Methylmercury in Natural and Disturbed Wetlands

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June, 1996

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements of Doctor of Philosophy.

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ISBN 0-612-19730-1

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Abstract

Methylmercury (MeHg) is the most toxic species of mercury (Hg), and is an important ecosystem contaminant. In wetlands on the Canadian Shield, in NW Ontario, MeHg concentrations in peat and peat porewater ranged from 0.3 to 53 ng g^{-1} and < 0.1 to 7.3 ng l^{-1}, respectively. The greatest concentrations of MeHg occurred just below the water table, emphasizing the importance of redox reactions in Hg methylation. Methylmercury partition coefficients between peat and peat porewater ranged from 1.6×10^3 to 8.6×10^5. No significant correlations between MeHg and concentrations of H^+, NH_4^+, NO_3^-, NO_2^-, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), SO_4^{2-} and dissolved organic carbon (DOC) in the porewater of the wetlands were found.

Following shallow impoundment of a wetland, MeHg concentrations in the upper metre of peat porewater increased from 0.2 ± 0.2 ng l^{-1} to 0.8 ± 0.8 ng l^{-1}. Total mercury (T-Hg) and MeHg concentrations were determined in decomposing sedge, spruce needles, and *Sphagnum* moss, placed in a headwater wetland and the impounded wetland. The amount of T-Hg decreased in all tissues regardless of location. The amount of MeHg increased by as much as an order of magnitude in the tissues placed in the impounded wetland and wet areas (hollows and lawns) of the headwater wetland, but decreased in tissue placed in the dry areas (hummocks) of the headwater wetland. Therefore, it is during anaerobic decomposition of plant material that MeHg is produced in wetlands.

Incubations of peat were performed with addition of Hg, molybdate, SO_4^{2-}, S^{2-}, NH_4NO_3, pyruvate, and upland DOC. Methylmercury production was increased only after addition of SO_4^{2-} and retarded only by NH_4NO_3. Although SO_4^{2-} may not be required to methylate Hg, the increased availability of SO_4^{2-} may influence the size and composition of the population of sulfate reducing bacteria in peat, thereby increasing the potential for Hg methylation.

Résumé

Le méthylmercure (MeHg), forme la plus toxique du mercure (Hg), est un important contaminant des écosystèmes. Dans des milieux humides du Bouclier canadien, (nord-ouest de l'Ontario), les concentrations de MeHg dans la tourbe et l'eau interstitielle de tourbe variaient de 0,3 à 53 ng g^{-1} et $< 0,1$ à 7,3 ng l^{-1}, respectivement. Les plus fortes concentrations de MeHg ont été observées juste sous la nappe phréatique, ce qui montre bien que les réactions d'oxydoréduction jouent un rôle important dans la méthylation du Hg. Les coefficients de partage du méthylmercure entre la tourbe et l'eau interstitielle de tourbe variaient de $1,64 \times 10^3$ à $8,6 \times 10^5$. Aucune corrélation significative n'a été observée entre le MeHg et les concentrations de H^+, NH_4^+, NO_3^-, NO_2^-, d'azote total dissous (ATD), de phosphore total dissous (PTD), de SO_4^{2-}, et de carbone organique dissous (COD) dans l'eau interstitielle des milieux humides.

Dans les milieux humides faiblement inondés, les concentrations de MeHg dans le premier mètre d'eau interstitielle de tourbe sont passées de $0,2 \pm 0,2$ ng l^{-1} à $0,8 \pm 0,8$ ng l^{-1}. Les concentrations totales de mercure (T-Hg) et de MeHg ont été déterminées dans les carex, aiguilles d'épinette et sphaignes en décomposition placés dans un milieu humide d'amont et dans le milieu humide inondé. La concentration totale de mercure a diminué dans tous les tissus sans égard à l'environnement. La teneur en MeHg a augmenté d'un plein ordre de grandeur dans les tissus placés dans les milieux humides inondés et les dépressions humides des milieux humides d'amont, mais a diminué dans les tissus placés dans les zones sèches de ces mêmes milieux. La production de MeHg dans les milieux humides a donc lieu durant la décomposition anaérobique de la matière végétale.

On a procédé à des incubations de tourbe additionnée de Hg, de molybdate, de SO_4^{2-}, de S^{2-}, de NH_4NO_3, de pyruvate et de COD d'amont. La

production de méthylmercure n'a augmenté qu'après ajout de SO_4^{2-} et n'a été retardée que par ajout de NH_4NO_3. Bien que le SO_4^{2-} ne soit pas nécessaire à la méthylation du Hg, sa disponibilité accrue pourrait avoir un effet sur la taille et la composition de la population de bactéries sulfo-réductrices dans la tourbe et ainsi accroître le potentiel de méthylation du mercure.

Acknowledgements

I would like to thank Dr. John Rudd and Dr. Carol Kelly for including me in the Experimental Lakes Area Reservoir Project.

In support of my field research, I would like to thank: Dr. Nigel Roulet for the use of the piezometer network; Jan Slavajeck for the manufacture of the Teflon® wells and sipper; Brian Branfireun, Jean-Jacques Dogoua, Pauline Gerrard, Eric Mewhinney, Kathleen Savage, and Dr. Vince St.Louis, for sampling help; Ken Beaty and Mark Lyng for hydrology data and operating the reservoir; Dr. Jill Bubier and Dr. Tim Moore for the construction of litter bags.

In support of my laboratory work, I would like to thank: Dr. Robert Flett and Nicolas Bloom for aid in the development of analytical techniques; Barbara Gooding, Dinean McKay, and Alison MacHutchon for performing some of the T-Hg and MeHg analysis; Eva Schindler and the staff of the ELA chemistry laboratory for the analysis of nutrient chemistry; and Laud Matos and Mike Dalva for DOC measurements.

For their helpful discussions during the compilation of my thesis, I would like to thank Brian Branfireun, Neil Comer, Dr. Carol Kelly, Patricia Sellers, Dr. Vince St.Louis and especially my supervisory committee of Dr. Tim Moore, Dr. Alfonso Mucci and Dr. John Rudd. For editorial comments, I thank Neil Comer, Dr. Tim Moore and Dr. John Rudd.

This research would not have been possible without the financial support of Manitoba Hydro, and for that support I would especially like to thank Dennis Windsor. I would also like to thank Hydro Quebec for supporting the study of Hg speciation in decomposing plant tissues.

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Contributions to Knowledge

This thesis makes original contributions in four areas of our knowledge about MeHg cycling in wetlands: 1) the distribution of MeHg in pristine wetlands, 2) the impact of impoundment on the concentration and amount of MeHg in peat porewater, 3) the methylation of Hg during plant decomposition in pristine and impounded wetlands, and 4) the controls on the availability of MeHg for transport from wetlands and the production of MeHg in peat.

1) When this thesis was initiated, no measurement of MeHg concentration in "pristine" wetlands had been made. Even today, only one other study containing porewater concentrations of MeHg has been published. Methylmercury concentrations in peat and porewater vary by three orders of magnitude within and amongst wetlands. The highest MeHg concentrations in peat and porewater are located at the oxic/anoxic interface clearly indicating the importance of redox reactions in Hg methylation. Little was learned about the controls on MeHg production in the wetlands by correlating peat porewater chemistry variables with MeHg concentration. This result indicates just how complicated MeHg cycling is and emphasises the need for mechanistic studies.

2) Although impoundment is recognized as a cause of the increased MeHg burden of fish, the mechanism behind the increase is not known. In this thesis, the impact of impoundment on the porewater MeHg concentrations of a wetland is presented. Concentrations of MeHg increased by a factor of 5, clearly demonstrating that more Hg was methylated in the peat as a result of impoundment. Although the reason for the increase is not well understood, this thesis contains the only record of pre- and post-impoundment porewater MeHg concentrations in the literature and, as such, will be a valuable asset to modelling MeHg cycling in newly impounded systems.

3) The impounded biomass has been identified as a potentially important site of Hg methylation in new reservoirs. In this thesis, the change in the amounts of T-Hg and MeHg in decomposing plant tissue were observed in pristine and impounded wetlands. This is the first study of its kind and demonstrates that MeHg is produced during anaerobic decomposition in both wetlands and reservoirs.

4) Two important controls on MeHg availability and production were identified. It was found that with increasing peat MeHg concentration, the amount of MeHg partitioned to the peat porewater increased disproportionately. This means that in wetlands where MeHg concentrations in peat are high, more MeHg, and perhaps even T-Hg, is available for export. By experimentation, Hg methylation in peat could only be stimulated by addition of SO_4^{2-}. Although the idea that sulfate reducing bacteria (SRB) are important methylators of Hg is not new, this is the first evidence which implicates SRB as the most important group of Hg methylating bacteria in pristine and impounded wetlands.

Chapter 1: The Importance of Wetlands in Cycling Mercury and Methylmercury in Catchments

1.1 Introduction

1.1.1 Sources of Mercury

Mercury (Hg) is a trace element that occurs as a variety of species and valences (e.g., Hg^o, Hg^{2+}, CH_3Hg^+, HgS) in the atmosphere, rocks, soils, and living organisms (Andren and Nriagu, 1979). Hg^o is volatile and gaseous Hg^o is readily dispersed through the atmosphere (Ferrara et al., 1982; Matheson, 1979; Schroeder, 1992; Schroeder et al., 1992). As Hg^{2+}, Hg readily binds with organic complexes and accumulates in the organic horizons of soils and lake sediments (Andersson, 1979; Bargagli et al., 1988; Bothner et al., 1980; Rada et al., 1989; Sorensen et al., 1990). The association of Hg with organic carbon is important because it facilitates the incorporation of Hg into the food chain, particularly in the form of CH_3Hg^+ (monomethylmercury) (Meile, 1991a). Although monomethylmercury (MeHg) can form abiotically, environmental occurrences are primarily the result of microbial production (Winfrey and Rudd, 1990; Xu and Allard, 1991). Where oxidized sulfur species are reduced, such as in lake and estuarine sediments, reduced sulfur species effectively scavenge Hg, forming HgS which remains stable in low redox environments (Dyrssen and Wedborg, 1991).

Prior to industrialization, additions to the pool of Hg cycling at the earth's surface were limited to mineral weathering and volcanoes (Varekamp and Buseck, 1981). Industrial activities, such as those involving coal burning, cement production, human cremation, dental amalgams and garbage incineration, release large amounts of Hg to the atmosphere (e.g., Fukuzaki et al., 1986; Lindberg, 1980; SEPA, 1991; Shieh, 1992). The amount of Hg released by industry has led many to suggest the annual atmospheric load of Hg has doubled in the past few decades (e.g., Appleqvist et al., 1978; Carr and Wilkniss, 1973; D'Itri, 1972;

Engstrom et al., 1992; Jensen and Jensen, 1991; Vandall et al., 1991; Weiss et al., 1971). Although some of these studies estimating the long term change in Hg deposition are suspect (Meile, 1995; Sheppard et al., 1991), together they form a strong case and indicate industrialization has greatly increased the amount of Hg cycling in the environment.

Atmospheric deposition of Hg over the earth's surface is not uniform. Deposition of Hg in areas far from industrial activity, such as northern Wisconsin (USA) and northwestern Ontario (the Experimental Lakes Area), is far less than in Europe (Håkanson, 1990; Iverfeldt, 1991; Meile, 1991b; Sorensen et al., 1992; St.Louis et al., 1995). However, remote areas have not entirely escaped anthropogenic Hg deposition. Lucotte et al. (1995) estimate industrial sources contribute 20% of the annual Hg input to northern Quebec, and St.Louis et al. (1995) found the largest Hg deposition events in N.W. Ontario (Experimental Lakes Area) occurred from storms which tracked across the industrial N.E. United States. Deposition of anthropogenic Hg is blamed for the contamination of fish in many lakes of Scandinavia (e.g., Håkanson et al., 1990; Hultberg and Iverfeldt, 1992). In eastern North America, where anthropogenic Hg deposition is suspected to be high, a large number of lakes also contain sport fish with high levels of Hg (OME, 1988; Wiener, 1987). However, Hecky (pers. comm., 1994) found Hg concentrations in fish of northern and central Canadian lakes had not increased in the past 20 years and there is no evidence for the Hg contamination of fish or marine mammals in the Canadian arctic (Wagemann et al., 1995).

Discerning the impact of anthropogenic Hg deposition on a region is confounded by the fact that Hg levels in organisms, and in particular fish, are not consistent among lakes of the same region (Bodaly et al., 1993; Driscoll et al., 1994). Therefore, local factors also influence the availability of Hg to the food chain. Despite the site to site variability, where anthropogenic Hg deposition is minimal, few organisms are endangered by the amount of Hg present. Therefore, prior to industrialization it is likely the amount of Hg cycling presented no threat to organisms at the upper end of the food chain.

Large but localized releases of Hg have also resulted in contamination of both terrestrial and aquatic ecosystems (e.g., Bertani et al., 1994; Lacerda, 1995; Maserti and Ferrara, 1991; Parks et al., 1986). Water bodies adjacent to large concentrations of industry such as Onondaga Lake, NY and Minimata Bay, Japan have been heavily contaminated with Hg (e.g., Klien and Jacobs, 1995; Mitra, 1986). In remote areas, pulp and paper mills have contaminated water ways such as the English-Wabigoon system in northwestern Ontario and Hg released from old and present gold mining operations have contaminated rivers in North Carolina, Nevada, and Amazonia (e.g., Callahan et al., 1994; Craig and Morten, 1983; Rudd et al., 1983; Veiga and Meech, 1995). In fact, it was the localized contamination of food sources with Hg which alerted scientists to the potential threat posed by the general increase in Hg levels in the environment (Mitra, 1986).

The majority of Hg released to the environment is in the form of inorganic Hg-X species (e.g., Lindqvist and Rodhe; 1985; Nriagu, 1989; Pleijel and Munte, 1995). However, up to 95% of the Hg in fish is MeHg (Bloom, 1992). Only in Scandinavia have large amounts of anthropogenic MeHg deposition been observed (Håkanson et al., 1990; Hultberg and Iverfeldt, 1992). In order for MeHg concentrations to become elevated in fish of catchments receiving primarily pluvial inorganic Hg, it must be converted to MeHg within catchments. Prior to 1988, lacking the ability to measure MeHg at environmental concentrations, researchers tried to link the total amount of Hg present in sediment or water with Hg concentrations or burdens in fish (e.g., Bodaly et al., 1991; Håkanson, 1990; Johnston et al., 1991). This approach required the assumption that all Hg species behave the same. Collectively the results from these studies are difficult to interpret. This is most likely because the total amount of Hg (T-Hg) is not always a good predictor of the presence of MeHg which is the Hg species that bioaccumulates in fish (Kelly et al., 1995). With the development of analytical methods, by Bloom (1989) and Horvat et al. (1993), to measure sun part per trillion MeHg concentrations in water and sediments, the biogeochemical cycling of MeHg can now be studied.

Lakes receiving large amounts of Hg through atmospheric deposition or from point sources are not the only water bodies where Hg levels in fish are high. It has been well documented by Bodaly et al. (1984) that MeHg concentrations in fish have risen significantly in Canadian water bodies enlarged to create reservoirs. Fish obtain the vast majority of MeHg from their food (Hall et al., 1996). Therefore, more MeHg must be present in the post impoundment food chain of fish than the pre impoundment food chain. This may be the result of a change in food chain structure and/or more MeHg entering the food chain below the level of the fish. It has been hypothesized by Hecky et al. (1991) that the MeHg in reservoir fish originated in the decomposing vegetation. But even with the obvious impact impoundment has on MeHg concentrations in fish, it remains unclear how and where MeHg is formed in reservoirs and how the MeHg is transferred to the fish.

1.1.2 Sites of Hg Methylation in Lakes: an evolution in methods

Mercury methylation occurs in both the water column and sediments of lakes (e.g., Gilmour et al., 1992; Matilainen, 1995; Rudd et al., 1983; Watras and Bloom, 1992; Winfrey and Rudd, 1990). The maximum rates of Hg methylation occur at the oxic/anoxic interface, which is most commonly the sediment water interface (e.g., Compeau and Bartha, 1984; Jackson, T.A. 1988; Regnell, 1990; Rudd et al., 1983; Xun et al., 1987). The traditional method of measuring the Hg methylation rate requires the addition of radioactive $^{203}Hg^{2+}$ (Furutani and Rudd, 1980). Until recently, the specific activity of the $^{203}Hg^{2+}$ used was low and, to detect its methylation, required the addition of $^{203}Hg^{2+}$ at concentrations much higher than background Hg concentrations. As the MeHg may have been produced in pathways not normally present in uncontaminated systems, the conclusions of these studies are limited to expressions of Hg methylating potential.

By applying essentially the same method as Furuteni and Rudd (1980), but high specific activity $^{203}Hg^{2+}$, methylation of Hg has been observed at Hg concentrations very close to ambient levels (Gilmour and Riedel, 1995; Matilainen, 1995; Stordal and Gill, 1995). These studies verified the conclusions of the earlier low specific activity $^{203}Hg^{2+}$ studies, but still have the undesirable requirement of adding Hg which may be more or less available for methylation than *in situ* Hg.

Measurement of net changes in the ambient MeHg concentration of incubated lake sediments and water have also demonstrated that both anaerobic sediment and water are sites of MeHg production (e.g., Gilmour and Henry, 1991; Gilmour et al., 1992; Watras and Bloom, 1992). This method verifies that it is *in situ* Hg that is methylated and identifies the degree to which a site is a source of MeHg, but yields less information about the dynamics of the methylation processes.

1.1.3 A Mechanism for Hg Methylation

A number of microorganisms have been identified that can methylate Hg, including sulfate reducing bacteria (SRB) and fungi (Compeau and Bartha, 1985; Fisher et al., 1995; Gilmour and Henry, 1992; Landner, 1971; Wood et al., 1968; Yamada and Tonomura, 1972). Of the organisms identified that methylate Hg, it has been demonstrated that some SRB are important Hg methylating organisms in lake and estuarine sediments (Choi and Bartha, 1994; Compeau and Bartha, 1985;

Gilmour et al., 1992). A pathway by which Hg is methylated in a common SRB (*Desulfovibrio desulfricans* LS.) has been proposed by Choi et al. (1994). During the fermentation of simple carbon compounds, a methyl group is transferred from methylcobalamine to inorganic Hg while synthesizing acetyl-CoA. The pathway identified is one in which acetyl-CoA is generally used to oxidize acetate but the pathway is bidirectional and is used in reverse by some SRB to manufacture acetyl-CoA. It appears that in *Desulfovibrio desulfricans* LS., Hg is methylated when the pathway is used to produce acetyl-CoA. However, SRB that do not use this pathway to produce acetyl-CoA have also produced MeHg (Sparling pers. comm., 1996). No pathway requiring SO_4^{2-} has been identified and in pure culture the presence of SO_4^{2-} can inhibit Hg methylation (Sparling pers. comm., 1996). In the environment, the opposite appears to be true. Increasing the supply of SO_4^{2-} to lake sediments initially increases MeHg concentration and production (Gilmour et al., 1992, Gilmour and Riedel, 1995). However, SO_4^{2-} need only be elevated a few mg l^{-1} before the availability of carbon is observed to limit Hg methylation (Choi and Bartha, 1994; Gilmour et al., 1992). Although SO_4^{2-} is not required to methylate Hg, increased SO_4^{2-} availability may allow some SRB to better compete for carbon (Lovley and Klug, 1983).

1.1.4 Demethylation of MeHg in Lakes

Once formed, MeHg is chemically stable, but MeHg may be destroyed photochemically and microbiologically (Ramlal et al., 1986; Sellers et al., 1996; Spangler and Spigarelli, 1973). Methylmercury is broken down by a wide range of microorganisms, with volatile end products being Hg°, CO_2, and CH_4 (e.g., Oremland et al., 1995; Ramlal et al., 1986; Spangler and Spigarelli, 1973). The end products of the UV photo degradation of MeHg have yet to be identified (Sellers et al., 1996). Whether photochemical or microbiological demethylation is more important depends on the medium in which the MeHg is located. It has been implied, but never proven, that MeHg demethylation is widespread in the water column. Sellers et al. (1996) suggest demethylation in the water column is primarily the result of photo degradation, which could explain the aerial extent over which MeHg demethylation is thought to occur in the water column. Since UV light rarely penetrates to the lake bottom, demethylation in sediments is most likely biological.

From the previous discussion, it is obvious that there are a number of ways to increase the amount of MeHg in the fish of a lake. The MeHg input to a lake may be increased, internal Hg methylation can be increased, the rate of MeHg demethylation can be reduced, or the food chain structure may be altered improving the transfer of MeHg to fish. Although the four are unlikely to be mutually exclusive, I will focus on the first two for the remainder of this discussion.

1.1.5 The Role of Wetlands in MeHg Cycling in Watersheds

Recent work on Hg and MeHg cycling in watersheds has focused on the water column and sediments of lakes, with little attention paid to the catchment area. It has been well documented that wetlands significantly influence downstream water chemistry through metal fixation, adding and modifying organic acids, and leaching organometallic complexes (Buffle, 1988; Gambrell, 1994; Gilliam, 1994; Thurman, 1985). Although it has been shown that peat traps Hg, and attempts to use this quality to quantify increases in anthropogenic inputs have been made (Lucotte et al., 1995; Madsen, 1981), no study has been made of the biogeochemistry of Hg in wetlands. From what has been learned about Hg methylation in lake sediments, it would appear wetlands have the biogeochemical setting suitable for the production of MeHg and the hydraulic conditions for its effective export (e.g., Boelter, 1965; Furutani and Rudd, 1980; Gilmour and Henry, 1991; Ponnamperuma, 1972; Winfrey and Rudd, 1990). Unfortunately, a lot less is known about the microbial communities that inhabit wetlands than those which inhabit lake sediments.

Despite not examining Hg speciation in wetlands specifically, a number of recent observations have indicated wetlands may be important sites of Hg methylation and sources of Hg and MeHg to downstream lakes. It has been observed that Hg concentrations are higher in lakes receiving large amounts of allocthonous dissolved organic carbon (DOC) (brown water lakes) than lakes receiving smaller amounts of DOC (clear water lakes) (Lee and Hutlberg, 1990; Meile, 1991; and Mierle, 1990). St.Louis et al. (1994) observed catchments containing wetlands released 17 times more MeHg than purely upland catchments and that this MeHg must have originated in the wetland. The importance of wetlands to Hg contamination of fish became apparent when Driscoll et al. (1994) reported the amount of shoreline wetland in the drainage basin of Adirondack lakes was positively correlated with MeHg in fish. Tranvik (1988) and Tulonen et al. (1992) have demonstrated that terrestrial DOC is an important carbon source for microorganisms. Therefore, MeHg exported from wetlands bound to DOC may represent a pathway for MeHg to enter the food chain. These observations are important to Canadian fish stocks. Approximately 14% of the Canadian land mass is classified as wetland and the vast majority is located in northern Canada where fish are an important food and income source (National Wetlands Working Group, 1989).

1.1.6 Methylmercury in Reservoirs

The decomposition of plant material following impoundment results in the depletion of oxygen from the lower water column and the formation of anoxic conditions in the newly impounded sediments (Jackson, 1988). The presence of a large amount of undecomposed plant material in anoxic conditions at the bottom of a reservoir is quite similar to the biogeochemical environment of a wetland (Ponnamperuma, 1972). Also, a significant portion of the land impounded by hydroelectric developments in northern Canada is wetland. Since wetlands are potential sources of MeHg, the obvious question is: do wetlands contain enough MeHg to contaminate the fish population of a reservoir?

A number of factors may promote the release of MeHg from peat to the overlying water column. Groundwater seepage through the submerged soils will be minimized because of the lack of any significant hydrologic gradient (Winter, 1995). Mixing between reservoir water and soil water will therefore depend on the degree of disturbance and the hydraulic conductivity of the mediums. Near surface peat has a greater hydraulic conductivity than most soils and lake sediments (e.g., Boetler, 1965; Lee et al., 1980; Munter and Anderson, 1981). Peat also has the potential to float, and is easily eroded (Rönkä and Uusinoka, 1976). Therefore, characteristics of peat are likely to better facilitate the transfer of MeHg to the water column, either affixed to DOC or to organic particles (POM), than other soils. Both DOC and POM are food for bacteria and benthos, and a means by which MeHg can be transferred to the food chain (Tranvick, 1988, Tulonen et al., 1992). It is also possible that the conditions created by impoundment accelerate Hg methylation in peat and increase the amount of MeHg affixed to these particles (Hecky et al., 1991).

1.2 The Goals and Location of Research

1.2.1 Summary of Research Objectives

Understanding MeHg dynamics in pristine and impounded wetlands will aid in understanding the larger problem of Hg contamination of fish. The specific aims of this research are:

i) determine the distribution of T-Hg and MeHg concentrations in peat and MeHg concentrations in peat porewater of a number of remote wetlands and examine the controls on this distribution by comparing porewater MeHg concentration with other porewater chemistry variables (Chapter 2);

ii) by determining the distribution and concentration of MeHg in the porewater of a riparian wetland before and after impoundment, assess the change in MeHg concentration in porewater brought on by impoundment and determine if the change in the MeHg concentration in peat porewater is related to other porewater chemistry variables (Chapter 3);

iii) examine how different biogeochemical environments affect T-Hg and MeHg concentrations of plant matter during decomposition by monitoring the amount of T-Hg and MeHg in three types of plant tissue as they decompose in different environments of a pristine and impounded wetland (Chapter 4);

iv) examine the controls on the production and release of MeHg from peat in laboratory incubations (Chapter 5).

In Chapter 6, the results of these studies will be synthesized and the importance of these results discussed in the broader context of MeHg cycling in natural and impounded wetlands, with the conclusions reiterated in Chapter 7.

1.2.2 Location and Framework of Research

This study was conducted as part of the Experimental Lakes Area Reservoir Project (ELARP) at the Experimental Lakes Area (ELA) located just east of Kenora, Ontario (49° 40' N, 93° 43' W) (Figure 1-1a). The ELA has a geology and ecology typical of the boreal forest on the Canadian Shield (Brunskill, 1971). Winters are cold and summers are warm, with mean daily temperatures in January of -18°C and July of 18°C. Winters are dry with an average snowfall of 25 mm per month contributing to an average annual snowpack of 150 cm. Summers are wetter with thunderstorms supplying most of the 80 mm of average monthly precipitation. Average total precipitation for the year is 623 mm (Beaty and Lyng, 1989).

The purpose of ELARP is to examine the effect of flooding of a wetland watershed on Hg and carbon cycling. A riparian wetland (Figure 1-1b) containing a central pond (Lake 979) was flooded in June, 1993 and the impact of impoundment has been monitored until 1995. Prior to impoundment, the riparian wetland was a treed bog with *Picea mariana* (black spruce) dominating the bulk of the canopy and some *Larix laricina* (tamarack) near the pond edge. The understorey was composed of hummocks, dominated by *Sphagnum fuscum* and shrubs (mainly *Ledum groenlandicum* but also *Chamaedaphne calyculata*), and open hollows, dominated by *Sphagnum magellanicum and Sphagnum angustifolium*. The wetland is surrounded on three sides by steep faces of open granite bedrock, which are interrupted by treed islands of *P. mariana* and *Pinus banksiana Lamb*. (jack pine). On the west side, shallow soils covered with *P. banksiana* and mosses extend up to 50 m from the peat margin to the granite rock face. A long arm isolated from the main part of the wetland extends 350 m to the NE. It contains essentially the same vegetation as the main part of the wetland although the presence of *Sphagnum fallax* in the bryophyte community indicated some areas of the arm were wetter (Bubier, pers. comm., 1994).

The hydrology of the central pond (Lake 979) is dominated by flow from Lake 240, and the water residence time in the pond is only a few days at normal flows. The reservoir is filled each spring and lowered each fall to prevent ice damage to the control structure and to mimic the operation of a hydroelectric reservoir. When impounded, the pond area increases from 23,800 to 155,000 m^2, the pond volume increases from 16,500 to 122,400 m^3 and the maximum depth in the central pond increases from 1.2 to 2.5 m. Measurements of water chemistry including MeHg and T-Hg concentrations in the water column and peat porewater, and physical parameters such as peat and water temperature and depth of light penetration, were made in 1991 and 1992 to provide background information.

A headwater wetland catchment containing a central pond (Lake 632) was monitored from 1991 to study MeHg and carbon cycling in a pristine wetland (Figure 1-1c). The wetland covers 42,500 m^2 and is located at the bottom of a 402,000 m^2 watershed. The peatland is mainly an ombrotrophic bog covered by *P. mariana* and the occasional *L. laricina*. The understorey is composed of *C. calyculata* and *L. groenlandicum* on *S. fuscum*-dominated dry hummocks with wet hollows dominated by *S. angustifolium*. Two small areas of the wetland are treeless poor fens. The fens were a mixture of the same hummocks and hollow terrain but contained large lawns of *S. angustifolium, S. magellanicum, and S. fallax* that were broken by sparse stands of *C. oligosperma*, in the inflow fen, but by a substantial stand of *C. rostrata* at the outflow fen (Figure 1-1). Surrounding the wetland, the upland is a complex mosaic of open granite bedrock areas with treed islands (*P. banksiana* , *P. mariana*) and mixed forests (*P. banksiana, P. mariana, Betula papyrifera*) on shallow soils derived from glacial deposits covered with *Sphagnum* and moss mats. In the middle of the wetland is a small central pond (Lake 632) having an average depth of 1 m, area of 8600 m^2, and total volume of 6300 m^3.

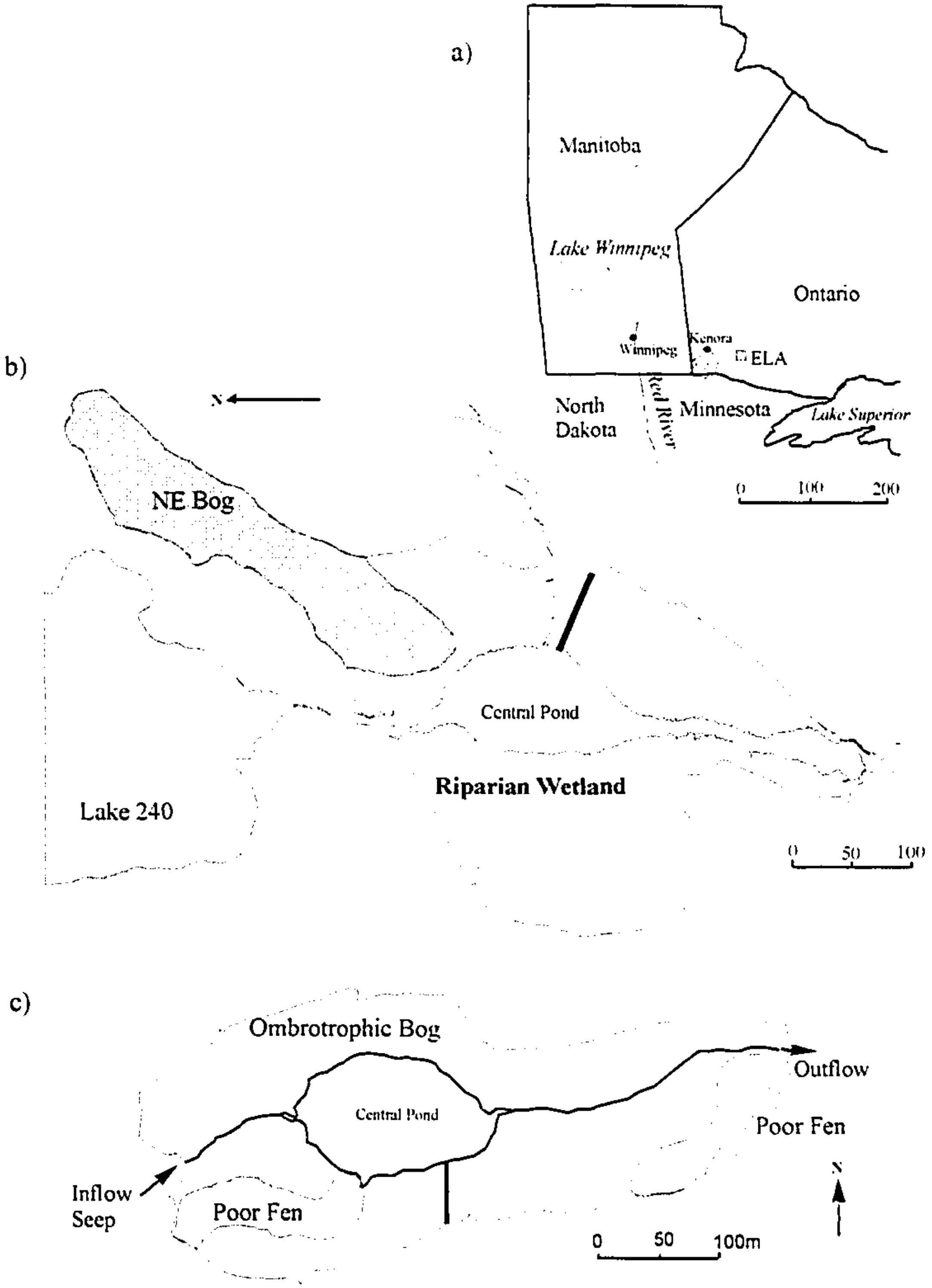

Figure 1-1) The location of a) the Experimental Lakes Area and sketch maps of b) the riparian wetland (catchment 979) and c) the headwater wetland (catchment 632).

Chapter 2: Distribution of Methylmercury in Remote Wetlands

2.1 Introduction

Wetlands influence many aspects of watershed chemistry such as modifying water pH (by contributing organic acids) and retaining metals and nutrients or changing their speciation (e.g., Eckhardt and Moore, 1990; Gambrell, 1994; Gilliam, 1994; Mitchell et al., 1995; Thurman, 1985). The presence of wetland in watersheds has also been linked to high methylmercury (MeHg) concentrations in fish, but the reason has not been established (Driscoll et al., 1994). Wetlands could be sources of MeHg or supply nutrients or Hg, thus stimulating Hg methylation in lakes (Miskimmin et al., 1992). Recently, wetlands have been shown to influence Hg export and Hg speciation (St. Louis et al., 1994; Westling, 1991). In a study of three boreal forest catchments at the Experimental Lakes Area (ELA), in northwestern Ontario, St.Louis et al. (1994) observed two wetland catchments released up to 17 times more MeHg than a catchment containing only upland. The MeHg exported from wetland catchments was greater than the combined inputs of precipitation and runoff. To continually export MeHg, wetlands must be a substantial source of MeHg or a site of Hg methylation.

Studies of MeHg cycling have focused on lakes and estuaries and principally the generation of MeHg in their sediment (e.g., Furutani and Rudd, 1980; Gilmour and Henry, 1991; Winfrey and Rudd, 1990). In lake and estuarine sediments, MeHg concentration and production is influenced by environmental factors such as redox potential, pH, temperature, chemistry, available nutrients, and amount and type of DOC present, although the influence of individual factors is not well known (Choi and Bartha, 1994; Compeau and Bartha, 1984; Craig and Morton, 1983; Gilmour et al., 1992; Miskimmin et al., 1992; Winfrey and Rudd, 1990; Wright and Hamilton, 1982). These same factors vary within individual wetlands, and even more so among different wetland types (e.g., Nörrstrom, 1994; Ponnamperuma, 1972; Rashid, 1974; Thurman, 1985). How these factors may influence MeHg concentration and production in wetlands is unknown.

In lake sediments and rivers, the majority of Hg is fixed to particles, and only small proportions of the T-Hg present exist in the dissolved form (Mucci et al., 1995; Parks et al., 1986). Likewise, MeHg will be partitioned between peat and peat porewater. For MeHg to be exported from wetlands at concentrations observed by St.Louis et al. (1994), a considerable amount of MeHg must be available for export, but the partition between the peat and peat porewater is not known. Wetlands also receive and redistribute nutrients through the porewater. Therefore, the peat porewater provides a means to examine the distribution of MeHg within and among wetlands, and provides insight into what controls the concentration and production of MeHg in wetlands.

In this chapter, I describe the distribution of MeHg and T-Hg in the peat and peat porewater of seven different Precambrian shield wetlands. I discuss the partition between MeHg in peat and peat porewater and correlations between the MeHg concentration and porewater chemistry (pH, DOC, TDP, NO_2^-, NO_3^-, NH_4^+, SO_4^{2-} and conductivity).

2.2 Study Sites

2.2.1 The Wetlands

Seven wetlands were studied at the Experimental Lakes Area (ELA), in northwestern Ontario, that are typical of the region. The wetlands will be referred to as: ombrotrophic bog, poor fen, riparian wetland, northeast bog (NE bog), riverine wetland, upland bog, and Eagle marsh. This group of wetlands does not cover all wetland types in the region but is typical of wetlands found in the ELA

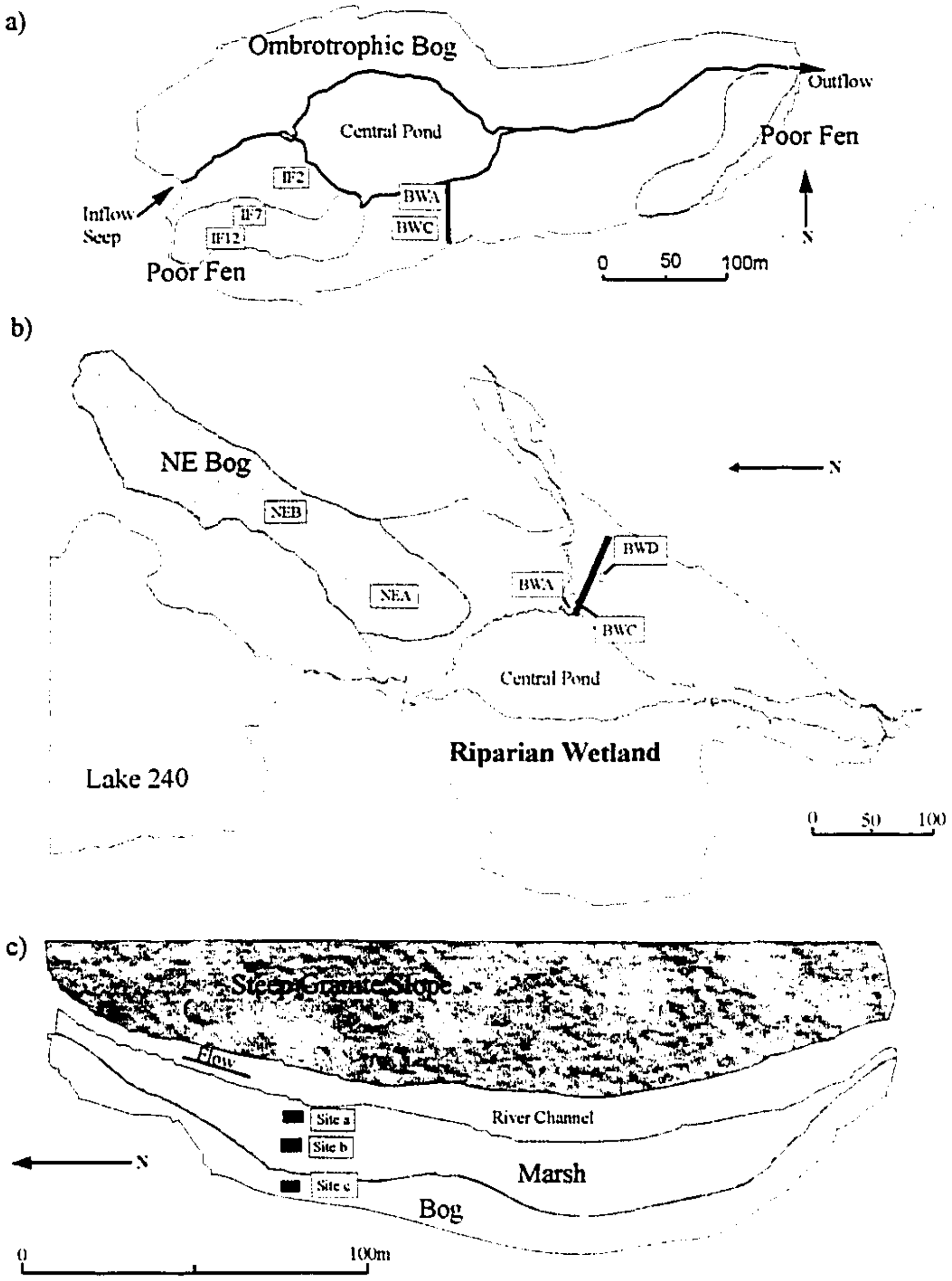

Figure 2-1) Sketch maps of: a) the headwater wetland, containing the poor fen and ombrotrophic bog, b) the riparian wetland and NE bog, and c) the riverine wetland.

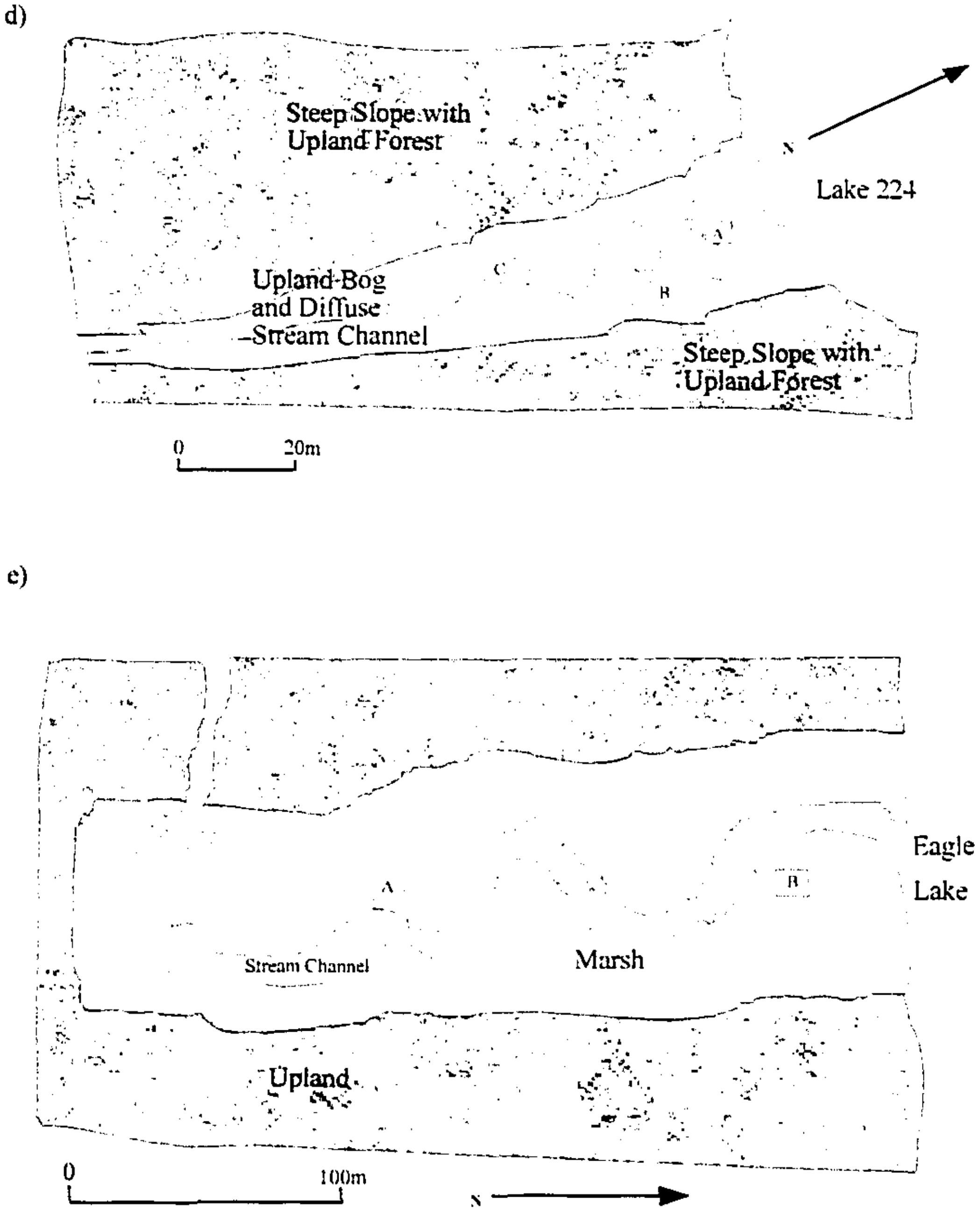

Figure Cont. 2-1) Sketch map of: d) upland bog, located at the S.W. inflow of lake 224, and d) the Eagle Marsh, located at the S inflow of Eagle Lake.

area.

A 42500 m^2 headwater wetland (Figure 2-1a) was the site of the ombrotrophic bog and the poor fen and contains a small central pond (lake 632). The wetland is fed by a 0.4 km^2 forested watershed. The headwater wetland is mainly an ombrotrophic treed bog (*P. mariana* and *L. laricina*). The bog surface is made up of *S. fuscum* hummocks, partially covered with *C. calyculata* and *L. groenlandicum*, and *S. magellanicum* hollows. The upper metre of ombrotrophic peat is poorly decomposed, regardless of whether it is beneath the hummocks or hollows.

In areas of the headwater wetland where groundwater emerges and/or where upland runoff flows across the wetland, poor fens have developed. A poor fen at the inflow of the headwater wetland covering about 5% of the wetland surface was studied (Fowle, 1995). The fen is treeless, and the surface made up of *S. fuscum* hummocks separated by small (< 1 m^2) hollows. The hollows are primarily covered by *S. magellanicum* and contain sparse stands of *C. oligosperma*. The hollows are inundated with water for much of the year and support the growth of algae. The peat is poorly decomposed beneath the hummocks but well decomposed beneath the hollows.

The riparian wetland is an ombrotrophic treed bog bisected by a 0.023 km^2 central pond (Lake 979) (Figure 2-1b). The pond is fed by Lake 240 and empties into Lake 663. The wetland, not including the NE arm, covers 0.12 km^2 and drains a 0.98 km^2 forested watershed. This wetland was impounded in the summer of 1993. Large areas of the bog are covered with *P. mariana* but the central pond is surrounded by *L. laricinia*. The wetland surface is composed of a series of hummocks and hollows primarily covered by *S. fuscum* and *S. magellanicum* respectively. The hummocks were often covered by *C. calyculata* and *L.*

groenlandicum.

The 0.04 km^2 NE bog is the north east arm of the riparian wetland. Precipitation, runoff from the steep rock faces, and groundwater from faults in the granite supply water to the wetland. The hydraulic gradient is from the arm to the central pond and therefore the biogeochemistry of the NE bog is unlikely to be influenced by the riparian wetland (Roulet, pers. comm. 1994). Although the composition of the vascular plant and bryophyte communities of the NE bog is the same as the riparian wetland, the distribution is not. Few *L. laricina* are present and *S. fallax*, which is of limited abundance in the riparian wetland, dominates the wet central axis of the arm (Bubier, pers. comm., 1994).

A riverine wetland consisting of a 50 m wide and 300 m long marsh/bog complex lies along the river connecting Lake 155 with Lake 208 (Figure 2-1c). The first 20 m of wetland extending from the forest margin is bog; and composed of large hummocks of *S. fuscum* and hollows of *S. magellanicum*. The hummocks support *C. calyculata* and *L. groenlandicum*. At 20 m from the forest margin, the bog rapidly grades into a *Carex*-dominated marsh. The marsh surface is inundated by 10 to 20 cm of water. Mats of *Sphagnum sp.* (most likely *S. fallax*) have developed along the edge of the wetland and extend onto the river. The peat is shallow and poorly decomposed in the bog but well humified in the marsh (Figure 2-1c).

An upland bog has developed around the stream at the S.W. inflow to lake 224. The bog is only 40 m wide at the streams outflow and tapers to a point some 100 m up stream. The bog is covered by a dense forest canopy (*Alnus sp.*, *P. mariana*, *L. laricina* and a few *Betula sp.*) and thick shrub layer of *C. calyculata* and *L. groenlandicum*. The bog surface is comprised of small hummocks dominated by *S. fuscum* and small hollows with *S. magellanicum* and larger hollows containing *S. fallax*. The hummocks support a wide diversity of other plants including grasses and bryophytes. Higher in the valley, the stream channel is essentially a series of linked hollows, through which water flows following rain events. Only following large rain events do the hollows further from the apex become linked. The stream channel is well defined at the valley mouth where it is covered by dense *Alnus sp.* thickets (Figure 2-1d). The peat is < 0.50 cm on average and is poorly decomposed beneath hummocks and well decomposed beneath hollows.

At the S.W. inflow to Eagle Lake, a marsh dominated by tall *Carex spp.*, grasses and *Equisetum arvense* has developed (Figure 2-1e). The marsh occupies a shallow flat basin and is approximately 50 m wide and 300 m long. A variety of rushes and reeds live along the stream channel that meanders through the marsh. The wetland surface is inundated with between 10 and 30 cm of water for up to 40 metres from the main channel. At the margins, the marsh vegetation gives way to bog vegetation like the riverine wetland. The peat is well humified beneath the sedge-dominated portions of the marsh but less decomposed at the margins.

2.3 Methods

2.3.1 Collection of Water Samples

Water samples for Hg analysis were collected using an ultra-clean protocol used for all T-Hg and MeHg sampling at the ELA, and described in St.Louis et al. (1994). This technique ensures that the water sample does not come in contact with unclean surfaces. Porewater was collected from the peat using either polychloroethene (PVC) wells or a Teflon® sipper (Figure 2-2). The water was pumped from wells through acid washed Teflon® tubing into a Teflon® transfer case using a peristaltic pump. The sample was immediately poured through 0.45 μm Nalgene® cellulose nitrate membrane filter, housed in a disposable filter case, and transferred into a Teflon® bottle. The Teflon® bottles and transfer cases had been acid washed for 6 hours in hot HNO_3 and stored filled with low-Hg water with 1% low-Hg HCl added. The samples were double bagged and transported to the lab in a cooler, where samples for MeHg analysis were frozen and T-Hg analysis were acidified.

The PVC wells were placed in the wetland one year prior to sampling to allow any exchange surfaces to become saturated. Further influences the well may have had on the porewater were minimized by pumping all resident water from the well and a sample obtained as soon as the well was refilled. To test if PVC affected the T-Hg or MeHg concentration of the porewater, water samples were collected from Teflon® and PVC wells installed side by side and analysed for T-Hg and MeHg concentration. The analytical variation in T-Hg and MeHg concentration (n = 8) of replicate samples taken from Teflon® wells was 5 and 10% respectively. The T-Hg and MeHg concentration in porewater taken from the Teflon® wells were both higher and lower than porewater from PVC wells. The variation in porewater T-Hg concentration from each well type was 17% (both Teflon® and PVC) and between well types was 21%. The variation in the porewater MeHg concentration within well types was 72% (Teflon®) and 65% (PVC) and between well types was 69%. Therefore, within the limits of spatial variability and analytical error, no systematic error is evident from using PVC wells.

In areas where no wells were installed, a Teflon® sipper (Figure 2-2) was used to collect samples. The sipper is comprised of a Teflon® rod through which a Teflon® tube is fed, the end of the tube rapped with Teflon® tape to prevent it from sliding back up the rod and to create a tight seal. A Teflon® sampling head is threaded onto the Teflon® tube and stainless steel outer shell threaded onto the sampling head to provide stability. Samples were also extracted by the peristaltic pump transfer case method.

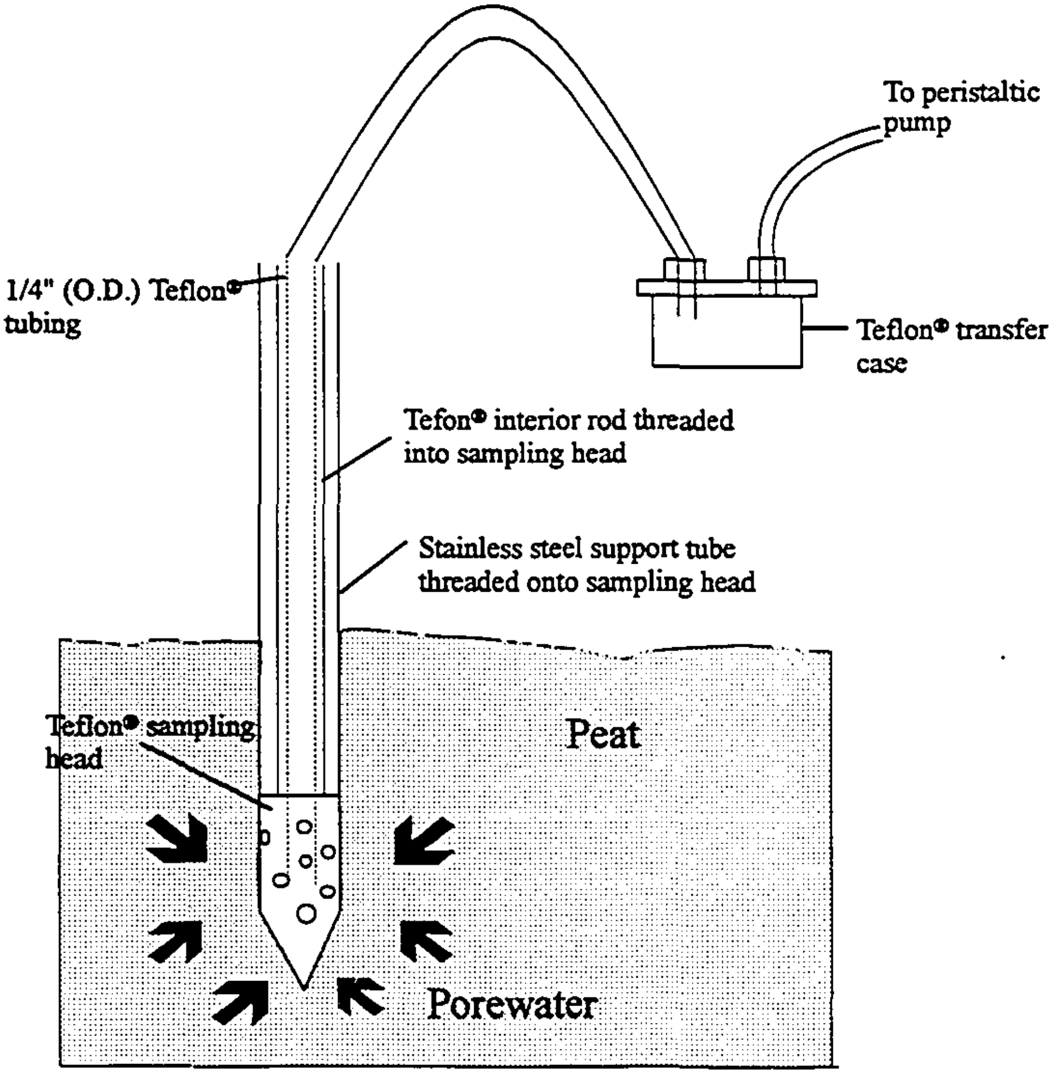

Figure 2-2) The peat porewater sipper. The head is made of Teflon® and threads into an inner Teflon® rod and outer stainless steel rod. A Teflon® tube is threaded through the inner tube and the tip wrapped with Teflon® tape to create a better seal inside the Teflon® head. The perforations in the sampling head covered a 5 cm length. Usually, a 250 ml water sample was collected. Ideally, this water would have come equally from a 7 cm^3 volume around the tip assuming the hydraulic conductivity was constant over the depth.

Filtration of peat porewater was deemed necessary because, despite covering the well inlets with 400μm Nitex® mesh, the particulate load of samples could not be controlled. Filtration through a 0.45μm membrane prevents the transfer of MeHg to potentially precipitating oxides (thereby increasing the variability of analysis), removes most bacteria, and is consistent with the protocols of the methods used to determine other aspects of the peat porewater chemistry (see below). Finally, the MeHg in the filtered fraction is perhaps more representative of the MeHg that fluxes between the porewater and adjacent water bodies.

Water for nutrient and DOC analyses were collected in Nalgene® bottles, capped with zero volume caps, and transported to the lab where they were filtered through Gelman AE® filters. The samples were refrigerated at 5°C until analysis could be performed. Water for the determination of pH was pumped from the well or sipper into C-flex tubing, sampled and stored in a syringe equipped with a stopcock until analysis.

The number of water samples that could be collected from each wetland was limited, therefore sampling was customized for each wetland in order to gather some idea of the spatial variability of MeHg in the peat porewater. In the poor fen, two profiles were taken so as to bisect the apparent flow path between the upland and the inflow stream (Figure 2-1a). Three profiles were taken in the ombrotrophic bog, two in a moderately forested area at 4 m (BWA) and 22 m (BWC) from the pond and the third in a heavily forested area (IF2) (Figure 2-1a). In the riparian and riverine wetlands, 3 profiles were taken along a transect between the forest margin to the adjacent water body (Figure 2-1b, 2-1c). In the NE bog three profiles were taken that traverse the long axis of the wetland. In the upland bog and Eagle marsh, three and two profiles, respectively, were taken along the basin. Unfortunately, this approach does not take into account temporal variations, but if nutrient concentrations in the peat porewater have a consistent influence on MeHg concentrations in the porewater, the relationship should be apparent in the spatial survey.

2.3.2 Collection of Peat Samples

Peat was extracted from four of the wetlands for determination of MeHg and T-Hg concentration. To extract peat, a small hole was cut into the peat surface and peat was quickly removed from the exposed wall with gloved (clean room grade PVC) hands. Water was removed from the base of the pit to minimize contamination. This method allowed for better stratigraphic control and cleanliness than any available corer. Peat samples were taken at 5 to 10 cm increments, placed in ziplock® plastic bags, and frozen upon returning to the lab.

2.3.3 Analyses of Water Samples

Methylmercury in water was measured using modifications of the methods of Horvat et al. (1993) and Bloom (1989). To separate MeHg from humic substances, water samples were distilled at a sub-boiling temperature (90°C) after addition of 500 μl 9N H_2SO_4 and 200 μl 20% KCl. The distillate was then ethylated, and the ethylated Hg species purged from solution onto a Tenax® trap using nitrogen gas. The Tenax® trap was flash heated in a stream of ultra high purity (UHP) helium, and the released ethylated Hg species separated chromatographically (OV3 on Chromasorb W-AW-DMCS, 60/80 mesh) prior to passing through a combustion tube (900°C) were all Hg species are converted to Hg^o for detection by atomic fluorescence. MeHg is reported as ng Hg l^{-1}. The extraction efficiency was monitored using the method of standard additions. The efficiency was generally 95% (± 10%) but could be 10 to 15% lower in sulfidic waters. The impact of sulfide was minimized by diluting the sample. All samples were analysed in duplicate, and the analytical error was generally < 20%. Where duplicates had a variance greater than 20%, samples were reanalysed if possible.

An artifact whereby MeHg is produced during distillation has recently been discovered (Bloom et al., 1996). In waters rich in DOC, X % but as much as 1% of the inorganic Hg can be methylated. This has ramifications were T-Hg concentrations are high and the MeHg concentration is a small component of the T-Hg concentration. In this study although DOC concentrations are very high, the proportion of T-Hg that is MeHg is well in access of any amount of MeHg that could have been produced during distillation and thus does not alter the results of this study.

T-Hg concentrations in water samples were measured at Flett Research of Winnipeg, Manitoba, using a modification of the method of Bloom and Fitzgerald (1988). Water samples were first oxidized with 1% BrCl and then placed in a bubbler with 0.5% $SnCl_2$ to reduce all Hg species to Hg^o. UHP nitrogen was used to purge Hg from solution onto a gold trap. The gold trap was flash heated in a stream of UHP helium and the elemental Hg measured by atomic fluorescence. The analytical error on duplicates was always < 10%.

The conductivity of peat porewater was measured with a Radiometer CD-M3 conductivity meter in a closed vessel using unfiltered water brought to 25°C. pH was measured in a closed vessel using a Fisher pH meter and Ag Ag/Cl miniature glass body combination electrode. The nutrients NH_4^+, NO_2^-, NO_3^-, and total dissolved phosphorus (TDP) were measured using the methods of Stainton et al. (1977) and SO_4^{2-} by suppressed ion chromatography, at the Fisheries and Oceans Laboratories in Winnipeg or at the ELA.

DOC was determined using a Shimadzu TOC-5050. The porewater sample was acidified and purged with nitrogen to remove inorganic carbon. A subsample of the porewater is sprayed onto a platinum catalyst heated to 680°C (high temperature combustion) and the released CO_2 measured with an infrared gas analyser.

2.3.4 Determination of T-Hg and MeHg Concentrations in Peat

The MeHg concentration in peat was determined using a method similar to Horvat et al. (1993). Peat samples were air dried until only moist and a homogenized subsample (< 1 g d.w.) was placed in a Teflon® vessel. A small amount of low Hg double-distilled and purified water (SQ), 500 μl of 9N H_2SO_4, and 200 μl of 20% KCl were added and left to digest for 4 hrs. The vessels were heated to 90°C and over a 3 hour period the extract distilled into a second clean Teflon® vessel. The Hg species in the distillate were ethylated and purged from solution by nitrogen onto a Tenax® trap. The Tenax® was flash heated in a stream of UHP helium, the Hg species separated chromatographically, converted into Hg°, and detected by atomic fluorescence. The MeHg concentrations are reported as ng Hg g^{-1} (d.w.). Extraction efficiency varied slightly between tissue types but was approximately 90% (±10%) and was monitored by standard addition. The extraction efficiency remained linear through three orders of magnitude and a simple correction factor was applied to the samples. Further attempts to extract MeHg from the residual tissue yielded <5% of the original MeHg extracted. The analytical error, defined by the coefficient of variance of triplicate samples, was typically <20%. In the cases where the variance exceeded 20%, the samples were analysed again. The limit of detection based on 3x the standard deviation of the blank was established as 1.2 pg g^{-1}.

Total-Hg concentrations in peat samples were measured at Flett Research of Winnipeg, Manitoba. Subsamples of the homogenized air dried peat were digested in warm HNO_3-H_2SO_4 mixture. A subsample of the extract was then oxidized with 1% BrCl and placed in a bubbler with 0.5% $SnCl_2$ to reduce all Hg species to Hg^o. The Hg species were purged from solution with UHP nitrogen onto a gold trap, which was flash heated in a stream of UHP helium and the elemental Hg measured by atomic fluorescence. The analytical error on duplicates was always <10%.

Because of the volatility of Hg species, samples could not be oven dried. To determine the MeHg and T-Hg concentrations on a dry weight basis (d.w.) a correction factor was therefore required. A large subsample of the peat was weighed at the time of analyses, oven dried at 50°C, and the water content determined.

2.4 Results

2.4.1 Total-Hg and MeHg Concentrations in Peat

Methylmercury concentrations of near-surface peat from hollows from 3 wetlands are presented in Table 2-1 and detailed profiles of MeHg and T-Hg in a hummock and hollow of the riparian wetland are presented in Figure 2-3. The MeHg concentration of peat is highly variable, ranging from 0.3 to 53 ng g^{-1} (d.w.). Within wetland variability is also large, as MeHg concentrations in upland bog peat range from 0.3 to 34.8 ng g^{-1} and in riparian wetland peat from 0.11 to 3.5 ng g^{-1}. In the riparian wetland, MeHg concentrations are greater in the hollows than hummocks (Figure 2-3). Also, MeHg concentrations are greatest below the lowest annual water table. The T-Hg concentrations in peat of the riparian wetland ranges from 10 to 111 ng g^{-1} (d.w.) and, like MeHg, the highest concentrations are also found in hollows. The proportion of T-Hg occurring as MeHg ranges from 0.2 to 47.4%.

Upland Bog			Poor Fen			Ombrotrophic Bog		
Depth (cm)	Conc. (ng g^{-1})	Std.	Depth (cm)	Conc. (ng g^{-1})	Std.	Depth (cm)	Conc. (ng g^{-1})	Std.
a10	28.6	8.1	pool	53	9.6	10-20	8.1	3.1
a25	11.2	3.8	10-20	14.3	1.0			
a40	34.8	7.1						
b10	2.6	0.2						
b25	8.5	1.4						
b40	13.1	3.0						
c10	0.30	0.1						
c25	0.44	0.1						
c40	13.1	1.4						

Table 2-1) The mean MeHg concentration in peat of the upland bog, poor fen, and ombrotrophic bog. The mean and standard deviation are of triplicate analysis. In the poor fen, the depth indicated as pool refers to a black *S. fallax*, found at the pool edges perhaps blackened by the precipitation of FeS·nH$_2$O species. In the upland bog column, the a, b, and c next to the depths refers to the profile locations on Figure 2-1d.

2.4.2 Methylmercury and Total Mercury Concentrations in Peat Porewater

MeHg concentrations in peat porewater of the 7 wetlands are summarized in Figures 2-4 and 2-5. Methylmercury concentrations range from below the limit of detection (0.02 ng l^{-1}) to 7.26 ng l^{-1} with the mean 0.48 ng l^{-1}. The highest MeHg concentrations were recorded in the poor fen and the upland bog (Figure 2-4). At all locations, the maximum MeHg concentration occurs near the surface, but below what appears to be the lowest annual water table, and decreases with depth (Figure 2-5h).

Eight samples of porewater were taken from the riparian wetland and ombrotrophic bog, and the porewater analysed for T-Hg concentration (Table 2-2). Little can be said about the distribution of T-Hg but concentrations ranged from

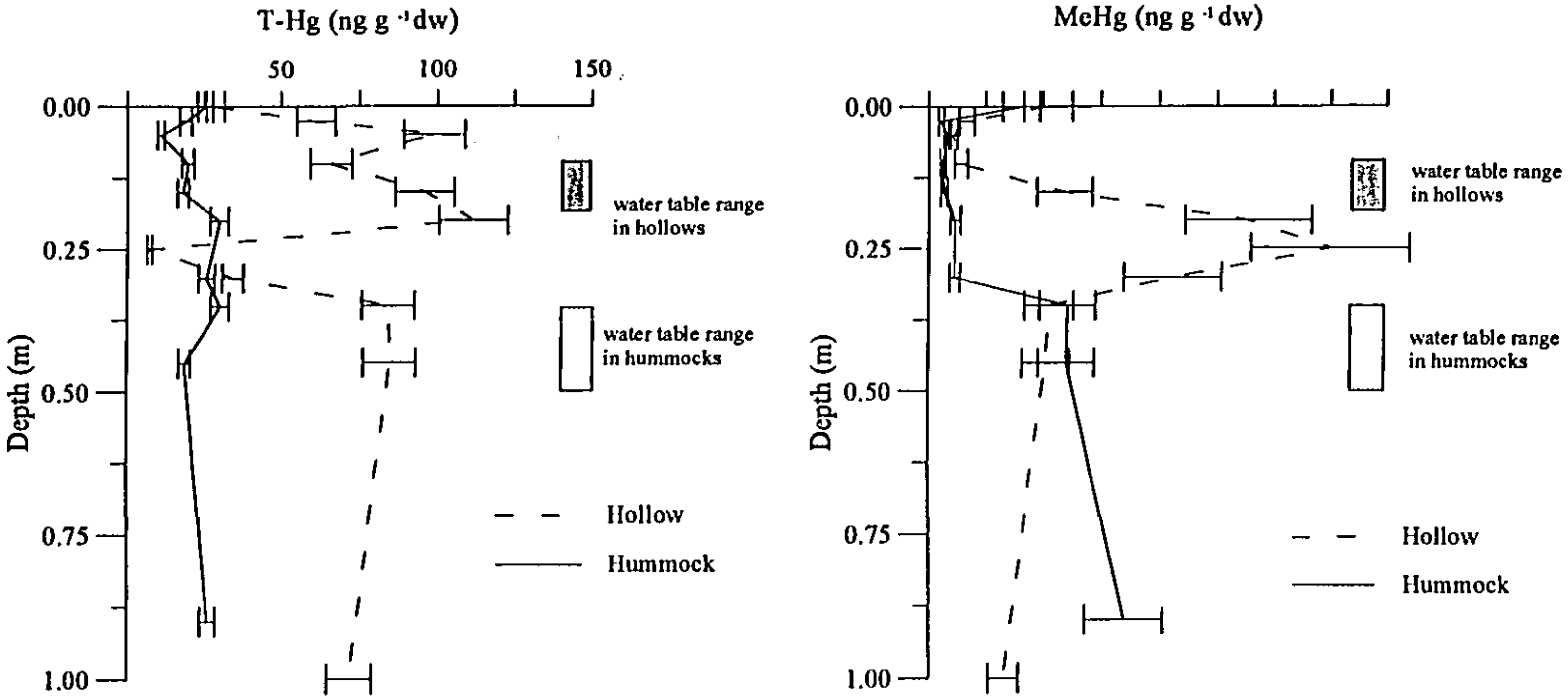

Figure 2-3) Total-Hg and MeHg profiles of a peat hummock and a peat hollow of the riparian wetland. The error bars indicate one standard deviation around the mean of duplicate (T-Hg) and triplicate (MeHg) analysis.

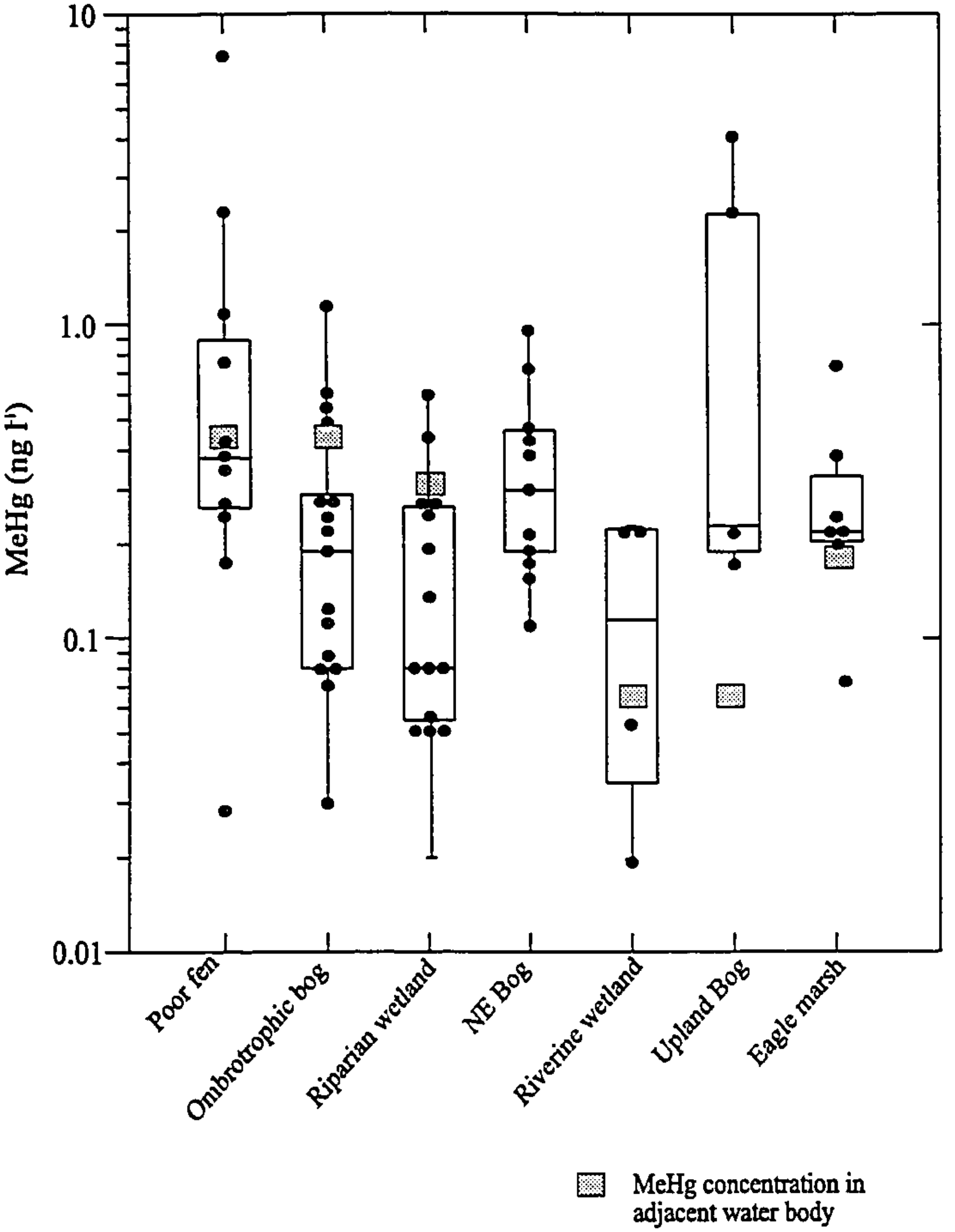

Figure 2-4) Box and whisker plot of MeHg concentrations in porewater of the 7 wetlands. The horizontal line in the box represents the median. The ends of the boxes represent the upper and lower quartiles. The whiskers are 1.5 x the hinge spread (1.5 x the upper and lower quartiles). The individual concentrations are depicted as circles and concentrations outside the whiskers are considered outliers.

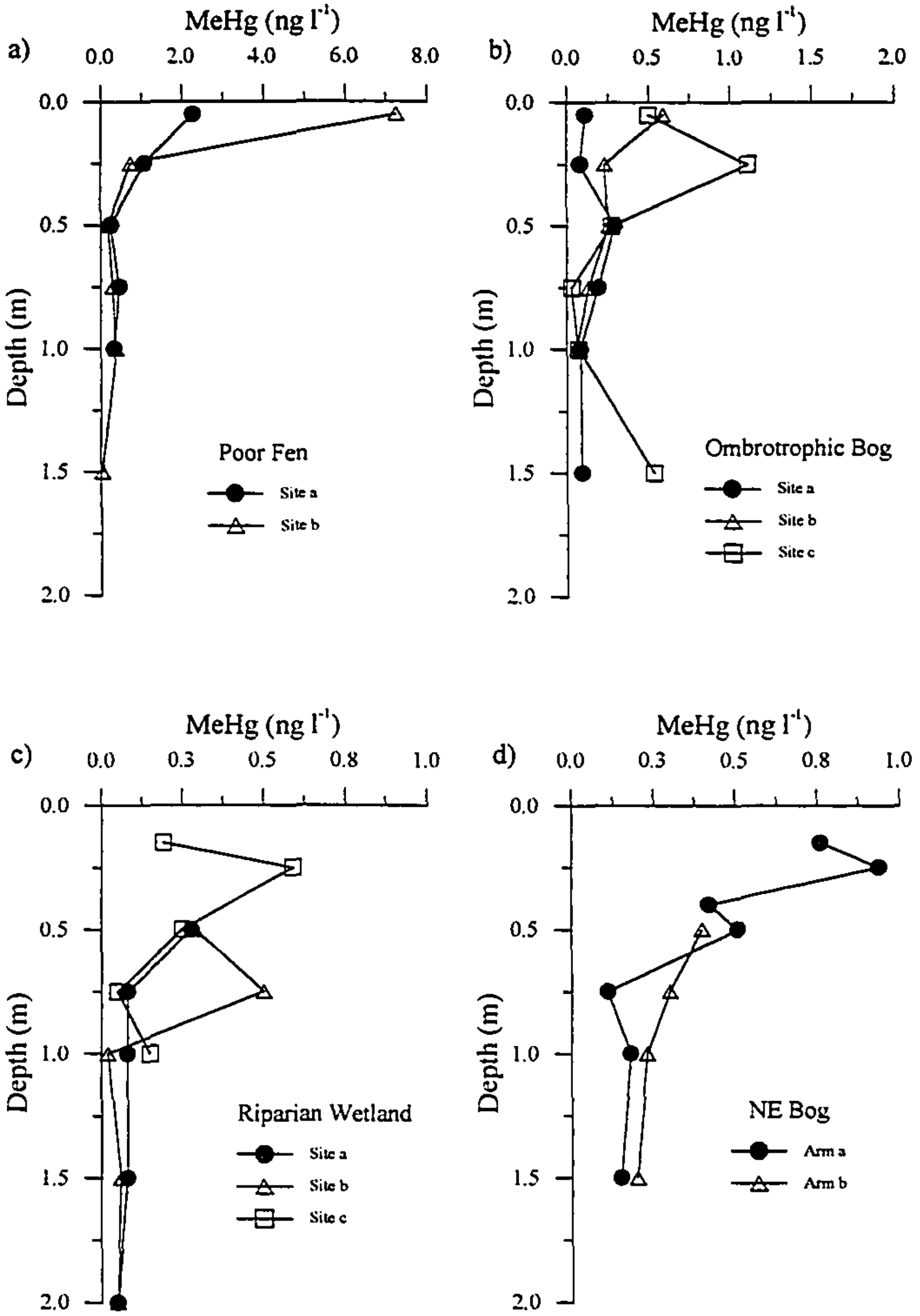

Figure 2-5) Methylmercury concentrations in peat porewater at the a) poor fen, b) ombrotropic bog, c) riparian wetland, and d) NE bog.

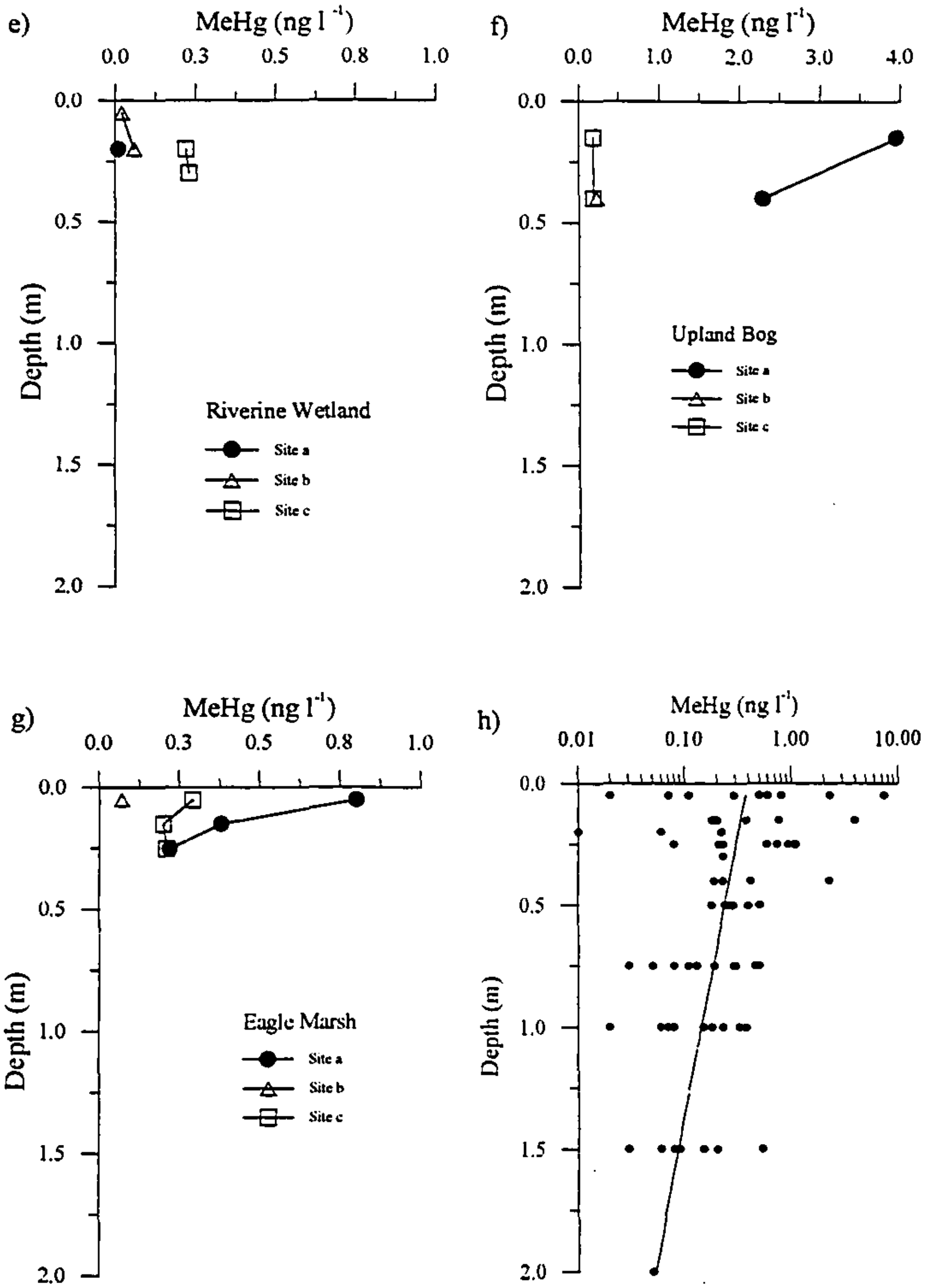

Figure 2-5 Cont.) MeHg concentrations in peat porewater of the e) riverine wetland, f) upland bog, g) Eagle marsh, and h) the combined data of the seven wetlands (plotted as log MeHg) with the line of best fit described by:
ln (MeHg) = -1 x (Depth) - 0.93, R^2= 0.21, n=70 p=0.000

0.60 to 8.63 ng l^{-1} of which between 1 and 26% occurred as MeHg.

%MeHg	T-Hg (ng l^{-1})	MeHg (ng l^{-1})	Depth (m)
26.15	1.95	0.51	0.50
10.66	8.63	0.92	0.40
7.92	6.19	0.49	0.25
6.58	1.67	0.11	0.75
4.07	6.88	0.28	0.25
3.59	6.86	0.25	0.75
2.36	3.38	0.08	1.25
1.06	3.76	0.04	1.00

Table 2-2) Percent of T-Hg that is MeHg in porewater from the ombrotrophic bog and riparian wetland. The % MeHg is unrelated to depth or T-Hg concentration.

2.4.3 The Relationship Between MeHg in Peat and Peat Porewater

In the four wetlands from where peat was sampled, porewater was collected prior to sampling the peat to determine MeHg concentration. Partition coefficients (K_D) between the peat and the porewater were calculated using

$$K_D = \mathrm{MeHg}_{peat}\ (\mathrm{ng\ g^{-1}}) / \mathrm{MeHg}_{aqua}\ (\mathrm{ng\ g^{-1}}).$$

The resulting partition coefficients range from 860 to 56,780 with the mean being 16,400 (Table 2-3). Therefore, a gram of peat contains more than 10,000 times the amount of MeHg in a gram of porewater.

The MeHg concentrations of peat and porewater are plotted in Figure 2-6. The relationship between the two is an exponential one, as concentrations of MeHg in peat at each end of the range approach the 1:1000 line, but in the middle of the range are well below it. This distribution may reflect a change in adsorption ability with increasing MeHg concentration. The line of best fit, can be used to estimate the MeHg concentration in peat or peat porewater (Figure 2-6).

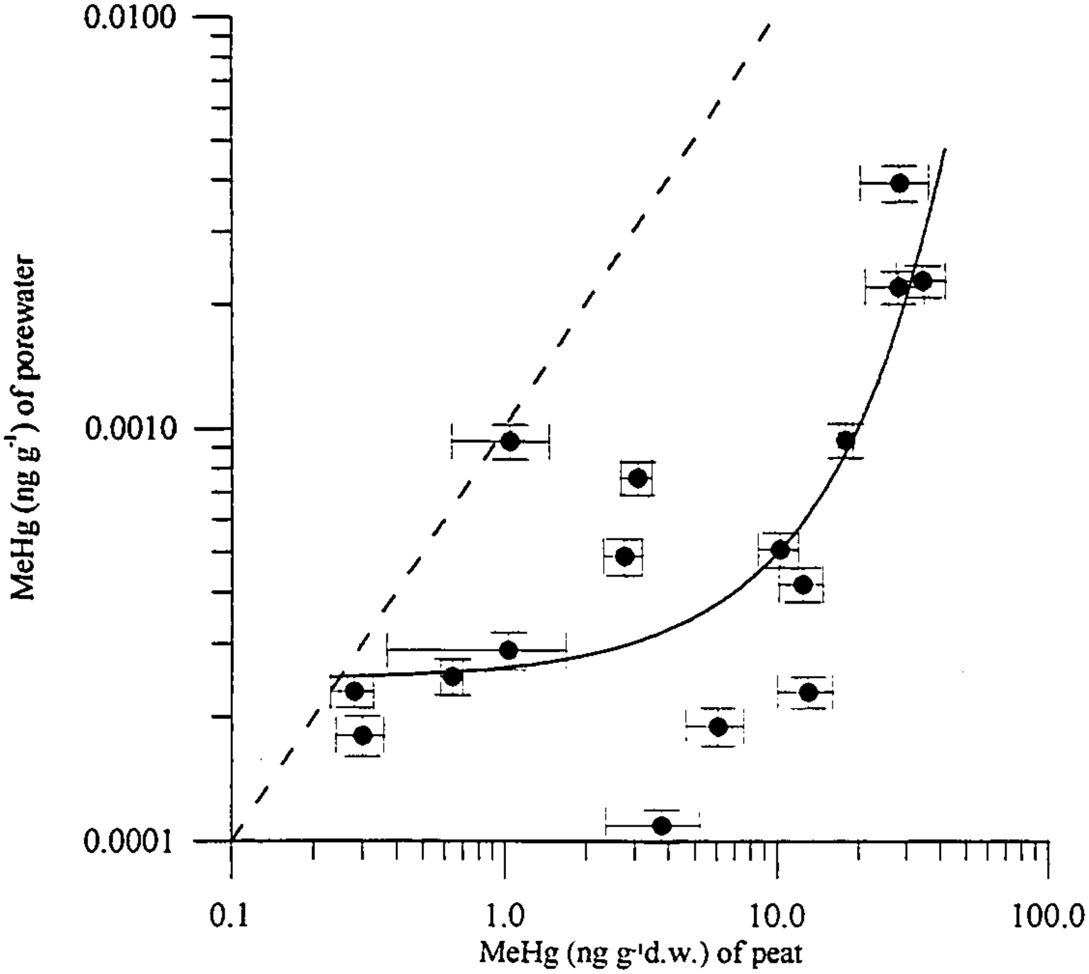

Figure 2-6) A log:log plot of the MeHg concentrations in peat and peat porewater of the wetlands at the ELA. The relationship is best described by the exponential equation Ln (MeHg in water ng g^{-1}) = 0.071 x (MeHg peat ng g^{-1}) - 8.31, R^2= 0.61 ($p<0.000$) The dashed line is the 1g:1000g line.

Peat (ng g^{-1})	Water (ng g^{-1})	K_D x10^3	Peat (ng g^{-1})	Water (ng g^{-1})	K_D x 10^3
34.8	2.28 x 10^{-3}	15.3	3.7	0.11 x 10^{-3}	33.9
28.6	3.95 x 10^{-3}	7.2	3.1	0.76 x 10^{-3}	4.1
28.3	2.21 x 10^{-3}	12.8	2.3	0.49 x 10^{-3}	4.7
18.0	0.94 x 10^{-3}	19.2	1.7	0.29 x 10^{-3}	5.9
13.1	0.23 x 10^{-3}	56.8	1.5	0.93 x 10^{-3}	1.6
12.5	0.42 x 10^{-3}	29.8	0.3	0.18 x 10^{-3}	1.7
10.3	0.51 x 10^{-3}	20.2	0.2	0.23 x 10^{-3}	0.9
6.1	0.19 x 10^{-3}	32.1			

Table 2-3) Peat and peat porewater MeHg partition coefficients (K_D).

2.4.4 Wetland Peat Porewater Chemistry

The peat porewater chemistry from the 7 wetlands is presented in Table 2-4 and the distribution of the chemistry portrayed in Figure 2-7. The range in pH across all the porewater samples is 4.04 to 6.64 with the mean 5.34, conductivity ranged from 17 to 135 μS cm^{-1} with the mean 49 μS cm^{-1}, TDP ranged from 3 to 328 $\mu g\ l^{-1}$ with the mean 48 $\mu g\ l^{-1}$, NH_4^+ ranged from 12 to 5140 $\mu g\ l^{-1}$ with the mean 735 $\mu g\ l^{-1}$, and DOC ranged from 7 to 97 mg l^{-1} with the mean 32 mg l^{-1}. Sulfate and NO_3^- concentrations in peat porewater were measured in only four of the wetlands. Concentrations of SO_4^{2-} ranged from 0.03 to 4.7 mg l^{-1} with the mean 0.4 mg l^{-1} and NO_3^- concentrations ranged from undetectable to 81$\mu g\ l^{-1}$ with the mean 6 $\mu g\ l^{-1}$.

Some of the individual porewater chemistry variables and porewater MeHg concentrations are plotted in Figure 2-8. The data is not normally distributed but logging the data improves the distribution. Only NH_4^+ concentration is significantly and negatively ($R^2 = 0.152$, $p = 0.004$) related to MeHg concentration among the 7 wetlands but this relationship is to weak for any predictive purpose. Using stepwise

Site : depth	**MeHg** ng l^{-1}	**DOC** mg l^{-1}	**pH**	**NO_3^-** $\mu g\ l^{-1}$	**NO_2^-** $\mu g\ l^{-1}$	**NH_4^+** $\mu g\ l^{-1}$	**TDP** $\mu g\ l^{-1}$	**SO_4^{2-}** mg l^{-1}	**Cond** $\mu S\ cm^{-1}$
Poor Fen									
Site IF12 0 cm	2.27	12.7	4.80	81	2	12	14	0.73	17
Site IF12 25 cm	1.08			6	4	21	27	0.35	
Site IF12 50 cm	0.24		5.07	1	4	37	35	0.29	19
Site IF12 75 cm	0.46	6.4	5.42	4	2	36	13	0.35	20
Site IF12 100 cm	0.33		5.45						26
Site IF7 0 cm	7.26	11.0	4.95	2	1	22	16	0.67	17
Site IF7 25 cm	0.74			22	8	58	63	0.29	
Site IF7 50 cm	0.18		5.62	3	23	339	178	0.29	24
Site IF7 75 cm	0.29		5.78	3	5	378	52	0.29	37
Site IF7 100 cm	0.38		5.62						40
Site IF7 150 cm	0.03	3.0	5.78						43
Ombro. Bog									
Site BWA 0 cm	0.11			2	5	26	23	0.04	
Site BWA 25 cm	0.08								
Site BWA 50 cm	0.29	36.2	4.36	<1	5	27	20	0.04	30
Site BWA 75 cm	0.19	44.4	4.36	1	6	29	24	0.16	30
Site BWA 100 cm	0.08	45.4	4.85	5	11	27	15	0.16	30
Site BWA 150 cm	0.09	17.0	5.61	15	31	602	16	0.03	64
Site BWC 0 cm	0.59			<1	22	76	28	0.16	
Site BWC 25 cm	0.23								
Site BWC 50 cm	0.25	67.6	4.51	<1	18	2136	179	0.03	41
Site BWC 75 cm	0.13	42.5	4.85	5	18	2448	80	0.03	37
Site BWC 100 cm	0.06	31.4	5.09	2	4	1155	27	0.03	39
Site IF2 0 cm	0.50			<1	10	40	46	0.03	
Site IF2 25 cm	1.11			<1	9	196	37	0.03	
Site IF2 50 cm	0.27	49.9	4.04	<1	8	418	61	0.03	52
Site IF2 75 cm	0.03	48.6	4.46	<1	15	1000	218	0.16	34
Site IF2 100 cm	0.07	29.2	4.92						26
Site IF2 150 cm	0.53	8.3	5.52						30
Riparian Wet.									
Site BWA 50 cm	0.28	24.3	5.52	4	2	860	42	0.27	37
Site BWA 75 cm	0.08	31.1	5.90	4	2	1660	52	0.11	77
Site BWA 100 cm	0.08	23.1	5.90	3	4	1920	52	0.11	87
Site BWA 150 cm	0.08	45.7	6.05	6	4	1760	57	0.11	118
Site BWA 200 cm	0.05	26.6	6.09	9	5	1660	52	0.11	120
Site BWC 50 cm	0.29	33.9	5.53	5	2	410	11	0.31	35
Site BWC 75 cm	0.50	31.4	5.51	9	1	490	10	0.35	36
Site BWC 100 cm	0.02	24.9	5.55	6	4	1100	11	0.23	52
Site BWC 150 cm	0.06	24.4	5.90	2	6	1800	9	0.11	86
Site BWC 200 cm	0.05	27.9	5.94	6	2	2170	58	0.11	117
Site BWD 15 cm	0.19	49.2	5.83						35
Site BWD 25 cm	0.59	56.3	5.16						33
Site BWD 50 cm	0.25	36.2	4.02	2	2	420	17	4.66	31
Site BWD 75 cm	0.05	20.7	5.22	8	2	890	12	4.18	46
Site BWD 100 cm	0.15	18.2	5.80	1	1	360	24	1.09	51

Table 2-4) Peat porewater chemistry of the 7 wetlands at the ELA.
Table codes: The letters beside sites denote location on figure 2-1. UND indicates undetectable.

Site : depth	MeHg ng l^{-1}	DOC mg l^{-1}	pH	NO_3^- $\mu g\ l^{-1}$	NO_2^- $\mu g\ l^{-1}$	NH_4^+ $\mu g\ l^{-1}$	TDP $\mu g\ l^{-1}$	SO_4^{2-} mg l^{-1}	Cond $\mu s\ cm^{-1}$
NE Bog									
Site NEA 15 cm	0.76								
Site NEA 25 cm	0.94								
Site NEA 40 cm	0.42								
Site NEA 50 cm	0.51	37.2	4.72	6	3	240	13	0.27	27
Site NEA 75 cm	0.11	76.6	4.52	4	9	1750	46	0.10	44
Site NEA 100 cm	0.18		4.62	3	10	2090	55	0.11	46
Site NEA 150 cm	0.15	97.2	4.74	2	14	5140	328	0.11	97
Site NEB 50 cm	0.40	55.6	4.68	9	8	590	31	0.16	55
Site NEB 75 cm	0.30	83.7	4.82	8	9	1010	62		83
Site NEB 100 cm	0.23	67.8	5.24	6	7	1170	48		67
Site NEB 150 cm	0.20	42.0	5.67	3	5	2124	83		42
Riverine Wet.									
Site a 0 cm	UND								
Site a 20 cm	UND	7.2	6.42		1	24	22		31
Site b 0 cm	0.02	8.5	6.64		2	31	29		34
Site b 20 cm	0.06	12.3	5.90		1	16	17		42
Site c 20 cm	0.22	64.0	6.04		2	38	24		64
Site c 30 cm	0.23								
Upland Bog									
Site a 15 cm	3.95	18.7	5.63		2	40	14		28
Site a 40 cm	2.28	24.1	5.54		13	433			23
Site b 40 cm	0.23	15.7	5.58		3	78	51		21
Site c 15 cm	0.18	12.5	5.81		10	243	112		12
Site c 40 cm	0.19	32.1	5.5		2	33	18		25
Eagle Marsh									
Site a 5 cm	0.80	7.6	5.57						26
Site a 15 cm	0.38								
Site a 25 cm	0.22	28.8	5.85						135
Site c 0 cm	0.07	14.7	6.26		2	35	3		23
Site c 5 cm	0.29		4.93		2	165	18		
Site c 15 cm	0.20		4.92		2	305	13		115
Site c 25 cm	0.21	7.1	5.08		2	320	10		115

Table 2-4 Cont.) Peat porewater chemistry of the 7 wetlands at the ELA.
Table codes: The letters beside sites denote location on figure 2-1. UND indicates undetectable

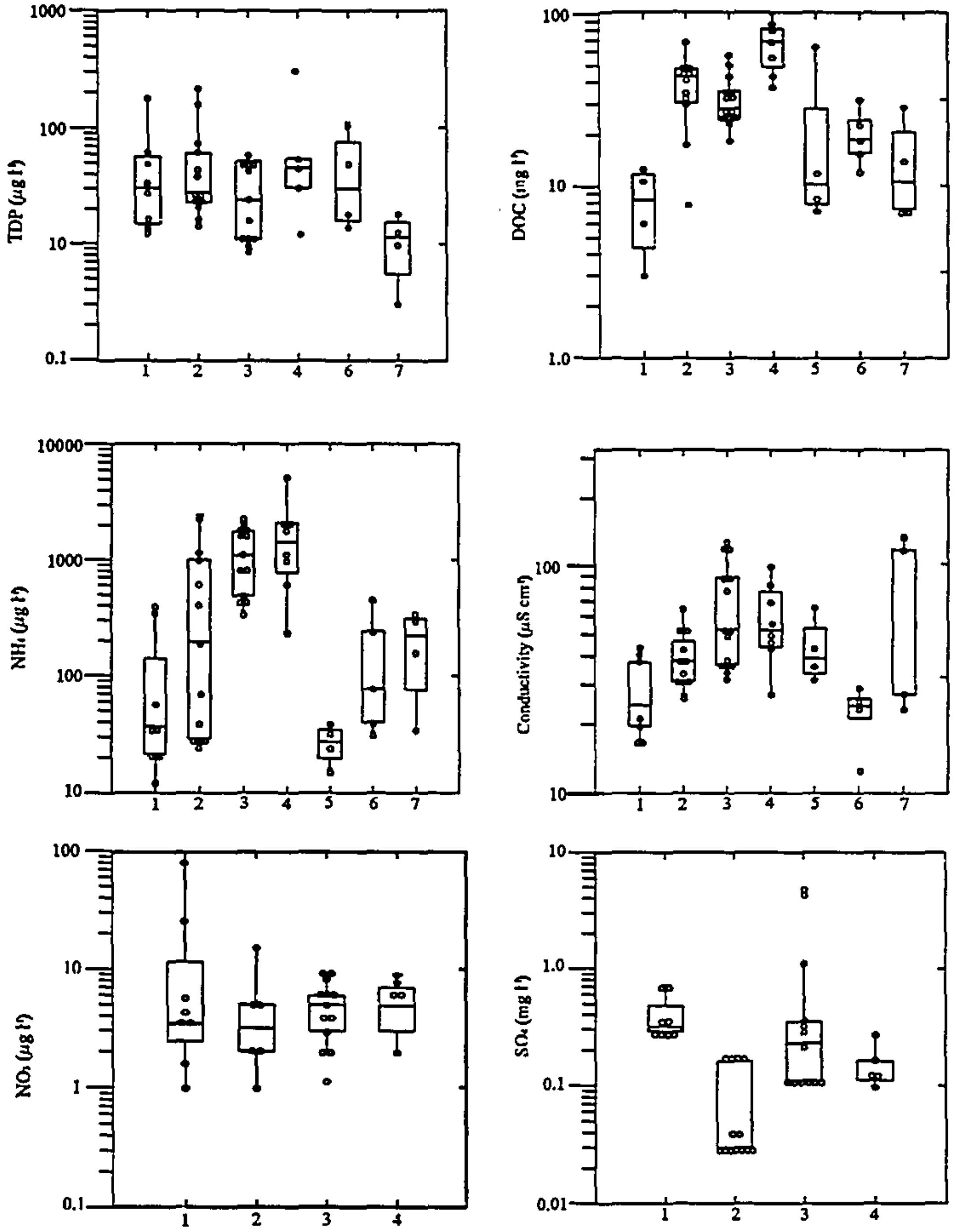

Figure 2-7) Box and whisker plots of nutrient concentrations in peat porewater of the 7 wetlands studied at the ELA. The sites are numbered: 1) poor fen, 2) ombrotrophic bog, 3) riparian wetland, 4) NE bog, 5) riverine bog, 6) upland bog, and 7) Eagle marsh. The horzontal line in each box represents the median and the ends of the box represent the first quartiles. The whiskers are 1.5 x the hinge spread (1.5 x the upper and lower quartile). The circles represent the actual concentrations and concentrations outside the span of the whiskers are considered outliers.

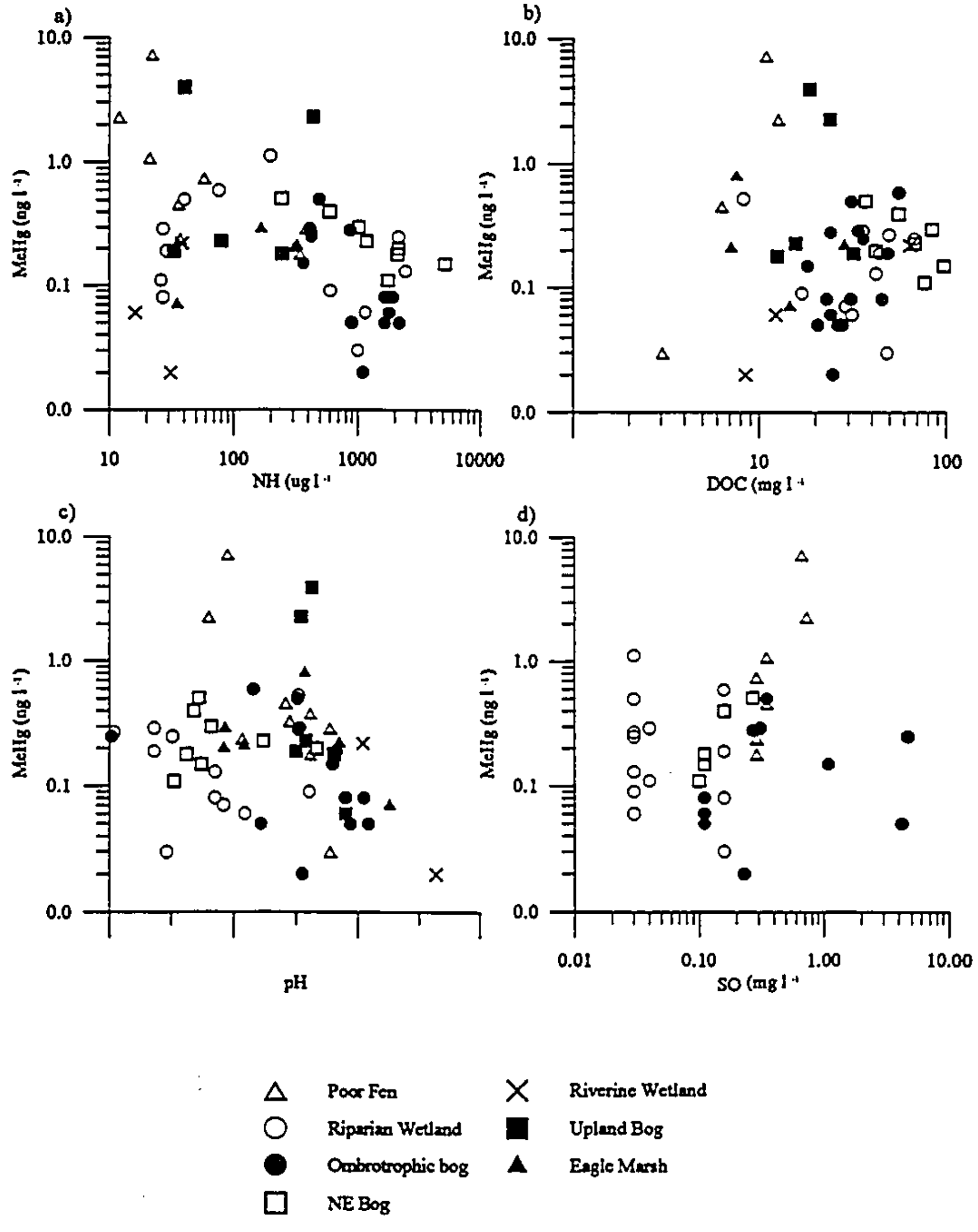

Figure 2-8) Scatter plots of porewater MeHg concentrations vs a) NH_4^+ concentrations, b) DOC concentrations, c) pH, and d) SO_4^{2-} concentrations, for the 7 wetlands.

multiple regressions of two or more (when the data set was large enough) variables only pH in combination with NH_4^+ concentration yielded a significant and negative relationship with the MeHg concentration (R^2 0.216, p = 0.002). Within single wetlands, significant negative relationships between MeHg concentration and pH (ombrotrophic bog R^2 0.621, p= 0.035 and poor fen R^2 0.595, p = 0.015) and MeHg concentration and NH_4^+ concentration (NE bog R^2 0.741, p = 0.006 and riparian wetland R^2 0.477, p = 0.009) and positive relationships between MeHg concentration and SO_4^{2-} concentration (poor fen R^2 0.765 p = 0.005 and NE Bog R^2 0.865, p = 0.022) are present.

2.5 Discussion

2.5.1 The Distribution of T-Hg and MeHg in Peat

Although only 2 profiles of T-Hg in peat were determined, there is a substantial difference in T-Hg concentration between the dry hummock and wet hollow. This is consistent with the observation of Moore et al. (1995) who observed an order of magnitude higher concentration of T-Hg in *Sphagnum* species occupying wet sites than dry sites. They also observed an increase in the concentration of T-Hg down the length of a single *S. angustifolium* strand. This pattern suggests either the dead portions of the *Sphagnum* strand are more effective in accumulating Hg than the live plant or a magnification resulting from T-Hg retention during decomposition.

The higher T-Hg concentration in peat and *Sphagnum* of wet areas may result from a greater long term exposure to Hg, or a greater Hg retention ability. Ombrotrophic bogs have been used to assess long term changes in the atmospheric deposition of Hg (Jensen and Jensen, 1991; Madsen, 1981). However, given the spatial variability of T-Hg in peat, and the uncertainty behind its mobility, great care should be taken in using ombrotrophic peat to assess long term changes in Hg deposition.

Methylmercury concentrations in peat are highly variable, ranging from 0.3 to 53 ng g^{-1} (d.w.) with the largest accumulation in hollows. Moore et al. (1995) also observed the highest concentrations of MeHg in *Sphagnum* species that occupy hollows. As in the case of T-Hg, the long term source of MeHg in peat could be from incoming water. However, exports of MeHg from the headwater wetland are greater than inputs, indicating the wetland is a source of MeHg (St.Louis et al., 1994). Therefore most of the MeHg in porewater must originate in the peat.

2.5.2 The relationship Between MeHg in Peat and Peat Porewater

A change in the MeHg concentration of peat will affect the amount of MeHg in the porewater (Figure 2-6, Table 2-3). However, as the partition of MeHg between peat and porewater is not consistent, the amount of change depends upon the MeHg concentration of the peat. The exponential pattern in Figure 2-6, of lower K_d's at higher and lower peat MeHg concentrations, suggests the efficiency of MeHg retention by peat diminishes after a concentration of 10 ng g^{-1} is exceeded. This may reflect a saturation of the high affinity MeHg adsorption sites on peat and the lower affinity sites are not as effective in competing with DOC for MeHg under anaerobic conditions. MeHg adsorption on peat will be discussed further in Chapter 5.

2.5.3 The Influence of Porewater Chemistry on Porewater MeHg Concentration: applying the lessons learned from lake studies.

In lakes, high concentrations of MeHg have been correlated with low pH, perhaps because more Hg is available for methylation or species of Hg methylating bacteria are favoured (Winfrey and Rudd, 1992). The pH of the wetlands in this study are lower than most lakes (mean 5.3) favouring both the above conditions, but pH alone is not a good predictor of porewater MeHg concentration (Figure 2-8c).

The concentration of DOC in lakes has been positively correlated with MeHg concentration in water but is often negatively correlated with the MeHg concentration in fish (Meile, 1991; Mierle, 1990). Miskimmin et al. (1992) reported that the addition of bog water to lake sediments did not increase the concentration of MeHg in lake sediments, despite increasing bacterial activity. In wetlands, concentrations of DOC and MeHg are unrelated (Figure 2-8b). It would seem that the correlation between high DOC and MeHg concentrations in lakes is because wetlands are large sources of both DOC and MeHg (St.Louis et al., 1994). However, stimulating in-lake Hg methylation may be dependent on the quality and not merely the quantity of carbon. Thus, the notion that terrestrial DOC entering lakes stimulates in-lake Hg methylation cannot not be entirely dismissed.

In lake and estuarine sediments, the oxic/anoxic interface, which generally corresponds to the sediment lake water interface, has been identified as the site where Hg methylation is greatest (e.g., Gilmour et al., 1992; Matilainen, 1995; Watras and Bloom, 1992). Within each wetland of this study, the highest porewater MeHg concentrations occurred near the surface, just below the water table (Figure 2-5). The water table of the wetland coincides or is close to the oxic/anoxic interface. The oxic/anoxic interface is the optimal location for anaerobic microorganisms to acquire nutrients and for electron acceptors to be reoxidized. From these observations, we may conclude that areas of intense redox reactions are areas where Hg methylation is likely to occur.

Although the nutrient supply appears to be an important control on Hg methylation, only a negative correlation between NH_4^+ and MeHg concentrations in peat porewater was found among the 7 wetlands. No relevant reason has been presented in the literature as to why high MeHg and NH_4^+ concentrations cannot coexist but the hydrological and biogeochemical setting which promotes the accumulation of each maybe different. Within individual wetlands, MeHg concentration is correlated with the SO_4^{2-} concentration in two cases and with pH in two cases but in most wetlands no correlations were found between the concentration of MeHg and other water chemistry parameters. The observed correlations maybe coincidental, given their infrequency, or indicate the conditions under which Hg is methylated is specific and controlled by a number of factors.

It is important to point out that inferring the *in situ* concentrations of other chemical species affect the production of MeHg has two underlying assumptions: the species being measured are important to Hg methylation and the species concentration at the time of measurement is the same as during Hg methylation. If factors promoting the production or destruction of MeHg are episodic and/or species consumption matches species enrichment at one point in time, concentration-based correlations are unlikely to be useful and give the false appearance that concentrations of other chemical species are not important to Hg methylation.

Sulfate reducing bacteria (SRB) have been implicated in methylating Hg in environmental samples (e.g., Choi and Bartha, 1994; Gilmour et al., 1992; Sparling pers. comm., 1995). There are a number of reasons why no relationship was found between the porewater concentrations of SO_4^{2-} and MeHg across the 7 wetlands in this study, and these reasons will be discussed in detail in Chapters 5 and 6. Interestingly, the largest MeHg concentrations occurred in "black" peat which is composed of oily black *Sphagnum fallax*. It is possible that the normally green *S. fallax* is blackened because of the formation of $FeS{\cdot}nH_2O$ during SO_4^{2-} reduction.

2.5 Conclusions

In peat, concentrations of T-Hg range from 10 to 120 ng g^{-1} and MeHg from 0.1 to 4.0 ng g^{-1} with the highest concentrations of both in hollows. In peat porewater, the concentrations of MeHg range from 0.02 to 7 ng l^{-1}. Partition coefficients between MeHg in peat and porewater range from 8.0 x 10^3 to 5.7 x 10^5, but proportionally more MeHg is partitioned to the porewater as the MeHg concentration increases. Concentrations of MeHg in peat and porewater are greatest just below the water table, suggesting that biologically mediated redox reactions are important in Hg methylation. No water chemistry variables were correlated with MeHg concentration, indicating the complexity of the Hg methylation process.

Chapter 3: MeHg Concentrations in the Peat Porewater of a Recently Impounded Wetland

3.1 Introduction

Although the principal methylators of Hg and mechanism for Hg methylation are are still under investigation, it is clear MeHg production can be enhanced by increasing the nutrient supply. In laboratory incubations, Wright and Hamilton (1982) enhanced MeHg production in sediments by addition of "Tryptic Soy Broth" (TRP) which is a soybean-casein digest. Their experiment is of limited application because TRP is much more bioavailable than lake water nutrients and Hg was added to enable detection of MeHg but they show that the MeHg concentration can be increased through the stimulation of microbial activity by increasing the nutrient supply. The addition of SO_4^{2-} to lake sediment has also increased the MeHg concentration in overlying lake water (Gilmour et al., 1992; Gilmour and Riedel, 1995). Where SO_4^{2-} availability does not limit SO_4^{2-} reduction, Hg methylation appears to be controlled by the availability of carbon (e.g., Choi and Bartha, 1994).

When land is impounded to create reservoirs, the supply of nutrients to bacteria is increased through the decomposition of the terrestrial vegetation (Hecky et al., 1991; Jackson, 1988). Not surprisingly, the contamination of fish stocks with MeHg is most acute in shallow reservoirs, which have the greatest land:water ratio (Bodaly et al., 1993). The hydroelectric reservoirs of northern Canada often flood wetland but the area of wetland flooded is unknown (National Wetlands Working Group, 1988). Wetlands also contain substantial amounts of MeHg in peat, peat porewater, and plants (Moore et al., 1995, Chapter 2). Therefore, impoundment of wetland may result in more MeHg becoming available to the food chain, by enhancing the release of MeHg from wetland stores (peat, peat porewater, and vegetation) or stimulating production of new MeHg in the anaerobic and nutrient-rich conditions.

In Chapter 2, it was found that the MeHg concentration in peat porewater is a relatively good indicator of the MeHg concentration in peat, although further development of this relationship is required. The peat porewater is also the means by which MeHg is transferred from pristine wetlands to adjacent water bodies, primarily during rain events (Bishop et al., 1995; Branfireun et al., 1996). The peat porewater of the impounded wetland was assumed to continue to play a similar role, but instead of relying on episodic flushing by storm water, the export of MeHg from the submerged wetland would occur by diffusion or through pond water mixing with porewater.

In this chapter, I will discuss the impact of impoundment on MeHg concentrations in peat porewater, and the importance of the pre and post-impoundment store of MeHg in peat porewater in the MeHg budget of the reservoir. I will also discuss how the changing chemistry (pH, and concentrations of SO_4^{2-}, NO_2^-, NO_3^-, NH_4^+, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and dissolved organic carbon (DOC) of the reservoir water and peat porewater may have influenced MeHg concentrations in peat porewater.

3.2 Methods

3.2.1 Sites

The small, 0.16 km^2, riparian wetland (979) described in Chapters 1 and 2 was flooded with water from the upstream oligotrophic lake 240. The water level in the old pond was increased by 1.2 m, which resulted in a maximum water depth of 2.5 in the old pond, 1.8 m over the peat adjacent the pond, and a few centimetres at the wetland margin. Surface water chemistry was monitored at the inflow from lake 240 (240IF), the east inflow (EIF) (Figure 3-1), centre buoy, and outflow of the reservoir (979OF). Atmospheric inputs of a large number of chemical species are routinely monitored by the ELA meteorological station (Linsey et al., 1987)

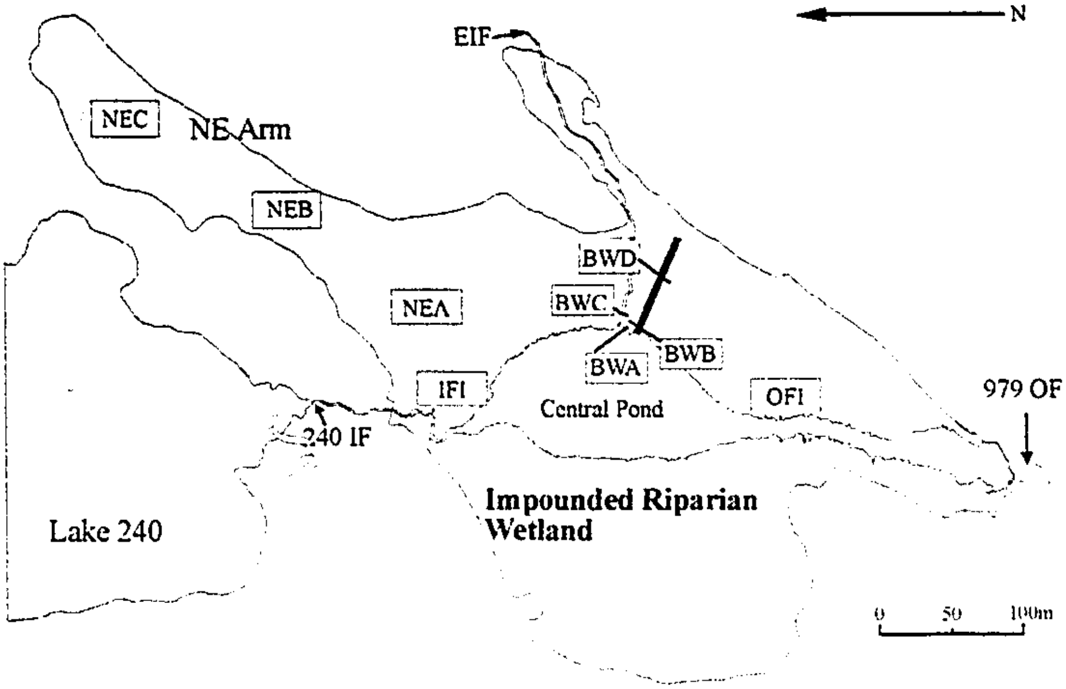

Figure 3-1) Peat porewater and surface water chemistry sampling locations in the experimental reservoir. The shaded area represents the extent of the impoundment.

In 1991, seven permanent sites from which to sample peat porewater were established along two of the hydro-geochemical gradients in the wetland portion of the catchment (Figure 3-1) (Roulet pers. comm., 1992). One of the gradients studied was between the pond and wetland margin on the east side of the basin. Here, four sampling sites were established at 0.4 m (BWA), 2.2 m (BWB), 5.4 m (BWC), and 56.5 m (BWD) from the pond. The stratigraphy of the transect was described by B. Warner (pers. comm., 1994). In simple terms, the stratigraphy consists of a silt bed, up to 2 m thick, overlain by *Sphagnum* peat. The *Sphagnum* unit increases in depth from a few centimetres at the wetland margin to 2 m at the pond edge (Bubier et al., 1993). A layer of gyttja and limnic peat, up to 2 m thick and extending about 20 m from the pond edge, forms a wedge between the peat and the silt. The second gradient studied was between the NE arm and the central pond. Three sampling sites along the central axis of the NE arm were located approximately 30 m (NEA), 90 m (NEB), and 150 m (NEC) from the old pond (Figure 3-1). The stratigraphy of this transect is not as well known. The peat was approximately 3 m deep at NEA, 1.5 m deep at NEB, and 2.0 m deep at NEC. The shallowing of the peat near NEB is the result of a bedrock shelf that also restricts the transfer of water out of the NE arm (Bubier et al., 1993, Roulet pers. comm. 1993). The peat is underlain by a thin silt layer but that can exceed 1 m in depth in some areas.

Prior to sampling, foot paths and board walks were constructed to all permanent sites to minimize disturbance. Each permanent site consisted of polychloroethene (PVC) wells and piezometers installed in nests to depths from 0.10 m to a maximum of 6.0 m where bedrock allowed. Porewater chemistry, including MeHg, was sampled from the wells at depths below 0.5 m. Between the interface and 0.50 cm, water was sampled using the Teflon® sipper described in Chapter 2. This initial sampling design was compromised when much larger than anticipated amounts of peat began to float. To characterize the chemistry of the porewater in the floating islands, two additional sampling sites were added. The sites were located near the reservoir inflow (IFI) and the reservoir outflow (OFI) to assess if water circulation in the reservoir may have affected the porewater chemistry (Figure 3-1). No permanent stations could be established on the islands. Therefore, samples of porewater were collected using the Teflon® sipper.

3.2.2 Collection of Peat Porewater Samples

Using the protocols described in section 2.3.1, porewater samples were collected for the analyses of pH and the concentrations of T-Hg, MeHg, NH_4^+, NO_3^-, NO_2^-, SO_4^{2-}, TDP, TDN, and DOC. The peat porewater chemistry, other than MeHg data, was collected as part of the ELARP monitoring program (Kelly et al., 1996; St.Louis et al., 1996). Peat porewater samples were collected and analysed for NH_4^+, NO_3^-, NO_2^-, SO_4^{2-}, TDP on 4 occasions preceding impoundment and 3 occasions following impoundment. The TDN content was only determined on two occasions following impoundment. pH and DOC were measured biweekly during the ice free seasons from 1992 to 1994, but reduced to monthly in 1995. Methylmercury was measured twice a year with the chemistry sampling from 1993 to 1995 at five sites (BWA, BWC, BWD, NEA, and NEB) and at a few sites and depths in the fall of 1992. MeHg, pH, and DOC concentrations were collected from the peat island sites in the fall of 1994 (only IFI) and July and September of 1995.

3.2.3 Collection of Surface Water Samples for Mass Balance Analysis

As part of the mass balance research of Kelly et al. (1996) the contributions of H^+ (as pH), T-Hg, MeHg, NH_4^+, NO_3^-, NO_2^-, SO_4^{2-}, TDP, TDN, and DOC from the upstream lake, east inflow, centre of the lake, outflow, and the atmosphere were monitored for two years prior to impoundment and for three years of impoundment. Water chemistry sampling protocols and budgets are described in detail in St.Louis et al. (1994, 1996). Total-Hg samples were not filtered, but care was taken not to collect particles or plankton. Surface water fluxes of chemical species into the reservoir basin were determined by extrapolating the concentration of each species over the continually recorded hydrograph. The atmospheric sampling protocol for Hg species is described in St.Louis et al. (1995) and other chemistry species in Linsey et al. (1987). Sampling is performed on a storm by storm basis, and fluxes calculated by multiplying concentration by water volume. Any missing events are prorated against the yearly mean.

3.2.4 Analytical Methods

The methods used in the analysis of pH and the T-Hg, MeHg and DOC concentration in water are described in section 2.3.3. MeHg concentrations will be reported as ng Hg g^{-1} or l^{-1}. The concentrations of SO_4^{2-}, NO_3^-, NO_2^-, NH_4^+, TDN, and TDP were determined at the Fisheries and Oceans laboratory at the Freshwater Institute in Winnipeg, Manitoba or the ELA chemistry laboratory.

3.3 Results

3.3.1 Contributions of MeHg and Nutrients from the Atmosphere and Lake 240 to the Experimental Reservoir

It is not the purpose of this chapter to present the water chemistry budget of the experimental reservoir, which is done in detail in Kelly et al. (1996) and St.Louis et al. (1996). The purpose of this chapter is to discuss the impact of impoundment on the concentrations of MeHg in the peat porewater. This is based however, on the premise that inputs of MeHg and chemical species, which may promote methylation of Hg, have not deviated from normal over the period of the experiment. Therefore, a brief summary of the atmospheric and watershed chemistry will be presented to demonstrate this premise is correct.

The contributions of MeHg, T-Hg, NO_3^-, NO_2^-, NH_4^+, TDN, SO_4^{2-}, TDP, H^+, and DOC from the atmosphere, watershed and upstream lake 240 determined by St.Louis et al. (1996) are summarized in Table 3-1. The mean annual atmospheric deposition of the

Species	Atm mM m^{-2} 1990-91	Lake and 979 EIF inputs (g) 1990-91	Atm mM m^{-2} 1991-92	Lake and 979 EIF input (g) 1991-92	Atm mM m^{2-} 1992-93	Lake and 979 EIF inputs (g) 1992-93	Atm mM m^{2-} 1993-94	Lake and 979 EIF inputs (g) 1993-94	Atm mean & STD 1970-82 mM m^{2-}	Atm mean & STD 1991-94 mM m^{2-}	Lake and 979 EIF mean & STD 1991-94 (g)
NO_3^-	13.46	11900	11.29	65324	9.31	12830	13.37	8798	12.52 ± 3.52	11.86 ± 1.53	24700 ± 23500
NH_4^+	14.03	17780	16.26	67940	10.93	20000	14.97	23100	14.00 ± 5.71	13.81± 1.69	32200 ± 20700
TDN	34.32	266600	38.78	849930	34.47	383550	34.36	376820	33.38 ± 10.37	35.73±2.09	469200 ± 224600
TDP	0.15	3488	0.21	8853	0.10	3850	0.07	3290	0.37± 0.27	0.13± 0.05	2400 ± 1100
SO_4^{2-}	0.0085	3500000	0.0083	13000000	0.0065	5200000	0.0069	4200000	0.011± 0.0045	0.0075± 0.00076	8118000 ± 6341000
DOC	141	4700000	164	18099000	144	7023130	115	6878000	229 ± 80.68	141.5 ± 17	7.04 ± 0.07
Mean pH		7.15		7.02		6.95		6.03			6.79 ± 0.44
T-Hg	3.01 (μg m^{-2})	1.258	3.50 (μg m^{-2})	6.268	2.67 (μg m^{-2})	1.414	2.83 (μg m^{-2})	1.477		3.00 ± 0.311	4.109 ± 1.818
MeHg	0.039 (μg m^{-2})	0.032	0.045 (μg m^{-2})	0.221	0.034 (μg m^{-2})	0.143	0.036 (μg m^{-2})	0.102		0.039 ± 0.004	0.124 ± 0.684
Precip (mm)	755.3		807.4		880		698		700 ± 130	785 ± 67	

Table 3-1) The annual contibution of chemical species from the atmosphere (mM m^2) and lake 240 and the east inflow (g). The atmospheric contribution of the same chemical species from 1970-1982 are provided for comparison (Linsey et al., 1987).The T-Hg and MeHg deposition is from St Louis et al. (1995) and surface water inputs from Kelly et al. (1996). The nutrient deposition and inflow data was provided by Stainton (1995 unpublised data) and Rudd (1995 unpublished data), respectively. The water budget was provided by Beaty and Lyng (1989, and unpublished data, 1995) and the hydrologic year starts Nov 30.

species measured during the years 1991-1994 was not different from the period 1970-82, being within one standard deviation (STD) of the 12 year mean. However, within the study period, the atmospheric deposition of most species was lower during the impoundment years of 1993 and 1994 than the pre-flood years of 1991 and 1992.

Annual fluxes of most species from lake 240 and the EIF to the experimental catchment remained within 1 STD of the 4 year mean but the flux of SO_4^{2-} and NH_4^+ were lower in the post-flood years 1993-1994 than the pre-flood years 1991-1992.

3.3.2 The MeHg Concentration in Peat Porewater of Pre- and Post- Impoundment Peat

In September of 1992 and June of 1993, the mean MeHg concentration (±STD) in filtered peat porewater of the upper metre of peat in the riparian wetland was 0.20 ± 0.25 ng l^{-1} (n = 14) with a maximum of 0.51 ng l^{-1} and a minimum of 0.02 ng l^{-1} (Figure 3-2; Table 3-2). Below 1 m, the mean MeHg concentration was 0.10 ± 0.05 ng l^{-1} (n= 8), the maximum 0.2 ng l^{-1}, and the minimum 0.05 ng l^{-1}. The distribution of MeHg in these peat profiles is typical of other wetlands described at ELA, with maximum concentrations occurring just below the lowest annual water table (Chapter 2).

Date	Mean	STD	Median	cv	n
Jun 93(Pre-flood)	0.27	0.22	0.16	0.79	22
Aug 93	1.06	0.97	0.69	0.91	25
July 94	1.02	1.39	0.49	1.38	25
Sept 94	0.85	0.76	0.71	0.89	25
July 95	0.86	1.25	0.38	1.45	25
Sept 95	0.70	0.83	0.56	1.19	21
Post-flood All	0.82	0.83	0.55	1.19	121

Table 3-2: Statistical summary of pre and post-flood MeHg concentrations (ng l^{-1}) in the upper metre of peat from the stations BWA, BWC, BWD, NEA and NEB.

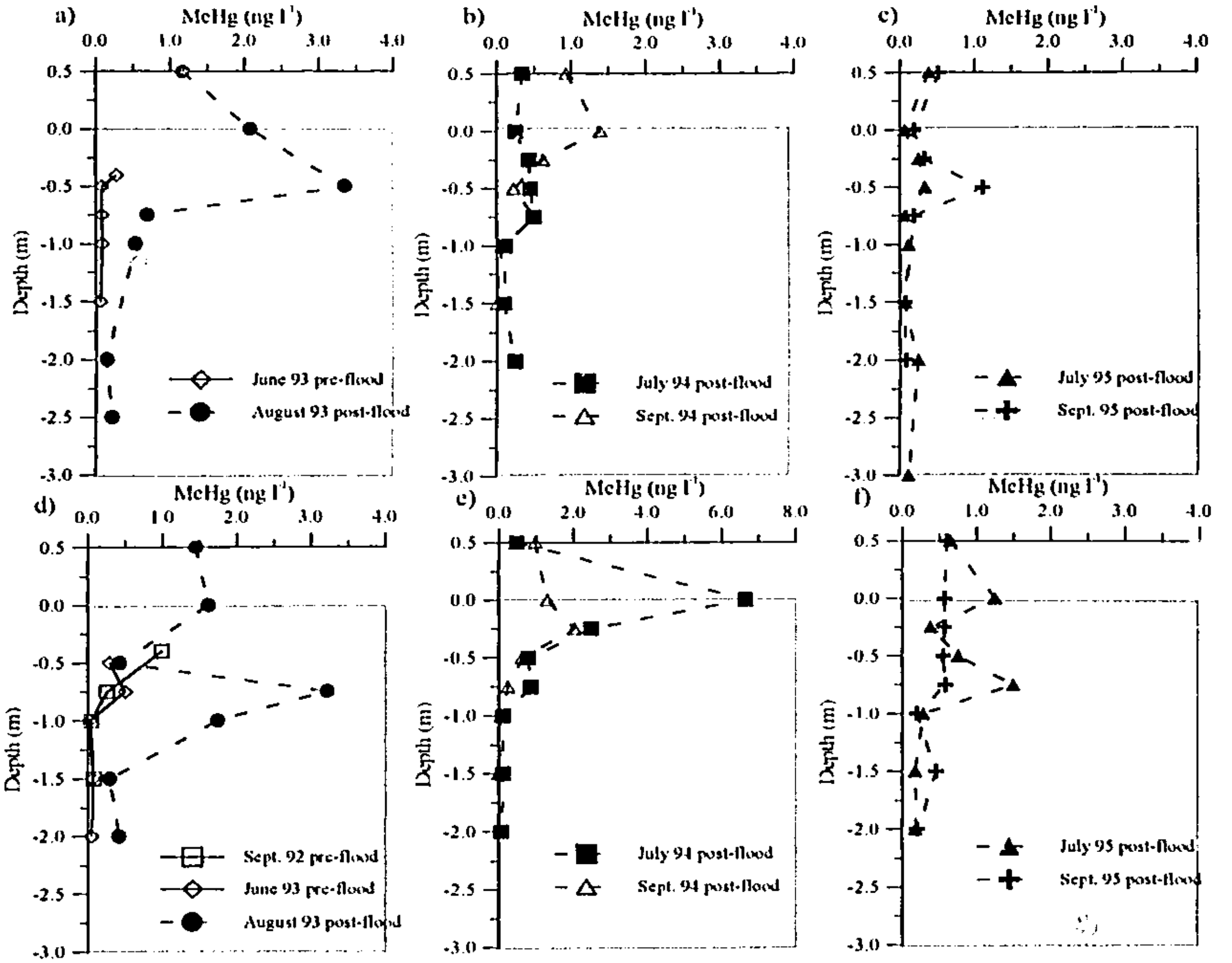

Figure 3-2) Methylmercury concentrations in peat porewater at sites BWA (a-c) and BWC (d-f). The profiles are divided into the pre-flood and initial impoundment year 1992-1993 (a and d) and the two subsequent impoundment years, 1994 (b and e), and 1995 (c and f). The shaded area represents the impounded peat and the white area the overlying water.

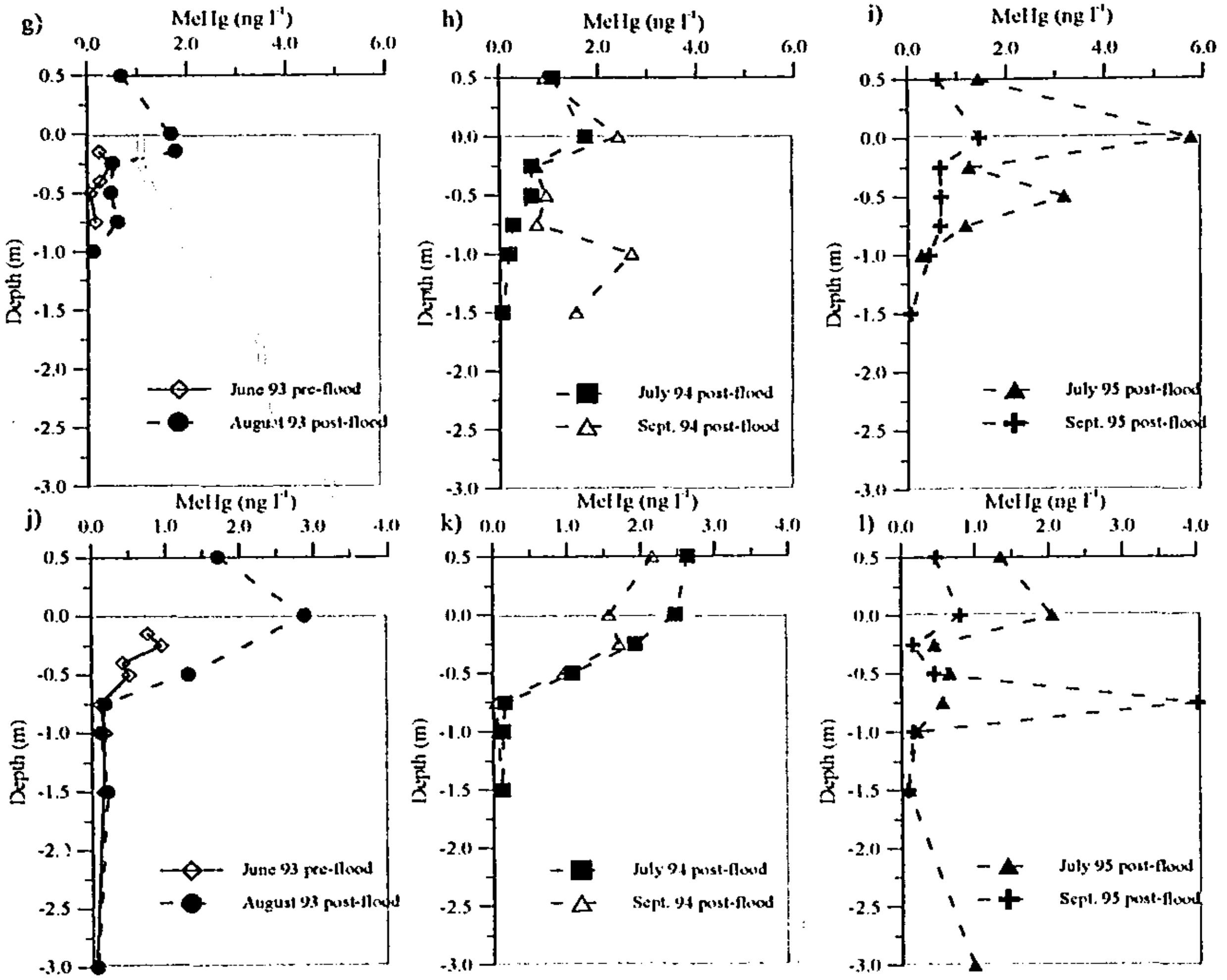

Figure 3-2 cont). Methylmercury concentrations in peat porewater at sites BWD (g-i) and NEA (j-l). The profiles are divided into the pre-flood and the initial impoundment year 1992-1993 (g and j) and the two subsequent impoundment years, 1994 (h and k), and 1995 (i and l). The shaded area represents the peat, and the white area represents the overlying water.

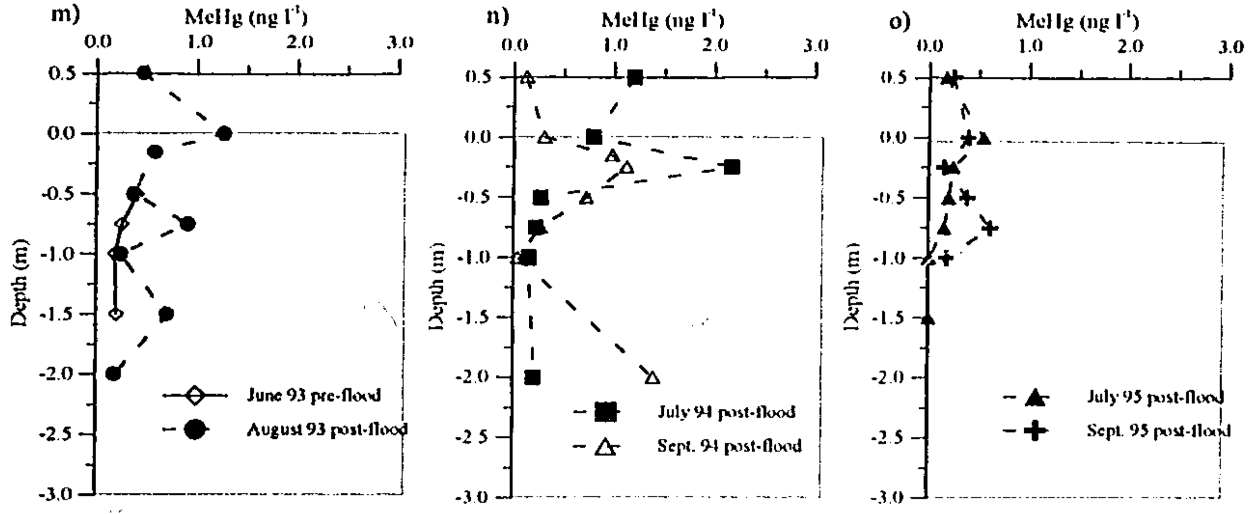

Figure 3-2 Cont.) Methylmercury concentrations in peat porewater at the site NEB (m-n). The profiles are divided into the pre-flood and initial impoundment year 1992-1993 (m), and the two subsequent impoundment years, 1994 (o) and 1995 (p). The shaded area represents the impounded peat and the white area the overlying water.

Following impoundment, the mean MeHg concentration in peat porwater was 0.90 ± 0.83 ng l^{-1} (n = 121) with a maximum of 6.7 ng l^{-1} and a minimum of 0.01 ng l^{-1} The largest MeHg concentrations occurred in the top 0.5 m of peat with the maximum concentration of 6.7 ng l^{-1} recorded at the peat/water interface (Figure 3-2). Over the three years following the initial impoundment, the MeHg concentration at the same depth in a profile could vary by as much as an order of magnitude. Such large spatial and temporal variabilities make the establishment of general trends difficult (Table 3-2). Still, shortly after impoundment, the maximum MeHg concentration in the profile appears to have shifted from the old water table to near the peat/water interface (Figure 3-2). This is clearly seen at site BWC and NEA. However, by 1995, this profile structure had disappeared and been replaced by two new profile structures. At sites BWC, BWD, and NEA MeHg concentrations in porewater remained high, but a bimodal profile structure developed with maxima at the peat/water interface and a depth of 1.0 m. At BWA and NEB, MeHg concentrations were lower than at the other three sites and the profiles have become almost homogeneous in appearance.

The porewater MeHg concentration profiles were greatly altered by the changing structural integrity of the peat. At site BWA, located adjacent to the pond, the buoyant peat became separated to such an extent that pondwater could easily mix with porewater. As a result, the MeHg concentrations in the porewater are the same as the adjacent pond water. At the sites BWC, BWD, and NEA, the top metre of peat became separated from the underlying peat surface at some time each summer. Although the separated peat did not reach the water surface, the peat porewater of the separated and underlying peat was more accessible to mixing with pond water. Therefore, the bimodal pattern of the MeHg concentration profile most likely reflects this pond water incursion. At site NEB, post-impoundment MeHg concentrations were generally lower than the other sites. Site NEB is located in the shallowly impounded NE arm of the wetland. Here, water circulation was poor, the peat did not exhibit a tendency to float, the peat thawed later in the year, and rates of decomposition were slower (Moore pers. comm., 1995). All of these factors may have contributed to the lower MeHg concentrations measured in the NE arm.

In porewater of the peat islands, the mean MeHg concentration was 0.72 ± 0.56 ng l^{-1} (n = 29) with the maximum 2.51 ng l^{-1} and the minimum 0.06 ng l^{-1} (Table 3-3). These concentrations are similar to those in the upper metre of submerged peat. The highest MeHg concentrations occur near the base of the peat island, which ranged between 0.75 and 1.0 m. Although this is opposite to the submerged peat, it is consistent with the observation that the maximum MeHg concentrations occur close to the interface between the peat and oxygenated pond water.

	Sept. 1994		July 1995		Sept.1995	
Depth (m)	Inflow (ng l^{-1})	Outflow (ng l^{-1})	Inflow (ng l^{-1})	Outflow (ng l^{-1})	Inflow (ng l^{-1})	Outflow (ng l^{-1})
Water	0.93					
0.00	1.39	-	0.18	0.65	0.33	0.39
0.25	0.62	-	0.27	0.38	1.33	0.45
0.50	0.22	-		0.77	0.92	1.34
0.75	0.51	-	0.36	2.51	0.84	1.44
1.00	0.06	-	0.49	0.30	0.62	0.17

Table 3-3: Methylmercury concentrations in the porewater of floating peat islands.

The large spatial and temporal variabilities in post-impoundment porewater concentrations of MeHg have made it difficult to clearly establish the magnitude and temporal stability of the change in porewater MeHg concentration induced by impoundment (Table 3-2). Furthermore, the data set is skewed, making it impossible to apply parametric statistics with confidence. The most effective way to compare pre and post-impoundment MeHg concentrations is through box and whisker plots (Figure 3-3). The overlap in porewater MeHg concentrations between pre-impoundment and post-impoundment dates is almost entirely limited to the upper quartile of pre-impoundment and the lower quartile of post-impoundment concentrations. Also, the pre-impoundment median MeHg concentration does not fall within the first lower quartile of any post-

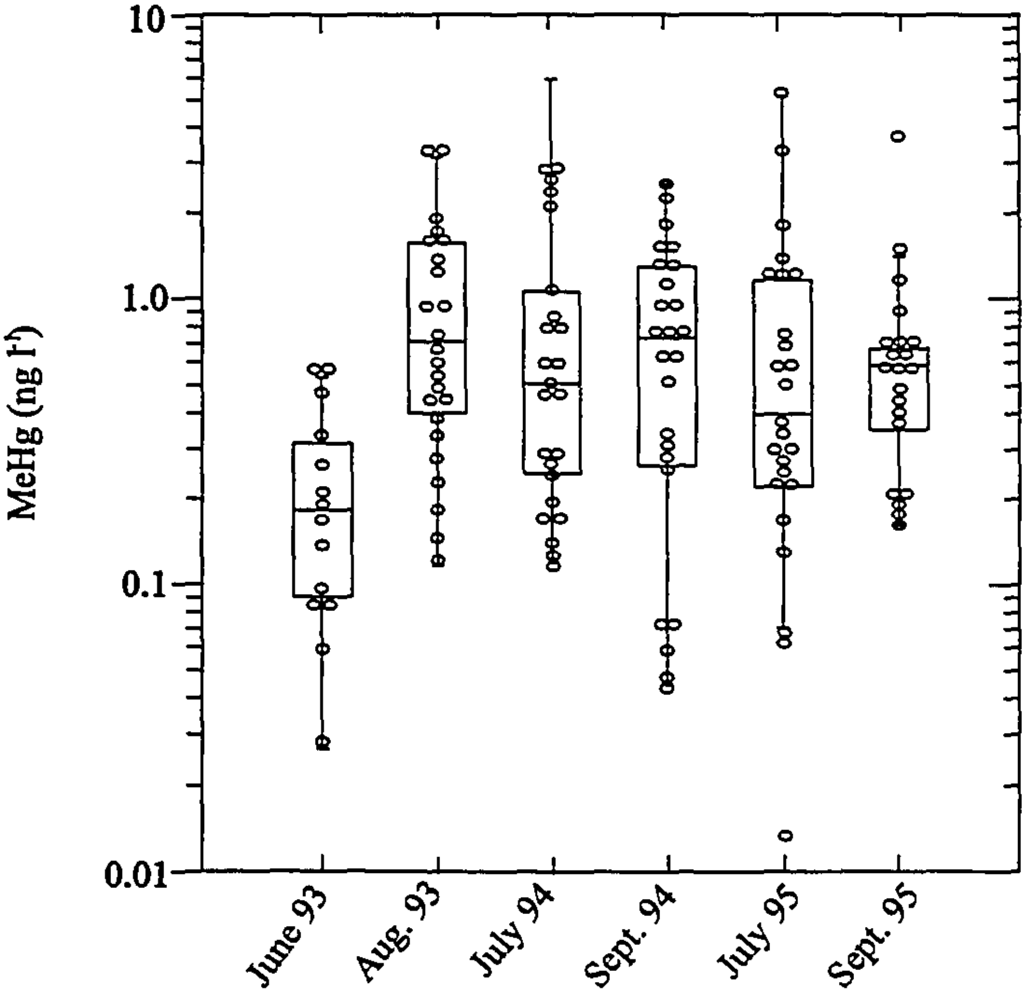

Figure 3-3) Box and whisker plot of pre-flood (June 1993) and post-flood (Aug 93-Sept. 95) log MeHg concentrations in the upper metre of peat porewater. The horizontal line in each box represents the median. The ends of the boxes represent the upper and lower quartiles. The whiskers are 1.5x the hinge spread (1.5 x the upper and lower quartiles). The individual concentrations are depicted as circles and the concentrations outside the whiskers are considered outliers.

impoundment sampling date. Therefore, even with the high degree of post impoundment variability (Table 3-3) the porewater MeHg concentrations in the wetland have increased substantially above the pre-impoundment concentration and have remained so for three years after impoundment.

3.3.3 The MeHg Concentration in Peat Porewater Compared to Surface Water.

The T-Hg and MeHg concentrations measured at the inflows, centre buoy, and outflow of the reservoirs are presented in Kelly et al. (1996) but for convenience, are briefly summarized here. Prior to impoundment, T-Hg concentrations of unfiltered water increased from 2.07 ± 0.71 ng l^{-1} to 2.62 ± 1.04 ng l^{-1} and MeHg concentrations increased from 0.04 ± 0.010 ng l^{-1} to 0.08 ± 0.054 ng l^{-1} on passing from the main outflow through the central pond (Kelly et al., 1996; St.Louis et al., 1996). Following impoundment, the mean T-Hg and MeHg concentrations of unfiltered water at the reservoir outflow were 3.41± 1.59 and 1.04 ± 0.61 ng l^{-1}, respectively, which is an increase of 26 % in T-Hg concentration and 1300% in MeHg concentration over the pre-impoundment values (Kelly et al., 1996; St.Louis et al., 1996).

The MeHg concentration of filtered surface water overlying the peat was always lower than the maximum concentration of the peat porewater (Figure 3-2). The MeHg concentration of unfiltered water at the centre of the reservoir was also lower than the maximum concentration in nearby peat porewater at the time the profiles were taken (Kelly et al., 1996). In the NE arm, the MeHg concentration of peat porewater was similar to, but rarely higher than, the water column in the centre of the reservoir (Figure 3-2). This is not surprising as the water in the NE arm is isolated from the water moving through the old pond channel and only mixes with the central pond during impoundment or draw down events (McCullough pers. comm., 1995).

3.3.4 The Surface Water Chemistry of the Reservoir

Impoundment had a measurable impact on some aspects of water column and peat porewater chemistry (Table 3-4 a,b). Using one STD as the criterion for a change, water column concentrations of NO_2^-, NH_4^+, TDN, TDP and DOC increased, NO_3^- remained the same, while the SO_4^{2-} concentration and the pH decreased. In the peat porewater, only an increase in the concentration of TDP is apparent, but the increase is spatially variable as the post-impoundment STD was twice that of the mean. The mean concentration of DOC in porewater decreased slightly, but remained within one STD of the mean. However, Matos and Moore (1996) found the spatial distribution and composition of DOC had changed.

Chemical fluxes between peat porewater and reservoir water column were not measured directly, but comparing the concentrations of chemical species between the porewater and reservoir, indicates the direction of flux. The NO_3^- and SO_4^{2-} concentrations in the upper 50 cm of peat porewater are generally lower than in the reservoir, therefore the water column would likely supply NO_3^- and SO_4^{2-} to the peat porewater. The concentrations of DOC, TDP, TDN, NH_4^+, and NO_2^- are greater in the peat porewater than the water column and therefore the peat porewater is a source of these chemical species to the reservoir water column.

Table 3.4a Pre-impoundment 1992-93 Post-impoundment 1993-94

Species	Water column mean	Water column std	Water column n	Water column mean	Water column std	Water column n
NH_4^+ ($\mu g\ l^{-1}$)	15.3	5.8	32	48.9	47.5	24
NO_3^- ($\mu g\ l^{-1}$)	10.2	15.3	32	5.5	8.2	24
NO_2^- ($\mu g\ l^{-1}$)	0.7	0.6	32	1.4	1.1	24
SO_4^{2-} ($mg\ l^{-1}$)	4.0	0.5	32	2.7	0.6	27
TDN ($\mu g\ l^{-1}$)	340	70	32	470	140	24
TDP ($\mu g\ l^{-1}$)	3.7	1.2	32	9.9	3.5	23
DOC ($mg\ l^{-1}$)	9.7	2.6	32	12.2	2.9	24
pH	6.6	0.2	32	6.3	0.3	23

Table 3.4b Pre-impoundment 1992-93 Post-impoundment 1993-94

Species	Porewater mean	Porewater std	Porewater n	Porewater mean	Porewater std	Porewater n
NH_4^+ ($\mu g\ l^{-1}$)	1200	700	117	1300	800	192
NO_3^- ($\mu g\ l^{-1}$)	3.4	4.2	117	3.1	4.4	193
NO_2^- ($\mu g\ l^{-1}$)	6.8	5.0	117	7.9	4.8	193
SO_4^{2-} ($mg\ l^{-1}$)	2.0	2.4	136	1.0	2.1	211
TDN ($\mu g\ l^{-1}$)				1500	3100	34
TDP ($\mu g\ l^{-1}$)	26.6	19.0	36	92.4	170.6	112
DOC ($mg\ l^{-1}$)	48.9	22.6	127	40.8	18.9	434
pH	5.6	0.5	129	5.2	0.5	335

Table 3.4: Concentrations of NH_4^+, NO_3^-, NO_2^-, SO_4^{2-},TDN, TDP, DOC, and the pH in the: a) surface water and b) upper metre of peat porewater. The pre-flood period is from January 1992 to June 1993 and post-flood period is from July 1993 to November 1994.

3.3.5 Relationships Between MeHg Concentration and Nutrients

The data set collected in this study is too small for most types of statistical analysis. Simple regressions between porewater concentrations of MeHg and the chemical species measured revealed no significant correlations, the largest r^2 being 0.053. Scatter plots of the data, such as those in Figure 2-8, also did not reveal any trends. The concentration of MeHg appears to depend on a number of variables, and even with a significant increase in MeHg concentration, the influence of single variables can not be elucidated.

3.4 Discussion

3.4.1 Methylmercury in Peat Porewater: an indicator of the change in the store of MeHg in the impounded wetland

The external loading of MeHg and other chemical species (which may stimulate Hg methylation) to the reservoir catchment did not change during the study period (Table 3-1). Therefore, the changes in water chemistry that occurred within the reservoir are assumed to be caused entirely by internal processes resulting from the impoundment. There are two potential reasons for the observed increase in water column and porewater MeHg concentrations. First, MeHg could be released from stores in the impounded wetland. Second the rate of Hg methylation in the reservoir could have increased relative to the rate of demethylation (the difference between methylation and demethylation will be called net MeHg production).

The pre-impoundment pool of MeHg in the peat porewater is of little importance to the post impoundment MeHg budget. Extrapolating the mean concentration of 0.2 ng l^{-1} over the upper metre of peat in the basin (142700 m^2) yields 0.02 g of MeHg, which represents only 2% of the 1.165 g of MeHg exported from the impounded wetland in 1993. However, following impoundment, the average MeHg concentration in the upper metre of peat porewater had increased to 1.0 ng l^{-1} and the estimated burden of MeHg in the peat porewater at any one time was approximately 0.12 g. The post-impoundment pool of MeHg in the peat porewater is now significant and represents 10%, 14%, and 39% of the 1.32 g, 0.92 g, and 0.33 g MeHg exported in the three impoundment years (Kelly et al., 1996).

The largest potential source of MeHg to peat porewater and the reservoir water is the vegetation and peat of the newly flooded wetland (Moore et al., 1995, Chapter 2). Leaching of MeHg from these pools could easily increase the MeHg concentration in both surface and peat porewater to observed levels. However, in Chapter 2, it was observed that MeHg concentration in peat porewater is only a reflection of the MeHg concentration in adjacent peat. Therefore, to maintain the partition between the peat and peat porewater, it is more probable that the MeHg concentration in the peat also increased (Chapter 4). It has been demonstrated that increasing nutrient availability increases net MeHg production in lake sediments (e.g., Gilmour et al., 1992; Wright and Hamilton, 1982). Therefore, the most likely reason for the increase in the amount of MeHg present in the reservoir waters is that microorganisms, stimulated by elevated nutrient concentrations in the reservoir, have methylated a portion of the inorganic Hg found in the peat and plant tissue during their decomposition (Moore et al., 1995; Hecky et al., 1991; Rasmussen et al., 1991). Increases in CO_2 and CH_4 emmisions from the wetland following impoundment indicate microbial activity has indeed been enhanced (Kelly et al., 1996). A portion of the newly produced MeHg is released to the water, maintaining the partition between peat and water. Changes in the speciation of Hg in decomposing plant tissue will be discussed in detail in Chapter 4.

3.4.2 Influence of Water Chemistry on the MeHg Concentration in Peat Porewater

Higher MeHg concentrations in water have been associated with warmer temperature, lower pH, increased nutrient supply, and anoxic conditions (Winfrey and Rudd, 1992). A number of changes in the reservoir may have promoted the higher concentrations of MeHg in the peat porewater. During the period of impoundment, the summer temperature in the upper 50 cm of peat increased by an average of 4°C (Roulet pers. comm., 1995). Although the increase in temperature would have assuredly increased the overall microbial activity, the impact would have been felt by both Hg methylating and MeHg demethylating bacteria. As MeHg concentrations remained high through the entire impoundment period, it is unlikely the increase in peat temperature alone was an important factor in net MeHg production.

Impoundment significantly increased the surface water concentrations of NH_4^+, TDP, and DOC and depleted the water of SO_4^{2-} and NO_3^-. However, impoundment had little impact on the concentrations of these same species in the peat porewater. This lack of a measurable change in the porewater chemistry may explain, in part, why the results of correlations between MeHg concentration and individual water chemistry parameters were uninformative. In both lake sediments and peat, microbially mediated processes often occur in layers often only a few centimetres thick (e.g., Brown and McQueen, 1985; Kelly, 1994; Lovley and Klug, 1983). Therefore, it is also likely that the gradients being sought were occurring at a scale much smaller than the sampling method was designed to measure. The establishment of measurable gradients was also confounded by the expansion and floating of peat.

Sulfate reducing bacteria have been identified as important Hg methylating bacteria in lake sediments (e.g., Choi and Bartha, 1994; Gilmour et al., 1992). In laboratory incubations, Gilmour et al. (1992) increased the MeHg concentration in the water overlying sediments by increasing the surface water SO_4^{2-} concentration by small amounts (1 to 10 mg l^{-1}). In the experimental reservoir, the mean water column SO_4^{2-} concentration was 2.7 mg l^{-1} which is much larger than the < 0.06 mg l^{-1} usually found in the peat porewater. Thus, the Hg methylation in peat may be a result of the increased availability of SO_4^{2-} to SRB.

Two observations have been made which contradict the above hypothesis. First, the addition of SO_4^{2-} has been shown not to stimulate SO_4^{2-} reduction in some wetland peats (Weider et al., 1990). Second, pure cultures of SRB can grow fermentatively and methylate Hg in the abscence of SO_4^{2-} (Berman et al., 1990; Choi et al., 1994; Sparling pers. comm., 1995). However, the term SRB encompasses a wide range of microorganisms. It may be that increasing the availability of water column derived SO_4^{2-} does not increase the overall rate of SO_4^{2-} reduction but stimulates some members of the SRB population or, if the flux is constant, changes its composition.

Choi and Bartha (1994) found that when SO_4^{2-} was not limited, MeHg production in sediments was limited by available carbon. In the peat porewater, the amount of carbon (DOC) did not increase as a result of impoundment, but the make up of the DOC changed, perhaps increasing its bioavailability (Matos and Moore, 1996). Using exchange resins (e.g., Leenheer, 1981; Richmond and Bourbonniere, 1987), DOC was fractionated into humic acid and 6 operationally defined fractions of fulvic acid (Table 3-5). In the first year of impoundment, the fulvic acid fraction increased from 70 to 90% of the DOC, and contained almost twice the amount of hydrophilic acids, hydrophilic neutrals, and hydrophobic acids as pre-impoundment DOC. These fractions contain compounds such as small carboxylic acids and oxidized carbohydrates, neutral sugars and polysaccharides, aromatic acids and polyphenols, rich in nitrogen and phosphorus (David et al., 1989; Leenheer, 1981; Thurman, 1985; Thurman et al., 1978).

Form of DOC	1992	1993	1994	1995
FA mg l^{-1} (%)	29.2 (73.2)	57.8 (98.3)	43.3 (99.6)	26.9 (96.0)
HA mg l^{-1} (%)	10.7 (26.8)	1.0 (1.7)	0.2 (0.4)	1.1 (4.0)
HPOA mg l^{-1} (%)	13.9 (34.9)	28.2 (47.9)	25.8 (59.3)	14.1 (50.4)
HPIA mg l^{-1} (%)	6.3 (15.7)	16.7 (28.4)	1.5 (3.5)	8.6 (30.6)
HPIB mg l^{-1} (%)	0.0 (0.0)	1.0 (1.7)	0.5 (1.2)	0.0 (0.2)
HPIN mg l^{-1} (%)	0.7 (1.8)	3.4 (5.7)	12.2 (28.0)	1.4 (5.2)
HPON+B mg l^{-1} (%)	8.3 (20.8)	8.6 (14.6)	3.3 (7.6)	2.7 (9.7)

Table 3-5: Form of DOC in water collected between 0.1-1.0 m between sites BWA and BWC. The forms are FA= fulvic acid, HA = humic acid, HPOA hydrophobic acid, HPIA = hydrophilic acid, HPIB = hydrophilic base, HPIN = hydrophilic neutral, and HPON+B = hydrophobic neutral and base (From Matos and Moore, 1996).

In 1995, the DOC returned to its pre-impoundment composition, whereas the concentration of MeHg in porewater remained high. The temporary change in the composition of the DOC may have served only as a catalyst to Hg methylation. Therefore, the increase in available carbon may also be partly responsible for the higher MeHg concentrations in the post-impoundment peat porewater.

3.4.3 Exchange of MeHg Between Peat Porewater and Pond Water

In general, the peat porewater contains higher concentrations of MeHg than the surface water, and therefore the impounded peat is a source of MeHg to the overlying water. The gradient between the peat porewater and the overlying water column is evident in profiles of MeHg concentration from the fall of 1993 (Figure 3-3a,d,g,j,k). From such gradients taken at a number of times and using the hydraulic conductivity of the peat it was hoped that the relative net MeHg productivity of each site could be determined. However, as the structural integrity of the peat began to fail and the upper metre of peat became buoyant, it was impossible to determine the hydraulic conductivity of the peat and extent of porewater and surface water mixing at any point in the profile. In such a scenario, a two dimensional profiling approach is of limited use, as MeHg may just as easily move laterally as vertically. The profiles in Figure 3-2 and results from the study of the peat islands (Table 3-3) reflect the penetration of surface water pockets into or under the peat. Therefore the sites of Hg methylation lie within anaerobic clusters surrounded by oxygenated water, rather than in a plane separating oxygenated water from anoxic porewater.

The floating of peat not only complicated the analysis of profile-scale processes but it also made it difficult to assess the relative importance of different areas of the impounded peat as sources of MeHg. The water budget of the system is dominated by flow through the middle of the old pond channel from the upstream lake 240 (McCullough and Beaty pers. comm., 1994). Mixing between the water flowing through the main channel and the water overlying the peat is dominated by wind action

(McCullough pers. comm., 1994). Once the amount of floating peat surrounding the old pond became large, the barrier further limited wind driven mixing and therefore limited the effect that the largest area of impounded peat had on the chemistry of exported water. Still, Kelly et al. (1996) observed a large change in the water chemistry at the outflow. It would therefore seem the floating peat surrounding the main channel must play a greater role in elevating MeHg concentrations in the central pond than most of the other impounded peat. However, directly adjacent to the pond (BWA), porewater concentrations of MeHg were almost the same as the pond water at the time of sampling. This paradox makes it imperative that a better understanding of *in situ* rates of net MeHg production and/or the rates of water mixing combined with a fine scale resolution of MeHg concentration are required to understand the relative importance of different areas of peat.

3.5 Conclusions

The pre-flood MeHg burden in the peat porewater is not important in the post impoundment MeHg budget of a reservoir. The MeHg concentration in peat porewater increased from an average of 0.2 to 0.8 ng l^{-1} following impoundment and shows little sign of diminishing three years after the initial impoundment. The increase in MeHg concentration in the porewater is most likely the result of an increase in net MeHg production in the peat, because of an overall increase in microbial activity. Although the peat porewater contains higher MeHg concentrations than the overlying water, the exchange between the two could not be directly assessed.

Chapter 4: Total-Hg and MeHg Concentrations in Decomposing Vegetation in a Precambrian Shield Headwater Wetland and an Impounded Riparian Wetland.

4.1 Introduction

Total-Hg concentrations in upland soils range from 10 to 250 ng g^{-1} (Aastrup et al., 1991; Lee et al., 1994; Mucci et al., 1995). Lee et al. (1994) reported less than 1 ng g^{-1} of the T-Hg in soil was MeHg, but concentrations of 4-5 ng g^{-1} have been reported in podzols of northern Quebec (Chaire de Recherche en Environnement, 1993). In both cases, only significant amounts of MeHg are found in the organic horizons. From the results of Chapter 2, it is clear peat contains a large amount of T-Hg (25 to 125 ng g^{-1}) and MeHg (0.2 to 50 ng g^{-1}). Perhaps of greatest environmental concern is that a significant amount of MeHg can be present in peat porewater and is therefore available for export (Bishop et al., 1995; Branfireun et al., 1996). Using a mass balance approach, St.Louis et al. (1994, 1996) have demonstrated that wetlands are net sources of MeHg but just how and where MeHg is produced in wetlands has not been well established.

Sphagnum is the dominant component of both the peat and surface vegetation of the ombrotrophic bogs at the ELA (Bayley et al., 1986; Rochefort et al., 1990). Moore et al. (1995) reported T-Hg concentrations between 25 and 75 ng g^{-1} (d.w.) and MeHg concentrations between 0.2 and 1.5 ng g^{-1} (d.w.) in the *Sphagnum* species that commonly occur in these bogs. The highest concentrations of MeHg were found in *Sphagnum fallax and Sphagnum angustifolium* which occupy wet areas in bogs such as hollows, and the lowest MeHg concentrations were found in *Sphagnum fuscum*, which occupies the drier hummocks. The MeHg concentration in peat of ombrotrophic bog hollows is between 1.0 and 3.2 ng g^{-1} (Chapter 2). Thus, the MeHg concentration in peat is generally greater than the concentration in the *Sphagnum* living at the surface. The MeHg concentration in peat of hummocks is 0.2 ng g^{-1}, and therefore the same concentration as the *S. fuscum*. However, the MeHg concentration of hummock peat that lies below the water table is similar to the concentration found in peat of hollows. In more nutrient rich wetlands at the ELA, MeHg concentrations in saturated peat were found in excess of 10 ng g^{-1}, which is five times greater than the MeHg concentration measured in any *Sphagnum spp.* (Chapter 2; Moore et al., 1995).

Peat is also composed of other plant tissues, such as leaves of shrubs and trees, which are more easily decomposed than *Sphagnum* (e.g., Johansson et al., 1986; Johnson and Damman, 1991; Ohlson, 1987). These plant tissues contain little MeHg (<0.3 ng g^{-1} d.w.), but they can contain considerable amounts of T-Hg (5 to 25 ng g^{-1}) (Moore et al., 1995; Rasmussen et al., 1991; Rasmussen, 1995). The fate of T-Hg and MeHg during the decomposition of plant matter in different wetland environments is not known. From the above observations, it would appear that wet areas favour the presence of MeHg. It is not clear whether the high MeHg concentrations at the wetter sites results from *in situ* MeHg production, perhaps by methylation of inorganic Hg stored in plant tissue, or from the accumulation of translocated MeHg.

Hecky et al. (1991) hypothesized that high MeHg burdens of reservoir fish were related to the decomposition of the impounded biomass. To assess this, Hecky et al. (1991) added different types of vegetation and soil to limnocorrals containing fish along with $^{203}Hg^{2+}$. More of the $^{203}Hg^{2+}$ ended up as MeHg in fish of corrals to which soil or plant materials were added, compared to controls containing only lake water. This experiment demonstrates that some of the added ^{203}Hg was methylated in the presence of decomposing vegetation but does not necessarily reflect the fate of inorganic Hg stored in the plant tissue because it may be more or less bioavailable. Furthermore, just where and how the MeHg was produced and how it ended up in the fish was not clear. In Chapter 3, it was clearly demonstrated that MeHg concentrations in peat porewater increased following impoundment and it is believed that this increase was the result of an increase in the amount of MeHg in peat and the decomposing biomass.

The purpose of this study is to examine the fate of T-Hg and MeHg during decomposition of fresh plant tissue in wetland and a shallowly impounded wetland environments. To accomplish this, litter bags containing three types of vegetation, typical of ombrotrophic bogs, were placed in the headwater wetland (632) and the impounded riparian wetland (979). Litter bags were retrieved periodically over 2.5 years and the change in mass and T-Hg and MeHg concentrations determined.

4.2 Methods

4.2.1 Materials

Large samples of whole *S. fuscum* (moss), *P. mariana* (black spruce) needles, and green *C. rostrata* (sedge) stems were collected from the riparian and headwater wetlands in the spring of 1993. All samples were air dried to remove excess water and minimize errors in weighing. One to 5 g of each tissue were placed in 10x10 cm 400 μm Nitex® bags (litter bags). Enough bags were made to place 18 bags of each type at each site, thus allowing 6 retrieval dates. The initial T-Hg concentration of each tissue type was determined from 3 replicate samples and initial MeHg concentration from 5 replicate samples (Table 4-1).

Material	T-Hg (ng g^{-1})	Std	MeHg (ng g^{-1})	Std
Picea mariana needles 1993	26.3	1.38	0.18	0.06
Carex rostrata Stem 1993	20.3	1.09	0.92	0.29
Sphagnum fuscum 1993	73.9	0.90	1.04	0.13

Table 4-1) Total-Hg and MeHg concentrations in the original plant tissue.

4.2.2 Sites

Litter bags containing spruce needles and sedge were placed on the peat surface and litter bags containing moss were inserted 5 to10 cm into the peat, to better simulate their true environment of decomposition. In the headwater wetland, a dry site near the wetland margin and a wet site adjacent the central pond were chosen (Figure 4-1a). Three sites were chosen in the riparian wetland catchment that would be covered by >2.0 m (deep), 1.0 m (intermediate) and 0.5 m (shallow) of water, following impoundment (Figure 4-1b). The deep site was located on pond sediment, and the intermediate and shallow sites were located on peat. It was hoped that any spatial differences in the physical or chemical environment of the reservoir, that would influence Hg chemistry of all decomposing plant tissues, would be reflected as differences in T-Hg and MeHg concentration of the tissue at these three sites. By midsummer 1994, the depth- based pattern was disrupted because substantial amounts of peat floated. The litter bags placed at the intermediate site were brought near or to the water surface. After this time, the intermediate site reflected decomposition in the floating peat environment. Although the depth relationship was destroyed, the new distribution perhaps better reflects the actual distribution of decomposition sites in the reservoir. The floating peat also affected the success of retrieving samples. No litter bags were recovered at the shallow impoundment site after the fall of 1994 and only litter bags containing sedge were recovered in the pond in the fall of 1995.

4.2.3 Sample Collection and Analysis

Triplicate samples (litter bags) of each plant type were retrieved biannually. Total-Hg and MeHg analyses can only be performed on tissue air dried until moist, as high temperatures are suspected to volatilize Hg from the plant tissue (Bloom pers. comm., 1993). Therefore a more complicated procedure to achieve oven dry

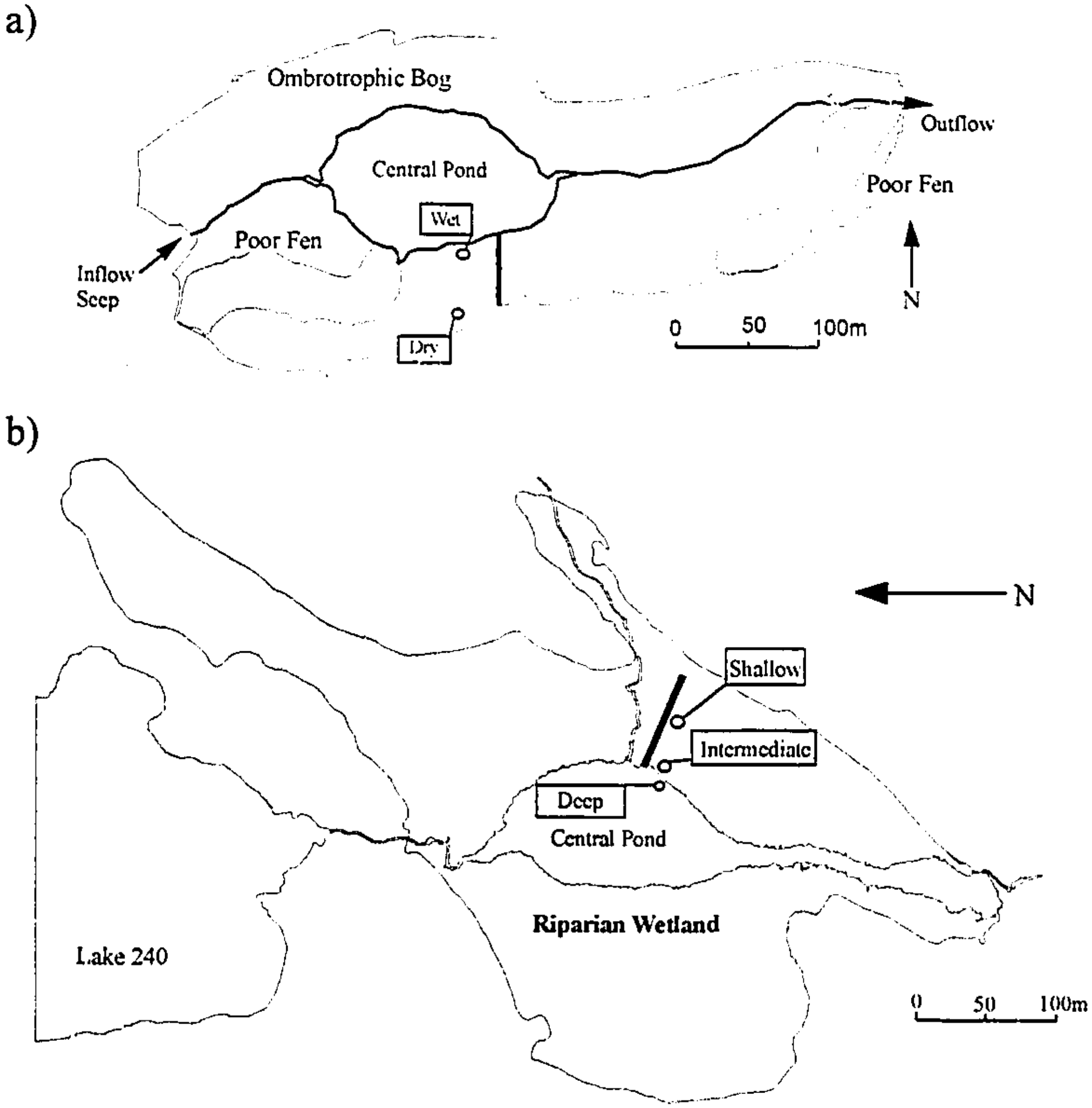

Figure 4-1) Location of litter bags in a) the headwater wetland (632 catchment), and b) the impounded riparian wetland (catchment 979).

weights was followed. First, the air dried mass of each litter bag was determined. The triplicate samples were then pooled and a large subsample oven dried. The subsequent loss in mass was applied as a correction factor to each litter bag. If the analyses of T-Hg and MeHg were not done immediately after air drying, the sample was frozen.

Total-Hg and MeHg concentrations were determined on subsamples of tissue taken from the homogenized pool of sample obtained from the three litter bags. The concentrations of T-Hg in the plant tissue were determined by using a modification of the method of Bloom and Fitzgerald (1988) at Flett Research, Winnipeg, Manitoba. The method is described in detail in Section 2.3.4. Analysis was performed on a minimum of two separate subsamples and concentrations are reported in ng Hg g^{-1} dry weight (d.w.). Methylmercury concentrations were determined at the ELA Hg lab using a method similar to Horvat et al. (1993). This method is also described in section 2.3.4. Analyses of MeHg concentrations were preformed on a minimum of three subsamples, and concentration are reported in ng Hg g^{-1} (d.w.) using the air dried oven dried correction factor.

Measuring mass loss and T-Hg and MeHg concentration allows not only an assessment of the relative change in T-Hg and MeHg concentrations but the change in the absolute amounts. This can be done by using the change in mass to normalize the T-Hg and MeHg concentrations from retrieved samples in terms of the original samples.

4.3 Results

4.3.1 Mass Loss from Plant Tissue in the Headwater Wetland and the Reservoir

The sedge mass was reduced by between 40 and 60% in the first year, and by a further 10 to 20% in the second year regardless of location (Figure 4-2a). The majority of mass loss from spruce needles (25% to 40%) also occurred in the first year of study but mass loss in years 2 and 3 is difficult to detect (Figure 4-2b). On average, no mass loss from moss can be confidently reported at any location (Figure 4-2c). To test if a tissue mass loss rate at any one site was significantly different than any other site, the samples of the mass loss measurements from each site were compared using a student t-test and the slopes of the decomposition rates compared using a modified t-test after linearizing the data using a log transformation of the *x* variable (time) (Zar, 1984). The rates of tissue mass loss were not significantly different between any of the five sites ($p < 0.05$).

4.3.2 Changes in the T-Hg and MeHg Concentrations of the Decomposing Plant Tissues

A summary of the T-Hg and MeHg concentrations in each tissue type are presented in Figures 4-3a-c and 4-4a-c. The T-Hg and MeHg masses in the decomposing tissue are expressed in terms of the original concentration in Figure 4-3d-f, 4-4d-f.

Upon examination of Figure 4-3 and 4-4 it is immediately apparent that the data set contains a large amount of spread which stems from a combination of analytical error and natural variability. The analytical error includes T-Hg and MeHg measurement error (<20%) and the unknown error in determining the dry weight of the tissue. The accuracy of the dry weight determination is dependent on the moisture content of the sample being uniform. Although care was taken to achieve and maintain a consistent moisture content, small differences were inevitable, particularly when the T-Hg concentration was measured after the MeHg concentration and in a different lab. Achieving and maintaining a consistent moisture content in the moss was most difficult and may be partially responsible for the variability in the T-Hg concentration in Figure 4-3e-f.

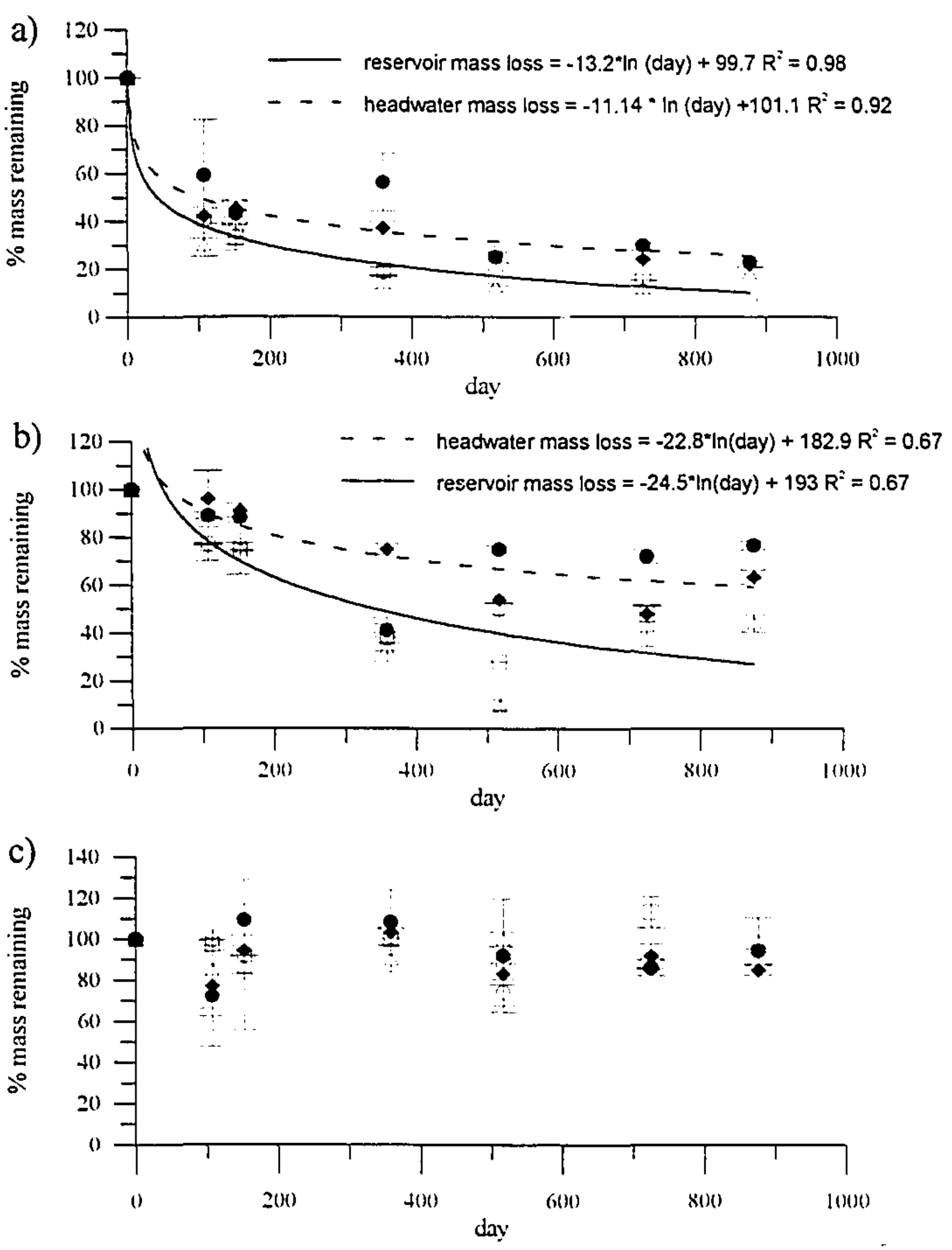

Figure 4-2) Mass loss (decomposition) of: a) sedge b) spruce needles, and c) moss; in the reservoir and headwater wetland. The decomposition of spruce needles and sedge was not linear with time which is apparent by the best fit lines in a and b.

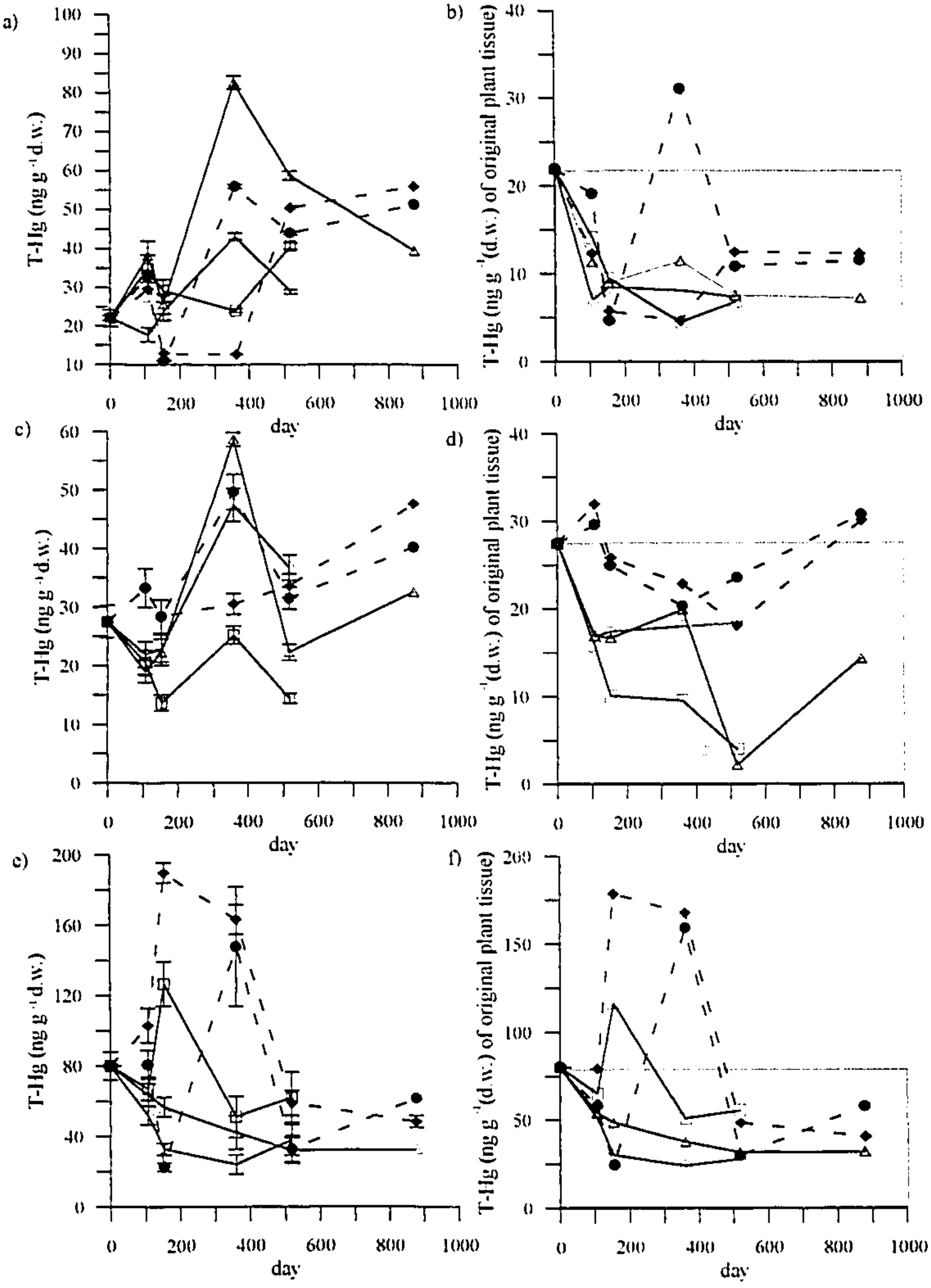

Figure 4-3) Concentration and the mass of T-Hg in a-b) sedge, c-d) spruce needles, and e-f) moss, in the headwater wetland and the reservoir from 1993-1995. The shaded area of figures b,d and f indicate when T-Hg was lost from the tissue. The standard deviation of duplicate measurements is expressed with error bars in a, c, and e.

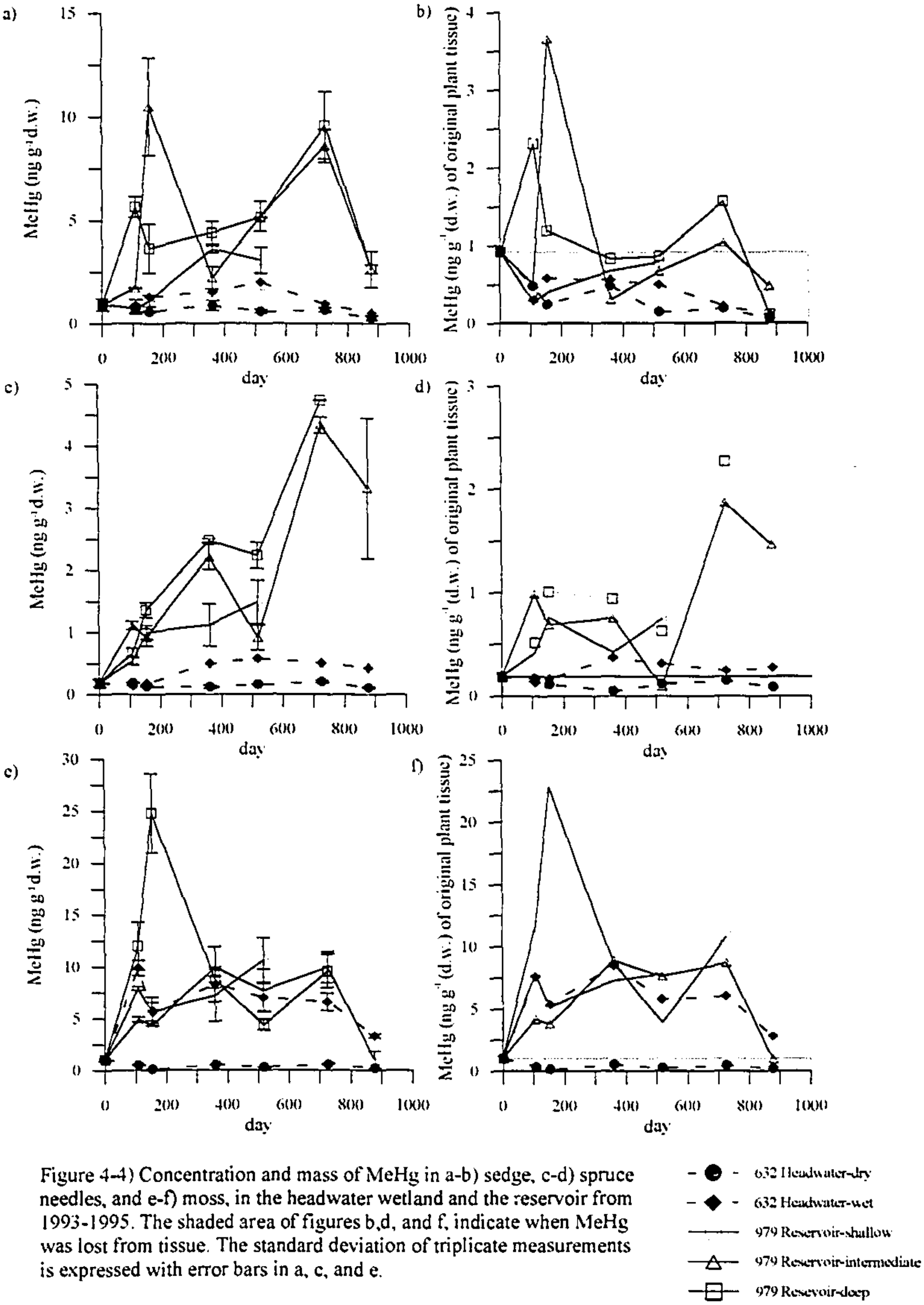

Figure 4-4) Concentration and mass of MeHg in a-b) sedge, c-d) spruce needles, and e-f) moss, in the headwater wetland and the reservoir from 1993-1995. The shaded area of figures b,d, and f, indicate when MeHg was lost from tissue. The standard deviation of triplicate measurements is expressed with error bars in a, c, and e.

The natural variability is the real spatial and temporal variability found at each site. For example, at the wet site, most bags remained saturated for most of the year, but all or parts of the tissue in some bags became dry for extended periods. Some litter bags were overgrown by *Sphagnum*, others covered in leaves, and others harbored insects. Therefore, this data set has the advantage of representing the broad range of concentrations that may occur at a site but the disadvantage of a large error term around each point. The general trends are more important than the inflections in the lines between measurements which may be caused by any number of factors.

In general, the T-Hg concentration increased by 220% in sedge, by 50% in the spruce needles, but decreased by 60% in the moss (Figure 4-3a-c). Although the concentration of T-Hg in sedge more than doubled, the amount of T-Hg remaining in the sedge was about half the original amount, as 60 to 80% of the sedge mass had been lost (Figure 4-3b). The loss of T-Hg from the spruce needles was less pronounced than that of the sedge. Although the spruce needles in the headwater wetland sites lost between 20 and 40% of their mass, the amount of T-Hg in the spruce needles remained unchanged (Figure 4-3d). In the reservoir, the amount of T-Hg in the spruce needles had decreased to approximately 50% of the original amount, therefore approximately 13 ng g^{-1} T-Hg had been lost. Despite an initial increase in the amount of T-Hg in moss, the amount of T-Hg in the moss had been reduced by 50%, or 40 ng g^{-1}, by the end of the experiment (Figure 4-3f).

Both amounts and concentrations of MeHg in decomposing tissue were also not consistent across sites or between types of decomposing tissue. In sedge, the MeHg concentration decreased from 0.93 to 0.30 ng g^{-1} at the dry site, remained unchanged at the wet site, and increased to an average of 2.7 ng g^{-1} in the reservoir. The sedge decomposing in the reservoir had been reduced to 20% of the original mass, yet contained the same amount of MeHg as it did at the onset of the experiment (Figure 4–4a). On average, the concentration of MeHg in spruce needles decreased from 0.19 to 0.11 ng g^{-1} at the dry site, increased to 0.5 ng g^{-1} at the wet site, and increased to 3.2 ng g^{-1} in the reservoir (Figure 4–4c). The amount of MeHg in the spruce needles decreased by an average of 50% at the dry site but increased by 50% at the wet site. In the reservoir, the amount of MeHg in the spruce needles steadily increased and after 2.5 years contained 10 times the original amount of MeHg (Figure 4–4d). With no significant change in the mass of the moss, the MeHg concentration of moss also reflects changes in the amount of MeHg in the moss. The concentration of MeHg in moss decomposing at the dry site decreased from 1.0 to 0.2 ng g^{-1}. At the wet site and all three sites in the reservoir, the MeHg concentration of moss increased to between 6.5 and 10 ng g^{-1}. Therefore, the amount of MeHg in moss at the wet site and in the reservoir increased by an average of 750% (Figure 4-4e-f).

The amount of MeHg in samples of sedge and moss collected from the reservoir in September 1995 is smaller than in most previous samples but the amount of MeHg in the spruce needles remained high. Being the last sampling date, it is not known if the decrease in the amount of MeHg in sedge and moss is indicative of a large drop in the amount of MeHg in the wetland or just part of the natural variability.

4.4 Discussion

4.4.1 Rates of Decomposition

The rates of mass loss (decomposition) from sedge stems and spruce needles of this study are similar to rates observed by others (e.g., Johansson et al., 1986; Ohlson, 1987). In this study, decomposition of *S. fuscum* could not be detected. In general, it is recognized that *Sphagnum* decomposition is slow, and annual mass loss rates of hummock forming species from 1 to 10% have been reported (e.g., Bartsch and Moore, 1985; Johnson and Damman, 1991; Reader and Stewart, 1972; Rochefort et al., 1990). It has also been reported that rates of plant decomposition are greatest under saturated or periodically saturated conditions and when nutrients are more available (Hogg et al., 1994; Johnson and Damman, 1993; Ohlson, 1987). In this study, despite differences in both the availability of nutrients and the degree of saturation, between site differences in rates of mass loss were not statistically significant (Chapters 2 and 3). With no apparent difference in the rate of decomposition of same tissue type across sites, between site differences in T-Hg and MeHg concentration can not be attributed to different rates of decomposition.

4.4.2 The Increase in the MeHg Concentration of the Plant Tissue: adsorption or Hg methylation?

As the amount of Hg decreased in all decomposing plant tissue, it is obvious the fresh plant matter is a source of Hg to the wetland. Two theories can explain the increase in MeHg concentration of the decomposing tissue. Either MeHg is adsorbed from the water (e.g. porewater, precipitation, reservoir water) or Hg is methylated in and/or on the tissue surfaces. As neither the input and output of Hg and MeHg to and from the litter bags nor *in situ* Hg methylation were measured, neither theory can be proven directly. However, there is evidence within this and other studies to suggest the latter is more likely.

The *in situ* methylation of Hg in peat and moss has been demonstrated in controlled anaerobic environments (e.g. Chapter 5; Morrison and Thérien, 1994; Povari and Verta, 1995) and observed in limnocorrals (Hecky et al., 1991) and reservoirs (Chaire de Recherche en Environnement, 1993; Matilainen, 1995). Using a mass balance approach, St.Louis et al.(1996) have shown wetlands are sources of MeHg. These studies suggest Hg methylation is likely to have occurred in the decomposing vegetation. However, the MeHg concentration in the moss will equilibrate with the concentration in the surrounding peat (Chapter 2 and 5). In the headwater wetland wet site, the concentration of MeHg in moss reached ≈ 7 ng g^{-1} which is equal to or higher than the concentration in the surrounding peat (4-8 ng g^{-1}). Therefore, the litter bags are likely to be a local source rather than sink of MeHg. Clearly, to finally resolve this issue, Hg methylation rates within the decomposing tissue at ambient concentrations needs to be measured.

4.4.3 Total-Hg Retention by Moss

About 30 to 40% of the T-Hg had been lost from moss placed at all sites. The loss of T-Hg from moss during decomposition is counter to that reported by Moore et al. (1995) for *Sphagnum angustifolium* and Satake and Miysaka (1984) reported for *Jungermannia vulcanicola*. They both found an enrichment of T-Hg toward the senescent part of the plant, but neither had accounted for any change in mass. Therefore, live *Sphagnum* may be more effective (per unit weight) in trapping Hg and other similar heavy metals than peat.

4.4.4 Factors Influencing the Amount of MeHg in Decomposing Plant Tissue

The site of decomposition affected the amount of MeHg in like tissues. Tissues decomposing at the dry site lost MeHg and tissue decomposing at the wet site or in the reservoir either gained MeHg or maintained a large amount of MeHg. Winfrey and Rudd (1992) suggested that anoxic conditions, high nutrient availability and low pH favour high MeHg concentrations. Being aerobic, the dry site and areas of the wetland such as hummocks do not favour the presence or production of MeHg. At the wet site, the litter bags were nearly always moist, if not saturated. Therefore, anaerobic conditions could develop in pockets, or throughout the decomposing plant mats (e.g., Norrström, 1994; Ponnamperuma, 1972). The fact that more MeHg was present in moss than any other tissue is perhaps due in part to the fibrous nature of moss, that allowed more anaerobic pockets to develop, and the fact that the moss was inserted into the peat rather than placed on the surface.

The simple presence of anoxic conditions cannot explain why MeHg concentrations in the litter became so high. Winfrey and Rudd (1992) also proposed that a greater availability of nutrients favoured Hg methylation. The fresh litter is a substantial source of C,N,P, and S that is readily available to microorganisms. The transfer of water through the oxic and anoxic zones further promotes the cycling of nutrients through microbial mediated redox reactions. Just what reactions are important in promoting Hg methylation are not clear but will be investigated further in Chapter 5.

4.4.5 Impoundment Effects upon MeHg Production in the Peatland

The MeHg concentration in moss of the reservoir was independent of site and on average (≈ 8 ng g^{-1}) was only slightly higher than moss at the wet site (≈ 7 ng g^{-1}) of the headwater wetland. The small difference between MeHg concentrations in the moss in the reservoir and moss at the wet site implies that impoundment has not created an environment in which rates of MeHg production are significantly higher. In the pre-existing wetland, high MeHg concentrations were restricted to hollows, but in the post impoundment environment, the condition of near permanent anoxia encompasses the entire peatland. Therefore, the increase in amount of MeHg in the porewater of the impounded riparian wetland (Chapter 3) perhaps has more to do with an increase in the area of MeHg production than an increase in the rate of Hg methylation at any particular site.

4.5 Conclusions

Over the 2.5 years of the experiment, sedge, spruce needles and moss lost 80%, 40%, and 0% mass, respectively. No significant difference in the rates of decomposition were apparent between the dry site and wet site in the headwater wetland and three sites in the experimental reservoir. T-Hg was released during the decomposition of all tissues, but the concentration of T-Hg increased in spruce needles and sedge.

Concentrations and amounts of MeHg in tissues decomposing at the headwater wetland dry site decreased. Methylmercury concentrations increased in all tissues decomposing at the wet and reservoir sites. At the wet site, the amount of MeHg in sedge and spruce needles did not change but increased by 600% in moss. In the reservoir, the amount of MeHg in the decomposing spruce needles and moss increased by 500 and 800%, respectively. These observations clearly indicate that during decomposition of vegetation under wet anaerobic conditions the amount of MeHg in the tissue increases. The increase in the amount of MeHg in the decomposing vegetation is most likely a result of *in situ* methylation of previously accumulated inorganic Hg.

Chapter 5: Controls on Mercury Methylation in Peat and its Release to Porewater

5.1 Introduction

Although methylmercury (MeHg) can be produced abiotically in the presence of very high concentrations of inorganic mercury (e.g., Bertilsson and Neujahr, 1971; Nagase et al., 1984; Ridley et al., 1977), the production of MeHg in uncontaminated systems is mainly, if not entirely, a biological process (e.g., Gilmour and Riedel, 1995; Jenson and Jernelov, 1969; Nagase et al., 1984). Pure cultures of a number of organisms have been shown to produce MeHg on exposure to large amounts of Hg (e.g., Campeau and Barth, 1985; Fisher et al., 1995; Gilmour and Henry, 1991; Landner, 1971; Wood et al., 1968; Yamada and Tonomura, 1972). Wood et al. (1968) and later Ridley et al. (1977) proposed the methylation of Hg required methylcobalamine, one of two metabolically active forms of vitamin B_{12}, to transfer a carbanion methyl-group to Hg^{2+}. Although methylcobalamine is still believed to be the primary methyl donor, just how the methyl group is transferred to the Hg^{2+} is not understood.

The presence of methylcobalamine in a microorganism is not enough to induce Hg methylation. For example, methanogens contain a large amount of methylcobalamine but they do not methylate Hg (Compeau and Bartha 1985, 1987). Choi and Bartha (1993) proposed that Hg methylation in the sulfur reducing bacteria (SRB) *Desulfovibrio desulfuricans* LS, was enzymatically mediated. Later, Choi et al. (1994) proposed that in *Desulfovibrio desulfuricans* LS, the transfer of the methyl group via methylcobalamine occurs in the acetyl-CoA pathway and occurs only when the bacteria is growing fermentatively. Within the group of bacteria loosely classified as SRB, there are species which do not methylate Hg and species which can methylate Hg but do not use the acetyl-CoA pathway in the manner proposed by Choi et al. (1994) (Sparling pers comm. 1996).

No pathway has been proposed for fungi such as *Neurospora crassa* and *Coprinus comatus* both of which have been shown to methylate Hg (Landner 1971; Fischer et al., 1995). None of the studies conducted to date have been performed at Hg concentrations similar to uncontaminated systems (Sparling pers. comm., 1995). Therefore in uncontaminated environments, it remains unclear which organisms are capable of methylating Hg and how they do it.

The identification of Hg methylation mechanisms is best achieved through studies of pure cultures even though it is doubtful that the few cultured organisms represent the behaviour of the countless unidentified microorganisms in natural systems. A different approach involves the manipulation of an entire microbial population within the medium in which they are found. For example, MeHg production in cores of estuarine and lake sediments has been observed by adding $^{203}Hg^{2+}$ (e.g., Furuteni and Rudd, 1980; Ramlal et al., 1986; Gilmour et al., 1995, Stordal and Gill, 1995). Similarly, the importance of functional groups of the microbial population (e.g. methanogens, denitrifiers) in the methylation of Hg can be assessed by the addition of bacterial metabolic inhibitors and stimuli. By using molybdate, a metabolic inhibitor of SRB (e.g., Compeau and Bartha, 1985; Gilmour et al., 1992; Gilmour and Riedel, 1995) and bromoethanesulfate, an inhibitor of methanogenesis (Campeau and Bartha, 1985), SRB have been identified as potentially important methylators of Hg in lake sediments. The addition of nutrients (e.g., Tryptic Soya Broth, Wright and Hamilton 1982) and small amounts of SO_4^{2-} (5 to 25 mg l^{-1}) (Gilmour et al., 1992) to lake sediments have increased the rate of MeHg production. However, maintenance of high SO_4^{2-} concentration (>50 mg l^{-1}) may slow or stop Hg methylation, perhaps because Hg is rendered unavailable being bound with the reduced sulfur forming HgS (Compeau and Bartha, 1985; Gilmour et al., 1992; Gilmour and Riedel, 1995).

Both pure culture and micro environment manipulation studies suggest that

SRB are potentially important methylators of Hg in lake and estuarine sediments. However, there appears to be a dilemma concerning the importance of sulfate in Hg methylation. In pure culture studies, the presence of SO_4^{2-} is unimportant but in the micro environment studies, SO_4^{2-} appears essential. Choi and Bartha (1994) observed that when excess H_2S was removed and SO_4^{2-} was not limited, the organic matter content of the sediment was correlated with the rate of Hg methylation. This observation indicates that, to some extent, both carbon and SO_4^{2-} maybe important.

Incubations of intact microbial populations have only been performed using lake and estuarine sediments. It has been clearly demonstrated in the previous chapters that wetlands are sites of Hg methylation. A large number of microorganisms, including fungi and bacteria, are known to exist in wetlands, but little is known about their numbers, species diversity, and activity (Benda, 1957). In the few freshwater wetlands in which sulfate reduction has been studied, the biologically mediated cycling of sulfur can be rapid, but the supply of SO_4^{2-} (that measured in surface or pore water) is not a good indicator of SO_4^{2-} reduction rates (e.g., Bayley et al., 1986; Giblin and Wieder, 1992; Spratt and Morgan, 1990; Wieder et al., 1990). Sulfate reducing bacteria are most active near the water table (Brown and MacQueen, 1985), which is also the location of high MeHg concentrations in the ELA wetlands (Chapter 2). Many other types of microorganisms are concentrated around the wetland water table (Benda, 1957). Other than this and other circumstantial evidence presented in previous chapters, SRB have not been linked to Hg methylation in wetlands.

In this chapter, I will discuss the results of experiments designed to:
i) examine the partition of MeHg between peat and peat porewater and
ii) determine if Hg methylation can be stimulated by addition of Hg and nutrients (SO_4^{2-}, NH_4^+, NO_3^-, and carbon as DOC and pyruvate), or inhibited by the addition

of H_2S or molybdate, a metabolic inhibitor of SRB.

5.2 Methods

5.2.1 General Approach

Peat was collected with gloved hands (PVC clean room quality gloves), packed into acid rinsed tupperware® containers to minimize exposure to oxygen, and transported to the laboratory. Under a nitrogen atmosphere, the peat was homogenized by gloved hands and 20 g (wet weight) subsamples of peat were placed in acid-washed 150 ml volumetric flasks along with 120 ml of "incubation water"and capped with a subba seal®. The flasks were incubated in the dark at 17°C. The composition of the incubation water depended on the experiment (see below) but all water was purged of oxygen with UHP nitrogen that had passed through an Oxisorb® scrubber. The unused "incubation water" water was retained in a large volumetric flask, capped with a subba seal®, and refrigerated at 4°C. Although the 5:1 peat:water ratio used does not reflect the peat:porewater ratio in the wetland, it was required to ensure enough water could be extracted for analysis.

Two sampling methods, destructive and sequential, were used. For the destructive sampling, a large number of flasks were set up with the same peat and incubation water. At the time of sampling, triplicate or duplicate flasks were opened, a water sample decanted from the flask and the flask discarded. In the sequential sampling experiments, water was extracted and replaced using an acid washed glass syringe. In both cases, the extracted water was immediately passed through a 0.45 μm cellulose nitrate filter, placed in a Teflon® bottle, and frozen. The sampling frequency used depended upon the experiment (see below).

5.2.2 Analytical Methods

The methods used to determine concentrations of MeHg in peat and concentrations of MeHg, DOC and SO_4^{2-} in water are described in Chapter 2. MeHg concentrations are reported as ng of Hg. Concentrations of molybdate were determined by capillary electrophoresis (Waters® Quanta 4000) with a detection limit of 2 μmol and error of ± 10% over the range of 2 and 60 μmol. Concentrations of CO_2 and CH_4 in head space gas were measured with a Shimadzu® mini 2 gas chromatograph equipped with a methanizer. The error in replicate measurements of CO_2 and CH_4 was <5%.

5.3 Experimental Design

5.3.1 Destructive Partition Experiments

To examine the peat-peat porewater partition, peat porewater or low Hg distilled deionized water (SQ) was combined with different types of peat and the subsequent concentration of MeHg in the incubation water determined. Peat was collected from the headwater wetland and the reservoir described in the previous chapters. In the headwater wetland, peat was taken from a depth of 10-25 cm (poor fen) and the surface (poor fen black) of hollows in the poor fen, and a depth of 10-25 cm in hollows of the ombrotrophic area (Chapter 2). In the reservoir, peat was collected from a depth of 0-5 cm (reservoir black peat), 10-20 cm (brown peat), and 1 m (deep peat). The reservoir black peat was found only in embayments along the edge of floating peat islands whereas the brown peat was common. The deep peat was sampled from the underside of peat islands.

In the first experiment, SQ water was combined with poor fen peat, ombrotrophic peat, brown peat, and deep peat. The incubation water was sampled after 24, 72, and 120 hr, to determine if MeHg could be released to SQ water. In the second experiment, peat was recombined with porewater extracted from the area from which the peat was collected. The porewater was collected as outlined in Chapter 2. The destructive sampling design was used to prevent any stimulation of Hg methylation by the replacement of incubation water.

5.3.2 Destructive Hg and Nutrient Addition Experiments using Poor Fen Peat

To test whether introduction of external stimuli would increase the MeHg concentration in the incubation water; peat porewater from the poor fen was enriched with 1 of 5 additions: 1) Hg (30 ng l^{-1} Hg added as HgCl above a T-Hg background of 6 ng l^{-1}), 2) NH_4^+ and NO_3^- (added as 80 mg l^{-1} NH_4NO_3 above a background of 35 μg l^{-1} NH_4^+ and 5 μg l^{-1} NO_3^-), 3 and 4) SO_4^{2-} (1 and 6 mg l^{-1} of SO_4^{2-} added as K_2SO_4 above a background of 0.8 mg l^{-1}), and 5) upland runoff water. The upland runoff addition was composed of an equal mix of porewater and upland runoff water yielding a final composition of 1.1 mg l^{-1} SO_4^{2-}, 26 mg l^{-1} DOC and ≈9 ng l^{-1} T-Hg. Also added to the peat in the upland runoff experiment was 2 g (d.w.) of wetland leaf litter (primarily *Chamaedaphne calyculata*). Therefore, although the sample will be called upland runoff addition, it is really a mixture that combines all the possible nutrient contributions of the poor fen. Control incubations consisted of poor fen peat combined with unenriched porewater, and with porewater in the absence of peat.

Not all nutrient addition experiments could be started at the same time, thus two sets of control incubations were required. The duration of Hg and NH_4NO_3 enrichment experiments as well as control set 1 was 120 hr, and were sampled after 24, 72, and 120 hr. The SO_4^{2-} enrichment, upland runoff addition, and second control (control set 2) experiments lasted 240 hr, and were sampled at 24, 72, 120 and 240 hr, with an additional sampling at 168 hr in the case of the SO_4^{2-} addition.

To examine if differences in rates and kinds of biological activity existed between types of additions, concentrations of CO_2 and CH_4 in the head space of the flasks were determined. Two 1 ml gas samples were extracted with a syringe and the head space was backfilled with 2 ml of UHP nitrogen.

5.3.3 Sequential Addition Experiments

To further examine if sulfur and carbon biochemistry is potentially important to MeHg production in peat, repeated additions of SO_4^{2-}, H_2S, MoO_4^-, and $CH_3COCOOH$ were made to incubated peat. Poor fen peat was combined with porewater collected from the same area and in the same manner as described in the previous incubations. The peat and porewater were allowed to equilibrate for 72 hr in the dark at 17°C prior to any enrichments. After the equilibration period (day3), 20 ml of water was removed and replaced by an equal amount of "treated porewater" using a syringe. Flasks were divided into groups of four, with each group receiving water enriched with a different agent. In one group, each flask received 20 ml of water containing 360 mg l^{-1} SO_4^{2-} (added as K_2SO_4) thereby elevating the SO_4^{2-} concentration in each flask by 60 mg l^{-1}. A second group received water enriched in H_2S. To achieve roughly the same amount of S^- ion as in the SO_4^{2-} addition (20 mg l^{-1} of S), 20 ml of a 259.2 mg l^{-1} solution of $Na_2S \cdot 9H_2O$ was added to each flask. A third group received water containing 48 mg l^{-1} molybdate, added as $NaMoO_4 \cdot 2H_2O$, resulting in a final concentration of 8 mg l^{-1} MoO_4. A fourth group of flasks received water enriched in pyruvate by 7.8 mg l^{-1}, added as $NaCH_3COCOOH$, thereby elevating the concentration of pyruvate in each flask to 1.3 mg l^{-1} (which is 10 times the rate of CO_2 emission). The final group of four flasks received unaltered peat porewater.

After 24 hours, a further 20 ml of sample was removed from each flask and replaced with the appropriately treated water. Therefore if the chemical species were not adsorbed or utilized, the concentration in the incubation water would double. This procedure was repeated a total of seven times with the experiment lasting 9 days. However, the flasks to which SO_4^{2-} was added were sampled for 3 more days (until day 12), but the replacement water was enriched in pyruvate as opposed to SO_4^{2-}. This was done to test if the SRB had become carbon limited. The 20 ml of sample removed daily was adequate for both MeHg and support chemistry analyses, but to maintain quality control, MeHg and chemistry analyses were performed on alternate samples from days 5 through 8. Concentrations of CO_2 and CH_4 in the head space were determined after day 4 and day 6.

5.4 Results

5.4.1 Methylmercury Partition Experiments

The results of the partition experiments are summarized in Table 5.1. The MeHg concentration in filtered "SQ" water exposed to the deep and brown peats remained near the limit of detection (0.02 ng l^{-1}), and increased only slightly when exposed to peat from the poor fen and ombrotrophic bog. The concentration of 0.42 ng l^{-1} is anomalous compared to all other measurements and may be unreliable. Addition of native peat porewater to peat produced more variable results. The MeHg concentration in the porewater decreased to near the detection limit when exposed to the deep peat, decreased by half when exposed to poor fen peat, but remained unchanged when exposed to the ombrotrophic peat. The MeHg concentration in the porewater increased by at least 5 times when water was exposed to either type of black peat. It is obvious that the higher the MeHg concentration in peat, the higher the MeHg concentration in the incubation water. The greatest change in the MeHg concentration of the water occurred in 24 hr, after which time the MeHg concentrations remained fairly consistent with variability most likely an artifact of the destructive sampling method.

Addition of Super "Q"

	Poor Fen Peat	Ombrotrophic Peat	Deep Peat	Brown Peat
	Peat (ng g^{-1})	Peat (ng g^{-1})	Peat (ng g^{-1})	Peat (ng g^{-1})
	20.9 ± 12.8	8.3 ± 1.0	4.4 ± 0.3	8.8 ± 3.3
Time (h)	water (ng l^{-1})	water (ng l^{-1})	water (ng l^{-1})	water (ng l^{-1})
0	0.00	0.00	0.00	0.00
24	0.07	0.42	0.07	0.08
72	0.14	0.09	UND	UND
120	0.16	0.07	UND	UND

Addition of Porewater

	Poor fen Peat	Ombrotrophic Peat	Deep Peat	Reservoir Black	Poor Fen Black
	Peat (ng g^{-1})	Peat (ng g^{-1})	Peat (ng g^{-1})	Peat (ng g^{-1})	Peat (ng g^{-1})
	20.9 ± 12.8	8.3 ± 1.0	4.4 ± 0.3	50.4 ± 8.9	53 ± 9.7
Time (h)	water (ng l^{-1})	water (ng l^{-1})	water (ng l^{-1})	water (ng l^{-1})	water (ng l^{-1})
0	1.73 ± 0.03	0.43 ± 0.02	0.93 ± 0.11	0.93 ± 0.11	0.38 ± 0.07
24	1.29 ± 1.16	0.27 ± 0.07	0.02 ± 0.02	8.75 ± 2.47	7.56 ± 0.76
72	0.47 ± 0.15	0.41 ± 0.15	0.02 ± 0.02	9.18 ± 2.11	6.85 ± 3.35
120	0.85 ± 0.20	0.50 ± 0.05			9.84 ± 1.83

Table 5-1) Methylmercury concentrations in peat (d.w.) and water of the partitioning incubations. UND indicates MeHg concentration was at or near the detection limit of 0.02 ng l^{-1}).

Methylmercury partition coefficients (K_D) between peat and peat porewater after 24 hrs of exposure were derived using

$$K_D = MeHg_{peat}\ (\text{ng g}^{-1}) / MeHg_{aqua}\ (\text{ng g}^{-1}).$$

The K_D increases as the MeHg concentration in peat decreases, which implies that as the MeHg concentration in peat increases, a greater proportion of MeHg is shifted to the water (Table 5-2).

Peat Type	$K_D \times 10^3$	STD x 10^3	MeHg in water (ng l^{-1})	MeHg in peat (ng g^{-1})
Deep	220	415	0.02	4.4
Ombrotrophic	30	4.6	0.27	8.3
Poor Fen	16	22.1	1.29	20.9
Poor Fen Black	7.01	1.9	7.56	53.0
Reservoir Black	5.76	1.0	8.75	50.4

Table 5-2) K_D's for MeHg in peat and incubation water and the measurement error, which is one standard deviation around the mean concentration of triplicate experiments. The concentrations of MeHg in peat are initial concentrations and the concentrations in porewater are from after 24 hrs of exposure (Table 5-1).

5.4.2 Destructive Hg and Nutrient Enrichment Experiments

The results of the "enrichment" incubations are presented in Figure 5-1. Because not all incubations could be run simultaneously, two sets of control incubations were required. The results of the two control sets were similar. At 24 hr the MeHg concentration in incubation water was between 0.6 to 3 ng l^{-1}. After 24 hr, the MeHg concentration was between 0.6 and 1.8 ng l^{-1}. Together, the controls provide a range of MeHg concentration against which to compare the effects of the treatments. Therefore, both control sets are plotted on all graphs of Figure 5-1 for direct comparison.

In flasks receiving Hg, the MeHg concentration in the incubation water was between 2.1 and 3.8 ng l^{-1} after 24 hr but decreased to between 0.3 and 1.8 ng l^{-1} after 120 hr (Figure 5-1a). The MeHg concentrations in the flasks receiving Hg are within the range of the controls. The addition of NH_4NO_3 to flasks resulted in MeHg concentrations in water of < 0.2 ng l^{-1} and are substantially lower than in the controls (Figure 5-1a).

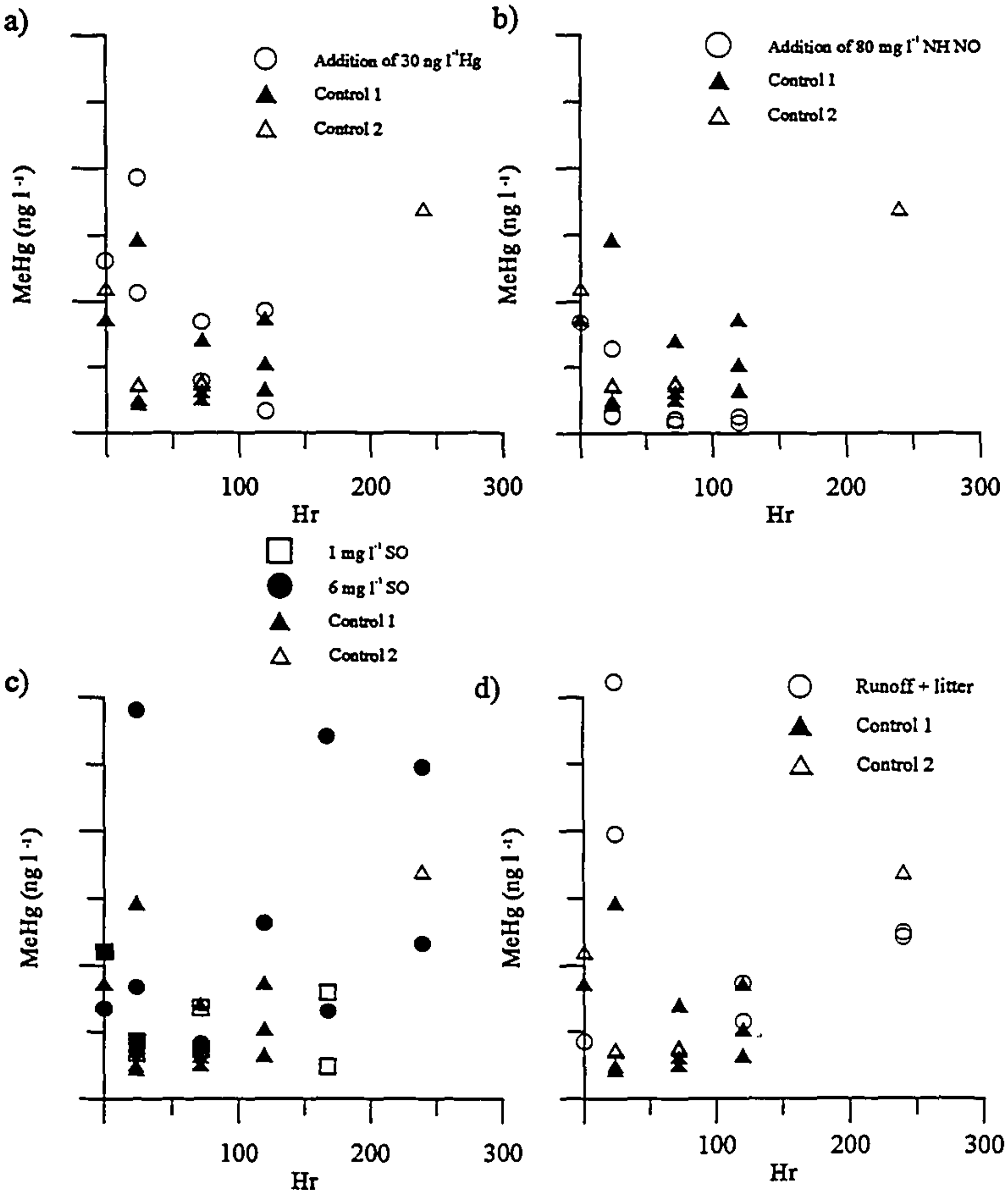

Figure 5-1) Methylmercury concentration in 0.45 μm filtered incubation water after addition of a) Hg, b) NH_4NO_3, c) K_2SO_4 and d) upland runoff water and leaf litter. Each concentration is from a different flask. Duplicate analyses were not performed on water from each flask, but the analytical error of duplicates measured at the time was typically 10%, or the size of the symbol. Control set 1 was run with the Hg and NH_4NO_3 additions and control set 2 was run with the SO_4^{2-} and runoff + leaf litter additions.

In flasks receiving 1 mg l^{-1} SO_4^{2-}, the MeHg concentrations ranged from 0.5 to 1.4 ng l^{-1} and therefore are within the range of the controls (Figure 5-1c). In flasks receiving 6 mg l^{-1} SO_4^{2-}, MeHg concentrations were very variable being as high as 6 ng l^{-1} and as low as 0.7 ng l^{-1}. No temporal trend is apparent, as flasks with MeHg concentrations > 5 ng l^{-1} are found at 3 of the 5 sampling dates, as are MeHg concentrations of < 1 ng l^{-1}. As each measurement is from a different flask, it appears the addition of SO_4^{2-} elevated the MeHg concentration in some flasks above the level of the control incubations, while it had no affect in others.

In the upland runoff flasks, the incubation water MeHg concentration was between 4 and 6 ng l^{-1} after only 24 hrs (Figure 5-1d). However, at subsequent sampling dates, the MeHg concentration was between 1.2 and 2.5 ng l^{-1} and within the range of the controls.

Although changes in the MeHg concentration of incubation water resulting from some of the treatments were evident, the lack of consistent results for any one treatment limits the significance of these findings. Hand homogenization did not eliminate the natural heterogeneity of the peat and, combined with the destructive sampling design, make temporal trends impossible to identify.

In flasks containing only 0.45 μm filtered peat porewater, no measurable change in the concentration of MeHg was recorded. This is not surprising because most bacteria would have been removed by the filter. In later experiments using unfiltered lake water and relatively low DOC concentrations, Sellers et al. (1996) found MeHg to be stable for many days.

Incubation Type	CH_4 (mg $g^{-1}d^{-1}$)	CO_2 (mg $g^{-1}d^{-1}$)
Control	0.00 ± 0.02	0.09 ± 0.02
NH_4NO_3	0.00 ± 0.02	0.12 ± 0.01
Sulfate 1 mg l^{-1}	0.04 ± 0.02	0.18 ± 0.06
Sulfate 6 mg l^{-1}	0.00 ± 0.01	0.24 ± 0.06
Upland runoff	0.06 ± 0.03	0.34 ± 0.13
30 mg l^{-1} Hg	0.00 ± 0.02	0.13 ± 0.01

Table 5-3) CO_2 and CH_4 production from the incubated peat, calculated from head space concentrations. To calculate the rates, the head space concentration after 24 hrs was considered time zero, and concentrations were recorded after 48, 72 and 120 hrs. The CO_2 rate is corrected for dissolved CO_2.

The rates of CO_2 and CH_4 production from the destructive incubations are presented in Table 5-3. The rate of CO_2 production was higher in all the amended flasks than in the controls, and the highest rates occurred in the SO_4^{2-} and runoff amended incubations. Although the flasks were anaerobic, very little CH_4 was produced during the incubation period. Only in the runoff and 1 mg l^{-1} SO_4^{2-} amended flasks was CH_4 production steady. No CO_2 or CH_4 was produced in the flasks containing only porewater.

5.4.3 Sequential Addition Experiments

In the destructive nutrient addition experiments, the variability in the initial partitioning of MeHg between peat and porewater (Table 5-1 and controls in Figure 5.1) was one factor which made it difficult to assess the impact of the enrichments. To avoid the influence of MeHg partitioning, the flasks were left to stabilize for three days prior to adding any enrichments. After 3 days, the MeHg concentrations ranged from 1.4 and 4.4 ng l^{-1} across all 16 flasks (Figure 5.2 and 5.3) and between 1.4 and 3.8 ng l^{-1} in the control subset. Therefore, the heterogeneity of the peat in the entire set is found in the controls. Over the remainder of the experiment, the MeHg concentrations declined at an average rate

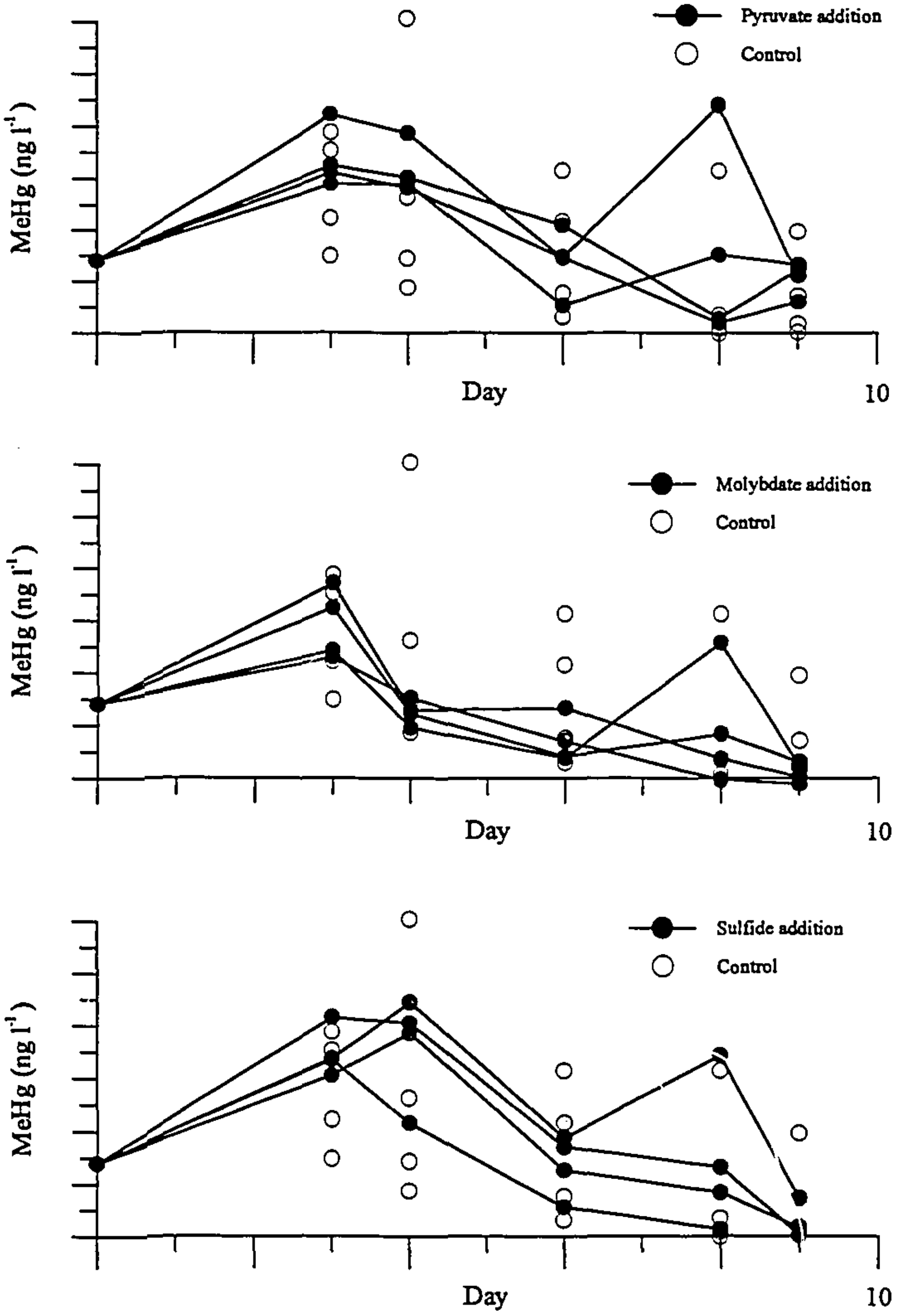

Figure 5-2) Dilution corrected MeHg concentrations in filtered water from incubations of rich fen peat receiving daily addition of a) 1.3 mg l^{-1}pyruvate, b) 8 mg l^{-1} molybdate, and c) 6.8 mg l^{-1} sulfide.

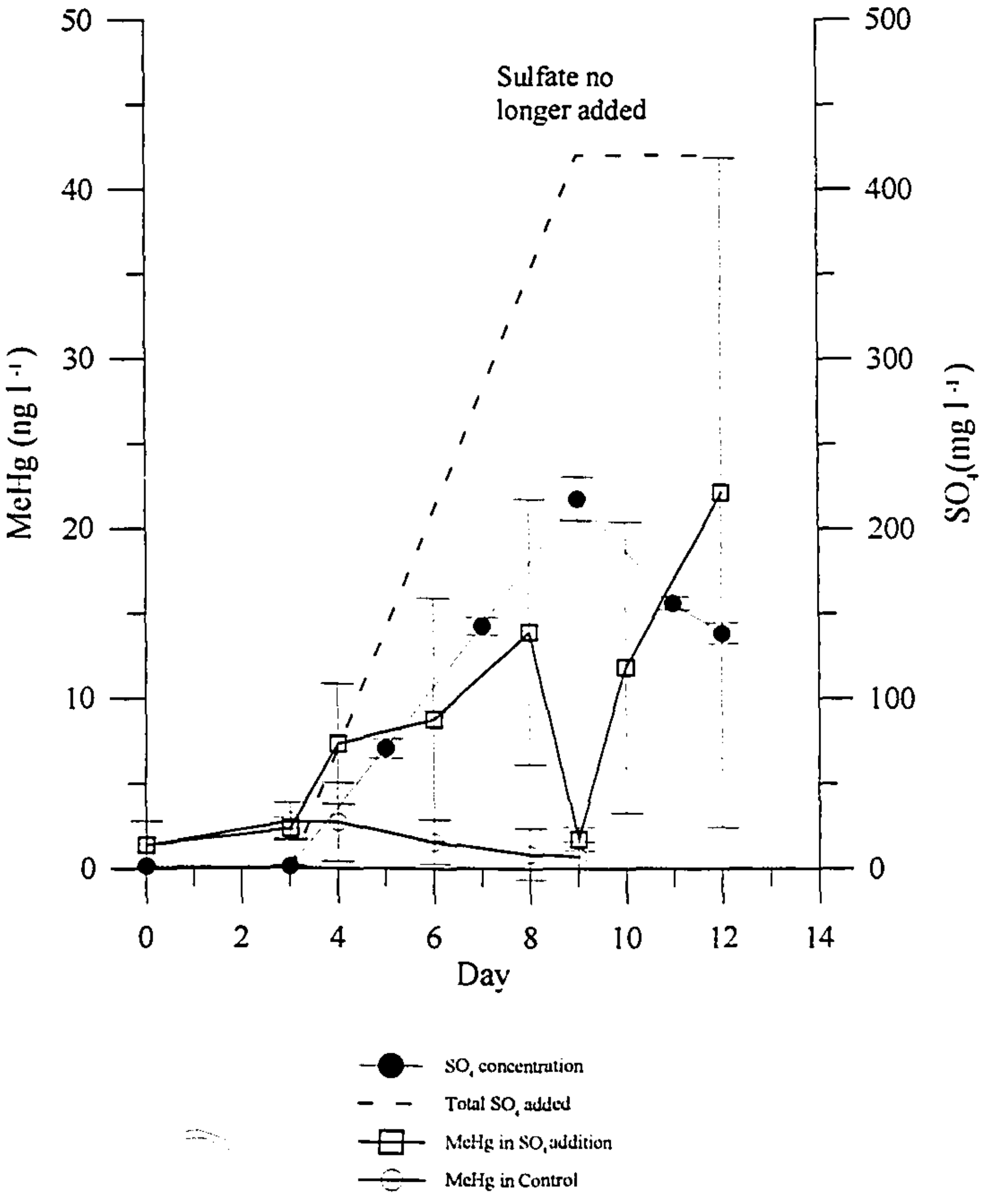

Figure 5-3) Dilution corrected MeHg and SO_4^{2-} concentrations in filtered water from incubations of poor fen peat receiving 60 mg l^{-1} SO_4^{2-} d^{-1}. The average methylation rate is 0.13 ng Hg g^{-1} d^{-1} and the SO_4^{2-} loss is 1.25 mg g^{-1} d^{-1} during the period of SO_4^{2-} addition and 2.8 mg g^{-1} d^{-1} during pyruvate addition. The error bars are the standard deviation around the mean of quadruplicate incubations.

of 0.3 ng l^{-1} d^{-1} resulting in MeHg concentrations between 0.2 and 2.0 ng l^{-1} at day 9. This range in MeHg concentration is consistent with the controls of the previous experiments.

In flasks receiving pyruvate, the MeHg concentration in the incubation water steadily decreased from between 2.8 and 4.0 ng l^{-1} to between 0.8 and 1.5 ng l^{-1} 6 days later (Figure 5-2a). In flasks receiving molybdate, the MeHg concentration in the incubation water decreased from between 2.0 and 4.0 ng l^{-1} to <0.5 ng l^{-1} (Figure 5-2b). In flasks receiving H_2S, the MeHg concentrations also decreased from an initial concentration of between 3.0 and 4.0 ng l^{-1} to between 1.0 and 0.05 ng l^{-1} (Figure 5-2c). In the pyruvate, molybdate, and H_2S experiments, the rates of MeHg depletion were all within the range of the control incubations. Concentrations of molybdate in the incubation water were barely detectable, and therefore all added molybdate was likely adsorbed onto the peat.

In three of the four flasks receiving SO_4^{2-}, the MeHg concentration in the incubation water increased steadily, and after 8 days, was 15 ± 6 ng l^{-1} (Figure 5-3). In the fourth flask, the MeHg concentration showed little change only increasing from 1.2 to 1.6 ng l^{-1}. On the 9th day of the incubation experiment, the MeHg concentrations decreased to 1.8 ± 0.7 ng l^{-1} in all the flasks receiving SO_4^{2-}. On the 10th day, the MeHg concentrations returned to levels comparable to day 8, being between 1.6 and 46 ng l^{-1}. At this time, SO_4^{2-} was no longer being added. The addition of pyruvate enriched water played no part in the decrease in the MeHg concentration as it was added after the day 9 samples were collected, but may have aided in the recovery of the MeHg concentrations.

The removal of SO_4^{2-} from the porewater occurred at a rate of 1.25 mg g^{-1} d^{-1} during SO_4^{2-} addition and 2.8 mg g^{-1} d^{-1} after SO_4^{2-} was no longer added (Figure 5-3). The concentration of SO_4^{2-} achieved a maximum of 217 ± 10 mg l^{-1} on day 9, which coincided with the apparent decrease in the MeHg concentration.

It is possible the decrease in the MeHg concentration in the incubation water is an artifact resulting from an analytical or experimental error. Water samples were diluted to alleviate a potential extraction problem created by the large amount of SO_4^{2-} in solution. But, as no other samples were apparently affected, an extraction problem appears unlikely. It is possible the flasks were contaminated with oxygen, and that MeHg was precipitated with sulfides only to be redissolved when the free oxygen was eliminated.

The concentrations of CO_2 and CH_4 were measured in the head space of the flasks on only two occasions. With not enough measurements to calculate rates of production, the amounts of CO_2 and CH_4 emitted as of the 6th day of the incubation are presented in Table 5-4. The CO_2 emitted from the pyruvate, SO_4^{2-}, and control treatments were similar. Emission from the flasks receiving molybdate and sulfide were slightly lower and considerably lower, respectively, than the controls. The amount of CH_4 produced from the control samples was: 1.5x the molybdate addition, 2x the pyruvate addition, 4x the SO_4^{2-} addition and 6x the H_2S addition.

Treatment	Mean CO_2 (mg)	Std	Mean CH_4 (mg)	Std
Molybdate	0.65	0.09	0.13	0.06
Sulfate	0.92	0.14	0.09	0.06
Pyruvate	0.93	0.15	0.17	0.07
Sulfide	0.13	0.04	0.05	0.01
Control	0.94	0.14	0.33	0.13

Table 5-4) The amount of CO_2 and CH_4 in the repeated addition incubation flasks after 6 days.

5.5 Discussion

5.5.1 The MeHg Concentration in Peat Controls the MeHg Concentration in Peat Porewater

In the partition experiments, the rapid change in the MeHg concentration of incubation water following exposure to peat demonstrates that the MeHg concentration of peat porewater is controlled by the MeHg concentration of the peat. Little MeHg was released to the SQ water when combined with peat. Therefore, MeHg must be strongly adsorbed onto peat and requires complexation by ligands, provided primarily by the DOC in peat porewater, for MeHg to be released and remain in porewater. Thus, the composition of the porewater will affect the partition of MeHg between porewater and peat (Hintelmann et al., 1995).

In chapter 2, the equation

$$\mathrm{Ln}\ (\mathrm{MeHg}_{\mathrm{aqua}}\ \mathrm{ng\ g}^{-1}) = 0.071 \times (\mathrm{MeHg}_{\mathrm{peat}}\ \mathrm{ng\ g}^{-1}) - 8.31\ (R^2 = 0.61)$$

was proposed to describe the partition of MeHg between *in situ* peat and peat porewater (Figure 5-4). Similar equations can be derived from the experimental data in Table 5-1, and both linear and exponential equations appear effective with R^2 of 0.94 and 0.85 respectively. Because the MeHg K_D's between the peat and peat porewater (Table 5-2) vary by more than an order of magnitude and are skewed, with K_D's being lowest at the highest peat concentrations, the relationship is unlikely to be linear despite the linear equation having a higher R^2. The equation that best describes the relationship is

$$\mathrm{Ln}\ (\mathrm{MeHg}_{\mathrm{aqua}}\ \mathrm{ng\ g}^{-1}) = 0.10 \times (\mathrm{MeHg}_{\mathrm{peat}}\ \mathrm{ng\ g}^{-1}) - 9.8\ (R^2 = 0.85).$$

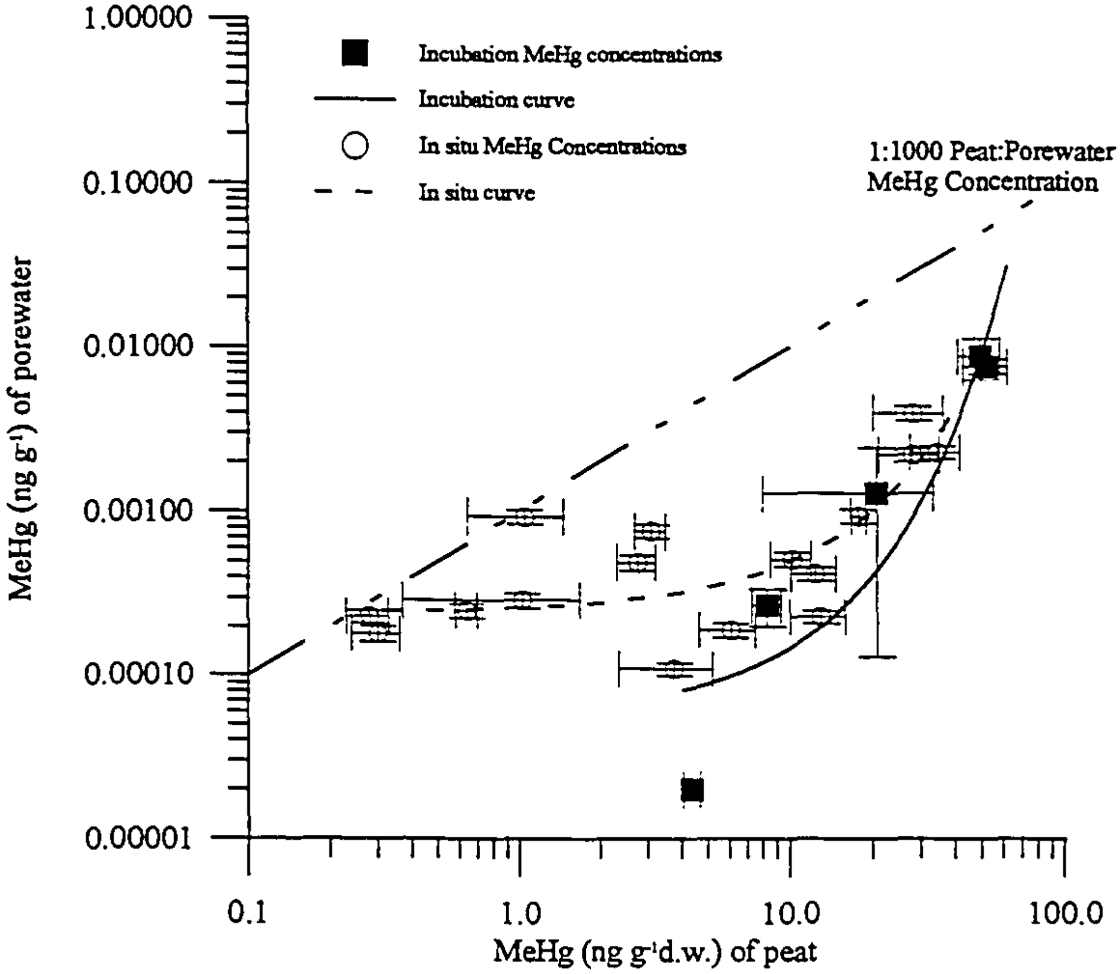

Figure 5-4) A log:log plot of the MeHg concentrations in peat and incubation water. The relationship is best described by the exponential equation

Ln (MeHg in water ng g^{-1}) = 0.10 x (MeHg peat ng g^{-1})- 9.8, R = 0.85 ($p<0.000$). The MeHg in peat and peat porewater relationship from figure 2- 6 is plotted in the background for comparison.

Despite different ratios in the amount of peat to water, the laboratory and *in situ* derived equations are similar and it is obvious that as the MeHg concentration in the peat increases a greater proportion of MeHg is partitioned to the porewater (Figure 5-4). This occurrence may be caused by a saturation of the most effective MeHg adsorption sites.

5.5.2 Stimulation and Inhibition of MeHg Production in Peat

The variability in MeHg concentration amongst controls and amongst flasks receiving the same treatment was large. The lack of a response in some flasks may have been the result of experimental problems such as exposure to O_2, but the consistently large degree of variability suggests this is not the major cause. The small scale heterogeneity found in *in situ* peat (Chapter 2) appears to be maintained through the laboratory homogenization. Variability may be reduced by slurrying the sample with a blender but in doing so, any hope of a comparison between laboratory and *in situ* conditions is lost. It could be argued that the flasks are not indicative of *in situ* conditions anyway, but the similarity between the *in situ* and laboratory K_D's suggest that the incubations are at least informative.

The addition of $HgCl_2$, at the beginning of the incubations did not result in higher MeHg concentrations in incubation water. Although the porewater concentration of T-Hg was increased by a factor of 6, the increase would have been temporary as 99% of the Hg would have been partitioned to the peat. The amount of Hg added was also small, being only 2.5 % of the Hg stored in the peat. Even so, the Hg added should have behaved like Hg that enters wetlands in runoff or precipitation and as such did not increase the porewater concentration of MeHg. This suggests that the amount of incoming Hg does not control the MeHg concentration in peat porewater. Across a number of environments, Kelly et al. (1995) observed that, apart from being lower, MeHg concentration is independent of the T-Hg concentration. Therefore, both field and laboratory observations suggest the availability of Hg is not the factor limiting MeHg production in wetlands.

After the addition of a large amount of SO_4^{2-} (> 60 mg l^{-1}) the MeHg concentration in incubation water could be clearly increased. The amount of sulfate added in the repeated addition incubations was much larger than what is usually found (<10 mg l^{-1}) in water entering the wetland (Linsey et al., 1987; Branfireun pers. comm., 1995). But these findings support the observations of the destructive addition experiments where an initial enrichment of incubation water by 6 mg l^{-1} SO_4^{2-} also elevated MeHg concentrations in some flasks. Together, these experiments indicate that the supply of SO_4^{2-} may be important in MeHg production. Any further link between total sulfate reduction and Hg methylation has not been established, as SO_4^{2-} loss rates, which are presumed to be mostly biological reduction (Brown, 1986), are the same in all flasks regardless of whether MeHg was produced.

As previously stated, I can support no explanation for the decrease in MeHg concentration of incubation water at day 9 of the sequential SO_4^{2-} addition experiment. It seems unlikely that binding of Hg by sulfides is responsible for the decrease, as concentrations of sulfide would have continued to increase beyond this date as SO_4^{2-} continued to be reduced. The experiment was designed to test carbon limitation and it is possible that addition of pyruvate stimulated MeHg production. However, the increase following pyruvate addition appears to only restore the previously existing trend, and therefore is unlikely to have stimulated the increase.

The methylation of Hg in pure cultures of *Desulfovibrio desulfuricans* L.S. has been linked to carbon biosynthetic reactions and not directly to SO_4^{2-} reduction (Berman et al., 1990; Choi et al., 1993). In this study, the addition of pyruvate had no impact on MeHg concentration and no apparent impact on CO_2 production (Figure 5-2, Table 5-3). The addition of upland runoff water and leaf litter, containing potentially bioavailable DOC (Matos pers. comm., 1995), initially increased the MeHg concentration in peat. The upland runoff water also contained 1.5 mg l^{-1} SO_4^{2-} and may have been the stimulatory agent and not the carbon. Therefore, either MeHg production in peat is not carbon limited or the pyruvate could not be utilized by the Hg methylating population (Devereux, et al., 1996) perhaps being adsorbed by peat and DOC.

Addition of H_2S did not result in lower MeHg concentrations than in the controls. Hintelmann et al. (1995) found two stability constants (Log K's of 2 and 10) existed between MeHg and DOC. Dryssen and Wedborg (1991) calculated log K's for MeHg and HS^- as15.4 and for Hg^{2+} with HS^-, 37.7. Therefore it is expected that upon addition of the sulfide, Hg and MeHg in solution should be transferred to the HS^- ligand. If this transfer did occur it did not affect the amount of MeHg in solution as the flasks receiving H_2S were in the range of the controls.

Addition of molybdate did not result in MeHg concentrations decreasing at a rate faster than the controls. It is possible that demethylation of MeHg in peat is slow at these MeHg concentrations or, SRB are also the most important demethylators of Hg (Oremland, 1991), and demethylation as well as methylation was inhibited by the addition of molybdate. Only in flasks receiving NH_4NO_3 did MeHg concentrations decrease significantly faster than the controls (Figure 5-1). It is most likely that denitrifying bacteria, that do not methylate Hg, out-competed the Hg methylating bacteria for carbon and in doing so, increased the rate of MeHg demethylation.

5.5.3 Sulfate Reduction and MeHg Production

Although in pure culture Hg methylation occurs fermentatively, and Choi and Bartha (1994) found no relationship between rates of Hg methylation and SO_4^{2-} reduction in lake sediments, the MeHg concentration in peat appears related to SO_4^{2-} supply. The supply of new SO_4^{2-} to wetlands can be intermittent. Unlike lake sediments, which receive sulfate from the water column (Lovley and Klug, 1983), SO_4^{2-} can be delivered to wetlands in precipitation, runoff, or via the groundwater. Bayley et al. (1986) found that SO_4^{2-} added to a wetland at the ELA, similar to the headwater wetland, was quickly removed and the majority biologically reduced (Spratt and Morgan, 1990; Wieder and Lang, 1988). However, increasing the sulfate supply to wetlands may not necessarily increase the bulk rate of SO_4^{2-} reduction as Wieder et al. (1990) found that the rate of sulfate reduction in peat was independent of SO_4^{2-} concentration.

In this study, the rate of SO_4^{2-} loss from solution averaged 1.25 mg g^{-1} d^{-1} or 33 mg l^{-1} d^{-1}, but the rate of SO_4^{2-} depletion from the water was not linear and had more than doubled by the end of the experiment. The change in the SO_4^{2-} depletion rate is indicative of a biological process with a growing population. This is inconsistent with the observations of Wieder et al. (1990) but debate is difficult given the SO_4^{2-} reduction studies in wetlands are of limited number. What remains unknown is if the bulk SO_4^{2-} reduction rates and respiration rates are similar across flasks, why is the Hg methylation rate different? It must be remembered that not all SRB methylate Hg, and the SRB population is made up of many kinds of microorganisms (Sparling pers. comm., 1995). Therefore, the makeup of the SRB population could greatly influence how, and if, Hg is methylated. In turn, the makeup of the SRB population maybe related to the speciation of SO_4^{2-} as incoming and recycled SO_4^{2-} may be available to different members of the population.

5.6 Conclusions

The concentration of MeHg in peat controls the initial MeHg concentration of peat porewater and knowing one the other can be predicted with some degree of confidence. Peat from a poor fen in a boreal headwater wetland becomes a net producer of MeHg only when stimulated by the addition of SO_4^{2-}. The addition of molybdate did not deplete MeHg concentrations faster than in controls. Therefore, unless SRB are also the primary demthylators of MeHg, demethylation of MeHg in peat is likely very slow. The addition of Hg and pyruvate did not enhance MeHg production suggesting that the availability of Hg and carbon do not appear to limit Hg methylation.

Chapter 6: Methylmercury Cycling in Pristine and Impounded Wetlands

6.1 Introduction

In the previous chapters, I have described the amount and distribution of MeHg in pristine and impounded wetlands, identified the probable location of MeHg production and, through laboratory experiments, attempted to explain what controls MeHg production in peat. In this chapter, I will discuss the limitations of the research and synthesize the observations in a discussion of what controls Hg methylation in both pristine and impounded wetlands.

6.2 The Study of MeHg in the Environment: still constrained by analytical limitations

Until the 1980's, concentrations of T-Hg in water and MeHg in water, soil, and plant material could not be accurately measured. Ideas of the behaviour of Hg and MeHg could only be obtained by observation of grossly contaminated sediments or by performing laboratory experiments in which concentrations of Hg orders of magnitude higher than background levels were introduced (e.g., Parks et al., 1986; Ramlal et al., 1986; Wright and Hamilton, 1982). Without knowing the true distribution of T-Hg and MeHg, it is difficult to interpret the results of these early experiments but it is clear the Hg methylation process is very complex (Winfrey and Rudd, 1992).

Although time consuming and exceedingly difficult to master, the analytical capability of MeHg and T-Hg measurement at the concentrations found in unpolluted environments is now possible (e.g., Bloom, 1989; Bloom and Fitzgerald, 1988; Horvat et al., 1993). One of the priorities of this study was to determine the concentration and distribution of MeHg in peat and porewater of wetlands to serve as a basis for future research. The number of measurements of MeHg concentration in both undisturbed and impounded wetlands made in this study is unequalled in the literature and alone is a significant contribution to our knowledge of MeHg in the environment.

One of the most noticeable attributes of this data set is the large spatial and temporal variability of the MeHg concentration in wetlands. The analytical error of individual measurements is well known, being less than 20%. Thus, the three orders of magnitude of variability in MeHg concentration within and between wetlands is a genuine distribution. High spatial and temporal variability of MeHg concentrations have been reported elsewhere, such as the water column of the experimental reservoir (Kelly et al., 1996), in peat porewaters of a Swedish wetland (Bishop et al., 1995), and in peat porewaters of the Florida Everglades (Gilmour pers., comm. 1996). Any one of a number of factors may influence the MeHg concentration at one point in time (Winfrey and Rudd, 1990). As a result, a high degree of variability will be an inherent part of any *in situ* experiment and research progress constrained by one's analytical capability.

6.3 Methylmercury in Pristine Wetlands

6.3.1 Methylmercury Production in Wetlands

In wetlands, the highest MeHg concentrations are located just below the water table, indicating that redox reactions are important in the Hg methylation processes. The importance of microbial activity around the oxic/anoxic interface to MeHg production was substantiated by the results of the decomposition experiment (Chapter 4). During the decomposition of fresh plant tissue (spruce needles, sedge stems and *Sphagnum* moss) in wet hollows, both the amount and concentration of MeHg increased, but in dry hummocks MeHg was lost. The increase in the amount of MeHg in tissues decomposing in the wet hollows is interpreted as being the result of methylation of Hg stored in the tissue under anaerobic conditions. As *in situ* Hg methylation in the decomposing tissue was not measured directly, the change in MeHg concentration in the tissue may not result entirely from *in situ* Hg methylation. MeHg could have been transferred to the vegetation from peat (Figure 2-4) or adsorbed from precipitation and runoff waters (Branfireun et al., 1996; St.Louis et al., 1995). Evidence was provided in Chapter 4 however, that suggests that the decomposing plant tissue is a source and not a sink of MeHg, but some doubt still remains.

6.3.2 The Availability of MeHg for Export

Mercury is lost from all decomposing plant tissues, but the speciation of the lost Hg is unknown (Figure 4-3, 4-4). There is evidence to suggest that much of the Hg lost from tissues decomposing under anaerobic conditions is MeHg. Methylmercury and other metals described as being dissolved in the $< 0.45\ \mu m$ fraction are not truly dissolved, but most likely associated with colloids (e.g., Ares and Ziechman, 1988; Koenings, 1976; Rashid, 1971). Still, being associated with fine particles or colloids will increase the mobility of MeHg.

Mucci et al. (1995) found that in aerobic reservoir water containing low T-Hg (<10 ng l^{-1}) and MeHg (<1 ng l^{-1}) concentrations, the solid/aqueous ($<0.45\ \mu m$) partition coefficients (K_D) for T-Hg were between 2.8×10^4 to 7.2×10^4 and for MeHg between 1.5×10^5 to 2.5×10^4. Others have reported solid/aqueous partition coefficients for MeHg ranging from 10^2 to 10^3 but at very high water concentrations (≈ 100 ng l^{-1}) (Akagi et al., 1979; Miskimmin, 1991).

In Chapters 2 and 5, the MeHg K_D's between anaerobic peat and porewater were estimated to lie between 1×10^3 and 2×10^5. The relationship between MeHg in peat and porewater appears to be exponential with proportionally more MeHg partitioned to the porewater as the MeHg concentration in peat increases. Above a peat MeHg concentration of 10 ng g^{-1}, between 6 and 24% of the 8 ng l^{-1} T-Hg in porewater is MeHg. Under the stimulation of SO_4^{2-} (Figure 5-2), the MeHg concentration in incubation water exceeded 40 ng l^{-1}. Thus, almost all the of the T-Hg in porewater is MeHg and more than half the 120 ng g^{-1} of T-Hg in peat is MeHg. The MeHg K_D for this experiment is $\approx 2 \times 10^3$, which is similar to the K_D's with high concentrations of MeHg in water reported by Miskimmin (1991) and Akagi et al. (1979) but 10 times lower than K_D's calculated for water with low MeHg concentrations by Mucci et al. (1995).

Others have observed an increase in the concentration of T-Hg under anaerobic conditions. In bottom waters of Onondaga Lake, New York, Jacobs et al. (1995) observed the T-Hg concentration increased from 5 to 18 ng l^{-1} following the formation of an anoxic hypolimnion. At the same time, the proportion of T-Hg occurring as MeHg increased from < 10% to as high as 50%. Thus under anaerobic conditions, as more Hg is methylated, the amount of T-Hg partitioned to the porewater increases.

The increased mobility of Hg resulting from its methylation under anaerobic conditions may be important in understanding the distribution of Hg and MeHg in impounded soils. Impounded podzols have higher T-Hg and MeHg concentrations than impounded peat (Chaire de Recherche en Environnement, 1993). One explanation for the higher MeHg concentration in the impounded podzols is that more MeHg is produced there. A second explanation may be the podzols retain more MeHg than peat. The submerged peat likely has a greater hydraulic conductivity than the submerged podzol, and combined with the increased mobility of MeHg at high MeHg concentration and under anaerobic conditions, more MeHg maybe exported from the submerged peat soils than the submerged podzols. In fact, partitioning of Hg from peat to water by methylation may limit the amount of

Hg that remains in impounded peat.

6.4 The Effect of Impoundment on the MeHg Store in Peat and Porewater

Porewater MeHg concentrations in the riparian wetland increased from between 0.05 and 1.0 ng l^{-1} to between 0.5 and 6.0 ng l^{-1} following impoundment (Table 3-4). The MeHg concentration in both peat and porewater of the impounded wetland have equals in pristine wetlands (Figure 2-6 and Table 5-1). In ombrotrophic wetlands however, high MeHg concentrations are restricted to hollows. Therefore, assuming Hg methylation in the water column is limited, I hypothesize that the increase in the MeHg concentration in the water column of the reservoir is largely the result of an increase in the areal extent of MeHg production as well as an increase in the rate of Hg methylation in the peat (Matilainen, 1995; Watras et al., 1995).

Showing just how much the pool of MeHg in the peat has changed with impoundment can be done by extrapolating the point measurements from Chapter 3 and Chapter 4 over the entire wetland (Figure 3-2 and Figure 4-4). Prior to impoundment, the MeHg concentration in the peat of the riparian wetland was less than 4 ng g^{-1} (Figure 2-4). The MeHg concentration in moss ($\approx$ 8 ng g^{-1}) exceeded this concentration but may only be indicative of the upper few centimetres of the peatland surface. Peat porewater concentrations were more frequently measured and cover more of the wetland (Figure 3-1, 3-2). Using the partition coefficients established in Chapters 2 and 5, the amount of MeHg in the peat can be estimated. Using an average porewater MeHg concentration of 0.8 ng l^{-1} and the equation in section 2.4.3, the average concentration of MeHg in peat is 16.7 ng g^{-1}. Extrapolating this concentration over the area of the wetland (131200 m^2) to a depth of 1 m (which is the depth affected) and using a peat bulk density (dry weight) of 0.1 g cm^{-3}, the post-impoundment burden of MeHg in the upper metre of peat is estimated at 219 g. With a porewater MeHg concentration of 0.3 ng g^{-1} (Table 3-4), the pre-impoundment burden in the peatland would have been 80 g MeHg. Therefore, the store of MeHg in the peatland has increased by 2.7 times following impoundment.

The accuracy of the assessment of the pool of MeHg in the peatland is limited by the reliance on peat/porewater partition and the temporal and spatial variability of MeHg concentrations, which could be better resolved by more peat and porewater sampling. Understanding the reasons for the variability is a more difficult problem. Spatial and temporal variations in MeHg concentration have been observed in surface water of lakes and ponds (e.g., Jacobs et al., 1995; St.Louis et al., 1994, 1996; Watras et al., 1994) and hydrological (e.g., Branfireun et al., 1996; Krabbenhoft et al., 1995), MeHg production (Gilmour et al., 1992; Korthals et al., 1987) and MeHg demethylation (Korthals et al., 1987; Oremland et al., 1995) arguments have been presented to explain these trends. In the reservoir, all of these explanations are likely to be valid at some location and point in time. Even if it were possible to establish the control on MeHg production at a single location, without knowing the overall impact MeHg production at that site has on the system as a whole, the immense effort required to obtain the result hardly justifies doing so.

6.5 Controls on MeHg Production

In isolation, many organisms have been found to methylate Hg (e.g., Berman et al., 1990; Choi et al., 1994; Compeau and Bartha, 1985; Fisher et al., 1995; Landner 1971; Wood et al., 1968; Yamada and Tonomura, 1972) but it has yet to be shown that any of these organisms are important methylators of Hg outside the laboratory. By stimulating and inhibiting the metabolic activity of different members of the microbial populations in sediment cores, sulfate reducing bacteria (SRB) have been identified as potentially important methylators of Hg (Gilmour et al., 1992; Gilmour and Riedel, 1995). Although this is a significant step, the number of bacterial species classified as SRB is very large and only some can methylate Hg (Sparling, pers. comm., 1995).

In pure culture, SO_4^{2-} is not required by SRB to produce MeHg (Choi and Bartha, 1994). Yet, to sustain a net increase in the MeHg concentration in environmental samples such as lake sediment (Choi and Bartha, 1994; Gilmour et al., 1993) and peat (Figure 5-3), a nominal supply of SO_4^{2-} is required. Even when large amounts of SO_4^{2-} are added, accumulation and perhaps even production of MeHg in peat is not assured (Figure 5-2). This may be because a Hg methylating population was not present, or another condition is required for MeHg to be produced.

There are two reasons why SO_4^{2-} may be required for Hg methylation in natural systems. The first reason is that SRB that methylate Hg at low Hg concentrations do not use the acetyl-CoA pathway proposed by Choi et al. (1994) but use a pathway that requires SO_4^{2-}. The second reason is that Hg methylating SRB require SO_4^{2-} to compete with other microorganisms for carbon. The idea that a link exists between the rate of SO_4^{2-} reduction and Hg methylation is not new (Gilmour et al., 1992). Choi and Bartha (1994) found no relationship between the gross rate of SO_4^{2-} reduction and Hg methylation in lake sediments, but this does not mean a more complicated relationship does not exist. Sulfate added to peat is reduced (Bayley et al., 1986) but the added SO_4^{2-} does not affect the gross rate of SO_4^{2-} reduction (Wieder and Lang, 1988). The gross rate of SO_4^{2-} reduction is controlled by the internal cycling of organic S complexes (Brown, 1986; Spratt and Morgan, 1990; Wieder et al., 1990). It would appear that inorganic SO_4^{2-}, such as K_2SO_4, is not the most available species of SO_4^{2-}, or at least not available to the SRB that control the gross rate of SO_4^{2-} reduction. The added SO_4^{2-} may be more available to the part of the SRB population that methylate Hg. This theory would explain why gross SO_4^{2-} reduction and Hg methylation rates are unrelated and suggests that the speciation of S is important in controlling Hg methylation.

6.6 Mercury Methylation in Wetlands

6.6.1 The Controlling Factors

I have identified a number of factors that may influence Hg methylation, MeHg concentration and transport in wetlands. In this section, I will summarize how I believe Hg methylation in wetlands occurs and how it is accentuated by impoundment.

In this study, the greatest amounts of MeHg were found at the oxic/anoxic interface: at the water table in wetlands and the peat surface/surface water interface in a reservoir. From this observation alone, the importance of redox reactions in Hg methylation is immediately obvious. Interestingly, concentrations of MeHg in peat and porewater of the poor fen equal the MeHg concentration in the reservoir. The common denominator between these two environments is a near constant supply of aerobic water, and therefore oxidized species, to an oxic/anoxic interface. In the reservoir, the source of oxidized species is the impoundment water, which is steadily replenished from the upstream lake 240 (Figure 5-1). In the poor fen, the source of fen water is not as obvious, but upland runoff and groundwater converge in the poor fen (Fowle, 1995), delivering oxidized species to the near-surface peat for a good portion of the year.

The incoming water provides not only DOC and SO_4^{2-}, but also Hg. Alone, increasing Hg did not stimulate Hg methylation in peat (Figure 5-1). Although Hg entering the wetland does not trigger Hg methylation, the incoming Hg may be readily available for methylation and should not be ignored. The importance of

SO_4^{2-} in Hg methylation has already been established elsewhere, but how it stimulates Hg methylation is not known. Sulfate may only be required to allow Hg methylating bacteria greater access to carbon, but the conceptual model I propose for the elevation of MeHg concentrations in peat is based on the primary need for SO_4^{2-}.

6.6.2 A Conceptualization of Hg Methylation in Wetlands

The concentration of SO_4^{2-} is between 3 - 4 mg l^{-1} in reservoir water (Rudd 1995 unpublished data), 1-10 mg l^{-1} in surface water entering the poor fen (Branfireun, pers. comm., 1995; Linsey et al., 1987) and 1-2 mg l^{-1} in upwelling groundwater (Branfireun pers. comm., 1995). These SO_4^{2-} concentrations are typical of waters in the ELA area (Stainton pers. comm., 1995), but it is the steady supply of SO_4^{2-} to a redox cline in peat which is unusual. The redox cline in the peat is not like the typical gradient found in lake sediments, where an aerobic layer is underlain by a steep unbroken redox gradient. The poor fen is best described as a series of small aerobic and anaerobic pockets (Heyes, unpublished redox data). In the reservoir, the circulation of surface water under and around floating peat is similar to the situation in the poor fen, but the anaerobic clusters may encompass entire peat islands. As a result, migratory water must pass in and out of anoxic areas and in doing so promotes the recycling of S, through repeated exposure to oxidizing and reducing conditions. Whether it is the increase in the availability of SO_4^{2-} as a recycled species or as an incoming species that promotes Hg methylation is not known, but if it was recycled SO_4^{2-} that was the most important, the production of MeHg should be more spatially consistent.

The amount of MeHg exported from the poor fen and reservoir peat is not dependent only on the rate of MeHg production. Two other important factors are the demethylation and adsorption of MeHg en route to adjacent surface water.

Most of the MeHg released from a site of MeHg production is likely adsorbed by peat containing less MeHg (Table 5-1). The adsorption of MeHg gives the apparently slow demethylation processes ample time to be effective. The export of MeHg is largely controlled by the distance between the site of production and the adjacent water body. Shortly after impoundment of the peat, the redox cline migrated from within the peat to the peat surface/surface water interface, thus shortening the path of newly produced MeHg to the water column. With the nonproductive sites no longer inhibiting the release of MeHg to the water column, the MeHg concentration in the overlying water quickly increased (Kelly et al., 1996). Once the peat began to float, the exchange of MeHg between peat and surface water became more complicated. Large areas of more humified peat (Bubier et al., 1993) containing little MeHg (Table 5-1) became exposed. Whether this peat becomes a site of MeHg production, upon colonization by bacteria, or lowers the MeHg concentration in the water column by adsorbing MeHg is not known.

To export significant amounts of MeHg from the poor fen, storm water is required to move through the wetland (Branfireun et al., 1996). When storm water carrying little MeHg passes through the poor fen, it not only flushes MeHg from highly productive areas, but it also introduces SO_4^{2-} and carbon that can stimulate Hg methylation. Desorption of MeHg from peat to porewater and the stimulation of Hg methylation by SO_4^{2-} addition both occur within the duration of a runoff event (Branfireun et al., 1996). It is not clear to what extent newly produced and desorbed MeHg contribute to the MeHg exported from the poor fen.

6.7 Future Research

Although this data set is the most substantial inventory of MeHg concentrations in wetlands collected to date, some obvious gaps need to be filled.

Peat porewater samples were not collected in winter. The amount of water passing through boreal wetlands is greatest during snow melt, thus if the concentration of MeHg in porewater builds up over the winter a large amount of MeHg may be exported in spring. I have suggested that storm events are important to both MeHg transport and production. To further address this issue, repeated measurements of MeHg concentration at a number of locations during a runoff event could be used to quantify the internal flux of MeHg through the peat, thereby identifying the sources and sinks of MeHg. Only when the local sources are identified can the *in situ* controls on MeHg production be addressed.

This data set is also limited to a sample of boreal wetlands found on the Canadian Shield. I suggest similar research should be performed on wetlands of other types and areas. The importance of hydrology in influencing MeHg concentration and export is now obvious. To better estimate the MeHg store and export of MeHg in other wetlands, a good understanding of the wetland hydrology is key. Sampling should be focused on hydrologically distinct zones, and on the inputs and outputs of nutrients from these zones. Once a number of wetlands have been studied, a means of identifying wetlands with high MeHg production and export potential can be devised.

Both SO_4^{2-} and carbon have been identified as important factors in controlling Hg methylation in wetlands. The speciation of S, and perhaps C, appears important in Hg methylation. Only a few studies of Hg methylation in freshwater wetlands have been conducted and many questions regarding the S cycle are unanswered. Even gaining a simple understanding of the speciation of S in Hg methylating and non Hg methylating wetland environments may considerably aid in understanding how sulfate reduction is related to Hg methylation.

Many other factors such as temperature and pH have also been identified as important controls on Hg methylation (Winfrey and Rudd, 1990). These factors may be secondary, but their impact on Hg methylation in wetlands needs to be investigated. Although not as scientifically rigorous as a step by step reductionist approach, the Experimental Lakes Area Reservoir Project has demonstrated that impoundment of a wetland increases MeHg production in the peat. In a complicated system such as peat, it is impossible to vary one factor while keeping the others constant. Thus, the most reasonable way of examining the effect of factors such as nutrient supply, temperature and water table frequency on Hg methylation in peat may be through *in situ* manipulation and the observation of the net ecosystem effect.

Methylation appears to be a way of increasing Hg mobility but the conditions under which MeHg becomes more mobile are not well known. Other than a change in the MeHg concentration, the redox potential, pH, nature of the particles and water chemistry undoubtedly affect the partitioning of MeHg between solid and dissolved (<0.45 μm fraction) phases. Understanding the conditions of MeHg partitioning will aid in determining when MeHg is most available for export from sediments. Once in the water column, MeHg may have a greater chance of entering the food chain through zooplankton than if it remained in the sediments (Patterson pers. comm., 1995).

Decomposing fresh plant tissues are important sites of Hg methylation. However, the circumstance surrounding Hg methylation in the plant tissue is not clear. Many questions still need be answered such as: i) where is Hg methylated (inside or outside the plant surface), ii) what external requirements are required by the decomposing population to methylate Hg, iii) does the internal nutrient composition of the plant tissue affect Hg methylation, iv) what is the speciation of Hg lost from the decomposing tissue, and v) how sensitive is the location of decomposition to MeHg production.

Although the MeHg production in peat has been clearly shown, the respective rates of Hg methylation and demethylation are not known. Potential Hg methylation rates can be measured by addition of high specific activity ^{203}Hg. Some would argue the added Hg may be more bioavailable, but addition of Hg has already been shown not to stimulate Hg methylation in peat. This method would not only be useful in assessing the relative Hg methylating activities of different kinds of peat but also in demonstrating that under anaerobic conditions, fresh plant material is an important site of Hg methylation.

On the analytical and methodological side, methods must be developed to measure fluxes of MeHg. We lack the tools to detect both hydrologic and chemical gradients in peat at the resolution necessary to observe biologically mediated processes.

Finally, the ability to interpret our results is limited by our knowledge of Hg methylating bacteria and our ability to detect them. Aside from general functional groups such as SRB and methanogens, we know little about the number and diversity of bacterial species present in most ecosystems. Until a better general understanding of microbial processes is obtained, we will not truly understand Hg methylation and MeHg demethylation.

Chapter 7: Conclusions

7.1 Conclusions

Methylmercury (MeHg) concentrations range from 4 to 60 ng g^{-1} in peat and from < 0.02 to 6 ng l^{-1} in peat porewater of wetlands on the Canadian Shield in NW Ontario. Methylmercury concentrations are variable within and amongst wetlands, and the highest MeHg concentrations are found just below the water table. The MeHg concentration in peat porewater is controlled by the MeHg concentration in peat. The partition coefficients (K_D) between MeHg in peat and porewater range from10^3 to 10^5. The amount of MeHg partitioned to the porewater increases disproportionally with increasing peat MeHg concentration. As MeHg can account for 50% of the total mercury (T-Hg) in peat and 25% of the T-Hg in peat porewater, the methylation of Hg in peat may be an important mechanism for increasing the mobility of Hg. The close proximity of high MeHg concentrations to the more hydraulically conductive peat near the water table likely aids in the export of MeHg from the wetlands especially during rain events.

Impoundment of the riparian wetland increased the mean porewater MeHg concentration from 0.2 to 0.8 ng l^{-1} and the store of MeHg in the riparian wetland is estimated to have increased by 2.7 times. The MeHg concentrations found in impounded peat porewater are not unique, but also exist in hollows of unmanipulated wetlands. What is unusual about the impounded peat porewater is the aerial extent of the high MeHg concentrations. Thus, the increase in the store of MeHg in the peat is likely caused more by the expansion of the area over which net MeHg production occurs than by an increase in the amount of MeHg produced at any particular site.

Total-Hg was lost from spruce needles, sedge stems and *Sphagnum* moss during decomposition under both aerobic and anaerobic conditions in the headwater wetland and under anaerobic conditions in the impounded wetland. Methylmercury was also lost from these tissues during aerobic decomposition in the headwater wetland but MeHg was gained during anaerobic decomposition in the wetland and the reservoir. The largest amounts of MeHg were present in the *Sphagnum* moss, where the MeHg concentration increased from 1 to 8 ng g^{-1} without a measurable loss in mass. These observations suggest that MeHg is produced only during anaerobic decomposition and that plant litter is an important site of Hg methylation in both pristine and newly impounded wetlands.

With the highest MeHg concentration in peat and porewater occurring at the oxic\anoxic interface, the importance of biologically mediated redox reactions in Hg methylation are obvious. However, no correlation was found between the porewater concentrations of MeHg and the concentrations of H^+, SO_4^{2-}, NH_4^+, NO_3^-, NO_2^-, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and dissolved organic carbon (DOC) amongst the pristine wetlands or in the impounded wetland. Of the additions: SO_4^{2-}, NH_4NO_3, DOC, pyruvate and Hg to peat, MeHg production was increased only by the addition of SO_4^{2-} and retarded only by the addition of NH_4NO_3. In wetlands, SO_4^{2-} is available at all sites of MeHg production, but the most consistent external SO_4^{2-} supplies are available to the poor fen and impounded peats which is where MeHg concentrations are highest. It appears the availability and the speciation of SO_4^{2-} is important in controlling MeHg production in pristine and impounded wetlands.

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