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Chapter 5

**Lysis induced decline of a Phaeocystis spring bloom
and coupling with the microbial foodweb**

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Lysis-induced decline of a *Phaeocystis* spring bloom and coupling with the microbial foodweb

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ABSTRACT: We studied the development and decline of the 1990 phytoplankton spring bloom in the Marsdiep area of the North Sea (The Netherlands) with emphasis on the cause of the decline of the *Phaeocystis* bloom, the role of microbial organisms and the utilization of organic material produced by the algae. At the top of the bloom *Phaeocystis* was nitrogen limited. The bloom declined through cell lysis. Sinking of colonies and grazing were found to be relatively unimportant as loss factors. Biomass in the microbial foodweb (bacteria and protozooplankton) remained low during the bloom but increased sharply as the bloom started to decline indicating that organic carbon released by the phytoplankton was rapidly utilized in the microbial foodweb. Results suggest that dissolved organic carbon produced by phytoplankton through excretion and lysis was the main source of carbon for the microbial foodweb including copepods.

INTRODUCTION

The spring development of phytoplankton in the Southern Bight of the North Sea is characterized by a diatom bloom, closely followed by a bloom of the colony-forming Prymnesiophycean *Phaeocystis* sp. (Lancelot et al. 1987). This spring bloom has been studied most thoroughly in the Marsdiep area near the Island of Texel, The Netherlands (Cadée & Hegeman 1986, 1991). Over the last 2 decades *Phaeocystis* has shown an increase in duration of the bloom and in maximum cell number reached during the bloom in this area. There is evidence that this trend is related to an increase of riverine nutrient input in the area (Cadée & Hegeman 1986, Lancelot et al. 1987).

Phaeocystis is usually the only non-diatom alga that takes advantage of the nutrients left after the diatom bloom. The success of this species in competing with other non-diatoms can be explained by its poor availability as a food source for herbivores. Colonies reach sizes of 2 mm in diameter and are mostly too large for predation by copepods and other zooplankton. Bivalves also prey inefficiently on *Phaeocystis* colonies (Beukema & Cadée 1991). Single cells of *Phaeocystis* are 3 to 8 µm in diameter and therefore at the lower limit of the food-size range found for copepods (Verity

& Smayda 1989, Estep et al. 1990). Even though some authors report predation on *Phaeocystis* single cells and colonies by copepods or ciliates (Weisse 1983, Admiraal & Venekamp 1986, Hansen et al. 1990, Weisse & Scheffel-Möser 1990), it is not yet clear if this predation has much quantitative significance. Hansen & van Boekel (1991) found that copepod grazing on phytoplankton during the 1990 *Phaeocystis* spring bloom in the Marsdiep removed less than 1 % of the phytoplankton standing stock per day.

Since grazing is probably not important as a loss factor during the *Phaeocystis* bloom, questions arise as to what causes the bloom to decline and what happens to the carbon fixed during the bloom. Sinking was an important loss factor during a *Phaeocystis* bloom in the Barents Sea (Wassmann et al. 1990). Also lysis of algal cells occurring at the end of the bloom, when nitrogen or phosphate limit further biomass increase, could result in decreasing cell densities. Riegman et al. (1990) showed that nutrient limitation occurred during a *Phaeocystis* bloom in the Marsdiep. Their results were not specific for *Phaeocystis* since the total phytoplankton community was studied. More than 1 nutrient was found to be limiting on several occasions during the bloom. It remained to be shown therefore, if, and by which nutrient, *Phaeocystis* growth was limited. The

aim of the present study was to determine what causes the breakdown of the *Phaeocystis* bloom in the Marsdiep and what happens with the *Phaeocystis* biomass. During the 1990 spring bloom we followed the development of phytoplankton and microbial organisms as well as parameters for nutrient limitation, phytoplankton cell lysis, and sinking of *Phaeocystis* colonies.

METHODS

Sampling procedure. Samples were collected at a jetty in the Marsdiep south of the Island of Texel, The Netherlands, between 21 March 1990 (Day 80) and 11 May 1990 (Day 131). All samples were collected at high water during the day. Sampling was most frequent during the *Phaeocystis* bloom period. Samples were taken to the laboratories and processed immediately.

Chemical analysis. Concentrations of silicate, inorganic phosphate (Pi), dissolved organic phosphate (DOP), ammonium, nitrite and nitrate were determined in water filtered through pre-rinsed 0.2 μm cellulose-acetate filters. Silicate and inorganic nitrogen compounds were determined according to Strickland & Parsons (1972) with an auto-analyzer and Pi according to Murphy & Riley (1962). DOP was determined as inorganic phosphate after persulfate oxidation (2 h, 120 °C) of filtered samples. This method was also used for determination of total phosphorus concentration in the *Phaeocystis* fraction (see below).

Phytoplankton. Cell numbers and species composition of phytoplankton were determined with the Utermöhl sedimentation technique (Utermöhl 1958) in samples fixed with buffered Lugol solution. Samples for chl *a* analysis were collected by filtering 0.5 to 1 l seawater over a GF/F Whatman filter. Filters were stored at -20 °C until analysis. Chlorophyll *a* and phaeopigment concentration were measured spectrophotometrically (Lorenzen 1967).

***Phaeocystis* colonies.** During the *Phaeocystis* bloom period (Days 92 to 114) colonies of *Phaeocystis* were separated from the other phytoplankton by filtration through a 300 μm mesh-size netting. This retained most colonies while other phytoplankton passed through. Colonies were resuspended in filtered seawater. Density of colonies in this resuspended sample (*Phaeocystis*-fraction) was in the same range as in the untreated field sample. Colonies showed no visual damage after treatment. The *Phaeocystis*-fraction was used to determine the P content and the maximum phosphate uptake rate ($V_{\text{max, Pi}}$) of *Phaeocystis* colony cells and the sinking rate of the colonies.

Phosphorus content of *Phaeocystis*. *Phaeocystis* P content was calculated by subtracting the dissolved

P fraction (Pi + DOP) from the total phosphorus content of the *Phaeocystis* fraction.

Maximum Pi uptake rate of *Phaeocystis*. The $V_{\text{max, Pi}}$ of *Phaeocystis* cells was determined according to Riegman et al. (1990). This parameter can be regarded as an indicator for phosphate limitation, since P-limited cells show strongly increased $V_{\text{max, Pi}}$ values compared with P-sufficient cells (Zevenboom et al. 1982). For P-sufficient *Phaeocystis* colony cells a $V_{\text{max, Pi}}$ value of 1.75 nmol Pi ($\mu\text{g chl } a$)⁻¹ h⁻¹ was found in laboratory experiments (van Boekel unpubl.). This value was used to distinguish between P-limited and P-sufficient colony cells during the *Phaeocystis* bloom.

Sinking rate of *Phaeocystis*. The sinking rate of *Phaeocystis* colonies was determined with the SETCOL method developed by Bienfang (1981). This method does not yield *in situ* sinking rates, but sinking rates of colonies at different stages of the *Phaeocystis* bloom can be compared since the experimental conditions (15 °C, dim light) were kept constant for all measurements.

Phytoplankton autolysis. Estimation of phytoplankton autolysis rates was based on the presence of esterase activity (EA) in the dissolved fraction of water samples. This parameter was chosen since esterases are strictly cytoplasmatic enzymes (Dixon & Webb 1979) and thus are expected to appear in the water only after cell breakage. In algal batch cultures a strong (up to 10-fold) increase of EA had been observed at the end of the exponential growth phase. This increase coincided with microscopically observed cytoplasm release by cells. Laboratory experiments also showed that grazing of phytoplankton by a calanoid copepod or the heterotrophic dinoflagellate *Oxyrrhis marina* did not stimulate EA release. Addition of bacteria (10^6 ml^{-1}) to algal cultures caused an increase of EA of less than 5 % (Riegman unpubl.). These preliminary experiments showed that EA is a sufficiently adequate parameter for the measurement of algal cell lysis.

Dissolved EA in field samples was measured by adding 30 μl fluorescein-diacetate (FDA, 2mM in 100 % acetone) and 30 μl 20 Mm EDTA (pH = 8.0) to a 3 ml sample which had been gently filtered through a 0.2 μm filter. EDTA was added for stabilization of FDA in sea water. Samples were incubated at 20 °C for 1 h, after which sample fluorescence was measured on a Hitachi F2000 fluorometer (excitation at 451 nm, emission at 510 nm with 10 nm bandwidth). Simultaneously, the stability of dissolved EA was tested by adding sonicated *Isochrysis* sp. culture samples to field samples and measuring EA for 24 h at field temperature. The addition of extra *Isochrysis* sp. EA was done in order to be able to follow EA breakdown more accurately. Within the temperature- and protease activity range (8 to 14 °C and 0.2 to 3 $\mu\text{mol naphthyl min}^{-1}$,

respectively) found during the sampling period, the degradation rate of the added EA was found to be constant at $25 \pm 5\% \text{ d}^{-1}$. From the net change of EA in field samples and a daily degradation rate of 25%, EA released over time was calculated. Released EA was related to particulate EA present in the phytoplankton yielding the specific daily phytoplankton autolysis rate (expressed as % particulate EA released d^{-1}).

Phytoplankton particulate EA could not be measured directly in field samples due to interference by particulate EA from other planktonic organisms (bacteria, zooplankters). Therefore, particulate phytoplankton EA was estimated from the chl *a* concentration in the field samples and an EA/chl *a* ratio, derived from exponentially growing algae in batch cultures. The EA/chl *a* ratio for *Thalassiosira* sp., *Rhodomonas* sp., *Chaetoceros socialis*, *Stephanopyxis* sp., *Isochrysis* sp., *Emiliana huxleyi*, *Lauderia borealis*, *Phaeocystis* sp. and *Ditylum brightwellii* was 5.5, 6.8, 19.1, 17.6, 21.6, 9.2, 9.0, 15.0 and 9.2 nmol fluorescein ($\mu\text{g chl a}$) $^{-1} \text{ h}^{-1}$, respectively. For estimation of particulate phytoplankton EA in field samples the mean of these values [$12.6 \text{ nmol fluorescein } (\mu\text{g chl a})^{-1} \text{ h}^{-1}$] was used.

Bacterioplankton. Bacteria were counted and measured with epifluorescence microscopy (Hobbie et al. 1977). For procedures see van Duyl et al. (1990). To convert to biomass we used $0.22 \times 10^{-12} \text{ g C } \mu\text{m}^{-3}$ biovolume (Bratbak & Dundas 1984).

Protozooplankton. Nanoflagellates, virtually representing all protists $<20 \mu\text{m}$, were fixed with glutaraldehyde (1% final concentration), stained with proflavine and 4 ml subsamples were gently filtered over $0.2 \mu\text{m}$ filters (for exact procedures see van Duyl et al. 1990, Bak & Nieuwland 1991). Flagellates were counted and measured with epifluorescence microscopy. Phototrophic nanoflagellates were distinguished through the presence of autofluorescence of chlorophyll. Conversion to biomass was based on a factor of $0.2 \times 10^{-12} \text{ g C } \mu\text{m}^{-3}$ biovolume, which is the mean of values determined by Fenchel (1982) and Børsheim & Bratbak (1987). To obtain densities of ciliates subsamples of 20 ml were fixed with acid Lugol solution. Ciliates were counted and measured between 2 and 24 h after collection using Utermöhl's sedimentation technique. The conversion factor to biomass was $0.19 \times 10^{-12} \text{ g C } \mu\text{m}^{-3}$ biovolume (Putt & Stoecker 1989).

Copepods. Sampling and determination procedures for copepods have been described by Hansen & van Boekel (1991). Copepod biomass was calculated using data from this paper and unpublished results. Since only *Temora longicornis* biomass was determined, total copepod biomass was calculated from data on copepod numbers and the *Temora* mean specific biomass. Thus, an equal mean specific weight for all copepod species was assumed. *Temora* presented

80% or more of total copepod number in most samples, so only a small deviation from real biomass values should be expected using this assumption.

RESULTS

Nutrients

As sampling started (Day 80) all nutrients were still present in high concentrations (Fig. 1). At Day 92 silicate concentration had dropped to $1.5 \mu\text{mol l}^{-1}$ and remained low thereafter. Pi concentration decreased to $0.25 \mu\text{mol l}^{-1}$ on Day 99. Concentration of nitrogenous nutrients (mostly nitrate) reached lowest levels on Day 105 ($1.81 \mu\text{mol l}^{-1}$ total dissolved inorganic nitrogen). Both Pi and nitrate concentration slowly increased again after Day 109. Ammonium was undetectable from Day 85 until Day 114, but was found again at relatively high concentration ($3.7 \mu\text{mol l}^{-1}$) on Days 117 and 121. DOP concentration was constant at ca $0.2 \mu\text{mol l}^{-1}$ during the sampling period (not shown).

Phytoplankton

Diatom cell number showed an initial peak on Day 92 (Fig. 2a); large species (*Biddulphia sinensis*, *Thalassiosira* sp.) dominated the population. Thereafter, diatom cell number fluctuated strongly, reflecting a succession of smaller species dominating the population. *Phaeocystis* cell number started to increase from Day 92, reaching highest levels on Day 105 ($67.7 \times 10^6 \text{ l}^{-1}$) (Fig. 2a). At that moment *Phaeocystis* comprised more than 80% of total phytoplankton biovolume (Hansen & van Boekel 1991). The *Phaeocystis* population then declined sharply. A second peak, consisting of *Phaeocystis* microflagellates together with the diatom *Asterionella glacialis*, was found on Day 114. The chl *a* concentration in the water reflected the 3 peaks (diatoms, *Phaeocystis* colonies and -microflagellates) in cell numbers (Fig. 2b). Phytoplankton cell lysis rate is given in Fig. 2c. Two peaks of high lysis rates were found on Days 108 and 120 following the peaks of *Phaeocystis* colonies and -microflagellates, respectively.

Phaeocystis colony fraction

The P content of *Phaeocystis* colony cells remained at a rather constant level of $0.045 \pm 0.017 \text{ pmol cell}^{-1}$ during the bloom period (not shown). The lowest value ($0.0245 \text{ pmol cell}^{-1}$) was found on Day 114. The maximum uptake rate for phosphate of *Phaeocystis* colony cells did not reach high values during the *Phaeocystis*

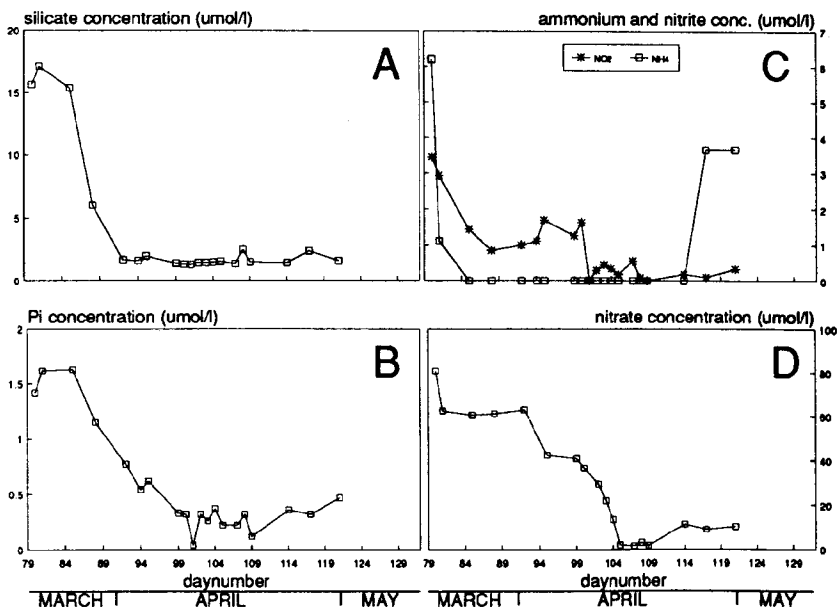


Fig. 1. Time course of the concentration of (A) silicate, (B) inorganic phosphate (Pi), (C) ammonium and nitrite, and (D) nitrate in the Marsdiep water during spring 1990

bloom (Fig. 3). At the top of the bloom (around Day 105), $V_{\max \text{ Pi}}$ values were indeed far below the threshold value for Pi limitation. High values were only found on Day 88 and 114, outside the *Phaeocystis* bloom period. Sinking rate measurements, made at regular intervals during the *Phaeocystis* bloom period, showed that the sinking rate of the colonies remained rather constant at $0.25 \pm 0.08 \text{ m h}^{-1}$ ($n = 16$) (Fig. 4). The lowest sinking rate was found at the maximum of the bloom. There was no clear relation between sinking rate and stage of the *Phaeocystis* bloom development.

Bacterioplankton

Bacterioplankton increased relatively slowly during the phytoplankton bloom period (Fig. 5a). A rapid increase occurred between Day 107 and 110 during the breakdown of the *Phaeocystis* bloom. After Day 114 bacterial biomass declined.

Protozooplankton

Nanoflagellate biomass showed the same development as bacterial biomass (Fig. 5b). Ciliate biomass also increased most rapidly after the *Phaeocystis* bloom period (Fig. 5c) reaching highest levels a few days after

bacteria and nanoflagellates. In contrast with bacteria and nanoflagellates the biomass of ciliates remained high for a period of ca 10 d (Day 110 to 121).

Copepods

Generally, copepod biomass developed steadily from Day 99 on, with 2 peaks at Days 110 and 121 (Fig. 5d). *Temora longicornis* comprised > 90 % of total copepod number until Day 121 (Hansen & van Boekel 1991).

DISCUSSION

Fate of *Phaeocystis*

Data suggest that limitation of the *Phaeocystis* spring bloom of 1990 in the Marsdiep area was caused by depletion of inorganic nitrogen in the water. This resulted in cell lysis (Figs. 1d, 2a & c). Nitrogen limitation of *Phaeocystis* was not shown directly, but substantial support for this statement is found in the results.

Firstly, sinking and grazing were relatively unimportant as loss factors. Sinking rates of *Phaeocystis* colonies did not change dramatically during the bloom

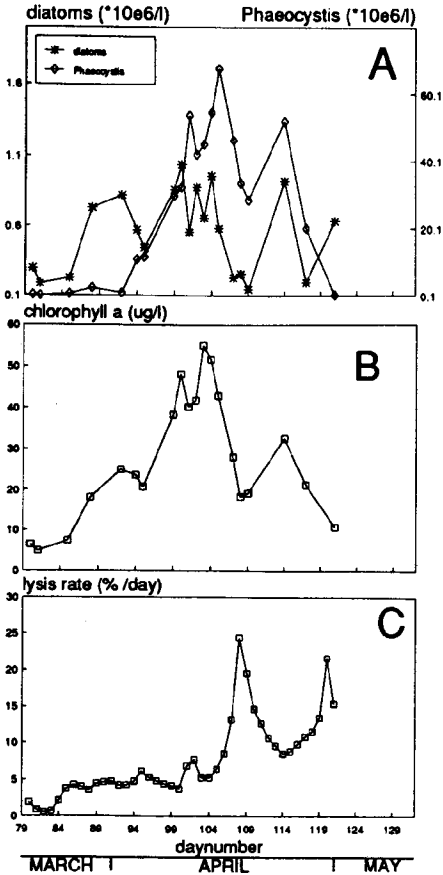


Fig. 2. Time course of (A) cell numbers of diatoms and *Phaeocystis*, (B) chlorophyll *a* concentration, and (C) lysis rate of phytoplankton in the Marsdiep water during spring 1990

period (Fig. 4), indicating that increased loss of colonies through sinking during the decline of the bloom is not likely. Also, in the turbulent Marsdiep water loss through sinking would have been minimal. In general, net sedimentation is relatively unimportant in Dutch coastal waters since high current velocities cause resuspension of deposited material (Jennes & Duineveld 1985). It has been postulated by Creutzberg et al. (1984) that phytoplankton biomass that is not consumed in the pelagic system is transported to less turbulent zones like the Frisian front where it ultimately settles. Grazing on *Phaeocystis* by copepods was shown to be insignificant by Hansen & van Boekel (1991). Ciliates, which can graze *Phaeocystis* single

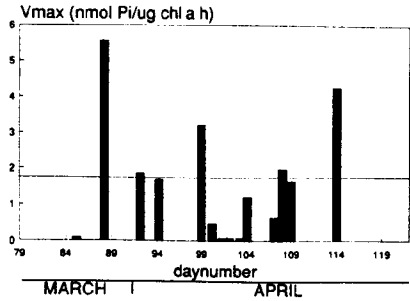


Fig. 3. Maximum uptake rate of inorganic phosphate ($V_{max, Pi}$) by *Phaeocystis* cells in the *Phaeocystis* fraction collected from the Marsdiep, spring 1990. Horizontal line indicates the $V_{max, Pi}$ value of P-sufficient *Phaeocystis* cells [$= 1.75 pmol Pi (\mu g chl a)^{-1} h^{-1}$]

cells (Admiraal & Venekamp 1986, Weisse & Scheffel-Möser 1990), remained low in biomass during the bloom period (Fig. 5c).

Lysis of phytoplankton cells showed a peak just after the top of the *Phaeocystis* bloom (Fig. 2). This suggests that the decline of the bloom was caused by cell death, most probably as a consequence of nutrient limitation. The 2 major nutrients for *Phaeocystis* are nitrogen and phosphate. Inorganic nitrogen in the water was depleted at the top of the *Phaeocystis* bloom while inorganic phosphate remained at relatively high concentrations (Fig. 1). Also, the P content of *Phaeocystis* cells ($0.045 pmol cell^{-1}$) was high compared with values found for P-limited cells ($0.01 pmol cell^{-1}$; van Boekel & Veldhuis 1990). The low $V_{max, Pi}$ values at the top of the bloom indicated that *Phaeocystis* was not P-limited (Fig. 3). Therefore, it is reasonable to state that nitrogen limitation caused the lysis of *Phaeocystis* cells. Nitrate limitation of phytoplankton was also

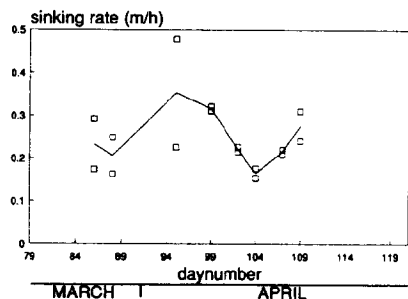


Fig. 4. Sinking rate of *Phaeocystis* colonies in the *Phaeocystis* fraction, determined with the SETCOL method. Line connects means of duplicates

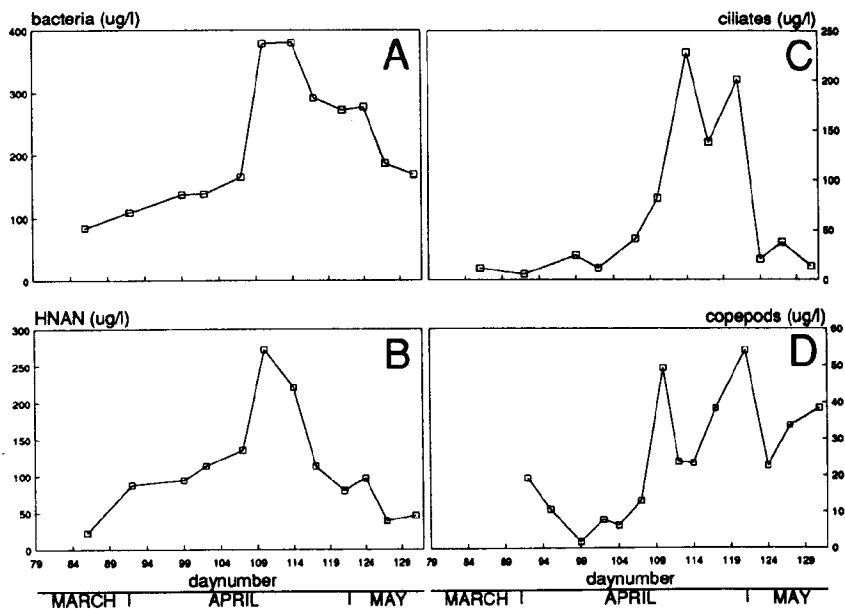


Fig. 5. Time course of biomass of (A) bacteria, (B) heterotrophic nanoflagellates (HNAN), (C) ciliates, and (D) copepods in the Marsdiep water during spring 1990

found by Riegman et al. (1990) during the 1989 *Phaeocystis* bloom in the Marsdiep. In earlier studies of the spring bloom in Dutch coastal waters Veldhuis et al. (1986, 1987) concluded that P_i was the limiting factor for *Phaeocystis*. In the Marsdiep P_i concentrations remained relatively high compared with values found in other Dutch coastal waters. This is probably caused by resupply of P_i from sediment suspended in the turbulent Marsdiep water (Froelich 1988) and by input of P_i from Lake IJsselmeer (van Raaphorst & van der Veer 1990). DOP concentration in the Marsdiep is also high throughout the year (de Jonge & Postma 1974). *Phaeocystis* can grow very efficiently using organic phosphates as P-source (van Boekel 1991). The relatively high DOP concentrations found during the bloom may have provided a substantial part of the P-demand of *Phaeocystis*.

Like the bloom of *Phaeocystis* colonies, the small bloom of *Phaeocystis* microflagellates and *Asterionella glacialis* was followed by an increased lysis rate. It is not clear whether the decline of both dominating algae was caused by lysis alone or that grazing on one or both species was important as a loss factor (see below).

The EA method of measuring lysis as it was used here should be regarded as a semi-quantitative method. It assumes that release of EA in the water can be exclu-

sively attributed to phytoplankton. Autolysis of bacteria and nanozooplankton was assumed to be negligible. Also, calculation of particulate EA concentration was based on EA/chl a values derived from cultures of algae. Since the biomass of bacteria and nanozooplankton was small compared to phytoplankton biomass the assumption that autolysis of these heterotrophs was negligible caused only a relative small error in the calculated lysis rate. The extent of the error introduced by taking the EA/chl a value from algal cultures is unknown. The range of values found for different algae (see 'Methods') indicates that in natural populations the EA/chl a value may fluctuate considerably depending on species composition. However, the mean EA/chl a value used for our calculations [$12.6 \text{ nmol fluorescein } (\mu\text{g chl } a)^{-1} \text{ h}^{-1}$] is close to the value found for *Phaeocystis* cultures [$15.0 \text{ nmol fluorescein } (\mu\text{g chl } a)^{-1} \text{ h}^{-1}$]. During the *Phaeocystis* dominated part of the spring bloom the calculated lysis rates will probably reflect the condition of the phytoplankton population reasonably well.

Role of bacteria and zooplankton

The strong increase of bacterio- and protozooplankton biomass over a relatively short period following the

decline of the *Phaeocystis* bloom contrasts sharply with the slow increase found during the bloom. This sudden change suggests that the microbial foodweb (bacterianaoflagellates-ciliates) was carbon-limited during the spring bloom. An increased release of dissolved organic carbon (DOC) through lysis during the decline of the *Phaeocystis* bloom would facilitate the strong increase of biomass in the microbial foodweb. The decrease of bacterial biomass after Day 114 despite the reduced grazing pressure by nanoflagellates also suggests carbon limitation of the bacteria, if nanoflagellates can be considered to be their main predators as is usually found (Riemann et al. 1990, van Duyl et al. 1990). Assuming a linear foodchain from DOC to bacteria to nanoflagellates to ciliates, it appears that the availability of carbon for bacteria (bottom-up control) rather than predation (top-down control) determined the total biomass in the microbial foodchain. Only during the decline of the *Phaeocystis* bloom (Day 107 to 110) was the bottom-up control released at least partially. Coupling of phytoplankton and microbial foodchain data suggests the following sequence: Bacteria consumed the organic matter released by the phytoplankton through excretion (until Day 105) and lysis (after Day 105). Bacteria were grazed by nanoflagellates that followed the bacterial development closely. Nanoflagellate biomass development was apparently controlled by ciliate grazing after Day 109. The reappearance of ammonium in the water after Day 114 was probably caused by the excretion of surplus nitrogen by bacteria, nanoflagellates or ciliates (Goldman et al. 1985, Caron et al. 1988). The assumption of a linear foodchain will probably not hold for ciliates, which are able to feed on both nanozooplankton and nanophytoplankton (Riemann et al. 1990, Weisse & Scheffel-Möser 1990). Since the peak in ciliate biomass coincided with the bloom of *Phaeocystis* microflagellates (Figs. 2a & 5c) it seems likely that ciliates grazed on both autotrophic and heterotrophic nanoplankton during this second bloom period. This is probably the most important short-cut in the foodchain since other groups of organisms that cause nonlinearities, like mixotrophic phyto- and zooplankton or picophytoplankton, are of little importance in the spring bloom situation (Riegman et al. 1990, van Boekel pers. obs.). Hansen & van Boekel (1991) suggested that ciliates served as major food source for *Temora longicornis* during the *Phaeocystis* dominated part of the spring bloom since the copepods clearly were not feeding on phytoplankton. Microzooplankton has been reported before to be an important food source for copepods (Stoecker & McDowell Capuzzo 1990, Kleppel et al. 1991, Nielsen & Kiørboe 1991). The decline of ciliate biomass after Day 121 might thus be explained as the combined effect of grazing by

copepods and reduced food supply (both nanoflagellates and *Phaeocystis* microflagellates). In this way copepods can be viewed as the next step following ciliates in the foodchain.

Although little direct evidence was found, the results strongly suggest that the decline of the *Phaeocystis* spring bloom in the Marsdiep area occurred through cell lysis as a result of nutrient limitation. The data also suggest that bacterio- and zooplankton growth depended largely upon DOC produced through lysis of *Phaeocystis*.

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