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Bioaccumulation of methylmercury in a marine diatom and the influence of dissolved organic matter

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A B S T R A C T

The largest bioconcentration step of most metals, including methylmercury (MeHg), in aquatic biota is from water to phytoplankton, but the extent to which dissolved organic matter (DOM) affects this process for MeHg largely remains unexplored in marine systems. This study investigated the influence of specific sulfur-containing organic compounds and naturally occurring DOM on the accumulation of MeHg in a marine diatom *Thalassiosira pseudonana*. Initial uptake rate constants and volume concentration factors (VCFs) of MeHg were calculated to evaluate MeHg enrichment in algal cells in the presence of a range of organic compound concentrations. At environmentally realistic and higher concentrations, the addition of glycine and methionine had no effect on algal MeHg uptake, but thiol-containing compounds such as cysteine and thioglycolic acid reduced MeHg accumulation in algal cells at high added concentrations (> 100 times higher than naturally occurring concentrations). However, environmentally realistic concentrations of glutathione, another thiol-containing compound as low as 10 nM, resulted in a decline of ~30% in VCFs, suggesting its possible importance in natural waters. Humic acid additions of 0.1 and 0.5 mg C/L also reduced MeHg VCFs by ~15% and ~25%, respectively. The bioaccumulation of MeHg for *T. pseudonana* in coastal waters with varying levels of dissolved organic carbon (DOC) was inversely correlated with bulk DOC concentrations. Generally, naturally occurring DOM, particularly certain thiol-containing compounds, can reduce MeHg uptake by phytoplankton.

1. Introduction

Methylmercury (MeHg) can cause adverse health effects on exposed wildlife and humans due to its neurotoxicity (Grandjean et al., 2010; Mason et al., 2012). In addition, it is one of the few metals that biomagnifies in aquatic food chains, resulting in much higher concentrations in organisms at upper trophic levels than primary producers (Morel et al., 1998). The largest bioconcentration step (often $> 10^5$, depending on the species) is from the aqueous phase to phytoplankton primary producers, which serve as the base of most marine food webs (Lee and Fisher, 2016). Consequently, these algal cells provide highly enriched sources of MeHg for herbivores. Thus, small or moderate differences in enrichment of MeHg at the base of aquatic food chains could lead to pronounced effects on the overall MeHg concentration in marine animals.

It has been suggested that MeHg is taken up by phytoplankton mainly through passive diffusion (Lee and Fisher, 2016; Mason et al., 1996), and its uptake rate and magnitude is governed by its chemical form. Many trace metals in seawater are known to form complexes with organic ligands which could potentially mitigate their bioavailability and toxic effects to marine microorganisms (Vraspir and Butler, 2009). However, little is known regarding MeHg complexation by naturally occurring organic compounds and its influence on bioaccumulation in microorganisms in marine environments. Mercury (Hg) as well as MeHg are known to bind strongly to sulfur (Fitzgerald et al., 2007; Ravichandran, 2004), an essential element required for protein synthesis. Biogenic compounds with sulfhydryl groups (also known as thiols) such as glutathione (GSH) and cysteine (Cys) can be produced and released into seawater by marine organisms (Dupont and Ahner, 2005; Swarr et al., 2016; Tang et al., 2000; Tang et al., 2005). Among many thiols species, GSH is thought to be a major ligand for many soft, B-type metals (e.g., Cu, Pb, Cd, Zn, Hg and MeHg) in neutral or basic conditions (Krezel and Bal, 1999). Field studies suggested that high-affinity ligands like GSH may account for most of the Hg complexation in estuarine waters (Han et al., 2006). A few studies have addressed how organic ligands affect Hg and MeHg bioavailability to bacterioplankton, motivated by their importance in MeHg production and demethylation (Hsu-Kim et al., 2013; Ndu et al., 2016; Ndu et al., 2012; Schaefer and Morel, 2009). For instance, the formation of a Hg-Cys complex has been shown to promote the uptake of Hg(II) by the bacterium, *Geobacter*

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sulfurreducens, and further enhance the enzymatic formation of MeHg (Schaefer and Morel, 2009). However, studies assessing how complexation of MeHg by naturally occurring organic compounds affect its bioavailability for marine phytoplankton are sparse.

In previous studies, Lawson and Mason (1998) examined the effect of organic and inorganic ligands to MeHg uptake by a marine diatom, and demonstrated inconsistent results among three ligands. Complexation of MeHg-bisulfide decreased the phytoplankton uptake rate and the uptake rate of MeHg-Cys and MeHg-thiourea complexes increased with increasing complexation by these ligands. Pickhardt and Fisher (2007) found that methionine (Met) had no significant effects on MeHg bioavailability to freshwater phytoplankton. Luengen et al. (2012) demonstrated that Cys at elevated concentrations diminished MeHg accumulation in the same diatom. In addition to the effects of specific organic compounds, MeHg accumulation in algae has been correlated with bulk dissolved organic carbon (DOC) concentrations. Generally, studies reported inverse relationships between DOC concentrations and MeHg enrichment in freshwater algae (Gorski et al., 2008; Luengen et al., 2012; Zhong and Wang, 2009), implying that binding of MeHg-DOC reduce MeHg bioavailability for phytoplankton. Variability among studies evaluating the effects of dissolved organic matter (DOM) on MeHg bioavailability may be attributable to variations in DOM composition and/or the effects of ionic strength in different water bodies. In marine environments, in addition to its high ionic strength, it is known that chloride ions (Cl^-) are important ligands for MeHg competing against other organic ligands (Fitzgerald et al., 2007). For this reason, the effects of marine DOM on MeHg bioavailability for algae need further exploration.

This study performed a series of experiments to systematically assess how concentrations of specific organic compounds affect the bioavailability of MeHg to a marine diatom. Selected organic compounds included glycine (Gly), cysteine (Cys), methionine (Met), thioglycolic acid (TGA), glutathione (GSH) and humic acid (HA). Gly is the most common and abundant amino acid in the ocean. Cys and Met are amino acids with sulfur content. TGA is not commonly seen and measured in marine environments, but because it contains a sulfhydryl group and has a similar size and structure to Gly (Table 1), we chose to use it in our study to demonstrate the importance of sulfur on MeHg-ligand complexation. GSH is widely observed in natural waters. HA is a principal component of humic substances and a major organic constituent of many natural waters. We also examined MeHg uptake in seawater containing different concentrations of naturally occurring DOM.

2. Methods

2.1. Phytoplankton cultures

We used a unicellular, centric marine diatom *Thalassiosira pseudonana* (clone CCMP1335) that has been widely used to evaluate metal bioaccumulation in marine environments, including MeHg (Lee and Fisher, 2016). Unialgal clonal cultures were maintained axenically with f/2 medium (Guillard and Ryther, 1962) at 18 °C under a light:dark cycle (14 h:10 h, 200 μmol quanta m^{-2} s^{-1}). Filter-sterilized (0.2 μm) surface seawater collected 8 km off Southampton, NY (Southampton seawater, SHSW, S = 35‰) was used to prepare f/2 medium for routine culture maintenance. To avoid the potential effects of ethylenediaminetetraacetic acid (EDTA) on MeHg speciation, experimental cultures were grown in separate flasks in artificial seawater (ASW) with amended f medium (nutrients at f/20 level) without adding EDTA for 7 d prior to all experiments.

2.2. Preparation of organic compounds and culture media

The chemical structures, stability constants with MeHg and other relevant information regarding the organic compounds added to the diatom cultures are presented in Table 1. Gly, Cys, Met, TGA and GSH are pure chemicals purchased from Sigma-Aldrich®. HA, also obtained from Sigma-Aldrich® (H16752) is a mixture of compounds from the decomposition of organic matter, particularly from dead plants, with a molecular weight range of 2000–500,000. Each organic compound except HA was dissolved in Milli-Q® water individually to make a stock solution (1 mM). To make a stock solution for HA where the molecular weight is uncertain, we dissolved 100 mg of HA in 100 mL of ASW (S = 35‰) and added sodium hydroxide to increase pH, enhancing its solubility. The solution's pH was then adjusted back to 8.2 and insoluble particulate HA was removed by passing through a 0.2 μm polycarbonate membrane. Total organic carbon (TOC) concentrations in the HA stock solution was determined using a Thermo Finnigan Flash EA 1112 elemental analyzer.

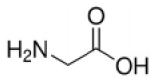
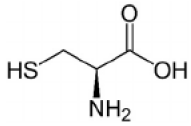
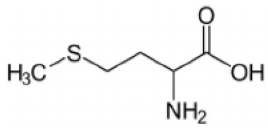
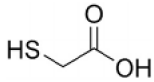
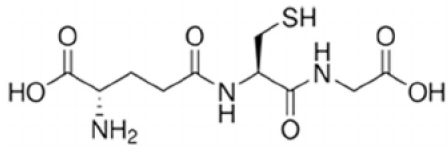
To minimize the potential effects of natural DOM on MeHg bioavailability, we prepared ASW with very low DOC concentrations as our experimental medium. First, all glassware and anhydrous salts (NaCl, Na_2SO_4, KCl, $NaHCO_3$, KBr and NaF) were combusted at 450 °C for 8 h and were then dissolved in Milli-Q® water subjected to UV-photooxidation of organic matter. The produced Milli-Q® water had a background DOC concentration below the detection limit (0.17 μM). Other noncombustible salts ($MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, $SrCl_2{\cdot}6H_2O$ and H_3BO_3) were added as a solution. The produced ASW was then filtered by passing through a combusted GF/F filter. The final salinity was 35‰ and the pH was adjusted to 8.2. Average DOC concentrations in our ASW were 40 ± 20 μM which is comparable to those in open ocean seawater (Hansell and Carlson, 2002; Kaiser and Benner, 2009).

2.3. MeHg uptake experiments

In general, the MeHg uptake experiments followed earlier studies described for mercury uptake by marine phytoplankton with a gamma-emitting radioisotope of Hg (^{203}Hg, $t_{½}$ = 46.6 d) to trace its partitioning between dissolved and algal cells (Fisher et al., 1984; Lee and Fisher, 2016). $^{203}Hg(II)$ was obtained from Eckert and Ziegler Isotope Products (Valencia, California) with a specific activity of 5 Ci g^{-1} (= 185 GBq g^{-1}). Methyl-^{203}Hg ($Me^{203}Hg$) was synthesized in the lab following established methods (Lee and Fisher, 2016; Rouleau and Block, 1997). In brief, ^{203}Hg was mixed with methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$, Sigma) at pH 5 with addition of acetate buffer. The reaction proceeded in the dark for 18 to 24 h and $Me^{203}Hg$, produced spontaneously, was then extracted by dichloromethane (CH_2Cl_2), purified, and re-dissolved in Milli-Q® water. The conversion yield (fraction of total ^{203}Hg recovered as $Me^{203}Hg$) was 99 ± 1% (n = 2). $Me^{203}Hg$ concentrations dissolved in seawater in our study ranged from 0.28 to 0.48 nM, corresponding to $Me^{203}Hg$ activities from 2.42 to 5.39 kBq L^{-1}. Radioactivity of $Me^{203}Hg$ in seawater or associated with algal cells was determined at 279 keV using an LKB Wallac 1282 Compugamma NaI (Tl) gamma detector. All measurements were counted with standards and decay-corrected. Propagated counting errors were below 5%. The experimental concentrations were below toxic levels to marine algae but were higher than those found in natural aquatic environments. However, previous studies have shown that bioconcentration factors and uptake rate constants for MeHg are not influenced at these concentrations (Lee and Fisher, 2016). For those organic compounds whose sulfur content were known, the MeHg to sulfur ratios used in the uptake experiments were all less than one. The MeHg to HA and MeHg to DOC ratios used in our study were also far below the estimated MeHg binding capacities reported from previous studies (Haitzer et al., 2002; Hintelmann et al., 1997; O'Driscoll and Evans, 2000).

To assess the influence of the specific organic compound concentrations on MeHg uptake by marine algae, we used trace metal clean and organic carbon free glass-stoppered Erlenmeyer flasks, each containing 140 mL of ASW and microliter quantities of $Me^{203}Hg$. Different levels of organic compounds from stock solutions were then added to

Table 1
Organic compounds used in this study and their chemical structures and stability constants with MeHg.

Ligand name	Chemical structure	log K	Reaction	Reference
Chloride		5.13[a]	$M^+ + L^-$ ML	[1]
Glycine (Gly)		7.52[a] 2.00[b]	$M^+ + L^-$ ML $M^+ + HL$ $M(HL)^+$	[1–3]
Cysteine (Cys)		16.46[c] 15.11[c]	$M^+ + L^{2-}$ ML^- $M^+ + HL^-$ M(HL)	[1, 3–6]
Methionine (Met)		7.17[a]	$M^+ + L^-$ ML	[1, 4, 6]
Thioglycolic acid (TGA)		16.93[c] 10.53[c]	$M^+ + L^{2-}$ ML^- $M^+ + HL^-$ M(HL)	[1, 3, 5]
Glutathione (GSH)		15.99[c] 15.55[c] 10.24[c]	$M^+ + L^{3-}$ ML^{2-} $M^+ + HL^{2-}$ $M(HL)^-$ $M^+ + H_2L^-$ $M(H_2L)$	[1, 5]
Humic acid (HA)		10–15		[7–9]

Reference: [1] NIST (2004), [2] Jawaid and Ingman (1981), [3] Alderighi et al. (2003), [4] Berthon (1995), [5] Arnold and Canty (1983), [6] Rabenstein (1978), [7] Fitzgerald and Lamborg (2003), [8] Amirbahman et al. (2002), [9] Hintelmann et al. (1997).

[a] Ionic strength = 1, temperature = 25 °C.

[b] Ionic strength = 0.15, temperature = 25 °C.

[c] Ionic strength = 0.1, temperature = 25 °C.

ASW (0, 10, 100, 1000 nM for Gly and TGA; 0, 1, 10, 100, 1000 nM for Cys, Met, and GSH; 0, 0.1, 0.5, 1.0, 5.0 mg C/L for HA). The pH in each flask was about ~8.2 and concentrations of nitrate, phosphate and silicate in each flask were fixed at 5 μM, 1 μM and 10 μM, respectively. Flasks containing $Me^{203}Hg$ and organic compounds were incubated at 18 °C in the dark for 8 h to allow equilibration before inoculation of algal cells. Inocula of diatom cells were concentrated by resuspending cells off 1 μm polycarbonate membranes from experimental cultures (without adding EDTA). Initial cell densities ranged from 6 to 8×10^4 cells mL^{-1}. Water and cell samples were collected periodically (t = 2, 4, 8, 12, 24, 48, 72) for radioassays. In brief, we measured the total radioactivity in 1 mL of unfiltered water (water plus cells) and 5 mL of suspension (cells only) collected onto a 1 μm polycarbonate membranes (washed with 5 mL of unlabeled, filtered ASW; vacuum pressure < 100 mmHg). Each type of treatment was run in duplicate, and control treatments (no algal cells) were performed simultaneously to correct the potential adsorption of dissolved $Me^{203}Hg$ onto membranes. Through this approach, the fractions of radioactivity in the dissolved phase and associated with the cells were determined. In addition, cell growth was monitored simultaneously over time using a Multisizer Coulter Counter®. Using the cellular radioactivity, cell density, and cell volume, we evaluated the degree of bioconcentration of the ^{203}Hg in the cells at each sample time by calculating volume concentration factors (VCFs), which expresses the enrichment of MeHg in algal cells relative to the ambient seawater as:

In the experiment to assess how bulk DOC concentrations affect MeHg uptake by marine algae, we prepared different seawater media with various DOC concentrations, including ASW, SHSW, and Long Island Sound seawater (LISSW). LISSW was collected from western Long Island Sound (S = 27.2‰, 40.96 N, 73.58 W), central LIS (S = 27.6‰, 41.14 N, 72.66 W), and eastern LIS (S = 30.4‰, 41.26 N, 72.07 W) and filtered through a 0.2 μm cartridge filter during a cruise on board the R/V Connecticut during September 10–12, 2014. Additionally, ASW and SHSW were mixed together in different ratios to prepare seawater with a DOC gradient between those of ASW and SHSW. The bioaccumulation of $Me^{203}Hg$ added to each water type, inoculated with *T. pseudonana,* followed the same protocols described above; for each medium, corresponding controls (without algal cells) were also examined in triplicate. DOC concentrations were measured using a Shimadzu TOC-L analyzer.

Sorption of MeHg onto flasks walls was examined by acid-rinsing (10% v/v HCl) the flask walls after each experiment. In general, adsorption of MeHg onto flask walls was very low (< 2%) and the mean mass balance of MeHg in each treatment was 97 ± 8% (n = 96), suggesting no MeHg loss during the experiments.

3. Results

After 72 h of exposure, 50–60% of MeHg was taken up by diatoms from the dissolved phase in the treatment without any addition of organic compounds (Fig. 1). Separate control treatments (no added organic compounds, diatoms exposed to MeHg) were tested in duplicate for each of 6 series of organic compounds, and all showed comparable results, indicating high reproducibility. MeHg uptake by algal cells was rapid in the first 12 h and reached steady-state between 12 h and 24 h. Growth of the diatoms was unaffected by any additions of organic matter other than HA additions ≥ 1 mg C/L, which stimulated growth (Fig. 2). Even though algal cells kept growing in all cultures, thereby providing more cell surfaces (Fig. 2), the total amount of MeHg bound to cells slowed down and leveled off, probably indicative of the decline in bioavailable MeHg remaining in the dissolved phase after 24 h.

Regardless of any given concentration added in Gly, Met and TGA treatments, there were no significant influences on the MeHg uptake (Fig. 1). For Cys treatments, no significant effects on the uptake were found when Cys levels were below 100 nM, whereas a high level of

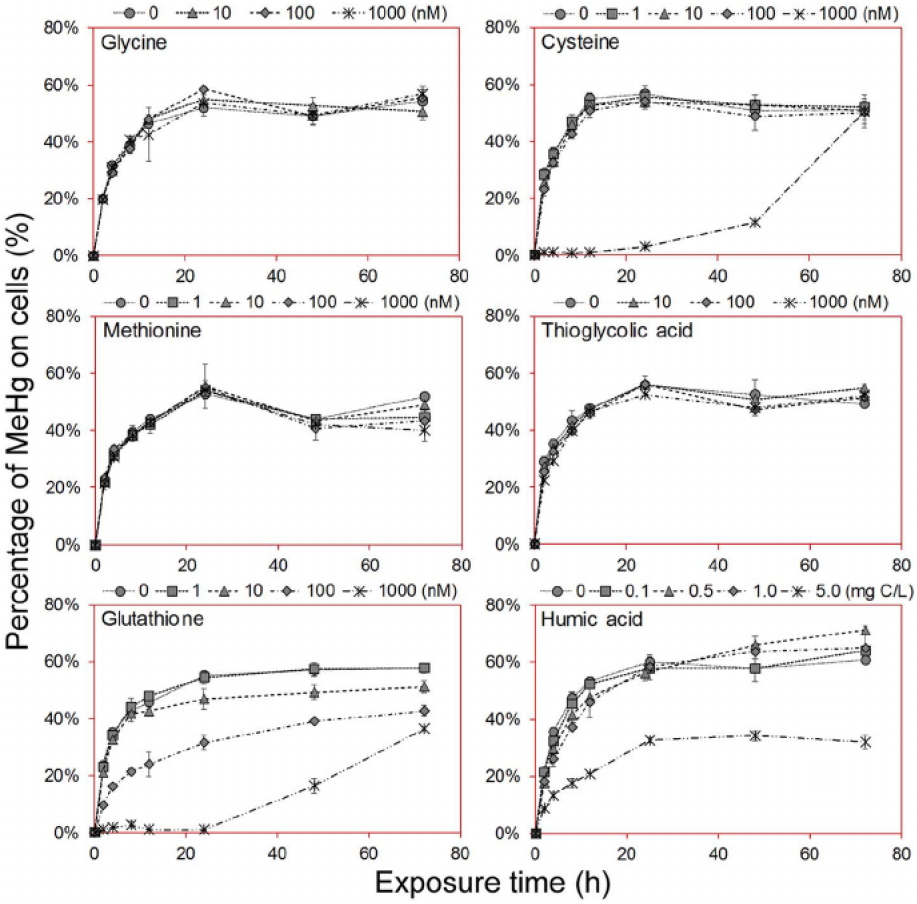


Fig. 1. Effects of varying concentrations of different dissolved organic compounds on the bioaccumulation of MeHg by cells of *Thalassiosira pseudonana*. Data points, the means from two replicates cultures ± 1 SD at each sample time, indicate the fraction of total MeHg associated with cells over time.

addition (1000 nM) greatly lowered the MeHg uptake by algal cells (Fig. 1). For GSH treatments, a significant decrease in the uptake was observed when GSH's concentrations were higher than 10 nM. For HA treatments, low levels (0.5 to 1.0 mg C/L) seemed to slightly decrease MeHg uptake and a high level (5.0 mg C/L) led to a significant decline in uptake (Fig. 3).

Combining the MeHg uptake with cell growth, MeHg content of the diatoms was normalized on a cell volume basis (Fig. 3). There were no substantial differences in cell growth among treatments (Fig. 2) except HA treatments. Therefore, VCFs generally showed a similar pattern with percentage of MeHg on cells (Fig. 1) and the difference in VCFs mainly reflect the influences from the added organic compounds. VCFs reached maximum values between 12 h and 24 h, after which they declined, reflecting subsequent cell growth and biological dilution of cellular MeHg content (Fig. 3). Briefly, biological dilution indicates that the total cell-bound MeHg stayed constant after the first 24 h of exposure but its concentration in cell biomass (as well as concentration factor) decreased as the cells continued to grow/divide.

Addition of Gly and Met had no significant effects on MeHg VCFs at all; high concentrations (1000 nM) of TGA and Cys lowered MeHg VCFs, but the decline was much greater in Cys treatments (> 85% decline) than in TGA treatments (~15% decline). For both GSH and HA treatments, an inverse relationship was found between the MeHg VCFs and the added concentrations of GSH and HA (Fig. 4). The short-term uptake rate constant, which was normalized to the dissolved MeHg concentrations in the media was calculated on per cell basis for the first 4 h of exposure (Fig. 4, Table 2). In general, high uptake rate constants corresponded to high concentration factors at steady state. The uptake rate constants and mean VCFs at steady state are given in Table 2.

In the experiment comparing different types of seawater, ASW exhibited the highest VCFs for MeHg ($11.7–23.1 \times 10^5$) and LISSW had the lowest VCFs ($2.15–8.59 \times 10^5$) over time (Fig. 5). SHSW and mixed seawater media (ASW + SHSW) had VCFs between those in ASW and LISSW. The mean DOC concentration in ASW was 40 ± 20 μM, in SHSW was 204 ± 16 μM, in LISSW (W) was 331 ± 17 μM, in LISSW (C) was 293 ± 51 μM, and in LISSE (E) was 254 ± 28 μM. Mixed seawater media had mean DOC concentrations of 71 ± 4 μM (L), 116 ± 13 μM (M) and 154 ± 5 μM (H) (Table 2). The uptake rate constants for each type of seawater medium were also calculated and listed in Table 2. Across all experiments, a linear relationship ($R^2 = 0.77$) can be found between the uptake rate constants and mean MeHg VCFs (Fig. 6). The MeHg uptake rate constants and mean MeHg VCFs were inversely related to the amount of DOC in each water type (Fig. 7).

4. Discussion

There are many organic compounds in seawater that can form complexes with MeHg. Some organic complexation may reduce MeHg's ability to associate with ligands on algal surfaces or inhibit its passage across membranes of algal cells, resulting in reduced accumulation in phytoplankton. Other organic complexes of MeHg may enhance its bioavailability, leading to enriched uptake by phytoplankton. Our results clearly showed that, at sufficiently high concentrations, some dissolved organic compounds can cause a considerable decline in MeHg uptake by algae (Fig. 4). Such a decline can be attributed to those organic compounds complexing with MeHg and competing with algae for MeHg. Gly has a comparable size and a similar chemical structure to TGA (Table 1) but TGA has a sulfhydryl functional group which has a strong binding affinity to MeHg. The difference in their stability constants with MeHg may explain the reduced MeHg uptake rate constant observed in the TGA treatment at 1000 nM (Fig. 4). Met also has a reduced sulfur, thioether functional group in its molecule. However, its stability constant for MeHg is relatively small (Table 1) and it had no effects on MeHg uptake. Our results were in accordance with Pickhardt

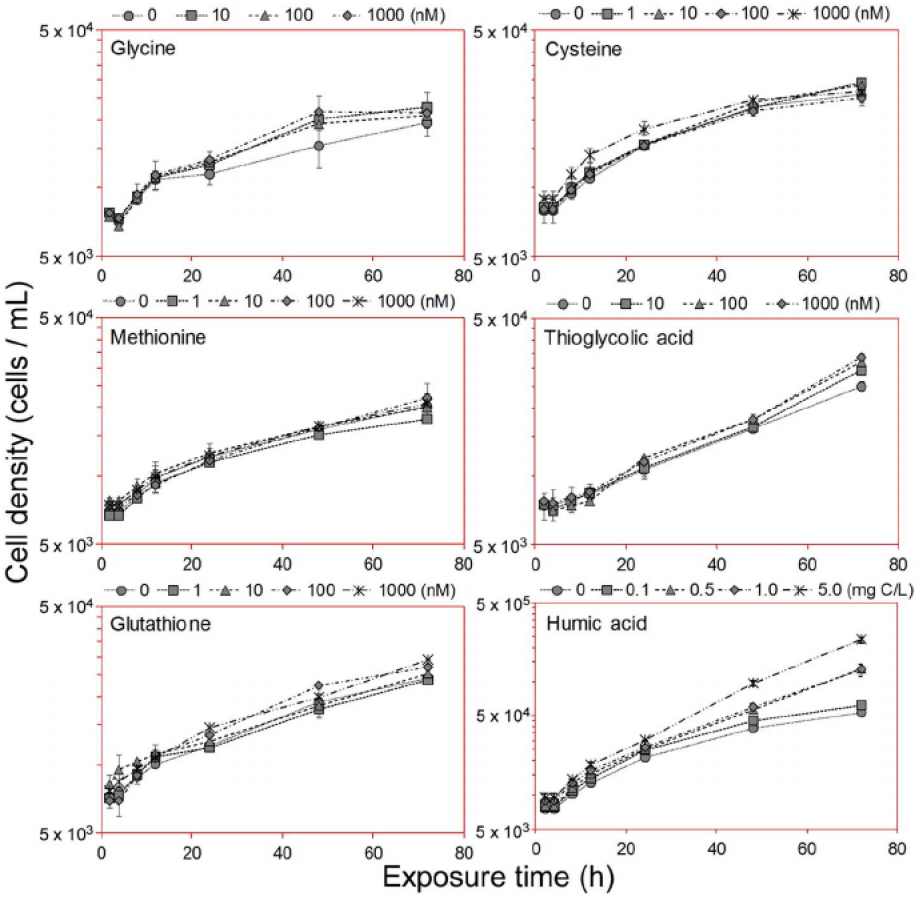


Fig. 2. Growth of *T. pseudonana* cells exposed to six different organic compounds at varying concentrations. Data points are the means from two replicates cultures ± 1 SD at each sample time.

and Fisher (2007) who found no effect of 0.75 nM of Met on MeHg uptake by a freshwater diatom.

At a concentration of 1 μM, Cys, with a sulfhydryl group, lowered the MeHg uptake rate constant and its VCF significantly, consistent with results of Luengen et al., 2012 who found greatly reduced MeHg accumulation in a freshwater diatom exposed to 67 μM of Cys. Lawson and Mason, 1998 showed that the MeHg uptake rate of a marine diatom decreased by > 50% as Cys concentrations increased from 0.5 to 10 μM, also comparable to our results. However, the rate then increased by > 100% when the concentrations increased from 10 to 50 μM in the study of Lawson and Mason, 1998. The highest Cys concentration used in our study, 1 μM, is still 2–3 orders of magnitude higher than common Cys concentration in most marine environments (Dupont et al., 2006; Swarr et al., 2016).

GSH, Cys and TGA all possess a sulfhydryl group and have comparable stability constants with MeHg (Table 1), but 10 nM of GSH had greater effects on MeHg uptake than the same concentration of Cys and TGA (Figs. 3 and 4). This discrepancy between GSH and the other compounds was probably caused by the differences in their speciation in seawater and/or their coordination chemistry. From the given acid dissociation constants at the common ocean pH, the dominant forms of GSH, Cys and TGA are $[H_2L_{GSH}]^-$ and $[HL_{GSH}]^{2-}$, $[HL_{Cys}]^-$, and $[HL_{TGA}]^-$, respectively (Alderighi et al., 2003; Jawaid and Ingman, 1981; Rabenstein, 1973; Sharma et al., 2006). Assuming MeHg only forms a 1:1 complex, the $[HL_{GSH}]^{2-}$ has the greatest stability constant with MeHg among these complexes (Table 1). Hg coordination chemistry tends to form linear, two-coordinate geometric patterns (e.g. CH_3-Hg-L), but it has the potential to form complexes with a higher coordination number (> 2) (Alderighi et al., 2003; Rabenstein, 1978). GSH has more potential donor atoms (S, N and two O) for MeHg than Cys (S, N and O) and TGA (S and O), which might allow for greater complexation (e.g., chelated complexes). Significant declines in algal MeHg uptake with GSH addition suggest that other similar thiols such as phytochelatin (PC, an oligomer of glutathione), glutamine-cysteine (Gln-Cys) and γ-glutamylcysteine (γEC, a precursor of glutathione) may also have comparable effects on reducing MeHg uptake by algae. Alternatively, it is possible that GSH is not accumulated from ambient seawater by phytoplankton cells to the same extent as other thiol-containing compounds and amino acids.

Reported stability constants of natural humic substances for MeHg range from 10^{10} to 10^{15}, probably reflecting the presence of thiols in this humic material (Amirbahman et al., 2002; Hintelmann et al., 1997). Humic substances may therefore provide important ligands for MeHg, consistent with the inverse relationship found between DOC concentrations and MeHg VCFs and uptake rate constants in HA treatments in this study. The concentrations used in our study overlap with the typical ranges reported for humic substances in seawater (0.06 to 0.6 mg C/L) (Thurman, 1985). Luengen et al. (2012) also found MeHg accumulation in a freshwater diatom was inversely related with the concentrations of humic substances isolated from natural DOM and Schartup et al. (2015) found terrestrial DOM (containing more humic-like substances) decreased MeHg uptake by both plankton and bacteria.

The decline in MeHg bioavailability after 1 d of exposure in all treatments may be attributable to its complexation with dissolved organic matter in the aqueous phase (< 0.2 μm) that greatly retards or prohibits its sorption to algal cell surfaces. As these laboratory experiments were conducted in ASW which contained very low levels of DOC (Table 2), the organic compounds to which the MeHg was bound – in addition to the organic compounds added to the ASW – may well have been released from the algal cells as they grew, as shown for other metals (Fisher and Fabris, 1982; Gonzalez-Davila et al., 1995; McIntyre and Guéguen, 2013). The composition of organic compounds released by cultured *T. pseudonana* into seawater during growth has been addressed (Longnecker et al., 2015) and include polypeptides and amino

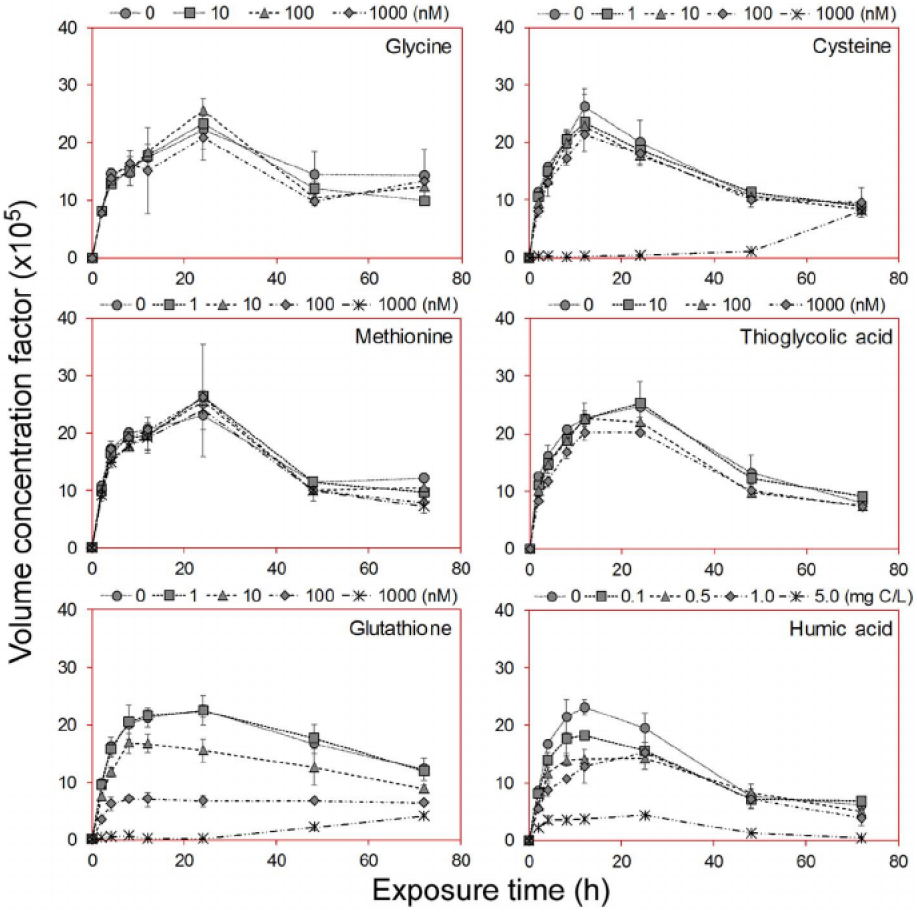


Fig. 3. Effects of varying concentrations of six different dissolved organic compounds on MeHg volume concentration factors (VCFs) in *T. pseudonana*. Data points are the means from two replicate cultures ± 1 SD at each sample time.

acids, some of which could be expected to bind with MeHg.

Many possible mechanisms of organic-metal complex uptake by algal cells have been proposed, including the direct transport of intact organic-metal complex into cells, followed by the intracellular release of metal (Campbell et al., 2002; Morel and Price, 2003; Simkiss and Taylor, 1995; Sunda, 1989). However, since DOM usually consists of many large and complex molecules, it is unlikely that the organic-MeHg complex can penetrate intact into cells directly. Another mechanism is the metal exchange between organic ligands and membrane transporters at the surface of cell membranes, which is more likely for essential metals like Fe (Hudson and Morel, 1990) than non-essential elements like Hg. Different from many other metals, both Hg and MeHg are lipid soluble and may penetrate membranes without specific membrane transporters (Gutknecht, 1981) and neutral MeHg species such as $MeHgCl^0$ can passively diffuse through a diatom's plasmalemma (Mason et al., 1996). It is plausible that formation of a strong organic-MeHg complex, which may carry an electric charge or be very large, could therefore lessen MeHg's permeability through cell membranes. Biological reduction near cell surfaces may be another mechanism to change metal availability to algal cells (Maldonado and Price, 2001). Assuming MeHg was demethylated and then further reduced to the form of Hg^0 (elemental Hg), the produced gaseous Hg^0 wouldn't accumulate in algal cells and would eventually be released into the overlying air. However, we did not observe any Hg loss across all treatments and the mean mass balance of MeHg in the seawater medium was 97 ± 8% (n = 96), as noted above.

In addition to the positive correlation between the uptake rate constants and mean MeHg VCFs (Fig. 6), the same correlation was also found between uptake rate constants and VCFs at every time point after steady state was reached (t = 12, 24, 48, and 72 h). Two groups of outliers shown in Fig. 6 were LISSW and high HA treatments (5 mg C/L). Such deviations may be attributable to the greater cell growth in these waters, leading to bio-dilution of MeHg and consequent lower VCFs.

As expected, MeHg mean VCFs and uptake rate constants both showed inverse relationships with bulk DOC concentrations (Fig. 7), consistent with previous studies (Gorski et al., 2008; Luengen et al., 2012). The decline in mean MeHg VCFs in freshwater diatoms with comparable increases in freshwater DOC (40 to 400 μM) was more pronounced than in seawater, ~6-fold difference (Luengen et al., 2012) vs. ~3.5-fold difference (this study). The differences may be caused by differences in the DOC composition between the waters used, and/or the chloride ions in seawater competing against DOC binding with MeHg. We did not measure the concentrations of thiols or humic substances in the SHSW and LISSW. Lamborg et al. (2004) reported that normalized mercury-binding ligand abundance (ligands to DOC ratio) in Long Island Sound (LIS) was higher than in the Mid-Atlantic Bight, which would include the collection site of the SHSW. They suggested that algal exudates and terrestrial input are the dominant sources of these ligands to the LIS, however the stability constants for MeHg were not measured.

A major role of thiols in living organisms is to defend against oxidative stress and to reduce the toxicity of metals (Ercal et al., 2001). Biogenic thiols produced within phytoplankton can be released into surrounding waters by exudation (Dupont and Ahner, 2005; Leal et al., 1999; Tang et al., 2005), cell lysis or sloppy grazing. Cys and GSH are common thiols that can be found in most aquatic systems, with GSH usually considered the dominant marine thiol. Relatively high concentrations of GSH (up to ~100 nM) can be found in anoxic and euxinic waters like the Black Sea (Mopper and Kieber, 1991), interstitial waters (Zhang et al., 2004) or lagoons (Chapman et al., 2009). In estuarine, coastal and open ocean waters, their concentrations typically fall in the

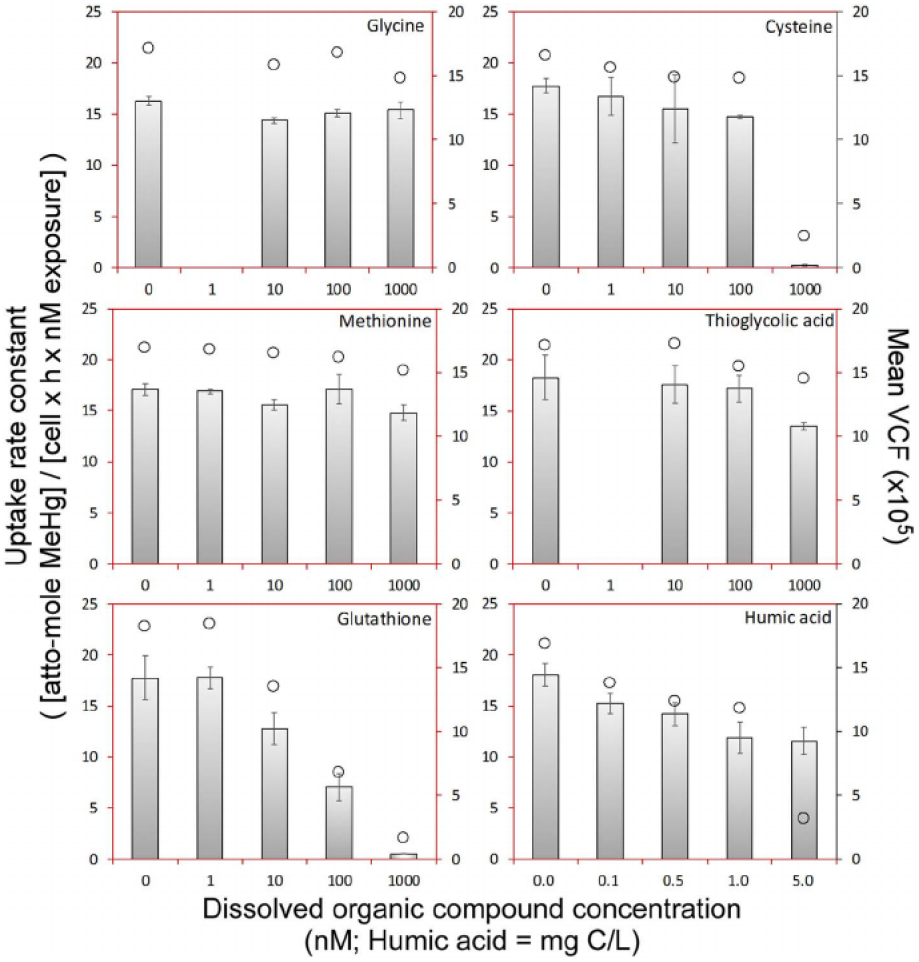


Fig. 4. Calculated MeHg uptake rate constants (bar) and mean volume concentration factors (VCFs, open circle) among varying concentrations of six organic compounds in ASW. All treatments shown are for 2 replicate cultures. The uptake rate constants were normalized to the ambient dissolved MeHg concentrations in the media and were expressed on a per cell basis for the first 4 h of exposure. The mean VCFs are the average of the 12-, 24-, 48-, and 72-h time points. Error bars are ± 1 SD.

low nM range. Cys concentrations in the marine environments are typically lower, for instance 0.3 to 2.11 nM in the North Pacific (Dupont et al., 2006) and < 0.1 to 1.5 nM in the North Atlantic (Kading, 2013; Swarr et al., 2016). These Cys concentrations are about 2 orders of magnitude lower than those (> 100 nM) that reduced MeHg uptake in our study.

In contrast to Cys, our study showed that MeHg uptake was decreased by 30% in the presence of 10 nM of GSH, a concentration approaching levels observed in marine environments, especially estuarine and coastal waters. Reported concentrations of GSH ranged from < 1 to 15 nM in the North-East Atlantic (Le Gall and van den Berg, 1998), 0.7 to 3.6 nM in coastal England (Al-Farawati and van den Berg, 2001), < 0.1 to 2.21 nM in the North Atlantic (Kading, 2013; Swarr et al., 2016), 0.01 to 0.76 in the North Pacific (Dupont et al., 2006), 0.23 to 6.23 nM in Galveston Bay, Texas (Tang et al., 2000), 0.17 to 0.79 nM in San Diego Bay, California (Tang et al., 2004), 0.08 to 0.13 nM in Cape Gear Estuary, North Carolina (Tang et al., 2004), and 0.37 to 0.45 nM in Norfolk Estuary, Virginia (Tang et al., 2004); however electroanalytical methods used in some of these studies may lead to overestimates of GSH concentrations due to indiscriminate quantification (the authors assumed GSH was the primary component in total thiols). Notably, rather than GSH, γEC (γ-glutamylcysteine, a precursor of GSH) was found to be the most abundant thiol species (2 to 15 nM) in the North Pacific (Dupont et al., 2006). Given that γEC has a similar chemical structure to GSH, we hypothesize that it could similarly affect MeHg's availability to algae. Since thiols are mainly of biological origin, their concentrations are presumed to be associated with primary production. Dissolved GSH profiles in water column usually exhibit a maximum at sub-surface depths but do not always coincide with the chlorophyll maximum. Al-Farawati and van den Berg, 2001 found that GSH-equivalent thiols were positively correlated with chlorophyll in coastal waters. However, this relationship was not observed in the North Pacific (Dupont et al., 2006), Northwest Atlantic (Kading, 2013; Swarr et al., 2016), and Galveston Bay, Texas (Tang et al., 2000). Dupont et al. (2006) suggested that biological exudation was the primary source of dissolved GSH and other thiols in the mixed layer, but this exudation process was mainly controlled by environmental conditions (e.g., light or metals) rather than primary productivity (Ahner et al., 2002; Dupont and Ahner, 2005; Dupont et al., 2004; Tang et al., 2004). Assuming a large amount of biogenic thiols was released into surrounding waters due to exudation, cell lysis or grazing after an algal bloom, MeHg uptake by phytoplankton may be reduced subsequently, resulting in overall reduction in MeHg biomagnification in this ecosystem.

The sharp increases in MeHg VCFs after 72 h in the high treatments of Cys and GSH (Fig. 3) were possibly due to the degradation of those organomercuric complexes, releasing the bioavailable MeHg. These results suggest that while assessing a thiol's impact on MeHg accumulation in the field, its turnover rate in the water column needs to be taken into consideration. Free dissolved thiols are known to be unstable; for example, the reported half-lives of thiols through photochemical degradation were < 1 d (Laglera and van den Berg, 2006; Moingt et al., 2010) and thiols can be readily oxidized (RSH RSSR, which has no thiol group) in the presence of strong oxidants (Winterbourn and Metodiewa, 1999). However, the formation of strong metal complexes like Hg-thiol retard degradation, lengthening their residence times to several days in the water column (Hsu-Kim, 2007; Moingt et al., 2010). Kading (2013) assumed that thiols are components

Table 2
MeHg uptake rate constants (per cell basis for the first 4 h of exposure) and mean volume concentration factors (VCFs, the average of the 12, 24, 48, and 72 h time points) in all experiments.

(a)

Type of organic compound	Added amount	Uptake rate constant	Mean VCF
	nM (mg C/L for humic acid)	atto-mole MeHg/cell/h/nM exposure	$\times 10^5$
Glycine (Gly)	0	16.3 ± 0.4	17.1
	10	14.3 ± 0.3	15.8
	100	15.0 ± 0.3	16.8
	1000	15.4 ± 0.8	14.8
Cysteine (Cys)	0	17.8 ± 0.7	16.6
	1	16.7 ± 1.9	15.6
	10	15.5 ± 3.3	14.8
	100	14.8 ± 0.2	14.8
	1000	0.3 ± 0.1	2.5
Methionine (Met)	0	17.1 ± 0.5	16.9
	1	16.9 ± 0.2	16.8
	10	15.5 ± 0.5	16.5
	100	17.1 ± 1.5	16.2
	1000	14.8 ± 0.7	15.1
Thioglycolic acid (TGA)	0	18.3 ± 2.2	17.1
	10	17.6 ± 1.8	17.3
	100	17.2 ± 1.3	15.5
	1000	13.5 ± 0.3	14.5
Glutathione (GSH)	0	17.7 ± 2.2	18.2
	1	17.8 ± 1.1	18.4
	10	12.8 ± 1.5	13.5
	100	7.1 ± 1.3	6.8
	1000	0.54 ± 0.03	1.7
Humic acid (HA)	0	18.1 ± 1.1	16.8
	0.1	15.2 ± 1.0	13.7
	0.5	14.2 ± 1.1	12.3
	1	11.9 ± 1.5	11.8
	5	11.6 ± 1.3	3.1

(b)

Type of seawater	Bulk DOC	Uptake rate constant	Mean VCF
	μM	atto-mole MeHg/cell/h/nM exposure	$\times 10^5$
ASW	40 ± 20	18.7 ± 2.6	17.0
ASW + SHSW (L)	71 ± 4	17.0 ± 0.9	14.1
ASW + SHSW (M)	116 ± 13	16.1 ± 1.4	13.8
ASW + SHSW (H)	154 ± 5	15.4 ± 0.1	12.8
SHSW	204 ± 16	15.6 ± 0.7	12.2
LISSW (W)	331 ± 17	9.2 ± 0.3	4.9
LISSW (C)	293 ± 51	12.3 ± 0.4	6.0
LISSW (E)	254 ± 28	11.4 ± 0.2	5.8

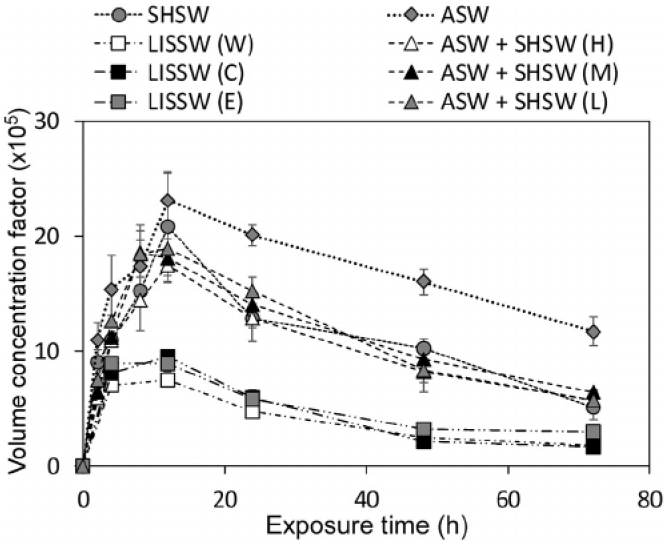


Fig. 5. Effects of different seawater media on MeHg volume concentration factors (VCFs) for *T. pseudonana*. Data points are the means from three replicates cultures ± 1 SD at each sample time. LISSW (W): Western LIS; LISSW (C): Central LIS; LISSW (E): Eastern LIS; ASW + SHSW (L): Low DOC; ASW + SHSW (M): Medium DOC; ASW + SHSW (H): High DOC; ASW: Artificial seawater; SHSW: Southampton seawater.

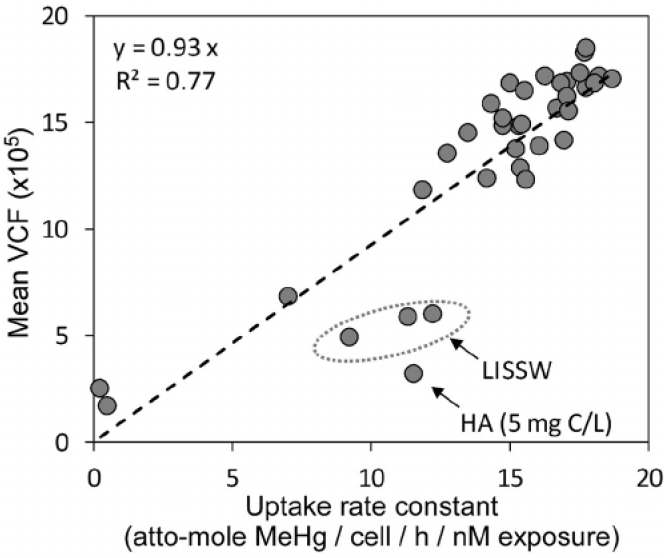


Fig. 6. Correlation between MeHg uptake rate constants and mean volume concentration factors (VCFs) across all experiments. The mean VCFs are the average of the 12, 24, 48, and 72 h time points. The dashed line and the equation shown reflect all data.

of the labile DOC in the ocean and suggested their turnover times may be on the order of hours to days based on the estimate from Carlson and Ducklow (1995). Dupont et al. (2006) used simple calculations based on the observed particulate and dissolved thiols concentrations and concluded that their turnover times were on the order of weeks or even longer assuming the system is at steady state.

Although thiols, HA (or humic substances), as well as bulk DOC, are capable of reducing MeHg uptake by phytoplankton, they can also lead to positive or negative feedbacks (directly or indirectly) on production, degradation and accumulation of MeHg in aquatic environments. For example, Schaefer and Morel (2009) reported that Cys was able to stimulate the production of methylmercury by the Fe-reducing bacterium *G. sulfurreducens*. However, binding of Cys with MeHg can increase its bioavailability for some Hg-resistant bacteria, leading to enhanced MeHg degradation (Ndu et al., 2012). The role of humic substances and DOC in the methylation of Hg and the degradation of MeHg remains more uncertain because of the variability in their concentrations and composition. DOM has been hypothesized to enhance methylation by stimulating the activity of heterotrophic bacteria or through direct abiotic methylation by humic or fluvic substances (Ullrich et al., 2001), but Hg methylation may also be inhibited due to Hg complexation with humic substances and DOC, reducing Hg bioavailability to bacteria (Ndu et al., 2012; Ullrich et al., 2001). A study in Arctic lakes further suggested that total Hg bioaccumulation was determined by binding thresholds on DOC, resulting in a bell-shaped relationship between total Hg accumulation and DOC concentrations (French et al., 2014). In brief, prediction of overall bioaccumulation and biomagnification of MeHg in an aquatic ecosystem cannot merely rely on measurements of bulk DOC or some ligands.

Thus, our study examined the effects of specific dissolved organic compounds (especially for thiols) and dissolved organic carbon (DOC) concentrations on the bioaccumulation of MeHg in a marine diatom. Overall the results indicated that under natural conditions glutathione and humic substances can play an important role on reducing MeHg

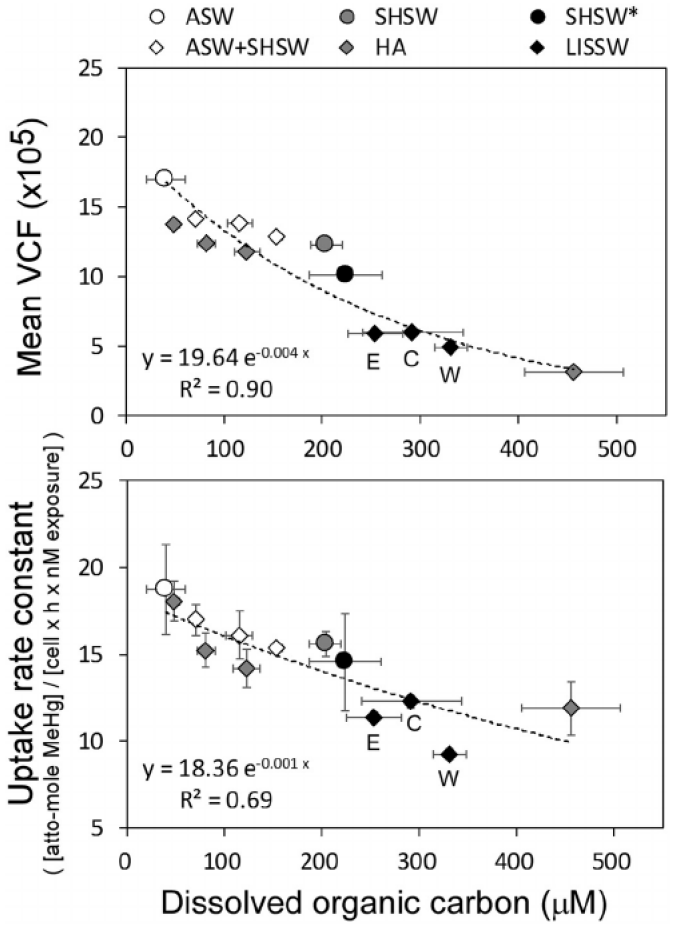


Fig. 7. MeHg uptake rate constants and volume concentration factors (VCFs) as a function of dissolved organic carbon (DOC) in different seawater media. SHSW* represents the data from Lee and Fisher (2016). E, C, and W indicate eastern, central, and western Long Island Sound samples.

uptake by phytoplankton, whereas glycine, methionine, cysteine, and thioglycolic acid at representative seawater concentrations do not affect MeHg bioaccumulation. Future studies involving other algal species, including natural plankton assemblages, may provide the extent to which these findings can be applied to natural ecosystems.

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