SHALLOW GROUNDWATER MERCURY SUPPLY IN A COASTAL PLAIN STREAM

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Supporting Information:

**Additional Surface-Water MeHg Sampling and Analysis Details**

USGS clean-sampling protocols and ultra-trace-level clean techniques were used to collect surface-water (SW) samples from the centroid of flow in the stream channels or from out-of-channel surface water locations (perennial wetlands, riparian pools, and seeps) and to collect hyporheic-water (HW) and groundwater (GW) samples from in-stream and floodplain piezometers \(^1\). Detailed descriptions of these procedures and techniques are provided in \(^2\)-\(^6\).

Surface-water samples were collected by dipping new polyethylene terephthalate copolyester, glycol-modified (PETG) containers in the center of flow or in center of the out-of-channel surface-water body using ultra trace-level clean techniques \(^4\), \(^5\), similar to EPA Method 1669 \(^3\). Hyporheic-water and groundwater samples were collected from in-stream and floodplain piezometers, respectively, by low flow pumping using a peristaltic pump and ultra-trace-level clean techniques \(^4\), \(^5\). Water samples were filtered through quartz fiber filters (47-mm diameter, 0.7-µm pore size) and included dissolved and colloidal fractions \(^7\). Filtrate was collected in Teflon\textregistered bottles, acidified to 1 percent HCl by volume, and stored in the dark until analysis. Hyporheic-water and groundwater samples were filtered in the field in a temporary glove box (see SI figures 4 and 6), stored on ice, and shipped overnight to the lab. Fresh gloves, glove box bags, and pre-cleaned filter assemblies were used for each sample. Peristaltic tubing was acid cleaned (5% HCl) and rinsed (distilled water) between samples.

Filtered water samples were analyzed for MeHg and FTHg by the USGS Wisconsin Mercury Research Laboratory (WMRL; Middleton, Wisconsin). FTHg was determined by cold vapor atomic fluorescence spectrometry (CVAFS) using a slight modification \(^5\) of USEPA Method
1631 (8) as described (3, 5, 6, 8-13). FMeHg was determined according to USEPA Method 1630 (14) by distillation, aqueous-phase ethylation, gas-phase separation, and cold vapor atomic fluorescence spectroscopy (CVAFS) (5, 12, 13, 15). Reporting limits for FMeHg were 0.04 ng/L.

Quality assurance quality control (QAQC) samples (15% of samples) for FTHg during April and July included field/method blanks (generally less than 5% of environmental sample concentrations, less than 10% in all cases), duplicate analyses (percent relative standard deviation (%RSD) = 4.4%), matrix spikes (recovery = 99.0% ± 7.3%) and check samples. Corresponding FMeHg QAQC samples included field/method blanks (less than detection in all cases), duplicate analyses (%RSD) = 4.6%), matrix spikes (recovery = 97.1% ± 21.6%) and check samples. Data quality objectives (DQO) were generally ±10% for precision and accuracy for all QAQC measures, and failure to reach any of these in a given run resulted in re-analysis until all DQO pass.

**Additional Sediment MeHg Concentration and Production Potential Analysis Details**

Methylmercury production potential (MPP) was quantified using a stable isotope incubation assay, previously detailed (16), and briefly described here. Three sub-samples of sediment (3.0±0.1 g wet weight) per site were transferred into 13-cm\(^3\) serum vials under anoxic (N\(_2\) atmosphere) conditions. An isotopically enriched solution (0.1 mL) of inorganic mercury (\(^{200}\text{HgCl}_2\)) was injected through the septa of each vial for a final amendment concentration of 45 ng of \(^{200}\text{Hg(II)}\) per g of sediment, wet weight (55–186 ng of \(^{200}\text{Hg(II)}\) per g of sediment, dry weight, depending on site). After vortexing for 1 minute, one of the three samples per set was immediately flash frozen in a mixture of dry ice and ethanol. This sample represented the killed control. The remaining two samples in each set were incubated at 23°C for 19 hours, after which
they too were flash frozen in dry ice and ethanol. All samples were stored at -80°C until further processing.

The isotopically enriched MeHg (Me\(^{200}\)Hg) produced from the \(^{200}\)Hg(II) during the incubation was extracted into KOH and methanol (CH\(_3\)OH) (17). Upon thawing, a sub-sample (ca. 0.5 g wet weight, exact weight recorded) was removed from each serum vial and transferred into a 15-mL plastic centrifuge tube. An internal standard of isotopically enriched Me\(^{199}\)Hg was added (0.15 ng) to each tube, followed by 2 mL of 25% KOH in methanol. After homogenizing (vortexing) for 1 minute, the samples were placed in a 60°C oven for 4 hours. Samples were then cooled to room temperature. DI water (8 mL) was added to each centrifuge tube, which was then shaken to mix and stored frozen at -80°C until further processing.

The Me\(^{200}\)Hg was subsequently quantified by a modified version of the ethylation assay (18), coupled with inductive coupled plasma mass spectrometry (ICP-MS) (19, 20), as previously detailed (16). Calibration standards were prepared with both non-enriched MeHg and isotopically enriched Me\(^{199}\)Hg and were assayed in the same way as the samples. Excess Me\(^{200}\)Hg (above natural abundance levels) produced during the sediment incubation was quantified on the basis of the calibration standards and the recovery of the internal standard (Me\(^{199}\)Hg) added during the KOH/CH\(_3\)OH extraction step. Any excess Me\(^{200}\)Hg measured in the killed controls was subtracted from the samples that had been incubated for 19 hours. A pseudo first-order rate constant for \(^{200}\)Hg(II)-methylation (k\(_{\text{meth}}\), units = 1/d) was then calculated from the ‘kill-corrected’ incubated samples as previously described for the radiotracer \(^{203}\)Hg(II)-methylation assay (21). Daily MPP rates (units = ng/g dry sediment/d) were then calculated as:

\[
\text{MPP} = \text{Hg(II)}_R - \text{Hg(II)}_R \cdot \exp(-k_{\text{meth}} \cdot t)
\]
where t=1.0 day and Hg(II)$_R$ (units = ng/g dry sediment) is the independently measured *in situ* concentration of inorganic ‘reactive’ mercury in ng g$^{-1}$ dry weight. Analysis of Hg(II)$_R$ from a separate sub-sample (from the same composited and homogenized depth interval) was conducted as previously described (21).

Quality assurance included a) killed controls (as described above), b) analytical duplicates, c) the use of an internal isotope enriched standards (Me$^{199}$Hg, as described above), and d) calibration standards prepared from commercial crystalline MeHgCl. For the current suite of samples, the percent deviation (from the mean) for duplicate analytical determinations of $k_{meth}$ and Hg(II)$_R$ ranged from 9–57% (n=4) and 9.2% (n=1), respectively.

Daily MPP rates expressed in terms of ‘ng g$^{-1}$ dry sediment d$^{-1}$, (as calculated above) were then converted to areal MPP rates (ng m$^{-2}$ d$^{-1}$) for the surface 0–2 cm sediment depth interval according to:

$$\text{Areal-MPP} = \text{MPP} \times (\%\text{dw}/100) \times \text{bd} \times 20,000$$

where ‘%dw’ = sediment percent dry weight (units = [g dry wt]/[g wet wt]), ‘bd’ = sediment bulk density (units = g wet wt/cm$^3$), and 20,000 converts units of cm$^3$ to m$^2$ for the top 0–2 cm depth interval.

*Additional Water Stable Isotope Analysis Details*

The ratios of naturally occurring stable isotopes of hydrogen ($^{2}$H/$^{1}$H) and oxygen ($^{18}$O/$^{16}$O) were analyzed by the USGS Stable Isotope Laboratory in Reston, Va., as described (22, 23) and reported relative to Vienna Standard Mean Ocean Water (VSMOW). Isotopic results are reported as $\delta^{2}$H and $\delta^{18}$O, the relative differences in parts per thousand (per mil; $\delta$/oo) between the sample isotope ratio and a known standard isotope ratio (22-24). Reach oxygen and hydrogen isotope...
compositions of precipitation, “deeper” groundwater, shallow groundwater, and surface water exhibited two distinct linear trends on scatterplots of $\delta^{18}$O versus $\delta^2$H (Figure). Precipitation and “deeper” groundwater, which had not undergone evaporation, fell along (but slightly higher than) a national meteoric water line (MWL) with a slope of about 8.11 (25). The national MWL has a slope that is relatively similar to the slope of the global meteoric water line (26). The isotopic composition of precipitation varied seasonally due to air-mass origin and temperature-dependent water-vapor condensation yielding isotopically heavier summer rain and isotopically lighter winter rain (24, 27). Meteoric waters impacted by evaporation have isotopic compositions which are enriched in $\delta^{18}$O and $\delta^2$H and which plot away from the MWL along evaporation lines with slopes of less than 8 (15, 24). That evaporative trend was observed for shallow groundwater and surface waters in McTier Creek, especially during July 2009. After correcting for the underlying evaporative signature, the $\delta^{18}$O and $\delta^2$H compositions of the mixed waters could be described by mixing between the two end-member compositions.

Isotopic compositions of “deeper” groundwater collected approximately 20 m below the water table (44 m below land surface) from well AK-2713 located in McTier Creek watershed (28) and of precipitation (National Atmospheric Deposition site SC06, Santee National Forest, Clarendon County, South Carolina; (29)) were used as end-members for mixing analysis. Isotopic compositions of waters collected during April and July 2009 (e.g., stream, shallow groundwater, wetlands, and streambed porewater) were assumed to be sourced from a mixture of the two end-members (24, 30). Estimated average fraction of “deeper” groundwater in the mixed water was computed by:

$$F_{gw} = \frac{\delta_{m} - \delta_{p}}{\delta_{gw} - \delta_{p}}$$
where $F_{gw}$ is the fraction of “deeper” groundwater in the mixture, $\bar{\delta}_m$ is the average $\delta^{18}$O or $\delta^2$H composition of the mixed water, $\bar{\delta}_p$ is the average $\delta^{18}$O or $\delta^2$H composition of precipitation collected in April and July 2009, and $\bar{\delta}_{gw}$ is the average $\delta^{18}$O or $\delta^2$H composition of “deeper” groundwater.

Limited data (i.e., “deeper” groundwater samples $n = 2$) prevented application of a robust sensitivity analysis to the mixing calculation (30). Instead, a modified sensitivity analysis based on the standard deviation of the mixed water isotopic composition and the difference in the isotope composition of the end-members was used. The uncertainty in the computation of the mixing fractions was estimated to be less than 10% using $\delta^2$H, because of the relatively large difference in end-member compositions (10.9 to 14.5 $\permil$). In contrast, $\delta^{18}$O compositions ranged 1.4 to 2.3 $\permil$, producing end-member mixing uncertainties of 15% or more. Therefore, only the contributions of “deeper” groundwater to samples collected within the reach estimated from $\delta^2$H data are presented here.
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Supplementary Figures and Tables:

SI Figure 1.
Relation of filtered methylmercury (FMeHg, green triangles, ▲) and filtered total mercury (FTHg, black triangles ▲) concentrations with discharge (Q) at the McTier Creek gage (02172305) for April-September of 2007-2008. Lines are simple linear regressions. Correlation coefficients (ρ) and p-values (p) are for Spearman Rank Correlations. Dotted line indicates reporting limit for FMeHg (0.04 ng L⁻¹).

ρ = -0.44; p = 0.027
ρ = 0.35; p = 0.091
SI Figure 2.

Transiently flooded riparian floodplain on east bank of McTier Creek study reach (upper photo).

Perennially flooded wetland on west bank of McTier Creek study reach (lower photo).

Corresponding subsurface stratigraphy shown at right.

Note, kaolin deposit about 30 cm bsl at west edge of E transect, only.
SI Figure 3.

A) Conceptual cross-section of McTier Creek shallow stratigraphy showing groundwater wells and in-stream piezometers at transect E (not to scale). Lined, white area on west side of channel denotes kaolin deposit underlying perennial wetland. Gray area denotes reduced, silty-sand (two uppermost strata in text). Underlying light gray strata denotes channel lag deposit.

B) Arrangement for sampling of in-stream piezometers. Top of screened interval (30 cm) for in-stream piezometers was 20 cm below the surface-water/bed sediment interface.

C) Arrangement for sampling of riparian groundwater wells. Tops of screened intervals (30 cm) for shallow and deep groundwater wells were approximately 30 cm and 150-170 cm below land surface, respectively.
McTier Creek hydrographs showing timing of recession limb sample collection following flood events in April and July of 2009. Insets indicate timing of surface- (SW), hyporheic- (HW), and ground-water (GW) samples relative to flood event (dashed line indicates approximate flood stage).
SI Figure 5.

USGS personnel using ultra trace techniques to sample groundwater from riparian groundwater wells at the McTier Creek study reach in July 2009.
SI Figure 6.

Water-level gradient profiles observed during seasonal flood events at the (A) E and (B) A groundwater well transects on the east bank of the McTier Creek sub-basin of the Edisto River basin. Locations of monitoring wells relative to McTier Creek are shown in the insets. Figure is from Bradley et al. 2010 (9).
SI Figure 7.
Lateral gradients in water elevation (● and ▲ indicate transects A and D, respectively) and FMeHg (upper graph) or FTHg (lower graph) concentrations (box plots; white, blue, and green indicate groundwater, stream surface water, and wetland surface water, respectively) during April 2009. Boxes and centerlines indicate interquartile ranges and medians, respectively. Red diamond indicates concentration of single sample collected from a riparian pool on the east bank of the reach. Locations of wetland and riparian pools are approximate.
Relation between δ²H and δ¹⁸O isotope ratios for stream-water (▲), wetland-water (♦), and groundwater (○) samples collected from the study reach in April (A.) and July (B.) 2009. Dashed line indicates local meteoric water line. Environmental end-members used for mixing analysis were groundwater collected within McTier basin from 20 m below the water table (■) and precipitation collected from nearby National Atmospheric Deposition site SC06 (□; error bars indicate interquartile range of precipitation data). Blue dotted lines indicate theoretical mixing line for selected end members. Departures of sample compositions from mixing line reflect evapotranspiration. Contribution of “deeper” groundwater to water samples estimated from end-member mixing analysis using δ²H isotope ratio data (lower graph).

Note: the increased influence of precipitation in July TOR and BOR samples is attributed to highly localized precipitation at the upper margins of the McTier Creek watershed (but not within the reach) approximately 8 hours before sample collection. This pulse of surface water is detectable as a slight bump in the recession curve during 0400-1800 h on July 14 (SI Figure 4).
SI Figure 9.
Groundwater discharging from bank to McTier Creek (July 2009).
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**SI Table 1.** Bottom-of-reach (BOR) to top-of-reach (TOR), distal (24 m) to proximal (6 m), and deep (150-200 cm below land surface) to shallow (30-60 cm below land surface) pairwise comparisons (Wilcoxon One-sided Signed Rank test) of FMeHg and FTHg concentrations in surface-water and groundwater samples collected from reach during April and July of 2009. Only the strongest directional relations (e.g. BOR > TOR stronger than TOR > BOR) are shown. Statistically significant relations are shown in Red Bold. Only paired data for a given time (BOR-TOR), transect (Distal-Proximal deep groundwater wells), or location (Shallow-Deep) were included. For n ≥ 5, α = 0.05. For n < 5, α = 0.1. No significant difference was observed between east- and west-bank groundwater samples (Wilcoxon One-sided Signed Rank; p > 0.3).

| Comparison                  | Matrix          | Parameter | Event(s) | n | p-value |
|-----------------------------|-----------------|-----------|----------|---|---------|
| **BOR greater than TOR**    | Surface Water   | FMeHg     | April    | 3 | 0.625   |
|                             |                 |           | July     | 3 | **0.087** |
|                             |                 |           | Both     | 6 | 0.147   |
|                             |                 | FTHg      | April    | 3 | 0.625   |
|                             |                 |           | July     | 3 | 0.125   |
|                             |                 |           | Both     | 6 | 0.219   |
| **Distal greater than Proximal** | Groundwater | FMeHg     | April    | 10| 0.138   |
|                             |                 |           | July     | 3 | 0.341   |
|                             |                 |           | Both     | 13| 0.051   |
|                             |                 | FTHg      | April    | 10| **0.032** |
|                             |                 |           | July     | 3 | 0.125   |
|                             |                 |           | Both     | 13| **0.016** |
| **Deep greater than Shallow** | Groundwater | FMeHg     | Both     | 6 | 0.166   |
|                             |                 | FTHg      | Both     | 6 | 0.078   |

&superscript;“n”&nbsp;indicates sample size.

&superscript;b 𝛼 = 0.1, for n < 5 (i.e. p-value < 0.1 is statistically significant for sample size less than five).