Methylmercury degradation and exposure pathways in streams and wetlands impacted by historical mining

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Abstract

We measured monomethyl mercury (MMHg) and total mercury (THg) concentrations and Hg stable isotope ratios (δ^{202}Hg and Δ^{199}Hg) in sediment and aquatic organisms from Cache Creek (California Coast Range) and Yolo Bypass (Sacramento Valley). Cache Creek sediment had a large range in THg (87 to 3,870 ng/g) and δ^{202}Hg (−1.69 to −0.20‰) reflecting the heterogeneity of Hg mining sources in sediment. The δ^{202}Hg of Yolo Bypass wetland sediment suggests a mixture of high and low THg sediment sources. We used relationships between %MMHg (the percent ratio of MMHg to THg) and Hg isotope values (δ^{202}Hg and Δ^{199}Hg) in fish and macroinvertebrates to identify and estimate the isotopic composition of MMHg. We found deviation from linear relationships between %MMHg and Hg isotope values and suggest this is indicative of the bioaccumulation of isotopically distinct pools of MMHg. We also estimated the isotopic composition of pre-photodegraded MMHg (i.e., subtracting fractionation from photochemical reactions) and found contrasting relationships between the estimated δ^{202}Hg of pre-photodegraded MMHg and sediment IHg. Cache Creek had mass dependent fractionation (MDF; δ^{202}Hg) of at least −0.4‰ whereas Yolo Bypass had MDF of +0.2 to +0.5‰. This result supports the hypothesis that Hg isotope fractionation between IHg and MMHg observed in rivers (−MDF) is unique compared to +MDF observed in non-flowing water environments such as wetlands, lakes, and the coastal ocean.

1. Introduction

Monomethyl mercury (MMHg) is a bioaccumulative developmental neurotoxin that is mainly produced from inorganic Hg (IHg) in aquatic environments.Donovan[1, 2] IHg has been released for hundreds of years predominantly from the mining of mercury sulfide (HgS) ores and combustion of coal, contaminating aquatic environments around the world.[3] Between the 1850's and 1970's approximately 100,000 Mg of mercury (Hg) was mined in the California Coast Ranges.[4] Metallic Hg (Hg(0)) was concentrated from Hg-ore by volatilizing (roasting) and then re-condensing the Hg(0) vapor.[5] Mine waste materials, including thermally processed ore (calcine, which contains residual Hg), were commonly disposed of near mining and processing sites.[5, 6] Cache Creek, in the California Coast Range, drains one of the most prolific Hg mining regions in North America, with over 30 former Hg mines in the watershed.[7] Studies have found high concentrations of IHg in Cache Creek sediment and water,[8] and have documented MMHg bioaccumulation in aquatic[9-12] and terrestrial[13] biota in the watershed. Sediment bound Hg in Cache Creek can be transported downstream through the Cache Creek Settling Basin (CCSB, a 1,450 ha leveed floodwater and sediment containment area) and into the Yolo Bypass, a larger floodwater conveyance area that drains into the San Francisco Bay Delta (SI Figure 1). Therefore IHg from Hg-mining in Cache Creek is a potential source of MMHg to both local and downstream food webs.

Yolo Bypass is a ~24,000 ha engineered flood bypass that diverts high river flows around the city of Sacramento, CA and uses a network of drainage and water supply channels to support agriculture and wildlife habitat. MMHg is thought to be produced in situ in Yolo Bypass wetlands,[14] and MMHg bioaccumulation has been documented

throughout Yolo Bypass in invertebrates, forage fish and salmonids.[15-17] During floods Yolo Bypass receives water and suspended sediment from Cache Creek and overflow from the Sacramento River (via the Fremont weir) and the Feather River (via Sutter Bypass),[18, 19] the latter of which drains multiple Au mining districts in the Sierra Nevada (e.g., Yuba and Bear Rivers; SI Figures 1, 3).[20, 21] Consequently, there are multiple potential upstream Hg sources (Coast Range Hg mining and Sierra Nevada Au mining) that might provide a labile source of IHg to Yolo Bypass.[19, 22] However, it is difficult to identify the relative contribution of these sources and their potential transformation to MMHg.[15, 19] In this study we measured natural variations in Hg stable isotope ratios in sediment and biota from Cache Creek and Yolo Bypass with the goal of differentiating between Hg sources, identifying biogeochemical transformations (e.g., IHg methylation and MMHg degradation), and tracking MMHg bioaccumulation.

Mercury has seven stable isotopes that are affected by mass-dependent fractionation (MDF; δ^{202}Hg) and mass-independent fractionation (MIF) of both odd-mass-number (Δ^{199}Hg, Δ^{201}Hg) and even-mass-number (Δ^{200}Hg, Δ^{204}Hg) Hg isotopes in the environment.[23] Experimental studies of Hg isotope fractionation have demonstrated MDF during biotic (i.e, Hg(II) methylation, MeHg degradation, Hg(II) reduction)[24-27] and abiotic (IHg sorption, coprecipitation, etc.)[28, 29] reactions while large magnitude odd-mass-number MIF (>0.5‰) occurs primarily during photochemical reactions.[23, 30] The Hg isotopic composition of sediment has previously been used to identify anthropogenic Hg sources and trace their transport and deposition in river and estuarine environments.[31-35] Hg isotopes have also been measured in a variety of Hg mine waste materials, including calcines and CA Coast Range Hg-ores.[36-40] Hg mine wastes can vary widely in isotopic

composition over very small spatial scales (e.g., δ^{202}Hg range of >5‰ within a single calcine sample),[31, 32] but sediment downstream of individual mines is thought to largely integrate these different mining sources.[35, 39, 41] We hypothesized that the isotopic composition of high THg sediment in Cache Creek downstream of individual mining districts would enable us to distinguish the contribution of Hg mining (Coast Range) vs. Au-mining (Sierra Nevada) Hg sources to Yolo Bypass. Furthermore, we hypothesized that this Hg mining signature would provide a fingerprint of the IHg that is methylated locally and bioaccumulated as MMHg in the Cache Creek food web.

To compare Hg biogeochemical processes between river and wetland environments we also measured Hg isotope ratios and THg and MMHg concentrations (to obtain %MMHg), in benthic macroinvertebrates and forage fish in Cache Creek and Yolo Bypass. This approach was previously used to 1) test whether the mixing of two isotopically distinct IHg and MMHg pools can explain the isotopic composition of biota and 2) to estimate the Hg isotopic composition of IHg and MMHg in food webs.[42-44] Fish feeding studies show essentially no isotopic fractionation of MMHg during trophic transfer,[45-49] and therefore the estimated isotopic composition of MMHg provides insight into MMHg biogeochemical transformations in the environment prior to bioaccumulation. For example, changes in Δ^{199}Hg values of MMHg have been used to identify spatial changes in the extent of MMHg photodegradation between different environments (streams, forests, etc.).[43, 50] Studies in the relatively less-contaminated Eel River (CA Coast Range), and in the gold mining contaminated Yuba River, have allowed comparison of the estimated isotopic composition of MMHg in food webs with IHg in sediment and other environmental reservoirs to infer MDF between these Hg pools.[42, 44]

This study, combined with the results of our previous work in the Yuba River,[42] is the first to measure and compare Hg isotopes in stream and wetland food webs downstream of both Hg and Au mining regions. We report THg and MMHg concentrations and Hg isotope ratios in sediment, benthic macroinvertebrates, and forage fish from five sites in Cache Creek and three wetlands in Yolo Bypass to identify Hg sources and Hg biogeochemical transformations. Our previous study in the nearby Yuba River compared sediment IHg and food web MMHg in a stream environment contaminated by Au mining alone and found higher δ^{202}Hg in sediment IHg compared to MMHg (−MDF from IHg to MMHg).[42] This result contrasted with lower δ^{202}Hg in IHg compared to MMHg (+MDF from IHg to MMHg) that was previously observed in lakes, estuaries and the coastal ocean.[51-54] In Cache Creek and Yolo Bypass, sediment is thought to be an important source of IHg that can be methylated, leading to MMHg bioaccumulation in local food webs.[11, 14] Based on previous studies in a variety of aquatic environments, hypothesized that in this study the MDF relationship between IHg and MMHg in Cache Creek would be consistent with the Yuba River, whereas the MDF between IHg and MMHg in Yolo Bypass wetlands would be more similar to that previously reported in lakes and the coastal ocean.

2. Methods and Materials

2.1 Sample Collection and Processing

2.1.1. Sediment

Sediment was collected between 2012 and 2013 from bars and terraces at two locations in Cache Creek ("Rumsey" and "Capay"; SI Figure 2) and in Bear Creek, one of three primary tributaries to Cache Creek. Surface sediment (0-10 cm) was also collected in

Yolo Bypass in 2013 and 2014 from three wetlands we refer to as Upper Wetland (UW), Permanent Wetland 2 (PW2), and Lower Wetland (LW). UW is upstream of the CCSB while PW2 and LW are downstream of the CCSB (SI Figure 3). All sediment samples were freeze-dried and multiple size fractions were analyzed. Three sediment fractions (<63μm, 1mm-63μm, and <1mm) were analyzed from the two locations in Cache Creek. In all Yolo Bypass wetlands the <1mm fraction was analyzed and in UW and LW the <63μm fraction was also analyzed. In Bear Creek only bulk un-sieved sediment samples were analyzed. Sediment was processed (dried, sieved, ground, analyzed) in the order of expected increasing THg concentration. Sediment was sieved to <1mm with stainless steel sieves that were cleaned thoroughly with a nylon brush between samples. A split of the <1mm sediment was ground and homogenized in an alumina ball mill and a separate split of <1mm sediment was sieved to <63μm. The fraction passing the <1mm sieve, but not the 63μm sieve, was retained and ground in an alumina ball mill (referred to as the "1mm-63μm fraction"). The fraction passing the <63μm sieve was retained and homogenized but not ground. All sediment samples were analyzed for THg concentration and Hg isotopic composition at the University of Michigan. The <63 μm sediment fractions collected in 2013 (two locations in Cache Creek and two locations in Yolo Bypass; SI Table 1) were also analyzed for THg (hot concentrated acid digestion followed by CV-AFS analysis) and MMHg (Section 2.2) at the US Geological Survey (USGS) in Menlo Park, CA.

2.1.2. Biota

Filamentous algae and aquatic organisms were collected from four sites in Cache Creek (Regional Park, Rumsey, Guinda, and Capay) during two separate sampling campaigns in March 2013 and June 2014 (SI Figure 2). In 2013 we collected

macroinvertebrates from riffle environments (e.g., *Megaloptera*, *Perlidae*, and *Hydropsychidae*) whereas in 2014 we collected macroinvertebrates (e.g., *Libellulidae*, *Gomphidae*, *Coenagrionidae*, etc) and filamentous algae (*Spirogyra* and *Hydrodictyon*) from slow moving water and pools at the exact same locations. The change in habitat and type of biota collected was due to lower streamflow in June 2014 (<0.1 m^3/s at the Rumsey Bridge USGS Gauging Station) than in March 2013 (1.4 to 2 m^3/s). In Yolo Bypass aquatic organisms were collected from the same wetlands where sediment was collected, in March 2013 (for UW and LW) and June 2014 (for UW and PW2; SI Figure 3). These wetlands contained similar types of organisms each year such as damselfly larva, dragonfly larva, and backswimmers (e.g., *Libellulidae*, *Gomphidae*, *Coenagrionidae, and Notonectidae*), along with two types of forage fish: mosquitofish (*Gambusia affinis)* and Mississippi silverside (*Menidia beryllina*). The organisms collected at each location are summarized in the Supporting Information (SI Figures 2 and 3 and SI Table 2).

All aquatic organisms were collected using a kick net, dip net, or by picking directly off of gravel cobbles or sediment. Individual organisms were removed with clean stainless steel tweezers and transferred into a secondary container with native water for field identification. Organisms were then composited by order, family, or species when possible, transferred into clean plastic tubes and immediately frozen on dry ice in the field. All biota samples are composites of 10 or more whole body individuals except for crayfish, which contain 1-3 individuals per sample. Biota were freeze-dried and then ground and homogenized with either an agate mortar and pestle (vigorously cleaned between each sample with laboratory wipes, double deionized water and isopropanol) or an alumina ball

mill (cleaned with double deionized water and isopropanol between each sample, and by grinding Hg-free quartz sand between sample types and locations) prior to analysis.

2.2 MMHg Concentration Analysis

The concentration of MMHg (dry wt.) in sediment and biota was measured at the USGS (Menlo Park, CA) simultaneously with samples from a previous study in the Yuba River.[42] Therefore QA/QC of MMHg analyses, reported here for the entire dataset, can also be found elsewhere.[42] Briefly, sediment was sub-sampled (20–30 mg) and extracted for MMHg using 25% KOH in methanol (25 g of KOH in 100 mL methanol) at 60°C for four hours.[55] Biota was sub-sampled (3–7 mg) and extracted for MMHg using 30% HNO_3 at 60°C (overnight, 12-16 hrs), as adapted from ([56]). Extract sub-samples were diluted, pH was adjusted to 4.9 with citrate buffer and they were assayed for MMHg by aqueous phase ethylation (with sodium tetraethylborate) on an automated MMHg analyzer (MERX system, Brooks Rand Laboratories).[57] For sediment, the relative percent deviation (RPD) of analytical duplicates was 8.4% (n=1 pair), matrix spike recovery was 107±1% (n = 2), and certified reference material (CRM) ERM-CC580 (estuarine sediment) recovery was 95% (n=1). For biota, the mean RPD of analytical duplicates was 3.0% (n=12 pairs), matrix spike recoveries were 105±1% (mean ± SE, n = 26), and CRM recoveries from NRC Tort-3 (lobster hepatopancreas) were 86±2% (mean ± SE, n=7) and from NIST-2967 (marine mussel tissue) were 94±3% (mean ± SE, n=7).

2.3 THg Concentration and Hg Isotope Analysis

Hg was separated from all samples for THg concentration and Hg stable isotope measurements by offline combustion, as described in detail elsewhere.[44, 58] Briefly, up to 1 g of sample was placed into the first furnace of a two furnace combustion system. The temperature of the first furnace was increased to 750°C over the course of 6 hours while the second furnace was held at 1000°C. The Hg released was carried in a flow of Hg-free O_2 through the second furnace and into a 1%$KMnO_4$ in 10% H_2SO_4 trapping solution ("1% $KMnO_4$ trap"). Trap solutions were partially reduced with 0.6% w/w hydroxylamine hydrochloride (NH_2OH•HCl) and an aliquot was measured for THg by CV-AAS (Nippon MA-2000). The dry weight THg concentration of samples reported in SI Tables 1, 2, and 3, were calculated based on the mass of Hg in the 1% $KMnO_4$ trap and the sample mass combusted. This offline combustion procedure recovered 112±17% (1SD; n=17) of Hg for a subset of 2013 biota samples that were independently analyzed for THg at the USGS in Menlo Park, CA.

Prior to isotopic analysis, contents of the 1%$KMnO_4$ trap solutions were treated with 0.3 ml 20% $SnCl_2$ and 0.3 ml 50% H_2SO_4 to reduce Hg(II) to Hg(0), which was purged into a secondary 1% $KMnO_4$ trap and reoxidized to Hg(II). This procedure was completed to isolate Hg from combustion residues and concentrate Hg for isotopic analysis. An aliquot of the secondary trap solution was analyzed by CV-AAS (Nippon MA-2000) with transfer recoveries averaging 95±5% (1SD; n= 59, minimum of 81%) for biota and 96±4% (1SD; n=27, minimum of 87%) for sediment. The Hg isotopic composition of the secondary trap solution was measured by cold vapor-multiple collector-inductively coupled plasma-mass spectrometry (CV-MC-ICP-MS; Nu Instruments). Final trap solutions were partially reduced with 0.6% w/w NH_2OH•HCl, diluted to a concentration between 0.9 and 5 ng/g, and Hg was

chemically reduced to Hg(0) online by the continuous addition of 2% (w/w) $SnCl_2$. The Hg(0) generated was separated from solution using a frosted tip gas-liquid separator and carried in a Hg-free stream of Ar gas to the MC-ICP-MS inlet. Instrumental mass bias was corrected by the introduction of an internal Tl standard (NIST 997) as a dry aerosol to the gas stream and by strict sample standard bracketing using NIST 3133 with a closely matched THg concentration and solution matrix.[59]

Mercury stable isotope compositions are reported in permil (‰) using delta notation ($\delta^{xxx}Hg$) relative to the NIST SRM 3133 (Eq. 1). MDF is reported using the $^{202}Hg/^{198}Hg$ ratio ($\delta^{202}Hg$) whereas MIF, the deviation from theoretically predicted MDF, is reported using capital delta notation ($\Delta^{xxx}Hg$; Eq. 2).[59] In this study, we use $\Delta^{199}Hg$ and $\Delta^{201}Hg$ to report MIF with $\beta = 0.252$ for $\Delta^{199}Hg$ and $\beta = 0.752$ for $\Delta^{201}Hg$.[59] All $\delta^{xxx}Hg$ and $\Delta^{xxx}Hg$ values for samples and SRMs are available in SI Tables 1, 2, and 3.

$$\text{Equation [1]: } \delta^{xxx}Hg\ (‰) = \{[(^{xxx}Hg/^{198}Hg)_{sample}/(^{xxx}Hg/^{198}Hg)_{NIST3133}]\text{-}1\}* 1000$$

$$\text{Equation [2]: } \Delta^{xxx}Hg = \delta^{xxx}Hg - (\delta^{202}Hg * \beta)$$

Procedural blanks and two CRMs (NRC Tort-2 and NIST 1944) were processed and analyzed in an identical manner alongside samples from this study and samples from a previous study in the Yuba River.[42] Therefore, CRM and process blank measurements are reported for the entire dataset here and can also be found elsewhere (see Donovan et al.[42]). Briefly, process blanks accounted for 0.2% to 1.8% of Hg in the final trap solutions, mean THg concentrations (±1SD) of CRMs were within 5% of certified values (SI Table 3)[42], and recoveries during secondary purge and trap procedures were 94±4% (1SD, n=6) and

96±7% (1SD, n=11) for NIST SRM 1944 and NRC Tort-2, respectively. The Hg isotopic composition of CRMs was consistent with previously reported values (SI Table 3).[32, 44, 46, 53, 60-65] Long-term analytical uncertainty of Hg isotope ratio measurements was estimated from the standard deviation (2SD) of the mean Hg isotopic composition of the UM-Almáden standard solution during analytical sessions between Jan. 2013 and Dec. 2014 when run concentrations were between 3 and 5 ng/g (SI Table 3). We estimated external reproducibility using the 2SD of mean Hg isotope values from replicate processing and analysis of NIST 1944 (n=6) and NRC Tort-2 (n=11). The 2SD of CRMs was greater than the 2SD associated with the long-term measurement of UM-Almáden. Therefore, we use CRMs to estimate the 2SD uncertainty of Hg isotope measurements in this study as ±0.08‰ for δ^{202}Hg and ±0.05‰ for Δ^{199}Hg (SI Table 3).

3. Results and Discussion

3.1 Regional Sediment Sources

Cache Creek sediment had variable THg concentrations (87 to 3,870 ng/g) and δ^{202}Hg values (−1.69‰ to −0.20 ‰) that reflect the heterogeneous distribution of Hg mine wastes in the watershed. The Hg isotopic composition of Cache Creek sediment did not change systematically with size class or THg concentration (Figure 1, SI Figure 4). Replicate analysis of <63μm sediment at Rumsey and Capay (n=3 for each site) resulted in highly variable THg (98 to 3870 ng/g) and δ^{202}Hg values (−1.42 to −0.20‰, respectively). These values overlapped with the THg and δ^{202}Hg of the <1mm and 1mm-63μm fractions at the same locations (87 to 1,480 ng/g and −1.69 to −0.55‰; n=5). A large range in δ^{202}Hg was similarly reported for sediment downstream of the New Idria (California USA) Hg mine

(−0.58 to 0.80‰) in another study and was attributed to the distribution of calcine and cinnabar particles.[35] Given the multiple Hg mining districts in the Cache Creek watershed, we think it is likely that similar Hg mining products persist in this catchment.[7] Additionally, sediment from Bear Creek, a primary upstream tributary of Cache Creek that contains both Hg mining and hydrothermal Hg sources,[66 9] had extremely high THg concentrations (23.7 to 468 μg/g). Therefore, a small mass of high THg sediment from upstream could significantly alter the isotopic composition of Cache Creek sediment. Bear Creek sediment had δ^{202}Hg (−0.31±0.17‰) and Δ^{199}Hg (0.08±0.01‰; mean±1SD, n=3) that was comparable to unroasted Hg mine waste from New Idria (δ^{202}Hg of −0.43 to +0.16‰)[39-41] and Hg ores from the CA Coast Range (δ^{202}Hg of −0.64±0.84‰, mean± 1SD, n=91).[38] Sediment collected in Cache Creek was located 13 river km downstream of Bear Creek, and further from individual Hg mining districts, and likely integrated multiple high THg tributary inputs and Hg sources. Therefore, its mean isotopic composition (δ^{202}Hg of −0.99±0.45‰; and Δ^{199}Hg of 0.10±0.07‰; mean±1sd, n=11) provides a reasonable estimate of the large quantity of IHg stored in sediment that could be methylated and accumulate as MMHg in local or regional food webs.

In Yolo Bypass, the δ^{202}Hg of wetland surface sediment changes as a function of THg concentration (r^2 = 0.91, $p<0.001$; Figure 1). This suggests that Yolo Bypass wetland sediment is a mixture of high and low THg sources with different δ^{202}Hg values. Yolo Bypass sediment with THg less than 60 ng/g, typical for pre-mining sediment in the Sierra Nevada,[67] had δ^{202}Hg between −0.67 and −1.03‰ and is consistent with low THg, pre-mining dated sediment in SF Bay sediment cores (δ^{202}Hg of −0.98±0.06‰, n=5).[33] With increasing THg the δ^{202}Hg of Yolo Bypass sediment trended towards −0.47±0.04‰ (y-

intercept ±1SE; Figure 1), which is indistinguishable from Yuba Fan sediment contaminated by Au mining (δ^{202}Hg of −0.38±0.17‰; mean ±1SD, n=7).[42] However, this value is also within the wide range of δ^{202}Hg values found in Cache Creek sediment (−1.69 to −0.11‰). Sediment THg and δ^{202}Hg also changed spatially from North to South in Yolo Bypass, with low THg and δ^{202}Hg values in UW and higher THg and δ^{202}Hg values in PW2 and LW. Sediment in PW2 and LW, downstream of CCSB, had a small range in δ^{202}Hg (−0.59±0.06; mean ±1SD, n=5) that is most similar to Sierra Nevada Au mining inputs, but due to the variability of Cache Creek sediment δ^{202}Hg, we are unable to rule out the presence of Cache Creek derived Hg. Nonetheless, these spatial changes might reflect erosional regions within Yolo Bypass or be related to the episodic timing of sediment delivery.[18, 19] For example, earlier work has shown that decadal floods deliver large volumes of Hg-laden sediment from the Yuba-Feather system into Yolo Bypass.[68] Future investigation of the isotopic composition of Yolo Bypass sediment, with greater spatial or temporal resolution, may prove valuable to understanding sediment transport in the region. Additionally, characterization of the suspended load in Cache Creek and the Yuba River might aid future studies that investigate the transport of Hg from Sierra Nevada Au mining vs. Hg mining sources in the Coast Ranges. Although we are unable to distinguish between the high THg mining sources (Sierra Nevada Au mining vs. Coast Range Hg mining), the isotopic composition of Yolo Bypass wetland sediment is best explained as a mixture of low THg, non-mining sediment with δ^{202}Hg of ~ −1‰ and high THg, mining-derived sediment with δ^{202}Hg of ~ −0.5‰.

3.2 Biota THg, MMHg and Hg Isotopic Compositions

3.2.1. Cache Creek

Aquatic organisms from Cache Creek had overlapping THg and MMHg concentrations between 2013 (104 to 334 ng/g and 45 to 220 ng/g, respectively; n=7) and 2014 (151 to 889 ng/g and 80 to 608 ng/g, respectively; n=25 excluding algae; SI Table 2). These concentrations were similar to values measured in other studies in Hg mine impacted rivers[69] and consistent with previous surveys in the Cache Creek watershed.[9-11, 66, 70] Filamentous algae (*Spirogyra* and *Hydrodicton*) from Cache Creek had somewhat higher MMHg levels (7 to 83 ng/g, n=4) than *Cladoraphora* measured in the Yuba River (2.4 to 17 ng/g)[42] but within the range of MMHg reported for various algal groups from the Eel River.[71, 72] The %MMHg (mean ±1SD) of organisms changed with general feeding group from filamentous algae (43±19%, n=4) to collector-gatherers and filtering organisms (54±15%, n=10; e.g., Asian clam, caddisfly larva, and burrowing mayfly larva) to predatory invertebrates (92±7%, n=12; e.g., dragonfly larva, damselfly larva, and creeping waterbug) and mosquitofish (82±6%; n=3). This trend is consistent with the preferential trophic transfer of MMHg via biomagnification, as reported previously in Cache Creek (e.g., [11, 13]).

Following the approach of Tsui et al. [44] and others ([42, 43, 60]), we evaluated relationships between %MMHg and Hg isotope values to determine whether the Hg isotopic composition of Cache Creek biota could be explained as linear mixtures of isotopically distinct IHg and MMHg pools. The Δ^{199}Hg of all biota generally increased with increasing %MMHg (r^2 of 0.34, $p<0.001$) and at 0% MMHg (i.e., 100% IHg) the Δ^{199}Hg (0.19±0.17‰, intercept ±1SE) was within error of bulk sediment (Δ^{199}Hg of 0.10±0.07‰, 1SD, n=11; Figure 2A). When samples were separated by year (2013 and 2014) the relationship for 2014 biota strengthened ($r^2=0.51$, $p<0.001$), but no significant relationship

existed for 2013 biota (r^2= 0.05, p = 0.64; Figure 2A). We estimate the Δ^{199}Hg of IHg and MMHg for each year by extrapolating these relationships to 0% MMHg (i.e., 100% IHg) and 100%MMHg (Figure 2A; Table 1A). The δ^{202}Hg of biota did not increase with increasing %MMHg in either 2013 or 2014, nor when annual data were combined (r^2 of 0.10, p =0.08, Figure 3A). Although positive relationships between δ^{202}Hg and %MMHg have been reported in lakes, forests and the coastal ocean,[43, 44, 60] the lack of such a relationship in Cache Creek is consistent with other California streams.[42, 44] Similar to Δ^{199}Hg estimates, we estimated the δ^{202}Hg of IHg and MMHg in the food web by extrapolation to 100% MMHg and 100% IHg. When there was no significant linear relationship between %MMHg and either Δ^{199}Hg or δ^{202}Hg, we also estimated Hg isotope values for MMHg by calculating the mean values of organisms with >80% MMHg (SI Table 4) following [44]. Estimates for the δ^{202}Hg and Δ^{199}Hg of MMHg using each method were within error and therefore, for consistency with other studies (e.g., [42]), we use linear estimates in the following discussion. The estimated isotopic composition (δ^{202}Hg and Δ^{199}Hg) of MMHg and IHg in the Cache Creek food web each year is summarized in Table 1A.

3.2.2. Yolo Bypass

In Yolo Bypass we did not observe significant differences in THg or MMHg concentrations between different wetlands or sampling years for benthic macroinvertebrates (67 to 524 ng/g and 60 to 426/g, respectively) or forage fish (125 to 573 ng/g and 114 to 630 ng/g, respectively). The reported THg and MMHg concentrations are similar to previous investigations of fish[16] and invertebrates[17] in Yolo Bypass, which identified such wetlands as potential hotspots of methylation. In this study we observed

relatively high MMHg concentrations in many invertebrate predators (e.g., water scavenger beetle, creeping waterbugs, and dragonfly larva) that also had high %MMHg (>81%). Although damselfly larva, midge larva and fairy shrimp had slightly lower MMHg concentrations, their %MMHg was relatively high (55 to 95% MMHg). Overall, 13 of 16 benthic macroinvertebrate samples collected contained greater than 80% MMHg. Yolo Bypass forage fish (Mosquitofish and Mississippi silverside) had a large range in MMHg (114 to 630 ng/g, n=6) but consistently high %MMHg (> 87%); similar to forage fish from elsewhere in the watershed (Cache Creek and the Yuba River).[42] Although we did not observe an increase in %MMHg across feeding groups (or presumed trophic levels), the diverse assemblage of aquatic organisms with elevated MMHg concentrations and consistently high %MMHg strongly suggest that MMHg bioaccumulation occurs in Yolo Bypass food webs, consistent with other studies carried out in this region.[10, 15-17]

The Δ^{199}Hg and δ^{202}Hg of all Yolo Bypass biota generally increase with increasing %MMHg (Figure 2B, 3B). To estimate MMHg isotopic compositions the biota was grouped by individual wetland, because sediment Hg isotopic compositions were different in each location. There were very few organisms with less than 80% MMHg in each wetland and, therefore, we could not test whether biota Hg isotopic compositions are explained by mixtures of IHg and MMHg pools (i.e., the required assumptions for linear regression between %MMHg and Hg isotope values were not met). Instead we estimated the isotopic composition of MMHg in each wetland from the mean Hg isotope values (both δ^{202}Hg and Δ^{199}Hg; ±1SD) for high %MMHg organisms (>80%), following Tsui et al. and Donovan et al.[42, 44]. The estimated isotopic composition of MMHg in each location is summarized in Table 1B.

3.3 MMHg Photodegradation

Large magnitude, odd mass number MIF (Δ^{199}Hg or Δ^{201}Hg) is thought to result from photochemical processes including inorganic Hg^{2+} photochemical reduction and MMHg photodegradation.[23, 30, 73, 74] The $\Delta^{199}Hg/\Delta^{201}Hg$ ratio of MMHg in the food web and measured in biota has been used to differentiate between Hg^{2+} photochemical reduction (ratio of ~1.0) and MMHg photodegradation (ratio between ~1.2 and ~1.4).[30, 75] Cache Creek and Yolo Bypass biota have $\Delta^{199}Hg/\Delta^{201}Hg$ ratios of 1.23±0.03 (1SE, n= 32; SI Figure 5) and 1.16±0.03 (1SE, n=22; SI Figure 6), respectively. The Cache Creek ratio falls between literature averages for freshwater fish (1.28±0.01; 1SE, n=135)[23] and marine fish (1.20±0.01; 1SE, n=60),[23] and is similar to biota from nearby rivers (Yuba R.=1.27±0.03 and Eel R.=1.28±0.08).[42, 44] The Yolo Bypass ratio is on the low end of the range reported for MMHg photodegradation experiments (1.17 to 1.38),[75] but still comparable to Cache Creek and also to forest biota from northern Michigan (1.21±0.03)[43] and northern California (1.15±0.06, n=10)[44]. The observed ratios strongly suggest that the MIF observed in biota, and therefore the estimated Δ^{199}Hg and Δ^{201}Hg of MMHg, results from MIF during photodegradation of MMHg.

The extent of MMHg photodegradation, prior to the MMHg entering the food web, can be quantified from experimental relationships that are sensitive to parameters such as dissolved organic carbon (DOC) concentration,[30, 75] MMHg:DOC ratios,[73] and the wavelength of incident radiation.[76] We use different experimental relationships for Cache Creek and Yolo Bypass to account for differences in reported DOC concentrations. In non-agricultural Yolo Bypass wetlands, the median porewater DOC was 12 mg/L (n=20)[14] and

wetland surface water DOC ranged from 6 to 10 mg/L.[22] Therefore, we use 10 mg/L experimental relationships[30] to estimate that between 9% and 12% of MMHg had undergone photodegradation in Yolo Bypass wetlands in this study. This finding is comparable to Florida Lakes where photodegradation was thought to be inhibited by low water clarity and high DOC.[53] In Cache Creek, surface water DOC measured downstream of Capay during a recent 4 year period (1999-2003) was 2.8±0.12 mg/L (1SE, n=104)[77] and at Rumsey surface water DOC was separately reported between 1 and 3 mg/L.[78] Therefore, we use 1 mg/L DOC experimental relationships to estimate that in 2014 ~31±4% of MMHg in Cache Creek had undergone photodegradation, which is higher than the estimated extent of MMHg photodegradation in 2013 (~17±3%). The estimates in Cache Creek are similar to observations nearby in the Yuba River (24-35%)[42] and the South Fork Eel River (27%)[44] and much greater than estimated MMHg photodegradation in Yolo Bypass wetlands.

The extent of MMHg photodegradation in Cache Creek was significantly greater in 2014 than in 2013. This result is consistent with a parallel study of the Yuba River where the extent of photodegradation was also higher in 2014 (35%) than in 2013 (24%).[42] The Yuba River and Cache Creek are on opposite sides of the Sacramento Valley (75 km apart) and contaminated by different Hg sources (Au mining vs. Hg mining) but experience relatively similar environmental conditions (e.g., high sunlight/low shading). Cache Creek and the Yuba River both had higher flows during sampling in 2013 than in 2014, due to a progressive drought that decreased discharge in many California rivers and streams. Regional changes in streamflow could control the extent of MMHg photodegradation by changing water depth, water clarity, and MMHg residence time. However, we should also note that the timing of sampling was different each year. Both streams were sampled in

early spring in 2013 compared to early summer in 2014, and seasonal streamflow and canopy cover are thought to be important factors in MMHg photodegradation.[50] Therefore, the results could suggest a greater extent of MMHg photodegradation occurred prior to sampling in June 2014 (i.e., during springtime) than prior to sampling in March 2013 (i.e., during winter). Thus, changes in stream conditions and/or the timing of sampling could explain the increase in MMHg photodegradtion between years for both Cache Creek and the Yuba River. This suggests that the isotopic composition MMHg in short-lived benthic macroinvertebrates is useful for identifying relatively quick (i.e., seasonal or annual) changes in MMHg photodegradation in stream environments.

3.4 MMHg Exposure Pathways

Previous studies have estimated the isotopic composition of MMHg and IHg in food webs (e.g., [43, 44, 60]) to understand Hg sources and biogeochemical transformations. This approach assumes that the Hg isotopic composition of biota sampled is a mixture of isotopically distinct IHg and MMHg pools. [44] This assumption and method of interpreting foodweb data can be tested by comparing %MMHg and Hg isotope values (δ^{202}Hg or Δ^{199}Hg). In Cache Creek, the deviation from a linear relationship between %MMHg and Hg isotope values, and the different estimated MMHg isotopic compositions each year, provide evidence for multiple isotopically distinct pools of IHg and MMHg. For example, Asian clam and filamentous algae exhibit a ~1‰ range in δ^{202}Hg (−1.15 to −0.18‰; Figure 3A) in Cache Creek. Asian clam are filter feeding bivalves that obtain particles from the water column and substrate[79] and filamentous algae trap suspended sediment. These feeding behaviors and physical characteristics probably lead to the accumulation of IHg from

sediment, with a 1.5‰ range in δ^{202}Hg, and explain the variation in some biota δ^{202}Hg values. The Δ^{199}Hg of Cache Creek biota may also be affected by feeding behaviors. For example, aquatic worm and burrowing mayfly larva, which non-selectively consume benthic detritus and sediment, fall below the linear relationship for %MMHg vs. Δ^{199}Hg (Figure 2A). Since the Δ^{199}Hg of MMHg is driven by the extent of MMHg photodegradation,[23, 30] these benthic organisms could have accumulated MMHg from the benthic substrate that has undergone less photochemical degradation (i.e., lower Δ^{199}Hg) than MMHg accumulated by other organisms.

In Yolo Bypass the measured Δ^{199}Hg of biota cannot be explained by a single MMHg isotopic composition because high %MMHg biota (>80%) had a 1.5‰ range in Δ^{199}Hg (Figure 2B). The lowest Δ^{199}Hg values were measured in UW and LW omnivorous crayfish (0.34 to 0.66‰), which typically forage near the sediment in wetland environments. Conversely, some of the highest Δ^{199}Hg values (0.76 to 1.81‰, n=6) were measured in mosquitofish and Mississippi silversides. Mosquitofish consume zooplankton and macroinvertebrates near the water surface[80] and Mississippi silversides are planktivores that consume zooplankton and particulates in the water column.[81] Thus, the wide range in Δ^{199}Hg among high %MMHg organisms in Yolo Bypass suggests that biota may be exposed to different pools of MMHg that have been more or less photodegraded (i.e., MMHg pools with higher or lower Δ^{199}Hg). Some evidence for multiple MMHg pools has been observed in previous studies. For example, zooplankton in arctic lakes had Δ^{199}Hg (1.5 to 3.4‰, n=6) that was much higher than co-located benthic organisms.[82] Therefore, in contrast to studies that have demonstrated binary mixing between two isotopically distinct IHg and MMHg pools, we suggest that the Hg isotope and %MMHg data from Cache Creek and Yolo Bypass

provide evidence for multiple MMHg isotopic compositions within a single habitat. Further, we suggest that the bioaccumulation of these isotopically distinct MMHg pools results from the differences in feeding behavior of organisms. Therefore future Hg isotope studies should carefully consider the feeding behavior of the aquatic organisms that are sampled whose diets might change with habitat, prey availability, and age. Overall, these findings suggest that Hg isotope measurements may aid in separating benthic vs. planktonic exposure pathways, similar to the past use of Hg isotopes to understand exchanges across the aquatic-riparian interface.[44, 62]

3.5 Linking IHg Sources to MMHg

To link IHg sources to MMHg in the food web we subtracted the known amount of MDF that occurs during photodegradation in proportion to the MIF that occurs exclusively during MMHg photodegradation. This approach has been used to estimate the δ^{202}Hg of MMHg prior to photodegradation ("pre-photodegraded MMHg") in previous studies (e.g., [51-54, 60]) and identify MDF between MMHg and potential IHg sources. Here, we assume that all Δ^{199}Hg of MMHg results from photochemical degradation, which would be valid if MMHg is formed from Yolo Bypass wetland sediment (Δ^{199}Hg of 0.09±0.03‰) or Cache Creek sediment (Δ^{199}Hg of 0.10±0.07‰). From the estimated isotopic composition of MMHg and DOC concentration in each location (Table 1A, 1B), we used experimentally derived Δ^{199}Hg vs. δ^{202}Hg slopes (2.43 for 1 mg/L DOC and 4.79 for 10 mg/L DOC)[30] to estimate the δ^{202}Hg of pre-photodegraded MMHg in Cache Creek to be between −1.40 and −1.45‰ in 2013 and 2014 (Figure 4). In Yolo Bypass we estimate the δ^{202}Hg of pre-photodegraded MMHg for

each individual wetland: −0.51‰, −0.13‰ and −0.37‰ for UW, PW2 and LW, respectively (Figure 5).

3.5.1. Yolo Bypass

The $\delta^{202}Hg$ of wetland sediment in Yolo Bypass varies as a function of THg concentration, suggesting a mixture of mining-derived and non-mining sediment (Figure 1). In each wetland the estimated $\delta^{202}Hg$ of pre-photodegraded MMHg is higher than the measured $\delta^{202}Hg$ of sediment, which consists of >95% IHg. Thus, we observe positive $\delta^{202}Hg$ offsets ($\delta^{202}Hg_{\text{pre-photodegraded MMHg}} - \delta^{202}Hg_{\text{IHg}}$) of +0.35‰, +0.49‰, and +0.16‰ for UW, PW2, and LW, respectively (Figure 5). These $\delta^{202}Hg$ offsets are similar in both direction and magnitude to previous studies of lakes, estuaries and the coastal ocean (+0.4 to +0.8‰),[51-53, 60] where it was suggested that IHg in sediment is biotically methylated (−MDF)[26, 27] followed by significant mer-mediated biotic degradation (+MDF)[24], such that the residual MMHg has higher $\delta^{202}Hg$ than the sediment (net positive biotic MDF).[51, 53] Positive $\delta^{202}Hg$ offsets in Yolo Bypass wetlands (0.16 to 0.49‰) are consistent with this interpretation, indicating that at least a portion of the MMHg in Yolo Bypass wetland food webs is formed in situ from sediment. As mentioned above (Section 3.4), the $\Delta^{199}Hg$ of biota in Yolo Bypass suggests that multiple pools of MMHg with different $\Delta^{199}Hg$ values might exist within these wetlands. However, we cannot discern whether the differences in the estimated $\Delta^{199}Hg$ of MMHg is related to the formation of MMHg from different Hg sources in these wetlands or if all MMHg originates from the same source but is photodegraded to varying extents. In either case, the positive $\delta^{202}Hg$ offsets link sediment IHg to MMHg in biota and suggest that the transport and deposition of IHg-enriched sediment from

upstream is an important process that supplies IHg, and eventually MMHg, to downstream wetland food webs.

3.5.2. Cache Creek

The negative δ^{202}Hg offset between MMHg and various IHg sources in Cache Creek (Figure 4) is similar to other studies of river systems (e.g., [42, 44]) but contrasts with positive δ^{202}Hg offsets in Yolo Bypass. In Cache Creek the Δ^{199}Hg of MMHg changed between 2013 (0.60±0.04 ‰) and 2014 (1.22±0.08‰), yet the estimated δ^{202}Hg of pre-photodegraded MMHg was nearly identical each year (−1.40 and −1.45‰). This suggests that the MMHg likely originated from the same source, but was photodegraded to a different extent each year yielding different Δ^{199}Hg values. There is an overlap between pre-photodegraded MMHg δ^{202}Hg and sediment δ^{202}Hg in Cache Creek. However, the δ^{202}Hg of pre-photodegraded MMHg is ~0.7‰ lower than the estimated δ^{202}Hg of IHg in the food web (−0.59 to −0.68‰; Table 1A) and ~0.4‰ lower than the mean δ^{202}Hg of co-located sediment (−0.99 ± 0.45‰). This relationship (δ^{202}Hg offset of −0.4 to −0.7‰) is consistent in both direction and magnitude with the nearby Yuba River (δ^{202}Hg offset of −0.4 to −0.9‰).[42] The negative δ^{202}Hg offset in Cache Creek could indicate that either (1) a labile IHg source (i.e., not bulk sediment or IHg in the food web) with δ^{202}Hg less than −1.4‰ is methylated or (2) in-situ methylation of sediment IHg results in net negative MDF in Cache Creek.

Labile IHg with a lower δ^{202}Hg value than bulk sediment could originate from external watershed sources or through biogeochemical processes within the stream. If we assume the δ^{202}Hg offset in Cache Creek is identical to previous non-stream studies (i.e.,

+0.4 to +0.8‰),[51-53, 60] then from the δ^{202}Hg of pre-photodegraded MMHg we would predict labile IHg to have δ^{202}Hg between −2.2 and −1.4‰. The presence of MMHg in streams is often considered a function of drainage basin landscape characteristics that promote Hg deposition and IHg-methylation within the watershed.[83-86] IHg is stored in terrestrial organic matter and soils within watersheds, and typically has low δ^{202}Hg (−1.0 to −2.5) and slightly negative Δ^{199}Hg (−0.1 to −0.4‰).[58,44] The δ^{202}Hg range for these IHg sources is consistent with predicted labile IHg values (−2.2 to −1.4‰), however Cache Creek's steep mountainous catchment has high erosion rates which does not allow for significant accumulation of surface organic matter.[87] The mass of IHg in surface organic matter is likely very small relative to Hg from mine waste in the watershed (e.g., ~100 Mg of Hg is stored upstream in Clear Lake).[70] It was previously observed that MMHg concentrations in Cache Creek biota increase with distance from upstream reservoirs[11] and methylation in Cache Creek is thought to be promoted by in situ geochemical conditions such as high sulfate.[11, 66, 88] Other studies have suggested IHg in streams can be methylated in hyporheic zones[89] or when associated with epilithic periphyton[90] or filamentous algae.[71] Therefore, we suggest it is more likely that in-stream IHg sources and biogeochemical processes in Cache Creek lead to MMHg in the Cache Creek food web.

It is likely that only a fraction of IHg in Cache Creek sediment is labile and available for methylation.[14, 86] If this fraction has lower δ^{202}Hg than the mean sediment δ^{202}Hg, then the negative δ^{202}Hg offset between sediment and pre-photodegraded MMHg would be an artifact of this difference. Earlier studies demonstrated systematic differences in the δ^{202}Hg of various sediment size fractions.[32, 35] We observed δ^{202}Hg in Cache Creek <1mm sediment (−1.69 and −1.45‰) that is similar to the pre-photodegraded MMHg δ^{202}Hg (−1.4 to

−1.45‰), but we cannot explain why comparably low δ^{202}Hg was not observed in any individual size fraction (1mm-63μm or <63 μm). Hg that is leached (e.g., water soluble, thiosulfate extractable, etc.) from Hg mine wastes (calcine, ore, sediment, etc.) is potentially labile and experiments have identified sediment leachates with δ^{202}Hg values up to 1.3‰ higher than bulk materials.[31, 37, 91] Sequential extraction of calcine mine wastes at the New Idira Hg mine (CA) similarly found higher δ^{202}Hg values in more soluble and easily extractable phases.[39] This result is not consistent with our prediction that a labile Hg fraction will have δ^{202}Hg values lower than bulk sediment. Alternatively, in situ co-precipitation or sorption reactions would be expected to fractionate Hg mass dependently resulting in lower δ^{202}Hg values for the reaction products (−MDF, e.g., HgS or Hg bound to colloids).[28, 29] Sequential extraction of residual Hg phases, presumably HgS, in New Idria calcine wastes had lower δ^{202}Hg than the bulk material,[39] however a separate study found that HgS species extracted from Hg contaminated sediment had higher δ^{202}Hg than bulk sediment.[31] Clearly it is possible for different pools of Hg within sediment to have δ^{202}Hg that deviates from bulk sediment values, depending on the source material, transport history, and in situ biogeochemical reactions. In this study, we cannot distinguish a particular sediment size-fraction or Hg-species that would have δ^{202}Hg between −1.40 and −1.45‰ and that would be preferentially methylated in Cache Creek. Below we consider the alternative scenario that the δ^{202}Hg offset results from net negative MDF of up to 0.7‰ between IHg and MMHg in Cache Creek.

3.6 Comparison of MDF in Streams and Wetlands

In this study we observed a negative offset between sediment IHg and pre-photodegraded MMHg in Cache Creek that contrasted with positive δ^{202}Hg offsets in three Yolo Bypass wetlands. Our results, along with the significant negative δ^{202}Hg offset observed in a previous study of the Yuba River,[42] suggests a fundamentally different MDF behavior in streams compared to other aquatic environments (e.g., coastal oceans, lakes, and wetlands). In the Yuba River, where a negative δ^{202}Hg offset was observed, we proposed that net negative MDF could result from either a lack of biotic MMHg degradation or a different biotic degradation pathway.[42] Since both mechanisms are partially controlled by physical and geochemical conditions that change between flowing and non-flowing water environments, we hypothesized that the negative δ^{202}Hg offset might be characteristic of stream environments. The results of this study are consistent with this hypothesis. In light of the results in Cache Creek and Yolo Bypass, we reexamine the possible mechanisms for net negative MDF in stream environments.

If MMHg is formed from sediment IHg then the negative δ^{202}Hg offset in the Yuba River and Cache Creek suggests a lack of +MDF during MMHg formation or degradation. In previous work, we suggested that biotic MMHg degradation might occur through a different pathway, such as oxidative MMHg degradation,[92] and thereby change the observed net biotic MDF. Although Hg isotope fractionation during oxidative degradation has not yet been measured, this mechanism was proposed because the product of oxidative MMHg degradation (Hg^{2+}) could be remethylated (i.e., additional −MDF)[26, 27] whereas the product of *mer*-mediated degradation (Hg(0)) is partially removed, resulting in +MDF.[24] It is generally thought that mer-mediated degradation is dominant in contaminated environments where bioavailable Hg is high and oxidative MMHg degradation is more

common in pristine environments.[92, 93] If this were the case, we would expect significant +MDF from *mer*-mediated degradation (i.e., a $+\delta^{202}$Hg offset) in both the Yuba River and Cache Creek where large quantities of IHg-enriched sediment persists. Instead, we observed $-\delta^{202}$Hg offsets in both streams. Similarly, we might expect minimal +MDF from mer-mediated degradation (i.e., small or no $+\delta^{202}$Hg offset) in locations where sediment is not enriched with Hg from mining sources. In contrast to these expectations, in Yolo Bypass UW where sediment THg concentrations are at pre-mining levels (36 to 62 ng/g) we observed a positive δ^{202}Hg offset (+0.35‰) and found that the magnitude of the δ^{202}Hg offset is not related to sediment THg concentration across the Yolo Bypass wetlands. This is consistent with a study of multiple estuaries on the NE USA coast where positive δ^{202}Hg offsets were measured regardless of sediment THg (which ranged from 6 to 2,960 ng/g).[60] In the future, measurements of geochemical parameters that control Hg bioavailability (i.e., DOC, redox, etc.) or *mer*-enzyme activity, in combination with Hg isotope measurements, may help to clarify whether changes in the biotic MMHg degradation pathways could affect net MDF of Hg in aquatic environments.

Without invoking a unique oxidative MMHg degradation process, the simplest explanation for the negative δ^{202}Hg offset observed in Yuba River and Cache Creek is a relative lack of biotic MMHg degradation in stream environments. An experimental study of biotic IHg-methylation and MMHg degradation suggested that turbulent diffusion of MMHg from the IHg substrate could increase the magnitude of negative MDF.[27] Following this idea, we propose that in situ methylation in flowing water likely advects MMHg to the water column, removing it from the substrate and decreasing its availability for biotic MMHg degradation.[42] This would result in pre-photodegraded MMHg with lower δ^{202}Hg

than the IHg substrate because it would have undergone relatively little +MDF. Conversely in standing water, such as in wetlands, MMHg is likely stored in sediment for a longer period of time leading to a greater extent of in situ biotic MMHg degradation and significant +MDF. The results of this study, with −MDF in Cache Creek and +MDF in Yolo Bypass wetlands, are consistent with the hypothesis that biotic MMHg degradation occurs to a lesser extent in streams than in standing water environments. Thus, photochemical MMHg degradation is a relatively more significant MMHg degradation pathway in streams (up to 35% in the Yuba River and 31% in Cache Creek) than in wetlands (e.g., 9–12% in Yolo Bypass) and other non-flowing water environments where biotic MMHg degradation likely occurs to a greater extent.

3.7 Conclusions

This study provides new insight that will aid in future tracing of Hg and MMHg in stream and wetland food webs. Analysis of THg, MMHg and Hg isotopes in benthic macroinvertebrates and forage fish proved valuable for estimating the isotopic composition of MMHg. Our comparisons of Hg isotopes and %MMHg values in biota provide evidence for multiple MMHg isotopic compositions or MMHg pools within a single habitat. This provides further evidence that the specific feeding behavior of aquatic organisms could help to identify benthic and planktonic MMHg exposure pathways in future Hg isotope studies. In this study, we compared the δ^{202}Hg of IHg (estimated in the food web or measured in sediment) with the δ^{202}Hg of pre-photodegraded MMHg. We found both positive (Yolo Bypass Wetlands) and negative (Cache Creek and Yuba River[42]) δ^{202}Hg offsets within the same watershed. We suggest that this different net MDF could

result from the absence of biotic MMHg degradation in streams compared to other non-flowing water environments (e.g., wetlands, lakes). This result implies that photochemical MMHg degradation is a more significant MMHg degradation pathway than biotic MMHg degradation in stream environments.

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Tables

Table 1: Estimated isotopic compositions of Hg pools in (A) Cache Creek and (B) Yolo Bypass. Cache Creek IHg and MMHg values shown here are estimated from linear relationships between %MMHg and Hg isotope values for each year (2013 and 2014; Figure 2A and3A). The errors reported are the 1SE of the y-intercept at 100% MMHg or 100% IHg. Yolo Bypass MMHg values are estimated from all organisms in each wetland across both years (2013 and 2014 not separate) that had greater than 80%MMHg. The reported errors in Yolo Bypass are the 1SD of the mean values for these organisms.

(A)		δ^{202}Hg	1SE	Δ^{199}Hg	1SE
		‰	‰	‰	‰
2013	IHg	-0.59	0.15	0.55	0.07
	MMHg	-1.16	0.08	0.60	0.04
2014	IHg	-0.68	0.16	0.06	0.18
	MMHg	-0.92	0.07	1.22	0.08

(B)		δ^{202}Hg	1SD	Δ^{199}Hg	1SD	n
		‰	‰	‰	‰	
Upper Wetland (UW)	MMHg	-0.30	0.16	0.99	0.44	9
Permanent Wetland 2 (PW2)	MMHg	0.08	0.28	0.96	0.18	7
Lower Wetland (LW)	MMHg	-0.22	0.10	0.70	0.21	3

Figures

Figure 1: Inverse sediment THg concentration (1/THg) vs. δ^{202}Hg for all sediment from this study, a previous study in the Yuba and Feather Rivers,[42] and an earlier study in San Francisco Bay (SF Bay)[33]. A full legend details all symbols. Diamonds represent Cache Creek and Bear Creek sediment samples with fill patterns representing different size fractions (filled = <63µm, half-filled = 1mm-63µm, empty = <1mm). Colored circles represent Yolo Bypass sediment from different locations (blue = UW, black = PW2, green = LW) and their fill denotes size fraction (filled = <63µm, empty = <1mm). Squares represent sediment previously analyzed from the Yuba and Feather Rivers (orange = Yuba, yellow = Feather).[42] X symbols represent pre-mining sediment previously analyzed from SF Bay subtidal sediment cores.[33] The dashed line and corresponding equation shows the linear relationship between 1/THg and δ^{202}Hg for Yolo Bypass wetland sediment (n=9).

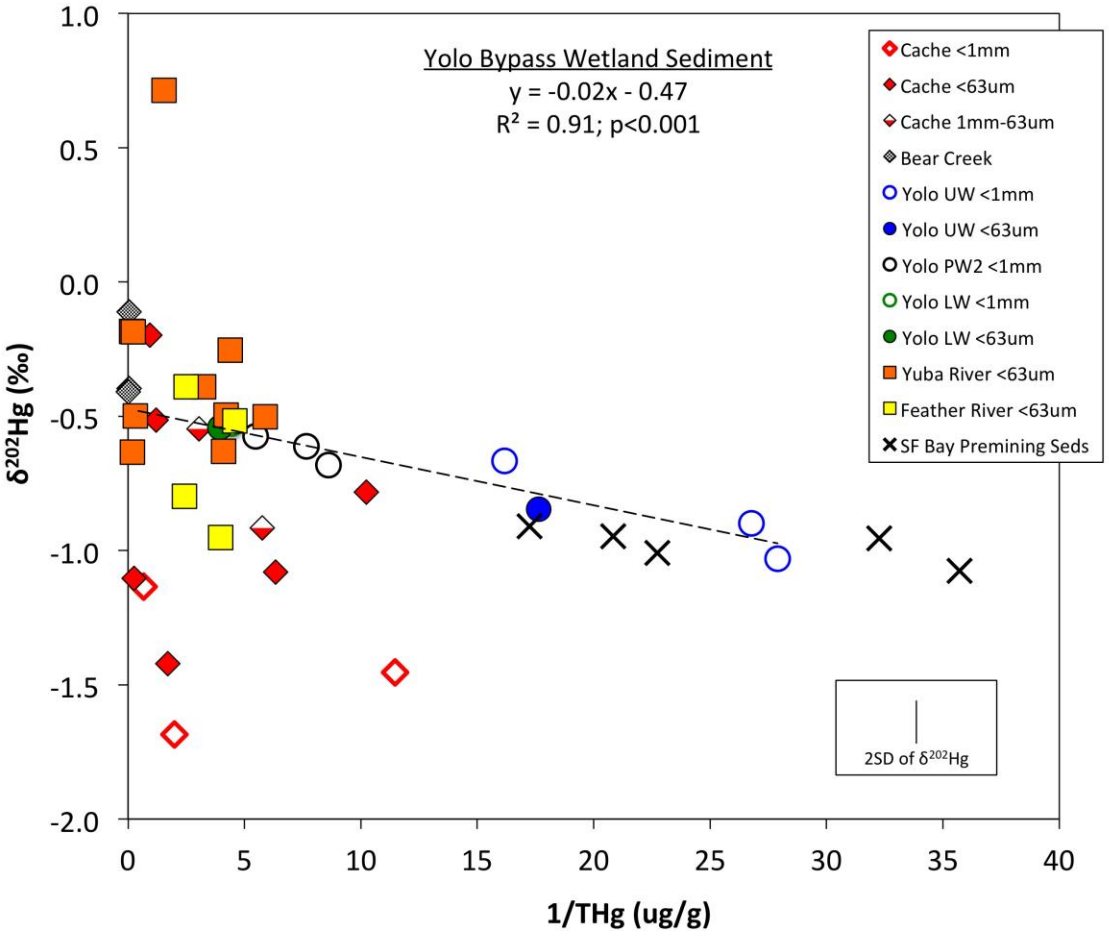
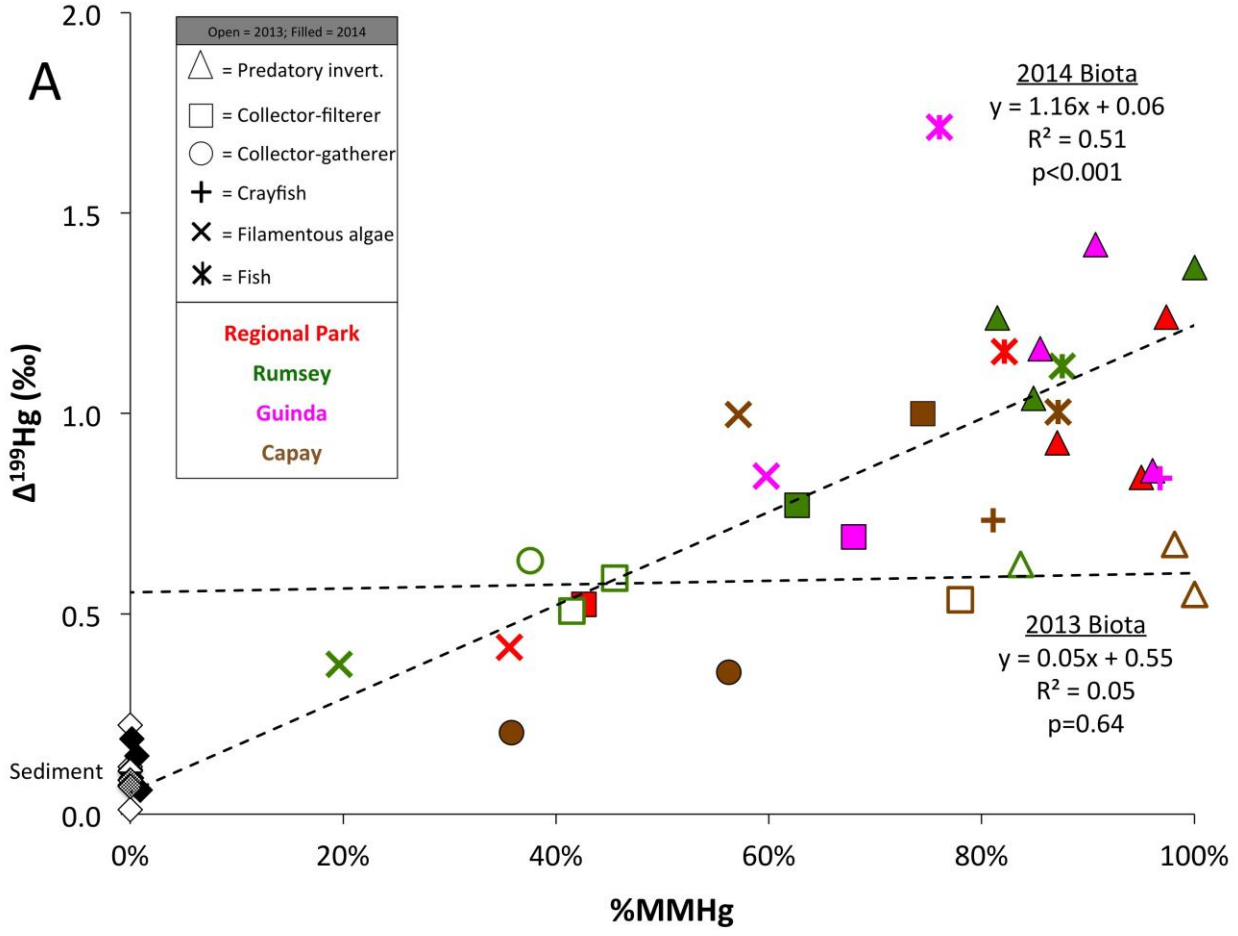


Figure 2: %MMHg vs. Δ^{199}Hg for biota from (A) Cache Creek and (B) Yolo Bypass Wetlands. A detailed legend in each figure explains symbol colors and types. Briefly, in (A) Cache Creek colors represent sampling location (red = Regional Park, green = Rumsey, pink = Guinda, and brown = Capay) and open/filled symbols denote the year of sampling (2013/2014, respectively). The type of symbol represents the sample type. Similarly, for (B) Yolo Bypass, the colors show different wetlands (blue = UW, black = PW2, green = LW) and symbols represent different types of biota. Co-located sediment (diamonds) is included for each location at representative %MMHg values (~<5%), however, sediment was not included in the linear relationships shown.



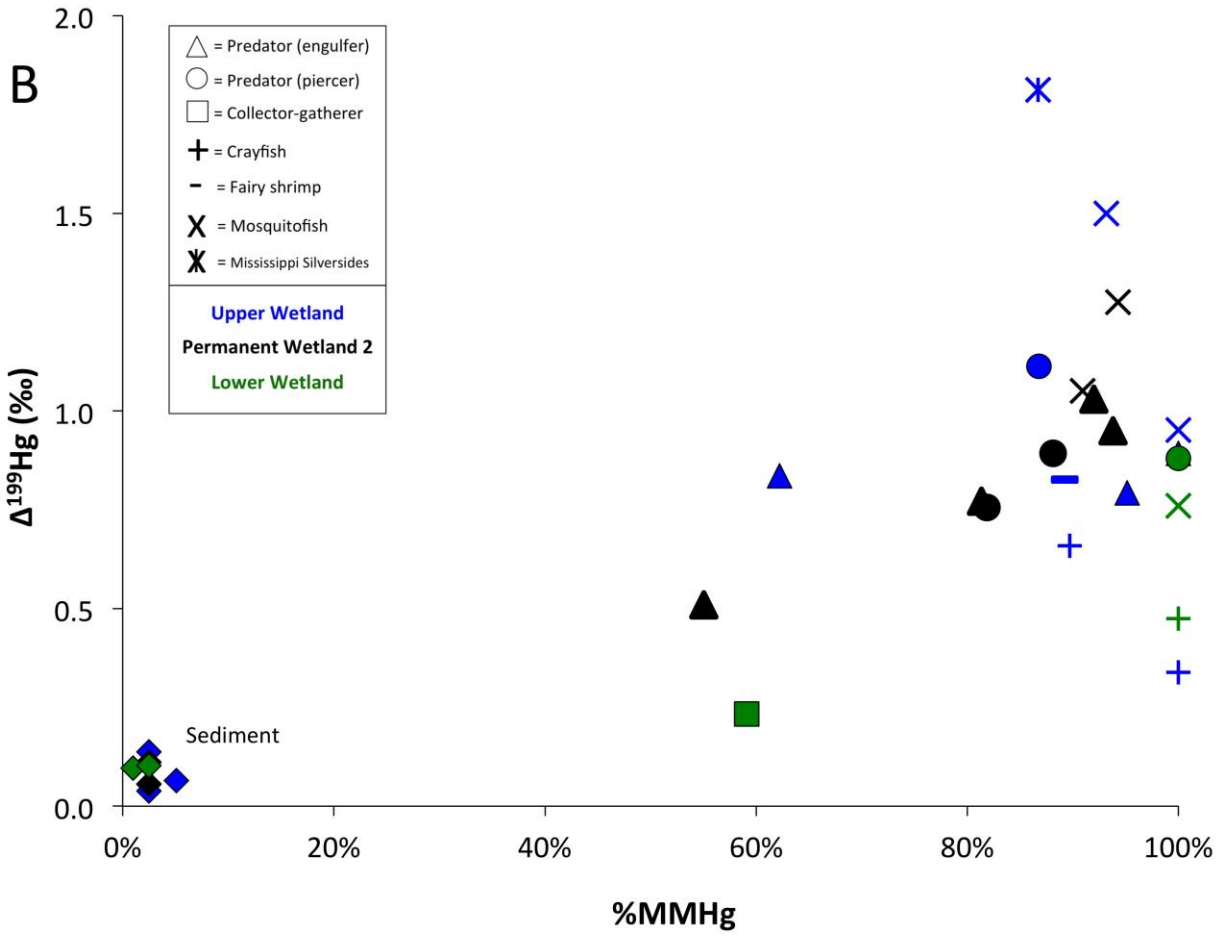
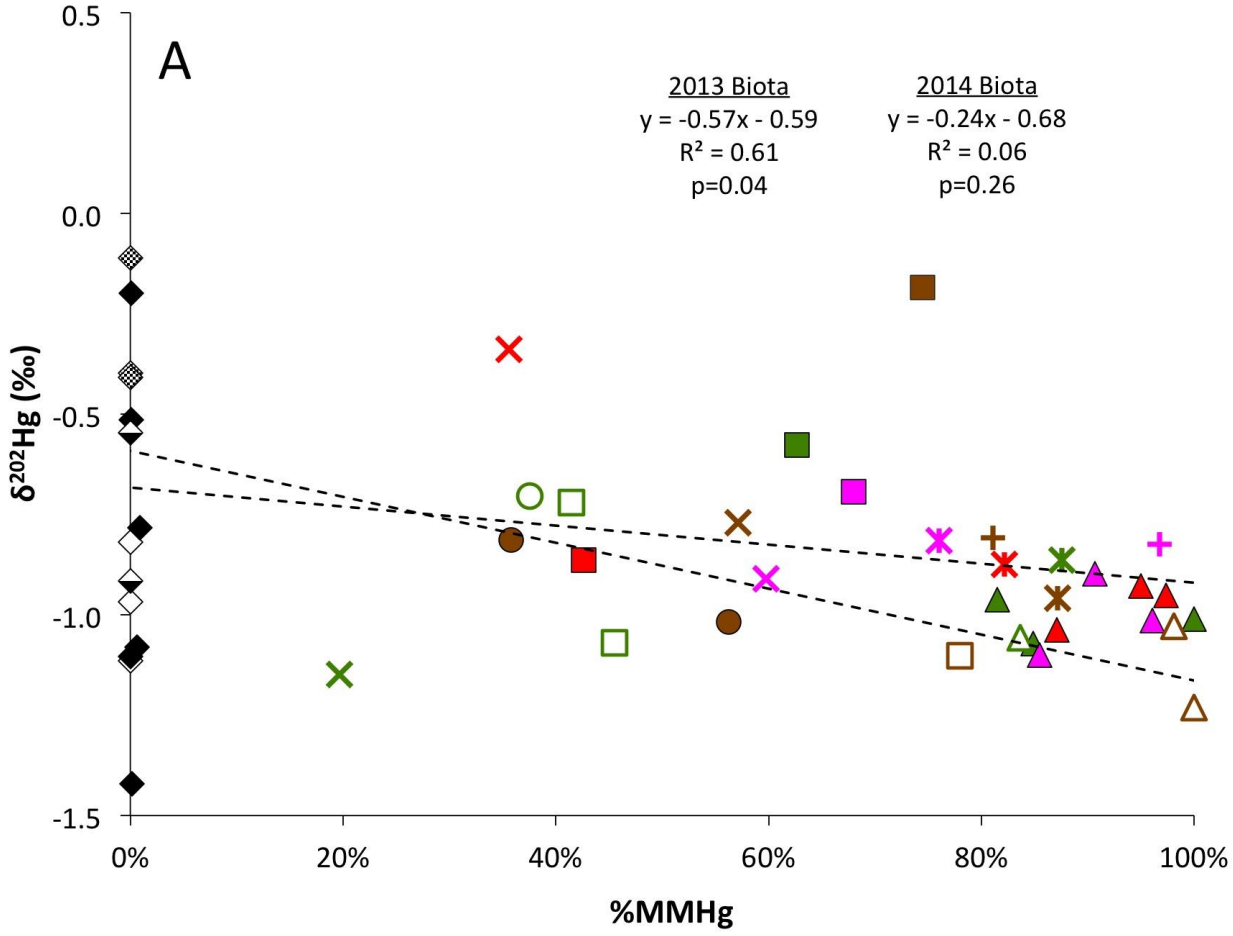

B
= Predator (engulfer)
= Predator (piercer)
= Collector-gatherer
= Crayfish
= Fairy shrimp
= Mosquitofish
= Mississippi Silversides
Upper Wetland
Permanent Wetland 2
Lower Wetland
Sediment
2.0
1.5
1.0
0.5
0.0
Δ199Hg (‰)
0%
20%
40%
60%
80%
100%
%MMHg

Figure 3: %MMHg vs. δ^{202}Hg for (A) Cache Creek and (B) Yolo Bypass Wetlands. Symbol types and colors in this figure (A, B) are identical to the description in Figure 2 (A, B) and presented in detail in the Figure 2 legends and SI Figure 7. Briefly, in (A) Cache Creek colors represent sampling location (red = Regional Park, green = Rumsey, pink = Guinda, and brown = Capay) and open/filled symbols denote the year of sampling (2013/2014, respectively). The type of symbol represents the sample type. Similarly, for (B) Yolo Bypass, the colors show different wetlands (blue = UW, black = PW2, green = LW) and symbols represent different types of biota. Co-located sediment (diamonds) is included for each location at representative %MMHg values (~<5%), however, sediment was not included in the linear relationships shown.



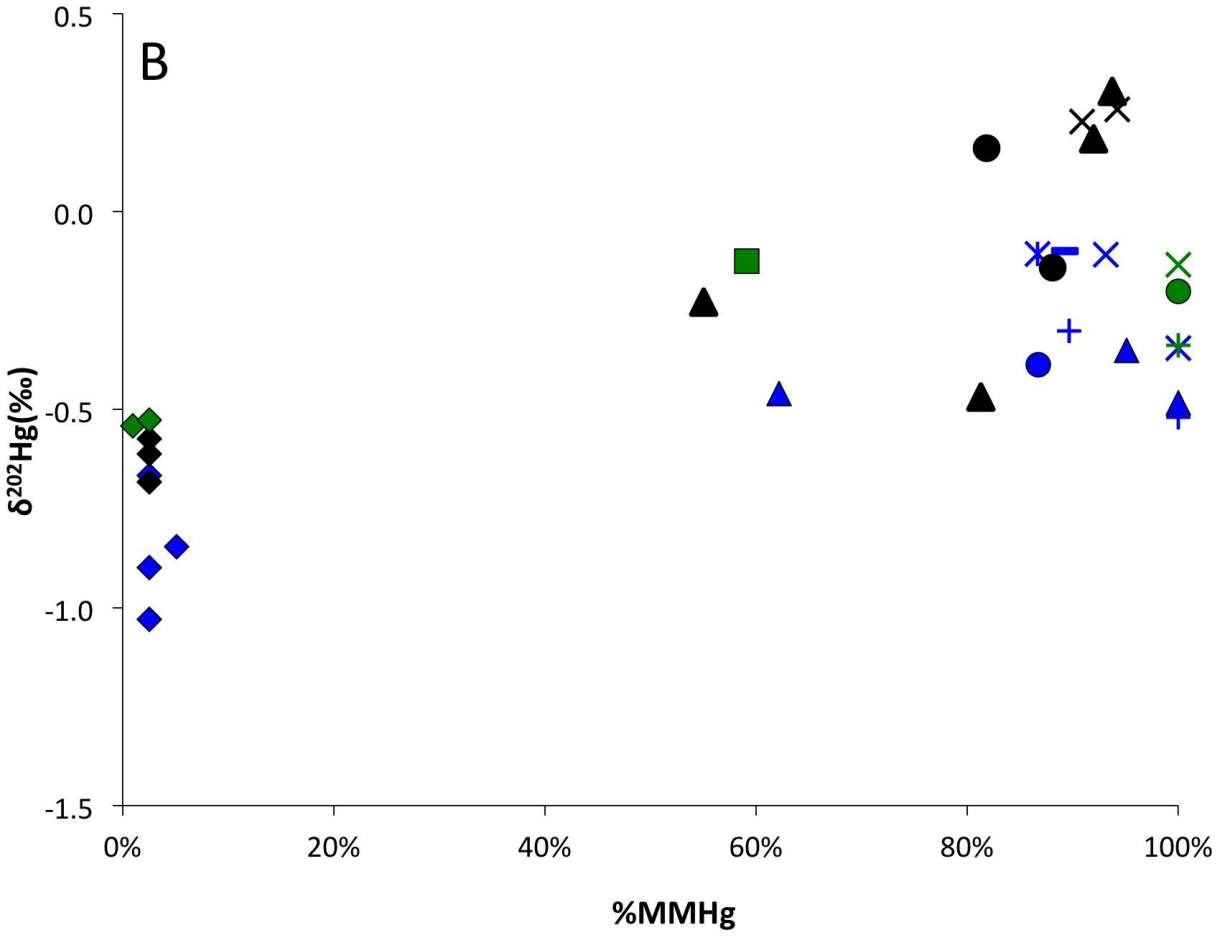

B
0.5
0.0
-0.5
-1.0
-1.5
δ202Hg(‰)
0%
20%
40%
60%
80%
100%
%MMHg

Figure 4: δ^{202}Hg vs. Δ^{199}Hg for biota and sediment in Cache Creek.

Cache Creek sediment is represented by black diamonds and the specific size fraction is indicated by the fill pattern (filled = <63µm, half-filled = 1mm-63µm, empty = <1mm). Bear Creek bulk (<1mm) sediment is shown with gray diamonds. The biota symbol types and colors are identical to those explained in Figures 2 and 3 and the detailed biota legend is included below. The estimated MMHg isotopic composition for each year is labeled ("2013 or 2014 MMHg") and the size of the cross represents the 1SE error associated with the estimated Hg isotope values. The experimental slope for MMHg photodegradation (1 mg/L DOC from[30]) is drawn from estimated MMHg values as a black dashed line to show the δ^{202}Hg of pre-photodegraded MMHg that was identified.

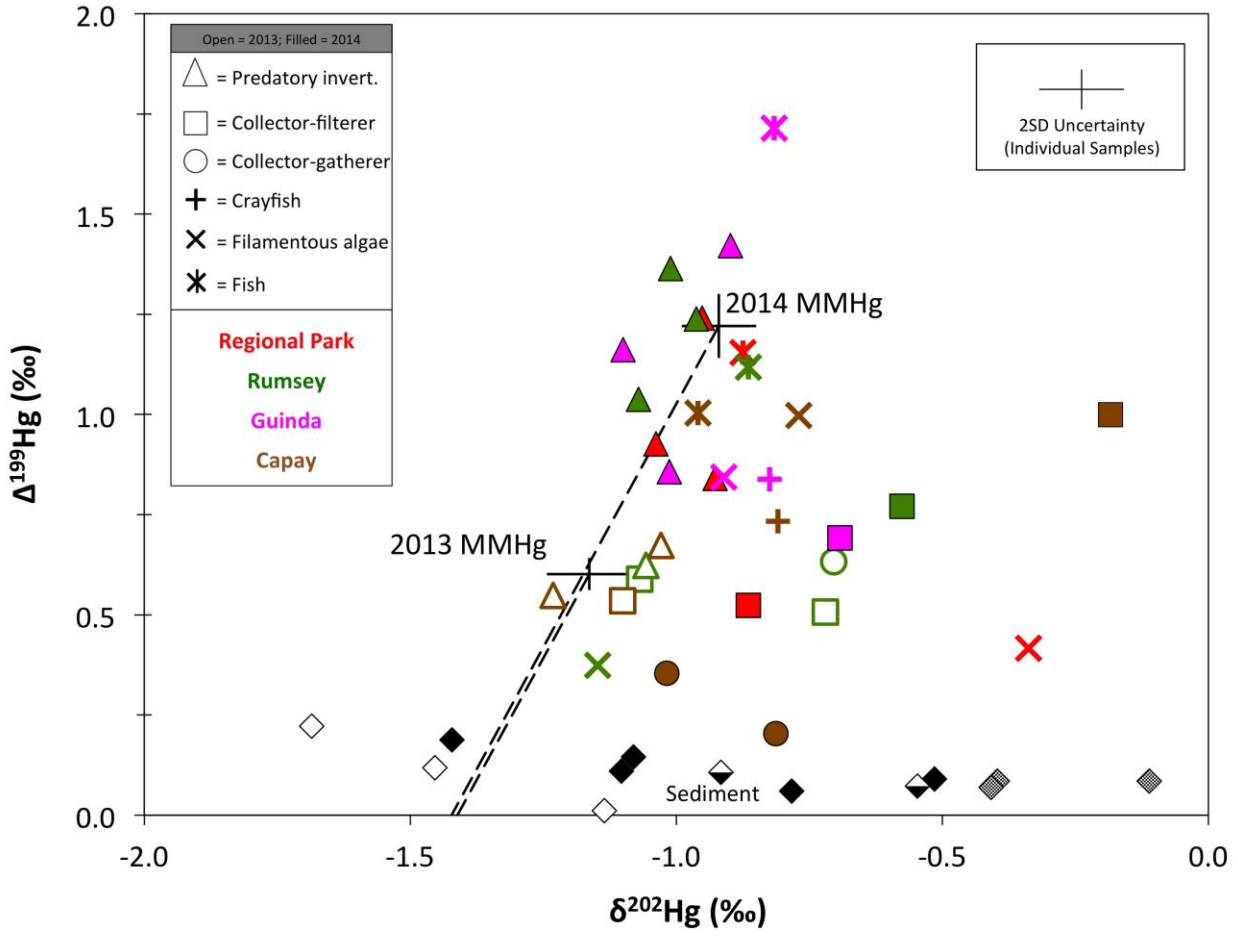
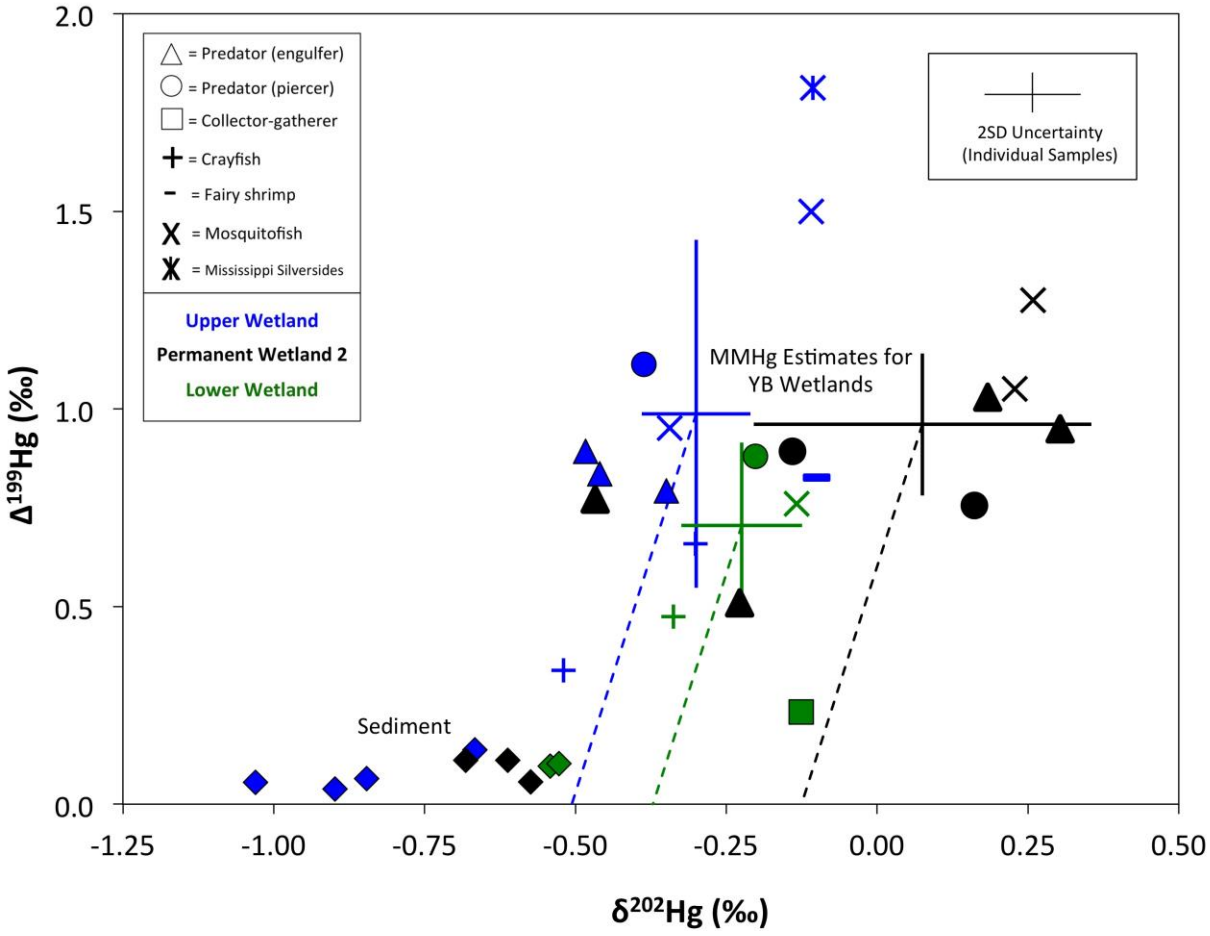


Figure 5: δ^{202}Hg vs. Δ^{199}Hg for Yolo Bypass Wetlands (Upper, PW2 and Lower). All symbols are colored according to the wetland location (blue = UW, black = PW2 and green = LW). The different types of sediment (diamonds) and biota (all other symbols) are identical to the symbols explained in Figures 2 and 3 and the detailed biota legend is included below. The estimated MMHg isotopic composition for each wetland is included as crosses and the size of the cross denotes the 1SE uncertainty for each estimated Hg isotope value. Experimental photochemical degradation slopes for 10 mg/L DOC concentrations (from [30]) are included as dashed lines and colored according to the individual wetland.



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