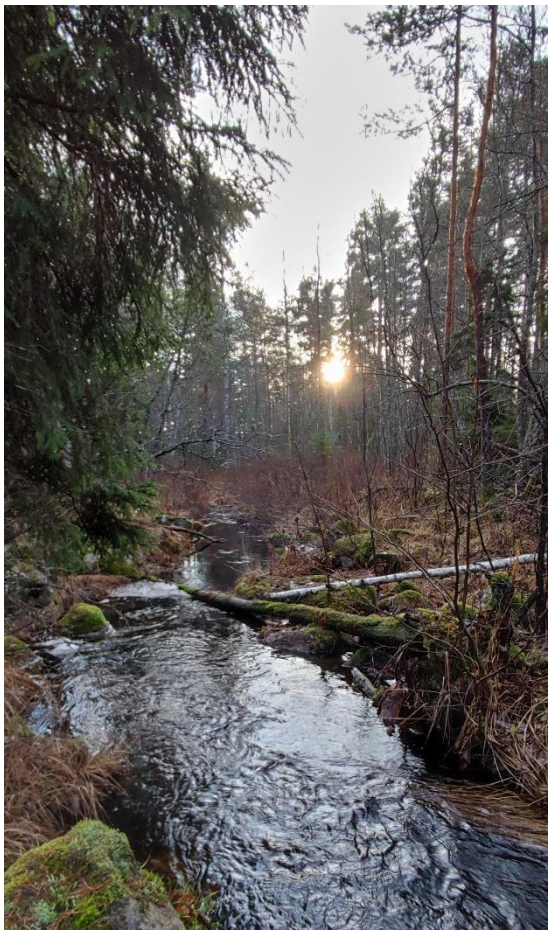
Newly Inundated Forest Landscapes - Hotspots for Mercury in Water and Benthic Fauna

Kirstine Evald Andersson



Master's degree project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of aquatic sciences and assessment
EnvEuro - soil, water, and biodiversity

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Credits: 30 Credits
Level: Second cycle, A2E
Course Title: **Independent Project in Environmental Science**
Course code: **EX0897**
Programme/education: **EnvEuro - soil, water, and biodiversity**
Course coordinating dept: **NJ faculty**
Cover picture: Natural wetland, site N11, Photo: David Kniha, 2022

Keywords: Wetland restoration, inundation, mercury cycling, beaver impoundments, aquatic biogeochemistry

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Abstract

Mercury (Hg) is a widespread contaminant in high-latitude aquatic ecosystems, where it can undergo microbial transformation into toxic methylmercury (MeHg). Due to its chemical properties, MeHg tends to accumulate in the aquatic food chain and will eventually end up in predatory species at the top of the terrestrial food web. This study aimed to assess the effects of new inundations in the forest landscape induced by beaver and human activity on the concentrations of Hg in water and benthic fauna. It was hypothesized that inundated systems would have elevated concentrations of THg and MeHg in water downstream of the flooded area, as well as concentrations of THg in benthic fauna. This study covered 72 sites across central Sweden including natural and restored wetlands, beaver ponds, and respective wetlands. Water was analysed for Hg, MeHg, and standard water chemistry and benthic fauna was analysed for THg. The results showed a significant increase in concentrations of THg and TOC at the outlet of restored wetlands, compared to both natural wetlands and previously drained wetlands (reference sites), indicating release of TOC and associated THg following restoration. MeHg concentrations and %MeHg of THg were significantly higher downstream of beaver impoundments, compared to their reference sites indicating new formation of MeHg. The results showed no significant effect of inundation on THg concentrations in the benthic fauna. The results suggest that new inundations caused by construction of beaver dams are hotspots for Hg methylation whereas, rewetting of previously drained wetlands promotes mobilisation of THg and organic matter (OM).

Keywords: Wetland restoration, inundation, mercury cycling, beaver impoundments, aquatic biogeochemistry

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List of Abbreviations

OM - Organic matter
DOM - Dissolved organic matter
TOC - Total organic carbon
BC - Beaver control
RC - Restored control
B - Beaver pond
R - Restored wetland
N - Natural wetland
GIS - Geographic Information Systems
Hg - mercury
THg - Total mercury
MeHg - Methylmercury
%MeHg - % methyl mercury of total mercury
Abs F 420/5 - Absorbance at wavelength 420 nm
Abs F 436/5 - Absorbance at wavelength 436 nm
Tot-N - Total nitrogen
NO2+NO3-N - nitrite + nitrate nitrogen
SO_4^{2-} - sulphate
Tot P - total phosphorous
Cl^- - chloride
Ca^{2+} - Calcium
Mg^{2+} - Magnesium
Na^+ - Sodium
K^+ - Potassium
Si^{4+} - Silicium

1. Introduction

Forest landscapes at higher latitudes are known to be hotspots for accumulation of anthropogenic mercury (Hg), originating from long range transboundary transport (Selin 2009). Research has mainly been focused on the accumulation of mercury in aquatic food webs, whilst less attention has been drawn to terrestrial food webs (Bishop et al. 2020).

Inundation of forest landscapes with Hg rich forest soils is of concern as methylmercury (MeHg) is mainly formed in aquatic systems with changing redox conditions (Jackson 1996). Reduced soil conditions favour microbial transformation of inorganic mercury into MeHg, a toxic and persistent organic compound which is highly bioavailable and tends to accumulate in food webs (Compeau & Bartha 1985; Ward et al. 2010). Research has shown that forest soil inundations following wetland restoration (Tjerngren et al. 2012b) as well as the creation of hydroelectric dams (Tremblay et al. 1996), experimental reservoirs (Paterson et al. 1998; St. Louis et al. 2004; Hall et al. 2005, 2009), and beaver ponds (Roy et al. 2009; Levanoni et al. 2015; Painter et al. 2015) cause elevated concentrations of MeHg in both in water and aquatic insects.

Deterioration of aquatic ecosystems is a major threat to water security and freshwater/aquatic biodiversity (Vörösmarty et al. 2010). In Sweden one-fourth of the wetlands have been drained since the early 19th century. The remaining wetlands are impacted by forestry operations, nitrogen deposition, and other human activity (Swedish Environmental Protection Agency 2018). Restoration of previously drained wetlands is a strategy to mitigate the effects of climate change, improve carbon sequestration and nutrient retention, as well as conservation of biodiversity. These ecosystem services are also supported by beaver-induced inundations. After being nearly extinct in both Europe and North America, the Eurasian beaver (*Castor fiber*) is now recolonizing large parts of its former distribution range, and the population density is still increasing (Halley et al. 2012). However, the ecosystem services related to rewetting and beaver activity come with the possible drawback of elevated levels of MeHg in water and biota(Tjerngren et al. 2012b; Levanoni et al. 2015; Painter et al. 2015). This stresses the need to understand and assess Hg's fate and distribution in newly inundated forest landscapes.

This master thesis is written as a part of the project INFORM-Hg (newly INundated FORrest landscapes as hotspots for Mercury bioaccumulation and transfer across the aquatic-terrestrial food web) conducted by the Swedish University of Agricultural Science (SLU). In this project, the role of human- and beaver-induced wetlands in forested landscapes on the MeHg accumulation in aquatic-terrestrial food webs will be assessed (Eklöf 2022). Thus, the aim of this thesis is to evaluate to which extent new inundations in the forest landscape, induced by humans and beavers, respectively, cause higher THg and MeHg concentrations in water and THg in benthic fauna, compared to natural wetlands and reference sites (without restorations or beaver dams). This was addressed by analyzing water samples collected from the respective wetlands for MeHg, THg, and standard water quality. Benthic fauna collected from the respective wetlands were analyzed for THg. The inundation effect will be assessed by comparing concentrations of

MeHg and THg and relevant water chemistry and landscape characteristics in beaver and restored wetlands to natural wetlands and to nearby reference sites. Finally, the results will be discussed in relation to implications for future nature management.

The following hypotheses will be tested:

- Newly inundated forest landscapes, either caused by wetland restoration or by the construction of beaver dams, will have higher concentrations of MeHg and THg in water, compared to natural wetlands.
- Newly inundated forest landscapes, either caused by wetland restoration or by the construction of beaver dams, will have higher concentrations of THg in benthic fauna in natural wetlands.
- Inundation will result in higher relative MeHg concentrations and %MeHg of THg in beaver ponds compared to restored wetlands due to the presence of easily degradable organic matter.
- Inundation will result in higher relative THg concentrations in beaver ponds compared to restored wetlands due to the high amount of peat in restored wetlands.

2. Mercury in boreal ecosystems

2.1 Sources of mercury

Hg ends up in the terrestrial environment via both natural and anthropogenic pathways. The natural sources include volcanic eruptions, geothermal activity, and weathering of Hg-rich rocks (10% of net emission). Hg is also introduced via point sources such as small-scale artisanal gold mining, industrial effluent, biomedical waste, and coal-fired electricity generation (30% of net emission). The major contributing factor is, however, atmospheric deposition and remobilization of legacy anthropogenic Hg previously released to terrestrial and aquatic environments (60% of net emission) (UN Environment 2019; Bishop et al. 2020). Human activities, that bring Hg from long-term sedimentary storage, have increased total atmospheric Hg concentrations by 450% above natural levels (UN Environment 2019). The Hg emissions have declined in the United States and Europe since the 1980s mainly due to regulations regarding sulphur emissions, while the emissions have increased in the Global South. Small-scale artisanal gold mining is the main contributor to the emissions from sub-Saharan Africa and South America accounting for 38% of the global emissions (UN Environment 2019).

Hg is emitted in the forms of either divalent oxidized Hg [Hg(II)], associated with particulate matter [Hg(P)], or as gaseous elementary Hg [Hg(0)]. The presence of Hg(0), which is the most abundant Hg-species (Selin 2009), facilitates long-range atmospheric transport before deposition to water and land. This is one of the major reasons why Hg emitted due to anthropogenic activities in the south ends up in high latitude ecosystems and contributes to increases in the amount of legacy Hg stored in boreal terrestrial environments (Johansson et al. 2001).

2.2 Biogeochemical cycling of mercury

As stated, the biogeochemical cycling of Hg involves atmospheric transport, deposition to land and water, and re-volatilization (Selin 2009). Hg is deposited onto soil and vegetation from the atmosphere. Dry deposition includes both Hg(0) and Hg(II) that attach directly to surfaces including particles in the atmosphere and vegetation, whereas wet deposition is primarily in the form of Hg(II). After deposition some Hg will rapidly re-volatize back into the atmosphere, and the remainder will be incorporated into the soil pool. The forest canopy takes up Hg(0) during the growing season, which makes litter fall the largest component of atmospheric deposition in boreal forests. As litter fall decomposes, the organic matter (OM) binds Hg in the forest soil (St. Louis et al. 2001; Bishop et al. 2020).

More than 90% of the terrestrial Hg is stored in the soil, with higher concentrations in organic soils than mineral soils (Bishop et al. 2020). Thus, peatlands and fens hold a substantial pool of Hg after thousands of years of atmospheric deposition (Grigal 2003; Mitchell et al. 2008) and are known to have high outputs of Hg to surface waters. Boreal soils are rich in OM, which makes them play an essential role in the cycling of Hg in high latitude ecosystems (Branfireun & Roulet 2002; Skyllberg et al. 2003; Tjerngren et al. 2012b). The total concentration of

Hg (HgT) is known be strongly related to the amount of OM in the soil (Lavoie et al. 2019; Branfireun et al. 2020), whereas the relationship between MeHg and OM is less distinct. Hg moves from the terrestrial to the aquatic environments both in particulate and dissolved form, and nearly all of it is bound to OM (Figure 1).

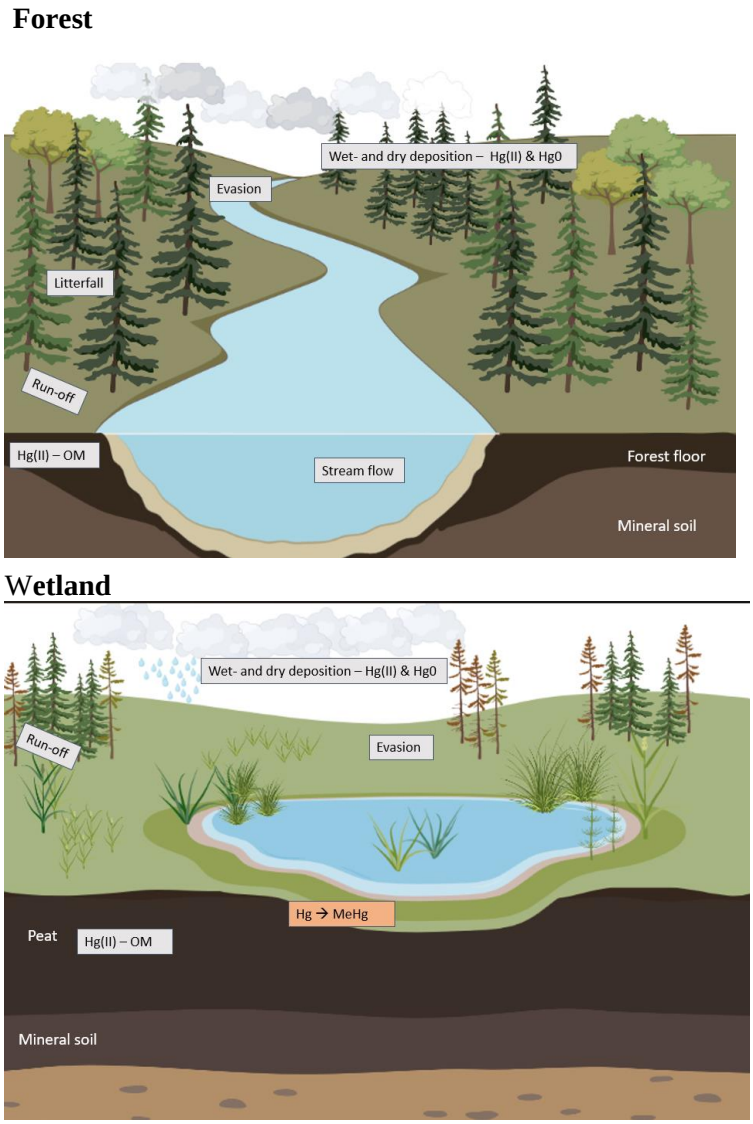


Figure 1. Movement of Hg in boreal forests and wetlands (Modified from Bishop et al 2020 and Branfireun 2020).

The aquatic-terrestrial cycling of Hg in boreal systems is mediated by the hydrological as well as several biogeochemical cycles (Bishop et al. 2020). The hydrological cycle facilitates the movement of Hg from the terrestrial environment to the aquatic, where it can undergo transformation to MeHg. Also, the carbon cycle as well as the sulphur cycle play essential roles in the methylation of Hg (Skyllberg et al. 2003; Wang et al. 2020). OM regulates the terrestrial pool of Hg, as it binds Hg, and Hg methylation, as it donates electrons to the microorganisms that

methylate Hg. Hg binds to reduced sulphur in thiol groups on OM substances and (SO_4^{2-}) is an important electron donor in the methylation process (Skyllberg et al. 2003)

The transformation of Hg is dominated by the processes of methylation and demethylation. Methylation of Hg occurs simultaneously with demethylation which proceeds both abiotically and biotically(Tjerngren et al. 2012a). In boreal wetlands the frequencies of these processes are affected by several environmental factors such as the availability of electron donors and acceptors (primarily OM) and the concentration of Hg within the cell (Tjerngren et al. 2012a). Methylation of Hg is carried out by a diverse set of microbial guilds and is linked to anoxic conditions, where SO_4^{2-} and iron (Fe^{3+}) reduction and fermentation occurs (Compeau & Bartha 1985; Gilmour & Henry 1991). The guilds include sulphate- and iron reducing bacteria, with sulphate reducing bacteria being identified most often.

2.3 Mercury mobilisation and transformation in inundated landscapes

Water saturated systems such as peatlands, fens, and other wetlands are hotspots for Hg methylation ($Hg(II) \rightarrow HgCH_3$) due to their anoxic conditions (Mitchell et al. 2008b). Several studies have identified wetlands to be sinks for THg and significant sources of MeHg to boreal forested ecosystems (St Louis et al. 1993; Louis et al. 1996; Galloway & Branfireun 2004). MeHg production and catchment export varies amongst different wetland types (Louis et al. 1996; Tjerngren et al. 2012b). Factors including hydrology, mineralogy and climate which regulate acidity, nutrient status and vegetation composition are considered to control the methylation of Hg. Previous large scale flooding experiments at the Experimental Lakes Area (Ontario, Canada) (Bodalay et al., 2004) of well drained forest soils and a wetland complex showed to promote methylation. MeHg formation increased rapidly within the first 2-3 years of flooding in both cases, with the highest increase in the wetland complex, whereas THg remained relatively stable.

Wetland restoration for ecological and climate purposes might affect the Hg cycling in wetlands, by increasing the mobilisation and transformation of Hg. As already stated, boreal wetlands have been shown to be sources of MeHg, and the formation of Hg is correlated with nutrient status and the presence of bioavailable Hg species (Tjerngren et al. 2012b). However, despite many years of research in the field of Hg dynamics in boreal wetlands, scientific knowledge about the effect of small-scale wetland restoration remains scarce. The increasing extent of wetland restoration only stresses the need for more research.

Wetland type and %wetland of the catchment are known to be related to the formation of MeHg, whereas they are less important for the retention of THg (Grigal 2003). Local hydrology plays a central role in the methylation of MeHg in wetlands, as they are found to be greater sources of MeHg during periods with high water yield (Louis et al. 1996). One Swedish study on THg and MeHg dynamics in boreal wetlands (Tjerngren et al. 2012) found that wetlands undergoing restoration, in general, tended to be sinks for THg and sources of MeHg. Wetlands with intermediate nutrients undergoing substantial inundation were the greatest sources of

MeHg and showed either temporal patterns or constant increase in methylation within the first 1-3 years after inundation. On the other hand, small scale flooding, which is commonly applied in the Swedish forest sector to restore the hydrology of previously drained wetlands, showed no or limited effect on methylation. This suggests that the physicochemical environment in the wetland primarily related to nutrient availability is more important than the inundation itself (Tjerngren et al. 2012b; a).

Inundation of boreal forest soils has been shown to be strongly correlated to the mobilization of Hg and to the formation of MeHg (St Louis et al. 1993; Louis et al. 1996; Bodalay et al. 2004; St. Louis et al. 2004; Hall et al. 2005). Nevertheless, they often lack hydrological connectivity to surface waters, making them less influential in the catchment export of MeHg. Changes in catchment hydrology, caused by for instance beaver activity, can however promote the export of MeHg to surface water systems (Bishop et al. 2009). Following the recovery of the beaver population in Europe and North America, many studies have been performed to evaluate the environmental effects of beaver activities. Despite the amount of data available, there is no consensus about the actual role of beavers in the cycling of nutrients and contaminants (Ecke et al. 2017). Especially, the effects of beaver dams on OM concentrations and composition in water are unclear. Previous studies find contradictory results, with both increases and decreases in DOC as well as different patterns in how carbon processing is affected by beaver impoundments (Catalán et al. 2017). Beaver dams have been shown to decrease the molecular weight, as well as increase the bioavailable fraction of dissolved organic matter (DOM). A greater extent of beaver activity increases discontinuity in water courses either by building new dams or recolonising abandoned systems (Halley et al. 2012; Ecke et al. 2017). Despite beaver impoundments in most cases only causing temporary a disturbance, the high frequency and density of beaver dams in boreal streams might have a great impact on the Hg dynamics. Inundation either caused by humans or beavers, creates favourable conditions for methylating microorganisms, causing increases in the availability of electron acceptors (SO_4^{2-}) and donors (labile DOM) and Hg species as well as creating appropriate redox, pH, and temperature conditions(Ecke et al. 2017). The wetland areas associated with beaver impoundments increase retention of sediment and OM, and the decomposition of flooded plant material might create anoxic conditions, which make beaver ponds an appropriate site for Hg methylation. These factors are regulated by the intrinsic properties of the flooded area and control the environmental effect of the impoundment (Levanoni et al. 2015).

Water level fluctuations in a flood-control reservoir increased both the release of THg from the solid phase to the pore water, as well as the concentrations of DOC and sulphate stimulating methylation (Eckley et al. 2017). However, when comparing beaver ponds to human made inundations such as reservoirs or artificial dams, one must consider the difference in scale and context; beaver ponds are often 1-2 ha and less extensive, whereas reservoirs can be up to 8000 ha (Ecke et al. 2017). Beaver ponds are abandoned when the food availability is no longer sufficient, and possibly recolonised when food resources recover. Studies from Canada and Sweden found that the methylation efficiency (%MeHg) in old and recolonised ponds is lower than those of new impoundments (Roy et al. 2009; Levanoni et al. 2015). Pond age also affects the processing of OM. Recent flooding enhances the mineralisation of OM which is attributed

to OMreleased from the flooded soil. Three different sources of OM were identified: continuous input of DOM from upstream systems, temporary input of DOM from recently inundated soil, and DOM from autochthonous and secondary processing triggered by increased nutrient availability (Catalán et al. 2017).

2.4 Bioaccumulation of Hg

MeHg is a potent neurotoxin, and accumulates in aquatic food webs as MeHg, posing a threat to humans and wildlife (Ward et al. 2010). The drivers of Hg accumulation in aquatic ecosystems are complex and associated with a large variety of environmental factors including hydrological connection to methylation sites, land use, catchment geology, and food web structure (Ward et al. 2010; Wu et al. 2019). The research on bioaccumulation of Hg in benthic fauna in boreal environments has mainly been focused on lakes and hydroelectric reservoirs in North America (Tremblay et al. 1996, 1998; Tremblay & Lucotte 1997; Hall et al. 2009). The attention of Swedish studies has been centered around freshwater fish, with only a few including organisms lower in the food web (Bishop et al. 2009; Åkerblom et al. 2014; Wu et al. 2019).

Previous studies have found significant increases in Hg in benthic food webs in hydroelectric reservoirs shortly after inundation of the area (Tremblay et al. 1996). The concentrations of MeHg in benthic fauna also showed to be positively correlated with the MeHg concentrations in the lake water (Hall et al. 2009). Experimental flooding of a wetland complex also resulted in increased concentrations of MeHg in zooplankton(St. Louis et al. 2004). However, MeHg produced in the peat did only make a small contribution to the MeHg concentration in zooplankton, which indicates that the levels of MeHg in zooplankton follow the production of MeHg in the open water. Increased uptake of MeHg in benthic fauna has also been associated with beaver impoundments (Painter et al. 2015; Ecke et al. 2017). Painter et al. (2015), studied in-stream beaver impoundments in the Rocky Mountains in Canada and found that MeHg and %MeHg increased downstream of beaver dams in both water and all compartments of the benthic food web. However, the concentrations were only significantly higher in predatory invertebrates downstream, compared to upstream (Painter et al. 2015). Painter et al. (2015) found large variations in MeHg among sites, which accredited to the concentration of DOC rather than the presence of beaver dams.

3. Materials and Methods

3.1 Site description

This study involves 30 new inundations (15 beaver ponds (B) and 15 restored wetlands (R)) and 12 natural wetlands (N) (Figure 2). Each of the beaver ponds and restored wetlands have been compared to near-by reference sites (BC and RC respectively). 72 sites were included in the study (Table A 1).

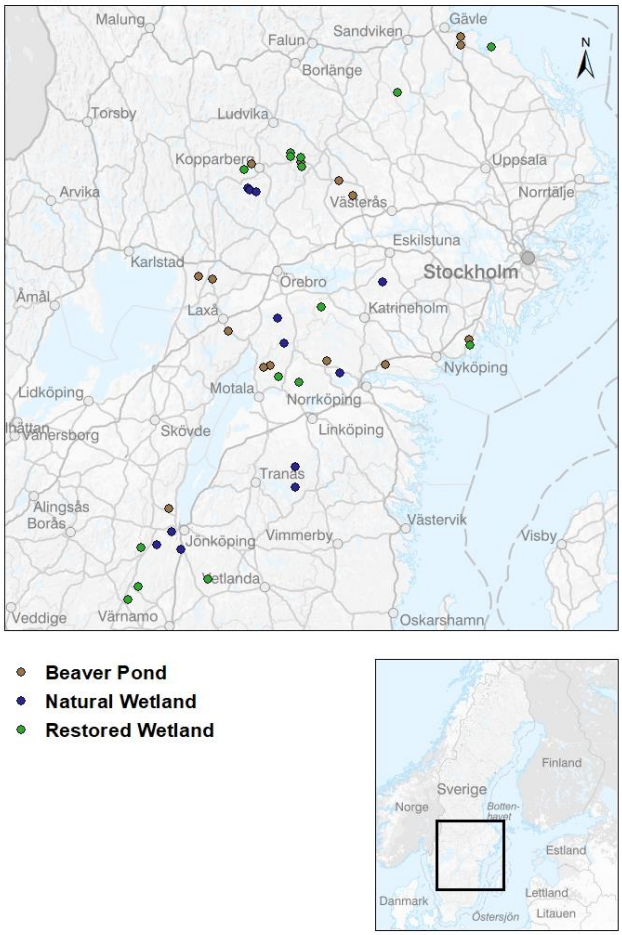


Figure 2. Extent of study area and sampling points.

Pictures of selected sites are found in Appendix 1 (Table A 2). RC and BC sites were as similar as possible to the catchments of B and R with regards to size, land use, and vegetation but without beaver ponds and restored wetlands. See Table 1 for specifications. This means that the

*Table 1. Catchment characteristics expressed as mean values of various landscape variables ± standard error, for all sites together, beaver ponds, beaver controls, restored wetlands, restored wetland controls, and natural wetlands. *(2013-2021)*

	All sites ± SE	Beaver Pond	Beaver Control	Restored Wetland	Restored Control	Natural Wetland
Catchment area (ha)	396.4 ± 90.4	1042 ± 347	227 ± 59	92.5 ± 44.2	237 ± 142	379 ± 140
% Open wetland	11.77 ± 2.51	3.15 ± 1.14	4.04 ± 1.21	18.79 ± 6.90	18.65 ± 8.60	14.83 ± 4.45
% Open water	1.49 ± 0.41	3.20 ± 1.17	2.08 ± 1.17	NA	0.46 ± 0.40	1.77 ± 0.95
% Arable land	0.86 ± 0.27	1.67 ± 0.74	1.28 ± 0.95	0.21 ± 0.14	0.51 ± 0.35	0.56 ± 0.38
% Forest	65.81 ± 2.29	66.87 ± 2.23	62.76 ± 3.32	71.71 ± 6.82	60.02 ± 7.00	68.16 ± 4.14
% Dry coniferous forest	49.72 ± 2.55	50.74 ± 2.86	47.38 ± 3.26	54.18 ± 8.55	45.50 ± 7.10	51.07 ± 4.57
% Dry deciduous forest	2.36 ± 0.42	4.41 ± 1.25	2.43 ± 0.94	1.42 ± 0.76	1.59 ± 0.79	1.84 ± 0.67
% Dry mixed forest	3.39 ± 0.42	5.20 ± 0.88	4.75 ± 0.78	1.61 ± 0.72	2.35 ± 1.00	2.94 ± 1.11
% Wet forest	10.41 ± 1.00	6.52 ± 1.04	8.20 ± 1.79	14.49 ± 2.88	10.58 ± 2.75	12.31 ± 1.54
% Wetland	22.11±2.85	9.67±1.94	12.24±2.67	33.29±8.06	29.23±8.55	27.14±5.13
% Dry forest	55.47 ± 2.55	60.34 ± 2.78	54.0 ± 3.22	57.21 ± 8.25	49.44 ± 7.23	55.86 ± 4.93
% Clear cut*	8.04 ± 0.75	7.28 ± 1.1	10.68 ± 1.41	8.97 ± 2.97	6.71 ± 1.5	4.62 ± 0.78

RC are previously drained wetlands without any restoration. Despite RC and BC being located close to B and R respectively, they were hydrologically disconnected from the study sites.
One important difference between the R and B is that the former represents previously drained wetlands, whereas, this is not necessarily the case for BC. N were included as a natural reference to compare to R to evaluate if the drained wetlands would return to the natural function of a wetland after restoration. The sites included in the study were all located within the distribution range of the Eurasian Beaver (*Castor fiber*). The catchments are situated at latitudes ranging from N 57° 20.5581' (R15) to N 60° 37.2401' (B3) and longitudes ranging from E 13° 35.1093' (R10) to E 17° 46.1189' (R15). The average area of the catchments is 396.4 ± 90.4 hectares (ha). The mean water temperature throughout the sampling campaign was 7.55± 0.35 °C.

All catchments are located in forested areas, with a forest cover accounting for an average 65.81 ± 2.29 % of the catchment area (Table 1). The forests in all catchments in the study are dominated by coniferous species (spruce and pine) with some deciduous forest. Open wetland and forested wetland each covered on average around 20% of the catchment. The average percentage of clear cuts in each catchment is approximately 8% (Figure A 1). Only clear cuts conducted between 2013 and 2021 have been taken into account. The geology of the catchments/ soil types comprises glacial deposits, clay/silt, exposed bedrock, and peat (SGU soil map). Overall, the studied catchments represent both peat and minerogenic soils within each

type of inundation. The thickness of the peat layer ranges from 30 cm to >50 cm (SLU peat map). Average annual precipitation was ranging from 400 mm in east to 800 mm in west and average annual temperature was between 6 in the south and 8 °C in the north (data from Swedish meteorological and Hydrological institute (SMHI), 1991-2020).

3.2 Site identification

R were selected in dialogue with county boards and other stakeholders. B were identified and selected using aerial photographs following the method described in Levanoni et al. (2015), or by contacting county boards, hunting teams, orienteering teams among others. BC and RC were identified by using map material within ArcGIS. N were located in protected areas, such as nature reserves or Natura 2000 areas, which was necessary for us to be able track the history of the wetland.

3.3 Field Sampling and sample preparation

At each of the sampling sites and their respective controls, grab water samples were collected in the outlet of the wetland or beaver pond following an ultra-clean sampling protocol. To minimise contamination, single use nitrile gloves were used when collecting water and fauna samples. Control samples were collected in a close-by ditch or stream. Dissolved oxygen was measured in the field using a portable sensor (Hach HQ40d Portable Multi-Parameter Meter with standard LDO electrode). Measurements were carried out approximately 20 cm below the water surface. Water for THg and MeHg analyses were sampled in acid washed Teflon bottles, while water samples for basic water chemistry and total organic carbon (TOC) measurements were collected in low-density polyethylene (LDPE) bottles. All bottles were rinsed three times in stream water before collecting the water sample. The samples were sent to the laboratory for MeHg and THg analyses the same or the next weekday. Samples collected Friday were shipped on Monday, to not be stacked in the post office on the weekend. In the field, water samples were kept dark and cold in a portable refrigerator. In the laboratory, samples were stored at 4 °C until analysis.

Sampling of benthic fauna was carried out using standardised kick sampling supplemented with manual collection (SS-EN 27828 1994). The fauna samples were stored in 15 ml centrifuge tubes and were frozen directly in the field and kept on dry ice until arrival at the laboratory where they were stored at -20°C. One individual from each group of benthic fauna was stored in ethanol for taxonomy. Samples were freeze dried until all water was removed. After freeze drying, the samples were stored at -20°C to avoid warm and humid air. The freeze-dried samples were ground to powder in the vial using an acid cleaned glass rod.

Individuals from the following taxa were sampled: *Zygoptera, Trichoptera, Anisoptera, Megaloptera, Asellota, Coleoptera, Senticaudata,* and *Hygrophila* (Tables A 3 & 4). Individuals from the groups *Asellota, Coleoptera, Senticaudata,* and *Hygrophila* were of low abundance and therefore treated as one group (*Other*) in the statistical analysis.

3.4 Chemical analyses

MeHg and THg in water

The analyses of total mercury (THg) and MeHg in water were carried out at the Swedish Environmental Research Institute (IVL). Both MeHg and THg were quantified using Cold Vapour Atomic Fluorescence Spectroscopy (CV-AFC). The analysis method for THg follows the standard of the US Environmental protection agency (EPA), EPA 1631 Version E. The analysis of MeHg is based on EPA-1630. The detection limit of the analysis method for THg is 0.06 ng/l and the limit of quantification is 0.2 ng/l with 8% analytical uncertainty. For the MeHg analysis the detection limit is 0.02 ng/l and the limit of quantification 0.06 ng/l with 12% analytical uncertainty (US EPA 2002). Both methods are accredited by the Swedish Board for accreditation and Conformity Assessment (SWEDAC). The percentage of THg comprised of MeHg (%MeHg) was used as a proxy for Hg methylation, as %MeHg has shown to be positively correlated to methylation rate (St Louis et al. 1993).

THg in benthic fauna

The quantification of THg in benthic fauna was carried out at the Department of Aquatic Sciences and Assessment, SLU. The analysis method used catalytic combustion and atomic absorbance spectroscopy with the aid of Milestone DMA-80 instrument. The analytical method had an uncertainty of 18%. THg was considered to be a sufficient predictor for Hg accumulation in benthic fauna, as THg concentrations were found to be somewhat similar to the MeHg concentration (Tremblay et al. 1996). Using THg as a predictor for MeHg, might however result an in overestimation of bioaccumulation in benthic fauna at low levels in the food chain, as previous studies found higher %MeHg in predator invertebrates.

Water quality analysis

The chemical analysis included quantification of TOC, ionic compounds, and nutrients (N, P, and S species), measures of pH, dissolved oxygen, alkalinity and conductivity, and absorbance at 420 and 436 nm. C/N ratio was calculated as TOC/Tot N. TOC was analyzed at the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala (SLU). TOC was quantified by combustion analysis using a Shimadzu TOC-VCPH with TNM-1 module and automatic sample changer ASI-V. Before analysis, the unfiltered samples were treated with HCl and CO_2 expulsion. Absorbance was measured at 420 and 436 nm (PerkinElmer Lambda 35 with 4 nm bandwidth and sample changer). pH, conductivity, alkalinity, Tot. N and P, Nitrate-N (NO_3^--N), Nitrite-N (NO_2^--N), ammonium (NH_4^+-N), phosphate-P (PO_4^{3-}-P), sulfate (SO_4^{2-}), Chloride (Cl^-), Calcium (Ca^{2+}), fluoride (Fl^-) potassium (K^+), Magnesium (Mg^{2+}), Sodium (Na^+), and Silicon (Si^{4+}) were analyzed at the Department of Aquatic Sciences and assessment at SLU applying SWEDAC accredited methods (see https://www.slu.se/en/departments/aquatic-sciences-assessment/laboratories/vattenlabb2/ for a detailed description).

3.5 Spatial analysis and geographic information sources

Catchment characterisation was carried out using geographical information systems (ArcMap 10.8.2). Catchment delineation was partly carried out using a model designed to delineate the Swedish monitoring sites for water by SLU. As the catchments in the present study are rather small, the modelled catchments were controlled and, if needed, edited by hand using a 2x2 m gridded national elevation model and a 50x50 m national topographic map (Swedish Land Survey, Lantmäteriet).

Land cover data were extracted from the National Land Cover Database (NMD) base map, which has 25 thematic classes and a resolution of 10x10 m (Olsson & Ledwith 2020). The mapping was carried out between 2017 and 2019. Information about where and when clear-cuts had been performed in the catchments was derived from the Swedish Forest Agency (Skogsstyrelsen). Only clear cuts conducted between 2013 and 2021 were considered, as the older clear-cuts were suggested to have a minor effect on the water quality. The vector data constituting the polygon shape file is based on satellite images (Skogsstyrelsen 2022).

The peat map shows mineral soil and open water bodies and divides the predicted thickness of the organic layer into three classes (≥ 30 cm peat depth, ≥ 40 cm peat depth, and ≥ 50 cm peat depth. The map is developed at the Department of Forest Ecology Management, SLU Umeå, and the Department of Land and Environment, SLU Uppsala. The map is based on map data from the Swedish Land Survey, the Swedish Meteorological and Hydrological Institute (SMHI), the Geological Survey of Sweden (SGU), and field observations from the Swedish Soil Inventory (Markinventeringen). It identifies 80% of the peatlands in Sweden with a high level of detail. The map is in raster format with a 2x2 m spatial resolution, and SWEREF$_{99}$TM projection (Ågren et al. 2022).

The soil map from SGU is a generalised and simplistic map of Sweden's soil geology. It has a spatial resolution of 1:1 million and is applied in the form of a vector file of polygons and is projected on SWEREF_99TM (EPSG:3006). The soil type map is based on already existing soil maps of various resolutions, which affects the accuracy, and gives the map a relatively high uncertainty. However, it is appropriate for giving a general overview of the soil type distribution in the studied catchments.

3.6 Data analysis

The data were checked for normality prior to statistical analysis using the Shapiro-Wilks test (R studio). As the data did not follow a normal distribution, a non-parametric test (Wilcoxon rank sum test) was applied to test if the MeHg and THg concentrations and %MeHg of THg in water differed significantly from each other between the treatment groups. P-values below 0.05

were considered statistically significant. The following tests were conducted (See Figure 3 for a graphical overview):

- Concentrations of THg and MeHg in the water from restored wetlands and beaver ponds were compared to natural wetlands.
- Concentrations of THg and MeHg in the water from restored wetlands were compared to their reference sites (drained wetlands without restoration).
- Concentrations of THg and MeHg in the water from beaver ponds were compared to their reference sites (water courses without beaver impoundment).
- Difference in THg and MeHg concentrations between restored wetlands and beaver ponds relative to their reference sites.

A Two-way analysis of variance (ANOVA) was applied to test if the THg concentrations in the different groups of benthic fauna differed significantly between the treatment groups. Both treatment, group, and their interaction, were used as explanatory variables in the two-way ANOVA. Concentrations of THg were log transformed prior to analysis to follow a normal distribution. The following tests were conducted:

- Concentrations of THg in the benthic fauna from restored wetlands and beaver ponds were compared to natural wetlands.
- Concentrations in the different groups of benthic fauna in restored wetlands were compared to their reference sites (drained wetlands without restoration).
- Concentrations in the different groups of benthic fauna in beaver ponds were compared to their reference sites (water courses without beaver impoundment).

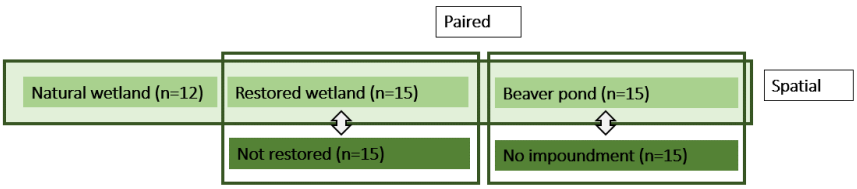


Figure 3. Experimental setup. "Not restored" and "No impoundment" are the reference sites for restored wetlands and beaver ponds respectively.

A Principal component analysis (PCA) was performed to explore any trends and correlations in the dataset. A partial least squares regression (PLS) was conducted to test to which extent differences in landscape characteristics and water quality explained the variance in the THg and MeHg in water. Variables with a VIP (Variable importance for the projection) value above one were considered significant for explaining the variation in the dependent variable (Varmuza & Filzmoser 2009).

Shapiro-Wilks test, Wilcoxon rank sum test, Two-way ANOVA, and PCA were conducted using R studio (RStudio 2023.03.0 Build 386 "Cherry Blossom" Release, 2023). PLS was carried out using SIMCA-17® (Version 17.0.2.34594, 2021). Graphics were created with BioRender.com ©.

4. Results and Discussion

4.1 Effect of inundation on THg and MeHg concentrations in water

The water samples were analysed for THg and MeHg. The average concentrations of THg in water ranged from 5.68±0.65 ng/l in N to 15.03±3.44 ng/l in R (Table A 5). The concentrations of THg were significantly higher in R, in comparison to RC (p=0.012) (Table 2), while THg concentrations in B did not differ significantly from those in BC (Figure 4). R had significantly higher concentrations of THg, compared to both B and N (p=0.03 and 0.05 respectively). THg concentrations did not differ significantly between B and N.

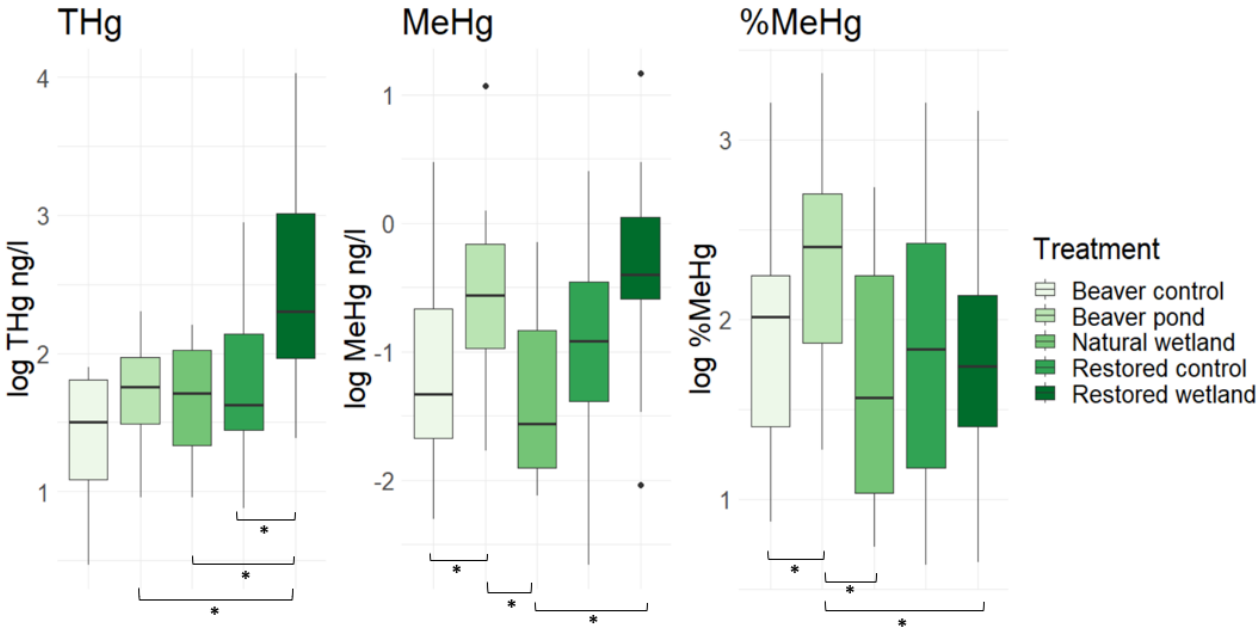


*Figure 4. Concentrations of THg and MeHg and %MeHg of THg in water sampled at the outlet of restored (R) and natural wetlands (N), beaver ponds (B), and the respective reference sites for restored wetlands (RC) and beaver ponds (BC). * Indicates significant difference between the two respective treatments. E.g. THg concentrations. in R and RC are significantly different (p<0.05).*

N had the lowest concentrations of MeHg (0.33±0.08 ng/l), whereas R had the highest concentrations of MeHg (0.91±0.19 ng/l). B had significantly higher concentrations of MeHg, compared to BC (p=0.019), while the concentrations of MeHg in R and RC showed no significant difference. Both R and B contained higher concentrations of MeHg, compared to N (p=0.003 and p=0.006). B and R did not show any significant difference with regards to MeHg. The relative concentrations of THg (ΔTHg) in R and B showed no significant difference (Figure 5,

Table A 7). The same was seen for MeHg. This shows a similar relative effect of inundation on THg mobilisation in beaver ponds and restored wetlands, as well as a similar relative effect of inundation on MeHg concentrations.

Table 2. Results Wilcoxon rank sum test for Hg in water, p-values < 0.05 are marked with green as they indicate statistical significance.

	THg	MeHg	%MeHg
B-BC	0.088	0.019	0.0344
R-RC	0.012	0.074	0.836
N-R	0.005	0.003	0.402
N-B	0.783	0.006	0.014
R-B	0.003	0.289	0.024

Wetlands as well as beaver impoundments are renowned to be sources of MeHg to downstream aquatic ecosystems (Louis et al. 1996; St. Louis et al. 2004; Roy et al. 2009; Tjerngren et al. 2012b; Levanoni et al. 2015; Ecke et al. 2017; Wang et al. 2020). A mass-balance study (input-output) from Tjerngren et al. (2012a) found that previously drained wetlands situated in Northern Sweden, subjected to small scale flooding were sinks for THg and sources of MeHg. They also experienced large variations in the production of MeHg, which was due to physicochemical factors. The results of the present study found high concentrations of THg at the outlet of restored wetlands, which, in contrast to the study of Tjerngren et al. (2012a), indicates release of THg.

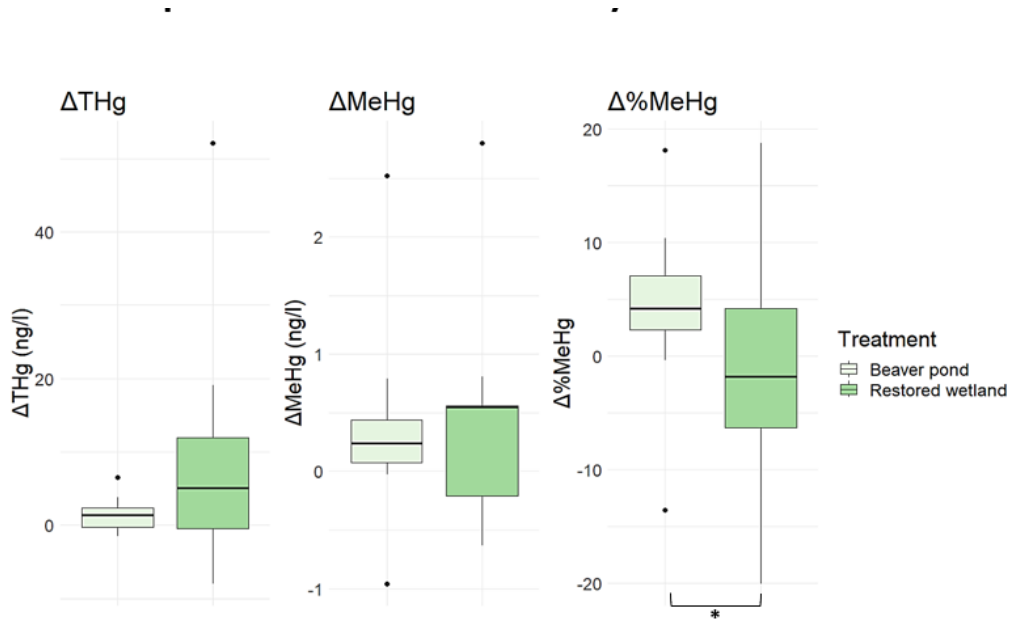


*Figure 5. Difference in THg, MeHg and %MeHg of THg between beaver ponds and restored wetlands and their respective control sites. * Indicates statistical difference (p<0.05).*

The concentration of MeHg relative to THg (%MeHg), can be used as an approximation of the methylation efficiency of Hg (St Louis et al. 1993). For %MeHg the lowest percentage was found in N (5.96%) and the highest in B (11.98%). The %MeHg in B was also significantly higher than the %MeHg in BC, which was not the case for R and RC. B also had higher %MeHg

than both R (p=0.024) and N (p=0.014). Δ%MeHg was significantly higher in B than R (Figure 2). This indicates a higher effect of inundation caused by beaver activity on MeHg formation than inundation due to restoration. Despite containing a larger pool of inorganic Hg, R did not have a higher Hg methylation potential (%MeHg). Other studies have also found that the total concentration of Hg does not solely control the formation of MeHg (Branfireun et al., 2020; Galloway & Branfireun, 2004; Mitchell et al., 2008; Wang et al., 2020). Both a Swedish and a Canadian study on the effect of beaver impoundments on water quality found elevated concentrations of THg and MeHg downstream of the ponds, as well as increases in %MeHg (Roy et al. 2009; Levanoni et al. 2015). The average MeHg concentration at the outlet of the beaver ponds included in this study was 0.73 ng/l which accounts for 11.98% of THg. At the reference sites, the average concentration was 0.41 ng/l, accounting for 0.41% of THg. MeHg concentrations and %MeHg were in the same range of the study from Sweden, but low in comparison to the Canadian study. One remarkable difference between the Canadian and Swedish studies is that the Canadian sites are in the northern temperate vegetation zone, whereas the Swedish are in the boreal zone where the temperature is lower. This might influence the conditions for methylating microorganisms, favouring methylation in Canadian beaver impoundments in comparison to Swedish.

Experimental flooding of a low-lying wetland complex, also at the Experimental Lakes Area (Ontario, Canada), showed a significant increase in Hg methylation in the flooded peat 2-3 years after flooding. The wetland was assumed to account for 97% of the MeHg produced in the reservoir within this period (St. Louis et al., 2004). They found that %MeHg of THg in the flooded peat increased from 5-10% prior to flooding to 60-80% after flooding. Compared to a similar experiment in the same area where upland forest sites were flooded (Hall et al., 2005), %MeHg was higher in the flooded wetland.

In this study R had significantly higher concentrations of MeHg in comparison N but did not differ significantly from RC neither with regards to MeHg nor to %MeHg. However, the variances (SE) in both were large. As stated earlier %MeHg increases remarkably in the years after inundation, hence the age of the restoration might also have an impact on the low methylation efficiency, compared to other studies. Also, the methylation efficiency might be impacted by the age of the peat in the wetland; Wang et al (2020) found that older, nutrient poor peatlands with strongly humified organic material had lower methylation efficiency, but larger storage of THg.

4.2 Effect of inundation on THg concentrations in benthic fauna

The results of the analysis of THg in benthic fauna (Figure 6, Table A 8), showed no influence of inundation on THg when taking the different groups of benthic fauna into account (ANOVA, F). However, there was a significant difference between the fauna groups.

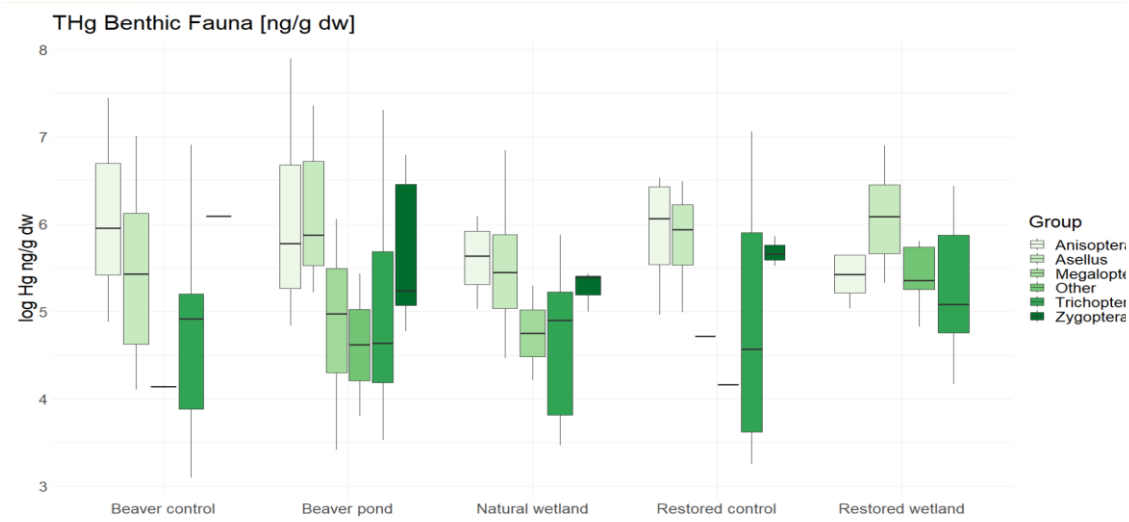


Figure 6. THg concentrations (ng/g dw) in each insect group for all treatments/types of wetlands. The group "other" include Hemiptera, Gammarus, Coleoptera and unidentified individuals.

This indicates that the THg concentrations in benthic fauna are not directly affected by increased MeHg concentrations in water related to inundation, but rather by the food web structure in the respective system. The benthic food web structure and species community might be affected by the disturbance of the system caused by either the construction of beaver dams or wetland restoration. These disturbances may result in changes in nutrient status, TOC, light conditions, and concentrations of dissolved oxygen (section 4.3) which is likely to affect the ability of benthic fauna to find food or hunt. Ecke et al. (2017) found a net decrease in diversity and/or abundance of macroinvertebrates downstream of beaver impoundments, compared to upstream and in the impoundment itself.

Previous research found elevated Hg concentrations in benthic food webs in hydroelectric reservoirs and downstream of beaver impoundments in North America (Tremblay et al. 1996; Painter et al. 2015). They also found that Hg concentrations increase with the trophic level. Studies on Hg uptake in zooplankton and insect larvae also show positive relationship between MeHg in the organism and the concentration of MeHg in water (Tremblay & Lucotte 1997; Tremblay et al. 1998; Hall et al. 2009). The lack of inundation effect in the present study, contrary to the North American studies (Tremblay et al. 1996; Painter et al. 2015), could be due to differences in environmental and climatic conditions, as well as experimental setup and objectives. In this study, concentrations of MeHg in benthic fauna were not quantified. Also, two different catchments, instead of up- downstream concentrations, were compared, which may increase the variation in data. Furthermore, flooding due to the establishment of hydroelectric reservoirs has a much larger spatial scale than flooding due to beaver activity. Also, controlled fluctuations in the water level in human made reservoirs also promote Hg methylation (Eckley et al. 2017).

Despite this study not finding a direct effect of inundation on THg concentrations in benthic fauna at the outlet of the flooded systems, the elevated concentrations of THg and MeHg in the water might pose a risk to receiving aquatic ecosystems further downstream.

4.3 Effect of inundation on water quality

The results of the water quality analysis showed differences in the physicochemical environment related to the inundation type. High concentrations of TOC and low concentrations of dissolved oxygen were characteristic for R. N sites were characterized by high C/N ratios, whereas the outlets of B were associated with intermediate C/N ratios and high concentrations of dissolved oxygen (Figure 7). See Appendix 3, Table A 9 for a full overview of water quality measurements.

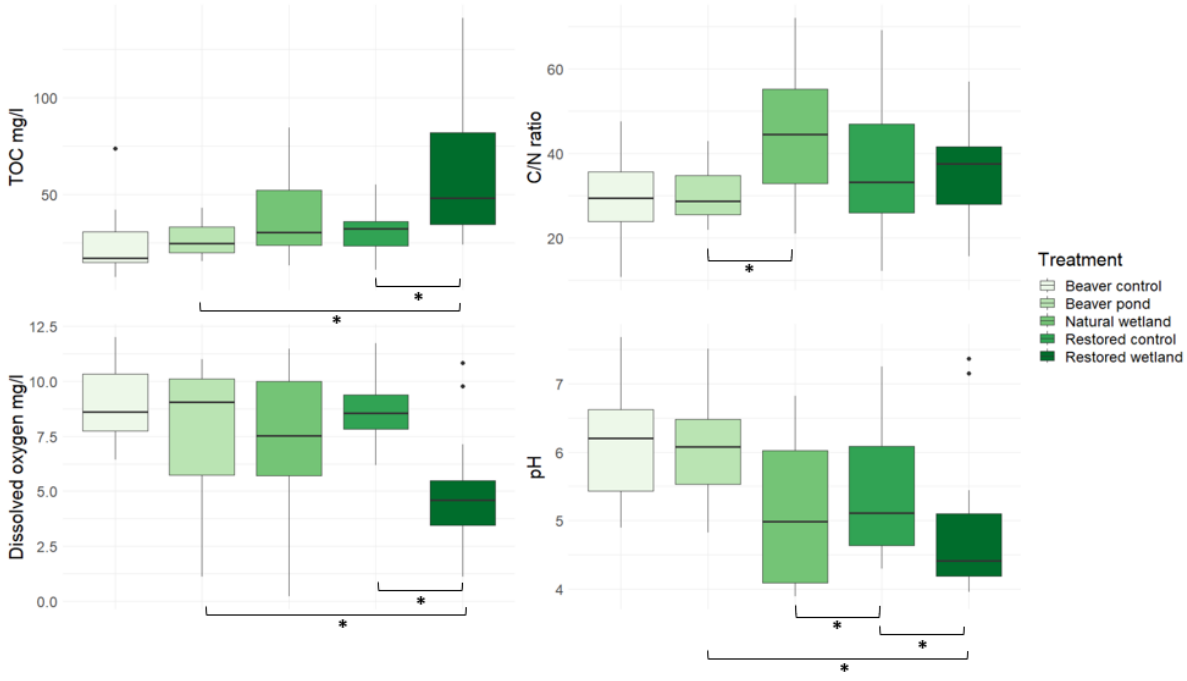


*Figure 7. Water chemical factors, which were shown to influence the variation between the inundated systems. * Indicates statistical difference ($p<0.05$).*

The C/N ratio in water was significantly lower in B, compared to N and the pH was significantly higher (Table 3). pH and dissolved oxygen were significantly lower in R, compared to B, and TOC was significantly higher. B did not differ significantly from BC neither with regards to concentrations of TOC or dissolved oxygen, C/N ratio, nor pH. TOC concentrations were significantly higher in R, compared to RC, whereas pH and concentrations of dissolved oxygen were significantly lower in R. In this study, no differences in neither concentrations of TOC, dissolved oxygen nor nutrients were observed in beaver ponds, compared to their reference sites (Table 3). Early studies have considered boreal beaver ponds as a type of wetland. Beaver ponds are remarkably different from wetlands. The open water pond facilitates longer water residence times and sedimentation, and the dams have substantial effects on the local hydrology (Catalán et al. 2017), which might explain the difference in water quality between the two systems.

Table 3. Results Wilcoxon rank sum test for selected variables for water quality, p-values < 0.05 are marked with green.

	pH	TOC mg/l	C/N ratio	Dissolved O_2
B-BC	0.756	0.146	0.933	0.303
R-RC	0.044	0.006	0.803	0.000
N-R	0.788	0.060	0.074	0.060
N-B	0.004	0.179	0.034	0.751
R-B	0.001	0.001	0.213	0.005

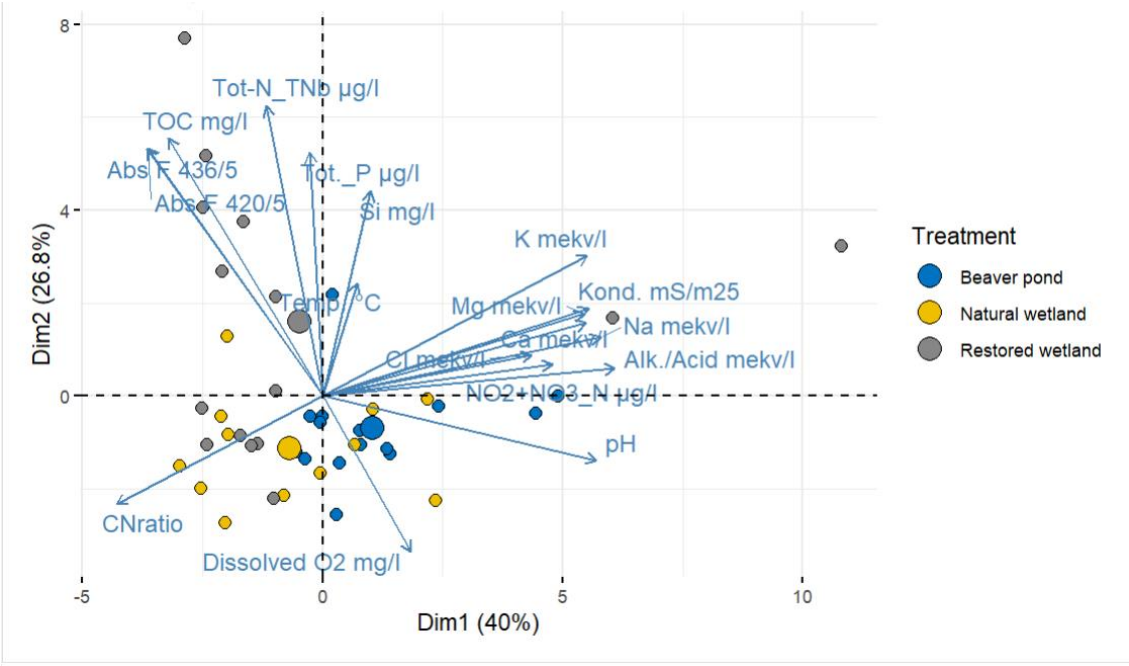


Figure 8. PCA of the chemical variables separating the three types of inundations. The plot shows PCA scores for beaver ponds (blue), natural wetlands (yellow), and restored wetlands (grey).

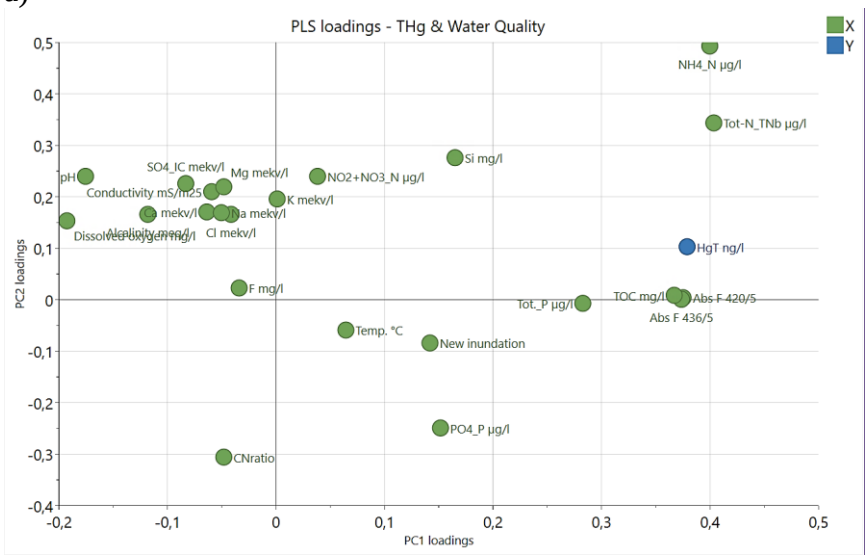
A PCA was conducted to explore the relationship between water quality and inundation type. The first principal component (PC1, 40% of total variance) separates the B from R (Figure 8). The second principal component (PC2, 28% of total variance) separates B and R from N. The two first components explain 68% of the variance. TOC and variables related to TOC (Abs, C/N ratio, nitrogen, and phosphate) contributed the most to the separation, dividing B ponds and N from R. The PCA shows negative correlation between dissolved oxygen and TOC, as well as pH and TOC. Dissolved oxygen separated N from B and R, and pH contributed to the separation of R and N from B.

A PLS model with water quality and inundation type (new inundation=1 for B and R/no inundation =0 for RC, BC, and N) as explanatory variables (X) explained 89% of the variation in THg (Y) concentrations with the first two components (R^2X=55%, Q^2=75%) (Figure 9a). The explanatory variables with VIP > 1 were positively correlated to THg and were all associated with the content of OM in the water (Table 4). When using MeHg as the response variable the PLS model explained 57% (R^2Y) of the variation in MeHg concentrations with the first two components (R^2X=50% and Q^2=36%). Explanatory variables associated with OM and nutrient status as well as dissolved oxygen had VIP > 1 (Table 4). Dissolved oxygen was negatively correlated with MeHg, whereas the rest showed positive correlation (Figure 9b). Recently inundated systems (B and R), were positively correlated to THg and MeHg but did not have a VIP above 1 (THg: 0.67, MeHg: 0.93). This indicates that the variation in THg and MeHg is not solely linked to the inundation itself, but also strongly correlated to other environmental factors. THg is proven to be positively correlated with the concentration of OM (Ullrich et al. 2001; Brigham et al. 2009; Dittman et al. 2009, 2010; Lavoie et al. 2019), which is also the case for this study (Figure A 2). We found no relationship between MeHg and THg, which is also supported by other studies, as the formation of MeHg is driven by additional factors than just the pool of inorganic Hg (Louis et al. 1996; Brigham et al. 2009; Lavoie et al. 2019).

Table 4. Statistics for PLS analysis with water chemistry. Shows VIP-values for the X variables having a significant influence on the variance in THg and MeHg.

Y variable	X variable	VIP	R^2X	R^2Y	Q^2
THg	NH_4-N	1.92	55%	89%	75%
	Tot N	1.90			
	Abs 420/5	1.75			
	Abs 436/5	1.74			
	TOC	1.71			
	Tot P	1.31			
MeHg	Tot P	1.88	50%	57%	36%
	Tot N	1.70			
	TOC	1.55			
	NH_4-N	1.54			
	Abs 420/5	1.51			
	Abs 436/5	1.51			
	Diss. O_2	1.2			
	PO_4-P	1.02			

a)



b)

Figure 9. PLS loading plots showing variables contributing to the variation in THg (a) and MeHg (b).

Beaver impoundments have been shown to influence the downstream water quality. In North American steams Roy et al. (2009) found concentrations of nutrients (total N and total P) and dissolved organic carbon (DOC) to increase, while the concentrations of dissolved oxygen decreased, wich indicate heterotrophic activity. Roy et al (2009) also found that low concentrations of dissolved oxygen in the ponds were the main determinant for MeHg concentrations in the outlets. The current study does not present oxygen concentrations measured in the impoundment, but in the outflow. It could be that oxygen levels in the outlet of the pond is different from that of the pond itself. The high concentrations of dissolved oxygen found at the outlet of the beaver ponds in this study might be due to the rapid reoxygenation of running water. A

recent study found that Hg methylation in the sediment of 9 beaver ponds in Sweden was controlled by the presence of fresh humic and algal-derived OM (Herrero Ortega et al. 2018). Upstream DOM was more humified, whereas DOM was more protein-like which indicates increased autotrophic activity in the pond, as well as more aromatic which indicate release of terrestrial DOM from the inundated soil (Herrero Ortega et al. 2018). Also, a second study found the fraction of bioavailable DOM was higher in the ponds than upstream, despite the total concentrations of DOM being similar (Catalán et al. 2017) .

Despite low concentrations of oxygen and low pH, which govern the redox conditions of the water and are said to promote methylation of Hg (Compeau & Bartha 1985), restored wetlands did not show any significant production of MeHg. This might be due to the composition of OM, which supposedly is too recalcitrant to facilitate favourable conditions for Hg methylating microorganisms. Also, the bioavailability of Hg is controlled by the composition of organic substances (Tjerngren et al. 2012b; Bravo et al. 2017). Tjerngren et al (2012a) found wetlands undergoing restoration with intermediate pH to have the highest net MeHg production (Tjerngren et al. 2012a; b). They concluded that the factors in charge of controlling Hg methylation were the concentrations of bioavailable Hg species and the availability of electron acceptors (SO_4^{2-}) and donors (low molecular organic substances). It is well-known that more labile OM in well drained upland soils supports higher MeHg formation after inundation than recalcitrant OM in peat (St Louis et al. 1993; St. Louis et al. 2004; Hall et al. 2005; Tjerngren et al. 2012b). This agrees with the lower C/N ratio found in beaver ponds (30.7±1.6), and the corresponding high methylation efficiency in beaver ponds, represented by %MeHg. Likewise, the high C/N ratio found in natural wetlands (43.8±4.6) supports the low methylation efficiency. Tjerngren et al. found the highest methylation at sites with intermediate OM content, which agreed with the findings of Hall et al (2005).

4.4 Effect of landscape characteristics on THg and MeHg concentrations in water

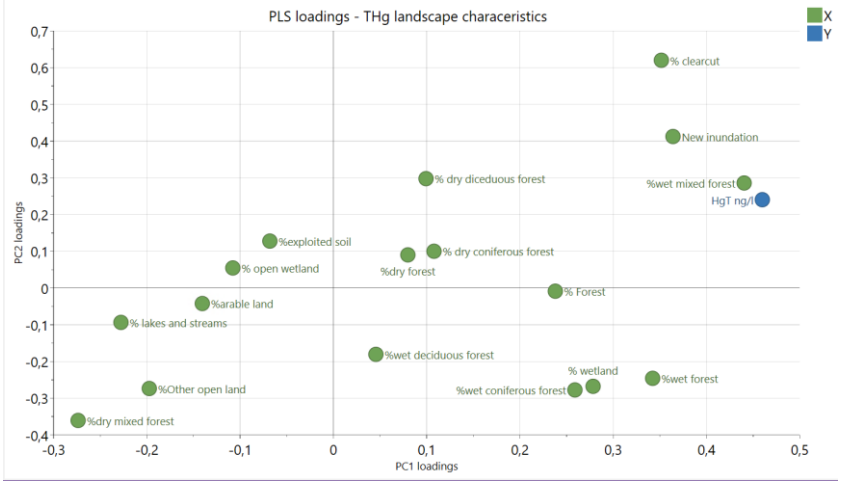
The landscape in the catchments included in this study is dominated by forest of which the main part is dry coniferous forest. Wetlands did, on average, account for less than ¼ of the catchment area (Section 3.1). Both %wetland and %coniferous forest have shown to be appropriate predictors for Hg methylation in boreal environments, due to their physicochemical conditions (St Louis et al. 1993; Hall et al. 2005). A PLS analysis using landscape characteristics and inundation type as explanatory variables was conducted to explore the impact of land cover and use on the variation in THg and MeHg. The PLS model using THg as the response variable explained 35% (R^2Y) of the variance in THg concentrations with the two first components (R^2X=31%, Q^2=-21%). The variables with VIP above one (Table 5) were positively correlated to THg and associated with the wetness of and amount of carbon rich soil/OM in the catchment, as well as the extent of forest (Figure 10). When using MeHg as the dependent variable the PLS model explained 40% (R^2Y) of the variance with the two first components (R^2X=38%, Q^2=-16%). The variables explaining the variation in MeHg were similar to the variables contributing to the variation in THg. Contemplating the results of the PLS models, the chemical factors tend

to be the better predictor for the variations in THg and MeHg concentrations. A recent study (Lam et al., 2022) also found a positive relation between THg- and MeHg export and % wet forest in boreal catchments. They also found that the wetness of the landscape supports the mobilisation of OM and associated Hg, which in fact result in a greater Hg export as well as favourable conditions for Hg methylation (Lam et al. 2022). However, they found %coniferous cover to be the most significant predictor of Hg methylation. Increased Hg methylation at coniferous sites can be linked to the larger throughfall of Hg as well as more readily leaching of acidic humic substances, which lowers the pH in receiving waters to promote methylation (Johansson et al. 2001; St. Louis et al. 2001). In areas with extensive wetland cover such as restored wetlands and natural wetlands, % wetland might though be a better metric for predicting THg and MeHg concentrations (Lam et al. 2022). Catalán et al. (2017) found strong variation between systems, with both increase and decrease in DOM in impoundments, compared to upstream, even in ponds of the same age. This suggests that catchment- and landscape specific characteristics have a greater influence on the DOM quantity and quality in the system than the impoundment itself.

Table 5. Statistics for PLS analysis with landscape characteristics. Shows VIP-values for the X variables having a significant influence on the variance in THg and MeHg.

Y variable	X variable	VIP	R^2X	R^2Y	Q^2
THg	% wet mixed forest	1.63	31%	35%	-21%
	% clearcut	1.55			
	% wet forest	1.45			
	New inundation	1.41			
	% wetland	1.26			
	%Wet coniferous forest	1.19			
	%Dry mixed forest	1.10			
MeHg	% wet mixed forest	2.13	38%	40%	-16%
	New inundation	1.65			
	% Forest	1.24			
	% wet forest	1.54			
	% open wetland	1.09			
	% wetland	1.08			

a)



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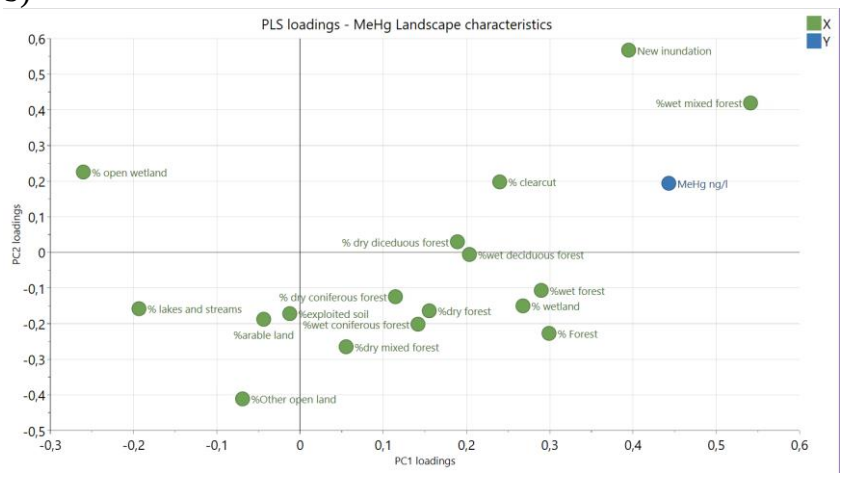

Figure 10. PLS loading plot showing how landscape characteristics influence the variation in THg (a) and MeHg (b).

5. Conclusions and Implications for Nature Management

The aim of this study was to assess the effect of new inundations in the forest landscape induced by respectively beaver and human activity on the concentrations of THg and MeHg in water and benthic fauna. Restored wetlands were found to release THg and TOC, whereas beaver impoundments were hotspots for the formation of new MeHg indicated by high %MeHg of THg. This indicates that the methylation efficiency in saturated systems is not controlled by the concentration of THg. A large variation in MeHg concentrations in restored wetlands also indicates that Hg methylation is impacted by several factors. The inundation itself did not seem to affect the Hg concentrations in benthic fauna, which implies that the variation was rather explained by differences in the trophic level among the groups of benthic fauna. The variation in THg and MeHg concentrations showed to be positively correlated to the quantity and quality of OM in the water as well as to the extent of wetland and coniferous forest in the catchment. Overall, this study shows that the processes of Hg mobilisation and transformation in newly inundated and rewetted forest systems are complex and regulated by the intrinsic properties of the catchment and the inundated site itself.

The conclusions made upon this study, suggests that the risk of elevated Hg methylation in restored wetlands is lower, compared to beaver impoundments than first anticipated, which is positive from a restoration point of view. Soils that have already been flooded are less sensitive to flooding than well drained upland soils. On the contrary, flooding of previously drained peat soils results in release of THg and TOC. The difference is most likely due to the quality of OM in the flooded area and the properties of the catchment. Flooding of upland soils releases easily degradable humic substances and promotes the formation of algal derived OM which tend to fuel Hg methylation (Catalán et al. 2017; Bravo et al. 2018; Herrero Ortega et al. 2018). The OM in already or previously water saturated organic soils is older, more humified, and thus less bioavailable.

This study did in accordance with previous research show that %wetland in a catchment is an important driver for MeHg methylation (%MeHg) (Louis et al. 1996; St. Louis et al. 2004; Lam et al. 2022). Natural wetlands showed to release both MeHg, THg, and TOC, however not in the same quantities as restored wetlands. It is hard to conclude whether the release of THg and MeHg from restored wetlands eventually will decrease to pre-drainage levels. The drained reference sites for restored wetlands retain THg, MeHg, and TOC, and might, in theory, be the environment with the lowest hazard related to Hg contamination. However, the drained soils are very sensitive to flooding, which will result in initial release of a large pool of Hg and associated OM, which might still put pressure on the aquatic environment, as THg will reach a system with conditions favourable for Hg methylation.

The increasing beaver population in the boreal landscape stresses the need for understanding the influence of beaver activity on Hg methylation and bioconcentration in the impoundments and receiving surface waters. Beaver inundations create new wetland-like environments following the flooding of both upland and lowland soils. Development and implementation of

beaver management strategies to prevent beavers from flooding upland soils might lower the risk of subsequent Hg methylation in the flooded area.

Despite decreasing deposition, the large terrestrial pool of Hg keeps increasing. This makes forest catchments sensitive to any activities that may result in mobilisation or methylation of Hg, and boreal waterbodies are in fact already impacted by Hg contamination. Hg levels in freshwater fish in Swedish lakes are far above the EU environmental quality standard of 0.02 mg/kg (Åkerblom et al. 2014) which might, in fact, have adverse effects on human health. This study showed that the environmental benefits of wetland restoration and beaver impoundments such as biodiversity conservation, improved carbon sequestration, water-, and nutrient retention, may come with the trade-off of deterioration of the water quality in and downstream of the flooded area. In order to provide the best future for humans and nature, further research is needed to understand how to minimise the negative effects related to inundation of boreal soils.

References

Ågren, A.M., Hasselquist, E.M., Stendahl, J., Nilsson, M.B. & Paul, S.S. (2022). Delineating the distribution of mineral and peat soils at the landscape scale in northern boreal regions. *SOIL*, 8 (2), 733–749. https://doi.org/10.5194/soil-8-733-2022

Åkerblom, S., Bignert, A., Meili, M., Sonesten, L. & Sundbom, M. (2014). Half a century of changing mercury levels in Swedish freshwater fish. *Ambio*, 43 (1), 91–103. https://doi.org/10.1007/s13280-014-0564-1

Bishop, K., Allan, C., Bringmark, L., Garcia, E., Hellsten, S., Högbom, L., Johansson, K., Lomander, A., Meili, M., Munthe, J., Nilsson, M., Porvari, P., Skyllberg, U., Sørensen, R. & Zetterberg, T. (2009). *The Effects of Forestry on Hg Bioaccumulation in Nemoral/Boreal Waters and Recommendations for Good Silvicultural Practice*

Bishop, K., Shanley, J.B., Riscassi, A., de Wit, H.A., Eklöf, K., Meng, B., Mitchell, C., Osterwalder, S., Schuster, P.F., Webster, J. & Zhu, W. (2020). Recent advances in understanding and measurement of mercury in the environment: Terrestrial Hg cycling. *Science of the Total Environment*, 721. https://doi.org/10.1016/j.scitotenv.2020.137647

Branfireun, B.A., Cosio, C., Poulain, A.J., Riise, G. & Bravo, A.G. (2020). Mercury cycling in freshwater systems - An updated conceptual model. *Science of the Total Environment*, 745. https://doi.org/10.1016/j.scitotenv.2020.140906

Branfireun, B.A. & Roulet, N.T. (2002). *Controls on the fate and transport of methylmercury in a boreal headwater catchment, northwestern Ontario, Canada*

Bravo, A.G., Bouchet, S., Tolu, J., Björn, E., Mateos-Rivera, A. & Bertilsson, S. (2017). Molecular composition of organic matter controls methylmercury formation in boreal lakes. *Nature Communications*, 8. https://doi.org/10.1038/ncomms14255

Bravo, A.G., Kothawala, D.N., Attermeyer, K., Tessier, E., Bodmer, P., Ledesma, J.L.J., Audet, J., Casas-Ruiz, J.P., Catalán, N., Cauvy-Fraunié, S., Colls, M., Deininger, A., Evtimova, V. V., Fonvielle, J.A., Fuß, T., Gilbert, P., Herrero Ortega, S., Liu, L., Mendoza-Lera, C., Monteiro, J., Mor, J.R., Nagler, M., Niedrist, G.H., Nydahl, A.C., Pastor, A., Pegg, J., Gutmann Roberts, C., Pilotto, F., Portela, A.P., González-Quijano, C.R., Romero, F., Rulík, M. & Amouroux, D. (2018). The interplay between total mercury, methylmercury and dissolved organic matter in fluvial systems: A latitudinal study across Europe. *Water Research*, 144, 172–182. https://doi.org/10.1016/j.watres.2018.06.064

Bringmark, L., Lundin, L., Augustaitis, A., Beudert, B., Dieffenbach-Fries, H., Dirnböck, T., Grabner, M.T., Hutchins, M., Kram, P., Lyulko, I., Ruoho-Airola, T. & Vana, M. (2013). Trace metal budgets for forested catchments in Europe-Pb, Cd, Hg, Cu and Zn. *Water, Air, and Soil Pollution*, 224 (4). https://doi.org/10.1007/s11270-013-1502-8

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. https://doi.org/10.1007/s10750-016-2766-y

Compeau, G.C. & Bartha, R. (1985). *Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sedimentt*. https://journals.asm.org/journal/aem

Ecke, F., Levanoni, O., Audet, J., Carlson, P., Eklöf, K., Hartman, G., McKie, B., Ledesma, J., Segersten, J., Truchy, A. & Futter, M. (2017). Meta-analysis of environmental effects of beaver in relation to artificial dams. *Environmental Research Letters*. Institute of Physics Publishing. https://doi.org/10.1088/1748-9326/aa8979

Eckley, C.S., Luxton, T.P., Goetz, J. & McKernan, J. (2017). Water-level fluctuations influence sediment porewater chemistry and methylmercury production in a flood-control reservoir. *Environmental Pollution*, 222, 32–41. https://doi.org/10.1016/J.ENVPOL.2017.01.010

Eklöf, K. (2022). *Newly inundated forest landscapes as hotspots for mercury bioaccumulation and transfer across the aquatic-terrestrial food web (INFORM-Hg)*. *Swedish University of Agricultural Science*. https://www.slu.se/en/departments/aquatic-sciences-assessment/research/forskningsprojekt/active-research-projects/gh/inform-hg/ [2023-03-16]

Galloway, M.E. & Branfireun, B.A. (2004). Mercury dynamics of a temperate forested wetland. *Science of The Total Environment*, 325 (1–3), 239–254. https://doi.org/10.1016/J.SCITOTENV.2003.11.010

Gilmour, C.C. & Henry, E.A. (1991). Mercury methylation in aquatic systems affected by acid deposition. *Environmental pollution (Barking, Essex : 1987)*, 71 (2–4), 131–169. https://doi.org/10.1016/0269-7491(91)90031-Q

Grigal, D.F. (2003). Mercury Sequestration in Forests and Peatlands. *Journal of Environmental Quality*, 32 (2), 393–405. https://doi.org/10.2134/jeq2003.3930

Hall, B.D., Cherewyk, K.A., Paterson, M.J., Drew, R. & Bodaly, A. (2009). Changes in methyl mercury concentrations in zooplankton from four experimental reservoirs with differing amounts of carbon in the flooded catchments. *Canadian Journal of Fisheries and Aquatic Sciences*, 66, 1910–1919. https://doi.org/10.1139/F09-123

Hall, B.D., St. Louis, V.L., Rolfhus, K.R., Bodaly, R.A., Beaty, K.G., Paterson, M.J. & Cherewyk, K.A.P. (2005). Impacts of reservoir creation on the biogeochemical cycling of methyl mercury and total mercury in boreal upland forests. *Ecosystems*, 8 (3), 248–266. https://doi.org/10.1007/s10021-003-0094-3

Halley, D., Rosell, F. & Saveljev, A. (2012). Population an distribution of Eurasian Beaver (Castor fiber). *Baltic Forestry*, 18 (1), 168–175

Herrero Ortega, S., Catalán, N., Björn, E., Gröntoft, H., Hilmarsson, T.G., Bertilsson, S., Wu, P., Bishop, K., Levanoni, O. & Bravo, A.G. (2018). High methylmercury formation in ponds fueled by fresh humic and algal derived organic matter. *Limnology and Oceanography*, 63, S44–S53. https://doi.org/10.1002/lno.10722

Jackson, T.A. (1996). *Mercury in Aquatic Ecosystems*

Johansson, K., Bergbäck, B. & Tyler, G. (2001). *IMPACT OF ATMOSPHERIC LONG RANGE TRANSPORT OF LEAD, MERCURY AND CADMIUM ON THE SWEDISH FOREST ENVIRONMENT*

Lam, W.Y., Mackereth, R.W. & Mitchell, C.P.J. (2022). Landscape controls on total mercury and methylmercury export from small boreal forest catchments. *Biogeochemistry*, 160 (1), 89–104. https://doi.org/10.1007/s10533-022-00941-9

Lavoie, R.A., Amyot, M. & Lapierre, J.F. (2019). Global Meta-Analysis on the Relationship Between Mercury and Dissolved Organic Carbon in Freshwater Environments. *Journal of Geophysical Research: Biogeosciences*, 124 (6), 1508–1523. https://doi.org/10.1029/2018JG004896

Levanoni, O., Bishop, K., Mckie, B.G., Hartman, G., Eklöf, K. & Ecke, F. (2015). Impact of Beaver Pond Colonization History on Methylmercury Concentrations in Surface Water. *Environmental Science and Technology*, 49 (21), 12679–12687. https://doi.org/10.1021/acs.est.5b03146

St. Louis, V.L., Rudd, J.W.M., Kelley, C.A., Rolfhus, K.R., Lindberg, S.E. & Dong, W. (2001). Importance of the Forest Canopy to Fluxes of Methyl Mercury and Total Mercury to Boreal Ecosystems. *Environmental Science and Technology*, 35, 3089–3098. https://doi.org/10.1021/es001924p

St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Bodaly, R.A., Paterson, M.J., Beaty, K.G., Hesslein, R.H., Heyes, A. & Majewski, A.R. (2004). The Rise and Fall of Mercury Methylation in an Experimental Reservoir. *Environmental Science and Technology*, 38 (5), 1348–1358. https://doi.org/10.1021/es034424f

Louis, V.L.S.T., Rudd, J.W.M., Kelly, C.A., Beaty, K.G., Flett, R.J. & Roulet, N.T. (1996). Production and loss of methylmercury and loss of total mercury from boreal forest catchments containing different types of wetlands. *Environmental Science and Technology*, 30 (9), 2719–2729. https://doi.org/10.1021/es950856h

Mitchell, C.P.J., Branfireun Brian A & Kolka, R.K. (2008). Spatial Characteristics of Net Methylmercury Production Hot Spots in Peatlands. *Environmental Science and Technology*, 42. https://doi.org/10.1021/es0704986

Olsson, B. & Ledwith, M. (2020). *NMD2018 Product Description ENG*

Painter, K.J., Westbrook, C.J., Hall, B.D., O'Driscoll, N.J., Jardine, T.D. & Peters, D.P.C. (2015). Effects of in-channel beaver impoundments on mercury bioaccumulation in Rocky Mountain stream food webs. *Ecosphere*, 6 (10). https://doi.org/10.1890/ES15-00167.1

Paterson, M.J., Rudd, J.W.M. & St. Louis, V. (1998). Increases in total and methylmercury in zooplankton following flooding of a peatland reservoir. *Environmental Science and Technology*, 32 (24), 3868–3874. https://doi.org/10.1021/es980343l

Roy, V., Amyot, M. & Carignan, R. (2009). Beaver ponds increase methylmercury concentrations in Canadian shield streams along vegetation and pond-age gradients. *Environmental Science and Technology*, 43 (15), 5605–5611. https://doi.org/10.1021/es901193x

Selin, N.E. (2009). Global biogeochemical cycling of mercury: A review. *Annual Review of Environment and Resources*, 34, 43–63. https://doi.org/10.1146/annurev.environ.051308.084314

Skogsstyrelsen (2022). *Utförda avverkningar - produktbeskrivning*. http://www.skogsstyrelsen.se/wms

Skyllberg, U., Qian, J., Frech, W., Xia, K. & Bleam, W.F. (2003). Distribution of mercury, methyl mercury and organic sulphur species in soil, soil solution and stream of a boreal forest catchment. [2023-04-11]

SS-EN 27828 (1994). Water quality - Methods for biological sampling - Guidance on hand-net sampling of aquatic benthic macro-invertebrates

St Louis, V., Rudd, john W., Kelly, C.A., Beaty, K.G., Bloom, N.S. & Flett, R.J. (1993). Importance of Wetlands as Sources of Methyl Mercury to Boreal Forest Ecosystems. *Can. J. Fish. Aquat. Sci*, 51, 1065–1076. www.nrcresearchpress.com

Swedish Environmental Protection Agency (2018). *SWEDEN'S ENVIRONMENTAL OBJECTIVES - AN INTRODUCTION*. Stockholm.

Tjerngren, I., Karlsson, T., Björn, E. & Skyllberg, U. (2012a). Potential Hg methylation and MeHg demethylation rates related to the nutrient status of different boreal wetlands. *Biogeochemistry*, 108 (1–3), 335–350. https://doi.org/10.1007/s10533-011-9603-1

Tjerngren, I., Meili, M., Björn, E. & Skyllberg, U. (2012b). Eight boreal wetlands as sources and sinks for methyl mercury in relation to soil acidity, C/N ratio, and small-scale flooding. *Environmental Science and Technology*, 46 (15), 8052–8060. https://doi.org/10.1021/es300845x

Tremblay, A., Cloutier, L. & Lucotte, M. (1998). *Total mercury and methylmercury fluxes via emerging insects in recently flooded hydroelectric reservoirs and a natural lake*

Tremblay, A. & Lucotte, M. (1997). Accumulation of total mercury and methyl mercury in insect larvae of hydroelectric reservoirs. *Canadian Journal of Fisheries and Aquatic Science*, 54, 832–841

Tremblay, A., Lucotte, M. & Rheault, I. (1996). Methylmercury in a benthic food web of two hydroelectric reservoirs and a natural lake of Northern Quebec (Canada). *Water, Air, and Soil Pollution*, 91, 255–269

UN Environment (2019). *Global Mercury Assessment 2018*. Geneva, Switzerland.

US EPA (2002). *Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*. [2023-04-27]

Varmuza, K. & Filzmoser, P. (2009). *Introduction to Multivariate Statistical Analysis in Chemometrics*. 1. ed. CRC Press, Taylor & Francis Group.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R. & Davies, P.M. (2010). Global threats to human water security and river biodiversity. *Nature*, 467 (7315), 555–561. https://doi.org/10.1038/nature09440

Wang, B., Nilsson, M.B., Eklöf, K., Hu, H., Ehnvall, B., Bravo, A.G., Zhong, S., Åkeblom, S., Björn, E., Bertilsson, S., Skyllberg, U. & Bishop, K. (2020). Opposing spatial trends in methylmercury and total mercury along a peatland chronosequence trophic gradient. *Science of the Total Environment*, 718. https://doi.org/10.1016/j.scitotenv.2020.137306

Ward, D.M., Nislow, K.H. & Folt, C.L. (2010). Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. *Annals of*

the New York Academy of Sciences. Blackwell Publishing Inc. https://doi.org/10.1111/j.1749-6632.2010.05456.x

Wu, P., Kainz, M., Åkerblom, S., Bravo, A.G., Sonesten, L., Branfireun, B., Deininger, A., Bergström, A.K. & Bishop, K. (2019). Terrestrial diet influences mercury bioaccumulation in zooplankton and macroinvertebrates in lakes with differing dissolved organic carbon concentrations. *Science of the Total Environment*, 669, 821–832. https://doi.org/10.1016/j.scitotenv.2019.03.171

Acknowledgements

First of all, I would like to thank my supervisor, researcher Karin Eklöf, Swedish University of Agricultural Sciences, for her dedicated involvement in the process of writing this thesis. I also thank her for sharing her excessive knowledge and expertise, which have made a great impression on me. I see her as a role model as a female researcher in an academic world full of men! Also, a special thanks to Professor Peter Engelund Holm, University of Copenhagen, my co-supervisor, for valuable advice and feedback during the writing process. I would also like to thank student assistants at SLU for helping to identify the study sites. This work could also not have been carried out without the research assistants, Kajsa and David. Thanks a lot to them for taking samples in all weathers wearing oversized waders, for taking beautiful photos of wetlands, and for bringing me on fieldwork.
Last but not least, I would like to thank my flatmates and friends for providing an excellent emotional support system and for being part of this experience. Without them, it would not have been fun at all! Not to mention my parents and my partner for their support and encouragement through all my years of studying. The project was funded by Formas (#2020-00962).

Kirstine Evald Andersson

Popular Science Summary

Mercury is a heavy metal, present in the air, soil, and water. Soils, especially the ones with a lot of organic material, can hold a lot of mercury, which has been emitted due to natural and human activities for thousands of years. At first glance the presence of mercury in soil and water might not sound like a serious problem. But in fact, it is, because a certain form of mercury; methylmercury, can be transferred from water to organisms like insects, fish, and birds and might end up in humans. Methylmercury is produced by specific groups of microorganisms thalivees in environments with a lack of (or low) oxygen. Water saturated systems such as lakes and wetlands offer good conditions for the microorganisms that produce methylmercury. Especially water saturated systems in boreal environments, that holds both a large pool of mercury and high availability of organic material, which is a source of energy for the microorganisms.

The mobilization and methylation of mercury in forest soils can also be impacted by disturbances. This study has evaluated how the mercury concentrations in water and benthic insects are affected by wetland restoration and beaver dams. During the past 100-150 years wetlands have been drained by digging ditches to increase forest production. Today many drained wetlands are being rewetted in order to favour biodiversity conservation, carbon sequestration and water holding capacity. The Swedish beaver population is recovering after being almost extinct. Beavers create impoundments where they can forage safely and build their lodges. The areas where beavers build their dams are often rich in organic matter, and thereby also mercury. One might obviously assume that flooding of well-drained, OM and possibly also mercury rich soils will result in release of inorganic mercury, as well as formation of methylmercury and increase the concentrations in both water and benthic fauna. To investigate this hypothesis we sampled water and benthic fauna at the outlet of 72 flooded sites across central Sweden: 15 restored wetlands, 15 beaver ponds, 12 natural wetlands, and 15 reference sites for restored wetlands and beaver ponds, respectively.

We found that mercury was methylated in beaver impoundments, but not in restored wetlands. The restored wetlands did instead release a large amount of the mercury that was stored in the soil, as well as the organic carbon it is bound to. Total mercury concentrations in restored wetlands were also higher than in natural wetlands. We did not find any increase in mercury concentrations in the insects downstream of either beaver ponds nor restored wetlands compared to their references. We did however find higher concentrations of mercury in insects higher up in the food chain. Overall, this study showed that the two different disturbances influence the fate of mercury in different ways, and that it depends on the properties of the flooded area itself.

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Appendix 1 - Site description

*Table A. 1 Sampling locations: coordinates (*SWEREF_99TM), Treatment, County, and Town*

Site	Latitude*	Longitude*	Treatment	County	Town
B1	6619427	561396	Beaver pond	Västmanland	Surahammar
B1C	6618814	561242	Control	Västmanland	Surahammar
B2	6628996	552508	Beaver pond	Västmanland	Skinnskatteberg
B2C	6626534	554067	Control	Västmanland	Skinnskatteberg
B3	6722938	631433	Beaver pond	Uppland	Älvkarleby
B3C	6724940	638181	Control	Uppland	Älvkarleby
B4	6525962	637225	Beaver pond	Södermanland	Nynäs
B4C	6522911	636284	Control	Södermanland	Nynäs
B5	6640031	495637	Beaver pond	Örebro	Ljusnaberg
B5C	6639267	495469	Control	Örebro	Ljusnaberg
B6	6508868	507684	Beaver pond	Östergötland	Motala
B6C	6512699	506593	Control	Östergötland	Motala
B7	6507632	503385	Beaver pond	Östergötland	Askersund
B7C	6507163	502813	Control	Östergötland	Askersund
B8	6564985	470328	Beaver pond	Örebro	Degerfors
B8C	6565911	461639	Control	Örebro	Degerfors
B9	6566529	460910	Beaver pond	Örebro	Degerfors
B9C	6566758	472441	Control	Örebro	Degerfors
B10	6531092	480744	Beaver pond	Örebro	Laxå
B10C	6532882	479765	Control	Örebro	Laxå
B11	6717388	631770	Beaver pond	Uppland	Älvkarleby
B11C	6717277	632429	Control	Uppland	Älvkarleby
B12	6640967	527893	Beaver pond	Västmanland	Skinnskatteberg
B12C	6640128	527205	Control	Västmanland	Skinnskatteberg
B13	6415860	441629	Beaver pond	Jönköping	Habo
B13C	6412564	439732	Control	Jönköping	Habo
B14	6511586	544839	Beaver pond	Östergötland	Finspång

B14C	6511799	543164	Control	Östergötland	Finspång
B15	6509425	582800	Beaver pond	Östergötland	Norrköping
B15C	6509637	581055	Control	Östergötland	Norrköping
N1	6623991	492900	Natural wetland	Örebro	Kindla
N2	6623191	494189	Natural wetland	Örebro	Kindla
N3	6621760	498695	Natural wetland	Örebro	Hällabomossen
N4	6523185	516742	Natural wetland	Östergötland	Motala
N5	6539403	512475	Natural wetland	Örebro	Skåle
N6	6388857	449533	Natural wetland	Jonköping	Farbergskärret
N7	6391924	434032	Natural wetland	Jönköping	Angerdshestra prästskog
N8	6400594	443269	Natural wetland	Jönköping	Dumme mosse point 2
N9	6442513	524162	Natural wetland	Östergötaland	Ycke_point_2
N10	6503958	552974	Natural wetland	Östergötaland	Ängenäs
N11	6429683	524012	Natural wetland	Östergötaland	Lillörens
N12	6563237	580601	Natural wetland	Södermanland	Varglyans
R1	6647092	520888	Restored wetland	Dalarna	Stormossen
R1C	6647530	520655	Control	Dalarna	Stormossen
R2	6644050	527608	Restored wetland	Dalarna	Kungamossen
R2C	6644860	527984	Control	Dalarna	Kungamossen
R3	6638305	528236	Restored wetland	Dalarna	Djurgårdsmossen
R3C	6638115	527929	Control	Dalarna	Djurgårdsmossen
R4	6522227	637919	Restored wetland	Södermanland	Nynäs
R4C	6526171	637019	Control	Södermanland	Nynäs
R5	6636591	490538	Restored wetland	Örebro	Nittälvens
R5C	6636306	491402	Control	Örebro	Nittälvens
R6	6644746	521171	Restored wetland	Dalarna	Blankmossen
R6C	6644199	519582	Control	Dalarna	Blankmossen
R7	6501543	513029	Restored wetland	Östergötaland	Bromossen
R7C	6502324	513761	Control	Östergötaland	Bromossen
R8	6497620	526518	Restored wetland	Östergötaland	Getmans rike

R8C	6497802	527130	Control	Östergötaland	Getmans rike
R9	6546907	541107	Restored wetland	Södermanland	Perstorpsskogen
R9C	6546742	541905	Control	Södermanland	Perstorpsskogen
R10	6716473	651802	Restored wetland	Uppsala län	Gölarna
R10C	6717535	654425	Control	Uppsala län	Gölarna
R11	6686334	590651	Restored wetland	Gävleborg	Stormosse
R11C	6688286	594692	Control	Gävleborg	Stormosse
R12	6369754	467380	Restored wetland	Jönköping	Västernarken
R12C	6370664	466547	Control	Jönköping	Västermarken
R13	6364781	421905	Restored wetland	Jönköping	Klosjöns
R13C	6364052	421480	Control	Jönköping	Klosjöns
R14	6389937	423405	Restored wetland	Jönköping	Komosse
R14C	6389414	423359	Control	Jönköping	Komosse
R15	6356401	414846	Restored wetland	Jönköping	Anderstorps Stormosse
R15C	6354846	414188	Control	Jönköping	Anderstorps Stormosse

Table A 2. Photos of selected sites (David Kniha & Kajsa Weslien, 2022).

Beaver ponds	 *B3*	 *B11*	 *B13*
Restored wetlands	 *R3*	 *R4*	 *R6*
Natural wetlands	 *N1*	 *N10*	 *N11*

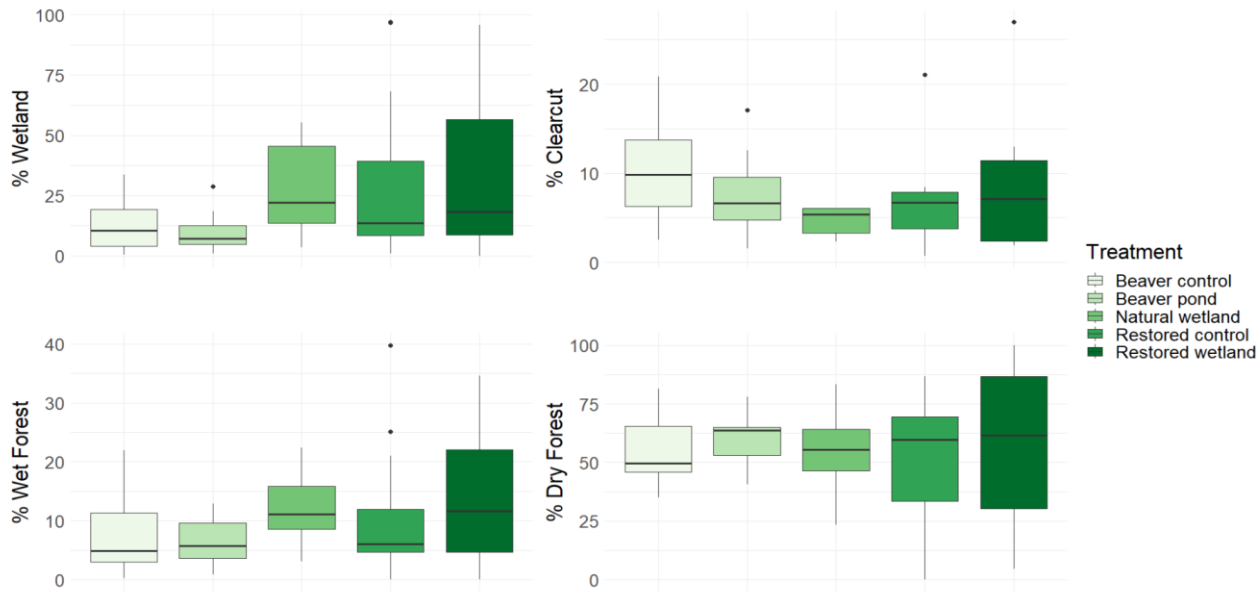

Figure A 1. Selected landscape characteristics

Appendix 2 - Description of Benthic Fauna

Table A 3. Taxonomy benthic fauna

Order	Sub order	Family	Genus/species
Odonata	*Zygoptera*	*Coenagrionidae*	*Pyrrhosoma nymphula*
		Calopterygidae	*Calopteryx splendens*
			Calopteryx sp.
	Anisoptera	*Aeshnidae*	*Aeshna cyanea*
			Aeshna sp.
		Corduliidae	*Somatochlora metallica*
			Somatochlora flavomaculata
			Cordulia aenea
		Libellulidae	*Libellula depressa*
		Gomphidae	*Onychogomphus forcipatus*
Isopoda	*Asellota*	*Asellidae*	*Asellus aquaticus*
Diptera	*Nematocera*	*Chiornomidae*	*Orthocladiinae*
Hemiptera	*Heteroptera*	*Corixinae*	*Sigara sp.*
Coleoptera	*Adephaga*	*Dytiscidae*	*Agabus bipustulatus*
			Hydroporus sp.
			Agabus melanarius
			Agabus sp.
Amphipoda	*Senticaudata*	*Gammaridae*	*Gammarus pulex*
Trichoptera	*Integripalpia*	*Limnephilidae*	*Glyphotaelius pellucidus*
	Annulipalpia	*Polycentropodidae*	*Plectrocnemia sp.*
			Polycentropus irroratus
			Holocentropus dubius
			Polycentropus flavomaculatus
	Integripalpia	*Phryganeidae*	*Oligotricha lapponica*
			Semblis atrata
			Oligostomis reticulata
Hygrophila		*Planorbidae*	*Bathyomphalus contortus*
Megaloptera		*Sialidae*	*Sialis lutaria*

Table A 4. Fauna found at each site.

Site	Fauna
B1	*Aeshna cyanea* *Asellus aquaticus* *Bathyomphalus contortus* *Coenagrionidae*
B1C	*Asellus aquaticus* *Plectrocnemia sp.*
B2	*Aeshna cyanea* *Asellus aquaticus* *Orthocladiinae* *Limnepilidae* *Polycentropus irroratus* *Coenagrionidae*
B2C	*Asellus aquaticus* *Oligotricha striata*
B3	*Asellus aquaticus* *Gammarus pulex* *Sialis lutaria*
B3C	*Asellus aquaticus* *Beaver Sialis lutaria*
B4	*Somatochlora metallica* *Sialis lutaria* *Calopteryx splendens*
B4C	Asellus aquaticus
B5	*Asellus aquaticus* *Limnepilidae*
B5C	*Aeshna cyanea* *Asellus aquaticus* *Plectrocnemia sp.*
B6	*Somatochlora flavomaculata* *Asellus aquaticus* *Sialis lutaria*
B6C	*Asellus aquaticus* *Plectrocnemia sp.*
B7	*Asellus aquaticus* *Limnepilidae* *Sialis lutaria*
B7C	*Limnepilidae*
B8C	*Plectrocnemia sp.*
B9	*Asellus aquaticus* *Limnepilidae* *Sialis lutaria*
B9C	*Aeshna sp.* *Asellus aquaticus* *Plectrocnemia sp.*
B10	*Somatochlora metallica* *Asellus aquaticus* *Limnepilidae* *Sialis lutaria*
B10C	*Asellus aquaticus* *Plectrocnemia sp.* *Pyrrhosoma nymphula*
B11	*Asellus aquaticus* *Limnepilidae*
B11C	*Asellus aquaticus* *Limnepilidae*
B12	*Asellus aquaticus* *Plectrocnemia sp.*
B12C	*Limnepilidae*
B13	*Aeshna sp.* *Asellus aquaticus* *Limnepilidae* *Sialis lutaria* *Pyrrhosoma nymphula*
B13C	*Glyphotaelius pellucidus*
N5	*Asellus aquaticus* *Plectrocnemia sp.*
N6	*Somatochlora metallica* *Asellus aquaticus* *Limnephilidae* *Sialis lutaria* *Pyrrhosoma nymphula*
N7	*Asellus aquaticus* *Plectrocnemia sp.*
N8	*Asellus aquaticus* *Limnephilidae* *Pyrrhosoma nymphula*
N9	*Asellus aquaticus* *Semblis atrata*
N10	*Limnephilidae*
N11	*Somatochlora metallica* *Asellus aquaticus* *Glyphotaelius pellucidus* *Sialis lutaria* *Calopteryx sp.*
N12	*Asellus aquaticus* *Limnephilidae*
R1	*Cordulia aenea* *Asellus aquaticus* *Oligotricha lapponica*
R1C	*Plectrocnemia sp.*
R2	*Asellus aquaticus* *Plectrocnemia sp.*
R2C	*Aeshna sp.* *Asellus aquaticus* *Pyrrhosoma nymphula*
R3	*Agabus bipustulatus* *Hydroporus sp.*
R3C	*Restored wetland*

Site	Fauna
R5C	*Asellus aquaticus*
	Agabus sp.
R6	*Aeshna sp.*
	Asellus aquaticus
	Plectrocnemia sp.
R6C	*Cordulia aenea*
	Asellus aquaticus
	Plectrocnemia sp.
	Pyrrhosoma nymphula
R7	*Limnephilidae*
R7C	*Asellus aquaticus*
	Plectrocnemia sp.
R8	*Hygrotus sp.*
	Semblis atrata
R8C	*Limnephilidae*
R9	*Asellus aquaticus*
	Limnephilidae
R9C	*Asellus aquaticus*
	Limnephilidae
R10	*Asellus aquaticus*
	Limnephilidae
R10C	*Limnephilidae*
R11	*Aeshna sp.*
	Asellus aquaticus
R11C	*Asellus aquaticus*
	Plectrocnemia sp.
R12	*Asellus aquaticus*
	Limnephilidae
R12C	*Aeshna sp.*
	Plectrocnemia sp.
	Sialis lutaria
R13	*Aeshna sp.*
	Limnephilidae
R13C	*Asellus aquaticus*
	Plectrocnemia sp.
R14	*Plectrocnemia sp.*
R14C	*Limnephilidae*
R15	*Semblis atrata*
R15C	

Appendix 3 - Mercury Results

Table A 5. Mean, standard error, min, max and median for THg (ng/l), MeHg (ng/l) and %MeHg in water.

	Mean	SE	Min	Max	Median
Beaver pond					
THg	5.88	0.55	2.60	10.0	5.80
MeHg	0.73	0.17	0.17	2.9	0.57
%MeHg	11.98	1.74	3.58	29.0	11.06
Beaver control					
THg	4.53	0.51	1.30	7.90	4.50
MeHg	0.41	0.10	0.07	1.60	0.26
%MeHg	8.18	0.49	2.40	24.62	7.53
Restored wetland					
THg	15.03	3.44	4.00	56.8	10.00
MeHg	0.91	0.19	0.13	3.2	0.67
%MeHg	7.29	1.41	1.91	23.5	5.70
Restored control					
THg	6.85	1.12	2.40	19.0	5.10
MeHg	0.52	0.10	0.10	1.50	0.40
%MeHg	9.10	1.94	1.89	24.58	6.25
Natural wetland					
THg	5.68	0.65	2.60	9.10	5.55
MeHg	0.33	0.08	0.12	0.86	0.21
%MeHg	5.96	1.35	<0.06	15.36	4.71

Table A 6. Difference in THg, MeHg and %MeHg for beaver ponds and restored wetlands and their respective control sites.

	ΔTHg (ng/l)	**ΔMeHg (ng/l)**	**Δ%MeHg**
B-BC	1.35±0.56	0.33±0.19	4.09±1.79
R-RC	7.19±3.17	0.36±0.21	-1.85±2.55

Table A 7. THg in benthic fauna

	Beaver pond	**Beaver control**	**Restored wetland**	**Restored control**	**Natural wetland**
Trichoptera	280±119	251±86	260±63	314±130	144±33
Anisoptera	806±350	741±488	350±140	434±126	292±65
Megaloptera	173±47	63±0.23	NA	112*	133±65
Asellus	605±125	340±105	510±115	383±65	330±102
Zygoptera	398±155	441*	NA	296±30	198±25
Other	137±92	NA	234±39	645*	NA

Appendix 4 - Water Quality Analysis

Table A 8. Results of water quality analysis (Mean and standard error)

	Beaver pond		**Beaver control**		**Restored wetland**		**Restored control**		**Natural wetland**	
	mean	***se***	***mean***	***se***	***mean***	***se***	***mean***	***se***	***mean***	***se***
Dissolved O_2 mg/l	7.89	0.73	9.00	0.42	5.02	0.67	8.76	0.36	7.19	1.00
pH	6.06	0.19	6.16	0.23	4.85	0.28	5.38	0.24	5.13	0.32
Conductivity mS/m25	9.00	2.30	15.3	5.45	9.42	3.20	7.91	2.72	6.51	0.82
Alkalinity meq/l	0.45	0.21	0.78	0.36	0.23	0.32	0.23	0.21	0.03	0.10
Abs F 420/5	0.50	0.05	0.41	0.08	1.29	0.21	0.57	0.06	0.69	0.09
Abs F 436/5	0.40	0.04	0.32	0.07	1.02	0.17	0.45	0.05	0.54	0.07
TOC mg/l	27.17	2.27	24.51	4.40	63.29	9.77	30.01	2.97	38.68	5.91
Tot-N µg/l	$9.16*10^2$	$0.94*10^2$	$8.90*10^2$	$1.71*10^2$	$2.04*10^3$	$3.99*10^2$	$1.01*10^3$	$1.96*10^2$	$9.04*10^2$	$1.08*10^2$
C/N ratio	30.7	1.63	29.7	2.65	34.6	2.3	37.2	4.1	43.8	4.51
NO2+NO3-N µg/l	89.60	20.61	$1.9*10^2$	98.45	80.33	29.14	$2.02*10^2$	$1.40*10^2$	65.3	26.0
SO4 meq/l	0.15	0.05	0.23	0.07	0.10	0.07	0.17	0.07	0.07	0.02
Tot_P µg/l	32.75	9.93	16.05	2.41	51.74	9.69	18.54	3.93	15.07	4.03
Cl meq/l	0.13	0.01	0.36	0.24	0.12	0.02	0.12	0.02	0.12	0.02
Ca meq/l	0.62	0.24	0.92	0.39	0.55	0.32	0.46	0.26	0.28	0.09
Mg meq/l	0.14	0.02	0.24	0.10	0.14	0.05	0.12	0.03	0.09	0.01
Na meq/l	0.17	0.01	0.36	0.19	0.15	0.02	0.17	0.03	0.14	0.02
K meq/l	0.03	0.00	0.04	0.01	0.03	0.01	0.03	0.01	0.01	0.00
Si mg/l	4.59	0.36	5.63	0.39	5.03	0.54	4.50	0.71	3.77	0.38

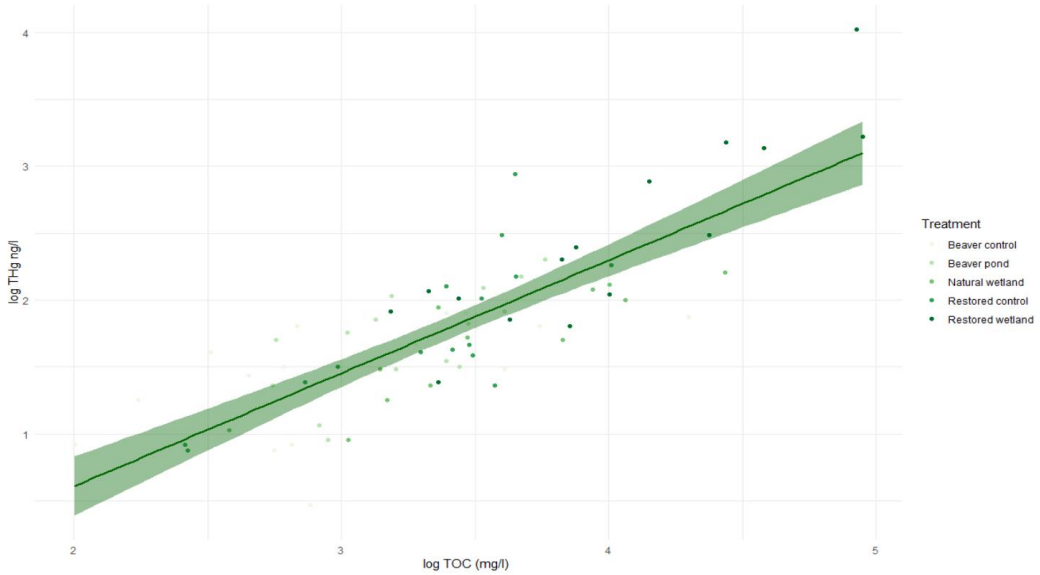

Figure A 2. Relationship between THg and TOC in water.