uOttawa

L'Université canadienne
Canada's university

FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

uOttawa

L'Université canadienne
Canada's university

FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

Jonathon R. Hill
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Biology spec. tox. chm/env)
GRADE / DEGREE

Department of Biology
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Factors Affecting Methyl Mercury Partitioning to DOC and UVB Photodegradation in Fresh Waters
TITRE DE LA THÈSE / TITLE OF THESIS

D. Lean
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

J. Blais

D. Fortin

A. Rencz

Gary W. Slater
LE DOYEN DE LA FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES /
DEAN OF THE FACULTY OF GRADUATE AND POSTDOCORAL STUDIES

FACTORS AFFECTING METHYL MERCURY PARTITIONING TO DOC AND UVB PHOTODEGRADATION IN FRESH WATERS

JONATHAN R. HILL

Thesis submitted to the
School of Graduate Studies and Research
University of Ottawa
In partial fulfillment of the requirements for the
M.Sc. degree in the

Ottawa-Carleton Institute of Biology

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-494-11292-1
Our file Notre référence
ISBN: 0-494-11292-1

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Abstract

The partitioning of methyl mercury (MeHg) to dissolved organic carbon (DOC) and the photodegradation of mercury by UVB radiation (280-320 nm) are two important processes that influence the availability of MeHg to the base of the aquatic food chain.

Water samples from 20 sites across Eastern Ontario and Western Quebec were filtered sequentially using tangential flow ultrafiltration to determine the size distribution of MeHg and DOC and to test whether the concentrations and distribution of these two variables varied in wetlands, lakes and rivers. These filtrates were also analyzed for DOC fluorescence and absorbance. The highest proportions of mean MeHg (47.3 ± 25.4%), DOC (56.8 ± 14.5%) and DOC FL (74.5 ± 11.4%) were found in the low molecular weight fractions (<5 kDa). Significant differences in the distribution and concentration of MeHg amongst filtered samples were found between wetlands, lakes and rivers. MeHg was related to DOC at all size fractions. The low molecular weight organic compounds may be an important contributor to MeHg biomagnification through uptake by bacteria and/or algae.

St. Lawrence river water was collected to test factors that affect the rate of photodegradation of MeHg. Samples exposed to UVB irradiance from a fluorescent lamp and spiked with MeHg (5 ng/L) illustrated significant decreases in concentration with a 31% average loss after 6 hours. No significant difference in photodegradation was found between samples with and without added Fe(II). MeHg concentrations decreased 35.4% and 41.7% after 6 hours of exposure at pH 3 and 5, respectively. It appears that photo-demethylation is a function of UVB exposure, is more rapid in acidic conditions and likely occurs slower under most natural freshwater conditions due to the attenuation of UVB.

Résumé

L'association du méthylmercure (MeHg) au carbone organique dissous (COD) et la photodégradation du mercure par les rayons UVB (280-320 nm) sont deux processus importants pouvant influencer la disponibilité de MeHg à la base de la chaîne alimentaire aquatique.

Des échantillons d'eau de 20 endroits differents en Ontario et au Quebec ont été filtrés séquentiellement à l'aide de l'ultrafiltration de flux pour déterminer la distribution de MeHg et du COD, afain de voir si les concentrations et la distribution de ces deux variables variaient entre les marécages, les lacs et les fleuves. Des filtrats ont été également analysés par fluorescence (FL) et l'absorbance (Abs) du COD a été déterminée. Les niveaux les plus élevés de MeHg (47.3 ± 25.4%), COD (56.8 ± 14.5%) et de fluorescence du COD (74.5 ± 11.4%) ont été observés dans les fractions à faible poids moléculaire (<5 kDa). Il y avait des différences significatives dans la distribution de MeHg entre les échantillons filtrés des marécages, lacs et fleuves. Le MeHg était présent dans toutes les fractions de COD. Les fractions de composés organiques faible poids moléculaire peuvent donc être un facteur important dans la biomagnification du MeHg, lors de la prise en charge par des bactéries ou des les algues.

L'eau du fleuve St. Laurent a été échantillonée pour examiner les facteurs affectant le taux de photodégradation de MeHg. Après l'addition de 5 ng/L de MeHg aux échantillons d'eau, ces derniers ont été exposés aux rayons UVB avec une lampe fluorescente. Les résultats ont démontré des diminutions significatives de concentration de MeHg avec une perte moyenne de 31% après 6 heures. Il n'y avait pas de différence significative de photodégradation entre les échantillons avec et sans Fe(II) supplémentaire. Les concentrations de MeHg ont diminué de 35.4% et de 41.7% après 6 heures d'exposition à un pH de 3 et de 5, respectivement. Il apparaît que la photo-demethylation est fonction de l'exposition aux UVB et qu'elle est plus rapide sous des conditions acides. Par consequant, la photodégradation du MeHg dans les échantillons d'eau naturelle est probablement lente care les eaux naturelles ont un pH près de la neutralité (pH 5-8).

Acknowledgements

First, I would like to thank my parents, David and Nancy, for their understanding, support and empathy throughout my pursuit of this degree. I would also like to extend special appreciation to Jennifer Gibson for her friendship as well as for her valuable contributions to this study. In the laboratory and out in the field, I am very grateful for the hard work and fellowship of the whole Lean lab team. Extra thanks to Susan Winch and Nelson O'Driscoll for their scientific guidance.

This study was financially supported through research grants awarded to my supervisor Dr. David Lean from the innovative and interdisciplinary Collaborative Mercury Research Network (COMERN). I would like to acknowledge Dr. Lean for his direction, trust, knowledge and enthusiasm about science. Thank you to my committee members Dr. Jules Blais and Dr. Andy Rencz for their patience, insightful questions and positive suggestions.

I am sincerely grateful to the Heather G. McMurter Scholarship Committee at the U of O for selecting me as the recipient of their award for 2003 as well as the COMERN board of directors for awarding me 3rd Prize at their 5th Annual Congress.

Finally, thank you to my colleagues at the St. Lawrence River Institute and the fishers of Stormont, Dundas and Glengarry counties, like my grandfather Allan and my cousins Emma and Thomas, who inspired me to investigate and appreciate but not to fear our environment.

Tables of Contents

Chapter 1. Introduction

Chapter 2. Methods

Chapter 3. Results

Chapter 4. Discussion

List of Tables

List of Figures

LIST OF ABBREVIATIONS

COMERN	Collaborative Mercury Research Network
C-DOC	chromophoric dissolved organic carbon
COC	colloidal organic carbon
DGM	dissolved gaseous mercury
DOC	dissolved organic carbon
DOC FL	DOC fluorescence
DOC Abs	DOC absorbance at 254 nm
GC-AFS	gas chromatography atomic fluorescence spectrometry
HDPE	high density polyethylene
HMW	high molecular weight
LMW	low molecular weight
MeHg	methyl mercury
Milli-Q	de-ionized water
TFU	tangential flow ultrafiltration
UVA	ultraviolet-A radiation (320-400 nm)
UVB	ultraviolet-B radiation (280-320 nm)
QSU	quinine sulfate units (units of DOCFL)
S.F.	Network for Sustainable Forestry Management
W.E.R.F.	Water Environment Research Foundation

Preface

As with most scientific research, very specific information is required in the study of environmental cycling of contaminants to fill critical gaps in our understanding of movements between substrates in ecosystems. The primary goal of this Master's Thesis is a modest one, to contribute solid data towards our comprehension of aspects of the mercury cycle that influence the bioavailability of methyl mercury to the base of the food chain. To this end, this work also represents bricks in the wall of COMERN Projects 3.1.2.2 "Methylmercury photodegradation and mercury recycling in aquatic ecosystems" and Case Study 3.2.4 "The St. Lawrence River Mercury dynamics in aquatic ecosystems". Insight and motivation for my research followed observations made in the survey of mercury in the lakes of northern Quebec supported by the Network for Sustainable Forestry Management (S.F.). This study also fits within a comprehensive mercury research program in the laboratory of Dr. Lean, who has enabled students to examine separate segments of the food chain and the global mercury cycle. It is my hope that cooperative initiatives such as collaborative research networks and targeted research programs will continue and encourage further progress in this field.

Growing up in the community of Cornwall on a section of the St. Lawrence River which is noted for being an "Environmental Area of Concern", I realize that the climate of environmental contamination can and does harm real people. Reliable information is required to separate problems that require attention from the many sensationalized issues which can be perpetuated. Contaminants deserve our serious attention in order to prevent further corruption of water, land, wildlife and air.

Chapter 1

Introduction

1.1 Rationale

The study of methyl mercury (MeHg) is important since it is a global contaminant known to bioaccumulate in food chains and cause neurological impairments in organisms at low concentrations. MeHg tends to bind to a collection of molecules called dissolved organic carbon (DOC). The binding of MeHg to DOC may influence how much MeHg is available to the base of the aquatic food chain (Choi et al. 1998). The determination of the dynamics of free and bound MeHg in association with DOC has been elusive due to the inability to accurately measure these very low concentrations. Small disparities in our perception of MeHg availability at the base of the food chain could result in models with inflated or inordinately low estimates of mercury contamination (Hickey et al. in press). The molecular size of DOC bound MeHg fractions may influence bioavailability to algae and bacteria. Therefore, environmental standards calculated by organizations such as the U.S. Environmental Protection Agency (E.P.A.) and Environment Canada may be improved by further study of MeHg and DOC partitioning in order to monitor and manage fresh water systems more effectively.

The majority of mercury research has been based in lakes and has been generalized to other aquatic environments. Due to the higher rate of hydraulic residence time and the hydrodynamic changes caused by current flow and channeling, more research into the differences in MeHg mercury flux in rivers is required. Wetlands are also an understudied system in the mercury cycle even though they are considered to be a significant source of MeHg. It is important to consider the unique processes that may change interactions between MeHg and DOC in wetlands where the concentration of DOC is often high, the pH low and the water depth shallow. Wetlands accumulate large amounts of slowly decaying plant material (Mitsch and Gosselink 1993) and have high rates of sulfate-reducing bacterial methylation in sediments. These characteristics can lead to increased levels of aqueous DOC and MeHg respectively (Boening 2000).

In addition to the MeHg partitioning, UVB photochemical reactions alter the bioavailability of mercury to the base of the food chain by initiating changes in speciation. Exposure to UVB irradiation can de-methylate MeHg and allow dissolved gaseous mercury to escape the system. However, the effect of other water chemistry variables such as acidity and the concentration of other dissolved ions on MeHg photodegradation is still unknown.

Since the world is undergoing constant change through continued anthropogenic industrialization and natural flux, it is imperative that we identify the fundamental processes in the mercury cycle so that we may mediate mercury problems as they arise. A nuanced appreciation of processes such as MeHg partitioning and UVB photochemical reactions are required for predictive modeling and to initiate best management practices. For instance, global climate change has been predicted to cause large fluctuations in DOC concentrations through the creation and elimination of wetlands. Also, the flooding of forests through the construction of new hydroelectric reservoirs will mobilize organic deposits. In both of these cases, the outcome of higher DOC concentrations for MeHg binding and bioavailability to the aquatic foodchain are unknown. There are also indications that a shift in global temperatures will result in changes to the acidity levels in lakes (Yan et al. 1998, Schindler et al. 1997). This process may free a greater percentage of bound MeHg to be taken up by the biota. If these large scale changes come to fruition, a more precise understanding of the biochemical interactions between MeHg and DOC will only become more important.

1.2 MeHg

Mercury is a naturally occurring metal with a well documented history. It is particularly curious due to unique chemical properties which can change dramatically depending on its speciation. Volumes of data are collected each year in regards to the biological damage it can inflict and the way in which it cycles through ecosystems all over the world. It is now recognized as a global contaminant of concern. Due to the harmful effects it can inflict upon human beings, this thesis will concentrate on the organic mercury species called methyl mercury (MeHg).

Methyl mercury is a neurotoxin present in terrestrial and aquatic environments. It is the most likely mercury species to cause severe damage to humans since it is persistent and bioavailable (Wren 1985). MeHg is known to disrupt the function of microtubules by initiating polymerization and depolymerization events which can interfere with cellular growth and the development of neurons (Sager 1988). Human exposure to MeHg is primarily through the consumption of food such as fish and aquatic mammals. MeHg bioaccumulates and biomagnifies in food chains which means that dosage concentrates in tissue and magnifies when it is consumed by a higher trophic level.

Aqueous MeHg usually exists as the neutral ions Ch_3HgCl and Ch_3HgOH after being converted from the inorganic form Hg(II) (Rand 1995). Due to the greater than 1 million times biomagnification which is estimated to occur between water and fish tissue, very small changes in aqueous MeHg concentration may have a tremendous impact on higher rungs of the food chain (Watras et al. 1994). Also, it is known that the proportion of MeHg increases dramatically at each step in the food chain. From zooplankton to fish there is more than a tripling in the percentage concentration of MeHg.

It is known that MeHg ions can adsorb to or be absorbed by the cell walls or membranes of phytoplankton and be accumulated (Kirkwood et al. 1999). Phytoplankton may also recycle mercury back into aquatic systems via the products of their decomposition (Boener 2000). Pickhardt et al. (2002) found that the number of algae which exist in a system may impact the amount of MeHg transmitted up to higher trophic levels in aquatic systems. Since algae take up MeHg from the surrounding waters, a high number of algae per unit volume seems to disperse the concentration of this toxin in such a way that individual algae have a low overall body burden (Pickhardt et al. 2002). If low numbers of algae per unit volume are present in a given system, the available MeHg in the surrounding waters is concentrated in fewer cells, which, given the same chemical conditions would translate into higher overall body burdens (Pickhardt et al. 2002). Subsequently, zooplankton in the aquatic system received more MeHg from a site with fewer algae (Pickhardt et al. 2002). This relationship may explain the disparity between the relationship of MeHg in natural waters with fish Hg concentrations.

Although correlations sometimes exist, there is not a strong relationship between the amount of total MeHg in fresh water and the concentration of MeHg in fish. For instance, Gorski et al. (2003) tested two lakes with different levels of MeHg in the water and in the fish. They found that high fish Hg concentrations were not related to unfiltered MeHg concentrations in the surface water but were correlated to zooplankton MeHg concentrations (Gorski et al. 2003). This scenario can occur when using the standard laboratory measurement of total MeHg in water which does not necessarily represent the bioavailable fraction. One system may have higher MeHg concentrations in the water yet have lower concentrations in fish. Organic molecules can bind and influence the uptake and assimilation of MeHg by organisms at the base of the food chain. The partitioning of MeHg to these compounds will be investigated in this thesis.

Dissolved organic carbon is known to bind MeHg and can transport it from catchments into aquatic systems, within the water column of a given system and between aquatic environments (Mierle and Ingram 1991, Wallschlager et al. 1996). DOC can form complexes with MeHg, rendering it less bioavailable to organisms such as phytoplankton (Nwobu unpublished). The complexation of MeHg and liberation from associations with DOC plays a large part in determining bioavailability (Amirbahman et al. 2002).

A number of laboratories have attempted to calculate accurate constant (pKa) values for binding between MeHg in aquatic solutions with thiol (-SH) functional groups. Thiol groups tend to outnumber MeHg molecules by an overwhelming margin, this has been estimated at a ratio of 2.7×10^5 RSH to MeHg (Karlsson and Skyllberg 2003). It has been found that the equilibrium binding constants between MeHg and thiol groups to be relatively similar in magnitude to the binding constants between MeHg and DOC molecules (Karlsson and

Skyllberg 2003). This finding reaffirms the importance of thiol groups as the primary binding sites for MeHg in aquatic environments.

High levels of acidity (low pH) can liberate MeHg from ligands such as particulate and colloidal organic carbon complexes (Amirbahman et al. 2002, Greenfield et al. 2001). It has been proposed that MeHg may be more available when there is increased competition from H+ ions for thiol sites (Karlsson and Skyllberg 2003). Another hypothesis is that higher acidities encourage increased methylation of mercury near the sediment and within the water column (Winfrey and Rudd 1990). HgII is incorporated into these bacteria cells by facilitated diffusion. Kelly et al. (2003) have found that this process is accelerated under acidic conditions because DOC develops a reduced negative charge and binds a lower number of ligands. This phenomenon would increase the amount of Hg(II) accumulated by bacteria which may translate into more methylated mercury in the system.

1.3 Shape, Structure and Size of DOC Molecules

At the chemical base of the mercury cycle in aquatic environments, the fate of MeHg and the form of DOC are inextricably linked. Therefore, it is imperative to discuss the relevant chemical aspects of these unique organic complexes in order gain an appreciation for its environmental associations.

DOC is the name given to a dynamic set of highly complex polyelectrolytic molecules which are present in fresh water and marine environments (Hayes et al. 1989). DOC molecules are responsible for the familiar yellow, dark brown and black tea-like colours that are found in many fresh water systems. DOC has been classically defined as the concentration of organic carbon found in the 0.45 micron filtrate (Hall et al. 1996). However, with the advent of better filtration technology, it is now known that a large portion of organic carbon in the 0.45 micron filtrate is not actually dissolved. The less than 0.45 fraction actually contains free metals, bound metals, complex ions and a distribution of colloidal molecules (Guéguen 2002). It is important to acknowledge that the diverse chemical qualities of DOC are just as important to aquatic environments as site specific concentrations (Babiarz et al. 2003).

Research has revealed that there are several kinds of aquatic organic carbon which can be distinguished by molecular size and chemical characteristics. Therefore, while the term DOC is still used, it is now understood that this represents a complex distribution of aquatic macromolecules with distinctive properties.

DOC is usually subdivided into 3 main groups; particulate organic carbon (POC), colloidal organic carbon (COC) and dissolved organic carbon (DOC). POC is often identified as the fraction of organic carbon with diameters above 1 µm (Sigg 2000), 0.7 µm (Babiarz et al. 1998), 0.2 µm (Lean personal communication), 0.45 µm (Eyrolle et al. 1996) or between 0.1 µm to 0.45 µm (Hill and Apler 2001) depending upon the author. The colloidal fraction has been identified as being between the molecular weight of 10 kDa to the diameter of 0.7 µm (Babiarz et al. 1998), 10 kDa to 0.45 µm (Sigg 2000), 5 kDa and 0.45 µm (Eyrolle et al. 1996) and, 1 kDa and 0.1 µm (Hill and Alpin, 2001). Low molecular weights tend to be reported as less than 10 kDa filtrates (Sigg 2000, Babiarz et al. 1998). In this thesis, the particulate will be described as greater than 0.2 μm, the colloidal fraction as between 5 kDa and 0.2 μm and low molecular weight fractions as less than 5 kDa.

The primary components of DOC are humic substances which comprise anywhere from 50-90% of the aggregate macromolecule (Hayes et al. 1989, Sachse et al. 2001, V.-Balogh et al. 2003). The composition of the humic substances can vary widely depending on the origin of the material. The weight of these molecules can range from a few hundred to several million Daltons (Perminova et al. 2003). Humic substances are generally formed by the decomposition of organic matter such as leaf litter and animal decay. Perminova et al. (2003) has reported that at least 77 different humic materials exist. This material can be transported into freshwater environments from allochtonous sources such as catchment soils and inflows, or autochthonous sources such as algae decay or the detritus of other aquatic organisms (Rasmussen et al. 1989). These humic substances are a heterogeneous complex of humic acids, fulvic acids and humin (Melamed et al. 2000).

Humic acids are the largest humic substances which can have a range of molecular weights from the thousands to the hundreds of thousands of Daltons. One author theorized a size range of 10 to 300 kDa (Lawrence 1980). Humic acids are insoluble in acids but soluble in bases (Rashid et al. 1985). They are defined as products that are extracted from sediments, soil and peat in alkaline conditions (Rashid et al. 1985) which form a dark amorphous organic precipitate at an acidic pH of 2.0 (O'Driscoll and Evans 2000). Humic acids have a high degree of polymerization and seem to be the condensation products of fulvic acids (Rashid et al. 1985).

Fulvic acids are compounds with low molecular weights which range from the hundreds to thousands of Daltons (Hintelmann et al. 1997). The molecular size limit has been approximated at 10 kDa (Lawrence 1980). Due to the prevalence of oxygen-rich functional groups in their structure, they are soluble at all pH values (Rashid et al. 1985). Some smaller molecular weight fulvic acids are even soluble in water. Fulvic compounds have a preponderance of aliphatic side chains, are weakly aromatic (Rashid et al. 1985) and are found in higher concentrations than humic acids in DOC molecules (V.-Balogh et al. 2003).

Humins are the smallest of the three compounds and are insoluble in weak acids and bases due to strong bonds with minerals (Rashid et al. 1985). It is thought that they are denatured or dehydrated humic acids.

Non-humic compounds may only comprise a few percent of the total molecular mass but are apt to play an important role in DOC molecules. Labile organic compounds such as carboxylic acids, carbohydrates and amino acids make up approximately 20% of the DOC molecules (Sache et al. 2001). Proteins can comprise up to approximately 10% of DOC mass and are important building blocks for the aliphatic sections of humic material (Rashid et al. 1985).

DOC molecules can have a variety of functional groups such as carboxylic acids, phenolics, keto- and thiols (Hintelmann et al. 1997). Carboxylic acids are amongst the most plentiful and important functional groups in DOC molecules. While both charges are present in humic substances, the dissociation of carboxylic groups seems to be responsible for the overall negative charge of the molecule. Lignins and phenolics have been found to comprise many chromophoric regions in DOC molecules (Grzybowski 2000). MeHg tends to bond strongly to sulfur-rich functional groups such as thiols. Solubility constants in a number of recent research papers have verified that MeHg has a great affinity for thiol-containing groups such as cysteine and glutathione (Amirbahman et al. 2002, O'Driscoll and Evans 2000). Although the constants range in magnitude they demonstrate that under relatively neutral conditions a very high percentage of MeHg in solution will bind to DOC via these thiol groups (O'Driscoll and Evans 2000).

Recently, in studies of individual lakes, researchers have found that the majority of DOC exists in the low molecular weight size fractions. Sachse et al. (2001) has claimed that the mass of DOC exceeded POC by one order of magnitude. Eyrolle et al. (1996) and Wu (2003) found that most of the DOC is located in the less than 20 kDa filtrate and 5 kDa filtrates respectively. Her et al. (2003) also found that the low size fraction between 5-1 kDa had the highest level of DOC aromaticity. River environments are theorized to have a higher proportion of high molecular weight humic substances than lakes since particulates can remain suspended for longer periods in a flowing system (Grzybowski 2000). Martin-Mousset et al. (1997) studied DOM in a range of rivers in France which showed that generally 70-80% of DOC existed in the higher than 1 kDa fraction but that the biggest river had 50% DOC in the 1 kDa filtrate. In contrast, Sigg et al. (2000) stated that most of the DOC molecules in the Thur river in Switzerland were found in the lower than 10 kDa fraction. It is important to keep in mind differences that are inherent in DOC molecules from different sites as well as subtle variations in laboratory techniques.

Few papers have attempted to quantify the proportion of DOC and MeHg in different size fractions for a variety of systems similar to this study. However, Babiarz et al. (2003) found that DOC concentrations averaged 48% of the total in the less than 10 kDa fraction with a range of 38 to 64 percent. The 10 kDa to 0.7 μm fraction contained an average DOC content of 49% with a range of 30 to 63 percent. MeHg concentrations averaged 40% of the total in the less than 10 kDa fraction with a range of 26 to 89 percent. The colloidal fraction (10 kDa to 0.7 μm) accounted for 60% of the total MeHg concentration with a range of 32 to 75 percent. An average of only 10% of MeHg was found in the particulate (>0.7 μm) phase.

Chromophoric DOC (CDOC) are chemically active regions within DOC molecules which are preferentially composed of aromatic functional groups (Winch, 2002). CDOC absorb visible radiation which can cause the excitation of electrons within functional groups (Korshin et al. 1997). CDOC molecules are most susceptible to UVB photobleaching which can cause CDOC regions to have low average molecular sizes (Grzybowski 2000).

DOC molecules will undergo electron excitation and fluoresce when activated by the UV spectrum (Winch 2002). Fresh water bodies with long water residence times generally contain DOC of low concentration, fluorescence (DOC FL) and absorbance (DOC Abs) (Gennings, 2001). This is important since UV damage to aquatic biota is reduced by a shielding or photoprotective property of DOC (Lean, 1997). High concentrations of DOC are known to attenuate UVA and UVB radiation, when DOC becomes photobleached, this ability is lessened (Lean, 1998). Fresh water biota such as phytoplankton seem to be at an increased risk when DOC concentrations are below 3 mg C/L (Lean, 1997). Since UV radiation can also degrade mercury compounds, DOC can reduce the amount of photooxidation and photoreduction of mercury that occurs by protecting MeHg-DOC bonds in molecules below the surface level in aquatic environments (Lean, 1997).

1.4 Photodegradation of MeHg

It is now known that UV irradiation can both photo-methylate and photo-demethylate mercury (Lean et al. 2003). The types of wave lengths that are known to be most important in photochemical reactions involving organic molecules and mercury species in aquatic environments are UVB (290-320 nm) and UVA (320-400 nm) (Lean 1998). Exposure to the UVB spectrum of radiation is the principle photo-initiator of MeHg degradation in fresh water (Sellers et al. 1998). The photodegradation of MeHg has been shown to proceed as an abiotic first order reaction (Sellers et al. 1998). Sterilization experiments which excluded bacteria from solutions prior to exposure have proven that MeHg losses in photodegradation experiments were not due to biotic demethylation and required the presence of light to progress (Sellers et al. 1998).

The mechanism of UVB photodegradation may occur through indirect photolysis with the formation of hydroxyl radicals in solution that react with $MeHg\text{-}Cl_2$ dissolved ions (Chen et al. 2003). It is known that alkyl groups can be stripped from MeHg and ethyl mercury in the presence of light by reactive oxygen species such as hydroxyl radicals and singlet oxygen (Chen et al. 2003). Freely dissolved MeHg is more likely to degrade due to the lack of protection by organic molecules. MeHg can be displaced from bonds with DOC functional groups by dissolved metals such as Fe(II) or H+ ions. The presence of Fe(II) in solution may also promote hydroxyl radical formation since it will quickly oxidize to Fe(III). This process may also encourage MeHg photodegradation. The product of MeHg and ethyl mercury photodegradation seems to be dissolved gaseous mercury (Hg^0) which volatilizes and escapes the system (Chen et al. 2003).

1.5 Objectives and Hypotheses

The objectives of this study were as follows:

1. To determine whether MeHg concentrations are related to DOC concentrations in un-fractionated and fractionated fresh water samples.

2. To determine whether differences exist between percentages of MeHg, DOC, DOC FL and DOC Abs in the particulate, colloidal and low molecular weight fractions of fresh water systems.

3. To determine if differences exist between wetland, river and lake environments in relation to the concentrations of MeHg, DOC and DOC FL in un-fractionated samples. Wetlands were predicted to have higher MeHg, DOC and DOC FL concentrations than lakes while rivers were expected to have intermediate concentrations.

4. To determine if differences exist between wetland, river and lake environments in relation to the distributions of MeHg, DOC and DOC FL in fractionated samples.

5. To determine whether either high or low molecular weight size fractions of MeHg are better predictors of fish mercury concentrations than total MeHg.

6. To test factors that influence the photo-demethylation of MeHg. Laboratory experiments using low DOC St. Lawrence River water were devised to test the effect of iron and pH change on MeHg when exposed to UVB irradiation.

The null hypotheses (H_0) to be tested in this study were as follows:

1. H_0: There is no significant linear relationship between MeHg and DOC concentrations in (a) un-fractionated and (b) fractionated fresh water samples.

2. H_0: There are no significant differences in the proportions of MeHg and DOC, DOC FL and DOC Abs in the particulate (>0.2), colloidal fractions (<0.2 μm, >5 kDa) and low molecular weight fractions (< 5 kDa).

3. H_0: Un-fractionated mean concentrations of MeHg, DOC, DOC FL and DOC Abs in wetlands are lower or equal to rivers or lakes. The un-fractionated mean concentrations of MeHg, DOC, DOC FL and DOC Abs in rivers are lower or equal to lakes.

4. H_0: There are no significant differences between the size distribution of MeHg, DOC, DOC FL and DOC Abs amongst wetlands, lakes and rivers.

5. $H_{0:}$ The low molecular weight size fractions of MeHg (< 5 kDa and <1 kDa) are less or equally predictive for Hg concentrations in fish as high molecular weight size fractions (> 5 kDa and >1 kDa) or total MeHg.

6. $H_{0:}$ The concentration of MeHg in St. Lawrence River water exposed to UVB radiation for 6 hours will increase or remain equal (a) when spiked with Fe(II) or (b) when pH is reduced to 5 or 3.

Chapter 2

Methods

2.1 Water Sampling

Water samples were collected from a total of 33 freshwater sites throughout Eastern Ontario and Western Quebec for this study from June 6th 2002 to September 13, 2003 (Table 1). Sample sites were selected to obtain a range of DOC concentrations in the fresh water categories of wetlands, rivers and lakes for the region of Eastern Ontario and Western Quebec (Figure 1). DOC values were known for a subset of sites (Hickey et al. in press and Winch 2003) in order to ensure that both high (e.g. Raisin River) and low (e.g. St. Lawrence River) concentrations would be acquired. A subset of these sample sites (6 wetland, 7 lake and 7 river sites) was chosen for MeHg and DOC partitioning tests (Figure 2).

Additional data originate from two mercury studies for which I performed the MeHg laboratory analysis and much of the field sampling within a two year period (2001-2003). This data is presented courtesy of the respective principle investigators and is analyzed in a new light for this study to illuminate the relationship between un-fractionated MeHg and DOC. The first study (Hickey et al. in press), which was commissioned by the Water Environment Research Foundation (W.E.R.F.) analyzed water chemistry and fish Hg concentrations from 30 lakes (Table 1) to calculate a guideline for MeHg in water acceptable for piscivorous wildlife. The second study, which was commissioned by the Sustainable Forestry Program (S.F.), was carried out by the laboratories of Dr. Lean (U of O) and Carignan of Université de Montréal (e.g. O'Driscoll et al. 2004, Garcia et al. 1999 and 2000) to analyze the affects of clearcut logging on MeHg in water, zooplankton and fish.

Table 1. Location of study sites with the date sampled and fresh water designation. Study sites are separated into three data sets: (a) Hill Thesis study sites, (b) Water Environment Research Foundation (W.E.R.F) study sites and (c) Sustainable Forestry (S.F.) study sites. In situ surface water chemistry data of pH and conductivity are also listed. Conductivity is estimated from alkalinity for the S.F. data set using the equation $y = 2.0x + 33.5$ ($r^2=0.95$) calculated from the W.E.R.F. study (Hickey et al. in press).

a)

Study Site	Designation	Province	Data Set	Date Sampled	Latitude	Longitude	pH	Conductivity (µS/cm)
Big Rideau*	Lake	ON	Hill Thesis	August 9, 2003	44°46	76°13	8.7	191.0
Commanda	Lake	ON	Hill Thesis	August 3, 2003	46°01	79°43	6.9	49.0
Cranberry	Lake	ON	Hill Thesis	August 10, 2003	44°45	75°42	8.8	190.0
Lac MacGregor	Lake	QB	Hill Thesis	September 6, 2003	45°42	75°48	7.9	104.0
Lac St. Pierre	Lake	QB	Hill Thesis	September 6, 2003	45°43	75°50	8.4	83.7
Loch Garry*	Lake	ON	Hill Thesis	June 22, 2003	45°15	74°43	7.7	209.2
Madawaska*	Lake	ON	Hill Thesis	August 9, 2003	45°28	76°21	7.8	111.0
Newboro*	Lake	ON	Hill Thesis	August 10, 2003	44°38	76°20	7.8	215.0
Opinicon*	Lake	ON	Hill Thesis	August 11, 2003	44°34	76°19	8.1	166.2
Otter	Lake	ON	Hill Thesis	August 10, 2003	44°30	76°32	8.3	279.0
Sharpes Bay*	Lake	ON	Hill Thesis	June 26, 2002	44°27	78°12	7.3	.
Temagami	Lake	ON	Hill Thesis	August 4, 2003	47°00	80°05	7.1	90.4
Black River	River	ON	Hill Thesis	September 13, 2003	45°06	74°52	7.3	339.0
Gatineau*	River	QB	Hill Thesis	September 6, 2003	45°36	75°27	6.8	38.0
Hooples Creek	River	ON	Hill Thesis	September 13, 2003	45°01	74°57	8.5	244.0
Ottawa*	River	ON	Hill Thesis	August 9, 2003	45°24	76°30	7.5	57.0
Raisin*	River	ON	Hill Thesis	June 22, 2003	45°08	74°30	8.3	446.0
Rideau *	River	ON	Hill Thesis	September 6, 2003	45°27	75°42	8.6	390.0
South Nation*	River	ON	Hill Thesis	July 16, 2002	45°19	75°07	7.8	173.7
St. Lawrence*	River	ON	Hill Thesis	October 3, 2002	45°01	74°42	8.7	280.0
Yamaska *	River	QB	Hill Thesis	May 30, 2003	46°09	72°48	7.4	348.0
Baie St. Francois*	Wetland	QB	Hill Thesis	May 30, 2003	46°12	72°48	6.7	169.0
Blackadder Creek	Wetland	ON	Hill Thesis	September 13, 2003	45°02	74°45	6.9	276.0
Blueberry*	Wetland	ON	Hill Thesis	August 10, 2003	44°54	76°18	6.7	306.0
Cooper's Marsh	Wetland	ON	Hill Thesis	September 13, 2003	45°07	74°30	7.0	319.0
Mer Bleue	Wetland	ON	Hill Thesis	September 15, 2003	45°24	75°30	5.8	112.7
Mississipi*	Wetland	ON	Hill Thesis	August 9, 2003	45°27	76°15	7.9	226.0
Odessa*	Wetland	ON	Hill Thesis	August 10, 2003	44°22	76°38	7.4	372.0
Otter*	Wetland	ON	Hill Thesis	August 10, 2003	44°30	76°32	7.3	289.0
Vieux Chemin*	Wetland	QB	Hill Thesis	September 6, 2003	45°42	75°52	7.0	289.0

b)

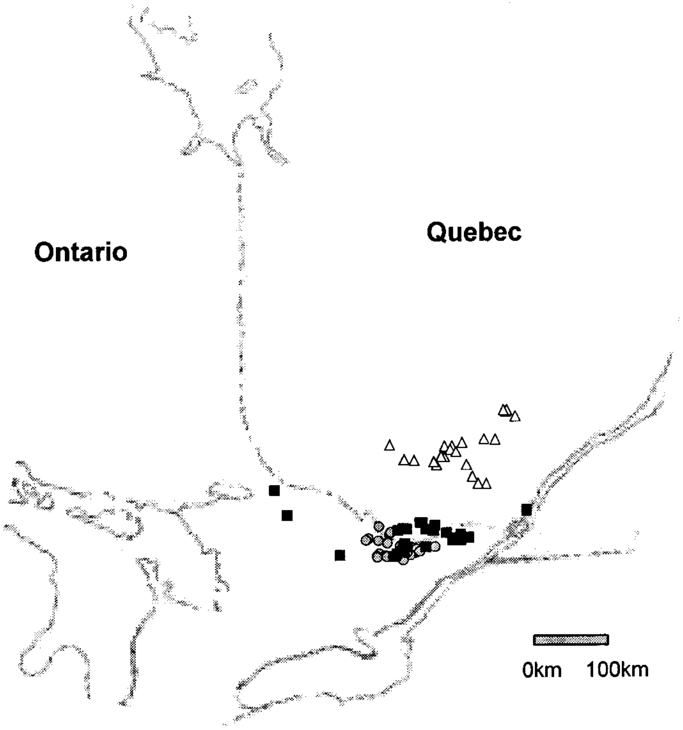
Study Site	Designation	Province	Data Set	Date Sampled	Latitude	Longitude	pH	Conductivity (µS/cm)
Ashby	Lake	ON	W.E.R.F.	July 31, 2001	45°05	77°21	7.3	48.1
Beaver	Lake	ON	W.E.R.F.	August 10, 2001	44°30	77°02	8.0	157.0
Bellamys	Lake	ON	W.E.R.F.	July 9, 2001	44°43	76°02	9.1	273.5
Buckshot	Lake	ON	W.E.R.F.	August 1, 2001	45°00	77°04	8.0	84.6
Calabogie	Lake	ON	W.E.R.F.	July 4, 2001	45°16	76°45	8.3	116.6
Camden	Lake	ON	W.E.R.F.	July 25, 2001	44°25	76°52	8.8	269.3
Charleston	Lake	ON	W.E.R.F.	July 11, 2001	44°32	76°00	8.9	232.7
Christie	Lake	ON	W.E.R.F.	July 18, 2001	44°48	76°26	8.5	151.2
Crotch	Lake	ON	W.E.R.F.	August 16, 2001	44°55	76°48	8.4	96.4
Devil	Lake	ON	W.E.R.F.	July 17, 2001	44°35	76°27	8.8	184.2
Dog	Lake	ON	W.E.R.F.	July 24, 2001	44°25	76°21	9.0	171.0
Eloida	Lake	ON	W.E.R.F.	July 18, 2001	44°40	75°58	8.9	282.3
Gananoque	Lake	ON	W.E.R.F.	July 6, 2001	44°27	76°09	9.0	248.3
Graham	Lake	ON	W.E.R.F.	July 10, 2001	44°34	75°53	8.6	189.8
Indian	Lake	ON	W.E.R.F.	July 12, 2001	44°36	76°20	9.0	221.2
Jonhstown Bay	Lake	ON	W.E.R.F.	September 6, 2001	44°45	75°27	8.6	301.5
Lake Ontario	Lake	ON	W.E.R.F.	August 30, 2001	44°15	76°21	8.8	301.9
Lime	Lake	ON	W.E.R.F.	July 17, 2001	44°24	77°07	8.5	361.1
Liittle Mellon	Lake	ON	W.E.R.F.	July 26, 2001	44°21	77°06	7.1	75.9
Loughborough	Lake	ON	W.E.R.F.	August 28, 2001	44°27	76°25	8.7	280.0
Mink	Lake	ON	W.E.R.F.	August 9, 2001	45°33	77°03	8.8	316.1
Norway	Lake	ON	W.E.R.F.	August 8, 2001	45°20	76°43	8.9	225.2
Pike	Lake	ON	W.E.R.F.	July 3, 2001	44°47	76°21	9.0	165.4
Sand	Lake	ON	W.E.R.F.	July 18, 2001	44°34	76°16	8.8	190.5
Troy	Lake	ON	W.E.R.F.	August 29, 2001	44°31	76°16	9.2	223.9
Varty	Lake	ON	W.E.R.F.	July 19, 2001	44°23	76°49	8.8	213.7
Weslemkoon	Lake	ON	W.E.R.F.	August 1, 2001	45°02	77°42	7.9	43.3
Whitefish	Lake	ON	W.E.R.F.	August 29, 2001	44°32	76°14	9.2	196.9

c)

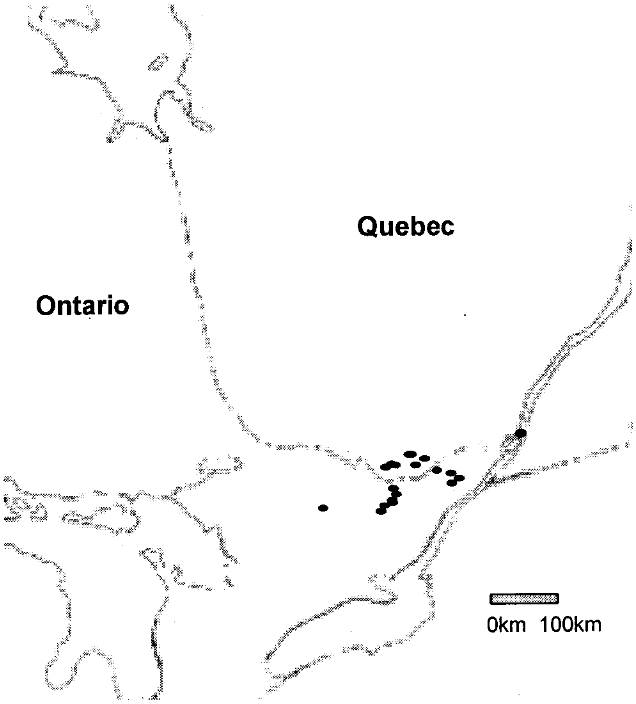
Study Site	Designation	Province	Data Set	Date Sampled	Latitude	Longitude	pH	Conductivity (µS/cm)
AB34	Lake	QB	S.F.	June 2001	47°13'53"	73°58'19"	6.5	203
AB35	Lake	QB	S.F.	June 2001	47°09'23"	74°13'11"	6.3	182
AB40	Lake	QB	S.F.	June 2001	47°31'17"	74°23'03"	6.4	91
CSL2	Lake	QB	S.F.	June 2001	48°51'13"	74°40'53"	6.4	316
CSL5	Lake	QB	S.F.	June 2001	48°43'04"	74°57'57"	6.6	181
DA2	Lake	QB	S.F.	June 2001	48°45'44"	76°43'00"	6.3	79
DA4	Lake	QB	S.F.	June 2001	48°11'23"	76°19'33"	5.1	47
DA9	Lake	QB	S.F.	June 2001	48°09'59"	76°01'47"	5.6	71
DF2	Lake	QB	S.F.	June 2001	50°04'29"	73°23'44"	6.7	356
DF4	Lake	QB	S.F.	June 2001	50°07'20"	73°21'22"	6.3	191
DF5	Lake	QB	S.F.	June 2001	50°07'07"	73°27'11"	6.8	538
DF7	Lake	QB	S.F.	June 2001	49°52'02"	73°06'57"	6.3	259
DF9	Lake	QB	S.F.	June 2001	48°42'31"	75°01'03"	6.3	155
K1	Lake	QB	S.F.	June 2001	48°19'43"	75°16'50"	6.0	99
K2	Lake	QB	S.F.	June 2001	48°17'56"	75°10'08"	5.7	74
K3	Lake	QB	S.F.	June 2001	48°18'26"	75°16'18"	6.1	116
K4	Lake	QB	S.F.	June 2001	48°35'24"	75°00'25"	6.2	94
K8	Lake	QB	S.F.	June 2001	48°29'04"	74°51'35"	5.8	78
N35	Lake	QB	S.F.	June 2001	47°59'23"	74°32'08"	6.5	129
N43	Lake	QB	S.F.	June 2001	48°57'31"	73°42'26"	6.2	121
N55	Lake	QB	S.F.	June 2001	47°59'23"	75°24'40"	6.5	241
N70	Lake	QB	S.F.	June 2001	48°05'12"	75°29'09"	6.4	109
N89	Lake	QB	S.F.	June 2001	48°58'17"	74°02'08"	6.4	199

Figure 1. (a) A map of all freshwater sites (n=81) used to elucidate the relationship between MeHg, DOC, pH and conductivity in Eastern Ontario and Western Quebec. W.E.R.F. project study sites (n=28) are represented by grey circles, study sites from the Sustainable Forestry project (n=23) are shown as white triangles and sites sampled during this Master's Thesis (n=30) are shown in black squares. (b) A map of all freshwater sites from Eastern Ontario and Western Quebec used in the partitioning experiments of Master's Thesis (n=20).

a)



b)



The MeHg and DOC water samples are compatible since they were collected in the same manner, from a common region (Figure 1) and processed using the same laboratory techniques. Surface water MeHg samples were taken in two 1 L HDPE bottles approximately 30 cm below the air-water interface. The mercury sampling protocol "Clean Hands, Dirty Hands" (Bloom, 1995) was followed with one field researcher reaching into the water to collect the initial sample with gloves, while a second field researcher closed the cap and sealed the bottles in zip-lock bags. Two additional surface water samples were collected in the same manner with 50 mL polypropylene tubes for DOC concentration, DOC FL and DOC Abs analysis.

With the help of a field partner, water samples for fractionation were collected in 25 L high density polyethylene (HDPE) carbuoys from approximately 5-15 feet off shore. Littoral zones were chosen for water collection since fish are known to feed prolifically in these areas. Care was taken in order to reduce disturbance to the surface sediments and to avoid collecting resuspended particles.

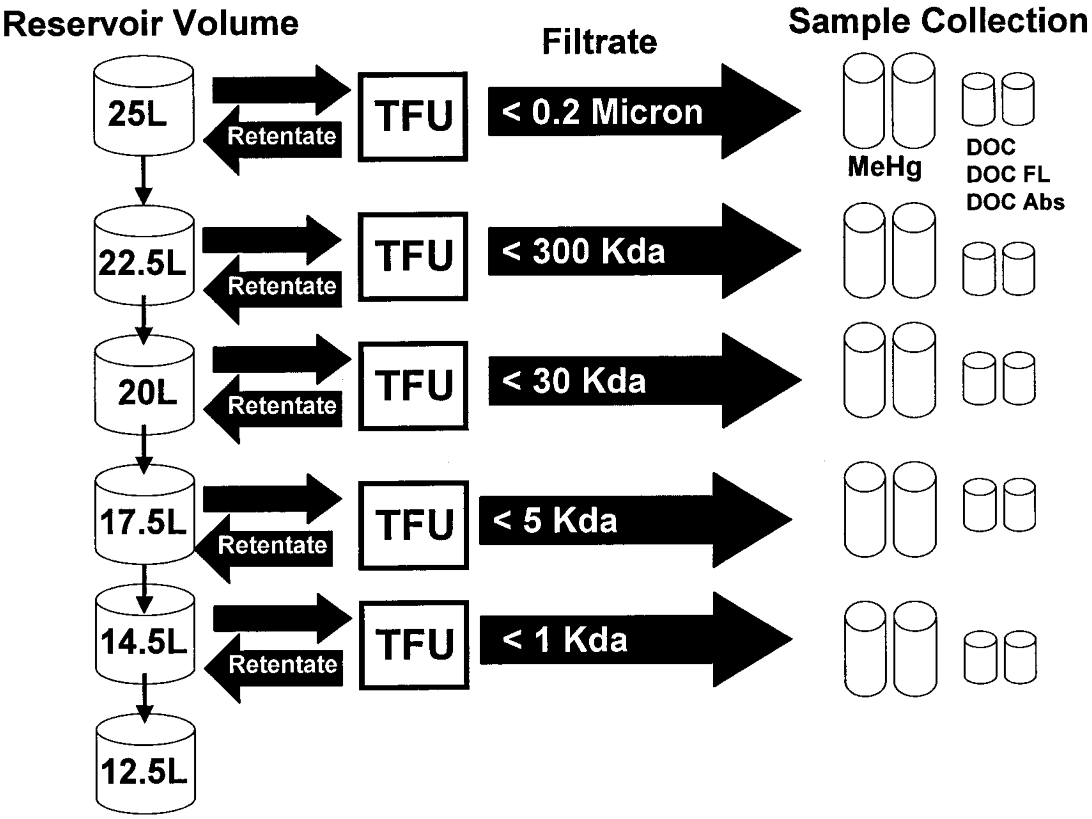
Surface water MeHg samples were preserved to prevent adsorption of mercury onto the bottles. Twenty milliliters of ultra-pure hydrochloric acid (to yield a 0.5% solution) were added on site or directly upon return to the laboratory. The 25 L carbuoy could not be acidified in order to maintain natural conditions for fractionation, however, all samples were refrigerated at 4^0C upon return to the laboratory and kept in the dark to avoid photodegradation. Conductivity, a general assessment of ionic activity measured in micro Siemens [µS] was taken with a VWR Traceable Hand Held Conductivity Meter while on site or directly upon return to the laboratory. pH, a log scale measure of H+ ions, was measured *in situ* with a VWR Scientific Products Symphony SP21 portable meter.

2.2 Ultrafiltration

A technique called Tangential Flow Ultrafiltration (TFU) was used to partition the 25 L carbuoys into a number of different fractions. First, the entire sample was prefiltered with a silk screen 100 micron in-line filter to remove large particles which may have clogged subsequent filters. Prefiltration also ensures that zooplankton and invertebrates do not contaminate the sample solution and provide an overestimate of MeHg concentration.

For this series of experiments, a PALL Tangential Flow Centramate™ Ultrafiltrator was used for fractionation. Ultrafiltration is a an alternate technique to convential in-line filtration which simply uses a force, typically generated by a vacuum and aided by gravity, to suction a solution through a specifically sized porous filter. Instead, an ultrafiltrator is attached to a peristaltic pump (Masterflex L/S standard drive model No. 7520-00) which directs the flow of the solution parallel to specifically size porous membrane cassettes. The tangential direction of solution flow allows molecules of sufficiently small size to penetrate the membrane as filtrate. Molecules which are too big to enter the membrane cassette as well as small molecules which are not at the "front of the line" continue to bypass the filter and are described as retentate. Retentate solution then recirculates back into the original sample (Figure 2).

Figure 2. Procedure for Tangential Flow Ultrafiltration (TFU) which illustrates a 25 L HDPE carbuoy filled with sample water which is prefiltered and then sequentially ultrafiltrated with 5 filter cassettes of different sizes. Retentate recirculates back into the original container. Filtrate is collected with 1 L HDPE bottles (MeHg) and 50 ml polypropylene tubes (DOC, DOC FL, DOC Abs).


Reservoir Volume
Filtrate
Sample Collection
25L
22.5L
20L
17.5L
14.5L
12.5L
Retentate
Retentate
Retentate
Retentate
Retentate
TFU
TFU
TFU
TFU
TFU
< 0.2 Micron
< 300 Kda
< 30 Kda
< 5 Kda
< 1 Kda
MeHg
DOC
DOC FL
DOC Abs

Tangential flow is a more sensitive method of filtration since less force needs to be applied to collect low molecular weight samples. When a large degree of force is used upon a solution, artifacts are often formed as a result of breaking down large molecules into smaller ones when they are compressed through the filter. Also, when large particles cake onto a conventional inline filter, molecules which are the desired size to be collected can often bind to or be blocked access to open pores by this sedimentary layer. The use of relatively low peristaltic pressure (<10 psi) and the ability to give desirable molecules multiple chances to pass through the membrane cassettes into sample collection bottles are highly advantageous in the fractionation process.

Membrane cassettes are available in a number of molecular weight sizes. In this series of experiments, 5 separate sizes were used to allow for a high resolution of fractionation: 0.2 microns [μm], 300 kilodaltons [kDa], 30 kDa , 5 kDa , and 1 kDa. Membrane cassettes were all categorized as OMEGA Suspended Screen which is specialized for higher retentate flow. All cassettes have large surface areas (approximately 0.09 m^2) that can filter very high volumes of solution (PALL Gelman Laboratory Guide page 106). The largest pore size is expressed as a diameter in microns which is equivalent to 0.001 millimeters while the lower size fractions are expressed in kilodaltons which are measured in 1000 dalton mass units. The membrane cassettes chosen for these experiments are made from a compound called polyethersulfone which is designed to limit adsorption such as protein binding to prevent hold-over particles from contaminating ensuing samples.

Samples were filtered sequentially through 0.2 μm, 300 kDa, 30 kDa, 5 kDa and 1 kDa TFU cartridges. Although exact conversions between μm's, a distance, and daltons, a mass, are not possible, the 0.2 μm filtrate has been estimated to be approximately 1333 kDa. As a corollary, mass units of 300, 30, 5 and 1 kDa have been estimated as 0.045 μm, 0.0045 μm 0.00075 μm and 0.00015 μm respectively. The 5 and 1 kDa size fractions both required back pressure to produce filtrate. MeHg, DOC, DOC fluorescence and DOC absorbance at 254 nm were collected and analyzed for each size fraction. Percentages were then calculated for each variable based on loss between each size fraction similar to Babiarz et al (2001).

Concentration factors are kept below 2 to avoid elevated proportions of solute relative to the original solution (Hoffman et al. 2000). This is accomplished by discarding the last half of the original water sample which is mostly retentate. Since contamination is always a potential issue in mercury research, all pieces of tubing used in the filtration process except for three small connecting elbows were coated with Teflon® since it is more repellent to mercury binding than other synthetics and approved by the E.P.A. (Bloom, 1995). When filter cassettes were not in use, they were stored in a 0.1 molar NaOH solution which desorbs particles and colour from the cassettes. After each filter was changed, they were left over night in a bath of deionized Milli-Q water to rinse. Also, deionized Milli-Q water was run through the whole filtering system after each cartridge was changed.

2.3 MeHg Analysis

Methyl mercury in water analysis in the laboratory of Dr. Lean uses the Cai et al (1996) method of extraction. A perastaltic pump and Teflon tubing was used to preconcentrate and adsorb the 1 liter MeHg samples onto sulfhydryl cotton columns. The cotton columns were infused with a MeHg binding mixture of thioglycolic acid ($HS.CH_2COOH$), acetic anhydride $(CH_3CO)_2O$, 36% acetic acid and sulfuric acid H_2SO_4 (Lee and Mowrer, 1989). Next, 2:1

solution of copper sulfate ($CuSO_4$) to acidic potassium bromide (KBr) was used to elute the MeHg into 7 ml L vials. An exact amount (300 µL) of methylene chloride (DCM, CH_2Cl_2) was added to the sample, shaken for 20 minutes and centrifuged at 3500 rpm for 10 minutes to form an organic bubble containing the MeHg. The DCM was subsequently removed and filtered through a drying agent (anhydrous sodium, $NaSO_4$) into GC vials for analysis.

Intermediate MeHg standards of 10 ppm, 200 ppb and 5 ppb were made from an original stock solution of 800 ppm CH_3HgCl. Sample concentrations were based upon standard curves which were derived from the analysis of samples with 0, 0.83, 1.6, 2.5, 3.3 and sometimes 6.6 ng/ml of MeHg. Machine check standards were tested after approximately every 8-10 samples. Duplicates of samples were run from which an average and a standard deviation are calculated. Blank samples and 1 ppt spikes were intermittently run through MeHg analysis process in order to ensure that no contamination exists and that sample recoveries are high. The machine detection limit has been calculated to be approximately 20 pg/L.

A gas chromatography absorption fluorescence spectrometer (GC-AFS) was used to test for MeHg concentrations. The GC-AFS uses argon as a carrier gas to transport the MeHg through a long capillary column and undergoes pyrolysis at 800^0C. The heating process converts the MeHg into Hg^0 before the AFS detector converts the signal into a peak which is displayed and quantified against standards using HP-CHEM software.

2.4 Water Quality Analysis

Water samples were vacuum filtered for DOC, DOC FL and DOC Abs analyses with a 0.45 μm hydrophilic polysulfone HT-450 Tuffryn ® membrane filter and kept in the dark at 4^0C until analysis. An IO Analytical 1010 total organic carbon analyzer which incorporates ultra-high purity nitrogen as a carrier gas was used to test the samples for DOC. Thirty ml of each sample are transferred into amber vials which are inserted into an autosampling tray. A small aliquot is sipped by the autosampler which uses persulfate to oxidize the organic material within the solution. It is heated by an oven and carried via nitrogen gas into the analytic unit. Organic carbon concentrations are calculated using WinTOC© software compared to KHP standards of 0, 5, 10, 25 and 50 mg C /L. All 0.45 μm un-fractionated water samples as well as all size fractions from the partitioning experiment were run this way with machine duplicates.

DOC FL, a measure of electron emission after excitation at a specific wavelength, was analyzed with a Turner Designs Model 10 fluorometer equipped with excitation filters #7-60 and 5G (excitation wavelength 365 nm), emission filter #47-B and reference filter 2A (emission wavelength 437 nm) (Scully et al. 1996). After prefiltration, each sample was poured into a clean test tube, covered to block background radiation and inserted into the fluorometer. Fluorescence values were measured against standards in units of quinine sulfate (QSU) that spanned the range of samples (0-200 QSU) where one quinine sulfate unit is equal to 1 μg/L in 0.1 M H_2SO_4.

A Varian CARY 100 Bio UV visible spectrophotometer was used to measure the absorbance wavelengths from 200 nm to 800 nm. DOC absorbance at the 254 nm was the primary wavelength chosen for this study since it is commonly used in monitoring by the waste water treatment industry due to a linear relationship with DOC (Korshin et al. 1997). Approximately 3 ml of each sample was poured into a clean quartz cuvette (pathlength = 1 cm) and inserted into an analysis chamber. A second cuvette filled with DI Milli-Q water is placed into another chamber as a reference blank. Each size fraction from the partitioning experiment as well as the majority of surface water samples were tested for absorbance in this manner.

2.5 Predicting Hg concentrations in fish with MeHg in water

A subset (n=5) of the partitioning data set which had been previously analyzed as part the W.E.R.F. study was chosen to compare different aqueous MeHg concentrations against fish mercury concentrations. Each sample site had Hg concentrations for at least 20 fish (blue gills, pumpkin seeds and perch) between 5-20 cm in length.

The fish mercury concentrations used in this comparison have been converted into log standard fish concentrations in order to represent the range of fish as one value for a given site. Log standard fish mercury concentrations were calculated by log-transforming the length and MeHg concentration of each fish and taking the equation of the line of these data points. A length of 10 cm was used since this value approached the average length of fish caught. The resulting individual scalar value from this equation provides the log standard fish mercury concentration for each site. The log standard fish mercury concentration was then compared by linear regression to five different aqueous MeHg values: total MeHg in water, large fraction MeHg (>5 kDa solution, >1 kDa solution) and small fraction MeHg (<5 kDa solution, <1 kDa solution).

2.6 UVB exposures

In order to test whether photo-demethylation can take place in St. Lawrence river water, four 25 litre HDPE carbuoys of water were collected approximately one foot below the air-water interface from the mid-channel near Cornwall, Ontario in October 2003.

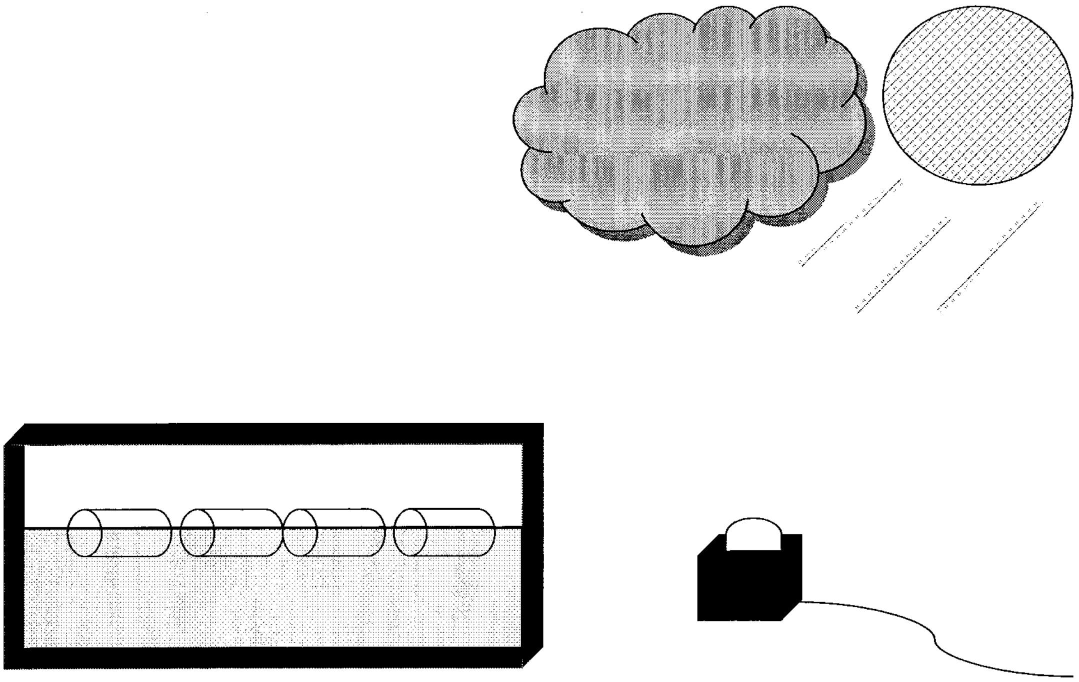
A first experiment was undertaken where both ambient (0.02 ng/L) and spiked (5 ng/L MeHg-Cl) samples were tested for photodegradation under incubation periods in natural light of 0, 3 and 6 hours (Figure 5). All samples were prefiltered with a 0.2 μm tangential flow cassette in order to remove all particulates and bacteria. After filtration, samples were collected in 1 litre Teflon® bottles and placed in a large basin filled with a continuous flow of water in order to maintain a controlled temperature. The basin was located on the roof of the Gendron biology building so that the samples could be exposed to an unrestricted regime of solar irradiation. UVB (280-320 nm) wavelengths were measured at 2 nm increments with an Optronics Optikon OL754-C spectroradiometer with dual monochromator, an OL IS470-W submersible sphere and an OL 730-7Q-WP waterproof fibre optic probe. Optolab 754 Software was used to calculate the cumulative UVB levels of exposure. The spectroradiometer was positioned close to the exposed samples and monitored in order to measure the level of spectral irradiance at 20 minute intervals. The manufacturer lists the wavelength accuracy of the Optikon system at ±0.1 nm in the UVB spectrum and ±0.2 nm for the UV visible spectrum.

A second experiment under laboratory conditions was undertaken with triplicate MeHg-Cl spiked samples (5 ng/L) as well as triplicate samples that were spiked with both MeHg and $FeSO_4$-$7H_2O$ in order to adjust the concentration of Fe(II) to 100 μM. UVB lamps (maximal energy at 310 nm) were used to expose samples for 0, 3 or 6 hours to 0, 7.2 and 13.8 kJm^{-2} of irradiance respectively.

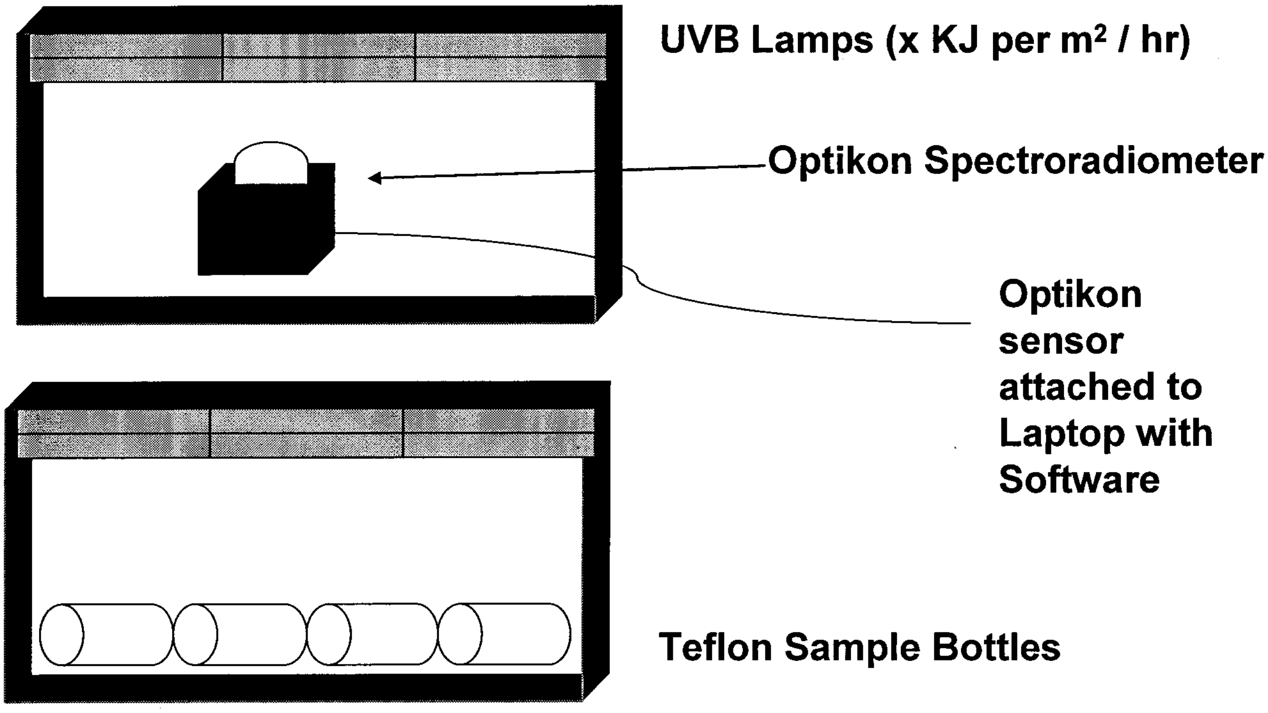
A third experiment was devised to incorporate pH differences in order to determine if low pH samples would photodegrade more quickly than neutral ones. A 20% perchloric acid ($HClO_4$) solution was used to adjust the pH of triplicate samples down to approximately pH 5 and pH 3. Perchloric acid was chosen since it will affect chloride ion concentrations less than hydrochloric acid and it will not degrade MeHg molecules as much as nitric acid. Dark spiked controls and DI water controls at all pH levels as well as neutral spiked samples were compared to the test samples. All samples were exposed to 0, 3 and 6 hours of UVB radiation under lamps in order to compare percentage MeHg photodegradation. The same UVB lamps and Optikon equipment were used in this experiment as previously described. The acidity of samples was monitored throughout the experiment. A few test dissolved gaseous mercury (DGM) samples were taken using a Tekran mercury air analyzer in an attempt to account for the loss of MeHg through the photodegradation process.

Figure 3. Photoincubation of St. Lawrence River Water samples in (a) natural conditions and under UVB lamps (b).

a)



b)


UVB Lamps (x KJ per m² / hr)
Optikon Spectroradiometer
Optikon sensor attached to Laptop with Software
Teflon Sample Bottles

2.7 Statistical Analysis

The statistical program S-Plus® 6.2 Academic Site edition from the University of Ottawa as well as Microsoft Excel 2003 were used for all data analyses. The default biological probability value of 95% was used for significance testing but a secondary level of 90% was sometimes indicated to emphasize noticeable relationships or differences between means. Tests for univariate normality included visual inspection of scatter-plot matrices and one-sample Kolmogorov-Smirnov tests. Analysis of variance (ANOVA) was used to test for significant differences among means. T-tests were used to test for significant differences between two means while one sided two sample t-tests were used to test whether one mean was statistically higher than another. Linear regressions were used to check relationships between variables. P values for significant t-tests and as well r^2 values for significant linear regressions are shown in the text. An extended set of statistics for selected tests (degrees of freedom, t values, r^2 and p values) are found in the Appendix section of this study.

Chapter 3

Results

Each water chemistry variable was graphed in histogram form in order to visibly inspect the distribution of the data. Due to the presence of some skews and discontinuity, it was difficult to determine from the histograms (Appendix) whether all of the distributions were consistent with statistical normality. Therefore, means and standard deviations were used to test for normality using a one-sample Kolgomorov-Smirnov Equation (Appendix). The test demonstrated that the majority of the samples, while their distributions were imperfect, agreed with the null hypothesis and met the statistical conditions of normality. The results of this study are subdivided into thematic sections related to the thesis objectives as follows:

3.1 The predictive relationship between MeHg and DOC

Un-fractionated water chemistry data including MeHg, DOC, DOC FL and DOC absorbance were analyzed for the combined data set (Table 2) and the Hill Thesis data set in isolation in order to determine relationships between variables across the study area and continuity between studies.

The Combined Data Set

The Hill Thesis, W.E.R.F. and Sustainable Forestry data sets number 30, 28 and 23 sites respectively. The majority of water chemistry variables in the merged data set (n=81) have at least two orders of magnitude of range which provides a wide representation of concentrations for this geographical area. The three data sets overlap each other in ranges for all variables but group means did show significant differences through analysis of variance for MeHg ($p<0.001$), DOC ($p<0.02$) and pH ($p<0.001$).

The Hill data set had the highest mean MeHg, DOC, and conductivity values (Appendix). This data set had statistically higher mean MeHg ($p<0.001$), DOC ($p=0.02$) and lower pH ($p<0.001$) than the W.E.R.F. study. These Hill sites are also significantly higher in pH ($p<0.001$) than the S.F. study values.

The W.E.R.F. data set had the lowest mean MeHg and DOC values with the highest pH while the S.F. data set had the lowest mean pH and conductivity values. The S.F. data had statistically higher mean MeHg ($p<0.001$), DOC ($p=0.02$) and lower pH ($p<0.001$) than the W.E.R.F. data set.

The Hill data had high range with the highest standard deviations of the three groups for MeHg and DOC. The W.E.R.F. data set range was more narrow with the lowest standard deviations for MeHg, DOC and conductivity. The S.F. data set had mostly intermediate mean concentrations and variability with the lowest standard deviation for pH.

All three data sets had ratios of more than 2 pg MeHg per mg of DOC. The S.F. data set had the highest ratio of MeHg: DOC while the W.E.R.F. data set had the lowest ratio.

The individual site with the highest MeHg concentration is Blueberry Marsh, a wetland which had the highest DOC concentration and level of DOC FL (Table 2). The individual

Table 2. Study sites with surface water concentrations of MeHg, DOC, DOC FL and DOC Abs at 254 nm. Comparative ratios are shown for MeHg, DOC and DOC FL. Sites are given designations (D) of lakes (L), rivers (R) or wetlands (W) and original dataset names of (a) Hill Master's Thesis, (b) W.E.R.F. and S.F.

a)

Study Site	DS	D	MeHg pg/L	S.D.	DOC mg C /L	DOC FL QSU	S.D.	DOC Abs 254 nm	MeHg: DOC pg / mg C	MeHg: DOC FL pg/L / QSU	DOC FL: DOC QSU / mg C /L
Big Rideau	1	L	151.1	12.5	6.8	27.8	0.9	.	22.3	5.4	4.1
Commanda	1	L	156.3	9.7	10.4	56.4	7.5	0.15	15.1	2.8	5.4
Cranberry	1	L	48.7	6.1	6.7	11.1	0.0	.	7.3	4.4	1.7
Lac MacGregor	1	L	93.6	10.9	4.8	15.1	0.0	0.11	19.6	6.2	3.2
Lac St. Pierre	1	L	67.4	18.6	5.5	16.1	0.5	0.09	12.2	4.2	2.9
Loch Garry	1	L	91.9	.	11.1	24.8	1.4	0.17	8.3	3.7	2.2
Madawaska	1	L	66.3	0.2	6.5	36.4	0.0	.	10.3	1.8	5.6
Newboro	1	L	172.1	.	7.4	24.4	0.0	0.11	23.4	7.0	3.3
Opinicon	1	L	32.3	10.3	5.5	21.8	0.0	0.12	5.9	1.5	4.0
Otter	1	L	141.3	31.5	7.7	15.1	0.0	.	18.4	9.4	2.0
Sharpes Bay	1	L	28.9	2.8	5.7	6.4	0.0	0.03	5.0	4.5	1.1
Temagami	1	L	70.5	5.6	4.0	10.8	0.5	0.09	17.7	6.6	2.7
Black River	1	R	372.4	7.9	38.4	138.4	4.7	1.37	9.7	2.7	3.6
Gatineau	1	R	138.1	6.3	7.2	42.4	0.9	0.26	19.1	3.3	5.9
Hooples Creek	1	R	61.0	.	5.4	16.1	0.5	0.07	11.3	3.8	3.0
Ottawa	1	R	89.6	.	7.1	37.8	0.0	.	12.6	2.4	5.3
Raisin	1	R	373.4	12.9	22.1	128.4	0.0	0.79	16.9	2.9	5.8
Rideau	1	R	85.2	9.9	11.4	77.1	0.9	0.31	7.5	1.1	6.8
South Nation	1	R	110.5	4.1	13.8	64.4	0.0	0.44	8.0	1.7	4.7
St. Lawrence	1	R	104.8	3.4	2.9	3.4	0.4	0.03	36.6	31.2	1.2
Yamaska	1	R	123.4	3.7	8.5	69.4	1.4	0.37	14.4	1.8	8.1
Baie St. Francois	1	W	280.4	6.7	12.2	91.0	0.2	0.40	23.0	3.1	7.5
Blackadder Creek	1	W	77.6	.	4.6	7.4	0.5	0.04	16.8	10.4	1.6
Blueberry	1	W	506.8	.	51.6	161.8	0.0	1.82	9.8	3.1	3.1
Cooper's Marsh	1	W	98.7	34.8	14.6	105.8	0.0	0.37	6.7	0.9	7.2
Mer Bleue	1	W	369.7	28.6	39.6	100.8	0.5	1.48	9.3	3.7	2.5
Mississipi	1	W	64.7	.	10.0	48.4	0.0	0.25	6.5	1.3	4.8
Odessa	1	W	220.5	40.0	11.4	71.4	1.4	0.28	19.3	3.1	6.2
Otter	1	W	181.9	4.8	8.4	45.8	0.0	0.17	21.6	4.0	5.4
Vieux Chemin	1	W	408.9	17.2	11.5	74.4	0.9	0.29	35.7	5.5	6.5

b)

Study Site	DS	D	MeHg pg/L	S.D.	DOC mg C /L	DOC FL QSU	S.D.	DOC Abs 254 nm	MeHg: DOC pg / mg C	MeHg: DOC FL pg/L / QSU	DOC FL: DOC QSU / mg C /L
Ashby	2	L	24.4	.	5.1	.	.	.	4.8	.	.
Beaver	2	L	28.7	.	7.6	.	.	.	3.8	.	.
Bellamys	2	L	57.6	.	9.9	.	.	.	5.8	.	.
Buckshot	2	L	29.4	.	6.2	.	.	.	4.7	.	.
Calabogie	2	L	37.4	.	6.2	.	.	.	6.0	.	.
Camden	2	L	151.4	.	13.8	.	.	.	11.0	.	.
Charleston	2	L	38.9	.	5.4	.	.	.	7.1	.	.
Christie	2	L	49.3	.	8.8	.	.	.	5.6	.	.
Crotch	2	L	51.0	.	5.9	.	.	.	8.7	.	.
Devil	2	L	21.4	.	4.6	.	.	.	4.6	.	.
Dog	2	L	22.7	.	5.8	.	.	.	3.9	.	.
Eloida	2	L	94.1	.	8.5	.	.	.	11.1	.	.
Gananoque	2	L	44.8	.	7.1	.	.	.	6.3	.	.
Graham	2	L	70.1	.	7.8	.	.	.	9.0	.	.
Indian	2	L	20.0	.	6.2	.	.	.	3.2	.	.
Jonhstown Bay	2	L	33.0	.	2.8	.	.	.	11.9	.	.
Kingston Basin	2	L	28.0	.	2.2	.	.	.	12.6	.	.
Lime	2	L	48.2	.	5.2	.	.	.	9.3	.	.
Liittle Mellon	2	L	38.3	.	6.2	.	.	.	6.2	.	.
Loughborough	2	L	94.9	.	6.0	.	.	.	15.7	.	.
Mink	2	L	47.2	.	8.5	.	.	.	5.6	.	.
Norway	2	L	33.8	.	8.6	.	.	.	3.9	.	.
Pike	2	L	24.2	.	10.6	.	.	.	2.3	.	.
Sand	2	L	45.1	.	7.0	.	.	.	6.5	.	.
Troy	2	L	20.0	.	6.5	.	.	.	3.1	.	.
Varty	2	L	70.5	.	7.1	.	.	.	9.9	.	.
Weslemkoon	2	L	30.6	.	5.8	.	.	.	5.3	.	.
Whitefish	2	L	66.1	.	5.5	.	.	.	12.1	.	.
AB34	3	L	126.1	.	6.7	.	.	.	18.9	.	.
AB35	3	L	95.1	.	6.3	.	.	.	15.2	.	.
AB40	3	L	64.8	.	3.4	.	.	.	18.9	.	.
CSL2	3	L	79.7	.	6.4	.	.	.	12.4	.	.
CSL5	3	L	246.3	.	11.1	.	.	.	22.1	.	.
DA2	3	L	106.0	.	7.5	.	.	.	14.1	.	.
DA4	3	L	113.6	.	10.8	.	.	.	10.5	.	.
DA9	3	L	169.4	.	11.3	.	.	.	14.9	.	.
DF2	3	L	111.1	.	9.2	.	.	.	12.1	.	.
DF4	3	L	112.9	.	10.5	.	.	.	10.7	.	.
DF5	3	L	69.1	.	8.9	.	.	.	7.8	.	.
DF7	3	L	101.2	.	13.1	.	.	.	7.7	.	.
DF9	3	L	237.6	.	15.6	.	.	.	15.2	.	.
K1	3	L	169.4	.	7.5	.	.	.	22.7	.	.
K2	3	L	167.2	.	10.5	.	.	.	15.9	.	.
K3	3	L	85.3	.	7.3	.	.	.	11.6	.	.
K4	3	L	192.2	.	9.3	.	.	.	20.8	.	.
K8	3	L	191.9	.	12.3	.	.	.	15.6	.	.
N35	3	L	80.0	.	4.7	.	.	.	16.9	.	.
N43	3	L	160.0	.	9.6	.	.	.	16.6	.	.
N55	3	L	68.0	.	5.6	.	.	.	12.2	.	.
N70	3	L	37.2	.	5.9	.	.	.	6.3	.	.
N89	3	L	20.1	.	4.4	.	.	.	4.6	.	.

site with the lowest MeHg concentration is Troy Lake while the lowest DOC concentration and DOC FL level were found in Kingston Basin. The lowest pH was found at lake DA4 in Quebec while the highest pH was found at Whitefish Lake (Table 1). The highest conductivity was found at lake DF5 while the lowest conductivity and alkalinity was found at the Gatineau River. The St. Lawrence River had the highest ratio of MeHg: DOC while Pike Lake had the lowest ratio of all sites.

Significance Testing of the Un-fractionated Data Set

MeHg was found to be significantly dependent on DOC (r^2=0.63, p<0.001) and pH (r^2=0.16, p=0.002) in the overall data set. DOC was found to be dependent upon pH (r^2=0.06, p=0.028) with a negative slope. Conductivity (r^2=0.1, p=0.005) and the ratio of MeHg to DOC (r^2=0.1, p=0.003) concentrations were found to be related to pH with negative slopes.

3.2 Concentrations of MeHg and DOC in lakes, rivers and wetlands

Un-fractionated water samples from the Hill data set were tested to determine whether significant mean differences in concentrations exist between aqueous MeHg, DOC, DOC FL, DOC Abs, and conductivity as well pH in the freshwater types of lakes, rivers and wetlands.

The highest mean surface water MeHg, DOC, DOC FL, and conductivity concentrations were found at wetland locations (Table 3). Wetlands had significantly higher mean MeHg (p=0.003), DOC (p=0.012), DOC FL (p<0.001), DOC Abs (p=0.032), conductivity (p=0.002) and lower pH (p=0.001) than lakes. Wetlands also had the highest standard deviations of the three freshwater types for mean MeHg and DOC.

Table 3. (a) Mean values and standard deviations for water samples of the Hill Master's Thesis, subdivided by freshwater type. MeHg, DOC, DOC FL, DOC Abs at 254 nm are shown. (b) Mean values for pH, conductivity and ratios of MeHg, DOC and DOC FL .

a)

	MeHg (pg/L)				DOC (mg C/L)				DOC FL (QSU)				DOC Abs 254 nm	
Designation	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**
Lakes	93.4	50.1	28.9	172.1	6.8	2.1	4.0	11.1	22.2	13.7	6.4	56.4	0.11	0.04
Rivers	162.0	121.6	61.0	373.4	13.0	11.1	2.9	38.4	64.2	46.1	3.4	138.4	0.46	0.44
Wetlands	245.5	157.6	64.7	506.8	18.2	16.0	4.6	51.6	78.5	43.9	7.4	161.8	0.57	0.63

b)

	pH				Conductivity (µS/cm)				MeHg:DOC (pg / mg)		MeHg:DOC FL (pg/L / QSU)		DOC FL:DOC (QSU / mg C /L)	
Designation	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Lakes	7.9	0.6	6.8	8.8	153.5	70.4	49.0	279.0	13.8	6.5	4.8	2.3	3.2	1.4
Rivers	7.9	0.7	6.8	8.7	257.3	143.2	38.0	446.0	15.1	8.9	5.6	9.6	4.9	2.1
Wetlands	7.0	0.6	6.7	7.9	262.1	80.0	112.7	372.0	16.5	9.6	3.9	2.8	5.0	2.1

Rivers had intermediate mean concentrations and variability for the majority of categories. River water mean pH was lower than wetlands ($p=0.004$) and statistically higher mean MeHg ($p=0.046$), DOC ($p=0.037$), DOC FL ($p=0.004$), DOC Abs ($p=0.023$) and conductivity ($p=0.024$) than lakes. The lowest mean MeHg, DOC, DOC FL and conductivity concentrations were found in lakes (Figure 4). Lakes also had the lowest standard deviations for each of these categories.

Linear Regressions (Hill Data Set)

In the Hill Thesis Data set, MeHg and DOC were found to have a significant linear relationship ($r^2=0.67$, $p<0.001$) with a positive slope. MeHg also was found to be significantly dependent upon DOC FL ($r^2=0.66$, $p<0.001$). DOC was found to have a strong positive linear relationship with DOC FL ($r^2=0.73$, $p<0.001$). DOC FL was found to have a significant linear relationship with the negative inverse of Incidence ($r^2=0.78$, $p<0.001$). MeHg, DOC, DOC FL, DOC Abs and conductivity all had negative but non-significant relationships with pH. DOC Abs had a significant linear relationship with DOC ($r^2=0.98$, $p<0.001$), and was highly related to DOC FL ($r^2=0.73$, $p<0.001$) and MeHg ($r^2=0.66$, $p<0.001$).

Figure 4. A comparison of MeHg and DOC FL mean values with standard deviations for lakes, rivers and wetlands for samples from the Hill Thesis Data set.

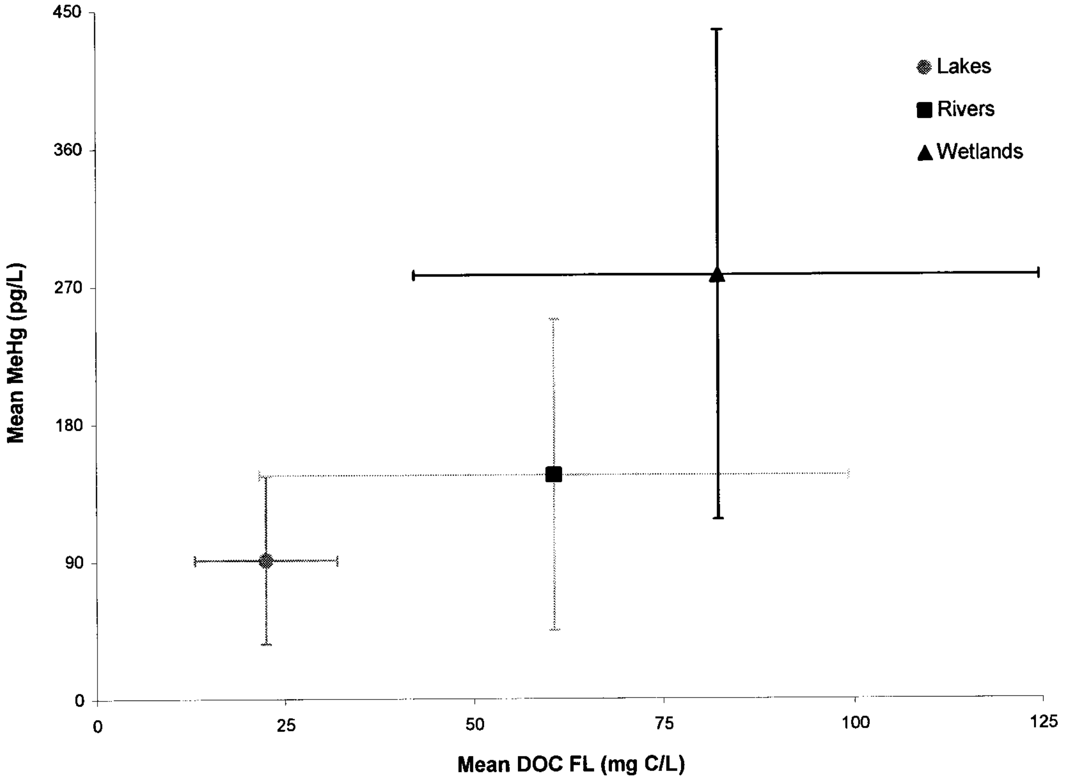
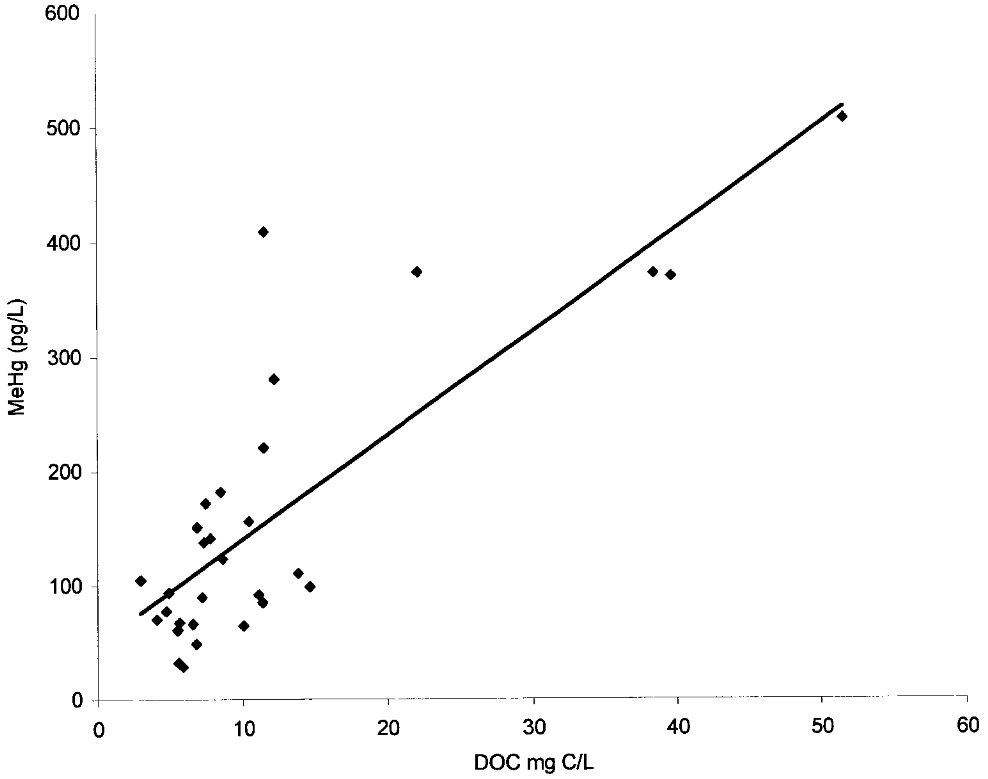
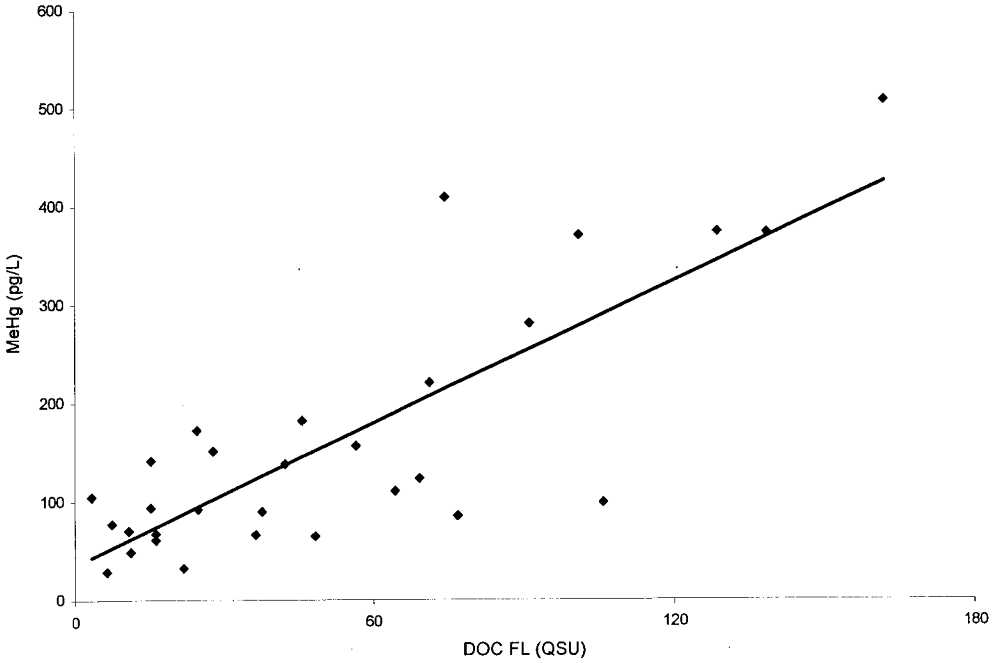

450
360
270
180
90
0
0
25
50
75
100
125
Mean MeHg (pg/L)
Mean DOC FL (mg C/L)
● Lakes
■ Rivers
▲ Wetlands

Figure 5. MeHg concentrations in unfiltered samples from the Hill Thesis data set compared to (a) DOC and (b) DOC FL concentrations.

a)


600
500
400
300
200
100
0
MeHg (pg/L)
0
10
20
30
40
50
60
DOC mg C/L

b)


600
500
400
300
200
100
0
MeHg (pg/L)
0
60
120
180
DOC FL (QSU)

Mean concentrations at different size fractions of aqueous MeHg, DOC, DOC Fl and DOC Abs were analyzed for freshwater sites in Eastern Ontario and Western Quebec using TFU to determine whether differences exist between lakes, rivers and wetlands (Tables 4).

In a series of one-sided t-tests between freshwater types, wetlands were found to have higher MeHg concentrations than lakes for all size fractions: unfiltered (p=0.007), 0.2 μm (p=0.003), 300 kDa (p=0.001), 30 kDa (p=0.002), 5 kDa (p=0.003) and 1 kDa (p=0.002). Wetlands were also found to have higher MeHg concentrations than rivers for all size fractions: surface water (p=0.05), 0.2 μm (p=0.003), 300 kDa (p=0.005), 30 kDa (p=0.008), 5 kDa (p=0.013) and 1 kDa (p=0.005). Rivers were found to have higher MeHg concentrations in the 300 kDa (p=0.047) and 5 kDa (p=0.1) size fraction than lakes.

DOC concentrations in wetlands were found to be higher than lakes for all size fractions (at a secondary level of confidence [90%]): surface water (p=0.06), 0.2 μm (p=0.067), 300 kDa (p=0.072), 30 kDa (p=0.068), 5 kDa (p=0.075) and 1 kDa (p=0.061).

DOC concentrations in rivers were found to be significantly higher than lakes for one size fraction at the primary level of confidence (95%; 1 kDa [p=0.022]) and three others at a secondary level of confidence: surface water (p=0.085), 0.2 μm (p=0.055) and 300 kDa (p=0.062).

Table 4. MeHg and DOC concentrations as well as DOC FL and DOC Abs with standard deviations (S.D.) are displayed for partitioned freshwater samples at size fractions of 0.2 μm, 300 kDa, 30 kDa, 5 kDa and 1 kDa. Data is shown for (a) lakes (L), (b) rivers (R) and (c) wetlands (W). Data marked with asterisks represent estimates based on lowest determinable MeHg concentration.

a)

Study Site	D	Fraction	MeHg pg/L	S.D.	DOC mg C/L	DOC FL (QSU)	S.D.	DOC 254 nm	MeHg: DOC pg MeHg/ mg C	MeHg: DOC FL pg/L MeHg / QSU	DOC FL: DOC QSU / mg C /L
Big Rideau	L	0.2 micron	55.8	0.0	4.8	24.4	0.0	0.13	11.7	2.3	5.1
Big Rideau	L	300 Kda	46.2	4.7	4.7	23.4	0.5	0.13	9.9	2.0	5.0
Big Rideau	L	30 Kda	45.3	2.2	3.2	19.1	0.0	0.10	14.3	2.4	6.0
Big Rideau	L	5 Kda	37.0	1.4	3.4	19.1	0.0	0.10	10.9	1.9	5.6
Big Rideau	L	1 Kda	20.0*	.	2.3	16.4	5.7	0.08	.	.	7.0
Lac McGregor	L	0.2 micron	26.4	.	2.8	12.4	0.0	0.10	9.4	2.1	4.4
Lac McGregor	L	300 Kda	15.0	7.0	2.5	12.4	0.0	0.10	5.9	1.2	4.9
Lac McGregor	L	30 Kda	15.0*	.	1.3	8.6	0.2	0.08	.	.	6.5
Lac McGregor	L	5 Kda	15.0*	.	1.4	11.4	0.5	0.08	.	.	8.2
Lac McGregor	L	1 Kda	15.0*	.	0.5	6.1	0.5	0.06	.	.	11.4
Loch Garry	L	0.2 micron	91.9	0.9	8.1	21.1	0.0	0.14	11.4	4.4	2.6
Loch Garry	L	300 Kda	82.2	1.1	8.1	21.8	0.9	0.15	10.2	3.8	2.7
Loch Garry	L	30 Kda	73.8	4.2	5.7	19.4	0.5	0.12	13.0	3.8	3.4
Loch Garry	L	5 Kda	70.6	0.9	6.3	20.4	1.9	0.13	11.2	3.5	3.2
Loch Garry	L	1 Kda	63.6	1.6	4.4	13.4	2.4	0.09	14.6	4.7	3.1
Madawaska	L	0.2 micron	43.2	2.4	5.1	35.1	1.9	0.16	8.4	1.2	6.9
Madawaska	L	300 Kda	37.3	2.8	4.8	33.1	0.9	0.16	7.8	1.1	6.9
Madawaska	L	30 Kda	20.0*	.	3.2	25.1	0.9	0.12	.	.	7.8
Madawaska	L	5 Kda	20.0*	.	3.3	24.4	0.0	0.11	.	.	7.3
Madawaska	L	1 Kda	20.0*	.	2.3	19.1	2.8	0.09	.	.	8.3
Newboro	L	0.2 micron	210.7	4.3	4.2	24.4	0.0	0.13	50.2	8.6	5.8
Newboro	L	300 Kda	108.4	0.0	3.8	22.4	0.9	0.12	28.9	4.8	6.0
Newboro	L	30 Kda	121.4	11.0	2.5	19.8	0.9	0.10	49.2	6.1	8.0
Newboro	L	5 Kda	11.1	0.2	2.7	18.1	3.3	0.10	4.2	0.6	6.8
Newboro	L	1 Kda	11.1*	.	2.0	16.8	6.1	0.09	.	.	8.6
Opinicon	L	0.2 micron	24.9	7.0	5.6	21.1	0.6	0.10	4.5	1.2	3.8
Opinicon	L	300 Kda	19.4	0.9	5.6	20.6	0.2	0.10	3.5	0.9	3.7
Opinicon	L	30 Kda	15.5	.	4.5	15.8	0.9	0.08	3.5	1.0	3.5
Opinicon	L	5 Kda	15.5*	.	4.2	19.4	0.5	0.07	.	.	4.6
Opinicon	L	1 Kda	15.5*	.	2.9	14.4	0.9	0.05	.	.	5.0
Sharpes Bay	L	0.2 micron	32.0	8.3	6.1	7.1	0.0	0.02	5.2	4.5	1.2
Sharpes Bay	L	300 Kda	24.8	.	5.9	6.4	0.0	0.02	4.2	3.9	1.1
Sharpes Bay	L	30 Kda	21.4	4.4	6.1	4.4	0.0	0.02	3.5	4.8	0.7
Sharpes Bay	L	5 Kda	19.2	1.7	4.0	4.4	0.0	0.02	4.8	4.3	1.1

b)

Study Site	D	Fraction	MeHg pg/L	S.D.	DOC mg C/L	DOC FL (QSU)	S.D.	DOC 254 nm	MeHg: DOC pg MeHg/ mg C	MeHg: DOC FL pg/L MeHg / QSU	DOC FL: DOC QSU / mg C /L
Gatineau	R	0.2 micron	87.3	8.1	5.3	36.8	0.1	0.23	16.6	2.4	7.0
Gatineau	R	300 Kda	82.4	12.3	5.3	35.8	0.0	0.23	15.7	2.3	6.8
Gatineau	R	30 Kda	30.6	9.6	1.8	19.4	0.0	0.09	17.4	1.6	11.0
Gatineau	R	5 Kda	14.9	8.4	1.4	17.1	0.0	0.10	10.6	0.9	12.2
Gatineau	R	1 Kda	14.9*	.	1.2	15.1	1.9	0.09	.	.	12.6
Ottawa	R	0.2 micron	78.2	.	5.9	37.8	0.0	0.20	13.3	2.1	6.4
Ottawa	R	300 Kda	78.4	.	4.0	32.4	0.0	0.16	19.8	2.4	8.2
Ottawa	R	30 Kda	71.1	.	3.2	23.1	0.0	0.11	22.3	3.1	7.2
Ottawa	R	5 Kda	29.1	0.2	3.6	24.4	0.0	0.12	8.2	1.2	6.9
Ottawa	R	1 Kda	20.3	4.7	3.2	23.1	0.0	0.12	6.4	0.9	7.3
Raisin	R	0.2 micron	160.4	2.4	16.5	127.1	0.0	0.57	9.7	1.3	7.7
Raisin	R	300 Kda	143.2	14.5	16.4	127.8	0.9	0.56	8.7	1.1	7.8
Raisin	R	30 Kda	132.4	2.2	13.8	127.8	0.9	0.48	9.6	1.0	9.3
Raisin	R	5 Kda	130.5	0.9	12.8	118.4	4.7	0.44	10.2	1.1	9.3
Raisin	R	1 Kda	92.1	1.6	8.2	93.8	0.0	0.27	11.3	1.0	11.5
Rideau	R	0.2 micron	17.8	7.5	11.1	73.1	0.0	0.33	1.6	0.2	6.6
Rideau	R	300 Kda	9.4	12.0	11.1	73.1	0.9	0.32	0.8	0.1	6.6
Rideau	R	30 Kda	9.6	10.5	9.0	63.8	0.9	0.27	1.1	0.2	7.1
Rideau	R	5 Kda	9.6*	.	8.1	56.4	1.9	0.24	.	.	7.0
Rideau	R	1 Kda	9.6*	.	6.1	41.8	1.9	0.18	.	.	6.9
South Nation	R	0.2 micron	90.7	8.3	14.3	63.1	0.0	0.42	6.4	1.4	4.4
South Nation	R	300 Kda	155.5	83.5	13.1	61.8	0.0	0.41	11.9	2.5	4.7
South Nation	R	5 Kda	66.7	1.0	9.0	51.1	0.0	0.29	7.4	1.3	5.7
South Nation	R	1 Kda	46.9	5.6	.	45.8	0.0	0.23	.	1.0	.
St. Lawrence	R	0.2 micron	70.6	3.1	1.6	2.4	0.0	0.03	45.2	29.1	1.6
St. Lawrence	R	300 Kda	87.5	7.7	1.9	3.0	0.2	0.03	46.0	29.6	1.6
St. Lawrence	R	30 Kda	73.4	0.7	1.5	1.8	0.2	0.03	49.0	41.7	1.2
St. Lawrence	R	5 Kda	54.3	3.1	1.3	2.4	0.0	0.03	43.3	22.4	1.9
St. Lawrence	R	1 Kda	54.0	.	1.3	1.0	0.1	0.03	41.5	52.6	0.8
Yamaska	R	0.2 micron	93.8	6.5	7.5	67.8	0.9	0.22	12.5	1.4	9.0
Yamaska	R	300 Kda	66.7	6.2	7.8	67.8	0.9	0.22	8.6	1.0	8.7
Yamaska	R	30 Kda	51.1	5.1	5.7	56.8	2.4	0.17	8.9	0.9	9.9
Yamaska	R	5 Kda	48.7	9.7	5.5	53.8	3.8	0.16	8.9	0.9	9.8
Yamaska	R	1 Kda	44.9	3.3	3.9	37.1	0.9	0.11	11.5	1.2	9.5

c)

Study Site	D	Fraction	MeHg pg/L	S.D.	DOC mg C/L	DOC FL (QSU)	S.D.	DOC 254 nm	MeHg: DOC pg MeHg/ mg C	MeHg: DOC FL pg/L MeHg / QSU	DOC FL: DOC QSU / mg C /L
Baie St. Francois	W	0.2 micron	227.7	9.7	11.0	91.0	0.2	0.29	20.8	2.5	8.3
Baie St. Francois	W	300 Kda	219.8	23.6	10.7	90.8	0.5	0.30	20.6	2.4	8.5
Baie St. Francois	W	30 Kda	134.0	3.0	7.8	78.4	0.9	0.22	17.1	1.7	10.0
Baie St. Francois	W	5 Kda	125.6	0.6	7.7	75.8	0.9	0.22	16.3	1.7	9.8
Baie St. Francois	W	1 Kda	117.9	0.7	5.6	55.8	0.9	0.15	21.1	2.1	10.0
Blueberry	W	0.2 micron	445.0	8.7	56.4	121.8	9.4	2.41	7.9	3.7	2.2
Blueberry	W	300 Kda	440.2	.	56.7	115.1	0.0	2.44	7.8	3.8	2.0
Blueberry	W	30 Kda	324.3	54.0	40.6	235.1	0.0	1.50	8.0	1.4	5.8
Blueberry	W	5 Kda	.	.	35.1	251.8	4.7	1.26	.	.	7.2
Blueberry	W	1 Kda	246.2	34.4	22.0	208.4	0.0	0.77	11.2	1.2	9.5
Mississippi	W	0.2 micron	78.8	1.3	9.1	48.4	0.0	0.25	8.7	1.6	5.3
Mississippi	W	300 Kda	63.4	3.1	9.1	47.8	0.9	0.24	7.0	1.3	5.3
Mississippi	W	30 Kda	55.7	2.3	6.6	40.1	0.5	0.19	8.5	1.4	6.1
Mississippi	W	5 Kda	51.6	1.4	5.9	37.4	0.5	0.18	8.8	1.4	6.4
Mississippi	W	1 Kda	64.8	.	4.8	29.8	0.0	0.15	13.5	2.2	6.2
Odessa	W	0.2 micron	239.7	3.1	10.1	71.2	2.1	0.30	23.8	3.4	7.1
Odessa	W	300 Kda	231.9	2.2	9.3	71.9	1.1	0.31	24.9	3.2	7.7
Odessa	W	30 Kda	196.4	10.4	7.7	64.1	0.5	0.26	25.5	3.1	8.3
Odessa	W	5 Kda	168.3	3.5	4.8	61.8	0.0	0.24	34.9	2.7	12.8
Odessa	W	1 Kda	150.1	3.3	4.1	45.8	0.0	0.18	36.6	3.3	11.1
Otter	W	0.2 micron	209.0	3.4	6.5	40.6	7.4	0.17	32.0	5.2	6.2
Otter	W	300 Kda	204.4	13.8	5.6	40.4	7.5	0.17	36.8	5.1	7.3
Otter	W	30 Kda	145.8	3.8	4.2	34.1	1.4	0.13	34.6	4.3	8.1
Otter	W	5 Kda	134.5	4.2	4.2	30.4	0.0	0.13	32.1	4.4	7.3
Otter	W	1 Kda	128.7	10.1	3.6	24.4	0.0	0.10	35.4	5.3	6.7
Vieux Chemin	W	0.2 micron	390.7	5.0	9.0	73.0	1.1	0.27	43.5	5.4	8.1
Vieux Chemin	W	300 Kda	387.4	0.7	9.0	69.8	0.0	0.27	43.1	5.6	7.8
Vieux Chemin	W	30 Kda	278.3	14.4	6.7	63.1	1.9	0.21	41.4	4.4	9.4
Vieux Chemin	W	5 Kda	332.5	.	6.2	59.1	0.0	0.19	53.4	5.6	9.5
Vieux Chemin	W	1 Kda	170.3	1.5	4.3	40.8	0.5	0.14	39.2	4.2	9.4

Once again, wetlands have higher DOC FL concentrations than lakes for surface water (p=0.002), 0.2 µm (p<0.001), 300 kDa (p<0.001), 30 kDa (p=0.016), 5 kDa (p=0.024) and 1 kDa (p=0.047) filtrates.

Rivers have higher DOC FL concentrations than lakes for surface water (p=0.014), 0.2 µm (p=0.014), 300 kDa (p=0.016), 30 kDa (p=0.042), 5 kDa (p=0.033) and 1 kDa (p=0.048) filtrates.

Wetlands have higher DOC Abs concentrations than lakes at a secondary confidence level (90%) for surface water (p=0.084), 30 kDa (p=0.1) and 5 kDa (p=0.09) filtrates. Rivers have higher DOC Abs concentrations than lakes for surface water (p=0.023), 0.2 µm (p=0.022), 300 kDa (p=0.022), 30 kDa (p=0.075) and 5 kDa (p=0.045) as well as at secondary confidence level (90%) for the 1 kDa (p=0.064) fraction.

The ratio of MeHg to DOC, MeHg to DOC FL and DOC to DOC FL were not significantly different in the surface water samples of wetlands, lakes and rivers. Wetlands had the highest MeHg to DOC and DOC to DOC FL ratios as well as the lowest MeHg to DOC FL ratios. Lakes had the lowest MeHg to DOC and DOC to DOC FL ratios as well as the highest MeHg to DOC FL ratios. Rivers had intermediate ratio levels.

3.3 Percentage MeHg and DOC in the particulate, colloidal and LMW fractions

Mean MeHg percentages were 47.3 ± 25.4% in the LMW fraction and 26.3 ± 25.1% and 27.3 ± 14.9%, in the particulate and colloidal fractions respectively. In an ANOVA of MeHg percentages in the particulate, colloidal and LMW fractions, it was found that significant differences exist (p<0.001). Using a two-sample t-test, it was revealed that a higher percentage of MeHg was found in the LMW size fraction than the particulate (p=0.012) and colloidal fractions (p=0.004).

Mean DOC percentages were 56.8 ± 14.5% in the LMW fraction, 18.3 ± 14.6% in the particulate fraction and 24.9 ± 12.8% in the colloidal fraction. Mean DOC FL were found at even higher percentages in the LMW fractions at 74.5 ± 11.4% with 7.1 ± 8.7% and 18.5 ± 11.8% in the particulate fraction and colloidal fractions respectively. In ANOVA's tests of the particulate, colloidal and LMW fractions, it was also determined that there were significant differences for both percentage DOC (<0.001) and DOC FL (p<0.001). Using two sample t-tests more percentage DOC existed in the LMW (p<0.001) and colloidal (p<0.001) fractions than in the particulate. Elevated percentages of DOC FL were also found in the LMW (p<0.001) and colloidal fractions (p<0.001) as compared to the particulate. Higher levels of DOC (p=0.07) and DOC FL (p<0.001) were found in the LMW fractions than the colloidal group at primary and secondary degrees of significance respectively.

3.4 Distribution of MeHg and DOC in lakes, rivers and wetlands

Mean molecular size distributions of aqueous MeHg, DOC, DOC Fl and DOC Abs were analyzed for freshwater sites in Eastern Ontario and Western Quebec using TFU to determine whether differences exist between lakes, rivers and wetlands (Tables 9-11).

The distribution of MeHg in different size fractions was found to vary greatly between different fresh water systems. ANOVA's for percentage MeHg at each size fraction [0.2 μm (p=0.047), 300 kDa (p=0.026), 30 kDa (p=0.019), 5 kDa (p=0.015) and 1 kDa (p=0.025)] revealed significant differences between the freshwater systems. All three freshwater types were only similar in that moderate to large proportions (>40%) were found in the <30 kDa filtrates for the percentage MeHg in the filtrates.

Wetlands were found to have higher mean percentage MeHg in filtrates than lakes at the primary level of significance (95% confidence) for 0.2 µm (p=0.045), 300 kDa (p=0.01), and 5 kDa (p=0.031). Wetland MeHg percentages in filtrates were also higher than lakes at a secondary level of significance (90% confidence) for 30 kDa (p=0.104) and 1 kDa (t-test, t=2, df=10, p=0.073).

Wetlands were also found to differ greatly from rivers with respect to MeHg percentage in the 300 kDa (p=0.017), 30 kDa (p=0.0429), 5 kDa (p=0.0429) and 1 kDa (p=0.0062) filtrates. Wetlands MeHg percentages in filtrates were also higher than rivers at 0.2 µm (p=0.102).

Although differences in concentration have been documented in the previous section, no significant differences were found between the distribution of DOC filtrate percentages of the overall total for the three freshwater types. All three freshwater types were similar in that large proportions (>60%) were found in the <30 kDa filtrates for the percentage DOC in the filtrates

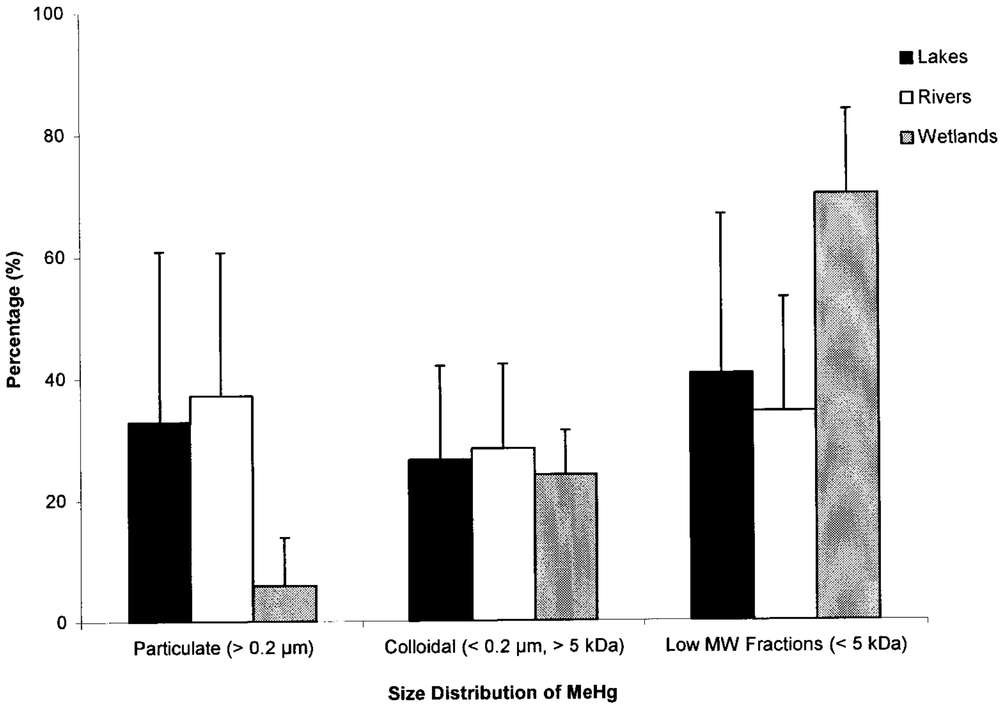
No significant differences in percentage DOC FL content were found at any size fraction. All three freshwater types were similar in that large proportions (>70%) were found in the <30 kDa filtrates for the percentage DOC FL in the filtrates.

Table 5. Mean values for percentage MeHg, DOC, DOC FL and DOC Abs (254 nm) for all sample sites as well as their standard deviations are listed and subdivided into the freshwater types of lakes, rivers and wetlands. Size fractions shown are surface water (SW), 0.2 micron as well as 300, 30, 5 and 1 kDa. Comparative ratios which use these percentage values for MeHg, DOC and DOC FL also shown.

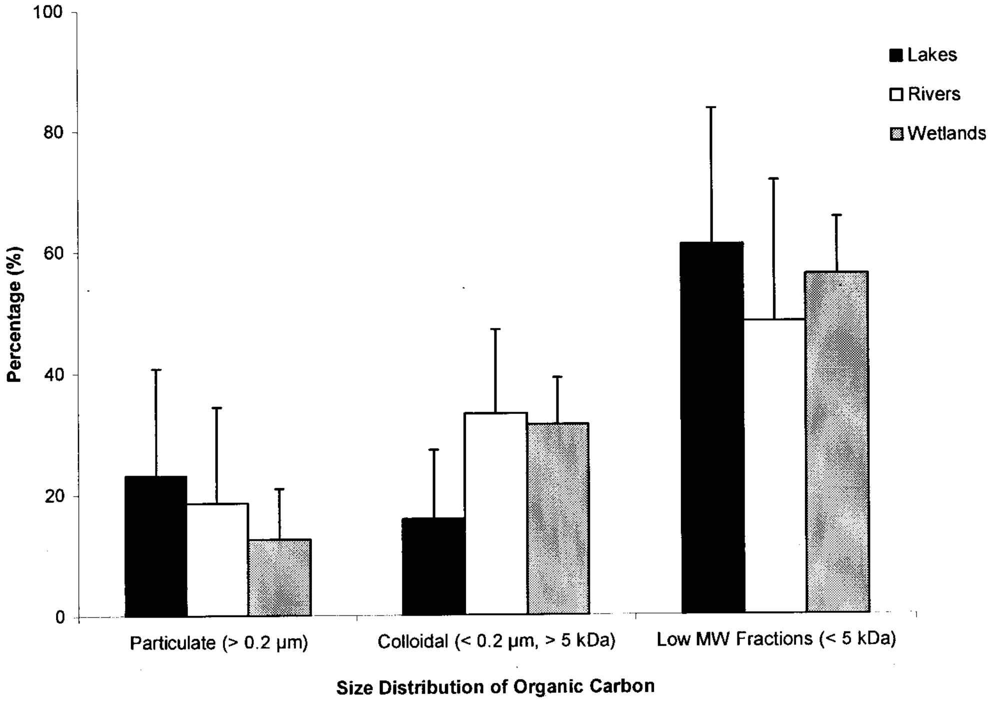
Designation	Fraction	MeHg %	S.D.	DOC %	S.D.	DOC FL %	S.D.	DOC Abs %	S.D.	MeHg: DOC %	MeHg: DOC FL %	DOC FL: DOC %
Lakes	SW	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	1.0	1.0	1.0
Lakes	0.2 micron	67.2	24.3	76.9	15.3	92.7	6.9	88.3	5.6	0.9	0.7	1.2
Lakes	300 Kda	57.3	26.8	74.3	17.1	90.3	5.8	89.1	3.1	0.8	0.6	1.2
Lakes	30 Kda	52.2	24.0	63.5	26.3	70.7	13.0	71.3	1.2	0.8	0.7	1.1
Lakes	5 Kda	40.6	26.1	61.0	22.4	75.2	16.9	70.1	5.4	0.7	0.5	1.2
Lakes	1 Kda	35.1	23.4	38.2	14.7	56.9	13.1	52.2	6.8	0.9	0.6	1.5
Rivers	SW	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	1.0	1.0	1.0
Rivers	0.2 micron	62.0	23.5	83.6	13.0	94.9	5.4	78.6	16.7	0.7	0.7	1.1
Rivers	300 Kda	55.7	28.0	73.4	19.1	84.9	15.2	77.8	14.3	0.8	0.7	1.2
Rivers	30 Kda	42.8	26.7	54.8	22.2	74.0	18.7	48.1	12.5	0.8	0.6	1.3
Rivers	5 Kda	31.4	18.7	48.3	23.3	60.8	25.0	50.1	12.4	0.7	0.5	1.3
Rivers	1 Kda	28.5	15.5	40.5	29.1	54.1	22.9	37.9	9.0	0.7	0.5	1.3
Wetlands	SW	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	1.0	1.0	1.0
Wetlands	0.2 micron	94.1	7.9	87.5	8.4	93.6	10.0	91.2	12.3	1.1	1.0	1.1
Wetlands	300 Kda	93.0	8.7	84.0	11.6	91.9	11.1	91.4	11.5	1.1	1.0	1.1
Wetlands	30 Kda	72.5	15.6	64.1	9.5	81.5	7.2	70.3	11.2	1.1	0.9	1.3
Wetlands	5 Kda	70.0	13.8	56.1	9.4	77.3	7.5	67.5	10.3	1.2	0.9	1.4
Wetlands	1 Kda	58.5	16.4	42.2	4.6	61.5	6.5	50.9	9.2	1.4	1.0	1.5

Figure 6. The percentage distribution of (a) MeHg, (b) DOC and (c) DOC FL in the Particulate (>2 μm), Colloidal (<2 μm, >5 kDa) and LMW (Low Molecular Weight) size fractions for lakes, rivers and wetlands.

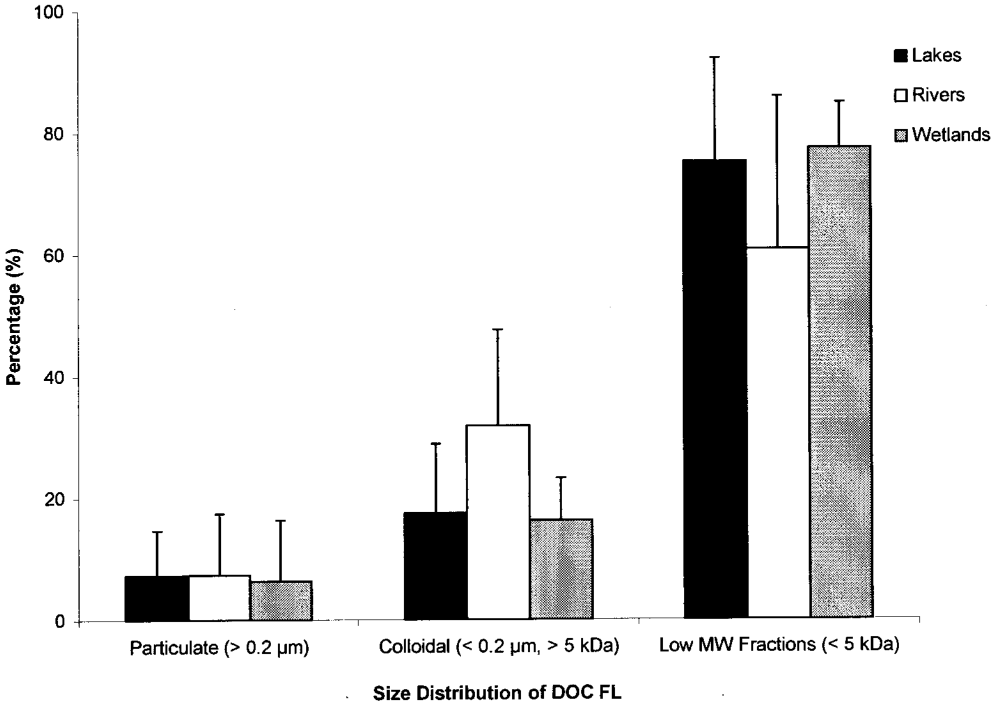
a)


100
80
60
40
20
0
Percentage (%)
■ Lakes
□ Rivers
▨ Wetlands
Particulate (> 0.2 μm)
Colloidal (< 0.2 μm, > 5 kDa)
Low MW Fractions (< 5 kDa)
Size Distribution of MeHg

b)


100
80
60
40
20
0
Percentage (%)
■ Lakes
□ Rivers
▨ Wetlands
Particulate (> 0.2 μm)
Colloidal (< 0.2 μm, > 5 kDa)
Low MW Fractions (< 5 kDa)
Size Distribution of Organic Carbon

c)


100
80
60
40
20
0
Percentage (%)
■ Lakes
□ Rivers
▨ Wetlands
Particulate (> 0.2 µm)
Colloidal (< 0.2 µm, > 5 kDa)
Low MW Fractions (< 5 kDa)
Size Distribution of DOC FL

Rivers were found to differ greatly at the level of 95% confidence from lakes with respect to DOC Abs percentage in the 30 kDa (p=0.012) and 5 kDa (p=0.026) filtrates. Rivers also varied from lakes with a higher level of DOC Abs at a 90% confidence level for the 1 kDa (p=0.07) filtrate.

Wetlands differed from lakes with a higher level of DOC Abs at a 90% confidence level for the 5 kDa (p=0.0752) and 1 kDa (p=0.0895) filtrates. All three freshwater types were similar in that large proportions (>60%) were found in the <30 kDa filtrates for the percentage DOC in the filtrates.

Wetlands were found to have significantly higher percentage ratio of MeHg to DOC in the 5 kDa size fraction as compared to lakes (p=0.014) and rivers (p= 0.012). Wetlands also differed from lakes (p= 0.07) and rivers (p= 0.008) at a secondary degree of significance in the 1 kDa size fraction.

Linear regressions between MeHg, DOC, DOC FL and DOC Abs

MeHg was found to have a significant linear relationship with DOC at 0.2 μm (r^2=0.43, p=0.002) as well as 300 kDa (r^2=0.46, p=0.001), 30 kDa (r^2=0.45, p=0.002) and 1 kDa (r^2=0.54, p<0.001) filtrates. MeHg also had significant and dependent relationships with DOC FL at 0.2 μm (r^2=0.39, p=0.003), as well as 300 kDa (r^2=0.39, p=0.003), 30 kDa (r^2=0.53, p<0.001), 5 kDa (r^2=0.28, p=0.02) and 1 kDa (r^2=0.55, p<0.001) filtrates.

DOC was found to have a strong linear relationship with DOC Abs at 0.2 μm (r^2=0.98, p=<0.001), 300 kDa (r^2=0.98, p<0.001), 30 kDa (r^2=0.97, p<0.001), 5 kDa (r^2=0.97,

$p<0.001$) and 1 kDa ($r^2=0.96$, $p<0.001$). DOC was also found to have significant positive association with DOC FL at 0.2 µm ($r^2=0.49$, $p<0.001$), 300 kDa ($r^2=0.45$, $p=0.001$), 30 kDa ($r^2=0.9$, $p<0.001$), 5 kDa ($r^2=0.93$, $p<0.001$) and 1 kDa ($r^2=0.96$, $p<0.001$) filtrates. DOC Abs had significant relationship with DOC FL at 0.2 ($r^2=0.45$, $p=0.001$), 300 kDa ($r^2=0.41$, $p=0.002$), 30 kDa ($r^2=0.91$, $p<0.001$), 5 kDa ($r^2=0.95$, $p<0.001$) and 1 kDa ($r^2=0.96$, $p<0.001$) filtrates.

MeHg in wetlands was found to have significantly linear relationship by regression with DOC ($r^2=0.61$, $p=0.065$) and DOC FL at 0.2 µm ($r^2=0.55$, $p=0.091$), as well as DOC FL at 30 ($r^2=0.52$, $p=0.106$) and 1 kDa ($r^2=0.69$, $p=0.042$). MeHg in wetlands also had a significant association with DOC Abs at 1 kDa ($r^2=0.66$, $p=0.05$). MeHg in rivers was determined to be related by regression with DOC ($r^2=0.45$, $p=0.098$), DOC FL ($r^2=0.52$, $p=0.065$) and DOC Abs ($r^2=0.5$, $p=0.075$) at 5 kDa. MeHg in lakes was found to have significant linear relationships with DOC at 5 kDa ($r^2=0.61$, $p=0.038$) and 1 kDa ($r^2=0.63$, $p=0.061$) filtrates.

DOC, DOC FL and DOC Abs data for rivers and wetlands were found to be highly related to each other. Regressions performed between DOC and DOC FL, DOC and DOC Abs and DOC FL and DOC Abs demonstrated significant linear relationships at each size fraction. Only DOC FL and DOC Abs were significantly associated at the 0.2 µm ($r^2=0.8$, $p=0.007$) 300 kDa ($r^2=0.78$, $p=0.008$) 30 kDa ($r^2=0.81$, $p=0.005$) and 5 kDa ($r^2=0.78$, $p=0.009$) size fractions for lake samples.

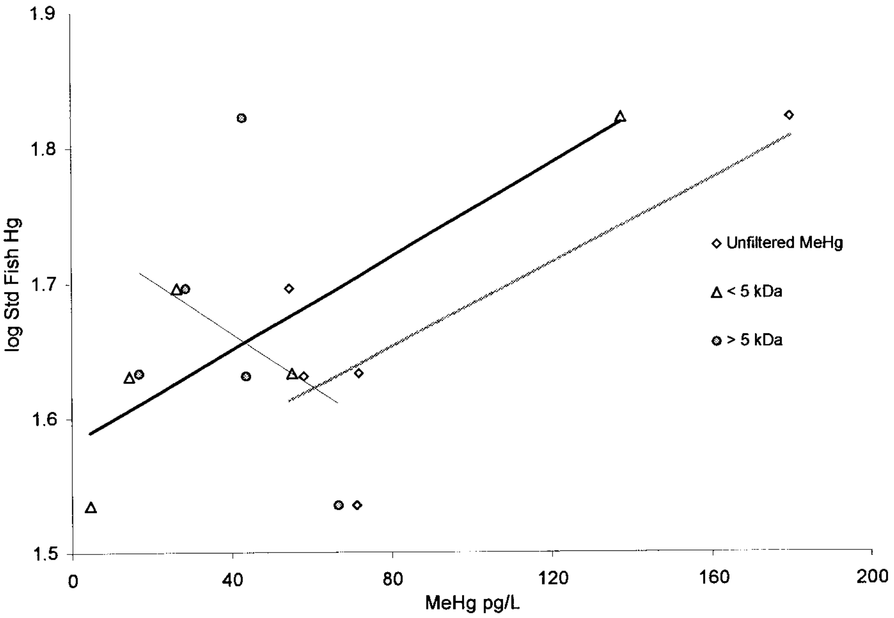
3.5 Predicting Hg concentrations in fish with MeHg in water

Total aqueous MeHg concentrations collected from a subset of sites (n=5) from the W.E.R.F. project were compared to high (>5 and 1 kDa) and LMW fraction (<5 and 1 kDa) concentrations of MeHg calculated using partitioning percentages from this study. These 5 variables were compared and regressed as independent variables with the log-transformed fish Hg concentrations from a sub sample of W.E.R.F. sites to determine which molecular fraction of MeHg explains the most variation in a linear correlation.

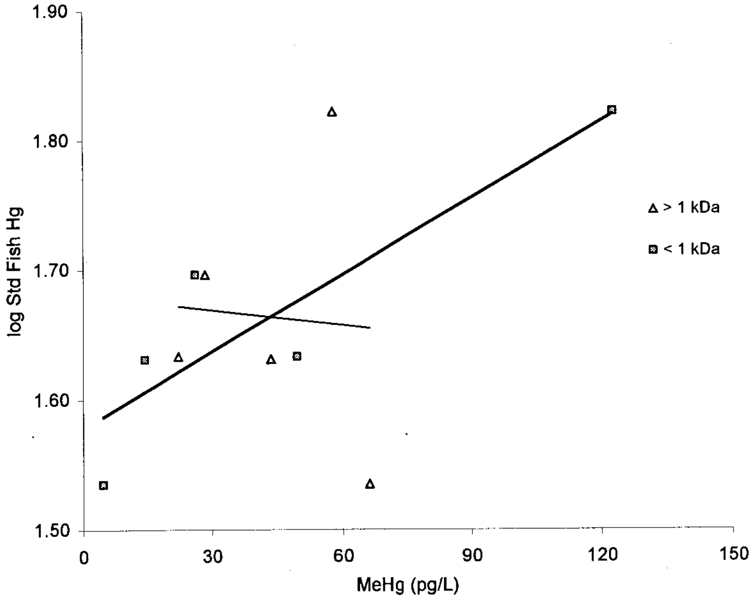
The highest correlated linear regression, which was found to be significant, existed between the <1 kDa molecular weight fraction (r^2=0.77, p=0.05) and log fish Hg concentrations (Figure 7). The second best regression was between the < 5 kDa size filtrate and the log fish Hg concentrations (r^2=0.76, p=0.056). The unfiltered total MeHg concentration also had a relatively predictive relationship with the log fish Hg concentrations (linear regression, r^2=0.59, p=0.129) but was not as effective as the LMW MeHg values. The HMW concentrations which were tested were not predictive.

Figure 7. Log-transformed standard fish Hg concentrations from the W.E.R.F. study compared to total aqueous MeHg concentrations as well as high and low molecular weight filtrates.

a)


1.9
1.8
1.7
1.6
1.5
log Std Fish Hg
0
40
80
120
160
200
MeHg pg/L
◇ Unfiltered MeHg
△ < 5 kDa
⊙ > 5 kDa

b)


1.90
1.80
1.70
1.60
1.50
log Std Fish Hg
0
30
60
90
120
150
MeHg (pg/L)
△ > 1 kDa
▣ < 1 kDa

3.6 UVB Photodegradation of MeHg in the St. Lawrence River

A number of variables were tested to observe and record photo-demethylation via UVB irradiation in the St. Lawrence River (Appendix).

Ambient (0.02 ng/L) and spiked (5 ng/L MeHg) samples were tested for photodegradation under incubation periods in natural light of 0, 3 and 6 hours (cumulative UVB levels of 0, 0.47, and 0.69 kJ/m^2 respectively). Triplicate ambient samples were near the detection limit and did not change significantly during the course of the experiment. Triplicate spiked samples as well as dark and de-ionized water controls also did not change significantly with UVB exposure. The intensity of radiation in natural light on a cloudy day in autumn was insufficient to show any sign of photodegradation (Figure 8).

A second experiment under laboratory conditions was undertaken with triplicate MeHg spiked samples (5 ng/L) as well as triplicate samples that were spiked with both MeHg and $FeSO_4 \cdot 7H_2O$ in order to adjust the concentration of Fe(II) to 100 μM. Samples were exposed for 0, 3 or 6 hours to a relatively high intensity of UVB irradiance which was emitted at cumulative levels of 0, 7.2, and 13.8 kJ/m^2 respectively. Consequently, samples spiked with MeHg did show significant decreases (r^2=0.63, $p<0.051$) in concentration with increases in UVB radiation with a 20% average loss after 3 hours and a 31% average loss after 6 hours of exposure. Photodegradation also occurred in samples spiked with MeHg and 100 μM of Fe(II) as average levels dropped 11% after 3 hours of exposure and a 17% after 6 hours of exposure. The loss of MeHg concentration in samples spiked with iron under increased UVB was also found to be significant (r^2=0.752, $p<0.029$). Dark and deionized water controls did not show significant change.

Using univariate analysis of variation, under these conditions the MeHg concentrations of samples with and without iron spikes were not found to be significantly different independent of UVB radiation ($p=0.014$). The addition of iron did not add or reduce MeHg in the solution. However, the rates of photodegradation of samples spiked with iron and those without were found to be approach a primary level of significance ($p<0.057$).

A third experiment demonstrated that the amount of MeHg loss increases when pH is lowered from 7.5 to 5 and 3 (Figure 9). The loss of MeHg concentration at pH 3 ($r^2=0.812$, $p<0.001$) and 5 ($r^2=0.8416$, $p<0.001$) under increased UVB was found to be significant. After 3 hours of exposure at pH 3 and 5, MeHg concentration decreased 35.4% and 41.7% respectively (Figure 10). After 6 hours of exposure at pH 3 and 5, MeHg concentration decreased 58.3% and 64.6% respectively (Figure 10). Although DGM analysis was not a major part of this experiment, measurements confirmed that more Hg was escaped as Hg^0 in samples exposed to 3 (496 pg/L) and 6 hours (284 pg/L) of UVB than those which were kept in the dark (45 pg/L).

Figure 8. Percentage MeHg remaining in water samples from the St. Lawrence River spiked to 5 ng/L MeHg under low and high UVB irradiation with one set additionally spiked with 100 μM Fe(II) for 0, 3, and 6 hours.

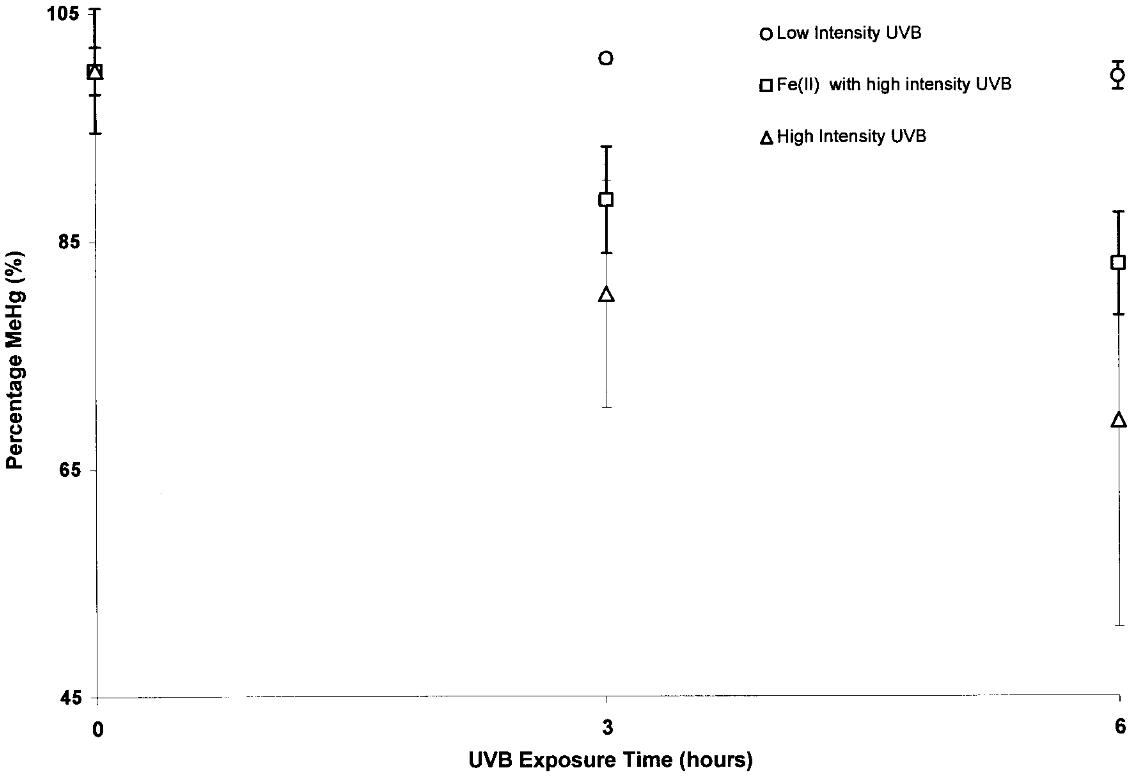
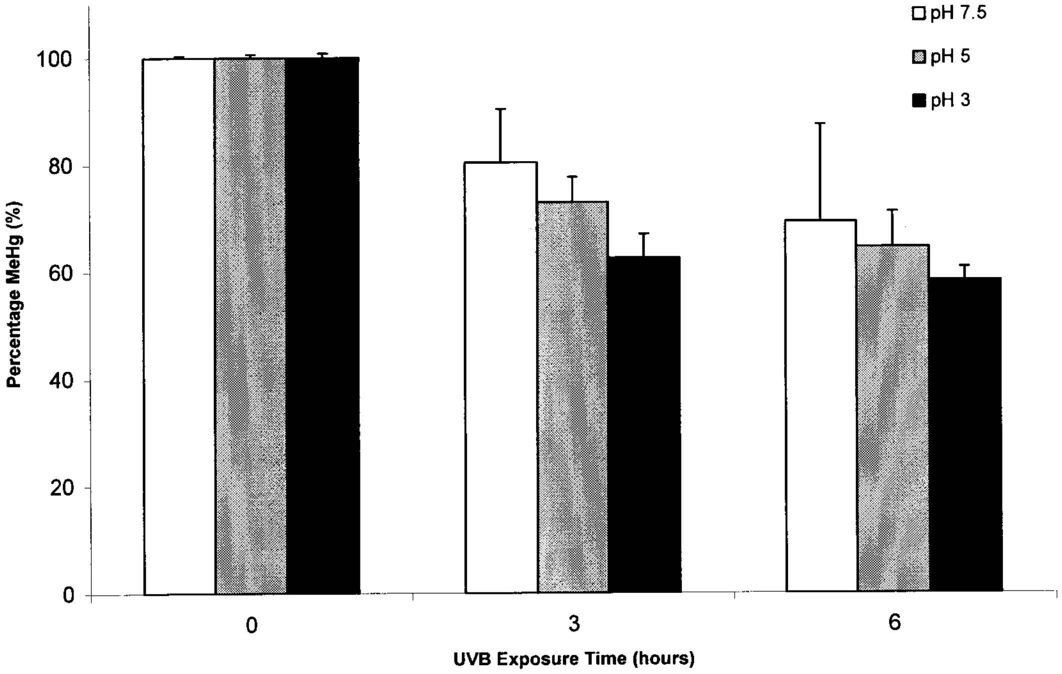

105
85
65
45
Percentage MeHg (%)
0
3
6
UVB Exposure Time (hours)
○ Low Intensity UVB
□ Fe(II) with high intensity UVB
△ High Intensity UVB

Figure 9. Triplicate samples of percentage MeHg at three levels of pH (7.5, 5 and 3) exposed to UVB radiation for 0, 3 and 6 hours.


□ pH 7.5
▨ pH 5
■ pH 3
Percentage MeHg (%)
0
20
40
60
80
100
0
3
6
UVB Exposure Time (hours)

Chapter 4

Discussion

The following interpretations are subdivided into thematic sections related to the thesis objectives and hypotheses as follows:

4.1 The predictive relationship between MeHg and DOC

In the Hill Thesis Data set, MeHg was significantly related to DOC, DOC FL, and DOC Abs in un-fractionated samples by linear regression. Therefore, null hypothesis 1a, that there is no significant linear relationship between MeHg and DOC in un-fractionated fresh water samples, is rejected. The linear relationship between MeHg and DOC is quite strong but outliers still exist. There are a variety of confounding factors which may have clouded the relationship between MeHg and DOC at some sites such as varying concentrations of thiol functional groups and pH. Consistent regression r^2 values between 65 and 70% suggest that DOC, DOC FL, and DOC Abs are equally good at predicting MeHg in freshwater systems. The functionality of DOC FL as a predictor for MeHg is notable since it is much less time consuming and inexpensive than testing for DOC or directly for mercury and may be a useful surrogate for management surveys in the future.

MeHg was negatively associated with pH in this thesis. This is consistent with current research (Kelly et al. 2003), however, the relationship was not significant. This is not surprising given the heavy influence of the limestone geology on the study area. Calcium and magnesium from limestone can buffer solutions and neutralize acids in lakes. Most lower pH samples were wetlands which did not make up a large portion of the data set.

Although a moderately large range of pH was achieved in this study, this was not sufficient to show major effects due to acidity.

A number of DOC absorbance parameters were calculated for this study but found to be autocorrelated. Although these parameters were equally related to MeHg in water, using more than one was not useful since they would not improve linear regressions. From the literature, different attributes of organic molecules were expected. For instance, the 280 and 285 nm wavelengths have been used to estimate the proportion of fulvic compounds in freshwater while the 300 nm wavelength has been linked to particulate organic carbon in fresh waters (Kalbitz et al. 2000). The ratio of wavelengths 390:355 has been found to be predictive for the proportion of fulvic acid in the solution (Kalbitz et al. 2000). Instead, the wavelength at 254 nm was used as the main absorbance parameter for the partitioning experiments to corroborate DOC loss in the filtrates. Korshin et al (1997) asserts that the absorbance wavelength at 254 nm is commonly used in monitoring by the waste water treatment industry due to a linear relationship with DOC.

Linear Regressions between Water Chemistry Parameters (all data sets)

Most of the significant regressions found in the Hill Thesis data set were found in the larger combined data set. The relationship between MeHg and DOC was quite strong with a positive slope, similar to the Hill subset. This finding provides further evidence that DOC is likely the strongest predictor of MeHg in freshwater environments. As well, MeHg had a relatively weak but significantly negative relationship with pH.

The DOC, conductivity and the ratio of MeHg to DOC were all found to have significant and negative linear regressions with pH. These findings are understandable since high acidities in solution can promote bacterial methylation of mercury through higher rates of uptake (Winfrey and Rudd 1990) and separate MeHg from bonds with DOC (Greenfield et al. 2001). Higher DOC and conductivity concentrations are apparent at low pH due to the degradation of organic compounds which perpetuate higher values of both characteristics.

4.2 Concentrations of MeHg and DOC in lakes, rivers and wetlands

Many significant differences were found between un-fractionated water chemistry values sampled from wetland, lake and rivers sites. The third null hypothesis of this study was rejected as wetlands had the highest levels of MeHg, DOC, DOC FL, DOC Abs, conductivity and the lowest pH levels. Wetlands are prone to these characteristics due to shallow depths and high biological activity and degradation which promotes acidity, increased levels of fresh aqueous organic compounds and the bacterial methylation of mercury (Mitsch et al. 1993). Wetlands had the highest level of variability for these characteristics. The highest MeHg and DOC concentrations in wetlands seem to have occurred at sites that undergo long periods of time with water levels that are very shallow (Blueberry Marsh, Mer Bleu). Others sites which appeared closely linked to a lake or river (Otter Marsh, Mississippi Marsh,) had lower levels of MeHg and DOC. It may be that the resuspension of organic particles during the flooding of dry areas has a positive impact on Hg production.

Also consistent with hypothesis 3, lakes were found to have significantly lower MeHg, DOC and DOC FL, DOC Abs, conductivity and higher pH levels than rivers and wetlands. This was expected due to the relatively larger volume of water present in lake systems which act to dilute organics and acidic detritus. Longer hydrologic renewal times in lakes allow DOC and MeHg in the water column to be irradiated and degraded over long periods by UVB. Rivers, as the transition zone from wetlands to lakes, had water chemistry concentrations that were lower but not significantly different than wetlands in most cases but significantly higher than lakes for notable parameters such as MeHg, DOC, DOC FL, DOC Abs, and conductivity. These results were expected since rivers receive a large amount of fresh organic compounds from tributaries and wetlands during the spring that can raise DOC and aromatic concentrations as well as lower pH. However, although some of these compounds will linger in river embayments or may form particulates that may rest in the water column or sediment, the flowing hydrology of these systems promotes expulsion and dilution. The bigger rivers where dilution was greater seemed to have lower MeHg and DOC values (St. Lawrence, Ottawa) while those which flowed across agricultural areas with a majority of water volume from small tributaries (Black, Raisin, South Nation) had much higher levels of these parameters.

Wetlands were also found to have higher MeHg, DOC, DOC FL and DOC Abs concentrations at the majority of filtrates than rivers or lakes. Rivers were found to have intermediate values while lakes had the lowest magnitudes of these parameters. These findings are indicative that the chemical differences between the three freshwater types extend from the largest to the smallest size fractions found in solution.

4.3 Percentage MeHg and DOC in the particulate, colloidal and LMW fractions

The primary objective for the examination of molecular size distributions in this study was to better appreciate the chemical associations between MeHg and DOC that may influence biomagnification at the base of the aquatic food chain. The second null hypothesis of this study is rejected since significant differences were detected between percentages of MeHg, DOC and DOC FL in the particulate, colloidal and LMW fractions. The largest proportion of MeHg, DOC and DOC FL was found in the LMW fractions while the lowest percentages were present in the particulate. Also, a relatively large amount of MeHg and DOC was left in the 1 kDa size fractions of many freshwater sites. This result is consistent with the few studies which have analyzed the location of MeHg (Babiarz et al. 2003), DOC (Wu 2003) and aromaticity (Her et al. 2003, Haitzer et al. 2003) by molecular size in lakes. Generally it has been assumed that up to 90 % of DOC is comprised of fulvic acids. Since fulvics are smaller than humic acids and they are known to have high levels DOC FL, it is likely aggregations of these compounds that we find in low molecular weight fractions.

MeHg may bind to small DOC fractions since a large number of small molecules would have surface area available (and hence binding sites). Large molecules may have many binding sites inflected inside the structure which would be less available for binding (Winch Pers. Convers. January 2004). Only sites at or near the surface of the large molecule would have effective binding sites.

4.4 Distribution of MeHg and DOC in lakes, rivers and wetlands

The fourth null hypothesis of this study, that there are no significant differences between the size distribution of MeHg, DOC, DOC FL and DOC Abs amongst wetlands, lakes and rivers was rejected. As predicted, hydro-geological, water chemistry and organic compound compositional differences amongst the three fresh water types produced significant variation in the binding of MeHg to organic compounds at the studied size fractions.

Wetlands had higher percentage MeHg, DOC, DOC FL and DOC Abs concentrations in the lower size fractions than rivers and lakes. While no large differences were found in the distribution of percentage DOC amongst the three freshwater types, and only the 30 kDa DOC FL size fractions illustrated statistical difference, a number of low molecular weight filtrates showed statistical difference in DOC Abs. These findings corroborate the prediction that it is the aromatic functional groups of organic molecules that bind MeHg. Where there are more fresh aromatic functional groups that are rich in sulfur containing thiols more MeHg will be present even in the smallest of size fractions.

Wetlands were found to have a significantly higher percentage MeHg to DOC in the 5 kDa and 1 kDa size fractions than lakes. This may be due to higher levels of bacterial methylation of Hg in wetlands or a different type of organic compounds in these environments. The ultimate molecular size of organic compounds in solution likely depends on the origin of degrading compounds such as lignins from different types of plant vegetation or algae cells. Wetlands likely receive a richer organic soup containing these proteins and because of increased degradation, these seem to be generally of smaller molecular size than lakes and rivers. All three freshwater types had the higher DOC FL per DOC molecule in the low molecular weight size fractions (<30 kDa). This may be a further indication that the low molecular weight size fractions are the primary location of important aromatic functional groups that can bind MeHg. Rivers were found to have generally larger molecular weight organic compounds than lakes which agreed with two previous studies (Grzybowski 2000, Martin-Mousset et al. 1997).

Linear Regressions between variables in each fraction

The percentage MeHg, DOC and DOC FL means in each size fraction were similar and seem to be excluded by filters in comparable proportions. Statistical tests illustrated that MeHg and DOC were significantly related in all size fractions except the 5 kDa filtrate. Therefore, null hypothesis 1b, that there is no significant linear relationship between MeHg and DOC in fractionated samples, is rejected. This finding demonstrates that MeHg seems to be bound to aromatic organic compounds even at extremely low molecular sizes. These small molecular associations containing MeHg may be proteins such as cysteine, fulvic acids or broken organic compounds that contain thiol functional groups.

When the percentage MeHg, DOC and DOC FL values for the three freshwater types were analyzed by regressions throughout all size fractions it was found that relationships between these variables existed but that some had become obscured. Wetlands still retained significant linear relationships between percentage MeHg and DOC, DOC FL or DOC Abs at the 0.2 μm, 30 and 1 kDa molecular fractions. However MeHg was only correlated to organic carbon indicators at 5 kDa for rivers and in the 5 and 1 kDa size fractions for lakes. One explanation for the lack of significant linear relationships between percentage MeHg and DOC when separated by freshwater type may simply be sample size. That some of these associations still exist when subdivided into smaller groups at extremely small molecular sizes reinforce the link between these compounds.

Partitioning Conclusions

TFU was determined to be a good technique for this series of experiments. Contamination was not a concern and MeHg replicates were reasonable. A series of papers by Hoffman et al. (2000) and Babiarz et al. (2000 and 2001) have also shown TFU to be a reliable filtration method. Common benefits of this technique include the ability to process large volumes of solution without the introduction of reagents, as well as being a generally nondestructive and inexpensive method (Cai 1999). It has been found that with proper cleaning between samples, DOC could be recovered effectively with very low blank values (Hoffman et al. 2000). The most recent mass balances for ultrafiltration of MeHg and DOC has yielded percentage recoveries of 97.3 and 99.8 respectively (Babiarz 2003). Another study provided percentage recoveries of 109 ± 12% for DOC and 106.9 ± 9% for DOC FL (Guéguen 2002).

It appears that DOC and its functional groups require a relatively unobstructed outside surface area to bind molecules such as MeHg. In a large molecular aggregation of functional groups, the majority of these would be inside the aggregation. Chemical groups inside the aggregation may capture some small molecules but many binding opportunities would be lost due to the lack of access to the surrounding solution. The surface of the molecule seems to be able to bind with free flowing molecules with more efficacy due to greater access. Therefore, if a large molecule was broken into a number of smaller structures, it seems that there would be a larger overall substrate area available for MeHg binding. Less substrate area would be wasted in the dense center of an organic molecule while more functional groups would be open to the outside solution.

Following this rationale, it would seem logical that MeHg has been observed in this thesis to be associated in very high percentages with small fraction organic carbon molecules. It seems easier for MeHg molecules to bind to the surface area of many small DOC molecules than to the surface and inside of a few larger fraction molecules.

4.5 Predicting Hg concentrations in fish with MeHg in water

The molecular size of DOC may be one of the most important factors contributing to the bioavailability of MeHg in fresh water systems (Cai 1999). In order to investigate whether the MeHg content of the LMW fractions (5 and 1 kDa) is suitable for predicting mercury in fish, these fractions were contrasted with normal total aqueous MeHg in surface water and related to fish concentrations values. The 5 and 1 kDa size fractions were found to be the best predictors of MeHg in fish in this small test set of samples. Therefore, null hypothesis 4, which stated that LMW size fractions of MeHg are less or equally predictive for Hg concentrations in fish as HMW size fractions or total MeHg was rejected. This preliminary study provides some circumstantial evidence that the bioavailable fraction of MeHg is located in the low molecular weight fractions. An explanation for this phenomenon may be that MeHg molecules are bound or loosely associated with these small compounds and are incorporated in filter feeding organisms such as algae and bacteria at the base of the food chain. This way, aqueous MeHg is concentrated and enters the food chain when it attaches to the food utilized by filter feeding biota. It is also possible that the LMW fraction MeHg molecules may be small enough to be actively transporting into the cells of bacteria and/or algae.

4.6 UVB Photodegradation of MeHg in the St. Lawrence River

St. Lawrence River water has a neutral pH and is known to be extremely low in dissolved organic carbon. It also has minimal concentrations of cations such as iron (0.07 µM from Quemerais et al. 1998) and low levels of aqueous MeHg despite relatively high levels of mercury in the food chain.

The intensity of radiation in natural light on a cloudy day in autumn was insufficient to show any sign of photodegradation in St. Lawrence River water. When UVB levels were increased under laboratory conditions, a significant decrease in MeHg concentration was found at both 3 and 6 hours. The longer the time of exposure to UVB, the greater the opportunity for the de-methylation of MeHg.

The addition of iron under UVB radiation did not reduce MeHg concentrations in the solution. Therefore, null hypothesis 5a, that the concentration of MeHg in St. Lawrence River water exposed to UVB radiation for 6 hours will increase or remain equal when spiked with Fe(II) was not rejected. The rates of photodegradation of samples spiked with iron and those without were found to be significantly lower ($p<0.057$). In other words, adding iron actually decreased the degree of photodegradation under these conditions. It is possible that Fe(II) ions oxidized to Fe(III) and formed iron oxides that sorbed with MeHg in solution. These iron oxides may have shielded some MeHg molecules from UVB radiation.

The concentrations of MeHg in water exposed to UVB radiation decreased as pH was lowered. Therefore, null hypothesis 5b, that the concentration of MeHg in St. Lawrence River water exposed to UVB radiation for 6 hours will increase or remain equal when pH is reduced to 5 or 3 was rejected. This may be due to acidic proliferation of H+ ions that dislodge MeHg from bonds with organic molecules. The majority of photodegradation took place in the first 3 hours of exposure. Photodegradation continued until the end of 6 hours but the rate decreased. It seems that photolytic reactions with pH affect MeHg and their binding opportunities quickly through the formation of hydroxyl radicals and decreases over time.

Photodegradation Conclusions

MeHg can degrade naturally but this is a complex process. Low pH and increased exposure to UVB irradiation can encourage photodegradation. Iron may not be a significant factor in UVB induced photo-demethylation. More research should be done with different UVB intensities, use of other wave lengths, change in acidities and increased concentrations of dissolved organic carbon in order to explore this question further.

4.7 Areas of Improvement and Future Work

At the end of any scientific research project, it is important to analyze problems that were encountered and how they may be improved for subsequent studies.

There is evidence to suggest that some of the environmental parameters measured (e.g. DOC and MeHg) fluctuate from higher levels during the spring run-off period to lower concentrations at the end of the summer. However, processing commitments made it difficult to avoid sampling across this period. Also, there is further evidence to suggest that there exists a certain amount of within site variation of MeHg and DOC at each individual location. For example, one may expect different DOC and MeHg concentrations in the open water and littoral zones. Additionally, there might be value to sampling water for MeHg at various depths in the water column. It is possible that MeHg concentrations may be different above and below the thermocline where fish spend the majority of their time. Regrettably, the length of time needed to process each site also made repeated sampling of particular sites and using different depths unrealistic. Fortunately, previous sampling experience with some of these sites (Hickey et al. in press) indicate that water chemistry values have not changed dramatically over a two year period. It was decided that time would be better spent including a greater number of sampling locations in order to determine variation between sites rather then continued study of one particular type of water body which may not be representative of the study area.

It is proposed that a larger study of this type analyze at least two or three sites within each water body for three years with special attention to sampling in both the spring and late summer in order to observe whether partitioning distributions change drastically with influx of runoff. This would include partitioning differences between littoral and pelagic zones as well as the effect of temperature. An important study which is simultaneously being completed at Trent University by Kristin Mueller (In preparation) has taken a full watershed approach for a series of partitioning experiments. This is also a good route of experimentation but should be combined with larger surveys in order to understand the hydrological and regional variations of MeHg partitioning.

It may also be important to further categorize each freshwater type into groups such as seepage and drainage lakes, acidic bog vs. neutral marsh systems and tributary vs. heavy flow rivers. It is likely that variation will decrease and MeHg partitioning means will be more accurate and of higher resolution. However, for this to be possible, sample size must be increased dramatically. One way that sample size could increase would be to reduce filtrate fractions to 0.2, 30 and 1 kDa. The most differences occurred at these molecular sizes and perhaps this could allow more time to be spent in the field and analyzing samples in the laboratory.

There are some problems with the TFU method. Personal experience has shown there is pressure induced DOC deterioration similar to in-line filtering when high pressures are exceeded at very low membrane filter sizes. Although these problems can be mediated, they are sometimes unavoidable at very high DOC concentrations and low filter sizes since back pressure is required to have a sufficient volume of filtrate. Some compounds can be rejected by the membrane which can lead to an underestimate of DOC at low molecular size fractions. Wear and the conglomeration of carbon material on the filter membrane head may cause increased variability in results with filter age (Guéguen 2002). Fortunately, fouling of the membrane surface is minimized at DOC concentrations less than 100 mg/L (Cai 1999). Further laboratory analysis is required in order to characterize the active filtering life-span of filter membranes. In recent sorption experiments under extreme conditions, it has been found that some losses of mercury can occur in polyethersulfone membranes (Babiarz et al. 2000).

Three relatively new techniques which may be used in future partitioning research include isotopic MeHg, nitrogen 15 analysis, thiol elucidation and lignin characterization. Isotopic Hg could be added to an ecosystem similar to the METALLICUS project to simulate inflows and atmospheric deposition in order to track the route of Hg into different size fractions. It may be possible to analyze the concentrations of isotopic MeHg in different size particles to see what becomes incorporated into small organisms (algae, zooplankton) at the base of the aquatic food chain. This could be combined with N15 analysis of invertebrates and fish to approximate trophic level. Detailed thiol analysis on DOC samples could also be done in order to check the relationships between thiol content and MeHg binding. DOC FL is a good indicator of fresh aromatic groups in DOC but thiol analysis would likely provide a more comprehensive and direct method for showing MeHg binding functional groups. Finally, the low molecular weight compounds from wetlands, rivers and lakes could be analyzed for their lignin content. It is quite possible that certain plant compounds degrade into forms that are more or less adept at binding MeHg due to their respective protein structures and molecular size.

Another important improvement which could have been made to this study would have been to expand the study region to incorporate more low pH systems. Acidity is an important factor in MeHg production and bioavailability (Amirbahman et al. 2002). It would be interesting to see if large differences in partitioning occur at pH levels <6 which may be low enough to free MeHg from bonds with organic substrates and increase the rate of bacterial methylation.

4.8 Recommendations for Watershed Management

It is imperative that the scientific process yield data that contributes to our appreciation of natural processes and offers at least subtle improvements in the way we interact with the environment. Therefore, this section of the thesis is devoted to a few general recommendations to the management, scientific and consumer communities concerning MeHg partitioning and photodegradation in aquatic systems.

To the Ecosystem Managers (E.C., DFO, MNR, OME, CEC)

It was confirmed in this thesis that both the magnitudes and distribution of MeHg are significantly different depending on whether the water body is a wetland, lake or river. Consequently, it is recommended that all three systems are considered when monitoring surveys or risk assessment occur. These systems do not exist in isolation since the outflow from wetlands can feed into lakes and rivers while rivers can contribute water into lakes and wetlands. Watershed approaches that anticipate flooding or drought due to climate change are imperative. In the near future, many areas of heavy fishing may undergo water chemistry changes that can drastically affect Hg concentrations in the aquatic biota due to rapid influxes of DOC or decreases in pH. Watershed perspectives should be considered even when the boundaries of country, province or state must be crossed.

Although it is not confirmed that atmospheric emissions from anthropogenic sources are the most substantial contributor to Hg in fish, this thesis asserts that even small changes to MeHg concentrations at the base of the food chain may be linked to substantial Hg fluctuations in higher trophic levels. If anthropogenic mercury can be linked to bioavailable MeHg in food chains, the precautionary principle would suggest that emissions should be strictly monitored if not restricted completely.

To the scientific community (Researchers, NSERC)

Studies should continue to discern what fraction of MeHg becomes bioavailable so that isotopic research can confirm the origin of these contaminants. Predictive models should be revised to include advances in our awareness of the processes which occur at the base of the aquatic food chain. Photo-demethylation and partitioning may be important additions to mercury cycling. Ecosystem studies such as COMERN and METALLICUS should evaluate whether the process of MeHg partitioning by TFU or other methods can be incorporated into their sampling regimes. If better predictions can be made regarding fish Hg based upon a filtered MeHg sample or even a DOC FL sample, perhaps it may be easier to prove to policy makers and regulators that improvements are possible.

To the consumers (General Public)

Consumers of fish should be aware that fish from brown water systems are generally higher in Hg compared to those of the same size from a clear water environment. People should know that lakes, rivers and wetlands may have different amounts of Hg depending on the chemistry of the water and the kind of particles which are present. Fish consumption guidelines should focus upon species of fish that are eaten regularly and possibly utilize a labeling process. These fish should include marine species such as tuna and shark which both tend to be particularly high in Hg.

4.9 Overall Conclusions

DOC is a highly predictive parameter for MeHg in fresh water systems. This relationship is likely due to bonds between MeHg and the aromatic functional groups of DOC. The concentration of H+ ions in freshwater systems is also important but not as predictive when the range of values does not include high levels of acidity. When surface water chemistry variables were compared between wetlands, rivers and lakes, it was found that significant differences were present for MeHg, DOC, DOC FL and pH concentrations. Wetlands had higher levels of MeHg and the DOC associated parameters as well as lower pH than rivers and lakes.

Partitioning experiments confirmed that water chemistry differences extended to all size fractions down the low molecular weights. The three freshwater types were found to have significantly different magnitudes at several filtrate sizes for many of the critical MeHg indicators even in the same geographical and geological area. Furthermore, as predicted, the size distributions of percentage MeHg, DOC, DOC Abs and DOC FL were different depending on the freshwater type. This observation indicates that wetlands, lakes and rivers may have varying chemical processes or contain different organic compounds which are responsible for these distributions. A common discovery to wetlands, lakes and rivers was that a large proportion of MeHg, DOC and DOC FL were found in the LMW weight fractions. This may be because small organic fragments may have increased binding opportunities as compared to larger molecules which may have more obstructed functional groups. Using data from the W.E.R.F. study it was shown that the low molecular weight DOC bound MeHg was highly correlated to fish (10-20 cm). I conclude that this may be the bioavailable form which can be incorporated into the base of the food chain.

MeHg photo-demethylation does take place in the waters of the St. Lawrence River. However, substantial photodegradation could only be observed at higher levels of UVB irradiation in the laboratory. Hydraulic mixing and ambient levels of UVB irradiation may make the process of natural photodegradation slower. Contrary to initial predictions, the addition of iron did not increase the level of MeHg photo-demethylation. This may be due to the difficulty in replicating the natural chemical conditions in solution. Further experiments with iron and UVB irradiation should include various periods of equilibration. Decreasing pH was found to have the most profound effect on the photo-demethylation of MeHg. When levels of acidity were increased, the rate of photodegradation increased dramatically. An increase in H+ ions can dislodge MeHg from bonds with DOC that is known to have protective qualities. If MeHg becomes dissolved in solution, DOC may not be able to provide protection from UVB wavelengths through absorption. MeHg may be more exposed to photolysis reactions and can become demethylated.

The processes of photo-demethylation and MeHg partitioning both affect the concentration and composition of mercury in the water column and the possibly the degree to which it is available to the lowest trophic levels. Studies should continue since these processes are important determinants for the extent of MeHg contamination in aquatic systems.

References

Amirbahman, A., A.L. Reid, T.A. Haines, J.S. Kahl and C. Arnold. 2002. Association of methylmercury with dissolved humic acids. Environmental Science and Technology. 36: 690-695.

Babiarz, C.L., S.R. Hoffman, M.M. Shafer, J.P. Hurley, A.W. Andren and D.E. Armstrong. 2000. A critical evaluation of tangential-flow ultrafiltration for trace metal studies in freshwater systems. 2. Total mercury and methylmercury. Environmental Science and Technology. 34: 3428-3434.

Babiarz, C.L., J.P. Hurley, S.R. Hoffmann, A.W. Andren, M.M. Shafer, and D.E. Armstrong. 2001. Partioning of total mercury and methylmercury to the colloidal phase in freshwaters. Environmental Science and Technology. 35: 4773-4782.

Babiarz, C.L., J.P. Hurley, D.P. Krabbenhoft, C. Gilmour, and B.A. Branfireun 2003. Application of ultrafiltration and stable isotopic amendments to field studies of methyl mercury partioning to filterable carbon in lake water and overland runoff. The Science of the Total Environment. 304: 295-303.

Bloom, N.S. 1995. Trace Metals and ultra-clean sample handling. Environmental Lab. 7: 20-25.

Boener, D.W. 2000. Ecological effects, transport, and fate of mercury: a general review. Chemosphere. 40: 1335-1351.

Cai, Y. 1999. Size distribution measurements of dissolved organic carbon in natural waters using ultrafiltration technique. Water Research. 33(13): 3056-3060.

Cai, Y., R.. Jaffe, A. Alli, and R.D. Jones. 1996. Determination of organomercury compounds in aqueous sample by capillary gas chromatography-atomic fluorescence spectrometry following solid phase extraction. Analytical Chemical Acta 334:251-259.

Chen, J., S.O. Pehkonen and C-J Lin. 2003. Degradation of monomethylmercury chloride by hydroxyl radicals in simulated natural waters. Water Research. 37: 2496-2504.

Choi, M.H., Chech, J.J. and M.C. Lagunas-Solar. 1998. Bioavailability of Methyl mercury to sacramento blackfish (*orthodon microlepidotus*): dissolved organic carbon effects. Enivornmental Toxicology and Chemistry. 17(4): 695-701.

Conte, P. and A. Piccolo. 1999. Conformational arrangement of dissolved humic substances. Influence of solution composition on association of humic molecules. Environmental Science and Technology. 33: 1682-1690.

Eyrolle, F., M.F. Benedetti, J.Y. Benaim and D. Fevrier. 1996. The distribution of colloidal and dissolved organic carbon, major elements, and trace elements in small tropical catchments. Geochimica et Cosmochimica Acta. 60(19): 3643-3656.

Fukushima, T., T. Ishibashi and A. Imai. 2001. Chemical Characterization of Dissolved Organic Matter in Hiroshima Bay, Japan. Estuarine, Coastal and Shelf Science. 53: 51-62.

Garcia, E. and R. Carignan. 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.

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

Gorski, P.R., L.B. Cleckner, J.P. Hurley, M.E. Sierszen, and D.E. Armstrong. 2003. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, U.S.A. The Science of the Total Environment. 304: 327-348.

Greenfield, B.K., T.R. Hrabik, C.J. Harvey, and S.R. Carpenter. 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.

Grzybowski, W. 2000. Effect of short-term sunlight irradiation on absorbance spectra of chromophoric organic matter dissolved in coastal and riverine water. Chemosphere. 40: 1313-1318.

Guéguen, C., C. Belin, and J. Dominik. 2002. Organic colloid separation in contrasting aquatic environments with tangential flow filtration. Water Research. 36: 1677-1684.A172

Hall, G.E.M., G.F. Bonham-cater, A.J. Horowitx, K.Lum, C.Lemieux, B. Quemerais and J.R. Garbarino. 1996. The effect of using different 0.45 μm filter membranes on 'dissolved' element concentrations in natural waters. Applied Geochemistry. 11: 243-249.

Hayes, M.H, P. MacCarthy, R.L. Malcolm, R.S. Swift. Humic Substances II: In Search of Structure, John Wiley: Chichester, 1989.

Hickey, M.B.C., J.R. Hill, J.J. Ridal, G.M. Richardson, J.C. Gibson, J.Holmes and D.R.S. Lean. 2002. Predicting mercury in fish with water chemistry. In press.

Hintelmann, H., P.M. Welbourn, and R. D. Evans. 1997. Measurement of complexation of methylmercury compounds by freshwater humic substances using equilibrium dialysis. 31: 489-495.

Hoffman, S.R., M.N. Shafer, C. L. Babiarrz and D.E. Armstrong. 2000. A critical evaluation of tangential-flow ultrafiltration for trace metal studies in freshwater systems. 1. Organic Carbon. Environmental Science and Technology. 34: 3420-3427.

Kalbitz, K., S. Geyer, and W. Geyer. 2000. A comparative characterization of dissolved organic matter by means of original aqueous samples and isolated humic substances. Chemosphere. 40: 1305-1312.

Karlsson, T. and U. Skyllberg. 2003. Bonding of ppb levels of methyl mercury to reduced sulfur groups to soil organic matter. Environmental Science and Technology. 37: 4912-4918.

Kirkwood, A.E., P. Chow-Fraser and G. Mierle. 1999. Seasonal mercury levels in phytoplankton and their relationship with algal biomass in two dystrophic shield lakes. Environmental Toxicology and Chemistry. 18 (3) 523-532.

Kelly, C.A., J.W.M. Rudd and M.H. Holoka. 2003. Effect of pH on Mercury uptake by an aquatic bacterium: Implications for Hg cycling. Environmental Science and Technology. 37: 2941-2946.

Korshin, G.V., C-W Li and M.M. Benjamin. 1997. Monitoring the properties of natural organic matter through UV spectroscopy: A consistent theory. Water Research. 31(7): 1787-1795.

Lawrence, J. 1980. Semi-quantitive determination of fulvic acid, tannin and lignin in natural waters. Water Research. 14: 373-377.

Lean, D.R.S. 1998. Influence of UV-B radiation on aquatic ecosystems. In: Environmental Toxicology and Risk Assessment: Seventh Volume ASTM STP 133, E.E. Little, A.J. Delonay, and B.M. Greenberg, Eds. American Society for Testing and Materials pp 1-20.

Lean, DRS and SD Siciliano. 2003. Production of methylmercury in lakes by solar radiation. J. Phys. IV France 107: 743-747.

Martin-Mousset, B., J.P. Croue, E. Lefebvre and B. Legube. 1997. Distribution and characterization of dissolved organic matter of surface waters. Water Research. 31(3): 541-553.

Melamed, R., F.E. Trigueiro and R.C. Villas Boas. 2000. The effect of humic acid on mercury solubility and complexation. Applied Organometallic Chemistry. 14: 473-476.

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

Mitsch, W.J. and J.G. Gosselink (eds.). Wetlands. Second Edition. John Wiley and Sons. Toronto. 1993.

Mueller, K. and P. Dillion. Spatial variability of dissolved organic matter (DOM) within Plastic lake watershed and its effect on mercury (Hg) binding affinity and capacity. M.Sc. Thesis. In preparation.

O'Driscoll, N.J., and R. D. Evans. 2000. Analysis of methyl mercury binding to freshwater humic and fulvic acids by gel permeation chromatography/hybride generation ICP-MS. Environmental Science and Technology. 34: 4039-4043.

O'Driscoll, N.J., T. A. Clair and A. Rencz (eds.) 2001. Cycling of Mercury in Kejimkujik National Park: Toxic Substance Research Initiative Project #124 Summary, December 2001 Occasional Report no. 18. Environment Canada, Sackville, New Brunswick, 93p.

O'Driscoll, N.J., D.R.S. Lean, L. Loseto, R. Carignan and S. Siciliano. 2004. Effect of dissolved organic carbon on the photoproduction of dissolved gaseous mercury in lakes: Potential impacts on Forestry. Environmental Science and Technology. 38: 2664-2672.

Perminova, I.V., F.H. Frimmel, A.V. Kudryavtsez, N.A. Kulikova, G. Abbt-Braun, S. Hesse and V.S. Petrosyan. 2003. Molecular Characteristics of humic substances from different environments as determined by size exclusion chromatography and their statistical evaluation. Environmental Science and Technology. 37: 2477-2485.

Pickhardt, P., C.L. Folt, C.Y. Chen, B. Klaue, J.D. Blum, 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. Proceedings of the National Academy of Sciences of the United States of America. 99(7): 4419-4423.

Rashid, M.A.(eds.) Geochemistry of Marine Humic Compounds. Springer-Verlag. New York. 1985.

Rasmussen, J.B., L. Godbout, M. Schallenberg. 1989. The humic content of lake water and its relationship to watershed and lake morphometry. Limnology and Oceanography. 34 (7): 1336-1343.

Sachse A., D. Babenzien, G. Ginzel, J. Gelbrecht and C.E.W. Steinberg. 2001. Characterization of dissolved organic carbon (DOC) in a dystrophic lake and an adjacent fen. Biogeochemistry. 54: 279-296.

Sager, P., 1988. Selectivity of methylmercury effects on cytoskeleton and mitotic progression in cultured cells. Toxicol. Appl. Pharm. 94: 473-486.

Schindler, D.W. and P.J. Curtis. 1997. The role of DOC in protecting freshwaters subjected to climatic warming and acidification from UV exposure. Biogeochemistry. 36: 1-8.

Sellers, P., Kelly, C.A., J.W.M. Rudd and A.R. MacHutchon. 1996. Photodegredation of methylmercury in lakes. Nature 380: 694-697.

Sigg, L. H. Xue, D. Kistler and R. Schoenenberger. 2000. Size Fractionation (Dissolved, Colloidal and Particulate) of Trace Metals in the Thur River, Switzerland. Aquatic Geochemistry. 6: 413-434.

U.S. EPA. 1997. Mercury Report to Congress, Volume VI: an Ecological Assessment for Anthropogenic Mercury Emissions in the United States. Report EPA-452/R-97-008, EPA, Washington.

V.-Balogh, K., L. Voros, N. Toth and M. Bokros. 2003. Changes of organic matter quality along the longitudinal axis of a large shallow lake (Lake Balaton). Hydrobiologia. 506-509: 67-74.

Wallschlager, D. M.V.M. Desai and R-D. Wilken. 1996. The role of humic substances in the aqueous mobilization of mercury from contaminated floodplain soils. Water, Air and Soil Pollution. 90: 507-520.

Watras, C.J., Bloom, N.S., Hudson, R.J.M., Gherini, S., Munson, R., Claas, S.A., Morrison, K.A., Hurley, J., Wiener, J.G., Fitzgerald, W.F., Mason, R., Vandal, G., Powell, D.E., Rada, R.G., Rislov, L., Winfrey, M., Elder, J., Krabbenhoft, D.P., Andren, A.W., Babiarz, C., D.B. Porcella, and J.W. Huckabee. 1994. Sources and Fates of Mercury and Methyl mercury in Wisconsin Lakes. In C. J. Watras, J. W. Huckabee, (eds.) Mercury Pollution Integration and Synthesis. United States of America. Lewis Publishers. Pp.153-177.

Winch, S. 2002. Influence of UVB radiation on the capacity of dissolved organic carbon to protect freshwater phytoplankton from metal toxicity. University of Ottawa Thesis.

Winfrey, J.R. and J.W.M. Rudd. 1990. Environmental factors affecting the formation of methyl mercury in low pH lakes. Environmental Toxicology and Chemistry 9: 853-869.

Wren, C.D. 1985. Probable case of mercury poisoning in a wild otter, Lutra canadensis, in northwestern Ontario. Canadian Field Naturalist 99: 112-114.

Wu, F.C., E. Tanque and C.Q. Liu. 2003. Fluorescence and amino acid characteristics of molecular size fractions of DOM in the waters of Lake Biwa. Biogeochemistry. 65: 245-257.

Yan, N.D., W. Keller, K.M. Somers, T.W. Pawson and R.E. Girard. 1996. Recovery of crustacean zooplankton communities from acid and metal contamination: comparing manipulated and referenced lakes. Canadian Journal of Fisheries and Aquatic Sciences. 53: 1301-1327.

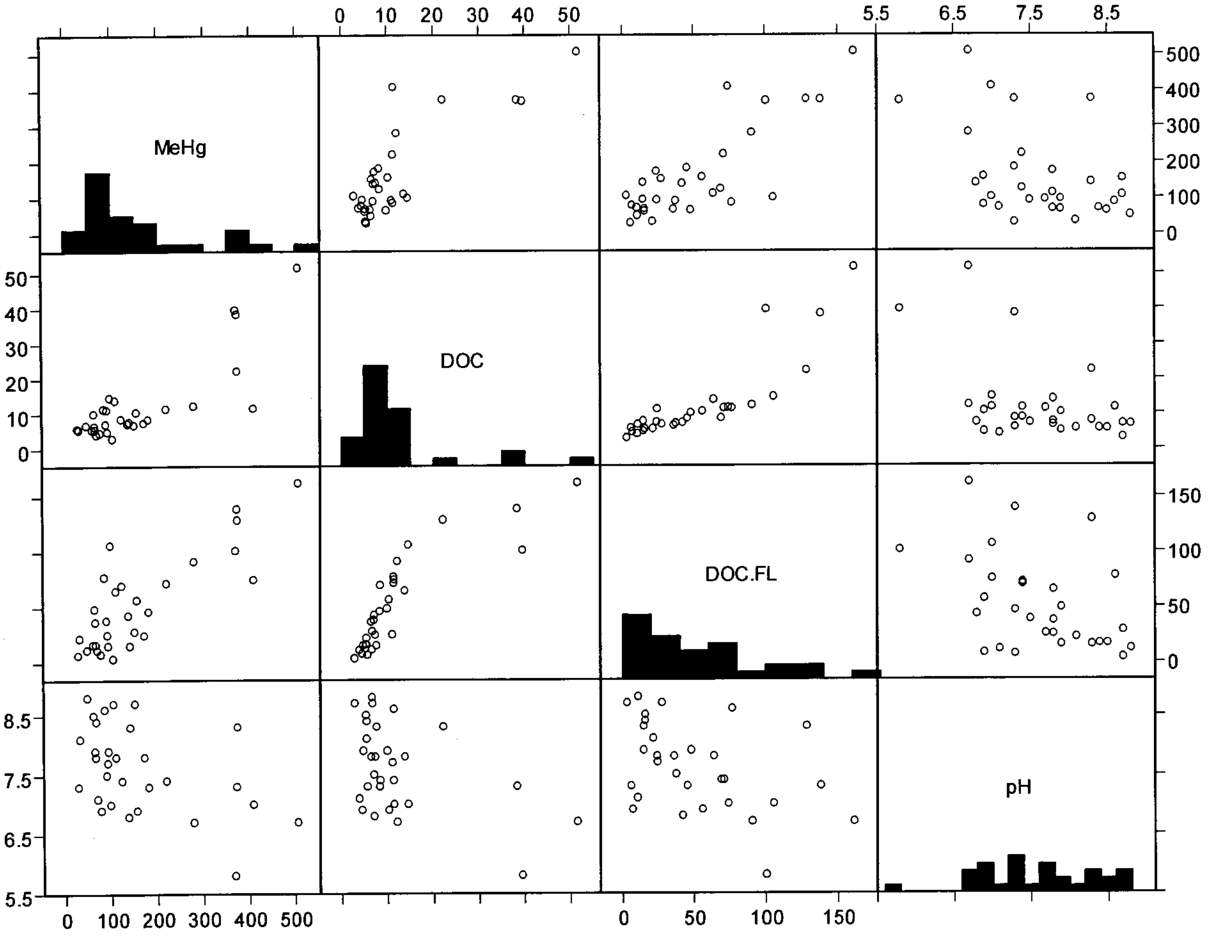
Yan, N.D., W. Keller, N.M. Scully, D.R.S. Lean and P.J. Dillon. 1997. Increased UV-B penetration in a lake owing to drought-induced acidification. Nature 381: 141-143.

Appendix

Supplementary Tables and Statistical Analyses

Table 1. Histograms of the Hill and combined data sets.

a)



b)

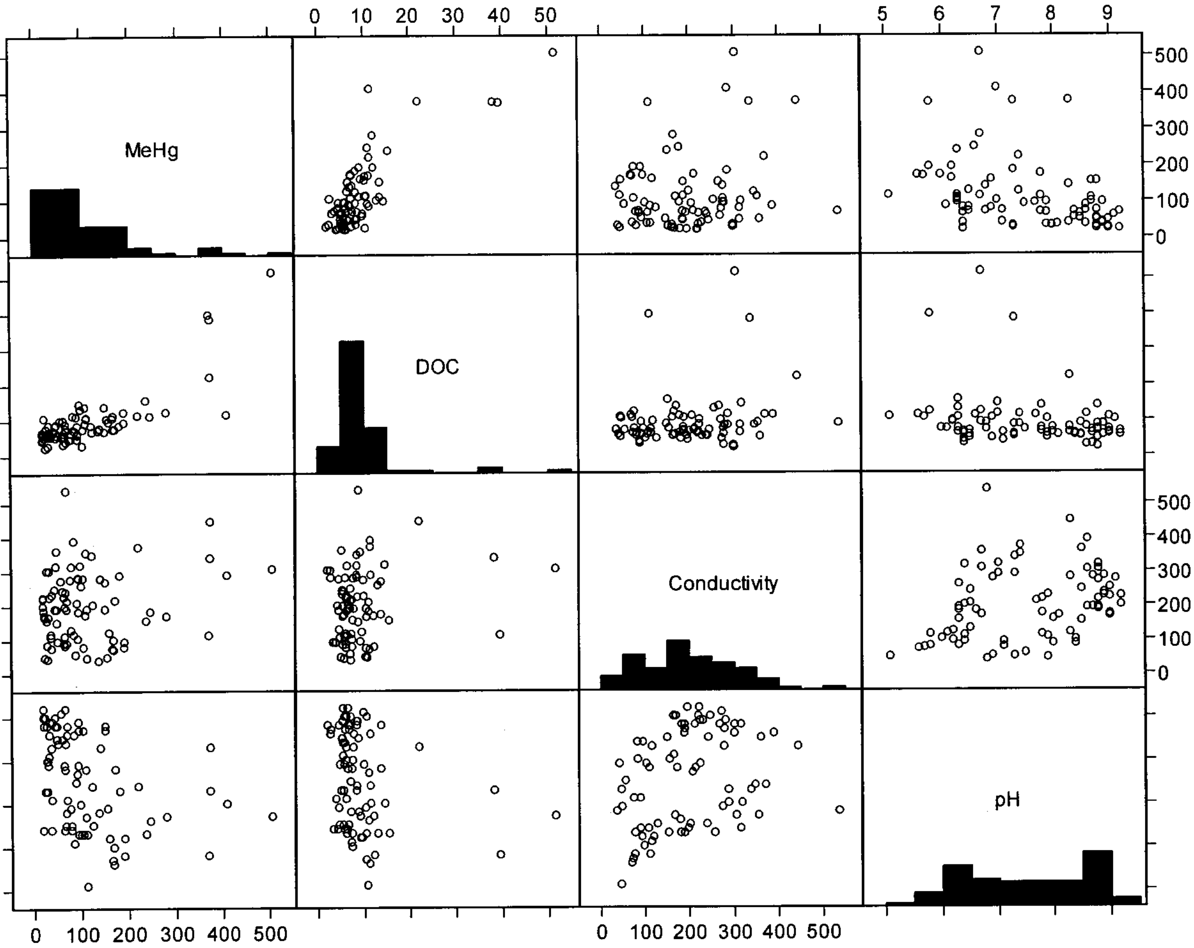

MeHg
DOC
Conductivity
pH
0
10
20
30
40
50
5
6
7
8
9
500
400
300
200
100
0
0
100
200
300
400
500

Table 2. Selected one sample Kolmogorov-Smirnov normality tests for MeHg, DOC, and DOC FL from the Hill Thesis (partitioning), W.E.R.F. and SF data sets, and subdivided by freshwater type. Having a probability of >0.05 does not reject the null hypothesis (~normal distribution).

Variable	Freshwater Designation	Kolmogorov-Smirnov	Probability
MeHg	Lake	0.18	0.79
DOC	Lake	0.18	0.77
DOC FL	Lake	0.18	0.80
MeHg	River	0.36	0.16
DOC	River	0.25	0.56
DOC FL	River	0.17	0.93
MeHg	Wetland	0.16	0.95
DOC	Wetland	0.23	0.65
DOC FL	Wetland	0.15	0.69
MeHg	Hill	0.20	0.35
DOC	Hill	0.32	0.03
DOC FL	Hill	0.15	0.50
MeHg	W.E.R.F.	0.20	0.19
DOC	W.E.R.F.	0.13	0.65
MeHg	S.F.	0.16	0.51
DOC	S.F.	0.12	0.88

Table 3. Mean surface water values are shown for (a) MeHg, DOC and DOC FL. (b) Conductivity (cond.), pH, MeHg:DOC, MeHg:DOC FL and DOC:DOC FL with their standard deviations are also shown. All variables are subdivided into four data sets: Hill Thesis, W.E.R.F. and Sustainable Forestry (S.F.) and the combination of these projects (ALL).

a)

	MeHg pg/L				DOC mg C/L				DOC FL (QSU)			
Data Set	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**
Hill Thesis	159.6	126.5	28.9	506.8	12.1	11.4	2.9	51.6	51.7	42.7	3.4	161.8
W.E.R.F.	47.2	28.9	20.0	151.4	6.8	2.3	2.2	13.8	.	.	.	.
S.F.	121.9	60.2	20.1	246.3	8.6	3.1	3.4	12.3	.	.	.	.
All	110.0	97.1	20.0	506.8	9.3	7.5	2.2	51.6	51.7	42.7	3.4	161.8

b)

	pH				Conductivity (µS/cm)				MeHg:DOC (pg/mg)		MeHg:DOC FL (pg/L/QSU)		DOC FL:DOC (QSU/mg/L)	
Data Set	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Hill Thesis	7.6	0.7	5.8	8.7	219.4	110.5	38.0	446.0	13.2	0.5	3.1	6.9	4.3	6.9
W.E.R.F.	8.6	0.5	7.1	9.2	200.8	83.8	43.3	361.1	6.9	#DIV/0!	.	.	.	.
S.F.	6.2	0.4	5.1	6.8	128.7	51.4	47.0	538.0	14.2	5.5	.	.	.	.
ALL	7.6	1.1	5.1	9.2	198.9	103.6	38.0	538.0	6.0	7.7	3.1	7.6	4.3	7.6

Table 4. Mean values from the Hill Master's Thesis partitioning sites for (a) MeHg, DOC, DOC FL, DOC Abs at 254 nm, and (b) pH, conductivity (cond.), MeHg:DOC, MeHg:DOC FL and DOC:DOC FL along with their standard deviations. Sample sites are subdivided into the freshwater types of lakes, rivers and wetlands.

a)

	MeHg (pg/L)				DOC (mg C/L)				DOC FL (QSU)				DOC Abs	S.D.
Designation	Mean	S.D.	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.	Min	Max	254 nm	
Lakes	90.9	54.9	28.9	172.1	6.8	2.1	4.8	11.1	22.4	9.5	6.4	36.4	0.11	0.04
Rivers	146.4	101.7	85.2	373.4	10.4	6.2	2.9	22.1	60.4	38.9	3.4	128.4	0.46	0.44
Wetlands	277.2	159.7	64.7	506.8	17.5	16.7	8.4	51.6	82.1	42.5	45.8	161.8	0.57	0.63
ALL	166.2	130.6	28.9	506.8	11.3	10.4	2.9	51.6	53.6	40.2	3.4	161.8	0.38	0.48

b)

Designation	pH	S.D.	Min	Max	Cond. (µS/cm)	S.D.	Min	Max	MeHg: DOC pg MeHg/ mg C	S.D.	MeHg: DOC FL pg/L MeHg / QSU	S.D.	DOC FL: DOC QSU / mg C /L	S.D.
Lakes	7.9	0.4	7.3	8.7	166.1	48.5	104.0	215.0	13.3	7.7	4.1	2.3	3.3	1.1
Rivers	7.9	0.7	6.8	8.7	247.5	161.5	38.0	446.0	14.0	9.9	2.4	11.0	5.8	2.2
Wetlands	7.2	0.5	6.7	7.9	275.2	69.9	169.0	372.0	15.8	10.4	3.4	1.4	4.7	1.5
ALL	7.7	0.6	6.7	8.7	230.5	113.4	38.0	446.0	16.3	9.2	4.7	6.5	4.7	2.0

Table 5. Percentages of the unfiltered total methyl mercury (MeHg), dissolved organic carbon (DOC) and DOC fluorescence (FL) with standard deviations (S.D.) are displayed for partitioned freshwater samples at size fractions of 0.2 um, 300 kDa, 30 kDa, 5 kDa and 1 kDa. Data is shown for designations (D) of wetlands (W), rivers (R) and lakes (L).

a)

Study Site	D	Fraction	MeHg %	S.D.	DOC %	DOC FL %	S.D.
Big Rideau	L	SW	100.0	8.3	100.0	100.0	3.4
Big Rideau	L	0.2 micron	36.9	0.1	70.7	88.0	0.0
Big Rideau	L	300 Kda	30.6	10.1	69.1	84.4	2.0
Big Rideau	L	30 Kda	30.0	4.8	46.9	68.8	0.0
Big Rideau	L	5 Kda	24.5	3.7	50.1	68.8	0.0
Big Rideau	L	1 Kda	**24.5**	.	34.5	59.2	34.4
Lac MacGregor	L	SW	100.0	11.7	100.0	100.0	0.0
Lac McGregor	L	0.2 micron	28.2	.	58.6	82.3	0.0
Lac McGregor	L	300 Kda	16.0	46.8	53.3	82.3	0.0
Lac McGregor	L	30 Kda	**16.0**	.	56.8	56.7	2.2
Lac McGregor	L	5 Kda	**16.0**	.	60.2	75.7	4.1
Lac McGregor	L	1 Kda	**16.0**	.	23.0	40.4	7.7
Loch Garry	L	SW	100.0	27.7	100.0	100.0	2.9
Loch Garry	L	0.2 micron	100.0	.	72.5	85.2	5.7
Loch Garry	L	300 Kda	89.5	1.0	72.6	87.9	0.0
Loch Garry	L	30 Kda	80.4	1.3	51.3	78.5	4.3
Loch Garry	L	5 Kda	76.8	5.7	56.7	82.5	2.4
Loch Garry	L	1 Kda	69.2	1.3	39.1	54.2	9.2
Madawaska	L	SW	100.0	2.5	100.0	100.0	17.6
Madawaska	L	0.2 micron	65.2	0.4	79.2	96.3	0.0
Madawaska	L	300 Kda	56.2	5.5	74.0	90.8	5.4
Madawaska	L	30 Kda	**46.3**	7.4	74.0	68.9	2.8
Madawaska	L	5 Kda	**46.3**	.	76.8	67.1	3.8
Madawaska	L	1 Kda	**46.3**	.	53.1	52.4	0.0
Newboro	L	SW	100.0	.	100.0	100.0	14.8
Newboro	L	0.2 micron	**63.0**	.	57.1	100.0	0.0
Newboro	L	300 Kda	63.0	2.0	51.0	91.8	0.0
Newboro	L	30 Kda	70.6	0.0	33.6	80.9	4.2
Newboro	L	5 Kda	6.5	10.9	36.4	74.1	4.8
Newboro	L	1 Kda	**6.5**	1.6	26.6	68.6	18.2
Opinicon	L	SW	100.0	.	100.0	100.0	36.6
Opinicon	L	0.2 micron	77.0	32.0	**100.0**	96.9	0.0
Opinicon	L	300 Kda	60.1	28.1	**100.0**	94.5	2.7
Opinicon	L	30 Kda	47.9	4.6	82.0	72.4	0.9
Opinicon	L	5 Kda	**47.9**	.	77.0	89.3	6.0
Opinicon	L	1 Kda	**47.9**	.	52.8	66.3	2.4
Sharpes Bay	L	SW	100.0	.	100.0	100.0	6.5
Sharpes Bay	L	0.2 micron	**100.0**	9.8	**100.0**	**100.0**	0.0
Sharpes Bay	L	300 Kda	85.6	25.9	**100.0**	**100.0**	0.0
Sharpes Bay	L	30 Kda	74.1	.	**100.0**	68.9	0.0
Sharpes Bay	L	5 Kda	66.5	20.4	70.2	68.9	0.0

b)

Study Site	D	Fraction	MeHg %	S.D.	DOC %	DOC FL %	S.D.
Gatineau	R	SW	100.0	4.6	100.0	100.0	2.2
Gatineau	R	0.2 micron	63.2	9.3	72.7	86.6	0.3
Gatineau	R	300 Kda	59.7	15.0	72.9	84.3	0.0
Gatineau	R	30 Kda	22.1	31.2	24.3	45.6	0.0
Gatineau	R	5 Kda	10.8	56.8	19.4	40.3	0.0
Gatineau	R	1 Kda	**10.8**	.	16.6	35.6	12.5
Ottawa	R	SW	100.0	.	100.0	100.0	0.0
Ottawa	R	0.2 micron	87.2	.	82.8	100.0	0.0
Ottawa	R	300 Kda	87.4	.	55.7	85.9	0.0
Ottawa	R	30 Kda	79.4	.	45.0	61.2	0.0
Ottawa	R	5 Kda	32.4	0.6	50.1	64.7	0.0
Ottawa	R	1 Kda	22.6	23.2	44.5	61.2	0.0
Raisin	R	SW	100.0	3.4	100.0	100.0	0.0
Raisin	R	0.2 micron	43.0	1.5	74.6	99.0	0.0
Raisin	R	300 Kda	38.4	10.1	74.0	99.5	0.7
Raisin	R	30 Kda	35.5	1.7	62.1	99.5	0.7
Raisin	R	5 Kda	35.0	0.7	57.7	92.2	4.0
Raisin	R	1 Kda	24.7	1.7	37.0	73.0	0.0
Rideau	R	SW	100.0	11.6	100.0	100.0	1.2
Rideau	R	0.2 micron	20.9	42.2	97.7	94.8	0.0
Rideau	R	300 Kda	11.0	127.7	97.7	94.8	1.3
Rideau	R	30 Kda	11.2	109.4	79.3	82.7	1.5
Rideau	R	5 Kda	**11.2**	.	71.1	73.2	3.3
Rideau	R	1 Kda	**11.2**	.	53.5	54.2	4.5
South Nation	R	SW	100.0	3.7	100.0	100.0	0.0
South Nation	R	0.2 micron	82.1	9.2	**100.0**	97.9	0.0
South Nation	R	300 Kda	82.1	53.7	94.4	95.9	0.0
South Nation	R	5 Kda	60.3	1.5	65.2	79.3	0.0
South Nation	R	1 Kda	42.5	12.0	.	71.0	0.0
St. Lawrence	R	SW	100.0	3.3	100.0	100.0	11.2
St. Lawrence	R	0.2 micron	67.3	4.3	54.6	72.2	0.0
St. Lawrence	R	300 Kda	83.5	8.8	66.4	88.1	6.4
St. Lawrence	R	30 Kda	70.1	1.0	52.4	52.4	10.7
St. Lawrence	R	5 Kda	51.8	5.7	43.8	72.2	0.0
St. Lawrence	R	1 Kda	51.5	.	45.4	30.6	9.2
Yamaska	R	SW	100.0	3.0	100.0	100.0	2.0
Yamaska	R	0.2 micron	76.0	6.9	88.0	97.6	1.4
Yamaska	R	300 Kda	54.0	9.3	90.8	97.6	1.4
Yamaska	R	30 Kda	41.4	10.0	66.9	81.8	4.2
Yamaska	R	5 Kda	39.5	20.0	64.1	77.4	7.0
Yamaska	R	1 Kda	36.3	7.4	45.8	53.4	2.5

c)

Study Site	D	Fraction	MeHg %	S.D.	DOC %	DOC FL %	S.D.
Baie St. Francois	W	SW	100.0	4.2	100.0	100.0	0.2
Baie St. Francois	W	0.2 micron	81.2	10.8	90.1	100.0	0.5
Baie St. Francois	W	300 Kda	78.4	2.2	87.8	99.8	1.2
Baie St. Francois	W	30 Kda	47.8	0.5	64.5	86.2	1.2
Baie St. Francois	W	5 Kda	44.8	0.6	63.4	83.3	1.7
Baie St. Francois	W	1 Kda	42.1	2.4	45.8	61.3	0.2
Blueberry	W	SW	100.0	.	100.0	100.0	0.0
Blueberry	W	0.2 micron	87.8	1.9	**100.0**	75.3	7.7
Blueberry	W	300 Kda	86.8	.	**100.0**	71.2	0.0
Blueberry	W	30 Kda	64.0	16.7	78.8	**71.2**	0.0
Blueberry	W	5 Kda	**64.0**	.	68.0	**71.2**	1.9
Blueberry	W	1 Kda	48.6	14.0	42.6	**71.2**	0.0
Mississipi	W	SW	100.0	.	100.0	100.0	0.0
Mississipi	W	0.2 micron	**100.0**	1.6	90.8	100.0	0.0
Mississipi	W	300 Kda	98.0	4.8	90.6	98.6	2.0
Mississipi	W	30 Kda	86.1	4.2	65.5	82.8	1.2
Mississipi	W	5 Kda	79.8	2.7	58.8	77.3	1.3
Mississipi	W	1 Kda	**79.8**	.	48.0	61.5	0.0
Odessa	W	SW	100.0	18.1	100.0	100.0	2.0
Odessa	W	0.2 micron	**100.0**	1.3	87.9	99.7	2.9
Odessa	W	300 Kda	**100.0**	1.0	81.4	**100.0**	1.6
Odessa	W	30 Kda	89.1	5.3	67.3	89.7	0.7
Odessa	W	5 Kda	76.3	2.1	42.2	86.5	0.0
Odessa	W	1 Kda	68.1	2.2	35.9	64.1	0.0
Otter	W	SW	100.0	2.6	100.0	100.0	0.0
Otter	W	0.2 micron	**100.0**	1.6	77.7	88.6	18.1
Otter	W	300 Kda	**100.0**	6.8	66.1	88.3	18.7
Otter	W	30 Kda	80.2	2.6	50.1	74.5	4.1
Otter	W	5 Kda	73.9	3.1	49.9	66.5	0.0
Otter	W	1 Kda	70.7	7.8	43.2	53.4	0.0
Vieux Chemin	W	SW	100.0	4.2	100.0	100.0	1.3
Vieux Chemin	W	0.2 micron	95.6	1.3	78.4	98.0	1.6
Vieux Chemin	W	300 Kda	94.7	0.2	78.3	93.7	0.0
Vieux Chemin	W	30 Kda	68.1	5.2	58.6	84.8	3.0
Vieux Chemin	W	5 Kda	81.3	.	54.4	79.4	0.0
Vieux Chemin	W	1 Kda	41.7	0.9	37.9	54.8	1.2

Table 6. Mean spectral data with standard deviations are shown for un-fractionated water samples. Absorbance wavelengths for DOC designated (D) as wetlands (W), rivers (R) and lakes (L) at 254, 280, 285 and 300 nm. Absorbance ratios of 250:365 (E2/E3) and 390:355 as well as the sum of wavelengths between 256-312 (indicative of C=C and C=O content) have also been shown.

		Aromatic Ratio			C=C, C=O		Fulvics		DOC	Fulvics	Fulvics	Particulate
Study Site		**250 (E2)**	**365 (E3)**	**E2/E3**	**Sum (256-312)**	**390**	**355**	**390/355**	**254**	**280**	**285**	**300**
Lakes	Mean	0.106	0.024	6.28	4.58	0.02	0.020	0.825	0.11	0.086	0.077	0.056
	S.D.	0.036	0.021	3.65	1.14	0.01	0.009	0.126	0.04	0.021	0.020	0.015
Rivers	Mean	0.472	0.095	5.75	18.57	0.07	0.109	0.745	0.46	0.343	0.318	0.253
	S.D.	0.454	0.090	2.97	17.98	0.06	0.106	0.250	0.44	0.331	0.309	0.246
Wetlands	Mean	0.585	0.113	6.45	23.01	0.08	0.131	0.947	0.57	0.424	0.393	0.312
	S.D.	0.650	0.136	1.94	26.01	0.09	0.161	1.002	0.63	0.477	0.445	0.360
ALL	Mean	0.585	0.113	6.45	23.01	0.08	0.131	0.947	0.57	0.424	0.393	0.312
	S.D.	0.494	0.101	2.80	19.60	0.07	0.120	0.604	0.48	0.360	0.336	0.271

Table 7. Log-transformed standard fish Hg concentrations from the W.E.R.F. study compared to total aqueous MeHg concentrations from the same study partitioned into high (>30, 5 and 1 kDa) and low (<30, 5 and 1 kDa) molecular weight filtrates using percentages from the Hill thesis.

				30 kDa				5 kDa				1 kDa			
			Standard	Percentage		Magnitude		Percentage		Magnitude		Percentage		Magnitude	
Study Site	D	MeHg pg/L	Fish Hg log[ng/g]	High %	Low %	high pg/L	low pg/L	High %	Low %	high pg/L	low pg/L	High %	Low %	high pg/L	low pg/L
Big Rideau	L	57.6	1.63	70.0	30.0	40.3	17.3	75.5	24.5	43.5	14.1	75.5	24.5	43.5	14.1
Loch Garry	L	71.4	1.63	19.6	80.4	14.0	57.4	23.2	76.8	16.5	54.8	30.8	69.2	22.0	49.4
Newboro	L	70.8	1.54	29.4	70.6	20.8	49.9	93.5	6.5	66.2	4.6	93.5	6.5	66.2	4.6
Opinicon	W	54.1	1.70	52.1	47.9	28.2	25.9	52.1	47.9	28.2	25.9	52.1	47.9	28.2	25.9
Odessa	L	180.0	1.82	10.9	89.1	19.7	160.3	23.7	76.3	42.6	137.4	31.9	68.1	57.5	122.5

Table 8. MeHg concentrations, cumulative UVB levels and % MeHg remaining in each sample with their SD (pH and DGM for some samples). Experiment 1 shows a comparison between spiked and unspiked, ambient and laboratory UVB exposures (Table 14 a), Experiment 2 shows a comparison between spiked and unspiked laboratory UVB exposures with and without Fe2+ and Experiment 3 shows a comparison between spiked and unspiked laboratory UVB exposures with varying pH levels.

Description	Experiment	pH	Cumulative UVB (kJ/m2)	MeHg (ng/L)	SD	MeHg (%)	SD	DGM (pg/L)
0 Ambient	1	7.6	0.00	0.02	0.00	100.0	0.0	.
3 Ambient	1	7.6	0.47	0.02	0.00	73.1	20.3	.
6 Ambient	1	7.6	0.69	0.02	0.00	86.4	11.9	.
0H spike	1	7.6	0.01	4.07	0.08	100.0	2.1	.
3H spike	1	7.6	0.47	4.11	0.02	101.0	0.4	.
6H spike	1	7.6	0.69	4.05	0.05	99.4	1.2	.
0H Dark Spike	1	7.6	0.00	4.07	0.00	100.0	0.0	.
3H Dark Spike	1	7.6	0.00	4.45	0.00	109.3	0.0	.
6H Dark Spike	1	7.6	0.00	4.65	0.00	114.2	0.0	.
0H DI Spike	1	7.6	0.00	4.36	0.00	100.0	1.8	.
3H DI Spike	1	7.6	0.47	3.91	0.00	89.8	0.0	.
6H DI Spike	1	7.6	0.69	4.02	0.00	92.2	0.0	.
0H spike	2	7.6	0.0	5.28	0.02	100.0	0.3	.
3H spike	2	7.6	7.2	4.24	0.53	80.3	10.0	.
6H spike	2	7.6	13.8	3.65	0.96	69.2	18.1	.
0H Spike + Fe	2	7.6	0.0	5.67	0.31	100.0	5.4	.
3H Spike + Fe	2	7.6	7.2	5.03	0.27	88.6	4.7	.
6H Spike + Fe	2	7.6	13.8	4.71	0.26	83.0	4.5	.
0H Dark Spike + Fe	2	7.6	0.0	5.67	0.31	100.0	5.4	.
3H Dark Spike + Fe	2	7.6	0.0	5.55	0.17	97.8	3.0	.
6H Dark Spike + Fe	2	7.6	0.0	5.29	0.03	93.2	0.6	.
0H DI Spike + Fe	2	7.6	0.0	5.59	0.00	100.0	0.0	.
3H DI Spike + Fe	2	7.6	7.2	5.06	0.00	90.6	0.0	.
6H DI Spike + Fe	2	7.6	13.8	5.02	0.00	89.9	0.0	.
0H spike	3	3	0.00	4.78	0.04	100.0	0.8	56.0
3H spike	3	3	7.18	3.04	0.21	62.5	4.5	247.0
6H spike	3	3	13.80	2.82	0.12	58.3	2.5	224.7
0H spike	3	5	0.00	4.74	0.03	100.0	0.6	33.9
3H spike	3	5	7.18	3.52	0.23	72.9	4.7	744.2
6H spike	3	5	13.80	3.13	0.32	64.6	6.6	343.5
3H Dark Control	3	7.6	0.00	4.80	0.07	100.0	1.4	.
6H Dark Control	3	7.6	0.00	4.80	0.08	100.0	1.6	.

Table 9. One sided two sample t-tests shown for (a) concentrations and (b) percentages at different fractions for partitioning data set of Hill Thesis (1 sided t-tests).

a)

		MeHg			DOC			DOC FL			Abs (254)		
		W	R	L	W	R	L	W	R	L	W	R	L
SW	W	.	t=1.7902	t=2.9105	.	t=1.0439	t=1.689	.	t=0.9608	t=3.6362	.	t=0.5978	t=1.496
	R	p=0.051	.	t=-1.271	p=0.1595	.	t=1.4637	p=0.1786	.	t=2.5111	p=0.2816	.	t=2.3037
	L	p=0.007	p=0.1139	.	p=0.0597	p=0.0845	.	p=0.002	p=0.0137	.	p=0.0844	p=0.0234	.
0.2 um	W	.	t=3.4107	t=3.4499	.	t=1.0727	t=1.6147	.	t=0.8231	t=4.5863	.	t=0.8616	t=1.3009
	R	p=0.003	.	t=0.5471	p=0.1532	.	t=1.7283	p=0.2134	.	t=2.4841	p=0.2045	.	t=2.3416
	L	p=0.003	p=0.2972	.	p=0.0673	p=0.0548	.	p=0.0004	p=0.0144	.	p=0.1128	p=0.022	.
300 kDa	W	.	t=3.0744	t=3.9592	.	t=1.0715	t=1.5769	.	t=0.7923	t=4.8212	.	t=0.8766	t=1.2943
	R	p=0.005	.	t=1.8195	p=0.1535	.	t=1.6546	p=0.2225	.	t=2.443	p=0.2006	.	t=2.3482
	L	p=0.0011	p=0.0469	.	p=0.0716	p=0.062	.	p=0.0003	p=0.0155	.	p=0.1139	p=0.0217	.
30 kDa	W	.	t=2.9051	t=3.5572	.	t=1.0724	t=1.609	.	t=1.0366	t=2.469	.	t=0.8299	t=1.4031
	R	p=0.0079	.	t=0.7299	p=0.1544	.	t=1.0535	p=0.1622	.	t=1.8979	p=0.214	.	t=1.5963
	L	p=0.0022	p=0.2404	.	p=0.068	p=0.1574	.	p=0.0156	p=0.0421	.	p=0.0971	p=0.0745	.
5 kDa	W	.	t=2.6161	t=3.4142	.	t=0.9754	t=1.5452	.	t=1.1445	t=2.22	.	t=0.8544	t=1.4576
	R	p=0.0129	.	t=1.3563	p=0.1752	.	t=1.3531	p=0.1384	.	t=2.0241	p=0.2064	.	t=1.8937
	L	p=0.0033	p=0.1	.	p=0.0753	p=0.1005	.	p=0.0242	p=0.0329	.	p=0.0895	p=0.0454	.
1 kDa	W	.	t=3.0699	t=3.7629	.	t=0.9614	t=1.6886	.	t=1.0605	t=1.856	.	t=0.8775	t=1.3359
	R	p=0.0053	.	t=1.0225	p=0.1795	.	t=2.2969	p=0.1558	.	t=1.821	p=0.2004	.	t=1.703
	L	p=0.0016	p=0.1653	.	p=0.0611	p=0.0222	.	p=0.0466	p=0.0479	.	p=0.1092	p=0.0635	.

b)

		MeHg			DOC			DOC FL			Abs (254)		
		W	R	L	W	R	L	W	R	L	W	R	L
0.2 um	W	.	t=3.097	t=2.264	.	t=0.8349	t=1.3465	.	t=0.1822	t=0.1923	.	t=1.2235	t=0.4362
	R	p=0.102	.	t=0.3163	p=0.4216	.	t=0.5137	p=0.8587	.	t=0.0191	p=0.267	.	t=1.1067
	L	p=0.0448	p=0.7572	.	p=0.2052	p=0.6168	.	p=0.851	p=0.985	.	p=0.6779	p=0.3108	.
300 kDa	W	.	t=2.8075	t=3.1137	.	t=0.6687	t=1.0599	.	t=0.0723	t=0.3474	.	t=1.4821	t=0.3822
	R	p=0.017	.	t=0.1474	p=0.5175	.	t=0.4752	p=0.9436	.	t=0.6297	p=0.1888	.	t=1.5474
	L	p=0.0099	p=0.8852	.	p=0.3119	p=0.6432	.	p=0.7349	p=0.5407	.	p=0.7135	p=0.1727	.
30 kDa	W	.	t=2.318	t=1.7725	.	t=1.0447	t=0.0601	.	t=1.2287	t=2.5628	.	t=2.4736	t=0.1796
	R	p=0.0429	.	t=0.6328	p=0.3207	.	t=0.7193	p=0.2473	.	t=0.0241	p=0.0563	.	t=3.8186
	L	p=0.104	p=0.5398	.	p=0.9531	p=0.4869	.	p=0.0264	p=0.9812	.	p=0.8634	p=0.0124	.
5 kDa	W	.	t=3.8436	t=2.466	.	t=0.3816	t=0.697	.	t=0.8388	t=0.4952	.	t=2.1486	t=0.4486
	R	p=0.0027	.	t=0.5119	p=0.71	.	t=0.9175	p=0.4194	.	t=0.5652	p=0.0752	.	t=2.9485
	L	p=0.0313	p=0.618	.	p=0.5002	p=0.3769	.	p=0.6302	p=0.5824	.	p=0.6695	p=0.0257	.
1 kDa	W	.	t=3.3787	t=2.0051	.	t=0.3177	t=0.7356	.	t=0.9672	t=0.8392	.	t=2.023	t=0.202
	R	p=0.0062	.	t=0.6021	p=0.7572	.	t=0.3129	p=0.3542	.	t=0.3514	p=0.0895	.	t=2.2871
	L	p=0.0728	p=0.5593	.	p=0.4789	p=0.7608	.	p=0.4209	p=0.7319	.	p=0.8479	p=0.0709	.

Table 10. Data set comparisons (Hill, W.E.R.F. and S.F.) with two sample t-tests with p and t values shown by water chemistry variable.

	MeHg			DOC		
	Hill	**WERF**	**SF**	**Hill**	**WERF**	**SF**
Hill	.	t=4.5911	t=1.3167	.	t=2.404	t=2.4026
WERF	p=0	.	t=5.8099	p=0.0195	.	t=1.4209
SF	p=0.1938	p=0	.	p=0.1654	p=0.0201	.

	pH			Conductivity		
	Hill	**WERF**	**SF**	**Hill**	**WERF**	**SF**
Hill	.	t=-5.8695	t=8.683	.	t=0.7158	t=1.553
WERF	p=0	.	t=-41.67	p=0.4772	.	t=1.0804
SF	p=0	p=0	.	p=0.1267	p=0.2852	.

Table 11. Linear Regressions for selected tests (only significant values shown).

		r^2, df	r^2, df	r^2, df
Designation		**y MeHg x DOC**	**y MeHg x DOC FL**	**y MeHg x DOC Abs**
Total DS	SW	0.63, 19	.	.
Part.	SW	0.67, 28	0.66, 28	.
Part.	0.2 μm	0.43, 18	0.39, 18	.
Part.	300 kDa	0.46, 18	0.39, 18	.
Part.	30 kDa	0.45, 17	0.53, 17	.
Part.	5 kDa	.	0.28, 17	.
Part.	1 kDa	0.54, 16	0.55, 17	.
Wetlands	SW		.	.
Wetlands	0.2 μm	0.61, 4	0.55, 4	.
Wetlands	300 kDa	.	.	.
Wetlands	30 kDa	.	0.52, 4	.
Wetlands	5 kDa	.	.	.
Wetlands	1 kDa	.	0.69, 4	0.66, 4
Rivers	SW	.	.	.
Rivers	0.2 μm	.	.	.
Rivers	300 kDa	.	.	.
Rivers	30 kDa	.	.	.
Rivers	5 kDa	0.45, 5	0.52, 5	0.5, 5
Rivers	1 kDa	.	.	.
Lakes	SW	.	.	.
Lakes	0.2 μm	.	.	.
Lakes	300 kDa	.	.	.
Lakes	30 kDa	.	.	.
Lakes	5 kDa	0.61, 5	0.63, 5	.
Lakes	1 kDa	.	.	.