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Concentrations of dimethylsulphoniopropionate and activities of dimethylsulphide-producing enzymes in batch cultures of nine dinoflagellate species

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Abstract Dinoflagellates are recognised as one of the major phytoplankton groups that produce dimethylsulphoniopropionate (DMSP), the precursor of the marine trace gas dimethylsulphide (DMS) which has climate-cooling potential. To improve the prospects for including dinoflagellates in global climate models that include DMSP-related processes, we increased the data base for this group by measuring DMSP, DMS-producing enzyme activity (DPEA), carbon, nitrogen and Chl *a* in nine clonal dinoflagellate cultures (1 heterotrophic and 8 phototrophic strains). Growth rates ranged from 0.11 to 1.92 day^{-1} with the highest value being for the heterotroph *Crypthecodinium cohnii*. Overall, we observed two orders of magnitude variability in DMSP content (11–364 mM) and detected DPEA in five of the nine strains (0.61–59.73 fmol $cell^{-1}$ h^{-1}). Cell volume varied between 454 and 18,439 μm^3 and whilst C and N content were proportional to the cell volume, DMSP content was not. The first DMSP measurements for a dinoflagellate from Antarctic waters and a species with diatom-like plastids are included. Lower DMSP concentrations were found in three small athecate species and a dinoflagellate with haptophyte-like plastids. The highest concentrations and production rates tended to be in globally distributed dinoflagellates and the heterotroph. Photosynthetic species that are distributed in temperate to tropical waters showed low DMSP concentrations and production rates and the polar representative showed moderate concentration and a low production rate. Estuarine species had the lowest concentrations and production rates. These data should help refine the inclusion of dinoflagellates as a functional group in future global climate models.

Keywords Batch cultures · Carbon · Dinoflagellates · DMSP · DMS-producing enzymes · Nitrogen

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Introduction

Dimethylsulphide (DMS) is derived from the degradation of the phytoplankton metabolite dimethylsulphoniopropionate (DMSP; see review by Stefels et al. 2007) and both compounds are key components of the sulphur cycle. DMS is transferred by sea-to-air gas exchange from the ocean to the atmosphere where it oxidises rapidly to form sulphate aerosols that can serve as cloud condensation nuclei (von Glasow and Crutzen 2004). The sulphur cycle is then completed by

the wet or dry deposition of sulphate and other sulphur-containing oxidation products back to the sea or to the Earth's surface. These aerosols and clouds increase the albedo and reduce the amount of solar radiation reaching the Earth's surface (Shaw 1983). The CLAW hypothesis, which proposed a feedback control of phytoplankton on climate through DMS emissions (Charlson et al. 1987), remains controversial.

Modelling DMS production and its effect on climate is a real challenge due to the complexity of the biological and physical processes involved (Stefels et al. 2007). The biological processes begin with DMSP production by various marine phytoplankton and the primary issues for modelling are: which taxa synthesise DMSP, what is the intracellular DMSP concentration and how does this vary with environmental conditions. Subsequently, DMSP is released to the environment by exudation and cell lysis due to senescence, grazing and viral infection (Yoch 2002; Stefels et al. 2007). Much of the dissolved DMSP is assimilated or catabolised by bacteria without the production of DMS, or exported via sedimentation of detritus (Kiene et al. 1999; Zubkov et al. 2001). Nonetheless, a portion of the DMSP is converted to DMS by the action of bacterial and algal enzymes (Steinke et al. 1996; Todd et al. 2009). In models, phytoplankton biomass is usually represented as carbon (C) or nitrogen (N) concentrations or chlorophyll *a* (Chl *a*). A few carbon-specific DMSP values have been estimated for some phytoplankton functional groups including dinoflagellates (Le Quéré et al. 2005; Stefels et al. 2007) but only a few papers give direct measurements for dinoflagellate cultures (Matrai and Keller 1994; Keller et al. 1999a, b). Chl *a* is often quoted in field data sets but rather few values are given alongside dinoflagellate culture measurements (Ginzburg et al. 1998; Harada et al. 2009; Zhuang et al. 2011).

Here, we focus on marine dinoflagellates which are recognised as one of the major DMSP-producing phytoplankton groups (Stefels et al. 2007). Dinoflagellates are common in aquatic ecosystems, benthic environments and sea-ice (Taylor et al. 2008). It is a very diverse taxonomic group with free-living, symbiotic and parasitic representatives. It is thought that about 50% of the 2,000 or so living dinoflagellate species are photosynthetic, whilst others are heterotrophic or mixotrophic. Some of the earliest DMSP and DMS production studies focused on cultures of the osmotrophic dinoflagellate *Crypthecodinium* (*Gyrodinium*) *cohnii* (Ishida 1968). Later the work of Keller et al. (Keller 1988/1989; Keller et al. 1989a, b) highlighted the wide variability in DMSP concentration between dinoflagellates. About 40 dinoflagellate species have been tested for their intracellular DMSP concentration and 9 species have also been assessed for the activity of DMS-producing enzymes (DPEA; we use this term rather than the previously used DMSP lyase activity/DLA given the recent discoveries of a wide range of bacterial DMS-producing enzymes: DPE, see Todd et al. 2011). However, there are rather few in-depth studies on dinoflagellate clones. Data from various field studies suggests that dinoflagellates may make a significant contribution to marine DMS production (e.g. Archer et al. 2009). Furthermore, dinoflagellates appear to be increasing in abundance and blooming earlier in the season in some marine waters as temperatures increase (Edwards et al. 2006; Mercado et al. 2007).

Here we present DMSP concentrations and DMS-producing enzyme activity (DPEA) data for nine diverse dinoflagellate species with associated C, N and Chl *a* measurements. Our overall aim was to increase the database available for describing and modelling the contribution of this important phytoplankton group to DMSP and DMS production.

Materials and methods

Dinoflagellate strains

Within the constraints of the range of cultures available from culture collections, we selected nine diverse dinoflagellate clones for this study. Eight cultures were purchased from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, Maine, USA) and *Lingulodinium polyedrum* LP2810 was kindly provided by Dr Débora Iglesias-Rodríguez (University of Southampton, Southampton, UK). Of the nine clonal cultures: four photosynthetic species with peridinin plastids (*Heterocapsa triquetra* CCMP449, *Scrippsiella trochoidea* CCMP1599, *Amphidinium carterae* CCMP1314, *Lingulodinium polyedrum* LP2810) and one heterotroph (*Crypthecodinium cohnii* CCMP316) are relatively common species and allow some comparison with published

DMSP content and DPEA values. In addition, we selected *Alexandrium minutum* (CCMP113) for which DMSP had previously been studied though only in field samples. It is a good example of a toxic dinoflagellate that can cause paralytic shellfish poisoning (PSP) in humans after consumption of contaminated seafood. *A. minutum* is globally distributed due to a recent expansion and blooms have been reported more frequently in recent decades (McCauley et al. 2009). *Polarella glacialis* (CCMP1138) is found in northern and southern polar waters, Antarctic sea-ice, and saline lakes (Montresor et al. 1999, 2003; Thomson et al. 2004; Rengefors et al. 2008).

The dinoflagellates are a unique group of species that harbour a range of different plastid types other than the common peridinin plastid, hence we also used the ichtyotoxic species *Kryptoperidinium foliaceum* (CCMP1326), which harbours diatom-like plastids (Withers and Haxo 1975; Chesnick et al. 1997), and *Karlodinium veneficum* (CCMP4151) which has haptophyte-like plastids (Tengs et al. 2000). Dinoflagellates also display highly diverse morphologies: some have an armour of cellulosic plates, others are naked. Among the species selected here, *A. carterae*, *P. glacialis* and *K. veneficum* are naked or athecate.

These nine species are coastal species that can also occur in estuaries, except *K. foliaceum* and *K. veneficum* which mainly occur in estuarine or brackish waters (Tomas 1997; Throndsen et al. 2007). *H. triquetra*, *S. trochoidea*, *A. minutum*, *A. carterae* and *K. veneficum* are considered to have a global distribution (Tomas 1997; Throndsen et al. 2007; Hoppenrath et al. 2009 and World Register of Marine Species, http://www.marinespecies.org), *Polarella glacialis* is a polar species and *C. cohnii*, *K. foliaceum*, *L. polyedrum* occur in temperate and tropical zones (Tomas 1997; Litaker et al. 2002; Bachvaroff et al. 2009). *C. cohnii* is a euryhaline, heterotrophic dinoflagellate that is often associated with decomposing seaweeds (Tomas 1997; Mendes et al. 2009).

Culture conditions

Basic information for the nine dinoflagellate species and the growth conditions used are given in Table 1. Enriched seawater media (f/2, L1, f/2 + NPM and Prov.) were prepared as recommended by the culture collection and full details can be found on the CCMP website (https://ccmp.bigelow.org/). Silicate was omitted from the original formulae as it is not needed for dinoflagellates. Growth conditions were at or close to the recommendations for optimal growth but were not specifically optimized for all parameters. Stock cultures were maintained in environmental chambers (MLR-351 Plant Growth Chamber, Sanyo, Loughborough, UK) using a light:dark cycle of 14:10 h and with light supplied by fluorescent tubes (FL40SS W/37, Sanyo, Loughborough, UK). Precultures were grown in glass Erlenmeyer flasks and cells in exponential phase were always used for inoculation of new medium. Batch cultures for the experiments were grown in the same medium and environmental conditions as stock cultures and precultures. The flasks were filled to 1/3rd of the flask volume and unsealed. They were capped with a cotton bung to allow air exchange, swirled daily and opened (using sterile technique) during sampling. Four species were axenic and axenity was checked at the end of each experiment by microscopic observation of samples stained with DAPI (Sigma-Aldrich, D9542).

Experimental approach

Three replicate cultures were grown for each species. Triplicate samples were taken for DMSP, C, N and Chl *a* analyses in early to mid-exponential phase. The time of sampling was based on increase in cell density and the total cell volume ranged between 20 and 60 $\mu L_{cell}\ L^{-1}$ (cell volume varied substantially between species). Samplings during the exponential phase were aimed at taking cells in balanced growth and avoiding nutrient limitation (Wood et al. 2005). However, pH was not monitored so we cannot dismiss the possibility of CO_2 limitation given the high cell densities (1.5×10^3–4.6×10^4 cell mL^{-1}). Samplings were performed at mid-way-through of the photoperiod to limit any variation due to the diel cycle. The average and relative standard deviation (RSD) for all samples ($n = 9$) is given for each parameter in the results.

Cell counts and cell volumes

A particle counter (Coulter Multisizer 3, Beckman Coulter (UK) Ltd., High Wycombe, UK), with a 100 μm aperture tube, was used to monitor growth. Cell density (cell mL^{-1}), total cell volume ($\mu L_{cell}\ L^{-1}$ equivalent to $10^6\ \mu m^3\ mL^{-1}$) and mean cell volume

Table 1 A summary table of the clonal dinoflagellate cultures investigated

Latin name	Synonyms	Strain code	Collection site	Medium[a]	LI	*T* (°C)
[T]*Alexandrium minutum*	*Alexandrium ibericum*	CCMP113	Ria de Vigo, Spain	L1	156	15
[A]*Amphidinium carterae*		CCMP1314	Falmouth, MA, USA	f/2	204	22
[A]*Crypthecodinium cohnii*	*Glenodinium cohnii* *Gyrodinium cohnii*	CCMP316	Unknown	f/2 + NPM	127	22
[A]*Heterocapsa triquetra*	*Perdinium triquetra*	CCMP449	St Lawrence Estuary, Canada	f/2	120	15
[T]*Karlodinium veneficum*	*Karlodinium micrum*	CCMP415	Norway, North Sea	f/2	122	15
Kryptoperidinium foliaceum	*Peridinium foliaceum*, *Glenodinium foliaceum*	CCMP1326	La Jolla, California Bight, USA	L1	204	22
[PT]*Lingulodinium polyedrum*	*Gonyaulax polyedra*	LP2810	San Diego, California, USA	f/2	204	22
Polarella glacialis		CCMP1138	McMurdo Sound, Antarctica	Prov	101	4
[A]*Scrippsiella trochoidea*	*Perdinium trochoideum*	CCMP1599	Falmouth, MA, USA	f/2	102	15

Species names, synonyms, strain codes, origin, toxicity, axenity and culture conditions are described (*LI* light intensity in µmol photons m^{-2} s^{-1} and *T* temperature in °C). Three species are athecate (*A. carterae*, *P. glacialis* and *K. veneficum*), others are thecate. *K. foliaceum* contains diatom-like plastids, *K. veneficum* has haptophyte-like plastids, the heterotroph *C. cohnii* is thought to have a remnant plastid and the remaining clones have peridinin plastids

[A] axenic, [T] toxic, [PT] potentially toxic

[a] Media were prepared as described in "Materials and methods" section

(μm^3) were measured in three replicate cultures ($n = 3$) daily, or less frequently depending upon the growth rate of each species. Growth rate was obtained by applying a logistic model as shown in Fig. 1. The logistic equation was $y = A/(1 + Be^{-kx})$, where k was the maximum growth rate, A the maximum cell density and $B = A/N_0 - 1$, where N_0 was the initial cell density (Stefels et al. 2009). The equation was solved with Microsoft Office Excel (version 2003) and the fitted values A and k were used to calculate the growth rate on the day of sampling according to the equation $\mu = k - (N_t \times k/A)$.

Carbon and nitrogen analyses

An elemental analyzer (CE440, Exeter Analytical, Coventry, UK) was used to determine the C and N composition of cells harvested by filtration. Two milliliters culture aliquots were filtered through a 13 mm diameter Whatman GF/F filter (pre-combusted at 450°C for 4 h) in a Swinnex filter holder (Millipore, Watford, UK) using a hand vacuum pump (<5 cm Hg). The filters were folded in half, wrapped in foil and rapidly frozen in liquid nitrogen prior to storage at −80°C. All samples were analysed within 1 month. Prior to analysis, the samples and blank filters were dried at 35°C for 48 h before being placed in nickel capsules (pre-combusted at 1,000°C for 1 h). As suggested by Goldman and Dennett (1985) and Collos (2002), rinsing and acidification steps were avoided in order to limit cell disruption and C losses. Thus, C was considered originating essentially from POC (Karl et al. 1991; Menden-Deuer and Lessard 2000).

Chlorophyll analyses

Five milliliters culture aliquots were gravity filtered through 25 mm GF/F filters. The use of a glass filtration unit without the glass frit support allowed rapid filtration (maximum 2 min). The filter was folded in half, wrapped in foil, rapidly frozen in liquid nitrogen and stored in a freezer at −80°C for up to a month (Arar and Collins 1997). Chlorophyll was extracted by putting each filter in a vial with 10 mL of 90% acetone in the dark at 4°C for 20–24 h. Fluorescence was measured before and after acidification with three drops of 8% HCl and the Chl *a* concentration was calculated as described by Parsons et al. (1984). Chlorophyll fluorescence was measured with a Turner fluorometer (AU-10, Turner Designs, California,

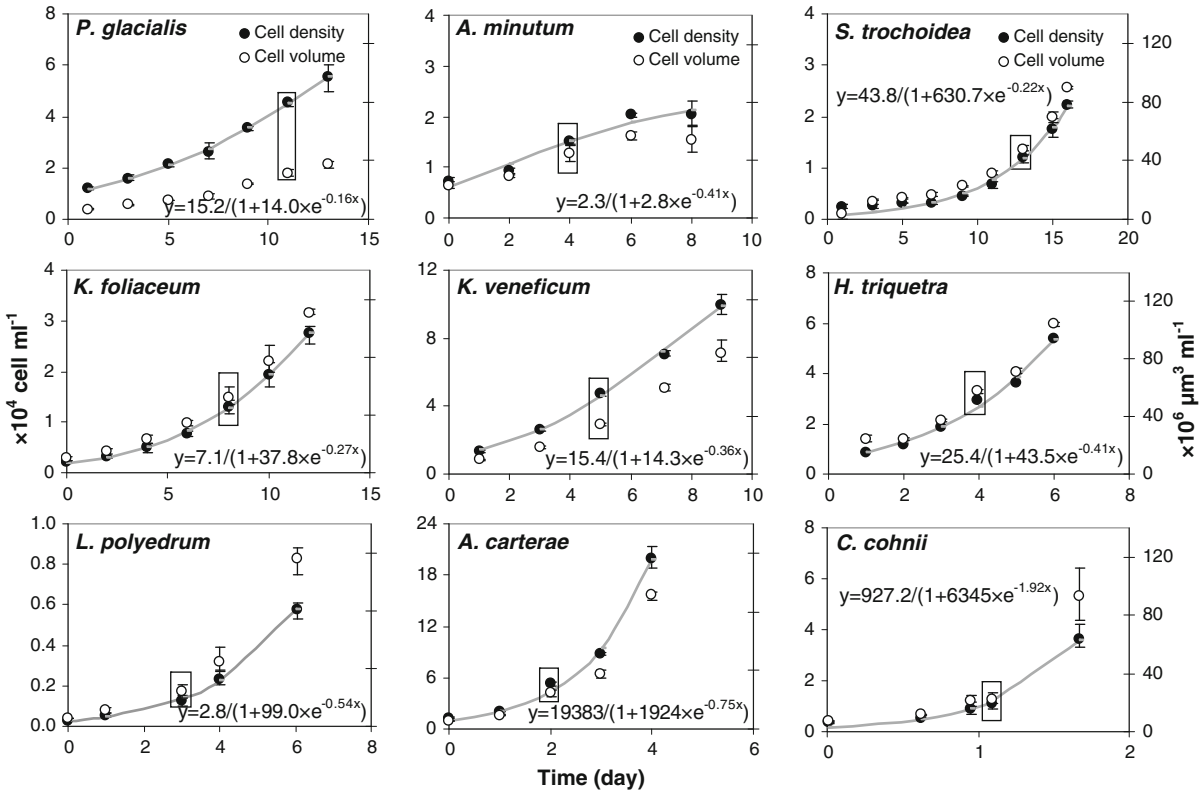

Fig. 1 Growth curves for nine dinoflagellate species. The data are presented in terms of cell density in cell mL^{-1} ($\times 10^4$) and cell volume in μm^3 mL^{-1} ($\times 10^6$). The average value for three replicate cultures is given with range bars to show the minimum and maximum values. Logistic curves were fitted to the cell density data according to the equation $y = A/(1 + Be^{-kx})$, where A is the maximum biomass; $B = A/N_0 - 1$ and k = max specific growth rate. The resulting equations are shown on each graph and the associated R^2 correlation coefficients were between 0.97 and 1. Samples were taken for analysis during the exponential phase of growth as indicated by the rectangles. Species are in order of increasing growth rates

USA) equipped with a 10-045 lamp, a 340–500 nm excitation filter and a >650 nm emission filter. Freshly prepared pure Chl *a* in 90% acetone solutions (*Anacystis nidulans*, Cyanophyceae, Sigma-Aldrich, C6144) of known concentrations (0.006–0.227 mg L^{-1}) were used for calibration. The concentration of the Chl *a* standard was verified using a spectrophotometer (Lambda 25, Perkin Elmer, Massachusetts, USA) using the average value of the results obtained using the SCOR (U.N.E.S.C.O. 1966) and Lorenzen methods (Lorenzen 1967).

$DMSP_T$ analyses

Total DMSP ($DMSP_T$: includes particulate and dissolved DMSP plus DMS) was taken as a conservative approximation of intracellular DMSP (Keller et al. 1989a; Yost and Mitchelmore 2009). We adopted $DMSP_T$ analysis here because some dinoflagellates are sensitive to filtration and we found that this technique gave the most consistent data. Analysis involved using a digital pipette to introduce 1 mL of culture into a 5 mL vial containing 2 mL of 0.75 M NaOH. Vials were rapidly sealed with a screw cap and a Teflon septum, agitated and left in the dark to react (>12 h and less than 2 weeks storage) at ambient temperature. The NaOH reacts with DMSP to release DMS, which was quantified using a headspace method (Steinke et al. 2000). A gas chromatograph (GC-2010 Shimadzu UK Ltd., Milton Keynes, UK), equipped with a 30 m × 0.53 mm CP-SIL 5CB fused-silica capillary column (Varian, Oxford, UK) and a flame photometric detector (FPD) was used. After equilibration overnight in a heater block at 30°C, 80 µL aliquots of headspace were injected into the GC with a 100 µL heated glass syringe by means of an autosampler (Multipurpose

sampler MPS, Gerstel, Mülheim an der Ruhr, Germany). Duplicate DMSP standards in the concentration range 0.07–25 μM prepared in identical vials were used to calibrate the GC. The DMSP detection limit was 0.07 μM based on the lowest concentration used for calibration. Concentrations in the samples were always in excess of that in the lowest calibration standard. The estimated DMSP production rate was calculated as the DMSP concentration per cell volume multiplied by the specific growth rate.

DPEA analyses

To estimate in vitro DPEA cultures were sampled in mid-to late exponential growth phase at a total cell volume between 40 and 100 $\mu L_{cell}\ L^{-1}$. These higher cell densities were favourable for detection of the enzyme activity. Cells were harvested by gently filtering 100 mL through a polycarbonate filter (47 mm diameter, 2.0 μm pore size). The filtration was gently assisted with a hand-operated vacuum pump (<15 cm Hg). Culture extracts were prepared from the filter by sonication in pH 8.2 tris buffer (Steinke et al. 2000) and duplicate analyses were done. Extracts were put in cryogenic vials, rapidly frozen in liquid nitrogen and stored at −80°C for later analysis. After thawing at room temperature, extracts (295 μL) were transferred to gas-tight vials. DMSP was added (5 μL, 1.2 M) and DMS production was monitored over time with 4 sequential measurements of DMS in the headspace of the vial. DPEA was corrected for the average activity found in buffer control vials (7.34 nmol $L^{-1}\ h^{-1}$) and the average and standard deviation of three replicate cultures calculated.

Data analyses

Statistical tests were performed using Microsoft Office Excel (version 2003) and SPSS (version 16.0). Data normality was assessed by performing Kolmogorov–Smirnov and Shapiro–Wilk tests. In some cases data were normalized by $\log_{10}$ transformation prior to regression analysis. The carbon and nitrogen to volume relationships were determined by model I regression as described by Menden-Deuer and Lessard (2000). Least-squares regression analyses were done and the statistical significance of the regressions was tested by ANOVA. Correlations between two variables were tested with the Pearson tests for normal variables and the non-parametric Spearman test for non-normal variables. The non-parametric Mann–Whitney *U* test was also applied for the comparison of two independent groups of non-normal data.

Results

Growth rates varied between 0.11 and 1.92 day^{-1} and the average growth rate for the phototrophic dinoflagellates was 0.32 day^{-1} (Table 2). The polar species *P. glacialis* grew the slowest, *A. carterae* was the fastest growing phototrophic species at 0.75 day^{-1} and the growth rate of the heterotrophic species *C. cohnii* was the highest overall. These data are comparable with dinoflagellate growth rates reported in the literature (e.g. 0.16–1.28 day^{-1}, Tang 1996). Growth curves for the nine dinoflagellate species are shown in Fig. 1.

The mean cell volume was extremely variable between species (Table 2). There was a 40-fold difference between the smallest (*A. carterae*, 454 μm^3) and the biggest species (*L. polyedrum*, 18,439 μm^3) and the three athecate species (*A. carterae*, *P. glacialis* and *K. veneficum*) were the smallest of the group (454–680 μm^3). These cell volumes fall in the range reported for dinoflagellates by Menden-Deuer and Lessard (2000). The average 0.06 RSD for the cell volumes we measured with the Coulter Multisizer 3 compares favourably with 0.22–0.56 RSD values that other workers have derived from microscopy data (Verity et al. 1992; Menden-Deuer and Lessard 2000).

The dinoflagellate cells contained on average 1,233 ± 1.46 pg C $cell^{-1}$ ± RSD and 277 ± 1.61 pg N $cell^{-1}$ (Table 2; Fig. 2a). The average concentrations on a cell volume basis were 0.330 ± 0.76 pg C μm^{-3} and 0.067 ± 0.61 pg N μm^{-3}. *C. cohnii* contained higher C and N concentrations than photosynthetic species. The average RSD for the C and N measurements per cell and per cell volume were 0.19 and 0.20 respectively. The C:N ratio varied between 3.6 and 6.3 g g^{-1} (Table 2) and there was no significant difference between the C and N concentrations per cell volume for the thecate versus athecate dinoflagellates (Mann–Whitney *U* test, $P > 0.05$).

A least-squares regression analysis for the $\log_{10}$ transformed C per cell versus cell volume data (Fig. 2b) showed a significant relationship (ANOVA, $P < 0.01$). The correlation was further supported with the Pearson test ($P < 0.01$). The N per cell values

Table 2 Growth rate, mean cell volume, C, N and Chl *a* concentrations measured in nine dinoflagellate species

Species	Growth rate (day^{-1})	Volume (μm^3)	C		N		C:N ratio	Chl *a*	
			pg $cell^{-1}$	pg μm^{-3}	pg $cell^{-1}$	pg μm^{-3}		fg $cell^{-1}$	fg μm^{-3}
H. triquetra	0.39	1879 (0.03)	344 (0.14)	0.173 (0.13)	55 (0.10)	0.028 (0.09)	6.3 (0.15)	2873 (0.31)	1.45 (0.30)
S. trochoidea	0.21	3339 (0.11)	649 (0.23)	0.163 (0.19)	153 (0.31)	0.038 (0.28)	4.3 (0.30)	2424 (0.52)	0.61 (0.50)
A. carterae	0.75	454 (0.04)	139 (0.18)	0.301 (0.20)	31 (0.12)	0.066 (0.14)	4.5 (0.19)	2311 (0.16)	4.94 (0.18)
A. minutum	0.15	2712 (0.07)	745 (0.11)	0.251 (0.21)	160 (0.11)	0.054 (0.20)	4.7 (0.07)	3309 (0.17)	1.11 (0.24)
C. cohnii	1.92	1726 (0.05)	2051 (0.22)	0.987 (0.24)	352 (0.23)	0.169 (0.24)	5.8 (0.15)	– (–)	–
P. glacialis	0.11	658 (0.11)	199 (0.18)	0.285 (0.21)	37 (0.12)	0.052 (0.17)	5.5 (0.21)	1005 (0.54)	1.43 (0.55)
K. foliaceum	0.22	3389 (0.05)	1037 (0.17)	0.260 (0.18)	225 (0.14)	0.057 (0.15)	4.6 (0.17)	14771 (0.14)	3.71 (0.15)
L. polyedrum	0.52	18439 (0.04)	5760 (0.32)	0.308 (0.30)	1429 (0.29)	0.076 (0.27)	4.0 (0.25)	57352 (0.37)	3.07 (0.35)
K. veneficum	0.25	680 (0.01)	176 (0.18)	0.244 (0.13)	48 (0.27)	0.067 (0.27)	3.6 (0.32)	1879 (0.18)	2.60 (0.18)
Averages									
Phototrophs	0.32 (0.66)	3944 (1.52)	1131 (1.68)	0.248 (0.22)	267 (1.78)	0.055 (0.29)	4.7 (0.18)	10740 (1.80)	2.37 (0.63)
All species	0.50 (1.13)	3697 (1.53)	1,233 (1.46)	0.330 (0.76)	277 (1.61)	0.067 (0.61)	4.8 (0.18)	– (–)	– (–)

The C:N ratio was calculated with C and N expressed per litre of culture. For each species, the mean value (± relative standard deviation) for all replicates is shown. The mean value (± relative standard deviation) calculated for the eight phototrophs or all nine dinoflagellate species is also given. *Crypthecodinium cohnii* is a heterotrophic species that has no Chl *a* so it is excluded in the average values given for the phototrophs

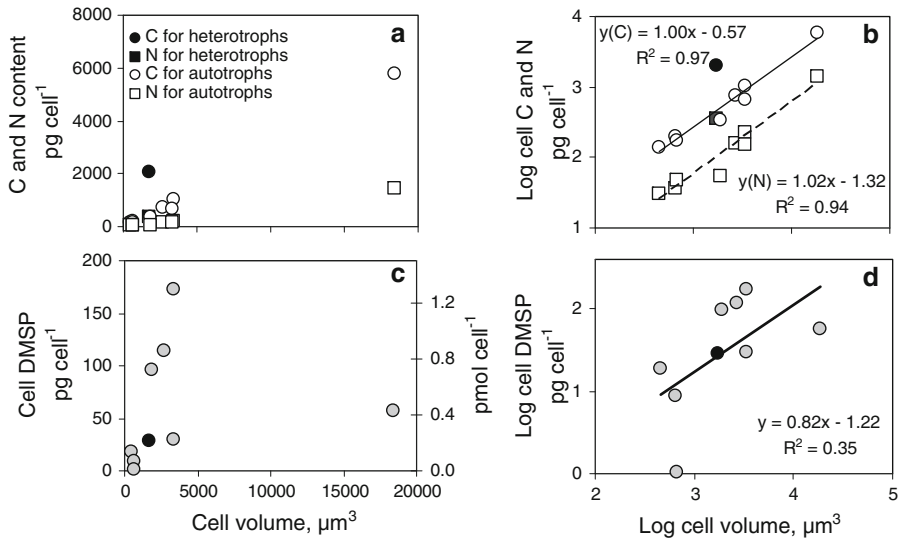

Fig. 2 Cell carbon, cell nitrogen and DMSP per cell versus cell volume data for eight autotrophic dinoflagellates (*open symbols*) and the heterotroph *C. cohnii* (*black symbols*). **a** Amount of carbon and nitrogen per cell, **b** Log_{10}-transformed C and N data, **c** Amount of DMSP per cell in pg and pmol per cell units and **d** Log_{10}-transformed DMSP per cell data. The data for *C. cohnii* are not included in the equations

showed a similar relationship with cell volume (Fig. 2b). Therefore the C and N content may be predicted from the following equations. For the eight autotrophs:

$$Log_{10}C\ (pg\ cell^{-1}) = -0.572 + (0.997 \times Log_{10}\text{-}Vol(\mu m^3),\ r^2 = 0.97,\ P < 0.01$$
$$Log_{10}N\ (pg\ cell^{-1}) = -1.321 + (1.023 \times Log_{10}\text{-}Vol(\mu m^3),\ r^2 = 0.94,\ P < 0.001$$

For all nine species including the heterotroph:

$$Log_{10}C\ (pg\ cell^{-1}) = -0.445 + (0.981 \times Log_{10}\text{-}Vol(\mu m^3),\ r^2 = 0.81,\ P < 0.001$$
$$Log_{10}N\ (pg\ cell^{-1}) = -1.213 + (1.009 \times Log_{10}\text{-}Vol(\mu m^3),\ r^2 = 0.81,\ P < 0.001.$$

Table 2 also shows that Chl *a* content ranged from 1,005 fg $cell^{-1}$ in *P. glacialis* to 57,352 fg $cell^{-1}$ in *L. polyedrum* with an average of 10,740 ± 1.80 fg $cell^{-1}$. The high value substantially exceeds the 190–23,000 fg $cell^{-1}$ range reported for 35 dinoflagellate species by Tang (1996). Expressed on a per cell volume basis, the Chl *a* concentrations ranged from 0.61 fg μm^{-3} in *S. trochoidea* to 4.94 pg μm^{-3} in *A. carterae*, with an average of 2.37 ± 0.63 pg μm^{-3}. The chlorophyll data showed an RSD between replicates of 0.30 which was larger than for other parameters but of the same order observed in other phytoplankton culture studies (e.g. Steinke et al. 1998).

For $DMSP_T$ analysis, the average RSD obtained was 0.14 per cell and 0.16 per cell volume (Table 3) which is lower than the RSD of 0.52 for $DMSP_T$ analysis given by Yost and Mitchelmore (2009) for strains of *Symbiodinium*. $DMSP_T$ concentrations per cell varied from 1.0 pg $cell^{-1}$ (0.008 pmol $cell^{-1}$) in *K. veneficum* to 174.4 pg $cell^{-1}$ (1.3 pmol $cell^{-1}$) in *S. trochoidea* with an average of 59 ± 0.99 pg $cell^{-1}$ (0.44 pmol $cell^{-1}$). Given that the cell volume was highly variable between species, the $DMSP_T$ values expressed per cell volume and per cell did not give the same species order. $DMSP_T$ concentrations per cell volume ranged from 11 mM in *K. veneficum* to 364 mM in *H. triquetra*, with an average of 174 ± 0.82 mM (Table 3).

Three groups of species can be distinguished: (1) *A. minutum*, *A. carterae*, *S. trochoidea* and *H. triquetra* had DMSP concentrations between 290 and 364 mM, strongly above the average; (2) *C. cohnii* and *P. glacialis* had medium DMSP concentrations of 103 and 94 mM respectively and (3) *K. veneficum*, *L. polyedrum* and *K. foliaceum* had low DMSP concentrations ranging from 11 to 56 mM (Table 3). The thecate species we tested contained significantly higher DMSP concentrations per cell volume than the athecate ones (Mann–Whitney *U* test, $P < 0.05$). Given that the athecate species had smaller cell volumes, we tested the correlation between the cell volume and the amount of DMSP per cell. However, opposite conclusions were obtained with different statistical tests: correlation was significant ($P < 0.05$) with a Spearman's test but not significant ($P > 0.05$) using a Pearson's test with log_{10} normalised data (Fig. 2d shows the regression analysis of these normalised data). Finally, dividing the nine

Table 3 DMSP and in vitro DPEA data for nine dinoflagellate species

Species	$DMSP_T$:CV (mM)	$DMSP_T$:cell		$DMSP_T$:C (mmol mol^{-1})	$DMSP_T$:N mmol mol^{-1}	$DMSP_T$:Chl*a* (mmol g^{-1})	C-DMSP:C (%)	S-DMSP:N (g g^{-1})	DPEA (nmol L^{-1} $h^{-1)}$	DPEA: cell (fmol $cell^{-1}$ h^{-1})	DPEA: CV (mmol L^{-1} h^{-1})	Prod. rate $DMSP_T$:CV (mM day^{-1})
		pg $cell^{-1}$	pmol $cell^{-1}$									
H. triquetra	364 (0.06)	96.5	0.719 (0.07)	25.1 (0.13)	184.5 (0.09)	250 (0.30)	12.6 (0.16)	0.422 (0.13)	BDL	BDL	BDL	142 (0.04)
S. trochoidea	326 (0.13)	174.4	1.300 (0.19)	24.0 (0.16)	119.3 (0.26)	536 (0.49)	12.0 (0.30)	0.273 (0.37)	15.7 (0.30)	0.61 (0.37)	0.15 (0.34)	70 (0.07)
A. carterae	300 (0.12)	18.8	0.140 (0.09)	12.1 (0.18)	63.8 (0.11)	61 (0.16)	6.0 (0.20)	0.146 (0.15)	613.0 (0.02)	3.07 (0.08)	6.70 (0.06)	225 (0.05)
A. minutum	290 (0.20)	115.6	0.861 (0.10)	13.9 (0.06)	75.4 (0.05)	260 (0.14)	6.9 (0.15)	0.173 (0.15)	1180.5 (0.07)	59.73 (0.07)	22.56 (0.10)	44 (0.14)
C. cohnii	103 (0.24)	28.6	0.213 (0.22)	1.2 (0.13)	8.5 (0.14)	– (–)	0.6 (0.31)	0.019 (0.32)	239.8 (0.17)	7.27 (0.27)	2.86 (0.33)	203 (0.17)
P. glacialis	94 (0.12)	8.9	0.066 (0.05)	4.0 (0.18)	25.3 (0.12)	66 (0.54)	2.0 (0.19)	0.058 (0.13)	BDL	BDL	BDL	11 (0.10)
K. foliaceum	56 (0.13)	29.9	0.223 (0.12)	2.6 (0.15)	13.8 (0.12)	15 (0.12)	1.3 (0.21)	0.032 (0.19)	BDL	BDL	BDL	12 (0.06)
L. polyedrum	23 (0.32)	57.2	0.426 (0.33)	0.9 (0.30)	4.2 (0.27)	7 (0.36)	0.4 (0.46)	0.010 (0.44)	164.8 (0.10)	27.73 (0.10)	1.38 (0.11)	12 (0.19)
K. veneficum	11 (0.09)	1.0	0.008 (0.09)	0.5 (0.19)	2.3 (0.27)	4 (0.19)	0.3 (0.20)	0.005 (0.28)	BDL	BDL	BDL	3 (0.06)
Averages												
Phototrophs	183 (0.82)	62.8	0.468 (0.97)	10.4 (0.97)	61.1 (1.05)	150 (1.25)	5.2 (0.97)	0.14 (1.05)	493.5 (1.06)	22.78 (1.21)	7.70 (1.34)	65 (1.23)
All species	174 (0.82)	59.0	0.440 (0.99)	9.4 (1.05)	55.2 (1.14)	– (–)	4.7 (1.05)	0.13 (1.14)	442.8 (1.06)	19.68 (1.26)	6.73 (1.36)	80 (1.09)
Stefels et al. 2007	$n = 32$			22 (1.45)		111 (1.51)	11.1 (1.42)					

$DMSP_T$ is expressed per total cell volume (CV), per cell, per carbon, per nitrogen and per chlorophyll *a*. C-DMSP:C indicates the DMSP-carbon to C ratio and S-DMSP:N indicates the DMSP-sulphur to N ratio. The species are given in decreasing order of their $DMSP_T$ per cell volume values. The estimated production (Prod.) rate for $DMSP_T$ per cell volume (CV) is expressed in mM day^{-1}. For DPEA DMS production data are expressed per litre of culture, per cell and per total cell volume (CV). For each species, the mean value (± relative standard deviation) for all replicates is shown. The mean value (± relative standard deviation) calculated for the eight phototrophs or nine all dinoflagellate species is also given. The phototroph averages exclude the heterotroph *Crypthecodinium cohnii*. Data from Stefels et al. (2007) is provided for comparison

BDL below detection limit

Table 4 Comparison of DMSP and DPEA results from this study and published data

Species/strain	Data source	DMSP:CV (mM)	DMSP:cell (pmol cell^{-1})	DPEA:CV (mmol L^{-1} h^{-1})	DPEA:cell (fmol cell^{-1} h^{-1})
H. triquetra					
CCMP449	This study	364	0.719	BDL	BDL
NIES-7	Niki et al. (2000)	300	0.600	20.00	30.00
S. trochoidea					
CCMP1599	This study	326	1.300	0.15	0.61
CCAP1134/1	Hatton and Wilson (2007)	169	0.329	NT	NT
CCMP1131	Keller et al. (1989a)	350	2.861	NT	NT
NIES-369	Niki et al. (2000)	600	1.300	8.00	18.00
Not given	Zhuang et al. (2011)	ND	0.541	NT	NT
A. carterae					
CCMP1314	This study	300	0.140	6.70	3.07
CCAP1102/1	Hatton and Wilson (2007)	57	0.015	NT	NT
CCMP1314	Spiese et al. (2009)	109	ND	NT	NT
Not given	Harada (2007)	288	0.098	13260.00	4488.00
CCMP1314	Harada (2007)	326	0.133	3.61	1.48
CCMP1314	Keller et al. (1999a)	219	0.024	NT	NT
CCMP1314	Keller (1988/1989)	377	0.144	NT	NT
A. minutum					
CCMP113	This study	290	0.861	22.56	59.73
Field sample	Jean et al. (2005)	3388	14.200	NT	NT
C. cohnii					
CCMP316	This study	103	0.213	2.86	7.27
CCMP316	Keller et al. (1989a)	377	0.341	NT	NT
L. polyedrum					
Lp2810	This study	23	0.435	1.38	27.73
CCMP1738	Harada (2007)	13	0.119	11.52	102.60
CCAP1121/2	Hatton and Wilson (2007)	5	0.054	NT	NT

DMSP and DPEA data are given per cell volume (CV) and per cell. DMSP values expressed in pg cell^{-1} were converted to mol cell^{-1} units using the DMSP zwitterion molecular weight of 134.2 g

BDL DPEA was below the buffer only control value, *NT* DPEA was not tested, *ND* no data

species in two groups according to cell size: large cells (1,726–18,439 μm^3) and small cells (454–680 μm^3), also divides the species into thecate and athecate species respectively and these groups show a significant difference in DMSP concentrations. The two estuarine species (*K. foliaceum* and *K. veneficum*) fall into the low DMSP group and have significantly lower DMSP concentrations than the coastal species (Mann–Whitney *U* test, $P < 0.001$). A significant difference in DMSP concentrations was observed depending on latitudinal distribution (Kruskal–Wallis test, $P < 0.001$). Temperate to tropical species appeared to contain medium to low DMSP concentrations and the polar species showed medium concentration. Most globally distributed species had high DMSP concentrations with the exception of *K. veneficum* which had the lowest concentration. Finally, considering coastal species only, those that have a global distribution such as *H. triquetra*, *S. trochoidea*, *A. carterae* and *A. minutum* have significantly higher DMSP concentrations per cell volume (Mann–Whitney *U* test, $P < 0.05$).

We compared the DMSP and DPEA data obtained in this study with data available in the literature

(summarized in Table 4). In the following section we consider each of the species investigated in turn.

We obtained similar DMSP content values for *H. triquetra* CCMP449 isolated from Canadian waters to those for the Japanese strain NIES (Niki et al. 2000; Table 4). Using similar medium but a higher temperature (20°C) and shorter photoperiod (12:12 h) for culturing this species, Niki et al. (2000) detected substantial DPEA suggesting that it might also directly contribute to the DMS pool in the sea. However, we were unable to detect any DPEA in CCMP449.

For *S. trochoidea*, several studies suggest intra-specific variation in DMSP concentration in the range 169–600 mM (Table 4). Here, CCMP1599 had 326 mM DMSP when grown at 15°C which is close to the 350 mM value reported for CCMP1131 grown at 20°C (Keller et al. 1989a). We detected DPEA of 0.61 fmol cell^{-1} h^{-1} in *S. trochoidea* culture extracts which was lower than the 18 fmol cell^{-1} h^{-1} obtained by in vivo assay on NIES-369 (Niki et al. 2000). This difference is likely to be due to differences in analytical methods but intra-specific variability between strains of *S. trochoidea* is also possible.

Four studies have looked at DMSP in *A. carterae* CCMP314 and the 57–377 mM range found suggests intra-specific variation (Table 4). We obtained a DPEA of 3.07 fmol cell^{-1} h^{-1} in our study which is about twice the in vitro activity value of 1.48 fmol cell^{-1} h^{-1} reported for the same strain by Harada (2007). However, the very high DPEA values of 4488 fmol cell^{-1} h^{-1} for what we assume to be a different strain (strain code not provided; Harada 2007) suggests wide intra-specific variation in DPEA.

Jean et al. (2005) analysed DMSP in 80 *A. minutum* cells picked directly from a seawater sample from the Mediterranean Sea and found the DMSP concentration to be 3388 mM or 14.2 pmol cell^{-1} (Table 4). This is by far the highest DMSP level reported for any phytoplankton. We found 10-fold lower concentration in *A. minutum* CCMP113 originally isolated on the Atlantic coast of Spain, which is still a substantial DMSP concentration. Moreover the high DPEA (59.73 fmol cell^{-1} h^{-1}) associated with this species makes it a potent DMS producer.

Here, *C. cohnii* CCMP316 showed a lower DMSP concentration than previous results suggest (Table 4). However, we have seen considerable variation in DMSP content during different growth phases and depending on nutrient availability (Caruana et al. in prep). Given that *C. cohnii* is a fast growing species, we monitored growth carefully to ensure sampling during its short exponential phase. This might account for the higher values reported by Keller et al. (1989a). DPEA in *C. cohnii* was reported over 40 years ago by Kadota and Ishida (1968) but it was not quantified. Hence, our value of 7.27 fmol cell^{-1} h^{-1} represents the first in vitro DPEA value for this species (Table 4) and suggests that this heterotroph is a DMS producer.

Dinoflagellates have been suggested as a major source of DMSP in Antarctic coastal waters (Gibson et al. 1996) and *P. glacialis* was reported as dominant in an Antarctic fast ice phytoplankton community (Thomson et al. 2006). It is the first polar dinoflagellate tested for DMSP content. It has a moderate DMSP concentration of 94 mM or 0.066 pmol cell^{-1}, but DPEA was not detected.

K. foliaceum had a low DMSP concentration (56 mM and 0.223 pmol cell^{-1}) and no DPEA was detected. No DMSP data are available for comparison with this species or other dinoflagellates with diatom-like plastids.

L. polyedrum LP2810 had a low DMSP concentration (23 mM) though slightly higher than values obtained for other strains (5–13 mM, Table 4). The sulphonium compounds gonyauline and gonyol have been identified in *L polyedrum* species and may possibly cause an overestimation of DMSP concentration (Nakamura et al. 1996). *L. polyedrum* LP2810 showed some DPEA but substantially lower than reported for strain CCMP1738 (Harada 2007).

The DMSP concentration in *K. veneficum* was low and no DPEA was detected with our assay conditions. Only a few dinoflagellates with haptophyte-like plastids have been tested for DMSP. Harada (2007) also found low DMSP levels in *Karenia brevis* (18 mM) though in this case DPEA was detected.

The estimated DMSP production rate (Table 3) gives an indication of the production capacity for each species. These data divide the species into three groups: (1) *H. triquetra*, *C. cohnii* and *A. carterae* which have a high DMSP production rate of 142–225 mM day^{-1}, (2) *A. minutum* and *S. trochoidea* with medium rate of 44–70 mM day^{-1} and (3) *K. veneficum*, *P. glacialis*, *L. polyedrum*, *K. foliaceum* at 3–12 mM day^{-1}. Large celled thecate species had a DMSP production rate significantly higher than small celled athecate species (Mann–Whitney *U* test, $P < 0.05$), but overall the athecate *A. carterae*

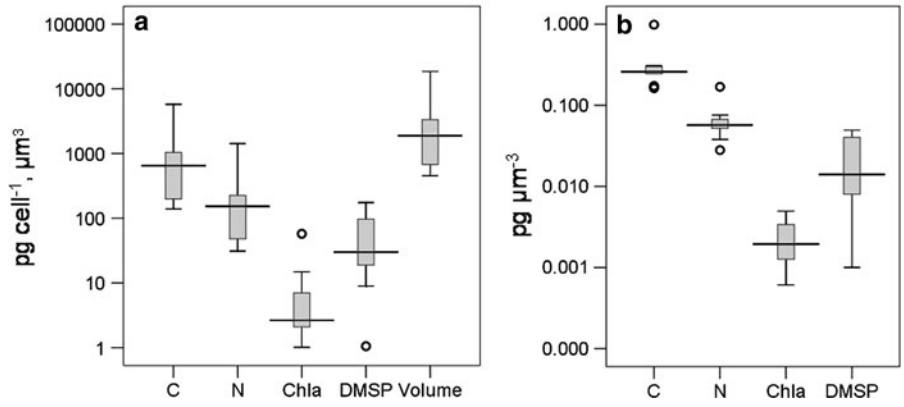

Fig. 3 **a** The quantity of C, N, Chl *a* and DMSP in pg cell^{-1} plus cell volume in µm^3 for nine dinoflagellate species and **b** concentrations of C, N, Chl *a*, DMSP per cell volume in pg µm^{-3}. The *box and whisker plots* show the range of the data (*error bar*), the 1st and 3rd quartile (*box*) and the median value (*horizontal bar*) obtained for each parameter measured. The outlier data (*open circle*) are **a** Chl *a*: *L. polyedrum*, DMSP: *K. veneficum*; **b** C upper: *C. cohnii*, C lower: *S. trochoidea* and *H. triquetra*; N upper: *C. cohnii*, N lower *H. triquetra*

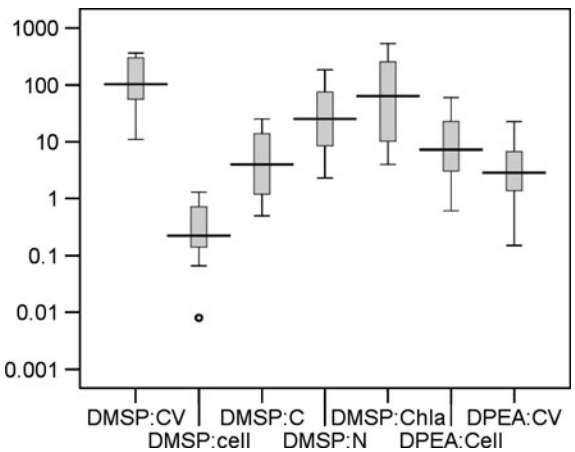

Fig. 4 The DMSP and DPEA data for nine dinoflagellate species. The *box and whisker plots* show the data range (*error bar*), the 1st and 3rd quartile (*box*) and the median value (*horizontal bar*) obtained. The DMSP data are presented in the following units: per cell volume (CV) DMSP:CV = mM, DMSP:cell = pmol cell^{-1}, DMSP:C = mmol mol^{-1}, DMSP:N = mmol mol^{-1} and DMSP:Chl *a* = mmol g^{-1}. DPEA is expressed in DPEA:cell = fmol cell^{-1} h^{-1} and DPEA:CV = mmol L^{-1} h^{-1}. The outlier data (*open circle*) are DMSP:cell data: *K. veneficum* and DPEA:CV data *S. trochoidea*

presented the highest DMSP production rate. Estuarine species had a production rate significantly lower than coastal species (Mann–Whitney *U* test, $P < 0.001$). There appeared to be significant difference in DMSP production rate versus latitudinal distribution with higher rates for globally distributed species and lower rates for polar species (Kruskal–Wallis test, $P < 0.01$).

The $DMSP_T$ per C quotas ranged from 0.5 to 25.1 mmol mol^{-1} with an average of 9.4 ± 1.05 mmol mol^{-1} (Table 3). The $DMSP_T$ per N values ranged from 2.3 to 184.5 mmol mol^{-1} with an average of 55.2 ± 1.14 mmol mol^{-1}. Using the $DMSP_T$ values as an approximation of the intracellular DMSP, the C in DMSP would represent between 0.3 and 12.6% of the total cell C across this group of dinoflagellates. The sulphur in DMSP to N ratio (S-DMSP:N, Table 3), which is a key parameter for DMS models (Gabric et al. 1993; Cropp et al. 2007), ranged from 0.005 to 0.422 g g^{-1} with an average of 0.13 ± 1.14 g g^{-1}. $DMSP_T$:Chl *a* is also a key modelling parameter and this ratio ranged from 4 mmol g^{-1} in *K. veneficum* to 536 mmol g^{-1} in *S. trochoidea* with an average of 150 ± 1.25 mmol g^{-1}.

Five of the nine dinoflagellate species presented detectable DPEA with DMS production in culture extracts ranging from 15.7 to 1180.5 nmol L^{-1} h^{-1} (Table 3). The highest DPEA was found in *A. minutum* (1180.5 nmol L^{-1} h^{-1}). This activity was just under twice that observed for *A. carterae*, 5- and 7-fold higher than in the *C. cohnii* and *L. polyedrum* cultures. DPEA was low in *S. trochoidea* (15.7 nmol L^{-1} h^{-1}) and no activity was detected in *H. triquetra*, *P. glacialis*, *K. foliaceum* and *K. veneficum*.

The variability among the nine species obtained for each parameter is visualised in the box and whisker plots shown in Figs. 3 and 4. The C and N concentrations showed little variation per cell volume and variation per cell over two orders of magnitude

(Fig. 3). This difference arises from the variation in cell volume that also spreads over two orders of magnitude. The variation in DMSP concentrations expressed per cell volume, per cell, per C, N and Chl *a* each extended over two orders of magnitude (Fig. 4). The DPEA showed the same order of variability per cell and slightly less per cell volume.

Discussion

In order to compare the DMSP levels found in different phytoplankton groups and with a view to providing values for inclusion in ecosystem and global climate models, it is necessary to express DMSP data in terms of C, N and Chl *a* (Stefels et al. 2007). Menden-Deuer and Lessard (2000) developed an equation to estimate carbon content from cellular volume using a database of 34 dinoflagellate species. DMSP values expressed per C often come from estimates derived from such equations (Le Quéré et al. 2005; Stefels et al. 2007) and only rarely from actual measurements (Matrai and Keller 1994; Keller et al. 1999a, 1999b). DMSP values from field studies are sometimes expressed in terms of readily available Chl *a* data. Whilst it is difficult to apportion Chl *a* to specific phytoplankton in the field, it is an attractive parameter for modelling studies as a proxy for phytoplankton biomass and because of the ready availability of satellite Chl *a* data. In this study, our approach was to measure these parameters directly.

C and N content

The amount of C and N per cell and the C:N ratio (3.6–6.3 g g^{-1}) obtained in our study are within the 3.44–6.45 g g^{-1} range for 20 dinoflagellates sampled in exponential phase as published by Menden-Deuer and Lessard (2000). A higher range of C:N values was obtained by Verity et al. (1992) for three phototrophic dinoflagellates sampled in late exponential phase (5.1–8.8 g g^{-1}) whereas similar mean values were reported by Meksumpun et al. (1994) for *Alexandrium catenella* and *Scrippsiella trochoidea* over the growth (5.83 and 6.52 g g^{-1} respectively, equivalent to 6.8 and 7.6 mol mol^{-1}). However, in these two species, the C:N ratio varied with growth stage: it increased in early exponential phase, decreased in late exponential phase and in *A. catenella* alone, C:N increased again during the stationary phase. Such variations between and within growth stages might explain some of the variability between studies. We found that the photosynthetic species showed a C:N ratio of 4.7 ± 0.18 g g^{-1}, slightly lower than the Redfield ratio of 5.7 g g^{-1} (equivalent to 6.625 mol mol^{-1}).

Whilst *C. cohnii* showed a higher C concentration per cell volume (0.987 pg μm^{-3}) than the phototrophic species (average of 0.248 ± 0.22 pg μm^{-3}), its carbon quota of 2,051 pg C $cell^{-1}$ fits well within the range of 223–35,349 pg $cell^{-1}$ published for other heterotrophic species (Menden-Deuer and Lessard 2000). The N content of *C. cohnii* (352 pg $cell^{-1}$) was also higher than that of the photosynthetic species and values obtained for heterotrophic dinoflagellates in another study (48.43 and 89.09 pg $cell^{-1}$, Menden-Deuer and Lessard 2000). We are not aware of any further data for comparison. The C content measured in *L. polyedrum* (5,760 pg $cell^{-1}$) was six times higher than the 990 pg $cell^{-1}$ value reported by Mullin et al. (1966) despite similar cell volumes of 18,439 and 16,800 μm^3 respectively. However it is possible that these differences reflect the potential for variation between the many strains of this species.

We observed a significant positive relationship between cell volume and C and N content and derived the equation $Log_{10}\ C = -0.445 + (0.981 \times Log_{10}\ Vol)$ for predicting C content from the mean cell volume. This is close to the relationship proposed by Menden-Deuer and Lessard (2000) on the basis of all previous published values $Log_{10}\ C = -0.353 + (0.864 \times Log_{10}\ Vol)$. Moreover in agreement with their findings and using comparative statistical tests, we also failed to obtain any significant difference in C and N concentrations between thecate and athecate dinoflagellates. However, Menden-Deuer and Lessard (2000) obtained higher C concentrations per cell volume values for thecate species using of regression-based predictions.

DMSP and DPEA

All the dinoflagellates assessed in this study produced DMSP, but there is considerable variability between species. This is in agreement with the earlier observations of Keller et al. (1989a). *H. triquetra*, *S. trochoidea*, *A. carterae* and *A. minutum* showed high DMSP concentrations (290–364 mM) that are comparable with or higher than those reported for the well-studied

prymnesiophyte *Emiliania huxleyi* (50–304 mM from Franklin et al. 2010; Harada 2007; Keller 1988/1989; Keller et al. 1989a; Steinke et al. 1998; van Rijssel and Gieskes 2002; Wolfe et al. 1997). These dinoflagellates are common species that may act as strong sources of DMS especially in coastal waters during bloom events.

The batch cultures may have experienced CO_2 limitation due to high cell density (1.5×10^3–4.6×10^4 cell mL^{-1}) and C concentrations (0.6–1.1 mM). CO_2 limitation can enhance DMSP synthesis (Sunda et al. 2002; Bucciarelli and Sunda 2003) and could lead to an overestimation of DMSP content and production. Moderate CO_2 limitation may also increase Chl *a* concentrations (Bucciarelli and Sunda 2003). However, our cultures were in exponential growth and the particulate C concentration in most of them was below the likely dissolved inorganic carbon concentration of 2.5 mM. Additionally the cotton bungs used to cover the culture flasks would allow some gas exchange that would be enhanced by the daily sampling.

Over our whole dataset, DMSP-carbon was a variable fraction of the total cell carbon: 6–13% in species containing high concentrations of DMSP (*H. triquetra*, *S. trochoidea*, *A. carterae*, *A. minutum*), and 0.3–2% for the low to medium DMSP concentration groups (*K. veneficum*, *L. polyedrum*, *K. foliaceum*, *P. glacialis* and *C. cohnii*). The higher range results agree with the calculations of Matrai and Keller (1994) for two dinoflagellates (12–20%), Stefels et al. (2007) based on 32 dinoflagellate species (11%) and the 8% value from published data given by Archer et al. (2002b). In the latter case, the value was used in a model to predict DMSP conversion into DMS in a phytoplankton bloom. The DMSP to C ratio was also variable between species. We obtained an average DMSP:C of 0.0094 mol mol^{-1} ($n = 9$) which is lower than the 0.022 mol mol^{-1} calculated by Stefels et al. (2007) using the Menden-Deuer and Lessard (2000) C conversion equation. Our value of 12.1 mmol mol^{-1} for *A. carterae* CCMP1314 is well above the average 1.8 mmol mol^{-1} reported for the same strain in exponential phase by Keller et al. (1999a); this might result from the large variation in DMSP concentration observed for this strain and the different growth conditions applied by Keller et al. (lower N supply and continuous light exposure).

The DMSP:Chl *a* ratios are provided to allow comparison with field data and provide values for modellers. We obtained an average of 150 mmol g^{-1} (Table 3) which is in agreement with the 111 mmol g^{-1} value calculated by Stefels et al. (2007). Our value of 536 mmol g^{-1} for *S. trochoidea* is above the 177 mmol g^{-1} value obtained by Zhuang et al. (2011) and our average is close to their 105 mmol g^{-1} value for the dinoflagellate *Prorocentrum minimum*, for both cultures sampled in exponential phase (5th day). For *A. carterae*, we obtained 61 mmol g^{-1} which is close to the approximative range of 30–50 mmol g^{-1} plotted by Harada et al. (2009). The DMSP:Chl *a* range (4–536 mmol g^{-1}, Table 3) we found is broader than those for mixed field populations (40–280 mmol g^{-1} in Belviso et al. 2000; Lee et al. 2009 and Archer et al. 2009) and higher than field data for other taxonomic groups (diatoms 2–97 mmol g^{-1} and *Phaeocystis pouchetii* 11–134 mmol g^{-1}, reviewed by Matrai and Vernet 1997). Our DMSP:Chl *a* ratios are also higher than those for *Emiliania huxleyi* cultures (40 and 85 mol - mol^{-1} or 45 and 95 mmol g^{-1}; Bucciarelli et al. 2007). The higher dinoflagellate values may stem from the 55% lower Chl *a* content per C unit reported by Tang (1996) for this taxon in comparison to that for diatoms, prymnesiophytes and chrysophytes. In this context, methods are being developed for the identification of phytoplankton groups from satellite data (Alvain et al. 2008), and this might eventually allow the prediction of phytoplankton-associated DMSP concentrations in the field. However, as yet attempts to relate DMS:Chl ratio to dominant phytoplankton groups have been unsuccessful (Masotti et al. 2010) because the dominant groups identified in satellite pigment data were mistaken for either high or low producers. No increase in DMS:Chl was observed in cruise datasets when the satellite data suggested high producer groups and in several oceanic regions, suggested low producers were associated with higher DMS:Chl ratios. Several reasons may explain this gap: the contribution of non-dominant groups is not considered (Masotti et al. 2010), the phytoplankton groups may be incorrectly divided (e.g. dinoflagellates have been excluded, see Alvain et al. 2005), environmental conditions such as temperature and solar irradiance could affect plankton physiology and DMSP production, and DMS production and removal processes are not taken in account.

The DPEA we measured in these dinoflagellate cultures was variable (0.61–59.73 fmol $cell^{-1}$ h^{-1},

Table 3), though well within the lower end of the range observed for strains of *Emiliania huxleyi* (0.12–750 fmol cell^{-1} h^{-1}; Steinke et al. 1998). We did not optimize the pH for maximal enzyme activity as these authors did, but rather measured DPEA at pH 8.2 which is close to pH of seawater and in accord with the intracellular pH of 8 reported for dinoflagellates (Dason and Colman 2004). Different pH optima have been reported for DPEA in dinoflagellate species: 6–6.5 in *C. cohnii* (Kadota and Ishida 1968), 6.0 in *Alexandrium* cultures (Wolfe et al. 2002), 8.0 in *Symbiodinium microadriaticum* (Yost and Mitchelmore 2009) and 8–8.5 for several cultured species (Harada 2007). The variability in DPEA from different studies may be influenced by the use of different pH conditions, so we recommend the use of a pH close to that of seawater for future phytoplankton screening studies.

Three of the five species which displayed DPEA were axenic. The presence of bacteria in *A. minutum* and *K. foliaceum* cultures means that we cannot exclude the possibility of bacterial DPEA in these cultures. For DPEA measurement in this study, the preparation of the cellular extract started with a filtration through a 2.0 µm pore filter which would have reduced the unattached bacterial population. We note that Wolfe et al. (2002) observed similar DPEA in axenic and non-axenic strains of *Alexandrium*. Though, ideally, confirmation of the origin of the enzymatic activity would necessitate testing axenic cultures of the same strains, we note that DMS production in the sea will always be associated with the whole microbial community and not the dinoflagellates alone.

All globally distributed coastal species, which are of most concern in terms of aquaculture and human health, showed the highest DMSP concentrations and production rates but highly variable DPEA. The multifunctional compound DMSP might benefit the biogeographic expansion of some dinoflagellates. For instance, the osmolyte role of DMSP might be advantageous to species adapting to various saline to brackish environments (Vairavamurthy et al. 1985; Dickson and Kirst 1986), its grazer deterrent properties might induce better resistance to predation (Wolfe et al. 1997), and antioxidant potential might support survival under adverse environmental conditions (Sunda et al. 2002). The temperate to tropical photosynthetic species we studied showed low DMSP concentrations and production rates and low to nil DPEA. The temperate to tropical heterotrophic representative *C. cohnii* had medium DMSP concentration, high DMSP production rate and moderate DPEA. We found the dinoflagellates from estuarine habitats to have the lowest DMSP concentrations and production rates, and no detectable DPEA. The lower salinity of estuarine environments may in part explain a lesser osmolyte requirement and the lower DMSP concentration reported previously (Iverson et al. 1989). The polar dinoflagellate had no detectable DPEA, medium DMSP concentration and low DMSP production rate. Melting sea-ice and increasing polar seawater temperature could affect polar dinoflagellates and their DMSP production in the future. Jin et al. (2007) report on ice melt observations and modelling that demonstrates stratification induced by low salinity of melt waters and the subsequent bloom of phytoplankton released from sea ice. More research on dinoflagellates from polar habitats is clearly warranted.

In this study the large thecate species contained significantly higher DMSP concentrations than the smaller athecate ones. However this difference between thecate and athecate was not verified in our recent synthesis of all the published data for dinoflagellates (Caruana et al. in prep.). Small thecate species showed variable DMSP production rates and lower DPEA than large thecate ones. In contrast with coccolithophores where cell volume is significantly correlated with the quantity of DMSP per cell (Franklin et al. 2010), no obvious correlation was found for the nine dinoflagellate species we studied (Fig. 2c, d). A significant correlation obtained with the non-parametric Spearman test between cell volume and the amount of DMSP per cell for the nine dinoflagellate species was not confirmed by the parametric Pearson test using $\log_{10}$-transformed data.

Input for models

Various scientists have used diagnostic (empirical) models to simulate global DMS emission based on remote sensing data such as Chl *a*, mixed layer depth and solar radiation (e.g. Simó and Dachs 2002; Vallina and Simó 2007). They have been successful in realistically predicting the DMS emission in comparison with observed DMS concentrations and have also reproduced the DMS seasonality (Simó and Dachs 2002; Halloran et al. 2010), including the DMS summer paradox of high DMS and low Chl

a concentrations seen at low latitudes (Vallina and Simó 2007). Moreover a strong, global scale, positive correlation between the solar radiation dose and the DMS concentrations in the upper mixed layer has been identified (Vallina and Simó 2007). However, this global relationship has been questioned by Belviso and Caniaux (2009) who did not find a strong relationship between these two parameters in the northeast Atlantic Ocean. Additionally, they underlined the role of the biological processes involved in DMS production including high plankton-associated DPEA and, for coastal Mediterranean waters, the impact of phytoplankton succession on summer DMS concentrations. Also, these diagnostic models appear limited in simulating DMS concentrations in shelf-seas, equatorial and Antarctic regions (Halloran et al. 2010). This is perhaps not surprising given the decoupling between Chl *a* and DMS (Kettle et al. 1999) and the general lack of correlation between these parameters in field data (e.g. Holligan et al. 1987). The oceanic DMS data accumulated during last decade has recently been used by Lana et al. (2011) to update the database of global DMS concentrations and air-sea fluxes and establish monthly maps for these parameters. This new climatology represents the latest tool for validating DMS models and challenging assessments of the relationship between DMS and environmental and/or biological parameters.

Prognostic (mechanistic) models have been developed to improve the description and understanding of the underlying ecosystem processes that control DMSP and DMS production. The approach has generally been to couple existing ecological models which implement the nutrient-phytoplankton-zooplankton food chain, to DMSP and DMS production. In Gabric et al. (1993, 1999) and Cropp et al. (2007), the phytoplankton component is described as a whole and the DMSP production is parameterized by the γ factor (γ = S-DMSP:N). The γ factors of 0.3 (Gabric et al. 1993) and 0.358 (Cropp et al. 2007) were based on limited data for a few phytoplankton species or measurements of a specific Arctic phytoplankton population (γ = 0.04 and 0.2, Gabric et al. 1999). To achieve better prediction of DMSP concentrations for different phytoplankton populations and areas γ factors are needed that encompass a much wider range of culture and field measurements. Here, we report additional values for this factor (average S-DMSP:N = γ = 0.13, range = 0.005–0.422, Table 3) for the nine dinoflagellate species we studied. At present these types of model do not take the phytoplankton taxonomic variability into account, but this would be necessary to represent a more realistic phytoplankton diversity and succession.

The phytoplankton modules of DMS models have improved with the implementation of different DMSP quotas for various phytoplankton groups. In 1996, van den Berg et al. succeeded in representing the DMS production and variation in the southern North Sea by including six phytoplankton groups, three of which produced DMSP: diatoms, non-grazed flagellates (*Phaeocystsis*) and grazed flagellates. Other parameters needed for the gradual improvement of models are a description of the conversion of DMSP to DMS, exudation rates (Laroche et al. 1999), DPEA (van den Berg et al. 1996), bacterial cleavage and microzooplankton grazing (Archer et al. 2002a). In 2005, Le Quéré et al. proposed a model with 10 plankton functional types (PFT) including phytoplankton and zooplankton classified according to their biogeochemical roles (e.g. coccolithophores as calcifiers, diatoms as silicifiers). In this scheme autotrophic dinoflagellates fall into the "mixed phytoplankton" PFT group and parameterization of this group is based on a DMSP:C production value of 12 mmol mol^{-1} which is the same as that used for the "DMS producers". Our measurement for eight phototrophic dinoflagellates gave an average of 10.4 $\pm$ 0.97 mmol mol^{-1} which supports the estimate of Le Quéré et al. (2005). The autotrophic dinoflagellates in this PFT are considered as inefficient DMS producers lacking DPEA, having no direct impact on the S cycle and no defined biogeochemical role, whereas, here, five of the nine species we tested showed substantial DPEA. Moreover some dinoflagellates contain DMSP at levels comparable to or higher than in *Phaeocystis*, which Le Quéré et al. (2005) included in the "DMS producers" group or "*Emiliania*" which is not included but recognized as affecting the DMS cycle. In view of our results, we suggest the implementation of a separate group of efficient DMSP- and DMS-producing dinoflagellates. DMS cleavage by DMS-producing enzymes could also be included. Similar mechanistic models have been developed for toxin production by dinoflagellates (John and Flynn 2002), and these could be further developed to investigate DMSP and DMS production by dinoflagellate species in coastal areas where blooms occur.

A DMS model was tested by Vogt et al. (2010) with the implementation of five PFTs from Le Quéré et al. (2005) including coccolithophores, diatoms, nanoflagellates (N_2-fixers, DMS producers represented mainly by *Phaeocystis* and mixed phytoplankton represented by autotrophic dinoflagellates and chrysophytes) micro- and mesozooplankton for the prediction of DMSP production. This model was successful in predicting mean DMS concentrations and seasonality at mid and high latitudes and was in reasonable agreement with correlations of 0.47 and 0.62 for the Kettle and Andreae (2000) database and the Global Surface Sea databases (http://saga.pmel.noaa.gov/dms/). Chlorophyll concentrations were underestimated in various areas including the Arctic, North Atlantic and most upwelling regions, and DMS concentrations were underestimated in the Southern Ocean, equatorial Pacific and northern North Atlantic. These limitations might be reduced by further developing the biological component of the DMS model with all the DMSP-containing phytoplankton groups described by Stefels et al. (2007). This would require an increase in the database of DMSP:C data for the key phytoplankton groups and other species-specific data would be needed for various parameters of the model, for example: grazing, cleavage by free DMS-producing enzymes and photolysis (see Vogt et al. 2010).

Heterotrophic dinoflagellates are thought to represent about 50% of the living dinoflagellates i.e. about 1,000 species. A few heterotrophs have been shown to contain DMSP including *Crypthecodinium cohnii*, *Pfiesteria piscicida*, *Pfiesteria schumwayae* (Miller and Belas 2004) and *Protoperidinium pellucidum* (Jean et al. 2005). We found that the DMSP:C ratio of *C. cohnii* was substantially lower than the photosynthetic species investigated here (Tables 2, 3). The capacity for DMSP production in heterotrophic dinoflagellates certainly warrants further investigation given that they may be a large source of DMSP and DMS that needs to be better understood and eventually parameterised in models.

Conclusion

Here, we have provided additional data for C, N, Chl *a*, DMSP content and DPEA for nine dinoflagellate species. We address these data to modellers with a view to implementing the dinoflagellate group in future DMS model investigations.

We confirmed the generally low dinoflagellate growth rates previously described, the exception being *Crypthecodinium cohnii*, an osmotrophic species that grows rapidly on a medium containing glucose as the dominant C source. The relationship between cell volume and C and N content proposed for dinoflagellates was also verified by adding new species to the available dataset.

Wide variability in cell volume and Chl *a* content was evident among the nine species. DMSP content varied over two orders of magnitude and some species contain DMSP at concentrations equivalent to those observed in the well-studied DMSP-producers *Phaeocystis* and *Emiliania*. Some trends have been observed in the DMSP production capacity between the nine dinoflagellates we considered, relative to morphological characteristics, trophic modes and zonal distribution. Small athecate dinoflagellates and a species that harbours haptophyte-like plastids tended to have lower DMSP concentrations than the other species. The dinoflagellate species that have expanded at all latitudes, though not in estuaries, had the highest DMSP concentrations and production rates. More research will be needed to ascertain whether the multifunctional compound DMSP might advantage these dinoflagellates in competition with non-DMSP producing phytoplankton. Temperate to tropical photosynthetic dinoflagellates showed low DMSP concentrations and low production rates and the heterotroph had a medium DMSP concentration and high production rate. The polar representative showed moderate concentration and low production rate. In our dataset the estuarine species tended to contain lower DMSP concentration than the coastal species. A large variability in DPEA was observed irrespective of zonal distribution, though lower activity was found in small athecate species. Comparison with published data highlights the potential for intra- and inter-specific variation in DMSP concentrations and DPEA. It is possible that contrasting dinoflagellate species, with for example 12 or 0.3% of total carbon content as DMSP, may not be using this metabolite in the same way. Further research to elucidate the multifunctional role of this compound for dinoflagellates and other types of phytoplankton is warranted and might explain some of the inter-specific variability observed.

We have presented data here for dinoflagellate species that had not been considered previously in the context of DMSP and DMS-production. In a parallel study we have compiled all the published data on DMSP content and DPEA in dinoflagellates which should allow further exploration of the contribution of this plankton group to DMSP and the sulphur cycle (Caruana et al. in prep.). The dinoflagellates may be a difficult group to parameterise in DMSP and DMS models due to their extreme biological diversity and the wide variability in the DMSP concentrations observed. Further work on this group is warranted to increase our knowledge base. In this regard, the heterotrophic dinoflagellates deserve particular attention because there is insufficient DMSP and DMS-production data available to dictate whether a division between photosynthetic and heterotrophic dinoflagellates will be required.

Dinoflagellate populations are being affected by global change as evidenced by an increase in abundance in the Mediterranean and North Seas (Edwards et al. 2006; Mercado et al. 2007) and earlier seasonal blooming (Edwards and Richardson 2004). Large celled dinoflagellates are more carbon dense than diatoms (Menden-Deuer and Lessard 2000) and their proliferation might alter carbon sequestration patterns in the ocean. Additionally, we currently lack knowledge on DMSP-production by polar dinoflagellates and how this may be affected in the future by melting sea ice. With more species-specific data implemented in mechanistic models it would be possible to predict the likely consequences of the proliferation or extinction of one or more species. The overall consequences for DMSP production, DMS emissions and climate need to be addressed.

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