

Environmental Origins of Methylmercury Accumulated in Subarctic Estuarine Fish Indicated by Mercury Stable Isotopes

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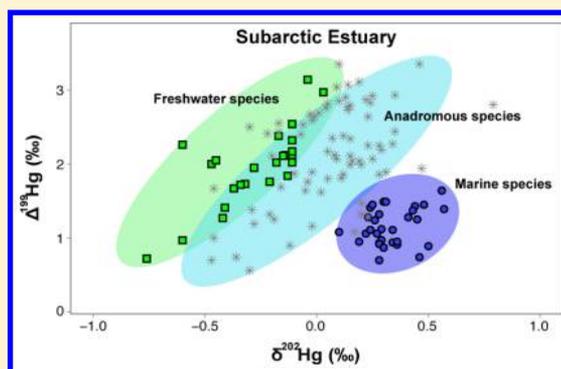
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Supporting Information

ABSTRACT: Methylmercury (MeHg) exposure can cause adverse reproductive and neurodevelopmental health effects. Estuarine fish may be exposed to MeHg produced in rivers and their watersheds, benthic sediment, and the marine water column, but the relative importance of each source is poorly understood. We measured stable isotopes of mercury ($\delta^{202}\text{Hg}$, $\Delta^{199}\text{Hg}$, and $\Delta^{201}\text{Hg}$), carbon ($\delta^{13}\text{C}$), and nitrogen ($\delta^{15}\text{N}$) in fish with contrasting habitats from a large subarctic coastal ecosystem to better understand MeHg exposure sources. We identify two distinct food chains exposed to predominantly freshwater and marine MeHg sources but do not find evidence for a benthic marine MeHg signature. This is consistent with our previous research showing benthic sediment is a net sink for MeHg in the estuary. Marine fish display lower and less variable $\Delta^{199}\text{Hg}$ values (0.78‰ to 1.77‰) than freshwater fish (0.72‰ to 3.14‰) and higher $\delta^{202}\text{Hg}$ values (marine: 0.1‰ to 0.57‰; freshwater: -0.76‰ to 0.15‰). We observe a shift in the Hg isotopic composition of juvenile and adult rainbow smelt (*Osmerus mordax*) when they transition between the freshwater and marine environment as their dominant foraging territory. The Hg isotopic composition of Atlantic salmon (*Salmo salar*) indicates they receive most of their MeHg from the marine environment despite a similar or longer duration spent in freshwater regions. We conclude that stable Hg isotopes effectively track fish MeHg exposure sources across different ontogenic stages.



INTRODUCTION

Methylmercury (MeHg) is a bioaccumulative neurotoxicant produced from divalent inorganic mercury (Hg^{II}) in aquatic ecosystems that adversely affects the health of humans and wildlife.^{1–3} Benthic sediment, rivers, wetlands, and the marine water column are all potential locations for MeHg formation and uptake into estuarine foodwebs.^{4–8} The origin of environmental MeHg sources accumulated by fish affects their responses to changes in Hg^{II} inputs. For example, there may be a substantial temporal lag between atmospheric Hg inputs and MeHg levels in benthic sediment but a more rapid response in surface seawater.⁹ Tracing the environmental origins of MeHg in fish is challenging due to their diverse foraging preferences and migration patterns. Stable Hg isotopes show promise as a new empirical tracer for fish foraging behavior and corresponding MeHg exposure sources.^{10–13} Here we use stable isotopes of Hg, carbon, and nitrogen in

subarctic estuarine fish with diverse habitats to characterize their predominant environmental MeHg exposure sources.

Bioaccumulation modeling for MeHg suggests dietary ingestion accounts for >90% of the body burden in fish.^{14,15} Stable isotopes of carbon (reported as $\delta^{13}\text{C}$) and nitrogen (reported as $\delta^{15}\text{N}$) provide information on fish habitat type and trophic position.¹⁶ However, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ change more rapidly in fish than MeHg because their half-lives (5–173 days) are generally much shorter than persistent and bioaccumulative contaminants like MeHg (~1–4 years).^{17–22} Understanding long-term foraging patterns is particularly important for anticipating MeHg levels in species such as salmon and trout that shift their diet and/or foraging territory over different

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Table 1. Habitat and Prey Preferences of Fish and Shellfish and MeHg Production Sources Inferred Based on Hg Stable Isotope Composition

common name (scientific name)	N	ecological zones ^a	prey preference ^b	inferred MeHg production sources ^c
Capelin (<i>Mallotus villosus</i>)	6	pelagic	zooplankton	marine water column
Herring (<i>Clupea harengus</i>)	1	pelagic	zooplankton, zoobenthos	marine water column
Blue mussel (<i>Mytilus edulis</i>)	6	intertidal	detritus and plankton	marine water column
Atlantic cod (<i>Gadus morhua</i>)	5	benthopelagic	finfish, zoobenthos	marine water column
Atlantic salmon (<i>Salmo salar</i>)	12	benthopelagic	finfish, zoobenthos	marine water column
Shorthorn sculpin (<i>Myoxocephalus scorpius</i>)	10	benthic	finfish ^d , zoobenthos, crustaceans ^d , molluscs (bivalves) ^d	marine water column
Flatfish (<i>Pleuronectoide sp.</i>)	20	benthic	zoobenthos, plants, zooplankton, molluscs (bivalves) ^d , crustaceans ^d	mixed
Arctic char (<i>Salvelinus alpinus alpinus</i>)	4	benthopelagic	finfish, zoobenthos	mixed
Brook trout (<i>Salvelinus fontinalis</i>)	48	benthopelagic	zoobenthos, finfish ^d , zooplankton, insects ^d , crustaceans ^d	mixed
Lake whitefish (<i>Coregonus clupeaformis</i>)	27	benthopelagic	zooplankton, zoobenthos, molluscs (bivalves) ^d	mixed
Rainbow smelt (adult) (<i>Osmerus mordax</i>)	18	pelagic	zooplankton, finfish	mixed
Three-spine stickleback (<i>Gasterosteus aculeatus</i>)	14	benthopelagic	zooplankton, zoobenthos	mixed
Lake chub (<i>Couesius plumbeus</i>)	8	benthopelagic	zoobenthos, zooplankton, plants	terrestrial/river
Longnose sucker (<i>Catostomus catostomus</i>)	10	benthic	detritus, zoobenthos, plants ^d	terrestrial/river
Northern pike (<i>Esox lucius</i>)	3	benthopelagic	finfish ^d , zoobenthos	terrestrial/river
Rainbow smelt (juv.) (<i>Osmerus mordax</i>)	7	pelagic	zooplankton	terrestrial/river

^aBased on compilation of literature data from Fishbase.⁵⁹ ^bBased on literature data.^{59,92} ^cBased on stable Hg isotope composition (Figures 1–3). ^dIdentified by stomach content analysis as part of this study.

ontogenic stages. Thus, naturally occurring Hg stable isotopes in fish can potentially provide complementary information to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.^{10,12,23}

All seven stable Hg isotopes undergo mass-dependent fractionation (MDF, reported as $\delta^{202}\text{Hg}$) as the result of many physical, chemical, and biological processes.^{24–30} Mass-independent fractionation (MIF, reported as $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$) occurs predominantly during photochemical reactions, wherein the odd-mass-number isotopes are either enriched or depleted in reaction products relative to the even-mass-number isotopes.^{26,29,31} MIF is widely observed in aquatic organisms.^{26,32,33} However, no substantial isotopic fractionation has been observed between MeHg in plankton and higher trophic level fish.^{11,34,35} This allows MIF signatures to serve as a conservative tracer for environmental MeHg sources accumulated by fish.

Prior work suggests different sources and/or reaction pathways of Hg species drive the variable Hg isotopic composition measured in terrestrial, coastal, and oceanic biota. Tsui et al.¹³ found riverine and terrestrial food webs have distinct $\delta^{202}\text{Hg}$ values, which they attributed to different MeHg sources. In aquatic ecosystems, MIF is primarily a function of photochemistry and varies with the extent of light penetration in different foraging regions. Blum et al.¹² showed that pelagic marine fish foraging in deeper waters with limited light penetration had lower $\Delta^{199}\text{Hg}$ values relative to those feeding in surface waters. Senn et al.¹⁰ showed that the $\Delta^{199}\text{Hg}$ values in pelagic fish were greater than those in coastal fish from the Gulf of Mexico, presumably due to reduced water turbidity, enhanced photochemistry, and faster demethylation in the open ocean water column.

The main objective of this study is to investigate whether stable Hg isotopes can elucidate the environmental origins of MeHg accumulated in fish from coastal ecosystems with multiple potential exposure sources (rivers, benthic sediment, and the marine water column). Prior work has focused on midlatitude ecosystems and demonstrated differences in Hg

isotopic signatures in biota from two contrasting food webs (oceanic vs coastal, terrestrial vs riverine)^{10,13} but has not extended these findings to the diversity of MeHg exposure sources and contrasting food webs within a single coastal ecosystem. Here we report new measurements of stable Hg, C, and N isotopes and tissue Hg and MeHg burdens in fish and shellfish with contrasting benthic, benthopelagic, and pelagic habitats from the region within and surrounding Lake Melville, a large subarctic fjord in Labrador, Canada (Supporting Information, Figure S1).

■ MATERIALS AND METHODS

Sample Collection. Table 1 lists the fish and shellfish species analyzed in this study and their predominant habitats. We reviewed the literature on the indigenous composition of benthic, benthopelagic, and pelagic marine and freshwater fish commonly found in the Lake Melville region to identify species that represent each habitat type. Inuit community members harvested 15 types of fish and shellfish ($n = 202$) from the main freshwater tributary to Lake Melville (lower Churchill River), the estuary (Lake Melville), and the Labrador Sea between June and August of 2014 and 2015 (Table 1). Smaller fish were captured by minnow traps and larger fish were obtained by rod or directly from commercial fishing vessels in Lake Melville. Standard length and weight of each whole fish was measured in the field or lab. Each fish was frozen after collection and shipped to Harvard University for analysis.

In the laboratory, we collected subsamples of axial muscle tissue and freeze-dried and homogenized samples prior to analysis for Hg, MeHg, and stable isotopes. Minnow species (stickleback, juvenile smelt, and chub) were freeze-dried, and whole fish samples were homogenized using a blender because they were too small to fillet muscle tissue. Surface sediment samples (0–3 cm) were taken from a box corer or stainless steel gravity corer in Goose Bay, Groswater Bay, and the Churchill River (September 2012 and June 2013) (Supporting

Information, Figure S1), as described in Schartup et al.,⁵ and analyzed for Hg, MeHg, and Hg isotopes in this work.

Fish Species Age, Habitat, and Diet Classification. We harvested available fish scales and otoliths for age determination at NOAA's Northeast Fisheries Science Center in Woods Hole, MA. We determined the age of Atlantic salmon and brook trout by analyzing their scales, following established methods.^{36,37} Otoliths were used to age other species following the "thin-section and bake" technique.^{38,39} Stomach contents of all fish were dissected and examined prior to chemical analysis. Qualitative diet composition from this analysis was combined with a literature synthesis of the feeding ecology and life history for all fish to determine the a priori habitat of all species (Table 1), which we compared to information from Hg isotopes in this analysis.

Total Hg and MeHg Analysis. We measured total Hg concentrations in all fish samples by thermal decomposition, amalgamation, and atomic absorption spectrophotometry (EPA method 7473) using a Nippon MA-3000 Mercury Analyzer. At least one method blank and one certified fish tissue reference material (CRM: TORT 3) were tested every 10 samples. The average Hg recovery for the TORT 3 CRM was $100.7 \pm 2.2\%$ ($n = 22$).

We measured MeHg concentrations in all minnows, mussels, and benthic fish following a modified EPA 1630 method established in prior work.^{39–42} The tissue Hg burden of predatory fish (cod, salmon, trout, pike, and whitefish) is widely assumed to be close to 100% MeHg,⁴³ but lower trophic level organisms have more variable MeHg fractions.^{44,45} Samples were spiked with 1 mL of enriched Me^{201}Hg (2 ng/mL) and then digested with 5N HNO_3 solution at 70 °C overnight prior to MeHg analysis. Two CRMs (TORT 3 and DORM 4) were included in each digestion cycle. Acid was neutralized with 8 N KOH and buffered with a 2 M acetate buffer. Aqueous MeHg was ethylated using sodium tetraethylborate (NaBEt_4). Ethylated MeHg was purged onto a Tenax packed column and separated by gas chromatography using a Tekran 2700 MeHg autoanalyzer coupled to a Thermo iCAP-Q ICP-MS with Teflon tubing for MeHg detection.^{8,46} We analyzed ongoing precision and recovery (OPR) standards with different concentrations every 10 samples. Mean recovery was $105.5 \pm 5.7\%$ (SD; $n = 8$). The average recoveries for TORT 3 and DORM 4 were $92.4 \pm 5.0\%$ (SD; $n = 8$) and $99.7 \pm 9.8\%$ (SD; $n = 8$). Precision, estimated by replicate analysis of CRMs and duplicate fish samples, was better than 7% and 8%, respectively.

Carbon and Nitrogen Isotope Analysis. Freeze-dried and homogenized fish tissue samples were analyzed for stable isotopes of carbon and nitrogen at Boston University's Stable Isotope Laboratory and the University of Hawaii at Manoa's Isotope Biogeochemistry Laboratory.⁴⁷ Isotopic values are reported in conventional δ -notation relative to international standards (C, Pee Dee Belemnite; N, atmospheric nitrogen). A secondary standard (glycine) was included to ensure instrumental accuracy and precision in both laboratories. The standard deviation around the expected values for the secondary standards was within 0.3% and 0.2% for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. As lipids have more depleted $\delta^{13}\text{C}$ values relative to other tissues, aquatic samples with high lipid content (i.e., C/N ratio > 3.5) are generally reported as a normalized $\delta^{13}\text{C}$ value by either lipid extraction or mathematical methods.^{48,49} We used the C/N ratio as a proxy for lipid content to estimate $\delta^{13}\text{C}$ values of lipid-free muscle and whole-

body tissue, following the relationships derived by Logan et al.⁴⁹

Stable Hg Isotope Analysis. Approximately 0.1–0.2 g of freeze-dried and homogenized fish ($n = 135$) and sediment samples ($n = 7$) were digested at 120 °C for 6 h using a 2 mL acid mixture ($\text{HCl}/\text{HNO}_3 = 1:3$, v/v) for stable Hg isotope analysis, following the protocol established by others.^{33,50} The digest solutions were diluted to 1 ng mL^{-1} for most samples. For samples with low available mass or low tissue total Hg concentrations, digest solutions were diluted to 0.4–0.5 ng mL^{-1} . Samples were analyzed using a Neptune Plus multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) housed at the Wisconsin State Laboratory of Hygiene.

The sample introduction system and analytical methods for stable Hg isotope analysis follow previous studies.^{51,52} Briefly, samples containing Hg were continuously mixed and reduced with 3% SnCl_2 . The cold vapor generator was custom designed, as described in Yin et al.⁵² Volatile $\text{Hg}(0)$ was separated by a frosted glass phase separator and introduced to the MC-ICP-MS with argon gas. Instrumental mass bias was corrected using an internal Tl standard (NIST SRM 997) and sample-standard bracketing. The Hg concentrations and acid matrices of the bracketing standard (NIST SRM 3133) were systematically matched to the neighboring samples. Data nominations follow the protocols suggested by Blum and Bergquist.⁵³ MDF is expressed using $\delta^{202}\text{Hg}$ notation (eq 1). MIF is expressed as the difference between the measured $\delta^{\text{xxx}}\text{Hg}$ value, the value predicted based on MDF, and the $\delta^{202}\text{Hg}$ value (eqs 2 and 3).⁵³

$$\delta^{202}\text{Hg} = \left[\frac{{}^{202/198}\text{Hg}_{\text{sample}}}{{}^{202/198}\text{Hg}_{\text{NIST3133}}} - 1 \right] \times 10^3\text{‰} \quad (1)$$

$$\Delta^{199}\text{Hg} \approx \delta^{199}\text{Hg} - (\delta^{202}\text{Hg} \times 0.2520) \quad (2)$$

$$\Delta^{201}\text{Hg} \approx \delta^{201}\text{Hg} - (\delta^{202}\text{Hg} \times 0.7520) \quad (3)$$

All CRMs (TORT-2, TORT-3, DORM-2, DORM-4, and MESS-1) were prepared and analyzed in the same way as the samples. Total Hg in all solutions was monitored by MC-ICP-MS using ^{202}Hg signals, which yielded mean recoveries of 94.0% for fish samples and CRMs. The UM-Almadén standard solution (0.5–1.0 ng mL^{-1} , diluted in 10% aqua regia) was measured once every 10 samples. We found the overall average and uncertainty of UM-Almadén and CRMs agreed well with prior work (Supporting Information, Section 1).^{13,51,54}

Data Analysis. Differences in isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\Delta^{199}\text{Hg}$, $\Delta^{201}\text{Hg}$, and $\delta^{202}\text{Hg}$) among fish with different habitats and ecological zones were examined using two-way ANOVA and Tukey's Honest Significant Difference (HSD) test. The $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ ratio was calculated from the slope of York regression which accounts for error in both the dependent and independent variables.⁵⁵ All statistical analyses were performed using R.⁵⁶

RESULTS AND DISCUSSION

Fish Habitats and Hypothesized MeHg Sources. Fish species collected as part of this work span predominantly freshwater and marine habitats. Within these environments, we collected species that represent benthic, benthopelagic, and pelagic habitats. Many species are exposed to a mixture of MeHg sources (benthopelagic and anadromous fish). We

hypothesized that the predominant habitat type of each fish would dictate their environmental MeHg source and, in turn, would be recorded by distinguishable differences in tissue Hg isotopic composition.

Table 1 shows the literature and $\delta^{13}\text{C}$ derived habitats (Supporting Information, Figure S2) and prey of different fish and shellfish included in this study. Lake chub, northern pike, and juvenile rainbow smelt have low tolerance for high-salinity conditions^{57–59} and thus primarily forage in the freshwater environment. Potential MeHg sources in the freshwater environment include benthic sediment in rivers, terrestrial soils, and/or wetlands.^{13,60} In brackish sections of the Churchill River (the main freshwater tributary to Lake Melville estuary), some MeHg may also be transported inland from the estuarine river mouth during seasonal intrusion of tidal waters.^{61,62}

Herring, capelin, and Atlantic cod are generally considered offshore marine species, although they may migrate into estuaries for food and/or spawning (Table 1).⁵⁹ In the marine environment, MeHg is produced both in the water column and in benthic sediment.^{5,6,8,63–65} Species with a strong link to the benthic environment across different habitats include shorthorn sculpin (marine), flatfish (anadromous), and longnose sucker (freshwater).⁵⁹ All other species are anadromous or benthopelagic and are likely to be exposed to MeHg from multiple sources (Table 1).

Distinct Freshwater and Marine Food Chains. Distinct freshwater and marine food webs are evident from the Hg isotopic composition of fish and shellfish species measured in this study (Figure 1a). Marine species from this study (capelin, herring, and cod) cluster together based on their Hg isotope composition and are enriched in $\delta^{202}\text{Hg}$ relative to freshwater species (chub, pike, and juvenile smelt). Anadromous fish generally fall between the marine and freshwater food webs (Figure 1a). We expected the Hg isotopic composition of benthic species (shorthorn sculpin, flatfish, and longnose sucker) to also be distinct. Instead, benthic species cluster with pelagic and benthopelagic species across marine, anadromous, and freshwater food webs. The lack of a distinct benthic signature suggests a common MeHg source across benthic and pelagic environments (Table 1 and Figure 1).

Further evidence for a common MeHg source is provided in Figure 2, which shows that mean $\Delta^{199}\text{Hg}$ and $\delta^{202}\text{Hg}$ values in species within each foraging region (freshwater, mixed, and marine) do not differ significantly among benthic, benthopelagic, and pelagic habitats. By contrast, mean $\delta^{13}\text{C}$ values in benthic species are significantly higher (Tukey's HSD, $p < 0.05$) than others (Figure 2), confirming that their dietary substrate is different. Species that feed on benthic microalgae and vegetation generally have higher $\delta^{13}\text{C}$ values than those feeding on phytoplankton.^{44,66,67} Together, these data suggest that marine and freshwater fish in the Lake Melville region each have a single environmental MeHg production source across benthic, benthopelagic, and pelagic habitats.

MeHg Biomagnification. Figure 1b shows the MeHg concentration in each fish and shellfish species plotted against their $\delta^{15}\text{N}$ values. Values of $\delta^{15}\text{N}$ in anadromous species fall between the freshwater and marine food chains for all species except flatfish. Flatfish cluster with marine species based on their $\delta^{15}\text{N}$ but are clearly anadromous based on their Hg isotopic composition. We attribute this difference to the longer lifetime of Hg in fish (years) compared to N (<70 days),^{14,21,68,69} thus recording foraging behavior over a longer period.^{17–22} Freshwater species have much lower $\delta^{15}\text{N}$ values

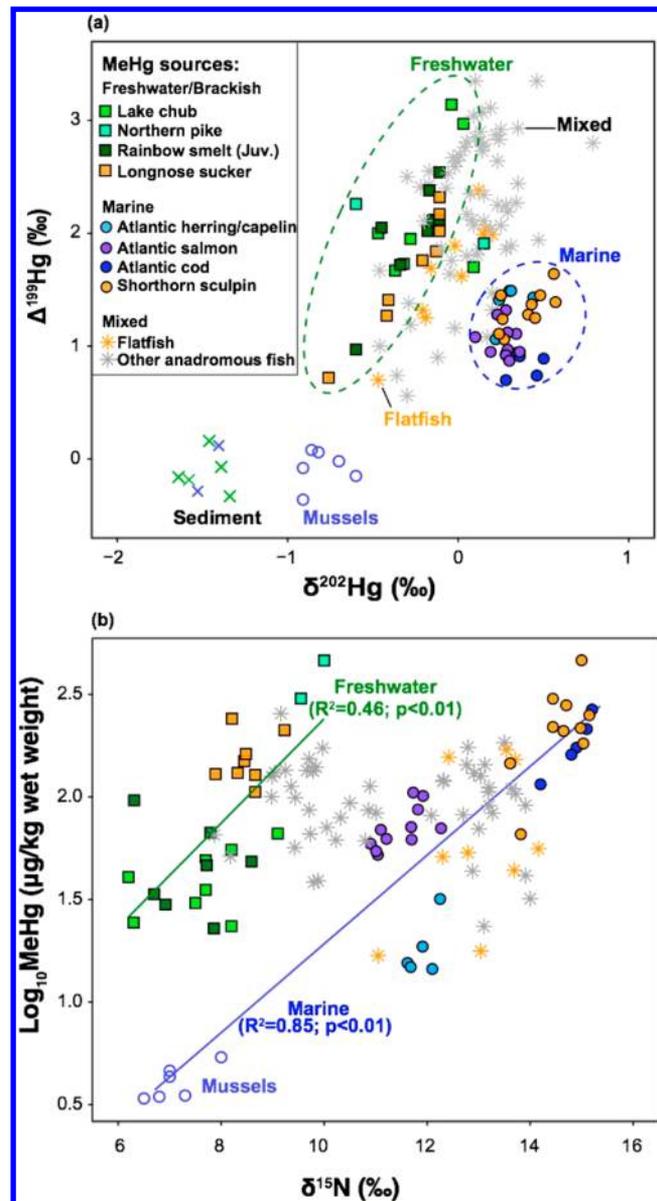


Figure 1. (a) Stable Hg isotope composition of individual subarctic fish/shellfish from the Lake Melville, Labrador region. (b) Methylmercury (MeHg) in each fish/shellfish plotted against their nitrogen isotope composition. Species are grouped by their dominant habitat (Table 1) and inferred MeHg sources based on Hg isotopes. Square symbols denote species that obtain their MeHg burden from freshwater/terrestrial sources. Circles indicate MeHg from the marine environment. Mixed MeHg sources are indicated by asterisks, and benthic species are colored in yellow. Benthic sediment samples are indicated by crosses with freshwater regions in green and marine regions in blue.

than those of anadromous and marine species at comparable trophic levels (Figure 1b and Table 2). This likely reflects variability in baseline $\delta^{15}\text{N}$ values among different food webs rather than a true difference in trophic position, as reported in other studies.^{10,16,70}

Pre-photodegraded MeHg Sources. Ratios of $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ for marine, freshwater, and anadromous food webs in this study range between 1.25 and 1.33 and are useful for identifying reaction pathways leading to Hg MIF (Supporting Information, Figure S3). Values observed here are closer to the

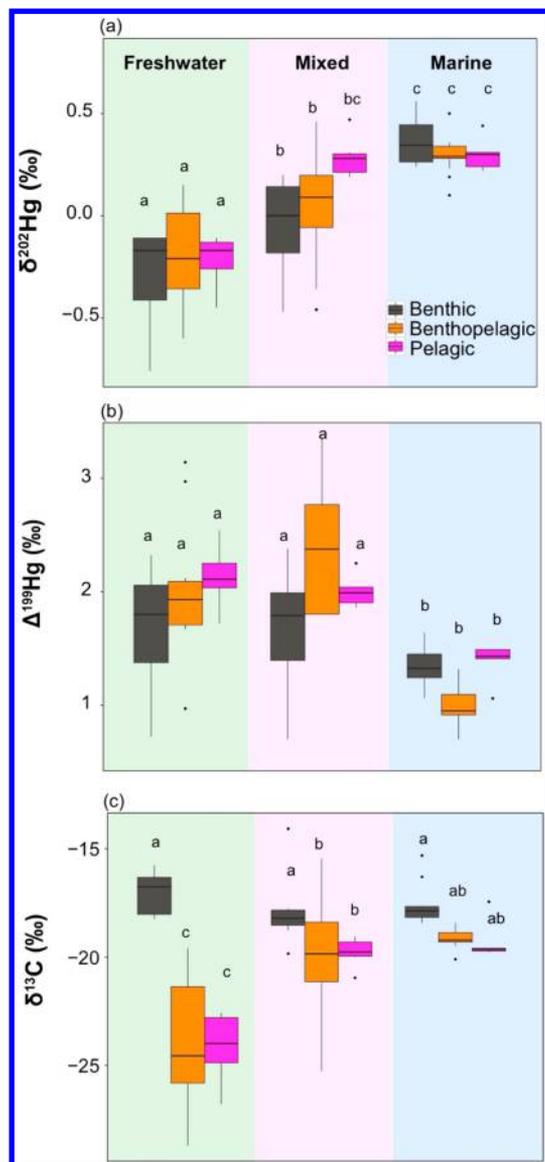


Figure 2. Box and whisker plots of means and variance in Hg and C isotope composition among benthic, benthopelagic, and pelagic fish and shellfish species from freshwater, mixed, and marine-harvesting regions. Letters above boxes indicate similarities among groups (same letters indicate no statistical difference, Tukey's HSD, $p > 0.05$).

experimentally derived ratio for photochemical demethylation of MeHg (1.36 ± 0.02 , 2 SE) than the ratio for photoreduction of divalent Hg^{II} (1.00 ± 0.02 , 2 SE).²⁶ MIFs in fish from this study thus likely reflect photochemical demethylation prior to MeHg uptake, as has been suggested for food webs from other regions.^{10–12}

We calculated the pre-photodegraded Hg isotope signatures for fish/shellfish from the Lake Melville region (Figure 3, blue and green shaded regions) based on the slope of the experimentally derived relationship between $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ for MeHg conversion to elemental Hg.²⁶ Pre-photodegraded MeHg sources to the freshwater and marine food webs are clearly separated, even after considering potential variability in the slope due to the presence of dissolved organic carbon (DOC) in freshwater ecosystems (Figure 3). Negative MIF of MeHg has not been reported in any study⁷¹ and would be required to explain the isotopic composition of the marine

food chain if it was derived from the same MeHg source as the freshwater food chain. These results reinforce the notion of distinct environmental MeHg production sources that have accumulated in freshwater and marine food webs.

Different Hg^{II} sources that provide the substrate for methylation most likely explain distinct pre-photodegraded $\delta^{202}\text{Hg}$ values measured in freshwater and marine fish. Freshwater ecosystems receive more inorganic Hg from the watershed compared to offshore marine areas where atmospheric deposition is well-established as the main source.^{72–74} Prior work indicates that the $\delta^{202}\text{Hg}$ signatures of watershed/terrestrial Hg sources tend to be more negative than precipitation and gaseous elemental mercury.^{51,75,76} For example, negative $\delta^{202}\text{Hg}$ values in peat and soil (-3 to -1%) are lower than those for Hg in precipitation (-1 to 1%) and gaseous elemental Hg (0.5 to 1%).^{75–79}

Other processes leading to variable $\delta^{202}\text{Hg}$ include the balance between methylation and demethylation reactions^{24,25} and partitioning to suspended particles.^{80,81} The range in MDF resulting from variable methylation/demethylation reactions reported by previous studies report is smaller ($<0.5\%$)^{6,11} than the difference between pre-photodegraded MeHg in freshwater and marine fish observed here ($>1\%$). Similar Hg isotopic compositions of surface sediment samples across marine and freshwater regions observed here (Figure 3) suggest that partitioning to suspended solids does not play an important role in distinguishing pre-photodegraded MeHg sources in the freshwater and marine environment. Distinct $\delta^{202}\text{Hg}$ signatures of Hg^{II} sources are thus needed to explain the observed differences between pre-photodegraded MeHg in fish from freshwater and marine habitats observed here.

In the marine environment, slopes based on laboratory measurements of aqueous photochemical demethylation (blue shaded region in Figure 3) encompass the Hg isotopic composition of almost all marine fish in this study and previous work. These results are suggestive of a similar pre-photodegraded marine MeHg source in the Lake Melville region (Labrador Sea), Gulf of Mexico, and the Atlantic and Pacific Oceans. Cossa et al.⁸² similarly suggested that high MeHg concentrations in Atlantic hake from the continental shelf could be explained by a water column MeHg source.

Many studies report methylation of Hg^{II} (primarily from the atmosphere) in the marine water column.^{64,65,83} Similar isotopic composition of precipitation has been reported across many regions globally in areas that are not directly impacted by local point sources (e.g., $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values: 0.2% and 0.7% in France,⁷⁵ -0.3% and 0.3 – 0.5% Great Lakes, U.S.A.,^{76,84} and 0.1% and 0.2% , Florida, U.S.A.⁸⁵). We thus infer that atmospherically deposited Hg is the likely origin of the common pre-photodegraded MeHg source observed here across different marine systems.

Factors Affecting Variability in $\Delta^{199}\text{Hg}$ in Marine Fish.

Marine species in this study have more tightly clustered $\Delta^{199}\text{Hg}$ values (0.78% to 1.77%) than freshwater and anadromous fish (0.56% to 3.35%), indicating more limited MeHg photodegradation prior to entering the marine food web (Figure 1a). Figure 3 shows that values observed here are similar to those for migratory species from the Gulf of Mexico and deep-sea fish and squid from the North Pacific and Atlantic Oceans^{10,12} but lower than those for offshore Gulf of Mexico species and North Pacific species that forage near the surface.^{10,12}

Table 2. Descriptive Statistics (Mean \pm Standard Deviation) for Fish, Shellfish, and Sediment Samples Included in This Study

sample type	N	length (cm)	age (years)	total Hg (ng/g wet weight)	MeHg fraction	$\delta^{15}\text{N}$	trophic level ^a
Fish							
Arctic char	4	42.5 \pm 3.5	NA	62.4 \pm 41.8	NA	13.4 \pm 0.4	4.4
Atlantic cod	5	NA	NA	186.3 \pm 57.4	NA	14.8 \pm 0.4	4.1
Atlantic salmon	12	NA	3.7 \pm 2.1	73.2 \pm 20.1	NA	11.5 \pm 0.4	4.5
Blue mussel	6	NA	NA	17.3 \pm 2.5	0.2 \pm 0.04	7.1 \pm 0.5	2.0
Brook trout	48	32.2 \pm 7.0	5.3 \pm 0.9	104.8 \pm 34.4	NA	12.6 \pm 1.4	3.3
Capelin	6	18.1 \pm 0.4	NA	18.6 \pm 2.7	0.9 \pm 0.1	11.9 \pm 0.2	3.2
Flatfish	20	20.2 \pm 4.7	4.9 \pm 1.6	67.8 \pm 40.9	1.0 \pm 0.1	13.1 \pm 1.0	3.6
Herring	1	32	NA	35.7	0.9	12.3	3.4
Lake chub	8	7.4 \pm 1.8	NA	50.0 \pm 24.1	0.9 \pm 0.2	7.6 \pm 1.0	3.4
Lake whitefish	27	34.9 \pm 4.0	6.5 \pm 3.2	122.2 \pm 43.6	NA	9.5 \pm 0.8	3.2
Longnose sucker	10	32.4 \pm 2.2	6.6 \pm 1.9	142.9 \pm 42.3	1.0 \pm 0.1	8.5 \pm 0.4	2.5
Northern pike	3	67.0 \pm 2.8	NA	343.4 \pm 104.9	NA	9.8 \pm 0.2	4.1
Rainbow smelt (adult)	18	19.7 \pm 2.4	NA	103.9 \pm 44.7	1.1 \pm 0.1	12.8 \pm 0.4	3.0
Rainbow smelt (juvenile)	7	9.9 \pm 1.1	NA	44.8 \pm 21.6	1.0 \pm 0.2	7.4 \pm 0.8	NA
Shorthorn sculpin	10	26.3 \pm 3.1	8.9 \pm 4.4	209.4 \pm 83.4	1.1 \pm 0.1	14.6 \pm 0.5	3.9
Three-spine stickleback	14	5.4 \pm 0.7	NA	77.8 \pm 36.0	0.8 \pm 0.2	9.5 \pm 1.1	3.3
Sediment ^b							
Churchill River	1			25.2	0.04		
Goose Bay	4			18.0	0.004 \pm 0.003		
Groswater Bay	2			12.7	0.07 \pm 0.002		

^aOn the basis of diet composition.⁵⁹ ^bTotal Hg concentration of sediment is reported as ng/g dry weight.

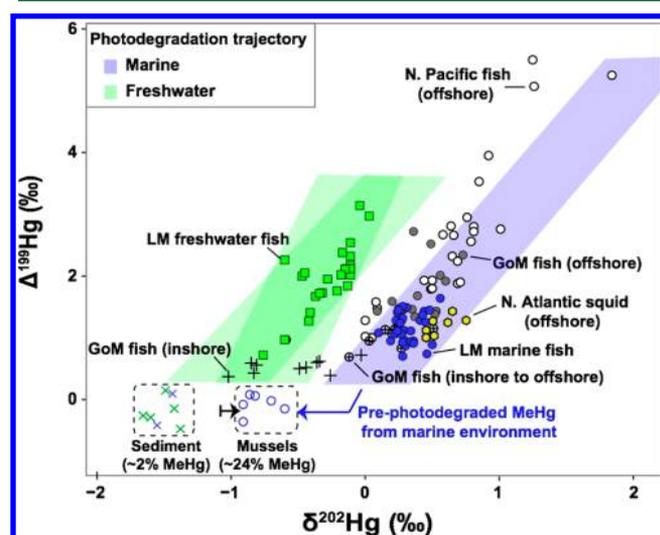


Figure 3. Stable Hg isotope composition of Lake Melville (LM) fish and North Atlantic (N. Atlantic) squid from this study, compared to those harvested from the Gulf of Mexico (GoM)¹⁰ and North Pacific Ocean (N. Pacific).¹² Crosses indicate benthic sediment samples from the river and estuarine river mouth (green) and outer marine regions (blue). Blue and green shaded regions indicate the photodegradation trajectories for MeHg based on experimental data.²⁶ Different slopes in the freshwater environment consider the shifts resulting from 10 mg/L (steeper slope) and 1 mg/L (shallow slope) dissolved organic carbon (DOC) in solution. This range encompasses the mean DOC concentration of 5 mg/L measured in the freshwater regions of Lake Melville.⁵

Blum et al.¹² reported lower $\Delta^{199}\text{Hg}$ values in offshore marine fish species that forage in deeper waters due to lower light penetration and photochemical reaction rates. We find that the simple relationship between $\Delta^{199}\text{Hg}$ and feeding depth for open-ocean fish¹² does not hold for species harvested near ocean margins. For example, Atlantic salmon commonly feed in surface waters of pelagic regions⁵⁹ but their isotopic

composition is similar to that of offshore species that feed at several hundred meters depth (Figure 3). This is likely due to the diversity of processes affecting photochemical degradation in nearshore areas. Lower photochemical degradation rates in surface waters of coastal ecosystems compared to the open ocean are driven by higher suspended particle loads and DOC concentrations that reduce light penetration.^{10,86} Sorption to DOC and particles may also stabilize MeHg, making it more resistant to degradation.⁸⁷

MeHg Photodegradation within Diverse Freshwater Habitats. Species from freshwater environments in this study exhibit a wide range of $\Delta^{199}\text{Hg}$ values (0.72‰ to 3.14‰). Maximum $\Delta^{199}\text{Hg}$ values for freshwater fish observed here are higher than previously reported for biota from small streams (up to ~ 1.5 ‰).^{13,88,89} There is no significant correlation between the fraction of total Hg measured as MeHg and the Hg isotopic composition of freshwater species (Supporting Information, Figure S4). These results contrast with other work showing that the majority of variability in Hg isotopes within food webs can be explained by differences in MeHg content.^{11,13} Variability in $\Delta^{199}\text{Hg}$ values in the freshwater food chain from this study must therefore be driven by differing degrees of MeHg photodegradation in the freshwater environment.

Diverse habitats of freshwater fish in the Lake Melville region likely explain their wide range in $\Delta^{199}\text{Hg}$ values. Photochemical MeHg demethylation will vary depending on light intensity as a function of water turbidity, depth, and canopy.^{12,54,89} The terrain along the Churchill River is diverse with variable water depths and solids loads. Some regions have a wide (>500 m) channel with minimal canopy, contrasting with the field locations of previous studies (small forested stream locations).^{13,89}

Blue Mussels and Sediment Hg Isotopic Composition. Blue mussels have the lowest $\delta^{202}\text{Hg}$ (-0.91 ‰ to -0.60 ‰) and $\Delta^{199}\text{Hg}$ (-0.38 ‰ to 0.08 ‰) values among all species included in this study. Mussel $\Delta^{199}\text{Hg}$ values are similar to

those for benthic sediment, but $\delta^{202}\text{Hg}$ values are on average 0.7‰ higher (Figure 3). We attribute the low Hg isotopic values in mussels to their high Hg^{II} content, which makes up ~80% of their total tissue burden (Table 2). Benthic sediment also contains mainly inorganic Hg (mean 98%, Table 2) and has low Hg isotopic values ($\Delta^{199}\text{Hg}$: -0.33 to 0.16‰; $\delta^{202}\text{Hg}$: -1.34 to -1.64‰). Previous studies have inferred based on results from isotope-mixing models that $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values for Hg^{II} are lower than those of MeHg.^{11,13}

We used an isotope-mixing model (eq 4) to investigate potential sources of Hg^{II} and MeHg in mussels based on Hg isotope ratios measured in mussels, marine sediment, and marine fish.

$$\begin{aligned} & [\delta^{202}\text{Hg}^{\text{II}} \times f_{\text{Hg}^{\text{II}}} + \delta\text{Me}^{202}\text{Hg} \times (1 - f_{\text{MeHg}})] \\ & = \delta^{202}\text{Hg}_{\text{mussel}} \end{aligned} \quad (4)$$

In eq 4, $f_{\text{Hg}^{\text{II}}}$ and f_{MeHg} are the measured fractions of Hg^{II} and MeHg (76% and 24%, respectively). $\delta^{202}\text{Hg}_{\text{mussel}}$ is the measured average $\delta^{202}\text{Hg}$ value for total Hg in mussels (-0.8‰). $\delta^{202}\text{Hg}^{\text{II}}$ and $\delta\text{Me}^{202}\text{Hg}$ represent the $\delta^{202}\text{Hg}$ values of Hg^{II} and MeHg in mussels, respectively.

If marine sediment (average $\delta^{202}\text{Hg} = -1.5‰$) was the primary Hg^{II} exposure source, eq 4 suggests the $\delta^{202}\text{Hg}$ for MeHg would have a value of ~1.4‰. Because this greatly exceeds the $\delta^{202}\text{Hg}$ values for all other fish species (<0.6‰), we infer that bulk sediment is not the primary Hg^{II} source for mussels.

If we assume the MeHg source for mussels is the same as other marine species from Lake Melville (pre-photodegraded MeHg average $\delta^{202}\text{Hg} \approx 0‰$), eq 4 suggests the $\delta^{202}\text{Hg}$ value of Hg^{II} in mussels is approximately -1‰. This is on average 0.5‰ higher than that for bulk sediment (Figure 3), which is again inconsistent with benthic sediment as the main source of Hg^{II} to mussels. We postulate based on these data that Hg^{II} in blue mussels is predominantly derived from settling organic debris for these filter feeders rather than benthic sediment.

Stable Hg Isotopes Indicate Life History of Different Fish Species. Figure 4 shows the Hg, C, and N isotope composition of rainbow smelt (*Osmerus mordax*) across a range of sizes indicating different fish ages. A similar plot for flatfish is shown in the Supporting Information, Figure S5. Mature rainbow smelt are enriched in $\delta^{202}\text{Hg}$, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ relative to juveniles, corresponding to their well-documented migration from the freshwater to marine environment.^{58,59} Higher $\delta^{202}\text{Hg}$ and $\delta^{13}\text{C}$ in adults are consistent with elevated values in the marine environment compared to freshwater regions (Figure 2). The shift in $\delta^{15}\text{N}$ likely results from higher baseline values in the marine ecosystem and potentially a higher trophic level occupied by mature smelt.

Atlantic salmon (*Salmo salar*) migrate between the Churchill River and the offshore marine environment over their life cycle and are exposed to diverse MeHg sources. Aging analysis indicates that Atlantic salmon included in this study spent 1–2 years in the ocean before returning to their freshwater spawning grounds where they previously spent 1–4 years as juveniles (see Supporting Information, Section 3). Despite this relatively long duration spent in the freshwater environment, stable Hg isotopes indicate that the majority of MeHg in Atlantic salmon is derived from the marine environment (Figure 1a and Figure 3). Salmon grow rapidly during an ontogenetic stage in the marine environment and consume large quantities of prey to

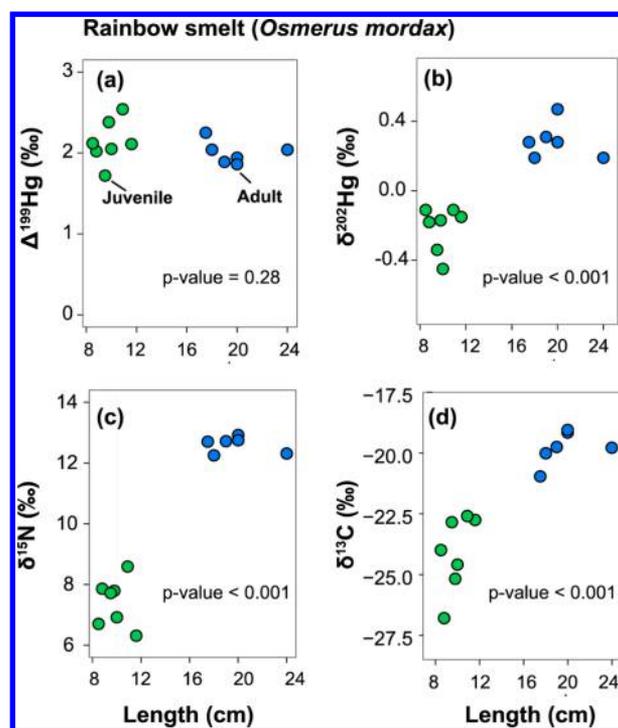


Figure 4. Stable isotope ratios measured in juvenile and adult smelt. Statistically significant differences between ontogenetic stages are denoted by p -values (t test, two-sided).

meet their bioenergetic demands, thus exposing them to large quantities of dietary MeHg with a marine origin.⁹⁰

These examples illustrate that, when combined, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and stable Hg isotopes provide insights into environmental MeHg sources, short- and long-term feeding habitats of fish, and species migration patterns. In this study, we identified two distinct sources of MeHg to freshwater and marine food webs and a notable absence of a benthic MeHg signature in the estuarine ecosystem. Isotopic measurements empirically confirm the results of previous modeling work suggesting that estuarine sediments are not the main source of water column MeHg for some coastal ecosystems.^{9,44,91} We find that stable Hg isotopes are a powerful tool for elucidating the diverse environmental origins of MeHg accumulated in fish within a complex coastal ecosystem.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03206.

Supplemental statistics for Hg isotope ratios, supplemental figures, and photos of salmon scales collected in this study (PDF)

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Notes

The authors declare no competing financial interest.

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