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Mercury Biomagnification through Food Webs Is Affected by Physical and Chemical Characteristics of Lakes

Meredith G. Clayden,*[,†] Karen A. Kidd,[†] Brianna Wyn,[‡] Jane L. Kirk,[§] Derek C. G. Muir,[§] and Nelson J. O'Driscoll[∥]

[†]Canadian Rivers Institute and Biology Department, University of New Brunswick, Saint John, New Brunswick E2L 4L5, Canada

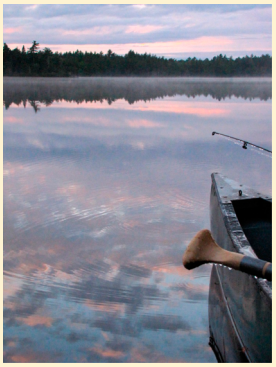
[‡]North/South Consultants, Incorporated, Winnipeg, Manitoba R3Y 1G4, Canada

[§]Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, Ontario L7R 4A6, Canada

[∥]Department of Earth and Environmental Sciences, Acadia University, Wolfville, Nova Scotia B4P 2R6, Canada

Supporting Information

ABSTRACT: Mercury (Hg) contamination in aquatic systems remains a global concern because the organic form, methyl Hg (MeHg), can biomagnify to harmful concentrations in fish, fish-eating wildlife, and humans. Food web transfer of MeHg has been explored using models of log MeHg versus relative trophic position (nitrogen isotopes, δ^{15}N), but regression slopes vary across systems for unknown reasons. In this study, MeHg biomagnification was determined for 11 lake food webs in Kejimkujik National Park, Nova Scotia, Canada, and compared to physical and chemical lake characteristics using principal component and multiple regression analyses. MeHg biomagnification (regression slopes of log MeHg versus baseline-adjusted δ^{15}N for fishes and invertebrates) varied significantly across lakes and was higher in systems with lower aqueous nutrient/MeHg/chloride scores. This is one of the largest, consistent data sets available on MeHg biomagnification through temperate lake food webs and the first study to use a principal component and multiple regression approach to understand how lake chemical and physical characteristics interact to affect biomagnification among systems. Overall, our results show that the magnitude of MeHg biomagnification through lake food webs is related to the chemical and physical characteristics of the systems, but the underlying mechanisms warrant further investigation.



INTRODUCTION

Biomagnification of the organic form of mercury (Hg), methyl Hg (MeHg), through aquatic food webs leads to potentially toxic concentrations of this contaminant in the tissues of fish and fish-eating wildlife.[1] The average trophic transfer of MeHg has been quantified using stable isotopes of nitrogen (δ^{15}N) because the heavier isotope (^{15}N) increases from prey to predator and provides a continuous measure of relative trophic position within a food web.[2] Within lakes, concentrations of MeHg are consistently positively and significantly related to the δ^{15}N of biota.[3] Slopes of the regression between $\log_{10}$-transformed MeHg [or total Hg (THg)] concentrations and δ^{15}N quantify the average Hg transfer through the food web,[2] and these slopes range from 0.16 to 0.26 in Arctic lakes, from 0.18 to 0.26 in temperate lakes, and from 0.16 to 0.28 in tropical lakes.[3] Mercury biomagnification has typically been quantified in one or a few lakes per study, with different experimental designs across locations and relatively few samples of lower-trophic-level organisms, making it difficult for broader comparisons to understand the nearly 2-fold differences in biomagnification slopes within and between geographic regions. Although biomagnification slopes differ statistically across a small number of lakes,[4] it is not known whether there are any effects of ecosystem characteristics on the biomagnification of MeHg because larger, systematic comparisons of slopes with higher sample sizes (numbers of lakes and taxonomic groups of lower-trophic-level organisms) are lacking.

Although most of the previous focus in the literature has been on the regression slopes, the y intercepts of these Hg biomagnification models also vary across lakes after standardizing for differences in basal δ^{15}N values.[4,5] It is not known whether variation in the intercepts reflects differences in concentrations of MeHg in primary consumers, although these intercepts are higher in some lakes with higher MeHg in bulk zooplankton.[6] It is unclear whether the intercepts are related to any other biological, physical, or chemical characteristics of ecosystems.

Ecosystem characteristics do, however, play an important role in the bioaccumulation of Hg by aquatic organisms. In particular, low pH promotes Hg bioavailability and methylation; therefore, biota in acidic systems tend to have relatively high MeHg concentrations.[7,8] Wetlands are both exporters of organic matter (which facilitates particulate and dissolved Hg and MeHg transport into downstream waters) and act as areas of MeHg production.[9] As such, lakes with more surrounding

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wetland area can have higher Hg concentrations in organisms.[10] Furthermore, Hg concentrations in biota at the base of the food web are relatively dilute in systems with more nutrients and higher primary productivity (biomass dilution),[11] and fish also tend to grow faster in such conditions,[12] leading to lower Hg concentrations in their tissues. Because diet is the main source of MeHg to fish,[13] both biomass and growth dilution may lead to lower overall MeHg bioaccumulation in biota of more nutrient-rich systems when compared to those from oligotrophic systems.[11] Given the predictive relationships between MeHg concentrations in organisms and ecosystem characteristics, it is possible that the magnitude of MeHg biomagnification through food webs (slopes) and/or the y intercepts of the log MeHg versus δ^{15}N relationships are also related to the chemical or physical conditions of lakes.

Both MeHg and δ^{15}N in aquatic biota are linked to protein content or amino acid composition and cycling. More specifically, MeHg in muscle protein is bound to the thiol (−SH) group on the amino acid cysteine,[14] a dominant source of sulfur in living cells,[15] and this amino acid also facilitates Hg bioaccumulation into microorganisms.[16] In addition, the fractionation of δ^{15}N from prey to predator (Δ^{15}N) is affected by dietary protein quality (i.e., presence of essential sulfur-containing amino acids) and content.[17] We examined whether tissue nitrogen (% N) and sulfur (% S) contents, as proxies for total protein and cysteine contents,[15,18] respectively, predicted MeHg concentrations in biota from these food webs. To our knowledge, these relationships have not been explored previously and contrasted to MeHg biomagnification models developed with δ^{15}N.

Kejimkujik National Park and National Historic Site (KNPNHS) in southwestern Nova Scotia, Canada, is a biological hotspot for Hg contamination in common loons (*Gavia immer*) and their prey, yellow perch (*Perca flavescens*).[19] Concentrations of Hg in these fish have increased in the majority of lakes sampled between 1996 and 2006, despite relatively constant Hg deposition to the area.[20] Lakes in KNPNHS have been culturally acidified[21] and have many characteristics that promote Hg methylation and bioaccumulation in aquatic organisms, including low pH and high levels of dissolved organic matter.[22] Significant among-system differences in the slopes of log MeHg versus δ^{15}N were found for four lakes within KNPNHS, and slopes were within the range found for near-neutral systems.[4]

In this study, primary through tertiary consumers from seven additional lakes in KNPNHS were analyzed for MeHg, relative trophic position (δ^{15}N adjusted to a common primary consumer in each lake),[23] % N, and % S. Data were combined with those of the four lakes studied by Wyn et al.,[4] providing a large and consistent data set over which to assess how Hg biomagnification is affected by different ecosystem characteristics. Our objectives were as follows: (1) to examine whether MeHg biomagnification slopes and intercepts of the log MeHg versus δ^{15}N regressions were related to ecosystem characteristics using a novel principal component and multiple regression approach, (2) to determine whether measured MeHg concentrations in organisms at the base of the food web (three lower-trophic-level groups: Limnephilidae caddisflies, Heptageniidae mayflies, and bulk zooplankton) were related to these ecosystem characteristics and whether their correlates reflected those of the regression intercepts, and (3) to determine whether biotic MeHg concentrations were related to measures of protein content (% N and % S) of the biota and whether these elements were better predictors of MeHg concentrations than δ^{15}N.

METHODS

Field Sampling. Lakes were chosen to capture a range of physical and chemical characteristics in KNPNHS (see Figure S1 and Tables S1 and S2 of the Supporting Information) and were sampled for the same taxa and following the same procedures as Wyn et al.[4] Bulk zooplankton and benthic invertebrates from each lake were collected in 1 year, in June, July, and August of 2009 or 2010. Benthic invertebrates were kept in aerated water overnight prior to being live-sorted into major taxa and frozen for further identification. Yellow perch (*P. flavescens*; n = 25−30 individuals/lake), banded killifish, and golden shiner (*Fundulus diaphanus* and *Notemigonus crysoleucas*, respectively; 8−10 individuals per species per lake) were collected using fyke nets and by angling in September of the same year as the invertebrate collections; weights and fork lengths were measured, and dorsal muscle tissues were removed and frozen immediately after collection. Whole bodies of killifish and shiner were also retained. Unfiltered surface and profundal water samples for THg and MeHg analyses were collected, preserved, and analyzed, as described in the Supporting Information. Samples for other water chemistry analyses, including pH, alkalinity, calcium (Ca), chloride (Cl), sulfate (SO_4), total organic carbon (TOC), total nitrogen (TN) and phosphorus (TP), iron (Fe), and aluminum (Al) were collected in the spring and fall over several years by Environment Canada; additional details on these analyses are provided in the Supporting Information.

Laboratory Analyses. Laboratory processing and analysis followed the same procedures as Wyn et al.[4] Individual fish muscle, whole-body fish samples, and pooled invertebrates (≥3 individuals per taxon within dates) were freeze-dried, homogenized, and subsampled for each analysis. Invertebrates were identified to the genus level (except Chironomidae) but had to be grouped to a higher level to obtain sufficient biomass for analysis; however, genera were only grouped when they had similar functional feeding habits.[24] For samples of Chironomidae, the predatory subfamily Tanypodinae was excluded but other individuals were grouped to gather enough biomass for analysis, because the other major subfamilies of Chironomidae (Chironominae and Orthocladiinae) generally belong to the gatherer−collector or filter−collector functional feeding groups.[24] Previous analyses of fish from these lakes found that MeHg accounted for more than 90% of THg;[4] for this reason, THg concentrations were measured in fish. Approximately 10 mg (±0.1 mg) of homogenized fish muscle (yellow perch) or whole body (other fish species) was analyzed on a Milestone DMA-80 direct mercury analyzer at the University of New Brunswick (Saint John, New Brunswick, Canada). A factor of 0.6 was used to calculate whole-body equivalent THg concentrations from dorsal muscle concentrations for yellow perch.[4] Whole-body fish THg concentrations were used for all analyses. MeHg analyses of invertebrates (individuals pooled within taxa and sampling date) were performed at Acadia University (Wolfville, Nova Scotia, Canada; additional details in the Supporting Information). All Hg data are expressed on a dry weight (dw) basis. δ^{15}N and nitrogen content of biological tissues were analyzed at the Stable Isotopes in Nature Laboratory (Fredericton, New Brunswick, Canada). Sulfur analysis was conducted at Iso-Analytical (Sandbach, U.K.).

Further details on the lab analyses and quality assurance procedures are provided in the Supporting Information.

Data Analyses. All data were examined for normality and homoscedasticity prior to statistical analysis, and data points with studentized residuals >3 were excluded. Fish lengths, weights, and Hg data for all organisms were $\log_{10}$-transformed to meet assumptions of normality.

Fishes in the lakes of KNPNHS feed primarily on littoral organisms, particularly those species sampled for the current study.[4] For this reason, the δ^{15}N of Limnephilidae, a littoral primary consumer found in all study lakes, was used to standardize the δ^{15}N of the other organisms and to correct for among-system differences in basal δ^{15}N.[25] Mean δ^{15}N of Limnephilidae from each lake was subtracted from the δ^{15}N of all individual organisms from that system to obtain $\delta^{15}N_{adj}$. These adjusted values were used in all subsequent analyses.

Results from the seven lakes were combined with the data for four lakes sampled in 2006−2007.[4] Analysis of variance (ANOVA) and Tukey's multiple comparisons (α = 0.05) were used to compare log Hg concentrations, log fork lengths (for fishes), $\delta^{15}N_{adj}$, % N, and % S within taxonomic groups across lakes.

Mercury biomagnification for each lake was calculated as the slope of the regression between log Hg (MeHg in invertebrates, Limnephilidae, Heptageniidae, littoral Chironomidae, Amphipoda, Aeshnidae, and zooplankton; THg in fishes, banded killifish, golden shiner, yellow perch; same taxa used for all lakes) and $\delta^{15}N_{adj}$. Residuals of all regressions were examined for normality (Kolmogorov−Smirnov tests; $p > 0.150$). Slopes were compared across lakes using analysis of covariance (ANCOVA) and manual multiple comparisons, whereas y intercepts were compared within groups of lakes with similar slopes using a Tukey's test in Minitab 16 software (see the Supporting Information for further details).

Multiple linear regression analyses using % N, % S, and $\delta^{15}N_{adj}$ of food web organisms were used to determine whether % N and % S explained additional variation in their log Hg concentrations. These analyses were confined to the seven lakes sampled in 2009 and 2010 (see Table S1 of the Supporting Information). An Akaike Information Criterion (AIC) adjusted for small sample sizes (AIC_c) was used to identify the best model for each lake.[26]

Principal component analysis (PCA) of the physical and chemical characteristics of the lakes (see Tables S1 and S2 of the Supporting Information) followed by multiple linear regression with the principal components[27] were used to examine whether selected ecosystem characteristics (see Table S3 of the Supporting Information) predicted Hg biomagnification slopes, y intercepts of these regressions, or lake-mean log Hg concentrations in lower-trophic-level organisms (Limnephilidae, Heptageniidae, and zooplankton) or yellow perch across lakes. This principal component regression approach was chosen as a statistically robust way of examining Hg biomagnification through food webs in relation to a large set of intercorrelated lake characteristics;[27] it allowed us to avoid arbitrarily excluding individual lake characteristics prior to regression analyses and to avoid potentially overinterpreting the importance of some variables in these analyses.[28] All lake characteristics were log-transformed and standardized prior to PCA. To account for variability in trophic position within taxa across lakes, $\delta^{15}N_{adj}$ was included as a variable in the models of log Hg in perch or lower-trophic-level organisms (raw mean δ^{15}N values were used for Limnephilidae; see above). AIC_c was again used to identify the best model in each case. To confirm the multiple regression analyses using principle components, multivariate canonical redundancy analysis (RDA) was conducted on the log Hg versus $\delta^{15}N_{adj}$ regression slopes and intercepts (dependent variables) using lake chemical and physical characteristics as independent variables. Principal component and multiple regression analyses were performed using IBM SPSS 19 software, whereas ANOVA, ANCOVA, simple linear regression, and correlation analyses were conducted using Minitab 16 software. RDA was conducted in R statistical software 3.0.0, using the Vegan package version 2.0-7. Additional details on data analyses are provided in the Supporting Information.

RESULTS AND DISCUSSION

Lake Chemical and Physical Characteristics. PCA grouped lakes by four main factors. The first factor (hereafter referred to as the "pH/metals/lake morphometry" factor) was most strongly influenced by lake water pH, metals (THg, Fe, and Al), and physical features (surface and catchment area and maximum depth; see Table S3 of the Supporting Information). Aqueous MeHg, Cl, and nutrients (Ca, TOC, TN, and TP) had the highest loadings in the second factor (hereafter the "nutrients/MeHg/chloride" factor). Wetland area (as a percentage of total catchment area) was dominant in the third factor (hereafter the "wetlands" factor), and lake water SO_4 concentrations had the highest loading in the fourth factor (hereafter the "sulfate" factor). Because of the multicollinearity between lake characteristics (see Table S4 of the Supporting Information), these groupings (rather than individual variables) were used in subsequent analyses (see the Methods) to examine among-lake variability in the log MeHg versus $\delta^{15}N_{adj}$ regression slopes or intercepts and concentrations of MeHg or THg in biota (see Tables S5 and S6 of the Supporting Information).

MeHg in Invertebrates. As in other studies, MeHg concentrations in invertebrate taxa varied significantly across lakes in KNPNHS (see Table S5 of the Supporting Information). For example, mean MeHg in Aeshnidae dragonflies varied by approximately 3-fold, from 0.17 ± 0.03 to 0.54 ± 0.34 $\mu g\ g^{-1}$ of dw (i.e., Peskowesk > Upper Silver and Pebbleloggitch; ANOVA, p = 0.002). Across lakes, log MeHg concentrations in lower-trophic-level biota (Limnephilidae, Heptageniidae, and zooplankton) were most strongly related (positively) to the pH/metals/lake morphometry factor and the sulfate factor accounted for additional variability among Limnephilidae (R_{adj}^2 = 0.348−0.730; see Table S7 of the Supporting Information). While it was not possible to attribute the variability in invertebrate MeHg concentrations to individual variables within the factors, these factors include variables shown in other studies to increase Hg methylation and bioavailability and MeHg concentrations in organisms. For instance, pH and lake size and depth strongly influenced the pH/metals/lake morphometry factor (see Table S3 of the Supporting Information), and lower lake pH and greater depth have been related to higher Hg concentrations in freshwater invertebrates.[6,29] Higher sulfate concentrations may also stimulate Hg methylation by sulfate-reducing bacteria, thereby providing more MeHg to the base of aquatic food webs,[30] which might account for the higher mean MeHg concentrations in Limnephilidae from lakes with higher sulfate scores.

THg in Fish. Mercury concentrations within fish species in KNPNHS also differed across lakes. For example, despite

Table 1. Regression Slopes (±SE) and Intercepts (±SE) of Log Hg (THg for Fish and MeHg for Invertebrates; $\mu g\ g^{-1}$ of dw) versus $\delta^{15}N_{adj}$ (‰) for 11 Lake Food Webs in Kejimkujik National Park, Nova Scotia, Canada, Sampled in 2006–2010[a]

lake name	slope	intercept	R^2	n
Big Dam West	0.128 ± 0.010 a	−0.980 ± 0.058	0.792	47
George	0.142 ± 0.010 ab	−1.046 ± 0.054	0.807	54
Peskowesk	0.145 ± 0.010 ab	−1.045 ± 0.063	0.807	48
Hilchemakaar	0.150 ± 0.010 abc	−1.379 ± 0.072	0.796	64
Upper Silver	0.151 ± 0.009 bc	−1.423 ± 0.057	0.831	64
Big Dam East	0.157 ± 0.007 abcd	−1.394 ± 0.049	0.906	52
Cobrielle	0.167 ± 0.009 cde	−1.242 ± 0.058	0.832	68
North Cranberry	0.184 ± 0.008 def	−1.262 ± 0.059	0.899	55
Puzzle	0.206 ± 0.010 fg	−1.467 ± 0.065	0.891	54
Pebbleloggitch	0.217 ± 0.013 g	−1.554 ± 0.072	0.880	42
Beaverskin	0.229 ± 0.009 g	−1.705 ± 0.064	0.924	52

[a]The slopes differed significantly among lakes (ANCOVA, $p < 0.001$); $p < 0.001$ for all regressions. Pairwise differences are indicated by lakes that do not share a letter; Tukey's multiple comparisons, $\alpha = 0.05$ (see the Methods). See Table S10 of the Supporting Information for pairwise differences in intercepts within groups of lakes with similar slopes.

having similar mean fork lengths (ANOVA, $p > 0.05$), mean THg concentrations in yellow perch ranged more than 2-fold across lakes, from 0.72 ± 0.28 to 1.93 ± 0.62 $\mu g\ g^{-1}$ of dw (see Table S6 of the Supporting Information). AIC_c scores indicated that mean log THg concentrations in perch were best predicted by the wetlands factor, but this model did not account for a lot of the variability in perch log THg across lakes ($R_{adj}^2 = 0.020$; see Table S7 of the Supporting Information)

Whereas there were some relationships between MeHg or THg in biota and lake chemistry and watershed characteristics across 10 of the lakes (see Table S7 of the Supporting Information), one lake (Pebbleloggitch), which has unusual physical and chemical characteristics (see Tables S1 and S2 of the Supporting Information), did not follow these trends. For instance, it is much shallower than other lakes (maximum depth of 2.5 m) and has the lowest pH and among the highest aqueous concentrations of THg, MeHg, and TOC; however, THg and MeHg concentrations in fishes and invertebrates, respectively, from this lake were among the lowest overall (see Tables S1, S2, S5, and S6 of the Supporting Information). Because of this, data from Pebbleloggitch were outliers, and although this lake was included in comparisons of Hg biomagnification slopes and intercepts across lakes (see below), it was excluded from the principal component and multiple regression analyses with lake characteristics (see Tables S3, S7, and S8 and Figure S2 of the Supporting Information). The chemical nature of TOC in Pebbleloggitch may cause Hg species to be sequestered and rendered unavailable to biota.[10]

Hg Biomagnification through Food Webs. The regression slopes of log MeHg versus $\delta^{15}N_{adj}$ of the KNPNHS food webs ranged from 0.128 ± 0.010 to 0.229 ± 0.009 (Table 1 and Figure 1) and were significantly different among the 11 lakes (ANCOVA, $p < 0.001$). In general, slopes were higher in lakes that had lower nutrients/MeHg/chloride factors (slope = −0.613 × nutrients/MeHg/chloride; $R_{adj}^2 = 0.298$; see Table S8 of the Supporting Information). This negative relationship suggests higher Hg biomagnification in lakes with lower trophic status and supports other studies showing that biotic mercury concentrations can undergo biomass and growth dilution in more nutrient-rich systems.[11,12] Furthermore, aqueous Ca (which had a strong influence on the nutrients/MeHg/chloride factor; see Table S3 of the Supporting Information) is known to limit the permeability of biological membranes and may,

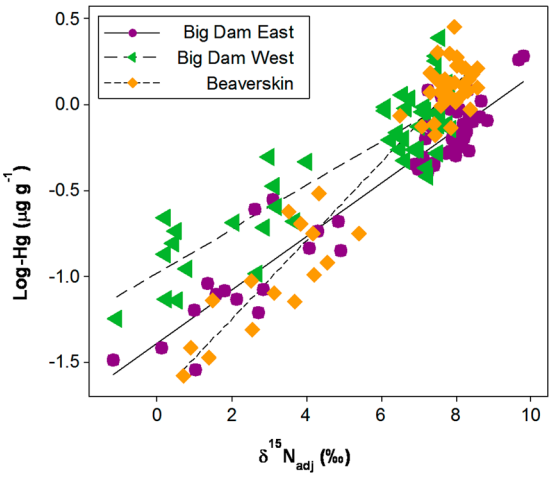

Figure 1. Log Hg (THg for fish and MeHg for invertebrates; $\mu g\ g^{-1}$ of dw) versus $\delta^{15}N_{adj}$ (‰) of fishes and invertebrates from three selected lakes in KNPNHS that illustrate the range of Hg biomagnification (regression slopes) observed in this study (see Table 1 for regression models).

therefore, decrease uptake of Hg into organisms, such as fish[31,32] and bacteria.[33] However, results from the current study contrast with those for food webs with lake trout as top predators in Ontario, Saskatchewan, and Alberta, Canada, which had higher Hg biomagnification in systems with higher aqueous TP.[5] Although it is unclear why our results differ from the lake trout study in this regard, the yellow perch in the food webs of KNPNHS feed primarily on littoral organisms,[4] whereas the lake trout were found to feed more equally on littoral and pelagic organisms.[5] Most of the individual variables with strong loadings in the nutrients/MeHg/chloride factor were also highly correlated with TOC (e.g., TN, TP, and MeHg; see Table S4 of the Supporting Information), and this is likely because organic matter is a source of nutrients in the water column.[34] The role of OC in Hg bioaccumulation is anomalous and varies among organisms and systems.[3] However, it is plausible that TOC affects the delivery of Hg to the aquatic systems in our study and its availability to lower-trophic-level organisms, as other studies have shown.[35] Thus, the higher Hg biomagnification slopes that we have seen in lower-nutrient, lower-TOC systems (see Figure S2 of the Supporting Information) could be due to enhanced uptake of

Hg by lower-trophic-level organisms (thereby driving up the intercepts of the regressions of log Hg versus $\delta^{15}N_{adj}$; see the discussion of the interdependence of slopes and intercepts below) under these conditions, although this does not explain the greater biomagnification of Hg through these food webs. Given that most lakes in KNPNHS are considered oligotrophic or mesotrophic and have higher TOC, studies of Hg biomagnification in eutrophic systems or those with lower aqueous OC would be of interest.

In the current study, we assumed that prey to predator fractionation of $\delta^{15}N$ ($\Delta^{15}N$) did not vary across lakes, but some among-system differences in Hg biomagnification may be related to variable $\Delta^{15}N$ across food webs. There is evidence that the quality of dietary protein is inversely related to $\delta^{15}N$ fractionation between an organism and its food and that the protein content of prey also contributes to variable $\Delta^{15}N$.[17] Furthermore, although little is known about how aqueous nutrient content affects the amount or quality of protein in low trophic-level organisms, nutrient content is related to the community composition of vegetation and other primary producers in lakes.[36] As such, differences in Hg biomagnification slopes between lakes that vary in their aqueous nutrient content could, in part, be related to differences in the availability or quality of proteins in prey.

Nitrogen and Sulfur Contents as Predictors of Biotic Hg Concentrations. In general, among invertebrates in KNPNHS lakes, the collector−gatherer taxa (Asellidae isopods and Limnephilidae caddisflies) had the lowest % N and % S contents in each lake, whereas the predatory Aeshnidae dragonflies had the highest % N and % S; some between-lake differences in % S but not % N were found (Aeshnidae and Heptageniidae; see Table S5 of the Supporting Information). The relative percentages of N and S among fish species were more variable and differed significantly between some lakes for yellow perch (see Table S6 of the Supporting Information). Within each food web, there were strong positive relationships between log MeHg concentrations in biota and their % N (R^2 = 0.600−0.785 across lakes) or % S (R_{adj}^2 = 0.404−0.731; see Table S9 of the Supporting Information). However, in six of the seven lakes, $\delta^{15}N_{adj}$ was a better predictor of biotic Hg concentrations than either % N or % S, although % N did account for additional variability in log Hg concentrations in some of these lakes (Big Dam West, George, and Upper Silver; see Table S9 of the Supporting Information). It is not clear why % N and % S were better predictors of log Hg concentrations than $\delta^{15}N_{adj}$ in one lake (Peskowesk; see Table S9 of the Supporting Information). Although we expected that % S might explain additional variation in log Hg concentrations of organisms because of the importance of sulfur amino acids in tissue storage of Hg, % S only predicted more variation in log Hg concentrations than $\delta^{15}N_{adj}$ in one lake (Peskowesk; see Table S9 of the Supporting Information). Therefore, it seems that the strong individual relationships between log Hg and % S in each lake may be more a function of the covariation of % S with $\delta^{15}N_{adj}$ and % N content.[15,18] Relationships between MeHg and measures of protein content are perhaps not surprising, given that MeHg is known to accumulate in proteins through its binding to the sulfur-containing amino acid cysteine[14] and that % N and % S are proxies of total protein and sulfur contents, respectively. However, overall, these results suggest that tissue nitrogen turnover and relative trophic level, as indicated by $\delta^{15}N$,[37] is more closely related to Hg accumulation in organisms than measures of their protein content.

Intercepts of Log Hg versus $\delta^{15}N$ Regressions. Within the lakes that had similar biomagnification slopes, there were some significant differences in the *y* intercepts (see Table S10 of the Supporting Information). Intercepts were positively correlated with MeHg concentrations in zooplankton and Heptageniidae across 10 lakes (see Table S4 of the Supporting Information; Pebbleloggitch excluded), which suggests that the intercepts reflect concentrations of MeHg in organisms at the base of the food web[5] (but see caveat below). In contrast to the negative relationships with Hg biomagnification slopes, the intercepts were positively related to the pH/metals/lake morphometry and nutrients/MeHg/chloride factors across 10 lakes (see Table S8 of the Supporting Information). As discussed above, it is possible that the relationships of the pH/metals/lake morphometry and nutrients/MeHg/chloride factors with the intercepts arise from the collinearity of TOC with many of the individual variables that influence these factors. More specifically, organic matter can transport metals and act as a source of nutrients in lake water.[33] It is also likely that several variables interact to affect Hg fate and uptake into the food web; for example, Hg photoreduction is diminished at greater depths in lakes with high iron and TOC, potentially leading to greater retention and bioavailability of Hg.[38] Although we expected that regression intercepts and MeHg concentrations in lower-trophic-level organisms would be influenced by similar factors, the intercepts were strongly (positively) predicted by both the pH/metals/lake morphometry and nutrients/MeHg/chloride factors, whereas MeHg in lower-trophic-level organisms was more strongly influenced by the pH/metals/lake morphometry factor alone (except for Limnephilidae, where the sulfate factor was also important; see Tables S7 and S8 of the Supporting Information). The reason for this difference is unclear, but it may relate to the interdependence of the regression slopes and intercepts, because the slopes were also predicted by the nutrients/MeHg/chloride factor

The fact that the MeHg versus $\delta^{15}N_{adj}$ regression slopes and intercepts were both predicted by the same factors in this study is not surprising because regression models with higher slopes have lower intercepts ($r = -0.800$; $p < 0.01$; see Table S4 of the Supporting Information). This is confirmed by the results of multivariate canonical redundancy analysis (RDA), showing that the slopes and intercepts are similarly influenced (albeit in opposite directions) by lake characteristics (see Figure S3 of the Supporting Information). However, the RDA was not significant (permutation test; $p > 0.4$); therefore, it was only possible to make very general comparisons between these results and those of principal component regression analyses. Nonetheless, the interdependence of the slopes and intercepts and their explanatory variables warrants further study as more estimates of these quantities become available for different ecosystem types and geographic areas.[5]

Overall, results from this study suggest that the concentration of Hg in taxa and its biomagnification through the food web were affected by lake characteristics. Although the biomagnification slopes herein were similar to those of other studies, we found that lakes in KNPNHS with lower nutrients/MeHg/Cl scores had higher slopes, possibly because of biomass and growth dilution or effects of nutrients on Hg bioavailability. Mercury concentrations in yellow perch were not related to biomagnification slopes across lakes ($r = 0.587$; $p > 0.05$; see

Table S4 of the Supporting Information), suggesting that elevated trophic transfer cannot account for the high Hg in perch reported by others for lakes in KNPNHS.[19,20] Given that the current study examined lakes within a more narrow range of nutrients (particularly lower calcium, TN, and TP and higher TOC and iron), studies of eutrophic or lower TOC systems are needed to determine whether the relationships observed herein are more widespread. We also recommend that future studies of Hg biomagnification slopes and intercepts consider a principal component regression approach to evaluate the influence of ecosystem characteristics, particularly when large sets of collinear physical and chemical data are available. Although the mechanisms behind the relationships identified herein are not fully understood, they are partially supported by more focused studies on individual lake characteristics and their effects on biotic Hg concentrations and food web biomagnification. Further studies using comparable experimental designs and across a broader range of lake characteristics would be beneficial in identifying common factors affecting Hg biomagnification in aquatic ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

Lake chemical and physical characteristics, along with details of field, laboratory, and statistical procedures. Detailed results of principal component, multiple regression, and redundancy analyses of Hg biomagnification slopes and biotic Hg concentrations, along with a map of the study lakes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +1 506 648 5809. E-mail: meredith.clayden@gmail.com.

Notes

The authors declare no competing financial interest.

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