

**Untersuchungen zur Produktion und zum mikrobiellen
Umsatz von β -Dimethylsulphoniopropionat**

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(Wilhelm Gaul)

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1 Einleitung

1.1 Die geochemische Bedeutung des DMSP

Die Produktion des Zellstoffwechsel-kompatiblen Osmolyten β -Dimethylsulphoniopropionat (DMSP, $(\text{CH}_3)_2\text{S}^+(\text{CH}_2)_2\text{COO}^-$) durch marine Planktonalgen hat in den vergangenen Jahrzehnten ein stark zunehmendes Interesse erfahren (Stefels 2000, Kiene *et al.* 2000, Simó 2001), vor allem weil eines der enzymatischen Spaltprodukte des DMSP, das Dimethylsulfid (DMS, $\text{CH}_3)_2\text{S}$) als die häufigste leicht flüchtige Schwefelverbindung des Ozeans identifiziert wurde (Lovelock *et al.* 1972, Andreae 1990). DMS ist die wichtigste biogene Schwefelquelle für die Bildung von Aerosolen und Kondensationskeimen in der Troposphäre und leistet im globalen Schwefelkreislauf den größten Teil des Schwefeltransportes vom Ozean zum Land (Andreae 1990, Brimblecombe und Lein 1989). Die Dichte der im Laufe der DMS-Oxidation in der Atmosphäre gebildeten Schwefel-Aerosole ist ein bedeutender Faktor im globalen Strahlungshaushalt, da sie sowohl die Streuung des kurzwelligen Lichtes in der Troposphäre, als auch die Kondensation und Strahlungseigenschaften von Wolken beeinflusst (Charlson *et al.* 1987, Andreae und Crutzen 1997). Auch steht die atmosphärische Oxidation des DMS durch den Umsatz von Nitrat- und Hydroxyl-Radikalen in engem Zusammenhang mit der Ozon-Bildung in der marinen Grenzschicht (Platt und Le Bras, 1997).

In diesen Eigenschaften des DMS liegt ein großes Potential für Wechselwirkungen zwischen der biologischen Produktion im Ozean und Klimaänderungen. Die Ergebnisse paläoklimatischer Untersuchungen an Eiskernen aus den Polargebieten legen eine generell enge Beziehung zwischen der DMS-Emission des Ozeans und den starken Temperaturschwankungen im Quartär nahe (Legrand *et al.* 1988, 1991, 1997). Ein rezenter Hinweis für die Existenz von Wechselwirkungen, findet sich in der ausgeprägten Saisonalität und der engen Beziehung von DMS-Emissionen und den Aerosol-Konzentrationen in der anthropogen wenig beeinflussten Südhemisphäre (Ayers and Gras 1991, Yvon und Saltzman, 1996).

1.2 Die biologische Funktion des DMSP

Das Interesse am DMSP richtet sich jedoch in jüngerer Zeit nicht nur auf die Rolle als Vorläufer des DMS, sondern auch auf seine zellphysiologische Funktion und seine Bedeutung im mikrobiellen Nahrungsnetz. Hier gibt es bedeutende Fortschritte im Verständnis der Funktion des DMSP in der Zelle (Stefels 2000) und im mikrobiellen Nahrungsnetz (Simó 2001). Zu nennen sind insbesondere die Entdeckung seiner herausragenden Rolle als Kohlenstoff- und Schwefel-Quelle der bakteriellen Produktion (Kiene *et al.* 2000) und der Wirkung des DMSP und einiger seiner Abbauprodukte als intrazellulärer Schutz gegen Sauerstoff- und Hydroxyl-Radikale (Sunda *et al.* 2002).

Viele Stoffwechselleistungen der Zelle werden durch die hohen Konzentrationen von anorganischen Ionen im Meerwasser gehemmt. Unter solchen Bedingungen ersetzen zellstoffwechsel-kompatible organische Osmolyte im Cytosol einen großen Teil der anorganischen Ionen und stabilisieren dadurch die Enzymfunktionen (Kirst, 1989; Groene und Kirst, 1991). Die chemische und die strukturelle Ähnlichkeit zu anderen pflanzlichen Osmolyten wie Glycin-betain und Prolin legt eine Primärfunktion des DMSP in der Osmoregulation sehr nahe (Vairavamurthy *et al.*, 1985; Dickson und Kirst, 1987). Die Bedeutung des DMSP für die Osmoregulation wird besonders deutlich in den hohen sehr veränderlichen DMSP-Gehalten bei Eisalgen, die in ihrem Lebensraum extremen Salzgehaltsschwankungen ausgesetzt sind (Kirst *et al.*, 1991; Kasten *et al.* 1992). Dabei ist die Stabilisierung der Zellfunktionen und Strukturen durch das DMSP auch nahe dem Gefrierpunkt noch wirksam (Nishiguchi und Somero, 1992 in Groene, 1995). Von Stefels (2000) wurde eine interessante Hypothese zur Funktion der DMSP-Produktion als „Überlauf-Mechanismus“ zur Ableitung überschüssiger Mengen an energiereichen organischen Verbindungen vorgeschlagen. Schließlich wurde bei Gröne (1995) eine mögliche Funktion des DMSP in der Methyl-Gruppen Übertragung z.B. bei der Methionin Synthese diskutiert. In diesem Zusammenhang konnten Kiene *et al.* (1999) das DMSP und eines seiner mikrobiellen Abbauprodukte, das Methanethiol als Vorläufer in der bakteriellen Methionin und Protein-Synthese nachweisen.

Darüber hinaus wurde in mehreren Laborstudien für das DMSP eine Wirkung als chemische Abwehr gegen den Fraß durch herbivores Mikrozooplankton beobachtet (Wolfe *et al.* 1997, Strom *et al.* 2003). So wurde bei einigen Stämmen der Coccolithophoride *Emiliana huxleyi* mit hoher DMSP-Lyaseaktivität im Vergleich zu Stämmen mit geringerer Aktivität eine erhebliche Verringerung des Fraßdruckes durch Protozoen beobachtet (Wolfe *et al.* 1997). Die vermutete

Hemmwirkung wurde dabei der Acrylsäure, die bei der enzymatischen Spaltung von DMSP frei wird zugeschrieben. Während einer Blüte von *E. huxleyi* im Nord-Atlantik wurde jedoch beobachtet, dass diese Alge vom Mikrozooplankton bevorzugt konsumiert wurde (Holligan *et al.* 1993). Bei den im Rahmen dieser Studie durchgeführten Experimenten zur Herbivorie des Mikrozooplankton fanden sich jedoch keine Hinweise auf eine Vermeidung des DMSP-produzierenden Phytoplankton in natürlichen Planktongemeinschaften.

Außerhalb des produzierenden Organismus kommt dem DMSP und praktisch sämtlichen Abbauprodukten des DMSP eine Bedeutung als Substrat für den bakteriellen Stoffwechsel zu (Taylor und Kiene 1989, Kiene und Taylor 1989, Yoch 2002). In mehreren Untersuchungen konnte gelöstes DMSP als eine bedeutende organische Kohlenstoff und Schwefel Quelle für Bakterien beschrieben werden, wobei angenommen werden kann, dass der mikrobielle Abbau des gelösten DMSP bis zu 15% des Bedarfs an organischem Kohlenstoff und den nahezu gesamten Bedarf an Schwefel deckte (Kiene und Linn 2000, Zubkov *et al.* 2001, Simó *et al.* 2002). Damit ist das DMSP angesichts der Menge und Komplexität der Zusammensetzung gelöster organischer Stoffe im Meerwasser eines der bedeutendsten bisher bekannten Substrate für das marine Bakterioplankton (Kiene *et al.* 2000).

Erstaunlicherweise gibt es jedoch trotz der Vielfalt der beschriebenen Funktionen des DMSP bislang keine befriedigende Erklärung für die hohen Art-spezifischen Unterschiede in der DMSP-Produktion bei marinen Planktonalgen. Haptophyta und Dinoflagellen wurden häufig als besonders starke DMSP-Produzenten angesehen, jedoch übertreffen die Art- und Stammspezifischen Unterschiede der intrazellulären DMSP-Konzentrationen bei weitem die Klassenspezifischen Unterschiede (Keller *et al.* 1989). Für Diatomeen wurden bislang mit wenigen Ausnahmen nur relativ geringe DMSP-Konzentrationen beschrieben (Keller *et al.* 1989). Hierbei ist jedoch zu berücksichtigen, dass die tatsächlichen zytosolischen Konzentrationen bei vakuolisierten Diatomeen um ein vielfaches höher sein könnten, wenn das intrazelluläre DMSP überwiegend im plasmatischen Teil der Zelle gelöst wäre. Für die Annahme das auch Cyanobakterien DMSP produzieren (Belviso *et al.* 1993, Wilson *et al.* 1998) finden sich jedoch weder bei Keller *et al.* (1989) noch in dieser Untersuchung Nachweise. Auf Grund der hohen Art-spezifischen Variation ist das Verhältnis zwischen den klassischen Indikatoren der Phytoplankton Biomasse und dem partikulären DMSP in der Regel sehr variabel (Leck *et al.* 1990, Belviso *et al.* 2000 und 2001). Ausnahmen bilden Situationen, in denen bedeutende

DMSP-produzierende Arten wie *Phaeocystis globosa* oder *Emiliania huxleyi* die Bestände dominieren oder Blüten bilden (Liss et al. 1994, Malin et al. 1993).

1.3 Ziel der Untersuchungen

Das übergeordnete Ziel aller hier durchgeführten Untersuchungen war es, zu einem besseren Verständnis der Veränderlichkeit der DMS Produktion in natürlichen Planktongemeinschaften beizutragen.

Ausgehend von der DMSP-Produktion des marinen Phytoplanktons wurde ein vereinfachtes konzeptionelles Modell, die „Biologische Schwefel Pumpe“ (Abbildung 1), zur Beschreibung der biologische Prozesskette DMS-Produktion verwendet. Die Schlüssel-Prozesse in der produktiven Linie dieses Modells sind die pflanzliche Produktion, die Feisetzung durch Herbivore und die enzymatische Spaltung zu DMS durch Bakterien. Auf dem Weg vom intrazellulären DMSP zum DMS gibt es jedoch in allen Phasen bedeutende Verluste.

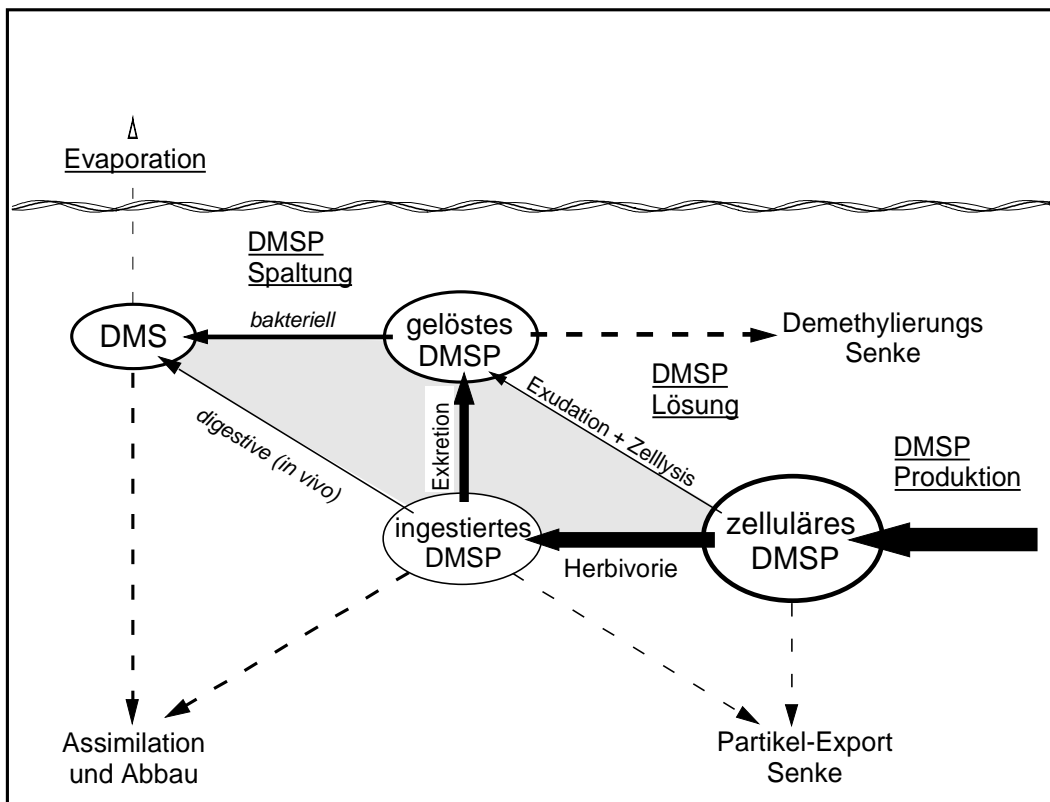


Abbildung 1. Konzeptionelles Model der „Biologischen Schwefel Pumpe“. Durch den Unterhalt des Schwefel-Rücktransportes vom Ozean zum Land ist diese Prozesskette der Produktion, Lösung und Spaltung von DMSP, die biologische Grundlage für einen geschlossenen globalen Schwefelkreislauf.

Ein bislang weitgehend unbekannter Teil des partikulären DMSP geht verloren durch sinkende Zellen, Aggregate und Kotballen des Metazooplanktons (Hatton 2002). Ein ebenfalls kaum bekannter Anteil des von Herbivoren ingestierten DMSP wird assimiliert und abgebaut, ohne dass DMS oder gelöstes DMSP dabei frei werden (Wolfe *et al.* 1994). Und schließlich kann ein sehr variabler Teil sowohl des freigewordenen DMSP als auch des zu DMS gespaltenen DMSP von Bakterien assimiliert oder metabolisiert werden (Kiene 1996, Kiene und Linn 2000, Simó 2000). Im Rahmen dieser Studie wurden die drei Prozessstufen der produktiven Linie im Hinblick auf die vermuteten Schlüsselfaktoren und die Effizienz der Umsätze untersucht. Auf der 1. Stufe wurde besonders die artspezifische und nährstoffbedingte Veränderlichkeit der pflanzlichen DMSP Produktion untersucht. Auf der Stufe der Freisetzung des zellulären DMSP lag der Schwerpunkt auf dem Umsatz durch das herbivore Mikroplankton und auf der 3. Stufe wurde der Einfluss alternativer organischer Kohlenstoff- und Schwefel-Quellen auf den mikrobiellen DMSP-Abbau und die Freisetzung von DMS durch Bakterien untersucht.

Die Ergebnisse der Studie werden im Folgenden in drei Manuskripten in englischer Sprache vorgestellt. Das Kapitel 2 behandelt die Ergebnisse von Laboruntersuchungen zur stickstoffbedingten Veränderlichkeit der DMSP-Produktion in marinen Planktonalgen. Im Kapitel 3 werden die Ergebnisse einer saisonalen Feldstudie zur Produktion und zum mikrobiellen Umsatz von DMSP in natürlichen Planktongemeinschaften der Nordsee vorgestellt und das Kapitel 4 befasst sich mit den Ergebnissen von Feldexperimenten zur nährstoffbedingten Änderungen des mikrobiellen Umsatzes von gelöstem DMSP zu DMS im tropischen Atlantik. Die Ergebnisse dieser Studie werden im Kapitel 5 in einer allgemeinen Zusammenfassung und Schlussbetrachtung dargestellt. Das Kapitel 6 enthält einen kurzen Überblick in deutscher und englischer Sprache.

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2 Nitrogen-related *versus* species-specific variability of the DMSP production in marine phytoplankton

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2.1 Abstract

A total of 37 strains of marine phytoplankton algae were investigated in batch culture experiments for changes of their intracellular concentration of dimethylsulphoniopropionate (DMSP) in response to a decreasing supply of dissolved inorganic nitrogen (DIN). In nine of these strains the production of particulate DMSP was examined in relation to cellular contents of particulate organic carbon (POC) and particulate organic nitrogen (PON) and decreasing concentrations of DIN. In 25 of 32 DMSP-producing algae the intracellular DMSP concentration increased under N-deficient growth conditions. The increase of the intracellular DMSP concentration was, on average, three to four times stronger in diatoms (+ 235%, $n = 10$) than in dinoflagellates (+ 76%, $n = 14$) and coccolithophorids (+ 51% $n = 5$). When cells grew under N-limited conditions, the amount of particulate DMSP they produced per unit PON (DMSP_p/PON ratio) was, on average, 4.5 times higher than under N-replete conditions. The transition from an N-replete to an N-deficient state of the intracellular DMSP concentration and the DMSP_p/PON ratio was quite sharp, and occurred at about 2.5 μM DIN. The N-specific DMSP production (*i.e.* the amount of DMSP produced per micromole PON per day) showed two different responses to decreasing DIN concentration. In six of nine cases it first increased in response to decreasing DIN concentration until a pronounced intermediate maximum was reached between about 0.7 and 2.8 μM DIN and then it sharply decreased. This response was caused by a continuous decline in the growth rate and a relatively stronger increase of the DMSP_p/PON ratio. In the other three cases the decrease in the growth rate was always stronger than the increase of the DMSP_p/PON ratio and resulted in an steady decrease of N-specific DMSP production. Overall we found an enormous species-specific variability (SSV) in the intracellular DMSP concentration (0.3 - 1521 mM), of the DMSP_p/PON ratio (0.014 - 1.16 $\mu\text{mol S } \mu\text{mol}^{-1} \text{N}$) and of the N-specific DMSP production (maxima: 0.007 - 0.603 $\mu\text{mol S } \mu\text{mol}^{-1} \text{N day}^{-1}$), which was about one to two orders of magnitude higher than their corresponding nitrogen-related variabilities (NRV).

However, at the community level the impact of the SSV decreases exponentially with increasing species diversity, and it fully applies only to mono-species blooms. The relatively small NRV, which in general displayed a systematic unidirectional response, would therefore, on a global scale, clearly dominate over the high, but almost entirely random, SSV.

2.2 Introduction

Dimethylsulphoniopropionate (DMSP) is a virtually omnipresent compound throughout the euphotic zone of the ocean because it constitutes a major intracellular solute of most Prymnesiophyceae, Dinophyta and some other marine phytoplankton algae (Keller *et al.* 1989). Dimethylsulphoniopropionate has received increasing interdisciplinary interest, because it is the principal precursor of dimethylsulfide (DMS) (Challenger and Simpson 1948), which dominates the oceanic emission of volatile organic sulphur to the atmosphere (Andreae 1990) and therefore contributes the most of the reactive sulphur to the remote unpolluted atmosphere (Andreae 1990; Ayers *et al.* 1991). The oceanic emissions of DMS constitute a major biogeochemical link between surface ocean processes and the chemistry of the marine atmosphere, due to a high reductive potential of DMS for the sequestration of OH⁻ and NO₃ radicals (Platt and Le Bras 1997) and the subsequent generation of sulphate aerosols and cloud condensation nuclei (Ayers and Gras 1991; Yvon and Saltzman 1996; Andreae and Crutzen 1997).

DMSP has multiple physiological functions; in marine phytoplankton it is one of the major compatible solutes that stabilises enzymatic processes, and regulates osmotic pressure and also serves as a methyl donor in cell metabolism (Kirst 1996, Randal *et al.* 1996, Stefels 2000). It was also found to have a grazing repellent function (Wolfe *et al.* 1997, Strom *et al.* 2003) and might have an ecological side effect as an info-chemical (Steinke *et al.* 2002). Furthermore, DMSP and most of its breakdown products are strong antioxidants that can efficiently scavenge toxic intra cellular hydroxyl and oxygen radicals (Sunda *et al.* 2002). Within marine microbial food webs dissolved DMSP can be a major source of reduced sulphur and carbon for bacterial production (Kiene *et al.* 2000, Simo *et al.* 2002). However, despite progress in the understanding of the physiological significance of DMSP for marine phytoplankton, there are considerable uncertainties in predicting both the variability of its production and the oceanic emissions of DMS to the atmosphere (Kettle *et al.* 1999). This is partly because there is no direct relation between DMS and primary production (Leck *et al.* 1990), chlorophyll-*a* concentration (Andreae 1990; Kiene *et al.* 2000), or taxon-specific marker pigments such as the 19'-hexanoyloxyfucoxanthin of prymnesiophytes (Dacey *et al.* 1998) but primarily because of great uncertainties about the response in DMSP production to changing growth conditions. Nevertheless, ice core records of the past glacial-interglacial cycle show that oceanic DMS emissions were highly sensitive to climatic variability (Legrand *et al.* 1991; Saltzman 1995), although they did not follow the trend proposed in the "planetary thermostat hypothesis" by

Charlson *et al.* (1987). However, only few studies have been addressed to the feedback potential of the major environmental factors on the DMS(P) production of marine phytoplankton (Stefels and van Leeuwe 1998, Keller *et al.* 1999 a, b, van Rijssel and Gieskes 2002, Slezak and Herndl 2003).

The nitrogen concentration was early assumed to affect DMS(P) production, because elevated DMS concentrations were frequently found under meso- and oligotrophic growth conditions. It was suggested that under nitrogen-deficient growth conditions DMSP might substitute its nitrogen-analog osmolytes (*e.g.* glycine-betaine) (Andreae 1986). However, no clear reciprocal relation was found between the cellular content of glycine-betaine and DMSP which is also due to the dominance of DMSP in most investigated algal species (Keller *et al.* 1999 a, b). Nevertheless, the synthesis of DMSP is closely related to the cellular nitrogen metabolism, since the desamination of its amino acid precursor methionine releases reduced nitrogen for other cell functions (Greene 1962; Gage *et al.* 1997, Stefels 2000). Increased levels of intracellular DMSP were frequently reported from cultures of marine phytoplankton under nitrogen-deficient growth conditions (Turner *et al.* 1988; Gröne and Kirst 1992; Matrai and Keller 1994; Stefels and van Boekel 1993). Therefore it appears that the nitrogen supply of phytoplankton growth plays a significant role in the DMSP production.

In this study, we present data on the responses of the specific production of DMSP to decreasing nitrogen concentrations in batch culture experiments for nine different species of marine plankton algae, as well as the nitrogen-related variability (NRV) of the intracellular DMSP concentration for 37 strains of marine plankton algae including dinophytes, haptophytes, diatoms, cyanophytes and one strain each of a cryptophyte, raphidophyte and chrysophyte.

2.3 Materials and methods

Experimental setup. Prior to the experiments all cultures were pre-conditioned for 2 wk to moderate light ($<100 \mu\text{E m}^{-2} \text{s}^{-1}$) and nutrient conditions (about $75 \mu\text{M NO}_3$ and $5 \mu\text{M PO}_4$). The experiments were conducted with non-axenic batch cultures in natural seawater; these were started with inoculates (2.5 % v/v) from the pre-conditioned culture in the exponential growth phase. The experiments were repeated two to four times by starting a new nutrient-replete batch with an inoculate from the nutrient-depleted stationary culture (Figure 1). Cells were kept in suspension by slow rotation of the incubation bottles (2.2 dm³ polycarbonate (Nalgene)). As culture media we used a stock of sterile filtered and autoclaved natural seawater from the North Sea, with a salinity of 32‰. The medium was moderately enriched with nutrient- and vitamin supplements (Guillard 1975), adapted to an N/P ratio of 15:1 according to Redfield *et al.* (1963),

i.e. 30 μM NO_3 and 2 μM PO_4 . Cultures of Bacillariophyceae additionally received 28 μM $\text{Si}(\text{OH})_4$. This amount of nutrients was sufficient for virtually nutrient-replete growth during the early growth phases, while the nitrogen was still exhaustible by the end of the exponential growth phase. Most algae were grown at 16°C in a 12h:12h light-dark cycle. Strains of *Trichodesmium*, *Synechococcus* and *Chromulina* were grown at 22°C in the artificial seawater medium YBCII (Chen *et al.* 1996), with a salinity of 35 ‰. The light intensity inside a medium filled incubation bottle was 84 $\mu\text{E m}^{-2} \text{s}^{-1}$, as measured by a quasi scalar 4π sensor (QSL, Biospherical Instruments Inc.) in the photosynthetically active range. Samples for nutrient analysis, cell cytometry and particulate DMSP (DMSP_p) quantification were taken with syringes *via* screw-lock valve connections on the incubation bottles at intervals of 22 to 74 h. For a subset of 9 algae we additionally monitored the particulate organic carbon (POC) and nitrogen (PON) through the whole growth cycle and replicated the experiments 2 to 4 times for each strain. With each sampling the cultures were checked for contamination by means of phase contrast and epifluorescence microscopy.

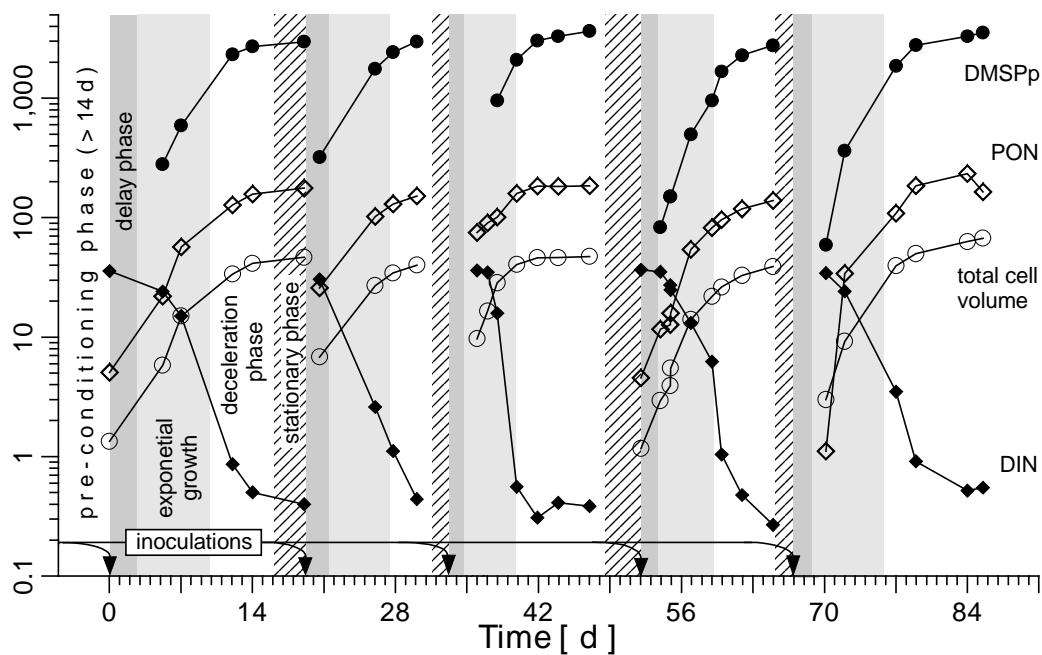


Fig. 1 The coccolithophoride *Calyptrosphaera sphaeroidea* (LB1940): An example for the development of particulate DMSP (DMSP_p , nM), particulate organic nitrogen (PON, μM), dissolved inorganic nitrogen (DIN, μM) and total cell volume ($\mu\text{L dm}^{-3}$) in a series of re-started batch culture growth cycles. The cycles were re-started with inoculates (2.5% volume) from the stationary culture to nutrient-replete medium. **Shadings** show the approximate extension of the growth cycle phases: delay phase (**grey**), exponential phase (**light grey**), deceleration or log phase (**white**) and stationary phase (**hatched**). The time courses of the DMSP_p and DIN and the cell size spectrum were monitored in batch cultures of overall 37 strains of phytoplankton to determine the nutrient-related changes of their intracellular DMSP concentration (Table 1, Figure 5). Nine strains were additionally monitored for PON and POC to determine their N-specific rates and ratios.

Nutrient analysis, cell size and elemental composition. The concentrations of dissolved inorganic nitrogen (DIN, *i.e.* $\Sigma \text{NO}_3, \text{NO}_2, \text{NH}_4$) and dissolved inorganic phosphate (DIP) were analysed manually according to Hansen and Kororleff (1999). Nutrient samples were passed through pre-combusted GF/F filters and were stored frozen in polypropylene bottles at -25°C till analysis. PON and POC were analysed according to Erhard and Koeve (1999). Samples of 30 – 180 ml were filtered on pre-combusted glassfiber-filters (Whatman GF/F). Filters were rinsed for 5 seconds with 10 ml milli-Q water to remove seasalt (without any significant losses in chlorophyll-*a* or particulate DMSP); they were then frozen in liquid N_2 and stored at -25°C till analysis. Carbonate-rich samples (coccolithophorids) were exposed to fuming HCl for 24h to volatilise inorganic carbon. Filters were dried for 24h at 50°C and sealed in tin vials before analysis on a CHN-analyser (EURO EA 3000, HEKA-tech) calibrated with acetanilid. Cell number and cell volume were measured by an electronic particle analyser (Beckmann-Coulter MULTISIZER-II) calibrated with spherical latex beads of 10 and 20 μm diameter. Three sub-samples with a minimum count of 1000 cells were analysed. Some diatoms exceeded the dimensions of the flow capillary and were counted and measured microscopically according to Smetacek (1981).

Analysis of (cell) particulate and intracellular DMSP. The DMSP_p was measured as DMS that releases to the gas phase after alkaline hydrolysis, according to Steinke *et al.* (1998). Sub-samples of 10 - 60 ml from the cell cultures were filtered onto cellulose-acetate membranes (0.2 μm pore size ST 68, Schleicher & Schuell) and were hydrolysed with 4ml of 2N NaOH for 8 - 24h in Teflon-sealed screw cap vials (25ml). The vials were then incubated in a water bath at 60°C for 30 min and were equilibrated by sonication before replicate gas samples of 25 – 250 μl were taken from the headspace by gastight microlitre syringes. The gas samples were directly injected via a Teflon-sealed injection-liner on a gas chromatograph (Varian, GC-6000) equipped with an isothermally (60°C) operated fused silica capillary (Chrompack, CP-Sil 5 CB, 15m x 0,32mm) and a flame photometric detector (Thermoquest, FPD-800). A dilution series of ultra-pure DMSP-HCl (CASS, University of Groningen) dissolved in milli-Q water was used for calibration. The diluted DMSP standards (1 ml aliquots) were hydrolysed together with a blank filter by 3 ml of 2N NaOH in Teflon-sealed screw cap vials (25ml) and were analysed as described above. The calibration coefficients were determined according to Uher (1999). The detection limit for a 250 μl headspace injection was 0.5 pmol DMS. The intracellular DMSP concentration (DMSP_{cell}) was calculated as femtomoles of particulate DMSP per cubic micrometer of cell volume, which is equivalent in short to millimolar values of DMSP.

Osmolytic contribution of intracellular DMSP (ϕ_{DMSP}). This estimate is based on the ratio of the partial osmotic pressure of the cellular DMSP (π_{DMSP}) and the total osmotic pressure of the intracellular solutes (π_i) $\phi_{DMSP} = \pi_{DMSP} / \pi_i$. Osmotic pressure was calculated based on the van't Hoff osmotic equation $\pi = C \cdot R \cdot T \cdot z$ where C = concentration of the solutes, R = gas constant, T = absolute temperature and z = osmotic coefficient of the solute ($z = 1$ for DMSP and $z = 1.9$ for the major solutes of seawater). However, in the following calculation we considered for the true intracellular concentration of DMSP that about 15 % of the cell volume of microalgae consist of non-osmotic and water-insoluble lipophilic cell structures (Dickson and Kirst 1987). We assumed that the total concentration of intracellular solutes is equivalent to the concentration of the major solutes in the growth medium (*i.e.* $0.535 \text{ mol dm}^{-3}$ for natural seawater of 32‰ salinity) plus an excess concentration that causes cell turgor and which is about 10% in marine microalgae (Dickson and Kirst 1987). The estimated osmolytic contribution of intracellular DMSP for growth in natural seawater of 32‰ salinity is therefore calculated by $\phi_{DMSP} = 1.056 \text{ mol}^{-1} \cdot DMSP_{Cell}$.

Responses to changing DIN concentration. At times of high production the nutrient concentration in a batch culture decreases fast and exponentially due to an intense nutrient uptake (Fig. 1). Growth- and cell parameters were therefore usually more closely related to the average DIN concentrations of the previous incubation interval (Equation 2, Appendix). For the cell physiological parameters (intracellular DMSP, C/N ratio, DMSP/PON ratio), this is because they need time for the adaptation to changing growth conditions and display memory effects caused by intracellular nutrient reserves. But, in case of the growth rate and DMSP production, this is simply because their determination is based on a sample interval (in contrast to instantaneous rates based on a point in time). We tried the regression analysis of growth- and cell parameters using DIN concentrations averaged over the past 12h, 24h and 48h of incubation, and received the best fits for DIN concentrations averaged over the preceding 24 h incubation periods. We therefore applied time averaged DIN concentrations in all regression analyses of growth- and cell parameters. Only the DIP/DIN ratio was directly related to the coincident DIN concentration (Fig.2), since both DIN and DIP are instantly and synchronously affected by changes due to nutrient uptake. Equations used in the regression analysis are summarised in the Appendix. Regressions were carried out with the statistic program *SigmaPlot*® version 5.0 (SPSS Inc.).

2.4 Results

The batch cultures were all started with moderate nutrient enrichments at near-optimum ratios of dissolved inorganic nitrogen and phosphorus (Fig. 2). This created virtually nutrient-replete growth conditions during early exponential growth, while the amount of nutrients was still low enough to be exhausted and to terminate the exponential growth phase (Fig. 4). Under nitrogen replete-growth conditions the molar ratio of the particulate organic carbon and nitrogen (C/N ratio) of the cells was generally close to the Redfield C/N ratio (Redfield *et al.* 1963). The C/N ratios show high fluctuations, but started to increase significantly at DIN concentrations $<2 \mu\text{M}$, which indicated the transition to nitrogen-limited growth conditions.

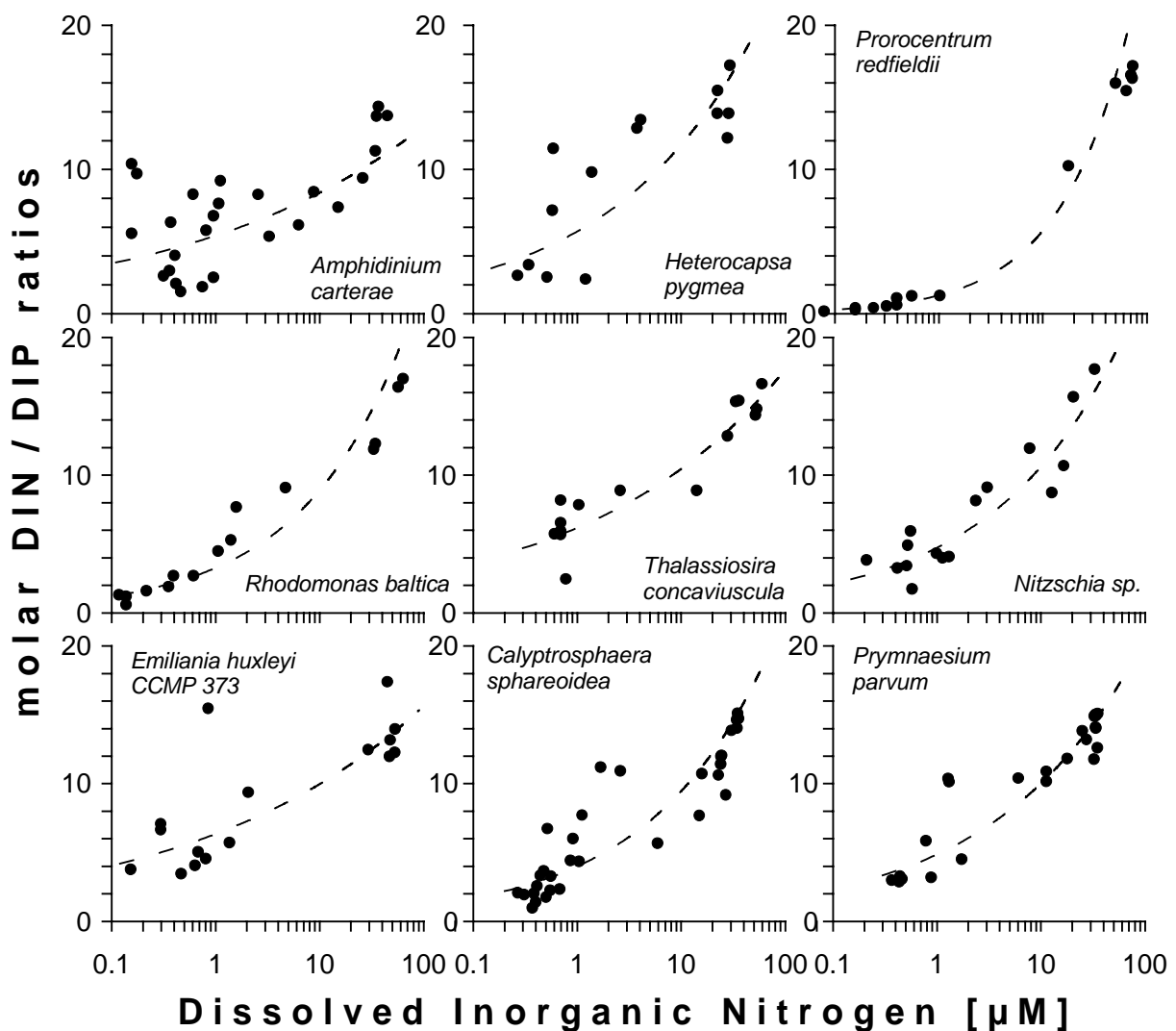


Fig. 2 Elemental composition of the culture medium: The molar ratio of dissolved inorganic nitrogen and phosphorus (*DIN/DIP*) in batch culture media in relation to the (coincident) DIN concentration. Trend lines are regressions by a power function [$y = a x^b$]. Data from the delay and senescence phase are excluded

However, the molar ratio of dissolved inorganic nitrogen to phosphorus appeared to decline immediately without any apparent offset during the depletion of the DIN pool and its accumulation into biomass (Fig. 2). The intracellular concentration of DMSP (DMSP_{cell}) showed a high species-specific variability (SSV) and ranged for eukaryotic algae over three orders of magnitude, from 0.34 mM in *Coscinodiscus wailesii* to 1521 mM in *Prorocentrum redfieldii* (Table 1). The DMSP_{cell} reached almost 100% of the estimated total demand of osmolytes (about 948 mM) in two *Alexandrium* strains and significantly exceeded the required amount of osmolytes in *P. redfieldii* and in swarmers of a *Gyrodinium* strain (ME-127).

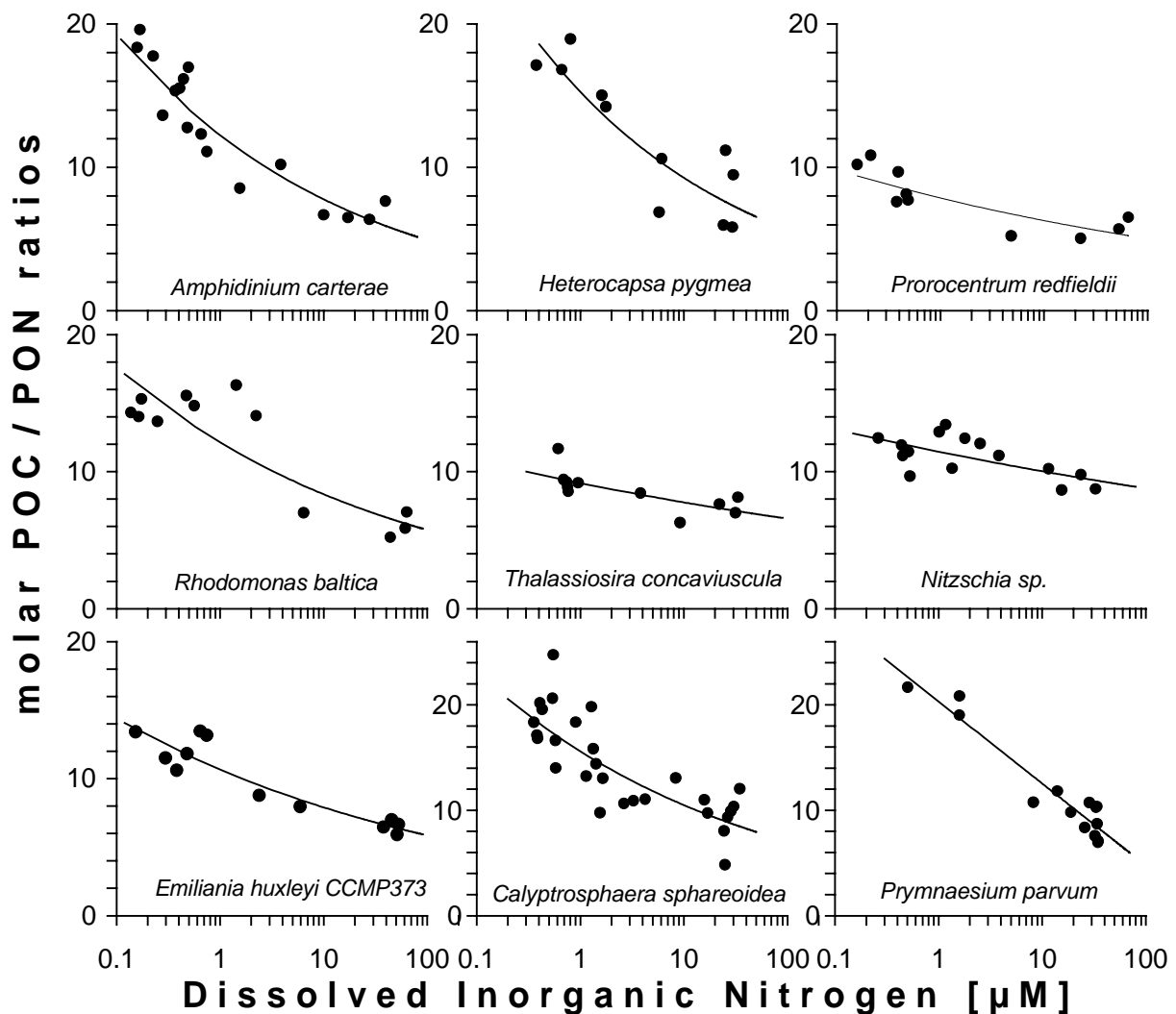


Fig. 3 Elemental composition of the microalgae: The molar ratio of particulate organic carbon and nitrogen (POC/PON) in relation to concentrations of dissolved inorganic nitrogen (DIN), which were averaged over 24h preceding sampling (see “Materials and methods” and Appendix). Trend lines are regressions by a power function [$y = a \cdot x^b$]. Data from the delay and senescence phase are excluded.

Table 1 Taxon-specific variability of the intracellular DMSP concentration (DMSP_{cell}) at high (20μM) and low (0.2 μM) concentrations of dissolved inorganic nitrogen (DIN) and the estimated contribution of DMSP to total cell requirements of osmolytes at high DIN concentration. For calculations see text.

Abbreviations: **CCMP** = Provasoli-Guillard Center for the Culture of Marine Phytoplankton, Bigelow Laboratory, Main USA, **CCAP** = Culture Collection of Algae and Protozoa, Scottish Marine Biological Association, Oban, Scotland, UK, **CCAC** = Culture Collection of Algae at Cologne-University, **ME** = Dr. Malte Elbrächters dinoflagellate collection at the Alfred-Wegener-Institut für Polar- und Meeresforschung (AWI), Germany, **PPC** = Plymouth Marine Laboratory, U.K. **SAG** = Culture Collection of Algae at the University of Göttingen, Germany, **UTEX** = Culture Collection of Algae at the University of Texas at Austin. **n.d.** = not detectable. **Bold printed** strains have been used in the following nitrogen-related experiments.

	strain origin	Intracellular concentration of DMSP		Osmotic contribution of DMSP
		at high DIN	at low DIN	at high DIN
Dinophyta		mM DMSP (fmol DMSP / μm ³)		% of osmotically required solutes
<i>Alexandrium catenella</i>	ME-217, Mediterranean	601	918	63.5
<i>Alexandrium tamarense</i>	ME-181, Orkneys	685	901	72.3
<i>Amphidinium cartearae</i>	SAG 37.80, English Canal	113	317	11.9
<i>Gymnodinium chlorophorum</i>	ME-100, North-Sea	61.9	118	6.5
<i>G. chlorophorum</i> “swarmers”		-	687 ^(a)	72.5 ^(a)
<i>Gyrodinium sp.</i>	ME-127, North-Sea IX. 1999	48.6	44.6	5.1
<i>Gyrodinium sp.</i> “swarmers”		-	1215 ^(a)	128 ^(a)
<i>Heterocapsa pygmea</i>	UTEX LB 2421, Mediterranean	372	782	39.2
<i>Prorocentrum balticum</i>	CCMP 1787, S-Pacific	308	369	32.5
<i>Prorocentrum cassubicum</i>	ME 134, SE-Pacific	127	481	13.4
<i>Prorocentrum mexicanum</i>	ME-268, SE-Indic	28.5	44.5	3.0
<i>Prorocentrum minimum</i>	PPC 18 B, English Canal	199	435	21.0
<i>Prorocentrum minimum</i>	ME-172, NE-Atlantic	231	394	24.4
<i>Prorocentrum redfieldii</i>	ME-138, North-Sea	1521	896	161
<i>Prorocentrum triestinum</i>	ME-142, NE-Atlantic	432	633	45.6
<i>Prorocentrum triestinum</i>	ME-147, Mediterranean	581	872	61.4
Prymnesiophyceae				
<i>Calyptrosphaera sphaeroidea</i>	UTEX LB1940, NE-Atlantic	39.8	65.2	4.2
<i>Emiliana huxleyi</i>	CCMP 373, Sargasso Sea	464	165	49.0
<i>Emiliana huxleyi</i>	CCMP 2112, North-Sea	77.6	134	8.2
<i>Micromonas pusilla</i>	SAG 39.85, English Channel	59	157	6.2
<i>Prymnesium parvum</i>	SAG 127.79, North-Sea	27.4	32.4	2.9
Bacillariophyceae^(c)				
<i>Coscinodiscus granii</i>	North-Sea, III 2001	4.97	9.32	0.52
<i>Coscinodiscus wailesii</i>	North-Sea, V. 2001	0.34	0.67	0.036
<i>Cylindrotheca closterium</i>	North-Sea, III 2001	52.7	180	5.6
<i>Nitzschia spec.</i> ^(b)	North-Sea, III 2001	19.9	28.3	2.1
<i>Odontella aurita</i>	North-Sea, V 2001	7.36	36.8	0.78
<i>Odontella longicornis</i>	North-Sea, III 2001	1.46	3.31	0.15
<i>Thalassiosira angulata</i>	North-Sea, XII 2000	23.9	68.2	2.52
<i>Thalassiosira anguste-lineata</i>	North-Sea, I 2001	9.12	80.8	0.96
<i>Thalassiosira concaviuscula</i>	North-Sea, XII 2000	28.3	120	2.99
<i>Thalassiosira punctigera</i>	North-Sea, XII 2000	4.50	7.20	0.48
<i>Thalassiosira cf. minima</i>	North-Sea, VIII 2001	2.94	-	0.31
<i>Thalassiosira rotula</i>	North-Sea, XII 2000	30.4	-	3.21
Cryptophyta				
<i>Rhodomonas baltica</i>	CCAP 979, English Channel	10.9	45.6	1.2
Raphidophyta				
<i>Haramonas viridis</i>	North-Sea, IX. 2000 ^(d)	2.76	0.81	0.29
Chrysophyceae				
<i>Ochromonas cf. bourellyi</i>	CCAC 1891, Sargasso Sea ^(e)	113	91.4	11.9
Cyanophyceae				
<i>Trichodesmium erytraeum</i>	Sargasso Sea, VIII. 2000	n.d.	n.d.	-
<i>Trichodesmium sp.</i>	CCMP1985, NW-Atlantic	n.d.	n.d.	-
<i>Synechococcus sp.</i>	UTEX LB 2626, Gulf of Mex.	n.d.	n.d.	-

(a) Value refers to stationary growth phase

(b) Epiphytic diatom isolated from a colony of *Phaeocystis globosa*

(c) Isolated and held by H. Halliger, **AWI** Germany

(d) Isolated, described and maintained by M. Hoppenrath, **AWI**, Germany

(e) Isolated from a rough-culture of a *Trichodesmium erytraeum* bloom.

Table 2 Comparison of the maxima of the specific growth rate (μ_{vol}) and the instantaneous rate of DMSP production (μ_{DMSP}). The results are mean values (\pm SD) determined from a minimum of two growth cycles by non-linear regression according to Equations 3 and 4 (Appendix).

	maximum specific rates	
	μ_{vol}	μ_{DMSP}
	d ⁻¹	d ⁻¹
<i>A. cartearae</i>	0.288 \pm 0.03	0.308 \pm 0.05
<i>H. pygmea</i>	0.497 \pm 0.05	0.438 \pm 0.04
<i>P. redfieldii</i>	0.416 \pm 0.05	0.530 \pm 0.07
<i>Rh. baltica</i>	0.926 \pm 0.09	0.746 \pm 0.18
<i>C. closterium</i>	1.19 \pm 0.19	1.11 \pm 0.08
<i>Nitzschia sp.</i>	0.464 \pm 0.10	0.474 \pm 0.10
<i>Th. concaviuscula</i>	1.66 \pm 0.25	1.53 \pm 0.14
<i>E. huxleyi</i> CCMP373	1.27 \pm 0.13	1.95 \pm 0.54
<i>P. parvum</i>	0.535 \pm 0.08	0.67 \pm 0.05
<i>C. sphaeroidea</i>	0.710 \pm 0.09	0.693 \pm 0.08

Table 3 Molar DMSP to particulate organic nitrogen ratio ($DMSP_p/PON$) at high (20 μ M) and low (0.2 μ M) DIN concentrations determined according to Equation 7 and 8 (Appendix).

	$DMSP_p/PON$ (μ mol S / μ mol N)	
	at 20 μ M DIN	at 0.2 μ M DIN
<i>A. carterae</i>	0.039 \pm 0.03	0.403 \pm 0.08
<i>H. pygmea</i>	0.180 \pm 0.05	0.815 \pm 0.12
<i>P. redfieldii</i>	1.163 \pm 0.28	0.629 \pm 0.11
<i>Rh. baltica</i>	0.071 \pm 0.04	0.359 \pm 0.10
<i>Nitzschia spec.</i>	0.011 \pm 0.001	0.061 \pm 0.003
<i>Th. concaviuscula</i>	0.211 \pm 0.06	0.820 \pm 0.14
<i>E. huxleyi</i> CCMP373	0.138 \pm 0.04	0.348 \pm 0.03
<i>P. parvum</i>	0.014 \pm 0.002	0.045 \pm 0.003
<i>C. sphaeroidea</i>	0.027 \pm 0.001	0.112 \pm 0.005

The investigated cyanophyta strains of *Trichodesmium* and *Synechococcus* did not produce any detectable amounts of DMSP. In 26 of the 32 DMSP-producing strains, the $DMSP_{cell}$ significantly increased under low DIN concentrations in the log phase compared to the nitrogen-replete growth conditions in the exponential growth phase. The highest increases of 400 % and 790 % were observed in the diatoms *Odontella aurita* and *Thalassiosira anguste-lineata* (Table 1). However, in 4 of 32 strains, the $DMSP_{cell}$ under nitrogen-replete growth conditions was significantly higher than under nitrogen limited conditions (Table 1 and Fig. 5).

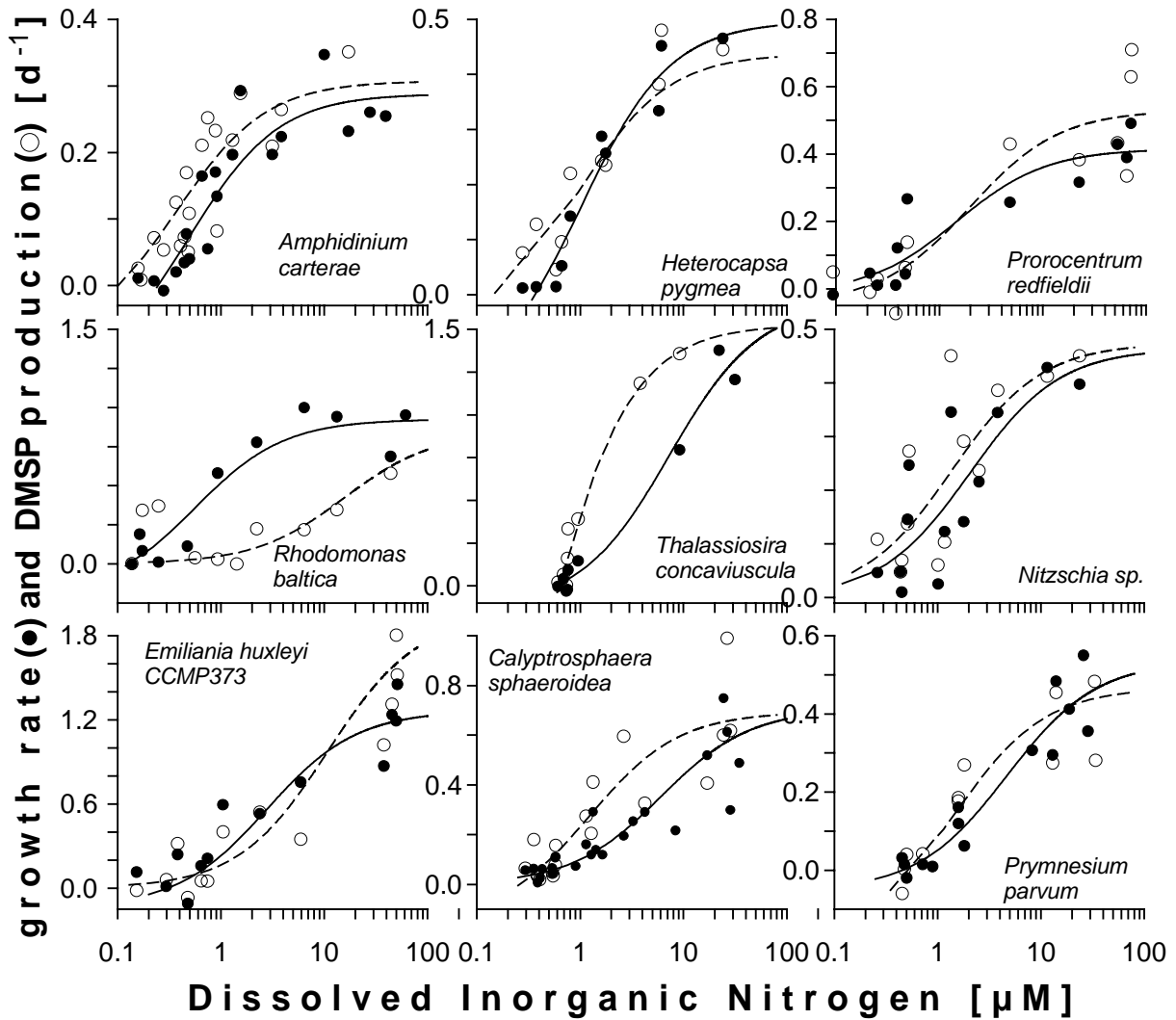


Fig. 4 The specific growth rate (μ_{vol}) (closed circles) and the instantaneous rate of DMSP production (μ_{DMSP}) (open circles) in relation to time-averaged concentrations of dissolved inorganic nitrogen (calculation see “Materials and methods” and Appendix). The calculation of μ_{vol} is based on the increase of total cell-volume (V) during the incubation interval t_n-t_i such that: $\mu_{vol} = 1 / (t_i - t_n) \cdot \ln(V_i / V_n)$, the calculation of μ_{DMSP} is analogously based on the increase of particulate DMSP. *Regression lines:* The relations to the time-averaged DIN concentration (\tilde{N}) are described for both rates by a MONOD-function $\mu(\tilde{N}) = \mu_{max} \cdot (\tilde{N} - s) / K_{1/2} + (\tilde{N} - s)$, which consist of a maximum rate of growth or DMSP production (μ_{max}) as well as a half-saturation concentration ($K_{1/2}$) and a threshold concentration of DIN (s) for growth or DMSP production.

Among these exceptions were *P. redfieldii* and *Emiliana huxleyi* CCMP 373, where the $DMSP_{cell}$ at low DIN concentration decreases to about 59% and 36 % of the $DMSP_{cell}$ under nitrogen-replete growth conditions. The other strain of *E. huxleyi*, CCMP 2112, responded as the majority of the other species and increased its $DMSP_{cell}$ under low DIN concentrations (by a factor of 1.73) compared to under nitrogen-replete conditions.

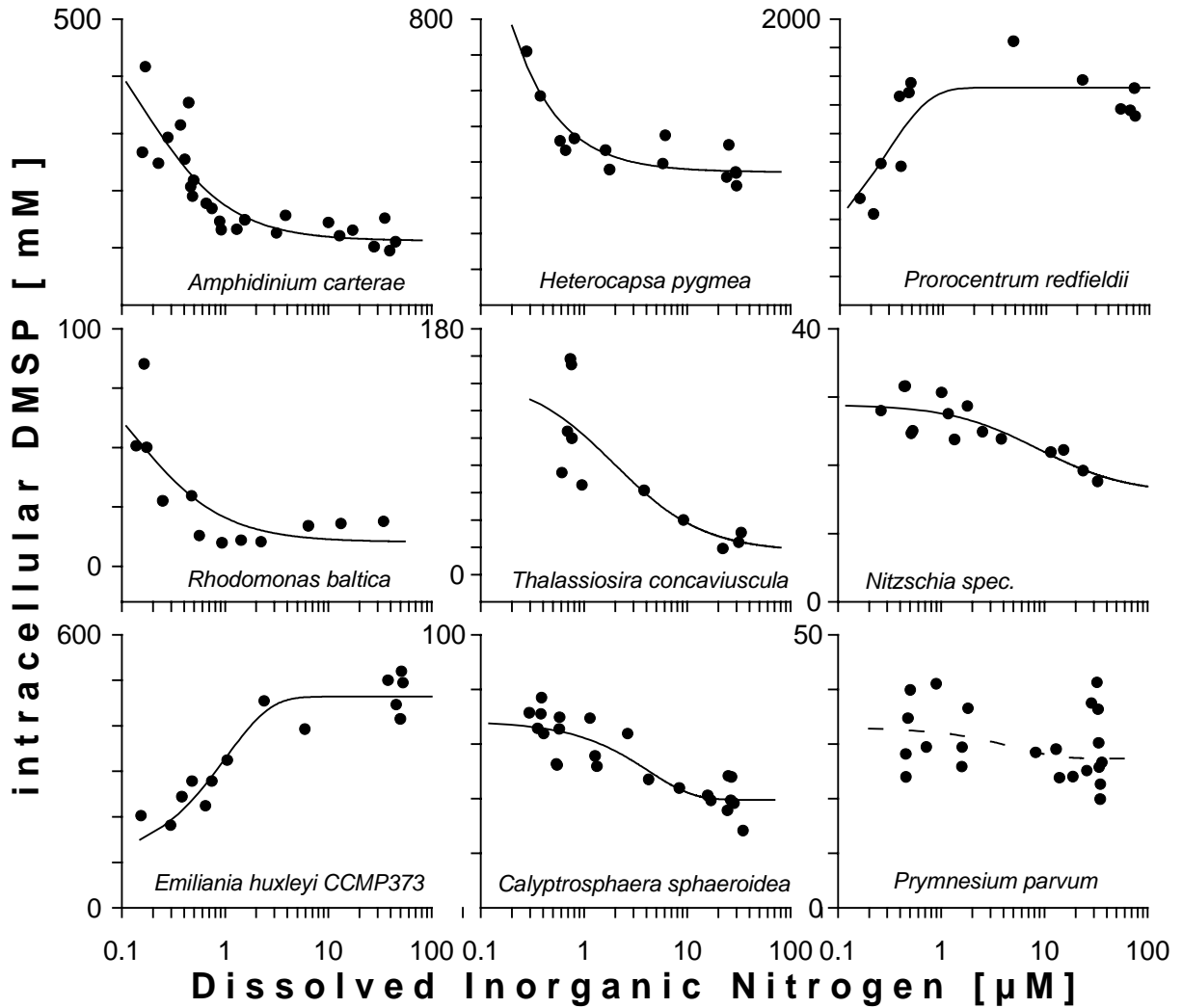


Fig. 5 Intracellular DMSP concentration ($DMSP_{cell}$) in relation to concentrations of dissolved inorganic nitrogen (DIN) which were time-averaged over the 24h prior to sampling (see “Materials and methods” and Appendix). Data from the delay and senescence phase are excluded. **Regression lines:** For a $DMSP_{cell}$ maximum at low nitrogen concentrations (e.g. *Amphidinium carterae*), the increase of $DMSP_{cell}$ at decreasing time-averaged DIN (\tilde{N}), is described by a modified MONOD-function, including a $DMSP_{cell}$ minimum ($DMSP_{min\ cell}$) and a maximum $DMSP_{cell}$ change ($\delta DMSP_{cell}$) between nitrogen-replete and nitrogen-deficient growth: $DMSP_{cell}(\tilde{N}) = DMSP_{min\ cell} + \delta DMSP_{cell} \cdot \left(\frac{k}{\tilde{N} + k} \right)$. For a $DMSP_{cell}$ maximum under nitrogen-replete conditions (e.g. *Prorocentrum redfieldii*), the decline of $DMSP_{cell}$ at decreasing DIN is described by an exponential function: $DMSP_{cell}(\tilde{N}) = DMSP_{min\ cell} + \delta DMSP_{cell} \cdot (1 - e^{-k \cdot \tilde{N}})$.

In the dinoflagellates *Gymnodinium chlorophorum* and *Gyrodinium spec.* (ME-127) swarmers were formed under nutrient-depleted conditions in the stationary growth phase. These swarmers had significantly higher intracellular DMSP concentrations than the usual (asexual) dinoflagellates. The $DMSP_{cell}$ in the swarmers of *Gyrodinium spec.* (ME-127) even exceeded the osmotically required amount of intracellular solutes (Table 1). Our estimates of the rate of

DMSP production are exclusively based on changes in the particulate DMSP, and did not include the exudation of dissolved DMSP to the medium. They are therefore a minimum estimates for the total DMSP production.

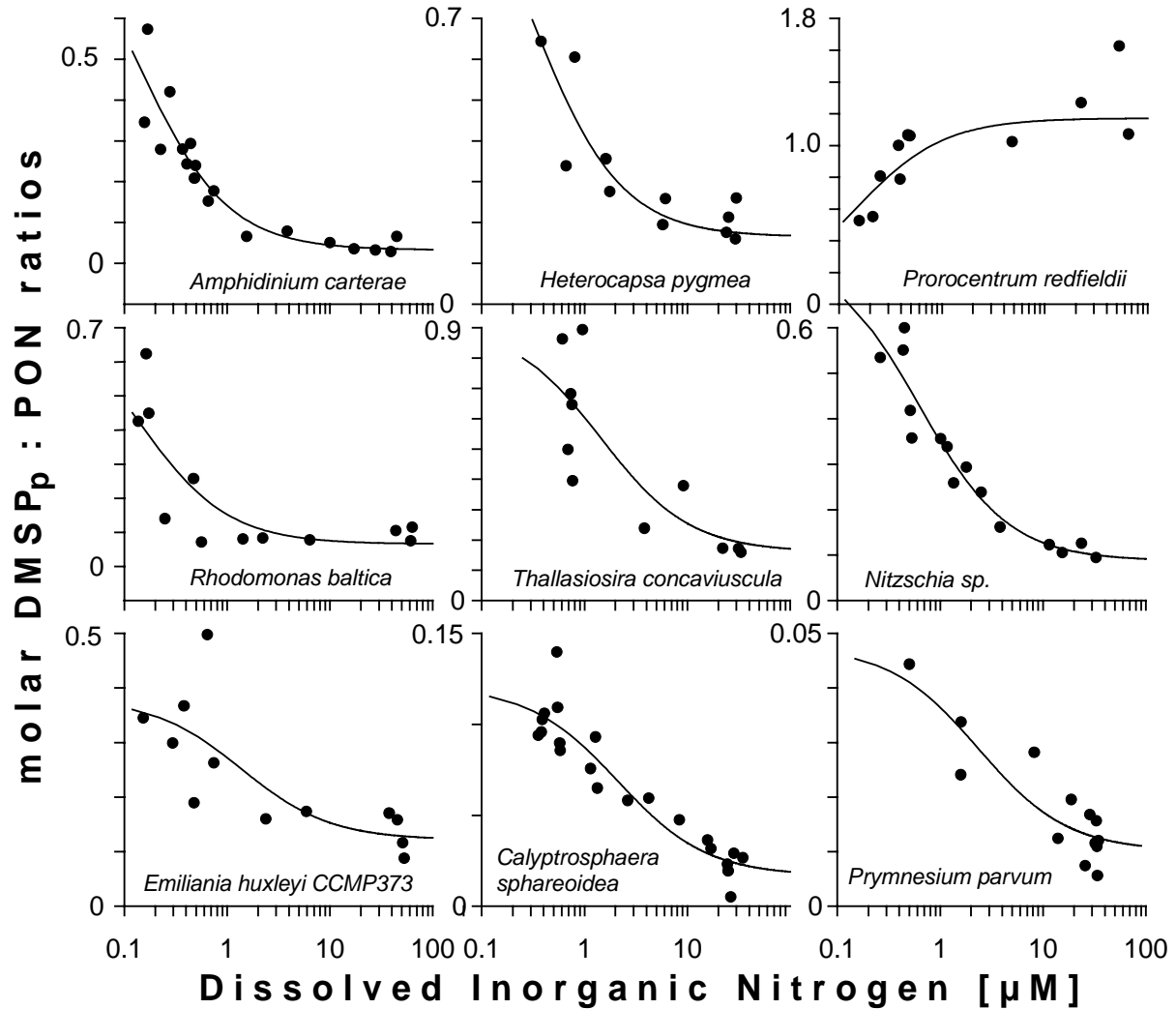


Fig. 6 Molar ratio of the particulate DMSP and particulate organic nitrogen ($DMSP_p / PON$) in relation to concentrations of dissolved inorganic nitrogen (DIN) averaged over the 24h prior to sampling (see “Materials and methods” and Appendix). Data from the delay and senescence phase are excluded. *Regression lines*: the two different responses are described by modified MONOD-functions. For a minimum ratio (R_{min}) at high DIN concentrations, the response is described by $DMSP_p / PON(\tilde{N}) = R_{min} + \delta R \cdot k / (k + \tilde{N})$ where δR is the maximum increase of the ratio. If the minimum was at a low DIN concentration (*Prorocentrum redfieldii*), the response is described by $DMSP_p / PON(\tilde{N}) = R_{min} + \delta R \cdot \tilde{N} / (k + \tilde{N})$.

However, significant exudation was observed only during the late stationary phases (data not presented) which was generally excluded from the regression analyses of growth- and cell parameters presented in this study. The maxima of the specific growth rate and the instantaneous rate of DMSP production in the nine algal strains selected for the cell-nitrogen-specific experiments ranged from 0.29 d^{-1} to 1.66 d^{-1} and from 0.39 d^{-1} to 1.95 d^{-1} respectively (Table 2).

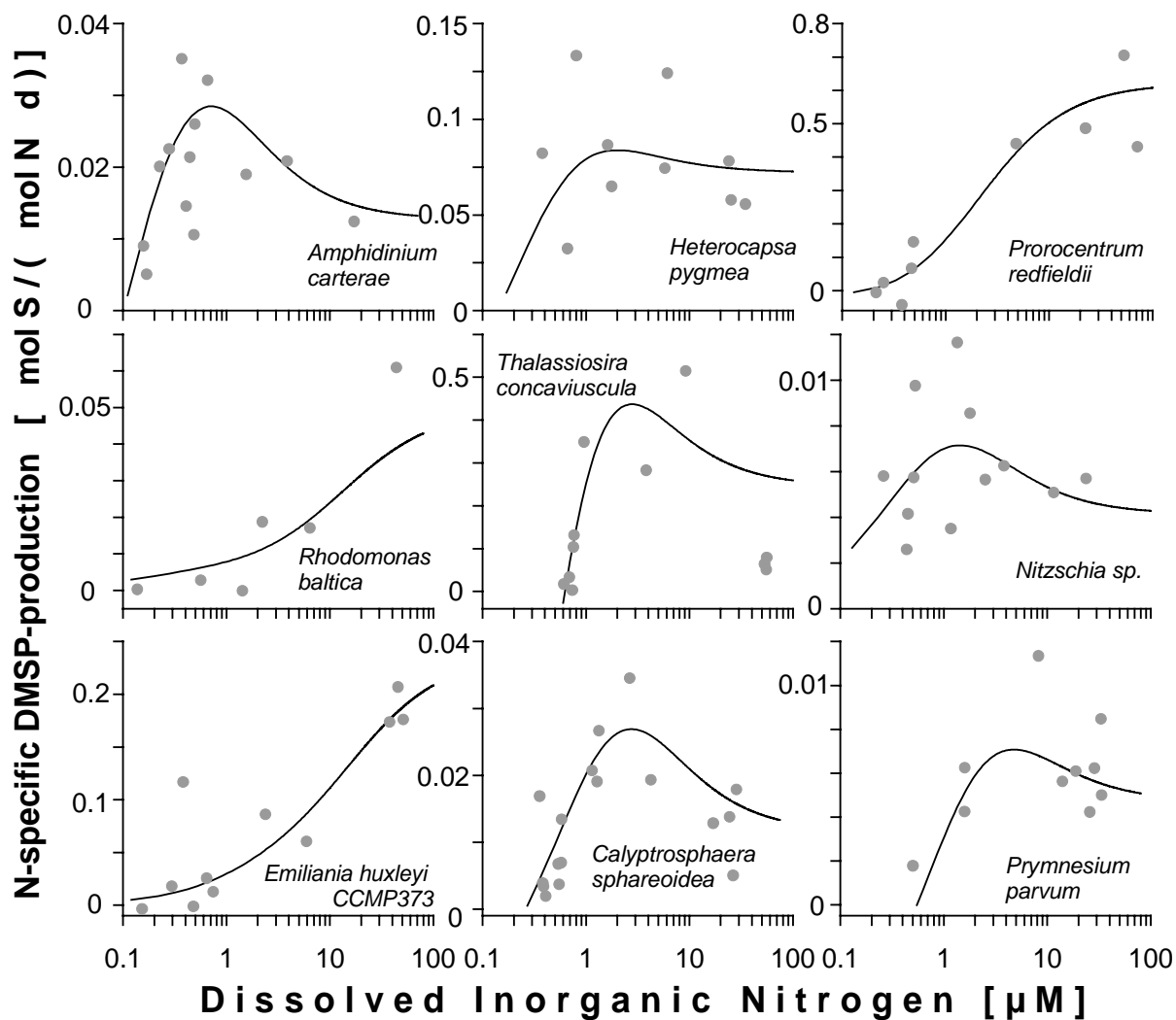


Fig. 7 Nitrogen-specific production of DMSP in response to concentrations of dissolved inorganic nitrogen: (1) The single data points are direct observations which were achieved when both the DMSP production rate and the DMSP/PON ratio were available ($n = 97$). (2) The *plotted line* is a prediction from regressions of these two parameters (Fig. 6 and 4 respectively), which together completely determine the nitrogen-specific DMSP production (Equation 9, Appendix). These regressions are based on a total number of $n = 122$ and $n = 117$ observations respectively.

Table 4 Estimates for the maximum of the nitrogen-specific production of particulate DMSP ($P_{DMSP/N}$) and for the corresponding DIN concentration at the $P_{DMSP/N}$ maximum (based on regression analysis according to Equation 9, Appendix). And estimates for the amount of particulate DMSP produced during the depletion of 20 μ M DIN and its total assimilation into PON (based on regression analysis according to Equation 7 or 8, Appendix).

	$P_{DMSP/N}$ maximum	DIN concentration at $P_{DMSP/N}$ maximum	DMSP _p produced during total assimilation of 20 μ M DIN
	$\mu\text{mol S} / (\mu\text{mol N}\cdot\text{d})$	$\mu\text{M DIN}$	$\mu\text{M DMSP}_p$
<i>A. cartearae</i>	0.029 ± 0.001	0.7	1.2 ± 0.04
<i>H. pygmaea</i>	0.084 ± 0.005	2.0	4.5 ± 0.27
<i>P. redfieldii</i>	0.603 ± 0.082	> 50	23 ± 1.85
<i>Rh. baltica</i>	0.042 ± 0.007	> 50	1.7 ± 0.28
<i>Nitzschia sp.</i>	0.007 ± 0.001	1.4	0.3 ± 0.04
<i>Th. concaviuscula</i>	0.437 ± 0.065	2.8	6.0 ± 0.89
<i>E. huxleyi</i> CCMP373	0.189 ± 0.013	> 50	3.3 ± 0.23
<i>P. parvum</i>	0.007 ± 0.001	4.8	0.4 ± 0.06
<i>C. sphaeroidea</i>	0.027 ± 0.001	2.8	0.8 ± 0.03

The DMSP production was overall closely related to the growth rate; however, at the species level, considerable differences were found, especially in the low and intermediate ranges of nitrogen concentrations, where changes in the intracellular DMSP concentrations were high (Fig. 4 and 5). Threshold concentrations of DIN for growth and DMSP production were either not significant or fell into the nano-molar range of the DIN concentration (Fig. 4). The molar ratio of the particulate DMSP to particulate organic nitrogen (DMSP_p/PON ratio) increased in eight out of nine cases in response to decreasing DIN concentrations (Fig. 6). The average increase at a decrease of the DIN concentration from 20 μ M DIN to 0.2 μ M DIN was by a factor of 4.4 (± 2.7 , $n = 9$) (Table 3, Fig. 6). The only exception to this response was found for *P. redfieldii*, where the DMSP_p/PON considerably decreased under low DIN concentrations to about 54% of the values under nitrogen-replete conditions. The decrease of the DMSP_p/PON ratio in *P. redfieldii* was mainly attributable to the decrease of the intracellular DMSP (Fig. 5). The N-specific production of DMSP and its relation to concentrations of DIN are presented in Figure 7 with direct observations and predictions based on the regression analysis of the inherent parameters (DMSP_p/PON ratio and rate of DMSP production). The maxima of the N-specific DMSP production ranged from 7.2 mmol DMSP mol⁻¹ N day⁻¹ for *Nitzschia sp.* to 603 mmol DMSP mol⁻¹ N day⁻¹ for *P. redfieldii*. In contrast to the DMSP production, which continuously declined in response to decreasing nitrogen concentration (Fig. 4), the N-specific DMSP production showed intermediate maxima in six out of nine investigated strains (Fig. 7, Table 4). These intermediate maxima occurred at DIN concentrations between 0.7 μ M and 4.8 μ M. However, in the other cases, the N-specific DMSP production had saturation responses

with estimated maxima at DIN concentrations above 50 μM DIN and with a continuous decline in the course of nutrient depletion (Fig. 7).

2.5 Discussion

With regard to nutrient conditions, the conducted batch culture experiments are best comparable to fast-developing new production systems like a vernal algal bloom or the margin of an upwelling system, which are both important sources for the emission of DMS to the atmosphere (Belviso *et al.* 1993, Malin *et al.* 1993, Matrai *et al.* 1996). However, a nutrient-depleted batch culture is not directly comparable with a natural oligotrophic system, since the nutrient supply there can be based on intense regeneration. Nevertheless, the growth conditions for most of the upper ocean are characterised by a fast seasonal or vertical depletion of dissolved inorganic nitrogen. During this development most of the investigated algae showed a considerable increase in their intracellular DMSP concentrations (Table 1, Fig. 5). In two of the dinoflagellates, a substantial increase in the intracellular DMSP concentration was also observed during the formation of swarmers (Table 1). This compares to the two phase increase of $\text{DMSP}_{\text{cell}}$ observed in *Tetraselmis subcordiformis* during nitrogen-deficient growth and during cyst formation by Gröne and Kirst (1992). Interestingly the diatoms investigated in our study showed, on average, a significantly greater increase in the $\text{DMSP}_{\text{cell}}$ under nitrogen deficiency (+235%) than both dinoflagellates (+75%) and Prymnesiophyceae (+51%). However, the observed SSV of intracellular DMSP concentrations was about two orders of magnitude higher than the NRV. Significant strain-specific differences of the intracellular DMSP concentration were found for all three strain couples of *Prorocentrum minimum*, *Prorocentrum triestinum* and *Emiliana huxleyi* investigated in this study (Table 1), as well as for the two strain couples of *Micromonas pusilla* and *Imantonia rotunda* in Keller *et al.* (1989) and for at least 3 of 6 strains of *E. huxleyi* in Steinke *et al.* (1998). The observed strain-specific range of the intracellular DMSP concentrations for nutrient-replete conditions in *E. huxleyi* presently reaches from 50 mM in CCMP 1516 (Steinke *et al.* 1998) to 428 mM in CCMP 8613C (=CCMP 376) (Matrai and Keller 1994) and 464 mM in CCMP 373 (this study). There are also considerable inter-laboratory variations in the nutrient-replete intracellular DMSP concentration of *E. huxleyi*, e.g. Strain L: 157 to 250 mM, strain CCMP 370: 113 to 232 mM, strain CCMP 373 (=BT6): 107 to 242 mM and strain CCMP 378: 59 to 145 mM (Keller *et al.* 1989, Wolfe and Steinke 1996, Steinke *et al.* 1998, Keller *et al.* 1999a, b van Rijssel and Buma 2002, van Rijssel and Gieskes 2002, Slezak and Herndl 2003), which apparently relate to relatively small differences in the growth conditions. Furthermore, we observed opposing strain-specific responses to nitrogen

deficiency between two different strains of *E. huxleyi* (Table 1). In strain CCMP 2112 the intracellular DMSP concentration increased under nitrogen deficiency, as previously observed for a different strain by Turner *et al.* (1988) but strain CCMP 373 responded in just the opposite manner and decreased its intracellular DMSP concentration at low nitrogen concentrations, as previously observed for CCMP 8613C by Matrai and Keller (1994) and CCMP 378 by Keller *et al.* (1999a). All this points to an amazing intra-specific variability, which, for a species of such outstanding biogeochemical importance (Holligan *et al.* 1993, Malin *et al.* 1993), deserves more attention in future research, to provide adequate biological information for improved models of biogeochemical cycles in the upper ocean (Archer *et al.* 2002, Gabric *et al.* 2004).

The manifestation of class-specific differences in levels of intracellular DMSP between major phytoplankton groups is partly obscured by high SSV and is potentially biased by the selection of several vigorously DMSP-producing dinoflagellate genera. However, the main trends outlined by Keller *et al.* (1989) are apparent also in this investigation. But care should be taken, especially in the case of diatoms, not to make a direct connection between the level of intracellular concentration and the productivity of DMSP. The actual concentration in the diatom cytoplasm may be much higher than that calculated for the whole cell, since there is no DMSP synthesis in the cell vacuole and membranes are not freely permeable for DMSP. Furthermore, under stable growth conditions, the instantaneous rate of DMSP production does not depend on the level of intracellular DMSP, but is directly equivalent to the specific growth rate.

Interestingly the intracellular DMSP concentration in two of the investigated dinoflagellates (*Prorocentrum redfieldii* and the swarmers of a *Gyrodinium sp.*) significantly exceeded the estimated amount of osmotically required solutes (Table 1). Likewise Matrai and Keller (1994) presented intracellular DMSP concentrations for *Amphidinium carterae* (CCMP AMPHI) of >1565 mM, and also Keller *et al.* (1989) found intracellular concentrations as high as 1082 mM and 2201 mM for an unspecified *Prorocentrum* strain and *A. carterae* (CCMP AMPHI) respectively. If we account for all the other vital intracellular solutes, these concentrations certainly exceed the required amount of osmolytes. With regard to the osmotic pressure and the excessive cell turgor that would arise, it seems unlikely that in these cases the total cellular DMSP is freely dissolved in the cytoplasm. We conclude that the apparent osmotic excess of cellular DMSP has either a reduced osmotic activity or an extra cellular location. Reduced osmotic activity of DMSP may result from some sort of aggregation, complex binding, or a close membrane association, but such forms of DMSP are presently unknown. On the other hand, some dinoflagellates actually have temporarily protruding cell structures *e.g.* extensive

feeding veils (Schnepf and Elbrächter 1992), non-plasmatic extracellular structures or exopolymer particles (Passow 2002) that could accumulate DMSP and outwit the cell volume measurement are unknown. However, we do not presently know what actually causes this apparent osmotic excess.

Turning back to the NRV of the DMSP production, it appeared that the effect of nitrogen limitation was most pronounced in the molar ratio of DMSP and particulate organic nitrogen (DMSP_P / PON) and its response to decreasing concentrations of DIN (Fig. 6). In eight out of nine cases the DMSP_P/PON increased under nitrogen-deficient growth conditions. For a decrease in the DIN concentration from 20 μM to 0.2 μM, the DMSP_P/PON increased, on average, by a factor of 4.4 (±2.7, n = 9) (Table 3, Fig. 6). The only case in which the DMSP_P/PON decreased under nitrogen-limited conditions compared to nitrogen-replete conditions was *P. redfieldii*, which had the highest DMSP_P/PON and DMSP_{cell} values observed in this study (Table 3, Fig. 6). Apart from this exceptional case, the observed range of DMSP_P/PON ratios under nitrogen-replete conditions (0.014 to 0.211 μmol S μmol⁻¹ N, n=8) was comparable to values re-calculated from data presented by Matrai and Keller (1994) (0.036 – 0.263 μmol S / μmol N, n=4), by Keller *et al.* (1999b) (0.075 to 0.486 μmol S / μmol N, n=6) and Steinke *et al.* (1998) (0.023 to 0.205 μmol S / μmol N, n=6). The transition of the DMSP / PON ratio from the N-replete to the N-deficient state was quite sharp and occurred at a DIN concentration of about 2.5 μM (Fig. 6). Such transitions were also apparent in all cell- and growth parameters at about the same level of DIN. It is notable that comparable nitrogen concentrations are also critical for the transition in natural plankton communities from the new production to the regenerating system (Dortch and Packard 1989; Taylor *et al.* 1993; Longhurst 1995).

The published data on the effect of N limitation on the levels of intracellular DMSP are conflicting (Stefels 2000). However including this study (n=32) a total of 45 strains of DMSP-producing phytoplankton have now been investigated as to their response to N limitation (excluding overlaps and 0-producers); 4 strains responded indifferently, 11 strains decreased- and 29 strains increased their intracellular DMSP concentrations under-nitrogen deficient growth conditions (Turner *et al.* 1988, Gröne and Kirst 1992, Keller and Korjef-Bellows 1996, Keller *et al.* 1999a,b, Bucciarelli and Sunda 2003).

The ultimate physiological cause for the overall increase of the intracellular DMSP concentration under nitrogen-deficient growth conditions, especially in relation to cellular nitrogen, is presently not fully understood. Andreae (1986) was the first to suggest a role of nitrogen supply in the DMSP production and to propose that DMSP, as a nitrogen-free

osmolyte, might be preferentially used and substituted for nitrogen-containing osmolytes (glycine betaine, free amino acids, proline, homarine) under nitrogen-deficient growth conditions. The observed increase of the $\text{DMSP}_P / \text{PON}$ ratio under nitrogen limitation (Fig. 6) supports this hypothesis. However, Keller *et al.* (1999a,b), who addressed a series of elaborate experiments to the substitution hypothesis, did not find a reciprocal relation between major N-containing osmolytes and DMSP, and further concluded that the dominance of DMSP makes a reciprocal relation unlikely in most species. Stefels (2000) suggested that increased DMSP production may function as an overflow mechanism, allowing continued carbon and sulphate assimilation and a re-allocation of nitrogen from the DMSP-precursor methionine to other amino acids under nitrogen limitation and other physiological stress conditions. Consistent with the overflow hypothesis, Gröne and Kirst (1992) found that additions of methionine caused a significant increase of DMSP synthesis in *Tetraselmis subcordiformis*. Furthermore, there seems to be a link between nutrient limitation, oxidative stress on the cell metabolism and the antioxidant function of DMSP, its enzymatic cleavage product DMS and acrylate as well as its oxidation products dimethylsulphoxide and methane sulphonic acid (Sunda *et al.* 2002). However, the NRV of the intracellular DMSP had usually only a limited effect on the overall decrease of the rate of DMSP production at decreasing DIN concentrations. The DMSP production followed in most cases broadly the decline of the specific growth rate (Fig. 4). Nevertheless in five of nine cases DMSP production at low DIN concentrations was significantly higher than the growth rate, and in three of these cases (*A. carterae*, *H. pygmaea* and *R. baltica*) the DMSP production continued at DIN concentrations at which the cell growth had ceased (Fig. 4). Since a phytoplankton bloom is often dominated by a few or just one species we considered what would theoretically happen if 99 % of an initial DIN pool of 20 μM would be assimilated into phytoplankton PON by different mono-species blooms of the investigated phytoplankton strains. We therefore determined the relation between the $\text{DMSP}_P / \text{PON}$ ratio and the DIN concentration (Equation 7 and 8, Appendix) and integrated over the range of 0.2 to 20 μM DIN (Table 4). For example, a bloom of *A. carterae* starts with a production of about 0.015 $\mu\text{mol DMSP } \mu\text{mol}^{-1} \text{PON day}^{-1}$ and increases its production to 0.028 $\mu\text{mol DMSP } \mu\text{mol}^{-1} \text{PON day}^{-1}$ by the end of the bloom (Fig. 7); overall it would overall yield about 1.2 μM particulate DMSP. A bloom of *E. huxleyi* (CCMP373) would similarly produce about 0.029 $\mu\text{mol DMSP } \mu\text{mol}^{-1} \text{PON day}^{-1}$ by the end of the bloom but would end up with a yield of about 3.3 μM DMSP, because it has a much higher N-specific DMSP production at high DIN concentration than *A. carterae*. The highest yield of about 23 μM DMSP would be achieved by *P. redfieldii*. But interestingly a bloom of *Thalassiosira concaviuscula*, which has a relatively

low intracellular DMSP concentration but a high DMSP_p/PON ratio, would even yield about 6.0 μM particulate DMSP. This comparison should clearly point out the potential underevaluation of diatoms when productivity is inferred just from the levels of intracellular DMSP. Nevertheless, in view of the enormous SSV of both the intracellular DMSP levels as well as the N-specific rates and ratios, it seems initially that total whole productivity in natural phytoplankton is driven primarily by species composition and succession. However the more diverse a community is, the less relevant individual differences are (SSV) and, hence, the more relevant the average NRV becomes. Since the community smoothing of individual differences applies, of course, also to the N-specific DMSP production (Fig. 7) we can expect that the more diverse and evenly distributed the phytoplankton is, the closer it comes to the average NRV, *i.e.* a constant increase of the DMSP production at decreasing DIN up to a critical concentration of about 2.5 μM DIN (Table 4) and a sharp decline in the nano-molar range. The full magnitude of SSV only applies to mono-species blooms. Nevertheless, on a global scale, the overall regular and unidirectional NRV of 1 order of magnitude would clearly dominate over 3 orders of random SSV. The observed NRV can also explain some of the natural variability of DMS(P) between different nutrient regimes in the upper ocean (Andreae 1990, Kettle *et al.* 1999). The overall predictable NRV of the intracellular DMSP and the DMSP_p/PON ratio can be easily parameterised (Equation 3 and 5, Appendix) and might be useful in nitrogen-based biogeochemical models of DMS(P) production (Anderson *et al.* 2001, Gabric *et al.* 2001, Archer *et al.* 2002). However, understanding and predicting the DMSP production of natural phytoplankton communities clearly requires further investigation of the response to their physical environment.

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Appendix. Equations used for the regression analyses of cell physiological responses to a decreasing concentration of dissolved inorganic nitrogen (DIN).

Dissolved inorganic nitrogen (DIN)		
(1)	$r_i = \frac{1}{t_i - t_n} \cdot \ln\left(\frac{DIN_i}{DIN_n}\right)$	The equation describes the exponential decrease of the DIN concentration in a batch culture medium due to the nutrient uptake by microalgae. DIN_n and DIN_i are the start and end concentrations of a sample interval in a series of intervals at the sample time t_n and t_i respectively. Unit: [d^{-1}]
(2)	$\tilde{N}_i = \frac{DIN_i \cdot r_i \cdot \delta t}{e^{r_i \cdot \delta t} - 1}$	The time averaged DIN concentration of (\tilde{N}) is the mean DIN concentration of the previous diurnal growth cycle and thus refers to an incubation interval of $\delta t = 24h$. Unit: [μM]
Cell-physiology in relation to decreasing DIN		
(3)	$\mu_{vol}(\tilde{N}) = \frac{\mu_{max} \cdot (\tilde{N} - s)}{K_{1/2} + (\tilde{N} - s)}$	The Growth rate (μ_{vol}) is described as a function of \tilde{N} by the maximum growth rate (μ_{max}), the half saturation constant ($K_{1/2}$) and the threshold concentration (s) for growth. Unit: [d^{-1}]
(4)	$\mu_{DMSP}(\tilde{N}) = \frac{\mu_{max} \cdot (\tilde{N} - s)}{K_{1/2} + (\tilde{N} - s)}$	The DMSP production rate (μ_{DMSP}) is described as a function of \tilde{N} by the maximum DMSP production rate (μ_{max}), the half saturation concentration ($K_{1/2}$) and the threshold concentration (s) for DMSP production. Unit: [d^{-1}]
Intracellular DMSP ($DMSP_{cell}$) Unit: [mM]		
(5)	$DMSP_{cell}(\tilde{N}) = DMSP_{min cell} + \left(\frac{\delta DMSP_{cell} \cdot k}{\tilde{N} + k}\right)$	Case 1: If the $DMSP_{cell}$ increases at decreasing \tilde{N} and has its minimum $DMSP_{min cell}$ at high \tilde{N} . The amplitude of $DMSP_{cell}$ is $\delta DMSP_{cell}$. Case 2: If the $DMSP_{cell}$ decreases at decreasing \tilde{N} and reaches its minimum $DMSP_{min cell}$ at low \tilde{N} . (k : regression coefficient).
(6)	$DMSP_{cell}(\tilde{N}) = DMSP_{min cell} + \delta DMSP_{cell} \cdot \left(1 - e^{k \cdot \tilde{N}}\right)$	
Molar ratio of intracellular DMSP and particulate organic nitrogen ($DMSP_P / PON$) Unit: [$\frac{n mol S}{\mu mol N}$]		
(7)	$DMSP_P / PON(\tilde{N}) = R_{min} + \frac{\delta R \cdot k}{k + \tilde{N}}$	Case 1, The ratio increases at decreasing \tilde{N} and has its minimum R_{min} at high \tilde{N} . The ratios amplitude is δR . (k : regression coefficient).
(8)	$DMSP_P / PON(\tilde{N}) = R_{min} + \frac{\delta R \cdot \tilde{N}}{k + \tilde{N}}$	Case 2, The ratio decreases at decreasing \tilde{N} and reaches its minimum R_{min} at low \tilde{N} .
Nitrogen specific DMSP production ($P_{DMSP/N}$) Unit: [$\frac{n mol S}{\mu mol N \cdot d}$]		
(9)	$P_{DMSP/N}(\tilde{N}) = \mu_{DMSP}(\tilde{N}) \cdot \frac{DMSP_P}{PON(\tilde{N})}$	The Nitrogen specific DMSP production describes the production of particulate DMSP per mole particulate organic nitrogen, which is the product of the DMSP production rate (Equation 4) and the DMSP / PON ratio (Equation 7 and 8).

3 Seasonal dynamics in production and turnover of DMSP in the North-Sea

Wilhelm Gaul¹ and Justus van Beusekom².

3.1 Abstract

A series of twelve *in situ* incubation experiments was conducted between October 2000 and April 2002 at a coastal time series station in the North Sea. The aim was to assess the seasonality of the production- and turnover of β -dimethylsulphoniopropionate (DMSP) in relation to the dynamics and growth conditions of phytoplankton. The seasonal cycle started 2001 with a massive diatom bloom dominated by *Odontella aurita* (Bacillariophyceae) which depleted dissolved silicate and phosphate within 2.5 weeks. However, DMSP-production during this phase (518 nM in 35d) was of minor importance. A second bloom followed with a mass development of *Phaeocystis globosa* (Haptophyta) which depleted the residual nitrate. The rapid decline of the *Phaeocystis*-bloom was associated with a massive increase of cryptophytes. These mixotrophic flagellates apparently utilized free single cells of *Phaeocystis* as a nutrient source when masses of them left the gelatinous colonies probably due to phosphate deficiency. The production of particulate DMSP (DMSP_p) during *Phaeocystis*- and cryptophyta bloom (4433 nM DMSP_p in 45d) accounted for about 73% of the total DMSP production between March and October. The highest surface concentrations of 14 nM DMS and 67 nM dissolved DMSP (DMSP_D) occurred with an offset of about one week after the DMSP_p maximum of 603 nM, which coincided with the *Phaeocystis* maximum, a minimum grazing activity and severe nutrient limitation of DMSP-producing phytoplankton. After the spring bloom the surface concentrations of DMS and DMSP remained relatively low, except for a short increase mid July which was associated with the occurrence of *Emiliania huxleyi* (Haptophyta). A brief autumnal diatom bloom 2001 in contrast to the extended vernal diatom bloom 2002, did not cause any concentration increase of DMS or particulate DMSP. The consumption of DMSP_p by microherbivores was overall closely related to its production by phytoplankton ($R^2=0.778$) and on average accounted for 94 % of the DMSP_p production. Evidence for selective grazing of DMSP-producers by microherbivores was found in three of ten experiments. However, avoidance was rare compared to indiscriminate and preferential grazing of DMSP-producers, this investigation does therefore not confirm the idea that DMSP acts as a chemical defence against herbivores. The consumption of DMSP_p was also closely related ($R^2=0.991$) to the release of DMSP_D which on average accounted for 77 % of the consumption of DMSP_p by microherbivores. Most of the observed release of DMSP_D is therefore not an additional DMSP production besides the particulate DMSP production but rather the reoccurrence of the later in the extracellular phase due to a release mediated by the grazing of microherbivores. Since virtually all of the released DMSP was rapidly decomposed to DMS or other sulphur compounds by microbial and enzymatic processes, it appears that microzooplankton herbivory controls the first step in the DMSP conversion from the cell particulate phase to the sea surface evaporation of DMS.

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3.2 Introduction

The algal osmolyte β -dimethylsulphoniopropionate (DMSP) is the major precursor of dimethylsulfide (DMS) (Chalenger and Simpson 1948) which is a climatically important trace gas and the main natural source of reduced sulphur in the atmosphere (Charlson *et al.* 1987, Andreae 1990). The DMSP-production of marine phytoplankton is therefore the basis of the oceanic DMS production. However, since the DMSP content of marine phytoplankton algae is highly species-specific (Keller *et al.* 1989), the concentrations of particulate DMSP in the sea are only weakly related to bulk parameters of algal biomass (Belviso *et al.* 2001). A compilation of over 15000 DMS measurements by Kettle *et al.* (1999), suggest that hot-spots of DMS production are located in regions with a seasonal occurrence of phytoplankton blooms. Some of the highest surface concentrations of DMSP were found associated with blooms of *Emiliania huxleyi* in the North Atlantic (Burkill *et al.*, 2002) and *Phaeocystis* blooms in the North Sea (Turner *et al.*, 1996, Kwint and Kramer 1996). The North Sea is an ideal environment to study the natural production and the microbial turnover of DMSP due to a strong seasonality of the growth conditions and the associated succession of the plankton communities from eutrophic coldwater systems to quasi-subtropical oligotrophic nutrient regenerating systems. The conversion and phase transfer of DMSP from the cell particulate phase of phytoplankton to extracellular solution and the subsequent volatilisation as methanethiol (MeSH) and DMS show a strong dependence on microbial food web processes and in particular to grazing by microherbivores (Wolfe *et al.* 1994, Christaki *et al.* 1996) and bacterial metabolism (Kiene and Linn, 2000). We chose a coastal time series station in the North Sea as a site to study the production of particulate DMSP and its turnover by microherbivores in an environment characterized by a strong seasonality of the ambient nutrient conditions.

3.3 Methods

Sampling

The sampling was carried out at a coastal time series station (55° 02' N, 008° 27' E) in the Northfrisian Wadden Sea (North Sea). Seawater for chemical analysis was collected with Niskin-bottles (5 dm³) once or twice per week. Samples for the analysis of DMS and dissolved DMSP were collected from the top inlet of the Niskin-bottles by syringes equipped with a filter capsule (GF/F, 25mm), a 40cm PTFE tubing and a screw lock valve. This

technique excluded particulate DMSP, avoided air contact and allowed a closed sample transfer to the gas extraction. Samples for nutrients analysis were filtered (nucleopore, 0.45 μm , 47mm \varnothing) and were analysed within a few hours after sampling or were kept frozen at -20°C for a later analysis. Samples for the analysis of phytoplankton pigments and particulate DMSP (DMSP_P) were gently filtered on glass fibre filters (Whatman, GF/F) and were immediately shock frozen in liquid nitrogen and stored at -20°C till analysis (pigment samples) or enclosed in gastight vials for hydrolysis as described below (DMSP_P). Samples for the conduction of incubation experiments were collected with a large Niskin-bottle (25dm³) and were transferred *via* a wide bore silicone tubing directly into the incubation bottles (short term inhibition experiments) or into polycarbonate tanks pre-filled with sterile filtered seawater (serial dilution experiments). All sampling and incubation equipment was previously cleaned with a dilute detergent (Mucosol) and was rinsed with HCl-solution and milli-Q water.

Conduction of *in situ* incubations.

One experiment (DEX-1) was conducted in the centre of an oligotrophic warm water core in the German bight at $54^{\circ} 22' \text{ N}$, $07^{\circ} 34' \text{ E}$. The other *in situ* experiments (DEX-2 to DEX-14) were conducted at a mooring near the coastal time series station. All serial dilution experiments were carried out *in situ* by means of a special incubator equipped with a rotation device for the incubation bottles. The incubator was flexibly linked to a moored surface buoy and set to a floating depth of 5m. Incubations were started at noon and were terminated after 24h incubation. Additional short term inhibition experiments were carried out in parallel and were terminated after 6h incubation.

Serial dilution experiments

Twelve *in situ* experiments were carried out (Table1) following the protocol of Landry (1993) with modifications described in Gaul and Antia (2001) to determine the specific growth rates and microzooplankton herbivory of total phytoplankton (based on chlorophyll-*a*) as well as the production of and consumption of DMSP-producing phytoplankton (based on particulate DMSP). Five dilution levels (100% to 12% unfiltered seawater) were prepared in Polyethylene-carboys pre-filled with filtered seawater collected from same depth and location. Seawater samples were gently pre-screened and transferred to the pre-filled carboys by a submerged silicon tubing equipped with a meshbag (200 μm) on the inlet to exclude mesozooplankton. The filtered seawater was prepared in three steps: pre-screening through

net gauze of 20µm pore size, pre-filtration through glass fibre filter (GF/F, Whatman) and sterile filtration through a cellulose acetate membrane (0.2µm pore size). Two subsamples of each dilution were transferred to polycarbonate bottles (2.2 dm³, Nalgene®) and received a full set of nutrient-enrichments (20 µM NaNO₃, 0.5 µM NH₄Cl₂, 2 µM KH₂PO₄, 5 µM Si(OH)₄, 1 µM Na₂EDTA, 0,1 µM FeSO₄ and 0,01 µM MnCl₂). To determine the effect of selective nutrient enrichment, six additional subsamples of undiluted seawater were prepared of which two received no enrichments, two received a selective nitrate-enrichment and two received a selective silicate-enrichment. Samples of 0.25 to 1 dm³ for the determination of the initial concentration of particulate DMSP, chlorophyll-*a* and accessory marker pigments were taken immediately after dilution from each carboy and were filtered through GF/F filters (25 mm Ø). DMSP_p samples were incubated for hydrolysis in vials with strong base as described below, pigment samples were immediately frozen in liquid N₂ and stored at -25°C until analysis. Experiments were terminated after about 24 h *in situ* incubation when samples of 0.25 to 1 dm³ were filtered as above from each incubation bottle. Initial samplings were generally completed within 1 hour after the collection of seawater and were taken as the start points of the incubations. The experiment duration was determined individually for each dilution level as the time interval from the initial (t_0) sampling of diluted seawater from carboys, to the final (t_f) sampling of seawater from incubation bottles.

Short term inhibition experiments

Four additional subsamples of the above undiluted seawater (200µm screened) were used to determine the total release of dissolved DMSP by exudation, cell-lysis and herbivory (**R**) and the microbial and enzymatic decomposition of dissolved DMSP (**D**). Two of these subsamples were untreated and used to determine the net production of dissolved DMSP. The other two subsamples were treated with 50 µM glycine betaine (Sigma, B-3501) which is a structural analogue of DMSP and a potent short-term inhibitor of DMSP decomposition (Kiene and Gerard, 1995) and with the broad spectrum antibiotics (tetracycline, Fluka 87130 and chloramphenicol, Fluka 23275) according to Kiene (1990) to inhibit both microbial and enzymatic decomposition of dissolved DMSP. The total release of dissolved DMSP was determined from the liberation of dissolved DMSP after 6h incubation with inhibitors. The microbial and enzymatic decomposition of dissolved DMSP was calculated from the difference of the total release of dissolved DMSP and its net production in the untreated incubations.

Nutrient analysis

Nutrient samples of the experiments were passed through Whatman GF/F filters and were analysed according to Hansen and Koroleff (1999). Samples were frozen immediately in 250ml PE-bottles and stored at -25° C until analysis. Ammonia, phosphate and silicate samples from coastal time series station were analysed within 4h after the collection, samples for nitrate and nitrite were kept frozen as above until analysis in series by an automated system after Hansen and Koroleff (1999). Nutrient samples from the time series station were passed through 0.45 µm Nuclepore filters. All samples were frozen at -20°C until analysis except for dissolved silicate (kept at 4°C until analysis).

Phytoplankton pigments

High performance liquid chromatography (HPLC) was used to analysis the composition of phytoplankton pigments following the protocol of Mantoura and Llewellyn (1983) with modifications according to Barlow et al. (1997). Pigments were extracted in 90% acetone with glass pearls in a cooled sonicator. Extracts were cleared by ultra-centrifugation and filtration through a Teflon membrane. A temperature controlled autosampling- and injection system (WATERS 717 *plus*) was used to mix the purified extract and ammonium acetate buffer solution immediately before the injection into the WATERS 996 chromatographic system, equipped with a C₁₈-NUCLEOSIL column (4.6 x 250mm, MACHERY-NAGEL). Pigments were detected spectrophotometrically by a photodiode array and peak areas were integrated at 440 nm. Pigments were identified according to retention time and absorption spectra by comparison with references obtained from highly purified pigment standards (Water Quality Inst., Hørsholm, DK).

DMS analysis

The measurements basically followed the protocol of Kiene (1993) and were carried out on a Varian gas-chromatograph (GC-6000) equipped with a flame photometric detector (Thermoquest FPD-800) and a fused silica capillary column (CP-Sil 5 CB Chrompack: 30m x 0,32mm). Samples were cryofocused in a closed PTFE purge and trap system without internal glass or metal surfaces. The filtered seawater samples (10 – 50 ml) were purged for 5min with a flow of finely dispersed He (80 ml · min⁻¹). The gas stream was efficiently dried by condensation of water vapour in lopes of PTFE tubing which were tightly fitted into an aluminium block and cooled by Peltier elements to -20°C to condense and freeze water vapour during the gas extraction. The block was heated to +60°C after extraction to melt and

drain of the condensed water. Additionally a PTFE tubing of 15cm length and ¼ in o.d. packed with K₂CO₃ was used to extract residual water vapour from the gas stream. A loop of PTFE tubing of 1/16 in o.d. submerged in liquid nitrogen was used to cryofocus DMS and was subsequently submerged in a water bath of 60°C to release the extracted gas sample onto the isothermally (60°C) operated GC column. The system was calibrated according to Turner *et al.* (1990) and Uher (1999) with dilutions of DMS standards (Merck No. S28759949) dissolved in ethylene glycol (Merck No. K27686321017). The detection level was about 0.5 pmol DMS.

Dissolved DMSP (DMSP_d)

DMSP was determined as DMS which is released in an equimolar ratio after cold hydrolysis with a strong base according to Kiene (1993). Seawater samples were filtered (GF/F) and purged to remove DMS and particulate DMSP prior to hydrolysis and were analysed by the purge and trap procedure as described above for DMS.

Particulate DMSP (DMSP_p)

Gas phase measurements were applied to determine particulate DMSP as DMS which is released in an equimolar ratio after cold alkaline hydrolysis according to Steinke *et al.* (1998). Sub-samples of 10 - 60 ml from the cell cultures were filtered on glass fibre filters (Whatman, GF/F) and were hydrolysed with 4ml of 2N NaOH for 8 - 24h in Teflon sealed screw cap vials (25ml). The vials were then incubated in a water bath at 60°C for 30 min and were equilibrated by sonication before replicate gas samples of 25 – 250 µl were taken from the headspace by gastight microlitre syringes. The gas samples were directly injected via a Teflon-sealed injection-liner on a gas chromatograph (Varian, GC-6000) equipped with a isothermally (60°C) operated fused silica capillary (Chrompack, CP-Sil 5 CB 15m x 0,32mm) and a flame photometric detector (Thermoquest, FPD-800). A dilution series of ultra pure DMSP-HCl (CASS, University of Groningen) dissolved in mili-Q water was used for calibration. The diluted DMSP standards (1 ml aliquots) were hydrolysed together with a blank filter by 3 ml of 2N NaOH in Teflon sealed screw cap vials (25ml) and were analysed as described above. The calibration coefficients were determined according to Uher (1999). The detection limit for a 250 µl headspace injection was 0.5 pmol DMS.

3.4 Results

Growth conditions

Nitrogen stocks at the onset of the vernal diatom blooms 2001 and 2002 were 52 μM and 70 μM dissolved inorganic nitrogen (DIN) respectively (Figure 1). The corresponding stocks of dissolved silicate and dissolved inorganic phosphorous (DIP) were 31 μM and 35 μM and 1.1 and 0.97 μM and were depleted within 2.5 weeks after the onset of the diatom blooms. Residual nitrogen pools of 20.7 μM and 34.7 μM DIN were present after the vernal diatom blooms which were depleted by the following *Phaeocystis* bloom. In 2001, there was a intermediate nutrient increase during the decline of the *Phaeocystis* bloom (day 120 to day 150) of about 9 μM DIN and 0.15 μM DIP which was virtually depleted till the end of the bloom. A second minor nutrient increase of about 1 μM DIN happened after day 162 and was likewise soon depleted. However, after this input the phosphate concentrations constantly increased from about 100 nM to 610 nM and growth conditions shifted from a phosphate to a nitrogen limited system till the nitrogen concentrations also began to increase in mid August (day 225) (Figure 1). A short increase of about 15 μM of silicate in July (day 200) was associated with a decrease of about 1.3 μM DIN but did not stimulate a second bloom of diatoms.

experiment	date	<i>in situ</i> temperature	NO_3^-	NH_4^+	DIN	PO_4^{-3}	Si(OH)_4	dominant micro-phytoplankton
		$^\circ\text{C}$	μM					
DEX-1	04.10.2000	16.8	0.65	0.09	0.79	0.35	3.62	(not determined)
DEX-2	06.10.2000	14.5	4.10	0.53	5.20	1.06	0.26	divers <u>diatom</u> flora and cryptophyta
DEX-4	08.04.2001	6.0	25.1	0.40	26.4	0.17	0.38	<i>Odontella aurita</i>
DEX-5	08.05.2001	10.9	7.31	0.58	8.22	0.64	1.31	<i>Phaeocystis globosa</i>
DEX-6	15.05.2001	12.5	14.7	0.36	15.5	0.55	0.27	(<i>Cylindrotheca closterium</i> , <i>Pseudo-nitzschia spec</i>)
DEX-7	26.05.2001	13.9	2.93	0.24	3.43	0.22	0.16	cryptophyta and <i>Ph. globosa</i>
DEX-9	20.06.2001	14.6	0.23	0.12	0.43	0.34	1.36	highly divers <u>diatom</u> and
DEX-10	14.08.2001	19.5	0.74	0.26	1.04	0.59	0.71	dinoflagellate flora and cryptophyta
DEX-11	12.10.2001	12.8	8.71	6.06	16.3	1.45	8.58	<i>Odontella sinensis</i> , cryptophyta
DEX-12	21.03.2002	5.4	60.2	2.64	64.4	0.73	30.5	diverse <u>diatom</u> and flagellate flora
DEX-13	04.04.2002	7.0	58.1	0.38	59.3	0.44	21.3	<i>Odontella aurita</i> and cryptophyta
DEX-14	10.04.2002	7.9	42.1	0.18	43.7	0.01	2.6	(<i>Thalassiosira punctigera</i> , <i>Skeletonema costatum</i> <i>Gyrodinium undulans</i>)

Table 1 Growth conditions during the conduction of *in situ* experiments. DEX-1 was located in the centre of an oligotrophic warm water core in the German bight at 54° 22' N, 07° 34' E. The other experiments were conducted at the time series station at 55° 02' N, 008° 27' E.

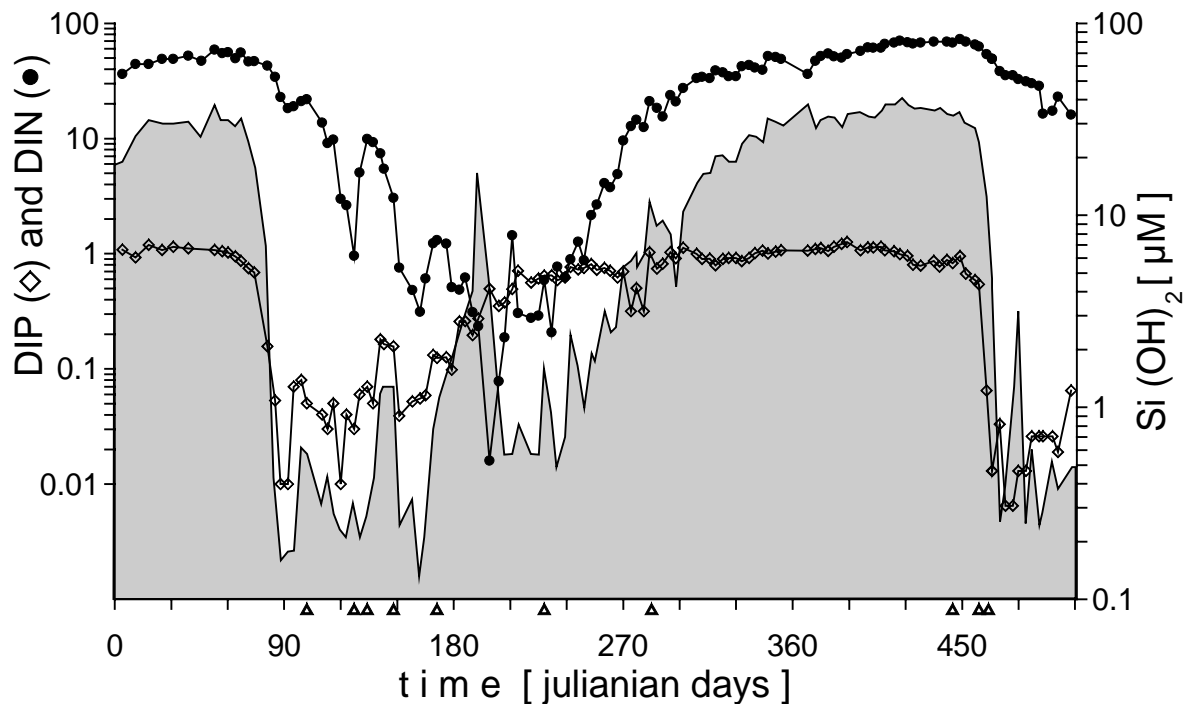


Fig. 1 Surface concentrations of dissolved inorganic nitrogen (DIN) (●), -phosphate (DIP) (◇) and silicate (shaded) between January 2001 and May 2002. Triangles (▲) on x-axis indicate dates of the *in situ* experiments DEX-4 to Dex-14.

Phytoplankton succession

After a relatively mild winter the vernal diatom bloom 2001 started relatively early and was exceptionally strong due to calm and clear water and good light conditions. The vernal diatom bloom 2002 started about 3 weeks later and was weaker than in 2001 (Figure 2). Both blooms were dominated by *Odontella aurita* and various *Thalassiosira sp.*. After the onset in the 2nd week of March [day 71], the vernal diatom bloom 2001 increased within two weeks [day 85] to a maximum of $64 \mu\text{g Chl-}a \text{ dm}^{-3}$ (Figure 2) and consumed almost the entire winter stocks of silicate and phosphate. The bloom rapidly declined within one week to $12.8 \mu\text{g Chl-}a \text{ dm}^{-3}$ [day 92] and was followed by a slowly developing bloom of *Phaeocystis globosa* and small pennate diatoms (*Cylindrotheca closterium*, *Pseudo-nitzschia spec.*). The *Phaeocystis* bloom reached its maximum in the 1th week of May [day 127] and assimilated virtually all residual nitrate ($20 \mu\text{mol NO}_3^- \text{ dm}^{-3}$) but did not cause a significant increase of the chlorophyll-*a* concentration (Figure 2). A short bloom of cryptophytes was associated with the release of free *Phaeocystis* flagellates from the gelatinous colony housings during the decline of the *Phaeocystis* bloom. During summer phytoplankton stocks remained relatively low but small blooms were observed of cyanophyta at the end of June and of haptophyta at the end of June

and early in July. However apart from the small haptophyta bloom in July no further events were associated with an increase of the particulate DMSP.

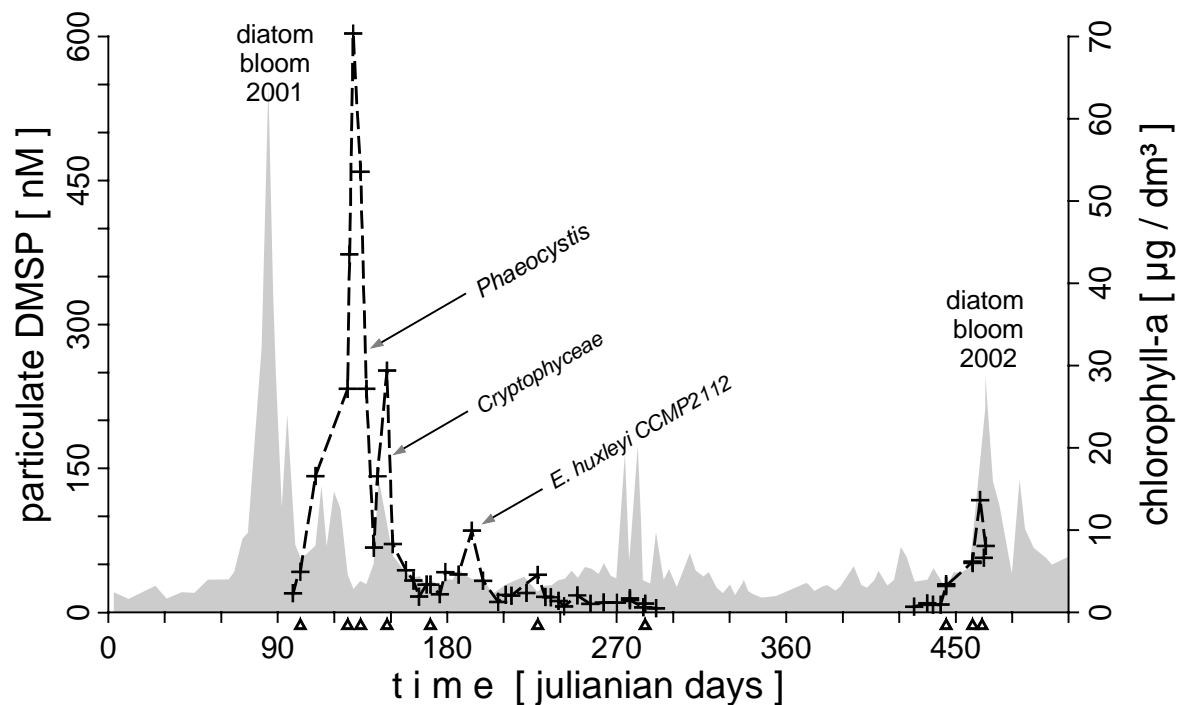


Fig. 2 Mixed layer temperature and concentrations of particulate DMSP (---) and chlorophyll-*a* (shaded area) between January 2001 and May 2002. Triangles (▲) on x-axis indicate dates of the *in situ* experiments DEX-4 to DEX-14. (DEX-1 and 2 were conducted in X. 2000 and DEX-3 and 8 were lost in storm events).

Growth and grazing loss of total and DMSP-producing phytoplankton

The specific growth rates of the DMSP-producing phytoplankton (based on $DMSP_p$) ranged from $0.02\ d^{-1}$ (DEX-11) to $0.94\ d^{-1}$ (DEX-10) and were in most cases not significantly different from those of the whole phytoplankton community (based on chlorophyll-*a*) (Table 2). The two exceptions were DEX-7 and DEX-11 where the growth of DMSP-producers was significantly lower than those of the total phytoplankton. In both cases the growth was severely nutrient limited but the DMSP-producers were stronger nutrient limited than the whole phytoplankton community. In DEX-5, DEX-6 and DEX-11 the maximum growth rates of DMSP-producing phytoplankton were significantly higher than those of the total phytoplankton, *i.e.* the growth of the DMSP-producers responded significantly stronger to a full nutrient enrichment than the community. The specific grazing loss of the DMSP-producing phytoplankton ranged from $0.05\ d^{-1}$ (DEX-13) to $0.87\ d^{-1}$ (DEX-5 and -10) and were in most of the cases (6 of 10) also not significantly different from the specific grazing losses of the total community (Table 3). However two cases each were observed where herbivorous

microzooplankton avoided DMSP-producing phytoplankton (DEX-7 and -14) and where they preferential grazed DMSP-producers (DEX-4 and -11).

	Specific growth rates of			
	DMSP-producers		Total phytoplankton	
	μ_0 d ⁻¹	μ_{\max} d ⁻¹	μ_0 d ⁻¹	μ_{\max} d ⁻¹
DEX-1	0.22 ± 0.070	0.24 ± 0.018	<i>n. d.</i>	<i>n. d.</i>
DEX-2	0.21 ± 0.022	0.21 ± 0.015	<i>n. d.</i>	<i>n. d.</i>
DEX-4	0.52 ± 0.058	0.56 ± 0.058	0.097 ± 0.010	0.11 ± 0.017
DEX-5	0.63 ± 0.013	0.85 ± 0.029	0.66 ± 0.040	0.78 ± 0.056
DEX-6	0.25 ± 0.080	0.68 ± 0.021	0.19 ± 0.003	0.31 ± 0.022
DEX-7	0.09 ± 0.069	0.46 ± 0.049	0.60 ± 0.036	0.62 ± 0.078
DEX-9	0.87 ± 0.032	1.08 ± 0.058	0.93 ± 0.038	0.90 ± 0.029
DEX-10	0.94 ± 0.177	0.91 ± 0.102	0.64 ± 0.061	0.82 ± 0.085
DEX-11	0.02 ± 0.009	0.44 ± 0.023	0.29 ± 0.024	0.37 ± 0.031
DEX-12	0.25 ± 0.004	0.24 ± 0.020	0.20 ± 0.014	0.23 ± 0.019
DEX-13	0.42 ± 0.013	0.49 ± 0.035	0.34 ± 0.018	0.39 ± 0.027
DEX-14	0.41 ± 0.044	0.41 ± 0.044	0.32 ± 0.019	0.46 ± 0.039

Table 2 Estimates of the specific growth rates of DMSP-producing and total (eukaryotic) phytoplankton in experiments without nutrient enrichment (μ_0) and with a full nutrient enrichment (μ_{\max}) ± SD. Abbreviation: *n.d.*: not determined. (Estimates for eukaryotic phytoplankton are based on monovenyl-chlorophyll-*a*.)

	Specific grazing loss of	
	DMSP-producers	Total phytoplankton
	g_{DMSP} d ⁻¹	$g_{\text{Chl-}a}$ d ⁻¹
DEX-1	0.17 ± 0.031	<i>n. d.</i>
DEX-2	0.20 ± 0.024	<i>n. d.</i>
DEX-4	0.50 ± 0.098	0.04 ± 0.028
DEX-5	0.87 ± 0.048	0.83 ± 0.089
DEX-6	0.06 ± 0.030	0.08 ± 0.038
DEX-7	0.25 ± 0.079*	0.52 ± 0.121
DEX-9	0.53 ± 0.097	0.52 ± 0.054
DEX-10	0.87 ± 0.160	0.75 ± 0.149
DEX-11	0.57 ± 0.06	0.24 ± 0.054
DEX-12	0.19 ± 0.036	0.21 ± 0.031
DEX-13	0.05 ± 0.052	0.07 ± 0.046
DEX-14	0.36 ± 0.076	0.59 ± 0.059

Table 3 Estimates of the specific microplankton grazing loss of the DMSP-producing (g_{DMSP}) and total (eukaryotic) phytoplankton ($g_{\text{Chl-}a}$) by herbivorous microzooplankton ± SD. Rate estimates for eukaryotic phytoplankton are based on monovenyl-chlorophyll-*a* which is specific for eukaryotic algae. Abbreviation: *n.d.*: not determined. *This rate is an underestimate due to the presence of mixotrophic cryptophytes (see text).

DMS, dissolved DMSP and particulate DMSP

The coastal waters of the North Sea are efficiently mixed by strong tidal currents and wind stress facilitates an intensive gas exchange with the atmosphere resulting in relatively low surface concentrations of DMS. The DMS concentrations at the time-series station ranged from 0.31 nM at day 172 to a maximum of 14.1 nM at day 134. The maximum concentrations of DMS, dissolved and particulate DMSP were observed during the *Phaeocystis* bloom (DEX-5, -6, -7) (Figure 3, Table 4). The concentration of particulate DMSP had further maxima which were associated with a maximum of alloxanthin (a specific marker pigment of cryptophytes) at the end of May and again in July where the coccolithophoride *Emilaina huxleyi* and relatively high concentrations of 19'-hexanoyloxyfucoxanthin (a typical haptophyta pigment) were observed (Figure 3). The coccolithophoride was isolated to determine its intracellular DMSP concentration and is deposited as strain **CCMP 2112** in the Provasoli-Guillard Centre for the Culture of Marine Phytoplankton, Bigelow, Main USA. Due to the highly seasonal variability of the ratio of particulate DMSP and Chl-*a* (Table 4), the concentration of DMSP was virtually independent of the chlorophyll-*a* ($R^2 = 0.005$; $n = 43$). However, a weak but significant relation was found between the concentration of particulate DMSP and 19'-hexanoyloxyfucoxanthin (Table 5).

Experiment			DMSP _p				DMSP _p / Chl- <i>a</i>	
	DMS	DMSP _D	Total	< 200 μm	< 20 μm	< 5 μm	> 20 μm	< 20 μm
	nM						nmol / μg	
DEX-1	1.0	3.6	20	15	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>
DEX-2	0.8	3.1	5.3	4.6	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>	<i>n. d.</i>
DEX-4	0.8	1.3	20	18	15	4.3	0.8	6.3
DEX-5	0.8	23	373	240	137	36	92	40
DEX-6	10	48	602	358	183	108	99	51
DEX-7	2.7	19	252	188	142	72	5.5	17
DEX-9	0.9	11	16	16	7.8	2.7	12	3.6
DEX-10	0.8	3.0	20	7.9	1.1	0.8	21	4.4
DEX-11	2.7	1.0	9.2	8.1	5.1	<i>n. d.</i>	1.0	0.7
DEX-12	< 0.2	6.3	29	22	18	<i>n. d.</i>	16	26
DEX-13	< 0.2	6.9	52	46	37	<i>n. d.</i>	29	11
DEX-14	0.4	3.6	57	55	23	<i>n. d.</i>	10	3.2

Table 4 Concentrations of DMS, dissolved DMSP, particulate DMSP in four size groups and DMSP / Chlorophyll-*a* ratio in two size groups.

	DMSP _p (n=41)	Chl- <i>a</i> (n=45)	Chl- <i>a</i> (<20µm) (n=45)
19-But	0.003	0.610	0.080
Fucoxanthin	0.013	0.813	0.727
19-Hex	0.417	0.014	0.026
Prasincoxanthin	0.002	0.003	0.012
Alloxanthin	0.097	0.714	0.474
Zeaxanthin	0.011	0.049	0.015
Lutein	0.011	0.013	0.002
Chl- <i>b</i>	0.016	0.190	0.179
DMS	0.417	0.002	0.042
DMSP _D	0.736	0.001	0.045

Table 5 Coefficients of determination for linear regressions (R^2) between particulate DMSP (DMSP_p), chlorophyll-*a* and major phytoplankton pigments, DMS and dissolved DMSP (DMSP_D).

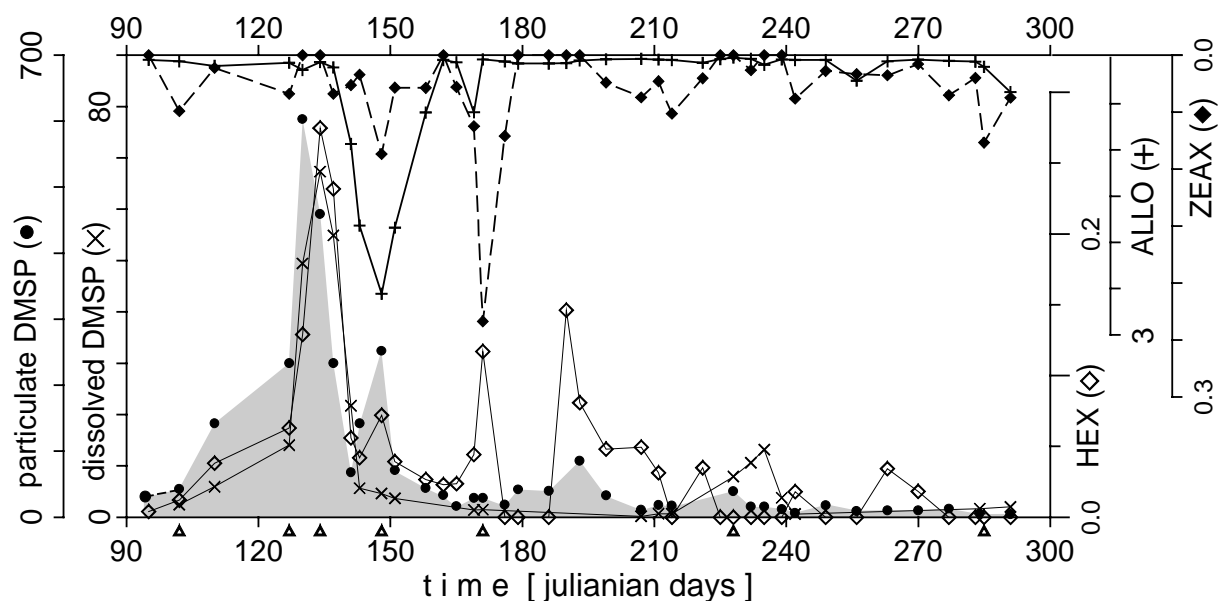


Fig. 3 Surface concentrations of particulate- (●) and dissolved DMSP (×) and major phytoplankton pigments between April and October 2001. **Abbreviations:** HEX: 19'-hexanoyloxyfucoxanthin (◇), ALLO: alloxanthin (+), ZEAX: zeaxanthin (◆). Triangles (▲) on x-axis indicate dates of the *in situ* experiments DEX-4 to DEX-10.

Production and turnover of particulate DMSP

The production of particulate DMSP, its response to nutrient enrichment and the consumption of particulate DMSP by microzooplankton grazing were studied in serial dilution experiments (Figure 4). Estimates for the specific rates of DMSP production without nutrient enrichment ranged from 0.09 d^{-1} at the end of May (DEX-7) to 0.94 d^{-1} at mid August (DEX-10) (Table 2).

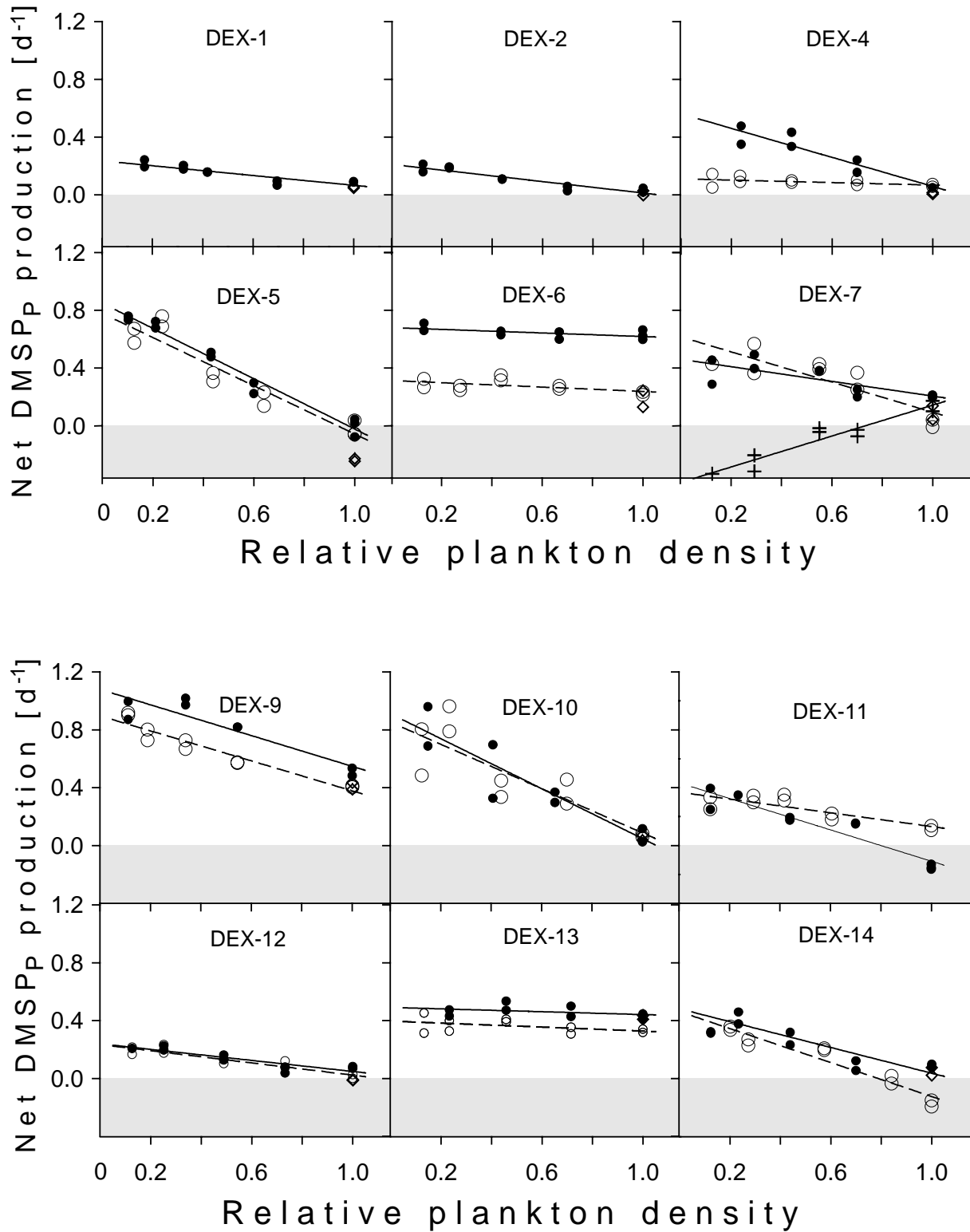


Fig. 4 Net growth rate of DMSP-producing (●) and total eukaryotic phytoplankton (○) at *in situ* incubation experiments with full nutrient enrichment as a function of the relative plankton density and net production in undiluted incubations without nutrient enrichment (◇). For DEX-7 there are also net growth rates of alloxanthin (specific pigment of cryptophytes) presented (+). Results of regression analyses are presented in Table 2 and 3.

Nutrient enrichment had no significant effect on DMSP production in DEX-1 and DEX-2 where growth was probably light limited and in DEX-4 where the ambient nutrient concentration were high. However, nutrient enrichment had also no effect on DMSP production in DEX-9 where the ambient nutrient concentrations were not expected to saturate phytoplankton growth. Significant increases of the DMSP production after nutrient enrichment were observed in DEX-5 to DEX-9.

The consumption of particulate DMSP by microzooplankton grazing ranged from 0.92 nM DMSP (DEX-2) to 185 nM DMSP (DEX-5) per day and accounted for 6.8 % (DEX-6) to 89.6 % (DEX-10) of the particulate DMSP in the size fraction $< 200 \mu\text{m}$ (Table 6 and 4). At the peak of the *Phaeocystis* bloom (DEX-6) the consumption of particulate DMSP by microzooplankton grazing was $24.2 \text{ nM}\cdot\text{d}^{-1}$ which accounted for 24.9 % of the gross production of particulate DMSP and was significantly lower than during the exponential increase ($185 \text{ nM}\cdot\text{d}^{-1}$, DEX-5) and during the decline ($53.2 \text{ nM}\cdot\text{d}^{-1}$, DEX-7) of the *Phaeocystis* bloom (Table 6).

Turnover of dissolved DMSP

The total release of dissolved DMSP and DMS (DMS(P)) by microzooplankton grazing, exudation and cell-lysis (R) and its microbial and enzymatic decomposition (D) was studied in selective inhibition experiments with glycine betaine as a competitive substrate of DMSP-lyase and antibiotics. Dissolved DMS(P) was released at a rate $0.45 \text{ nM}\cdot\text{d}^{-1}$ (DEX-2) to $144 \text{ nM}\cdot\text{d}^{-1}$ (DEX-5) which is equivalent to a turnover of the dissolved DMSP pool of 14.4 % d^{-1} and 640 % d^{-1} respectively. The total release of dissolved DMS(P) was significantly related to the amount of the particulate DMSP consumed by microzooplankton grazing (Figure 5) and accounted for 36.1 % (DEX-4) to >100 % (DEX-11 and -13) of the particulate DMSP consumed by microzooplankton grazing (Table 6). DEX-13 was the only case where the release of dissolved DMS(P) significantly exceeded the consumption of particulate DMSP by microherbivores. The majority of the dissolved DMS(P) released by microzooplankton grazing, exudation and cell-lysis was ultimately decomposed by microbial and enzymatic processes (Table 6). The highest turnover of the dissolved DMSP pool by microbial and enzymatic decomposition was observed before the maximum of the *Phaeocystis* bloom (DEX-5) (Table 6).

Experiment:	<i>P</i>	<i>C</i>	<i>R</i>	<i>D</i>	δt
	nM DMSP _p · d ⁻¹		nM DMS(P) _d · d ⁻¹		d
DEX-1	3.4 ± 0.1	2.6 ± 0.5	1.0 ± 0.3	0.8 ± 0.5	26
DEX-2	0.98 ± 0.1	0.92 ± 0.1	0.5 ± 0.4	0.3 ± 0.4	3.5
DEX-4	9.3 ± 1.0	9.0 ± 1.8	3.3 ± 0.4	2.8 ± 0.6	14
DEX-5	135 ± 2.7	185 ± 10	144 ± 15	142 ± 16	16
DEX-6	97 ± 13	24 ± 12	22 ± 9.1	17 ± 11	10.5
DEX-7	68 ± 14	53 ± 17	36 ± 1.7	36 ± 3.7	18.5
DEX-9	17 ± 0.6	9.1 ± 0.8	6.0 ± 0.6	6.0 ± 0.7	40
DEX-10	7.7 ± 1.5	7.1 ± 1.3	4.5 ± 0.7	3.9 ± 0.9	53.5
DEX-11	0.13 ± 0.3	4.3 ± 0.4	5.1 ± 1.6	5.7 ± 2.3	2.5
DEX-12	5.6 ± 0.1	4.3 ± 0.8	2.5 ± 0.6	2.6 ± 1.1	7
DEX-13	27 ± 1.1	2.8 ± 2.9	11 ± 4.7	14 ± 4.9	9.5
DEX-14	23 ± 2.1	21 ± 4.4	7.2 ± 1.5	9.7 ± 1.7	4
Season:	nM DMSP _p		nM DMS(P) _d		
Diatom-bloom (DEX-4,-12,-13,-14)	518	267	197	229	35
Phaeocystis-bloom (DEX-5,-6,-7)	4433	4193	3201	3117	45
Summer (DEX-9,-10)	1071	744	481	449	94
Autumn (DEX-1,-2,-11)	92	82	41	36	32

Table 6 Estimates (\pm SD) of the production (*P*) of particulate DMSP and its consumption by herbivorous microzooplankton (*C*) and estimates for the release of dissolved DMSP by exudation and herbivory (*R*) and its microbial decomposition (*D*). The estimates of different seasons are based on the rates *P*, *C*, *R* and *D* integrated over the number of days (δt) between the midpoints of the neighbour experiments. The experiments from different years were therefore sorted after the Julian calendar day, *i.e.* from day 80 (DEX-12 in 2002) to day 285 (DEX-11 in 2001).

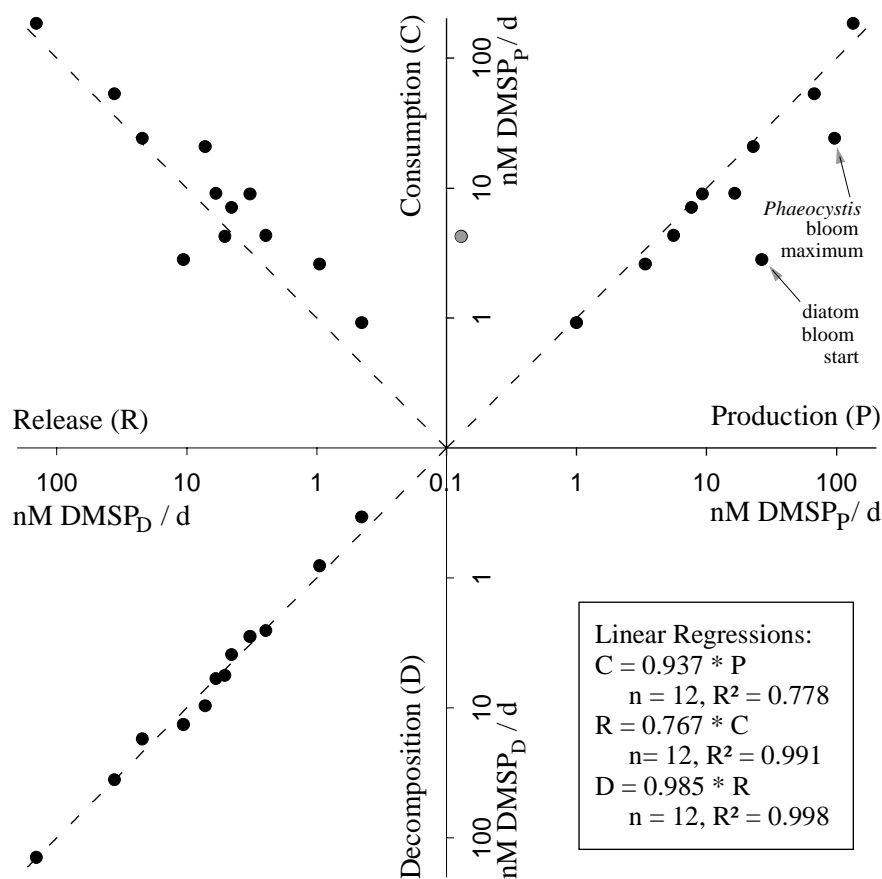


Fig. 5 Relation between the production of particulate DMSP (P), its consumption by herbivorous microzooplankton (C), the total release of dissolved DMSP (including exudation, and herbivory) (R) and the total decomposition of dissolved DMSP (D) (including bacterial decomposition and enzymatic cleavage by free DMSP-lyase). Arrows indicates the maximum of the *Phaeocystis* bloom (DEX-6) and the onset of the vernal diatom bloom (DEX 13).

3.5 Discussion

Phytoplankton succession and DMSP-production

The development of phytoplankton during spring was characterised by 3 distinct bloom phases. It started with a massive bloom of diatoms which was followed by a *Phaeocystis* bloom and it finished with a bloom of cryptophytes. The diatom blooms 2001 and 2002 started quite differently at the 10th March and 1th April and at mixed layer temperatures of 1 °C and 7 °C respectively. Nutrients were sufficiently available long before the bloom initiation. Such high temperature and timing variation therefore indicate that the bloom initiation most likely depends on the light intensity or daily light doses which usually became sufficient for some diatoms during March. However the depletion of dissolved silicate

terminated the diatom blooms in both years after about 2.5 weeks. Phosphate was likewise depleted by the end of the bloom but it seemed that its regeneration had already started because the N:P-ratios between the amount of DIN and DIP consumed during the blooms were relatively high (2001: 31.3 μM DIN / 1.1 μM DIP and 2002: 35.3 μM DIN / 0.97 μM DIP). The decrease of DIN could therefore continue after silicate and DIP were depleted (Figure 1).

Interestingly according to the constant molar ratio between the silicate- and nitrogen consumption, the silicate requirements of the vernal diatom blooms 2001 and 2002 were the same (2001: 31.0 $\mu\text{mol Si}$ / 31.3 $\mu\text{mol DIN}$ and 2002: 34.6 $\mu\text{mol Si}$ / 35.3 $\mu\text{mol DIN}$). The silicate concentration at the onset of the diatom bloom therefore largely determined the residual nitrogen after the diatom bloom and hence constrains the magnitude of the *Phaeocystis* bloom which subsequently depleted all winter nitrogen. Such silicate defined partitioning of new nitrogen between diatom and flagellate dominated communities potentially applies - except for polar regions and subtropical gyres - to all seasonally affected ocean regions and upwelling systems. In our study both blooms were temporally clearly separated, however the partitioning would also apply to cases with a partial or total overlap of the diatom- and flagellate bloom as long as only silicate depletes before nitrogen.

The partitioning has far reaching consequences for the ocean biogeochemistry in general (*e.g.* elemental composition of exported particles) and for the DMSP production in particular since diatom dominated communities have usually much lower DMSP/Chl-*a* ratios than flagellate dominated communities. In our study the *Phaeocystis* dominated communities (DEX-5 and -6) had up to 29 times higher DMSP/Chl-*a* ratios than diatom dominated communities (DEX 4 and -14) (Table 4) and a similar ratio can be assumed for the DMSP-production per unit nitrogen. Nevertheless the DMSP-production rates during the onset (DEX 13) and near the maximum of the diatom bloom 2002 (DEX 14) were the second highest after the *Phaeocystis* bloom (Table 2 and 6). Furthermore an estimate for the DMSP_p concentration at the maxima of the diatom blooms 2001 and 2002 (based on their Chlorophyll-*a* concentrations and the DMSP/Chl-*a* ratio near the maximum of the diatom bloom 2002 (DEX 14)) gave concentrations of 348 nM and 157 nM respectively which were exceeded only during the *Phaeocystis* bloom. Surface concentrations and production rates during summer and autumn were insignificant compared to these events. The spring is therefore overall the most important season for DMSP-production and the diatom bloom considerably contributes to the vernal DMSP-production.

Nutrient saturation of growth and DMSP-production

Although silicate and phosphate were depleted after the diatom bloom (DEX-4) and again after the *Phaeocystis* bloom (DEX-7) nutrient enrichment could not stimulate phytoplankton growth in these cases (Table 2). Nutrient additions did also not increase phytoplankton growth in an early summer experiment (DEX-9) although the main nutrients were virtually depleted. An efficient nutrient regeneration could explain nutrient saturated growth in DEX-7 and DEX-9 but growth in DEX-4 appeared to be suppressed ($\mu = 0.097$) rather than nutrient saturated also because the DMSP-producing subgroup grew faster ($\mu = 0.52$) and almost nutrient saturated ($\mu_{max} = 0.56$). The other two cases of nutrient saturated growth and DMSP-production (DEX-12 and DEX-13) are in accordance with high ambient nutrient concentrations. Significant nutrient limitation at the community level was found before and during the *Phaeocystis* bloom (DEX-5 and DEX-6 respectively), in late summer (DEX-10) and autumn (DEX-11) 2001 and at the end of the diatom bloom 2002 (DEX-14). Growth of DMSP-producing phytoplankton was also nutrient limited at DEX-5, 6 and -11 and additionally in the early summer experiments (DEX-7 and -9) but not in DEX-10 and -14. The restoration of DIP began in June and was finished in August while DIN and silicate only began in September and approached winter maxima in December (Figure 2). After the diatom bloom (DEX-5 to DEX-7 and DEX-14) the limiting nutrient elements were therefore phosphorus and silicate whereas during summer (DEX-9 and -10) nutrient limitation most likely shifted to nitrogen. In autumn (DEX-11) a trace metal (Fe or Mn) limited growth since the main nutrients were sufficiently present but growth and even more DMSP-production was stimulated by nutrient enrichments which included FeSO_4 , MnCl_2 and Na_2EDTA (Table 2). However, light limitation is probably the major factor determining the end of the growth season which mostly occurs between September and October.

Microzooplankton herbivory and DMSP-consumption

The intensity of microzooplankton grazing seemed to play an important role in the vernal bloom dynamics. A relatively intense microzooplankton grazing during the diatom bloom 2002 (DEX-14) is most likely the reason for the lower phytoplankton biomass of the bloom maximum compared to 2001 ($64.2 \mu\text{g Chl-}a\text{-dm}^{-3}$ and $28.9 \mu\text{g Chl-}a\text{-dm}^{-3}$ respectively). This explains also why the DIN consumption from the start to the maximum of the bloom was higher in 2002 ($20.6 \mu\text{M}$) than in 2001 ($17.7 \mu\text{M}$) especially in relation to the increase of phytoplankton biomass (2002: $20.6 / 24.1 = 0.86 \mu\text{mol N} / \mu\text{g Chl-}a$) (2001: $17.7 / 61.7 = 0.29 \mu\text{mol N} / \mu\text{g Chl-}a$) which indicates stronger transfer of nitrogen into heterotrophic biomass

and hence higher herbivorous grazing intensity. Unfortunately we have no estimates of grazing intensity during the diatom bloom 2001, however right after the bloom (DEX-4) herbivorous microzooplankton grazing was insignificant except for DMSP-producing phytoplankton which formed only a minor part of the phytoplankton by this time.

The subsequent *Phaeocystis* bloom encountered intensive herbivorous grazing at the onset (DEX-5) both at the community level and for DMSP-producing phytoplankton. However a relatively large fraction of DMSP-producers could probably escape consumption since about 36% was found in the size fraction $> 200\mu\text{m}$ primarily including *Phaeocystis* colonies. The *Phaeocystis* bloom reached its maximum (in terms of the particulate DMSP and 19-hexanoyl-oxo-fucoxanthin concentration; DEX-6) at a time where grazing by herbivorous microplankton had almost completely ceased and growth of phytoplankton and DMSP-producers was severely nutrient limited but nevertheless persisted at a low level. The breakdown of the *Phaeocystis* bloom was associated with a massive development of cryptophytes which could have profited from the degradation of the *Phaeocystis* bloom because these protists typically include herbi- and bacterivorous mixotrophs. The assumption, that the cryptophytes in DEX-7 were mixotrophes, is supported by the negative dilution response of the net growth rate of the taxon-specific pigment alloxanthin. The negative dilution response is expected for mixotrophic protists because their growth depends on the density of food particles which decreases linearly with dilution (while the common positive response of autotrophic organisms is caused by the synchronous relieve from grazing pressure. Due to these inverse dilution responses of the growth of mixo- and autotrophic protists the occurrence of mixotrophes caused an underestimation of the growth- and grazing estimates at the level of the phytoplankton community. If these mixotrophs also produced DMSP, the estimates for the community of DMSP-producers will likewise have been underestimated. According to the phytoplankton pigment composition (and microscopic observations), cryptophytes were present in low quantities throughout the whole growth period but formed a sizeable fraction of phytoplankton only right after the *Phaeocystis* bloom (DEX-7). Some cryptophytes are fairly strong DMSP-producers (Keller *et al.* 1989) and they formed a major DMSP-producing group in DEX-7, but they contributed only relatively little to the total stock of phytoplankton. The expected suppression of the dilution response by inverse dilution response of the mixotrophic cryptophytes in DEX-7 is therefore stronger for the DMSP-producing phytoplankton than for the whole phytoplankton and might partly explain the differences in the growth and grazing estimates.

In the following we briefly consider possible causes for the remarkably fast decline of the *Phaeocystis* bloom. Sinking of cells and colonies is a mechanism that can not be ruled out but the water column in the Wadden Sea is generally completely mixed. Fast advection of the water masses by the residual coastal boundary stream in the German Bight cannot be ruled out for the outer part of the Sylt Rømø Bight, but in general the exchange rates are low: about 10% per tide (Gätje and Reise, 1998). Viral infection was frequently documented in association with algal blooms in the North Sea although its significance for the collapse of *Phaeocystis* blooms is presently unknown (Larsen *et al.* 2004). However the following observations may provide an alternative nutrient and grazing related explanation for the rapid bloom decline. First observation: phosphate was virtually depleted during the decline of the *Phaeocystis* bloom. Second observation: masses of gelatinous colony housings of *Phaeocystis globosa* were found partly or completely evacuated during the decline of the *Phaeocystis* bloom (direct microscopic observations on fresh, unpreserved bottle samples). And finally, the associated blooming of cryptophytes was apparently not effected by the DIP depletion and must therefore be based on the utilization of recycled phosphate.

If the *Phaeocystis* flagellates had escaped due to severe phosphate starvation from their gelatinous colony housings, which so far gave them shelter against micro-herbivore grazers but which now certainly hindered a sufficient nutrient transport, they might have become a direct phosphate source for the mixotrophic cryptophytes.

This hypothesis is supported by (I.) the associated shift in the size distribution of the particulate DMSP, *i.e.* the fraction with predominantly single cells ($< 5\mu\text{m}$) had increased from 9.7% (DEX-5) to 29% (DEX-7) while the fraction of colony sized DMSP ($>200\mu\text{m}$) decreased from 41% (DEX-6) to 25% (DEX-7) (II.) by the inverse changes of the 19-Hexanoyl-oxo-fucoxanthine (from 0.27 to $<0.01 \mu\text{g}\cdot\text{dm}^3$) and alloxanthin concentration (from 0.11 to $1.82 \mu\text{g}\cdot\text{dm}^3$) during the decline of the bloom (between day 137 and day 143 respectively), (III.) by the decrease of the growth of DMSP-producing phytoplankton in the course from the initiation to the decline of the *Phaeocystis* bloom, (VI.) by the severe nutrient limitation of the DMSP-producing phytoplankton during the decline of the bloom and (V.) by a significant increase of microplankton grazing of DMSP-producing phytoplankton after the bloom maximum which is actually higher than it appears from the data in Table 3 due to the above discussed underestimate of grazing during DEX-7 caused by the interference of mixo- with autotrophic microplankton.

A chemical defence effect of DMSP-producing against herbivorous protists was reported from various laboratory experiments (Wolfe and Steinke 1996, Strom *et al.* 2003) and during a

coccolithophoride bloom in the North Sea when grazing loss of DMSP producers was significantly lower than at the community level (Archer *et al.* 2001). The grazer repellent effect of DMSP is assumed to be indirectly due to a suggested function as a warning messenger for the presence of a toxic compound (Strom *et al.* 2003). The cleavage of DMSP could theoretically have a negative effect on the digestion process due to the release of DMS, acrylate and H⁺. Nevertheless, in the gut of mammals DMSP was found to have a protective effect under acid stressed digestion (Ishida *et al.* 1990). Acrylate has only a moderate antibiotic effect and can be easily metabolised by marine bacteria (Kiene 1990). The existence of a toxic co-product of the DMSP-synthesis in marine phytoplankton is presently unknown. The existence of most toxins of marine protista are clearly restricted to a single class or genus (Hallegraeff 1993) while DMSP is produced in all classes of eukaryotic protists but has an almost completely random strain- and species-specific variability of the intracellular DMSP-concentration (Keller *et al.* 1989, Gaul and Antia 2004). Therefore, a function of DMSP as warning messenger for a specific toxic compound seems to be unlikely. Furthermore, the chemical defence hypothesis seems to be in contradiction with recent findings that DMSP as well as most of its derivatives and cleavage products are efficient antioxidants which function as scavengers of intracellular oxygen radicals (Sunda *et al.* 2002). A possible detection of the level of toxic oxygen radicals by the level of intracellular or cell-peripheral DMSP would certainly be lost in the extremely high taxon-specific variability of intracellular DMSP (Keller *et al.* 1989).

Nevertheless, apart from the challenging question how the observed chemical defence of DMSP against micro-herbivorous may work, it is worthwhile to examine its effect on grazing in natural plankton communities. If DMSP-producing phytoplankton is avoided or less intensively grazed by microherbivores compared to other eukaryotic phytoplankton, the specific grazing rates should be lower than for the total eukaryotic community. In two series of experiments, the one presented here which is conducted in the North Sea and another series which we conducted in different regions of the tropical Atlantic (data presented elsewhere), the rate of microplankton grazing on DMSP-producing phytoplankton was on average 2.3 times higher than at the community level. In 4 of 16 cases (here: 2 of 10 cases) the herbivorous microplankton preferentially grazed DMSP-producing phytoplankton and in further 4 cases they avoided DMSP-producers. Therefore grazing rates for DMSP-producing phytoplankton were not significantly related to grazing rates of total phytoplankton ($R^2 = 0.039$; $n = 16$). The observed ambivalence in the selectivity of herbivorous microplankton for DMSP-producing phytoplankton could be due to an ambivalence of DMSP as a selection

factor itself. The cases of preference might be due to an attraction of grazers in the plumes of dissolved DMSP which can most likely be easily tracked by protists and small metazoa (Steinke *et al.* 2002).

Conversion of particulate DMSP to dissolved DMSP and DMS.

The conversion efficiency of the processes which release DMSP from the cell-particulate pool to solution is an inherently limiting factor for the production of DMS and its ultimate release to the atmosphere. Cell-lysis and exudation of DMSP are processes which have *per se* a conversion efficiency of 1. In contrast the fraction of the ingested cell-particulate DMSP that is released by microherbivores as DMS or dissolved DMSP is variable (Wolf *et al.*, 1994). The intensity and the conversion efficiency of herbivorous grazing are therefore important determinants for the transition of DMSP from the cell-particulate to the dissolved phase and its further conversion to DMS. Considering that healthy phytoplankton cells exude only a minor fraction of their intracellular DMSP (Laroche *et al.*, 1999) and that their most likely fate in natural plankton communities is not auto-lysis but consumption by microherbivores, most of the observed increase of dissolved DMSP in the inhibition experiments can be attributed to a release which is mediated by herbivory and excretion. The ratio of the release of dissolved DMSP (R) and the consumption of particulate DMSP (C) by microherbivores (R / C ratio) may therefore be a first estimate for the conversion efficiency of particulate DMSP by microherbivores. The release of dissolved DMSP significantly exceeded the consumption only during DEX-13 and accounted on average for 77 % of the DMSP consumed by microherbivores. This is comparable to conversion ratios previously observed in laboratory experiments for *Oxyrrhis marina* at low food density (Wolfe *et al.* 1994) and indicates that microherbivores do not assimilate or decompose significant amounts of the ingested DMSP into biomass or other sulphur compounds but rather efficiently release DMSP from the particulate phase into solution.

Conclusions

Phytoplankton development followed a seasonal cycle of light-, silicate-, phosphate- and nitrogen-limited growth conditions. The spring bloom had three distinct succession phases characterized by a predominance of centric diatoms, *Phaeocystis globosa* and cryptophytes. The seasonally highest surface concentration of dissolved (48 nM) and particulate DMSP (602 nM) coincided with the maximum of the *Phaeocystis* bloom. But the highest rates for the production of particulate DMSP (135 nM·d⁻¹) and the release of dissolved DMSP (144 nM·d⁻¹)

¹) were observed about 1 week before the *Phaeocystis* maximum was reached. The maximum coincided with the lowest grazing loss (0.06 d^{-1}) and the strongest nutrient limitation of DMSP-producing phytoplankton observed during spring and summer. The release of dissolved DMSP was closely related to the amount of particulate DMSP consumed by microzooplankton grazing. No evidence for a general avoidance of DMSP-producing phytoplankton by herbivorous microzooplankton was found, but a selective grazing was observed in 4 of 10 experiments including both avoidance as well as preference of DMSP-producers.

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4 The microbial decomposition of dissolved DMSP and its nutrient dependent DMS yields in the tropical Atlantic

Wilhelm Gaul and Karin Lochte

4.1 Abstract

A series of nutrient enrichment experiments was conducted with natural size-fractionated bacterial communities collected in the Amazon influenced western Atlantic, the central and the eastern subequatorial region of the Atlantic. The aim was to study the nutrient related changes of the DMS yield of the microbial decomposition of dissolved DMSP (conversion efficiency) in the tropical Atlantic Ocean. The growth conditions in the upper mixed layer were extremely oligotrophic at all stations where samples for experiments were collected. Ambient concentrations of dissolved inorganic nitrogen (DIN) were $\leq 0.3 \mu\text{mol N}\cdot\text{dm}^{-3}$ and the rates of bacterial carbon production without nutrient enrichments were $\leq 0.4 \mu\text{gC}\cdot\text{dm}^{-3}\cdot\text{d}^{-1}$. However after several hours of growth under exclusion of grazer control, the bacterial carbon production, the microbial degradation of dissolved DMSP and the production of DMS was much higher in most samples with organic as well as inorganic nutrient amendments compared to controls. The overall strongest stimulation was achieved by the combined enrichment of dissolved organic nitrogen and sulphur (DONS). The DONS enrichment caused a maximal short term increase of consumption of dissolved DMSP and production of DMS by a factor of 4.1 and 44.7 respectively compared to controls without nutrient enrichments. The DMS yield in the controls was on average 21% of the microbial DMSP degradation but significantly increased due to organic and inorganic nutrient enrichments. The overall highest DMS yields were observed in DONS enriched incubations where on average 72% of the microbially consumed DMSP was converted to DMS. We conclude that the availability of dissolved organic sulphur and carbon controls the bacterial utilization of DMSP as a sulphur and carbon source and the amount of DMSP-sulphur which can be released *via* enzymatic cleavage as DMS.

4.2 Introduction

The production of the algal osmolyte DMSP has received considerable attention in the past decade of research (Stefels 2000, Kiene *et al.* 2000, Simó 2001), primarily because it is the principal precursor of the volatile sulphur species DMS in the ocean (Challenger and Simpson 1948, Gage *et al.* 1997) which was identified as an important player in the global S-cycle (Lovelock *et al.* 1972), in the Earth's backscatter of solar radiation (Charlson *et al.* 1987) and atmospheric chemistry (Platt and Le Bras 1997). But the DMSP production receives

increasing attention also because of the discovery of new cell- and eco-physiological functions (Wolfe *et al.* 1997, Stefels 2000, Sunda *et al.* 2002) and its importance as a bacterial C- and S-source (Kiene *et al.* 2000). However, even though there is only little information available about the *in situ* production of particulate DMSP by phytoplankton (Archer *et al.* 2002), it appears that actually just a small fraction of the algal DMSP is converted to DMS (Kiene and Linn 2000). This is because two biological transformations are required before the cellular DMSP-sulphur can escape as DMS to the atmosphere, *i.e.* the release from the intracellular phase to the ambient aquatic environment and the conversion of the dissolved non-volatile precursor to DMS. The release is mediated by four different biological processes: exudation (cell leakage), cell autolysis (of senescent cells), viral lysis and herbivory (Laroche *et al.* 1999, Stefels 2000, Wilson *et al.* 2002, Wolfe *et al.* 1994 Christaki *et al.* 1996). However since dissolved DMSP is chemically fairly stable in sterile seawater (Dacey and Blough 1987) and not liable to photochemical cleavage (Slezak *et al.* 2001), the conversion to the volatile sulphur species DMS is obviously exclusively mediated by enzymatic cleavage with DMSP-lyases of either algal (Stefels and Dijkhuizen 1996, Steinke *et al.* 1998) or bacterial origin (Yoch 2002). Among the releasing processes significant losses in the transformation from cellular DMSP to DMS are caused by herbivory, since not all of the ingested cellular DMSP is excreted as dissolved DMSP or DMS (Wolfe *et al.* 1994), whereas exudation and cell lysis are processes which release DMSP directly from the intracellular pool. However, the greatest losses occur after the cellular release due to bacterial degradation of dissolved DMSP *via* the demethylation pathway and by bacterial consumption of DMS (Yoch *et al.* 1997, Kiene and Linn 2000, Yoch 2002). The net yield of DMS from the biological DMSP production is therefore significantly affected by the bacterial utilization of DMS and dissolved DMSP. The demethylation pathway is a major link for the bacterial sulphur assimilation from DMSP (Kiene *et al.* 1999) and it acts as a major sink of the DMS production. Therefore it was suggested that the DMS yield might be closely linked to the bacterial sulphur demand (Kiene and Linn 2000).

Our experiments focussed on the nutrient related changes of the DMS yields of the DMSP decomposition in oligotrophic microbial communities in the tropical Atlantic. We applied incubation experiments with natural size-fractionated bacterial communities which were selectively enriched with organic and inorganic nutrients, to test the hypothesis that the utilization of DMSP as a sulphur- and carbon-source decreases and the DMS yield increases if alternative organic sulphur- or carbon-sources are abundantly available.

4.3 Materials and Methods

Four sets of incubation experiments were conducted with natural size-fractionated bacterial communities collected in the tropical Atlantic in 20th October and 02nd November 2002 (Figure 1). The investigation aimed on the bacterial turnover of DMSP and the efficiency of its conversion to DMS under natural and experimentally manipulated nutrient conditions.

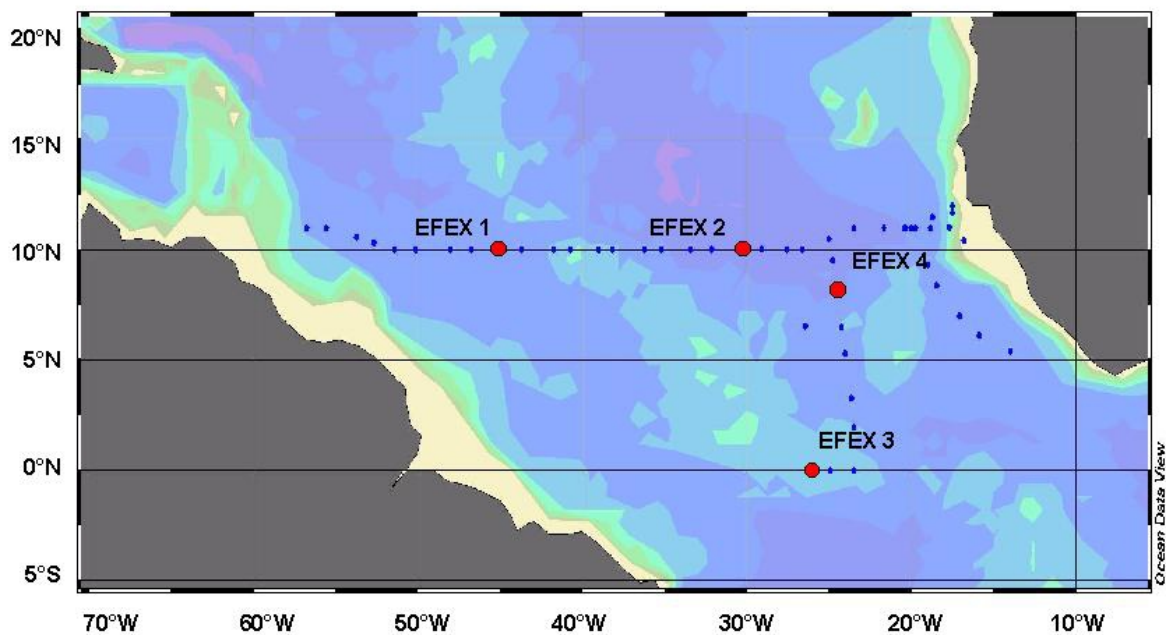


Figure 1. Working area of the M55 expedition in the tropical Atlantic with the position of field experiments in the Amazon influenced western Atlantic (EFEX 1), central subequatorial Atlantic (EFEX 2), central equatorial Atlantic (EFEX 3) and in the eastern subequatorial Atlantic (EFEX 4).

Experimental set up

Seawater was collected with Niskin bottles attached to a CTD probe (Seabird, GO) from a standard depth of about 20m (Figure 2). The samples were gently pre-screened during transfer from the Niskin bottles to Polyethylene carboys (25litre) by a silicon tubing equipped with a meshbag of 10 μ m net gauze at the inlet to reduce the micro- and nanoplankton content. Free bacteria were separated from the larger eukaryotic plankton organisms by size fractionation with gentle pressure filtration through cellulose acetate membranes of 1 μ m pore size and 15 cm diameter. Previous fluorescence microscopic examination of the filtrate stained with 3,6-

Diamidino-acridin-hemisulfate (*Proflavin*[®]/SIGMA) and sampled on black nucleopore membranes of 0.2µm pore size revealed that cells > 2.5µm were efficiently retained by fractionation, *i.e.* small single-celled cyanobacteria and the smallest eukaryotic algae with cell diameters less than 2.5µm passed the filter. The filtrate therefore basically contained free suspended natural picoplankton *sensu* Sieburth *et al.* (1978). The amount of particulate DMSP in the 1µm size-fractionated filtrate which could be sampled on cellulose acetate membranes of 0.2µm pore size was < 0.25nM (*i.e.* not detectable).

Twelve subsamples of the size-fractionated filtrate were distributed to two sets of six incubation bottles each (2.15dm³, high density polyethylene (HDPE)) which received five different experimental treatments including antibiotics, dissolved DMSP and organic or inorganic nutrients. 10 subsamples were spiked with 100nM DMSP-HCl (CASS, University of Groningen), two subsamples additionally received dissolved inorganic nitrogen and phosphorus (**DINP**) (10µM NaNO₃, 1µM NH₄-Cl and 1.5 µM KH₂PO₄), two were amended with dissolved organic carbon (**DOC**) (100µM Amylopectin), two were additionally enriched with dissolved organic sulphur nitrogen and carbon (**DONS**) (100µM methionine), two were additionally treated with antibiotics (**AB**) (1.25 mg·ml⁻¹ tetracycline, Fluka 87130 and 2.5 mg·ml⁻¹ chloramphenicol, Fluka 23275) and two subsamples served as controls. The remaining two bottles were used to take samples for the start concentrations of DMS, dissolved DMSP and bacterial production.

The whole sample preparation (size fractionation, transfers and nutrient addition) was generally completed and incubations started within 1 hour after the collection of seawater. The samples were incubated for 6h to 28h at a constant temperature of 28°C which was in all cases close to the *in situ* temperature (Table 1). During incubation the bottles were constantly rotated in a roller incubator to keep the cells in suspension. To facilitate a gastight sample extraction from the incubation bottles, the screw caps were equipped with screw lock valves with a syringe connection and an internal prolongation of polytetrafluorethylen (PTFE) tubing

Sampling

Subsamples were taken with gastight syringes *via* the screw lock valve of the incubation bottles. At the start of the experiment (*i.e.* at the addition of the nutrient supplements) samples were taken to determine the initial concentration of dissolved inorganic nitrogen and phosphate, DMS and dissolved DMSP as well as the bacterial production. The first bottle of each treatment was sampled after 6 to 19h and the duplicate bottle after 25 to 28h of

incubation. The experiment duration was defined as the time interval from the initial sampling at the time point of nutrient addition (t_0) to the intermediate (t_1) or final sampling (t_2). For bacterial production the time points were defined by the incubation start with H_3 Thymidine.

Nutrients and chlorophyll-a

All nutrient concentrations were determined according to Hansen and Koroleff (1999). Nitrate, nitrite and phosphate were measured by an autoanalyser whereas the concentration of ammonia was determined manually to lower the detection level. Total chlorophyll was determined by acetone extraction and fluorescence measurement in a turner design fluorometer before and after acidification according to Herbland *et al.* (1985).

DMS analysis

The measurements basically followed the protocol of Kiene (1993) and were carried out on a Varian gas-chromatograph (GC-6000) equipped with a flame photometric detector (Thermoquest FPD-800) and a fused silica capillary column (CP-Sil 5 CB Chrompack: 30m x 0,32mm). Samples were cryofocused in a closed PTFE purge and trap system without internal glass or metal surfaces. Filtered (GF/F) seawater samples (10 – 50 ml) were purged for 5min with a flow of finely dispersed He ($80 \text{ ml} \cdot \text{min}^{-1}$). The gas stream was dried by condensation of water vapour in lopes of PTFE tubing which were tightly fitted into an aluminium block and cooled by Peltier elements to -20°C to condense and freeze water vapour during the gas extraction. The block was heated to $+60^\circ\text{C}$ after extraction to melt and drain of the condensed water. Additionally a PTFE tubing of 15cm length and $\frac{1}{4}$ in o.d. packed with K_2CO_3 was used to extract residual water vapour from the gas stream. A loop of PTFE tubing of $\frac{1}{16}$ in o.d. submerged in liquid nitrogen was used to cryofocus DMS and was subsequently submerged in a water bath of 60°C to release the extracted gas sample onto the isothermally (60°C) operated GC column. The system was calibrated according to Turner *et al.* (1990) and Uher (1999) with a dilution series of DMS (Merck No. S28759949) dissolved in ethylene glycol (Merck No. K27686321017). The detection level was about 0.5 pmol DMS.

Dissolved DMSP (DMSP_d)

DMSP was determined as DMS which is released in an equimolar ratio after cold hydrolysis with a strong base according to Kiene (1993). Seawater samples were filtered (GF/F) and purged to remove DMS and particulate DMSP prior to hydrolysis and were analysed by the purge and trap procedure as described above for DMS.

Particulate DMSP (DMSP_p)

Gas phase measurements were applied to determine particulate DMSP as DMS which is released after hydrolysis with strong base according to Steinke *et al.* (1998). Sub-samples of 10 - 60 ml were filtered on cellulose-acetate membranes (0.2 µm pore size ST 68, Schleicher & Schuell) and were hydrolysed with 4ml of 2N NaOH for 8 - 24h in Teflon sealed screw cap vials (25ml). The vials were then incubated in a water bath at 60°C for 30 min and were equilibrated by sonication before replicate gas samples of 25 – 250 µl were taken from the headspace by gastight microlitre syringes. The gas samples were directly injected via a Teflon-sealed injection-liner on a gas chromatograph (Varian, GC-6000) equipped with a isothermally (60°C) operated fused silica capillary (Chrompack, CP-Sil 5 CB 15m x 0,32mm) and a flame photometric detector (Thermoquest, FPD-800). A dilution series of ultra pure DMSP-HCl (CASS, University of Groningen) dissolved in mili-Q water was used for calibration. The diluted DMSP standards (1 ml aliquots) were hydrolysed together with a blank filter by 3 ml of 2N NaOH in Teflon sealed screw cap vials (25ml) and were analysed as described above. The calibration coefficients were determined according to Uher (1999). The detection limit for a 250 µl headspace injection was 0.5 pmol DMS.

Bacterial production

Bacterial production was determined by incorporation of [³H]-labeled thymidine into macromolecules precipitated by cold trichloroacetic acid (TCA) according to Bjørnsen and Kuparinen (1991). Four 10ml aliquots of water sample were dispensed into plastic vials. One of these subsamples was fixed by addition of 100 µl 39% formalin amended with non-radioactive thymidine and served as a blank. All samples received methyl-[³H]-thymidine, specific activity 3.11 TBq mmol⁻¹ (Amersham), to a final concentration of 2 nM. The samples were incubated for 2-3 h at 28°C, and the incubation was stopped by addition of 100µl 39% formalin amended with thymidine. The samples were filtered through 0.22 µm poresize cellulose acetate filters (pre-soaked in a non-radioactive thymidine solution) and rinsed 10 times with 2 ml 5 % ice cold TCA. The filters were placed in scintillation cocktail (Lumagel SB, Baker Chemicals), and the radioactivity incorporated in the cold TCA precipitable material was measured onboard by a Packard Tricarb 1900TR Liquid Scintillation Analyzer. Corrections for quench were made by transformed spectral indices of external standards and stored quench curves.

DMSP consumption and DMS production

The microbial decomposition of dissolved DMSP (D_{DMSP}) was determined under exclusion of virtually all DMSP-producing or -releasing organisms and under exclusion of UV-light in incubations of $< 1.0\mu\text{m}$ fractionated samples which contain mainly free suspended bacteria and no detectable amounts of particulate DMSP. We assume that under these conditions no other processes than the microbial metabolism produced or degraded significant amounts of dissolved DMSP and DMS. The decomposition rate of dissolved DMSP is based on the change of the concentration of dissolved DMSP ($\delta [DMSP_d]$) during the incubation interval $[t_0, t_n]$ of the length δt and was calculated by $D_{DMSP_d} = -1 \cdot \delta [DMSP_d] / \delta t$.

The microbial production of DMS (P_{DMS}) is determined here as the mean net production of DMS of natural picoplankton communities in DMSP spiked incubations of about 25h to 28h length. The microbial DMS production is based on the observed net change (increase) of the DMS concentration ($\delta [DMS]$) which is the result of both DMS generating and DMS degrading processes (*i.e.* basically DMSP cleavage, bacterial DMS assimilation and its oxidation to dimethylsulfoxide and sulphate) and was calculated by $P_{DMS} = \delta [DMS] / \delta t$.

DMS yield of microbial DMSP degradation

The DMS yield of the microbial DMSP degradation (conversion efficiency) should be most adequately defined as the fraction of microbially consumed DMSP which is converted to DMS. However, for practical reason we determined the DMS yield as the ratio of the microbial DMS production and the microbial consumption of dissolved DMSP (DMS-yield = $P_{DMS} : D_{DMSP}$). This is because the observed DMS production is, as mentioned above, a net production which also includes the bacterial consumption of DMS and hence potentially underestimates the DMS release from DMSP cleavage.

4.4 Results

Water column conditions

The seawater from the upper mixed layer at all stations, where samples were taken for incubation experiments, was virtually nitrogen and phosphate depleted but had residual silicate between 1.1 and 5.0 $\mu\text{mol}\cdot\text{dm}^{-3}$ (Table 1). The chlorophyll maxima were mostly associated with the nitracline while the maxima of DMS and particulate DMSP were all

located within the upper mixed layer (Figure 2 and 3). In the Amazon influenced western Atlantic (EFEX-1) we additionally found a pronounced second maximum of DMS and particulate DMSP at the surface. In the eastern equatorial Atlantic (EFEX-3) a weak second DMSP_P maximum occurred near the thermocline but this was not apparent in the DMS concentration. The *in situ* concentrations of dissolved DMSP at the depth were seawater samples for the experiments were taken ranged from 4.9 nmol·dm⁻³ in the Amazon influenced western Atlantic (EFEX-1) to 30.8 nmol·dm⁻³ in the eastern subequatorial region (EFEX-4), the *in situ* DMS concentrations ranged from 2.1 nmol·dm⁻³ in the eastern subequatorial region (EFEX-4) to 5.8 nmol·dm⁻³ in the central subequatorial Atlantic (Table 2).

Table 1 Sampling depth, *in situ* temperature and concentration of inorganic nutrients for experiments conducted in the Amazon influenced western Atlantic (EFEX-1), the central subequatorial Atlantic (EFEX-2), the central equatorial Atlantic (EFEX-3) and the eastern subequatorial Atlantic (EFEX-4). The Location of the sampling stations are presented in Figure 1.

experiment	Sample depth m	<i>In situ</i> temp °C	Ammonia μ mol · dm ⁻³	Nitrate	Nitrite	Phosphate	Silicate
EFEX-1	22.4	29.1	0.06	0.05	0.00	0.06	5.0
EFEX-2	21.3	27.8	0.26	0.06	0.00	0.01	1.2
EFEX-3	21.9	26.2	0.12	0.03	0.00	0.09	1.1
EFEX-4	22.1	28.3	0.09	0.02	0.00	0.03	1.3

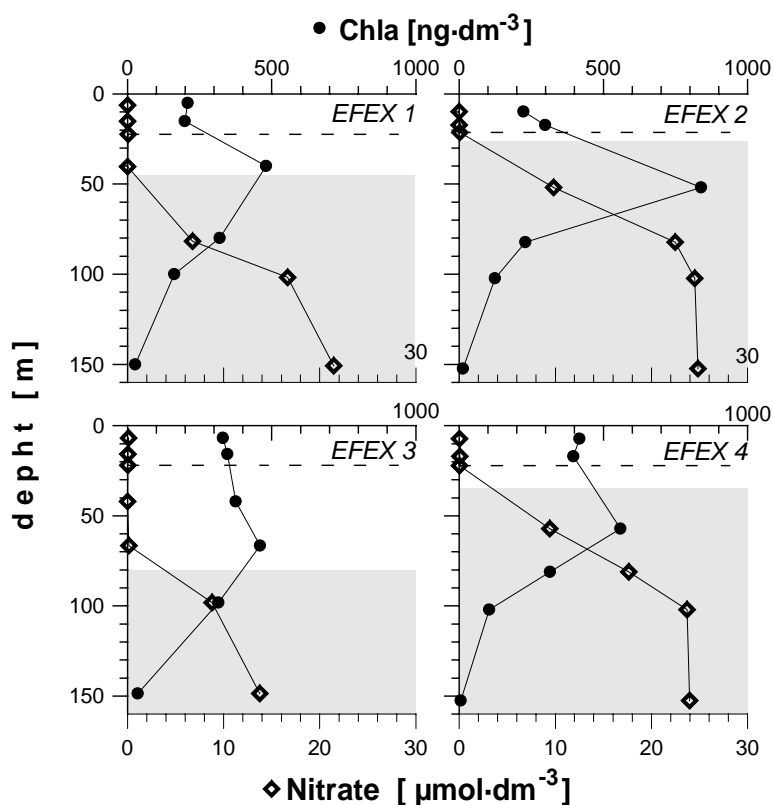


Figure 2. Vertical distribution of phytoplankton biomass (concentration of chlorophyll-*a*) and depth profiles of nitrate concentration at stations where seawater samples for incubation experiments were collected. The approximate extension of the upper mixed layer is indicated by the white unshaded area, the sampling depth for dilution experiments is marked by a dashed line.

Table 2 *In situ* concentrations of DMS, dissolved and particulate DMSP (DMSP_d and DMSP_p) as well as the initial bacterial production (BP) at the depth where seawater was collected for the incubation experiments. The location of the sampling stations are presented in Figure 1.

experiment	DMS n mol · dm ⁻³	DMSP _d	DMSP _p	BP μgC·dm ⁻³ ·d ⁻¹
EFEX-1	2.7	4.9	45.1	0.33 ± 0.05
EFEX-2	5.8	7.3	54.6	0.37 ± 0.05
EFEX-3	4.5	16.6	70.1	0.37 ± 0.05
EFEX-4	2.1	30.8	36.2	0.12 ± 0.02

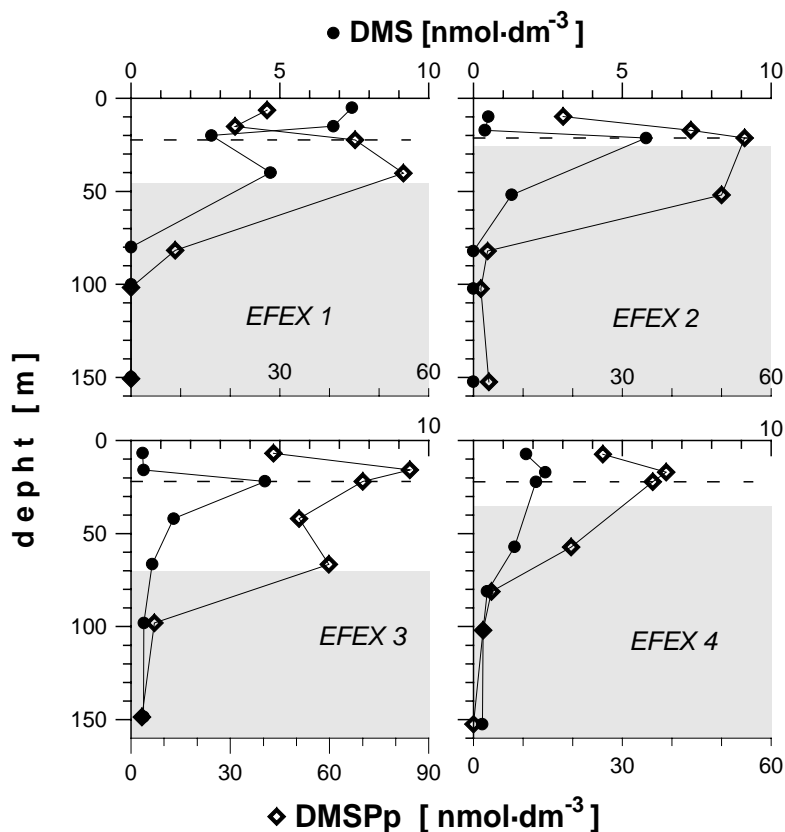


Figure 3. Vertical distribution of particulate DMSP and depth profiles of the DMS concentration at stations where seawater samples for incubation experiments were collected. The approximate extension of the mixed layer is indicated by the white unshaded area, the sampling depth for dilution experiments is marked by a dashed line. Dissolved DMSP was not determined in the vertical profiles.

Bacterial carbon production

The ambient rates of the bacterial carbon production in the whole plankton as well as the initial experimental rates in the fractionated samples were extremely low (Table 2 and Figure 4 respectively). The bacterial production in incubation experiments with natural size-fractionated bacterial communities increased significantly in all nutrient amended treatments,

in all controls and till the end of the incubation also in two samples (EFEX 2 and EFEX 3) treated with antibiotics (Figure 4, bottom row). In the Amazon influenced western Atlantic (EFEX-1) and in the eastern subequatorial Atlantic (EFEX-4) the increase in the bacterial production was relatively slow or delayed in controls and incubations with incomplete nutrient enrichments (DINP and DOC) compared to the DONS enriched incubations.

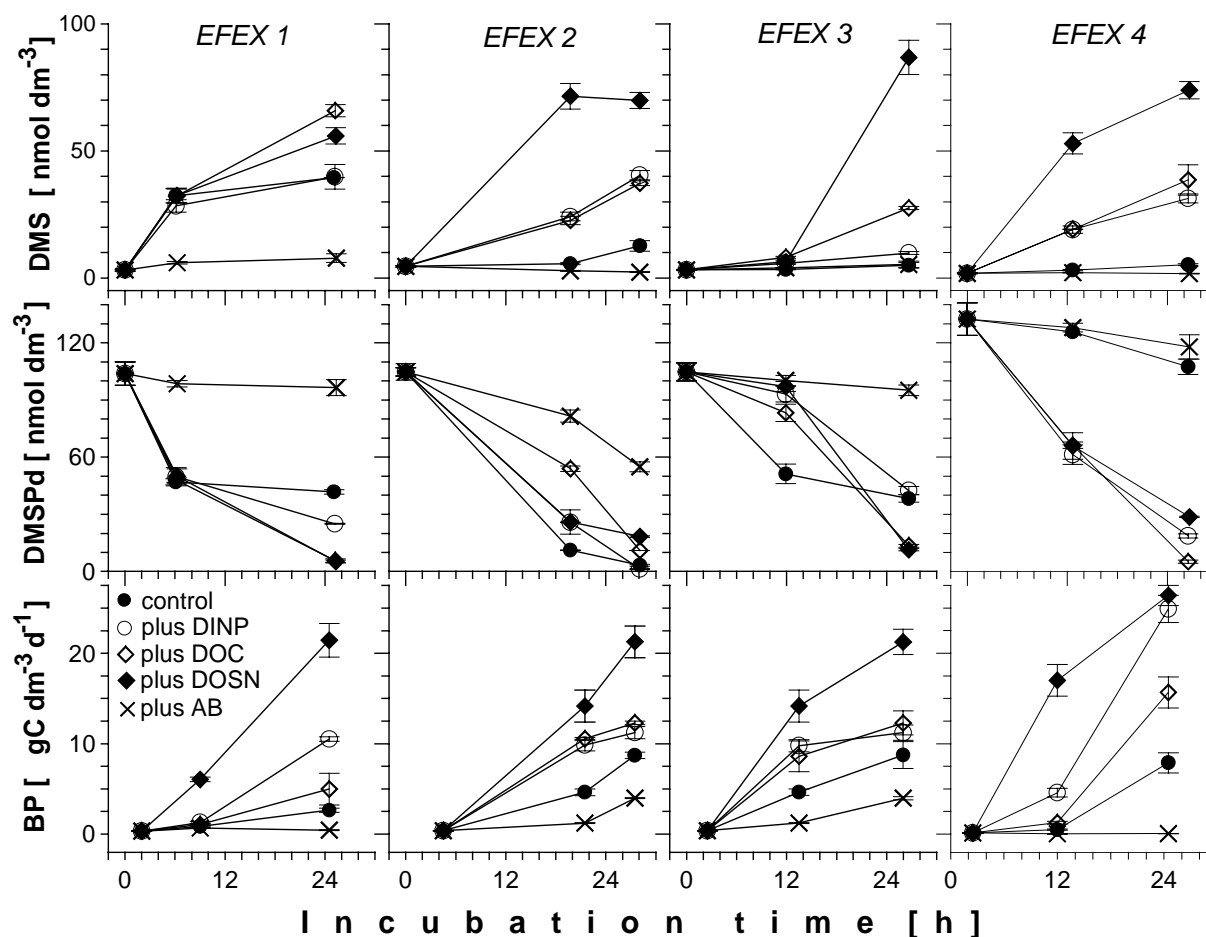


Figure 4. Time courses of the concentration of DMS and dissolved DMSP (DMSP_d) as well as for the bacterial production in seawater samples incubated with different nutrient enrichments: untreated control (●), plus DINP (○), plus DOC (◇) plus DONS (◆) and plus antibiotics (×). Abbreviations and treatments are as described in Table 3. Note that all samples were amended with 100nM DMSP_d. Data points show average values and vertical bars show ranges of duplicate (DMS and DMSP_d) or triplicate (bacterial production) measurements.

DMS production

Handling of seawater samples during preparation for the incubation experiments, especially size fractionation and four sample transfers *via* silicon tubing from the Niskin bottles to the incubation bottles caused net changes to the *in vitro* content of DMS between +0.4 to -1.3 nmol DMS dm⁻³. These are substantial disturbances in relation to the ambient DMS

concentration but they are of minor importance compared to most of the observed changes during incubation.

The DMS concentration strongly increased in all incubation experiments with natural picoplankton which were enriched with DOC and DONS and except for samples collected in the central equatorial Atlantic (EFEX-3) it also increased in DINP enriched incubations (Figure 4). A strong increase of the DMS concentration was observed also in unenriched picoplankton samples from the Amazon influenced western Atlantic (EFEX-1) and the central subequatorial Atlantic (EFEX-2). The antibiotic treatment completely inhibited the production of DMS in all experiments and it depressed or significantly reduced (central subequatorial Atlantic (EFEX-2)) the consumption of dissolved DMSP. The bacterial production was efficiently depressed for at least 12h but greatly increased till the end in incubations of unenriched picoplankton from the Amazon influenced western Atlantic (EFEX-1) and the central equatorial Atlantic region (EFEX-3) (Figure 4).

The microbial DMS production in incubations without nutrient additions ranged from 0.07 to 1.45 $\text{nmol}\cdot\text{dm}^{-3}\cdot\text{h}^{-1}$ but increased (up to 3.13 $\text{nmol}\cdot\text{dm}^{-3}\cdot\text{h}^{-1}$) in most experiments due to various nutrient enrichments. The only case where the microbial production of DMS did not significantly increase in relation to the control was DINP enriched picoplankton from the Amazon influenced western Atlantic (EFEX-1) (Table 3). The overall strongest increase of the microbial DMS production was caused by DONS enrichments, followed by enrichments of DOC and DINP. Likewise the consumption of dissolved DMSP increased in most experiments due to organic and inorganic nutrient enrichments but differences between DONS, DOC and DINP enrichments had no clear trends and were not similar to the responses of the microbial DMS production (Table 4). However, the microbial DMSP consumption in DINP enriched incubations of picoplankton from the central equatorial region (EFEX-3) and in DINP or DOC enriched incubations from the central subequatorial region (EFEX-2) were not significantly different from the controls. The DONS enrichment in the central subequatorial region (EFEX-2) was the only case where the microbial DMSP consumption in a nutrient enriched picoplankton sample was significantly lower than in the control (Table 4). Overall, the microbial consumption of DMSP always exceeded the production of DMS.

Tab. 3 DMS production (\pm SD of analytical replicates) in seawater samples incubated with selective nutrient enrichments: Dissolved inorganic nitrogen and phosphor (**DINP**, *i.e.* +10 μ M NO₃, 1 μ M NH₄ and 1.5 μ M PO₄), dissolved organic carbon (**DOC**, *i.e.* +100 μ M Amylopectin), dissolved organic sulphur and nitrogen (**DONS**, *i.e.* +100 μ M Methionin) or antibiotics (**AB**, *i.e.* +1.25 mg·ml⁻¹ tetracycline and 2.5 mg·ml⁻¹ chloramphenicol). All treatments were additionally enriched with 100nM DMSP_d. Estimates are based on 25h to 28h. The Location of the sampling stations are presented in Figure 1.

experiment	Control nmol · dm ⁻³ · h ⁻¹	+DINP	+DOC	+DONS	+AB
EFEX-1	1.45 ± 0.02	1.46 ± 0.21	2.49 ± 0.11	2.09 ± 0.14	0.19 ± 0.08
EFEX-2	0.29 ± 0.09	1.28 ± 0.07	1.17 ± 0.04	2.33 ± 0.12	-0.08 ± 0.01
EFEX-3	0.07 ± 0.05	0.25 ± 0.03	0.91 ± 0.03	3.13 ± 0.26	0.08 ± 0.05
EFEX-4	0.13 ± 0.02	1.11 ± 0.07	1.39 ± 0.23	2.71 ± 0.13	0.00 ± 0.01

Table 4 DMSP consumption (\pm SD of analytical replicates) in incubations with selective nutrient additions. Abbreviations of treatments are as described in Tab. 3. The Location of the sampling stations are presented in Figure 1.

experiment	Control nmol · dm ⁻³ · h ⁻¹	+DINP	+DOC	+DONS	+AB
EFEX-1	2.48 ± 0.30	3.13 ± 0.26	3.89 ± 0.28	3.90 ± 0.28	0.29 ± 0.42
EFEX-2	3.62 ± 0.09	3.68 ± 0.09	3.33 ± 0.08	3.07 ± 0.09	1.77 ± 0.12
EFEX-3	2.49 ± 0.26	2.33 ± 0.27	3.42 ± 0.21	3.50 ± 0.21	0.36 ± 0.29
EFEX-4	0.95 ± 0.32	4.29 ± 0.36	4.81 ± 0.35	3.90 ± 0.34	0.55 ± 0.56

DMS yield of the microbial DMSP decomposition

The DMS yield describes the fraction of the dissolved DMSP which was microbially degraded and converted to DMS. The DMS yield varied over a wide range from a minimum of 2.7 % in nutrient unamended samples from the central equatorial region (EFEX 3) to 89.5% in DONS enriched samples at the same station (Table 5). The average DMS yield in unenriched samples was 20.8%. Apart from samples collected at the Amazon influenced region (EFEX-1) where the microbially conversion of dissolved DMSP to DMS was relatively stable regardless of the kind of nutrient addition, the DMS yields in unenriched samples were overall significantly lower than in the DINP, DOC or DONS enriched samples. The DMS yields in DOC and DONS enriched incubations was equal (EFEX 2) or higher (EFEX 1, 3 and 4) than in DINP enriched samples. And except for the experiment in the Amazon influenced region (EFEX-1) DONS enrichments achieved higher DMS yields than DOC enrichments.

Table 5 DMS yield of the microbial DMSP decomposition. *i.e.* the fraction of the microbially consumed DMSP which is converted to DMS. Abbreviation of treatments is as described above (Tab. 3). The location of the sampling stations are presented in Figure 1.

experiment	Control	+DINP	+DOC	+DONS
	DMS production / DMSP consumption [%]			
EFEX-1	58.7	46.6	63.9	53.5
EFEX-2	8.0	34.7	35.0	75.6
EFEX-3	2.7	10.6	26.7	89.5
EFEX-4	13.8	25.9	28.9	69.5
Average:	20.8	29.4	38.6	72.0

4.5 Discussion

Even though there are several alternative pathways for the microbial degradation of DMSP, all DMSP-sulphur will be ultimately oxidised to sulphate. But it makes a difference if the final oxidation happens in the ocean or after the emission of volatile sulphur species in the atmosphere (Charlson *et al.* 1987). Our investigation therefore focused on the channelling of the DMSP-sulphur to the atmospheric oxidation and hence to that part of the microbial DMSP degradation that yields the volatile sulphur species DMS which seems to be the major link of the reduced sulphur to the atmosphere (Andreae 1999).

Considering that the algal production of DMSP in the euphotic zone of the ocean (Archer *et al.* 2001, Simo *et al.* 2002) is on average about two to three orders of magnitude higher than the emission of DMS to the atmosphere (Andreae *et al.* 1994, Kiene and Bates 1990, Simó and Pedrós-Alio 1999, Archer *et al.* 2002), it becomes clear that the losses in the productive line from the cellular DMSP to the volatile DMS are as important as the production itself. The overall greatest sink for the DMSP after its release from the phytoplankton cells which is caused by herbivory, cell lysis or exudation (Wolfe *et al.* 1994, Christaki *et al.* 1996 - Stefels 2000, Wilson *et al.* 2002 - Laroche *et al.* 1999) seems to be the bacterial decomposition through the demethylation / demethiolation pathway and the uptake and assimilation into bacterial biomass (Kiene and Linn 2000). But even the fraction which is converted to DMS is subsequently liable to further oxidation or assimilation by bacteria (Wolfe and Kiene 1993, Kiene and Linn 2000). The partitioning of the microbial degradation between the DMS producing pathway and the demethylation-, assimilation or oxidation of the DMSP-sulphur is therefore a major factor in the biological DMS production of the ocean. The observed DMS yields of the microbial DMSP degradation in natural plankton communities range from virtually 0% to 100% (Table 6).

Table 6 Published data on DMS yields of the microbial DMSP degradation.

location	DMS yield	Authors
Tropic E-Pacific	5 %	Kiene 1992
Duplin River estuary	28 %	Kiene and Service 1991
Coastal North Sea	0 – 5 %	Kwint et al 1996
Gulf of Mexico	22 – 66 %	Kiene 1996
Gulf of Main	12 - 15 %	Kiene 1996
Sargasso Sea	20 – 67 %	Ledyard and Dacey 1996
Subpolar N-Atlantic	6-100 %	Simó and Pedrós-Alió (1999)
Coastal and Shelf	< 21 %	Kiene and Linn 2000
Oceanic sites	< 13 %	Kiene and Linn 2000
Labrador Sea	60 %	Schultes <i>et al.</i> 2000
Mediterranean	87 %	Simó et al. 2000
North Sea	24 %	Simó et al. 2000
Subpolar N-Atlantic	9 – 95 %	Simó et al. 2000
North Sea	9.5 – 14.2 %	Zubkov <i>et al.</i> 2001

However, these losses of DMSP from the DMS producing pathway supply significant amounts of energy, organic carbon and organic sulphur for bacterial production which may provide up to 15% of their carbon and up to 100 % of their sulphur demand (Kiene and Linn 2000, Simo *et al.* 2002). The value of DMSP as an energy source for bacterial growth is questionable since the expenditure of energy for cellular transport may exceed the energy yield (Jansen and Hansen 2001) while its cleavage product acrylate is readily utilized by marine bacteria as a growth substrate (Kiene 1990). The DMSP-sulphur seems to be provided primarily *via* the demethylation pathway for bacterial biomass production, as methanethiol (MeSH) serves as a major source of reduced sulphur for bacterioplankton (Kiene *et al.* 1999). Kiene and Linn (2000) were the first to suggest, that the DMS yield of the microbial DMSP degradation might be related to bacterial sulphur demand and assumed that the yield would increase to about 100% if the supply of dissolved DMSP exceeds the bacterial sulphur demand. However in natural communities DMSP acts in concert with all other organic sulphur- and carbon compounds and even if DMSP is the dominant organic sulphur species some less abundant but preferentially assimilated compounds may effect the partitioning between the demethylation- and the cleavage-pathway, *i.e.* between the utilization as a sulphur source or the release of DMS. Our experiments were therefore designed to test the slightly modified hypothesis that the DMS yield increases, if alternative sources of dissolved organic carbon- and sulphur are readily and more abundantly available than DMSP. The assumption is made, that the DMSP assimilating bacteria primarily use DMSP as a source of organic carbon and sulphur (*via* demethylation / demethiolation) if other carbon- and sulphur sources (cysteine, methionine, glutathione), do not satisfy the whole carbon- or sulphur demand or require far more energy for uptake or assimilation (such as SO_4^{2-}).

The investigation required to uncouple the production and release of dissolved DMSP from the bacterial consumption which was achieved by the isolation of free bacteria from both bacterivorous grazers and DMSP-producing algae. The exclusion of bacterivorous grazers favoured in all experiments a rapid increase of the bacterial production (Figure 4), which indicated a severe grazer control within the whole microbial community. The observed rates for the microbial consumption of DMSP and the production of DMS are therefore overestimates compared to *in situ* conditions.

However the grazer exclusion revealed the differences between the communities and magnified the responses due to selective nutrient enrichments which indicated that the bacterial production was after 6h to 18h limited by the availability of organic sulphur.

Sulphur is always abundantly available in the form of sulphate whereas its uptake and assimilation is energetically quite costly (Stefels 2000). Apparently the bacterial sulphur assimilation is a thermodynamic rather than an availability problem. The demethylation / demethiolation of DMSP provides reduced sulphur in the form of methanethiol which can be readily assimilated into protein *via* methionine (Kiene et al. 1999), while the cleavage pathway provides DMS which is rather of minor importance as a bacterial sulphur source (Kiene and Linn 2000).

Therefore, we expected in the above hypothesis that bacteria which grow under sulphur limited conditions would degrade relatively more DMSP *via* the demethylation pathway and less *via* the cleavage pathway than under sulphur replete conditions. Consequently high DMS yields would only be achieved if the DMSP utilizing bacteria either predominantly use other sulphur sources than DMSP or if the supply of DMSP greatly exceeds the bacterial sulphur demand.

This assumption is consistent with the observation that the overall highest increases of the bacterial production as well as the highest DMS yields were achieved in incubations which were enriched with an alternative source of dissolved organic sulphur and nitrogen (Figure 4, table 5). Except for EFEX 4 in the eastern tropical Atlantic the changes of the microbial DMSP-consumption in response to nutrient enrichment appeared to be relatively small compared to increases of the BP. This indicates that the DMSP-degrading bacteria did either not profit from the enrichments to the same degree as the major producers or that the significance of DMSP as a carbon- and sulphur source decreased due to the greater availability of alternative substrates. The relatively strong synchronous increase of the DMS production in response to the nutrient enrichments, especially organic carbon and sulphur,

rather points to a reduced utilization of DMSP as a sulphur and carbon source than to a weak or no response of the DMSP-degrading bacteria to nutrient additions.

However the conversion efficiencies all remained far below 100% although the enrichments were several times in excess of the estimated sulphur demand of the bacterial production. This might be due to bacteria which used DMSP as the preferred or additional source of organic sulphur or by a relatively high uptake and retention *e.g.* as compatible solute. Interestingly the DMS yields increased to a lower degree also in response to an excess supply of DINP or readily digestible DOC. A possible explanation for the effect of DOC enrichments on the DMS yield is that the availability of extra energy might increase the availability and utilization of alternative sulphur compounds which otherwise require too much energy for uptake and assimilation.

We conclude, that the nutrient availability, especially readily degradable organic carbon and sulphur, has a strong impact on the DMS yield of the microbial DMSP degradation and therefore constitutes major limiting factor for the DMS production in the ocean.

Acknowledgments

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5 Zusammenfassung und Schlussbetrachtungen

Diese Studie war darauf ausgerichtet, zu einer besseren Vorhersagbarkeit von Veränderungen der DMS Produktion im Ozean beizutragen.

Die Komplexität mariner Planktongemeinschaften, die Vielzahl der beteiligten Prozesse und ihrer Beeinflussbarkeit durch eine Reihe abiotischer Faktoren, setzt der Vorhersagbarkeit jedoch natürliche, technische und ökonomische Grenzen. Andererseits, begründet die Regelmäßigkeit der saisonalen Zyklen in den DMS-Emissionen (Yvon und Saltzman, 1996, Ayers and Gillett 2000), der enge Zusammenhang der DMS-Emissionen mit Klimaänderungen im Tertiär (Legrand *et al.* 1988, 1991, 1997), die relativ klaren Verteilungsmuster des DMS im Ozean (Kettle *et al.* 1999) und vor allem eine anhaltend lebhaft und innovative Forschungsaktivität die Hoffnung, dass man diesem Ziel aus unterschiedlichen Richtungen näher kommt.

Charlson *et al.* (1987) nahmen an, dass, wenn Licht und Temperatur die DMS Produktion positiv beeinflussen, durch die vermehrte Produktion von Schwefel-Aerosolen, eine negative Rückwirkung auf die Einstrahlung entstände, die als Antagonist zum CO₂ möglicherweise wie eine „Globale Thermostat Regelung“ wirke. Mit dieser Hypothese regten Charlson *et al.* (1987) eine der kontroversesten und fruchtbarsten Diskussionen in den Naturwissenschaften der vergangen Jahrzehnte an.

Die biologische DMS-Produktion, P_{DMS} ist im Prinzip sehr einfach beschreibbar durch die pflanzliche DMSP-Produktion, P_{DMSP} und den dimensionslosen Ertragskoeffizienten, k , der den Anteil beschreibt, der von P_{DMSP} zu DMS abgebaut wird. Dabei wird P_{DMSP} vollständig bestimmt durch den intrazellulären DMSP-Gehalt der Planktonalgen (a) und deren Wachstumsrate (b) und Abundanz (c), so dass gilt,

$$P_{DMS} = P_{DMSP} \cdot k = a \cdot b \cdot c \cdot k \text{ (Gleichung 1).}$$

Der Einfluss des Lichtes und des Nährstoffangebotes auf P_{DMSP} .

Im photosynthetisch aktiven Wellenlängenbereich (PAR) hat die Lichtintensität, im Einklang mit der Thermostat-Hypothese von Charlson *et al.* (1987), innerhalb gewisser Schwellenwerte, einen positiven Einfluss auf das Wachstum und den intrazellulären DMSP-Gehalt der Planktonalgen (das Produkt $a \cdot b$) (Vetter und Sharp 1993, Matrai *et al.* 1995,

Stefels und van Leeuwe 1998, Slezak und Herndl 2003). Die Temperatur und überschwellige Lichtintensitäten haben jedoch einen entgegengesetzten Effekt, weil bei steigender Temperatur und bei hohen UV-Strahlungsintensitäten die Konzentration von intrazellulärem DMSP (Faktor *a*) abnimmt, und bei hohen Strahlungsintensitäten im PAR das Wachstum und die DMSP-Produktion gehemmt werden (Produkt *a·b*) (Hefu und Kirst 1997, van Rijssel und Buma 2002). Das Licht begrenzt in erster Linie die Zeit und den Raum (euphotische Zone), in der die pflanzliche Produktion einschließlich der DMSP-Synthese überhaupt möglich ist. Letztlich wird die Größe der Produktion jedoch bestimmt durch die Menge der Nährstoffe, die in diesem Licht-Zeit-Raum verfügbar ist. So weit diese Nährstoffmenge erschöpfbar ist, bestimmen Temperatur und Lichtintensität zunächst nur die Geschwindigkeit mit der dies geschieht. Bei der pflanzlichen DMSP-Produktion wurde daher in dieser Studie das Augenmerk vor allem auf den Einfluss des Nährstoff- bzw Stickstoffangebotes gelegt. Zum einen, weil der Stickstoff der am häufigsten limitierende Nährstoff im Ozean ist (Dugdale und Wilkerson 1992, Graziano *et al.* 1996). Zum anderen weil in einer Reihe von Untersuchungen bei Stickstoffmangel erhöhte zelluläre DMPS-Gehalte beobachtet wurden (Turner *et al.* 1988; Gröne und Kirst 1992; Matrai und Keller 1994). Da Wachstumsraten in der Regel positiv mit dem Nährstoffangebot korreliert sind, wurde in dieser Studie für die Faktoren *a* und *b* gegenläufige Reaktionen auf Änderungen im Stickstoffangebot vermutet, deren Gesamteffekt auf die spezifische DMSP Produktionsrate (dem Produkt *a · b*) bislang unbekannt waren. In den hierzu durchgeführten Labormessungen an einer Auswahl von Algenstämmen aus den wichtigsten eukarionten Klassen des marinen Phytoplanktons (Kapitel 2), dominierte bei abnehmenden Konzentrationen von gelöstem anorganischen Stickstoff (DIN) in allen Fällen die Abnahme der Wachstumsrate (*b*) über die Zunahme der intrazellulären DMSP-Konzentration (*a*). Die spezifische DMSP Produktionsrate (*a · b*) nahm also bei sinkender DIN-Konzentration ab. Wurde jedoch die DMSP-Produktion auf den partikulären organischen Zellstickstoff (PON) bezogen, *d.h.* auf die hier so genannte N-spezifische DMSP-Produktion, die beschreibt wie viel partikuläres DMSP pro partikulärem Zellstickstoff und pro Tag produziert wird, so zeigten sich zwei unterschiedliche Reaktionen auf abnehmende DIN-Konzentrationen.

In 6 von 9 Fällen nahm die N-spezifische DMSP-Produktion bei Abnahme der DIN Konzentration zunächst zu, steigerte sich bis zu einem intermediärem Maximum zwischen 0.7 und 2.8 μM DIN und sank darunter rasch auf null ab. Dieser Verlauf ergab sich aus einer kontinuierlichen Abnahme der Wachstumsrate bei einer gleichzeitig relativ stärkeren Zunahme des $\text{DMSP}_p / \text{PON}$ Verhältnisses. In den anderen Fällen überwog bei hohen wie bei

niedrigen DIN Konzentration die Abnahme der Wachstumsrate, und bewirkte eine kontinuierliche Abnahme der N-spezifischen DMSP-Produktion bei abnehmender der DIN Konzentration. Die hier beobachteten artspezifischen Unterschiede in den intrazellulären DMSP-Konzentrationen (0.3 bis 1521 mM), den P_{DMSP} / PON Verhältnissen (0.014 – 1.16 $\mu\text{mol S} / \mu\text{mol N}$) und den Maxima der N-spezifischen DMSP-Produktion (0.007 bis 0.603 $\mu\text{mol S} \cdot \mu\text{mol}^{-1} \text{N} \cdot \text{d}^{-1}$), lagen um 1 bis 2 Größenordnungen über ihren stickstoffbedingten Veränderlichkeiten.

Auf den ersten Blick könnte man daher annehmen, dass P_{DMSP} vollkommen von den artspezifischen Unterschieden bestimmt würde. Auf dem Niveau einer natürlichen Planktongemeinschaft nimmt jedoch der Einfluss der artspezifischen Unterschiede mit steigender Diversität exponentiell ab und kommt in vollem Umfang erst in Algenblüten zur Geltung, die von nur einer Art dominiert werden.

Auf globaler Ebene wird daher der Trend in der stickstoffbedingten Veränderlichkeit der DMSP-Produktion über die weitgehend unvorhersagbare artspezifische Veränderlichkeit der DMSP-Produktion dominieren.

Im Kapitel 3 konnte darüberhinaus am Beispiel der Haptophyce *Phaeocystis globosa* auch das Auftreten einer blütenbildenden Schlüssel-Art der DMS-Produktion in Beziehung gesetzt werden zu Änderungen im Nährstoffangebot, in diesem Falle die Erschöpfung des gelösten Silikates durch die vorangehende Diatomeenblüte. Auch die ausgedehnten Blüten der Haptophyce *Emiliania huxleyi*, die wahrscheinlich die bedeutendste Schlüssel-Art der DMS-Produktion im borealen und gemäßigten Nord Atlantik ist, entwickeln sich in der Regel aus dem Rest-Nitrat, das nach der Erschöpfung des Silikat durch Diatomeen übrig bleibt (Holligan *et al.* 1993). Das Nährstoffangebot beeinflusst daher potentiell alle Einzelfaktoren der DMSP-Produktion; d.h. auf der Ebene der Zellphysiologie die Faktoren **a** und **b**, auf der Ebene der Artzusammensetzung vor allem den Faktor **a** und schließlich in Situationen mit relativ geringen Fraß- und Sinkverlusten, auch die Bestandsgröße der pflanzlichen Produzenten (Faktor **c**).

Bedeutung der Herbivorie

Im Gegensatz zur Wachstumsrate wird der Faktor **c** jedoch häufig nicht allein von den abiotischen Wachstumsbedingungen (Licht, Temperatur und Nährstoffe) bestimmt, sondern eher von den dichteregulierenden Verlustprozessen, insbesondere von der Fraßaktivität des herbivoren Mikrozooplanktons (filtrierende Metazoen, Protozoen und mixotrophe Algen). Wichtige Ausnahmen von dieser sogenannten *Top-down*-Kontrolle durch die Herbivoren

bilden die Anfangsphasen von Frühjahrsblüten, wenn die Fraßaktivität der Herbivoren im Vergleich zum Wachstum des Phytoplanktons noch gering ist, und möglicherweise auch Virusepidemien im Phytoplankton (Bratbak *et al.* 1993, Wilson *et al.* 2002).

Da die Planktonalgen einerseits, wie oben beschrieben, mit sehr unterschiedlicher Intensität DMSP produzieren und da andererseits die meisten Herbivoren, einschließlich des Mikrozooplanktons eine zum Teil hochselektive Nahrungsauswahl betreiben (Gaul und Antia 2001), kann die Bestandsgröße des DMSP-produzierenden Phytoplanktons auch durch einen selektiven Fraßdruck stark beeinflusst werden, insbesondere dann, wenn die DMSP-Produktion selbst ein Kriterium der Nahrungsauswahl wäre. Unter kontrollierten Laborbedingungen konnte für einige wichtige Vertreter des Mikrozooplanktons ein verringerter Fraßdruck auf DMSP-produzierende Algen nachgewiesen werden (Wolfe *et al.* 1997). Dabei kam auch der Aktivität der beim Fraß freiwerdenden pflanzeigenen DMSP-spaltenden Enzyme (DMSP-Lyasen) eine wichtige Rolle zu. In den Feldexperimenten, die im Rahmen dieser Studie in sehr unterschiedlichen Planktongemeinschaften der Nordsee (Kapitel 3) und des Tropischen Atlantik (hier nicht dokumentiert) durchgeführt wurden, konnte jedoch nur in drei von sechzehn Experimenten ein im Vergleich zum Chlorophyll-*a* verminderter Fraßdruck auf DMSP-produzierendes Phytoplankton festgestellt werden. Da andererseits in vier Fällen auch eine Bevorzugung des DMSP-produzierenden Phytoplanktons nachgewiesen werden konnte, wie sie zuvor bereits während einer Blüte von *E. huxleyi* für eben diese Alge beobachtet wurde (Holligan *et al.* 1993), stützt diese Untersuchung nicht die, aus den Laborexperimenten abgeleitete Annahme einer allgemeinen chemischen Fraßschutzfunktion des DMSP (Strom *et al.* 2003).

Dies schmälert jedoch keineswegs die Bedeutung der Herbivorie für die DMS-Produktion im allgemeinen, denn sie beeinflusst diese sowohl auf der Produktionsebene (P_{DMSP}) als auch auf der Ertragsebene (Faktor k);

Zum einen kann sie bei entsprechender Intensität und Selektivität die Bestandsgröße und die Zusammensetzung des Phytoplanktons stark beeinflussen (Faktor a und c , Gleichung 1) und zum anderen ist sie einer der wichtigsten Prozesse, die zellpartikuläres DMSP freisetzen, wobei durch eine teilweise enzymatische Spaltung zu DMS während der Verdauung auch der DMS-Ertrag (Faktor k , Gleichung 1) mit beinflusst wird (Wolfe *et al.* 1994). In den hier vorgestellten experimentellen Untersuchungen von natürlichen Planktongemeinschaften in der Nordsee (Kapitel 3) betrug der Konsum von partikulärem DMSP durch das herbivore Mikrozooplankton im Durchschnitt etwa 94% der pflanzlichen Produktion, wovon im Mittel 77% wieder als gelöstes DMSP freigesetzt wurde.

Der Faktor k

Der DMS-Ertrag ist einer der komplexesten Größen in der oben aufgestellten Gleichung der DMS-Produktion. Er kann bereits bei der Freisetzung des zellpartikulären DMSP aus der Pflanzenzelle durch die Herbivoren erheblich gemindert werden (Wolfe *et al.* 1994), und er wird nach der Freisetzung weiter gemindert durch den bakteriellen Abbau von gelöstem DMSP und DMS, sowie durch Photolyse unter dem Einfluß von UV(B)-Licht. Licht hat zwar keinen direkten Einfluss auf den DMS-Ertrag, weil DMSP unter den Lichtbedingungen in der euphotischen Zone des Ozeans nicht photolysiert wird (Slezak *et al.* 2001). Jedoch ist das DMS bei hohen Intensitäten im UV(B)-Bereich einer bedeutenden Photooxidation ausgesetzt (Brimblecombe und Shooter 1986, Brugger *et al.* 1998, Toole *et al.* 2003), wobei dies an das Vorhandensein von Photosensitizern gebunden ist (Brugger *et al.* 1998, Toole *et al.* 2003), weil DMS das Licht in diesem Wellenlängenbereich nicht absorbieren kann (Brimblecombe und Shooter 1986). Indirekt könnten hohe Lichtintensitäten auch den DMS-Ertrag durch die Photoinhibition des mikrobiellen Abbaus von DMSP zu DMS verringern (Slezak *et al.* 2001).

Im Rahmen dieser Studie war vor allem der DMS-Ertrag des bakteriellen DMSP-Umsatz von Interesse. Dieser hat einen so großen Einfluß auf die DMS-Produktion, dass in den Extermfällen gelöstes DMSP einerseits beinahe vollständig zu DMS umgesetzt, oder andererseits nahezu ohne DMS-Ertrag abgebaut wird (Kapitel 4, Tabelle 6). Für den DMS-Ertrag des bakteriellen DMSP-Umsatzes ist entscheidend, in welcher Weise das gelöste DMSP als Substrat des bakteriellen Wachstums genutzt wird. Wenn das gelöste DMSP vollständig über den Weg der Demethylierung und Demethiolierung abgebaut und dabei als Quelle für reduzierten Schwefel und Kohlenstoff genutzt wird, entsteht kein DMS. Wird das DMSP hingegen vollständig enzymatisch gespalten, so wird zwar mit der Acrylsäure organischer Kohlenstoff verfügbar, der DMSP-Schwefel der dabei als DMS frei wird, ist jedoch nicht mehr direkt als organische Schwefelquelle nutzbar. Auf diesem Umstand basiert die hier gemachte Annahme, dass der mikrobielle DMS-Ertrag abhängig ist von der Verfügbarkeit und dem Bedarf an gelösten organischen Schwefelverbindungen, und dass bei einem Überangebot an alternativen organischen Schwefelquellen der DMS-Ertrag steigt durch die Bevorzugung der enzymatischen Spaltung gegenüber dem Schwefel-assimilierenden Abbauweg. In den zur Prüfung dieser Hypothese durchgeführten Experimenten (Kapitel 4) betrug der DMS-Ertrag ohne zusätzliche Nährstoffe im Mittel 21% des mikrobiellen Abbaus an gelöstem DMSP. Die Erträge konnten sowohl durch Anreicherung mit organischen als auch anorganischen Nährstoffen gesteigert werden. Die DMS-Erträge konnten dabei, durch

kombinierte Anreicherung von organischem Schwefel und Stickstoff in Form von Methionin, bis auf maximal 90% gesteigert werden. Die Anreicherung mit organischem Schwefel übertraf in allen Fällen die Effekte der Anreicherung mit gelöstem anorganischem Stickstoff und Phosphor, oder mit gelöstem organischem Kohlenstoff. Hieraus wurde geschlossen, dass die Bedeutung des DMSP als Substrat für bakterielles Wachstum in erster Linie in seiner Funktion als Schwefelquelle liegt, und dass der Umfang dieser Nutzung einen entscheidenden Einfluß auf den DMS-Ertrag hat.

Hieraus kann zusammenfassend geschlossen werden, dass praktisch alle Einzelfaktoren der DMS-Produktion durch die Nährstoffversorgung des Phyto- und Bakterioplanktons beeinflusst werden, wobei gelöster anorganischer Stickstoff auf der Produktionsseite und gelöster organischer Schwefel auf der Ertragsseite im allgemeinen die DMS-Produktion positiv beeinflussen. Das schließt nicht aus, dass bei einer Änderung in der Artenzusammensetzung der Faktor *a* durch das dominante Auftreten einer besonders produktiven Form seine Macht zurückerlangt. Da am Umsatz des DMSP im Grunde die gesamte Planktongemeinschaft beteiligt ist, erfordert eine echte Vorhersagbarkeit der DMS-Produktion noch eine wesentliche Verbesserung des Verständnisses der Schlüssel-Prozesse und des Auftretens der Schlüssel-Arten in Zeit und Raum.

6 Kurzfassung

Die Studie umfasst drei experimentelle Untersuchungen; Labormessungen zur DMSP-Produktion in Kulturen mariner Planktonalgen, eine Serie von *In situ* Experimenten zur Produktion und Umsatz von partikulärem DMSP in Nordsee, und eine Serie von selektiven Nährstoffanreicherungs-Experimenten zum mikrobiellen Umsatz von gelöstem DMSP im tropischen Atlantik.

In den Kulturexperimenten wurde schwerpunktmäßig der Einfluss der artspezifischen Unterschiede und des Stickstoffangebotes auf die DMSP-Produktion untersucht. Die beobachteten artspezifischen Unterschiede überwogen dabei um ein bis zwei Größenordnungen über die stickstoffbedingten Änderungen in der intrazellulären DMSP-Konzentrationen und der DMSP-Produktion.

Die überragende Bedeutung von Schlüssel-Arten und der Einfluss des Nährstoffangebotes auf die DMSP-Produktion geht auch aus den *In situ* Experimenten in der Nordsee deutlich hervor. Etwa $\frac{3}{4}$ der gesamten DMSP-Produktion zwischen März und Oktober ereignete sich nach der vernalen Diatomeenblüte in einer 45 Tage dauernden Blütephase von *Phaeocystis globosa* (Haptophyta) und einer nachfolgenden Massenentwicklung von mixotrophen Flagellaten (Cryptophyceen). Die Maxima der *Phaeocystis*-Blüte und der Oberflächenkonzentration von partikulärem DMSP trafen zusammen mit einem lokalen Minimum der Fraßaktivität des Mikrozooplanktons und ihres Konsums von partikulärem DMSP. Ansonsten war der Konsum von partikulärem DMSP durch herbivores Mikrozooplankton eng mit der pflanzlichen DMSP Produktion korreliert, und entsprach im Mittel 94 % eben dieser Produktion. Im Durchschnitt wurde etwa 77% des vom Mikrozooplankton konsumierten partikulären DMSP wieder als gelöstes DMSP freigesetzt und in den meisten Fällen schon in weniger als 24 Stunden vollständig mikrobiell abgebaut.

Die selektiven Nährstoffanreicherungs-Experimente mit natürlichen Bakterien-Gemeinschaften aus dem tropischen Atlantik galten vor allem der nährstoffbedingten Veränderlichkeit des DMS-Ertrages beim mikrobiellen DMSP-Abbau. Ohne Nährstoffanreicherungen betrug der DMS-Ertrag durchschnittlich 21 % des Abbaus von gelöstem DMSP. Durch anorganische und organische Nährstoffanreicherungen konnte der Ertrag bis auf maximal 90 % gesteigert werden. Die höchsten Erträge wurden erzielt mit kombinierten Anreicherungen von gelöstem organischen Schwefel und Stickstoff in Form von Methionin.

7 Summary

The study contains three experimental investigations; laboratory experiments about the DMSP-Produktion in cultures of marine planktonalgae, a series of field experiments about the production and turnover of particulate DMSP in the North-Sea and a series of nutrient enrichment experiments about the microbial turnover of dissolved DMSP in the tropical Atlantic.

The culture experiments focused on the species-specific and the nitrogen-related variability of the DMSP-production. The observed species-specific variability of the intracellular DMSP concentration and the DMSP-production were one to two orders of magnitude higher than the range of their nitrogen-related changes and highlighted the role of key species.

The importance of key species and the effect of the nutrient supply on the DMSP-production was also apparent in the series of *in situ* experiments in the North-Sea. About $\frac{3}{4}$ of the total DMSP-production between March and October happened during a phase of 45 days after the vernal diatom bloom during a bloom of *Phaeocystis globosa* (Haptophyta) and a subsequent development of mixotrophic flagellates (cryptophyta). The maxima of the *Phaeocystis* bloom and of the surface concentrations of particulate DMSP coincided with a temporal minimum of the consumption of particulate DMSP by microzooplankton grazing. Apart from the *Phaeocystis* bloom, the consumption of particulate DMSP by microzooplankton was closely related with its production by phytoplankton and accounted on average for about 94 % of the production. About 77% of the consumed DMSP was on average released as dissolved DMSP and was in most cases microbially degraded within less than 24h.

The nutrient enrichment experiments with natural size-fractionated bacteria communities in the tropical Atlantic aimed at the nutrient related variability of the DMS-yield of the decomposition of dissolved DMSP. In experiments without nutrient enrichments the DMS yield was on average 21 %. The DMS yield increased in most experiments due to organic or inorganic nutrient enrichments. The highest DMS-yields were achieved by the combined enrichments of dissolved organic sulphur and nitrogen by methionine.

8 Literatur

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9 Anhang

Veröffentlichungen

Gaul W, Antia A N, Koeve W (1999) Microzooplankton grazing and nitrogen supply of phytoplankton growth in the temperate and subtropical North East Atlantic. Marine Ecology Progress Series 189: 93-104

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Gaul W und van Beusekom J (...) Seasonal dynamics in production and turnover of DMSP in the North-Sea. Zur Begutachtung eingereicht bei Journal of Sea Research

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