.055	58.5	2.240	0.195
53.0	2.888	0.066	38.0	0.519	0.030	59.4	2.225	0.228
53.4	1.922	0.061	38.5	0.629	0.047	60.2	2.159	0.227
54.4	2.706	0.055	38.5	0.513	0.039	61.1	2.140	0.185
54.7	2.243	0.053	38.8	0.493	0.039	63.6	2.381	0.218
55.0	3.429	0.110	38.9	0.591	0.033	64.9	2.783	0.121
55.0	1.827	0.060	39.7	0.617	0.040	67.3	3.048	0.154
55.2	1.713	0.054	40.4	0.605	0.047	70.6	4.166	0.223
56.4	1.825	0.073	40.8	0.699	0.050	77.0	2.881	0.288
57.0	3.979	0.090	40.9	0.662	0.047	81.0	5.556	0.283
57.4	2.077	0.090	41.0	0.628	0.035			
57.7	1.945	0.067	41.7	0.722	0.051			
57.8	2.978	0.060	42.0	0.701	0.045			
58.3	3.403	0.078	43.6	0.712	0.051			
58.6	6.553	0.038	44.0	0.692	0.025			
59.2	2.167	0.087	45.4	0.817	0.022			
60.6	4.702	0.069	49.1	1.053	0.088			
60.7	2.244	0.051	51.6	1.529	0.062			
61.9	2.399	0.068	53.3	1.468	0.092			
62.9	2.539	0.113	55.1	1.602	0.105			
64.6	2.713	0.057	58.9	2.005	0.116			
65.2	2.962	0.116						
65.8	3.143	0.086						
67.4	3.375	0.100						
68.8	3.624	0.081						
71.1	3.945	0.072						

Table H2 continued...

Peenaquim Park reference			Pavan Park			Coaldale bridge		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
37.0	0.622	0.036	32.3	0.324	0.039	41.2	0.706	0.047
39.4	0.727	0.045	36.4	0.426	0.032	42.4	0.704	0.045
40.2	0.664	0.049	37.0	0.423	0.041	43.6	0.974	0.063
41.5	0.885	0.052	37.0	0.509	0.042	46.4	1.133	0.066
41.7	0.939	0.037	37.1	0.453	0.041	47.4	1.283	0.079
41.9	0.922	0.033	38.9	0.500	0.041	47.4	1.169	0.063
42.0	0.771	0.033	38.9	0.554	0.051	47.5	1.135	0.071
42.1	0.863	0.052	39.9	0.554	0.050	47.5	1.212	0.076
42.2	0.909	0.053	40.4	0.609	0.046	48.1	1.371	0.062
42.7	0.954	0.036	41.5	0.674	0.038	49.7	1.176	0.047
43.9	1.038	0.034	42.5	0.706	0.060	50.0	1.272	0.071
44.0	1.022	0.051	49.9	1.289	0.080	50.5	1.378	0.059
44.3	1.044	0.032	50.0	1.280	0.056	52.1	1.624	0.092
44.7	0.858	0.041	50.7	1.249	0.069	52.1	1.460	0.090
45.0	1.142	0.048	51.9	1.270	0.114	53.4	1.801	0.110
45.3	1.133	0.053	52.1	1.297	0.117	54.6	1.630	0.153
45.4	1.401	0.034	52.1	1.210	0.120	55.4	1.244	0.119
45.8	1.337	0.032	53.0	1.289	0.121	56.4	1.686	0.135
46.8	1.027	0.047	54.3	1.387	0.136	56.6	1.905	0.079
47.4	1.161	0.035	55.3	1.588	0.111	57.3	1.996	0.145
47.5	1.314	0.038	55.4	1.335	0.136	59.8	2.385	0.218
48.1	1.388	0.063	55.8	1.549	0.156	60.4	1.891	0.203
49.7	1.192	0.063	57.9	1.720	0.169	60.7	1.810	0.137
51.3	1.427	0.096	58.1	1.533	0.142	60.9	2.409	0.181
53.1	1.515	0.088	58.5	1.935	0.186	61.3	2.032	0.187
53.2	1.836	0.119	59.1	1.994	0.129	62.8	2.680	0.190
53.5	1.535	0.101	59.4	1.975	0.145	64.6	3.322	0.109
60.9	2.748	0.169	60.1	1.920	0.168	65.0	2.995	0.173
61.0	2.904	0.153	60.9	1.776	0.170	66.9	2.843	0.202
61.4	2.222	0.152	61.6	2.202	0.124	70.1	2.971	0.170
66.2	2.919	0.141	62.8	2.435	0.123	70.6	3.885	0.232
67.6	3.459	0.162	65.0	2.817	0.219	72.2	3.894	0.196
68.5	3.857	0.172	65.1	2.410	0.170	75.7	4.397	0.212

Table H2 continued...

Hwy 36		
Fork length (mm)	Weight (g)	THg (ppm)
31.6	0.321	0.036
37.8	0.533	0.033
43.0	0.864	0.075
43.1	0.758	0.072
43.2	0.589	0.043
44.5	0.842	0.071
44.8	0.889	0.091
44.8	0.845	0.070
46.3	1.019	0.079
46.8	0.971	0.085
47.0	1.138	0.073
47.3	0.952	0.062
47.5	1.090	0.085
47.9	0.827	0.098
48.0	1.107	0.082
48.0	1.115	0.070
48.4	1.245	0.090
50.3	1.237	0.076
50.3	1.319	0.095
50.7	1.226	0.090
51.8	1.405	0.118
52.1	1.359	0.120
54.1	1.685	0.151
55.4	1.790	0.121
56.5	1.489	0.163
57.8	1.901	0.104
58.0	1.843	0.102
58.3	1.667	0.147
58.4	1.891	0.095
63.9	2.092	0.231
64.4	2.383	0.203
65.3	2.661	0.220
66.6	3.195	0.202

Table H3: Mercury concentrations in longnose dace from urban waste water outfalls within the City of Lethbridge (2006 samples).

Paradise Canyon storm outfall			Lethbridge sewage			Peenaquim storm outfall		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
20.7	0.082	0.051	39.8	0.822	0.043	42.3	0.803	0.046
23.3	0.134	0.049	39.9	0.644	0.048	43.6	1.002	0.043
24.1	0.161	0.020	40.3	0.685	0.045	44.0	1.126	0.055
25.6	0.145	0.052	40.8	0.830	0.042	46.6	1.269	0.071
28.6	0.234	0.042	40.9	0.764	0.053	46.9	1.115	0.078
28.9	0.237	0.029	41.1	0.920	0.028	47.0	1.155	0.030
29.4	0.243	0.054	41.2	0.796	0.019	47.3	1.283	0.048
32.1	0.389	0.016	41.6	0.868	0.042	48.3	1.236	0.047
32.1	0.324	0.031	42.9	0.921	0.017	49.1	1.522	0.080
32.6	0.383	0.027	43.2	0.900	0.032	49.2	1.314	0.073
34.7	0.472	0.037	43.3	0.908	0.036	49.6	1.295	0.060
40.2	1.565	0.086	43.7	1.025	0.037	50.5	1.631	0.078
45.0	0.820	0.066	44.3	1.007	0.032	50.6	1.386	0.084
47.6	0.963	0.070	44.3	1.050	0.030	50.9	1.641	0.071
49.6	1.311	0.087	44.7	1.111	0.043	51.1	1.894	0.059
49.8	1.263	0.092	44.8	1.231	0.026	52.8	1.660	0.067
50.4	1.229	0.062	45.7	0.979	0.031	53.3	1.732	0.140
51.1	1.133	0.118	45.8	1.055	0.036	53.8	1.856	0.154
52.1	1.484	0.161	45.9	1.042	0.045	53.9	2.670	0.117
54.2	1.496	0.069	46.1	1.266	0.022	55.5	2.066	0.138
54.2	1.529	0.178	46.3	1.120	0.029	59.4	2.441	0.124
55.0	1.671	0.139	47.2	1.293	0.031	60.9	2.700	0.106
56.8	1.935	0.126	48.4	1.379	0.040	64.3	2.988	0.199
57.5	2.187	0.193	48.7	1.367	0.067	64.6	3.210	0.169
58.0	2.126	0.139	50.2	1.398	0.022	68.1	3.676	0.125
59.2	2.081	0.163	50.6	1.571	0.044	71.2	3.642	0.154
60.1	2.327	0.184	51.8	1.555	0.063	72.8	4.480	0.161
60.3	2.266	0.165	53.1	1.740	0.056	72.9	4.933	0.146
60.5	2.179	0.144	62.0	2.602	0.080	73.6	4.214	0.190
61.4	2.351	0.137	62.9	2.592	0.133	77.3	5.355	0.244
67.4	3.962	0.228				78.0	6.067	0.191
68.6	3.567	0.143				80.3	6.104	0.155
						81.9	5.593	0.174

Table H4: Mercury concentrations in longnose and white suckers from Popson Park (reference) and urban waste water outfalls within the City of Lethbridge (2006 samples).

Popson Park		
Fork length (mm)	Weight (g)	THg (ppm)
29.0	0.272	0.027
29.0	0.248	0.014
33.7	0.433	0.039
36.5	0.561	0.047
37.6	0.602	0.029
38.5	0.613	0.028
38.6	0.633	0.045
41.2	0.746	0.055
43.5	0.774	0.029
43.5	0.985	0.017
43.6	0.976	0.020
43.8	0.762	0.026
46.2	0.991	0.028
49.2	1.199	0.035
53.0	1.586	0.057
53.5	1.488	0.051
54.8	1.688	0.037
55.2	1.894	0.073
55.8	1.914	0.033
55.9	1.781	0.045
56.2	1.646	0.055
56.2	1.782	0.033
56.7	1.894	0.040
57.1	1.847	0.051
57.1	1.999	0.072
57.8	2.012	0.053
57.8	2.022	0.055
61.8	2.535	0.031
66.8	3.335	0.053
69.1	3.299	0.054
69.3	3.467	0.047
76.5	4.563	0.071
93.3	0.735	0.033

Ridgewood storm outfall		
Fork length (mm)	Weight (g)	THg (ppm)
30.7	0.252	0.048
30.9	0.276	0.042
32.1	0.322	0.036
34.3	0.416	0.042
35.1	0.351	0.044
35.6	0.488	0.044
36.5	0.411	0.029
36.9	0.439	0.036
38.8	0.508	0.031
39.2	0.618	0.024
41.6	0.692	0.027
43.2	0.734	0.033
43.9	0.798	0.056
44.1	0.872	0.035
44.1	0.828	0.047
44.1	0.897	0.030
44.4	0.859	0.055
44.6	0.821	0.112
46.2	0.974	0.033
46.7	0.907	0.037
46.7	1.057	0.036
47.4	1.166	0.036
48.1	1.173	0.027
49.2	1.231	0.028
49.9	1.145	0.027
50.5	1.220	0.082
50.5	1.219	0.099
50.6	1.460	0.137
50.7	1.425	0.068
50.8	1.268	0.023
51.1	1.223	0.111
51.2	1.408	0.019
51.5	1.157	0.039
52.0	1.574	0.060
52.3	1.414	0.037
54.8	1.732	0.019
55.7	1.991	0.027
56.5	2.027	0.056
57.1	1.842	0.027
59.6	2.230	0.067

Ridgewood storm outfall		
Fork length (mm)	Weight (g)	THg (ppm)
60.7	2.355	0.028
61.7	2.354	0.032
62.0	2.979	0.020
63.4	2.855	0.026
63.6	3.325	0.049
63.9	1.915	0.036
64.4	2.766	0.038
64.6	2.963	0.046
65.3	3.178	0.047
68.0	3.423	0.066
68.7	3.823	0.035
76.8	5.468	0.050
60.2	2.471	0.029
60.3	2.032	0.067
60.4	2.208	0.058
60.6	2.467	0.048

Coalhurst sewage		
Fork length (mm)	Weight (g)	THg (ppm)
55.2	1.874	0.032
58.0	2.243	0.030
59.9	2.706	0.022
61.8	2.665	0.048
62.0	2.792	0.021
62.0	2.978	0.022
63.0	9.355	0.045
63.9	2.888	0.015
64.8	3.429	0.017
67.6	3.403	0.030
68.9	3.979	0.034
71.5	4.702	0.018
79.6	6.547	0.034
82.6	7.443	0.044
82.8	6.553	0.024
86.0	7.617	0.023
87.9	8.576	0.029
88.8	8.904	0.036

Table H5: Mercury concentrations in longnose and white suckers from sites along the Oldman River, irrigation drainage canals, and the Little Bow River above Twin Valley reservoir (2005 samples).

Ft. Macleod			Pearce Rd.			Popson Park		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
114	16.7	0.133	160	42.2	0.076	177	56.9	0.117
105	13.2	0.134	140	34.5	0.078	172	63.3	0.129
126	21	0.086	134	26.6	0.038	128	19.7	0.191
123	19.8	0.152	158	37.3	0.057	150	3737	0.193
122	22.8	0.136	108	13	0.017	156	44.3	0.137
148	38	0.138	122	20.1	0.016	160	42.8	0.090
121	19.6	0.130	136	27.2	0.042	151	47.9	0.150
147	37.3	0.155	148	37.5	0.030	132	22.4	0.154
135	25.9	0.102	170	59.3	0.069	142	30.4	0.117
95	9.3	0.158	166	39.8	0.128	136	26.4	0.220
130	25.9	0.153	190	69	0.080	132	24.4	0.171
122	19.9	0.083	146	39.8	0.060	110	13	0.097
116	16.4	0.100	121	20.2	0.037	131	21.9	0.138
102	12.1	0.140	136	29.4	0.073	143	30.3	0.170
114	16.3	0.133	168	55.1	0.101	141	27.9	0.111
107	12.5	0.117	132	25.9	0.062	135	26	0.170
						139	28.9	0.246

Table H5 continued...

Pavan Park			Monarch canal			BASF canal		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
85	7.8	0.080	86	8.9	0.095	72	5.3	0.029
95	8.8	0.054	88	8.1	0.040	75	5.7	0.051
96	10.4	0.111	97	11.3	0.105	84	7.6	0.055
105	n/a	0.065	100	11.1	0.115	89	8.5	0.055
107	13.4	0.034	103	14.8	0.181	95	10.5	0.075
109	15.0	0.072	107	14.1	0.213	97	13.7	0.051
116	16.3	0.049	110	19.0	0.065	98	11.2	0.056
123	17.6	0.051	114	18.4	0.188	101	13.4	0.051
123	20.7	0.088	124	22.0	0.117	130	26.0	0.068
124	20.2	0.050	125	23.4	0.070	136	25.8	0.074
127	20.5	0.047	125	26.2	0.142	159	57.3	0.092
130	25.5	0.045	126	25.9	0.200	160	46.5	0.079
133	24.0	0.051	134	24.0	0.093	172	70.8	0.080
139	37.2	0.067	135	27.9	0.119	177	72.2	0.052
140	30.0	0.039	137	35.0	0.073	187	62.0	0.065
155	38.9	0.107	156	38.6	0.116	196	83.1	0.073
156	38.5	0.046	164	54.6	0.141			
158	42.4	0.070						
165	11.0	0.045						
197	82.3	0.202						

Table H5 continued...

Pyami canal			Battersea canal			Little Bow River		
Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)	Fork length (mm)	Weight (g)	THg (ppm)
79	5.5	0.054	130	30.1	0.058	88	8.1	0.060
81	6.4	0.049	134	33.3	0.050	96	9.4	0.048
84	6.5	0.037	137	34	0.065	96	11.3	0.060
86	6.6	0.076	138	34.9	0.058	106	14.3	0.057
88	7.9	0.061	138	38.1	0.059	108	10.8	0.043
90	8.7	0.060	144	42.5	0.064	112	17.1	0.063
94	9.4	0.071	144	38.7	0.055	113	17.5	0.057
97	12.0	0.059	146	26.9	0.048	114	16.6	0.053
105	14.2	0.056	147	47.8	0.054	118	20.1	0.046
105	14.7	0.067	149	42.3	0.052	118	18.2	0.053
107	14.5	0.070	156	53.9	0.057	122	19.8	0.046
112	17.8	0.072	158	53.3	0.050	124	22.0	0.044
115	20.5	0.089	159	53.9	0.053	126	12.6	0.034
119	19.5	0.068	165	59.1	0.039	147	38.1	0.039
130	27.1	0.069	166	48.4	0.048	175	66.8	0.034
155	47.5	0.054	167	63.3	0.043	193	99.6	0.034
157	52.1	0.060				208	12.3	0.032

Table H6: Mercury concentrations in northern pike from Twin Valley reservoir (2005 samples).

Fork length (mm)	Weight (g)	Age (yrs)	THg (ppm)
350	310	3	0.368
367	366	2	0.683
377	464	4	0.697
379	419	2	0.615
420	620	4	0.387
420	611	4	0.487
427	598	3	0.338
437	708	5	0.824
440	526	3	0.930
444	736	5	1.090
446	652	4	1.283
457	794	5	1.178
458	755	5	0.535
459	758	3	0.877
460	682	4	1.543
465	768	4	0.405
468	805	3	0.902
470	815	4	0.456
491	924	4	0.525
492	838	5	1.270
498	883	4	1.152
499	1049	4	0.781
510	979	5	1.154
517	1046	5	1.395
539	1185	5	1.418
546	1418	5	1.380
553	1135	4	0.816
590	1693	6	1.461

Table H7: Mercury concentration in lake trout from Waterton Lakes National Park (2005 samples).

Basin	Fork length (mm)	Weight (g)	Age (yrs)	THg (ppm)
Upper	216	93	5	0.128
	246	141	6	0.126
	254	156	5	0.126
	254	156	6	0.231
	272	194	3	0.180
	283	220	5	0.165
	295	252	6	0.335
	297	257	6	0.158
	315	311	8	0.211
	322	333	8	0.184
	343	408	9	0.310
	370	521	6	0.243
	390	616	5	0.155
	410	724	8	0.391
	415	752	9	0.288
	428	830	12	0.574
	484	1230	12	0.887
	490	1280	6	0.273
	510	1450	10	1.406
	510	1450	9	0.319
	520	1550	11	0.846
	540	1750	7	0.227
	540	1750	11	0.312
Middle	695	3900	12	0.620
	720	4400	12	0.549
	733	4600	11	0.434
	740	4800	10	0.507
	750	5000	11	0.463
	770	5500	12	1.087

Table H8: Mercury concentrations in lake whitefish from Waterton Lakes National Park (2005 samples).

Fork length (mm)	Weight (g)	Age (yrs)	THg (ppm)
139	30	1	0.039
144	35	2	0.035
185	79	2	0.048
191	95	3	0.048
197	84	2	0.042
200	96	3	0.054
200	103	3	0.056
203	114	3	0.062
209	102	3	0.046
212	115	3	0.055
213	125	3	0.104
214	124	3	0.059
215	122	4	0.060
216	122	3	0.046
220	119	3	0.049
220	136	3	0.058
223	133	3	0.055
225	128	3	0.042
228	146	3	0.054
238	152	4	0.060
241	173	4	0.070
247	197	4	0.109
248	184	4	0.110
249	193	3	0.061
251	183	4	0.082
251	183	4	0.053
260	188*	4	0.040
261	179	5	0.065
264	224	4	0.048
265	227	3	0.057
267	237	4	0.067
267	238	4	0.060
270	237	4	0.082
280	236*	5	0.047
283	268	5	0.063
298	349	6	0.127
305	307*	4	0.052
305	307*	5	0.063
310	323*	4	0.059
315	340*	7	0.076
317	412	6	0.127
320	356*	5	0.101
325	374*	6	0.071

* weight data reconstructed using regression model $7x10^{-6} \bullet$ fork length$^{3.0764}$, $R^2 = 0.99$

Table F8 continued...

Fork length (mm)	Weight (g)	Age (yrs)	THg (ppm)
335	410*	5	0.105
335	410*	6	0.054
342	437*	6	0.072
343	518	3	0.131
345	449*	4	0.195
345	449*	6	0.102
345	449*	7	0.083
350	470*	5	0.180
353	482*	7	0.095
355	490*	7	0.078
367	543*	4	0.209
380	605*	5	0.145
390	655*	5	0.135
400	708*	7	0.094
405	736*	8	0.173
410	764*	6	0.133
415	793*	9	0.063
415	793*	7	0.148
415	793*	7	0.106
415	793*	7	0.125
420	823*	6	0.171
420	823*	8	0.140
420	823*	7	0.125
425	853*	8	0.179
425	853*	8	0.177
430	885*	8	0.115
430	885*	8	0.111
430	885*	7	0.142
440	949*	8	0.261
440	949*	10	0.117
440	949*	8	0.135
440	949*	8	0.088
440	949*	5	0.240
445	983*	9	0.179
450	1017	9	0.116
452	1130	7	0.188
460	1088*	9	0.084
460	1088*	9	0.103
465	1125*	8	0.147
470	1163*	11	0.130
470	1163*	10	0.192
480	1241*	10	0.161
490	1322*	14	0.138
495	1364*	9	0.203
495	1364*	7	0.222
575	2162*	15	0.160