

Impact of phytoplankton bloom magnitude on a pelagic microbial food web

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ABSTRACT: The impact of phytoplankton bloom magnitude on the microbial food web of the Dutch pelagic coastal zone (Southern Bight of the North Sea) was studied during spring 1993. Data were compared to a study carried out in 1992 at the same location. Light availability and temperature were the key factors for the timing of the diatom spring bloom. Specific algal cell lysis rates during the diatom bloom were always $<0.1 \text{ d}^{-1}$. Due to sinking of the diatoms no effect of the difference in bloom magnitude between the 2 years on the pelagic microbial food web was detected. A high onset level of nitrate ($55 \mu\text{M}$) resulted in a dense bloom of the colonial *Phaeocystis*, with maximum cell numbers of $1.8 \times 10^8 \text{ cells l}^{-1}$. During the development of the bloom, the pH of the seawater increased from 7.9 to 8.7. The bloom of *Phaeocystis* declined through cell lysis (up to 0.3 d^{-1}). Due to the enhanced biomass of *Phaeocystis* the amounts of released algal cellular carbon were high (up to $400 \mu\text{g C l}^{-1} \text{ d}^{-1}$). Differences in *Phaeocystis* bloom magnitude were, as a consequence of algal cell lysis, reflected in bacterial production (up to $90 \mu\text{g C l}^{-1} \text{ d}^{-1}$ in 1993) and grazing activity of members of the microbial food web. The elevated importance of the microbial food web resulted in a high standing stock of microzooplankton ($850 \mu\text{g C l}^{-1}$) and accumulation of regenerated inorganic nutrients. Calculations on both carbon and nitrogen budgets indicate that most of the *Phaeocystis* bloom was mineralized within the water column.

KEY WORDS: Phytoplankton bloom magnitude · Diatoms · *Phaeocystis* · Cell lysis · Bacterial production · Microbial food web · Mineralization

INTRODUCTION

The occurrence of phytoplankton blooms is well known for nutrient-rich and well-mixed coastal areas (Pennock 1985, Lancelot et al. 1987, Lignell et al. 1993). Because algal blooms accumulate high amounts of organic carbon and nutrients in the particulate fraction, they are important in determining the flow of energy, carbon and organic nutrients in marine ecosystems (Holligan et al. 1984, Lignell et al. 1993, Brussaard et al. 1995). Termination of an algal bloom by sedimentation results in extensive export of particulate organic matter from the pelagic to the benthic ecosystem. Reports on the importance of sinking as a loss process frequently concern diatom blooms (Riebesell 1989, Passow 1991). In the continental coastal pelagic ecosystems in the Southern Bight of the North Sea, a

bloom of diatoms in early spring is generally followed by a bloom of the colony-forming *Phaeocystis* (Haptophyta, Prymnesiophyceae). Phytoplankton cell lysis following mortality appeared the most important loss factor for the *Phaeocystis* blooms (Van Boekel 1992, Brussaard et al. 1995). The released algal cellular carbon was the main source of carbon for bacteria (Brussaard et al. 1995). Blooming of *Phaeocystis* in the Dutch coastal area directed the flow of energy, carbon and organic nutrients towards the microbial food web. It can be expected that most of the photosynthetically fixed carbon will be mineralized within the microbial food web. Secondly, the release of relatively nutrient-rich organic components through cell lysis will stimulate nutrient regeneration, which may affect phytoplankton succession.

Algal blooms differ in magnitude from one year to the other (Cadée & Hegeman 1986, Lancelot et al. 1987) depending on environmental variables such as irradi-

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ance, temperature, nutrient supply, and grazing (Parsons et al. 1978, Rhee & Gotham 1981a, b, Riegman et al. 1992, 1993, Peperzak 1993). Differences in the biomass of the algal bloom between years will be reflected in the magnitude of the loss factors and thus the energy flow. We hypothesize that the effect on the pelagic microbial food web will be most explicitly noticed whenever cell lysis is the major loss factor. Variations in phytoplankton bloom magnitude will influence bacterial production, which in turn affects the grazing activity and the rate of nutrient remineralization within the microbial food web.

In this study we report on pelagic plankton dynamics in the Dutch coastal zone (Southern Bight of the North Sea) during spring 1993. To determine the impact of phytoplankton bloom magnitude (diatoms and *Phaeocystis*) on the microbial food web, we compare our data of 1993 with those of a field study carried out at the same location in 1992 (Brussaard et al. 1995). We furthermore describe the factors underlying the differences in algal bloom biomass.

MATERIAL AND METHODS

Samples were taken frequently during spring 1993 at high tide from the NIOZ (Netherlands Institute for Sea Research) pier in the Marsdiep tidal inlet, Texel, The Netherlands. To study algal cell lysis and plankton dynamics, a good time series is essential. The NIOZ pier allows frequent sampling without restrictions on the availability of research ships. We are, however, aware that by sampling from the NIOZ pier a proportion of the water sampled may have originated from the tidal inlets. We tried to overcome this by sampling only at high tide when the water was still incoming and therefore representative of the Dutch coastal zone. Salinities were between 28.5 and 31.5‰, normal values for the nutrient-enriched continental Dutch coastal area.

Reactive silicate, dissolved orthophosphate and nitrogen were analyzed by means of an autoanalyzer. Samples for total chlorophyll *a* (chl *a*) were, after gentle filtration over Whatman GF/F filters (20×10^3 Pa), extracted in 90% acetone and analyzed fluorimetrically. Phytoplankton and microzooplankton were counted in buffered Lugol-preserved samples using a Zeiss inverted microscope. *Phaeocystis* cell numbers were converted into carbon using a factor of 12.15 pg C per flagellate cell and 14.2 pg C per colonial cell (Lancelot et al. 1991). Bacterial production was estimated from incorporation rates of ^3H -leucine [L-(4,5- ^3H -leucine); 150 to 164 Ci mmol $^{-1}$, Amersham] into bacterial protein. Aliquots of sea water (each 10 ml, made 10 nM with leucine) were incubated at field tem-

perature for 1 h, after which they were treated according to Simon & Azam (1989). We used an intracellular isotope dilution factor of 1, being the most conservative. Bacteria were counted on an epifluorescence microscope after collection on 0.2 μm polycarbonate membrane filters (Poretics) using acridine orange. Cell numbers were converted into biomass using a factor of 0.22×10^{-12} g C μm^{-3} biovolume (Bratbak & Dundas 1984). Heterotrophic nanoflagellates (HNFs) were fixed with glutaraldehyde (1% final concentration) and, after collection on 0.2 μm Nucleopore filters, stained with proflavine and counted with epifluorescence microscopy. A factor of 0.2×10^{-12} g C μm^{-3} biovolume was used to convert cell numbers into biomass (Fenchel 1982, Børsheim & Bratbak 1987). Biovolume of microzooplankton was calculated from numbers per size category and converted into biomass using a factor of 0.19×10^{-12} g C μm^{-3} biovolume (Putt & Stoecker 1989). Mesozooplankton was concentrated by means of a 50 μm mesh sieve, fixed with an equal volume of 4% formalin and counted under a binocular dissecting microscope. For carbon conversion factors see Brussaard et al. (1995). We used gross growth efficiencies of 0.35 for bacteria and 0.3 for heterotrophic nanoflagellates and micro- and mesozooplankton.

Specific daily phytoplankton autolysis rates were estimated based on the presence of esterase activity (EA) in the dissolved fraction of the water samples (EAD). Esterases are strictly cytoplasmatic enzymes which will be released into the water during cell leakage and can therefore be used to measure cell lysis. EAD was measured fluorimetrically (Hitachi F2000 fluorometer, excitation at 451 nm and emission at 510 nm with 10 nm bandwidth) in triplicate at 20°C over 1 h in gently filtered field samples (3 ml sample over a 0.2 μm Polysulfone Acrodisc syringe filter at 15×10^3 Pa) using fluorescein-diacetate (30 μl 2 mM FDA in 100% acetone, Sigma) as substrate and EDTA (30 μl 20 mM EDTA, pH = 8.0) as stabilizer of FDA in sea water. Increased pressure during filtration (up to 45×10^3 Pa) did not alter the results on algal cell lysis, implying that the pressure used throughout this study (15×10^3 Pa) did not cause additional cell leakage. Because the pH of the seawater fluctuated significantly during spring (Fig. 1) we buffered the samples for the esterase assay with 8 mM HEPES-buffer (final concentration, pH = 7.9, Aldrich). HEPES had no effect on the fluorescence signal. We assessed the stability of EAD by measuring the EA of 0.2 μm filtered unbuffered seawater incubated at *in situ* temperature for 24 h. Contrary to Van Boekel et al. (1992) we filtered the field samples in order to avoid lysis of algal cells present in the samples during incubation. Cell lysis of phytoplankton within the samples would have resulted in overestimated EA at the end of the

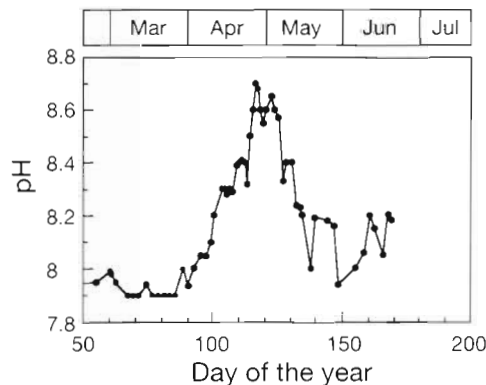


Fig. 1. Fluctuations in pH in the water of the Marsdiep during late winter and spring 1993

incubation and therefore in underestimated daily breakdown rates of EAD. To obtain the specific daily phytoplankton cell lysis rate, the net change of EAD over time was corrected for the daily degradation of EA and divided by the EA in the particulate algal fraction (EAP). Phytoplankton EAP could not be measured directly in the field samples due to interference by EAP from other organisms and therefore was estimated from chl *a* using a mean EAP to chl *a* ratio of 5 ± 3 nmol fluorescein $(\mu\text{g chl } a)^{-1} \text{ h}^{-1}$ (Brussaard et al. 1995).

RESULTS

Nutrients

Concentrations of the 4 macro-nutrients (Fig. 2) reduced strongly during late winter and early spring 1993. Silicate concentration declined to $<1 \mu\text{M}$ at Day 92. At Day 109 the concentration of phosphate had dropped to $0.94 \mu\text{M}$. Ammonium decreased to concentrations $<2 \mu\text{M}$ at Day 85 and fluctuated between 0.73 and $2.2 \mu\text{M}$ until Day 126. Nitrate concentration dropped to $1.2 \mu\text{M}$ on Day 125. Ammonium, phosphate and nitrate levels peaked again during a 3 wk period between Days 140 and 160. Ammonium reached a maximum value of $21 \mu\text{M}$ on Day 155.

Phytoplankton

The maximum concentration of diatom cells (Fig. 3A) was found on Day 88 ($2.3 \times 10^6 \text{ l}^{-1}$). *Phaeocystis* was abundant from Days 100 to 170 (Fig. 3B), during which the major blooming period was found from Days 100 to 134. Highest numbers of *Phaeocystis* colonies of $5\text{--}8 \times 10^4 \text{ l}^{-1}$ were counted between Days 107 and 118.

Phaeocystis total cells reached maximum numbers of $1.8 \times 10^8 \text{ l}^{-1}$ on Day 119. *Phaeocystis* flagellate cell numbers were very low ($<0.4 \times 10^7 \text{ l}^{-1}$) until Day 116. Whereas *Phaeocystis* colonial cell numbers were already declining (since Day 119), flagellate cell number still increased until Day 123. The bloom of *Phaeocystis* is reflected in the chl *a* concentration (Fig. 3C). The high production of *Phaeocystis* biomass coincided with an increase in pH (Fig. 1) up to 8.7 at the peak of the bloom.

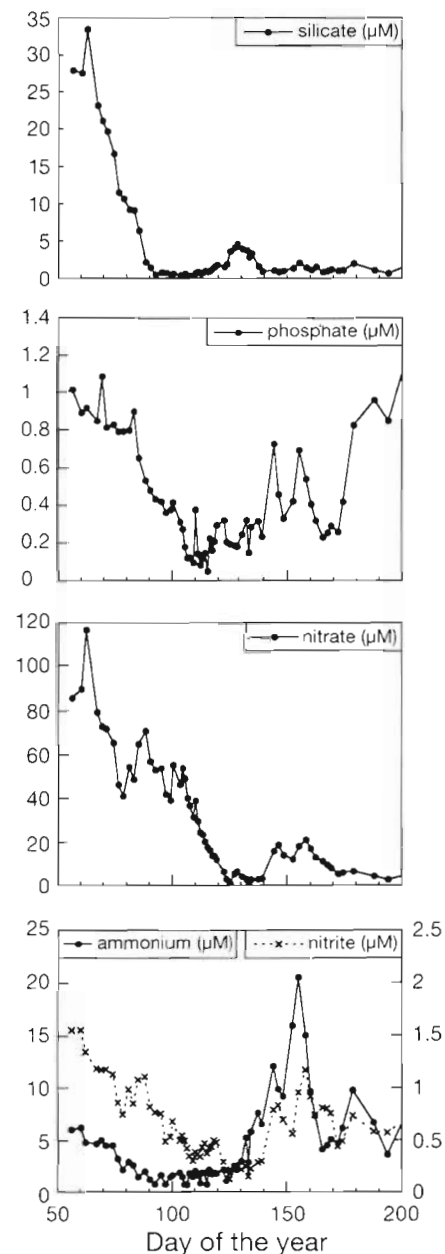


Fig. 2. Variations in the concentrations of silicate, phosphate, nitrate, ammonium and nitrite (μM) in the water of the Marsdiep during 1993

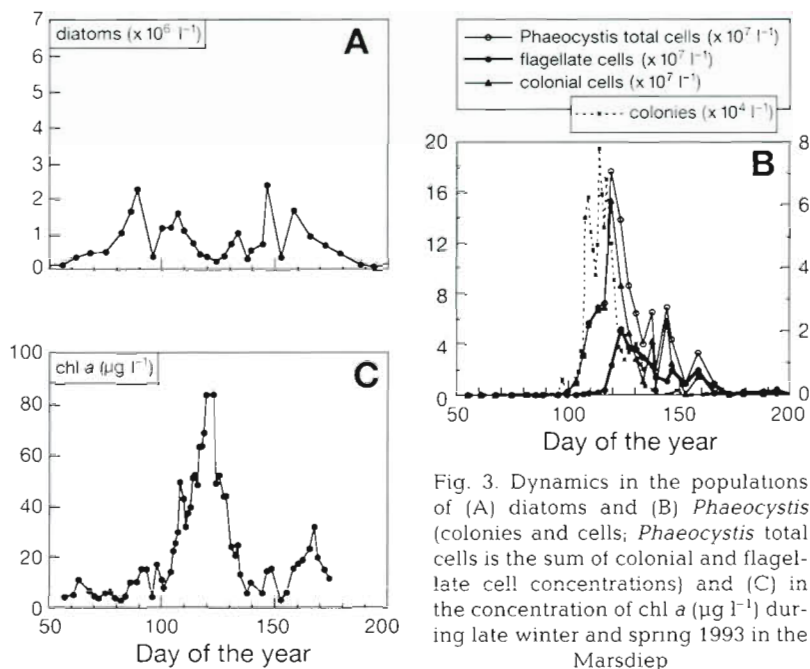


Fig. 3. Dynamics in the populations of (A) diatoms and (B) *Phaeocystis* (colonies and cells; *Phaeocystis* total cells is the sum of colonial and flagellate cell concentrations) and (C) in the concentration of chl *a* ($\mu\text{g l}^{-1}$) during late winter and spring 1993 in the Marsdiep

Phytoplankton cell lysis

During the diatom spring bloom, the specific daily algal cell lysis rates (Fig. 4; expressed as percentage of EAP released daily) were always less than $10\% \text{ d}^{-1}$. Highest lysis rates (up to $33\% \text{ d}^{-1}$) were observed during *Phaeocystis* dominance, and corresponded to either reduced increases or decreases in *Phaeocystis* colonial cell number.

In order to correct for the pH in the field, HEPES-buffer was added to the samples during the esterase assay. Specific daily lysis rates obtained from samples incubated with HEPES buffer were in general lower ($12 \pm 20\%$ lower, $n = 54$) than the lysis rates derived without the addition of HEPES (Fig. 4A).

The *in situ* degradation rates of EAD ranged between 29 and $59\% \text{ d}^{-1}$, with an average rate of $43 \pm 11\% \text{ d}^{-1}$ ($n = 15$). No significant relationship between

the stability of EAD in the field samples and the pH in the ambient water (within the range of 7.9 to 8.7) was found. Using sonificated cultures of the diatom *Skeletonema costatum*, we found the rates of EA degradation independent of pH. Neither temperature (3.5 to 20°C) nor bacteria affected the stability of EA in sonificated cultures of *Phaeocystis*. Compared to the lysis rates based on an EA degradation rate of $25\% \text{ d}^{-1}$ (Brussaard et al. 1995), the algal lysis rates in this study were higher (on average 1.6 ± 0.5 times, $n = 15$) due to the lower stability of EAD. The higher daily breakdown rates resulted in higher amounts of released EA over time and thus in higher specific cell lysis rates, as is illustrated in Fig. 4B. Because of the large variation in the stability of EA, we were unable to correct the majority of data on algal cell lysis for which no *in situ* degradation rates were obtained. To compare the data with a study executed in 1992 (Brussaard et al. 1995), we used lysis rates based on a daily EA degradation rate of 25% to calculate the amounts of released cellular carbon released. However, for future studies we recommend the estimation of EA stability for every sample.

At the peak of the bloom, when the algal biomass consisted mainly of *Phaeocystis*, the ratio of *Phaeocystis* cellular C to chl *a* was $29 (\pm 8.5, n = 5)$. This value is equal to those commonly reported for *Phaeocystis* (Lancelot et al. 1991, Davies et al. 1992) and was used to calculate the amounts of algal cellular carbon released daily (Fig. 5). The release of algal cellular carbon during the diatom bloom was below $10 \mu\text{g C l}^{-1} \text{ d}^{-1}$. Due to the high rates of algal cell lysis and the high biomass of *Phaeocystis*, most algal cellular carbon was released during the bloom of *Phaeocystis* (up to almost $400 \mu\text{g C l}^{-1} \text{ d}^{-1}$).

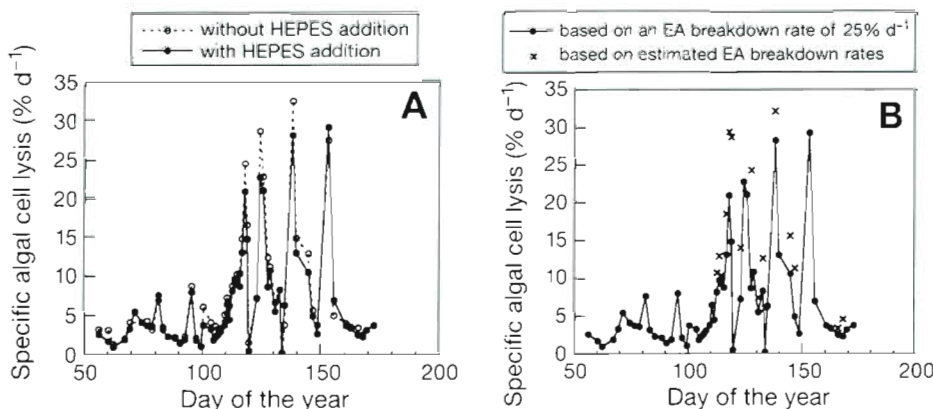


Fig. 4. Fluctuations in the level of specific daily phytoplankton cell lysis [% esterase activity (EA) in the particulate algal fraction released per day] in the water of the Marsdiep during spring 1993. (A) Algal cell lysis with and without addition of HEPES-buffer using an EA breakdown rate of $25\% \text{ d}^{-1}$, and (B) algal lysis rates estimated in the samples with HEPES addition using a fixed EA degradation rate of $25\% \text{ d}^{-1}$ or variable rates estimated *in situ*

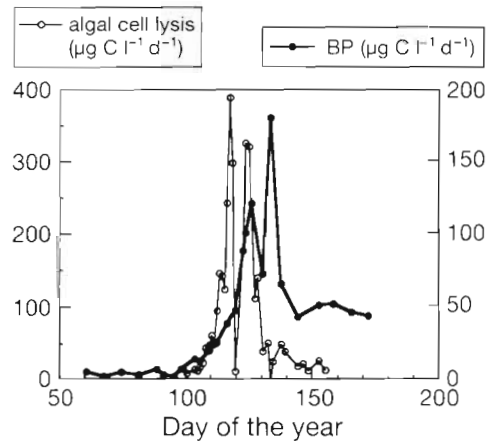


Fig. 5. Variations in the amount of algal cellular carbon released daily by phytoplankton cell lysis ($\mu\text{g C released l}^{-1} \text{d}^{-1}$), and bacterial production (BP, $\mu\text{g C l}^{-1} \text{d}^{-1}$) in the Marsdiep during spring 1993

Bacterial production and biomass

For 1993, as well as for 2 other years (van Duyl unpubl.), bacterial production was significantly related to temperature as long as the *Phaeocystis* bloom did not decline (Fig. 6; $r < 0.9$ and $p < 0.001$ for the slope, using ANOVA).

During the early spring diatom bloom bacterial production was below $5 \mu\text{g C l}^{-1} \text{d}^{-1}$ (Fig. 5). Bacterial production followed the released algal cellular carbon during the bloom of *Phaeocystis*, and reached rates up to $90 \mu\text{g C l}^{-1} \text{d}^{-1}$ (Day 134). After the bloom collapsed, bacterial production stayed on a higher level than before the bloom (around $25 \mu\text{g C l}^{-1} \text{d}^{-1}$).

Bacterial biomass increased with bacterial production and peaked on Day 133 ($311 \mu\text{g C l}^{-1}$, Fig. 7A). A second peak in bacterial biomass was observed on Day 165 ($279 \mu\text{g C l}^{-1}$), during which the bacterial production was constant.

Zooplankton biomass

Heterotrophic nanoflagellates (Fig. 7A) showed a comparable trend in biomass to the bacteria. The first peak (Day 126, $291 \mu\text{g C l}^{-1}$) was a few days before and the second (Day 172, $287 \mu\text{g C l}^{-1}$) just after peaks in bacterial biomass. Before Day 126, small ($< 5 \mu\text{m}$) HNFs dominated in cell number, whereas thereafter no dominance in cell number of the small or larger HNFs was found.

Microzooplankton (Fig. 7B) consisted of 2 groups: tintinnid ciliates and other microzooplankton (predominantly oligotrichous ciliates). Tintinnids became significant after Day 130 and caused the high peak in microzooplankton biomass on Day 137 (857 mg C l^{-1}).

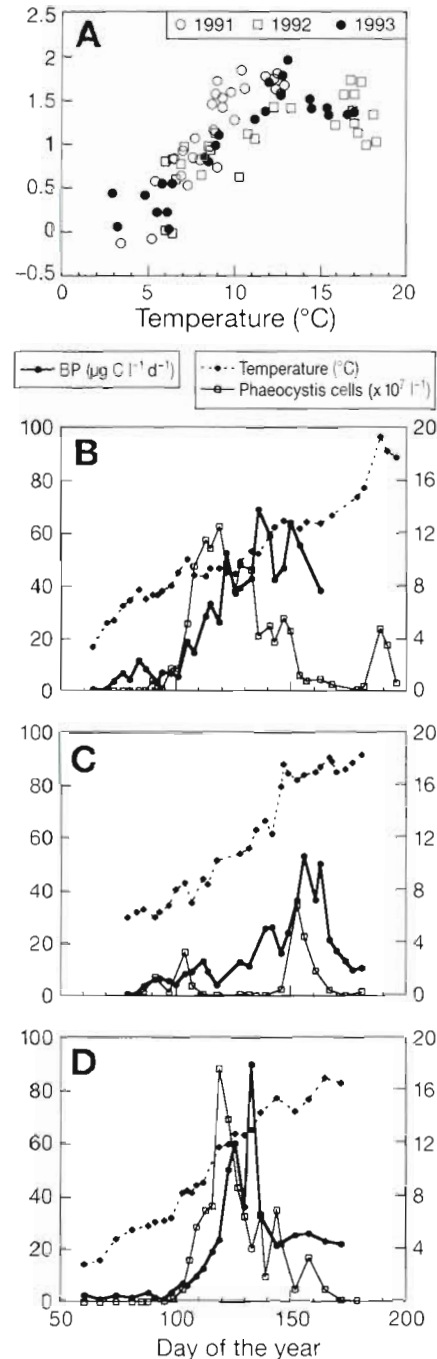


Fig. 6. (A) Log bacterial production (log BP) versus temperature for the years 1991, 1992, and 1993. Dynamics of BP ($\mu\text{g C l}^{-1} \text{d}^{-1}$), *Phaeocystis* cell concentration ($\times 10^7 \text{ l}^{-1}$), and temperature ($^{\circ}\text{C}$) for (B) 1991, (C) 1992, and (D) 1993

Naked ciliates peaked on Day 116 ($498 \mu\text{g C l}^{-1}$), and declined afterwards to $< 50 \mu\text{g C l}^{-1}$ on Day 144.

Mesozooplankton biomass (Fig. 7B) peaked on Day 130 ($157 \mu\text{g C l}^{-1}$), which was 2 wk after the peak in naked oligotrichous ciliates. A second peak occurred on Day 158 ($313 \mu\text{g C l}^{-1}$) 3 wk after the

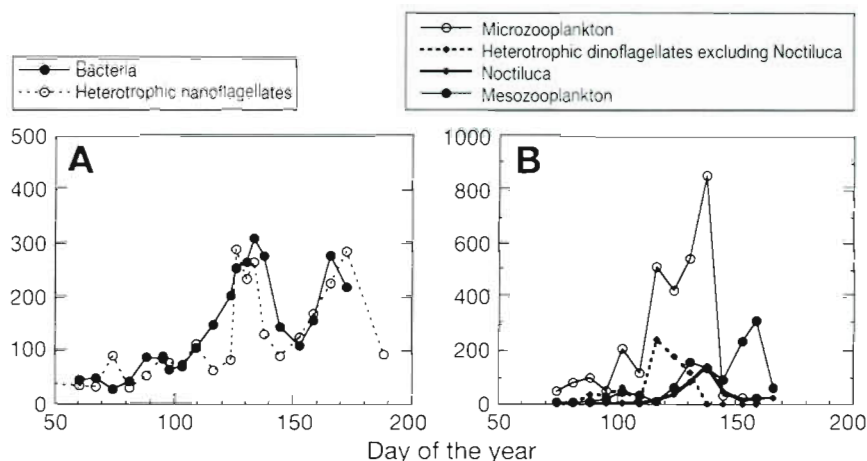


Fig. 7. Dynamics in biomass ($\mu\text{g C l}^{-1}$) of (A) bacteria and heterotrophic nanoflagellates and (B) of microzooplankton, *Noctiluca miliaris*, heterotrophic dinoflagellates other than *N. miliaris*, and mesozooplankton during 1993 in the Marsdiep area. Note the different scales in (A) and (B)

peak in tintinnid biomass. Mesozooplankton consisted mainly of copepods, with the share of adult copepods increasing in time.

The heterotrophic dinoflagellate *Noctiluca miliaris* (Fig. 7B) peaked once on Day 137 and reached a maximum biomass of $143 \mu\text{g C l}^{-1}$. Heterotrophic dinoflagellates other than *N. miliaris* were significantly present from Days 88 to 130, with a maximum biomass of $243 \mu\text{g C l}^{-1}$ on Day 116 (Fig. 7B).

DISCUSSION

Diatom bloom

The onset of the diatom bloom in early spring was delayed and the increase in biomass less rapid compared to 1992 (Brussaard et al. 1995). Light availability and temperature influence the growth of phytoplankton (Yoder 1979, Rhee & Gotham 1981a, b). Compared to other years we found a low mean water-column radiation ($<22 \text{ Wh m}^{-2} \text{ d}^{-1}$, estimated according to Peperzak 1993 and Brussaard et al. 1995) from Days 40 to 56, which was due to low solar radiation ($<200 \text{ J cm}^{-2} \text{ d}^{-1}$). From Days 55 to 70, temperature was relatively low ($<4^\circ\text{C}$). Low radiation, accompanied by low temperatures delayed the onset of the diatom bloom. Size-selective grazing (Riegman et al. 1993) may explain the sharp decline in silicate concentration from Day 60 without an accompanying increase in phytoplankton biomass during the next 2 wk. With maximum diatom cell concentrations half those in 1992, a comparable species composition (G. C. Cadée pers. comm.) and a stronger decline in silicate concentration, the diatom bloom must have been the result of higher loss rates. Grazing by mesozooplankton was unlikely due to its low standing stock in early spring, and low numerical response at the prevailing tempera-

tures (Klein Breteler et al. 1982). Diatom cell lysis appeared to be a loss factor of only minor importance ($<0.1 \text{ d}^{-1}$). Sedimentation very likely controlled the height of the diatom standing stock (Moloney et al. 1986). Passow (1991) found species-specific aggregation to be responsible for a rapid differential disappearance of diatom species blooming during spring. As the amounts of algal cellular components released by lysis were low ($<10 \mu\text{g C l}^{-1} \text{ d}^{-1}$) and most of the particulate matter was lost from the pelagic ecosystem by sedimentation, pelagic bacteria showed no response to the diatom bloom.

With bacterial production being half that of 1992, differences in diatom bloom biomass between the 2 years were reflected in bacterial production. However, the impact on the microbial food web is negligible because of the low bacterial production rate.

Phaeocystis bloom

As long as the concentration of silicate is above $2 \mu\text{M}$, *Phaeocystis* loses the competition for both nitrogen and phosphate from diatoms (Egge & Aksnes 1992). At the time silicate became depleted, mean water-column photosynthetically active radiation was about $200 \text{ Wh m}^{-2} \text{ d}^{-1}$, which is well above the threshold level for colony formation ($100 \text{ Wh m}^{-2} \text{ d}^{-1}$, Peperzak 1993) and therefore did not prevent the blooming of *Phaeocystis* colonies as it did in 1992 (Brussaard et al. 1995). The higher levels of nitrate at the start of the bloom in 1993 ($55 \mu\text{M}$ compared to $20 \mu\text{M}$ in 1992) allowed *Phaeocystis* to reach higher cell numbers (~ 3 times higher than in 1992). This is in good agreement with the recently reported significant relationship between the nitrate concentrations at the onset of a *Phaeocystis* bloom and the cell density at the top of the bloom (Lancelot 1995). During the build-up of the *Phaeocystis*

bloom, pH increased from 7.9 to 8.7 as a result of CO₂ net fixation into the alga. Apparently, CO₂ uptake could not be balanced by diffusion from surrounding waters or the atmosphere during the increase in *Phaeocystis* biomass. Comparable CO₂ dynamics has been reported for the lower part of the Narraganset Bay (Hinga 1992).

A decline in *Phaeocystis* colony number, and the occurrence of 'ghost' colonies (Verity et al. 1988) only 1 wk after the bloom started can be explained by a dip in mean water column radiation from Days 106 to 109 to levels below 100 Wh m⁻² d⁻¹. Despite high solar radiation (>5000 Wh m⁻² d⁻¹, data supplied by Royal Dutch Meteorological Institute) *Phaeocystis* probably also experienced light limited growth during the period of bloom decline (Days 119 to 134) due to self-shading, as was reflected in a strong decline in secchi disk values (G. C. Cadée pers. comm.). Our data suggest that beside potential light limitation, the *Phaeocystis* bloom became depleted by nitrate ($K_s = 4 \mu\text{M}$ for inorganic nitrogen, Lancelot et al. 1986). The formation rate of colonies is reduced when there is low availability of nitrate (Riegman et al. 1992). The number of colonies declined due to desintegration of the colony matrix, whereas the decline in colonial cell number was caused by cell lysis and transformation into flagellate cells by the rest of the colonial cells. A rise in number of flagellate cells during the decline of colonial cells was observed in our study. The increasing concentration of ammonium recorded at the end of the bloom prevented the formation of new colonies even more (Riegman et al. 1992). Ammonium inhibited the uptake of nitrate (McCarthy et al. 1975), whereas low light may have negatively influenced the reduction of nitrate to ammonium within the algal cells (Syrett 1981). The high pH in the ambient water during the *Phaeocystis* bloom (up to 8.7) probably also affected the development of the bloom. Photosynthesis induces not only a rise in external pH, but also inside *Phaeocystis* colonies high values of pH have been reported (up to 9, Lubbers et al. 1990). This may have restricted the rate of primary production in *Phaeocystis* (Riebesell et al. 1993) and the nitrogen metabolism within the cells. We found no significant relationship between specific algal cell lysis and pH during the bloom of *Phaeocystis*. We hypothesize that as a result of nitrogen and energy deficiency, synthesis of structural and functional components was blocked which resulted in osmolytic deregulation and subsequently cell death through plasmolysis.

Although viruses can also be potential mortality agents for algal blooms (Bratbak et al. 1993, Brussaard et al. 1996), the complete absence of any viral infection within cells of *Phaeocystis* sampled at different stages of the bloom during spring 1994 (authors' unpubl.

results) indicate that viruses may at least not always be important for the decline of *Phaeocystis* blooms in the Dutch coastal area.

The decline of the major peak in *Phaeocystis* colonial cells (Days 119 to 134) was equal to 160 $\mu\text{mol C l}^{-1}$. The integrated release of algal cellular carbon due to cell lysis was 158 $\mu\text{mol C l}^{-1}$, which indicates that the *Phaeocystis* bloom declined through cell lysis. Released algal cellular carbon was 2.9 times higher than was estimated during the *Phaeocystis* bloom in 1992 (cell lysis recalculated using a C:chl *a* ratio of 29), reflecting a comparable enhancement of *Phaeocystis* biomass.

Based on carbon standing stock we calculated the net variation of the population of heterotrophs (the sum of bacteria, HNFs, microzooplankton, heterotrophic dinoflagellates, and mesozooplankton) over this period. The heterotrophic carbon demand (estimated from net production and a gross growth efficiency of 0.3) was 167 $\mu\text{mol C l}^{-1}$. The close match with the losses of *Phaeocystis* suggest that the bloom of *Phaeocystis* was mineralized within the water column. We are aware that this conclusion largely depends on the conversion factors used. The conversion factors yielding *Phaeocystis* cellular carbon were based on cell concentrations and had standard deviations of 35% (Lancelot et al. 1991). Applying these to our data still resulted in 78% of the *Phaeocystis* bloom being mineralized within the water column. Our data on the stability of EAD indicated that the actual specific algal cell lysis rates, and thus the amount of released algal cellular carbon, were probably higher (on average 1.6 times). On the other hand, the ratio of EAP to chl *a* in senescent colony-containing cultures of *Phaeocystis* (comparable to the decline of the bloom in the field) was found to be up to 2-fold higher than the mean ratio of 5 used in this study. The consequently 2-fold lower algal lysis rates will compensate for the positive effect of the lower stability of EAD on algal cell lysis. The extracellular polysaccharides of the *Phaeocystis* colony mucus were thought to be largely resistant to biodegradation (Thingstad & Billen 1994, Lancelot 1995) and were therefore not included in the C budget calculated for Days 119 to 134.

From Days 134 to 170, cell lysis was not the only loss factor responsible for the declines in *Phaeocystis* cell numbers. Algal cell lysis equalled 60 $\mu\text{mol C l}^{-1}$, which was only 40% of the cumulative losses of *Phaeocystis* during this period. The rates of losses other than cell lysis were estimated by taking the net result of specific algal cell lysis and specific net growth rate of *Phaeocystis*, and were between 0.1 and 0.4 d⁻¹. Since sinking of *Phaeocystis* seems to be not important in the turbulent Dutch coastal area (Van Boekel et al. 1992, Brus-

saard et al. 1995), these rates most probably indicated grazing pressure. It is very likely that the tintinnids grazed on the fast growing *Phaeocystis* flagellate cells. Grazing on *Phaeocystis* single cells by tintinnids has been observed by Admiraal & Venekamp (1986). The succession of naked ciliates by tintinnids can be explained by the size of *Phaeocystis* cells (3 to 8 μm). Tintinnids consume most actively nanoplankton in the size range 3 to 10 μm (Rassoulzadegan et al. 1988), whereas oligotrichs feed most efficiently on prey in the 2.5 μm size range (Bernard & Rassoulzadegan 1990).

Microbial food web

At low substrate concentrations, pronounced effects of temperature on bacterial growth are reported (Pomeroy et al. 1991). Such conditions applied in the Dutch coastal zone in early spring when diatoms dominated the algal biomass. At higher substrate concentrations temperature was shown to have little effect on bacterial growth and respiration (Pomeroy & Wiebe 1993). The relatively high amounts of algal cellular components released through cell lysis during the *Phaeocystis* bloom masked the relationship between bacterial production and temperature.

During the major part of the bloom (Days 100 to 134), the cellular carbon released by *Phaeocystis* due to cell lysis was equal to $305 \mu\text{mol C l}^{-1}$, sufficient to account for the bacterial carbon demand of $240 \mu\text{g C l}^{-1}$ during this period. After Day 140, the bacterial carbon demand was higher than could be sustained by algal cell lysis. It is likely that additional food sources such as by-products of animal ingestion and digestion (Jumars et al. 1989) and the large amount of slowly biodegradable extracellular polysaccharides of the *Phaeocystis* colony mucus (Thingstad & Billen 1994) supplied the bacteria with carbon.

The enhanced amounts of released algal cellular carbon compared to 1992 were reflected in bacterial production (2.3 times higher). Enhanced bacterial production following a relatively large bloom of *Phaeocystis* was also found for 1991 (Fig. 6), suggesting a general validity of the coupling of bacterial production to the magnitude of the *Phaeocystis* bloom owing to lysis of *Phaeocystis* cells.

The enhanced bacterial production rates were not reflected in bacterial biomass implying higher loss rates (mortality rates up to 0.28 d^{-1} compared to maximum rates of 0.1 d^{-1} in 1992). Though grazing by HNFs (Weisse 1990) seemed responsible for the mortality of bacteria, viral lysis of bacteria cannot be ruled out as a potential loss factor (Proctor et al. 1993). The rise in biomass of HNFs (Days 116 to 126) coincided with

the decline in biomass of naked oligotrichous ciliates, suggesting that HNFs were controlled by grazing of microzooplankton (Weisse 1990). Heterotrophic dinoflagellates are not very selective for prey type (Hansen 1992) and probably competed with ciliates for prey. Unlike heterotrophic dinoflagellates, HNFs (Fenchel 1982) and microzooplankton (Verity 1986), mesozooplankton cannot respond very fast to an increase of prey (Klein Breteler et al. 1982). This allows the prey of mesozooplankton to reach high biomasses. Differences in *Phaeocystis* bloom magnitude between the 2 years under study were, as a consequence of algal cell lysis and bacterial production, reflected in the standing stock of microzooplankton.

The high grazing activity on all trophic levels of the microbial food web resulted in substantial accumulation of regenerated inorganic nutrients, especially ammonium. It has already been known for a long time that mesozooplankton excrete both nitrogen and phosphorus (Ikeda 1977). Only in the last decade has it become more clear that Protozoa have to be considered as the more important mineralizers of nutrients, as is clearly illustrated by the computer simulation model of Moloney et al. (1986). Verity (1985) reported high excretion rates of ammonium by tintinnids, which are thought to be typical for temperate coastal waters. Ageing cells of *Noctiluca miliaris* rise to the water's surface and release, due to cell lysis, ammonium and phosphate (Schaumann et al. 1988). Although depending on the C:N:P ratio of the algal lysis components (Caron et al. 1988, Jürgens & Güde 1990), it is likely that bacteria also contributed to the excretion of ammonium and phosphorus as they were found to be carbon limited during this study.

Calculations on the nitrogen budget (Table 1) show that the shift in biomass composition between Days 119 and 155 was equivalent to a decrease of $27 \mu\text{mol N l}^{-1}$. Correction of the *in situ* concentrations of ammonium, nitrate and nitrite for conservative mixing of fresh water (IJsselmeer, $S \approx 0.3\text{‰}$) and offshore North Sea water ($S \approx 33.5\text{‰}$) end members resulted during this period in a net accumulation of $29 \mu\text{mol N l}^{-1}$ (maximum concentration on Day 155). The strongest incline in the concentration of inorganic nitrogen was found after the major decline of the *Phaeocystis* bloom (Days 119 to 134), during which the net variation of the heterotrophic nitrogen became negative. Ammonium and nitrate are released from the sediments in the Dutch coastal waters at rates of approximately 12 and $5 \text{ mg N m}^{-2} \text{ d}^{-1}$, respectively (Billen et al. 1990). Between Days 119 and 155 this would have resulted theoretically in an accumulation of only $2 \mu\text{M}$ ammonium and $1 \mu\text{M}$ nitrate. Not only most of the remineralization of ammonium, but also most of the nitrification took place within the water column. The

Table 1 Nitrogen standing stocks ($\mu\text{mol N l}^{-1}$) of the populations of *Phaeocystis*, bacteria, HNFs, microzooplankton, heterotrophic dinoflagellates (HD, excluding *Noctiluca miliaris*), *N. miliaris*, mesozooplankton, and the concentration of ammonium (μM) in the Marsdiep area on Days 120, 134 and 155. Total heterotrophic biomass is the sum of bacteria, HNFs, microzooplankton, heterotrophic dinoflagellates, and mesozooplankton. Nitrogen was calculated from carbon biomass using a C:N ratio of 10 for *Phaeocystis* and 4 for heterotrophs. Concentrations of the considered nitrogen compound after correction for conservative mixing are given in parentheses. This was done by subtracting the fraction determined by conservative mixing of coastal North Sea water and IJsselmeer water from the *in situ* concentration

| Day | 119 | 134 | 155 |
|---|------------|------------|-------------|
| Particulate organic N (μM) | | | |
| <i>Phaeocystis</i> | 20.5 | 4.6 | 2.0 |
| Bacteria | 3.5 | 6.4 | 2.7 |
| HNFs | 1.5 | 5.4 | 3.0 |
| Microzooplankton | 9.8 | 14.9 | 0.6 |
| HD | 4.5 | 1.1 | 0 |
| <i>Noctiluca miliaris</i> | 0.7 | 2.5 | 0.6 |
| Mesozooplankton | 0.8 | 3.2 | 5.8 |
| Total for heterotrophs | 20.8 | 33.5 | 12.7 |
| Dissolved inorganic N (μM) | | | |
| Ammonium | 1.8 (0.9) | 4.5 (3.6) | 20.3 (16.7) |
| Nitrate | 12.4 (1.1) | 2.2 (-3.0) | 17.7 (13.8) |
| Nitrite | 0.5 (0.3) | 0.2 (0) | 0.9 (0.7) |
| Total nitrogen salts | 14.7 (2.3) | 6.9 (0.6) | 38.9 (31.2) |

potential importance of nitrification in coastal marine ecosystems has been reported also by Berounsky & Nixon (1985). The actual concentration of regenerated ammonium would have been higher because growth of diatoms at the end of this period had lowered the actual concentration of regenerated ammonium. In conclusion, not only the calculations based on the carbon but also on the nitrogen budget clearly indicate that the major part, if not all, of the *Phaeocystis* bloom was mineralized within the water column. As stated by Wassmann (1994), mineralization within the water column of most of the *Phaeocystis* bloom can be expected in areas like the Dutch coastal zone with high turbulence energy supply.

Summarizing, the hypothesis that phytoplankton bloom magnitude affects the importance of the microbial food web in the Dutch coastal pelagic ecosystem whenever algal cell lysis is the major loss factor was confirmed by the blooming of *Phaeocystis*. An enhanced bloom of the colonial *Phaeocystis* could have developed in spring 1993 due to a high standing stock of nitrate at the onset of the bloom. Cell lysis was the major loss factor for the *Phaeocystis* bloom and the large amount of released cellular components resulted in enhanced bacterial production. Elevated grazing activity by members of the microbial food web and the

relatively low numerical response by mesozooplankton explained why only microzooplankton biomass was affected by variation in *Phaeocystis* bloom magnitude. In contrast to the preceding diatom bloom, most of the *Phaeocystis* bloom was mineralized within the water column. The utilization of the high concentration of released nutrient-rich algal organic matter by heterotrophs led to substantial accumulations of regenerated inorganic nutrients.

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