High resolution porewater profiling of methylmercury with a novel equilibrium passive sampler

Supporting Information

Jada Damond[1], Cynthia Gilmour[2], and Upal Ghosh[1,*]

University of Maryland Baltimore County

Smithsonian Environmental Research Center

Table of Contents

Analytical Methods

All aqueous samples (overlying water and porewater) were filtered to 0.45 µm with a pre-rinsed (with DI water) glass microfiber (GD/X) filter before being aliquoted to the sample container and preserved respective to the analytical method detailed below. Sediment samples were lyophilized and stored frozen until analyses. Passive sampler sections were stored frozen until MeHg analysis. Analyses of total Hg, MeHg, and most other parameters were conducted at SERC in Edgewater, MD.

Sulfide

Freshly filtered aqueous samples were immediately preserved in freshly made sulfide antioxidant buffer (SAOB) (Brouwer and Murphy, 1995) and analyzed using an ion-selective electrode, on the day of sampling. Standard calibration curves were performed on each day of sampling. The electrode was calibrated against dilutions of saturated Na_2S in SOAB. The saturated Na_2S solution is calibrated by titration against a (0.1 M) lead perchlorate standard solution.

pH

Filtered aqueous samples were measured with an ion-selective electrode and meter (Orion Star™) on the day of sampling. Standard calibration curves were performed on each day of sampling, using three pH reference solutions as calibration points.

Dissolved organic carbon (DOC)

Filtered aqueous samples were stored frozen in a 40 mL glass vials until analysis. On the day of analysis, samples were thawed at room temperature then immediately analyzed for DOC using a Shimadzu TOC-L. Standard calibration was performed, using potassium hydrogen phthalate (KHP) standard solution, on the day of analysis.

Anions (Sulfate, Nitrate, and Chloride)

Filtered aqueous samples were stored frozen in 15-mL polypropylene (PP) centrifuge tubes. Sulfate, nitrate and chloride were measured via ion chromatography (Dionex™ Integrion™ HPIC™ System), using an IonPac AS18-4µm (2 x 150mm) column and isocratic 32 mM KOH eluant. Standard calibration was performed using Dionex™ Seven Anion Standard II on the day of analysis.

Redox-Active Elements (Iron, Manganese, Sulfur)

Filtered aqueous samples were preserved in 0.5 vol% trace-metal grade hydrochloric acid and stored refrigerated, then measured with an Optima 8300 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Standard calibration was performed using an in-house multiple metal cation solution, which was tested against a customized solution made and analyzed by Analytichem (formerly SCP Science) and NIST Standard Reference Material®

1643f. The internal standard was made in-house; it was a solution of 20 ppm gallium, 1 ppm scandium, and 10 ppm yttrium in dilute nitric acid (2 vol% HNO_3).

Total Mercury (THg)

Analysis of both aqueous and solid samples used modifications of EPA Method 1631e (Gilmour et al., 2018; U.S. Environmental Protection Agency, 2002). using isotope-dilution techniques. THg analysis was performed using a Tekran 2600 purge-and-trap system (Toronto, Canada) for sample introduction to an Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). THg was measured in porewater, overlying water, and sediments. Filtered aqueous samples were preserved in 0.5 vol% trace-metal grade HCl and refrigerated in PETG bottles until digestion and analysis. Sediment samples were lyophilized and frozen. PS were sectioned and stored frozen in 15mL polypropylene centrifuge tubes.

Porewater and surface water analysis. Approximately 0.2 mL porewater or 1 mL surface water was placed a 40-mL clear VOA vials (Thomas Scientific), adjusted to ~25 mL with in-house ultrapure water (>22 MΩ), and digested overnight at room temperature with 0.5% v/v BrCl. Immediately prior to analysis, 0.1 mL 30% hydroxylamine hydrochloride (Acros Organics) was added to quench free chlorine, followed by reduction with 0.1 mL 20% $SnCl_2$ (J.T. Baker).

Sediment digests. Approximately 0.05-0.1 g subsamples of lyophilized and ground material were digested with 5 mL of 7:4 v/v HNO_3/H_2SO_4 and heated until vapors cleared. Digestates were diluted to ~50 mL with deionized water, and Hg was oxidized to Hg(II) by adding 0.5 mL BrCl.

Reagents were prepared following Method 1631e, with KBr and $KBrO_3$ (Beantown Chemicals, Hudson, NH) muffled at 300 °C to minimize Hg contamination. Hydroxylamine hydrochloride was purged with N_2 for 30 min after addition of $SnCl_2$ to 0.1%, then stored refrigerated. Blank subtraction was performed using the sample matrix. Primary Hg standards (1 ppm in 2% HNO_3) were obtained from Brooks Rand. Working 1 ppb dilutions were prepared in 0.5% BrCl and refrigerated. Each run also included two NIST-traceable QCS standards (SCP Science, Montreal, QC; Inorganic Ventures, Christiansburg, VA). Certified reference materials (CRMs) were digested and analyzed with all sample sets. A dilution of NIST 1641e was used for aqueous samples, and NIST NJ 2706 was used for sediment samples. All analytical runs included digest and instrument blanks.

Sample concentrations were quantified by isotope dilution using enriched ^{199}Hg isotope spikes prior to digestion. Working isotope spike stocks were 1 ppb, preserved in 0.5 vol% BrCl and refrigerated, diluted from a 100 ppb stock up to 1 week prior to analyses. The target spike mass ratio was 5:1 spiked ^{199}Hg to ambient ^{202}Hg levels. The concentration of the spike was routinely measured using reverse isotope-dilution (Hintelmann and Ogrinc, 2002). Enriched isotopes were obtained from Oak Ridge National Laboratory.

Methylmercury (MeHg)

MeHg concentrations were measured using modifications of EPA Method 1630 (Gilmour et al., 2018; U.S. Environmental Protection Agency, 1998), using isotope-dilution techniques with dual inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900) and CVAF detection.

MeHg was measured in porewater, overlying water, sediments, and passive samplers (subtracting the weight of the protective mesh).

MeHg samples were prepared for analysis by steam distillation in 60-mL PFA jars (Savillex) using an aluminum heating block at 120 °C. Prior to distillation, samples were adjusted to 22.5 mL with ultrapure water and amended with 0.2 mL 20% KCl (source), 0.2 mL 1 wt % ammonium pyrrolidinedithiocarbamate (APDC, Acros Organics), and 1 mL 9 M H_2SO_4 (Millipore). Distillates were collected in a wet ice bath with a target recovery of 65–70% at an N_2 flow rate of 45 mL/min. Sample and distillate weights were recorded. Distillation equipment was cleaned by scrubbing with 409® cleaner followed by boiling in 20% HCl.

MeHg analysis was performed by isotope-dilution using a Tekran 2700 purge-and-trap GC system (Toronto, Canada) for ethylation and sample introduction to the ICP-MS. For aqueous samples, distillate aliquots were diluted to 27 mL with ultrapure water in 40-mL amber VOA vials (Thomas Scientific), buffered with 0.3 mL 5M sodium acetate, and derivatized by aqueous-phase ethylation with 0.03 mL 1% sodium tetraethylborate (Strem), following EPA Method 1630. Working 1 ppb stock solutions were made from 1ppm MeHgCl (BrooksRand) were stored refrigerated in 0.5% trace-metal grade HCl, 0.5% acetate buffer, and 10 µM glutathione to limit degradation. 1 ppb MeHg Quality Control Sample (QCS) standards were made from 1 ppm MeHgOH (BrooksRand) and stored refrigerated in 0.5% acetic acid, 0.2% HCl, 10 µM glutathione. Both the working standards and QCS standards were prepared every 3 months and stored refrigerated. MeHg standards were verified against certified inorganic Hg standards before and after BrCl digestion to monitor stability. For sediments and PS samples, NIST 1566b oyster tissue was distilled alongside samples as the certified reference material (CRM). Distillation and instrument run blanks were also included in each run.

Similar to THg samples, sample concentrations were quantified by isotope dilution. An enriched isotopic $Me^{199}Hg$ spike (~0.1 ppb, preserved in 0.5% trace-metal grade HCl, 0.5% acetate buffer, and 10 µM glutathione) was added to samples prior to distillation, at a spike ratio of 5:1 of spike to expected ambient levels. $Me^{199}Hg$ spikes were synthesized in-house using methylcobalamin (Bancon-Montigny et al., 2004; Hintelmann and Ogrinc, 2002)$Me^{199}Hg$ stocks were preserved in 0.5% trace-metal grade HCl, 0.5% acetate buffer, and 10 µM glutathione, and stored frozen. The concentration of the spike was routinely verified using reverse isotope-dilution (Hintelmann and Ogrinc, 2002). Enriched isotopes were obtained from Oak Ridge National Laboratory.

Detection limits were based on three times the standard deviation of distillation blanks. Across all runs, MeHg concentration in distillation blanks averaged 0.066 ng/L, with a standard deviation of 0.023 ng/L. Our QA/QC strategy for sampling and analysis included 5-10% blanks, lab and field duplicates and CRMs (where available). QC data are summarized below (Table S1).

Supplemental Table S1. QC summary data, showing average ± 1 standard deviation (SD) MeHg isotope spike recovery of each matrix distilled, and average (± 1 SD) duplicate relative percent differences (RPD) for distillation duplicates and instrument run duplicates, with their corresponding counts. N/A = not applicable. Distillation duplicates were not performed for Passive Samplers, and run duplicates were not performed for the CRM (as other matrices were used as a run duplicate). Due to the low SW concentrations (unable to dilute distillate), no instrument duplicates were performed on the surface water samples

Matrix	**Average Spike Recovery ± 1 SD (%)**	**Average Distillation Duplicate RPD ± 1 SD (%)**	**Average Instrument Run Duplicate RPD ± 1 SD (%)**
DI	73 ± 10%	n/a	n/a
CRM - NIST 1566b	71 ± 10%	5.3 ± 5.0% (count = 15)	n/a
Passive Sampler	77 ± 11%	n/a	5.3 ± 5.2% (count = 9)
Porewater	76 ± 17%	8.5 ± 12.9% (count = 4)	1.5 ± 0.5% (count = 3)
Sediment	76 ± 5%	7.0 ± 5.5% (count = 13)	1.4 ± 1.0% (count = 5)
Surface Water	80 ± 5%	11.9 ± 9.9% (count = 2)	n/a

Supplemental Figures

Photo Gallery

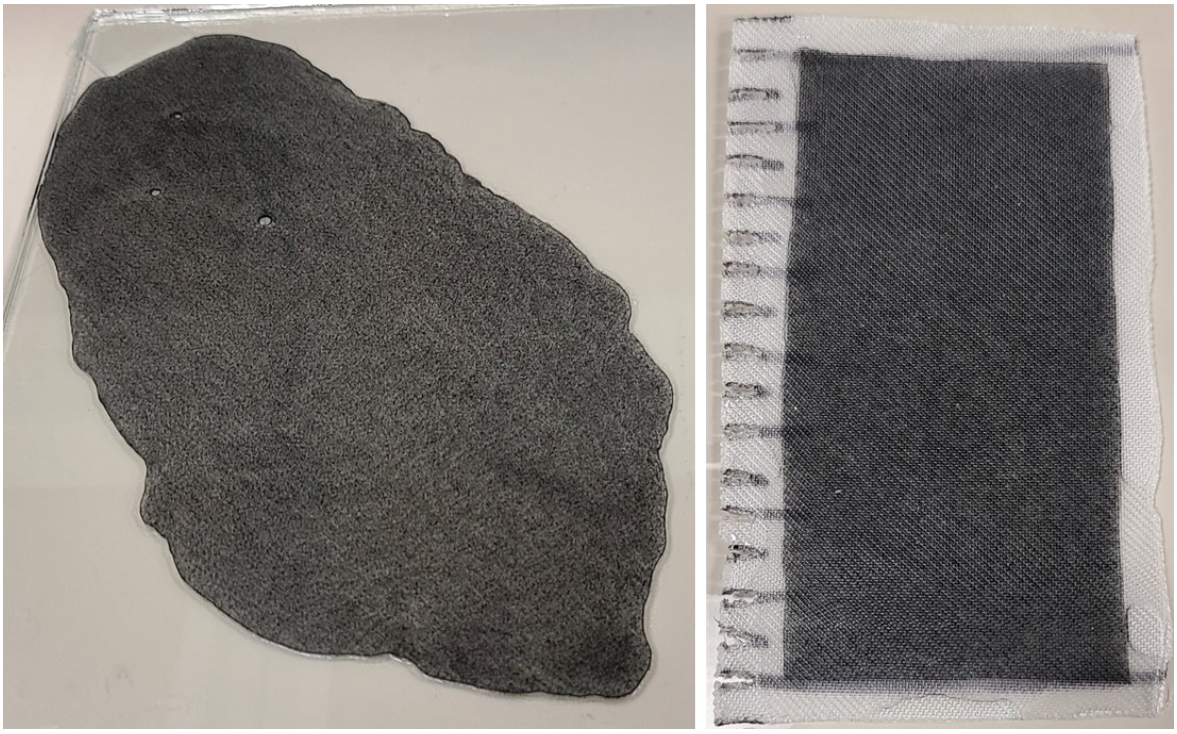

Figure S1 (left) Ag+AC polymer after casting onto a glass plate and (right) a cut passive sampler in polypropylene mesh ready to be deployed. Note that figures are not to scale. The passive sampler is 4 cm by 8 cm.

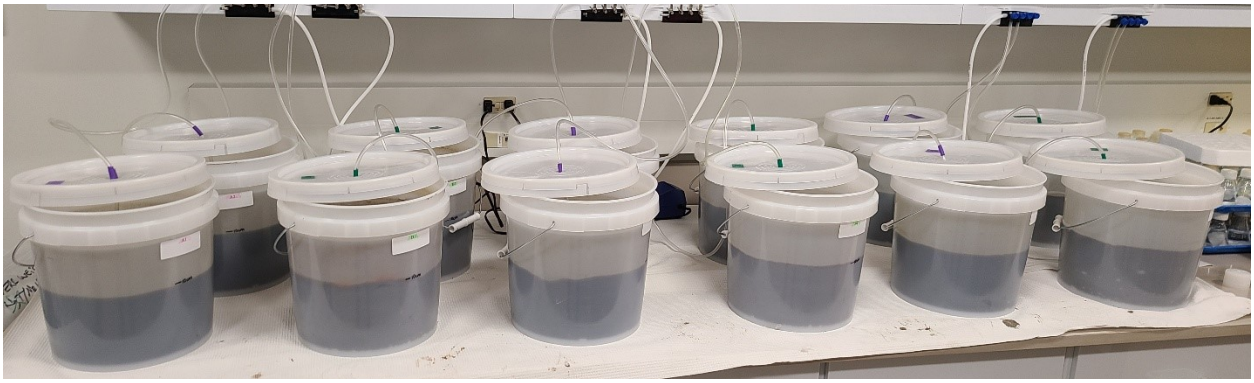

Figure S2 Full setup of microcosms used in the study.

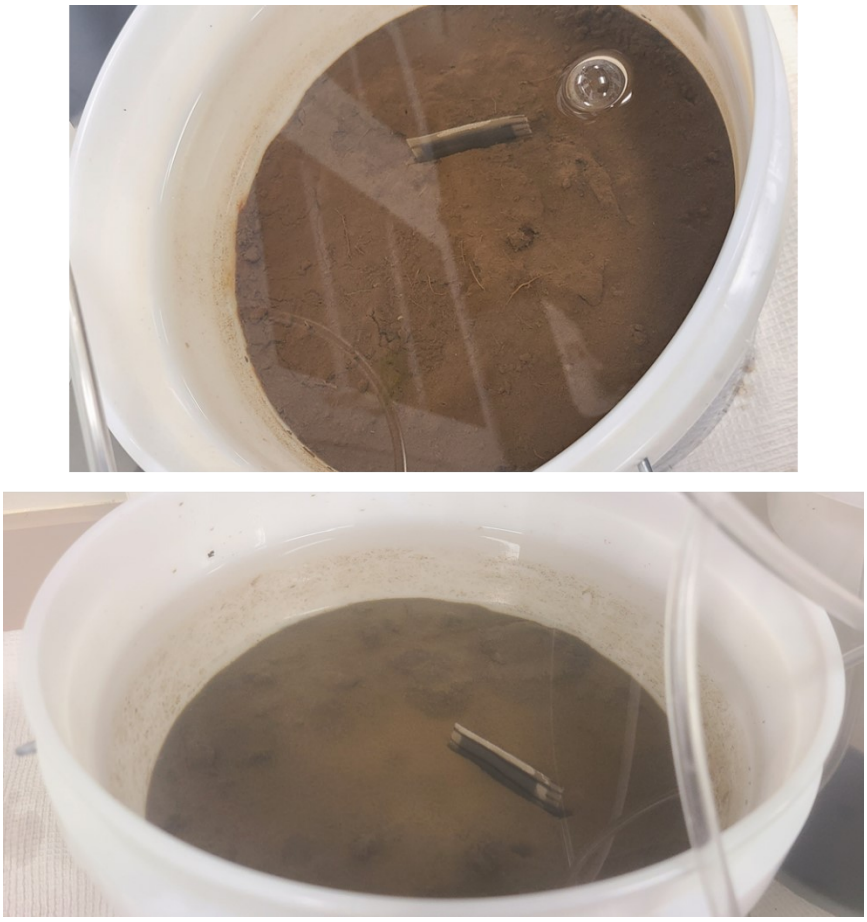

Figure S3 Passive sampler deployed in (top) freshwater sediment microcosms and (bottom) estuarine sediment microcosms.

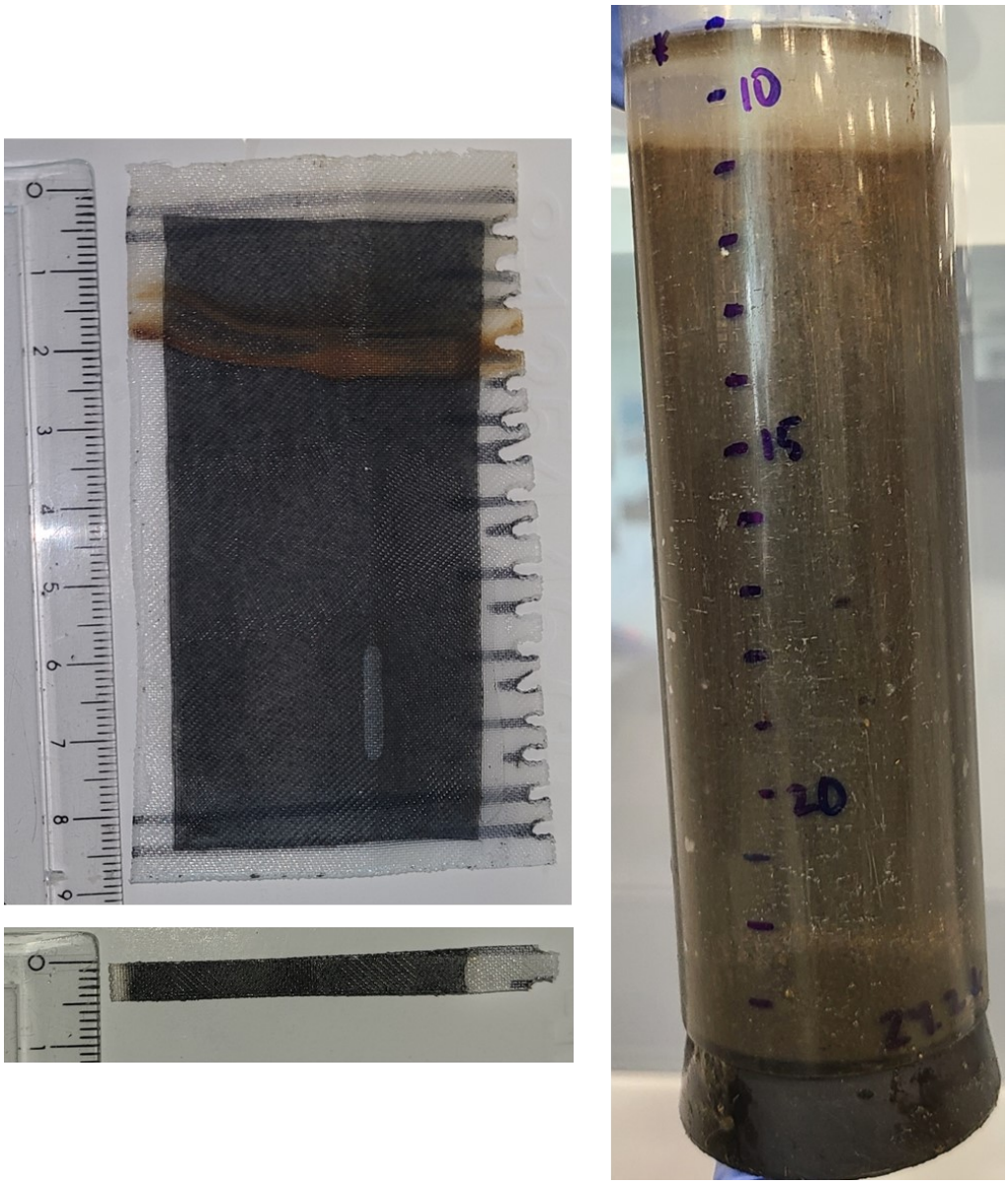

Figure S4 (left) Passive Sampler after retrieval (top) and a 0.5 cm section cut from the PS right after retrieval (bottom), and (right) a picture of a core taken from a sediment microcosm to be transported to the glovebox. Centimeter markings on the core were hand marked for ease of lab work. Please note these figures are not to scale.

Supplemental Graphs

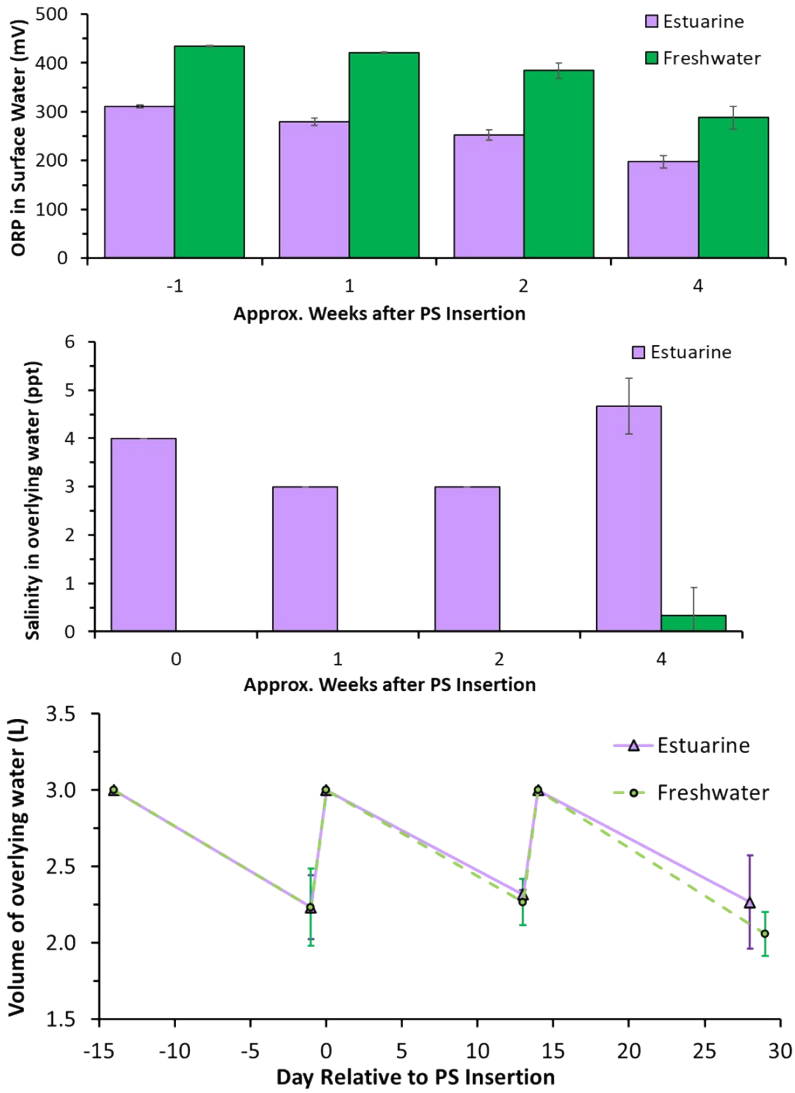

Figure S5. Overlying water chemistry through time. Right before PS insertion (t0), overlying water was replaced with source water to lower NH_4 levels. Evaporation of the overlying water was observed, so 14 days after PS insertion (t+14), deionized water was added to the surface water so that overlying water volume reached 3 L.

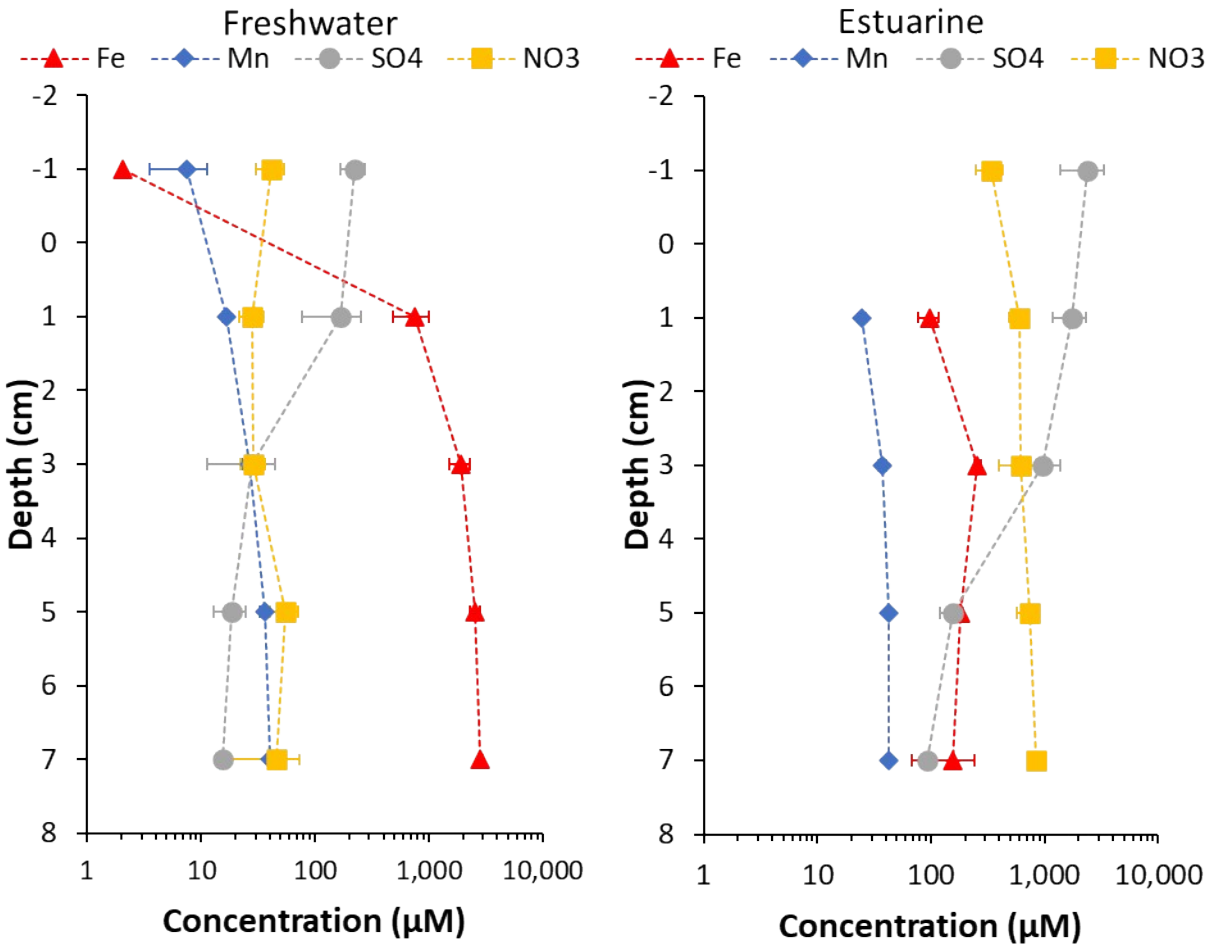

Figure S6. Direct aqueous depth profiles of measured electron donors and acceptors in the freshwater (left) and estuarine (right) microcosms at takedown, 28 days after PS insertion. Points represent averages across 3 replicate microcosms and error bars represent one standard deviation. In the estuarine microcosms, surface water Fe and Mn were below detection limits in all three replicates.

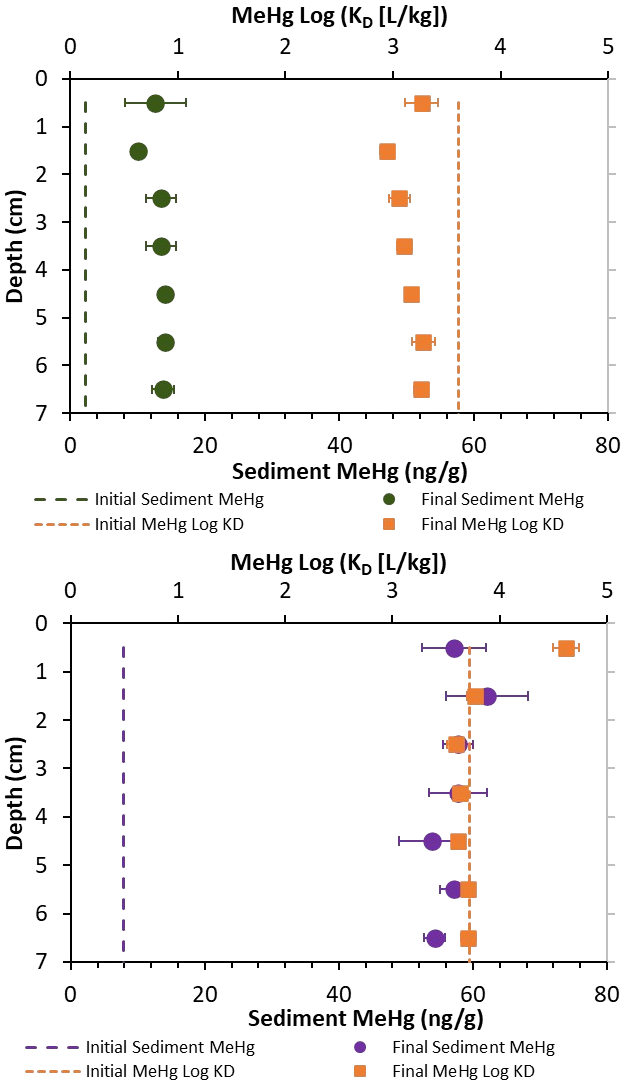

Figure S7. Sediment MeHg profiles and sediment-porewater partitioning (log K_D) in freshwater (top) and estuarine (bottom) sediment microcosms at takedown. Points show averages (n=3) with error bars showing one standard deviation. Initial MeHg sediment concentrations are plotted as a vertical dashed line.

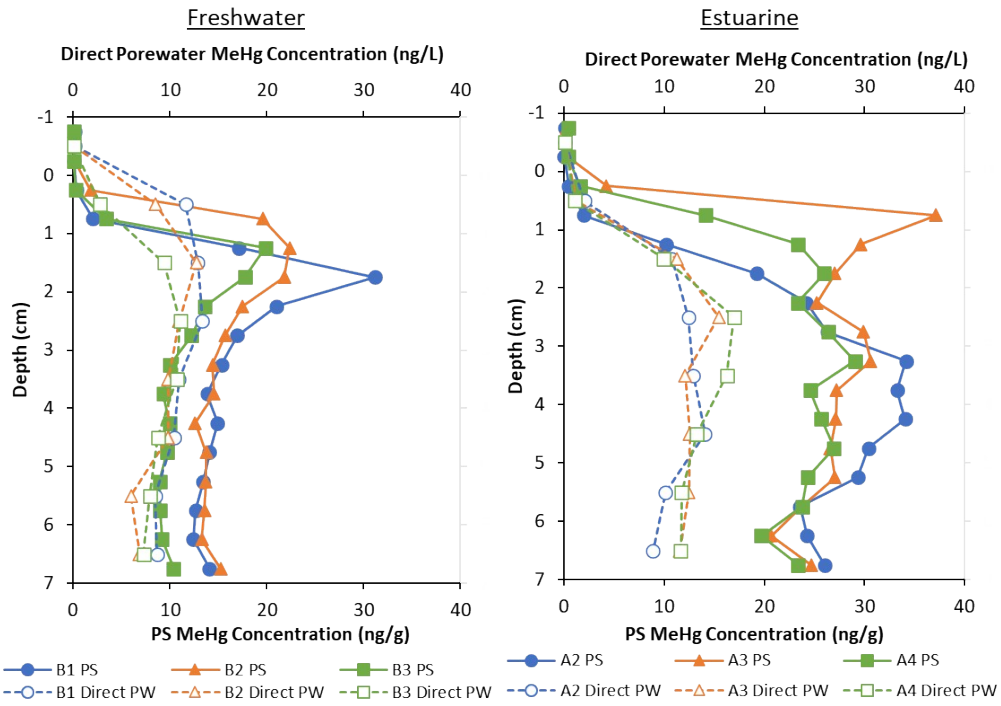

Figure S8. Plotting for each replicate, depth profiles of passive sampler (closed shapes) MeHg concentrations and direct porewater (open shapes) MeHg concentrations in freshwater (left) and estuarine (left) microcosms

Supplemental Tables

Please note a comprehensive table of initial measurements and takedown measurements are provided as a supplemental excel spreadsheet.

Supplemental Table S2. Sediment mixture composition for each microcosm type. Wet:dry ratios (gww/gdw) for the final sediment mixtures report the average ± standard deviation of 6 replicate samples. Measured THg of the final mixtures report the average ± standard deviation of 3 replicate samples. For the freshwater microcosm, contaminated soils from the Berry's Creek Study Area (BCSA, (VENTRON/VELSICOL | Superfund Site Profile | Superfund Site Information | US EPA, [SD1] [SD2] [GC3], (U.S. Environmental Protection Agency, n.d.)) were mixed with sediments collected at Smithsonian Environmental Research Center (SERC). The BCSA samples were collected from an oligohaline *Phragmites australis* marsh and a nearby drainage ditch. Both had been collected for use in prior experiments (e.g. (Driscoll et al., 2025; Washburn et al., 2022). The SERC sediment was collected from a freshwater pond in watershed 101 (Correll et al., 2001). THg levels in the SERC freshwater pond mud were not determined prior to mixing (noted as "n.d.") but assumed to be at regional background levels. For the estuarine microcosms, a BCSA-SERC soil mixture from a prior study (Driscoll et al., 2025) was used and amended with additional fresh marsh soil. The mix included surface soils from an oligohaline BCSA *P. australis* marsh (Nevertouch) plus creek and marsh sediment from an intertidal mesohaline *P. australis* marsh at SERC, the Global Change Research Wetland (GCREW). Mercury and MeHg data for this site can be found in (Mitchell and Gilmour, 2008) .

Microcosm Type	Source	Volume (mL)	Wet:dry ratio (gww/gdw)	THg (ng/kgdw)
Freshwater	North Ditch (BCSA)	1600	2.6	47
	Patterson Plank (BCSA)	3100	5.3	30
	SERC freshwater pond mud	27000	2.2	n.d.
	Total freshwater sediment mixture	**31700**	**2.3 ± 0.0**	**2.8 ± 0.8**
Estuarine	*1 part Nevertouch Phrag Marsh Soil* *2 parts SERC Phrag Soil* *1 part SERC tidal creek sediment*	18400	5.9	19
	SERC intertidal *P. australis* marsh soil	18400	5.8	0.2
	Total estuarine sediment mixture	**36800**	**5.9 ± 0.1**	**9.6 ± 0.7**

Supplemental Table S3. Summary of benchtop measurements of overlying water during microcosm incubation. Oxidation-Reduction Potential (ORP) measurements were taken with a AgCl based InLab Redox Micro probe (Mettler Toledo), ammonia (NH_4) measurements were taken with test strips, and salinity measurements were taken via refractometer.

Sediment Type	Bucket ID	Sampler insertion date	Measurement Date	Time (days after insert)	Time (~weeks after insert)	Overlying Water ORP (mV)	NH_4 (mg/L)	Salinity (ppt)
Estuarine	A2	4/18/2022	4/13/2022	-5	-1	308	0.5	
Estuarine	A3	4/19/2022	4/13/2022	-6	-1	313	0.5	
Estuarine	A4	4/19/2022	4/13/2022	-6	-1	313	0.5	
Freshwater	B1	4/18/2022	4/13/2022	-5	-1	436	6	
Freshwater	B2	4/18/2022	4/13/2022	-5	-1	433	6	
Freshwater	B3	4/19/2022	4/13/2022	-6	-1	433	6	
Estuarine	A2	4/18/2022	4/18/2022	0	0			
Estuarine	A3	4/19/2022	4/19/2022	0	0		1	4
Estuarine	A4	4/19/2022	4/19/2022	0	0			
Freshwater	B1	4/18/2022	4/18/2022	0	0		6	0
Freshwater	B2	4/18/2022	4/18/2022	0	0			
Freshwater	B3	4/19/2022	4/19/2022	0	0		6	0
Estuarine	A2	4/18/2022	4/22/2022	4	1	271		3
Estuarine	A3	4/19/2022	4/22/2022	3	1	281		3
Estuarine	A4	4/19/2022	4/22/2022	3	1	287		3
Freshwater	B1	4/18/2022	4/22/2022	4	1	422	0.5	0
Freshwater	B2	4/18/2022	4/22/2022	4	1	419		0
Freshwater	B3	4/19/2022	4/22/2022	3	1	420		0
Estuarine	A2	4/18/2022	5/2/2022	14	2	241	0	3
Estuarine	A3	4/19/2022	5/3/2022	14	2	263	0	3
Estuarine	A4	4/19/2022	5/3/2022	14	2	253	0	3
Freshwater	B1	4/18/2022	5/2/2022	14	2	375	0	0
Freshwater	B2	4/18/2022	5/2/2022	14	2	375	0	0
Freshwater	B3	4/19/2022	5/3/2022	14	2	403	0	0
Estuarine	A2	4/18/2022	5/16/2022	28	4	212	0	5
Estuarine	A3	4/19/2022	5/17/2022	28	4	190	0.5	4
Estuarine	A4	4/19/2022	5/17/2022	28	4	191	0.5	5
Freshwater	B1	4/18/2022	5/17/2022	29	4	305	0	0
Freshwater	B2	4/18/2022	5/17/2022	29	4	262	0	0
Freshwater	B3	4/19/2022	5/18/2022	29	4	297	0.5	1

Supplemental Table S4. Table of Log K_{PS} values determined across experiments detailed in (Sanders et al., 2020; Washburn et al., 2022). Acronyms for MeHg complexes: Cys = cysteine, DOM = dissolved organic matter, (7) MeHg DOM Species are ESHA = Elliott Soil humic acid; LHA = Leonardite humic acid; PPHA = Pahoke Peat humic acid; SRFA = Suwanee River fulvic acid; SRNOM = Suwanee River natural organic matter; UMRNOM = Upper Mississippi River natural organic matter; SRHA = Suwannee River humic acid.

Log KPS	**MeHg complex or Soil Type**	**Experiment Details**	**Source**
3.01	MeHgCys	MeHgCys sorption isotherms (14 day)	Washburn et al (2022)
2.96 (recommended)	Average Log KPS between SRNOM (Log KPS = 2.76) and UMRNOM (Log KPS = 3.15	MeHgDOM sorption isotherms (14 day)	Washburn et al (2022)
1.98 – 3.15	Range of Log KPS across all (7) MeHgDOM species	MeHgDOM sorption isotherms (14 day)	Washburn et al (2022)
3.41	Berry's creek soil slurries (MeHgSH dominated at Day 0, but MeHgDOM dominated at day 20)	Berry's Creek Soil slurries (20 day)	Sanders et al (2020)
3.59	Berry's creek sediment microcosms	Berry's creek sediment microcosms (28 day)	Sanders et al (2020)

References

Bancon-Montigny, C., Yang, L., Sturgeon, R.E., Colombini, V., Mester, Z., 2004. High-yield synthesis of milligram amounts of isotopically enriched methylmercury (CH3198HgCl). Appl. Organomet. Chem. 18, 57–64. https://doi.org/10.1002/aoc.572

Brouwer, H., Murphy, T., 1995. Volatile sulfides and their toxicity in freshwater sediments. Environ. Toxicol. Chem. 14, 203–208. https://doi.org/10.1002/etc.5620140204

Correll, D.L., Jordan, T.E., Weller, D.E., 2001. Effects of Precipitation, Air Temperature, and Land Use on Organic Carbon Discharges from Rhode River Watersheds. Water, Air, Soil Pollut. 128, 139–159. https://doi.org/10.1023/a:1010337623092

Driscoll, S.K., Gilmour, C.C., Brown, S.S., Nedrich, S., Lotufo, G.R., Farrar, J.D., Steevens, J.A., Schwartz, G., Sanders, J.P., 2025. Influence of activated carbon on the bioaccumulation of methylmercury from sediment by the amphipod Leptocheirus plumulosus. Environ. Toxicol. Chem. https://doi.org/10.1093/etojnl/vgaf259

Gilmour, C.C., Bell, J.T., Soren, A.B., Riedel, Georgia, Riedel, Gerhardt, Kopec, A.D., Bodaly, R.A., 2018. Distribution and biogeochemical controls on net methylmercury production in Penobscot River marshes and sediment. Sci Total Environ 640–641, 555–569. https://doi.org/10.1016/j.scitotenv.2018.05.276

Hintelmann, H., Ogrinc, N., 2002. Determination of Stable Mercury Isotopes by ICP/MS and Their Application in Environmental Studies. Acs Sym Ser 835, 321–338. https://doi.org/10.1021/bk-2003-0835.ch021

Mitchell, C.P.J., Gilmour, C.C., 2008. Methylmercury production in a Chesapeake Bay salt marsh. J. Geophys. Res.: Biogeosciences 113. https://doi.org/10.1029/2008jg000765

Sanders, J.P., McBurney, A., Gilmour, C.C., Schwartz, G.E., Washburn, S., Driscoll, S.B.K., Brown, S.S., Ghosh, U., 2020. Development of a Novel Equilibrium Passive Sampling Device for Methylmercury in Sediment and Soil Porewaters. Environ Toxicol Chem 39, 323–334. https://doi.org/10.1002/etc.4631

U.S. Environmental Protection Agency, 2002. Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Office of Water.

U.S. Environmental Protection Agency, 1998. Method 1630. Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. Office of Water.

U.S. Environmental Protection Agency, n.d. VENTRON/VELSICOL | Superfund Site Profile | Superfund Site Information | US EPA [WWW Document]. URL https://cumulis.epa.gov/supercpad/cursites/csitinfo.cfm?id=0200674 (accessed 12.16.25).

Washburn, S.J., Damond, J., Sanders, J.P., Gilmour, C.C., Ghosh, U., 2022. Uptake Mechanisms of a Novel, Activated Carbon-Based Equilibrium Passive Sampler for Estimating Porewater Methylmercury. Environ Toxicol Chem. https://doi.org/10.1002/etc.5406