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RESEARCH ARTICLE

Terrestrial carbon inputs drive methylmercury accumulation in zooplankton of boreal and subarctic lakes

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Abstract

Boreal and subarctic lakes are subject to the climate-sensitive process of browning, whereby transport of terrestrial dissolved organic matter (tDOM) to lakes results in greater dissolved organic carbon (DOC) concentrations and associated darker water color. Increasing tDOM will increase mercury (Hg) transport to these lakes, but whether this leads to greater methylHg (MeHg) bioaccumulation in food webs remains unclear. We determined whether increasing DOC increased MeHg bioaccumulation in the lower food web (i.e., zooplankton) by measuring a suite of water chemistry characteristics (including aqueous MeHg and DOC) along with stable isotopes of C ($\delta^{13}C$) and N ($\delta^{15}N$), fatty acid (FA) profiles, and MeHg content of zooplankton from 16 Scandinavian boreal and subarctic lakes along a DOC gradient in the Fall of 2016. We found that both aqueous and zooplankton MeHg were positively correlated with DOC concentration, and that DOC and zooplankton MeHg both increased with the bacterial FA marker 18:1n-7 and decreased with docosahexaenoic acid (DHA) : arachidonic acid and DHA : eicosapentaenoic acid ratios in zooplankton, which are indicators of diet or taxonomic composition. Zooplankton MeHg content was best predicted by $\delta^{13}C$ and the FA 18:1n-7, indicating that zooplankton MeHg bioaccumulation in zooplankton was associated with changes in their resource use along a DOC gradient. Our results suggest that lake browning will likely lead to an increase in MeHg bioaccumulation in zooplankton by affecting aqueous MeHg exposure and lower food web dynamics. In turn, this may lead to increased MeHg contamination in fish and other wildlife.

Over the last several decades, boreal lakes in Scandinavia have undergone lake browning—a process driven by increased transport of terrestrially derived dissolved organic matter (tDOM) from surrounding catchments, which increases dissolved organic carbon (DOC) concentrations and associated water color in lakes (Monteith et al. 2007; Kritzberg 2017). Several factors contribute to lake browning, including recovery from acidification (Monteith et al. 2007; de Wit et al. 2021), climate change (e.g., increased precipitation and higher temperature; de Wit et al. 2016), and land use changes (e.g., afforestation or reforestation; Glaz et al. 2015; Finstad et al. 2016; Kritzberg 2017). In heavily forested and/or wetland-dominated boreal and subarctic catchments, mercury (Hg) transport and biogeochemical cycling are strongly linked to organic carbon (OC) dynamics (Grigal 2002; Braaten et al. 2018; Lavoie et al. 2019). Mercury binds to tDOM, which is transported to downstream aquatic ecosystems via runoff and is typically the main source of DOC in oligotrophic boreal lakes (Mattsson et al. 2005; Wilkinson et al. 2013). Although tDOM is strongly and positively associated with aqueous methyl Hg (MeHg) in lakes (Lavoie et al. 2019), the putative effects of increasing tDOM on trophic transfer and assimilation of MeHg by consumers are not well understood (French et al. 2014; Poste et al. 2019; Wu et al. 2021; McClelland et al. 2024).

Ecosystem changes driven by increased tDOM can directly and indirectly influence MeHg bioaccumulation in the lower

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food web (Wu et al. 2019a, 2021; Poste et al. 2019). MeHg transport to lakes increases with tDOM, which can directly increase dietary exposure and uptake of MeHg in zooplankton. In humic lakes with high tDOM, zooplankton may assimilate greater allochthonous or heterotrophic C, resulting in greater MeHg exposure (Chételat and Amyot 2009; Taipale et al. 2016b; Hiltunen et al. 2017; Wu et al. 2019a). These allochthonous C sources are typically lower in nutritional quality relative to autochthonous C and can decrease energy transfer and increase MeHg bioaccumulation indirectly via reduced trophic efficiency and lower growth dilution (Poste et al. 2019). Though associations between zooplankton MeHg and tDOM have been observed recently, the mechanism(s) by which tDOM may influence MeHg bioaccumulation in the base of aquatic food webs and zooplankton are not fully understood (Jonsson et al. 2017; Wu et al. 2019b; Poste et al. 2019). Under a changing climate and with greater afforestation, where tDOM continues to increase in boreal lakes (de Wit et al. 2016), more research is needed across broad spatial scales and a range of physico-chemical conditions to predict how increasing tDOM will affect MeHg bioaccumulation in these freshwater food webs.

Biochemical indicators of dietary composition such as stable isotopes and fatty acids (FAs) can provide insight into how DOC, including tDOM, affects bioaccumulation of MeHg in zooplankton (Kainz et al. 2008; Poste et al. 2019; Wu et al. 2021). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are commonly used to infer food web structure, carbon source (allochthonous vs. autochthonous C), and trophic position (Peterson and Fry 1987). Stable isotopes of C and N have also been used to infer the degree of terrestrial subsidy and herbivory, respectively, in zooplankton diets, as well as estimate trophic niches (Cole et al. 2011). Diet exerts a strong influence on FA profiles in zooplankton, and quantifying zooplankton FA composition helps infer their relative use of terrestrial, bacterial, and algal C sources (Kainz and Fisk 2009). Terrestrial sources are indicated by long-chain saturated FA (SFA; i.e., 22:0 and 24:0); bacterial sources by specific monounsaturated FA (MUFA; i.e., 18:1n-7) and odd-chain-length FA (i.e., 15:0 and 17:0); and algal sources by n-3 polyunsaturated FA (PUFA) like eicosapentaenoic acid (EPA; 20:5n-3) and/or docosahexaenoic acid (DHA; 22:6n-3), which reflect high nutritional quality food sources (Kainz and Fisk 2009; Jardine et al. 2020). As such, these stable isotopes and FA can help elucidate processes by which tDOM influences diet, resource use, and MeHg bioaccumulation in the lower food web.

We investigated how environmental and food web factors linked to tDOM influence zooplankton MeHg bioaccumulation in boreal and subarctic lakes. Using DOC as a proxy for tDOM (Mattsson et al. 2005; Wilkinson et al. 2013), we sampled lakes along an extensive 10° latitudinal gradient and examined the relationships among DOC, zooplankton MeHg content, and various physico-chemical (e.g., watershed size, lake depth, Secchi depth, aqueous MeHg, pH, DOC, chlorophyll *a* [Chl *a*], total P) and food web variables (carbon source and trophic position via $\delta^{13}C$ and $\delta^{15}N$, and zooplankton FA composition). We tested multiple alternative (non-mutually exclusive) hypotheses for the mechanisms through which higher DOC could be associated with increased zooplankton MeHg content: (i) zooplankton MeHg content could increase directly due to increased aqueous MeHg exposure; (ii) zooplankton MeHg could increase via greater reliance on terrestrial or bacterial carbon sources—specifically, greater reliance on allochthonous or heterotrophic (i.e., terrestrial or bacterial) C would increase zooplankton MeHg content due to the strong binding of MeHg with tDOM; and (iii) elevated tDOM could increase zooplankton MeHg content by reducing food quality due to the shift from autotrophy to heterotrophy, leading to lower consumer biomass and lower growth dilution of MeHg by zooplankton. We predicted that (i) aqueous and zooplankton MeHg would be directly correlated with DOC; (ii) both DOC and zooplankton MeHg would be positively correlated with dietary markers indicating reliance on allochthonous or heterotrophic C (i.e., saturated and bacterial FA, and $\delta^{13}C$), and (iii) both DOC and zooplankton MeHg would be negatively correlated with ΣPUFA proportions and lower essential FA proportions, and positively associated with saturated and bacterial FA.

Materials and methods

Study lakes and sampling

A total of 16 lakes in Norway and Sweden were sampled on a single occasion during September or early October 2016 (Fig. 1). Lakes were selected based on the availability of historical data on Hg in fish and to capture a gradient in DOC (operationally defined as carbon < 0.7 μm) and nutrient concentrations as well as latitude (ranging from 59.0°N to 69.4°N). Selected lakes were clustered into three groups according to latitude: Subarctic lakes in Northern Scandinavia, and boreal/temperate lakes in central or southern Scandinavia, and regions will henceforth be referred to as North, Central, and South (*see* Supporting Information Table S1 for lake characteristics). Lakes without local sources of Hg were ideally selected for study, although some lakes (particularly in southern Norway) were impacted by agriculture and logging in the surrounding catchment. We also included data from two lakes (Royjso and Store Oyvannet) that were originally presented in a parallel study by Poste et al. (2019).

For each lake, Secchi depth was measured, and surface water was collected from a single station in the center of the lake. Using a surface grab sample, 5 L of lake water was collected into a black polyethylene jug, and subsamples were removed for total organic carbon (TOC) (in acid-washed amber glass bottles, preserved with 1 mL 4 N H_2SO_4), total nutrients (in acid-washed polyethylene bottles, preserved with 1 mL 4 N H_2SO_4) and general water chemistry analysis

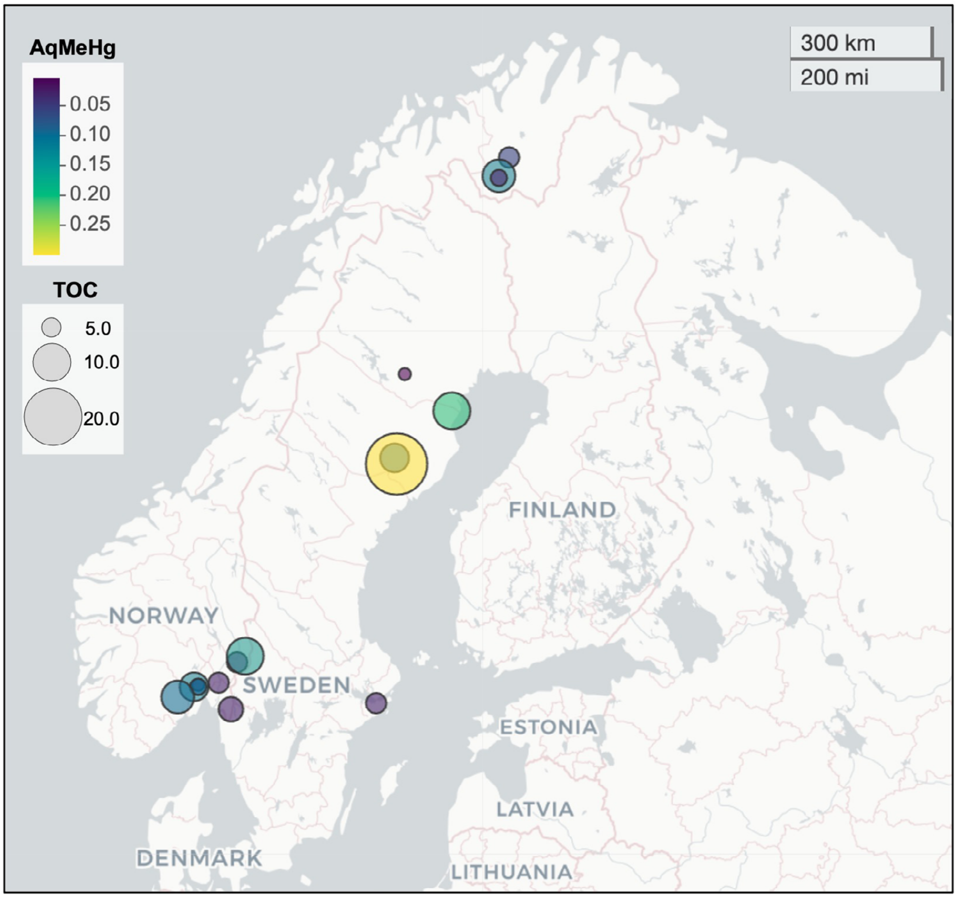


Fig. 1. Map of 16 boreal lakes throughout Norway and Sweden sampled in September 2016. Lakes were chosen to range in dissolved organic carbon (DOC) and nutrients within each of the latitudinal clusters (southern Norway, Mid-Scandinavia, and Subarctic Scandinavia). DOC concentrations in study lakes ranged from 2.0 to 21.6 mg L^{-1} (shown as size of points from small to large) while aqueous MeHg ranged from 0.01 to 0.30 ng L^{-1} (shown as color gradient from purple to yellow).

(in acid-washed polyethylene bottles, unpreserved, kept cold and dark). The remaining water was kept dark and cold until filtration upon reaching the lab. Samples for aqueous total Hg and MeHg were collected directly into trace-metal clean HDPE bottles, and samples for aqueous MeHg were preserved in the field with 1 mL trace-metal clean HCl (as described in Braaten et al. 2014b). Upon arrival to the lab, a subsample was filtered through a GF/F filter (nominal pore size 0.7 μm) for analysis of DOC and dissolved inorganic nutrients. Subsamples of the 5 L surface grab sample were filtered through GF/F filters for Chl *a* analysis and particulate organic matter (POM) stable isotope analysis. Filters were wrapped in aluminum foil and stored at −20°C until further processing and analysis.

Zooplankton was collected at a mid-lake station in each lake by multiple vertical hauls using a 150-μm mesh tow net. Hauls were passed through 100–500-μm mesh to provide a clean zooplankton sample and remove unwanted phytoplankton/detritus. Subsamples of collected zooplankton for MeHg and stable isotope analysis were transferred to a 20-mL polyethylene (PE) vial and frozen at −20°C, while subsamples for FA analysis were transferred to a 2-mL cryovial and stored at −80°C.

Sample analyses

Water chemistry and Chl a

Analysis of water chemistry was carried out at the Norwegian Institute for Water Research water quality lab, using standard and accredited methods, including analysis of nutrients (total P and N, PO_4, $NO_2 + NO_3$ [mostly NO_3 in these oligotrophic lakes], NH_4, and SiO_2), total and dissolved OC (TOC, DOC), and pH, Cl^-, and SO_4^{2-} (Braaten et al. 2014a). Analytical methods are described in detail in Kaste et al. (2018) for a national monitoring program using the same protocols.

Chlorophyll *a* analysis was carried out at UiT–The Arctic University of Norway. Briefly, filters were extracted using methanol, and chlorophyll and pheophytin were determined fluorometrically on a Turner 10-AU fluorometer by measuring fluorescence both before and after acidification with HCl (as described in Parsons 1984). Given the high degree of seasonal variability in Chl *a* concentrations, we also estimated areal PP for all study lakes as described in (Thrane et al. 2014) based on TOC and TP concentrations measured during the Fall sampling event and corrected for incident irradiation based on latitude to assess effects of primary production on MeHg bioaccumulation in zooplankton.

Mercury analysis (water and zooplankton)

Mercury analysis of water (total Hg and MeHg) and zooplankton (MeHg) was done at the Norwegian Institute for Water Research (Oslo, Norway). Total Hg in water was determined using US EPA method 1631 (oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry; US EPA 2002), while MeHg in water was determined as described by Braaten et al. (2014b, 2014a) (based on US EPA method 1630: distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry; US EPA 1998). Method detection limits were 0.02 ng L^{-1} for MeHg and 0.2 ng L^{-1} for total Hg. For MeHg analysis in zooplankton, samples were lyophilized and acid-digested in 30% HNO_3 (12 h at 60°C) and analyzed as described in Braaten et al. (2014b; based on US EPA method 1630). Quality control/assurance measures for each sample run: (1) method blanks ($n = 6$; all < 0.1 pg MeHg); (2) certified reference materials including TORT-2 (lobster hepatopancreas, $n = 2$, recovery: 69–102%) and/or SRM 2976 (mussel tissue, $n = 4$, recovery: 91–110%). Zooplankton MeHg bioaccumulation factors (BAFs) relative to water were calculated by dividing zooplankton MeHg content (ng kg^{-1} dm) by aqueous MeHg concentrations (ng L^{-1}).

Stable isotope analysis (POM and zooplankton)

Stable carbon and nitrogen isotope analysis ($\delta^{13}C$, $\delta^{15}N$) of freeze-dried POM filters and bulk, homogenized zooplankton was done at the UC Davis Stable Isotope Facility (University of California Davis, USA) using an Elemental Analyzer—Isotope Ratio Mass Spectrometer. For every 10 samples, a replicate sample was prepared and analyzed (excluding filters, where replicates were not available). Long-term standard deviation at UC Davis is ± 0.2‰ and ±0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and relative percent difference for replicate samples analyzed in the current study was 0.05–1.2% for $\delta^{13}C$ and 1.1–8.7% for $\delta^{15}N$. Molar C : N ratios in zooplankton ranged from 4.97 to 8.82, suggesting that lipid content could have an effect on $\delta^{13}C$ values. Therefore, we mathematically lipid-corrected zooplankton $\delta^{13}C$ values according to Post et al. (2007):

$$\delta^{13}C_{corr} = \delta^{13}C_{raw} - 3.32 + 0.99 \times C:N$$

where $\delta^{13}C_{corr}$ is the lipid corrected $\delta^{13}C$ value, $\delta^{13}C_{raw}$ is the original $\delta^{13}C$ value, and C : N is the molar C : N ratio of zooplankton.

Due to the seasonal and spatial variability in POM $\delta^{15}N$, and the degree to which selective feeding by zooplankton typically means that POM is not representative as their main basal food source (Cabana and Rasmussen 1996; Post 2002), we did not attempt to calculate a trophic position using $\delta^{15}N$ values of zooplankton and POM. Instead, we examined relationships between zooplankton $\delta^{15}N$ values and FA or FA biomarkers (FABM) directly.

Fatty acid analysis (zooplankton)

Fatty acid analysis for zooplankton was done at Toronto Metropolitan University (Canada; previously Ryerson University) based on methods described by Folch et al. (1957). Methods used for sample extraction, analysis, and quality control were the same as those described in detail in a parallel seasonal study including two of our study lakes (Poste et al. 2019) and Supporting Information Part C. Individual FA and FABM were identified based on previous research by scanning the literature for typical biomarkers of zooplankton diet items, indications of zooplankton taxonomic composition, or previously recognized associations with DOC and/or MeHg content. All FA and FABM are defined in Supporting Information Table S2.

Statistical analyses

The R software environment and associated base packages, along with several additional packages ("vegan," "ggplot2," "factorMineR," "factoextra," and others) were used for statistical analyses and data visualization (Posit Team 2024). For all statistical tests, α was set at 0.05. This dataset included many (> 50) variables, but not all variables were obtained for each lake due to the opportunistic sampling of some lakes. For multivariate analysis and structural equation modeling of stable isotopes and FA (described in detail below), only lakes that contained concurrent measurements of TOC, zooplankton MeHg, stable isotopes, and FA composition were used (16 lakes). For other variables that contained missing data, we used an impute function in the package "missMDA" to predict the missing data and make use of the full dataset in our analyses (Josse and Husson 2016).

All non-proportional variables in the dataset (i.e., aqueous MeHg, DOC, zooplankton MeHg, total FA, etc.) were log-transformed to satisfy the assumptions of normal distribution for linear regression (LR) analyses. Outliers in the dataset were identified systematically using the percentiles of each variable, where values outside the lower (2.5) and upper (97.5) percentiles for each variable were flagged. Using this method, three zooplankton MeHg datapoints (Sidensjön lake, 911 ng g^{-1}; Løken lake, 0.65 ng g^{-1}; and Ellentjørn lake, 0.39 ng g^{-1}) and two DOC datapoints (Loken lake, 21 mg L^{-1} and Norra Reivo, 2.4 mg L^{-1}) were identified as potential outliers. Statistical analyses were conducted with and without the values; and if they significantly impacted the outcome of the analyses, they were excluded. Additionally, Holsvatten lake, a lake in the central Scandinavia region with a DOC concentration of 9.9 mg L^{-1} was excluded from analyses because zooplankton had a FA profile that differed substantially from all other lakes and was characterized by particularly low levels of DHA (< 1%), likely indicating a pronounced difference in taxonomic composition (namely, a lack of calanoid copepods; Brett et al. 2009).

Multivariate approaches were used to assess patterns in zooplankton MeHg content, stable isotope ratios, and FA composition across the study lakes. Principal component analysis was used to understand the inter-correlations between water physico-chemical parameters among the 16 study lakes in the three study regions and to identify parameters that contributed most to the variation among lakes. Patterns in stable isotope ratios, FA composition, and MeHg content in zooplankton and their relation to the environmental variables aqueous DOC, aqueous MeHg, Chl *a*, lake area, and region were assessed through redundancy analysis (RDA). ANOVA-like permutation tests were used to determine the significance of individual environmental variables.

For variables that contributed most to the variation across lakes in the RDA, LR analysis was used to further describe the individual relationships between physico-chemical parameters (i.e., aqueous MeHg, DOC) and zooplankton MeHg, stable isotope ratios, individual FA, and FABM. We used LR to determine whether zooplankton BAFs were related to DOC. Because aqueous MeHg and DOC were strongly correlated and BAF is calculated using the former, we also evaluated BAFs along the DOC gradient by regressing the residuals of zooplankton MeHg vs. aqueous MeHg against DOC (Braaten et al. 2018).

We used structural equation modeling (SEM) to test whether our hypothesized conceptual model of zooplankton MeHg bioaccumulation in boreal and subarctic lakes (Supporting Information Fig. S1) was supported by our observations in the current study. A detailed description of the SEM approach and model checks is in Supporting Information Part D. For comparison to SEM results, we then used multiple LR to test multiple model structures and used the Akaike Information Criterion selection method to determine the model that best described zooplankton MeHg among lakes. Results of SEM and multiple LR were compared to confirm the validity of results.

Results

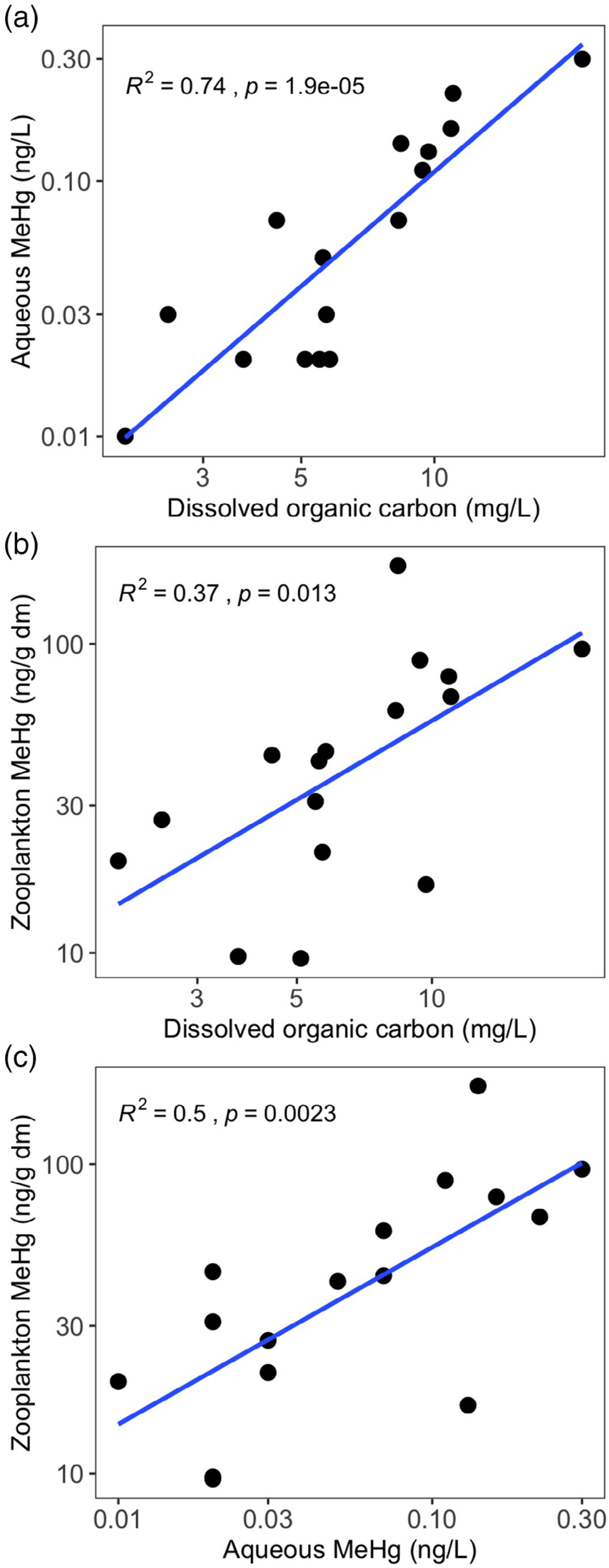
Relationships between DOC, aqueous MeHg, and zooplankton MeHg

Among lakes, aqueous MeHg concentrations ranged 30-fold from 0.01 to 0.30 ng L^{-1}, while DOC concentrations ranged 9-fold from 2.0 to 21.6 mg L^{-1}. Log transformed aqueous MeHg increased with log DOC concentrations (LR, $p < 0.001$, $F_{1,14} = 40.03$, $R^2 = 0.74$, Fig. 2). Aqueous MeHg was most closely associated with DOC or TOC and was positively associated with the first principle component (PC1; principal component analysis, Fig. 3). The percent aqueous MeHg (of total Hg) ranged from 1.5% to 15% among lakes. Percent aqueous MeHg, total N, and total P were also positively associated with PC1 and with aqueous MeHg and DOC. Chl *a* and pheophytin, as proxies of trophic status, were positively associated with PC2 and were not significantly related to aqueous MeHg or DOC (LR, $p > 0.05$ for all relationships, Supporting Information Figs. S2, S3). Most OC in the study lakes was present as dissolved OC, with DOC : TOC in surface water ranging from 0.91 to 1.0 across lakes. Secchi depth was strongly negatively related to DOC (LR, $p < 0.001$, $F_{1,14} = 73.73$, $R^2 = 0.83$, Supporting Information Fig. S3), confirming that DOC is a good proxy for water color in these lakes. Lake surface area ranged from 0.02 km^2 (Mångsrettjärnen) to 7.27 km^2 (Lyseren) and was inversely related to both DOC (LR, $p = 0.033$, $F_{1,14} = 5.604$, $R^2 =$ 0.29) and aqueous MeHg (LR, $p = 0.014$, $F_{1,14} = 7.919$, $R^2 = 0.36$, Supporting Information Figs. S2, S3). Larger lakes and catchment areas were negatively associated with aqueous MeHg and DOC (principal component analysis, Fig. 3). Most physico-chemical parameters did not differ significantly between regions; therefore, regions were pooled for all subsequent analyses and reporting (regional data presented in Supporting Information Part E, Fig. S9).

Zooplankton MeHg content ranged from 9.59 ng g^{-1} dm in Lyseren (Southern Scandinavia) to 911 ng g^{-1} dm in Sidensjön (Central Scandinavia). Log zooplankton MeHg was positively related to both log DOC (LR, $p = 0.013$, $F_{1,14} = 8.085$, $R^2 = 0.37$, Fig. 2) and log aqueous MeHg (LR, $p = 0.002$, $F_{1,14} = 13.8$, $R^2 = 0.50$, Fig. 2) across lakes, but was not predicted by trophic status as inferred from total P or Chl *a* (LR, $p > 0.05$ for all comparisons, Supporting Information Fig. S4). Lake surface area was inversely related to zooplankton MeHg (LR, $p = 0.011$, $F_{1,14} = 8.513$, $R^2 = 0.38$, Supporting Information Fig. S4). Zooplankton BAF ranged from 127,890 to 2,245,000 L kg^{-1} among lakes and BAF decreased with increasing DOC concentration (LR, $p = 0.041$, $F_{1,14} = 5.055$, $R^2 = 0.27$, Supporting Information Fig. S5A). However, the residuals of zooplankton MeHg vs. aqueous MeHg (our proxy for BAF) had no significant relationship with DOC (LR, $p = 0.99$, $F_{1,14} = 0.058$, Supporting Information Fig. S5B).

Zooplankton dietary patterns along DOC gradient

Lipid-corrected zooplankton $\delta^{13}C$ values ranged from −36.7 to −26.2‰, indicating a range in food sources and resource use across lakes. Zooplankton $\delta^{13}C$ was positively correlated with lake area (LR, $p = 0.005$, $F_{1,14} = 10.36$, $R^2 = 0.34$), $\delta^{13}C$ of POM (LR, $p = 0.043$, $F_{1,10} = 5.383$, $R^2 = 0.28$) and terrestrial FA (ΣTerrFA) in zooplankton (LR, $p = 0.033$, $F_{1,14} = 5.598$, $R^2 = 0.29$), and was inversely related to zooplankton MeHg (LR, $p = 0.030$, $F_{1,14} = 5.866$, $R^2 = 0.30$, Fig. 4g). However, there were no significant relationships between DOC and $\delta^{13}C$ of zooplankton (LR, $p = 0.357$, $F_{1,14} = 0.909$) or $\delta^{13}C$ of POM (LR, $p = 0.357$, $F_{1,12} = 0.909$). Zooplankton was consistently depleted in ^{13}C relative to POM, suggesting that POM was not entirely representative of



the zooplankton diet. Despite the association with ΣTerrFA, zooplankton $\delta^{13}C$ values were not correlated with any other individual FA or FABM (LR, $p > 0.05$ for all variables, Supporting Information Fig. S6).

Zooplankton $\delta^{15}N$ values ranged from 0.23 to 5.2‰ across the study lakes and were negatively related to NO_3 levels (LR, $p < 0.001$, $F_{1,16} = 22.14$, $R^2 = 0.55$), suggesting that this range in zooplankton $\delta^{15}N$ might be related to differences in N deposition of lakes rather than differences in trophic position. Due to the seasonal and spatial variability in POM $\delta^{15}N$, and the degree to which selective feeding by zooplankton typically means that POM is not representative as their main basal food source, we did not attempt to calculate a trophic position using $\delta^{15}N$ values of zooplankton and POM. Instead, we examined relationships between zooplankton $\delta^{15}N$ values and FA or FABM directly. Zooplankton $\delta^{15}N$ values were negatively related to the MUFA 18:1n-9 (LR, $p < 0.001$, $F_{1,14} = 6.836$, $R^2 = 0.33$, Supporting Information Fig. S6), but no other significant relationships with FA or FABM were observed (LR, $p > 0.05$ for all variables, Supporting Information Fig. S6).

The proportions of saturated FA (ΣSAFA), monounsaturated FA (ΣMUFA), polyunsaturated FA (ΣPUFA), odd chain length FA (ΣOddChainFA), and ΣTerrFA relative to total FA varied among lakes, implying variation in diet or resource use of zooplankton, and/or differences in species composition of collected samples. The ΣPUFA was inversely related to ΣSFA (LR, $p < 0.001$, $F_{1,14} = 31.51$, $R^2 = 0.69$) and ΣMUFA (LR, $p = 0.030$, $F_{1,14} = 5.807$, $R^2 = 0.29$). ΣOddChainFA and ΣTerrFA were generally unrelated to other FA and FABM (LR, $p > 0.05$ for all variables, Supporting Information Fig. S6). Zooplankton total FA content ranged from 22.16 to 1255 $\mu g\ mg^{-1}$ dm and was not related to any stable isotope, FA, or FABM variable (LR, $p > 0.05$ for all variables, Supporting Information Fig. S6). Of all the zooplankton FA and FABM analyzed, only 18:2n-6 (LA) was significantly different among regions (ANOVA, $p = 0.025$, $F_{2,13} = 4.973$; Supporting Information Fig. S7), suggesting that region was not a main factor influencing dietary variables among lakes.

Associations between DOC, zooplankton MeHg, and FA composition

Redundancy analysis showed that zooplankton MeHg was closely associated with the bacterial biomarker 18:1n-7 as well as the essential FA EPA (20:5n-3) and arachidonic acid (ARA; 20:4n-6), and the FABM for diatoms (RDA, Fig. 4). In contrast, the essential FA DHA and the ratios of DHA : EPA and DHA :

Fig. 2. Linear regression analysis of the relationships between log-transformed aqueous MeHg and dissolved organic carbon (DOC) (**a**), zooplankton MeHg and DOC (**b**), and zooplankton MeHg and aqueous MeHg (**c**). Statistically significant (p-value < 0.05) linear regressions are shown with the line of fit (blue line) on the figure.

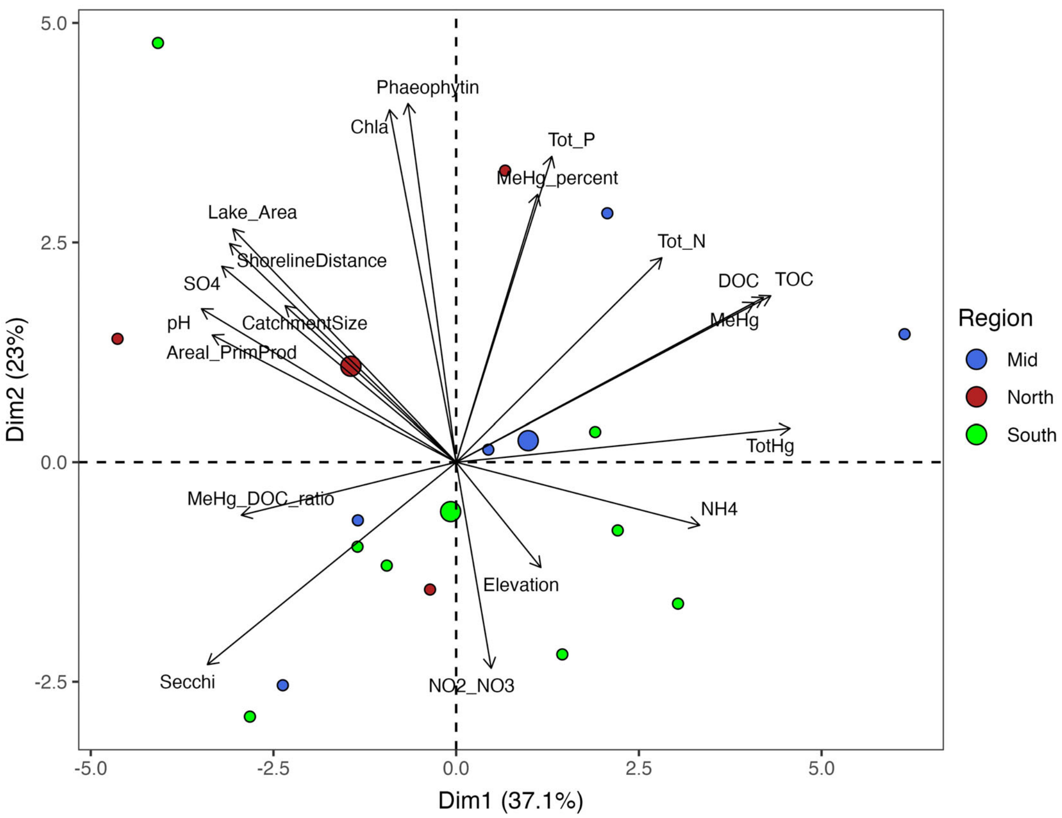


Fig. 3. Principal component analysis biplot of the physico-chemical characteristics of 16 Scandinavian lakes selected along a gradient of dissolved organic carbon using type 2 scaling to demonstrate correlations among variables in the plot. Lakes spanned three regions (southern Norway; green, Mid-Scandinavia; blue, and subarctic; red). The first two axes of the principal component analysis explained 60.1% of the variance in physico-chemistry among lakes. Areal_PrimProd, areal primary production; Chl *a*, chlorophyll *a*; DOC, dissolved organic carbon; MeHg, methylmercury; MeHg_percent, percent aqueous MeHg; NH_4, ammonium, NO_2_NO_3, nitrite + nitrate; TOC, total organic carbon; TotHg, total mercury; Tot_N, total nitrogen; Tot_P, total phosphorus.

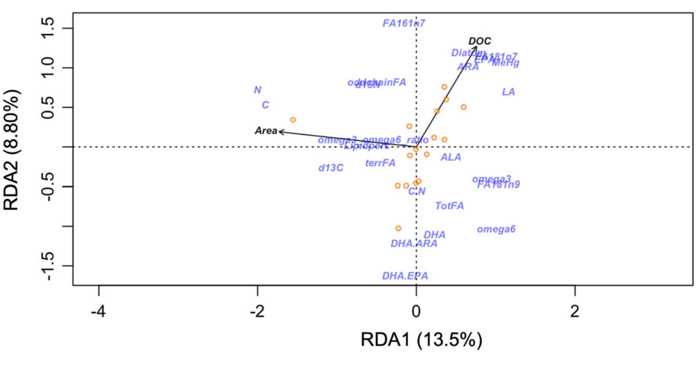


Fig. 4. Redundancy analysis showing the relationships between environmental variables (DOC and lake area) and dietary markers measured in zooplankton (stable isotope ratios, fatty acids, and fatty acid biomarkers) using type 1 scaling to demonstrate similarities among variables. The first two axes of the RDA explained just 22.3% of the variance in dietary markers among lakes and no environmental variables were statistically significant in the RDA, although DOC was closely associated with zooplankton MeHg content, 18:1n-7, ARA, EPA, and the diatom FABM, while lake area was closely associated with zooplankton $\delta^{13}C$ and ΣterrFA. ARA, arachidonic acid; DOC, dissolved organic carbon; EPA, eicosapentaenoic acid; FABM, fatty acid biomarkers; RDA, redundancy analysis.

ARA, two indicators of species/diet composition, were negatively associated with zooplankton MeHg and DOC in the RDA (RDA, Fig. 4). Saturated FAs, including ΣTerrFA and ΣOddChainFA biomarkers, were not associated with zooplankton MeHg or DOC (RDA, Fig. 4). While the RDA was useful in visualizing the correlations among variables, only a small portion of the total variance in zooplankton MeHg content, stable isotope values, and FA/FABM was explained by lake physico-chemistry. Lake area and DOC were the physico-chemical variables most closely associated with zooplankton MeHg, stable isotopes, and FA/FABM, with the first two axes of the RDA explaining 22.3% of the variance in dietary metrics among lakes (RDA, Fig. 4).

Linear regressions confirmed several significant relationships between zooplankton MeHg content, stable isotopes, and FA composition (Fig. 5). Zooplankton MeHg was most strongly and positively related to 18:1n-7 (LR, $p = 0.002$, $F_{1,14} = 12.96$, $R^2 = 0.48$; Fig. 5f) and was also positively related to ARA (LR, $p = 0.036$, $F_{1,14} = 5.403$, $R^2 = 0.28$; Fig. 5a). Zooplankton MeHg was inversely related to their DHA : ARA (LR, $p = 0.027$, $F_{1,14} = 6.055$, $R^2 = 0.30$) and DHA : EPA (LR,

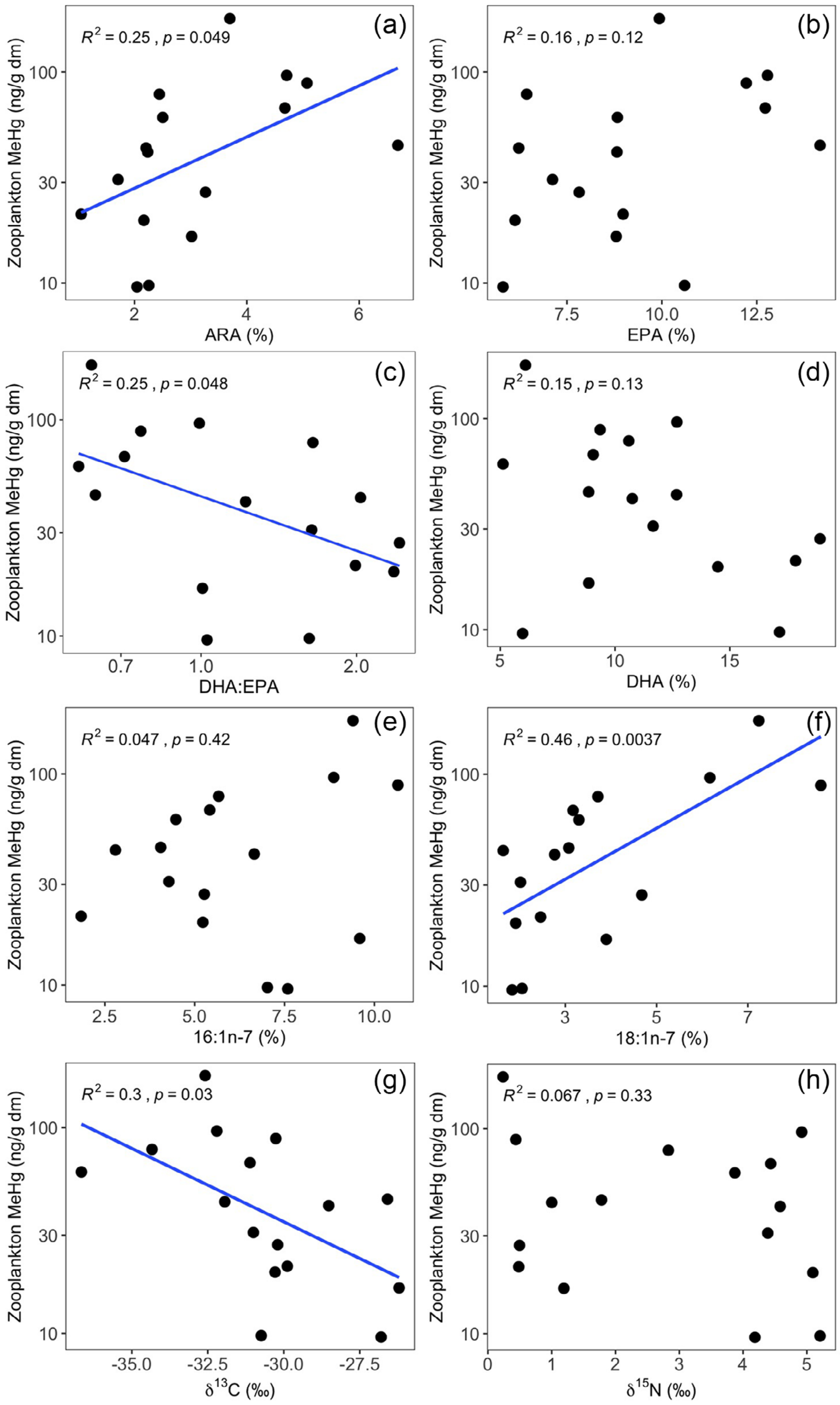


Fig. 5. Linear regression relationships between log-transformed zooplankton MeHg content and fatty acids and stable isotope ratios in zooplankton: ARA and EPA are essential fatty acids (**a**, **b**), DHA:EPA is a biomarker of copepod vs. cladoceran dominance in bulk zooplankton samples that likely changes due to concomitant increases in EPA and decreases in DHA (**c**), DHA is an essential fatty acid (**d**), 16:1n7 is a biomarker of a diatom diet (**e**), 18:1n-7 is a bacterial FA biomarker (**f**), δ^{13}C indicates carbon resource use (**g**), and δ^{15}N indicates trophic position (**h**). Statistically significant ($p < 0.05$) linear regressions are shown with the line of fit (blue line) on the figure. ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

$p = 0.048$, $F_{1,14} = 4.681$, $R^2 = 0.25$; Fig. 5c). Similarly, DOC was positively related to zooplankton 18:1n-7 (LR, $p = 0.022$, $F_{1,14} = 6.652$, $R^2 = 0.32$, Supporting Information Fig. S8), and inversely related to their DHA : ARA (LR, $p = 0.040$, $F_{1,14} = 5.123$, $R^2 = 0.27$) and DHA : EPA (LR, $p = 0.010$, $F_{1,14} = 8.936$, $R^2 = 0.39$; Supporting Information Fig. S8). DOC was not related to zooplankton ARA (LR, $p = 0.117$, $F_{1,14} = 2.799$, Supporting Information Fig. S8) but it was significantly and positively related to their EPA (LR, $p = 0.048$, $F_{1,14} = 4.693$, $R^2 = 0.25$, Supporting Information Fig. S8).

Neither zooplankton MeHg nor DOC were correlated with the FA and FABM for diatoms (LR, $p > 0.05$ for all comparisons), but both 16:1n7 and the diatom FABM were positively related to ARA (LR, $p = 0.016$ and 0.036, $F_{1,14} = 7.423$ and 5.387, $R^2 = 0.35$ and 0.28) and 18:1n7 (LR, $p = 0.004$ and 0.018, $F_{1,14} = 7.249$, $R^2 = 0.34$, Supporting Information Fig. S6). The FA ARA, EPA, 18:1n7 in zooplankton were all positively correlated with each other (LR, $p < 0.05$ for all comparisons, R^2 ranged from 0.31 to 0.48, Supporting Information Fig. S6). Despite lake area being a significant predictor of zooplankton MeHg content, DOC, and zooplankton $\delta^{13}C$ (reported above), it was not significantly related to any FA or FABM in zooplankton (LR, $p > 0.05$ for all variables, Supporting Information Fig. S6).

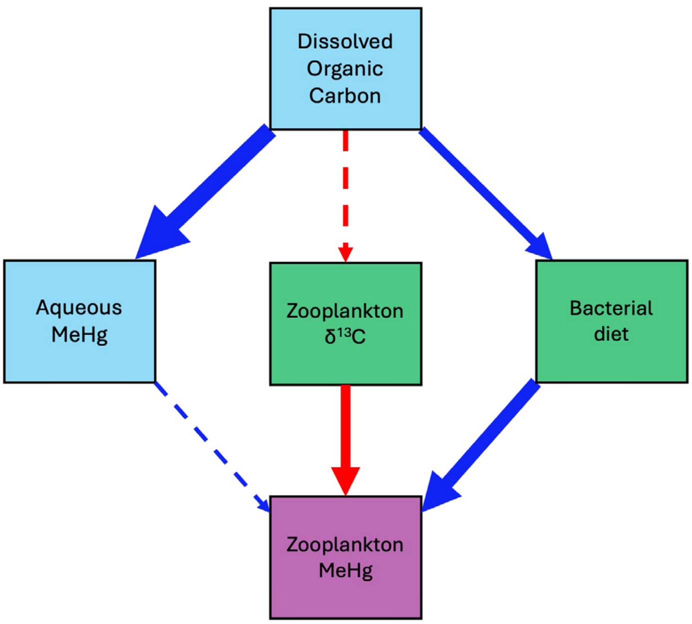


Fig. 6. Structural equation model (SEM) showing how physico-chemical characteristics (represented by blue boxes) and zooplankton diet metrics (represented by green boxes) relate to zooplankton MeHg content. Boxes represent exogenous (independent) and endogenous (dependent) measured variables, with solid arrows pointing from independent to dependent variables. Thickness of arrows indicates strength of relationships between variables (thicker lines are stronger relationships) and color of arrows indicates direction of relationships (blue is positive, red is negative). Dashed arrows were statistically non-significant in the model.

Modeling zooplankton MeHg bioaccumulation

The structural equation model to predict zooplankton MeHg summarized our evidence for a direct influence of DOC on aqueous MeHg and an indirect influence of DOC on zooplankton MeHg accumulation (Fig. 6; Supporting Information Table S3a,b). Our model included DOC, aqueous MeHg, and the 18:1n-7 and $\delta^{13}C$ of zooplankton (SEM, $\chi^2 = 1.462$, $p = 0.833$, Fig. 6; Supporting Information Table S3a,b). Aqueous MeHg was positively influenced by DOC, and DOC alone explained 85% of its variation among lakes (SEM, Fig. 6; Supporting Information Table S3a,b). Zooplankton MeHg was positively associated with their 18:1n-7 (bacterial diet biomarker) and negatively associated with their $\delta^{13}C$, and combined, these two variables explained 66% of the variation in zooplankton MeHg among lakes (SEM, Fig. 6; Supporting Information Table S3a,b). There was a nonsignificant positive association between aqueous MeHg and zooplankton MeHg (SEM, $p = 0.181$, Fig. 6; Supporting Information Table S3a,b). There was also a negative, but weak, association between $\delta^{13}C$ of zooplankton and DOC (SEM, Fig. 6; Supporting Information Table S3b). The bacterial diet biomarker 18:1n-7 was positively associated with DOC, explaining 28% of the variation in 18:1n-7 among lakes (SEM, Fig. 6; Supporting Information Table S3a,b).

We confirmed the results of this SEM using multiple regression analysis (Supporting Information Table S4). Zooplankton MeHg content across lakes was best predicted by zooplankton $\delta^{13}C$ values and 18:1n-7 (MLR, $p < 0.001$, $F_{2,13} = 12.59$, $R^2 = 0.66$, Fig. 4F,G) with the model structure:

$$\log \text{zooplankton MeHg}\left(\text{ng g}^{-1}\text{ dm}\right) = 1.02 \times \log 18:1\text{n7}\,(\%) - 0.13 \times \delta^{13}\text{C}\,(‰) - 1.53$$

Discussion

Our results highlight that DOC (our proxy for tDOM) was strongly associated with aqueous MeHg, and both parameters were positively related to zooplankton MeHg content. Fatty acid and stable isotope biomarkers provided additional insight into dietary pathways linking DOC to MeHg content in zooplankton. Higher proportions of the bacterial FA 18:1n-7 and lower $\delta^{13}C$ values in zooplankton with higher DOC and/or higher zooplankton MeHg content suggest increased heterotrophy in brown water lakes, while increased proportions of algal PUFA like ARA and EPA with increasing zooplankton MeHg content or DOC may reflect taxonomic shifts in zooplankton along the DOC gradient. These results suggest that elevated aqueous MeHg, in combination with changes in resource use and/or taxonomic responses in zooplankton, may lead to their increased MeHg exposure in browning lakes.

Diet and resource use relate to zooplankton MeHg along the DOC gradient

Zooplankton MeHg content was positively related to aqueous MeHg and DOC in this study, which is consistent with findings from Scandinavian lakes (Braaten et al. 2018; Poste et al. 2019) and the Canadian High Arctic (Chételat and Amyot 2009). However, large variation in zooplankton MeHg content was observed among lakes, and it was best predicted by 18:1n-7 (positively; a biomarker of heterotrophic bacteria), and $\delta^{13}C$ (negatively; an indicator of C resource use), supporting our hypothesis that there is a dietary shift in zooplankton from autotrophy to heterotrophy along the DOC gradient. The MUFA 18:1n-7 is characteristic of gram-negative bacteria and can indicate heterotrophic resource use in zooplankton under high tDOM conditions (Pace et al. 2007; Bodelier et al. 2009; Hiltunen et al. 2017). Similarly, low zooplankton $\delta^{13}C$ values can be attributed to increased heterotrophic activity in humic lakes (France et al. 1997; Persaud et al. 2009), or increased use of autochthonous DOC or methane, both of which have been broadly observed in humic boreal lakes across the global north (Bastviken et al. 2003; Kankaala et al. 2006; Taipale et al. 2008). Positive relationships between lower food web MeHg content and heterotrophy have been observed elsewhere and indicate that resource use can drive MeHg bioaccumulation in brownwater lakes. In coastal lake ecosystems, zooplankton MeHg content was better predicted by bacterial diet content (inferred using ΣOddChainFA) than algal diet (inferred using ΣPUFA; Kainz and Mazumder 2005). Similarly, in stream-dwelling aquatic insect larvae, ingestion of bacterial FA was associated with greater MeHg bioaccumulation, and a PUFA-rich algal diet was associated with lower MeHg bioaccumulation (de Wit et al. 2014). Experimentally increasing tDOM in estuarine mesocosms induced a shift in the pelagic food web from autotrophy to heterotrophy and resulted in zooplankton MeHg BAFs increasing by 2- to 7-fold, also supporting results observed herein (Jonsson et al. 2017).

Although 18:1n7 had the strongest relationship with zooplankton MeHg content, positive associations between heterotrophic (18:1n-7), autotrophic (16:1n-7), and essential (EPA and ARA) zooplankton FA biomarkers suggest a common uptake route. Both EPA and 16:1n-7 can be biomarkers of diatoms in freshwater systems, and these associations with zooplankton MeHg content could indicate an increasing diatom diet for zooplankton with increasing DOC (Berggren et al. 2015; Strandberg et al. 2023). It is likely that zooplankton were feeding on both heterotrophic and autotrophic resources—ingesting essential FA from diatoms but subsidizing their diet with tDOM via the bacterial food web (McMeans et al. 2015a; Taipale et al. 2016a). These lakes were sampled in the fall when Chl *a* concentrations were generally low ($< 2.5\ \mu g\ L^{-1}$), and Chl *a* was not related to any of the FA measured, making it challenging to evaluate the role of phytoplankton biomass in driving zooplankton MeHg and FA differences across lakes. Zooplankton can retain algal-derived FA, and they are often not related to PUFA in seston, particularly in the Fall and Winter when these resources may no longer be available. Therefore, the lack of relationships between algal-derived FA and Chl *a* is likely due to physiological regulation by zooplankton (McMeans et al. 2015b; Grosbois et al. 2017). Overall, our findings support the hypothesis that in higher DOC lakes, a shift or subsidization of autotrophic to heterotrophic resources may be associated with greater MeHg exposure, but we interpret this relationship with caution because the changes in FA observed along the DOC gradient may also indicate shifts in zooplankton or phytoplankton taxonomy, as discussed below.

Zooplankton community composition may influence FA profiles along the DOC gradient

The increase in proportions of 18:1n-7 and EPA, and decrease in DHA, along the DOC gradient could suggest either shifts in zooplankton community composition or shifts in zooplankton FA composition in response to lake browning. EPA and DHA are physiologically required for growth and development of zooplankton and cannot be synthesized de novo, thus forming an important part of zooplankton diet (Kainz et al. 2004). The relative proportions of these essential FA vary taxonomically, as cladocerans retain little DHA, while copepods characteristically retain both EPA and DHA. A decrease in DHA : EPA could indicate that the relative proportion of cladocerans in our bulk zooplankton samples increased along the DOC gradient (Kainz and Fisk 2009; Burns et al. 2011). Alternatively, in previous studies of zooplankton FA in boreal and subarctic Scandinavian lakes, ARA and EPA increased in copepods but not cladocerans, while MUFA and DHA decreased in cladocerans but not copepods along a DOC gradient (Lau et al. 2021; Chaguaceda et al. 2024). It is possible that our results reflect changes in FA composition of both cladocerans and copepods along a DOC gradient, rather than shifts in community composition (Lau et al. 2021, Chaguaceda et al. 2024). We did not identify the taxonomic composition of zooplankton samples; instead, we used bulk zooplankton samples representing both cladocerans and copepods, so we cannot confirm which of these hypotheses could be true. However, in our parallel study, the relative proportion of cladocerans was higher, and the proportion of calanoid copepods was lower, in a brown vs. clear water lake (Poste et al. 2019), suggesting that a shift toward cladoceran-dominant communities in higher DOC lakes could be the reason for the results observed herein.

Cladocerans and copepods vary in FA composition and dietary feeding modes/preferences, with cladocerans being relatively non-selective filter-feeders that are capable of supplementing autochthonous food sources with bacterial or detrital OM, while copepods (especially calanoids) more selectively feed on high nutritional quality autochthonous food sources (Kainz and Fisk 2009). A taxonomic shift to cladoceran-dominated communities may explain the increased heterotrophy of zooplankton along the DOC gradient in our study. For example, in the Canadian Arctic, *Daphnia* density was positively related to DOC concentration, and *Daphnia*-dominated communities had 2- to 5-fold higher MeHg content relative to copepod-dominated communities (Chételat and Amyot 2009). Similarly, in western Arctic lakes, zooplankton MeHg content increased with DOC concentration, and cladoceran-dominated communities had higher MeHg BAF relative to copepod-dominated communities (Guernon 2019). Cladocerans could be a more effective conduit/vector of MeHg relative to copepods, and bacterial productivity may be related to zooplankton MeHg bioaccumulation indirectly through its effects on zooplankton community composition (Chételat and Amyot 2009). Given the strong potential effect of zooplankton community composition on their MeHg content, further separation of taxonomic groups in future studies will help to determine the relative importance of changes in resource use versus changes in community composition in zooplankton MeHg bioaccumulation.

Role of nutritional quality in MeHg bioaccumulation

We tested the hypothesis that lower nutritional quality of dietary items for zooplankton in high-DOC lakes can lead to reduced growth dilution of MeHg, leading to greater MeHg bioaccumulation in these systems. Allochthonous C, even when trophically upgraded via the microbial food web, is considered a lower nutritional quality food source for zooplankton that lacks essential FA relative to phytoplankton (Kelly and Scheibling 2012; Brett et al. 2017; Tanentzap et al. 2017). Here, we found no indications of lower nutritional quality in the zooplankton diet in high-DOC lakes: there was no change in ΣPUFA content with increasing DOC/MeHg, no relationships with ΣterrFA or ΣOddChainFA, no evidence of direct terrestrial C assimilation ($\delta^{13}C$ was consistently lower than −28‰), no decrease in n3:n6 ratio, and some essential FA (EPA and ARA) were positively associated with DOC and zooplankton MeHg content. This further suggests that there was still an autotrophic food web pathway in the zooplankton diet, even in high-DOC lakes, where tDOM has been found to subsidize but not fully replace autotrophic resources for zooplankton (Bastviken et al. 2003; Pace et al. 2007; Taipale et al. 2008). Another possible mechanism for the increase in zooplankton MeHg is that their consumption rates increase with reliance on lower nutritional quality food (i.e., allochthonous C) to meet nutritional needs, but this hypothesis has not been explored to our knowledge; and again, we found no indication of decreasing nutritional quality in the zooplankton diet with increasing DOC. Although we cannot determine the specific impact of changes in nutritional quality and biomass dilution vs. dietary shifts on zooplankton MeHg content herein, we highlight these factors as important variables that can be studied in the future to improve our understanding of lower food web MeHg dynamics in browning lakes.

Implications for boreal and subarctic lakes

Lake browning is expected to continue in boreal and subarctic regions due to climate change and it will have ecological implications for lake ecosystems (de Wit et al. 2016, 2021; Blanchet et al. 2022). We demonstrated herein that lake tDOM drives MeHg exposure in the lower food web of boreal and subarctic lakes both directly, by increasing in-lake aqueous MeHg concentrations, and indirectly, by changing zooplankton resource use and/or taxonomic composition. In these boreal and subarctic lakes, heterotrophy and associated changes in zooplankton composition and diet were potential drivers of zooplankton MeHg content. These findings help to understand the potential mechanisms by which DOC influences MeHg exposure and help to refine and support our conceptual model of MeHg bioaccumulation in these ecosystems. Due to the significant effect of DOC on zooplankton MeHg content observed, future studies could focus on incorporating specific qualities of DOM that promote its use by microbes, as well as better taxonomic resolution, into a more refined model to predict MeHg uptake in boreal and subarctic lake zooplankton.

Author Contributions

Conceptualization: Amanda E. Poste, Karen A. Kidd, Staffan Åkerblom, Hans Fredrik Veiteberg Braaten, Heleen A. de Wit. Methodology: Amanda E. Poste, Michael T. Arts, Staffan Åkerblom, Hans Fredrik Veiteberg Braaten, Heleen A. de Wit. Formal analysis: Stephanie D. Graves, Amanda E. Poste. Investigation: Amanda E. Poste, Katrine Borgå, Karen A. Kidd, Stephanie D. Graves. Resources: Amanda E. Poste, Karen A. Kidd, Michael T. Arts. *Data curation*: Stephanie D. Graves, Amanda E. Poste. Writing – original draft: Stephanie D. Graves. Writing – review and editing: all authors. Visualization: Stephanie D. Graves, Amanda E. Poste. Supervision: Amanda E. Poste, Karen A. Kidd.

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Conflicts of Interest

None declared.

Data Availability Statement

Replication data and code are available in the online data repository Borealis: https://doi.org/10.5683/SP3/CID3WA.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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