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Dimethylsulphoniopropionate (DMSP), DMSP-lyase activity (DLA) and dimethylsulphide (DMS) in 10 species of coccolithophore

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ABSTRACT: We investigated dimethylsulphoniopropionate (DMSP) quota (pg DMSP cell^{-1}), intracellular DMSP concentration (mM), *in vitro* and *in vivo* DMSP-lyase activity (DLA) and dimethylsulphide (DMS) concentration in batch cultures of 10 species of coccolithophore. Species were chosen to span the phylogenetic and size range that exists within the coccolithophores. Our overall objective was to examine if *Emiliania huxleyi*, considered a 'model' coccolithophore species, is representative in terms of DMSP, DLA and DMS, because other coccolithophores contribute substantially to phytoplankton biomass and carbon fluxes in temperate and tropical waters. DMSP was found in all species, and DMSP quotas correlated significantly with cell volume, reflecting the fundamental physiological role of DMSP as a compatible solute in this group. This DMSP quota–cell volume relationship can be used to calculate the relative contribution of different species to total DMSP. Lowered nutrient availability (batch growth at a 10-fold lower nutrient concentration) did not significantly affect DMSP quota. In contrast to DMSP, DLA and DMS concentration were variable between the subset of species investigated. Coccolithophore DLA is known only in *E. huxleyi* and *Gephyrocapsa oceanica*, and we found DLA to be restricted to these closely related species. If DLA is restricted to a subset of coccolithophores, then those species are more likely to emit DMS directly in the sea. Our results indicate that in ecosystems where coccolithophores form stable populations, species other than *E. huxleyi* can make significant contributions to the particulate DMSP pool and hence to the amount of DMSP potentially available to the microbial loop.

KEY WORDS: Marine sulphur cycle · Calcifying haptophytes · Biogeochemistry · *Emiliania* · *Gephyrocapsa* · *Calcidiscus* · Coccolithophores

INTRODUCTION

Coccolithophores, along with the dinoflagellates, have the highest intracellular concentrations of dimethylsulphoniopropionate (DMSP) found in phytoplankton (Keller et al. 1989). DMSP acts as a compatible solute and may have other important physiological roles as part of an overflow mechanism (Stefels 2000), as the basis of an antioxidant cascade (Sunda et al. 2002), and also as an infochemical (Steinke et al. 2002a). DMSP is the molecular precursor of the volatile trace gas dimethylsulphide (DMS). DMS is an important sulphur source to the remote marine atmosphere that may form aerosol particles that directly scatter incoming radiation back into space. These aerosols can also act as cloud condensation nuclei and thereby influence global albedo indirectly following cloud formation (Charlson et al. 1987). In addition to the direct production of DMS by some phytoplankton, bacteria can catabolise DMSP to DMS, with variable amounts of

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the available DMSP being metabolised to sulphate, DMS and methanethiol (Kiene et al. 2000). Due to this bacterial metabolism, which is dependent not just on the species composition of the bacterioplankton, but also on the physiological state of both bacteria and algae, the relationship between microalgal DMSP and DMS release from the oceans is complex and highly variable in space and time (Malin & Kirst 1997). Nevertheless, major factors that govern how much algal DMSP ends up as DMS potentially available for sea–air gas exchange include: (1) the amount of algal DMSP, (2) the activity of algal DMSP-lyase enzymes in DMSP-producing algae, (3) the physiological and photosynthetic state of DMSP producers, (4) the demise of DMSP producers due to natural cell death, grazing and viral lysis, and (5) the composition and metabolic activity of associated bacterioplankton (see Stefels et al. 2007 for review).

Knowledge of some of these factors can be gained using laboratory cultures. *Emiliania huxleyi* is the best studied coccolithophore due to its ubiquity, its capacity to form blooms visible from space, its ease of culture and the ready availability of a range of clonal strains (Malin & Steinke 2004). In *E. huxleyi* batch cultures, DMSP quota and concentration remains relatively constant during batch growth (Wolfe & Steinke 1996), and this pattern is consistent between strains (Steinke et al. 1998). In contrast, DMSP-lyase activity (DLA) is highly variable between strains of *E. huxleyi* (Steinke et al. 1998), suggesting that differences in DLA could contribute to strain-specific differences within this species. Data on DLA in coccolithophores other than *E. huxleyi* is currently restricted to one measurement for *Gephyrocapsa oceanica* where no *in vivo* activity was detectable (Niki et al. 2000). Most other investigations of DLA to date have concentrated on community, or size-fractionated, measurements of DLA in field samples (Steinke et al. 2002b, Harada et al. 2004), but such approaches cannot resolve the potential role of individual species in DMSP and DMS cycling.

Models examining the transformation of DMSP to DMS have parameterised differences in DMSP content between *Emiliania huxleyi* and other algal taxa (Archer et al. 2002), but the extent of variation in DMSP and DMS characteristics within the coccolithophores is poorly known. Addressing this gap in our knowledge is important given the fact that coccolithophores other than *E. huxleyi* are significant members of the oceanic food webs where 75% of global aquatic productivity occurs (Pauly & Christensen 1995). Community data from field studies indicates that what are sometimes assumed to be single-species coccolithophore blooms are in reality assemblages of various coccolithophores and other taxa. For example, Malin et al. (1993) found that coccolithophores other than *E. huxleyi* formed a substantial component of the coccolithophore biomass in a NE Atlantic bloom and calculated that they made a significant contribution to the DMSP pool. Of these other species, very little is known of DMSP content, DMSP-lyase activity and potential DMS emission. Taxonomic analyses of natural coccolithophore populations have revealed diverse and abundant communities in subtropical waters at the Hawaii Ocean Time-series (HOT) (Cortés et al. 2001) and Bermuda Atlantic Time-series Study (BATS) (Haidar & Thierstein 2001) sites. These assemblages show marked seasonal variability with coccolithophore biomass typically greatest in late winter and early spring. *Emiliania huxleyi* is often numerically dominant at these sites, but due to its small cell size, larger-celled species can also be major contributors to the coccolithophore biomass at certain times. Despite the ecological importance of coccolithophores, DMSP and DMS characteristics across the phylogenetic range of the coccolithophores are poorly known. This lack of basic data extended to growth rates until the recent study by Buitenhuis et al. (2008) who found that the small-celled species *E. huxleyi* and *Gephyrocapsa oceanica* had higher potential growth rates than several other species, and that temperature optima for growth broadly reflected biogeographical ranges. In part, the lack of basic data on coccolithophores has been due to this being a difficult phytoplankton group to culture. However, many more coccolithophores have now been brought into culture, including representatives of the larger subtropical species and different life-history stages of the same species (Probert & Houdan 2004). Here we investigate several of these newly available cultures to provide a baseline comparison of the DMSP, DLA and DMS characteristics of a range of coccolithophore species and strains.

MATERIALS AND METHODS

Coccolithophore culture. The diploid coccolithophores *Emiliania huxleyi*, *Gephyrocapsa oceanica*, *Calcidiscus leptoporus*, *C. quadriperforatus*, *Helicosphaera carteri*, *Umbilicosphaera hulburtiana*, *U. sibogae*, *U. foliosa*, *Oolithotus fragilis* and *Coccolithus braarudii* (Fig. 1, Table 1) were selected to span the phylogenetic and cell size range found in this group. In one strain of *C. leptoporus* (RCC1154), haploid and diploid cultures were examined simultaneously as both stages showed stable growth under the same culture conditions. Not all measurements were collected for all species listed in Table 1; for example, *Scyphosphaera apsteinii* was only investigated for *in vitro* DMSP-lyase activity, and DMS measurements were made only on a subset of species. Cells were grown in

batch culture in K/5 media (Keller et al. 1987) prepared using aged oceanic seawater. Si was omitted and inorganic phosphate ($NaH_2PO_4.H_2O$) replaced glycerophosphate at a final concentration of 7 µM in the medium. *E. huxleyi* (CCMP 370) was obtained from the Provasoli-Guillard culture collection (http://ccmp.bigelow.org). All other cultures were supplied by the Algobank Culture Collection (U. Caen, France) and are now available from the Roscoff Culture Collection (www.sb-roscoff.fr/Phyto/RCC/). Cultures were grown in cotton wool-stoppered glass conical flasks at 17°C with a 14 h light:10 h dark cycle. Light intensity was 85 µmol m^{-2} s^{-1}, measured in seawater in the culture vessel with a spherical collector (Biospherical Instru-

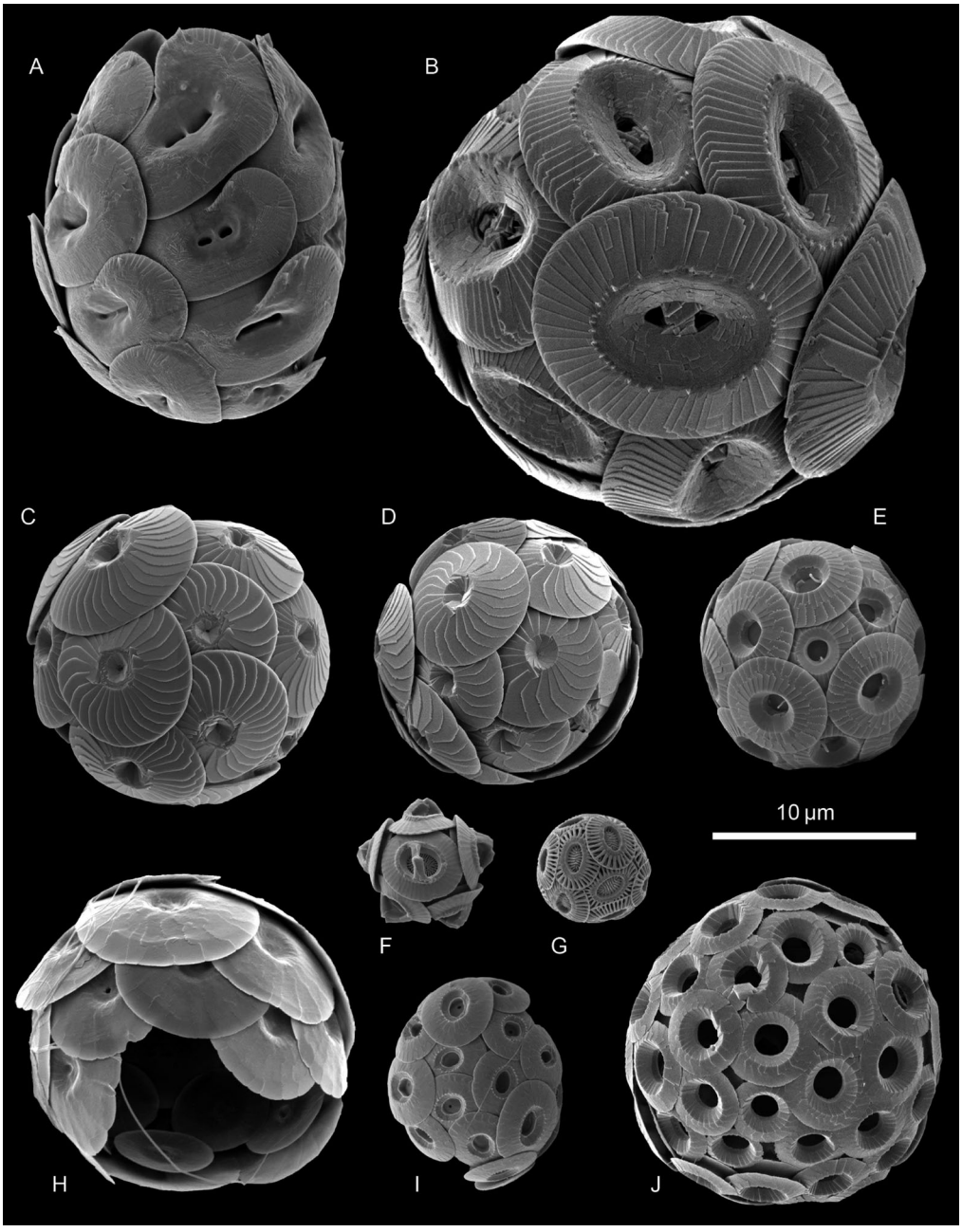

Fig. 1. Coccolithophores at relative size to illustrate the size and morphological range of the species studied. (A) *Helicosphaera carteri*, (B) *Coccolithus braarudii*, (C) *Calcidiscus quadriperforatus*, (D) *Calcidiscus leptoporus* (diploid, heterococcolith), (E) *Umbilicosphaera foliosa*, (F) *Gephyrocapsa oceanica*, (G) *Emiliania huxleyi*, (H) *Oolithotus fragilis*, (I) *U. hulburtiana* and (J) *U. sibogae*. The images are scanning electron micrographs of specimens from natural seawater samples and were taken by J. Young

Table 1. Strain information for coccolithophores investigated in the present study. For further information please see www.sb-roscoff.fr/Phyto/RCC/

Species (strain)	Origin	Strain synonyms
Emiliania huxleyi (CCMP 370)	North Sea	451 B, F451
Emiliania huxleyi (RCC1216)	W. New Zealand	AC472, TQ26
Gephyrocapsa oceanica (RCC1291)	W. Mediterranean (Alboran Sea)	AC330, AS62C
Gephyrocapsa oceanica (RCC1319)	S. Atlantic (South Africa)	AC335, NS6-2
Gephyrocapsa oceanica (RCC1247)	W. Mediterranean (Spain)	AC313 ESP7410
Gephyrocapsa oceanica (RCC1313)	W. Mediterranean (Alboran Sea)	AC284, JS1
Calcidiscus quadriperforatus (RCC1159)	W. Mediterranean (Alboran Sea)	*C. leptoporus*, *C. leptoporus* ssp. *quadriperforatus* AC356, ASM40
Calcidiscus leptoporus (RCC1130)	S. Atlantic (South Africa)	AC370, NS10-2
Calcidiscus leptoporus (RCC1154) haploid and diploid	W. Mediterranean (Alboran Sea)	AC360, AS31
Calcidiscus leptoporus (RCC1143)	S. Atlantic	AC383, N482-1
Scyphosphaera apsteinii (RCC1455)	Mediterranean (Spanish coast)	AC505, TW16
Umbilicosphaera hulburtiana (RCC1474)	S. Atlantic (South Africa)	AC437, NS3A
Umbilicosphaera sibogae (RCC1469)	W. Mediterranean (Alboran Sea)	AC435, ASM39
Umbilicosphaera foliosa (RCC1471)	W. Mediterranean (Spain)	*U. sibogae* var. *foliosa* AC436, ESP6M1
Oolithotus fragilis (RCC1376)	S. Atlantic (South Africa)	AC498, NS10D
Helicosphaera carteri (RCC1333)	S. Atlantic (South Africa)	AC428, NS10-8
Coccolithus braarudii (RCC1200)	S. Atlantic (Namibia)	*C. pelagicus*, *C. pelagicus* ssp. *braarudii* AC400, N476-2

ments QSL 2101). Cell counts were made with a Coulter Multisizer (Beckman Coulter MS3) and photosynthetic capacity was monitored using a chlorophyll fluorometer (Heinz-Walz, PHYTO-PAM equipped with a PHYTO-ED measuring head). Cultures were sampled in mid to late exponential phase. Growth rate was calculated using counts or *in vivo* fluorescence during exponential growth. Extensive trials using antibiotics (Provasoli's antibiotic mixture, Sigma-Aldrich P8029) on *G. oceanica* cultures achieved only temporary reductions in bacterial numbers; we could not totally eliminate bacteria from these cultures. Given that the application of antibiotics can have non-specific physiological effects on algal metabolism (Cottrell & Suttle 1993), we continued the work with unialgal (non-axenic) cultures, but with frequent sub-culturing to keep bacterial numbers to a minimum.

Cell number and volume. A Coulter multisizer (Beckman Coulter MS3) was used to quantify cell numbers and cell volume. Coccolithophores have an extracellular layer of calcium carbonate coccoliths and these, plus the cell itself, is sometimes referred to as the coccosphere. To determine cell volume, as opposed to coccosphere volume, we removed coccoliths by adding HCl to culture samples and then immediately measured cell size. We found that adding 5 µl of 10% HCl per ml of culture (final concentration of HCl = 58 mM) caused total lith dissolution. Fig. 2 shows how the apparent particle diameter of *Calcidiscus leptoporus* (RCC 1130) is reduced upon acid decalcification as analysed by the Coulter multisizer. The effect of coccolith removal on cell diameter was similar in other species (data not shown). We found that the addition of acid had no effect on cell number or cell diameter (*t*-test, $p > 0.05$) in the time frame of the measurement (<2 min). The possible effect of HCl addition on cell size was investigated independently measuring the diameter of cells with and without coccoliths using light and epifluorescence microscopy. Cell diameter

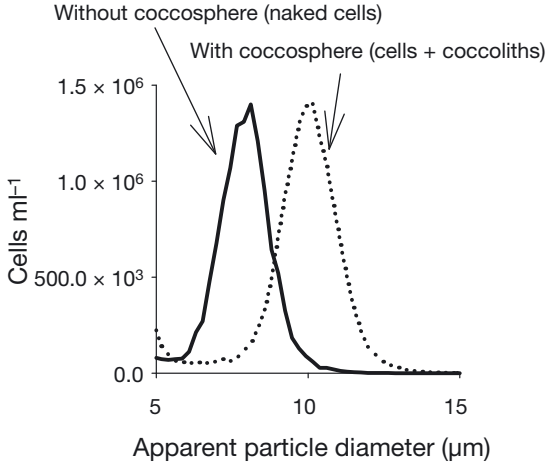

Fig. 2. *Calcidiscus leptoporus*. Decalcifying cells with HCl for assessment of cell, rather than coccosphere (cells + $CaCO_3$ coccoliths), size. A 10% HCl solution was added to cultures at 5 µl ml^{-1} of culture. The dotted line shows the Coulter counter plot before acidification, and the solid line approximately 1 min after, in samples of *C. leptoporus* (RCC1130)

was used to calculate cell volume assuming spherical cells.

Particulate DMSP. In mid-exponential phase, cells were gently filtered (<6 kPa) onto a 25 mm GF/F filter (Whatman, 0.7 µm nominal pore size) using a glass filter tower and a hand-operated vacuum pump. Culture volumes of 2 to 8 ml were used depending on culture population density and filtering typically took 2 to 3 min per filter. Immediately after filtration, the filter was folded and placed at the top of a 4 ml glass vial containing 2 or 3 ml of 0.5 M NaOH. The gas-tight vial lid was screwed on and the filter was then flicked down into the NaOH, causing alkaline hydrolysis of cell DMSP to DMS. Sample vials were stored at room temperature in the dark for up to 1 mo before analysis. A gas chromatograph (Shimadzu GC2010) with a fused-silica column (Varian CP SIL 5CB) and a flame photometric detector was used to measure DMS in the headspace of the vials. Vials were incubated at 30°C for >6 h to ensure consistent partition between the liquid and gas phases and an autoinjector/autosampler system (Shimadzu AOC 20s and 20i, or Gerstel MPS) was used to sample the vial, and to inject vial headspace gas onto the column of the gas chromatograph. Standards were analysed before each batch of analyses using DMSP standards (Centre for Analysis, Spectroscopy & Synthesis, University of Groningen) with the same liquid to head space ratio as the batch of samples being analysed. The DMS detection limit was lower than 0.12 µM (lowest analysed standard concentration). The DMSP quota (pg DMSP cell^{-1}) and intracellular DMSP concentration (mM) of the cells were calculated using the cell count and volume data.

***In vitro* DLA assay.** We used the protocol of Steinke et al. (2000). Cultures (1.5 l) were concentrated by 2-step centrifugation (4°C, ~4500 × *g*, 2 × 20 min) to pellets of less than 0.5 ml volume. Cell losses in the supernatants of the centrifugation steps were quantified and corrected for using Coulter multisizer counts. Buffer (see below) was added to the cell pellets and the resulting suspension was snap-frozen in liquid N_2. The suspension then underwent five 5 s cycles of sonication on ice, snap-freezing and thawing. The resulting crude extract was stored at –80°C. The extraction buffer was a citric acid-phosphate buffer adjusted to constant ionic strength with NaCl (Elving et al. 1956). A pH range of 2 to 7.4 was used in reaction assays which were incubated at 30°C in gas-tight vials with 20 mM DMSP added as a substrate. At defined intervals the DMS in the headspace of the vial was analysed as described above, and a rate of DMS production was calculated, expressed as DMS production cell^{-1} min^{-1}. This production rate was corrected for abiotic DMS production using buffer plus substrate controls which were analysed simultaneously. Outgassing from the extract was assessed prior to substrate addition and the substrate was purged with helium before use.

***In vivo* DLA assay.** Cells were concentrated with gentle centrifugation (2000 × *g*, 20 min, at the growth temperature of 17°C) and then resuspended directly in buffer without sonication in 2 ml gas-tight vials. The assay was started with the addition of 20 mM DMSP, and reaction mixtures were incubated at 17°C for 1.25 h. DMS in the headspace of the vial was analysed as above. The reaction mixture consisted of small volumes of concentrated culture (200 µl) amended with a buffer (95 µl), along with 5 µl of DMSP stock solution to give a total reaction volume of 300 µl. The buffer used was 300 mM 1,3 bis[tris(Hydroxymethyl) methylamino]propane (bis-tris propane), amended with 500 mM NaCl at a pH of 8.2.

DMS. We used a cryogenic purge-and-trap apparatus in-line with the GC2010 gas chromatograph (see above) to measure DMS (Turner et al. 1990). Ten ml of GF/F filtrates were purged for 15 min using oxygen-free nitrogen. Glass-wool aerosol water traps plus Nafion counter-flow driers (Permapure) were used to eliminate water from the sample stream before concentrating the DMS onto a Teflon trap at –150°C. Purge efficiency was checked and the system was calibrated prior to each batch of analyses using DMSP standards hydrolysed by adding 10 M NaOH. The DMS detection limit was below 0.02 nM (lowest analysed standard concentration).

K/50 media/low nutrient experiments. Single strains of 5 species — *Calcidiscus leptoporus* (RCC1154; diploid), *Umbilicosphaera sibogae* (RCC1469), *Helicosphaera carteri* (RCC1333), *Gephyrocapsa oceanica* (RCC1291) and *Coccolithus braarudii* (RCC1200) — were grown in K/50 media under the light and temperature conditions described above. Cultures were grown over 2 complete batch cycles to allow for acclimation to the lower nutrient concentrations before samples for DMSP analyses were taken on the third exponential growth phase. All experimental procedures were otherwise identical to those described above, with duplicate cultures analysed for each species.

RESULTS

Culture growth and cell characteristics in K/5 and K/50 media

K/5 medium

The average specific growth rate (µ) was 0.4 d^{-1} across the 10 species of coccolithophores, and ranged from 0.2 d^{-1} in *Helicosphaera carteri* to 0.7 d^{-1} in

Table 2. Growth and dimethylsulphoniopropionate (DMSP) concentration (mM; in mid-log phase) of 10 species of coccolithophore in K/5 and K/50 media batch cultures. Data are means of duplicate cultures; numbers in parentheses refer to range of data

Species	Growth rate (μ d^{-1})	Cell diameter (μm)	Cell volume (μm^3)	DMSP (mM)
K/5 media				
Emiliania huxleyi[a]	0.7 (0.21)	3.6 (0.6)	25 (13)	295 (69)
Umbilicosphaera hulburtiana[b]	0.44 (0.01)	4.25 (0)	40 (0)	715 (70)
Gephyrocapsa oceanica[c]	0.34 (0.28)	5.2 (3.5)[d]	99 (154)[d]	174 (190)[d]
U. sibogae[b]	0.4 (0.05)	7.1 (0)	187 (0)	291 (5)
U. foliosa[b]	0.56 (0.17)	7.5 (0)	221 (0)	479 (20)
Calcidiscus leptoporus[c]	0.46 (0.26)	8.1 (0.2)	278 (20)	412 (62)
Calcidiscus leptoporus (haploid)[b]	0.47 (0.01)	7.2 (0)	195 (0)	187 (49)
Oolithotus fragilis[b]	0.25 (0.06)	9.5 (0)	449 (0)	386 (41)
Helicosphaera carteri[b]	0.19 (0.01)	10.3 (0)	572 (0)	628 (105)
C. quadriperforatus[b]	0.41 (0.04)	11.6 (0)	817 (0)	596 (39)
Coccolithus braarudii[b]	0.49 (0.14)	13.6 (1.1)	1316 (320)	541 (34)
K/50 media				
Gephyrocapsa oceanica (RCC1291)	0.32 (0.02)	5.2 (0)	74 (0)	350 (70)
Umbilicosphaera sibogae	0.27 (0.01)	7.3 (0)	204 (0)	312 (178)
Calcidiscus leptoporus (RCC1154)	0.23 (0.02)	7.5 (0)	221 (0)	409 (150)
Helicosphaera carteri[e]	0.23	11.6	817	523
Coccolithus braarudii	0.42 (0.003)	12 (0)	905 (0)	613 (67)

[a]Mean of 2 strains (single cultures)
[b]Mean of duplicate cultures (single strain)
[c]Mean of 3 strains (single cultures)
[d]Includes 2 calcified strains
[e]Duplicate culture failed to grow

Emiliania huxleyi (Table 2). Growth rates of the haploid and diploid phases of *Calcidiscus leptoporus* (0.5 d^{-1}) were not significantly different (Mann-Whitney *U*-test, $p > 0.05$). Photosynthetic capacity (dark-adapted F_V/F_M) was always ~0.6 during culture growth and upon harvest for all of the species examined (Table 2). Mean cell volume ranged from 20.5 μm^3 in *E. huxleyi* (RCC1216) to 1316 μm^3 in *Coccolithus braarudii* (RCC1200).

K/50 medium

Growth rates of the 5 species grown in K/50 media (Table 2) were similar to growth rates achieved in K/5 media, but due to Liebig limitation, K/50 culture biomass yield was between 20 and 35% of the biomass accumulated in K/5 media.

DMSP quota and concentration in K/5 and K/50 media

K/5 medium

DMSP quota (pg $cell^{-1}$) showed a significant correlation with cell volume ($r^2 = 0.97$, $p < 0.01$, $n = 15$; Fig. 3A). Thus the lowest quota was found in a strain of *Emiliania huxleyi* (0.8 pg DMSP $cell^{-1}$) and the highest quota in *Coccolithus braarudii* (95 pg DMSP $cell^{-1}$). Intracellular DMSP concentration ranged between 261 mM (*Gephyrocapsa oceanica* RCC1247) to 715 mM (*Umbilicosphaera hulburtiana*). The mean DMSP concentration across all species and strains was 458 mM (SD = 155, n = 14). Given the relationship in Fig. 3A, DMSP quota can be predicted from cell volume using the equation: DMSP $quota_{K/5}$ (pg $cell^{-1}$) = $-4.3 + (0.078 \times$ cell volume). The relationship in Fig. 3A includes all available data. The mean DMSP quota and concentration were 1.1 pg DMSP $cell^{-1}$ and 295 mM, respectively, for *E. huxleyi* strains and 1.2 pg $cell^{-1}$ and 174 mM for *G. oceanica*. In diploid *C. leptoporus* cells, the mean DMSP quota and concentration were 15.2 pg $cell^{-1}$ and 412 mM, respectively. The haploid phase of *C. leptoporus* had a lower DMSP quota and concentration (4.2 pg $cell^{-1}$ and 192 mM, respectively) than the diploid phase.

K/50 medium

Cells grown in K/50 had similar DMSP levels to those grown in K/5, both in DMSP quota (pg $cell^{-1}$), which remained a function of cell volume, and also in DMSP intracellular concentration (mM). DMSP quota showed a significant relationship with cell volume ($r^2 = 0.97$, $p <$

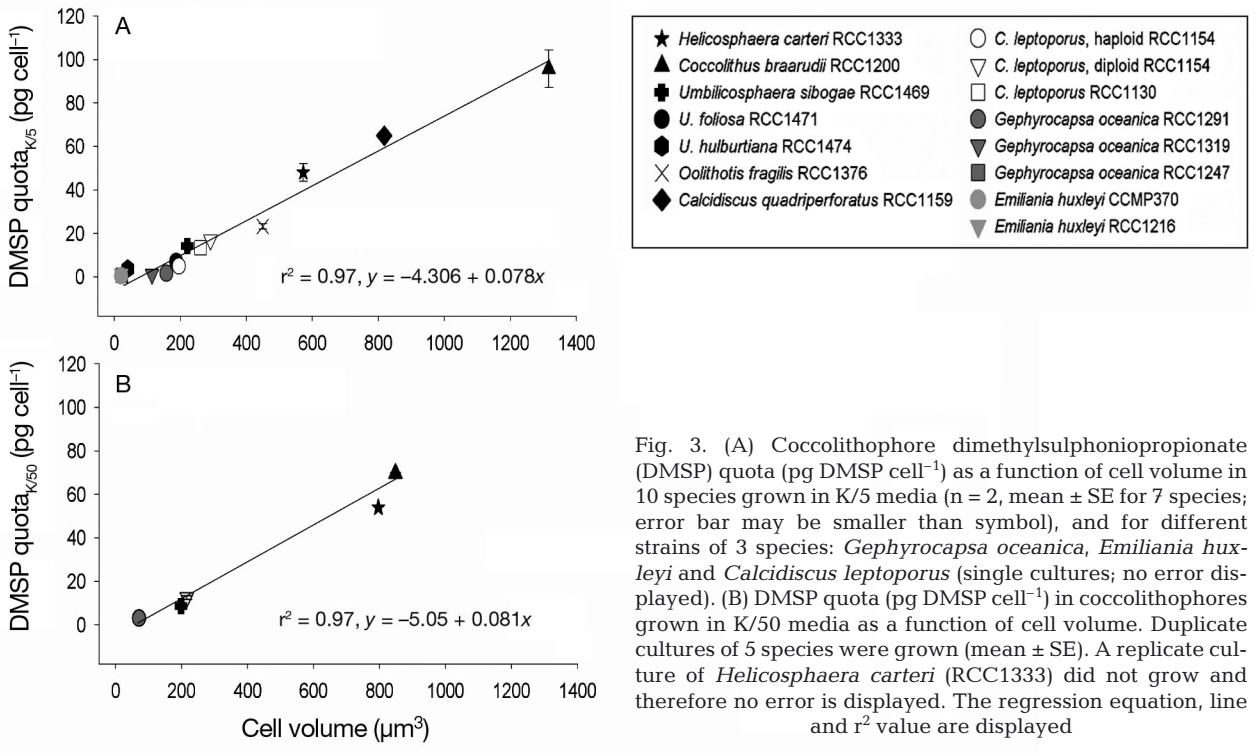

Fig. 3. (A) Coccolithophore dimethylsulphoniopropionate (DMSP) quota (pg DMSP cell^{-1}) as a function of cell volume in 10 species grown in K/5 media (n = 2, mean ± SE for 7 species; error bar may be smaller than symbol), and for different strains of 3 species: *Gephyrocapsa oceanica*, *Emiliania huxleyi* and *Calcidiscus leptoporus* (single cultures; no error displayed). (B) DMSP quota (pg DMSP cell^{-1}) in coccolithophores grown in K/50 media as a function of cell volume. Duplicate cultures of 5 species were grown (mean ± SE). A replicate culture of *Helicosphaera carteri* (RCC1333) did not grow and therefore no error is displayed. The regression equation, line and r^2 value are displayed

0.01, n = 5; Fig. 3B). Given the relationship shown in Fig. 3B, DMSP quota can be predicted from cell volume using the equation: DMSP quota$_{K/50}$ (pg cell^{-1}) = –5.05 + (0.081 × cell volume). DMSP intracellular concentrations ranged between 312 mM (*Umbilicosphaera sibogae* RCC1469) and 690 mM (*Coccolithus braarudii*). There was no significant difference between the slopes of the DMSP quota–cell volume relationship in cells grown in K/5 and K/50 media (*t*-test, p > 0.05).

DMS

DMS concentration in the culture media ranged from 0.2 to 480 nM during exponential growth (Table 3, Fig. 4). In general, DMS concentration increased with culture biomass. Some of the smallest species showed the highest DMS concentrations in the media, though this was not consistent. Since small cells have higher surface area to volume ratios, and the exchange of metabolites is therefore more efficient in small cells, we calculated the ratio of culture DMS to cellular DMSP (DMS:DMSP) for all species and examined if there was an association between the DMS:DMSP ratio and cell volume (and thus surface area to volume ratio). Two of the smallest strains (*Emiliania huxleyi* RCC1216 and *Gephyrocapsa oceanica* RCC1247) had the highest DMS:DMSP ratio; however, other small-celled species showed no difference with large-celled species (data not shown).

In vitro and *in vivo* DLA

A subset of species and strains were examined for *in vitro* DLA. In 3 out of 4 *Gephyrocapsa oceanica* strains, levels of *in vitro* activity were similar to 'low-lyase' *Emiliania huxleyi* strains (Steinke et al. 1998, 2000), with maximum activity at pH 5 (Fig. 5). Values of *G. oceanica* DLA ranged between 0.5 and 5.9 fmol DMS cell^{-1} h^{-1}, with all strains showing maximum activity at pH 5. Parallel assessments of DLA in the previ-

Table 3. Culture filtrate dimethylsulphide (DMS) concentration during exponential growth of 4 species of coccolithophore

Species (strain)	Calcification status	DMS, range (nM)
Emiliania huxleyi (CCMP 370)	Naked	1.5–37.4
Emiliania huxleyi (RCC1216)	Naked	2.9–480
Gephyrocapsa oceanica (RCC1247)	Naked	4.2–108
Gephyrocapsa oceanica (RCC1291)	Calcified	0.4–5.8
Gephyrocapsa oceanica (RCC1319)	Calcified	0.2–1.6
Calcidiscus leptoporus (RCC1154)	Calcified	0.3–0.5
Calcidiscus leptoporus (RCC1130)	Calcified	0.3–0.5
Calcidiscus quadriperforatus (RCC1159)	Calcified	1–17

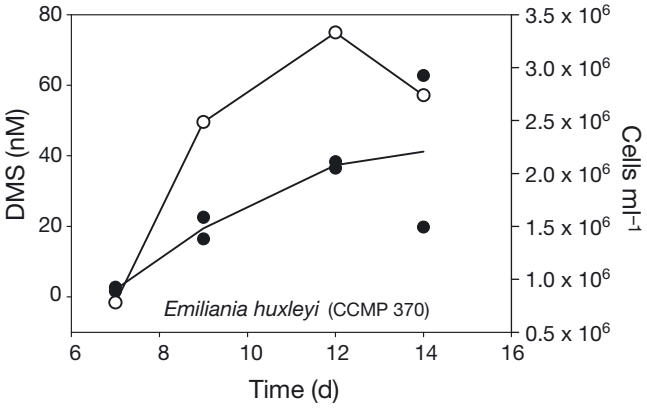

Fig. 4. *Emiliania huxleyi*. Dimethylsulphide (DMS) concentration (●) in a single culture of *E. huxleyi* (CCMP 370) during batch culture growth (cells ml^{-1}; ○). DMS and cell number measurements were technical duplicates with the average shown by the solid line

ously investigated *E. huxleyi* strains CCMP373 and CCMP379 gave levels of activity comparable to that previously recorded for these strains (Steinke et al. 1998). No *in vitro* activity was found in diploid *Calcidiscus leptoporus* (RCC1154 and RCC1143) or *C. quadriperforatus* (RCC1159) at pH 2.2, 4, 5 or 6. In *Coccolithus braarudii* (RCC1200) and *Scyphosphaera apsteinii* (RCC1455), no *in vitro* activity was found at pH 5. No *in vivo* DLA was found in *C. leptoporus* (RCC1154), *Umbilicosphaera sibogae* (RCC1469), *Gephyrocapsa oceanica* (RCC1291) or *G. oceanica* (RCC1319). *E. huxleyi* (RCC1216) had an *in vivo* activity of 0.22 fmol DMS cell^{-1} min^{-1} at pH 8.2. Previous measurements of *in vivo* DLA ranged from 0.002 to 1.7 fmol DMS cell^{-1} min^{-1} in *E. huxleyi* strains CCMP379 and CCMP373, respectively (Steinke et al. 2000).

DISCUSSION

In the present study, we tested the idea that extensive work on one coccolithophore (*Emiliania huxleyi*) can be used to characterise the coccolithophores as a group. Such an approach is problematic since the coccolithophores exploit a wide variety of ecological niches and the potential physiological diversity between species and strains is great. Therefore, the assumption that the physiological characteristics of one 'model' species can be representative of all species in that taxa is a questionable, although pragmatic, approach. Our findings of variable DLA and DMS levels between different species make generalisations on intrinsic DMS emission very difficult. However, particulate DMSP (DMSPp), the source of DMS in microbial foodwebs, is likely to be much more predictable, since DMSPp is a function of cell volume. Predicting DMSPp from species requires good taxonomic distinction and an awareness of the potential impact of environmental pressures on cellular DMSP, such as irradiation, which is at present poorly constrained with contradictory studies in the literature (e.g. van Rijssel & Buma 2002, Slezak & Herndl 2003). The multiple species of coccolithophore which we tested span a wide phylogenetic range and have disparate ecologies, including mesotrophic forms (*Calcidiscus leptoporus*), oligotrophic forms (*Umbilicosphaera sibogae*; haploid phase of *C. leptoporus*) and deep-photic species (*Oolithotus fragilis*). These large differences in species ecology were not reflected in differences in DMSP content, indicating the fundamental physiological role of DMSP across the group.

Keller (1989) and Keller et al. (1989) pioneered research into how phytoplankton vary in their accumu-

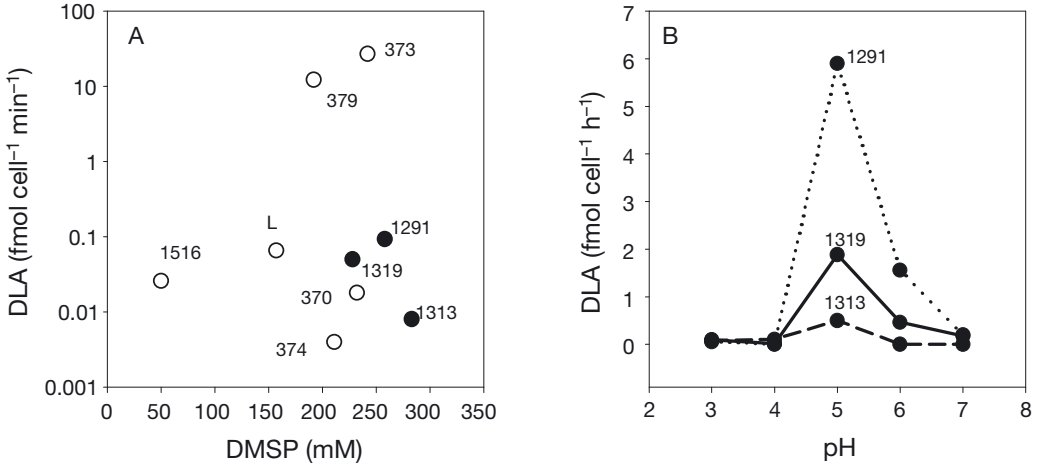

Fig. 5. *Gephyrocapsa oceanica* and *Emiliania huxleyi*. (A) Comparison of *in vitro* DMSP-lyase activity (DLA) and dimethylsulphoniopropionate (DMSP) concentration in 3 strains of *G. oceanica* (●) with 6 strains of *E. huxleyi* (○; data from Steinke et al. 1998). RCC (*G. oceanica*) or CCMP (*E. huxleyi*) strain codes are given alongside the data point, and the DLA value shown is at the *in vitro* pH optima. (B) pH optima of DLA in 3 strains of *G. oceanica*

lation of DMSP and, comparing across taxa, concluded that smaller forms are more likely to produce an equivalent or greater amount of DMSP per unit of cell volume than many larger forms. In coccolithophores, DMSP quota ranges from 0.75 pg DMSP cell^{-1} in *Emiliania huxleyi* to 19.8 pg DMSP cell^{-1} in *Syracosphaera elongata* (now *Pleurochrysis carterae*), with intracellular DMSP concentrations between 35 and 195 mM for 6 coccolithophore strains (Keller et al. 1989). When comparing studies, some taxonomic aspects have to be taken into account. A few coccolithophores, most notably *Umbilicosphaera sibogae*, form multi-cell coccospheres in nature (Young et al. 2003), with up to 4 cells coexisting within one coccosphere and taking up only a small proportion of the total coccosphere volume. In other species, the holococcolith (haploid) coccosphere can have 4 to 8 cells, although this may be an artefact of culture (I. Probert pers. obs.). In such species, direct prediction of the size of the species-specific DMSP pool from count data would be biased without an adequate determination of the mean number of cells per coccosphere. In the present study, coccolith removal and cell dispersion likely led to the lower cell DMSP quota found in *U. sibogae* (7.2 pg cell^{-1}) compared to Keller (1989), who found 13.8 pg cell^{-1}. Nonetheless, our results conform to the general rule established by Keller et al. (1989): the coccolithophores, like the dinoflagellates, accumulate relatively high amounts of DMSP compared with published values for other phytoplankton taxa. The DMSP quota–cell volume relationship remained when cells were grown at lower nutrient concentrations, indicating that species with disparate ecologies in nature do not respond differently to lowered nutrient availability during laboratory growth in our experiments. However, we would point out that interactions between light, temperature and culture mode could influence DMSP content and, therefore, the specific form of the relationship described here will likely be unique to our culture conditions.

An aim of the present study was to determine the potential for DMSP production in coccolithophores that had not been examined previously. Genera such as *Calcidiscus* and *Helicosphaera* are important members of oceanic assemblages, and our data can be combined with count data to estimate the relative contribution of different coccolithophores to the DMSP pool. Using BATS coccolithophore count data sets (from 1991 and 1994) for 1, 25 and 100 m depths (Haidar & Thierstein 2001), we estimate that *Calcidiscus leptoporus* can account for up to 12% of the coccolithophore DMSPp pool and *Helicosphaera carteri* up to 11%, whilst the other 7 species we examined never provide more than 6% of coccolithophore DMSPp. Our DMSP data set indicates that at the BATS site, where *Emiliania huxleyi* is dominant in terms of numbers and biomass, *E. huxleyi* will dominate the coccolithophore DMSPp pool by providing 74 to 82% of DMSPp. This analysis is limited to the species for which we have laboratory data. Overall, the differences in population size achieved by these different species reflect adaptations to exploit variable nutrient regimes, i.e. r versus K selection strategies (Young 1994). Calculating the contribution of different species to the DMSP pool is dependent on the availability of high-quality taxonomic data sets, and these are limited. Both the BATS and HOT data sets are taken to represent oligotrophic, oceanic conditions. Knowing the contribution of coccolithophores other than *E. huxleyi* to the DMSP pool and potential DMS release in other systems requires better data coverage on the relative biomasses of different coccolithophore species in different systems.

The enzymatic conversion of DMSP to DMS by DLA is thought to be an important metabolic pathway in many haptophytes and dinophytes. DLA contributes to the multiple metabolic functions of cellular DMSP (Stefels 2000). Currently, detailed molecular understanding of the enzyme, or family of enzymes, that cleaves DMSP to DMS and acrylate is lacking. Further genetic and biochemical investigations are needed to advance knowledge of the ecophysiological role of DLA. In the present study we used an established protocol (Steinke et al. 2000) to survey newly cultured coccolithophores for DLA, and found DLA in only 2 species, *Emiliania huxleyi* and *Gephyrocapsa oceanica*, restricted to one family, the Noelaerhabdaceae. Niki et al. (2000) found no *in vivo* activity in *G. oceanica*, whereas we found *in vitro* activity in 3 out of the 4 *G. oceanica* strains that we tested. DLA may exist in different places even within the same species: Steinke et al. (2007) found *in vitro* activity in 2 strains of *E. huxleyi*, but *in vivo* activity was only present in one of these strains. We found no evidence of *in vivo* DLA in 2 strains of *G. oceanica*, in agreement with Niki et al. (2000), who used a similar *in vivo* assay to the present study. Our *in vitro* results for 3 strains of *G. oceanica* are broadly similar to those described for low DLA strains of *E. huxleyi* with high DLA strains of *E. huxleyi* also recognised (Steinke et al. 1998). The conclusion that *G. oceanica* DLA was broadly similar to *E. huxleyi* DLA was only possible by examining multiple strains, underlining the difficulty of making interspecific generalisations about DLA when intraspecific variability in DLA is so high. The absence of DLA (using both *in vitro* and *in vivo* assays) in the other coccolithophores we examined is puzzling. However, the absence of activity using this activity-based assay is not definitive: there could have been no expression due to our culture conditions, or no activity due to the conditions of the assay. A next step would be to test if DLA is 'switched on' in

these other species by environmental pressures such as increases in light and UV intensity, or grazing. An ecophysiological role for DMSP has been proposed whereby oxidative stress is reduced via DMSP breakdown providing a quenching role in the cell (Sunda et al. 2002). A corollary of this idea is that those species which possess DLA should show upregulation of enzymatic activity under the environmental conditions which lead to oxidative stress. In the present study, only *E. huxleyi* and *G. oceanica* showed DLA. It is difficult to attribute this finding to oxidative stress having a greater impact on these 2 species compared with the other species, since the light level we used for all species was relatively low (<100 µmol m^{-2} s^{-1}) and DLA was measured during the nutrient-replete exponential phase. Since *E. huxleyi* has an exceptional resistance to photoinhibition (Paasche 2001), which is not dependent on the presence of coccoliths, at least in diploid cells (Harris et al. 2005, Houdan et al. 2005), light stress is unlikely to cause oxidative stress in diploid *E. huxleyi*, and the same could be true for diploid cells of the closely related *G. oceanica*. Therefore, our data set supports the idea that these 2 species have an inherent tendency for high DLA expression, unrelated to environmental stress; i.e. a high constitutive DLA. Contamination from bacterial DLA is also a possibility since our *G. oceanica* cultures were non-axenic. However, since the majority of biomass in our homogenates will have been algal, any potential bacterial signal would likely have been low. In addition, our *E. huxleyi* and *G. oceanica* cultures would have needed substantially higher bacterial DLA levels than the other species in order to contribute to the clear DLA signal observed which seems unlikely. Lastly, a non-axenic *E. huxleyi* strain (RCC1216) had a similar *in vivo* DLA to an axenic *E. huxleyi* strain (CCMP 373; Steinke et al. 2000), suggesting that the bacterial DLA signal, in this species at least, was not significant. On the other hand, the high intraspecific variability makes comparisons of this nature difficult. Based on our findings and experience, we would argue that the DLA assay as it stands is limited since it represents an enzymatic 'black box'; further efforts to complete the molecular characterisation of the enzyme(s) responsible for the DMSP cleavage pathway are needed.

Measurements of DMS during coccolithophore culture growth are limited. Wolfe & Steinke (1996) found DMS increased during the batch growth of *Emiliania huxleyi* strains CCMP 370 and 373 to maxima of 250 and 12 nM, respectively. Amongst the species and strains we examined, *E. huxleyi* (RCC1216) produced the highest levels of culture DMS. Using the solid phase microextraction method, Yassaa et al. (2006) noted exceptionally high levels of DMS in cultures of *Calcidiscus leptoporus* (RCC1135) of up to 2134 nM, in contrast to a strain of *Emiliania huxleyi* (CCMP 371) in the same study (up to 67 nM). This finding led Yassaa et al. (2006) to speculate that *C. leptoporus* could be responsible for unaccounted DMS emissions in natural assemblages, and that *C. leptoporus* must have a high DMSP content per cell, as well as a high DLA. We showed here that DMSP content is proportional to cell volume, as is the case throughout all the coccolithophores we examined, and that no *in vitro* or *in vivo* DLA was detectable with our protocols in 2 strains of *C. leptoporus*. Therefore, our data indicate that it is somewhat premature to propose that *C. leptoporus* is an 'exceptional' DMS producer. The physiological condition of the cells is likely the most important factor influencing culture DMS. Thus measurements of batch culture DMS will only be reliable during the confirmed exponential phase of growth under controlled conditions, as bacterial activity, cell lysis and cell stress can all lead to elevated levels of DMS. In this regard, chemostat culture of coccolithophores would also help to investigate the potential for DMS release between coccolithophores under different nutrient and irradiance regimes.

In conclusion, our data set demonstrates that DMSP synthesis is ubiquitous throughout coccolithophores which have disparate ecologies in nature, and that coccolithophore DMSP quota correlates significantly with cell volume. Thus *Emiliania huxleyi* is a good model species for the coccolithophores as a whole in terms of DMSP, but appears to be atypical in its DLA and, therefore, potential DMS release.

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