HHS Public Access

Author manuscript

Limnol Oceanogr. Author manuscript; available in PMC 2018 August 15.

Published in final edited form as:

Limnol Oceanogr. 2016 September ; 61(5): 1626–1639. doi:10.1002/lno.10318.

Methylmercury uptake by diverse marine phytoplankton

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Luiz Drude de Lacerda [Associate editor:]

Abstract

Phytoplankton may serve as a key entry for methylmercury (MeHg) into aquatic food webs however very few studies have quantified the bioconcentration of MeHg in marine phytoplankton from seawater, particularly for non-diatoms. Experiments using ^{203}Hg to measure MeHg uptake rates and concentration factors in six marine phytoplankton species belonging to different algal classes were conducted and the influence of light, temperature, and nutrient conditions on MeHg bioaccumulation were determined. All algal species greatly concentrated MeHg out of seawater, with volume concentration factors (VCFs) ranging from 0.2×10^5 to 6.4×10^6. VCFs were directly related to cellular surface area-to-volume ratios. Most of the cellular MeHg was found in the cytoplasm. Temperature, light, and nutrient additions did not directly affect MeHg uptake in most species, with the exception that the dinoflagellate *Prorocentrum minimum* displayed significantly greater uptake per cell at 18°C than at 4°C, suggesting an active uptake for this species. Passive transport seemed to be the major pathway for most phytoplankton to acquire MeHg and was related to the surface area-to-volume ratio of algal cells. Environmental conditions that promoted cell growth resulted in more total MeHg associated with cells, but with lower concentrations per unit biomass due to biodilution. The very high bioconcentration of MeHg in marine phytoplankton is by far the largest bioconcentration step in marine food chains and variations in algal uptake may account for differences in the amount of MeHg that ultimately builds up in different marine ecosystems.

Introduction

Interest in the biogeochemical cycling of methylmercury (MeHg) in marine ecosystems stems in part from the fact that its neurotoxicity can cause adverse health effects to exposed wildlife and humans (Wiener et al. 2003; Grandjean et al. 2010; Mason et al. 2012). Human exposure to MeHg is primarily from diet, especially seafood consumption (Sunderland 2007). Although MeHg concentrations in seawater are extremely low, typically in the subpico- to femto- molar range (Fitzgerald et al. 2007; Lamborg et al. 2014; Bowman et al. 2015), MeHg is the only mercury species to build up in aquatic food chains and display biomagnification, resulting in high MeHg levels in some common seafood items.

However, our understanding of the factors controlling MeHg accumulation in marine food webs is surprisingly limited. Most research in marine environments has focused on Hg (total

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Hg or MeHg) levels in commercial fish consumed by humans due to public health concerns (Morel et al. 1998; Selin 2009; Karimi et al. 2012). Very few studies have investigated Hg and MeHg interactions with primary producers at the base of marine food webs (Mason et al. 2012). Phytoplankton are the most critical entry where metals enter marine food webs (Fisher and Reinfelder 1995). What little lab and field data exist on this issue show that MeHg is concentrated from ambient water into phytoplankton by at least a factor of 10^5 (Watras et al. 1998; Pickhardt and Fisher 2007; Hammerschmidt et al. 2013; Gosnell and Mason 2015), which is by far the largest bioconcentration step in aquatic food webs. Phytoplankton then serve as a highly enriched source of MeHg for herbivores which can pass this compound on to animals higher in the food chain. Subsequent enrichment with increasing trophic levels in aquatic food chains is much smaller, almost always less than a factor of 10.

To date, most studies involving algal uptake of MeHg have considered freshwater phytoplankton (Watras et al. 1998; Miles et al. 2001; Gorski et al. 2006; Pickhardt and Fisher 2007; Luengen et al. 2012), and its trophic transfer in freshwater ecosystems (Pickhardt et al. 2002; Pickhardt et al. 2005). The few studies on marine environments are confined to a coastal diatom and cyanobacterium (Mason et al. 1996; Lawson and Mason 1998; Zhong and Wang 2009; Kim et al. 2014). Published data on MeHg interactions with other marine phytoplankton species are sparse. Generally, bioconcentration factors of metals can vary substantially among different algal species, and for any given metal generally increase with surface area-to-volume ratios (SA/V) of cells (increasing inversely with cell size) (Fisher 1986; Fisher and Reinfelder 1995). Since the composition of phytoplankton assemblages often shifts seasonally, MeHg uptake by the prevailing algal assemblage might also vary, consequently affecting overall MeHg accumulation in organisms at higher trophic levels.

In this study, we examined the uptake of MeHg by six marine phytoplankton species belonging to different algal classes to determine how biological attributes (e.g., size, surface area-to-volume ratio, cell wall composition, and metabolic rate) and environmental conditions (light, temperature, salinity, and nutrient levels) affect MeHg bioaccumulation in diverse marine algae. This information, combined with field data, could ultimately be used by global biogeochemical models describing Hg cycling in the marine environment.

Materials and methods

Six phytoplankton species *Thalassiosira pseudonana* (diatom), *Dunaliella tertiolecta* (chlorophyte), *Rhodomonas salina* (cryptophyte), *Prorocentrum minimum* (dinoflagellate), *Emiliania huxleyi* (coccolithophore), and *Synechococcus bacillaris* (cyanobacterium), belonging to different algal classes, were used in this study (Table 1). All species were held in clonal, unialgal cultures maintained axenically for generations in an incubator at 18°C under a light:dark cycle (14 h:10 h, 200 μmol quanta m^{-2} s^{-1}) provided by cool white fluorescent lamps. The f/2 medium (Guillard and Ryther 1962) was prepared with sterile filtered (0.2 μm, Nuclepore polycarbonate membrane) surface seawater (35 psu, DOC = ~2 mg L^{-1}) collected 8 km off Southampton, New York (Lat. 40.77°N, Long. 72.43°W), and was used for maintaining routine cultures. However, to avoid the potential effects on MeHg

speciation due to the presence of ethylenediaminetetraacetic acid (EDTA), inocula for experimental algal cultures were grown in separate flasks with amended f medium (nutrients at f/20 level and without EDTA) for 7 to 10 d prior to experiments.

The gamma-emitting radioisotope, ^{203}Hg ($t_{½}$ = 46.6 d) was used to trace the transfer of Hg between dissolved and particulate phases in all cultures. Commercially available $^{203}Hg(II)$ was converted to monomethylmercury ($CH_3{}^{203}Hg^+$ or $Me^{203}Hg$) from inorganic $^{203}HgCl_2$ following established methods (Rouleau and Block 1997; Luengen et al. 2012). Briefly, inorganic ^{203}Hg solution purchased from Eckert and Ziegler Isotope Products (Valencia, California) (specific activity: 5 Ci g^{-1}) was mixed with methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) and acetate buffer at pH 5, allowing the reaction to proceed in the dark for 18 h to 24 h and then forming $Me^{203}Hg$. Following extraction by dichloromethane (CH_2Cl_2) and purification procedures, $Me^{203}Hg$ was then re-dissolved in Milli-Q® water and was ready to use. The conversion yield (fraction of total ^{203}Hg recovered as $Me^{203}Hg$) was 95 ± 3% (n = 6). A series of experiments was conducted using $Me^{203}Hg$ to track the partitioning of MeHg between water and phytoplankton. $Me^{203}Hg$ activities in experiments ranged from 4.46 kBq L^{-1} to 9.29 kBq L^{-1}, corresponding to concentrations of 0.29 nM to 0.42 nM. These MeHg concentrations were at the high end of those found in natural waters, however previous studies have shown that bioconcentration factors and uptake rate constants for metals (including Hg) in algal cells are not affected by modestly elevated metal concentrations that are below toxic levels (Fisher et al. 1984). As noted in numerous previous gamma-emitting radiotracer studies, this approach provides a nondestructive, noninvasive and direct measurement of metal bioaccumulation while using low metal concentrations that are in the range of naturally occurring levels (Fisher 2002).

The MeHg uptake experiments generally followed protocols described for metal uptake by marine phytoplankton (Fisher et al. 1984; Stewart and Fisher 2003b). The seawater used in all experiments except those involving nitrate or chloride additions was 0.2 μm filtered surface seawater (35 psu, collected 8 km off Southampton, New York), without addition of any nutrients. Trace metal clean glass-stoppered Erlenmeyer flasks, each containing 150 mL of seawater and microliter quantities of $Me^{203}Hg$ solution (added 1 h prior to inoculation with phytoplankton cells to reach equilibrium) were incubated at 18°C under the light:dark cycle. Inocula of algal cells were concentrated by resuspending cells off 1 μm membranes or by centrifugation at 1000 g for 10 min from late log-phase cultures (without EDTA). Considering the difference in algal biomasses, initial cell densities ranged from 6×10^3 to 6×10^4 cells mL^{-1}, depending on algal species. Water and cell samples were periodically collected into glass tubes and onto 1 μm polycarbonate membranes, respectively for radioassay. This approach (Fisher et al. 1983a) measures the total radioactivity in 1 mL of suspension (water plus cells) and, in a separate sample taken at the same time, the radioactivity in 10-mL of suspension caught on a 1 μm polycarbonate membrane (vacuum pressure < 100 mmHg) that was then washed with 2 × 5 mL of unlabeled, filtered seawater. With this approach, the fraction of total radioactivity in suspension associated with cells and the fraction in the dissolved phase could be determined. We performed both short term (t = 1, 2, 3, 4, 8 h) and long term (t = 2, 6, 12, 24, 48, 72 h) experiments to evaluate the uptake rate constants and bioconcentration factors of MeHg for each species. For each culture, cell density and volume were monitored simultaneously over time using a Multisizer™ Coulter

Counter® and cell surface area was calculated using appropriate geometric equations (Table 1).

All experiments included control flasks without algal cells which were used to correct for the potential adsorption of $Me^{203}Hg$ onto filter membranes. In addition, sorption of MeHg onto culture flasks was examined by acid washing the flask walls after cell exposures. Sample activities were determined using an LKB Wallac 1282 Compugamma NaI(Tl) gamma detector. ^{203}Hg activity was assessed at 279 keV. All samples were counted with standards and decay-corrected. Propagated counting errors were < 5%.

To examine the effect of low temperature (4°C) on MeHg uptake by phytoplankton, algal cells cultured in medium without EDTA were acclimated at 4°C for 6 h prior to experimental inoculation, after which the experimental procedures described above were followed. To assess MeHg uptake by phytoplankton without light, experimental cultures were held in the dark (18°C). In an experiment evaluating the effects of nitrate on MeHg uptake, different levels of nitrate additions (0, 5, 10, 50 μM) to artificial seawater (prepared by adding salts to Milli-Q® water; 35 psu; DOC = ~1 mg L^{-1}) (Kester et al. 1967) were used as the experimental media; phosphate and silicate concentrations were fixed at 1 μM and 10 μM, respectively. The nutrient concentrations were representative of nutrient levels reported for Long Island Sound, New York (Gobler et al. 2006). Four algal species, including the diatom, the chlorophyte, the dinoflagellate, and the coccolithophore were tested in the nutrient experiment. In a parallel experiment assessing the influence of the culture medium's ionic strength on MeHg uptake, the euryhaline diatom, *T. pseudonana*, was exposed to MeHg under four different salinities, reflected by chloride concentrations of 5.90, 27.9, 55.4, and 550 mM, prepared by mixing WCL-1 medium (Guillard 1975) and artificial seawater. The highest chloride concentration corresponds with 35 psu seawater. The diatoms were cultured in these media for more than two generations to make sure they were fully acclimated to low salinity environments.

The cytoplasmic distribution of MeHg in phytoplankton cells was examined in all six species after exposure to $Me^{203}Hg$ for 3 d (two to three cell divisions) to uniformly radiolabel the cells (Fisher et al. 1983b; Reinfelder and Fisher 1991; Stewart and Fisher 2003b).

Results

Algal growth at 18°C under a 14:10 light:dark cycle varied among species, with population doublings during the 72 h exposure period ranging from one for the dinoflagellate *P. minimum* to four for the diatom *T. pseudonana* (Fig. 1). All algal species accumulated MeHg (Fig. 1), however two patterns emerged. For those cultures (cryptophyte *R. salina*, chlorophyte *D. tertiolecta*, coccolithophore *E. huxleyi*) where- < 40% of the total MeHg was associated with the cells over the 72 h period, uptake generally followed increases in algal biomass, whereas when > 60% of the MeHg was associated with cells (diatom *T. pseudonana*, dinoflagellate *P. minimum*, cyanobacterium *S. bacillaris*), uptake slowed over time even as growth continued (Fig. 1), probably because there was little bioavailable MeHg remaining in the dissolved phase to support further uptake. For all species, most of the

MeHg was found in the cytoplasmic fraction of the cells (Table 2). Among the algal species, no general patterns were observed for MeHg penetration into the cytoplasm among the various culture treatments.

In each flask, sorption of $Me^{203}Hg$ to the glass walls accounted for < 5% of the total $Me^{203}Hg$, and at least 90% of the total $Me^{203}Hg$ could be accounted for by summing dissolved, particulate, and flask wall activities of the added $Me^{203}Hg$ (Fig. 2). The exception was for the coccolithophore (*E. huxleyi*) cultures, where a substantial loss of $Me^{203}Hg$ (~30%) was consistently observed, implying strong volatilization from the cultures. The cryptophyte *R. salina* and the cyanobacterium *S. bacillaris* cells were prone to attach to flask walls and may not have been completely removed by acid rinsing of the walls. This may explain that ^{203}Hg recoveries for these cultures were such that mass balances were not able to achieve ~100%.

Volume concentration factors (VCFs) were calculated ($[Bq\ Me^{203}Hg\ \mu m^{-3}]_{cell}/[Bq\ Me^{203}Hg\ \mu m^{-3}]_{solution}$), showing the relative degree of enrichment in the algal cells relative to the ambient water. Figure 3 shows VCFs of six species over time under different environmental conditions. At 18°C, VCFs ranged from 0.2×10^5 for the chlorophyte *D. tertiolecta* to 6.4×10^6 for the cyanobacterium *S. bacillaris*. Within the first 12 h, there were no differences in MeHg VCFs among environmental conditions for all species but the dinoflagellate. After 12 h of exposure, VCFs in the dark and low temperature treatments leveled off. However, VCFs at 18°C and light/dark conditions decreased slightly and then leveled off. This decreasing trend was particularly obvious in the cultures of the diatom *T. pseudonana* and the cyanobacterium *S. bacillaris* for which growth was most pronounced. To compare MeHg sorption to abiotic and living particles, the MeHg accumulation by suspended glass beads (diameter ~5 μm), added at a density of 10^5 beads mL^{-1}, was determined. Only about 1% of the MeHg adsorbed onto the surface of the glass beads, resulting in a VCF of about 10^3 (data not shown).

In the short term exposure experiments, it was clear that light had no significant effect on MeHg uptake rates for any species (Fig. 4) but temperature did have a pronounced effect on MeHg uptake rates in the dinoflagellate *P. minimum* and to a lesser extent in the cyanobacterium *S. bacillaris*. The short term uptake rate constant, which was normalized to the initial dissolved MeHg concentrations in the media, was calculated on per cell, per μm^2 cell surface, and per μm^3 cell volume bases for the first 4 h of exposure during which time MeHg uptake was linear (Fig. 5; Table 3). At 18°C, the uptake rate constants on cell surface area or cell volume bases were comparable for the diatom, the cyanobacterium, and the dinoflagellate, all of which were significantly higher than the other species, sometimes by more than an order of magnitude.

Experiments to assess the effects of nitrate level on MeHg uptake showed no significant effect in four algal species. MeHg was accumulated over time but no differences were found between high and low nutrient treatments (Fig. 6). However, higher nutrient levels led to significant cell growth after 48 h to 72 h (Fig. 7a–d). Such increases in algal biomass would result in a “dilution” of MeHg in algal cells, and lower VCFs in high nutrient treatments were indeed observed at the end of the experiment (Fig. 7e–h). For the ionic strength

(salinity) experiment involving the diatom *T. pseudonana*, VCF values increased with chloride concentrations (Fig. 8a). Uptake of MeHg was essentially linear over the first 4 h exposure, and at this time the MeHg VCFs in the diatoms was linearly related to the log chloride concentration (Fig. 8b).

Discussion

This is the first report describing MeHg uptake by diverse marine phytoplankton cells, and how environmental conditions affect the uptake process. While all species greatly concentrated MeHg out of ambient seawater, significant differences among the species can be explained. VCFs among the different algal species significantly increased with cell surface area-to-volume ratios (Fig. 9) except for the dinoflagellate which may have a different uptake pathway. In general, the greater MeHg enrichment in phytoplankton for smaller cells with greater surface area-to-volume ratios is consistent with findings for many other metals and algal taxa (Fisher and Reinfelder 1995).

These findings are also consistent with the conclusion that passive sorption of dissolved MeHg to cell surfaces dominates the uptake pathway. Most other metals that speciate as cations in seawater behave similarly (Fisher 1986; Fisher and Reinfelder 1995). The comparable uptake rate constants of MeHg by most species at 4°C, when cell growth and metabolic activity were minimal, and at 18°C when growth rates and metabolic activity were high, is also consistent with the passive uptake of MeHg. Like temperature's effects, the only effect of light on MeHg uptake by phytoplankton was an indirect one. Thus, uptake rate constants for short exposure periods were unaffected by light, but after several days, illuminated cells were able to grow more than cells held in constant darkness, leading to greater biomass and hence more total particulate MeHg. VCFs eventually declined in illuminated cultures held at 18°C because biodilution of cell-bound MeHg occurred when cell biomasses increased to such an extent that most of the bioavailable MeHg in the dissolved phase was exhausted. This biodilution has been observed for MeHg in cultures (Karimi et al. 2007) and natural assemblages (Pickhardt et al. 2002) of freshwater algae. It would not be expected to occur in most marine waters because plankton biomass densities are typically low enough to preclude exhausting ambient MeHg. Further, because it is retained by aquatic organisms much more effectively than most other metals (including inorganic mercury), MeHg is more prone to biodilution than other metals (Karimi et al. 2010).

The pronounced loss of ^{203}Hg from the coccolithophore cultures, but not from uninoculated medium or other algal cultures, indicates that once the Me^{203}Hg was taken up by the coccolithophore (*E. huxleyi*), it was converted to a gaseous form of Hg, either Hg^0 or dimethylmercury (Me_2Hg). Henry's law constants of either of these products would suggest that they would display evasive properties. The production of mercury gas in the coccolithophore cultures was probably attributable to bacterial cells that were observed to be growing in cultures of this clone. Bacteria containing the Hg resistance (*mer*) operon might explain the demethylation and reduction of MeHg (Barkay et al. 2003) in the coccolithophore culture. We are unaware of reports demonstrating conversion of MeHg into an evasive form of mercury, although marine microorganisms smaller than 3 μm have been

shown to reduce dissolved Hg(II) to elemental Hg^0, an evasive form of mercury (Mason et al. 1995).

As expected, the MeHg VCFs were positively related to the surface area-to-volume ratios of the various algal cells (inversely related to cell size) (Fig. 9). Expressed on a surface to volume ratio, the MeHg concentration factors were comparable to that found for the freshwater diatom *Cyclotella meneghiniana* (SA/V = 0.94 μm^{-1}) exposed under similar conditions (Pickhardt and Fisher 2007; Luengen et al. 2012). This is broadly consistent with patterns shown for many other particle-reactive metals that sorb onto cell surfaces (Fisher and Reinfelder 1995) and is also consistent with the idea that MeHg binds passively to them. This finding would suggest that the degree of enrichment of MeHg in algal cells in natural communities could depend on the size of the predominant cells in plankton assemblages, which in turn may vary seasonally and regionally.

The unique MeHg uptake pattern in the dinoflagellate *P. minimum* showed a significantly higher MeHg uptake rate at 18°C than at 4°C. This suggests another pathway of MeHg acquisition in this species rather than solely passive transport. As this dinoflagellate can be mixtrophic, it may be possible that MeHg bound to organic matter or other nanoparticles was acquired via an energy-requiring process such as phagocytosis. Tranvik et al. (1993) suggested that metals bound to colloidal matter might be an important source of metals to heterotrophic flagellates. Wang and Guo (2000) found that marine colloidal materials added to cultures of a diatom and dinoflagellate affected uptake of some metals. However, there was no related study for either Hg or MeHg.

Nitrogen addition to the algal cultures increased the algal biomass in cultures after 2 d, and this led to more total particulate MeHg and eventually lower VCFs. However, the MeHg uptake rate constants over the first 4 h of exposure (during which growth differences between treatments were minimal) were unaffected by nitrogen additions. In contrast, Wang and Dei (2001a, 2001b) demonstrated significant increases in short-term Cd and Zn uptake by phytoplankton enriched with nitrogen. As with light and temperature, the principal effect of nutrient additions on MeHg uptake was an indirect one, where the greater biomass eventually resulting from higher nitrogen levels led to more total particulate MeHg in algal cells, but with each cell being less enriched in MeHg. Consequently, in eutrophic waters where algal biomasses are high, MeHg concentrations in cells may be expected to be lower, consistent with observations in lakes (Pickhardt et al. 2002) and the NW Atlantic Ocean (Hammerschmidt et al. 2013). This could be expected to result in lower MeHg concentrations in animals in eutrophic ecosystems.

The penetration of MeHg into the cytoplasm of the cells is consistent with findings for MeHg in marine and freshwater diatoms (Mason et al. 1996; Pickhardt and Fisher 2007). This has implications for the likelihood of trophic transfer of MeHg to herbivorous zooplankton, as shown for MeHg with a marine diatom (Mason et al. 1996) and for other metals for crustacean zooplankton and molluscan larvae (Reinfelder and Fisher 1991; Reinfelder and Fisher 1994; Stewart and Fisher 2003a). To our knowledge, the distribution of MeHg in other algal cell types has not been reported. Because the VCFs and uptake rate constants of MeHg were not affected by light and temperature in most algal species (Figs. 3,

4), it appears that metabolic activities did not lead to active MeHg uptake, consistent with earlier conclusions (Mason et al. 1996).

The relationship of MeHg uptake by the diatom *T. pseudonana* over a range of chloride concentrations coincides with observations of previous studies reported for the diatoms *Thalassiosira weissflogii* (Mason et al. 1996) and *Ditylum brightwellii* (Kim et al. 2014). The increased lipophilicity of chloro-complexed MeHg should increase the passive transport of MeHg with increasing Cl concentration and may therefore lead to greater MeHg enrichment in marine food chains than in freshwater food chains.

Overall, the range of VCFs of MeHg in the six algal species (log VCF = 4.3–6.8) was comparable to findings for previous lab studies involving freshwater or marine phytoplankton (Table 4). Pickhardt and Fisher (2007) reported log VCF ranged from 5.1 to 6.2 and Miles et al. (2001) reported log VCF ranged from 5.4 to 6.9 for freshwater algae. For marine algae, Kim et al. (2014) reported log VCF values ranging from 4.9 to 6.2. Our data also agree with bioaccumulation factors of MeHg obtained from field studies (Table 4), suggesting that the experimental conditions used in this study are generally applicable to natural waters. Thus, even though the experimental MeHg concentrations exceeded typical MeHg concentrations in marine ecosystems, the degree of bioconcentration in phytoplankton is comparable. The field measurements cited in Table 4 were originally expressed on a dry weight basis, and these were converted to a volume basis by using a mean ratio of cell volume:dry weight of 5.0 (Fisher et al. 1983a). The field measurements were reported for microseston in the Northeast Atlantic (log VCF = 4.9) (Hammerschmidt et al. 2013), the subtropical North Pacific (log VCF = 5.9) (Hammerschmidt and Bowman 2012), and in the central Pacific (log VCF = 6.3) (Gosnell and Mason 2015). Relatively low VCFs were found in the North Sea (log VCF = 4.2) (Baeyens et al. 2003), and in Long Island Sound (log VCF = 4.2) (Hammerschmidt and Fitzgerald 2006a). Given the variability of VCFs noted among algal cultures in this study, the concentration factors of MeHg in natural phytoplankton assemblages in the field may be expected to vary seasonally and spatially with the phytoplankton composition and predominant cell size. This variation in MeHg VCFs among phytoplankton may be one order of magnitude or more (Fig. 9), and these differences in MeHg bioaccumulation at the base of the food web may be reflected in variations in higher trophic level animals, such as in fish. Moreover, the fraction of lithogenic materials might also influence the overall concentration factor, particularly in coastal regions, since MeHg has relatively low affinity for abiotic particles, as shown with the glass beads. VCFs in phytoplankton were about 100-fold higher than in glass beads, suggesting that MeHg binds principally to biochemical compounds (especially S and N-rich compounds such as proteins) (Vallee and Ulmer 1972; Onyido et al. 2004) associated with living cells. These findings indicate that MeHg will be more enriched in living than in abiotic particles, and consequently inversely related to the abundance of lithogenic particles (Bloom et al. 1999; Hammerschmidt et al. 2004; Hammerschmidt and Fitzgerald 2006b). Thus the relatively low VCFs seen in coastal regions such as the North Sea and Belgian coastal waters were probably due to the higher fraction of lithogenic materials collected in suspended particulate material. In contrast, high VCFs were observed in open ocean regions such as the central Pacific where biogenic particles are dominant.

The VCFs of MeHg reported here are higher than for most other metals, even particle-reactive metals such as Zn, Cd, Ag, Pb, Po, and inorganic Hg (Fisher et al. 1984; Fisher et al. 1987; Stewart and Fisher 2003b), and about the same as the transuranic elements Pu and Am (Fisher et al. 1983a). Importantly, MeHg penetrates into the cytoplasm of algal cells to a much greater than the other metals (particularly true for Pb, Pu, Am, and inorganic Hg), which largely remain sorbed on the surface of cells. Consequently, the MeHg is assimilated by herbivores that feed on these phytoplankton (and subsequently passed up food chains) to a much greater extent than occurs with other metals (Reinfelder and Fisher 1991).

Acknowledgments

We appreciate helpful discussions with R. Mason and J. Reinfelder and helpful comments from two anonymous reviewers. Research reported in this publication was supported by NSF Award PLR 1260345, NIEHS Award P42ES007373, and NYSERDA Award 34357

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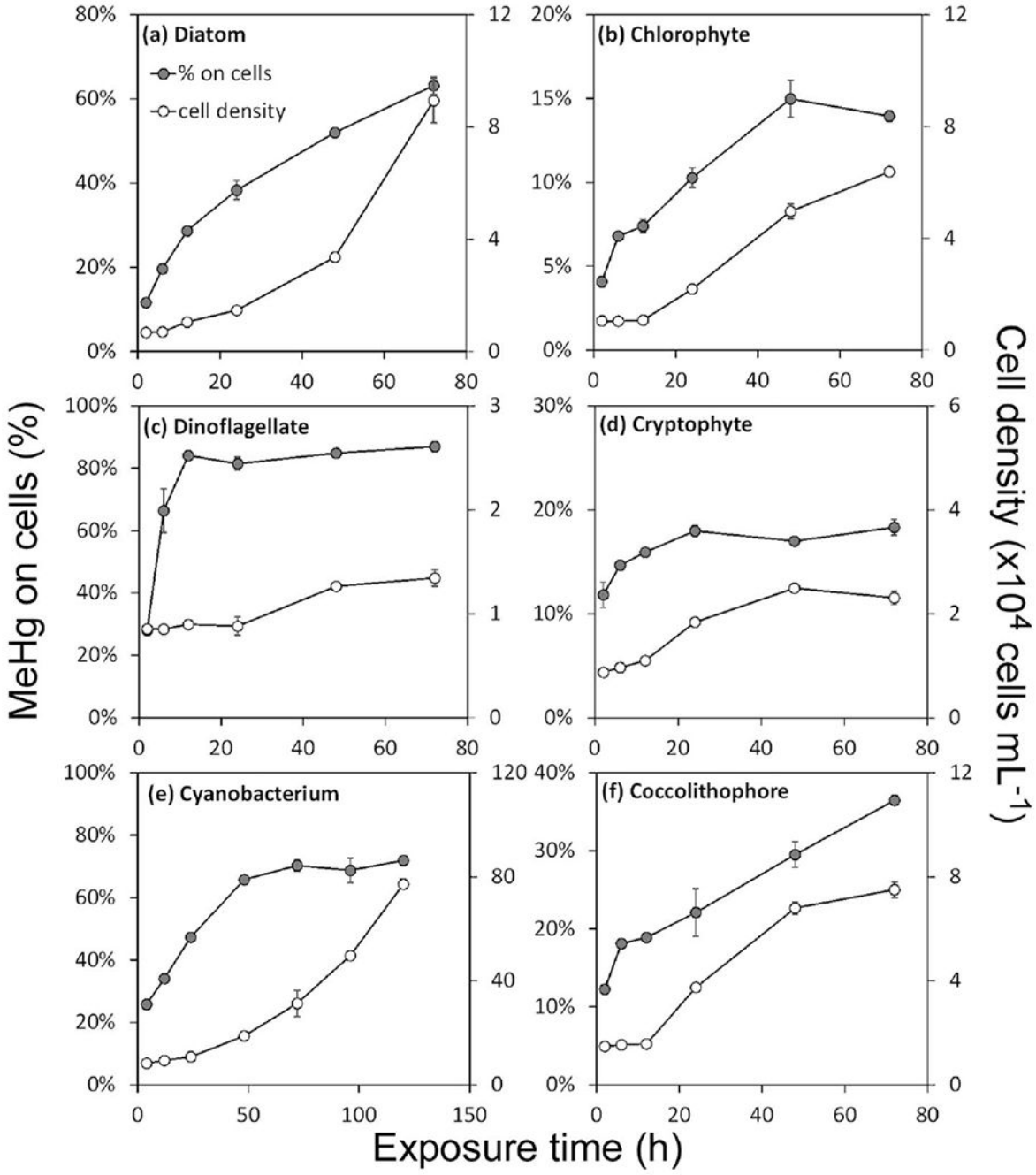

Fig. 1.

MeHg uptake and growth of algal cells among six phytoplankton species over time. The solid circles (left axis) represent the percentage (%) of total MeHg in the cultures associated with the particulate (>1 μm) phase. The open circles (right axis) represent the cell densities ($\times 10^4$ cells mL^{-1}). Data points are the means from three replicates cultures with error bars of one standard deviation. Note the different scales on each graph.

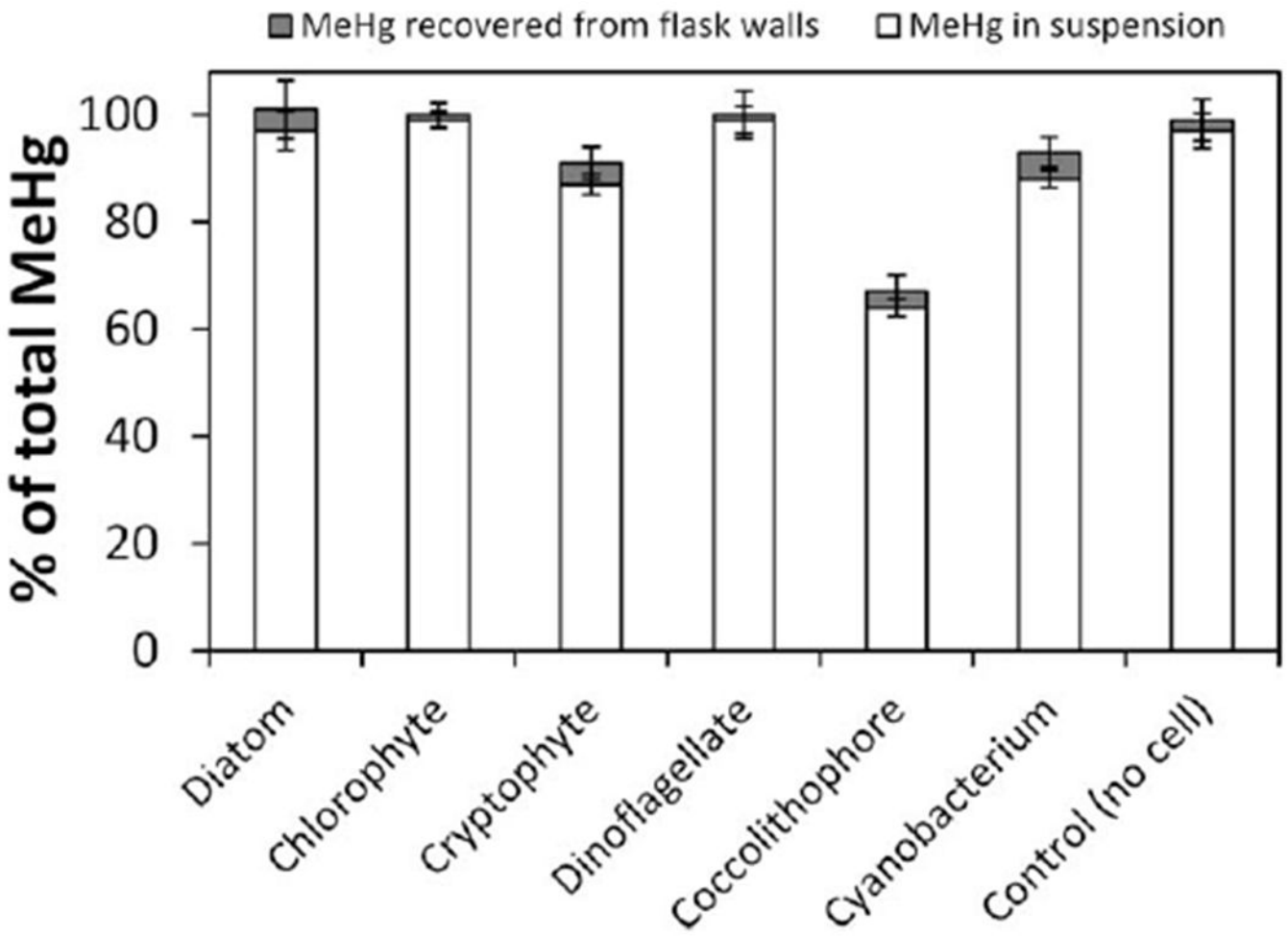

Fig. 2.

MeHg mass balance in cultures at 18°C and 14:10 light:dark cycle. The white bar represents the percentage of total MeHg remaining in suspension (dissolved plus particulate fractions after 72 h exposure). The gray bar represents the MeHg recovered from the flask wall by acid rinsing at 72 h. Recovery of less than 100% of total $Me^{203}Hg$ from each culture is presumed attributable to evasion into the air.

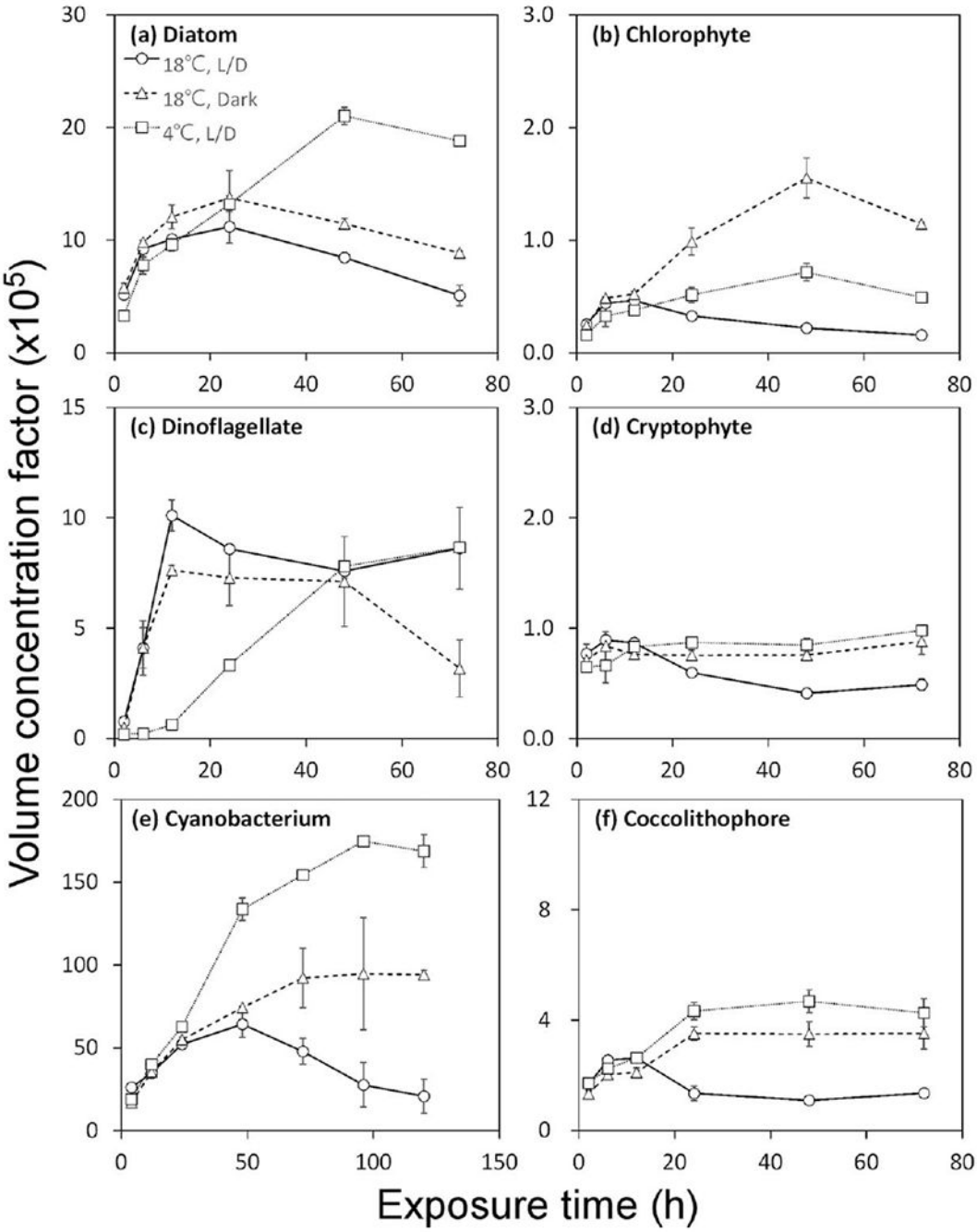

Fig. 3.

MeHg volume concentration factors over time under different environmental conditions. L/D represents 14:10 light:dark cycle. Data points are the means from two replicate cultures shown with 1 SD error bars. Note the different scales on each graph.

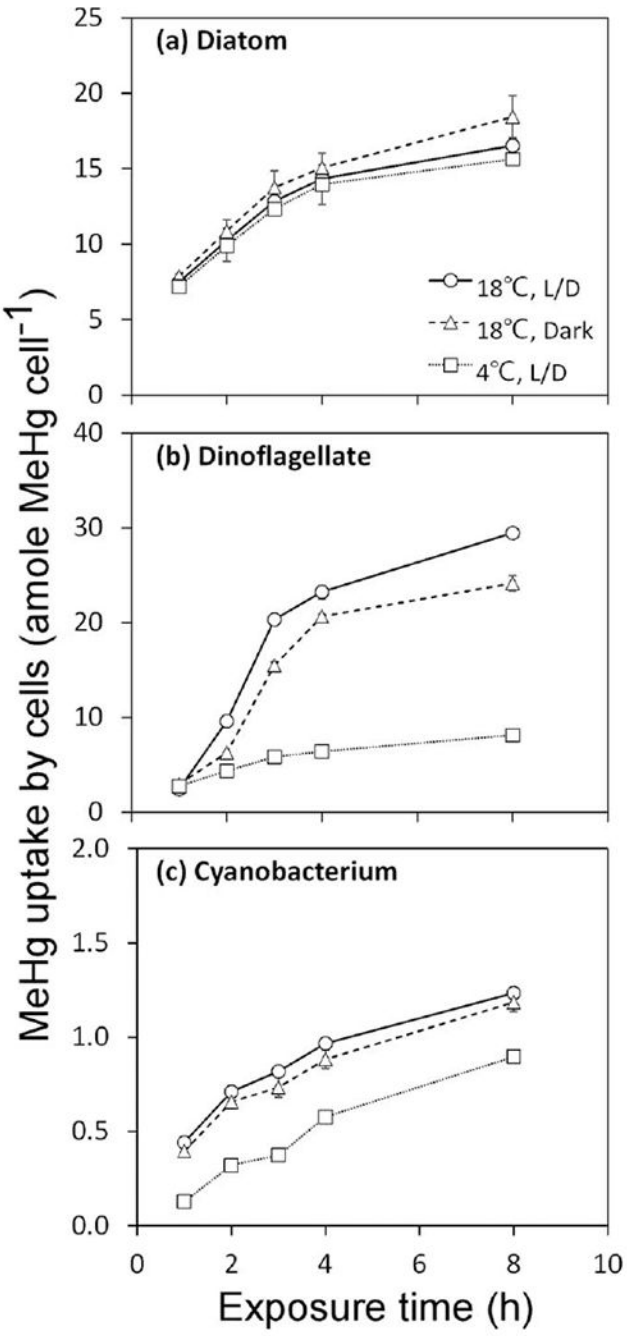

Fig. 4.

Uptake of MeHg in cells over short-term exposures for *T. pseudonana* (diatom), *P. minimum* (dinoflagellate), and *S. bacillaris* (cyanobacterium) under different environmental conditions. L/D represents 14:10 light:dark cycle. Data points are the means from two replicate cultures shown with 1 SD error bars.

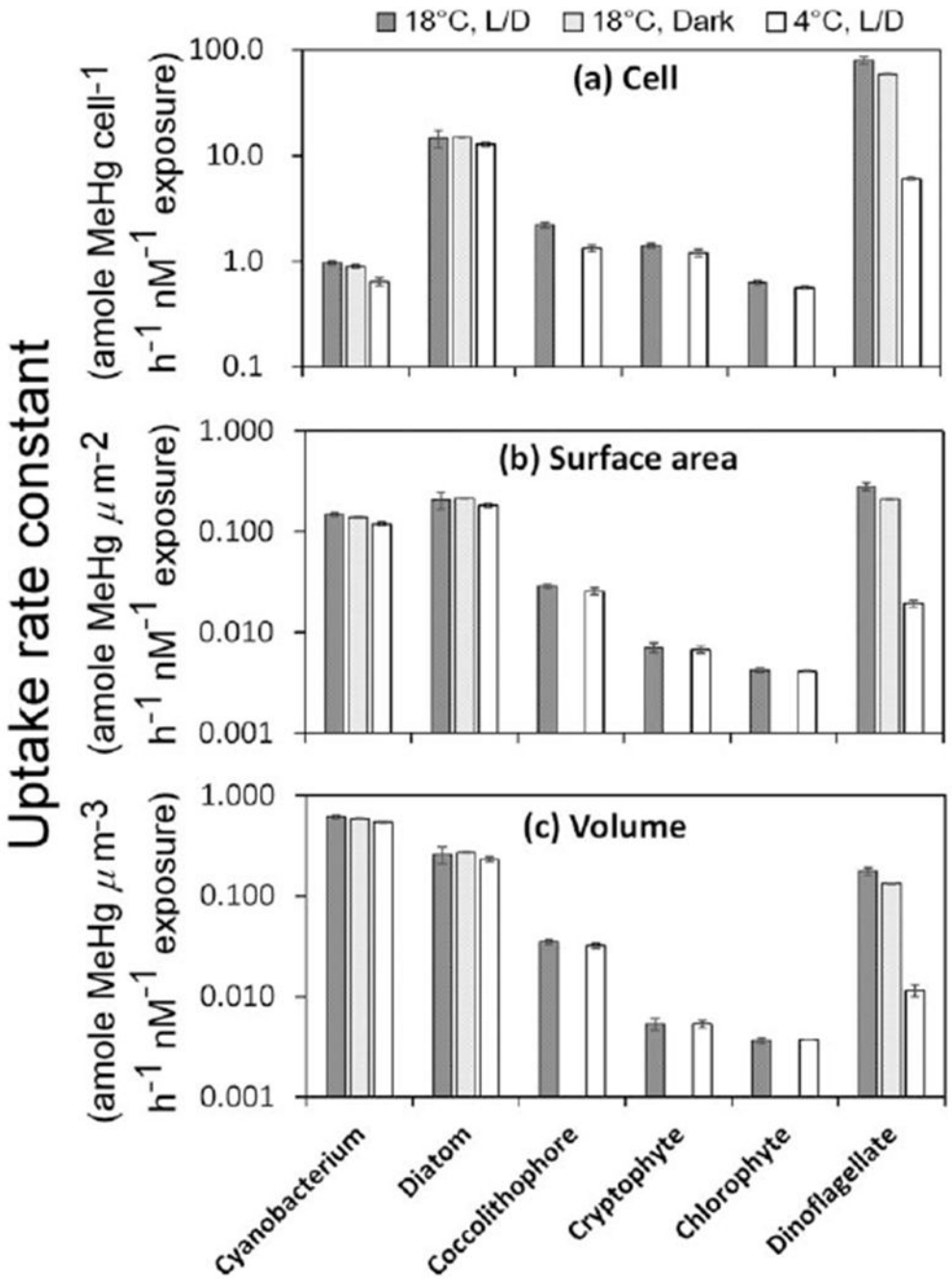

Fig. 5.

Uptake rate constants of MeHg in six different algal species over short-term exposures. Values are normalized to the initial dissolved MeHg concentrations in the media and are expressed on per cell, per μm^2 cell surface area, and per μm^3 cell volume bases for the first 4 h of exposure. Algal species on the *x* axis are arranged in increasing size order, with the cyanobacterium being the smallest cell and the dinoflagellate the largest cell.

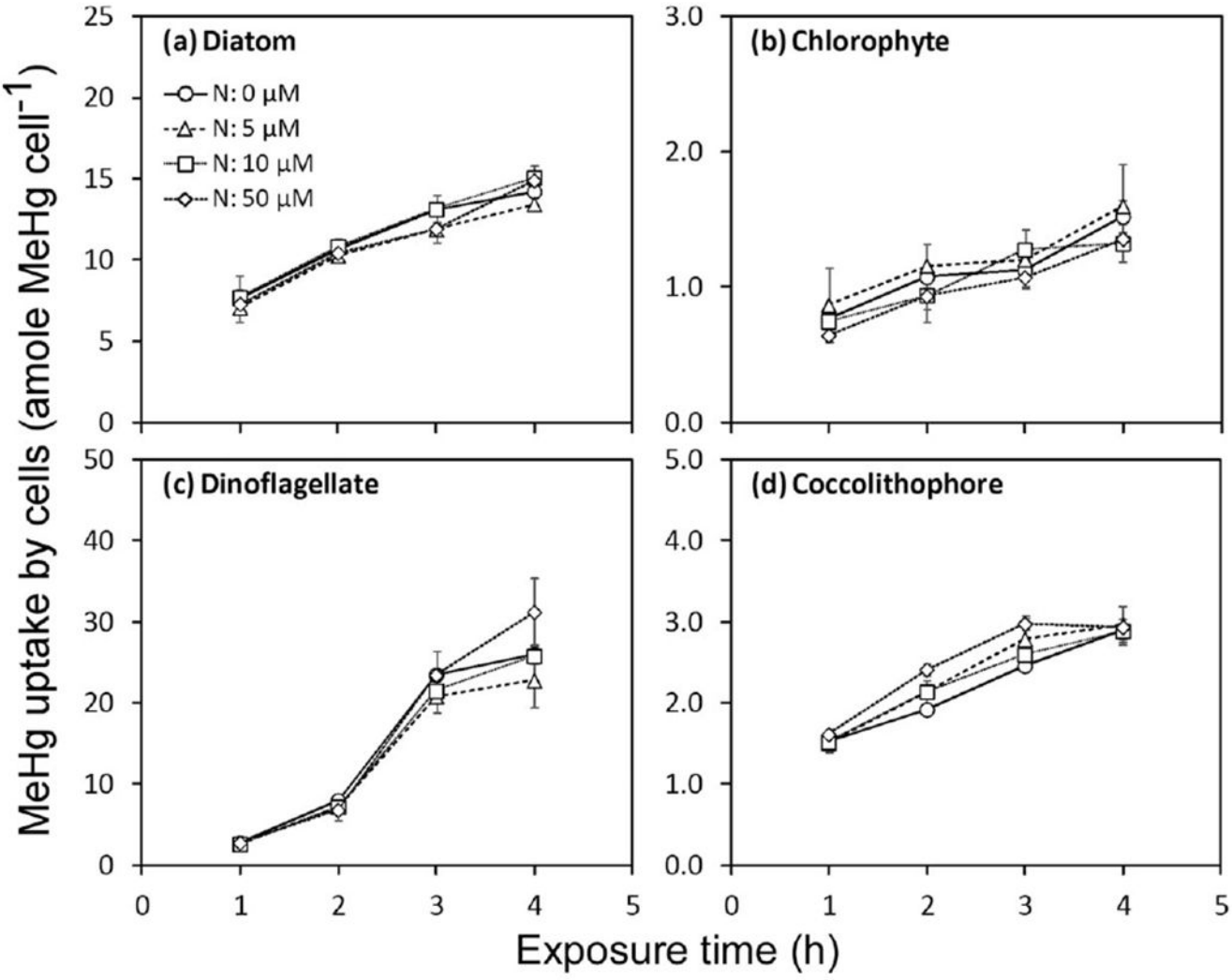

Fig. 6.

Uptake of MeHg by cells exposed under four nitrate treatments for *T. pseudonana* (diatom), *D. tertiolecta* (chlorophyte), *P. minimum* (dinoflagellate), and *E. huxleyi* (coccolithophore). Data points are the means from two replicate cultures shown with 1 SD error bars.

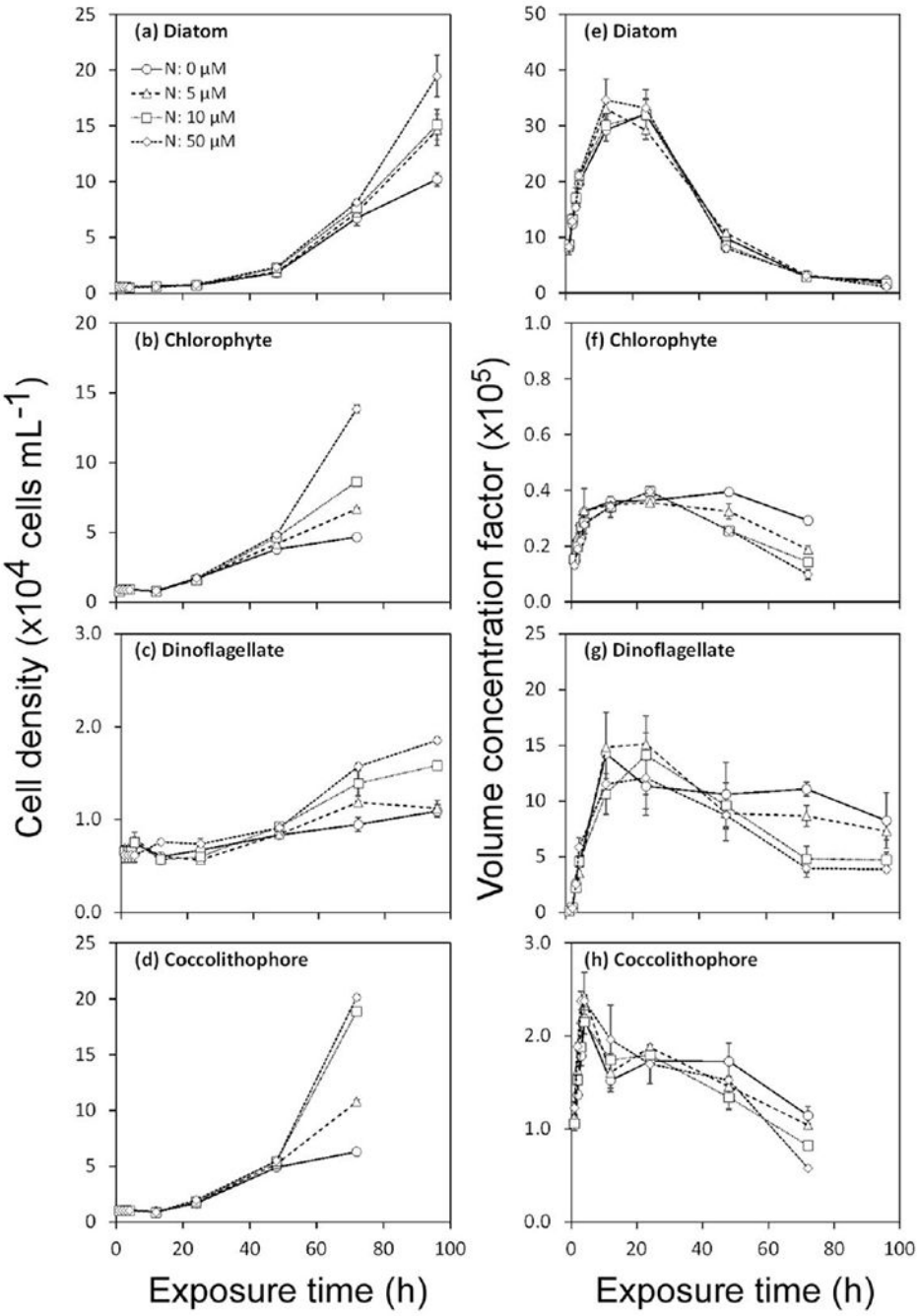

Fig. 7.

Cell growth **(a–d)** and MeHg volume concentration factors (×10^5, **e–h**) over time in cultures exposed to four different nitrate treatments. Data points are the means from two replicate cultures shown with 1 SD error bars.

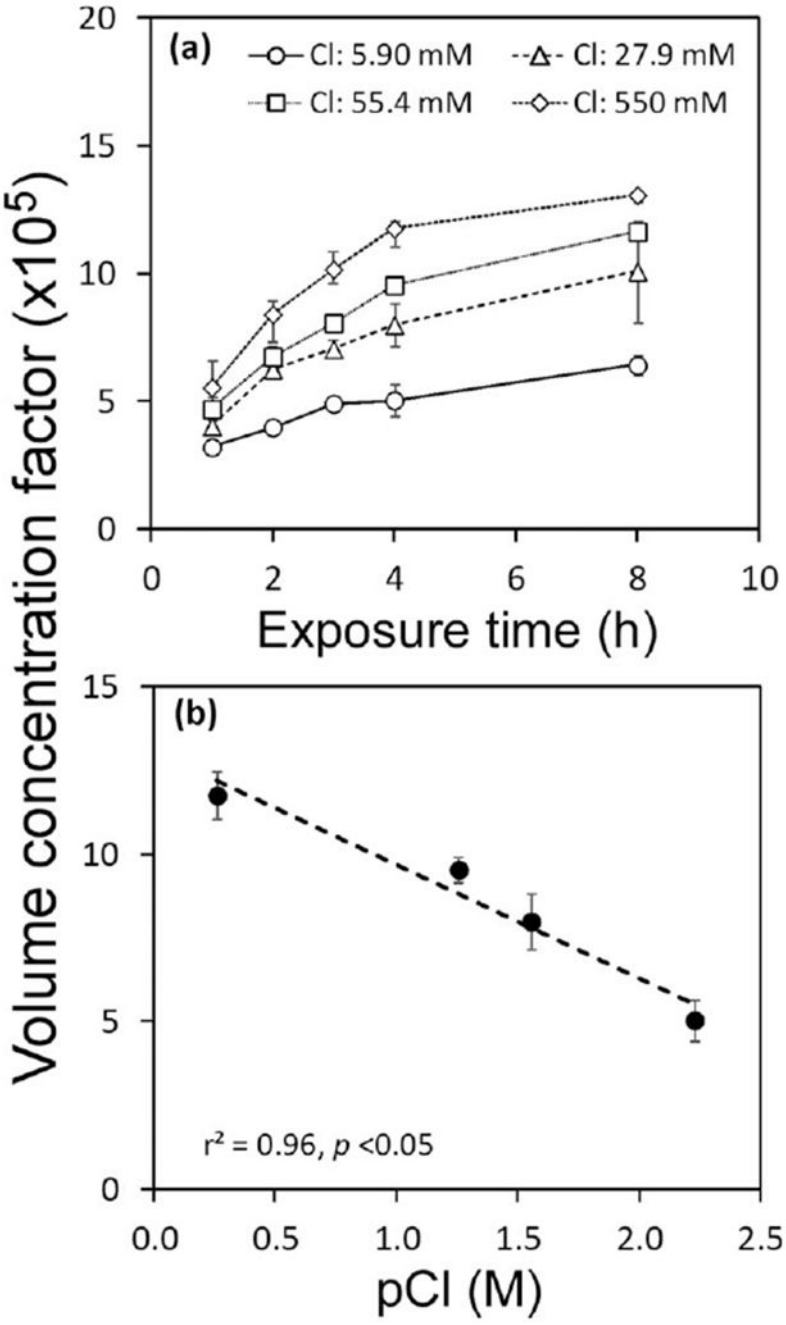

Fig. 8.

(a) Volume concentration factors (×10^5) for the diatom, *T. pseudonana*, over time at four different chloride concentrations. **(b)** The VCFs (t = 4 h) vs. pCl (−log Cl concentration). The dotted line represents the linear regression relating VCF to pCl (r^2 = 0.96, p < 0.05). Data points are the means from three replicate cultures shown with 1 SD error bars.

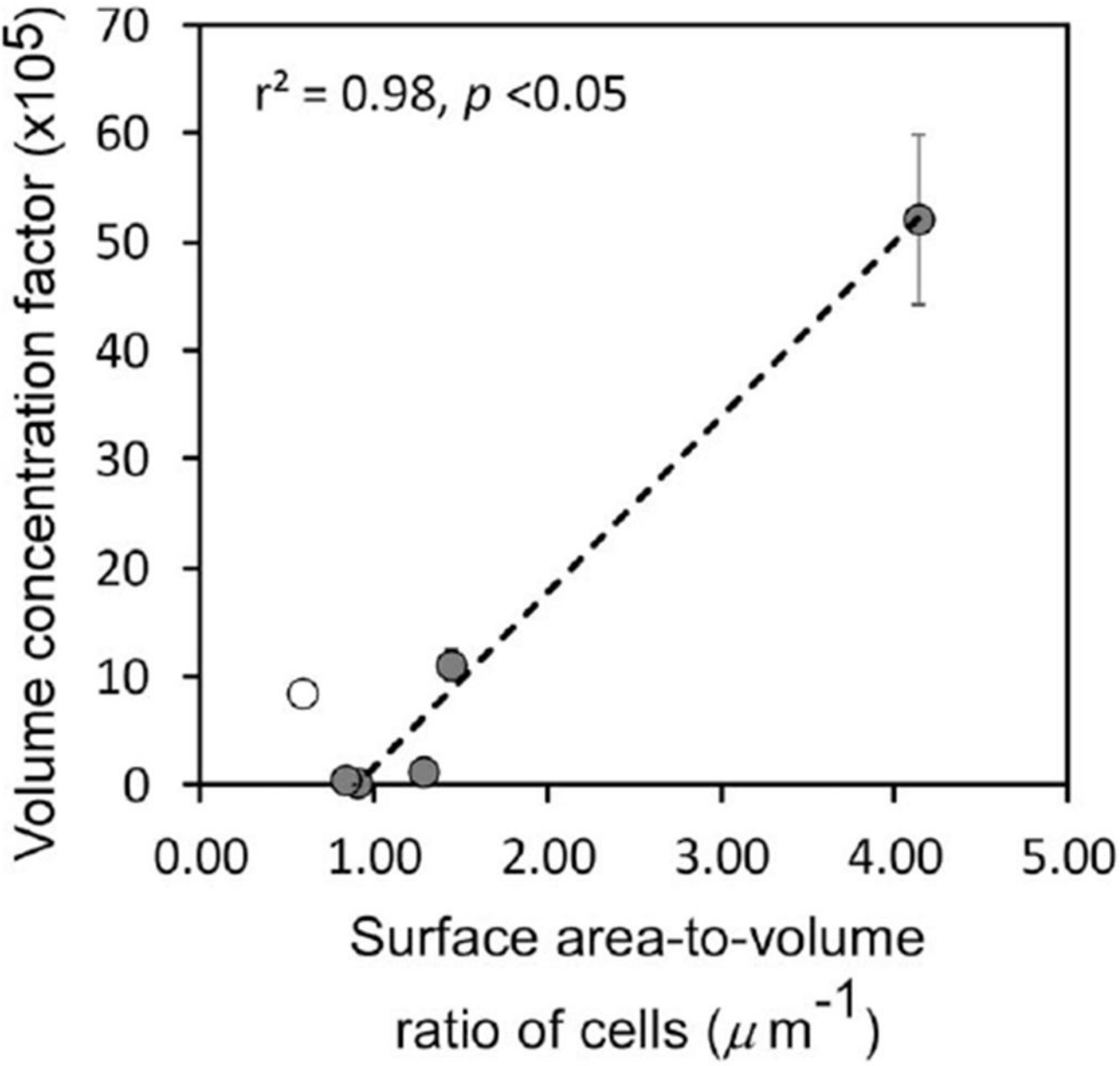

Fig. 9.

Correlation between the mean surface area-to-volume ratio of algal cells from each culture and the MeHg volume concentration factors (VCFs). Data points are the mean VCFs at 24 h (n = 3) shown with 1 SD error bars. The linear regression excludes the dinoflagellate *P. minimum* (open circle).

Table 1.

Phytoplankton species used in this study; ranges of measured cell volumes (μm^3), calculated surface areas (μm^2), and mean surface area-to-volume ratios (μm^{-1}).

Phytoplankton type	Species	Clone	Volume μm^3	Surface area μm^2	SA/V ratio* μm^{-1}
Diatom	*Thalassiosira pseudonana*	CCMP1335	43–62	63–89	1.44
Chlorophyte	*Dunaliella tertiolecta*	CCMP1320	141–227	127–204	0.90
Cryptophyte	*Rhodomonas salina*	CCMP1319	160–231	133–192	0.83
Dinoflagellate	*Prorocentrum minimum*	CCMP696	540–778	313–451	0.58
Coccolithophore	*Emiliania huxleyi*	CCMP375	40–75	51–97	1.28
Cyanobacterium	*Synechococcus bacillaris*	CCMP1333	1.1–1.6	4.4–6.6	4.13

*Surface area-to-volume ratio.

Table 2.

Percentage of total cellular MeHg in the cytoplasmic fraction under three environmental conditions.

	Conditions		
Algal species	**18°C, L/D***	**18°C Dark**	**4°C, L/D**
T. pseudonana	26	53	61
D. tertiolecta	75	73	53
R. salina	77	74	79
P. minimum	27	39	57
E. huxleyi	78	88	82
S. bacillaris	38	91	73

* L/D: 14:10 light-dark cycle.

Table 3.

MeHg uptake rate constants (normalized to the initial dissolved MeHg concentrations in the media) on per cell, per μm^2 cell surface area, and per μm^3 cell volume bases for the first 4 h of exposure.

		Algal species					
Uptake rate constant	**Conditions**	***T. pseudonana***	***D. tertiolecta***	***R. salina***	***P. minimum***	***E. huxleyi***	***S. bacillaris***
Cell basis *	18°C, L/D	15±3	0.63±0.03	1.4±0.1	80±6	2.2±0.1	0.97±0.03
	18°C, Dark	15±0.1			59±0.2		0.89±0.03
	4°C, L/D	13±1	0.56±0.02	1.2±0.1	6.1±0.1	1.3±0.1	0.64±0.06
Surface area basis †	18°C, L/D	0.21±0.04	0.0042±0.0002	0.0071±0.0007	0.28±0.02	0.029±0.001	0.15±0.01
	18°C, Dark	0.21±0.00			0.21±0.00		0.14±0.00
	4°C, L/D	0.18±0.01	0.0041±0.0000	0.0067±0.0005	0.02±0.00	0.026±0.002	0.12±0.01
Volume basis ‡	18°C, L/D	0.26±0.05	0.0036±0.0002	0.0053±0.0007	0.18±0.01	0.035±0.002	0.62±0.03
	18°C, Dark	0.27±0.00			0.13±0.01		0.59±0.01
	4°C, L/D	0.23±0.01	0.0037±0.0000	0.0054±0.0004	0.01±0.00	0.032±0.002	0.55±0.01

unit:

*amole MeHg $cell^{-1}$ h^{-1} nM^{-1} exposure

†amole MeHg μm^{-2} h^{-1} nM^{-1} exposure

‡amole MeHg μm^{-3} h^{-1} nM^{-1} exposure

Table 4.

MeHg VCFs from culture experiments (a) and field studies (b).

(a) Lab study

Particle type	Diameter range* (μm)	Log VCF	References
Marine algae	1.0–12 (6)	4.3–6.8	This study
	5.0–32 (5)	4.9–6.2	Kim et al. (2014)
Freshwater algae	8.0 (1)	4.2–5.4	Luengen et al. (2012)
	2.0-8.2 (4)	5.1–6.2	Pickhardt and Fisher (2007)
	4.0-120 (4)	5.4–6.9	Miles et al. (2001)
Glass beads	5.0	3.1	This study

(b) Field data

Sampling site	Size range (μm)	Log VCF†	References
North Sea		4.2	Baeyens et al. (2003)
Belgian coast		4.2	Baeyens et al. (2003)
Long Island Sound	>0.2	4.5	Hammerschmidt and Fitzgerald (2006*a*)
Northeast Atlantic	0.2–200	4.9	Hammerschmidt et al. (2013)
North Pacific	1.0–51	5.9	Hammerschmidt and Bowman (2012)
Central Pacific	0.2–200	6.3	Gosnell and Mason (2015)

*Mean diameter of algal cells. The numbers in parentheses indicate the number of algal species tested in the study.

†Literature values were originally on a dry wt basis; they were converted to VCFs by using a mean cell volume:dry wt ratio of 5.0, based on measured values from 6 marine phytoplankton cells (Fisher et al. 1983*a*)