Review of stable mercury isotopes in ecology and biogeochemistry

By: Martin Tsz-Ki Tsui, Joel D. Blum, and Sae Yun Kwon


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Abstract:

Due to the advent of cold vapor-multicollector-inductively coupled plasma mass spectrometry (CV-MC-ICP-MS) in the past two decades, many research groups studying mercury (Hg) biogeochemistry have integrated stable Hg isotopes into their research. Currently, >200 studies using this technique have been published and this has greatly enhanced our understanding of the Hg biogeochemical cycle beyond what Hg concentration and speciation analyses alone can provide. These studies are largely divided into two groups: (i) controlled experiments investigating fractionation of Hg isotopes and refining tools of isotopic analyses, and (ii) studies of natural variations of Hg isotopes. It is now known that Hg isotopes undergo both mass dependent fractionation (MDF; reported as the ratio of mass $^{202}$Hg to $^{198}$Hg) and mass independent fractionation (MIF), with MIF occurring at odd masses ($^{199}$Hg, $^{201}$Hg) to a larger magnitude and at even masses ($^{200}$Hg, $^{204}$Hg) to a much smaller magnitude. The two types of MIF are controlled by different photochemical processes. The range of isotopic variations of MDF, odd-MIF, and even-MIF are now well documented in a diverse set of environmental samples, and researchers are continuing to explore how the field of Hg isotope biogeochemistry can be further developed and taken to the next level of understanding. One application that has received considerable attention is the use of Hg isotopes to examine the environmental controls...
on the production and degradation of methylmercury (MeHg), the most toxic and bioaccumulative form of Hg. Since MeHg is efficiently assimilated and biomagnified along food chains, MeHg has the potential to be a robust ecological tracer. In this review, we give an updated overview of the field of Hg isotopes and focus on how Hg isotopes of MeHg can be used to address fundamental ecological questions, including energy transfer across ecosystem interfaces and as a tracer for animal movements.

**Keywords:** Isotopic fractionation | Methylmercury | Trophic transfer | Energy tracers

**Article:**

1. **Use of isotopes in Hg biogeochemical studies**

1.1. Overview of environmental Hg studies

After about five decades of intensive research on mercury (Hg) in the environment we have learned that this heavy metal is a globally distributed pollutant and has a very complex, but still incompletely understood, biogeochemical cycle (Fitzgerald et al., 2007). Mercury poses a serious health threat to humans and wildlife especially when they are exposed to elevated levels of the neurotoxin monomethylmercury (MeHg) through dietary sources (e.g., fish and rice; Mergler et al., 2007, Feng et al., 2008) or gaseous elemental Hg [GEM or Hg(0)] through occupational exposure (e.g., artisanal gold mining; Tomicic et al., 2011). In environmental Hg research samples are commonly analyzed for the total concentration of Hg (total-Hg or THg) and/or the concentration of specific chemical species of Hg [i.e., gaseous elemental Hg (GEM), as Hg(0); particulate bound Hg, as Hg(II); inorganic oxidized Hg, as Hg(II); monomethyl-Hg, as MeHg; or dimethyl-Hg, as Me2Hg]. This remains the most common data collected in studies of the distribution, transport, fluxes, and pools of Hg in diverse environments including terrestrial, freshwater, and marine ecosystems (Fitzgerald et al., 2007).

As an additional aid to studying the biogeochemical Hg cycle, isotopic measurements have also been applied in many environmental and ecotoxicological studies on Hg. These can be largely divided into five types: (I) the use of radioactive Hg isotopes (e.g., 203Hg), (II) the spiking of enriched stable Hg isotopes (e.g., highly enriched 199Hg, 200Hg or 201Hg), (III) the use of light isotopes to resolve food web complexities (e.g., δ13C, δ15N, δ34S) that can help interpret sources and biomagnification of MeHg, (IV) the analysis of δ13C in the methyl (CH3) group of MeHg, and (V) the measurement of natural abundance isotope ratios of Hg (see their general classification and examples of use in Table 1), with the last type being the major focus of this review.

**Table 1.** Five types of isotopes used in different Hg biogeochemical studies.

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| I       | Radiolabeled Hg isotopes      | 203Hg    | γ-counter       | • Experiments tracing uptake and efflux of Hg in small aquatic organisms (Tsui and Wang, 2004)  
• Experiments quantifying Hg methylation in sediment cores (Gilmour and Riedel, 1995) and periphyton (Cleckner et al., 1999) | Relatively short half-life (~47 days), popular in earlier studies but its use has declined drastically in recent years |

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| II   | Food web resolution with light stable isotopes | $^{13}$C, $^{15}$N, $^{34}$S, D | Elemental analyzer-IR-MS | • Aquatic vs. terrestrial dietary sources as related to different Hg levels (Bartrons et al., 2015)  
• Trophic position estimation for calculating trophic magnification slope for MeHg (Lavoie et al., 2013)  
• Distinguishing freshwater vs. marine based food webs and Hg levels (Fry and Chumchal, 2012) | Widespread use in food web studies investigating sources and trophic positions, to investigate dietary sources for MeHg and its biomagnification |
| III  | Spiking of enriched stable Hg isotopes | $^{199}$Hg, $^{200}$Hg, $^{201}$Hg | Quadrupole ICP-MS | • Methylation and demethylation rates in sediment cores (Hammerschmidt and Fitzgerald, 2006)  
• METAALICUS project to understand large-scale Hg biogeochemical cycling and transport in watersheds (Harris et al., 2007) | Relative rates and source tracking. Spike Hg may be different from native Hg, still popular for methylation/demethylation measurements |
| IV   | Natural abundance stable non-Hg isotopes | $^{13}$C of CH$_3$Hg | GC-C-IRMS | • Understanding the sources of the methyl group that produces MeHg (Masbou et al., 2015) | In its infancy |
| V    | Natural abundance stable Hg isotopes | $^{198}$Hg, $^{199}$Hg, $^{200}$Hg, $^{201}$Hg, $^{202}$Hg, $^{204}$Hg | MC-ICP-MS | • Estimating photodemethylation of MeHg in aquatic ecosystems (Bergquist and Blum, 2007)  
• Distinguishing environmental and dietary MeHg routes to aquatic organisms and humans (Laffont et al., 2009)  
• Delineating the contribution of dry vs. wet deposition of Hg export via streamflow (Woerndle et al., 2018) | Rapidly growing number of applications |

METALLICUS = The Mercury Experiment to Assess Atmospheric Loadings in Canada and the US.  
ICP-MS = Inductively-coupled-plasma mass spectrometry.  
IR-MS = Isotope-ratio mass spectrometry.  
GC-C-IRMS = Gas chromatography-combustion-isotope ratio mass spectrometry  
MC-ICP-MS = Multi-collector inductively-coupled-plasma mass spectrometry.

1.2. Expansion of the field of Hg isotope studies

Due to advancement of multicollector-inductively coupled plasma mass spectrometry (MC-ICP-MS) from the 1990s to the 2000s (Walder and Freedman, 1992, Halliday et al., 1998, Albarède et al., 2004), several research groups in the early 2000s began method development for Hg introduction into MC-ICP-MS instruments in order to allow high precision measurements of Hg isotope ratios. While an early study used quadrupole ICP-MS to analyze Hg isotope ratios in environmental samples (e.g., lake sediments; Jackson, 2001), the precision obtained was criticized for being too low to discern environmental variations (Hintelmann et al., 2001). It should be noted that the natural range of Hg isotope variations is relatively small (e.g., <10‰; Blum et al., 2014), especially compared to many light element isotopes such as δD (e.g., 700‰) and δ$^{13}$C (e.g., 110‰) (Fry, 2006), owing to the much larger percent mass differences among light element isotopes compared to Hg isotopes.
The most important technique developed for high-precision analysis of Hg isotopes was the continuous generation of a cold vapor Hg(0) stream using SnCl₂ as an on-line reducing agent, and introduction of this Hg(0) into the plasma source. Ion beam intensities for the 6 major Hg isotopes (198Hg, 199Hg, 200Hg, 201Hg, 202Hg, and 204Hg; excluding 196Hg due to its very low natural abundance, i.e., 0.16%) are measured simultaneously using Faraday Cup collectors (Blum and Bergquist, 2007). The nomenclature for reporting Hg isotope ratios follows the common practice in isotope geochemistry of using delta (δ) notation in permil (‰) (Blum and Bergquist, 2007), which has been widely adopted within the Hg isotope community beginning in 2007. Measurements of the natural abundance of Hg isotope ratios has received much attention and interest, and an increasing number of research groups around the world, whether originally working on Hg or not, are acquiring MC-ICP-MS instruments and have built or purchased the specialized inlet systems required for Hg isotope measurements. We note that three geographic regions around the world have the majority of established laboratories for high-precision Hg isotope analyses using MC-ICP-MS: North America (United States and Canada), East Asia (e.g., China, Korea, and Japan), and Western Europe (e.g., France and Switzerland). We expect the number of laboratories capable of performing high-precision Hg analysis and the number of geographic regions with these laboratories will increase rapidly in the next decade.

Due to the power of having multiple isotope ratios (Δ₁⁹⁹Hg, Δ₂⁰⁰Hg, Δ₂⁰¹Hg, δ²⁰²Hg, Δ₂⁰⁴Hg; nomenclature to be explained below) and the demonstrated versatility to probe into complexities of Hg cycling in the environment, the number of papers published reporting natural abundance Hg isotope ratios (i.e., technical development, lab/control studies, environmental studies, and review/data synthesis) have increased rapidly, as shown in Fig. 1, where we plot the number of papers published or in press as of October 16, 2019. As noted in Fig. 1, ~72% of published papers (total = 231) are based on analyses of environmental field samples.

![Fig. 1. Number of papers published each year as that are related to stable Hg isotopes, by October 16, 2019. Papers just published or in press are considered in year 2019. The type of paper is mainly based on the main emphasis of each paper, divided into field collection / environmental samples, method development and improvements, lab/control studies of isotopic fractionation of Hg isotopes, and papers on review, data synthesis and model development. Database was derived from Web of Science and Google Scholar.](image-url)
Among almost all published studies, it has become general practice to report mass spectrometry results relative to the primary Hg isotope bracketing standard solution (SRM 3133) and to report results for the secondary Hg isotope standard solution (UM-Almadeń; RM 8610). Both standards are available from the National Institute of Standards and Technology (NIST). The primary Hg isotope standard solution is also available as SRM NIMS-1 (as a dilution from NIST SRM 3133) from the National Research Council of Canada (NRCC) (Meija et al., 2010). In addition, many commonly adopted standard reference materials (SRMs) are available from NIST, NRCC, International Atomic Energy Agency (IAEA), European Reference Materials (ERM), etc. to match a variety of sample matrices (e.g., sediment/soil, plant, animal, ash, etc.). All Hg isotope research groups have routinely measured these matrix-matched SRMs to facilitate quality control/quality assurance, and Hg isotope results for a variety of SRMs were recently compiled by Blum and Johnson (2017).

1.3. Scope of this review

The number of published studies utilizing Hg isotopes has grown far too large to include all of them in this review (Fig. 1). For each topic, we have selected just a few papers that are both recent and relevant to our discussions. Thus, this paper is not meant to provide an exhaustive review of all published studies nor a complete data synthesis. We refer readers to a number of previous reviews and data synthesis papers on Hg isotopes for such compilations (e.g., Bergquist and Blum, 2009, Yin et al., 2010, Blum, 2011, Kritee et al., 2013, Blum et al., 2014, Yin et al., 2014, Sun et al., 2016a, Sun et al., 2016b, Blum and Johnson, 2017, Buchachenko, 2018). In the following sections, we will summarize: i) the main “signatures” of Hg isotope variation discovered to date and the associated biogeochemical processes that lead to them (Section 2), ii) the major ecosystem types that have been studied using Hg isotopes and the major findings from selected papers (Section 3), and iii) the ecological applications of Hg isotopes as demonstrated in previous and ongoing studies (Section 4).

2. Isotopic fractionation of Hg

2.1. Mass-dependent fractionation (MDF)

Similar to the stable isotopes of light elements such as C and N, Hg isotopes undergo kinetic isotope fractionation that is dependent on nuclide mass. MDF of Hg isotopes is defined as given below (following Blum and Bergquist, 2007):

$$\delta^{202}\text{Hg} = \left\{ \left[ \frac{^{202}\text{Hg} / ^{198}\text{Hg}}{^{202}\text{Hg} / ^{198}\text{Hg}}_{\text{sample}} \right] - 1 \right\} \times 1000$$

It has been demonstrated by laboratory studies that many redox reactions involving Hg cause MDF including microbial reduction of Hg(II) (Kritee et al., 2008), dark abiotic reduction of Hg(II) (Zheng and Hintelmann, 2010a), microbial demethylation of MeHg (Kritee et al., 2009), photoreduction of Hg(II) (Bergquist and Blum, 2007, Zheng and Hintelmann, 2009), photodemethylation of MeHg (Bergquist and Blum, 2007), photomicrobial reduction of Hg(II) (Kritee et al., 2018), photomicrobial demethylation of MeHg (Kritee et al., 2018), abiotic dark oxidation of dissolved Hg(0) in the presence of dissolved organic matter (DOM) (Zheng et al.,
dark microbial methylation of Hg(II) (Rodriguez-González et al., 2009, Janssen et al., 2016), binding of aqueous Hg(II) with thiol groups (Wiederhold et al., 2010), sorption of Hg(II) to goethite surfaces (Jiskra et al., 2012), abiotic dark reduction of Hg(II) using SnCl₂, ethylation of Hg(II) using NaBEt₄ (Yang and Sturgeon, 2009), and photooxidation of Hg(0) in the presence of halogens (Sun et al., 2016a, Sun et al., 2016b). However, trophic transfer has been found not to cause significant MDF of Hg(II) or MeHg when it is transferred from the diets to the muscle tissue of fish in controlled feeding experiments (Kwon et al., 2012, Kwon et al., 2013, Feng et al., 2015, Kwon et al., 2016). However, internal metabolic fractionation has been reported to cause MDF of Hg(II) or MeHg mainly in high trophic level animals (e.g., Laffont et al., 2009) when specific tissue types (e.g., human hair) are analyzed for Hg isotopic composition. Thus, there may be Hg isotope fractionation between higher trophic levels, but this is not yet completely understood.

With regards to ecosystem Hg cycling, the foliar uptake of gaseous Hg(0) induces a large-magnitude MDF (~−2.0 to −3.0‰) and this represents one of the largest and most important processes causing MDF (Demers et al., 2013, Enrico et al., 2016, Obrist et al., 2017). Most importantly, this leads to large variations in the MDF of Hg isotopes in natural vegetated ecosystems receiving dry deposition of Hg (e.g., distinct isotopic signatures of δ²⁰²Hg between gaseous Hg(0) and organic matter), which allows tracing of Hg that enters forested ecosystems via atmospheric deposition, which is one of the most important pathways for deposition of atmospheric Hg(0) to the Earth’s surface (St. Louis et al., 2001).

2.2. Mass-independent fractionation (MIF)

Large-magnitude MIF of Hg isotopes has been observed to be associated with photochemical reactions (Bergquist and Blum, 2007, Chandan et al., 2015, Sherman et al., 2010, Zheng and Hintelmann, 2010b). We consider this to represent another important discovery related to Hg isotopes, in addition to the large-magnitude MDF caused by foliar uptake of Hg(0). The MIF of Hg isotopes occurs both in the odd-mass (¹⁹⁹Hg and ²⁰¹Hg) and even-mass (²⁰⁰Hg and ²⁰⁴Hg) isotopes, but with a much larger magnitude for the odd masses. Capital delta values for MIF are calculated as shown below (Blum and Bergquist, 2007):

\[
\Delta^{199}\text{Hg} \approx \delta^{199}\text{Hg}_{\text{measured}} - (\delta^{202}\text{Hg}_{\text{measured}} \times 0.2520)
\]

\[
\Delta^{200}\text{Hg} \approx \delta^{202}\text{Hg}_{\text{measured}} - (\delta^{202}\text{Hg}_{\text{measured}} \times 0.5024)
\]

\[
\Delta^{201}\text{Hg} \approx \delta^{201}\text{Hg}_{\text{measured}} - (\delta^{202}\text{Hg}_{\text{measured}} \times 0.7520)
\]

\[
\Delta^{204}\text{Hg} \approx \delta^{204}\text{Hg}_{\text{measured}} - (\delta^{202}\text{Hg}_{\text{measured}} \times 1.4930)
\]

Large-magnitude odd-MIF (\(\Delta^{199}\text{Hg}\) or \(\Delta^{201}\text{Hg}\)) has been experimentally demonstrated to be caused by photochemical reduction of Hg(II) and photodemethylation of MeHg in the presence of DOM (Bergquist and Blum, 2007, Chandan et al., 2015, Zheng and Hintelmann, 2010b), photochemical reduction of Hg(II) in snow (Sherman et al., 2010), photooxidation of Hg(0) in the presence of halogens (Sun et al., 2016a, Sun et al., 2016b), and recently by photochemical reduction of Hg(II) and photodemethylation of MeHg inside marine phytoplankton cells (Kritee et al., 2018). This odd-MIF is mainly attributed to the magnetic isotope effect (MIE), but odd-MIF can also be caused by the nuclear volume effect (NVE) during processes such as abiotic dark reduction of Hg(II) in the presence of DOM and SnCl₂ causing relatively large odd-MIF (up
to ~1.0‰) (Zheng and Hintelmann, 2010a), binding of Hg(II) to thiol groups which can cause small-magnitude odd-MIF (<0.1‰) (Wiederhold et al., 2010) and volatilization of metallic Hg which can also cause small magnitude odd-MIF (~0.1‰) (Estrade et al., 2009, Ghosh et al., 2013).

The ratio of Δ^{199}Hg/Δ^{201}Hg has been found to be an important diagnostic for reactions involving different Hg species undergoing photochemical transformations (i.e., photoreduction of Hg(II) vs. photodemethylation of MeHg) and of different mechanisms causing MIF (i.e., MIE vs. NVE). Bergquist and Blum (2007) reported Δ^{199}Hg/Δ^{201}Hg of 1.00 for photoreduction of Hg(II) and 1.36 for photodemethylation of MeHg in controlled experiments, with the MIE being the dominant mechanism leading to the MIF signature. Many environmental studies have analyzed samples with predominantly Hg(II) (e.g., foliage, sediment, precipitation) that had Δ^{199}Hg/Δ^{201}Hg close to 1.0 (e.g., Gratz et al., 2010, Demers et al., 2013, Yu et al., 2016, Zheng et al., 2016), but for samples with predominantly MeHg (e.g., biota) Δ^{199}Hg/Δ^{201}Hg is usually somewhat higher than 1.0 (e.g., ~1.15–1.33; Point et al., 2011, Tsui et al., 2012, Blum et al., 2013, Yin et al., 2016). Further, when the odd-MIF is caused by the NVE, it has been found that the Δ^{199}Hg/Δ^{201}Hg ratio equals ~1.54 based on equilibrium experimental data (Wiederhold et al., 2010, Ghosh et al., 2013) and ~1.61 from dark kinetic experimental data (Zheng and Hintelmann, 2010a).

Currently, we do not know the specific mechanism causing MIF of even-mass Hg isotopes, but it has been suggested that the self-shielding effect, which was found to occur in Hg vapor fluorescent light bulbs (Mead et al., 2013), may contribute to even-MIF in the atmosphere (e.g., tropopause; Chen et al., 2012) through processes such as photooxidation of Hg(0) to Hg(II). This may lead to the unique Δ^{200}Hg/Δ^{204}Hg ratios of Hg in atmospheric samples including Hg(0) (GEM) and Hg(II) (precipitation) which have been found to be around ~0.5 (e.g., Δ^{200}Hg/Δ^{204}Hg ratios: ~0.49 in a temperate forest; Demers et al., 2013; ~0.58 at French Pyrenees; Enrico et al., 2016). Further studies are needed to better understand and characterize the mechanisms causing this unique even-MIF signature.

2.3. Use of Hg isotopes in environmental studies

A great strength of Hg isotopes as a tracer is that they commonly undergo both MDF and MIF during transformations in the environment. This essentially yields “dual isotope” (i.e., MDF and odd-MIF) or “triple isotope” (i.e., MDF, odd-MIF, and even-MIF) signatures that can be used to trace Hg in ecosystems, much like the commonly used approach of creating dual isotope plots for δ^{13}C and δ^{15}N in food web isotope studies (Jardine et al., 2006) and other environmental studies that measure two stable isotopes for the same chemical compound such as for PCBs (δ^{13}C and δ^{37}Cl; Drenzek et al., 2002) and nitrate (δ^{15}N and δ^{18}O; Finlay et al., 2007). The majority of Hg isotope studies plot MDF vs. odd-MIF (δ^{202}Hg on x-axis vs. Δ^{199}Hg on y-axis) to reveal isotopic differences in environmental reservoirs, which are similar to other stable isotopes exhibiting both MDF and MIF (e.g., sulfur isotopes plotted as δ^{34}S vs. Δ^{33}S; Han et al., 2017).

Since the main constraint controlling the odd-MIF signature for Hg(II) and MeHg is the availability of sunlight to drive photochemical reactions, this provides unique information on Hg cycling, especially in ecosystems with light attenuation gradients that are caused by i) water
depth in lakes (Gantner et al., 2009, Lepak et al., 2018) and the ocean (Blum et al., 2013, Motta et al., 2019), ii) turbidity from pollution or suspended matter in coastal, lake and river systems (Sherman and Blum, 2013, Yin et al., 2016), and iii) varying canopy density within stream networks and forests (Tsui et al., 2013, Kwon et al., 2015). The majority of environmental samples that have been measured for Hg isotopic composition and that have large magnitude (>0.5‰) odd-MIF have been related to photodemethylation of MeHg, most often revealed in high-trophic level aquatic biota samples that contain predominantly MeHg (e.g., > 90% of THg as MeHg in fish; Bloom, 1992).

Recent investigations have also demonstrated subtle, but measurable, even-MIF in environmental samples containing predominantly Hg(II) or Hg(0) that originated from the atmosphere (e.g., precipitation or GEM; Gratz et al., 2010, Chen et al., 2012, Sherman et al., 2012, Demers et al., 2013, Demers et al., 2015, Sherman et al., 2015a, Wang et al., 2015, Yuan et al., 2015, Enrico et al., 2016). Studies measuring both Δ200Hg and Δ204Hg in atmospheric samples (precipitation and GEM) have shown that these two isotopic signatures are often negatively correlated, with the magnitude of Δ204Hg being about twice as large as Δ200Hg and with the opposite signs (Demers et al., 2013, Enrico et al., 2016). Specifically, correlated positive Δ200Hg and negative Δ204Hg has been found in most precipitation samples measured, but negative Δ200Hg with positive Δ204Hg has been observed in only a few instances. These include: Hg(0) samples in a temperate forest in Wisconsin, USA (precipitation: Δ204Hg = −0.25 ± 0.21‰ and Δ200Hg = 0.18 ± 0.05‰, n = 5; GEM above the forest canopy: Δ200Hg = 0.13 ± 0.05‰ and Δ204Hg = −0.10 ± 0.02‰, n = 3; Demers et al., 2013) and in two peatland systems in mountainous region of French Pyrenees (precipitation: Δ204Hg = −0.31 ± 0.10‰ and Δ200Hg = 0.21 ± 0.04‰, n = 9; GEM at Pic du Midi: Δ204Hg = 0.03 ± 0.10‰ and Δ200Hg = −0.05 ± 0.04‰, n = 10; GEM at Pinet: Δ204Hg = 0.03 ± 0.05‰ and Δ200Hg = −0.05 ± 0.02‰, n = 10; Enrico et al., 2016).

Thus, even-MIF provides a unique way to trace the inputs of atmospheric Hg species into the surface environment, and helps distinguish wet deposited Hg (i.e., precipitation) from dry deposited Hg (GEM through foliar uptake or oxidation of Hg(0) by organic matter) entering aquatic systems, which subsequently become the substrate for MeHg production. For example, slightly positive Δ200Hg (from +0.07 to +0.16‰; n = 23; Lepak et al., 2015) has been observed in pelagic fish (mostly MeHg) from the Laurentian Great Lakes, and their source of MeHg has been linked to Hg(II) derived from wet deposition on the lake surface because precipitation collected in the Great Lakes region has been found to have a comparable magnitude of positive Δ200Hg (e.g., Δ200Hg = 0.16 ± 0.06‰; n = 20; Gratz et al., 2010; Δ200Hg range: 0.21 to 1.24‰, n = 23; Chen et al., 2012; Δ200Hg = 0.18 ± 0.05‰, n = 5; Demers et al., 2013). Interestingly, a recent study in a coastal food web in Bohai Sea, China, showed that the food webs were imprinted with slightly negative Δ200Hg and opposite sign, positive Δ204Hg, with an overall slope of −0.48 ± 0.04 (Meng et al., 2019), similar to those observed in GEM and precipitation (Demers et al., 2013, Enrico et al., 2016). Similar findings as in the Great Lakes have been observed in the open ocean. For example, fish samples collected at various depths of the Northern Pacific Ocean exhibited slightly positive Δ200Hg (from +0.05 to +0.13‰; n = 28; Blum et al., 2013; averaged at +0.07 ± 0.05‰; n = 9; Motta et al., 2019) while precipitation collected at or near Hawaii has been also shown to have slightly positive Δ200Hg of +0.14 ± 0.05‰; n = 7 (Motta et al., 2019). Large lakes and the open ocean can be expected to receive significant inputs of precipitation
Hg(II), which can then become the substrate for subsequent microbial methylation, and the produced MeHg can then be incorporated into the aquatic food webs.

3. Examples of environmental studies using Hg isotopes

3.1. Environments highly contaminated with Hg

Anthropogenic releases of Hg to the environment have dwarfed natural inputs since the industrial revolution and have made it a pollutant of global concern, as evidenced in the historic human Hg poisoning in Minamata, Japan (Harada, 1995). It is well known that point source pollution such as industry and mining activities can result in severe air, soil, and water pollution of Hg (Pacyna and Pacyna, 2002). Many Hg research studies have applied Hg isotope measurements to characterize Hg sources in highly contaminated environments around the world, and have shown that this isotopic tool can offer information not revealed by THg and MeHg concentration analyses alone. This includes

i) specific sources of Hg responsible for contamination,

ii) distribution of contaminant Hg sources in the environment, and

iii) redox processes that may have affected the chemical forms and bioavailability of Hg in the environment.

Thus far, most contaminated systems examined have been aquatic systems with legacy or recent point sources of Hg. These include, but are not limited to, historical Hg discharges from industry into Minamata Bay, Japan (Balogh et al., 2015), mining of Hg in Idrija, Slovenia (Foucher et al., 2009), historical Au and Hg mining inputs to northern California rivers, USA (Donovan et al., 2016a, Donovan et al., 2016b) leading to contamination of San Francisco Bay, California, USA (Donovan et al., 2013, Gehrke et al., 2011a, Gehrke et al., 2011b), artisanal Au mining in French Guiana (Guédron et al., 2018, Goix et al., 2019), Hg mining in southwestern China (Feng et al., 2013, Yin et al., 2013a, Yin et al., 2013b, Li et al., 2017), industrial legacy Hg in the Hackensack River, New Jersey, USA (Reinfelder and Janssen, 2019), Hg derived from a large coal ash spill in Kingston, Tennessee, USA (Bartov et al., 2013), legacy Hg from industrial sources in the South River, Virginia, USA (Washburn et al., 2017, Washburn et al., 2018), industrial sources to East Fork Poplar Creek at Oak Ridge, Tennessee, USA (Donovan et al., 2014, Demers et al., 2018), tracking of Hg from chlor-alkali plants in Russia (Perrot et al., 2010) and Cuba (Feng et al., 2019), and accidental Hg spills in a historical shipwreck (Rua-Ibarz et al., 2016). A few investigations have also examined isotopic ratios of Hg from atmospheric point sources such as coal-fired power plants in Florida, USA (Sherman et al., 2012, Demers et al., 2015) and Inner Mongolia, China (Tang et al., 2017), historical Pb-Zn smelting (Sonke et al., 2010, Ma et al., 2013), and mining regions in southwestern China (Wang et al., 2015).

It should be noted that industrial sources of Hg(II) often have $\delta^{202}$Hg between −1 and 0‰, and in most cases industrial Hg(II) does not exhibit significant odd-MIF (i.e., near-zero odd-MIF). For example, Hg ore from Idrija, Slovenia had $\delta^{202}$Hg close to 0‰ (Idrija ore-black: $\delta^{203}$Hg = 0.23‰; Idrija ore-red: $\delta^{202}$Hg = −0.26‰; no reported MIF$^2$ values; Foucher et al., 2009), cinnabar from Almadén Mine, Spain, had largely negative $\delta^{202}$Hg values (from −0.92 to 0.15‰) and near zero $\Delta^{199}$Hg values (from −0.12 to −0.02‰) (Emslie et al., 2015), legacy Hg from Minamata Bay, Japan had $\delta^{202}$Hg estimated to be −0.67‰ and $\Delta^{199}$Hg to be close to −0.05‰ (Balogh et al., 2015), and phenyl-Hg (derived from the paper industry in Sweden) had $\delta^{202}$Hg ranging from −0.50 to −0.20‰ and $\Delta^{199}$Hg ranging from −0.10 to −0.05‰ (Wiederhold et al.,
In studies of these contaminated sites, it has been a common practice to designate a relatively unpolluted reference site for comparison. The environmental samples collected at reference sites (e.g., surface sediment in rivers upstream of a point source discharge) generally have $\delta^{202}\text{Hg}$ (and sometimes $\Delta^{199}\text{Hg}$) values distinct from industrial sources (e.g., background Hg isotope values often have considerably lower $\delta^{202}\text{Hg}$ values), and one can use a binary mixing model to estimate the relative Hg contribution from industrial sources versus background sediments using $\delta^{202}\text{Hg}$ and/or $\Delta^{199}\text{Hg}$. One can also estimate the Hg isotopic compositions of the point source by plotting $\delta^{202}\text{Hg}$ vs. $1/\text{THg}$ in sediments downstream of point source discharges. Theoretically when $1/\text{THg} \approx 0$, the $\delta^{202}\text{Hg}$ value should represent the isotopic composition of the original point source of Hg (Foucher et al., 2009, Balogh et al., 2015, Feng et al., 2019).

When a small fraction of Hg(II) present in the environment is microbially methylated (e.g., in surface sediment), the isotopic composition of the produced MeHg will be fractionated, first by methylation and then by partial demethylation (microbial and/or photochemical). This imparts MDF and/or odd-MIF (in the case of photodemethylation) in the residual pool of MeHg, which is subjected to biological uptake (Bergquist and Blum, 2007, Kwon et al., 2012). Among studies investigating highly Hg-contaminated systems, not surprisingly the Hg isotopic compositions of MeHg (as measured in biota, especially fish) were significantly different from those of Hg(II) measured in contaminated sediment samples. For example, in a study of San Francisco Bay (California, USA), Gehrke et al. (2011b) measured more positive values of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ in two different species of fish (comprised mostly of MeHg) compared to those in the sediment at each sampling location within the bay. The authors corrected $\delta^{202}\text{Hg}$ values in fish to estimated values prior to photodemethylation of MeHg using the known experimental relationship between $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ (Bergquist and Blum, 2007) and showed that there was a consistent, positive offset from $\delta^{202}\text{Hg}$ of sediment to the “corrected” $\delta^{202}\text{Hg}$ in fish among sites, suggesting that sediment is the main source of Hg(II) for the bioaccumulated MeHg in the local food webs. In contrast, from a study of biota in a river polluted with Hg from Au mining, Donovan et al. (2016a) observed that the “corrected” $\delta^{202}\text{Hg}$ in MeHg from the Yuba River (northern California, USA) was lower than $\delta^{202}\text{Hg}$ in sediment. These authors suggested that such differences represent the environment of flowing water ecosystems as opposed to lentic ecosystems. Thus, it appears that there may be ecosystem attributes (e.g., flowing vs. lentic) that can lead to unique isotopic fractionation of Hg in contaminated environments.

### 3.2. Background environment

Given the high global anthropogenic emissions and long-range atmospheric transport and deposition of Hg, virtually all ecosystems are influenced by both natural and anthropogenic Hg. We regard ecosystems predominantly receiving Hg from the globally mixed atmospheric pool to be “background” even though we acknowledge that the majority of Hg in these ecosystems is derived from anthropogenic sources (Swain et al., 1992, Selin et al., 2008). Compared to highly contaminated environments, Hg cycling in natural ecosystems is less well understood as concentration and speciation analyses do not always offer a clear picture of the various natural sources, depositional pathways, and biogeochemical processes affecting Hg. Additionally, background ecosystems can be difficult to study due to the analytical challenges of low Hg concentrations (e.g., trace quantities of MeHg in seawater, living foliage, and precipitation).
There are now almost two hundred of Hg isotope environmental studies investigating Hg biogeochemical processes around the globe. These can be classified as forested and non-forested (e.g., wetlands and Arctic tundra) terrestrial ecosystems, and freshwater and marine aquatic ecosystems. However, there are at least two obstacles to using Hg isotope measurements in comparison to Hg concentration and speciation analyses alone. First, it requires a considerable amount of time and expense to process samples for Hg isotope analysis, especially if one chooses to use thermal combustion followed by matrix clean-up (Blum and Johnson, 2017). There have, however, been some recent efforts to develop methods to reduce the processing time (e.g., Janssen et al., 2019a). Second is the amount of Hg required for high-precision analysis by MC-ICP-MS; this is especially a problem when measuring precious samples with limited Hg levels in background ecosystems. In our experience at the University of Michigan laboratory, it typically requires at least 5–10 ng of Hg for a high-precision measurement (over 24 individual measurements), while there are a few other laboratories that typically use a minimum of ~4 ng of Hg for high-precision measurements (R. Yin and S. Janssen, personal communications). In comparison, as little as ~0.05 ng of Hg is needed for THg concentration analysis using CVAFS.

The precision of Hg isotope measurement decreases sharply with the decreasing Hg concentration of run solutions (e.g., from 5 ng/g to 0.5 ng/g; Tsui et al., 2013); thus measuring the isotopic composition of samples with very low Hg concentration or small sample mass can compromise data precision. To solve these issues, recent studies have developed preconcentration methods for surface water samples with low Hg levels using ion-exchange columns (e.g., Strok et al., 2014, Washburn et al., 2019), purge and trap techniques (e.g., Lin et al., 2015, Woerndle et al., 2018), and ultrafiltration (e.g., Jiskra et al., 2017). In addition, a recent study attempted to circumvent this issue by adding a Hg standard with known isotopic compositions into such samples with limited Hg (i.e., seawater) and used a binary mixing model to determine the actual sample Hg isotope compositions (Huang et al., 2019).

3.2.1. Terrestrial ecosystems

Among different terrestrial ecosystems, forests are a net sink for atmospheric Hg due to the foliar uptake of gaseous Hg(0) (Ericksen et al., 2003, Jiskra et al., 2018). Forested ecosystems typically receive much higher atmospheric deposition (dry and wet) than non-forested areas (wet only) (St. Louis et al., 2001). Interestingly, foliar uptake followed by oxidation of GEM [from Hg(0) to Hg(II)] has been shown to induce a significant MDF in deciduous forests (Demers et al., 2013), evergreen forests (Yuan et al., 2019), and Arctic tundra (Obrist et al., 2017). Specifically, gaseous Hg(0) is thought to be oxidized by reduced thiol ligands in foliar tissues (e.g., δ^{202}Hg from ~0‰ in gaseous samples to about −2.0‰ in foliage; Demers et al., 2013). Obrist et al. (2017) also showed that Hg(0) oxidation by tundra vegetation caused large-magnitude MDF, while Douglas and Blum (2019) recently reported a similar fractionation of δ^{202}Hg between Hg(0) and Hg(II) in the Arctic snowpack. These oxidation processes may be analogous to the oxidation of Hg(0) by DOM in the aqueous environment (Zheng et al., 2012, Zheng et al., 2013). The large-magnitude MDF that occurs during foliar uptake enhances the power of Hg isotopes in studies of forested ecosystems as there are often multiple distinct isotopic endmembers including foliage/litter Hg(II), soil Hg(II), precipitation Hg(II), and gaseous Hg(0) in a single ecosystem (e.g., a temperate forest; Demers et al., 2013) (see Fig. 2).
Fig. 2. Range of reported Hg isotope values in forested ecosystems and/or non-contaminated sites in the continental United States, including foliage and litter samples in a forest in Wisconsin (Demers et al., 2013) and multiple forests across the U.S. (Zheng et al., 2016), streamwater in a small catchment in northern Minnesota (Woerndle et al., 2018), forest floor and mineral soil in a forest in Wisconsin (Demers et al., 2013), precipitation in the Midwest (Gratz et al., 2010), Florida (Sherman et al., 2012), and a forest in Wisconsin (Demers et al., 2013), and gaseous elemental Hg (GEM) in the Midwest (Gratz et al., 2010) and a forest in Wisconsin (Demers et al., 2013).

Dry deposited Hg, defined as Hg sequestered by foliage and also found in fresh litter, is characterized by a more negative $\delta^{202}\text{Hg}$ (from $-3$ to $-2\%_o$) while wet deposited Hg (except from point sources which can have highly variable and very negative $\delta^{202}\text{Hg}$; Sherman et al., 2012) usually has near zero $\delta^{202}\text{Hg}$ (e.g., Gratz et al., 2010, Demers et al., 2013, Enrico et al., 2016). Recent studies monitoring the export of Hg via streamflow in forested ecosystems have also shown that the majority of Hg exported was derived from previously dry deposited Hg in the forested watersheds, as their isotopic signatures resemble those of foliage/litter and forest floor rather than direct precipitation (see Fig. 2) (Jiskra et al., 2017, Woerndle et al., 2018).

There are several recent Hg isotope studies that provide new insight into Hg cycling in forested ecosystems. Among them, Yuan et al. (2019) showed that $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ decrease (and $\text{THg}$ increases) as foliage matures, and the authors suggested that foliar Hg(II) is photoreduced and Hg(0) is emitted from the interior of leaves by sunlight. This raises interesting questions regarding the Hg photochemical processes that occur within leaf tissue, perhaps similar to the observation of photoreduction of Hg(II) and photodemethylation of MeHg within marine phytoplankton cells recently observed in a laboratory study (Kritee et al., 2018). Along a litter decomposition gradient among multiple temperate forests, Zheng et al. (2016) showed that during litter decomposition $\delta^{202}\text{Hg}$ increased, potentially due to the direct oxidation and sequestration of Hg(0) by the decomposing organic matter. In mineral soil, $\delta^{202}\text{Hg}$ is more positive than that in litter (Demers et al., 2013, Zheng et al., 2016) (Fig. 2) as it represents a
mixing of atmospheric Hg deposition (from litterfall and precipitation) and geogenic sources, while only subtle changes in Δ¹⁹⁹Hg occur vertically in soils (Zheng et al. 2016).

The majority, if not all, of Hg in litter and organic soil is considered to be associated with thiol groups in organic matter (Ravichandran, 2004). Once desorbed from the solid phase (e.g., soil organic matter), Hg associated with DOM can be mobilized by runoff into streams (Grigal, 2002). It is hypothesized that once Hg-DOM is desorbed from the solid phase, Hg does not necessarily dissociate from DOM, and thus MDF may not occur during this desorption step from the solid phase. This is an important assumption and was supported by a study reporting similar δ²⁰²Hg in soil (Hg bound to soil OM) and surface runoff (Hg bound to DOM) in a boreal forest catchment in northern Sweden (Jiskra et al., 2017). Another recent study in an upland-peatland catchment in northern Minnesota (USA) found that Hg in streamflow from a catchment can have significant variation in δ²⁰²Hg (−2.12 to −1.32‰) and less (but significant) variation in Δ¹⁹⁹Hg (−0.35 to −0.12‰) as a function of streamflow over 2 years of monitoring (see the variations of streamwater isotope data in Fig. 2). The authors of that study suggested that the isotopic variation was mainly due to the varying input of wet deposited Hg vs. dry deposited Hg (via foliage) over different seasons as controlled by hydrological conditions (Woerndle et al., 2018).

Recently Hg isotope studies have been carried out in other vegetated terrestrial ecosystems such as the Arctic tundra where the uptake of Hg(0) is highly enhanced during the short summers in the Arctic. This was estimated via a mass-balance study and Hg isotope analysis showed that ~70% of Hg inputs were derived from dry deposition to the Arctic inland regions (Obrist et al., 2017). The isotopic signatures of Hg in vegetation, organic soil, rock, GEM, and wet deposition in the Arctic tundra (Obrist et al., 2017) were similar to those observed for temperate forest studies (Fig. 2). Interestingly, lichens, being important components of the tundra systems, have sometimes been found to imprint slightly positive odd-MIF on Hg (Blum et al., 2012, Olson et al., 2019) while other studies of lichen have found them to have negative odd-MIF of Hg (Carignan et al., 2009, Estrade et al., 2010, Jimenez-Moreno et al., 2016, Barre et al., 2018) similar to those found in tree foliage and litter (Demers et al., 2013, Zheng et al., 2016). The variable odd-MIF values among different studies of lichens may be attributable to differences in photochemical re-emission of non-thiol bound Hg (Zheng and Hintelmann, 2010b) and mixing between dry-deposited Hg(0) and wet-deposited Hg(II) (of different odd-MIF values) (Gratz et al., 2010, Enrico et al., 2016). Olson et al. (2019) also found that grass species (Tussock grass or Carex aquatic), small vascular plants (Dwarf birch or Betula nana), and lichen species (White lichen or Cetraria islandica, and Brown lichen or Masonhalea richardsonnii) had slightly positive Δ²⁰⁰Hg in the Arctic, which may be caused by wet Hg(II) deposition. Clearly, the relative importance of atmospheric Hg species is different among the Arctic tundra and forested ecosystems, and is in need for further investigation.

3.2.2. Freshwater environment

Among different aquatic ecosystems, freshwater environments including lakes, reservoirs, wetlands, streams, and rivers have been widely studied for Hg cycling due to their extensive contamination by both point and non-point sources (e.g., Wiener et al., 2006, Brigham et al., 2009). Analyzing stable Hg isotopes in these ecosystems has provided new insight into the transport, transformation, and bioaccumulation of Hg (e.g., Blum et al., 2014). Also, many
studies of forested watersheds focus on both the landscapes (i.e., forest canopy and floor) and the adjacent water bodies (e.g., Kwon et al., 2015, Jiskra et al., 2017, Woemidle et al., 2018). Thus far, the majority of Hg isotope studies have mainly sampled two types of environmental media in these freshwater systems, which include sediment and fish (e.g., Gantner et al., 2009, Sherman and Blum, 2013, Feng et al., 2019). These two sample types represent endmembers of Hg speciation, with sediment Hg occurring as almost all Hg(II) and Hg in fish muscle tissue as almost all MeHg.

Isotopic measurements of sediment and fish have provided insights into Hg beyond that gained from concentration and speciation analysis alone. However, there are many limitations if only those media are analyzed, and if they are only analyzed for the Hg isotope composition of total Hg. For instance, only a small fraction of Hg(II) in surface sediment is available for microbial Hg methylation to MeHg (i.e., only 1–5% of THg as reactive Hg(II) in sediment; e.g., Marvin-DiPasquale et al., 2009), and thus it may not be straightforward to relate THg isotopic compositions in bulk sediment to the MeHg isotopic composition measured in biota samples. In fact, there is often a mismatch in $\delta^{202}$Hg observed between sediment and fish, even after accounting for MDF caused by photodemethylation of MeHg (e.g., Gehrke et al., 2011b). Therefore, approaches such as sequential extraction of bioavailable Hg(II) as well as direct isolation of MeHg from sediments are sometimes warranted for isotopic analysis. These methods have, however, thus far only been tested for highly contaminated sediments (e.g., Janssen et al., 2015, Grigg et al., 2018, Qin et al., 2018). There are some current efforts being made to separate MeHg from biological tissues using ion-exchange columns (S. Janssen, personal communications).

It is also often assumed that fish muscle tissues collected from a particular aquatic habitat represent MeHg formed at that location, but in some cases (see following examples) this assumption may not be valid. For example, a Hg isotope study in headwater streams has demonstrated that some fish individuals may acquire terrestrially derived MeHg (from forest invertebrates) when stream productivity is low (Tsui et al., 2014), while a recent study in a river–lake system (Madenjian et al. 2019) has shown that upstream–downstream movement can be significant for fish (e.g., walleye) and their MeHg isotopic composition may be inconsistent with those of other resident fish. Thus, the possibility of fish movement must be considered in the interpretation of fish Hg isotopic signatures.

For biota samples, it can be very useful to collect a suite of samples of members of a freshwater food web in order to determine the isotopic compositions of Hg(II) and MeHg in the specific habitats, since MeHg in fish can sometimes be derived from “external” sources. In particular, we have found that analyzing Hg isotope compositions among invertebrate consumers of different trophic levels and feeding guilds (also resulting in different percent of THg as MeHg, i.e., $\%_{\text{MeHg}}$) can allow quantitative derivation of Hg(II) and MeHg isotopic compositions in the specific habitats through linear regression analysis. Specifically, Tsui et al. (2012) found that Hg(II) isotopic compositions within the food webs of a northern California river (USA) derived using this approach had $\delta^{202}$Hg of $-1.30\%$ and $\Delta^{199}$Hg of $-0.10\%$. The authors found those to be different from the isotopic values found in the terrestrial soil in the surrounding forest ($\delta^{202}$Hg of $-1.54\%$ and $\Delta^{199}$Hg of $-0.27\%$), implying that there may be some modification of this bioavailable Hg(II) once released to the aqueous phase (e.g., microbial processing and/or
photochemical reactions). Kwon et al. (2015) also observed measurable differences in the estimated Hg(II) isotopic composition from the linear regression of biota and bulk sediment in a temperate lake in Michigan, USA. That study found that the estimated Hg(II) isotopic composition converted to MeHg and accumulated by biota was similar to the isotopic composition of terrestrial soil, introduced via runoff. However, we do not know if this pool of bioaccumulated Hg(II) is the same as the pool of Hg(II) in sediment that is bioavailable to methylating microbes, which is an important research question that needs additional investigation.

The inputs of Hg into freshwater ecosystems typically involves direct dry and wet deposition (the latter is especially true over large lake surfaces), and watershed input through suspended solids and DOM. Thus, it is useful to collect different sample types for Hg isotope analysis, which can represent major inputs including gaseous Hg(0), wet precipitation Hg(II) (e.g., Kurz et al, 2019), suspended sediment particles (e.g., Washburn et al. 2019), and DOM-bound Hg in water (e.g., Jiskra et al., 2017, Woerndle et al., 2018). Due to the high binding affinity of Hg with DOM, the analysis of water samples (e.g., streamwater) must involve complete breakdown of DOM and other binding ligands before concentration of Hg for isotopic analysis (e.g., ultracentrifugation, purge and trap, sorption–desorption resin, etc.). For example, Woerndle et al. (2018) recently validated an approach of using an acidic mixture of permanganate/persulfate at elevated temperature (65–90 °C) for the complete breakdown of DOM prior to Hg isotopic analysis. However, one challenge is to concentrate a sufficient amount of Hg from relatively pristine fresh surface water (or seawater) with low THg levels, especially in areas with very low DOM (e.g., THg < 1 ng/L).

With growing numbers of Hg isotope studies of lakes and river/stream systems, a pattern has started to emerge with fish in lakes showing higher values of both $\delta^{202}$Hg and $\Delta^{199}$Hg compared to those in rivers, owing to higher degrees of photodemethylation of MeHg in lakes than in flowing systems (Bergquist and Blum, 2007, Tsui et al., 2013, Lepak et al., 2015, Madenjian et al., 2019). However, such a simple pattern may be restricted to lake systems with high water clarity because lakes receiving high anthropogenic loadings of suspended particulates (high turbidity) can have significantly reduced photodemethylation of MeHg (Sherman and Blum, 2013). In stream networks it has been demonstrated that Hg isotope signatures, especially odd-MIF, can increase with river size due to reduction in canopy cover and accompanying increases in photoreduction of Hg(II) and photodemethylation of MeHg (Tsui et al., 2013). It has recently become evident that in non-point source (forested) vs. point source (urban/industrial) streams fish have contrasting values of $\delta^{202}$Hg. In the northeastern USA forested streams median values for $\delta^{202}$Hg ($n = 7$) are $-0.95$ and $-0.83\%$ for prey and game fish, respectively and for urban/industrial streams median values of $\delta^{202}$Hg ($n = 16$) are $-0.26$ and $-0.38\%$ for prey and game fish, respectively) (Janssen et al., 2019b).

3.2.3. Marine environment

There are an increasing number of Hg isotope studies examining coastal ecosystems that are known to receive large anthropogenic Hg inputs from inland sources; for example the Pearl River Delta in south China (Liu et al., 2011) and the Gulf of Mexico in the southern USA (Senn et al., 2010, Perrot et al., 2019). The latter two studies in the Gulf of Mexico have shown that
there is a gradient of Hg isotope compositions in fish from the Mississippi River delta to the open ocean, as a result of mixing of terrestrial and oceanic Hg and a gradient of turbidity that can drive photodemethylation of MeHg (i.e. the MIF signature; see Section 4.3 on the ecological applications of Hg isotopes). In a study of coastal systems in the northeastern USA, Kwon et al. (2014) demonstrated that in the majority of coastal sites MeHg accumulated in the organisms was likely derived from the local coastal sediments, which is similar to the findings from the more contaminated San Francisco Bay, California, USA (Gehrke et al., 2011b). Interestingly, the MIF signatures associated with MeHg were found to be quite small in this northeastern USA coastal study (e.g., Δ^{199}Hg up to 1.0‰; Kwon et al., 2014) and similar results were found in fish tissue from the coastal areas of the Gulf of Mexico (e.g., Δ^{201}Hg up to 0.6‰; Senn et al., 2010; Δ^{199}Hg up to 0.9‰; Perrot et al., 2019) as well as along the Norwegian coast (e.g., Δ^{199}Hg up to 0.8‰; Rua-Ibarz et al., 2019). This is also similar to other coastal fish samples from the Pearl River estuary, south China (with the majority of fish having Δ^{199}Hg below 0.5‰; Yin et al., 2016) and in the much more contaminated Minamata Bay, Japan (with the majority of fish having Δ^{199}Hg below 1.0‰; Balogh et al., 2015). The source of Hg(II) from which MeHg is produced can also have effects on the Δ^{199}Hg values of MeHg. For example, Hg(II) derived from dry deposition can be imprinted with slightly negative Δ^{199}Hg (Zheng et al., 2016), but Hg(II) from industrial sources can have near-zero Δ^{199}Hg (Wiederhold et al., 2015) while Hg(II) from wet deposition (e.g., into large lakes and ocean) can have variable positive Δ^{199}Hg (Gratz et al., 2010). The large differences in Δ^{199}Hg among ecosystem types suggest that the high turbidity from inland sources can significantly reduce photodemethylation of MeHg largely formed in the local coastal sediments (Hammerschmidt and Fitzgerald, 2006).

The last type of aquatic system that we consider is the open ocean ecosystem, which represents the largest ecosystem on the Earth’s surface but has been studied very little using Hg isotopes. In the last two decades, there have been an increasing number of oceanic Hg studies examining Hg concentration and speciation (e.g., Hammerschmidt and Bowman, 2012, Lamborg et al., 2014). There are only three published studies examining depth profiles of Hg isotopes in the open ocean; one in fish from the north Pacific Ocean (Blum et al., 2013), another in sinking particles and zooplankton (Motta et al., 2019) in the north Pacific Ocean, and another in Pacific bluefin tuna from off the coast of southern California (USA) and their potential prey species from both the eastern and western Pacific Ocean (Madigan et al., 2018). While we will discuss the ecological applications of Hg isotope measurements from these two north Pacific Ocean studies in Section 4.4, each of these studies have demonstrated a strong decline of photodemethylation of MeHg along depth profiles in the open ocean, starting with very high MIF in the fish residing in surface waters (e.g., Δ^{199}Hg up to 5.5‰; Blum et al., 2013), with much lower MIF (e.g., Δ^{199}Hg ~1.0‰; Blum et al., 2013) in fish residing below the photic zone. This is due to the fact that MeHg produced in the photic zone undergoes a high degree of photodemethylation whereas MeHg produced at depth is not as extensively photodemethylated. In the open ocean atmospheric deposition (dry and wet deposition) represents the major input of Hg into the water column (Fitzgerald et al., 2007). There have only been a few studies that examined Hg isotopes in marine top-predatory animals across spatial scales or with depth and they have all been carried out in polar regions including the Arctic (Point et al., 2011, Masbou et al., 2018), the Antarctic (Zheng et al., 2015), and in the Faroe Islands (Li et al. 2014). There is a great need for additional studies to probe the complexities of Hg cycling in oceanic systems using Hg isotopes, especially in other less studied geographic areas.
4. Ecological applications of Hg isotopes

There are many areas of Hg research that are benefiting from the use of stable Hg isotopes to solve or add new insight to longstanding questions. Researchers are also beginning to use stable Hg isotopes to tackle research questions unrelated to Hg; for example offering insight into air-pollution, the history of sea and lake ice-cover, insect movement, organic matter sources, and energy transfers across ecosystem boundaries. Among these, there have been a few recent studies focusing on paleobiology and the relationship between volcanic eruptions and extinction events (e.g., Zheng et al., 2018, Wang et al., 2018, Grasby et al., 2019), which show promise for the use of Hg and Hg isotopes to add new insights into Earth history.

While Hg isotopes can be used to trace biogeochemical reactions that fractionate Hg leading to unique signatures of MDF and MIF (odd- and even-mass), we know that Hg isotopes can also be used to trace the movement of MeHg (as sequestered in animal tissues) from specific sources through food webs and between ecosystem compartments. This is made feasible by the lack of MIF of Hg isotopes during trophic transfer and metabolic processes, and the minimal MDF of Hg isotopes (at least at lower trophic levels) during trophic transfer (Kwon et al., 2012). The degree to which MDF of Hg isotopes occurs at higher trophic levels is in need of further study. In the following sections we highlight several examples where researchers have already observed and utilized the uniqueness of Hg isotopes in providing novel ecological information. However, before doing this we will discuss some of the limitations (and opportunities) of using Hg isotopes in an ecological context.

4.1. Limitations and opportunities of analyzing Hg isotopes of MeHg

Isotopic compositions of MeHg in an ecosystem are often inferred from analyzing organisms at the top of natural food webs, such as the muscle tissue of fish (most common) or the blood of birds. It should be noted that internal fractionation of Hg isotopes (causing MDF) may occur leading to modification of the Hg isotopic composition in some tissues (Kwon et al., 2014, Xu and Wang, 2015), especially for animals of higher trophic levels and samples not analyzed as whole body. Therefore, it is best to measure multiple types of tissues in top predator animals (e.g., fish liver and muscle: Xu and Wang, 2015, Perrot et al., 2019, Rua-Ibarz et al., 2019; e.g., different tissues of marine mammals: Perrot et al., 2016, Masbou et al., 2018) in order to constrain the amount of metabolic fractionation of Hg isotopes, if any. As a well-studied example, Hg in human hair has been observed to have much higher δ²⁰²Hg than the diets of the human subjects (offset ~2‰; Laffont et al., 2009, Sherman et al., 2015b), while fish liver has been shown to often have different MDF (but not MIF) from muscle tissue mainly due to internal demethylation of MeHg (Kwon et al., 2013, Rua-Ibarz et al., 2019).

For many aquatic food webs, fish muscle is a good choice of tissue to analyze for Hg isotopes as it often has high %MeHg (Bloom, 1992) and the Hg isotopic compositions have been found to be closely related to those observed at the lower trophic levels in which the Hg isotopic compositions of whole body invertebrate samples are often also analyzed (Tsui et al., 2012, Kwon et al., 2015), implying that Hg isotope values of MeHg measured in fish muscle tissue may reflect MeHg at lower trophic levels and even the ambient environment (e.g., water); but
this will require further studies to validate. However, %MeHg is not necessarily high among all invertebrate organisms or forage fish, and it largely depends on the trophic position of the organisms (Chasar et al., 2009) as well as the methylation potential (i.e., hotspot) of the local habitats (Mitchell et al., 2008); the latter can be inferred from %MeHg in the water column or sediment porewater (Drott et al., 2008, Mitchell et al., 2008). Thus, one would need to (i) regress the THg isotopic composition against %MeHg to derive the endmember “pure” MeHg isotopic compositions of the food web that is being investigated (e.g., Tsui et al., 2012, Kwon et al., 2015, Donovan et al., 2016a, Meng et al., 2019), or (ii) use a fixed endmember value of Hg(II) and estimate Hg isotope values of MeHg for individual food web samples (Tsui et al., 2012). An alternative approach is to isolate and measure the isotopic composition of MeHg from biota samples without high %MeHg (i.e., well below 90%) using solvent extraction (Masbou et al., 2013) or separation of MeHg by gas chromatography (Epov et al., 2008, Bouchet et al., 2018; Janssen et al., 2015), but this remains technically difficult and has not yet become a commonly adopted approach.

There are advantages to analyzing Hg isotope compositions in lower trophic level organisms rather than top predators (e.g., fish and birds) in some ecosystem types, as it is thought that invertebrate organisms largely acquire MeHg from several different routes (e.g., herbivore, carnivore, and detritivore) and thus their MeHg isotopic compositions (measured or estimated) may reflect different pools of MeHg in the ambient environment (e.g., water, sediment or soil) (Kwon et al., 2014, Tsui et al., 2018). In contrast, there is the potential, although it is poorly quantified, for the top predator animals to acquire MeHg from external habitats such as through large animal movements (Tsui et al., 2018) and feeding on externally supplied diets (Tsui et al., 2014), which may provide unique opportunities to use Hg isotopes for additional ecological applications (see below). However, it should be realized that it remains technically challenging to measure Hg isotopes in lower trophic level invertebrates regardless of the method, due mainly to the low masses of organisms (e.g., smaller size and low abundances) and low tissue THg concentrations.

4.2. Absence of trophic fractionation in lower food webs and potential ecological applications of Hg isotopes

In organic matter, it is widely regarded that Hg(II) and MeHg preferentially bind with thiols and other S-containing compounds due to their high binding affinity constants (e.g., Hg(II)-S2- has a stability binding constant of 10^{52.4}; Ravichandran, 2004). During trophic transfer, MeHg has a high assimilation efficiency (e.g., >90%) and is strongly retained in the tissues of consumers with a long internal half-life, leading to biomagnification of MeHg (Tsui and Wang, 2004). Thus, MeHg is concentrated at the top trophic levels of food webs while Hg(II) “biodiminishes” due to the low assimilation efficiency and the much higher efflux rate than MeHg, leading to the general increase of %MeHg with increasing trophic level in both aquatic (Mason et al., 2000, Chasar et al., 2009) and terrestrial food webs (Tsui et al., 2019). The use of Hg isotopes in high-trophic level biota to reflect the Hg isotope values of MeHg in the ambient environment (e.g., water, sediment, and soil) is supported by the absence of significant trophic fractionation of Hg isotopes of Hg(II) and MeHg in the lower trophic levels of aquatic food webs, as shown by several feeding experiments with fish (Kwon et al., 2012, Kwon et al., 2013, Feng et al., 2015, Kwon et al., 2016). This supports the use of Hg isotopes of MeHg to explore Hg biogeochemical
cycling in the ambient environment and also as a robust tracer of food web trophic transfer. However, the current consensus is that significant MDF of Hg isotopes may occur in the higher trophic levels of food webs, and thus caution is needed in the interpretation of Hg isotope data in animal tissues.

The MDF and MIF of Hg isotopes create a situation where one can use both isotope signatures to trace the sources of organic matter and food web interactions over space, and potentially over time. For example, with respect to space, when MeHg occurs in sunlight illuminated ecosystem compartments, it can acquire significant odd-MIF (resulting in more positive $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ values) and this can be used to trace dietary sources exposed to various light levels. With respect to time, Kwon et al. (2016) assessed the timescale required for a changing MeHg source for both $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ to be reflected in tuna muscle tissues. Also, animals having a large range of movements (e.g., migratory birds and fish) can take up MeHg from one source (contaminated or background) and move into another with a different isotopic signature of MeHg. We have compiled a number of studies (both published and preliminary) that we deem to successfully demonstrate the usefulness of Hg isotope compositions of MeHg in tracing food webs over space, and more importantly we demonstrate their complimentary nature to other commonly used light stable isotope tracers (H, C, N, S; Fry, 2006) in food web ecology. In some cases, we have found that Hg isotopes of MeHg can be a superior tracer to other commonly used light element ecological isotope tracers. Nevertheless we strongly encourage the simultaneous measurements of common food web tracers (e.g., $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) along with Hg isotopes for individual food web samples in order to provide the most useful interpretation of Hg isotope measurements.

4.3. Example I: Coastal and oceanic mixing of dietary sources

Two studies of fish from the Gulf of Mexico revealed significant differences in $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ on a large spatial scale (Senn et al., 2010, Perrot et al., 2019) (Fig. 3A). Specifically, data showed that fish living closer to the mouth of the Mississippi River have lower $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ presumably because they obtain MeHg that has undergone less photodemethylation of MeHg (due to high turbidity). In contrast, fish inhabiting the open ocean far from the mouth of the Mississippi River have higher $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$. Fig. 3B shows the values of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$, which display a clear distinction in isotopic compositions of MeHg between coastal and oceanic species in the Gulf of Mexico, especially for odd-MIF, as this is strongly controlled by water clarity as shown by a lake study (Sherman and Blum, 2013). Consistent with this trend, transitional species all exhibit intermediate $\Delta^{199}\text{Hg}$ values. Compared to $\delta^{13}\text{C}$, which is widely used for tracing energy (carbon) sources (Fry, 2006), there are clearly advantages to using $\Delta^{199}\text{Hg}$ over $\delta^{13}\text{C}$ to trace the spatial locations where fish acquire MeHg (or their diets) in the Gulf of Mexico (Fig. 3C). It is also interesting to note that there is a strong negative relationship between $\Delta^{199}\text{Hg}$ and $\delta^{15}\text{N}$ (Fig. 3D), suggesting that there is a different baseline $\delta^{15}\text{N}$ among coastal and oceanic ecosystems as human sources of N (e.g., agricultural fertilizer) are known to have higher $\delta^{15}\text{N}$ (Cabana and Rasmussen, 1996). Thus, the use of odd-MIF (and MDF) is very useful for identifying the locations where long-range aquatic (e.g., fish and mammals) and terrestrial (e.g., seabirds) animals obtain most of their diets (and MeHg). This is particularly useful in ecosystems with a strong gradient of turbidity and/or sources of MeHg (terrestrial vs. oceanic).
4.4. Example II: Vertical profiling of animal migration

In deep lakes and in the ocean, vertical movements and differences in typical feeding depths by animal consumers are common but not always easy to resolve (Roe, 1974, Pearre, 2003). Blum et al., 2013, Sackett et al., 2017 showed that in the north Pacific Ocean and the coastal Hawaiian Islands, respectively, $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ are both excellent indicators for distinguishing the feeding depth of fish in the oceanic water column (Fig. 4A and B). Sackett et al. (2017) found $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values in Giant Trevally that were inconsistent with their presumed feeding depth and were able to attribute this discrepancy to acquisition of MeHg from terrestrial sources (also supported by different $\delta^{13}\text{C}$) (Fig. 4C). For the two measured light element isotopes (C and N), the authors found somewhat weaker relationships with their mean foraging depth for $\delta^{13}\text{C}$ (Fig. 4C) and essentially no relationship for $\delta^{15}\text{N}$ (Fig. 4D).
Fig. 4. Reported values for (A) δ^{202}Hg (MDF) and (B) Δ^{199}Hg (odd-MIF), (C) δ^{13}C, and (D) δ^{15}N from two marine fish studies on multiple foraging depths in North Pacific Subtropical Gyre (Blum et al., 2013) (shown as O) and multiple species of bottomfish near Hawaiian Islands (Sackett et al., 2017) (shown as ▽). Dashed line represents linear regression analysis (except for Fig. 4D in which the linear regression is not statistically significant), and data on *Caranx ignobilis* (common name: giant trevally) is excluded from regression analysis (inside the red rectangle) because Sackett et al. (2017) demonstrated that this fish species obtained a substantial portion of MeHg from terrestrially derived sources (runoff). For the fish in North Pacific Subtropical Gyre, foraging depth profile data was obtained from Choy et al. (2009). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Similar results were observed in a study of Arctic lakes (Gantner et al., 2009) in which Arctic char feeding on benthic chironomids (Δ^{199}Hg: 0.32–1.31‰) exhibited two times lower MIF values compared to those measured in pelagic zooplankton (Δ^{199}Hg: 1.51–3.40‰) in these lakes. However, the exact %MeHg in these intermediate trophic organisms was not reported so we do not know if these Hg isotope signatures are directly comparable among fish and invertebrates (i.e., whether they are mainly MeHg).

We suggest that the same principles can be applied to the study of other “deep” ecological systems such as extensive riverine floodplains and hyporheic zones (e.g., Flathead and Tobacco rivers, Montana; Stanford and Gaufin, 1974). In these cases, Hg isotopes may serve as a unique tracer of surface exposure of groundwater (MeHg gaining positive odd-MIF) and the tracing of organic matter can be assisted with odd-MIF signatures, which are insensitive to non-photochemical reactions (e.g., dark microbial Hg transformations; Kritee et al., 2013). However, it should be noted that non-photochemical reduction of Hg(II) may occur in the presence of organic matter without light and this may imprint Hg with MIF signatures, mediated through NVE (Zheng and Hintelmann, 2010a). Thus it is important to examine diagnostic markers such as the ratio of Δ^{201}Hg/Δ^{199}Hg to ascertain whether the MIF was caused by MIE or NVE (Blum et al., 2014).

4.5. Example III: Spatial variations within stream networks
Another important ecological research area that can be addressed using Hg isotopes is that of spatial food web interactions within stream networks. Hg isotopes are a welcome addition to the methods used in this research area due to the complexity of the system including upstream–downstream movement of animal consumers (e.g., fish migration; upstream drift of emerged insects) and large, yet poorly characterized, exchanges among food webs along land–water interfaces (Nakano and Murakami, 2001, Baxter et al., 2005). In a study of a northern California (USA) stream network, Tsui et al., 2013, Tsui et al., 2014 found that Hg isotope signatures of MeHg are highly dependent on the stream network position for different aquatic consumers such as macroinvertebrates (stream insect larvae of different functional feeding groups) and fish. They found that the MIF signature is mediated by the effect of canopy cover on in-stream photodemethylation of MeHg (Tsui et al., 2013). Additionally, in small headwater streams it is observed that fish consumers can acquire MeHg from adjacent terrestrial diet items with different MeHg isotopic compositions from their aquatic counterparts (Tsui et al., 2014).

As shown in Fig. 5A, it is apparent that δ¹³C generally increases for different consumers along a stream size gradient. This is due mainly to the water velocity effect on C isotope fractionation in autotrophs (Finlay et al., 1999), but there is a large mismatch between fish (steelhead trout) and other macroinvertebrates due to the fact that fish obtain most of their energy sources from the pool habitat while the macroinvertebrates mainly feed in riffles (Finlay et al., 2002). For another potential energy tracer, δD (Fig. 5B), it was also observed that there was a large mismatch between macroinvertebrates and fish, suggesting that fish may obtain H differently than macroinvertebrates (e.g., water; Solomon et al., 2009). However, we found for both δ²⁰²Hg (Fig. 5C) and Δ¹⁹⁹Hg (Fig. 5D) that both groups of consumers are similar among sites and more importantly that they show systematic variations with stream size (expressed as watershed area) as expected for δ¹³C and δD (Finlay et al., 1999, Finlay et al., 2010). Thus, Hg isotopes of MeHg are shown to be a robust spatial identifier of animal consumer positions in a complex stream network, regardless of specific habitat locations such as whether the animal consumers are residing mainly in riffles vs. pools, which can be easily distinguished by δ¹³C due to differing water velocity effects (Finlay et al., 2002).

In the same stream network, Tsui et al. (2014) observed that some steelhead trout individuals obtain terrestrial sources of MeHg in small tributaries, because their Hg isotope signatures of MeHg are very different from the local stream insect larvae. Thus, even such subtle differences can be demonstrated using Hg isotopes, and this conclusion could not be reached with C, N, or D isotope tracers in this case. In a downstream river location of the study watershed, Tsui et al. (2012) also found that two riparian spider species had Hg isotope ratios of MeHg intermediate between river and forest food webs. They also found that neither C, N, nor D isotopes could provide adequate resolving power, providing solid evidence that Hg isotopes of MeHg can be complementary to light stable isotope tracers and can even be superior tracers under some circumstances.
Fig. 5. Reported values of (A) $\delta^{13}$C, (B) $\delta$D, (C) $\delta^{202}$Hg (MDF), and (D) $\Delta^{199}$Hg (odd-MIF) in macroinvertebrates of different feeding guilds (whole body from multiple individuals) (Tsui et al., 2013) and juvenile steelhead trout (fillet only) (Tsui et al., 2014) in streams of different sizes (expressed as watershed area) in a forested watershed in northern California.

Another potential, yet mostly unexplored, aspect of Hg isotopes is the study of upstream–downstream movement of animal consumers. In the same northern California watershed discussed above, Tsui et al. (unpublished data) found that emerged adult mayfly (*Ephemerella maculata*) collected in a small tributary (drainage area ~2 km$^2$) had whole body $\Delta^{199}$Hg values that were higher than expected for this small stream, which has high tree canopy cover. The $\Delta^{199}$Hg values of mayfly were 2.07 and 2.76‰ for two pooled samples, but this was before correction for MeHg content, and so the actual $\Delta^{199}$Hg for their MeHg may be even higher (Tsui et al., unpublished data). In this tributary, other stream insect larvae had $\Delta^{199}$Hg values of MeHg from 0.82 to 1.50‰, suggesting that these emerged adult flies likely migrated from further downstream locations where there is lower tree canopy cover density (Tsui et al., 2013). This
result is consistent with observations of adult mayfly migration upstream after emergence observed in an Arctic river (Hershey et al., 1993) and adult stonefly upstream migration in a temperate forest catchment (Macneale et al., 2004). In Fig. 6, we illustrate and summarize the ecological applications of Hg isotopes of MeHg, corresponding to both applications above on coastal and open ocean (Fig. 6A) as well as to application in stream networks (Fig. 6B).

Fig. 6. Illustration on different ecological applications of Hg isotopes in (A) large surface lake or ocean (spatial and depth differences) and (B) complex forested watersheds (land–water interactions, and upstream–downstream movement) as discussed in the paper. Note: Diagrams were not drawn to scale.

4.6. Limitations and future development of Hg isotopes as energy tracers

As demonstrated above, there are many potential applications of stable Hg isotopes as energy tracers, either complimentary to other light isotope tracers or as superior stand-alone tracers. However, it appears that odd-MIF, predominantly caused by photochemical reactions, is the main reason that Hg isotopes can be used effectively as robust energy tracers because the MIF
signatures are not altered by dark or abiotic secondary processes. Even-MIF is often of very small magnitude in ambient samples (e.g., rainfall) and once incorporated into food webs, the even-MIF magnitude is often very small ($\Delta^{200}\text{Hg} \sim 0.2\%\text{oo}$ or less; Lepak et al., 2015, Motta et al., 2019; $\Delta^{208}\text{Hg} \sim 0.2\%\text{oo}$; Meng et al., 2019). Thus, the utility of even-MIF as an ecological tracer is unlikely to yield a precise quantitative resolution compared to $\Delta^{199}\text{Hg}$ or $\Delta^{201}\text{Hg}$ unless the precision of analytical measurements is greatly improved. There are many potential areas for further development of Hg isotopes as ecological tracers, in particular the study of intracellular/in vivo MIF and applications of Hg isotopes in studies of human health. Here we identify six research areas that we believe show promise for future applications.

1) Phytoplankton with light-transparent bodies have been shown to produce MIF in intracellular Hg (Kritee et al., 2018). This is an understudied area, and depending on its magnitude, it could affect our interpretation of MIF signatures observed in top predators (e.g., piscivorous fish). Our current paradigm is that fish Hg isotope values of MeHg reflect the isotope values of MeHg in the ambient water or sediments, which is almost certainly correct for MIF but may not be true for MDF. If intracellular fractionation turns out to be extensive it could change that interpretation of MIF as well. Additionally, we need to ascertain whether other animal consumers with transparent bodies living in the photic zone of the ocean and lakes, such as copepods and Daphnia, produce intracellular MIF. These processes should be studied in controlled experiments such as those carried out by Kritee et al. (2018) for phytoplankton.

2) Internal MDF has been observed for some fish and mammalian species for Hg isotopes, and can be significant (Ma et al., 2018, Rua-Ibarz et al., 2019). While it is clear that there is an absence of MIF produced in these organisms, small increases in MDF have been observed even in animals (Kwon et al., 2013, Kwon et al., 2016). Studying Hg isotopes in different organs within the bodies of animals as well as for different species of Hg can offer insight into the complicated processing and redistribution of Hg within animal bodies, especially through excretion (if it is a rate-limiting step) and internal demethylation of MeHg (e.g., fish liver; Rua-Ibarz et al., 2019). While many studies have focused on the internal dynamics of MeHg, in highly contaminated ecosystems the exposure and bioaccumulation of Hg(II) may be significant enough to cause health impacts (e.g., in artisanal gold mining; Tomicic et al., 2001). For long-lived organisms, there is a need for additional study of Hg turnover rates in various tissues (Kwon et al., 2016), along the lines of previous studies that have observed organ/tissue-specific turnover rates for $\delta^{13}\text{C}$ isotopes (e.g., Hobson and Clark, 1992).

3) Animal movements can be substantial and hard to discern in nature and this is an important research area in ecology. Most Hg and Hg isotope studies have assumed that the food web items collected in the same system have direct trophic linkages. However, this is not always the case and even for headwater streams surrounded by forests there can be complex trophic exchanges between land and water (Nakano and Murakami, 2001). For animals that have a wide range of movement, we suggest that Hg isotopes can uniquely offer insights into their feeding sources and pathways. For example, Tsui et al. (2018) found that in a small watershed at Acadia National Park, Maine, USA, there was a wide range of Hg isotope values in blood from different songbird species caught at the same location, which could not be accounted for by the range of Hg isotope compositions observed among the locally caught invertebrates (aquatic and terrestrial). This
suggests that songbirds moved into the studied area from other areas with contrasting Hg isotopic compositions of their food sources.

4) Additional study of isotope fractionation of Hg in terrestrial food webs would benefit from further research. Tsui et al. (2012) provided the first demonstration of the Hg isotope signature of a forest floor food web in a northern California (USA) watershed and showed a large contrast in both $\delta^{202}$Hg and $\Delta^{199}$Hg compared to the adjacent river food web. Ongoing research (Tsui et al., *unpublished data*) has also demonstrated that in other temperate forest ecosystems certain groups of invertebrate species (e.g., moths) often have distinct and much higher odd-MIF values than their counterparts collected in the same forest habitat. Moths mainly feed on detrital materials in temperate forests, which often contain Hg with slightly negative odd-MIF values (Demers et al., 2013, Zheng et al., 2016; Tsui et al., *unpublished data*). Thus their higher, positive $\Delta^{199}$Hg values are puzzling and prompt us to suggest further investigation of their physiology and specific diets, which can help better define the different routes of Hg(II) and MeHg entering the base of forest food webs.

5) So far, based on a few studies at lower trophic levels in aquatic food webs, we have not observed significant MDF (within analytical error) of MeHg among these biota (basal resources, invertebrates, and fish) in the same habitat (i.e., with direct trophic relationships) including a river study (Tsui et al., 2012) and a coastal study (Kwon et al. 2014). However, in order to better understand the MeHg production and trophic transfer at lower trophic levels, it is important to develop a better approach to examine isotopic compositions of MeHg in these basal resources and invertebrate consumers. Since these biota samples often have lower THg concentrations and much lower %MeHg compared to most fish species (Chasar et al., 2009), we recommend further studies designed to optimize isolation of MeHg from these biological materials for high-precision Hg isotope analysis. Approaches using solvent extraction, gas chromatography and ion exchange chromatography are potential solutions (e.g., Masbou et al., 2013, Janssen et al., 2015, Bouchet et al., 2018, Grigg et al., 2018, Qin et al., 2018) for biological samples, especially those with lower MeHg levels.

6) Paleoecology may potentially benefit from examining Hg isotope compositions in archived sediment and fossil records. For example, Zheng et al. (2018) showed different Hg isotope compositions (odd-MIF) for sedimentary rocks deposited under oxic vs. H2S-rich euxinic conditions, which may have important implications for Earth history and ancient biological evolution. In another study, Grasby et al. (2017) used Hg isotopes to distinguish the ancient inputs of Hg into deep and nearshore marine environments, as evidenced by the different odd-MIF compositions due to volcanic inputs and biomass/terrestrial sources. Interestingly, Zheng et al. (2015) used Hg isotopes to show that penguin and seal feces were the dominant sources of Hg to the sediments over time in Ross Sea, Antarctica.

The topics listed above are just a few examples of research areas that may result in the more extensive use of Hg isotopes for ecological and Earth history applications, and for understanding of the complexities of Hg cycling in natural food webs. As with all areas of Hg isotope research, the only limit to the application of Hg isotopes is the imaginations of researchers in the field, and we hope that our discussions here will help to open more avenues for Hg isotope research, and that it will further develop and be expanded in the following decades.
Declaration of Competing Interest

The authors declare no conflict of interest.

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