Methylmercury in Boreal Freshwater Food Webs

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Metylkvicksilver i näringsväv av boreala sötvatten

Sammanfattning

Förekomst av kvicksilver (Hg) i akvatiska miljöer och bioackumulering av den organiska formen metylkvicksilver (MeHg) kan ge upphov till skador hos både människor och vilda djur. Områden med skogsavverkningar och våtmarker kan verka som "hotspots" för MeHg-export till sjöar och vattendrag. Syftet med denna avhandling är att förbättra förståelsen för hur miljöförändringar påverkar biotillgänglighet och bioackumulering av MeHg i svenska sötvatten med fokus på basen av den akvatiska näringsväven.

I en studie kring effekter av skogsavverkning på bioackumulering av Hg i fisk detekterades en stor variation mellan olika sjöarna. Studien betonar behovet av långsiktiga studier för att bedöma påverkan på kvicksilvernivåerna i biota. Boreala bäverdammar studerades för att förstå processerna som kan öka MeHg produktionen i sedimenten vid nyetablering av bäverdammar. Ökad MeHg-produktion samt förändringar av kolkvaliteten i sedimenten detekterades kort efter översvämning av skogsmark vid uppbyggnad av nya bäverdammen.

För att ta reda på varför det finns så stora skillnader mellan MeHg-koncentrationen i vatten och MeHg-bioackumulering i olika akvatiska ekosystem genomfördes en omfattande metaanalys. Slutsatsen var att kvoten mellan koncentrationen av MeHg i vattnet och plankton, så kallad biokoncentrationsfaktorn, var avgörande för vidare bioackumulering. För att identifiera kopplingen mellan MeHg i akvatiska biota och näringsupptaget från olika källor i dieten, har fettsyror använts som markör för specifika kostkällor för vattenlevande primära konsumenter av plankton och makroinvertebrater. Genom att korrelera Hg-bioackumulering med specifika fettsyror bekräftade jag att Hg-upptaget ökade samtidigt med fleromättade fettsyror hos primära konsumenter.

Sammanfattningsvis kan dessa resultat bidra till en ökad förståelse för den roll som basen av en näringsväv har för överföring av MeHg högre upp i näringsvävar, samt betydelsen av landskapsfaktorer för bioackumulering av MeHg. Det finns ett behov av vidare forskning med fokus på strukturen och funktionen i botten av akvatiska näringsvävar, för att bättre kunna prediktera hur förändringar i landskapet, klimatet och vattenkemin leder till förändringar i bioackumulation av MeHg i fisk.

Nyckelord: kvicksilver, metylkvicksilver, bioackumulering, biokoncentration, biotillgänglighet, planktonisk näringsväv, naturlig organisk substans, primära konsumenter, metylering, fettsyror

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Methylmercury in Boreal Freshwater Food Webs

Abstract

Mercury (Hg) and bioaccumulation of its organic form methylmercury (MeHg) has long been regarded as a global issue for human and wildlife health. This is of particular concern for Sweden, where forest harvests and wetlands remain hotspots of MeHg export to boreal freshwaters. The aim of the study was to better understand how environmental change impacts MeHg bioavailability and bioaccumulation with a focus on the base of the food web in Swedish freshwaters.

In the thesis, I first examined the impacts of forest harvest on Hg bioaccumulation in fish. From analysis of more than a thousand fish samples from six lakes, it was clear that there was a large variation of fish Hg levels over time and between lakes. The study emphasizes the need for long-term studies to assess the influences on Hg levels in biota. Beaver ponds were also studied to understand the processes influencing MeHg concentrations in water. Increased MeHg production in the sediment was stimulated shortly after flooding by new beaver dams, and that stimulation was related to the quality of the organic carbon.

To explore the basis for large discrepancies between aqueous MeHg exposure and the ultimate degree of MeHg bioaccumulation in different aquatic ecosystems, a literature review was conducted on MeHg bioaccumulation from water into the base of the food web. The bioconcentration factor for MeHg, that is the ratio of MeHg concentrations in water and seston or zooplankton, was found to be critical for subsequent MeHg bioaccumulation. To better identify linkages between MeHg uptake and different nutrient sources at the base of the food web, fatty acids were used to distinguish specific diet sources in aquatic primary consumers of plankton and macroinvertebrates. Correlation of Hg bioaccumulation with specific fatty acids confirmed that Hg content increased simultaneously with the retention of polyunsaturated fatty acids in primary consumers.

These findings contribute to a better focus on the role of the base of aquatic food webs in transferring MeHg upwards along trophic cascades, as well as landscape influences on elevated MeHg bioaccumulation. Further advances in understanding how the structures at the base of aquatic food webs function with respect to mercury cycling are needed to better predict how changes in the landscape, climate and water chemistry will alter Hg bioaccumulation in fish.

Keywords: mercury, methylmercury, bioaccumulation, bioconcentration, bioavailability, planktonic food web, natural organic matter, primary consumers, methylation, fatty acids

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Dedication

献给一路培养我成长的父亲
To my father, for always supporting me no matter where…

子非鱼，焉知鱼之乐？
　惠子问庄子
You are not a fish, so whence do you know the happiness of fish?
　Huizi asked Zhuangzi

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Wu P.**, Bishop K., von Brömssen C., Eklöf K., Futter M., Hultberg H., Martin J., Åkerblom S. Does forest harvest increase the mercury concentrations in fish? Evidence from Swedish lakes. Under revision in *Science of the Total Environment*

II Catalán N., Herrero Ortega S., Gröntoft H., Hilmarsson T.G., Bertilsson S., **Wu P.**, Levanoni O., Bishop K., Bravo A.G. (2017). Effects of beaver impoundments on dissolved organic matter quality and biodegradability in boreal riverine systems. *Hydrobiologia*, 793(1), 135-148

III Herrero Ortega S., Catalán N., Björn E., Gröntoft H., Hilmarsson T.G., Bertilsson S., **Wu P.**, Bishop K., Levanoni O., Bravo A.G. High methylmercury formation in ponds fueled by fresh humic and algal derived organic matter. Bioconcentration of aqueous methylmercury in seston predicts methylmercury in fish. Under review after revision in *Limnology and Oceanography*

IV **Wu P.**, Kainz M.J., Bravo A.G., Åkerblom S., Sonesten L., Bishop K. Bioconcentration of aqueous methylmercury in seston predicts methylmercury in fish. Under revision in *Environmental Science and Technology*

V **Wu P.**, Kainz M.J., Åkerblom S., Sonesten L., Bravo A.G., Branfireun B., Deininger A., Bergström A.-K., Bishop K. Diet source matters for mercury bioaccumulation by primary consumers in the freshwater food webs of Swedish lakes. (manuscript)

Paper III is reproduced with kind permission from the publisher Springer International Publishing AG

The contribution of PW to the papers included in this thesis was as follows:

I PW wrote the paper with contributions from all authors. SÅ conceived the study. PW performed much of the field work, total mercury, and data analysis with contributions from HH and JM. CB and MF also contributed to statistical analysis of data.

II NC wrote the paper with contributions from all authors. PW conceived and planned the field sampling campaign. AGB conceived the specific study on carbon quality. SHO, HG, AGB, PW, and OL performed the field work.

III SHO wrote the paper with contributions from all authors. PW conceived and planned the field sampling campaign. AGB conceived the specific study on carbon quality and mercury methylation. SHO, HG, AGB, PW, and OL performed the field work. SHO and HG performed the mercury methylation analysis and organic matter analysis. SHO and AGB performed the statistical analysis of data.

IV PW conceived the study and wrote the paper with contributions from all authors. PW collected the literature data and performed the data analysis.

V PW wrote the paper with contributions from AGB, MJK and KB. PW, KB, and AKB conceived the study. PW, AD, SÅ performed the field work. BB contributed to the mercury analysis. PW performed fatty acids analysis with contributions from MJK. MJK, AKB, and AD contributed to phytoplankton sampling and analysis. LS contributed to the data analysis.

Abbreviations

%MeHg	Fraction of methylmercury to total mercury
%RPD	Relative percent difference
ALA	α-linoleic acid; 18:3(*n*-3)
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARA	Arachidonic acid; 20:4(*n*-6)
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BDOM	Biodegradable dissolved organic matter
BFA	Bacterial fatty acids
BIX	Biological index
Chl *a*	Chlorophyll *a* in water
CRM	Certified reference material
CVAAS	Cold vapour atomic absorption spectrometry
CVAFS	Cold vapour atomic fluorescence spectrometry
DHA	Docosahexaenoic acid; 22:6(*n*-3)
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EEM	Emission-excitation matrix
EFA	Essential fatty acids
EMP	Environmental Monitoring Programme
EPA	Eicosapentaenoic acid; 20:5(*n*-3)
EQS	Ecological quality standard
FAME	Fatty acid methyl esters
Fe	Total iron ions in water
FT	Total fluorescence
GC-ICPMS	Gas chromatography-inductively coupled plasma mass spectrometry

Hg	Mercury
Hg(0)	Elemental mercury
Hg(II)	Inorganic divalent mercury
HIX	Humidification index
IRB	Iron reducing bacteria
k_d	Potential demethylation rate constant
k_m	Potential methylation rate constant
LIN	Linoleic acid; 18:2(*n*-6)
MDL	Method detection limit
MeHg	Methylmercury
MQL	Method quantification limit
MUFA	Monounsaturated fatty acids
NMDS	Non-metric dimensional scaling
NOM	Natural organic matter
OM	Organic matter
PERMANOVA	Permutational multivariate analysis of variance
pH	Measure of hydronium ion concentration
PLS	Partial Least Squares
PUFA	Polyunsaturated fatty acids
QA/QC	Quality assurance/quality control
R.U.	Fluorescence peaks
SAFA	Saturated fatty acids
SDA	Stearidonic acid; 18:4(*n*-3)
SRB	Sulphur reducing bacteria
SUVA	Specific UV-absorbance
TDA AAS	Thermal decomposition amalgamation, and atomic absorption spectrometry
terr.FA	Terrestrial fatty acids
THg	Total mercury
TLP	Total lipids
TMF	Trophic magnification factor
TOC	Total organic carbon
totN	Total nitrogen in water
totP	Total phosphorous in water
UNEP	United Nations of Environmental Programme
USEPA	United States of Environmental Protection Agency
WHO	World Health Organization
$\delta^{13}C$	Ratio of heavier to lighter stable isotopes of carbon; $^{13}C/^{12}C$
$\delta^{15}N$	Ratio of heavier to lighter stable isotopes of nitrogen; $^{15}N/^{14}N$

1 Introduction

Mercury (Hg, *hydragyrum*, quicksilver) is ubiquitously spread in the environment by both natural processes and pollution. The tragic health problems discovered in the vicinity of Minamata, Japan in the 1960s were due to illegal industrial waste water discharge containing highly concentrated, neurotoxic alkylmercuric compounds (organic forms of Hg, CH_3Hg^+ known as methylmercury, or MeHg). The discovery that these dreadful consequences resulted from Hg exposure stimulated research on managing the Hg problem. In addition to waste water discharges, increased Hg emissions to the atmosphere as a result of industrialization since the mide-1800s have had major impacts on Hg cycling in the world (Selin, 2014; Pirrone *et al.*, 2010). The Minamata Convention on Mercury, an international treaty adopted in October 2013 by the United Nations of Environmental Programme (UNEP), marks a major step forward in the global efforts to tackle the enduring legacy of Hg pollution (UNEP, 2013). However, the fate of Hg, particularly more bioavailable organic forms, involve great complexities that remain to be understood. This contributes to large uncertainties in identifying the environmental risks and management options related to human activities in different environmental settings.

1.1 Mercury in the environment

Biogeochemical cycling of mercury involves various forms with distinct physicochemical characteristics (Figure 1): Elemental mercury (Hg(0)), inorganic mercury (Hg(II)) and organic mercury (the majority being MeHg). Gaseous elemental Hg(0) can be transported long distances. Oxidation of Hg(0) results in inorganic divalent Hg (Hg(II)) that combines with metalloids to form inorganic Hg, e.g. HgS, $HgCl_2$. Elemental and inorganic forms of Hg can further be methylated by sulphur reducing bacteria (SRB), iron reducing bacteria (IRB) and other microbes in suboxic conditions in aquatic and terrestrial environments (Gilmour *et al.*, 2013; Hu *et al.*, 2013; King *et al.*, 2002; Benoit *et al.*, 1999).

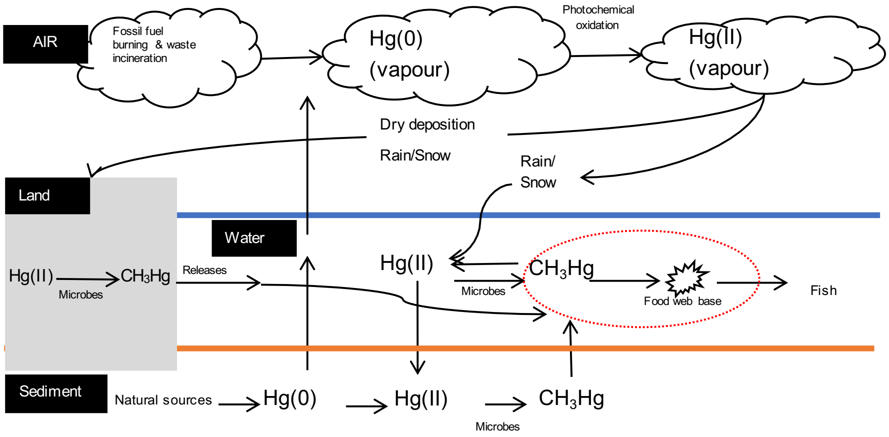

Figure 1. Biogeochemical cycling of Hg at the interface of water, air and land. Elemental mercury (Hg(0)) is released/emitted to the atmosphere by human activities and natural events; Wet/Dry deposition of Hg(0) and inorganic mercury (Hg(II)) to the ground; Microbes form organic mercury, or MeHg, utilizing inorganic mercury under suboxic conditions; Plankton at the base of the aquatic food web efficiently takes up MeHg; MeHg biomagnified to higher trophic levels, eventually ending up on our dinner tables. The area circled with the red dashed line marks the focus of this thesis: Transfer of aqueous MeHg to biota at the base of the food web. Adapted from Barkay *et al.* (2011).

Increases in Hg emissions associated with industrialization have impacted Hg cycling in the world by increasing atmospheric Hg (Pirrone *et al.*, 2010). This has resulted in a 10 % increase of Hg in oceanic surface water and a three-fold increase of oceanic fish Hg concentrations (Lamborg *et al.*, 2014). Increases in fish Hg concentrations due to atmospheric Hg increases are found even in aquatic ecosystems located far from industrial sites (Engstrom, 2007; Lindqvist *et al.*, 1991). Fish are a major route of exposure to MeHg for both humans and other biota according to reports from the World Health Organization (WHO)

(IPCS, 1990). Consideration of the risks from Hg in fish prompted the EU to set up an Ecological Quality Standard (EQS) for Hg and its compounds in aquatic biota of 0.02 µg g^{-1} wet weight (w.w.), which is intended to protect humans and wildlife from Hg risks (EC, 2013; EC, 2008).

In Sweden, almost all lakes have fish Hg concentrations that is higher than the EU EQS of 0.02 µg g^{-1} w.w. (Åkerblom *et al.*, 2014; Miller *et al.*, 2013; Danielsson *et al.*, 2011; Bignert *et al.*, 2010). The EU EQS is 25 times lower than the 0.5 µg g^{-1} w.w. maximum safe consumption levels previously allowed by the EU for Hg in fishery products (EC, 2006). High Hg levels in freshwater biota are also not rare. In the boreal zone and the Amazon region, freshwater fish contains high levels of Hg in some lakes and rivers contains levels of Hg well over 0.5 µg g^{-1} w.w. (Azevedo-Silva *et al.*, 2016; Åkerblom *et al.*, 2014)

How difficult is it to remediate fish Hg when the levels are too high for safe consumption? In Sweden, it has been estimated that it will take at least 200 years even if atmospheric emissions are reduced (Meili *et al.*, 2003). The recovery period is long despite the fact that Sweden has been one of the leading countries in phasing out mercury usage since the 1990s (KEMI, 2011).

1.2 Mercury in planktonic food webs

While Hg in fish poses a threat to consumers regardless of its chemical speciation, MeHg is the mercury species that bioaccumulates most efficiently (Clarkson *et al.*, 2003). Many studies that analysed MeHg concentrations in aquatic biota with a focus on MeHg bioaccumulation in piscivorous wildlife have shown that MeHg concentrations increase steadily with each trophic level (Clayden *et al.*, 2013; Munthe *et al.*, 2007; Mason *et al.*, 1995). A comprehensive review by Lavoie *et al.* (2013) on total Hg (THg) and MeHg concentrations along aquatic food chains across the globe has put much of the existing data in perspective. They found that bioaccumulation patterns above the base of the food web were relatively similar with some consistent differences between regions.

Using seston (i.e. suspended particles, mostly composed of small algae and bacteria) as the base of the pelagic food web, Rolfhus *et al.* (2011) found that dissolved organic matter (DOM) limited MeHg uptake from water by seston, zooplankton, and fish, yielding lower bioconcentration factors (BCF, defined as the ratio between the MeHg concentrations in biota and water), while the trophic magnification factor (TMF) from the base of the food web upwards remained stable across different food webs. This finding is an indication of the complexity of Hg bioconcentration from water into the biota due to the influence of different environmental factors, but also the importance of the variability of bioconcentration into the base of the pelagic food web for the cumulative bioaccumulation at higher trophic levels (Figure 2).

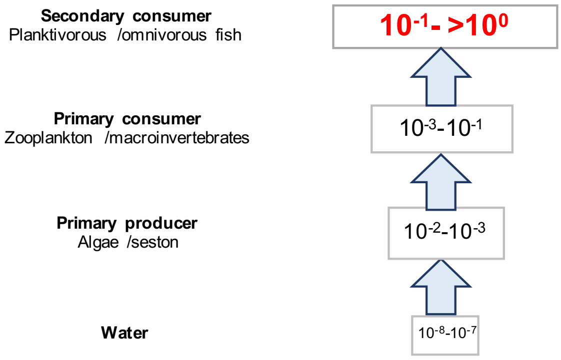

Figure 2. Conceptual model of MeHg bioaccumulation and biomagnification in aquatic food webs. All units in µg g^{-1}(wet weight concentration for biota) or mg L^{-1} (aqueous concentration). Adapted from Lehnherr (2014) with kind permission from NRC Research Press.

Correlations between catchment characteristics and fish Hg concentrations have also been utilized to examine the influences on Hg concentrations in aquatic biota. Low pH surface water (pH <6, generally oligotrophic) is associated with higher Hg concentrations in freshwater ecosystems (Le Faucheur *et al.*, 2014). On the other hand, aquatic ecosystems with eutrophication are often associated with increased plankton biomass and consequently lower Hg concentrations in the aquatic biota, which is described as "biodilution" (Karimi *et al.*, 2007; Pickhardt *et al.*, 2002). Some more eutrophic aquatic ecosystems in China and Eastern Europe have relatively high concentrations of aqueous MeHg (>1 ng L^{-1}), but these are accompanied by lower MeHg in biota (<0.2 µg g^{-1} w.w.) (Liu *et al.*, 2012; Suchanek *et al.*, 2008; Nguyen *et al.*, 2005; Farkas *et al.*, 2000). This stands in contrast to the relatively high MeHg concentrations in the biota of oligotrophic boreal ecosystems (often >0.5 µg g^{-1} w.w.) despite aqueous MeHg concentrations that are often well below 1 ng L^{-1} (Clayden *et al.*, 2013; Watras & Bloom, 1992). Mason *et al.* (2012) and Le Faucheur *et al.* (2014) have summarized experimental approaches to studying Hg bioconcentration from water into biota. They found that the transfer of bioavailable MeHg fractions from water into seston is a critical step for subsequent MeHg bioaccumulation in aquatic food webs.

1.3 Impact of forest harvest and beaver impoundment on methylmercury

1.3.1 Forest harvest

The raw materials provided by forest harvest are of great socioeconomic value. In parallel to the socioeconomic benefits, there are concerns about how forestry operations influence water quality (Futter *et al.*, 2016; Roberge *et al.*, 2016). One of these concerns is the possible effect on Hg concentrations in freshwater biota in boreal waters that already pose a risk to human and wildlife health (Åkerblom *et al.*, 2014). A number of studies have evaluated the effects of forest harvest on Hg concentrations and exports in surface waters, but few studies have tested the direct effect from forest harvest on fish Hg concentrations. Since there is increasing evidence that the export of total mercury (THg) and the bioaccumulative MeHg is affected by forest operations (Eklöf *et al.*, 2016), the limited information on the biotic effects of forest harvest on fish Hg concentration is a concern in itself. Further investigations on the connection between forestry and Hg concentrations in biota, especially fish (of which dorsal muscle THg is generally >90% MeHg (Munthe *et al.*, 2004)), are thus needed to provide a better basis for managing the many trade-offs inherent in forest management.

The forestry effects on Hg in runoff water and biota have been attributed to the generally wetter conditions in soils when trees are harvested due to transpiration decreases in combination with more snow accumulation in clear-cut areas (Shanley & Bishop, 2012). This in turn increases runoff and changes hydrologic flow paths in ways that may increase the mobilization of Hg (Eklöf *et al.*, 2016). Furthermore, suboxic conditions form in water logged and disturbed areas that promote methylation of inorganic Hg (Kronberg *et al.*, 2016).

Several studies have since evaluated the effect of forestry operations on THg and MeHg concentrations, as well as fluxes in runoff water: Sorensen *et al.* (2009) found a 15% increase in THg concentrations in the output of water from two boreal catchments after forestry harvest. Skyllberg *et al.* (2009) showed a significant increase in MeHg concentrations in runoff from 0-4 years' post-harvest forest for forest areas above the highest post-glacial shoreline in Sweden (but not below). Eklöf *et al.* (2014) found that MeHg loadings to surface waters were 30-50% higher after a combination of logging and site preparation. Kronberg *et al.* (2016) estimated that there will be a 12-20% increase in MeHg concentrations in Swedish forest runoff if forest harvest effects persist for a decade. However, there are also studies that did not find significant increases in either THg and/or MeHg concentrations in runoff water after logging (de Wit *et al.*, 2014; Allan *et al.*, 2009) and stump harvest (Ukonmaanaho *et al.*, 2016; Eklöf *et al.*, 2013; Eklöf *et al.*, 2012). As pointed out by Eklöf *et al.* (2016), most of the studies since those pioneering works (pre-2006) have focused on Hg concentrations in water (and aquatic loadings), rather than on the biota, with some notable exceptions, e.g. Garcia *et al.* (2007); Desrosiers *et al.* (2006) and de Wit *et al.* (2014). Desrosiers *et al.* (2006) and Garcia *et al.* (2007) both observed decreases in biomass and concurrent increases in Hg concentrations in periphyton and zooplankton. de Wit *et al.* (2014) reported no significant impacts of forest harvest on Hg concentrations in macroinvertebrates (and stream water) from a catchment with about 30% forest harvest although the forestry operations resulted in considerable disturbance of the soils in the harvested area. So far, there has been no study on the direct connection between different areal extents of forest clear-cut across several catchments over a period of several years to examine how fish Hg concentrations respond to commercial forestry operations in a boreal setting.

1.3.2 Beaver impoundments

Increasing number of Eurasian beaver (*Castor fibre*) populations now recovering from near extinction are one of the main causes of ponding in Swedish streams (Halley *et al.*, 2012). Besides increasing wetland habitat heterogeneity (Naiman *et al.*, 1986) and plant richness over the long term (Law *et al.*, 2017), the recovery of beaver populations can stimulate primary production and nutrient loads (Margolis *et al.*, 2001; Naiman *et al.*, 1986). This also poses a pressure to environmental health due to elevated risks of MeHg in the stream network, particularly during the first few years after the initial flooding created by a new beaver pond. The finding of over three-fold MeHg increase in water downstream of some beaver impoundments can be critical to MeHg bioaccumulation and biomagnification in stream food webs (Levanoni *et al.*, 2015), eventually affecting wildlife and human health (Scheuhammer *et al.*, 2007). There is some evidence that beaver ponds along boreal streams can enhance natural organic matter (NOM) bioavailability and degradability (Lapierre *et al.*, 2013), as well as elevated aqueous MeHg (Levanoni *et al.*, 2015; Roy *et al.*, 2009). The processes leading to increases of aqueous MeHg shortly after beaver impoundment, and whether this is related to altered NOM bioavailability are, however, less known.

NOM composition couples with environmental factors (e.g. temperature, redox potential, pH, and sulphur speciation) to influence the biological methylation rate of inorganic divalent Hg (Hg(II)) at methylation hotspots in inland waters (Drott *et al.*, 2007; Ullrich *et al.*, 2001). In aquatic systems, NOM is a mixture of autochthonous and allochthonous compounds that might affect the formation of MeHg in contrasting ways, such as by controlling the overall activity of microbial communities (Bravo *et al.*, 2017) or by affecting Hg(II) solubility, availability for bacterial uptake and subsequent methylation (Jonsson *et al.*, 2014; Jonsson *et al.*, 2012; Schaefer & Morel, 2009). The role of NOM for Hg methylation processes is critical, depending on NOM molecular composition as revealed by Bravo *et al.* (2017) from a study investigating Hg(II) methylation rates in lake sediments. Autochthonous algal-derived NOM enhances Hg(II) methylation by boosting the activity of microbial communities. Enhancement of Hg(II) methylation can also be achieved by NOM providing low molecular mass thiols which form bioavailable complexes with Hg(II) (Leclerc *et al.*, 2015). However, the components of NOM controlling Hg(II) methylation processes in boreal streams, which are normally poor in autochthonous NOM but enriched in allochthonous terrigenous NOM, remain unclear.

1.4 Impact of diet sources on methylmercury bioaccumulation

The variety of lipids, particularly fatty acids (FA), and their preservation in food webs make them useful as dietary tracers. Thus, FA have been used extensively as biomarkers for what is retained in the food web. One example is the use of FA to successfully examine the bioaccumulation of persistent organic pollutants (Lynn *et al.*, 2007; Fisk *et al.*, 2001; Swackhamer & Skoglund, 1993). The FA can distinguish dietary sources lower down in the food web, in particular separating algal-derived organic matter in consumers, such as zooplankton, macroinvertebrates, and fish, from bacterial origin organic matter and dietary organic matter of terrestrial origin (Kainz & Mazumder, 2005). But while both the bioaccumulation patterns of toxic MeHg and lipids distribution in the food web have been extensively studied, these have often been done separately (Lavoie *et al.*, 2013; Syvaranta & Rautio, 2010). There have been few efforts to link MeHg with lipids, specifically polyunsaturated fatty acids (PUFA) that are useful for resolving processes at lower trophic levels. Several FA were considered essential to the somatic growth of primary consumers, abbreviated as EFA (essential fatty acids), as they are integral parts of cell membranes: linoleic acid (LIN; 18:2*n*-6), α-linoleic acid (ALA; 18:3*n*-3), arachidonic acid (ARA; 20:4*n*-6), eicosapentaenoic acid (EPA; 20:5*n*-3), stearidonic acid (SDA; 18:4*n*-3), and docosahexaenoic acid (DHA; 22:6*n*-3). LIN and ALA can only be obtained by diets due to lack of related enzymes in animals for *de novo* synthesis

(Cook & McMaster, 2002). Among the few existing joint investigations of Hg and FA in freshwater planktonic food webs, Kainz and Mazumder (2004) as well as Kainz *et al.* (2006) found that MeHg increase was not related to the PUFA distribution patterns in zooplankton in general. Yet so far there is no study available showing the relationship of somatic growth related FA (e.g. EPA) with MeHg bioaccumulation in aquatic food webs, despite the fact that these particular FA might be helpful as dietary biomarkers to link utilization of biomass with the cycling of an organic pollutant such as MeHg.

Many Swedish freshwater lakes are small (a few hectares), unproductive, oligotrophic, acidic, and humic (high dissolved organic carbon concentrations, e.g. 5-10 mg L^{-1} and even higher). Such acidic and humic waters have been found to be generally more efficient at bioaccumulation of Hg than less acid and/or less humic lakes (Clayden *et al.*, 2014; Edmonds *et al.*, 2012; Lindqvist *et al.*, 1991). For such lakes, an increase in nutrient loading, such as elevated nitrogen and/or phosphorous availability will generally increase plankton biomass, which can in turn biodilute fish Hg concentrations (Karimi *et al.*, 2007; Pickhardt *et al.*, 2002). But the biological processes related to the nutrient and contaminant uptake (specifically uptake of mercury) at the base of food web within boreal inland waters are not well understood. Whether MeHg bioaccumulation with simultaneous uptake of FA is linked to environmental factors such as dissolved organic carbon (DOC), pH, total phosphorous (totP), or total nitrogen (totN) remains worth investigating further.

1.5 Objectives

This thesis consists of five papers (paper I-V below) which focus on MeHg bioavailability in freshwaters and relevant environmental factors, including bioaccumulation from primary producers (phytoplankton) to primary consumers (zooplankton and macroinvertebrates) and secondary consumers (fish). The objectives of the five papers are as follows:

- To determine whether forestry impacts fish Hg concentrations in the years after forest harvest as compared to the pre-harvest situation (paper I).
- To evaluate the changes in natural organic matter quality and biodegradation patterns in beaver ponds considering the age of the ponds (paper II).
- To assess the impacts of natural organic matter on MeHg formation after beaver impoundment (paper III).
- To identify the relationships between MeHg bioconcentration and bioaccumulation in different aquatic ecosystems (paper IV).
- To examine the effects of water chemistry and fatty acid composition on MeHg bioaccumulation in primary consumers (paper V).

2 Materials and methods

2.1 Study sites

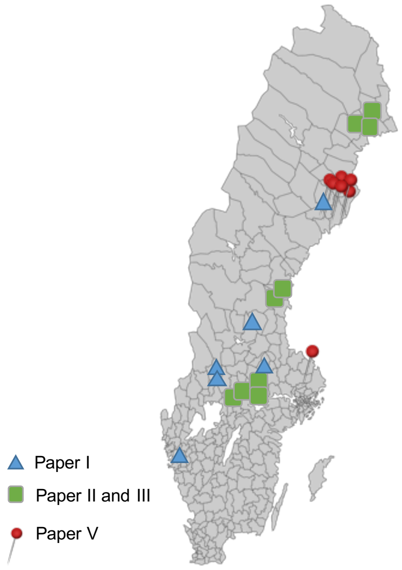

Figure 3. Map of Sweden marking the locations of study sites included in this thesis: lakes from Paper I (blue triangles), beaver ponds from Paper II and III (green squares), and lakes from Paper V (red pins).

Six forest lakes with clear-cuts planned for 2012 were identified in advance of the harvest (paper I) (Figure 3 and 4). Beaver ponds visited for sampling of water and sediments were clustered in the north, middle, and south of Sweden (paper II) (Figure 3 & 5, Table 1). To examine the relationship between aqueous MeHg, bioconcentration into the base of the food web, and MeHg bioaccumulation in fish, published literature on aquatic ecosystems from around the world was reviewed (Figure 6). Lastly, to use diet source biomarkers in predicting MeHg bioaccumulation by primary consumers, a set of experimental lakes in the north of Sweden as well as a contrasting lake in the south were selected (Figure 3).

2.1.1 Forest harvest

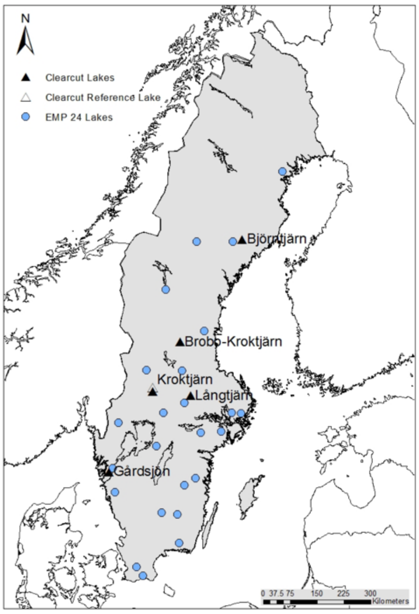

Figure 4. Fish sampling sites from lakes with forest harvest in portions of their catchments (n=5, filled black triangles), one clear-cut reference lake (Svultentjärn, hollow triangle) and the larger reference set, 24 National Environmental Monitoring Programme (EMP) lakes spread across Sweden (blue dots).

For paper I, six small headwater lakes were selected for the study with information collected from the Swedish Forest Agency (Skogsstyrelsen, http://www.skogsstyrelsen.se/). The five lake catchments that were subject to conventional forest harvesting are referred to as clear-cut lakes. One small catchment was not harvested despite the initial plan to do so, and this is referred to as the clear-cut reference lake) (Figure 4). The lakes are all situated in managed forest catchments across Sweden (Table 1).

To provide a larger pool of "references" without forest harvest during the study period, data from the Swedish National Environmental Monitoring Programme (EMP) was included. EMP is a long-term freshwater environmental monitoring programme, commissioned by the Swedish Environmental Protection Agency (www.swedishepa.se). The EMP lakes selected for paper I represent small, forested, headwater, remote, and relatively pristine freshwaters across the nation (Gotland excluded), with sufficient samples (>10 fish sampled between August-September from each lake every year since 2010). These criteria were met by 24 EMP lakes (Figure 4).

Table 1. Catchment characteristics of the six lakes sampled in this study.

Lake (abbreviation)	Location		Lake area	Catchment size	Clear-cut area	Relative clear-cut area
	Latitude (N)	Longitude (E)	ha	ha	ha	%
Björntjärn (BJ)	63°54′	18°49′	3.9	195.4	55.3	28
Brobo-kroktjärn (BK)	61°22′	15°20′	10.9	154.0	19.4	13
Gårdsjön (GA)	58°3′	12°1′	31.3	180.0	11.9	6
Kroktjärn (KR)	60°7′	13°58′	4.8	199.9	72.7	36
Långtjärn (LA)	60°1′	15°52′	6.5	236.3	93.9	40
[1]Svultentjärn (SV)	60°12′	13°58′	3.8	95.4	0.0	0

[1] Fish sampled from this lake are used as a reference for the clear-cut group in the data analysis.

2.1.2 Beaver impoundments

Study sites in paper II and III were selected from those studied by Levanoni *et al.* (2015) (Figure 4). More than half of the beaver impoundment sites were rebuilt over already-existing beaver ponds during the course of almost two decades (Table 1). Flooded areas and increased water elevation related to the situation downstream of beaver dams can be clearly noticed in most of the sites (Figure 5). Sampling started in late September and was finished by early October 2014 at locations distributed between the hemi-boreal and boreal vegetation zone in naturally acidic catchments mostly covered by coniferous forest (Table 2).

Figure 5. From left to right: pictures from beaver ponds sampled in Västerås, Örebo, Sundsvall, and Luleå during the autumn o2014 field campaign (Photo: Pianpian Wu, SLU).

Table 2. *Location and land use scenario of the studied beaver pond sites. The land use (%) and age (N: New < 18 years old and O: Old > 18 years old) of the beaver system was previously described elsewhere (Levanoni et al., 2015), in parentheses is the nomenclature of the site in their paper.*

Site	Coordinates		Geographic location	Land use (%)			Age
	Latitude (N)	Longitude (E)		Wetlands	Arable land	Forest	
1	59°42'31"	16°5'35"	Västerås	0.4	4.4	95	7-10, N (23)
2	59°41'18"	16°1'53"	Västerås	5	0	86	2-7, N (24)
3	59°14'52"	14°50'25"	Örebo	3.7	5.3	83.2	10-18, N (21)
4	59°14'11"	14°52'6"	Örebo	3.5	4.3	87	2-7, N (22)
5	62°19'5"	16°49'45"	Sundsvall	1.4	1	91.4	> 18, O (13)
6	62°13'12"	16°48'38"	Sundsvall	5.8	0.7	91.5	> 18, O (14)
7	66°3'18"	22°5'18"	Luleå	6.8	0.4	76.7	2-8, N (1)
8	66°12'54"	21°53'9"	Luleå	12.8	0	80.2	>18, O (2)
9	66°13'37"	22°1'37"	Luleå	26.1	0	65.9	2-8, N (3)

2.1.3 MeHg bioaccumulation review

Paper IV took “samples” all over the world from publications over the past four decades. The literature selection identified publications listed in Web of Science^{TM} and PubMed (pubmed.gov) that contained MeHg concentrations in water, plankton (seston and/or zooplankton), as well as planktivorous fish from pelagic food webs in lakes, reservoirs, and estuaries, since 1975.

More than 8,000 studies were screened through searching for matches with “mercury/Hg” and “accumulation” in all fields (includes Title/ Keywords/ Abstracts). Eventually, 32 papers from the 1970s to 2015 matched the selection criteria. These papers reported data from 59 aquatic ecosystems at a total of 22 sites (Figure 6). The spatial expanse of the studies ranged from the tropics in the southern hemisphere to the boreal region, with most of them coming from temperate latitudes.

Figure 6. Map of the world marked with red pins showing the aquatic ecosystem sites investigated in paper IV, the literature review of MeHg bioconcentration and bioaccumulation.

2.1.4 Diet sources in experimental lakes

In paper V, seven lakes were selected for examining diet sources and the corresponding Hg levels in primary consumers of zooplankton and macroinvertebrates (Figure 3). Six of the lakes are located close to each other in the Västerbotten region of northern Sweden. One lake is further south, in eastern Sweden within the Stockholm region. The lakes were selected along a gradient of dissolved organic carbon (DOC) concentrations to represent the typical variety of mostly unproductive, oligotrophic lakes in the Swedish boreal landscape (Parkman & Meili, 1993; Meili, 1991) (Table 3). The catchment areas, particularly those situated in the Västerbotten region, consist mainly of coniferous forest (*Picea abies*, *Pinus sylvestris*) and open *Sphagnum* dominated mires.

Whole lake fertilization of nitrogen (N) addition was implemented in Nedre Björntjärn in 2012 and 2013, while forest clear-cut activity took place in the beginning of 2013 in the catchments of Lillsjöliden and Struptjärn. The catchments of lakes Övre Björntjärn and Nedre Björntjärn were clear-cut in the beginning and June of 2014, respectively. Later in 2014, the harvested areas around lakes Lillsjöliden and Struptjärn were subjected to site preparation.

Paper V is part of the ongoing research collaboration, Sino-Swedish Mercury Research Framework (SMaReF), between China and Sweden with sampling lakes within both countries. Besides samples of primary producers and consumers, secondary consumers (fish) were also collected and analysed for Hg and FA.

Table 3. *Physical lake characteristics and summer water column chemistry from the sampling campaign in 2015. DOC: dissolved organic carbon, Chl-a: Chlorophyl a.*

Lake (Abbreviation; Location)	Lake type	Trophic status	Surface area (ha)	Catchment area (ha)	DOC (mg L^{-1})	pH	Chl-*a* (mg L^{-1})
Övre Björntjärn (OBT; 64.1N, 18.8E)	Humic	Oligotrophic	4.8	324.9	27.2	4.89	1.5
Nedre Björntjärn (NBT; 64.1N, 18.8E)	Humic	Oligotrophic	3.2	371	23	4.99	1.5
Mångstenstjärn (MST; 64.3N, 18.8E)	Clear	Oligotrophic	1.8	14.2	10.3	6.51	2.3
Stortjärn (STOT; 64.3N, 19.8E)	Humic	Oligotrophic	3.9	86.6	19.6	5.28	2.5
Lillsjöliden (LSL; 63.8N, 18.6E)	Clear	Oligotrophic	0.8	29.2	13.5	6.24	2.4
Struptjärn (STRP; 64.0N, 19.5E)	*	Oligotrophic	3.1	1183	25.1	5.24	34
Erken (ER; 59.8N, 18.6E)	Clear	Mesotrophic	2400	14100	11.2	7.87	8

*Lake Struptjärn was undergoing an invasive species algal bloom during the sampling campaign in 2015.

2.2 Sampling and analysis

Lake and stream water was sampled to evaluate the elements and compounds related to the Hg biogeochemical cycle (papers I, II, III, and V), while sediments were collected mainly to assess Hg methylation and MeHg demethylation processes. Biological samples of primary producers and consumers (paper V), and fish (paper I) were collected to identify THg (paper I) and MeHg concentrations (paper V), FA compositions (paper V), as well as stable isotope content of ^{13}C and ^{15}N (papers I and V).

2.2.1 Net fishing and fish muscle analysis

The sampling for Paper I was done annually (2010, 2011, and 2013, 2014, 2015) during late summer, August – September (Figure 7). For collecting fish samples, two benthic multi-mesh gillnets (Lundgrens Fiskredskap AB) (Champ *et al.*, 2009; CEN, 2005) were applied to each lake and left standing in the water overnight (≈12 hours). The nets were then taken and rinsed as soon as possible, usually within 6 hours. The captured fish, perch (*Perca fluviatilis* L.), were measured directly after sampling for their weight and total length (from the tip of the snout to the tip of the caudal fin) and archived individually at -20° C.

Figure 7. From left to right: Fish sampling campaign in September 2013 in the clear-cut lakes with preparation of fishing and water sampling equipment, deploying gillnets from the boat, and captured perch in gillnets (Photo: Pianpian Wu, SLU).

Fish muscle samples were obtained later by dissecting the dorsal fin muscle from the thawed frozen whole-fish samples. Small muscle pieces (~1 g wet weight) were sectioned into 2 ml polypropylene plastic vials and stored (-20° C) until they were freeze-dried (≈72 hours). For the determination of fish sample wet content both the wet and dried weights of each muscle sample were measured. Freeze-dried samples were kept frozen at -20° C until THg analysis. One pair of fish gill covers, or operculum, were cut off from both sides of the fish head and stored at -20° C for age identification by reading the "growth rings" (annuli) in wash-dried operculum (Das, 1994; Le Cren, 1947).

Total mercury concentrations in fish muscle samples were analysed with a SMS 100 Total Mercury Analyser (PerkinElmer Inc.) through thermal decomposition (750°C) followed by amalgamation on a gold-trap, thermal desorption, and analysis of Hg vapour by atomic absorption spectroscopy (TDA AAS) according to EPA method 7473 (USEPA, 2007). Absorbance is measured at the 253.7 nm wavelength as a function of THg concentration in the sample. A detection limit as low as 0.005 ng of THg is guaranteed by the manufacturer. The method quantification limit (MQL) for fish muscle samples is around 1 ng g^{-1} dry weight (d.w.). 5 to 10 mg of freeze-dried fish muscle samples were used for THg analysis. Blanks and standards of certified reference materials (CRM) (either DOLT-4 with THg concentration of 2.58 ± 0.22 µg g^{-1} d.w., or DORM-4 with THg concentration of 0.410 ± 0.055 µg g^{-1} d.w.) were run at least once per every 10 samples to assess accuracy of the analysis. The percent recoveries of CRMs were within the range of 90-110%. Precision was assessed by doing duplicates of at least every 10th sample. The coefficient of variation from duplicate analyses was under 3%.

A subset of fish muscle samples was selected for stable isotope analysis to identify the natural abundance of ^{13}C and ^{15}N. The amount of muscle tissue used for stable isotope analysis was approximately 1 mg. The analysis was performed by the Stable Isotope Facility (SIF) at the University of California, Davis, using an elemental analyser interfaced to a continuous flow isotope ratio mass spectrometer. ^{13}C and ^{15}N were analysed in the same sample. Stable isotope values are expressed in δ notation as parts per mille deviation from a standard reference (^{13}C vs. ^{12}C; ^{15}N vs. ^{14}N).

Water samples were taken concurrently with fish sampling events in each lake from lake surface water at the pelagic zone. For each sample the following water chemistry parameters were analysed: total organic carbon (TOC), chlorophyll *a* (Chl *a*), totN, totP, pH, and iron (Fe).

Data on THg concentrations in perch muscles from the EMP lakes captured at similar times of the year during 2010-2015 were retrieved from the Swedish national data host for Hg in fish at that time, the Swedish Environmental Research Institute (IVL, https://dvsb.ivl.se, latest accessed in April 2017).

2.2.2 Water and sediment sampling in beaver ponds

For paper II, water samples were collected from nine river systems distributed in a latitudinal gradient along the distribution range of beavers in Sweden. Specifically, there were two types of water samples collected: in the open water section of the ponds, close to the dam (referred as “Pond”), and in the upstream running waters, at least 100 m before the inlet of the pond (referred as “Upstream”). Triplicate water samples (0.5 m depth) for chemical characterization were collected in acid washed and pre-combusted glass bottles. Dissolved oxygen (DO), conductivity and temperature were measured both at the surface water and the water overlying the sediment of each sampled pond. A water subsample from each site was used to measure pH in the laboratory. Another subsample was used to measure Chl *a* and dissolved organic matter (DOC) after filtering through 0.7 µm pre-combusted Whatman® GF/F filters, while the unfiltered subset was analysed for totP. Water samples for optical dissolved organic matter (DOM) characterization and degradation dynamics were filtered within 24-hour of sampling from the ponds.

Figure 8. Sediment core sampling in a beaver impoundment near Sundsvall (Photo: Pianpian Wu).

Besides water samples, sediment cores were sampled in paper III to investigate sediment DOM characteristics (Figure 8). From each pond, 3 replicates of sediment cores of about 30 cm depth with 30 cm of overlying water were collected using a 6-cm diameter UWITEC gravity corer. Cores were kept upright at 4 °C until further processing within 12 hours of sampling. A subset of freeze-dried sediment samples of 7 to 10 mg was analysed for elemental content of C and N using a COTECH ECS 4010 elemental analyser calibrated with sulphanilamide standard.

In paper III, Surface water samples were only taken from the impoundment area above the dam and analysed for aqueous MeHg, Chl *a*, totP, sulphate

($SO4^{2-}$), and DOC. Analysis of MeHg in water was performed by the Swedish Environmental Research Institute (IVL), which determined MeHg in water by cold vapour atomic fluorescence spectrometry (CVAFS) following US EPA 1630 method (USEPA, 1998), with a method detection limit (MDL) of 0.02 ng L^{-1}, and method quantification limit (MQL) of 0.06 ng L^{-1}.

DOM characterization and degradation dynamics

In papers II and III, absorbance spectra (200 to 800 nm) were measured with a Lambda 40 spectrophotometer (Perkin-Elmer) following the method described by Kothawala *et al.* (2013). Specific UV absorption coefficients at wavelength λ a_λ (m^{-1}) were calculated according to the Beer Lambert law: $a_\lambda = A_\lambda ln10/L$, where A_λ is absorbance and L is the path length (m). The $SUVA_{254}$ was then calculated as the ratio of a_{254} and DOC (mg C L^{-1}). Fluorescence emission-excitation matrices (EEM) were obtained with a fluorescence spectrophotometer using a 1-cm quartz cuvette and Milli-Q water as a blank. The area underneath the water Raman scan was used to normalize sample intensities. Corrections for instrument specific biases, Raman scattering and inner filter effect were applied using the $FDOM_{corr}$ toolbox for MATLAB (Mathworks, Natick, MA, USA). We obtained the main fluorescent peaks associated with DOM (Peaks A, T and C, and the total fluorescence (FT)) together with several optical indices that provide information about the composition and origin of DOM (Biological index (BIX), Humidification index (HIX) and $SUVA_{254}$).

Paper II investigated biodegradable DOM (BDOM) in water samples from beaver ponds, which was determined in dark incubations as described in Guillemette and del Giorgio (2011). Water from the Ponds and Upstream sections from four systems classified as New (i.e. S1, S2, S7 and S9), were filtered through pre-combusted Whatman® GF/C filters and distributed into 40 ml wrapped glass vials. Each vial was filled to the brim, closed to avoid air bubbles and incubated in the dark at 20ºC. For each site, triplicate vials were sacrificed for analyses at time zero and after 1, 2, 4, 7, 14, 21, 28, 35, 42 and 57 days. Incubated samples were filtered through 0.2 µm membrane filters and acidified with 10 µl of 10% v/v HCl. The reported total BDOM was the difference between the final and the initial DOC of the samples.

DOM degradation kinetics in paper II were studied by applying a reactivity continuum model following a similar approach as described in Koehler *et al.* (2012). The relative DOC concentration (DOC_t/DOC_0) over time was modelled, from which the decay coefficients (*k)* over the incubation time can be calculated and fitted to a linear regression.

Mercury methylation assay

For sediment samples in paper III, the upper 2 cm of the sediment cores were sectioned in a glove box under an inert N_2-atmosphere. Sediment slurries were prepared following Bravo *et al.* (2017), which involved a Hg(II) isotope tracer ($^{198}HgCl_2$). The tracer was added to sediment slurries at concentrations similar to ambient Hg(II) values. One replicate was immediately frozen (t_0) and another three replicates (t_f) were incubated for 24 h in the glove box at 18 °C and subsequently frozen. Hg(II) and MeHg were extracted from sediment with focused microwave assisted acid extraction for 4 minutes at 80 W and subsequently analysed by species specific isotope dilution using capillary gas chromatography connected to inductively coupled plasma mass spectrometry (GC-ICPMS) (Rodriguez-Gonzalez *et al.*, 2013). Eventually, Hg(II) methylation rate constants (k_m) were calculated from the initial and final concentrations of the $Me^{198}Hg$ species.

2.2.3 Literature screening

In Paper IV, the MeHg concentrations in water, seston and/or zooplankton, and fish (measured in muscle tissues from low-trophic-level fishes that are mostly planktivorous) were extracted from the selected 32 publications. Seston was defined as the base of the food web, mostly consisting of algae and bacteria. Bioconcentration factors between MeHg concentrations in water and seston were calculated. The MeHg concentrations in zooplankton were used to calculate bioaccumulation factors, since this MeHg represents food uptake from seston as well as that derived from both facilitated transport and passive transfer through cell membranes (Mason *et al.*, 1996). It was not always possible to differentiate between herbivorous and predatory zooplankton, thus it is possible that some zooplankton also ingested other consumers. The MeHg concentrations in planktivorous and small omnivorous fish (i.e. preyfish) were extracted from the articles to define a consistent trophic level for “fish” across the sites. Although we focused on pelagic lower trophic-level fish, benthivorous and occasionally piscivorous feeding could not be entirely ruled out.

Information and measurements on catchment characteristics, including trophic status, acidity, and biome were also extracted from the site descriptions in both selected papers and related publications.

2.2.4 Plankton and macroinvertebrate sampling in experimental lakes

In paper V, water, plankton, and macroinvertebrate samples were taken concurrently in the summer of 2015. Plankton was sampled by vertical hauls from near bottom to the surface of the lake pelagic zone with a limnology water sampler. This water was then filtered sequentially through non-metallic plankton nets of three different mesh sizes (500 µm, 100 µm, and 25 µm) to obtain different size fractions of plankton samples: seston (<25 µm), microplankton (25-100 µm), mesozooplankton (100-500 µm in Sweden) and macrozooplankton (>500 µm). These plankton samples were stored on dry ice within 15 min and then moved to a -80°C freezer before freeze-drying at under -20°C. We took additional plankton samples from both lake epilimnion and hypolimnion and stored them at 4 °C before sending them off for zooplankton and phytoplankton taxonomical analysis.

Similar to paper III, we also took water samples from each lake for aqueous MeHg analysis, which was performed by IVL. The MeHg in water was determined by CVAFS, following US EPA 1630 method (USEPA, 1998), with a detection limit of 0.02 ng L^{-1}. The THg in water was also identified by CVAFS, but following US EPA 1631 method (USEPA, 2002) performed by IVL (a detection limit of 0.05 ng L^{-1}). The pH, totN, totP, TOC, and Chl *a* in water samples were analysed by the Department of Aquatic Sciences and Assessment (Swedish University of Agricultural Sciences, Uppsala). It has previously been shown in majority of Swedish surface waters that DOC and TOC differ on average by < 5% (Köhler, 1999; Ivarsson & Jansson, 1994), so we interpreted TOC as very similar to DOC (Gadmar *et al.*, 2002).

For taxonomical analysis, zooplankton samples (plankton size fraction of 100-500 µm and > 500 µm) were also analysed at the Department of Aquatic Sciences and Assessment (Swedish University of Agricultural Sciences, Uppsala), while the phytoplankton samples (plankton size fraction of 25-100 µm and < 25 µm) were analysed taxonomically at WasserCluster Lunz (Lunz am See, Austria).

Littoral benthic macroinvertebrates were collected with sieving hand nets (0.5 mm mesh size) and kick-sampling trials in shallow stony and/or sandy habitats (0-1 m depth). Macroinvertebrate taxonomy identification was performed by the Department of Aquatic Sciences and Assessment (Swedish University of Agricultural Sciences, Uppsala). Individuals of abundant taxa were sorted and identified to the lowest possible taxonomic unit (i.e. species, genus, or, in some cases, family or order level). These sorted samples were stored at -20°C before freeze-drying. They were then pulverized using an agate mortar and pestle for further analysis of Hg and FA.

The THg and MeHg analysis for plankton and macroinvertebrate samples were performed by Department of Biology, Western University (London, Ontario, Canada). THg were analysed with a DMA-80 Total Mercury Analyser (Milestone Srl) employing US EPA method 7473 (USEPA, 2007), or TDA AAS. The equipment's MDL was documented to as low as 0.1 ng. Blanks and CRMs were run at least once per every 10 samples to assess accuracy of the analysis (CRMs relative percent difference (%RPD) <20%, blanks less than 10% of the lowest sample THg concentration). MeHg concentrations in plankton and macroinvertebrate samples were analysed with a Tekran® 2700 Methyl Mercury Auto-Analysis System (Tekran Instrument Corporation). The Model 2700 is based on atomic fluorescence detection of CVAFS following EPA Method 1630 (USEPA, 1998), with a MDL as low as 0.002 ng L^{-1}. Mean method blanks were 0.045 ng L^{-1} with variation under 0.01 ng L^{-1}. Sample replicates and CRM were run at least once per every 15 samples to assess accuracy of the analysis (%RPD <35%).

Fatty acids analysis in plankton and macroinvertebrate samples

Lipids were extracted and analyzed from freeze-dried, homogenized samples (ca. >1 mg plankton, or 3-10 mg macroinvertebrate dry weight) using chloroform:methanol (2:1 v/v), following Heissenberger *et al.* (2010). First, total lipids (TLP) were quantified as mass fractions (mg lipids g^{-1} d.w.) gravimetrically using duplicate measurements. Known volumes of total lipid extracts were derivatized to fatty acid methyl esters (FAME) using H_2SO_4-methanol (incubated at 50°C for 16 h). FAME were then dried under N_2 before being re-dissolved in hexane and run on a gas chromatograph (Thermo Scientific^{TM} TRACE^{TM} Gas Chromatograph coupled to flame ionization detection) with a Supelco^{TM} SP-2560 column used for separation of FAME. FAME were identified by comparison of their retention times with known standards (37-component FAME mix, Supelco^{TM} 47885-U; bacterial fatty acids, Supelco^{TM} 47080-U; and the following individual FAME standards: stearidonic acid, O5130 SIGMA^{TM}; and n-3 docosapentaenoic acid, Supelco^{TM} 47563-U) and quantified with reference to seven-point calibration curves derived from 2.5, 50, 100, 250, 500, 1000, 2000 ng μL^{-1} solutions of the FAME standard for each identified FA. FAME were expressed as mass fractions (mg FA g $d.w.^{-1}$) and as individual FA relative proportions (% of total identified FA).

2.3 Data analysis and statistics

The effect of forest harvest on fish Hg concentrations were estimated using general linear models (GLM), which took consideration of fish size (weight) and clear-cut period (paper I). In paper II, linear mixed effects models were applied to test for significant differences in the DOM descriptors between Age groups (New vs Old systems), Section (Upstream vs Pond of each system) and the interaction of both factors. To investigate the correlation of pond characteristics with DOC quality and further Hg(II) methylation, principal component analyses (PCA) were applied to the nutrients (Chl *a*, SO_4^{2-}, totP), DOC concentration and NOM optical properties This was followed by non-linear regression analyses between k_m and DOC and MeHg (paper III). In both paper IV and V, partial least square (PLS) regressions were applied to investigate the correlation of MeHg bioconcentration factors with environmental water chemistry, e.g. pH, nutrient conditions, and DOC, as well as biotic factors of FA composition (paper V). THg and MeHg concentrations in biota (paper I, IV, and V) and FA concentrations (paper V) were log-10 transformed to satisfy the requirement of normal data distribution.

In papers I and V, the statistical analyses of the data were performed or processed using the software JMP 10 (© SAS Institute Inc.). Special cases of GLM, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) for THg concentrations, $\delta^{13}C$ and $\delta^{15}N$ were used to test for statistical differences in the fish Hg concentrations and stable isotopes at different time periods before or after clear-cut in 2012. Years 2010-2011 were combined as the reference period, since some of the clear-cut lakes were sampled only once during these two years prior to forest harvest.

Data analyses in paper II were performed in R version 3.1.2 (R Core team, 2014). Significance of the fixed effect, pond colonization age, was evaluated using ANOVA. Non-metric dimensional scaling (NMDS) was used to ordinate the samples according to their DOM properties. A permutational multivariate analysis of variance (PERMANOVA) was performed over the same data set used in the NMDS to analyse the influence of Age and Section on the ensemble of DOM properties, with Site as a grouping factor.

Both PCA (paper III) and PLS (paper IV and V) were conducted using SIMCA (Umetrics, MKS Instruments AB, Umeå). In paper III, the non-linear regression analyses between k_m, DOC and MeHg concentrations were assessed using SigmaPlot 13 (Systat Software Inc.).

Data extraction for paper IV from selected publications was completed by screening data tables, as well as deploying WebPlotDigitizer for data plots (Ankit Rohatgi). We used PLS regression analysis in paper IV to assess the relative importance of different environmental factors in explaining the variation of MeHg transfer in biota. Similarly, PLS regression in paper V was used to assess the relative importance of different environmental factors and FA in explaining the variation of THg concentrations, MeHg concentrations, and the proportion of MeHg in THg (%MeHg) in primary producers and primary consumers.

VIP scores were calculated for each variable based on the PLS loading weights. A variable with a VIP score close to or greater than 1 (one) can be considered important in the model (Eriksson *et al.*, 2006). Thus, we applied the criteria of VIP scores >1 to identify highly influential predictor variables. We used regression coefficients and VIP to evaluate the contribution of each predictor for an individual response. The greater the coefficient size, the stronger the response (Y-variable) is correlated to the predictor (X-variable).

3 Major results and discussion

3.1 Forestry harvest impacts on fish mercury

Since the feeding habits of perch change with age, particularly in the boreal landscape (Rask, 1986), with young perch (age<2) feeding on zooplankton and small macroinvertebrates while bigger perch are mostly piscivorous (Thorpe, 1977), we divided the fish into two size groups, small (length<100 mm) and large (length ≥100 mm).

We noticed a large degree of variation in fish from these lakes over the years. For fish samples collected in 2013, one year after the forest harvest, there was an increase in large fish THg concentrations (length ≥100 mm) in Lake Brobo-Kroktjärn (22%, $p<0.0001$), Kroktjärn (43%, $p=0.02$), and Långtjärn (121%, $p<0.0001$) when compared with fish THg in 2010-2011. In 2014, only Lake Brobo-Kroktjärn fish THg concentrations increased significantly (15%, $p=0.02$) compared to before forest harvest. On the contrary, there was a significant decrease in large fish THg from Lake Gårdsjön (-14%, $p=0.004$). The clearcut reference lake Svultentjärn's large fish [Hg] had a slight increase in 2013(5%, $p=0.05$), and a 49% increase in 2014 compared to 2010-2011 ($p<0.0001$). No fish were successfully sampled from Lake Kroktjärn after 2013.

The degree of fluctuation in perch from lakes subject to forest clear-cut was consistent with a review of studies on how forestry affects Hg concentrations in runoff (Eklöf *et al.*, 2016). That review found that there was a large degree of variation, with a number of "no" or "low" forestry effects being reported alongside reports of larger effects for both MeHg and THg. In our clear-cut lakes, there was a low degree of response in some catchments, and a larger response in some, at some times, most notably in the large perch THg in the first year after the forest harvest. Even the direction of change was not consistent.

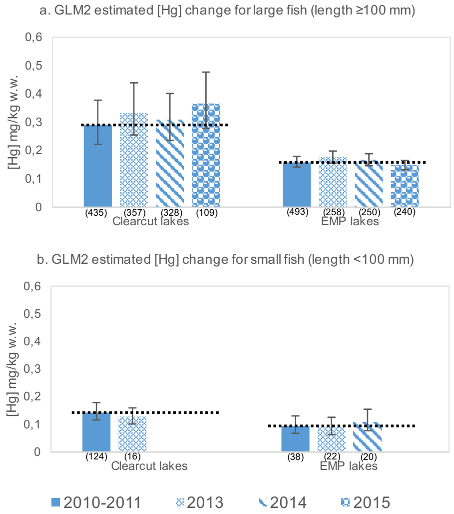

Figure 9. Predictions of fish THg using GLM in clear-cut lakes and EMP lakes in the time before forest harvest (2010-2011, in solid blue pillars) and in each individual year afterwards. Dashed lines indicate fish THg concentrations at the 2010-2011 level in corresponding lake groups. Data on small fish from the clear-cut lakes are only available until 2013, and for the EMP lakes until 2014. Predictions of the temporal changes were done separately for large (length ≥100 mm; upper panel) and small (length <100 mm; lower panel) fish, in the following order from left to right for each lake: Before forest harvest in 2010-2011; One year after forest harvest in 2013; Two years after forest harvest in 2014; Three years after forest harvest in 2015. Numbers in brackets indicate sample numbers. Small fish in EMP lakes in 2010-2011, 2013, and 2014 come exclusively from two of the twenty-four lakes: Övre Skärsjön and Stensjön.

The change in fish THg was more obvious when the GLM analysis was conducted for the five clear-cut lakes and EMP lakes (Figure 9). In 2013, one year after the forest harvest, both clear-cut lakes and unharvested EMP lakes had significant THg increases in the large fish (length ≥100 mm) THg (Clear-cut lakes: 16%, $p<0.0001$; EMP lakes: 10%, $p=0.002$). In 2014, two years after the 2010-2011 period, there was 7% higher THg ($p=0.1$) in large fish THg from the clear-cut lakes, while there was no significant change for the EMP lakes (4%, $p>0.35$). There was a significant increase (17%, $p=0.052$) in the 2014 small fish THg of the EMP lakes as compared to 2010-2011. The 2015 large fish THg were 26% ($p<0.0001$) higher in the clear-cut lakes compared to the pre-harvest period. On the contrary, the concentrations in the EMP lakes were 7% ($p=0.03$) lower in 2015 compared to 2010-2011 in the EMP lakes. In the small fish (length <100 mm) THg there was no significant change in either the clear-cut or the EMP lakes.

Our study suggests that the area of the catchment subject to forestry operations may influence the degree of response in the accumulation of Hg in fish. According to the GLM analysis, the two largest increases in large perch THg one year after the forest harvest occurred in Lake Långtjärn (121%) and Kroktjärn (43%), which were also the lakes with the largest proportion of harvested area in the lake catchments (40% and 36%, respectively). However, while the THg of large fish in Lake Långtjärn during the subsequent two years (2014 and 2015) remained larger than before harvest, the relative increase was much smaller. The variation seen in the response in fish THg over time in Lake Långtjärn strengthens the view that effects from forest clear-cut needs to be followed over long periods.

The significant increase in fish THg (mainly large fish) in the clear-cut lakes for several years after the forest harvest indicated by the GLM analysis, contrasted with the general trend in the EMP lakes (Figure 9). Three years after forest harvest in 2012, the fish THg in the clear-cut lakes had increased by 26% for large fishes, compared to the period before forest harvest. Though the large fish THg increased in EMP lakes during the first year after 2012, the fish THg returned to the earlier level a year later, i.e. in 2014 when the THg of large fish in the EMP lakes was not significantly different from the 2010-2011 level.

No increase in the [Hg] of small fish after forest harvest was observed in this study, possibly link to biodilution after forest harvest. Increased total phosphorus and DOC (Winkler *et al.*, 2009; Carignan *et al.*, 2000), as well as higher zooplankton abundance (Leclerc *et al.*, 2011) have been observed in forestry impacted lakes. Leclerc *et al.* (2011) demonstrated that higher prey availability of zooplankton abundance elevated growth rate of age-0 yellow perch in Canadian Shield Lakes two years after forest harvest. We therefore suspect that the absence of a significant increase in smaller fish [Hg] shortly after forest harvest might be linked to increased feeding success and fish growth rate, a scenario similar to biodilution (Rypel, 2010).

It is likely that the changes in fish [Hg] in clearcut lakes could not be attributed entirely to an effect from forest harvest on the concentration and loading of Hg in runoff from the catchment. While clearcuts and/or subsequent forest harvest activities (e.g. site preparation) often do exacerbate Hg concentrations and loadings in runoff from the terrestrial to the aquatic environment, the degree of change in Hg loadings and aqueous concentration varies considerably, and is sometimes not present at all (Eklöf *et al.*, 2016). Even when there is increased loading of Hg to the lake following forestry practices, the biomagnification of Hg in biota are determined by numerous other factors, including indirect effects of the forest harvest on feeding behavior and food web structure, but also weather induced changes on both water quality and food webs (Rask *et al.*, 2007; Rask *et al.*, 1998).

3.2 Beaver pond colonization history influence on dissolved organic matter quality and mercury methylation

3.2.1 Remobilization of fresher DOM in pioneer beaver ponds

Changes in DOM spectroscopic properties and biodegradability in beaver ponds are complex and depend both on the ageing and specific features of the beaver systems. In order to determine the changes in DOM linked to this process, we compared the differences of DOM descriptors between the Upstream and the Pond sections of beaver systems. We predicted higher differences of DOM variables between Upstream and Pond for the youngest beaver dams, as mobilization of biologically available DOM was expected to be substantial only for a limited time after damming. Accordingly, differences in the DOM descriptors between Upstream and Pond were only found for New sites.

While we did not observe significant differences in concentration, the changes observed in spectroscopic DOM descriptors provided information on the processes affecting the DOM. Upstream DOM had a higher humification index compared to samples from the Ponds. In contrast, we observed a significant increase between Upstream and Pond in fluorescence peak B, related to the presence of protein-like materials. Protein-like fluorescence is linked to unprocessed materials (Lapierre & del Giorgio, 2014), enhanced microbial activity or autochthonous production (Fellman *et al.*, 2010). In the studied sites, this is probably derived from an increased autotrophic planktonic community (i.e. significantly higher Chl *a* levels) and longer water residence time in the Pond compared to the Upstream. In contrast with this, Pond samples had significantly higher values of SUVA_{254} an indicator of aromaticity (Weishaar *et al.*, 2003). The EEMs of the Old sites showed more prominent protein-like fluorescence than the EEMs of the new ponds and significantly higher values of the A:C ratio. This increase could be related to the release of terrigenous DOM from recently inundated soils (Mladenov *et al.*, 2005) and the decomposition of wood in the bottom of the ponds.

Significantly higher BDOM was found in Ponds compared to Upstream samples, suggesting that beaver ponds are important sites for carbon processing. In the case of Pond sites, the broader spectrum of DOM reactivity types signals a variable origin of the material. Considering that beaver ponds receive primarily the terrestrial DOM from the upstream sections, the recently inundated soils constitute a second temporary source of organic matter (OM) that can shift both the fluorescence and absorbance patterns of the OM (Mladenov *et al.*, 2005). A third source of DOM derives from autochthonous production and secondary organic matter processing (Weissenberger *et al.*, 2012). In support of this, water from beaver dams had significantly higher representation of the protein-like peaks and a decrease in longer-wavelength fluorescent DOM.

Though there was a great variability in DOM concentration found in beaver ponds and upstream, the spectroscopic DOM descriptors (i.e. $SUVA_{254}$, HIX, BIX) clearly separated the samples according to age group, showing that DOM quality can be indicative of an old pond. New ponds had higher fluorescence peaks (R.U.) and total fluorescence than Old ponds. Comparing our observations to earlier work, DOM in the New ponds seems to be less processed (Catalán *et al.*, 2013; Kothawala *et al.*, 2012) and more humic in nature than DOM in the Old ponds.

To summarize, the damming (i.e. Upstream vs Pond) and ageing (i.e. New vs Old systems) processes have analogous effects on DOM quality. Hence, when the beaver pond age reaches the last stages of the succession towards a lentic system, initial differences in DOM quality between Upstream and Pond sites dissipate. Beaver impoundments within the first few decades of existence provide a boost of terrigenous organic matter from the inundated surrounding soils and a more extended DOM input from enhanced autochthonous production.

3.2.2 Increased inputs of fresh humic and algal derived NOM control Hg methylation in new ponds

The role of molecular composition of NOM on Hg(II) methylation processes in boreal lake sediments has been recently highlighted (Bravo *et al.*, 2017). In this study, we employed a set of optical spectroscopy parameters widely applied to assess the source (produced within the aquatic system versus imported from the terrestrial environment) and degradation status (e.g. due to photochemical and/or biological processing) of dissolved NOM (Fellman *et al.*, 2010) to reveal whether increased inputs of fresh humic and algal derived NOM influence the Hg(II) methylation rate.

The NOM composition in recently established ponds was enriched in algal-derived NOM (*e.g.* shown as high Chl *a*) and was more humic and less processed (denoted from now onwards “fresh”) compared to older ponds. Our PCA statistics indicated the presence of nutrients (i.e. totP and SO_4^{2-}) and internally produced algal-derived NOM (i.e. Chl *a*) were associated with the presence of terrigenous humic acids (shown as FT) (Jaffé *et al.*, 2008) and aromatic compounds (shown as $SUVA_{254}$) (Weishaar *et al.*, 2003). These groups of compounds were particularly abundant in two of our New ponds featuring with high k_m. It is likely that the initial flooding of soils entails a resuspension and transport of soil humic NOM and nutrients into the water as previously reported in other studies (Roy *et al.*, 2009; Hall & St. Louis, 2004). Besides, we found that highly processed humics dominated the NOM composition in our Old ponds, which were characterized by low k_{m}. Overall, our New ponds' sediment samples were characterized by higher algal-derived NOM, fresh humic NOM, or degraded NOM. Algal exudates might also have raised the concentration of low molecular mass thiols and augment Hg(II) availability for Hg(II) methylating bacteria as previously suggested (Leclerc *et al.*, 2015). And it is likely that, enriched phytoplankton-derived NOM in New ponds facilitated Hg(II) methylation by enhanced microbial activities. This is similar to what has been reported for boreal lake sediments (Bravo *et al.*, 2017).

Therefore, we summarize that the reason pioneer beaver ponds are associated with higher MeHg in water is that the changes in organic matter quality increase the methylation rate, and thus the net methylation. Whether this will lead to increased bioaccumulation of Hg in the biota in and around pioneer beaver ponds remains to be addressed. Analysis of additional data on the Hg in macroinvertebrates from these ponds has so far not revealed a connection to either the increased MeHg concentrations in water observed by (Levanoni *et al.*, 2015), or the increased k_{m} observed in the laboratory (Wu *in prep*).

3.3 Methylmercury bioconcentration factors in planktonic food webs predict fish mercury bioaccumulation

We did not find a significant correlation between aqueous MeHg concentrations and MeHg concentrations in preyfish (small planktivorous/omnivorous fish) ($R^2<0.01$, $p>0.9$). Additionally, aqueous MeHg concentrations could neither predict seston MeHg concentrations ($R^2=0.01$, $p>0.5$) nor zooplankton MeHg concentrations. ($R^2<0.01$, $p>0.5$). On the contrary, MeHg bioconcentration factors from water to seston ($BCF_{w\text{-}s}$) significantly predicted fish MeHg concentrations ($R^2=0.27$, $p=0.002$).

A comparison of $BCF_{w\text{-}s}$ with the MeHg bioaccumulation factor from zooplankton to fish ($BAF_{z\text{-}f}$), showed that the former is the major control on the MeHg concentrations in preyfish (Figure 10). This is in accordance with what Mackay *et al.* (2013) predicted from the algebraic relationships between BCF, BAF, and trophic magnification factor (TMF) for chemical bioaccumulation in fish: BCF (without dietary input of a chemical) can be regarded as the fundamental determinant of bioaccumulation and biomagnification in aquatic food webs. Furthermore, MeHg concentrations in fish correlated significantly, and could be explained by $BCF_{w\text{-}s}$, while $BAF_{z\text{-}f}$ did not show any predictive power. Further bioaccumulation along trophic levels will increase MeHg concentrations, but more steadily and predictably as shown by Lavoie *et al.*

(2013). Wyn *et al.* (2009) have also stressed the prominence of water-seston MeHg uptake in food webs over trophic factors in low-pH lakes of Nova Scotia, Canada.

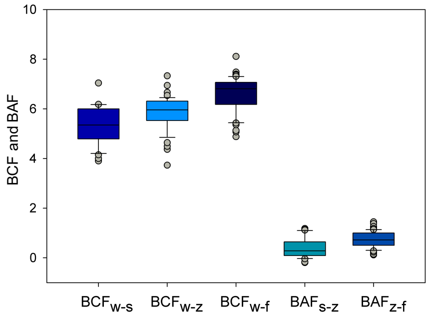

Figure 10. Boxplot of bioconcentration factors (BCF) and bioaccumulation factors (BAF) (mean ± standard deviation) representing, from left to right, bioconcentration factors of MeHg transfer from water to seston (BCF_{w-s}, 5.32±0.73), from water to zooplankton (BCF_{w-z}, 5.82±0.69), and water to preyfish (BCF_{w-f}, 6.60±0.69), then MeHg bioaccumulation factors within the food web from seston to zooplankton (BAF_{s-z}, 0.38±0.38), and finally the MeHg trophic transfer from zooplankton to preyfish (BAF_{z-f}, 0.75±0.31).

The importance of BCF_{w-s} relative to the bioaccumulation from zooplankton into preyfish (BAF_{z-f}) leads us to conceptualize the understanding of MeHg biomagnification in aquatic ecosystems (the total increase in MeHg from water to subsequent trophic levels) as having two distinct steps. The first step is transferring MeHg from water into the base of the pelagic food web, represented by the BCF_{w-s}. The next step is MeHg bioaccumulation from one trophic level to the next (BAF_{s-z}, then BAF_{z-f}), and onwards along the trophic levels in the food web (i.e. MeHg biomagnification). We conceptualized this trophic transfer as an inclined "staircase" of MeHg bioaccumulation (Figure 11). On the other hand, the movement from water into the base of the pelagic food web (BCF_{w-s}) may be described as a vertical "elevator", being an indicator of that first decisive step MeHg takes from water into the base of the food web. This "elevator" (BCF_{w-s}) takes the aqueous MeHg and increases it by anywhere from three to six orders of magnitude, which is decisive for MeHg concentrations at subsequent trophic levels.

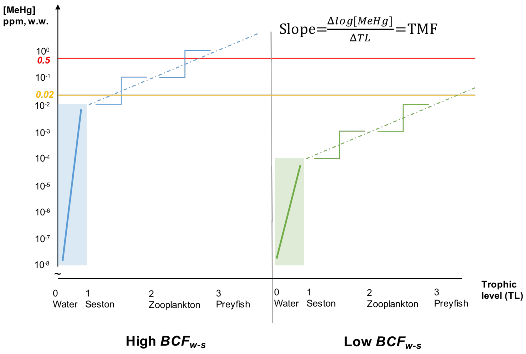

Figure 11. Conceptualization of the "MeHg elevator" from water to the base of the pelagic food web (the $\mathrm{BCF}_{\text{w-s}}$), and the subsequent "MeHg staircase" of increase along the trophic levels in a pelagic food web. The X-axis is the conceptualized trophic level. Y-axis is MeHg concentration (ppm, equivalent to µg MeHg ml^{-1} in aqueous phase, µg MeHg g^{-1} in solid phase). Blue solid lines indicate changes of MeHg concentrations in aquatic ecosystems with high $\mathrm{BCF}_{\text{w-s}}$, while green solid lines indicate the changes in low $\mathrm{BCF}_{\text{w-s}}$ systems. Shaded blue and green areas highlight the contrasts for aquatic food webs with different $\mathrm{BCF}_{\text{w-s}}$. The slopes of the dashed lines represent the TMF, similar to BAF. The horizontal orange solid line is EU's EQS of 0.02 µg g^{-1} w.w. for freshwater biota (EC, 2008). The horizontal red solid line is the more often adopted safe consumption WHO EQS for fish Hg concentrations (assuming 100% MeHg) of 0.5 µg MeHg g^{-1} w.w. (USEPA, 2012; IPCS, 1990).

Our conceptualization indicates that the magnitude of the $\mathrm{BCF}_{\text{w-s}}$, rather than the aqueous MeHg concentrations, is the key determinant of MeHg concentrations in planktivorous fish. Then the number of stairs in the trophic transfer between the start and end of the bioaccumulation "staircases" further determines the concentrations at the top of the aquatic food web.

The understanding of the interaction between environmental factors and functional organism groups at the base of the food web is crucial to improving the understanding and predictability of overall Hg biomagnification. Nutrient-poor aquatic ecosystems, many also low in pH, such as the those in remote areas of North America and northern Europe and Asia, exhibited both the highest $\mathrm{BCF}_{\text{w-s}}$, and the highest fish MeHg concentrations. Increases in nutrients will result in higher primary production, thus the aqueous MeHg present is taken up by higher phytoplankton biomass (i.e., MeHg biodilution; Pickhardt *et al.* (2002)). This lowers the $\mathrm{BCF}_{\text{w-s}}$, leading to lower MeHg in consumers at higher trophic levels. Thus, the trophic status of aquatic ecosystems can suppress or enhance growth of primary producers and consumers, and therefore trophic MeHg transfer within the planktonic food web. However, the effect of DOC on MeHg bioconcentration is complex. Despite a well-known chemical coupling between DOC and Hg in freshwaters (Ravichandran, 2004), DOC can also interact with biota to decrease MeHg bioconcentration through passive uptake in zooplankton because of Hg binding to aquatic humic substances (Gorski *et al.*, 2006). Such influences may contribute to why we found that DOC was more important than pH and biome regions for BCF, especially $BCF_{w\text{-}s}$ (DOC vs. $BCF_{w\text{-}s}$: $R^2=0.45$, $p< 0.001$; pH vs. $BCFw\text{-}s$: $p>0.05$).

3.4 Specific fatty acids matter for mercury bioaccumulation in plankton and macroinvertebrates

We utilized partial least square (PLS) regression analysis to understand the correlation of Hg bioaccumulation with both biotic factors (FA groups) and abiotic factors (water chemistry parameters). Different PLS models were tested with for plankton (PLSpk), macroinvertebrates (PLSma), as well as a focus on MeHg bioconcentration factors (BCF) in primary consumers (PLSbcf).

In the PLSpk model for plankton of all size fractions, pH, totP, and $\delta^{13}C$ predicted plankton THg and MeHg. Higher %MeHg was predicted by Chl *a* was higher, but %MeHg correlated negatively with EPA and terrestrial FA. We had expected to see increasing Hg or FA contents with increasing plankton size-fraction classes, as described in Kainz *et al.* (2006). However, different size-fractions of plankton did not seem to differ in either Hg contents or FA groups, but we found higher Hg contents in plankton from oligotrophic lakes than the more eutrophic one (Erken is the only mesotrophic lake). This indicated boreal freshwater plankton accumulate Hg and FA regardless of size, and tend to accumulate more Hg in oligotrophic waters.

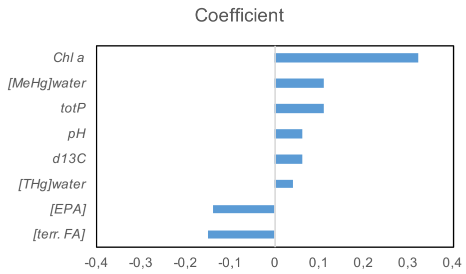

Figure 12. Highly influential predictor variables (VIP scores >1.0) from our PLS analysis of PLSpk model to predict Hg contents (THg and MeHg concentrations, %MeHg) in plankton, explained with regression model coefficients (coefficient values: >0 for positive correlation; <0 for negative correlation): Chl *a* =Chlorophyll *a* in mg m^{-3}; d13C=$\delta^{13}C$; totP=total phosphorous in µg L^{-1}; [EPA]=log-transformed concentration of eicosapentaenoic acid; $[MeHg]_{water}$=log-transformed aqueous MeHg concentration; $[THg]_{water}$=log-transformed aqueous total Hg concentration; [terr. FA]=log-transformed concentration of terrestrial fatty acids.

It was clear that water chemistry parameters (aqueous Hg, TOC, Chl *a*, and totP) were important for Hg contents in primary consumers. FA groups, particularly terrestrial FA and EPA correlate to differences in zooplankton MeHg between lakes. This suggested that increasing EPA (a somatic growth enhancing, algae-derived FA) and terrestrial FA are associated with decreasing Hg content in plankton, while Chl *a* is associated with increasing Hg content. It is likely that increasing EPA stimulated plankton growth and facilitated biodilution of Hg concentrations (Goedkoop *et al.*, 2007; Campbell *et al.*, 2005). Decreased Hg content under increased retention of terrestrial FA suggests that Hg is diluted in ingested terrigenous organic matter, or due to decreased Hg bioavailability to plankton in humic waters (French *et al.*, 2014).

The PLSma model for macroinvertebrates predicted that Chl *a*, TOC, and aqueous Hg concentrations positively correlated with Hg contents (THg and MeHg concentration, as well as %MeHg) (Figure 13). The totP, δ^{13}C, and pH were negatively correlated with THg and MeHg concentrations. The TLP (total lipids) and bacterial FA concentrations were negatively related to %MeHg.

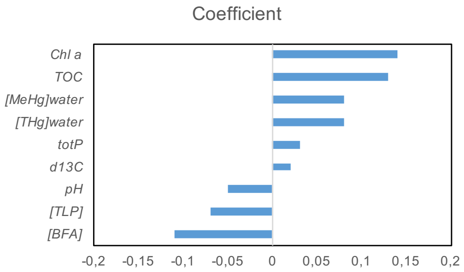

Figure 13. Highly influential predictor variables (VIP scores >1.0) from our PLS analysis of PLSma model to predict Hg contents (THg and MeHg concentrations, %MeHg) in macroinvertebrates, explained with regression model coefficients (coefficient values: >0 for positive correlation; <0 for negative correlation): Chl *a* =Chlorophyll *a* in mg m^{-3}; d13C=δ^{13}C; totP=total phosphorous in µg L^{-1}; TOC=total organic carbon in mg L^{-1}; [TLP]=log-transformed concentration of total lipids (mg lipids g^{-1} sample); [BFA]=log-transformed concentration of bacterial fatty acids; [MeHg]$_{water}$=log-transformed aqueous MeHg concentration; [THg]$_{water}$=log-transformed aqueous total Hg concentration.

For macroinvertebrates, water chemistry, remained a better predictor of Hg bioaccumulation compared to FA. Increasing total lipids and bacterial FAs were associated with decreasing Hg, suggesting a Hg biodilution in macroinvertebrates due to ingestion of nutrient-rich bacterial-derived diet, similar to what has been described in de Wit *et al.* (2012) about macroinvertebrates sampled in Norwegian streams during the summer. On the other hand, DOC likely lowered pH and contributed to Hg bioavailability that resulted in increased uptake of Hg contents by aquatic consumers (Kelly *et al.*, 2003). In high DOC waters, MeHg is up to 6-fold more efficiently adsorbed and transfused across cell membranes than inorganic Hg (Pickhardt & Fisher, 2007). Thus, if MeHg bioavailability to macroinvertebrates was promoted by high DOC, this can significantly contribute to elevated Hg contents in aquatic consumers.

The predictive power of abiotic and biotic factors on the MeHg bioconcentration factor (BCF_{MeHg}) was tested using PLS in primary consumers of both plankton and macroinvertebrates (Figure 14). BCF_{MeHg} evaluates the uptake of MeHg by primary consumers and has been applied earlier by aquatic scientists with respect to Hg in aquatic food webs (Rolfhus *et al.*, 2011). Unlike the previous PLS models predicting Hg contents, most of the water chemistry parameters, except Chl *a*, failed to predict BCF_{MeHg} as highly influential variables. Instead, it was one of the PUFA, LIN contents (concentrations and % of LIN) that correlated positively with BCF_{MeHg}.

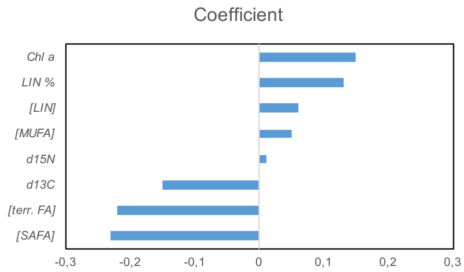

Figure 14. Highly influential predictor variables (VIP scores >1.0) from our PLS analysis of PLSbcf model to predict log-transformed MeHg bioconcentration factor ([BCF_{MeHg}]) in primary consumers of plankton and macroinvertebrates, explained with regression model coefficients (coefficient values: >0 for positive correlation; <0 for negative correlation): Chl *a* =Chlorophyll *a* in mg m^{-3}; d13C=$\delta^{13}C$; d15N=$\delta^{15}N$; [SAFA]=log-transformed concentration of saturated fatty acids; [MUFA]=log-transformed concentration of monounsaturated fatty acids; [LIN]=log-transformed concentration of linoleic acid; %LIN=proportion of linoleic acids among all FAME compounds; [terr. FA]=log-transformed concentration of terrestrial fatty acids.

The transfer of MeHg into aquatic consumers relied more on the physiological characteristics of FA composition than water chemistry, according to our PLSbcf model. This is in accordance with previous study of MeHg and FA correlation in macroinvertebrates from two different Norwegian streams in two seasons (de Wit *et al.*, 2012).

The MeHg bioconcentration factor in primary consumers was influenced more by the FA composition rather than by water chemistry. Both Chl *a* and LIN influenced the MeHg partition in zooplankton and macroinvertebrates. As stated in Napolitano (1999), LIN is an essential PUFA of algal origin. This suggested that zooplankton and macroinvertebrates in boreal freshwaters ingested algae as an important food source, making it a prominent Hg exposure route. Ingesting LIN as an essential substrate is of great importance to primary consumers as this 18-carbon PUFA compound from the base of the food web is a precursor of other physiologically required PUFA, providing correlative evidence that algal diet provide Hg to primary consumers.

For MeHg retention in primary consumers, terrestrial FA and SAFA were less favoured as compared to the uptake of MUFA and PUFA in zooplankton and macroinvertebrates, regardless of total lipids availability. Yet terrestrial FA can contribute to total Hg bioaccumulation in plankton (linear correlation between terrestrial FA and total Hg in phytoplankton: $R^2=0.55$, $p=0.004$; in zooplankton: $R^2=0.42$, $p=0.004$). This suggests that allochthonous organic matter sources, although not supporting zooplankton growth (Brett *et al.*, 2009), were associated with Hg bioaccumulation in boreal freshwater lakes, especially those with poor nutrient conditions.

4 Concluding remarks and future perspectives

4.1 Summary of contributions

Based on extensive field investigation results, this thesis provided new insights into Hg bioavailability and bioaccumulation in boreal freshwaters from clear-cut lakes to beaver pond river systems. This was complemented by a meta-analysis of MeHg bioconcentration and bioaccumulation data from around the world. The majority of the studies considered the influence of environmental factors, which allowed us to make statistical analyses of the influences on Hg bioaccumulation, with a focus on MeHg retention at the base of the food web.

The thesis contributed to the empirical knowledge on Hg cycling from terrestrial landscapes into biota within the food web as a result of forest harvest activities (paper I). That work emphasized the need for long term, comprehensive ecosystem studies to define the eventual effects of land use changes on Hg bioaccumulation in biota. We also showed how beaver ponds modified the lotic system in a way that led to changes in DOM sources and processing (paper II). This laid the groundwork for further research on Hg bioaccumulation in boreal beaver pond systems where the effects of new impoundments on DOM and Hg methylation appear to be transient (paper III).

Our literature review complemented the important synthesis work on Hg TMF by Lavoie *et al.* (2013) with a novel synthesis of ecosystem observations on Hg transfer from water into the base of the food web and onwards (paper IV). Our meta-analysis succeeded in showing the importance of mercury accumulation in the base of the food web for the overall bioaccumulation further up in the ecosystem with field observations from many different regions of the world. This supports what others had surmised from laboratory studies, more localized field studies, and theoretical considerations.

To go beyond identifying the base of the food web as having a key role in Hg bioaccumulation to actually unravelling the interrelated processes will require focussed efforts, aided by appropriate techniques. We took a step in this direction by operationally defining components at the base of the food web using plankton size, and then measuring FA groups to reveal the dietary sources and Hg in those different parts of the food web base (paper V). From this we considered whether dietary biotic markers (represented by FA groups), or abiotic factors (e.g. water chemistry), matter the most for MeHg bioconcentration and bioaccumulation at the base of the food web in boreal freshwaters.

4.2 Future work

The thesis highlighted the importance of the base of the food web for MeHg uptake from water into the biota. We have done this through both original field work in boreal freshwaters and synthesis of field data from other aquatic ecosystems in the world. Much more remains to be done in the directions explored by this thesis. Several paths forward seem particularly intriguing for exploration. The Sino-Swedish Mercury Research Framework (SMaReF) provides opportunities for comparative ecosystem scale investigations. The strong contrasts between the dominant surface water ecosystems of these two countries can be analysed with the help of sophisticated techniques, including fatty acid analyses. This could help resolve the long-standing conundrum of why aquatic biota in China (and some other parts of the world such as Eastern Africa) have relatively low MeHg concentrations in the biota, despite relatively high aqueous MeHg concentrations, compared to the situation in many high-latitude aquatic ecosystems where biotic MeHg concentrations are higher despite much lower MeHg concentrations in water. This could hopefully lead to a better understanding of ecological and biogeochemical processes relevant to MeHg bioaccumulation, as well as provide clues to management strategies.

Carefully planned mesocosm studies are another potential pathway for elucidating the complexities at the food web base of many natural aquatic ecosystems. With manipulated mesocosm systems, tracers of mercury and diet can be applied with greater precision in different food web structures than is possible in the natural environment. But the challenge of simulating relevant ecological processes and structures in the laboratory are considerable. The rewards for success however, are more than commensurate, given the threat that Hg continues to pose to the health of people and wildlife.

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Popular science summary

The ubiquitous spread of mercury (Hg) in the environment by pollution has long been considered as a major threat to the health of people and wildlife. The risks are mainly due to potent neurotoxic effects of the very bioavailable organic form of Hg, methylmercury (MeHg). This has been of particular concern in Sweden due to persistently high Hg concentrations in freshwater fish, even though regional mercury emissions to the environment have decreased markedly in recent decades. Nowadays, forest harvest activities and wetlands remain hotspots of MeHg export to boreal freshwaters. The effects of forest harvest, however, appear more variable than previously recognized. Furthermore, after half a century of concerted study, it remains something of a puzzle that MeHg in water contrasts so greatly with MeHg in fish, from almost undetectable in water with current analytical methods to routinely exceeding EU's guideline level (>0.02 µg g^{-1} wet weight) by an order of magnitude in Swedish freshwaters. The aim of this study was to better understand how environmental change impacts MeHg bioavailability and bioaccumulation with a focus on the base of the food web in Swedish freshwaters.

The study started with an investigation into the impacts of forest harvest on Hg bioaccumulation in fish. Six small boreal lakes were sampled annually, of which five were visited both before and after forest clear-cuts. From analysis of more than a thousand fish samples, it was clear that there was a large variation of fish Hg concentrations over time and between the study lakes. This variability emphasizes the need for long-term studies to assess the influences on Hg concentrations in biota.

Boreal beaver ponds were also studied to understand the processes influencing MeHg concentration increases in water after flooding induced by the establishment of beaver dams. Two age classes of beaver ponds were identified based on the years since the initial impoundment - old ponds and "pioneer" ponds. The damming process, which transforms a stream into a lentic system, shifts the sources and quality of dissolved organic matter. Mercury methylation experiments using sediments obtained from beaver ponds suggested that the increase in pond water MeHg concentration was mainly stimulated by increased MeHg production in the sediment shortly after flooding, and that this stimulation was related to the quality of the organic carbon.

To better understand the large discrepancies between aqueous MeHg exposure and the ultimate degree of MeHg bioaccumulation in different aquatic ecosystems, a literature review was conducted on MeHg bioaccumulation from water into the base of the food web. The bioconcentration factor for MeHg, i.e. the ratio of MeHg in water and seston or zooplankton, was found to be critical for subsequent MeHg bioaccumulation. Several environmental parameters were closely related to MeHg bioconcentration factors, including dissolved organic carbon, pH, and nutrient status of the aquatic ecosystems.

To better identify linkages between MeHg uptake and trophic structures at the base of the food web, fatty acids were used to distinguish specific diet sources in aquatic primary consumers of plankton and macroinvertebrates. By correlating Hg bioaccumulation with specific fatty acids, it was confirmed that Hg content increased simultaneously with polyunsaturated fatty acids in primary consumers. The MeHg bioconcentration factors in primary consumers were less correlated with water chemistry parameters, as compared to fatty acids.

These findings contribute to a better understanding of the role of the base of aquatic food webs in transferring MeHg along trophic cascades, as well as landscape influences on elevated MeHg bioaccumulation. Further advances in understanding the structure and function at the base of aquatic food webs with respect to mercury cycling, including the bioconcentration processes for different species of Hg, are needed to better predict how changes in the landscape, climate and water chemistry will alter the bioaccumulation of Hg in fish.

Populärvetenskaplig sammanfattning

Kvicksilver (Hg) är ett allmänt förekommande grundämne i naturen och exponering av dess organiska form metylkvicksilver (MeHg) kan ge upphov till skador på människors och djurs hälsa. Riskerna är beroende av MeHgs neurotoxiska egenskaper. I Sverige är koncentrationerna av Hg i sötvattenfisk fortfarande höga, även om utsläppen av Hg har minskat. Områden med skogsbruk och våtmarker bidrar till betydande transporter av MeHg till boreala sötvattenekosystem. Effekter från skogsbruk på Hg-transporter eller förändringar i förmågan att bioackumulera MeHg varierar. Dessutom kontraherar koncentrationer av MeHg i vatten kraftigt med MeHg-koncentrationen i fisk, från mycket låga halter i vatten till halter i fisk som överstiger FAO/WHOs riktlinjer för att skydda mänsklig hälsa (0,5 $\mu g\ g^{-1}$ våtvikt) och EU:s riktlinje som är satt för att skydda vilda djur (0,02 $\mu g\ g^{-1}$ våtvikt). Syftet med detta arbete var att bättre förstå hur miljöförändringar påverkar MeHg biotillgänglighet och bioackumulering med fokus på basen av näringsväven i svenska sötvatten.

Studien inleddes med en undersökning av effekterna från skogsbruk på förändringar av Hg-koncentrationer i fisk. Sex små boreala sjöar provfiskades varje år, varav fem besöktes både före och efter skogsavverkningar. Mer än tusen fiskprover analyserades och visade på en stor variation av Hg-koncentrationen i fisk över tid men också mellan sjöar. Studien betonar behovet av långsiktiga studier för att bedöma effekterna på variationen i Hg-koncentrationen i fisk över tid.

Bäverdammar undersöktes också för att förstå de processer som påverkar ökningen av MeHg-koncentrationer i vatten efter översvämning från bäverdammar. Bäverdammar skapar dämmen som omvandlar bäckar till ett lentiskt ekosystem, och både källor och nedbrytningen av löst organiska material förändras men också metyleringen av Hg. Metylering av oorganisk Hg i sediment från bäverdammar indikerade att ökningen i MeHg-koncentrationer i vatten stimulerades genom ökad MeHg-produktion i sedimentet kort efter översvämningen och att denna stimulering var relaterad till kvaliteten på det organiska kolet.

För att bättre förstå skillnaderna mellan halterna av MeHg i vatten och bioackumulering av MeHg i olika akvatiska ekosystem, genomfördes en litteraturöversyn av bioackumulering av MeHg från vatten till basen av näringsväven. Biokoncentrationsfaktorn för MeHg, det vill säga förhållandet MeHg i vatten och fytoplankton eller djurplankton, visade sig vara avgörande för bioackumuleringen av MeHg. Variationen i biokoncentrationsfaktorer kunde förklaras med löst organiskt kol, pH och näringsstatus för de akvatiska ekosystemen.

Genom att särskilja specifika födokällor hos vattenlevande primära konsumenter (djurplankton och bentiska makroinvertebrater) med hjälp av fettsyror kunde vi studera upptag av MeHg vid basen av näringsväven. Upptaget av Hg ökade med en ökad tillgång av fleromättade fettsyror hos primära konsumenter. I synnerhet var biokoncentrationsfaktorer för MeHg i primära konsumenter bättre korrelerade med innehållet av fettsyror än med vattenkemiska faktorer.

Dessa resultat bidrar till att bättre förstå hur basen i den akvatiska näringsväven stimulerar ackumuleringen av MeHg mellan trofiska nivåer, samt hur markanvändning påverkar bioackumulering av MeHg i fisk. Framsteg i förståelse av hur strukturen och funktionen vid basen av akvatiska näringsvävar påverkar omsättningen av Hg behövas för att bättre förutsäga hur förändringar i landskap, klimat och vattenkemi förändrar Hg-koncentrationen i fisk.

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