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Light-dependence of carbon and sulfur production by polar clones of the genus Phaeocystis

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Abstract Blooms of the marine prymnesiophyte genus Phaeocystis link the oceanic and atmospheric compartments of the carbon and sulfur cycles. Modeling the fluxes of dimethylsulfide from the ocean to the atmosphere has been limited due to a lack of information on functional responses to environmental variables. In this study, the light-dependence of extracellular carbon production and dimethyl sulfide (DMS) production by non-axenic polar clones of Phaeocystis spp. was examined at different growth stages. Comparative experiments were run with non-axenic Arctic clones of the diatoms Thalassiosira nordenskioldii and Skeletonema costatum. A large portion of carbon incorporated by the colonial stage of Phaeocystis spp. is released extracellularly, in particular in stationary colonies. This extracellular production can be modeled as a function of irradiance, as for carbon incorporation. In Phaeocystis spp., cellular and extracellular carbon incorporation represent different uptake rates, indicating the formation of two distinct carbon pools. The release of extracellular carbon by polar Phaeocystis spp. was not a constant fraction of total production over the irradiance range used. We observed little extracellular carbon production by cells at high irradiance, and maximal rates were observed at intermediate irradiance. Newly incorporated carbon that accumulates in the mucilage of the colonial stage of antarctic Phaeocystis sp. during photosynthesis was not reutilized for cellular growth during the dark period, as observed for temperate clones. In contrast, only a minor fraction of the radiocarbon incorporated by the diatoms was released extracellularly for all growth stages. The production of DMS was an order of magnitude higher for Phaeocystis spp. than for diatoms. The chlorophyll-specific production of DMS and DMSP (dimethylsulphonio-propionate, the precursor to DMS) by Phaeocystis spp. showed a hyperbolic response to irradiance, while Arctic diatoms (weak or non-producers of DMS), on the other hand, did not show any light-dependency of DMS production. An inverse relationship between DMS and DMSP production in stationary clones of Arcti P. pouchetii was observed, but not for the exponentially growing antarctic clone. Stationary colonies also had higher DMS and dissolved DMSP production rates than exponentially growing ones. These relationships can be extrapolated to the field in areas where Phaeocystis spp. dominates.

Introduction

Blooms of the marine prymnesiophyte genus Phaeocystis link the oceanic and atmospheric compartments of the carbon and sulfur cycles. This genus is highly productive and abundant in all oceans, contributing to > 80% of the annual phytoplankton biomass in certain areas (Verity et al. 1988). This marine phytoplankter has a complex heteromorphic life cycle, during which it alternates between free-living 3 to 9 μm cells and mucilaginous colonies of several millimeters with imbedded non-motile cells. The colonial palmelloid stage largely dominates the Phaeocystis population, but free-living cells are always present in low numbers. Rapid growth, biomass accumulation, and sinking of P. pouchetii colonies have been observed during arctic
blooms (Wassmann et al. 1990; Smith et al. 1991); this is especially important in North Atlantic and Weddell Sea waters, where the introduction of carbon-rich organic matter into deep waters may sequester atmospheric CO$_2$ (Fogelqvist 1991; Smith et al. 1992). The biological activity of *Phaeocystis* spp. may affect global climate through production of dimethyl sulfide (DMS), especially in polar seas (Berresheim 1987; Gibson et al. 1988). This volatile sulfur compound is known to be a source of aerosols which can affect the Earth’s albedo through cloud formation over the oceans (Charlson et al. 1987; Ayers et al. 1990; Putaud et al. 1992). Several authors have noted that since the genus *Phaeocystis* is a substantial DMSP (dimethylsulfiniopropionate, the precursor to DMS)- and DMS-producer (Keller et al. 1989), higher levels of these sulfur compounds should be observed where it dominates (Barnard et al. 1984; Gibson et al. 1990).

Modeling the fluxes of DMS from the ocean to the atmosphere has been limited to correlation of DMS concentrations with phytoplankton biomass (e.g. Thompson et al. 1990). Erickson et al. (1990) used a relationship between irradiance and DMS concentrations published by Bates et al. (1987) to produce global, seasonal maps of DMS fluxes. However, such fluxes of DMS into the atmosphere lack biological information as to the processes affecting production rates of DMS. Our lack of understanding of what controls the phytoplankton production of DMS and DMSP has prevented the development of analytical models which can be used to predict DMS distributions in space and time. Based on the relationship between DMS and DMSP production and growth irradiance which has been observed for macroalgae (Karsten et al. 1990a, b) and more recently a diatom (Vetter and Sharp 1993), we hypothesize that DMS concentration is related to irradiance and that a light-dependent relationship of DMS and DMSP production exists for *Phaeocystis* spp.

In general, diatoms are believed to be the first algae to bloom in the Arctic, followed by the colonial form of *Phaeocystis puchetii* or *P. globosa* (Jonsson 1986; Veldhuis et al. 1986; Cadée and Hegeman 1991). The genus *Phaeocystis* is known to form blooms in shelf areas of the Barents Sea, Bering Sea, and Antarctica as well as in temperate waters (e.g. Barnard et al. 1984; Davidson and Marchant 1987; Holligan et al. 1987; Wassmann et al. 1990). Although photosynthetic rates of polar *Phaeocystis* spp. are highly dependent on irradiance, there is a large discrepancy among their reported rates of maximal production ($P_{max}$, $\mu$g C (mg chlorophyll a)$^{-1}$h$^{-1}$); from an average of 1 to 2 $\mu$g C (mg chlorophyll a)$^{-1}$h$^{-1}$ (Platt et al. 1982; Verity et al. 1990) to high values of 8 to 12 $\mu$g C (mg chlorophyll a)$^{-1}$h$^{-1}$ (Palmisano et al. 1986; Costa et al. 1994). This apparent conflict may be due to extracellular carbon incorporation into the mucilaginous matrix forming each colony of *Phaeocystis* spp. rather than into the embedded cells. Temperate clones of this genus have been observed to either reutilize this extracellular carbon for protein synthesis (Lancelot and Mathot 1985) or excrete it as dissolved organic carbon (DOC) (Guillard and Helbling 1971). The proportion of newly incorporated CO$_2$ that goes into cellular carbon, mucilage, or extracellular DOC has very important implications in the food web and carbon cycle.

In this paper we report experiments with polar clones of *Phaeocystis* spp., at different growth stages to test (1) the light-dependence of DMS and/or DMSP production by *Phaeocystis* spp. grown under environmental conditions simulating polar oceans, (2) whether high $P_{max}$ estimates for *Phaeocystis* are due to extracellular carbon production, and (3) the hypothesis that newly incorporated carbon in the mucilage of the colonial stage of polar *Phaeocystis* spp. is reutilized for cellular growth during the dark period, as observed for temperate clones. Comparative experiments were run with arctic clones of the diatoms *Thalassiosira nordenskioeldii* and *Skeletonema costatum*, two species of polar phytoplankton.

Materials and methods

Sampling

Experiments were carried out from 11 to 27 May 1992 at the Kårkiva Marine Station, University of Tromsø, Norway, with *Phaeocystis puchetii* material obtained from a mesocosm maintained at the University of Bergen with water from Raunesfjord (courtesy of J. Egge). Also present were the coccolithophore *Emiliania huxleyi* and several diatoms (i.e. 85% *P. puchetii* by number). This material was flown from Bergen and kept at 6°C, 10 $\mu$mol quanta m$^{-2}$s$^{-1}$ of irradiance, and in continuous light for several days at Tromsø. A second set of experiments was performed in December 1992 at the University of Miami with an antarctic *Phaeocystis* sp. clone (CCMP # 1374) from the Provasoli-Guillard Culture Collection of Marine Plankton (no arctic clone was available at the time), grown in f/2 culture medium, and kept at 5°C ± 1°C, 35 $\mu$mol quanta m$^{-2}$s$^{-1}$ of irradiance, and a 16 h:8 h dark light cycle (according to the effective day length at Kårkiva in May). Experiments with a mixture of the arctic diatoms *Thalassiosira nordenskioeldii* and *Skeletonema costatum* (courtesy of H.C. Eliertsen, University of Tromsø) were performed at both localities, and were kept at the same temperature, irradiance, and light cycle as the *P. puchetii* and *Phaeocystis* sp. cultures. The cultures were not axenic.

The Arctic species was observed with an inverted microscope and image-analysis system as described in Noji et al. (1990) and the antarctic clone with an epifluorescent Olympus microscope. Visual observations provided a qualitative definition of the growth stages in the *Phaeocystis* cultures. The nomenclature of *Phaeocystis* in this paper follows Baumann et al. (1994), with the arctic clone referred to as *P. puchetii* and the antarctic clone as *Phaeocystis sp*.

Experiments

The light response of the *Phaeocystis* spp. and arctic diatoms [rate of carbon incorporation or production ($P$) vs irradiance ($I$) curves] was measured using two modified, temperature-controlled, incubation
systems (redesigned from Lewis and Smith 1983). For all the P vs I experiments 300 to 400 ml samples were inoculated with pre-cleaned H2CO3 to give a final activity of either 37 kBq ml-1 (for mesocosm experiments) or 18.5 kBq ml-1 (for culture experiments). Then, 80 aliquots of either 5 or 10 ml volume were incubated at 4°C for 1 or 12 h in blue light (fungus-halogen source filtered through 1 cm glass, 1 cm of unicellular algae, and 2 cm of phytoplankton, R. Potter Plastics No. 2069 and 2424, Plexiglas) at intensities between 0 and 3000 μmol quanta m-2 s-1. The larger volume and longer incubation period were used to increase the sensitivity of the rate of DMS or DMSP production [DMS(P)] versus irradiance (I) experiments.  

For our carbon experiments, we define particulate carbon (POC) as that present in the cells and the mucilage, and dissolved organic carbon (DOC) as extracellular. Given the colonial physiology of *Phaeocystis* spp., carbon also needs to be operationally separated into cellular and extracellular fractions. Carbon cellular refers to that present in the cells and collected onto a glass-fiber Whatman GF/C filter (see following paragraph). Extracellular carbon refers to the filtrate passing through the GF/C filter which includes, in the case of *Phaeocystis* spp., the carbon in the mucilage and the extracellular DOC (Gieskes and van Bennekum 1973; Lancelot et al. 1986; Lancelot personal communication). This extracellular fraction also includes bacterial carbon.

Two methods were used to determine total, cellular, and extracellular carbon production as a function of irradiance. In some of the P vs I experiments (for example, those run in parallel with the DMS(P) vs I experiments) all three rates were measured in each of the 20 aliquots from one P vs I incubator. After incubation, each aliquot was mixed in a vortex and sub-sampled as follows: First, a 5 ml subsample was taken and acidified by adding 0.2 ml of 5% HCl. The remaining 5 ml were hand-filtered onto a Whatman GF/C glass-fiber filter through which 5 ml of air were then forced. The filter was placed in 5 ml of 0.2 μm-filtered seawater and acidified. The 5 ml that passed the filter were caught and acidified also. All three subsamples were then ventilated for 24 h and counted as a gel in 10 ml of Universal (ICN) scintillation cocktail. The activity of the samples was determined using a liquid scintillation counter, all corrected by quench curve using the external standard method.

In all other P vs I experiments, only total and cellular production were determined using two incubators. Filtrations were done in a filter rack using ~10 mm Hg vacuum pressure. Extracellular production was calculated as the difference between the fitted curves for total and particulate carbon incorporation. The accuracy of the difference method was verified at the beginning of the experiment, when the three fractions were measured as described above (data not shown).

Experiments were done to test the effectiveness of filtration through GF/C filters at separating mucilage from cells, following Lancelot et al. (1986). Chlorophyll was used as an indicator of cell loss through a GF/C filter (with > 5 mm Hg vacuum) but retained on a Whatman GF/F filter at a lower vacuum pressure. In addition, we tested the effectiveness of two fixatives, formalin and aqueous HCl, at stopping photosynthesis prior to filtration. These fixatives were added at the end of the experiment to a final concentration of 2% formalin or 5% HCl. Samples were stored for about 1 h and subsequently filtered through Whatman GF/C filters. These experiments were performed with cultures of *Phaeocystis* spp. and a mixture of arctic diatoms. A control was obtained by immediate filtration of the samples without fixation.

The chlorophyll-specific P vs I curve parameters \( P_{\text{max}} \), \( \alpha \), and \( \beta \) were estimated by fitting a hyperbolic function (Platt et al. 1982).

\[
P_{\text{max}} (\mu g \text{ C} \cdot \mu l^{-1} \text{ chlorophyll} \cdot h^{-1}) \text{ represents the maximum chlorophyll-specific photosynthetic rate, } \alpha (\mu g \text{ C} \cdot \mu l^{-1} \text{ chlorophyll} \cdot h^{-1}) \text{ represents the slope of the curve at low light intensities, and } \beta (\mu l^{-1}) \text{ represents the degree of photoinhibition at high light intensities. All statistical analyses and curve fittings were performed using SYSTAT software.}
\]

A time-series experiment of 14C incorporation into the cellular and extracellular (mucilage plus DOC) fractions was run over 24 h to test the potential exchange of low molecular weight compounds between the mucilage and the cells in the colonial *Phaeocystis* spp. (Lancelot and Mathot 1985). A CCMP *Phaeocystis* sp. culture in exponential phase was incubated at 5°C ± 1°C, 35 μmol quanta m-2 s-1 of irradiance, and a 16 h light:8 h day light cycle (according to the effective day length at Karvika). The experiment started at the beginning of the light period.

DMS analysis was done with a purge and trap system in line, as described in Matrai and Keller (1993). DMS, particulate DMSP and dissolved DMSP were determined in separate fractions. DMS was measured in unfiltered and filtered seawater samples. Only DMS values from filtered samples are presented in the irradiance experiments; no significant difference was observed between filtered and unfiltered DMS samples (data not shown). Samples were first withdrawn with a Teflon tube attached to a glass syringe, then gently filtered through a filter unit with a Whatman GF/C filter. DMS was stripped from the water (2 to 3 ml) with helium, and trapped in Teflon tubing immersed in liquid nitrogen. Next, DMS was desorbed by immersing the Teflon tubing in warm water. Chromatographic separation was achieved with a Supelco Chromosil 330 column kept at 85°C, and the carrier gas was He at 40 ml/min-1. Particulate DMSP was analyzed in the material collected on the filters (cells and mucilage) and dissolved DMSP was measured in the filtrate. The filter was placed in a 14 ml serum bottle filled with artificial seawater, and the bottle was capped with a Teflon-faced septum; particulate DMSP was converted to DMS by addition of 5N NaOH, pH 12, and incubation of the sample for at least 6 h. A subsample was then sparged for the DMS evolved. Dissolved DMSP samples (2.5 ml of filtrate) were placed in the sparger, 5N NaOH was added, and the DMS evolved was trapped and determined as above. Liquid standards for DMS and DMSP were treated as described above.

DMS(P) vs I experiments were done parallel to the photosynthesis experiments under identical conditions, except that no isotope was added. Samples were incubated in tightly closed 20 ml scintillation vials. Experiments in Tromsø lasted for 2 to 3 h (Experiments 2 to 9), while in Miami experiments lasted from 3 to 12 h (Experiments 20 to 23). Incubations were terminated by immediate gentle filtration. Replicate experimental determinations of DMS and DMSP in natural samples incubated at one irradiance were within 6 and 11% (n = 2 or 3, coefficient of variation), respectively. Analytical precision is < 5% for most of the working range and < 10% for the lowest concentration of DMSP (Matrai and Keller 1993).

Chlorophyll was extracted in 90% acetone over 24 h, centrifuged prior to reading, and determined according to Holm-Hansen et al. (1965) with a Turner Designs fluorometer, calibrated with pure chlorophyll a (Sigma Biochemicals). Underwater photosynthetically available radiation (PAR) was measured by a PUV-2000 from Biospherical Instruments Inc. In the laboratory, we used a Biospherical Instrument Inc. Model QSL-100.

**Results**

Different growth stages of the colonial phase of *Phaeocystis* spp. were observed. The *P. pouchetii* colonies from the mesocosm had begun to disintegrate, and the cells in the mucilage were clumped and yellowish; a few days after our experiments, flagellated cells were observed leaving the colonies. These properties characterize the colonies as stationary (K. Estep, University of Amsterdam, personal communication). Samples from the CCMP *Phaeocystis* sp. culture were in exponential phase, with green cells evenly distributed around the periphery of the colony. DMS/DMSP measurements were obtained from a senescent culture having colonies with a more marked clumping of cells, broken colonies
Table 1 Phaeocystis spp. and diatoms. Photosynthesis vs irradiance parameters, where $P_{\text{max}}$ is maximum photosynthetic rate (µg C µg$^{-1}$ chlorophyll a h$^{-1}$), $\alpha$ is initial slope of light saturation curve [µg C µg$^{-1}$ chlorophyll a h$^{-1}$ (µmol quanta m$^{-2}$s$^{-1}$)$^{-1}$], $\beta$ is photoinhibition constant (same units as $\alpha$), and $I_s$ is saturation onset irradiance (µmol quanta m$^{-2}$s$^{-1}$), following Platt et al. (1982). Experiments presented here include arctic P. pouchetti from a mesocosm (Bergen, Norway), antarctic Phaeocystis sp. from Provasoli-Guillard Culture Collection of Marine Phytoplankton, and diatoms from a Norwegian fjord (Thalassiosira nordenskioldii and Skeletonema costatum). Experiments were maintained at 5°C (Phaeocystis sp.) or 6°C (P. pouchetti and diatoms). Experiments 5 to 14 were stopped using 2% formalin or 2% HCl; this would cause overestimate of extracellular carbon release (see “Results – Carbon production” for further details).

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<th>Particulate carbon incorporation</th>
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as well as colonies devoid of cells, and flagellated cells. Similar phases were described by Rousseau et al. (1994) and correspond to stages of bloom development.

Carbon production

Phaeocystis spp. showed maximum rates of total carbon incorporation ($P_{\text{max}}$) varying from 0.76 to 4.19 μg C μg$^{-1}$ chlorophyll$^{-1}$, light-dependent photosynthetic rates ($\alpha$) from 0.03 to 0.22 μg C μg$^{-1}$ chlorophyll$^{-1}$ (μmol quanta m$^{-2}$ s$^{-1}$), and saturation onset irradiance ($I_o$) from 3.6 to 57.4 μmol quanta m$^{-2}$ s$^{-1}$ (Table 1, Fig. 1a). On the average, $P_{\text{max}}$ from the stationary culture grown in the Bergen mesocosm was lower than the value for the exponentially growing, CCMP culture (1.08 vs 2.34 μg C μg$^{-1}$ chlorophyll$^{-1}$, respectively).

Of the total carbon incorporated by Phaeocystis spp., 5 to 46% was released extracellularly (Table 1). This release was not a constant fraction of the total carbon incorporated over the whole range of irradiance. Maximum rates of extracellular carbon incorporation were observed at intermediate irradiance, between 50 and 900 μmol quanta m$^{-2}$ s$^{-1}$. Diatoms showed similar photosynthetic parameters for total carbon incorporation to those of Phaeocystis spp., although on the average, the latter showed higher maximum rates of carbon incorporation and higher $\alpha$, normalized to chlorophyll$^{-1}$. In contrast to the prymnesiophyte, only between 1 and 11% of the radiocarbon incorporated was released extracellularly by the diatoms (Table 1, Fig. 1b) for all growth stages.

Extracellular production will be overestimated if autotrophic cells pass or rupture on the filter. We assessed the magnitude of this potential artifact by measuring the amount of chlorophyll $a$ retained on a Whatman GF/F filter (with < 5 mm Hg vacuum) after passing a Whatman GF/C at a higher vacuum pressure. Experiments showed that GF/C filters retained > 99% of Phaeocystis spp. cells while the mucilage passed through (Lancelot et al. 1986).

In our initial $P$ vs $I$ experiments, formalin or HCl was used to stop photosynthetic uptake before filtering to determine cellular production. Although this procedure is acceptable for measuring total production versus irradiance (e.g. Lewis and Smith 1983), it can create a serious artifact when estimating carbon incorporation into the cellular fraction by filtration. Addition of these agents to live samples initiates leakage of organic carbon from autotrophic cells. Thus, depending upon the time elapsed between “killing” and filtering, the procedure will cause cellular production to be underestimated. Both fixatives induced the release of labeled compounds into the extracellular fraction by as much as 50 and 65% in the case of Phaeocystis sp. and the diatoms, respectively. Similar losses of $^{14}$C into DOC from diatoms treated with formalin (30% over 24 h at 4 °C) have been reported by Lee and Fisher (1992). This loss seems to be independent of temperature (Cole et al. 1984; Lee and Fisher 1992), and the physiological state of the cells may significantly affect the composition of the DOC released (Fisher and Fabris 1982).

Reutilization of extracellular carbon

During the 24 h time-course experiments, carbon was incorporated linearly with time during the light hours,
and reached a plateau during darkness (Fig. 2). Similar radiocarbon incorporation was observed in the extracellular fraction, which was ~20% of the total carbon incorporated. With a daylength of 18 h, no decrease in radiocarbon activity was observed in the extracellular pool during the night.

Sulfur production

The production of DMS was an order of magnitude higher for the colonial prymnesiophyte than for the diatoms. The chlorophyll-specific production of DMS by *Phaeocystis* spp. showed a hyperbolic response to irradiance, similar to the carbon relationship. Maximum production rates, DMS_{max} ranged from 1.0 to 2.94 nM DMS μg⁻¹ chlorophyll a h⁻¹ (Fig. 3a). The response at light-limiting levels ranged from 0.129 to 0.27 nM DMS μg⁻¹ chlorophyll a h⁻¹ (μmol quanta m⁻² s⁻¹). An experiment with arctic diatoms, on the other hand, did not show any light-dependency of DMS production (0.09 nM DMS μg⁻¹ chlorophyll a h⁻¹, Table 2; open squares in Fig. 3a). This diatom mixture had a corresponding maximum carbon incorporation of 1.47 μg C μg⁻¹ chlorophyll a h⁻¹ (Fig. 3b).

The chlorophyll-specific rate of particulate DMSP production also showed a relationship with irradiance (Fig. 4a). This relationship was different from DMS production and carbon incorporation, in that *Phaeocystis* spp. colonies exposed to irradiance < 3 μmol quanta m⁻² s⁻¹ showed higher production of particulate DMSP than colonies exposed to irradiance > 3 μmol quanta m⁻² s⁻¹. DMSP production increased linearly with irradiance from 3 μmol quanta m⁻² s⁻¹ to ~50 μmol quanta m⁻² s⁻¹, with an apparent saturation thereafter, at similar levels to the dark production (Fig. 2a).

The average production of DMSP and dissolved DMSP [nM DMS(P) μg⁻¹ chlorophyll a h⁻¹] by *Phaeocystis* spp. was highest in the stationary phase and lowest in the exponential phase (6-15 fold higher in the stationary than in the exponential phase) (Table 2). There was no clear trend in the case of particulate DMSP; experiments with stationary cells gave a 4-fold range in production rates among several experiments without a strong physiological or analytical
Table 2. *Phaeocystis* spp. and diatoms. Average production of dimethylsulphide (DMS) and dimethylsulphoniopropionate (DMSP) by *arctic P. poucheti* from a mesocosm (Bergen, Norway); by *antarctic Phaeocystis* sp. clone from Provasoli-Guillard Culture Collection of Marine Phytoplankton, and by diatom mixture (*Thalassiosira nordenskioldii* and *Skeletonema costatum*), grown or maintained at 5°C (*Phaeocystis* sp.) or 6°C (*P. poucheti* and diatoms) and at irradiance of 10 to 50 μmol quanta m^{-2}s^{-1}. Values are in units of nM DMS or DMSP μg^{-1} chlorophyll a h^{-1} (DMS, DMSP measured after filtration through GF/C glass-fiber filter; DMSP_{part}, DMSP measured in particles retained by GF/C glass-fiber filter; DMSP_{diss}, DMSP measured in water after filtration of sample through GF/C glass-fiber filter; na not analyzed).

<table>
<thead>
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<th>Experiment</th>
<th>Culture</th>
<th>Growth stage</th>
<th>DMS</th>
<th>DMSP_{part}</th>
<th>DMSP_{diss}</th>
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<td><em>P. poucheti</em></td>
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<td>1.46</td>
<td>10.32</td>
<td>10.34</td>
</tr>
<tr>
<td>9</td>
<td><em>P. poucheti</em></td>
<td>stationary</td>
<td>1.67</td>
<td>2.50</td>
<td>na</td>
</tr>
<tr>
<td>7</td>
<td>Diatoms</td>
<td>stationary</td>
<td>0.09</td>
<td>0.33</td>
<td>na</td>
</tr>
<tr>
<td>21</td>
<td><em>Phaeocystis</em> sp.</td>
<td>exponential</td>
<td>0.11</td>
<td>2.60</td>
<td>na</td>
</tr>
<tr>
<td>22</td>
<td><em>Phaeocystis</em> sp.</td>
<td>exponential</td>
<td>0.22</td>
<td>1.08</td>
<td>0.55</td>
</tr>
<tr>
<td>23</td>
<td><em>Phaeocystis</em> sp.</td>
<td>senescent</td>
<td>0.91</td>
<td>2.11</td>
<td>3.89</td>
</tr>
</tbody>
</table>

![Fig. 4](image.png)

Fig. 4. *Phaeocystis* sp. a Rate of production of particulate dimethylsulphoniopropionate (DMSP) as function of irradiance (DMSP_{part} = 1.58, α = 0.033, β = 3110, τ_{0} = 47.87, r^2 = 0.68). At irradiance < 3 μmol quanta m^{-2}s^{-1}, particulate DMSP production is independent of light intensity. b Rate of carbon incorporation measured simultaneously to sulfur production (□ total carbon; △ cellular carbon; ▲ extracellular carbon).

reason to discriminate between them, other than natural variability. The average production rates of DMS and particulate DMSP by diatoms in the stationary phase were consistently lower than those of the prynnesiophytes at all growth stages.

There was an inverse relationship between DMS and particulate DMSP production by the *Phaeocystis poucheti* cells from the Bergen mesocosm (Fig. 3c). Although both experiments had stationary cells, the second experiment was done with cells kept longer in the cold room and, as such, may have been older. We could not reproduce these results with the exponentially growing CCMP culture.

Based on the rates of total carbon incorporation (C_{T}) and DMS(P) production by *Phaeocystis* spp., it is clear that the overall rate of carbon incorporation to particulate DMSP (DMSP_{p}) production is high. It is approximately three times as high for light-saturating conditions (C_{T}:DMSP_{p} = 59 ± 8, molar ratio) than for light-limiting conditions (C_{T}:DMSP_{p} = 21 ± 14, molar ratio). On the other hand, this ratio was equivalent in the case of DMS for light-limiting and light-saturating conditions (C_{T}:DMS = 34 ± 8 and 36 ± 17, molar ratio, respectively).

**Discussion**

**Carbon**

Our results show that a large portion of carbon incorporated into the colonial stage of *Phaeocystis* spp. can be released extracellularly, in particular in old colonies. This extracellular production can be modeled as a function of irradiance, similar to carbon uptake by cells (standard primary production measurements based on plankton retained by filters, Steeman Nielsen 1952) and to total carbon incorporation (based on non-filtered samples, Lewis and Smith 1983). Extracellular carbon incorporation can be estimated as the difference between total and cellular carbon uptake. In the past, results from these two experimental designs were considered equivalent despite evidence to the contrary (Riemann and Jensen 1991). In colonial *Phaeocystis* spp., cellular and total carbon incorporation represent different measurements, indicating the formation of two distinct carbon pools.

The release of extracellular carbon by the polar *Phaeocystis* spp. was not a constant fraction of total
production over the range of irradiance used. We observed little extracellular carbon production in photo-
inhibited cells, while maximal rates (up to 46%) were observed at intermediate irradiance levels. These results differ from Zlotnik and Dubinsky's (1989); these authors observed up to 12% excretion in three different phytoplankton species. Their values are closer to what we observed in the diatom assemblage (up to 10% excretion). Nonetheless, our finding that extracellular carbon release as a function of irradiance was of similar magnitude to carbon incorporation into the cellular fraction supports the suggestion of Verity (1981) and Verity et al. (1990) that excretion rates and photosynthetic production of organic matter are linked.

Polar phytoplankton show $P_{\text{max}}$ rates between 1 and 2 μg C μg$^{-1}$ chlorophyll a h$^{-1}$ on average (Platt et al. 1982; Sakshaug and Holm-Hansen 1986; Smith and Sakshaug 1990). However, higher photosynthetic values have been reported for polar clones of *Phaeocystis* spp., varying from 3–8 μg C μg$^{-1}$ chlorophyll a h$^{-1}$ (Palmisano et al. 1986) to 10–12 μg C μg$^{-1}$ chlorophyll a h$^{-1}$ (Cota et al. 1994). Our results do not agree with these high rates, showing $P_{\text{max}}$ rates within the polar averages. Our estimates are based on algae maintained under light limitation at irradiance < 50 μmol quanta m$^{-2}$s$^{-1}$ (see Fig. 1 and Table 1). Higher $P_{\text{max}}$ and $z$ values for *P. pouchetii* are balanced by a lower C:chlorophyll, so that photosynthetic parameters recalculated per unit carbon do not show a higher efficiency (Verity et al. 1990).

Our preliminary results for polar clones of *Phaeocystis* spp. do not support the hypothesis that low molecular weight carbohydrates which accumulate in the mucilage during photosynthesis can be used as a source of carbon in the dark period, unlike temperate clones which do, and are thus able to maintain nocturnal cellular growth (Lancelot and Mathot 1985; Veldhuis and Admiraal 1985). This difference might be attributable to genetic differences among clones (Baumann et al. 1994) or to adaptation of the algae to a longer daylength in high latitudes (15 to 18 h) (Gilstad and Sakshaug 1990), which could be expected to affect physiological processes as well. Independent of the reason, the lack of utilization of these low molecular weight compounds during the dark hours may imply overall lower growth rates for polar than for temperate cells of *Phaeocystis* spp. In addition, all extracellular carbon remained unutilized by polar *Phaeocystis* spp. cells.

The role of bacterial activity in our experiments is unknown. Bacterial degradation of the mucilage produced by *Phaeocystis* spp. might be greater in the stationary phase of cultures and late-bloom stages in natural environments than in exponentially growing cultures or early and senescent phases of blooms in temperate waters (Thingstad and Billen 1994). In polar waters, there is conflicting evidence of possible inhibition of bacterial growth at low temperatures (Pomeroy and Deibel 1986; Thingstad and Martinussen 1991) which may be reconciled when higher substrate concentrations are available (Pomeroy and Wiebe 1993). During an antarctic bloom of *Phaeocystis* spp., organic substrates, particularly DOC, were available in excess of their apparent utilization (Davidson and Marchant 1992); this might explain the higher bacterial activities associated with the late stage of an arctic *P. pouchetii* bloom (Thingstad and Martinussen 1991). Thus, we can speculate that bacteria were abundant and active in our natural samples and, knowing that in our stationary samples extracellular carbon production was high, bacteria may have consumed labeled mucilaginous carbon or extracellular DOC and converted it to labeled bacterial POC. This carbon would still be considered extracellular in our experimental design (i.e., it passes through a Whatman GF/C filter). The overall effect would be that our extracellular carbon production was underestimated due to the $^{14}$C respired by bacteria. In the case of exponentially growing colonies, other studies have indicated that bacterial growth and colonization is extremely low (Rousseau et al. 1994) and that mucilaginous material is slowly broken down by bacteria (Thingstad and Billen 1994); thus, any bacterial effect in our culture samples would be minimal. There may also be slow microbial degradation of the extracellular carbon in senescent cultures or blooms due to nutrient limitation of bacterial growth (Thingstad and Billen 1994). Thus, including the portion of $^{14}$CO$_2$ respired by bacteria, the magnitude of the extracellular carbon production as reported here is at most an underestimate of the true rate.

Our experimental design did not solve for the difference between carbon associated with the mucilage and real DOC. Previous studies indicate that a large fraction of the extracellular carbon remains as colonial mucilage rather than being excreted (Lancelot and Mathot 1985). It is only after the colony becomes stationary that the carbon attached to the mucilage is most likely to enter the food chain, either through colonial excretion (Thingstad and Billen 1994), zooplankton grazing (Huntley et al. 1987; Estep et al. 1990; Davidson and Marchant 1992), bacterial colonization and consumption (Thingstad and Martinussen 1991; Thingstad and Billen 1994), dissolution (Smith et al. 1992), or flocculation after attachment to other particles (Passow and Wassmann 1994).

Our results highlight that >50% of the primary productivity can be allocated extracellularly in the colonial mucilage of *Phaeocystis* spp. The implications for the carbon cycle in polar waters are that a large fraction of the primary production can be associated with extracellular carbon and potentially become DOC. In addition, conventional methods for measuring primary production determine the amount of newly incorporated carbon into the particulate fraction, i.e., carbon retained by a Whatman glass-fiber filter (GF/F). If the carbon retained by the filters underestimates total
carbon fixation by as much as 50%, this suggests that
polar phytoplankton may be able to utilize a much
larger proportion of atmospheric carbon than pre-
viously thought.

Sulfur

Our results suggest that DMS and particulate DMSP
production by *Phaeocystis* spp. can be modeled as
a function of irradiance, similar to the process of
carbon incorporation. The response of DMS (a) to
light-limiting irradiance is higher than for carbon in-
corporation, pointing to an uncoupling of these two
processes. Saturation at irradiance lower than 5 µmol
quanta m⁻² s⁻¹ suggest a high DMS production in the
upper part of the euphotic zone during the growth
season in polar and sub-polar areas. Particulate DMSP
production, on the other hand, was sustained at a high-
er rate in samples kept in the dark than at low ir-
radiance. Particulate DMSP production in the water
column can then be modeled as a function of irradiance
at depths at which I > 3 µmol quanta m⁻² s⁻¹. The
particulate DMSP produced and accumulated in the
dark does not seem to be released as DMS, as parallel
production of DMSP was not observed at very low
irradiance. This enhanced dark DMSP production
could explain the higher intracellular DMSP concen-
tration observed by Karsten et al. (1990b) in macroal-
gae kept in the dark versus algae grown at irradiance
from 3 to 50 µmol quanta m⁻² s⁻¹.

One pathway of carbon export usually not con-
considered is that of carbon attached to DMS and DMSP
(Holligan and Bacleh 1991). For every mole of DMS
released, 2 mol of carbon are also released. If so,
a range of DMS production rates (equivalent to release)
of up to 3 nM DMS µg⁻¹ chlorophyll h⁻¹ (Fig. 3a)
would imply an equivalent release of carbon of up to
0.07 µg C-DMS µg⁻¹ chlorophyll h⁻¹, representing as
much as 7% of the total carbon incorporated by the
cells. Similarly, carbon incorporation into DMSP (as-
suming cells were uniformly labeled) would represent as
much as 13% of the total carbon uptake; this is carbon
which might potentially be lost from the water column
as DMS. Assuming balanced growth, the possibility
exists that as much as 20% of the total carbon incor-
porated may be found in DMS and DMSP in polar
*Phaeocystis* spp.

We observed higher DMS and dissolved DMSP
production rates in stationary colonies than in expon-
entially growing ones. Production rates of DMS and
DMSP are scarce in the literature, and cannot be
compared with ours because they are expressed in
different units (Vaivaramurthy et al. 1985; Dacey and
algal physiological stage and DMS and DMSP concen-
trations, rather than production, has been described
for another significant DMS producer, the coc-

apparently contradictory results clearly point to the need for more work in this area and make it difficult to speculate on the effect of bacterial activity on our results.

The concentration of DMS in seawater is strongly influenced by the interactions between phytoplankton, macro- and micro-grazers, and bacteria. Phytoplankton production of DMS and DMS production of DMS by *Phaeocystis* spp. allows for mathematical formulation of this process in polar waters. The lack of such a response by Arctic diatoms will constrain the spatial and temporal modeling of this process. This information, currently not included in any climate model, will improve the recent attempts to relate DMS or a proxy to parameters that can be remotely sensed (Eriksen et al. 1996; Thompson et al. 1990; Falkowski et al. 1992; Matrai et al. 1993). Oceanic DMS emissions constitute nearly half of the total biogenic sulfur entering the troposphere (Andreae 1989) and are a major source for sulfate aerosols which are now known to play an active role in climate control (Charlson et al. 1987).

In conclusion, our experiments with *Phaeocystis* spp. show that the rate of DMS and extracellular carbon production can be modeled as a function of irradiance. In the field, these relationships can be extrapolated to areas where *Phaeocystis* spp. dominates, namely spring blooms in the North Sea (e.g. Lancelot and Mathot 1985; Veldhuis et al. 1986), Norwegian fjords, and in the ice edge in the Arctic (e.g. Wassmann et al. 1990) and Antarctica (e.g. Fryxell 1989). We need information on phytoplankton composition as well as irradiance and biomass in order to assess the relative contribution of DMS-producing phytoplankton in the community and their role in CO₂ and S cycles.

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