**University of Alberta**

# Influence of forest canopies on the deposition of methylmercury to boreal ecosystem watersheds

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Ecology

Department of Biological Sciences

Fall 2010
Edmonton, Alberta

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***To my Mom,***

*who instilled in me the faith*
*that one can accomplish anything they set their heart on,*
*and whose ever giving spirit is thoroughly missed*

~

# *Abstract*

Methylmercury (MeHg) is a potent vertebrate neurotoxin and a contaminant of global concern. Increased anthropogenic emissions of mercury (Hg) to the atmosphere have led to increased bioaccumulation of MeHg in top predatory organisms such as fish, the consumption of which is the main exposure pathway of this toxin to humans and other animals. Forest canopies significantly increase the deposition of Hg in general to watersheds, but sources and fates of MeHg deposition in particular remain poorly understood. In this study, wet and dry loadings of MeHg to a watershed were quantified, and the retention and (photo)reduction of MeHg on foliage were measured using unique stable isotope experiments. We found that traditional methods of quantifying net deposition underestimate incoming sources of MeHg, and that retention of MeHg on forest canopies results in delayed transport of a significant portion of newly deposited MeHg from terrestrial catchments into adjacent lakes.

# *Acknowledgements*

First and foremost I wish to thank my supervisor, Dr. Vincent St. Louis, for the opportunity to take part in the Mercury Experiment To Assess Atmospheric Loadings in Canada and the US (METAALICUS), one of the largest and most comprehensive whole-ecosystem studies to date. I consider it a great privilege to have spent several summers conducting research with both the METAALICUS team and the larger Experimental Lakes Area (ELA) community. This experience has greatly increased my breadth of knowledge and understanding of both ecology and the scientific process. These summers will long be remembered as times of both personal and professional growth, and some of the best times of my life. Additionally, I am grateful for Vince's guidance and dedication to producing top quality research and publishable results. He is to be commended for the time and commitment he provides to ensuring the success of each of his students. I would also like to thank Dr. J.C. Cahill and Dr. J.D. Wilson of my supervisory committee for their time and encouragement through all stages of my research program.

My lab mates have also been critical to the success of this project and I appreciate the time and effort they have put forth on my behalf. Dr. Jennifer Graydon is owed an enormous debt of gratitude for the insights and guidance provided throughout my graduate program, and for always being willing to share her thoughts and expertise on the dynamics of mercury cycling within the boreal forest. Many thanks are also extended to Igor Lehnherr for providing advice and helpful comments with regards to all aspects of my research program but especially for those relating to lab work and data analyses. His help with the preparation and application of mercury spikes is also appreciated. Last, but certainly not least, Dr. Jane Kirk has been an excellent mentor, acting as both a source of inspiration and a great friend.

I am additionally thankful for the analytical assistance of Sara Berkel, Po Yee Chan, Megan Hawkins and Caroline Lee, the field support of Ken Sandilands and Michael Tate, and the statistical input of Dr. Jens Roland and Alana Clason.

GIS figures are the result of much appreciated time and effort from Charlene Neilson and Craig Emmerton. Many thanks are also owed to support staff in the 3rd and 4th floor offices that make the path to success a lot easier to navigate.

Over the course of my studies I am fortunate to have met many helpful, inspiring and motivating individuals. In particular, I would like to acknowledge the love and support of the Raab and Hrenchuk families who always made me feel welcome and at home on trips to and from the field and during much needed vacations. They have surely influenced me in more ways than they realise. I would especially like to thank Lee Hrenchuk for always lending an ear, initiating dance parties and reminding me that science and art can exist in unison. To Dustin Raab, thank you for completing this journey with me – through good times and bad, your unwavering support and delicious dinners (not to mention breakfasts, lunches, and COFFEE) made this all the more enjoyable.

I am grateful for my friends and family who have been a constant source of support and, often times, a much needed break from academic life. It is in large part due to their continued encouragement, patience, and general interest with regards to this endeavor that I was able to complete my studies - I hope that you are all proud.

Funding for this research was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Circumpolar Institute. Personal funding was provided by NSERC, and the University of Alberta.

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# *Chapter 1. General Introduction*

## Mercury as a global pollutant

Mercury (Hg) is a naturally occurring heavy metal that is mobilized in the environment from both natural (e.g., volcanic eruptions) and anthropogenic (e.g., industrial combustion and mining) sources, the latter having greatly increased since the onset of industrialization (Swain et al., 1992). While some uncertainty still exists as to the full extent to which humans have impacted the global cycling of Hg (Lindberg et al., 2007), it is well established that increased atmospheric emissions have resulted in increased Hg deposition of up to 10 fold in industrialized regions, and 3-5 fold in more remote regions, compared to pre-industrial times (Munthe et al., 2007; Selin, 2009). Increased deposition of Hg is a concern as, despite the fact that it is naturally occurring, its unique physico-chemical and bioaccumulative properties make Hg a persistent and highly toxic contaminant. For these reasons, Hg is one of the most commonly studied trace elements in the environment (Morel et al., 1998).

Of primary concern is the bioaccumulation and biomagnification of the methylated and neurotoxic form of Hg, methylmercury (MeHg), through food webs to potentially toxic levels in top predatory organisms, especially humans. Consumption of contaminated fish is the most significant source of MeHg exposure for humans (Inskip and Piotrowski, 1985; Fitzgerald and Clarkson, 1991; Fitzgerald et al., 1998; Hightower and Moore, 2003; Mergler et al., 2007 and references therein). Fish and wildlife Hg advisories in Canada and the United States (U.S.) have been steadily increasing and MeHg is consistently responsible for the largest number of fish consumption advisories for North American freshwaters of any contaminant (EPA fact sheet, 2009; Ontario Ministry for the Environment, 2009). While analytical limitations have previously made it difficult to prove a direct relationship between atmospheric Hg deposition rates and MeHg concentrations in fish (Hintelmann et al., 2002), it is now generally accepted that the increased deposition of Hg released from human activities is the primary cause of this rise in MeHg contamination (Harris et al., 2007).

Several sources of MeHg to lakes exist, including direct atmospheric deposition, runoff from terrestrial catchments, and in-lake production. The relative importance of these sources relies on a number of different factors (Rudd, 1995; Munthe et al., 2007). While our understanding of the Hg biogeochemical cycle has vastly improved over the last few decades, the ability of Hg to readily undergo chemical transformations under normal environmental conditions makes the biogeochemical cycling of Hg within and between environmental compartments incredibly complex, and as a result, many uncertainties still exist (Downs et al., 1998; Grigal 2002; Lindberg et al., 2007; Selin, 2009). Due to its physical and chemical features, the atmosphere provides the primary medium for Hg mobilization and exchange in the environment (Lindberg et al., 2007), and affects all three of the above-mentioned sources.

Three main forms of Hg exist in the environment: gaseous elemental Hg (Hg(0)), inorganic divalent Hg (Hg(II)), and organomercury (namely MeHg but also dimethyl DMHg). While the non-methylated forms are more abundant, less toxic, and more commonly associated with anthropogenic emissions than methylated Hg (Downs et al., 1998 and references therein), all forms of Hg have the potential to be converted to MeHg via natural processes in the environment. In the atmosphere, Hg(0) comprises approximately 98% of all the Hg species (Downs et al., 1998). Gaseous Hg(II) (operationally defined as reactive gaseous Hg, RGM) and particulate bound Hg (p-Hg) comprise the bulk of the rest of the atmospheric Hg budget (Downs et al., 1998). DMHg and MeHg are also known to exist in the atmosphere but are present in much smaller quantities in comparison to the other species (Lamborg et al., 1995; Lee et al., 2003), and their sources are not yet well understood (Lindberg et al., 1991; Hall et al., 1995; Bloom et al., 1996; Mester and Sturgeon, 2002; Gårdfeldt et al., 2003; St. Louis., et al., 2005; Hammerschmidt et al., 2007). The atmospheric fate of Hg, especially deposition, is strongly linked to its speciation in the atmosphere (Lindberg et al., 2007).

## Influence of the forest canopy on Hg deposition

One of the greatest uncertainties in our understanding of the movement of Hg from the atmosphere to the biosphere, and ultimately into the food-web, involves the effects of terrestrial plants on the deposition and migration of Hg through the terrestrial compartments of an ecosystem. Atmospheric Hg is deposited to the landscape via both wet and dry deposition processes (Bloom and Watras, 1989). Deposition of Hg to open areas, including lake surfaces, is governed primarily by wet deposition, whereas, deposition to terrestrial compartments of watersheds is affected by both wet and dry sources. Due to its high vapor pressure and low oxidation potential, Hg(0) is quite stable and has a relatively long atmospheric residence time of 6 to 12 months. This long atmospheric residence time allows Hg(0) to be widely dispersed from point sources to remote regions, making it a truly global pollutant (Fitzgerald et al., 1998; Schroeder and Munthe 1998; Downs et al., 1998; Lindberg et al., 2007). Ionic Hg species and p-Hg, are more soluble and have higher deposition velocities than Hg(0), resulting in much shorter atmospheric residence times than Hg(0) (Downs et al., 1998). As a result, these ionic Hg species are the main ones deposited to both terrestrial and aquatic ecosystems in precipitation (Fitzgerald and Mason, 1996). Conversely, Hg(0) dominates the dry deposition flux of Hg to terrestrial landscapes, which also includes deposition of RGM and, to a lesser extent, p-Hg, as it is both oxidized on the surface of wet foliage to Hg(II) and taken up directly by foliage via stomata (Iverfeldt, 1991; Munthe et al., 1995; Lindberg, 1996; Frescholtz et al., 2003; Ericksen et al., 2003; Millhollen et al., 2006). The combined effect of wet and dry deposition to forested sites results in much higher loadings of Hg to terrestrial compartments of watersheds in comparison to their aquatic counterparts in the open (Grigal, 2002).

As with other forms of Hg, MeHg is deposited in precipitation and experiences enhanced loadings at forested sites. While this is now well documented in the literature, the sources of MeHg and the implications of this enhanced deposition on fish MeHg levels are poorly understood in comparison to other aspects of the Hg biogeochemical cycle. Higher loadings of MeHg at forested sites are primarily the result of MeHg deposition in litterfall. It has been suggested that dry deposition of MeHg, primarily particulate bound MeHg (p-MeHg), is the source of this deposition although, as of yet, it has not been measured directly and remains purely speculative (Hultberg et al., 1994; Munthe et al., 1995). In-canopy abiotic methylation of Hg(II) on and within canopy foliage have also been proposed as potential sources of MeHg to terrestrial sites (Munthe et al., 1995; Rudd et al., 1995), although, a recent enriched Hg isotope study could account for only a small fraction of the MeHg in thoughfall (precipitation passing through the canopy) via this process (Graydon et al., 2008). Soil contributions and root uptake of MeHg are thought to be minimal outside of heavily contaminated areas (Lindberg et al., 1979; Godbold and Hüttermann, 1988; Bishop et al., 1998).

## Mercury Experiment to Assess Atmospheric Loading in Canada and the U.S. (METAALICUS)

Our general understanding of Hg biogeochemical cycling and the relationship between atmospheric Hg deposition and MeHg concentrations in fish has recently been improved by the use of enriched stable Hg isotopes. Enriched stable Hg isotope tracers alter the abundance ratios of natural isotope fractionation in the environment, allowing the experimentally applied Hg to be quantifiably distinguished from already present “ambient” Hg. As a result, enriched stable Hg isotopes can be used to track Hg over time as it moves through different ecosystem compartments and undergoes transformations in the biogeochemical cycle (Hintelmann et al., 2002).

METAALICUS is the largest project to date making use of enriched stable Hg isotope loading. Stable Hg isotopes were experimentally applied at the whole ecosystem level to simulate the impact of Hg contaminated deposition on biogeochemical processes leading to elevated MeHg concentrations in fish. Application of isotopes at this scale is necessary because Hg is transported through ecosystem compartments in many complex, interconnected, and poorly understood pathways, limiting the real-world predictive value of laboratory and mesocosm-scale experiments (Harris et al., 2007). Different isotopic Hg spikes were loaded onto the wetland ($^{198}$Hg), upland ($^{200}$Hg), and lake surface ($^{202}$Hg) compartments of the watershed because each of these compartments was expected to respond differently to Hg loadings. Addition of the different Hg isotopes allowed researchers to investigate how route of entry affects MeHg accumulation in fish. Experimental loading of Hg increased the atmospheric Hg load to the watershed by approximately six times, simulating a deposition rate similar to that experienced in more polluted regions such as the northeastern U.S. and Europe (Harris et al., 2007).

Early results from METAALICUS have already benefitted our understanding of Hg biogeochemical cycling. For example, within a month of addition, “spike” $^{202}$Hg(II) added directly to the lake had already been methylated and Me$^{202}$Hg had entered the food-web; after two months, it was found in several fish species. Contrastingly, although significant amounts of Hg were exported from the upland and wetland compartments, less than 1% was spike Hg. After 3 years of loading, $^{200}$Hg(II) remained equally distributed between vegetation and soils in the upland and the majority of $^{198}$Hg(II) remained bound to vegetation in the wetland (Harris et al., 2007).

In addition to being valuable at the whole-ecosystem scale, enriched stable isotopes can also be used for mechanistic studies of Hg cycling at a smaller and more controlled scale. For example, in a microcatchment study at the ELA, different enriched stable Hg(II) isotope spikes were applied sequentially over four years to ground vegetation to examine retention of Hg(II) in soil and vegetation following deposition (Hintelmann et al., 2002; Graydon et al., 2009). In a similar study, Graydon et al. (2006) applied spike Hg(II) to canopy foliage to monitor mechanisms of loss and retention following deposition. Furthermore, enriched Hg isotope experiments are not limited to Hg(II) and have also been used for mechanistic studies of MeHg behavior. For example, Lehnherr and St. Louis (2009) recently demonstrated that ultraviolet radiation is the primary mediator of MeHg photodemethylation in freshwater lakes, an important biogeochemical sink that limits the availability of MeHg to lake biota. Using the enriched MeHg isotopes, they were also able to show that this process was the same for both spike and ambient MeHg.

This thesis presents research conducted within the framework of the METAALICUS project, with the objective to further support studies examining the influence of terrestrial vegetation on deposition and retention of atmospheric Hg within boreal forest watersheds, specifically, the role of MeHg within these processes. In addition to measuring incoming sources of MeHg, unique enriched stable isotope experiments were designed to examine the fate of MeHg following deposition.

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# *Chapter 2. Sources and fates of methylmercury deposited to forest canopies*

## Introduction

Methylmercury (MeHg) is a potent vertebrate neurotoxin and a contaminant of global concern. Several recent studies, including the whole-ecosystem Mercury Experiment To Assess Atmospheric Loadings In Canada and the US (METAALICUS) at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada, have demonstrated that increased anthropogenic emissions of mercury (Hg) to the atmosphere have led to increased bioaccumulation of MeHg in top predatory organisms such as fish (Harris et al., 2007). While deposition of inorganic Hg(II) and subsequent microbial methylation in anaerobic lake sediments (Gilmour et al., 1992) and wetlands (St. Louis et al., 1994), for example, is likely the main source of MeHg to food-webs, it has been suggested that direct atmospheric deposition of MeHg and run-off from terrestrial compartments of the watershed might also be important sources of MeHg in fish (Hultberg et al., 1994; Rudd 1995).

Terrestrial compartments of watersheds typically receive more Hg from the atmosphere than do their aquatic counterparts due to the relatively large amount of surface area available for dry deposition and efficient scavenging of atmospheric Hg by foliage in forest canopies (Iverfeldt, 1991; Lindberg et al., 1992; Hanson et al., 1995; Munthe et al., 1995a; Lindberg, 1996; Ericksen et al., 2003; Milhollen et al., 2006). Dry deposition is thought to represent an equivalent, if not larger, source of Hg to watersheds than wet deposition (Munthe et al., 2004). However, to date, there have been no studies simultaneously measuring both wet and dry deposition to allow for direct comparison, and the net effect of the canopy on Hg deposition is often determined by calculating the sum of throughfall (precipitation passing through the canopy) and litterfall (senescent foliage) loadings and subtracting loadings from open area precipitation (Lindberg et al., 2007). Recent research demonstrating photoreduction and emission to the atmosphere of a portion of newly wet-deposited Hg from foliage suggests that this indirect mathematical approach is associated with large uncertainties and is likely to underestimate the contribution of the canopy and dry deposition to total loadings of Hg to watersheds (Graydon et al., 2006; Lindberg et al., 1998). While forest canopies significantly increase the deposition of all forms of Hg (total Hg, THg) to watersheds, sources and fates of MeHg deposition in particular remain poorly understood (Schroeder and Munthe, 1998).

Biogeochemical cycling of Hg has been studied for a number of years at the ELA. Past whole ecosystem experiments there (Hall et al., 2005; Harris et al., 2007) have required concurrent measurements of net Hg loadings in this region. As a result, quantification of wet deposition of THg and MeHg in open areas has been on-going at the ELA since 1992 and was expanded in 1998 to include measurements of THg and MeHg under forest canopies for estimation of dry deposition (St. Louis et al., 2001), yielding the most long-term record of Hg deposition of any remote site world-wide (St. Louis et al., 1995; Graydon et al., 2008). The ELA has historically received low atmospheric loadings of both THg (36 ± 17 mg $ha^{-1}$ $yr^{-1}$) and MeHg (0.5 ± 0.2 mg $ha^{-1}$ $yr^{-1}$) in open area precipitation, however, as with other sites, elevated deposition of both THg and MeHg under forest canopies suggests that dry deposition is an important source of Hg to the region (Graydon et al., 2008),

In this study, we continued to quantify wet and dry loadings of MeHg and THg at the ELA to examine the influence of terrestrial vegetation on deposition and retention of atmospheric MeHg in comparison to THg. In addition to estimating dry deposition using the indirect mathematical approach described above, we attempted for the first time to measure particulate bound MeHg (p-MeHg) in the atmosphere using a high volume air filtering technique. In addition, we used enriched stable Hg isotope experiments to examine the fate of MeHg on canopy foliage following deposition.

## Methods

### *General site description*

Our research was conducted in a number of different watersheds throughout the ELA in the remote Boreal ecoregion of northwestern Ontario, Canada (Figure 2.1). This area is underlain by Precambrian Shield geology with upland forests dominated by stands of jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), and white birch (*Betula papyrifera*) of varying age due to fire succession. Wetland regions typically contain mixed black spruce, jack pine, and tamarack (*Larix laricina*) canopies with alder (*Alnus rugosa*) shrub understory.

### *Sources of MeHg to canopy foliage*

#### *Concentrations and annual loadings of MeHg and THg in open area precipitation and throughfall*

Precipitation and throughfall samples were collected during the ice-free season (May through October) in 2007 using automated wet deposition collectors (Aerochem Metrics, Figure 2.2). One collector was installed in an open area on a peninsula in the METAALICUS watershed. Throughfall collectors were deployed under an upland deciduous red maple/birch canopy, an upland old growth balsam fir/black spruce canopy, an upland fire regenerated jack pine canopy, and a wetland black spruce/alder canopy in the METAALICUS watershed. We duplicated collectors under the old growth and fire regenerated canopies for a total of six throughfall collection sites (Figure 2.3).

In each automated collector, one acid-washed glass funnel (15 cm diameter) was attached via a piece of Teflon tube and Teflon compression fitting to a closed acid-washed 2-L Teflon jar containing 1 mL of concentrated trace-metal grade (TMG) HCl to keep deposited Hg in solution. An acid-washed, circular Teflon grid placed at the base of the funnel filtered out coarse debris. Samples were collected following a minimum deposition of 20-30 mm, allowing sufficient volume for both MeHg and THg analyses. As a result, samples were sometimes a composite of 2-3 smaller deposition events. Following collection,

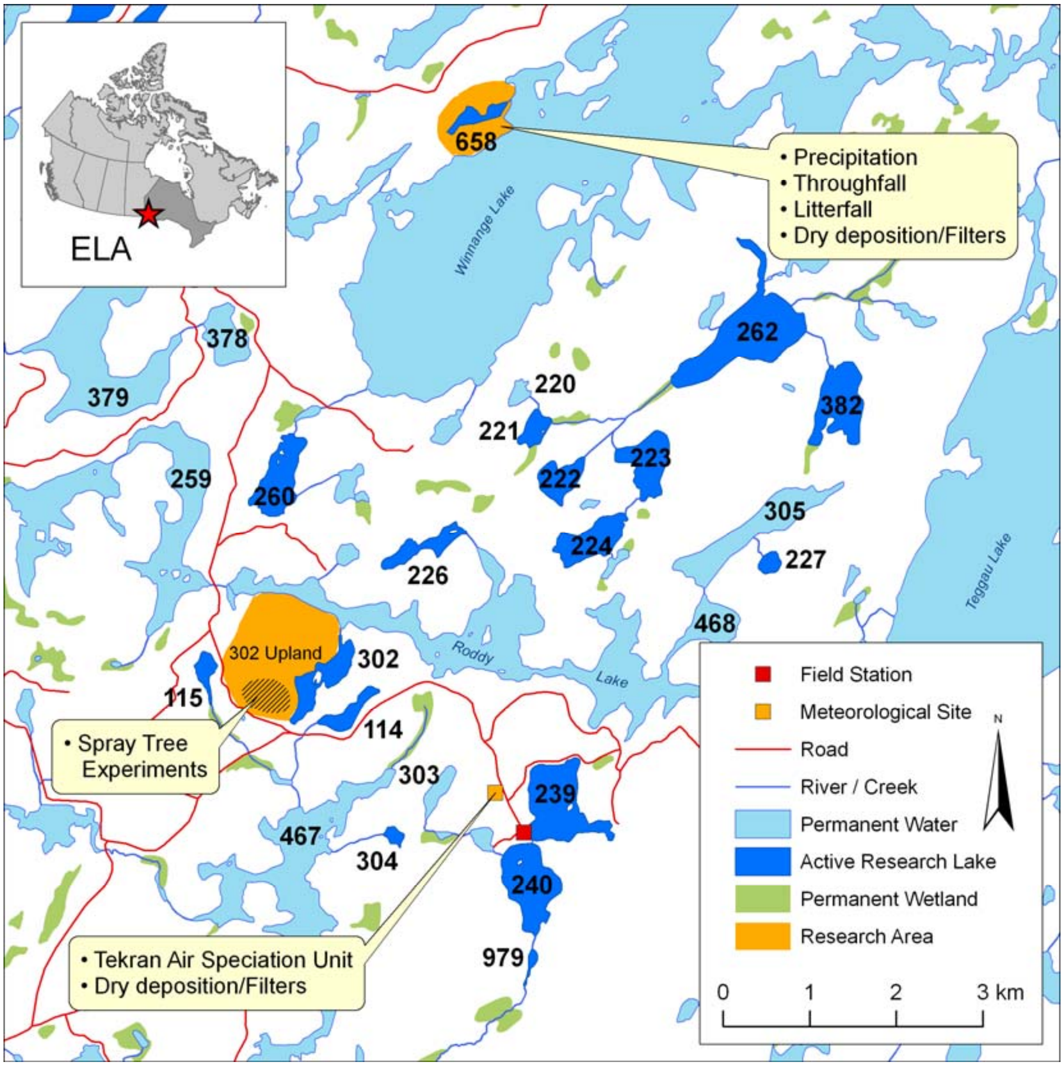

Figure 2.1 Map of the Experimental Lakes Area (ELA, NW Ontario, Canada) highlighting specific areas where research and sampling activities were conducted for this study.

Figure 2.2 Automated wet deposition collectors (Aerochem Metrics) situated in the open (left panel) and under the forest canopy (upland jack pine; right panel) in the METAALICUS watershed.

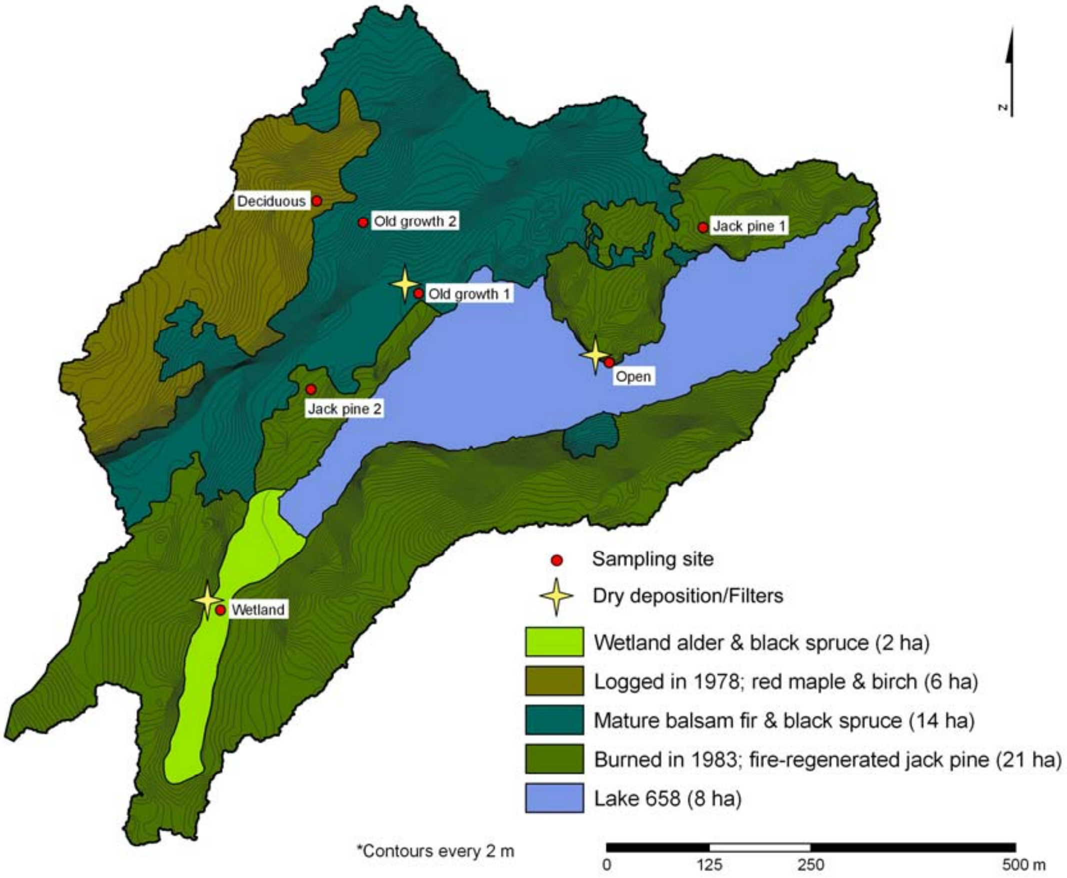

Figure 2.3 Distribution of the four general forest types and all sampling sites within the METAALICUS watershed. Each sampling site represents the location of an automated open precipitation or throughfall collector and, with the exception of the open site, litterfall collectors. Sites at which air filtering units were set out to sample for p-MeHg are also indicated.

samples were transferred to clean amber glass bottles (Environmental Sampling Supply, QC class), further preserved with TMG HCl equal to 0.2% of the final volume, and stored refrigerated until analysis. If after 2 weeks there was insufficient sample volume for analyses, funnels and Teflonware were switched out of the collectors. Strict clean-hands, dirty-hands protocol (St. Louis et al., 1994) was used during all handling of sampling equipment and samples.

To estimate net winter MeHg and THg loadings, integrated snowpack samples were collected near the time of maximum snowpack depth on 21 March 2007. Winter sampling was conducted at this time, as opposed to on a per-event basis, because concentrations of MeHg and THg in snow at the time of maximum snow pack depth provide a good estimate of the amount of Hg that will ultimately 'enter' the watershed during snowmelt (Lalonde et al., 2003; Nelson et al., 2008). Open area snow samples were collected from the surface of Lake 658, whereas throughfall snow samples were collected adjacent to automated throughfall collectors, using an acid-washed Teflon core tube (3.5 cm diameter). Snow was then transferred from the core tube into an acid-washed 2-L Teflon jar. Snow samples were stored frozen until analysis.

During the open-water season, water deposition (mm of rain) in the open was quantified using a standard tipping bucket rain gauge whereas water deposition under the canopy was measured using standard B-type rain gauges positioned adjacent to the automated collectors. Areal water volume of snow deposited in the open was obtained using a Nipher snow gauge at the ELA meteorological site. Under-canopy snowpack water equivalent (SWE) data were obtained from a survey of the throughfall sites in the METAALICUS watershed at the time of snow sampling. Near each automated collector, average areal water volume of the snowpack (L $m^{-2}$) was determined from the weight of one snow core (1 g = 1 mL) collected using a 12.2 cm diameter core tube.

Annual MeHg and THg loadings were calculated for the water year (November 1-October 31) because snowfall following freeze-up in early November contributes to runoff the following spring. Winter net areal loads of

MeHg and THg in open area and throughfall snowpacks were calculated by multiplying the total areal water volume by the measured snowpack MeHg and THg concentrations. During the open-water season, measured MeHg and THg concentrations were multiplied by areal water volumes of rain associated with those sampling events and then summed to calculate the total annual loading. For rain events that occurred before deployment of the collectors in the spring and after they were shut down in the fall, open loadings were estimated by multiplying areal water volume measured at the meteorological site during those periods by the average volume-weighted THg or MeHg concentration in rain for the year. For throughfall sites, open areal water volume for the missing periods was corrected by the average interception capacity of the canopy at each site before estimating the loading.

### *Concentrations and annual loadings of MeHg and THg in litterfall*

Litterfall was captured in 625 $cm^2$ collectors deployed in duplicate near each automated throughfall collector. Litter fell onto a piece of mesh fiberglass screening laminated between upper and lower portions of an untreated wooden frame so that samples remained dry until collection. Samples were collected in late October 2007 after the majority of deciduous leaves had fallen and once again in early May 2008 to collect any litterfall that occurred over winter. Litter remained in the collectors for only a short time before collection in October because there is minimal mass flux before senescence of deciduous tree leaves. During winter, conifer needles and small branches that broke under the weight of snow fell directly onto the snowpack where they remained frozen until deposited into collectors during spring melt. Samples were collected using clean-hands, dirty-hands protocol and stored frozen in Ziploc bags. Fall and spring litterfall samples from individual collectors were freeze-dried, weighed, pooled into a composite sample and homogenized using stainless steel coffee grinders rinsed with deionized water and cleaned with paper towels between samples (St. Louis et al., 2001; Graydon et al., 2008).

For each litterfall collector, MeHg and THg concentrations were multiplied by corresponding litter mass loadings. Average annual litterfall MeHg and THg loadings at each site were calculated from the two replicate collectors.

### *Dry deposition of particulate bound MeHg*

In addition to wet-deposition, dry deposition of atmospheric Hg species represents a significant source of Hg to forested watersheds where the large number of receptor sites on foliage can result in significant scavenging of Hg from the atmosphere. Dry deposition is thought to be largely responsible for the increased loadings of both MeHg and Hg(II) under forest canopies compared to open sites (Grigal, 2002), and, in the case of Hg(II), it is suspected that a portion of the extra Hg is derived from dry deposition of particulate bound Hg (p-Hg) (Rea et al., 2000; Poissant et al., 2004). It has been proposed that the same could be true for MeHg (Munthe et al., 1995a), although few attempts have been made to directly quantify atmospheric concentrations of particulate bound MeHg (p-MeHg).

We sampled for p-MeHg using air sampling units consisting of a quartz microfiber air sampling filter (Whatman QM-A 47mm diameter, >99.95% retention of particles ≥ 0.3 μm; Whatman technical note, 2002) housed in an acid-washed Teflon filter pack connected to a constant flow high volume air sampling pump (SKC Inc., 224-PCXR8 Universal Sample Pump; Figure 2.4). The filter packs were set 1.5 m above the ground and ~20 $m^3$ of air was pulled through each filter at a rate of 5 L $min^{-1}$ for ~3 days. The inlet port of each filter pack was capped with Nytex screening (200 μm mesh opening) to prevent insects from entering the sampling stream and collecting on filters. Plastic domes were mounted above filter packs to prevent filters from being wetted during precipitation events, although we attempted whenever possible to sample during rain-free intervals when atmospheric concentrations of particulate matter should have been higher.

Prior to use, all filters were pre-combusted at 450 °C for 6 hrs, and stored individually in acid washed plastic filter cases sealed with Parafilm. Sets of six

Figure 2.4 Example of p-MeHg sampling under the old growth canopy. Shown here is the sampling unit mounted 1.5 m above ground with the Teflon filter pack housing a QM-A filter capped with Nytex screening and sheltered under a plastic dome to prevent filters from being wetted during precipitation events. Tubing is connected to a constant flow high volume air sampling pump (SKC Inc., 224-PCXR8 Universal Sample Pump; not shown).

filters (four samples and two travel blanks) were further stored double bagged under positive pressure in sealed acid-cleaned glass mason jars purged with UHP $N_2$. Air sampling units ($n$ = 4) were deployed within the METAALICUS watershed (2 under forest canopies and one in the open) and at an open area reference station located at the ELA meteorological site (Figure 2.3**)**. The ELA meteorological site is situated on a bedrock knoll surrounded by a fire regenerated jack pine/white birch forest ~0.5 km west of the ELA base station on Lake 239, where a Tekran® automated air Hg speciation unit was also deployed and measuring p-Hg (Figure 2.1). Sampling units were deployed in this manner because we hypothesized that higher concentrations of p-MeHg would be measured in open air as opposed to under the canopy due to the efficiency of canopy foliage at scrubbing and binding particles from the atmosphere.

Following collection from the field, filters were placed back in their individual filter cases sealed in Parafilm, double-bagged and stored frozen under positive pressure in sealed acid-cleaned glass mason jars purged with UHP $N_2$ until MeHg analysis.

### ***Fate of MeHg on Canopy Foliage***

#### *(Photo)reduction of MeHg and evasion as Hg(0)*

Previous studies found that a significant portion (~40-80%) of Hg(II) wet deposited and initially retained on foliage is rapidly photoreduced to Hg(0), which then fluxes back to the atmosphere (Graydon et al., 2006; Lindberg et al., 1998; Lindberg et al., 2003). However, it is unknown whether wet deposited MeHg is also photoreduced on foliar surfaces and subsequently returned to the atmosphere as Hg(0). To quantify the potential photoreduction of MeHg on foliage to Hg(0), we sprayed a birch, jack pine, and spruce tree with an ~1 $\mu g\ L^{-1}$ $Me^{199}Hg$ solution using a hand-held plastic garden sprayer to simulate a wet deposition event and measured flux rates of $^{199}Hg(0)$ using a dynamic flux chamber (DFC) that allowed enclosed foliage to maintain normal physiological function. Oligotrophic Winnange Lake water was used to mix the spray solution to simulate rain water by providing ligands to which the isotopes could bind before application to the trees. The lake water was acidified using TMG HCl to pH=4, as was done for the METAALICUS loading experiment, to ensure that the Hg remained in solution during the spray event (Sandilands et al., 2005).

The DFC consisted of a rectangular PFA Teflon bag (Welch Fluorocarbon Inc.; 15 x 15 x 40 cm, ~9 L) mounted on an external stainless steel frame with an outlet sampling port located at the bottom center of the chamber and four inlet holes (6 mm diameter) evenly spaced on the front of the chamber (

Figure 2.5). PFA Teflon was chosen for the chamber bag because it is inert to Hg binding and allows high transmissivity of UV radiation (Figure A.1). The chamber was pulled over the sample branch and sealed with a cable tie. Two Brailsford TD-2NA vacuum pumps connected to the outlet port via Teflon tubing and a straight Teflon compression fitting created air flow through the chamber. The flow rate through the DFC was measured using a GFM17S Aalborg stainless steel thermal mass flow meter (0-30 L min$^{-1}$ capacity, 0.1 L min$^{-1}$ precision). Flows were maintained between 7-9 L min$^{-1}$, establishing a complete exchange of air within the chamber approximately every 1 minute. As a result, there was no visible condensation buildup on the inside of the chamber, nor any signs of foliar stress such as leaf wilting. A Brailsford TD-2NA vacuum pump sub-sampled the outlet line between 0.5 and 2 L min$^{-1}$ through a gold-coated glass bead trap (gold trap) that stripped Hg(0) completely from the air. A second sampling stream was used to quantify spike Hg(0) concentrations entering the chamber through inlet holes. Aalborg GFM17 thermal mass flow meters (0-5 L min$^{-1}$ capacity, 0.01 L min$^{-1}$ precision) and TOT10 totalizers measured total air flow through the gold traps. Soda lime traps (8-14 mesh, Alfa Aesar) were used upstream of gold traps to remove humidity (

Figure 2.5).

Flux measurements were performed twice on the day trees were sprayed and repeated 1, 6 and 17 days post-spray. A new flux chamber was used for each measurement (*n*=15), however, six were run as blank chambers prior to use on tree foliage. All flux measurements lasted 1 hour. Prior to each flux measurement, all components of the sampling line upstream of gold traps were switched out with new or acid washed replacements. All flux measurements on a given tree

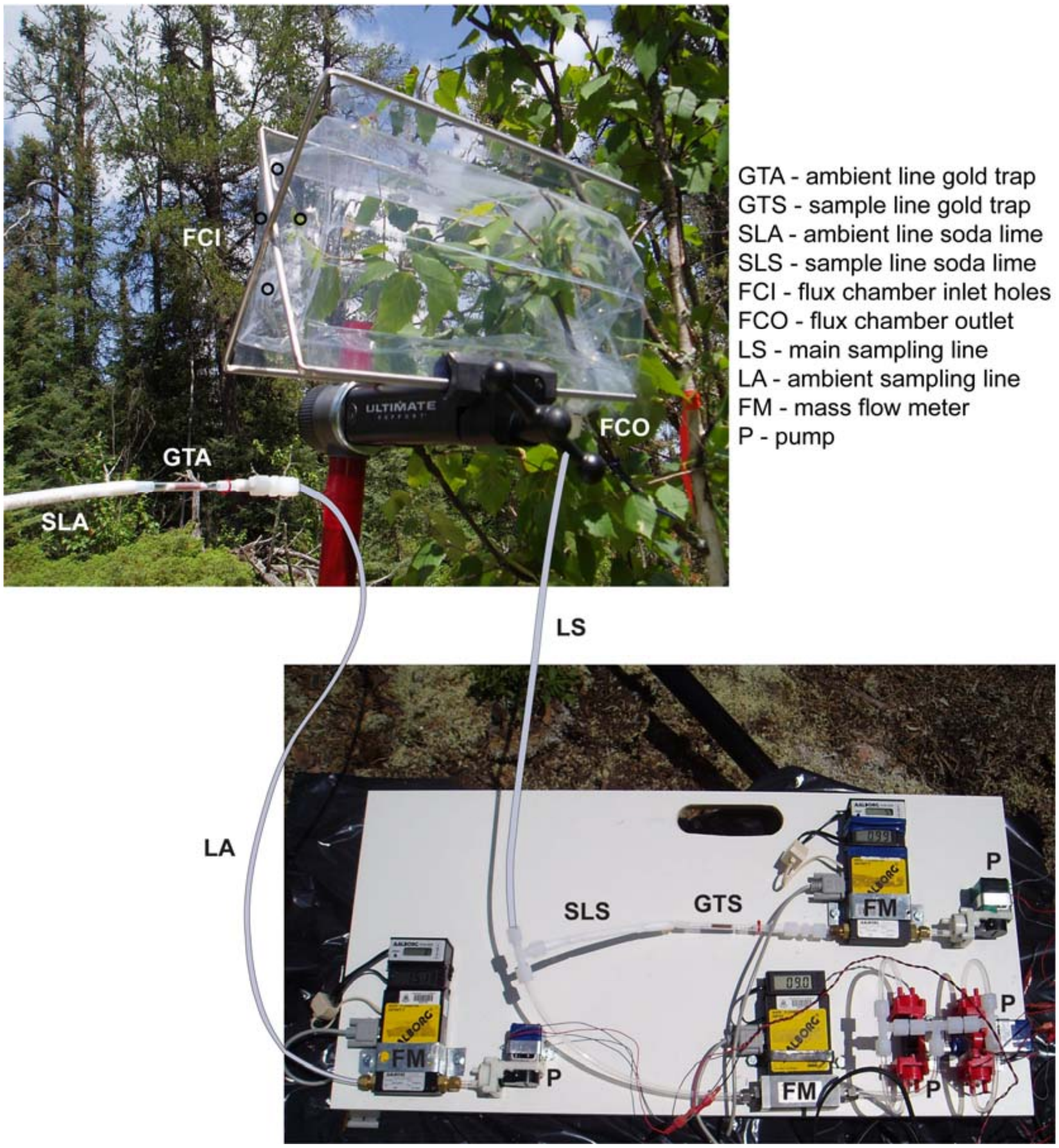

Figure 2.5 In-situ sampling arrangement of the dynamic flux chamber (DFC). Upper image shows healthy birch foliage contained within the DFC during a flux measurement, with no build-up of condensation. Lower image shows sampling line set-ups with connected pumps and flow meters. The main sampling line (right) creates air flow through the DFC and also connects to the sub-sampling line where the soda-lime trap removes moisture and the gold trap collects Hg(0) to measure concentrations in air exiting the chamber. The ambient sampling line (left) is used to measure concentrations of Hg(0) in air entering the chamber.

were performed on the same branch. Prior to the first flux measurement, branches adjacent to the flux branch were collected to provide a general sense of how much Me$^{199}$Hg spike was initially retained on each tree.

All gold traps were heated to 400 $^{o}$C in a stream of UHP $N_2$ just prior to flux measurements to desorb any residual Hg, then sealed using Teflon plugs and tape, and individually bagged. Gold traps were stored under positive pressure in sealed acid-cleaned glass mason jars purged with UHP $N_2$ both before and after use. Travel blank gold traps ($n$ = 3) accompanied sample traps to and from our field site.

Flux rates of $^{199}$Hg(0) ($F$; ng g$^{-1}$ h$^{-1}$ or ng m$^{-2}$ h$^{-1}$) from foliage were calculated using the following equation:

$$F = \frac{(^{199}\mathrm{Hg}(0)_o / \beta_o - ^{199}\mathrm{Hg}(0)_i / \beta_i) \times f_m}{W} \tag{1}$$

where $^{199}$Hg(0)$_o$ = total spike $^{199}$Hg(0) on the outlet gold trap (ng); $\beta_o$ = total air flow through the outlet gold trap (L); $^{199}$Hg(0)$_i$ = total spike $^{199}$Hg(0) on the inlet gold trap (ng); $\beta_i$ = total air flow through the inlet gold trap (L); and $f_{\mathrm{m}}$ = volume of air exiting the chamber (L hr$^{-1}$). As $^{199}$Hg(0) was below detection in air exiting all blank chambers and was never detected on travel blank traps, it was unnecessary to include these potential background sources in the flux rate calculation.

To further standardize flux rates among the different trees, and account for any differences in the initial concentration of Me$^{199}$Hg on flux branches, the initial flux rate was set to 100%, and subsequent fluxes were expressed as a % of that initial flux.

### *Long-term retention of MeHg*

While a portion of the Hg(II) wet-deposited to forest canopies can be rapidly photoreduced, some remains bound to canopy foliage, contributing to loads of Hg(II) in litterfall (Grigal, 2002; Graydon et al., 2006). To determine if the same is true of MeHg, and to compare the long-term retention of MeHg and

Hg(II) on foliage, nine trees (3 each of birch, jack pine, and black spruce) in the upland portion of the Lake 302 watershed were sprayed with both Me$^{199}$Hg and $^{198}$Hg(II) to simulate a wet deposition event. This was done in the same manner as was described for the flux trees.

On 18 July 2006, a designated portion of each tree received 1L of solution (~1 µg $^{198}$Hg L$^{-1}$, ~0.4 µg Me$^{199}$Hg L$^{-1}$), such that foliage was dripping following spray. After complete drying (~0.5 hrs), a foliage sample was clipped from each tree and bagged using clean-hands, dirty-hands protocol. All nine trees were sampled again 1, 3 and 6 days post spray, and weekly thereafter to determine rate loss constants and retention half-lives of the Hg isotopes on foliage. Sampling of spruce and jack pine lasted for a total of 35 days post-spray while the birch trees were not sampled beyond 2 weeks post-spray due to drought-induced early senescence that year. Additionally, the spruce and jack pine trees were sampled again in May 2007 for a longer term determination of Hg retention. Individual samples were stored and processed as described for litterfall.

To better compare spike Hg retention among our 9 trees, we first standardized for variability in post-spray spike concentrations of canopy foliage by setting the initial spike concentration for each tree at 100% and expressing the foliar concentration of each subsequent sample as a % of the initial concentration. A rate constant for the decline of foliar spike concentrations was calculated for each tree by modeling the spike concentration data using first-order chemical kinetics:

$$\ln\,(\%\,\mathit{initial\,spike})_t = \ln\,(\%\,\mathit{initial\,spike})_0 - k\,\mathrm{t} \qquad (2)$$

where (% *initial spike*)$_t$ and (% *initial spike*)$_0$ are the % of initial foliar concentration of either Me$^{199}$Hg or $^{198}$Hg(II) remaining at time t and time 0 respectively, and $k$ is the first-order rate constant. To determine if decline rates varied between Me$^{199}$Hg and $^{198}$Hg(II) and/or among foliage types, we used a mixed effects model (Proc Mixed) in SAS v. 9.2 (SAS Institute Inc. 2007). In this analysis, values of $k_{\mathrm{MeHg}}$ and $k_{\mathrm{Hg(II)}}$ obtained for each tree served as the response variable, tree ID served as the random effect, and foliage species and Hg species served as the fixed effects. Average $k_{\mathrm{MeHg}}$ and $k_{\mathrm{Hg(II)}}$ values for each tree species were then used to calculate half lives ($t_{1/2}$) of the spike retention:

$$t_{1/2} = \frac{\ln(2)}{k} \tag{3}$$

### *Ambient and Hg Isotope Spike Analyses*

Throughout this paper, *ambient Hg* refers to any Hg present that was not experimentally added whereas *spike* Hg refers to enriched Hg stable isotopes either loaded onto the METAALICUS watershed or used in spike experiments. Although spikes were highly enriched for a given Hg isotope, they still contained minor quantities of all other Hg isotopes. Therefore, reported values of enriched Hg *spikes* refer to *all experimentally applied* Hg. All sample analyses were completed in the University of Alberta Biogeochemical Laboratory (Edmonton, AB, Canada) using inductively coupled plasma mass spectrometry (ICP-MS; Elan DRC-e, PerkinElmer Sciex). Because ICP-MS quantifies amounts of individual Hg isotopes, it was possible to distinguish ambient Hg from experimentally applied Hg spikes when analyzing samples (Hintelmann and Ogrinc, 2003).

#### *Precipitation and Throughfall:*

For MeHg analysis, 100 ml of precipitation/throughfall sample was distilled at 125°C for approximately 5 hours. Volatile Hg species were then purged from the distillate and trapped onto Tenax following ethylation using NaBEt4. Trapped Hg species were thermally desorbed and separated using a GC glass column packed with 15% OV-3 Chromosorb (60/80 Mesh) heated to 105 °C and delivered to the ICP-MS for detection. $\mathrm{Me^{201}Hg}$, proportional to the amount expected to be found in each sample, was added as an internal standard prior to distillation to correct for procedural recoveries. Concentrations of ambient and spike MeHg were calculated from the measured isotope ratios as described in Hintelmann and Ogrinc (2003).

Precipitation and throughfall samples were analyzed for THg using an automated Tekran 2600 total Hg analyzer interfaced with the ICP-MS. Briefly, all Hg species in a sample were oxidized to Hg(II) by the addition of BrCl, reduced to Hg(0) using $SnCl_2$, purged onto gold traps, and analyzed after thermal desorption by ICP-MS.

*Foliage and Filters:*

MeHg content of foliage and filters was quantified in a similar manner as the precipitation/throughfall samples; however, prior to distillation, samples were digested overnight in 500 uL of 9 M $H_2SO_4$, 200 uL of 20 % KCl and 40 mL of Milli-Q water in 50 mL Teflon vials. For litterfall samples, ~250 mg of ground litterfall was digested, whereas, for spray tree foliage, only ~100 mg of ground tissue was digested due to the high concentration of the applied spike. In the case of filters, one filter was digested. $Me^{201}Hg$ was added prior to the digestion as an internal standard.

For THg analysis, ~250 mg of ground foliage was digested in 60 mL Teflon bombs using 7 mL of 7:3 (vol/vol) $HNO_3/H_2SO_4$. Bombs were heated in a vented oven for two hours at 125 °C. Samples were allowed to cool and 19 mL of Milli-Q water and 1 mL of BrCl were added. Bombs were closed and heated overnight at 60 °C. A 0.5 mL subsample of the digest was diluted with Milli-Q water and hydroxylamine hydrochloride (0.04 %, for neutralization) to a final volume of 50 mL. Sample reduction, delivery, and detection were as described above for precipitation/throughfall samples.

Successful inter-laboratory comparisons of duplicate water and foliage samples were conducted (Graydon et al., 2008) with Flett Research Ltd. (Winnipeg, MB) and the Trent University Worlsford Water Quality Center (Peterborough, ON). Limits of detection (LODs) for ambient MeHg and THg analyses were calculated as 3 x S.D. of analytical blanks and were between 0.05 to 0.3 ng $L^{-1}$ and 0.01 to 0.03 ng $L^{-1}$ respectively for precipitation/throughfall and 3 pg $g^{-1}$ and 0.4 ng $g^{-1}$ respectively for litterfall. The LODs for the experimentally applied Hg isotopes varied with the precision of the isotope ratio measurement, ranging between 0.5-2.0 % of the ambient Hg concentration of a sample.

*Gold Traps:*

Hg(0) was desorbed from sample traps at 400 °C into UHP Ar carrier gas and delivered to the ICP-MS. To ensure the quality and consistency of peak signals amongst samples, sample traps were first desorbed onto an analytical trap, which was then desorbed into the ICP-MS. Spike Hg(0) concentrations were determined to be above detection limit when the amount of $^{199}$Hg(0) was greater than 0.25% of the ambient Hg(0) desorbed from a trap (Hintelmann and Ogrinc, 2003).

## Results and Discussion

### ***Sources of MeHg to canopy foliage***

#### *Concentrations and annual loadings of MeHg and THg in open area precipitation and throughfall*

Average concentrations (±SD) of MeHg and THg measured in open area precipitation in 2007 were $0.06 \pm 0.04$ ng $L^{-1}$ and $5.9 \pm 3.6$ ng $L^{-1}$ respectively, with annual volume weighted averages of 0.05 ng $L^{-1}$ and 5.2 ng $L^{-1}$. These average concentrations are similar to those previously recorded between 1992-2006 at the ELA for MeHg ($0.08 \pm 0.04$ ng $L^{-1}$) and THg ($6.1 \pm 2.6$ ng $L^{-1}$) (Graydon et al., 2008). In general, open area MeHg and THg concentrations varied similarly amongst sampling events (co-efficient of variation = 0.62 for both) and exhibited a slight increasing trend until early fall when the highest concentrations were recorded (0.12 ng $L^{-1}$ and 14 ng $L^{-1}$ respectively; Figure 2.6).

Throughfall MeHg concentrations in 2007 were sometimes lower than the $0.06 \pm 0.04$ ng $L^{-1}$ measured in open precipitation, whereas THg concentrations were typically higher in throughfall than the $5.9 \pm 3.6$ ng $L^{-1}$ measured in open precipitation (Figure 2.6). On average, volume weighted concentrations of MeHg in throughfall were generally highest under the old growth canopy ($0.11 \pm 0.04$ ng $L^{-1}$), followed by the jack pine ($0.09 \pm 0.002$ ng $L^{-1}$), wetland (0.07 ng $L^{-1}$) and

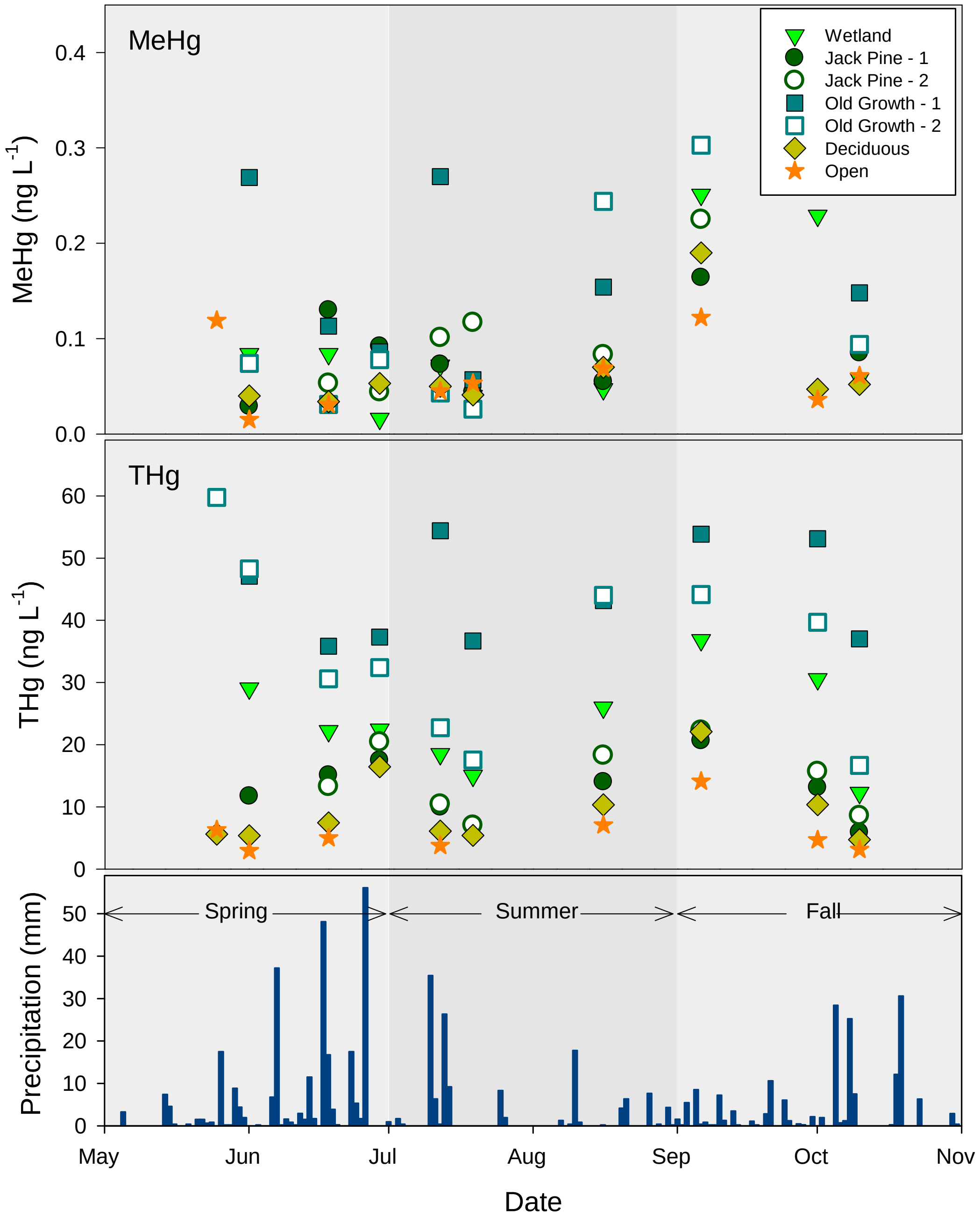

Figure 2.6 Concentrations of MeHg and THg in open area precipitation and throughfall collected under different forest canopy types within the METAALICUS watershed during the open-water season. A daily record of open area precipitation (lower panel) is included for general reference of precipitation trends over this period.

deciduous (0.06 ng $L^{-1}$) canopies. Average THg throughfall concentrations were more variable among canopy types than MeHg but similarly highest under the old growth canopy (35.5 ± 7.9 ng $L^{-1}$) followed by the wetland (22.0 ng $L^{-1}$), jack pine (14.3 ± 1.1 ng $L^{-1}$) and deciduous (9.9 ng $L^{-1}$) canopies. The %MeHg in throughfall (0.3-0.6%) was lower than that in open area precipitation (1%) as a result of the higher amounts of Hg(II) in throughfall, and no comparable increase of MeHg, compared to open area precipitation. The lack of corresponding increase in MeHg is likely a result of the relatively small amount of MeHg in the atmosphere that is potentially available for dry deposition in comparison to Hg(II) or Hg(0), although it has also been suggested that vegetation might preferentially adsorb MeHg over other forms of Hg (Grigal 2002). Both the trends and concentrations for throughfall MeHg and THg measured in 2007 were consistent with those observed over the long term at the ELA (Graydon et al., 2008).

Measures of Hg concentrations in wet deposition are a function of not only the amount of Hg accumulating in water as it passes through the atmosphere and, in the case of throughfall, the forest canopy, but also the volume of water deposited. As a result, concentrations of Hg in throughfall are influenced by both washoff of Hg from foliage increasing the Hg mass, and the evaporation of water from the canopy foliage reducing the volume of water passing through the forest canopy. Hg loadings, which take into account both of these processes, are therefore more useful measures for comparison between open and forested sites than Hg concentrations.

As with previous years, in 2007 the majority (83% and 96% respectively) of open area MeHg (0.41 mg $ha^{-1}$) and THg (34.9 mg $ha^{-1}$) loading occurred during the open-water season (Figure 2.7). In both instances this pattern was driven primarily by lower water deposition during the winter (142 mm) than during the open-water season (647 mm). Low snowpack concentrations were also a contributing factor for THg (0.9 ng $L^{-1}$, ~0.2x average concentration in rain) but not for MeHg (0.05 ng $L^{-1}$, ~1x average concentration in rain).

Average annual throughfall loadings of MeHg did not differ much among

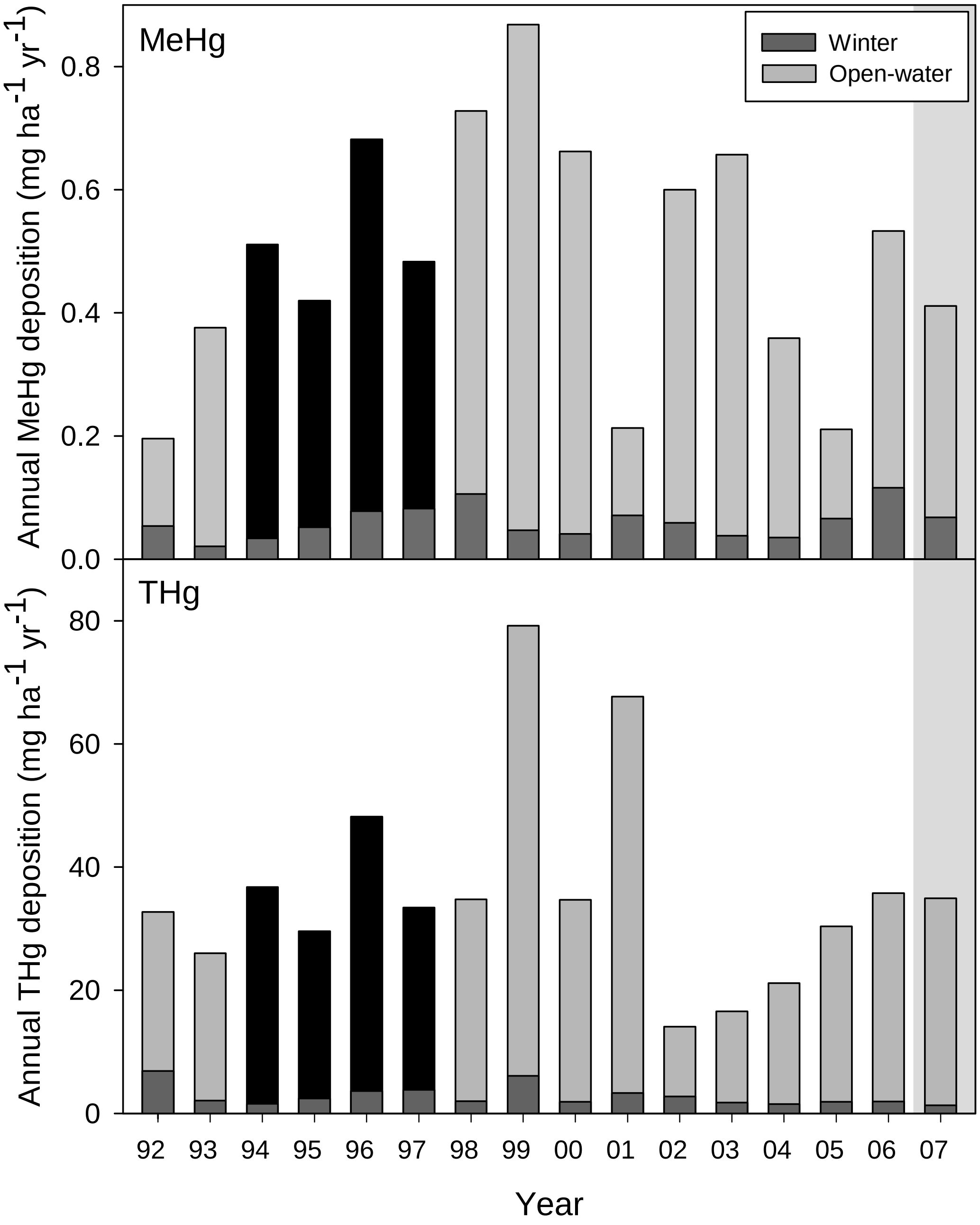

Figure 2.7 Annual winter and open-water season wet deposition loadings of MeHg and THg in open areas at the ELA. Black bars represent years in which rain and snow were not collected for Hg analysis. Loadings for these years were estimated using the long-term average Hg concentrations and measured wet deposition volumes at the ELA. Data collected prior to 2001 was from outside of the METAALICUS watershed and collected on a per event basis (For a full description, please refer to Graydon et al. (2008)).

the various canopy types (wetland = 0.44 mg ha$^{-1}$; jack pine = 0.34 ± 0.01 mg ha$^{-1}$; deciduous = 0.35 mg ha$^{-1}$) and the open, except for under the old growth canopy (0.60 ± 0.16 mg ha$^{-1}$) where deposition was somewhat higher (Figure 2.8). Although the difference was small, throughfall MeHg loadings at the jack pine and deciduous sites were actually lower than MeHg loading in the open, suggesting that some portion of newly deposited MeHg may remain bound to foliage or returned to the atmosphere following deposition. Throughfall loadings of THg under all canopy types were greater than THg loadings in the open, and were nearly 3 times larger under the old growth (141 ± 15 mg ha$^{-1}$) and wetland (114 mg ha$^{-1}$) canopies than under the jack pine (50 ± 4 mg ha$^{-1}$) and deciduous (49 mg ha$^{-1}$) canopies (Figure 2.8).

### *Concentrations and annual loadings of MeHg and THg in litterfall*

In 2007, average litterfall MeHg and THg concentrations at the ELA were 0.35 ± 0.17 ng g$^{-1}$ and 35 ± 14 ng g$^{-1}$ respectively. Concentrations were slightly higher under the old growth canopy (0.49 ± 0.25 ng g$^{-1}$, 49 ± 9 ng g$^{-1}$ ) in comparison to the wetland (0.31 ± 0.09 ng g$^{-1}$, 37 ± 15 ng g$^{-1}$), jack pine (0.27 ± 0.01 ng g$^{-1}$, 25 ± 2 ng g$^{-1}$), and deciduous (0.24 ± 0.02 ng g$^{-1}$, 22 ± 1 ng g$^{-1}$) canopies (Figure 2.8). MeHg represented approximately 1% of THg in litterfall at all sites.

The concentrations of MeHg and THg are typical of those previously measured at the ELA. Long term monitoring of litterfall concentrations at the METAALICUS sites has established that, in the case of MeHg, the only significant difference among sites is higher litterfall concentrations under the old growth canopy than under all other canopy types (Graydon et al., 2008). In comparison, a previous study conducted at the ELA (St. Louis et al., 2001) found that wetland canopy litterfall had higher concentrations of MeHg (0.38 ± 0.12 ng g$^{-1}$) than did litterfall from a young jack pine canopy (0.20 ± 0.18 ng g$^{-1}$). It was suggested that this pattern may be the result of a larger pool of MeHg available for uptake by plants in wetlands. Hg(II) methylation has been shown to naturally occur in saturated anaerobic wetland soils, but not in drier aerobic upland soils

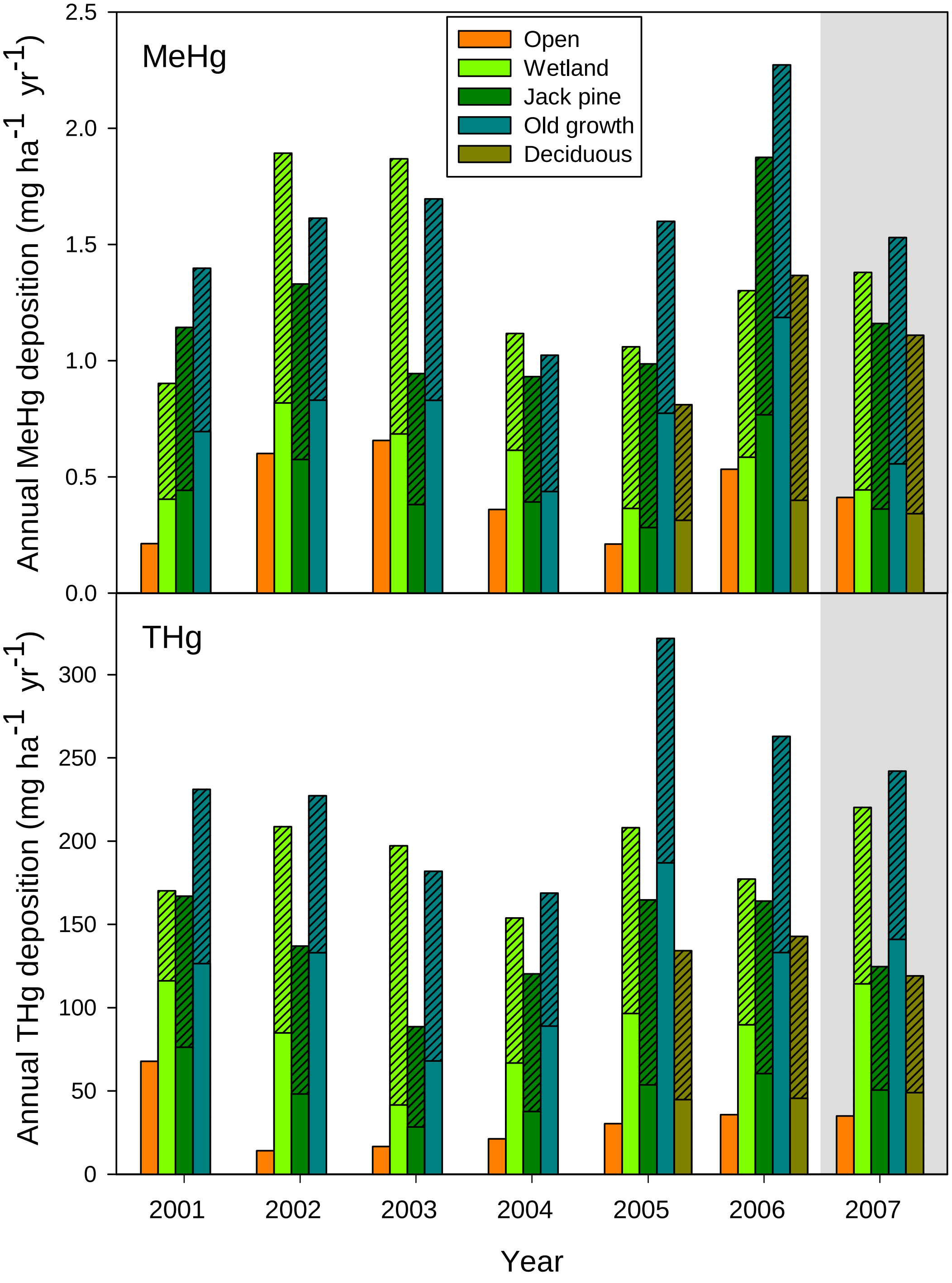

Figure 2.8 Long-term record of annual MeHg and THg loadings in open area precipitation, as well as throughfall (stacked solid) and litterfall (stacked cross-hatched) under four different forest types in the METAALICUS watershed at the ELA.

(St. Louis et al., 1994; St. Louis et al., 1996; Hurley et al., 1995). Although litterfall collected at our wetland site had slightly higher concentrations of MeHg than litterfall collected at the upland jack pine sites, the difference was not as large as that measured by St Louis et al. (2001).

This difference in findings between the two studies is likely the result of different site characteristics, as both age and foliage type are known to have an effect on Hg concentrations (Rasmussen et al., 1991; Moore et al., 1995; Rasmussen, 1995; Rea et al., 2002; Ericksen et al., 2003). In our study, the young jack pine and wetland canopies were of similar age and predominantly composed of the same tree species (as a result of a previous forest fire) whereas, in St Louis et al. (2001), the young jack pine and wetland sites being compared had different canopy compositions in terms of both age and dominant tree species. However, the small number of replicate collectors under each canopy type in both studies limits our ability to draw any firm conclusions given that the complexity and heterogeneity of forest canopy structure could also be affecting results. Furthermore, as jack pine does not grow in saturated wetland soils it is not the ideal species for this comparison. Alder (*Alnus spp.)* which grow readily in both well and poorly drained soils would make better study species.

The mean annual mass flux of litterfall at all of our sites was 2770 ± 910 kg ha$^{-1}$, 70 ± 14% of which occurred before the end of October. For both MeHg and THg, the resulting litterfall loadings were slightly higher under the old growth (0.97 ± 0.34 mg ha$^{-1}$, 101 ± 13 mg ha$^{-1}$) and wetland (0.94 ± 0.34, 106 ± 25 mg ha$^{-1}$) canopies than under the jack pine (0.80 ± 0.16, 74 ± 15 mg ha$^{-1}$) and deciduous (0.77 ± 0.07, 70 ± 16 mg ha$^{-1}$) canopies. On average, litterfall loadings in 2007 resulted in a 2-3 fold increase in MeHg and THg deposition at forested sites compared to the open. Historically, a similar pattern has been found at the ELA (Table 2.1); however, neither differences in annual mass flux of litterfall or differences in annual litterfall MeHg and THg loadings among forest types were significant (Graydon et al., 2008).

Table 2.1 Average (± S.D.) annual loadings of MeHg and THg in the open and in throughfall and litterfall under four canopy types at the ELA. Long-term averages are provided for comparison to the 2007 loadings.

| | Average MeHg deposition (mg ha$^{-1}$) | | | | Average THg deposition (mg ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| Site | Precipitation | Throughfall | Litterfall | Total | Precipitation | Throughfall | Litterfall | Total |
| *1992-2006* | | | | | | | | |
| Open | 0.50 ± 0.20 | | | | 36 ± 17 | | | |
| *2001-2006* | | | | | | | | |
| Open | 0.43 ± 0.20 | | | 0.43 ± 0.20 | 31 ± 20 | | | 31 ± 20 |
| Old Growth[†] | | 0.79 ± 0.41 | 0.81 ± 0.17 | 1.60 ± 0.44 | | 123 ± 41 | 110 ± 21 | 233 ± 46 |
| Wetland | | 0.59 ± 0.17 | 0.78 ± 0.29 | 1.37 ± 0.34 | | 83 ± 26 | 103 ± 35 | 186 ± 44 |
| Jack Pine[†] | | 0.47 ± 0.16 | 0.73 ± 0.20 | 1.20 ± 0.26 | | 51 ± 17 | 89 ± 17 | 141 ± 25 |
| Deciduous[*] | | 0.36 ± 0.06 | 0.65 ± 0.18 | 1.00 ± 0.19 | | 45 ± 1 | 93 ± 6 | 138 ± 6 |
| *2007* | | | | | | | | |
| Open | 0.41 | | | 0.41 | 35 | | | 35 |
| Old Growth | | 0.56 ± 0.16 | 0.97 ± 0.34 | 1.53 ± 0.38 | | 141 ± 15 | 101 ± 13 | 242 ± 20 |
| Wetland | | 0.44 | 0.94 ± 0.34 | 1.38 ± 0.34 | | 114 | 106 ± 25 | 220 ± 25 |
| Jack Pine | | 0.37 ± 0.01 | 0.80 ± 0.16 | 1.16 ± 0.16 | | 50 ± 4 | 74 ± 15 | 125 ± 16 |
| Deciduous | | 0.34 | 0.77 ± 0.07 | 1.11 ± 0.07 | | 49 | 70 ± 16 | 119 ± 16 |

[†] 2001-2006 averages for the old growth and jack pine canopies include duplicate automated throughfall collectors that were not deployed until 2005.

* Litterfall was collected at the deciduous site from 2001-2006, however throughfall monitoring at this site was from 2005-2006. The 2005-2006 throughfall average was used here as a proxy for the long term average as there was no significant increasing or decreasing trend in THg or MeHg loadings observed from 2001-2006 (Graydon et al., 2008).

Annual loadings for 2007 were calculated in part as a contribution to on-going METAALICUS research and a long-term record of Hg deposition at the ELA. A detailed account of MeHg and THg deposition in open precipitation from 1992-2006, and throughfall and litterfall from 2001-2006 in the METAALICUS watershed can be found in Graydon et al. (2008). In all instances, the 2007 loadings fit well within the long-term trends (Table 2.1; Figure 2.7; Figure 2.8).

The above loadings support previous findings, both at the ELA and elsewhere, that forest canopies enhance deposition of MeHg and THg to watersheds (Iverfeldt, 1991; Munthe et al., 1995b; Schwesig and Matzner, 2000; St. Louis et al., 2001; Porvari and Verta, 2003; Graydon et al., 2008; Witt et al., 2009). While deposition of Hg to open areas is primarily in wet forms, deposition at forested sites is the cumulative result of throughfall and litterfall loadings, both of which can be affected by dry- as well as wet deposition processes. Throughfall and litterfall loadings must therefore be added together to determine the net effect of forest canopies on MeHg and THg deposition (Table 2.1). In 2007, total loadings of MeHg under the forest canopy ranged from 3 to 4 times higher than loading in the open, with litterfall loadings comprising ~2/3 of the total load under all canopy types. The enhancing effect of the canopy on deposition was generally greater for THg than for MeHg and ranged from 3 (under the deciduous canopy) to 7 (under the old growth canopy) times higher than open loadings. In the case of THg, litterfall and throughfall contributed nearly equally to net loadings.

Although all potential sources of MeHg to forest canopies are not yet fully understood, efficient scavenging of particulate and other reactive species of Hg from the atmosphere by foliage has been proposed as a primary source of the enhanced loading of both MeHg and THg at forested sites (Iverfeldt, 1991; Munthe et al., 1995a; Lindberg, 1996; Graydon et al., 2008). Dry deposition to the canopy occurs via several processes, and can result in Hg both on the surface and inside of canopy foliage. Hg *on* the surface may be washed-off, contributing to throughfall loadings, whereas Hg *in* foliage is likely to be retained, contributing to litterfall loadings (Iverfeldt 1991). The difference in relative contributions of litterfall and throughfall to total loadings of MeHg and THg is likely the result of differences in the predominant source of dry deposition for each Hg species.

In the case of THg, throughfall loadings are enhanced by the wash-off of previously dry-deposited Hg from foliage during precipitation events, namely reactive gaseous Hg(II) and atmospheric Hg(0) which is oxidized on the surface of wet foliage to Hg(II) (Iverfeldt, 1991; Lindberg et al., 1994). As Hg(0) is the predominant species of Hg in the atmosphere it has potential to greatly enhance deposition of Hg to forested sites. In addition to settling on foliage, Hg(0) is exchanged between foliage and the atmosphere via stomata. Although it is known to be a dynamic process, with foliage both taking in and releasing Hg(0) (Hanson et al., 1995; Lindberg, 1996), the net result of this exchange is that canopy foliage acts as a sink for atmospheric Hg (Grigal, 2002 and references therein; Ericksen et al., 2003). Thus, Hg(0) contributes not only to the THg in throughfall loadings, as described above, but contributes to litterfall loadings as well. However, as the concentration of gaseous MeHg in the atmosphere is substantially lower than that of Hg(0), it is unlikely that such a source would have a significant effect on MeHg loadings. In the case of MeHg, p-MeHg is likely to be the principle ‘dry’ source enhancing deposition at forest sites. As p-MeHg has potential to be washed from foliage surfaces and end up in throughfall, adsorption to foliage would need to be very efficient for such a source to have a greater effect on litterfall, in comparison to throughfall, loadings (Munthe et al., 1995a). Some portion of deposited p-MeHg may remain bound to foliage or retained in the wax layer of leaves and needles due to the strong affinity of MeHg for organic matter (Hultberg et al., 1994).

### *Dry deposition of MeHg*

Rates of Hg dry deposition can be estimated using several different methods (Lindberg et al., 2007). Using the “direct” method, which involves subtracting wet-deposition loadings in the open from the sum of loadings in throughfall and litterfall, we estimated that annual deposition of dry-deposited MeHg in 2007 ranged from 0.70 ± 0.07 mg ha$^{-1}$ (deciduous canopy) to 1.1 ± 0.38

mg ha$^{-1}$ (old growth canopy). However, of the 54 filters deployed to collect p-MeHg over a series of 16 sampling intervals, not one had detectable levels of MeHg on it (LOD = 5 pg filter$^{-1}$, calculated as 3 * S.D. of travel blanks, *n*=33) despite the fact that particulate matter was visually present. This finding does not mean that p-MeHg does not exist; only that it was either not detectable using our current sampling protocols or that it exists in concentrations lower than we were able to quantify.

Given that ~20 m$^3$ of air was pulled through each filter, atmospheric concentrations of p-MeHg could be as high as 0.25 pg m$^{-3}$ and remain at or below the 5 pg detection limit for our filters. It is reasonable to assume that this might be the case as summertime p-Hg concentrations measured by the Tekran® air Hg speciation units at the ELA meteorological site are quite low, often between 5-20 pg m$^{-3}$ (V. St Louis/J. Graydon, unpublished data), and MeHg is thought to represent only a small fraction of THg in the atmosphere (~0.1% of the global atmospheric budget of Hg ; Lamborg et al., 1995). Assuming that this is a suitable method for quantifying p-MeHg, and using our detection limit as an upper limit of atmospheric concentrations, we conclude that p-MeHg accounts for less than 5% of all particulate bound Hg species in air at the ELA.

Although little work has been published on the deposition of p-Hg, deposition velocities ($V_d$) are thought to be in the range of 0.02–2.0 cm s$^{-1}$ and are consistent with theoretical and field studies conducted for other fine particle species (Zhang et al., 2009). We assume here that $V_d$ for p-MeHg is similar. Based on the above $V_d$ and presumably low atmospheric concentrations of p-MeHg, the annual dry depositional flux of p-MeHg is not likely to exceed ~0.015–1.5 mg ha$^{-1}$ and could be substantially lower given that no p-MeHg was detected on our filters.

If we are to trust our calculated deposition estimate, at its upper limits, dry deposition of p-MeHg could account for the differences observed between loadings of MeHg in the open and under forested sites in the METAALICUS watershed (0.7-1.1 mg ha$^{-1}$), lending support to previously made assumptions.

Additional support for this theory was provided by Lamborg et al. (1995) and Hall et al. (2005, unpublished results) who proposed that the association of a portion of MeHg with particles in rainwater samples is evidence of atmospheric wash-out of p-MeHg. While this is certainly a possibility, Bloom and Watras (1989) failed to observe a decreasing trend in the concentration of MeHg in consecutive samples collected during a single rain event, a phenomenon that would typically be observed during washout events. Additionally, lack of correlation between rainfall amount and MeHg concentrations reported by Mason et al. (1997) and Graydon et al. (2008) does not support washout of atmospheric p-MeHg. Alternatively, Hammershmidt et al. (2007) suggested abiotic methylation of reactive Hg(II) species ($Hg_R$) as a likely source of MeHg in rain and further suggested a positive relationship between $Hg_R$ associated with particulate matter and production of MeHg. If the latter is true, then p-MeHg in rainwater may simply be the result of aqueous phase methylation of p-Hg and no longer lends support to the former argument. Clearly, further effort and more rigorous testing are required to fully elucidate sources of MeHg in atmospheric deposition and subsequently sources of MeHg to forest canopies.

In addition to atmospheric deposition, other sources of Hg to the canopy have been proposed, although they are often thought to be less important, especially in the case of THg. Previous studies have indicated that, except in areas with heavily Hg contaminated soils (Lindberg et al., 1979), uptake and transport of both MeHg and Hg(II) from soils to plant foliage is limited as roots provide a strong adsorption site for Hg (Beauford et al., 1977; Godbold and Hutterman, 1988). Root uptake and subsequent sequestration in leaves likely accounts for less than 10% of THg and 3% of MeHg measured in litterfall (Johnson and Lindberg, 1995; Lindberg, 1996; Bishop et al., 1998). Mechanical weathering and/or leaching of Hg from foliage have been suggested as possible sources of MeHg and THg in throughfall; however, the leaching process is thought to contribute minimal amounts of Hg in comparison to washoff (Rea et al., 2000) and the contribution from mechanical weathering has yet to be quantified. Furthermore, on an annual basis this source likely would not have a large impact on total Hg loadings as anything not being accrued in throughfall from foliage would likely eventually contribute to the litterfall flux.

In-canopy abiotic methylation of Hg(II), which could then either be rinsed off in throughfall or be retained on foliage and contribute to litterfall, has been speculated as another potentially important source of MeHg at forested sites (Rudd, 1995; Munthe et al., 1995a). However, in throughfall samples collected under canopies in the METAALICUS watershed, Graydon et al. (2008) found that less than 0.5% of spike THg had been methylated, whereas up to 3% of ambient THg was in a methylated form. Unless ambient and/or previously deposited Hg is more readily methylated than spike and/or newly deposited Hg, abiotic methylation of Hg(II) on foliage is not likely to be a significant source of MeHg in the forest canopy. Methylation of Hg(II) might also occur on decomposing litterfall following leaf drop, however, this is less likely given that the majority of litterfall was collected soon after abscission and the remainder was elevated and kept dry and/or frozen prior to collection. Furthermore, Hall and St. Louis (2004) found that MeHg mass decreased, rather than increased, in litterbag samples placed on forest soils at the ELA.

Alternatively, the MeHg deposited in litterfall might simply be the result of retention of small amounts of MeHg from past deposition events. For example, loadings of MeHg are typically highest under the old growth forest, where the canopy is composed primarily of long lived species that retain foliage for several growing seasons (Bonan, 2002; Apple et al., 2002), and lowest under the deciduous and jack pine canopies, where the tree stands are younger and retain their foliage and needles for much shorter periods of time (Romberger, 1993; Richardson and Rundle, 1995). Foliage is known to be a net sink of Hg with concentrations increasing over the length of a growing season in coniferous (Rasmussen et al., 1995) and deciduous (Lindberg et al., 1996) species and over multiple growing seasons as trees and foliage age. It is typically believed that the commonly higher concentrations of Hg in conifer needles in comparison to nearby deciduous leaves is a result of the older foliage retained on conifers (Rasmussen et al., 1991). It could also be a result of canopy structure and the effects it might have on mechanisms of Hg loss from the canopy (Graydon et al., 2009). For example, a denser canopy would provide greater shading from UV radiation and shelter from the effects of wind erosion. Further understanding of the fate of MeHg following deposition, as we describe below, is required before similar assumptions can be made about this potential source of MeHg in litterfall loadings.

### ***Fate of MeHg on Canopy Foliage***

#### *(Photo)reduction of MeHg and evasion as Hg(0)*

We detected fluxes of $^{199}$Hg(0) from all three trees sprayed with Me$^{199}$Hg using our DFC. Spike Hg(0) evasion rates were highest immediately after spray application and declined rapidly thereafter, leveling off to near detection limits within one week post-spray (Figure 2.9). The initial flux rate was greatest from the jack pine branch (97 pg hr$^{-1}$) followed by the birch (45 pg hr$^{-1}$) and spruce (41 pg hr$^{-1}$) branches. In contrast, the branches collected adjacent to our flux branch suggest that the initial concentration of Me$^{199}$Hg on foliage was highest on the birch (~0.8 ng g-1) followed by the jack pine (~0.5 ng g-1) and finally the spruce (~0.3 ng g-1).

This is contrary to what we expected to find based on a similar study that monitored volatilization of Hg(0) following the application of Hg(II) spike to foliage. There, the highest initial spike Hg(0) flux rates were measured from foliage with the highest initial spike Hg concentrations (Graydon et al., 2006). There are several possible explanations for why we did not observe a similar pattern. For example, possible heterogeneity of spray application could lead to inaccurate estimates of how much spike was retained on our sample branches. Additionally, the time that lapsed between spray and initial flux measurement may have affected the initial measured flux rates. For example, the birch tree experienced the largest delay between spray application and initial flux measurement (~5 hrs) which resulted in a nearly identical flux rate to the second flux measured from the jack pine (43 pg hr$^{-1}$) at a similar time post spray. Finally, possible differences in the intensity of solar radiation at the time of flux

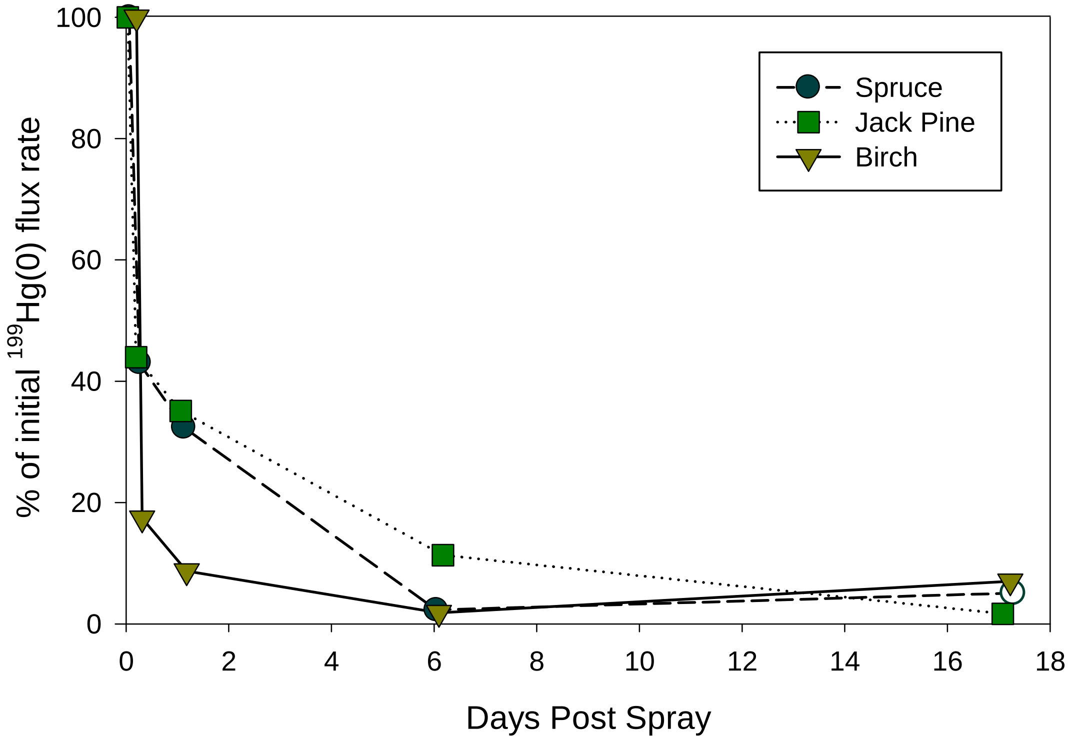

Figure 2.9 Relative fluxes of $^{199}$Hg(0) from the foliage of spruce, jack pine and birch trees sprayed with Me$^{199}$Hg to simulate a wet deposition event. Each flux rate is presented as a % of the initial flux rate for easy comparison of flux pattern amongst the trees over the two week sampling period. The flux rate measured from the spruce tree on day 17 was below detection limit and is indicated as such by an open, as opposed to shaded, symbol.

measurements might explain why the birch and spruce branches, which were fluxed back to back on the same day, had similar flux rates despite the different foliar concentrations, but lower than the flux rate from the jack pine branch, which was sprayed at a later date and therefore might have experienced different radiative conditions.

This study did not examine the mechanism behind the observed demethylation of $\mathrm{Me^{199}Hg}$ and subsequent volatilization of $^{199}\mathrm{Hg}(0)$ from plant foliage; however, the results of similar studies suggest that a photochemical reaction is largely responsible. MeHg in lake water is degraded primarily by solar radiation in the ultraviolet region (100-400 nm), and to a lesser extent by wavelengths in the visible region (400-700 nm; Sellers et al., 1996; Sellers et al., 2001; Hammerschmidt and Fitzgerald, 2006; Lehnherr and St. Louis, 2009). Furthermore, photodemethylation (PD) is thought to be the largest sink of MeHg in many lakes (Sellers et al., 2001; Hammerschmidt et al., 2006), although MeHg can also be microbially demethylated (Oremland et al., 1991; Marvin-DiPasquale et al., 2000; Schaefer et al., 2004). Microbial demethylation is unlikely to be of significance, in comparison to PD, on plant foliage that sustain relatively large amounts of incident solar radiation and harbor relatively low numbers of demethylating bacteria. A lack of lag-time prior to observed volatilization of $^{199}\mathrm{Hg}(0)$, that would be required for enzyme induction in a biotic reaction, further suggests that the acting mechanism is abiotic (Grigal, 2002).

Although previous studies have been able to demonstrate that photodemethylation is an important mechanism for loss of MeHg in lakes, it has not yet been established exactly how this reaction proceeds, nor have the end-products of the reaction been clearly identified (Sellers et al., 1996; Gårdfeldt et al., 2001). In our study, the use of stable isotopes allowed us to clearly demonstrate that, regardless of the reaction sequence, the end product of the above described (photo)demethylation of MeHg on canopy foliage is Hg(0), thereby making the overall process (photo)reductive. Because Hg(0) is rapidly evaded, (photo)reduction of MeHg represents a net loss of Hg from the canopy.

As our primary objective was not to determine absolute flux rates with the DFC, but rather to establish whether MeHg wet deposited to canopy foliage is volatilized from the canopy as Hg(0), and to determine whether there exist any differences in the pattern of this loss between different tree species, flux rates were standardized to the initial flux rate measured for each tree and only relative comparisons of how flux rates decreased over time were made between the three trees. We found that the two coniferous species, despite being fluxed on different days and therefore likely under different radiative conditions, showed a more similar flux rate *pattern* in comparison to the birch tree. Flux rates from the birch branch decreased more rapidly than those from either jack pine or spruce (Figure 2.9). Although our ability to make inferences about any differences associated with foliage type is limited by our small sample size, it does appear that foliage type has an effect on the rate of (photo)reduction of spike MeHg.

MeHg PD in water is known to be mediated by photosensitizing species such as nitrate or dissolved organic matter (Chen et al., 2003; I. Lehnherr, unpublished data) and the same is likely true on wet foliage surfaces following spray application. Similarly, dissolved organic ligands, such as humic acids, are involved in the photoreduction of Hg(II) in aqueous solution (Alberts et al., 1974; Allard and Arsenie, 1991), and Graydon et al. (2006) found that some component(s) of foliar surfaces likely acts as the required electron donor in this reaction thereby enhancing photoreduction and volatilization of Hg(II) from canopy foliage. Graydon (2003) further suggests that the reducing potential of foliar surfaces may be affected by foliage type. Foliar surfaces should likewise be a suitable source of the required organic matter and/or nitrates in PD reactions. Given the vastly different structures of birch leaves and coniferous needles it is possible that different amounts or types of photosensitizing agents and, in general, different micro-environments might arise on the foliage surfaces subsequently affecting PD rates. Foliage structure can also affect the amount of direct light received by foliar surfaces. The needle structure of coniferous trees is much more complex and the needles more densely packed than leaves on deciduous trees, creating more shading from direct radiation. In effect, dense foliage on conifers might reduce initial loss of MeHg, leaving more available for prolonged and elevated flux rates.

Because we could not simultaneously measure flux rates and the amount of spike remaining on each flux branch, it is not known whether the more rapid decline in flux rate measured from birch foliage results in less loss of spike MeHg via this pathway over time or, alternatively, whether the rapid reduction in the magnitude of the flux rate is a result of lower concentrations of spike remaining on foliage. However, previous studies that have measured photoreduction and evasion of Hg(II) spike from foliage (Graydon et al., 2006), as well as studies measuring PD rates in the water column (Sellers et al., 1996; Lehnherr and St. Louis, 2009), suggest that the observed rapid decline in flux rate over time is most likely representative of a first order chemical kinetics model whereby the magnitude of the flux is proportional to the amount of MeHg remaining on foliage and the amount of solar radiation received. If this is the case, the data suggest that (photo) reduction of newly wet-deposited MeHg and subsequent evasion as Hg(0) is initially more efficient on birch foliage than on either of the coniferous species, resulting in less available $Me^{199}Hg$ to be demethylated and evaded from birch foliage after a shorter period of time.

### *Long-term retention of MeHg*

To better understand the net effect of MeHg (photo)reduction, and any other potential mechanisms of loss, on MeHg retention in forest canopies following deposition, we monitored the long-term retention of $Me^{199}Hg$ on foliage of an additional 9 trees sprayed with $Me^{199}Hg$, and with $^{198}Hg(II)$ for comparison. The amount of spike Hg initially retained on foliage following spray application was similar for all trees (avg. $Me^{199}Hg = 6.3 \pm 1.5$ ng $g^{-1}$, avg. $^{198}Hg(II) = 5.7 \pm 1.1$ ng $g^{-1}$) and declined exponentially over the course of the study. Previous studies using stable Hg isotopes found the same first-order decline pattern for Hg(II) spike applied to both canopy (Graydon et al., 2006; Lindberg et al., 2003) and ground (Graydon et al., 2009) vegetation, as well as for ambient and spike MeHg in lake water (Sellers et al., 1996; Lehnherr and St. Louis, 2009). Prior to this study, however, a similar pattern had not been confirmed for MeHg on vegetation.

Short-term retention rate constants, $k_{\mathrm{MeHg}}$ and $k_{\mathrm{Hg(II)}}$, were obtained for each tree type from the slope of typical first-order kinetic plots (Figure 2.10). Average $k_{\mathrm{MeHg}}$ and $k_{\mathrm{Hg(II)}}$ ranged from -0.026 to -0.10 day$^{-1}$ and -0.031 to -0.098 day$^{-1}$ for Me$^{199}$Hg and $^{198}$Hg(II) respectively, with corresponding $t_{1/2}$ of spike retention on foliage of 27 ± 4.0 to 6.7 ± 2.0 days for Me$^{199}$Hg and 22 ± 3.7 to 7.1 ± 1.1 days for $^{198}$Hg(II) (Table 2.2). We found that Hg type did not have a significant effect on the retention rate of spike Hg on foliage (p=0.4516). However, reduction of foliar concentration of both Me$^{199}$Hg and $^{198}$Hg(II) occurred significantly faster for birch foliage than for either jack pine or spruce needles (p<0.001). Rates of decline of both Me$^{199}$Hg and $^{198}$Hg(II) on jack pine and spruce were similar (p=0.071). These results are consistent with previous studies that found Hg(II) spike was retained longer in coniferous canopies compared to deciduous canopies (Graydon et al., 2006; Graydon 2009).

By the end of the 2006 sampling season in late August, 34 ± 11% and 26 ± 12% of the initially retained Me$^{199}$Hg remained on spruce and jack pine foliage respectively. A similar portion, 26 ± 14%, remained on birch foliage at the time of leaf drop, 2 weeks earlier. Retention of $^{198}$Hg(II) was similar with 26 ± 5%, 14 ± 8%, and 31 ± 6%, remaining on spruce, jack pine and birch foliage, respectively. Comparing foliage collected from the spruce and jack pine trees early in the 2007 growing season with that collected at the end of the 2006 season, we found that the percentage of initially applied MeHg spike remaining on foliage had decreased slightly (spruce = 27 ± 9%, jack pine = 16 ± 6%), but not significantly so (paired t-test, *t*=2.182, DF=5, p=0.081). The amount of THg spike remaining on foliage had similarly decreased (spruce = 18 ± 7%, jack pine = 8 ± 3%); however, the difference in retention between the two seasons was significant (paired t-test, *t*=3.300, DF=5, p=0.021). Although, based on a typical exponential decline pattern, we were not expecting a large decline in foliar concentrations of either Hg spikes between these sampling events; the measured loss was less than expected given the calculated short-term rate constants and half lives. As a result,

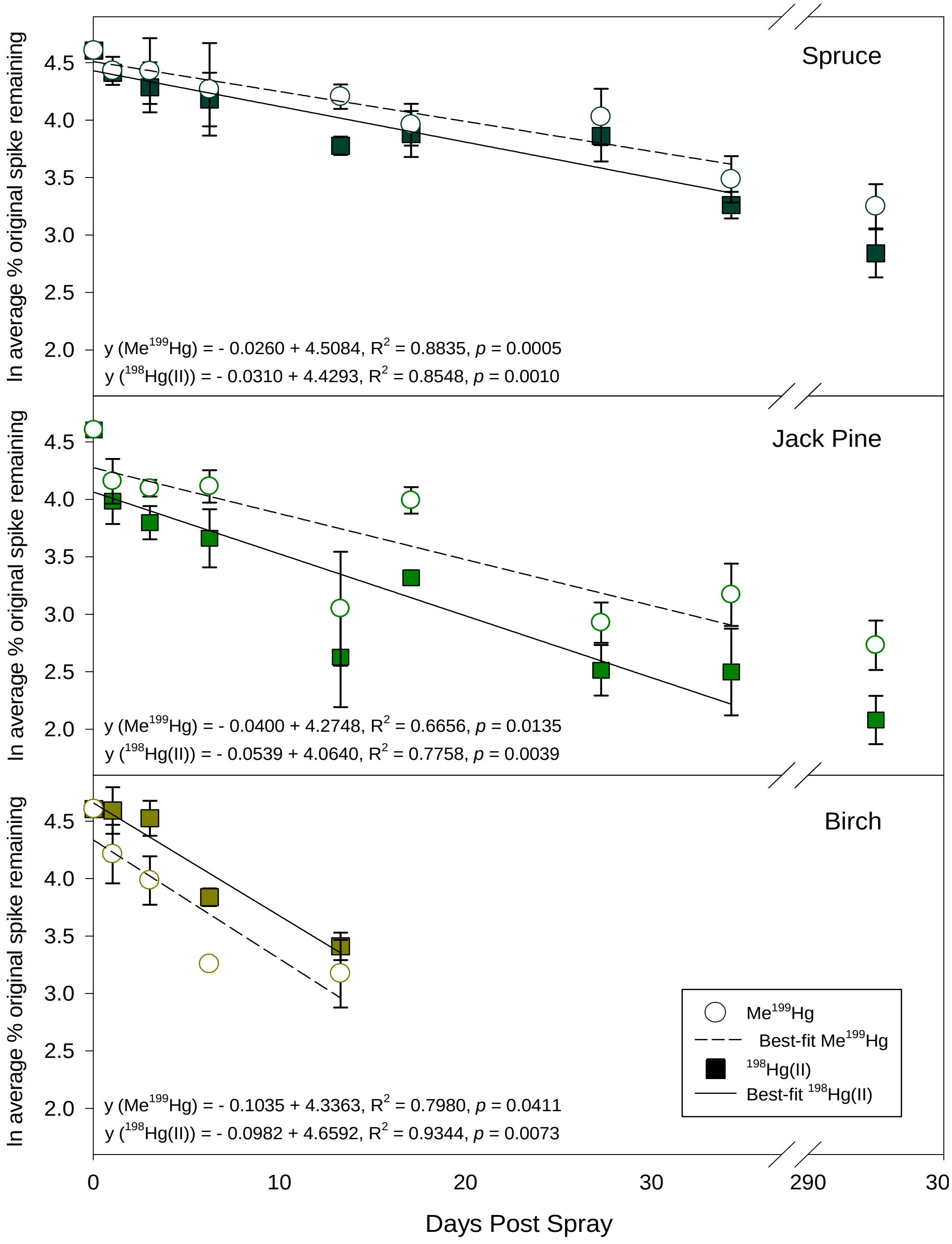

Figure 2.10 Retention of Me$^{199}$Hg and $^{198}$Hg(II) on spruce (n=3), jack pine (n=3), and birch (n=3) foliage in 2006. Data points are ln of % of initial post-spray spike Hg concentration retained on foliage at time of sampling (mean +/- S.D.). Data points from the single sampling event in 2007 for spruce and jack pine are included here for reference but were not included in the regression analyses.

Table 2.2 Retention rate constants (*k*) and half lives ($t_{1/2}$) of Me$^{199}$Hg and $^{198}$Hg(II) on foliage of 3 tree species (mean +/- S.D.) - based on 35 days (short-term) and 295 days (long-term) of monitoring following a simulated wet-deposition event.

| | Short-term | | Long-term | |
|---|---|---|---|---|
| Tree species | *k* (day$^{-1}$) | $t_{1/2}$ (days) | *k* (day$^{-1}$) | $t_{1/2}$ (days) |
| *Me$^{199}$Hg* | | | | |
| Birch | -0.10 | 7 ± 2 | - | - |
| Jack Pine | -0.040 | 17 ± 5 | -0.0042 | 165 ± 86 |
| Spruce | -0.026 | 27 ± 4 | -0.0036 | 193 ± 64 |
| *$^{198}$Hg(II)* | | | | |
| Birch | -0.098 | 7 ± 1 | - | - |
| Jack Pine | -0.054 | 13 ± 3 | -0.0054 | 128 ± 64 |
| Spruce | -0.031 | 22 ± 4 | -0.0046 | 151 ± 46 |

when the 2007 data are included in our regression models, the rate constants decrease and the half-lives increase substantially (Table 2.2). These long-term $t_{1/2}$s for both $Me^{199}Hg$ (spruce = 193 ± 64 days, jack pine = 165 ± 86 days) and $^{198}THg$ (spruce = 151 ± 46 days, jack pine = 128 ± 64 days) are consistent with previously reported half-lives for Hg(II) spike retention on foliage (Graydon 2009).

The prolonged retention of spike Hg on foliage following our initial sampling season is most likely a result of seasonal changes in environmental conditions. For the majority of time between the 2006 and 2007 sampling events, foliage experienced winter conditions. During this period, not only are the typically low temperatures likely to reduce chemical activity on plant surfaces, and therefore any chemical transformations and evasion of Hg spikes (Landa, 1978; Kim et al., 1997; Grigal 2002), but the reduced amount of solar radiation incident on foliage (from both lower sun angles and snow cover) could further decrease photoreduction rates. A comparison of the flux pattern shown above with that of foliar retention of MeHg suggests that photoreduction and subsequent evasion of Hg(0) is likely a significant mechanism of loss of MeHg from the canopy and could be affected by seasonal conditions.

In addition to any structural and/or chemical processes that might be causing a short-term difference in retention of Hg on birch leaves in comparison to jack pine and spruce needles, the annual senescence and loss of foliage by birch at the end of each growing season is responsible for the much larger long-term differences in retention of Hg between these species. While the short-term $k$ and $t_{1/2}$ values likely provide an accurate estimate of MeHg and Hg(II) retention on birch foliage, the long-term $k$ and $t_{1/2}$ values are a more accurate representation of retention on jack pine and spruce foliage. In contrast to deciduous species, such as birch, conifers retain foliage longer, dropping needles asynchronously after life spans of up to several years in most species (Richardson and Rundle, 1995; Bonan, 2002; Apple et al., 2002).

However, while our long-term $t_{1/2}$s for MeHg and Hg(II) agreed well with previous findings of Graydon (2009) for Hg(II) retention on coniferous trees, the approximately 7 day half-life of both MeHg and Hg(II) retention calculated here for birch is much shorter than the 110 ± 30 days previously reported for Hg(II) retention in deciduous canopies in the METAALICUS watershed. The difference in reported values between these two studies is likely a result of a combination of factors. For one, the METAALICUS watershed was typically sprayed just after leaf out in the spring (Sandilands et al., 2005) whereas our trees were not sprayed until mid summer. Furthermore, as previously mentioned, 2006 was a summer of extreme drought that caused early senescence of our birch foliage. The above combined resulted in a much shorter and highly skewed monitoring of Hg retention on our trees compared to the previous study. Secondly, while Graydon (2009) measured the Hg remaining on foliage of both deciduous and coniferous species over multiple growing seasons, we did not return to sample our birch trees beyond the first sampling season. While it was expected that the majority of $Me^{199}Hg$ and $^{198}Hg(II)$ initially retained on foliage would be lost at the time of leaf abscission, Graydon's (2009) results suggest that a portion of the applied spike was retained on the branches extending the retention of Hg in the canopy. Finally, the trees for our study were specifically selected due to their exposed locations and subsequent high exposure to incoming radiation. Study trees in the METAALICUS watershed were selected along transects and were generally shaded by neighbouring trees and foliage. If, as is suspected, photoreduction is an important mechanism of loss of Hg from canopy foliage, this could account for a potentially large portion of the differences observed between these two studies.

As a result, the short-term retention values reported here for birch foliage are likely underestimates of both MeHg and Hg(II) retention in the forest canopy. However, in all cases, coniferous foliage is shown to retain Hg longer than deciduous foliage. As suggested above, different rates in the photoreduction and retention of MeHg on canopy foliage can explain some of the differences observed among loadings under the different canopy types.

As the magnitude of $^{199}$Hg(0) fluxes declined more rapidly and tapered off more quickly than the observed decline in spike Hg retention on foliage, it is plausible that mechanisms of loss besides photoreduction and evasion might also be removing Hg from the canopy pool. As rain water and wind pass through the canopy, mechanical weathering could remove some portion of the MeHg and Hg(II) bound to the surface of foliage and/or pieces of leaves and needles with associated Hg. If this was occurring as rain passed though the canopy it could account for some of the MeHg and Hg(II) found in throughfall. Previous studies have found that in the initial period post deposition, this form of loss is extremely small for Hg(II) (Graydon et al., 2006, Graydon et al., 2009), however, this mechanism of loss may become more important over time as Hg and foliage become increasingly weathered.

If we assume that the spike Hg applied in this study behaves like newly deposited atmospheric Hg, then we can predict similar retention times for newly deposited atmospheric ambient MeHg and Hg(II) on foliage following deposition events. However, while our study followed retention of spike after a single deposition event, ambient Hg is intermittently deposited from multiple rain events throughout the year, and the portion of ambient Hg remaining on foliage at the time of abscission would depend on the balance between new inputs and loss mechanisms. Any retention of new atmospheric Hg on canopy foliage following deposition would result in delayed movement of both new MeHg and Hg(II) into the soil profile, into runoff, and finally into lakes. As litterfall is the predominant conduit of atmospheric Hg deposition at forested sites, movement into the soil profile of any remaining portion of newly deposited Hg will likely occur slowly via senescence of litterfall, further delaying movement from the atmosphere into aquatic food-webs.

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# *Chapter 3. General Conclusion*

The main goal of my research was to investigate the influence of terrestrial vegetation on the deposition and retention of atmospheric MeHg within boreal forest watersheds. Measures of MeHg in open area precipitation and throughfall have led some researchers to suggest that atmospheric deposition of MeHg may represent an important contribution to the MeHg loads observed in fish (Hultberg et al., 1994). However, our understanding of how terrestrial components of the watershed might affect this deposition has previously been limited to conclusions drawn from differences between loadings of MeHg in the open and under forest canopies. Prior to this study, our knowledge of the sources and fates of MeHg on canopy foliage has relied heavily on speculation.

Here, I have supported previous findings that MeHg deposition is enhanced under forest canopies compared to nearby open sites. Furthermore, I was able to show that the deposition enhancing effect of the canopy is underestimated by the standard comparison of loadings in throughfall + litterfall to that in the open as a result of the retention and subsequent photoreduction and evasion of a portion of newly deposited MeHg. Additionally, the retention of a portion of newly deposited MeHg in the canopy, and subsequently in litterfall prior to its decomposition, will delay movement of MeHg from the atmosphere to the soil pool, run-off, and ultimately into aquatic food-webs. However, our attempt to quantify p-MeHg as a source of MeHg to the forest canopy was unsuccessful and further work is required to understand all potential sources of MeHg being deposited to terrestrial landscapes.

Advancing our understanding of Hg speciation and mechanistics of the Hg biogeochemical cycle remains a challenge, especially in the atmosphere and in relation to MeHg (Lindberg et al., 2007; Selin, 2009). However, any increased knowledge in these areas would vastly improve our understanding of the role forests play in enhancing Hg deposition and, perhaps most importantly, how reductions in emissions of Hg will affect MeHg concentrations in fish. While ecosystem scale projects like METAALICUS, and its smaller sub-catchment counterpart at the ELA (Hintelmann et al., 2002; Graydon et al., 2009), are making use of enhanced stable isotopes of Hg(II) to elucidate the movement of Hg through various ecosystem compartments, they are also producing large amounts of samples that could further benefit our understanding of MeHg production and cycling within these same compartments. For example, a large number of archived foliage, litterfall, and ground vegetation samples that have previously only been analyzed for isotopic THg content could be analyzed for isotopic MeHg to further our understanding of sources and fates of both Hg(II) and MeHg within various forested ecosystem compartments. Specifically, it would be beneficial to know what portion of the MeHg in litterfall was also present on canopy foliage versus what might have been formed or adsorbed following abscission. Additionally, quantification of isotopic MeHg, if present, in foliage from the aforementioned sub-catchment experiment would also help to determine what portion of MeHg comes from a ground based, or recycled input source, as Hg isotopes were applied directly to the ground and not to the canopy as was done with METAALICUS. Making use of these archived samples has potential to greatly enhance our understanding of MeHg cycling at a limited cost as it would not require any further funding to accommodate the monetary demands of field based research.

To date, findings from METAALICUS suggest that the MeHg concentrations in fish may respond slowly to reductions in atmospheric emissions of Hg in watersheds with large watershed-to-lake area ratios (Harris et al., 2007). As proposed restrictions on Hg emissions are implemented, the dynamics of Hg cycling between the terrestrial and aquatic compartments of watersheds are likely to become increasingly important in terms of their overall effect on MeHg levels in fish due to the large pools of Hg and MeHg stored in soils. Furthermore, land-use changes as humans continue to migrate further into remote regions for recreation and resource exploitation will also have an effect on mobilization of both Hg and MeHg in forested catchments. For instance, forest harvesting activities are known to drastically increase Hg levels, particularly MeHg, in run-off water – a result of both increased mobilization of MeHg and increased methylation of Hg stored in soils (Porvari et al., 2003; Munthe et al., 2004). Projected climate changes in the boreal ecoregion are also likely to affect Hg export from uplands and wetlands into lakes. For example, extreme weather events, such as storms and heavy rainfalls, will have a similar effect to forestry practices, increasing both Hg and MeHg export (Munthe et al., 2007). Alternatively a projected increase in the incidence of forest fires in the boreal ecoregion would likely result in a return of the large stores of Hg retained in wetlands and upland soils to the atmosphere (Turetsky et al., 2006), reducing the run-off and MeHg export associated with these sites, but potentially causing large pulses of atmospheric deposition directly to the lake surface.

It is important to understand the role of canopy foliage in the net deposition of MeHg to watersheds, as a clear understanding of all aspects of the Hg biogeochemical cycle will be critical to effectively manage bioaccumulation of toxic MeHg in aquatic ecosystems.

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# *Appendix*

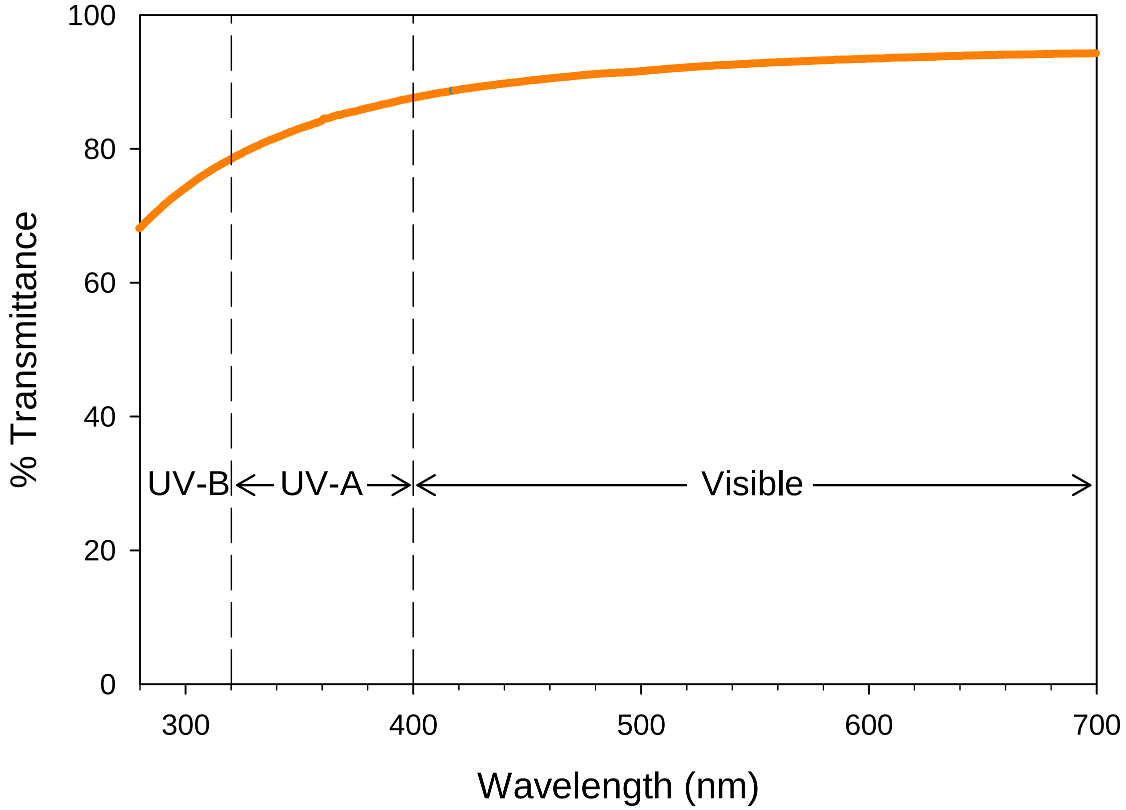

Figure A.1 Transmittance of UV-B (280-320 nm), UV-A (320-400 nm) and visible (400-700 nm) radiation through PFA-Teflon of DFC walls.