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Contrasting Effects of Marine and Terrestrially Derived Dissolved Organic Matter on Mercury Speciation and Bioavailability in Seawater

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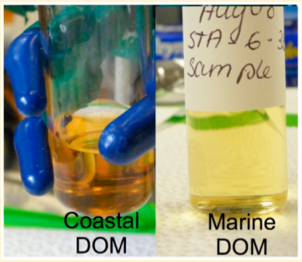
Supporting Information

ABSTRACT: Methylmercury (MeHg) is the only species of mercury (Hg) to biomagnify in aquatic food-webs to levels that are a widespread concern for human and ecological health. Here we investigate the association between dissolved organic matter (DOM) in seawater and Hg speciation and uptake using experimental data and field measurements from Long Island Sound (LIS) and the Northwestern Atlantic continental margin. We measured differences in DOM composition across sampling stations using excitation emission matrix fluorescence spectroscopy and further separated DOM into terrestrial and marine components using Parallel Factor Analysis (PARAFAC). Highest MeHg concentrations were found in the estuarine stations (LIS) with highest DOM concentrations due to enhanced external inputs from the watershed and rivers. For stations on the shelf and slope, MeHg in plankton increased linearly with a decreasing fraction of fluorescence attributable to DOM components with a terrestrial rather than marine origin. These results are corroborated by experimental data showing higher MeHg uptake by cells in the presence of predominantly marine DOM compared to terrestrial DOM. Highest fractions of dissolved gaseous mercury were also found at stations with the highest marine DOM content, suggesting a greater reducible fraction of divalent inorganic Hg. These data suggest DOM composition is a critical driver of Hg reactivity and bioavailability in offshore marine waters.



INTRODUCTION

Mercury (Hg) is a naturally occurring heavy metal with a biogeochemical cycle that has been substantially perturbed by human activity.[1,2] Methylmercury (MeHg) is the only Hg species to biomagnify in aquatic food-webs and accumulate at levels that are a widespread concern for human and ecological health.[3,4] Dissolved organic matter (DOM) binds strongly with both Hg and MeHg in natural ecosystems and affects bioavailability.[5] Freshwater systems exhibit much wider gradients in DOM concentrations (<80 to >1700 μM)[6] than in marine systems (34−80 μM).[7] Molecular structure and primary sources marine DOM are much more diverse than in terrestrial systems.[8−10] Here we investigate how differences in marine DOM composition affect Hg reactivity and biological uptake of MeHg in estuarine and continental shelf waters.

In aquatic ecosystems, divalent inorganic mercury (Hg^{II}) can be converted to MeHg by a variety of microbial claves or reduced to elemental mercury (Hg^0) through a combination of photolytic and biological reactions.[11−14] Strong binding of Hg^{II} to DOM has been hypothesized to affect the reactive pool available for both net methylation and net reduction.[8,9,15,16] DOM may also enhance the activity of methylating microbes in oligotrophic ecosystems by providing a substrate for bacterial activity, while under eutrophic conditions, impacts on binding to Hg^{II} may be more important.[17−20] Previous experimental and field studies report mixed effects of DOM on biological uptake of MeHg.[21−24] We hypothesize here that variability in DOM composition helps to explain these results.

Excitation emission matrix (EEM) fluorescence spectroscopy is widely used to characterize differences in the composition of DOM. These data can be used to identify DOM that originates from soils, rivers, and marine productivity according to their characteristic fluorescence intensities and differences in the wavelength maxima.[25] Parallel Factor Analysis (PARAFAC) has been widely used to further separate DOM into chemically distinct components, based on spectra of known compounds or organic matter structure, that are difficult to distinguish in the EEMs.[26] Here we use such an analysis to distinguish broadly between sources with a terrestrial and marine origin in order to minimize over interpretation of the individual PARAFAC components.[27−29]

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The main objective of this work is to better understand how DOM composition affects Hg reactivity and MeHg bioavailability in marine ecosystems. To do this, we collected data on Hg and DOM concentrations and composition in filtered seawater from two estuarine stations in Long Island Sound (LIS), and along a gradient of terrestrial- and marine-influenced locations of the Northwest Atlantic continental margin (abbreviated as NWA from hereon). We compare identified components of DOM using EEM and PARAFAC analysis to Hg concentrations measured at each site and uptake of MeHg by plankton. We complement these data with laboratory experiments that further investigate how DOM composition affects Hg binding using a titration experiment and biological uptake using a bioreporter.

METHODS

Field Sampling. Figure 1 shows seawater sampling locations for DOM and Hg species in LIS and the NWA,

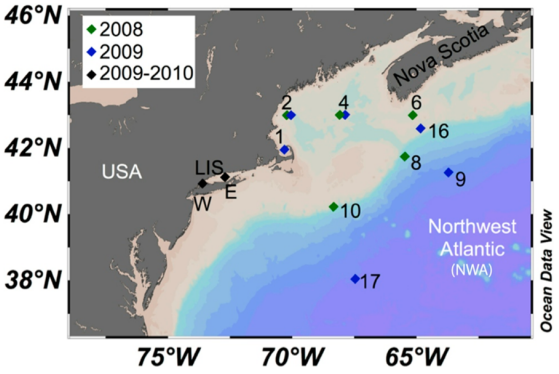

Figure 1. Seawater sampling locations in Long Island Sound (LIS) and on the Northwest Atlantic (NWA) continental margin. Station coordinates are available in SI Table S1.

which includes the Gulf of Maine, shelf, and slope. We collected Long Island Sound seawater using acid-washed, Teflon-lined General Oceanics GO-FLO sampling bottles and Teflon coated messengers deployed on a Kevlar line following trace-metal clean protocols.[30]

LIS samples were obtained from two sites on three cruises: (1) 6–7 August 2009, (2) 09 November to 14 December 2009, and (3) 24–27 May 2010 (see Supporting Information (SI) Table S1 for further details of sampling locations). Samples from the NWA were collected between 17 and 26 August 2008 and 29 September to 7 October 2009. Total Hg, MeHg, and dissolved gaseous mercury (DGM) concentrations in seawater and MeHg in plankton were measured at stations shown in Figure 1. We use particulate MeHg measured in LIS as a proxy for concentrations in microseston (>0.2 μm).[31]

We measured dissolved organic carbon (DOC) concentrations and DOM composition for all LIS cruises and at stations shown in Figure 1 on the NWA cruise in 2008 (SI Table S2). We measured Hg species concentrations for LIS across seasons. For the NWA, we used 2008–2009 data from Soerensen et al.[8] on total Hg and DGM and 2008–2009 data from Hammerschmidt et al.[31] on MeHg in seawater and MeHg in plankton.

Total Mercury and Methylmercury Analyses. All LIS seawater for Hg species analysis was transferred into acid washed FEP bottles in the field prior to filtration, and stored double-bagged inside iced coolers. We filtered samples in a laminar flow hood <6 h after collection using acid-washed vacuum filtration units. Filtrates were acidified and refrigerated, and quartz fiber filters (QFFs) were frozen inside acid-washed plastic dishes.

LIS seawater samples for total Hg analysis were digested with bromine monochloride (BrCl) and prereduced with hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) prior to analysis. Hg^{II} was reduced with tin chloride ($SnCl_2$) to Hg^0 in a bubbler, purged onto traps containing gold-coated beads, heated, and detected using cold vapor atomic fluorescence spectroscopy (CVAFS).[32,33] Samples for MeHg analysis were digested and distilled following Horvat et al.[34] Spike recoveries (105% for distillation spike recoveries, 95 and 97% for aqueous standard recoveries) did not indicate any recovery artifact associated with the levels of DOM present for our samples.[35] The distillates were then ethylated using sodium tetraethylborate[36] and separated using packed-column gas chromatography prior to detection.[33] Filters for particulate MeHg analysis were handled as described in Hammerschmidt et al.[31] Total Hg and MeHg were measured using a PerkinElmer ELAN DRC II Inductively Coupled Plasma Mass Spectrometer or CVAFS.

Dissolved Organic Matter and Nutrients Analyses. We preconcentrated DOM in 25 L of seawater into a 10 mL methanol solution for titration and uptake experiments. Duplicate 2 × 25 L surface water samples were taken at 1 m depth from the ship's seawater inflow at NWA stations sampled in August 2008 (Figure 1) following Dittmar et al.[37] Seawater was filtered using thoroughly cleaned Whatman Polycap capsule filters (sequentially 1, 0.45, and 0.2 μm) and acidified with HCl to a pH of 2. A medium flow peristaltic pump (40 mL min^{-1}) was connected to a modified styrene divinylbenzene polymer (PPL) type sorbent cartridge (Varian), prerinsed with 0.01 M hydrochloric acid (HCl) and methanol. Each sample was eluted through a PPL cartridge, rinsed with 0.01 M HCl, and dried with nitrogen gas. Finally, the cartridge was eluted with methanol (~10 mL) into a muffled amber glass vial (550 °C) at a flow rate of 2 mL min^{-1}. The eluate was frozen and freeze-dried.

LIS seawater samples for DOC, dissolved total nitrogen (TN), and fluorescence intensity measurements were initially filtered through muffled (550 °C) Glass Fiber Filters (GFFs) and 0.22 μm (Durapore Millipore) filters into muffled amber glass vials and frozen until analysis. Water for nutrient analyses was collected in muffled scintillation vials. Muffled GFFs (25 mm) with particulate material for carbon, nitrogen, and sulfur measurement were immediately frozen. Seawater was filtered through a preweighed GFF (47 mm) for total suspended solids (TSS) and through a muffled QFF for particulate MeHg (SI Table S3). Muffled GFFs with particulate material were oven-dried at 70 °C and analyzed for carbon, nitrogen, and sulfur (CNS) content using a CNS elemental analyzer (Fisons NA 1500 series 2). DOC and TN were determined using a Shimadzu TOC-V and TN auto analyzer.

We analyzed the composition of marine DOM in seawater using three-dimensional excitation–emission fluorescence spectroscopy (3D-EEM). For all samples and blanks, we measured fluorescence at room temperature associated with excitations ranging from 220 to 450 nm every 5 nm and emissions ranging from 230 to 700 nm every 1 nm (PMT Voltage of 700 V, and emission and excitation slits of 5 nm) using a Hitachi F2000 fluorometer with 1 cm quartz cells. Reabsorption of fluorescence by neighboring molecules within each solution

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Table 1. Excitation and Emission of each PARAFAC Component and Previously Identified Characteristics[42−45]

	excitation (nm)	emission (nm)	literature description	classification for this work
C1	275−290	330−350	protein-like, tyrosine	marine
C2	240−325	370−420	humic-like, terrestrially derived, microbial humic	terrestrial
C3	250−380 (320−375)[a]	425−500	humic-like, large molecular size, hydrophobic compounds, microbial humic	terrestrial
C4	245−275	350−400 (280−300)	protein-like, tryptophan	marine
C5	290−325	380−450	humic-like and marine-like, medium size compounds	terrestrial
C6	220−225 (270)	320−360	protein-like	marine
C7	230−240 (350−375)	435−500	humic-like, terrestrial, small molecular size compounds	terrestrial

[a]Secondary peak locations ranges in parentheses.

(also known as the inner filter effect) was reduced by maintaining the absorption coefficients of each solution below 0.04 cm^{-1} at 220 nm.[38]

Excitation−emission spectra were corrected by subtracting the intensities of the matrix used for DOM solution (Milli-Q water or phosphate buffer solutions). Scatter in the analytical results was reduced by inserting zeros into the matrix for instances where no signal above background could be detected. Calibrated fluorescence intensities (SI Figure S1) are presented in Raman Units (R.U.). Additional spectra processing details are given in the SI.[39]

We used PARAFAC modeling[40] to group fluorescence spectra (n = 57) for samples from LIS and the NWA in 2008 into seven distinct components designated C1−C7 (Table 1) using the DOMFluor toolbox in MATLAB.[41] We categorized five of the seven components as terrestrial or marine by comparing their excitation and emission loading plots to previously identified components published in the literature.[42−45] For the remaining two components, this designation was based on statistical association with the identified components. SI Figures S2 and S3 show individual excitation and emission loading plots used in PARAFAC modeling, and the relative component fluorescence is summarized in SI Table S4.

Complexation and Uptake Experiments. DOM fluorescence is quenched when Hg binds to ligands closely associated with fluorophores.[46−49] To examine the Hg binding sites of DOM, we added $Hg(NO_3)_2$ at concentrations between 0 and 0.75 μM to a solution containing 30 mg L^{-1} DOM from Station 8 on the NWA (Figure 1), 0.04 M phosphate buffer (pH 6), and 0.1 N sodium chloride.[15] Station 8 was chosen because it contains all seven terrestrial and marine DOM components. A variety of studies indicate that complexation of Hg and MeHg with DOM occurs preferentially to thiol groups that can be saturated.[50−52] The ratio of Hg to carbon (on a molar basis) for these addition experiments ranged from 4×10^{-5} to 7.5×10^{-4}. This is at the high end of the ratios found in sediments (10^{-5} to 10^{-8})[53,54] but within the range where binding of Hg to the high affinity natural DOM sites dominates.[55] All solutions were prepared in duplicate and incubated in the dark for 24 h to allow equilibrium to be reached.[56] We measured fluorescence of each solution using the same methods as field samples. Differences between experimental replicates were insignificant based on one-way ANOVA and showed high reproducibility for all components.

To examine DOM impacts on MeHg bioavailability, we used the *Escherichia coli* (*E. coli*) mer-lux biosensor to investigate the effects of terrestrial and marine DOM sources on cellular uptake. The biosensor is described in detail elsewhere[57] and produces light proportionally to the amount of MeHg inside cells but not the exterior solution. We incubated 5 nM MeHg solutions containing terrestrial (Suwannee River terrestrial reference material purchased from the International Humic Substance Society) or marine (NWA Station 10, Figure 1) DOM between 0 to 100 mg L^{-1} in 40 mL liquid scintillation vials for 24 h in the dark and then added biosensor cells. We selected NWA Station 10 DOM as the representative marine material because it did not contain terrestrial DOM components. Four replicates were prepared for each solution. We measured bioluminescence of resulting solutions using a Packard Tri-Carb 3100 TR Liquid Scintillation Analyzer in the single photon mode.

RESULTS AND DISCUSSION

Identification of Terrestrial and Marine DOM Components. Figure 2 contrasts the EEMs characteristic of terrestrial and marine DOM. The terrestrial reference material is from the Suwannee River (Figure 2A) and no marine DOM reference material is available, so we used offshore seawater from NWA Station 6 for comparison. The terrestrial DOM has a much

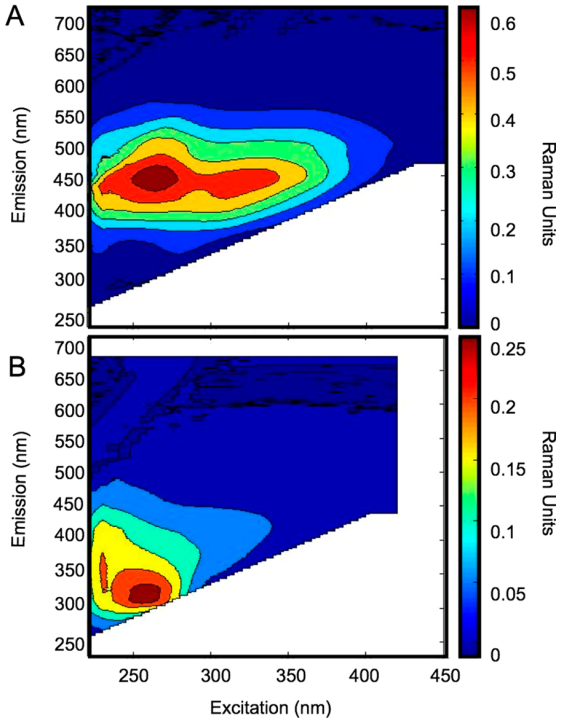

Figure 2. Three-dimensional excitation emission matrix (3D-EEM) for Panel (A) Suwannee River reference material obtained from the International Humic Substance Society and Panel (B) marine DOM from Station 6 on the Northwest Atlantic (NWA) continental margin.

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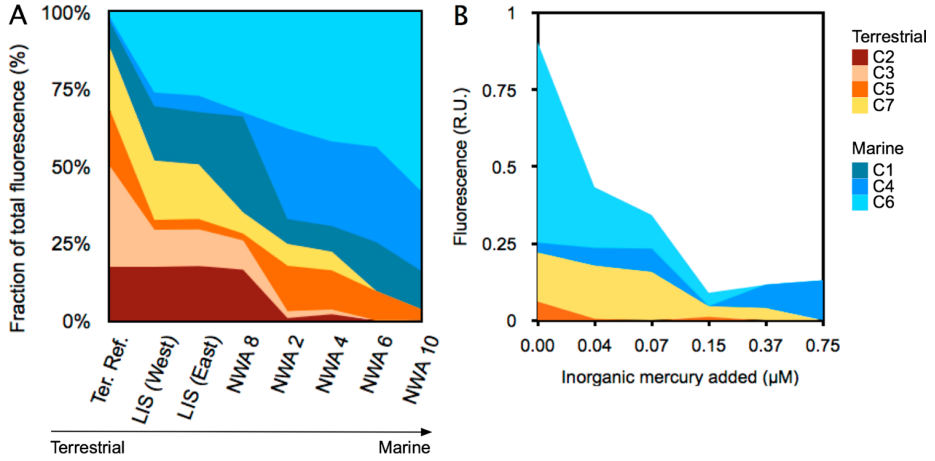

Figure 3. Panel (A) shows the fraction of fluorescence intensity attributable to terrestrial and marine dissolved organic matter (DOM) components across a range of terrestrial to marine sampling stations. SWR = Suwannee River terrestrial reference material; LIS = Long Island Sound; NWA = Northwest Atlantic continental margin stations (Figure 1). Panel (B) Experimental results showing change in DOM fluorescence intensity (NWA Station 8) with increasing inorganic mercury concentrations. DOM from Station 8 displayed fluorescence from all seven DOM components (Panel A), but only those with changes greater than experimental variability are reported here (SI Figure S5).

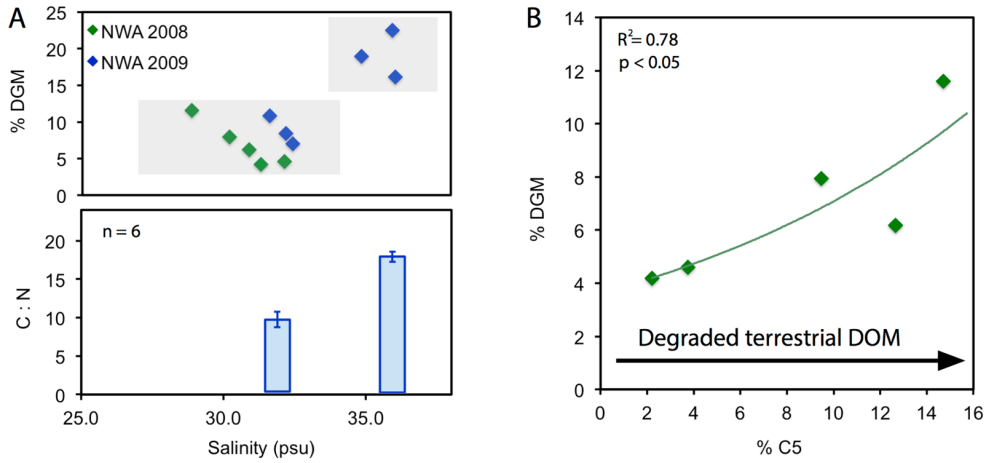

Figure 4. Relationship between DOM composition and dissolved gaseous mercury as a fraction of total mercury (%DGM) in offshore marine waters. Panel (A) shows higher %DGM in offshore seawater (salinity ≥35) compared to nearshore stations (salinity <35) in the Northwest Atlantic (NWA) (data from Soerensen et al.[8]) and corresponding carbon to nitrogen molar ratios (C/N) measured in this work. Bars represent average C/N ratios for nearshore and offshore NWA stations from 2009. Panel (B) shows increasing %DGM with a higher fraction of the DOM component that reflects degraded terrestrial organic matter (C5) during the 2008 NWA cruise.

broader range of excitations and higher characteristic emission wavelengths than the marine DOM (Figure 2B). SI Figure S4 shows the hybrid fluorescence spectra obtained from the estuarine site in LIS. These data illustrate how differences in the characteristic fluorescence of terrestrial and marine sources of DOM can be used for their identification.

Figure 3A shows the seven DOM components identified in PARAFAC modeling for all sampling sites. We used data from Table 1 to classify each DOM component as either terrestrial (C2, C3, C5, and C7) or marine (C1, C4, and C6). Generally, humic components were classified as terrestrial and protein-like components as marine.[25,58] For C1 and C5, these designations are less clear. We grouped C1 into the marine category based on its strong association with C6 (pairwise correlation $r = 0.96$, $p < 0.001$, $n = 17$) and C5 into the terrestrial category due to its association with C2 ($r = 0.73$, $p = 0.001$, $n = 17$) in LIS seawater. C5 is also known to be the product of degradation of terrestrially derived organic matter,[42,43] and increases at sites with a higher C:N ratio in seawater ($R^2 = 0.39$, $p = 0.01$, $n = 16$).

Hg Binding to Terrestrial and Marine DOM Components. Figure 3B shows results of our titration experiment that progressively added Hg^{II} to seawater containing DOM from NWA Station 8. Results illustrate that both the terrestrial and marine DOM components designated here bind to Hg^{II}, resulting in changes in fluorescence. Metal binding can enhance the fluorescence of some DOM components while quenching others.[59,46] For components C5, C6, and C7, emission peaks are quenched (red-shifted) and become broader and less defined by cation binding to ligands closely associated with fluorophores, which is consistent with previous literature.[46−49] As the amount of Hg added to NWA Station 8 seawater is increased from zero to 0.75 μM (Figure 3B), total fluorescence decreases significantly (one-way ANOVA, $p < 0.05$) by approximately 40%. By contrast, fluorescence increases over 400% at higher Hg concentrations for the C4 component which

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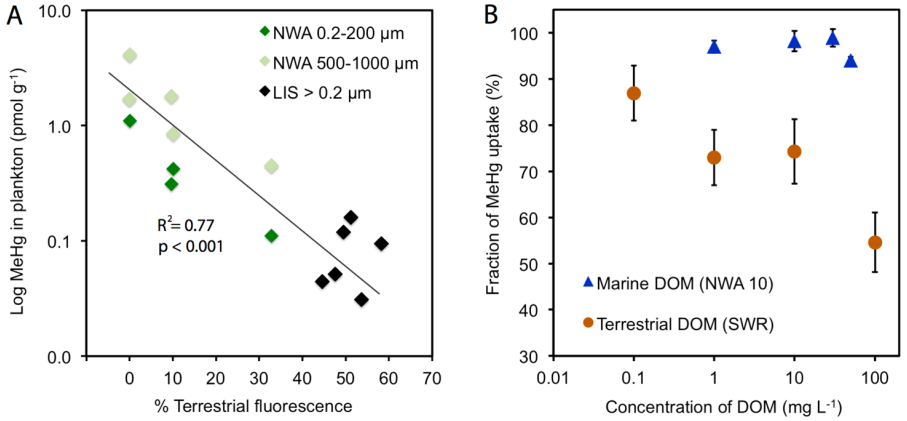

Figure 5. Field (Panel A) and experimental (Panel B) measurements of the effects of DOM composition on MeHg uptake by plankton. Panel (A) shows relationship between fluorescence attributable to terrestrial DOM components and MeHg concentrations in plankton from Long Island Sound (LIS) (data from this study) and the Northwest Atlantic continental margin (NWA) (data from Hammerschmidt et al.[31]). Panel (B) shows MeHg uptake in the *E. coli* bioreporter[52] in the presence of marine (NWA Station 10) and terrestrial (Suwannee River) DOM.

is also indicative of binding.[46,59] These results reinforce that Hg has a strong affinity for both marine and terrestrial DOM components. Ndu et al. (2012)[57] showed that MeHg and Hg^{II} behave similarly in the presence of DOM. Differences in Hg speciation and uptake discussed in later sections therefore likely reflect DOM quality and bioavailability rather than lack of binding by marine DOM.

MeHg Concentrations in Marine Seawater. Measured MeHg concentrations were 5-fold lower in the Atlantic (~50 fM) than in LIS (~250 fM). We found no significant relationship between MeHg and DOM concentration or composition across all sites but this is likely due to the influence of external MeHg sources in LIS. Observed variability in field concentrations in LIS can be explained by large MeHg inputs from the watershed and freshwater tributaries.[16,60,61]

Within the 2008−2009 Atlantic margin cruises, we observed a significant increase in %MeHg with salinity (SI Figure S6; R^2 = 0.5, p = 0.02). Since salinity and terrestrial DOM content are inversely correlated (R^2 = 0.73, p < 0.01) this is suggestive of increasing methylation in offshore waters with lower terrestrial organic matter content. Further data are needed to test this hypothesis since we only measured data on DOM composition for the 2008 NWA cruise (SI Figure S7).

Impact of DOM on Dissolved Gaseous Hg (DGM) Concentrations. Soerensen et al.[8] showed that %DGM was significantly higher in offshore NWA seawater (salinity ≥35) compared to nearshore stations (salinity <35). The authors lacked direct measurements of DOM but hypothesized that a higher fraction of marine DOM may increase the reducible pool of Hg^{II}.[8] We measured differences in DOM across stations used in the Soerensen et al.[8] analysis and nearshore and offshore concentrations to be similar (77 ± 13 μM) but carbon to nitrogen (C/N) molar ratios are suggestive of differences in terrestrial DOM. Measured C/N ratios in filtered waters were significantly higher at the offshore stations and are consistent with DOC/DON ratios found in surface seawater (Figure 4).[62] Low nitrate levels in seawater from our sampling stations (<2 μM) mean most of the dissolved nitrogen is organic (DON).[63,64] Selective mineralization of DON over DOC leads to an increase in C/N ratios as decomposition progresses.[65−67] Thus, increases in C/N ratios at offshore stations likely reflect aging of organic matter (Figure 4A).

Figure 4B shows increasing %DGM in NWA seawater with a higher fraction of degraded terrestrial DOM indicated by the C5 component (R^2 = 0.78, p < 0.05).[42,43] This supports the hypothesis that turnover of labile terrestrial organic reduces the stability of Hg bound to DOM, which enhances Hg^{II} reduction rates. Aging of terrestrial DOM rather than marine DOM may drive the increase in %DGM in offshore marine waters observed by Soerensen et al.[8] Baeyens and Leermakers[68] also observed a higher %DGM in more saline waters of the North Sea but did not present any data on DOM composition.

Terrestrial DOM Decreases MeHg Uptake by Plankton and Bacteria. Figure 5A shows MeHg in NWA and LIS plankton declines with decreasing contribution to total fluorescence from terrestrial DOM sources (increasing fluorescence from marine DOM). This relationship holds across all size fractions despite trophic interactions in larger size classes (SI Figure S7), suggesting that the effect of DOM on MeHg uptake propagates through the food-web. Lowest levels of MeHg in plankton were observed in LIS despite highest ambient MeHg concentrations, where the fraction and concentrations of terrestrial DOM were also highest (~150 μM). Plankton MeHg concentrations were more variable in LIS than other sites, likely due to more heterogeneous MeHg sources, as discussed above. Total fluorescence from terrestrial sources across NWA stations varied from zero to >30% and was also inversely associated with MeHg concentrations in plankton (Figure 5A and SI Figure S7). We did not observe a statistically significant association between total DOC concentrations and plankton MeHg content but this likely reflects the narrow range in DOC concentrations between LIS and the NWA. For example, Luengen et al.[69] observed declines in planktonic MeHg uptake across a much wider gradient in DOC concentrations than measured in our study.

Figure 5B shows experimental results of MeHg uptake into a bioreporter (*E. coli*)[57] measured in the presence of contrasting marine and terrestrial DOM sources. Similar to field measurements, uptake of MeHg was less efficient in the presence of high terrestrial DOM concentrations, but unaffected by marine DOM. Both field and experimental results thus suggest marine DOM does not affect cellular uptake of MeHg but terrestrial DOM can inhibit uptake. Results of our titration experiment (Figure 3B) show Hg binds to both terrestrial and marine DOM. We thus hypothesize that differences in MeHg uptake in

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the presence of marine and terrestrial DOM are driven by the larger molecular weight of terrestrial DOM, which may restrict passive transport through the cellular membrane.[57]

Results of this research show that DOM composition has a large impact on reactivity of Hg and biological uptake of MeHg. Hg^{II} binds to both terrestrial and marine DOM components, as illustrated by the titration experiment (Figure 3). We found a positive relationship between the DOM components that reflects degraded terrestrial material (C5) and %DGM in offshore marine waters (Figure 4). These results, in combination with higher C/N ratios in offshore waters, suggest that the reducible pool of Hg^{II} is linked to degradation of terrestrial DOM in marine water. This may also imply that the pool available for methylation is increased in offshore marine waters but needs to be resolved with additional measurements. Both experimental and field results suggest terrestrial DOM has an inhibitory effect on MeHg uptake by bacteria and phytoplankton, but marine DOM does not (Figure 5). Differences in Hg reactivity and MeHg uptake in the presence of marine and terrestrial DOM help to explain higher bioaccumulation factors often found in marine systems compared to terrestrial sites.[70,71]

■ ASSOCIATED CONTENT

Supporting Information

Additional information on PARAFAC analysis, 4 tables, and 7 figures. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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