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Effect of Algal and Bacterial Diet on Methyl Mercury Concentrations in Zooplankton

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We studied the effect of zooplankton diet on MeHg accumulation in different zooplankton size-fractions from lakes of different trophic status. Using fatty acid biomarkers, we tested the hypotheses that (a) variations of MeHg concentrations are determined by the taxonomic composition of zooplankton and (b) concentrations of dietary algal and bacterial compounds can predict MeHg concentrations of seston (10−64 μm), micro- (100−200 μm), meso- (200−500 μm), and macrozooplankton (>500 μm) in lakes on Vancouver Island, Canada. MeHg concentrations increased from seston (4−48 ng g dry weight^{-1}) to macrozooplankton (94−240 ng g dry weight^{-1}), indicating that MeHg accumulated as a function of plankton size. Results from linear regression analysis showed that MeHg concentrations were not significantly related to the taxonomic composition of zooplankton. However, using dietary lipid biomarkers, we demonstrated that bacterial diet ($R^2 = 0.50$; $p < 0.01$) could better predict variations of MeHg concentrations in zooplankton than essential algal diet ($R^2 = 0.35$; $p < 0.01$). Because MeHg accumulation within the planktonic food web was higher (20×) than the observed accumulation of total bacterial (6.5×) and algal (4.7×) diet biomarkers, zooplankton retained dietary MeHg more efficiently than bacterial and algal diet compounds. These results indicate that MeHg of macrozooplankton, the preferred prey size of planktivorous fish, is more efficiently transferred than essential diet compounds to organisms at higher trophic levels.

Introduction

Methyl mercury (MeHg) bioaccumulates in phagotrophic organisms of aquatic food webs. The planktonic food web is of particular interest as it conveys MeHg from the base of the food chain to organisms at higher trophic levels as a result of dietary uptake (e.g., *1*−*3*). The body size of prey has important implications for the transfer of nutrients as well as MeHg to higher trophic levels because macrozooplankton are the preferred prey size for planktivorous fish (*4*). Recent field studies reported increasing MeHg concentrations with increasing plankton size and suggested that MeHg is preferentially accumulated in larger-bodied adult zooplankton species such as *Holopedium gibberum* (*5*) and calanoid copepods (*6*).

Although it is important to measure plankton size, it is known that different genera can have similar body size, but higher body mass. This distinction is critical for prey consumption as heavy zooplankton species may transfer higher MeHg concentrations to predators than lighter ones. Therefore, in addition to knowing the taxonomic composition of zooplankton in lakes (*6*), we need to understand how MeHg concentrations are related to biomass shares of zooplankton genera within the planktonic food web.

There is correlative evidence that MeHg uptake from edible size-fractions is the principal route of MeHg in zooplankton in lakes (*7*−*9*). In recent laboratory experiments, while demonstrating that algal food is an important diet source of MeHg in *Daphnia magna*, Tsui and Wang (*10*) found that assimilation efficiencies of MeHg in this cladoceran decreased with increasing carbon content of algae. This finding raises the question whether higher algal biomass in natural systems, as the case for lakes of higher trophic status (algal biomass), conveys MeHg less efficiently to primary consumers than in lakes with lower algal biomass? Because the quantity and taxonomic composition of algal diet in zooplankton may differ among lakes of different, but also similar nutrient levels, the identification and quantification of zooplankton diet are crucial to a more detailed understanding of MeHg patterns in planktonic food webs.

To address these gaps, it is clear that more information on the dietary status of zooplankton is needed. For identification of zooplankton diet sources in lakes, analyses of stable C (δ^{13}C) and N (δ^{15}N) isotopes (e.g., *11*−*13*) and lipid biomarkers (e.g., *6*, *14*, *15*) have been applied. The application of lipid biomarkers, especially some fatty acids (FA), is particularly informative because it is possible to quantify algal- and bacteria-specific FA compounds. Napolitano (*16*) described polyunsaturated fatty acids (PUFA) in plankton as FA markers to assess algal biomass, and odd-saturated and branched-chain FA as bacterial biomarkers. Although it has only been demonstrated for daphnids that de novo FA synthesis rates are very low (<2%; *17*), there is, to our knowledge, no clear evidence that FA synthesis rates are higher in other zooplankton. It is thus assumed that most FA in zooplankton are largely dietary in origin and can be used as their diet indicators. Hence, we suggest that measuring the quantity of algal and bacterial diet in terms of selected FA in zooplankton provides more detailed information on how MeHg concentrations in zooplankton are related to their diet.

To address these objectives, we conducted a field study to (a) measure MeHg concentrations of different plankton size classes (i.e., 10−64, 100−200, 200−500, >500 μm), (b) identify their taxonomic composition and to calculate the standing-stock biomass of size-specific zooplankton genera, and (c) quantify algal- and bacterial-defined FA biomarkers within planktonic food webs. In this study, we tested the hypotheses that (i) plankton size, the taxonomic composition, and biomass of zooplankton genera, and (ii) the quantity of algal and bacterial biomarkers can predict MeHg concentrations in organisms of planktonic food webs. We demonstrate that MeHg accumulation of planktonic food webs is largely determined by increasing body size of zooplankton and by retention of bacterial and, to a lesser degree, algal diet, and propose that diet quantity rather than diet quality determines MeHg concentrations in zooplankton.

Materials and Methods

The study was conducted in June 2002 in six monomictic coastal lake systems on southern Vancouver Island, British Columbia, Canada. Shawnigan Lake (SHL; 48°37′ N, 123°38′ W) and Elk Lake (ELL; 48°31′ N, 123°23′ W) are natural lakes

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TABLE 1. Water Chemistry Measured in Natural Lakes and Drinking-Water Reservoirs of Southern Vancouver Island, British Columbia (*T* = Temperature; DO = Dissolved Oxygen; DOC = Dissolved Organic Carbon; Chl *a* = Chlorophyll *a* Data Are Mean Values of Epi-, Meta-, and Hypolimnetic Chl *a* Values ±Standard Deviation (SD; from Kainz et al., *27*))

station	station-depth(m)	Secchi-depth(m)	T^a (°C)	pH	DO[a](mg L^{-1})	Chl *a*mean ± SD(μg L^{-1})	DOC (mg L^{-1})
Natural Lakes							
SHL-A	24.0	4.5	20.8	7.1	7.9	1.7 ± 0.5	3.7
SHL-B	49.0	6.0	21.3	7.1	7.6	1.3 ± 1.2	3.7
COL	20.0	7.0	20.6	6.7	7.3	1.0 ± 0.3	2.5
ELL[b]	13.0	4.5	22.7	7.5	9.1	3.2 ± 0.6	6.0
Reservoirs							
SOL-A	15.5	6.5	21.5	7.1	7.5	0.5 ± 0.2	2.9
SOL-B	67.0	7.0	19.3	7.1	7.6	0.7 ± 0.3	3.1
GOL	28.0	7.5	20.8	6.7	8.5	0.4 ± 0.2	2.4
BUL	41.0?	8.0	20.9	6.9	6.7	0.5 ± 0.4	2.4

[a] Epilimnetic values. [b] ELL turned anoxic below 9 m water depth.

used for recreational activities including sport fishing. Council Lake (COL; 48°31′ N, 123°40′ W), Sooke Reservoir (SOL; 48°33′ N, 123°41′ W), Goldstream Reservoir (GOL; 48°30′ N, 123°38′ W), and Butchard Reservoir (BUL; 48°32′ N, 123°39′ W) are located in the protected Capital Regional District watershed area. SOL, GOL, and BUL are drinking-water reservoirs, established in 1914 and upgraded in 1970 (SOL; dam was raised by 6 m) and 1995 (GOL, BUL; no water-level changes), in which artificial drawdown of water occurs. The lake morphometry of SHL and SOL is very similar; both lakes have a shallow (-A) and a deep (-B) basin, and their mean water retention times are 2.0 and 1.4 yrs, respectively (*18*). Some physicochemical characteristics of these lakes are listed in Table 1.

For Chl *a* sampling, 1 L of epi-, meta-, and hypolimnetic lake water, using a Van Dorn water sampler, was filtered through a Gelman GF/F filter (0.45-μm pore size). The samples were kept frozen until extraction. For dissolved organic carbon (DOC) samples, epilimnetic lake water was taken with a plastic syringe and filtered (GN-6 mixed cellulose ester Gelman membranes, 0.45-μm pore size). All samples were kept without headspace in precombusted glass vials at 4 °C until analysis.

Zooplankton was collected by vertical tows from the deepest stations in each lake basin using a 64-μm plankton net. The organisms were rinsed with filtered (0.45-μm) lake water to remove adhered matter and size-fractionated using 100-, 200-, and 500-μm Nitex meshes. For logistic reasons, zooplankton were not sorted by genus. For seston, lake water was sampled using an integrated sampling tube (10-m length), filtered through a 64-μm mesh, and retained in a 10-μm mesh-size filter cup. This method was chosen to be consistent with the zooplankton size separation to store all samples immediately under the same conditions. The "seston fraction" (10−64 μm) excluded pico- and smaller nanoplanktonic algae and exceeded what is generally considered to be the most edible size-fraction (<30 μm; *19*) in the diet of cladocerans. To minimize the risk of oxidative and/or lipolytic degradation of lipids and their moieties, which may occur during prolonged handling of organisms at ambient temperatures, size-fractionated zooplankton and seston were transferred to polypropylene vials and immediately put on dry ice. The samples were kept frozen at −80 °C in a cryogenic freezer until lyophilization, following which they were again stored at −80 °C until FAME analysis could be performed.

Analyses

Zooplankton Classification, Chl *a*, pH, and DOC. Zooplankton were transferred to a plankton counting wheel under a microscope for identification, enumeration, measurement, and subsequent biomass (body weight per liter) estimation using Z-Counts software (Version 2.3, Voila Data Inc., Gloucester, Ontario). For Chl *a*, samples were extracted with 95% ethanol, followed by spectrophotometer measurements. The pH of epilimnetic lake and reservoir water was determined with a VWR Scientific Products model 2000 pH-meter. For DOC analysis, triplicate samples (8 mL) of filtered lake water were acidified with HCl (2 N) before analysis in a Shimadzu TOC-5000A analyzer (Shimadzu Corp., Kyoto, Japan). Dissolved oxygen (DO) and temperature profiles were measured using an YSI model 3800 multisampler (YSI Yellow Springs, OH).

Lipid and Fatty Acid Analysis. Lipids from homogenized, freeze-dried zooplankton samples (5−10 mg) were extracted as described by Parrish (*20*). Briefly, the samples were sonicated and vortexed four times in a 4:2:1 chloroform−methanol−water mixture, and the organic layers were removed and pooled.

FA were analyzed as FA methyl esters (FAME) using a gas-chromatograph (GC; Varian CP-3800, Varian, Inc., Palo Alto, Calif.) and analyzed with a flame-ionization detector (FID). The methyl esters were prepared by transesterifying the lipid extract in BF_3/CH_3OH; see Kainz et al. (*6*) for details on FAME formation. The FAME were analyzed on a 2560 Capillary Column (100 m, 0.25 mm i.d., 0.2 μm film thickness; Supelco, Sigma-Aldrich Inc., Bellefonte, PA). Helium was used as the carrier gas (1 mL min^{-1} flow rate). The following temperature ramp was employed: 65 °C for 0.5 min, hold at 195 °C for 15 min after ramping at 40 °C min^{-1}, and hold at 240 °C for 10 min after ramping at 2 °C min^{-1}. Helium (makeup gas) and air (combustion) had flow rates of 30 and 300 mL min^{-1}, respectively. The FID was isothermal at 260 °C, whereas the injector was programmed to rise to 250 °C at a rate of 200 °C min^{-1} after holding at 150 °C for 0.5 min. FAME were identified by comparison of their retention times with known standards (37-component FAME mix, Supelco). Quantification of individual FAME components was calculated on the basis of known amounts of injected standard dilutions (2000, 1000, 500, 250, 100, 50, and 2.5 ng μL^{-1}).

Methyl Mercury Analysis. Each freeze-dried zooplankton fraction was ground to a powder using a glass rod, and 0.5−1 mg (DW) of a sample was digested in 0.5 mL of a KOH/MeOH (1 g 4 mL^{-1}) solution for 8 h at 68 °C. The detailed method of this MeHg analysis is given by Pichet et al. (*21*). In brief, MeHg was separated by GC and then quantified using atomic fluorescence spectrometry. Each sample was analyzed three times, and analytical mean values (±SD) were reported. The detection limit for this method was about 0.6 pg of MeHg, which corresponds to 0.3 ng g^{-1} for a typical 2 mg sample, and the accuracy was positively tested by analyzing different National Research Council of Canada standards (DORM-1 and TORT-1).

Data Analysis. Paired *t*-test analysis was used to test (a) the effect of hydrographical differences between lakes and reservoirs on plankton size and (b) the level of difference for MeHg, algal, and bacterial FA concentrations of plankton between lakes and reservoirs (water body effect). Factors of plankton size difference were calculated by comparing the mean plankton sizes among the different size-fractions. To examine the effect of zooplankton taxonomy on differences in MeHg concentrations among the sampled lakes, we performed nearest neighbor, hierarchical cluster analysis. This numerical test grouped zooplankton of different taxa into classes so that similar ones formed clusters. The level of similarity of clusters was expressed by distance (taxonomy effect). To test the effect of zooplankton biomass on MeHg concentrations, we used analysis of variance (ANOVA) for meso- (200−500 μm) and macrozooplankton (>500 μm) fractions. We applied linear regression analysis, using analysis

of covariance (ANCOVA) to correct for the effect of sampling station, to examine the relationship between size of planktonic organisms and MeHg concentrations (plankton size effect); using the same method, we tested the relationships between algal and bacterial biomarkers and MeHg concentrations of planktonic organisms.

Results

Lake and Reservoir Characteristics. The mean (±SD) epilimnetic water temperature was 21 °C (±1), and all lakes and reservoirs were thermally stratified (thermocline started on average at 6 m depth). The water columns were generally well oxygenated (>2 mg DO L^{-1}) with the exception of ELL, which was anoxic below 9 m. The lakes and reservoirs had pH values of ~7. Mean Chl *a* concentrations in reservoirs (<1 $\mu g\ L^{-1}$) were lower than those of natural lakes (2.1 ± 1.1 $\mu g\ L^{-1}$; Table 1).

Size-Fraction and Taxonomic Composition. The mean length of macrozooplankton was 1144 and 1102 μm for the natural lakes and reservoirs, respectively. Although meso- and microzooplankton were collected using mesh sizes between 200 and 500 μm and 100−200 μm, respectively, their mean lengths were larger (i.e., 633 and 205 μm; 623 and 237 μm for natural lakes and reservoirs, respectively) because they could probably pass, head-first, through the smaller mesh size. We found no significant ($p > 0.05$) difference in zooplankton size in any size-fractions between natural lakes and reservoirs. Organisms of the seston size-class (10−64 μm) were not counted microscopically.

Macrozooplankton was mainly comprised of Calanoid copepods and *Daphnia* spp. In addition, *Holopedium gibberum* was identified in COL, SHL-A, and -B, GOL, BUL, and SOL-A. The mesozooplankton size-fraction mainly consisted of Calanoid and cyclopoid (missing at ELL) copepods, *Daphnia* spp., and copepod nauplii. The microplankton size-fraction was composed of copepod nauplii, *Keratella* spp., and some phytoplankton (*Asterionella formosa*, *Tabellaria fenestrata* et *T. flocculosa*, *Cyclotella* spp., *Ceratium hirundinella*, and *Dinobryon divergens*). The sestonic size-fraction was mainly comprised of algae (e.g., *A. formosa, T. fenestrata,* and *Chrysosphaerella longispina*). Results from nearest neighbor, hierarchic cluster analyses showed that the taxonomic composition of macrozooplankton was similar among ELL, SHL, SOL, and GOL as they shared clusters; however, the zooplankton communities of BUL and COL formed a cluster by themselves. Communities of mesozooplankton from ELL, SHL, SOL, GOL, and BUL shared clusters, and the community structure of COL created its own cluster. The biomass within the macrozooplankton size-fraction was highest for *H. gibberum* and *Daphnia* spp. (Figure 1). For the mesozooplankton size class, biomass shares differed among the lakes; however, the biomass of *H. gibberum* dominated in COL.

MeHg and FAMEs in Zooplankton. Mean MeHg concentrations increased from seston (7 ± 3 and 19 ± 20 ng g^{-1}) to the macrozooplankton size class (105 ± 9 and 178 ± 60 ng g^{-1}) in natural lakes and drinking-water reservoirs, respectively, and were significantly ($p = 0.013$) lower in planktonic organisms of natural lakes than in those of drinking-water reservoirs. Variations of the measured plankton size-fractions significantly predicted individual MeHg concentrations of planktonic organisms in both natural lakes ($R^2 = 0.78$, $p < 0.0001$) and drinking-water reservoirs ($R^2 = 0.74$, $p < 0.0001$; Figure 2). In macrozooplankton, mean MeHg concentrations were lowest in mesotrophic ELL (94 ± 2 ng g^{-1}) and highest in oligotrophic GOL (240 ± 19 ng g^{-1}). Using linear correlation analysis, MeHg concentrations of each plankton size-fraction were not significantly ($p > 0.1$) related with pH-values or concentrations of DOC and Chl *a* of lake water. Moreover, MeHg concentrations were not significantly

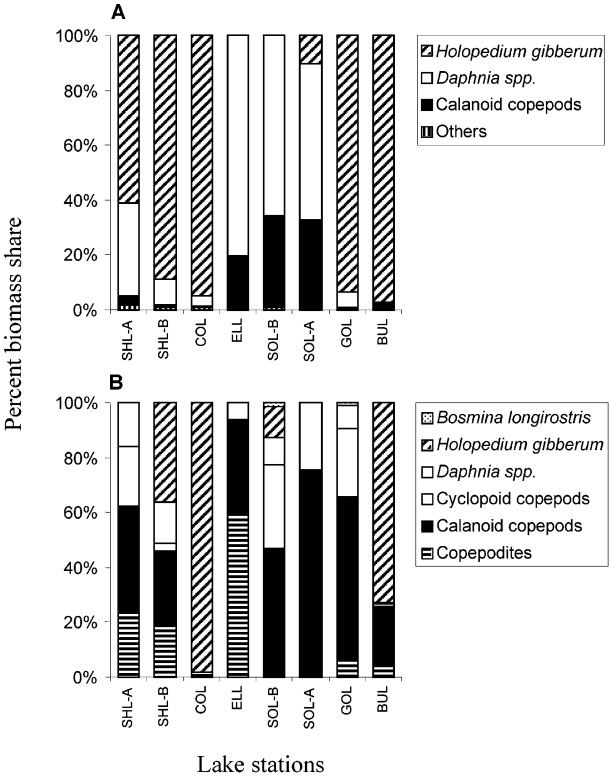

FIGURE 1. Percentages of biomass shares (cumulative bars) of (A) macrozooplankton (>500 μm mesh size) and (B) mesozooplankton (200−500 μm mesh size) from the natural lakes Shawnigan Lake, Stations A and B (SHL-A and -B), Council Lake (COL), and Elk Lake (ELL), and from the drinking-water reservoirs Sooke Lake, Stations A and B (SOL-A and -B), Goldstream Lake (GOL), and Butchard Lake (BUL). Figure modified from Kainz et al. (*27*).

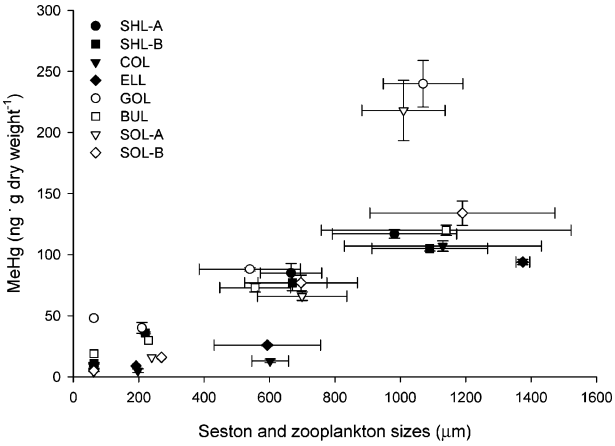

FIGURE 2. Methyl mercury concentrations in seston (10−64 μm), micro- (100−200 μm), meso- (200−500 μm), and macrozooplankton size-fraction (>500 μm) of natural lakes (dark symbols) and drinking-water reservoirs (light symbols).

($p > 0.05$) correlated with the biomass distribution of macro- (*H. gibberum*, *Daphnia* spp., and Calanoid copepods) and mesozooplankton (*H. gibberum*, *Daphnia* spp., Cyclopoid and Calanoid copepods, and copepodites).

Plankton size correlated significantly with MeHg; therefore, plankton size was not included as a covariate (for ANCOVA investigating relationships between concentrations of MeHg and FA biomarkers) to avoid problems with collinearity. Total PUFA concentrations, used as algal biomarkers, increased from seston (9.5 ± 1.7 mg g^{-1}) to macrozooplankton (24.3 ± 3.6 mg g^{-1}) and showed the highest

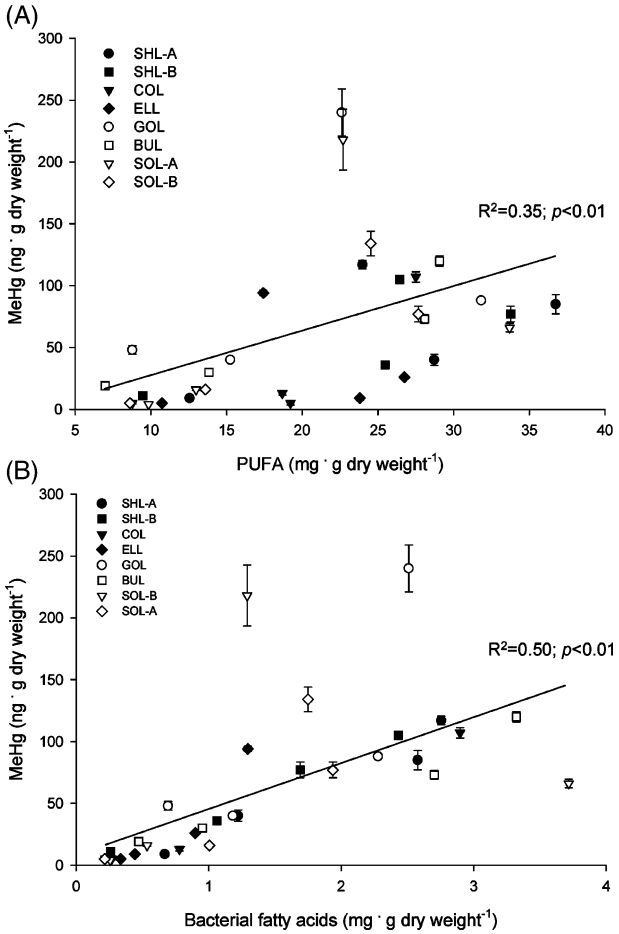

FIGURE 3. Methyl mercury concentrations related to (A) algal-derived polyunsaturated fatty acid (PUFA) concentrations, and (B) total bacterial fatty acid concentrations of seston (10−64 μm), micro- (100−200 μm), meso- (200−500 μm), and macrozooplankton size-fraction (>500 μm) of natural lakes (dark symbols) and drinking-water reservoirs (light symbols).

concentrations in mesozooplankton (30.0 ± 5.7 mg g^{-1}). Concentrations of these identified algal biomarkers did not differ significantly between lakes and reservoirs ($p = 0.4$) or between lakes with highest (GOL) and lowest (ELL) MeHg concentrations ($p = 0.9$). In macrozooplankton, highest PUFA concentrations were identified in BUL (29.1 mg g^{-1}) and lowest in ELL (17.4 mg g^{-1}). MeHg concentrations were significantly ($p < 0.01$) linearly correlated with concentrations of PUFA in organisms of the planktonic food web ($R^2 = 0.35$; Figure 3A). Because PUFA concentrations decreased in macrozooplankton, we examined the effect of body size on the accumulation of PUFA; results from quadratic regression analysis ($y = 9978.9 + 55.38x - 0.0369x^2$ and $y = 3445.3 + 64.799x - 0.0403x^2$) showed that PUFA concentrations started to significantly ($p < 0.001$) decrease with plankton sizes >750 and >804 μm, in lakes and reservoirs, respectively.

Concentrations of bacterial biomarkers, identified as the sum of odd-saturated and branched-chain FA (i.e., C15:0 and C17:0 and their iso and anteiso series), increased from seston (0.4 ± 0.2 mg g^{-1}) to macrozooplankton (2.3 ± 0.7 mg g^{-1}). In macrozooplankton, highest bacterial FA concentrations were measured in BUL (3.3 mg g^{-1}) and lowest in ELL (1.3 mg g^{-1}). MeHg concentrations were significantly ($p < 0.01$) linearly correlated with concentrations of total bacterial FA ($R^2 = 0.50$; Figure 3B). Using quadratic regression models, concentrations of total bacterial FA decreased significantly ($p < 0.001$) for zooplankton body sizes >1133 and >842 μm in lakes and reservoirs, respectively. Concentrations of total identified bacterial FA did not differ significantly ($p = 0.2$) between lakes and reservoirs. However, bacterial FA concentrations in macrozooplankton with highest MeHg concentrations (GOL) were significantly higher ($p = 0.029$) than in macrozooplankton with lowest MeHg concentrations (ELL).

TABLE 2. Accumulation Factors for Concentrations of Methyl Mercury (MeHg), Algal (PUFA), and Total Bacterial ($BAFA_{tot}$) Fatty Acid Compounds (mean ± SD) within the Planktonic Food Web: From Seston (seston; 10−64 μm), to Micro- (micro; 100−200 μm), to Meso- (meso; 200−500 μm), and to Macrozooplankton (macro; >500 μm)

	seston−micro	micro−meso	meso−macro	seston−macro
MeHg	2.5 (±1.3)	2.9 (±1.1)	3.0 (±2.4)	20.0 (±16.0)
PUFA	2.1 (±0.7)	2.1 (±0.6)	1.2 (±0.4)	4.7 (±0.8)
$BAFA_{tot}$	2.3 (±1.3)	2.9 (±1.7)	1.4 (±1.0)	6.5 (±2.7)

We defined "accumulation" as the increase of MeHg and FA concentrations from smaller to larger plankton sizes and "accumulation factors" as the quotients of MeHg and FA concentrations between different larger and smaller plankton size-fractions. Between seston and macrozooplankton, the mean accumulation factors were highest for MeHg (20), followed by total bacterial (6.5) and total algal fatty acids (4.7; Table 2).

Discussion

MeHg in Zooplankton. It is important to understand how MeHg concentrations are distributed within different size-fractions of the planktonic food web because MeHg concentrations in organisms at higher trophic levels are largely derived from dietary uptake of plankton. Results from field studies suggested that Hg concentrations in the piscivorous Northern pike (*Esox lucius*) were significantly related to MeHg concentrations of zooplankton >200 μm (*1*). Here, we examine variations of MeHg concentrations in plankton of different size classes and demonstrate that MeHg concentrations can be significantly predicted by concentrations of dietary bacterial and, to a lesser degree, by dietary algal biomarkers of planktonic food webs, but not by the taxonomic composition and biomass of larger (>200 μm body size) zooplankton.

MeHg concentrations increased significantly with increasing plankton size-fractions in both natural lakes and drinking-water reservoirs. Such size-dependent increases of MeHg concentrations in pelagic plankton indicate biomagnification of MeHg along the planktonic food web and are consistent with previous MeHg studies on zooplankton of temperate lakes (*5*, *6*). MeHg concentrations were higher in plankton of reservoirs than in natural lakes as observed in other studies of recently constructed Canadian reservoirs (*8*, *9*). It has been reported that in northern Wisconsin lakes (e.g., *2*), MeHg concentrations in non-size-fractionated zooplankton were negatively correlated with pH and positively correlated with DOC concentrations. In the present study, these water chemistry variables could not significantly predict the variability of MeHg concentrations in zooplankton, indicating that circumneutral lake water and the narrow range of DOC concentrations had little effect on patterns of MeHg concentration of these planktonic food webs.

MeHg concentrations can vary with the taxonomic composition of zooplankton (*22*). To examine the effect of the taxonomic composition on MeHg concentrations of zooplankton, we identified genera of meso- and macrozooplankton, the size-fractions closely related to MeHg concentrations in fish (*1*). Results from nearest neighbor analysis clearly indicate that the taxonomic composition of zooplankton is similar between the study lakes and reservoirs;

thus, there is no statistical evidence that the observed variation of MeHg concentrations in these zooplankton size-fractions, particularly between lakes and reservoirs, is associated with zooplankton taxonomy of these lake systems.

In addition to the analysis of taxonomic zooplankton composition, we estimated the standing stock biomass of these zooplankton size-fractions because it is possible that the variation of MeHg concentrations is related to the body mass of zooplankton genera rather than to individual counts. There is no significant correlative evidence that biomass of zooplankton genera can predict MeHg concentrations within the meso- and macrozooplankton. In macrozooplankton of lakes with similar biomass shares of the cladoceran *H. gibberum*, MeHg concentrations of this size-fraction differed significantly between GOL and SHL-B, COL, and BUL. On the basis of these results, we suggest that MeHg concentrations within these planktonic food webs are not related to particular zooplankton genera or to their body mass. There may be several explanations for these observations. First, retention and depuration dynamics of MeHg may be similar within the analyzed herbivorous zooplankton. However, it is difficult, if at all possible, to separate the effect of MeHg retention from MeHg depuration of lake samples. Alternatively, variations of MeHg concentrations in zooplankton may depend on the amount of ingested food. Because MeHg concentrations of the planktonic food web are significantly related to plankton size, increasing MeHg concentrations are possibly related with increasing uptake and perhaps retention of zooplankton diet. To test the effect of diet on MeHg in zooplankton, we examined relationships between algal and bacterial biomarkers, and MeHg concentrations of seston and different zooplankton size-fractions.

Diet Quality and MeHg in the Planktonic Food Web. Although it has been demonstrated in the laboratory that diet is a major source of MeHg in zooplankton (e.g., *10*, *22*), sources of ingested food in lake zooplankton have not been identified and quantified yet with regard to MeHg research in planktonic food webs. We used algal and bacterial lipid biomarkers to quantify the presence of algae and bacteria in lake seston and zooplankton. Although algal-derived lipid biomarkers can be used to assess algal diet, we emphasize that the sum of these algal biomarkers cannot be used as a proxy for growth-enhancing nutrients for zooplankton. It has been recently demonstrated that some algal-derived nutrients, docosahexaenoic acid (DHA) in particular, have little effect on somatic growth on laboratory-raised daphnids (*23*). We demonstrate correlative evidence that concentrations of algal and bacterial biomarkers can significantly and positively predict MeHg concentrations in zooplankton, reflecting the importance of diet identification as well as diet quantification to account for different MeHg patterns of zooplankton.

The significant relationship between concentrations of MeHg and algal FA along the planktonic food web indicates that MeHg accumulation patterns in lake zooplankton are directly related to the amount of ingested algae. This result suggests that variations of MeHg concentrations in different zooplankton size-fractions can be predicted by the uptake of algal diet rather than by variations of biomass shares of zooplankton. Hence, the accumulation of MeHg in zooplankton is related to retention dynamics of algal diet and therefore to some essential dietary nutrients in zooplankton. In addition to zooplankton diet, it has been reported that MeHg concentrations in zooplankton can be predicted by dissolved MeHg concentrations in lake water (e.g., *8*). We argue, however, that algal MeHg concentrations are more efficiently transferred across the plant−animal interface than MeHg across the water−animal interface because MeHg of the aquatic food web is most efficiently enriched between water and algae (*24*).

Concentrations of algal biomarkers decreased significantly in macrozooplankton, while MeHg concentrations continued to increase. These different accumulation patterns suggest that retention of algal diet decreases in larger-bodied zooplankton possibly because dietary energy is allocated into reproduction and transferred to offsprings (*25*). Alternatively, decreasing concentrations of algal biomarkers in macrozooplankton may be associated with inefficient retention of DHA, a FA compound used as algal biomarker, as previously demonstrated for cladoceran zooplankton (*26*, *27*). Therefore, the dietary retention of some ingested essential algal compounds in zooplankton is less effective than the dietary accumulation of nonessential MeHg in zooplankton.

Although less nutritious than algae (*28*), bacteria have been shown to comprise a small but significant portion of the herbivorous zooplankton diet (*6*, *14*). In this study, concentrations of bacterial FA biomarkers could significantly predict 50% of the variation of MeHg concentrations within the planktonic food web. This indicates that MeHg concentrations in zooplankton are more closely related with less nutritious bacterial than algal diet. Moreover, the significant decrease of both dietary algal and bacterial FA concentrations in the macrozooplankton size-fraction clearly demonstrates that the continuous accumulation of MeHg in zooplankton does not linearly depend on the dietary retention efficiency of bacterial or algal diet in macrozooplankton. These results show that MeHg concentrations in zooplankton are surprisingly more closely associated with less nutritious bacterial diet than with essential algal diet. Moreover, because diet accumulation in zooplankton was similar in all of the sampled lakes, higher MeHg concentrations in macrozooplankton of reservoirs were probably related to higher MeHg concentrations of reservoir diet. Therefore, on the basis of correlation models, we propose that the quality of zooplankton nutrition does not determine MeHg concentrations in zooplankton.

MeHg concentrations of macrozooplankton were significantly lower in mesotrophic ELL than in oligotrophic lakes. These observed differences are in line with findings of another study in northwestern Ontario (*29*) and suggest that MeHg in zooplankton is lower in lakes with higher nutrient levels because the MeHg partitioning coefficient between water and algae decreases with increasing algal biomass (biodilution effect). However, although algal biomass was highest in ELL, macrozooplankton contained the lowest concentrations of both algal biomarkers and MeHg. Lower MeHg concentrations may have been conveyed across the plant−animal interface in the mesotrophic ELL because (a) algal diet retained in zooplankton contained less MeHg (diet effect) and/or (b) less algal diet was required as lower molar C:P ratios from unfiltered lake water (Mazumder, unpublished) may have satisfied dietary and thus physiological requirements for zooplankton (lake trophic effect). It is possible that the lowest MeHg concentrations observed in macrozooplankton of ELL have been caused by faster growth rates and greater growth dilution as is generally observed for fish in meso- and eutrophic lakes (e.g., *30*); such a biodilution effect would also suggest that algal-derived FAs are not the only growth-enhancing nutrients for zooplankton. Finally, it is also possible that more MeHg was adsorbed to the larger DOC pool of ELL, assuming that DOC competes successfully against algae for available MeHg. In addition to the lowest concentrations of MeHg and algal biomarkers, macrozooplankton of ELL contained the lowest concentrations of bacterial FA biomarkers. This indicates that MeHg concentrations in zooplankton of the meso- and oligotrophic study lakes co-vary with diet retention in zooplankton. Finally, the taxonomic composition of zooplankton in mesotrophic ELL does not account for the observed differences in MeHg concentrations or dietary FA biomarkers because the taxo-

nomic composition of zooplankton genera in ELL is similar to that of other lakes with higher MeHg concentrations.

Implication for Higher Trophic Levels. Accumulation patterns of MeHg and highly nutritious PUFA in planktonic organisms have important toxicological and dietary implications for higher trophic levels. Macrozooplankton are the preferred prey size for planktivorous fish because of the generally accepted size-selective feeding concept for planktivores, by which large-bodied zooplankton are favored over smaller ones (*4*). MeHg concentrations accumulate at different rates than algal and bacterial FA compounds along the planktonic food web. The mean accumulation factor of MeHg between seston and macrozooplankton is higher than that of algal PUFA and bacterial FA. While MeHg accumulation increases steadily with increasing body size, mean accumulation factors of algal and bacterial FA compounds increase from seston to mesozooplankton, but decrease toward the cladoceran-dominated macrozooplankton size class. These different accumulation patterns indicate that MeHg (nonessential substance) is retained more efficiently than zooplankton diet (essential substances) in planktonic organisms. Therefore, following Brooks and Dodson's size-selective feeding concept, MeHg will be most efficiently conveyed to planktivorous fish by feeding on macrozooplankton.

In contrast to MeHg, some algal-derived PUFA are essential to optimal development and reproduction success for fish, and the physiological demand for PUFA is therefore high. However, because PUFA concentrations were highest in mesozooplankton, the most efficient uptake of algal PUFA by planktivorous fish would be achieved by ingesting organisms of the mesozooplankton size-class. Consequently, from a nutritious point of view, mesozooplankton represent the most favorable prey size for PUFA uptake, whereas it is macrozooplankton for MeHg uptake. Because macrozooplankton is the generally preferred diet size for planktivorous fish, MeHg will be more efficiently accumulated than algal-derived PUFA from these planktonic food webs.

This study shows that MeHg is highly retained and accumulated as a function of plankton size in zooplankton. Correlative evidence suggests that bacterial diet can better predict variations of MeHg concentrations in zooplankton than essential algal diet from lakes of different trophic status. The ranges of taxonomic composition and biomass of zooplankton were not significantly related to variations of MeHg concentrations, indicating that retention of dietary MeHg is similar among the different zooplankton genera of these study lakes. Although different genera of zooplankton contain higher concentrations of algal and bacterial diet, they accumulate MeHg more efficiently than essential diet compounds. Consequently, we conclude that macrozooplankton, as the preferred prey items for planktivorous fish, transfer MeHg, a conservative contaminant, more efficiently than essential diet compounds, that is, nonconservative substances, from the planktonic food web to organisms at higher trophic levels.

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