Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of *Emiliania huxleyi* (Prymnesiophyceae)

Marion van Rijssel*, Winfried W.C. Gieskes

Department of Marine Biology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands

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Abstract

The precursor of the volatile S-compound dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP), is produced by marine microalgae, notably by Prymnesiophyceae. The production of DMSP by an axenic isolate of *Emiliania huxleyi* (Lohm.) Hay et Mohler under different temperature and light conditions was studied as a first step towards understanding the role of DMSP-producing algae in climate regulation. Both light and temperature affected growth rate and cell size in batch cultures, but the concentration of DMSP in the cells was dependent on temperature only: at low temperature DMSP accumulated. This physiological response, assumed to be characteristic of DMSP-producing microalgae in general, is in line with the correlation that has been found between elevated concentrations of the DMS oxidation product MSA in ice core slices corresponding with low sea surface temperatures. Apparently, DMS does not play the role in climate regulation formulated in the CLAW hypothesis that proposes a feedback mechanism in which elevated temperatures lead to an increase in albedo via DMS-derived cloud condensation nuclei.

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1. Introduction

Marine phytoplankton are the primary source of dimethylsulfoniopropionate (DMSP), the precursor of the volatile sulphur compound dimethylsulfide DMS (Bates et al., 1987). In the atmosphere, oxidation products give rise to cloud condensation nuclei that affect cloud albedo. Charlson et al. (1987) hypothesised that global warming as a consequence of increasing emissions of CO₂ and other greenhouse gases could enhance the production of algal biomass and thereby the amount of DMSP and DMS produced (the so-called CLAW hypothesis). The consequent higher ocean emissions of DMS would counteract global warming since an increase in the Earth’s albedo would result in a reduction of surface solar irradiance and thus cooling at the Earth surface.

The effect of changes in temperature and irradiance on pelagic ecosystems has hardly been studied, however. More specifically, data to answer the question what the reaction of DMSP-producing algae would be in a warmer ocean are lacking. Here we focus on effects of temperature and irradiance on growth and DMSP content of *Emiliania huxleyi*. This species was selected because it occasionally dominates the phyto-
plankton as massive blooms in the ocean (Brown and Yoder, 1994) and it is known to produce copious amounts of DMSP (Keller, 1991).

DMSP is a compatible solute that contributes to the osmotic balance of algal cells in sea water (Kirst, 1996) and cellular DMSP content is often found to be coupled to salinity (Dickson et al., 1982; Reed, 1983; Vairavamurthy et al., 1985; Dickson and Kirst, 1987a,b; Karsten et al., 1991a, 1992; Kirst, 1996; Stefels, 2000; Van Rijssel and Buma, 2002). DMSP is most effective as a compatible solute (Groene and Kirst, 1991) at low temperatures (Nishiguchi and Somero, 1992; Karsten et al., 1996), and macroalgae are known to accumulate DMSP in their tissues at low temperatures (Karsten et al., 1990a, 1992, 1996). Microalgae seem to have a similar response to temperature: the amounts of DMSP per cell in *E. huxleyi* (Meyerdiers, 1997) and *Tetraselmis subcordiformis* (Sheets and Rhodes, 1996) are higher at low temperatures. In all these studies the DMSP content is unfortunately expressed on a per cell or per unit chlorophyll-a basis. To understand the role of DMSP as a compatible solute, an expression per cell volume gives more insight into the concentration in the cell and its physiological functions (Keller, 1991; Stefels, 2000).

Higher light intensity and longer day lengths induce elevated DMSP contents in macroalgae (Karsten et al., 1990b, 1991b, 1992), but for microalgae the relation with light is less clear because different expressions for DMSP content (or production rates) are reported in the literature. An example is the study of Wilhelm et al. (1997), who found increasing amounts of DMSP per unit chl-a in *Prymnesium parvum* with increasing irradiance, while the amount of DMSP per cell was independent of irradiance. The production rates of DMSP per unit chl-a by *Phaeocystis* sp. have been reported to be a function of irradiance (Matrai et al., 1995); different light intensities used in the culturing of four other species, including *E. huxleyi*, did not yield significant differences in the amount of DMSP per cell (Keller and Korjeff-Bellows, 1996).

In the field, phytoplankton are usually subjected to short-term fluctuations in light and temperature in the course of a day because of the combination of the rhythm of solar elevation and vertical mixing. Long-term changes in climate are superimposed on this short-term time scale. Here the response of *E. huxleyi* to different temperature and light conditions on short time scales was studied with emphasis on the DMSP concentration of the cells.

2. Methods

2.1. Experimental set-up

An axenic *Emiliania huxleyi* strain L (NIOZ culture collection, Texel), originally isolated from the Oslo Fjord by O.K. Anderson, was inoculated in 0.5 dm$^3$ Erlenmeyer flasks filled with 250 cm$^3$ of the artificial seawater medium described by Veldhuis and Admiraal (1987), except for the vitamins and concentrations of NO$_3^-$ and PO$_4^{3-}$ (88.3 and 3.6 $\mu$M, resp.), which were taken from Guillard (1975). The concentration of bicarbonate was doubled to 4.5 mM to prevent carbon limitation of this coccolithophorid. In one experiment (day-night regime) cotton-plugged Erlenmeyers were placed two by two in cabinets with different temperatures and a photon flux density of 20 $\mu$mol photons m$^{-2}$ s$^{-1}$ (provided by Philips TL20W/33R and measured with a Li-Cor photometer equipped with a Quantum cosine collector) during a 14 h light period each day. To minimise the risk of location artefacts, cabinets were placed in random order and the temperature of the cultures was monitored with Tinytag (Gemini Data) temperature loggers placed next to the cultures. In another experiment (continuous light regime) the Erlenmeyer flasks were placed in two cabinets (set points 8 and 12 °C) with continuous light. Light tubes were mounted high in the cabinet and pairs of flasks were positioned so that they received different irradiance levels. Since the light tubes generated some heat that resulted in a temperature gradient in the cabinet, the temperature of the cultures was monitored as well.

Every day at the same time samples were taken aseptically for analyses of biomass and DMS(P). For comparison some samples were taken at steady state (see Section 3.5.) from a light-limited turbidostat at 15 °C (Stolte et al., 2000). Stock cultures were kept at 15 °C in continuous light or at 12 °C with a L:D 14:10 cycle and checked regularly for contamination with bacteria using Hoechst dye no 33258 (Paul, 1982).
2.2. Analytical procedures

Samples for analysis with an electronic particle analyser (Coulter Counter ZM equipped with channelyser 256 and a counting tube with a 30 μm inlet, Coulter Electronics LTD, Luton, England) were diluted in 0.2 μm filtered medium (2–40 ×) and placed in the dark. Analysis took place within one hour. Particle size was calibrated with latex particles 8.7 μm in diameter (Coulter Electronics LTD, Luton, England). Measurement of the diameter of cells in one sample using a microscope revealed that the coulter counter measurements did not include the coccolithosphere. Growth rates were determined in the early phase of growth by fitting an exponential curve through the biomass versus time data (minimum of 4 measurements) in the exponential phase of growth.

For DMS and DMSP analyses 5 cm³ samples were transferred in 20 cm³ crimp top vials (Chrompack) containing 50 mm³ phosphoric acid (85%). The acid brings the pH below 1.0, which prevents conversion of DMSP into DMS. Vials were immediately sealed with a teflon-lined, butyl rubber septum (Chrompack) and stored at 4 °C in the dark until analysis of the headspace. Vials were incubated in a 30 °C water bath and shaken firmly before injection of a 0.5 cm³ headspace sample into a Packard 437 gas chromatograph with FID detector (Visscher and Van Gemerden, 1991); a Supelpak S column was used instead of a Porapak column. This value gives DMSconc. Then 0.5 cm³ 10 M NaOH was injected into the vial to convert all DMSP into DMS and acrylate overnight. Again, DMS was measured in the headspace (DMStotal). For calibration, DMSP (prepared according to Chambers et al., 1987) standard (0–10 μM) was converted into DMS in the same way as the samples and measured. The detection limit was 0.1 μM and duplicate measurements differed less than 5%. To calibrate DMSconc 0.5 cm³ 10 M HCL in addition to phosphoric acid (0.9%) was added to the calibration solution after alkali conversion to obtain similar conditions as in the samples (same total volume was used). The concentration of DMS was calculated according to DMSP = DMStotal – DMSconc. Since the contribution of DMSconc to DMStotal was less than 3% in the day-night regime experiment (see Results) this value was not measured again for the continuous light regime experiment and only DMStotal was used. For the calculations of DMSP per cell and per cell volume we used the DMS measured in the total culture on the assumption that the contribution of dissolved DMSP to the total was low (Wolfe and Steinke, 1996). Two duplicate control experiments at 4 and 15 °C revealed that less than 6% of the DMSP was in the filtrate of GF/F filtered samples (gravity only).

Regression analysis was performed on growth rate, average cell volume, DMSP per cell and DMSP per volume data. In our experiments, cultures are the primary measurement units (first level). Duplicate cultures were placed side by side in cabinets, with the risk of introducing artefacts caused by unknown factors at the cabinet level (second level). If so, duplicates would in fact be ‘pseudoreplicates’ (and as a result sample size n would collapse to half the number of measurements). Therefore, a type of linear modelling was used (ML3 for Windows, Prosser et al., 1991) that is designed for data with a hierarchical structure and that is able to detect non-random effects due to nesting of data. Only one value per culture was used in a backward procedure with all possible interactions in the first model including non-random effects at the second level. Temperature and light intensity were entered both as linear and as quadratic terms to investigate possible non-linearity. The day-night regime and the continuous light regime were compared using a dummy variable. For testing the significance of variables in the full model the deviance test was used: the change in deviance between the full model and the model without the variable was used as a test statistic with an approximate χ²-distribution with one degree of freedom (Van Duijn, 1999).

3. Results

3.1. Batch culture results

_Emiliania huxleyi_ was inoculated in batch cultures that were incubated at two regimes: a day-night regime (different temperatures) and a continuous light regime (different combinations of both temperature and irradiance). The parameters cell density, cell volume, and the concentrations of DMS and DMSP were measured for each culture. The values for the two batch cultures at 12 °C day-night regime are
presented in Fig. 1. Biomass increased exponentially ($\mu = 0.48 \, \text{d}^{-1}$) until the stationary phase was entered. The concentration of DMSP in the cultures (sum of particulate and soluble DMSP) increased during the exponential phase from 0.4 to 11.7 $\mu$M DMSP and an additional 1 $\mu$M was produced during the stationary phase of growth. The concentration of DMS was low and reached a maximum of 0.9 $\mu$M at the onset of the stationary phase. Since DMS can escape via the cotton plugs in the Erlenmeyer flasks, its concentration has relevance only for the calculation of the amount of DMSP (attempts to estimate the total DMS produced by measuring the non-volatile by-product acrylate in the supernatant according to Noordkamp et al. (2000) failed, probably due to the detection limit (0.5 $\mu$M)). During the exponential phase of growth of all 14 batch cultures with the day-night regime, the contribution of the DMS$_{\text{conc}}$ was only 2.60 ± 0.45% (mean ± SE, $n = 72$) of the DMS$_{\text{total}}$ measured after alkali treatment. The contribution in the end-values was 2.97 ± 0.47% (mean ± SE, $n = 14$) but at the onset of the stationary phase occasionally up to 8% was present as DMS.

3.2. Growth rates

There was a clear effect of temperature on growth of *Emiliania huxleyi* in the day-night regime (Figs. 2A and 3). The growth rate in the exponential phase increased from 0.12 d$^{-1}$ at 4 °C to 0.85 d$^{-1}$ at 23.2 °C. At both 2 and 25 °C there was no growth observed within three weeks (data not shown). In the experiment with the continuous light regime the cultures had different combinations of light intensity and temperature. Cultures in the high temperature cabinet (dark symbols) grew faster than the ones in the lower temperature cabinet (Fig. 2B). Higher light intensities coincided with slightly elevated temperatures. Analysis of variance revealed that there was a significant effect of temperature ($\chi^2 = 27.154, p < 0.00001$) but no effect of light intensity on growth rate ($\chi^2 = 0.041, p = 0.840$). Therefore, the two regimes (continuous light and the day-night regime) could be modeled together to analyse the effect of temperature and regime on growth rate (Fig. 3, Table 1). The best fit was obtained by adding both temperature and its quadratic term (temp$^2$) in the statistical model (temp; $\chi^2 = 33.91, p < 0.0001$, temp$^2$; $\chi^2 = 5.28, p < 0.05$). The growth rates of cultures grown in the continuous light regime were lower than the ones with a day-night regime ($\chi^2 = 9.87, p < 0.005$). The effect of temperature in both regimes did not differ: $\chi^2 = 0.674$, df=2, $p = 0.714$.

3.3. DMSP and cell size in the day-night regime

For the day-night regime cultures the cell size changed quickly after addition of the inoculum (Fig. 2C). For cultures incubated at temperatures above inoculum temperature (12 °C) the cell size decreased

![Fig. 1. *Emiliania huxleyi* grown in batch cultures at 12 °C in day-night regime (14 h, 20 $\mu$mol photons m$^{-2}$ s$^{-1}$ d$^{-1}$). (A) Development of biomass in terms of cell density (●) and total biovolume (○). (B) Concentration of DMSP (●) and DMS (○) in the culture. (C) Average cell volume (▲). Error bars represent standard deviation of duplicate cultures.](image-url)
Fig. 2. Batch cultures of *Emiliania huxleyi* at different regimes. Left panel, day-night regime (symbols key for temperatures in (A)), right panel batches in continuous light regime with different combinations of light intensity and temperature (symbols key for light intensity-temperature combinations in (B)). (A,B) cell density, (C,D) average cell volume, (E,F) DMSP content of cells, (G,H) concentration of DMSP in the cells. Each point represents the average of two batch cultures.
while cultures below this temperature started to produce bigger cells, especially at low temperatures. After the initial change in cell size the cell volume gradually decreased until the stationary phase was reached and the mean cell size started to increase in the stationary phase of growth. The amount of DMSP per cell (Fig. 2E) for the day-night regime cultures was clearly affected by temperature: DMSP per cell decreased five-fold with increasing temperature. With a rather constant amount of DMSP per cell and a changing cell size during growth in batch cultures it is obvious that DMSP per volume was changing, too (Fig. 2G).

It is hard to predict if or when adaptation in these cultures was complete and a constant DMSP per volume was reached. Therefore, the end values of the cultures were used for statistical analysis (Fig. 4). For cell volume, DMSP per cell, and DMSP per volume, significant effects were observed by incorporating both temperature and temperature$^2$ in the statistical models (Table 1, Fig. 4). It is clear that in the stationary phase of growth of these batch cultures the high amount of DMSP in the cells at low temperature is accompanied by a large cell volume. Nevertheless, the DMSP per volume at the low end of the temperature range is twice the concentration found at high temperatures.

The amount of biomass produced (end values) in the cultures that had reached the stationary phase (all except the 4 °C) did not significantly decrease with temperature ($n=12$, $\chi^2=3.73$, $p=0.06$) and was $7.7 \times 10^{10} \pm 0.2 \mu m^3 dm^{-3}$ (mean ± SE).

### 3.4. DMSP and cell size in the continuous light regime

For the continuous light regime there are indications that both temperature and light intensity act on cell size in opposite manners. Cells in the low temperature cabinet were bigger than those in the higher temperature cabinet (Fig. 2D), consistent with the temperature effect observed on cell size in the day-night regime. However, for the low temperature cabinet the culture with 30 $\mu$mol photons m$^{-2}$ s$^{-1}$ at 6 °C (30–6 combination) always had bigger cells than the culture with 17 $\mu$mol photons m$^{-2}$ s$^{-1}$ at the same temperature. In addition, similar cell sizes were

### Table 1

Results of the hierarchical linear modelling of $\mu$, cell volume, amount of DMSP per cell and DMSP per volume

<table>
<thead>
<tr>
<th>$\mu$ (d$^{-1}$)</th>
<th>Regime</th>
<th>Constant</th>
<th>Temp</th>
<th>Temp$^2$</th>
<th>Light</th>
<th>Light$^2$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>L:D</td>
<td>-0.089</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Continuous</td>
<td>-0.145</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume ($\mu m^3$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L:D</td>
<td>96.19</td>
<td>-5.81</td>
<td>0.150</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Continuous</td>
<td>57.30</td>
<td>-1.194</td>
<td>Ns</td>
<td>0.431</td>
<td>-2.5 $\times$ 10$^{-3}$</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>f mol DMSP per cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L:D</td>
<td>37.68</td>
<td>-3.503</td>
<td>9.45 $\times$ 10$^{-2}$</td>
<td></td>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>23.32</td>
<td>-1.196</td>
<td>Ns</td>
<td>0.015</td>
<td>Ns</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>mM DMSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L:D</td>
<td>501.67</td>
<td>-40.60</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
</tr>
</tbody>
</table>

When there was no significant effect of light intensity in the data of the continuous light regime, data of the continuous and L:D regime were pooled and tested together. An effect of regime was then tested using a dummy variable. Tests for significance were at the 0.05 level. No significant interactions between variables were found, nor evidence for non-random effects at the cabinet level. Temp, temperature (°C); light, (μmol photons m$^{-2}$ s$^{-1}$); &, not relevant; Ns, not significant when added to the model.
observed for the 140–8.3 and 17–6 combinations, indicating that a higher light intensity can compensate for the effect of a higher temperature. In the other cabinet the 120–15.2 combination had bigger cells than the 9–12 combination despite the higher temperature. For DMSP per cell the values in the low temperature cabinet were higher than in the higher temperature cabinet (Fig. 2F) again indicating an effect of temperature. Within the cabinets, however, some results suggested that high light intensities have an opposite effect on DMSP per cell: the combination 9–12 had much lower values than the 120–15.2 combination despite the lower temperature. Differences between the cultures in the cabinets became smaller towards the stationary phase of growth (Fig. 2F). The values obtained for DMSP per volume were again higher for the low temperature cabinet than the values in the high temperature cabinet. The different light-temperature combinations had different patterns in time (Fig. 2H).

Statistical analysis of the end values was also performed for the continuous light regimes. For cell volume and DMSP per cell both temperature and light variables were involved (Table 1, Fig. 4). For DMSP per volume only temperature was of importance in a way that was not different from the day-night regime.

![Fig. 4](image-url)

**Fig. 4.** The effect of temperature and light intensity on average cell volume, DMSP per cell and DMSP per volume at the end of the batch culture experiments. The dependent variables were standardised for 20 μmol photons m⁻² s⁻¹ (temperature graphs) and 15 °C (light intensity graphs) using the hierarchical models (Table 1). Each point represents one culture value, (●) day-night regime and (○) continuous light regime.
No effects of light intensity or light regime were found.

### 3.5. Turbidostat steady states

To be able to study the effects of low and high irradiance levels in continuous light on the DMSP content in *Emiliania huxleyi* without temperature effects, two steady states of a light-limited turbidostat at 15 °C were compared (Table 2). Similar to the data obtained in batch cultures the amount of DMSP per cell at 15 °C in low light conditions was about 5 fmol per cell, but at a light intensity of 100 μmol photons m⁻² s⁻¹ there was more DMSP per cell (8.9 versus the 6.9 fmol per cell calculated from the batch culture model) than in batch culture at the same light intensity. Again, with high light intensity, the increase of the amount of DMSP per cell was observed with a concomitant increase in cell size. The DMSP per volume, therefore, did not change as much. DMSP per volume values in the turbidostat were higher than the values obtained in batch cultures and the cell volumes lower.

### 4. Discussion

#### 4.1. Growth rate and cell size

Both temperature and light affected growth rate and cell size of *Emiliania huxleyi* strain L. Within the growth range, temperature had a stimulating effect on growth rates and cells became smaller at higher temperatures as found also by Meyerdierks (1997) for the same *E. huxleyi* strain L. The response of growth was also similar to strain BT-6 described by Watabe and Wilbur (1966). The continuous light regime had a negative influence on the growth rate of strain L compared to the day-night regime (14:10 L:D cycle). The growth rate response of *E. huxleyi* on day length seems to be rather strain specific; Paasche (1967) found a stimulating effect with his strain, whereas Nielsen (1997) measured no effect of day length on the growth rate in strain B92/317. Price et al. (1998) described different responses of strains to continuous light versus a 14:10 L:D regime. The observation of Brand and Guillard (1981) that coastal isolates benefit from continuous light whereas oceanic isolates are harmed by these conditions clearly does not apply for our coastal isolate. There are a number of reports on the stimulating effect of light intensity on growth rate and/or cell volume in *E. huxleyi* (Paasche, 1967, 1999; Brand and Guillard, 1981; Muggli and Harrison, 1996; Nielsen, 1997). Curiously, there was no measurable stimulating effect of light intensity on growth rate in our batch culture experiments. Small differences were perhaps obscured by temperature differences that had a higher impact. However, the growth rate in the turbidostat was clearly higher with high light intensities. Celluar volumes increased with increasing light intensity until an optimum around 80 μmol photons m⁻² s⁻¹ in batch cultures.

<table>
<thead>
<tr>
<th>Light (μmol photons m⁻² s⁻¹)</th>
<th>μ (d⁻¹)</th>
<th>average cell volume (μm³)</th>
<th>DMSP per cell (fmol per cell)</th>
<th>DMSP per volume (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
<td>0.14</td>
<td>25.5</td>
<td>5.03</td>
<td>197</td>
</tr>
<tr>
<td>100</td>
<td>0.65</td>
<td>38.0</td>
<td>8.86</td>
<td>233</td>
</tr>
</tbody>
</table>

#### 4.2. Effects of light on DMSP content

Apparently, DMSP per cell was influenced by light conditions, but this was mostly due to cell size differences. For DMSP expressed as amount per biovolume neither light regime nor light intensity was found to influence batch cultures. Only a small increase was found in the turbidostat data. There is an effect of light on DMSP content in macroalgae (Karsten et al., 1990b, 1991b, 1992) but this does not have to be the case for microalgae. Effects of different light intensities on DMSP per cell were not found in *Prymnesium parvum* (Wilhelm et al., 1997) or in four microalgae, including *E. huxleyi* (Keller and Korjeff-Bellows, 1996; no changes in cell volume were observed). In addition, light is not necessarily coupled to DMSP synthesis, as has been shown for another prymnesiophyte (*Phaeocystis globosa*) that produced DMSP in the dark (Stefels, 1996).

#### 4.3. Effects of temperature on DMSP content

There was a negative effect of temperature on the DMSP content expressed per volume. *Emiliania hux-
*E. huxleyi* seems to adjust its internal DMSP concentration according to the environmental temperature similar to findings in macroalgae (Karsten et al., 1992, 1996). A higher amount of DMSP per cell was reported earlier at low temperatures for *E. huxleyi* (Meyerdierks, 1997) and for the green microalga *Tetraselmis subcordiformis* (Sheets and Rhodes, 1996) although DMSP per volume was not measured. This could indicate that at low temperatures accumulation of DMSP, which is an excellent compatible solute especially at low temperatures (Nishiguchi and Somero, 1992; Karsten et al., 1996), is a mechanism for microalgae as well. Adaptation of the internal concentration upon a temperature change was a rather slow process most likely because DMSP cannot be rapidly synthesised (Stefels, 2000). Perhaps this is the reason why at steady state conditions in the turbidostat the concentrations were slightly higher than observed for the batch cultures that were probably still adapting to the new temperature.

Both the amounts of DMSP per cell and DMSP per volume in this study are in the range of values observed earlier in prymnesiophytes (Keller, 1991; Keller et al., 1999a,b; Stefels, 2000). Values included dissolved DMSP and in the case of the continuous light regime experiment also dissolved DMS. As expected, the amount of dissolved DMS present was usually low (less than 3%) as was found earlier in closed bottle experiments by Wolfe and Steinke (1996). Although not relevant for estimation of the cellular DMSP content, it must be noted that an unknown amount of DMS escaped from our open cultures. In addition, some of the DMSP measured was not part of the cells, also resulting in an overestimation of the cellular DMSP content. A fraction of 6% (not temperature dependent) of the total DMSP passed GF/F filters in control experiments indicating the maximal error since the filtration procedure is also likely to release DMSP from disrupted cells.

The amount of DMSP per cell was rather constant over time in the day-night regime batch cultures. This observation is similar to the observations of a constant amount of DMSP per cell by Turner et al. (1988), Keller (1991) and Matrai and Keller (1994), for other *Emiliania* strains. The steep decline in DMSP per cell observed by Keller and Korjef-Bellows (1996) and by Keller et al. (1999a) at the beginning of batch cultures can be explained if inocula have been used from cultures that were incubated at lower temperatures, and consequently contained cells with a high DMSP concentration. In that case DMSP production will stop at the higher growth temperatures and the DMSP concentration will drop quickly by dilution over the newly formed biomass.

### 4.4. DMS production

DMSP-lyase (the enzyme that cleaves DMSP into DMS and acrylate) and its substrate DMSP are located in different compartments of the cell in *Emiliania huxleyi* (Steinke et al., 1998). This is probably the reason for relatively little DMS being produced directly by *E. huxleyi* (Wolfe and Steinke, 1996). The role of *E. huxleyi* in DMS production in the ecosystem seems to be confined to the production of the precursor. Most of the DMS is produced from DMSP after cell disruption as a result of decay (at the end of the bloom), viral mediated lysis (Bratbak et al., 1995) or grazing (Levasseur et al., 1996; Wolfe and Steinke, 1996). Especially when the bloom collapses and the DMSP is released into the water column, a mismatch in DMSP availability and the bacterial reduced sulphur demand occurs and superfluous DMSP is cleaved into DMS and acrylate facilitating escape of DMS to the atmosphere (Kiene et al., 2000).

### 4.5. Implications for global warming

Since the internal DMSP concentration of *Emiliania huxleyi* was affected by temperature while the total amount of biomass formed was not, the amount of DMSP produced by an *E. huxleyi* bloom is likely to be temperature dependent. The two-fold drop in DMSP per volume found in the temperature range 5–15 °C in cultures is representative of the natural situation: it is the temperature range of the surface waters where *Emiliania* blooms occur (Brown and Yoder, 1994). On the assumption that the response of *E. huxleyi* is characteristic of DMSP-producing microalgae, a decrease rather than an increase of the DMSP concentration in the cells must be expected upon the temperature rise of the ocean now so often discussed under the heading ‘Global Warming’. This result seems to contradict the feedback mechanism proposed by Charlson et al. (1987) that implies Earth’s self regulation (GAIA hypothesis). Note that
only a small fraction of the total DMSP produced in microalgal blooms will escape to the atmosphere, potentially available for cloud induction. The CLAW hypothesis is hard to maintain anyway. For example, changes in processes that determine the abundance of DMSP-producing algae, the activity of DMSP-consuming bacteria, and physical sea-to-air transfer of DMS, all potentially the result of ocean warming, are hard to predict. In other words, ‘Global Warming’ does certainly not necessarily result in increased sea-to-air emission of DMS. Interestingly, our observation of less DMSP production by marine phytoplankton at elevated temperatures is in line with observations of low concentrations of the major oxidation product of DMS methanesulphonate (MSA), in ice cores taken in Antarctica from interglacial periods (Legrand, 1997).

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