New York State Energy Research and Development Authority

Mercury Stable Isotope Analysis in Long Island and Adirondack Songbirds

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Mercury Stable Isotope Analysis in Long Island and Adirondack Songbirds

Final Draft

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Keywords

Songbirds, mercury isotopes, mercury origins, Long Island, Adirondack Mountains

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Executive Summary

Using archival data from past NYSERDA songbird mercury (Hg) monitoring projects, we explored the hypothesis that Hg source types are different across New York State and can be differentiated using Hg isotopes, a relatively new analytical technique. Songbird blood samples were collected to compare Hg isotope ratios from tidal marsh and pine barren habitat on Long Island, and forested wetland and upland forest habitat in the Adirondack Mountains.

We found a large amount of variation in Hg isotope ratios that generally corresponded with habitat type. Isotope signatures from the tidal marsh habitat suggest that Hg in this area is derived from different sources than all the other habitats. Tidal marsh isotopes appeared more similar to the expected signatures of industrial effluent while the other habitats were more similar to expected signatures from coal utility boilers. However, a more focused study is needed to confirm this hypothesis.

This study is the first attempt to differentiate Hg sources in songbirds using Hg stable isotopes and also the first study using Hg stable isotopes in New York State. This methodology shows promise in identifying and differentiating sources of Hg as well as Hg methylation pathways across a wide range of New York habitats.

1 Introduction

Atmospherically deposited mercury (Hg) is a contaminant that has adverse effects on wildlife (Scheuhammer et al. 2007) and can have local, regional, continental, and global origins. Mercury is released into the atmosphere by burning fossil fuels, garbage, and other materials (e.g., via coal-fired power plants, mining facilities, and municipal incinerators), then travels on the prevailing winds, and is deposited in distant habitats (Driscoll et al. 2007). Microorganisms primarily residing in soil and sediment convert inorganic Hg into the more biologically toxic methylmercury (MeHg; Ullrich et al. 2001). These microorganisms also interact with local sources of Hg that are not necessarily deposited via the atmosphere and can originate from various industrial sources (e.g., chlor-alkali plants and wastewater treatment plants). Thus, elevated Hg in an ecosystem can be a result of many different sources, each with different management or mitigation strategies. As MeHg is a global concern for wildlife and human health, finding effective means to monitor ecosystems and determine sources of MeHg is crucial to assessing risk.

An emerging technique that quantifies the natural variation of Hg isotope ratios in environmental samples give new insight into the origin of Hg in the environment and ecosystem food webs (Berquist and Blum 2007, Blum 2011, Tsui et al. 2012). A recent Hg isotope study found it feasible to distinguish sources of Hg in precipitation from local (a coal-fired power plant) vs. global (well mixed, distant sources) pools (Sherman et al. 2012). Further, another study in San Francisco Bay showed that even after being deposited in the environment and methylated the resultant MeHg in the food webs could be traced to two different sources of industrial Hg pollution (Gerkhe et al. 2011). These examples, along with others, indicate that source-typing of Hg in the environment is becoming feasible with this new approach. This method is an important new tool for distinguishing different sources of Hg and evaluating its potential for methylation and entering the food webs.

To achieve accurate source-typing and effectively monitor a wide variety of ecosystems, strong indicators of Hg levels and Hg origins must be determined. Most wildlife Hg studies to date have focused on top predators like fish-eating birds because Hg bioaccumulation to high levels was first documented in their tissues (Scheuhammer et al. 2007, Evers et al. 2008). Despite this trend, emerging research is demonstrating that songbirds consuming other types of aquatic and terrestrial invertebrates can accumulate Hg at concentrations similar to or greater than fish-eating birds (Evers et al. 2005, Cristol et al. 2008, Jackson et al. 2011a). Because much of the methylation of Hg across the landscape is thought to occur in wetlands that do not have fish, songbirds are particularly useful for determining risk to Hg pollution in smaller waterbodies and aquatic/terrestrial interfaces (Ullrich et al. 2001). Moreover, songbirds may be at even greater risk to Hg toxicity because they are potentially more sensitive to the toxicological effects of Hg, including endocrine and immune system disruption and reproductive impairment (Heinz et al. 2009, Jackson et al. 2011b). The complicating factor for using this species

group is their diverse foraging habitats and poorly described food webs in comparison to piscivores. Thus Hg source-typing is illuminating when used with songbirds because it more clearly defines the local and global origins of their Hg (via aquatic or terrestrial food webs) exposure.

The Mercury and Air Toxics Standard (MATS) rule, which was passed by the U.S. Environmental Protection Agency (EPA) on December 16, 2011, is expected to reduce Hg emissions from coal-fired power plants by approximately 90% over the next few years. However, global Hg levels are still expected to increase due in large part to anthropogenic emissions associated with subsequent long-range atmospheric transport and deposition of fossil fuels burned in Asia (Lindberg et al. 2007). Thus the amount of Hg introduced into a given ecosystem will be dependent upon the proportion that is global versus regional or local point-source. By documenting changes in both atmospheric Hg deposition amounts and sources, the United States can make more informed decisions for managing Hg risk in ecosystems and human populations.

In this study, we compare Hg stable isotope values among songbirds captured at two locations in New York State: the Adirondack Mountains and Long Island. Within each of these ecosystems, we collected samples from two different habitat types. In the Adirondacks, we sampled birds in bog wetlands and upland forests and on Long Island we sampled birds in tidal marsh and the pine barrens. Our primary hypothesis was the source of Hg is different between the two regions. Specifically, a local source of Hg is suspected in the tidal marsh habitat, which is close to an industrial incinerator. We also expected to observe differences in Hg isotope ratios among species because of differences in diet and movement. This study is the first attempt to differentiate Hg sources in songbirds using Hg stable isotopes and also the first study using Hg stable isotopes in New York State.

2 Methods

2.1 Study Sites

Two primary locations were selected for this research: the Adirondack Mountains and Long Island. In the Adirondacks, we sampled Bloomingdale Bog, Madawaska Flow, Massawepie Mire, and Spring Pond Bog (Figure 1). Bloomingdale Bog and Massawepie Mire were relatively dry bog and surrounding upland forest and Madawaska Flow and Spring Pond Bog were much larger wetland complexes with more water. On Long Island, we sampled at Hempstead and Riverhead pine barrens. Hempstead is series of mesic tidal marshes and the Riverhead pine barrens are dry, open forest (Figure 1). All data used in this study are archival and the original objectives of the associated projects were to quantify Hg levels in songbird species.

Figure 1. Songbird Capture Sites in Adirondacks and on Long Island

2.2 Bird Capture and Tissue Sampling

Bird capture and blood sampling on Long Island occurred in July 2012. Adirondack bird capture and sampling occurred in June and July 2011. At both sites, we used 12-meter mist nets with 30 millimeter mesh to safely capture songbirds. In tidal marsh, we flushed birds from the vegetation into the nets. In all other habitats, we erected nets along flyways or in otherwise high activity habitat and used conspecifics playback to lure birds into the nets. All birds were banded with a U.S. Geological Survey aluminum band. We determined sex, age (adult or hatching year), and breeding status for each bird. Morphometrics like wing chord, body mass, tarsus length, and fat score were also measured. Venipuncture of the cutaneous ulnar vein (Figure 2) with a 27-gauge sterile disposable needle allowed collection of 50-70 microliters of whole blood into heparinized Mylar-wrapped tubes for Hg measurements (concentration and isotope ratios) and light stable isotope analysis. The capillary tubes were sealed with Critocaps® and stored in plastic vacutainers on ice for up to 6 hours before freezing at -17° Celsius. All birds were released unharmed within 10-25 minutes of capture.

Figure 2. Blood Sample Collection from a Saltmarsh Sparrow

Source: Oksana Lane, Biodiversity Research Institute

2.3 Lab Analysis

2.3.1 Avian Tissues Mercury Analysis

All blood samples were analyzed for total Hg concentrations as whole blood. MeHg was not measured because it has been shown that approximately 95% of total Hg in songbird blood is MeHg (Rimmer et al. 2005, Edmonds et al. 2010). All blood Hg concentrations are expressed in micrograms per gram (µg/g), wet weight (ww). All blood samples were analyzed at Biodiversity Research Institute's Wildlife Mercury Research Laboratory in Gorham, ME, using the direct combustion/trapping atomic absorption (AA) method on a Milestone DMA 80.

This approach has been incorporated by the U.S. EPA in SW-846 Method 7473. The calibration utilized a blank and two calibration standards (DORM-3 and DOLT-4), one for each of the two detector cells. Instrument response was evaluated immediately following calibration, and thereafter, following every 20 samples and at the end of each analytical run by running two certified reference materials and a check blank.

2.3.2 Mercury Stable Isotope Analyses

Mercury isotopic composition analyses were conducted for 34 samples across 10 species at the Biogeochemistry and Environmental Isotope Geochemistry Laboratory under the supervision of Joel Blum at the University of Michigan, using the standard protocol established to provide high-precision Hg isotope data (Blum and Bergquist 2007, Blum 2011). Samples did not need to be aggregated to provide sufficient amounts of Hg to conduct this analysis. Fourteen samples analyzed were from Long Island, and 20 samples were from the Adirondacks.

The blood was thermally combusted to release sample Hg as Hg(0) gas. Mercury was trapped in an oxidizing solution (1% $KMnO_4$) as Hg(II). The matrix was separated by purge and trap methods, and the concentration was adjusted to a constant value along with bracketing standards. Hg isotope ratios were quantified using a Nu Instruments multicollector inductively coupled plasma mass spectrometer using thallium that is introduced as an aerosol, and sample-standard bracketing to correct for mass bias. Isotope compositions were referenced to the bracketing Hg standard (NIST 3133 solution). Compositions were reported in per mil (‰) as either mass-dependent fractionation (denoted as "del" or $\delta^{202}Hg$) or mass-independent fractionation (denoted as "delta," or $\Delta^{199}Hg$ or $\Delta^{201}Hg$). This analysis included quality assurance and quality control procedures of measuring standards and blanks.

2.4 Statistical Analyses

Hierarchical clustering analysis was used to determine natural differences in Hg isotope signatures among data points. Using the Ward technique, the data were ordinated and then organized by similarity. Using a broken stick plot, the appropriate number of groups for the data is estimated then each individual data point is assigned to a group. Otherwise, all analysis is a general comparison of means with standard error and or a simple two variable biplot. All analyses were conducted in JMP 9.03 (SAS Institute, Cary, NC).

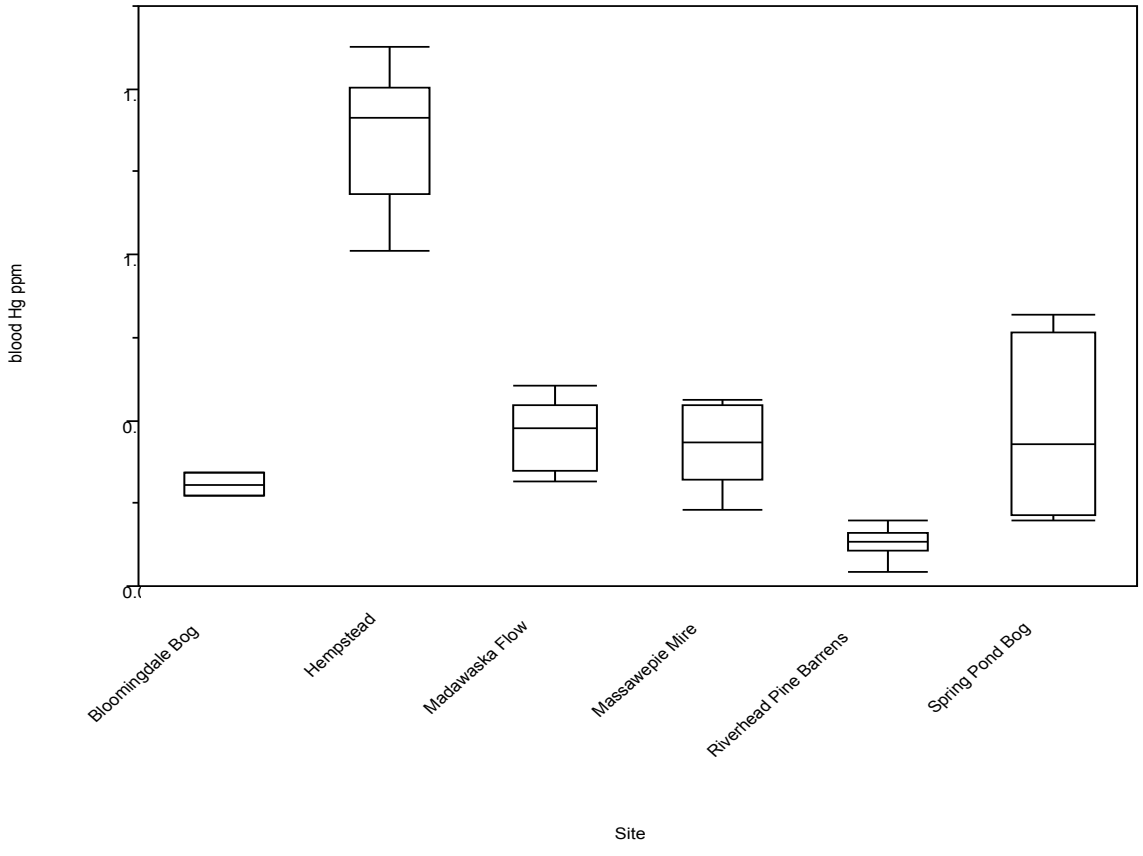
3 Results

3.1 Avian Mercury Exposure

The individuals selected for Hg isotope analysis had more than an estimated 10 nanograms (ng) of Hg in their blood sample. This cutoff removes a significant portion of the samples available to measure, thus habitats with consistently high Hg like Hempstead tidal marshes are sampled more evenly than those that have lower Hg like the pine barrens (Figure 3).

Figure 3. Box and Whisker Plots of Total Mercury Levels Over Study Sites

The box represents the inner 50% of the data, the horizontal line through the box is the mean, and the whiskers show the minimum and maximum values. Hempstead and Riverhead are on Long Island. Bloomingdale Bog, Madawaska Flow, Massawepie Mire, and Spring Pond Bog are in the Adirondack Mountains.

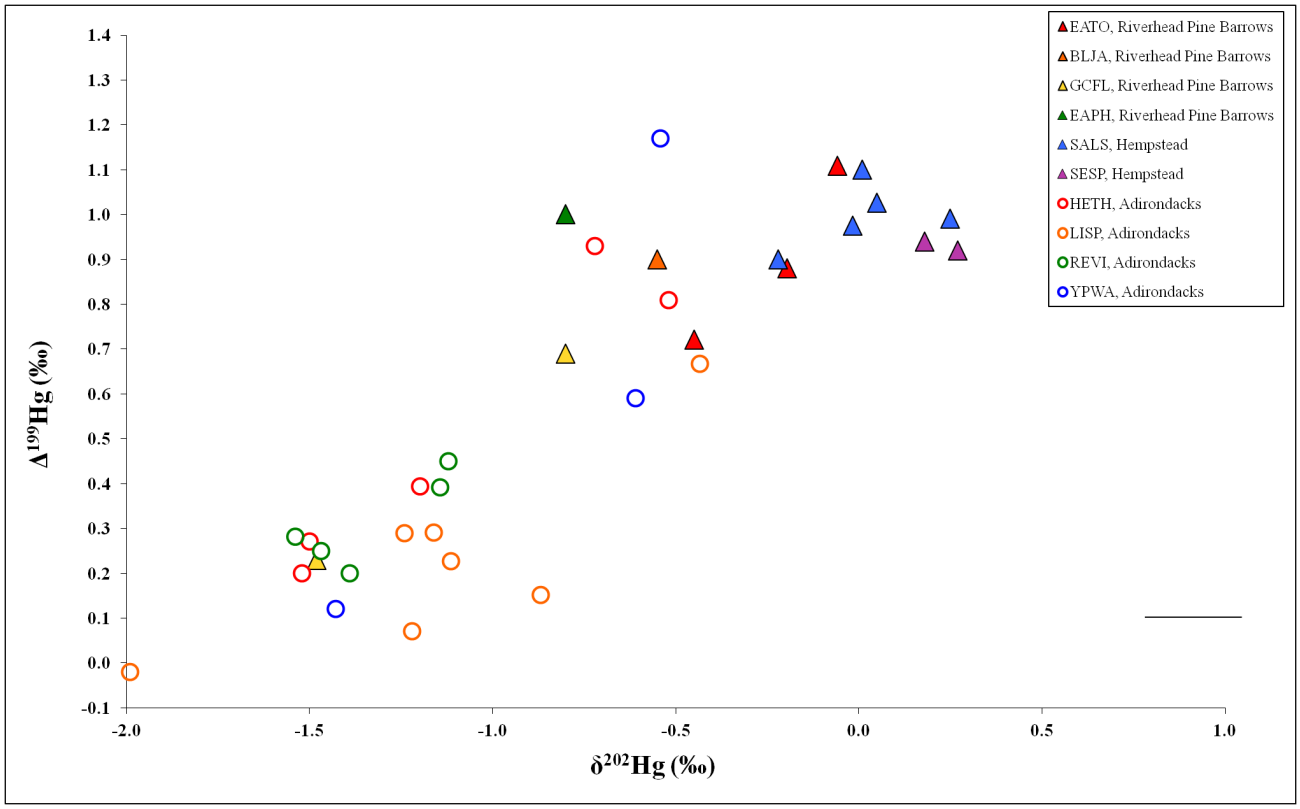


3.2 Avian Mercury Stable Isotopes

We found that Δ^{199}Hg and δ^{202}Hg values differed between regions such that individuals from the Adirondacks (open circles) showed depleted levels of ^{199}Hg and ^{202}Hg and those at Long Island (closed triangles) showed enriched levels (Figure 4). Mercury isotope levels also varied by species within habitat which is presumably related to differences in diet.

Figure 4. Δ^{199}Hg and δ^{202}Hg Isotope Biplot Categorized by Sampling Site and Species

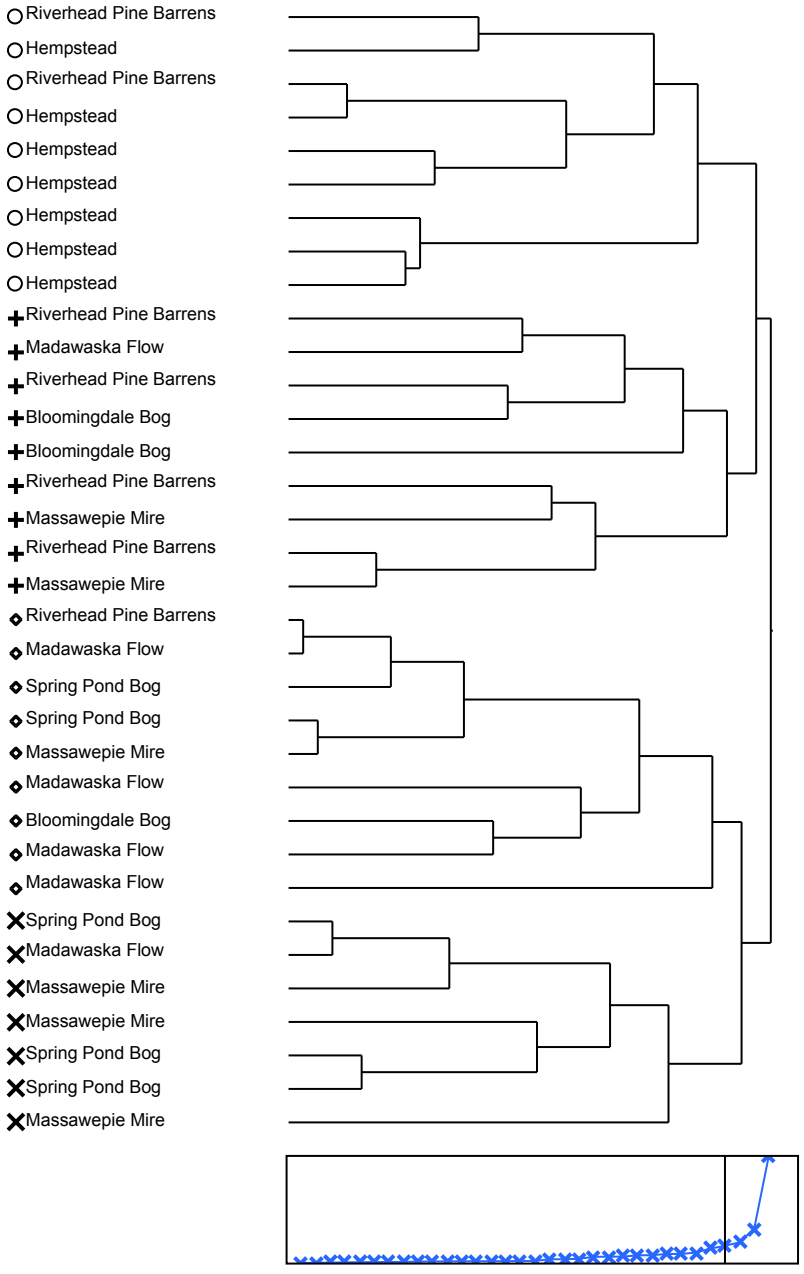
Open circles are from the Adirondacks while closed triangles are from Long Island. Colors vary by species (see legend for details).



We conducted a hierarchical clustering analysis to determine what the natural groupings of Hg stable isotopes are in the data. This technique explores the variation in Hg isotopes at each sites and attempts to find the most parsimonious grouping of the sites based on that variation. The broken stick plots at the bottom of Figure 5 and Figure 6 are used as a tool to determine the optimal number of groups in the data set. The symbols to the left of the site and species names indicate which of the four clusters each data point belongs to. The clusters tend to fall along site boundaries. In these cases, four was considered the optimal number of groupings for this data set, which generally corresponded to (1) Long Island tidal marshes, (2) upland forests on Long Island and the Adirondacks, and (3) and (4) are both groupings of mixed-species from Adirondack Mountain bogs and upland forests (Figure 5).

Figure 5. Dendrogram Constructed Using Ward Hierarchical Clustering with a Broken Stick Plot at the Bottom

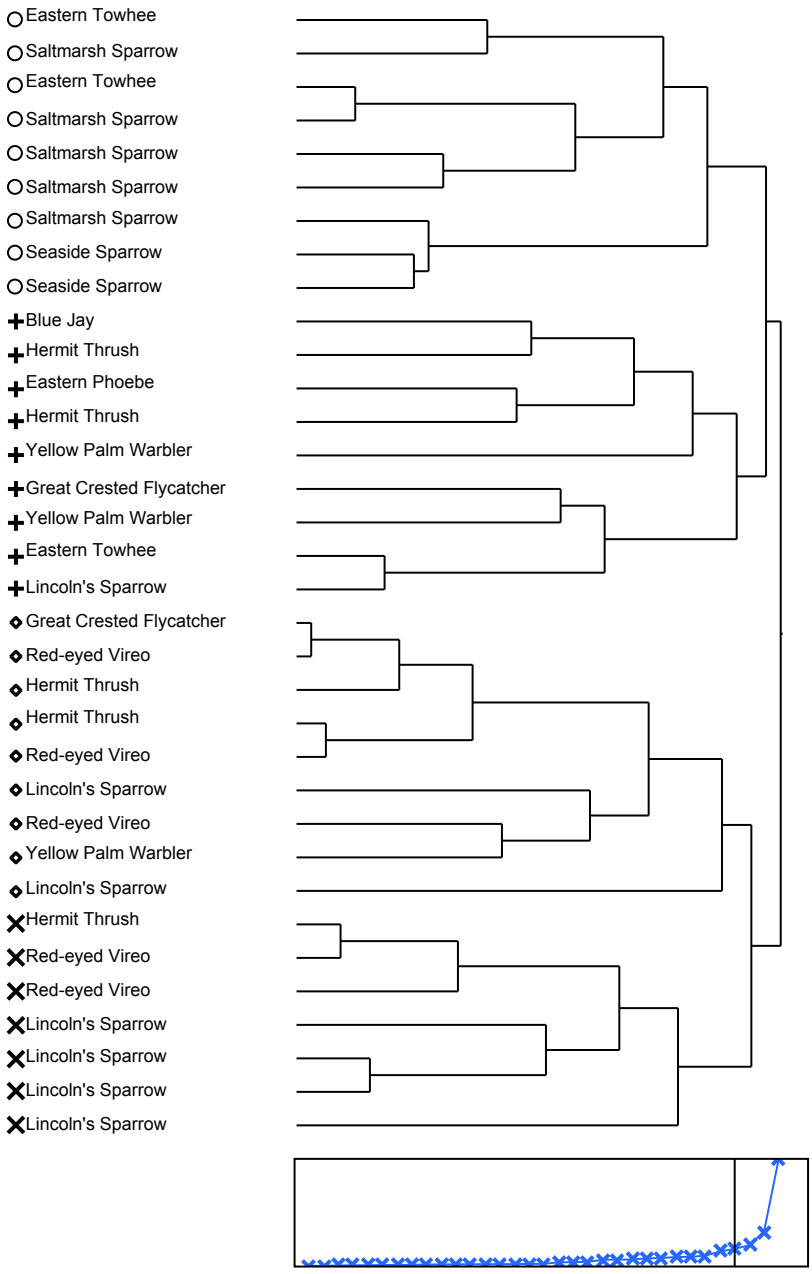
Points are classified using the four main categories and identified using the symbols on the far left. Points are labeled by sampling site.



Species also appears to be a useful factor for explain variance in Hg isotopes. Some species are habitat specialist and more likely to have a narrow range of exposure to Hg isotopes. For example, we have a number of Saltmarsh Sparrows sampled in this study (a saltmarsh habitat endemic), there is relatively little variation in Hg isotopes signatures, and they are found in only one of our cluster groups (Fig. 6). Other species like Hermit Thrush are habitat generalists and are found in both Long Island and the Adirondacks. Within species, they show a wide range of isotopic signatures within species and falls within three different clusters. Mercury isotope signatures can also be used to differentiate among habitat specialists and explain why species that use the same habitats accumulate different amounts of Hg. Yellow Palm Warblers, a bog habitat specialist, actually appear to have isotope signatures fairly closely related to Long Island upland and saltmarsh than some of the wetter Adirondack habitats. Lincoln's Sparrows, another bog specialist, tended to have the isotope signatures that fell out closely with bog habitats. These differences are likely due to differences in diet and this study helps us identify reasons for why Palm Warblers and Lincoln's Sparrows can use the same habitat but have differing Hg exposure levels.

Figure 6. Dendrogram Constructed Using Ward Hierarchical Clustering with a Broken Stick Plot at the Bottom.

Points are classified using the four main categories and identified using the symbols on the far left. Points are labeled by study species.

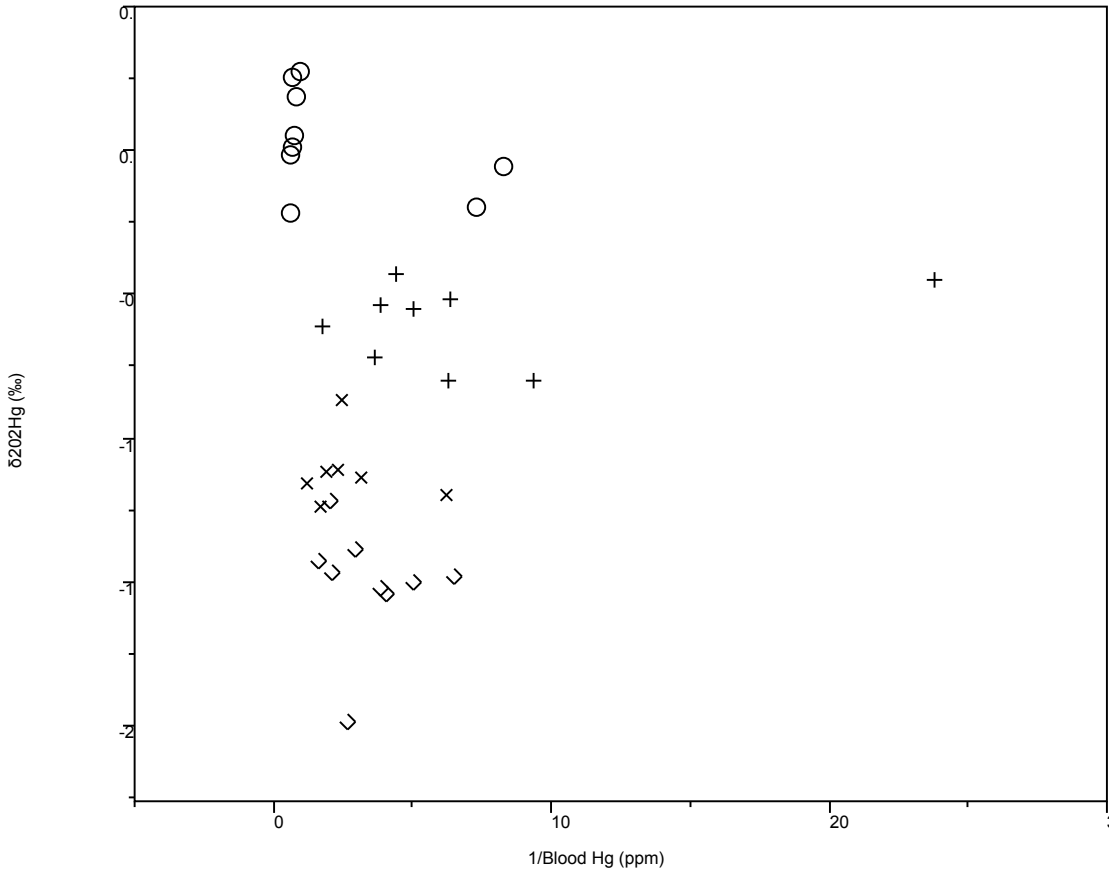


The two groups that showed the greatest difference is the group composed of saltmarsh, pine barren and some Adirondack habitats (open circles and filled pluses) with mostly wetland habitats in the Adirondacks (open diamonds and filled crosses). Given that saltmarsh sites are the furthers to the right and top of the mixing line in Figure 4 while the Adirondack forested wetlands are the further bottom and left along the same graph the differences in these clusters appear to make sense. Some Adirondack forest habitats and Long Island forests appeared to be in the middle of the mixing line but the cluster hierarchy appeared to find them more closely related to the salt marsh isotope signature.

Lastly, we compared Hg levels in the original blood sample to the δ^{202}Hg (Figure 7). No clear mixing lines were present in the data as a whole though within the assigned clusters there were perhaps trends of increasing Hg with decreasing δ^{202}Hg in tidal marsh and the pine barrens and increasing Hg with increase δ^{202}Hg in the Adirondacks. Generally, it appears samples with high Hg levels could have differing δ^{202}Hg signatures, which suggests that while there are differing pathways for Hg exposure between sites the end exposure levels are similar.

Figure 7. Blood Hg levels as a function of Δ^{202}Hg and Δ^{199}Hg

Points are symbolized using the cluster analysis previously conducted (see Figures 5 and 6).



4 Discussion

While our sampling regime had biases, we think our data accurately represent Hg stable isotope signatures from the songbirds with the highest Hg levels of samples collected from a variety of habitats in New York. In this study, we tended to use samples that we already knew to have high levels of blood Hg (the vast majority of which is 95% MeHg in songbirds). This means that our description of Hg isotopes for the songbirds in these habitats really only represent the isotopic signatures of individuals that achieved high exposure levels. There could be other types of Hg isotopes signatures in these habitats that represent less significant sources of Hg that we did not measure. However, we do think that this analysis has characterized the Hg isotope signatures that are created via the dominant Hg methylation pathways in those environments. We found large differences in Δ^{199}Hg and δ^{202}Hg among species and sites in our survey, enough so that these isotopes were useful in categorizing the data set into four distinct groups that closely aligned with their habitat.

Tidal marsh and pine barren habitats mostly exhibited highly positive Δ^{199}Hg values. This result indicates that there is a large amount of photo-demethylation occurring in these habitats (Bergquist and Blum 2007). This photochemical process occurs when Hg in aqueous solutions is exposed to light and has little to do with the original source of Hg in the ecosystem. δ^{202}Hg ratios, however, are related to emissions origin with coal fired utility boilers showing more negative values and industrial effluent showing more positive values. Correcting for the effect of photodemethylation on Δ^{199}Hg and δ^{202}Hg values (there is a small but predictable effect on δ^{202}Hg) then these data suggest that tidal marsh isotope signatures, in particular, could be derived in large part from industrial effluents whereas all other samples could be predominantly derived from coal combustion. Although this result is consistent with our hypothesis entering the study, this study is preliminary and it is possible that this relationship is confounded by other factors.

Variation in δ^{202}Hg values is not always due entirely to source type, as there can also be variations in δ^{202}Hg due to mixing of inorganic Hg and MeHg in organisms (Tsui et al. 2012). It has also been suggested that aquatic systems may be dominated by in situ methylation of Hg whereas atmospherically deposited MeHg may be significant in forests. In both locations, the drier habitats tended to occupy the more central portion of the distribution of both Δ^{199}Hg and δ^{202}Hg, though there are outliers in both locations. On the whole, wet habitats tended toward the fringes of the distribution; specifically, Long Island tended toward the positive side and Adirondacks toward the negative side. For Δ^{199}Hg these differences can be attributed to the prevalence of sunlight in tidal marshes and open canopy forests, and the photochemical degradation of methylmercury. But with δ^{202}Hg values, the habitat related differences are less obvious. In past research, aquatically derived Hg isotope signatures have tended to look like what we see in the Adirondacks with low Δ^{199}Hg and δ^{202}Hg values (Tsui et al. 2012, Tsui and Adams unpublished data). Although the tidal marsh data differ from this trend, tidal marsh ecosystems have not been previously characterized by Hg isotopes values.

Overall, that lack of similar signal in Long Island suggests one of two possibilities: (1) these differences are due to a different Hg isotopic composition entering the system (i.e., multiple sources of Hg), and/or (2) Hg methylation processes are fundamentally different in estuarine or marine systems as compared to freshwater aquatic systems. Given the magnitude of the measured differences, it is unlikely that this result is entirely due to processes occurring in the habitat. Thus, it is likely that multiple, different Hg sources impact these habitats. A study designed specifically to trace sources of Hg would tell us much more about the possibility of multiple sources across these habitats, but for now we would argue that this interpretation is likely, but not certain. A large incinerator that is known to burn Hg-containing thermostats in Hempstead is near the tidal marsh site; Hg isotope signatures consistent with these results would be expected if that facility is the source of Hg in the nearby tidal marsh.

Despite not defining a clear causal mechanism, this study shows that species, habitat, and location explain significant portions of the Hg isotope signatures in songbirds and that they are an effective tool for a priori categorization. We observed a large of amount of variation in isotope signatures, enough so that future work could reasonably partition Hg source types. Such variation is useful for understanding the movements of Hg throughout the landscape and the bioaccumulation process, in addition to identifying multiple sources of Hg in the ecosystems. Still, source partitioning can be challenging using stable isotopes, more information is required to make accurate mixing models that can predict sources (e.g., Phillips and Gregg 2003).

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