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Impact of rainfall on the Diatom - *Phaeocystis* succession in the eutrophicated coastal waters of the North Sea.

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Abstract

Eutrophication in the continental coastal waters of the North Sea is characterized by the massive development of *Phaeocystis* colonies sustained by anthropogenic sources of nitrate (NO_3) unused by the early spring growth of a dissolved silicate-controlled diatom community. The influence of meteorological conditions on eutrophication in the Belgian coastal zone was studied through a high resolution time series of hydrological, nutrients and phytoplankton data collected during two meteorologically contrasting years: 1) in 1993 characterized by a relatively dry winter - early spring season and 2) in 1994 characterized by high rainfall in winter - early spring. Attention was given to the mechanisms driving the diatom - *Phaeocystis* colonies succession, particularly the winter value of the nitrate : silicate (NO_3 : DSi) molar ratio as an indicator of nutrient enrichment. This comparative analysis was supplemented with data collected in the same area since 1988. The analysis of meteorological and hydrological data indicate that changes in rainfall can be perceived in Belgian coastal waters with a delay of 50 days. It is demonstrated that rainfall prevailing during the winter - early spring period (December - March) are structuring the spring phytoplankton community by determining DSi and NO_3 concentrations from land-based sources. Low winter rainfall is expected to allow for accumulations of gelatinous *Phaeocystis* colonies. These results support the hypothesis that diatoms are able to outcompete *Phaeocystis* when sufficient DSi is available.

Key words: eutrophication, diatoms, *Phaeocystis*, nutrient, rainfall, anthropogenic activity.

Introduction

Explosive phytoplankton bloom development is recorded every spring in the eutrophicated continental coastal waters of the North Sea, an area receiving the discharge of 7 major Western European rivers draining regions characterized by high population densities, intensive industrial activities and agricultural practices (Fig. 1). This spring bloom event is commonly characterized by the occurrence of a moderate diatom bloom controlled by dissolved silicate (DSi) concentrations followed by an exponential development of non-siliceous phytoplankton almost entirely dominated by large gelatinous colonies of *Phaeocystis* (Bätje & Michaelis, 1986; Cadée, 1986; Cadée & Hegeman, 1986; Lancelot *et al.*, 1987; Cadée & Hegeman, 1991; Lancelot, 1995). The *Phaeocystis* bloom represents usually, at their maximum development, more than 90% of the phytoplankton carbon biomass (Rousseau *et al.*, 1990). However, the extent and magnitude of *Phaeocystis* blooms varies greatly from year to year and has been shown to be sustained by nitrate availability at the end of the early spring diatom bloom (Lancelot, 1995; Rousseau *et al.*, in preparation). The ability of *Phaeocystis* colonies to utilize nitrate (NO_3) as N source was demonstrated through competitive experiments under laboratory-controlled conditions (Riegman *et al.*, 1992) and was also confirmed by the elevated f_{NO3} ratio (0.5-0.8) characterizing the N metabolism of field *Phaeocystis* colonies, both in the coastal North Sea (Lancelot & Mathot, 1987) and Greenland Sea (Smith *et al.*, 1991; Smith, 1993). These observations suggest that the amplitude of the diatom - *Phaeocystis* succession is determined by the DSi and NO_3 level of these coastal waters.

The nutrient environment of the North Sea coastal phytoplankton is strongly determined by the quantitative and qualitative composition (N:P:Si ratio) of the river inputs. The latter is driven by changes in both human activities in the watershed and rainfall. Human activity largely influences the N and P content of land-based sources. For instance, the use of fertilizers and phosphate-containing detergents, domestic

sewage, industrial waste and inland hydraulic management were shown to be responsible for N and P excess delivery into the Dutch coastal waters (Bennekom *et al.*, 1975; Gieskes & Schaub, 1990; Riegman *et al.*, 1992). Contrasting, silicate delivery by rivers originates from the weathering of alumino-silicate soil minerals and is affected by human activity especially with the construction of dams on rivers (Bennekom *et al.*, 1975; Gieskes & Schaub, 1990). Rainfall affects quantitative and qualitative nutrient inputs into coastal waters by modifying the relative contribution of point (N and P from industrial and urban waste waters) and diffuse (mostly nitrate and silicate from agricultural and soil mineral leaching respectively) sources of nutrients. Point sources usually remain constant when diffuse nutrient sources are directly affected by rainfall.

Contrasting rainfall recorded in the North Western European catchment basin in the winter-spring period of 1993 and 1994 provides an opportunity to study the influence of meteorological conditions on coastal eutrophication of the North Sea. The winter-spring period of 1993 was rather dry while in 1994 exceptionally high rainfall prevailed from December to April. The impact of rainfall on the nutrient environment and its consequence on the magnitude of *Phaeocystis* colony blooms is documented in this paper through the analysis of phytoplankton, nutrient and hydrological (rainfall, Scheldt flow rate, salinity) data seasonally collected in the Belgian coastal waters in 1993 and 1994. Particular emphasis is given to the relationship between 1) hydrology and NO_3 and DSi delivery and 2) between the diatom - *Phaeocystis* succession and nutrient enrichment of the coastal zone. These relationships are further examinated by the use of monitoring data on nutrients and phytoplankton in the continental coastal waters of the North Sea during the period 1988-1994.

Material and methods

1. Sampling station and procedure

Sampling was performed at station 330 in the Belgian coastal waters of the North Sea (N 51°26.05; E 002° 48.50; Fig. 1). This station is characterized by waters well-mixed to the bottom (20 m depth) and was chosen as a reference survey station for *Phaeocystis* bloom development since 1984. Sampling occurred monthly in winter and weekly to twice-weekly during spring. A bucket was used for sampling in order to avoid *Phaeocystis* colony disruption or damage.

2. Hydrology and meteorology

The salinity was measured by conductivimetry using a Beckman salinometer. Monthly and 10-days average Scheldt flow rates at the catchment basin outlet (station Schelle) were provided by Ministerie van Vlaamse Gemeenschap - Antwerpse Zeehavendienst. Rainfall data were documented by the Institut Royal de Météorologie (Uccle, Belgium). Monthly and 10-days values represent average for 20 meteorological stations distributed on the whole Belgian Scheldt catchment basin.

3. Chemical measurements

NO_3^-+NO_2^- and DSi concentrations were measured on 0.45 µm membrane (Sartorius) filtered sea water. NO_3^-+NO_2^- concentration measurements were made with Griess reagent in acid medium after reduction of nitrate into nitrite on a Cd/Cu column at pH 8.5 (Grasshof, 1983). DSi concentrations were determined spectrophotometrically after formation of a silico-molybdic complex and subsequent reduction into molybdate blue by ascorbic acid (Koroleff, 1983).

Chlorophyll a concentrations were measured on 90 % (v:v) acetone extracted particulate material using glass fiber filters (GF/C) for 12h at 4°C. Chl a concentration was determined spectrophotometrically (Lorenzen, 1967).

4. Biological measurements

Phytoplankton was analysed under inverted microscope (Leitz Fluovert) according to Utermöhl method (Hasle, 1978) after a 12h concentration in counting chambers (Hydrobios) of preserved samples with 1% lugol-glutaraldehyde solution. Sedimented volumes (10 to 100 ml) were adjusted in order to count 500 cells in total with at least 100 cells of the most abundant genus or species. Diatoms were enumerated at a magnitude of 100X or 200X and identified until genus level unless a species was easily identifiable or dominant. *Phaeocystis* colony and colony cells enumeration were performed according to Rousseau *et al.* (1990).

Diatom and *Phaeocystis* carbon biomass was calculated from microscopic determination of number and biovolume, the latter being calculated from cell or colony dimension measurement by comparison to a graduated reticule. *Phaeocystis* colony carbon biomass was calculated by using equations recommended by Rousseau *et al.* (1990). Diatom carbon biomass was calculated for each species or genus by using a specific average conversion factor calculated from biovolumes measured on a cell population along the whole period of its development. Biovolumes are then converted using a carbon content factor of 0.11 $pgC.\mu m^{-3}$ of plasmavolume recommended by Edler (1979) for diatoms.

Results.

I. Seasonal changes of phytoplankton and nutrient concentrations in the Belgian coastal waters during 1993 and 1994.

Total phytoplankton biomass, as expressed by Chl a concentrations (Fig. 2) exhibited a similar seasonal patterns in 1993 and 1994 , both in amplitude and timing, suggesting a similar phytoplankton development. A spring bloom reaching Chl a concentrations of 45 $mg.m^{-3}$ was recorded by the end of April. Spring bloom termination occurred

suddenly by the end-May when Chl a concentrations reached their minimum levels (< 0.5 $mg.m^{-3}$). In summer, some moderate phytoplankton bloom developments were observed, reaching similar amplitudes in 1993 and 1994. The phytoplankton growth period ended at the end of October-November when winter Chl a levels less than 1 $mg.m^{-3}$ were recorded.

Diatoms (Fig. 3a) and *Phaeocystis* colonies (Fig. 3b) contributed quite differently to phytoplankton spring biomass with *Phaeocystis* colonies largely predominant in 1993 and diatoms in 1994. Two periods with high diatom biomass were recorded: in early spring and in summer (Fig. 3a). The phytoplankton spring succession began with increases in diatom biomass in early March both in 1993 and 1994. However, the magnitude reached by both diatom blooms differed greatly by more than a factor 3, reaching 200 $mgC.m^{-3}$ in 1993 and more than 700 $mgC.m^{-3}$ in 1994. Seasonal diatom species succession was similar for both years. Neritic species such as *Thalassiosira* spp., *Melosira* sp., *Skeletonema costatum*, *Thalassionema nitschzoïdes*, *Plagiogramma brockmanni*, *Asterionella glacialis* and the large *Coscinodiscus* spp. were the dominant species in late winter-early spring. The spring diatom bloom was characterized by the successive development of *Chaetoceros* spp. and *Rhizosolenia* spp., mainly *R. delicatula*, the latter dominating to nearly 100 % of the diatom population at the height of the diatom bloom. *R. delicatula* continued to dominate throughout the summer. The autumn diatom community was composed primarily of the winter species assemblage described above, *Coscinodiscus* spp. and *Chaetoceros* spp., with some *Rhizosolenia* spp. in 1993. *Phaeocystis* colonies reached their maximum biomass between the spring and summer diatom blooms (Fig. 3b). While co-occurring with diatoms, large accumulations of *Phaeocystis* colonies developed in 1993, reaching a maximum of 4300 $mgC.m^{-3}$ whereas in 1994 a low biomass of 530 $mgC.m^{-3}$ was recorded, what is about one order of magnitude lower than in 1993.

NO_3 and DSi concentrations (Fig. 3c) were fluctuating similarly in 1993 and 1994. Maximum nutrient concentrations were reached during winter months when biological activities are at their minimum level. These winter concentrations constitute the nutrient stock available for coastal phytoplankton bloom development and result from the mixing of Atlantic waters with the cumulative nutrient input by the rivers Seine and Scheldt. No clear differences between the 1993 and 1994 NO_3 and DSi winter stocks were observed (Fig. 3c). However, the relative abundance of NO_3 and DSi expressed by the winter NO_3^- : DSi molar ratio decreased from 1.6 in 1993 to 1.2 in 1994, indicating a higher availability of silicate in 1994 compared to 1993. The lowest NO_3 and DSi concentrations were reached by the end of March each year.

The ratio of NO_3 to DSi uptake by the early spring diatom community in 1993 and 1994 was indirectely estimated by plotting NO_3 and DSi concentrations (Fig. 4). The slope of the regression line gives an estimate of the NO_3^- : DSi cellular ratio for the diatom community. The diatom community was characterized by a similar NO_3^- : Si molar ratio of 1.4 for both 1993 and 1994, in agreement with the reported range of values for coastal diatoms (0.8-1.3; Brzezinski, 1985). The zero ordinate can be used to determine the winter NO_3 concentration left over after the diatom decline and available for *Phaeocystis* development. The available NO_3 concentrations for *Phaeocystis* colonies was estimated at 11 $mmol.m^{-3}$ in 1993 while undetectable in 1994, suggesting that no nitrate originating from the winter stock was available for *Phaeocystis* colony development in spring 1994. The dependance of *Phaeocystis* bloom magnitude to the NO_3 stock left over after the diatom decline has been previously established, from phytoplankton and nutrient data, for different stations of the Southern Bight of the North Sea for the period 1988-1993 (Lancelot, 1995). These stations (Fig. 1) are located along a SW-NE axis along the coast, from the French zone to the German Bight. Due to a residual circulation, these stations are gradually submitted to the cumulative influence of the different rivers discharging in these coastal areas. Accordingly, the *Phaeocystis* and nutrient data obtained in 1993 and

1994 are adjusting very well the exponential relationship between the maximum Phaeocystis colonial cell density and NO_3 concentrations observed at the early spring diatom decline (Fig.5).

DSi concentrations of 3.9 mmol.mm^{-3} in 1993 and 2.3 mmol.mm^{-3} in 1994 were recorded during the spring bloom period dominated by *Chaetoceros* spp. and *Rhizosolenia* spp. species. Consequently, the exceptionally high diatom biomass recorded in spring 1994 was necessarily sustained by remineralisation processes and / or by sustained riverine delivery. As a general trend, the lowest nitrate concentrations were observed at *Phaeocystis* decline (Fig. 3a). Unexpectedly, a peak of nitrate was recorded in 1994 at the time of diatom decline and before *Phaeocystis* development. This high nitrate concentration could originate from rapid remineralisation of diatom-derived organic matter. Reasons for the observed delay between nitrate accumulation and *Phaeocystis* development are not understood; it could be due to bottom-up control by herbivorous protozooplankton.

II. Meteorology, hydrology and nutrient delivery to the coastal zone

Monthly rainfall recorded over the Belgian Scheldt catchment basin (Fig. 6) shows that contrasting meteorological conditions were occurring during the winter-spring period 1993 and 1994. The winter-spring rainfall in 1993 was low compared to the normal, currently defined as the monthly mean calculated for the last 35 years. In contrast, rainfall was exceptionally high in 1994 reaching 160 % of the normal. Particularly intense rainfall prevailed in December 1993 with values 250 % higher than the normal.

The direct incidence of rainfall conditions on the Scheldt flow rate is evidenced by the positive relationship existing between the monthly average Scheldt flow rate and monthly rainfall over the Scheldt catchment basin recorded during cold and rainy months, from November to April (Fig. 7a). At this time, evapotranspiration in the watershed is reduced to its minimum and the hydric stocks of soil and aquifers are restored.

The relationship between meteorological conditions, river discharge and nutrient delivery was than established by analysing salinity and nutrient data collected at station 330 during the period 1988-1994 in relationship with the 10-day averaged Scheldt flow rate. This analysis shows that the influence of the Scheldt discharge and hence of rainfall conditions can be observed at station 330 with a delay of 50 days (Fig. 7b). Such a delay was best estimated by relating Scheldt flow rate and observed salinities at station 330 with varying delays. These data clearly shows that the influence of freshwater inputs is more sensitive for high flow rate (400 $m^3.sec^{-1}$).

The riverine origin of nutrient enrichment in this coastal area is evidenced by the significant negative linear relationship between NO_3 (Fig. 7c) and DSi (Fig. 7d) concentrations in winter when no phytoplankton growth is occurring, and salinity. Extrapolation of these data to zero salinity gives an estimate of nutrient enrichment from freshwater origin. The lack of significant interannual fluctuations in the NO_3 / salinity relationship suggests that no major change in land use and human activities (industrialization, urbanization, agricultural practices), affecting NO_3 inputs, did occur during the last 8 years as seen for the river Rhine for the period 1961 to 1978 (Bennekom & Wetsteijn, 1990).

This series of empirical relationships evidences the impact, with a delay of 50 days, of rainfall conditions over the Scheldt catchment area on the nutrient environment of the coastal area. It is deduced, from these results that the huge *R. delicatula* development

observed in April 1994 (Fig. 3a) is sustained by riverine nutrient inputs driven by the abundant rainfall recorded 50 days before (Fig.4).

Discussion

The coastal waters of the North Sea are heavily enriched by nutrients of anthropogenic origin through the discharge of 7 major rivers (Lancelot *et al.*, 1987). These continental sources are deficient in DSi compared to N with respect to diatom requirements (Brzezinski, 1985; Lancelot, 1995). Long-term records of phytoplankton blooms in this area gives indication that the spring phytoplankton succession is characterized by the development of a first DSi-controlled diatom community followed by the massive outburst of *Phaeocystis* colonies sustained by NO_3 left over at the diatom bloom declines (Veldhuis *et al.*, 1986; Weisse *et al.*, 1986; Reid *et al.*, 1990). High resolution time series of phytoplankton and nutrient data recorded in Belgian coastal waters during two contrasting meteorological years 1993 and 1994 provide evidence that meteorological conditions, in particular rainfall, prevailing in the winter - early spring period could contribute to the regulation of the diatom - *Phaeocystis* succession in this coastal area.

Rainfall act on the quantity and quality of riverine nutrient inputs and determine the nutrient environment of coastal phytoplankton. In the Belgian coastal zone, a period of 50 days is necessary to detect the influence of rainfall on the salinity (Fig. 7b) and nutrient (Fig. 7c & 7d) signature. The qualitative changes in the winter nutrient status of coastal phytoplankton induced by changes in rainfall is evidenced by Fig. 8a relating the winter NO_3^- : DSi molar ratio to the 10-days averaged rainfall measured 50 days before. Winter NO_3^- : DSi is shown to exponentially decrease from 3 at rainfall lower than 2 $mm.d^{-1}$ to 1 at rainfall higher than 5 $mm.d^{-1}$. This dramatic lowering of NO_3^- : Si ratio is explained by a rainfall-driven modification of the relative contribution of diffuse (agricultural leaching) and point (industrial and urban

waste water) sources of nutrients (Billen *et al.*, 1990). High rainfall indeed increases the contribution of diffuse sources by increasing DSi leaching from rock minerals and NO_3 used as fertilizer (in average 70 % of NO_3 delivery to river system in North Western Europe catchment basin; Bennekom *et al.*, 1975). On the contrary, point sources releasing N and P, are not affected by rainfall conditions. Rainfall, thus, by acting preferentially on DSi delivery modifies the nutrient environment of the coastal zone. Assuming that NO_3 and DSi requirements of North coastal diatoms are in a ratio of 1.4 as determined from the early spring decrease of nutrients (Fig. 4), it can be savely concluded that persistent winter rainfall conditions less than 2 $mm.d^{-1}$ would disadvantage diatoms compared to *Phaeocystis* colonies while persistent high rainfall conditions could sustain the development of diatom blooms. Such an impact of rainfall on the relative proportion of the diatom and *Phaeocystis* colony spring bloom has been assessed by relating the magnitude of these blooms to the 3-month cumulative rainfall recorded over the Scheldt catchment basin with a delay of 50 days (Fig. 8b). Accordingly, a positive relationship exists between 3-months cumulative rainfall and the integrated diatom biomass over the whole spring bloom period (March, April and May). This suggests that, in absence of changes in nutrient sources from anthropogenic origin, diatom biomass is partly determined by rainfall prevailing during the late winter - spring. On the contrary, a highly negative relationship established between 3-months cumulative rainfall and the integrated *Phaeocystis* colony biomass (Fig. 8b). Contrary to diatoms which constitute a suitable source of food for meta- and protozooplankton, *Phaeocystis* colonies largely resist grazing pressure, most probably due to their large size (several mm) reached at their maximum development (Lancelot & Rousseau, 1994; Weisse *et al.*, 1994). Consequently, *Phaeocystis* colony bloom magnitude is better indicatory than diatoms to assess the effect of meteorological condition changes on phytoplankton community composition.

Conclusion

In the eutrophic coastal water of the North Sea, *Phaeocystis* colony blooms are reported to be controlled by the excess of nutrients from anthropogenic origin . This study, however shows that the magnitude of *Phaeocystis* colony blooms is controlled by the combined effect of land use and rainfall conditions acting on the early development of diatoms. The relative importance of diatom and *Phaeocystis*, without altering their succession, is driven by nutrient quality discharged into coastal area, under control of combined effects of anthropogenic activities and meteorological conditions.

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Figure captions

Figure 1: Map of the continental coastal waters of the North Sea. Station 330 in the Belgian coastal waters is indicated as well as the different stations sampled from the French coastal zone to the German Bight. Arrows indicate the residual circulation.

⩙ indicates the limit of the North European rivers watershed.

Figure 2: Seasonal changes in Chl a at station 330 during 1993 and 1994.

Figure 3: Seasonal changes in a) Diatom biomass ; b) *Phaeocystis* colony biomass and c) NO_3 and DSi concentrations at station 330 during 1993 and 1994.

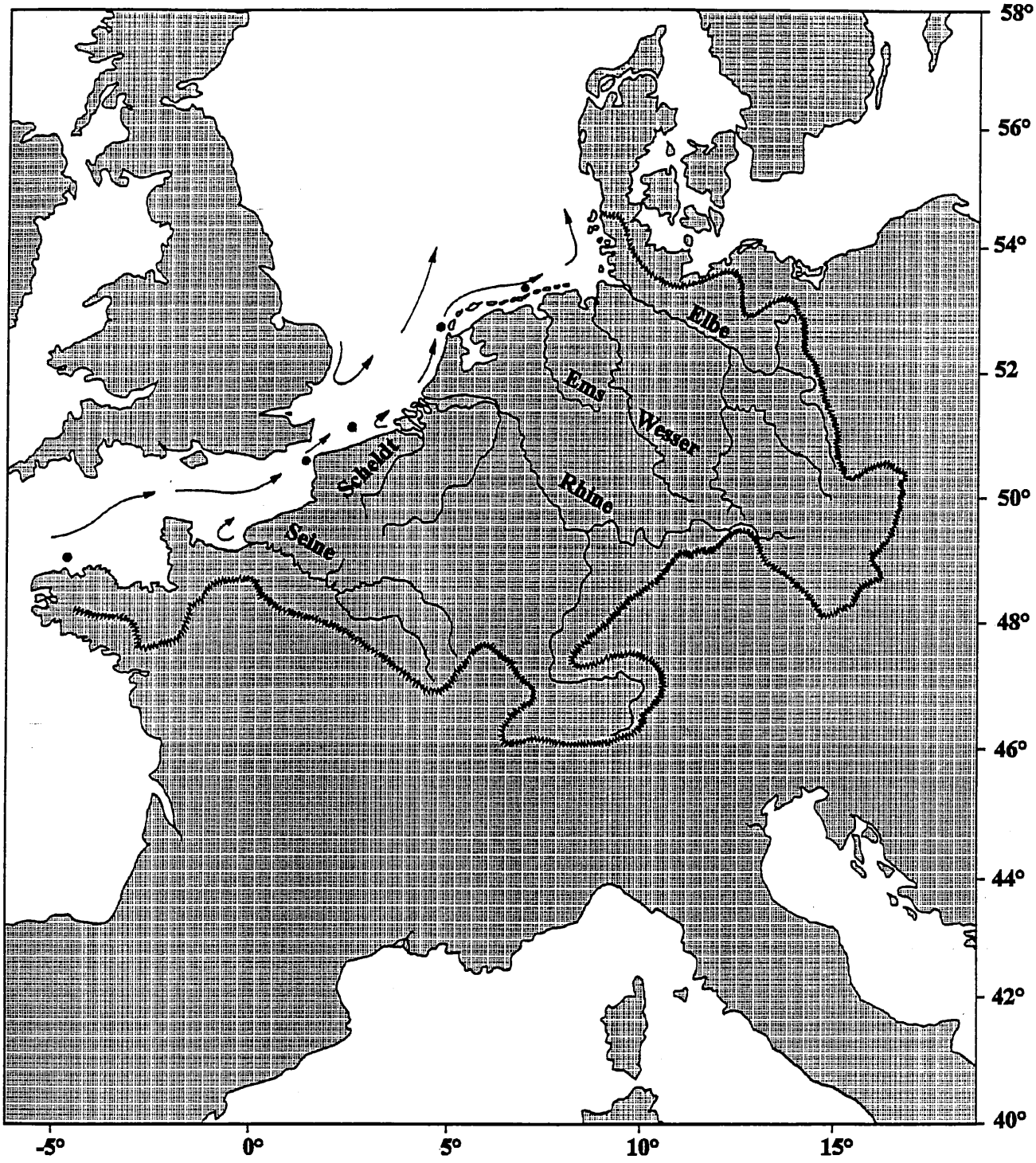
Figure 4: NO_3 *versus* DSi concentrations during the diatom early spring development in March 1993 and 1994.

Figure 5: Relationship between maximum *Phaeocystis* colonial cell density and ambient NO_3 at the bloom onset for the different stations sampled along the coast of the Sothern Bight of the North Sea. Data 1988-1994

Figure 6: Seasonal changes in monthly rainfall over Scheldt watershed (columns) compared to monthly seasonal normal *i. e.* mean calculated for the last 35 years (line).

Figure 7: Relationships between a) Monthly rainfall over Scheldt watershed and monthly average Scheldt flow rate measured during cold and rainy months; b) 10-days average Scheldt flow rate and salinity recorded at station 330 with a delay of 50 days; c) winter NO_3 concentrations and salinity at station 330; d) winter DSi concentrations and salinity at station 330. Data 1988 - 1994.

Figure 8: Relationship between a) winter NO3 : DSi molar ratio and 10-day average rainfall recorded 50 days before; b) Diatom and *Phaeocystis* biomass integrated on the blooming spring period (March to May) and 3-month cumulative rainfall on the Scheldt watershed occurring with a delay of 50 days.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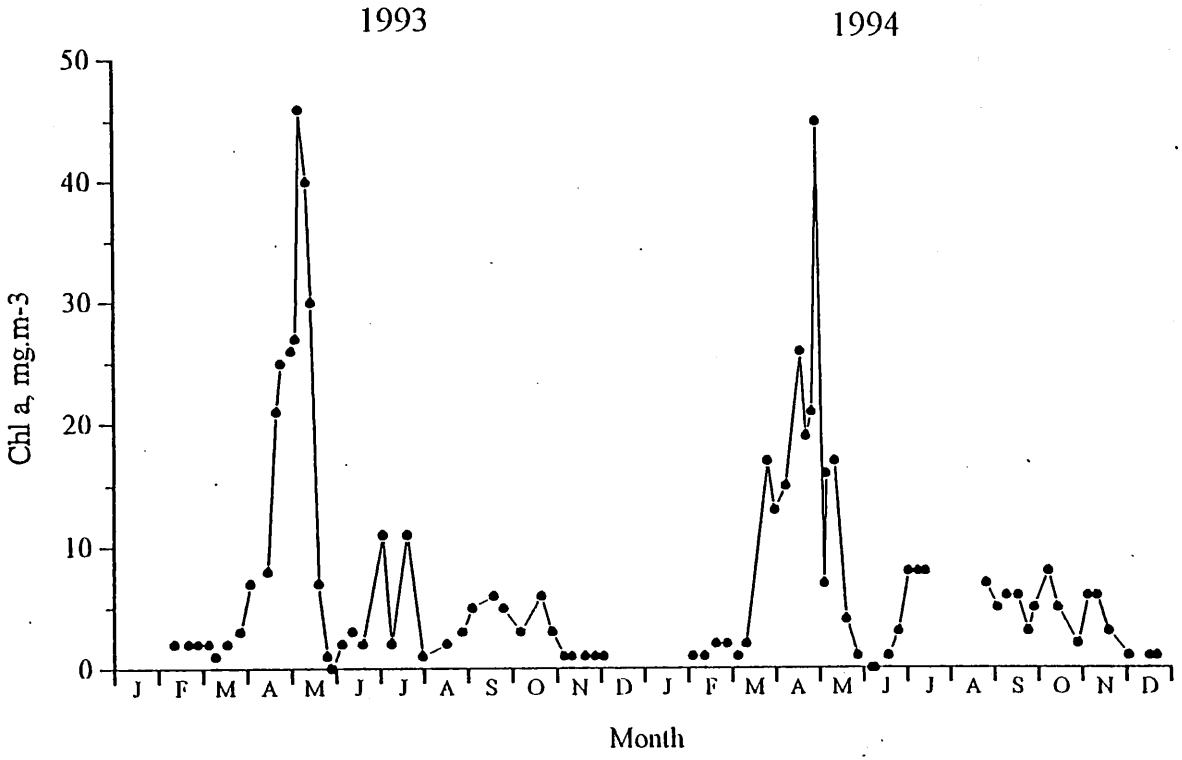
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Month

Fig 2

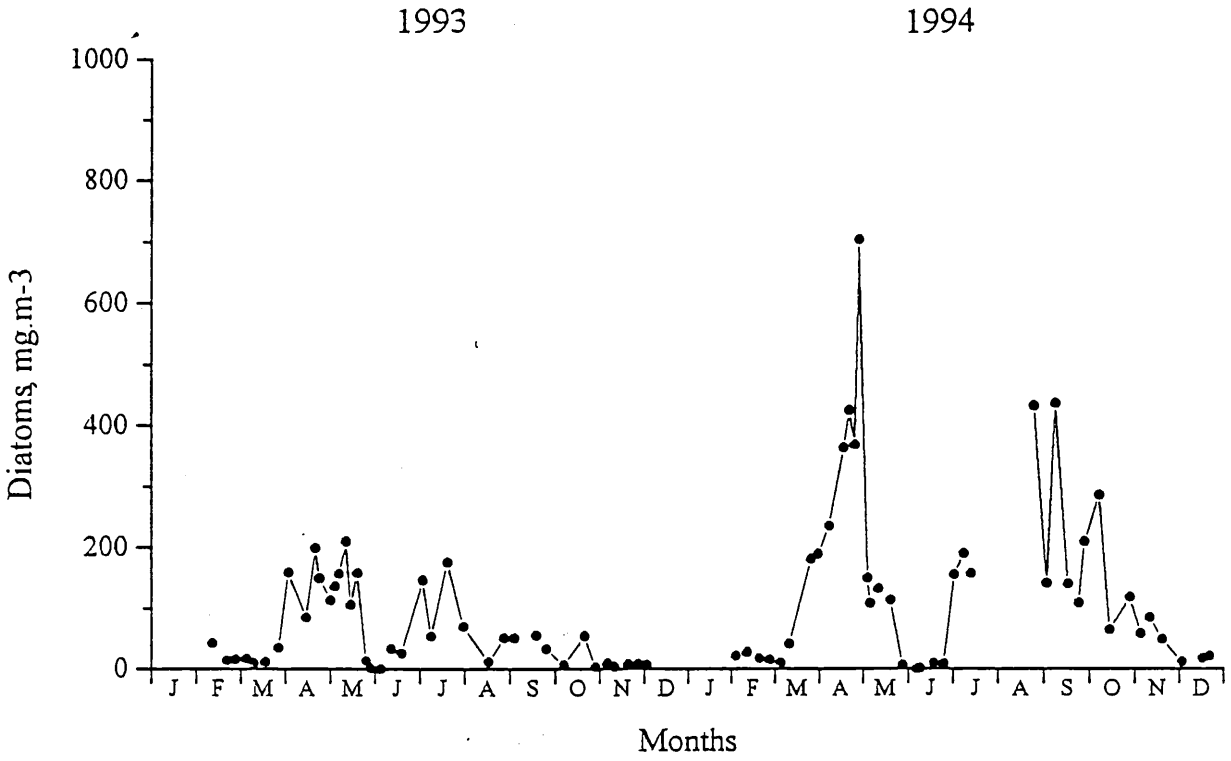

1993
1994
1000
800
600
400
200
0
Diatoms, mg.m-3
J F M A M J J A S O N D J F M A M J J A S O N D
Months

fig 3a

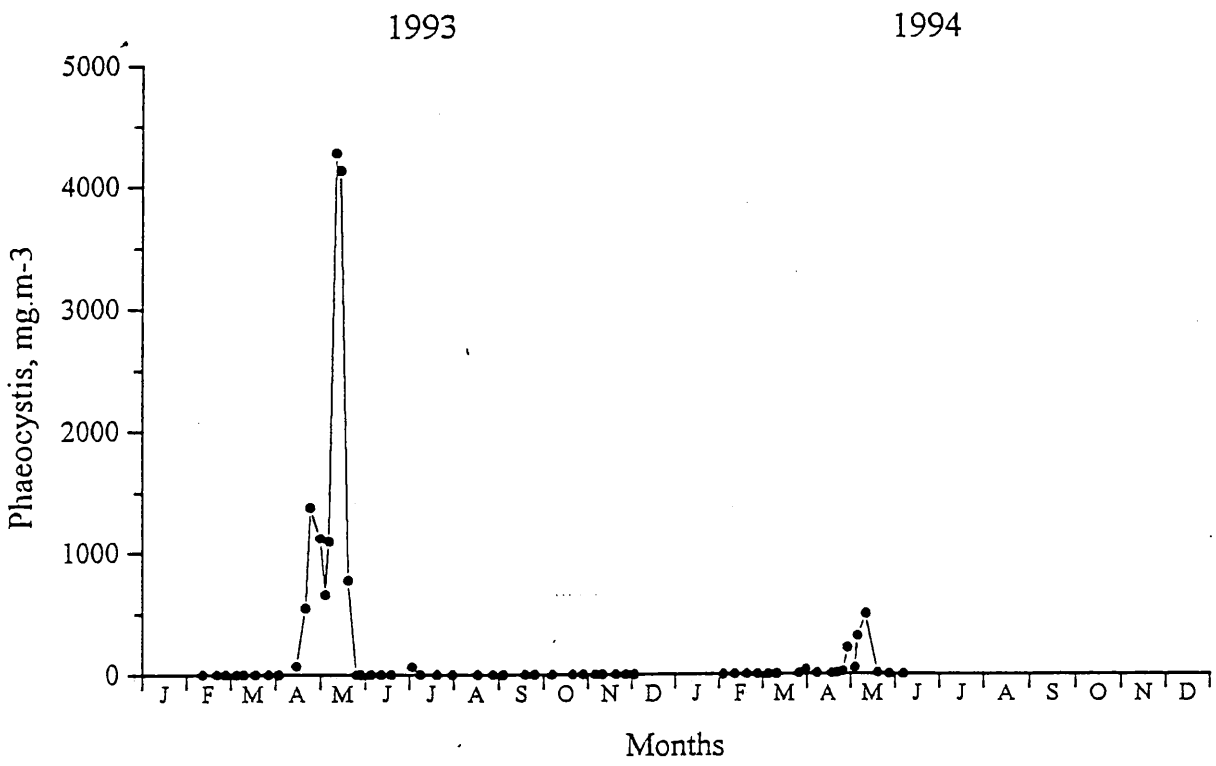

1993
1994
5000
4000
3000
2000
1000
0
Phaeocystis, mg.m-3
J F M A M J J A S O N D J F M A M J J A S O N D
Months

Fig 3b

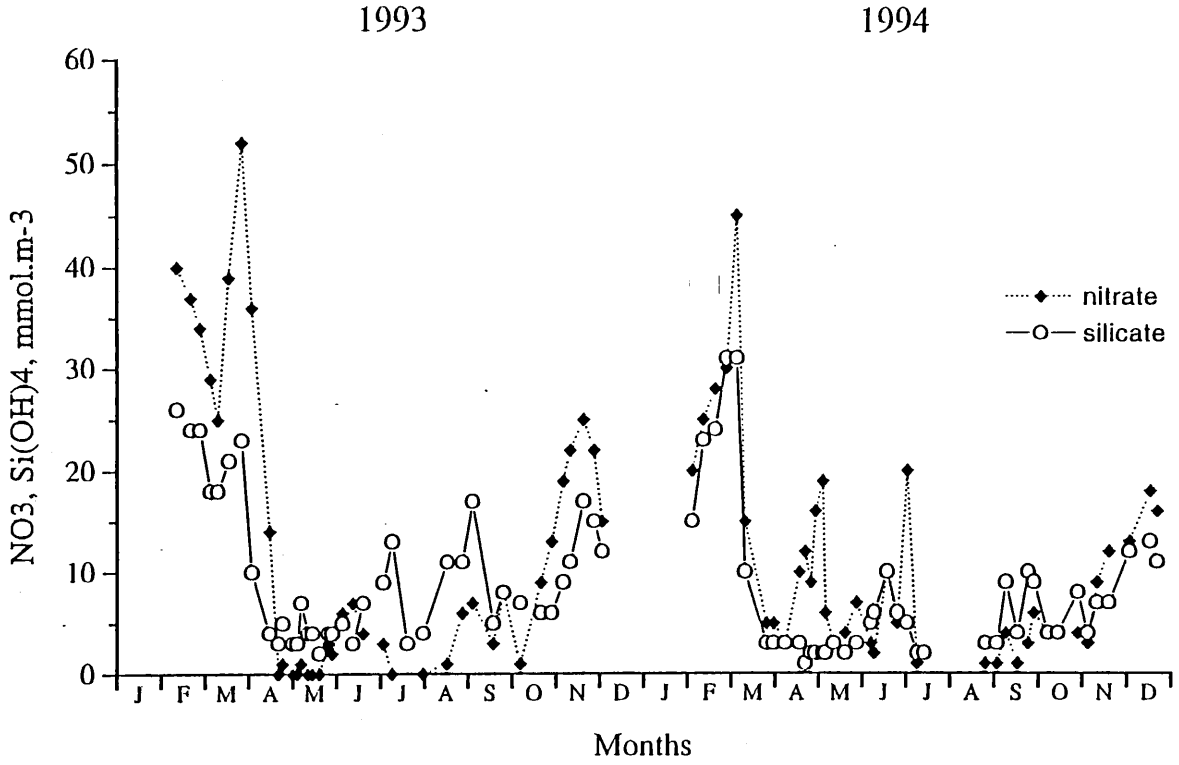

1993
1994
60
50
40
30
20
10
0
NO3, Si(OH)4, mmol.m-3
nitrate
silicate
J F M A M J J A S O N D J F M A M J J A S O N D
Months

fig 2c

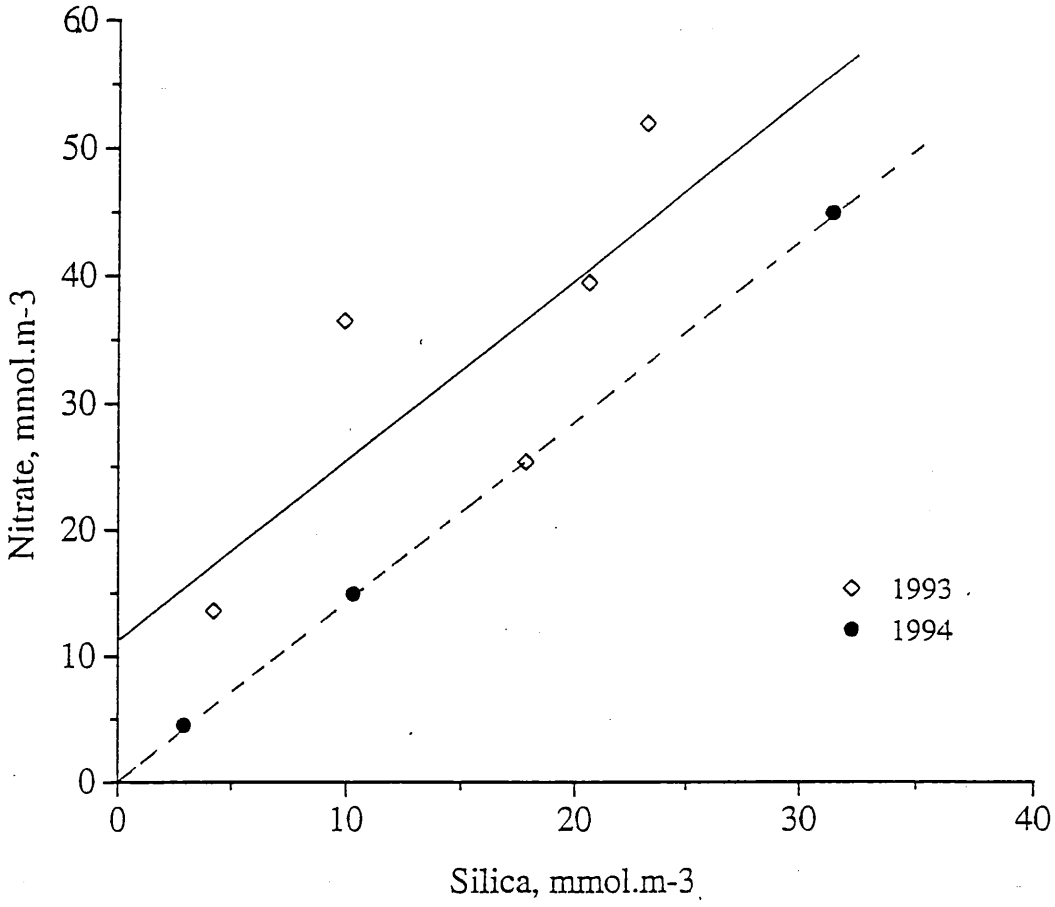
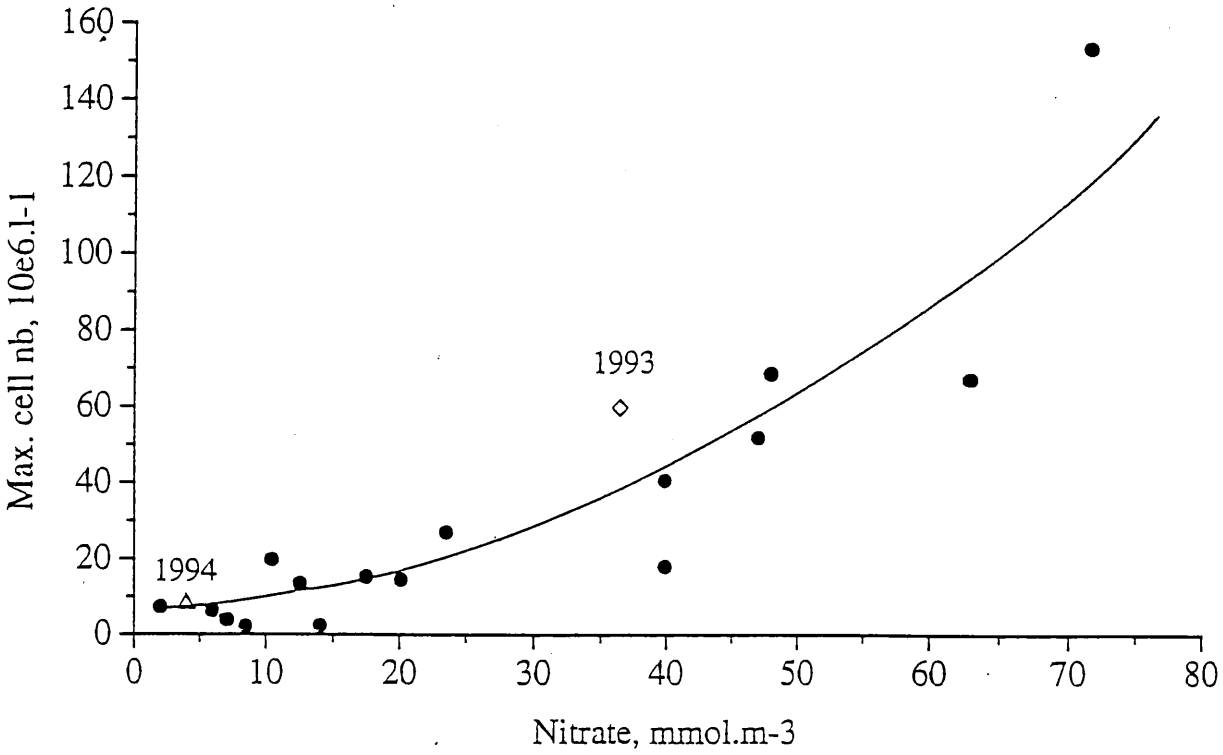

60
50
40
30
20
10
0
Nitrate, mmol.m-3
0
10
20
30
40
Silica, mmol.m-3
1993
1994

Fig 4


Max. cell nb, 10e6.l-1
160
140
120
100
80
60
40
20
0
1993
1994
0
10
20
30
40
50
60
70
80
Nitrate, mmol.m-3

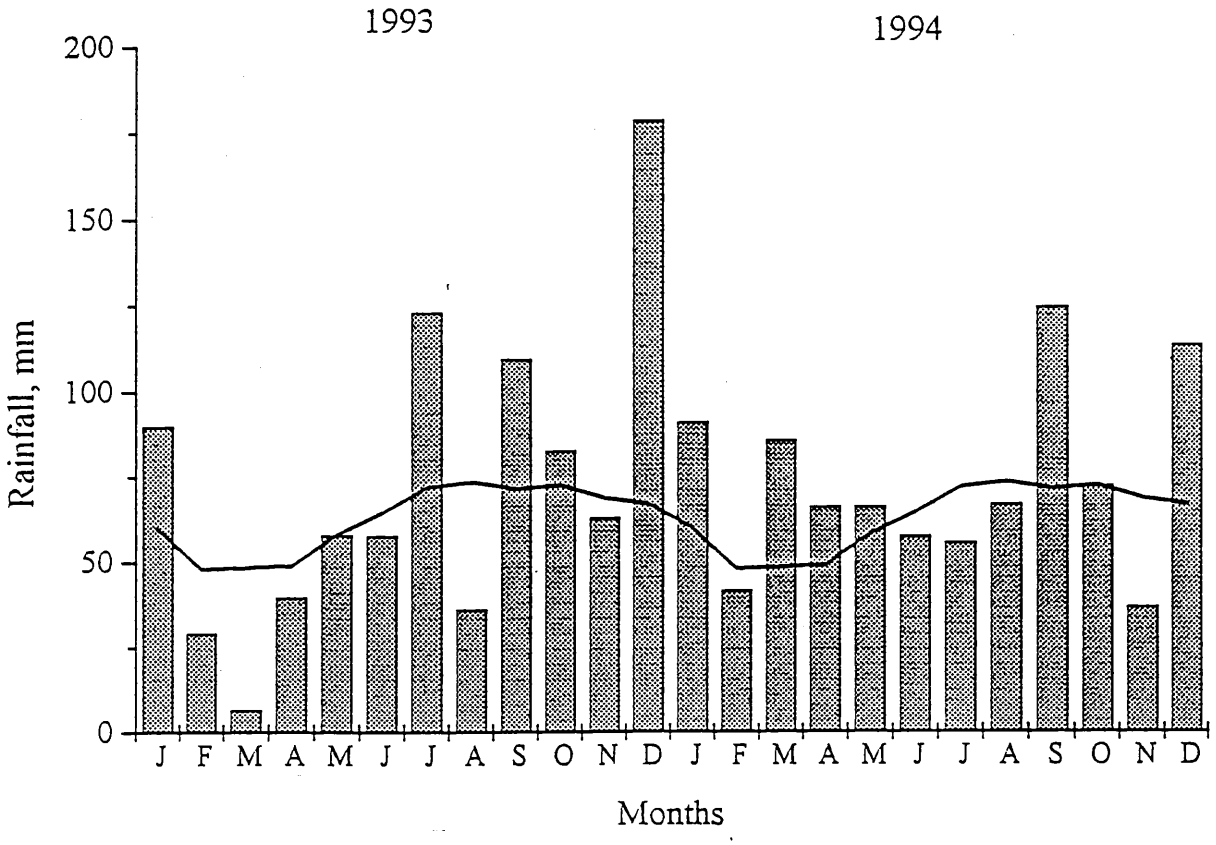

1993
1994
200
150
100
50
0
Rainfall, mm
J F M A M J J A S O N D J F M A M J J A S O N D
Months

Fig 6

a

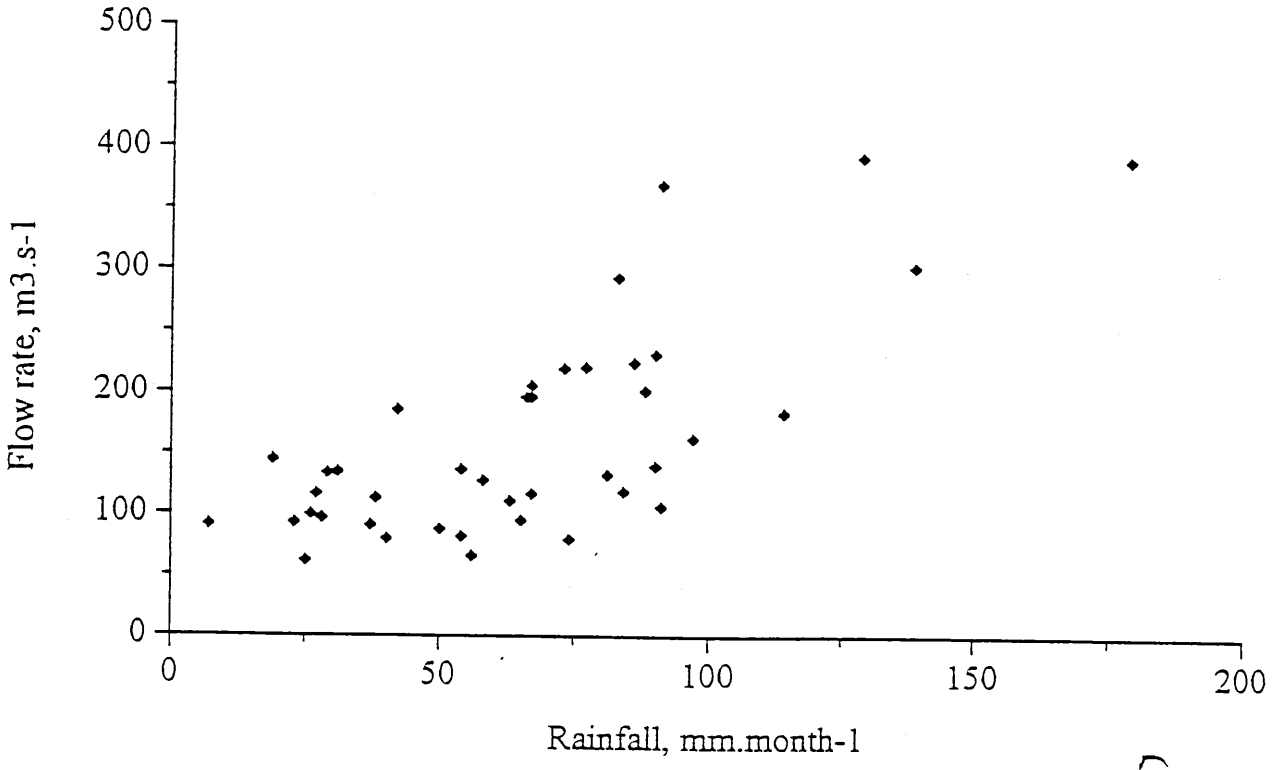


Fig 7a

b

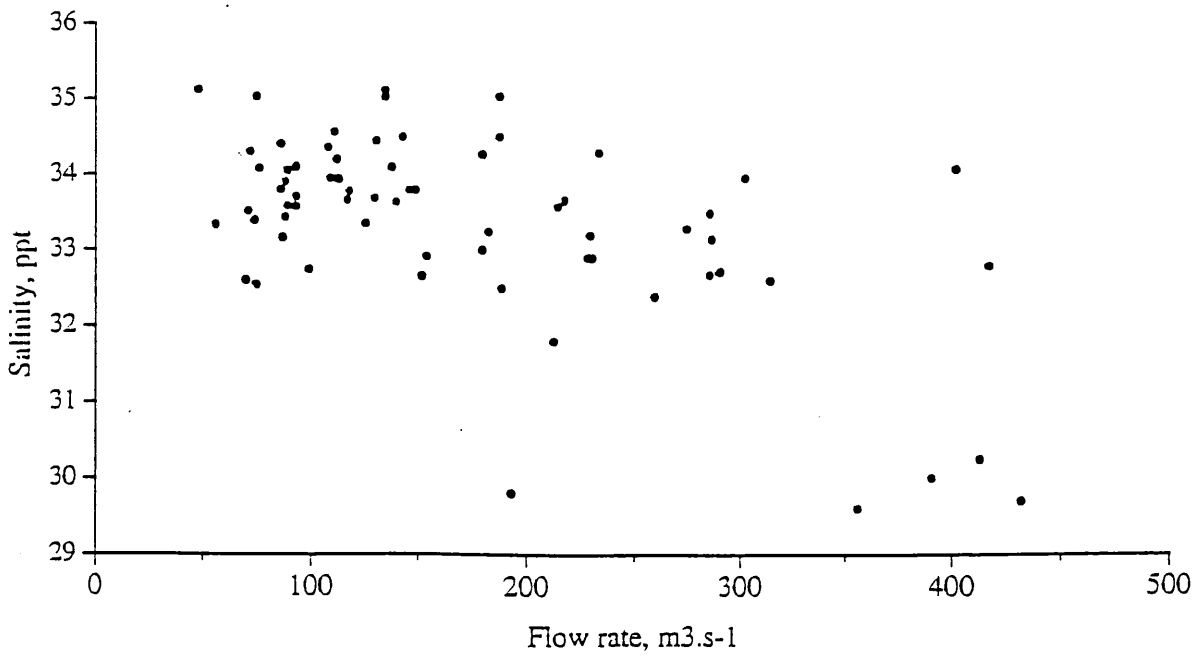


Fig 7b

c

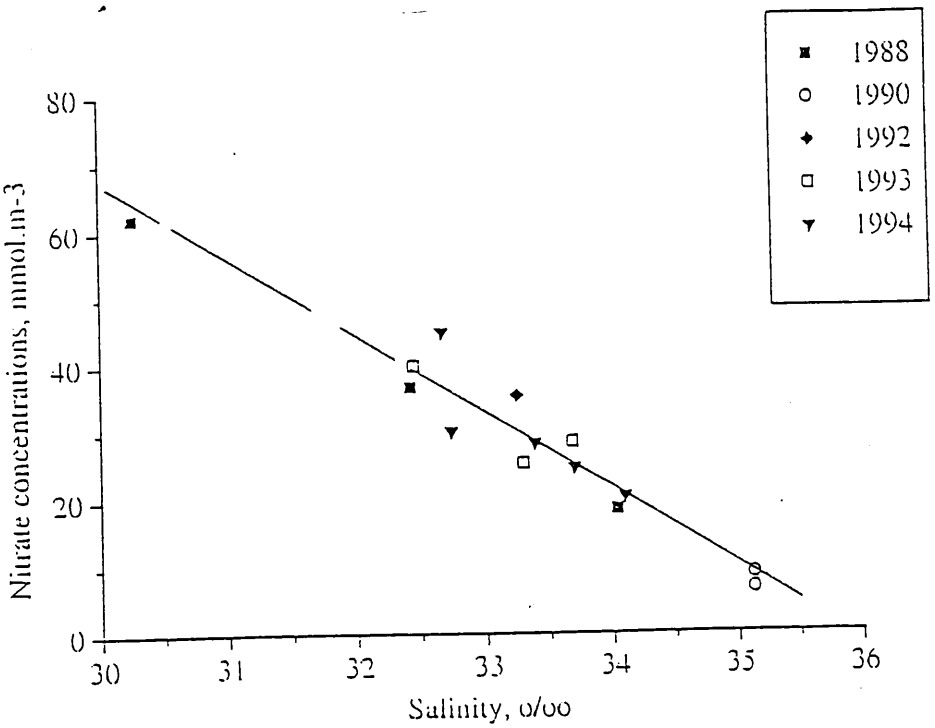


Fig 7c

d.

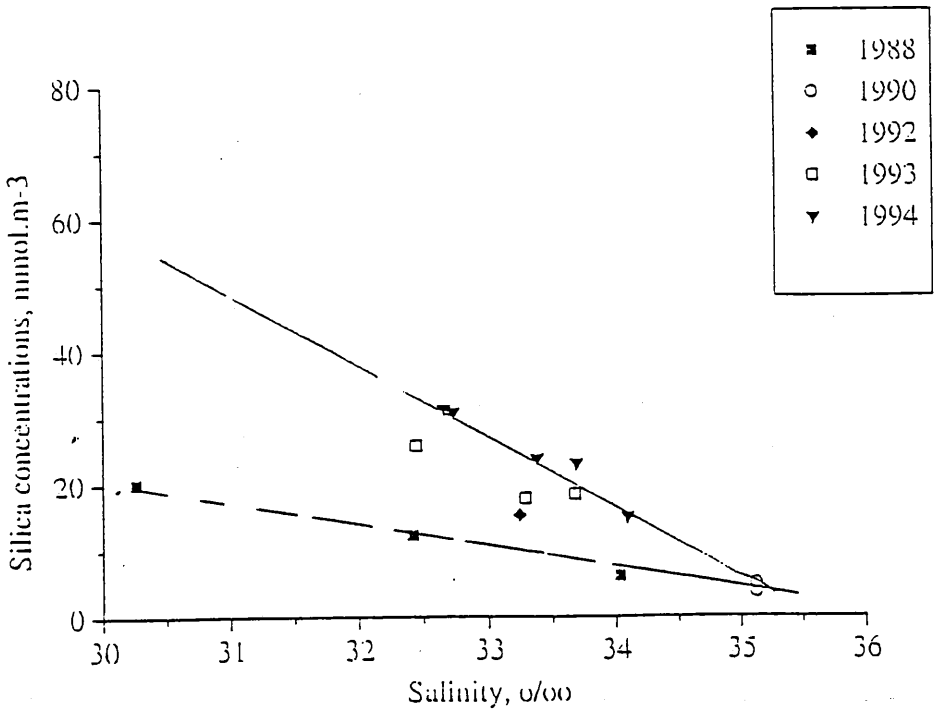
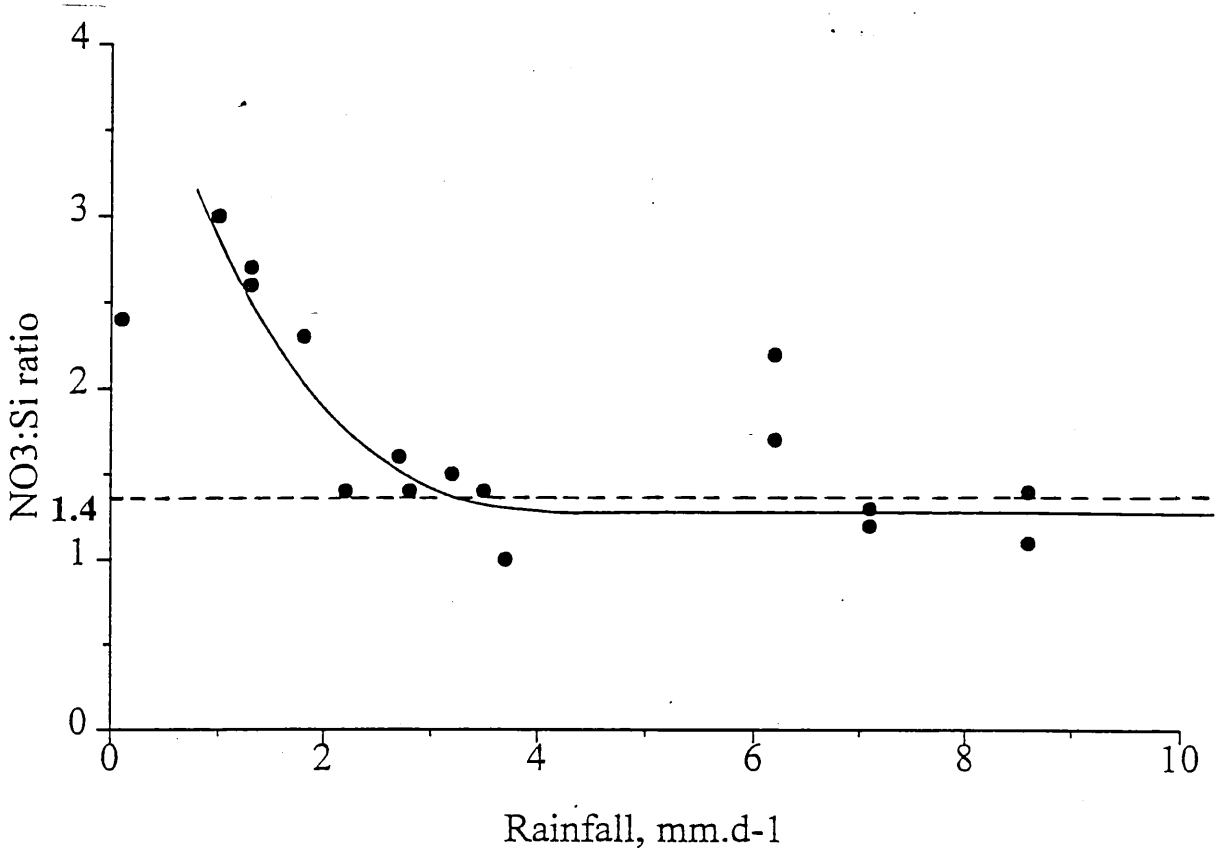


Fig 7d

a


NO3:Si ratio
1.4
Rainfall, mm.d-1

b.

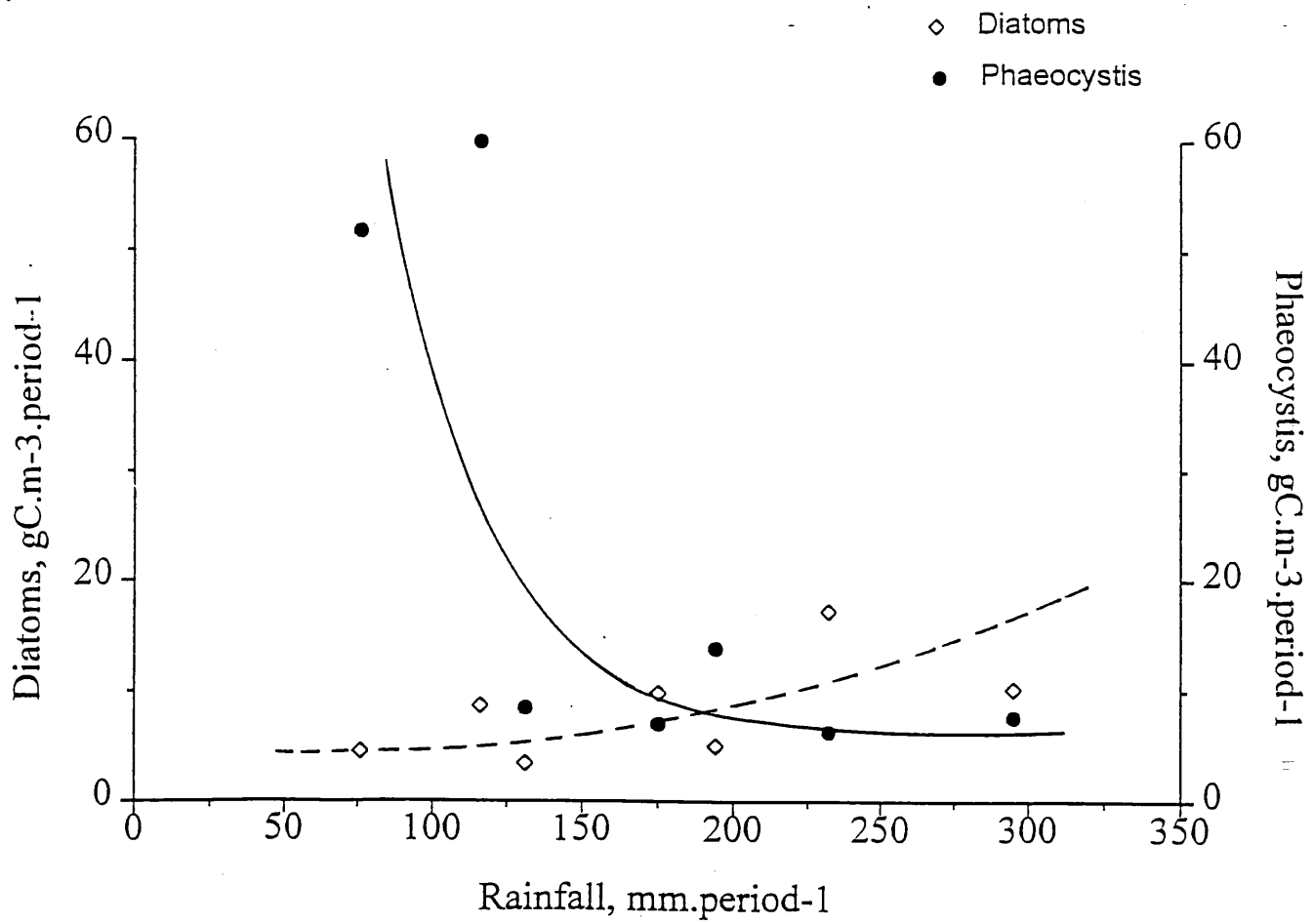

Diatoms
Phaeocystis
Diatoms, gC.m-3.period-1
Phaeocystis, gC.m-3.period-1
Rainfall, mm.period-1

fig 8.a, b

Manuscript 2

Major and comparable roles for free-living and attached bacteria in the degradation of *Phaeocystis*-derived organic matter in Belgian coastal waters of the North Sea.

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ABSTRACT: Microbial degradation of *Phaeocystis* colonies and their derived organic matter by free-living and attached bacteria was investigated in the Belgian coastal waters during the spring development diatom - *Phaeocystis* colonies in 1994. Results obtained show concomitant evolution of hydrolytic ectoprotease and β-ectoglucosidase ectoenzymatic activities with respect to the phytoplankton bloom, suggesting that the low biodegradability of *Phaeocystis* colonies leading to transient accumulations of *Phaeocystis* derived material in the coastal North Sea was not due to a lag phase required for the induction of β-ectoglucosidase. Up to 66 % of total bacterial biomass was found attached to particles larger than 10 μm. While occurring always in low abundance compared to free-living bacteria, both the average specific biomass and growth rate of particle-attached bacteria were very high i.e. 60 fgC $cell^{-1}$ and 0.28 h^{-1}, respectively. Similarly specific ectoenzymatic activities of particle-attached bacteria were on average about 5 times higher than those characterising free-living bacteria. Budget calculations show a 53 % contribution of *Phaeocystis*-attached bacteria to the mineralisation of *Phaeocystis*-associated production i.e. a 53%:47% role for attached and free-living bacteria, respectively.

KEY WORDS: *Phaeocystis* degradation · Free-living and particle-attached bacteria · Ectoenzymatic activity · Growth rate.

INTRODUCTION

The explosive blooms of large *Phaeocystis globosa* colonies are a common spring event in the eutrophic continental coastal waters of the North Sea (Lancelot *et al.* 1987, Lancelot & Rousseau 1994). The successful development of *Phaeocystis* colonies in nitrate-rich marine environments (Smith 1993; Lancelot & Rousseau 1994) has been attributed to the high ability of the colony form to take advantage of large concentrations of ambient nitrate (Riegman *et al.* 1992), the resistance against mortality by metazooplankton grazing pressure (Weisse *et al.* 1994) and sedimentation due to gelling properties of their mucilaginous matrix (Lancelot & Rousseau 1994). However, little is known about the mechanisms driving the sudden termination of *Phaeocystis* blooms usually observed in *Phaeocystis*-dominated environments (Wassmann 1994). Three mechanisms possibly determine the fate of *Phaeocystis* biomass: colony disintegration producing large amounts of dissolved organic matter (DOM) and *Phaeocystis* cells, aggregation and mass sedimentation.

Colony disruption and cell autolysis, the latter under control of nutrient depletion at *Phaeocystis* maximum density, have been suggested as the main factor controlling *Phaeocystis* decline (van Boeckel *et al.* 1992). *Phaeocystis* colony disintegration produces free-living cells and DOM in the surrounding water. Released *Phaeocystis* cells are likely maintained at low density due to intense protozoans grazing pressure (Weisse & Scheffel- Möser 1990). On the other hand, DOM derived from colony disruption, exudation and autolysis constitutes substrates to support bacterial growth. However, due to the large contribution to this pool of the *Phaeocystis* colony matrix (up to 90 % of *Phaeocystis* derived organic matter; Rousseau *et al.* 1990) which is an homogeneous polysaccharide composed of different monosaccharides (Thingstad & Billen 1994, Janse *et al.* 1996), *Phaeocystis*

derived DOM is likely deficient in N and P with respect to bacterial needs (Eberlein *et al.* 1985, Thingstad & Billen 1994). Accordingly, transient accumulation of DOM (Eberlein *et al.* 1985, Billen & Fontigny 1987) and foam (Lancelot & Rousseau 1994) has been ascribed to nutrient limitation observed at the senescent phase of *Phaeocystis* blooms rather than to a poor biodegradability of this material.

Current knowledge of the aggregation potential of *Phaeocystis* colonies during their development and contribution to vertical flux is still limited (Riebesell 1993, Rousseau *et al.* 1994, Lancelot & Rousseau 1994). The mucilaginous nature of the colony matrix should confer high surface stickiness to *Phaeocystis* colonies, therefore enhancing rapid aggregation and subsequent sedimentation. In contrast, recent field (Riebesell 1993) and laboratory controlled experiments (Passow & Wassmann 1994) suggest that compared to diatoms *Phaeocystis* colonies largely resist aggregation with other suspended living and non-living particles and do not contribute significantly to the vertical flux of particles (Riebesell *et al.* 1995). Individual large senescent *Phaeocystis* colonies observed in both temperate and polar waters (Verity *et al.* 1988, Thingstad & Martinussen 1991, Rousseau *et al.* 1994, Putt *et al.* 1994) were rapidly colonized by dead and living auto-and heterotrophic micro-organisms.

To what extent do attached bacteria contribute to the degradation and trophic fate of *Phaeocystis*-derived material is not known. Current knowledge on the dynamics of attached and free-living bacteria indicates that by colonizing phytodetritus bacteria give rise to highly dynamic micro-environments which form and transform through microbial processes. These microaggregates (phytoplankton colonized by microorganisms) in turn actively interact with free-living bacteria and particle feeders by providing them with labile substrates (Azam & Cho 1987, Herndl 1988) and particles with high nutritional value (Chröst 1991).

Despite the presence of attached bacteria on senescent *Phaeocystis* colonies, information is lacking about the growth characteristics of *Phaeocystis*-attached bacteria and their contribution to the degradation of *Phaeocystis*-derived material. Yet the question is of prime ecological and biogeochemical importance: a close association between *Phaeocystis* colonies and bacteria enhances remineralization in the water column, increases the transfer of organic matter to higher trophic levels, and thus reduces the flux of organic matter to the sediment.

This work specifically addresses the question of microbial degradation of *Phaeocystis*-derived material and its contribution to *Phaeocystis* bloom decline. It reports first field evidence of the significant contribution of attached bacteria in the degradation process of *Phaeocystis*-derived material. The study was conducted in spring 1994 in the Belgian coastal waters of the North sea where *Phaeocystis* colony blooms are observed each year in spring. The dynamics of free-living versus particle-attached bacteria (biomass and number; ectoenzymatic activity; growth rate) was investigated at a fixed station in the Belgian coastal waters during a seasonal cycle.

MATERIALS AND METHODS

Study site. Sampling was carried out at station 330 (Lat. 51°26.05 N, Long. 2°48.50 E), *Phaeocystis*-reference station in the Belgian coastal waters, regularly monitored since 1988. This site is under influence of the Scheldt river with salinity ranging between 30 ‰ and 35 ‰. The average depth is 22 m and the water column is permanently well mixed resulting from very strong tidal currents. Sampling covered the period from February till end of June 1994. Sampling frequency was daily to weekly during spring and monthly during winter and summer. All water samples were carefully collected at the surface with a bucket, in order to avoid damaging or disrupting *Phaeocystis* colonies.

Phytoplankton and aggregate biomass. Phytoplankton biomass (diatoms, *Phaeocystis* free-living cells and colonies) was calculated on basis of number and biovolume determination under an inverted microscope (Leitz Fluovert) according to the Utermöhl method (Hasle 1969). Ten to 100 ml samples were preserved with acid lugol solution (1 % final concentration) and allowed to settle for 12 h in counting chambers (Hydrobios). A maximum of 500 cells was counted in total, with at least 100 cells of the most abundant genus or species. Diatoms were enumerated at a magnification of 100x or 200x. Diatom carbon biomass was calculated for each species or genus using a specific average cellular biovolume. The latter was established from biovolumes measured on one population along the whole period of its development. Biovolumes were then converted using a carbon content factor of 0.11 $pgC.\mu m^{-3}$ of plasma volume as recommended by Edler (1979) for diatoms. *Phaeocystis* colony and *Phaeocystis*-derived aggregate enumeration and biomass determination were performed according to the method described in Rousseau *et al.* (1990). *Phaeocystis*-derived aggregates were defined as senescent *Phaeocystis* colonies invaded by auto-and/or heterotrophic microorganisms. Estimates of the

carbon content of *Phaeocystis*-derived aggregates do not include the biomasses of microorganisms attached to the aggregates.

Phytoplanktonic production. Daily primary production rates were calculated from phytoplankton biomass, nutrient concentrations, light availability, ambient temperature using the AQUAPHY set of equations of Lancelot *et al.* (1991) supported by experimentally determined photosynthesis and growth parameters for the diatoms and *Phaeocystis* communities.

Bacterial number and biomass. Water samples were preserved with formaldehyde (2 % final concentration). A 10 µm-pore size Nuclepore membrane was used to filter 5 to 10 ml of sample in order to separate free-living bacteria from bacteria attached to *Phaeocystis* colonies and its derived particles. Accordingly, 10 µm is the minimum size of recorded *Phaeocystis* colonies (Rousseau *et al.* 1990). Numbers and biomasses of *Phaeocystis*-attached bacteria were estimated directly over *Phaeocystis* colonies or *Phaeocystis* derived aggregates. Possible overestimation of attached bacteria due to scavenging of the free-living bacteria over *Phaeocystis* colonies during the filtration was considered. Ten µm prefiltered samples (1 ml aliquots) were filtered through 0.2 µm Nuclepore membrane to estimate abundance and biomass of free-living bacteria.

Bacteria numbers and sizes were determined by epifluorescence microscopy after DAPI staining following the procedure of Porter and Feig (1980). Bacteria were measured and classified according to shape and size allowing calculation of biovolume for each size and shape (Garnier *et al.* 1992). Biomass was estimated from abundance and biovolume distribution using the carbon/biovolume factor depending on biovolume as proposed by Simon and Azam (1989).

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Bacterial production. Bacterial production was estimated by incorporation of ^{3}H-leucine into bacterial proteins (Kirchman *et al.* 1985, Simon & Azam 1989) and by incorporation of ^{3}H-thymidine into bacterial DNA (Fuhrman & Azam 1982). Reverse filtration as proposed by Dodson and Thomas (1978) using 10 µm Nuclepore membrane was chosen to distinguish between the free-living bacteria and the total bacterial community. The radioactivity incorporated by the total bacterial community was measured on the unfiltered water sample. The radioactivity incorporated by free-living bacteria was measured in the filtrate. The radioactivity incorporated by attached bacteria was then estimated by difference. The procedures were as follows:

Incorporation of ^{3}H-leucine was measured at four concentrations of L-(3,4,5 - ^{3}H(N))-leucine (Amersham, 120-140 Ci mmole^{-1}) between 2 and 77 nM (2 nM ^{3}H-leucine in each experiment but enriched with 0 to 75 nM non-radioactive leucine). For each experiment, two times 20 ml of spiked sample were incubated during 30 min in the dark at *in situ* temperature. Incubation was stopped by adding formaldehyde (final concentration 1 %) and reverse filtration was applied on one set of incubation flasks. Radioactivity incorporated into protein of the unfiltered community and of 10 µm-prefiltered sample was measured after protein precipitation. Protein was precipitated with cold (0°C) trichloroacetic acid (TCA) (5 % final concentration) and after heating at 85 °C for 30 min. Reaction was stopped by cooling and precipitated protein was collected by filtration on 0.2 µm pore size cellulose acetate membrane (Sartorius). Radioactivity of precipitated protein was measured with a Packard Tri-Carb scintillation counter. The incorporation rate was estimated as described in Servais (1990).

Incorporation of ^{3}H-thymidine was measured at 20 nM of methyl-(^{3}H)-thymidine (Amersham 40-50 Ci mmol^{-1}). Two times 20 ml sample were incubated in the presence of ^{3}H-thymidine for 60 min (Servais 1992). The incubation was stopped by

adding formaldehyde (final concentration 1 %). The same post-incubation procedure described for leucine incorporation was applied to distinguish radiotracer incorporation by free-living bacteria from total bacteria. Samples were then filtered on 0.2 μm pore size cellulose acetate membrane (Sartorius) and macromolecules were precipitated by adding three times 5 ml ice cold TCA (10 %). Radioactivity associated with the filters was measured with a Packard Tri-Carb scintillation counter.

Leucine and thymidine incorporation were converted into bacterial production using conversion factors of Servais (1990), established for the North Sea bacterial communities i.e. 3950 gC produced per mole of leucine incorporated into proteins and 2.66×10^{18} bacteria produced per mole of thymidine incorporated in the cold TCA insoluble material. The conversion factors for attached bacteria are assumed to be the same as those estimated for free-living bacteria.

Ectoenzymatic activity. Potential ectoenzymatic activity was measured at 20 °C after adding to the sample a saturating concentration of artificial substrates that produce fluorescent products when hydrolysed by ectoenzymes present in the sample. Two ectoenzymatic activities were tested: ectoprotease and ecto-β-glucosidase. The former was measured because it has been suggested of being a constitutive property of bacteria in aquatic environments (Billen 1991), the latter as an indicator of the degradation of *Phaeocystis*-colony-derived material degradation, through the specific cleavage of β-glucoside links characteristic of the *Phaeocystis* gel-producing polysaccharides (Thingstad & Billen 1994, Lancelot & Rousseau 1994). Activity of free-living bacteria was distinguished from that of particles-attached bacteria by measurement in unfiltered samples and comparing with the activity after reverse filtration through 10 μm pore size membranes (Nuclepore).

Ectoprotease activity: L-leucyl-2β-naphthylamide hydrochloride (LLβN, Sigma, St. Louise, USA) was used as substrate for proteolytic ectoenzymes. It produces fluorescent naphtylamine after hydrolysis of the peptide-like bond. The experimental procedure was that of Somville and Billen (1983). Two ml water samples were transferred into a sterile quartz fluorimeter cell kept at 20 °C; 50 µl of a sterile 40 mM solution (1000 µM final concentration) of L-leucyl-2β-naphthylamide was added, and the increase of fluorescence at 410 nm under 340 nm excitation (KONTRON SFM 25 fluorimeter) was measured as a function of time over 25-50 min. Enzyme activity was estimated from the initial slope. Fluorescence of a standard naphthylamine solution was used for calibration

Ectoglucosidase activity: 4-methylumbelliferyl-β-glucoside (MUF-GLU, Sigma, St. Louis, USA) was used as substrate for β-glucosidase, which produces fluorescent 4-methylumbelliferone after hydrolysis of β-linked (1-2, 1-3, 1-4, 1-6) disaccharides of glucose, celluhexose, and carboxymethylcellulose (Barman 1969). The procedure was adapted from the protocol of Hoppe *et al.* (1983) and Somville (1984). Two ml water samples were transferred into a sterile quartz fluorimeter cells kept at about 20 °C; 250 µl of a sterile 6 mM solution of 4-methylumbelliferyl-dβ-glucoside was added. The increase of fluorescence at 445 nm under 360 nm excitation was measured as a function of time over 25-50 min. Enzyme activity was estimated from the initial slope. Fluorescence of a standard 4-Methylumbelliferone solution was used for calibration.

RESULTS

Phaeocystis colonies and *Phaeocystis* -derived aggregates

Phaeocystis colonies bloomed in late April-early May 1994, at our station 330 in the Belgian coastal waters (Fig. 1A). The species observed in the North Sea was *Phaeocystis globosa* (Baumann *et al.* 1994). The maximum colony biomass, 500 mgC m^{-3}, was unexpectedly low compared to the previous years (Lancelot *et al.* 1991) or the early spring diatom bloom (Fig. 1A). The successful development of the spring diatom bloom has been explained by the extremely high rainfall in the Scheldt river watershed in late winter 1994 (Rousseau and Lancelot 1996, Lancelot et al. 1997). *Phaeocystis*-derived aggregates appeared in the water column shortly after the appearance of *Phaeocystis* colonies and increased in biomass concomitantly (Fig. 1B). However the aggregates carbon remained lower than the carbon of *Phaeocystis* colonies, representing a maximum of 20 % of total *Phaeocystis* carbon (Fig. 1B). Both *Phaeocystis*-derived aggregates and colonies disappeared from the water column in mid-May.

Bacterial abundance and biomass

Spring variations of abundance and biomass of free-living bacteria and bacteria attached to *Phaeocystis* colonies or derived aggregates are illustrated in figure 2A.

Particle-attached bacteria were recorded at the time of occurrence of *Phaeocystis*-derived aggregates (Fig. 1B & 2A) but were always less abundant than free-living bacteria (Fig. 2A). In contrast, maximum biomass reached by both bacterial communities was equivalent, around 60 mgC m^{-3} (Fig. 2C). At the peak of the bloom, 12 to 66 % of total bacterial biomass was attached to *Phaeocystis* colonies or derived aggregates (Fig. 2C). This discrepancy between bacterial density and biomass

suggests a marked difference between the specific biomass of the two bacterial communities. The specific biomass of free-living bacteria (15 fgC $cell^{-1}$) was low compared to attached bacteria (15-80 fgC $cell^{-1}$) and displayed little seasonal variation (Fig. 2B). The specific biomass of attached bacteria increased along with its growth, reaching more than 5 times the specific biomass characteristic of free-living bacteria, at their maximum development.

As noted by Billen (1990), a delay of 10 days was observed between the occurrence of *Phaeocystis* maximum development and the development of free-living bacteria. In contrast, maximum biomass reached by attached bacteria coincided with maximum *Phaeocystis* development.

Bacterial production

Biomass and cell production by attached bacteria comprised 10-73 % and 2-68 % of total bacterial community, respectively (Fig. 3 A & B). As observed for biomass, maximum production rates by attached bacteria were observed earlier in the season compared to free-living bacteria. Biomass and cell production rates of attached bacteria did not display concomitant seasonal variations because of the large range of biovolume reached by attached bacteria. More interesting was the marked difference in specific growth rate displayed by the two bacterial communities (Table 1). Average specific growth rate of attached bacteria (0.28 h^{-1}) was one order of magnitude higher than that of free-living bacteria (0.018 h^{-1}).

Ectoenzymatic activity

The seasonal evolution of potential ectoenzymatic activities was closely related to phytoplankton and bacterioplankton bloom development with maxima in April-May (Fig. 4 A & B). Attached bacteria contributed to 20-60 % of total ectoprotease and 10-50 % of ecto-β-glucosidase. Ecto-β-glucosidase activity was generally one order of magnitude lower than ectoprotease activity (Fig. 4 A & B). Both enzymatic activities, however, varied concomitantly as reflected in the significant correlation between them observed for both attached and free-living bacteria (Fig. 5). The slopes of the regression lines differ, however, by a factor two between the two bacterial communities with free-living bacteria expressing higher β-glucosidase compared to ectoprotease. This difference is somewhat reflected in the ratio of specific activities (Table 1). More remarkable are the significantly higher specific enzymatic activities exhibited by attached bacteria compared to free-living bacteria for both ectoprotease and β-glucosidase activities (Table 1).

DISCUSSION

Biodegradability of *Phaeocystis*-derived organic matter

Although bacterial permeases recognize only monomeric or oligomeric substrates, dissolved organic matter in aquatic environment is mainly polymeric (Cole *et al.* 1984, Billen & Servais 1989, Münster & Chröst 1990). Biodegradability of organic matter relies thus on both the presence of specific enzymes for cleaving the polymeric bonds, and on the secondary and the tertiary structure of the polymer, determining the accessibility for the active site of the enzyme. *Phaeocystis*-derived organic matter released after colony matrix disruption has long been considered as refractory owing to the presence of foam accumulation on the beaches bordering the North Sea at the time of *Phaeocystis* blooms (Lancelot 1995). However, the transient

nature of this organic matter accumulation on the one hand, and the simple sugar composition of the colony matrix (70 % glucose, 15 % xylose, 15 % acidic sugar linked by β-glucosidic bonds; Thingstad & Billen 1994) on the other hand, would suggest that *Phaeocystis*-derived organic matter is readily biodegradable. The observed slow biodegradability of *Phaeocystis* colony-derived organic matter in *Phaeocystis*-dominated ecosystem would possibly result from a delay required for the induction of a non constitutive β-glycosidase (ectoenzymes hydrolysing the polymers of mucus) and/or by nutrient shortage due to the N and P deficiency of *Phaeocystis*-derived material with respect to bacterial needs.

Data presented in this paper on the concomitant evolution of ectoprotease and β-glucosidase activities with respect to diatom and *Phaeocystis* development and the strong correlation between the enzymatic activities, suggest that β-glucosidase activity is constitutive as ectoprotease activity and is not induced by the presence of *Phaeocystis* colonies. Consequently, transient foam accumulation observed at the time of *Phaeocystis* blooms does not reflect a lag phase required for the induction of β-glucosidase. Furthermore, the experiments on the biodegradability of various organic matter derived from *Phaeocystis* (cells, colonies and mucilaginous matrix) do not exhibit significant differences when nutrients are not limiting (Tallier 1994). Altogether, this suggests that *Phaeocystis* mucus derived organic matter would constitute an easily biodegradable substrate for ambient bacteria, provided nutrients are not limiting. This has still to be demonstrated by laboratory-controlled experiments.

Contribution of attached bacteria to the degradation of *Phaeocystis*-derived organic matter

Like in other *Phaeocystis*-dominated ecosystems e.g. Ross Sea (Putt *et al.* 1994), bacteria were found to colonize *Phaeocystis* colonies and their derived aggregates at the peak of the bloom of the continental coastal waters of the North Sea. Early stages of actively growing *Phaeocystis* colonies were not colonized by bacteria suggesting that attachment properties of *Phaeocystis* colonies may change according to their physiological stage. Several mechanisms have been suggested to explain the high potential of senescent phytoplankton for invasion by bacteria. The release of nutrients by senescent phytoplankton may cause a stimulation in bacterial activities which cause bacterial clumping and attachment (Albright *et al.* 1986). Recent biochemical and microscopic analysis of *Phaeocystis* colonies using confocal microscopy (van Ryssel, submitted) give indirect evidence of the decrease of *Phaeocystis* colony compactness when growing in size and age. This decrease in gel compactness might well explain the high vulnerability of large senescent *Phaeocystis* colonies to invasion by bacteria, other microorganisms or/and dead particles.

In agreement with the previous studies (Biddanda 1986, Biddanda & Pomeroy 1988, Azam *et al.* 1993), the size of attached bacteria was significantly larger than that of free-living bacteria. According to Kanopka (1992), high values of attached bacteria biovolume could reflect the capability of bacteria to store carbon such as carbohydrates during N- and/or P-limiting conditions when cell division is no longer possible. Moreover, specific (per cell) activities (growth rates and ectoenzymatic activities) of particle-attached bacteria were significantly higher than those of free-living bacteria. Other studies have shown that bacteria inhabiting marine snow have cell-specific ectoenzymatic activities up to 3 orders of magnitude higher than bacteria

in the surrounding water (Karner & Herndl 1992, Smith *et al.* 1992, Griffith 1994, Martinez *et al.* 1996). Delong et al. (1993) found that particle-attached bacteria were phylogenetically distinct from free-living bacteria and were able to degrade a wide range of polymeric compounds. However, the relative contribution of free-living and particle-attached bacteria to the degradation of organic matter relies on the size and organic composition of aggregates.

The contribution of attached bacteria to the degradation of organic matter derived from *Phaeocystis* was estimated from carbon budget calculations comparing the carbon requirements of attached and free-living bacteria with the spring production of organic carbon by diatoms and *Phaeocystis* colonies. Bacterial organic carbon requirement was calculated by integration of daily bacterial productions measured at the station 330 during the spring bloom period (March-May) assuming an assimilation efficiency of 30 % (Billen *et al.* 1990) (Fig. 6B). This calculation shows that attached bacteria require more than 53 % (60 gC m^{-2}) of the carbon demand of the total bacterial community (113 gC m^{-2}). This calculation highlights the important ecological role of attached bacteria in the dynamics of *Phaeocystis*-dominated ecosystem. Phytoplankton production was estimated by integration of daily total phytoplanktonic production during the bloom period (Fig. 6A). In 1994, *Phaeocystis* production (110 gC m^{-2}) contributed only 49 % to the total spring primary production (222 gC m^{-2}) in contrast to previous years where its contribution was more than 80 % (Lancelot & Mathot 1987). Assuming that bacteria attached to *Phaeocystis* colonies and derived aggregates utilize only labile organic carbon derived from *Phaeocystis* colonies, the transfer of organic matter from *Phaeocystis* to attached bacteria was estimated at 60 gC m^{-2} i.e. 55 % of *Phaeocystis* colony production. Assuming that, on the average, 80 % of *Phaeocystis* colony production consists of carbohydrates and using an average *Phaeocystis* colony C/N ratio of 27 (Rousseau *et al.* 1990), it can be

demonstrated that *Phaeocystis* derived substrates available to particle-attached bacteria would be severely nutrient limited compared to nitrogen requirement of bacteria (C/N = 4, Lancelot & Billen 1985). This suggests that an additional nitrogen source is required for bacterial utilization of *Phaeocystis*-derived material. The observed concomitant development of protozoa during *Phaeocystis* blooms (Becquevort, unpublished data) and their regeneration might supply the required nitrogen as well as phosphate. During years of very large *Phaeocystis* blooms, the nutrient limitation of bacterial mineralization would be more important, resulting in added accumulation of *Phaeocystis*-derived organic matter in the coastal waters of the North Sea.

In conclusion, *Phaeocystis*-attached bacteria biomass was low compared to free-living bacteria, yet it was responsible for roughly one-half of total bacterial mineralization of *Phaeocystis*-associated production in Belgian coastal zone of the North Sea during 1994 spring-summer. The fate of attached bacteria is presently unknown. They could be released from the decomposing colonies in the water column, consumed during metazoan grazing on the *Phaeocystis* aggregates, or exported to the sea floor along with sedimenting *Phaeocystis* aggregates.

Acknowledgements. The authors are indebted to the crew and scientists working on R/V Belgica for the weekly water sampling at the station 330. This works is a part of thesis of P. Thallier and F. Morri. We thank J.M. Dandois and P. Duponcheel for their technical assistance. This work was supported by EC Environment and Climate Programme (contract n°EVSV-CT94-0511) and national SSTC Impulse Programme in Marine Sciences (contract s/11/070). All our gratitude to G. Billen for helpful discussions during the course of this study and to V. Schoemann , J deJong, F. Azam and two anonymous reviewers for their constructive comments on this manuscript.

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Figure captions:

Figure 1: Temporal evolution of (A) phytoplankton biomass (diatoms and *Phaeocystis*) and (B) *Phaeocystis* biomass (colonies and aggregates) at station 330 in the Belgian coastal waters in 1994.

Figure 2: Temporal evolution of (A) abundance, (B) specific biomass and (C) biomass of free-living and particle-attached bacteria at station 330 of the Belgian coastal waters in 1994.

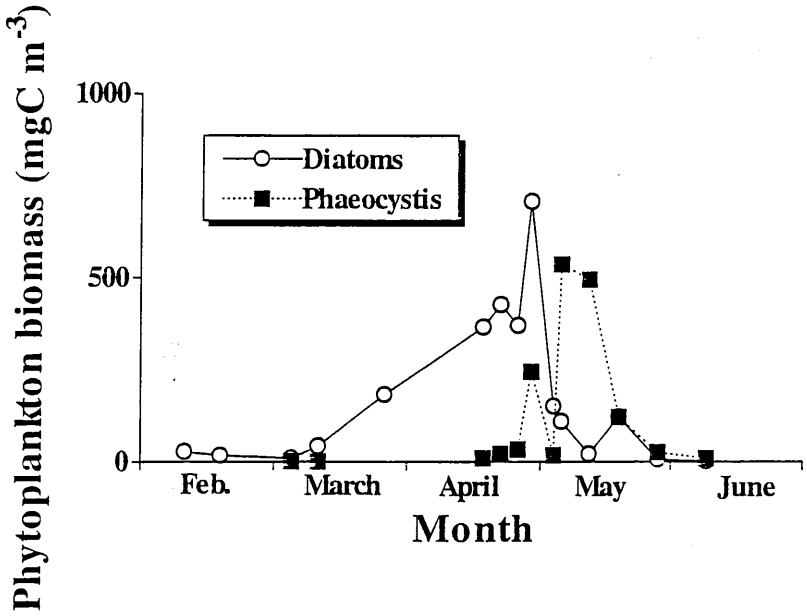
Figure 3: Temporal evolution of (A) cell production and (B) biomass production of attached and free-living bacteria at station 330 of the Belgian coastal waters in 1994.

Figure 4: Temporal evolution of (A) ectoprotease and (B) ecto-β-glucosidase activities (free-living and attached bacteria) at station 330 of the Belgian coastal waters in 1994.

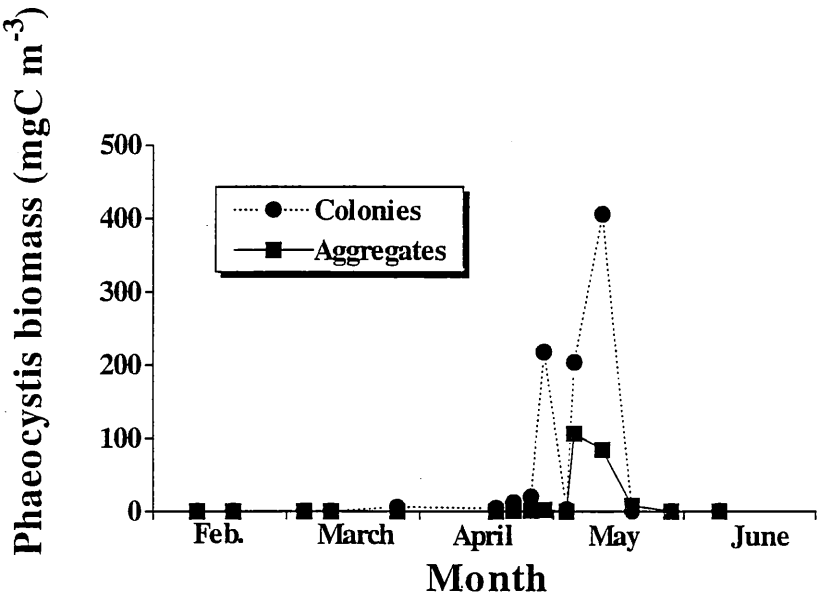
Figure 5: Relationship between ectoprotease and ecto-β-glucosidase activities of (A) free-living bacteria ($y = 0.054 \cdot x$, $R^2 = 0.93$) and (B) attached bacteria ($y = 0.025 \cdot x$, $R^2 = 0.85$).

Figure 6: Temporal evolution of (A) primary production (total phytoplankton ■, *Phaeocystis* ❍) and (B) bacterial carbon demand (total bacteria ■, attached bacteria ❍) at station 330 of the Belgian coastal waters in 1994.

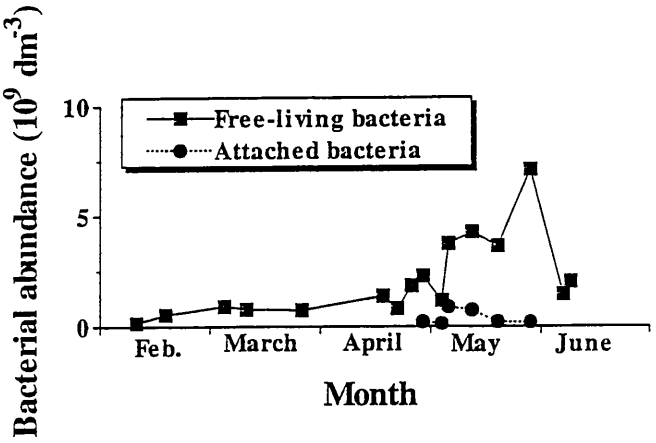
A.


Phytoplankton biomass (mgC m-3)
1000
500
0
Diatoms
Phaeocystis
Feb.
March
April
May
June
Month

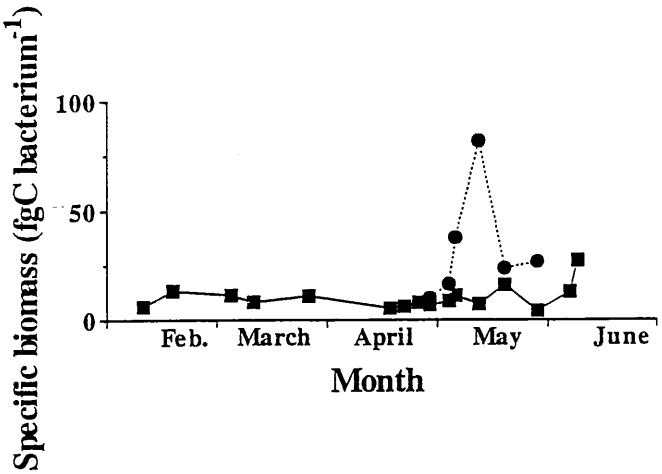
B.


Phaeocystis biomass (mgC m-3)
500
400
300
200
100
0
Colonies
Aggregates
Feb.
March
April
May
June
Month

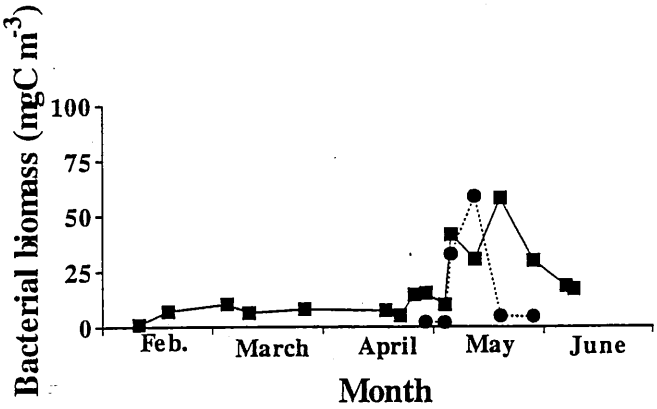
A.


Bacterial abundance (10^9 dm^{-3})
10
5
0
Free-living bacteria
Attached bacteria
Feb.
March
April
May
June
Month

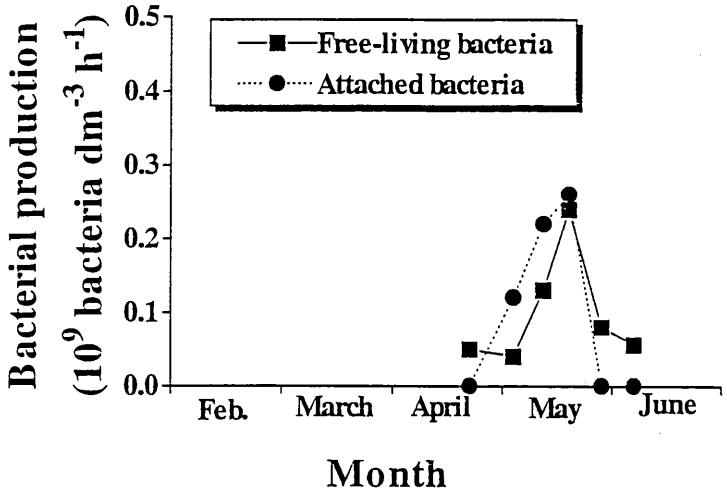
B.


Specific biomass (fgC bacterium^{-1})
100
50
0
Feb.
March
April
May
June
Month

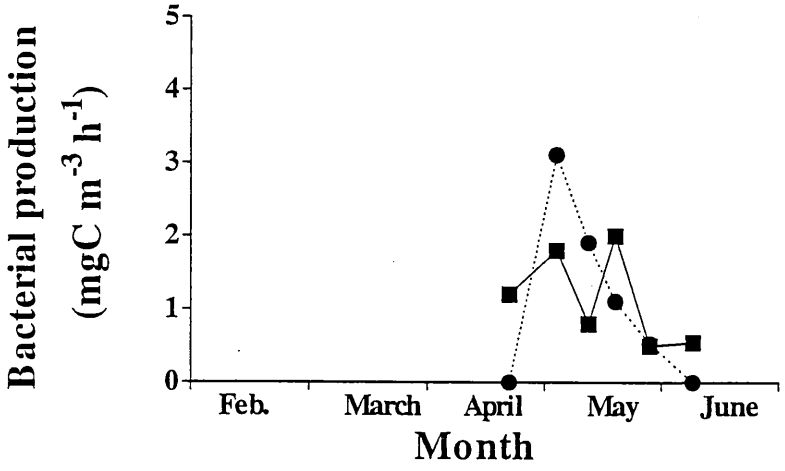
C.


Bacterial biomass (mgC m^{-3})
100
75
50
25
0
Feb.
March
April
May
June
Month

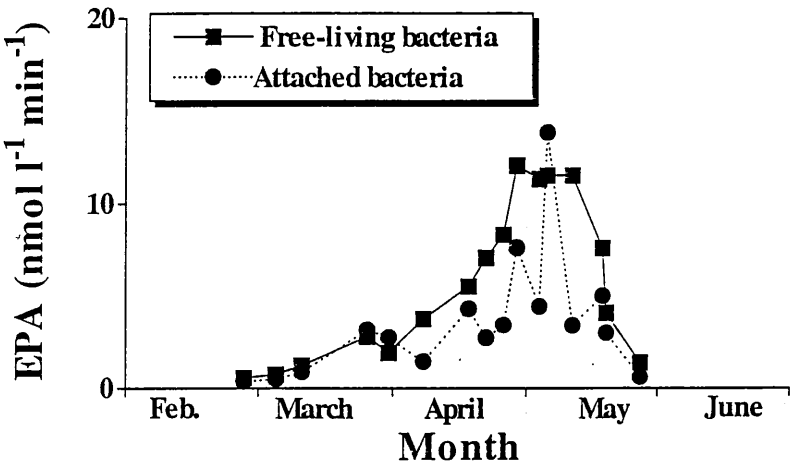
A.


Bacterial production
(10^9 bacteria dm^{-3} h^{-1})
0.5
0.4
0.3
0.2
0.1
0.0
Free-living bacteria
Attached bacteria
Feb.
March
April
May
June
Month

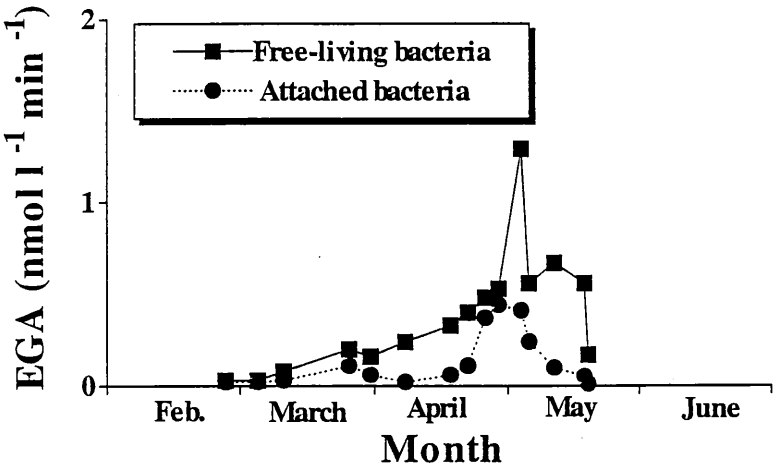
B.


Bacterial production
(mgC m^{-3} h^{-1})
5
4
3
2
1
0
Feb.
March
April
May
June
Month

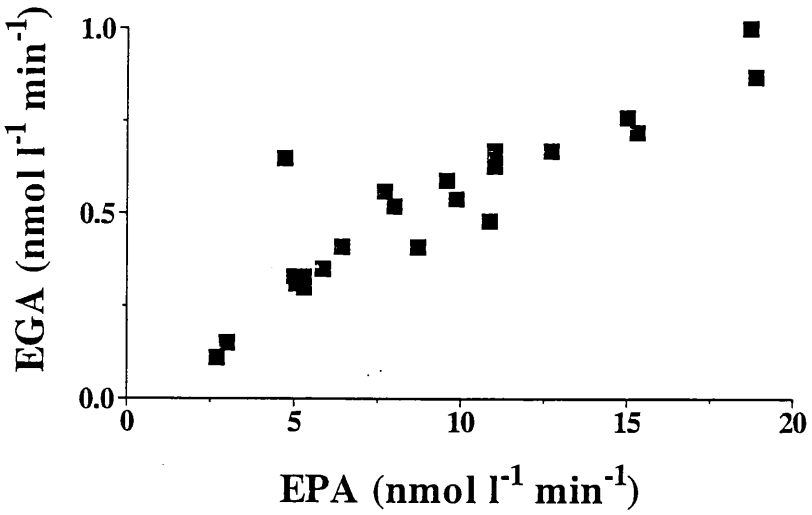
A.


Free-living bacteria
Attached bacteria
EPA (nmol l-1 min-1)
20
10
0
Feb.
March
April
May
June
Month

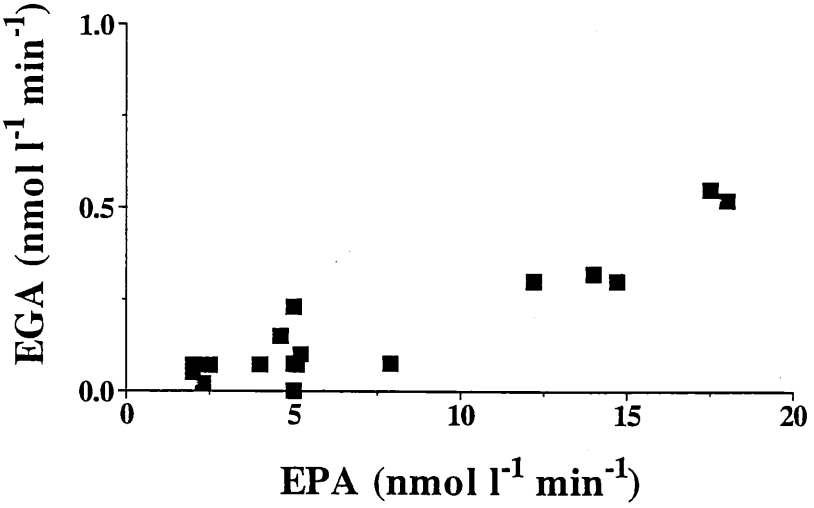
B.


Free-living bacteria
Attached bacteria
EGA (nmol l -1 min -1)
2
1
0
Feb.
March
April
May
June
Month

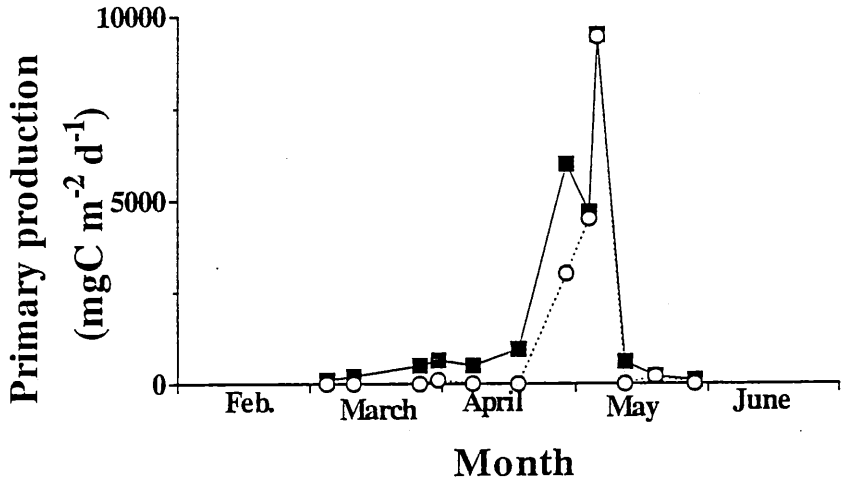
A.


EGA (nmol l-1 min-1)
1.0
0.5
0.0
0
5
10
15
20
EPA (nmol l-1 min-1)

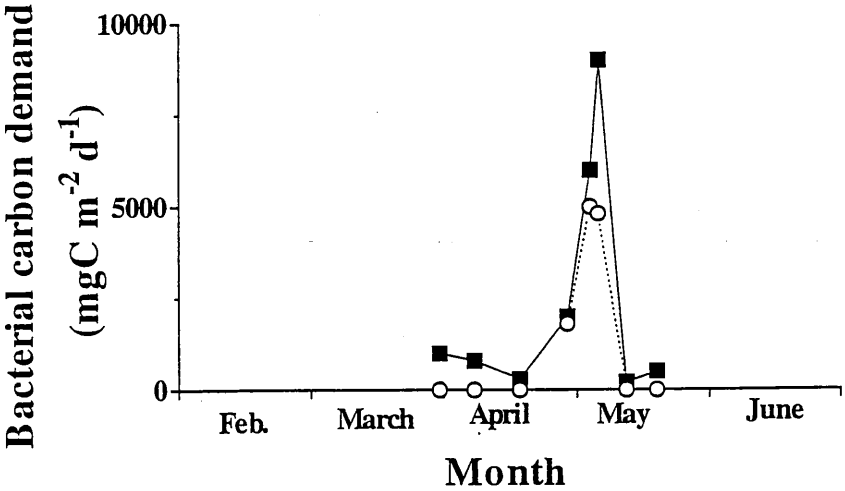
B.


EGA (nmol l-1 min-1)
1.0
0.5
0.0
0
5
10
15
20
EPA (nmol l-1 min-1)

A.


Primary production
(mgC m-2 d-1)
10000
5000
0
Feb.
March
April
May
June
Month

B.


Bacterial carbon demand
(mgC m-2 d-1)
10000
5000
0
Feb.
March
April
May
June
Month

	Free-living bacteria min-max (average)	Attached bacteria min-max (average)
growth rate (h^{-1})	0.007 - 0.040 (0.018)	0.005 - 0.850 (0.280)
specific ectoproteolytic activity(EPA) (nmol $cell^{-1}$ min^{-1})	2.11 - 9.9 (5.3)	17.00 - 40.18 (26.4)
specific ectoglucosidasic activity(EGA) (nmol $cell^{-1}$ min^{-1})	0.02 - 1.11 (0.34)	0.06 - 3.7 (1.36)
EGA/EPA	0.06	0.05

Table 1: Growth rate, specific ectoprotease activity (EPA), specific ecto-β-glucosidase activity (EGA) and EGA/EPA ratio for free-living bacteria and bacteria attached to *Phaeocystis* colonies or derived aggregates.

Manuscript 3

Effects of phytoplankton blooms on the cycling of manganese and iron in coastal waters

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Running head: *Mn and Fe cycling*

Abstract

The spring sequence of intense plankton developments controls the solubility of Mn and Fe in eutrophic shallow coastal waters of the North Sea. Proliferation of diatoms, *Phaeocystis* colonies and *Noctiluca* was accompanied by changes of particulate and dissolved Mn and Fe in the water column. The latter parameters were measured simultaneously with the associated physical, chemical and biological variables at two sites in the coastal waters of the Southern North Sea, in 1993 at one site and in 1994 at both sites. We observe a seasonal increase of dissolved Mn and Fe and progressive changes in the composition of the particulate Mn and Fe in the water column, after the top of the development of diatoms and *Phaeocystis* colonies. This can be explained by the following sequence of events. The organic matter produced in spring by the phytoplankton blooms is degraded by the heterotrophic organisms in the water column and the sediment. This leads to anoxic conditions near the surficial layer in the shallow coastal sediments. First, Mn oxides and then Fe oxides are used as oxidants for the degradation of organic matter resulting in the reduction and dissolution of Mn and Fe. Dissolved Mn and Fe diffuse to the oxic overlying water and a part of it is adsorbed or precipitated on the suspended particulate matter. The other part remains in solution longer due to a decrease in the oxidation rate. This decrease results from a lowering of the pH and oxygen concentrations in the water column that occurs due to the development of heterotrophs in the water column and sediment. Overall, the seasonal evolution of dissolved and particulate Mn and Fe is consistent with the successive autotrophic and heterotrophic activities.

Introduction

Studies on the impact of phytoplankton blooms on the biogeochemical cycling of Mn and Fe in coastal waters are limited. Mn and Fe are two essential elements for phytoplankton growth (Sunda 1989). Uptake of dissolved Mn and Fe by phytoplankton is enhanced in coastal eutrophic waters, where primary productivity and associated Mn and Fe cellular requirements are particularly high (Brand et al. 1983; Sunda et al. 1991; Sunda and Huntsman 1995). Some bloom forming algae like *Chlorella* sp. in freshwater (Richardson et al. 1988; Richardson and Stolzenbach 1995) and *Phaeocystis* sp. in seawater (Lubbers et al. 1990), are known to produce microenvironments of high pH (>9.0) due to photosynthetic activity. Subsequent accumulation of precipitated Mn has been observed on cultured *Phaeocystis* sp. collected from the Southern Ocean (Davidson and Marchant 1987) and the North Sea (Lubbers et al. 1990). The pH sustained in the colonies is thought to be also favourable for Fe precipitation (Lancelot and Rousseau 1994), although it has not yet been investigated. *Phaeocystis* sp. is a cosmopolitan species with a unique life cycle in which the ecological function of the colonial stage is subject of vigorous debate (Lancelot and Rousseau 1994; Rousseau et al. 1994; Lancelot 1995; Riegman and Van Boekel 1996). Nowadays *Phaeocystis* sp. produces massive blooms of large (100-8000 μm) gelatinous colonies in spring and has become the major bloom forming species in the eutrophic continental coastal waters of the North Sea (Bätje and Michaelis 1986; Cadée and Hegeman 1986; Lancelot et al. 1987; Lancelot 1995; Cadée 1996). It is also a major bloom forming species in polar waters, where it may account for part of the oceanic carbon uptake (Wassmann et al. 1990; Smith et al. 1991; Smith Jr. et al. 1996). The brownish colour of colonies observed in Antarctic (Davidson and Marchant 1987) and North Sea waters (Bätje and Michaelis 1986) was thought to be due to the Mn-precipitates within the matrix. It is therefore hypothesised that *Phaeocystis* may play a key role as a vector for Mn and Fe transport in the coastal waters of the North Sea, where it constitutes the major

component of the phytoplankton community (Lubbers et al. 1990; Lancelot and Rousseau 1994; Riegman and Van Boekel 1996).

Phytoplankton can affect the Mn and Fe concentrations in the water column, not only *via* uptake. Increase of dissolved Mn and Fe in the water column may indirectly originate from the phytoplankton blooms. Large amounts of organic matter produced in the euphotic zone may sediment and the degradation of this phytoplankton derived material in the sediments may lead to anoxic conditions. In this case, bacteria use Mn(IV) and Fe(III) solid phases as oxidants. If Fe and Mn are reduced near the sediment-water interface, Mn(II) and Fe(II) will diffuse from the anoxic interstitial waters to the overlying water (Sundby et al. 1981; Kremling 1983; Sundby et al. 1986; Slomp et al. 1997).

Phytoplankton blooms may thus affect the biogeochemical cycles of Mn and Fe in several ways due to:

(i) removal of dissolved Mn and Fe by adsorption and biological uptake;

(ii) precipitation and accumulation of Mn and Fe within phytoplankton oxidising microenvironments, such as *Phaeocystis* colonies;

(iii) deposition of phytoplankton derived material and subsequently decomposition of the sedimented material which may in turn lead to release of dissolved Mn and Fe from anoxic sediments.

The aim of this work was to study the impact of phytoplankton blooms on the cycling of Mn and Fe in coastal waters dominated by *Phaeocystis* sp. blooms. In this context, we measured particulate and dissolved manganese and iron concentrations during seasonal cycles, simultaneous with the associated physical, chemical and biological variables at two sampling stations in the shallow coastal area of the North Sea, where *Phaeocystis globosa* is regularly blooming.

Materials and methods

Sampling- Surface samples were collected at the stations indicated in figure 1: station 330 (51°26'05" N; 02°48'50" E) in the Belgian coastal zone, and the Marsdiep station (53° 02'00" N; 04° 58'00" E) located at the NIOZ (Netherlands Institute for Sea Research) jetty in the Marsdiep tidal inlet of the Dutch Wadden Sea. Both stations are characterized by waters which are well-mixed to the surface sediment, therefore sampling at a certain depth is representative of the whole water column. In order to sample North Sea water at the Marsdiep station, sampling was carried out at high tide. Station 330 is under the influence of the river Scheldt and the Marsdiep station under the influence of the river Rhine.

Samples were collected using a polyethylene (HDPE) bucket (cleaned with acid) with a wide pouring lip (to avoid disruption of the fragile *Phaeocystis* colonies) attached to a kevlar cable. Samples were taken at the upwind and upstream sides of either ship or jetty and directly carefully transferred to a clean 10 liters HDPE bottle. This method was compared with GoFlo bottle sampling method and was shown not to contaminate. Samples were taken for the whole year from the RV Belgica in 1993 and 1994 at station 330. At the Marsdiep station, samples were collected from the jetty from March to August 1994. During the spring phytoplankton blooms, water was sampled once or twice a week.

Manganese, iron and aluminium- Laboratory materials used for trace metal analysis were made of Teflon, polycarbonate or high density polyethylene. All containers were previously washed in 7N HNO_3 (48 hours) except for the less resistant polycarbonate materials which were washed with 1N HCl. After acid washing, all material was thoroughly rinsed with ultra pure water (Barnstead Nanopure system). All procedures were carried out in a class-100 laminar flow bench.

Samples for trace metal analyses were filtered in a polycarbonate device (Sartorius) refitted with Teflon O-rings. The samples were successively filtered over 10, 0.8 and 0.1 μm Nuclepore polycarbonate membrane filters of 47 mm diameter. The fraction larger than 10 μm is assumed to contain the large phytoplankton, including

Phaeocystis colonies and large diatoms. The fraction retained on the 0.8 μm filter included small-sized phytoplankton such as the *Phaeocystis* single cells. Since particulate Mn, Fe and Al concentrations on the 0.1 μm filter were either below detection limit or less than 5% of the total particulate concentration, this fraction was not taken into account. The filtrate passing 0.1 μm was considered to contain dissolved Mn and Fe. No vacuum was applied during the filtration over 10 μm to avoid squeezing and disruption of the *Phaeocystis* colonies. Vacuum pressure applied to the second filtration was kept lower than 0.3 atm in order to keep phytoplankton cells intact as much as possible (Goldman and Dennett 1985).

Filtered water samples were acidified to pH<2 with three times quartz distilled (3Q) 14N HNO_3 and stored in Teflon bottles at 4°C until analysis. Filters were stored in polycarbonate Petri dishes at -18°C until further treatment.

Particulate Mn, Fe and Al, collected on the 10 μm filters in 1994 were subjected to a three step extraction method (Lewis and Landing 1991). During the first step, filters were submerged in 25% (4.5 M) acetic acid (3Q-HAc) at room temperature to extract the most reactive fraction. Filters were then soaked in a mixture of 2M 3Q-HCl and 1M 3Q-HNO_3 also at room temperature. Finally, the most refractory fraction retained on the filters was completely hydrolysed with 750 μl 12N 3Q-HCl, 250 μl 14N 3Q-HNO_3 and 250 μl 40% HF (Merck Suprapur) in Teflon bombs at 100-120°C. The last step of the digestion procedure was directly applied to the 10 μm filters collected in 1993, and to all the 0.8 μm and 0.1 μm filters, to measure the total particulate Mn, Fe and Al retained on these filters. These extractions were also applied to a marine reference sediment material for trace metals analysis (BCSS-1, available from the National Research Council of Canada) to check the accuracy of the methods. Results of the three step extraction (summing results obtained for each step) and results of the total digestion alone, were within the range of values certified by the National Research Council of Canada. Blank values were below 5% of the measured sample concentrations.

Dissolved and extracted Mn, Fe and Al were measured by Flame Atomic Absorption Spectrophotometry (Perkin Elmer 2380) for high concentrations (> 50 μg/l

for Mn and Fe and > 500 μg/l for Al). Samples with lower concentrations were analysed by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS, Perkin Elmer Zeeman 5100 PC) using calibration by standard addition. Multiple injection of the sample was applied for Mn and Fe concentrations below 20 nM. Accuracy of the analytical method for seawater samples was checked by measuring CASS-3 (for Mn and Fe) and NASS-4 (Mn only, below detection limit for Fe) seawater reference material for trace metals (National Research Council of Canada). The relative standard deviation of the method was better than 5%. A detection limit of three times the standard deviation of the procedural blanks was applied to the data.

Physical, chemical and biological parameters- Salinity was determined by conductivity using a Beckman salinometer. Total suspended matter was analysed by weighing a preweighed GF/F filter after the filtration of 200 to 1000 ml of water sample and drying at 105°C (4 hours). Alkalinity was determined by acid titration in a closed vessel, upon which the titration curve was fitted with a modified Gran plot (Stoll et al. 1993). Total inorganic carbon (TIC) was measured on an automated coulometer modified after Johnson et al. (1987) according to the method described in DOE (1991). The pH and carbon dioxide concentrations were calculated from alkalinity and TIC, using the dissociation constants given by Goyet & Poisson (1989). Dissolved oxygen was measured according to the Winkler method and oxygen saturation percentages were calculated taking into account water temperature and salinity. Nutrients were determined in 0.45 μm membrane (Sartorius cellulose-acetate) filtered sea water according to the methods described in Grasshoff et al. (1983). Chlorophyll *a* was quantified spectrophotometrically following Lorenzen (1967) after 90% v:v acetone extraction (12 hours) in the dark at 4°C of the particulate material retained on glass fibre filters (Whatman GF/C).

Phytoplankton samples were fixed with a 1% lugol-glutaraldehyde solution. The species composition, cell density and biovolumes of diatoms and *Phaeocystis* colonies were determined under an inverted microscope (Leitz Fluovert) after 12h sedimentation

of 5-10 ml samples in Utermöhl chambers (Hasle 1978). A carbon content of 0.11 pg C μm^{-3} of plasmavolume (Edler 1979) was used to convert the diatoms biovolumes measured on a cell population during their development period. *Phaeocystis* colony sizes were converted into carbon biomass using the equations established by Rousseau et al. (1990). Biomass of the giant heterotrophic dinoflagellate, *Noctiluca miliaris*, was determined by counting the cells under a darkfield illuminated stereomicroscope directly after sampling and using a factor of 0.2 μg C $cell^{-1}$ (Uhlig and Sahling 1990).

Results

Physical, chemical and biological parameters- At both stations, the temperature increased from about 5°C in early March to about 20°C in early July (Fig. 2a-c). The salinity varied between 31.8 and 34.9 at station 330, and between 23.7 and 30.5 at the Marsdiep station (Fig. 2a-c). No regular trend was observed at station 330 in 1994 (Fig. 2b), while salinities tended to increase at the Marsdiep station between March and July (Fig. 2c). Excluding the high values in April-May, suspended particulate matter was low at station 330 compared with that at Marsdiep station (Fig. 3d-f).

Average winter levels of nutrients (Fig. 3) as well as the plankton developments (Fig. 4) were higher at the Marsdiep station than at station 330. Nutrient concentrations decreased in February-March and increased again in May-June. At station 330, chlorophyll-*a* concentrations (Fig. 4a and 4b) were at similar maxima (45 mg m^{-3}) in early May 1993 and 1994. At the Marsdiep station, the phytoplankton development showed three chlorophyll maxima of 75, 45 and 40 mg m^{-3} from late April to early July (Fig. 4c). Diatoms developed from March to the end of May (Fig. 4d-f). *Phaeocystis globosa* blooms started later in April and also ended at the end of May (Fig. 4d-f). *Phaeocystis* dominated the spring phytoplankton biomasses in 1993 at station 330 (Fig. 3d; note the difference of scale for diatoms and *Phaeocystis*), whereas diatoms were dominant in 1994 at both stations (Fig. 4e and 4f). In late May-early June, *Noctiluca*

miliaris biomass was highest at both stations (Fig. 4g-i). These organisms suddenly disappeared in late June-early July.

During the development of the diatoms at the Marsdiep station, oxygen saturation increased from 100 to 125% (Fig. 5a). It reached undersaturated values in late May and was at minimum in early June when the *Noctiluca* peaked (Fig. 4i). Dissolved carbon dioxide concentrations decreased (27 μM to 6 μM) and the pH increased (8.1 to 8.6) during the development of the diatoms and the beginning of the bloom of *Phaeocystis* (Fig. 5b and 4f). From late May to late June, when diatoms and *Phaeocystis* disappeared from the water column and *Noctiluca* developed, dissolved carbon dioxide concentrations were again higher (13 μM) with pH of about 8.2.

Manganese, iron and aluminium concentrations- At both stations, total and dissolved Mn concentrations increased from May to early June (Fig. 6). Particulate Fe represented more than 80% of the total Fe and decreased from February-March till August following the same trend as particulate Al (Fig. 7). Aluminium can be regarded as a good indicator for the presence of inorganic terrigeneous particles. Particulate Mn, Fe and Al were at minimum in late May-early June (Fig. 6d-f and 7).

Concentrations of Mn, Fe and Al were much higher at the Marsdiep station than at station 330 (Fig. 6 and 7). At the Marsdiep station, dissolved Fe concentrations also increased from May to early June (Fig. 7c); while at station 330, there was no trend in the concentrations of dissolved Fe (Fig. 7a and 7b).

Composition of the particulate suspended matter- The composition of the suspended particulate matter changed progressively from May to the end of July at the Marsdiep station (Fig. 8b, 8d, 8f and 8h). The molar ratios of Mn/Al and Fe/Al were stable in March-April and close to the average soil ratio (Fig. 8b; Mn/Al = 0.0069 and Fe/Al = 0.27) as calculated from the average content of Mn, Fe and Al in soils (Chester 1990). From May to the end of July, we observed an enrichment of Mn and Fe compared to Al in the suspended particulate matter (Fig. 8b). At that time there was a

steady increase of "exchangeable" Mn and Fe (HAc) relative to the Al content (Fig. 8d) and relative to the more refractory fraction (AR + REF; Fig. 8f). Mn and Fe extracted in the second and the third step also increased relative to Al (Fig. 8h). At station 330, there was no trend in the temporal evolution of the composition of the suspended matter (Fig. 8a, 8c, 8e and 8g).

Discussion

Behaviour of manganese and iron- This study suggests that planktonic developments can induce changes in the concentrations of dissolved Mn and Fe and in the composition of the suspended particulate matter. The observed temporal variations in total Mn, dissolved Mn and dissolved Fe concentrations as well as the temporal changes in the composition of the suspended particulate matter all occurred close after the first spring peak of chlorophyll *a* concentrations. Maxima in dissolved Mn and Fe concentrations coincided with minima in chlorophyll *a* concentrations and maxima in *Noctiluca* biomass. At that time, oxygen was undersaturated and at its lowest and dissolved carbon dioxide concentrations at a maximum, showing the dominance of the heterotrophic activity over the autotrophic activity.

The concentrations of total and dissolved Mn increased just after the maximum of chlorophyll *a* concentrations and peaked when chlorophyll *a* concentrations were at low concentrations. This shows the presence of a source of dissolved Mn to the water column which could be linked to the decline of the phytoplankton blooms. The relative progressive enrichment in "exchangeable" Mn (HAc fraction) in the particulate matter also occurred at the same time. It is likely due to a transfer of the imported dissolved Mn to the suspended particles, which would explain the enrichment in Mn relative to Al in the particulate suspended matter. The similar changes observed for Fe in the composition of the particulate suspended matter could also be due to the same process.

Role of the rivers- Dissolved Mn and Fe can be delivered by rivers to coastal waters (Duinker and Nolting 1976; Duinker and Nolting 1978; Duinker et al. 1979; Turner et al. 1991) but there was no correlation between salinities and the increase in dissolved Mn. On the contrary, at the Marsdiep station, we observed gradually higher salinities, reflecting the lower discharge of the river Rhine (Van Der Giessen et al. 1990; Visser et al. 1991) from April to July. Our results were therefore not explained by variations in run-off supply from the surrounding rivers.

Role of phytoplankton blooms in Mn and Fe removal- This study shows that phytoplankton developments did not have a significant direct effect on the Mn and Fe content of the suspended particulate matter at the studied sites. At the Marsdiep station, Mn/Al and Fe/Al ratios were stable and close to average soil ratios even when the peak of chlorophyll *a* concentrations was observed. This suggests that the particulate Mn and Fe content of the suspended matter was mainly controlled by the presence of terrigeneous materials and not directly affected by the phytoplankton blooms. The relative enrichments in Mn and Fe compared to Al were observed after the phytoplanktonic developments and could thus not be explained by the selective removal of the biologically essential Mn and Fe compared to Al. Mn/Al and Fe/Al molar ratios were scattered at station 330 and did not show any co-variation with the phytoplankton development.

Several authors proposed that *Phaeocystis* colonies could play an important role in the cycling of Mn and Fe by producing oxidising and accumulating microenvironments for these trace metals (Davidson and Marchant 1987; Riegman and Van Boekel 1996; Lubbers et al. 1990). Contrary to Morris (1971, 1974) who observed variations in the dissolved and particulate Mn concentrations concomitant with a bloom of *Phaeocystis* in the Menai Straits, our results show no direct effect of the development of *Phaeocystis* on Mn and Fe concentrations at the studied stations. In 1993, *Phaeocystis* dominated the total phytoplankton cell number and biomass which is usually the case in the coastal waters of the Southern North Sea (Cadée and Hegeman

1993). In 1994, *Phaeocystis* development in spring was exceptionally low (Cadée 1996) and diatoms dominated the spring phytoplankton community instead. Even so, particulate Mn and Fe concentrations as well as Mn/Al and Fe/Al were similarly scattered during the spring phytoplankton blooms of 1993 and 1994 at station 330. There was no significant accumulation of Mn and Fe in the suspended particulate matter caused by the *Phaeocystis* colonies.

Regeneration and recycling of dissolved Mn and Fe occur during the remineralization of the phytoplankton (Hutchins and Bruland 1994). At station 330 in late May-early June 1993 and 1994 (during and following the decline of the spring phytoplankton blooms), we observed an increase of dissolved Mn of about 4 nM d^{-1} in the surface waters for a period of 10-15 days . If we consider the spring primary production of 222 g C m^{-2} as calculated by Becquevort et al. (1997) for station 330 in 1994, an average depth of 22 m and a range of Mn:C ratio of 6-32 μmol Mn mol^{-1} C (Sunda 1989; Sunda and Huntsman 1996) for phytoplankton from coastal areas with the same range of concentrations as in the present study, the phytoplankton could have only accumulated about 6-27 nM during the whole spring bloom. The regeneration of dissolved Mn from the phytoplankton could therefore only account for a small part of the release in dissolved Mn observed after the spring phytoplankton bloom.

Both diatoms and *Phaeocystis globosa* have been shown to produce aggregates which may scavenge other micro-organisms and inorganic particles such as clays containing aluminosilicates (Avnimelech et al. 1982; Lancelot et al. 1990; Riebesell 1991; Riebesell 1993; Lancelot 1995). The scavenged materials of these aggregates increase the density of the phytoplankton debris and may speed up their exportation to the sediments (Avnimelech et al. 1982; Wassmann 1994). Aggregation during the decline of the diatoms and *Phaeocystis* blooms could thus contribute to the rapid removal of both organic and inorganic materials from the water column and to their exportation to the sediments. This mechanism could explain the observed simultaneous decrease of chlorophyll *a* and particulate Al, particulate Mn and particulate Fe concentrations in late May-early June in the water column. It could decrease the amount

of suspended particles and hence decrease the amount of adsorptive sites for Mn and Fe and of autocatalytic sites for the oxidation of Mn (Kessick and Morgan 1975; Stumm and Morgan 1981; Sung and Morgan 1981; Yeats and Strain 1990; Turner et al. 1992).

Role of the sediment- Our results confirmed, with a high time resolution, that seasonal variations occur in the concentrations of dissolved Mn and Fe in the North Sea with increases in spring and summer compared to lower values in winter (Burton et al. 1993; Tappin et al. 1993; Tappin et al. 1995). Like Burton et al. (1993), our results of Mn and Fe in the suspended particulate matter showed enrichments earlier than Dehairs et al. (1989) who only observed higher values of Mn/Al and Fe/Al in late summer and autumn. These authors attributed these variations to Mn and Fe inputs by benthic recycling. Diagenetic benthic flux of dissolved Mn and Fe is indeed a potential source of dissolved Mn and Fe to the water column in organic rich shallow coastal areas (Sundby et al. 1981; Kremling 1983; Sundby et al. 1986). Dissolved Mn and Fe are produced when aerobic biological remineralization of freshly sedimented organic matter depletes or strongly reduces oxygen in the sediments. Mn and Fe oxides are then used as secondary oxidants for further degradation of the organic matter and become reduced and solubilized. When this is happening at, or close to, the sediment-water interface, dissolved Mn and Fe may escape by diffusion to the water column because of the formation of a high concentration gradient between porewaters and the overlying water column (Sundby et al. 1981; Slomp et al. 1997). This process may thus provide dissolved Mn and Fe to the water column during the decline of spring blooms when high quantities of phytoplanktonic derived material are transported to the sediment.

Fluxes of dissolved Mn and Fe were estimated from sandy sediment cores collected at the end of July 1994 at an intertidal shallow station close to and contributing to the Marsdiep station (Epping et al. 1997). In absence of photosynthetic activity by benthic diatoms, an average flux of 0.79 mmol m^{-2} d^{-1} was calculated for dissolved Mn, while for Fe a high variability was recorded, likely due to the high removal rate of Fe by adsorption and oxidation. The average Fe flux was 0.59 mmol m^{-2} d^{-1}. Assuming an

average water depth of the Dutch coastal zone of 18 m and a well mixed water column, these fluxes could supply 44 μmol m^{-3} d^{-1} Mn and 33 μmol m^{-3} d^{-1} Fe to the water column. These results show the existence of fluxes high enough to explain the observed late spring net increases of 17 μmol m^{-3} d^{-1} for Mn and 27 μmol m^{-3} d^{-1} for Fe.

Seasonal changes in the dissolved Mn and Fe benthic fluxes, with higher values in spring and summer have been observed in estuarine and coastal waters (Aller and Benninger 1981; Aller 1994) as well as in mesocosms experiments (Hunt 1983). These variations were attributed to several factors. Hunt (1983) showed the dependence of these fluxes on temperature and on the rates of primary production in the water column. The quantity but also the quality of the organic matter accumulating in the sediments as well as the magnitude of the benthic heterotrophic development will determine the oxygen and nitrate penetration depth. This is the depth below which Mn and Fe will become reduced in the sediments and determines the possibility for dissolved Mn and Fe to escape from the sediment to the water column (Chester 1990 and references therein; Slomp et al. 1997).

The higher increases in dissolved Mn and Fe at the Marsdiep station than at station 330 could be partially explained by the differences in primary production rates between the two stations. Due to higher nutrient inputs from the Rhine compared to the Scheldt, higher primary production can be sustained in the Dutch than in the Belgian coastal area of the North Sea (Cadée and Hegeman 1986; Veldhuis et al. 1986; Lancelot 1995). As shown by the data of chlorophyll *a* and phytoplankton biomass, the phytoplankton development was higher at the Marsdiep station than at station 330 and hence more phytoplankton-derived material could have reached the sediment in the Marsdiep area and induced higher benthic fluxes of dissolved Mn and Fe.

Even if in 1993 and 1994 the phytoplankton biomass differed at station 330, we still observed similar increases in dissolved Mn. In 1993, *Phaeocystis* colonies dominated whereas in 1994, it was the diatoms. Due to specific biodegradability and fate of these two types of algae at the end of their development, a similar quantity of

easily biodegradable phytoplanktonic material could have reached the sediments in 1993 and 1994.

Slomp et al. (1997) showed that most of the North Sea sediments are relatively poor in Mn and Fe oxides. The quantity of Mn and Fe oxides already present in the sediments and imported during the deposition of the phytoplanktonic material, in particular with the aggregates, could also influence the benthic fluxes and explain on one hand the difference in maxima of dissolved Mn between the two stations and on the other hand the similarity in the maxima of dissolved Mn at the station 330 in 1993 and in 1994.

Influence of the pelagic heterotrophs on the oxidation and reduction of Mn and Fe- Heterotrophs can favour the dissolution of Mn and Fe not only via the remineralization of the phytoplankton (Hutchins and Bruland 1994) but also by producing reducing microenvironments (Hutchins and Bruland 1994; Wells et al. 1995; Barbeau et al. 1996); by changing chemical conditions like the pH and the dissolved oxygen concentrations in the water, and by "cleaning up" the water column from suspended matter. It has been suggested that the acidic digestive systems of plankton grazers are reducing microenvironments which could play a major role in the Mn and Fe speciation (Hutchins and Bruland 1994; Wells et al. 1995; Barbeau et al. 1996). The enzymatic degradation combined with low pH could be favourable to transform organically-bound cellular Mn and Fe to inorganic dissolved Mn and Fe (Frey and Small 1979; Hutchins and Bruland 1994). The pH of *Noctiluca* vacuoles is as low as 4.35 (Taylor 1987). Among heterotrophic micro-organisms, *Noctiluca miliaris* was dominant in biomass at station 330 in late May-early June 1993 and 1994 (S. Becquevort, pers. comm.). They also developed at the Marsdiep station at the time we observed increases of dissolved Mn and Fe concentrations. Our results suggest that *Noctiluca* could have played a role in the dissolution of Mn and Fe. *Noctiluca* activity can be very intense (Weisse et al. 1994). They are large omnivorous dinoflagellates feeding on phytoplankton, like *Phaeocystis* cells, microprotozoa and bacteria (Weisse and Scheffel-

Möser 1990; Weisse et al. 1994). The very high net production of ammonia observed simultaneously with their development could be due to their intense regenerating activity together with the decline of the autotrophs which no longer consumed nitrogen. *Noctiluca* could also be mainly responsible for the strong decrease in dissolved oxygen concentrations to undersaturated values coupled with the increase in carbon dioxide concentrations and decrease in pH. Bätje and Michaelis (1986) found similar results of oxygen saturation, decreasing to 60% as a consequence of disintegrating *Phaeocystis* material in the Frisian coastal waters of the North Sea. At the Marsdiep station in 1993, Brussaard et al. (1996) measured a comparable decrease of pH from 8.7 at the top of the bloom of *Phaeocystis* to 8.0 when chlorophyll *a* concentrations were at minimum and when *Noctiluca* as well as other microzooplankton peaked. In accordance with the following kinetic equations:

$$-\frac{d[Fe(II)]}{dt} = k[Fe(II)][OH^-]^2[O_2]$$ (Millero et al. 1987)

$$-\frac{d[Mn(II)]}{dt} = k[Mn(II)][Mn_p][O_2]$$ (Yeats and Strain 1990),

the observed changes in pH (pH = -log[H^+] and K_w = [OH^-][H^+]) and oxygen concentrations would slow down the oxidation rates of both Mn and Fe, thus maintaining Mn and Fe longer into solution. The fact that *Noctiluca* are opportunist organisms feeding on all kinds of micro-organisms will decrease the amount of suspended particles and hence decrease the transfer of dissolved Mn and Fe to the particles.

Conclusions

The following sequence of events is proposed to explain our results. The observed increase of dissolved Mn and Fe at the end of spring would be the consequence of sedimentation favoured by aggregation processes of large amounts of phytoplankton derived material. The deposit of fresh organic matter would have

stimulated benthic microbial activity, depleting oxygen at or close to the surface of the sediments. Bacterial degradation of organic matter in suboxic condition is then accompanied by reduction and dissolution of Mn and Fe. Dissolved Mn and to a lesser extent Fe (due to its rapid oxidation rate) are released in the water column by diffusion from the porewater. Part of this dissolved Mn and Fe is adsorbed or precipitated on suspended particles and will enrich the particulate matter in Mn and Fe compare to Al. The rest remains longer in solution because of the heterotrophic activity, in particular of *Noctiluca* and to the lowered amount of particles.

In shallow turbulent eutrophic coastal areas, the direct removal of Mn and Fe by the phytoplankton and the role of *Phaeocystis* colonies as sequestrating agent of Mn and Fe are not major processes compared to the benthic diagenetic release of dissolved Mn and Fe from the sediments. The biogeochemical cycles of Mn and Fe are driven by the eutrophication-dependent magnitude of phytoplankton blooms, the heterotrophic development that follows the spring bloom, and the quality of phytoplankton-derived material reaching the sediment.

Similar sequences of plankton development and biogeochemical cycling of Mn and Fe are likely to be significant in all shallow coastal waters experiencing seasonality. Of course in other regions different plankton species may prevail, while the intensity also depends on supply of nutrients.

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FIGURE CAPTIONS

Fig. 1: The Southern North Sea. The two locations that were sampled are indicated: station 330 (A) in the Belgian coastal zone and the Marsdiep station (B) in the Dutch coastal zone.

Fig. 2: Temporal evolution of salinity (♦), temperature (×) and particulate suspended matter (▲) in 1993 and in 1994 at station 330 and in 1994 at the Marsdiep station.

Fig. 3: Temporal evolution of silicate (♦), phosphate (×), ammonium (●) and nitrate (○) in 1993 and in 1994 at station 330 and in 1994 at the Marsdiep station.

Fig. 4: Temporal evolution of dissolved oxygen saturation (●), dissolved oxygen concentrations (○), pH (■) and dissolved CO_2 (□) in 1993 and in 1994 at station 330 and in 1994 at the Marsdiep station.

Fig. 5: Temporal evolution of chlorophyll *a* (▲), diatoms biomass (●), *Phaeocystis globosa* biomass (○) and of *Noctiluca miliaris* biomass (♦) in 1993 and in 1994 at station 330 and in 1994 at the Marsdiep station.

Fig. 6: Temporal evolution of total Mn (Mn_{tot}; ■), dissolved Mn (Mn_d; ●) and particulate Mn (Mn_p; ○) in 1993 and in 1994 at station 330 and in 1994 at the Marsdiep station.

Fig. 7: Temporal evolution of dissolved Fe (Fe_d; ●), particulate Fe (Fe_p; ○) and particulate Al (×) in 1993 and in 1994 at station 330 and in 1994 at the Marsdiep station.

Fig. 8: Temporal evolution of molar ratios of: (a, b) total particulate Mn (closed symbols) and Fe (open symbols) over total particulate Al in 1993 (triangle) and in 1994 (circle); particulate Mn (closed symbols) and Fe (open symbols) dissolved in 25% acetic acid (HAc) over the total particulate Al (c, d) and over particulate Mn and Fe hydrolysed in the second and third step (AR+REF) of the sequential extraction (e, f) in 1994; Mn and Fe hydrolysed in the second and third step (AR+REF) of the sequential extraction over total particulate Al in 1994 (g, h) at station 330 and at the Marsdiep station.

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Fig. 1

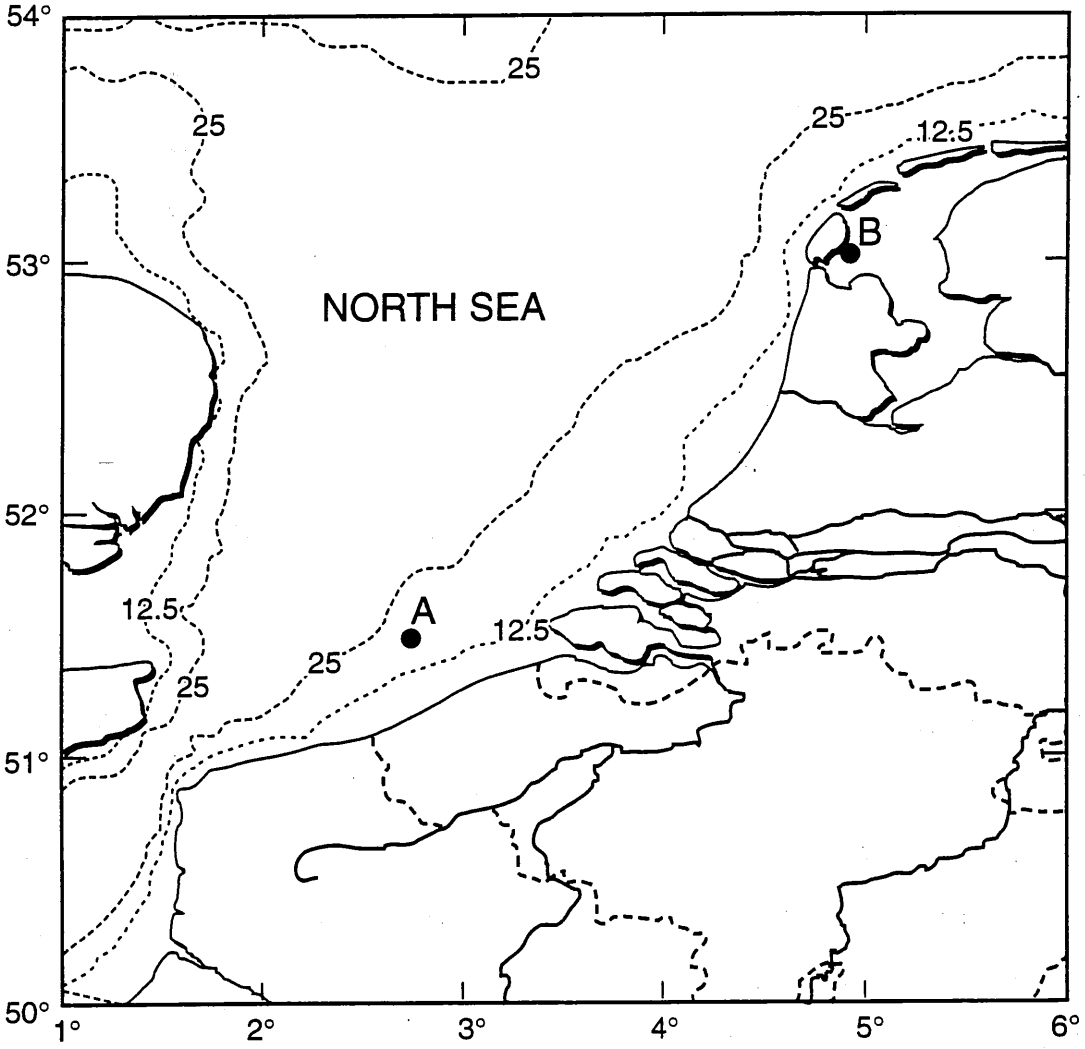


Fig. 2

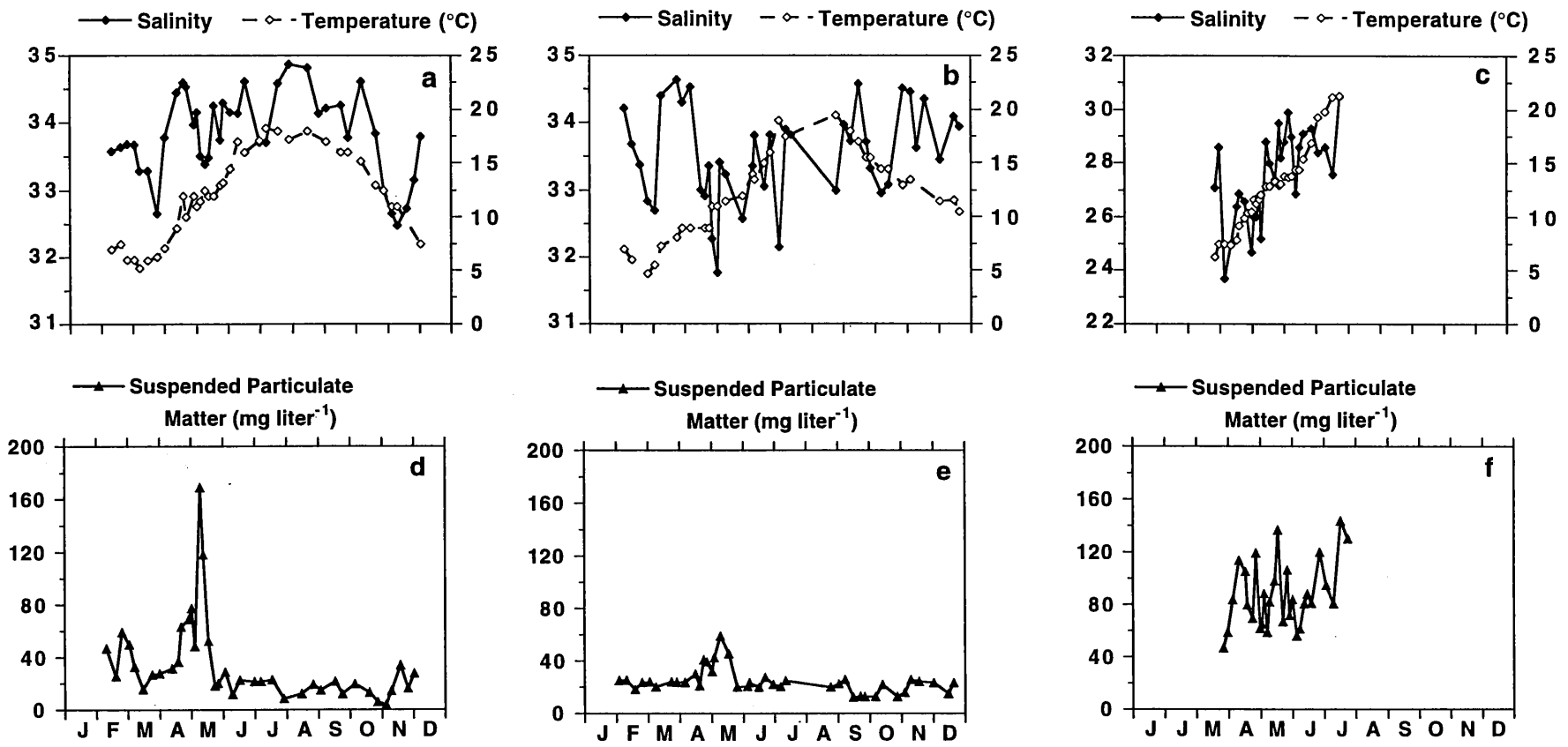

'330' 1993
'330' 1994
'Marsdiep' 1994
Salinity
Temperature (°C)
Suspended Particulate Matter (mg liter-1)
a
b
c
d
e
f
J F M A M J J A S O N D

Fig. 5 'Marsdiep' 1994

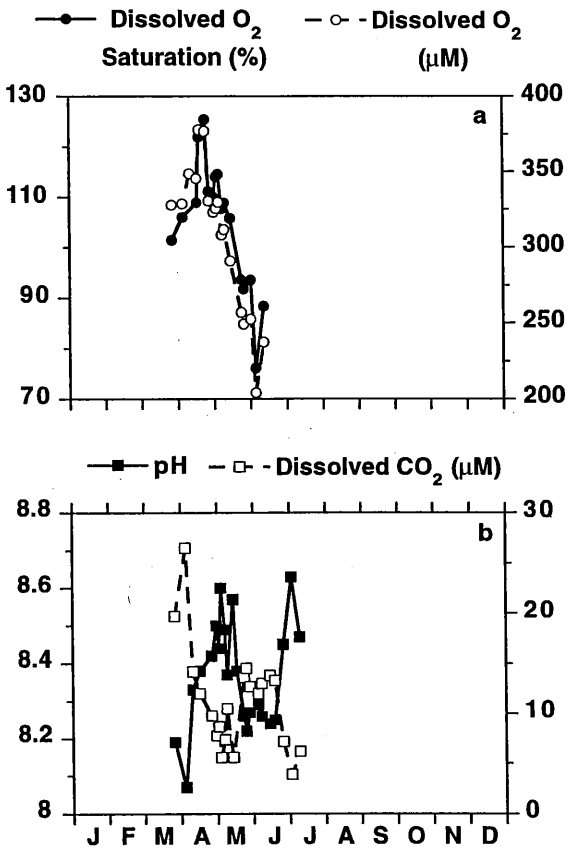


Fig. 8

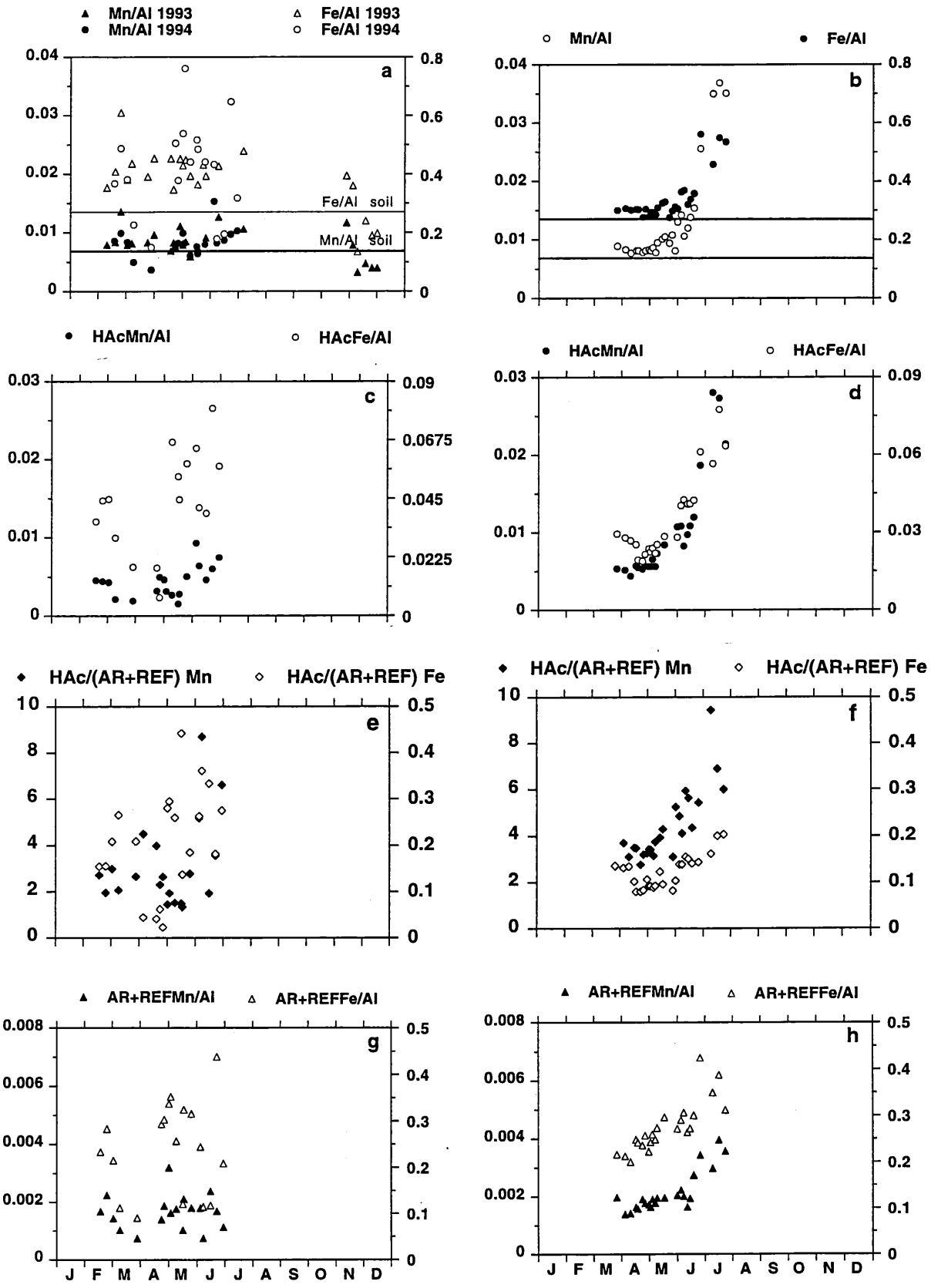

'330'
'Marsdiep'
Mn/Al 1993
Mn/Al 1994
Fe/Al 1993
Fe/Al 1994
a
Fe/Al soil
Mn/Al soil
Mn/Al
Fe/Al
b
HAcMn/Al
HAcFe/Al
c
d
HAc/(AR+REF) Mn
HAc/(AR+REF) Fe
e
f
AR+REFMn/Al
AR+REFFe/Al
g
h
J F M A M J J A S O N D

Fig. 3 '330' 1993 '330' 1994 'Marsdiep' 1994

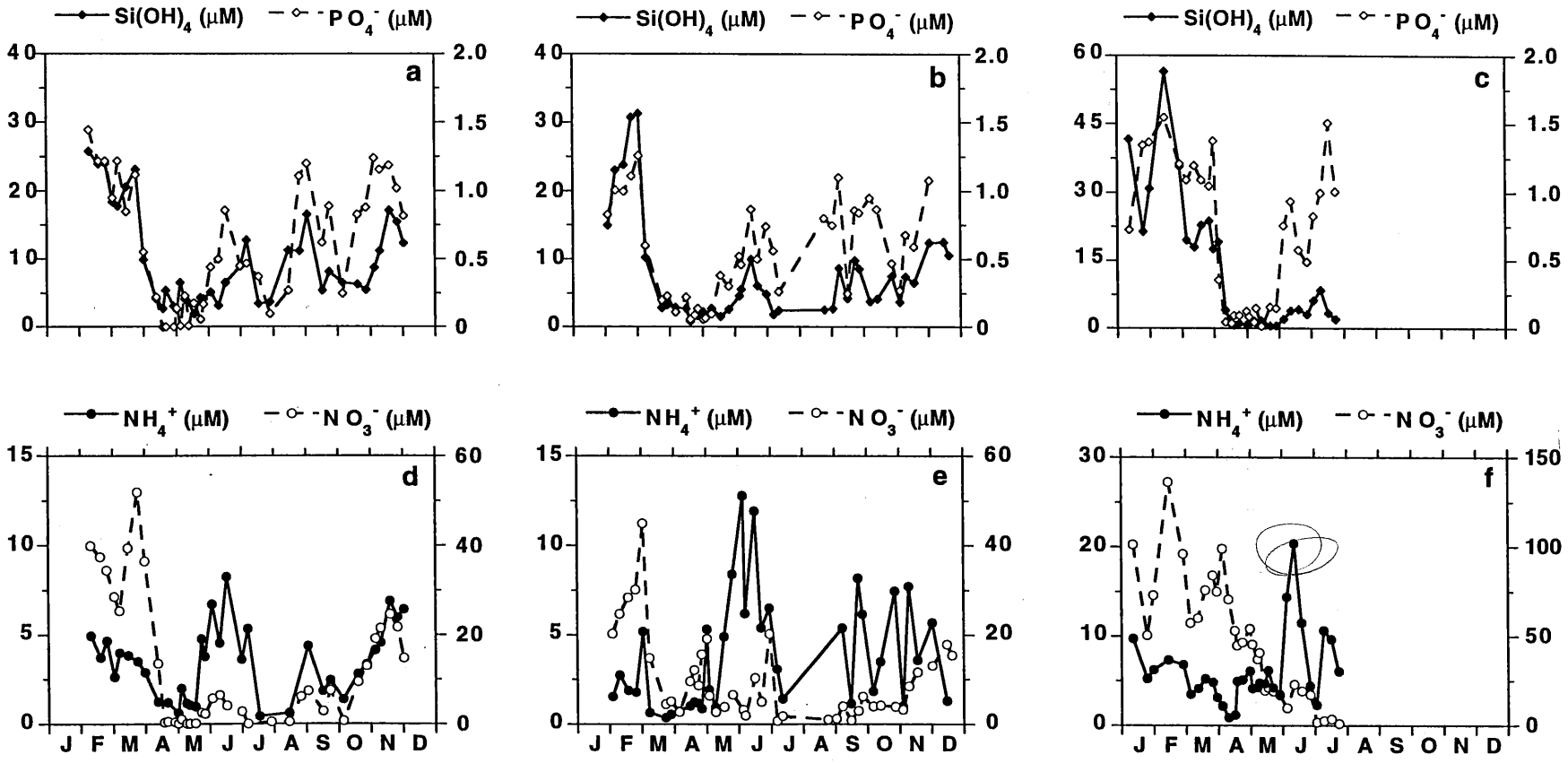


Fig. 4

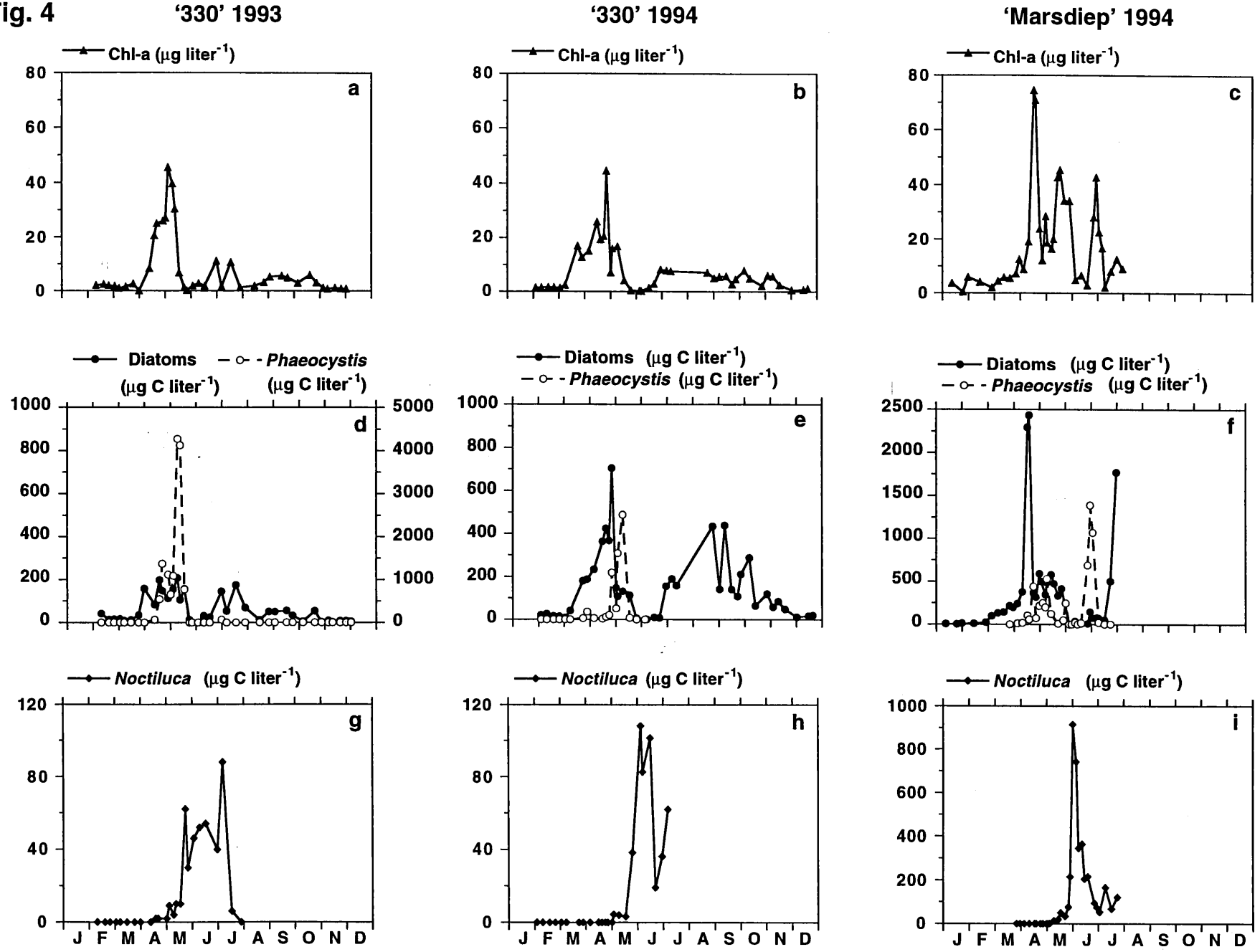

'330' 1993
'330' 1994
'Marsdiep' 1994
Chl-a (µg liter-1)
a
b
c
Diatoms (µg C liter-1)
Phaeocystis (µg C liter-1)
d
e
f
Noctiluca (µg C liter-1)
g
h
i
J F M A M J J A S O N D

Fig. 6

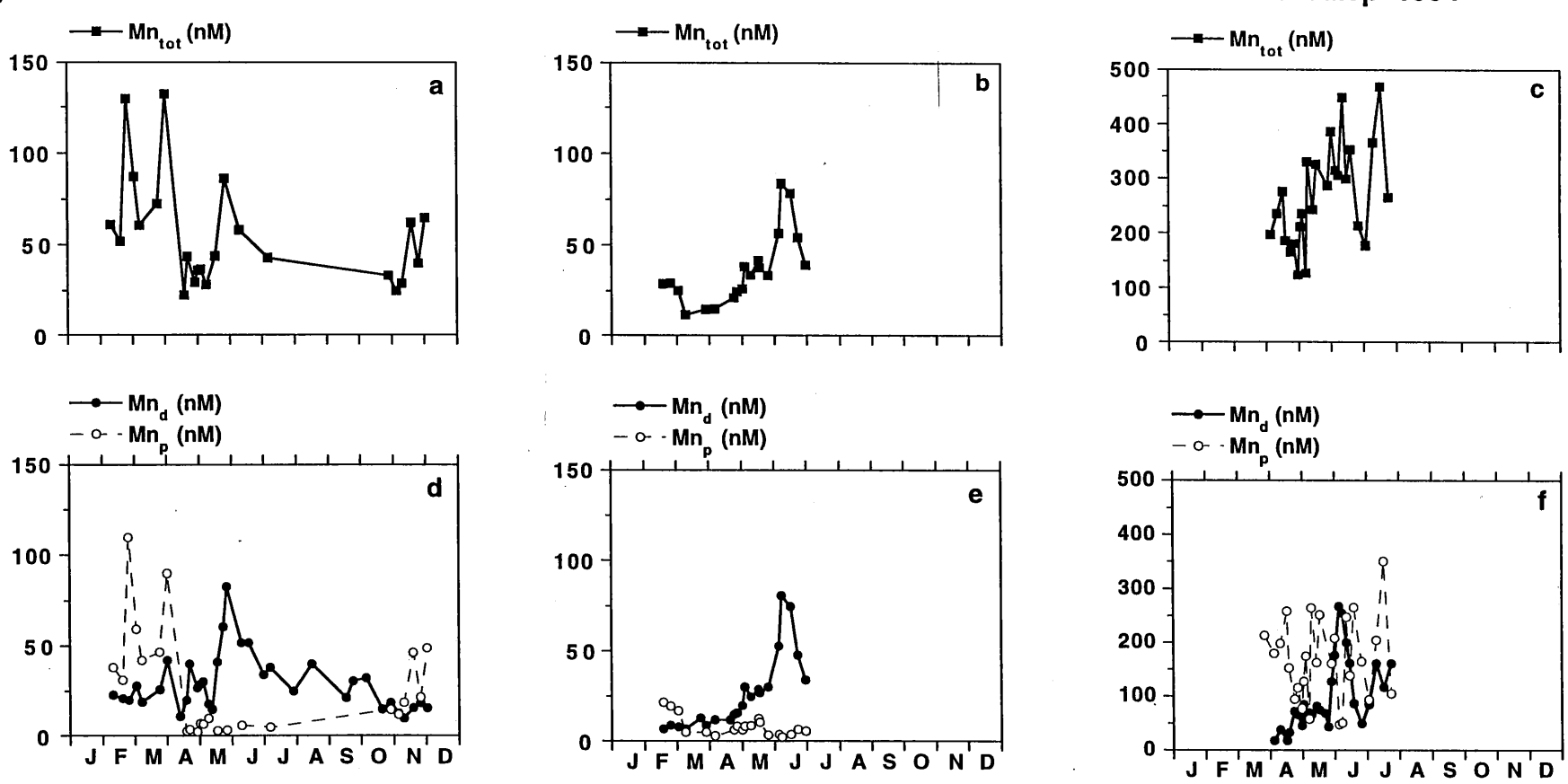
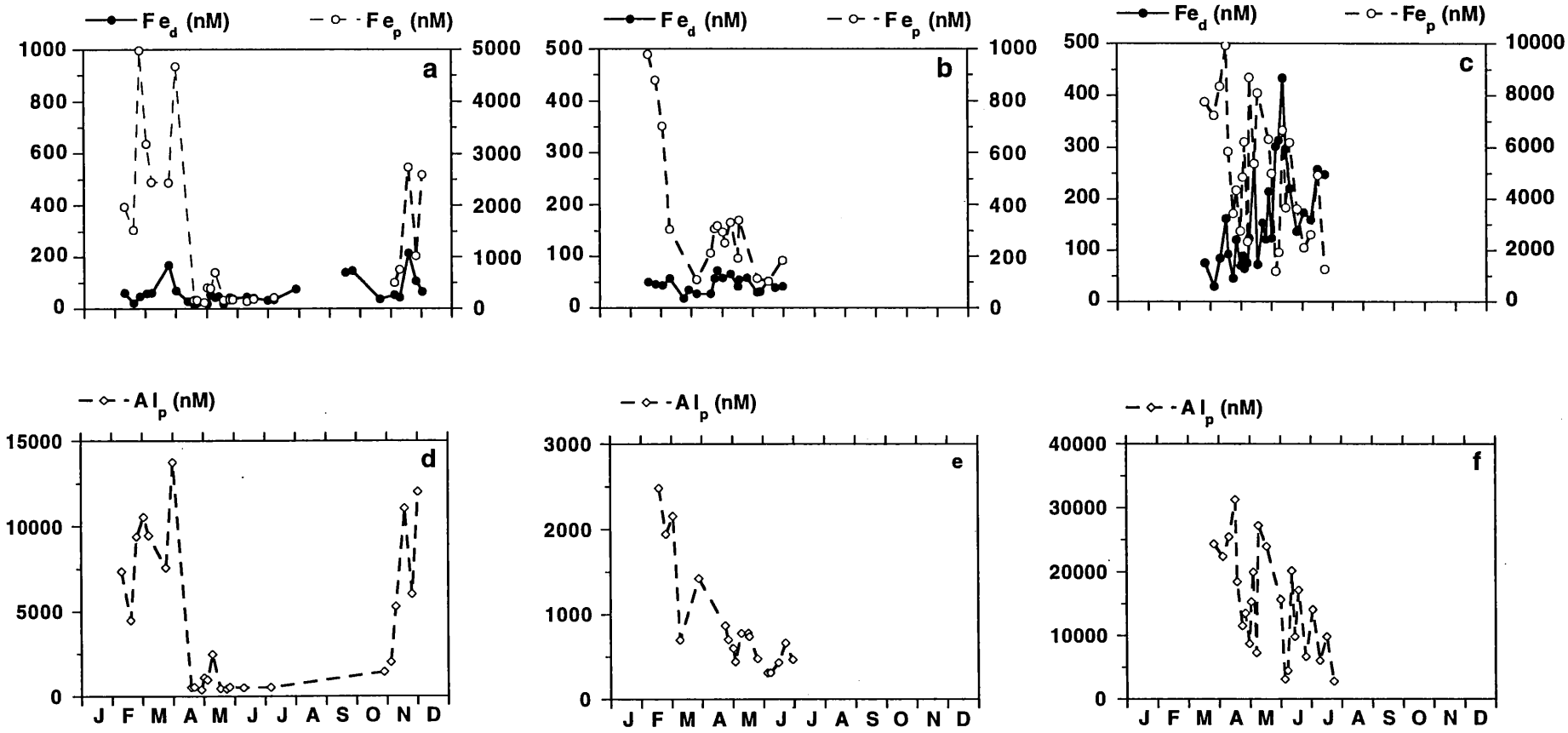

'330' 1993
'330' 1994
'Marsdiep' 1994
Mn_tot (nM)
Mn_d (nM)
Mn_p (nM)
a
b
c
d
e
f
0
50
100
150
200
300
400
500
J F M A M J J A S O N D

Fig. 7


'330' 1993
'330' 1994
'Marsdiep' 1994
Fe_d (nM)
Fe_p (nM)
Al_p (nM)
a
b
c
d
e
f
J F M A M J J A S O N D

Manuscript 4

Autecology of the marine haptophyte *Phaeocystis* sp.

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1. *Phaeocystis* physiological ecology

1.1. Life forms

The eurythermal and euryhaline genus *Phaeocystis* is one of the most widespread marine haptophytes, with most species sharing the ability to produce nearly monospecific blooms in many environments. Its unusual heteromorphic life cycle, which alternates between gelatinous colonies and different types of free-living cells (vegetative non-motile, vegetative flagellate and microzoospores), sets it apart from other members of the class (Fig.9 in Rousseau *et al.* 1994). The colonies - composed of thousands of cells embedded in a mucilaginous matrix - occasionally reach several mm in diameter. Individual cells, 3-10 µm in diameter, are distributed within the gel matrix of the colonies, which vary in form from little (20 µm) to large (1 mm) homogeneous spheres and to large ill-formed colonies invaded by bacteria and protists. This variety in colony form appears to be largely a function of life stage.

The dominance of one form over the other in natural environments has dramatic consequences for planktonic and benthic ecosystem structure and functioning (Lancelot and Rousseau 1994; Weisse *et al.* 1994) and can have severe environmental (Lancelot *et al.* 1987) and biogeochemical (Wassmann 1994) consequences. Free-living cells are heavily grazed by protozoa (Weisse *et al.* 1994) and stimulate the development of an active microbial food-web (Lancelot 1995) which retains most *Phaeocystis*-derived material in the surface waters ('regeneration-based food chain'). However a linear 'export'-food chain, with mesozooplankton grazing on protozoa (Hansen and van Boekel 1991; Bautista *et al.* 1992; van Boekel *et al.* 1992) may also develop. The trophic and geochemical role of the colonial form is more complex and depends on the colony size (Weisse 1983), the microbial colonization of senescent *Phaeocystis* colonies (Estep *et al.* 1990) and the feeding behavior and life strategy of indigenous mesozooplankton (Weisse *et al.* 1994; Wassmann 1994). The large size of colonies lowers the risk of being eaten because of the considerable time-lag in the response of large herbivores. However, the presence of overwintering meso- and meta- zooplankton in deep water

environments can result in sustained grazing. In shallow, turbid environments, colony grazing is limited due to the prevalence of immature mesozooplankton (Weisse *et al.* 1994). In addition, the gel properties of the *Phaeocystis* mucilaginous matrix (Lancelot and Rousseau 1994) combined with low aggregation properties compared to those of diatoms (Riebesell 1993) tend to maintain healthy *Phaeocystis* colonies in surface waters. *Phaeocystis* supply to the deep ocean and the benthos thus relies on the capacity of colonies to resist microbial degradation and sedimentation i.e., a compromise between the environmental characteristics and the intrinsic features of *Phaeocystis* (colony size and density) determined by the gel properties and the colonization by bacteria and protists.

Phaeocystis colonies, if present in sufficient density, are a nuisance occurrence. The mucopolysaccharide matrix of the colonies is extremely viscous and odorous, clogs nets and upon colony death, either sinks or breaks down into an organic foam. Impressive banks of this foam are regularly observed on North Sea beaches (Lancelot *et al.* 1987 and references therein). There are also many reports of fish avoiding areas of *Phaeocystis* blooms (e.g. Hurley 1982 and references therein) and of deleterious effects on shellfish (Moestrup, 1994 and references therein). In addition, there is one recent report of fish mortalities associated with *Phaeocystis*, with a substantial crop of farmed salmon lost in 1992 in Norway during a bloom period (Tangen pers. comm. in Moestrup 1994). Furthermore *Phaeocystis* is a major planktonic source of the atmospherically-important gases, dimethyl sulfide (e.g. Barnard *et al.* 1984) and methyl bromide (Saemundsdottir and Matrai, 1997).

1.2 Biogeographical distribution

1.2.1 Species number and distribution

There has been considerable confusion in the literature regarding the number of valid *Phaeocystis* species due to the lack of taxonomic criteria (see review by Sournia 1988). Recent molecular data indicate the existence of at least three colony-producing species : *P. pouchetii*, *P. globosa*, and *P. antarctica* (Medlin *et al.* 1994), in addition to the distinctive, *P. scrobiculata*, which has only been observed in the single cell phase (Moestrup 1979). The latter is ultrastructurally different from the colony-forming *Phaeocystis* species, especially in the occurrence of a nine ray pattern in its filaments and the fine structure of its scales. Aside from resolution at the molecular level, the only criteria for separation of the three colonial species are the original diagnostic features, colony shape and geographic distribution (Baumann *et al.* 1994). In both *P. globosa* and *P. antarctica*, individual cells are uniformly distributed around the periphery of the colony, whereas in *P. pouchetii*, the cells are grouped in clusters, usually of four cells, in lobes of the colony. *Phaeocystis antarctica* is present only in Antarctic coastal waters, whereas *P. globosa* is present in more temperate waters, with a growth temperature optimum of 15°C. The temperature range of *Phaeocystis pouchetii* is intermediate, being present in boreal and cold, temperate waters (Bauman *et al.* 1994).

1.2.2. Life forms distribution: the importance of inorganic nitrogen sources

Strain-related morphological and physiological characteristics appear to be of little significance with respect to the autecology and dynamics of *Phaeocystis* blooms. The shared ability to form large gelatinous colonies, demonstrated for all strains except *P. scrobiculata* constitutes the key ecological factor. Conditions prevailing for the existence of free-living and colonial *Phaeocystis* forms are thus examined irrespective of species.

Despite intensive research efforts, factors controlling the occurrence and dominance of *Phaeocystis* life forms in natural environments, and in particular the transition from the free-living to the colonial stage are not fully understood (Rousseau *et al.* 1994). The nutrient status, in particular phosphate limitation, is now believed to be a major factor driving colony formation from free-living cells (Veldhuis and Admiraal 1987). Furthermore, the dominant form of inorganic nitrogen is likely an important clue for understanding the dominance and the biogeographical distribution of the colonial stage. Experiments performed with cultures of *Phaeocystis* (Riegman *et al.* 1992) demonstrate that free-living cells outcompete colonial forms in ammonium- and phosphate-limited conditions whereas colonies dominate in nitrate-replete cultures. This suggests that free-living *Phaeocystis* cells would be prevalent in environments which rely on regenerated nitrogen and that colonial forms would rely on nitrate supply and thus would be associated with new production. The geographical distribution of free-living cells and colonies supports this hypothesis. Solitary cells are cosmopolitan in distribution, and are an important component of the haptophycean assemblage which dominates oceanic nanophytoplankton in many areas (e.g. Thomsen *et al.* 1994). They are also a seasonal dominant in some relatively pristine coastal areas including the Gulf of Maine (Keller and Haugen 1996) and the Gulf of Alaska (Booth *et al.* 1982). The abundances recorded in these areas however are up to an order of magnitude lower (e.g., *ca.* 2 10^6 cells l^{-1} in the Gulf of Maine, Keller and Haugen 1996) than the bloom concentrations typical of more eutrophic environments. The biomass of free-living cells of *Phaeocystis* is presumably kept in check by protozoan grazing pressure which in turn regenerates ammonium and phosphate.

Massive blooms of *Phaeocystis* colonies have been observed in turbulent, nutrient-rich environments at all latitudes. These dense, near-monospecific blooms regularly occur in spring, in nitrate-rich temperate and polar areas of the world ocean. In the North Atlantic, colonial *Phaeocystis* blooms have been recorded in such physically contrasting areas as temperate estuaries (e.g. Roger and Lockwood 1990), coastal bays (e.g. Jones and Haq 1963; Verity *et al.* 1988), the tidally-mixed continental coastal waters of the North Sea (e.g. Lancelot *et al.* 1987); the Norwegian (e.g. Egge and Asknes 1992) and Danish coastal waters (Rieman unpublished) and most Norwegian fjords (e.g. Sakshaug 1972; Eilertsen *et al.* 1981). In boreal and austral polar waters, *Phaeocystis* blooms have been recorded at the receding ice-edge of the Barents Sea (e.g. Rey and Loeng 1985; Wassmann *et al.* 1990), Greenland Sea (e.g. Smith *et al.* 1991), Islandic waters (e.g. Stefanson and Olafsson, 1991), Bering Sea (e.g. Barnard *et al.* 1984), Ross Sea (e.g. El-Sayed *et al.* 1983; Palmisano *et al.* 1986); Weddell Sea (e.g. Buck and Garrison 1983), Prydz Bay (e.g. Davidson and

Marchant 1992) and Bransfield Strait (e.g. Bodungen *et al.* 1986). Scattered colonies of *Phaeocystis* were also recorded in the permanently ice-free portion of the Barents Sea (Rey and Loeng 1985; Wassmann *et al.* 1990).

In all of these areas, the colonial form largely dominates. Its rapid development is sustained by new sources of nitrate of natural (winter deep convection) or anthropogenic (coastal areas under the influence of river discharge) origin as showed by the positive relationship between the maximum Chl.*a* concentration reached by colonies in each *Phaeocystis*-dominated environment and the nitrate reduction observed during the bloom (Fig.1).

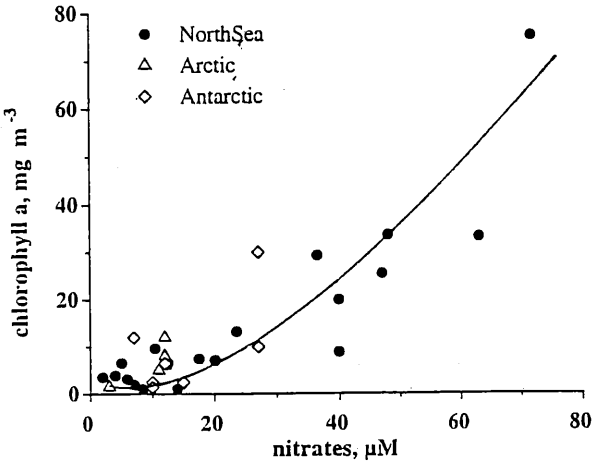

Figure 1: Empirical relationship between maximum *Phaeocystis*-Chl.*a* and nitrate reduction. Data from Rey and Loeng 1985; Wassmann *et al.* 1990; Vernet, 1991; Smith unpublished; Palmisano *et al.* 1986; El-Sayed *et al.* 1988; Rousseau *et al.* unpublished

Accordingly elevated f_{NO3} ratios (the ratio of nitrate uptake to the total inorganic nitrogen uptake rate) have been measured in the Greenland Sea (mean: 0.56, range: 0.09-0.9; Smith 1993) as well as in the continental coastal waters of the North Sea (mean: 0.62, range: 0.5 -0.8; Lancelot *et al.* 1986). In both areas, f_{NO3} decreased from 0.8-0.9 at the beginning of the *Phaeocystis* bloom to 0.4-0.5 at its decline.

1.3. Ecophysiology of *Phaeocystis* colonies

The above characteristics place *Phaeocystis* colonies at an ecological position similar to the spring diatom population which often blooms at the same time. However, the position of the maximum development of *Phaeocystis* colonies with respect to that of diatoms in the spring phytoplankton succession varies among systems and between years. Reasons for this variation - resource versus predator based competition - are not well understood, although the unique ability of *Phaeocystis* to form colonies is a common element of most hypotheses.

The gel-forming exopolysaccharides of the colonial matrix, may enable *Phaeocystis* colonies to outcompete other phytoplankters in turbulent waters by

increasing buoyancy and retention in surface waters and by avoiding consumption by indigenous mesozooplankton due to their large size (Lancelot and Rousseau 1994). In addition, the palatability of *Phaeocystis* colonies is still questionable, as large metazooplankton will feed selectively on diatoms when offered a choice between *Phaeocystis* colonies and diatoms (Verity and Smayda 1989). Some diatoms (e.g. *Chaetoceros socialis*, a species which often co-dominates with *Phaeocystis*) have developed similar adaptive mechanisms to resist sinking and grazing.

Table I : Photosynthetic characteristics - photosynthetic capacity K_{max} and light adaptation parameter I_K - of *Phaeocystis* and spring diatoms

	K_{max} mgC mgChl.a^{-1} h^{-1}	I_K µmol m^{-2} s^{-1}	Reference
BOREAL POLAR WATERS:			
Phaeocystis cells	0.9-1.5	4-29	Matrai *et al.* 1995
Phaeocystis colonies	0.8-4.2	16-57	Matrai *et al.* 1995
	5.3-13.3	32-140	Cota *et al.* 1994
			Verity *et al.* 1991
Diatoms	0.8-1.2	14-104	Cota *et al.* 1994
	0.6-7.5	9-48	Matrai *et al.* 1995
TEMPERATE WATERS:			
Phaeocystis cells	0.8-3	10-120	Lancelot, Mathot 1987
Phaeocystis colonies	2-14	120-180	Lancelot, Mathot 1987
			Lancelot unpublished
	5-15	91-144	Colijn 1983
	3-8.5	250	Verity *et al.* 1988
Diatoms	1.6-4	125-236	Lancelot, Mathot 1987
			Lancelot unpublished
ANTARCTIC WATERS:			
Phaeocystis colonies	3.5-8.1	47-144	Palmisano *et al.* 1986

Springtime populations and cultures of *Phaeocystis* colonies and diatoms display similar photosynthetic properties (Table I) suggesting that both taxonomic groups are able to adapt their photophysiology to the low light conditions prevailing in early spring. The superior photosynthetic efficiency of *Phaeocystis* free-living cells at lower light levels (Table I) may indirectly promote the prevalence of *Phaeocystis* colonies by seeding the water column with large numbers of cells for colony initiation. Furthermore, the considerable flexibility of *Phaeocystis* colonies to adapt their photosynthetic characteristics to ambient light conditions, as evidenced in the Southern Ocean (Palmisano *et al.* 1986) and in the continental coastal waters of the North Sea (Lancelot unpublished) offers an alternate explanation for the competitive success of *Phaeocystis* colonies in turbulent and turbid systems. In the Ross Sea, *Phaeocystis* colonies associated with sea-ice doubled their photosynthetic efficiency by lowering the typical value of the light adaptation parameter I_k (Platt *et al.* 1980) from about 100 to 50 µmol m^{-2} s^{-1} when drifting underneath the ice from well-

illuminated ice-free waters (Palmisano *et al.* 1986). Similarly in the near-shore continental coastal waters of the North Sea, I_k values for *Phaeocystis* exponentially decrease from 250 to 10 μ mol $m^{-2} s^{-1}$ along the SW-NW turbidity gradient (Lancelot, unpublished).

Finally, the energy (Lancelot and Mathot 1985), phosphate (Veldhuis and Admiraal 1987) and trace element (Davidson and Marchant 1987) storage capacity of the colonial matrix may also impart a competitive advantage to *Phaeocystis* colonies over diatoms when energy-costly nitrate is the dominant nitrogen source and/or ambient trace elements concentrations are depleted.

Keeping in mind the peculiar physiology of *Phaeocystis*, we now present the autecology of *Phaeocystis* colony blooms in key areas. Particular attention is given to the diatom-*Phaeocystis* colony succession and to physical and chemical conditions initiating, maintaining and limiting blooms of *Phaeocystis* colonies. For this comparison, we have not included zooplankton grazing pressure, although we acknowledge that in some systems and at certain times, it may be critical.

2. Auto-ecology of *Phaeocystis* colony blooms : case studies

2.1. Boreal and Austral polar waters

Blooms of *Phaeocystis* colonies reaching 6-8 mg Chl.*a* m^{-3} (about 7 10^6 cells per liter) are regularly observed in subarctic and arctic shelf seas at the beginning of the vernal period (April-May). In these sea-ice associated areas, *Phaeocystis* colonies are often associated with the retreating ice-edge. Bloom development is triggered by the stability induced in the upper 15-40 m surface waters by ice melt. An exception, however, is the Atlantic Current region where *Phaeocystis* colonies flourish later in the season, after water column stabilization due to surface heating (Rey and Loeng 1985; Vernet 1991; Wassmann *et al.* 1990).

The sequence of phytoplankton succession at the receding ice-edge is similar for diverse sea-ice environments. In most areas, *Phaeocystis* colonies appear first, contributing up to 95% of cell density (Vernet 1991). Populations peak in late April-early May and are distributed homogeneously in the upper mixed layer at depths of 15-40 m (e.g. Rey and Loeng 1985). The biomass maximum is limited by the winter stock of nitrate (about 12 μM). Winter silicate levels of 5-6 μM remain unutilized during this period (Smith *et al.* 1991; Stefansson and Olafson 1991). Depending on the degree of turbulence, *Phaeocystis* colonies accumulate at the pycnocline, reaching biomass levels of 12 mg Chl.*a* m^{-3} (Vernet, 1991) or densities up to 27 10^6 cells l^{-1} (Thingstad and Martunissen 1991). These deep maxima tend to be very transient however and often sediment abruptly (Wassmann 1994). Remineralization typically is completed within the aphotic water column and little *Phaeocystis*-derived material reaches the bottom (Wassmann *et al.* 1990).

Low concentrations of *Chaetoceros socialis* and *Pseudo-Nitzschia delicatissima* are present at the time of the *Phaeocystis* bloom, but the main diatom population, composed of *Chaetoceros* spp., *Thalasiossira* sp., *P. delicatissima* and *N. cylindrus,* develops later, as *Phaeocystis* declines, reaching maximum density at the depth of the nutricline (Rey and Loeng 1985). Occasionally, the diatom community blooms

before *Phaeocystis*, as low silicate concentrations (~ 1 μM) have been observed at the time of *Phaeocystis* blooms (Wassmann *et al.* 1990). Reasons for this reverse succession are not known.

In the Southern Ocean, blooms of *Phaeocystis* colonies have been recorded in waters influenced by a receding ice-edge as well. *Phaeocystis* blooms are particularly well documented in the Ross Sea where they dominate in the extreme southern (El-Sayed *et al.* 1983) and southeastern areas (e.g. Palmisano *et al.* 1986). The southwestern region of the Ross Sea is characterized by diatom-dominated blooms, which are also enhanced by ice melt (Smith and Nelson 1985). Melting ice not only increases water column stability but also supplies a significant inoculum of viable sea-ice diatoms into the water column. The sea-ice community in the southwestern Ross Sea is composed largely of diatoms (Smith and Nelson 1985).

The seasonal pattern in phytoplankton biomass in the Ross Sea follows the retreating ice edge which is driven in the southeastern region by catabatic winds and by solar heating at the northern ice-edge. Phytoplankton community succession at the ice-edge from south to north shows a shift from a *Phaeocystis*-dominated to a diatom-dominated population (Fig. 2). The reasons for this change are not known, although changes in the degree of vertical stability may be important. The observed ice-edge diatom bloom in the southwestern Ross Sea typically coincides with the formation and persistence of a sharp halocline and pycnocline at a depth of 20-30 m (Smith and Nelson 1985). High intensity winds, typical in the area adjacent to Ross Island, prevent the establishment of vertical stability. We suggest that *Phaeocystis* colonies, with the buoyancy attributable to the mucilaginous matrix, are better able to maintain themselves in the surface waters than diatoms. We also believe that *Phaeocystis* is better able to adapt to the lower ambient light conditions associated with deep vertical mixing.

Maximum recorded *Phaeocystis* biomass in the Southern Ocean is 12 mgChl.*a* m^{-3} (30 10^6 cells l^{-1}; Palmisano *et al.* 1986). Although ca. 31 μM of nitrate has been consistently measured in this area, it has not been fully utilized during the bloom period. Based on this nitrate level, the observed biomass is *ca.* 50% lower than might be expected (30 mg Chl.*a* m^{-3} or 60 10^6 cells l^{-1}; Fig.1). Nitrate concentrations of 10 to 19 μM have been measured in the water column at the peak of the *Phaeocystis* bloom (e.g. Palmisano *et al.* 1986). Light and/or iron limitation have been suggested to explain this paradigm (de Baar *et al.* 1997). The expected 30 mg Chl.*a* m^{-3} level of biomass has been observed in the spring beneath the annual sea ice in Prydz Bay (Davidson and Marchant 1987). In this area, as in the Ross Sea (Palmisano *et al.* 1986), high densities of *Phaeocystis* colonies were found beneath the sea ice, advected from ice-free surface waters where their growth was initiated. Populations appear to be maintained in this dim environment both from the buoyant properties of the *Phaeocystis* matrix, which keep the colonies just beneath the sea ice, and the rapid photoadaptative capability of *Phaeocystis* to the variable light environment (Palmisano *et al.* 1986). The exceptionally high concentrations of *Phaeocystis* recorded beneath the ice in Prydz Bay (Davidson and Marchant 1992) may have been sustained by additional iron present in the ice. Iron released in this

way during sea ice melt has been observed in the Atlantic sector of the Southern Ocean (de Baar *et al.* 1997)

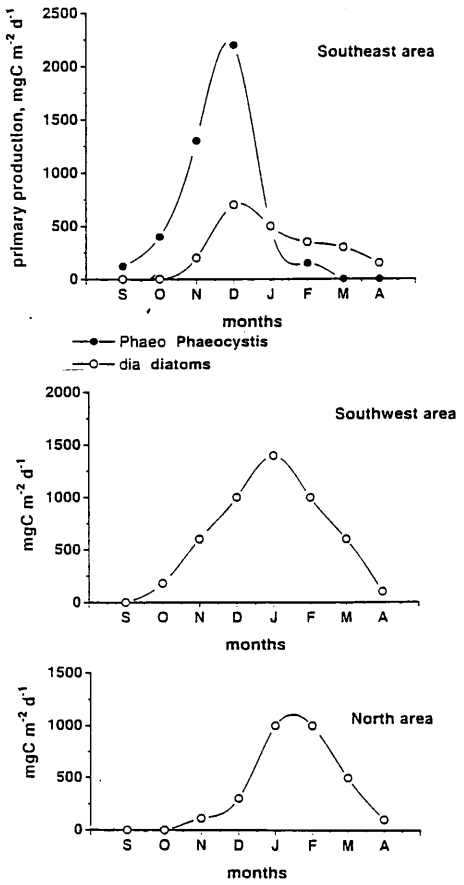

Figure 2: *Phaeocystis*- and diatom- daily growth in the Ross Sea

2.2. North Atlantic coastal waters under the influence of riverine inputs

Large blooms of *Phaeocystis* colonies are recurrent phenomena in the fjords and coastal environments of Norway, occurring in early spring, between early March and late May, depending on the latitude. In these systems, the spring bloom utilizes winter stocks of nutrients while later spring-summer blooms rely on the riverine supply of nutrients enriched in snow melt from the mountains. Phytoplankton seasonal succession is particularly well documented in Trondheimfjord (1963-1966; Sakshaug 1972) and Balsfjorden (1977-1978; Eilertsen *et al.* 1981), respectively at 64°N and 69°N. In both fjords, the spring bloom is initiated by increasing light levels, rather than water column stability, which occurs later in the spring as freshwater inputs increase (Sakshaug 1972; Eilertsen *et al.* 1981). The composition of the spring bloom exhibits considerable interannual variability, sometimes it is

dominated by diatoms, other years by *Phaeocystis*, or by co-occurrence (Fig.3). In years when *Phaeocystis* blooms are co-incident with diatoms, the main diatom is typically *Chaetoceros socialis*.

The amplitude and extent of the colonial *Phaeocystis* bloom also show significant interannual variation. In 1977, *Phaeocystis* cell numbers ranged from 0.5 and 2 10^6 cells l^{-1} in Balsfjorden throughout the spring and summer, from March to September. In contrast, the 1978 *Phaeocystis* bloom was short in duration (April), but very intense, with maxima up to an order of magnitude higher than observed in 1977 (Fig.3). Furthermore, the relative abundance of diatoms and *Phaeocystis* in the spring bloom period was quite different, with diatoms dominant in 1977 and *Phaeocystis* in 1978. The reasons for this are not known, although freshwater inputs may contribute to water column and nutrient conditions which may favor one form over the other.

Similar *Chaetoceros-Phaeocystis* successions are typical in the Trondheimfjord as well, although *Phaeocystis* maxima typically persist only for one month (March-April; Sakshaug 1972). As in the northern fjord, large interannual variation occurs with cell densities ranging from 1 to 8 10^6 cells l^{-1}. Higher levels have been observed in areas under river influence (Sakshaug 1972).

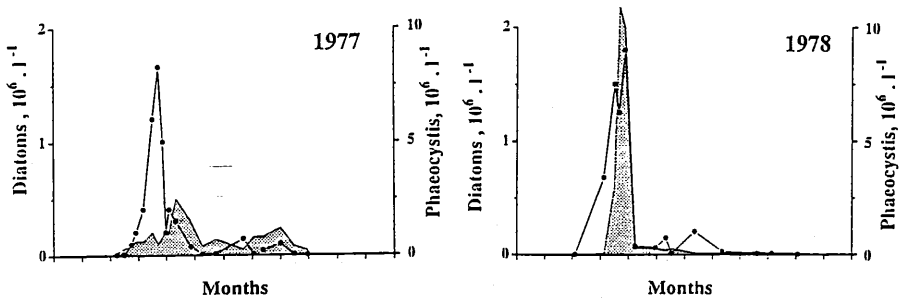

Figure 3: Diatom-*Phaeocystis* (gray area) colonies succession in the Balmsfjord in 1977 and 1978 (redrawn from Eilertsen *et al.* 1981).

Massive blooms of *Phaeocystis* colonies, with cell numbers up to 10^8 cells l^{-1}, are observed every spring in the continental coastal waters of the North Sea, which receives the discharge of seven major west-European rivers. The fluvial basins, characterized by high population densities and intense industrial and farming activities, have introduced new and unbalanced sources of nutrients into coastal waters (Lancelot 1995and references therein). The general N, P, Si enrichment of the coastal area is characterized by winter concentrations an order of magnitude higher than those in adjacent Atlantic waters (Lancelot 1995). Qualitative changes in the nutrient ratios supplied by the freshwater sources have resulted in an excess of nitrate with respect to silicate, which has implications for the growth of coastal diatoms (Lancelot 1995).

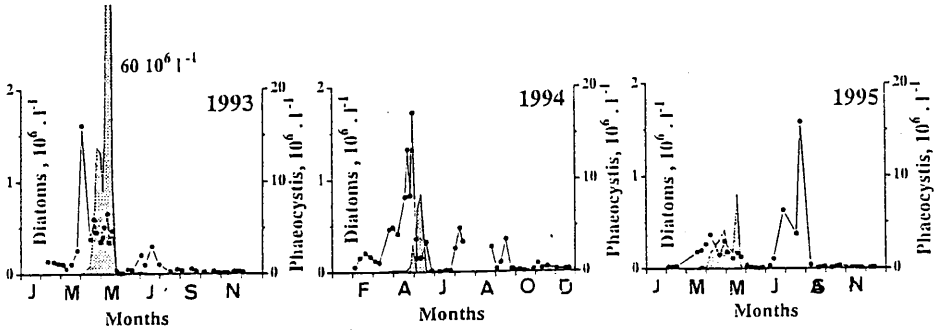

Figure 4: Diatom-*Phaeocystis* (grey area) colonies succession in the continental coastal waters of the North Sea (station 330). Rousseau *et al.* unpublished

Since 1988, the spring phytoplankton community at station 330 (N 51°26.05, E 02°9.08) located in Belgian coastal waters, has been monitored extensively. The general phytoplankton succession is similar to that characterizing Norwegian fjords. Diatoms initiate the vernal bloom in early spring (February-March) (Fig.4). *Phaeocystis* colonies appear somewhat later. Large interannual variations in *Phaeocystis* biomass are also evident in this system, with *Phaeocystis* cell maxima varying over two orders of magnitude (Fig.4). The early-spring diatom community is composed of small, neritic species including *Thalassiosira nordenskoldii*, *T. rotula*, *Asterionella glacialis*, *Thalassionema nitzschoïdes*, *Plagiogramma brockmanii*, and *Skeletonema costatum*. This diatom community, although typical of the North Sea, is not observed in the Norwegian fjords; its growth is controlled by the winter concentration of silicate (Rousseau *et al.* unpublished). *Phaeocystis* colonies appear as the early spring diatom community declines. The later diatom community composed of *Chaetoceros* spp. and *Schroederella* sp., both of which require relatively low levels of silicate, appear at the same time. As the *Phaeocystis* bloom develops, additional diatoms, *Cerataulina* sp. and *Rhizosolenia* spp., mainly *R. delicatula,* become abundant as well. The fluctuations in this community of larger diatoms and *Phaeocystis* colonies (Fig.4) appear to result from the competition for nitrate, suggesting that both occupy the same ecological niche. The extreme differences in the abundance of diatoms and *Phaeocystis* colonies recorded in 1993 and 1994, with *Phaeocystis* dominating in 1993 and diatoms in 1994 (Fig.4) has been correlated to differences in late winter meteorological conditions prevailing during 1993 (cold and dry) and 1994 (temperate, high rainfall). Rousseau *et al.* (unpublished) show evidence that rainfall - strength, frequency and duration - controls the amount and the relative contributions of nitrate and silicate of freshwater origin, with high silicate associated with high rainfall. When silicate is available, the *Rhizosolenia* sp. and *Cerataulina* sp. diatom community may be able to outcompete *Phaeocystis* colonies.

It appears that under non-limiting concentrations of silicate and nitrate, diatoms outcompete *Phaeocystis* colonies in temperate North Atlantic coastal waters. This contrasts with the succession from *Phaeocystis* colonies to diatoms observed in boreal and austral polar waters. Based on its apparently superior photoadaptative properties, *Phaeocystis* should have an advantage in all systems in early spring. However, temperature-dependent growth experiments performed on diatom and

Phaeocystis communities sampled throughout the winter-spring in the coastal North Sea (Fig.5) demonstrate that the early spring diatom community grows better than *Phaeocystis* at the temperatures typical of early spring (5-8°C).

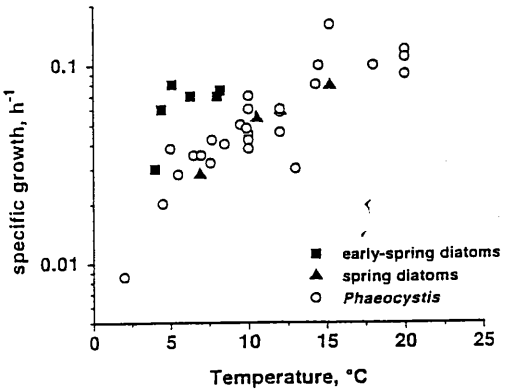

Figure 5: Relationship between temperature and specific growth of diatoms and *Phaeocystis* colonies. (Lancelot *et al.*, unpublished data)

3. Conclusions and perspectives

There are many questions which remain about the physiological ecology of the genus *Phaeocystis*. The success of *Phaeocystis* in marine systems has been attributed to its ability to form large gelatinous colonies during its life cycle (Lancelot and Rousseau, 1994). These colonies are functionally similar to the large, chain-forming or colonial diatoms that occupy the same spring bloom niche in turbulent, tidally- or seasonally-mixed water columns. In most environments, the magnitude of the *Phaeocystis* colony bloom appears to be regulated by nitrate availability. An exception is the Southern Ocean where iron shortage may prevent optimal utilization of the high nitrate resources. An analysis of the diatom-colonial *Phaeocystis* succession in contrasting *Phaeocystis*-dominated ecosystems demonstrates that, while there are large interannual and spatial variations, there appear to be consistent differences between ecosystem types. In polar waters, *Phaeocystis* precedes the main diatom bloom in most areas; in temperate waters, the reverse is true. *Chaetoceros socialis* emerges as a common co-dominant with *Phaeocystis* colonies in each *Phaeocystis*-dominated ecosystem, typically blooming slightly before but never achieving biomass comparable to *Phaeocystis* colonies.

The efficient and adaptable photophysiology of *Phaeocystis*, combined with superior buoyant properties imparted by the colonial matrix, make *Phaeocystis* extremely competitive in turbulent environments or under low light conditions. These conditions are typical in polar waters where *Phaeocystis* colonies initiate the vernal season. Diatoms appear to dominate early spring blooms in these waters only when vertical stratification occurs (Smith and Nelson, 1985). This scenario is not typical of Northeast Atlantic coastal waters and bays where the spring bloom is initiated by a diatom community composed of small neretic species, which also do well at low light levels but grow better than *Phaeocystis* colonies at the temperatures

of 5-8°C prevailing in early spring. While a comparison of regional differences in bloom formation and succession is helpful, the differences observed between regions may be simply a result of biogeography, i.e. the occurrence of different species. These observed differences reinforce the need to establish the systematics of this genus in a comprehensive way. In polar environments, the dominant species appear to be *Phaeocystis pouchetii* and *P. antarctica*, in Arctic and Antarctic coastal waters respectively. In Northeast Atlantic temperate waters (North Sea), the species appears to be *P. globosa.* In Northwest Atlantic temperate waters (Gulf of Maine, Narragansett Bay), the species appears to be *P. pouchetii.* The temperature tolerances of these species are different enough to account for at least some of the reported contradictions in autecology from different environments. Thus, it is not unreasonable to conclude that *Phaeocystis* precedes diatoms in polar waters due to its superior photophysiology, while in temperate environments, where the less eurythermal *P. globosa* dominates, diatoms have the edge in early spring. Although what we know about the physiology of these algae is consistent with the empirical observations of diatom-*Phaeocystis* colony succession, our knowledge is incomplete. We need to develop appropriate mechanistic models which consider the unique physiological characteristics of diatoms and of *Phaeocystis.* Comparative laboratory experiments of the physiology of the different species of *Phaeocystis* are also required.

Little can be said about the autecology of the solitary cells of *Phaeocystis. Phaeocystis* colonies are rare in regions with a permanently stratified water column. In these areas, solitary cells of *Phaeocystis*, which appear to be more competitive at low nutrient concentrations, are more prevalent. Changes in trophic function and structure result from these alterations in life stage. With the flagellate stage, an entirely different community develops, based on a microbial food web, with regeneration of nutrients and carbon in surface waters. Understanding the life cycle of *Phaeocystis* is critical to understanding the ecosystems where it occurs. How do the cells overwinter? Is there a benthic stage? Is the flagellate stage linked to sexuality? These are all questions which need attention. The role of other trophic levels in bloom dynamics also warrants further investigation. Do microzooplankton play a critical role by controlling the single cell phase and is bloom development ultimately controlled by metazooplankton grazing upon microzooplankton? Is there any basis for allelopathy in these blooms and if not, why are bacteria and protozoa not associated with healthy colonies? Finally, is there any basis for the observations that *Phaeocystis* is toxic? If so, can this toxicity be induced or are the toxic effects associated with anoxia or hypoxia, due to the viscous character of the mucilage ?

To date efforts have concentrated on understanding the physico-chemical conditions enhancing the exponential development of *Phaeocystis* colonies, rather than the fate of *Phaeocystis* colonies and *Phaeocystis* bloom termination. Grazing and sedimentation in particular appear to rely on the presence (deep environments) or absence (shallow water environments) of overwintering meso- and meta-zooplankton (Weisse *et al.*, 1994) and on the water column characteristics (Wassmann, 1994). The sudden termination of *Phaeocystis* blooms in all *Phaeocystis*-dominated systems highlights the need for additional investigations on the relationship between turbulence and the formation of *Phaeocystis*-derived

aggregates. Although *Phaeocystis* colonies do not apparently readily aggregate (Riebesell, 1993), changes in water column vertical structure, especially if driven by salinity change, may be important. The gelling properties of the colony matrix, which equilibrate colony density to that of seawater, may cause *Phaeocystis* colonies to rise in response to salinity increases or sink when salinity decreases. Further investigations should focus on the interaction of physics and *Phaeocystis* colonies at different stages of their development, particularly in frontal structures such as river plumes and the receding ice edge in polar systems.

The unique heteromorphic life cycle of *Phaeocystis* imparts versatility and adaptive abilities to this genus that are not shared by other co-occurring phytoplankters. The colonial life stage is an obvious and important factor in the structure and function of coastal ecosystems, but the ubiquity of the less-well studied solitary flagellate stage may make it an important contributor to oligotrophic environments as well. *Phaeocystis* is present and important as a primary producer in almost every oceanic environment. Its occurrence as a nuisance species is directly linked to eutrophication, and as such, is one of the few species where such a clear, causal relationship is apparent.

4. Acknowledgments

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Manuscript 5

12. Ecology of *Phaeocystis*: the key role of colony forms

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Abstract

Species of *Phaeocystis* exhibit phase alternation between individual cells and gelatinous colonies. They regularly form dense, nearly specific blooms, in very contrasting nutrient-rich areas of the world's oceans. The uniqueness of this genus of marine phytoplankters rests not only in its ubiquity but mostly in its peculiar physiology and ecology. No other marine phytoplankter has ever been shown to dominate an entire ecosystem; no other marine species distinguishes itself by a complex polymorphic life cycle that induces dramatic changes in the structure and functioning of planktonic and benthic food-webs as well as in the biogeochemistry of trace elements. The main features of the ecology of *Phaeocystis*-dominated ecosystems are analysed with regard to the *Phaeocystis* life cycle, and to recent data on the biochemistry and nutrient (major and trace element) metabolism of the different morphological forms that succeed each other during *Phaeocystis* bloom development, in relationship to the behaviour of bacteria and micro-, meso-, and meta-zooplankton and the physical structure of the marine habitat. Particular emphasis is given to the biological functioning of *Phaeocystis* colonies that constitute by far the most important morphological forms in natural environments, as determined from the analysis of the structure and function of the mucilaginous matrix embedding the cells. Evidence is presented that the most remarkable ecological and biogeochemical properties of *Phaeocystis*-dominated ecosystems are attributable to the capacity of *Phaeocystis* colonial cells to synthesize, in nutrient-deprived conditions, exopolysaccharides capable of gelation.

The Haptophyte Algae (ed. J. C. Green and B. S. C. Leadbeater), Systematics Association Special Volume No. 51, pp. 229–45. Clarendon Press, Oxford, 1994.

Phaeocystis: a very widespread genus but still poorly understood

Phaeocystis is one of the most widespread marine genera and also one of the most intriguing. *Phaeocystis* species are among the few phytoplankters exhibiting phase alternation between free-living solitary cells (3–9 μm in diameter) and gelatinous colonies (palmelloid stage, reaching several millimetres in diameter) (Kornmann 1955; Verity *et al.* 1988, 1992; Rousseau *et al.* 1994). Species of *Phaeocystis* are euryhaline and eurythermal, and, in the spring, they regularly forms dense, near monospecific blooms in very contrasting nutrient-rich areas of the world ocean (Davidson and Marchant 1992). In most of these areas, the colonial forms largely dominate and are sustained by new sources of nutrient which are either of natural (Buck and Garrison 1983; Smith *et al.* 1991) or anthropogenic origin (Al Hassan 1990; Cadée and Hegeman 1990, 1991; Lancelot *et al.* 1992).

The uniqueness of *Phaeocystis* lies not only in its massive blooms, but also in its exceptional physiology and ecology. No other marine phytoplankters have been ever shown to dominate an entire ecosystem, or to distinguish themselves by a complex polymorphic life cycle (Kornmann 1955; Rousseau *et al.* 1994) that induces dramatic changes in the structure and functioning of the planktonic (e.g. Lancelot *et al.* 1987; Davidson and Marchant 1992; Weisse *et al.* 1994) and benthic food-webs (e.g. Pieters *et al.* 1980; Rogers and Lockwood 1990), as well as in the biogeochemistry of trace elements (Davidson and Marchant 1987; Lubbers *et al.* 1990) and sulfur (Liss *et al.* 1994).

The biology of *Phaeocystis* and the ecology of *Phaeocystis*-dominated systems have recently been reviewed by Davidson and Marchant (1992). Other recent publications have dealt with more specific aspects of *Phaeocystis* ecology such as species diversity and associated biochemistry (Baumann *et al.* 1994); the life cycle (Rousseau *et al.* 1994), the fate of *Phaeocystis* colonies (Weisse *et al.* 1994; Thingstad and Billen 1994; Wassmann 1994), and DMS production (Liss *et al.* 1994). All these authors concluded that the main features of *Phaeocystis*-dominated ecosystems are driven by the physiology, biochemistry, and peculiar life cycle of this marine phytoplankter. Surprisingly, our basic knowledge in this field is still limited and fragmentary due to uncertainties surrounding species identity, and the incomplete morphological and biochemical descriptions of *Phaeocystis* life forms found during bloom development.

This paper constitutes an attempt to establish the cause and effect relationship between the peculiar physiology of *Phaeocystis*, and the structure and functioning of *Phaeocystis*-dominated ecosystems. This will be done on the basis of recent investigations on the *Phaeocystis* life cycle, and on the carbon and nutrient (major and trace element) metabolism of the different successional morphotypes occurring during a *Phaeocystis* bloom.

Particular emphasis is given to the biological functioning of *Phaeocystis* colonies since they constitute by far the most important morphological form in natural environments. In order to avoid confusion due to inter-population variability, and so leave aside the unresolved species diversity problem, all the reported data refer to microscopic descriptions, chemical analyses, and process-oriented studies carried out on *Phaeocystis* populations originating from one area, the southern North Sea. On the basis of this analysis, current knowledge on *Phaeocystis* in the world ocean is briefly re-appraised and some general features of the factors controlling the structure and functioning of *Phaeocystis*-dominated ecosystems are put forward.

The sequence of *Phaeocystis* life forms in the southern North Sea and its ecological implications

The complex sequence of morphological forms exhibited in a pure culture of *Phaeocystis* colonies (German Bight strain) during their growth (Kornmann 1955) has been shown to correspond to those during a *Phaeocystis* bloom development in the southern North Sea (Rousseau *et al.* 1994; Fig. 12.1). Under natural conditions, however, the successive phases of a *Phaeocystis* bloom are accompanied by the development of a large variety of heterotrophic organisms feeding selectively on some *Phaeocystis* morphotypes, so producing complex and dynamic food webs (Fig. 12.2). The main features of the *Phaeocystis* bloom in the southern North Sea can be summarized as follows.

Phaeocystis succeeds an early spring diatom bloom and dominates the phytoplankton community at more than 90% of cell number, nearly wholly of the colonial form (Lancelot and Mathot 1987). Colonies originate from the transformation of free-living cells and multiply by budding or division (Fig. 12.1; Kornmann 1955). A low density of free-living cells is always present, being controlled by colonial lysis and grazing by microzooplankton (Fig. 12.2; Martens 1981; Admiraal and Venekamp 1986; Weisse and Scheffel-Möser 1990).

Colony forms exhibit a marked temporal evolution, from small (20–50 μm in diameter) spherical colonies, often localized on *Chaetoceros* setae, to large (mm) healthy colonies of various sphere-derived forms devoid of attached heterotrophic microorganisms during the exponential phase of the bloom development (Fig. 12.1), to senescent irregular colonies (Fig. 12.1) progressively invaded by protozoa actively grazing on colonial cells (Fig. 12.3) and, finally, at the end of the bloom, to the formation of sticky aggregates (Fig. 12.1) colonized by various heterotrophic organisms developing complex microbial networks (Fig. 12.3). This progressive transformation of homogeneous biological entities to heterogeneous

Some events of *Phaeocystis* sp. life cycle in natural environments

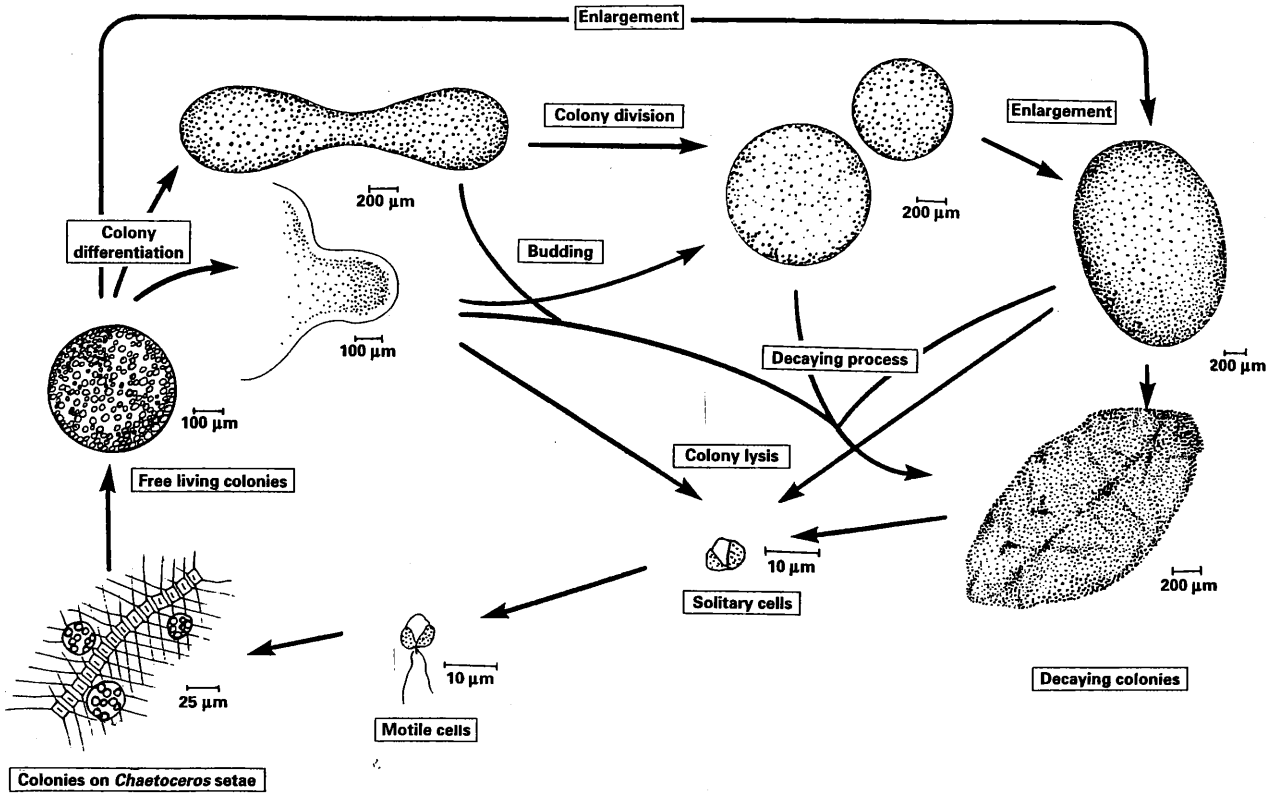


Fig. 12.1 The sequence of *Phaeocystis* morphotypes during spring bloom development in Belgian coastal waters (southern North Sea). Redrawn from Rousseou *et al.* (1994).

microbial aggregates appears to be driven by the maturation of the colony itself. This is made possible by the unpalatibility of healthy colonies for co-occurring mesozooplankton (Hansen and Boekel 1992; Fransz *et al.* 1992). Knowledge of environmental factors controlling these transformations is very scarce.

In the southern North Sea, as in most *Phaeocystis*-dominated environments, the termination of the bloom is characterized by the sudden complete disappearance of senescent colonies and their derived aggregates due to either accelerated sedimentation (possibly resulting from increasing density due to colonization), microbial disintegration in the water column, consumption by mesozooplankton, or advective export. Little is known at present about the relative importance of these mechanisms which depend on the physical characteristics of the marine habitat, the food quality and density of the aggregates, the feeding behaviour of mesozooplankton, and the biodegradability of the organic matter from decaying colonies. Thus, the biochemical composition of the primary colonies may be important. Evidence for low biodegradability of the *Phaeocystis*-derived polymeric material is given by the large accumulation of sea foam observed at bloom decline in the open sea (Rogers and Lockwood 1990), and on the beaches (Bätje and Michaelis 1986; Lancelot *et al.* 1987) of the shallow turbulent southern North Sea.

This simple visual description of the *Phaeocystis* event in the southern North Sea highlights the key role of *Phaeocystis* colonies in determining ecosystem structure and functioning. The appraisal of its ecological function is now approached through the determination of the biological functioning of *Phaeocystis* colonies.

The biological functioning of *Phaeocystis* colonies

A *Phaeocystis* colony originates from the transformation of one free-living cell (Kornmann 1955; Rousseau *et al.* 1994). Once formed, each colony constitutes an entity inside which the non-motile cells grow and divide (Lancelot and Mathot 1985). How far this aggregation makes *Phaeocystis* colonies particularly well adapted to growth in nutrient-rich conditions and to outcompete other phytoplankters is examined on the basis of the biological functioning of *Phaeocystis* colonies (Fig. 12.4). This in turn is determined from an analysis of the structure and function of the mucilaginous matrix.

Planktonic food-web of *Phaeocystis*-dominated ecosystem

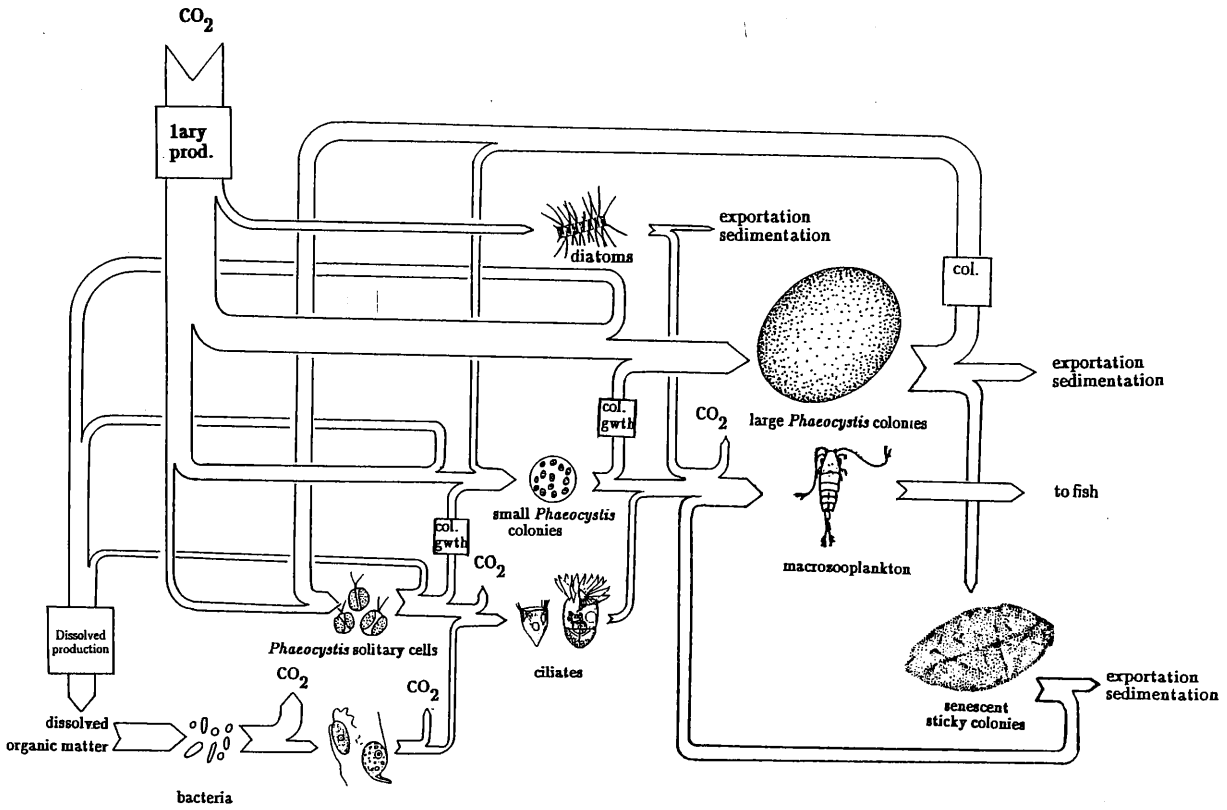


Fig. 12.2 Schematic representation of the structure of the planktonic food-web of the *Phaeocystis*-dominated ecosystem of the southern North Sea.

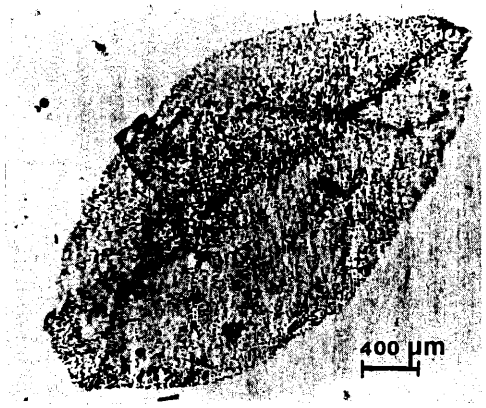
The mucilaginous matrix: chemical characterization and biosynthesis

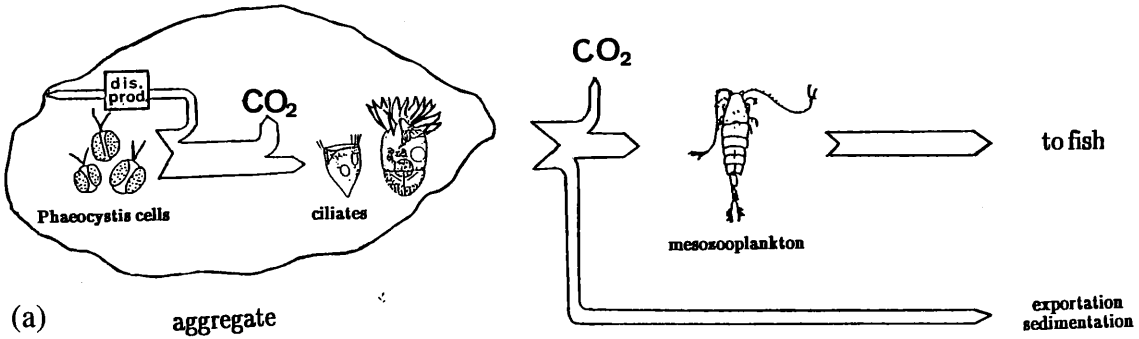
Various microscopic and chemical methods have been used to determine the biochemical composition of *Phaeocystis* cells and colony matrix. Current knowledge, although preliminary, indicates that the mucilaginous matrix is formed through gelation of carboxylated and sulphated polysaccharide chains promoted by salt (calcium and magnesium) bridges (Boekel 1992). These polysaccharides are actively secreted by the colony cells, under the control of light and inorganic nutrients (Lancelot and Billen 1985; Lancelot *et al.* 1986). The exopolymeric synthesis is, however, not triggered by nutrient depletion (Lancelot 1983). At the height of the bloom, when nutrients are depleted, more than 80% of the photoassimilated carbon is devoted to the synthesis of exopolymeric substances, compared with about 50% when nutrients are not limiting. Thus, the contribution of the mucilaginous matrix to the *Phaeocystis* colony biomass increases dramatically from about 50% to 90% during bloom development (Rousseau *et al.* 1990). Also, the seawater content of the gel increases with the size of the colony, suggesting that the gel compactness decreases with colony growth (Fig. 12.1). To what extent this apparent modification of gel consistency is accompanied by changes in gel properties (e.g. gel strength, swelling ability) has not been determined, but might be a clue to understanding the progressive transformation of healthy colonies to aggregates as observed under natural conditions (Fig. 12.1). Determination of gel firmness requires, however, a complete analysis of the composition and structure of the polysaccharide chains, i.e. the type and sequence of sugars in the polymeric units that make up the mucilaginous matrix. Basic knowledge in this field is, however, still limited and would benefit from further investigations.

The physiological function of the mucilaginous matrix.

The energy storage function of the mucilaginous matrix is now well agreed. Numerous process studies have demonstrated that the polysaccharides composing the matrix constitute an energy-storing substrate, which is catabolized by the colony cells during the light-limited period to meet their biosynthetic requirements (Fig. 12.4; Lancelot and Mathot 1985; Veldhuis and Admiraal 1985; Lancelot *et al.* 1986; Veldhuis *et al.* 1991). This reservoir thus gives to colonial cells a selective advantage over free-living cells to benefit from high nutrient concentrations in low-light environments by increasing the energy storage capacity of each cell. The

Food-web in *Phaeocystis*-derived aggregates





(a) aggregate

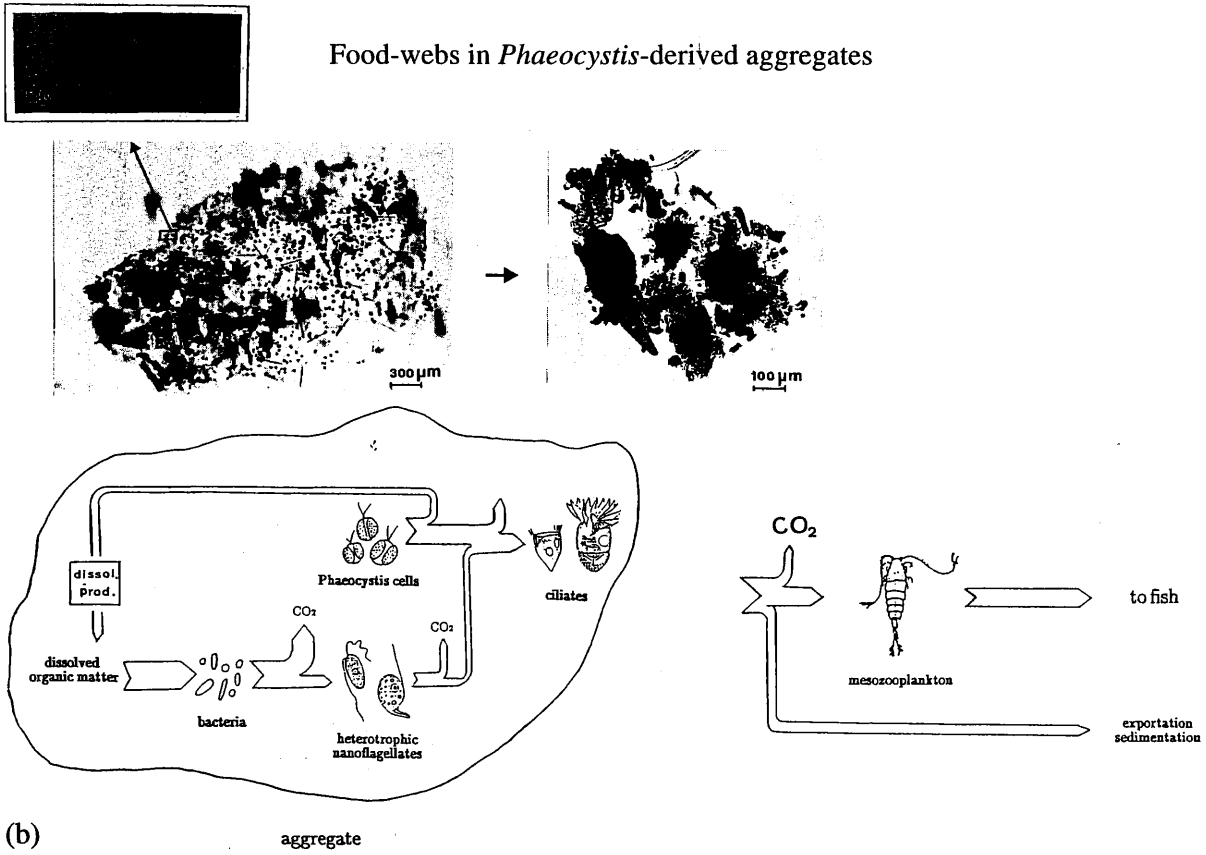

Food-webs in *Phaeocystis*-derived aggregates
300 µm
100 µm
dissol. prod.
dissolved organic matter
bacteria
heterotrophic nanoflagellates
Phaeocystis cells
ciliates
CO₂
CO₂
CO₂
mesozooplankton
to fish
exportation sedimentation
(b)
aggregate

Fig. 12.3 Schematic representation of the structure of the microbial food-web in aggregates derived from *Phaeocystis* colonies in the southern North Sea, illustrating the progressive invasion of a senescent colony by various heterotrophic micro-organisms. (**a**) Invasion of a decaying *Phaeocystis* colony by protozoa (mostly ciliates) grazing on colony cells, and (**b**) subsequent development of complex and changing microbial food-webs involving *Phaeocystis* cells, bacteria, and bacterivorous and herbivorous protozoa. Photographs by V. Rousseau and S. Becquevort.

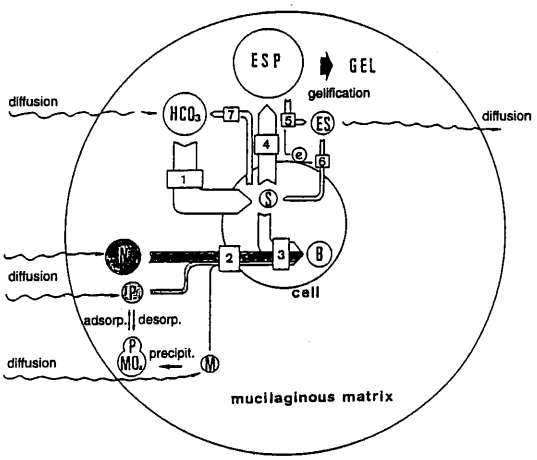
energetic gain depends on the colony size. Over an order of magnitude increase to a colony of 1 mm^3 (the average colony size at the height of a *Phaeocystis* bloom, Rousseau *et al.* 1990), the pool of energetic substrates available to the colony has been estimated to increase by a factor of 20.

On the other hand, experimental evidence suggests that the catabolism of colonial polysaccharides greatly modifies the chemical structure of the gel by providing intermediates of lower molecular weight inside the colonial matrix (Fig. 12.4; Veldhuis and Admiraal 1985). These chemical changes modify gel firmness and could indirectly be the cause of the progressive transformation of healthy homogeneous colonies to microbe-invaded decaying colonies. Indeed this transition mostly occurs at the height of the bloom when light is possibly limited due to the high *Phaeocystis* biomass.

The nutrient and trace element sequestering function of the mucilaginous matrix

Besides its structural role and energy storage function, the mucilaginous matrix of *Phaeocystis* colonies has been shown recently to act as a reservoir for phosphorus (Veldhuis *et al.* 1991) and trace elements, especially manganese (Davidson and Marchant 1987; Lubbers *et al.* 1990) and probably iron. These sequestration mechanisms result from a suite of abiotic chemical reactions, resulting from both the gel properties and the biological activity of colonial cells (Fig. 12.4; Lubbers *et al.* 1990). The colonial matrix constitutes a 3-dimensional network, embedding cells and seawater, and acts as diffusion barrier for solute molecules (Lubbers *et al.* 1990). Consequently, physico-chemical conditions (nutrients, pH, Eh) inside the colonies can be significantly different from those of the external medium due to the biological activity of colonial cells. For example, Lubbers *et al.* (1990) demonstrated that high pH conditions, corresponding to that of Mn/Fe oxyhydroxide precipitation, can be reached inside *Phaeocystis* colonies in culture when exposed to optimal light conditions. Manganese precipitation is strongly light-dependent and is slightly reversible under prolonged dark periods, making dissolved manganese available for colonial cells (Fig. 12.4; Lubbers *et al.* 1990). Deposits of Mn/Fe oxyhydroxides inside the mucilaginous matrix in turn drive the

(a) light metabolism and chemical reactions



(b) dark metabolism and chemical reactions

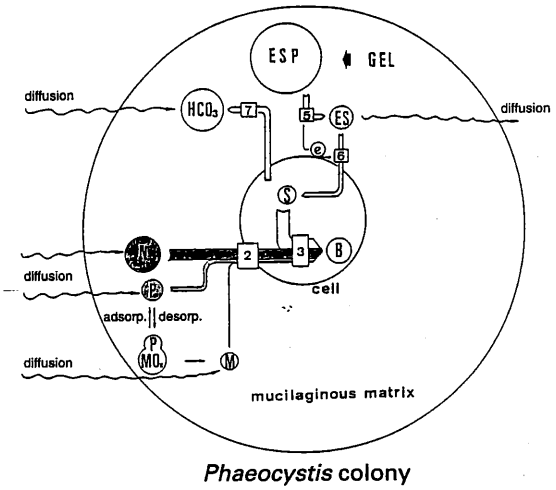


Fig. 12.4 Schematic representation of: (a) the light and (b) the dark metabolism of *Phaeocystis* colonies. *Intracellular and intracolonial pools*: B = cellular biomass; e = exoglucosidase; ES = extracellular oligosaccharide; ESP = extracellular polysaccharide; M = trace metals (mainly Mn and Fe); MO_x = trace metal oxy-hydroxide; N = inorganic nitrogen (nitrate and ammonium); P = phosphate; S = monomeric precursor. *Processes*: 1 = photosynthesis; 2 = nutrient uptake; 3 = cellular growth; 4 = polysaccharide secretion; 5 = polysaccharide hydrolysis; 6 = polysaccharide catabolism.

sequestering of other metals and of phosphate by adsorption from the enclosed seawater. This reaction is reversible, making phosphate available to colonial cells when phosphate in the external medium is depleted (Fig. 12.4; Veldhuis *et al.* 1991). Rough calculations, based on the potential amount of manganese precipitate inside a 1 mm^3 *Phaeocystis* colony (Lubbers *et al.* 1990), suggest that this nutrient storage mechanism can meet trace metal and phosphorus requirements of individual colonial cells. This makes colonial cells more competitive than free-living cells when ambient concentrations of these nutrients reach limiting values. Interestingly, nitrate and ammonium are not adsorbed on Mn/Fe oxyhydroxides, and no mechanism of nitrogen sequestration in *Phaeocystis* colonies has yet been demonstrated.

The biological functioning of *Phaeocystis* colonies: its implication for the structure and functioning of *Phaeocystis*-dominated ecosystems

The near-complete dominance of colony forms in *Phaeocystis*-dominated ecosystems all over the world ocean indicates that similar mechanisms have developed both to decrease losses through grazing, sinking, and degradation, and to outcompete free-living *Phaeocystis* cells as well as other phytoplankters. Thus, the biological functioning of *Phaeocystis* colonies outlined above from southern North Sea data (Fig. 12.4) might be extended to the genus worldwide. Each *Phaeocystis* colony can be regarded as a biofilm in which several related and mutually-dependent biological and chemical processes are occurring for the benefit of the aggregated biological entity. Biogeographical and ecological features of *Phaeocystis*-dominated systems are here reappraised on this basis.

Our analysis suggests that the most remarkable physiological and ecological properties of *Phaeocystis* colonies are attributed to their capacity to synthesize gel-forming nutrient-deprived exopolysaccharides. Firstly, this mechanism leads either directly or indirectly to the building of significant supplementary intracolonial reserves of energetic substrates, phosphate, and trace elements for the benefit of colonial cells. The intracolonial energy reservoir might well explain the competitive edge *Phaeocystis* colonies have over free-living cells and other phytoplankters when energy-costly nitrates constitute the nitrogen source (Riegman *et al.* 1992). It may also explain the dominance of colonial forms in nitrate-enriched environments. To what extent the phosphate and micro-nutrient sequestrating mechanisms allow *Phaeocystis* to outcompete other algae by depriving them of essential elements is difficult to assess properly at present and will depend on the ambient chemistry of the marine system. Presumably, the role of trace metal sequestration by *Phaeocystis* colonies as mediators of species succession would be of less significance in the

polluted coastal waters of the southern North Sea than in the Southern Ocean, which is typically characterized by low trace metal availability (Nolting *et al.* 1991; Westerlund and Ohman 1991). Much has yet to be known, however, about the regulation of Mn/Fe precipitation and dissolution inside the colony and the subsequent phosphate adsorption/desorption, and about the diffusion properties of the gelatinous matrix, but there is no doubt that *Phaeocystis* blooms, due to their importance and the dominance of colonial forms, play an important role in biogeochemical cycles. The fate of sequestrated trace elements is then strongly linked to the fate of *Phaeocystis* colonies.

Secondly, the nutrient-deprived mucous secretion, by rapidly increasing *Phaeocystis* colony size while lowering significantly its nutritional value, can be seen as causing the general unpalatibility of *Phaeocystis* colonies to most grazers (Weisse *et al.* 1994). Related to this, polysaccharides produced through colony lysis have been shown to be refractory to bacterial degradation (Thingstad and Billen 1994), causing accumulation of dissolved organic matter in the water column (Billen and Fontigny 1987).

Thirdly, the gel characteristics of the mucilaginous matrix and its rapid turnover rate considerably reduce the average density of the whole colony bringing it closer to that of surrounding seawater, with attendant benefits for suspension. The firmness of the gel is determined by the sugar residue composition of the exopolysaccharide and the Ca/Mg content of seawater (both possibly varying between *Phaeocystis*-dominated ecosystems). Simple calculation shows that this method of determining density would prevent *Phaeocystis* colonies from sinking in stratified seas where stratification is due to salinity differences. Such areas are typically coastal and polar seas, where massive blooms of *Phaeocystis* colonies have been observed (Davidson and Marchant 1992), stratification being due to river outflow and ice melting, respectively. On the other hand, gelation offers no particular advantage in the fully mixed tidal areas like the southern North Sea. Counteracting this mechanism, Mn/Fe oxyhydroxide deposits inside the colony modify the sinking characteristics of *Phaeocystis* colonies by significantly increasing colonial density. The present-day failure to appreciate properly how these opposite mechanisms regulate the density of *Phaeocystis* colonies might well explain the general confusion surrounding an appreciation of the sedimentation of *Phaeocystis* colonies in natural environments of contrasting hydrodynamics (Riebesell 1993; Wassmann 1994).

The sudden termination of *Phaeocystis* blooms, commonly characterized by the formation of senescent colonies and aggregates (Fig. 12.1) followed by their massive disappearance through either specific grazing on aggregates (Estep *et al.* 1990), sedimentation (Wassmann *et al.* 1990; Wassmann 1994), or dissolution in the water column (Billen and Fontigny 1987), while perceived as a perturbance, can also be seen as a consequence of

Phaeocystis physiology. Alteration of the chemical composition and structure of the mucilaginous matrix, due to *Phaeocystis* dark catabolism, constitutes one possible autogeneous mechanism triggering the decay of mature colonies and their subsequent colonization by various microorganisms through the modification of their attachment properties. The successive colonization of decaying colonies by attached auto- and heterotrophic microbial communities creates microenvironments based on regenerated production. This production, through nutrient regeneration, possibly enhances the bacterial degradation of the nitrogen-deficient polymers of mucus. Aggregate formation and transformation can thus considerably modify the density and food quality of the primary *Phaeocystis* colonies.

The subsequent fate of *Phaeocystis*-derived aggregates greatly varies between shallow and deep-sea environments, according to the physical structure of the marine habitat and the zooplankton present (Wassmann 1994). Trophodynamically, the formation of *Phaeocystis*-derived aggregates can be seen as a subtle mechanism to induce low grazing pressure on healthy colonies by copepods, and would constitute one explanation of the often high secondary biological production associated with *Phaeocystis*-dominated ecosystems. Mesozooplankton grazing on *Phaeocystis*-derived aggregates has, however, no impact on bloom regulation. Only the selective grazing by protozoa on *Phaeocystis* cells could be of significance for bloom regulation (Hansen and Boekel 1991). In this way, colony division and budding, as commonly observed during *Phaeocystis* bloom development (Verity *et al.* 1988; Rousseau *et al.* 1994; Fig. 12.2), might be seen as a subtle way of delaying aggregate formation and so escaping grazing by mesozooplankton. In addition, colony division, by providing smaller-sized healthy colonies, probably also reduces *Phaeocystis* colony losses by sedimentation. Thus, it is not only the ability to form gelatinous colonies of high biological competitivity that contributes to the success of *Phaeocystis* as a bloom-forming genus, but also the occurrence in their life history of various colony division events (Fig. 12.1) which are probably driven by physical conditions.

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The life cycle of *Phaeocystis* (Prymnesiophyceae): evidence and hypotheses

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Abstract

The present paper reviews the literature related to the life cycle of the prymnesiophyte *Phaeocystis* and its controlling factors and proposes novel hypotheses based on unpublished observations in culture and in the field. We chiefly refer to *P. globosa* Scherffel as most of the observations concern this species. *P. globosa* exhibits a complex alternation between several types of free-living cells (non-motile, flagellates, microzoospores and possibly macrozoospores) and colonies for which neither forms nor pathways have been completely identified and described. The different types of *Phaeocystis* cells were reappraised on the basis of existing microscopic descriptions complemented by unpublished flow cytometric investigations. This analysis revealed the existence of at least three different types of free-living cells identified on the basis of a combination of size, motility and ploidy characteristics: non-motile cells, flagellates and microzoospores. Their respective function within *Phaeocystis* life cycle, and in particular their involvement in colony formation is not completely understood. Observational evidence shows that *Phaeocystis* colonies are initiated at the early stage of their bloom each by one free-living cell. The mechanisms controlling this cellular transformation are still uncertain due to the lack of information on the overwintering *Phaeocystis* forms and on the cell type responsible for colony induction. The existence of haploid microzoospores released from senescent colonies gives however some support to sexuality involvement at some stages of colony formation. Once colonies are formed, at least two mechanisms were identified as responsible of the spreading of colony form: colony multiplication by colonial division or budding and induction of new colony from colonial cells released in the external medium after colony disruption. The latter mechanism was clearly identified, involving at least two successive cell differentiations in the following sequence: motility development, subsequent flagella loss and settlement to a surface, mucus secretion and colony formation, colonial cell division and colony growth. Aggregate formation, cell motility development and subsequent emigration from the colonies, release of non-motile cells after colony lysis on the other hand, were identified as characteristic for termination of *Phaeocystis* colony development. These pathways were shown to occur similarly in natural environments. In the early stages of the bloom however, many recently-formed colonies were found on the setae of *Chaetoceros* spp, suggesting this diatom could play a key-rôle in *Phaeocystis* bloom inception. Analysis of the possible environmental factors regulating the transition between the different phases of the life cycle, suggested that nutrient status and requirement of a substrate for attachment of free-living cells would be essential for initiation of the colonial form. Physical constraints obviously would be important in

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determining colony shape and fragmentation although autogenic factors cannot be excluded. Some evidence exists that nutrients regulate colony division, while temperature and nutrient stress would stimulate cell emigration from the colonies.

1. Introduction

Phaeocystis is one of the few marine phytoplankters exhibiting an heteromorphic life history. While two different cell types — vegetative cells and flagellates (zoids) — were already identified in the early beginning of this century as *P. globosa* Scherffel (Scherffel, 1900) and *P. pouchetii* (Hariot) Lagerheim (Ostenfeld, 1904), the first description of the general feature of the *Phaeocystis* life cycle is due to the detailed microscopic work of Kornmann (1955). This morphological study conducted on a cultured *P. globosa* strain isolated from Dutch coastal waters in the North Sea, evidenced the high complexity of the cycle, characterized by the alternance between different free-living cells and mucilaginous colonies of non-motile coccoid cells (the palmelloid stage). Colonies were shown to widely vary in shape and size, reaching several mm at the stationary stage of their growth. Apart from colonial cells, three different types of *Phaeocystis* flagellate free-living cells with likely different functions in the cycle were identified by Kornmann (1955): the swarmers, the microzoospores and the macrozoospores, varying between 3 and 9 μm in diameter. The occurrence of these various morphological cell types with additional reference to non-flagellate free-living cells, was later reported by Kayser (1970) and Parke et al. (1971).

Both morphological forms — free-living cells and colonies — have been reported to occur in the natural environment. Among the different species, the flagellate stage has been commonly recorded in absence of any colonies in oligotrophic waters of the Atlantic (Parke et al., 1971; Estep et al., 1984), Pacific (Moestrup, 1979; Booth et al., 1982; Hallegraeff, 1983; Hoepffner and Haas, 1990) and Mediterranean Sea (Delgado and Fortuño, 1991). Reversely, colony forms are predominant in nutrient enriched waters and are responsible for massive developments (El-Sayed et al., 1983; Eilertsen and Taasen, 1984; Rey and Loeng, 1985; Bätje and Michaelis, 1986; Weisse et al., 1986; Davidson and Marchant, 1987; Lancelot et al., 1987; Gunkel, 1988; Al-Hasan et al., 1990). The predominance of one morphological form on the other has been shown to have a strong influence on the trophodynamic structure of *Phaeocystis*-dominated ecosystems, due to the large size difference existing between both forms (Lancelot et al., 1987; Davidson and Marchant, 1992). *Phaeocystis* free-living cells, due to their small size, have been shown to be actively grazed by protozoa (Admiraal and Venekamp, 1986; Weisse and Scheffel-Möser, 1990) emphasizing the importance of the microbial food-web. Colonies, on the other hand, while little grazed in shallow environments (Hansen and van Boekel, 1991; Weisse et al., 1994), were shown to constitute a source of food for some mesozooplankton and metazoa species in deep-waters environments (Weisse et al., 1994).

The full knowledge of *Phaeocystis* life cycle, including the detailed description of all morphological forms as well as the factors controlling the transition from one form to another is thus prerequisite for understanding the ecological structure and functioning of *Phaeocystis*-dominated ecosystems. Numerous morphological studies were conducted for this purpose under laboratory conditions, using unialgal *Phaeocystis* cultures (Kayser, 1970; Parke et al., 1971; Veldhuis and Admiraal, 1987; Rousseau et al., 1990; Cariou, 1991; Riegman et al., 1992), mesocosm (Verity et al., 1988a, b) and field (Bätje and Michaelis, 1986; Veldhuis et al., 1986; Cadée, 1991) conditions. Surprisingly, nothing really new could be deduced from these investigations since the morphological description of some stages of *Phaeocystis* life cycle by Kornmann (1955). Even in recent laboratory studies, a great deal of confusion subsists about the different life forms, since they can be quite difficult to distinguish using conventional observation techniques. Flagellates are mentioned in numerous papers on *Phaeocys-*

tis (e.g. Riegman et al., 1992): in most cases, it is not clear, however, whether authors observed flagellates (swarmers) sensu Kornmann (1955) or microzoospores. An additional difficulty stems for the considerable taxonomic confusion and uncertainties about identity of *Phaeocystis* species or strains (Sournia, 1988; Baumann et al., 1994). In field observations, data interpretation is sometimes difficult due to the possible presence of different *Phaeocystis* species and of selective grazers feeding preferentially on one morphological stage.

In this paper, existing data on *Phaeocystis* life cycle are reappraised in the light of unpublished microscopic and flow cytometric observations in culture and field conditions. On this basis, evidence and new hypotheses about the *Phaeocystis* life cycle and its controlling factors are presented.

Referring to the criteria developed by Jahnke and Baumann (1987) and Baumann et al. (1994) for identifying the different *Phaeocystis* species, nearly all investigations made on cultured material refer to the only *P. globosa* Scherffel species. Although it is the most widely used taxa in literature, very few informations concern indeed the life cycle of *P. pouchetii* (Hariot) Lagerheim (Ostenfeld, 1904; Gunkel, 1988) and no reference to the life cycle of *P. scrobiculata* Moestrup, has been made in literature. Even, the colonial stage of this latter species, has, at the present time, never been observed. It is therefore questionable whether the sequence of events and regulating factors are the same for the different identified species. Here, we will always refer to *P. globosa* Scherffel, unless mentioned otherwise. Moreover, in order to avoid extending the confusion that already exists in literature, we will always use Kornmann's (1955) nomenclature for referring to the different cell types, despite the warning made by Sournia (1988) for a blind use of words such as spore, zoid, swarmer,... This deliberate choice is justified by the fact that Kornmann's (1955) observations constitute still today the most complete and the only comparative study of the different *Phaeocystis* cell types.

2. Observations in culture

2.1. The different Phaeocystis cell types

Beside colonial cells, four different *Phaeocystis* free-living cells have been described, based on their size, motility and DNA content:

Free-living cells derived from the transformation of colonial cells released into the external medium

At least two morphotypes of free-living cells originating from the transformation of colonial cells have been identified on basis of their size and motility:

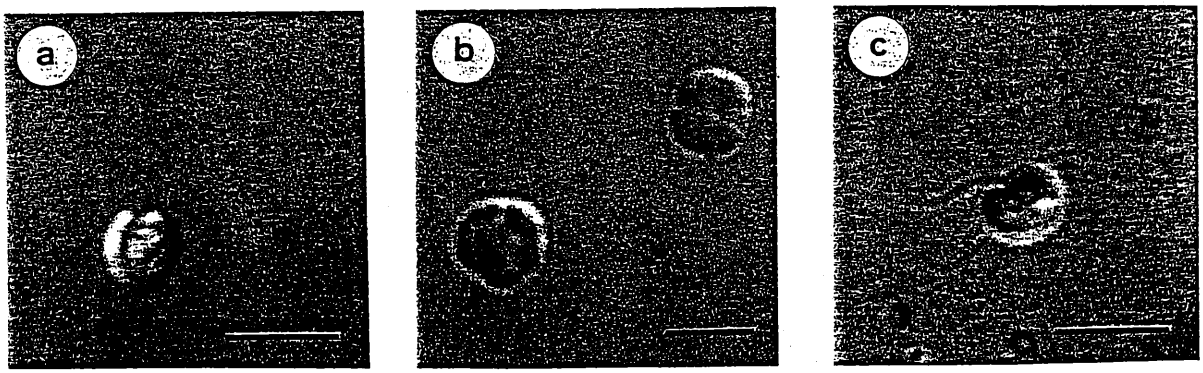

Fig. 1. Free-living cell types of *P. globosa*. Cells were fixed with glutaraldehyde 1% and viewed under Nomarski interference contrast. Scale bar = 5 μm. (a) Flagellate (swarmer) that appeared a few hours after the release of non-motile colonial cells due to colony disruption (strain ROSKO-A); (b) Non-motile cells (Strain NIOZ); (c) Microzoospore that appeared in a 2 month-old culture (strain PCC540) (photogr. by R. Casotti).

Flagellates or swarmers

These cells (Fig. 1a) were identified as flagellates produced after colony disruption, when initially non-motile colonial cells released from the colonial matrix in the culture medium, develop flagella within a few hours (Kornmann, 1955; Cariou, 1991). The life span of these flagellates is however very short (Kornmann, 1955: "Das kurzzeitige Schwärmerstadium...") and it is not clear whether these cells are capable of cellular division. These motile cells possess two flagella, one haptonema and their size is quite similar to that of original colonial cells, i.e. 4.5–8 μm (Kornmann, 1955).

Non-motile free-living cells

Visual observation gives evidence of the short life span of the swarmers: within 24 to 48 h, their majority (90%; Cariou, 1991) disperse in the culture flasks, lose their motility and settle usually on the walls and the bottom of the culture flasks (Kayser, 1970; Cariou, 1991). These non-motile free-living cells (Fig. 1b) are similar to colonial cells, in particular with respect to the cell size (Rousseau et al., 1990) and cannot be differentiated from colonial cells released into the medium immediately after colony disruption. The size similarity of swarmers and non-motile free-living cells is confirmed by data of light scattering (size index) measured by flow cytometry (Table 1). Moreover, comparison of flow cytometric signature (Table 1) indicates that both cell types are characterized by the same ploidy level. These cells have been shown capable of vegetative division (Kayser, 1970; D. Vaulot and R. Casotti, unpublished data) and have a strong ability to generate new colonies by secreting the polysaccharidic substances composing the colony matrix (Kayser, 1970). By successive divisions, the cell number increases in the colony while this latter is increasing in size (Kornmann, 1955). This sequential pathway constitutes the most common mechanism to induce the formation and growth of new colonies. However, there is presently not enough evidence that the flagellate cell stage is a necessary intermediate for initiating colony formation and that the above pathway constitutes the only mechanism to generate *Phaeocystis* colonies.

Table 1
Flow cytometric signatures of the different free-living cell types of *P. globosa*. Forward and right angle scatters (FALS and RALS respectively) are relative indexes of size, while the DNA level of G1 phase indicates the ploidy level of the cells. Mean ± standard error of each parameter are expressed relative to 2.07 μm fluorescent beads (Pandex). n = number of samples analysed. Method: Analytical protocol slightly modified from Boucher et al. (1991) as follows: Preservation of cells in liquid nitrogen after fixation with glutaraldehyde 1% or paraformaldehyde 0.5%. Staining with 25 μg/ml of Chromomycin A3 (Sigma) and flow cytometric analysis at 457 nm and 100 mW [EPICS V (Coulter Electronics)]. (R. Casotti, unpubl. data).

	Non-motile cells	Flagellates (swarmers)	Microzoospores
FALS	8.51 ± 0.21	12.84 ± 0.54	4.51 ± 0.79
RALS	0.47 ± 0.01	00.44 ± 0.00	0.24 ± 0.05
DNA level of G_1 phase	1.14 ± 0.02	01.04 ± 0.09	0.58 ± 0.03
n	128	2	6
Strains	DCZ02 NIOZ PCC540 ROSKO-A ROSKO-E	PCC540	PCC540 ROSKO-A ROSKO-E

Strains: DCZ02 and NIOZ (provided by M. Veldhuis and W. van Boeckel, Texel, The Netherlands); PCC540 (provided by the Plymouth Culture Collection, Plymouth, U.K.); ROSKO-A and ROSKO-E (isolated in Roscoff, France).

The property of these cells to adhere to solid surfaces explains their label "benthic stage" (Kayser, 1970; Parke et al., 1971; Sieburth, 1979; Chang, 1984; Sournia, 1988). There is nevertheless absolutely no evidence for a truly differentiated benthic stage, as observed in *Hymenomonas carterae* Braarud, another prymnesiophyte (von Stosch, 1967).

Microzoospores

Kornmann (1955) identified a second type of flagellate cells, called microzoospores because of their smaller size (3–5 μm). Microzoospores (Fig. 1c) have been identified in senescent cultures after colony disappearance (Kornmann, 1955) or in conjunction with non-motile cells and colonies (R. Casotti, unpubl. obs.). The process of microzoospore formation is presently unknown. Interestingly, flow cytometric signature indicates that

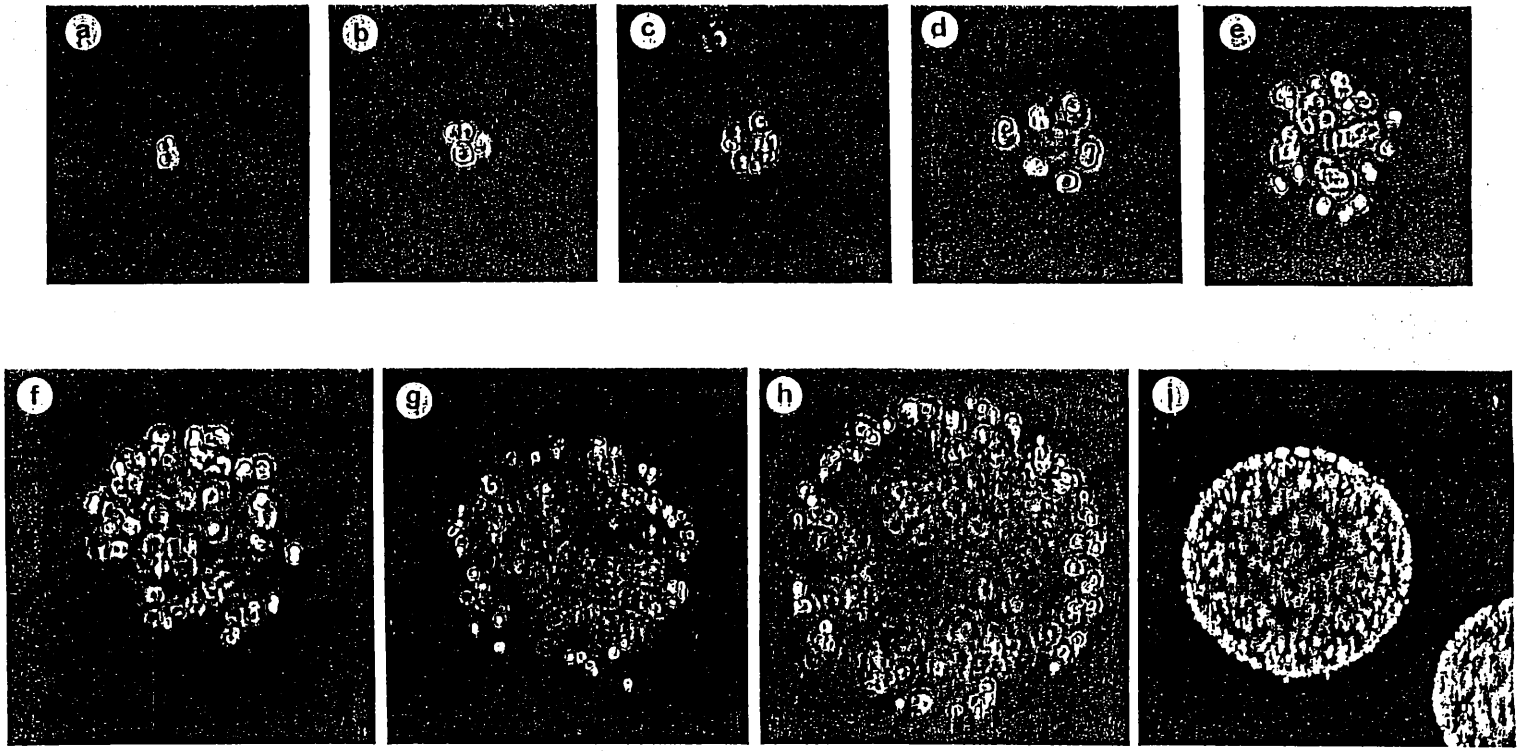

Fig. 2. Different development stages of *P. globosa* colonies (strain from Plymouth Culture Collection). Culture conditions: inoculum with colonial cells released from their matrix by mechanical disruption; culture medium of Veldhuis and Admiraal's (1987) with NO_3^-, NH_4^+ and PO_4^{3-} concentrations: 50, 25 and 5 μM, respectively; temperature: 10°C; irradiance: 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ under 12 h light: 12 h dark cycle. Microphotographs were taken under inverted microscope (Leitz Fluovert) from living specimens sampled daily and representing the predominant stages of colony development in culture during a 10-day period. Colony diameters are respectively: a. 14 μm; b. 28 μm; c. 43 μm; d. 61 μm; e. 108 μm; f. 148 μm; g. 232 μm; h. 290 μm; i. 925 μm (photogr. by V. Rousseau).

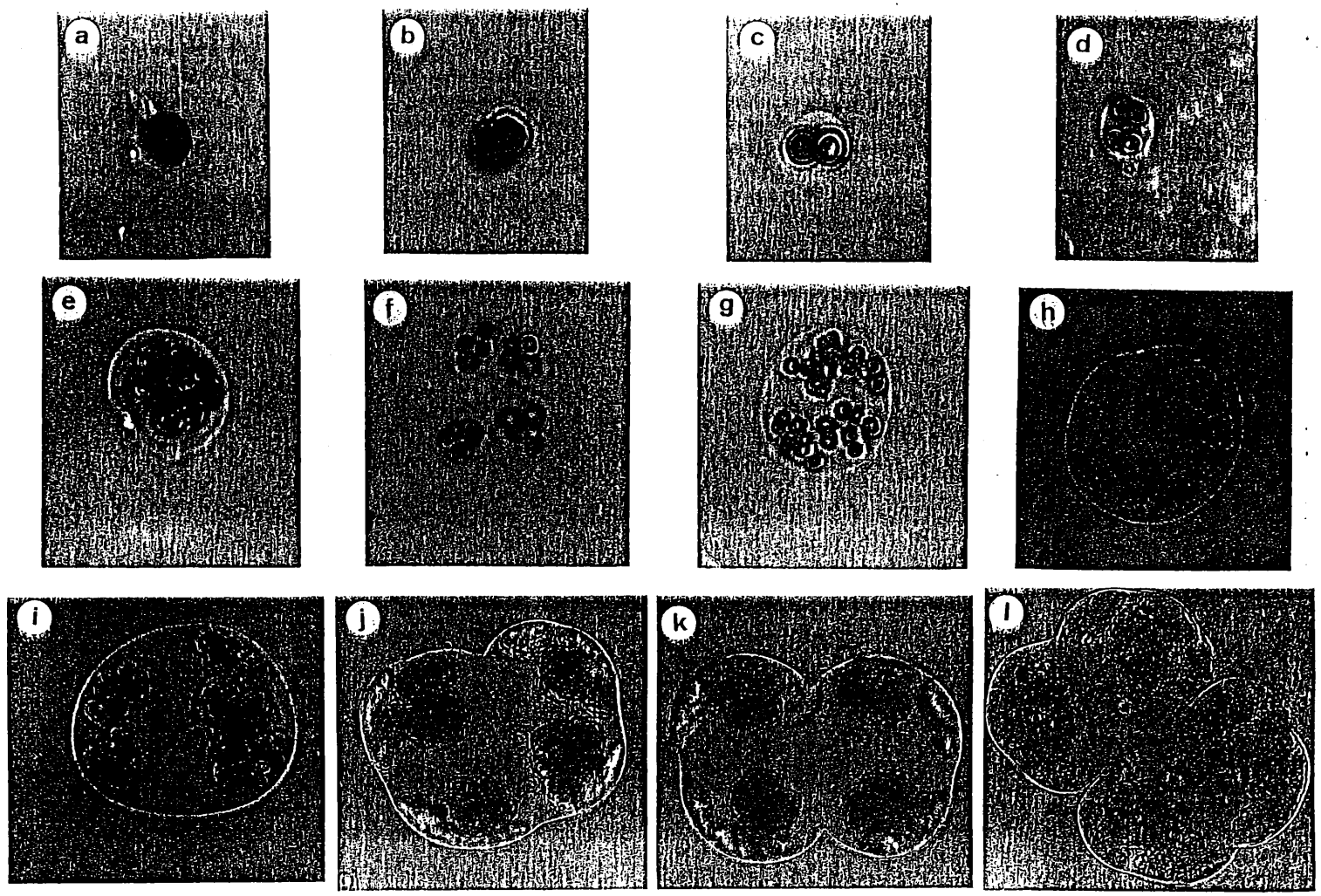

Fig. 3. Sequential development of *P. pouchetii* (strain isolated from the Greenland Sea) from free-living cells to cloud-like colonies. The characteristic grouping of cells within the mucilaginous matrix is clearly visible since the 16-cell stage (picture f). Culture conditions: inoculum with free-living cells originating from a culture in exponential phase; culture medium of Jahnke and Baumann (1987); temperature: 0°–2°C; continuous irradiance of 30–40 $\mu E \cdot m^{-2} \cdot s^{-1}$. Photographs were taken under inverted microscope from living specimens sampled daily and representing the dominant stages of colony development in culture during a 10-day period. Colony sizes are respectively: a. 8 μm; b. 12 μm; c. 15/18 μm; d. 16/24 μm; e. 40/48 μm; f. 68/68 μm; g. 60/75 μm; h. 125/140 μm; i. 150/180 μm; j. 370/470 μm; k. 400/560 μm; l. 700/890 μm (photogr. by J. Gunkel).

microzoospores distinguish themselves from above cell types by their significantly smaller size, and by their half DNA content (Table 1). Cells have been found in either G_1, S or G_2 phases of the cell cycle (R. Casotti, unpubl. data) confirming that they are capable of vegetative division (Kornmann, 1955). In 1971, Parke et al. published a very detailed ultrastructural study of cells referred as zoids, the most likely Kornmann's microzoospores, owing to their size range. Their work revealed two types of cells. Both types possess two equal heterodynamic flagella, a short stout haptonema with a distal swelling, an anterior depression, two types of organic body scales, chrysolaminarin vesicles and two chloroplasts. They differ by the presence, in only one type, of

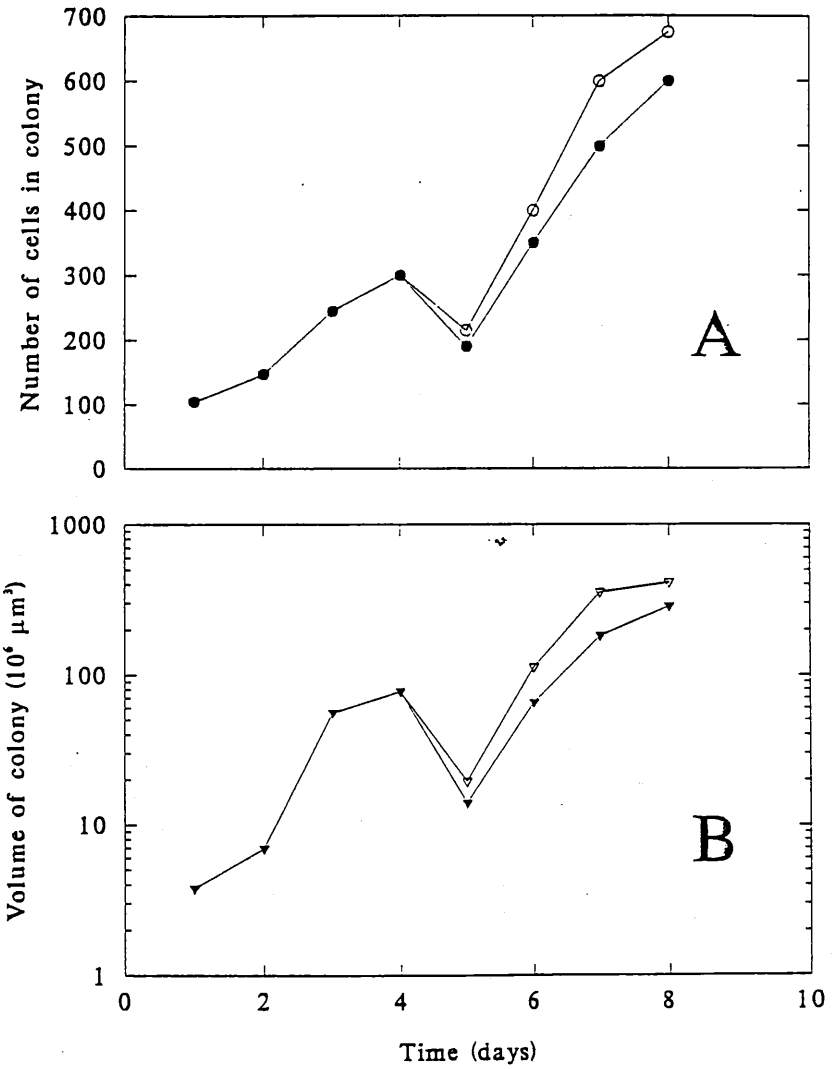

Fig. 4. Cleavage of a large colony into two daughters, each containing nearly the same number of cells. (A) Colony cell number and (B) Colony volume. Sum of volumes of daughter colonies is about 40% of the volume of the mother colony as an indirect evidence of colony matrix loss following colony cleavage. Culture conditions: inoculum with an isolated *P. globosa* colony (strain PCC540); culture medium of Veldhuis and Admiraal's (1987) with PO_4^{3-} concentration of 2.5 μM; continuous illumination of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; temperature: 15°C. Daily monitoring of colony cell number and volume (V. Cariou, unpubl. data).

superficial vesicles that release a thread-like material forming a five rays star pattern. This feature has been used as an important taxonomic criterion to identify different species among *Phaeocystis* free-living cells (see in particular Moestrup, 1979).

Macrozoospores

In addition to the swarmers and the microzoospores, Kornmann (1955) observed in his cultures, a third type of flagellates: the macrozoospores. These cells were shown to appear inside colonies of 50 to 150 μm in diameter that did not further increase in size. Some of these cells regenerated new colonies, either inside the colonies themselves or, after emigration from the colonies into the external medium. It is not clear whether these cells were morphologically different from either swarmers or microzoospores. Their formation seems to be linked to inadequate growth conditions for colonial stage and would constitute an anomaly in colony development. Accordingly, macrozoospores have never been mentioned as such after Kornmann's description, although development of flagellate cells within colonies followed by emigration has been subsequently reported (Verity et al., 1988b).

Colonial cells

Colonial cells are non-motile cells which size ranges between 4.5 and 8 μm. They have two or four chloroplasts and contain a vesicle of chrysolaminarin (formerly leucosin) that can be stained with cresyl blue (Scherffel, 1900; Kornmann, 1955). Their ultrastructure has been studied by electron microscopy (Chang, 1984) although taxonomic identity of the described species is not clear (Baumann et al., 1994). This microscopic analysis showed that colonial cells are deprived of flagella and haptonema, possess a longitudinal groove, lack the organic scales covering microzoospores and are surrounded by a mucilage envelope composed of about 10 layers roughly 0.5 μm wide.

2.2. Colonial stage development

The sequential development of a colony from a free-living cell itself originating from colony disruption has been observed in culture for both *P. globosa* (Kornmann, 1955; Fig. 2) and *P. pouchetii* (Gunkel, 1988; Fig. 3). In its earliest stage, the colonial development is similar for both species with dividing cells remaining located in the centre of the colony. Differentiation in colony development occurs at the 16 cell-stage. At this stage, *P. pouchetii* colony transforms from a spherical to a cloud-like shape showing the well-known typical group arrangement of the cells within the mucilaginous matrix (Fig. 3). In a *P. globosa* colony, cells progressively migrate towards the edge of the colony and remain located on a spherical surface, 15–20 μm away from the border of the colonial matrix (Fig. 2). Usually, cells are regularly distributed on the periphery of the colony. However, polarized colonies with cells accumulated on one side have been occasionally observed in both undisturbed cultures (Kornmann, 1955; Cariou, 1991) and mesocosms (Verity et al., 1988b). As *P. globosa* colonies grow in size, some of them may progressively lose their spherical shape and become elongate, digitate or bladder-like (Kornmann, 1955; Rousseau et al., 1990). The division of a large colony into two smaller daughters of either similar sizes containing nearly the same numbers of cells (Fig. 4) or into several colonies of different sizes has been observed both in pure cultures (Kornmann, 1955; Rousseau et al., 1990; Cariou, 1991) and in mesocosms (Verity et al., 1988a). The regeneration of entire colonies from fragments has also been observed by Kornmann (1955).

Colony diameter varies from 10 μm up to 8 mm (Kornmann, 1955) or even 20 mm (Kayser, 1970) for *P. globosa*. The maximum size recorded for *P. pouchetii* does not exceed 2 mm (Baumann et al., 1994). A significant logarithmic relationship between cell number per colony and colony volume (Fig. 5) has been established for several *Phaeocystis* species from diverse origins: *P. globosa* (Kornmann, 1955; Rousseau et al., 1990), *P. pouchetii* (Gunkel, 1988) and *Phaeocystis* sp. from Antarctica (Davidson and Marchant, 1987). Fig. 5, which compares these data with the regression line established in culture for *P. globosa* by Rousseau et al. (1990), suggests that the calculated relationship is globally valid for the differ-

ent *Phaeocystis* species. The 1.96 exponent of the relationship indicates that the relative importance of the mucilaginous matrix dramatically increases with the size of the colony.

Within the colony, cell division occurs by usually synchronous binary fission (Kornmann, 1955). The resulting number of colonial cells is then expected to be a power of two. Some evidence of asynchronous colonial cells division has been

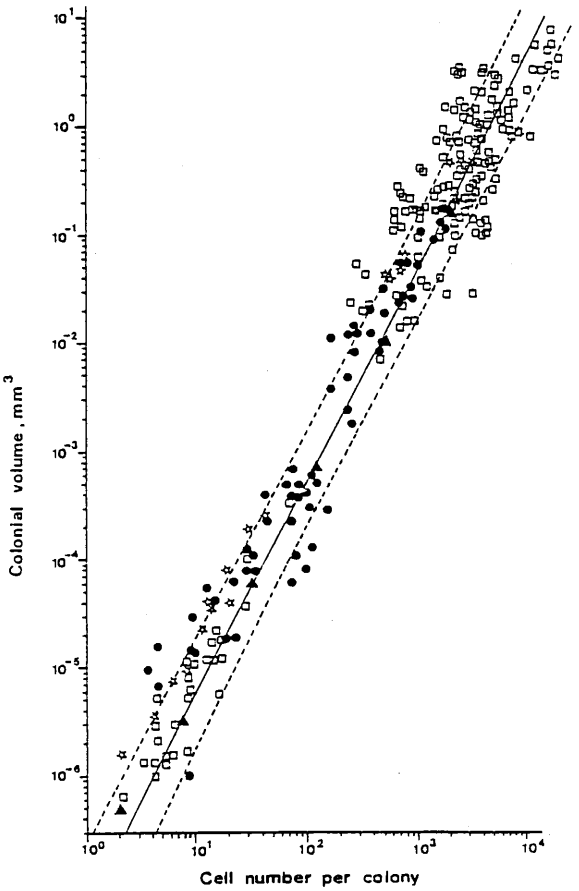

Fig. 5. Relationship between colony cell number and colony volume established for different *Phaeocystis* strains and compared with the regression line and its confidence interval at 99% calculated for a growing *P. globosa* culture (Rousseau et al., 1990); ▲ *P. globosa* (Kornmann, 1955), □ *P. globosa* (Rousseau et al., 1990), ● *Phaeocystis* sp. Antarctic strain (Davidson and Marchant, 1987); ☆ *P. pouchetii* (Gunkel, 1988). Equation of the regression line is: $\log C = 0.51 \log V + 3.67$ where C is the colony cell number and V is the colonial volume expressed in mm^3.

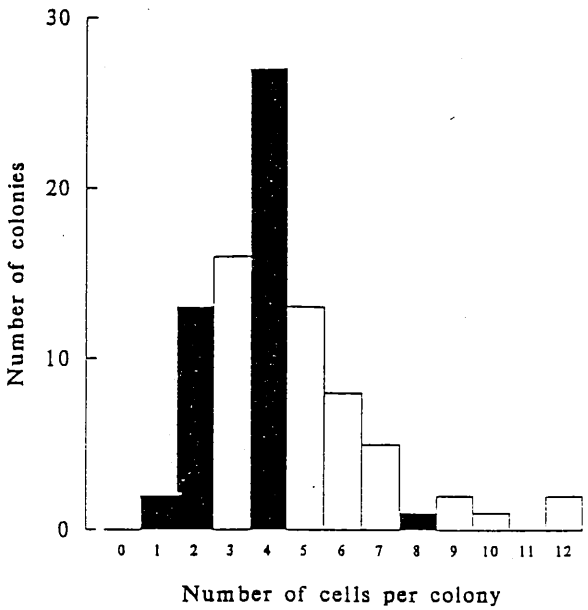

Fig. 6. Frequency histogram of cell number per colony in a *P. globosa* (strain PCC540) culture. The dark bars correspond to the numbers of cells per colony expected for synchronous cell division. Culture conditions: in K culture medium (Keller et al., 1987); temperature: 13°C; continuous illumination of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. *Phaeocystis* colony sampling in the exponential growth phase. Staining with Alcian blue and inverted microscopic examination (D. Vaulot, unpubl. data).

however reported for *Phaeocystis* examinated either under light microscopy (Kornmann, 1955; Fig. 6) or time lapse video microscopy (J.-L. Birrien, unpubl. data). From these observations, it is suggested that synchrony of the division inside colonies would be induced by the light regime.

Two phenomena are generally observed at the decline of a colony culture growth: (i) microaggregate formation through the progressive invasion of colony mucus by bacteria (Guillard and Hellebust, 1971; Davidson and Marchant, 1987) leading ultimately to the complete degradation of the colonies and (ii) "ghosts" colonies formation due to the emigration into the external medium of flagellates issued from the transformation of non-motile cells within healthy spherical colonies (Kornmann, 1955; Verity et al., 1988b; Cariou, 1991). It is not clear, however, whether these cells are diploid flagellates or/and haploid microzoospores.

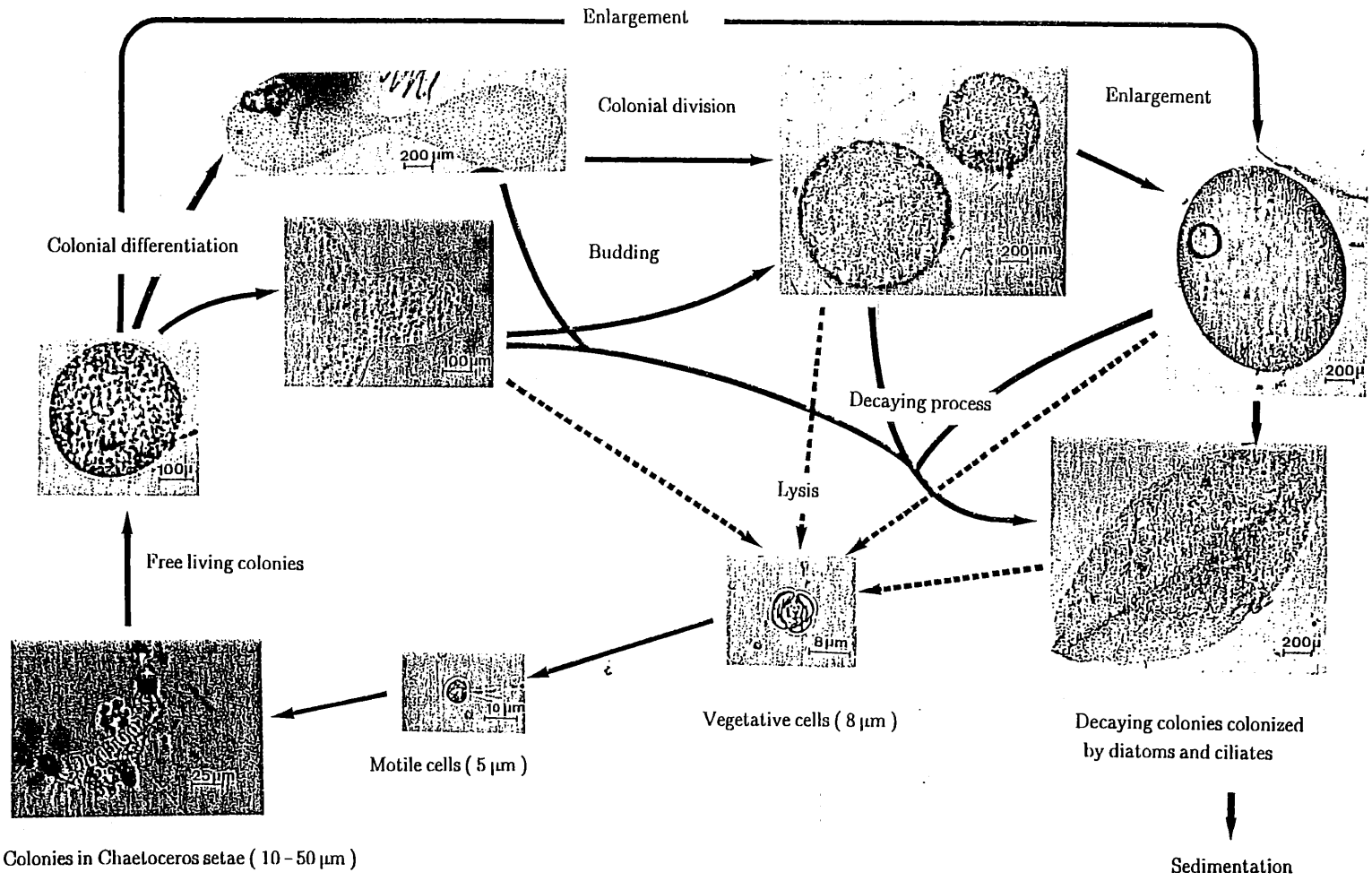

Fig. 7. Sequence of events occurring during a spring bloom of *P. globosa* in 1988 in the Belgian coastal waters of the North Sea as identified by a microscopic morphometric analysis of free-living cells and colonies. Surface seawater samples were collected 2–3 times/week at station N 51°26.05; E 002°49.08 with a bucket in order to avoid colony disruption and fixed with a lugol-glutaraldehyde solution. *Phaeocystis* colonies and free-living cells were enumerated under light microscope (Leitz Fluovert) using Utermöhl concentration method, at a magnitude of 40 or 100 and 1000 respectively. Morphological analysis was conducted as described in Rousseau et al. (1990) (V. Rousseau, unpubl. data).

3. Field observations

The sequence of events characterizing a *P. globosa* bloom development in natural environment has been identified through a detailed light microscopy analysis of morphological stages that succeeded each other during the spring bloom 1988 in the Belgian coastal waters (Fig. 7). This

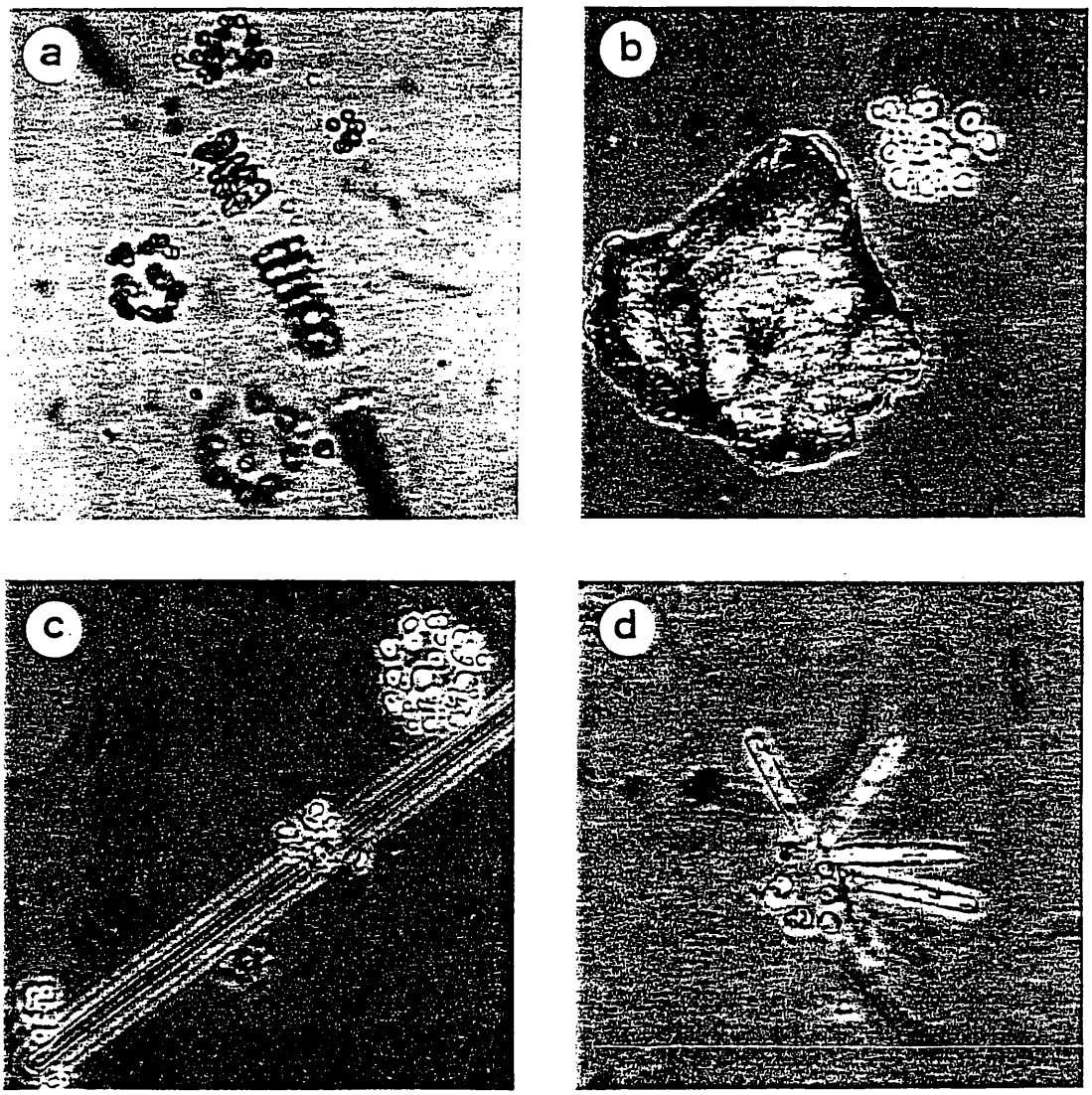

Fig. 8. Young spherical *P. globosa* colonies less than 50 μm in diameter attached to different solid substrates: (A) on *Chaetoceros* sp. setae as observed in the Belgian coastal waters during the early stage of the spring bloom 1988 (methods as in Fig. 7). (B) on living diatom *Asterionella* sp. (C) on a sand particle and (D) on a glasswool fiber. Culture conditions: inoculum with free-living cells obtained by mechanical disruption of colonies; culture medium of Veldhuis and Admiraal's (1987) with NO_3^-, NH_4^+ and PO_4^{3-} concentrations: 50, 25 and 5 μM, respectively; temperature: 10°C; illumination of 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ under a 12 h light–12 h dark cycle (V. Rousseau and T. Davies, unpubl. data).

study shows that the complex events evidenced in pure culture of *Phaeocystis* are also occurring under natural conditions.

3.1. Colony growth

The early stage of the bloom development is dominated by young healthy spherical colonies that succeed to a *Chaetoceros*-dominated diatom community. These colonies, less than 50 μm in diameter, are usually located on the setae of the diatom *Chaetoceros* spp. (Fig. 8a) whereas free-living colonies of this size are seldom observed. The formation of these small colonies seems to be strictly linked to the occurrence of *Chaetoceros* spp. This phenomenon, also observed in other *Phaeocystis*-dominated populations (Boalch, 1987), suggests that some *Chaetoceros* species would play a key-rôle in the development of *Phaeocystis* bloom by acting as a solid substrate. However, according to Cadée and Hegeman (1991), *Chaetoceros* cells would be too sparse to support all colonies, suggesting that, if required, other species or particles could act as substrate for colony development. As the bloom evolves, spherical colonies released from *Chaetoceros* setae undergo differing development. Part of them keeps spherical form and increase in size, covering a large range of diameter (50 μm–2 mm). Others change from spherical to elongate form and produce new daughter colonies by budding or division. This differentiation results, at the top of the bloom, in the complex coexistence of a high diversity of colony shapes and sizes, also observed in German coastal waters (Bätje and Michaelis, 1986).

3.2. Senescent stage and bloom termination

Decaying colonies are very scarce in the early stage of the bloom but appear in great numbers during the course of the bloom development. Senescent colonies are irregular in shape, less turgid and have a sticky mucus which appears less consistent compared to healthy colonies. Their large range of size (200 μm–2 mm) indicates that they originate from healthy colonies of different age. Senescent colonies are progressively invaded by various auto- and heterotrophic microorganisms and are covered by inorganic detritus, leading to the formation of aggregates of various size and composition at the end of the bloom. *Phaeocystis*-derived aggregates composed of mucus, *Phaeocystis* cells, diatoms, ciliates, dinoflagellates and heterotrophic nanoflagellates constitute micro-environments where a complete trophic food-web develops (Weisse et al., 1994). Their sudden disappearance from the water column may result from sedimentation, consumption, desintegration in the water column (Thingstad and Billen, 1994; Wassmann, 1994; Weisse et al., 1994) or advective transportation. Concomitantly with aggregate formation, at the end of the bloom, small flagellate cells similar to the microzoospores described in cultures, were observed to develop inside colonies and subsequently migrate outside. This has been observed for both *P. globosa* (Scherffel, 1900; Jones and Haq, 1963; Parke et al., 1971; Cadée and Hegeman, 1986; Veldhuis et al., 1986a) and for *P. pouchetii* (Gunkel, 1988).

3.3. The free-living cell stage

In the field, low densities of free-living flagellate cells were observed to precede the formation of the colonial form (Tande and Båmstedt, 1987; Davies et al., 1992) and persisted along the *Phaeocystis* colony development. The nature of the initial colony-forming cells could, however, not be identified due to the inadequacy of the light microscopy technique usually used in field studies for identifying the *Phaeocystis* cell types present in the water. In the same way, the nature of the over-wintering *Phaeocystis* form remains unidentified. Kornmann (1955) hypothesized that *Phaeocystis* survives as motile form throughout the year. Alternatively, Cadée (1991) regularly observed *Phaeocystis* colonies during winter in Dutch coastal waters of the North Sea. He suggested that these colonies could constitute the wintering form of *Phaeocystis* providing the inoculum for the next spring bloom through the release of motile cells. The presence, during the course of the bloom, of low density of free-living cells of the same size as colonial cells, whether motile or not, (Rousseau et al., 1990; Weisse and

Scheffel-Möser, 1990) suggests that part of *Phaeocystis* colonies are continuously disrupted along the course of the bloom development.

4. Factors regulating the different phases of *Phaeocystis* life cycle

4.1. Colony formation

Existing data on the factors controlling colony formation are very scarce. The nutient status is now believed to constitute a major factor driving colony formation from free-living cells. Phosphate concentration less than 1 μM has been suggested to induce massive formation of colonies from free-living cells in batch unialgal cultures of *Phaeocystis*. Actually, a careful reexamination of these data (figs. 1 and 2 in Veldhuis and Admiraal, 1987) indicates that colonies were yet present at a wide range of phosphate concentrations (0 to 70 μM). More recently, Cariou (1991) gave experimental evidence that a threshold phosphate concentration of 0.5–1 μM was a necessary condition to generate colonies from released colonial cells. Contrasting with these results, competitive experiments carried out by Riegman et al. (1992) under laboratory controlled conditions clearly showed that *P. globosa* colony forms were absent under phosphate or ammonium limitation but dominant under nitrate control. This indicates that massive blooms of *Phaeocystis* colonies may be expected in N-controlled environments with a high new production relative to regenerated production. Accordingly, *Phaeocystis* colonies are generally blooming in marine systems enriched in nutrients either naturally (El-Sayed, 1984; Smith et al., 1991) or through anthropogenic inputs (Lancelot et al., 1987; Al-Hasan et al., 1990). The rôle nutrients could play in colony initiation is still unclear. Several hypotheses have been suggested among which the induction of cellular differentiation (e.g. from free-living cell type to colonial type) and the selective enhancement pre-existing colonial-type cells (Riegman et al., 1992) are the most probable.

The requirement of a solid substrate has been suggested by several authors as triggering factor for colony formation in batch cultures and in natural environment. Both Kornmann (1955) and Kayser (1970) found that, in cultures, the flagellate cells liberated from disrupted colonies became attached before forming new colonies. From field observations, several authors (Boalch, 1987; V. Rousseau, this work) concluded that some diatoms and more particularly some *Chaetoceros* spp. may fulfill the rôle of substrate. However, recent experimental work under controlled laboratory conditions (Rousseau and Davies, unpubl. data), gives strong evidence that any microscopic particle, either biological (e.g. diatoms), organic or mineral (sand, glasswool) may act as substrate for colony development (Fig. 8b, c, d). Supporting this, young colonies attached to the diatom *Biddulphia* sp. were observed in the German coastal waters (T. Weisse, pers. commun.). Selective grazing of the small free-living colonies, preferential adhesion of diatoms to colonies due to specific attachment properties of surface polymers or release of attracting substances are hypotheses to be tested for explaining the localization of small colonies on diatoms and more specifically on *Chaetoceros* spp. setae in the natural environment.

4.2. Colony differentiation

The factors that regulate colony shapes (from spherical to elongate and budding colonies) are still unknown but the influence of physical forcing (water turbulence, particles) are strongly suspected (Kornmann, 1955). The process of colony division seems to be, at least partially, regulated by nutrient concentrations although autogenic factors cannot be excluded (Verity et al., 1988a). At the end of the bloom, nutrient limitation would induce physiological changes leading to the senescence of colonies and their invasion by various microorganisms.

4.3. Motility development and emigration of cells from the colonies

Motility development within the colony and subsequent release of cells from colonies have

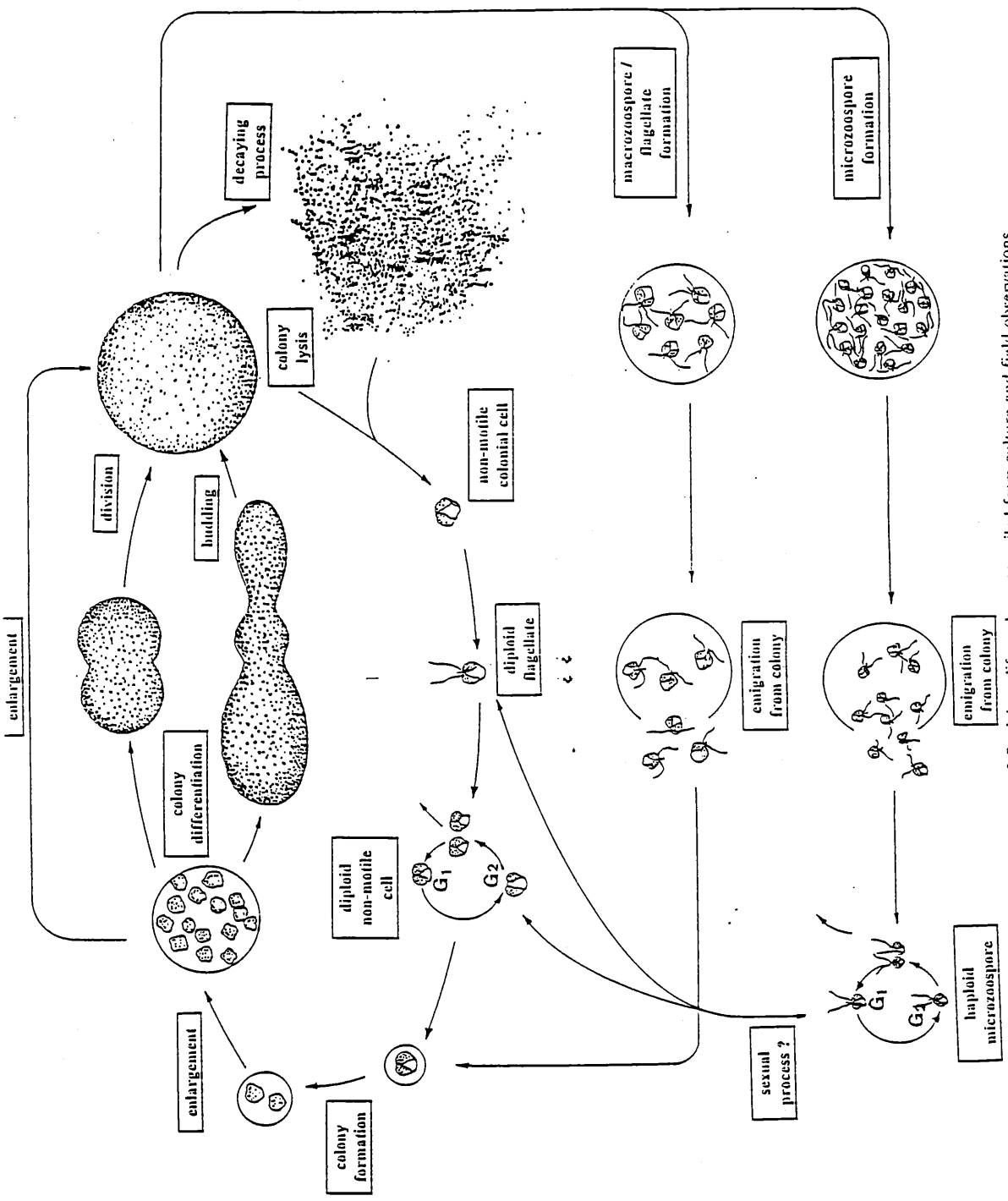

Fig. 9. Current status of *P. globosa* life cycle as compiled from culture and field observations.

been observed for colonies under stressed conditions. Nutrient limitation (Kornmann, 1955) possibly accompanied by significant temperature change (Verity et al., 1988b) have been showed to generate motility development within *Phaeocystis* colonies either under mesocosm or laboratory conditions.

5. Conclusions

The current knowledge of *P. globosa* life cycle synthetized from field and culture observations is illustrated by Fig. 9. At this stage, however, it is difficult to propose a coherent scheme for the place of the different cell types within *Phaeocystis* life cycle and to elucidate the pathways leading from one type to another. More has to be known about the mechanisms initiating *Phaeocystis* cellular differentiation and colony formation. As a first step in this direction, flow cytometric studies, by demonstrating ploidy differences between non-motile solitary cells and flagellates (diploid) and microzoospores (haploid), give strong support for the involvement of these latter in sexuality as already suggested by Kornmann (1955). Such alternation of haploid and diploid generations has also been observed in *Hymenomonas carterae* Braarud (von Stosch, 1967). Whether this give rise to an alternation of diploid and haploid colonies (the former originating from non-motile diploid cells and the latter from haploid microzoospores) or whether all colonies are diploid has not been demonstrated. However, no change in ploidy has been observed when diploid cells released from colonies give rise to new colonies (R. Casotti, unpubl. data), so corresponding to a vegetative multiplication of this alga favouring the further spreading of the colonial stage once initiated. This opens several questions that could be solved through further cytofluorometric investigations. Indeed, assuming all colonies are diploid, then colony formation from microzoospore-dominated cultures (Kornmann, 1955) implies either the sexual conjugation of haploid cells or alternatively the presence of a background of diploid cells in microzoospore cultures. Conversely, meiosis must intervene when microzoospores are formed in senescent cultures. None of these processes has been observed yet, suggesting that sexuality may involve a tiny percentage of the vegetative populations. In the meantime, the potential common occurrence of sexuality in *Phaeocystis*, resulting in high genetic plasticity, could be an explanation for its worldwide distribution.

Identification of the over-wintering form and of the first active form preceding colony formation, as well as the mechanisms involved in the transition between free-living cell stage and colony are essential for understanding the occurrence of *Phaeocystis* blooms. A better knowledge of processes of colony division and cell release from colony would allow to estimate the spreading of the colonial stage once initiated. Finally, the relative importance of motility development, of senescent colony and aggregate formation should be further investigated owing to their different ecological rôle in the bloom termination.

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Microbial degradation of *Phaeocystis* material in the water column

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Abstract

Observational evidence shows that the large amounts of mucilaginous substances produced by blooms of *Phaeocystis* colonies largely resist rapid microbial degradation in surface waters of most *Phaeocystis*-dominated ecosystems. In this paper the biodegradability of *Phaeocystis* colony-derived material is analysed with respect to current knowledge and novel data on the chemical nature of *Phaeocystis* material in relationship with specific bacterial enzymatic activities. Particular emphasis is given to the chemical nature of *Phaeocystis* colony matrix which constitutes more than 80% of total colony biomass at maximum development. This analysis gives evidence of the potential biodegradability of this mucilaginous material made of nutrient-deprived polysaccharides. Other factors controlling microbial degradation as the production of antibacterial substances by *Phaeocystis* colonies, cold temperature and lack of inorganic nitrogen and phosphate are further considered. It is concluded that nutrient limitation currently observed at the senescent stage of *Phaeocystis* blooms might well explain the low biodegradability of *Phaeocystis* material. However the lack of bacteria attached to colonies during the exponential phase of *Phaeocystis* bloom development are not clearly understood and needs further investigations.

1. Introduction

One of the characteristic features of *Phaeocystis* blooms is the production of large amounts of mucoid colonial material which may represent up to 90% of total algal biomass (Rousseau et al., 1990). One of the more spectacular consequences of this input of organic material to the marine environment is the formation of large amounts of foam on the beaches during blooms of this algae (Lancelot et al., 1987). Of greater ecological significance is, however, the fate within the marine ecosystem of the organic material produced by these blooms. As for any other algal bloom, the organic material produced may either be ingested by zooplankton or degraded by bacteria. The relative magnitude of these two rates will determine whether the material is channeled into the food web at the microbial, or at the mesozooplankton level, presumably with large consequences for the relative importance of "classical" versus "microbial" parts of the ecosystem (Fig. 1). The rate of these two processes relative to the sinking rate of the material from senescent blooms will determine whether the primary degradation takes place (1) in or close to the photic zone, (2) in the aphotic part of the water column or (3) reaches the bottom. Bacterial degradation of the colony may thus be important, not only as a clean-up process in areas like the southern part

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of the North Sea where the blooms of *Phaeocystis* may be a nuisance, but also as a potential structuring process in the marine food webs of this (Joiris et al., 1982) and in other areas sustaining large commercial fisheries such as the Barents Sea (Wassmann et al., 1990; Thingstad and Martinussen, 1991). A model eventually aiming at assessing the role of *Phaeocystis* in the marine ecosystem therefore requires a proper understanding, not only of how bacterial degradation of the mucilaginous material is controlled, but also of the relative importance of this process compared to the rates of other processes moving and transforming the material.

In bacterial degradation, macromolecules have to pass through a two-step process (Billen and Servais, 1989), with an initial hydrolysis followed by a subsequent bacterial uptake and utilization of the monomers. A variety of factors such as temperature, antibiotics, nutrient concentrations, competition and predation, will in a consortium influence these processes and thereby regulate the rate of degradation.

The importance of various mechanisms in regulating the degradation process is reflected in three observations on bacterial growth related to *Phaeocystis*:

- Colonies in early stages of blooms are almost entirely free of attached bacteria (Lancelot and Rousseau, 1994; Thingstad, pers. obs. Barents Sea). (See Fig. 2).
- Late stationary phases of cultures (Davidson and Marchant, 1987) and late bloom stages in natural environments (Veldhuis et al., 1986; Billen

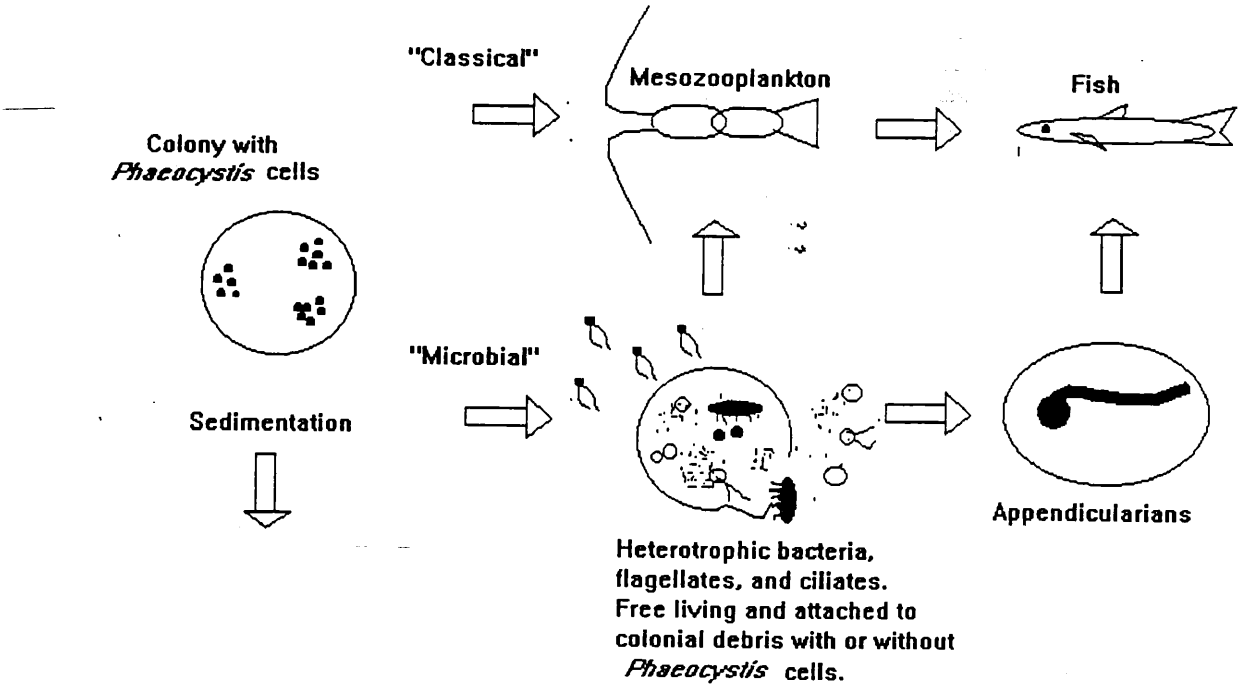

Fig. 1. Main fates of *Phaeocystis* material. A "competition" exists between the "classical" food chain where healthy colonies enter a food chain via mesozooplankton to fish, and a "microbial" food chain where a consortium of heterotrophic microorganisms (bacteria, heterotrophic flagellates, ciliates, etc.) may develop both as free living and attached to the remains of decaying colonies. The secondary production of this microbial complex may be fed back via mesozooplankton into the food chain to fish, either via filter feeders with fine meshed filters such as e.g. appendicularians, or via e.g. copepods feeding on colonized aggregates of remains of *Phaeocystis* colonies. The rates of transfer into the two food chains structures the food chain of *Phaeocystis*-dominated ecosystems and influences the depth to which particulate organic material from senescent blooms will sediment (organisms not drawn to scale).

and Fontigny, 1987, Lancelot and Rousseau, 1994) are accompanied by increased growth of free living bacteria, along with colonization of the mucus by attached bacteria (Thingstad and Martinussen, 1991). (See Fig. 3).

– Organic material may accumulate in the water during the senescent phase of blooms (Eberlein et al., 1985; Billen and Fontigny, 1987).

Together these observations seem to indicate that a substantial part of the material is of a chemical nature that makes it available to bacterial attack, but also that the degradation process may be slow both in the early and in the senescent phase of blooms. The reasons for a slow degradation may be widely different in early and senescent stages of the bloom.

2. Type of material

Organic material produced by marine microorganisms are not necessarily of a chemical nature that make them readily available to bacterial attack. Using seawater bacterial communities, Pett (1989) found 29% of the material from *Skeletonema costatum* cells to have a half-life on the order of months, and 4% to have a half-life of years, and Brophy and Carlson (1989) found glucose and leucine added to natural water to be transformed into high molecular weight compounds that persisted 6 months of incubation.

As was shown by Guillard and Hellebust (1974), the material excreted by colonial forms of *Phaeocystis pouchetii* is predominantly carbohydrates. Qualitative sugar composition of the excreted material was similar in the two strains investigated, and was dominated by glucose, mannose and rhamnose. Chromatography of the material indicated that the same sugars were present in the matrix material. Molecular weight determination showed that most of the liberated material was of high molecular weight (less than 20% had MW < 700), but with a wide spectrum in sizes. More than two-thirds of the material consisted of oligo- and poly-saccharides with molecular weights equivalent to 4–40 hexose residues. In

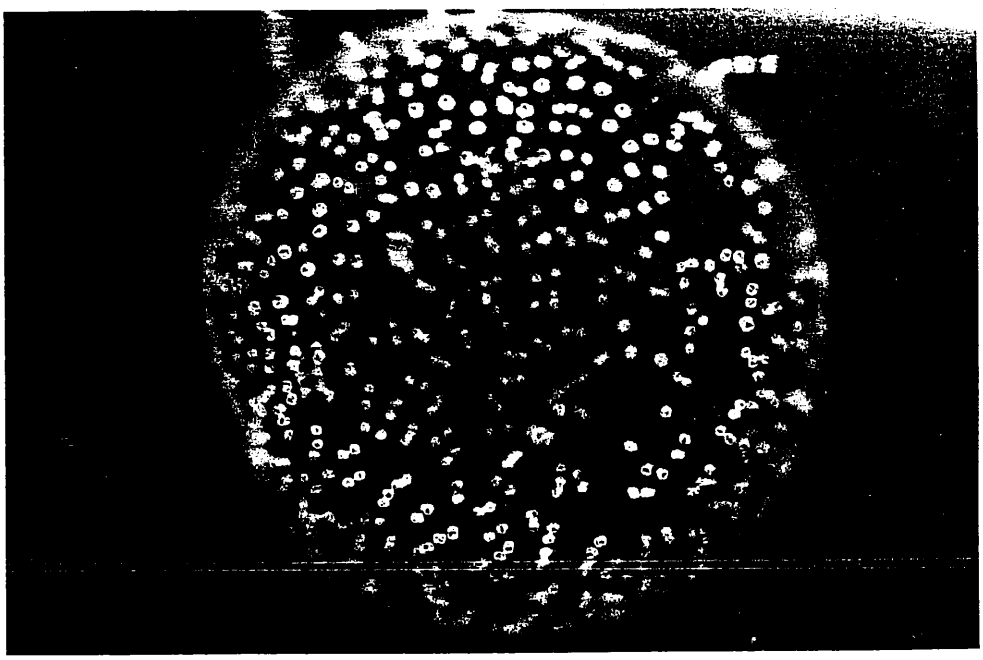

Fig. 2. Healthy *Phaeocystis globosa*-type colony sampled in the Belgian coastal zone at the early development of the spring bloom. (Inverted microscopy; photograph: V. Rousseau).

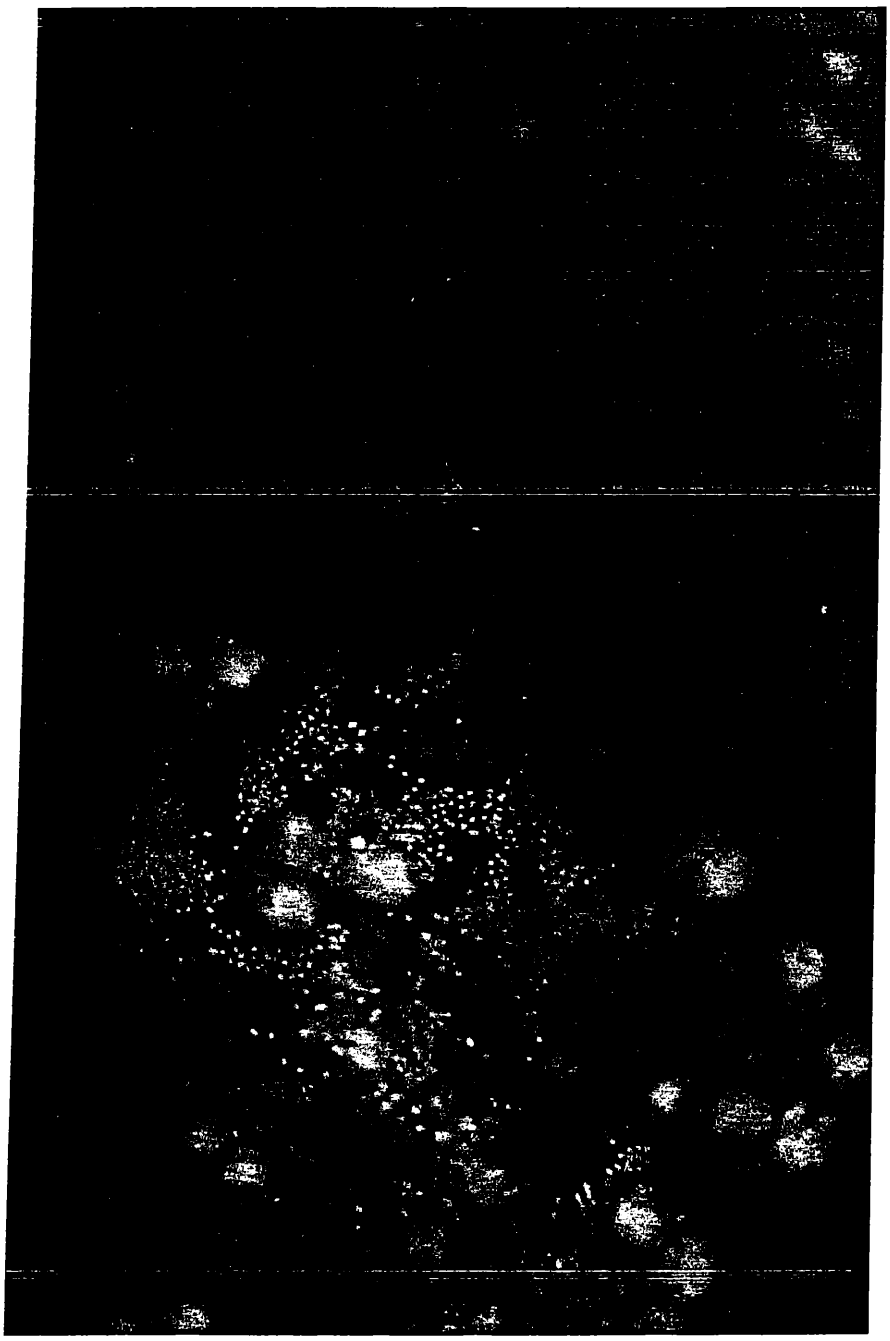

Fig. 3. Decaying colony of *Phaeocystis* from the aphotic zone in the Barents Sea. Bacteria have colonized the mucoid colonial material with some *Phaeocystis* cells (bright) still remaining in the matrix. Preparation stained by DAPI and primuline photographed under UV excitation in epifluorescence microscopy. (photograph: I. Martinussen).

recent investigations, Lancelot et al. (unpublished data) provide further indications concerning the structure of *Phaeocystis* mucus. Gas chromatography of a hydrolysate of mucus revealed a presence of 70% glucose, 15% xylose and an unidentified derivative, possibly acidic owing to the positive response to alcian blue staining.

Concentrated mucus was found to act as a competitive inhibitor to 4MUF-β-glucoside, an artificial fluorogenic substrate of β-glucosidases, indicating that at least parts of the polysaccharides are polymers linked by β-glucosidic bonds. No effects were found on the hydrolysis of 4MUF-α-glucoside. The susceptibility of polymers to enzymatic hydrolysis depends on both the presence of suitable enzymes recognizing the polymeric bond involved, and on the secondary and tertiary structure of the polymer, determining their accessibility for the active site of enzymes.

Intuitively, one would expect that degradability is correlated to the functional role of the polysaccharide with storage polymers being easy to hydrolyze, while polymers with a structural function need to be more resistant. It has been shown that at least part of the colony material has a storage function allowing energy requiring processes such as protein synthesis (Lancelot and Mathot, 1985; Lancelot et al., 1986) and phosphate uptake (Veldhuis et al., 1991) to continue in the dark. That part of the mucus should therefore be easily mobilizable, at least by the algae themselves. Other parts may however be more resistant. A high degree of side chain formation, or other "irregularities" in the polymer are known to cause considerable loss of degradability. No information is, however, available on the degree of side chain structure of *Phaeocystis* mucus. Lack of water solubility and close stacking of individual chains caused by hydrophobic bonds between them is another cause for low biodegradability, as e.g. in the case of cellulose (β-1,3-glucan). *Phaeocystis* mucus appears like as a gel-like material, with high degree of hydration. Electron microscopic studies of the colony material (Chang, 1984) revealed a multilayered mucilaginous envelope without any signs of structurally supporting fibers. The basis for a low biodegradability of *Phaeocystis* seems therefore not to be found in its structure. β-glucosidase activity is present among the bacterial communities of marine waters, including those were *Phaeocystis* are present (Thingstad and Martinussen, 1991), but apparently at low levels when compared to e.g. proteolytic activity (Lancelot et al., unpubl. data). These enzymes are susceptible to rapid induction (Chrost, 1991) so that the lack of ability to produce this enzyme is not an explanation for low biodegradability of mucus.

3. Antibiotics

Colonies of *Phaeocystis* may be stained for fluorescence microscopy such that both the algal cells with their chloroplast and nucleus, the mucoid material, and any bacteria attached to the mucus may be observed (Martinussen and Thingstad, 1991). Using this technique, we have found colonies from early stages of blooms in the Barents Sea to be amazingly free of bacteria. In the *Phaeocystis pouchetii*-type, where algal cells are grouped in patches on the colonial surface, the protective mechanism would seem to extend to parts of the colony not in immediate contact with the vegetative cells. The acrylic acid produced by *Phaeocystis* (Sieburth, 1961) is a potential candidate as the anti-bacterial agent. As shown by Sieburth (1961), however, the concentrations of acrylic acid required to give significant bacteriostatic/bacteriosidic effects are high (order of $g \cdot l^{-1}$).

In non-axenic batch cultures of *Phaeocystis* sp. originating from the Southern Ocean, the correlation between acrylic acid concentration and bacterial numbers was negative but low (-0.4) (Davidson and Marchant, 1987). Correlation with growth rate was not reported in these experiments.

Grossel and Delesmont (1984) reported the presence of acrylic acid during *Phaeocystis* blooms in the eastern Channel. The maximum concentration recorded averaged 13 $\mu g \cdot l^{-1}$, at least three orders of magnitude below the concentrations reported to inhibit marine bacteria. Acrylic acid is produced in equimolecular amounts with

dimethyl-sulphide (DMS) during the enzymatic splitting of β-dimethylpropiothetin (DMPT) (See Liss et al., 1994). DMPT has been suggested to be important in the osmoregulation of algae (Vairavamurthy et al., 1985). Production of DMS during bloom (not *Phaeocystis*) simulations in tanks has been found to be 7–26 times higher during the senescent phase than during the growth phase (Nguyen et al., 1988). This pattern seems difficult to reconcile with the hypothesis that acrylic acid is predominantly protecting young and healthy colonies.

Another hypothetical mechanism for colony protection could be a frequent peeling of the outermost membrane of the colonies. To our knowledge, this has not been specifically tested, but such a mechanism would be expected to give ample growth of bacteria outside the colonies in the exponential growth phase of *Phaeocystis* cultures. This does not seem to fit with the experimental results either in batch cultures where bacterial growth was associated with the stationary and not the exponential phase of *Phaeocystis* (Davidson and Marchant, 1987), or in the field, where a distinct lag is observed between the development of *Phaeocystis* blooms and that of planktonic bacteria (Laanbroek et al., 1985; Veldhuis et al., 1986; Billen and Fontigny, 1987).

4. C:N:P-ratios of *Phaeocystis*

When blooms of *Phaeocystis* collapse due to depletion of either available N- or available P-sources from the water, supply of N or P for subsequent formation of bacterial biomass is restricted to the content of these elements in the organic matter degraded, and to remineralized

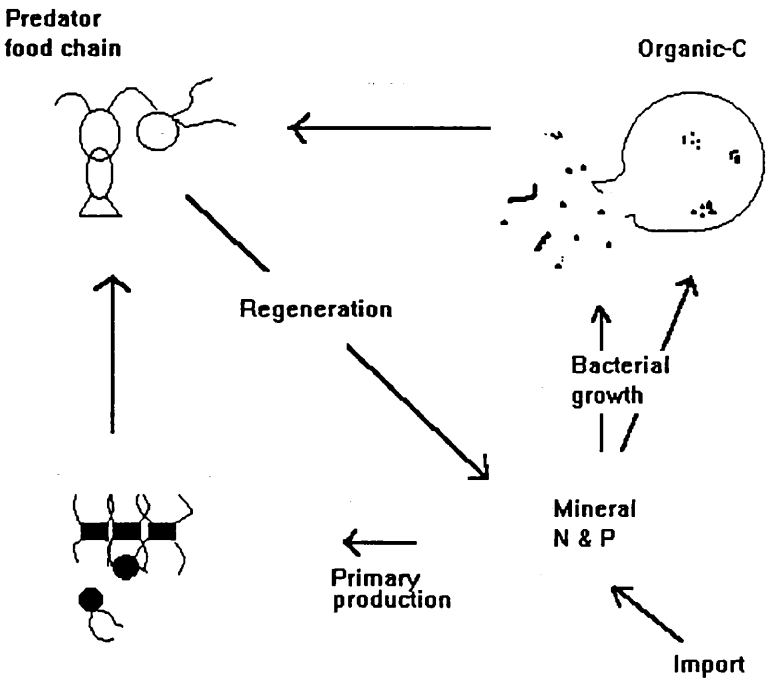

Fig. 4. Trophodynamic interactions presumably controlling the degradation of *Phaeocystis* material in the photic zone of waters where blooms collapse due to N or P-depletion. Formation of bacterial biomass on C-rich carbohydrates from mucus requires the supply of dissolved sources of N and P for which the bacteria must compete with phytoplankton. The rate of supply of these nutrient by import is controlled by hydrographic processes, and the internal regeneration by the processes in the predator food chain (organisms not drawn to scale).

and "new" inputs of these elements. Consumption of readily degradable substances like glucose may under such conditions be controlled by trophodynamic processes of mineral cycling such as competition, predation and remineralization (Pengerud et al., 1987) (Fig. 4). Nutrient limitation of the degradation process would be particularly be expected when C:N and/or C:P ratios of the organic substrates are high (Martinussen and Thingstad, 1987). Veldhuis et al. (1986) found protein to be the dominant photosynthetic product during early stages, while carbohydrate synthesis was dominant during late stages of the *Phaeocystis* spring bloom in Dutch coastal waters.

C:N-ratios of *Phaeocystis* has been found to increase as the bloom progresses (Hickel, 1984) and values more than 5 times the Redfield ratio has been found at low concentrations of ambient nitrogen sources (Lancelot et al., 1991; Baumann et al., 1994). *Phaeocystis globosa*-type was found by Jahnke (1989) to have an atomic C:N:P-ratio of $568 \pm 102 : 59 \pm 9 : 1$ under phosphate deficiency, an increase in C:P of about 5 relative to the Redfield ratio.

Mineral nutrient limitation of the degradation of C-rich parts such as the carbohydrates could therefore be expected. Of interest in this respect is the observation by Eberlein et al. (1985) of high concentrations of carbohydrates following a *Phaeocystis* bloom in the German Bight. No specific information of phosphate concentrations during this particular event was reported, but due to the high nitrate : phosphate content in the river runoff from the northwestern part of the European continent to the North Sea (Lancelot et al., 1991), blooms in this coastal area may become P-limited (van Bennekom et al., 1975; Veldhuis et al., 1986), Since bacterial P-content is high relative to Redfield's ratio (Goldman et al., 1987; Vadstein et al., 1988), one may speculate that bacterial development is particularly restricted under P-limitation. In this scenario, organic matter from *Phaeocystis* blooms in the German Bight would be conserved due to P-limitation, and the probability would increase for alternative fates such as zooplankton ingestion, sedimentation or advective transport into the Jutland Current where nitrate-rich/phosphate-poor water masses are transported northwards along the western coast of southern Scandinavia (Thingstad et al., unpubl. data). In stable water columns such as the polar waters of the Barents Sea, the particulate part the organic matter would sediment from the deep chlorophyll maxima into the nutrient replete water masses below (Wassmann et al., 1990). Nutrient limitation would then be expected to be a problem mainly for degradation of dissolved components left in the cold, oligotrophic melt layer of this region.

5. Temperature

Phaeocystis is common species both in Antarctic (El-Sayed et al., 1983) and in Arctic (Skjoldal and Rey, 1989) waters where blooms may occur at water temperatures below 0°C. There has been suggestions in the literature that bacterial growth is inhibited at low temperatures (Pomeroy and Deibel, 1986; Autio, 1990). Following the arguments presented in the introduction, large differences in the temperature sensitivity of key processes such as primary production, zooplankton grazing, particle sedimentation and bacterial degradation would have the important consequence of promoting different ecosystem structures in cold and temperate waters. Other investigators have, however, found bacterial activities in polar regions comparable in magnitude to that in temperate waters (Cota et al., 1990). Possibly such apparently conflicting observations may be reconciled using a more elaborate hypothesis where higher substrate concentrations are required to compensate for lower temperatures (Pomeroy and Wiebe, 1993). Thingstad and Martinussen (1993) found high bacterial numbers and activities associated with deep chlorophyll maxima formed by *Phaeocystis* at late stages of the ice edge bloom in the Barents Sea. Similarly, differences in temperature sensitivity of the different processes of bacterial degradation (hydrolysis by extracellular enzymes, uptake, bacterial growth, etc.) could qualitatively alter the degradation at different water temperatures. The extracellular enzymatic hydrolysis of macromolecules has been suggested to be rate limiting

in temperate waters (Somville and Billen, 1983; Billen, 1991). Studies of the temperature sensitivity of extracellular proteases in natural Barents Sea water down to the freezing point (−1.9°C) did not reveal any lower limit for the functioning of these enzymes (Thingstad and Martinussen, 1991). Psychrophilic bacteria may grow well at such low temperatures on monomeric organic substrates (Harder and Veldkamp, 1971). When estimates of in-situ bacterial growth rates are compared to growth rates of natural bacterial communities to which substrates have been added, the in-situ growth rates seem to be far below the maximum obtainable for any given temperature (range investigated −1.9 to +25°C, Billen and Servais, 1989), indicating that temperature is not the major limiting factor.

6. Secondary effects

Subsequent to bacterial assimilation of organic carbon originally fixed by *Phaeocystis* primary production, the fate of this material is linked to the fate of the bacterial biomass. In some environments, only a minor fraction of the bacterial biomass is apparently transferred to higher trophic levels (Ducklow et al., 1986). In the case of invaded *Phaeocystis*, the situation may be different since the attachment to floculate changes the functional size of bacteria in the predator chain. It has been shown that old colonies of *Phaeocystis* are more susceptible to mesozooplankton grazing than actively growing colonies (Estep et al., 1990). Zooplankton in the Barents Sea are also often found to concentrate in layers

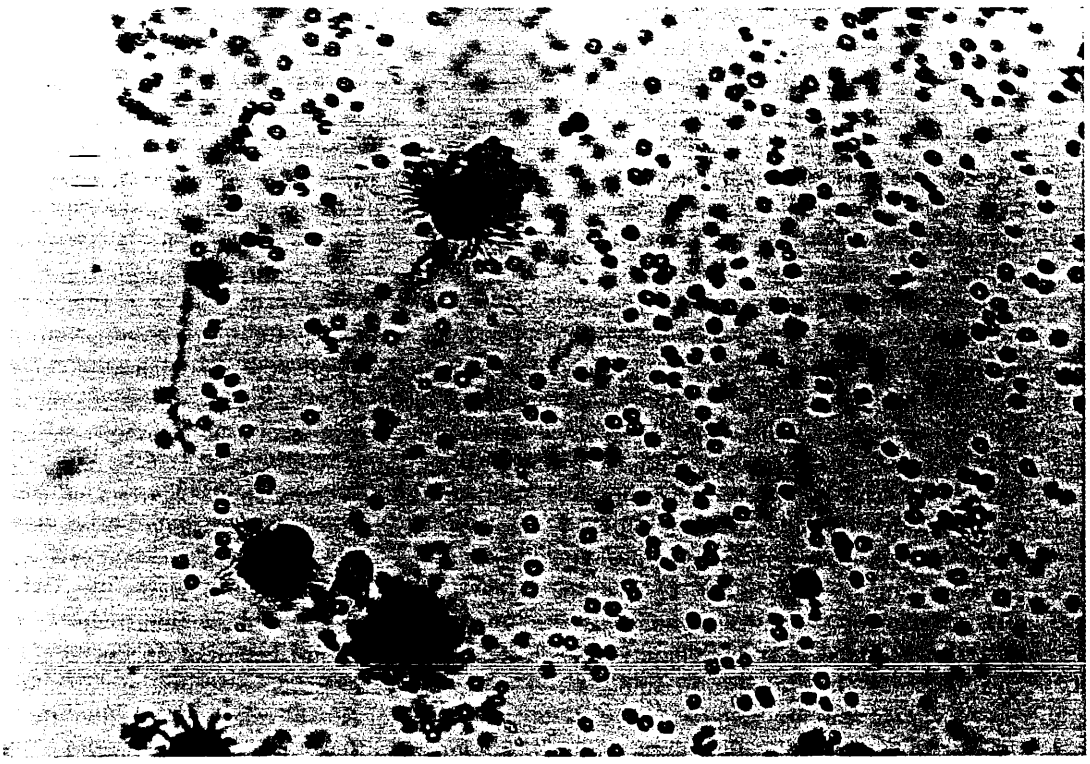

Fig. 5. Ciliates-invaded senescent *Phaeocystis* colony sampled in the Belgian coastal zone at the stationary stage of the bloom development. (Inverted microscopy; photograph: V. Rousseau).

below the deep chlorophyll maximum (Eilertsen et al., 1989). In summer situations, capelin stomach content has been found to be dominated by appendicularians (Hassel et al., 1984). With their fine-meshed filtering nets, these organisms may harvest bacteria directly (Deibel and Powell, 1987), and thus potentially constitute a short link from the microbial community of bacteria and small flagellates to commercially valuable fish stocks.

Bacteria invading the mucoid layer of *Skeletonema* cells during late spring bloom have been shown to be subject to viral attack (Bratbak et al., 1990). Increases in the count of free viruses coinciding with ageing of *Phaeocystis* colonies in Belgian coastal waters has also been observed (Heldal and Bratbak, unpubl. data). In the period following these blooms, Billen and Fontigny (1987) found, however, that the major fraction of bacterial mortality could be attributed to predation, leaving only a minor fraction to viral lysis and other causes of mortality and suggesting the predator food chain to be the major fate of bacterial biomass formed on *Phaeocystis* material.

Decaying colonies have been shown to be first invaded by ciliates and heterotrophic dinoflagellates (Fig. 5), probably grazing on liberated single *Phaeocystis* cells (Lancelot et al., 1991; Becquevort, unpubl. data). Bacterial colonization occurs later and coincides with the development of large populations of heterotrophic flagellates. Decaying aggregates formed at the wane of *Phaeocystis* blooms thus appear as microcosms with well developed grazing food chains.

7. Conclusion

Phaeocystis blooms produce an ample input of organic material to the pelagic environment.

Although the detailed chemistry of the carbohydrates produced are still not entirely known, it appears that this material should be potentially biodegradable. Despite this, it seems to largely resist rapid microbial breakdown. This is particularly the case during active growth of the colonies which are efficiently protected against bacterial colonization by mechanisms not clearly understood.

Slow microbial degradation may also be the case in the senescent phase where the skewed C:N:P-ratio of the material offered to the microbial food chain is suggested as a mechanism slowing down degradation due to nutrient limitation of bacterial growth.

When effective, such mechanisms would shift the trophic structure of *Phaeocystis* based food chains by increasing the probability of mesozooplankton grazing and/or increasing the depth to which sedimentation of colonies and mucus may occur.

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Manuscript 8

Kinetics of Protozoan Predation on two Types of Prey ; Characterisation of Food Selectivity.

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Introduction

Protozooplankton holds a key trophic position as a link between the microbial food web and metazooplankton-based food chain. Dissolved organic matter, bacteria, autotrophic flagellates, diatoms, other protozoa constitute food items of protozoa. Observational evidence of size, motility, nutritional quality and relative concentration of prey cells (Stoecker *et al.* 1981, 1986; Sherr *et al.* 1983, Andersson *et al.* 1986) as food selectivity factors by protozooplankton has been reported in the literature. Accurate description of protozooplankton predation must then take into account the ability of protozoa of switching between different feeding modes when different food ressources are available, but laboratory demonstrations and mathematical formulations of food selectivity are rare.

It has been demonstrated by Menon *et al.* (1996) that the presence of one group of prey is not affecting the maximum protozoan ingestion rate on the other group of prey rather would decrease the affinity of the predator for this latter prey when selectivity between both groups of prey is significant. Physiologically, this decrease in affinity for the considered prey is expressed as demonstrated below by a change in the apparent half-saturation constant describing the protozoan ingestion rate in the presence of different concentrations of the competitive prey.

For one single food prey, the relationship between ingestion rates and food concentration has been already studied, and in the most cases, the protozoan ingestion obeys to Holling type II model (Holling, 1959; Hassell 1978). Fenchel (1982) provided a simple theoretical interpretation of this kinetic based on the idea that predator stops its filtration activity during the time required for the digestion of the prey. The ingestion rate I, expressed in number of prey ingested per predator per unit time is then described by the following equation:

$$I = \frac{F.N}{(1+F.N.T)} \qquad (1)$$

in which T is the digestion time of one prey, F the clearance rate (filtered volume per predator and per unit time) and N the biomass of prey per unit volume.

Dividing all terms by F.T, the equation become an hyperbolic function analogous to that of Michaelis-Menten kinetics:

$$I = Im\,ax.\frac{N}{N+Kg} \qquad (2)$$

defining the maximum ingestion rate (Imax) as $\frac{1}{T}$ and the half-saturation food concentration constant as $\frac{1}{F.T}$.

In the case, of two kind of prey are offered, protozoan ingestion rate I is expressed by the sum of the two feeding activities:

$$I = I_1 + I_2 \tag{3}$$

in which the reciprocal influence of $prey_{1,2}$ digestion time on feeding activities I_1 and I_2 is mathematically described as follows:

$$I1 = \frac{F1.N1}{1 + F1.N1.T1 + F2.N2.T2} \tag{4}$$

$$I2 = \frac{F2.N2}{1 + F2.N2.T2 + F1.N1.T1} \tag{5}$$

Combining (4) and (5) gives the following expressions for I_1 and I_2:

$$I1 = \left(\frac{N1}{N1 + K1g.\left(1 + \frac{N2}{K2g}\right)} \right) \tag{6}$$

$$I2 = \left(\frac{N2}{N2 + K2g.\left(1 + \frac{N1}{K1g}\right)} \right) \tag{7}$$

in which $K1g\left(1 + \frac{N2}{K2g}\right)$ and $K2g\left(1 + \frac{N1}{K1g}\right)$ are defined as apparent values of K'1g and K'2g respectively, thus:

$$I1 = I1\,max.\left(\frac{N1}{N1 + K'1g}\right) \tag{8}$$

$$I2 = I2\,max.\left(\frac{N2}{N2 + K'2g}\right) \tag{9}$$

According to the above model, the protozoan food selectivity between has been studied in the Belgian coastal waters of the North Sea. It consisted in measuring the

kinetics parameters describing the ingestion rate of natural assemblages of protozoa in the presence of various concentrations of competitive prey: bacteria and flagellates (*Phaeocystis* solitary cells).
Protozoan ingestion were estimated by the method based on the uptake of fluorescently prey developed by Sherr *et al.* (1987).

Material and Methods

Material:

Water samples were collected in May at the station 330 in the Belgian coastal waters of the North Sea (N 51°26.05; E 02° 48.50). This station was chosen as a Belgian reference station for *Phaeocystis* bloom development since 1984. This station is under influence of Scheldt river with the salinity ranging between 30 % and 35 %. The average depth is 22 m and the water column is permanently well mixed resulting from very strong tidal currents.

Methods:

Microorganism numbers and biomasses

Bacteria were fixed with formaldehyde 40% (2% final concentration). Glutaraldehyde (0.5% final concentration) was used to preserve phototrophic and heterotrophic flagellates. Biological samples were stored at 4°C before staining (within 12 hours after collection).
Cell density and biovolume of photo- and heterotrophic microorganisms were determined by epifluorescence microscopy (Leitz, Laborlux D) after DAPI staining (Porter and Feig, 1980).
Fixed samples (2 to 5 ml for bacteria and 5 to 20 ml for flagellates) were stained with 4'6 diamidino-2-phenylindole (DAPI) and isolated by filtration on 0.2 µm Nuclepore polycarbonate black membranes under gentle vacuum (<50 mmHg) (Haas, 1982). The filters were rinsed with sterile filtered sea water, air-dried for 30 seconds and mounted in Olympus immersion oil. Microscopic slides were stored at -40 °C until examination.
Bacteria were enumerated on a minimum of 20 different fields at 1000x magnification. Cell sizes were measured visually by comparison with an ocular micrometer and biovolumes were calculated by considering rods and cocci, respectively, as cylinders and spheres (Watson *et al.*, 1977). Biovolumes were converted to cell carbon by using the biovolume-dependent conversion factor established by Simon and Azam (1989).
A minimum of 100 flagellates per filter were counted and phototrophs were discriminated from heterotrophs by the presence of photosynthetic pigments. Cell sizes were measured visually by means of an ocular micrometer. Biovolumes were calculated from cell dimensions and shapes. The Edler (1979) coefficient of 0.11 pgC μm^{-3} was used to estimate carbon biomass from biovolume estimate.

Protozoan ingestion rate

The technique based on the uptake of fluorescently labeled prey (FLB-fluorescently labeled bacteria and FLA-fluorescently labeled algae) proposed by Sherr *et al.* (1987) and by Rublee and Gallegos (1989) was used to estimate protozoan ingestion rate.
Natural protozoan populations are incubated in the presence of added prey (bacteria and flagellates), previously stained with DTAF (5-(4,6-dichlorotriazin-2-yl) aminofluorescein). Fluorescently labeled bacteria were prepared from natural assemblages of bacterioplankton according the procedure de Sherr *et al.* (1987).

Fluorescently labeled algae were supplied from cultures of North Sea *Phaeocystis* sp. using the procedure of Rublee and Gallegos (1989).
The number of prey ingested by protozoa is kinetically measured during the course of the incubation experiment through the microscopic identification of fluorescent prey in protozoan digestive vacuoles. Sub-samples were removed every 15 to 60 min. along the incubation period (2 to 8 h). Biological activity was immediately stopped through the sequential addition of the following preservatives: alkaline lugol solution (0.5% final concentration), borate buffered formalin (3% final concentration), and a drop of 3 % sodium thiosulfate (Sherr *et al.*, 1989). Samples were stored in glass vials at 4°C in the dark until preparation for microscopic analyses. Ingestion rate (number of prey ingested by protozoa per hour) is deduced from the initial slope of the time-dependence curve.

Maximum ingestion rate and half-saturation constant for the ingestion of one prey

The dependence of protozoan ingestion rate on prey abundance was determinate by incubating at *in situ* temperature, natural assemblages of protozooplankton at various concentrations of fluorescent prey between 0.04 10^9 and 0.45 10^9 bacteria l^{-1} (5.5 and 39 µgC l^{-1}), and between 1.1 and 5.3 10^6 *Phaeocystis* solitary cells l^{-1} (8 and 80 µgC l^{-1}). Ingestion rates were plotted against the abundance of bacteria and flagellates. The maximum ingestion rates and the half-saturation constants were estimated by fitting a hyperbolic function to the experimental results, using a program based on the least squares criterion.

Maximum ingestion rate and half-saturation constant for the ingestion of one prey in presence of other prey.

The influence of the presence of one type of prey (auto and heterotrophic flagellates) on the ingestion of the other type of prey (bacteria) was experimentally studied using the set-up of competitive experiments (seen fig. 1, Menon et al., 1996). By dilution or concentration, natural microorganism assemblages of several densities were obtained in order to cover a large range of flagellate prey ($Np_{1,4}$). These assemblages were incubated in the presence of four concentrations of FLB ($Nb_{1,4}$). The maximum ingestion rates and the apparent half-saturation constants were estimated for each assemblage, as described above.

Results

Oligotrich ciliates dominated the protozoan community in the water sample. They ingested bacteria as well as *Phaeocystis* solitary cells. Therefore, the kinetic parameters presented in this paper are those of this protozoan taxon. As represented in figures 2 and 3, the kinetics of protozoan ingestion on both prey followed hyperbolic functions characterized by maximum ingestion rates and half-saturation food concentration constants. Maximum ingestion rates were , respectively, 9.8 cells protozoa^{-1} h^{-1} or 0.015 h^{-1} for *Phaeocystis* solitary cells and 11.18 cells protozoa^{-1} h^{-1} or 0.005 h^{-1} for bacteria. Half-saturation food concentrations were 0.55 10^6 cells l^{-1} or 9.1 µgC l^{-1} for *Phaeocystis* solitary cells and 0.12 10^9 cells l^{-1} or 10.7 µgC l^{-1} for bacteria.

The feeding selectivity between bacteria and *Phaeocystis* solitary cells was studied in the following experiment; the maximum ingestion rate and the apparent half-saturation constant characterizing the protozoan ingestion of *Phaeocystis* solitary cells were estimated in the presence of four bacterial concentrations ranged between 0.001 and 0.83 10^9 cells l^{-1} (Fig. 4) or 0.1 and 75 µgC l^{-1} (Fig. 5). The maximum ingestion rate was ranged between 9.05 and 9.95 *Phaeocystis* solitary cells protozoa^{-1} h^{-1} (mean value: 9.51 cells protozoa^{-1} h^{-1}) or between 0.013 and 0.015 h^{-1} (mean value: 0.014 h^{-1}), and thus was found to be independent of the concentration of bacteria (Fig. 6). On the other hand, the apparent half-saturation food concentration increased with increasing the bacterial biomass (Fig. 7), from 0.27 to 1.30 10^6 *Phaeocystis* solitary cells l^{-1} (6.3 to 20 µgC l^{-1}). According to the competitive model developed above, extrapolation to zero bacterial biomass yields the true value of the half-saturation food concentration constant for *Phaeocystis* solitary cells ingestion by protozoa. Indeed, K'1g = K1g (1 + N2/K2g), and then K1g (half-saturation food concentration for *Phaeocystis* solitary cells) was 0.36 10^6 cells l^{-1} or 6.0 µgC l^{-1}.

Discussion

Protozoa observed in the experiments was principally oligotrich ciliates. Fenchel (1980a), Jonsson(1986) suggest that the oligotrichous ciliates are structurally adapted for the capture of flagellates and no of bacteria. Other studies have demonstrated the occurrence of small oligotrich ciliates which ingest bacteria more or less efficiently (Borsheim, 1984; Riever *et al.*, 1985; Lessard and Swift, 1985; Sherr & Sherr, 1987; Sherr *et al.* 1989..). Today it is widely recognized that planktonic ciliates feed mainly on pico- and nanosized organic particles, including bacteria (Borsheim, 1984; Riever et *al.*, 1985; Lessard and Swift, 1985; Sherr & Sherr, 1987; Sherr *et al.* 1989), coccoid cyanobacteria (Rassoulzadegan *et al.* 1988; Rublee & Gallegos, 1989), auto- and heterotrophic nanoflagellates and small dinoflagellates (Bernard & Rassoulzadegan, 1990; Kivi & Setälä, 1995) and detritus (Posch & Arndt, 1996).

In this study, oligotrich ciliates (ESD, µm) are fed on both bacteria and flagellates. Their functional responses (i.e. the ingestion rates as a function of food concentration) appears to be quite similar to those of protozoa studied previously (Heinbokel 1978, Goldman and Caron 1985, Caron *et al.* 1985, 1986, Parslow *et* al. 1986, Fenchel 1987). When ingesting bacteria as well as flagellates, their functional feeding response is described by an hyperbolic function (Holling type II model, Holling 1959) analogous to the Michaelis-Menten kinetics. The maximal ingestion rate (Imax) of *Phaeocystis* solitary cells was three times higher than of bacteria but ingestion of both saturated at the same food biomasses. 9.1 and 10.7 µgC l^{-1} for the half-saturation constant respectively for *Phaeocystis* solitary cells and bacteria are well within the range of values expected to encounter in the Belgian coastal zone waters (Becquevort et al.,). Few data of Imax and Kg are reported in the literature, however, the values measured in this study are in the same range (Table 1). According to Fenchel (1987) defining the maximum ingestion rate (Imax) as $\frac{1}{T}$, the digestion time (T) for bacteria will be 3 times longer than these for

Phaeocystis single cells. On the other hand, Kg being $\frac{1}{F.T}$, the clearance rate would be smaller for bacteria than for flagellates.

The presence of one type of prey on the functional feeding response of protozooplankton ingesting an other prey has been studied by the generalisation of Holling type II model. This process has been followed in the case of two bacterial prey (Menon et al., 1996). The presence of one population does not affect the maximum ingestion rate of the other prey but decreases the affinity of the predator for this other prey when competition for the two prey is significant. Physiologically, this decrease in affinity for the considered prey is expressed by a competitive-related change in the apparent half-saturation constant describing the protozoan ingestion rate in the presence of different concentration of the competitive prey. Data presented in this paper show that a significant competitive inhibition of the two kinds of tested prey could be evidenced. Consequently, the bacterial prey increase reduced the ciliate affinity for *Phaeocystis* solitary cells.
In the Belgian coastal zone of the North Sea, bacterioplankton responded to phytoplankton development with a delay of 10 days (Billen . Their biomass reached 60 µgC l-1. At this time, the *Phaeocystis* solitary cell biomass was low. Maximum of *Phaeocystis* solitary cells were observed just before or/and after the phytoplankton bloom (Rousseau et al.). Therefore, the bacterial concentration became significant and competitive inhibitor with *Phaeocystis* solitary cells , only when *Phaeocystis* solitary cell biomass was low.

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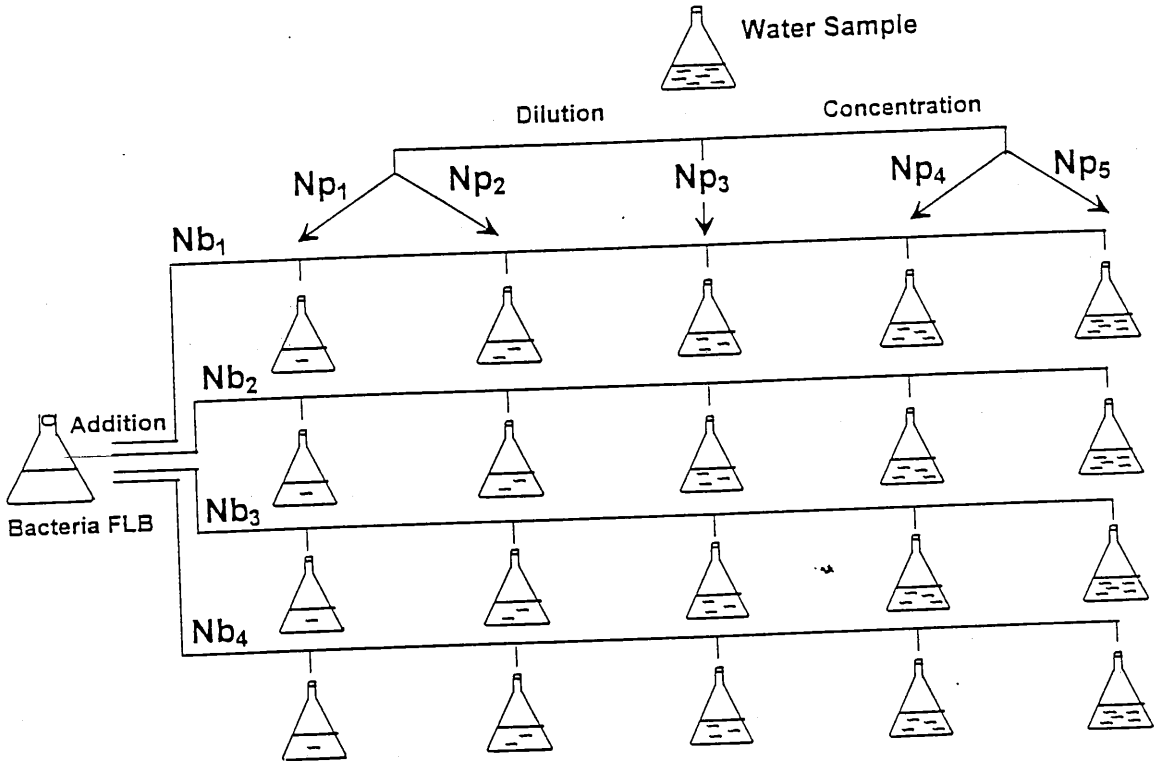

Water Sample
Dilution
Concentration
Np1
Np2
Np3
Np4
Np5
Nb1
Nb2
Nb3
Nb4
Addition
Bacteria FLB

Fig. 1

Figure 1: Schematic representation of the procedure for determining the protozoan kinetics on flagellates (*Phaeocystis* solitary cells,P) and bacteria (B) present simultaneous at various concentrations.

Figure 2: Michaelis-Menten kinetic describing the functional response of protozoan ingestion on bacteria. (A) ingestion rates (bacteria ingested per protozoa per hour) in function of bacterial abundances and (B) ingestion rates (per hour) in function of bacterial biomasses.

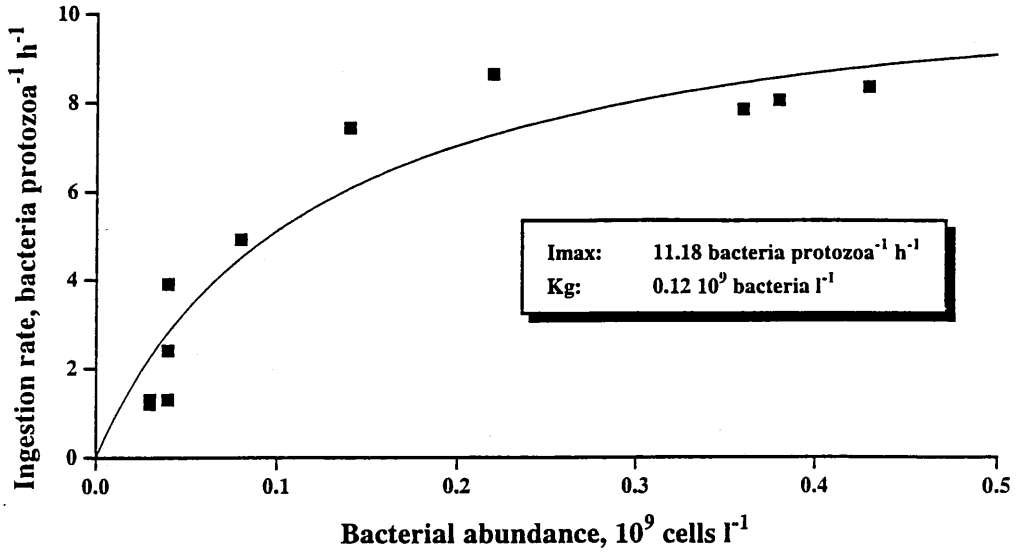
Figure 3: Michaelis-Menten kinetic describing the functional response of protozoan ingestion on flagellates (*Phaeocystis* solitary cells). A) ingestion rates (flagellates ingested per protozoa per hour) in function of flagellate abundances and (B) ingestion rates (per hour) in function of flagellate biomasses.

Figure 4: Michaelis-Menten kinetics describing the functional response of protozoan ingestion on flagellates (*Phaeocystis* solitary cells) in presence of different bacterial abundances.

Figure 5: Michaelis-Menten kinetics describing the functional response of protozoan ingestion on flagellates (*Phaeocystis* solitary cells) in presence of different bacterial biomasses.

Figure 6: Protozoan maximum ingestion rates on flagellates (*Phaeocystis* solitary cells) as a function of bacterial abundances (a) and biomasses (b).

Figure 7: Protozoan apparent half-saturation constant for flagellates (*Phaeocystis* solitary cells) as a function of bacterial abundances (a) and biomasses (b).


Ingestion rate, bacteria protozoa^{-1} h^{-1}
10
8
6
4
2
0
0.0
0.1
0.2
0.3
0.4
0.5
Bacterial abundance, 10^9 cells l^{-1}
Imax: 11.18 bacteria protozoa^{-1} h^{-1}
Kg: 0.12 10^9 bacteria l^{-1}

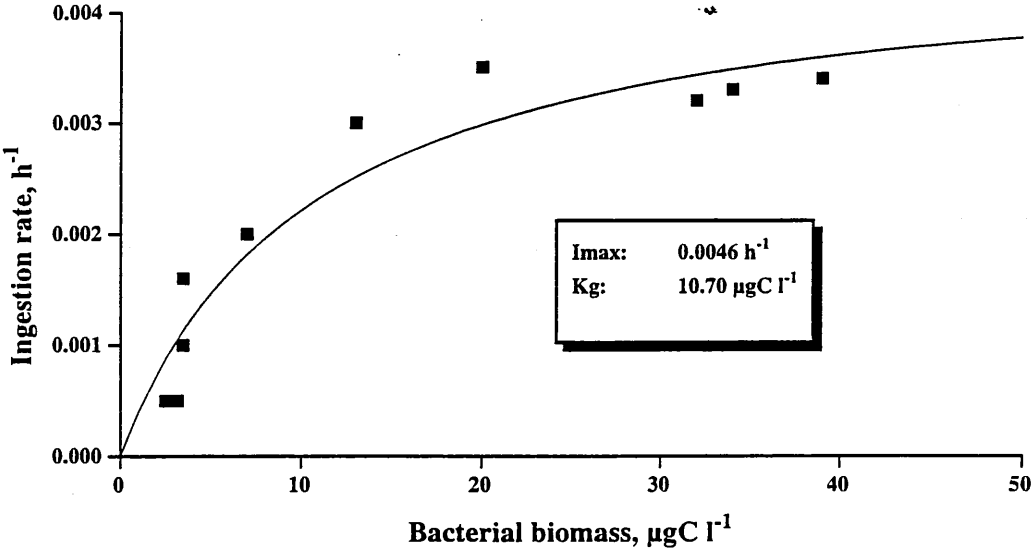
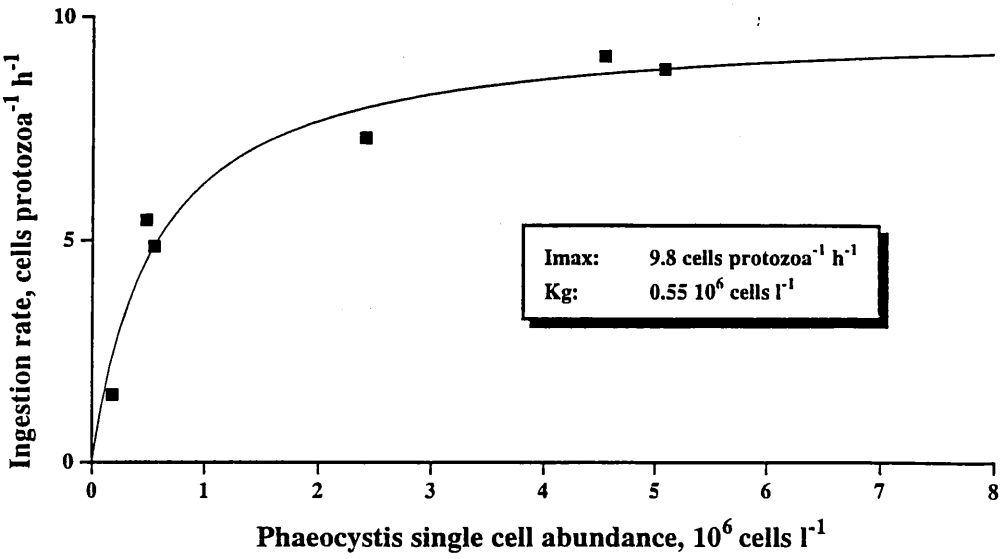

Ingestion rate, h^{-1}
0.004
0.003
0.002
0.001
0.000
0
10
20
30
40
50
Bacterial biomass, µgC l^{-1}
Imax: 0.0046 h^{-1}
Kg: 10.70 µgC l^{-1}

fig.1


Ingestion rate, cells protozoa^{-1} h^{-1}
10
5
0
0
1
2
3
4
5
6
7
8
Imax: 9.8 cells protozoa^{-1} h^{-1}
Kg: 0.55 10^{6} cells l^{-1}
Phaeocystis single cell abundance, 10^{6} cells l^{-1}

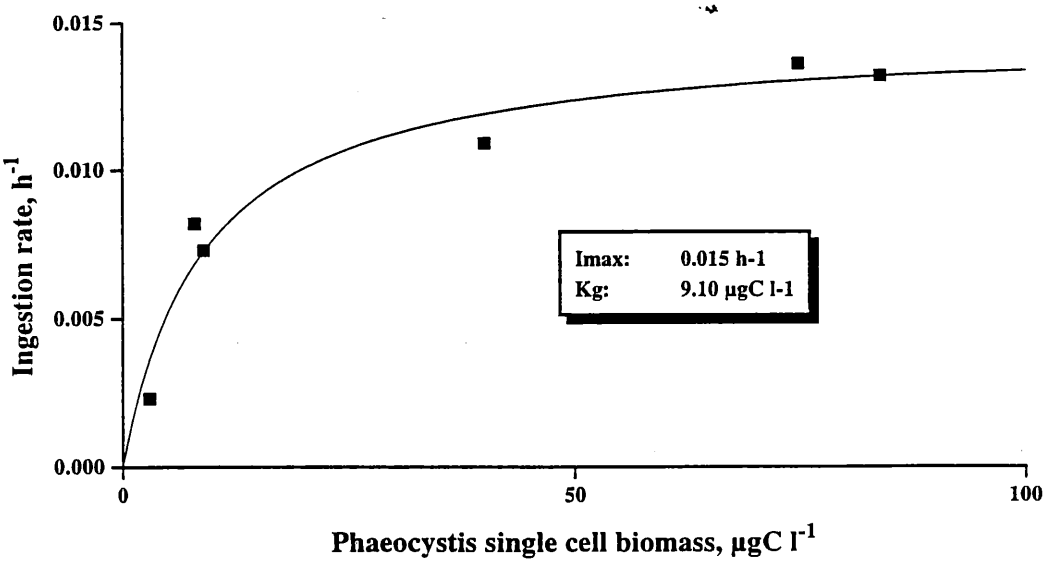
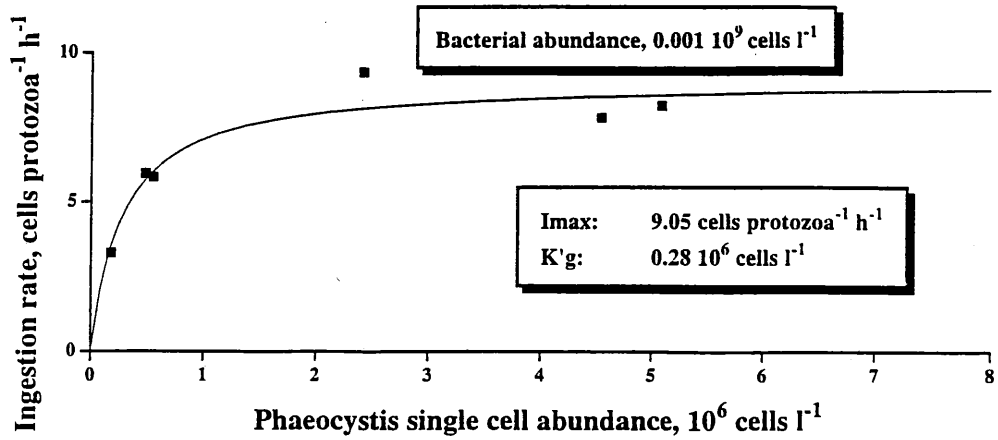
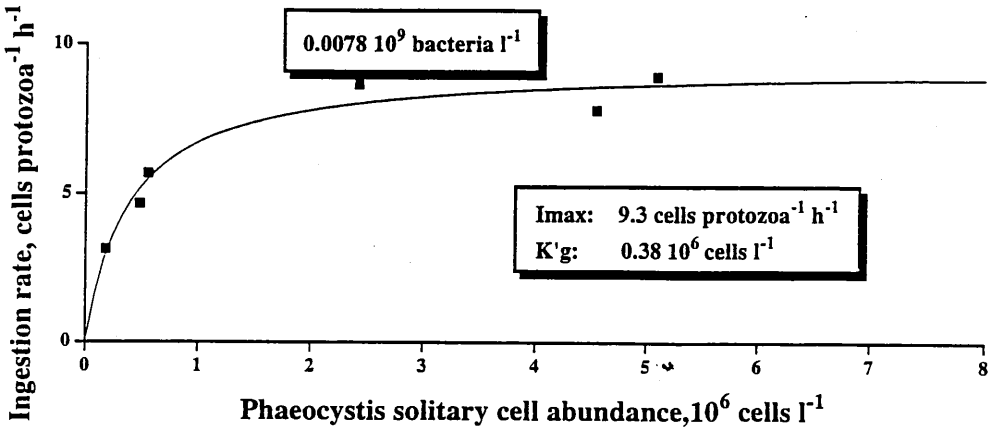
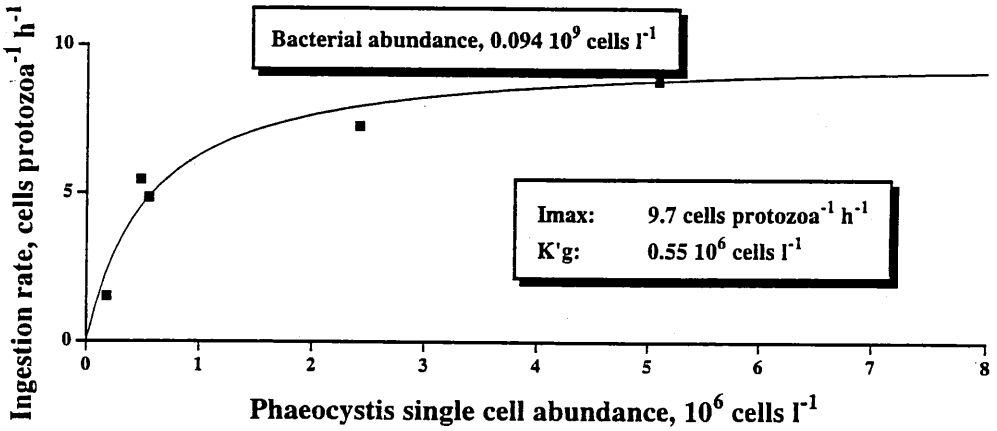

Ingestion rate, h^{-1}
0.015
0.010
0.005
0.000
0
50
100
Imax: 0.015 h-1
Kg: 9.10 µgC l-1
Phaeocystis single cell biomass, µgC l^{-1}

Fig.3


Bacterial abundance, 0.001 10^9 cells l^-1
Imax: 9.05 cells protozoa^-1 h^-1
K'g: 0.28 10^6 cells l^-1
Ingestion rate, cells protozoa^-1 h^-1
Phaeocystis single cell abundance, 10^6 cells l^-1


0.0078 10^9 bacteria l^-1
Imax: 9.3 cells protozoa^-1 h^-1
K'g: 0.38 10^6 cells l^-1
Ingestion rate, cells protozoa^-1 h^-1
Phaeocystis solitary cell abundance,10^6 cells l^-1


Bacterial abundance, 0.094 10^9 cells l^-1
Imax: 9.7 cells protozoa^-1 h^-1
K'g: 0.55 10^6 cells l^-1
Ingestion rate, cells protozoa^-1 h^-1
Phaeocystis single cell abundance, 10^6 cells l^-1

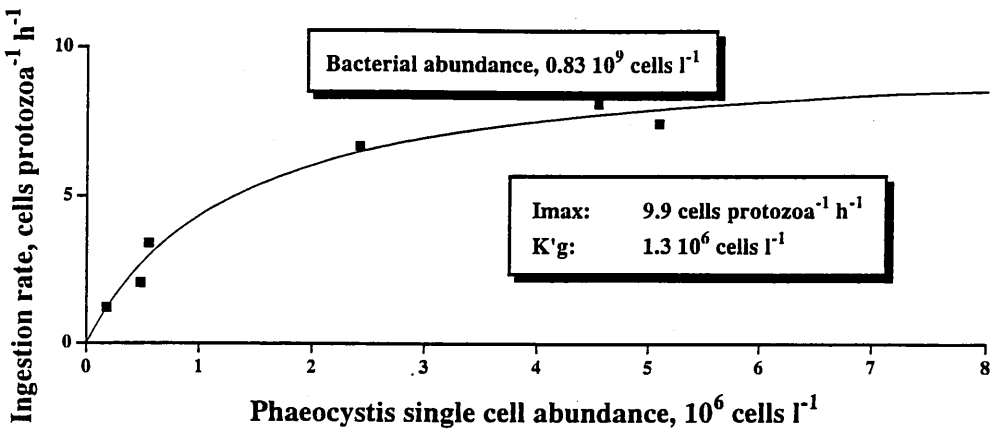
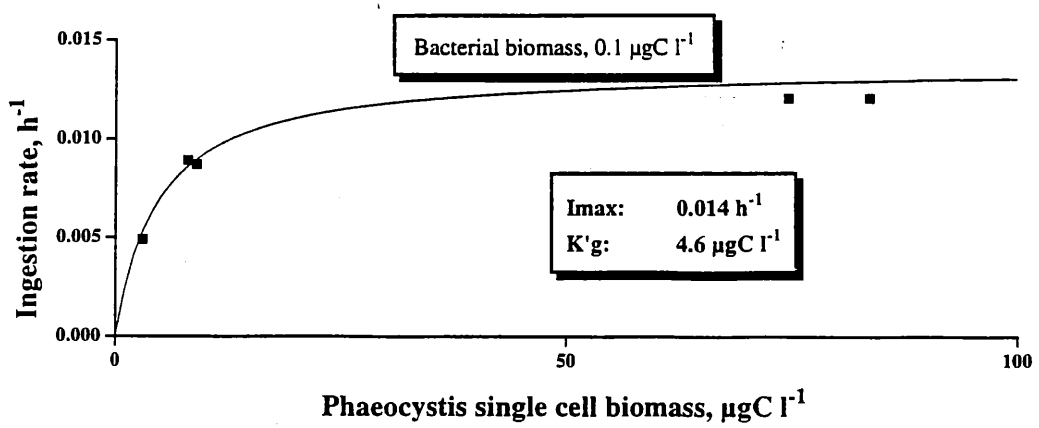
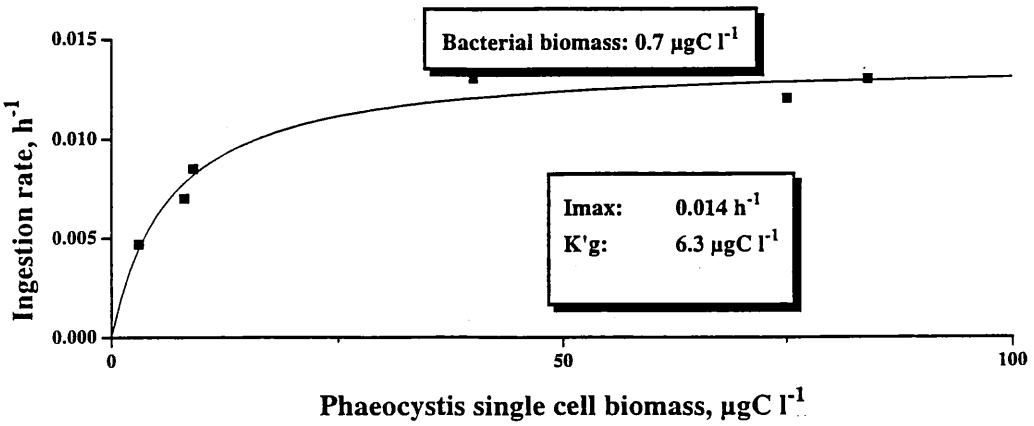
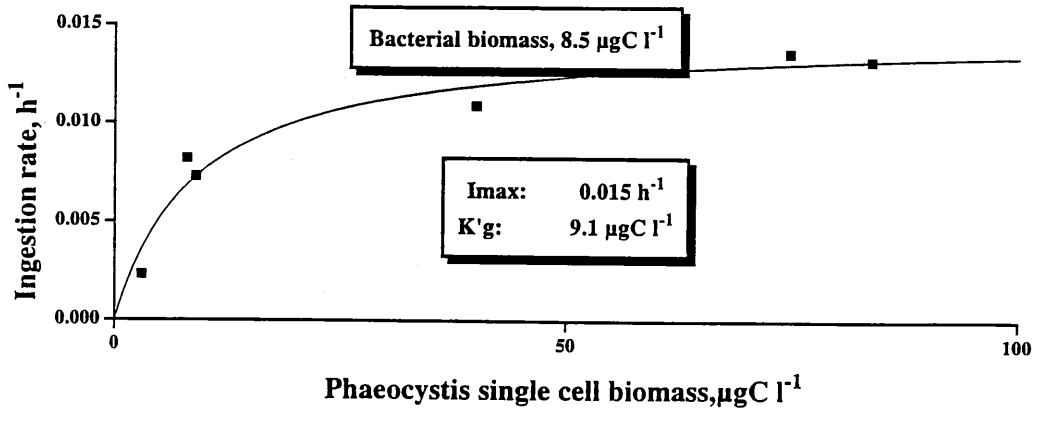
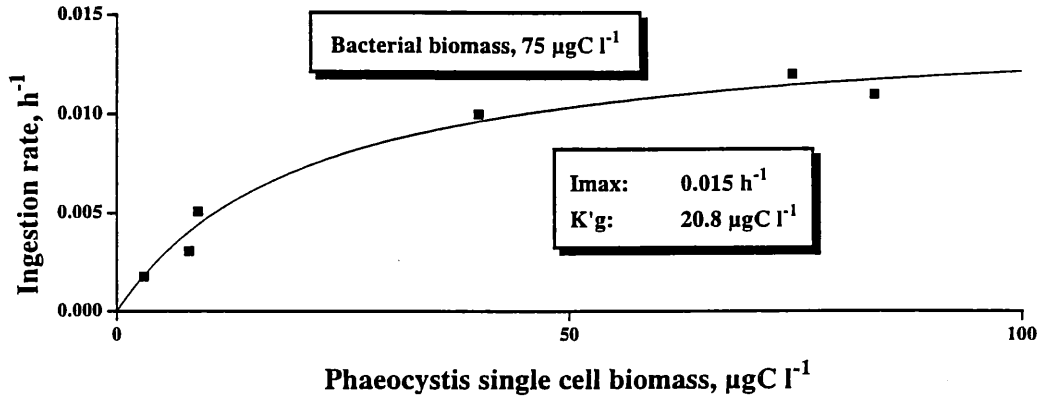

Bacterial abundance, 0.83 10^9 cells l^-1
Imax: 9.9 cells protozoa^-1 h^-1
K'g: 1.3 10^6 cells l^-1
Ingestion rate, cells protozoa^-1 h^-1
Phaeocystis single cell abundance, 10^6 cells l^-1

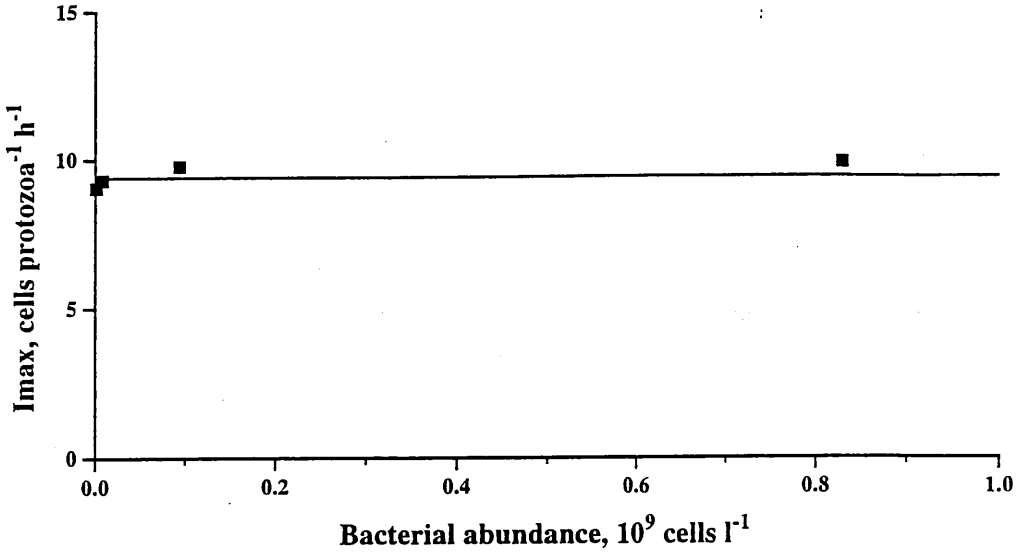
fig. 4


Bacterial biomass, 0.1 µgC l-1
Imax: 0.014 h-1
K'g: 4.6 µgC l-1
Ingestion rate, h-1
0.015
0.010
0.005
0.000
0
50
100
Phaeocystis single cell biomass, µgC l-1


Bacterial biomass: 0.7 µgC l-1
Imax: 0.014 h-1
K'g: 6.3 µgC l-1
Ingestion rate, h-1
0.015
0.010
0.005
0.000
0
50
100
Phaeocystis single cell biomass, µgC l-1


Bacterial biomass, 8.5 µgC l-1
Imax: 0.015 h-1
K'g: 9.1 µgC l-1
Ingestion rate, h-1
0.015
0.010
0.005
0.000
0
50
100
Phaeocystis single cell biomass,µgC l-1


Bacterial biomass, 75 µgC l-1
Imax: 0.015 h-1
K'g: 20.8 µgC l-1
Ingestion rate, h-1
0.015
0.010
0.005
0.000
0
50
100
Phaeocystis single cell biomass, µgC l-1


15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
Imax, cells protozoa^{-1} h^{-1}
Bacterial abundance, 10^9 cells l^{-1}

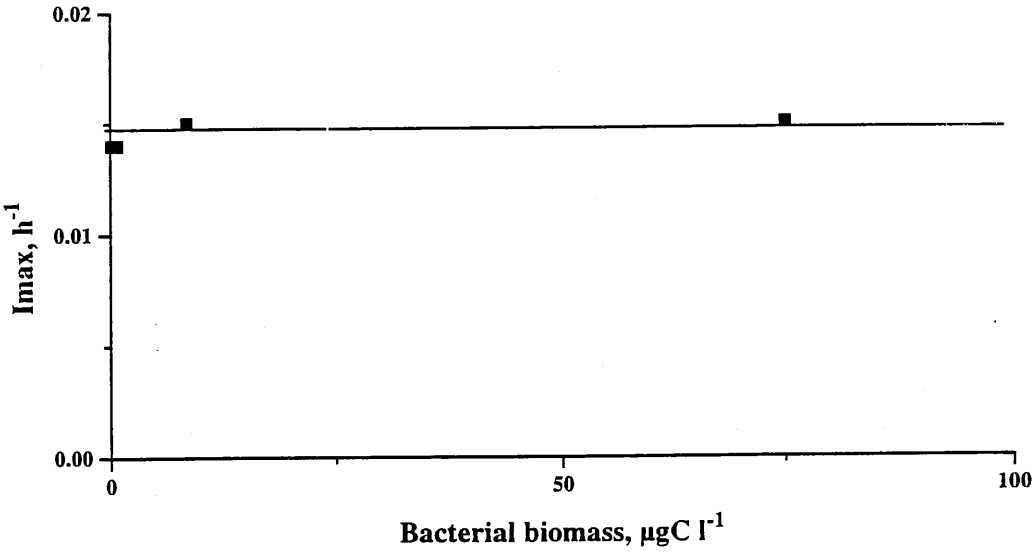
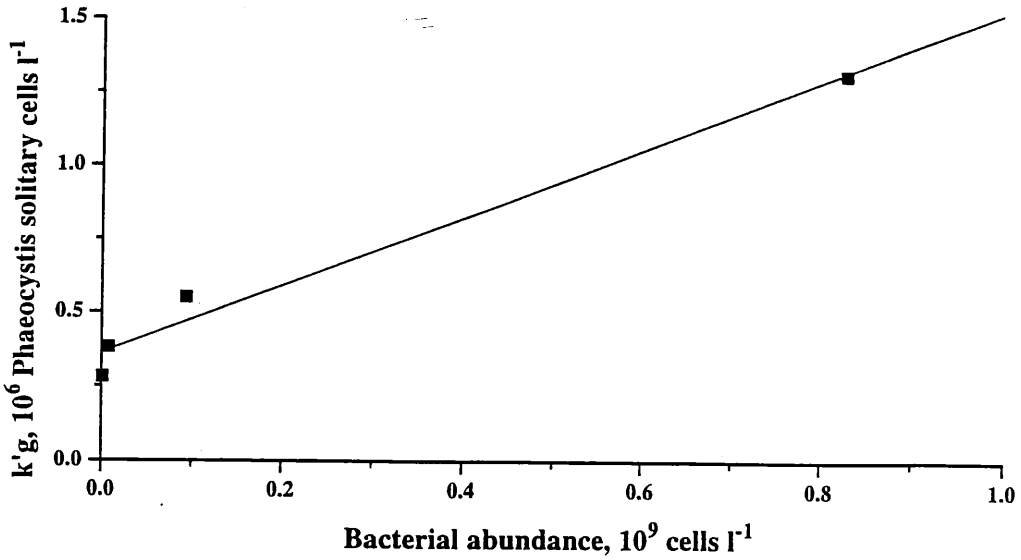

0.02
0.01
0.00
0
50
100
Imax, h^{-1}
Bacterial biomass, µgC l^{-1}

fig. 6


k'g, 10^6 Phaeocystis solitary cells l^{-1}
1.5
1.0
0.5
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Bacterial abundance, 10^9 cells l^{-1}

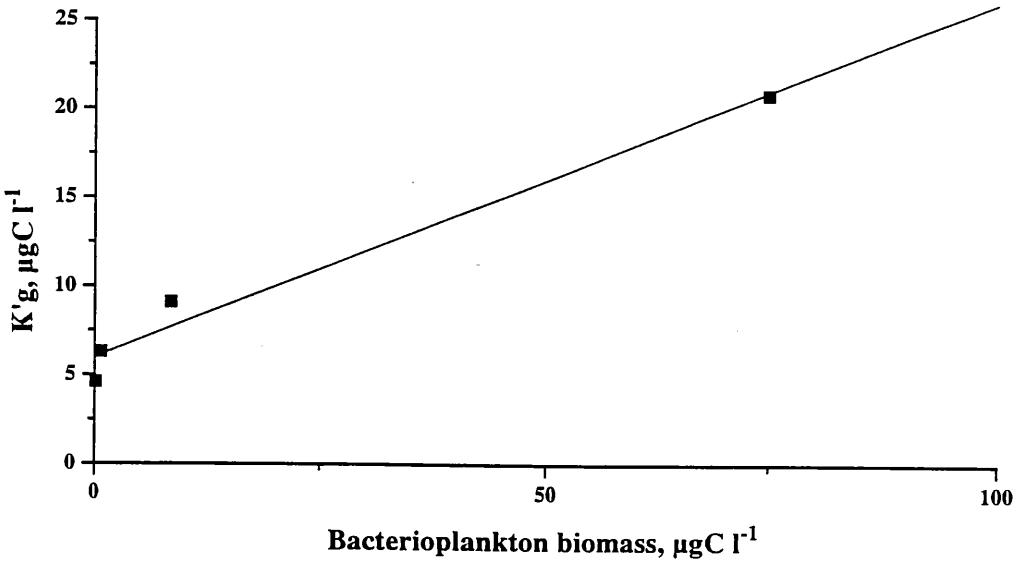

K'g, µgC l^{-1}
25
20
15
10
5
0
0
50
100
Bacterioplankton biomass, µgC l^{-1}

fig 7c

Manuscript 9

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MICROBIAL ECOLOGY

Kinetics of Flagellate Grazing in the Presence of Two Types of Bacterial Prey

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Abstract. Grazing rates of mixed cultures of freshwater, heterotrophic nanoflagellates on two populations of bacterial prey present together at varying concentrations were measured by using fluorescently labeled bacteria. The effect of one population on the ingestion kinetics of the other was consistent with a theory based on competitive inhibition of enzymatic reactions. However, allochthonous bacteria, when present in low concentrations within a much larger population of small autochthonous bacteria, may be preferentially grazed, which is due to their large size.

Introduction

Grazing by phagotrophic flagellates is the major cause of bacterial mortality in aquatic environments [17, 21, 23, 24, 28]. Since the early work of Fenchel [7, 8], it has been recognized that flagellate ingestion rate obeys hyperbolic kinetics as a function of the concentration of bacterial prey. Fenchel provided a simple theoretical interpretation of this kinetics based on the idea that the predator stops its filtration activity during the time required for digestion of a captured prey. If T represents the digestion time of one prey, and F is the clearance rate (volume filtered by one predator per unit time), the ingestion rate (I, in number of prey ingested per predator per unit time) can then be written:

$$I = \frac{F \cdot N}{1 + F \cdot N \cdot T} \tag{1}$$

where N is the abundance of prey per unit volume.

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Dividing all terms by $F \cdot T$, yields

$$I = I_{max} \cdot \frac{N}{N + K_g} \tag{2}$$

if I_{max} is defined as $1/T$ and K_g as $1/(F \cdot T)$.

In this form, relation 2 is analogous to a Michaelis-Menten relationship between ingestion rate and prey concentration, with maximum ingestion rate (I_{max}) inversely depending on the digestion time, and half-saturation prey concentration (K_g) depending on both digestion time and clearance rate. While the clearance rate (F) is a characteristic of the predator and its filtration mechanism, and depends on food size, shape, and motility, the digestion time (T) also depends on the size and biochemical composition of the prey. This provides a simple theoretical background for interpreting "preferences" of predators toward some kind of prey [4, 12, 15, 19, 31, 32].

The problem we address in this report is that of the kinetics of flagellate bacterivory in the presence of several prey with different morphological or biochemical characteristics, hence the possibility of different prey "preferences." This question is important, for example, in the case of allochthonous bacteria with specific morphological characteristics introduced into an autochthonous community of bacteria and protozoans. Allochthonous bacteria can be fecal bacteria or other heterotrophic bacteria accompanying treated or untreated wastewater [10, 11]. Allochthonous bacteria are known to be exposed to grazing by autochthonous flagellates [9, 13, 14, 27], but it is unclear how the presence of one population of prey interferes with the grazing of others. How can we predict the effect of the often overwhelmingly dominant autochthonous population on the grazing rate of the allochthonous bacteria?

This question can be answered theoretically by extending Fenchel's theory and its analogy to Michaelis-Menten kinetics to the case of simultaneous ingestion of two distinct populations of prey (populations 1 and 2). We will show that in this case an analogy holds with the theory of competitive inhibition of enzymatic reactions.

Extending expression 1 by taking into account the effect of the digestion time of each prey on the grazing activity of the predator yields [16, 20]:

$$I_1 = \frac{F_1 \cdot N_1}{1 + F_1 \cdot N_1 \cdot T_1 + F_2 \cdot N_2 \cdot T_2} \tag{3}$$

and

$$I_2 = \frac{F_2 \cdot N_2}{1 + F_2 \cdot N_2 \cdot T_2 + F_1 \cdot N_1 \cdot T_1}$$

Defining, as above,

$$I_{1max} = 1/T_1 \quad \text{and} \quad I_{2max} = 1/T_2 \tag{4}$$

and

$$K_{1g} = 1/(F_1 \cdot T_1) \quad \text{and} \quad K_{2g} = 1/(F_2 \cdot T_2) \tag{5}$$

relation 3 can be rearranged as follows

$$I_1 = I_{1\max} \cdot \left(\frac{N_1}{N_1 + K_{1g} \cdot \left(1 + \frac{N_2}{K_{2g}}\right)} \right) \tag{6}$$

Similarly, it can be shown that

$$I_2 = I_{2\max} \cdot \left(\frac{N_2}{N_2 + K_{2g} \cdot \left(1 + \frac{N_1}{K_{1g}}\right)} \right) \tag{7}$$

Relations 6 and 7 can be rewritten,

$$I_1 = I_{1\max} \cdot \left(\frac{N_1}{N_1 + K'_{1g}} \right) \text{ and } I_2 = I_{2\max} \cdot \left(\frac{N_2}{N_2 + K'_{2g}} \right) \tag{8}$$

by defining "apparent" values of K_{1g} and K_{2g} as (apparent half-saturation constant are noted K′)

$$K'_{1g} = K'_{1g} \cdot \left(1 + \frac{N_2}{K_{2g}}\right) \text{ and } K'_{2g} = K_{2g} \cdot \left(1 + \frac{N_1}{K_{1g}}\right) \tag{9}$$

The presence of a second population of prey therefore acts on the ingestion rate of the first by altering the apparent value of the half-saturation constant but without modifying the maximum ingestion rate. This is the exact characteristic of the competitive inhibition of an enzymatic reaction in the presence of a substance recognized by the enzyme as an alternative substrate.

Note that when the ingestion parameters ($I_{\max}$, K_g) of both populations are identical, relations 8 can simply be written

$$I_1 = I_{\max} \cdot \left(\frac{N_1}{N_1 + N_2 + K_g} \right) \text{ and } I_2 = I_{\max} \cdot \left(\frac{N_2}{N_1 + N_2 + K_g} \right)$$

hence

$$I_1 = I_t \cdot \left(\frac{N_1}{N_1 + N_2} \right) \text{ and } I_2 = I_t \cdot \left(\frac{N_2}{N_1 + N_2} \right)$$

with

$$I_t = I_{\max} \cdot \left(\frac{N_1 + N_2}{N_1 + N_2 + K_g} \right)$$

In this case only, the rate of ingestion of each population is simply proportional to its relative abundance with respect to the other.

The purpose of this report is to experimentally demonstrate the validity of this analogy between the kinetics of flagellate grazing in the presence of two distinct populations of prey and the theory of competitive inhibition of enzymatic reactions. As an experimental model system, we used enriched cultures of bacteria and

phagotrophic protozoans obtained from Seine river water, with addition of a culture of *Escherichia coli* as a second, easily distinguishable, bacterial prey.

Materials and Methods

Enriched Cultures of Bacteria and Protozoans

River water samples were collected in the river Seine downstream from Paris (France). They were enriched in protozoa by incubating them at 20°C for a few days in the presence of wheat grains, following the procedure proposed by Fenchel [6]. Bacteria present in the enriched cultures were considered to be autochthonous bacteria (0.075–0.15 μm^3). A strain of *E. coli* (1–1.6 μm^3), recently isolated in our laboratory from human feces and identified using API 20 E system, was used as model of allochthonous bacteria.

Bacterial Numbers and Biomass

Bacterial numbers were determined by epifluorescence microscopy at 1000× magnification after DAPI (4′-6 Diaminophenylindol) staining, following the procedure of Porter and Feig [22]. Bacteria were counted in 10 fields on one preparation. Bacterial cell size was determined by measuring with an eyepiece graticule the length and width of 100 randomly selected bacteria in each sample. Biovolumes were calculated by treating rods and cocci as cylinders and spheres, respectively [34]. Biovolumes were converted into carbon units using the biovolume-dependent conversion factors proposed by Simon and Azam [33], which ranges from 4.10^{-13} g C μm^{-3} for the smaller bacteria (0.026 μm^3) to $1.3.10^{-13}$ g C μm^{-3} for the larger ones (>0.4 μm^3).

Nanoflagellate Numbers and Biomass

Enumeration of heterotrophic nanoflagellates (2–20 μm) was performed by epifluorescence microscopy after DAPI staining, following the procedure developed by Porter and Feig [22]. In each sample, 100–150 organisms were enumerated. Phototrophs were discriminated from heterotrophs by the red chlorophyll autofluorescence. Cell sizes were usually measured with an eyepiece graticule, and cell volumes (15–26 μm^3) were calculated from stereometric shapes of the cells [5]. Biovolumes were converted into biomass using the conversion factor proposed by Edler [5]: $1.1.10^{-13}$ g C μm^{-3}.

Grazing Rate Estimation

The technique based on the uptake of fluorescently labeled bacteria (FLB) proposed by Sherr et al. (29, 30) was used to estimate flagellate grazing rates. Bacteria previously stained with 5-(4,6-dichlorotriazin-2-Y1)amino] fluorescein (DTAF) were directly visualized within flagellate vacuoles by epifluorescence microscopy. After addition of FLB to the sample, the number of FLB ingested by protozoans was followed as a function of time for short incubation periods (around 1 h) (Fig. 1). The initial slope of increase of ingested FLB per flagellate was taken as a measure of grazing. In our study, FLB were prepared with autochthonous bacteria (auto-FLB) and *E. coli* (*E. coli* FLB) to estimate the grazing rates of these two types of bacteria.

Maximum Rates and Half-Saturation Constant for the Grazing of Autochthonous Bacteria

Using an enriched culture of autochthonous bacteria and protozoa, a range of six ($0.9.10^9$, $1.8.10^9$, $2.8.10^9$, $3.3.10^9$, $4.3.10^9$, 8.10^9 bacteria per liter) (experiment 1) or five ($2.4.10^9$, $4.1.10^9$, $7.6.10^9$,

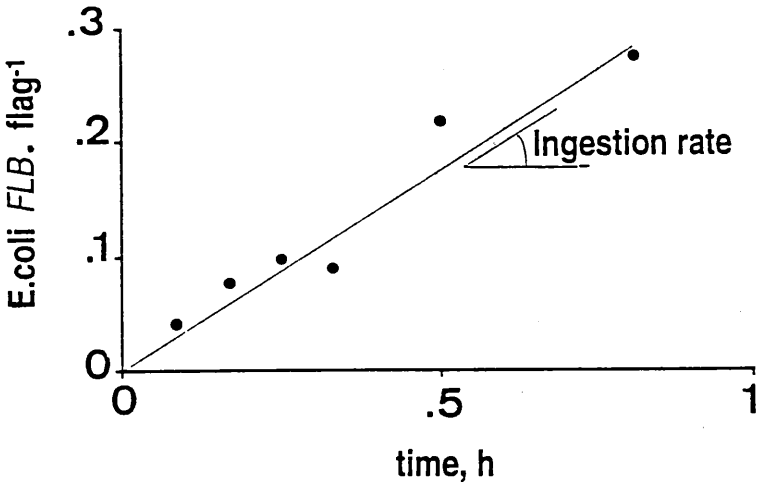


Fig. 1. Ingestion kinetics of *E. coli* FLB by heterotrophic nanoflagellate. Determination of the grazing rate (*E. coli* FLB/flagellate · h) by the initial slope of the curve.

$11.1.10^9$, 30.10^9 bacteria per liter) (experiment 2) concentrations of autochthonous bacteria was prepared by either dilution in 0.2 μm filtered water or concentration by centrifugation. Figure 2a schematically illustrates the measurement carried out in experiment 2. Auto-FLB were added in tracer concentration (around 1% of autochthonous bacterial abundance), and the grazing rate on these labeled prey (I_{FLB}) was estimated. The in situ grazing rate on the total pool of autochthonous bacteria (I) was calculated from I_{FLB}, taking into account the presence of auto-FLB and nonlabeled autochthonous bacteria: $I = I_{FLB} \cdot (N_{FLB} + N)/N_{FLB}$, [29], where N_{FLB} is the abundance in auto-FLB, and N is the abundance of nonlabeled autochthonous bacteria [30, 33]. In situ grazing rates were plotted against the abundance of autochthonous bacteria. The maximum grazing rates and the half-saturation constants were estimated by fitting a hyperbolic function to the experimental results, using a program based on the least squares criterion.

Maximum Rates and Half-Saturation Constant of E. coli *Grazing*

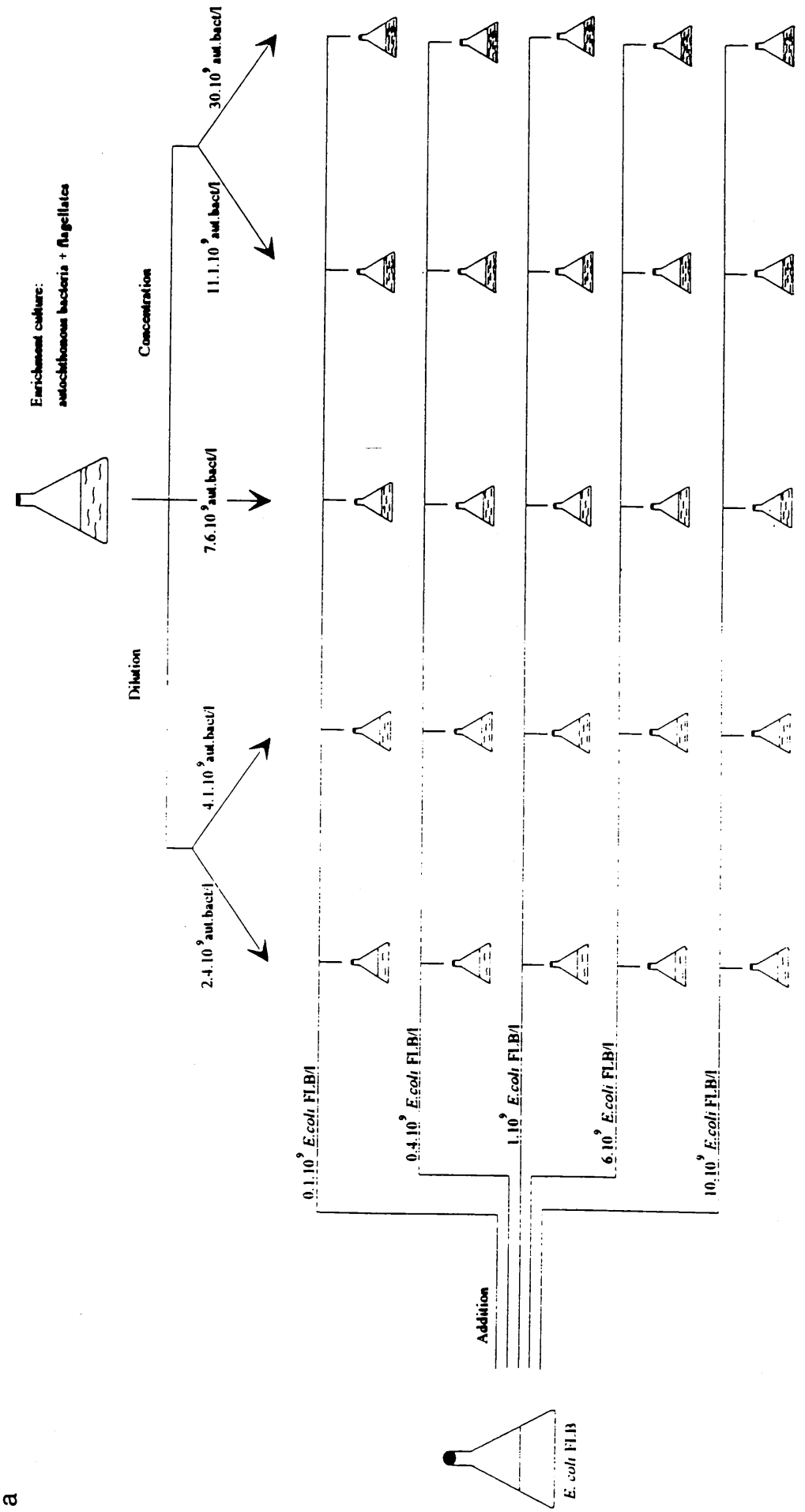
The maximum grazing rate and the apparent half-saturation constant of *E. coli* were determined in the presence of various concentrations of autochtonous bacteria by estimating the grazing rate of *E. coli* FLB added at various concentrations (experiment 1: $0.11.10^9$, $0.36.10^9$, $0.7.10^9$, 1.10^9, $5.5.10^9$ *E. coli* FLB per liter; experiment 2: $0.1.10^9$, $0.4.10^9$, 1.10^9, 6.10^9, 10.10^9 *E. coli* FLB per liter) in different autochthonous bacteria concentrations (experiment 1: $0.9.10^9$, 2.10^9, 3.10^9 autochthonous bacteria per liter; experiment 2: $2.4.10^9$, $4.1.10^9$, $7.6.10^9$, $11.1.10^9$, 30.10^9 autochthonous bacteria per liter). Figure 2b schematically represents the measurement carried out in experiment 2.

The maximum grazing rates and the apparent half-saturation constants were estimated, as for autochthonous bacteria, by adjustment of a hyperbolic function to the experimental results of grazing rates as function of *E. coli* concentration.

Results

Maximum ingestion rates, estimated from experiments 1 and 2 (Fig. 3a, b, respectively), were, respectively, 5.5 and 9.1 autochthonous bacteria grazed per flagellate per hour (Table 1). Half-saturation constants, deduced by best-fitting were $2.55.10^9$ and $11.80.10^9$ autochthonous bacteria per liter for experiments 1 and 2, respectively.

The maximum grazing rate and the apparent half-saturation constant for *E. coli* were determined in the presence of various concentrations of autochthonous bacteria by estimating the grazing rate on *E. coli* FLB added at various concentrations in


Enrichment culture:
autochthonous bacteria + flagellates
Concentration
Dilution
30.10⁹ aut.bact/l
11.1.10⁹ aut.bact/l
7.6.10⁹ aut.bact/l
4.1.10⁹ aut.bact/l
2.4.10⁹ aut.bact/l
0.1.10⁹ E.coli FLB/l
0.4.10⁹ E.coli FLB/l
1.10⁹ E.coli FLB/l
6.10⁹ E.coli FLB/l
10.10⁹ E.coli FLB/l
Addition
E. coli FLB

a

b

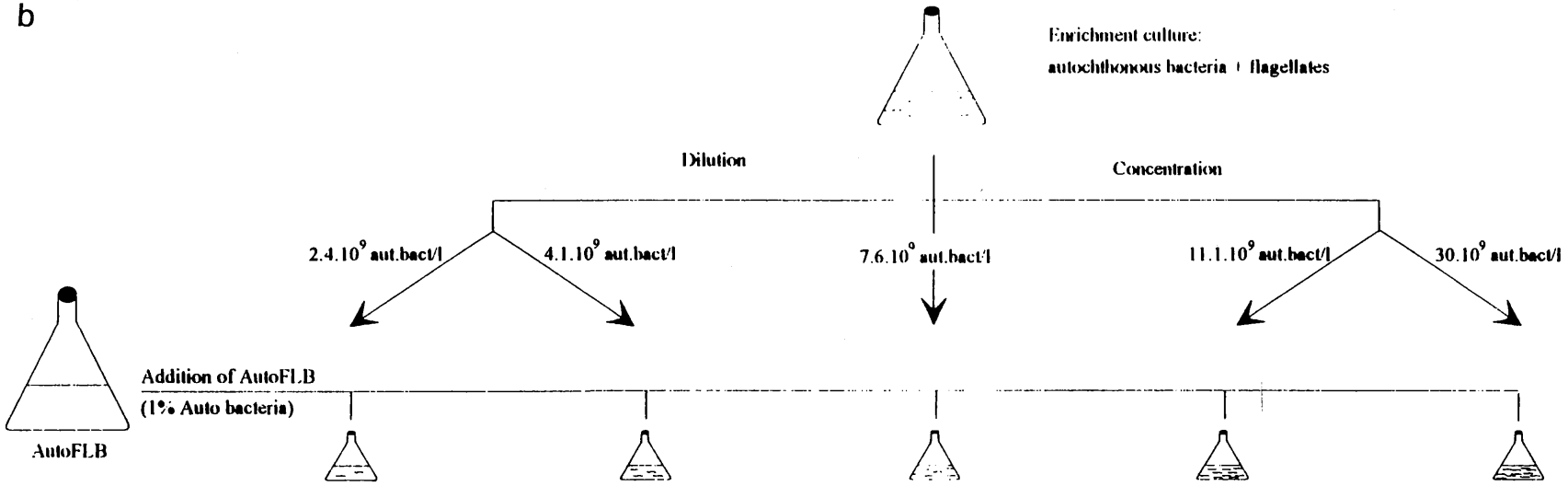


Fig. 2. Schematic representation of the procedure used for determining the kinetics of flagellate grazing on **a,** autochthonous bacteria and, **b,** *E. coli* present simultaneously at various concentrations (experiment 2, see text).

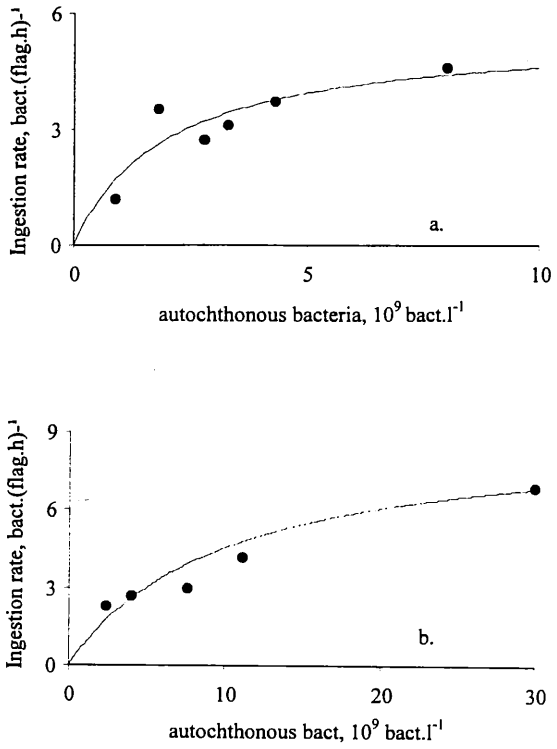


Fig. 3. Ingestion rates (bacteria · flagellate^{-1} · h^{-1}) of autochthonous bacteria by nanoflagellates. **a,** Experiment 1. **b,** Experiment 2.

each of the autochthonous bacteria concentrations (Fig. 4). Maximum rate of ingestion of *E. coli* was found to be independent of the concentration of autochthonous bacteria (Fig. 5a, b). By contrast, the apparent half-saturation constant for *E. coli* ingestion linearly increased with increasing concentrations of autochthonous bacteria (Fig. 5c, d). Extrapolation to zero autochthonous bacterial concentrations yields the true value of the half-saturation constant for *E. coli*. The slopes of the regression lines were 0.22 and 0.24 for experiments 1 and 2, respectively. These values are very close to the ratios of the half-saturation constants for *E. coli* and for autochthonous bacteria (0.23 and 0.22 for experiments 1 and 2, respectively), as deduced from our kinetic determinations (Table 1). The relationships represented in Fig. 5c and d thus strictly obey relation 9 above and relation 10 below:

$$K'_{\mathrm{gEcoli}} = K_{\mathrm{gEcoli}} \cdot \left(1 + \frac{N_{\mathrm{auto}}}{K_{\mathrm{gauto}}}\right) \tag{10}$$

or

$$K'_{\mathrm{gEcoli}} = K_{\mathrm{gEcoli}} + \left(\frac{K_{\mathrm{gEcoli}}}{K_{\mathrm{gauto}}} \cdot N_{\mathrm{auto}}\right)$$

Discussion

The observed effect of increasing concentrations of autochthonous bacteria on the ingestion rate of allochthonous bacteria is consistent with the theory of competitive inhibition developed above: The presence of one population of prey does not affect the maximum ingestion rate of the other but decreases the affinity of the predator for this other prey, as measured by the apparent half-saturation constant of ingestion, according to equation 10.

Table 1. Kinetic parameters of flagellates grazing on autochthonous bacteria (aut. bact.) and *E. coli*, determined in two experiments carried out with enrichment cultures obtained from river water

			I_{max}		K_g	
Experiment	Predator	Prey	bact.flag.$^{-1}$ h^{-1}	mgC.mgC^{-1} h^{-1}	10^9 bact. liter^{-1}	mgC.liter^{-1}
1	Flagellate (1.67)[a]	Aut. bact. (0.06)[a]	5.5 (±2.1)	0.189	2.55 (±0.55)	0.146
		E. coli (0.12)[a]	1.3 (±0.0)	0.093	0.6[b]	0.078
2	Flagellate (2.88)[a]	Aut. bact (0.03)[a]	9.1 (±2.6)	0.091	11.80 (±0.10)	0.341
		E. coli (0.08)[a]	3.5 (±0.7)	0.102	2.6[b]	0.218

[a] Specific biomass in pgC/organism.

[b] Values obtained by extrapolation to zero autochthonous bacteria (see Fig. 5).

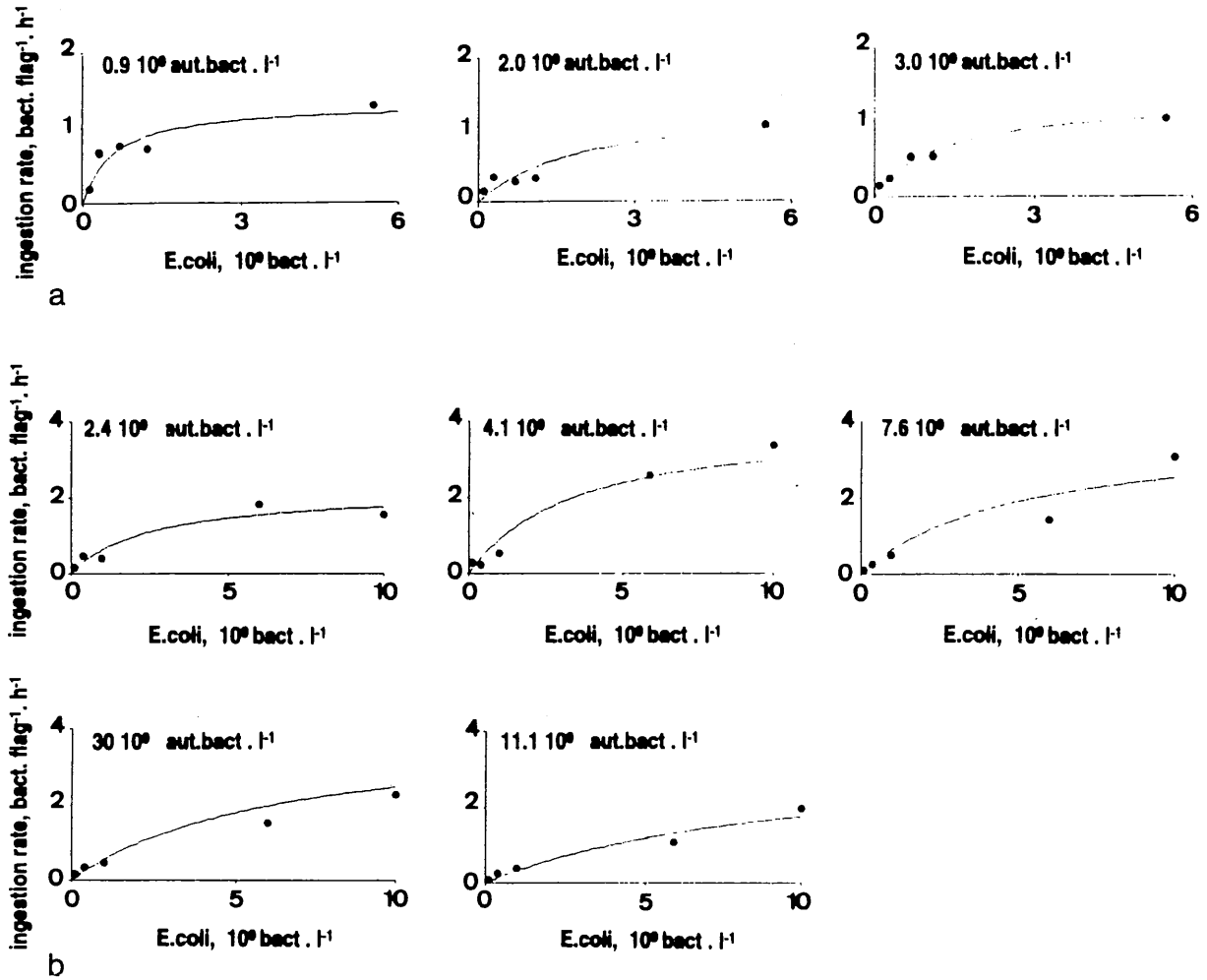


Fig. 4. Ingestion rates of *E. coli* (*E. coli* · flagellate^{-1} · h^{-1}) in the presence of autochthonous bacteria by nanoflagellates. **a,** Experiment 1. **b,** Experiment 2.

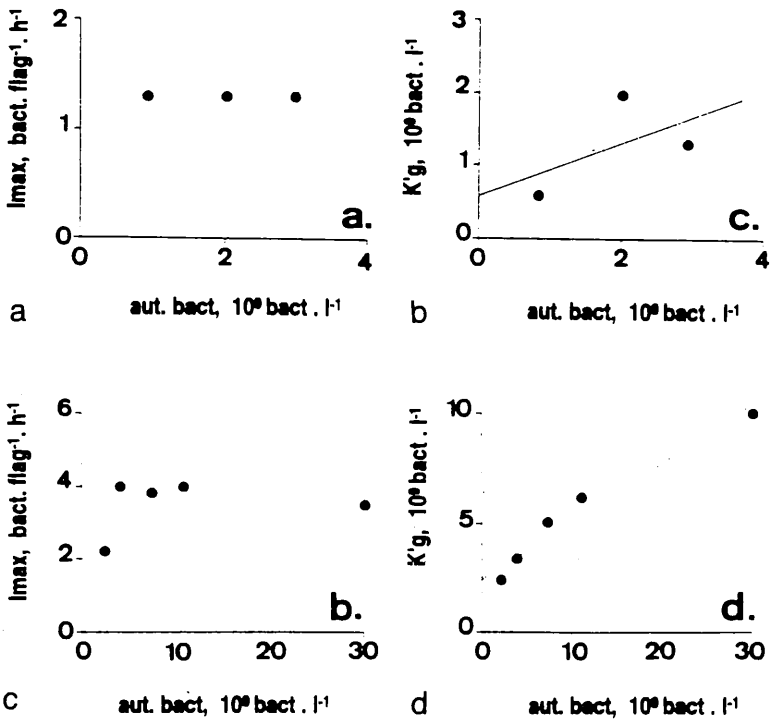


Fig. 5. Kinetic parameters of the ingestion of *E. coli* by nanoflagellates in the presence of autochthonous bacteria (aut.bact.) at various concentrations. **a and b,** Maximum grazing rate, experiments 1 and 2, respectively. **c and d,** Apparent half-saturation constant for *E. coli* grazing as a function of autochthonous bacterial concentration for experiment 1 (**c**) (regression line $y = 0.6.10^9 + 0.3\ x$) and experiment 2 (**d**) (regression line: $y = 2.6\ 10^9 + 0.26\ x$).

This result is important for predicting ingestion rates in the presence of two distinct populations of prey, differing in their characteristics with respect to grazing. Autochthonous and allochthonous bacteria, as defined in our experiments, are good examples. Table 1 illustrates a considerable difference between the grazing characteristics of the two bacterial prey in each of the two experiments performed with different heterospecific populations of predators. As expressed in terms of number of prey ingested per flagellate, maximum ingestion rates are smaller by a factor 3 to 4 for *E. coli* than for autochthonous bacteria. This difference is much smaller when expressed in terms of prey biomass ingested per unit biomass predator: In this case, the maximum ingestion rates vary from 0.09 to 0.18 mg C · mg $C^{-1} \cdot h^{-1}$ in both experiments for both prey. This is consistent with Fenchel's interpretation of the maximum rate of ingestion being equal to the reciprocal of the time required for prey digestion.

In experiments 1 and 2, the half-saturation constant for ingestion is smaller for *E. coli* than for autochthonous bacteria, even when expressed in mg C · $liter^{-1}$. According to Fenchel's interpretation of the half-saturation constant, inversely depending on digestion time and clearance rate, this implies that both populations of flagellates in our experiments have a higher clearance rate for the large allochthonous bacteria than for the smaller autochthonous bacteria. Several authors have indeed shown a selectivity of flagellate grazing for larger prey. Thus, Anderson et al. [1] observed that grazing by the flagellate *Ochromonas* sp. led to a 47% reduction of the mean bacterial biovolume. Gonzales et al. [15] showed that the clearance rate of a natural community of heterotrophic nanoflagellates is greater by a factor of 4 in the presence of bacteria with a mean biovolume of 0.08 μm^3 than in bacteria with a mean biovolume of 0.03 μm^3. Chrzanowski and Šimek [4] showed that freshwater heterotrophic flagellates preferentially select bacteria larger than 0.8 μm^3. Fenchel [7] and Monger and Landry [18, 19] established this dependence from theoretical hydrodynamical considerations. Although their models differ in the details—the former predicting a clearance rate proportional to the square of bacterial radius and the latter to the radius to the power 0.8—both agree in predicting higher clearance rate for greater sizes of prey.

Our results therefore confirm the theoretical considerations developed in the introduction. The kinetics of bacterial predation by phagotrophic flagellates can be represented by simple Michaelis-Menten kinetics, with a very simple interpretation for the maximum rate and half-saturation constant. Competitive inhibition occurs when two prey, with differing size and hence different specificity for grazing, are present together.

As an illustration, let us consider the case of a very small number of allochthonous *E. coli* present within a natural community of autochthonous bacteria with accompanying heterotrophic flagellates. The theory developed in this report and the kinetic parameters determined in our experiments allow us to predict the rate of grazing on the allochthonous population with respect to the autochthonous one. Total grazing rate (G) on autochthonous bacteria (B_{aut}) can be expressed as

$$G = I_{max} \cdot \frac{B_{aut}}{B_{aut} + K_{gaut}} \cdot Z$$

where B_{aut} is the biomass of autochthonous bacteria and Z is the biomass of predators.

The relative grazing rate of autochthonous bacteria (k_{aut}) is defined by the ratio G/B_{aut}, hence

$$k_{aut} = I_{max} \cdot \frac{1}{B_{aut} + K_{gaut}} \cdot Z$$

Direct estimations of k_{aut} in natural aquatic environments are generally in the range of 0.005–0.05 h^{-1} [2, 3, 25, 26].

Similarly, the relative grazing rate of allochthonous bacteria can be written

$$k_{Ecoli} = I_{max} \cdot \frac{1}{B_{Ecoli} + K'_{gEcoli}} \cdot Z$$

Considering that *E. coli* are generally present in natural aquatic environment in concentrations much lower than the half-saturation constants determined in Table 1 ($B_{Ecoli} \ll K_{gEcoli}$), and expressing K'_{gEcoli} as in equation 10 above, the ratio of the relative rate of grazing of allochthonous to autochthonous bacteria can simply be expressed as

$$k_{Ecoli}/k_{aut} \approx \frac{K_{gaut}}{K_{gEcoli}}$$

which is independent of the concentration of both autochthonous bacteria and protozoans. According to the values found in our experiments for the half-saturation constants, with K_{gEcoli} being about half the value of K_{gaut}, the relative rate of grazing on *E. coli* can be predicted to be about twice the grazing rate on autochthonous populations.

Acknowledgments. This work was made possible by a doctoral grant to P. Menon from the French Ministry for Research and Technology. G. Billen is Research Associate of the Fonds National de la Recherche Scientifique (Belgium).

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Manuscript 10

COASTAL EUTROPHICATION OF THE SOUTHERN BIGHT OF THE NORTH SEA : ASSESMENT AND MODELLING

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Abstract

Massive blooms of the colony-forming Haptophyte *Phaeocystis* sp. are a recurrent phenomenon in the eutrophicated continental coastal waters of the North Sea. This coastal zone is receiving the discharge of seven major west-european rivers the watershed of which is characterized by high population densities and intense industrial and agricultural activities. The basic mechanisms of *Phaeocystis* blooms development in response to riverine nutrient enrichment are being investigated since 1988 in the scope of national and EC research projects on *Phaeocystis* bloom dynamics. The ultimate objective is to set up a computer tool that could provide guidance for making selection among the control actions available for counteracting coastal eutrophication in this area. For this purpose, an integrated research methodology has been implemented, combining field observations of *Phaeocystis* blooms development and associated physico-chemical and biological variables at reference coastal stations, physiological studies of *Phaeocystis* nutrient metabolism and the development of the mechanistic biogeochemical MIRO model. Results of this long-term study are presented here. Observational and mathematical evidence is given that the present-day extent of *Phaeocystis* colony development observed in the whole area, mainly results from changes in riverine nitrate and silicate delivery, driven by changes in both anthropogenic activities and rainfall conditions on the watershed. Furthermore, realistic nutrient reduction scenarios as scheduled for North-Western Europe for the next 25 years, making use of the current MIRO model, gives additional illustration of the complex interaction between the continent and coastal sea systems and of the appropriateness of the mechanistic model as a decision support for selecting the most appropriate measure to counteract coastal eutrophication.

NATO Advanced Research Workshop on "Sensitivity of North Sea, Baltic Sea and Black Sea to anthropogenic and climatic changes" (14-18 November 1995). NATO-ASI Series, in press.

1. Introduction

The coastal zone, at the interface between land, ocean and atmosphere, plays a major role as recipient of large amounts of nutrients from human activities, including industrial effluents, agricultural runoff, and municipal sewage. Manifest effects of man-induced coastal eutrophication usually appear as qualitative changes of the structure and functioning of the pelagic and benthic food-web with resulting undesirable effects (e. g. invasions of undesirable or toxic phytoplankton species, extinctions of key species on higher trophic levels, reduced yields of harvestable fish or invertebrate populations...). This alteration of the ecosystem structure and functioning is primarily driven by changes in nutrient ratios available to coastal phytoplankton with respect to silicon availability - severely deficient compared to nitrogen and phosphorus -, resulting from the interplay of several factors as anthropogenic activities (land use modification, waste water purification treatments, hydraulic managments, farming practices,...), biogeochemical transformations occuring in the river systems and meteorological conditions [1].

Such harmfull events are occurring in the eutrophicated continental coastal waters of the North Sea. This area receives the discharge of 7 major west-european rivers draining regions characterized by high population densities and intensive industrial and agricultural activities (Fig.1). Transient foam accumulations observed, indeed, every spring at sea surface and on the beaches are resulting from food chain disruption due to the proliferation of one single non-siliceous species, the gelatinous colony-forming *Phaeocystis*, largely unpalatable for mesozooplankton and apparently hardly biodegradable [2]. Even if no toxicity is attributable to *Phaeocystis* to date, its dramatic invasion is being seriously considered regarding the nuisances it causes to the coastal ecosystem and to recreative and aquaculture activities. Most of these - including their impact on the higher trophic levels of the planktonic and benthic food chain, the exportation of organic matter to the oxygen depleted bottom waters of the Danish coastal area, the accumulation of foam on the beaches and the production of volatile sulphur compounds into the atmosphere - are the consequence of the peculiar physiology and life cycle of *Phaeocystis*, especially in its capacity to form large unpalatable gelatinous colonies causing food chain disruption [3] and to synthetize dimethyl-sulphide precursors [4].

Reduction of harmful *Phaeocystis* blooms in the continental coastal waters of the North Sea through the formulation of national and international regulations on sewage treatment facilities and farming practices aiming at the reduction of riverine nutrient delivery to the coastal sea is nowadays a main concern of public authorities. However the basic scientific knowledge required to properly assess the extent of nutrient reduction as well as its priority target (ammonium, nitrate, phosphorus) was up to now lacking. Yet, the choice between phosphorus or nitrogen reduction for controlling coastal eutrophication has considerable economical consequence.

In the scope of national and EC research projects, an integrated land-coastal sea system approach that combines field observations, process-level studies and numerical experimentation has been implemented since 1988. The purpose is to improve

knowledge on eutrophication mechanisms in the coastal North Sea and give guidance for making selections among the possible measures to counteract eutrophication in this coastal sea. The feasibility and the appropriateness of this integrated research methodology to identify and solve coastal eutrophication problems is illustrated in this paper which synthetizes (i) knowledge on the present-day eutrophication level of the continental coastal waters of the North Sea and its natural and man-induced controlling mechanisms; (ii) shortly describes the mechanistic biogeochemical model MIRO; (iii) appraises the model performance in its ability to predict present-day *Phaeocystis* blooms; and (iv) explore the response of the current *Phaeocystis*-dominated coastal North Sea ecosystem to environmental policies scheduled for the next decade by North-Western European countries with respect to EC guidelines.

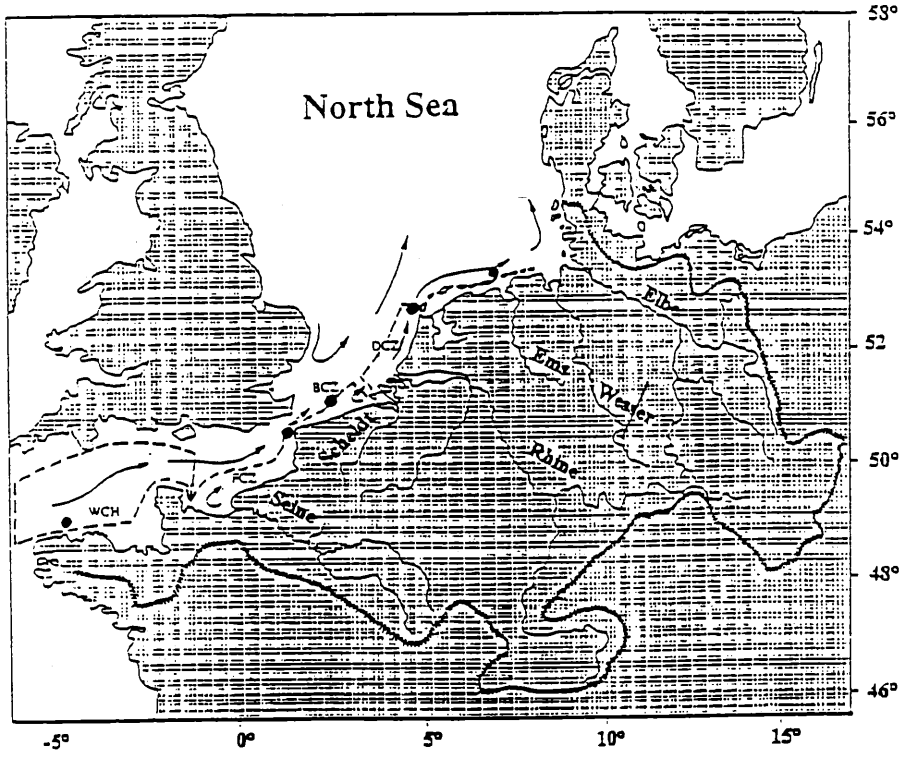

Figure 1. Map showing the watershed of the main rivers discharging in the continental coastal waters of the North Sea; the location of the monitoring stations (●); and the sub-areas (dotted contours) considered for the application of the MIRO model (WCH Western Channel; FCZ : French coastal zone; BCZ : Belgian coastal zone; DCZ : Dutch coastal zone)

2. The eutrophication phenomenon of the continental coastal waters of the North Sea : assesment and mechanisms

The present-day features of coastal North Sea eutrophication were appraised from the comprehensive analysis of high resolution time-series data on nutrients, phytoplankton and heterotrophic organisms yearly collected during the period 1988-1993 at selected reference stations within the area extending from the Western Channel to the Dutch coastal waters (Fig.1) [5], in relation to nutrients riverine loads and meteorological conditions. When combined with physiological studies on *Phaeocystis* nutrient metabolism [3, 6] and on the biodegradability of *Phaeocystis*-derived material [7], this comprehensive analysis allows to identify the basic mechanisms behind eutrophication of coastal North sea, in particular those ecological events that lead in spring to transient accumulations of foams on beaches bordering the North Sea.

2.1. THE NUTRIENT ENVIRONMENT OF COASTAL NORTH SEA PHYTOPLANKTON

The general nutrient enrichment of the coastal area from continental sources is reflected by N, P, Si winter concentrations (Table 1) which display a one-order-of-magnitude increase from the Western Channel to the Dutch coastal waters. Riverine nutrients discharging in the North Sea are indeed cumulating along a SW-NE axis as a result of the mean residual circulation of the water masses. Qualitative changes induced by freshwater sources of nutrients as exhibited by the N:P, N:Si and P:Si molar ratios of the winter inorganic nutrient pool (Table 1) clearly evidence in the whole area a nitrate excess over silicate and phosphate with respect to coastal diatom silicon requirements (average N:Si molar ratio of 1.2) [8] and phytoplankton phosphorus needs (Redfield's N:P molar ratio of 16).

TABLE 1. Nutrient discharge and winter concentration in the coastal North Sea

Coastal area	WCH	FCZ	BCZ	DCZ
River inputs :				
nitrogen : 10^3 T y^{-1} (% nitrates)		125 (80)	58 (44)	500 (70)
phosphorus :10^3 T y^{-1}(% phosphate)		12 (77)	7.3 (45)	29 (70)
silicates : 10^3 T y^{-1}		67	27	191
molar N:P - N:Si - P:Si		24 - 3 - 0.13	29 - 3.4 - 0.11	31 - 4.2 - 0.14
Average winter concentrations				
nitrate : µM	8	25	35	80
phosphate : µM	0.6	1.5	1.2	2
silicate : µM	6	9	12	40
molar N:P - N:Si - P:Si	17 - 1.4 - 0.11	17 - 2.7 - 0.17	30 - 3 - 0.11	28 - 2.7 - 0.1
Maximum spring chl a : mg m^{-3}	4	10	45	75

2.2. STRUCTURE AND FUNCTIONING OF THE COASTAL NORTH SEA PLANKTONIC FOOD-WEB

2.2.1. *The phytoplankton community*

Nutrient enrichment of coastal waters stimulates in spring the development of phytoplankton of which the biomass exhibits a one-order-of-magnitude increase from the Western Channel to the Dutch coastal waters (Table 1). The severely unbalanced nutrient enrichment with respect to diatoms requirements, however, stimulates, after the early spring development of a silicate-controlled diatom bloom (Fig.2a), the explosive development of the colony-forming *Phaeocystis*, sustained by new sources of nitrate of anthropogenic orgin (Fig.2b). The link between *Phaeocystis* colony blooms and the anthropogenic nitrate enrichment of the continental coastal waters of the North Sea is clearly evidenced by the positive relationship between the maximum *Phaeocystis* cells density reached in spring and the nitrate standing stock available at diatom decline (Fig.3).

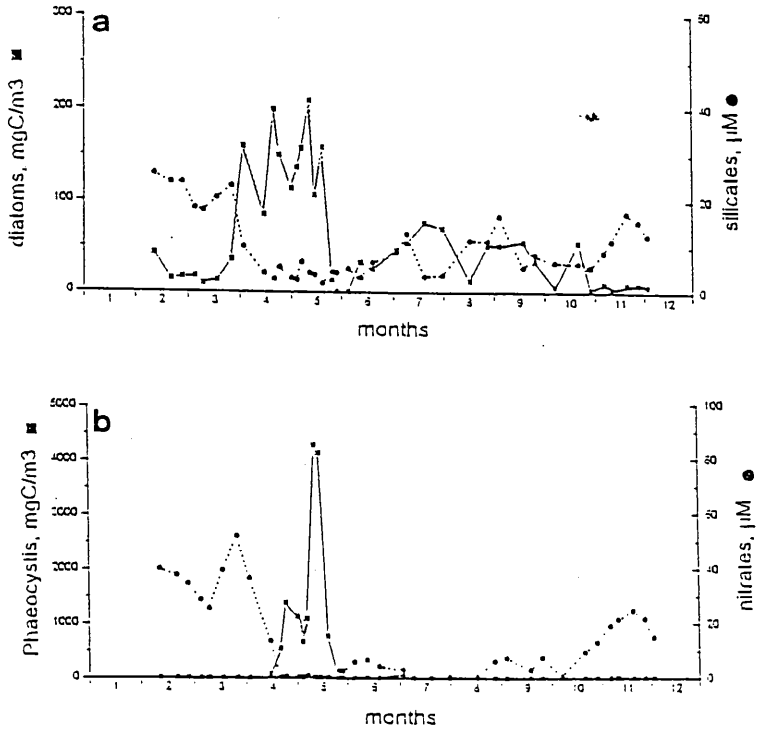

Figure 2 Typical spring phytoplankton succession and nutrients cycle in the Belgian coastal waters Monitoring data 1993 of Rousseau et *al.*[9]

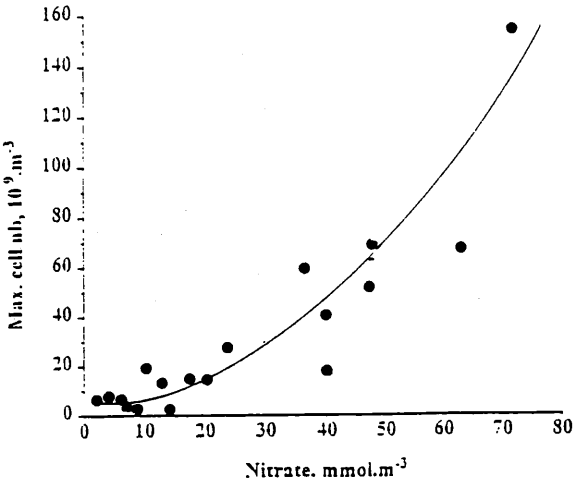

Figure 3 Relationship between *Phaeocystis* colony bloom magnitude and nitrate concentration at *Phaeocystis* onset. 1988-1995 data of Rousseau *et al.* [9]

Superimposing changes in anthropogenic activities - in particular those supplying nitrates to the freshwater and marine systems -, as driving forcing of *Phaeocystis* colonies development in the coastal North Sea, variations of late winter meteorological conditions on the watershed, in particular rainfall, were shown to induce contrasting changes in the spring diatom-*Phaeocystis* dominance as well [9]. Accordingly, the relative importance of diatom *versus Phaeocystis* colony blooms is positively related to late winter rainfall conditions (Fig.4) with persistant intense precipitations in winter driving the development of a diatom-dominated spring bloom while less intense rainfall promoting *Phaeocystis* colonies. To which extent this alternation in phytoplankton species dominance is affecting the trophic efficiency and the geochemical significance of the coastal North Sea is not known yet.

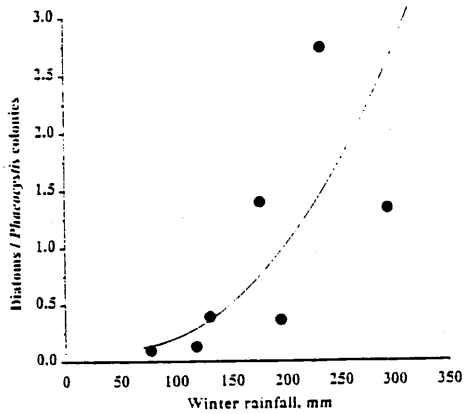

Figure 4 Relationship between late winter rainfall on the Scheldt watershed and the relative importance of the diatom :*Phaeocystis* carbon biomass. After Rousseau *et al.* [9]

2.2.2. *Planktonic food-web structure*

Phaeocystis is one of the few marine phytoplankters exhibiting phase alternation between free-living cells of 3-9 µm in diameter and 1 mm gelatinous colonies typically composed of thousands of cells embedded in a gel-like matrix made of polysaccharides [e.g. 3, 10, 11]. These *Phaeocystis* life forms are characterized by different ecological and geochemical fate [12]. The successfull development of *Phaeocystis* colonies is explained by an apparent higher ability of the colonial form tu use nitrate as nitrogen source [12, 13], and by their high resistance to grazing by indigeneous mesozooplankton [14]. The dominance of mesozooplankton-unpalatable *Phaeocystis* colonies has been deviating the classical linear food chain towards a complex microbial network. The latter is primarily stimulated by the release of *Phaeocystis* free-living cells and dissolved organic matter after disruption of non-grazed colonies. The outstanding aggregation of foam deposited on beaches during periods of onshore winds then results from transient accumulation of *Phaeocystis*-derived organic matter resistant to immediate microbial degradation. Interestingly enough, a positive relationship exists between the magnitude of *Phaeocystis* blooms and mesozooplankton [15]. This positive feedback might be explained by the existence of an active microbial food-web in which protozoa play a key role as grazers of *Phaeocystis* free-living [16, 17] and as food ressources for indigeneous mesozooplankton [18]. This stresses the high flexibility of this coastal ecosystem and its adaptability to environmental changes.

3. Modelling the eutrophication of the continental coastal waters of the North Sea

An integrated network of mechanistic biogeochemical models, consisting of the offline coupling of a biogeochemical model of *Phaeocystis* blooms development in the coastal North Sea - the MIRO model [5] - with a river system model calculating riverine nutrient delivery to the coastal zone, as a function of hydrology, land use and waste water purification policies - the RIVERSTRAHLER model [9] - is being developed for predicting the response of the *Phaeocystis*-dominated coastal ecosystem to changes in riverine nutrient loads resulting from changes occuring in the watershed. Basically these mechanistic models are describing and predicting the cycling of carbon, nitrogen, phosphorus and silicon through aggregated key components of the freshwater and coastal ecosystems over seasons and years in response to the physical and nutrient forcing. Such mathematical models, therefore, are based on physiological and geochemical principles. The numerical code synthetizes knowledge on the kinetics and the factors controlling the main auto- and heterotrophic processes involved in the functioning of the river and marine coastal ecosystem. The code is continuously in development relying on progress in experimental aquatic ecology. Current development of the MIRO model, in particular its ability to reproduce the present-day eutrophication in the continental coastal waters of the North Sea, in particular the magnitude and extent of *Phaeocystis* blooms, and explore the coastal ecosystem

response to nutrient reduction scenarios scheduled for the next 25 years is shortly presented below.

3.1. THE MIRO MODEL : MODELLING PRESENT-DAY *PHAEOCYSTIS* BLOOMS DEVELOPMENT IN THE COASTAL NORTH SEA.

3.1.1. *Description of the ecological model*

The MIRO ecological model describes the cycling of carbon, nitrogen, phosphorus and silicon through aggregated chemical and biological compartments of the planktonic and benthic components of the coastal area (Fig.5). Thirty-two state variables and twenty-six processes linking them were identified as important from the knowledge of the structure and functioning of *Phaeocystis*-dominated ecosystems. The model results of the assemblage of 4 modules describing the dynamics of phytoplankon, zooplankton, organic matter degradation and nutrient regeneration in the water column and the sediment. Mathematical formulation of kinetics, parameters and forcing functions are described in [5].

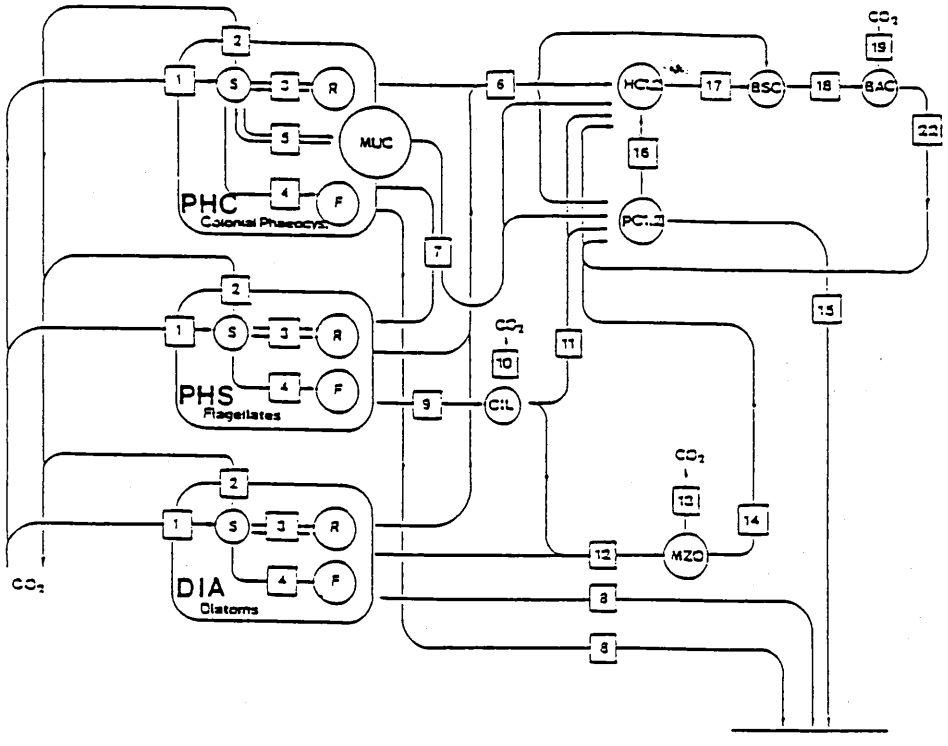

Figure 5 Structure of the MIRO model : carbon cycling. Legend for state variables (circles) and processes linking them (numbers) in text.

The phytoplankton module considers 3 phytoplankton groups: diatoms (DIA), free-living nanoflagellates (PHS) and *Phaeocystis* colonies (PHC). Due to their trophic fate, *Phaeocystis* free-living cells and colonies are considered as separate state variables, even though they constitute two different stages of the life cycle of the same phytoplankter (e.g. Rousseau *et al.*, 1994). The kinetics of phytoplankton activities is described according to the AQUAPHY model of Lancelot *et al* [20]. It considers 3 intracellular pools - monomers (S); reserve material (R); functional and structural metabolites (F) - and distinguishes the processes of photosynthesis (1) directly dependent on light availability, from the process of growth (4) controlled by the availability of intracellular monomers and ambiant nutrients. One single nutrient is limiting phytoplankton growth, according to the Liebig law of minimum. With respect to *Phaeocystis* colonies, an additional pool of extrapolymers (MUC) has been added to consider the mucilaginous matrix in which the cells are embedded and which serves as reserve of energetic material (5). Beside respiration (3), loss processes include excretion, cellular lysis (6), under control of nutrient stress; sedimentation (diatoms and *Phaeocystis* colonies) (8); zooplankton grazing (12).

The zooplankton module of the MIRO model considers two groups of zooplankton : the microzooplankton (CIL) feeding on free-living flagellates (9) and the mesozooplankton (MZO) grazing (12) on diatoms and microzooplankton according to a preference for the latters (F. Hansen, pers. com.). *Phaeocystis* colonies escape grazing. Colonies are however submitted to a nutrient stress-dependent process of colonial disruption (7) which releases in the water column free-living cells and dissolved organic polymers. A simplified description of zooplankton dynamics has been chosen, considering grazing as an hyperbolic Michaelis-Menten function of food items with however a threshold value below which no grazing is possible. Zooplankton growth and excretion (14) are calculated from grazing rates based on growth efficiency, zooplankton stoechiometry and the calculated nutrient composition of food. First-order mortality is considered.

Organic matter degradation by planktonic bacteria is described according to the HSB model of Billen[21], considering two classes of biodegradability for both dissolved (HC1 and HC2) and particulate (PC1 and PC2) organic matter. Ectoenzymatic hydrolysis of these polymers (17) produces monomers (BS) that can be taken up by bacteria (18). These processes are described by standard Michaelis-Menten kinetics. According to their origin, carbon and nitrogen contribute in variable proportions to the pools of organic matter. This proportion, compared to the bacterial C:N ratio determines whether net ammonification or ammonium uptake accompanies bacterial activity. All organic phosphorus is assumed to be released directly as ortho-phosphate during hydrolysis of polymeric organic matter and phosphorus is taken up by bacteria in its inorganic form only.

Benthic organic matter degradation and nutrient (N, P, Si) recycling is calculated making use of the algorythms developed by Lancelot and Billen [22] and Billen *et al.*, [23]. These algorythms, by solving steady-state diagenetic equations expressing the mass balance of organic carbon, oxygen, inorganic forms of nitrogen and phosphorus in the sedimentary column, calculate the fluxes of nitrate, ammonium and phosphate

accross the sediment-water interface resulting from a given sedimentation flux of particulate organic matter. Furthermore, a first-order kinetics describes benthic silicon redissolution and release of silicic acid to the water column.

3.1.2. *The multi-box MIRO model*

For a first application of the MIRO model to the continental coastal waters of the North Sea, a multi-box model has been considered on the basis of the hydrological regime. Such a low resolution of the hydrodynamics is reasonable in this tidally-well-mixed area. Three successive boxes , assumed to be homogeneous, have been chosen from the Baie de Seine to the Wadden Sea area of the Dutch coastal zone, on the basis of the hydrological regime (Fig.1). The offshore limit of the boxes is taken along a residual streamline so that inshore-offshore exchanges can be neglected. Each successive box is treated as an open system, receiving waters from the upwards adjacent box and exporting water to the downwards box. The seasonal variation of the state variables are calculated by solving the different equations expressing mass conservation in the system according to the Euler procedure.

The boundary conditions are provided by the results of the calculations performed for the conditions existing in the western Channel area, considered as a quasi oceanic closed system. Forcing functions are observed seasonal irradiance and seawater temperature and monthly riverine nutrient discharges of 1985 (source : North Sea Task Force, 1992).

The prediction capability of the MIRO model can be appraised from Fig.6 and 7 which compares respectively predicted chlorophyll a and *Phaeocystis* cellular density and nutrients concentration in the 3 sub-areas of the continental coastal waters of the North Sea with observations at the respective reference stations of the observational network, from 1988 to 1993. In spite of a reasonable general agreement between predictions and observations, in particular in the timing and magnitude of *Phaeocystis* blooms, the model does not predict properly the fast decline of *Phaeocystis* blooms, especially in the northern part of the simulated area where non-observed elevated biomass is predicted along the summer season. This discrepancy between predictions and measured data could originate either from the oversimplification of the hydrological regime, neglecting for instances the influence of the Wadden Sea in the Dutch coastal area or from a low description of mechanisms prevailing for *Phaeocystis* blooms termination and degradation. Further research in this field is in progress.

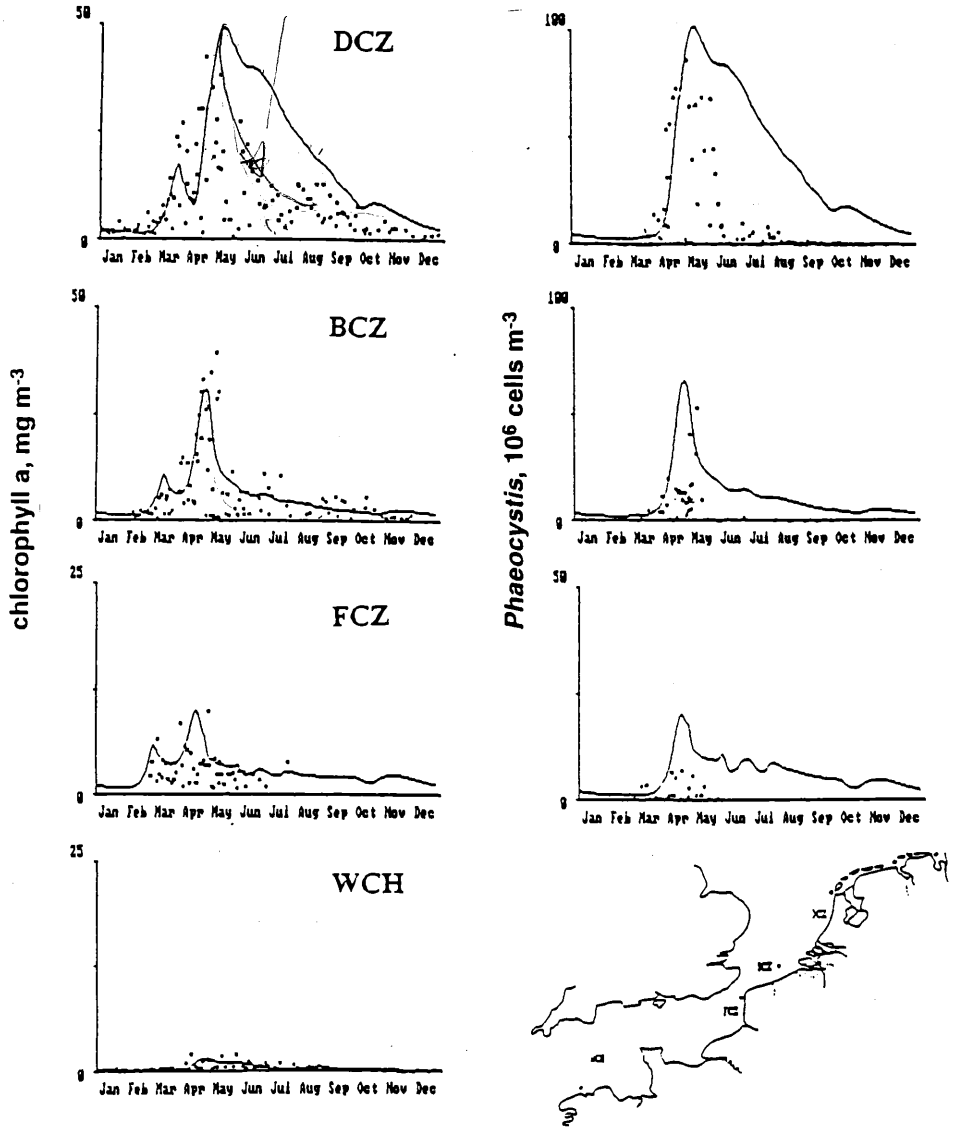

Figure 6 Observed (dots) and predicted (line) phytoplankton development in the coastal North Sea. Total chlorophyll a (a) and *Phaeocystis* cells (b)

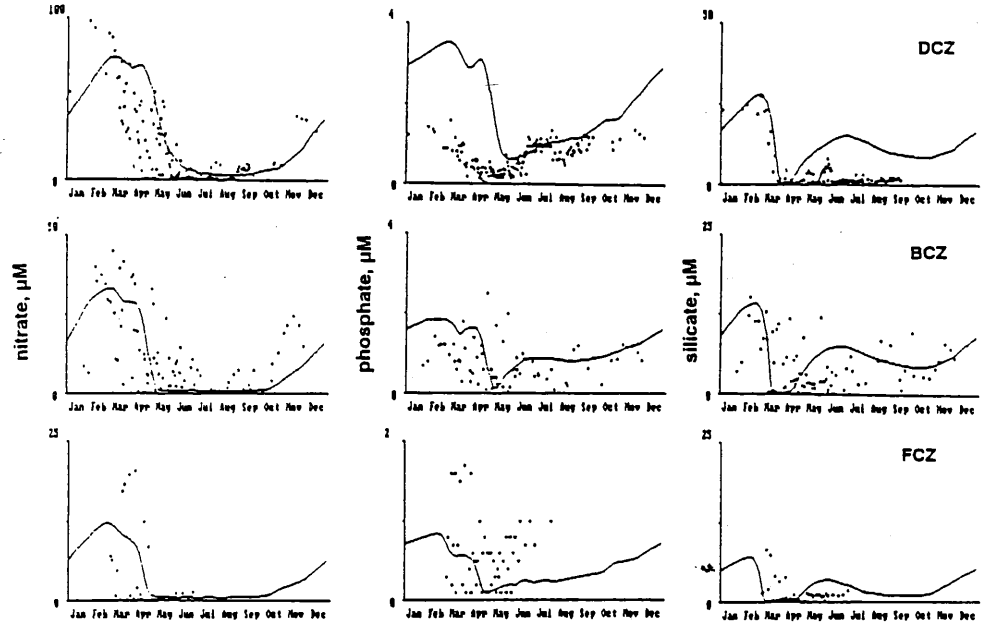

Figure 7 Observed (dots) and predicted (line) nutrients concentration in the coastal North Sea.

3.2. MODELLING *PHAEOCYSTIS* BLOOMS IN RESPONSE TO NUTRIENT REDUCTION SCENARIOS

The appropriateness of the integrated modelling approach for coastal management purpose is shown by exploring the response of the MIRO model to reduction scenarios of riverine nutrient delivery of the coastal zone consequent to the application of the EC guideline of May 1991 on urban waste water treatment for sensitive areas, *i.e.* 90 % phosphate removal and/or 75 % denitrification.

Nutrient reduction scenarios were established by modifying nutrient riverine inputs of 1985, according to the results generated by application of the RIVERSTRAHLER model to watersheds similar to those of the rivers Seine, Scheldt and Rhine and for various scenarios of urban waste water treatment. These calculations were based on published level of urban waste water treatment reached in the different watersheds. Due to the marked seasonality of both river flow discharge and nutrient transformation within the river system, a summer (April-September) and a winter (October-March) nutrient reduction factors were considered.

The extent of *Phaeocystis* colony blooms reduction expected from the application of the EC guideline of May 1991 can be appraised on Fig.8 which compares predicted *Phaeocystis* blooms after nutrient abatement scenarios with present-day predictions. Slight differences in the response of the coastal ecosystem are to be observed between subareas. Severe reductions of *Phaeocystis* blooms colonies are predicted after 90 % P removal. By contrast, very little bloom reduction is achieved in the scenario involving the denitrification treatment of waste waters. This is explained by the importance of diffuse sources of nitrogen. Interestingly enough, the simultaneous application of phosphorus removal and denitrification treatment of urban waste waters is not required for obtaining the largest reduction of *Phaeocystis* blooms in the coastal North Sea.

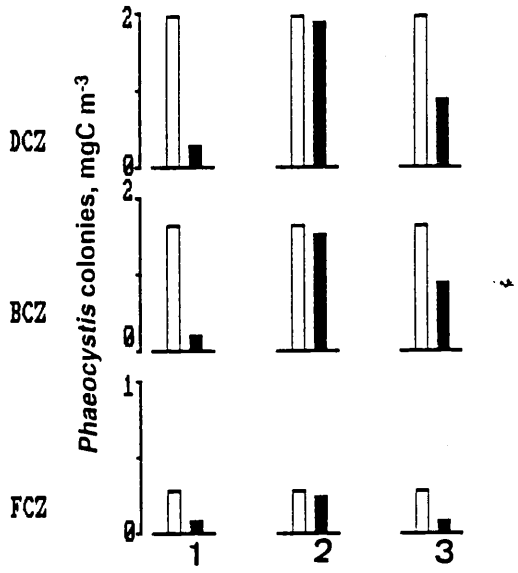

Figure 8 Predicted present-day magnitude of *Phaeocystis* blooms (white block) and after nutrient reduction scenarios (black block) : 1 = 90% P removal; 2 = 75% denitrification; 3 = 90% P removal and 75% denitrification

These exploratory nutrient reduction scenarios highlight the complex interactions between the continental and coastal marine systems and provide guidance to better select the available control actions on the watershed in order to reduce the development of *Phaeocystis* colony blooms. Further numerical work has to be done in this direction and should be supported by infrastructure development i.e. the setting up of permanent monitoring stations of *Phaeocystis* colony bloom development in the key sites of the coastal North Sea for assessing long-term changes of the ecosystem functioning resulting of waste water purification policies and climate change and verify model predictions.

Acknowledgements

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Seasonal succession of diatoms and Chlorophyceae in the drainage network of the Seine River: Observations and modeling

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Abstract

Seasonal succession of diatoms and Chlorophyceae have been analyzed in the Seine River system (France), which is characterized by a temperate oceanic hydrological regime and high nutrient enrichment. Phytoplankton development is invariably initiated by the decrease of discharge in spring. When this occurs in early spring, the bloom is dominated by diatoms that severely deplete silica, and a regular increase of their biomass is observed along the river continuum from headwaters to the estuary. The bloom occurs earlier downstream than upstream. Chlorophyceae succeed the diatoms by the end of May and represent a significant component of the summer phytoplankton population. Fluctuations of the phytoplankton biomass are observed within the continuum in summer, with high biomass in 6th-order rivers, low biomass in 7th-order rivers, and again high biomass in 8th-order rivers. These seasonal and spatial variations are interpreted with the aid of a mathematical model (the RIVERSTRAHLER model); the model calculates the development of diatoms and Chlorophyceae within the whole drainage network which is represented as a regular pattern of confluences of tributaries with increasing stream order. The model, taking into account both bottom-up and top-down regulating factors of phytoplankton, has proved to be a powerful tool in understanding the dynamics of a large drainage network.

Seasonal periodicity of phytoplankton has largely been described in lakes. Conceptual models (e.g. the PEG model, Sommer et al. 1986) have emerged from the synthesis of many studies carried out in a large number of lentic systems, and they describe the seasonal events in function of regulating factors: physical and energetic (hydrology, mixing, light, and temperature), nutritional (nutrients availability), and biological (grazing, sinking, and parasitism). A similar synthesis is not available for lotic systems. In most large rivers with temperate oceanic hydrological regimes, a bloom dominated by diatoms occurs after the decrease of discharge in spring, whereas a mixed population of Chlorophyceae and diatoms composes the summer phytoplankton. This pattern has been observed in the Loire (Champ 1980) and Lot Rivers, France (Capblancq and Dauta 1978), in the Meuse River, Belgium (Descy 1987), in the Thames River, U.K. (Lack 1971), and in the Sacramento River, California (Greenberg 1964). However, factors regulating phytoplankton development and seasonal periodicity in rivers are much less documented than those in lakes and are still poorly understood (Reynolds 1988).

Large river systems, from headwaters to estuaries, represent a continuum of interdependent ecosystems, so understanding phytoplankton development at any place in the river requires understanding its development upstream. Most previously published models of phytoplankton development in rivers, however, take into account only a limited river stretch (Admiraal et al. 1993). A model that uses the concept of stream order (Strahler 1957) was therefore developed (the RIVERSTRAHLER model, Billen et al. 1994) to describe the ecological functioning of a whole drainage network. The model is based on a rather fine description of the kinetics of the basic physiological processes (algal photosynthesis and growth, bacterial organic matter degradation, and zooplankton grazing), thus allowing a link to be made between these microscopic processes and their macroscopic manifestations at the scale of the whole drainage network. The model reasonably describes the conditions of phytoplankton development in two tributaries of the Seine basin (Billen et al. 1994). In the first version, however, phy-

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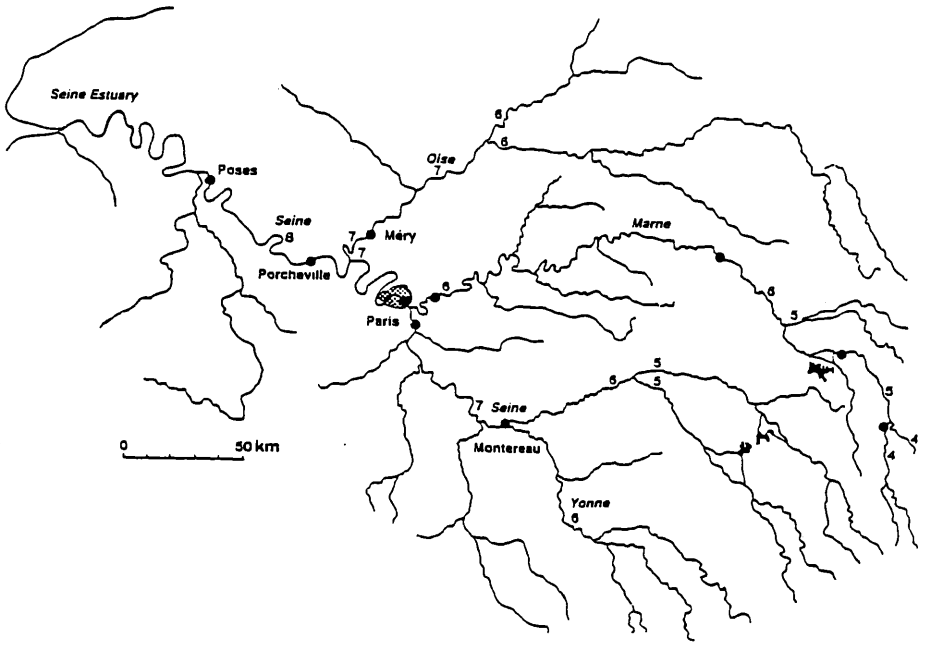

Fig. 1. General map of the Seine River system; sampling stations—●.

toplankton as a whole was taken as a sole compartment, and the possible role of silica as a limiting nutrient was ignored.

In the present paper, we further explore the ecological understanding of the seasonal periodicity of phytoplankton in the Seine River. We have extended the model by taking into consideration two distinct phytoplankton populations, the diatoms and the Chlorophyceae, which dominate in the river. Silica is considered an additional variable and we have investigated its possible role as a limiting nutrient controlling the spring bloom. A main objective was to explore the role of biotic and abiotic controls of seasonal periodicity of these two major groups of algae in the whole drainage basin. Finally, this mathematical analysis has been summarized in the form of a few statements that describe the main events of phytoplankton development in river systems by analogy with the PEG word model established for lake systems.

Study site

The Seine River, down to the entry of its estuarine zone at Poses, has a drainage area covering 64,500 km^2 that corresponds to the major part of the Parisian basin. According to Strahler's (1957) ordination, the Seine is 8th order at its mouth, below its confluence with the Oise River (Fig. 1). The Seine receives its longest tributary, the Marne River, just upstream of Paris. The Seine and all its tributaries have a pluvio-oceanic regime with a drop of flow in summer; the natural flow can decrease to 30 m^3 s^{-1} at Paris, but since the construction of reservoirs upstream, summer flow is sustained near 100 m^3 s^{-1}. High waters occur in winter (January) and can reach 2,400 m^3 s^{-1}. The annual flow averages 500 m^3 s^{-1} at the mouth. Due to intense human activities in the whole basin, but especially in the Parisian area, the Seine is considerably enriched in nutrients (Table 1).

Methods

Sampling program—A large sampling program, from the headwaters to large rivers, was carried out in 1991 at a seasonal scale in order to analyze phytoplankton biomass and nutrients. Sampling stations are indicated in Fig. 1. The database of phytoplankton composition comprises seasonal cycles on the Seine at Paris in 1991, at Porcheville in 1990, and on the Oise tributary at Méry

Table 1. Maximal values of main variables characterizing water quality in rivers of different stream order in the Seine drainage network (1991).

Variables	8	7	6	5	4
Nitrate (mg N liter^{-1})	6	5	4	4.5	5
Phosphate (mg P liter^{-1})	1	0.4	0.4	0.25	0.080
Silica (mg SiO_2 liter^{-1})	11	11	10	2–14*	2–14*
Chlorophyll *a* (μg liter^{-1})	80	100	90	20	10
Rotifers (ind. liter^{-1})	1,000	500	200	100	10

* Range of maximal values, depending on lithology of the subbasins.

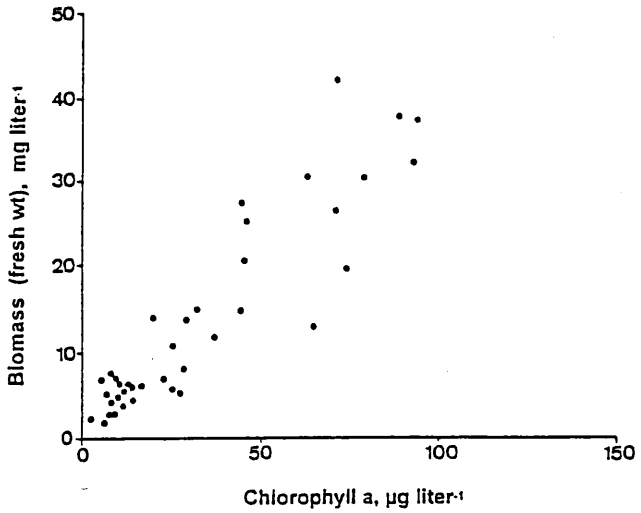

Fig. 2. Relationship between Chl *a* and fresh weight biomass of phytoplankton.

in 1991 and 1992. Sampling frequency varied from weekly to monthly.

Phytoplankton and nutrients analysis—Chlorophyll *a* was analyzed according to Lorenzen (1967) and phytoplankton counted with an inverted microscope (Utermöhl 1958). Phosphates, nitrates, and silica were measured spectrophotometrically (Eberlein and Kattner 1987; Jones 1984; Rodier 1984).

Calculations of phyto- and zooplankton biomass—Algal cells were assumed to be simple geometric figures, the dimensions of which were measured to estimate cell volume and biomass in fresh weight. A significant relationship was found between biomass in fresh weight and Chl *a* (Fig. 2). Because most data on phytoplankton biomass are expressed as chlorophyll (μg liter^{-1}), phytoplankton biomass was also calculated as chlorophyll for the different taxonomic groups, taking into account their respective contribution to total fresh weight biomass. For the requirements of the model, in which fluxes and stocks of phytoplankton must be expressed in carbon units, Chl *a* data were converted into carbon units by using a factor of 35 μg C (μg Chl *a*)$^{-1}$, determined for the Seine River according to the method of Garnier et al. (1989).

Zooplankton abundances have been determined at the Porcheville, Méry, and Paris stations (Testard and Francez pers. comm.). Values have been roughly converted into biomass by using a mean carbon content of rotifers of 0.6×10^{-3} mg C ind^{-1}.

Model description

The general structure of the model initially developed on the Seine River (Billen et al. 1994), which considered phytoplankton as a sole physiological entity, has been modified to take into account two algal groups—diatoms and Chlorophyceae—which differ in their physiological characteristics. Briefly, the RIVERSTRAHLER model consists in coupling a hydrological model (HYDROSTRAHLER) with an ecological model (RIVE) that takes into account the kinetics of C, N, and P exchange between the different compartments of the system. The HYDROSTRAHLER model is based on an idealized morphological description of the drainage network based on the concept of stream order as defined by Strahler (1957). Discharge, depth, and flow rate are calculated for each stream order from hydrometeorological data.

The RIVE model (Fig. 3, Billen et al. 1994) consists of a series of equations describing the processes of phytoplankton photosynthesis and growth in response to light and nutrients (AQUAPHY, Lancelot et al. 1991), the processes of bacterial utilization of organic matter in response to autochthonous and allochtonous sources of organic matter (HSB, Billen 1991), the processes of grazing and growth of zooplankton (ZOLA, Garnier and Billen 1994), and the processes of benthic nutrient recycling (Venice, Billen et al. 1989; Billen unpubl.) as well as nitrification, phosphate adsorption, and oxygen exchanges. The forcing functions consist of climatic constraints and point and nonpoint sources of organic matter and nutrients from the watershed. The model has proven adequate to simulate spatial, seasonal, and interannual variations of phytoplankton biomass and associated variables within the entire drainage network of the Seine (Billen et al. 1994).

In the model applied to both the diatoms and the Chlorophyceae, the phytoplankton biomass is split into two variables; silica constitutes an additional state variable (Fig. 3). Silica inputs, from the weathering of rocks, depend only on lithology. The model assumes a constant concentration of silica in headwaters (13.5 mg SiO_2 liter^{-1}, Thibert and Meybeck pers. comm.). Uptake of silica by diatoms is calculated from a constant Si:C ratio. Silica remineralization in the planktonic phase is assumed to occur according to first-order kinetics. In contrast to the previous version of the model, the process of direct sedimentation of algal cells is taken into account along with the sedimentation of dead particulate organic matter occurring after lysis or grazing. The sedimentation constant (h^{-1}) is calculated as the ratio between a constant sinking rate value (distinct for diatoms and Chlorophyceae) and depth, in accordance with the theory developed and experimentally demonstrated by Reynolds et al. (1990). The flux of dissolved silica diffusing from sediments to the water column is calculated as the mean of the sedimentation of particulate biogenic silica over the preceding month.

As the zooplankton assemblage is dominated by rotifers and protozoa, the dynamics of zooplankton is adequately represented by a continuous growth of an homogeneous population. Grazing characteristics are assumed identical for each algal group. For simulating the rapid bloom collapse observed in summer, the occurrence of an explosive mortality caused by fungal, bacterial, and

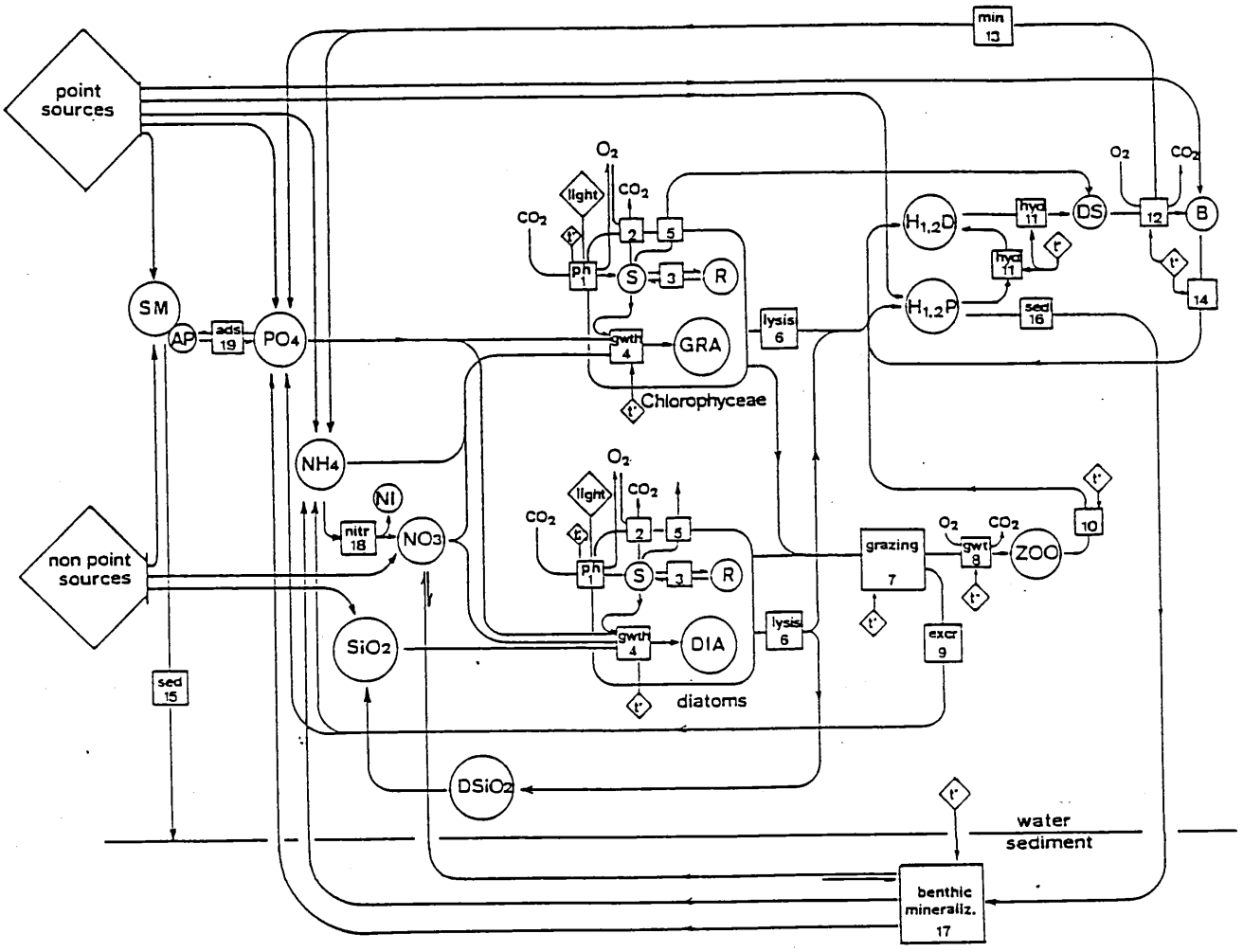

Fig. 3. Schematic representation of the model of ecological functioning of river systems, including the dynamics of two phytoplankton populations (*see* Billen et al. 1994 for more details on the general description of the model). State variables include: DIA—diatoms and GRA—green algae (Chlorophyceae) (both with S—intracellular low-molecular-weight organic metabolites and R—polymeric reserve compounds; GRA or DIA refers only to functional and structural metabolites); ZOO—zooplankton; H_1D—rapidly hydrolyzable dissolved organic matter; H_2D—slowly hydrolyzable dissolved organic matter; H_1P—rapidly hydrolyzable particulate organic matter; H_2P—slowly hydrolyzable particulate organic matter; DS—directly usable monomeric substrates; B—bacteria; NH_4—ammonium; NO_3—nitrate; NI—nitrifying bacteria; SiO_2—dissolved silica; $DSiO_2$—detrital particulate silica; PO_4—dissolved orthophosphate; AP—adsorbed orthophosphate; SM—suspended inorganic material. Processes taken into account are: 1—carbon fixation through photosynthesis; 2—algal respiration; 3—reserve synthesis and catabolism; 4—growth (i.e. synthesis of functional metabolites); 5—excretion; 6—lysis; 7—zooplankton grazing; 8—zooplankton growth and respiration; 9—zooplankton excretion; 10—zooplankton mortality; 11—hydrolysis of polymeric organic matter; 12—bacterial growth and respiration; 13—bacterial excretion of ammonium and phosphate; 14—bacterial mortality; 15—sedimentation of particulate inorganic material; 16—sedimentation of particulate organic matter; 17—benthic mineralization of organic matter and nutrient recycling; 18—water column nitrification; 19—phosphate adsorption-desorption on suspended matter.

viral parasitism is postulated, as discussed in the previous version of the RIVERSTRAHLER model (Billen et al. 1994). A 20-fold increase of lysis rate is considered once either diatom or Chlorophyceae biomass reaches a critical level corresponding to 65 μg Chl *a* liter^{-1} and temperature >15°C.

Results

Typical feature of phytoplankton in the Seine River—We have shown that in the Seine drainage network the onset of phytoplankton development invariably coincides with the decrease in flow and that the development occurs

Table 2. Physiological parameters of diatoms and Chlorophyceae drawn from the literature. μ_{max} (h^{-1})—maximum growth rate; t_{opt} (°C)—optimum temperature; *dti* (°C)—width of the temperature distribution; K_pSi, K_pP, K_pN (μg liter^{-1})—mean values of the half-saturation constants for silica, phosphate, and nitrate uptake.

	Diatoms	Chlorophyceae
μ_{max}* (h^{-1})	0.05	0.1
t_{opt}* (°C)	21	37
*dti** (°C)	13	17
K_pSi* (mg SiO_2 liter^{-1})	0.25 (SD = 0.26, n = 37)	—
K_pP† (μg P liter^{-1})	7.1 (SD = 15.8, n = 32)	46.1 (SD = 55.2, n = 20)
K_pN† (μg N liter^{-1})	5 (SD = 2, n = 8) (marine *Cyclotella*)	3 (SD = 1, n = 3) (marine *Dunaliella*)
K_pN‡ (μg N liter^{-1})	14 (independently on species)	

* Data from Paasche 1980; Dauta 1982; Dauta et al. 1982; Tilman et al. 1982; Ahlgren 1987; Grover 1989; Belkoura and Dauta 1992.
† Data from Caperon and Meyer 1972.
‡ Data from Sommer 1989; Reynolds 1984.

in 5th-order rivers where phytoplankton growth rate exceeds dilution rate (Billen et al. 1994).

In the larger tributaries (Marne, Oise, Seine), spring phytoplankton usually reaches a maximum in April or May (80–120 μg Chl *a* liter^{-1}) and is dominated by diatoms (Fig. 4). The spring peak of diatoms leads to depletion in silica that invariably falls from 15 to 1.5 mg SiO_2 liter^{-1} but occasionally to undetectable values in 6th- and 7th-order rivers (Fig. 4). Silica returns to high concentrations soon after the bloom (Fig. 4). Green algae succeed diatoms by the end of May but reach relatively low biomasses (Fig. 4). For the spring period (late March–late June), diatoms dominate the biomass, averaging 76% of the total (21% of the total is Chlorophyceae).

In summer (late June–late September), the phytoplankton is composed of a mixed population of diatoms and Chlorophyceae. Averaging the data over the summer period, diatoms represent 51% of total biomass (44% for the Chlorophyceae). Summer species of diatoms can, however, occasionally form high biomass and lead to silica depletion (Fig. 4).

About 200 taxa were usually found in the river; among them, 90 belong to the diatoms, 60 to the Chlorophyceae, and the others are equally distributed among the Xanthophyceae, Cyanophyceae, Euglenophyceae, Dinophyceae, and Chrysophyceae.

Centric diatoms (*Stephanodiscus hantzschii*) and, secondarily, *Stephanodiscus tenuis, Aulacoseira ambigua, Aulacoseira granulata*) invariably dominate the spring bloom but are occasionally accompanied by a high biomass of pennates (*Fragilaria ulna, Diatoma tenue*). Chlorophyceae are represented by several species of *Scenedesmus* (*S. acuminatus, S. bicaudatus, S. intermedius, S. opoliensis, S. quadricauda*) and many others (*Pediastrum boryanum, Monoraphidium contortum, Coelastrum microporum, Dictyosphaerium pulchellum*, etc.). The centric diatom *A. ambigua* seems to be a constant in the summer diatom populations.

Physiological characteristics of Chlorophyceae and diatoms—For the purpose of the RIVERSTRAHLER model, the physiology of the phytoplankton of the Seine River was investigated at the scale of the whole community (Billen et al. 1994). In this paper, we attempt to understand the dynamics of the populations of the two algal groups and the factors which regulate their occurrence and succession by analyzing the differences in the physiological characteristics of diatoms and Chlorophyceae on the basis of the results on pure culture studies reported in the literature (Table 2).

Data on maximal growth rates (μ_{max}) were plotted as a function of temperature (Fig. 5); they conform to a classical sigmoid relationship from which optimal temperature (t_{opt}) and sigmoid width (*dti*) can be determined. The optimum temperature is much less for the diatoms (21°C) than for the Chlorophyceae (37°C) (Fig. 5). At optimal temperature, the diatoms apparently have a lower μ_{max} than the Chlorophyceae do (0.05 vs. 0.1 h^{-1}).

For the kinetics of nutrient uptake, we averaged the values of half-saturation constant found in the literature for the diatoms and Chlorophyceae despite great variations among species (Table 2). A value of 0.25 mg SiO_2 liter^{-1} has thus been taken for the half-saturation constant for silica uptake of the diatoms. The half-saturation constant for phosphate uptake appears to be lower for diatoms (7.1 μg PO_4-P liter^{-1}) than for green algae (46.1 μg PO_4-P liter^{-1}). The half-saturation constants for nitrogen uptake are not significantly different for marine species of diatoms and Chlorophyceae (~4 μg NO_3-N liter^{-1}, Caperon and Meyer 1972). In freshwater, values of ~14 μg N liter^{-1} are reported for phytoplankton of eutrophic waters independent of species (Reynolds 1984; Sommer 1989). Such a value can therefore reasonably be accepted for both diatoms and Chlorophyceae of the Seine. The half-saturation constant found for nitrogen uptake is in any case much lower than the natural concentration of NO_3-N observed everywhere in the drainage network of the Seine.

A value of 0.4 mol Si per mol C has been taken for the Si:C uptake ratio of diatoms, according to Conley and Kilham (1989). A mean sinking rate of 0.4 m h^{-1} has been chosen for diatoms and one of 0.05 m h^{-1} for Chlo-

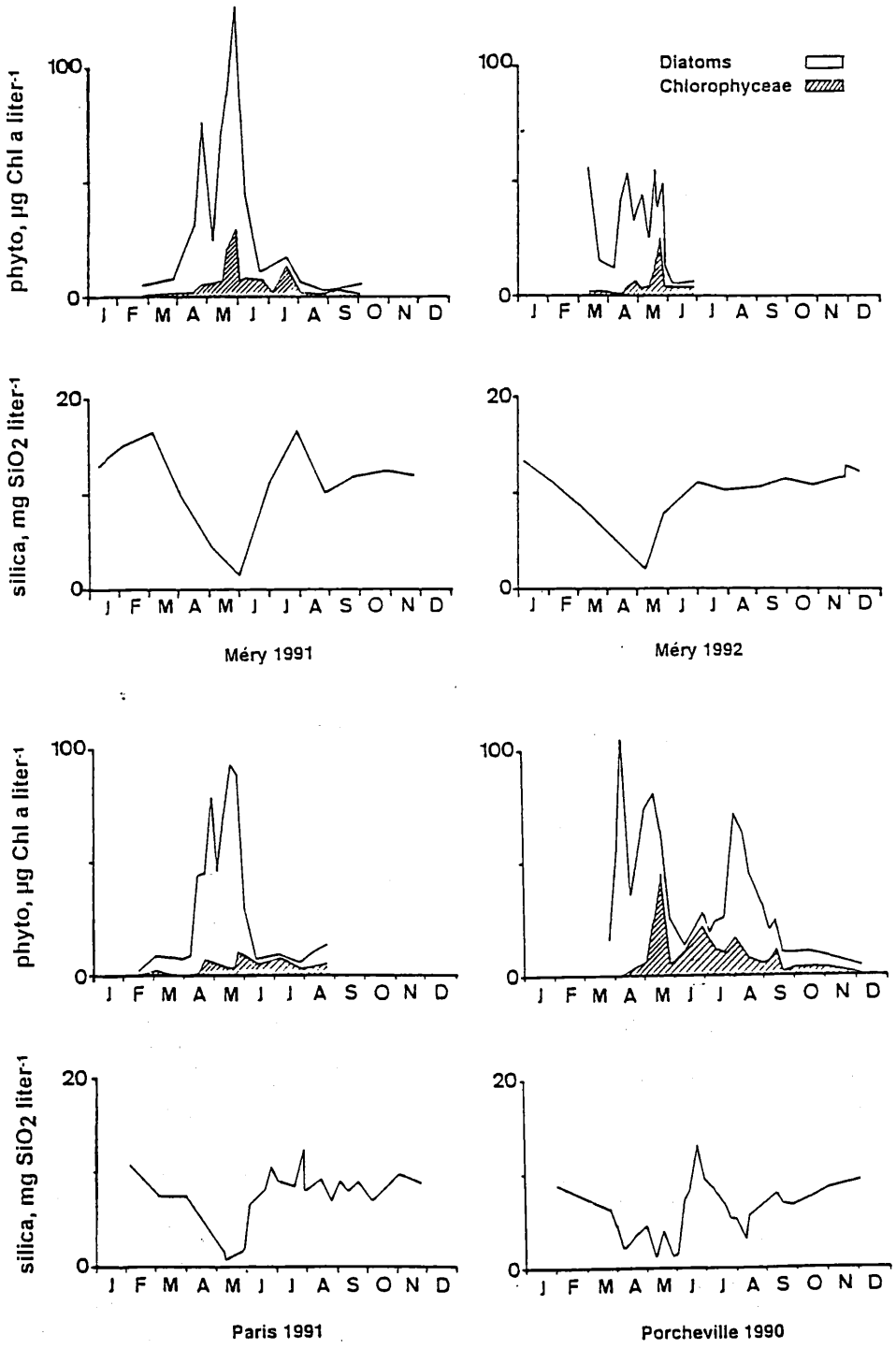

Fig. 4. Seasonal variations of the biomass of diatoms and Chlorophyceae and the concentration of silica at four stations in the Seine drainage network.

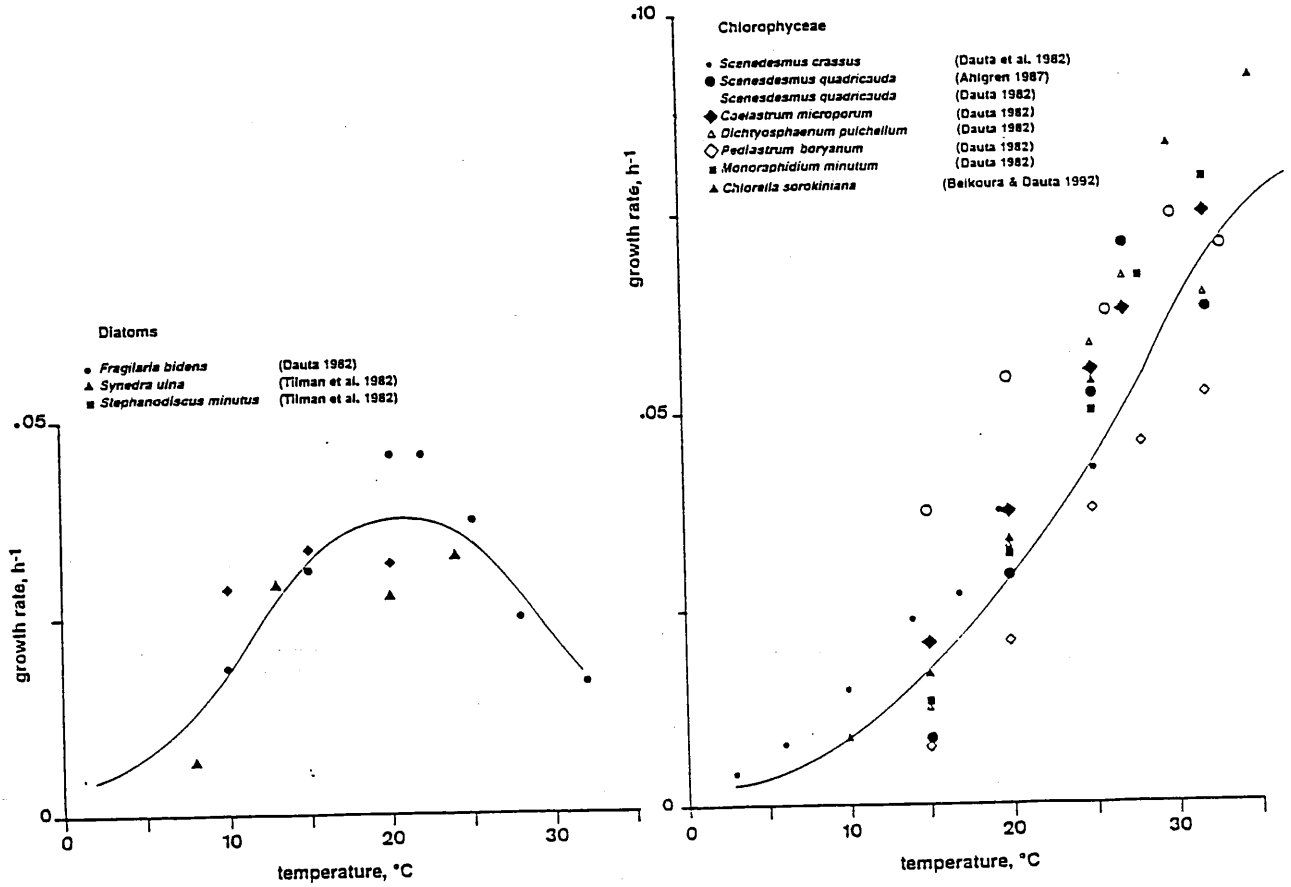

Fig. 5. Relationship between growth rate and temperature for diatoms and Chlorophyceae. The curve shows results from best fitting a sigmoid relationship.

rophyceae on the basis of values cited by Reynolds (1984). A lysis constant of 0.003 h^{-1} for diatoms and Chlorophyceae is assumed. The first-order rate constant of dissolution of detritic biogenic particulate silica has been taken as 0.001 h^{-1}, which is in the middle of the range cited in the literature (Lewin 1961; Nelson et al. 1976; Paasche and Ostergren 1980).

Table 3 summarizes the kinetics of the processes and the values of the parameters adopted in the model.

Modeling the dynamics of diatoms and Chlorophyceae in the Seine River—The model was run for calculating the seasonal variations of diatoms and Chlorophyceae in the whole Seine drainage network in the hydrometeorological conditions of 1991. A fairly good agreement between calculated and observed values of Chl *a*, silica, and phosphate was achieved at all stations in the drainage basin for which observed data were available for comparison (Fig. 6). Hydrological and climatic conditions were similar in 1991 and 1990, so data from 1990 at Porcheville are included for comparison with the data calculated for 1991 (Fig. 6). In most cases, the calculations nicely predict both the spring bloom and the variations in the summer level of phytoplankton biomass along the river continuum. When Figs. 3 and 6 are compared, it appears that the model adequately reproduces the biomass level of diatoms and Chlorophyceae at Paris and Méry and also their timing. However, at Porcheville in August, it appears that the model underestimates the biomass of diatoms (and overestimates the biomass of Chlorophyceae); consequently, the depletion in silica observed at that time is not adequately simulated by the model. The tendency is confirmed at Poses, for which the model does not predict the development of diatoms as shown by the observed silica depletion in August.

A general agreement is also found between calculated and estimated zooplankton biomass. The hypothesis that mortality and lysis of phytoplankton are enhanced explosively beyond a certain density thus allows realistic simulations of both phyto- and zooplankton biomass.

The model makes it possible to calculate phytoplankton growth (i.e. net primary production) and loss fluxes (sedimentation, lysis, and grazing) in the tributaries of different stream order in the drainage network. Table 4 presents the results of these calculations for periods in spring (April and May) and summer (July and August). In spring,

Table 3. The AQUAPHY submodel: kinetics of the processes taken into account in the description of algal physiology and value of the rate parameters involved for diatoms and Chlorophyceae.

Process	Rate expression*		Parameter	Diatoms	Chloro-phyceae
Photosynthesis (phot)	$k_{max}[1 - \exp - (\alpha I/k_{max})]PHY$	k_{max}†	max rate of photosynthesis, h^{-1}	0.2	0.4
		α	initial slope of the P/I curve, $h^{-1}(\mu Einst\ m^{-2}s^{-1})^{-1}$	0.0012	0.0012
Reserve synthesis	$sr_{max}M(S/PHY, K_s)PHY$	sr_{max}†	max rate of reserve synthesis, h^{-1}	0.13	0.26
		K_s	half-saturation constant	0.06	0.06
Reserve catabolism	$k_{cr}R$	k_{cr}†	1st-order constant of R catabolism, h^{-1}	0.2	0.2
Phyto growth (phygrwth)	$muf_{max}M(S/PHY, K_s)1fPHY$	muf_{max}†	max growth rate, h^{-1}	0.05	0.1
nutrient limitation factor	with $1f = M(PO_4, K_pP)$	K_pP	half-saturation constant for P uptake, μg P $liter^{-1}$	7	46
	or $M(NO_3 + NH_4, K_pN)$	K_pN	half-saturation constant for N uptake, μg N $liter^{-1}$	14	14
	or $M(SiO_2, K_pSi)$	K_pSi	half-saturation constant for Si uptake, mg SiO_2 $liter^{-1}$	0.25	—
Phyto respiration	maint PHY + ecbs phygrwth	maint†	maintenance coefficient, h^{-1}	0.002	0.002
		ecbs	energetic cost of biosynthesis	0.5	0.5
Phyto excretion	exp phot + exb PHY	exp	"income tax" excretion coef.	0.0006	0.0006
		exb	"property tax" excretion coef., h^{-1}	0.001	0.001
Phyto lysis	$k_d f + k_d f\ (1 + vf)$	$k_d f$†	mortality rate constant, h^{-1}	0.0075	0.005
		vf‡	parasitic lysis factor	20	20
Phyto NH_4 uptake	phygrwth/CN $NH_4/(NH_4 + NO_3)$	CN	algal C:N ratio, g C (g N)$^{-1}$	7	7
Phyto NO_3 uptake	phygrwth/CN $NO_3/(NH_4 + NO_3)$				
Phyto PO_4 uptake	phygrwth/CP	CP	algal C:P ratio, g C (g P)$^{-1}$	40	40
Phyto SiO_2 uptake	phygrwth/CSi	CSi	algal C:Si ratio, g C (g SiO_2)$^{-1}$	2	—

* $M(C, K_c) = C/(C + K_c)$; Michaelis-Menten hyperbolic function.
† These parameters are temperature-dependent. The values given are for optimum temperature. The temperature dependence is according to the sigmoid relationship: $p(T) = p(T_{opt}) \exp[-(T - T_{opt})^2/dti^2]$ with T_{opt} = 21°C for diatoms and 37°C for chlorophyceae and dti = 13°C for diatoms and 17°C for chlorophyceae.
‡ Parasitic enhancement mortality factor. Set at zero as long as algal density is lower than a threshold of 65 μg Chl a $liter^{-1}$ and temperature is <15°C.

primary production is dominated by diatoms in the whole drainage network and culminates in 7th-order rivers, with values between 1 and 2 g C m^{-2} d^{-1}. Sedimentation represents the major fate of primary production (beyond downstream exportation) in lower stream orders (up to 6th order), while lysis is the dominant loss flux in 7th- and 8th-order streams. Grazing is only significant downstream from 7th-order streams. In summer, primary production is dominated by Chlorophyceae from 6th-order streams downward and reaches values as high as those observed in spring for 7th-order streams. Lysis represents the major fate of both Chlorophyceae and diatoms, although grazing may cause a significant additional loss downstream. Direct sedimentation of algae is much less important at that time.

Exploring hypothetical scenarios—Several scenarios were tested to explore the role of hydrological, bottom-up, and top-down controls on the occurrence and succession of diatoms and Chlorophyceae.

The role of hydrology was explored by simulating the effects of rainy conditions (3.5 mm d^{-1}) that persist until the end of May. Such conditions are not unusual in the oceanic meteorological regime of the Seine basin. Compared to the reference situation, which corresponds to a drier period, the spring decrease of discharge is slower and the onset of the bloom is delayed until June (Fig. 7). Moreover, the scenario shows a lower amplitude of the bloom, the discharge remaining higher because of the larger contribution of groundwater. Regarding the composition of phytoplankton, Chlorophyceae succeed diatoms before silica is completely depleted (Fig. 7); at the onset of the bloom, the already higher temperature indeed favors Chlorophyceae, which have a higher growth rate. In addition, because zooplankton development is much reduced due to higher discharge and lower algal biomass upstream, the summer development of Chlorophyceae is less affected by grazing (Fig. 7).

We explored the role of top-down controls by running the model without zooplankton grazing and without lysis

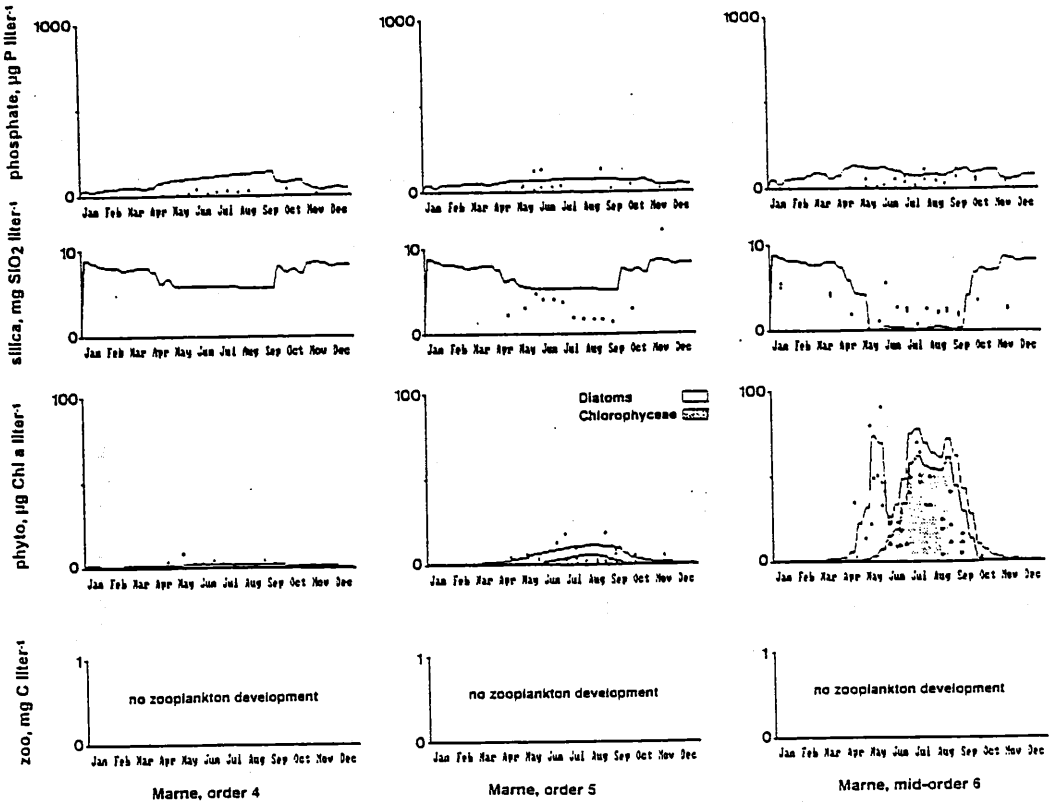

Fig. 6. Simulation, for the hydrological conditions of 1991, of the seasonal variations of diatoms and Chlorophyceae biomass, of phosphates and silica concentrations, and of zooplankton biomass at different locations in the Seine drainage network. Experimental data are shown for comparison.

of phytoplankton under infection. Under these conditions, the spring bloom is followed by a further increase in phytoplankton biomass without drastic termination. A high level of biomass is maintained until late summer (Fig. 8).

The role of silica concentrations on the seasonal development of phytoplankton was also tested. The model was run with values of silica concentration in headwater ranging from 10 to 150% of the reference concentration (13.5 mg SiO_2 liter^{-1}). Figure 9 shows the results for a 6th-order river. Increasing silica inputs to 150% of the reference value allowed the diatoms to bloom at a higher level, leading to higher zooplankton biomass that controlled the development of Chlorophyceae (Fig. 9). Conversely, reducing silica inputs led to a decrease in the amplitude of the diatom peak (Fig. 9). As a consequence, zooplankton development was reduced in lower order streams. Chlorophyceae thus can reach higher biomass which in turn sustains zooplankton development. In total, both phytoplankton and zooplankton developments are delayed.

Discussion

Phytoplankton development in the entire drainage network—Our model for the Seine River system considers two algal compartments and their controlling factors (bottom-up and top-down controls) as well as the circulation of C, N, P, and Si through all compartments of the river system. Moreover, owing to the use of the stream order concept, the model is able to describe phytoplankton development at the scale of the whole drainage network.

In spite of some simple assumptions (i.e. the physiology of the whole algal community reduced to two taxonomic groups, the top-down control not fully understood), agreement is found between calculated and observed variations of total phytoplankton biomass and nutrients in time and space. Simulation of a series of scenarios with different forcing functions or controls leads to a better understanding of typical features of phytoplankton development in the river. The hydrological control of the onset of the spring bloom, the distribution of spring biomass as a function of stream order, and the top-down control of spring populations have been discussed previously (Billen et al. 1994). The new results from the present version of the model, comprising two algal populations differing in their ecological characteristics, concern understanding the summer pattern of phytoplankton development along the river continuum and elucidation of the role of silica as a limiting factor in spring. The latter relates to diatom-Chlorophyceae succession and is discussed below.

The fluctuations of summer phytoplankton biomass

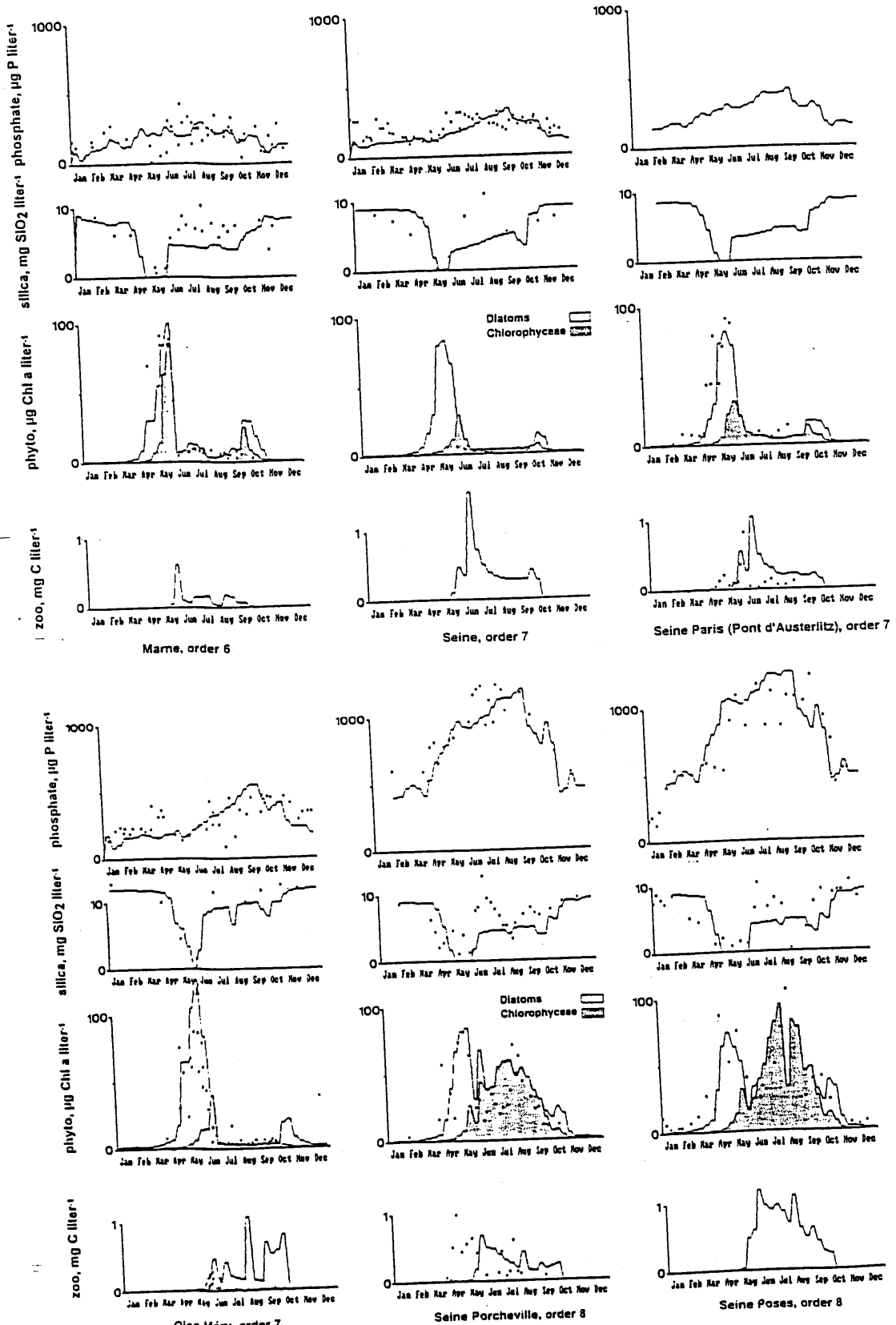

Fig. 6. Continued.

Table 4. Growth (net primary production), sedimentation, lysis, and grazing fluxes, expressed in mg C m^{-2} d^{-1} (1); losses are also expressed in percentage of growth (2). Calculations by the RIVERSTRAHLER model in tributaries of the Seine drainage network for the conditions of 1991.

	Spring (Apr–May)				Summer (Jul–Aug)			
	Diatoms		Chlorophyceae		Diatoms		Chlorophyceae	
	1	2	1	2	1	2	1	2
Marne (4th order)								
Growth	10.1	100	1.3	100	9.8	100	5.2	100
Sedimentation	3.9	39	0.2	13	4.1	42	0.3	5
Lysis	0.8	7	0.1	5	0.7	7	0.2	4
Grazing	0.0	0	0.0	0	0.0	0	0.0	0
Marne (5th order)								
Growth	33.7	100	3.2	100	44.9	100	29.9	100
Sedimentation	7.6	22	0.2	7	11.4	25	1.1	3
Lysis	2.6	7	0.2	5	3.3	7	1.4	4
Grazing	0.0	0	0.0	0	0.0	0	0.0	0
Marne (1st half of 6th order)								
Growth	413.0	100	63.8	100	489.4	100	1,322.3	100
Sedimentation	81.0	19	1.4	2	54.8	11	16.6	1
Lysis	64.1	15	2.7	4	411.4	84	1,005.3	76
Grazing	0.3	0	0.1	0	0.3	0	3.0	0
Marne (2nd half of 6th order)								
Growth	431.0	100	563.5	100	8.2	100	2,012.1	100
Sedimentation	276.0	64	40.4	7	1.0	12	29.1	1
Lysis	210.0	48	84.0	14	19.3	233	2,383.5	118
Grazing	20.6	0	103.6	18	0.5	6	173.7	8
Seine upstream from Paris (7th order)								
Growth	1,290.6	100	138.2	100	19.0	100	386.0	100
Sedimentation	137.5	10	1.2	0	0.5	2	1.4	0
Lysis	320.8	24	6.9	4	29.0	152	326.8	84
Grazing	278.6	21	37.7	27	9.2	48	196.4	50
Oise (7th order)								
Growth	2,035.8	100	109.5	100	1,139.9	100	1,440.3	100
Sedimentation	123.3	6	1.2	1	62.7	5	9.5	0
Lysis	288.6	14	6.8	6	1,097.3	96	1,562.6	108
Grazing	50.7	2	5.5	5	230.9	20	173.4	12
Seine from Paris to Porcheville (7th–8th order)								
Growth	950.7	100	279.6	100	29.4	100	1,952.0	100
Sedimentation	121.5	12	4.0	1	1.4	4	11.2	0
Lysis	334.1	35	27.7	9	4.6	15	144.6	7
Grazing	233.0	24	212.3	75	6.8	23	412.9	21
Seine from Porcheville to Poses (8th order)								
Growth	641.5	100	390.3	100	30.3	100	2,889.2	100
Sedimentation	139.6	21	4.2	1	2.6	8	29.7	1
Lysis	382.8	59	29.5	7	9.2	30	415.6	14
Grazing	193.4	30	214.4	54	22.9	75	417.1	14

within the entire drainage network (i.e. high summer biomass in mid-6th-order streams, low summer phytoplankton biomass in 7th-order streams, and high biomass again in 8th-order streams, *see Fig. 6*) can be interpreted as the result of classical predator-prey interactions which, in rivers, produce fluctuations in both time and space. In early spring, due to low temperature, low growth rate, and low residence time, phytoplankton biomass only gradually increases from the headwaters to the estuary (Fig. 10a). Top-down control follows phytoplankton development, with a similar pattern, leading to the decay of the spring bloom (Fig. 10b). In summer, in a nutrient-enriched environment, longer residence times and higher temperatures imply high growth rates and allow phyto-

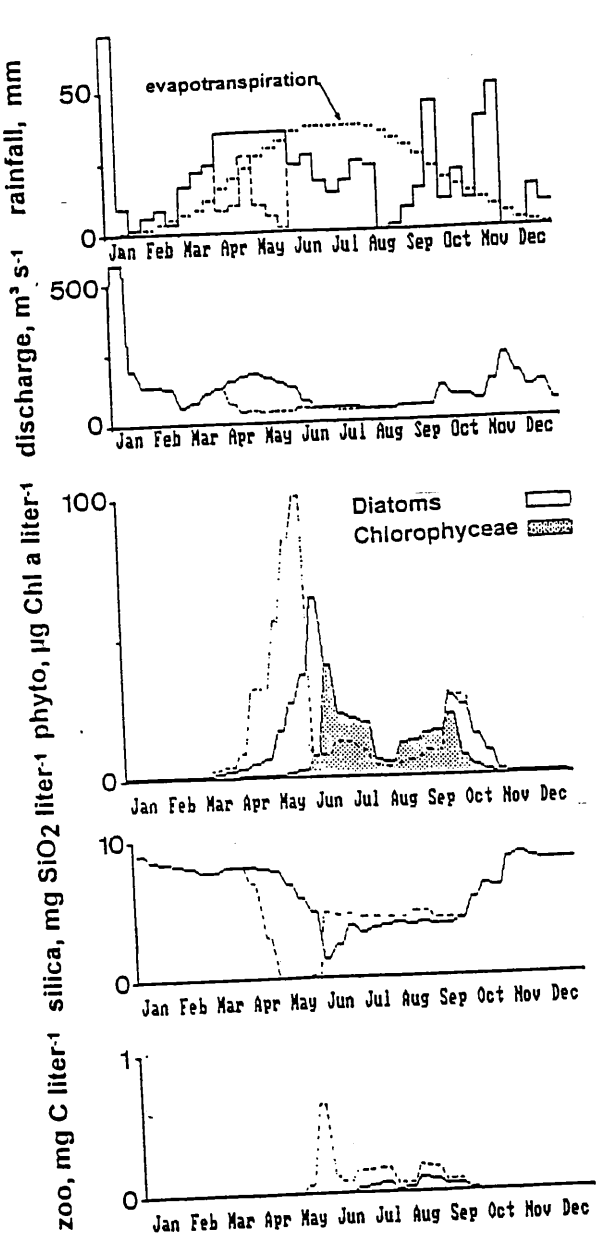

Fig. 7. Simulation of the seasonal variations of diatoms and Chlorophyceae biomass and silica concentration for a hypothetical rainy spring situation with pluviometry maintained at high level until late May. Resulting discharges are also presented. Reference conditions (*see Fig. 6*) are shown as dashed lines.

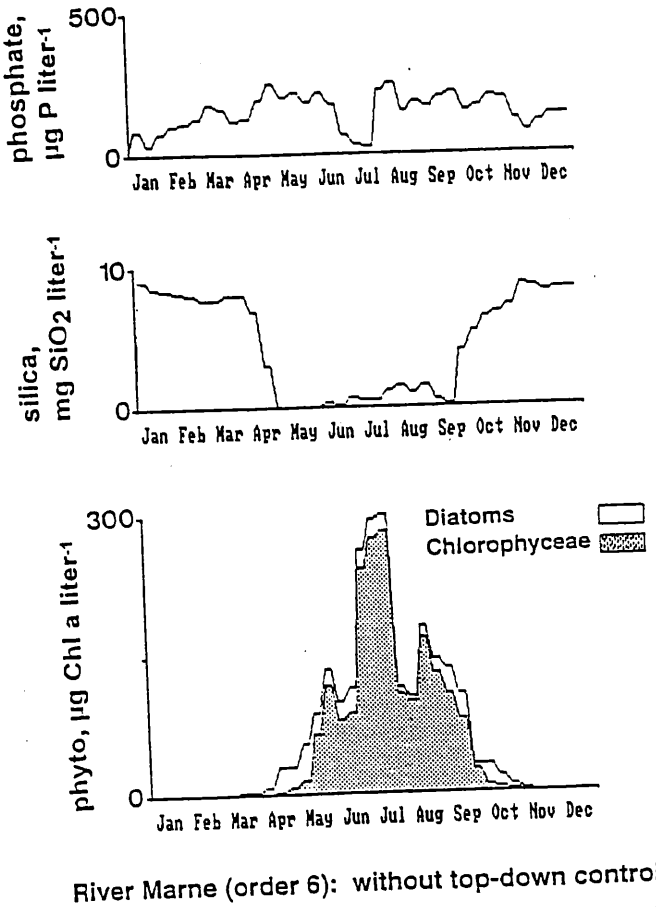

Fig. 8. Simulation of the seasonal variations of diatoms and Chlorophyceae biomass and silica concentration for a hypothetical scenario where top-down controls have been suppressed.

plankton to develop within a shorter course of the river (mid-6th order) provided that top-down control remains low; after that, the development of top-down control follows, which in turn rapidly decreases phytoplankton biomass (end 6th order and 7th order). Top-down controls decrease so that phytoplankton develops again, initiating a new sequence (8th order) (Fig. 10c).

Schematically, seasonal fluctuations of plankton interactions in rivers resemble those described in lake systems, except that the added longitudinal dimension leads to simplification of the interactions. In rivers, the zooplankton community is mainly composed of organisms with a short generation time, such as rotifers (Pourriot et al. 1982; Testard et al. 1993); large grazers, which have a generation time exceeding the residence time of the water, even in summer, cannot develop in the river. "Complex interactions" commonly reported in lake systems are therefore minimized by the reduction of taxa (Carpenter 1988). Moreover, in contrast to stratified lakes (Sommer et al. 1986), nutrient limitation in rivers does not so se-

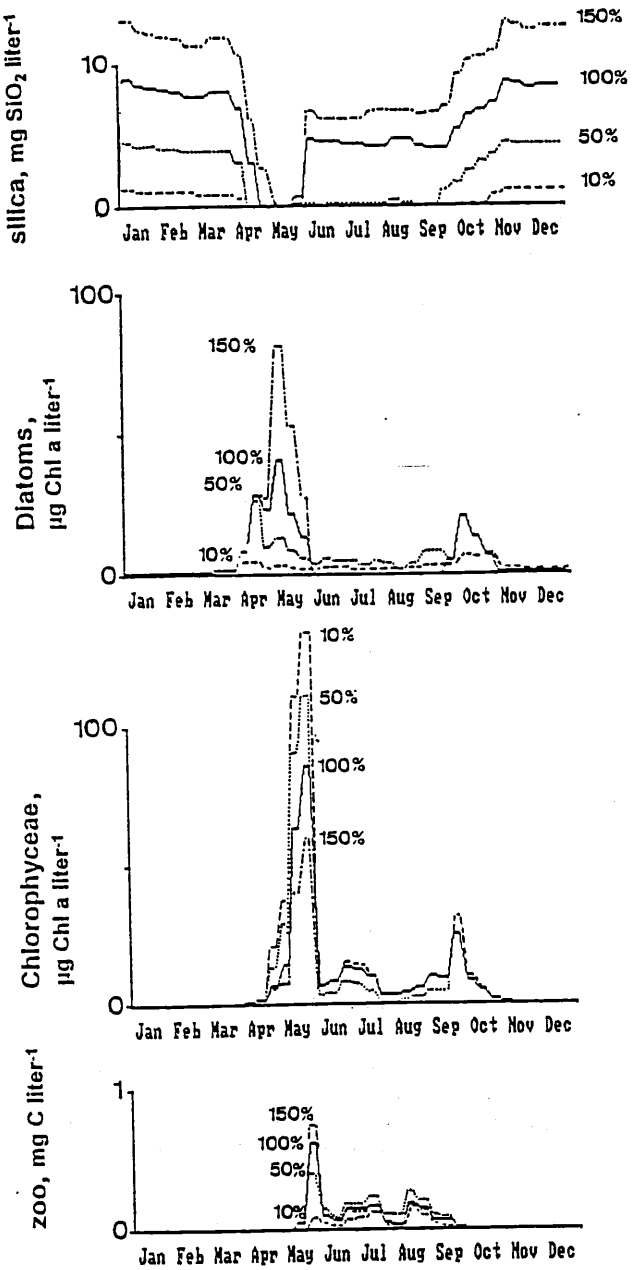

Fig. 9. Simulation of the seasonal variations of diatoms, Chlorophyceae, and zooplankton biomass for hypothetical scenarios where silica input has been taken at 150, 100, 50, and 10% of its actual value for the Seine catchment.

verely control the successional sequences of algae due to continuous replenishment of nutrients in headwaters by soil leaching and by anthropogenic input all along the course of the river.

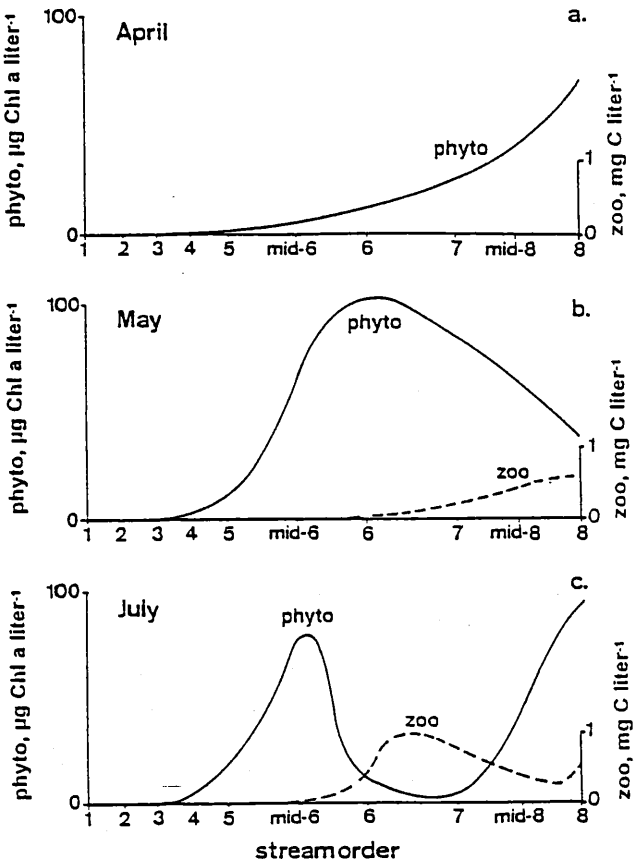

Fig. 10. Schematic representation of phytoplankton and zooplankton development along the river continuum for early spring (a), late spring (b), and summer (c) situations.

Diatoms-Chlorophyceae succession—Cyanobacteria, known for their inability to grow in turbulent or frequently mixed systems (Reynolds et al. 1983; Steinberg and Hartmann 1988), never form large populations in the Seine River, despite the eutrophic conditions encountered there. Large populations of Cyanobacteria have, however, been found in rivers, either under low discharge or when they develop in stagnant waters (Köhler 1993). Cryptophyceae, although known to be opportunistic (Stewart and Wetzel 1986), appear only occasionally. The Seine River provides the best conditions for the development of diatoms and Chlorophyceae, which are the dominant components of the phytoplankton.

Silica must be considered a first controlling factor of the diatom-Chlorophyceae succession because its availability is of prime importance for the occurrence of silicified organisms, including diatoms. In the Seine River, provided that favorable hydrological conditions are met in early spring, a high level of silica leads to a diatom spring bloom that depletes silica to limiting concentrations. Silica level clearly acts on the maximal diatom biomass reached during the spring bloom. However, the

sudden drop in spring biomass that occurs by early June cannot be explained by silica limitation. The model therefore questions the role of a composite effect of top-down control that includes both zooplankton grazing and parasite infection. The good agreement between observed and calculated values during the wax and wane of the spring bloom justifies the hypothesis made for representing the top-down composite control by zooplankton and algal parasites and the simplification still made for algal physiology (all diatoms species being taken as a sole population).

After the spring bloom, Chlorophyceae develop in a N- and P-rich environment (Si limited) with favorable temperature. In summer, the model nicely simulates the total biomass in the entire drainage network, but observed data show a mixed phytoplankton composed of Chlorophyceae and diatoms, which is not adequately predicted by the model. Taking into account a third algal group of summer diatoms, characterized by a different relationship between growth rate and temperature, would likely improve the simulations. Analyzing diatom species along a thermal gradient, Vinson and Rushforth (1989) showed that certain diatom species showed a preference for temperature >20°C, whereas others had a preference for temperature <15°C. However, more data are needed on the Seine River to adequately identify the composition of the summer community of diatoms and its associated physiology. It is interesting to note that whereas planktonic centric diatoms dominate in large rivers, including the Seine, large benthic pennates might represent a larger proportion in small rivers, especially when swept away from the bottom by increasing flow rates (Lack 1971; Jones and Barrington 1985).

Exploring the role of regulating factors—The scenarios tested with the model have shown that in the Seine River the amplitude of the spring phytoplankton peak can be strongly affected by the amount of silica. The extent to which diatoms dominate the phytoplankton biomass in a river system should therefore be related to the silica level found in the drainage basin. In the Ter River (Spain), where silica concentrations are very low (0.03–0.06 mg Si liter^{-1}), diatoms represent only 1–2% of the total algal abundance (Sabater 1990). Such low silica concentrations may be the result of retention in upstream lentic systems. The Ter system indeed includes a reservoir that could represent a sink for silica through diatom growth and sedimentation. Several other examples are reported by Conley et al. (1993). Obviously, the lithology and intensity of rock alteration, which constitutes the main silica input, must represent a natural selective factor for the occurrence of diatoms because silica concentration <3 mg SiO_2 liter^{-1} can be found in waters draining schist, marl, and limestone areas (Meybeck 1986).

The role of grazers as a regulating factor of phytoplankton biomass and seasonal succession has been generally neglected in rivers, compared to lakes, probably because zooplankton is essentially represented by rotifers, which are not efficient grazers and appear in low numbers: a maximum abundance of 360 ind liter^{-1} was found in the lower part of the Loire River (Pourriot et al. 1982). Large grazers, which cannot grow in the river, nevertheless locally appear as a result of inoculation from stagnant water bodies connected to rivers (Pourriot et al. 1982; Krocker *cited by* Köhler 1993); however, they rapidly disappear under fish predation (Pourriot et al. 1982). In the Seine, we hypothesize that algal size and biomass together with residence time of the water could sustain the development of grazers, possibly regulating phytoplankton populations. Zooplankton dynamics is described in the model by a continuous growth of the organisms. This description is a simplification of the behavior of a zooplankton community when it is comprised mostly of organisms with development stages (copepods, cladocerans); the representation is, however, acceptable in rivers, where rotifers (no development stages) dominate the grazing community. The model predicts zooplankton biomass in the correct range (a rough estimation of 1 mg C liter^{-1} at maximum).

Zooplankton grazing, however, constitutes only one aspect of phytoplankton losses. Protozoa that show low biomass (100 μg C liter^{-1}) could exert a significant grazing pressure (Becquevort pers. comm.). *Dreissena polymorpha*, which reaches high numbers in the Seine, could have a significant impact on the phytoplankton as well (Testard 1990; Testard et al. 1993). At this stage of development of the model, besides top-down control by zooplankton grazing, we postulate an additional effect by lysis under viral, fungal, or bacterial infection to adequately simulate the observed abrupt termination of blooms in the Seine. This result does agree with quantitative evidence that viruses can infect a variety of phytoplankton populations in the oceans (Suttle and Chan 1993; Bratbak et al. 1993). Algal-lysing bacteria (Reynolds 1984) and fungal parasites (Canter 1979; Bruning et al. 1992) are also known to cause rapid decline of phytoplankton populations. Besides grazing and lysis, calculation by the model shows that sedimentation may also represent a significant loss process of phytoplankton in the Seine River, particularly in lower and intermediate stream orders, where and when diatoms represent the major component of phytoplankton. This flux of sedimenting algae possibly sustains a significant benthic community. Sedimentation losses cannot, however, explain the drastic termination of the diatom spring bloom observed in late spring.

Conclusions

Sommer et al. (1986) summarized the main events characterizing the seasonal periodicity of plankton in stratified lakes in the form of a series of statements known as the PEG model. By reference to this model, using the present mathematical model, we can also describe the major features of planktonic seasonal events in large rivers with pluvio-oceanic regimes (Fig. 10).

Toward the end of winter, decreasing discharge and nutrient availability and increasing light allow the development of a spring diatom bloom during which the

phytoplankton growth rate exceeds the dilution rate; this occurs from the 5th-order rivers downward, phytoplankton biomass regularly increasing up to 8th-order streams, downstream in the river (Fig. 10a). The spring bloom of diatoms depletes silica, which can become a limiting nutrient. Sedimentation of diatoms represents a significant flux, mainly in mid-order rivers.

The dominance of edible centric diatoms leads to development of a rapidly growing zooplankton population that reaches sufficient biomass to control the phytoplankton. The dense population of diatoms may also become infected by microorganisms (virus, bacteria, fungii). As a consequence of grazing and infection, diatoms decrease rapidly in late spring, downstream of 6th-order streams (Fig. 10). A decrease in zooplankton then follows.

From the beginning of summer, higher temperature, increased residence time, lowered grazing pressure, and suitable nutrient concentrations (high N and P, low Si) represent favorable conditions for the development of Chlorophyceae that succeed the diatoms. The summer diatom populations can, however, reach a significant biomass due to rapid replenishment of silica from headwaters. Summer phytoplankton is thus characterized by a mixed population of Chlorophyceae and diatoms. The summer situation is characterized by spatial oscillations of planktonic biomass resulting from rapid temporal fluctuations. A high phytoplankton biomass is reached in a short distance along 6th-order streams. Zooplankton development follows rapidly and, together with parasites, decreases phytoplankton, resulting in a low plankton biomass equilibrium phase in 7th-order rivers. A lower grazing pressure and reduced infection permit the phytoplankton to develop again in 8th-order rivers, still followed by zooplankton (Fig. 10c).

In autumn, decreasing light and temperature result in a decline of phyto- and zooplankton biomass toward a winter minimum.

Due to conditions in river systems (continuous replenishment in nutrients from headwaters, permanent mixing, shorter residence time of the water, etc.), the successional sequence of phytoplankton described in stratified lakes as a result of a complex set of feedback mechanisms (nutrient limitation, trophic interactions) is shortened to the diatom-Chlorophyceae alternation, with the zooplankton community restricted to organisms with short generation times. From this point of view, the composition and dynamics of riverine phytoplankton tend to converge to those observed in shallow, intermittently stratified lakes, where successional sequences are frequently interrupted by wind effects (Reynolds 1988). The model developed in this paper helps to better understand these complex ecosystem dynamics.

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