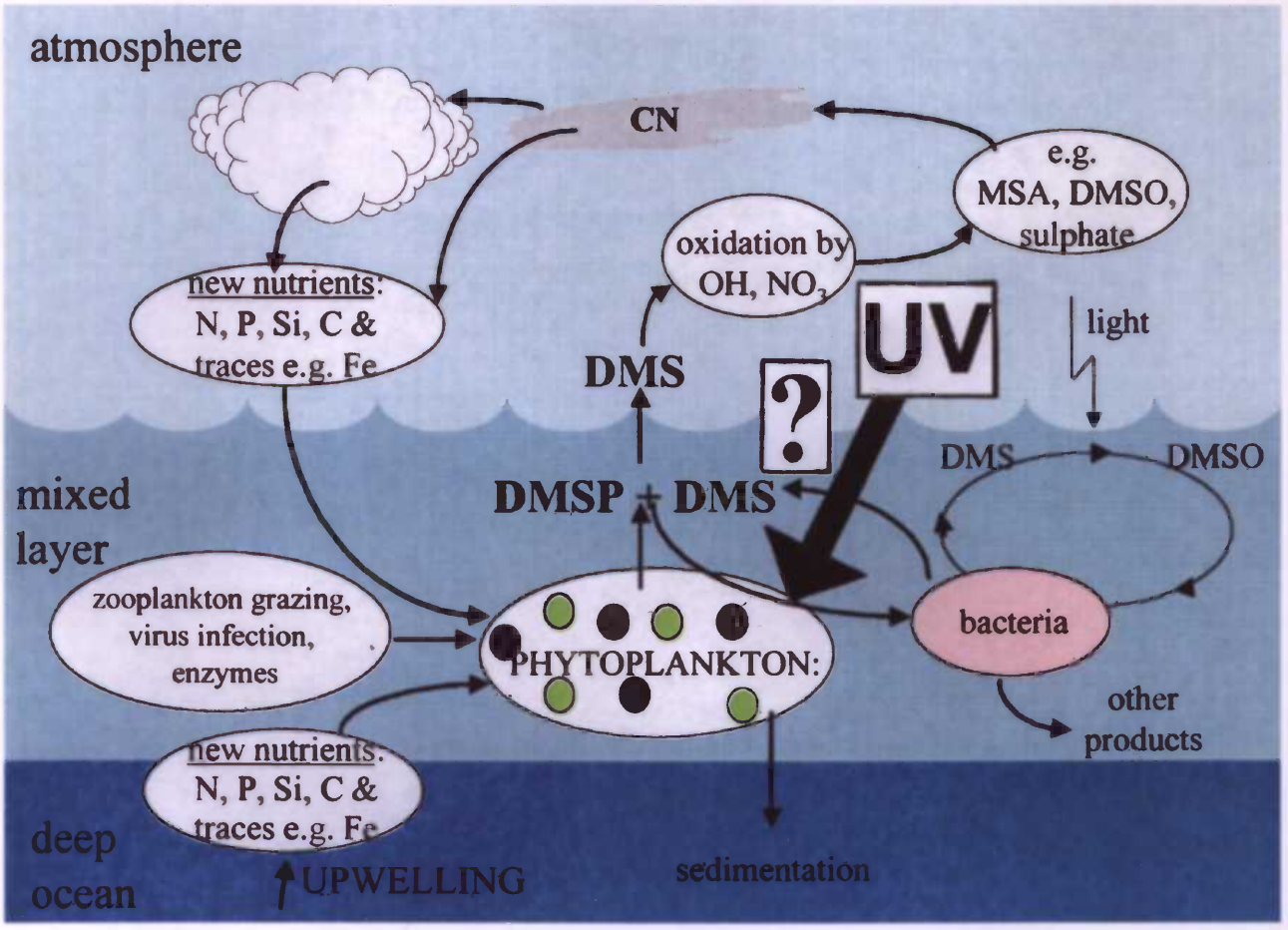
Effect of UV-B radiation on DMSP contents of the coccolithophorid *Emiliania huxleyi*

Sanna Kipinä *)



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Cover picture taken from p. 9 of "IRONAGES", part (B), Application to the 5th Framework programme of the EU. The figure represents DMS and DMSP cycling between ocean and atmosphere.

ABSTRACT

Emiliania huxleyi plays an important role in the global cycle of CO_2, the major greenhouse gas. It is a member of the *Prymnesiophyceae*, the class of marine microalgae that is notorious for its high level of DMSP, an osmolyte that is the precursor of dimethylsulfide (DMS). DMS is a volatile sulphur compound: it is released to the atmosphere when the balance of production, decomposition and transport of DMSP under water is positive. In the atmosphere DMS contributes to cloud formation because its oxidation products are cloud condensation nuclei. *Emiliania* has been said to counteract the greenhouse effect of CO_2 in this way. The other "global change" phenomenon discussed nearly as much as global warming in the last decade is the increase in ultraviolet-B radiation because of depletion of atmospheric ozone. UV-B has a negative effect on phytoplankton performance, therewith limiting its CO_2 uptake potential, but it was not known if the contents of DMSP in algal cells would change under enhanced UV-B radiation. The results of our laboratory experiments suggest that UV-B has no effect on the DMSP contents of *Emiliania huxleyi* cells. It is possible that the longer-wave UV component UV-A has counteracted UV-B effects on growth, cell division and DMSP production. The conclusion from the experiment is that increases in UV-B, expected to continue in the next decades, do not have to be considered in the global sulphur budget regarding DMSP-DMS cycling between sea and atmosphere as far as a major source of DMS is concerned: the DMSP production of *Emiliania huxleyi.*

INTRODUCTION

Global warming and increasing ultraviolet-B radiation ("the hole in the ozone layer") have been major topics of discussion in science and even more so in television programs, on the "Web" and in the popular press during the last few years. Global warming, the consequence of the release of greenhouse gases such as CO_2 by fossil fuel combustion and forest burning, has been said to be counteracted by emission of a natural gas from ocean to the atmosphere, the biogenic dimethylsulfide. This is a volatile sulphur component that enhances cloud formation (Ayers and Gras, 1991); clouds intercept sunlight, so solar warming of the globe decreases. This is in line with the famous "Gaia hypothesis" of Lovelock, who suggested already in 1972 (Lovelock *et al.*, 1972) that an increase in temperature ("global warming" due to over-emission of carbon dioxide) would enhance algal growth in the ocean and therefore production of DMSP, a microalgal cell component that is the precursor of DMS.

Many marine algae are producers of DMSP (dimethylsulfonioprionate). DMSP is a so-called "osmolyte", a substance that helps algae to survive in salt water (see van Rijssel, 2000: Kirst 1989). The DMSP in the algal cell can eventually be released into the water, for example during cell lysis (van Boekel *et al.*, 1992) at the end of a bloom (often induced by viral infection) or by grazing of zooplankton (Kwint *et al.*, 1996). Usually, at the end of an algal bloom bacteria become active. Some are specialists, converting DMSP to acrylate and DMS with lyases (Kiene and Bates, 1990); algae themselves also have these enzymes (Stefels *et al.*, 1995). DMS is soluble in seawater: part of it can escape to the air above the sea, where it produces the typical "smell of the sea" (Stefels, 1997). In the atmosphere, DMS is oxidised to sulfate aerosols, which contribute to the formation of cloud condensation nuclei (CCN) and thus eventually affect cloud albedo (Liss *et al.*, 1994; van Himbergen, 1999). Clouds regulate the amount of sunlight that reaches the Earth, so they have a cooling effect, which in turn should counteract the "greenhouse warming" of CO_2.

Just a few groups of marine phytoplankton produce by far the most DMSP (Keller *et al.*, 1989). The class Prymnesiophyceae harbours species that are notorious for this: *Emiliania huxleyi* and *Phaeocystis globosa,* of which *Emiliania huxleyi* is the most

important in the open ocean. Coccolithophorids, such as *Emiliania huxleyi,* have a world-wide distribution. They are well known for their seasonal abundance, covering vast areas of ocean surface, especially in the North Atlantic (Brown, 1995).

The cells of these microalgae are covered by elaborate calcified structures called coccoliths made of Ca_2CO_3, which can give the ocean a milky-white appearance when there are many, so blooms can be seen in the visible domain of the spectrum by satellite remote sensing due to light scatter from the coccolith platelets (Brown, 1995)(Fig. 1). By this means it has been found that blooms of *Emiliania huxleyi* are typically present during spring and summer time, especially at temperate and subpolar regions and (as already mentioned) particularly in surface waters of the North Atlantic (Ackleson et al. 1988). During bloom events, *Emiliania huxleyi* produce huge amounts of particulate carbon both in organic form (carbohydrates, proteins, lipids and, by the way, DMSP) and as calcium carbonate. After a bloom, cells sink and carbon is partly dissolved underway and partly stored in the sediment. This is known as "the carbon pump". This "pump" transports the carbon fixed by the growing cells near the surface (where light and other conditions favour photosynthesis) to the deep ocean, so in fact atmospheric CO_2 disappears from there into the sea. Thus, phytoplankton (and especially *Emiliania huxleyi*) play a role in climatic regulation: they moderate the greenhouse effect of CO_2 (de Vrind-de Jong *et al.*, 1988).

The ocean output of DMS, which also regulates climate as I have argued, is less well known. One factor thought to regulate DMSP contents of phytoplankton is temperature, an assumption on which the notion of "Gaia" is based, but this is not necessarily a correct assumption; for example what if the amount of DMS and DMSP consuming bacteria increases when the ocean's temperature increases (as expected, the so-called "global warming")? In that case DMS emission would decrease! Also, results have recently been presented that indicate that at higher temperatures the production of DMSP in cells of *Emiliania huxleyi* is lower (van Rijssel *et al.*, 1999).

The other factor mentioned so often in "global change" research is UV radiation. However, the effect of UV-B increase (expected because of stratospheric ozone depletion, which will continue until at least the middle of the next century) has

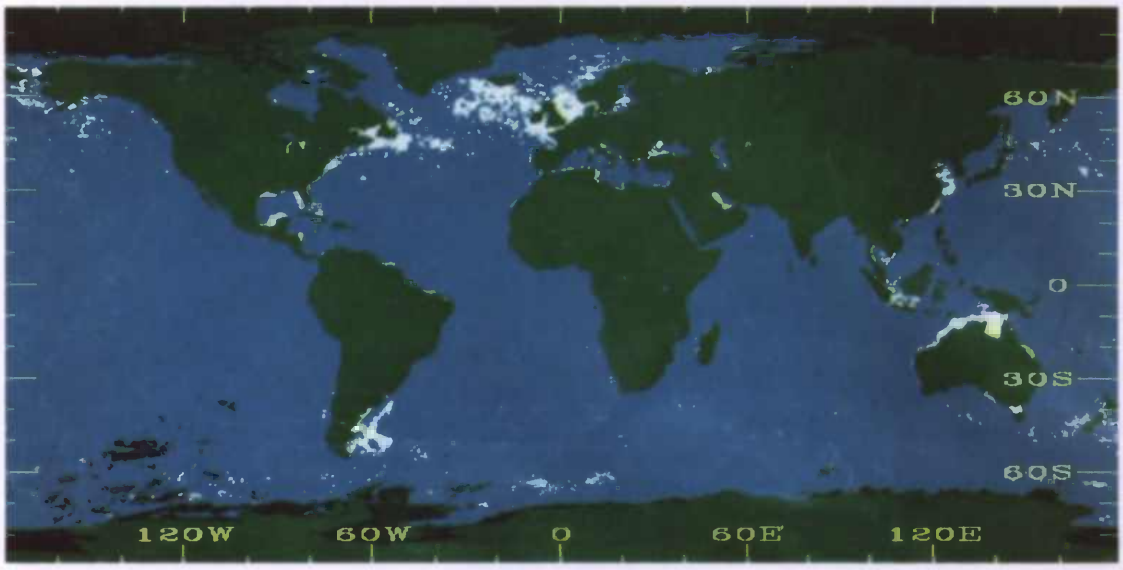

Fig.1. Global distribution of Coccolithophorids. Satellite imagery processed by Christopher W. Brown (Brown and Yoder, 1994)

Fig.2. *Emiliania huxleyi*, mean cell size 5 µm. Electron micrograph from webpage: http://www.soc.soton.ac.uk/SUDO/tt/eh/ (courtesy Todd Tyrrel)

received only a little attention with regard to its influence on DMSP content of microalgae. Hefu and Kirst (1997) have studied the effect of UV-radiation on DMSP content and DMS formation of *Phaecystis antarctica* (Prymnesiophyta) and the results indicate that DMSP contents of that species were reduced by UV- radiation.

We wanted to study effects of UV-B radiation on DMSP contents in *Emiliania* cells. Our hypothesis was that the production of DMSP may depend not only on temperature, but also on that other "global change" phenomenon, UV-B radiation increase. The investigations reported here were done in the framework of the "Dutch Programme for Studies of Climate Change in Relation to Air Pollution" within which a co-operative project is run between different universities and institutes in the Netherlands. Marion van Rijssel and Winfried Gieskes represent the University of Groningen. They contribute to a study of the role of marine phytoplankton (especially *Emiliania huxleyi*) in climate forcing through their influence on DMS emission from ocean to atmosphere.

As is clear from the cover picture, pathways of sulphur cycling are highly complicated. Nearly every step in transfer can conceivably be altered by UV-radiation. In the present report, attention is only paid to the direct influence on DMSP production in algal cells – the central part of the cycling illustrated on the cover.

MATERIAL AND METHODS

Culturing conditions

For comparison of species, algae were cultured in erlenmeyer flasks (100 ml) in a culture cabinet (16 °C, 16 light/8 dark). The medium used was the same as in supplement 1 except that the concentrations of Nitrogen and Phosphorus were 10 times higher. Also an amount of silicate was added (final concentration 10 µM).

Emiliania huxleyi was cultured (not axenically) at 15°C temperature and under continuous light. 9 quartz tubes, each containing 180 ml culture medium (see supplement 1), were inoculated with $1.7x10^8$ cells and placed in a cabinet, with 6 lamps of visible light, "PAR" (photosynthetically active radiation, 140 $\mu mol^{-2}.s^{-1}$), 1 UVB lamp (Philips TL 12) and 3 UVA lamps (Philips 09 N, UVAR ± 5 Wm^{-2}). Cells were exposed PAR lamps were under the cultures and UVA and UVB above, placed in such a way that the UVB lamp was further from the tubes than UVA lamps (Figure 3). Three different kind of cut-off filters were used to cover the cultures: 295 nm, 320 nm and 400 nm, so three of them received only PAR (from 400 nm upwards), three PAR plus UV-A-radiation (from 320 nm up) and the remaining culture tubes PAR, UV-A and UV-B radiation (from 295 nm upwards). In a first experiment, the quartz tubes containing cultures were put on a shelf of the cabinet, in a second experiment a waterbath was used in which the tubes were placed under the water. Experiments lasted four days, with three hours of UV-B treatment per day ($BED_{DNA300nm}$ ± 400 $J.m^{-2}.d^{-1}$), in the first experiment; and three days and four hours of UV-radiation per day in the other. In the second experiment $BED_{DNA300nm}$ was ± 500 $J.m^{-2}.d^{-1}$. Samples were taken immediately after the UV-B radiation was switched off and every day, after taken the samples, places of tubes were changed in such a way that every tube was alternately in the middle of the waterbath.

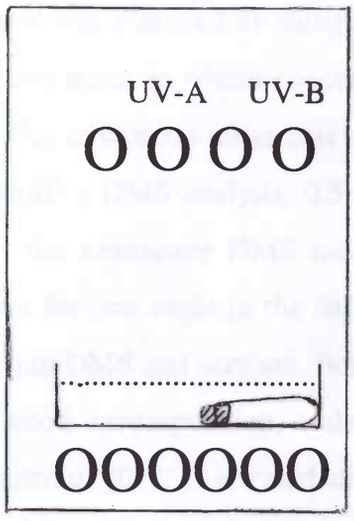

Figure 3. Experiment arrangement. See text for details.

In the third experiment 8 quartz tubes were placed in a waterbath and they received different doses of UV-A radiation (only one UV-A lamp: 1.6 Wm^{-2}) and continuous PAR light (visible light). UV-B radiation was switched off completely. The tubes received UV-A radiation for 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours per day and the experiment lasted two days. Samples for Coulter counter and DMSP measurements were taken before the experiment started and immediately after UV-A radiation treatments (twice, once per day).

Amounts, volume and size of cells were counted in every experiment using an electronic particle counter (Coulter Counter, model ZM, equipped with a Chanalyzer 256) equipped with a 30 µ aperture.

DMS and DMSP analysis

All DMS measurements were carried out using 5 ml samples of the cultures stored in the dark at 4 °C until analysis, in 60 ml glass vials with teflon Mininert valves. 50 µl phosphoric acid was added to the samples immediately after they were taken. This lowers the pH to 1, which is sufficient to prevent enzymatic or chemical conversion of DMSP into DMS (Noordkamp *et al*, 1998). Vials were placed in the dark in a refrigerator (4 °C). DMS (blank) was analysed by taking 0.5 ml of the headspace, which was injected into a Packard 437 gas chromatograph with FID detection equipped with a Supelpak S column (Porapak; Visscher and Van Gemerden, 1991). A

calibration series was prepared by using a 20 µM stock of DMSP, which was diluted with artificial sea water to obtain concentrations of 0.25, 1.0, 2.5, 5.0, 7.5 and 10.0 µM (DMSP). The calibration series was handled in the same way as the samples.

For "total" DMSP + DMS analysis, 0.5 ml 10 M NaOH (final pH 13) was added to the vials after the headspace DMS measurements (see above). The samples were allowed to react for one night in the dark at 4 °C. At pH 13, DMSP is decomposed quantitatively into DMS and acrylate. Before the measurement of DMS resulting from the NaOH-induced decomposition, vials were shaken to establish gas equilibrium; they were adjusted to 30 °C in a waterbath. Notice that DMSP is calculated as being in the particulate form, in other words, in the cells of *Emiliania huxleyi.* The DMSP-dissolved in the cultures was assumed to be ineligible.

The DMSP concentrations were calculated in the following way:

$DMSP = DMSP_{total} - DMS_{blank}$

RESULTS

Eight different species of microalgae were cultured to measure DMSP content of cells. Table 1 shows that there are considerable interspecific differences in DMSP contents. *Emiliania huxleyi* ranks amongst the lowest, as expected on the basis of its size; less expected is the fact that also the diatoms that were tested contain DMSP, as well as the dinoflagellate, *Prorocentrum micans* (cf. Keller *et al.*, 1989).

Table 1. DMSP contents per cell in different kind of species of algae.

SPECIES	DMSP/cell (pmol/cell)
Nitzschia closterium	6956.5
Thalassiosira weissflogii	123.5
Nitzschia sigma	14657.6
Amphiprora paludosa	5450.8
Navicula salinarum	2704
Prorocentrum micans	953626
Navicula spec.	137511
Emiliania huxleyi	265.3

Emiliania huxleyi was cultured for 6 days in continuous light to get an impression of the growth curve of this particular strain. Figure 4 suggests that *Emiliania huxleyi* reaches the exponential growth phase after five days of culturing. From then on it is possible to actually calculate the growth rate by exponential fitting, which gives a culture's growth rate, μ ($y=e^{\mu}$). Figure 4 also summarizes data on the concentration of DMSP (µmol) in the same culture at various growth stages. The concentration of DMSP increases from 0.04 µmol to 10.7 µmol and cell number from 7.2×10^6 to 2.16×10^9 in 6 days (Fig. 4). Using this "total" concentration of DMSP I calculated DMSP content per cell (fmol/cell) and DMSP per unit of cell volume (mM). It can be seen that the concentration of DMSP and amount of cells grow in the same proportion; when cell number suddenly increase in the phase of exponential growth, the concentration of DMSP also rises (Figure 4).

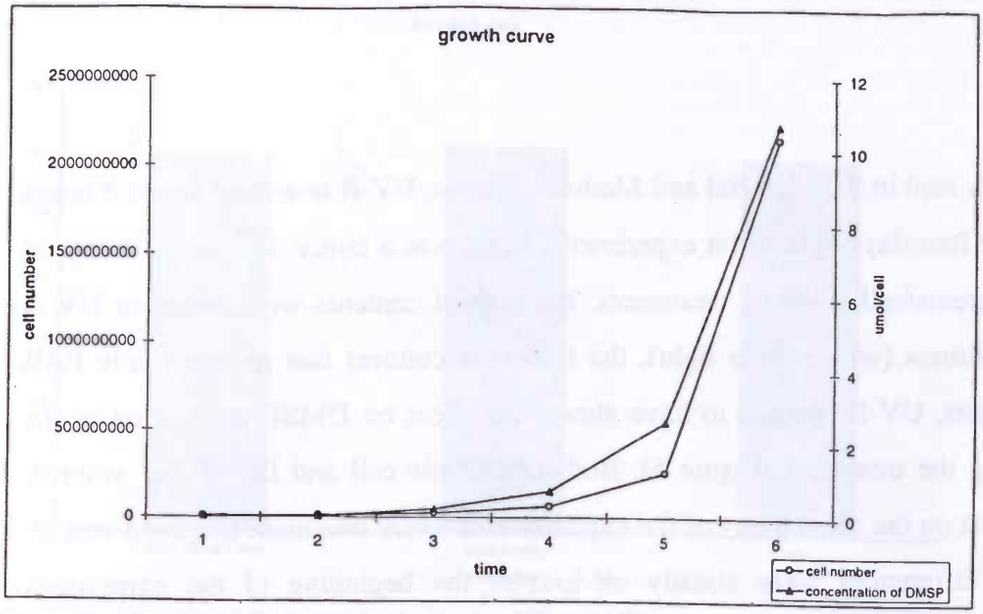

Fig. 4. Growth and DMSP production *Emiliania huxleyi* in batch culture.

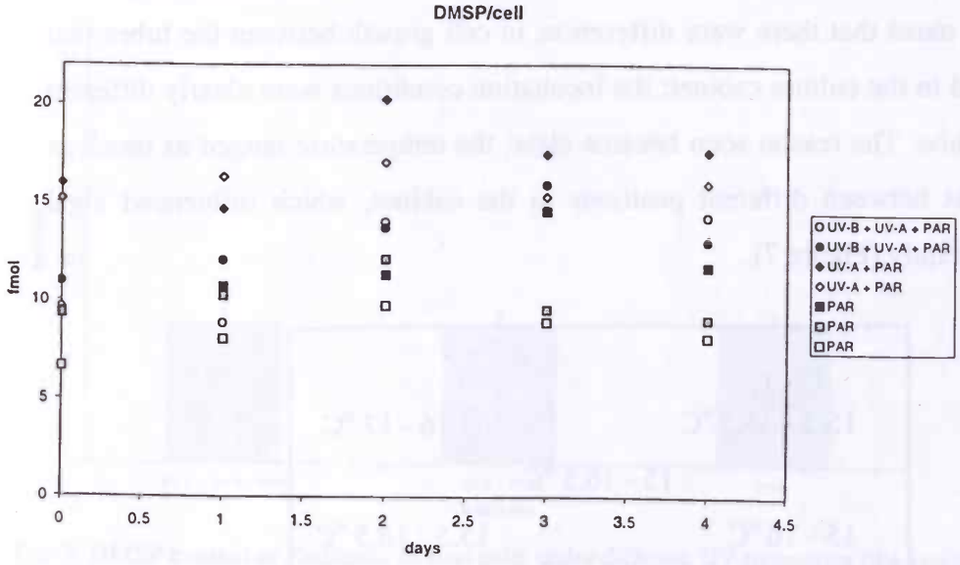

Fig. 5. DMSP content of *Emiliania huxleyi* cells in different UV-treatments.

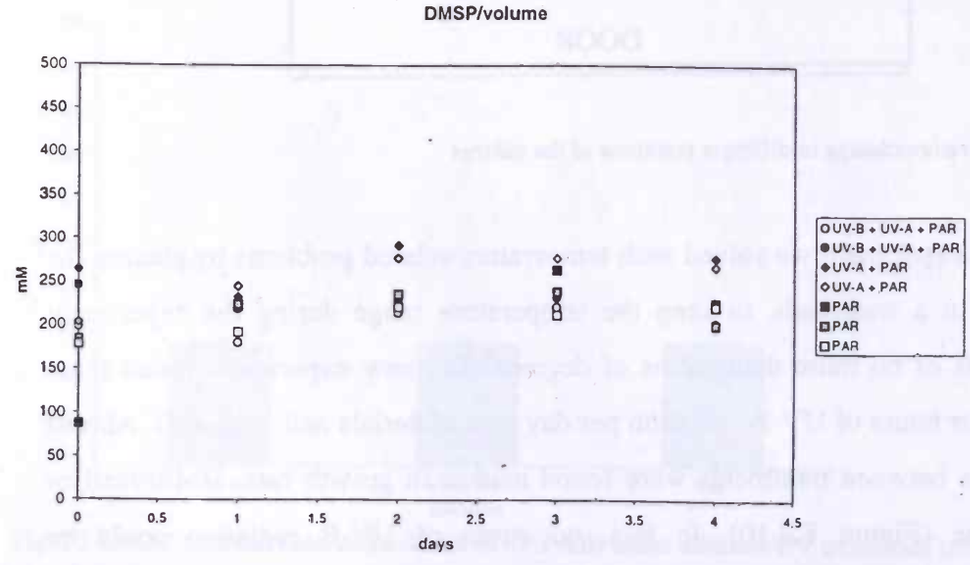

Fig. 6. DMSP concentration in *Emiliania huxleyi* cells in different UV-treatments.

As I already said in the Material and Methods section, UV-B treatment lasted 3 hours per day for four days in the first experiment. There was a minor difference, if any, in DMSP contents/cell between treatments: the highest contents were found in UV-A exposed cultures (with visible light), the lowest in cultures that received only PAR (visible light). UV-B seemed to have almost no effect on DMSP production in the cells during the treatment (Figure 5). Both DMSP per cell and DMSP per volume, were highest on the second day of the experiment in every treatment (Figure 5 and 6). However, differences were already obvious at the beginning of the experiment between treatments, even before the cells were irradiated (Figure 5). The same was noticed also after inspection of changes in DMSP contents per cell volume (Figure 6). That can only mean that there were differences in cell growth between the tubes that were incubated in the culture cabinet: the incubation conditions were clearly different from tube to tube. The reason soon became clear: the temperature ranged as much as several degrees between different positions in the cabinet, which influenced algal growth significantly (Figure 7).

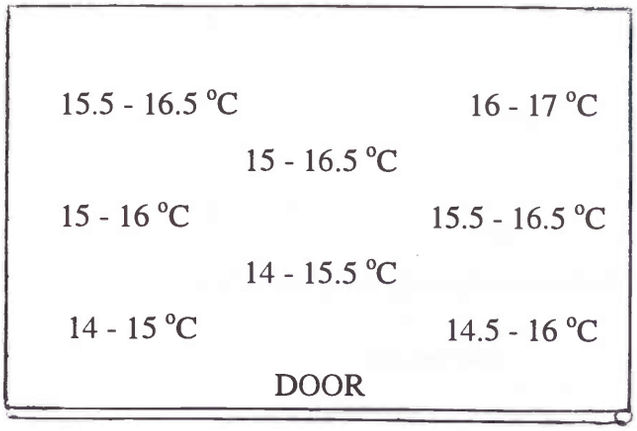

Figure 7. Temperature change in different positions of the cabinet

In the second experiment we solved such temperature-related problems by placing the quartz tubes in a waterbath, to keep the temperature range during the experiment between limits of no more than tenths of degrees. The new experiment lasted three days, with four hours of UV-B radiation per day (see Materials and methods). Almost no differences between treatments were found neither in growth rate, DMSP/cell or DMSP/volume (Figure 8,9,10). In fact, no stress of UV-B radiation could be registered at all - very unexpected because it is known that *Emiliania huxleyi* cells do

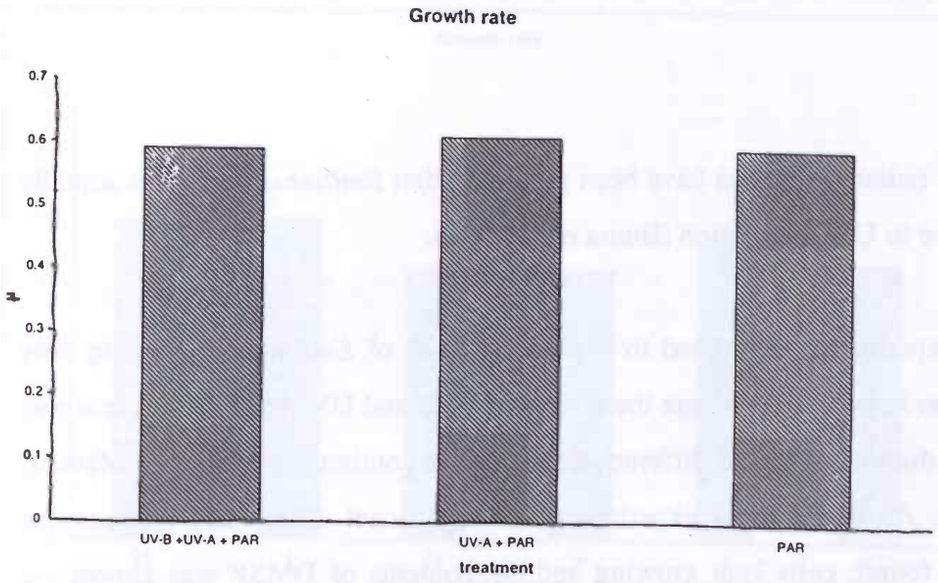

Fig. 8. Growth rates of *Emiliania huxleyi* under different UV treatments (second experiment).

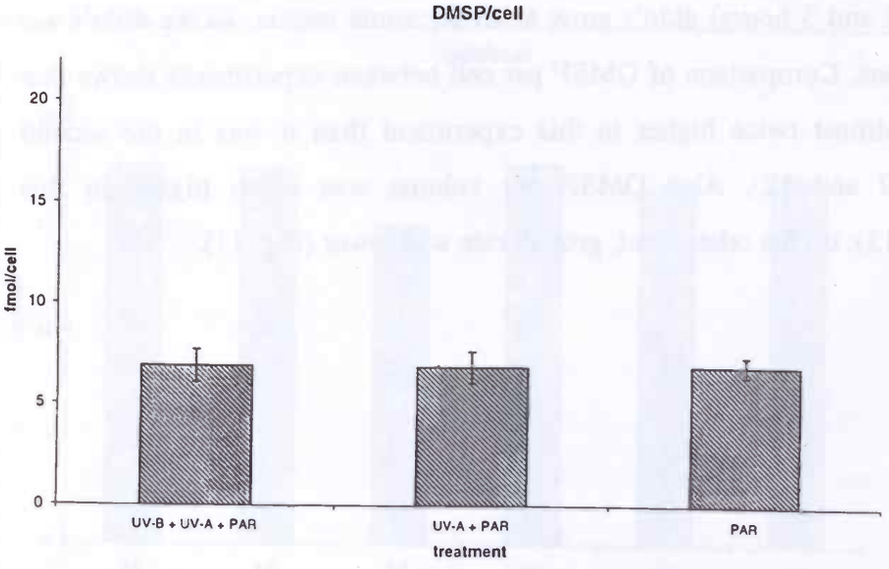

Fig. 9. DMSP content of *Emiliania huxleyi* cells under different UV treatments (the second experiment).

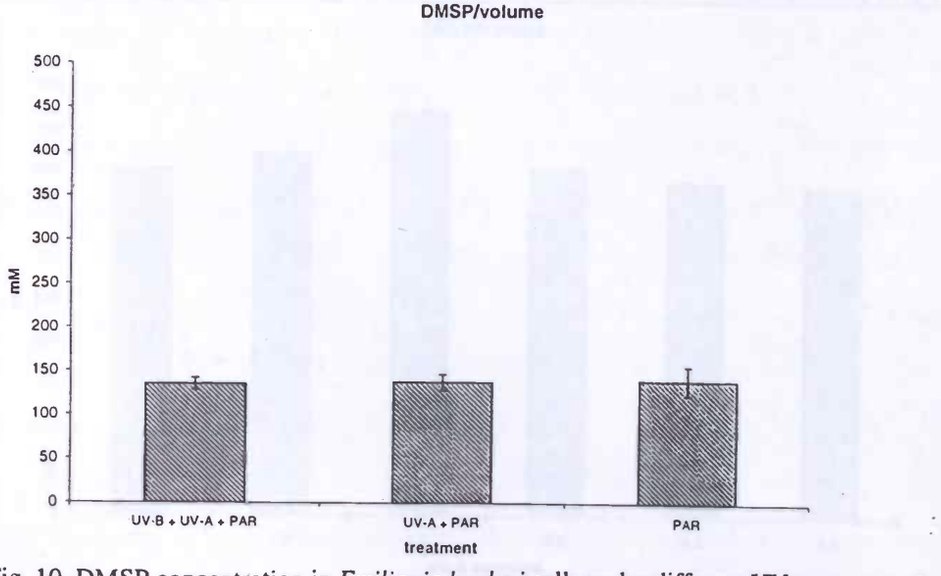

Fig. 10. DMSP concentration in *Emiliania huxleyi* cells under different UV treatments (second experiment).

react to UV-B radiation: results have been presented that *Emiliania huxleyi* is actually highly sensitive to UV-B radiation (Buma *et al.* 1998).

In the third experiment we wanted to expose the cells of *Emiliania huxleyi* to only UV-A radiation in order to compare the effect of UV-B and UV-A. The cells received only UV-A radiation, but with different doses, under continuous PAR (see Material and Methods). As in the other experiments, no significant differences between the cultures were found; cells kept growing and the contents of DMSP was almost the same, no matter if the tubes received 6 or 0.5 hours of UV-A light (Fig. 11, 12 and 13). Two tubes (2 and 3 hours) didn't grow at all for some reason, so we didn't use them in calculations. Comparison of DMSP per cell between experiments shows that DMSP/cell was almost twice higher in this experiment than it was in the second experiment (Fig.9 and 12). Also DMSP per volume was much higher in this experiment (Fig. 13); on the other hand, growth rate was lower (Fig. 11).

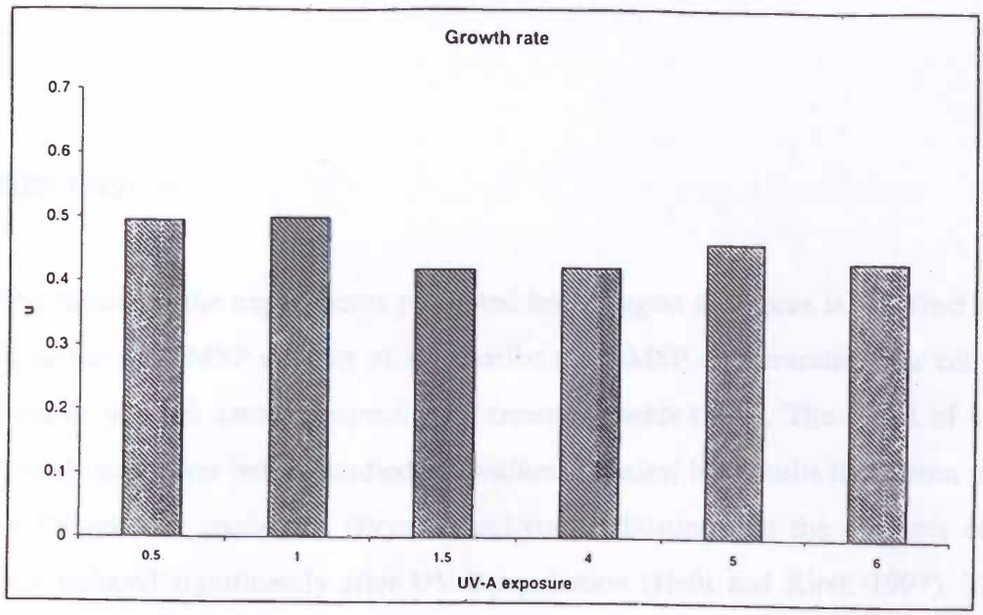

Fig. 11. Growth rate of *Emiliania huxleyi* with different exposure to of UV-A radiation.

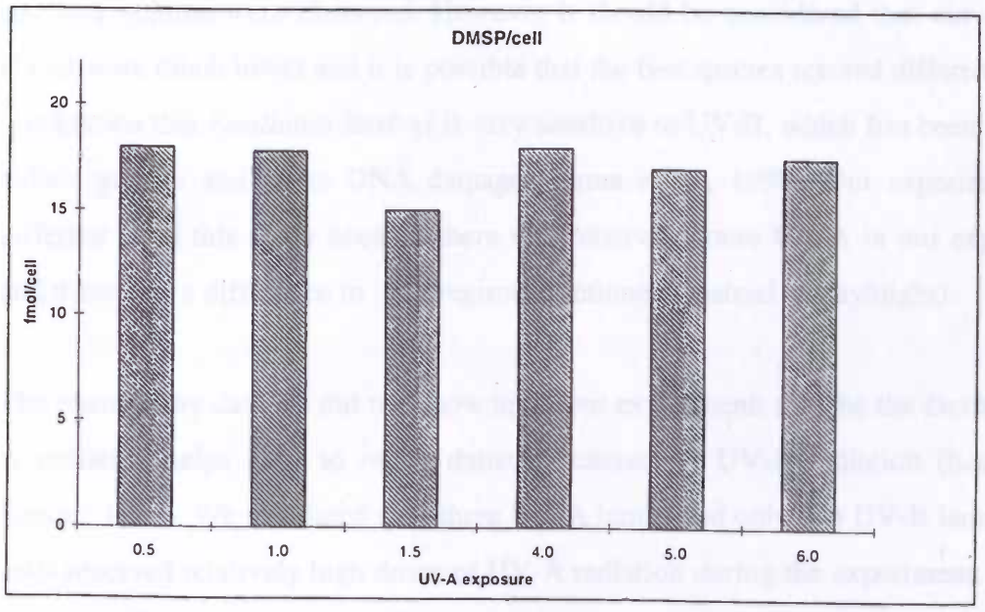

Fig. 12. DMSP content of *Emiliania huxleyi* cells after exposure to different doses of UV-A radiation.

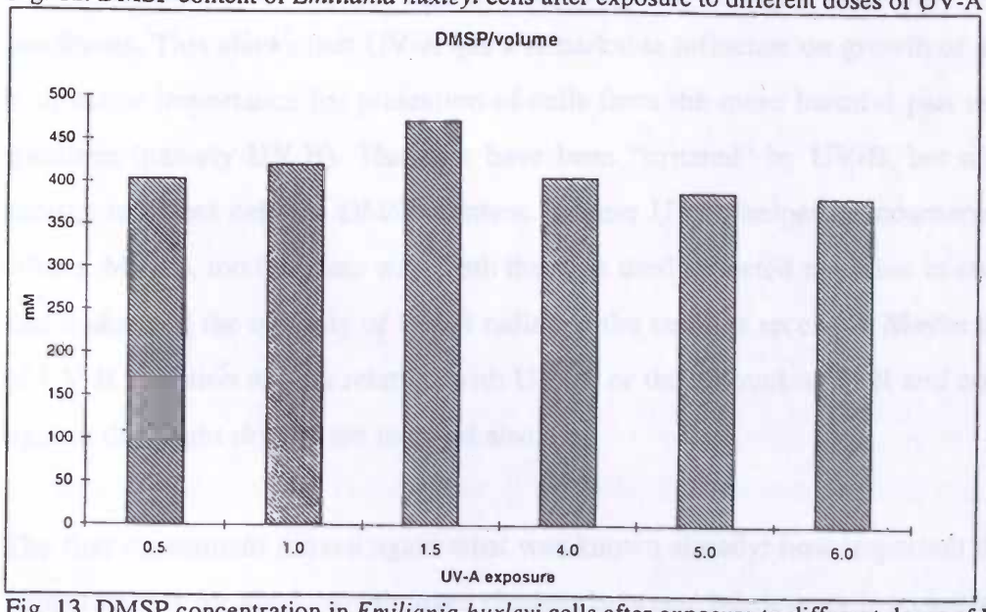

Fig. 13. DMSP concentration in *Emiliania huxleyi* cells after exposure to different doses of UV-A radiation.

DISCUSSION

The results of the experiments presented here suggest that there is no effect of UV-B radiation on DMSP content of algal cells: the DMSP concentration per cell and per volume was the same irrespective of treatment with UV-B. The effect of UV-B on DMSP was never before studied in *Emiliania huxleyi* but results have been presented in *Phaeocystis antarctica* (Prymnesiophyta), indicating that the contents of DMSP was reduced significantly after UV-B irradiation (Hefu and Kirst, 1997). This is in striking contrast with my results: no UV-B effect on DMSP, and even growth, cell size and volume were observed. However it should be considered that our doses of UV-B were much lower and it is possible that the two species reacted differently. Still it is known that *Emiliania huxleyi* is very sensitive to UV-B, which has been found to reduce growth and cause DNA damage (Buma *et al.*, 1998). Our experiment was different from this study because there was relatively more UV-A in our experiment and there was a difference in light regime (continuous instead of day/night).

The reason why damage did not show up in our experiment, may be the fact that UV-A radiation helps cells to repair damages caused by UV-B radiation (Sancar and Sancar, 1988). We irradiated with three UV-A lamps and only one UV-B lamp, so the cells received relatively high doses of UV-A radiation during the experiment. But still UV-A radiation was not as high in this experiment as it would be in natural conditions. This shows that UV-A has a remarkable influence on growth of algae and is of major importance for protection of cells from the more harmful part in the UV spectrum (namely UV-B). The cells have been "irritated" by UV-B, but maybe not enough to affect cellular DMSP content because UV-A helped to counteract UV-B effects. Maybe, too the glass waterbath that was used reflected radiation in such a way that it changed the quantity of UV-B radiation the cultures received. Maybe the doses of UV-B radiation and its relation with UV-A, or the amount of PAR and continuous light or day/night rhythm are relevant also.

The first experiment proved again what was known already: how important the factor temperature is for algal growth; even changes between 1-2 degrees in temperature had much effect, creating differences between cultures. M. van Rijssel (pers. comm.)

noticed before that at lower temperatures DMSP per cell (fmol) and DMSP per volume (mM) are higher with a drop in temperature (see also van Rijssel *et al.*, 1999).

In the third experiment only one UV-A lamp was on (and of course the PAR lamps). The reason why DMSP per cell and per volume were so high compared to the earlier experiments is unclear; this difference cannot be ascribed to UV-A exposure, because the DMSP contents remained almost the same no matter how many hours of UV-A radiation was received by the cells. The calibration curve for DMS(P) in the gas chromatograph looked however, different and values were consequently higher than in the earlier measurements, which may have contributed to relatively high concentrations of DMSP. Also the growth rate was different than in the earlier experiments. It was much lower, which shows that there still might be some problems with the culture cabinet we used, or in experimental set-up. This should be cleared up in future experiments.

In conclusion, with the dose of UV-B radiation that was applied no effect on DMSP content of *Emiliania huxleyi* could be registered. On the other hand, since we were not able to induce any measurable stress caused by the UV-radiation, effects should be studied in more detail before final conclusions can be drawn with respect to changes in DMSP contents of cells elicited by UV radiation. The doses that we applied considering the amount of UV-B were realistic, even the amount of UV-A was not that high as in the field, but obviously still high enough to compensate for the damage by the UV-A radiation dependent repair mechanisms. As long as the cells do not seem to have problems with this regime a stress response related to DMSP, metabolism is not expected and is still unknown if it exists or not.

Acknowledgements

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Supplement 1

MEDIUM FOR *EMILIANIA HUXLEYI*

Seawater substitutes (Veldhuis and admiraal 1987)

	Weight	mw	conc	end conc
NaCl	24.5	58.44	419 mM	419 mM
$MgCl_2 \cdot 6H_2O$	9.8	203.3	48.2	48.2
$CaCl_2 \cdot 2H_2O$	0.53	147.02	3.61	3.61
Na_2SO_4	3.21	142.04	22.6	22.6
K_2SO_4	0.85	174.27	4.88	4.88
All in 1 liter				

Trace elements 1

$Na_2EDTA \cdot 2H_2O$	3.942	336.24	11.72	5.86uM
$FeCl_3 \cdot 6H_2O$	3.15	270.3	11.65	5.83
$CuSO_4$	0.007	159.6	0.04	0.02
$ZnSO_4 \cdot 7H_2O$	0.022	287.54	0.077	0.038
$CoCl_2 \cdot 6H_2O$	0.01	237.93	0.042	0.021
$MnCl_2 \cdot 4H_2O$	0.18	197.91	0.910	0.455
$Na_2MoO_4 \cdot 2H_2O$	0.006	241.95	0.025	0.012
All in 1 liter				

Trace elements 2 (minor salts)

KBr	22	119.01	185	92.4
$SrCl \cdot 6H_2O$	6.39	266.62	23.96	11.98
$AlCl_3$	0.028	133.34	0.21	0.105
RbCl	0.061	120.92	0.50	0.252
LiCl	0.006	42.39	0.14	0.070
KI	0.02	166.01	0.12	0.060
H_3BO_3	1.0	61.83	16.02	8.09
All in 1 liter				

Vitamins (Guillard 1975)

Biotin	0.0002 g/l	0.5 ug/l
Cyanocobalamine B12	0.0002 F/2	0.5
Thiamine HCL	0.04	100

Other stocks (Guillard 1975)

$NaH_2PO_4 \cdot H_2O$	F/20	0.497	137.99	3.6	3.6
KNO_3	F/20	8.928	101.11	88.3	88.3
$NaHCO_3$		56	84	595666.7	4760

Sterilization
All the stocks are autoclaved separately except for the vitamins which are heat liable. The vitamins should be filter sterilized over a 0.2 μ filter.

Composition of the media proceeds as follows:

Prepare a mix containing:

* 5 ml Trace elements 1
* 5 ml Minor salts
* 5 ml Vitamins
* 10 ml NO_3 stock
* 10 ml phosphate stock

For 1 liter medium 3.5 ml mix

Also add carbonate:
For 1 liter medium 8 ml HCO_3