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Terrestrial organic matter increases zooplankton methylmercury accumulation in a brown-water boreal lake

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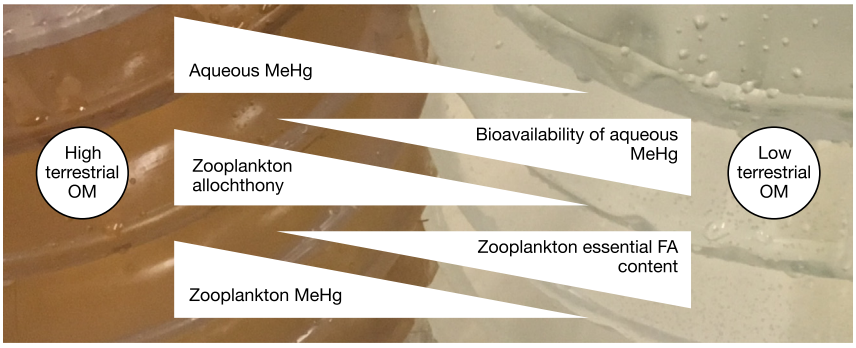
HIGHLIGHTS

- Strong differences in lower food web MeHg dynamics between a clear- and brown-water lake
- Between-lake differences were much more pronounced than seasonal variation.
- Terrestrial OM was linked to higher MeHg in water and zooplankton in the brown-water lake.
- Increased zooplankton allochthony in the brown-water lake led to lower essential fatty acid content.

GRAPHICAL ABSTRACT



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ABSTRACT

Increases in terrestrial organic matter (tOM) transport from catchments to boreal lakes can affect methylmercury (MeHg) accumulation in aquatic biota both directly by increasing concentrations of aqueous MeHg, and indirectly through effects on MeHg bioavailability and on energy pathways in the lower food web. We carried out a detailed seasonal study of water chemistry, zooplankton diet, and MeHg accumulation in zooplankton in two lakes with contrasting tOM concentrations. Between-lake differences explained 51% of the variability in our water chemistry data, with no observed effect of season or sampling depth, contrary to our expectations. Higher tOM was correlated with higher aqueous Hg concentrations, lower areal pelagic primary productivity, and an increased contribution of terrestrial particles to pelagic particulate organic matter. Based on dietary marker analysis ($\delta^{13}C$, $\delta^{15}N$, and fatty acid [FA] composition), zooplankton diet was strongly linked to feeding mechanism, with dietary reliance on phytoplankton highest in the selective-feeding calanoid copepods, and lowest in filter feeding cladocerans. Zooplankton dietary reliance on phytoplankton and their concentrations of high-quality lipids, including polyunsaturated fatty acids, were higher in the clear-water lake than in the brown-water lake, where bacterial and terrestrial food sources were more prevalent. MeHg was highest in zooplankton from the brown-water lake, with highest concentrations in the 200–500 μm zooplankton size fraction for both lakes. Contrary to our expectations, there was no effect of season on zooplankton dietary markers or MeHg. Our results suggest that, overall, higher tOM results in higher MeHg concentrations in water and zooplankton, and reduces

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zooplankton dietary reliance on phytoplankton. Increased tOM thus leads to a decrease in the nutritional quality of zooplankton (i.e. higher MeHg concentrations, and lower concentrations of essential fatty acids), which may cascade up the food web with negative implications for higher trophic levels.

1. Introduction

Mercury (Hg) accumulated in the soils of boreal regions is an important source of Hg to aquatic ecosystems (Ravichandran, 2004; Pirrone et al., 2010). Mercury's methylated, and more toxic, form (methylmercury; MeHg) is highly bioaccumulative and biomagnifies through aquatic food webs (Lavoie et al., 2013), resulting in potentially high concentrations and harmful effects in organisms at high trophic levels (Wolfe et al., 1998; Scheuhammer et al., 2007). A wide range of factors influence Hg cycling and food web uptake in aquatic systems, such as catchment characteristics, water chemistry, food web structure, climate, atmospheric deposition and local sources (Ravichandran, 2004; Clayden et al., 2013; Lavoie et al., 2013).

In lakes, increased "browning" of surface waters has been hypothesized to lead to higher Hg concentrations in water and fish (Jonsson et al., 2017; Creed et al., 2018). Surface water browning is caused by increased transport of terrestrial dissolved organic matter (tDOM) from catchments to lakes, and is particularly related to inputs of highly chromophoric humic compounds and Fe-DOM complexes (Creed et al., 2018). Increased delivery of terrestrial OM to northern surface waters has been attributed to reduced acid deposition via acid rain (de Wit et al., 2007; Monteith et al., 2007; de Wit et al., 2016) as well as climate change driven increases in precipitation (de Wit et al., 2016). Ongoing increases in vegetation cover in many northern regions ("greening"; Finstad et al., 2016) may also lead to increased tDOM inputs from terrestrial to aquatic systems by increasing catchment soil OM pools. These changes will lead to higher inputs of OM-associated nutrients and contaminants, including Hg, from catchments to surface waters (Korosi et al., 2015; Braaten et al., 2018).

Increased tDOM in aquatic systems can affect MeHg accumulation in freshwater food webs both directly, by increasing concentrations of aqueous MeHg, and indirectly, by altering the bioavailability of aqueous MeHg through sorption to tDOM, by supporting in situ MeHg production, and by altering trophic interactions (and primary energy sources) at the base of the food web. Indeed, MeHg concentrations in water, zooplankton, benthic invertebrates, and fish tend to increase with OM concentrations (Driscoll et al., 1994; Bravo et al., 2017; Braaten et al., 2018). However, terrestrially-derived (allochthonous) OM (tOM) may also be associated with reduced bioavailability of MeHg for uptake at the base of the food web, due to sorption to large refractory OM molecules (French et al., 2014; Jeremiason et al., 2016; Braaten et al., 2018).

tOM can be utilized as an energy source by the aquatic microbial community, which is an important carbon source for several species of zooplankton, particularly in oligotrophic lakes (Berggren et al., 2014; Karlsson et al., 2015; Tanentzap et al., 2017). However, a dietary shift toward increased reliance on microbial food sources may lead to reduced nutritional quality of food available for zooplankton since bacteria have lower concentrations of essential fatty acids than phytoplankton (Arts et al., 2009). Reliance on the microbial loop can also add additional trophic levels between the base of the food web and zooplankton (Karlsson et al., 2012, 2015). Combined, this shift from "green" to "brown" trophic pathways can reduce trophic efficiency in aquatic food webs, leading to increased bioaccumulation of MeHg in zooplankton (Jonsson et al., 2017; Creed et al., 2018).

Lower food web structure, and dietary reliance on allochthonous and microbial food sources by zooplankton can be assessed through analysis of dietary markers such as stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as well as fatty acid (FA) composition (Taipale et al., 2014; Tanentzap et al., 2017). In particular, differences in $\delta^{13}C$ values between allochthonous and autochthonous C-sources can be used to estimate the degree of terrestrial C-dependence (del Giorgio and France, 1996; Rautio et al., 2011; Berggren et al., 2014). Meanwhile, FA composition of zooplankton can provide additional insight into zooplankton diet (and dietary quality) with several individual FA and groups of FA acting as trophic markers indicating dietary reliance on specific food sources such as diatoms, bacteria, and terrestrial detritus (Arts et al., 2009). FA analysis can also provide information on the nutritional quality of the zooplankton themselves as prey for higher trophic levels (Arts et al., 2009). Some FA produced by phytoplankton, including the polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA), are essential for heterotrophic organisms that are not able to produce them de novo in quantities sufficient to meet their needs (Kainz et al., 2006; Hixson et al., 2015). In addition to being important for somatic growth and metabolism, these PUFA play a critical role in sustaining zooplankton reproduction (Kainz et al., 2006; Taipale et al., 2014), and are transferred upwards through the food web, providing a critical source of these essential FA for higher trophic level consumers, including humans (Hixson et al., 2015).

Food web studies of Hg accumulation often focus on higher trophic levels, and there is a relative scarcity of detailed studies of Hg uptake and transfer in the lower food web of freshwater ecosystems (Lavoie et al., 2013). Given the ongoing increase in tDOM concentrations in many northern freshwater ecosystems (Monteith et al., 2007; de Wit et al., 2016), and the potential for tDOM to affect MeHg cycling in water and the lower food web, there is a need for detailed lower trophic level understanding on the effects of tDOM on MeHg accumulation and trophic transfer. Furthermore, many studies focusing on MeHg accumulation only provide a "snapshot" of water chemistry, food web structure and MeHg accumulation, however, boreal lakes have strong seasonality in physicochemical conditions and productivity. This drives strong seasonal changes in aqueous tDOM and MeHg, as well as zooplankton community structure, growth and diet, highlighting the importance of understanding the effect of seasonality on drivers of MeHg uptake and trophic transfer in the lower food web.

Here, we focus on how tDOM and lower food web structure influence MeHg levels in zooplankton by carrying out a detailed seasonal study of water chemistry, pelagic lower food web structure and bioaccumulation of MeHg by zooplankton in two lakes with contrasting tDOM concentrations (one "brown" and one "clear" water lake). We tested the following hypotheses: 1) higher tDOM will be associated with higher concentrations of aqueous MeHg and therefore higher MeHg exposure (from water and diet) to zooplankton; 2) higher tDOM will lead to increased dietary reliance on allochthonous energy sources by the lower food web (both through direct uptake and via the microbial loop). As this will lead to lower trophic efficiency and/or additional trophic steps, we expect this to result in increased MeHg concentrations in zooplankton; and, 3) tDOM will reduce bioavailability of aqueous MeHg, which may act to moderate the net impact of tDOM on MeHg concentrations in zooplankton by reducing MeHg bioaccumulation efficiency.

2. Materials and methods

2.1. Study sites and sample collection

Samples were collected approximately every fourth week from a clear-water lake (Røysjø) and brown-water lake (Store Øyvannet) in

Table 1
Geographic location, and key physicochemical properties for the two study lakes. Values reported are means (±standard deviation). For both lakes, water chemistry data are for n = 14 samples (pooled data for surface and deep water samples).

Parameter	Clear-water lake (Røysjø)	Brown-water lake (Store Øyvannet)
Latitude	59.66 N	59.64 N
Longitude	10.30 E	10.10 E
Site depth (m)	21	15
pH	6.4 ± 0.1	5.8 ± 0.2
PAR attenuation coefficient (K_d; m^{-1})	0.29 ± 0.03	1.7 ± 0.49
Total organic carbon (TOC; mg L^{-1})	2.6 ± 0.5	8.1 ± 0.4
Total nitrogen (TN; μg L^{-1})	323 ± 140	340 ± 27
Total phosphorus (TP; μg L^{-1})	4.0 ± 1.8	9.7 ± 1.9
Chlorophyll *a* (chl *a*; μg L^{-1})	0.37 ± 0.19	0.45 ± 0.35
Total Hg (TotHg; pmol L^{-1})	3 ± 0.5	17 ± 3.5
Methyl Hg (MeHg; pmol L^{-1})	0.08 ± 0.07	0.58 ± 0.08

the south boreal region of Norway (Tables 1, S1, Fig. S1). The lakes were chosen to provide contrasting total organic carbon (TOC) concentrations, and because previous studies revealed much higher Hg concentrations in European Perch (*Perca fluviatilis*) from the brown-water than the clear-water lake (Fjeld and Rognerud, 2009).

For each sampling we measured Secchi depth and carried out vertical profiles of photosynthetically active radiation (PAR) as well as conductivity, temperature, density and chlorophyll fluorescence (CTD RBR XRX-620) (Fig. S2). We collected pelagic water and zooplankton samples from the centre of the lakes, and which were kept on ice during transport back to the Norwegian Institute for Water Research (NIVA) for further processing and analysis. Briefly, water was collected from just under surface and from 8 to 10 m (hereafter referred to as "surface" and "deep" water) using a Niskin water sampler. Aqueous total Hg (TotHg) and MeHg samples were collected in 250 mL fluorinated polyethylene (FLPE) bottles, following ultraclean sampling procedure to avoid contamination (Braaten et al., 2014b). All bottles were previously unused and pre-tested for traces of TotHg (quality tested by Brook Rand Labs; mean TotHg concentration = 0.1 pmol L^{-1}). TotHg and MeHg were sampled in separate bottles and kept in double plastic bags to avoid contamination during storage (Braaten et al., 2014b). Sample bottles for MeHg were pre-loaded with 1 mL concentrated hydrochloric acid (trace metal grade) to yield a 0.4% solution (by volume).

Zooplankton were collected by multiple vertical net hauls with 50 and 150 μm nets. Haul material from all hauls and both nets was pooled and size fractioned in the field by screening through a sieving tower yielding size fractions of 50–200 μm, 200–500 μm and >500 μm. Subsamples from each size fraction were collected for stable isotope analysis, MeHg analysis, fatty acid analysis and identification. Stable isotope and MeHg samples were stored at −20 °C, while samples for fatty acid analysis were stored at −80 °C until analysis. Samples for identification were preserved in the field with formalin, with a final concentration of 4% by volume. Zooplankton were identified through microscopy to genus, and species level where possible. Zooplankton data reflect relative abundance and biovolumes, rather than absolute values, since filtered water volume is unknown.

2.2. Sample preparation and analysis

2.2.1. Water chemistry and chlorophyll a measurements

Immediately upon arrival at NIVA, water for DOC analysis was filtered through a 0.2 μm polycarbonate membrane and water for analysis of dissolved nutrients, total nitrogen (TN), total phosphorus (TP), TOC and DOC was preserved with concentrated H_2SO_4 (final concentration of 1% by volume). Samples for particulate phosphorus (PartP) were collected on 47 mm Whatman glass fibre filters (GF/F) and stored at −20 °C until analysis. Nutrient and TOC/DOC analyses were carried out at NIVA using standard and accredited methods (Skarbøvik et al., 2016). Analytes included: pH, TOC, DOC, TN, TP, ammonium (NH_4^+), chloride (Cl^-), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), nitrite + nitrate (NO_2^-/NO_3^-), silicate (SiO_2) and PartP.

Water for the determination of chlorophyll *a* (chl *a*) was filtered through triplicate GF/F filters, and filters were stored at −20 °C until analysis at the University of Tromsø, Norway. Chlorophyll *a* was determined fluorometrically (on a Turner 10-AU fluorometer) after methanol extraction (Parsons, 2013). Water passed through a 0.2 μm polycarbonate filter was used for optical characterization of DOM through absorption spectroscopy. Briefly, absorbance was measured at 1 nm intervals over a wavelength range of 200 to 900 nm using a Perkin-Elmer Lambda 40P UV/VIS Spectrophotometer.

2.2.2. Mercury analysis

All Hg analyses were carried out at NIVA. TotHg in water was determined through oxidation, purge and trap and cold vapor atomic fluorescence spectrometry (CVAFS) based on USEPA method 1631. Meanwhile MeHg in water was determined as described by Braaten et al. (2014b, 2014c). For MeHg analysis of particulate organic matter (POM), water was filtered onto pre-combusted 47 mm Whatman quartz fibre filters (QMA; nominal pore size: 2.2 μm) using an acid-washed Teflon™ filtration manifold and filters were stored at −20 °C until analysis. Zooplankton and POM samples for MeHg analysis were lyophilized, acid-digested and analyzed as described in Braaten et al. (2014a). QA/QC measures for zooplankton analysis included a replicate sample for every 10th sample, method blanks (n = 3), certified reference materials (NIST SRM 2976 (mussel tissue) and TORT-2 (lobster hepatopancreas); n = 3, of which one replicate was spiked with 0.5 mL 1 ng mL^{-1} MeHgCl). QA/QC for POM analysis included filter blanks (n = 3), acid blanks (n = 3) and certified reference material (as for zooplankton analysis). CRMs were always within the certified concentration range for MeHg, and relative percent differences for replicate samples were <10% (range 1–9.8%).

2.2.3. Stable isotope analysis

Stable carbon and nitrogen isotope analysis (SIA) was carried out for zooplankton and POM (collected on pre-combusted GF/F filters and stored at −20 °C) at the University of California, Davis (UC Davis Stable Isotope Facility, USA). Zooplankton samples were lyophilized, homogenized and weighed out into tin capsules for analysis, with a replicate included for every 10th sample. POM filters were lyophilized and packed in tin capsules. $\delta^{13}C$, $\delta^{15}N$, as well as total C and N content were measured using an elemental analyzer interfaced to an isotope ratio mass spectrometer. Long-term standard deviation at UC Davis is ±0.2‰ for ^{13}C and 0.3‰ for ^{15}N. Stable carbon and nitrogen isotope values are expressed using delta notation, relative to international standards (Vienna PeeDee Belemnite for C, and atmospheric N for nitrogen) (Peterson and Fry, 1987).

2.2.4. Fatty acid analysis

Fatty acid (FA) analysis was carried out for zooplankton at Ryerson University (Canada) using methods outlined in Folch et al. (1957). Briefly, subsamples of zooplankton were lyophilized and moisture content and dry weight determined. Lyophilized samples were then homogenized in a mortar and pestle to which liquid nitrogen was added. At the beginning of the procedure 4 mL of (2:1) chloroform methanol was added, as well as 800 μL of 0.88% KCl (in Milli-Q water), and unmethylated tricosanoic acid (23:0) to act as an internal standard for evaluation of recovery and methylation efficiency. Lipids were extracted three time: once with 2:1 (v/v) chloroform:methanol, and twice with 86:14:1 chloroform:methanol:MilliQ water (v:v:v). After evaporating the extract under a gentle stream of N_2 gas, 2 mL of hexanes was added. To derivatize the FA to FA methyl esters (FAME), 2 mL of H_2SO_4 in methanol (1% v/v) was added as a catalyst. The samples were then heated at 90 °C for 90 min. FAME were extracted in 4 mL of hexane three times, then evaporated and concentrated. Extracts were then analyzed with gas chromatography (GC) using either a "split" or

"splitless" approach (with 2010 Dual AOCi Shimadzu GC-Plus), depending on the lipid content. The inlet parameters were as follows: pressure was 278.6 kPa, total flow: 60.1 mL/min, column flow: 1.12 mL/min, linear velocity: 20.0 cm/s, with a split ratio of 1/50. The temperature program was as follows: starting temperature was 140 °C and was held for 5 min, after which the temperature was increased 4 °C/min until reaching 240 °C, and was held for 15 min at 240 °C. The total run time was 45.0 min. Calibration curves were prepared using Nu-Chek mixed FA standards (GLC68E), methyl tricosanoate (methylated 23:0), and Nu-Chek FA Standard (GLC 463) was used for peak identification. Additionally, 18:4n−3 (SDA), purchased from Sigma Aldrich in acid form and then methylated, was used as an individual standard to identify SDA within samples.

2.3. Data treatment

Attenuation coefficients for photosynthetically active radiation (PAR) were calculated based on Beer's law using data from vertical light profiles (Eq. (1)):

$$\ln(E_z) = \ln(E_0) - K_d/z \quad (1)$$

where E_0 represents light just below surface, E_z represents light at depth z, and K_d is the attenuation coefficient for PAR. Specific UV absorption at 254 nm ($SUVA_{254}$; in L mg C^{-1} m^{-1}) is positively related to aromaticity and contribution of terrestrially-derived humic compounds to the DOM pool (Weishaar et al., 2003), and was calculated as absorption at 254 nm divided by DOC concentrations.

We calculated several summary metrics based on FA composition data (on both a proportional and mass-fraction basis; details in Table S3) for use in our data analysis, including sums of n−3 and n−6 FA (Σn−3, Σn−6, Σn−3 + n−6), saturated FA (ΣSFA), monounsaturated FA (ΣMUFA), polyunsaturated FA (ΣPUFA), as well as FA known to be bacterial and terrestrial markers (ΣBactFA (sum of odd-chain FA) and ΣTerrFA respectively). We also calculated the n−3 to n−6 FA ratio.

Bioaccumulation factors (BAFs; Arnot and Gobas, 2006) for MeHg were calculated as in Eq. (2).

$$BAF_{zooplankton} = MeHg_{zooplankton}/MeHg_{water} \quad (2)$$

where $BAF_{zooplankton}$ (in L kg^{-1}) represents the concentration of MeHg in zooplankton compared to their ambient environment (i.e. water), $MeHg_{zooplankton}$ is the measured MeHg concentration in the zooplankton size fractions (in pmol kg^{-1} wet weight) and $MeHg_{water}$ is the estimated concentration of dissolved MeHg in water (MeHg in whole water minus MeHg in POM; in pmol L^{-1}).

All statistical analyses were carried out using R (version 3.4.3, R Core Team, 2017). For the majority of water chemistry parameters, data were still non-normal after $\log_{10}$ transformation, thus the non-parametric Kruskal-Wallis chi-squared test was used for between lake and between depth comparisons for water chemistry. MeHg concentrations, BAFs and stable isotope values for zooplankton also had non-normal distributions after transformation, so Kruskal-Wallis tests were also used for between lake and size fraction comparisons of zooplankton stable isotope values and MeHg concentrations.

For FA summary metrics (Table S3) and individual FA (DHA), assumptions of normality and homoscedasticity were met (based on Levene's test for homoscedasticity, and the Shapiro-Wilks test for normal distribution of ANOVA residuals). We thus used analysis of variance (ANOVA) to test for significant differences in FA summary metrics (for both proportional and mass fraction data) by site and zooplankton size fraction. ANOVAs did not include FA data from the 50–200 μm size fraction since data were only available for a small number of samples for this size fraction (n = 3 of 29 samples).

We used principal components analysis (PCA) to explore correlations, similarities and differences between the multiple measured water chemistry parameters at the two study lakes (and for the two sampling depths). We also used redundancy analysis (RDA) in order to determine the amount of variance in the data set that could be explained by the explanatory variables lake, sampling depth, and season (sampling date). Similarly, we used PCA to characterize relationships between zooplankton taxonomy, dietary marker values ($\delta^{13}C$, $\delta^{15}N$, FA composition), and MeHg concentrations for the two study lakes (and for the zooplankton size fractions), and used RDA to determine the amount of variance in the zooplankton data set that was attributable to lake, size fraction, and sampling date. Multivariate analyses were carried out using the *vegan* package in R on centred and scaled (but otherwise untransformed) data.

3. Results and discussion

3.1. Effects of OM on physicochemical conditions and primary production

The two study lakes differed in physicochemical conditions, primarily related to their contrasting DOM concentrations (Figs. 1 & 2). Physical differences between the lakes included a deeper thermocline (Fig. S2) and increased light penetration (i.e. significantly lower attenuation coefficient and deeper Secchi depth; Kruskal-Wallis, both $p < 0.05$) in the clear-water lake compared to the brown-water lake (Tables 1 & S1). Water chemistry differed strongly between lakes, with higher concentrations of TOC, DOC, TP, MeHg, and TotHg in the brown-water lake, and higher pH, SO_4^{2-} and Cl^- concentrations in the clear-water lake, illustrated by strong separation of the two lakes along the first principle component (PC1) extracted by principle component analysis (Fig. 2). Within the clear-water lake, surface and deep-water samples were closely aligned, indicating no difference, while the surface and deep-water samples from the brown-water lake were separated along PC2, with higher dissolved nutrient and TN concentrations in the deep water, and higher concentrations of chl *a* in the surface water (Fig. 2). Julian day increased along PC2, reflecting higher chl *a*, and lower dissolved nutrient concentrations as the sampling season progressed (Figs. 2, S3). However, lake (and not depth or seasonality) was the only significant explanatory variable, accounting for 51.1% of the total variance in the data set (RDA, $p = 0.001$).

Mean concentrations of chl *a* did not differ between the clear and brown-water lakes ($p = 0.85$, Table 1, Fig. 1). However, while chl *a* concentrations were higher in the surface compared to deep water in the brown-water lake ($p = 0.013$), chl *a* did not differ between the two sampling depths for the clear-water lake ($p = 0.34$; Fig. 1).

High concentrations of DOM lead to rapid attenuation of PAR, which reduces light availability in the water column and may limit photosynthesis (Thrane et al., 2014). Indeed, several recent studies have shown decreased primary production with increasing DOC concentrations in lakes (Ask et al., 2009, 2012; Thrane et al., 2014). However, nutrient concentrations (including TP) were often higher in our brown-water lake (Tables 1, S2, Fig. 2), potentially supporting higher primary production in the photic zone, and explaining the lack of difference in surface chl *a* between the study lakes. This lack of difference may also be attributable to higher cellular chl *a* content in phytoplankton from the brown-water lake than in the clear-water lake, as a response to low light conditions (Felip and Catalan, 2000). Meanwhile, the low deep water chl *a* in the brown-water lake reflects the role of DOM in limiting light availability and primary production in the lower water column. This suggests that although surface chl *a* concentrations are similar between lakes, when integrated for the entire water column, areal phytoplankton production is highest in the clear-water lake (because light penetrates much deeper in this lake). This is also supported by modelled estimates of gross areal primary productivity (PP) based on Thrane et al. (2014; $\log(PP_a / PAR) = -0.7(\log DOC) + 0.48(\log TP)$; for 75 boreal Scandinavian lakes). Applying this model yields estimates of 1.11 and

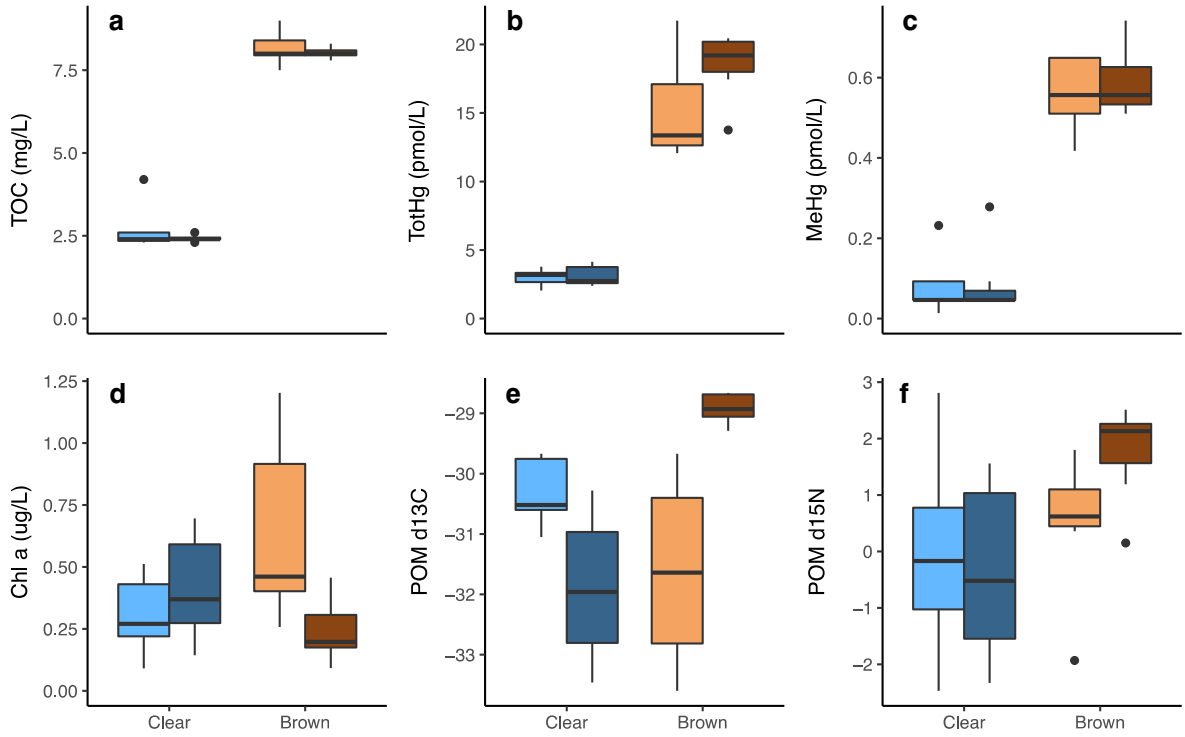

Fig. 1. Boxplots for selected water chemistry parameters for the clear water (blue fill) and brown water (brown fill) study lakes. Surface water data are shown using light blue/light brown fill, while deep water values are shown using dark blue/dark brown fill. Data shown includes aqueous TOC, TotHg and MeHg concentrations, chl *a* concentrations, and $\delta^{13}C$ and $\delta^{15}N$ of POM (in ‰). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.69 mg C mol photons^{-1} m^{-2} d^{-1} for PP_a/PAR for the clear and brown-water lakes respectively, which, given the close proximity of these two study sites (and therefore similar average daily incident PAR), suggests that gross areal PP is ~1.6 times higher in the clear-water lake than in the brown-water lake.

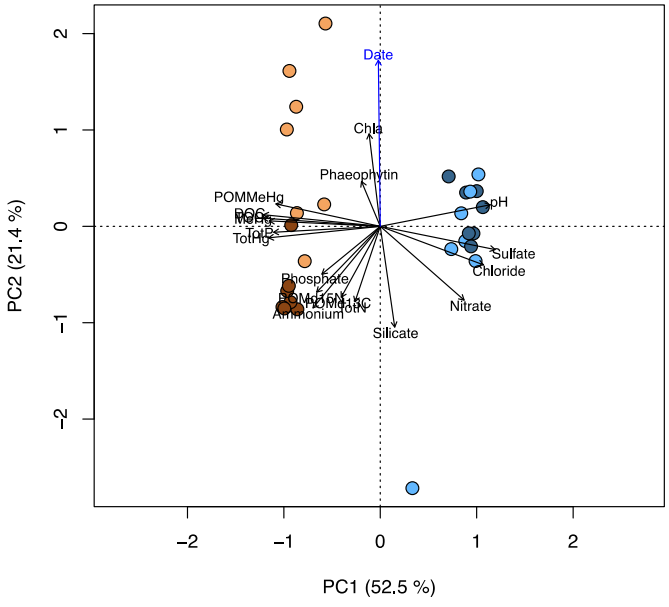

Fig. 2. Principal component analysis (PCA) triplot of water chemistry parameters (response variables, shown as black vectors), samples (shown as points), and passively overlaid explanatory variables (lake, depth and sampling date). Explanatory variables are indicated by colour of individual score points for lake and depth (blue/brown for clear-/brown-water lake, light/dark for surface/deep water respectively) and by a blue vector for date. The first two PC axes explain 73.7% of the variance in water chemistry, and when explanatory variables (lake, depth, date) are overlaid, these axes explain 83.3% of the variance (based on RDA variance partitioning results). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Terrestrial OM related to higher concentrations of aqueous Hg

TOC and DOC concentrations were ~3-fold higher in the brown-water lake than in the clear-water lake, regardless of depth (both $p < 0.001$; Fig. 1). In both lakes, the DOC concentrations were ~90% of TOC concentrations, as is typical of boreal Norwegian lakes (Hessen, 2005). Specific UV absorption ($SUVA_{254}$) values were 5.0 ± 0.2 L mg C^{-1} m^{-1} (Table S2) in the brown-water lake, suggesting that the OM pool was highly aromatic and dominated by terrestrially-derived material (Weishaar et al., 2003). $SUVA_{254}$ was lower in the clear-water lake (1.9 ± 0.4 L mg C^{-1} m^{-1}; Table S2), reflecting that the OM included a larger proportion of low molecular weight aliphatic compounds than in the brown-water lake (Hansen et al., 2016).

TotHg and MeHg in water were also higher in the brown-water lake (both $p < 0.001$; Fig. 1). This is consistent with results from other studies in unproductive forested boreal lakes, where OC concentrations are typically good predictors of aqueous TotHg and MeHg, due to the importance of catchment inputs of OM-associated Hg in determining aqueous Hg concentrations (Korosi et al., 2015; Braaten et al., 2018). Higher aqueous MeHg concentrations in the brown-water lake may also reflect decreased photodegradation of MeHg due to reduced light penetration (Poste et al., 2015; Klapstein et al., 2018). Despite the potential for higher OM-availability in the brown-water lake to fuel higher in situ Hg methylation, we did not observe strong evidence of this, with similar proportions of Hg present as MeHg in both study lakes (3.0 ± 3.3% in the clear-water lake, and 3.5 ± 0.9% for the brown-water lake), and no apparent accumulation of MeHg in the hypolimnion throughout the stratified season (no within lake differences based on sampling depth; $p > 0.2$ for both lakes).

Despite the higher aqueous MeHg concentrations in the brown-water lake due to increased catchment inputs and possibly reduced photodemethylation, and the potential for OM to act as a substrate for

in situ methylation, the higher OC concentrations and prevalence of high molecular weight and highly aromatic OM (as indicated by $SUVA_{254}$) may reduce the bioavailability and food web uptake of aqueous MeHg through sorption to the larger, more recalcitrant terrestrial-OM molecules (Zhong and Wang, 2009; French et al., 2014; Schartup et al., 2015; Jeremiason et al., 2016; Braaten et al., 2018), potentially moderating the effects of increased aqueous MeHg concentrations in this brown-water lake.

3.3. Increased reliance on allochthonous food sources by zooplankton in the brown-water lake

3.3.1. Terrestrial and phytoplankton contributions to the particulate organic matter (POM)

$\delta^{13}C$ and $\delta^{15}N$ values in POM are often interpreted as being representative of the base of the pelagic food web (i.e. in phytoplankton). However, bulk POM includes a mixture of detrital particles, bacteria and protozoa, terrestrial particles and phytoplankton (Jones et al., 1998); particularly in low productivity humic lakes (del Giorgio and France, 1996; Wilkinson et al., 2013). $\delta^{13}C$ and $\delta^{15}N$ of POM also vary seasonally and spatially, depending on phytoplankton bloom situation, catchment inputs of terrestrial particles, as well as changes in $\delta^{13}C$ and $\delta^{15}N$ of the inorganic nutrient pool upon which phytoplankton and bacteria rely (del Giorgio and France, 1996; Gu, 2009; Syvaranta et al., 2006; Jankowski et al., 2012). As such, in our low productivity systems, we do not expect POM to accurately represent the isotopic signature of phytoplankton (or zooplankton diet). Rather, we infer that these monthly measurements of the POM pool reflect the relative contribution of phytoplankton vs. terrestrial particles; with potential implications for dietary reliance on these food sources by zooplankton.

The $\delta^{13}C$ values for allochthonous POM are relatively consistent in boreal lakes, ranging from −28 to −25‰ (Karlsson et al., 2003; Kritzberg et al., 2004; Rautio et al., 2011; Berggren et al., 2015), in close agreement with estimated terrestrial $\delta^{13}C$ of −27‰ for the boreal ecoregion (Lajtha and Michener, 1994). We observed seasonally stable $\delta^{13}C$ values for deep water POM in the brown-water lake (−28.9 ± 0.2‰; Fig. 1, Tables 1, S2), which, given the low chl *a* concentrations observed, and the similarity to previously published estimates of terrestrial $\delta^{13}C$, suggests a dominance of terrestrially-derived C in the POM for the lower water column in this lake. Based on PCA, $\delta^{13}C$ of POM and chl *a* were negatively associated (Fig. 2), suggesting that seasonal changes in phytoplankton abundance drive changes in $\delta^{13}C$ of POM. This is consistent with previous studies that have demonstrated that $\delta^{13}C$ of phytoplankton is typically lower than for bulk POM or terrestrial particles (del Giorgio and France, 1996; Karlsson et al., 2003; Berggren et al., 2015). $\delta^{13}C$ of POM was significantly higher in the deep water than in the surface water of the brown-water lake, as well as both sampling depths in the clear-water lake (mean values ranging from −30.2 to −31.9‰), likely indicating increased contribution of phytoplankton to the POM for these samples (Fig. 1, Table S2). However, the lowest mean $\delta^{13}C$ values observed (for the deep water of the clear-water lake) were only 3‰ lower than the strongly terrestrial-C dominated deep water from the brown-water lake, suggesting that terrestrial C remains an important component of the POM across both study lakes, sampling depths and seasons.

POM $\delta^{15}N$ values were low and seasonally variable (Fig. 1, Table 1), particularly for the clear-water lake. A broad range of factors can influence the $\delta^{15}N$ of POM, including the source of the POM, phytoplankton community composition and growth rate, and inorganic nitrogen cycling (i.e. nitrogen fixation, nitrification, denitrification; Lehmann et al., 2004; Gu, 2009), complicating the interpretation of these data, and of resulting $\delta^{15}N$ data at consumer trophic levels. The particularly low, and often negative, $\delta^{15}N$ values observed may reflect the importance of long-range atmospheric transport and deposition of inorganic N for the inorganic nitrogen budget of these undisturbed forested lakes (Hessen et al., 2009).

3.3.2. Differences in zooplankton community between lakes and size fractions

The zooplankton species assemblages in the two study lakes both included three main cladoceran taxa (*Bosmina longispina*, *Holopedium glacialis*, and *Ceriodaphnia* sp.) and the cyclopoid copepod *Cyclops scutifer* (Table S4, Fig. S4). The main differences were the presence of the cladoceran *Bythotrephes* sp. in the clear-water lake, a visual predator likely favored by the higher light availability, and different dominant calanoid copepod taxa between the lakes (*Eudiaptomus* spp. dominating in the brown-water lake, and *Mixidiaptomus lacinatus* in the clear-water lake) (Table S4, Fig. S4). The brown-water lake also had higher rotifer abundances than the clear-water lake.

The biovolume of the 200–500 μm zooplankton size fraction was strongly dominated by calanoid copepods in the clear-water lake and a mix of *Ceriodaphnia*, cyclopoid and calanoid copepods in the brown-water lake (with fewer cyclopoids and more calanoids as the sampling season progressed) (Figs. 3 & S4). For the >500 μm fraction, *H. glacialis* was a dominant contributor to the total biovolume in both lakes, but with higher contribution of calanoid copepods and cladocerans in the clear-water lake (and in October for both lakes). The 50–200 μm fraction was dominated by copepod nauplii and copepodites for both lakes.

3.3.3. Zooplankton had lower quality diet in the brown-water lake

The relative importance of allochthonous vs. autochthonous energy sources has important implications with respect to dietary quality and trophic efficiency, with algal (autochthonous) food sources typically having higher nutritional value (higher lipid and essential fatty acid content, lower C:N and C:P ratios) than allochthonous food sources (i.e. terrestrial detritus, and energy derived through the microbial loop) (Arts et al., 2009). The degree to which zooplankton rely on allochthonous resources (i.e. terrestrial POM and DOM) vs. autochthonous resources (i.e. phytoplankton) depends on the availability of these resources in a system as well as zooplankton feeding strategy (Rautio et al., 2011; Berggren et al., 2014; Taipale et al., 2016; Tanentzap et al., 2017). Cyclopoid copepods are selective raptorial feeders, and may utilize allochthonous energy via the microbial loop, through selective feeding on bacterivorous microzooplankton such as ciliates, heterotrophic nanoflagellates and rotifers (Berggren et al., 2014). Calanoid copepods are typically selective suspension feeders, and able to selectively feed on phytoplankton (where sufficiently present) with allochthonous resource use at times of very low autochthonous resource availability (Berggren et al., 2014). Cladocerans are relatively non-selective filter feeders, and likely incorporate allochthonous nutrients and energy through ingestion of terrestrial particles and particle-associated microbes linked to POM, with the degree of allochthony inversely related to the relative contribution by phytoplankton to the POM (Berggren et al., 2014; Tanentzap et al., 2017). Thus, our expectation was that allochthony would be most prevalent in the brown-water lake due to a higher terrestrial contribution to the POM and DOM pools, and a lower primary productivity, and that, for both lakes, allochthony would be highest in filter-feeding cladocerans and cyclopoid copepods (due to their reliance on microzooplankton supported by the microbial loop).

We aimed to use $\delta^{13}C$ as a proxy for dietary reliance on terrestrial carbon, with higher values (enriched in ^{13}C) reflecting increased allochthonous resource use, and lower values (depleted in ^{13}C) reflecting increased reliance on phytoplankton. $\delta^{13}C$ values in zooplankton were consistently lower than the expected $\delta^{13}C$ of terrestrial OM (−27‰; Lajtha and Michener, 1994) and were often lower than $\delta^{13}C$ of POM (Table S5). Lower $\delta^{13}C$ in zooplankton relative to POM in lakes has previously been attributed to the contribution of higher $\delta^{13}C$ terrestrial particles and detritus to the POM paired with selective feeding by zooplankton on lower $\delta^{13}C$ phytoplankton (del Giorgio and France, 1996; Rautio et al., 2011).

For both study lakes, the lowest $\delta^{13}C$ values in zooplankton were in the 200–500 μm zooplankton size fraction, which was the size fraction

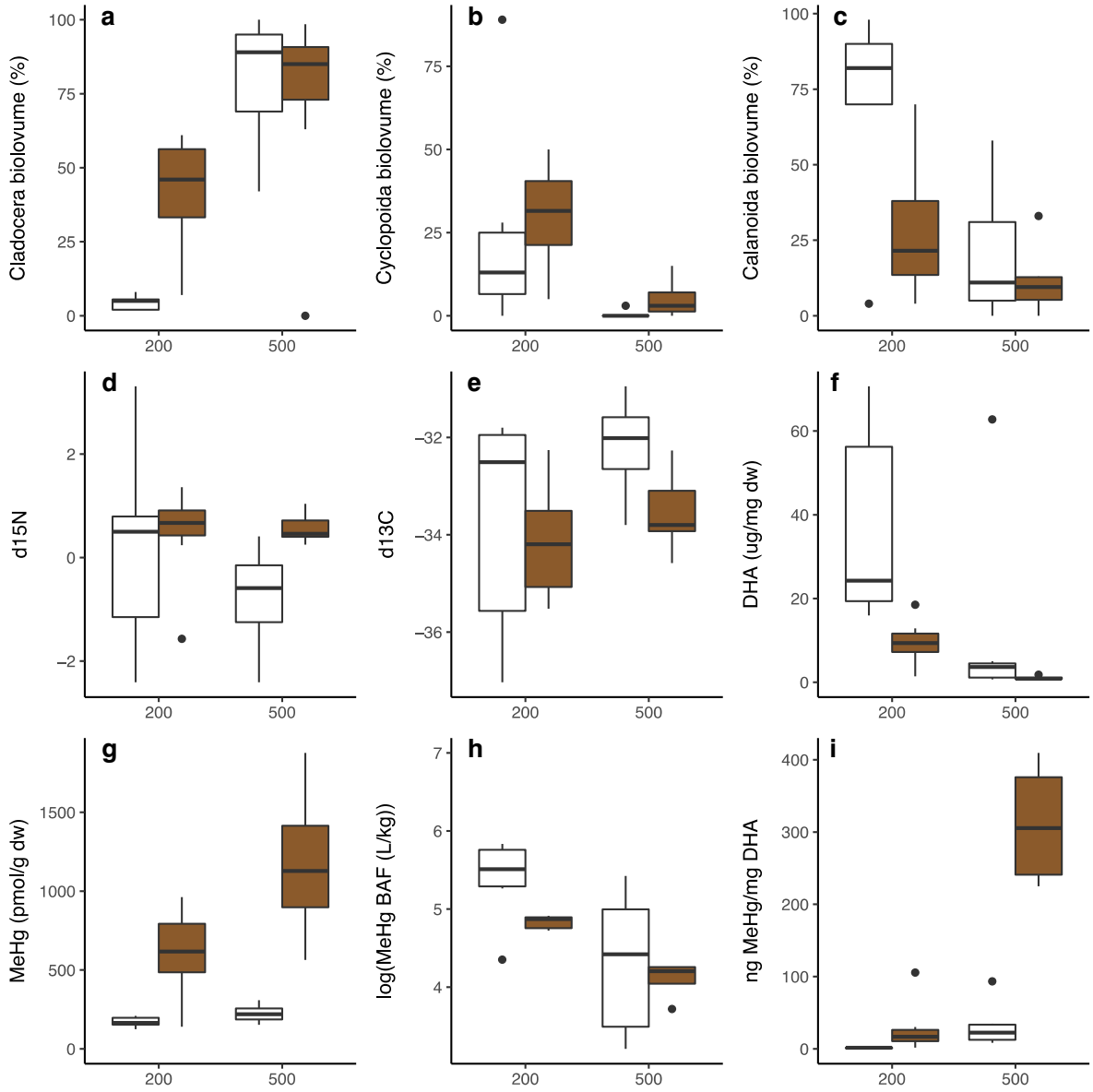

Fig. 3. Boxplots summarizing key data on zooplankton collected in the 200–500 μm and >500 μm size fractions for the clear water (boxplots with white fill) and brown water (boxplots with brown fill) lakes. Data displayed includes relative contribution of major zooplankton taxonomic groups to the biovolume of the sampled size fractions (for Cladocera, Cyclopoida and Calanoida), selected results for dietary marker analysis ($\delta^{13}C$, $\delta^{15}N$, DHA content), and MeHg data (MeHg concentrations, log-transformed MeHg bioaccumulation factors (MeHg BAF), and MeHg content in zooplankton per unit DHA). Data for the 50–200 μm size fraction are not shown here since for several of these metrics (including DHA and MeHg) data were only available for a small number of samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the highest proportion of calanoid copepods, supporting high selectivity for a phytoplankton diet by these zooplankton. $\delta^{13}C$ values in zooplankton did not differ significantly between study lakes (Fig. 3, Table S5), suggesting that, despite our expectations, there was no apparent difference in the relative importance of terrestrial-C sources between the two study lakes. However, other studies have also shown high terrestrial resource use by zooplankton in low productivity clear lakes where phytoplankton availability is low (Karlsson et al., 2003; Cole et al., 2011). For both study lakes, the low primary productivity and the high apparent contribution of terrestrial particles to POM could suggest a potential for ingestion of terrestrial POM, either directly by filter-feeding cladocerans, or indirectly by cyclopoid copepods through dietary reliance the microbial loop. However, the interpretation of $\delta^{13}C$ values as a proxy for allochthony, and in particular our ability to compare $\delta^{13}C$ values between lakes, is complicated by the fact that we do not have $\delta^{13}C$ measurements for either phytoplankton or data on baseline $\delta^{13}C$ of dissolved inorganic carbon from these lakes. Furthermore, in humic boreal lakes, there is evidence that dietary reliance on methane oxidizing bacteria is known to lead to lower than expected $\delta^{13}C$ in zooplankton (Jones et al., 1999; Jones and Grey, 2011), which, if occurring, would lead to an underestimation of terrestrial-resource use in our brown-water study lake and further complicate between-lake comparisons.

$\delta^{15}N$ values did not differ among zooplankton size fractions within the study lakes (Fig. 3, Table S5), suggesting no significant differences in trophic level between the zooplankton size fractions. This is consistent with the lack of significant contribution by predatory zooplankton observed in our samples, but may also reflect the high seasonal and depth-related variability in $\delta^{15}N$ of POM in both of our study lakes (Fig. 1), therefore limiting the utility of $\delta^{15}N$ values as an indicator of trophic level for these lower trophic level organisms.

We found no strong seasonal patterns for the FA composition of zooplankton and sampling date was not a significant explanatory variable for zooplankton taxonomy or dietary marker data (RDA, $p = 0.363$).

On both a proportional and mass-fraction basis, FA markers for terrestrial resources (high saturated and terrestrial FA) were higher in the >500 μm size fraction than in the 200–500 μm fraction for both lakes (ANOVA, both $p < 0.01$), while FA markers for algal resources (high ΣPUFA, DHA, and n−3:n−6 ratios) were higher in the 200–500 μm size fraction than in the >500 μm fraction (ANOVA, all with $p < 0.01$). These results suggest a higher dietary reliance on phytoplankton in the 200–500 μm size fraction, and a higher reliance on terrestrial resources in the >500 μm fraction. Differences in dietary sources are also illustrated by the separation of the zooplankton size fractions along the first principle component axis (PC1) extracted by principle component analysis which included taxonomic composition, $\delta^{13}C$, $\delta^{15}N$, FA composition and MeHg concentrations for the 200–500 and >500 μm size fractions (Fig. 4). Samples clustered based on lake and size fraction (Fig. 4), with the 200–500 μm size fraction from the clear-water lake most closely associated with algal FAs (and calanoid copepod biovolume), and with the >500 μm fraction from both lakes most associated with terrestrial FA (and cladocerans, in particular *H. glacialis*). The 200–500 μm size fraction for the brown-water lake was associated with bacterial FA (and *Daphnia* sp. as well as cyclopoid copepod biovolume). Both lake as well as zooplankton size fraction were significant explanatory variables for zooplankton taxonomy and dietary marker data, explaining 20.7 and 29.5% of the variance in the dataset respectively (RDA; $p = 0.003$ for lake and $p = 0.001$ for size fraction).

Differences in FA composition between size fractions are likely attributable to taxonomic differences, with the 200–500 μm size fraction including a higher proportion of calanoid copepods (particularly in the clear-water lake), leading to higher dietary selectivity and increased reliance on phytoplankton (as was also observed based on $\delta^{13}C$ values). Higher DHA concentrations in the copepod-dominated 200–500 μm size fraction also likely reflects the increased ability of copepods to retain and accumulate DHA relative to cladocerans (Arts et al., 2009).

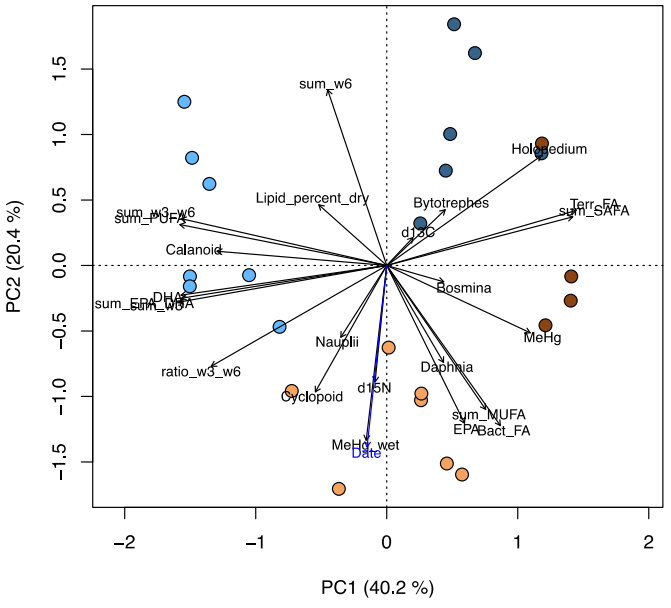

Fig. 4. Principal component analysis (PCA) triplot of zooplankton taxonomy, dietary marker and MeHg data (response variables shown as black vectors), samples (shown as points) and passively overlaid explanatory variables (lake, size fraction and sampling date). Explanatory variables are indicated by colour of individual score points for lake and size fraction (blue/brown for clear-/brown-water lake, light/dark for the 200–500 μm and >500 μm size fractions respectively) and by a blue vector for date. The first two PC axes explain 60.6% of the variance in water chemistry, and when explanatory variables (lake, size fraction, date) are overlaid, these axes explain 76% of the variance (based on RDA variance partitioning results). Data for the 50–200 μm size fraction are not shown here since for several of these metrics (including FA composition and MeHg) data were only available for a small number of samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Meanwhile the >500 μm size fraction was dominated by cladocerans in both lakes (Fig. 3), which is consistent with our expectation that these indiscriminate filter feeders are likely to ingest terrestrial POM, particularly in low productivity lakes where phytoplankton abundances are low.

For both size fractions, zooplankton from the brown-water lake had higher ΣMUFA and ΣBactFA, and lower ΣPUFA and DHA, than in the clear-water lake (ANOVA, all with $p < 0.05$ on both a proportional and mass fraction basis). On a mass fraction basis, ΣTerrFA was also higher in the brown-water lake (ANOVA, $p = 0.04$), indicating a higher degree of reliance on phytoplankton food-sources in the clear-water lake than in the brown-water lake. We also observed higher ratios of α-linoleic acid (ALA; 18:3n−3) to linoleic acid (LNA; 18:2n−6) in large zooplankton from the clear-water lake than from the brown-water lake ($p = 0.05$). This ratio has been shown to indicate terrestrial vs. aquatic resource use, with higher values indicating higher reliance on phytoplankton and lower values indicating reliance on terrestrial energy sources (Hixson et al., 2015). These results are consistent with our expectation that in the brown-water lake, where primary productivity is low due to reduced light availability, and where terrestrial particles and bacteria reliant on terrestrial DOM are likely to be more prevalent, allochthonous energy pathways to zooplankton will be more common.

An allochthonous vs. autochthonous energy pathway to zooplankton has important implications with respect to MeHg bioaccumulation, since zooplankton reliant on higher quality food sources, such as those from the clear-water lake, may be expected to have higher trophic efficiency (i.e. growth relative to mass of food consumed) and therefore lower bioaccumulation due to growth dilution (Karimi et al., 2007, 2016). Furthermore, zooplankton in the clear-water lake, which have higher mass fractions of Σ PUFA (and the essential fatty acid DHA), represent a higher nutritional quality food source for higher trophic levels than zooplankton from the brown-water lake, which may also reduce MeHg bioaccumulation at higher trophic levels through increased trophic efficiency and growth dilution (Karimi et al., 2007, 2016).

3.4. Higher MeHg in zooplankton from brown-water lake, but lower bioaccumulation factors

MeHg concentrations on a dry-weight basis (dw) were higher in all three zooplankton size fractions in the brown-water lake compared to the clear-water lake ($p < 0.05$). MeHg dw in the >500 μm fraction was higher than in the 50–200 μm fraction for both the clear- and brown-water lakes ($p = 0.01$ and 0.005 respectively; Fig. 3). Although consistent with observations from a wide range of studies (Kainz et al., 2002; Kainz and Mazumder, 2005; Kainz et al., 2008), our observed increase in MeHg dw with size appears to be driven by large differences in water content between the size fractions. The gelatinous cladoceran *H. glacialis* was an important contributor to the total biovolume in the >500 μm size fraction in both study lakes, leading to a particularly high moisture content for these zooplankton samples (mean of both lakes: 99.5 ± 0.4%) relative to the smaller size fractions (range of 81–97% for the 200–500 μm fraction). MeHg concentrations on a wet-weight basis (ww) were still higher in the brown-water lake than in the clear-water lake for both the 200–500 and >500 μm size fractions ($p = 0.002$ and 0.011 respectively). However, MeHg ww in zooplankton was higher in the 200–500 μm fraction than the >500 μm fraction for both lakes (both $p < 0.05$).

Our observed differences in MeHg ww concentrations between the size fractions do not seem to be related to differences in zooplankton trophic level between size fractions since the 200–500 and >500 μm size fractions do not include a significant contribution by predatory zooplankton, have no apparent differences in $\delta^{15}N$ values and are dominated by filter feeding cladocerans and largely herbivorous calanoids. On the other hand, it is possible that the higher MeHg ww in the 200–500 μm fraction may reflect the contribution of cyclopoids, particularly in the brown-water lake. Cyclopoid predation on

microzooplankton reliant on microbial food sources could add additional trophic levels, thus increasing effective trophic level and reducing trophic efficiency, both of which may increase accumulation of MeHg. Furthermore, the high abundances of rotifers in the brown-water lake suggest high availability of potential microzooplankton prey for cyclopoid copepods. This potential link between cyclopoid abundance, zooplankton trophic level and MeHg accumulation is also supported by the results of the PCA (Fig. 4), where cyclopoid contribution to the zooplankton biovolume was positively associated with both $\delta^{15}N$ and MeHg ww. A similar "browning"-mediated shift in dominant trophic pathways from a one-step phytoplankton-cladoceran zooplankton food web toward a two (or more)-step food web including increased rotifer abundance and predation on rotifers by cyclopoid zooplankton has been reported in a long-term study of lakes with increasing terrestrial OM (Williamson et al., 2015). Based on PCA, MeHg dw increased with increasing terrestrial and bacterial FA markers and Cladocera biovolume, and decreased with increasing algal FA markers. Meanwhile, MeHg ww increased with $\delta^{15}N$ and bacterial FA markers as well as contribution of Cyclopoida and *Daphnia* sp. to the zooplankton biovolume (Fig. 4).

MeHg bioaccumulation factors (BAFs; calculated based on MeHg ww) were higher in the clear-water lake than in the brown-water lake for the 200–500 μm zooplankton size fractions ($p = 0.025$; Fig. 3), but did not differ between lakes for the 500 μm size fraction ($p = 0.67$). The seasonal variability in BAFs for all zooplankton sizes was higher in the clear-water lake than the brown-water lake. However, aqueous MeHg concentrations in the clear-water lake were extremely low, so a very small change in MeHg concentrations, e.g. from 0.1 pmol L^{-1} to 0.05 ng L^{-1}, would lead to a doubling of BAF (i.e. a high sensitivity of BAF to small changes in aqueous MeHg). This leads to higher uncertainty in the BAFs for the clear-water lake and is likely the driver for the high between-date variability in BAFs in this lake. This uncertainty complicates comparison of BAFs between the two study lakes. MeHg BAFs were ~5-fold higher for the 200–500 μm zooplankton size fraction than for the >500 μm size fraction for the clear water study lake ($p = 0.005$) and did not differ between size fractions for the brown water lake ($p = 0.10$).

In summary, the BAFs suggest less efficient uptake of MeHg into the calanoid dominated 200–500 μm size fraction in the brown-water lake than in the clear-water lake (Fig. 3). Lower MeHg BAFs in the brown-water lake is contrary to our expectations based on evidence of higher dietary reliance on lower quality allochthonous and microbial food sources by zooplankton from the brown-water lake (which could be expected to reduce energy and nutrient transfer efficiency, thus increasing MeHg bioaccumulation). However, the lower BAFs in the brown-water lake likely reflect reduced bioavailability of aqueous MeHg in the brown-water lake due to sorption to larger and less bioavailable terrestrial OM molecules, which are prevalent in humic lakes, including our brown-water study lake, where $SUVA_{254}$ values indicate high concentrations of terrestrially-derived and highly aromatic OM. Reduced uptake of MeHg due to sorption to terrestrially-derived OM has been observed for both coastal and freshwater plankton (Zhong and Wang, 2009; Luengen et al., 2012; French et al., 2014; Schartup et al., 2015; Braaten et al., 2018). Despite higher concentrations of MeHg in the brown-water lake, reduced bioavailability due to higher terrestrial OM concentrations may play a role in limiting uptake into the base of the food web, acting to moderate food web accumulation of MeHg. However, given the higher MeHg concentrations in zooplankton from the brown-water lake, the net effect of higher terrestrial OM and aqueous MeHg concentrations was still positive (i.e. higher MeHg in zooplankton) in this lake.

MeHg per unit DHA in the brown-water lake zooplankton was higher than in the clear-water lake for both size fractions (both $p < 0.01$), and was higher in the >500 μm size fraction than in the 200–500 μm size fraction for both lakes (both $p < 0.05$; Fig. 3). Thus, zooplankton from the brown-water lake represent a poorer food source to higher trophic levels due to the combination of higher MeHg contamination and lower concentrations of PUFA. This is likely to be reflected in MeHg and essential FA concentrations at higher trophic levels (including fish).

3.5. Conclusions

Given the ongoing "browning" of boreal surface waters, there is a need for detailed knowledge on the potential effects of terrestrial OM on MeHg transport, uptake and food web transfer in aquatic ecosystems. Our results, contrasting a clear- and brown-water lake, suggest that higher terrestrial OM concentrations are linked to higher MeHg concentrations in water and zooplankton, reduced zooplankton reliance on phytoplankton as a food source, and lower concentrations of essential fatty acids such as DHA in zooplankton. Taken together, this suggests that high concentrations of terrestrial OM result in decreased nutritional quality of zooplankton, i.e. higher MeHg concentrations, and lower content of essential FA. Surprisingly, we found no effect of season on the above relationships and between-lake differences. This study emphasizes the importance of terrestrial OM concentrations in shaping aquatic biogeochemistry, ecology and contaminant dynamics.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.03.446.

References

Arnot, J.A., Gobas, F.A., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14, 257–297.
Arts, M.T., Brett, M.T., Kainz, M.J., 2009. Lipids in Aquatic Ecosystems. Springer, New York.
Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P., Jansson, M., 2009. Terrestrial organic matter and light penetration: effects on bacterial and primary production in lakes. Limnol. Oceanogr. 54, 2034–2040.
Ask, J., Karlsson, J., Jansson, M., 2012. Net ecosystem production in clear-water and brown-water lakes. Glob. Biogeochem. Cycles 26, GB1017.
Berggren, M., Ziegler, S.E., St-Gelais, N.F., Beisner, B.E., del Giorgio, P.A., 2014. Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes. Ecology 95, 1947–1959.
Berggren, M., Bergström, A.-K., Karlsson, J., 2015. Intraspecific autochthonous and allochthonous resource use by zooplankton in a humic lake during the transitions between winter, summer and fall. PLoS One 10, e0120575.
Braaten, H.F.V., Harman, C., Øverjordet, I.B., Larssen, T., 2014a. Effects of sample preparation on methylmercury concentrations in Arctic organisms. Int. J. Environ. Anal. Chem. 94, 863–873.
Braaten, H.F.V., de Wit, H.A., Harman, C., Hageström, U., Larssen, T., 2014b. Effects of sample preservation and storage on mercury speciation in natural stream water. Int. J. Environ. Anal. Chem. 94, 381–384.
Braaten, H.F.V., de Wit, H.A., Fjeld, E., Rognerud, S., Lydersen, E., Larssen, T., 2014c. Environmental factors influencing mercury speciation in Subarctic and Boreal lakes. Sci. Total Environ. 476, 336–345.
Braaten, H.F.V., de Wit, H.A., Larssen, T., Poste, A.E., 2018. Mercury in fish from Norwegian lakes: the complex influence of aqueous organic carbon. Sci. Total Environ. 627, 1–8.
Bravo, A.G., Bouchet, S., Tolu, J., Björn, E., Mateos-Rivera, A., Bertilsson, S., 2017. Molecular composition of organic matter controls methylmercury formation in boreal lakes. Nat. Commun. 8, 14255.
Clayden, M.G., Kidd, K.A., Wyn, B., Kirk, J.L., Muir, D.C.G., O'Driscoll, N.J., 2013. Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. Environ. Sci. Technol. 47, 12047–12053.
Cole, J.J., Carpenter, S.R., Kitchell, J., Pace, M.L., Solomon, C.T., Weidel, B., 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proc. Natl. Acad. Sci. 108 (5), 1975–1980.

Creed, I.F., Bergström, A.-K., Trick, C.G., Grimm, N.B., Hessen, D.O., Karlsson, J., et al., 2018. Global change-driven effects on dissolved organic matter composition: implications for food webs of northern lakes. Glob. Chang. Biol. 11, 34014–34069.
de Wit, H.A., Mulder, J., Hindar, A., Hole, L., 2007. Long-term increase in dissolved organic carbon in streamwaters in Norway is response to reduced acid deposition. Environ. Sci. Technol. 41, 7706–7713.
de Wit, H.A., Valinia, S., Weyhenmeyer, G.A., Futter, M.N., Kortelainen, P., Austnes, K., et al., 2016. Current browning of surface waters will be further promoted by wetter climate. Environ. Sci. Technol. Letters 3, 430–435.
del Giorgio, P.A., France, R.L., 1996. Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. Limnol. Oceanogr. 41, 359–365.
Driscoll, C.T., Yan, C., Schofield, C.L., Munson, R., Holsapple, J., 1994. The mercury cycle and fish in the Adirondack lakes. Environ. Sci. Technol. 28, 136–143.
Felip, M., Catalan, J., 2000. The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima. J. Plankton Res. 22, 91–106.
Finstad, A.G., Andersen, T., Larsen, S., Tominaga, K., Blumentrath, S., de Wit, H.A., et al., 2016. From greening to browning: catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. Sci. Rep. 24, 31944.
Fjeld, E., Rognerud, S., 2009. Miljøgiffter i ferskvannsfisk, 2008. Kvikksølv i abbor og organiske miljøgifter i ørret. Norwegian Institute for Water Research, Report number 1056/2009 (in Norwegian).
Folch, J., Lees, M., Sloane, S.G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
French, T.D., Houben, A.J., Desforges, J.-P.W., Kimpe, L.E., Kokelj, S.V., Poulain, A.J., et al., 2014. Dissolved organic carbon thresholds affect mercury bioaccumulation in Arctic lakes. Environ. Sci. Technol. 48, 3162–3168.
Gu, B., 2009. Variations and controls of nitrogen stable isotopes in particulate organic matter of lakes. Oecologia 160 (3), 421–431.
Hansen, A.M., Kraus, T.E.C., Pellerin, B.A., Fleck, J.A., Downing, B.D., Bergamaschi, B.A., 2016. Optical properties of dissolved organic matter (DOM): effects of biological and photolytic degradation. Limnol. Oceanogr. 61, 1015–1032.
Hessen, D.O., 2005. Aquatic food webs: stoichiometric regulation of flux and fate of carbon. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen. 29 pp. 39–49.
Hessen, D.O., Andersen, T., Larsen, S., Skjelkvåle, B.L., de Wit, H.A., 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnol. Oceanogr. 54, 2520–2528.
Hixson, S.M., Sharma, B., Kainz, M.J., Wacker, A., Arts, M.T., 2015. Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. Environ. Rev. 23, 414–424.
Jankowski, K., Schindler, D.E., Holtgrieve, G.W., 2012. Assessing nonpoint-source nitrogen loading and nitrogen fixation in lakes using $\delta^{15}N$ and nutrient stoichiometry. Limnol. Oceanogr. 57, 671–683.
Jeremiason, J.D., Reiser, T.K., Weitz, R.A., Berndt, M.E., Aiken, G.R., 2016. Aeshnid dragonfly larvae as bioindicators of methylmercury contamination in aquatic systems impacted by elevated sulfate loading. Ecotoxicology 25, 456–468.
Jones, R.I., Grey, J., 2011. Biogenic methane in freshwater food webs. Freshw. Biol. 56, 213–229.
Jones, R.I., Grey, J., Sleep, D., Quarmby, C., 1998. An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. Proc. R. Soc. Lond. Ser. B: Biol. Sci. https://doi.org/10.1098/rspb.1998.0270.
Jones, R.I., Grey, J., Sleep, D., Arvola, L., 1999. Stable isotope analysis of zooplankton carbon nutrition in humic lakes. Oikos 97–104.
Jonsson, S., Andersson, A., Nilsson, M.B., Skyllberg, U., Lundberg, E., Schaefer, J.K., et al., 2017. Terrestrial discharges mediate trophic shifts and enhance methylmercury accumulation in estuarine biota. Sci. Adv. 3, e1601239.
Kainz, M., Mazumder, A., 2005. Effect of algal and bacterial diet on methyl mercury concentrations in zooplankton. Environ. Sci. Technol. 39, 1666–1672.
Kainz, M., Lucotte, M., Parrish, C.C., 2002. Methyl mercury in zooplankton—the role of size, habitat, and food quality. Can. J. Fish. Aquat. Sci. 59, 1606–1615.
Kainz, M., Telmer, K., Mazumder, A., 2006. Bioaccumulation patterns of methyl mercury and essential fatty acids in lacustrine planktonic food webs and fish. Sci. Total Environ. 368, 271–282.
Kainz, M., Arts, M.T., Mazumder, A., 2008. Essential versus potentially toxic dietary substances: a seasonal comparison of essential fatty acids and methyl mercury concentrations in the planktonic food web. Environ. Pollut. 155, 262–270.
Karimi, R., Chen, C.Y., Pickhardt, P.C., Fisher, N.S., Folt, C.L., 2007. Stoichiometric controls of mercury dilution by growth. Proc. Natl. Acad. Sci. 104, 7477–7482.
Karimi, R., Chen, C.Y., Folt, C.L., 2016. Comparing nearshore benthic and pelagic prey as mercury sources to lake fish: the importance of prey quality and mercury content. Sci. Total Environ. 565, 211–221.
Karlsson, J., Jonsson, A., Meili, M., Jansson, M., 2003. Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. Limnol. Oceanogr. 48, 269–276.
Karlsson, J., Berggren, M., Ask, J., Byström, P., Jonsson, A., Laudon, H., et al., 2012. Terrestrial organic matter support of lake food webs: evidence from lake metabolism and stable hydrogen isotopes of consumers. Limnol. Oceanogr. 57, 1042–1048.
Karlsson, J., Bergström, A.-K., Byström, P., Gudasz, C., Rodríguez, P., Hein, C., 2015. Terrestrial organic matter input suppresses biomass production in lake ecosystems. Ecology 96, 2870–2876.
Klapstein, S.J., Ziegler, S.E., O'Driscoll, N.J., 2018. Methylmercury photodemethylation is inhibited in lakes with high dissolved organic matter. Environ. Poll. 232, 392–401.
Korosi, J.B., McDonald, J., Coleman, K.A., Palmer, M.J., Smol, J.P., Simpson, M.J., et al., 2015. Long-term changes in organic matter and mercury transport to lakes in the sporadic discontinuous permafrost zone related to peat subsidence. Limnol. Oceanogr. 60, 1550–1561.
Kritzberg, E.S., Cole, J.J., Pace, M.L., Granéli, W., Bade, D.L., 2004. Autochthonous versus allochthonous carbon sources of bacteria: results from whole-lake ^{13}C addition experiments. Limnol. Oceanogr. 49, 588–596.
Lajtha, K., Michener, R.H., 1994. Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications.
Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environ. Sci. Technol. 47, 13385–13394.
Lehmann, M.F., Bernasconi, S.M., McKenzie, J.A., Barbieri, A., Simona, M., Veronesi, M., 2004. Seasonal variation of the $\delta^{13}C$ and $\delta^{15}N$ of particulate and dissolved carbon and nitrogen in Lake Lugano: constraints on biogeochemical cycling in a eutrophic lake. Limnol. Oceanogr. 49, 415–429.
Luengen, A.C., Fisher, N.S., Bergamaschi, B., 2012. Dissolved organic matter reduces algal accumulation of methylmercury. Environ. Sci. Technol. 31, 1712–1719.
Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Høgåsen, T., et al., 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature 450, 537–540.
Parsons, T.R., 2013. A Manual of Chemical & Biological Methods for Seawater Analysis. Elsevier. Kent.
Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293–320.
Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R.B., Friedli, H.R., Leaner, J., et al., 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmospheric Chemistry and Physics Discussions 10, 4719–4752.
Poste, A.E., Braaten, H.F.V., de Wit, H.A., Sørensen, K., Larssen, T., 2015. Effects of photodemethylation on the methylmercury budget of boreal Norwegian lakes. Environ. Toxicol. Chem. 34, 1213–1223.
R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rautio, M., Mariash, H., Forsström, L., 2011. Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. Limnol. Oceanogr. 56, 1513–1524.
Ravichandran, M., 2004. Interactions between mercury and dissolved organic matter––a review. Chemosphere 55, 319–331.
Schartup, A.T., Ndu, U.C., Balcom, P.H., Mason, R.P., Sunderland, E.M., 2015. Contrasting effects of marine and terrestrially derived dissolved organic matter on mercury speciation and bioavailability in seawater. Environ. Sci. Technol. 49, 5965–5972.
Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W., 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36, 12–19.
Skarbøvik, E., Allan, I., Stålnacke, P., Hagen, A.G., Greipsland, I., Høgåsen, T., Selvik, J.R., Skanke, L.B., Beldring, S., 2016. Riverine inputs and direct discharges to Norwegian coastal waters – 2015. Norwegian Environment Agency Report number M634–2016 (86pp).
Syvaranta, J., Hamalainen, H., Jones, R.I., 2006. Within-lake variability in carbon and nitrogen stable isotope signatures. Freshw. Biol. 51, 1090–1102.
Taipale, S.J., Brett, M.T., Hahn, M.W., Martin-Creuzburg, D., Yeung, S., Hiltunen, M., et al., 2014. Differing Daphnia magna assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids. Ecology 95, 563–576.
Taipale, S.J., Galloway, A.W.E., Aalto, S.L., Kahilainen, K.K., Strandberg, U., Kankaala, P., 2016. Terrestrial carbohydrates support freshwater zooplankton during phytoplankton deficiency. Sci. Rep. 30, 1–15.
Tanentzap, A.J., Kielstra, B.W., Wilkinson, G.M., Berggren, M., Craig, N., del Giorgio, P.A., et al., 2017. Terrestrial support of lake food webs: synthesis reveals controls over cross-ecosystem resource use. Sci. Adv. 3, e1601765.
Thrane, J.-E., Hessen, D.O., Andersen, T., 2014. The absorption of light in lakes: negative impact of dissolved organic carbon on primary productivity. Ecosystems 17, 1040–1052.
Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. 37, 4702–4708.
Wilkinson, G.M., Pace, M.L., Cole, J.J., 2013. Terrestrial dominance of organic matter in north temperate lakes. Glob. Biogeochem. Cycles 27, 43–51.
Williamson, C.E., Overholt, E.P., Pilla, R.M., Leach, T.H., Brentrup, J.A., Knoll, L.B., et al., 2015. Ecological consequences of long-term browning in lakes. Sci. Rep. 5, 18666.
Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of mercury on wildlife: a comprehensive review. Environ. Toxicol. Chem. 17, 146–160.
Zhong, H., Wang, W.-X., 2009. Controls of dissolved organic matter and chloride on mercury uptake by a marine diatom. Environ. Sci. Technol. 43, 8998–9003.