Isotope Fractionation from *In Vivo* Methylmercury Detoxification in Waterbirds

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Abstract

The robust application of stable mercury (Hg) isotopes for mercury source apportionment and risk assessment necessitates the understanding of mass-dependent fractionation (MDF) due to internal transformations within organisms. Here, we used high energy-resolution XANES spectroscopy and isotope ratios of total mercury (δ^{202}THg) and methylmercury (δ^{202}MeHg) to elucidate the chemical speciation of Hg and the resultant MDF due to internal MeHg demethylation in waterbirds. In three waterbirds (Clark's grebe, Forster's tern, south polar skua), between 17-86% of the MeHg was demethylated to inorganic mercury (iHg) species primarily in the liver and kidneys as Hg-tetraselenolate ($Hg(Sec)_4$) and minor Hg-dithiolate ($Hg(SR)_2$) complexes. Tissular differences between δ^{202}THg and δ^{202}MeHg correlated linearly with %iHg ($Hg(Sec)_4$ + $Hg(SR)_2$), and were interpreted to reflect a kinetic

isotope effect during *in vivo* MeHg demethylation. The product-reactant isotopic enrichment factor ($\varepsilon_{p/r}$) for the demethylation of MeHg → $Hg(Sec)_4$ was −2.2 ± 0.1‰. δ^{202}MeHg values were unvarying within each bird regardless of $Hg(Sec)_4$ abundance, indicating fast internal cycling or replenishment of MeHg relative to demethylation. Our findings document a universal selenium-dependent demethylation reaction in birds, provide new insights on the internal transformations and cycling of MeHg and $Hg(Sec)_4$, and allow for mathematical correction of δ^{202}THg values due to the MeHg → $Hg(Sec)_4$ reaction.

Keywords: Mercury, demethylation, isotopes, MDF, birds

Introduction

Mercury (Hg) is a neurotoxin that impacts the health of aquatic and terrestrial animals worldwide.[1] Higher trophic level organisms (e.g., birds, fish, mammals) are exposed to methylmercury (MeHg) through dietary sources, which is assimilated in the digestive tract, circulated in the bloodstream, and retained in the protein of tissues as a MeHg-cysteine complex (MeHg-Cys).[2–4] The toxicological risks of MeHg to aquatic and terrestrial organisms are governed by *in vivo* transformations, inter-tissular exchanges, and depuration rates and pathways of MeHg and other biologically-relevant forms of mercury.[1] In birds, MeHg can be demethylated in the liver,[5,6] depurated into feathers during molt or to offspring by maternal transfer,[7] and excreted.[1] Stable isotope ratios of mercury are a central tool for ecologic risk assessment and mercury source apportionment to organisms,[8–14] yet critical questions remain on the isotopic fractionation of mercury by *in vivo* transformations.

The *in vivo* demethylation of MeHg induces mass-dependent fractionation (MDF) of mercury isotopes (denoted by δ^{202}Hg) as reported in birds,[6] fish,[15] and mammals.[9,16–18] The development[19,20] and

application[16,17] of methods for species-specific mercury isotope ratio measurements show promise for determining the effect of *in vivo* transformations on mercury isotope ratios. However, a chief barrier is quantifying the chemical speciation of inorganic Hg (iHg) with high precision in natural tissues. High energy-resolution X-ray absorption near-edge structure (HR-XANES) spectroscopy can identify and quantify mixtures of biologically-relevant mercury species at sub-parts-per-million concentration.[3,4,21–23] Recent application of HR-XANES in terrestrial bird and freshwater fish tissues revealed that MeHg-Cys is detoxified to a Hg-tetraselenolate ($Hg(Sec)_4$) complex, likely by selenoprotein P (SelP).[4] $Hg(Sec)_4$ was shown to be the organic precursor to nanoparticulate HgSe,[4] which has been observed with $Hg(Sec)_4$ by HR-XANES in marine birds[22] and by normal resolution XANES in birds and mammals.[24–26] Linking the chemical speciation of iHg (that indicate specific internal reactions) and MDF of stable mercury isotopes is needed to inform on the internal transformations and trafficking of mercury.

Here, tissues and feathers of piscivorous waterbirds from lacustrine (Clark's grebe, *Aechmophorus clarkia*),[4,27] estuarine (Forster's tern, *Sterna forsteri forsteri*),[28] and marine (south polar skua, *Stercorarius maccormicki*) environments were measured for mercury speciation by HR-XANES spectroscopy and species-specific isotope ratios. Previous research indicates internal MeHg demethylation in these birds.[4,5] Study goals were to determine the product-reactant isotopic enrichment factor ($\varepsilon_{p/r}$) for the *in vivo* detoxification of MeHg to $Hg(Sec)_4$ and investigate the internal cycling of biologically-relevant mercury species. The findings are discussed in context of MeHg detoxification in vertebrates and implications of *in vivo* MDF of mercury isotopes on environmental isotope applications.

Materials and Methods

Biologic Tissues

Tissues and feathers from three birds were analyzed including a Clark's grebe (*A. clarkia*; adult male) from Lake Berryessa (California, USA; collected September 11, 2012), a Forster's tern (*S. forsteri forsteri*; adult female) from the south San Francisco Bay (California, USA; collected June 13, 2018), and a south polar skua (*S. maccormicki;* adult female) from Cape Hallett located in the northern Victoria Land coast of the Ross Sea (Antarctica; collected November 22, 2016). The Clark's grebe and Forster's tern were necropsied to obtain the following tissues: breast feather, brain, pectoral muscle, kidneys, and liver. The south polar skua was necropsied to obtain the muscle, kidneys, and liver. Tissues were lyophilized and homogenized. Clark's grebe tissues were analyzed previously for mercury speciation by HR-XANES and mercury and selenium association with selenoproteins.[4]

HR-XANES Measurements

HR-XANES spectra of the Clark's grebe tissues are published[4,29] and were measured identically during the same experimental session on the Forster's tern and south polar skua samples. The south polar skua kidney tissue was not measured by HR-XANES. Complete details are provided in the SI. Briefly, mercury L_3-edge HR-XANES spectra were measured on freeze-dried samples with high-reflectivity analyzer crystals[30] (beamline ID26, European Synchrotron Radiation Facility). Proportions of Hg were quantified using least-squares fitting of data with linear combinations of diverse reference spectra.[4,21,22] The reference spectrum of MeHg-Cys was represented using the Clark's grebe breast feather spectrum, which was suitable based on a spectral comparison to previously analyzed biological samples with exclusively MeHg-Cys.[4] The spectrum of $Hg(Sec)_4$ was determined by iterative

transformation factor analysis.[4] The spectrum of Hg-dithiolate ($Hg(SR)_2$) complex in biota[3] was represented using Hg(L-glutathione)$_2$ at pH 7.4.[31,32]

Chemical and Isotope Analyses

Details on chemical and isotope measurements are provided in the SI. Briefly, tissues and feathers were measured for total mercury (THg), MeHg, and total selenium concentration.[33] Stable mercury isotope ratios were measured on THg acid digests[11,12] and resin separated MeHg fractions[20] of all samples from the Clark's grebe and Forster's tern. For the south polar skua, THg isotope ratios were measured on all tissues (muscle, kidneys, and liver) and on the MeHg fraction of the kidneys. Isotope analyses were performed using a multi-collector inductively coupled plasma mass spectrometer following established protocols[34,35] on material previously exposed to the X-ray beam for HR-XANES analysis, which had no effect on mercury isotope ratios (Figure S1). Delta values of MDF and mass independent fractionation (MIF) are expressed as $\delta^{XXX}Hg$ and $\Delta^{XXX}Hg$, respectively, in reference to NIST 3133. Isotopic data on certified reference materials and standards are provided in the SI (Table S1).

Results and Discussion

Mercury Speciation in Tissues

The Hg L_3-edge HR-XANES spectra from the three birds show distinct and consistent shifts among tissues that are diagnostic of differences in mercury speciation (Figure 1). The Clark's grebe tissues exhibit the most dramatic differences with mercury present as 100% MeHg-Cys in the brain (indicated by the sharp near-edge peak at 12,279.8 eV unique to MeHg-Cys)[23,36] and a progressive decrease in the amplitude of the near-edge MeHg-Cys peak in the muscle, kidneys, and liver spectra. As detailed

previously,[4] spectral shifts in the Clark's grebe tissues are due to an increasing percentage of mercury as $Hg(Sec)_4$ (0%, 11%, 59%, and 86% in brain, muscle, kidneys, and liver, respectively; Table 1, Table S2). A minor component of Hg-dithiolate complex ($Hg(SR)_2$) is observed in the muscle (23%) and kidneys (12%) (Table 1). In the Forster's tern, mercury is present solely as MeHg-Cys in the brain and muscle (100% MeHg-Cys), and the kidneys and liver exhibit increasing proportions of mercury as $Hg(Sec)_4$ (85% MeHg-Cys + 15% $Hg(Sec)_4$ and 75% MeHg-Cys + 25% $Hg(Sec)_4$, respectively; Table 1). Similarly, the south polar skua shows comparable differences between muscle (100% MeHg-Cys) and liver tissues (83% MeHg-Cys + 17% $Hg(Sec)_4$). There is no spectroscopic evidence for nanoparticulate HgSe, as observed in southern giant petrel by HR-XANES.[22] All tissues were modeled with high precision (Table S2) due to excellent species resolution of HR-XANES (e.g., see reference spectra in Figure 1). Good agreement is observed between %MeHg-Cys measured by HR-XANES and %MeHg measured by chemical measurements (Figure S2), consistent with a previous comparison.[4]

The iHg speciation correlates to the THg concentration between bird tissues. For the Clark's grebe and Forster's tern, THg concentrations of tissues (muscle, kidneys, liver) were normalized to that of the brain, which exhibited 100% MeHg-Cys. A robust positive correlation is observed between $\%Hg(Sec)_4$ and the relative THg concentration of each tissue to the brain (Figure S3). Molar concentrations of Se to Hg as $Hg(Sec)_4$ (Se:$Hg(Sec)_4$ ratio) are >4 in the Forster's tern kidneys and liver and south polar skua liver, consistent with the spectroscopic evidence that tissues contain $Hg(Sec)_4$ (Figure S4). The Clark's grebe kidneys and liver tissues exhibit $1 < Se:Hg(Sec)_4 < 4$, suggesting the co-presence of mononuclear $Hg(Sec)_4$ complexes and disordered $Hg_x(Se,Sec)_y$ clusters.[4]

Mass-Dependent Fractionation via Biotic Demethylation

Mercury isotope ratios showed clear evidence for MDF in tissues that have a mixture of MeHg-Cys and iHg species ($Hg(Sec)_4$, $Hg(SR)_2$) (Figure 2a; Table 1, Tables S3-S4). Tissular differences between δ^{202}THg and δ^{202}MeHg linearly correlated with the %MeHg-Cys (and hence 100-%iHg), as determined by HR-XANES (Figure 2a), suggesting that variation in δ^{202}THg is the result of mixing of two isotope endmembers (δ^{202}MeHg and δ^{202}iHg). For the Clark's grebe and Forster's tern, δ^{202}THg and δ^{202}MeHg were measured on each tissue (Table 1). For the south polar skua, δ^{202}THg was measured on the muscle and liver and both δ^{202}THg and δ^{202}MeHg were measured on the kidneys. The south polar skua kidneys δ^{202}MeHg (1.25 ± 0.02‰) matched the muscle δ^{202}THg (δ^{202}THg = 1.25 ± 0.10‰; 100% MeHg-Cys), and therefore was representative of δ^{202}MeHg in the muscle and liver. Differences between δ^{202}THg and δ^{202}MeHg were greatest in the Clark's grebe tissues (δ^{202}THg - δ^{202}MeHg = -1.90‰, -1.55‰, and -0.91‰ for the liver, kidneys, and muscle, respectively) followed by Foster's tern liver and kidneys tissues (−0.59‰ and −0.28‰, respectively) and the south polar skua liver (−0.45‰). In the Forster's tern, a modest difference was observed between δ^{202}THg and δ^{202}MeHg values of the muscle (0.22‰) despite no evidence of demethylation, and the δ^{202}MeHg of the liver was within 0.08‰ of the δ^{202}THg of the muscle. Although the Clark's grebe muscle and kidneys contained varying proportions of $Hg(SR)_2$ and $Hg(Sec)_4$, δ^{202}Hg values were consistently light compared to δ^{202}MeHg and statistically aligns with the regression line between δ^{202}THg– δ^{202}MeHg versus %MeHg-Cys (Figure 2a, Figure S5) of tissues where $Hg(Sec)_4$ is the dominant iHg species. Therefore, δ^{202}iHg is considered representative of the dominant $Hg(Sec)_4$ species.

Within each bird, variations of Δ^{199}Hg values and Δ^{199}Hg/Δ^{201}Hg ratios for both the THg and the MeHg fractions were largely within measurement precision regardless of mercury speciation (Figure 2b; Table 1; Figure S6), consistent with previous observations of the absence of MIF during internal partitioning and transformations of Hg within organisms.[6,9,17,18] Uniformity in Δ^{199}Hg and slope of

$\Delta^{199}Hg/\Delta^{201}Hg$ between the MeHg and iHg species indicates photochemical demethylation occurs within the food web prior to dietary assimilation of MeHg and likely reflect the bird's prey habitat and foraging behavior.[13,14,37,38]

We interpret isotopic differences between MeHg and $Hg(Sec)_4$ to be the result of a kinetic isotope effect during the *in vivo* demethylation of MeHg → $Hg(Sec)_4$. δ^{202}MeHg exhibited little variation within the Clark's grebe (−0.05 ± 0.18‰, average ± standard deviation, *n*=4) and Forster's tern (0.49 ± 0.14‰, *n*=4) regardless of differences in mercury speciation (Table 1, Figure 2b). Therefore, the isotopic fractionation of mercury in the birds behaved as an open system with an infinite reservoir of reactant (i.e., MeHg). Assuming a unidirectional reaction and instantaneous product,[39] the product-reactant isotopic enrichment factor ($\varepsilon_{Hg(Sec)4/MeHg}$) was determined as the y-intercept of the linear regression between δ^{202}THg– δ^{202}MeHg versus %MeHg-Cys ($\varepsilon_{Hg(Sec)4/MeHg}$ = −2.2 ± 0.1‰; slope ± 95% confidence intervals of fit; Figure 2a). The linear regression weighted each data point to measurement uncertainties.[40] MDF of mercury likely occurs during demethylation of MeHg to $Hg(Sec)_4$, likely by SelP. SelP is rich in selenocysteine residues (*n*≥10 for vertebrates)[4,41] that can facilitate MeHg demethylation[42,43] and was associated with $Hg(Sec)_4$ in the Clark's grebe tissues.[4] Notably, the Clark's grebe kidneys and muscle tissues contained $Hg(SR)_2$ along with $Hg(Sec)_4$. Consistent MDF of tissues that contain $Hg(SR)_2$ and $Hg(Sec)_4$ and those with only $Hg(Sec)_4$ support that $Hg(SR)_2$ is also a byproduct of *in vivo* demethylation of MeHg (Figures 2a, S5), though through an unknown pathway.

We report the first isotopic enrichment factor for *in vivo* demethylation of MeHg by selenium in vertebrates. The magnitude of $\varepsilon_{Hg(Sec)4/MeHg}$ in birds (−2.2 ± 0.1‰) is similar to isotopic differences observed in a range of aquatic mammals (detailed below) but markedly greater than the microbial *mer* pathway ($\varepsilon_{p/r}$ = −0.40 ± 0.20‰).[44] In mammal tissues where species-specific isotopic ratios were determined (δ^{202}MeHg and δ^{202}THg),[16,17] differences between δ^{202}MeHg and δ^{202}iHg pools were estimated to range from −2.1 to ~−3‰ (beluga whale and freshwater seal[16] muscle versus liver, and

pilot whale brain tissues).[17] In a study where only δ^{202}THg was measured,[18] the maximum difference in δ^{202}THg between muscle (~100% MeHg) and liver (~6% MeHg) in juvenile pilot whales was ~−2.3‰. Consistent MDF by MeHg demethylation across birds and mammals could be explained by a universal reaction mechanism involving SelP,[4] which is central to selenium homeostasis.[41] A more detailed comparison of $\varepsilon_{Hg(Sec)4/MeHg}$ to isotope measurements of other birds,[6] fish,[15,19,20] or mammals[9,16–18] would require species-specific isotope ratios and HR-XANES speciation, and knowledge of possible isotope effects from poorly understood processes (e.g., biomineralization of nanoparticulate HgSe from $Hg(Sec)_4$).[22,24] The expression of selenoproteins and insertion efficiency of selenocysteine residues during protein translation can vary between organisms, between tissues, and based on selenium availability,[41,45] and may influence the extent of MeHg demethylation across different organisms[6] and associated isotopic fractionation in environments that differ in selenium availability. Future research efforts are needed to evaluate the mechanisms and isotopic fractionation for MeHg demethylation by SelP, other selenoproteins,[46] and low molecular weight selenium-containing molecule[47], and quantify the variation in $\varepsilon_{Hg(Sec)4/MeHg}$ across diverse organisms and environmental settings (e.g., terrestrial versus marine).

Complementary spectroscopic and isotopic findings shed new light on the toxicokinetics of mercury in birds. Regarding MeHg, tissular δ^{202}MeHg values were not influenced by the local kinetic isotopic effect for the MeHg → $Hg(Sec)_4$ reaction (Figure 2b, Table 1) as would be predicted in a closed system. This observation likely reflects the fast internal cycling of MeHg relative to the demethylation reaction, consistent with observations in birds[6] and marine mammals,[16,17] and dilution of residual heavy δ^{202}MeHg with new dietary MeHg. The δ^{202}MeHg values of the Clark's grebe and Forster's tern feathers, which fingerprint blood mercury isotope ratios during feather growth,[48] were within the narrow range of tissular δ^{202}MeHg values (Table 1). Internal exchange of MeHg leading to uniform δ^{202}MeHg in the birds

is consistent with the dynamic nature of MeHg levels in birds due to physiological (e.g., molting, age) and environmental factors (e.g., dietary exposure).[10,27,28,49]

Regarding the toxicokinetics of $Hg(Sec)_4$, correlation between tissular concentrations of THg and $\%Hg(Sec)_4$ (Figure S3) indicates that $Hg(Sec)_4$ is depurated considerably slower than MeHg, consistent with observations between fish muscle versus liver.[4] It is unclear if $Hg(Sec)_4$ and $Hg(SR)_2$ in non-hepatic tissues were demethylated locally or are the result of inter-tissular exchange. Inter-tissular exchange of $Hg(Sec)_4$ or $Hg(SR)_2$ cannot be discounted, has been proposed in birds[6,22] and mammals,[16–18] and is represented in toxicokinetic models,[50] but there is a lack of mechanistic studies in nature. More broadly, *in vivo* demethylation of MeHg has been attributed to positive MDF between dietary MeHg and organism MeHg.[9,17,38,51,52] Quantifying the contribution of MeHg → $Hg(Sec)_4$ or $Hg(SR)_2$ on MDF between dietary and organism MeHg cannot be carried out here and necessitates an improved mechanistic understanding of isotopic fractionation from additional processes (e.g., ligand exchange,[53] $Hg(Sec)_4$ → nanoparticulate HgSe biomineralization).[22] Toxicokinetic models for mercury in birds[54] and mammals[50] will benefit from advancements from emerging techniques described here and elsewhere[4,20,22] that provide a foundation to understand the transformations and redistribution of biologically-relevant mercury species (MeHg, $Hg(SR)_2$, $Hg(Sec)_4$, nanoparticulate HgSe).

Implications on Environmental Applications of Stable Mercury Isotope Ratios

This study demonstrates significant MDF of mercury in bird tissues due to the demethylation of MeHg to primarily $Hg(Sec)_4$.[4] δ^{202}MeHg values were relatively unaffected by MeHg demethylation and therefore direct measurement of δ^{202}MeHg on tissues[20] is recommended for use of δ^{202}Hg for contaminant source apportionment[8–11] in higher-trophic level organisms and on liver or kidney tissues that are not predominantly MeHg.[1,6,9,17,18] It is unknown if isotopically light products of *in vivo*

demethylation ($Hg(Sec)_4$ and $Hg(SR)_2$) are transferred within foodwebs (e.g., scavenging of high trophic level organisms at the base of foodwebs).[13,20] Where direct isotopic analysis of the MeHg pool is not feasible, mathematical correction of δ^{202}THg using $\varepsilon_{Hg(Sec)4/MeHg}$ may be warranted in determining the isotopic composition of dietary MeHg sources prior to *in vivo* demethylation. When applying the $\varepsilon_{Hg(Sec)4/MeHg}$ (−2.2 ± 0.1‰), spectroscopic characterization of tissues is encouraged under two scenarios. First, in tissues with high %MeHg (e.g., >80%), HR-XANES analysis should be used to accurately quantify %MeHg due to incomplete recovery of MeHg using traditional chemical techniques (Figure S2 in SI, Figure S4 in Manceau et al. 2021,[4] Figure S2 in Bolea-Fernandez et al. 2019).[18] Second, in tissues with low %MeHg (e.g., <30%), HR-XANES analysis is necessary to detect co-occurrence of $Hg(Sec)_4$ and nanoparticulate HgSe.[22] It remains unknown if the biomineralization of nanoparticulate HgSe from $Hg(Sec)_4$ induces positive or negative MDF based on observation in marine bird[6] and mammal tissues with very low %MeHg.[16–18,24]

Supporting Information

Descriptions of measurements; mercury isotope ratios for CRMs, standards, and samples (Table S1, S3, and S4; Figure S1 and S6); HR-XANES spectra fit results (Table S2); comparison of %MeHg by HR-XANES and chemical analysis (Figure S2); correlations between THg concentration and iHg speciation (Figure S3); ratios of Se to Hg (Figure S4); comparison of isotope versus $\%Hg(Sec)_4$ by HR-XANES results (Figure S5) (PDF). HR-XANES spectra (XLXS).

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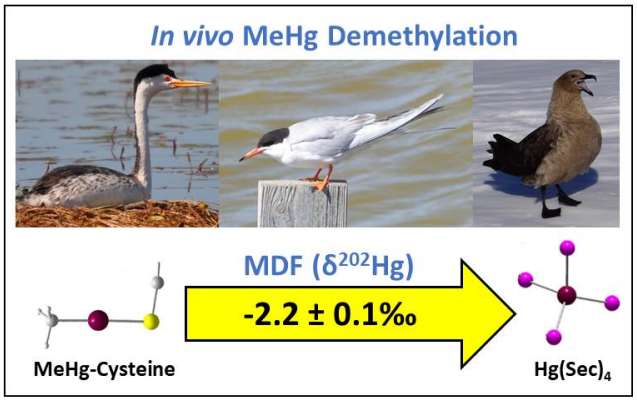

Figures and Tables

Table 1. Chemical, spectroscopic, and isotopic data of bird tissue and feather samples.

	--------- Chemical Meas. ---------[a]			---------- HR-XANES fit results ----------[b]			---------- Species-specific isotope ratios ----------			
Tissue	**THg**	**MeHg**	**Se**	**%MeHg-Cys**	**%Hg(Sec)$_4$**	**%Hg(SR)$_2$**	**δ^{202}THg**	**Δ^{199}THg**	**δ^{202}MeHg**	**Δ^{199}MeHg**
	(mg/kg)	**(mg/kg)**	**(mg/kg)**				**(±1SD)**	**(±1SD)**	**(±1SD)**	**(±1SD)**
Clark's grebe (*A. clarkii*)										
Brain	3.18	2.98	1.55	100	0	0	0.07 (0.03)	1.51 (0.03)	0.06 (0.04)	1.49 (0.02)
Muscle	7.10	3.71	2.31	66	11	23	-1.13 (0.04)	1.46 (0.04)	-0.22 (0.03)	1.43 (0.02)
Kidneys	21.6	6.38	10.6	28	59	12	-1.41 (0.03)	1.42 (0.04)	0.14 (0.02)	1.45 (0.03)
Liver	43.1	7.86	19.3	14	86	0	-2.07 (0.04)	1.49 (0.04)	-0.17 (0.02)	1.42 (0.02)
Breast Feather	41.4	32.7	1.04	100	0	0	0.15 (0.03)	1.77 (0.02)	0.13 (0.05)	2.04 (0.03)
Forster's tern (*S. forsteri*)										
Brain	5.28	4.48	3.31	100	0	0	0.51 (0.02)	0.68 (0.02)	0.57 (0.01)	0.81 (0.02)
Muscle	6.39	5.65	3.33	100	0	0	0.53 (0.03)	0.72 (0.03)	0.31 (0.02)	0.79 (0.02)
Kidneys	12.6	9.21	9.64	85	15	0	0.34 (0.02)	0.70 (0.03)	0.62 (0.03)	0.66 (0.02)
Liver	13.8	9.22	6.21	75	25	0	-0.14 (0.02)	0.67 (0.03)	0.45 (0.04)	0.71 (0.08)
Breast Feather	28.6	18.2	1.41	100	0	0	0.70 (0.03)	1.65 (0.04)	0.72 (0.02)	1.81 (0.04)
south polar skua (*S. maccormicki*)										
Muscle	1.75	1.39	19.4	100	0	0	1.25 (0.05)	1.99 (0.03)	--	--
Kidneys	8.61	4.56	--	--	--	--	0.17 (0.03)	2.00 (0.05)	1.25 (0.01)	1.92 (0.01)
Liver	8.19	6.39	29.7	83	17	0	0.80 (0.01)	2.02 (0.04)	--	--

[a]reported on a dry weight basis.
[b]precision of fit results are 5% for the Clark's grebe tissues[4] and 10% for Forster's tern and south polar skua tissues (see SI Table S2).

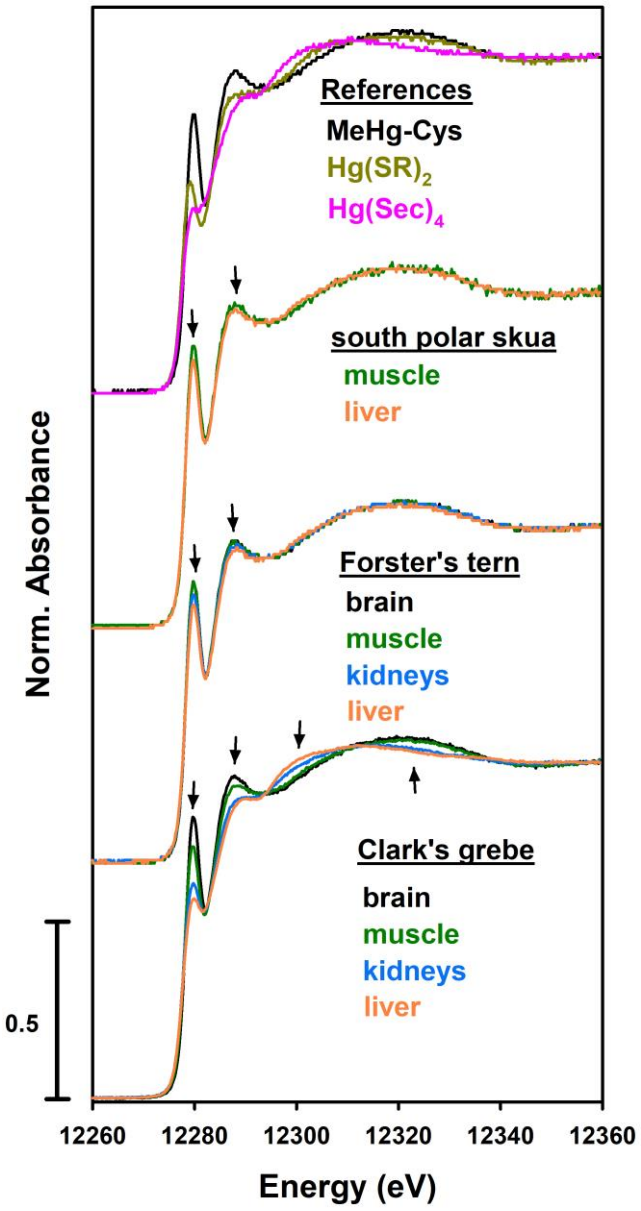

Figure 1. Hg L_3-edge HR-XANES spectra of tissues from a Clark's grebe, Forster's tern, and south polar skua (brain, black; muscle, green; kidneys, blue; liver, orange). Black arrows identify regions of the spectra that differ with shifts in mercury speciation primarily in the proportion of MeHg-Cys and $Hg(Sec)_4$. References spectra are shown for the three species observed in the tissues (MeHg-Cys; $Hg(SR)_2$; $Hg(Sec)_4$).

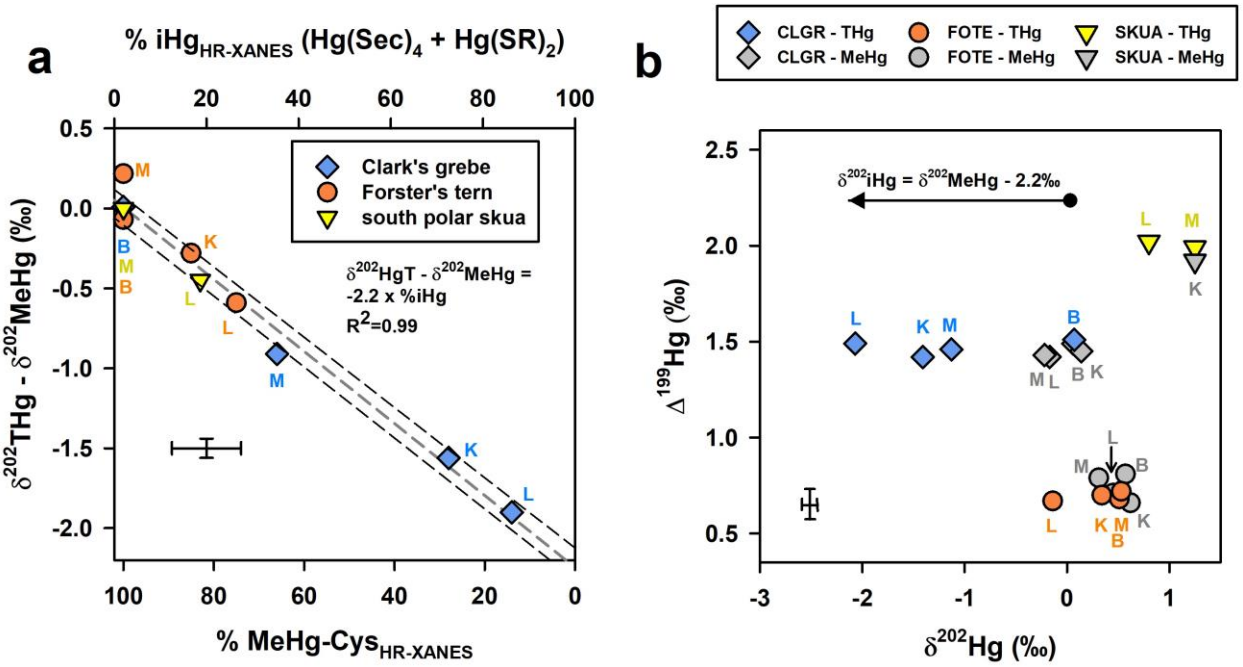

Figure 2. (**a**) Relationship between the difference in δ^{202}Hg values of total mercury minus methylmercury (δ^{202}THg – δ^{202}MeHg; Table 1) and the speciation of mercury as determined by HR-XANES of bird tissues; data are weighted to uncertainties of both X and Y variables. (**b**) Biplot of Δ^{199}Hg versus δ^{202}Hg of total mercury (THg; color-filled symbols) and methylmercury (MeHg; gray-filled symbols) for bird tissues. Single letters abbreviations identify the tissue type (B, brain; K, kidneys; L, liver; M, muscle). Generic error bars present uncertainties of isotope measurements (2SD) and HR-XANES fits (Table S2). In plot **a** the dashed gray and black lines present the fit of data and 95% confidence interval of the fit, respectively.