Mercury in fish from Norwegian lakes:

The complex influence of aqueous organic carbon

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Abstract

Mercury (Hg) concentrations in water and biota are often positively correlated to organic matter (OM), typically measured as total or dissolved organic carbon (TOC/DOC). However, recent evidence suggests that higher OM concentrations inhibit bioaccumulation of Hg. Here, we test how TOC impacts the Hg accumulation in fish in a synoptic study of Methyl-Hg (MeHg) in water and total Hg (THg) in perch (*Perca fluviatilis*) in 34 boreal lakes in southern Norway. We found that aqueous MeHg (r^2=0.49, p<0.0001) and THg (r^2 = 0.69, p<0.0001), and fish THg (r^2=0.26, p<0.01) were all positively related with TOC. However, we found declining MeHg bioaccumulation factors (BAF_{MeHg}) for fish with increasing TOC concentrations. The significant correlation between fish THg concentrations and aqueous TOC suggests that elevated fish Hg levels in boreal regions are associated with humic lakes. The declining BAF_{MeHg} with increasing TOC suggest that increased OM promotes increased aqueous Hg concentrations, but lowers relative MeHg bioaccumulation. A mechanistic understanding of the response from OM on BAF_{MeHg} might be found in the metal-complexation properties of OM, where OM complexation of metals reduces their bioavailability. Hence, suggesting that MeHg bioaccumulation becomes less effective at higher TOC, which is particularly relevant when assessing potential responses of fish Hg to predicted future changes in OM inputs to boreal ecosystems. Increased browning of waters may affect fish Hg in opposite directions: an increase of foodweb exposure to aqueous Hg, and reduced bioavailability of Hg species. However, the negative relationship between BAF_{MeHg} and TOC is challenging to interpret, and carries a great deal of uncertainty, since this relationship may be driven by the underlying correlation between TOC and MeHg (i.e. spurious correlations). Our results suggest that the trade-off between Hg exposure and accumulation will have important implications for the effects of lake browning on Hg transport, bioavailability, and trophodynamics.

Keywords

Bioaccumulation factor, boreal, freshwater, methylmercury, organic matter, perch

1 Introduction

Mercury (Hg) can undergo long-range transport in the atmosphere, and nearly two centuries of elevated Hg deposition from anthropogenic activities (Streets et al., 2011) have led to considerable stores of Hg in catchment soils, even in remote locations (Fitzgerald et al., 1998). Inorganic forms of Hg can be methylated into the neurotoxic Methyl-Hg (MeHg), which is biomagnified in aquatic food webs with potential harmful effects on aquatic organisms (Wolfe et al., 1998) and their consumers (Scheulhammer et al., 2007), including humans (Driscoll et al., 2013; Zahir et al., 2005). Although the toxic effects of Hg have been known for more than half a century (Kurland et al., 1960), researchers still struggle to understand and describe many of the complex processes involved in the biogeochemical cycling of the element, including mechanisms controlling accumulation and biomagnification of Hg in aquatic food webs.

Throughout the boreal zone, Hg concentrations in freshwater fish often exceed national and international dietary advisory limits, typically defined from 0.5-1.0 ppm (FAO UN, 1995, UNEP, 2002, Depew et al., 2012). Elevated concentrations are confirmed for both North American (e.g. Gandhi et al., 2014) and Scandinavian lakes (e.g. Åkerblom et al., 2014, Braaten et al., 2017), with levels posing a potential risk to ecosystem and human health. Recent studies have documented widespread increases in concentrations of aqueous organic matter (OM), normally measured as dissolved organic carbon (DOC) or total organic carbon (TOC), in freshwater lakes throughout the boreal forest zone (Monteith et al., 2007), and concentrations are predicted to change markedly with future climate change (de Wit et al., 2016, i.e. altered precipitation patterns). Browning of lakes may have strong impacts on Hg transport, availability and bioaccumulation and a better understanding of the interactions between climate, biogeochemistry and bioaccumulation is needed. In particular there is a need to increase our knowledge about the most decisive factors and processes for Hg accumulation in fish, and how these processes interact. This knowledge gap limits our ability to predict future levels of Hg in fish under various environmental changes.

Aqueous OM is known to affect the cycling of Hg in aquatic environments through chemical complexation (Ravichandran, 2004), through direct and indirect impacts on photochemical degradation of MeHg (Lehnherr and Louis, 2009, Klapstein et al., 2018), and through microbial production and de-methylation mechanisms and processes (Gilmour et al., 1998). In recent decades, many studies have shown significant, positive correlations between aqueous concentrations of OM and Hg, both in water and biota (Braaten et al., 2014a; Chasar et al., 2009; Driscoll et al., 1995; Meili et al., 1991). However, more recent investigations suggest that at higher OM concentrations, Hg may be less available for uptake into aquatic food webs (e.g. French et al., 2014, Jeremiason et al., 2016, Tsui and Finlay, 2011). French et al. (2014) showed reduced bioaccumulation of both total Hg (THg) and MeHg in aquatic invertebrates (amphipods of different size classes: 250-2000 µm and >2000 µm) in Arctic lakes at DOC concentrations > 8.6 mg C L^{-1}, while increased accumulation occurred at DOC < 8.6 mg C L^{-1}. Amphipods from lakes with higher DOC concentrations had lower Hg bioaccumulation factors (BAFs), defined as the ratio between the concentration of Hg in an organism and the concentration of Hg in its surrounding environment, i.e. water (French et al., 2014). A similar threshold value (≈ 8 mg C L^{-1}) was proposed by Driscoll et al. (1994) from a limited data set of North American fish populations. French et al. (2014) suggest that the mechanism responsible for the change in BAF with OM concentrations relates to the complexation of Hg by OM, where high presence of humic acids make Hg species less bioavailable.

Herein, we wanted to investigate the relationship between aqueous concentrations of MeHg and TOC in 34 boreal lakes in southern Norway and assess the effects of TOC concentrations on Hg in perch (*Perca fluviatilis*). We explore various modelling approaches, including a BAF approach similar to that of French et al. (2014), and discuss the limitations and potential future environmental implications of our results. Hg concentrations in aquatic food webs depend strongly on the uptake of Hg at the base of the food web (Chasar et al., 2009), implying that a potential TOC threshold for Hg bioaccumulation in fish prey will also be reflected as a threshold response in fish Hg. We hypothesise that reduced bioavailability of MeHg occurs at higher TOC concentrations, leading to lower BAFs for Hg in fish.

2 Experimental

2.1 Study sites and field sampling

Our investigation is based on 34 lakes from southeast Norway, located between 58.84° N and 60.51° N, 7.96° E and 12.51° E (WGS84, see *Figure 1* and *Supporting Information* for details). All lakes are located within the boreal forest ecotone, dominated by coniferous forest and wetlands, without direct influence from agricultural activities. Lake-specific information on geographical location is attached as *Supporting Information* (*Table S1*), while water chemistry (THg, MeHg and TOC) and fish Hg concentrations are shown in *Table 1*.

Samples were collected in the early autumn (August-September) between 2008 and 2012. Perch (*Perca fluviatilis*) is the most abundant fish species in all lakes, and was collected using gill nets composed of different mesh sizes for a broad distribution of fish size. For further details regarding sampling and sample handling we refer to Braaten et al. (2014b). To be included in our calculations, fish data from each of the 34 lakes had to comprise of at least n = 10 specimens, with the mean (± one standard deviation) being n = 23 ± 7 fish (*Table 1*).

Water sampling for chemical analysis was conducted as described in Braaten et al. (2014a). In short, samples of surface water for Hg speciation were collected using 250 mL fluoropolymer bottles, following ultraclean sampling procedures to avoid contamination (USEPA, 1996). All bottles were previously unused, and water for determination of THg and MeHg was sampled in separate bottles to avoid errors resulting from loss of Hg during preservation (Creswell et al., 2016, Braaten et al., 2014c). Samples for TOC determination were collected at the same time as the samples for Hg analysis in an high-density polyethylene (HDPE) bottle.

2.2 Analytical methods

Hg concentrations in fish were determined as THg. Wet samples of muscle tissue were analysed by thermal decomposition and direct atomic absorption spectrophotometry (Lumex Mercury Analyser

RA915). Quality assurance and quality control (QA/QC) are described in detail in Braaten et al. (2014b). Relative standard deviation (RSD) of sample duplicates was < 10 % and recovery of certified reference material (DORM-3 fish protein) within 90-110 %.

The analytical method for MeHg in water was based on USEPA method 1630 (USEPA, 1998) by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry (CVAFS). The method for THg in water followed USEPA method 1631 (USEPA, 2002) by oxidation, purge and trap, and CVAFS. Method detection levels (MDLs) were 0.02 and 0.1 ng L^{-1} for MeHg and THg, respectively. For both Hg species, automated systems were used for analysis (Brooks Rand Instruments MERX). QA/QC are described in detail in Braaten et al. (2014a). RSD of sample duplicates was < 10 % and < 20 % for THg and MeHg, respectively. Recoveries of blank spikes and matrix spikes were 80-120 % for MeHg and 90-110 % for THg. Both THg and MeHg were determined on unfiltered water to allow for comparison with levels of TOC.

TOC was measured by infrared spectrophotometry according to Norwegian and European Standard NS-EN1484 with a measurement uncertainty of ± 20 % and a MDL of 0.1 mg L^{-1}.

2.3 Data collection of fish Hg measurements

Fish Hg data were collected from previous publications by Fjeld and Rognerud (2009) and Braaten et al. (2017). Mean observed fish Hg concentrations (measured as THg) in the lakes varied from 0.16 ± 0.08 to 0.68 ± 0.12 ppm wet weight (w.w., *Table 1*). Based on monitoring data for perch from Scandinavian lakes (n = 80 lakes, n = 2026 specimens), ratios of MeHg to THg are typically above 0.95 in fish muscle tissue (Braaten et al., 2017), and as such we used concentrations of THg as an estimate of MeHg. Mean fish weight and total length (± one standard deviation), including ranges (min, max), in the complete dataset (n = 776) were 75.8 ± 113.3 g (1.0 – 1141.0 g) and 16.3 ± 5.9 cm (4.7 – 44.3 cm), respectively (Data for individual lakes are shown in the *Supplementary Information*, *Table S2*).

2.4 Treatment of fish Hg data

For comparison of Hg concentration in fish between lakes, a length and/or age adjustment is needed due to the strong co-variation between Hg concentration and fish size (i.e. length and weight; Sonesten, 2003, Chasar et al., 2009) as well as age (Braaten et al., 2014b). In our dataset, strong significant positive relationships were found for fish Hg concentrations (observed values) with both weight (r^2=0.46, p<0.0001) and length (r^2=0.35, p<0.0001). To investigate the between-lake fish Hg concentration variations, we carried out an analysis of covariance (ANCOVA) creating a general linear model. Explanatory variables included in the model included the fish characteristics available for all lakes; total length and weight. To evaluate potential changes in the relationship between fish length and weight, and Hg concentrations between the different lakes (length*lake and weight*lake), interaction terms were also included in the model (all p < 0.0001). The final model's summary of fit and analysis of variance are shown in *Supporting Information* (*Table S3*), together with residual and actual (i.e. measured) versus predicted plots (*Figure S1 and S2*). The final adjusted fish Hg concentrations model specifications include r^2 = 0.79 and root mean square error (RMSE) = 0.14.

All statistical analyses and calculations were performed in JMP 13.0.0 with a significance level α = 0.05, unless otherwise mentioned.

2.5 Calculation of bioaccumulation factors

Estimation of BAFs for MeHg (BAF_{MeHg}) in each lake were performed by dividing the mean adjusted concentrations of Hg in the perch populations (ppm, w.w.) by the concentrations of MeHg in water (ng L^{-1}), by the following function:

$$BAF_{MeHg} = [Hg]_{perch\ adjusted}/[aqueous\ MeHg] \quad (2)$$

BAFs should ideally rely on aqueous dissolved MeHg concentrations, however, given the oligotrophic nature of our study lakes (low nutrient concentrations, Braaten et al., 2014a, sustaining low algal productivity, and therefore low particulate matter), we calculated BAFs based on the total MeHg

concentrations (including dissolved and potential particle associated MeHg). Although the BAF_{MeHg} are calculated from one measurement of MeHg in water from each lake, aqueous MeHg concentrations measured in autumn in south-east Norwegian boreal lakes tend to be relatively stable from year to year (i.e. low inter-annual variability, Braaten et al., 2014a). Lake-specific estimates of BAFs are presented in the *Supporting Information* (*Table S2*).

Obtained BAF_{MeHg} were tested for outliers and all values were found to be within the 75 % quartile plus 1.5 * interquartile range.

3 Results and Discussion

3.1 Hg concentrations positively related to aqueous organic carbon

Aqueous concentrations of TOC, THg and MeHg ranged between 3.6 – 20.1 mg C L^{-1}, 1.3 – 6.6 ng Hg L^{-1} and 0.04 – 0.53 ng MeHg L^{-1}, respectively (*Table 1*). As expected, a significant positive linear relationship was found between aqueous concentrations of TOC and both THg (r^2 = 0.69; p < 0.0001, *Figure 2A*) and MeHg (data transformed to a logarithmic scale, r^2 = 0.49; p < 0.0001, *Figure 2B*). For THg, this relationship reflects the importance of OM as transport vector of Hg species from terrestrial to aquatic systems, a pattern well described for boreal areas in Scandinavia (Braaten et al., 2014a; Eklof et al., 2012) as well as North America (Dennis et al., 2005; Driscoll et al., 1995). In boreal humic lakes, where DOC typically constitutes more than 90% of TOC, particulate organic carbon (POC) is only a minor constituent of aqueous OM (Hessen, 2005; Wetzel, 2001). Therefore, TOC is usually a good proxy for DOC in these systems, and we present only TOC data in this manuscript. TOC in boreal forested lakes is often of predominantly terrestrial origin (allochthonous OM) with minor contribution from in-lake derived sources (autochthonous OM, Hessen, 1992), a factor of significant importance for the OM quality (Bravo et al., 2017). For MeHg, the positive correlation with TOC is likely to be related to TOC as a transport vector, but is also consistent with reduced loss of MeHg through photolytic de-methylation (Lehnherr and Louis, 2009; Poste et al., 2015) and increased OM availability for in-lake methylation (Ullrich et al., 2001).

206 The positive relationships between TOC concentrations and mean observed (linear regression:
207 $r^2 = 0.26$; $p < 0.01$, *Figure 3*) and adjusted (linear regression: $r^2 = 0.21$; $p < 0.01$) fish THg concentrations
208 were significant. Thus, our study confirms earlier findings demonstrating that elevated levels of Hg in
209 fish in boreal regions are associated with humic lakes (Driscoll et al., 1994; Hakanson et al., 1988). The
210 strong relationship between aqueous TOC concentrations and both aqueous Hg species and THg levels
211 in fish (*Figures 2 and 3*, respectively), reflects the dominant effects of OM on aquatic Hg cycling.
212 However, the positive relationship between observed fish THg concentrations and aqueous TOC
213 concentrations ($r^2 = 0.26$) leaves a considerable amount of variation unexplained, and disguises
214 complex relationships between bioaccumulation of MeHg in biota and aqueous OM in humic waters
215 (Driscoll et al., 1994; French et al., 2014), as well as other inter-lake differences in biogeochemistry and
216 ecology. We also observed a great deal of within-lake variability in fish THg concentrations (observed
217 concentrations, *Figure 3*), likely driven by between-fish differences in size, age and diet, further
218 supporting our decision to use adjusted fish THg data in our further analyses.
219
220 ***3.2 Complex interactions between aqueous organic matter and MeHg bioaccumulation***
221 French et al. (2014) suggested that at lower DOC concentrations (< 8.6 mg L^{-1}), aqueous Hg species are
222 primarily bound to smaller organic molecules (i.e. fulvic acids), while at higher OM concentrations (>
223 8.6 mg L^{-1}), higher molecular weight OM with higher affinity for Hg is more prevalent (i.e. humic acids).
224 Accordingly, in humic lakes, where the Hg to OM ratios are very low (*Table 1*), relatively more Hg is
225 bound to OM and therefore less available for uptake into the lower levels of aquatic food webs. The
226 relatively higher molecular weight and larger radius of OM in boreal lakes with higher TOC
227 concentrations has been confirmed by Vogt et al. (2004).
228 In the present dataset, BAF_{MeHg} appeared to have a unimodal response to increasing aqueous
229 TOC concentrations (*Figure 4*, green continuous line, $r^2 = 0.46$). The bell-shaped pattern indicates a
230 threshold peak TOC concentration (Lorentzian peak model inflection point, see *Supporting Information*
231 for details, *Table S4*), at 5.8 ± 0.6 mg L^{-1} (95 % confidence interval: 4.6 – 6.9 mg L^{-1}). Our modelled TOC

threshold value of 5.8 mg L^{-1} was lower than the threshold value found by French et al. (2014) for aquatic invertebrates, i.e. 8.6 mg L^{-1}. However, our maximum BAF_{MeHg} was found in a lake with a TOC concentration of 7.9 mg L^{-1}. This result was similar to the maximum DOC concentration (≈ 8 mg L^{-1}) obtained for the maximum fish Hg concentration reported by Driscoll et al. (1994) in lakes from the Adirondack region, a predominantly forested area in northern New York State, USA.

However, it can also be argued that the relationship between BAF_{MeHg} and aqueous TOC concentrations is simply described by a significantly negative linear relationship (*Figure 4*, $r^2 = 0.35$, $p < 0.001$, blue broken line). Jeremiason et al. (2016) found a lower BAF_{MeHg} with increasing levels of OM for lower trophic levels (i.e. dragonfly larvae), but over a much larger DOC range (9.6 – 55.7 mg L^{-1}) than in French et al. (2014, 6.8 – 30.0 mg L^{-1}) and than we present here (3.6 – 20.1 mg L^{-1}). Both the threshold (*Figure 4*, green continuous line) and negative linear relationship (*Figure 4*, blue broken line) support the view that bioaccumulation of MeHg in boreal aquatic freshwater food webs is strongly controlled by uptake at the base of the food web (Chasar et al., 2009), and that the uptake is highly influenced by lake TOC concentrations. Thus, the quality and/or quantity of OM is likely to be linked to the physico-chemical properties of MeHg and its bioavailability at the base of the food web. For example, Jeremiason et al. (2016) demonstrates, in contrast to French et al. (2014), that at higher DOC, relatively more MeHg is present in the dissolved phase and associated with DOM, making it less available for accumulation in algae at the base of the food web. It is also shown that reduced uptake of MeHg (estimated as bioconcentration factors for hydropsychid caddisflies) with increased DOC concentrations is related to reduced partitioning of dissolved MeHg to seston (which transfers MeHg to primary consumers, Tsui and Finlay, 2011).

Additionally, increased concentrations of terrestrial OM can lead to a shift in the balance between primary and bacterial production in lakes, with increased bacterial production and importance of microbial trophic pathways in lakes with higher allochthonous OM inputs (Karlsson et al., 2012; Karlsson et al., 2015). A shift from algal to more bacterial energy sources can be expected to

have important implications for MeHg uptake, trophic efficiency, and MeHg bioaccumulation in consumers (de Wit et al., 2012).

Figure 4 suggests that there is a threshold response of MeHg bioaccumulation to aqueous OM concentrations, with highest bioaccumulation at intermediate levels of OM (~7-10 mg C L^{-1}), which is reflected in Hg levels in aquatic biota from lower (i.e. phytoplankton, Gorski et al., 2008; invertebrates, French et al., 2014) to higher trophic levels (i.e. fish, *this study*). The threshold response in relative Hg bioaccumulation to OM implies that expected higher future TOC levels (de Wit et al., 2016) will promote Hg bioaccumulation in lakes with low TOC concentrations (< 5.8 ± 0.6 mg L^{-1}), and potentially reduce Hg bioaccumulation in lakes with high TOC concentrations (> 5.8 ± 0.6 mg L^{-1}). A similar threshold value (5.0 mg L^{-1}) is suggested for the lower trophic levels (i.e. algae) by Gorski et al. (2008). However, the functions in *Figure 4* could also imply that expected higher future TOC concentrations will simply lead to reduced Hg bioaccumulation over the complete OM concentration gradient (3.6 – 20.1 mg C L^{-1}) in the boreal ecozone. The two models (Lorentzian and linear) are both possible descriptions of the relation between BAF_{MeHg} and TOC, and both support a similar reduction of relative bioaccumulation of MeHg above the threshold value of 5.8 mg L^{-1}. However, the Lorentzian model has a slightly better fit (r^2 = 0.46) than the linear model (r^2 = 0.35).

For 30 of the 34 lakes included in this study, additional water chemistry parameters were available, including measurements of total nitrogen (Tot-N), total phosphorous (Tot-P), pH, and sulphate (SO_4^{2-}, *Table S5*, Supporting Information). We tested the influence of these parameters on both fish Hg concentrations and BAF_{MeHg} (as for TOC in chapter 3.1 and 3.2, respectively), by running both multiple regression analysis and one-way regressions. None of the parameters were significantly related to fish Hg concentrations (all $p > 0.15$), and they did not improve explanatory power when included in a multiple regression analysis with TOC (all $p > 0.2$).

3.3 Confounding factors and potential for spurious correlations

Although the present data show significant effects on MeHg bioaccumulation from aqueous OM concentrations (*Figure 4*), we should not eliminate other confounding factors. For example, perch from different lakes may feed at different trophic levels, with direct effects on the BAF_{MeHg}, and also perch diet may vary with lake TOC concentrations (e.g. preferred prey, pelagic and/or benthic feeding strategies, nutritional quality of prey, trophic position). If so, this could have a strong influence on the observed relationship between BAF_{MeHg} and TOC. Furthermore, inter-lake comparisons of BAFs may also be complicated by differences in fish growth rates between lakes, with higher BAFs expected for lakes with slower growing perch.

Increased concentrations of TOC have potentially large effects on lake conditions, including reduced light penetration, changing temperature regimes, and oxygen depletion (Read and Rose, 2013, Couture et al., 2015). Given that perch are cool-water adapted visual predators (Diehl, 1988), such changes in physicochemical conditions can be expected to have strong effects on perch foraging and growth, which in turn will affect Hg accumulation.

When assessing relationships between ratios, e.g. BAFs (fish [THg]/aqueous [MeHg]), and their denominators (e.g. aqueous MeHg) or parameters strongly correlated with their denominators (e.g. TOC), so-called spurious correlations may arise (Dunlap et al., 1997). Spurious correlations potentially lead to misleading or incorrect conclusions (Kronmal, 1993). Historically, ratios have been widely used to incorporate more than one variable into a single measure suitable for bivariate analyses. However, Jackson and Somers (1991) used several statistical examples to demonstrate that the use of ratios often lead to artifical correlations due to a lack of independence between ratios and denominators. Pollman and Axelrad (2014) refer to examples of unsupported conclusions from peer-reviewed publications where BAF is significantly correlated to aqueous contaminant concentrations, failing to recognise the real underlying statistical relationship. An example of such a spurious correlation is when BAF_{MeHg} is plotted against aqueous MeHg concentrations, i.e. when the dependent variable is plotted against the variable used to calculate the independent variable. In our data set, such a plot shows a strong exponential negative relationship ($r^2 = 0.82$, *Supporting Information, Figure S3*). In other words,

when THg in fish is divided by MeHg in water, low MeHg in water promotes high BAF_{MeHg}, and high MeHg in water promotes low BAF_{MeHg}. However, we also see a significant positive correlation between adjusted fish THg concentrations and aqueous MeHg concentrations ($r^2 = 0.16$, $p = 0.02$).

The relationships between BAF_{MeHg} and TOC (*Figure 4*) is, however, not as obvious an example of a spurious correlation, as TOC is not used to calculate BAF_{MeHg}. Because of the strong positive relationship between TOC and log-transformed MeHg in water (*Figure 2*), the strong relationship between BAF_{MeHg} and TOC (*Figure 4*) may be driven by the underlying correlation between TOC and MeHg.

In order to avoid a possible spurious correlation, we tested if TOC had additional explanatory power for fish THg concentrations when the effect of aqueous MeHg was accounted for. We found that there was no significant relationship between TOC and the residuals of the regression between adjusted fish THg and aqueous MeHg ($r^2 = 0.03$, $p = 0.29$, *Supporting Information*, *Figure S4*). This lack of relationship lends further weight to the possibility that the relationships between BAF_{MeHg} and TOC that we found (*Figure 4*) may by complicated by the underlying relationship between MeHg and TOC (Pollman and Axelrad, 2014). This highlights the challenges of assessing the effects of OM on Hg bioaccumulation, and the need for care in interpreting relationships that arise between these parameters.

3.4 Mechanistic explanations for the influence of TOC on BAF

We hypothesise, if we assume the reality of a threshold response of TOC on BAF_{MeHg}, that at lower TOC concentrations, two processes contribute to high uptake and bioaccumulation of MeHg: *1*) less OM is available to bind MeHg; and *2*) the OM available is typically present as smaller and more bioavailable molecules (French et al., 2014). Correspondingly, with increasing concentrations of TOC, more OM is available to bind MeHg and more OM is present as larger molecules (Vogt et al., 2004), potentially decreasing Hg bioavailability (as modelled by French et al., 2014). Thus, bioaccumulation of organic forms of Hg is reduced with increasing TOC concentrations. Hence, in our boreal lakes, increased OM

promotes increased aqueous Hg concentrations (*Figure 2*), but lowers Hg bioaccumulation (*Figure 4*). If the relationship between BAF_{MeHg} and TOC is simply linear (*Figure 4*), the same arguments hold, but for the full TOC concentration range, and not restricted to levels above 5.8 ± 0.6 mg L^{-1}.

A mechanistic understanding of the threshold response from OM on BAF_{MeHg} and the linear relationship between the two parameters (*Figure 4*) might be found in the metal-complexation properties of OM. Complexation of metals with OM usually reduces their bioavailability (Stockdale et al., 2010), which implies lower metal-specific BAFs, although there is some evidence that binding to smaller more labile OM may increase the availability of Hg for uptake into bacteria capable of Hg methylation (Chiasson-Gould et al., 2014). The OM-metals complexation is strongly affected by the affinity for specific metals (Haitzer et al., 2002), in addition to the molecular charge, which in turn depends on water chemical conditions such as pH and ionic strength (Haitzer et al., 2003, Tipping, 1993). If we apply these insights to Hg species chemistry, it is likely that OM concentrations and water chemical conditions, such as acidity, will affect distribution of MeHg binding and bioavailability. In agreement with the above, Driscoll et al. (1994) proposed that aluminium (Al) may compete with MeHg for organic binding sites, leading to greater bioavailability of MeHg in lakes with a high Al/DOC ratio.

3.5 Future implications on MeHg bioaccumulation following browning of surface waters

The current browning trend of surface waters has been explained by declining deposition of sulphur (Monteith et al., 2007), which leads to higher solubility of OM through increased charge density (De Wit et al., 2007), and might have implications also for the distribution of MeHg complexes. If intermediate TOC concentrations are indeed associated with the highest bioavailability of MeHg (*Figure 4*), future browning will significantly affect the BAF for Hg in fish. Hongve et al. (2004) showed that the increase in water colour is much larger than the increase in DOC concentrations, and suggests that this is related to the properties of the OM. The alteration of the OM includes an increase in the relative quantity of high-molecular weight compounds (Hongve et al., 2004). Our study documents ameliorating effects on Hg accumulation in fish at TOC concentrations lower than that of other studies,

possibly a consequence of qualitative differences in water chemistry between the Norwegian boreal lakes and the American boreal and Canadian Arctic lakes (Driscoll et al., 1994; French et al., 2014). This again might rely on differences in chemical properties of OM between Europe and North America, including differences in cation/DOC ratios, e.g. the Al/DOC ratio, possibly related to different levels of sulphate deposition and associated acidification (Garmo et al., 2014). Accordingly, more attention on relations between OM quality and fish Hg is warranted.

Our study highlights the complexity of the relationship between OM and Hg transport, bioavailability and bioaccumulation, as well as the challenges of quantitatively assessing the effects of OM on Hg bioaccumulation. However, gaining a comprehensive understanding of these complex relationships and processes is critical in the context of understanding how ongoing and future browning of boreal lakes can be expected to affect fish Hg concentrations and related risks to ecosystem and human health.

4 Conclusions

Given the complex ways in which OM and MeHg interact in boreal freshwater-catchment systems, and the importance of OM in Hg cycling in these systems, future climate-change driven shifts in OM loading to aquatic systems can be expected to affect Hg concentrations in water and aquatic food webs. Our results suggest that aqueous OM is important for the bioavailability and bioaccumulation of MeHg in aquatic food webs, and show that differences in OM concentrations are also reflected in fish Hg concentrations, with important implications for risk to human consumers. In the future, the trade-off between Hg exposure and accumulation will have important implications with respect to the effects of lake browning on Hg transport, bioavailability, and trophodynamics.

Acknowledgements

This work was supported by The Research Council of Norway (projects *Climer*, Grant Number 243644 and *Merino*, Grant Number 196295) and the International Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes (ICP Waters).

Literature

Åkerblom S, Bignert A, Meili M, Sonesten L, Sundbom M. *Half a century of changing mercury levels in Swedish freshwater fish*. Ambio 2014; 43 Suppl 1: 91-103.

Braaten HFV, Åkerblom S, de Wit HA, Skotte G, Rask M, Vuorenmaa J, Kahilainen KK, Malinen T, Rognerud S, Lydersen E, Amundsen PA, Kashulin N, Kashulina T, Terentyev P, Christensen G. *Spatial patterns and temporal trends of mercury in freshwater fish in Fennoscandia*, NIVA-report 7179/2017, ICP Waters report 132/2017, 2017.

Braaten HFV, de Wit HA, Fjeld E, Rognerud S, Lydersen E, Larssen T. *Environmental factors influencing mercury speciation in Subarctic and Boreal lakes*. Science of the Total Environment 2014a; 476: 336-345.

Braaten HFV, Fjeld E, Rognerud S, Lund E, Larssen T. *Seasonal and year-to-year variation of mercury concentration in perch (Perca fluviatilis) in boreal lakes*. Environmental Toxicology and Chemistry / SETAC 2014b; 33: 2661-70.

Braaten HFV, de Wit HA, Harman C, Hageström U, Larssen T. *Effects of sample preservation and storage on mercury speciation in natural stream water*. International Journal of Environmental Analytical Chemistry 2014c; 94: 381-384.

Bravo AG, Bouchet S, Tolu J, Björn E, Mateos-Rivera A, Bertilsson S. *Molecular composition of organic matter controls methylmercury formation in boreal lakes*. Nature Communications, 2017; 8, 14255.

Chasar LC, Scudder BC, Stewart AR, Bell AH, Aiken GR. *Mercury Cycling in Stream Ecosystems. 3. Trophic Dynamics and Methylmercury Bioaccumulation*. Environmental Science & Technology 2009; 43: 2733-2739.

Chiasson-Gould SA, Blais JM, Poulain AJ. *Dissolved Organic Matter Kinetically Controls Mercury Bioavailability to Bacteria*. Environmental Science & Technology 2014; 48: 3153-3161.

Couture RM, de Wit HA, Tominaga K, Kiuru P, Markelov I. *Oxygen dynamics in a boreal lake responds to long-term changes in climate, ice phenology, and DOC inputs*. Journal of Geophysical Research-Biogeosciences 2015; 120: 2441-2456.

de Wit HA, Mulder J, Hindar A, Hole L. *Long-term increase in dissolved organic carbon in streamwaters in Norway is response to reduced acid deposition*. Environmental Science & Technology 2007; 41: 7706-7713.

de Wit HA, Kainz MJ, Lindholm M. *Methylmercury bioaccumulation in invertebrates of boreal streams in Norway: Effects of aqueous methylmercury and diet retention*. Environmental Pollution 2012; 164**,** 235-241.

de Wit HA, Valinia S, Weyhenmeyer GA, Futter MN, Kortelainen P, Austnes K, et al. *Current Browning of Surface Waters Will Be Further Promoted by Wetter Climate*. Environmental Science & Technology Letters 2016; 3: 430-435.

Dennis IF, Clair TA, Driscoll CT, Kamman N, Chalmers A, Shanley J, et al. *Distribution patterns of mercury in lakes and rivers of northeastern North America*. Ecotoxicology 2005; 14: 113-123.

Depew DC, Basu N, Burgess NM, Campbell LM, Devlin EW, Drevnick PE, Hammerschmidt CR, Murphy CA, Sandheinrich MB, Wiener JG. *Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations*. Environmental Toxicology and Chemistry*,* 2012; 31**,** 1536-1547.

Diehl S. *Foraging efficiency of 3 fresh-water fishes - effects of structural complexity and light*. Oikos 1988; 53: 207-214.

Driscoll CT, Blette V, Yan C, Schofield CL, Munson R, Holsapple J. *The role of dissolved organic-carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes.* Water Air and Soil Pollution 1995; 80: 499-508.

435 Driscoll CT, Mason RP, Chan HM, Jacob DJ, Pirrone N. *Mercury as a Global Pollutant: Sources, Pathways,*
436 *and Effects*. Environmental Science & Technology 2013; 47: 4967-4983.
437 Driscoll CT, Yan C, Schofield CL, Munson R, Holsapple J. *The mercury cycle and fish in the Adirondack*
438 *lakes*. Environmental Science & Technology 1994; 28: A136-A143.
439 Dunlap WP, Dietz J, Cortina JM. *The spurious correlation of ratios that have common variables: A Monte*
440 *Carlo examination of Pearson's formula*. Journal of General Psychology, 1997; 124, 182-193.
441 Eklöf K, Folster J, Sonesten L, Bishop K. *Spatial and temporal variation of THg concentrations in run-off*
442 *water from 19 boreal catchments, 2000-2010*. Environmental Pollution 2012; 164: 102-109.
443 Fitzgerald WF, Engstrom DR, Mason RP, Nater EA*. The case for atmospheric mercury contamination in*
444 *remote areas*. Environmental Science & Technology 1998; 32: 1-7.
445 Fjeld E, Rognerud S. *Contaminants in freshwater fish, 2008. Mercury in perch and organic contaminants*
446 *in trout* (In Norwegian). Norwegian Institute for Water Research 2009, Report 1056/2009.
447 Food Agricultural Organisation of the United Nations (FAO UN). *Codex general standard for*
448 *contaminants and toxins in food and feed.* Codex Alimentarius. Codex Standard 1993–1995.
449 Food Agricultural Organisation of the United Nations, World Health Organisation (FAO/WHO):
450 Joint Publications, Geneva, 1995.
451 French TD, Houben AJ, Desforges J-PW, Kimpe LE, Kokelj SV, Poulain AJ, et al. *Dissolved Organic Carbon*
452 *Thresholds Affect Mercury Bioaccumulation in Arctic Lakes*. Environmental Science &
453 Technology 2014; 48: 3162-3168.
454 Gandhi N, Tang RWK, Bhavsar SP, Arhonditsis GB. *Fish Mercury Levels Appear to Be Increasing Lately:*
455 *A Report from 40 Years of Monitoring in the Province of Ontario, Canada*. Environmental
456 Science & Technology 2014; 48: 5404-5414.
457 Garmo ØA, Skjelkvale BL, de Wit HA, Colombo L, Curtis C, Fölster J, et al. *Trends in Surface Water*
458 *Chemistry in Acidified Areas in Europe and North America from 1990 to 2008*. Water Air and
459 Soil Pollution 2014; 225.

460 Gilmour CC, Riedel GS, Ederington MC, Bell JT, Benoit JM, Gill GA, et al. *Methylmercury concentrations*
461 *and production rates across a trophic gradient in the northern Everglades*. Biogeochemistry
462 1998; 40: 327-345.
463 Gorski PR, Armstrong DE, Hurley JP, Krabbenhoft DP. *Influence of natural dissolved organic carbon on*
464 *the bioavailability of mercury to a freshwater alga*. Environmental Pollution 2008; 154: 116-
465 123.
466 Haitzer M, Aiken GR, Ryan JN. *Binding of mercury(II) to dissolved organic matter: The role of the*
467 *mercury-to-DOM concentration ratio*. Environmental Science & Technology 2002; 36: 3564-
468 3570.
469 Haitzer M, Aiken GR, Ryan JN. *Binding of mercury(II) to aquatic humic substances: Influence of pH and*
470 *source of humic substances*. Environmental Science & Technology 2003; 37: 2436-2441.
471 Håkanson L, Nilsson A, Andersson T. *Mercury in fish in Swedish lakes*. Environmental Pollution 1988;
472 49: 145-162.
473 Hessen DO. *Aquatic food webs: stoichiometric regulation of flux and fate of carbon*. In: Jones J, editor.
474 International association of Theoretical and Applied Limnology, 2005.
475 Hessen DO. *Dissolved organic-carbon in a humic lake - effects on bacterial production and respiration*.
476 Hydrobiologia 1992; 229: 115-123.
477 Hongve D, Riise G, Kristiansen JF. *Increased colour and organic acid concentrations in Norwegian forest*
478 *lakes and drinking water - a result of increased precipitation?* Aquatic Sciences 2004; 66: 231-
479 238.
480 Jackson DA, Somers KM. *The specter of spurious correlations*. *Oecologia,* 1991, 86**,** 147-151.
481 Jeremiason JD, Reiser TK, Weitz RA, Berndt ME, Aiken GR. *Aeshnid dragonfly larvae as bioindicators of*
482 *methylmercury contamination in aquatic systems impacted by elevated sulfate loading*.
483 *Ecotoxicology,* 2016, 25**,** 456-468.

Karlsson J, Berggren M, Ask J, Bystrom P, Jonsson A, Laudon H, Jansson M. *Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers*. Limnology and Oceanography 2012; 57: 1042-1048.

Karlsson J, Bergstrom AK, Bystrom P, Gudasz C, Rodriguez P, Hein C. *Terrestrial organic matter input suppresses biomass production in lake ecosystems*. Ecology 2015; 96: 2870-2876.

Klapstein SJ, Ziegler SE, O'Driscoll NJ. *Methylmercury photodemethylation is inhibited in lakes with high dissolved organic matter*. Environmental Pollution 2018; 232: 392-401.

Kronmal RA. *Spurious correlation and the fallacy of the ratio standard revisited*. Journal of the Royal Statistical Society Series a-Statistics in Society 1993; 156: 379-392.

Kurland T, Faro S, Siedler H. *Minamata disease. The outbreak of a neurologic disorder in Minamata, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds*. 1, World Neurology, 1960, pp. 370-395.

Lehnherr I, Louis VLS. *Importance of Ultraviolet Radiation in the Photodemethylation of Methylmercury in Freshwater Ecosystems*. Environmental Science & Technology 2009; 43: 5692-5698.

Meili M, Iverfeldt A, Håkanson L. *Mercury in the surface-water of Swedish forest lakes - concentrations, speciation and controlling factors*. Water Air and Soil Pollution 1991; 56: 439-453.

Monteith DT, Stoddard JL, Evans CD, de Wit HA, Forsius M, Hogasen T, et al. *Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry*. Nature 2007; 450: 537-540.

Pollman CD, Axelrad DM. *Mercury bioaccumulation factors and spurious correlations*. Science of the Total Environment 2014; 496: VI-XII.

Poste AE, Braaten HFV, de Wit HA, Sorensen K, Larssen T. *Effects of photodemethylation on the methylmercury budget of boreal Norwegian lakes*. Environmental Toxicology and Chemistry 2015; 34: 1213-1223.

Ravichandran M. *Interactions between mercury and dissolved organic matter - a review*. Chemosphere 2004; 55: 319-331.

Read JS, Rose KC. *Physical responses of small temperate lakes to variation in dissolved organic carbon concentrations*. Limnology and Oceanography 2013; 58: 921-931.

Scheulhammer AM, Meyer MW, Sandheinrich MB, Murray MW. *Effects of environmental methylmercury on the health of wild birds, mammals, and fish*. Ambio 2007; 36: 12-18.

Sonesten L. *Fish mercury levels in lakes - adjusting for Hg and fish-size covariation*. Environmental Pollution 2003; 125: 255-265.

Stockdale A, Tipping E, Lofts S, Ormerod SJ, Clements WH, Blust R. *Toxicity of proton-metal mixtures in the field: Linking stream macroinvertebrate species diversity to chemical speciation and bioavailability*. Aquatic Toxicology 2010; 100: 112-119.

Streets DG, Devane MK, Lu Z, Bond TC, Sunderland EM, Jacob DJ. *All-Time Releases of Mercury to the Atmosphere from Human Activities*. Environmental Science & Technology 2011; 45: 10485-10491.

Tipping E. *Modelling ion-binding by humic acids*. Colloids and Surfaces 1993; 73: 117-131.

Tsui MTK, Finlay JC. *Influence of Dissolved Organic Carbon on Methylmercury Bioavailability across Minnesota Stream Ecosystems*. Environmental Science & Technology 2011; 45: 5981-5987.

Ullrich SM, Tanton TW, Abdrashitova SA. *Mercury in the aquatic environment: A review of factors affecting methylation*. Critical Reviews in Environmental Science and Technology 2001; 31: 241-293.

UNEP (United Nations Environment Programme). *Global Mercury Assessment*. UNEP Chemicals, Geneva Switzerland, 2002.

USEPA (United States Environmental Protection Agency). *Method 1669: sampling ambient water for trace metals at EPA water quality criteria level.* USEPA Office of Water; 1996.

USEPA. *Method 1630 Methylmercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*. USEPA Office of Water, 1998.

USEPA. *Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*. USEPA Office of Water, 2002.

536 Vogt RD, Akkanen J, Andersen DO, Bruggemann R, Chatterjee B, Gjessing E, et al. *Key site variables*
537 *governing the functional characteristics of Dissolved Natural Organic Matter (DNOM) in Nordic*
538 *forested catchments*. Aquatic Sciences 2004; 66: 195-210.
539 Wetzel R. *Limnology: Lake and River Ecosystems*. Academic Press, San Diego, 2001.
540 Wolfe MF, Schwarzbach S, Sulaiman RA. *Effects of mercury on wildlife: A comprehensive review*.
541 Environmental Toxicology and Chemistry 1998; 17: 146-160.
542 Zahir F, Rizwi SJ, Haq SK, Khan RH. *Low dose mercury toxicity and human health*. Environmental
543 Toxicology and Pharmacology 2005; 20: 351-360.
544