Environmental Science & Technology Letters

pubs.acs.org/journal/estlcu

Letter

Using Carbon, Nitrogen, and Mercury Isotope Values to Distinguish Mercury Sources to Alaskan Lake Trout

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Cite This: *Environ. Sci. Technol. Lett.* 2022, 9, 312−319

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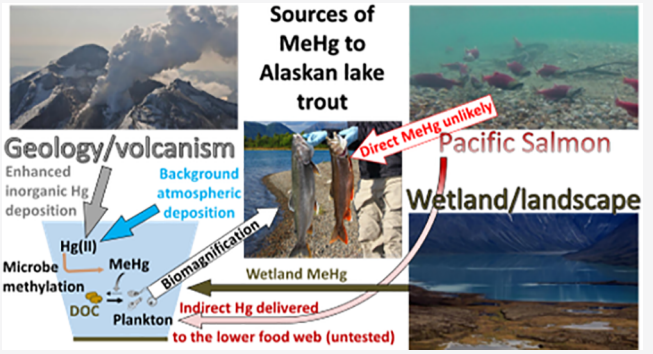
ABSTRACT: Lake trout (*Salvelinus namaycush*), collected from 13 remote lakes located in southwestern Alaska, were analyzed for carbon, nitrogen, and mercury (Hg) stable isotope values to assess the importance of migrating oceanic salmon, volcanic activity, and atmospheric deposition to fish Hg burden. Methylmercury (MeHg) bioaccumulation in phytoplankton (5.0−6.9 kg L^{-1}) was also measured to quantify the basal uptake of MeHg to these aquatic food webs. Hg isotope values in lake trout revealed that while the extent of precipitation-delivered Hg was similar across the entire study area, volcanic Hg is likely an important additional source to lake trout in proximate lakes. In contrast, migratory salmon (*Oncorhynchus nerka*) deliver little MeHg to lake trout directly, although indirect delivery processes via decay could exist. A high level of variability in carbon, nitrogen, and Hg isotope values indicates niche partitioning in lake trout populations within each lake and that a complex suite of ecological interactions is occurring, complicating the conceptually linear assessment of the contaminant source to the receiving organism. Without connecting energy and contaminant isotope axes, we would not have understood why lake trout from these pristine lakes have highly variable Hg burdens despite consistently low water Hg and comparable age-length dynamics.



KEYWORDS: *Mercury, isotopes, fish, volcanoes, salmon, lake trout, Minamata Convention*

INTRODUCTION

Mercury (Hg) concentrations in fish result from both ecosystem-scale and individual-scale factors. Ecosystem-scale variables include productivity,[1,2] land use or land cover (e.g., glaciation),[3] sources of Hg, favorable conditions for methylmercury (MeHg) production[4] or removal (e.g., reduction),[5−7] and the susceptibility of Hg to methylation or demethylation.[4,8,9] Individual-scale factors include foraging habits, trophic position, and growth rates.[10] The confounding effects of variables at both scales can complicate the interpretation of Hg concentrations in fish across heterogeneous ecosystems.

Stable isotope ratios of carbon and nitrogen on bulk tissue are a useful tool used to predict trophic positions and energy pathways. Carbon stable isotope values ($\delta^{13}C$) change very little through trophic linkages and act as indices of foraging behavior and fish habitat.[11] Stable isotope values of nitrogen ($\delta^{15}N$) undergo predictable trophic enrichment from prey to predators, allowing us to estimate trophic position.[12,13] While information regarding MeHg bioaccumulation and biomagnification can be inferred from $\delta^{13}C$ and $\delta^{15}N$ analyses, the connection of Hg sources through dietary pathways is best assessed by including Hg stable isotope analyses.[1,12,14,15]

Combining $\delta^{13}C$ and $\delta^{15}N$ with Hg stable isotope values facilitates determination of Hg sources and energy pathways to fish, but less commonly tested is the connection between fish habitat use and Hg sources in ecosystems where heterogeneity of Hg sources exists.[12] Mass-dependent fractionation (MDF) of Hg isotopes (represented as $\delta^{202}Hg$) enables inferences about Hg sources[16−18] and reaction processes.[5,16,19−22] In contrast, mass-independent fractionation (MIF) of Hg isotopes (represented by $\Delta^{###}Hg$) is observed in odd-isotopes and, by separate mechanisms, even-isotopes. In aquatic settings, odd-isotope fractionation (odd-MIF, reported as $\Delta^{199}Hg$) results from near-surface photoreduction or oxidation and has been leveraged as an indicator for depth.[5,16,23,24] Even-isotope fractionation (even-MIF, reported as $\Delta^{200}Hg$) occurs in the upper atmosphere and in fish marks the importance of precipitation-delivered Hg.[1,12,17,18,25−28] MeHg biomagnifica-

Received: February 8, 2022
Revised: March 11, 2022
Accepted: March 16, 2022
Published: March 21, 2022

tion processes conserve Δ^{199}Hg, Δ^{200}Hg, and to some extent δ^{202}Hg values through the food web.[29−31] Thus, Hg isotope values measured in fish provide information on the MeHg that enters at the base of the food web, and when heterogeneity in Hg isotope values exists between habitats (e.g., littoral, pelagic, and benthic zones) of an ecosystem, we can link fish habitats using Hg and carbon.[1,12,32] These multidimensional isotope values simultaneously allow us to track Hg reactionary processes[5−7,16,23] and sources[1,3,21,25,29,33−38] as well as fish habitat preference[11] and trophic placement.[12,13]

The pristine and rugged landscape of southwest Alaska is characterized by a marked spectrum of topography, glaciation, wetland cover, and connectivity to the ocean, all of which potentially influence how Hg inputs that are both shared (e.g., global Hg sources) and regional (e.g., proximity to volcanoes and prevalence of salmon) are received by lakes and the resident fish therein. To assess the relative importance of these variables, we selected 13 lakes and performed limnological measurements, assessment of watershed features, Hg concentration analyses in water, and biological sample collections (seston [plankton], lake trout [*Salvelinus namaycush*], and sockeye salmon [*Oncorhynchus nerka*]) for Hg concentration and isotopic analyses. Since direct deposition of long-range atmospheric Hg is uniform across the study area, we hypothesized that isotope values in lake trout inhabiting lakes that receive anadromous sockeye salmon (representing incoming flux of oceanic MeHg) and those located near volcanic Hg sources (deposited as inorganic Hg from particles and fumaroles[39,40]) would be isotopically distinct. We also hypothesized that variability in carbon isotope values of bulk tissue within a lake would be attributable to lake trout habitat preference and that this habitat partitioning[41] would result in variation in Hg isotope values, provided that differences in the Hg isoscape exist as shown previously.[12]

MATERIALS AND METHODS

Site Description. The study lakes are in southwest Alaska within the boundaries of two national parks and preserves (Lake Clark - LACL and Katmai - KATM), span three level III ecoregions, and feature landscapes shaped by glaciation and volcanism. Eighteen volcanoes exist within the study area, with 16 of them located in KATM. We selected 13 lakes according to four criteria: resident lake trout, quantified migratory salmon estimates, existing water quality monitoring efforts, and representative gradients in glacier and wetland cover. Lakes in these parks are oligotrophic and have low to moderate acid buffering capacity. Summertime measurements show that most of these lakes only weakly stratify. Further description of the lakes can be found in Table S1.

Field Collections and Preservation. In July of 2016, surface water samples[42] were collected from various open water depths using a Niskin sampler. Sample depth layers were chosen using a water quality profile measured by sonde upon arrival. Samples were temporarily held on ice in new Hg-clean 2-L bottles, filtered, acidified, and measured for total Hg (HgT, filter-passing as FHgT) and MeHg (FMeHg). Unacidified filter-passing water was collected, refrigerated, and analyzed for dissolved organic carbon (DOC; Tables S2−S3).[32] Details of ancillary water analyses and associated quality control procedures can be found on the U.S. Geological Survey Mercury Research Laboratory (USGS-MRL) website.[3,43,44] Seston were collected by vertical tows in the surficial 20 m with a 52 μm Nitex mesh net and subsequently size sieved (Table S4).[44]

Ten adult lake trout and three adult sockeye salmon (where present) were collected per lake by angling or gill net between 2011 and 2016. Sex, weight, and length were measured at capture, and sagittal otoliths and axial muscle tissue were removed from the carcass for age determination (Figure S1, Table S5)[45] and constituent analyses, respectively. All biological tissues were stored frozen, lyophilized, and homogenized prior to analysis.

Constituent Analyses. Fish tissue HgT concentrations were determined using direct combustion combined with atomic absorbance spectroscopy (Table S5).[34] A triplicate and standard reference material (SRM) analysis was performed once every 10 samples, with acceptable triplicate data achieving a relative standard deviation of less than 10% and an average SRM recovery of IAEA 407 (fish homogenate) measured at 100 ± 6%. Plankton MeHg concentrations were determined using 4.5 M nitric acid extraction, sodium tetraethylborate ethylation, gaseous purge and trap, thermal desorption, and atomic fluorescence spectroscopy (AFS). After successful MeHg analysis, plankton extracts were oxidized with bromine monochloride and analyzed for HgT concentration by AFS. For plankton analyses, SRM IAEA 452 (Scallop - *Pecten. Maximus*) recoveries were consistently within 10% of reported values, reagent blanks were negligible, and an in-house secondary standard to verify ongoing instrument calibration was within 10% of expected concentration.[44]

For HgT stable isotope analysis, approximately 0.1 g of fish tissue was digested in 5 mL of concentrated nitric acid (95 °C) overnight, oxidized with 5% bromine monochloride, and then heated for 4 h. Extracts were diluted to a 10% acid concentration, measured for HgT by AFS to assess recovery, and then analyzed for HgT stable isotopes.[1,46] IAEA 407 was used as the isotopic Hg SRM, and UM-Almadén was used as a secondary standard (Table S6).

Carbon and nitrogen stable isotope values in fish and plankton were analyzed by the University of California-Davis Stable Isotope Facility and reference material results met lab standards. Triplicates were added to determine precision (Table S7). To account for the δ^{13}C fractionation associated with lipid formation in fish, mathematical lipid corrections (lipid content approximated from molar C/N_{ratio}) were performed on lake trout δ^{13}C and labeled as $\delta^{13}C_{lipid\text{-}free}$.[47] Fatty acid content in zooplankton also fractionates δ^{13}C, so mathematical corrections approximated from sample $C{:}N_{ratio}$ were also applied to >118-μm size-sieved plankton δ^{13}C and labeled as $\delta^{13}C_{lipid\text{-}free}$.[48]

RESULTS AND DISCUSSION

Hg in Lake Water, the Lower Food Web, and Lake Trout. Analyzed constituents in filter-passing waters were among the lowest reported for surface waters in the literature. FHgT (0.18 ± 0.04 and 0.22 ± 0.11 ng L^{-1} for KATM and LACL, respectively; Table S2) in surface waters was similar between parks and lower, on average, than both the pelagic Pacific Ocean and Laurentian Great Lakes.[1,43,49] The FMeHg (often at or below method detection limits of 0.010 ng L^{-1}) and DOC (0.89 ± 0.39 and 0.62 ± 0.71 mg L^{-1} for KATM and LACL respectively) concentrations were extremely low, often lower than the open Pacific Ocean and Upper Great Lakes.[43,44,49]

https://doi.org/10.1021/acs.estlett.2c00096
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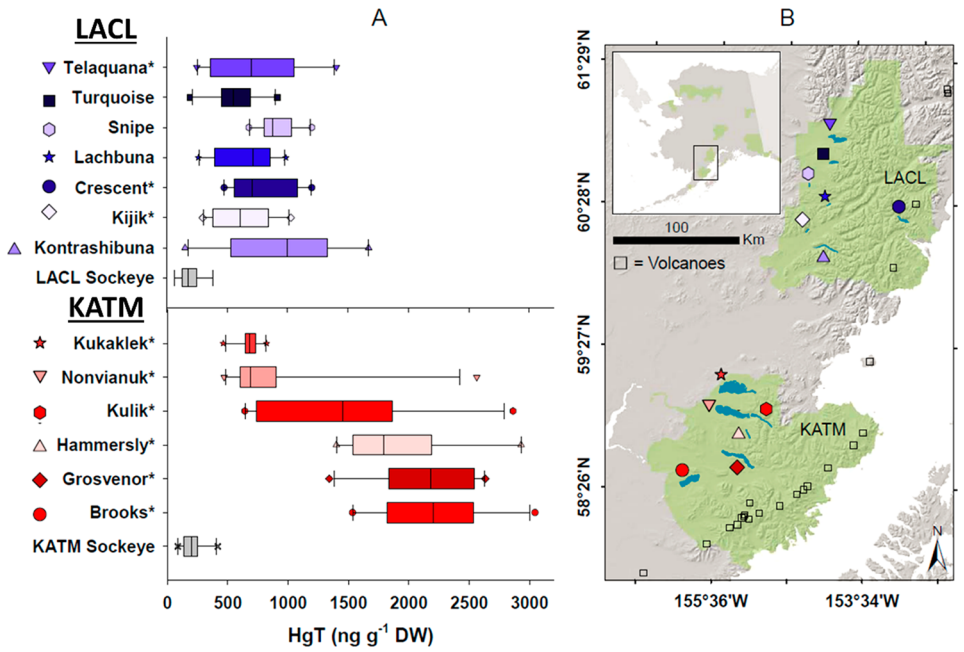

Figure 1. (A) Total mercury concentrations (HgT ng g^{-1} DW) in lake trout and sockeye salmon sampled from 13 lakes spanning two National Parks, Lake Clark (LACL), and Katmai (KATM). Box plots are distinguished by color (park), symbol (lake), and shading (water clarity, with lighter tones representing greater water clarity). Lakes are displayed along a latitudinal gradient, from north-most at the top to south-most at the bottom. Asterisks indicate the presence of sockeye salmon in lakes. Each red or blue box represents a collection of 10−11 lake trout, and each gray box includes all the sockeye salmon measured in a park (Tables S1 and S5). Whiskers mark the 25th and 75th quartiles, the center line the mean, and the symbols outliers. (B) Map of lake locations relative to nearby volcanoes (open squares on map). National Parks are depicted in green.

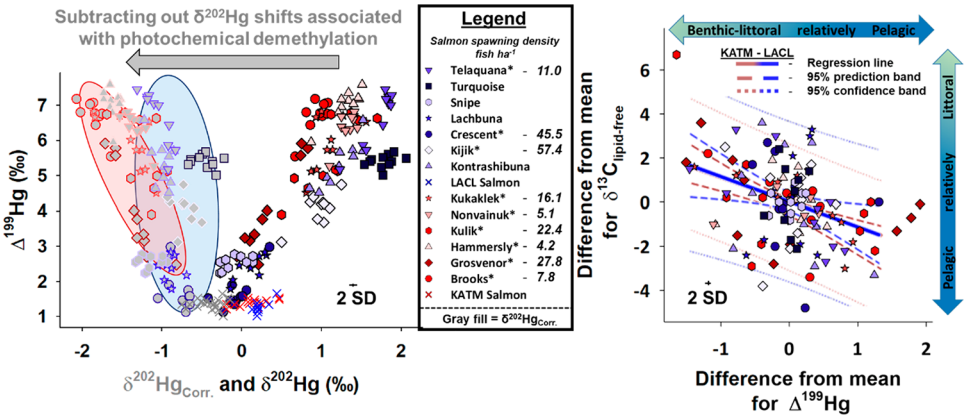

Figure 2. Left: Hg stable isotope values of δ^{202}Hg (colored) $\delta^{202}Hg_{Corr.}$ (gray-filled) versus Δ^{199}Hg in individual lake trout and anadromous sockeye salmon from 13 lakes in two parks (Tables S1 and S5). $\delta^{202}Hg_{Corr.}$ derivation is detailed in Supporting Information but represents a term corrected for the MDF produced during photochemical demethylation. Right: Data points represent individual lake trout that have been corrected to the lake population mean for $\delta^{13}C_{lipid\text{-}free}$ and Δ^{199}Hg. Solid, medium hashed, and dotted lines are color-coded and represent parks-specific regression lines, 95% prediction bands, and 95% confidence bands, respectively. Legend: Color formatting follows Figure 1A except for the gray-filled boxes. Asterisks indicate the presence of sockeye salmon in lakes. The legend is ordered by decreasing latitude from top to bottom. When italicized numbers are listed, those indicate that lakes' salmon spawning density (fish ha^{-1}).

The uptake of MeHg from water into plankton marks the baseline for MeHg entry into the food web in freshwater and marine systems and can be measured as the bioaccumulation factor (BAF [kg L^{-1}]). Bulk plankton, size-sieved at the 63−118 μm size fraction, was composed primarily of algae and contained very few zooplankton, as indicated by low δ^{15}N values and high C/N ratios (Table S4).[44] Focusing on bioaccumulation in phytoplankton allows us to estimate MeHg bioavailability while avoiding the complexities of biomagnification through higher trophic level zooplankton. BAFs, while commonly utilized in the literature to compare MeHg bioaccumulation across various water qualities and spatiotemporal ranges, are uncommonly measured in Alaska. We compared BAFs here to analogous oligotrophic systems with existing data, such as the open ocean and the Laurentian Great Lakes.[2,44] Although the 63−118-μm size fraction was consistently low in MeHg (6.1 ± 4.4 ng g^{-1}; Table S4) when compared to the larger size fractions collected here and algae reported elsewhere,[43,44] average BAFs are higher in these lakes (log 5.8 ± 0.5 L kg^{-1}) than typically found globally (log 2.4−5.9 in marine systems and log 5.5−6.0 in the Laurentian Great

https://doi.org/10.1021/acs.estlett.2c00096
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Lakes L kg^{-1}),[44,50] indicating more efficient MeHg bioaccumulation.

Lake trout HgT concentrations were widely variable within and across lakes (Figure 1), with fish from KATM often having higher HgT than fish from LACL. Variation in HgT concentrations within lakes were in part due to fish age and size differences, but systematic relationships were only present for some lakes which prevented us from performing corrections across the study. Great Lakes work[44] showed a significant and positive relationship between MeHg BAFs in phytoplankton and lake trout HgT, but here no relationship existed. This is likely because our seston-water data represents a single temporal snapshot, and lake trout were not size-age standardized.[51] However, C, N, and Hg isotope values in fish capture longer biological time spans that better integrate fish habitat, diet, and Hg content.

Hg Isotopes Reveal Hg Sources to Lake Trout. Lake trout spanned a large range in δ^{202}Hg and Δ^{199}Hg values (−0.42 to 2.06‰ and 1.13 to 7.59‰, respectively; Figure 2). These ranges were similar to those reported from the Great Lakes, where Hg isotope variability is primarily driven by trophic status and water clarity, and secondarily by the localized influence of anthropogenic inputs.[1] Although the Alaskan lakes are similarly oligotrophic to the least productive of the Great Lakes (like lake Superior), they lack a localized source of anthropogenic Hg and nutrient inputs (like lake Erie) that would drive variation in Δ^{199}Hg, so another mechanism, besides water clarity, must drive Δ^{199}Hg variability. The Δ^{199}Hg/Δ^{201}Hg[5,16,23] slopes (KATM = 1.27 ± 0.01, r^2 = 1.0; and LACL = 1.22 ± 0.01, r^2 = 0.99) measured in lake trout are indicative of photochemical demethylation,[5−7] so variability in lake trout Δ^{199}Hg values within a lake did not originate in the Hg source but rather from the extent of *in situ* photochemical processing. Secchi depth generally increased with mean lake trout Δ^{199}Hg across lakes, with some exceptions (Figures S3), in part supporting these conclusions. But some lakes had very low Secchi depth accompanied by high Δ^{199}Hg values, and we postulate that is related to the depth of chlorophyll maxima which for some lakes was proximate to the surface. Vertical placement of phytoplankton is important because phytoplankton are the entry point for MeHg, where pelagic Δ^{199}Hg values are captured initially. Phytoplankton can enhance photochemical demethylation when exposed to UV light,[52] which likely exacerbates Δ^{199}Hg formation in those anomalous lakes, and we propose vertical phytoplankton positioning might be influenced by unrealized variables (like glacial till shading) and should be investigated in the future.

The collection of Δ^{199}Hg and δ^{202}Hg values between the two parks are also offset by roughly 0.5‰ (Figure 2 left), likely due to differing proportions of distinct Hg sources between parks. The Δ^{199}Hg/δ^{202}Hg slope in LACL lake trout (2.28 ± 0.09, r^2 = 0.97) was like laboratory-derived photochemical demethylation in low DOC conditions (slope = 2.4),[3,6,7,29] indicating a similar Hg source among LACL lakes that is photochemically fractionated to varying degrees. In contrast, the Δ^{199}Hg/δ^{202}Hg slope for KATM lake trout (3.48 ± 0.33, r^2 = 0.90) was substantially higher than LACL, indicating that KATM lakes receive differing proportions of distinct Hg sources that were also photochemically demethylated to dissimilar extents (Figure S5). Finally, the lake trout Δ^{199}Hg/δ^{202}Hg values do not overlap with the anadromous salmon values (except at Crescent Lake, where later we will also rule out direct salmon consumption), indicating that direct consumption of salmon is not a dominant MeHg source.

Photochemical demethylation also changes δ^{202}Hg values, but Δ^{199}Hg can be used to estimate this fractionation.[3] The extent of photochemical demethylation (reflected by Δ^{199}Hg magnitude) differed among lakes making it challenging to compare δ^{202}Hg values between lakes. DOC and FHgT concentrations can influence δ^{202}Hg corrections, but here they are similar among lakes (Table S2), reducing concerns. We correct δ^{202}Hg using lake trout Δ^{199}Hg, the laboratory-derived Δ^{199}Hg:δ^{202}Hg slope (2.4) specific to these DOC and FHgT conditions and the assumption that Δ^{199}Hg values of incoming inorganic Hg are near-zero.[16,53,54] Although sources of Hg with nonzero Δ^{199}Hg values are typical from precipitation, we postulate that wet Hg deposition is relatively similar across all lakes, and this is supported by low Δ^{200}Hg variability among lake trout.[16,25,27,33,36,55] Furthermore, the lack of increased particulate or dissolved Hg below the thermocline indicates the sediments were not an appreciable Hg source to fish[1,12,42] (Table S2). Together, these factors and assumptions allowed us to estimate a $\delta^{202}Hg_{Corr.}$ for each fish, which we then compared across lakes.

We observed decreasing $\delta^{202}Hg_{Corr.}$ values and increasing lake trout HgT concentrations (Figure S6 - Pearson's r = −0.55 and p < 0.001 - Figure S6) as proximity to volcanoes increased (Figures 1B and 2 left). Isotope values from gaseous elemental Hg and fumarole-reactive Hg released from volcanoes are low in Δ^{199}Hg (−0.1 and −0.1 to 0.2‰, respectively) and δ^{202}Hg (−1.7 and −1.1 to −0.2‰ respectively) when compared to background gaseous elemental and precipitation-delivered Hg isotope values (Δ^{199}Hg; −0.3 to −0.1 and 0.2 to 0.6‰ are the interquartile ranges respectively and δ^{202}Hg; 0.0 to 0.7 and −1.1 to 0.0‰ are the interquartile ranges respectively).[33,36,53,56,57] Because the δ^{202}Hg values for fumarole-released Hg are lower than background measurements in precipitation and gaseous elemental Hg, and because we observed decreased $\delta^{202}Hg_{Corr.}$ values in lakes proximate to volcanoes, we have concluded that the southern KATM lake trout receive additional Hg from volcanism, likely as fumarole deposition directly to lakes or to the watershed.[39]

Determining the Importance of Salmon to Lake Trout Hg Accumulation. We found little evidence of direct contribution of salmon-derived Hg to lake trout. Lake trout $\delta^{202}Hg_{Corr.}$ values did not overlap with salmon $\delta^{202}Hg_{Corr.}$ values (Figure 2 left) in any lakes except Turquoise (which lacks salmon migration). Crescent Lake trout overlapped with salmon for paired δ^{202}Hg and Δ^{199}Hg values but only when uncorrected δ^{202}Hg values are used. Our study design does not account for indirect routes of Hg exposure from salmon. For example, lake trout may consume oceanic salmon MeHg through salmon eggs and newly hatched fry. Also, salmon decay as an exposure route was not considered. Decay results in the simultaneous liberation of Hg and a considerable influx of nutrients to these oligotrophic ecosystems.[10,58−61]

Although plankton would be the preferred sentinel to capture a δ^{15}N and $\delta^{13}C_{lipid\text{-}free}$ or Hg signal from decaying salmon, plankton collections slightly preceded salmon migration. So, we can only conclude that plankton δ^{15}N and $\delta^{13}C_{lipid\text{-}free}$ values do not differ between lakes with and without salmon migrations despite our assumption that salmon migrations would alter each lake's δ^{15}N and δ^{13}C baseline over time scales exceeding a year. We expected to observe a

https://doi.org/10.1021/acs.estlett.2c00096
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$\delta^{15}N$ and $\delta^{13}C_{lipid\text{-}free}$ or Hg shift in the lake trout too because migrating salmon deliver, *en masse*, large amounts of isotopically distinct sources of C, N, and Hg to relatively nutrient and Hg-poor lake ecosystems.[58,59,62] C, N, and Hg half-lives in long-lived lake trout are expected to extend beyond one year, allowing them to serve as sentinels to capture salmon migrations if salmon tissue is of direct and important sustenance.[12,63−65] While C and Hg isotope axes in lake trout were not distinguishable between lakes with and without salmon migrations, $\delta^{15}N$ values were higher in salmon-run lakes (t test; $t = 9.06$, $p \leq 0.001$ and Figure S2). Elevated $\delta^{15}N$ values indicate that senescent salmon might alter the nitrogen baselines; however, our study was not designed to accurately characterize this result in detail. Direct and indirect routes of trout exposure to salmon Hg warrant focused study, including a mass-balance approach using specimens collected over more frequent intervals, to better understand the influence of migratory salmon as a source of MeHg to resident fish in lakes and rivers.

Importance of Habitat Uses by Lake Trout. Using Hg stable isotope values to delineate Hg sources to fish can be complex when species undergo changes in life cycle[14] or dietary niche partitioning.[13] We propose that these lake trout niche-partition among preferred foraging habitats. In lakes where the largest observable differences in Hg and C isotope values exist between habitats, we can explore this partitioning. Trophic modifications and *in situ* reactions complicate interpreting $\delta^{15}N$ and $\delta^{202}Hg$ directly.[12,18,66] $\Delta^{199}Hg$ and $\delta^{13}C_{lipid\text{-}free}$ values are conserved along trophic processes, allowing us to trace foraging habits along axes of depth (via $\Delta^{199}Hg$)[1,12,32] and pelagic-versus littoral habitat types (via $\delta^{13}C_{lipid\text{-}free}$).[11] When we subtract the lake-specific lake trout mean $\Delta^{199}Hg$ and $\delta^{13}C_{lipid\text{-}free}$ values from each individual lake trout, we can compare $\Delta^{199}Hg$ and $\delta^{13}C_{lipid\text{-}free}$ values between lakes with differing baselines (Figure 2 right). We found many lakes where lake trout foraging habitat preferences exist. Some lake trout used relatively more littoral areas of the lake ($\Delta^{199}Hg$ decreased and $\delta^{13}C_{lipid\text{-}free}$ increased), and others used deep pelagic areas ($\Delta^{199}Hg$ increased and $\delta^{13}C_{lipid\text{-}free}$ decreased; Figure 2 right). More work would be useful to clarify whether this influences fish nutrition or Hg burden.[12] Together, these tools provide yet another line of evidence supporting the use of multi-isotope values to better understand energy pathways, contaminant sources, contaminant burdens, and variance therein.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.2c00096.

> Supplementary text on the research approach, site description, biological analyses and factors and, the soil collections performed. A series of figures that support points of discussion in the main manuscript as well as a diagram that helps communicate the process of correcting $\delta^{202}Hg$ for mass-dependent fractionation attributed to photochemical demethylation. Supporting data tables for the figures presented this manuscript. Those tables can also be found at 10.5066/P9UEP9C5. Citations pertinent to the Supporting Information (PDF)

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Author Contributions

K.K.B., C.A.E., and D.P.K. designed the study. R.F.L., J.M.O., and K.K.B. wrote this manuscript. C.A.E., D.P.K., J.P.H., Y.R., S.E.J., and D.B.Y. provided editorial input. R.F.L., K.K.B., J.M.O. collected samples. R.F.L., M.T.T., S.E.J., and J.M.O. provided substantial analytical support.

Notes

The authors declare no competing financial interest.
Data collected for this study are available at 1/P9UEP9C5.

■ ACKNOWLEDGMENTS

This work was supported by the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program, USGS Contaminant Biology Program, and the USGS - NPS Natural Resources Preservation Program. The views expressed in this

paper represent the views solely of the authors from the U.S. Environmental Protection Agency but do represent the views of the U.S. Geological Survey. Any use of trade, firm, or product names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government. All animals were collected following the protocols in the approved National Park Service Institutional Animal Care and Use Committee plan, under Protocol Number (AKR_SWAN_Bartz_Fish_2019.A3). Partial graduate student support was provided by the Wisconsin Alumni Research Foundation through the University of Wisconsin-Madison Graduate School (Award No. MSN165161), and the University of Wisconsin Water Resources Institute through a USGS-NIWR fellowship (Award No. MSN197848). Postdoctoral support was provided by the National Science Foundation Postdoctoral Fellowships for Research in Biology − Collection Program 2018 (Award No. 1812211). We thank Dr. Jessica Brandt and Dr. Sarah Laske, who both contributed a volunteer peer review of this work, and Evan Booher, Katie Junghans, and Jeff Nelson who provided critical field support.

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