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SHORT CONTRIBUTION

Uptake of methylmercury by marine microalgae and its bioaccumulation in them

Yuya Tada[1] · Kohji Marumoto[1]

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Abstract

Assessment of methylmercury (MeHg) accumulation by marine microalgae is critical to understand the dynamics of mercury (Hg) and MeHg in marine environments. We conducted incubation experiments with added MeHg to reveal its bioaccumulation by four marine microalgal lineages. Cyanophyceae had a higher cellular MeHg accumulation than Pelagophyceae, Prymnesiophyceae, and Bacillariophyceae (diatom). MeHg accumulation was higher in living (than dead) diatom cells. Moreover, diatom cells did not release cellular MeHg during cell division and the stationary phase. Our findings suggest that the community composition and metabolic activity of marine microalgae can be critical for MeHg biomagnification in marine food webs.

Keywords Marine microalgae · Methylmercury · Bioaccumulation · Living cell · Dead cell

1 Introduction

Fish consumption is the main source of toxic and bioaccumulative MeHg exposure in humans and marine mammals. High MeHg accumulation was observed in marine fish tissues (10^6–10^7 fold greater than that in seawater) (Wiener et al. 2007), whereas the concentration of MeHg in seawater was unexpectedly low (4–40 pg Hg) (Fitzgerald et al. 2007; Mason et al. 2012). Marine phytoplankton (microalgae) can take up and bioaccumulate MeHg from ambient seawater and thus serve as key entry points for MeHg in marine food webs. Several surveys on marine environments have revealed that the accumulation factor of MeHg from seawater to microalgae has reached up to 10^5, and that this value is relatively large compared to that of other organisms in marine ecosystems (Hammerschmidt et al. 2013; Gosnell and Mason 2015; Lee and Fisher 2016). Measurements of the uptake and accumulation rates of MeHg by marine microalgae are critical to understand the dynamics of mercury (Hg) and MeHg in marine environments. However, MeHg uptake and bioaccumulation by marine microalgae is still not fully understood. Information on the variation of the accumulation ratio among different marine microalgal lineages and the effect of metabolic activity on the MeHg accumulation rate is limited.

Multiple studies have investigated the MeHg uptake and bioaccumulation of several freshwater and seawater microalgal cultures (Mason et al. 1996; Moye et al. 2002; Gorski et al. 2006; Pickhardt and Fisher 2007; Zhong and Wang 2009; Wu and Wang 2011; Kim et al. 2014; Lee and Fisher 2016). Some studies have specifically reported on MeHg accumulation by marine diatoms under various environmental conditions (Mason et al. 1996; Zhong and Wang 2009; Kim et al. 2014; Lee and Fisher 2016, 2017). However, a few comparative studies have investigated MeHg bioaccumulation using several marine microalgal lineages. In the natural marine environments, dominant microalgal lineages shifts are known to occur from coastal areas to the open ocean (e.g., Shi et al. 2011). For instance, Bacillariophyceae (diatom) and Prymnesiophyceae are dominant lineages in the coastal environment, whereas Cyanophyceae and Pelagophyceae are reported to be the major lineages in the pelagic environment. Thus, to better understanding the MeHg biomagnification process of marine plankton, it is

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✉ Yuya Tada
tada@nimd.go.jp

[1] National Institute for Minamata Disease (NIMD), 4058-18 Hama, Minamata, Kumamoto 867-0008, Japan

critical to elucidate the MeHg uptake and accumulation of several marine microalgae.

To precisely quantify the capacity of marine microalgae to remove and bioaccumulate MeHg from ambient seawater, it is critical to evaluate if MeHg uptake occurs actively or passively (i.e., via absorption and adsorption). A previous study using freshwater diatoms showed that the MeHg incorporation rate of living cells was higher than that of experimentally killed cells (Pickhardt and Fisher 2007). This indicates that the MeHg uptake and accumulation rates of freshwater microalgae could depend on their metabolic activity. Lee and Fisher (2016) assessed marine microalgae and reported that there was no difference in the accumulation of MeHg in several microalgae under the distinct incubation temperatures (4–18 °C), which suggests that MeHg uptake does not depend on cellular metabolic activity. However, the difference in MeHg uptake and accumulation rates of marine microalgae between living and completely dead cells is still unclear.

The aim of this study was to determine the capacity of four microalgal lineages to accumulate and remove MeHg from ambient seawater, using incubation experiments with added MeHg. Moreover, we examined the MeHg uptake and accumulation by living and dead (experimentally killed) diatom cells. Finally, using a long-term incubation experiment, we examined if diatom cells released MeHg during cell division or the stationary phase.

2 Materials and methods

2.1 Experimental design and sample collection

In the present study, we used four marine microalgal species isolated from several marine environments, *Thalassiosira pseudonana* (Bacillariophyceae, CCMP1335), *Emiliania huxleyi* (Prymnesiophyceae, CCMP374), *Pelagomonas calceolata* (Pelagophyceae, CCMP1756), and *Synechococcus* sp. (Cyanophyceae, CCMP1336). *Thalassiosira pseudonana* was isolated from the coastal area in the North Atlantic, and has been widely used for incubation experiments with added Hg (e.g., Zhong and Wang 2009; Lee and Fisher 2016, 2017). *Emiliania huxleyi* is known as a ubiquitous marine alga that is widely distributed from coastal to pelagic environments (e.g., Ackleson et al. 1988). *Pelagomonas calceolata* was reported as one of the major microalgal lineages in the open ocean (Shi et al. 2011), and MeHg uptake or bioaccumulation of this lineage has never been examined. *Synechococcus* sp. CCMP1336 was isolated from the open ocean, and has been used in several incubation experiments as a prokaryotic photosynthesizer.

In the incubation experiments using *T. pseudonana*, precultured strains in the exponential phase (Fig. S1) were incubated in 1.5 l of L1 medium (Guillard 1975) with the addition of MeHg-cysteine (MeHg-cys) (final conc. ca. 1.2 ng Hg l^{-1}) at 22 °C under light conditions (100 µmol photon m^{-2} s^{-1}) in acid-washed 2 l FEP (fluorinated ethylene propylene) Teflon bottles for 0 h, 1 h, and 3 h. Prior to use, the Teflon bottles were washed vigorously by ultrapure water after 10 min of ultrasonic irradiation with an alkaline detergent and soaking in 2 M nitric acid solution for more than two nights. In this study, we used MeHg-cys as a standard solution, since this species is hydrophilic. The MeHg concentrations used in this study were approximately 50–100 times higher than that of the maximum values in subsurface seawater in the North Pacific Ocean (Hammerschmidt and Bowman 2012; Munson et al. 2015; Marumoto et al. 2018).

In the other microalgal cultures, precultured strains in the exponential phase (Fig. S1) were incubated in L1 medium with the addition of MeHg-cysteine (MeHg-cys) (final conc. ca. 0.7 ng Hg l^{-1}) at 22 °C under light conditions (100 µmol photon m^{-2} s^{-1}) in 250 mL acid-washed PFA (perfluoroalkoxy alkanes) Teflon bottles for 0 h, 1 h, and 3 h.

In the incubation experiments with living and dead cells, we used a marine diatom culture of *T. pseudonana* in the exponential phase (Fig. S2). To prepare the dead diatom cells, precultured cells were killed by freeze–thaw (− 60 °C) and heat (40 °C for 10 min) treatment. We confirmed the living status of cells (dead or alive) based on their growth or lack thereof during incubation. In the incubation experiment, dead and living diatom cells were cultured in 1.5 l of L1 medium in acid-washed 2 l FEP Teflon bottles with MeHg-cys (final conc. ca. 1.1 ng Hg l^{-1}) at 22 °C under light conditions (100 µmol photon m^{-2} s^{-1}) for up to 10 h. Three samples were incubated per treatment. During incubation experiments, we collected subsamples of dead and living cells at 0, 1, 5, and 10 h after microalgal inoculum.

For the long-term incubation experiment, precultured diatom cells were inoculated in 2.0 l of L1 medium in acid-washed Teflon bottles with MeHg-cys (final conc. ca. 1.0 ng Hg l^{-1}) at 22 °C under a light:dark cycle (12 h:12 h) for up to 2 weeks. Subsamples (200 ml) were collected on days 0, 1, 3, 7, and 14 during the incubation experiment. We incubated autoclaved L1 medium with added MeHg as the blank treatment in all experiments. These incubations were performed in duplicate.

To collect the cellular and dissolved MeHg samples, microalgal cells (particulate fraction) in the culture samples (200 ml volume) were collected using the GF-75 glass-fiber filter (47 mm diameter, ADVANTEC) (precombusted at 450 °C for 4 h), and stored at − 80 °C until further analysis. After collecting the microalgal cells, 160 ml filtrate (dissolved fraction) was amended with H_2SO_4 (final 0.5 M) and stored at 4 °C until further analysis.

2.2 Dissolved and cellular MeHg analyses

Dissolved MeHg concentrations were analyzed using a combined method of diphenylthiocarbazone (dithizone) extraction and the draft EPA method 1630 (Logar et al. 2002; Marumoto et al. 2018). Quantitative and efficient pre-concentration of MeHg was performed by extracting the Hg with 0.01% dithizone-toluene after pH adjustment to ca. 2.5. After pre-concentration, the extract with dithizone–toluene was back extracted into a water phase using Na_2S in 0.1 M NaOH with 50% (vol/vol) of EtOH solution (Ministry of the Environment Japan 2004). The MeHg concentration in the Na_2S solution was determined by the draft EPA method 1630 (US Environmental Protection Agency 2001), i.e., ethylation using $NaB(C_2H_5)_4$, preconcentrated onto a Tenax trap (Brooks-Rand Instruments), thermal desorption, gas chromatographic separation using a packed column (preconditioned 15% OV-3 GC column; Brooks-Rand Instruments), pyrolysis, and Hg measurement using an atomic fluorescence spectrometer (Tekran MODEL 2500; Tekran Inc.).

For analysis of MeHg in microalgal cells, we used a defined protocol for biological samples (Ministry of the Environment Japan 2004) with some modification in the cell lysis step. Before extracting MeHg with dithizone-toluene, microalgal cells were lysed with 1 M KOH-EtOH at 100 °C for 1 h and pH adjusted to ca. 2.5 using 1 M HCl and 20% EDTA solutions. The MeHg in the cell lysate was extracted with dithizone and analyzed using the draft EPA method 1630, as described previously.

The recovery of MeHg was $102 \pm 9\%$ ($n = 18$) for the dissolved fraction and $92 \pm 10\%$ ($n = 15$) for the particulate fraction, based on the recovery of a spike of known concentration of MeHg obtained from alkaline dissolution using Dorm-2 (National Research Council, Canada), i.e., a standard material for MeHg in dogfish.

2.3 Microalgal cell counts and image analyses

To count the microalgal cells, a 5 ml culture was fixed with 2% (vol/vol) paraformaldehyde and cells were counted under a microscope (ECLIPSE TE300, Nikon). To measure the abundance of *Synechococcus* sp., culture cells were filtered through a 0.2 µm pore-size polycarbonate membrane filter (25 mm diameter, Millipore). Cell abundance was counted under a fluorescence microscope (ECLIPSE TE300, Nikon). Microscopic images of the microalgal cells were captured as TIFF files using a charge-coupled device camera (ORCA-ER, Hamamatsu Photonics). The cellular length and width were measured using the image analysis software Image Pro-Plus 6.0 (Media Cybernetics) after edge detection using the Marr–Hildreth (Marr and Hildreth 1980) method and binarization. Cell volumes and surface areas of *T. pseudonana*, *E. huxleyi*, and other microalgal lineages were calculated from the cellular length and width using the equations for 'cylinders', 'spheres', and 'prolate spheroid' shapes, respectively, as described in Sun and Liu (2003).

Volume concentration factors (VCFs) (relative degree of MeHg enrichment in the algal cell to the ambient seawater) were calculated using the following equation:

$$\mathrm{VCF} = [\text{ag uptake} - \text{MeHg } \mu\text{m}^{-3}]_{\text{cell}} / [\text{ag added} - \text{MeHg } \mu\text{m}^{-3}]_{\text{dissolved fraction}}$$

which represents the ratio of intracellular (uptake) and dissolved MeHg concentrations (added) in medium seawater.

2.4 Statistical analysis

Data were analyzed using R software, version 3.4.3 (R Development Core Team 2017). The data were tested for normality with the Kolmogorov–Smirnov test. Statistical differences among treatments were analyzed using a Student's *t* test or one-way ANOVA followed by a Tukey–Kramer's post-hoc test.

3 Results and discussion

3.1 MeHg accumulation and removal capacity of several microalgal lineages

The incubation experiments with several microalgal lineages showed that the capacity of microalgae to accumulate and remove dissolved MeHg from ambient seawater varied among algal lineages (Table 1). These results are consistent with those of previous reports (Table 2), which suggests that the bioaccumulation of MeHg in marine microalgae vary among the dominant microalgal lineages and can be one of the critical factors for magnification rate of MeHg in marine food webs.

Among the microalgal lineages studied, the cellular MeHg concentration of *Synechococcus* sp., with a relatively smaller cell volume (ANOVA, $p < 0.001$; Tukey–Kramer post-hoc test, $p < 0.001$) (Table S1), was higher than those of other lineages, except *P. calceolata* (ANOVA, $p < 0.005$; Tukey–Kramer post-hoc test, $p < 0.01$ for both 1 h and 3 h incubation samples), and increased from 0.04 ag Hg μm^{-3} (average) in the initial stage to 0.63 and 0.48 ag Hg μm^{-3} (average) after the 1 h and 3 h incubation periods, respectively (Table 1). A similar trend was observed with the MeHg removal capacity from ambient seawater (absolute value of decreased dissolved MeHg [T1(T3) – T0] in the bottle/total microalgal volume in the bottle) of *Synechococcus* sp. The capacity of *P. calceolata*, with a large surface area to volume ratio (Table S1), was

Table 1 Cell abundance, particulate-, dissolved-MeHg concentration, total microalgal volume per bottle, cellular-MeHg concentration (per cell volume), MeHg-removal capacity from ambient seawater, and volume concentration factors of several microalgal lineages after incubation for 1 h and 3 h

Treatments	Cell abundance ($\times10^5$ cells ml^{-1})			Particulate MeHg (ng l^{-1})			Dissolved MeHg (ng l^{-1})			Total microalgal volume in the bottle[c] ($\times10^8$ μm^3)			Cellular MeHg concentration[d] (ag Hg μm^{-3})			MeHg-removal capacity from ambient seawater[e] (ag Hg μm^{-3})		Volume concentration factor[f] ($\times10^5$)	
	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T0	T1	T3	T1	T3	T1	T3
Experiment 1[a]																			
Blank 1				ND	ND	ND	0.98	1.05	0.99										
Blank 2				ND	0.02	0.01	1.03	1.03	1.00										
Bacillariophyceae 1	2.3	2.2	2.1	0.02	0.73	0.60		0.14	0.06	396	380	366	0.0004	0.019	0.016	0.024	0.025	1.33	2.67
Bacillariophyceae 2	2.4	2.5	2.3	ND	0.58	0.66		0.12	0.05	421	424	391	ND	0.014	0.017	0.021	0.024	1.15	3.07
Experiment 2[b]																			
Blank 1				ND	ND	ND	0.64	0.57	0.53										
Blank 2				ND	ND	ND	0.72	0.48	0.55										
Pelagophyceae 1	2.2	2.8	2.9	0.03	0.26	0.40		0.16	0.22	8.6	10.9	11.5	0.040	0.236	0.347	0.38	0.27	14.97	15.50
Pelagophyceae 2	2.2	2.3	2.6	0.05	0.38	0.27		0.19	0.21	8.6	8.8	10.0	0.061	0.431	0.271	0.33	0.34	22.75	12.63
Prymnesiophyceae 1	2.6	2.8	3.0	0.03	0.29	0.29		0.25	0.25	40.9	43.2	46.4	0.007	0.061	0.056	0.07	0.06	2.73	2.43
Prymnesiophyceae 2	2.7	2.9	2.6	0.04	0.08	0.21		0.27	0.26	42.2	45.4	39.9	0.009	0.02	0.049	0.05	0.07	0.64	2.06
Cyanophyceae 1	16.9	22.0	21.5	0.04	0.52	0.49		0.09	0.08	6.8	8.8	8.6	0.054	0.588	0.566	0.55	0.53	67.48	71.40
Cyanophyceae 2	18.0	23.3	22.7	0.02	0.63	0.36		0.09	0.09	7.2	9.3	9.1	0.026	0.681	0.396	0.42	0.51	77.75	45.79

T0, T1 and T3 indicate incubation time (0, 1 and 3 h incubation, respectively)

ND not detected

[a]Data from the treatments with *Thalassiosira pseudonana*

[b]Data from the treatments with several microalgal lineages

[c]Calculated with the cell abundance and the cell volume (Table S1 supplementary information)

[d]Calculated with the particulate MeHg and the total microalgal volume in the bottle

[e]Absolute value of decreased dissolved MeHg [T1(T3) − T0] (ag MeHg) in the bottle/total microalgal volume in the bottle ($\times10^8$ μm^3)

[f]Ratio of the cellular MeHg concentration (ag Hg μm^{-3}) and dissolved MeHg concentrations (ag Hg μm^{-3}) in medium seawater

Table 2 MeHg accumulation rates reported for several algal lineages

Marine algal species	Lineage	MeHg species	MeHg conc. (added for incubation) (ng)	Accumulation rate[a] (ag cell^{-1} h^{-1} ng^{-1})	References
Thalassiosira weissflogii	Bacillariophycea	CH_3HgCl	0.6–30	6–12	Mason et al. (1996), Le Faucheur et al. (2014)
Thalassiosira pseudonana clone 3H (CCMP1335)	Bacillariophycea	CH_3HgCl	200	5.7[b]	Zhong and Wang (2009)
Thalassiosira pseudonana (CCMP1335)	Bacillariophycea	CH_3HgCl	58–84	10[c]	Lee and Fisher (2016)
Dunaliella tertiolecta (CCMP1320)	Chlorophyceae			0.7[c]	
Rhodomonas salina (CCMP1319)	Cryptophyceae			4.0[c]	
Prorocentrum minimum (CCMP696)	Dinophyceae			70[c]	
Emiliania huxleyi (CCMP375)	Prymnesiophyceae			5.0[c]	
Synechococcus bacillaris (CCMP1333)	Cyanophyceae			1.0[c]	
Stephanopyxis palmeriana	Bacillariophycea	CH_3HgCl	4–20	2.6–10[d]	Kim et al. (2014)
Odontella regia	Bacillariophycea			3.0–12[d]	
Ditylum brightwellii	Bacillariophycea			2.8–11[d]	
Chaetoceros curvisetus	Bacillariophycea			1.9–7.6[d]	
Chroococcus minutus	Cyanophyceae			0.3–1.1[d]	
Thalassiosira pseudonana (CCMP1335)	Bacillariophycea	CH_3Hg-cysteine	1	2.4–3.2[e]	This study
Pelagomonas calceolata (CCMP175)	Pelagophyceae		0.7	1.1–2.1[e]	
Emiliania huxleyi (CCMP374)	Prymnesiophyceae			1.3[f]	
Synechococcus sp. (CCMP1334)	Cyanophyceae			0.3–0.4[e]	

[a]Normalized with the exposure MeHg concentration [(nM]), the algal cell abundance and the incubation time

[b]MeHg exposure = 200 ng l^{-1}, intracellula MeHg = 57% of total MeHg, algal cells = 10^5 cells ml^{-1}

[c]Read on Fig. 5a in the manuscript of Lee and Fisher (2016)

[d]Calculated from volume concentration factors in Fig. 2 (*S. palmeriana*, 7.3×10^4; *O. regia*, 2.0×10^5; *D. brightwellii*, 3.0×10^5; *C. curvisetus*, 1.2×10^6; *C. minutus*, 1.6×10^6), incubation time = 5 h

[e]Calculated using the data of 1 h incubation. Value range of duplicate samples

[f]Calculated using the data of 1 h incubation. Value of single sample

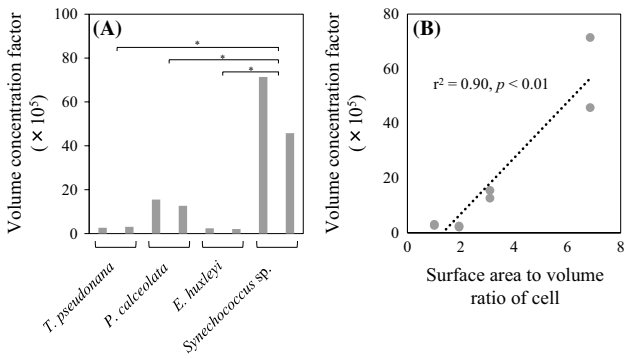

Fig. 1 Volume concentration factors (VCFs) of four microalgal lineages (**a**), and the correlation between the surface area to volume ratio and VCFs of MeHg (**b**). VCFs were calculated using cellular and dissolved MeHg concentration values after a 3 h incubation period. Asterisks denote significant differences among microalgal lineages

relatively higher than those of other lineages, except for *Synechococcus* sp. (ANOVA, $p < 0.005$; Tukey–Kramer post-hoc test, $p < 0.05$, for both 1 h and 3 h incubation samples). In addition, the MeHg VCF of this lineage was significantly higher than those of the other lineages after 3 h incubation (ANOVA, $p < 0.05$; Tukey–Kramer post-hoc test, $p < 0.05$) (Fig. 1 a; Table 1). Indeed, the VCFs of MeHg were positively correlated with the surface area to volume ratio ($r^2 = 0.90$, $p < 0.01$, Fig. 1 b). These results are consistent with the previous reports, which suggest that the cellular MeHg concentration factor of microalgae may be attributed to the surface to volume ratio (Kim et al. 2014; Lee and Fisher 2016). These findings suggest that the prominence of small microalgae, such as Cyanophyceae and Pelagophyceae, may lead to a high MeHg accumulation and removal capacity of marine plankton populations.

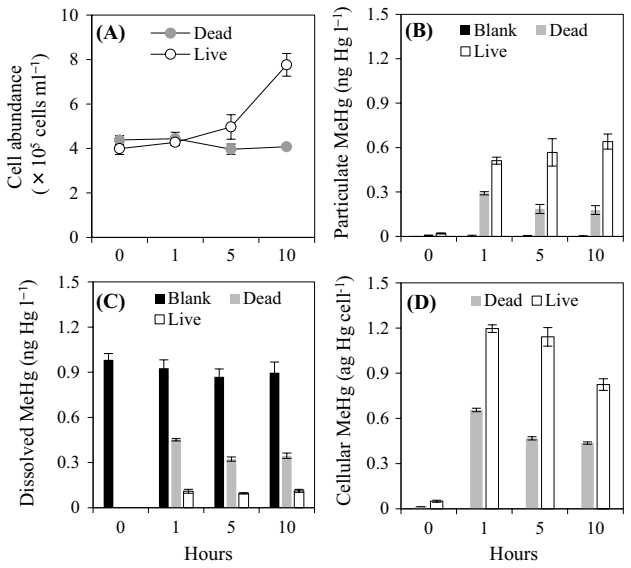

Fig. 2 Cell abundance (**a**), particulate (**b**), dissolved (**c**), and cellular (**d**) MeHg concentration in an incubation experiment with added MeHg using dead and living *Thalassiosira pseudonana* (diatom) cells. Error bars represent standard deviation

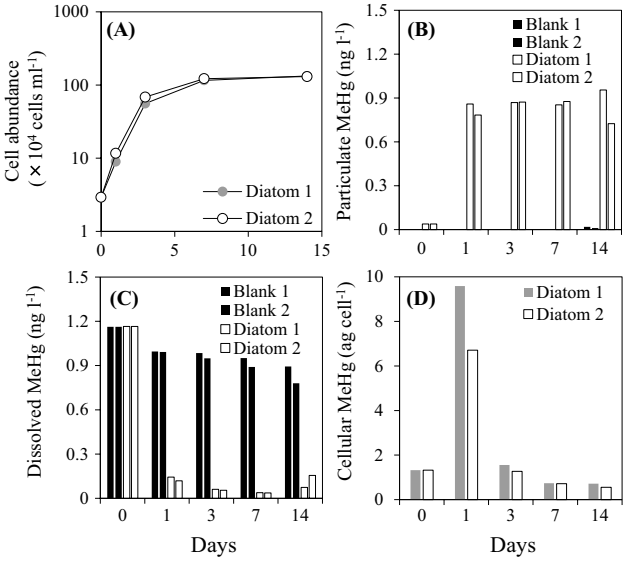

Fig. 3 Cell abundance (**a**), particulate (**b**), dissolved (**c**), and cellular (**d**) MeHg concentration in a long-term incubation experiment with added MeHg using *Thalassiosira pseudonana* (diatom) cells

3.2 MeHg uptake and accumulation by dead and living diatom cells

During the short-term experiment, the abundance of living diatom cells increased and reached a concentration of 7.8×10^5 cells ml^{-1} (average) after a 10 h incubation period, whereas the proliferation of dead cells was not observed (Fig. 2a). This indicated that the freeze–thaw and heat treatment used in this study effectively killed or debilitated the diatom cells.

The particulate MeHg concentrations revealed that the MeHg incorporation of living diatom cells (0.51 ± 0.01 ng Hg l^{-1}) were higher than those of dead cells (0.29 ± 0.02 ng Hg l^{-1}) (Student's *t* test; $p < 0.005$, $n = 3$) within 1 h of incubation (Fig. 2 b). In addition, the dissolved MeHg concentration in the treatments with living cells was lower than that of dead cells (0.11 ± 0.01 ng Hg l^{-1} and 0.45 ± 0.01 ng Hg l^{-1} for living and dead cells, respectively; Student's *t* test; $p < 0.001$, $n = 3$) (Fig. 2c). These findings suggest that the metabolic activity of the cells can affect the MeHg accumulation of the marine microalgal community.

The cellular MeHg concentration in living diatom cells was higher (1.8–3.6 times) than that of dead cells (Student's *t* test; $p < 0.001$, $n = 3$, for each incubation time) (Fig. 2d). These results were consistent with those of previous studies using freshwater microalgae, which demonstrated that MeHg accumulation in living cells was 1.5–5 times higher than that in dead cells (Pickhardt and Fisher 2007). The metabolic activity of microalgal cells should, therefore, be considered as one of the critical factors for the bioaccumulation of MeHg in marine microalgae. In natural marine environments, dead cells account for 20–70% of the total microalgal cells, and this proportion varies among seasons and water bodies (Agustí and Sanchez 2002; Hayakawa et al. 2008; Agustí and Duarte 2013). In addition, microalgal dead cells, as sinking particles, are important for metal transportation from the surface to the ocean depths (Fisher and Wente 1993). These reports suggest that the dead/living cell ratio may be an important factor when evaluating MeHg bioaccumulation and distribution.

Cellular MeHg accumulation was observed in dead diatom cells (41–55% of the living cells) (Fig. 2d), indicating that the MeHg in the dead cells was attributed to passive cellular incorporation (presumably absorption) or the adsorption onto the cell walls of the microalgal cells. In previous studies, adsorbed MeHg concentration of *T. pseudonana* (a different strain) accounted for 30–70% of the total cellular MeHg (Zhong and Wang 2009; Wu and Wang 2011). Another study that investigated freshwater microalgae showed that adsorbed MeHg on the cell walls accounted for 41–96% of the total cellular MeHg (Pickhardt and Fisher 2007). These data indicated that the contribution of MeHg accumulation by adsorption could be larger than that by absorption or uptake. Further experiments on MeHg uptake by dead and living cells of several microalgal lineages will elucidate the precise MeHg absorption, uptake, and adsorption rates of marine microalgae.

In previous studies, most marine microalgal cells passively accumulated MeHg from ambient seawater (Mason et al. 1996; Wu and Wang 2011; Lee and Fisher 2016). However, our experiments with living and dead cells

demonstrated that diatom cells actively took up MeHg. These inconsistencies could be attributed to the use of different MeHg species (cysteine-combined MeHg, a water-soluble species) in this study. Previous experiments with microalgae were conducted using MeHg-Cl, a lipophilic species of MeHg (Mason et al. 1996; Pickhardt and Fisher 2007; Wu and Wang 2011; Lee and Fisher 2016). Mason et al. (1996) highlighted that the high lipophilicity of the chloride mercury complex facilitates the passive uptake of MeHg into membranes. In natural aquatic environments, inorganic Hg or MeHg are normally complexed with dissolved organic matter, such as amino acids (Lamborg et al. 2004; Lee and Fisher 2017). Another study demonstrated that MeHg complexes with organic ligands (e.g., thiols containing amino acids) were more stable than those with inorganic ones, such as OH^-, SO_4^{2-}, and Cl^-, in aqueous solution (Rabenstein 1978). Collectively, the present and previous studies showed that the species and forms of MeHg in the natural seawater can be important factors that govern the active and passive uptake of MeHg by marine microalgae.

3.3 MeHg bioaccumulation by diatom cells

In the long-term incubation experiment, the abundance of diatom cells reached an average of 1.3×10^6 cells ml^{-1} at end of the incubation period (Fig. 3 a). An average of $86.0 \pm 8.2\%$ added MeHg was incorporated into the diatom cells after 1 day of incubation (Fig. 3b,c). Interestingly, the cellular MeHg concentration decreased with an increase in cell abundance (Fig. 3d) (ANOVA, $p < 0.005$; Tukey–Kramer post-hoc test, $p < 0.01$), which suggests that the MeHg inside or outside of the cells could be maintained through cell division. These results are consistent with those of previous reports on the biodilution of MeHg though microalgal proliferation (Pickhardt et al. 2002; Karimi et al. 2007; Lee and Fisher 2016). In this study, the long-term experiment revealed that the incorporated MeHg in the cells was not released for at least 2 weeks. This suggests that bioaccumulated MeHg in marine food webs will not move back to the ambient seawater except via cell lysis.

3.4 Total fraction of MeHg

In our experiments, the total (particulate + dissolved) MeHg concentration, including the control treatments (shown as blank), decreased with incubation time (Fig. S3). We used Teflon bottles to reduce the adsorption of MeHg onto the bottle surface wall. After incubation with *T. pseudonana* for 10 h, the total amount of Hg on the bottle wall ranged from 33 to 67 pg (data not shown), which indicates that the adsorption of MeHg to the Teflon bottles was relatively low. Another possible reason was the photochemical reduction (Hammerschmidt and Fitzgerald 2006) of MeHg attached to the bottle wall, which may have converted MeHg to volatile Hg during incubation. In addition, a recent study demonstrated microbial generation of gaseous elemental Hg from dissolved MeHg in seawater (Lee and Fisher 2019). To evaluate the effect of light and marine microbes on the chemical conversion of MeHg in seawater and its bioaccumulation by microalgae, it is essential to conduct further incubation experiments in dark or completely axenic conditions.

4 Conclusions

In conclusion, the present incubation experiments with several microalgal lineages revealed that *Synechococcus* sp. (with a smaller cell volume) had a higher capacity for MeHg accumulation and removal, and a higher volume concentration factor than those of *T. pseudonana*, *P. calceolata*, and *E. huxleyi*. The comparison of MeHg bioaccumulation between living and dead cells of *T. pseudonana* demonstrated that metabolic activity is important for the bioaccumulation of MeHg in marine microalgae. In addition, long-term experiments revealed that cell-incorporated MeHg is not released during cell division and the stationary phase (for at least 2 weeks). Our incubation experiments indicated that community structure and the metabolic condition of marine microalgae can affect MeHg bioaccumulation in marine food webs. Finally, further experiments using different MeHg species under various conditions are necessary for the precise evaluation of the MeHg uptake and absorption or adsorption ratio of marine microalgal cells.

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Compliance with ethical standards

Conflict of interest The authors declare there are no conflicts of interest.

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