Dynamics in carbohydrate composition of *Phaeocystis pouchetii* colonies during spring blooms in mesocosms

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Abstract

The colony-forming microalgae *Phaeocystis* produces two major pools of carbohydrates: mucopolysaccharides in the colony matrix and intracellular storage glucan. Both have different functions and separate degradation pathways in the ecosystem, so a partial precipitation method was developed to distinguish the dynamics of the two pools. Changes in concentration in response to variation in nutrients and irradiance were followed during a spring bloom of *Phaeocystis pouchetii* colonies in mesocosms near Bergen, Norway. Upon nutrient limitation, the carbohydrate to carbon ratio of the colonies increased from 15% during the growth phase, to more than 50% during the decline phase. During the growth phase of the bloom, the carbohydrate concentration and composition were influenced by irradiance: glucan concentrations showed strong diel dynamics and increased with higher light levels, whereas mucopolysaccharide concentrations were unaffected. During the exponential growth phase, glucan contributed 6–11% to *P. pouchetii* carbon, depending on the time of the day. During the decline of the bloom, the glucan contribution increased up to 60%. We provide further evidence for the concept that the *Phaeocystis* colony matrix is built with a relatively small but constant amount of carbohydrates, compared to the large quantities of glucan produced during *Phaeocystis* spring blooms. Since a major part of *Phaeocystis* primary production is recycled in the water column by bacteria, this vast glucan injection is a potential determinant of the magnitude and composition of the microbial community following a bloom.
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1. Introduction

Phaeocystis (Prymnesiophyceae), a microalgal genus with a world-wide distribution, is known for its massive blooms in coastal seas and in Arctic and Antarctic waters (Lancelot et al., 1987; Baumann et al., 1994; Mathot et al., 2000). During blooms most of the cells are present in a colonial life stage during which they are embedded in a transparent mucous matrix consisting of extracellular polymers, forming hollow spheres with an aqueous lumen (Van Rijssel et al., 1997). In North Sea coastal regions *Phaeocystis* blooms occur mainly in spring and are important in determining the flow of energy, carbon and nutrients in

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the ecosystem; *Phaeocystis* may contribute up to 90% of the total phytoplankton cell numbers (Joiris et al., 1982; Lancelot, 1984; Lancelot et al., 1987; Brussaard et al., 1996) and 65% of the local annual primary production (Joiris et al., 1982).

Carbohydrates contribute significantly (up to 90%) to the organic matter of *Phaeocystis* (Rousseau et al., 1990; Fernandez et al., 1992), with polysaccharides accounting for the bulk of the algal carbohydrates. Two major pools of polysaccharides in *Phaeocystis* are extracellular mucopolysaccharides, which are the main component of the mucous matrix of the colonies, and intracellular storage polysaccharides. Besides their different functions, these pools have separate degradation pathways in the ecosystem.

The colony matrix is generally not heavily colonised by bacteria during the growth phase of a bloom, but mucopolysaccharides can be degraded readily by bacteria (Janse et al., 1999). During senescence of a bloom, the colony matrix serves as the site of bacterial growth and remineralisation (Putt et al., 1994; Becquevort et al., 1998), indicating its importance in cycling of biogenic matter in the microbial loop. The mucopolysaccharides are large polysaccharides, consisting of at least nine different monosaccharides (Janse et al., 1996a). Given their nature, they may contribute to formation of transparent exopolymer particles (TEP) (Alldredge et al., 1993). Indeed, studies have shown that dissolved mucopolysaccharides formed TEP that either sediment (Riebesell et al., 1995) or act as a glue, sticking together other materials present during a bloom and thus enhancing their contribution to a vertical flux (Passow and Wassmann, 1994).

Besides a structural function in the mucous matrix, mucopolysaccharides were also reported to serve as storage polysaccharides (Lancelot and Mathot, 1985; Veldhuis and Admiraal, 1985). The principal storage polysaccharide in *Phaeocystis,* however, is an intracellular β-1,3-glucan with some branching at position 6, with an average size of 3.6 kDa, classified as chrysolaminaran (Janse et al., 1996b). It is produced in the light and consumed in the dark, when it is apparently used as a respiratory substrate. Beta-1,3-glucan is recognised to be the most abundant type of storage carbohydrate in marine phytoplankton (e.g. diatoms) and in various macroalgae (Painter, 1983). It can be degraded rapidly by bacteria in the water column as well as in the sediment (Arnosti, 2000; Keith and Arnosti, 2001).

The contribution of storage glucans to the total biomass of algae largely depends on growth conditions, nutrient status of the cells and light intensity. In the diatom *Chaetoceros affinis*, cellular glucan content accumulated markedly under nutrient deficiency (Myklestad and Haug, 1972; Myklestad, 1974). Also, in *Phaeocystis* colonies an increase in the ratio of total carbohydrate to carbon was reported when nutrient limitation occurred in batch cultures (Van Rijssel et al., 2000) and at the end of a spring bloom (Lancelot, 1984; Fernandez et al., 1992). In addition, carbohydrates oscillate in response to light variations over a diel cycle. In general, when nutrients are not limiting, and irradiance is sufficient to sustain high photosynthetic rates that exceed metabolic demands, glucan accumulates during the day. During the night, it can be respired as an energy supply to maintain cell metabolism and carbon for protein synthesis (Cuhel et al., 1984; Lancelot and Mathot, 1985; Granum et al., 2002).

If we wish to understand the growth of *Phaeocystis* and follow its carbon fluxes, it is important to differentiate between glucan and mucopolysaccharides and investigate the factors influencing their production. It is, however, difficult to separate *Phaeocystis* cells from the matrix, because *Phaeocystis* cells are very fragile and easily disrupted during filtration by commonly used methods. In this way, water soluble components such as glucan are released (Veldhuis and Admiraal, 1985; Van Rijssel et al., 1997; Mathot et al., 2000). Accordingly, there is considerable variation in the reported fraction of total carbon in *Phaeocystis* used to build the mucous matrix, ranging from 5 to 90% (Rousseau et al., 1990; Van Rijssel et al., 1997; Mathot et al., 2000). Measurements of the incorporation of labelled bicarbonate yielded estimates of 18–60% (Lancelot, 1984) and microscopic observations led to an estimate of up to 90% (Rousseau et al., 1990). More recent studies that did not use filtration methods, found 5–33% for field populations by using the relationship between colony size and carbon measurements (Mathot et al., 2000), or colony size, carbon and carbohydrate measurements (Van Rijssel et al., 1997). Because this approach assumes fixed contributions of cell carbon and matrix carbon, it does not allow analysis of dynamics of carbohydrate pools. In the present study we have developed a partial precipitation method to separate mucopolysaccharides from glucan, enabling us to circumvent problematic methods of separating cells from mucous matrix. This has allowed us to measure the dynamics of both pools during a bloom. To exclude the effect of sampling diverse water masses with a different bloom history, a spring bloom of *Phaeocystis pouchetii* was studied in mesocosms. In addition, short-term dynamics in response to variations in irradiance over diel cycles and in the water column were quanti-

fied. We provide further evidence for the concept that *Phaeocystis* colonies are built with a rather small but constant amount of carbohydrates (Van Rijssel et al., 1997; Hamm et al., 1999; Hamm, 2000), whereas the glucan pool shows considerable variation in response to external factors, such as nutrient status, stage of the bloom, and irradiance.

2. Materials and methods

2.1. Batch culture experiment

The method for partition of the particulate water-extractable carbohydrates into mucopolysaccharides and glucan was developed and tested in a batch culture experiment with *Phaeocystis globosa*. *P. globosa* (strain L) from the Dutch coastal zone of the North Sea was isolated by L. Peperzak in 1992. Cultures were grown in autoclaved seawater collected from the Dogger Bank, adjusted to 33‰ salinity with demineralised water, supplemented with sterile solutions of nutrients (Guillard and Helleburst, 1971), minor salts, trace elements and a vitamin solution (Veldhuis and Admiraal, 1987). Cultures of 250 ml were grown in 1 L Erlenmeyer flasks that were autoclaved and cotton plugged. Cultures were grown at $11°C$ at an irradiance of 40 μmol photons m^{-2} s^{-1} in a 14 h: 10 h light: dark cycle and shaken manually every day to keep the cells in suspension. Sampling was performed 4 h after the start of the light period. The culture was isolated and grown aseptically, but not completely bacteria free. During the exponential growth phase, however, bacterial numbers are usually less than 10% of the *Phaeocystis* population (own obs.).

2.2. Development of a protocol for partition of water-extractable carbohydrates into mucopolysaccharides and glucan

For analyses of particulate water-extractable carbohydrates, 200–250 ml samples of the batch culture were filtered onto precombusted (4 h, $450°C$) glass fibre filters (Whatman GF/F) under gravity pressure only. Filters were stored at $-20°C$ until further analysis. Particulate water-extractable carbohydrates consisting of mucopolysaccharides and glucan were extracted by addition of 5 ml MilliQ water for 30 min at $80°C$, followed by centrifugation ($10\,000 × g$ for 15 min). Carbohydrates were quantified in the supernatant by the modified phenol-sulphuric acid method described by Liu et al. (1973) with D(+)-glucose as reference. Subsequently, mucopolysaccharides were precipitated by

addition of two volumes of ice-cold 96% ethanol for 20 min at room temperature. Preliminary tests showed that laminarin (Sigma) did not precipitate in ethanol concentrations up to 85% (v/v). After vortexing and centrifugation ($14\,000 × g$ for 30 min) at $4°C$, the glucan fraction was quantified in the supernatant by the phenol-sulphuric acid method, with D(+)-glucose in 60% (v/v) ethanol as reference. Mucopolysaccharides were quantified by the phenol-sulphuric acid method after dissolving the pellet in 1 ml MilliQ water. Different precipitation times, including overnight precipitation, or precipitation at $-20°C$ did not affect the results. Precipitation was reproducible (S.D. <10% of glucan concentration in triplicate assays) and the sum of mucopolysaccharides and glucan equalled the water-extractable carbohydrates ($±10%$). Sometimes, however, quantification of mucopolysaccharides was irreproducible. Therefore, the quantification of glucan analyses was routinely used and the difference between the water-extractable carbohydrates and the glucan fraction was defined as mucopolysaccharides.

Glucose content was determined in the mucopolysaccharide and glucan fractions using the Boehringer D(+)-glucose test combination (Boehringer Mannheim) after overnight hydrolysis in 0.3 M HCl at $100°C$ (Myklestad et al., 1997) and subsequent neutralisation by addition of NaOH. Size distribution of the fractions was determined by ultrafiltration through 3 kD, 10 kD and 100 kD filters (Centricon, Millipore). To determine the laminarin content of glucan and mucopolysaccharides both fractions were digested with the enzyme laminarinase (E.C. 3.2.1.6 from *Trichoderma* sp., Sigma) as was described in Van Rijssel et al. (2000). It contains endo-β-1, 3-glucanase activity and some chitinase, cellulose and α-amylase activity and degrades laminarin from *Laminaria digitata* to monosaccharides (Sigma).

2.3. Mesocosm experiment

The dynamics of both mucopolysaccharides and glucan were studied in *P. pouchetii* blooms in mesocosms. The experiment was conducted from 28 February to 4 April 2003 at the Marine Biological Field Station in Raunefjorden, outside Bergen, Norway (60°16′ N, 05°14′ E), using three transparent floating polyethylene enclosures (4.5 m deep, 2 m diameter, ca. 11 m^3, 0.12 mm thick walls with 90% penetration of photosynthetically active radiation (PAR). Details of the location and general mesocosm design can be found in Svensen et al. (2001) and at the website of the University of Bergen (http://www.ifm.uib.no/LSF/

inst2.html). Surface irradiance (PAR) was registered continuously with a horizontally mounted Li-Cor cosine sensor (LI-COR, Lincoln, NE, USA). The data were averaged every 15 min and stored using a Li-Cor Li-1000 data logger. Light penetration in the mesocosms and the fjord water was measured daily with a Li-Cor cosine sensor horizontally mounted on a submersible frame.

The mesocosms were filled on 28 February by pumping nutrient-poor fjord water from 5 m depth. The mesocosm water column was well mixed with an airlift-system, pumping 40 L water min^{-1} (Jacobsen, 2000). In order to allow the introduction of new species, to avoid substantial pH changes due to primary production, and to replace water sampled during the mesocosm experiment, 10% of the water was renewed daily in each mesocosm from 3 March on, by pumping (peristaltic) fjord water from 1 m depth outside the mesocosms. On 3 March on, mesocosms M2 and M3 were enriched with nitrate ($NaNO_3$) and phosphate (NaH_2PO_4) to final concentrations of 16 μM and 1 μM, according to the Redfield ratio, to stimulate the development of a bloom of *P. pouchetii*. Mesocosm M1 received no nutrients and served as a control. Nutrient outflow with the renewal of water was replaced daily. In M2 daily replacement of nutrients was stopped on 20 March to induce a fast decline of the *P. pouchetii* bloom. Sampling was performed every third day, in the morning 4 h after sunrise, using 15 L carboys that were filled at the surface of the mesocosms. Samples were taken gently to avoid disruption of the colonies. To monitor carbohydrate and glucan concentration over a diel cycle, sampling was performed over the day at indicated times during the exponential growth phase (18 March) and during the decline phase (25 March) of the *P. pouchetii* bloom (Table 1).

To determine the influence of light intensity on the carbohydrates in *P. pouchetii* colonies, incubation experiments were carried out with samples from M2 during the exponential growth phase (19 March) and the decline phase (28 March) of the bloom (Table 1). From a 5 L sample of M2, *P. pouchetii* colonies were concentrated onto a 100 μm mesh net that was soaked

in HCl for 3 h and overnight in MilliQ water. While making sure that the mesh did not dry, we washed colonies with 0.2 μm filtered water from M2 to remove small algae and free bacteria, collected in a beaker glass and the volume was adjusted to 1 L with the same filtered water. At noon, the concentrated *P. pouchetii* colonies were incubated in 50 ml polystyrene tubes (TPP) in the fjord, at 1 m depth, 14 m depth (2% of surface irradiance) and wrapped in aluminium foil (dark control). Three 50 ml samples of the concentrated *P. pouchetii* colonies and three tubes of each treatment at T = 0 h, T = 3 h and T = 6 h were analysed for particulate water-extractable carbohydrate and glucan concentration.

2.4. Biological and chemical analyses

In the batch culture experiment, biomass was monitored by measuring autofluorescence on a Wallack, Victor 1420 plate reader (430 nm excitation, 660 nm emission, Janse et al., 1996b).

In the mesocosm experiment, phytoplankton abundance and species composition were determined in samples of 60 ml, which were fixed with glutaraldehyde (0.5% final concentration) and stored at 4°C until settled in 2, 10 or 50 ml chambers, and analysed at $200\times$, $400\times$ and/or $600\times$ magnification on a Olympus or LUMAM-P8 microscope. Cellular carbon of the various phytoplankton species was determined using the conversion described by Menden-Deuer and Lessard (2000). To determine the concentration of *P. pouchetii* colonies, samples were concentrated 5–10 times using reverse filtration through a 10 μm mesh. The concentrated sample was then placed into 3 ml well plates and allowed to settle for 1 h at 4°C. Colony concentrations were determined by averaging counts of at least four wells using a Nikon Diaphot inverted microscope at $200\times$ magnification.

Analyses of ammonium, phosphate and silicate were conducted on fresh samples. Ammonium was measured on a Turner Designs 10-AU fluorometer according to Holmes et al. (1999). Phosphate and silicate were measured using a spectrophotometer (Shimadzu UV-160)

Table 1
Overview of relevant parameters during the experiments

Experiment	Date (March)	*P. pouchetii* bloom phase	Radiation (MJ m^{-2} d^{-1})	Nitrate (μM)		Phosphate (μM)	
				M2	M3	M2	M3
Diel	18	exponential	4.0	10.04	9.74	0.79	0.79
	25	decline	3.4	0.02	0.01	0.09	0.07
Irradiance	19	exponential	1.5	7.14		0.96	
	28	decline	2.4	0.05		0.30	

according to Valderrama (1995). Samples (20 ml) for analysis of nitrate were fixed (0.2 ml chloroform) and stored at 4°C until analysis was performed using an autoanalyser (Hagebø and Rey, 1984).

For particulate organic carbon (POC) measurements triplicate 50–200 ml samples were filtered (<15 mPa) onto precombusted (4 h, 450°C) glass fibre filters (Whatman GF/F). Filters were stored at −20°C until analysis on an elemental-analyser type EA 110 (Interscience, Breda, The Netherlands). Samples for chlorophyll-a (chl-a) were collected each day, filtered onto 0.45 μm filters (Sartorius) and analysed according to Parsons et al. (1984) on a Turner Designs 10-AU fluorometer.

For particulate water extractable carbohydrate analysis 200–250 ml samples were filtered and analysed for carbohydrate and glucan concentration. At the time of *P. pouchetii* colony dominance, additional samples with an increased amount of colonies were obtained by gentle filtration of 500–1000 ml mesocosm water at gravity pressure only, onto a 100 μm mesh net that was previously soaked in HCl for 3 h and overnight in MilliQ water.

2.5. Statistical methods

The partition of carbohydrate fractions during different stages of the *P. pouchetii* bloom in M2 and M3 were compared using a paired student t-test. When it was confirmed they were different, differences in glu-can content between different bloom stages was tested in both mesocosms using ANOVA. The Tukey HSD post hoc test procedure was subsequently used to determine significant differences at a 95% confidence interval. Differences between the light treatments in the incubation experiment were tested using ANOVAs followed by the Tukey HSD post hoc test as described above.

3. Results

3.1. Carbohydrate partition and characterisation in a P. globosa batch culture

Water-extractable carbohydrates were extracted from *P. globosa* colonies and separated by ethanol precipitation into a mucopolysaccharide and a glucan fraction. Analysis of the glucose content of both fractions showed that mucopolysaccharides constituted 20% of glucose and 97% of glucan. Size fractionation showed that all mucopolysaccharides were >100 kDa. In the glucan fraction all polysaccharides were <100 kDa; 42% <3 kDa, 40% were 3 kDa–10 kDa, and 18% were 10 kDa–100 kDa. When an exponentially growing culture of *P. globosa* was placed in the dark, the glucan concentration decreased 70% within 24 h, whereas the mucopolysaccharide concentration remained constant for at least 6 d (Fig. 1). Because the β-1,3-glucan chrysolaminaran is the only known intracellular storage glucan of *Phaeocystis*
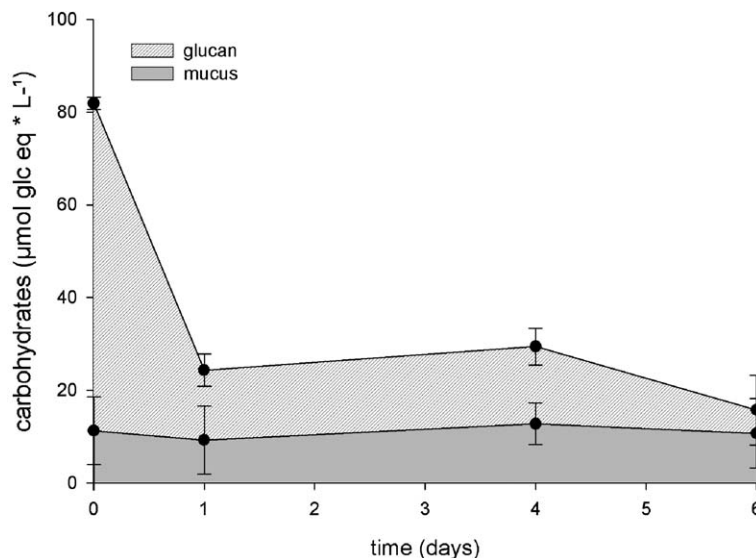

Fig. 1. Carbohydrate concentration and partitioning in mucopolysaccharides and glucan of an exponentially growing batch culture of *Phaeocystis globosa* that was placed in the dark at time 0. Carbohydrates are expressed as μmol glucose equivalents per liter. Error bars represent S.D. of triple carbohydrate analysis.

([Janse et al., 1996b](#)), it was tested whether both mucopolysaccharide and glucan fraction were susceptible to digestion by the enzyme laminarinase. Of the glucan fraction, 60% was quickly degraded to fragments <1 kDa, whereas the mucopolysaccharide fraction was not susceptible to laminarinase, even after prolonged incubation times.

3.2. P. pouchetii bloom development in the mesocosms

The phytoplankton species composition and numbers in each of the three mesocosms developed alike during the first thirteen days ([Fig. 2](#)). A diatom bloom dominated by *Chaetoceros socialis* was growing exponentially until 12 March, reaching a maximum of ap-
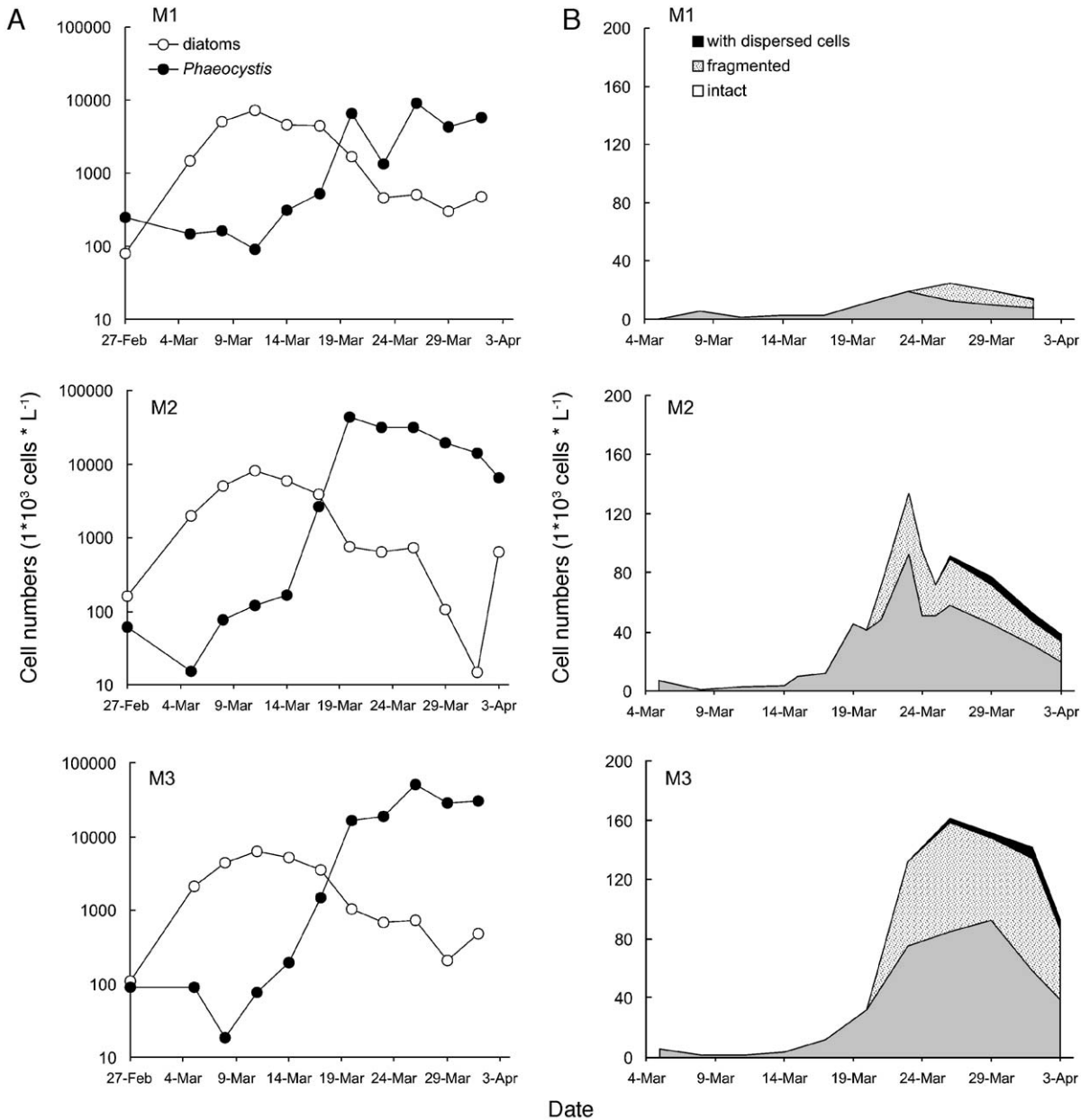
Fig. 2. Diatom and *Phaeocystis pouchetii* cell numbers (A), and *P. pouchetii* colony numbers (B) in the mesocosms during the spring bloom. (A) Diatoms were dominated by *Chaetoceros socialis*. (B) The different colony types were intact colonies (grey): with intact membrane, without tears or breaks; fragmented colonies (dotted): with compromised membrane, but still seemingly viable, i.e. cells are visible in usual patches inside the colony matrix; colonies with dispersed cells (black): intact colonies with dispersed cells; cells that were not in their usual patches within the colonies and were sometimes moving around within the colonies.

proximately $7 * 10^6$ cells L^{-1}. During this period the initial silicate concentration of 5 μM gradually decreased and was depleted on 7 March. The diatom bloom was followed by a *P. pouchetii* colony bloom in all mesocosms. The duration of the bloom and the appearance of different types of colonies were similar in all mesocosms, but colony numbers and cell numbers were approximately five times higher in the fertilised mesocosms M2 and M3 than in the unfertilised control M1 (Fig. 2). In M1, the *Phaeocystis* bloom reached a maximum of $9.2 * 10^6$ cells L^{-1} and $25 * 10^3$ colonies L^{-1} on 26 March. At that time *P. pouchetii* constituted 63% of the cell numbers and 55% of the algal biomass. Initial nitrate concentrations of 6.5 μM gradually decreased and were depleted from 19 March onwards. Initial phosphate concentrations of 0.34 μM gradually decreased and were depleted from 23 March onwards. In both fertilised mesocosms the *P. pouchetii* bloom development was similar. Based on the *P. pouchetii* cell numbers we distinguished three phases in this bloom: an exponential growth phase, a decline phase and a decay phase. *P. pouchetii* started to grow exponentially on 8 March, which lasted until 20 March, with maximum growth rates μ of 0.93 d^{-1} and 0.74 d^{-1} for M2 and M3, respectively. During this exponential growth phase all observed colonies were intact, but when exponential growth ceased, fragmented colonies started to develop in both mesocosms (Fig. 2). In M2, the bloom peaked at $42.8 * 10^6$ cells L^{-1} on 20 March, whereas colony numbers peaked a little later on 23 March at $134 * 10^3$ colonies L^{-1}. *P. pouchetii* cells constituted 89% of the cell numbers and 92% of the biomass. In M3 growth continued until 26 March, when the bloom peaked at $50.4 * 10^6$ cells L^{-1} and at $193 * 10^3$ colonies L^{-1}. *P. pouchetii* cells constituted 91% of the cell numbers and 84% of the biomass. In both mesocosms nitrate and phosphate concentrations gradually decreased during this period; nitrate was depleted on 20 March, phosphate on 23 March.

From 23 March onwards the bloom declined. At the beginning of the decline phase, chl-a concentrations were decreasing (Fig. 3A), whereas POC concentrations were still increasing (Fig. 3B). Nutrient concentrations of both nitrate and phosphate were below 0.1 μM. From 26 March on we began to observe intact colonies with dispersed cells; cells that were not in their usual patches within the colonies and were sometimes moving around within the colonies (Fig. 2). From 29 March on, the C/chl-a ratio increased strongly, from approximately 50 during the bloom to 300 for M2 and 250 for M3 (Fig. 3C), indicating the decay of the bloom. During this period nitrate concentrations

remained low, whereas phosphate increased slightly to 0.5 μM.

3.3. Carbohydrate dynamics over the course of the P. pouchetii bloom

The carbohydrate patterns in samples taken in the morning over the course of the *P. pouchetii* bloom
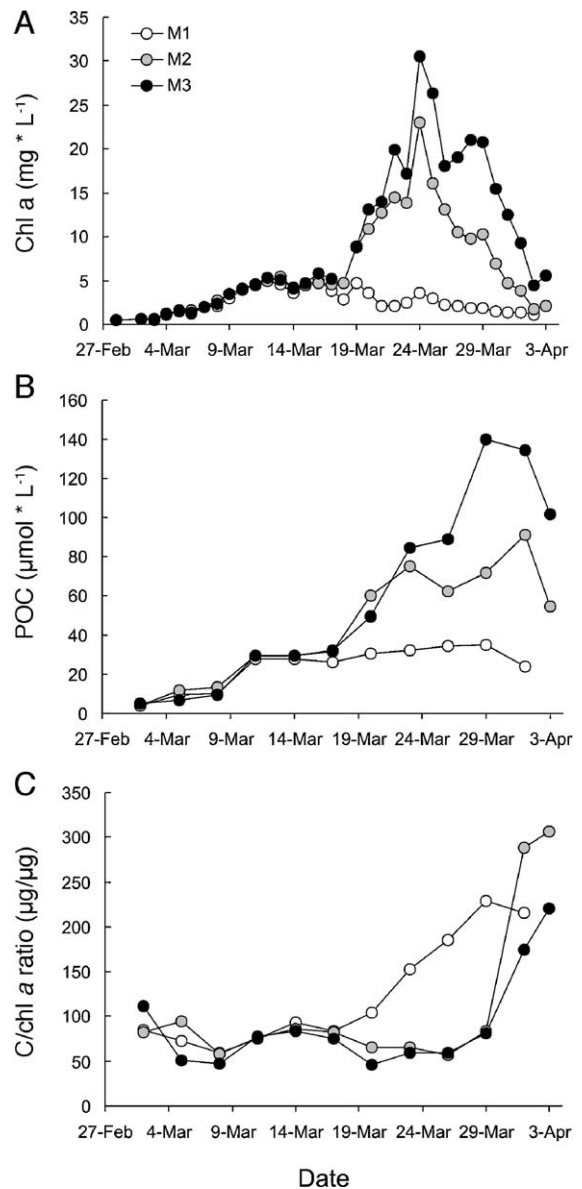

Fig. 3. Changes in chl-a concentration (A), POC concentration (B), and carbon to chl-a ratio (C) in the mesocosms M1; open circle, no nutrients added; M2, grey circle, initial nutrient addition and daily replacement of nutrient outflow until 20 March; M3 black circle, initial nutrient addition and daily replacement of nutrient outflow until the end of the experiment.

showed a similar trend in M2 and M3 (Fig. 4). During the exponential growth phase carbohydrates made up approximately 15 ($\pm$ 7.5)% of POC in both mesocosms, increasing to more than 50% when exponential growth ceased (Fig. 4A).

Most of the carbohydrates in *P. pouchetii* colonies (>100 μm fraction) were present as glucan during the entire bloom period (Fig. 4B and C). The contribution of

glucan was different during the various bloom stages (ANOVA, $P < 0.001$). During the exponential growth phase the contribution of glucan to the carbohydrates in *P. pouchetii* colonies was 63 ($\pm$ 1.4)%, which was lower than during the rest of the bloom (Tukey post-hoc test, $P < 0.05$). During the decline and decay phase of the bloom the contribution of glucan to the carbohydrates in *P. pouchetii* colonies was not different at 82 ($\pm$ 5.6)% (Tukey post-hoc test, $P = 0.28$).

3.4. Diel dynamics in carbohydrate pools

During the exponential growth phase of the *P. pouchetii* bloom (18 March), when nutrients were not limiting (Table 1), the carbohydrate concentration of the *P. pouchetii* colonies showed a strong diel pattern that was similar in both mesocosms (Fig. 5A and B). During the light period the carbohydrate concentration increased 3.9-fold in both mesocosms, and subsequently decreased 55% during the night. The increase in carbohydrate concentration was stronger after 8:30 a.m., when the irradiance increased. Partition of the carbohydrates showed the largest variation in the glucan pool. During the light period, glucan increased 5.6- and 6.5-fold for M2 and M3, respectively, and decreased 60% in both mesocosm during the night. During the day, mucopolysaccharides increased 2.2- and 3.4-fold in M2 and M3, respectively, during the night they decreased 44% and 35% for M2 and M3, respectively.

There was no variation in the carbohydrate concentration over a diel cycle during the beginning of the decline phase (25 March) of the bloom. The contribution of glucan was constant as well (Fig. 5C and D). The light conditions were similar during both experiments (Table 1).

3.5. Influence of light intensity

To determine the influence of light intensity on the carbohydrate concentration and glucan contribution in *P. pouchetii* colonies, samples from M2 in which colonies were concentrated were incubated at 1 m depth, 14 m depth (equivalent to 2% surface irradiance) and in the dark control, both during the growth phase and the decline phase of the bloom (Fig. 6). During the exponential growth phase of the bloom (19 March), the carbohydrate concentrations were positively influenced by irradiance, which was independent of the time of incubation (ANOVA, light $P < 0.001$, time $P = 0.54$). The total carbohydrate concentrations were different following each of the three light treatments (Tukey
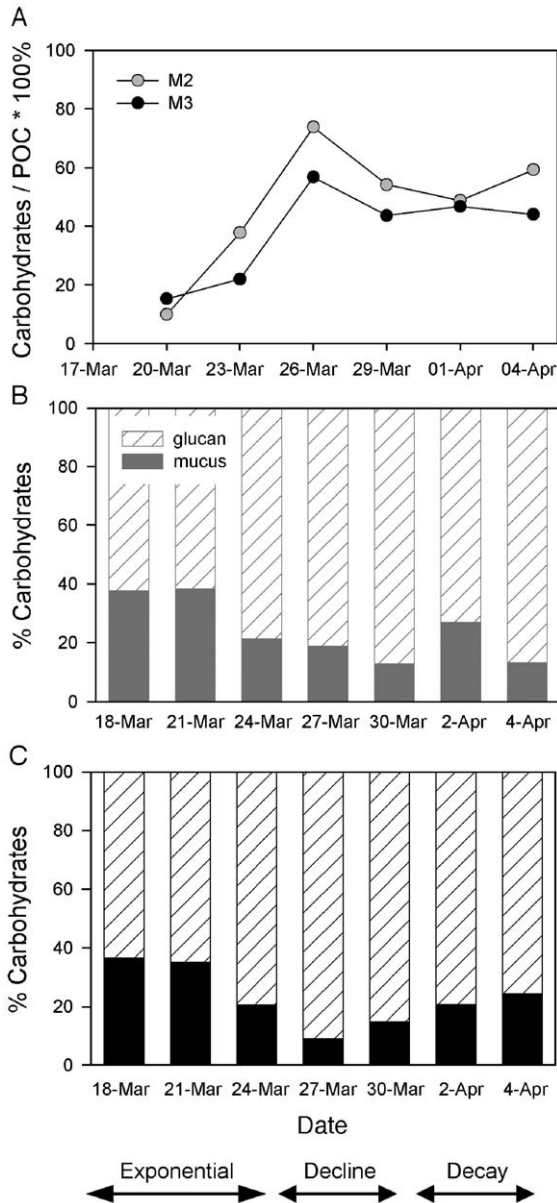

Fig. 4. Carbohydrate patterns over the course of the *Phaeocystis pouchetii* bloom. Percentage of POC in the form of particulate water-extractable carbohydrates (A) and partitioning of the carbohydrates in *P. pouchetii* colonies (>100 μ fraction) in mucopolysaccharides (plain bars) and glucan (arched bars) in M2 (B), and in M3 (C).
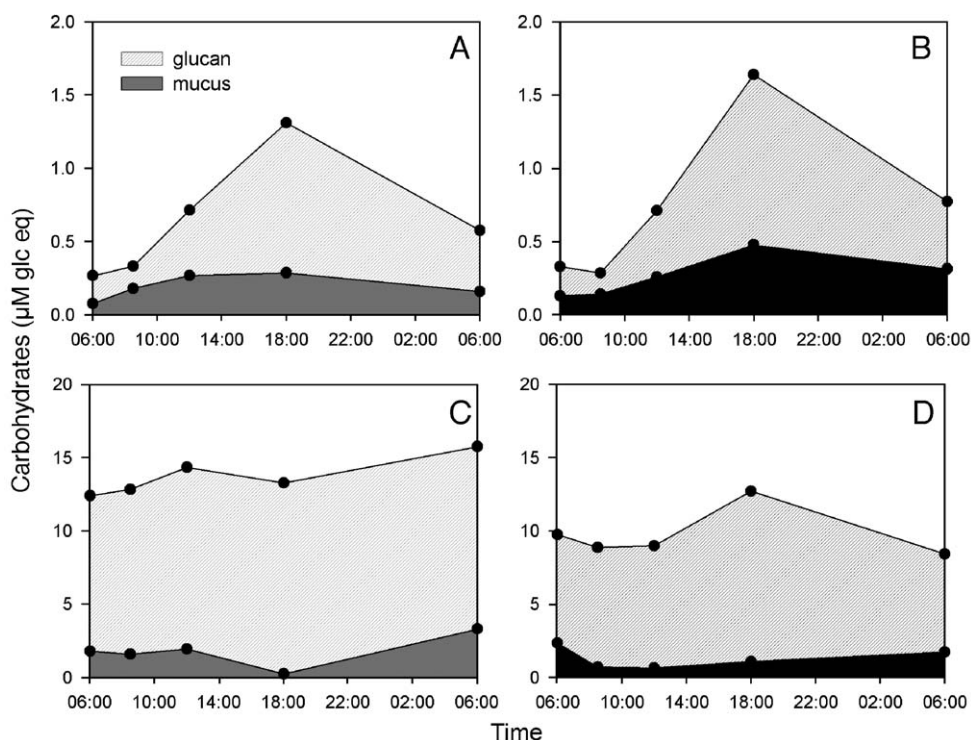
Fig. 5. Diel variations in carbohydrate concentration and composition in *Phaeocystis pouchetii* colonies of mesocosm M2 (A & C) and M3 (B & D) during the exponential growth phase, 18 March (A & B) and the decline phase, 25 March (C & D) of the *P. pouchetii* bloom.

post-hoc test, $P < 0.05$). Whereas neither the different light treatments nor the time of incubation had an effect on the concentration of mucopolysaccharides (ANOVA, light $P = 0.69$, time $P = 0.23$), glucan concentrations were positively influenced by irradiance, which was independent of the time of incubation (ANOVA, light $P = 0.001$, time $P = 0.48$).

During the decline phase of the bloom (28 March) neither the different light treatments, nor the time of incubation had an effect on the carbohydrate concentration (ANOVA, light $P = 0.89$, time $P = 0.11$). During this experiment the radiation was higher than during the experiment in the growth phase (Table 1).

4. Discussion

In this study the two major carbohydrate pools of *Phaeocystis* colonies, mucopolysaccharides and glucan, were successfully separated. The batch culture experiment showed that mucopolysaccharides were not consumed by the algae when cultures were placed in the dark (Fig. 1), in agreement with their structural role in the colony matrix. The glucan fraction, on the other hand, was readily consumed by the algae, demonstrating its storage function.

Chrysolaminaran was characterised as principle storage glucan in a non-colony-forming strain of *P. globosa* (Janse et al., 1996b). In this strain, 95% of water extractable carbohydrates consisted of glucose and all glucose polymers were digested by the enzyme laminarinase, whereas in a colony-forming strain no more than 40–70% of glucose polymers were digested (Van Rijssel et al., 2000). In concordance with this, our results showed that only 60% of the glucan fraction was digested. There may be differences in chrysolaminaran produced by single cells and colonial cells, e.g. in polymer size or in number of branches. Therefore, chrysolaminaran from colonial cells may not be fully degraded by the enzyme. Alternatively there may be a different type of glucan produced by colonial *Phaeocystis* cells. From the batch culture experiment, however, it can be concluded that the whole glucan pool has a storage function. If there is another type of storage glucan besides chrysolaminaran, it is not clear whether this is located in the cells or in the colony matrix, since our method did not separate *Phaeocystis* cells from the colony matrix. Nocturnal usage of carbohydrates from the colony matrix has been reported during blooms (Lancelot and Mathot, 1985), and in cultures of *P. globosa* (Veldhuis and Admiraal, 1985). Both studies
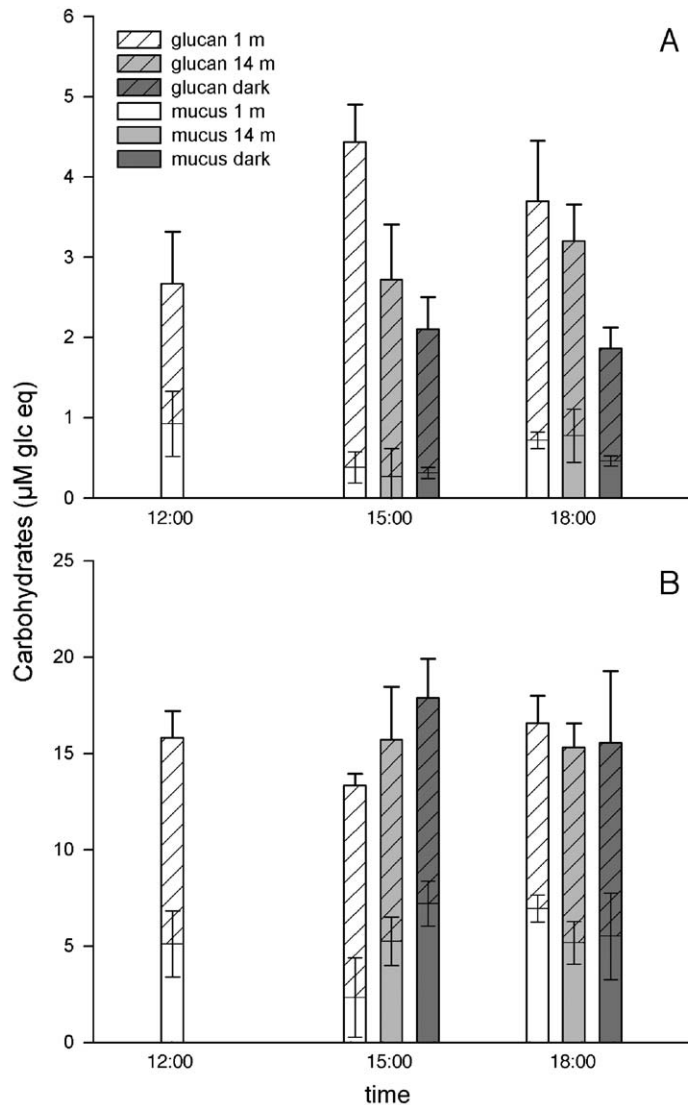
Fig. 6. Influence of light intensity on the carbohydrate concentration of *Phaeocystis pouchetii* colonies of mesocosm M2 during the exponential growth phase of the bloom (A, 19 March) and the decline phase of the bloom (B, 28 March). At noon (12:00) *P. pouchetii* colonies were incubated at depths of 1 m (white bars), 14 m (grey bars) and 14 m and dark controls (dark grey bars). The error bars represent S.D. of triple incubations.

used filtration to separate cellular carbon from the extracellular carbon fraction, containing the colony matrix and intra- as well as extra-colonial carbon. Since filtration could lead to the disruption of *Phaeocystis* cells, this fraction may have contained chrysolaminaran as well. Using a similar filtration method, however, Matrai et al. (1995) did not observe nocturnal usage of extracellular carbon in cultures of an Antarctic clone of *Phaeocystis* sp.

During the exponential growth phase of the *P. pouchetii* bloom, glucan synthesised during the day is used as a short-term storage to meet nocturnal consumption. In the incubation experiment we showed that daytime

synthesis of glucan was dependent on the light intensity. In addition, we hypothesised that glucan could be used during the light period in case photosynthetic irradiance was suboptimal, e.g. due to vertical mixing deep in the water column. Under normal bloom conditions, *Phaeocystis* colonies may be mixed down to several tens of metres and still actively grow (Joint and Pomroy, 1993; Arrigo et al., 1999; Wassmann et al., 2000). We could, however, not measure any significant decrease in the glucan fraction, or carbohydrate concentrations of the *P. pouchetii* colonies incubated at 14 m depth, or even in the dark control. In a recent model for the diatom *Chaetoceros brevis*, Van Oijen et

al. (2004) showed that the respiration rate of glucan during the day was 1.5 to 2.7 times lower than during the night. If this difference in respiration rate also applies to *P. pouchetii*, our procedure may not have been sensitive enough to measure a decrease of glucan during the applied incubation times.

In addition to short-term glucan dynamics, *P. pouchetii* accumulated glucan under nutrient-depleted conditions at the end of the bloom. Carbohydrate accumulation as a long-term storage of excess energy and carbon is commonly observed in algae under nitrogen or phosphorus limitation (Myklestad and Haug, 1972; Myklestad, 1974; Verity et al., 1988; Van Rijssel et al., 2000). Stored glucan may be used for survival until growth conditions improve. This may be relevant for *Phaeocystis*, since colonies of *P. globosa* have been found wintering in the water column in the Dutch North Sea (Cadée, 1991).

During the entire *P. pouchetii* bloom, mucopolysaccharides formed a minor part (<40%) of the carbohydrates. These results support the concept that the colony matrix is built with low amounts of carbohydrates (Van Rijssel et al., 1997; Hamm et al., 1999; Hamm, 2000). In the present study we did not measure directly which part of the *P. pouchetii* carbon is devoted to the mucus matrix, since the mesocosm POC was measured in >1 μm samples, whereas the carbohydrate partitioning into glucan and mucopolysaccharides was performed in >100 μm samples. However, if we assume that the contribution of carbohydrates to POC is the same in the two fractions, the contribution of mucopolysaccharides to the *P. pouchetii* carbon is less than 5% during the exponential growth phase of the bloom, and in the range of 5–13% during the decline and decay phase. These values are within the range reported for cultures of polar clones of *Phaeocystis* spp. (Matrai et al., 1995), *P. antarctica* in the Ross Sea (Mathot et al., 2000) and *P. globosa* in the North Sea (Van Rijssel et al., 1997), but much lower than values of up to 90% for *P. globosa* in the North Sea that were calculated based on microscopic observations by Rousseau et al. (1990).

There was a gradual increase in the contribution of glucan to the carbohydrates over the course of the *P. pouchetii* bloom. During a bloom of colonial *P. globosa* in the North Sea, however, the contribution of storage glucan was highly variable over the whole bloom (Janse et al., 1996a). Our results show that glucan contribution in *P. pouchetii* colonies during the exponential growth phase of a bloom is dependent on irradiance. Considering the variations in irradiance between days and over a diel cycle, this may explain the reported large variation of the contribution of glu-

can to the carbohydrates in *Phaeocystis* colonies in the field.

If we calculate the contribution of glucan to POC during the *P. pouchetii* bloom we find that this is low during the exponential phase (6–11%) depending on the time of the day. During the decline phase, however, this increases up to 60%. It must be noted that our study was conducted in completely mixed mesocosms of only 4.5 m depth, where the algae received much more light than under normal conditions in the field. Therefore, the contribution of glucan to colonies in the mesocosm may be higher than in the field. Still, it is evident that large quantities of glucan are produced during *Phaeocystis* spring blooms and subsequently channeled into the ecosystem. Since a major part of *Phaeocystis* primary production is recycled in the water column by bacteria (Wassmann, 1994; Brussaard et al., 1995; Rousseau et al., 2000), this vast carbon injection is a potential determinant of the magnitude and composition of the microbial community following a *Phaeocystis* bloom.

Acknowledgments

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