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Environmental Toxicology and Chemistry, Vol. 36, No. 3, pp. 661–670, 2017

Printed in the USA

USING SULFUR STABLE ISOTOPES TO ASSESS MERCURY BIOACCUMULATION AND BIOMAGNIFICATION IN TEMPERATE LAKE FOOD WEBS

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(Submitted 9 May 2016; Returned for Revision 13 June 2016; Accepted 5 September 2016)

Abstract: Nitrogen and carbon stable isotopes ($\delta^{15}N$, $\delta^{13}C$) are commonly used to understand mercury (Hg) bioaccumulation and biomagnification in freshwater food webs. Though sulfur isotopes ($\delta^{34}S$) can distinguish between energy sources from the water column (aqueous sulfate) and from sediments to freshwater organisms, little is known about whether $\delta^{34}S$ can help interpret variable Hg concentrations in aquatic species or food webs. Seven acidic lakes in Kejimkujik National Park (Nova Scotia, Canada) were sampled for biota, water, and sediments in 2009 and 2010. Fishes, zooplankton, and macroinvertebrates were analyzed for $\delta^{34}S$, $\delta^{15}N$, $\delta^{13}C$, and Hg (methyl Hg in invertebrates, total Hg in fishes); aqueous sulfate and profundal sediments were analyzed for $\delta^{34}S$. Within lakes, mean $\delta^{34}S$ values in sediments and sulfate differed between 0.53‰ and 1.98‰, limiting their use as tracers of energy sources to the food webs. However, log-Hg and $\delta^{34}S$ values were negatively related (slopes –0.14 to –0.35, R^2 0.20–0.39, $p < 0.001$–0.01) through each food web, and slopes were significantly different among lakes (analysis of covariance, lake × $\delta^{34}S$ interaction term $p = 0.04$). Despite these relationships, multiple regression analyses within each taxon showed that biotic Hg concentrations were generally better predicted by $\delta^{15}N$ and/or $\delta^{13}C$. The results indicate that $\delta^{34}S$ values are predictive of Hg concentrations in these food webs, although the mechanisms underlying these relationships warrant further study. *Environ Toxicol Chem* 2017;36:661–670. © 2016 SETAC

Keywords: Food web Trophic transfer Methylmercury Biomagnification Bioavailability Sulfur Stable isotope Acidic lake

INTRODUCTION

Methylmercury (MeHg) is a neurotoxic metal that is known to bioaccumulate in freshwater fishes. In Canada, MeHg concentrations (measured mainly as total Hg, [THg]) in fish frequently exceed government consumption guidelines and therefore pose a risk to people and wildlife that consume them. However, THg concentrations in fishes vary among ecosystems. Of particular importance are factors that affect Hg methylation and its uptake into the base of the food web because diet is the main route of Hg exposure for fish. Mercury is methylated during bacterial respiration by anaerobic microorganisms such as sulfate-reducing and iron (Fe)–reducing bacteria in sediments [1,2] and the water column [3,4] and by methanogenic microbes in periphyton mats [5,6]. The bioavailability of Hg(II) for methylation and subsequent uptake of MeHg by biota depend on redox and photochemical conditions as well as the speciation and concentration of organic matter, essential nutrients, and other metals (e.g., Fe [7,8]). Sulfur (S) compounds also affect Hg fate, with higher concentrations of sulfate associated with greater activity of sulfate-reducing bacteria and therefore MeHg production [1]. Furthermore, complexes of Hg with S compounds (e.g., neutral Hg-sulfide complexes and complexes of Hg to low–molecular weight thiols) are readily absorbed and methylated by sulfate-reducing bacteria [9,10] and algal biofilms [11].

Within aquatic ecosystems, different forms of S (e.g., aqueous sulfate, SO_4^{2-}, sulfides in sediment) tend to have distinct stable isotope values ($\delta^{34}S$) that have been used to understand its cycling [12]. More specifically, the reduction of aqueous SO_4^{2-} by sulfate-reducing bacteria typically results in deposited sulfide compounds that are lower in ^{34}S; as a result, $\delta^{34}S$ in the water column (as SO_4^{2-}) is generally more positive than that of bulk sediments [13]. Distinct $\delta^{34}S$ values of these abiotic compartments (including oxic and anoxic layers) can be used to estimate the relative importance of energy sources from the open water versus sediment to freshwater food webs [12,14,15]. Differences in the $\delta^{34}S$ values between water and sediment indicate the extent of SO_4^{2-} reduction (and therefore sulfate-reducing bacteria activity) within a system [13]. Not surprisingly, S isotope discrimination is typically more pronounced in anoxic areas with higher SO_4^{2-} concentrations, conditions favorable to sulfate-reducing bacteria growth and activity [13,16]. In lakes, such areas are generally located in deep profundal waters below oxyclines or in deeper, more oxygen-depleted sediments. There is also recent evidence that $\delta^{34}S$ values are related to sulfate-reducing bacteria activity in wetlands [17].

Although values of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are often used to assess an animal's carbon sources and trophic position, respectively, $\delta^{34}S$ values can provide complementary information that may improve our understanding of Hg fate in aquatic food webs. For instance, because little $\delta^{34}S$ discrimination (denoted as $\Delta^{34}S$) is thought to occur during assimilation of dietary S in animals [18], $\delta^{34}S$ values can elucidate energy sources supporting aquatic food webs (e.g., Croisetière et al. [12]). Furthermore, because

This article includes online-only Supplemental Data.

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Published online 20 September 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3615

MeHg readily binds to the S atom on the amino acid cysteine and is stored in proteins [19,20], its concentrations in aquatic biota are related to proxies of their cysteine and protein content (%S and %N, respectively [21–23]). Given these links between S compounds and the speciation, bioavailability, and binding of Hg in organismal tissues, $\delta^{34}S$ may help explain some of the within-system and among-system variability in Hg in freshwater biota.

Although $\delta^{34}S$ has been used to understand Hg contamination in birds [24] and fish communities [25–29], little is known about whether $\delta^{34}S$ predicts MeHg concentrations in lower trophic levels and its biomagnification in aquatic food webs. In the present study, we investigated whether $\delta^{34}S$ (and to a lesser extent total S content as a percentage of dry wt) explained patterns of MeHg bioaccumulation and biomagnification through lake food webs in a known biological Hg "hot spot," Kejimkujik National Park and National Historic Site (hereafter referred to as Kejimkujik), Nova Scotia, Canada [30,31]. The surface waters in this area are acidic and have relatively low SO_4^{2-} concentrations [32], so sulfate-reducing bacteria activity may be lower than in more neutral systems with higher SO_4^{2-}(e.g., Croisetière et al. [12]). However, wetlands in the Kejimkujik lake catchments have been shown to be important sources of MeHg [33], and MeHg production in these areas is related to a variety of wetland characteristics, including sulfate reduction rates and the abundance of certain species of sulfate-reducing bacteria [34]. Specifically, we measured $\delta^{34}S$, $\delta^{13}C$, C, and S content in biota, water, and sediment of 7 lakes and combined these data with previously reported $\delta^{15}N$ and %N values and THg or MeHg concentrations in fishes and invertebrates [23]. The present objectives were to determine whether Hg concentrations within and among freshwater taxa were related to their $\delta^{34}S$ values and whether $\delta^{34}S$ advances our understanding of how habitat and energy sources affect Hg accumulation in lake biota beyond that gained from using $\delta^{15}N$ or $\delta^{13}C$.

METHODS

Field collections

Seven temperate lakes in Kejimkujik were sampled for fishes, invertebrates, water, and sediments in 2009 and 2010. These lakes are in undisturbed catchments and remote from human settlement and point sources of pollution. The lakes are naturally acidic (pH 5.0–6.3), have low SO_4^{2-}concentrations (1.08–1.71 mg/L), and a range of total organic carbon concentrations (TOC 3.0–12.6 mg/L), lake surface area (24–685 ha), and maximum depth (4.2–13.0 m; Table 1 [23,35]); and support several fish species and a large number (>100) of macroinvertebrate taxa [36,37]. Of these, we collected primary consumers and detrivorous taxa (mayflies, family Heptageniidae; caddisflies, family Limnephilidae; isopods, family Asellidae) as well as predatory dragonflies (family Aeshnidae) from 3 or more sites within the littoral zone of each lake using dip and kick nets. Bulk zooplankton were collected by towing a 153-μm mesh Wisconsin net behind a canoe through the pelagic zone of each lake. When present, profundal midges (Chironomidae) were collected with an Ekman grab from the deepest part of each lake. All invertebrate samples were collected 3 times over the season from each lake (and pooled within lakes, sampling dates, and taxa) in June, July, and August of 2009 or 2010. Fish species collected included banded killifish (*Fundulus diaphanus*), golden shiner (*Notemigonus crysoleucas*), white sucker (*Catostomus commersoni*), and yellow perch (*Perca flavescens*); and all fishing was conducted in September of 2009 or 2010. Fish sampling was done using protocols approved by the Animal Care Committee of the University of New Brunswick (2009-03-01 and 2010-02-09). Additional details about the study lakes and their characteristics as well as field sampling methods and chemical analyses of the biota are given in Clayden et al. [23].

Water samples for analysis of $\delta^{34}S$ in sulfate were taken 3 times during the sampling season 2 m from the bottom in the deepest part of each lake ($n = 3$ replicates per lake), using a 2-L Teflon-lined Niskin sampler. Samples were kept on ice, and sulfate was extracted by ion-exchange as barium sulfate within a few hours of collection [38]. An Ekman grab was used to collect surface sediments (top 5 cm) from the deepest part of each lake, 3 times during the sampling season ($n = 3$ replicates per lake).

Mercury and sulfur isotope analysis

Previous analyses of fishes in Kejimkujik lakes showed that MeHg concentrations typically accounted for >90% of THg [23, and references therein]; therefore, THg concentrations are reported for all fish in the present study. Total Hg in fish was analyzed using a direct Hg analyzer at the Canadian Rivers Institute, University of New Brunswick Saint John; and MeHg analysis in macroinvertebrates and zooplankton was performed at the Center for Analytical Research on the Environment at Acadia University. Laboratory methods for Hg analyses, along with quality assurance procedures, are described in detail in Clayden et al. [23]. Briefly, THg analyses included certified reference materials (TORT-2 and DORM-2, National Research Council of Canada), blanks, and duplicates with each batch of

Table 1. Physical and chemical characteristics (mean ± standard deviation) of 7 study lakes in Kejimkujik National Park, Nova Scotia, Canada[a]

Lake	Surface area (ha)	Maximum depth (m)	pH ($n = 4$)	TOC (mg/L) ($n = 4$)	Chl-a (μg/L) ($n = 3$)	TN (mg/L) ($n = 4$)	TP (mg/L) ($n = 4$)	Sulfate (mg/L) ($n = 4$)	% Sulfur in sediments[b] ($n = 3$)
Big Dam East	45.5	4.2	6.24 ± 0.07	4.45 ± 0.83	1.37 ± 0.40	0.21 ± 0.05	0.008 ± 0.002	1.38 ± 0.05	0.25 ± 0.01
Big Dam West	105	9.5	5.10 ± 0.17	12.63 ± 5.43	3.65 ± 0.07	0.31 ± 0.10	0.012 ± 0.001	1.08 ± 0.21	0.13 ± 0.01
Cobrielle	132	6.25	5.62 ± 0.14	3.05 ± 0.42	0.97 ± 0.46	0.16 ± 0.03	0.005 ± 0.001	1.26 ± 0.12	0.26 ± 0.004
George	77.8	8.5	5.11 ± 0.09	9.63 ± 2.25	3.87 ± 2.32	0.26 ± 0.07	0.012 ± 0.002	1.37 ± 0.10	0.14 ± 0.01
Hilchemakaar	95.4	7.25	5.83 ± 0.17	5.68 ± 0.59	1.63 ± 0.55	0.23 ± 0.05	0.012 ± 0.001	1.71 ± 0.14	0.15 ± 0.06
Peskowesk	685	13	4.99 ± 0.17	6.90 ± 1.06	1.10 ± 0.14	0.22 ± 0.01	0.007 ± 0.001	1.38 ± 0.11	0.21 ± 0.005
Upper Silver	24.3	5.75	6.33 ± 0.02	3.60 ± 0.29	2.00 ± 0.35	0.17 ± 0.02	0.008 ± 0.001	1.49 ± 0.11	0.23 ± 0.02

[a]Clair [35] and Clayden et al. [23].
[b]Percent sulfur in sediments was measured as the proportion of sulfur by dry mass.
Chl-a = chlorophyll-a; TN = total nitrogen; TOC = total organic carbon; TP = total phosphorus.

samples. Recovery for TORT-2 was $107 \pm 6\%$ ($n = 113$) and that for DORM-2 was $93 \pm 4\%$ ($n = 77$). Mean THg in blanks was 0.0005 ± 0.0004 μg ($n = 198$), and the relative difference between duplicate samples was 5.4 % ($n = 78$; based on results from a larger set of samples from these lakes ($n =$ approximately 750 samples)). For MeHg mean percent recovery in DORM-2 was $103 \pm 7\%$ ($n = 34$) with a method detection limit of 2.1 pg. The relative difference between duplicates was 10%. All Hg concentrations are reported on a dry weight basis.

Sulfur isotope values of aqueous sulfate in lake water samples (as barium sulfate) were measured at the University of Waterloo Environmental Isotope Laboratory (Waterloo, ON, Canada). In sediment and biota $\delta^{34}S$ and %S (whole, pooled, and homogenized invertebrates and homogenized muscle from individual fish) were analyzed at Iso-Analytical (Crewe, United Kingdom) by elemental analysis–isotope ratio mass spectrometry. Certified reference materials (barium sulfate, International Atomic Energy Agency IAEA-SO-5, $\delta^{34}S_{VCDT} = 0.5‰$ relative to Vienna Canyon Diablo Troilite [VCDT]) and an in-house laboratory standard (barium sulfate, IA-R061, established $\delta^{34}S_{VCDT}$ value of 20.33‰) were analyzed with each batch of samples. The mean value for IA-R061 was $20.3 \pm 0.27‰$ (relative difference of 0.01% from established value, $n = 29$) and that for IAEA-SO-5 was $0.54 \pm 0.27‰$ (relative difference of 7.7% from certified value, $n = 26$). Approximately 10% of samples were analyzed in duplicate, with an average difference between duplicates of $6.9 \pm 5.0\%$ ($n = 34$).

Data analysis

All statistical analyses were conducted using SPSS Ver 21, SigmaPlot Ver 11, or R package Ver 3.3.1; and alpha was set at 0.05 for all tests. All fish Hg concentrations are reported as THg on a whole-body basis; when muscle samples were analyzed, whole-body estimates were calculated as in Clayden et al. [23]. All invertebrate Hg is reported as MeHg concentrations; samples consisted of freeze-dried, homogenized whole bodies pooled within taxa and collection dates. Isotope values ($\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$) in invertebrates and fishes within each lake were plotted to assess food web structure. Values of $\delta^{15}N$ for all biota were adjusted by subtracting the mean $\delta^{15}N$ value of Limnephilidae caddisflies within each lake (referred to as $\delta^{15}N_{adj}$) [23], whereas $\delta^{34}S$ and $\delta^{13}C$ of the biota were not adjusted. Linear mixed effects models were run between log-Hg (THg in fish, whole-body estimates, and MeHg in invertebrates) and $\delta^{34}S$ values within taxa (mayflies, caddisflies, isopods, dragonflies, banded killifish, golden shiner, and yellow perch) and across lakes. By including "lake" as a random categorical factor, it accounted for the lack of independence of the data from within a lake and for the effect of the system on MeHg concentrations. Analysis of covariance (ANCOVA) models were run with log-Hg as the dependent variable and lake, $\delta^{34}S$, and an interaction term as the independent variables. Further, backward stepwise multiple regressions were run using log-MeHg or THg concentrations in each benthic invertebrate taxon or fish species, respectively, as the dependant variable and $\delta^{15}N_{adj}$, $\delta^{13}C$, $\delta^{34}S$, %N, %C, and %S as predictor variables. Similar regression models were run using all THg food web data combined across lakes. Tolerance values and variance inflation factors were assessed per Tabachnick and Fidell [39] to ensure that there were no issues with multicollinearity in these models. For the full food web model, %N and %S were removed as predictors because of high variance inflation factor values (>10). Multiple regression models were assessed using Akaike's information criterion (AIC_c) adjusted for small sample sizes; the model with $\Delta AIC_c < 2$ (where ΔAIC_c is the absolute difference between the AIC_c value for a given model and the lowest overall AICc value of all candidate models) and the fewest number of parameters was selected [40]. In these models, all fish Hg concentrations were length-corrected using residuals from linear regressions similar to Swanson et al. [41] and Lescord et al. [42].

RESULTS

Stable isotope values in water, sediment, invertebrates, and fish

Mean values of $\delta^{34}S$ in bulk surface sediments and aqueous SO_4^{2-} ranged from 9.56‰ to 12.45‰ and from 10.07‰ to 10.65‰ across lakes, respectively (Figure 1; Supplemental Data, Table S1). All but 1 lake (George) had aqueous SO_4^{2-} with lower mean $\delta^{34}S$ values than those of sediments (Figure 1). This lake also had the highest surface chlorophyll-a concentrations, among the highest concentrations of TOC and nutrients, but among the lowest mean %S content in surface sediments, with %S varying from 0.13 to 0.26 across lakes (Table 1). The similarity of $\delta^{34}S$ values in aqueous SO_4^{2-} and sediments in each lake precluded their use in mixing models to assess sources of S to the food webs.

Values of $\delta^{34}S$ in littoral invertebrates overlapped with those of bulk pelagic zooplankton in 5 of the 7 lakes (Figure 1; Supplemental Data, Table S2). In fish, $\delta^{34}S$ was generally higher in yellow perch (9.52–10.38‰ across lakes) than golden shiner or banded killifish (9.17–9.92‰), and most fish had $\delta^{34}S$ values that were similar to those measured in invertebrates (mainly profundal chironomids and zooplankton but also littoral taxa; Figure 1; Supplemental Data, Table S2).

Littoral invertebrates, pelagic zooplankton, and fishes showed greater separation in their mean $\delta^{13}C$ values than was observed for $\delta^{34}S$. Specifically, mean $\delta^{13}C$ was consistently more positive in littoral invertebrates than pelagic zooplankton, by between 1.03‰ and 5.39‰ across lakes (Figure 2; Supplemental Data, Table S2). Similarly, values of $\delta^{13}C$ in fish were also relatively positive, overlapping with the range of $\delta^{13}C$ in littoral invertebrates but not pelagic zooplankton (Figure 2; Supplemental Data, Table S2).

Relationships between Hg concentrations and S isotopes

Linear mixed effects model results showed that relationships between log-MeHg and $\delta^{34}S$ were not significant across lakes in any invertebrate (isopods, mayflies, dragonflies, caddisflies) group ($p = 0.090$, 0.608, 0.422, and 0.681, respectively). Furthermore, in multiple regression models with all elements and isotope values, $\delta^{34}S$ was not a significant predictor of log-MeHg within any individual invertebrate taxon across lakes, but %S was a positive predictor of log-MeHg concentrations in caddisflies (Table 2). No significant isotopic or elemental predictors were found for log-MeHg concentrations in isopods or mayflies, whereas log-MeHg in dragonflies was predicted by both $\delta^{15}N_{adj}$ and %C (Table 2).

In fish, linear mixed effects model results showed that the relationship between log-Hg and $\delta^{34}S$ was significant across lakes only in yellow perch ($p = 0.033$; Supplemental Data, Figure S2) but not for any other fish species ($p = 0.872$ for banded killifish, $p = 0.980$ for golden shiner). However, within lakes, only Hilchemakaar showed a significant positive ($\log [THg] = 0.05 \times \delta^{34}S + 0.98$; $p = 0.006$) relationship between log [THg] and $\delta^{34}S$ in yellow perch. These relationships in yellow perch from the other 6 lakes were not significant ($p = 0.056$–0.489; data not shown). Generally, $\delta^{15}N_{adj}$, $\delta^{13}C$, %

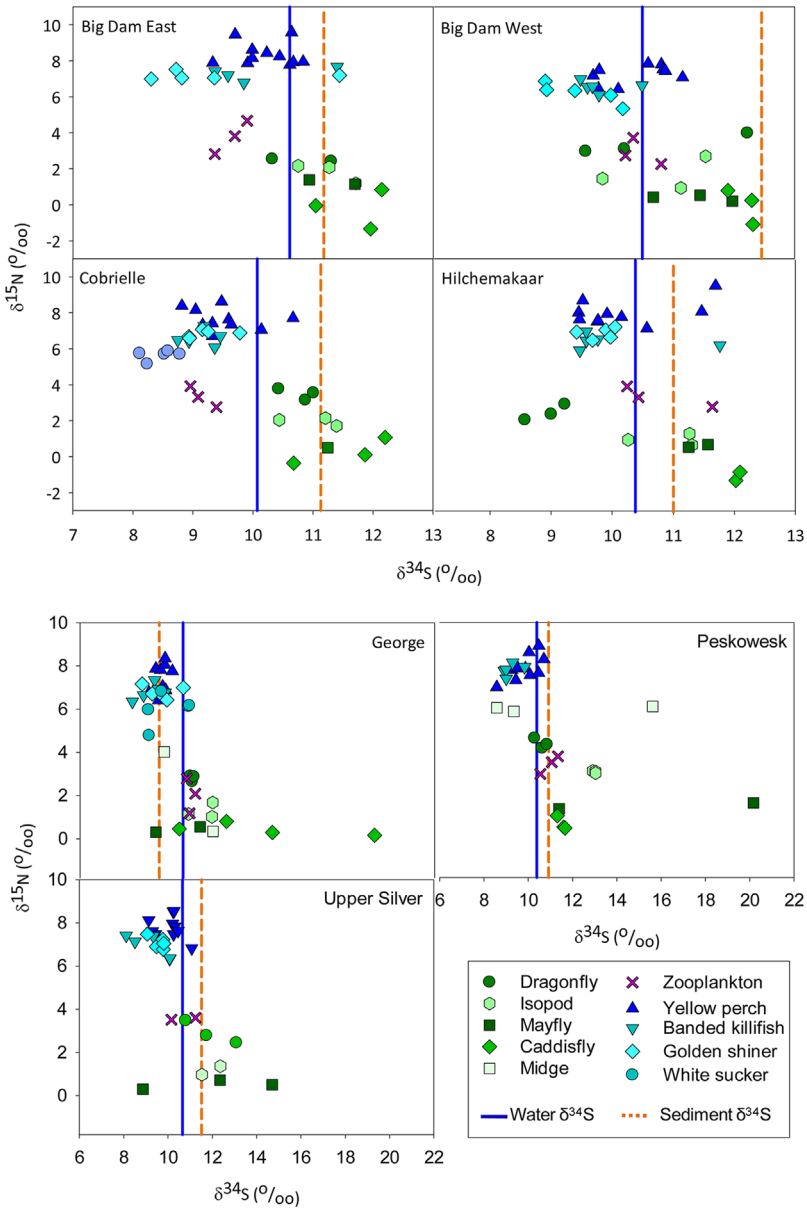

Figure 1. Sulfur and nitrogen stable isotope values ($\delta^{34}S$ and $\delta^{15}N$ in parts per thousand) through the food webs of 7 lakes in Kejimkujik National Park (NS, Canada). Note the differences in scale in the sulfur isotopes in biota from George, Peskowesk, and Upper Silver lakes. Mean $\delta^{34}S$ values in aqueous sulfate and sedimentary sulfur are represented by vertical lines.

N, and %C were better predictors of Hg concentrations than $\delta^{34}S$ or %S within fish species. More specifically, the best models for individual fish species included some combination of $\delta^{13}C$ and $\delta^{15}N_{adj}$ and elemental composition but excluded %S and $\delta^{34}S$ (Table 2).

When data for all fish and invertebrates (except zooplankton, which were not included because their $\delta^{13}C$ values were distinct from those of the fishes and littoral invertebrates in each lake; Figure 2) were combined within lakes, there were consistent negative relationships between log-THg or MeHg and $\delta^{34}S$ through each food web, and the slopes of these relationships were significantly different (Figure 3; ANCOVA: lake × $\delta^{34}S$ interaction term $p = 0.04$, $F = 2.26$). Likewise, when all food web data were considered simultaneously in linear mixed effects models to account for variability among lakes, a significant negative relationship between log-Hg and $\delta^{34}S$ was found ($p < 0.001$; Supplemental Data, Figure S1). A multiple regression of log-Hg concentrations for all food web organisms and lakes retained all predictors ($\delta^{15}N_{adj}$, $\delta^{13}C$, %C) but $\delta^{34}S$ ($R^2_{adj} = 0.135$, $p < 0.001$); %N and %S were not included because of their high collinearity with other variables (see *Methods*).

DISCUSSION

Sulfur isotope values in water, sediment, invertebrates, and fishes

Values of $\delta^{34}S$ in bulk sediments and aqueous SO_4^{2-} from our study lakes fell within the range typical for precipitation SO_4^{2-} [43], and these $\delta^{34}S$ data were more positive than results from other freshwater food webs [12,14,25,27,44,45]. In the lakes in the present study, most $\delta^{34}S$ measures of aqueous SO_4^{2-} (median 10.5‰), sediments (11.2‰), zooplankton (10.3‰), and all littoral invertebrate taxa (from 10.8‰ in Aeshnidae dragonflies to 12.0‰ in Limnephilidae caddis flies) were >10‰, a benchmark indicating dietary reliance on marine sources of S for birds (e.g., Ofukany et al. [24] and Lavoie

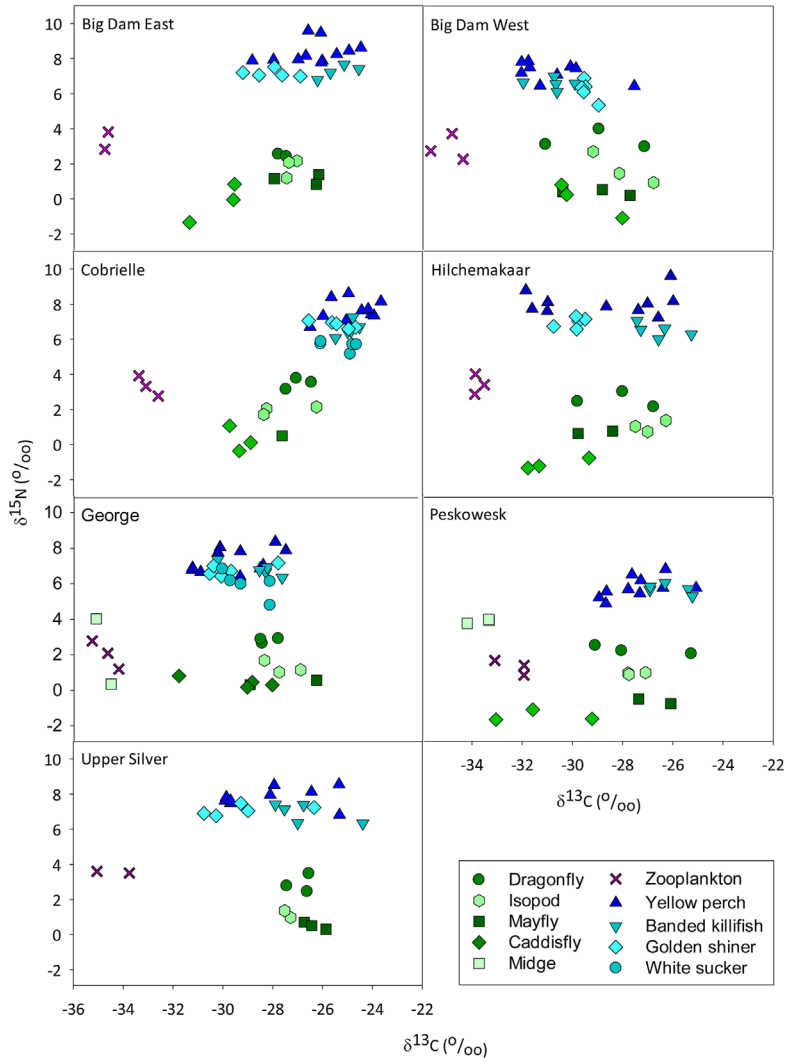

Figure 2. Carbon and nitrogen stable isotope values ($\delta^{13}C$ and $\delta^{15}N$ in parts per thousand) through the food webs of 7 lakes in Kejimkujik National Park (NS, Canada).

Table 2. A summary of multiple regression analyses of total or methyl mercury concentrations in fishes and invertebrates, respectively, against their stable isotope and elemental composition across 7 lakes in Kejimkujik National Park, Nova Scotia, Canada[a]

Taxonomic group	*n*	Variables included	Pearson correlation coefficients (*r*)	*p*	R^2_{adj}	AIC_c	ΔAIC_c
Invertebrates							
Dragonflies (family Aeshnidae)	20	$\delta^{15}N_{adj}$, %C	0.406, –0.259	0.012	0.325	–78.78	0.00
Isopods (*Caecidotea* spp.)	20	—	—	—	—	–83.61	0.49
Mayflies (family Heptageniidae)	16	—	—	—	—	–61.69	0.00
Caddis flies (family Limnephilidae)	19	%S	0.640	0.003	0.374	–74.29	0.00
Bulk zooplankton	20	$\delta^{13}C$, %C, $\delta^{15}N_{adj}$	0.545, 0.597, –0.066	<0.001	0.701	–85.58	0.00
Fishes							
Banded killifish	29	$\delta^{15}N_{adj}$, %N	0.158, –0.472	0.009	0.252	–87.95	0.00
Golden shiner	30	%N, %C	0.236, –0.097	0.066	0.122	–98.27	0.00
White sucker	15	$\delta^{15}N_{adj}$	0.592	0.020	0.301	–39.45	0.00
Yellow perch	71	$\delta^{15}N_{adj}$, %N, $\delta^{13}C$, %C	0.504, 0.230, 0.400, 0.048	<0.001	0.453	–234.17	0.00

[a]Dependent variables were log methyl mercury concentrations and length-corrected log total mercury concentrations in invertebrates and fishes, respectively. Independent variables included carbon, nitrogen, and sulfur isotope and elemental measures ($\delta^{34}S$, $\delta^{15}N_{adj}$, $\delta^{13}C$, %S, %N, %C). Note that correlation coefficients are listed respective to the final predictors. The best model for each taxonomic group is shown and was identified as that with the lowest Akaike information criterion value. Detailed model results for each taxon are given in the Supplemental Data.
AIC_c = Akaike information criterion adjusted for small sample size.

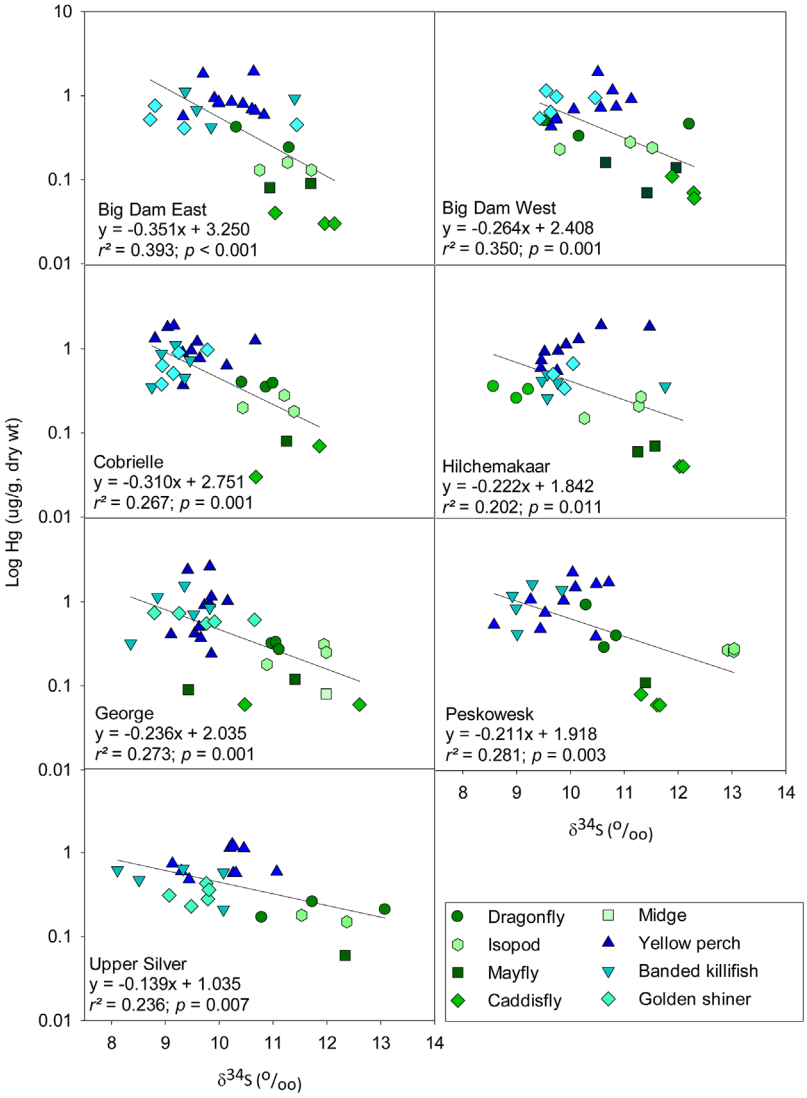

Figure 3. Relationships between log mercury (methylmercury in invertebrates and total mercury in fish) and sulfur stable isotope values ($\delta^{34}S$ in parts per thousand) through aquatic food webs from 7 lakes in Kejimkujik National Park (NS, Canada). Hg = mercury.

et al. [46]). In fact, fish $\delta^{34}S$ values from our temperate systems were higher and more consistent with values from estuarine and marine fishes [26,28] than values in freshwater ecosystems in other parts of North America [12,14,25,27,44,45,47]. In the present study, the similarity of $\delta^{34}S$ composition in aquatic ecosystems in this region to estimates from marine systems may be related to their proximity to the coast. Although the lakes are considered to be in the "interior" of southwestern Nova Scotia, Kejimkujik receives relatively high mean annual precipitation (1330 mm [48]), no point of mainland Nova Scotia is farther than 55 km from a coastline, and marine aerosols influence the chemistry of all surface waters [49]. The present results also suggest that there is not always a clear threshold $\delta^{34}S$ value that can distinguish between marine and freshwater S sources.

Contrary to expectations, all but 1 lake (George) had aqueous SO_4^{2-} with lower mean $\delta^{34}S$ values than those of sediments (Figure 1; Supplemental Data, Table S1). George Lake is among the deepest and most productive of our study systems (e.g., highest chlorophyll-a; Table 1) and therefore may undergo greater stratification than the other shallower and more oligotrophic lakes (temperature profiles were not available). These conditions in George Lake could potentially support greater sulfate-reducing bacterial activity and therefore might help to explain the distinct $\delta^{34}S$ values between water and sediments. Sulfate-reducing bacterial activity, and hence the discrimination of $\delta^{34}S$, tends to be lower in more oxygenated conditions and when SO_4^{2-} concentrations are relatively low [50]; and $\delta^{34}S$ discrimination has also been shown to be lower at intermediate temperatures compared with both lower and higher temperatures [51]. In these systems, the activity of Fe-reducing bacteria (or other microbes with non–sulfate-reducing metabolism) may dominate over sulfate-reducing bacteria given the acidic and Fe-rich conditions of these lakes [52,53].

Another consideration for the Kejimkujik lakes is that wetlands in the catchments have been shown to be important sources of Hg to downstream lake water and perhaps more important sources of MeHg than in situ Hg methylation in profundal lake sediments or anoxic bottom waters [54]. The $\delta^{34}S$ values of aqueous sulfate have been correlated with MeHg concentrations in other wetland waters, possibly reflecting sulfate-reducing bacteria activity; but other geochemical factors including Fe and manganese concentrations were also related to MeHg in wetland water [17]. The underlying geology and S

content of the bedrock also varies across the study lakes [54] but did not appear to influence the present results given the low variability in $\delta^{34}S$ values or total S content measured in sediments of the study lakes (Table 1; Supplemental Data, Table S1). Despite this, MeHg concentrations in wetland soils in Kejimkujik are related to bedrock lithology and to markers of sulfate-reducing bacteria activity [55]. It is not clear how baseline $\delta^{34}S$ values may vary in wetlands versus open lakes or whether $\delta^{34}S$ values might be more distinct between water and sediments in the former habitat. However, it is possible that MeHg concentrations in the biota from the study lakes may be more closely related to $\delta^{34}S$ in wetland samples than those in profundal lake sediments or the lake water column, given the importance of wetlands as sources of MeHg and possibly as sites of greater sulfate-reducing bacteria activity than the lakes in this region.

There was little separation in $\delta^{34}S$ between littoral and pelagic invertebrates in the present study (Figure 1; Supplemental Data, Table S2), and the overlap of $\delta^{34}S$ in sediment and water column SO_4^{2-} meant that they could not be used to infer the relative importance of either S source in the diets of food web organisms. Our findings contrast with those of Croisetière et al. [12] for boreal lakes, where $\delta^{34}S$ values of sediment and SO_4^{2-}, as well as benthic and pelagic invertebrates, differed by approximately 4‰ to 9‰ within lakes. These lakes also had higher concentrations of SO_4^{2-}(~6–24 mg/L across lakes at the sediment–water interface; estimated from figure 1 in Croisetière et al. [12]) than the lakes in Kejimkujik (means of 1.08–1.71 mg/L; Table 1), which may have promoted greater activity of sulfate-reducing bacteria and differences in $\delta^{34}S$ between sediment and water. In Lake Biwa, Japan, there was a greater distinction between $\delta^{34}S$ of biota that relied on water versus sediment S sources at sites that had anoxic conditions [14], and this is consistent with the finding that discrimination of S isotopes is inversely related to dissolved oxygen concentrations [41]. In addition, Karube et al. [14] inferred that the similar $\delta^{34}S$ values among invertebrate taxa that are known to feed on different S sources are indicative of oxic conditions. Similarly, relatively high oxygen concentrations in our study lakes (based on their relatively shallow depths) could also have limited the activity of sulfate-reducing bacteria and discrimination of $\delta^{34}S$ between SO_4^{2-}, sediments, and biota. However, data on dissolved oxygen concentrations in the water column of Kejimkujik lakes are needed to better assess this. Collectively, these results indicate that although $\delta^{34}S$ can be useful within some systems for differentiating between sources of S to food webs, it is important to keep in mind that differences in $\delta^{34}S$ values in food webs are not necessarily indicative of pelagic versus benthic sources of energy in systems with little sulfate-reducing bacterial activity.

The diversity of microorganisms known to methylate Hg is increasing [2,5,56], but further study is needed to understand the relative importance of groups with different metabolic habits (e.g., methanogens, sulfate-reducing bacteria, Fe-reducing bacteria) as net producers of MeHg. Kejimkujik is a well-known hot spot of biological Hg contamination [57–59], and our results hint at the possibility that other organisms and/or biogeochemical processes produce the high biological Hg concentrations in the apparent absence of significant in-lake sulfate-reducing bacteria activity. Additional research on the microbial ecology and biogeochemistry of these lakes and their surrounding wetlands could help us to better understand the processes that lead to the production of MeHg.

Relationships between Hg concentrations and C, N, and S isotopes in invertebrates

Only a few studies have examined relationships between Hg concentrations and $\delta^{34}S$ values in freshwater invertebrates. Whereas there were no relationships within invertebrate taxa between log-MeHg concentrations and $\delta^{34}S$ in the present study (see section, *Relationships between Hg concentrations and S isotopes*), results from Ozark mountain streams (AR, USA) were mixed, with a positive relationship between THg and $\delta^{34}S$ values in crayfish (*Orconectes* spp.) across sites but no relationship for Asian clams (*Corbicula fluminea* [44]) despite significant differences in $\delta^{34}S$ within both of these invertebrate groups across sites. It is possible that different microhabitat use by these invertebrates affected their $\delta^{34}S$ but not Hg. For example, in Lake Biwa, Japan, $\delta^{34}S$ values of water, sediments, and invertebrates varied within and outside of macrophyte beds in the littoral zone [14].

The importance of C, N, and S isotopes and elemental measures as predictors of log-MeHg concentrations varied among invertebrate taxa, and log-MeHg in isopods and mayflies was poorly described by all combinations of these variables (Table 2). We examined whether the variables that were significant predictors of MeHg concentrations in different taxa might have larger ranges and therefore potentially more influence on the total variability explained by each model, but this was not the case. For example, the ranges of each variable for isopods and mayflies were not smaller than those of other taxa, and the significant predictors of Hg in other taxa did not necessarily have the greatest range of values (Supplemental Data, Table S3). In dragonflies, $\delta^{15}N_{adj}$ was a significant positive predictor of their log-MeHg concentrations (Table 2), which may reflect the tendency of higher–trophic level organisms to accumulate more MeHg through biomagnification. In addition to $\delta^{15}N_{adj}$, log-MeHg in dragonflies was related (negatively) to %C, possibly indicating that their MeHg concentrations may be influenced by their relative body content of lipids and proteins, with higher protein content (and relatively lower C content) potentially leading to more accumulated MeHg [23]. In contrast, %C positively predicted log-MeHg in zooplankton (Table 2). Although it is difficult to account for the opposite direction of these relationships between dragonflies and zooplankton, %C and lipid content have been found to increase with the proportion of adult zooplankton biomass in Arctic lakes [60].

Whereas there were no individual taxa in the present study for which $\delta^{34}S$ was a significant predictor of MeHg, this metal was positively related to %S in caddisflies (Table 2). Previous analyses in these lakes also found significant positive relationships between log-Hg concentrations and %S content through each food web, but these relationships were not as strong as those with %N or $\delta^{15}N_{adj}$ [23]. The limited usefulness of $\delta^{34}S$ to predict MeHg within and among invertebrate taxa in the lakes of Kejimkujik may be related to its inability to distinguish S from open water versus sediment sources.

Relationships between Hg concentrations and C, N, and S isotopes in fishes

Although THg concentrations in the different fish species in the present study were best described by different combinations of elements and stable isotopes, neither $\delta^{34}S$ nor %S was a clear predictor of THg in any species. Linear mixed effects models examining the relationship between log-THg and $\delta^{34}S$ in yellow perch, although significant across lakes, had variable relationships between lakes (i.e., positive in Hilchemakaar and

nonsignificant in the other 6 lakes; Supplemental Data, Figure S2). Previous studies have also found mixed relationships between these measures in fish. For instance, Schmitt et al. [44] showed that $\delta^{34}S$ was a positive predictor of THg in smallmouth bass (*Micropterus dolomieu*) but a negative predictor in hog sucker (*Hypentelium nigricans*). Also, $\delta^{34}S$ was not a significant predictor of THg in either yellow perch or pumpkinseed sunfish (*Lepomis gibbosus*) in southeastern Ontario lakes, despite relatively strong positive correlations between $\delta^{34}S$ and $\delta^{15}N$ in these fish [27]. More studies have examined fish Hg in relation to $\delta^{34}S$ as a tracer of marine-derived sources of S in estuarine or coastal systems, but results from these studies are similarly ambiguous in terms of the strength of $\delta^{34}S$ as a predictor of fish Hg concentrations (e.g., Evans and Crumley [26] and Sluis et al. [29]). However, along a clear salinity gradient in coastal ponds and wetlands (San Francisco Bay delta, CA, USA) $\delta^{34}S$ was the strongest (positive) predictor of fish THg when compared to $\delta^{15}N$ and $\delta^{13}C$ [61]. These findings were attributed to favorable conditions for sulfate-reducing bacteria (standing water, high SO_4^{2-}, organic matter) and high levels of Hg methylation in the most landlocked systems, leading to clear discrimination of $\delta^{34}S$ between SO_4^{2-} and sedimentary sulfides and to distinct $\delta^{34}S$ values in the biota.

Comparing $\delta^{34}S$ and $\delta^{15}N_{adj}$ as predictors of Hg concentrations through lake food webs

Studies relating Hg concentrations to $\delta^{34}S$ values in freshwater biota representing a range of trophic levels are lacking, so it is not possible to compare the present findings to those from other food webs. However, selenium (Se) concentrations in yellow perch and their invertebrate prey were negatively related to $\delta^{34}S$ in lakes of northeastern Ontario and northwestern Québec [62]. Although the negative Se versus $\delta^{34}S$ relationships were similar to the present results for Hg versus $\delta^{34}S$ through food webs, the Quebec results were partly attributed to the higher $\delta^{34}S$ and lower Se concentrations in zooplankton compared with sediment-feeding invertebrates. However, in our study lakes the majority of pelagic zooplankton and littoral invertebrates had overlapping $\delta^{34}S$, so this is unlikely to have been a driver of the negative log-Hg versus $\delta^{34}S$ relationships.

Despite the significant negative relationships between log-Hg and $\delta^{34}S$ through each food web in Kejimkujik, $\delta^{15}N_{adj}$ was a much stronger predictor of Hg concentrations ($R^2 = 0.79$–0.92 [22]) than those using $\delta^{34}S$ ($R^2 = 0.20$–0.39; Figure 3). Although other studies for whole food webs are lacking, the mixed results among various fish and invertebrate taxa suggest that the relationship between Hg concentrations and $\delta^{34}S$ through aquatic food webs may be less consistent in strength and direction than the strong positive relationships between Hg concentrations and $\delta^{15}N$ through diverse systems worldwide [63].

In addition, $\delta^{13}C$ was more effective at distinguishing between pelagic and littoral taxa in the Kejimkujik lakes (Figure 2), whereas the overlap in $\delta^{34}S$ values among all invertebrate taxa, water, and sediments made it a relatively poor complement to the more commonly used $\delta^{15}N$ and $\delta^{13}C$ for understanding Hg trophic transfer and food web structure in these systems. Multiple regression models also showed $\delta^{13}C$ to be a significant predictor of log-Hg in zooplankton and yellow perch, whereas $\delta^{34}S$ was not significant in models of log-Hg concentrations in any taxa. These findings contrast with those of other studies [12,14] showing that $\delta^{34}S$ composition of biota, water, and sediments helped to identify sources of S to lake biota.

CONCLUSIONS

Despite the strong links between Hg and S cycling in aquatic systems, the present results were mixed with respect to the strength of $\delta^{34}S$ as a predictor of Hg concentrations in aquatic food webs. Within fish or invertebrate taxa, a combination of $\delta^{15}N_{adj}$, $\delta^{13}C$, %C, and/or %N generally better described variability in Hg concentrations than either $\delta^{34}S$ or %S. Similarly, $\delta^{34}S$ was a significant predictor of Hg through each food web but explained less of the variability than relationships with $\delta^{15}N_{adj}$. The overlapping ranges of values of $\delta^{34}S$ in the water column and sediments suggest that S stable isotopes cannot distinguish between different energy sources in these food webs. Rather, the lack of distinction between $\delta^{34}S$ in water and sediments also supports the idea that biogeochemical sources of MeHg and/or processes other than sulfate-reducing bacteria activity may be more important determinants of $\delta^{34}S$ values and MeHg concentrations in these Kejimkujik food webs.

Although a number of studies have examined relationships between C, N, and S isotopes among different freshwater and estuarine fishes, fewer studies have related Hg concentrations and $\delta^{34}S$ across a range of lower to higher trophic levels in freshwater food webs. A better understanding is needed of whether $\delta^{34}S$ might be more closely linked to Hg bioaccumulation and biomagnification in ecosystems where sulfate-reducing bacteria play a more important role in Hg biogeochemical cycling.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3615.

Acknowledgment—Funding for this study was provided by a Natural Sciences and Engineering Research Council of Canada Discovery Grant, the Canada Research Chair program and Environment Canada. We thank B. Fry for sharing his insight about S isotopes in food webs and Kejimkujik National Park and National Historic Site staff and Parks Canada for the logistical support. We also thank K. Batchelar, L. Del Bel, S. Edmonds, M. Gautreau, J. Kirk, A. Mercer, and A. Valois for their help in the field and laboratory, as well as E. Smenderovac for help with data analysis.

Data Availability—Data can be found in the Supplemental Data file. Additional data requests can be sent to the corresponding author (glescord@gmail.com).

REFERENCES

1. Compeau GC, Bartha R. 1985. Sulfate-reducing bacteria—Principal methylators of mercury in anoxic estuarine sediment. *Appl Environ Microbiol* 50:498–502.
2. Fleming EJ, Mack EE, Green PG, Nelson DC. 2006. Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Appl Environ Microbiol* 72:457–464.
3. Eckley CS, Hintelmann H. 2006. Determination of mercury methylation potentials in the water column of lakes across Canada. *Sci Total Environ* 368:111–125.
4. Acha D, Hintelmann H, Pabon CA. 2012. Sulfate-reducing bacteria and mercury methylation in the water column of the Lake 658 of the experimental lake area. *Geomicrobiol J* 29:667–674.
5. Hamelin S, Amyot M, Barkay T, Wang Y, Planas D. 2011. Methanogens: Principal methylators of mercury in lake periphyton. *Environ Sci Technol* 45:7693–7700.
6. Hamelin S, Planas D, Amyot M. 2015. Mercury methylation and demethylation by periphyton biofilms and their host in a fluvial wetland of the St. Lawrence River (QC, Canada). *Sci Total Environ* 512:464–471.
7. Ravichandran M. 2004. Interactions between mercury and dissolved organic matter—A review. *Chemosphere* 55:319–331.

8. Feyte S, Gobeil C, Tessier A, Cossa D. 2012. Mercury dynamics in lake sediments. *Geochim Cosmochim Acta* 82:92–112.
9. Benoit J, Gilmour C, Heyes A, Mason R, Miller C. 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. *ACS Symp Ser* 835:262–297.
10. Schaefer JK, Morel FMM. 2009. High methylation rates of mercury bound to cysteine by *Geobacter sulfurreducens*. *Nat Geosci* 2:123–126.
11. Leclerc M, Planas D, Amyot M. 2015. Relationship between extracellular low-molecular-weight thiols and mercury species in natural lake periphytic biofilms. *Environ Sci Technol* 49:7709–7716.
12. Croisetière L, Hare L, Tessier A, Cabana G. 2009. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshw Biol* 54:1006–1015.
13. Canfield DE. 2001. Isotope fractionation by natural populations of sulfate-reducing bacteria. *Geochim Cosmochim Acta* 65:1117–1124.
14. Karube Z, Okada N, Tayasu I. 2012. Sulfur stable isotope signature identifies the source of reduced sulfur in benthic communities in macrophyte zones of Lake Biwa, Japan. *Limnology* 13:269–280.
15. Proulx I, Hare L. 2014. Differences in feeding behaviour among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae. *Freshw Biol* 59:73–86.
16. Holmer M, Storkholm P. 2001. Sulphate reduction and sulphur cycling in lake sediments: A review. *Freshw Biol* 46:431–451.
17. Alpers CN, Fleck JA, Marvin-DiPasquale M, Stricker CA, Stephenson M, Taylor HE. 2014. Mercury cycling in agricultural and managed wetlands, Yolo Bypass, California: Spatial and seasonal variations in water quality. *Sci Total Environ* 484:276–287.
18. Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18:293–320.
19. Harris HH, Pickering IJ, George GN. 2003. The chemical form of mercury in fish. *Science* 301:1203–1203.
20. Lemes M, Wang F. 2009. Methylmercury speciation in fish muscle by HPLC-ICP-MS following enzymatic hydrolysis. *J Anal At Spectrom* 24:663–668.
21. Food and Agriculture Organization of the United Nations. 2003. Food energy—Methods of analysis and conversion factors. 77. Rome, Italy.
22. Tanz N, Schmidt H. 2010. Delta S-34-value measurements in food origin assignments and sulfur isotope fractionations in plants and animals. *J Agric Food Chem* 58:3139–3146.
23. Clayden MG, Kidd KA, Wyn B, Kirk JL, Muir DCG, O'Driscoll NJ. 2013. Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. *Environ Sci Technol* 47:12047–12053.
24. Ofukany AFA, Hobson KA, Wassenaar LI. 2012. Connecting breeding and wintering habitats of migratory piscivorous birds: Implications for tracking contaminants (Hg) using multiple stable isotopes. *Environ Sci Technol* 46:3263–3272.
25. Ofukany AFA, Wassenaar LI, Bond AL, Hobson KA. 2014. Defining fish community structure in Lake Winnipeg using stable isotopes (delta C-13, delta N-15, delta S-34): Implications for monitoring ecological responses and trophodynamics of mercury and other trace elements. *Sci Total Environ* 497:239–249.
26. Evans DW, Crumley PH. 2005. Mercury in Florida Bay fish: Spatial distribution of elevated concentrations and possible linkages to Everglades restoration. *Bull Mar Sci* 77:321–345.
27. Ethier ALM, Scheuhammer AM, Bond DE. 2008. Correlates of mercury in fish from lakes near Clyde Forks, Ontario, Canada. *Environ Pollut* 154:89–97.
28. Fry B, Chumchal MM. 2012. Mercury bioaccumulation in estuarine food webs. *Ecol Appl* 22:606–623.
29. Sluis MZ, Boswell KM, Chumchal MM, Wells RJD, Soulen B, Cowan JH Jr. 2013. Regional variation in mercury and stable isotopes of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico, USA. *Environ Toxicol Chem* 32:434–441.
30. Evers DC, Han Y, Driscoll CT, Kamman NC, Goodale MW, Lambert KF, Holsen TM, Chen CY, Clair TA, Butler T. 2007. Biological mercury hotspots in the northeastern United States and southeastern Canada. *Bioscience* 57:29–43.
31. Batchelar KL, Kidd KA, Drevnick PE, Munkittrick KR, Burgess NM, Roberts AP, Smith JD. 2013. Evidence of impaired health in yellow perch (*Perca flavescens*) from a biological mercury hotspot in northeastern North America. *Environ Toxicol Chem* 32:627–637.
32. Clair TA, Schwarcz HP, Kramer JR. 1989. The origins of sulfur in waters from four Nova Scotian basins, Canada. *Appl Geochem* 4:93–98.
33. O'Driscoll NJ, Lean DRS, Rencz AN. 2005. Review of factors affecting mercury fate in Kejimkujik Park, Nova Scotia. In O'Driscoll NJ, Rencz AN, Lean DRS, eds, *Mercury Cycling in a Wetland-Dominated Ecosystem: A Multidisciplinary Study*. Society of Environmental Toxicology and Chemistry, Boca Raton, FL, USA, pp 2–15.
34. Siciliano SD, Sangster A, Daughney CJ, Loseto L, Germida JJ, Rencz AN, O'Driscoll NJ, Lean DRS. 2003. Are methylmercury concentrations in the wetlands of Kejimkujik National Park, Nova Scotia, Canada, dependent on geology? *J Environ Qual* 32:2085–2094.
35. Clair TA. 2011. Water chemistry and dissolved organic carbon trends in lakes from Canada's Atlantic provinces: No recovery from acidification measured after 25 years of lake monitoring. *Can J Fish Aquat Sci* 68:953–953.
36. Bowman MF, Nussbaumer C, Burgess NM. 2014. Community composition of lake zooplankton, benthic macroinvertebrates and forage fish across a pH gradient in Kejimkujik National Park, Nova Scotia, Canada. *Water Air Soil Pollut* 225:2211.
37. Nussbaumer C, Burgess NM, Weeber RC. 2014. Distribution and abundance of benthic macroinvertebrates and zooplankton in lakes in Kejimkujik National Park and National Historic Site of Canada, Nova Scotia. *Canadian Field-Naturalist* 128:1–24.
38. Carmody RW, Plummer LN, Busenberg E, Coplen TB. 1998. Methods for collection of dissolved sulfate and sulfide and analysis of their sulfur isotopic composition. 97-234. Open File Report. US Geological Survey, Reston, VA.
39. Tabachnick BG, Fidell LS. 2007. *Using Multivariate Statistics*, 5th ed. Pearson, Boston, MA, USA.
40. Johnson JB, Omland KS. 2004. Model selection in ecology and evolution. *Trends Ecol Evol* 19:101–108.
41. Swanson HK, Kidd KA, Babaluk JA, Wastle RJ, Yang PP, Halden NM, Reist JD. 2010. Anadromy in Arctic populations of lake trout (*Salvelinus namaycush*): Otolith microchemistry, stable isotopes, and comparisons with Arctic char (*Salvelinus alpinus*). *Can J Fish Aquat Sci* 67:842–853.
42. Lescord GL, Kidd KA, Kirk JL, O'Driscoll NJ, Wang X, Muir DCG. 2015. Factors affecting biotic mercury concentrations and biomagnification through lake food webs in the Canadian high Arctic. *Sci Total Environ* 509:195–205.
43. Wadleigh M, Schwarcz H, Kramer J. 1996. Isotopic evidence for the origin of sulphate in coastal rain. *Tellus B Chem Phys Meteorol* 48:44–59.
44. Schmitt CJ, Stricker CA, Brumbaugh WG. 2011. Mercury bioaccumulation and biomagnification in Ozark stream ecosystems. *Ecotoxicol Environ Saf* 74:2215–2224.
45. Orr PL, Guiguer KR, Russel CK. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. *Ecotoxicol Environ Saf* 63:175–188.
46. Lavoie RA, Kyser TK, Friesen VL, Campbell LM. 2015. Tracking overwintering areas of fish-eating birds to identify mercury exposure. *Environ Sci Technol* 49:863–872.
47. Sanders TG Jr, Biddanda BA, Stricker CA, Nold SC. 2011. Benthic macroinvertebrate and fish communities in Lake Huron are linked to submerged groundwater vents. *Aquat Biol* 12:1–11.
48. Zhang J, Hudson J, Neal R, Sereda J, Clair T, Turner M, Jeffries D, Dillon P, Molot L, Somers K, Hesslein R. 2010. Long-term patterns of dissolved organic carbon in lakes across eastern Canada: Evidence of a pronounced climate effect. *Limnol Oceanogr* 55:30–42.
49. Underwood JK, Waller DH, Thirumurthi D. 1984. The influence of the ocean on the chemistry of precipitation in Nova Scotia. *Atmosphere-Ocean* 26(3):467–469.
50. Habicht KS, Gade M, Thamdrup B, Berg P, Canfield DE. 2002. Calibration of sulfate levels in the Archean Ocean. *Science* 298:2372–2374.
51. Canfield D, Olesen C, Cox R. 2006. Temperature and its control of isotope fractionation by a sulfate-reducing bacterium. *Geochim Cosmochim Acta* 70:548–561.
52. Kerekes J, Freedman B. 1989. Characteristics of three acidic lakes in Kejimkujik National Park, Nova Scotia, Canada. *Arch Environ Contam Toxicol* 18:183–200.
53. Koschorreck M. 2008. Microbial sulphate reduction at a low pH. *FEMS Microbiol Ecol* 64:329–342.
54. Siciliano SD, Sangster A, Daughney CJ, O'Driscoll NJ, Lean DRS. 2005. Geological dependence of MeHg concentrations in wetlands of Kejimkujik National Park. In O'Driscoll NJ, Rencz AN, Lean DRS, eds, *Mercury Cycling in a Wetland-Dominated Ecosystem: A Multidisciplinary Study*. Society of Environmental Toxicology and Chemistry, Pensacola, FL, USA, pp 197–228.
55. Siciliano S, Sangster A, Daughney C, Loseto L, Germida J, Rencz A, O'Driscoll N, Lean D. 2003. Are methylmercury concentrations in the wetlands of Kejimkujik National Park, Nova Scotia, Canada, dependent on geology? *J Environ Qual* 32:2085–2094.

56. Figueiredo NL, Canário J, O'Driscoll NJ, Duarte A, Carvalho C. 2016. Aerobic mercury-resistant bacteria alter mercury speciation and retention in the Tagus estuary (Portugal). *Ecotoxicol Environ Saf* 124:60–67.
57. Edmonds ST, Evers DC, Cristol DA, Mettke-Hofmann C, Powell LL, McGann AJ, Armiger JW, Lane OP, Tessler DF, Newell P, Heyden K, O'Driscoll NJ. 2010. Geographic and seasonal variation in mercury exposure of the declining rusty blackbird. *Condor* 112:789–799.
58. Burgess NM, Evers DC, Kaplan JD. 2005. Mercury and other contaminants in common loons breeding in Atlantic Canada. *Ecotoxicology* 14:241–252.
59. Wyn B, Kidd KA, Burgess NM, Curry RA, Munkittrick KR. 2010. Increasing mercury in yellow perch at a hotspot in Atlantic Canada, Kejimkujik National Park. *Environ Sci Technol* 44: 9176–9181.
60. Chételat J, Amyot M, Cloutier L. 2012. Shifts in elemental composition, methylmercury content and $\delta^{15}N$ ratio during growth of a high Arctic copepod. *Freshw Biol* 57:1228–1240.
61. Ackerman JT, Eagles-Smith CA, Heinz GH, De La Cruz SE, Takekawa JY, Miles AK, Adelsbach TL, Herzog MP, Bluso-Demers JD, Demers SA, Herring G, Hoffman DJ, Hartman CA, Willacker JJ, Suchanek TH, Schwarzbach SE, Maurer TC. 2014. Mercury in birds of San Francisco Bay-delta, California—Trophic pathways, bioaccumulation, and ecotoxicological risk to avian reproduction. 2014-1251. Open File Report. US Geological Survey, Reston, VA.
62. Ponton DE, Hare L. 2015. Using sulfur stable isotopes to understand feeding behavior and selenium concentrations in yellow perch (*Perca flavescens*). *Environ Sci Technol* 49:7633–7640.
63. Lavoie RA, Jardine TD, Chumchal MM, Kidd KA, Campbell LM. 2013. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environ Sci Technol* 47:13385–13394.