

Reduction of dimethylsulfoxide to dimethylsulfide by marine phytoplankton

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Abstract

Dimethylsulfoxide (DMSO) is an abundant but poorly understood methylated sulfur compound in the marine environment. One potentially significant loss pathway for DMSO is through its biological reduction to dimethylsulfide (DMS), which has been documented in a number of organisms, most notably bacteria. Here we present the first detailed study of DMSO reduction by several marine phytoplankton in axenic cultures. Reduction of DMSO was observed in four algal classes, with *in vivo* reduction rates ranging from 0.006 to 1.5 $\mu\text{mol} [\text{L cell volume}]^{-1} \text{s}^{-1}$ at 1.0 mmol L^{-1} DMSO. Corresponding turnover times for measured intracellular DMSO pools varied from hours to days. Michaelis–Menton kinetic parameters were estimated for *Isochrysis galbana*, *Thalassiosira pseudonana*, and *Amphidinium carterae*. The half-saturation constant (K_m) and maximal rate (V_{max}) for DMSO reduction ranged between 0.96 and 2.7 $\text{mmol} [\text{L cell volume}]^{-1}$ and 17–118 $\text{nmol} [\text{L cell volume}]^{-1} \text{s}^{-1}$, respectively. Our results suggest that DMSO reduction is a universal activity in marine phytoplankton, even in algae with no detectable dimethylsulfoniopropionate (DMSP). Although reduction of DMSO by marine eukaryotes may not contribute significantly to removal of DMSO from the dissolved phase, this reduction is likely to be a major source of DMS in species lacking detectable DMSP lyase activity. The ability of marine phytoplankton to reduce DMSO to DMS should allow algae to cycle these compounds as part of an antioxidant system.

Dimethylsulfoxide (DMSO) is often the dominant dissolved dimethylated sulfur species in the marine euphotic zone, with concentrations of 40–60 nmol L^{-1} in some cases (Simó et al. 1997). The main sources of dissolved DMSO (DMSO_d) are from the photochemical oxidation of dimethylsulfide (DMS) (Brimblecombe and Shooter 1986; Kieber et al. 1996) and the biological oxidation of DMS (Sunda et al. 2002; del Valle et al. 2007a). The biological consumption of DMSO_d is thought

to be very slow in surface seawater (del Valle et al. 2007b), although there are very few direct measurements of this process.

Dimethylsulfoxide has been shown to be associated with particulate matter. Simó et al. (1998) and Hatton and Wilson (2007) detected particulate DMSO (DMSO_p) in several phytoplankton cultures at μmol to $\text{mmol} [\text{L cell volume}]^{-1}$ concentrations. Hatton and Wilson (2007) determined that DMSO_p concentrations were consistently lower than particulate dimethylsulfoniopropionate (DMSP_p), with DMSO_p to DMSP_p ratios ranging from 0.082 to 0.91. Particle-associated production of DMSO was recently shown to be an important source of this compound to the dissolved pool in the euphotic zone of the Ross Sea, Antarctica (del Valle et al. 2007b). This process was stimulated by solar radiation and was proposed to be mediated by phytoplankton.

The physiological roles of DMSO in phytoplankton are poorly understood. DMSO has been hypothesized to perform several functions in algae, including cryoprotection, osmotic pressure regulation, and modification of intracellular electrolytes (Lee and de Mora 1999). However, these functions are not well described in the marine algal literature. DMSO may also be part of an oxidative stress defense mechanism (Sunda et al. 2002). An antioxidant role for DMSO is supported by a strong correlation with β -carotene in seawater, a known scavenger of singlet oxygen (Riseman and Ditullio 2004). DMSO is formed from reactions involving DMS with hydrogen peroxide, the hydroxyl radical or singlet oxygen (Foote and Peters 1971; Amels et al. 1997). The DMSO may react further with the

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Table 1. Axenic algal strains used for DMSO reduction studies. All cultures were obtained from the Provasoli-Guillard Center for the Culturing of Marine Phytoplankton (CCMP) (Boothbay Harbor, Maine).

Species	CCMP strain	Class	Collection site
<i>Thalassiosira oceanica</i>	1005	Coscinodiscophyceae	Sargasso Sea
<i>Thalassiosira pseudonana</i>	1335	Coscinodiscophyceae	Moriches Bay, New York
<i>Thalassiosira pseudonana</i>	1014	Coscinodiscophyceae	North Pacific Gyre
<i>Isochrysis galbana</i>	1323	Prymnesiophyceae	Marine Biological Station, Isle of Man
<i>Emiliana huxleyi</i>	374	Prymnesiophyceae	Gulf of Maine
<i>Rhodomonas lens</i>	739	Cryptophyceae	Gulf Stream (approximate)
<i>Amphidinium carterae</i>	1314	Dinophyceae	Falmouth Great Pond, Massachusetts
<i>Prorocentrum minimum</i>	1329	Dinophyceae	Great South Bay, New York

OH radical (Sunda et al. 2002) or be recycled back to DMS through its reduction. DMSO reductases are widespread in bacteria (see McCrindle et al. 2005 for review). In contrast, enzymes of the DMSO reductase family are unknown in eukaryotes, although the ability to reduce DMSO has been observed in a number of eukaryotes, most notably yeast (Hansen 1999). In eukaryotes, *S*-methionine sulfoxide (MetSO) reductases (MsrA) are thought to be the main enzymes responsible for DMSO reduction to DMS (Bamforth 1980).

Although DMSO reduction by bacteria and a few higher organisms have been examined, relatively little is known about this process in eukaryotic algae. Fuse et al. (1995) showed that five species of marine algae reduced DMSO to DMS over 7 d. They demonstrated that DMSO reduction occurred through a biological process, but they did not provide cell-normalized rates or rule out possible bacterial contamination of cultures as a mechanism for DMSO reduction.

In this study results are presented demonstrating the reduction of cellular DMSO in a number of different marine algal taxa grown under axenic conditions. Enzymatic kinetic parameters are presented, as well as time-resolved rates of DMSO reduction to DMS. A potential enzymatic mechanism for DMSO reduction to DMS is postulated by comparing sequence homology of known DMSO-reducing proteins to proteins encoded in the *Thalassiosira pseudonana* genome. The potential contribution of this activity to the overall DMS budget in the marine ecosystem is discussed, and we speculate on some novel roles for DMSO reduction in marine phytoplankton.

Methods

Chemicals—Unless otherwise noted, all chemicals used in this study were of the highest purity available. The DNA stain, 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), and DMSO ($\geq 99\%$) were purchased from Sigma-Aldrich. DMSP hydrochloride (99.995%) for DMS standards was obtained from Research Plus. Deuterated DMSO (DMSO- d_6 , 99 atom %) was obtained from Cambridge Isotopes. All solutions were prepared using 18.2 M Ω cm Milli-Q[®] water (Millipore Company).

Algal cultures—Axenic algal cultures (Table 1) were purchased from the Provasoli-Guillard Center for the Culturing of Marine Phytoplankton (CCMP). Heterotro-

phic bacterial contamination was monitored by inoculating culture subsamples into a yeast extract-tryptone broth medium and testing for bacterial growth by dark incubation for 3 d at ambient temperature (Andersen et al. 1997). The presence of bacterial cells in cultures was also examined in DAPI-stained filter preparations (Porter and Feig 1980). Cultures were grown in f/2 media, based on Tris-buffered (0.21 mmol L⁻¹ Tris, pH 7.8) artificial seawater (Goldman and McCarthy 1978), and grown under fluorescent lighting with a 14 : 10 light : dark cycle (120 μ mol quanta m⁻² s⁻¹ total irradiance). Diatom cultures were supplemented with 0.1 mmol L⁻¹ silica. All cultures except *Isochrysis galbana* and *T. pseudonana* CCMP 1014 were maintained at 22°C in 125-mL polycarbonate Erlenmeyer flasks. *I. galbana* and *T. pseudonana* CCMP 1014 were maintained at 14°C. Culture cell densities were determined with a Beckman-Coulter Z2 Particle Counter using 1% NaCl buffered with 50 mmol L⁻¹ sodium phosphate (pH 7.8) as the electrolyte diluent. Cells were diluted by a factor of 50 (200 μ L of culture into 10 mL of diluent). The buffered saline solution was filtered through a 0.2- μ m Polycap[™] 36 AS filter (Whatman) prior to use.

DMSO reduction experiments—For most experiments, triplicate 1-mL culture aliquots in mid- to late-exponential phase (approximately 10⁵–10⁶ cells mL⁻¹) were aseptically dispensed into sterile 20-mL crimp-top serum vials. Aliquots were amended with microliter additions of aqueous DMSO to final concentrations ranging from 0.01 to 10 mmol L⁻¹. Treated vials were sealed with Teflon-faced butyl rubber septa and placed in the incubator under the same conditions as used for growth of the cultures (see above). Cultures were generally sampled in the morning (approximately 3 h after the start of the light cycle) and allowed to sit in the incubator after addition of DMSO for 24 h before analysis. Controls consisted of 1-mL aliquots of a culture without added DMSO, 1 mL of autoclaved (25 min at 121°C and 1.22 atm) culture with or without added DMSO, and sterile media with DMSO.

For isotope labeling experiments, 20 μ L of 0.5 mol L⁻¹ aqueous DMSO- d_6 was added to duplicate 1-mL aliquots of a culture in 7-mL crimp-top vials and incubated for 2–6 d to ensure sufficient production of DMS- d_6 in the headspace. Cultures used for these experiments were taken from the early exponential phase. Controls consisted of cultures without added DMSO- d_6 and media with added DMSO- d_6 but without phytoplankton cells.

Sulfur analyses—DMS was measured using a Shimadzu gas chromatograph (GC-14A) equipped with a 2.4 m long \times 3.2 mm inner diameter (i.d.) Teflon column packed with Chromosil 330 (Supelco) and a flame photometric detector (FPD). The column was held at 60°C, with the injector and detector both set at 225°C. Under these conditions, DMS eluted at 1.4 min. DMS standards were prepared by adding 1 mL of 1 mol L⁻¹ NaOH to 20 μ L aqueous DMSP standards in sealed serum vials. Standards were maintained at the same temperature, volume, and approximate ionic strength as samples. The detection limit for this method with a signal-to-noise ratio of two was approximately 0.2 pmol DMS for an injection of 500 μ L of headspace.

Particulate DMSP was measured following capture of cells on a filter and subsequent addition of base to convert DMSP to DMS (Dacey and Blough 1987). All cultures (10 mL) were gravity filtered onto a 25-mm diameter GF/F filter (Whatman). Each filter was placed into a 20-mL crimp-top serum vial followed by the addition of 1 mL of 1 mol L⁻¹ NaOH. The vial was immediately sealed and allowed to incubate for at least 24 h in the dark at room temperature. Headspace injections of 100–500 μ L were then made using the GC-FPD as described above.

Unless otherwise noted, DMSO_p was calculated as the difference between DMSO measured in the total and dissolved culture samples by the TiCl₃ reduction technique (Kiene and Gerard 1994). Dissolved fractions were collected by gravity filtration of 10 mL of culture through a precombusted 25-mm diameter GF/F filter, of which only the first 3–4 mL was collected. Precombustion of the filters removed adsorbed DMSO (Kiene and Gerard 1994) but slightly reduced the nominal pore size of the filters (H. Loisel pers. comm.). However, this change is not sufficient to alter the retention of the much larger phytoplankton cells used in this study. For both total and dissolved DMSO, 1 mL of culture or filtrate was placed into a 20-mL crimp-top serum vial and 200 μ L 20% TiCl₃ was added. Sealed vials were incubated for 1 h at 55°C and then cooled to room temperature. Vials were sparged with ultra high purity (UHP) He for 4 min and the DMS collected on a cryotrap prior to injection into the GC (Kiene 1993). For dinoflagellate cultures, total and dissolved DMSO concentrations were sufficiently high to allow for detection from a 200–500 μ L injection of headspace rather than from the customary purge and trap method.

Isotopically labeled DMS-d₆ was quantified with a ThermoElectron Focus gas chromatograph equipped with a Thermo Polaris Q Mass Spectrometer (ThermoElectron) using a 30-m SPB-1 sulfur column (Supelco; 0.32 mm i.d., 4- μ m film thickness). The column was held at 32°C for 3 min, heated to 150°C at 20°C min⁻¹ and held at 150°C for 5 min. The injector and mass spectrometer transfer lines were both held at 170°C. The UHP He carrier gas was set to a flow rate of 2.0 mL min⁻¹. Mass spectra were averaged over the peak area (4.9–5.3 min, 43 scans). Injections were 300 μ L of culture headspace. Mass spectra were collected both in full scan (*m/z* 45–100) and in selected ion monitoring (*m/z* 50 and 68) modes, with a scan time of 0.51 s. The instrumental detection limit for DMS with a

signal-to-noise ratio of 2 was 0.4 pmol per 500- μ L injection of headspace.

Base addition to total DMSO samples from cultures was performed by addition of 100 μ L 5 mol L⁻¹ NaOH to 1 mL of sample. Samples were set aside in the dark for 24 h and sparged with UHP He to remove DMS. We added 100 μ L 5 mol L⁻¹ HCl, lowering the pH to approximately 4. Samples were then analyzed for DMSO employing the titanium chloride method.

Protein alignments—To determine a possible enzymatic mechanism by which DMSO was reduced to DMS in our cultures, basic local alignment search tool protein (BLASTP) algorithms were used to identify proteins from the *T. pseudonana* genome with high homology to either the *Saccharomyces cerevisiae* MsrA or *Rhodobacter sphaeroides* DMSO reductase. The *T. pseudonana* genome sequence was available from the Joint Genome Institute (JGI, <http://genome.jgi-psf.org/Thaps3/Thaps3.home.html>). *S. cerevisiae* sequences were accessed from an online genome library (<http://www.yeastgenome.org>). *Arabidopsis thaliana* sequences were obtained from Romero et al. (2006). The *R. sphaeroides* DMSO reductase sequence was obtained from Hille (1996). Protein sequences were downloaded in Fast-All (FASTA) format. Multiple sequence alignments were performed using the Clustal W algorithm.

DMSO uptake model—A kinetic uptake model was used to estimate the amount of time for the uptake of dissolved DMSO into algal cells and its equilibration with DMSO_d. The model used here was derived from a study by Tanaka et al. (2001) using the freshwater alga *Chlorococcum texanum*. Tanaka et al. (2001) determined model parameters using 300 or 500 mmol L⁻¹ sucrose and 200 mmol L⁻¹ DMSO as osmolytes. Tanaka et al. (2001) used these data to calculate values for model parameters including the reflection coefficient ($\sigma = 0.87$), hydraulic conductivity ($L_p = 5.82 \times 10^{-15}$ N s), and solute permeability ($\omega = 1.75 \times 10^{-17}$ mol N⁻¹ s⁻¹).

In the present study, all parameters were either calculated from the constants listed above or directly measured (i.e., cell volume and temperature). The model was run for *I. galbana*, and it was assumed that the cell volume (128 fL, mean value for *I. galbana*, Table 2) was constant throughout the equilibration of DMSO, and that DMSO and water were the only species that exchanged across the cell membrane.

Results

DMSO uptake model—For this study, mmol L⁻¹ levels of DMSO were added to the dissolved phase of the algal cultures to study the in vivo cellular reduction of DMSO. Therefore, DMSO uptake was modeled to ensure that the relative timescale of DMSO uptake by the algae was substantially shorter than its cellular reduction. The DMSO uptake model suggested that intracellular DMSO equilibrated with DMSO_d in our experiments within 15 s of the application of exogenous DMSO to axenic cultures of *I. galbana* (Fig. 1), and this process was much faster than the

Table 2. Cellular DMSO and DMSP concentrations, cell volumes, and cellular DMSO_p:DMSP_p ratios determined for axenic algal strains used in this study. DMSP concentrations represent the mean, plus or minus the range of duplicate samples, and DMSO concentrations are given as the mean, \pm SD of triplicate samples. All concentrations are in units of mmol [L cell volume]⁻¹. Cell volumes represent the mean, \pm SD, of triplicate measurements. BDL = below detection limit.

Species	DMSO _p	DMSP _p	Cell volume (fL cell ⁻¹)	DMSO _p :DMSP _p
<i>T. oceanica</i> 1005	0.86 \pm 0.20	2.8 \pm 0.6	166 \pm 10	0.303
<i>T. pseudonana</i> 1335	0.034 \pm 0.007	39.4 \pm 8.3	171 \pm 28	0.00085
<i>T. pseudonana</i> 1014	0.065 \pm 0.043	10.7 \pm 3.7	41.7 \pm 0.8	0.0061
<i>A. carterae</i> 1314	0.90 \pm 0.36	109 \pm 15	714 \pm 7	0.0082
<i>P. minimum</i> 1329	8.4 \pm 0.58	167 \pm 4	1173 \pm 37	0.050
<i>R. lens</i> 739	BDL	BDL	316 \pm 29	BDL
<i>E. huxleyi</i> 374	1.3 \pm 0.66	219 \pm 37	57.1 \pm 0.3	0.0060
<i>I. galbana</i> 1323	0.069 \pm 0.002	129 \pm 41	128 \pm 5	0.00053

DMSO reduction (\sim hours); similar results were obtained for the other algal species examined in this study. Increasing the cell size lengthens the equilibration time (\sim 5 min at 700 fL), though not significantly relative to the time scale for DMSO reduction (data not shown). The first-order rate constant for uptake, estimated from the kinetic model, was 0.4–0.5 s⁻¹, while that for enzymatic reduction was orders of magnitude smaller (10⁻⁵–10⁻⁴ s⁻¹), as estimated from Michaelis–Menton kinetics (see below).

Particulate DMSP and DMSO concentrations—DMSP_p and DMSO_p were measured for all strains tested (Table 2). Diatoms had low DMSO_p concentrations, ranging from 0.034 to 0.857 mmol [L cell volume]⁻¹. Dinoflagellates had high concentrations of both DMSO_p and DMSP_p (e.g., 0.9 mmol [L cell volume]⁻¹ and 109 mmol [L cell volume]⁻¹, respectively, for *Amphidinium carterae*; Table 2). Bacteria were absent from all strains used in this study as judged by lack of detectable bacterial cells by DAPI staining and lack of bacterial growth in the yeast extract-tryptone medium after inoculation with algal culture prior to the initiation of DMSO reduction experiments.

DMSO reduction activity—In vivo reduction of 1.0 mmol L⁻¹ DMSO to DMS was observed in all seven DMSP-containing axenic algal strains, as well as one strain with no detectable DMSP_p (Fig. 2). No significant differences were found in the cell density for cultures amended with 1.0 mmol L⁻¹ DMSO vs. cultures without added DMSO (data not shown). Additionally, over the course of the reduction experiments, algal growth rates in the serum vials were the same as growth rates observed in the batch cultures grown in 125-mL polycarbonate Erlenmeyer flasks. The highest concentration of DMSO added in our experiments (10 mmol L⁻¹) did not inhibit cell growth over the course of 6 d (data not shown). This is not surprising, since DMSO is generally considered nontoxic at the levels we used. Okumura et al. (2001) tested marine algae for DMSO toxicity and found no observable effects in *I. galbana* at 7.4 mmol L⁻¹ (95% confidence interval ranged from 3.3 to 15.8 mmol L⁻¹) and at 89.7 mmol L⁻¹ for *Prorocentrum minimum* (95% confidence interval ranged from 57.7 to 141 mmol L⁻¹). Thus, disruption of the normal growth of the algae is not

expected over the duration of the experiments presented here.

On average, dinoflagellates showed the highest net DMS production rates while prymnesiophytes had the lowest rates. Diatoms showed a high degree of variability between the species tested. *Thalassiosira oceanica* exhibited the highest net rate of reduction (1.5 \pm 0.2 μ mol [L cell volume]⁻¹ s⁻¹) of all species tested, whereas *Rhodomonas lens* had the lowest net rate of 0.0056 \pm 0.0017 μ mol [L cell volume]⁻¹ s⁻¹ (Fig. 2). Over the time course of growth, cell-specific DMSO reduction activity did not change significantly, remaining approximately 0.017–0.026 μ mol [L cell volume]⁻¹ s⁻¹ for *I. galbana* and 0.08–0.16 μ mol [L cell volume]⁻¹ s⁻¹ for *A. carterae* (Fig. 3A,B). Autoclaved cultures showed no DMSO reduction, with no increase in DMS concentrations observed after 24 h in either DMSO-amended samples or non-amended samples (data not shown).

Confirmation of the source of the DMS was obtained by addition of DMSO-*d*₆ to axenic cultures. Gas chromatography–mass spectrometry (GC-MS) analysis of the result-

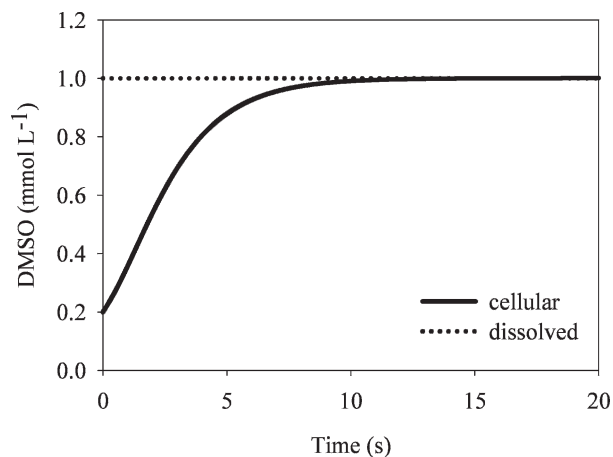


Fig. 1. Modeled time response of intracellular DMSO concentration in *I. galbana* to added 1 mmol L⁻¹ dissolved, extracellular DMSO. The model assumes a cellular volume of 130 fL, an initial DMSO_p concentration of 200 μ mol L⁻¹, and no loss of DMSO_p. Model parameters are based on Tanaka et al. (2001) as described in the Methods section.

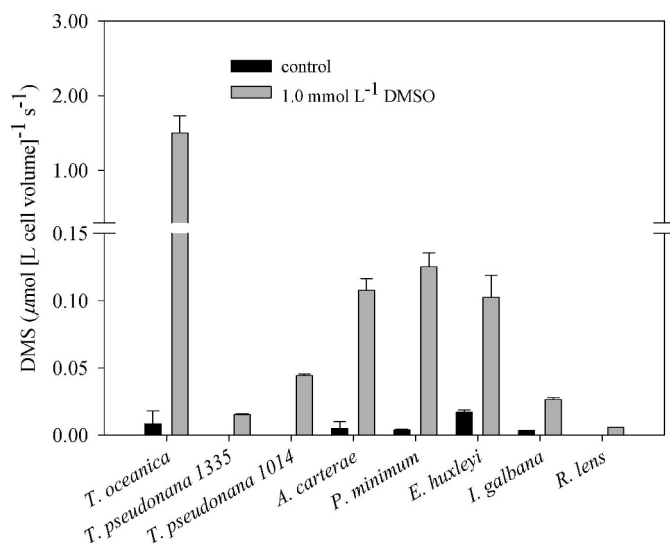


Fig. 2. Reduction of DMSO to DMS in axenic algal cultures after 24 h incubation in *f/2* media with 1.0 mmol L⁻¹ DMSO added (gray bars) or no additional DMSO added (black bars). Error bars represent the standard deviation (SD) of the analysis of triplicate samples. No DMS formation was observed in media or autoclaved controls. Cell volumes and ambient concentrations of particulate DMSO in the different cultures are reported in Table 2.

ing DMS after 2–6 d showed a single peak at the same retention time as an authentic DMS standard. Mass spectral data (Fig. 4) showed a molecular ion peak at m/z 68 (CD₃SCD₃) and fragments at m/z 50 (CD₃S), 48, and 46. The fragmentation pattern is consistent with the production of DMS-*d*₆ by the culture and its accumulation in the headspace. For strains where DMSP lyase activity was not detected (DLA-) (*T. oceanica*, *T. pseudonana* CCMP 1335 and CCMP 1014, and *I. galbana*; Niki et al. 2000; Harada 2007), GC-MS analysis showed that 92.6–96.8% of the total DMS production was due to the reduction of DMSO (Table 3). No peak associated with deuterated DMS was observed in media or culture controls. Strains with lyase activity (DLA+, *Emiliana huxleyi*, *P. minimum*, and *A. carterae*) showed significantly lower relative production of DMS from DMSO reduction (64.3–81.6%) compared with DLA- strains, presumably due to production of DMS from cellular DMSP.

In most strains, the quantity of nondeuterated DMS produced after addition of labeled DMSO was higher than could be accounted for by background DMS production (control values) and the amount of unlabeled DMSO present in the added substrate. For DLA+ strains, the unlabeled DMS excess was 18.4–35.7% of the total DMS production (mean of 27.1%, $n = 3$), while DLA- strains showed much lower percentage of excess unlabeled DMS, ranging from 3.2% to 7.4% of total production (mean of 6%, $n = 3$).

Both *I. galbana* and *T. pseudonana* CCMP 1335 showed a lag between the addition of exogenous DMSO and the first appearance of DMS in the headspace (Fig. 5A,B). The time delay between dosing and the detection of DMS in the headspace is likely due to the buildup of DMS to detectable

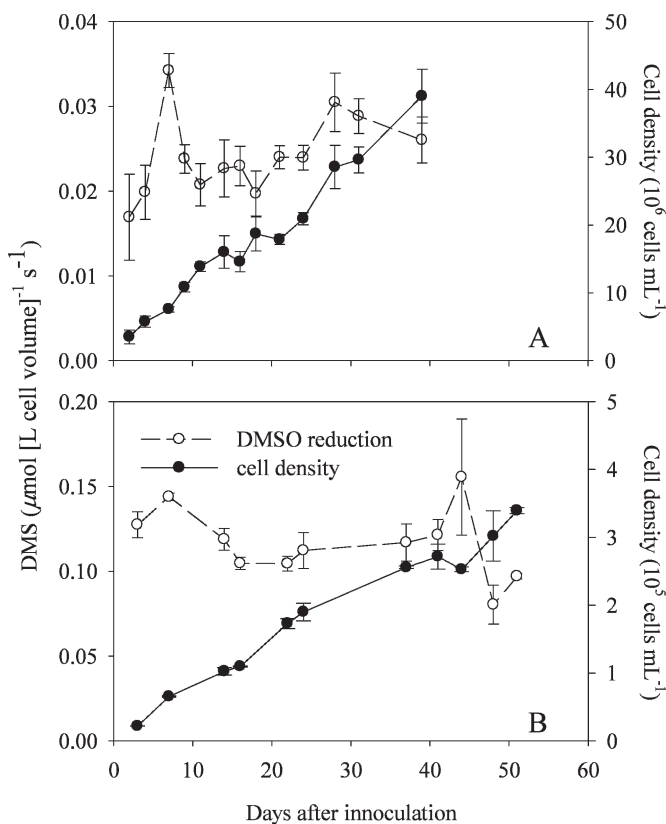


Fig. 3. Reduction rates of DMSO to DMS and cell densities in axenic cultures of (A) *I. galbana*, and (B) *A. carterae* during growth. Data points denote the mean value and vertical bars represent the SD of triplicate cell density samples and the range of duplicate DMS reduction rate measurement. For each time point during growth, duplicate DMSO reduction samples were taken from each batch culture and incubated with 10 mmol L⁻¹ added DMSO for 24 h as described in the Methods section. Cell volumes for both species are reported in Table 2.

levels in the headspace, rather than induction of the activity in the culture, although the latter cannot be ruled out.

Michaelis–Menton kinetics were determined for several phytoplankton strains (Fig. 6), and estimates of Michaelis–Menton kinetic constants for DMSO reduction were derived (Table 4). Values reported here are first approximations that reflect the overall process of DMSO uptake and reduction. *I. galbana* and *T. pseudonana* CCMP 1335 showed approximately the same maximal rate for DMSO reduction, with V_{\max} estimates (mean \pm standard error) of 29 ± 2 nmol [L cell volume]⁻¹ s⁻¹ and 17 ± 2 nmol [L cell volume]⁻¹ s⁻¹, respectively. However, the observed half-saturation constants differed by more than a factor of two, with K_m estimates of 0.96 ± 0.32 mmol L⁻¹ for *I. galbana* and 2.0 ± 0.7 mmol L⁻¹ for *T. pseudonana* CCMP 1335. *T. pseudonana* CCMP 1014 had approximately the same K_m (2.7 ± 1.1 mmol L⁻¹) but a much higher V_{\max} (55 ± 9 nmol [L cell volume]⁻¹ s⁻¹). Compared with these three species, *A. carterae* had a similar K_m (1.6 ± 0.4 mmol L⁻¹) and a much higher V_{\max} (118 ± 6 nmol [L cell volume]⁻¹ s⁻¹). Observed K_m values for *T. pseudonana* (both strains) and *A. carterae* were comparable with that reported for the

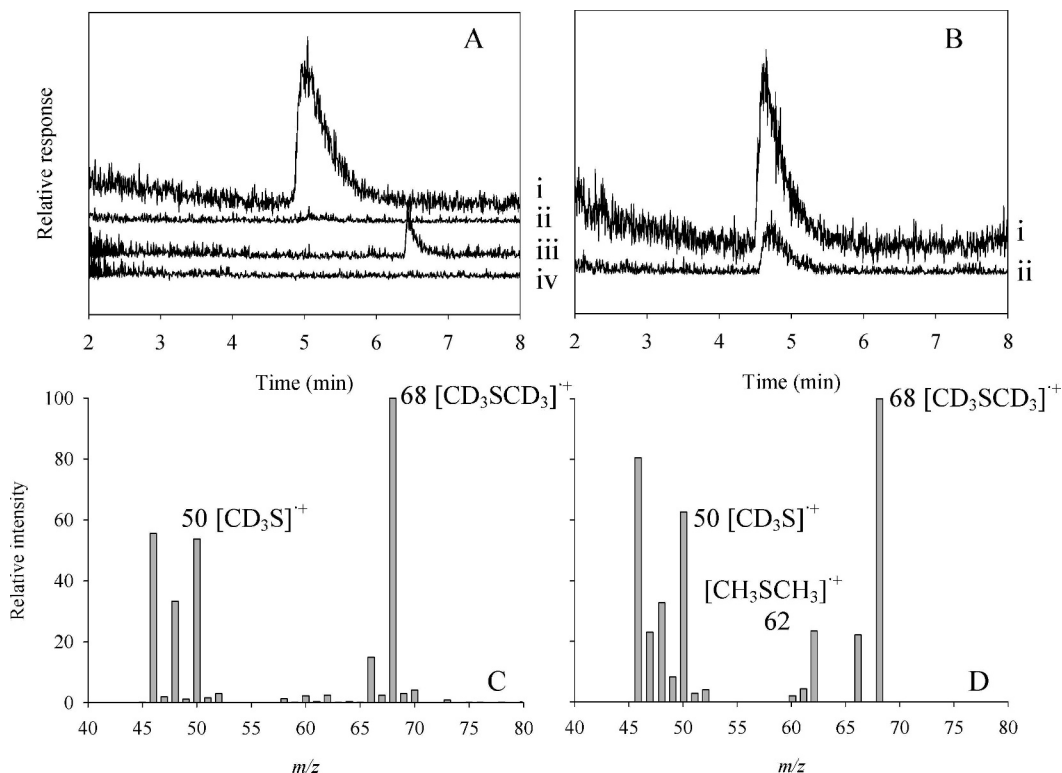


Fig. 4. GC-MS of DMS- d_6 produced by (A and C) *T. pseudonana* CCMP 1335 and (B and D) *E. huxleyi* CCMP 374. Representative chromatograms are shown in panels A and B for (i) extracted ion for m/z 68 in cultures with 10 mmol L $^{-1}$ added DMSO- d_6 , (ii) extracted ion for m/z 62 in cultures with added DMSO- d_6 , (iii) culture without added DMSO- d_6 , and (iv) media with 10 mmol L $^{-1}$ DMSO- d_6 , in (A) *T. pseudonana* and (B) *E. huxleyi*. Panels C and D depict mass spectra of DMS from GC-MS trace i for *T. pseudonana* and *E. huxleyi*, respectively.

reduction of MetSO by *Escherichia coli* (1.9 \pm 0.2 mmol L $^{-1}$; Boschi-Muller et al. 2001) but approximately an order of magnitude higher than K_m for *S. cerevisiae* K_m (0.2 mmol L $^{-1}$ for MetSO reduction, Black et al. 1960).

Discussion

Occurrence of DMSO reduction in phytoplankton—All eight algal strains tested here reduced DMSO to DMS, including *R. lens*, a non-DMSP and non-DMSO producing

Table 3. Contribution of DMSO reduction to total DMS production in axenic algal strains. The relative reduction rate is the ratio of abundance of DMS- d_6 to total DMS (CD_3SCD_3 and CH_3SCH_3) in the headspace of culture vials after 24 h exposure of algae to 10 mmol L $^{-1}$ DMSO- d_6 . Values are the mean of duplicate treatments \pm the range of values. Numbers following the species name correspond to the CCMP strain number.

Species	% contribution of DMSO reduction to total DMS production
<i>T. oceanica</i> 1005	92.6 \pm 8.1
<i>T. pseudonana</i> 1335	96.8 \pm 2.0
<i>I. galbana</i> 1323	92.6 \pm 0.7
<i>E. huxleyi</i> 374	64.3 \pm 3.1
<i>A. carterae</i> 1314	81.6 \pm 3.3
<i>P. minimum</i> 1329	72.8 \pm 5.0

strain. Autoclaved cultures and cell-free controls showed no DMS production from added DMSO, suggesting that the reduction we observed in live cells was biologically catalyzed. Taking our results together with those of Fuse et al. (1995), DMSO reduction is now known to occur in 13 strains across six classes of marine eukaryotic phytoplankton. The fact that this process has been observed in several algal taxa strongly suggests that this is a universal activity in marine phytoplankton. The widespread occurrence of this process in phytoplankton is perhaps not very surprising given that several eukaryotic taxa possess an analogous activity and a wide variety of enzymes are known to reduce DMSO.

The quantitative results presented in this study confirm the qualitative results of Fuse et al. (1995) and provide details regarding the cellular rates and kinetic constants for this process. Rates determined by Fuse et al. lack normalization to cellular parameters such as cell number, cell volume, or chlorophyll content, thereby precluding direct comparisons with our results. It is important to note that DMSO reduction activity was found in species known to produce large quantities of DMSP (e.g., *A. carterae* and *P. minimum*) as well as species known to produce little or no detectable DMSP (e.g., *R. lens*). While DMSO reduction activity for each algal species showed good agreement with a Michaelis-Menton kinetic model, no correlation was observed between DMSO reduction activity and cellular concentrations of DMSO (or DMSP) among the different

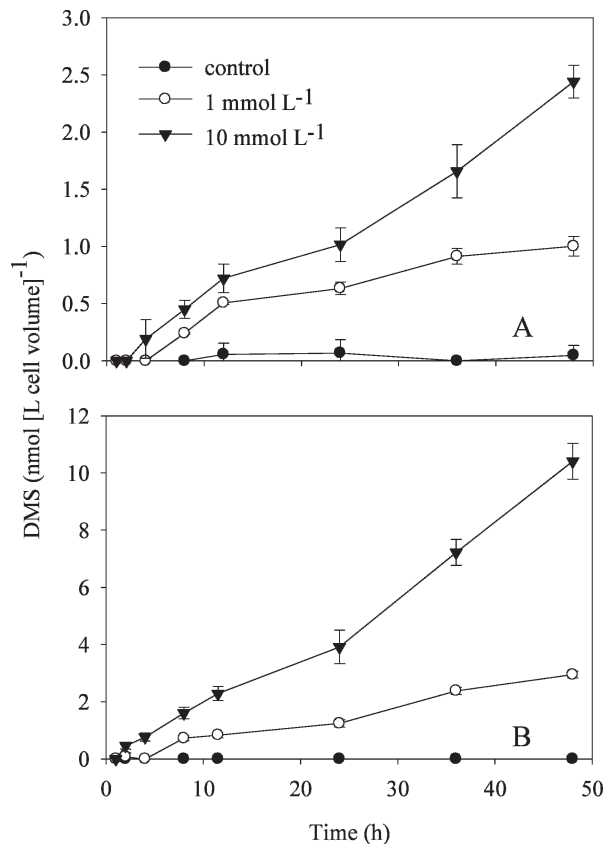


Fig. 5. Time course of DMSO reduction to DMS in (A) *I. galbana* and (B) *T. pseudonana* CCMP 1335 with 1 and 10 mmol L⁻¹ exogenous DMSO. Data points represent the mean value of triplicate samples. Error bars are 1 SD. The cell volume (mean \pm SD) for *I. galbana* is 146 ± 25 fL and 145 ± 11 fL for *T. pseudonana* CCMP 1335.

algal species tested (data not shown). This finding suggests that DMSO is not involved in the regulation of the enzyme(s) involved in its reduction, and DMSO reduction in algae is not mediated by a DMSO-specific reductase.

Putative mechanism—Although no mechanism can be assigned with certainty for the DMSO reduction activity, a putative enzyme system can be hypothesized. No homolog to the *R. sphaeroides* DMSO reductase was found in the *T. pseudonana* genome, indicating that the enzyme was not of the same family and thus not a DMSO reductase or a trimethylamine-*N*-oxide reductase. Four proteins were identified in the genome of *T. pseudonana* CCMP 1335 with high homology (*E* value $< 5 \times 10^{-6}$) to the *S. cerevisiae* *S*-methionine sulfoxide reductase (MsrA) protein, which reduces methionine sulfoxide found in peptides. MsrA in *A. thaliana* has been shown to reduce *S*-methionine sulfoxide in both the free and peptide-bound forms (Boschi-Muller et al. 2001). In *S. cerevisiae*, MsrA is thought to reduce DMSO (Bamforth 1980), and the removal of this gene has been shown to eliminate the production of DMS from DMSO (Hansen 1999). The analogous *T. pseudonana* proteins (TPMSRA1, TPMSRA 2, TPMSRA3, and TPMSRA4) were aligned with MsrAs

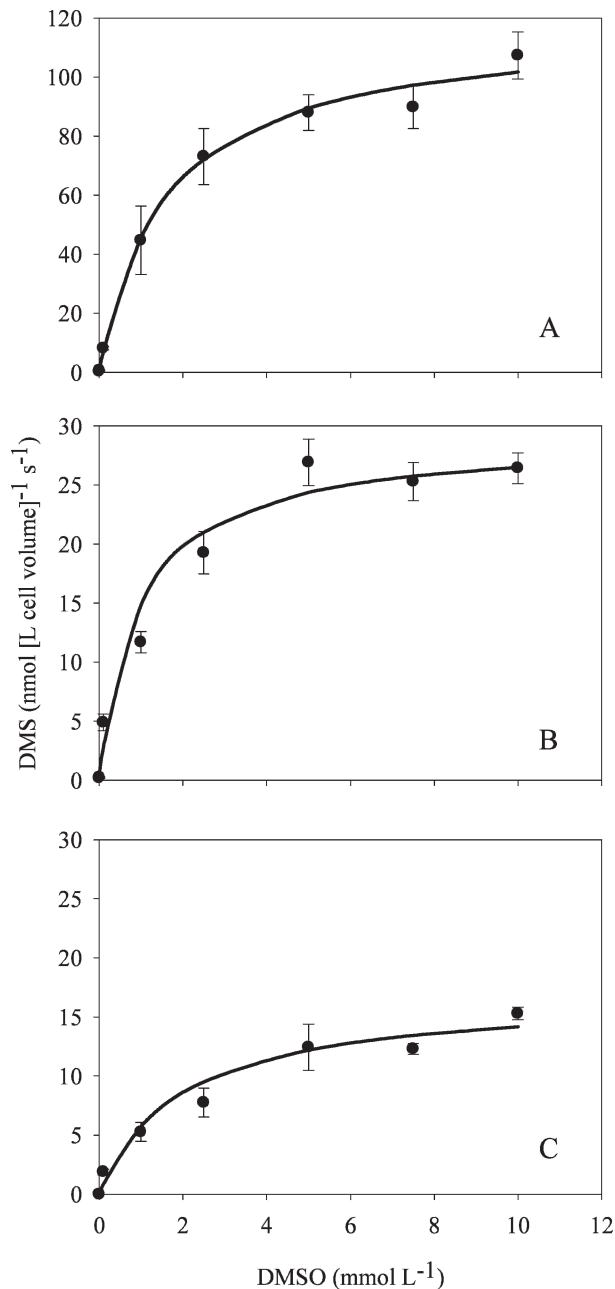


Fig. 6. Concentration dependence of DMSO reduction in (A) *A. carterae*, (B) *I. galbana*, and (C) *T. pseudonana* CCMP 1335. Data points represent the mean value and error bars denote SD of triplicate samples. Solid lines denote the theoretical rate of reduction, given K_m and V_{max} values in Table 4. Cell volumes (mean \pm SD) for *A. carterae*, *I. galbana*, and *T. pseudonana* CCMP 1335 were 978 ± 126 fL, 103 ± 8 fL, and 129 ± 13 fL, respectively, and did not change significantly with increasing DMSO concentration. Results for *T. pseudonana* CCMP 1014 are not shown here, but the Michaelis–Menton results for this species are given in Table 4.

from *A. thaliana* using the Clustal W algorithm (Fig. 7). All putative MsrAs except for TPMSRA3 showed the complete conserved catalytic domain, GCFWG (shaded in Fig. 7). The lack of the cysteine (Cys) residue in the catalytic site sheds doubt on the ability of TPMSRA3 to catalyze the

MetSO added at 10 mmol L⁻¹ did not inhibit DMSO reduction (data not shown). This dissolved concentration should be sufficient to fully saturate any transporters present on the cell surface while still maintaining the intracellular concentration of MetSO sufficiently low as to not inhibit intracellular MsrA enzymes. DMSO, on the other hand, is known to be freely diffusible across membranes (Narula 1967) and is expected to rapidly equilibrate with the dissolved phase. This same diffusibility ensures that regardless of the site of production, DMSO will be distributed throughout all subcellular compartments such that the localization of the putative MsrA is irrelevant to the ability of the cell to reduce its own endogenous DMSO. Based on this evidence, we conclude that most of the DMSO reduction activity we observed occurred intracellularly.

Particulate DMSO and DMSP—The DMSP concentrations reported here are similar to published concentrations in these algal species (Keller et al. 1989; Keller et al. 1999; Hatton and Wilson 2007). However, the cellular DMSO concentration we determined in *A. carterae* using the TiCl₃ method (0.9 mmol [L cell volume]⁻¹) is approximately a factor of six lower than the 5.4 mmol [L cell volume]⁻¹ obtained by Simó et al. (1998) with the NaBH₄ method, assuming a per cell volume of 715 fL. Likewise, our value is a factor of four lower than the 6.12 mmol [L cell volume]⁻¹ obtained by Hatton and Wilson (2007) with the enzymatic reduction method. Because of this discrepancy in intracellular DMSO concentrations, our DMSO_p:DMSP_p ratio for *A. carterae* (0.008, Table 2) is approximately six times smaller than the 0.04 reported by Simó et al. (1998). Similarly, our ratios for dinoflagellates (mean of 0.03) are about 5-fold lower than those reported by Hatton and Wilson (2007) for dinoflagellates (mean of 0.14). Ratios observed in our study for diatoms and prymnesiophytes were also significantly lower (0.103 and 0.0033, respectively) compared with corresponding average values (0.233 and 0.227, respectively) published by Hatton and Wilson (2007). Although differences could be attributed to the use of different strains or to lower light doses for growth, they are more likely due to methodological differences. Tests with *I. galbana* cultures showed that the direct treatment of unfiltered samples with TiCl₃ consistently resulted in lower particulate DMSO concentrations compared with that obtained with the NaBH₄ reduction method of Simó et al. (1998) (C. Spiese unpubl. results). This can explain why our DMSO_p concentrations are consistently lower than those reported by Simó et al. (1998). Direct comparisons of our approach with the enzymatic reduction method showed good agreement when unfiltered culture samples were not treated with alkali prior to the reduction step (Brinkley 2008). However, the addition of alkali (200 μL 5 mol L⁻¹ NaOH) to 1 mL of unfiltered algal culture samples (*I. galbana*) prior to TiCl₃ reduction increased total DMSO concentrations from 90 ± 10 nmol L⁻¹ DMSO (without base addition) to 280 ± 110 nmol L⁻¹ DMSO ($p < 0.01$, $n = 5$ samples per treatment). Together these results suggest that the source of the higher DMSO concentrations in unfiltered culture

samples is the basification step and not the reduction reaction. Although the origin of this difference is unknown, this is likely why Hatton and Wilson (2007) observed higher particulate DMSO concentrations than we observed here for the same algal species; they added 1 mL 10 mol L⁻¹ NaOH and 19 mL 50 mmol L⁻¹ Tris buffer to a vial containing a filter through which 20–50 mL of sample had been passed, then sealed and incubated the sample for 24 h prior to neutralization to pH 7.0 and reduction by the enzymatic technique.

Significance of DMSO reduction—In vivo DMS production rates from DMSO reduction were estimated for two species (*I. galbana* and *A. carterae*) using the Michaelis–Menton kinetic parameters (Table 4) and measured intracellular DMSO concentrations. Calculated DMS production rates were 0.021 and 2.62 fmol cell⁻¹ d⁻¹, respectively. These rates are comparable to or slower than DMS production rates reported for similar species. In particular, *Hymenomonas carterae* (now reassigned as *Pleurochrysis carterae* Class Prymnesiophyceae) produced 1.3 ± 0.1 fmol DMS cell⁻¹ d⁻¹ (Vairavamurthy et al. 1985), similar to our rates. Dacey and Wakeham (1986) reported a DMS production rate of 23 ± 16 fmol cell⁻¹ d⁻¹ for *Gymnodinium nelsoni* (now reassigned as *Akashiwo sanguinea* Class Dinophyceae), which is an order of magnitude or more higher than our estimated in vivo rates for *I. galbana* or *A. carterae*. Growth conditions for *P. carterae* were approximately the same as for the cultures used in our study, while *G. nelsoni* was grown at a slightly lower light intensity and lower temperature. An implicit assumption of these earlier studies is that DMSP was the main source of the DMS. Our results suggest that DMSO_p reduction may have been an important, if not main, source of DMS in these algal species.

As noted above, the main source of DMS in algae is generally assumed to be from the enzymatic cleavage of DMSP, since rates of abiotic DMSP hydrolysis are very slow (Dacey and Blough 1987). Our results suggest that DMSP may not always be the main precursor of DMS; the main source of DMS in some DMSP-containing algae may be due to the cellular reduction of DMSO. With the exception of the dinoflagellates (*A. carterae* and *P. minimum*) and *E. huxleyi* CCMP 374 (which has been shown to have very low potential lyase activity (<0.01 fmol cell⁻¹ min⁻¹; Wolfe et al. 1997)), no strain tested in our study had measurable DMSP lyase activity (Niki et al. 2000; Harada 2007). With no measurable lyase activity and at a DMSP_p concentration typical of *I. galbana* (129 mmol [L cell volume]⁻¹, Table 2), the rate of abiotic DMSP hydrolysis to form DMS (4.9 μmol [L cell volume]⁻¹ d⁻¹ at pH 7) is approximately 30 times slower than our calculated rate of DMS production (165 μmol [L cell volume]⁻¹ d⁻¹) from DMSO reduction at the DMSO_p concentration measured in *I. galbana* (0.069 mmol [L cell volume]⁻¹, Table 2).

For species with significant lyase activity, it is difficult to compare rates of cellular DMS production, most notably because of the lack of estimates of V_{\max} and K_m for DMSP lyase in most algal species. At saturating levels of DMSP, *A. carterae* had a maximum DLA of 86.5 mmol [L cell

volume] $^{-1}$ d $^{-1}$ (Harada 2007). In contrast, the maximum DMSO reduction rate we observed was 10.1 mmol [L cell volume] $^{-1}$ d $^{-1}$ at 10 mmol L $^{-1}$ exogenous DMSO (Fig. 6). This would suggest that DMSO reduction is less significant than DLA as a source of DMS in high lyase species. However, without better in vivo estimates of the relative contributions of these two processes, a more realistic comparison cannot be made. In general for those algae with little or no lyase activity, DMSO reduction is likely the main source of DMS in the cell. Furthermore, based on our results with *R. lens*, we suggest that even algae with no DMSP can act as a source of DMS through reduction of DMSO that diffuses into the cell, and this may be one reason why DMS is ubiquitous in oceanic surface waters.

Turnover of DMSO—From the in vivo DMSO reduction data presented in our study, turnover times for cellular DMSO in *I. galbana* (68.8 μ mol [L cell volume] $^{-1}$ DMSO) and *T. pseudonana* CCMP 1335 (33.8 μ mol [L cell volume] $^{-1}$ DMSO) are approximately 15.8 and 38.2 h, respectively. *A. carterae*, with a much higher DMSO reduction rate, could turn over the cellular DMSO pool (900 μ mol [L cell volume] $^{-1}$) in approximately 5.9 h, assuming a constitutive activity.

The role of eukaryotes in DMSO_d cycling would be as net producers (Simó et al. 1998; del Valle et al 2007b), with cells losing DMSO due to diffusion across the cell membrane. This would not be unexpected since cellular concentrations of DMSO are up to a factor of 10⁶ higher than DMSO_d in seawater (Simó et al. 1998; Hatton and Wilson 2007; this work). With this concentration gradient, the kinetic model predicts a net outward flux of DMSO into the surrounding seawater. This would not be true for non-DMSP-containing, non-DMSO-containing species such as *R. lens*, which may behave as net sinks for DMSO_d, especially during a bloom. Generally, though, algae are not expected to outcompete the much more numerous prokaryotes for DMSO_d. As such, bacteria are expected to be the largest contributors to DMSO removal from the water column.

Cellular roles of DMSO reduction—DMSO reduction could serve an important role in the cell. Sunda et al. (2002) demonstrated the potential for DMSP and DMS to function as antioxidants in the cell. However, the fate of these compounds was not investigated. Our study provides a potential mechanism for the cell to create a redox-coupled antioxidant cycle via DMSO reduction. It is possible that cellular DMS oxidation to form DMSO can signal the onset of oxidative stress and induce transcription of the genes encoding the enzymes involved in its reduction. MsrA isozymes have been shown to be up-regulated in response to oxidative stressors such as ozone and high UV flux (Romero et al. 2006).

It is interesting to note that added DMSO caused greater DMS production in several phytoplankton cultures tested (Fig. 2). Because DMSO is an excellent oxidant scavenger, it is possible that added DMSO scavenged oxidative radicals that would otherwise have reacted with DMS, leading to higher DMS release in the presence of DMSO. The source of the excess unlabeled DMS found in the

isotope labeling experiment is as yet unknown, although it is possible that DMSP lyase is involved given the differences between strains with and without lyase activity (<5% mean excess from DLA– strains vs. approximately 20% mean excess for DLA+ strains).

The ubiquity of DMSO reduction by marine phytoplankton suggests that it could be an important source of DMS in the marine environment. The potential for this process to be mediated by a repair enzyme—MsrA—points to a possible link between DMSO reduction and oxidative stress. Further understanding of the factors influencing the reduction of DMSO by phytoplankton will better constrain the in situ rates of this process and its contribution to the overall DMS cycle.

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