25/8/2000.

Université Libre de Bruxelles
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Dynamique des efflorescences de *Phaeocystis* et de diatomées dans les eaux côtières eutrophisées de la Baie Sud de la Mer du Nord.

Rousseau Véronique

Année académique 1999-2000

Thèse présentée pour l'obtention du grade de Docteur en Sciences Agronomiques

Sous la direction de C. Lancelot

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Contents

Abstract

Marine coastal eutrophication is generally described as a shift from a diatom- to a non siliceous species-dominated phytoplankton community in response to the N and P anthropogenically-enriched nutrient delivery to the coastal zone. Eutrophication of the coastal waters of the Southern Bight of the North Sea is characterized by the occurrence of recurrent short-living spring blooms of the non siliceous colony-forming haptophyte *Phaeocystis* succeeding to a moderate early spring diatom growth. The more evident manifestation of these blooms is the accumulation of ugly foam on the beaches bordering the continental coastal North Sea due to a trophic food chain disruption. The objective of this thesis was to provide a deeper insight on the biology and ecology of the genus *Phaeocystis* and to assess its ecological role in the ecosystem of the continental coastal North Sea and in the Belgian coastal waters in particular. The final aim was a better understanding of the mechanisms behind eutrophication in this area. The life cycle of *Phaeocystis* alternates several types of nanoplanktonic cells and large colonies. The prodigious success of *Phaeocystis* as blooming species was however related to its ability to form large gelatinous colonies. The eco-physiological properties of the colonial stage allow indeed this genus to take benefit of the large excess of NO_3 over $Si(OH)_4$ and PO_4 relative to N:P:Si diatom requirements, that characterises the nutrient environment of these coastal waters. The colonial structure of *Phaeocystis* is also responsible for its unpalatability for indigenous mesozooplankton. Ungrazed *Phaeocystis* colonies stimulate the establishment of a very active microbial network which indirectly resumes the linear food chain through microzooplankton grazing on *Phaeocystis* free-living cells. However, the trophic effiency of this microbial food chain is very low. The huge biomass produced by *Phaeocystis* colony bloom has therefore little benefit for the higher trophic levels. The very low trophic efficiency of the planktonic linear food chain and of the microbial network together with the potential contribution of ungrazed *Phaeocystis*-derived production to the bacterial carbon demand suggest that most of the *Phaeocystis*-derived production in the Belgian coastal waters is remineralised in the water column. The analysis of time series data on diatoms and *Phaeocystis* and their environmental control evidences some of the mechanisms controlling the succession and co-occurrence of these two taxa. So, the three diatom communities succeeding in the Belgian coastal waters are characterized by a silicification level (Si:C) positively related to the $Si(OH)_4$ availability suggesting a good adaptation of the diatoms to their Si environment. The particularly low Si:C characterizing the *Rhizosolenia* spp.-dominated diatom community, co-occurring with *Phaeocystis* colonies, suggests these diatoms are well adapted to the very low $Si(OH)_4$ levels prevailing during the spring bloom. A preferential distribution of this diatom community is elsewhere observed in well-balanced water masses. All together, these results suggest that diatoms are not necessary Si-limited but also probably PO_4 limited or Si and PO_4 co-limited. *Phaeocystis* colonies are characterized by a mixed nutrient behaviour, growing on new sources of NO_3 and remineralised PO_4. These results, coupled to results of ecological MIRO model runs, reveal the inefficiency of measures proposed by national and international authorities to achieve a substantial reduction of harmful *Phaeocystis* bloom. Only an integrated land-coastal sea modeling approach would be able to provide guidance to better select the available control actions on the watershed in order to reduce the development of *Phaeocystis* colony blooms.

Résumé

Le phénomène d'eutrophisation des eaux côtières est généralement décrit comme une modification de la proportion relative des diatomées dans la communauté phytoplanctonique au profit d'espèces non silicifiées, en réponse aux apports de nutriments anthropogéniques considérablement enrichis en N et P. L'eutrophisation des eaux côtières de la Baie Sud de la Mer du Nord se caractérise par le développement d'efflorescences printanières de l'espèce non silicifiée *Phaeocystis* qui succède à une croissance précoce des diatomées. La manifestation la plus évidente de ces efflorescences est l'accumulation de mousses nauséabondes sur les plages de cette zone côtière lié à une rupture de la chaîne trophique. L'objectif de cette thèse est d'assurer une meilleure connaissance fondamentale de la biologie et de l'écologie du genre *Phaeocystis* mais aussi de son rôle dans l'écosystème de la Baie Sud de la Mer du Nord et plus particulièrement de la zone côtière belge. Le but final de ce travail vise à une meilleure compréhension des mécanismes d'eutrophisation côtière. Le cycle de vie de *Phaeocystis* alterne plusieurs types de cellules nanophytoplanctoniques et des colonies volumineuses. Le succès prodigieux de cette algue dans les milieux enrichis est cependant lié à sa capacité de former des colonies. Les caractéristiques éco-physiologiques du stade colonial lui permettent en effet de tirer avantage des excédents de NO_3 en regard des besoins de $Si(OH)_4$ et PO_4 des diatomées que présentent ces eaux côtières. La structure coloniale de *Phaeocystis* lui permet également d'échapper à la pression de broutage du mésozooplancton indigène. Les colonies de *Phaeocystis* stimulent cependant l'établissement d'un réseau microbien très actif. Celui-ci réintègre la chaîne trophique linéaire par l'ingestion des cellules isolées de *Phaeocystis* par le microzooplancton, proie de choix pour le mésozooplancton. Cependant, l'efficience trophique de ce transfert est très peu élevée. La production des efflorescences de *Phaeocystis* est donc très peu bénéfique pour les niveaux trophiques supérieurs. Cette faible efficience trophique ainsi que la demande en carbone considérable des bactéries printanières suggèrent que la production primaire provenant de *Phaeocystis* est majoritairement reminéralisée dans la colonne d'eau.

L'analyse de données temporelles de diatomées et de *Phaeocystis* ainsi que de leurs facteurs de contôle, met en évidence certains mécanismes contrôlant la succession et la co-occurrence de ces deux taxons. Ainsi, les 3 communautés de diatomées se succédant au printemps dans la zone côtière belge sont caractérisées par un niveau de silicification (Si/C) positivement correlé à la disponibilité en silice. Plus particulièrement, le très faible rapport Si:C de la communauté de diatomées dominée par *Rhizosolenia* spp., co-occurrente de *Phaeocystis*, suggère une bonne adaptation de ces diatomées aux faibles niveaux de $Si(OH)_4$. Une distribution préférentielle de cette communauté de diatomées est par ailleurs observée dans des masses d'eau équilibrées en nutriments. Ensemble, ces résultats suggèrent que les diatomées ne sont pas nécessairement limitées par la silice mais aussi probablement par les phosphates ou co-limitées par les 2 nutriments. Les colonies de *Phaeocystis* sont, elles, caractérisées par un comportement nutritif basé sur des sources nouvelles de NO_3 et du PO_4 régénéré. Ces résultats, couplés à ceux du modèle écologique MIRO, révèlent l'inefficacité des mesures proposées par les autorités afin de réduire substantiellement les efflorescences indésirables de *Phaeocystis*. Seule une approche intégrée de modélisation des interactions continent-zone côtière pourra fournir la guidance nécessaire pour décider au mieux des mesures à prendre sur le bassin versant afin de réduire ces efflorescences indésirables.

Introduction

Introduction

1. Marine coastal eutrophication

1.1. Definition

Traditionally, the term eutrophication refers to "the process of enrichment of waters with nutrients, primarily nitrogen (N) and phosphorus (P), that stimulates aquatic production". Its most serious manifestations leads to visible algal blooms, either planktonic or benthic, either micro- or macrophytes (Vollenweider, 1992).

Marine coastal areas, at the interface between land, ocean and atmosphere are particularly vulnerable to human-made eutrophication, especially those with only limited interactions with adjoining oceanic waters and large freshwater influence.

Human-eutrophicated coastal zones contrasts from coastal shelf areas submitted to upwelling of deep waters at the shelf break. In naturally enriched coastal systems, seasonal upwelling of deep waters at the shelf break supply new diagenetically remineralised nutrients to coastal phytoplankton. These nutrient sources, well-balanced in terms of N:P:Si with regards to the diatoms (N:Si = 1 in average; Brzezinski, 1985) and phytoplankton (N:P = 16; Redfield *et al.*, 1963) needs, stimulate the growth of diatoms. In these naturally enriched coastal environment, the diatoms are efficiently controlled by grazers, inititing the linear food-web 'diatom-mesozooplankton-fish' (Claustre *et al.*, 1994). In human-eutrophicated coastal areas, the nutrient environment of phytoplankton is strongly modified, both quantitatively and qualitatively, by anthropogenic "new" sources of nutrients from continental origin. These terrestrial nutrient sources generally present large amounts of N and P resulting from human activities (use of fertilizers, intensive livestock farming, use of phosphate-containing detergents, land use modification). Silicon (Si) delivery which depends on rock weathering, is not directly affected by human activities.

These freshwater sources of nutrients to the coastal zone present therefore a strong unbalance with large excess of N and P over Si with regards to coastal diatom requirement (Billen *et al.*, 1991). In addition to these changes in proportion of inorganic nutrients, a quantitative shift might occur among the oxidation degree of N compounds delivered to coastal areas with oxidized forms dominant over reduced ones (Billen *et al.*, 1991; Lancelot, 1995). As a result of the freshwater influence in coastal areas, these new sources of nutrients generate therefore marked modifications of the amount and balance of nutrient environment of coastal phytoplankton.

The modification of N:P:Si ratio and particularly the increase of N:Si and P:Si, induces a shift in the floristic composition of phytoplankton with the occurrence of additional blooms of non-siliceous species (Officer & Ryther, 1980; Smayda, 1990, Billen *et al.*, 1991; Conley *et al.*, 1993). The non-siliceous species blooming are the most often undesirable flagellates, either toxic or poorly edible, causing harmful effects on the environment (Smayda, 1990; Billen *et al.*, 1991; Smayda, 1997).

1.2. Terrestrial sources of nutrients

Nutrient transport (N, P, Si) into coastal areas, either inorganic and organic forms, occurs along 3 major pathways: the riverine run-off, direct emissions and atmospheric deposition.

Riverine nutrient transport to the coastal zone depends of the amount of nutrients discharged into the river system either from point or diffuse sources. Point sources (N, P) depends on urban (household) and industrial sewage as well as the level of waste water purification treatments. Diffuse sources of nutrients depends on lithology (Si), land use such as the existence of a permanent or intermittent vegetation cover (N, P, Si) but also on fertilizing practices (N). The link between nutrient delivery in the watershed and nutrient inputs to the coastal marine area is, however, far for being direct due to the biogeochemical processes that eliminate, immobilize or transform the nutrients within the aquatic continuum formed by rivers, lakes, estuaries (Billen *et al.*, 1991). Biological uptake, adsorption of NH_4 and PO_4 onto suspended matter, mineralisation in the sediment, nitrification, denitrification eliminating NO_3 very efficiently in anoxic zones, are riverine and estuarine processes modifying the transfer of nutrients to the coastal areas (*e.g.* Billen *et al.*, 1985; Zwolman, 1994; Billen *et al.*, 1995; Ogilvie *et al.*, 1997; Robinson *et al.*, 1998). In addition, hydraulic managements such as damming of rivers, has an indirect impact on nutrient inputs to the coastal zone. Modifications of stream flows, are indeed deeply modifying the biogeochemical processes in the system.

Direct emissions from coastal cities and industries contribute also to the nutrient budget of coastal areas. These nutrient sources are particularly significant in tourist areas when the population density increases dramatically during the spring-summer period, in the absence of waste water treatment.

Atmospheric dry and wet deposition on the sea surface of anthropogenic N compounds emitted from agricultural (livestock farming, cattle and liquid manure), urban and industrial (combustion of fossil fuels) origin is an additional source of NO_3, NH_3, NH_4 but also urea, amino acids and organo-nitrate in the nutrient budget of coastal waters (Paerl, 1995; Asman & Larsen, 1996; Stalnacke *et al.*, 1996; Paerl, 1997). Atmospheric deposition was even suspected to exceed estuarine transport in some north western European shelf area downwind of urban, industrial and agricultural emissions (Graneli *et al.*, 1999). Atmospheric inputs of P, mainly related to high temperature combustion of organic matter, seaspray and soil particules (Stalnacke *et al.*, 1996), are negligible as are those of Si (Reid *et al.*, 1988).

1.3. Quantitative and qualitative changes of the nutrient environment of coastal areas.

In many temperate regions of the northern hemisphere, both quantitative and qualitative modification of the nutrient environment of the coastal phytoplankton has been related to continental or anthropogenic sources of nutrient, enriched in N and P (*e.g.* van Bennekom *et al.*, 1975; Lancelot *et al.*, 1987; Brockmann *et al.*, 1988; Conley *et al.*, 1993; Ragueneau *et al.*, 1994; Justic *et al.*, 1995; Nelson & Dortch, 1996). Quantitative changes are mostly related to the increasing inputs of N and P to estuarine and marine waters over the last 50 years due to increasing urbanization,

industrialization and intensification of agriculture. In this way, the N load of the North Sea has increased by a factor 2.5 from 1950 to 1980 (van Bennekom & Wetsteijn, 1990, Radach *et al.*, 1990; Graneli *et al.*, 1999). In Dutch coastal waters, N and P concentrations has been shown to increase by a factor 7 since 1932 (Van Bennekom *et al.*, 1975). In the Baltic, an upward trends in the concentration of N and P was observed during the last 30 years (Stalnacke *et al.*, 1996). Since the 60's and until the late 80's, riverine N and P inputs in the northwestern Black Sea has increased by a factor 5 and 3 respectively (Cociasu *et al.*, 1996) as a result of the development of economic activities and urbanization of the watershed.

While N and P inputs have dramatically increased in most of human-influenced coastal areas, Si loads either remained constant or decreased. Si load is indirectly affected by man through the combined effects of modifications of the hydraulic regimes (Officer & Ryther, 1980) and higher retention in freshwaters due to N and P enrichment (van Bennekom *et al.*, 1975; Admiraal *et al.*, 1990; Billen *et al.*, 1991). Decreasing Si riverine inputs resulting from numerous hydraulic management programmes was reported for the western Black Sea (Humborg *et al.*, 1997).

1.4. Manifestation of eutrophication

The manifestations of eutrophication are very variable and regionally specific. The effect of a given enrichment on the complex coastal ecosystem depends on the receiving water body, *i.e.* the loading tolerance, the geomorphological and hydrodynamical characteristics but also of the trophic structure of the ecosystem determined by the planktonic and benthic communities.

Beyond the diversity of its local manifestations, the common feature of coastal eutrophication lies in the shift in species composition of the planktonic community, with the occurrence of undesirable non-siliceous phytoplankton. The manifest effects on human-induced coastal eutrophication usually appear as qualitative changes of the structure and functioning of the pelagic and benthic food web with resulting adverse effects. Few beneficial effects of human-made eutrophication, such as increase of biological resources, have indeed been reported (Nehring, 1992).

The non-siliceous species sustained by additional anthropogenic nutrients includes most often harmful organisms, toxic or unpalatable for indigenous zooplankton with resulting adverse effects (Smayda, 1990; Lancelot, 1995). Among them, oxygen deficiency or depletion with associated fish and benthos mortality, fish and shellfish killing, reduction of species diversity, shortening of the trophic chains, hindrance for recreation (bathing, water sport, tourism) due to reduced transparency and unsightliness are the most often reported (Vollenweider, 1992).

Among the problems of widest concern is the apparent spread and increase in frequency of blooms of toxic algae (Richardson & Jorgensen, 1996; Graneli *et al.*, 1999). Some dinoflagellates and chrysophyceae are involved in causing Paralytic and Diarrhetic or Amnesic Shellfish Poisoning (PSP and DSP, ASP). These algal toxins accumulated in the food web and constitute a danger even when toxin producers are present at low cell concentrations. Ichtyotoxic species such as dinoflagellates and haptophyceae (*Chrysochromulina*, *Prymnesium*) are not toxic by themselves but

produce water soluble toxins causing mass mortality of fish and bottom fauna with serious economical consequences for the aquaculture industry. The episodic blooms of the toxic *Chrysochromulina polylepis* in the Danish, Swedish and Norwegian waters of the Skagerrak et Kattegat in 1988/1989 caused death of tons of farmed fish and bottom fauna kill (Rosenberg *et al.*, 1988; 1991). The observed increase in frequency, extent and duration of such blooms could be however caused by both technological development increased capacity of detection (satellite imagery) and change in cultural habits (consumption of shellfish during summer when they are more likely contaminated) or increasing fish concentrations in sea farming.

Some non-siliceous phytoplankton species, while not toxic, induce however indirect adverse effects on the marine ecosystem such as foam accumulation, anoxia, clogging of fish gills, reduced yield of harvestable marine resources and loss of the recreational value of coastal area (Smayda, 1977). These negative impacts are the visible manifestations of a disruption of the marine food web due to the unbalance between biomass production and consumption by the higher trophic levels. Two well-known examples of such deleterious effects are the *Phaeocystis* blooms and mucilaginous phenomenon in the Adriatic sea. The recurrent blooms of *Phaeocystis* colony in the coastal waters of the Southern Bight of the North Sea produce indeed enormous amounts of unpleasant foam which accumulates along the beaches (*e.g.* Lancelot *et al.*, 1987). The huge phytoplankton biomass due to river Po discharges beaches in the North Adriatic coastal waters was responsible for the ugly mucilage floating near the beaches during summer 1988 and 1989 (Herndl, 1992).

The distinction between direct eutrophication-related effects and those engendered by other anthropogenic factors could not be easily assessed. Pollution, introduction of alien species through ballast waters, over-fishing and hydraulic management are among factors whose effects superimposed to those of anthropogenic nutrient enrichment (Brockmann *et al.*, 1988). Over-fishing of piscivorous fishes strongly modifies the food-web structure, causing indirect accumulation of phytoplankton biomass by suppressing their control by herbivorous zooplankton. Increasing inputs to marine waters of trace metals essential or inhibiting algal growth (Cu, Co, Mn, Fe) were related to increasing mobility and leaching of these metals from the soils due to acid precipitation (Graneli *et al.*, 1999).

Moreover, eutrophication-related changes observed in the marine ecosystem occur against a background of meteorological and climatic variations without any possible distinction between both driving forces. Their complex interplay could result in intensification of eutrophication effects or unexpected manifestations. In this way, the red tides observed during spring 1998 in south China, causing huge fish damages, were related to the combined effects of El-Nino and eutrophied conditions (Yin *et al.*, 1999). The *Chrysochromulina* event constitutes another example of such complex interplay. The toxin production by *Chrysochromulina*, an usual component of phytoplankton community in this area, was related to unusually high N:P ratio prevailing at the time of the bloom due to the combined effect of extreme meterological conditions and nutrient enrichment (Maestrini & Graneli, 1991, Richardson & Jorgensen, 1996).

2. *Eutrophication of the Southern Bight of the North Sea*

2.1. Biotope description

The continental coastal zone of the North Sea constitutes one example of a human-induced eutrophicated coastal ecosystem in response to anthropogenic nutrient change. This area receives indeed the discharges of 7 large west-European rivers : the Seine, Scheldt, Meuse, Rhine, Ems, Weser and Weser. The watersheds of these rivers draining an area of 850.000 km^2 (Fig. 1), are very densely populated, highly industrialised and used for intensive agricultural practices.

Figure 1: Map showing the watershed of the main rivers discharging in the continental coastal waters of the North Sea. Arrows indicate the general residual circulation of water masses flowing northeastward.

The Southern Bight of the North Sea, extending from the Straight of Dover to the German Bight, includes the coastal waters of France, Belgium, the Netherlands and Germany (Fig.1). It is limited by the thermic stratification of Atlantic waters in the south-west and in the German Bight in the north-east. This area is a shallow continental coastal shelf with a maximum depth of 40 m. The combination of strong tidal current maintaining a high turbulent regime, with shallow water depths ensures a general vertical mixing throughout the year (Reid *et al.*, 1988, Otto *et al.*, 1990; Simpson, 1994). Along the Dutch and German coast, haline stratification often occurs in the river plumes with associated fronts between stratified and well mixed regions. The Southern Bight of the North Sea is an open system characterized by an inflow of Atlantic waters entering the Southern North Sea through the Dover Straits. This water mass flows alongshore along a southwest-northeast axis due to the general residual circulation (Fig. 1). Moreover, the physical feature of these shallow tidal waters is considerably influenced by wind which induces significant residual currents (Otto *et al.*, 1990). The progressive dilution of Atlantic waters by the various sources of freshwaters from rivers Seine, Scheldt, Rhine, Meuse, Elbe, Weser and Ems, results in a marked SW-NE salinity gradient. As a consequence, the Atlantic waters flowing in the Southern Bight of the North Sea are progressively enriched by nutrient river discharges.

2.2. Enrichment of the Southern Bight of the North Sea

The riverine inputs to the continental coastal waters of the North Sea constitute by far the main sources of nutrients. The atmospheric deposition of N has been indeed estimated to only 9 % of the total N input into Dutch coastal waters (Klein & van Buuren, 1992). The riverine loads of nutrients discharged into the Southern Bight are characterized by large amounts of anthropogenic N and P. Some 720 000 T of inorganic N (whose 80 % NO_3) and 50.000 TP (70 % PO_4) are discharged every year in the Southern Bight of the North Sea (Lancelot, 1995). The flux of Si was estimated to 290.000 TSi per year.

The excess of N over P and N over Si of North European river discarges, with respect to phytoplankton and diatom nutrient requirements are evidenced on figure 2 (Lancelot *et al.*, 1991). These data compiled for the period 1978 to 1988, evidenced a much higher N excess over P in northern rivers (Rhine, Ems, Weser, Elbe) than in southern (Seine, Scheldt) while a 3-4 times excess of N over Si is evident for all the rivers (Fig. 2).

Historically, the anthropogenic N and P riverine loads in the area shown a marked increase from the thirties (Radach *et al.*, 1990; van Bennekom & Wetsteijn, 1990) while, in the same period, silicate was not or poorly affected by human activities (van Bennekom *et al.*, 1975). From the eighties, an increase in N:P ratios in northern rivers (Rhine, Elbe) was recorded resulting both from a decrease in P and an increase or stagnation of N loads. The lowering of the P discharge observed is a consequence of the decreasing load of P to Dutch and German coastal waters due to the introduction of secondary treatment in sewage purification plants and regulation about the use of P-free detergents (Klein & van Buuren, 1992; Hickel *et al.*, 1993). During this period,

the inputs of total N and Si has remained unchanged (source: international Commission for the protection of the Rhine against pollution).

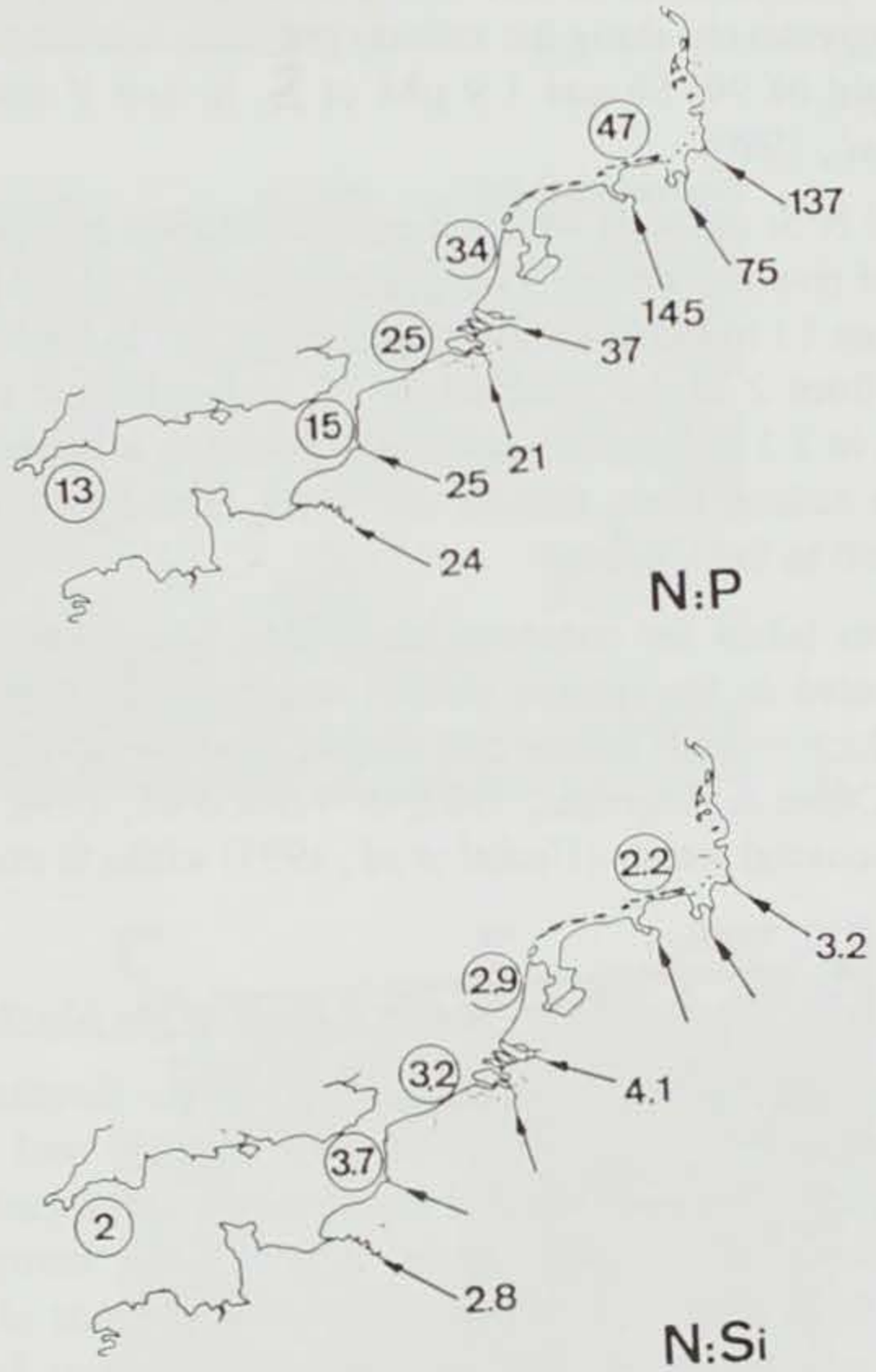


Figure 2 : N:P and N:Si ratio of the winter inorganic nutrient pool of the coastal areas of the Southern Bight of the North Sea (circles) and in the annual river discharges of the major rivers (reprinted from Lancelot *et al.*, 1991).

The quantitative and qualitative enrichment of the Southern Bight of the North Sea can be assessed from the winter concentrations and ratios of inorganic nutrients N, P, Si. In January-February, nutrients reached their highest level due to the completion of mineralisation processes and very reduced phytoplankton activities. Winter nutrient concentrations display one-order-of magnitude increase from the French to the German coastal waters as a result of the mean residual circulation of the water masses (van Bennekom *et al.*, 1975; Lancelot *et al.*, 1991). At the entrance of the Channel, at 35 salinity, inorganic N, Si and P are present at concentrations of 8, 4 and 0.6 µM respectively (Brockmann *et al.*, 1988; Lancelot *et al.*, 1990). These concentrations increase progressively along the salinity gradient, reaching average winter levels in the German Bight of 90, 50 and 1.9 µM of N, Si and P respectively (data 1988-1989; Lancelot *et al.*, 1990).

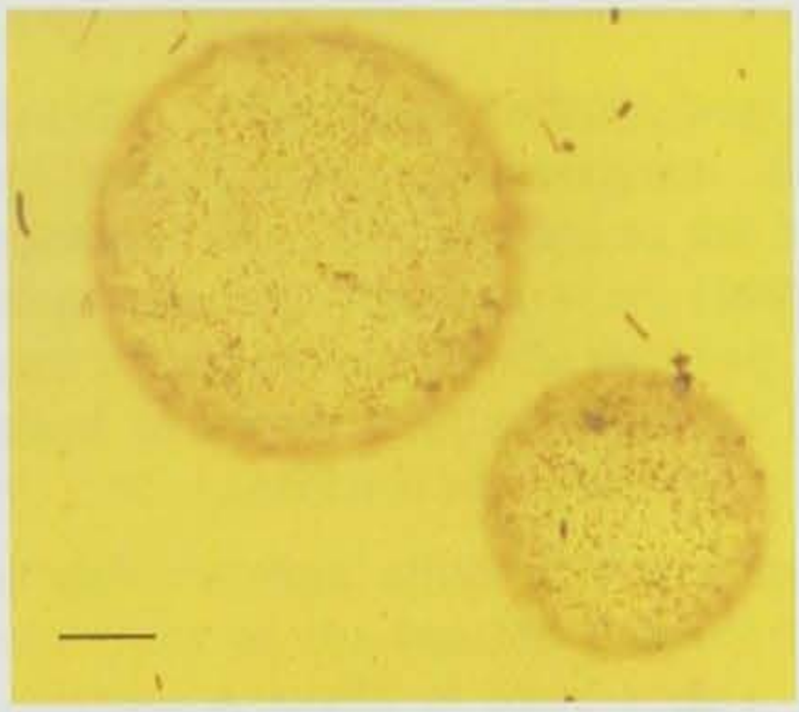
The N:P and N:Si ratios of winter inorganic nutrient concentrations clearly reflects the unbalance of riverine waters (Fig. 2; Lancelot *et al.*, 1991). N:P ratios are increasing regularly from 13 in the Western Channel up to 47 in the German Bight (Fig. 2). N:Si ratios vary from 2 in the Channel to 3.7 in the French coastal zone then decreases again down to 2.2 in the German Bight, showing a larger excess of Si in the French and Belgian coastal areas than in the North. These data also suggest a deficit of Si compared to P in the Channel.

The measures taken for reducing land-based sources of nutrient in the eigthies are clearly reflected in the marine coastal waters. PO_4 concentrations decrease due to efficient reduction of P inputs and related increase of N:P were indeed reported, for the Dutch (Cadee & Hegeman, 1993; de Vries *et al.*, 1998; Philippart *et al.*, 2000) and the German coastal waters (Hickel *et al.*, 1993) while Si concentrations remain stable.

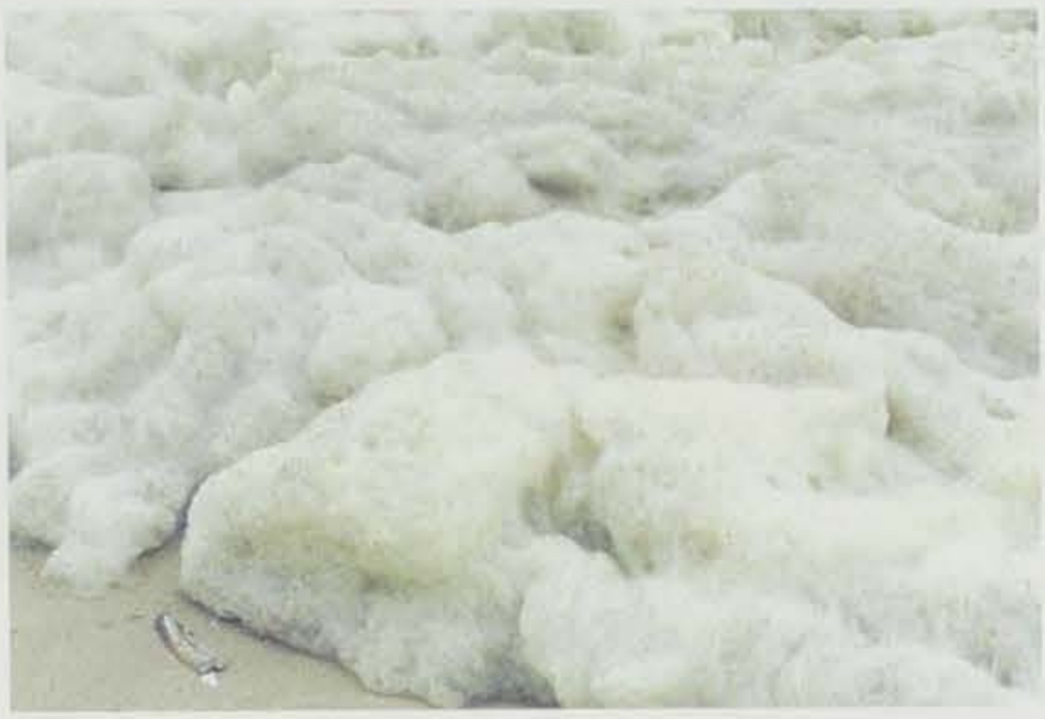
2.3. Phaeocystis blooms in the Southern Bight of the North Sea

The nutrient signature of the coastal waters of the Southern Bight of the North Sea presents therefore a large excess of NO_3 over Si and P (Lancelot *et al.*, 1991; Lancelot, 1995). This unbalanced enrichment is responsible for a peculiar structure and functioning of the ecosystem with visible harmful environmental effects (Lancelot, 1995). Clearly transient foam accumulations observed every spring at sea surface and on the beaches (Fig. 3b) are resulting from food chain disruption due to the proliferation of one single non-siliceous species, the gelatinous colonial haptophyte *Phaeocystis* sp. (Fig. 3a). In contrast to the episodic *Chrysochromulina* sp. bloom, *Phaeocystis* proliferation is a well known phenomenon which is observed every year from mid-April to mid-May in the whole Southern Bight of the North Sea (Gieskes & Kraay, 1977, Cadée & Hegeman, 1986, Lancelot *et al.*, 1987). *Phaeocystis* colony bloom occurs after a moderate diatom growth in late winter-early spring often reported as silicate-limited. *Phaeocystis* colony bloom occurs in association or not with a late spring diatom community dominated by larger species (Weisse *et al.*, 1986; Cadee & Hegeman, 1991; Rousseau *et al.*, in preparation).

Largely unpalatable for the indigeneous mesozooplankton (Gasparini *et al.*, 2000), *Phaeocystis* is responsible for the accumulation of large gelatinous colonies (Bätje & Michaelis, 1986; Cadée, 1986; Cadée & Hegeman, 1986; Lancelot *et al.*, 1987;



a.



b.

c.

Figure 3: a.) *Phaeocystis globosa* colonies (scale bar = 200 µm); b.) *Phaeocystis* foam accumulated on a Belgian beach (Ostende, May 1998); c.) Sledge for benthos sampling (250 µm size mesh) clogged by *Phaeocystis* colonies (Belgica, May 1999). Photographs by V. Rousseau.

Lancelot, 1995). *Phaeocystis* outburst, with cell density reaching 50-120 10^6 cells.l^{-1} represents usually, at their maximum development, more than 90% of the phytoplankton carbon biomass due to the high contribution of exopolysaccharides constituting the matrix (Rousseau *et al.*, 1990). However, the extent and magnitude of *Phaeocystis* colony blooms significantly vary in the whole area and has been shown to be sustained by nitrate availability at the end of the early spring diatom bloom (Lancelot, 1995; Lancelot *et al.*, 1998).

The most visible harmful effect of *Phaeocystis* blooms is the deposition of thick layers of odorous foam on the beaches that constitutes an hindrance to recreational and tourist activities specially in some popular resorts (Fig. 3b; Lancelot *et al.*, 1987; Cadée, 1990). Exportation of organic material to the Wadden Sea, the stratified German and Danish coastal waters resulting in anoxic bottom waters is another adverse effects associated to the massive blooms of *Phaeocystis* colonies (Lancelot, 1995). Besides, *Phaeocystis* mucilage was shown to affect aquaculture by clogging the gills of fish and shellfish, affecting their feeding and reproduction (Pieters *et al.*, 1980). Indirect effects on fisheries by clogging the fishermen's nets (Grossel, 1985) or influencing fish migration (Savage, 1930) were also reported. The presence of anoxic sediment due to massive *Phaeocystis* colonies sedimentation and bacterial degradation was also shown to reduce fish nursery ground in some areas (Rogers & Lockwood, 1990). *Phaeocystis* colonies could also affect scientific work by clogging sampling net (Fig. 3c).

3. The genus Phaeocystis

Phaeocystis sp. belongs to the class of Prymnesiophyceae Hibberd, order Phaeocystales Medlin, family of Phaeocystaceae Lagerheim. It is characterized by an unusual heteromorphic life cycle wich alternates gelatinous colonies whose size vary from 10 μm to 2-3 mm (Fig. 3a), and different morphotypes of nanoplanktonic free-living cells (Rousseau *et al.*, 1994). *Phaeocystis* colonies are characterized by thousands of cells embedded in a mucilaginous matrix composed of exopolyssacharides secreted by the cells themselves (Lancelot & Mathot, 1987; Lancelot & Rousseau, 1994). This genus, whose success as blooming species is clearly associated to its colonial stage (Lancelot *et al.*, 1998), has an almost worldwide distribution. Recurrent spring blooms of *Phaeocystis* sp. have been reported from the Arctic (*e.g.* Barnard *et al.*, 1984, Wassmann *et al.*, 1990, Smith *et al.*, 1991, Cota *et al.*, 1994), the Antarctic (*e.g.* Palmisano *et al.*, 1986, Gibson *et al.*, 1990; Rogers and Lockwood, 1990; Smith *et al.*, 1998; DiTullio *et al.*, 2000), temperate (*e.g.* Lancelot *et al.*, 1987; Davies *et al.*, 1992; Cadée & Hegeman, 1986) and subtropical areas (Guillard & Hellebust, 1971, Estep *et al.*, 1984).

Despite its worldwide distribution and its significance as blooming species, there is still controversy about the taxonomy of the genus *Phaeocystis* due to the lack of true taxonomic criteria (Sournia, 1988). Some species were identified on the basis of their free-living cell stage such as *P. scrobiculata* (Moestrup, 1979), *P. cordata* and *P. jahnii* (Zingone *et al.*, 1999). Bloom-forming species recognized up to now are however differentiated on the basis of morphology of the colony, the chemical

composition and genome characteristics (Baumann *et al.*, 1994, Medlin *et al.*, 1994, Vaulot *et al.*, 1994). On the basis of these studies, it is generally admitted that the North Sea strain is *Phaeocystis globosa*, the northern strain is *P. pouchetii* and the Antarctic strain is *P. antarctica*.

Irrespective of the colony-forming species, the dynamics of *Phaeocystis* blooms, their growth and wane, is driven by the dominance of its colonial stage (Lancelot & Rousseau, 1994). The ability of *Phaeocystis* to take benefit from eutrophication or blooming in nutrient-rich environment, was attributed to its high ability of to utilize nitrate as nitrogen source under colonial stage (Riegman *et al.*, 1992; Smith *et al.*, 1991; Lancelot & Rousseau, 1994; Lancelot *et al.*, 1998).

The fate of *Phaeocystis* bloom depends on the environment and is determined by the physical and biological characteristics of the ecosystem (Wassmann, 1994; Weisse *et al.*, 1994). Although largely debated, it is generally admitted that *Phaeocystis* colonies are not grazed by mesozooplankton in shallow environments, deviating therefore the classical planktonic food web (Weisse *et al.*, 1994; Lancelot & Rousseau, 1994; Gasparini *et al.*, 2000). Complex changing planktonic food-webs were however evidenced with a complex microbial food-web initiated by microprotozoa actively grazing on *Phaeocystis* cells originating from disrupted colonies (Weisse & Scheffel-Möser, 1994). Part of the *Phaeocystis*-derived production is however resuming the classical food-web through mesozooplankton feeding activity on protozoa (Hansen *et al.*, 1993; Gasparini *et al.*, 2000; Rousseau *et al.*, 2000). Massive sedimentation of *Phaeocystis* blooms were observed both in shallow and deep environments (Wassmann *et al.*, 1990, Riebesell, 1993, Peperzak *et al.*, 1998). Its role in carbon sequestration through rapid and early export to deep water and sediment was particularly evidenced in the Ross Sea in the Southern Ocean (Smith *et al.*, 1998; DiTullio *et al.*, 2000). On the contrary, cell and colonial lysis with further bacterial degradation could be the major process in the shallow turbulent waters of the Southern Bight of the North Sea (Van Boeckel *et al.*, 1992; Brussaard *et al.*, 1995; Osinga *et al.*, 1997; Rousseau *et al.*, 2000).

4. Outline of this thesis

The different chapters presented in this thesis aim to contribute to the fundamental knowledge of the biology, ecology and eco-physiology of *Phaeocystis globosa*. The final objective of the thesis is to better understand the mechanisms behind eutrophication in the Southern Bight of the North Sea and, more particularly, in the Belgian coastal waters.

This manuscript is made up of two main parts. The first part (chapters 1 - 4) is devoted to a better knowledge of the biology and ecology of genus *Phaeocystis*. The second part (chapters 5 - 8) is dedicated to the role of *Phaeocystis* and to the spring phytoplankton succession in eutrophicated coastal areas of the Southern Bight of the North Sea.

The knowledge on *Phaeocystis* life cycle and mechanisms determining the occurrence of its different life forms in natural environments were reviewed and reappraised in

chapter 1. Particularly, the transition from the free-living to the colonial stage and its environmental control was studied on basis on microscopic observations and flow cytofluorimetry analysis conducted on natural populations and cultures.

The assessment of *Phaeocystis* abundance and biomass is essential for understanding the dynamics of the blooms and their ecological role. In chapter 2, we present conversion factors allowing to calculate *Phaeocystis* free-living cells and colony carbon biomass determined from microscopic observations and chemical analysis conducted on natural and cultures *Phaeocystis* strain.

The biogeographical distribution, global significance and ecophysiology of both free-living and colonial stages of *Phaeocystis* are examined in chapter 3. On basis of this extensive review, some key elements of the autoecology of *Phaeocystis* are presented and discussed through case studies.

The dominance of one form over the other in natural environments has dramatic consequences for planktonic and benthic ecosystem structure and functioning. As the most important morphological form occurring in the natural environment, the biological functioning and ecological role of *Phaeocystis* colonies are more specifically studied in chapter 4.

The eutrophicated Belgian coastal waters are characterized by *Phaeocystis* colony-dominated spring blooms succeeding to a moderate silicate-limited diatom growth and co-occurring with a late spring diatom community. A long-term monitoring of phytoplankton successions and their environmental control was undertaken in Belgian coastal waters with, as main objective, the assessment of the interannual variability of the diatom-*Phaeocystis* colony succession. Chapter 5 presents the results of this phytoplankton and environmental data time series.

Diatoms, requiring Si for their growth, are essential components of the spring phytoplankton community of the eutrophicated Belgian coastal waters. Three main diatom communities were identified during spring bloom. The silica requirement of these 3 diatom aseemblages was investigated on basis of silica cellular content and tracer experiments. This study, presented in chapter 6, suggests that silicate availability is a key factor for regulating the intrinsic diatom succession.

The dominance of the coastal ecosystem by *Phaeocystis* colonies affects the structure and functioning of the pelagic food web. Analysis of a carbon flow network of the planktonic system subdivided into its different trophodynamic groups, allows to estimate the trophic efficiency of the planktonic food web in the *Phaeocystis*-dominated ecosystem of the Belgian coastal waters. This analysis is presented in chapter 7.

Finally, an integrated research methodology combining field observations of *Phaeocystis* blooms and associated physico-chemical and biological variables, field and laboratory physiological studies of nutrient metabolism, and development of the mechanistic biogeochemical model MIRO is presented in chapter 8. This methodology was used for assessing trend in the eutrophication status of the Southern Bight of the North Sea.

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Chapter 1

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

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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:

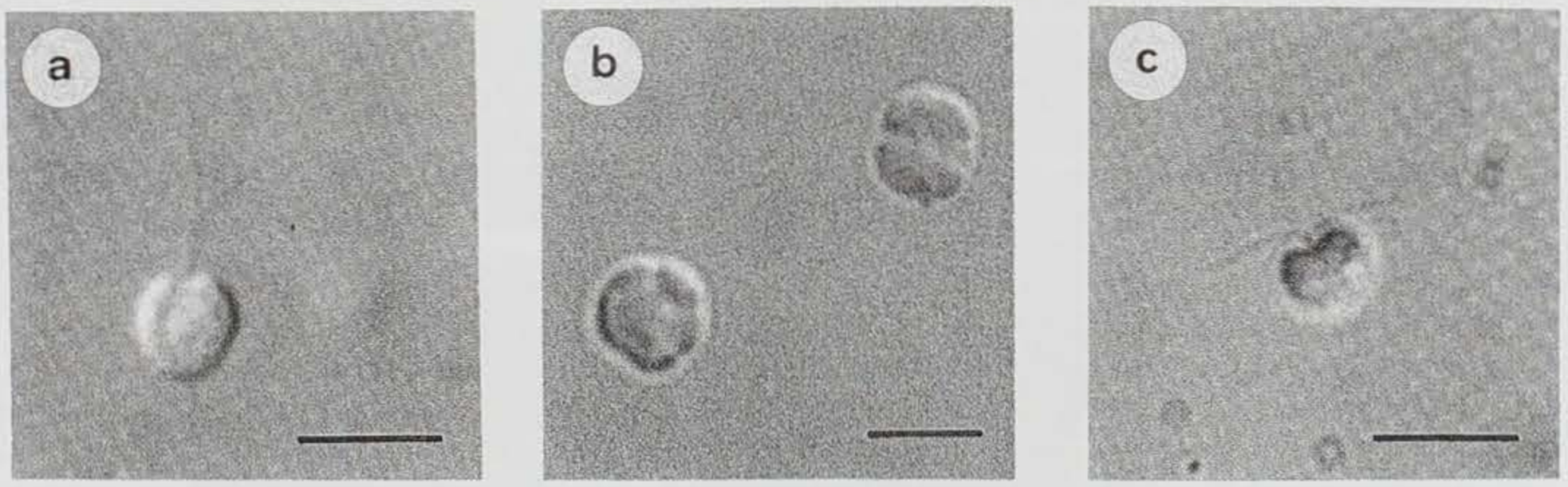

Fig. 1. Free-living cell types of *P. globosa*. Cells were fixed with glutaraldehyde 1% and viewed under Nomarski interference contrast. Scale bar = 5 μm. (a) Flagellate (swarmer) that appeared a few hours after the release of non-motile colonial cells due to colony disruption (strain ROSKO-A); (b) Non-motile 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

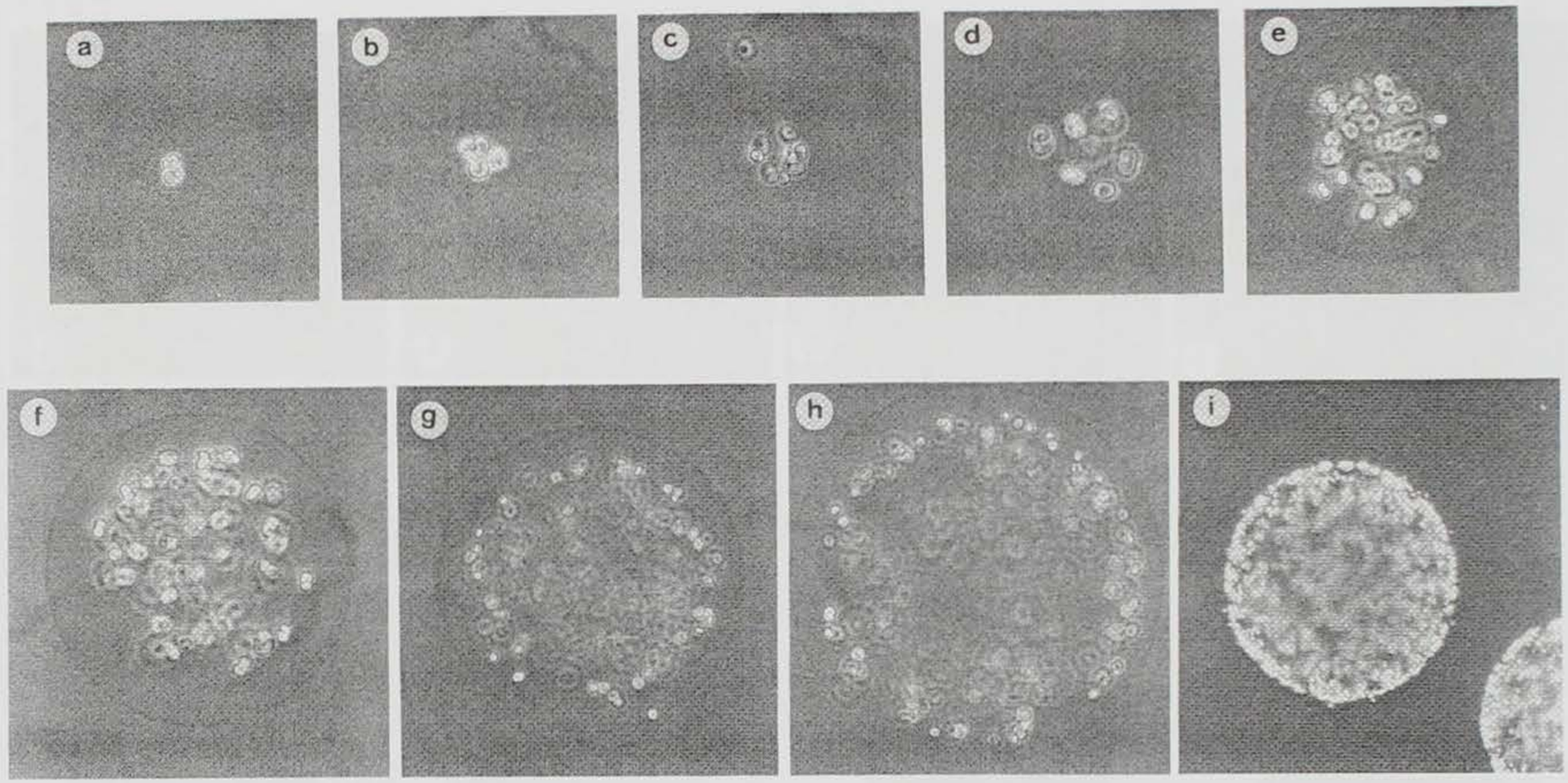

Fig. 2. Different development stages of *P. globosa* colonies (strain from Plymouth Culture Collection). Culture conditions: inoculum with colonial cells released from their matrix by mechanical disruption; culture medium of Veldhuis and Admiraal's (1987) with NO_3^-, NH_4^+ and PO_4^{3-} concentrations: 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).

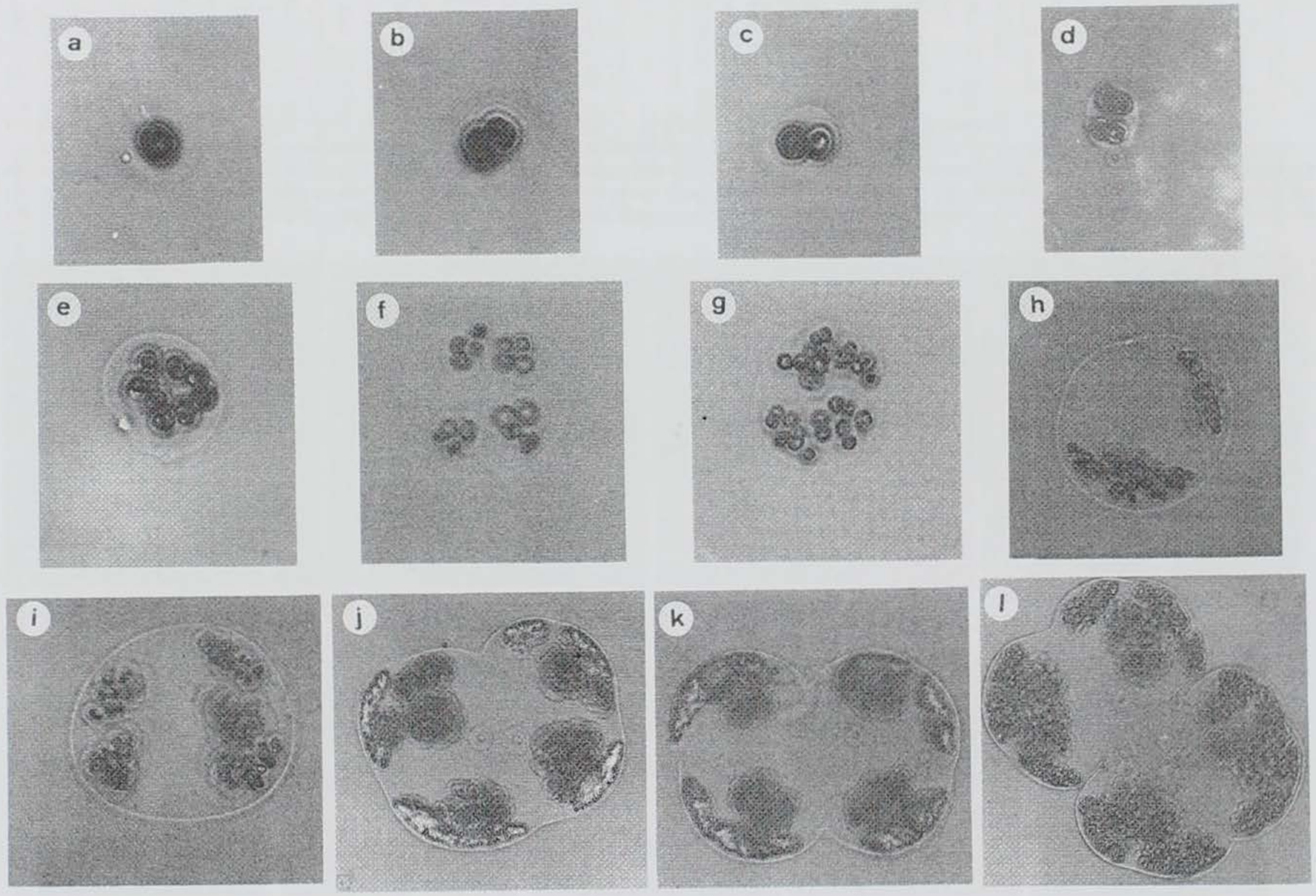

Fig. 3. Sequential development of *P. pouchetii* (strain isolated from the Greenland Sea) from free-living cells to cloud-like colonies. The characteristic grouping of cells within the mucilaginous matrix is clearly visible since the 16-cell stage (picture f). Culture conditions: inoculum with free-living 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

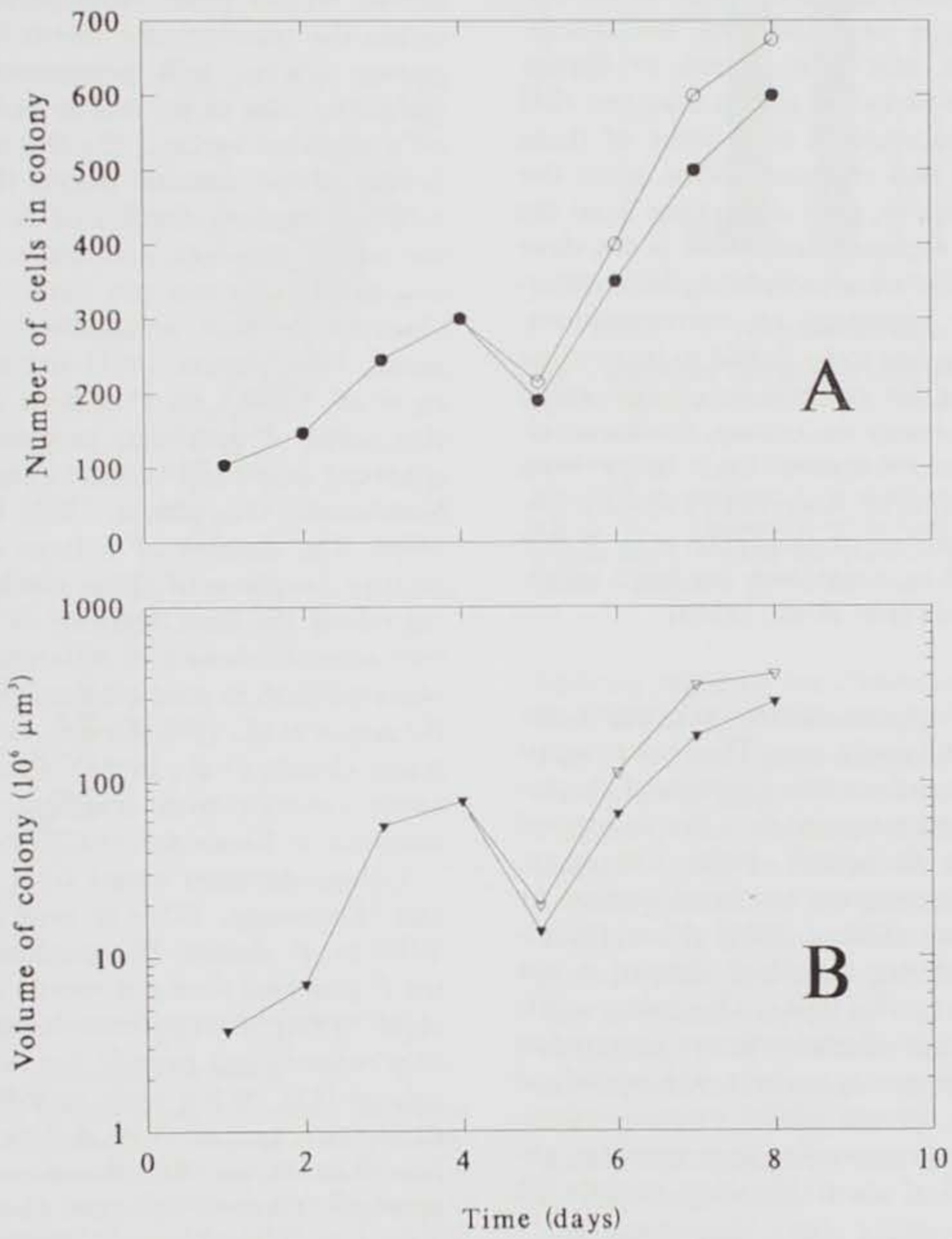

Fig. 4. Cleavage of a large colony into two daughters, each containing nearly the same number of cells. (A) Colony cell number and (B) Colony volume. Sum of volumes of daughter colonies is about 40% of the volume of the mother colony as an indirect evidence of colony matrix loss following colony cleavage. 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

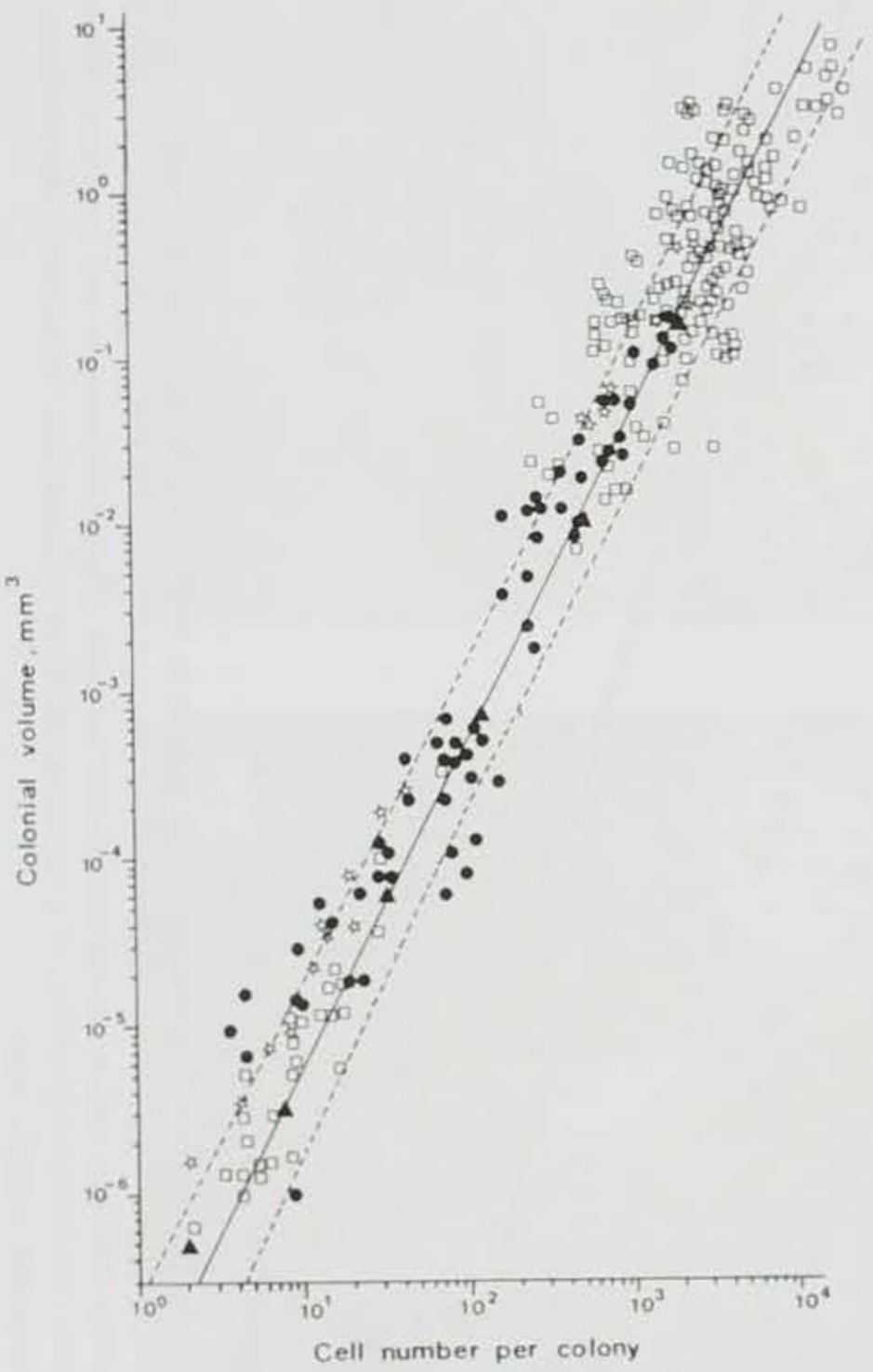

Fig. 5. Relationship between colony cell number and colony volume established for different *Phaeocystis* strains and compared with the regression line and its confidence interval at 99% calculated for a growing *P. globosa* culture (Rousseau et al., 1990); ▲ *P. globosa* (Kornmann, 1955), □ *P. globosa* (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.

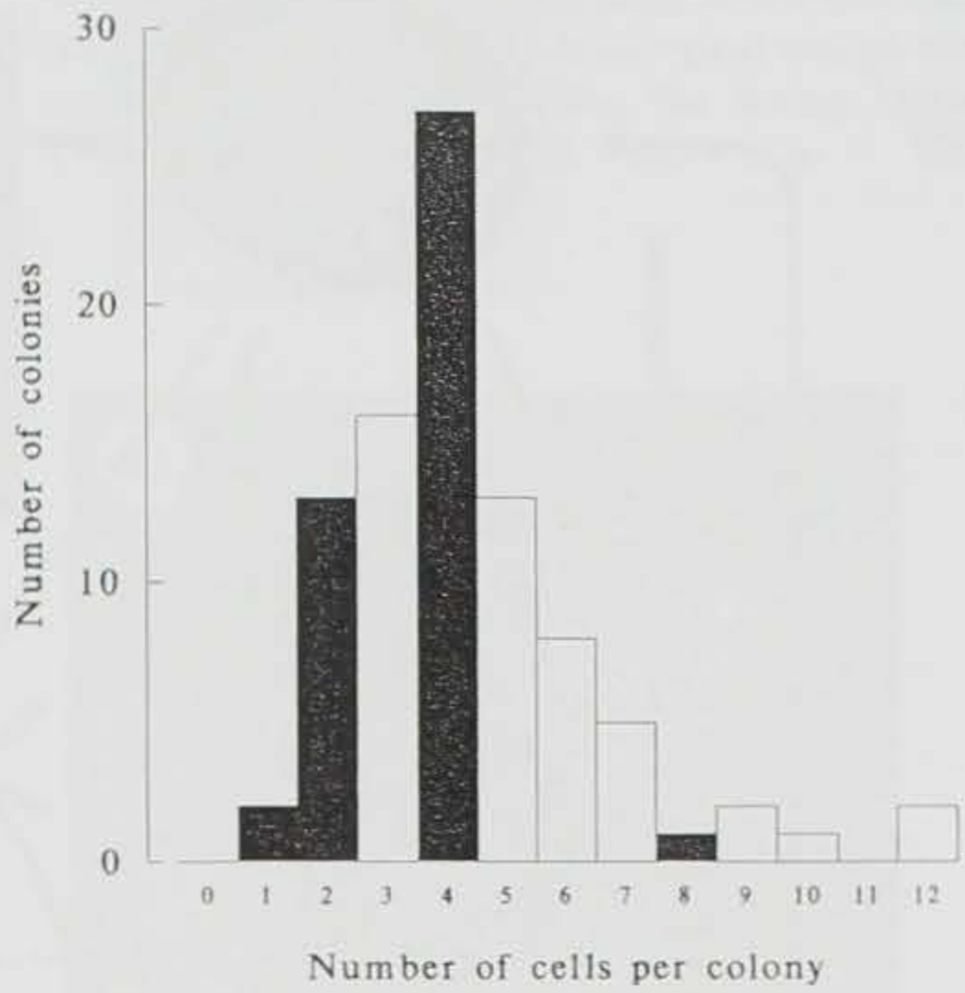

Fig. 6. Frequency histogram of cell number per colony in a *P. globosa* (strain PCC540) culture. The dark bars correspond to the numbers of cells per colony expected for synchronous cell division. Culture conditions: in K culture medium (Keller et al., 1987); temperature: 13°C; continuous illumination 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.

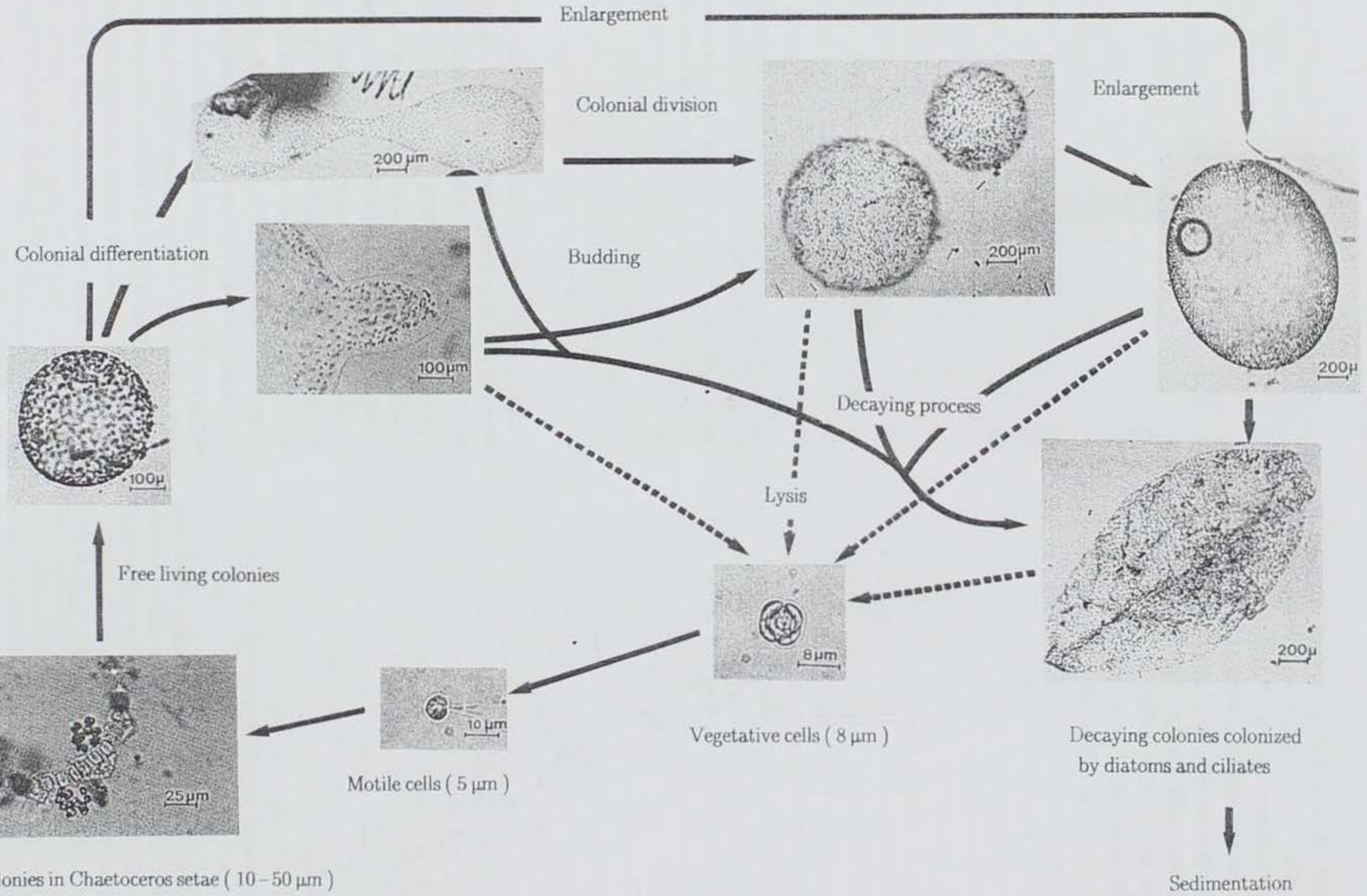

Fig. 7. Sequence of events occurring during a spring bloom of *P. globosa* in 1988 in the Belgian coastal waters of the North Sea as identified by a microscopic morphometric analysis of free-living cells and colonies. Surface seawater samples were collected 2–3 times/week at station N 51°26.05; E 002°49.08 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

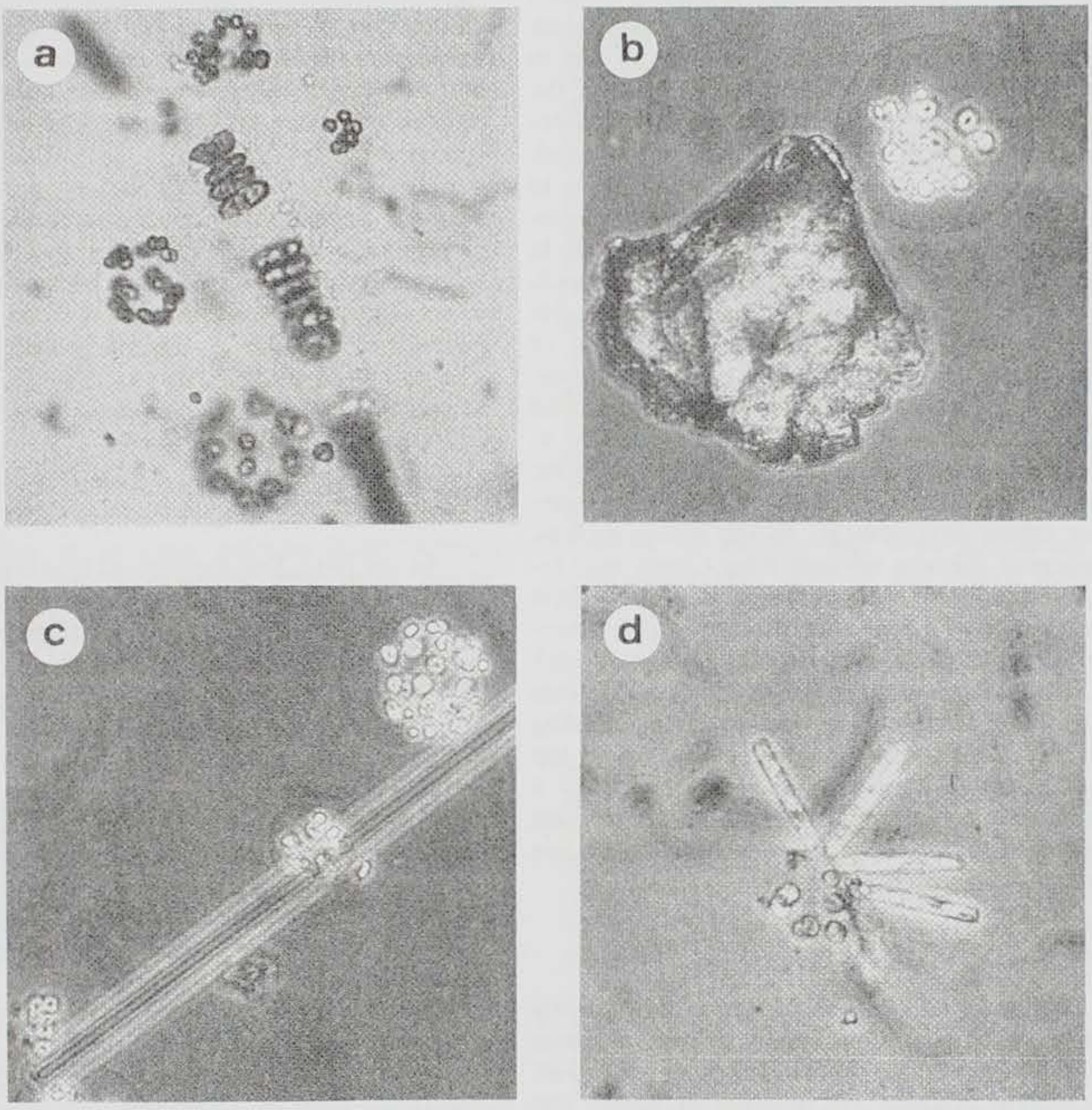

Fig. 8. Young spherical *P. globosa* colonies less than 50 μm in diameter attached to different solid substrates: (A) on *Chaetoceros* sp. setae as observed in the Belgian coastal waters during the early stage of the spring bloom 1988 (methods as in Fig. 7). (B) on living diatom *Asterionella* sp. (C) 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

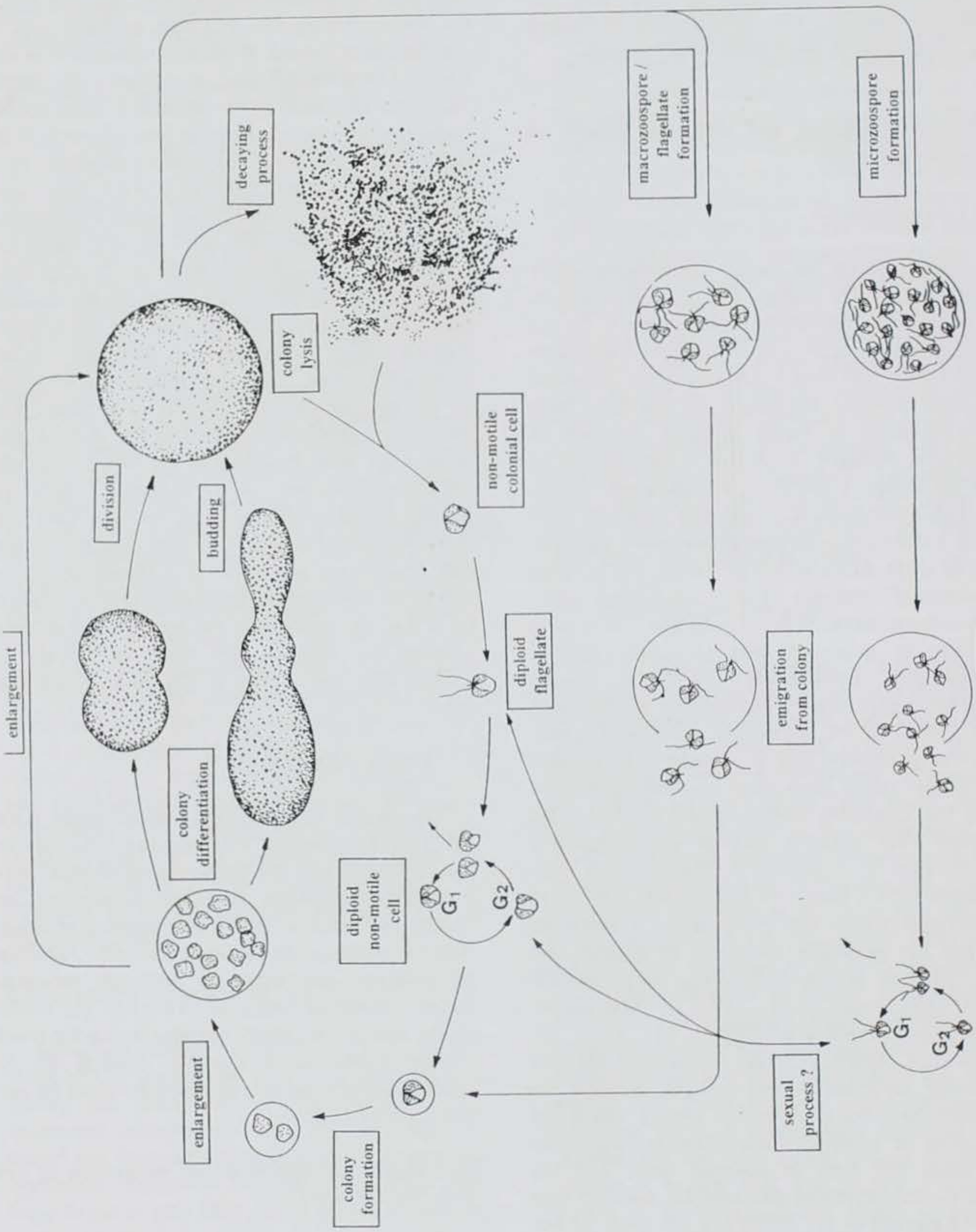

Fig. 9. Current status of *P globosa* life cycle as compiled from culture and field observations.

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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Chapter 2

Calculating carbon biomass of Phaeocystis sp. from microscopic observations

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Marine Biology

Calculating carbon biomass of *Phaeocystis* sp. from microscopic observations

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Abstract. Conversion factors for calculating carbon biomass of *Phaeocystis* sp. colonies and free-living cells were determined from microscopic observations and chemical analysis conducted on cultured and natural *Phaeocystis* sp. populations originating from the Southern Bight of the North Sea in 1986 and 1987. They allow calculation, in terms of carbon biomass, of the different forms of *Phaeocystis* sp. that succeed each other when the population is growing, on the basis of microscopic observations. The latter include enumerations of free-living cells (flagellated and non-motile) and colonies, as well as colonial biovolume measurement. Specific application to natural populations from Dutch coastal waters during spring 1986 shows that more than 90% of *Phaeocystis* sp. carbon biomass is under colonial form, most of it exceeding the grazing characteristics of current zooplankton at this period of the year. Detailed analysis of seasonal changes shows in addition that the size of the colonies greatly increases during the course of *Phaeocystis* sp. flowering, reaching sizes as high as 1 mm diameter at the top of the bloom when nutrients are depleted. Physiologically this corresponds to an enhanced synthesis of mucilaginous substances, with the decrease of available nutrients leading to an increasing contribution of the matrix to the total colonial carbon during the course of the bloom. Carbon content of *Phaeocystis* sp. colonies therefore greatly varies with their size, ranging from 0.3 to 1430 ngC colony^{-1}.

Introduction

Phaeocystis sp. is a very widespread phytoplanktonic alga that massively blooms mainly in temperate (Gieskes and Kraay 1975, Cadée and Hegeman 1986, Lancelot et al. 1987) and polar waters (Kashkin 1963, El-Sayed et al. 1983, Chang 1984, Eilertsen and Taasen 1984, Palmisano et al. 1986, Davidson and Marchant 1987). This prymnesiophycean is characterized by a complex polymorphic life cycle and occurs under at least two different morphological stages: unicellular and colonial. The former is characterized by free-living cells of 3 to 8 μm either flagellated or non-motile. The latter is composed of cells devoid of flagella, embedded in a mucilaginous matrix mainly composed of polysaccharides (Chang 1984, Lancelot unpublished data). These colonies originate either from one motile single cell that loses motility, secretes its mucilaginous substances (Kornmann 1955, Lancelot and Mathot 1985) and divides inside the colony or, later in the course of the life cycle, from true colonial division (Kornmann 1955, Verity et al. 1988). Colony size varies therefore by more than two orders of magnitude during the course of their development, ranging from 10 μm to 3 mm under natural conditions. Their cellular content varies accordingly, from 2 to about 10 000 cells, and their shape greatly changes with age (Bätje and Michaelis 1986). The two morphological forms cohabit in natural environments but feed different planktonic organisms: single cells are ingested by protozoa like tintinnids and other ciliates (Admiraal and Venekamp 1986), whereas colonies in the 10 to 300 μm size range can sometimes be grazed by zooplankton, depending on the species and its development stage (Weisse 1983, Daro 1985, Verity and Smayda 1989). Ungrazed, large colonies deposit onto the bottom (Wassman 1984, Jenness and Duineveld 1985), cover beaches as layers of seafoam (Bätje and Michaelis 1986, Lancelot et al. 1987), or are degraded in the water column by planktonic bacteria (Billen and Fontigny 1987).

Regarding the trophic rôle of *Phaeocystis* sp., and its importance as a dominant species in eutroph environments, either natural like Antarctic waters or anthropogenically influenced like the well known eutrophicated North European continental coastal zones, the accurate determination of single cell and colony numbers together with their respective carbon content is of prime importance for understanding of the dynamics of *Phaeocystis* sp. blooms.

Unfortunately, there is still presently no unifying criteria for accurately assessing *Phaeocystis* sp. abundance. This is explained by the lack of adequate proce-

dures for the sampling and preservation of intact colonies and for the mechanical separation of free-living cells and colonies. Current literature reports *Phaeocystis* sp. as single cell numbers (Eilertsen et al. 1981, Cadée and Hegeman 1986, Veldhuis et al. 1986, Weisse et al. 1986), as chlorophyll *a* (Gieskes and Kraay 1975, Cadée and Hegeman 1986, Lancelot and Mathot 1987) or as colony numbers (Jones and Haq 1963, Bätje and Michaelis 1986). The first two methods, because they do not distinguish between unicellular and colonial forms, greatly overestimate *Phaeocystis* sp. single cell abundance when colonial forms are predominant, as is usual in natural environments (Lancelot et al. 1987). Indeed chlorophyll *a* concentrations refer to both colonial and free-living *Phaeocystis* sp. cells as isolated by classical filtration procedures, whilst cell counts from classical Lugol's iodine or formalin preserved samples enumerate colonial cells as well because these preserving agents dissolve the mucilaginous matrix (Chang 1984, Admiraal and Venekamp 1986). Colony enumeration, on the other hand, is of minor ecological interest when not combined with colonial size and cell content evaluation.

An important step was, however, recently achieved by Davidson (1985) and Davidson and Marchant (1987), who established an experimental procedure for the estimate of free-living and colonial cell numbers. These authors not only used a suitable preserving agent for colonial forms but established a log/log regression between the diameter of *Phaeocystis* sp. colonies growing in the Southern Ocean and their number of cells. This relationship allows the assessment of the cell content of *Phaeocystis* sp. colonies from the knowledge of their size. From this, carbon content of colonies on the one hand, and of free-living cells on the other hand, should now be estimated provided the above relationship is general for *Phaeocystis* sp. and the conversion factors for the transformation of individual cells and mucilaginous matrix are determined.

In the framework of a joint EEC Research Project on the dynamics of *Phaeocystis* sp. blooms in the North European continental coastal zones of the North Sea, we have determined conversion factors that allow one to easily calculate *Phaeocystis* sp. single cell and colony biomass on the basis of single cell and colony counts and colonial volume measurements. These factors were statistically determined by combining numerous microscopic observations including cellular and colonial volume measurements and chemical and biochemical analysis. They were established from both pure cultures and field communities dominated at more than 95% by *Phaeocystis* sp. Ecological interest of this methodology is given by application to pure culture and field populations of *Phaeocystis* sp. at different stages of their development.

Materials and methods

Phaeocystis sp. samples

Phaeocystis sp. samples originated from both field populations and unialgal cultures. Field populations were sampled in Dutch and Belgian coastal waters respectively during spring 1986 and 1987 and

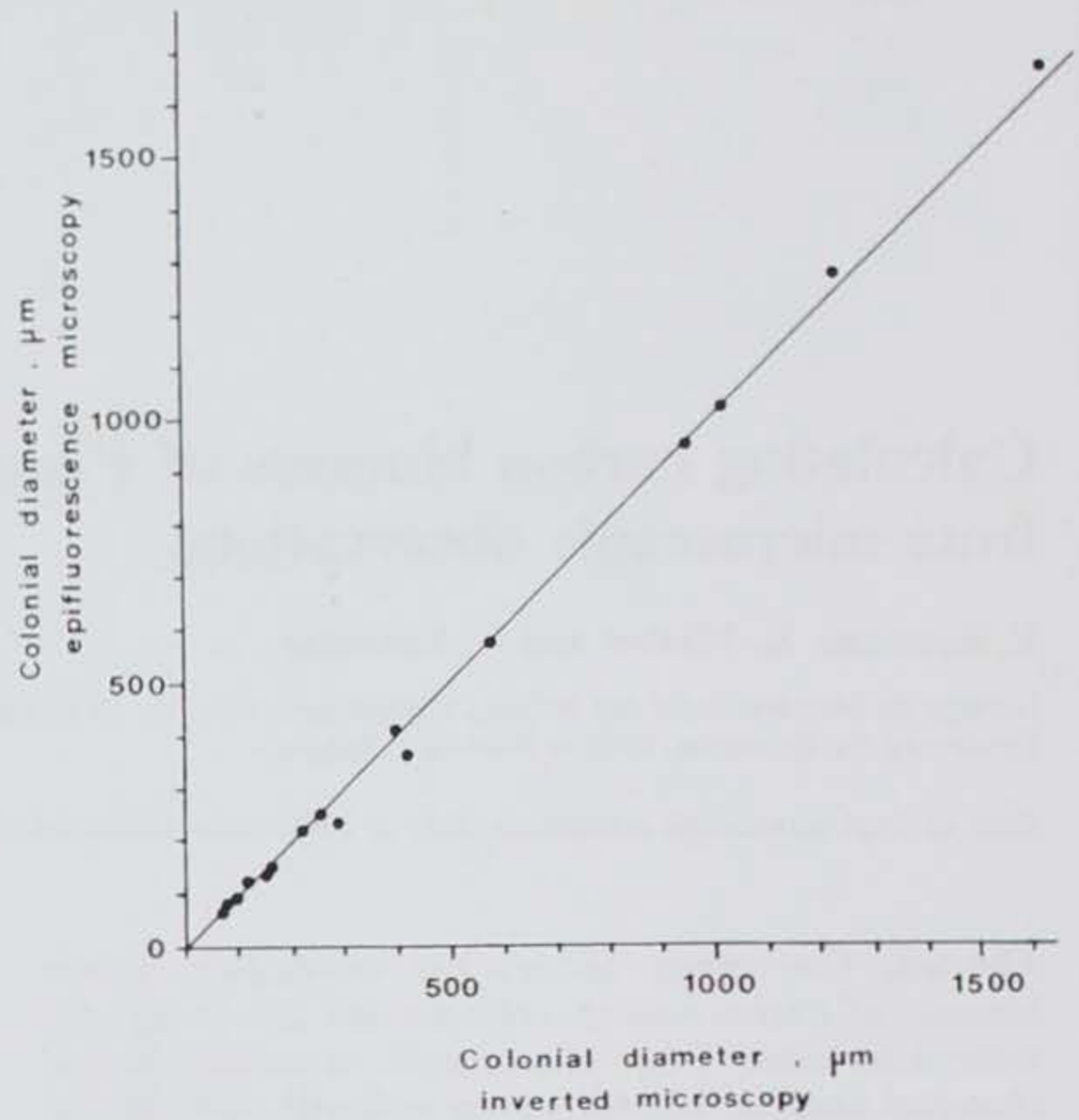

Fig. 1. *Phaeocystis* sp. Comparison between colony diameters measured by inverted and epifluorescence microscopy ($r^2 = 0.99$; $n = 16$; $y = 1.03\ x$)

were directly fixed for microscopic analysis. Subsamples were treated for chlorophyll *a* and dissolved organic carbon (DOC) analysis according to methods described below.

Cultures of *Phaeocystis* sp. colonies were first inoculated with a suspension of free-living cells. The latter had previously been obtained by gentle filtration through a 10 µm sterile net of unialgal *Phaeocystis* sp. culture originating from the Channel (strain from Plymouth Marine Laboratory, UK). Culture medium was prepared with sterile, filtered seawater enriched as in Veldhuis and Admiraal (1987) except for nitrate, ammonium and phosphate whose concentrations were respectively 50, 25 and 5 µ*M*. Cultures were grown at 11 °C in an illuminated growth cabinet (Luminincube II, Analys) under a 12 h light : 12 h dark cycle at 120 µE m^{-2} s^{-1}. Subsampling for microscopic and chemical (DOC and chl *a*) analysis was performed at short intervals during 2 wk in order to follow appearance of colonies and change in colony and single cell numbers.

Microscopic analysis

Cell and colony enumerations and biovolume measurements were carried out either by inverted (Leitz Fluovert) or epifluorescence microscopy (Leitz Laborlux D). The two methods are highly comparable as shown in Fig. 1 which compares the diameters of a large range of colony sizes measured by inverted and epifluorescence microscopy. Experimental procedures were the following: (1) Samples for inverted microscopy were preserved in glass bottles with a glutardialdehyde-lugol (35‰, v/v) solution (1 ml for 100 ml seawater) as prepared according to Thomas (personal communication) and stored in the dark at 4 °C. Fixed samples (10 ml) were sedimented for 12 h in Utermöhl plankton chambers. (2) Samples for epifluorescence microscopy (2 to 10 ml seawater) were stained with acridine orange solution as recommended by Hobbie et al. (1977). Colonies were sampled with open-end pipettes (Sterilin), collected on black-stained 0.2 µm filters using a gentle vacuum in order to avoid colony disruption. After filtration, filters were placed on microscope slides and stored in the dark at 4 °C until analysis.

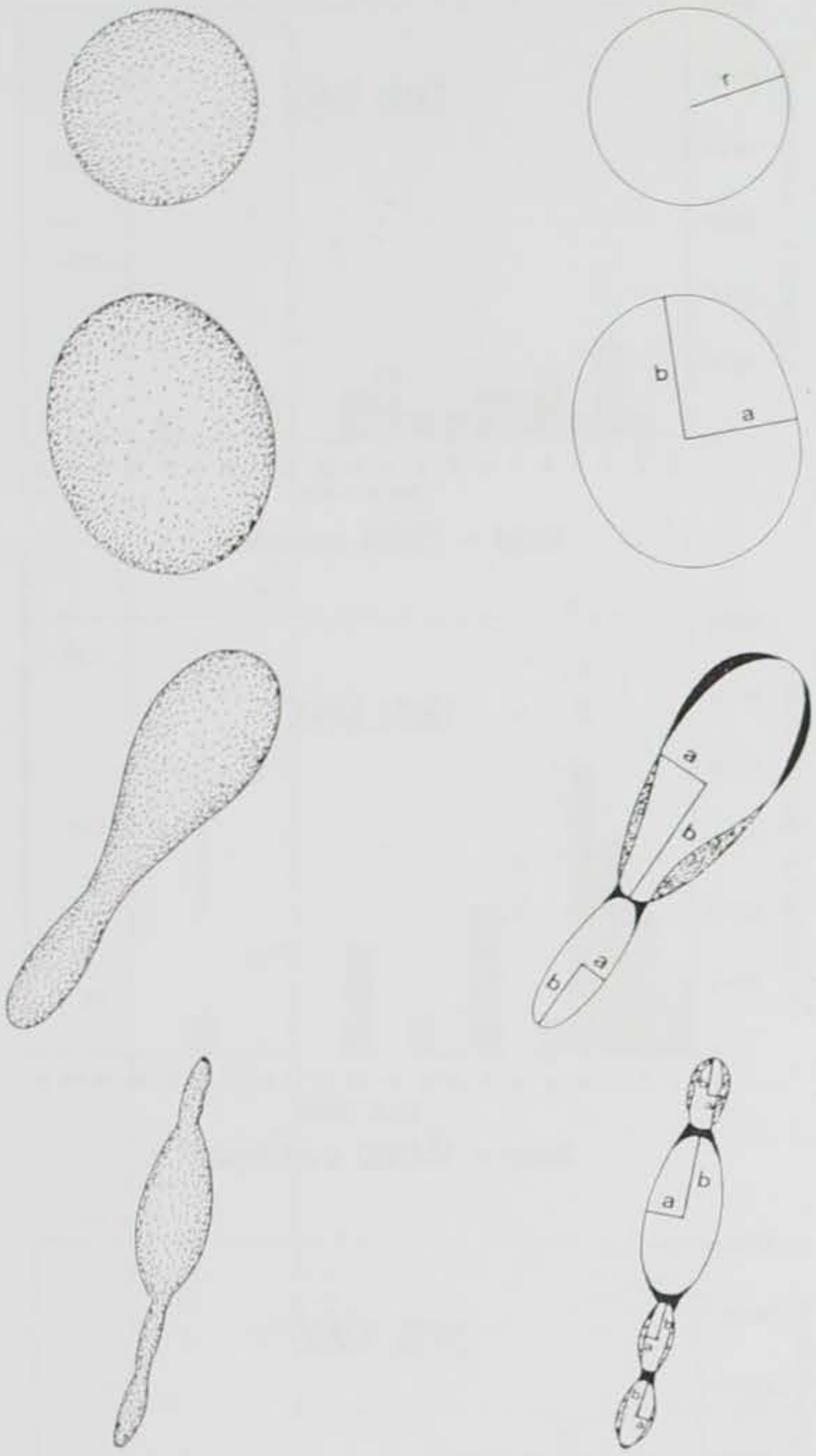

Fig. 2. *Phaeocystis* sp. Typical shapes of colonies observed in culture and field populations and their transformation in simplified geometrical forms. Volume over-estimations (stippled areas) and under-estimations (filled areas) are equivalent. Dimensions to be measured are also indicated. Ellipsoids are considered as rotating around their major axis; their volumes are then calculated by $V=4/3\,\pi\,a^2\,b$

In each case, all colonies ($n=10$ to 150) were enumerated and their size measured with a precision of $\pm 0.5\,\mu$m. Single cells were counted on several (10 to 30) randomly chosen fields (10 to 50 cells field^{-1}). Colony cell content determination was performed only by epifluorescence microscopy. Indeed, this method allows easy enumeration of stained cells because of their two-dimensional location. Cell and colony sizes were estimated visually by comparison to a calibrated grid. Cell volume was calculated by considering *Phaeocystis* sp. cells as ellipsoids, whilst colonial volume was calculated by considering colonies as spheres, ellipsoids or an arrangement of both. The hypothesis was formulated that ellipsoids are elongated, i.e. rotating around their major axis. Typical shapes of *Phaeocystis* sp. colonies that can be observed in culture and field populations and their transformation in a *collage* of geometrical forms are illustrated in Fig. 2.

Chemical analysis

Samples for chlorophyll *a* and DOC analysis were collected by filtration of small volumes on pre-ashed fiberglass filters (Whatman GF/C) using a high vacuum pressure in order to achieve complete colony disruption. Chlorophyll *a* was measured either by spectrophotometry (Lorenzen 1967) or by fluorescence (Yentsch and Menzel 1963) and dissolved organic carbon was determined by a persulfate, UV assisted wet oxidation, followed by infrared determination of CO_2 using Dohrmann 180 equipment.

Conversion factors

Relationship between colonial cell number and biovolume

The relationship between the colonial volume and cell number per colony was established on the basis of microscopic observations performed during the development of a pure *Phaeocystis* sp. culture, initially inoculated with single cells. Fig. 3 shows the size distribution of colonies at different stages of culture growth together with the change in colony number. Colony size, expressed in equivalent spherical diameter unit, increased with the age of the culture from 10 to 2000 μm. At the same time, a progressive change in dominant shapes from spheres to ellipsoids and irregular forms was observed. Detailed examination of temporal change in colony size and number clearly indicated that colonies are not only continuously initiated by free-living cells but also originate from the division of colonies themselves, in perfect agreement with Verity et al.'s (1988) data.

Fig. 4 shows a highly significant log/log relationship ($r^2=0.95$, $n=244$) between colonial volume and cell number per colony for the large range of colony sizes and shapes illustrated in Fig. 3. This relationship allowed us to estimate the cell content C of a colony from the knowledge of its volume V expressed in mm^3, according to the following equation:

$$\log C=0.51 \log V+3.67.$$

The slope of the regression line indicated that the relative importance of the mucilaginous matrix with regard to the cell number was increasing with the size of the colony.

The zero ordinate of the regression line, on the other hand, estimated at 8 μm the minimum size of a starting colony from a single free-living cell.

Validation of this empirical relationship and its applicability to *Phaeocystis* sp. colonies from several origins were examined in Fig. 5 where similar data relative to both natural populations and unialgal strains isolated from temperate and polar waters are compared with the regression line and its 99% confidence interval as calculated from data illustrated in Fig. 4. Examination of Fig. 5 suggests that the empirical log/log relationship is valuable for *Phaeocystis* sp. colonies from several origins. The higher deviation to the regression line for Southern Bight field data might be explained by the presence at the top of the *Phaeocystis* sp. bloom of large colonies characterized by highly diversified shapes whose geometrical form is sometimes difficult to define properly.

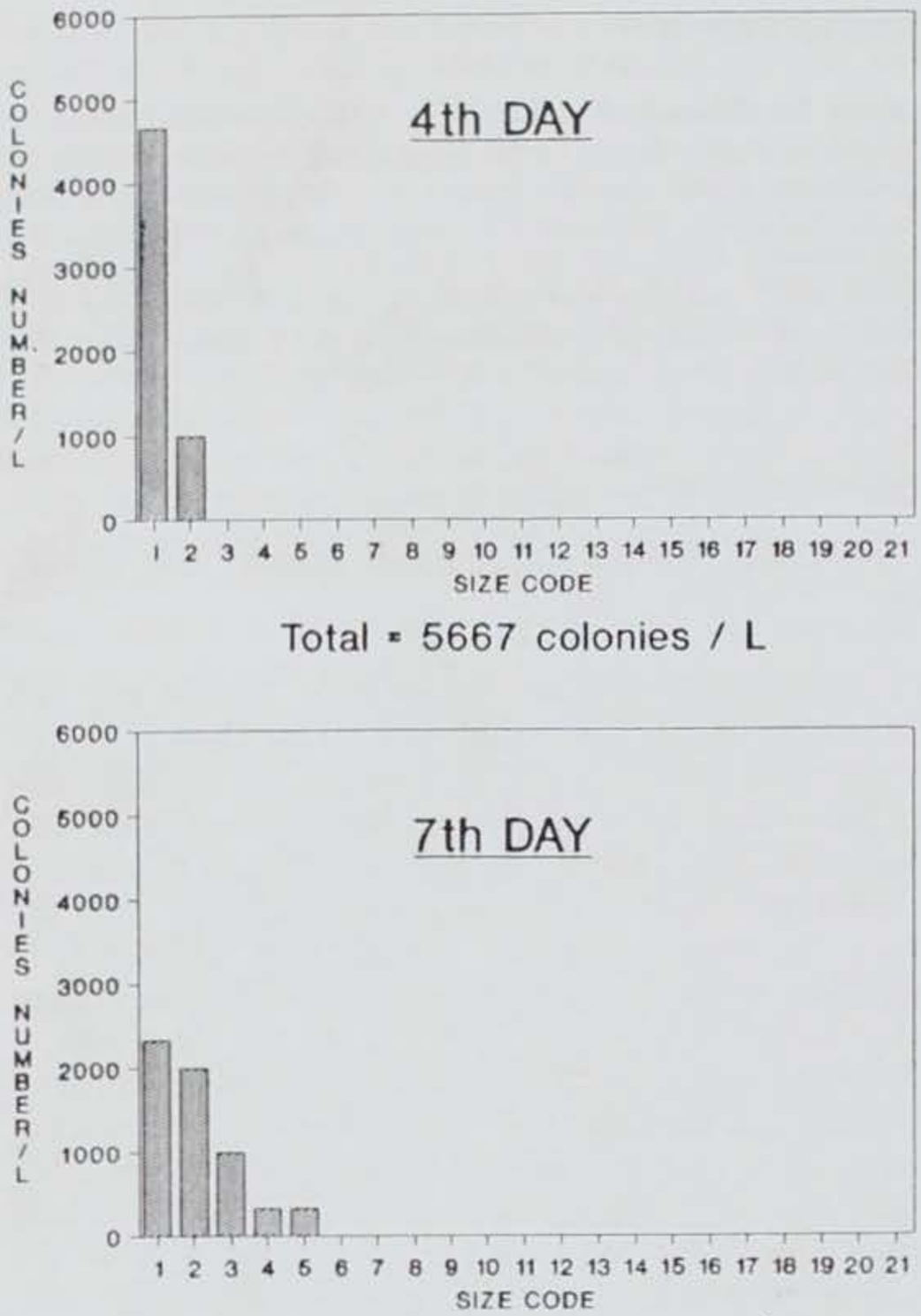

Fig. 3. *Phaeocystis* sp. Size distribution of colony number at six different stages of development of a pure culture. Sizes are expressed in terms of equivalent spherical diameter. Colonial diameter size code is as follows: (1) <50; (2) 50–100; (3) 100–200; (4) 200–300; (5) 300–400; (6) 400–500; (7) 500–600; (8) 600–700; (9) 700–800; (10) 800–900; (11) 900–1000; (12) 1000–1100; (13) 1100–1200; (14) 1200–1300; (15) 1300–1400; (16) 1400–1500; (17) 1500–1600; (18) 1600–1700; (19) 1700–1800; (20) 1800–1900; (21) 1900–2000 µm

Carbon content of free-living and colonial cells

Two procedures were used and compared to determine conversion factors for calculating carbon content of *Phaeocystis* sp. free-living and colonial cells from microscopic counts.

The most classical one is based on the determination of plasma volume from cell volume measurement and its transformation to carbon using the conversion factor 0.11 (pgC μm^{-3}) recommended by Edler (1979) for flagellates. Table 1 reports average value and standard deviation of *Phaeocystis* sp. cell carbon content calculat-

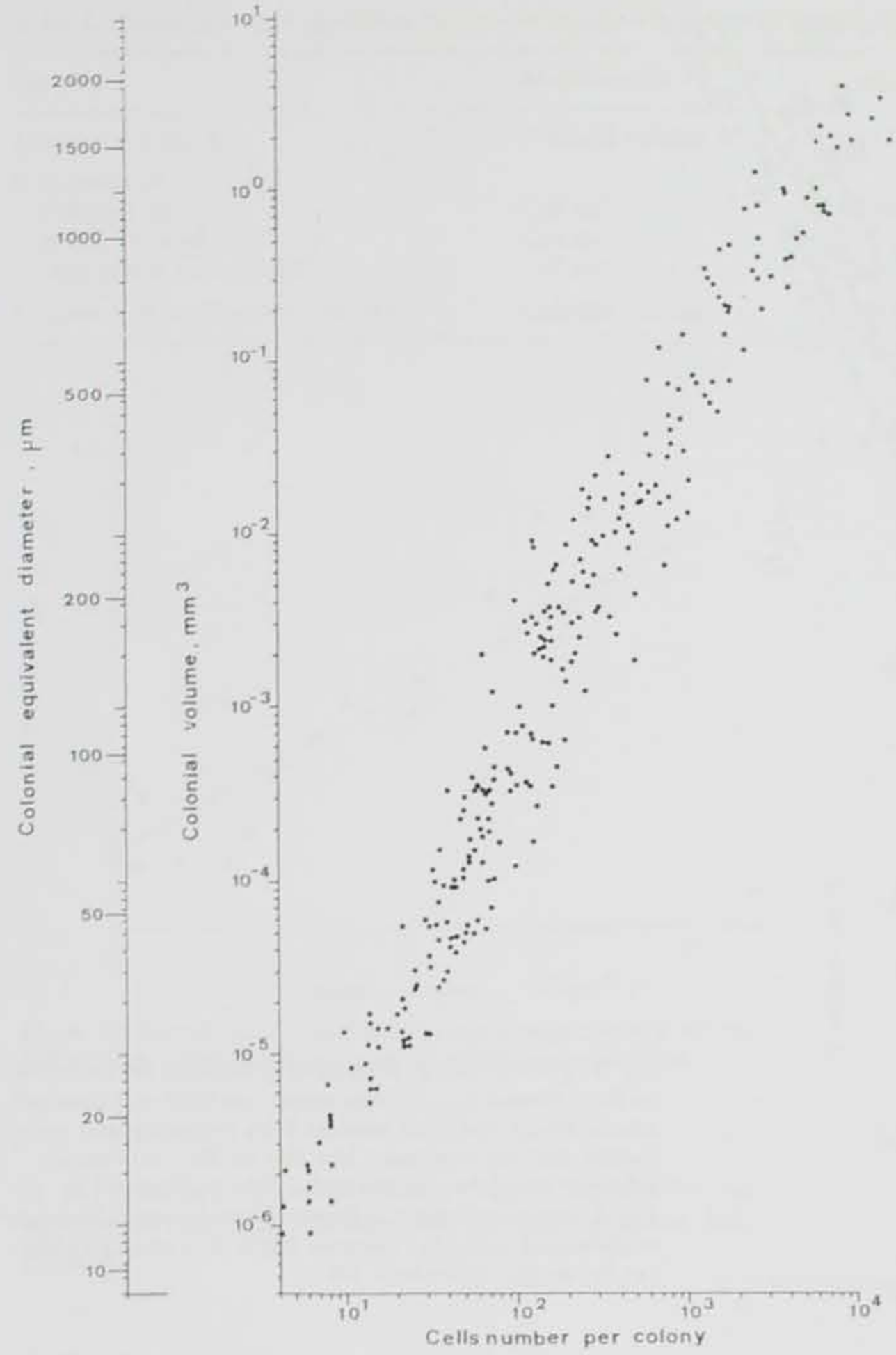

Fig. 4. *Phaeocystis* sp. Relationship between colonial volume and cell number per colony for the large range of colony sizes and shapes sampled during the batch culture development illustrated in Fig. 3. Colonial equivalent spherical diameter scale is indicated on left of volume scale

Table 1. *Phaeocystis* sp. Cell carbon content (pgC cell^{-1}) calculated from cell volume measurements

Cells	No. of observations	C content ±SD
Colonial cells	220	14.15±5.34
Free-living cells		
Flagellated	85	10.80±3.47
Non-motile	260	15.91±4.86

ed from cellular volumes relative to a large number of *Phaeocystis* sp. cells originating from pure culture and sampled in the Belgian coastal waters in spring 1988 and 1989, at different stages of the spring bloom development (Rousseau unpublished data). Examination of Table 1 shows that carbon associated with non-motile free-living cells is very close to that associated with colonial cells indicating their colonial origin. On the other hand, mean carbon content of flagellated single cells is lower, as expected from their smaller size.

The second procedure estimates a factor for the conversion of cell counts to carbon unit on the basis of the statistical regression analysis between biochemical carbon and cell number. Biochemical carbon is defined by the cell content in proteins, polysaccharides and lipids and is calculated from chlorophyll *a* concentrations using a 29 C:chl*a* (w:w) conversion factor as recommended in Lancelot-Van Beveren (1980) when *Phaeocystis* sp. dominates the community. This procedure does not discriminate, however, between colonial and free-living cells, as chlorophyll *a* concentrations refer to the whole population isolated by filtration. This statistical analysis was performed on *Phaeocystis* sp. populations sampled in the Belgian and Dutch coastal zones for different stages of the bloom development. The slope of the regression line estimates at 13.5±1.8 pg the *Phaeocystis* sp. cell carbon content ($r^2=0.81$, $n=29$, $p<0.01$). Good agreement was observed between the latter value and the carbon content of colonial and non-motile free-living cells calculated by means of the plasma volume method.

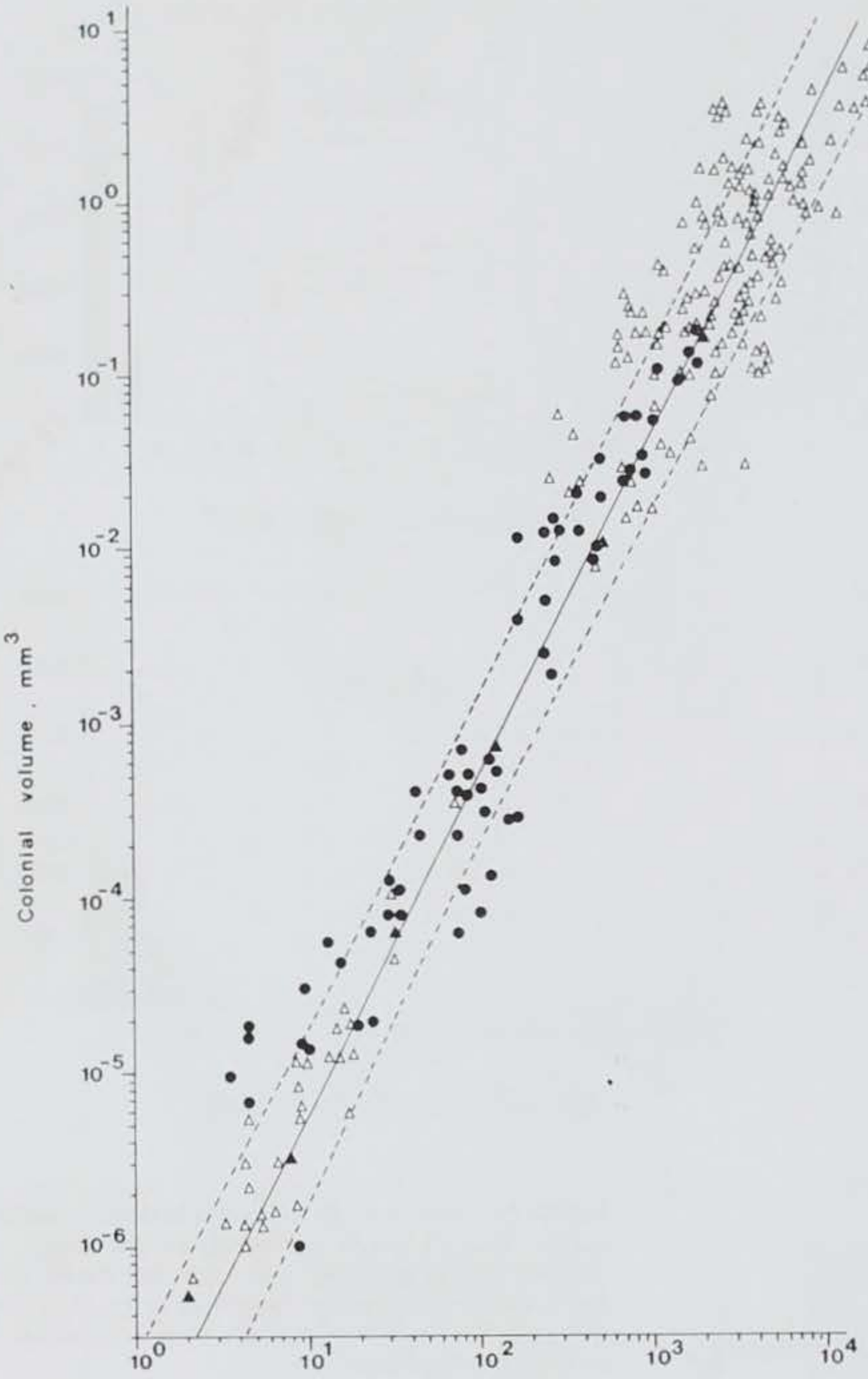

Fig. 5. *Phaeocystis* sp. Comparison between data relative to both natural populations (open symbols) and unialgal strains (filled symbols) isolated from temperate and polar waters, and the regression line and its 99% confidence interval calculated on the basis of data illustrated in Fig. 4. Data are from: (△) Belgian coastal waters (Rousseau unpublished data); (▲) German Bight (Kornmann 1955); (●) Prydz Bay (Davidson 1985)

Carbon content of mucilaginous matrix

Mucilaginous substances that compose the colonial matrix are currently separated from cells by vacuum filtration at high pressure through glassfiber (Whatman GF/C) filters (Lancelot and Mathot 1985). This mechanical procedure disrupts the colonies and solubilizes the colonial matrix into seawater. However, under these conditions, the dissolved mucilaginous compounds arising from living colonies cannot be distinguished from DOC of other origin without laborious chemical treatment including desalting, concentration of organic matter by dialysis and chemical precipitation of the mucilaginous substances.

This chemical procedure, unsuitable for routine analysis, was bypassed by utilizing statistical regression analysis between DOC concentration – a variable characterizing the total pool of dissolved organic substances – and the total colonial volume – a variable specific of the colonies. This statistical analysis was applied on several couples of data originating from both pure *Phaeocystis* sp. cultures and field communities dominated at 95% (in terms of cell number) by *Phaeocystis* sp. colonies. DOC data were, however, previously corrected for a background DOC value due either to culture medium initially enriched with vitamins or to refractory dissolved organic matter always present in natural seawater. The former has an average value of 2.8 ± 0.4 mgC l^{-1} whilst the latter reaches a mean background value of 1.1 ± 0.5 mgC l^{-1} in early spring in the coastal waters of the Southern Bight of the North Sea.

The relationship between DOC and total colonial volume of *Phaeocystis* sp. is illustrated in Fig. 6. A good correlation ($r^2 = 0.74$, $n = 24$, $p < 0.01$) is observed between total colonial volume of *Phaeocystis* sp. and its organic carbon content. The positive Y-intercept observed in Fig. 6 is due to the high variability of the background value. The slope of the regression line estimates

Table 2. *Phaeocystis* sp. Conversion factors for the determination of carbon biomass

Variable	Measurement	Conversion	Source
Colonial cell no. C	Colonial volume V	$\log C = 0.51 \log V + 3.67$	This paper Fig. 4
C biomass of			
Colonial cell	Cell no.	14.2 pgC cell^{-1}	Edler conversion factor (1979)
Motile isolated cell	Cell no.	10.8 pgC cell^{-1}	Edler conversion factor (1979)
Non-motile isolated cell	Cell no.	15.9 pgC cell^{-1}	Edler conversion factor (1979)
C content of mucilaginous matrix	Colonial volume	335 ngC mm^{-3}	This paper Fig. 6

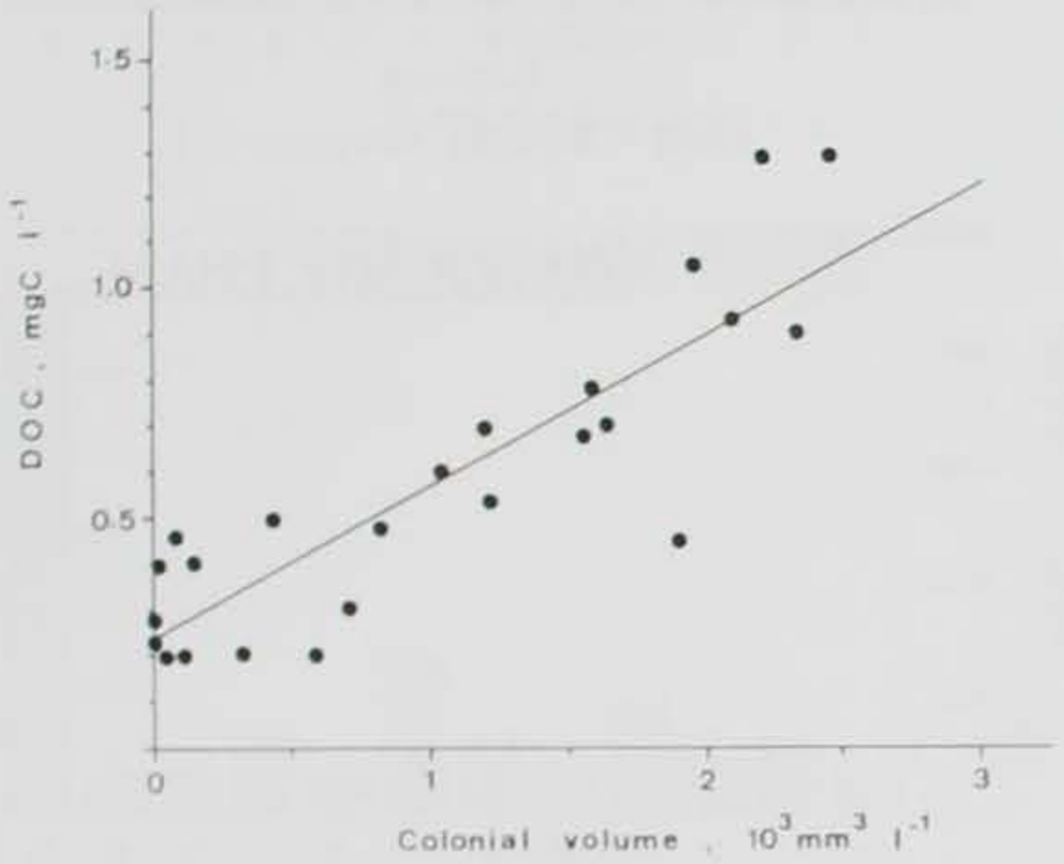

Fig. 6. *Phaeocystis* sp. Relationship between total colonial volume and corresponding dissolved organic carbon concentrations

at 335 ± 42 ngC (mm^3 colonial vol.)$^{-1}$ the factor for the conversion into carbon unit of mucilaginous biovolume.

Applications

The conversion factors recommended in the previous section are summarized in Table 2. They were used for calculating and comparing *Phaeocystis* biomass changes during its development in a pure culture and in the natural environment.

Fig. 7 shows the variations in carbon biomass of respectively free-living, colonial cells and colonies as calculated from microscopic counts and colonial volume measurements during the development of a batch culture of *Phaeocystis* sp. of which the frequency diagram of colonies is illustrated in Fig. 3. As indicated by Fig. 7, colonies develop and immediately dominate the population in terms of carbon biomass. Colonial biomass contributes indeed to 50 to 95% of total *Phaeocystis* sp. biomass during the course of culture development. Examination of Fig. 7 shows, on the other hand, that free-living cells and colonies have an identical carbon turnover rate. It indicates in addition that the specific growth rate of *Phaeocystis* sp. free-living and colonial cells, respectively 0.62 and 0.58 d^{-1}, are very close.

Fig. 7. *Phaeocystis* sp. Temporal change in calculated carbon biomass of free-living cells (●, continuous line), colonial cells (▲, continuous line) and colonies (■, dashed line) during the batch culture development illustrated in Fig. 3

A highly different feature is however observed when carbon data relative to *Phaeocystis* sp. colonies are discussed in terms of their size distribution. Fig. 8 shows the relative contribution per colony of different sizes of colonial cells and mucilaginous matrix to the total *Phaeocystis* sp. colonial carbon for four typical stages of the batch culture development. It clearly indicates that the specific carbon content of the colonies increases according to their size allowing a highly variable carbon/colony ratio ranging from 0.3 to 1436 (ngC colony^{-1}) for colonies whose diameters are respectively 10 µm and 2 mm. Although cellular carbon rises, the contribution of mucous carbon to total *Phaeocystis* sp. biomass is increasing much more rapidly when the size of the colonies is getting larger and becomes dominant once colonial diameter is higher than 400 µm. When colony diameter is higher than

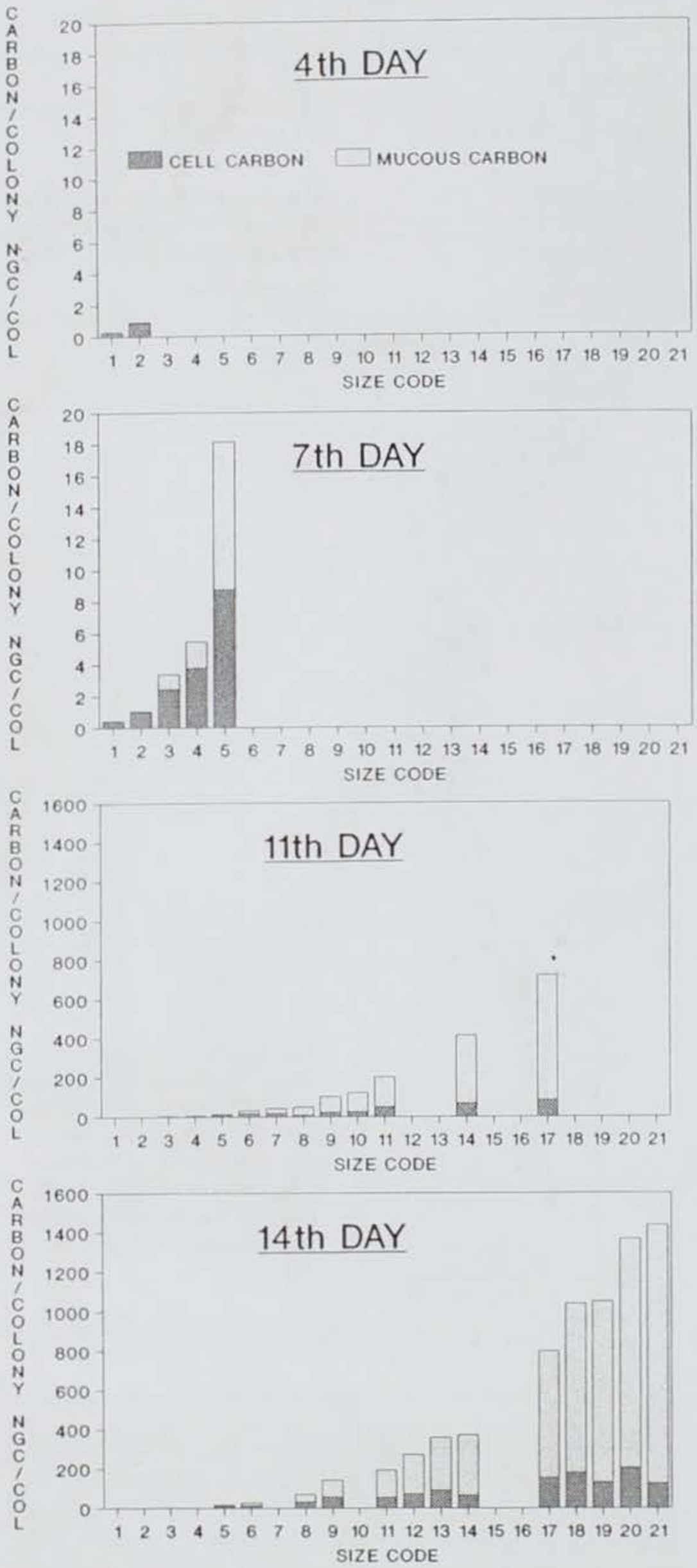

Fig. 8. *Phaeocystis* sp. Size distribution of colony carbon content with relative contribution of cellular and mucous carbon at four stages of batch culture development as illustrated in Fig. 3. For size code, see Fig. 3

1 mm, mucous contributes up to 90% of *Phaeocystis* sp. colony biomass. This has important ecological implications for organisms of higher trophic level that feed on *Phaeocystis* sp. Cells and mucous are indeed of very different nutritional quality. The mucilaginous substances of the matrix being mainly composed of polysaccharides

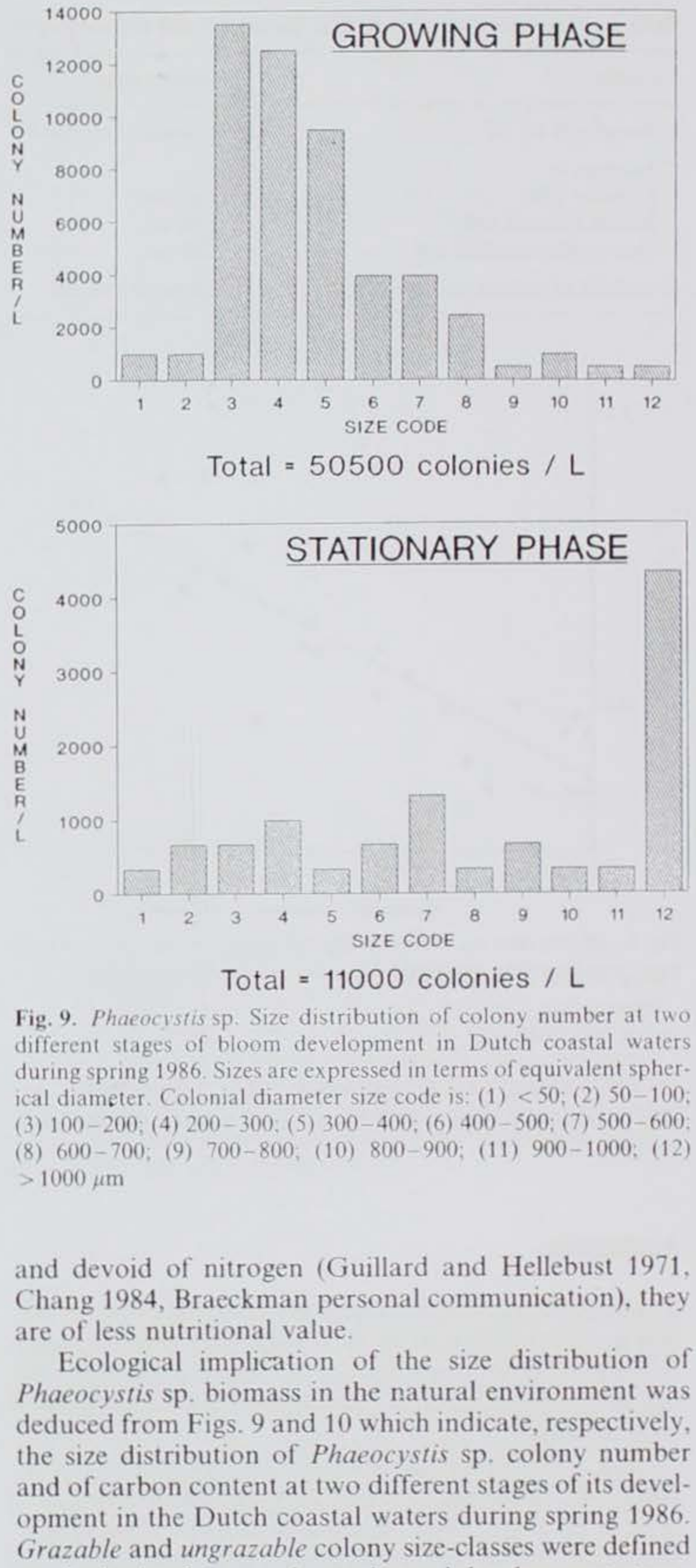

Fig. 9. *Phaeocystis* sp. Size distribution of colony number at two different stages of bloom development in Dutch coastal waters during spring 1986. Sizes are expressed in terms of equivalent spherical diameter. Colonial diameter size code is: (1) < 50; (2) 50–100; (3) 100–200; (4) 200–300; (5) 300–400; (6) 400–500; (7) 500–600; (8) 600–700; (9) 700–800; (10) 800–900; (11) 900–1000; (12) > 1000 μm

and devoid of nitrogen (Guillard and Hellebust 1971, Chang 1984, Braeckman personal communication), they are of less nutritional value.

Ecological implication of the size distribution of *Phaeocystis* sp. biomass in the natural environment was deduced from Figs. 9 and 10 which indicate, respectively, the size distribution of *Phaeocystis* sp. colony number and of carbon content at two different stages of its development in the Dutch coastal waters during spring 1986. *Grazable* and *ungrazable* colony size-classes were defined on the basis of copepods species and development stages present at the time of the *Phaeocystis* sp. bloom (Daro 1985) as well as their grazing characteristics determined under controlled conditions (Weisse 1983). According to this, a size limit of 300 μm of equivalent diameter was chosen.

Similarly to its development in batch culture, *Phaeocystis* sp. population is dominated by numerous small sized colonies during its growing phase, when nutrients are sufficient. At this time, the population is dominated by *grazable* forms of *Phaeocystis* sp. (Fig. 9, Table 3).

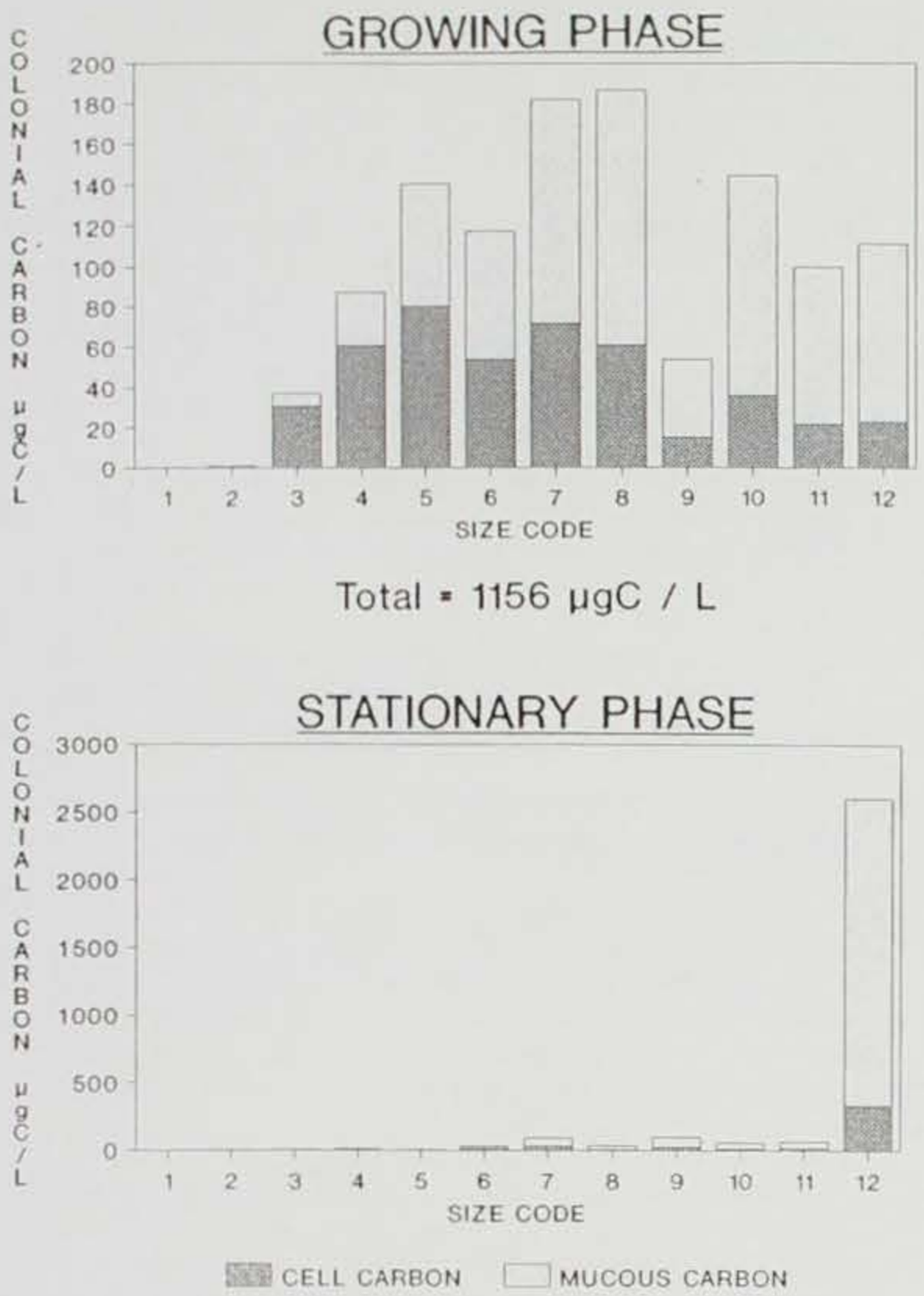

Fig. 10. *Phaeocystis* sp. Size distribution of colony carbon content with relative contribution of cellular and mucous carbon, at two different stages of bloom development in Dutch coastal waters during spring 1986. For size code, see Fig. 9

Table 3. *Phaeocystis* sp. Carbon content ($\mu g\ C\ l^{-1}$) of grazable and ungrazable forms at two periods of the spring bloom in 1986 in Dutch coastal waters. Numbers and percent mucous also given

Date	Free-living cells		Grazable (<300 µm)			Ungrazable (>300 µm)		
	No. $10^6\ l^{-1}$	C	No. $10^3\ l^{-1}$	C	Mucous %	No. $10^3\ l^{-1}$	C	Mucous %
May 06	7.56	107	28	125	26	22.5	1031	65
May 13	1.82	26	2.7	23	38	8.4	2984	85

Reversely, at the stationary phase of its development, the population has decreased in terms of colony number but is dominated at 75% by big colonies whose size is greater than 1 mm.

A different feature can be observed when data of Fig. 9 are converted into carbon unit as illustrated by Fig. 10. Carbon content of small colonies is insignificant compared with the large size ones, these latter being composed of more than 50% of mucilaginous substances. At the top of the bloom when large colonies dominate the population, *Phaeocystis* sp. biomass is composed of 85% of mucilaginous substances. This corresponds physiologically to the enhanced synthesis of mucilaginous substances that compose the colonial matrix when available nutrients become scarce (Lancelot and Billen 1985). According to this, colony carbon content ranges between 0.4 and 813 ng in the Dutch coastal waters during the course of *Phaeocystis* sp. flowering. The ecological impact of the size distribution of *Phaeocystis* sp. biomass on heterotrophic growth in the Dutch coastal waters during spring 1986 is clearly shown by Table 3, which gives available food for specific grazers (ciliates and zooplankton) in terms of carbon and cell or colony numbers for the two stages of the bloom. Examination of Table 3 highlights the fact that most *Phaeocystis* sp. biomass is not *grazable* by zooplankton and thus escapes this trophic pathway. Ungrazed *Phaeocystis* sp. colonies can, however, be degraded by planktonic bacteria. The efficiency of this microbial trophic pathway is however highly questionable, as more than 50% of *Phaeocystis* sp. biomass is composed of mucilaginous polysaccharides, the biodegradability of which remains to be determined.

Conclusion

The conversion factors recommended in this paper for the calculation of *Phaeocystis* sp. carbon biomass, on the basis of microscopic observations, were shown to be useful both for determining the life cycle of *Phaeocystis* sp. during the course of its flowering, and for evaluating available food for ciliates and zooplankton that graze, respectively, on cells and colonies – provided that microscopic observations are detailed as follows: (1) counts of free-living cells and distinction between flagellated and non-motile cells; (2) counts of colonies and measurement of their respective biovolume for different colony size-ranges, determined on the basis of grazing characteristics of zooplankton present at the time of *Phaeocystis* sp. bloom. These colony size-classes vary, therefore, from one biotope to another.

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Chapter 3

Autoecology of the marine haptophyte Phaeocystis sp.

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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

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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).

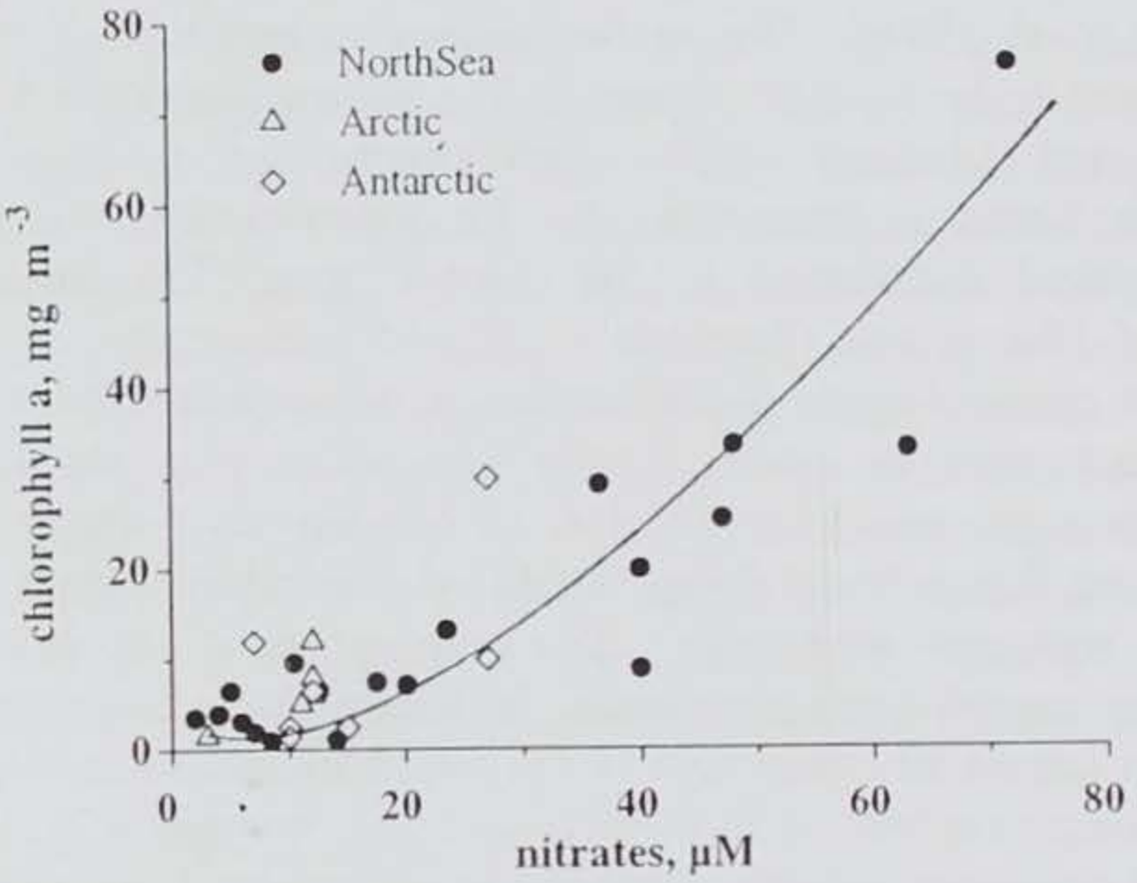

Figure 1: Empirical relationship between maximum *Phaeocystis*-Chl.*a* and nitrate reduction. Data from Rey and Loeng 1985; Wassmann *et al.* 1990; Vernet, 1991; Smith unpublished; Palmisano *et al.* 1986; El-Sayed *et al.* 1988; Rousseau *et al.* unpublished

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)

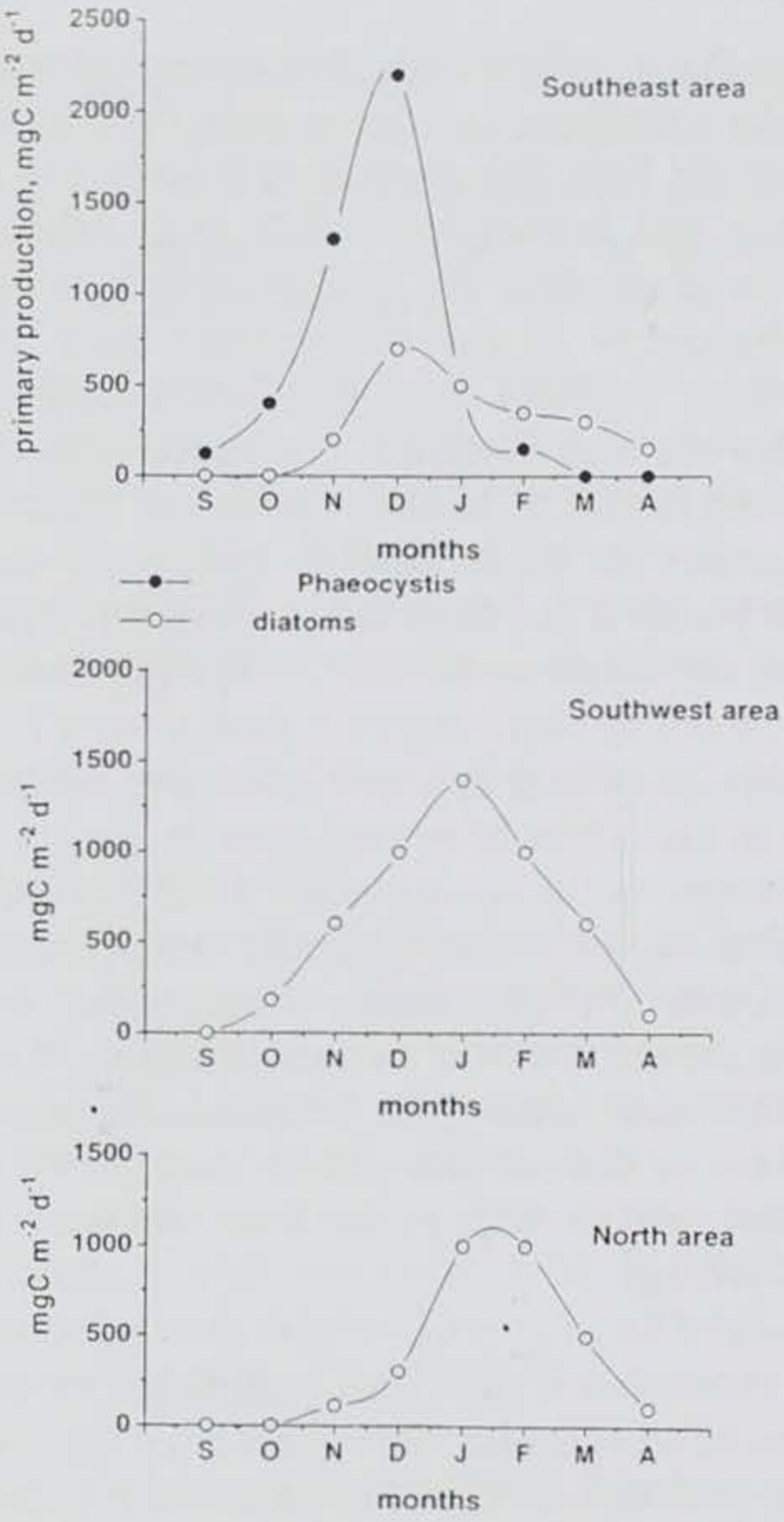

Figure 2: *Phaeocystis*- and diatom- daily growth in the Ross Sea

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).

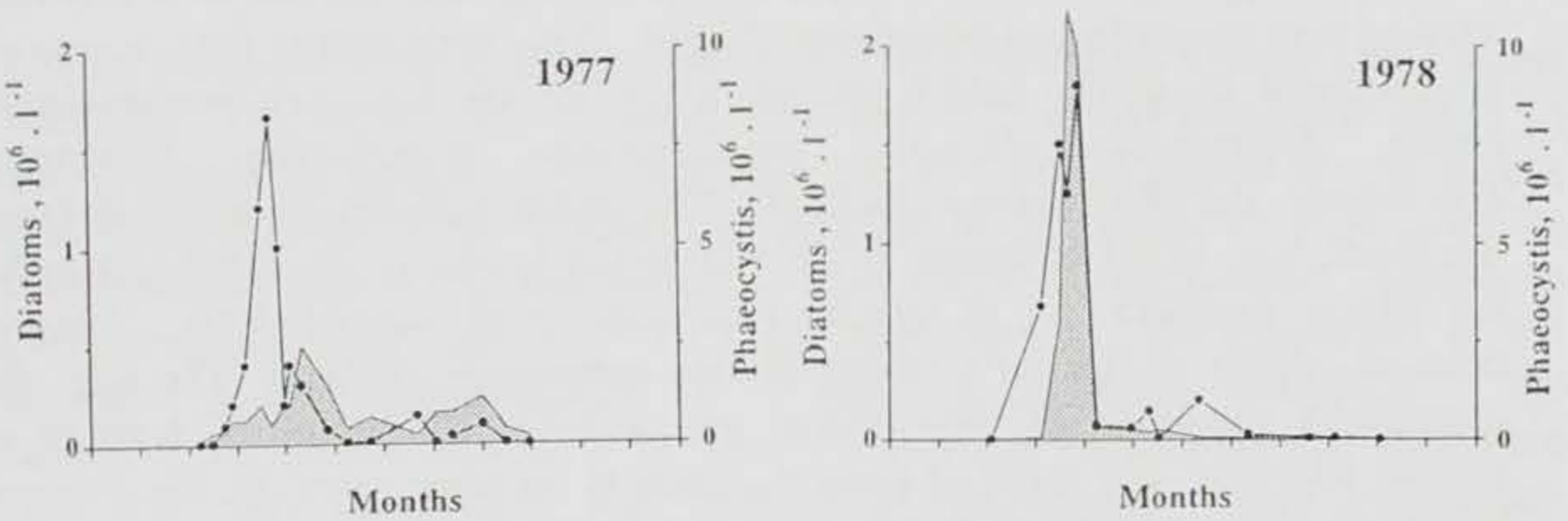

Figure 3: Diatom-*Phaeocystis* (gray area) colonies succession in the Balmsfjord in 1977 and 1978 (redrawn from Eilertsen *et al.* 1981).

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).

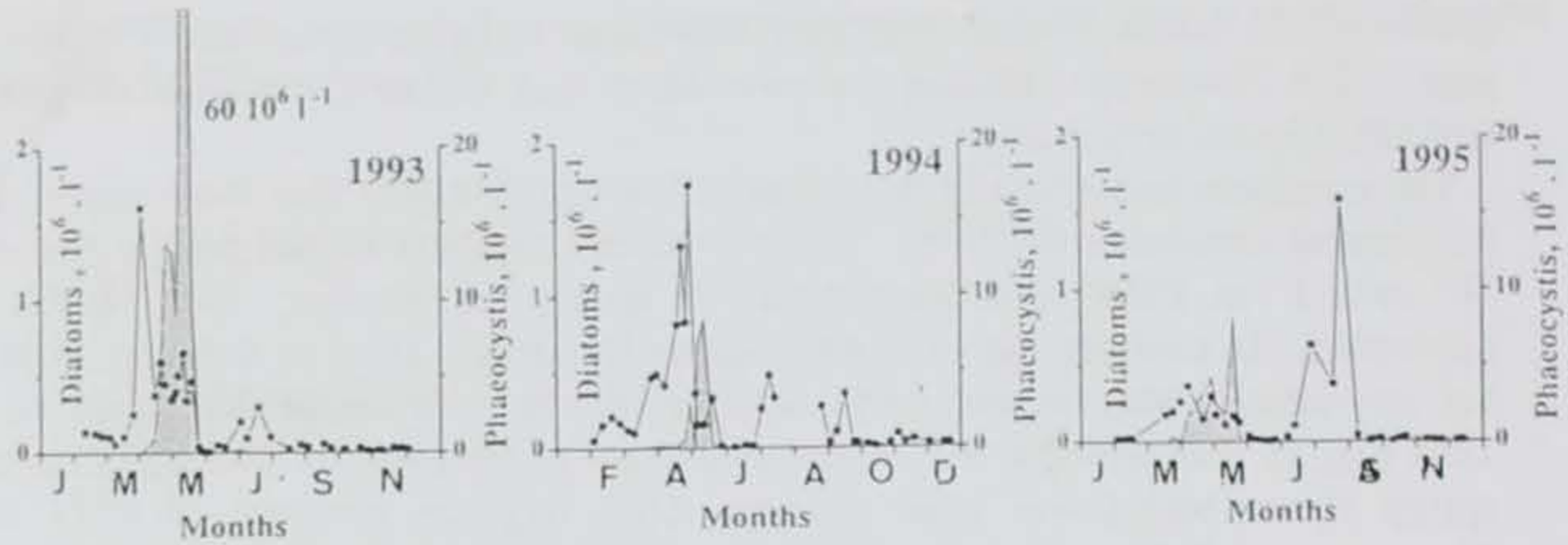

Figure 4: Diatom-*Phaeocystis* (grey area) colonies succession in the continental coastal waters of the North Sea (station 330). Rousseau *et al.* unpublished

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).

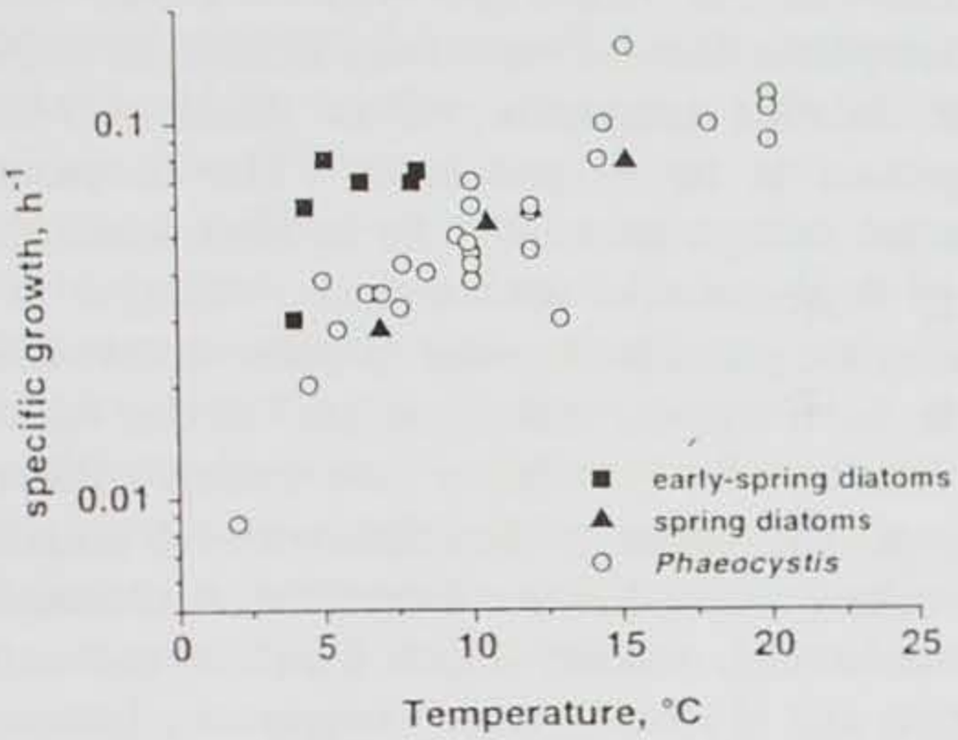

Figure 5: Relationship between temperature and specific growth of diatoms and *Phaeocystis* colonies. (Lancelot *et al.*, unpublished data)

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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Chapter 4

Ecology of Phaeocystis: the key role of colony forms

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In: The Haptophyte Algae, Systematics Association 51: 229-245 (1994)

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 micro-zooplankton (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

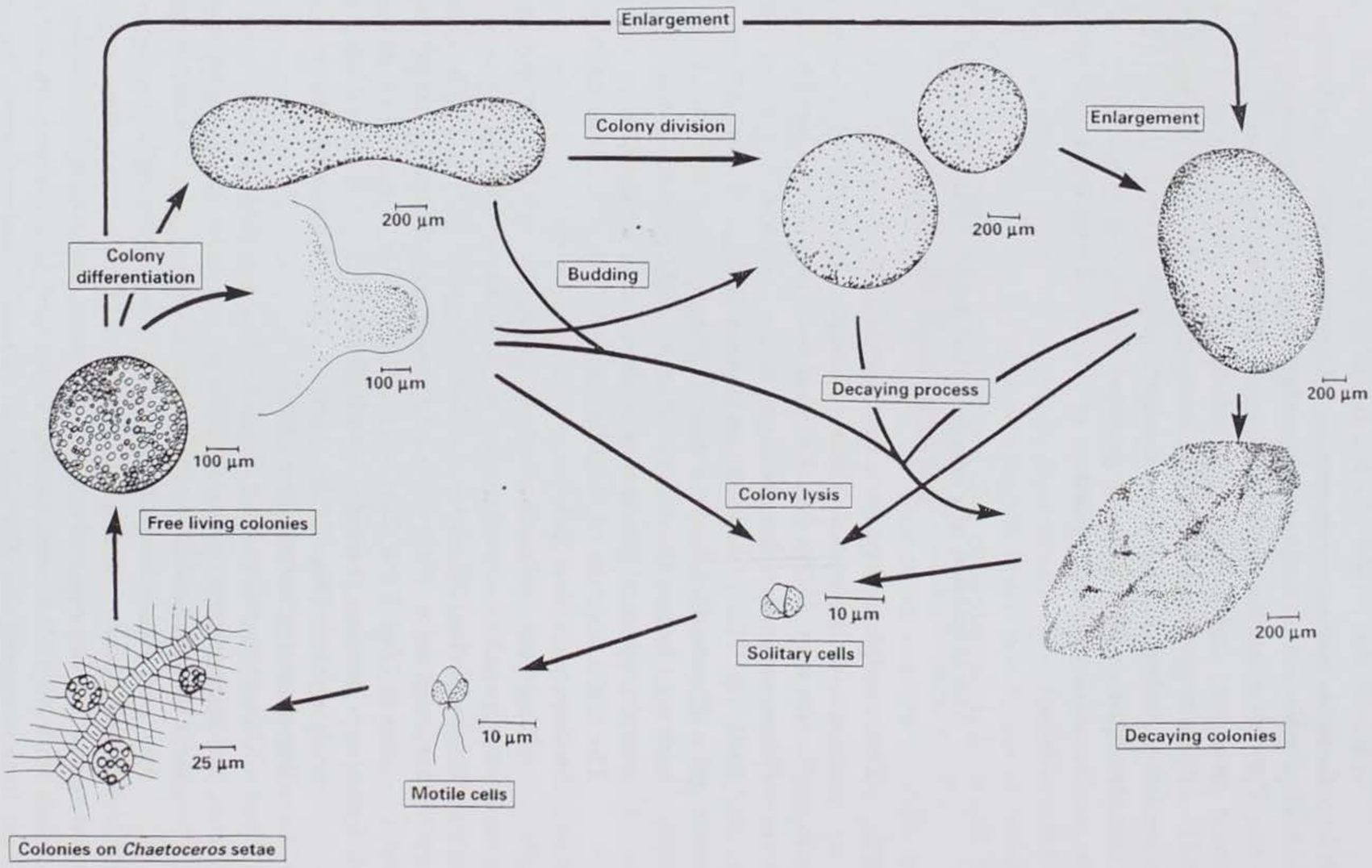


Fig. 12.1 The sequence of *Phaeocystis* morphotypes during spring bloom development in Belgian coastal waters (southern North Sea). Redrawn from Rousseou *et al.* (1994).

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

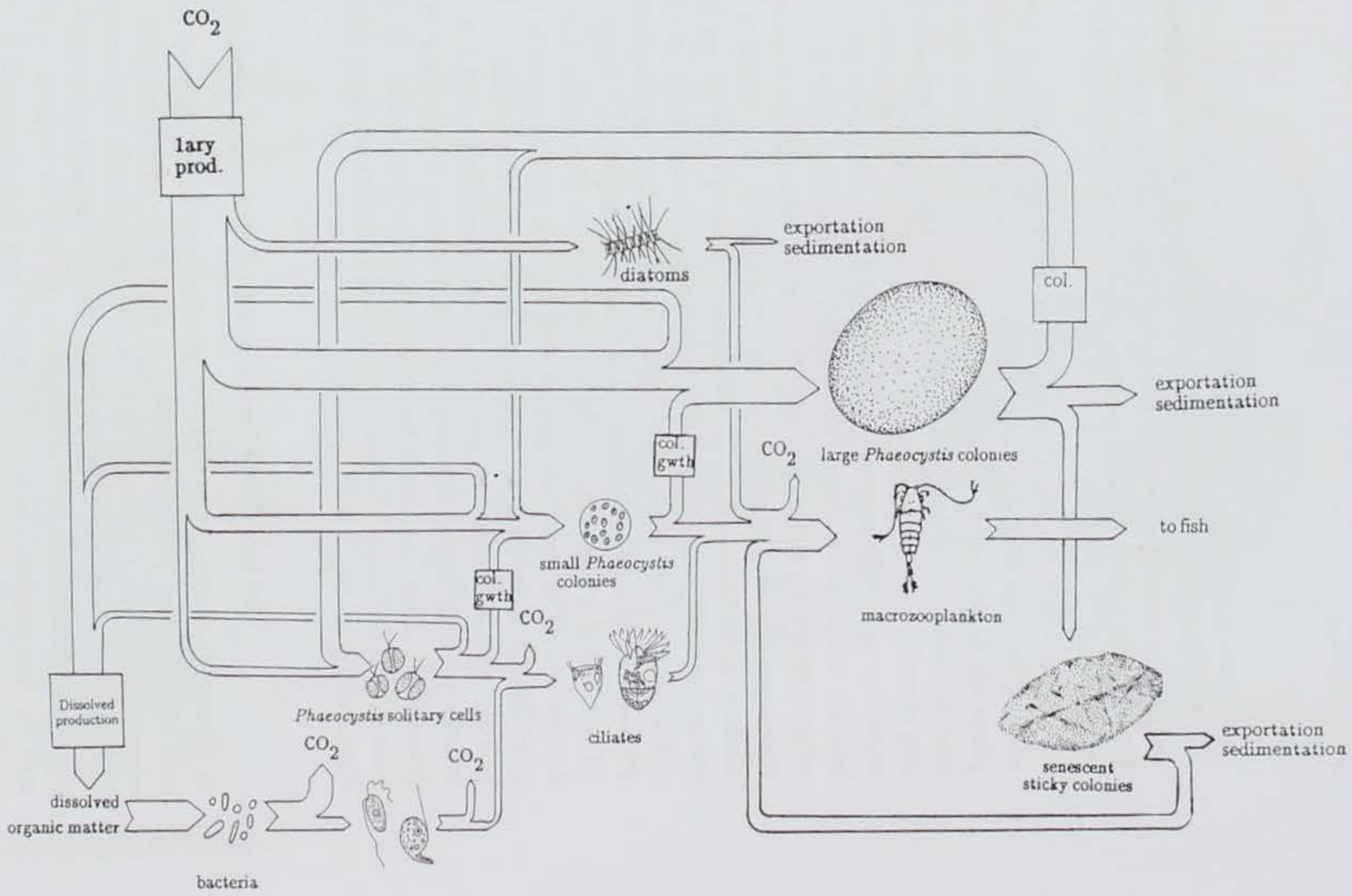


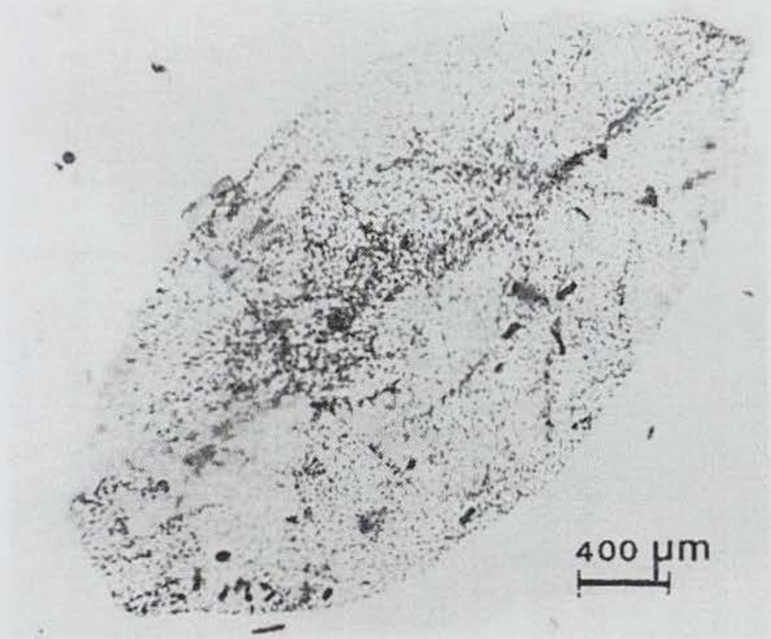
Fig. 12.2 Schematic representation of the structure of the planktonic food-web of the *Phaeocystis*-dominated ecosystem of the southern North Sea.

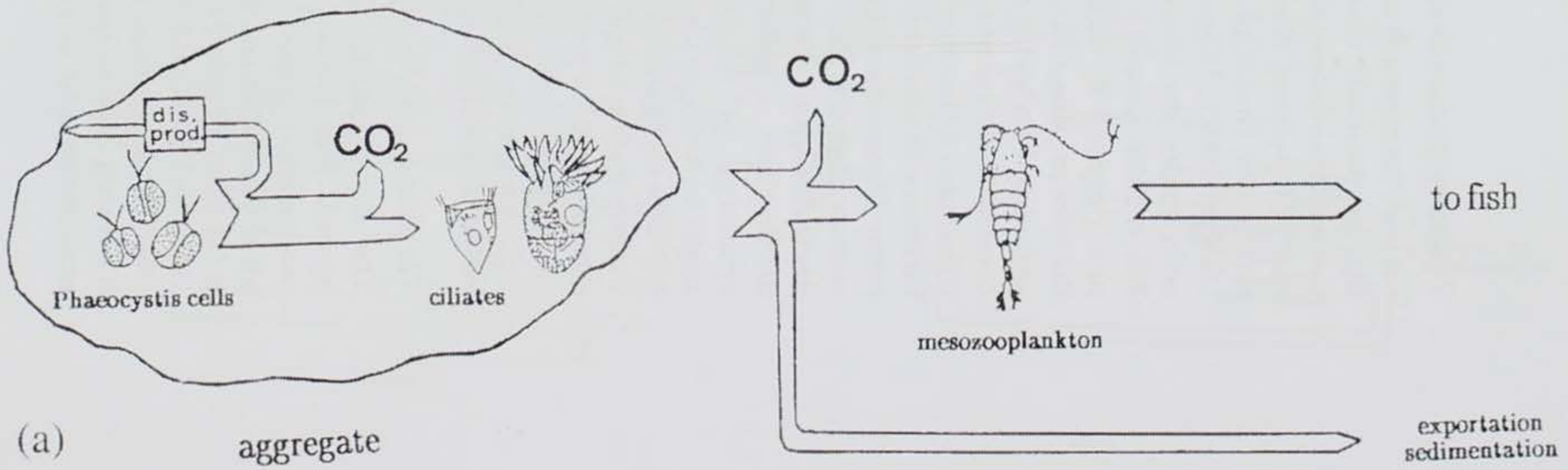
The mucilaginous matrix: chemical characterization and biosynthesis

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.

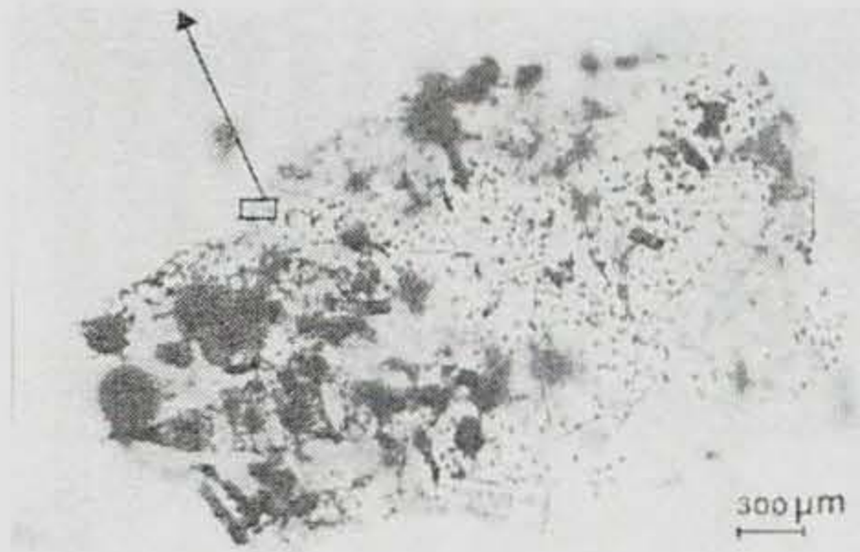
The physiological function of the mucilaginous matrix.

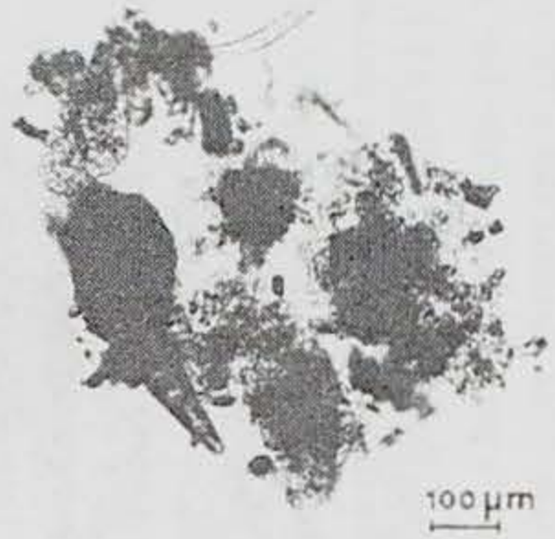
The energy storage function of the mucilaginous matrix is now well agreed. Numerous process studies have demonstrated that the polysaccharides composing the matrix constitute an energy-storing substrate, which is catabolized by the colony cells during the light-limited period to meet their biosynthetic 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

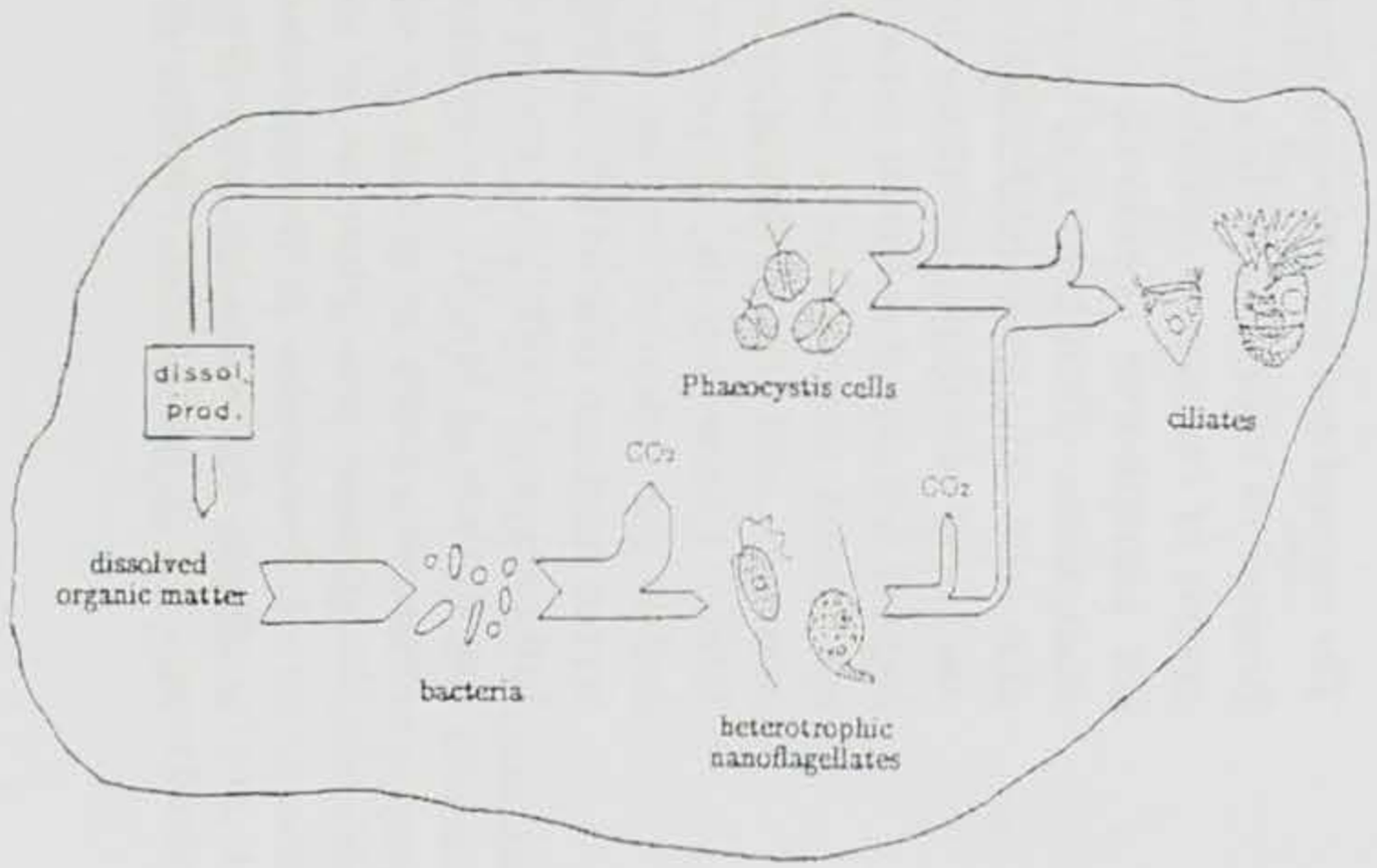

400 µm

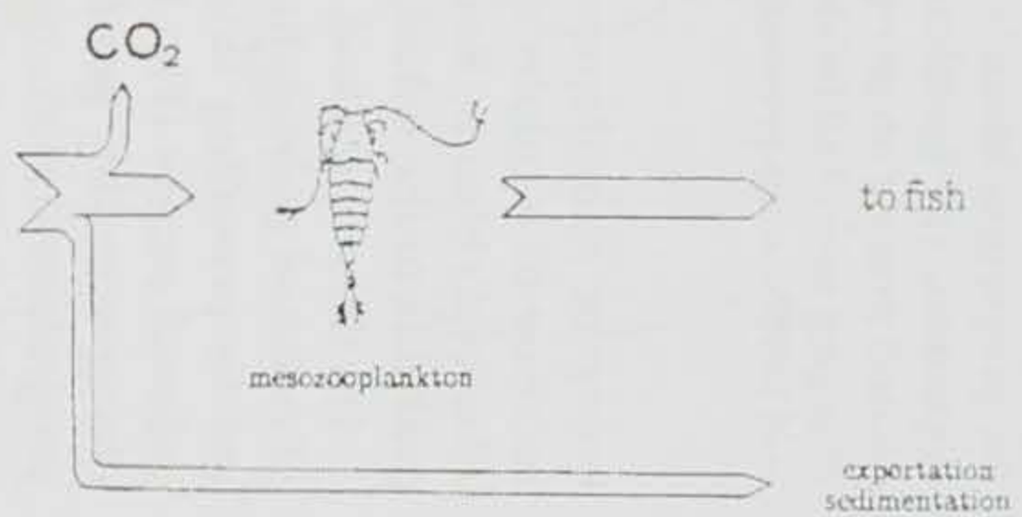

dis. prod.
CO_2
Phaeocystis cells
ciliates
(a)
aggregate
CO_2
mesozooplankton
to fish
exportation
sedimentation


10µm


300µm


100µm


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.

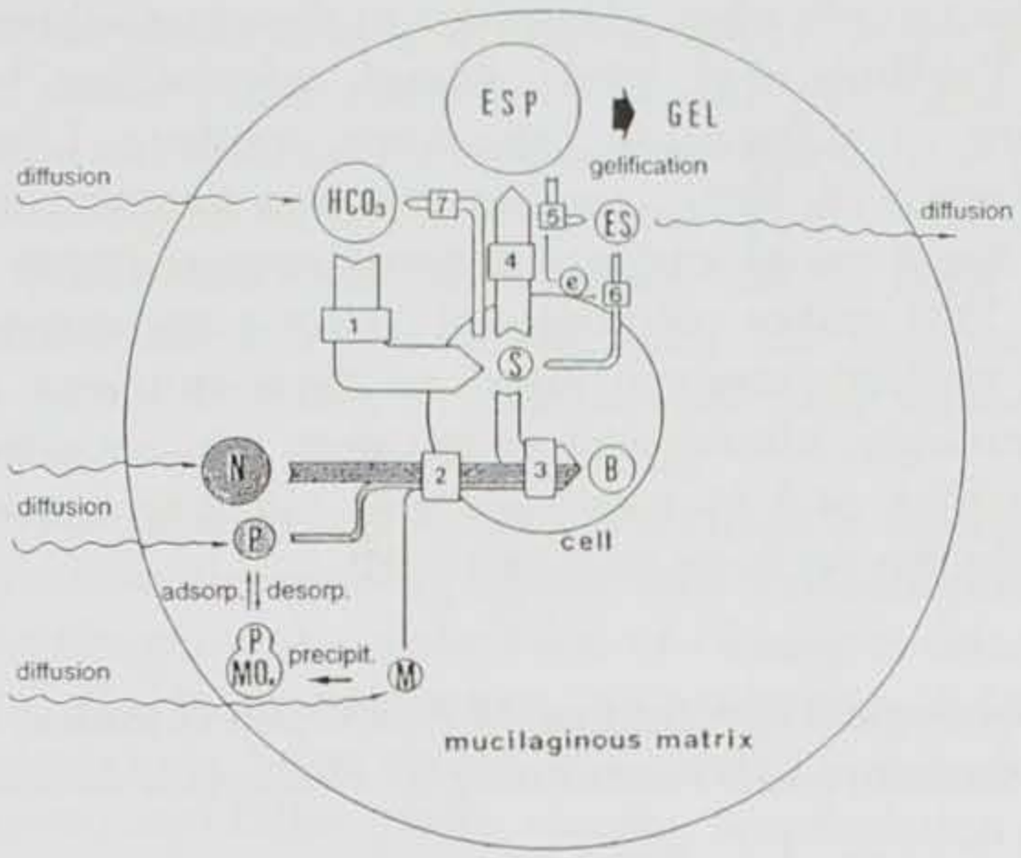
energetic gain depends on the colony size. Over an order of magnitude increase to a colony of 1 mm^3 (the average colony size at the height of a *Phaeocystis* bloom, Rousseau *et al.* 1990), the pool of energetic substrates available to the colony has been estimated to increase by a factor of 20.

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

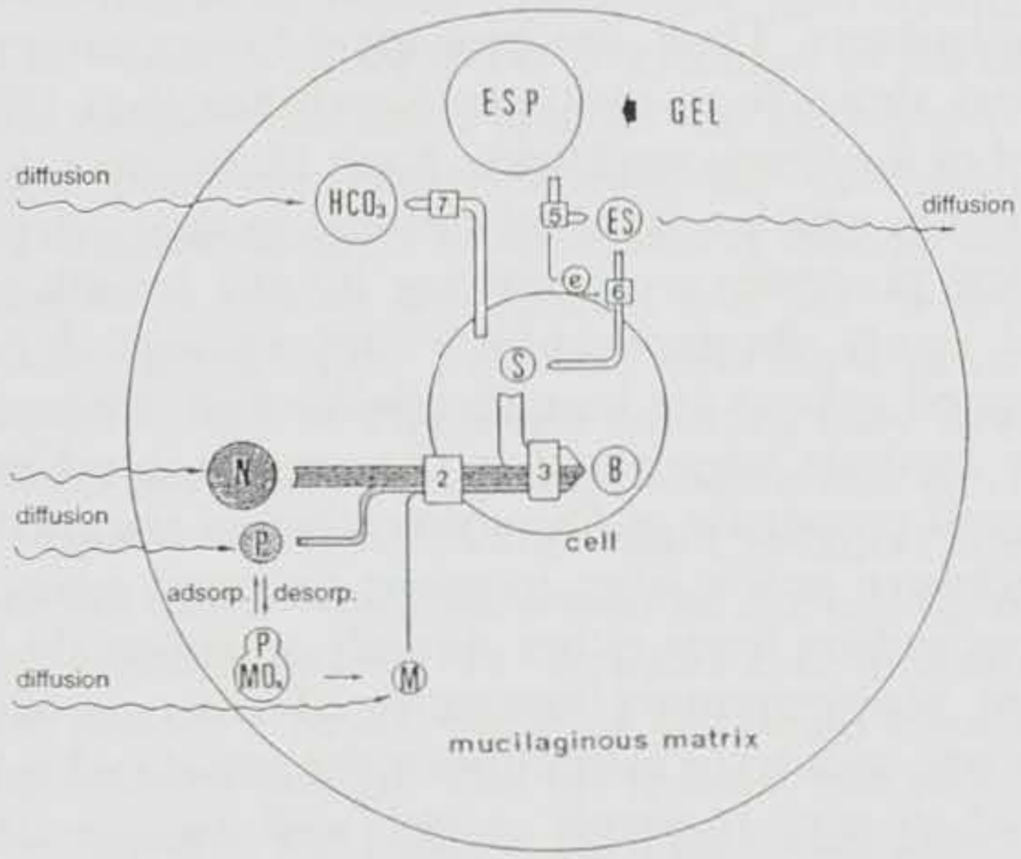


Fig. 12.4 Schematic representation of: (a) the light and (b) the dark metabolism of *Phaeocystis* colonies. *Intracellular and intracolonial pools*: B = cellular biomass; e = exoglucosidase; ES = extracellular oligosaccharide; ESP = extracellular polysaccharide; M = trace metals (mainly Mn and Fe); MO_x = 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.

Acknowledgments

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Chapter 5

On the interannual variability of diatoms and Phaeocystis colonies blooms in the eutrophicated Belgian coastal waters (Southern Bight of the North Sea) between 1988 and 1999.

V. Rousseau, J.-Y. Parent and C. Lancelot

In preparation

On the interannual variability of diatoms and *Phaeocystis* colonies blooms in the eutrophicated Belgian coastal waters (Southern Bight of the North Sea) between 1988 and 1999.

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Abstract

Current knowledge describes coastal eutrophication in the Southern bight of the North Sea and in the Belgian coastal waters in particular, as massive blooms of gelatinous *Phaeocystis* colonies growing on the nitrate excess left over after a first silicate-controlled diatom bloom of moderate amplitude. Long-term monitoring of phytoplankton successions and their environmental control (temperature, light and nutrients) was undertaken at reference station 330 of the Belgian coastal waters in 1988-1999. The main objective was the assessment of the interannual variability of the diatom-*Phaeocystis* colony succession as a result of naturally- and/or human-induced environmental changes. This time series data set was complemented with winter geographical surveys of inorganic nutrient concentrations in order to estimate the variability of nutrient loads. In spite of the variable hydrodynamic conditions prevailing at station 330, the phytoplankton succession was recurrent from year to year. Three main diatom communites were identified on basis of statistical analysis (TWINSPAN). In this way, the spring bloom is initiated by small colony-forming species *Thalassiosira* spp., *Skeletonema costatum*, *Thalassionema nitzschioides*, *Plagiogramma brockmannii*, *Asterionella glacialis*, *A. kariana*, *Melosira sulcata*. *Biddulphia* spp. and the larger *Coscinodiscus* spp. This moderate growth is followed by an ephemeral assemblage composed of *Chaetoceros* spp. (mainly the colonial *C. socialis*), and *Schroederella* sp., invariably associated to the onset of the blooming of *Phaeocystis* colonies. Co-occuring with these latter, a third diatom community blooms, essentially composed of larger diatoms *Rhizosolenia* spp., *Guinardia* sp. and *Cerataulina* sp. but largely dominated by *Rhizosolenia* spp. and constitutes the bulk of summer diatoms from June to about mid-September. A significant interannual variability was observed in the timing and duration of spring phytoplankton blooms. A one-month shift was observed in the onset of phytoplankton spring bloom while the seasonal succession is not modified. The onset of the spring bloom is driven by available light in the water column with a light threshold of 12 µmol quanta. m^{-2} s^{-1} required for phytoplankton growth. Duration varied from about a factor 2, ranging between 56 and 97 days. The contribution of *Rhizosolenia* spp.-dominated community and *Phaeocystis* colonies biomass to the phytoplankton spring bloom was highly variable with diatoms contributing from about 30 to 90 % of the cellular carbon biomass. This varying contribution of both taxa to the phytoplankton spring bloom was not related to changing nutrient loads. The main source of variability at the time scale of 12 years was rather related to changing hydrodynamic conditions due to different meteorological driving forces. The relative proportion of both taxa could indeed be related to the average salinity prevailing during their flowering, adding a spatial variability component to the temporal changes. The preferential flowering of diatoms at low salinities (below 31) was related to the nutrient quality change observed along salinity gradient with well-balanced nutrient environment compared to diatom requirements prevailing at low salinity. The results of this monitoring data set are discussed and compared with phytoplankton succession and controlling factors observed in adjacent areas.

Key words : eutrophication, phytoplankton variability, diatoms, *Phaeocystis*, nutrients, light, temperature.

In preparation

Introduction

The temporal variability of phytoplankton is governed by physical, chemical and biological factors according to various patterns such as cycles, trends, fluctuations at different frequencies (Smayda, 1998). Natural variability is driven primarily by external wind- and weather-driven forcing events which lead to fluctuation in temperature, irradiance, rainfall. These natural events, by stabilizing the ocean surface or upwelling deep nutrient-enriched waters, determine the light and nutrient environment of phytoplankton. The nutrient environment of phytoplankton in coastal waters is additionally modified both quantitatively and qualitatively by anthropogenic sources of nutrients brought through river discharges and/or atmospheric deposition (*e.g.* Lancelot *et al.*, 1991; Paerl, 1995). This alteration constitutes a major driving force behind natural change (Cadée, 1986; Hickel, 1998). However, the distinction between the effects of human-induced variations from those attributable to natural variation is difficult to assess (Colijn *et al.*, 1998).

The nutrient signature of coastal waters results from anthropogenic inputs of nitrogen and phosphorus, inducing a modification of nutrient ratios available to coastal phytoplankton with a severe deficiency in silicon compared to diatom silica requirements (Brzezinski, 1985). As a consequence, the anthropogenic nutrient sources result in the dominance of often poorly edible non-siliceous algae with harmful environmental consequences (Smayda, 1990;Billen *et al.*, 1991).

Receiving the discharge of 7 major west-European rivers, the Southern Bight of the North Sea is one example of human-induced eutrophicated coastal ecosystem. The unbalanced nutrient environment of these coastal waters results from the large influence of river inputs characterized by a large excess of nitrate over silicate and phosphate (Lancelot, 1995; Lancelot *et al.*, 1998) compared to diatom silica requirements (Brzezinski, 1985) and nitrogen and phosphorus phytoplankton needs (Redfield *et al.*, 1963). As a consequence, the spring phytoplankton bloom is recurrently characterized by the occurrence of a moderate diatom outburst in early spring, followed by a massive bloom of the non-siliceous colony-forming haptophyte *Phaeocystis* sp. in association or not with late spring diatoms (Gieskes & Kraay, 1975; Bätje & Michaelis, 1986; Cadée, 1986; Cadée & Hegeman, 1986; Veldhuis *et al.*, 1986; Weisse *et al.*, 1986; Lancelot *et al.*, 1987; Cadée & Hegeman, 1991; Hickel *et al.*, 1994; Lancelot, 1995; Lancelot *et al.*, 1998). Apart from these spring events, moderate blooms often dominated by diatoms but also ciliates and dinoflagellates are observed during summer and fall (Gieskes & Kraay, 1975; Cadée, 1986; Cadée & Hegeman, 1986).

The magnitude of *Phaeocystis* colony blooms in these coastal waters is determined by the nitrate excess left over at the end of the early spring silicate-controlled diatom flowering (Lancelot, 1995; Lancelot *et al.*, 1998). The high ability of *Phaeocystis* colonies to utilize nitrate as nitrogen source was demonstrated through competitive experiments under laboratory-controlled conditions (Riegman *et al.*, 1992) and confirmed by the elevated f_{NO3} ratio (0.5-0.8) characterizing the nitrogen metabolism of field *Phaeocystis* colonies (Lancelot *et al.*, 1986; Lancelot *et al.*, 1998). The low palatability of the large *Phaeocystis* colonies to coastal mesozooplankton, due to a size mismatch, (Weisse *et al.*, 1994; Gasparini *et al.*, 2000) results in the pelagic food chain disruption (Lancelot & Rousseau, 1994; Rousseau *et al.*, 2000). The foam deposit observed every year on the beaches of the Southern Bight of the North Sea, represents the most manifest effect of this food chain disruption and resulting biomass accumulation in the water column (Lancelot, 1995).

Long-term monitoring of phytoplankton successions and their environmental control (temperature, light, nutrients) constitutes one approach to assess the changing quality of these coastal waters and its possible variation in relation to anthropogenic and natural impact. Time series provide indeed a phenomenological description of the temporal pattern of phytoplankton communities, allowing identification of the interannual variability and its driven factors. Such monitoring was undertaken in the eutrophicated Belgian coastal waters of the Southern Bight of the North Sea with for main objective the assessment of interannual variability of the diatom-*Phaeocystis* colony succession as a result of naturally- and/or human-induced environmental changes.

The high resolution time series monitoring at one single location representative of the area was preferred to a low time resolution grid due to the high dynamics of phytoplankton blooms in Belgian coastal waters (Lancelot *et al.*, 1998; Ruddick *et al.*, 1998). Station 330 was chosen among stations of the national monitoring network as a possible observatory of eutrophication-related changes in the Belgian coastal waters because of its average physico-chemical and biological characteristics (Ruddick *et al.*, 1999). Belgian coastal waters are mainly composed of Atlantic water mass enriched by nutrients discharged by rivers Seine and Somme and inflowing north-eastward from the Straits of Dover (Lancelot *et al.*, 1991). These waters mix with freshwater from the Scheldt (Yang, 1998) and to some extent, the Rhine and the Meuse (van Bennekom *et al.*, 1990). An inherent problem related to sampling at one location in such hydrodynamical area, consists in the changing water mass distribution which add a spatial variability component to the observed temporal variability (Colijn *et al.*, 1998). Due to the difficulty to distinguish the changes related to the sequence of different water masses from those due to the intrinsic succession within the water mass, our time series monitoring at station 330 provides an average picture of the ecological functioning in this area, rather than a population dynamics.

In accordance, this paper presents results of phytoplankton time series recorded from 1988 to 1999 at station 330 in the Belgian coastal waters. Particular attention was given to the two major phytoplankton groups: the diatoms and the *Phaeocystis* colonies. The seasonal and interannual pattern of the phytoplankton succession was investigated in relation with environmental physico-chemical parameters (temperature, light, nutrients, salinity). This time series data set at one location was complemented with winter geographical surveys of inorganic nutrient concentrations in order to assess the interannual variability of nutrient loads in the investigated area.

Material & Methods

Sampling stations and procedure

The time series monitoring was conducted at station 330 (N 51°26.05; E 002° 48.50; Fig. 1) in the Belgian coastal waters. Surface seawater was sampled with a bucket aboard R.V. Belgica or when not available with a rubber boat. Seawater was analysed for major nutrients, suspended matter, temperature, salinity, Chl a and phytoplankton. Sampling occurred usually at a weekly frequency but fortnightly during winter and summer months. From 1989 to 1992, sampling was restricted to the spring period.

The winter geographical grid was sampled aboard R.V. Belgica at the 19 stations of the Belgian monitoring network (JMP-OSPAR; Fig. 1) during 3-days cruises between late January and early February for the period 1989-1999. Seawater for major inorganic nutrient concentrations and salinity analysis was collected at 3 m depth with Niskin bottles.

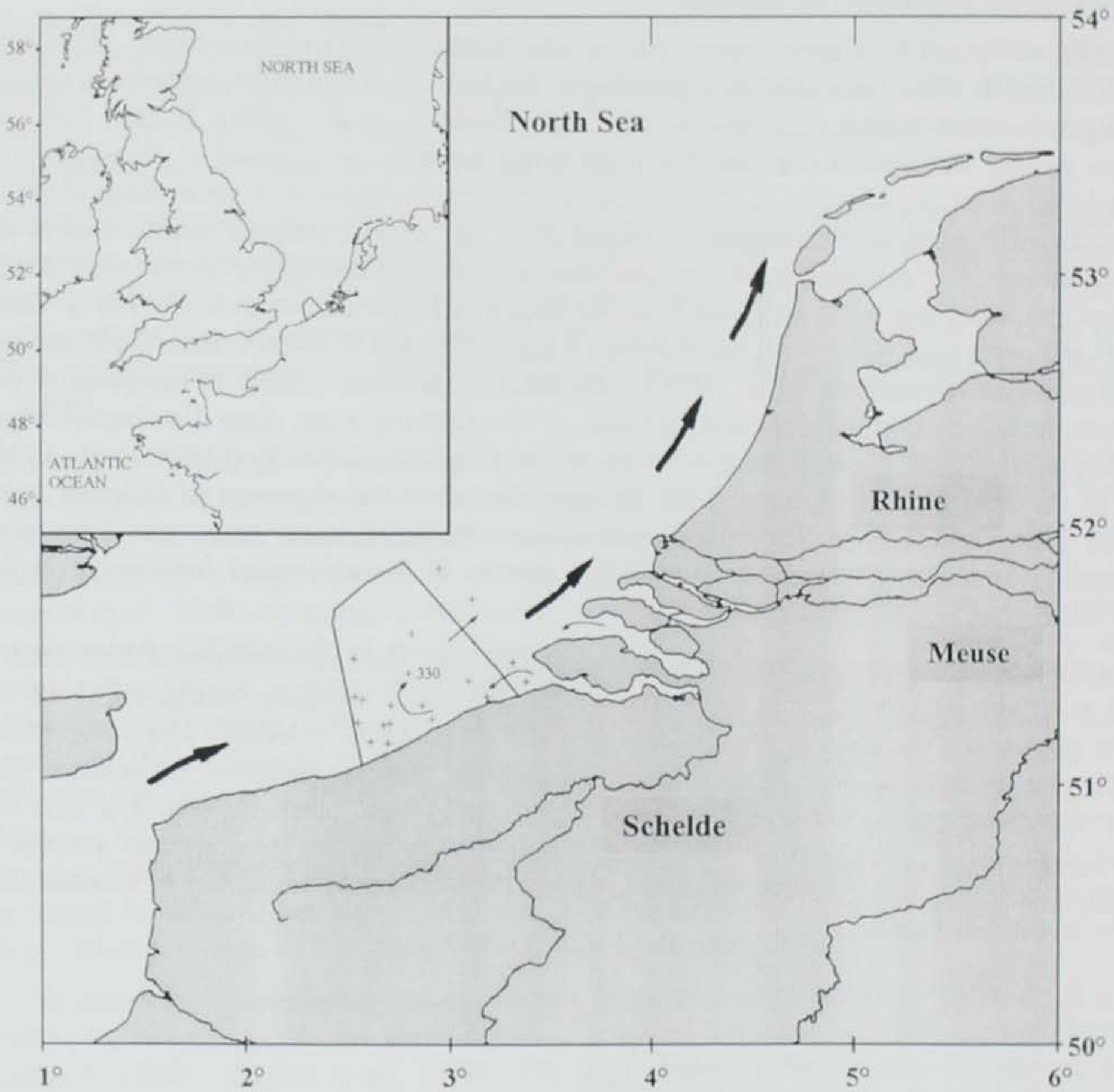


Figure 1: Map of the Southern Bight of the North Sea focusing on the Belgian coastal waters. Station 330 and JMP-OSPAR monitoring network stations are indicated. Arrows represent the general circulation of Atlantic (bold) and Scheldt (thin) water masses.

Physico-chemical measurements

Seawater temperature and salinity were measured directly after sampling with a thermosalinometer (Beckman). Suspended matter content was determined by weighting particulate matter collected by filtration on a GF/F (Whatman) pre-weighted filter and dried for 4 hours at 105 °C.

Global solar radiation GR ($J.cm^{-2}.d^{-1}$) was recorded by the meteorological station Ostende (Institut Royal de Météorologie de Belgique). These data were converted into daily-averaged photosynthetically active radiation *PAR* using the empirical relationship derived from the direct calibration of the global radiation instrument with a *PAR* sensor (Li-Cor):

$$GR = 3.43\ 10^{-6}\ PAR^{\ 2} + 0.0805\ PAR$$

in which GR is the global radiation ($J.cm^{-2}.30min^{-1}$) and *PAR* is the photosynthetically active radiation ($\mu mol\ quanta.\ m^{-2}.\ s^{-1}$).

The averaged light available to phytoplankton cells in the water column was assumed to be uniform in these vertically mixed waters. It was calculated by the equation of Riley (1957) :

$$I = I_0\ \frac{1 - e^{-K_e z}}{K_e\ z}$$

in which I is averaged *PAR* in the water column ($\mu mol\ quanta.\ m^{-2}.\ s^{-1}$), I_0 is the subsurface *PAR*, K_e is the vertical light attenuation coefficient (m^{-1}) and z is the average depth of the station (17 m).

The vertical light attenuation coefficient K_e was calculated from the suspended matter content (SM; $mg.l^{-1}$) according to the empirical relationship established for the Belgian coastal waters:

$$K_e = 0.024\ SM\ + 0.188\ (n = 38;\ r = 0.89)$$

where K_e was derived from optical vertical profiles.

Major nutrient (NO_3, NH_4, $Si(OH)_4$ and PO_4) concentrations were determined on 0.45 µm filtered seawater according to the colorimetric methods described in Grasshof *et al.* (1983).

Chlorophyll a (Chla) was measured on 90 % (v:v) aceton extracted particulate material isolated by filtration on GF/C filters (Whatman). Chla concentration was spectrophotometrically determined using the method and equations recommended by Lorenzen (1967).

Phytoplankton analysis

Phytoplankton samples were preserved with 1% (final concentration) lugol-glutaraldehyde solution and stored at 4°C in the dark. Diatoms and *Phaeocystis* colonies were enumerated under an inverted microscope (Leitz Fluovert) according to the Utermöhl method (Hasle, 1978). Magnification was chosen according to cell or colony size: 40 or 100 X for *Phaeocystis* colonies and 100 or 200 X for diatoms. At least 400 cells were enumerated in total with at least 100 cells of the most abundant genus or species. Diatoms were enumerated and identified according to the genus level unless a species was easily identifiable or dominant. Their carbon biomass (C-biomass) was calculated on the basis of cell density and biometric factors determined for each species or genus. A specific average conversion factor

was calculated from biovolumes measured on a cell population along the whole period of its growth. Biovolumes were then converted using a carbon content factor of 0.11 pgC μm^{-3} of plasma volume (Edler, 1979). *Phaeocystis* was identified as *P. globosa* and will here be referred as *Phaeocystis*. Colony cell number was determined according to the method described in Rousseau *et al.* (1990). *Phaeocystis* colony C-biomass was estimated from biovolume measurement according to the empirical procedures of Rousseau *et al.* (1990).

The phytoplankton community composition was statistically analysed with the 'Two Way Indicator Species Analysis' TWINSPAN (Hill, 1979). This analysis was performed on the phytoplankton C-biomasses matrix of all the individual samples collected during the 1988-1998 period. The C-biomasses were expressed as relative proportion prior to the statistical analysis.

Results

Hydrological regime and nutrient enrichment of the Belgian coastal waters.

The temporal changes of salinity recorded at station 330 during the period 1988-1999, shows variations from 30.8 to 35.5 with an average of 33.5 (Fig. 2). Physical factors determining salinity distribution are tidal dispersion, river discharge, wind stress and advection. The salinity variations observed during the investigated period, with a maximum amplitude of 4.7, is much higher than that observed during a tidal cycle (maximum amplitude of 0.5) as revealed by a 24h-cycle measurement (not shown). The salinity variations observed reflect therefore changing hydrographic conditions due to the varying mixing of freshwater sources originating from rivers Scheldt, Meuse and Rhine with Atlantic waters. Hydrodynamical modelling shows that, at the time scale involved in eutrophication processes, persisting wind direction strongly affect the spreading of the Scheldt plume in the Belgian coastal waters (Verlaan & Groenendijk, 1993; Yang, 1998). In particular, salinity decreases are associated with persisting northeastward winds deflecting the Scheldt plume to the north-east through station 330 (Yang, 1998).

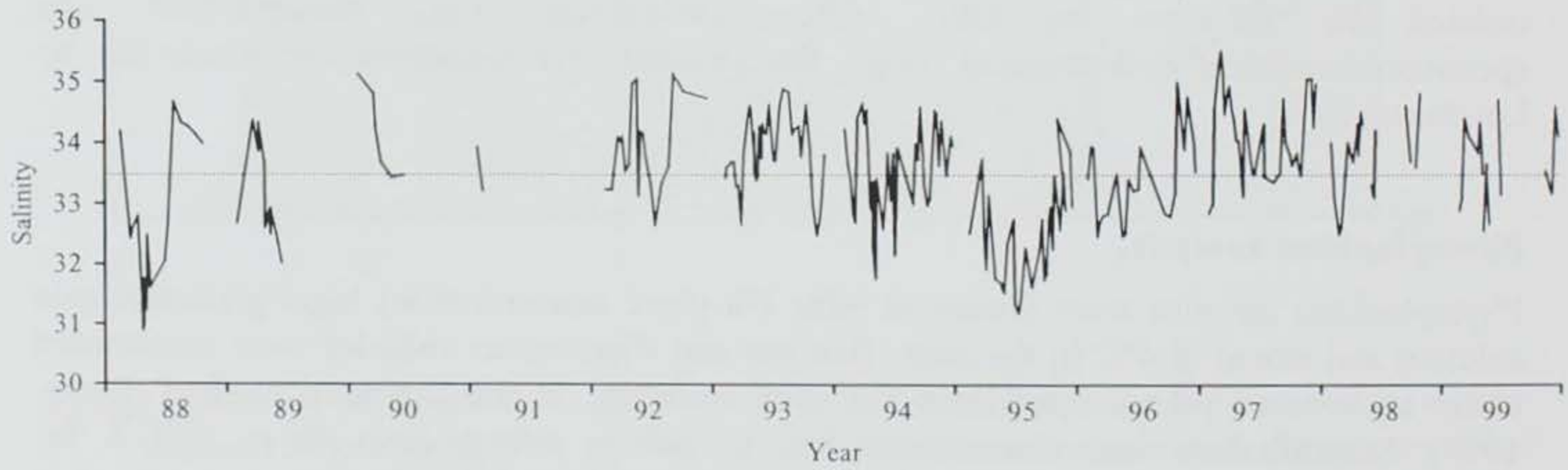


Figure 2: Temporal changes of salinity recorded at station 330 during the period 1988-1999. Dotted line represents the average salinity for the whole period.

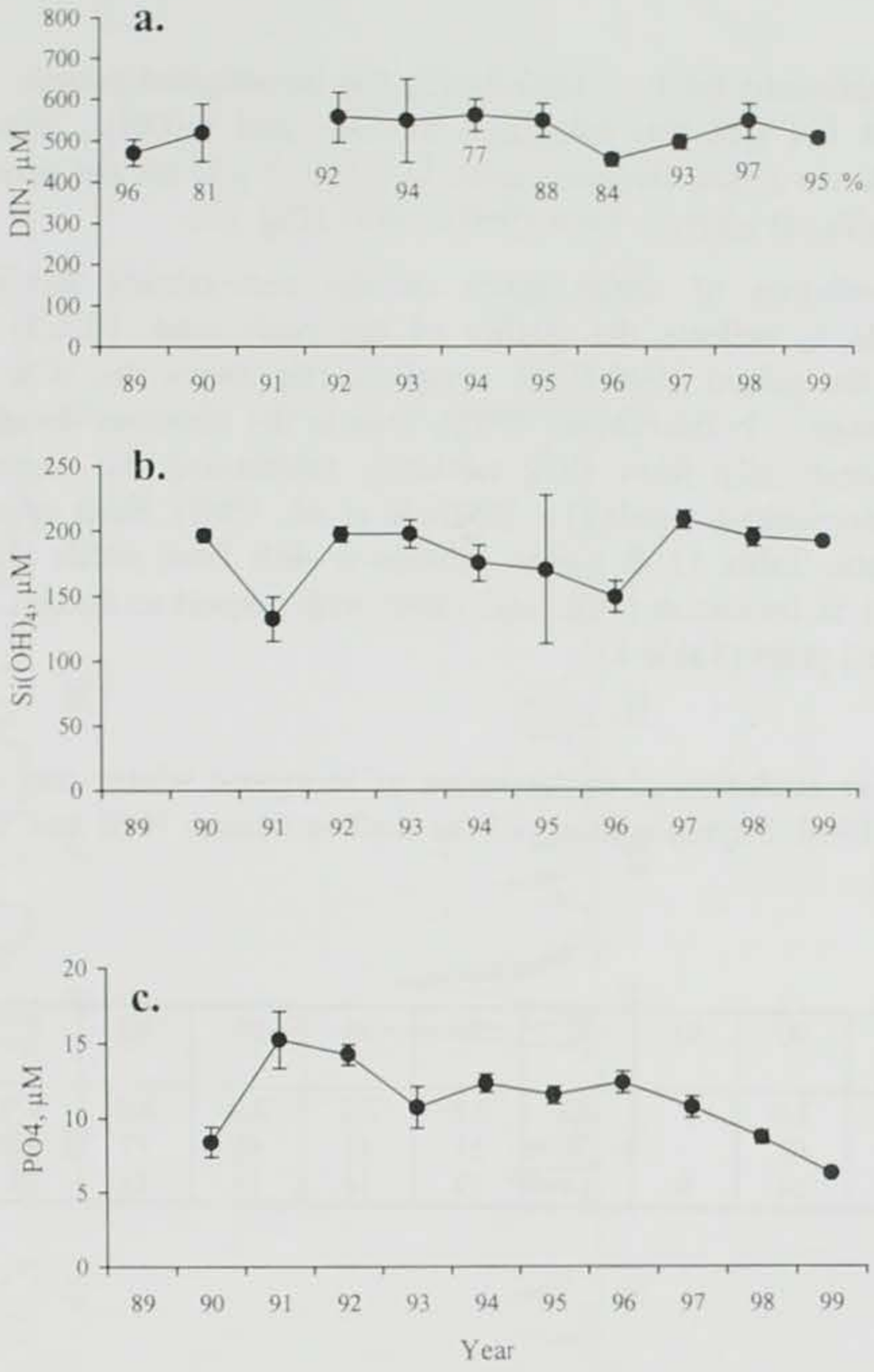


Figure 3: Evolution of inorganic nutrient loads (µM) for the period 1989-1990. a.) Dissolved inorganic nitrogen with indication of the percentage of NO_3 ; b.) Silicate; c.) Phosphate. Vertical bars represent the standard deviation of the Y-intercept at IC = 95 %.

The nutrient load of the investigated area was indirectly assessed on the basis of winter geographic surveys of nutrient concentrations and salinities. At the end of January and early February, nutrients reach their highest level due to completion of mineralisation processes and very reduced phytoplanktonic activities. Under such conditions, nutrients behave essentially in a conservative way, as reflected by the linearity of the recorded dilution curves (not shown). The extrapolation of each nutrient dilution curve to zero-salinity reflects the actual nutrient enrichment of the coastal area, taking into account advective transport, freshwaters input and benthic contribution in these shallow coastal waters. Statistically derived Y-intercept was calculated from least-squares regression for each winter data set and used here to assess the possible evolution of inorganic nutrient loads for the period 1989-1999 (Fig. 3). Clearly, extrapolated concentrations of DIN - mainly composed of NO_3 (Fig. 3a) - and $Si(OH)_4$ (Fig.

3b) did not vary significantly (at $P < 0.01$) during the investigated period. Average nutrient load of 520 µM and 181 µM was calculated for DIN and $Si(OH)_4$, respectively. On the contrary, PO_4 load shows a clear decrease from 15.2 to 6.2 µM for the period 1991 to 1999 after, however, a significant increase from 1990 to 1991 (Fig. 3c).

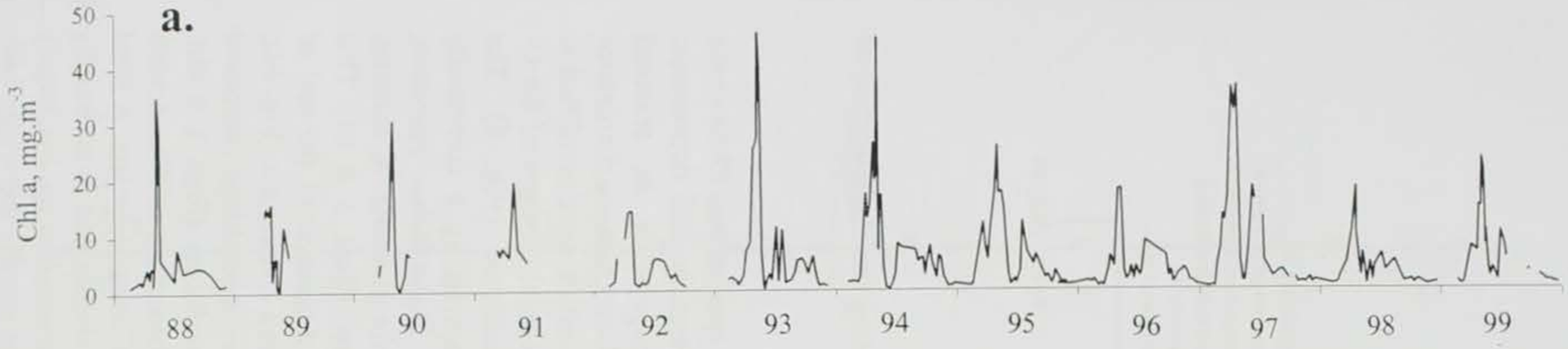
The year-to-year evolution of molar ratios of the zero-salinity extrapolated nutrient concentrations (Table 1) reflects the quality of the river load. DIN:Si did not change significantly during the period 1990-1999, remaining far above the N:Si requirements of coastal diatoms (average = 1; Brzezinski, 1985). Due to the observed decrease of PO_4 load, DIN:P increased dramatically from 1992 to 1999, reinforcing the excess of N over P compared to the phytoplankton needs (16; Redfield *et al.*, 1963). Such upward trend is also observed for Si:P ratio Table 1). It would indicate a shift from rather well-balanced Si:P (1992-1995) or slight Si limitation (1991 and 1996) with respect to diatom requirement to P limitation for the 3 last years (Table 1).

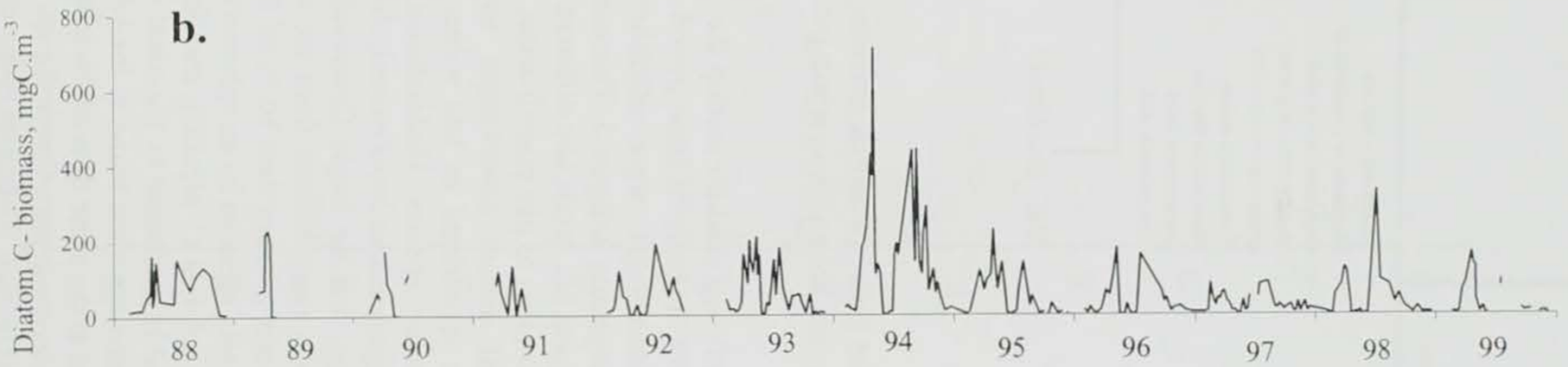
Table 1: Year-to-year evolution of molar ratios of inorganic winter nutrient loads for the period 1990-1999. Phytoplankton N:P as well as diatom N:Si and Si:P requirements are indicated.

	Phyto-Plankton	90	91	92	93	94	95	96	97	98	99
DIN:Si	1	2.6	-	2.8	2.8	3.2	3.2	3.0	2.4	2.8	2.6
DIN:P	16	62	-	39	51	45	48	37	46	63	81
Si:P	16	24	9	14	19	14	15	12	20	23	31

Phytoplankton succession in the Belgian coastal waters between 1988 and 1999.

Seasonality and recurrence are the most striking features of phytoplankton blooms recorded at station 330 during period 1988-1999 (Fig. 4). The seasonality is reflected by the level of Chla concentrations reached (Fig. 4a): a well pronounced spring outburst followed by further fluctuating summer and fall accumulations before reaching the winter level in mid-November, at the end of the vegetative season. These characteristics are also visible in the C-biomass pattern of the two main phytoplankton community components, the diatoms and *Phaeocystis* colonies (Fig. 4b, c). One single short-living bloom of *Phaeocystis* colonies occurs repetitively during spring after a first diatom growth. Diatoms are present throughout the vegetative period including that of *Phaeocystis* bloom (Fig. 4b, c) and are the main phytoplankter during early spring, summer and fall periods (Fig. 4b).





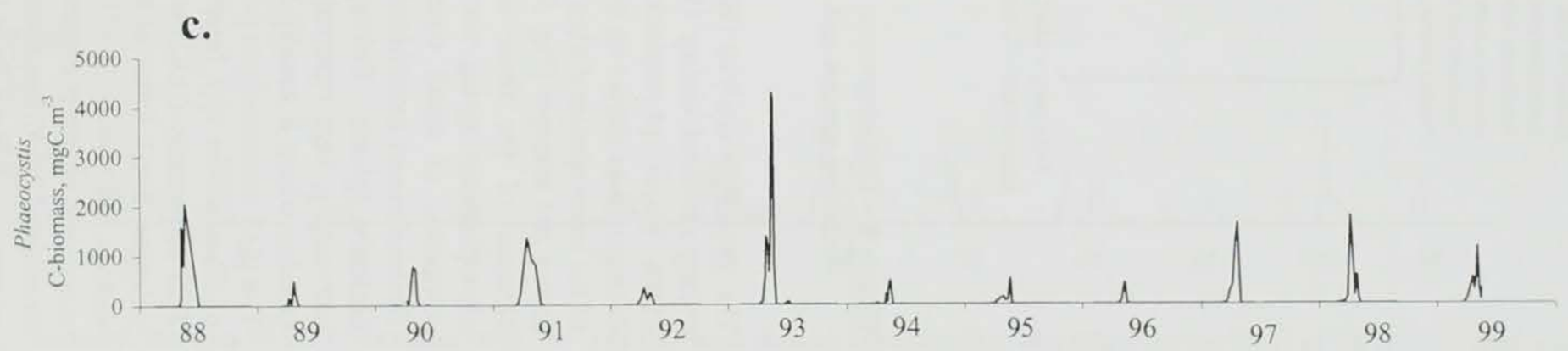


Figure 4: Seasonal changes in phytoplankton biomass at station 330 for the period 1988- 1999: a) Chl a concentrations ($mg.m^{-3}$); b) C-biomass of diatoms ($mgC.m^{-3}$) and c.) *Phaeocystis* colonies ($mgC.m^{-3}$).

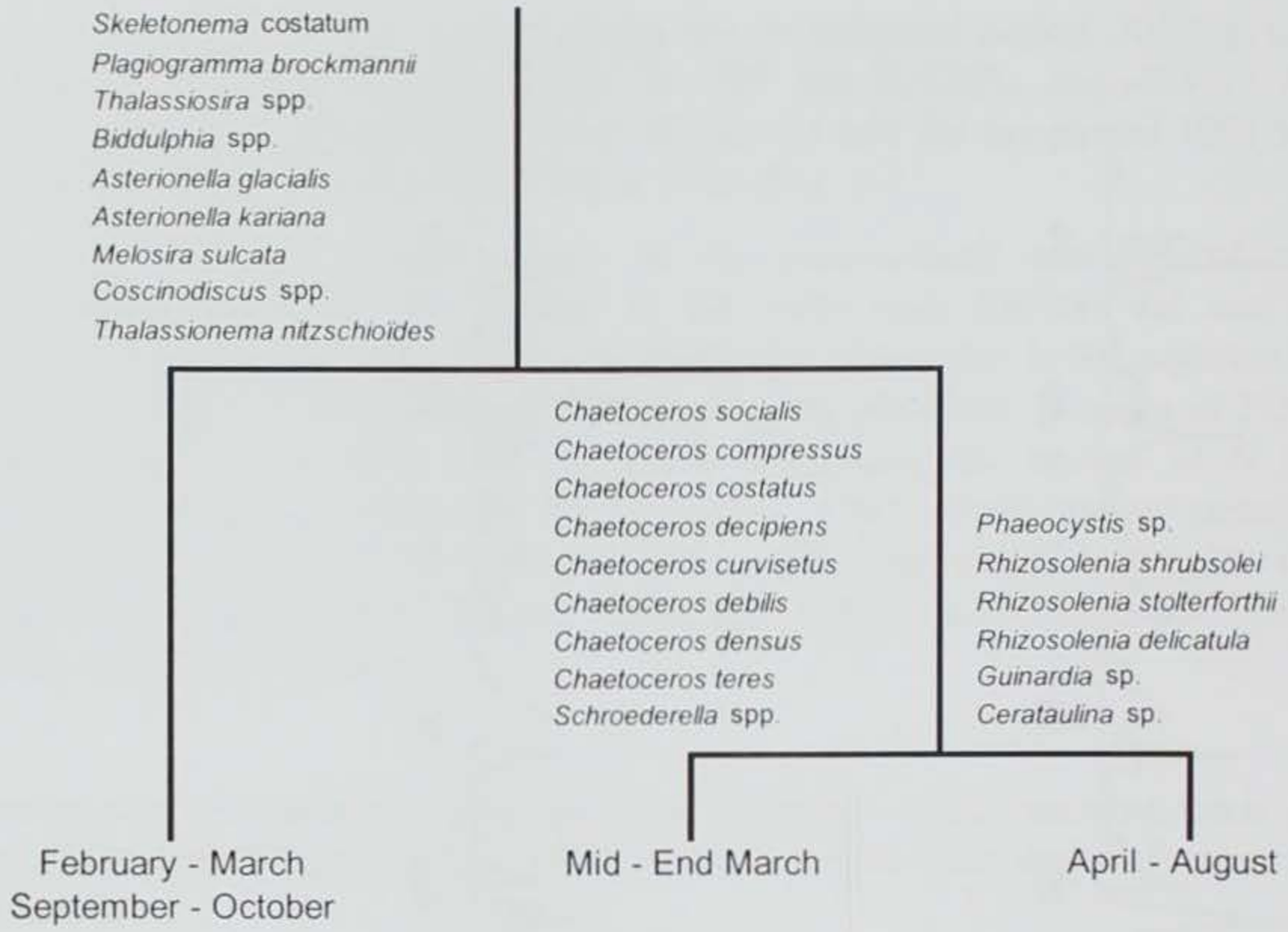


Figure 5 : Dendrogramm, obtained from TWINSPAN analysis, presenting the classification of diatom species and *Phaeocystis* colony into various periods.

The TWINSPAN analysis (Hill, 1979) was used for sorting the diatom species and *Phaeocystis* colony recorded during 1988-1999 on basis of their period of occurrence and dominance. Three major phytoplankton assemblages were identified corresponding to seasonal distribution as shown on the dendogramm in figure 5. This analysis shows that the dominance of the same species during spring and fall, while two different communities were identified in spring and summer (Fig. 5). According to this classification, the seasonal changes of the C-biomass of the three identified diatom assemblages were considered (Fig. 6). The phytoplankton spring succession is initiated in late winter-early spring by a community composed of small colony-forming species *Thalassiosira* spp., *Skeletonema costatum*, *Thalassionema nitzschioïdes*, *Plagiogramma brockmannii*, *Asterionella glacialis*, *A. kariana*, *Melosira sulcata*, *Biddulphia* spp. and the larger *Coscinodiscus* spp. (Figs. 5 & 6a). This community, here reported as DIA1, again blooms from the end of August until the end of October where it usually contributes to more than 90 % of the diatom C-biomass (Fig. 6a). The DIA1 community is progressively replaced by an ephemeral diatom assemblage composed of *Chaetoceros* spp. (mainly the colonial *C. socialis*), and *Schroederella* sp. (Figs. 5 & 6b). This community (DIA2) is invariably associated to the onset of the blooming of *Phaeocystis* colonies. Small forms of these latter are often found in the setae of *Chaetoceros* spp. A third diatom community (DIA3) blooms at the same time of *Phaeocystis* colonies and is essentially composed of larger diatoms *Rhizosolenia* spp., *Guinardia* sp. and *Cerataulina* sp. but largely dominated by *Rhizosolenia* spp. (Fig. 6c). The latter genus composes usually the whole bulk of summer diatoms from June to about mid-September (Figs. 5 & 6c).

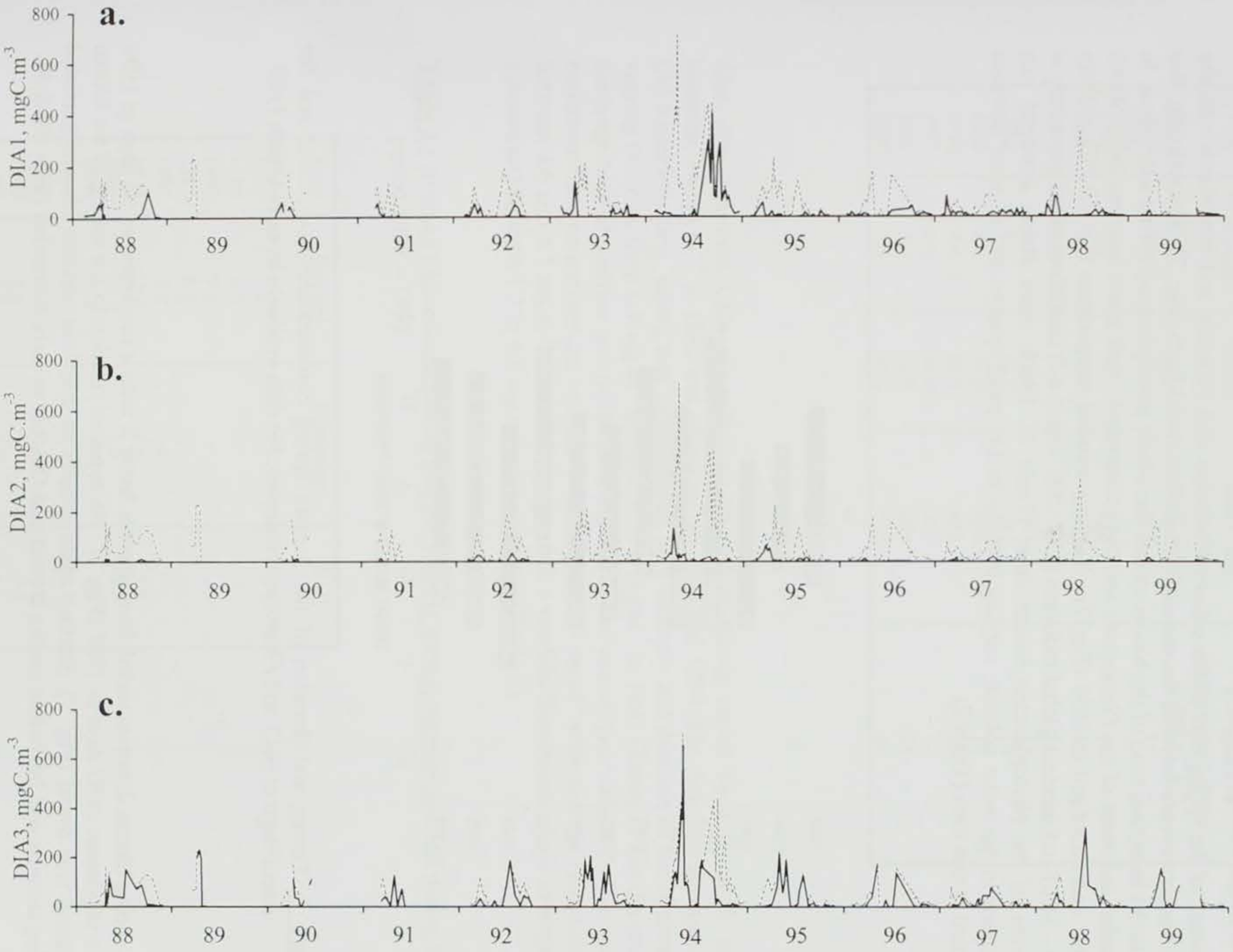


Figure 6: Seasonal changes of the C-biomass ($mgC.m^{-3}$) of the three diatom communities identified on the basis of TWINSPAN analysis, at station 330 for the period 1988- 1999: a) DIA1: *Thalassiosira* spp., *Skeletonema costatum*, *Thalassionema nitzschioïdes*, *Plagiogramma brockmannii*, *Asterionella glacialis*, *A. kariana*, *Melosira sulcata*, *Biddulphia* spp. and *Coscinodiscus* spp.; b.) DIA2: *Chaetoceros* spp. and *Schroederella* sp.; c.) DIA3: *Rhizosolenia* spp., *Guinardia* sp. and *Cerataulina* sp.

Phytoplankton bloom timing, duration and magnitude.

While the phytoplankton seasonal succession was remarkably repeated from 1988 to 1999, a significant interannual variability was observed in the timing and duration of phytoplankton blooms (Figs. 4 & 6).

The onset of the spring succession occurred between mid-February and late-March with the more frequent events between the end of February and mid-March (Fig. 7). Interestingly, this shift in the inception time of the bloom moves the whole phytoplankton spring succession. In particular, the onset of the *Phaeocystis* and DIA3 community took place between early-March in 1997 and mid-April in 1996 (Fig. 7). Varying ambient temperature (4 - 8.2 °C), salinity (32.2 - 34.9) and incident global radiation (253 - 597 $J.cm^{-2}.d^{-1}$) conditions were prevailing at the time of the phytoplankton bloom onset (Table 2). Much more stable averaged PAR conditions in the water column, varying from 10 to 14 µmol quanta. $m^{-2}.s^{-1}$ were however recorded at that time (Table 2).

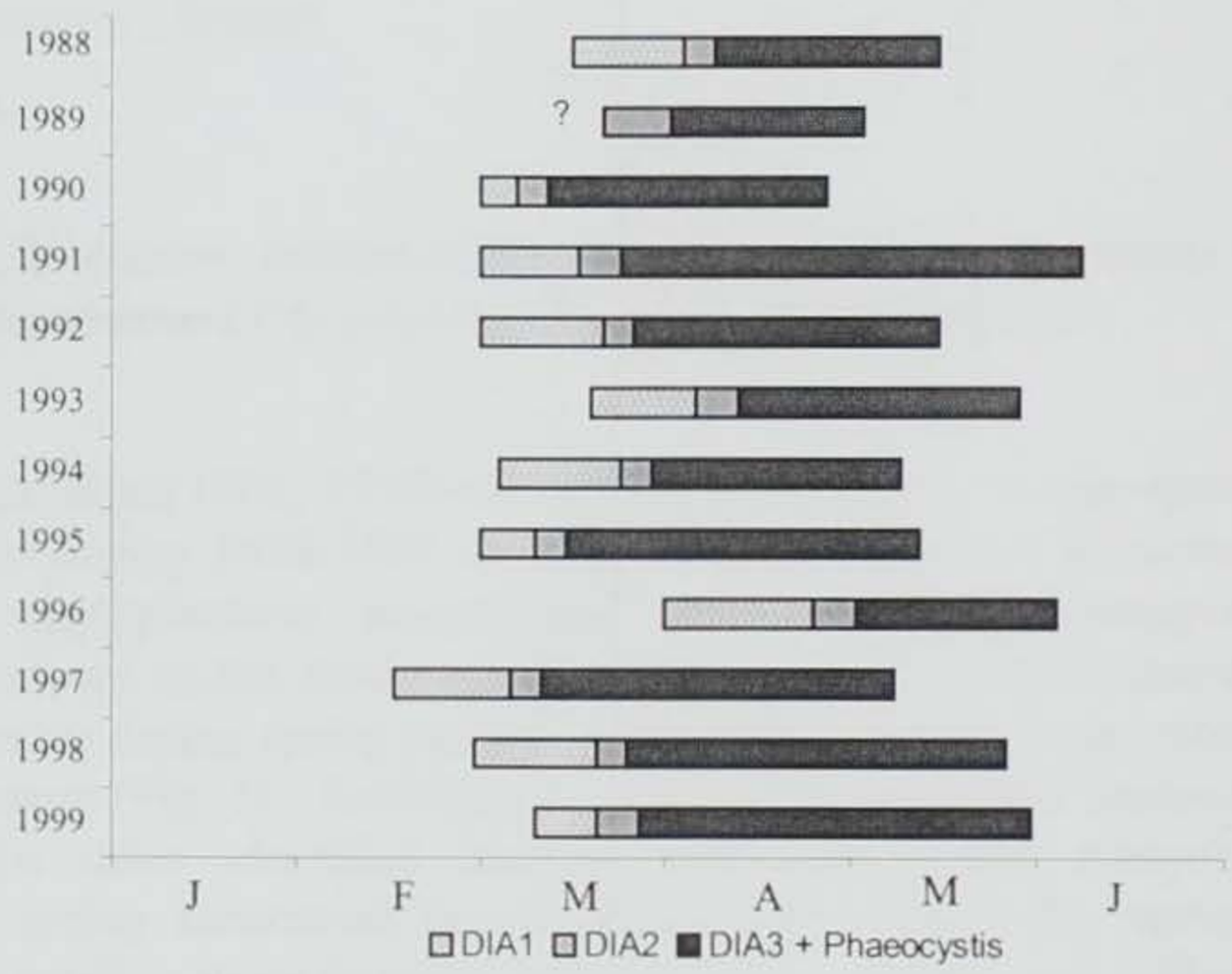


Figure 7: Timing and duration of the 3 main spring communities: DIA1, DIA2 and the assemblage of DIA3 and *Phaeocystis* colonies. No data available in early March 1989.

The spring bloom duration varied from about a factor 2 with a minimum of 56 days in 1996 and a maximum of 97 days in 1995 (Fig. 7). No apparent relation to the timing of the bloom inception was found (Fig. 7). Starting again usually in mid-June, diatom blooms persisted during summer and fall before ending around mid-October to early November (Figs. 4b & 6).

Table 2: Interannual variations of temperature, salinity, global radiation and averaged PAR in the water column at the onset of the spring bloom.

Year	Temperature (°C)	Salinity	Global Solar Radiation ($J.\ m^{-2}.d^{-1}$)	Averaged PAR in the water column ($\mu mol.m^{-2}.s^{-1}$)
1988	5.8	32.2	384	14
1989	-	-	-	-
1990	8.2	34.6	414	14
1991	6.0	33.6	377	13
1992	5.9	34.0	323	11
1993	6.0	33.5	597	12
1994	5.5	33.9	351	10
1995	8.0	32.8	323	10
1996	4.0	32.8	421	13
1997	4.0	34.9	253	12
1998	5.9	33.0	294	12
1999	7.0	34.1	573	14

Also, the maximum Chla concentration reached in spring varied by a factor 3, ranging between 14 $mg.m^{-3}$ in 1992 and 45 $mg.m^{-3}$ in 1993 and 1994 (Fig. 4a). The average spring Chl a concentration, taking into consideration both intensity and duration of the blooms, was varying twofold, from 6.9 $mg.m^{-3}$ in 1989 to 17.4 $mg.m^{-3}$ in 1997 (Table 3). On the contrary, the magnitude of summer and fall blooms showed lower year-to-year variability. In summer, maximum Chla concentrations varied between 10 and 16 $mg.m^{-3}$ with average values, ranging between 3.6 and 5.7 $mg.m^{-3}$ (Table 3). During fall, a twofold fluctuation of the average Chla concentrations, from 1.7 to 4.1 $mg.m^{-3}$ was estimated (Table 3).

Table 3 : Average Chla concentrations ($mg.m^{-3}$) during spring, summer and fall during the period 1988 – 1999.

Year	Spring	Summer	Fall
1988	7.8	3.7	1.7
1989	6.7	-	-
1990	9.9	-	-
1991	9.2	-	-
1992	8.6	3.6	-
1993	16.0	4.8	3.9
1994	16.9	5.6	4.1
1995	10.4	4.5	1.8
1996	9.1	5.7	2.7
1997	17.4	5.6	1.6
1998	8.6	3.7	1.7
1999	8.9	4.2	2.5

The spring diatom-*Phaeocystis* colonies succession

Every year, the maximum Chl a concentration reached during spring corresponded to the blooming of both *Phaeocystis* colonies and DIA3 community (Figs. 4a-c & 6c). The maximum C-biomass reached by *Phaeocystis* colonies ranged from 340 $mgC.m^{-3}$ in 1992 to 4275 $mgC.m^{-3}$ in 1993 while the maximum C-biomass of DIA3 varied from 117 $mgC.m^{-3}$ in 1991 to 705 $mgC.m^{-3}$ in 1994 (Figs. 4c & 6c).

The observed variation of the maximum Chla concentration reflects both a difference in the magnitude of the two taxa but also their varying contribution to the phytoplankton community. This is evidenced when considering the C-biomass of both taxa integrated over the whole spring period. In this way, DIA3 contributed from only 3 % in 1997 to 94 % in 1994 of the total phytoplankton C-biomass (not shown).

Phaeocystis colonies are biological entities composed of cells secreting an exopolysaccharidic matrix deprived of nutrients. The relative proportion of the mucilaginous component of *Phaeocystis* colony C-biomass increases significantly during the course of the bloom and could represent up to 90 % of the colony C-biomass (Rousseau *et al.*, 1990). Calculated on a cellular basis, the relative proportion of DIA3 and *Phaeocystis* colonies average C-biomass changes dramatically with DIA3 C-biomass contributing between 30 and 96 % of the total C-biomass of spring phytoplankton community (Fig. 8).

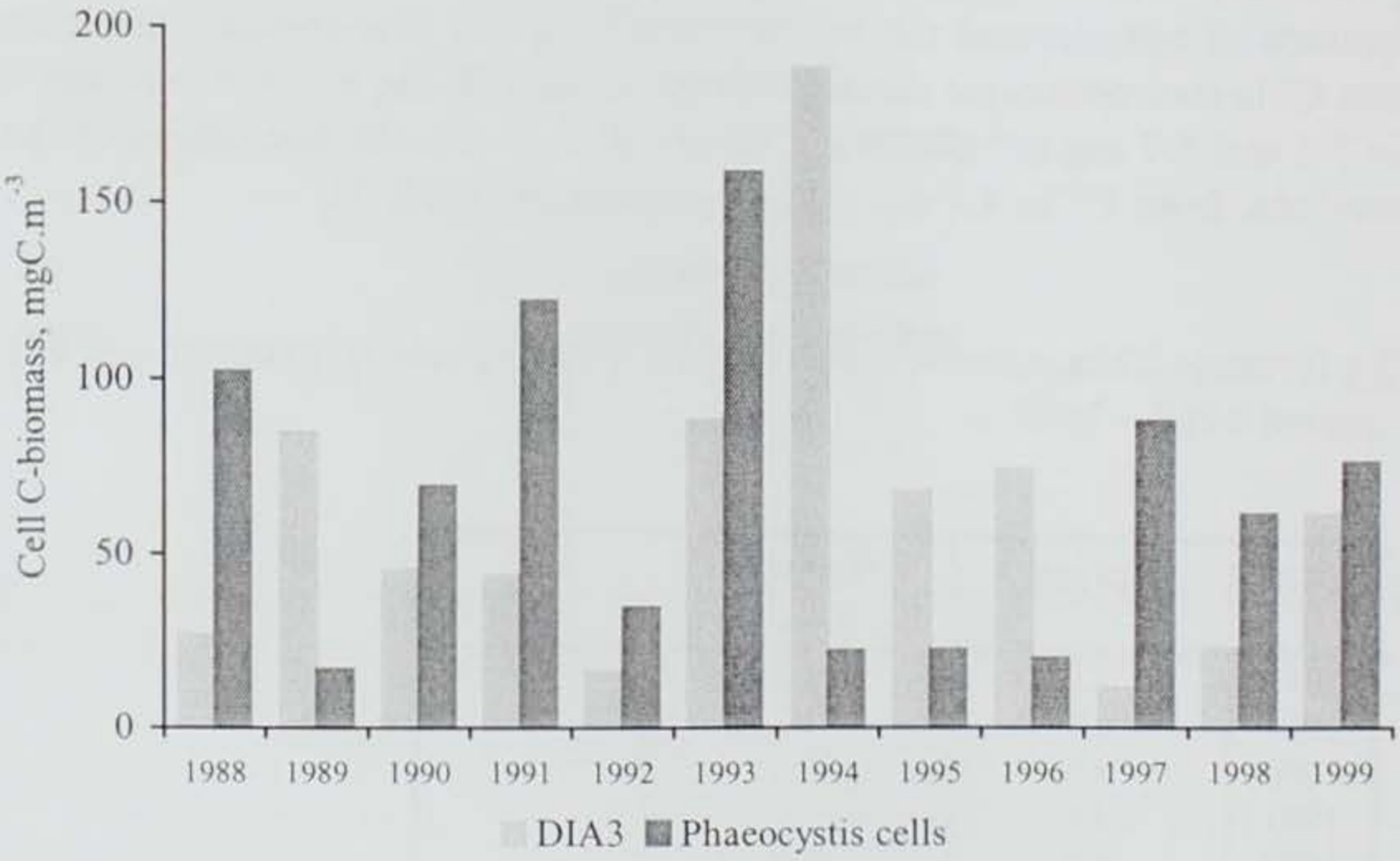


Figure 8: Interannual variations observed in the averaged spring C-biomass of the DIA3 community and *Phaeocystis* colonial cells.

Seasonal variations of inorganic nutrient concentrations.

The seasonal changes of dissolved inorganic nutrient (NH_4, NO_3, $Si(OH)_4$) and PO_4) concentrations are shown on Fig. 9. Nutrient concentrations reached at the end of the winter period fluctuated from year-to-year as a result of the combined effects of varying mixing of freshwater sources and remineralisation. For the 1988-1999 period, the winter concentrations were in average 3 µM, 33 µM, 18 µM and 1.2 µM for NH_4, NO_3, $Si(OH)_4$ and PO_4, respectively (Fig. 9). Compared to diatom and phytoplankton nutrient requirements (N:P:Si 16:1:16), these winter concentrations indicated a large excess of inorganic nitrogen (dominated at more than 90 % by NO_3) over $Si(OH)_4$ and PO_4 while these latter are well balanced. As a general trend, NO_3 and $Si(OH)_4$ varied similarly with a maximum in winter and a minimum during the spring bloom. A delay is however observed in the time of the NO_3 and $Si(OH)_4$ minimum concentrations. Later, during late spring and summer, NO_3 and $Si(OH)_4$ concentrations increased again and alternated between ephemeral peaks and very low values (Figs. 9b-c). From October, both NO_3 and $Si(OH)_4$ concentrations increased again progressively up to their maximum winter levels (Figs. 9b-c). NH_4 and PO_4 concentrations also showed a spring decrease but significant accumulations were recorded during late spring and summer due to very intensive heterotrophic activities occurring at that time (Figs. 9a & d; see Schoemann *et al.*, 1998). The concentrations of these nutrients largely fluctuated during the whole vegetative period before progressively increasing again during fall. During summer and fall, nutrient concentrations are generally anticorrelated to the diatom occurrence (Figs. 4 & 9), suggesting that top-down control could regulate phytoplankton dynamic at that time.

Spring nutrient dynamics

The spring dynamics of nutrients and phytoplankton was highlighted by zooming on the spring period 1996 (Fig. 10). This year presents indeed a typical pattern of phytoplankton succession and nutrient. Interannual variations are however recorded in the amplitude of both phytoplankton C-biomass and nutrients concentrations (Fig. 9).

In February, nutrient concentrations fluctuated around average winter levels of 32 µM, 6 µM, 22 µM and 1 µM of NO_3, NH_4, $Si(OH)_4$ and PO_4 respectively (Fig. 10 b-d). The decrease of nutrient concentrations started from early-March, except for NO_3 which exhibited a higher concentrations in Mid-March (Fig. 10 b-d). The spring nutrient decrease was associated to the very moderate blooming of DIA1-2 assemblage (Fig.10 a-d). In the same time, however, an increase of NO_3, $Si(OH)_4$ and PO_4 concentrations was correlated to a salinity decrease. At the end of the flowering of DIA1-2, NH_4, $Si(OH)_4$ and PO_4 reached a minimum values of 0.5, 1.8 and 0.6 µM, respectively while 28 µM of NO_3 were left over (Fig. 10a-d). From mid-April to early-May, *Phaeocystis* colonies and DIA3 community consumed the remaining stock of NO_3 while NH_4, $Si(OH)_4$ and PO_4 concentrations remained at low levels during their flowering (Fig. 10a-d). From their decline, the observed increase of nutrient concentrations suggests the predominance of heterotrophic activities, remineralisation and grazing (Fig.10a-d). Particularly, NO3 increased from 6 to 12 µM suggesting that high nitrification processes occurred at that time (Fig. 10 ab).

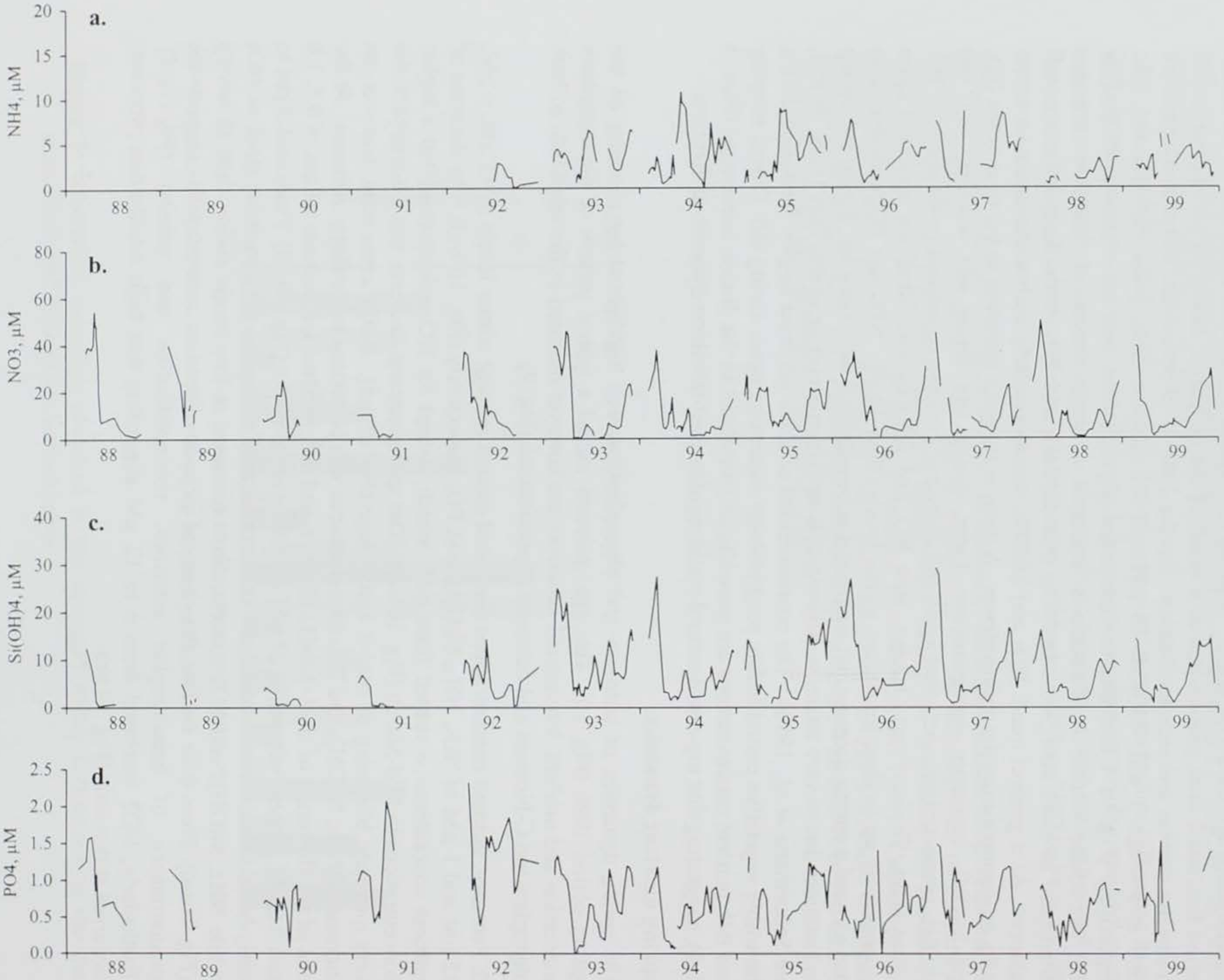


Figure 9: Temporal changes of the dissolved inorganic nutrient concentrations (µM) at station 330 for the period 1988- 1999: a) NH_4; b.) NO_3; c.) $Si(OH)_4$ and d.) PO_4.

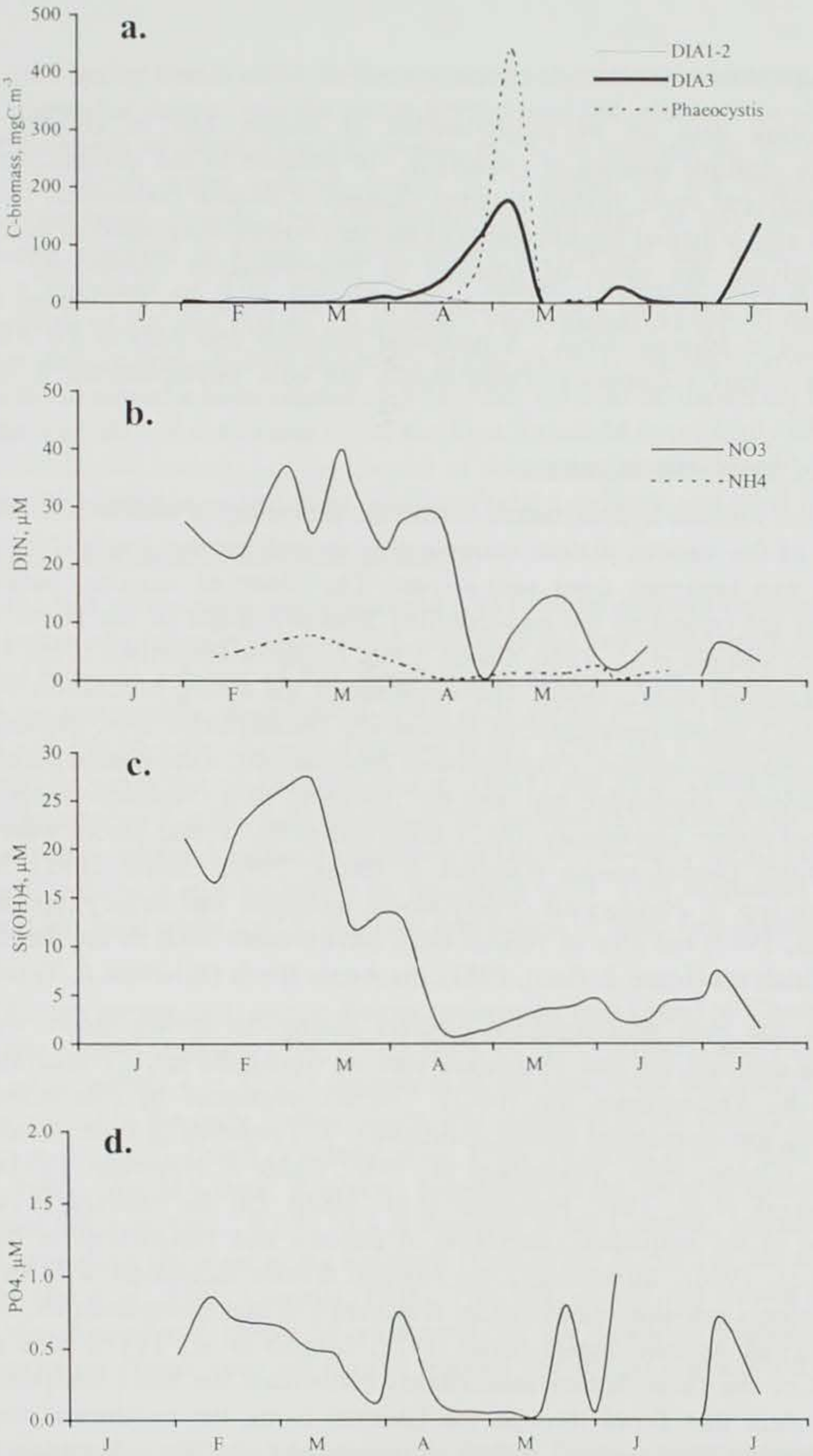


Figure 10: Temporal changes observed at station 330 during spring 1996 of: a.) phytoplankton C-biomass ($mgC.m^{-3}$) : DIA1 + 2, DIA3 and *Phaeocystis* colonies; and dissolved inorganic nutrient concentrations (µM): b.) NO_3 and NH_4; c.) $Si(OH)_4$ and d.) PO_4.

Discussion

The monitoring data set of phytoplankton at station 330 of Belgian coastal waters encompasses multiple sources of variability. In addition to the intrinsic succession pattern within the seasonal cycle, highly dynamic changes of natural conditions, *i.e.* meteorological changes but also potential modifications of nutrient conditions related to human activities are processes driving the observed changes in phytoplankton community. These different elements will be discussed here and compared to the dynamics of diatoms and *Phaeocystis* colony bloom in adjacent areas. A particular attention was paid to the *Rhizosolenia* spp.-*Phaeocystis* colony community which makes the bulk phytoplankton biomass during the spring bloom.

The diatom-Phaeocystis succession

In spite of the variable hydrodynamic conditions prevailing at station 330, the phytoplankton succession of the various diatom communities as well as the diatom-*Phaeocystis* colonies succession was recurrent from year-to-year. The observed seasonal pattern and floristic composition are typical of the non-stratified Southern Bight of the North Sea (Gieskes & Kraay, 1975; Velhuis *et al.*, 1986, Cadée, 1986; Cadée & Hegeman, 1986; 1991b; Bakker *et al.*, 1990; Lancelot *et al.*, 1998). The initiation of the spring succession by small colonial diatoms (DIA1) whose representative species are *Thalassiosira* spp., *Skeletonema costatum*, *Thalassionema nitzschioïdes*, *Plagiogramma brockmannii*, *Asterionella glacialis, A. kariana, Melosira sulcata, Biddulphia* spp. and the flowering of a *Chaetoceros* spp.- *Schroederella* sp.dominated diatom community (DIA2) at the end of this vernal bloom were also reported in adjacent Dutch coastal waters (Gieskes & Kraay, 1975; Cadée, 1991). The presence of *Chaetoceros* spp. at *Phaeocystis* colony bloom inception was largely reported for the North Sea (Boalch, 1987) but also in various other environment such as the Tasman Bay (Chang, 1983), the Irish sea (Jones & Hacq, 1963), the Arctic fjords (Eilertsen & Taasen, 1984).

Every year, the bulk phytoplankton biomass during the spring bloom was composed of *Phaeocystis* colonies but also of diatoms such as *Guinardia* sp., *Cerataulina* sp. but largely dominated by *Rhizosolenia* spp (DIA3). The co-occurrence of *Phaeocystis* colonies and *Rhizosolenia* spp.-dominated diatom community was reported in other areas of the Southern North Sea (Cadée, 1986; Veldhuis et al., 1986; Cadée & Hegeman, 1991a; Rahmel *et al.*, 1995; Peperzak *et al.*, 1998; Philippart *et al.*, 2000). On the contrary, it contrasts with the observation of the mutualistic exclusion of diatoms and *Phaeocystis* in the German Bight (Weisse *et al.*, 1986). Interestingly, the reported diatom succession, with an accumulation of the *Rhizosolenia* spp.-dominated diatom community in late spring-summer, is also recorded in poorly enriched Atlantic waters (Grall, 1973; Sournia *et al.*, 1987). This suggests that the succession of the three diatom assemblages constitutes the basic component of the North Atlantic waters that flows through the Channel along the continental coast. *Phaeocystis* colonies appear then as an additional component of the phytoplankton community, superimposing on the natural diatom succession in response to the continental sources of anthropogenic nutrients (Lancelot *et al.*, 1987).

Factors controlling the bloom inception

The onset of the spring bloom showed significant interannual variability which did not correlate with ambient temperature or incident global radiation. Temperatures ranging between 4°C and 8.2 °C and solar radiations between 253 and 597 $J.cm^{-2}.d^{-1}$ were indeed

recorded at the DIA1 spring bloom onset. Rather our results show that the ambient light, driven by the load of suspended matter, was the factor determining the onset of the phytoplankton bloom. A light threshold of about 12 µmol quanta. $m^{-2}.s^{-1}$ in average for the period 1988-1999, in this 17m-depth water column, would be required for the beginning of the phytoplankton exponential growth. The light control of the start of phytoplankton spring bloom was already reported in Dutch coastal waters by Gieskes and Kraay (1975) who estimated that a threshold of 0.03 gcal.$cm^{-2}.min^{-1}$ (about 10 µmole quanta.$m^{-2}.s^{-1}$) was necessary for growth of the first diatom community. These authors observed that the bloom was starting later inshore than offshore due to the higher turbidity caused by river discharges. Also Cadée (1986) observed a close relationship between the start of the spring bloom in the Marsdiep and the turbidity. For all these areas, the light threshold is primarily determined by the turbidity of the coastal waters, *i.e.* the content of suspended matter driven by river loads and resuspension of suspended matter under control of tidal amplitude and wind forcing.

Factors controlling the diatom-Phaeocystis succession

The growth of the late winter-early spring diatoms (DIA1) under nutrient repleted conditions, suggests that a better adaptation of these diatom species to the low temperature and irradiance prevailing at that time. As a matter of fact, these species are characterized by higher specific growth rates in the temperature range typical of the early spring (4-8°C) compared to the late spring diatoms and *Phaeocystis* colonies (Lancelot *et al.*, 1998). In a comparative study on the photoacclimation behaviour of *Thalassiosira* sp. (DIA1) and *Phaeocystis* colonies, Meyer *et al.* (2000) demonstrated that the light saturation for optimal cell division rate was reached for the diatom at water column irradiance prevailing in late winter-early spring (10 µE.$m^{-2}.s^{-1}$) while *Phaeocystis* colony is still far below its saturation level. This adaptation to both low temperature and light level could well explain the dominance of these first diatom community in early spring.

The growth of the late winter-early spring diatom community (DIA1) and of *Chaetoceros* spp.-*Schroederella* spp. (DIA2) consumed the winter stock of NH_4, $Si(OH)_4$ and PO_4 while NO_3 concentrations ranging from 4 to 32 µM depending of the year, were left over. Such nutrient spring decrease related to the early diatom bloom was observed in the French (Grossel, pers. comm.; Sournia *et al.*, 1987), Dutch (Veldhuis *et al.*, 1986; Peperzak *et al.*, 1998) and German (Weisse *et al.*, 1986) coastal waters. However, depending on the level of enrichment of the area, NO_3 amount left over vary. While NO_3 is depleted in the Channel, increasing amounts of NO_3 are left over along the coast of the Southern Bight of the North Sea due to the residual circulation (Lancelot *et al.*, 1991; Lancelot, 1995). This NO_3 excess was shown to govern the amplitude of the *Phaeocystis* bloom as evidenced by the positive relationship existing between the maximum *Phaeocystis* cell density reached and the NO_3 availability (Lancelot, 1995).

Very low PO4 concentrations (0.08 µM) persisting during *Phaeocystis* growth were reported for the Southern North Sea (van Bennekom *et al.*, 1975; Veldhuis *et al.*, 1986, Weisse *et al.*, 1986; Rahmel *et al.*, 1995). The low PO4 concentrations recorded at the time of *Phaeocystis* inception was suspected to induce the process of colony formation from solitary cells present in the water (Veldhuis *et al.*, 1987b; Cariou *et al.*, 1994). The possible role of *Chaetoceros* spp. in triggering the *Phaeocystis* colony formation, through mechanical or allelopathic effects, was also suspected but never proved (Boalch, 1987; Cadée, 1991; Rousseau *et al.*, 1994). It was also suggested that light could act as a trigger for colony formation (Peperzak,

1993). *Phaeocystis* colony bloom onset was indeed related to a rapid light increase in the water column (Peperzak *et al.*, 1988). This observation is compatible with recent experimental work demonstrating the ability of *Phaeocystis* colonies to respond to a rapid light increase through xanthophyll cycling (Meyer *et al.*, 2000).

The varying contribution of Rhizosolenia–dominated community and Phaeocystis colonies to the spring bloom

The observation of sometimes high diatom biomass at the time of *Phaeocystis* colony bloom contrasts with the general assumption that the spring bloom is entirely dominated by *Phaeocystis* colonies after diatoms are limited by $Si(OH)_4$.

The relative contribution of *Phaeocystis* colonies and *Rhizosolenia*-dominated community to the phytoplankton spring bloom was changing significantly from year to year (Fig. 8). This high variability recorded in the relative proportion of the *Rhizosolenia* community and *Phaeocystis* was not corresponding to a drastic interannual modification of the nutrient loads for the investigated period (Table 1). A significant ($n = 11$; $r = 0.81$ at $P<0.01$) negative correlation is however observed between the proportion of the *Rhizosolenia* spp.-dominated community (DIA3) to the total phytoplankton cell (DIA3 + *Phaeocystis* colonial cell) C-biomass averaged over the spring bloom, and the average salinity prevailing during their flowering (Fig. 11). This relation suggests that the main source of variability at the time scale of 12 years is related to changes in hydrodynamic conditions prevailing at station 330 due to different meteorological driving forces. Clearly, the *Rhizosolenia* spp.-dominated community represented a larger proportion of the phytoplankton C-biomass at lower salinity while *Phaeocystis* colonies reach higher C-biomass when salinity is high during the spring bloom (Fig. 11). This suggests a different spatial distribution of both taxa with diatoms blooming closer to freshwater source and *Phaeocystis* colonies more offshore.

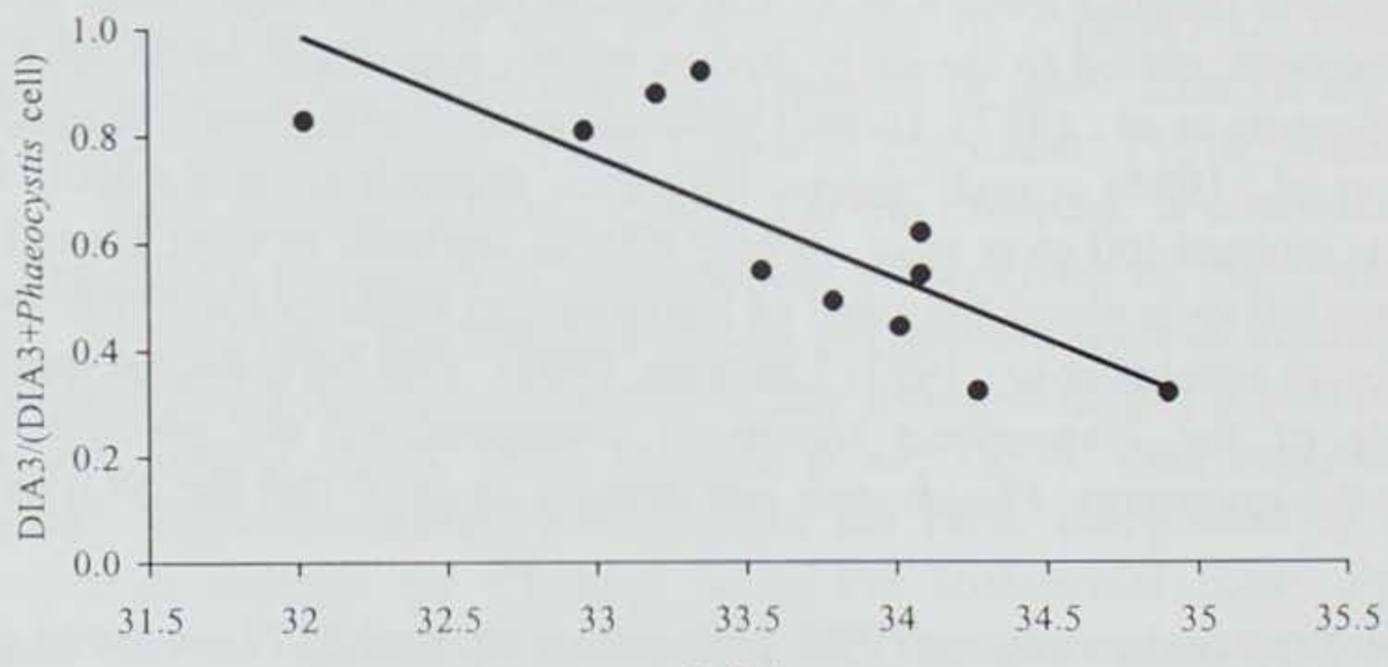


Figure 11: Relationship between the proportion of average C-biomass of DIA3 to (DIA3 + *Phaeocystis* colonial cell) and the average salinity prevailing at station 330 during their growth.

The virtual geographical distribution of diatoms and *Phaeocystis* colonies observed could be related to the nutrient signature of the water masses. The distribution of the winter nutrient ratios along salinity gradient (Fig. 12) calculated on basis of the data used in Fig. 3, shows a clear significant (at $P < 0.01$) decrease of N:P (Fig. 12 a) and Si:P (Fig. 12 c) while N:Si (Fig. 12 b) do not show any trend. The nutrient quality gradient shows the excess of N over P and Si with regards to phytoplankton needs is present in the whole Belgian coastal waters. On the contrary, a deficit of Si over P compared to diatom requirement is observed above salinity 31. This suggests that only below salinity 31, a well-balanced nutrient environment compared to the diatom nutrient requirements *i.e.* N:P:Si = 16:1:16, was prevailing. This could explain the dominance of diatoms over *Phaeocystis* colonies under these nutrient conditions.

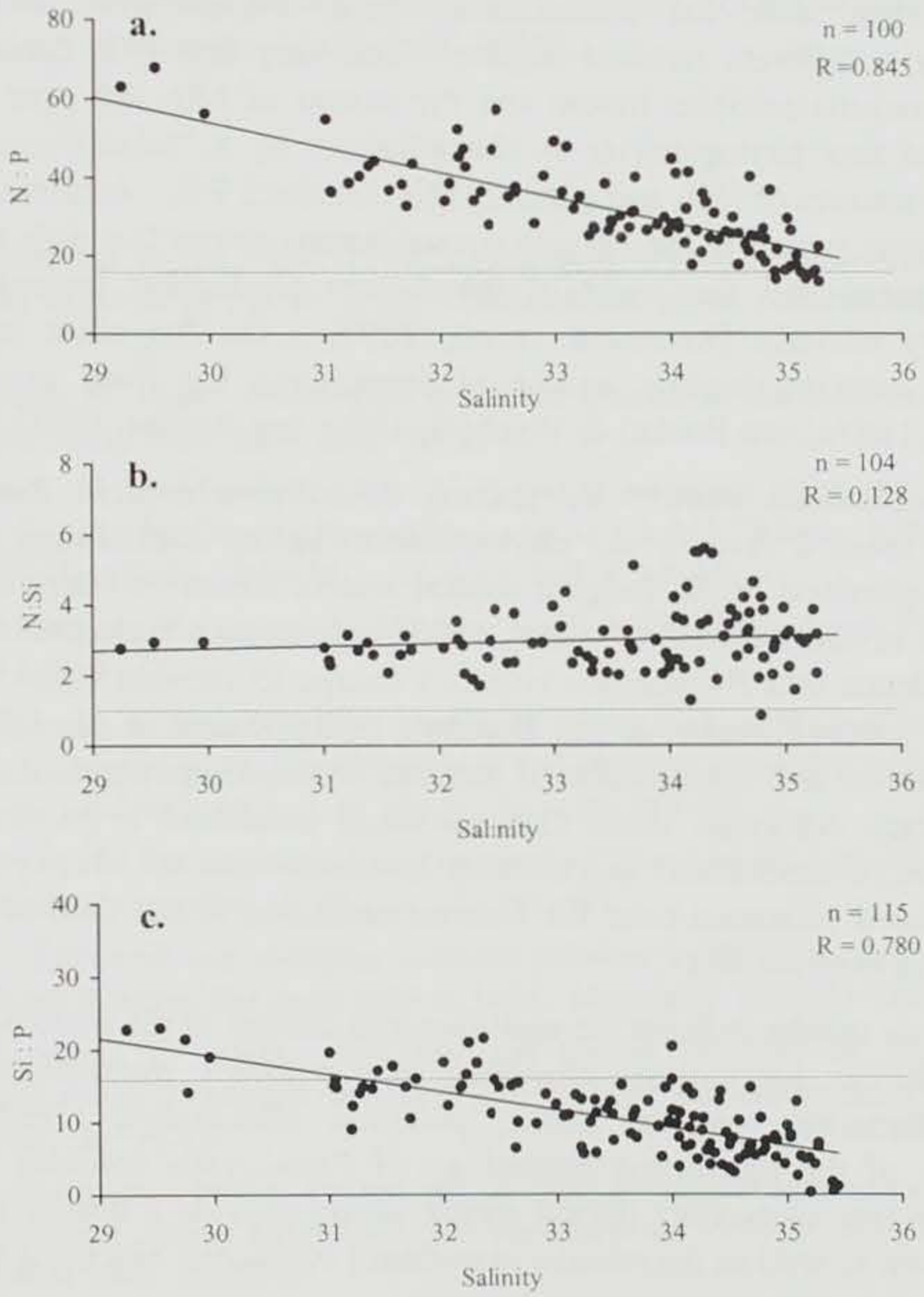


Figure 12: Distribution of winter nutrient ratios along salinity gradient: a.) N:P; b.) N:Si and c.) Si:P. Dotted lines represent the diatom and phytoplankton nutrient requirement.

The additional impact of the decreasing trend observed in the PO_4 concentrations which superimposes on the spatial variability could however not be evidenced in this study (Fig. 3; Table 1).

The occurrence of important *Rhizosolenia delicatula* biomass, with cell density reaching more than 1.2 10^6 cells.l^{-1} (3 times the maximum concentrations recorded at station 330), in the Channel was associated to very low nutrient concentrations (Sournia *et al.*, 1987). Their growth occurred indeed at NO_3, NH_4, PO_4 and $Si(OH)_4$ concentrations of 0.3, 0.4, 0.1 and 1.5 µM, respectively (Sournia *et al.*, 1997). Well adapted to grow at these low nutrient concentrations, this species is sustained by either remineralisation or riverine inputs (del Amo *et al.*, 1997) provided they are well-balanced. The demonstrated low $Si(OH)_4$ requirement of the *Rhizosolenia* community confirms their ability to grow at low Si supply (Rousseau *et al.*, submitted).

While co-occurring with the *Rhizosolenia* spp., *Phaeocystis* colonies are however characterized by a different nutrient regime. The very low PO_4 concentrations recorded during the *Phaeocystis* colonies bloom and the excess of NO_3 left over by the early spring diatoms suggests this phytoplankter is characterized by a "mixed" nutritional behaviour, growing on new sources of NO_3 and partially remineralised PO_4. A carbon budget established for the spring bloom period in the Belgian coastal waters shows that only a third of the diatom biomass was grazed and that most of the diatom production underwent lysis and was remineralised by bacteria (Rousseau *et al.*, 2000). On the other hand, the ability of *Phaeocystis* to grow on organic source of phosphorus has been established in cultures (Veldhuis *et al.*, 1987a; van Boekel & Veldhuis, 1990; van Boekel, 1991).

A selective zooplankton control by grazing could also explain the variability of the contribution of these 2 taxa to the phytoplankton spring community. Seasonal cycle of herbivorous zooplankton in the Belgian coastal waters shows that high density are recorded from early April to end September (Hecq, 1982) suggesting a high grazing pressure. There is now strong evidence that *Phaeocystis* colonies escape to mesozooplankton grazing in these shallow coastal waters (Hansen & van Boeckel, 1991; Weisse *et al.*, 1994; Gasparini *et al.*, 2000). It is however still questionable if diatoms of the *Rhizosolenia*-dominated community are grazed or not. Although these diatoms could constitute a good source of food for mesozooplankton, a mechanical or chemical hindrance due to *Phaeocystis* mucilage could explain the reduced clearance rates for diatoms measured at the time of *Phaeocystis* colony bloom (Gasparini *et al.*, 2000).

All together, these results indicate the multifactorial control of the diatom-diatom and diatom-*Phaeocystis* colonies succession, consisting in a complex interaction between light and temperature adaptation, nutrient status, potential allelopathic effects. Only a careful characterization of the nutritional physiology of *Phaeocystis* colonies and the three main diatom communities succeeding during spring would provide a deeper understanding of the succession pattern as well as the relative importance of the two blooming taxa.

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Chapter 6

Diatom succession and dissolved silicate availability in the Belgian coastal waters (Southern North Sea)

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submitted to Aquatic Microbial Ecology

Diatom succession and dissolved silicate availability in the Belgian coastal waters (Southern North Sea)

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Abstract

The significance of silicon in the diatom bloom dynamics in the eutrophicated coastal waters of the Belgian coastal waters (Southern Bight of the North Sea) was assessed by investigating phytoplankton succession and nutrient changes during the seasonal cycle of 1995. Particular attention was paid to establish the link between dissolved silicate availability, diatom species succession and their silica content. The silica cellular content (silicification level) of natural diatom communities was determined on basis of two methods. The measurement of biogenic silica after alkaline digestion of particulate material and estimate of diatom carbon biomass derived from microscopic observations was performed on weekly samples from the reference station 330 of the Belgian coastal waters. As an alternative method, parallel 24 hour-kinetics experiments of ^{14}C incorporation into proteins, as an index of phytoplankton growth, and ^{32}Si incorporation into diatom-dominated natural communities were conducted in semi-situ conditions. The seasonal pattern of diatom growth was characterized by three diatom assemblages dominated by small neritic species, *Chaetoceros* spp.- *Schroederella* sp. and *Rhizosolenia* spp, respectively. Superimposing the two latter diatom communities, *Phaeocystis* colonies bloomed during about one month. The two methods evidenced that the 3 diatom assemblages were characterised by distinct silica content with Si:C values decreasing from 0.68 to 0.04 along the course of the spring bloom and ambient dissolved silicate decreasing from about 13 µM to a minimum of 1.3 µM. The positive relationship observed between the diatom Si:C ratio and ambient dissolved silicate would suggest that dissolved silicate availability play a major role in shaping the diatom succession in the Belgian coastal waters. The twofold variability of the diatom Si:C ratio recorded within each assemblage would, however, suggest that intra-specific variability might be important as well. Our data are compared with available information on the inter- and intra-specific variability of individual marine diatom species and their potential controlling factors. An additional laboratory experiment with pure *Skeletonema costatum* cultures evidenced that detrital diatoms could well represent an important proportion of the extracted BSi and hence significantly overestimate the calculation of Si : C ratio of diatoms.

Key words : Diatoms, succession, silicate, silicification level

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Introduction

Diatoms have an absolute requirement for silicon and the availability of dissolved silicate can limit diatom growth. Depletion of dissolved silicate relative to inorganic nitrogen and phosphate has been observed to select for non-siliceous algae. As conceptually discussed in Officer & Ryther (1980) and Billen *et al.* (1991), this shift is exacerbated in coastal waters receiving anthropogenic inputs of nitrogen and phosphorus and results in the dominance of often poorly edible non-siliceous algae with harmful consequences.

Such is the case of the Belgian coastal waters (Southern Bight of the North Sea) largely influenced by freshwater sources with high concentrations of nutrients of anthropogenic origin. The winter signature of these coastal waters (Lancelot *et al.*, 1991; Lancelot 1995) shows nitrogen (mostly nitrate) excess, and silicate and phosphate deficiency relative to diatom silica requirements (Bzrezinski, 1985) and nitrogen and phosphorus phytoplankton needs (Redfield *et al.*, 1963). As a consequence, the spring phytoplankton bloom is recurrently dominated by large unpalatable *Phaeocystis* colonies succeeding to an early spring diatom bloom (Lancelot *et al.*, 1987 ; Cadée & Hegeman, 1991; Lancelot, 1995, Lancelot *et al.*, 1998). Nutrient changes along the spring bloom suggest that the growth of early spring diatoms is limited by dissolved silicate availability (Billen *et al.*, 1991; Schoemann *et al.*, 1998; Rousseau *et al.*, in preparation) while the magnitude of the *Phaeocystis* bloom is determined by the excess of nitrate (Lancelot, 1995; Lancelot *et al.*, 1998). The bloom of *Phaeocystis* colonies in the Belgian coastal waters is generally unique and persists during 20-40 days in spring (Rousseau *et al.*, in preparation). On the contrary, diatoms are always present and contribute significantly to the phytoplankton community throughout the vegetative season including periods of *Phaeocystis* occurrence when ambient dissolved silica is very low (Lancelot *et al.*, 1998). At this time, two consecutive diatom assemblages with different species composition succeed to the early spring diatom community (Lancelot *et al.*, 1998). This suggests that freshwater input and re-mineralisation processes are sustaining the growth of post-spring diatoms making more complex the link between silicate availability and diatom succession and coastal eutrophication in general, as already reported for other coastal areas (Ragueneau *et al.*, 1994; Del Amo *et al.*, 1997). Hence a highly relevant question with this respect is whether the ambient silicate is an important factor in the selection of the diatom species. Earlier microscopic observations in the English Channel (Cooper, 1933) reported indeed that a thin-walled species (*Cerataulina pelagica*) succeeded to a thick-walled one (*Coscinodiscus gravii*) when dissolved silicate declined during the spring bloom. Unfortunately the diatom silica content was not measured. On the other hand, an extensive laboratory study conducted on 27 marine species reported an interspecific variability of the diatom silicification level Si:C of about one order of magnitude (0.04-0.42; Brzezinski, 1985). Yet the link between diatom succession and dissolved silicate availability in natural environments is probably more complex due to the intraspecific variability with silica content of species decreasing under silicon limitation (Tilman & Kilham, 1976).

In order to better understand the significance of silicon in eutrophicated coastal seas, phytoplankton succession and nutrient changes were investigated in the Belgian coastal waters of the North Sea during the seasonal cycle of 1995. Particular attention was paid to establish the link between dissolved silicate availability, diatom species succession and their silica content. The determination of the diatom silica content was estimated indirectly based on the combination of different methods (chemical and microscopic, radiotracers) in order to circumscribe the interference of non-diatom carbon and detrital particulate silicon of various

origin (lithogenic material and detrital diatom frustules). In the first method, the silica cellular content of the natural diatom communities was determined on basis of the measurement of biogenic silica (BSi) after alkaline digestion of particulate material (Paasche, 1980; Krausse *et al.*, 1983) and estimate of diatom carbon biomass (C-biomass) derived from microscopic observations. The protocol of Ragueneau & Tréguer (1994) was used to remove the interference of lithogenic silica (LSi). Additional laboratory experiments with pure diatom cultures were conducted to estimate the interference of dead diatoms.

As an alternative method to estimate the silicification level of natural diatoms, we conducted parallel time-course studies of ^{14}C and ^{32}Si incorporation into diatom-dominated natural communities. Indeed the recent ^{32}Si technique (Tréguer *et al.*, 1991; Brzezinski & Phillips, 1997) has been applied successfully in various marine environments to measure the BSi production associated to living diatoms (*e.g.* Tréguer *et al.*, 1991; Nelson & Dortch, 1996; Brzezinski *et al.*, 1997; 1998; Ragueneau *et al.*, 2000).

In this paper, we present data on the silica content of coastal diatom communities succeeding in the Belgian coastal waters of the North Sea along the 1995 seasonal cycle, as obtained by the application of both methods. Results are discussed in relationship with silicate availability and species dominance.

Material and methods

Diatom cultures. A *Skeletonema costatum* monospecific culture (North Sea strain) was grown in polycarbonate carboy in the modified F20 medium (Rousseau *et al.*, 1990) where 50 µM of $Na_2SiO_3.9H_2O$ (final concentration) were added. The culture was maintained under a 12h:12h light:dark cycle with an illumination intensity of 200 $\mu mole.m^{-2}.s^{-1}$ at a constant temperature of 8°C. In order to estimate the interference of detrital BSi, two sub-cultures of the exponentially growing culture were considered : one pure *S. costatum* and one enriched with empty frustules of *S. costatum* ($6.5\ 10^5$ $cell.l^{-1}$). The empty frustules were obtained by pasteurisation by heating at 70°C for 1 hour an aliquot of the stock culture. After a 24h delay, samples of each sub-culture were taken for diatom enumeration and C-biomass determination as well as BSi and nutrient analysis.

Field sampling. Measurements were conducted on samples collected at the station 330 (N 51° 26.00 - E 002° 48.50). This station is part of the Belgian network. It constitutes since 1988 the reference station for monitoring seasonal and interannual changes in the Belgian coastal waters (Southern Bight of the North Sea) owing to its average physico-chemical characteristics (depth, temperature, salinity, nutrient enrichment level). These continental coastal waters flowing northeastward are influenced by freshwater discharges of the Scheldt (Yang, 1998) and to a less extent, by the Rhine/Meuse (van Bennekom & Wetsteijn, 1990). The strong tidal currents combined to a shallow water column ensure a complete vertical mixing of the water column (Simpson, 1994). The discharge of continental suspended matter together with a quasi-permanent resuspension of sandy and silt sediments due to the high turbulent regime, supply these coastal waters with large amounts of particulate matter.

Surface seawater samples were collected with a bucket aboard R.V. Belgica. Sampling was conducted at a weekly frequency between February and December 1995. Sub-samples were

analysed for major nutrients (NH_4, NO_3, PO_4 and $Si(OH)_4$), phytoplankton and BSi. Radiotracer experiments were run with four diatom-dominated phytoplankton communities, three collected in March 1995 and one in October 1995.

Analytical methods

Major nutrients (NH_4, NO_3, PO_4 and $Si(OH)_4$) concentrations were determined on 0.45 µm filtered seawater according to the colorimetric methods described in Grasshof *et al.* (1983).

Diatoms as well as the non-siliceous *Phaeocystis* colonies and free-living cells were enumerated under inverted microscope (Leitz Fluovert) according to Utermöhl's method (Hasle, 1978). Samples were preserved with 1% (final concentration) lugol-glutaraldehyde solution and stored at 4°C in the dark until analysis. Magnification was chosen according to cell or colony size: 40 or 100 X for *Phaeocystis* colonies; 100 or 200 X for diatoms and 320 X for cells of size less than 50 µm. At least 400 cells were enumerated in total with 100 cells of the most abundant genus or species. Diatoms were enumerated and identified according to the genus level unless a species was easily identifiable or dominant. Their C-biomass was calculated on the basis of cell density and biometric factors determined for each species or genus. A specific average conversion factor was calculated from biovolumes measured on a cell population throughout the period of its development. Biovolumes were then converted using a carbon content factor of 0.11 pgC μm^{-3} of plasma volume (Edler, 1979). *Phaeocystis* colony and free-living C-biomass was calculated according to Rousseau *et al.* (1990).

BSi was determined on particulate material collected on 47 mm diameter polycarbonate membrane (0.6 µm pore size; Nucleopore). Filters were dried immediately after filtration at 50°C for 12h. Filters were stored at room temperature in sealed Petri dishes until analysis. Two methods were applied for determination of BSi of culture and field samples. The BSi from the *S. costatum* cultures was determined on basis of time course experiments on the dissolution of BSi in boiling 0.2 M NaOH (Ragueneau & Tréguer, 1994). BSi concentrations of the samples are then calculated from the Y-extrapolated intercept at the plateau (de Master, 1981). BSi concentrations from field samples were determined according to the sequential Na(OH)/HF digestion method (Ragueneau & Tréguer, 1994). This method is based on the determination of a factor correcting the interference of LSi leaching with BSi extraction during the NaOH digestion. A linear relationship holds between lithogenic material extracted and the BSi of samples collected during period of low biological activity. The slope of this regression represents the proportion of LSi extracted with BSi. Application of this method on samples from the Belgian coastal waters during winter and low diatom occurrence periods, estimates that the contribution of LSi to BSi was 0.28 ($n = 6$; $r^2 = 0.74$).

Tracer experiments

The silicification level of diatoms was estimated by running parallel 24 hour time-course kinetics of ^{14}C and ^{32} Si radiotracer experiments on natural communities sampled at station 330. Incubations were conducted at *in situ* temperature under saturating light intensity (200 $\mu mole.m^{-2}.s^{-1}$) under a 12h:12h light:dark cycle.

Incorporation of ^{14}C into proteins was measured kinetically. Protein synthesis is indeed considered as good index of phytoplankton growth (Lancelot *et al.*, 1986). Natural communities were inoculated with 100 $\mu Ci.l^{-1}$ of a solution of ^{14}C-bicarbonate (45 mCi. $mmole^{-1}$, Amersham). After incubation, samples were filtered according to the procedure described by Mathot *et al.* (1992). Proteins were then specifically isolated from other cellular

constituents after TCA precipitation (protocol of Lancelot and Mathot, 1985). Radioactivity incorporated into proteins was measured by liquid scintillation (Packard Tri-Carb) after dissolution of proteins in 10 ml Ready Safe (Beckman).

In parallel to ^{14}C experiments, time course of ^{32}Si incorporation by natural diatom communities was measured making use of the protocol of Tréguer *et al.* (1991). Natural samples were inoculated with 0.6 $\mu Ci.l^{-1}$ of a $^{32}Si(OH)_4$ solution (1.125 $\mu Ci.\mu gSi^{-1}$; Los Alamos, US) in NaOH 0.1N and incubated in 200 ml polycarbonate flask. After each incubation time, phytoplankton was collected by filtration on 0.6 µm polycarbonate membranes (Nucleopore) and rinsed with 0.2 µm-filtered seawater. The diatom ^{32}Si incorporation was estimated by detection of the Cerenkov radiation (Packard Tri-Carb) of the daughter ^{32}P after secular equilibrium was reached (about 4 months).

Results

Phytoplankton succession in the Belgian coastal waters in 1995.

Diatoms and *Phaeocystis* are major contributors of phytoplankton in the Belgian coastal waters (Rousseau *et al.*, in preparation). As reported by Fig.1, diatoms were almost permanently present in the Belgian coastal waters throughout the 1995 vegetative period. On the contrary the occurrence of *Phaeocystis* colonies, although impressive in biomass, was limited to two months in spring (Fig.1). Diatoms initiated the phytoplanktonic succession at mid-February, when a light threshold of about 12 µmole $m^{-2}s^{-1}$ was reached in the water column (Rousseau *et al.*, in preparation). Their biomass increased from a background level of 10 $mgC.m^{-3}$ up to the end of April when the maximum was reached (226 $mgC.m^{-3}$, Fig.1).

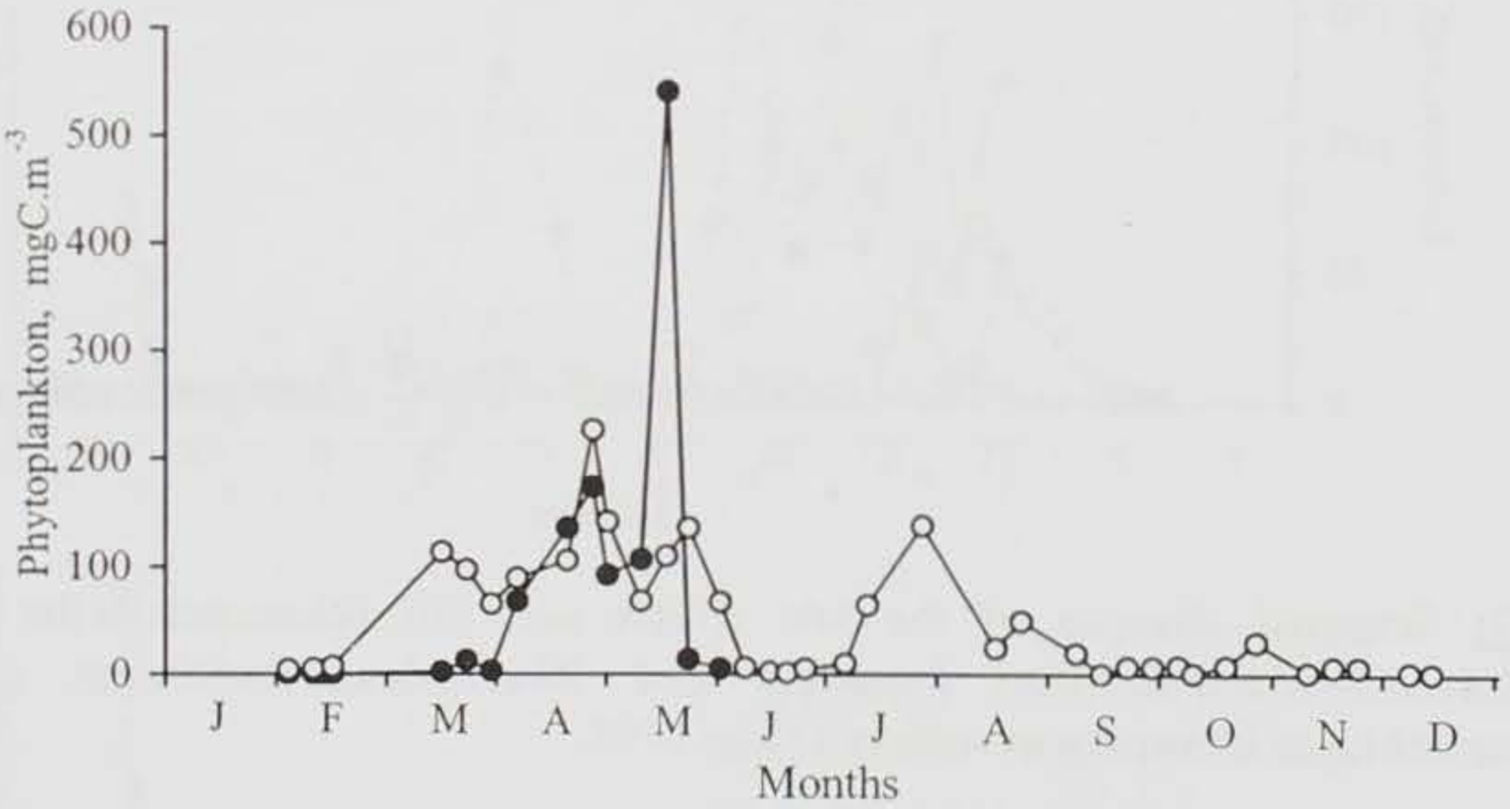

Figure 1: Seasonal changes of diatoms (open dots) and *Phaeocystis* (black dots) C-biomass observed at station 330 in 1995.

Diatoms maintained at level as high as 136 mgC.m^{-3} during the *Phaeocystis* bloom. At the end of May, diatoms nearly disappeared from the water column for a one-month period. A summer diatom bloom (138 mgC.m-3) occurred in July-August and a late rather modest diatom growth occurred in October. *Phaeocystis* colonies bloomed between late March and early June (Fig.1). As an average, *Phaeocystis* biomass reached was similar to that of diatoms except in mid-May when a maximum colony biomass of 540 mgC.m^{-3} was recorded (Fig. 1).

Three seasonally distinct diatom assemblages that represented together more than 95 % of the C-biomass could be identified from the taxonomic analysis (Fig. 2; Rousseau *et al.*, in preparation). The early diatoms (assemblage 1) were characterised by small colony-forming neritic species: *Asterionella glacialis, A. kariana, Thalassiosira* spp., *Thalassionema nitzschioïdes, Skeletonema costatum, Melosira sulcata, Plagiogramma brockmanii* (Fig. 2). Consecutive to this group a second diatom assemblage composed of *Chaetoceros* spp. and *Schroederella* sp. bloomed in mid-March (Fig.2). This community partly co-occurred with *Phaeocystis* colonies (Figs.1 & 2) but was rapidly replaced by a *Rhizosolenia*-dominated community (mainly R. *stolterfothii* and *R. delicatula*). The latter diatoms (assemblage 3) were present during the whole *Phaeocytis* bloom, reaching in April the maximum diatom biomass ever recorded in 1995 (Fig. 2). During summer, the diatom community was again dominated by the genus *Rhisozolenia*, mainly *R. delicatula* and *R. shrubsolei,* which composed the bulk diatom at the end of July (Fig. 2). The autumn diatom community was basically similar to the early assemblage with however the additional occurrence of large *Coscinodiscus* spp.

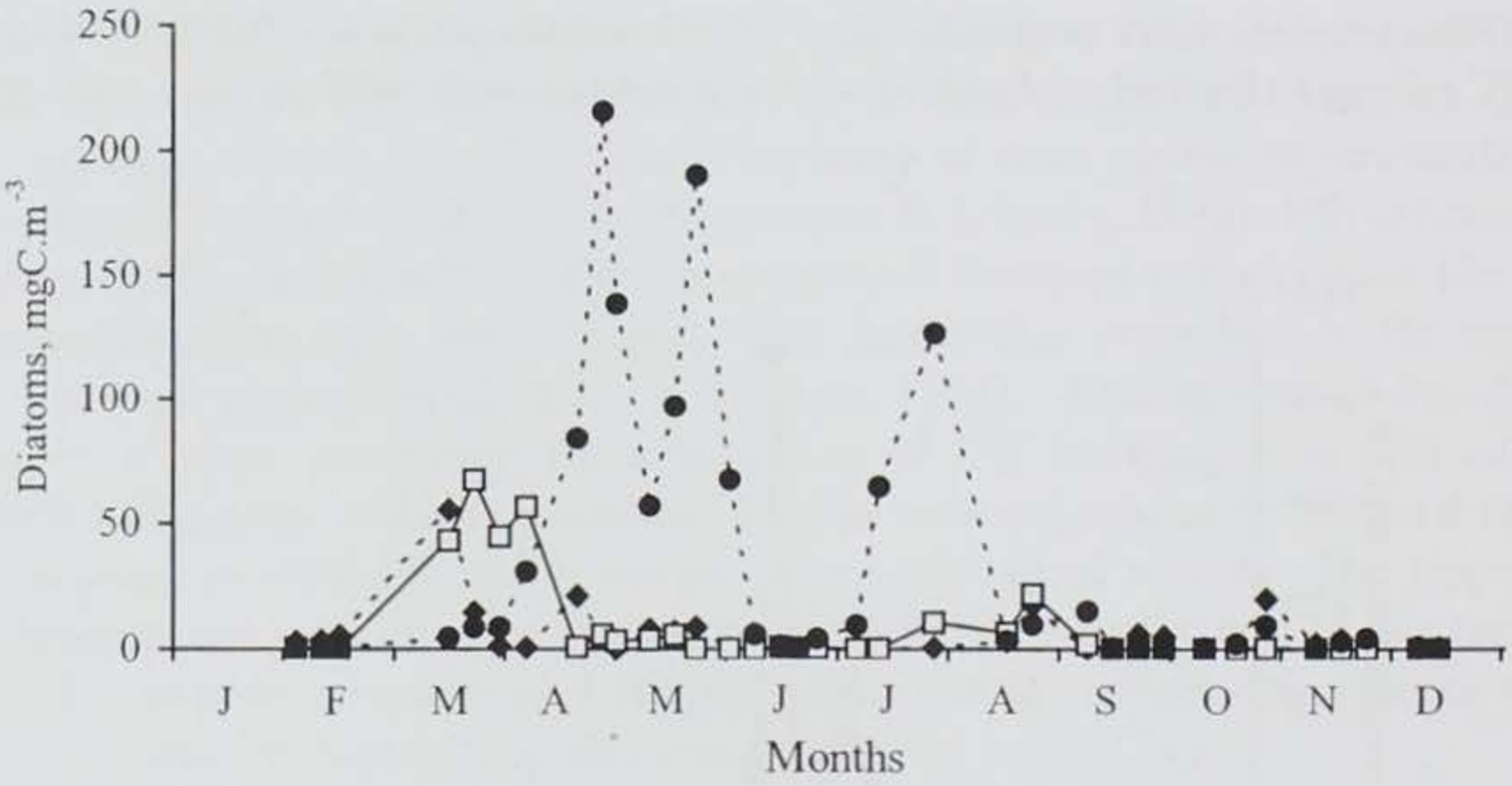

Figure 2: Seasonal changes of the late winter and fall (diamonds), the *Chaetoceros-Schroederella*-dominated (squares) and *Rhizosolenia*-dominated (dots) diatom assemblages blooming at station 330 in 1995.

Phytoplankton dominance and inorganic nutrient concentrations

In February, the early diatom community was responsible for the consumption of the $Si(OH)_4$ and PO_4 winter stocks, whose concentrations were in average 13 µM and 2.7 µM respectively (Figs. 2 & 3b,c). Some 3.9 µM $Si(OH)_4$ and 0.3 µM PO_4 were left over at the end of March when the diatom population shifted towards assemblage 2 (*Chaetoceros* spp. and

Schroederella sp.). The latter community maintained at low ambient $Si(OH)_4$ *i.e.* 1.5 µM up to mid-April, (Figs. 2 & 3b,c). At that time, a peak of 1.1 µM of PO_4 was recorded probably due to re-mineralisation of the early diatom-derived material. In contrast, NO_3 concentrations remained at an average level of 19 µM until early April (Fig. 3a). This large excess of NO_3 over $Si(OH)_4$ and PO_4 was nearly depleted at the time of *Phaeocystis* colony bloom maximum (Figs. 2 & 3a). In the second half of May, the decline of the *Phaeocystis* and *Rhizosolenia* spp. bloom corresponded to low NO_3, $Si(OH)_4$ and PO_4 concentrations, *i.e.* 1.3, 4.8 and 0.2 µM, respectively (Figs. 2 & 3a-c). The increase in nutrient concentrations observed in June was due to important re-mineralisation processes (Billen & Fontigny, 1987) in the nearly absence of diatoms. The latter was explained by the presence of high biomass of the giant omnivorous dinoflagellate *Noctiluca* (Schoemann *et al.*, 1998). As a consequence, a high transient NH_4 accumulation of 8.8 µM was observed in early June (Fig. 3a). These regenerated nutrient concentrations persisted before being taken up by summer *Rhizosolenia* spp. bloom (Figs. 2 & 3a-c). From early September, nutrient concentrations were progressively increasing up to their winter levels while phytoplankton biomass was decreasing due to light-limitation and grazing pressure.

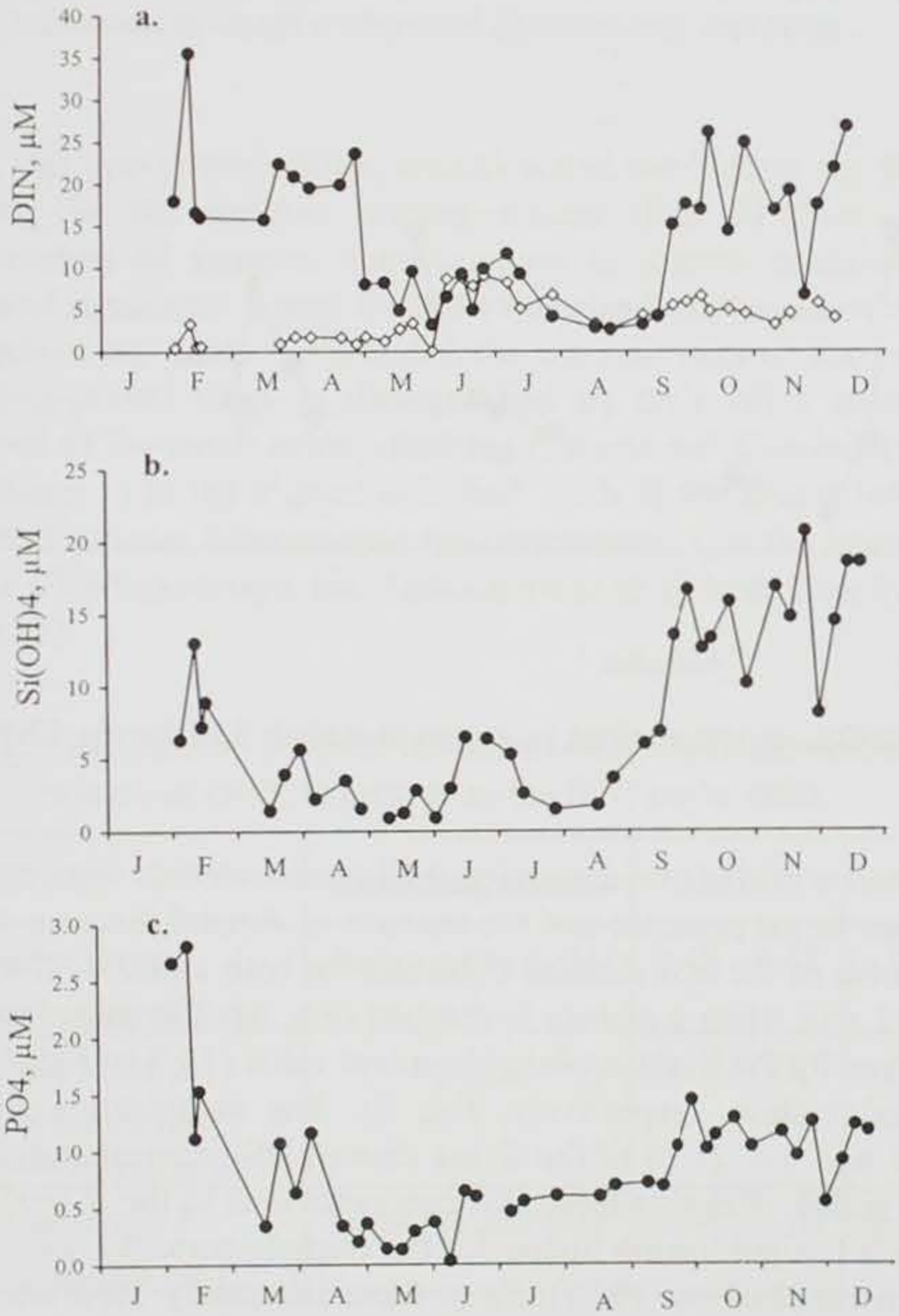

Figure 3: Seasonal changes of major inorganic nutrient concentrations: a) NO_3 (black dots), NH_4 (open dots); b.) $Si(OH)_4$; c) PO_4 recorded at station 330 during 1995.

Biogenic silica and the silicification level of diatom assemblages

The seasonal evolution of BSi (Fig. 4) shows a clear bimodal pattern which contrasts with that of diatom (Fig.2). So, BSi concentrations as high as 3.7 µM were associated to the modest bloom of early diatoms (Figs. 2 & 4). The sharp decrease observed at the end of April corresponded to the occurrence of *Rhizosolenia* spp. Low BSi concentrations of 0.6 µM persisted during the whole spring and summer period as long as *Rhizosolenia* was present in the water column (Figs. 2 & 4). Again, high BSi concentrations of about 2 µM were measured in fall but these were not clearly associated with diatom biomass maxima (Figs. 2 & 4). Clearly minimal values of BSi corresponded with the highest accumulation of diatom C-biomass and reversibly. As a whole, this suggests a large variability of the silica content of the different diatom assemblages but also a variable contribution to the BSi pool of dead diatoms and faecal pellets. Indeed, our analytical method for BSi determination measures all the particulate silicon associated to siliceous algae and their derived matter as cell debris and faecal pellets. When significant, this would overestimate the silica content of field diatoms.

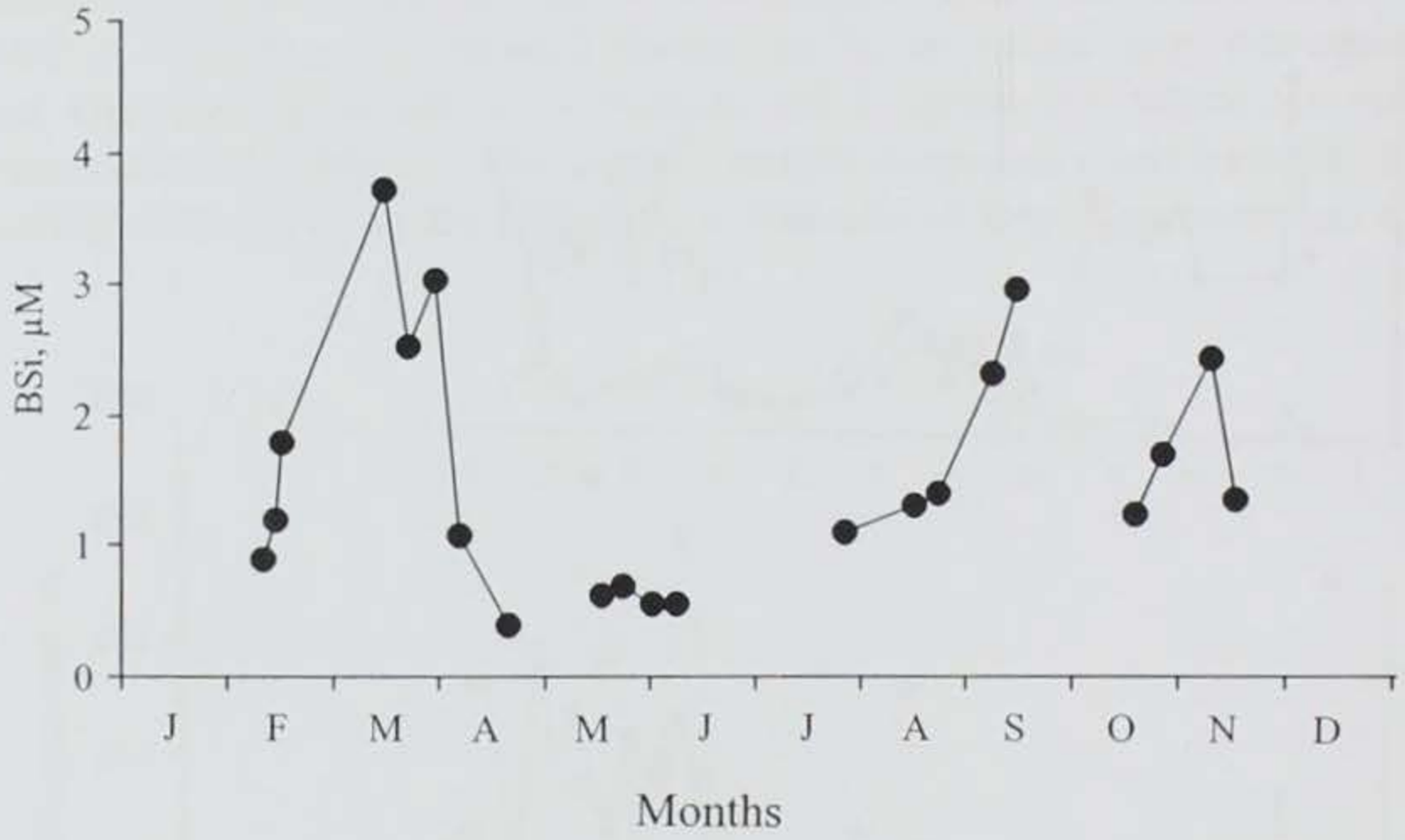

Figure 4: Seasonal changes of BSi concentrations recorded at station 330 during 1995.

In order to evaluate the importance of this overestimation, BSi measurements were conducted on a pure culture of *S. costatum* in the presence and the absence of detrital diatoms. Figure 5 compares time course experiment of the BSi alkaline digestion for both cultures. A complete dissolution is obtained after 12 min when a plateau is reached (Fig. 5). The silica content of each *S. costatum* culture is given by the Y-extrapolated intercept value (13.2 and 16.4 µM for the pure and frustule-enriched culture, respectively; Fig. 5). The contribution of empty frustules was estimated to 3.2 µM, *i.e.* 21 % of the living diatom BSi, corresponding to the proportion of empty frustules added. Relating these BSi concentrations to the *S. costatum* C-biomass gives a molar ratio BSi:C significantly higher for the frustule-enriched culture (0.31, µM:µM) compared to the non-enriched one (0.25). From these laboratory experiments, one may conclude that diatom silica content based on BSi measurement and C-diatom estimate can be significantly biased for natural diatom communities when not growing exponentially.

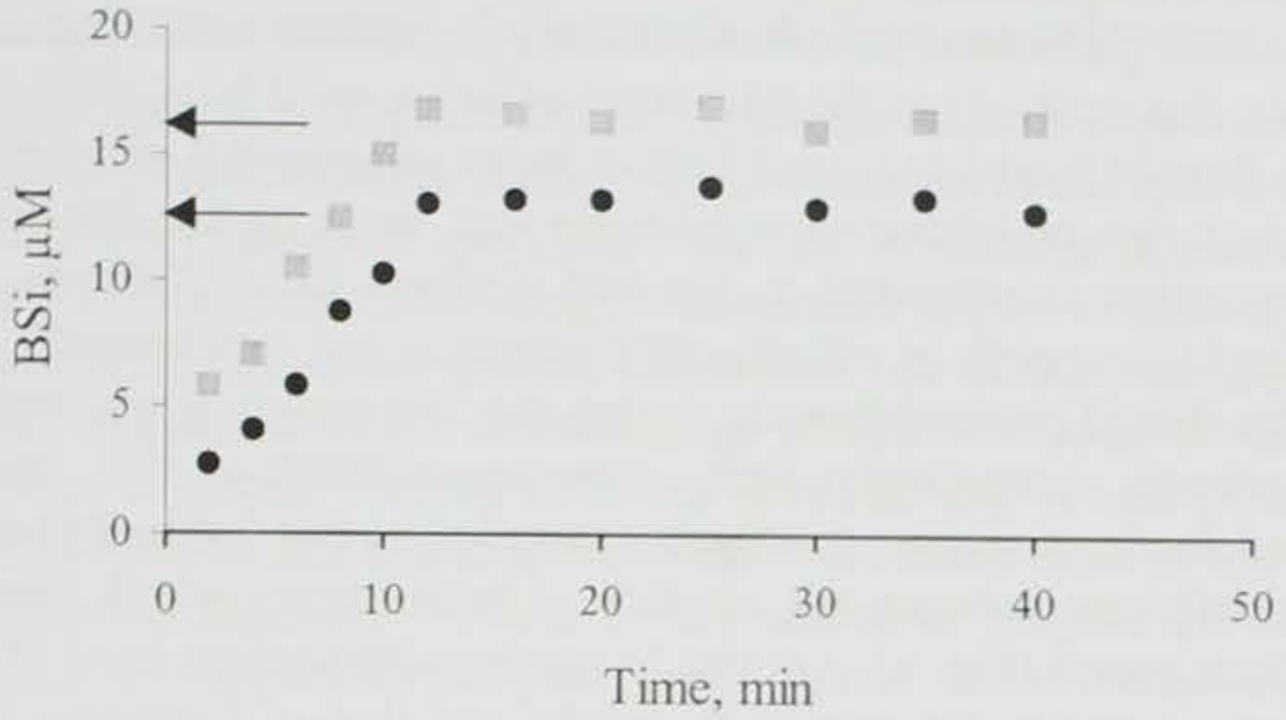

Figure 5: Time course experiment of the BSi alkaline digestion for a pure (dots) and frustule-enriched (squares) cultures of *Skeletonema costatum*.

Taking this into consideration, we calculated the Si:C of the three main diatom assemblages blooming in the Belgian coastal waters (Fig. 2) from BSi and C-biomass diatom measurements of samples corresponding to diatom communities in growing phase. The absence of significant empty frustules was checked by microscopy. Results of this calculation are reported in Table 1. Clearly, the various diatom assemblages succeeding along the vegetative period (Fig. 2) distinguished by their silica content. The diatom population composed of the small colony-forming diatoms and *Coscinodiscus* sp. of late winter and fall (assemblage 1) is the highest silicified with Si:C varying between 0.49 and 0.68. The late spring and summer *Rhizosolenia* spp.(assemblage 3) is the lowest silicified (0.04 - 0.10; Table 1) while *Chaetoceros* spp. and *Schroederella* sp. (assemblage 2) show intermediate Si:C ratios (0.27-0.14).

Table 1: Silicification level of the natural diatom communities blooming in Belgian coastal waters in 1995, expressed as the Si:C molar ratio.

Diatoms	**Blooming season**	**Si:C** (1)	**Si:C** (2)
Assemblage 1 (small neritic diatoms)	early spring & fall	0.49 - 0.68	0.30 - 0.52
Assemblage 2 (*Chaetoceros* spp./ *Schroederella* sp.)	spring	0.14 - 0.27	0.20 - 0.24
Assemblage 3 (*Rhizosolenia* spp.)	spring & summer	0.04 - 0.10	undetermined

(1) calculated on basis of BSi and microscopically-based C-biomass determinations.
(2) calculated from the tracer experiments.

As an alternative to this method based on stock measurements, the silicification level of field diatoms was determined from parallel 24h-time course experiments of ^{32}Si cell uptake and ^{14}C incorporation into proteins performed on natural diatom-dominated communities. Compared to stock measurements, this method has the advantage of being specific to siliceous algae and active phytoplankton. Protein synthesis is used here as index of growth (Lancelot *et al.*, 1986). Although it has been shown in laboratory conditions that most of the diatom silicic acid uptake and silica deposition is restricted to the cell division time (Sullivan, 1977), it is assumed that phytoplankton growth in natural environment is not synchronous. Under these conditions, ^{32}Si uptake should proceed linearly during the 24h period of incubation and with respect to protein synthesis. Accordingly, time-course experiments of ^{32}Si uptake and ^{14}C incorporation into proteins by a diatom community sampled in late February (assemblage 1) showed significant parallelism between the kinetics of both tracers which proceed linearly during the light and dark period (Fig. 6). On this basis, the silicification level of diatoms was estimated from the ratio between the rate of Si uptake and that of C-protein synthesis, the latter converted in cellular growth. A protein contribution of 72% to the diatom C-biomass was considered as typical for coastal North Sea communities (Lancelot *et al.*, 1986). Similar experiments conducted on diatom assemblages 1 and 2 during spring and fall allowed to calculate their silicification levels (Table 1). Such estimate could not be secured for assemblage 3 in spring due to the co-occurrence of *Phaeocystis* which contributes to protein synthesis. As shown in Table 1, the diatom Si:C ratio calculated from rate processes varies between 0.30 and 0.52 for assemblage 1 and between 0.20 and 0.24 for assemblage 2, in good agreement with those estimated from the biomass-based method.

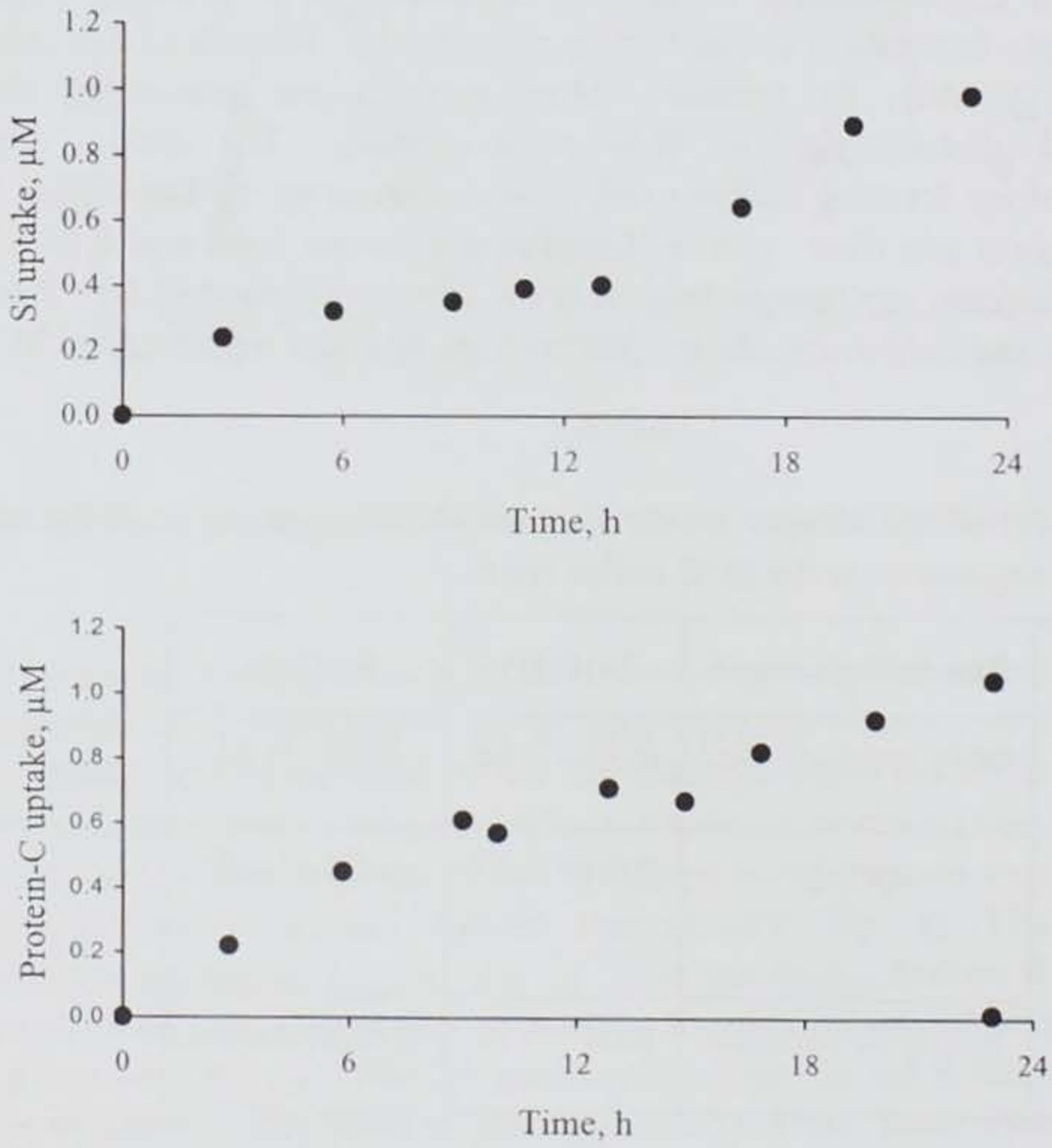

Figure 6: Time-course experiments of a.) ^{32}Si uptake and b.) ^{14}C incorporation into proteins by a diatom community (assemblage 1) sampled in late February 1995 at station 330.

Discussion

Determination of silica content of diatoms in shallow coastal waters

Accurate determination of the silica content of marine diatoms constitutes one prerequisite for assessing the role of silicon in the diatom bloom dynamics of eutrophicated coastal waters. No direct method exists to measure specifically the Si:C of diatoms in natural aquatic ecosystems due to several interferences. Comparing measurement of BSi to diatom C-biomass, the latter derived from microscopic observations, constitutes one of the best approaches. However, a major problem associated to the determination of BSi in coastal waters when applying the classical alkaline digestion method (Krausse *et al.*, 1983), results from the contribution of detrital BSi and of some LSi silica. In this work, the contribution of LSi could be assessed by using the sequential digestion method of Ragueneau & Tréguer (1994) and determining a correction factor specific for the Belgian coastal waters. These authors showed indeed that the interference of LSi is related to its concentration modulated by a correction factor that is typical for the area.

More complicated is the estimate of the contribution of detrital BSi which includes both empty frustules, diatom-derived aggregates and faecal pellets, all of them greatly varying along the season. This is particularly important during periods of diatom bloom decline and high mesozooplankton activity (from mid-May to September in the Belgian coastal waters; Hecq, 1980). For instances, the presence, revealed by microscopic observation, of empty diatom frustules and copepod faecal pellets in the samples of summer and fall 1995 when BSi was maximum and the diatom biomass not that important (Figs. 2 & 4) argues for the significant contribution of detrital BSi to the pool of BSi. On the other hand, the possible contribution of empty frustules was quantitatively supported by our laboratory experiment on pure diatom culture. This experiment showed that empty frustules could well represent an important proportion of the extracted BSi and hence significantly overestimate the further calculation of Si:C ratio of diatoms. This situation occurs in the natural environment, at bloom decline, especially in the well-mixed water column of the Southern Bight of the North Sea where re-suspension of sedimented frustules is expected. Under such conditions, it is save to conclude that the silica content estimates of field diatom communities based on BSi and diatom C-biomass measurement, are highly valuable when communities are in the growing phase but most probably overestimated outside these periods.

The second approach we used to determine the silica content of marine diatoms was based on radiotracer technology, by measuring the slope between the silicic acid (^{32}Si) uptake rate and C-protein synthesis (^{14}C) by natural diatom communities. The parallelism observed between the 24h-kinetics of ^{32}Si uptake and ^{14}C incorporation into proteins of diatoms gives support to a close coupling between silicic acid uptake, silica deposition and protein synthesis (Sullivan, 1986). This contrasts however with other observations (*e.g.* Brzezinsky *et al.*, 1990) reporting that diatom silicic acid uptake is a discontinuous process, tightly geared to the formation of cell wall (G2 phase of the cell cycle). Under such conditions, the linear ^{32}Si uptake observed for our 24h experiments would reflect asynchronous growth of field diatoms rather than a continuous uptake of silicic acid by siliceous algae. More has to be known on the mechanisms controlling Si transport, silica deposition and valve formation before safely concluding. Nevertheless relating the uptake rate of silicic acid to the diatom growth (here measured as protein synthesis) seems to be a valuable approach to estimate the silica content of field diatoms providing that diatoms are the dominant carbon producers. If not, other algae

will interfere with the measurement of protein synthesis and the Si:C ratio of diatoms will be underestimated.

Diatoms succession and silica availability

The phytoplankton seasonal pattern in the Belgian coastal waters in 1995 was identical to that recorded every year since 1988 (Lancelot *et al.*, 1998; Rousseau *et al.*, in preparation). It showed the occurrence in spring of three consecutive assemblages of diatoms respectively dominated by small neritic species (assemblage 1), *Chaetoceros* spp. - *Schroederella* sp. (assemblage 2) and *Rhizosolenia* spp. (assemblage 3). Superimposing the two latter diatoms, the colonial Haptophycea *Phaeocystis* bloomed during about one month. This spring pattern has been reported in the French (H. Grossel, pers. comm.) and Dutch (Gieskes & Kraay, 1975, Cadée & Hegeman, 1986; Philippart *et al.*, 2000) coastal waters and seems thus typical for the Southern Bight of the North Sea.

The three diatom assemblages observed in the Belgian coastal waters in 1995 were characterised by distinct silica content with Si:C values decreasing from 0.68 to 0.04 (Table 1) along the course of the spring bloom. At the same time, the ambient dissolved silicate was decreasing from about 13 µM to a minimum of 1.3 µM (Fig.3) suggesting that the observed seasonal pattern of diatoms might be due to difference in their silica requirement. Accordingly, a positive relationship is observed between the diatom Si:C ratio and ambient dissolved silicate (Fig. 7). Altogether this would suggest that dissolved silicate availability play a major role in shaping the diatom succession in the Belgian coastal waters. The twofold variability of the diatom Si:C ratio recorded within each assemblage (Table 1) would however suggest that intra-specific variability might be important as well.

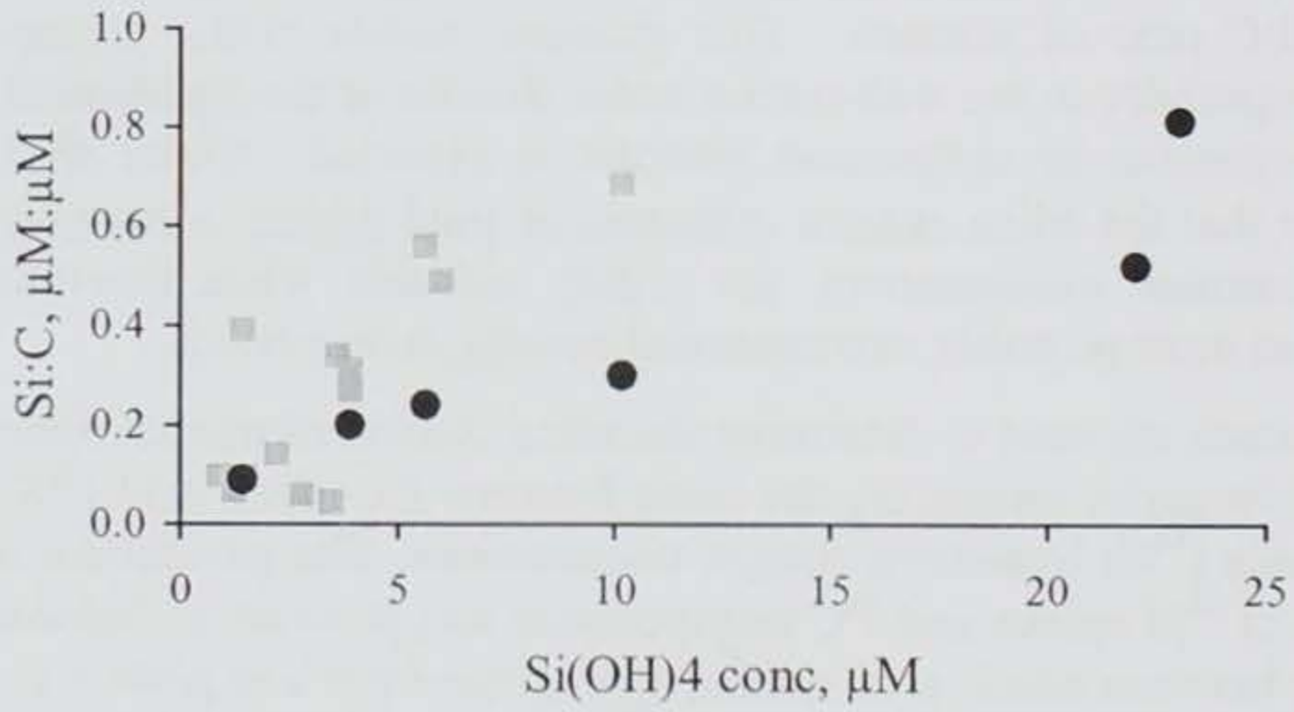

Figure 7: Relationship between the diatom Si:C ratio and ambient dissolved silicate from biomass- (squares) and process-based (dots) measurements.

In order to better understand the link between diatom succession and dissolved silicate availability in the Belgian coastal waters, we compared our data with available information on

the inter- and intra-specific variability of individual diatom species. Table 2 gathers the available information for cultured marine diatoms selected with respect to the three diatom assemblages identified in the Belgian coastal waters. All data correspond to diatoms in exponential growing phase but no distinction was made between light and temperature conditions. Globally, literature values report an inter-specific variation of the diatom Si:C between 0.04 and 0.68 and so give strong support to our field data (Table 1). However, the variability of the Si:C ratio between the different assemblages (Table 2) is not as distinct as measured in the Belgian coastal waters (Table 1). In average, the silicification level of diatoms of assemblage 1 is significantly higher than the one of assemblages 2 and 3. Some diatoms of assemblage 1 such as *Thalassionema nitszchioïdes* and *Coscinodiscus* sp. are well characterised by silica content as high as those measured in the Belgian coastal waters (0.3-0.68, Table 1). However, the silicification level of other species of assemblage 1 such as *e.g. Skeletonema costatum* is significantly lower (0.07-0.25; Table 2) with lowest values comparable to published values for assemblages 1 and 2. Also, and contrary to what has been measured in the field (Table 1), no clear distinction can be made between cultured diatoms of assemblages 2 and 3 based on their silica content (Table 2). The inter-specific variability of the silica content within assemblage 2 ranges between 0.04 and 0.16 while *Rhizosolenia sp* and *Ditylum brightwelii* shows variations between 0.11 to 0.20 (Table 2).

Part of the variability reported by Table 2 is most probably due to intra-specific variability. This particularly suggested when several Si:C values are available for a same species (Table 2). An intra-specific variability of about 2-3 is reported for the selected species of Table 2 which fits well with the current knowledge (Paasche, 1980). Intra-specific variability of the diatom Si:C has been attributed to changes in light intensity, photoperiod, temperature and nutrient availability (Eppley *et al.*, 1967; Harrison *et al.*, 1976; Paasche, 1980; Brzezinski, 1985; Takeda, 1998; De la Rocha *et al.*, 2000). The effect of changing light and temperature on the diatom Si:C was revisited by Brzezinski (1985). No obvious generic trend was observed when changing the photoperiod and temperature although a twofold variation of Si:C has been reported. On the contrary, low light (Furnas, 1978) and/or nitrogen (Harrison *et al.*, 1976) and/or iron (Takeda, 1998) and/or zinc (De La Rocha *et al.*, 2000) limiting conditions had a net effect of increasing the silica content of diatoms provide dissolved silicate is sufficient. Although alteration of kinetics of silica production under iron and zinc stress was recently evidenced (De La Rocha et al., 2000), the increase of Si:C could result from a slow-down of the growth process without affecting the diatom silicic acid uptake and silica deposition. In accordance, a two-three-fold increase of the Si:C was reported for a natural *Chaetoceros*-dominated diatom population when nitrate was decreasing to depletion and dissolved silicate was abundant (Kudo *et al.*, 2000). Yet inconclusive results were obtained by Llewellyn & Gibb (2000) who compared the silica content of 10 diatom species at the exponential and stationary stage of their growth. A increase of Si:C was well observed for low-silicified diatoms along the course of their growth. The reverse was observed for highly silicified diatoms such as *Thalassionema, Navicula* (Llewellyn & Gibb, 2000).

These results suggest a more complex pattern of nutrient interactions on the silicification level of diatoms with opposite effect of silicate and nitrogen/iron depletion. Indeed the ability of diatoms to vary their cell-wall thickness and consequently their silica content with $Si(OH)_4$ availability has been demonstrated by Paasche (1980). The physiological adaptation to low $Si(OH)_4$ concentrations by individual species was reported by Nelson & Dortch (1996).

Table 2: Diatom Si:C ratio: literature review

Species	Si:C	Reference
Assemblage 1		
Asterionella glacialis	0.11-0.12	Brzezinski (1985)
Skeletonema costatum	0.07-0.15	Brzezinski (1985)
	0.10	Paasche (1980)
	0.11	Harrison *et al.* (1976)
	0.20	Parsons *et al.* (1961)
	0.29	Llewellyn and Gibb (2000)
Stephanopyxis sp.	0.19	Brzezinski (1985)
Thalassionema nitzschioïdes	0.12-0.14	Brzezinski (1985)
	0.52	Llewellyn and Gibb (2000)
Thalassiosira sp.	0.05-0.15	Brzezinski (1985)
Thalassiosira weiss.	0.13	Llewellyn and Gibb (2000)
Coscinodiscus	0.22	Brzezinski (1985)
Navicula hansenii	0.44	Llewellyn and Gibb (2000)
Fragilaria striatula	0.68	Llewellyn and Gibb (2000)
Assemblage 2		
Chaetoceros sp.	0.04-0.16	Brzezinski (1985)
	0.13-0.15	Llewellyn and Gibb (2000)
Lauderia boralis	0.08	Brzezinski (1985)
L. danicus	0.08-0.11	Brzezinski (1985)
Assemblage 3		
Rhizosolenia sp.	0.14	Brzezinski (1985)
Ditylum brightwellii	0.11-0.20	Brzezinski (1985)

Based on this information, it seems plausible that the high ambient dissolved silicate of late winter and fall, in combination with sufficient inorganic nitrogen and phosphate and low light lead to the early-spring dominance of highly silicified diatoms in the Belgian coastal waters (Table 1). The pattern is more complex for the spring and summer diatom communities (assemblages 2 and 3) which grow under sufficient light but low silicate and phosphate conditions. Indeed high light and low silicate would decrease the diatom Si:C ratio while an opposite effect would result from phosphate depletion. Although the nutrient limitation status of the coastal diatom communities was not assessed during this study, the positive relationship existing between the silicification level of the different diatom community and the $Si(OH)_4$ availability suggests that the succeeding diatom communities in the Belgian coastal waters are well adapted to their $Si(OH)_4$ environment. This has particularly well documented for the large diatom genus *Rhizosolenia* which composed the bulk diatom during late spring and summer in the Belgian coastal waters (Fig.2; Lancelot *et al.*, 1998). This diatom genus is known to form huge blooms of 400 10^3 cells. l^{-1} in May-June in the Channel (Grall, 1972;

Sournia *et al.*, 1987) and in the Bay of Brest (Ragueneau *et al.*, 1994). It was also documented in other marine ecosystems such as in the Louisiana shelf (Dortch & Whitledge, 1992) and in the North Pacific (Shipe *et al.*, 1999). In all these environments, this diatom genus is associated to low $Si(OH)_4$ concentrations. In the Bay of Brest, the ability of *Rhizosolenia* spp. to grow on diagenetically remineralized silicon from sedimented early-spring diatoms was evidenced by Del Amo *et al.* (1997). This suggests that the genus *Rhizosolenia* is particularly well adapted to take advantage of low regenerated $Si(OH)_4$ concentrations and support to the hypothesis that ambient dissolved silicate is an important factor in the selection of diatom species.

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Chapter 7

Trophic efficiency of the planktonic food web in a coastal ecosystem dominated by Phaeocystis colonies

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Trophic efficiency of the planktonic food web in a coastal ecosystem dominated by *Phaeocystis* colonies

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Abstract

The trophic efficiency of the planktonic food web in the *Phaeocystis*-dominated ecosystem of the Belgian coastal waters was deduced from the analysis of the carbon flow network of the planktonic system subdivided into its different trophodynamic groups. A carbon budget was constructed on the basis of process-level field experiments conducted during the spring bloom period 1998. Biomasses and major metabolic activities of auto- and hetero-trophic planktonic communities (primary production, bacterial production, nanoproto-, micro- and meso-zooplankton feeding activities) were determined in 9 field assemblages collected along spring at reference station 330. In 1998, the phytoplankton spring flowering was characterized by a moderate diatom bloom followed by a massive *Phaeocystis* colony bloom. *Phaeocystis* colonies, contributing 70 % of the net primary production, escaped the linear food chain while the early spring diatom production supplied 74 % of mesozooplankton carbon uptake. The rest of mesozooplankton food requirement was, at the time of *Phaeocystis* colony bloom, partially fulfilled by microzooplankton. Only one-third of the microzooplankton production, however, was controlled by mesozooplankton grazing pressure. Ungrazed *Phaeocystis* colonies were stimulating the establishment of a very active microbial network. On the one hand, the release of free-living cells from ungrazed colonies has been shown to stimulate the growth of microzooplankton, which was controlling 97 % of the nanophytoplankton production. On the other hand, the disruption of ungrazed *Phaeocystis* colonies supplied the water column with large amounts of dissolved organic matter available for planktonic bacteria. The budget calculation suggests that ungrazed colonies contributed up to 60 % to the bacterial carbon demand, while alternative sources (exudation, zooplankton egestion and lysis of other organisms) provided some 30 % of bacterial carbon requirements. This suggests that the spring carbon demand of planktonic bacteria was satisfied largely by the autogenic production. The trophic efficiency was defined as the ratio between mesozooplankton grazing on a given source and food production. In spite of its major contribution to mesozooplankton feeding, the trophic efficiency of the linear food chain, restricted to the grazing on diatoms, represented only 5.6 % of the available net primary production. The trophic efficiency of the microbial food chain, the ratio between mesozooplankton grazing on microzooplankton and the resource inflow (the bacterial carbon demand plus the nanophytoplankton production) amounted to only 1.6 %. These low trophic efficiencies together with the potential contribution of ungrazed *Phaeocystis*-derived production to the bacterial carbon demand suggest that most of *Phaeocystis*-derived production during spring 1998 in the Belgian coastal area was remineralised in the water column.

Key words : *Phaeocystis* colonies, diatoms, planktonic food chain, microbial network, trophic efficiency.

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Introduction

The Southern Bight of the North Sea is dominated in spring by recurrent *Phaeocystis* colonies blooms which occur after a silicate-controlled vernal diatom bloom (Cadée, 1992 ; Lancelot *et al.*, 1998). Thereby, *Phaeocystis* colonies take advantage of the excess nitrate that characterises these eutrophicated coastal waters (Riegman *et al.*, 1992; Lancelot, 1995; Lancelot *et al.*, 1998). These blooms, which cumulate high biomass (Cadée & Hegeman,1986; Cadée, 1990; Rousseau *et al.*, 1990), last for about 4-6 weeks and then suddenly disappear. In spite of numerous investigations, the trophic efficiency and more generally the fate of the huge amount of organic matter produced by these blooms are not yet entirely understood. Sedimentation/exportation, colony disruption releasing cells and organic matter in the surrounding medium, cell lysis and grazing constitute the processes that could explain the sudden termination typical of *Phaeocystis* colony blooms. The relative importance of these loss processes in the shallow coastal waters of the Southern Bight of the North Sea is still controversial (Wassmann, 1994). It is generally accepted that healthy *Phaeocystis* colonies are not grazed upon by the indigenous mesozooplankton. Cell lysis was identified as the major loss factor for *Phaeocystis* at the decline of the bloom in the Marsdiep area in the Wadden Sea with further remineralisation of the lytic organic components by bacterioplankton (van Boekel *et al.*, 1992 ; Brussaard *et al.*, 1995). In these shallow turbulent coastal waters, sedimentation and direct grazing on *Phaeocystis* colonies were not found to be significant processes (van Boekel *et al.*, 1992; Brussaard *et al.*, 1995). On the contrary, Riebesell (1993) found that sedimentation of *Phaeocystis* colonies was the dominant loss factor from the surface layer in the German Bight. In the Dutch open waters, Peperzak *et al.* (1998) also observed some colony sedimentation but concluded that microprotozooplankton grazing was the dominant loss process of *Phaeocystis* cellular biomass.

The trophic fate of *Phaeocystis*-derived material, especially the transfer to higher trophic levels, is also uncertain. *Phaeocystis* biomass accumulation in the water column clearly indicates a lack of top-down control. In these shallow coastal waters, *Phaeocystis* colonies seem indeed to escape direct grazing due to a mismatch between the size of colonies and most indigenous mesozooplankton (Joiris *et al.*, 1982; Daro & van Gijsseghem, 1984; Daro, 1985; Hansen & van Boekel, 1991; Weisse *et al.*, 1994; Gasparini *et al.*, 2000). Part of the *Phaeocystis* production is assumed to be transferred to higher trophic levels via a complex microbial food web in which microprotozooplankton occupies a key trophic position as food item for mesozooplankton (Bautista *et al.*, 1992; Hansen *et al.*, 1993). Direct microprotozooplankton grazing on *Phaeocystis* free-living cells released after colonial disruption at the end of the bloom was observed (Admiraal & Venekamp, 1986; van Boekel *et al.*, 1992) and quantified (Weisse & Scheffel-Möser, 1990; Peperzak *et al.*, 1998). Furthermore, bacterial production sustained by *Phaeocystis* cell lysis products and polysaccharides released after disruption of ungrazed colonies could transfer *Phaeocystis*-derived material towards higher trophic levels via complex pathways involving heterotrophic nanoflagellates and microprotozooplankton. However, the efficiency of this trophic pathway is not known yet (Weisse *et al.*, 1994). Also the biodegradability of dissolved organic matter derived from the colony matrix is still

questionable and seems to depend on nutrient availability (Thingstad & Billen, 1994; Janse *et al.*, 1999).

Finally, transient *Phaeocystis*-derived agregates formed after colony senescence or by fragmentation have been recurrently observed at bloom termination (Lancelot & Rousseau, 1994 ; Becquevort *et al.*, 1998). Their colonization by bacteria (Becquevort *et al.*, 1998) and other microorganisms (Lancelot & Rousseau, 1994) makes them a possible valuable food resource for mesozooplankton. This trophic pathway has not been investigated yet.

Despite these numerous studies on the various processes underlying the decline of the bloom, the trophic efficiency in the ecosystem dominated by *Phaeocystis* colonies still needs to be adressed in a synoptic view. Such questions as : does *Phaeocystis* constitute an efficient link towards higher trophic levels or does it represent a trophic loss for the coastal ecosystem still require an answer. With this objective, a carbon flow network of the planktonic system has been constructed on the basis of fieldwork performed at one station in the *Phaeocystis*-dominated Belgian coastal waters. This area consists in an open system characterized by an alongshore northward water mass transport additional to the tidal currents. As a consequence, the temporal developments observed reflect a change in water masses as well as the plankton dynamics. The strategy of sampling at this unique station was, however, justified by the estimated residence time of water in the zone, 60 days (Lancelot, 1990 ; Billen, pers. comm.), a duration close to that of the *Phaeocystis* bloom, combined to the homogeneity of processes occurring downwards and upwards. This is particularly well illustrated by the continuity in microscopical observations performed during a *Phaeocystis* bloom, *e.g.* the sequence of events from the young, small colony to the colonized agregate (Rousseau *et al.*, 1994).

The carbon budget was constructed on the basis of process-level field experiments conducted during the *Phaeocystis* bloom event in spring 1998. Biomasses and major metabolic activities of auto- and hetero-trophic planktonic communities (primary production, bacterial production, nanoproto-, microproto- and meso-zooplankton feeding activities) were experimentally determined on field assemblages collected at reference station 330 of the Belgian monitoring network at different key periods of the phytoplankton spring bloom or calculated on the basis of literature parameterisation.

Material and Methods

Sampling station. The 20m-deep station 330 (N 51°26.00; E 02° 48.50; Fig. 1) was chosen as a reference of the Belgian coastal zone for the physico-chemical characteristics (depth, temperature, salinity, nutrient enrichment level). This region is mainly influenced by Atlantic waters already enriched by nutrients discharged by rivers Seine and Somme as well as Scheldt (Yang, 1998) and, to a less extent, by the Rhine and the Meuse (van Bennekom & Wetsteijn, 1990). The strong alongshore tidal currents (1 m s^{-1}) combined to the shallow water depths ensure a permanent vertical mixing of the water column (Creutzberg & Postma, 1979; Simpson, 1994).

Sampling. Aboard R.V. Belgica , seawater was sampled every week from late January to mid-June 1998. Sampling was done with a bucket to avoid *Phaeocystis* colony disruption, and the seawater was transferred to polyethylene bottles. Biological activities were determined on a large amount (200 l) of seawater sampled at key stages of the spring bloom.

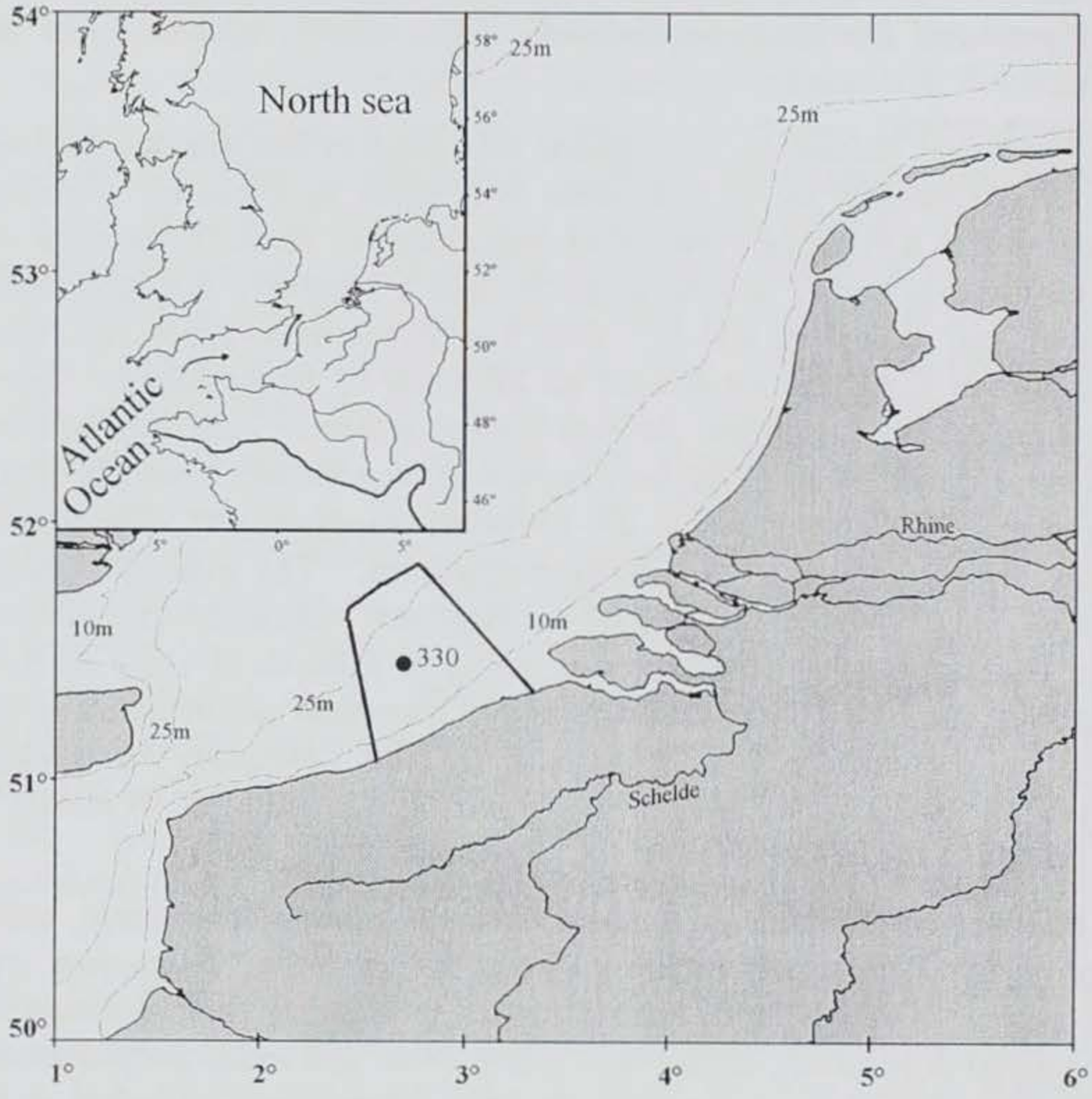

Figure 1 : Map of the Belgian coastal zone with location of station 330

Physico-chemical parameters. Seawater temperature and salinity were measured aboard with a thermosalinometer (Beckman). Major nutrient (NO_3, NH_4, $Si(OH)_4$ and PO_4) concentrations were determined on 0.45 µm filtered seawater according to the colorimetric methods described in Grasshof *et al.* (1983). Additionally, for days when biological processes were investigated, irradiance was continuously measured using a cosine sensor (LiCor). Chlorophyll a (Chl a) concentrations were measured on 90 % (v:v) acetone extracted particulate material isolated by filtration on glass fiber filters (GF/C, Whatman). Concentrations were spectrophotometrically determined using the method and equations recommended by Lorenzen (1967). Dissolved organic carbon (DOC) was

determined on ashed GF/F filtered seawater using the high-temperature catalytic oxidation (HTCO) technique (Sugimura & Suzuki, 1988; Suzuki, 1993).

Biomass of the trophodynamic groups. Depending on the organisms, specific procedures were used for sampling, preservation, storage and microscopic analysis. Diatoms, *Phaeocystis* colonies and microprotozooplankton were enumerated under an inverted microscope (Leitz Fluovert) according to Utermöhl method (Hasle, 1978). Samples were preserved with 1 % (final concentration) lugol-glutaraldehyde solution and stored at 4°C in the dark until analysis. Magnification was chosen according to cell or colony size: 40 or 100 X for *Phaeocystis* colonies; 100 or 200 X for diatoms or protozoa and 320 X for cells ranging in the size range 20-50 µm. In total, at least 400 cells were enumerated with 100 cells of the most abundant genus or species. Diatoms were enumerated and identified according to the genus level unless a species was easily identifiable or dominant. Their carbon biomass (C-biomass) was calculated on the basis of cell density and biometric factors determined for each species or genus. A specific average conversion factor was calculated from biovolumes measured on a cell population throughout the period of its development. Biovolumes were then converted using a carbon content factor of 0.11 pgC μm^{-3} of plasma volume (Edler, 1979). *Phaeocystis* was identified as *P. globosa* and will here be referred as *Phaeocystis*. Colony cell number was determined according to the method described in Rousseau *et al.* (1990). *Phaeocystis* colony C-biomass was estimated from biovolume measurement according to the empirical procedures of Rousseau *et al.* (1990) and van Rijssel *et al.* (1997). These two methods reflect different approaches of the colony structure based on the existing knowledge. In 1990, Rousseau *et al.* considered *Phaeocystis* colonies as jelly spheres with cells embedded in mucus and derived the measurement of cellular and mucilaginous C-biomass from colony biovolume. Later, with the development of confocal laser scanning imagery combined to biochemical analysis, van Rijssel *et al.* (1997) demonstrated the hollow structure of *Phaeocystis* colonies, also confirmed by Hamm *et al.* (1999), with cells embedded in a 7 µm membrane-like structure. On this basis, a constant carbon content of 57 pg C $cell^{-1}$ was experimentally determined for the Southern North Sea colonies (van Rijssel *et al.*, 1997). Analysis of both calculations (not shown) indicates that the two methods are comparable for natural colony assemblages with an average diameter of less than 700 µm. For higher average diameter, the method of Rousseau *et al.* (1990) estimates 30 % higher C concentrations. Therefore, the method of van Rijssel *et al.* (1997) was used in our further discussion.

Microprotozooplankton C-biomass was calculated by using the factor 0.19 pgC μm^{-3} (Putt & Stoecker, 1989) and 0.13 pgC μm^{-3} (Edler, 1979) for ciliates and dinoflagellates, respectively.

Bacteria, auto- and hetero-trophic nanoflagellates were enumerated by epifluorescence microscopy after DAPI staining following the method of Porter & Feig (1980). Samples were preserved with 40 % formaldehyde (final concentration 2 %) and 25 % glutaraldehyde (final concentration 0.5 %) for bacteria and flagellates, respectively. Bacteria were enumerated on 10 different fields at 1000 X magnification. Biovolumes were calculated by treating rods and cocci, respectively, as cylinders and spheres (Watson *et al.*, 1977) and converted to C-biomass by using the biovolume dependant conversion

factor established by Simon & Azam (1989). At least 100 flagellates were counted and autotrophs were discriminated from heterotrophs by the red chlorophyll autofluorescence. C-biomasses were calculated from cell density and biometric factors by using the factor 0.11 pgC μm^{-3} of Edler (1979).

About 50-100 liters of water were filtered through a 50 µm size mesh net for enumeration of copepod nauplii and copepodites I-II stages as well as other small metazooplankton. A 300 µm size mesh net equipped with a flow meter was used to sample larger stages of mesozooplankton. All samples were preserved with formaline (4 % final concentration). Small and large metazooplankton were identified to species level and enumerated under stereoscopic microscope. C-biomass was calculated using species- and stage- specific dry weight measured for the dominant metazooplankton species of the Belgian coastal waters (Daro & van Gijsegem, 1984).

Metabolic activities. Daily primary production, bacterial production and meso-zooplankton grazing were experimentally determined under simulated *in situ* conditions in 9 field assemblages collected along spring. Daily photosynthesis, growth, respiration and exudation rates of phytoplankton were determined from ^{14}C radiotracer experiments using the method described in Mathot *et al.* (1992) and the AQUAPHY set of equations of Lancelot *et al.* (1991). The experimental protocol involved two radiotracer experiments run in parallel for each sampled phytoplankton community. Photosynthetic characteristics were determined from short-term (2 h) experiments of ^{14}C uptake at different light intensities (P/I curve). Growth, exudation and respiration were assessed by running 24h-time course experiment of ^{14}C incorporation into phytoplanktonic cellular components (small metabolites, proteins, polysaccharides, lipids) during a natural day light cycle according to the protocol of Lancelot & Mathot (1985). AQUAPHY parameters were determined by mathematical fitting of experimental data using the equations described in Lancelot *et al.* (1991). Particular attention was given to select samples with a clear dominance of either diatoms or nanophytoplankton or *Phaeocystis* colonies. Daily rates of each community were then calculated by integration on the variations of light in time and within depth of the AQUAPHY set of equations with the relevant parameters, making use of *in situ* biomass, temperature and nutrients.

Bacterioplankton production was determined by ^{3}H-leucine incorporation into proteins and ^{3}H-thymidine into DNA according to the protocol described in Becquevort *et al.* (1998). Leucine and thymidine incorporation rates were converted into bacterial production using the following conversion factors established for the North Sea bacterial communities by Servais (1990): 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.

Mesozooplankton grazing on phyto- and protozoo-plankton was estimated from clearance or ingestion rates experimentally determined for each copepod species and stages and the respective prey biomass. Three methods, pigment gut content, radio-labelled prey and cell-count experiments, were used and combined (Gasparini *et al.*, 2000). The ranges of measured clearance rate are reported in Table 1 and discussed in Gasparini *et al.* (2000). No grazing was measured for *Phaeocystis* colonies (Gasparini *et al.*, 2000). The daily grazing rate of each species and stage of copepod was then

calculated on the basis of the specific biomass, the clearance rates reported in Table 1 and the prey biomass.

Table 1 : Range of clearance rates determined by Gasparini *et al.* (2000) for the different copepod species and stages on the various prey.

Copepod species & stage	Prey **Diatoms** ml ind^{-1} h^{-1}	Prey **Microzooplankton** ml ind^{-1} h^{-1}
Acartia clausi		
III-IV	0.2-0.4	0.2-0.5
V	0.3-0.6	0.3-0.8
Adult	0.6-1.1	0.5-1.4
Temora longicornis		
III-IV	0.4	0.2-0.5
V	0.6	0.2-0.7
Adult	0.8	0.3-1.0
Centropages hamatus		
III-IV	0.3-0.4	0.6-0.9
V	0.4-0.6	1.0-1.5
Adult	0.5-0.8	1.2-1.8

The grazing rates of nano- and micro-protozooplankton and of the small mesozooplankton (nanograzers) were calculated from prey concentrations using relationships describing the dependance of ingestion or clearance rates on prey. These relationships were derived from previous experiments performed on natural populations of prey and grazers in the Belgian coastal waters during the spring bloom period. The Holling type II functional response characterised by a maximum ingestion rate of 64.5 bacteria ingested per nanoprotozooplankton per hour and a half-saturation constant of 7.7 10^8 bacteria l^{-1} (Becquevort, 1987; Lancelot *et al.*, 1991) was used to calculate the nanoprotozooplankton grazing on bacteria. Microprotozooplankton grazing on nanoflagellates was calculated on basis of clearance rates ranging between 3.5 and 15.5 µl ind^{-1} h^{-1} for nanophytoplankton concentrations varying from 5 to 62 mgC m^{-3} measured by Weisse & Scheffel-Möser (1990). Grazing rates of metazoan nanograzers were considered as the sum of the grazing rate of nauplii and copepodite I-II, each with its own specific clearance rate. The following clearance rates–prey concentration relationships derived from Daro (1985) :

$$CR = 0.135\ 10^{-0.001\ p} \text{ and } CR = 0.442\ 10^{-0.003\ p}$$

where CR, the clearance rate, is expressed in ml ind^{-1} l^{-1} and p, the prey concentration, in μgC l^{-1} were used for nauplii and copepodite I-II stages, respectively.

The flow network model. The flow network model was designed on the basis of the current knowledge of the trophic interactions between the main auto- and hetero-trophic planktonic organisms. These latter were therefore grouped according to their trophic position. So, as already described in Lancelot & Rousseau (1994), phytoplankton was assembled as three trophodynamic groups with different trophic fates: *Phaeocystis* colonies, nanophytoplankton and diatoms. Diatoms are consumed by mesozooplankton or exported to the sediment; *Phaeocystis* colonies which are not grazed by mesozooplankton are susceptible either to sedimentation, or to colonial lysis producing dissolved organic matter and free-living cells. These free-living cells are then considered as nanophytoplankton together with unidentified autotrophic flagellates. Nanophytoplankton is grazed by microzooplankton but does not sediment due to its small size. The dissolved organic matter produced by lysis of organisms or mucus dissolution in the water column initiates a microbial network where bacteria are grazed by nanoprotozooplankton, being themselves a food item for microzooplankton. In this model, microzooplankton is composed of various nanograzers, which include the usual microprotozooplankton (the protists: ciliates and dinoflagellates) but also small metazoans such as copepod nauplii, copepodites I-II and pluteus larvae. These organisms represent a food resource for mesozooplankton here mainly composed of copepods.

Some metabolic flows, not determined experimentally, were additionally calculated on the basis of existing measurements and information available in the literature. For instance, the bacterial carbon demand (BCD) was estimated from the measured production rate assuming a growth efficiency of 0.28, the average reported for North Sea *Phaeocystis*-dominated ecosystem (Billen & Fontigny, 1987; Billen, 1990; Billen *et al.*, 1990; Brussaard *et al.*, 1995; Becquevort *et al.*, 1998). Furthermore, nano- and micro-zooplankton production rates were estimated from measured grazing rates using an average growth efficiency of 0.3 (Verity, 1985; Caron & Goldman, 1990; Hansen *et al.*, 1993; Riegman *et al.*, 1993). Also, protozoa and mesozooplankton egestion which releases dissolved organic matter, was estimated to 25 % and 15 % of the measured grazing activity, respectively (Eppley *et al.*, 1981; Elbrächter, 1991; Nagata, 2000).

Results

Seasonal evolution of inorganic nutrient concentrations. The seasonal evolution of dissolved inorganic nutrients (NO_3, NH_4, PO_4 and $Si(OH)_4$) is illustrated in Fig. 2. The significant dominance of dissolved inorganic nitrogen by NO_3 (more than 95 % during the winter period; Fig. 2a) is characteristic of the Southern Bight area as a consequence of the strong river influence. NO_3 winter concentrations (Fig. 2a) increased until end of February before dropping to detection limit value in mid-April at the time of the *Phaeocystis* colony bloom (Fig. 3d) and increased again up to 10 µM at the beginning of May. NH_4 concentrations, at a winter level of 0.7 µM, reached 2.1 µM in early April. Later, NH_4 concentrations increased progressively from 0.2 µM in mid-April up to 3.7

µM at the beginning of June, before dropping suddenly to 1.1 µM a few days later (Fig. 2a). PO_4 concentrations decreased from a winter value of 1 µM up to 0.09 µM in early April (Fig. 2b). Later concentrations fluctuated and a ephemeral peak of 0.6 µM was recorded in the second part of April. $Si(OH)_4$ concentrations showed the same variation as nitrate during the winter - early spring period and reached a minimum after mid-March (Fig. 2c). Later, in April and May, these concentrations remained at an average level of 3.6 µM.

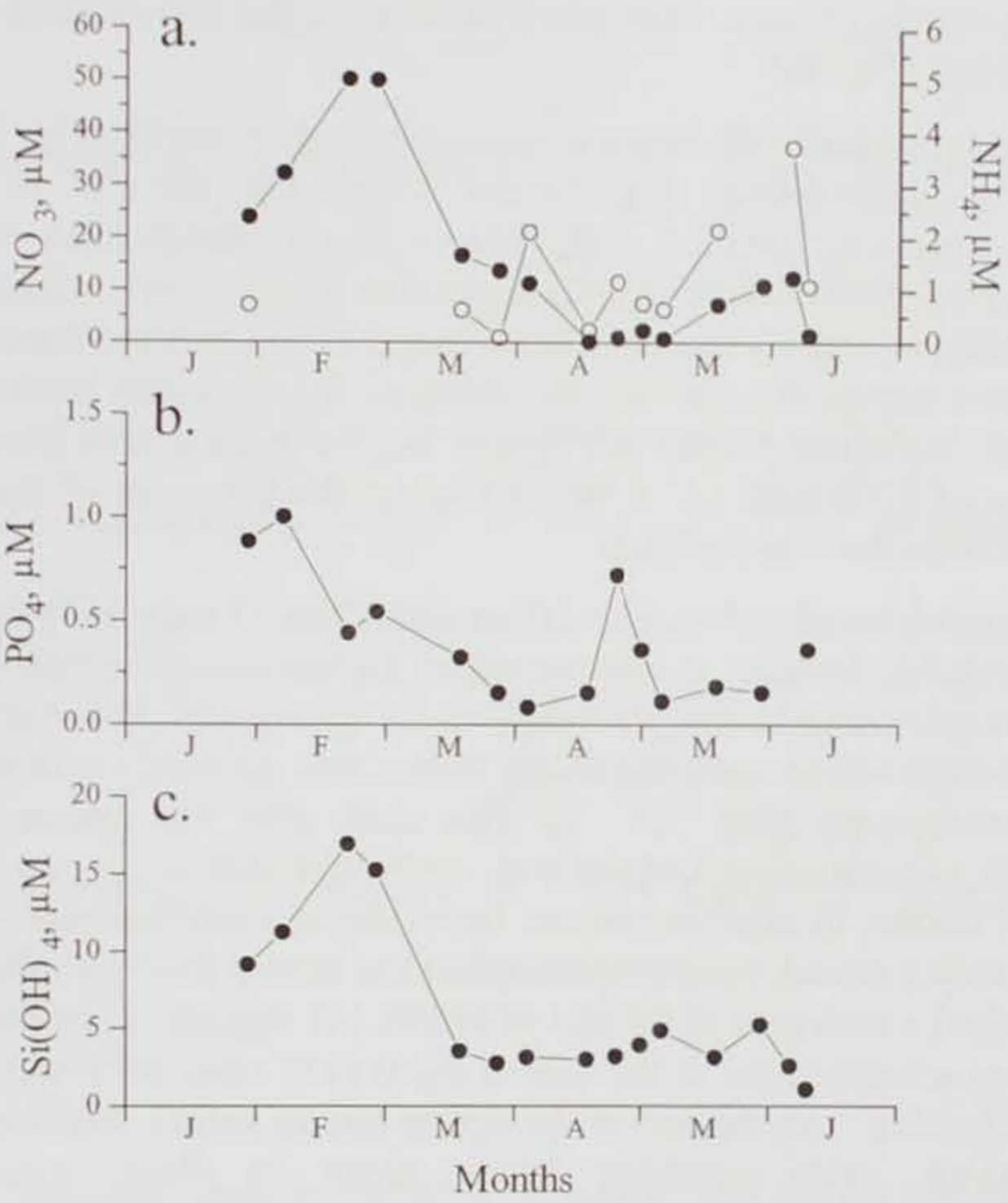

Figure 2 : Seasonal changes recorded at station 330 during spring 1998 of major inorganic nutrient concentrations (µM) a.) NO_3 (black dots), NH_4 (open dots); b.) PO_4 ; c.) $Si(OH)_4$.

Spring succession of auto- and heterotrophic planktonic organisms. The temporal variation of the biomass of auto- and hetero-trophic organisms during spring is shown in Fig. 3. In 1998, the onset of phytoplankton bloom occurred around the end of February and reached a maximum Chl a concentration of 18 mg m^{-3} (Fig. 3a) in mid-April. It decreased sharply during the second part of April down to 2 mg m^{-3} at the end of April. Two additional blooms of moderate amplitude were recorded from May (7 mg m^{-3}) to early June (5 mg m^{-3}). The spring phytoplankton bloom was initiated by a modest diatom bloom (124 mgC m^{-3}; Fig. 3b), consuming a large part of the winter stock of inorganic nutrients (Fig. 2), followed by a nearly monospecific *Phaeocystis* colony bloom (95 % in terms of cellular density) reaching 1100 mgC m^{-3} (Fig.3d). The *Phaeocystis* colony bloom ended in early May, probably due to NO_3 and/or PO_4 limitation (Fig. 2a,b), when a transient modest bloom of nanophytoplankton (150 mgC m^{-3}) was observed (Fig. 3c). At this time, nanophytoplankton was almost entirely dominated by *Phaeocystis* free-living cells. After the decline of the *Phaeocystis* bloom, diatoms accumulated again and reached a biomass of 350 mgC m^{-3} in late June (Fig. 3b).

Two successive maxima of bacterial biomass were observed in spring (Fig. 3e): in early April (20 mgC m^{-3}) at diatom decline (Fig. 3b) and in early May (61 mgC m^{-3}) during the decline of *Phaeocytis* colonies (Fig. 3d). The latter bacterial proliferation lasted for one month. The few available DOC data (Fig. 3e) suggest that these bacteria benefited from the substantial accumulation of organic matter in the water column observed during the second half of April. DOC concentrations were indeed increasing from a winter level of 1615 mgC m^{-3}, close to the DOC background level recorded in Belgian coastal area (Rousseau *et al.*, 1990) to a maximum of 4160 mgC m^{-3} at the end of April when most of the *Phaeocystis* colonies disappeared from the water column.

The biomass of nanoprotozooplankton (Fig. 3f) remained low (5 mgC m^{-3}) during most of the spring period, probably because it was controlled by microzooplankton (Fig. 3g). A transient peak of nanoprotozooplankton (56 mgC m^{-3}; Fig. 3f) was, however, recorded in mid-May. These bacterivorous nanoflagellates were most probably stimulated by the second bacterial development (Fig. 3e). At this time also, the grazing pressure of microzooplankton on nanoprotozooplankton was ineffective due to its low biomass (20 mgC m^{-3}; Fig. 3g). Contrary to small metazoans remaining at a low biomass (< 15 mgC m^{-3}) during the whole spring period, microprotozooplankton started their growth at the end of February. They reached a first peak at the end of March (49 mgC m^{-3}) but showed a much more intense biomass accumulation at the end of April (133 mgC m^{-3}) and disappeared very suddenly in early May. At the end of the spring bloom period, bacteria, nanoproto- and micro-zooplankton were reaching levels close to their winter biomass. Mesozooplankton (Fig. 3h) was composed of the usual coastal North Sea copepod species, mainly *Temora longicornis, Acartia clausi, Centropages hamatus* but also *Pseudo-* and *Para-calanus* and the tunicate *Oikopleura*. A moderate biomass of 48 mgC m^{-3} was reported at the end of March (Fig 3h), after the diatom bloom (Fig. 3b). At that time, *Oikopleura,* a gelatinous herbivorous, was contributing up to 50 % of the bulk mesozooplankton. The biomass of *Oikopleura* was much reduced during *Phaeocystis* colony growth, but a transient peak of 38 mgC m^{-3} was recorded at the end of April (Fig. 3h). Finally, a considerable increase in mesozooplankton biomass occurred in early June. At that time, the dinoflagellate *Noctiluca* appeared and contributed up to 23 % of the bulk mesozooplankton.

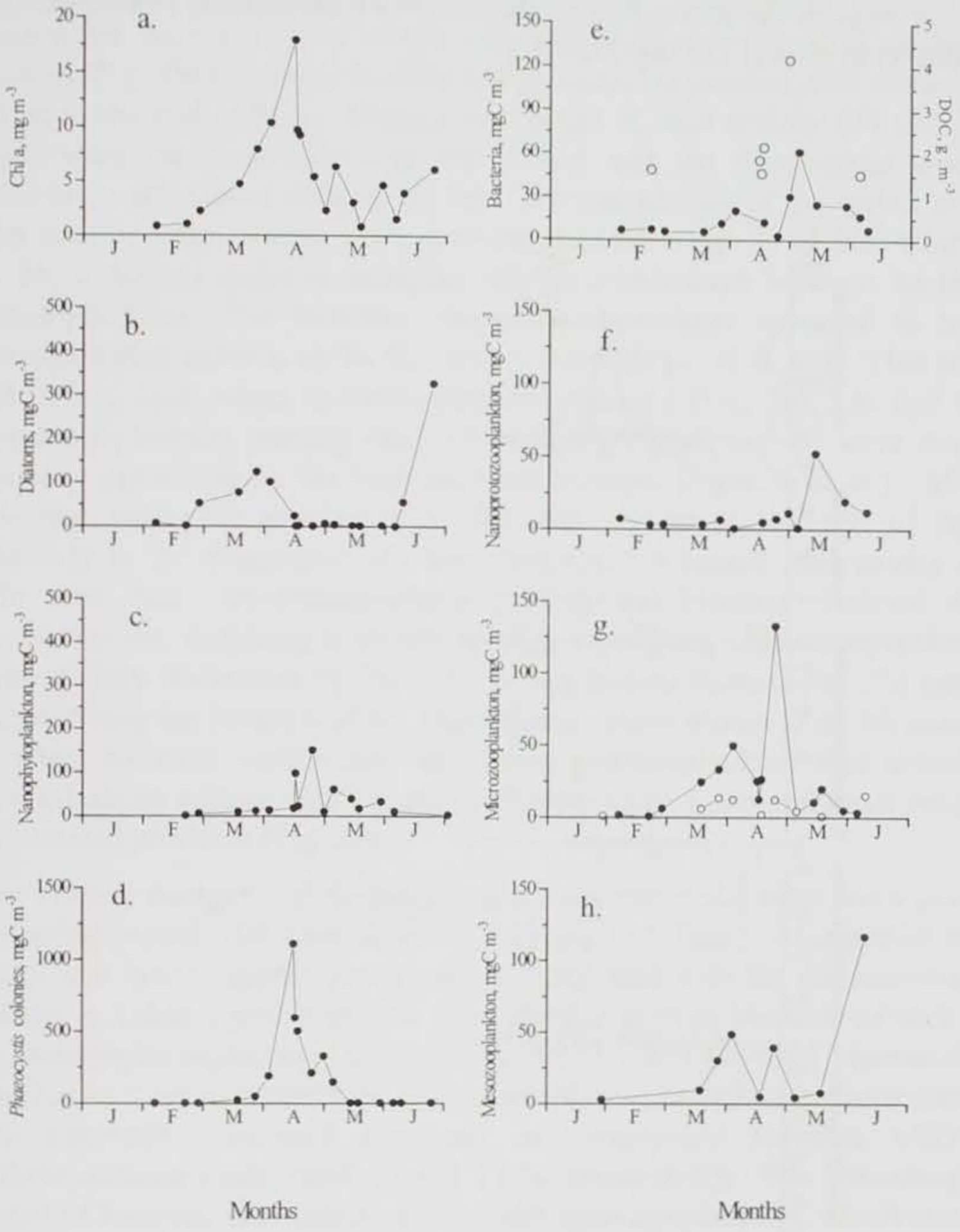

Figure 3 : Seasonal changes recorded at station 330 during spring 1998 of a.) Chl a concentrations (mg m^{-3}) and C-biomasses (mgC m^{-3}) of auto- and hetero-trophic planktonic organisms: b.) diatoms; c.) nanophytoplankton; d.) *Phaeocystis* colonies; e.) bacteria (black dots) and DOC (open dots); f.) nanoprotozooplankton; g.) microprotozooplankton (black dots); nauplii, copepodites I-II, various larvae (open dots); h.) mesozooplankton

Seasonal changes in metabolic activities. The temporal development of daily rates of auto- and hetero-trophic organisms is shown in Fig. 4. In 1998, daily phytoplankton growth (Fig. 4a) was highest in early April (220 mgC m^{-3} d^{-1}) at the time of *Phaeocystis* colony bloom. In the early stage of the spring bloom, diatoms were exclusively responsible for the moderate phytoplankton growth (25 mgC m^{-3} d^{-1}).

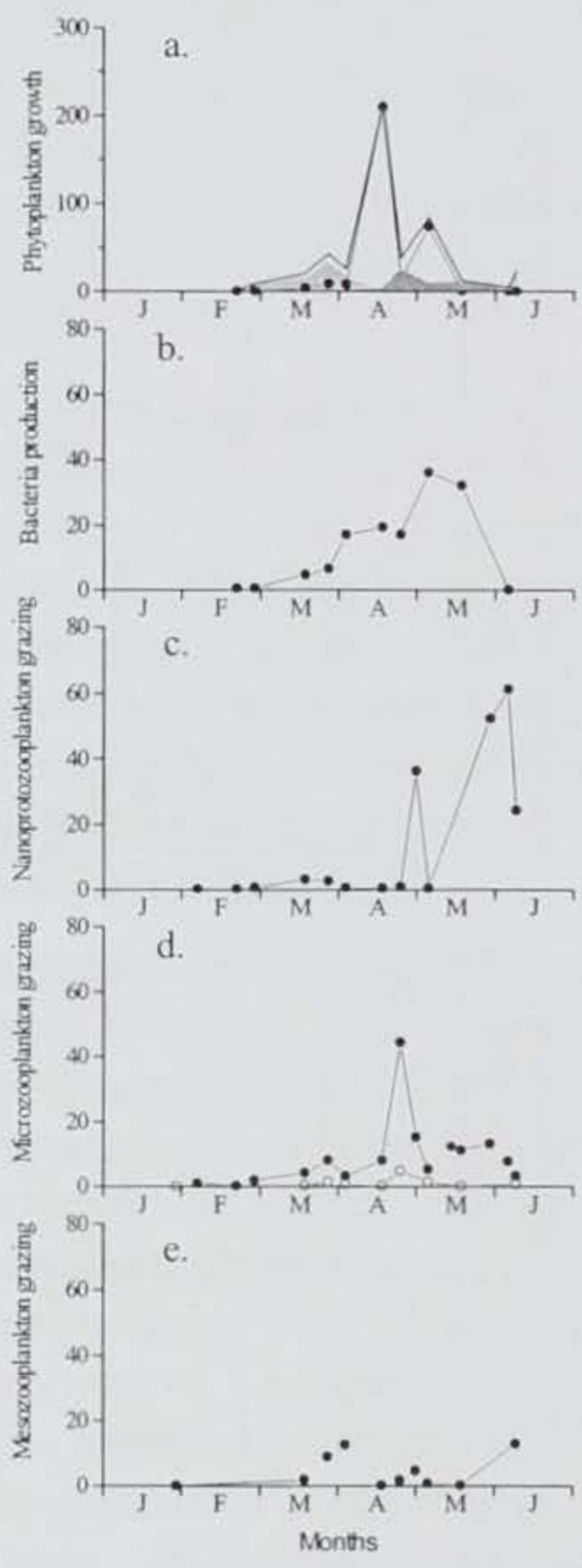

Figure 4: Seasonal evolution of major metabolic activities (mgC m^{-3} d^{-1}) of natural communities succeeding during the spring bloom 1998 at station 330: a.) phytoplankton net primary production (black dots: *Phaeocystis colonies*; light grey area : diatoms; dark grey area: nanophytoplankton); b.) bacterial production; c.) nanoprotozooplankton grazing; d.) microprotozooplankton (black dots); metazooplankton grazing (open dots) grazing; e.) mesozooplankton grazing.

After the decline of the *Phaeocystis* colony bloom, nanophytoplankton was the main primary producer (Figs. 4a & 3d). Heterotrophic activities increased with a delay of between a few days and a few weeks with respect to phytoplankton production. Bacterial production (Fig. 4b) increased in early March almost in concert with diatom production and lasted up to the end of May. Clearly, two peaks of high activity (20 and 35 mgC m^{-3} d^{-1}; Fig. 4b) were recorded following the diatom and the *Phaeocystis* colony production, respectively, with a short delay (Fig. 4a). The comparison of the spring evolution of daily grazing rates of nano-, micro- and meso-zooplankton (Figs. 4c-e) with their respective prey (Figs. 3b, c & e-g) suggests complex trophic interactions between bacterio-, nano- and microzoo-plankton. For instance, nanoprotozooplankton appeared to be controlled by microzooplankton grazing up to the end of April (Figs. 3f & 4d). This top-down control ceased in late April when microzooplankton declined (Fig. 3g). At this time, high daily nanoprotozooplankton grazing rates of 36 and 61 mgC m^{-3} d^{-1} were recorded (Fig. 4c) presumably stimulated by the high bacterial biomass (Figs. 3e & 4c). Microzooplankton (proto- and meta-zoa) grazing (Fig. 4d) was highest at the end of April responding immediately to the abundance of *Phaeocystis* cells released after colony disruption (Fig. 3c). In early May, microzooplankton grazing and biomass declined dramatically for unknown reasons, dropping to winter level in early June. Mesozooplankton grazing (Fig. 4e) was slightly stimulated by the early spring diatom bloom (Fig. 3b) but obviously was restricted during the duration of the *Phaeocystis* colony bloom (Fig. 3d) except at the end of April when colonies were scarce and microprotozooplankton was abundant. Actually, mesozooplankton grazing was effective when the second diatom bloom occurred stimulated by regenerated nutrients (Fig. 3b).

Spring carbon budget. C-biomasses and daily metabolic rates were integrated over the whole spring period (105 days, from 20 February to 6 June). This period included most of the auto- and hetero-trophic proliferations associated with the *Phaeocystis* event (Fig. 3). As shown in Table 2 which reports the weighted average biomass of each trophodynamic group, autotrophs represented the major part (72 %) of the total biomass (310 mgC m^{-3}). This indicates a net accumulation of phytoplanktonic matter in the ecosystem. Some 70 % of the autotrophic biomass consisted of *Phaeocystis* colonies while diatoms and nanophytoplankton contributed 17 and 13 %, respectively. The heterotrophic community consisted of bacteria, nanoproto-, micro- and meso-zooplankton, which contributed 20, 13, 40 and 27 % to the heterotrophic carbon, respectively.

The spring planktonic flow network of carbon (Fig. 5) has been constructed on the basis of the integration of the daily metabolic rates shown in Fig. 4. A total of 6035 mgC m^{-3} $period^{-1}$ was photosynthetised by the 3 groups of phytoplankton. From this, 15 % was respired and 5 % was directly excreted as small metabolites. Some 70 % of the spring net primary production (4846 mgC m^{-3} $period^{-1}$) was attributable to *Phaeocystis* colonies while spring diatoms and nanophytoplankton contributed 17 % and 13 %, respectively.
As *Phaeocystis* colonies were not grazed by the coastal North Sea mesozooplankton (Gasparini *et al.*, 2000), most of the net spring primary production escaped the linear food chain. Some 273 mgC m^{-3} $period^{-1}$, *i.e.* one third of the net diatom production (Fig. 5), fuelled the mesozooplankton directly and contributed 74 % to the total mesozooplankton ingestion. The rest of the mesozooplankton diet was provided by the microbial food web through the grazing of mesozooplankton on microzooplankton (97 mgC m^{-3} $period^{-1}$). This

transfer represented 37 % of the total production of the microbial food web (263 mgC m^{-3} $period^{-1}$; Fig. 5). This production issued from two distinct pathways corresponding to the production path of two possible prey items for microzooplankton. A direct one which consists in the direct grazing of microzooplankton on nanophytoplankton and a more complex one leading to the production of nanoprotozooplankton which involves bacterial production and the subsequent grazing of bacteria by nanoprotozooplankton (Fig. 5). Assuming no food selectivity, budget calculation of Fig. 5 shows a higher efficiency of the direct pathway with nanophytoplankton production supplying 71 % (623 mgC m^{-3} $period^{-1}$) of the total microzooplankton diet (875 mgC m^{-3} $period^{-1}$). This flow corresponded to a transfer of 97 % of the nanophytoplankton production to higher trophic levels, indicating a close coupling between microzooplankton and nanophytoplankton (Fig. 5). A lower proportion (64 %) of the nanoprotozooplankton production was ingested by microzooplankton and corresponded to 29 % of their needs. The nanoprotozooplankton was fuelled by bacterial production. The well-balanced budget between bacterial production (1584 mgC m^{-3} $period^{-1}$) and the nanoprotozooplankton grazing (1315 mgC m^{-3} $period^{-1}$) indicates a strong coupling between bacteria and their grazers.

Table 2 : Average carbon biomass of the different trophodynamic groups present at station 330 during the spring bloom period 1998 (105 days; 20 February-6 June).

Trophodynamic groups	Average biomass mgC m^{-3}
Diatoms	38.3
Nanophytoplankton	27.8
Phaeocystis colonies	156.5
Phaeocystis colonial cells	39.1
Bacteria	17.4
Nanoprotozooplankton	11.6
Microzooplankton	34.7
Mesozooplankton	23.4

Figure 5 : Carbon budget established on basis of integrated flows for the spring period (26 February- 6 June). Flows are expressed in mgC m^{-3} $period^{-1}$. Underlined figures correspond to metabolic flows not experimentally determined but calculated from existing measurements and information available in the literature (see text). O.M. represents the pool of organic matter.

CO_2

6035

Phaeocystis colonies

3397

888

Diatoms

805

273

Sedimentation?

Sedimentation?

370

Mesozooplankton

Microzooplankton

97

263

643

623

Nanophytoplankton

219

56

302

O.M.

5657

1584

1315

395

252

Bacteria

Nanoprotozooplankton

329

The bacterial carbon demand was estimated to 5657 mgC m^{-3} $period^{-1}$. The autogenic sources of carbon for planktonic bacteria originate from phytoplankton exudation, lysis of auto- and hetero-trophic organisms and zooplankton egestion. Budget calculation shows that in 1998, the direct production of small substrates by phytoplankton amounted to 302 mgC m^{-3} $period^{-1}$, *i.e.* 5 % of the bacterial needs. Zooplankton egestion contributed with 603 mgC m^{-3} $period^{-1}$, *i.e.* about 11 % of the BCD. Together these two processes represented therefore a minor contribution to the BCD. Ungrazed *Phaeocystis* colonies, diatoms and nanophytoplankton amounted to 3397, 532 and 20 mgC m^{-3} $period^{-1}$, respectively. Assuming a complete lysis of ungrazed primary production, autogenic carbon available for bacteria could amount to 4854 mgC m^{-3} $period^{-1}$ which compares very well with the estimated BCD, 5657 mgC m^{-3} $period^{-1}$. These results suggest that *Phaeocystis* colony loss by lysis might be the major process stimulating the bacterial activity. The high DOC concentration (Fig. 3e) observed after the *Phaeocystis* colony bloom at the end of April, constitutes an additional element suggesting the occurrence of massive lysis.

Discussion

The assessment of the fate of the large amount of organic matter produced during *Phaeocystis* blooms in the Belgian coastal waters is essential to determine the trophic status of *Phaeocystis*-dominated shallow coastal North Sea systems submitted to man-induced eutrophication. The beaches of these coastal seas are indeed often covered with transient foam layers of *Phaeocystis*-derived material (*e.g.* Lancelot *et al.*, 1987), thus suggesting that only little *Phaeocystis* material fuels the higher trophic levels. The planktonic carbon budget established on the basis of this time-resolution process study (Fig. 5) was intented to give a synoptic view of the trophic efficiency of the planktonic food web in the Belgian coastal ecosystem during the spring period of 1998. The main results of this carbon budget are discussed here and compared with previous studies in order to assess their relevance with respect to other *Phaeocystis*-dominated areas of the North Sea.

The spring carbon budget. As demonstrated by the 1998 results (Gasparini *et al.*, 2000) and in agreement with other existing data (Hansen & van Boekel, 1991; Weisse *et al.*, 1994), *Phaeocystis* colonies, which represented the bulk (70 %) of the net primary production during spring 1998, escaped the linear phytoplankton to mesozooplankton food chain. As nanophytoplankton has been shown non valuable food for North Sea mesozooplankton (Hansen *et al.*, 1993), diatoms were the only primary producers directly available as a food source to mesozooplankton in spring. In 1998, the early spring diatom bloom was particularly moderate in comparison with previous years (Becquevort *et al.*, 1998 ; Lancelot *et al.*, 1998). In spite of its small contribution to the total primary production, the vernal diatom bloom in 1998 supplied more than two-thirds of the mesozooplankton food. Later, during the *Phaeocystis* colony bloom, mesozooplankton grazing was food limited (Gasparini *et al.*, 2000). At that time however, some mesozooplankton food requirements were partially fulfilled by microzooplankton. Surprisingly, only one-third of the microzooplankton production was controlled by copepod grazing pressure. This proportion even drops to 22 % if the maximum microzooplankton growth yield of 0.5 measured by Hansen *et al.* (1993) is considered. This inefficient control of microzooplankton by mesozooplankton contrasts with reported laboratory (Hansen *et al.*, 1993) and field (Brussaard *et al.*, 1995 ; Gasparini *et al.*, 2000) grazing experiments which demonstrate indeed that microzooplankton is a valuable prey for copepods. Physical or

chemical stress due to the presence in the water column of large gelatinous *Phaeocystis* colonies affecting the feeding behaviour of copepods could be put forward (Gasparini *et al.*, 2000). On the other hand, the presence of other metazoans grazing on microzooplankton, not investigated in this study, cannot be excluded. This could explain the gap observed between microzooplankton production and its grazing by copepods. Fish larvae (Fukami *et al.*, 1999), ctenophore larvae (Stoecker *et al.*, 1987), cladocerans (Turner *et al.*, 1988) have indeed been reported to achieve an important link between microzooplankton and higher trophic levels in other coastal areas.

Budget calculations suggest that ungrazed *Phaeocystis* colonies were stimulating the development of a very active microbial network. The release of solitary cells from nutrient-stressed *Phaeocystis* colonies to the surrounding medium, which can be either controlled by *Phaeocystis* itself (Veldhuis *et al.*, 1986; Rousseau *et al.*, 1994) or be a result of colony disruption (Lancelot & Rousseau, 1994), has been shown to stimulate the growth of microprotozooplankton (Admiraal & Venekamp, 1986; Weisse & Scheffel-Möser, 1990). In spring 1998 as well, the microzooplankton largely dominated by protists, was controlling 97 % of the nanophytoplankton production. In addition to the release of free-living cells, *Phaeocystis* colony disruption supplies the water column with large amounts of dissolved organic matter available for bacteria (Thingstad & Billen, 1994; Lancelot, 1995). At that time, bacteria were already blooming (Fig. 3e), utilizing presumably organic products derived from the early spring diatom production. The additional supply of *Phaeocystis*-derived dissolved organic matter (Fig. 3e) enhanced the magnitude and extent of the bacterial bloom up to June. Assuming that the carbon of the *Phaeocystis* colony matrix is entirely biodegradable and nutrients were not limiting, budget calculation estimates the maximal contribution of ungrazed colonies to the BCD at 60 %. Alternative sources are provided by exudation, zooplankton egestion and lysis of other organisms. Altogether these processes contributed up to 90 % of the BCD in 1998. As such, this calculation indicates that the spring carbon demand of planktonic bacteria was almost entirely satisfied by the autogenic production with no carbon left for supply to the sediment. This conclusion would not be modified if the BCD was calculated with extreme reported values for bacteria growth efficiency 0.1 and 0.35.

The discharge of continental organic matter by the river Scheldt should therefore be considered as complementary source of biodegradable carbon in these coastal waters. Further work should be done in this direction.

The trophic efficiency of the planktonic food web. The trophic efficiency of the linear and microbial food webs was further defined as the ratio between the mesozooplankton grazing on a given food source and the food production. In spite of its major contribution to mesozooplankton feeding, the trophic efficiency of the linear food chain was only 34 % of the diatom net production. It drops to 5.6 % if the total available net primary production is considered. The trophic efficiency of the microbial food chain was calculated as the ratio between mesozooplankton grazing on microzooplankton and the resource inflow. The latter was calculated as the sum of the bacterial carbon demand and the nanophytoplankton production. This calculation gives a trophic efficiency of 1.5 % suggesting that the transfer of carbon to mesozooplankton through microzooplankton grazing was not significant.

The fate of *Phaeocystis* colony bloom. These low trophic efficiencies together with the high potential contribution of ungrazed primary production to the BCD suggest that most of

the *Phaeocystis*-derived production would be remineralised in the water column. Furthermore some investigations on bacterial activities in Belgian coastal waters during spring 1994 suggested that *Phaeocystis* production as a whole was sufficient to sustain the BCD shared partly by free-living and *Phaeocystis*-attached bacteria (Becquevort *et al.*, 1998). In the same line, Brussaard *et al.* (1995) concluded that the lysis products of ungrazed *Phaeocystis* accounted for 75 % of the decline of the bloom in the Dutch coastal waters.

Although no direct sedimentation of *Phaeocystis* material has been measured in the Belgian coastal waters, this result contrasts with visual observation (Cattrijsse, pers. comm.) and sediment spring release of dissolved Fe and Mn recorded in spring in the same area (Schoemann *et al.*, 1998) as indirect measurement of spring transfer of fresh organic matter from the water column to the sediment. In other areas of the Southern North Sea, Riebesell (1993) and Peperzak *et al.* (1998) also observed *Phaeocystis* colonies accumulated onto the bottom in the southern North Sea. This suggests that remineralisation is not always the dominant fate of the eutrophication-related *Phaeocystis* blooms of the Southern Bight of the North Sea. The occurrence of preferential sites for sedimentation depending on local turbulent strength could be an explanation of the different fate of *Phaeocystis* reported.

Even though the remineralisation of *Phaeocystis* in the water column was suggested to be the main fate of *Phaeocystis*-derived organic matter in spring 1998 in the Belgian coastal waters, several questions need still to be addressed. Sedimentation of *Phaeocystis* colonies seems to be a minor process in these coastal waters but should be definitely investigated in relation with benthic communities. Also nitrogen and phosphate regeneration processes accompanying the remineralisation *Phaeocystis*-derived material should be investigated as well their impact on the summer phytoplankton blooms.

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Chapter 8

Coastal eutrophication of the Southern Bight of the North Sea: assesment and modelling

C. Lancelot, V. Rousseau, G. Billen and D. van Eeckhout

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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.

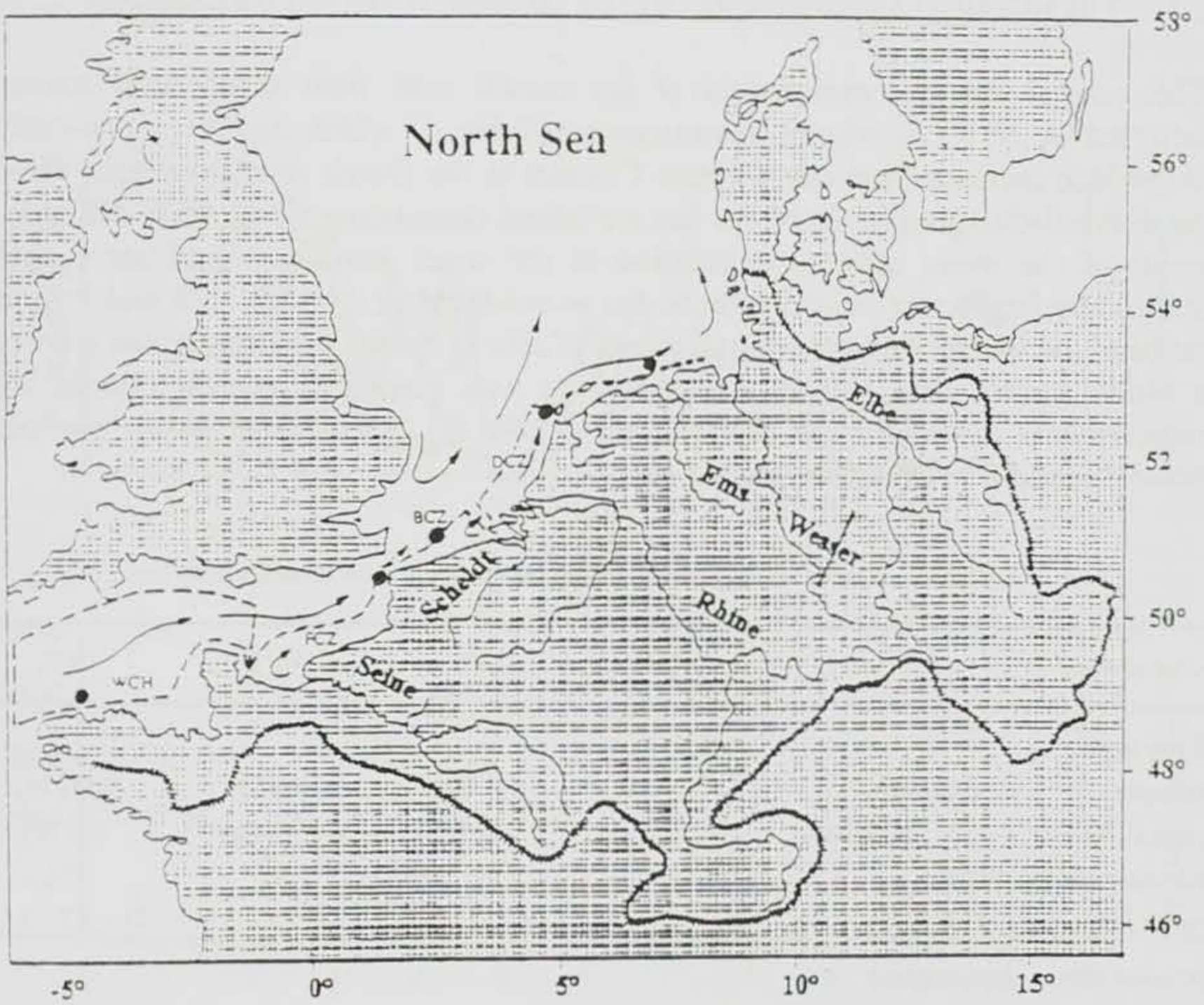

Figure 1. Map showing the watershed of the main rivers discharging in the continental coastal waters of the North Sea; the location of the monitoring stations (●); and the sub-areas (dotted contours) considered for the application of the MIRO model (WCH Western Channel; FCZ : French coastal zone; BCZ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

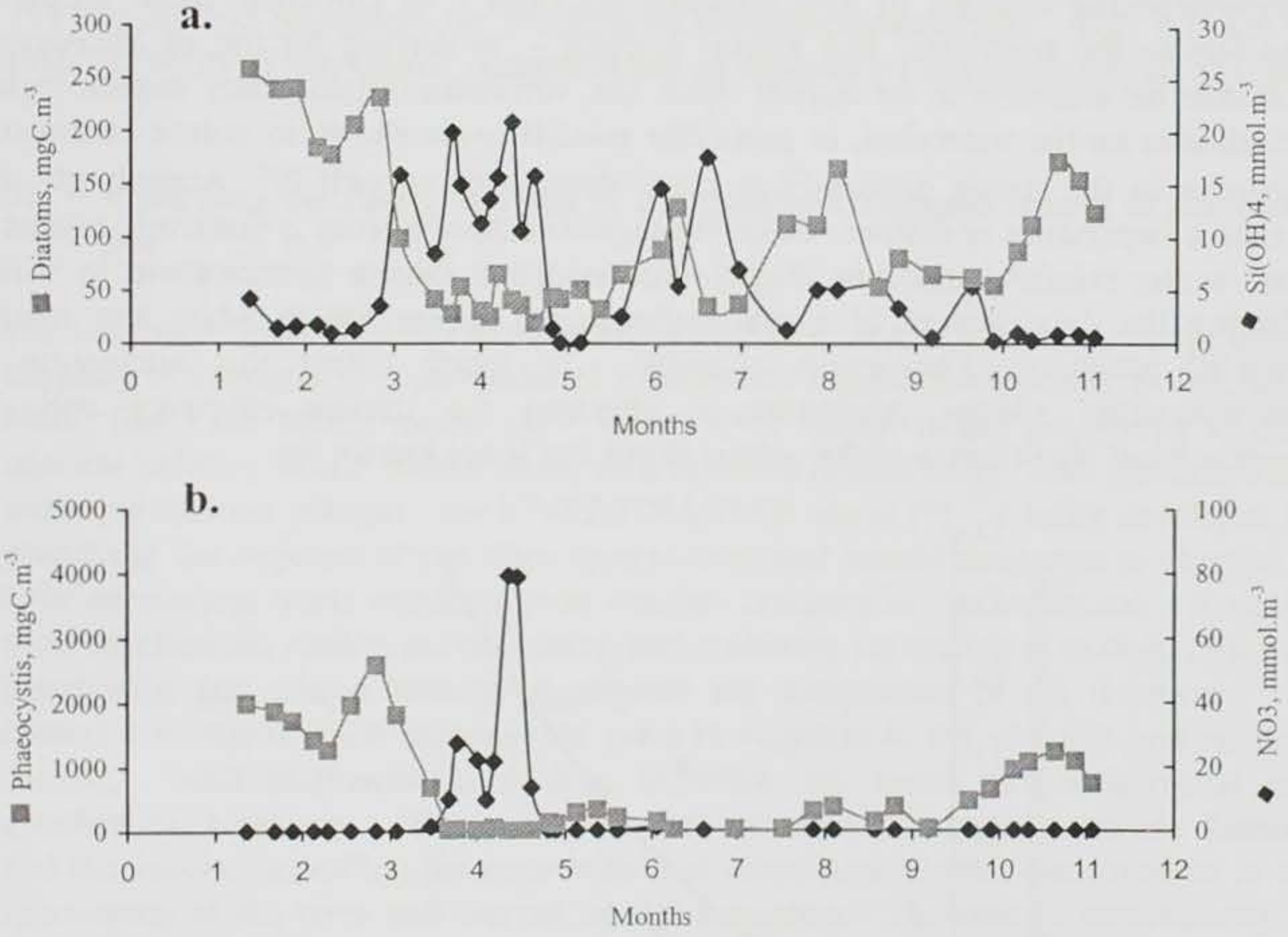

Figure 2 Typical spring phytoplankton succession and nutrients cycle in the Belgian coastal waters Monitoring data 1993 of Rousseau et *al.*[9]

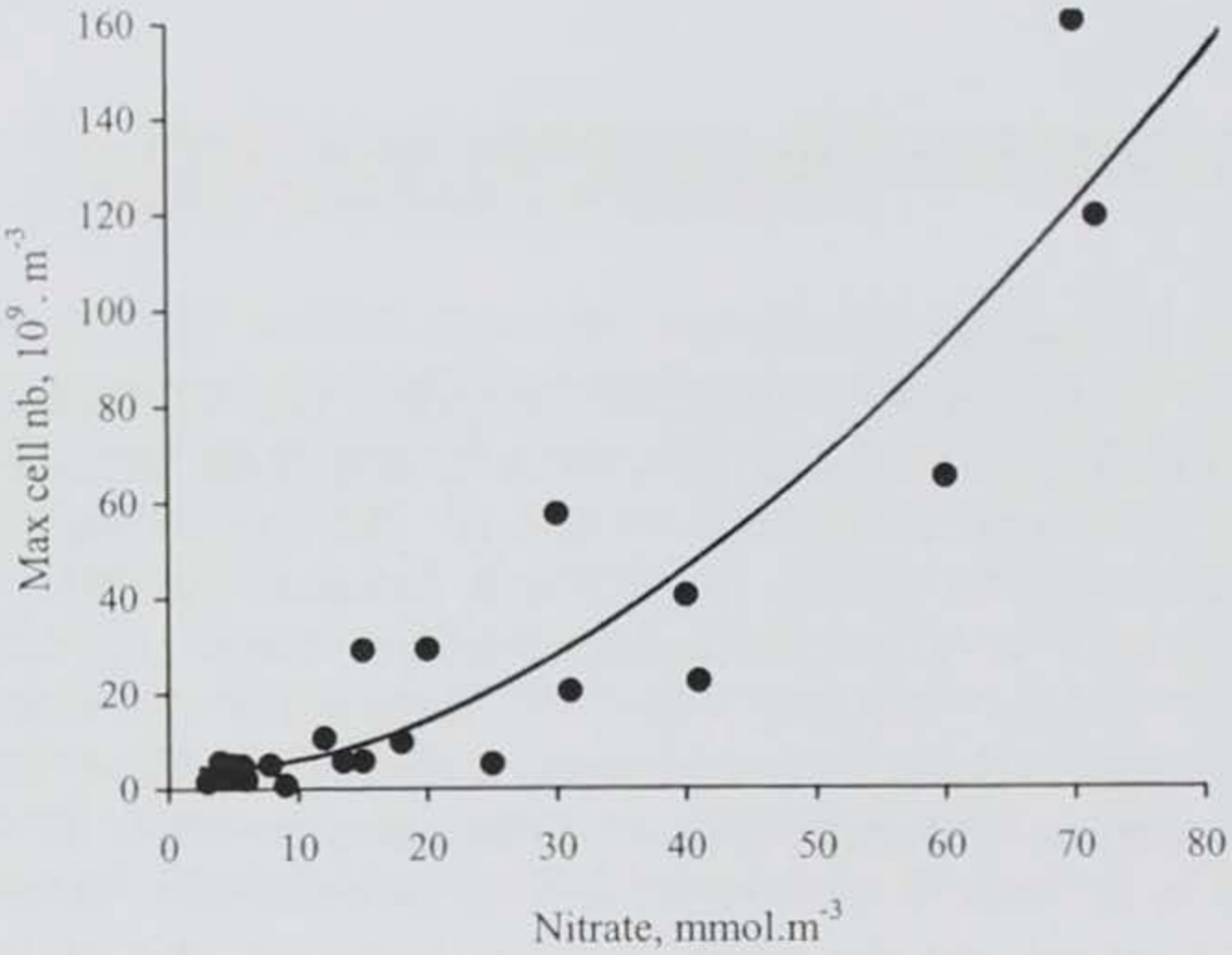

Figure 3 Relationship between *Phaeocystis* colony bloom magnitude and nitrate concentration at *Phaeocystis* onset. 1988-1995 data of Rousseau *et al.* [9]

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.

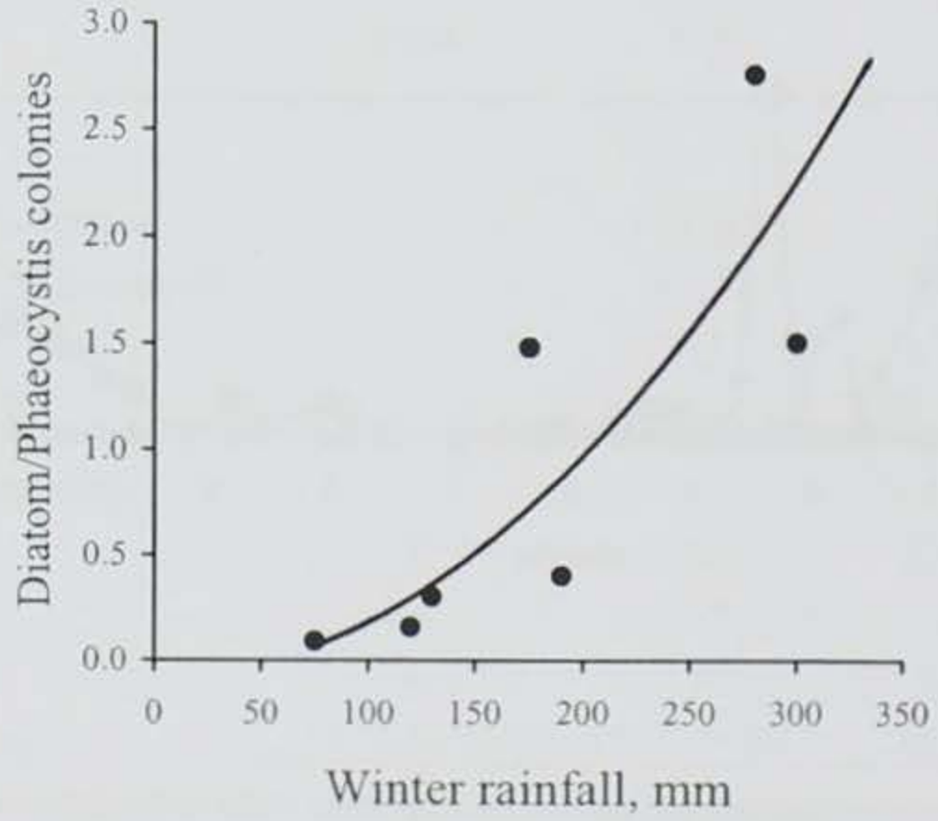

Figure 4 Relationship between late winter rainfall on the Scheldt watershed and the relative importance of the diatom :*Phaeocystis* carbon biomass. After Rousseau *et al.* [9]

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].

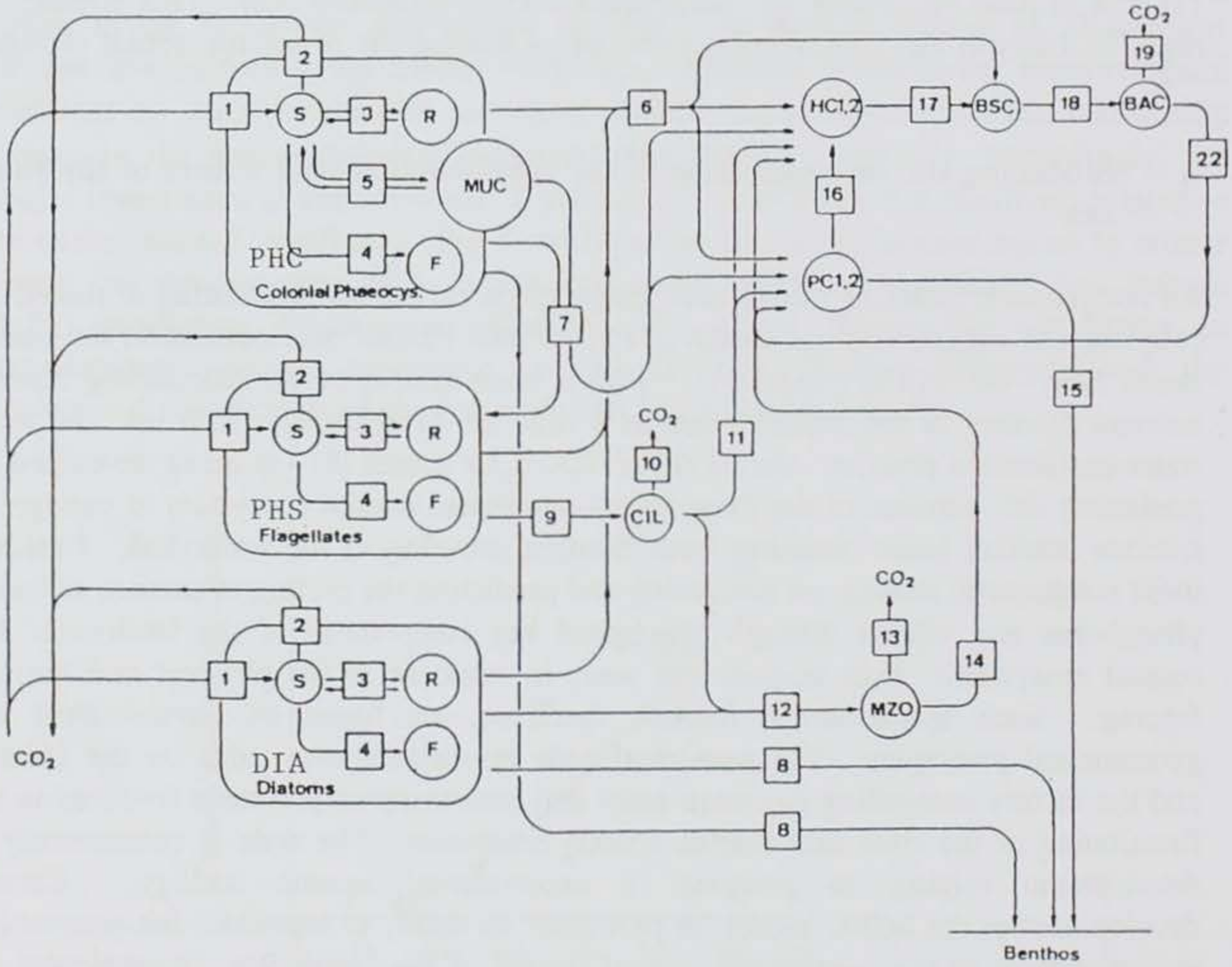

Figure 5 Structure of the MIRO model : carbon cycling. Legend for state variables (circles) and processes linking them (numbers) in text.

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.

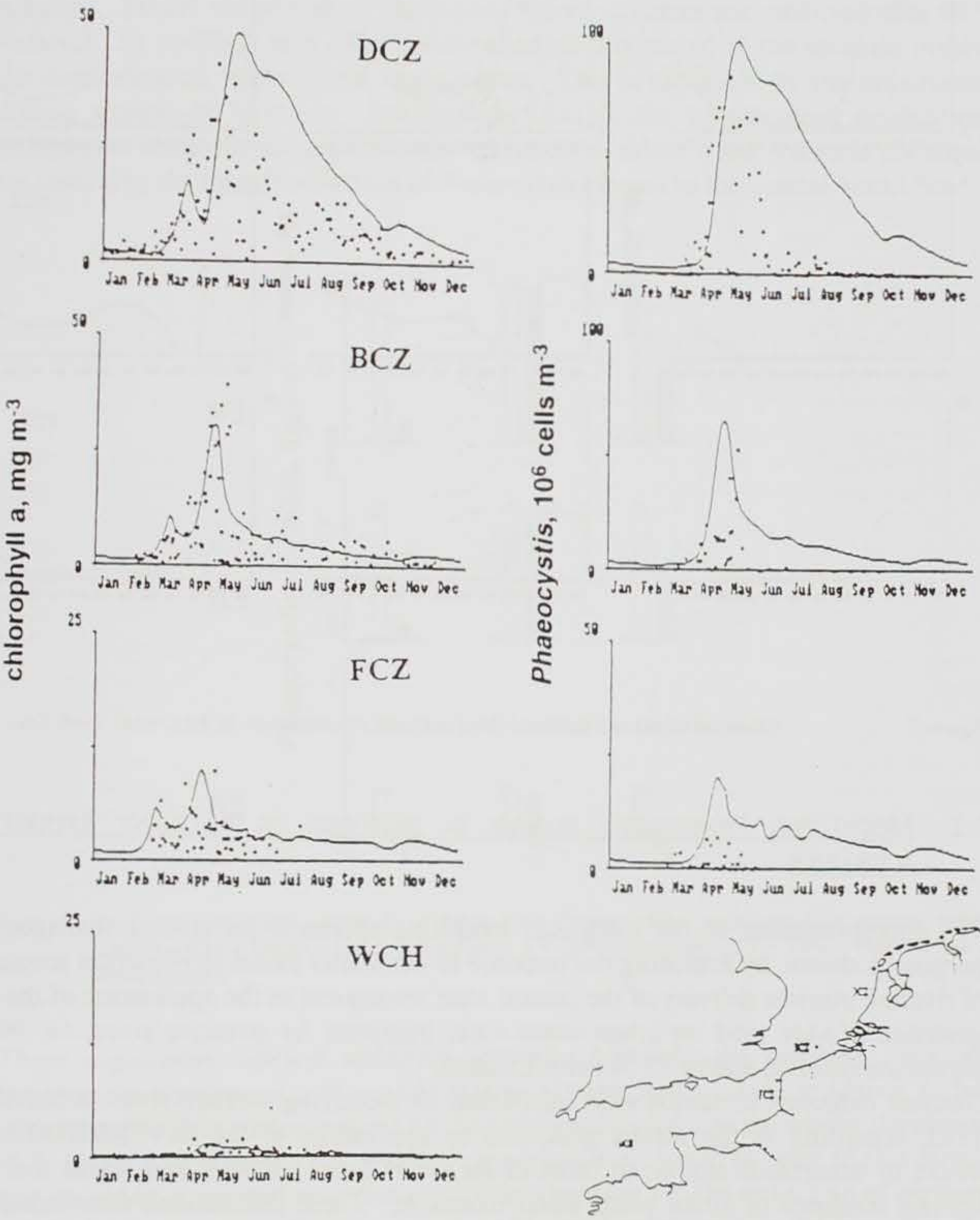

Figure 6 Observed (dots) and predicted (line) phytoplankton development in the coastal North Sea. Total chlorophyll a (a) and *Phaeocystis* cells (b)

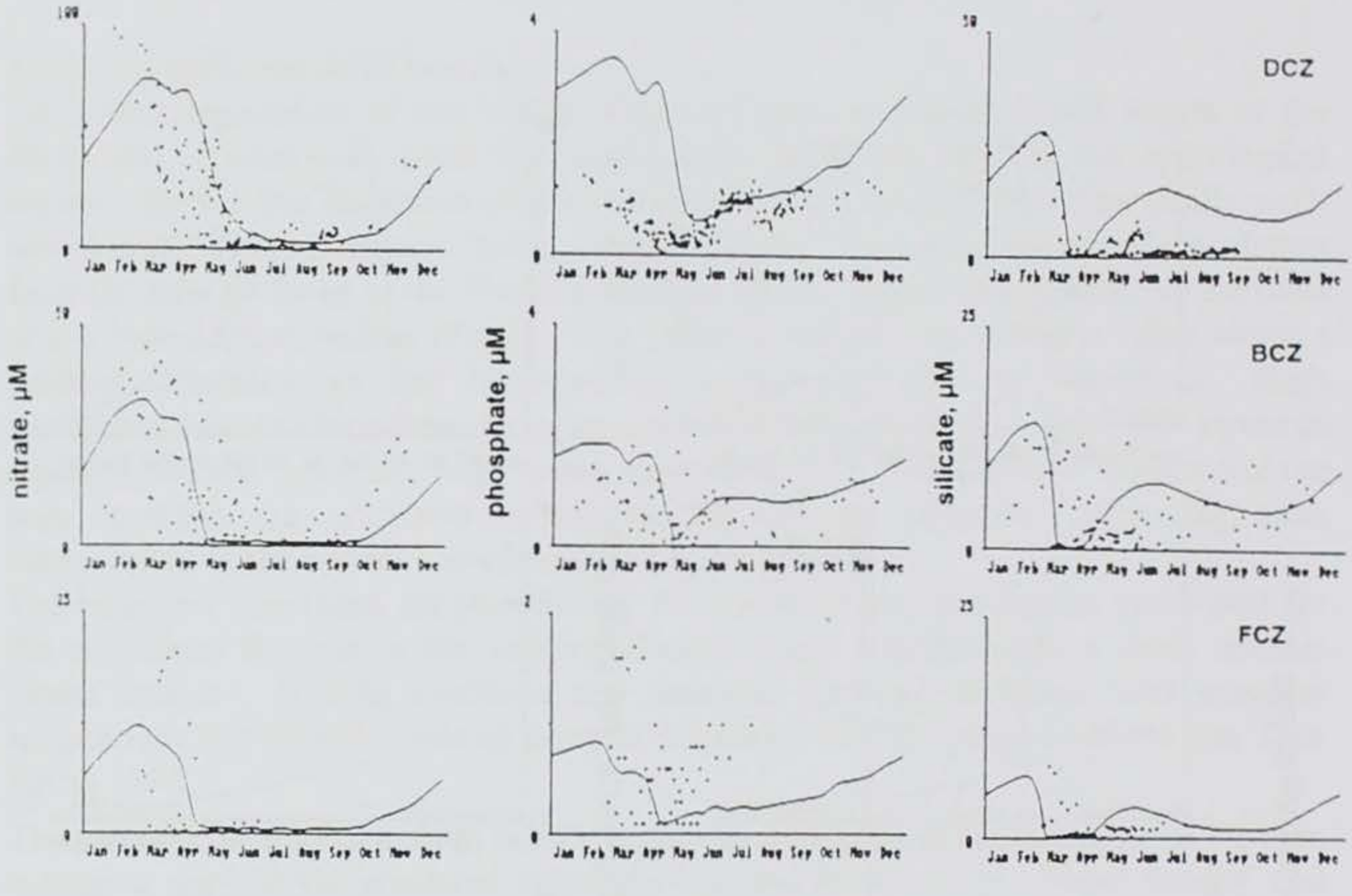

Figure 7 Observed (dots) and predicted (line) nutrients concentration in the coastal North Sea.

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.

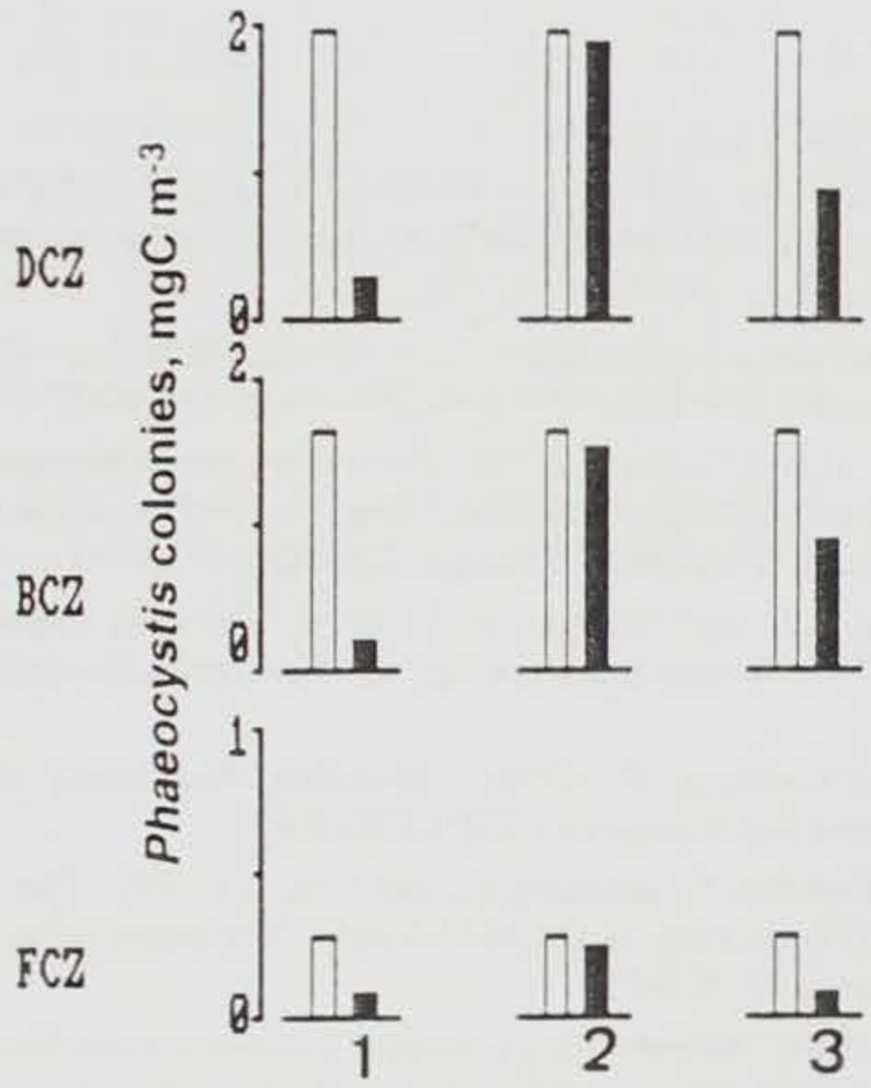

Figure 8 Predicted present-day magnitude of *Phaeocystis* blooms (white block) and after nutrient reduction scenarios (black block) : 1 = 90% P removal; 2 = 75% denitrification; 3 = 90% P removal and 75% denitrification

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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Chapter 9

General discussion

General discussion

Marine coastal eutrophication and phytoplankton physiology

Phytoplankton dynamics, competition and succession are the results of growth and loss processes. Phytoplankton growth is regulated by temperature, light and nutrient availability with large group- and species-specific differences. Loss processes are the fact of grazing, lysis and sedimentation. The dominance of one of the species depends on both growth characteristics and loss factors.

Human-induced coastal eutrophication appears usually as a shift from a diatom- towards non siliceous species-dominated phytoplankton community in response to nutrient delivery to the coastal zone. The continental nutrient sources present a large excess of anthropogenic N and P relative to Si availability compared to diatoms requirements (Officer & Ryther, 1980; Billen *et al.*, 1991; Conley *et al.*, 1993). This concept of eutrophication is based on the assumption of diatom limitation by Si availability.

The competitive superiority of diatoms has been related to their higher maximum growth rates compared to flagellates and other phytoplankters (Furnas, 1990; Smayda, 1997). The determination of environmental control of growth rate is therefore of capital importance for understanding the dominance of either diatom or non-siliceous phytoplankton in the phytoplankton community. Temperature adaptation is encompassed in the seasonal succession pattern. It is generally admitted that diatoms will outcompetiting other phytoplankton provided that well-balanced nutrients are supplied with regards to their requirements (Sommer, 1994), *i.e.* N:Si = 1 (Brzezinski, 1985) and N:P = 16 (Redfield *et al.*, 1963). The success of non siliceous species was attributed to nutrient physiological characteristics contrasting with those of diatoms (Smayda, 1997). The dominance of non siliceous species in anthropogenically-enriched coastal environment has been attributed to characteristic high values of half-saturation constant Ks for N and P uptake compared to those of diatoms (Smayda, 1997). The distinction of photo-adaptative characteristics proper to both taxa appears less evident (Smayda, 1997).

While diatoms are usually efficiently controlled by grazers, the non-siliceous species growing in anthropogenically enriched waters, are toxic or non edible by mesozooplankton (Smayda, 1990, 1997). These phytoplankton species consequently accumulate in the ecosystem, causing harmful algal bloom (HAB) event with resulting deleterious effects for the coastal ecosystem.

Phaeocystis colony blooms in the Southern Bight of the North Sea

Recurrent spring blooms of *Phaeocystis* colonies are recorded in the Southern Bight of the North Sea, succeeding to a late winter-early spring diatom community. The genus *Phaeocystis* presents the typical characteristics of non-siliceous HAB species with a high capacity to grow in anthropogenically-enriched waters and being

unpalatable for mesozooplankton. The life cycle of *Phaeocystis* alternates several types of nanoplanktonic cells and large colonies (Chapter 1). The prodigious success of *Phaeocystis* as blooming species in these eutrophicated waters was related to its ability to form large gelatinous colonies (Chapters 3, 4 & 5). The eco-physiological characteristics of the colonial stage allow *Phaeocystis* to take benefit of the large excess of NO_3 over $Si(OH)_4$ and PO_4 relative to N:P:Si diatom requirements, characterizing the nutrient environment of these coastal waters.

The higher ability of *Phaeocystis* colonies to utilize energy-consuming NO_3 is explained by the physiological function of the mucilaginous matrix. This latter increases indeed the energy storage capacity of each cell (Chapter 4). This was demonstrated elsewhere through competitive experiments in chemostats (Riegman *et al.*, 1992) and in the field where f-ratio of 0.5-0.8 are recorded (Lancelot *et al.*, 1986; Smith *et al.*, 1992). This is well evidenced by the empirical relationship existing between the NO_3 amount left over after the diatom decline (chapter 8).

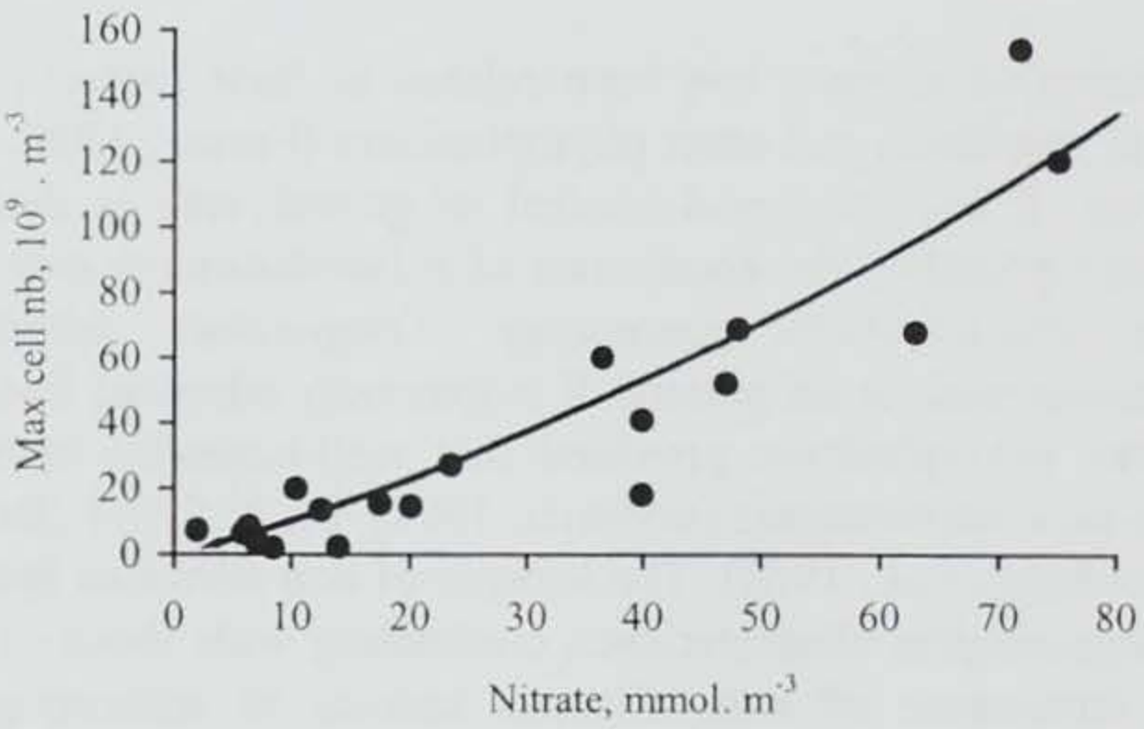

Figure 1: Relationship between *Phaeocystis* maximum cell density reached during the bloom and NO_3 concentrations at diatom decline.

The foam event observed every year on the beaches of the Southern Bight of the North Sea is the manifest consequence of a disruption of the trophic chain. The colonial structure of *Phaeocystis* is indeed responsible for massive accumulation of organic matter in the water column due to its unpalatability for indigenous mesozooplankton (Lancelot, 1995; Gasparini *et al.*, 2000). In these shallow coastal waters, *Phaeocystis* colonies were indeed shown to escape direct grazing due to a mismatch between the size of colonies and most of indigenous mesozooplankton.

Ungrazed *Phaeocystis* colonies stimulate the establishment of a very active microbial network. This latter indirectly resumes the linear food chain through microzooplankton grazing on *Phaeocystis* free-living cells and heterotrophic

nanoflagellates. However, the trophic effiency of this microbial food chain is very low, amounting to 1.6 %. The huge biomass produced by *Phaeocystis* colony bloom has therefore little benefit for the higher trophic levels (Chapter 7).

The very low trophic efficiency of the planktonic linear food chain and of the microbial network together with the potential contribution of ungrazed *Phaeocystis*-derived production to the bacterial carbon demand suggest that most of the *Phaeocystis*-derived production in the Belgian coastal waters is remineralised in the water column (Chapter 7). The *Phaeocystis* colony production would then acts as a additional, transient particulate nutrient reservoir with few usefulness for the ecosystem but rather negative effects. Some sedimentation of *Phaeocystis* colonies onto the bottom could however occur (Riebesell, 1993; Peperzak *et al.*, 1998) the most probably in preferential sites depending on the prevailing hydrodynamic characteristics. The northeastward exportation of a part of the *Phaeocystis* biomass, due to the residual circulation, could well be at the origin of the summer eutrophication problems recorded in this area (van Beusekom, 1999). The accumulation of ugly foam onto the beaches, resulting from exportation of *Phaeocystis* organic matter under coastward strong wind and stormy conditions, is another negative effect of these blooms.

The diatom-Phaeocystis succession

The blooming of *Phaeocystis* colonies succeeds to an early-spring diatom growth and co-occurs with late spring diatoms (Chapters 5 & 6). The diatom flowering is composed of a succession pattern of 3 main communities. A late winter-early spring diatom bloom occurs generally from mid-February to mid-March and is constituted of an assemblage of small neritic species whose the more common are *Skeletonema costatum, Asterionnella glacialis, Thalassionema nitzschioïdes, Thalassiosira* spp., *Plagiogramma brockmanii, Melosira sulcata*. This community is progressively replaced by a *Chaetoceros* spp. - *Schroederella* sp. assemblage. Later on, co-occurring with *Phaeocystis* colonies, a *Rhizosolenia* spp. - dominated community takes place (Chapters 5 & 6).

This reported diatom succession, with an accumulation of the *Rhizosolenia* spp.-dominated diatom community in late spring-summer, is also recorded in poorly enriched Atlantic waters (Grall, 1972; Sournia *et al.*, 1987). This suggests that the succession of the three diatom assemblages constitutes the basic component of the North Atlantic waters that flows through the Channel along the continental coast. *Phaeocystis* colonies appear then as an additional component of the phytoplankton succession pattern, grafting on the natural diatom succession in response to the continental sources of anthropogenic nutrients (Chapter 5 & 6).

The Si-limitation hypothesis

It is generally admitted that *Phaeocystis* colonies bloom after a first silica-limited diatoms blooms (Gieskes & Kraay, 1975; Lancelot & Mathot, 1987; Veldhuis *et al.*, 1986; Weisse *et al.*, 1986). Without direct experimental evidence, some of our results indicate, however, that silicate is not the only regulating factors of the diatom-*Phaeocystis* succession.

The positive relationship observed between the silicification level (Si:C) of diatoms and Si availability suggests that the different succeeding diatom communities are well adapted to their Si environment (chapter 6). Particularly, the low Si:C characterizing the *Rhizosolenia* spp.-dominated community suggests this diatom is particularly well adapted to grow on low silicate levels prevailing at the time of their flowering (chapters 5 & 6).

Our time-series data on phytoplankton in Belgian coastal waters indicate a higher contribution of the *Rhizosolenia* spp.-dominated community to the total cellular C-biomass of phytoplankton at low salinity. The winter nutrient ratios indicate elsewhere a pronounced change in nutrient quality along salinity gradient with a deficit of PO_4 over $Si(OH)_4$ while NO_3 is in large excess with regards to N:P:Si 16:1:16 ratio required by diatoms. This would suggest that only well-balanced nutrient environment in terms of Si but also PO_4 will stimulate diatom growth. The spectacular development of *Rhizosolenia delicatula* in the waters of the Channel (Sournia *et al.*, 1987; Lancelot *et al.*, 1991) would confirm the ability of this diatom to grow when nutrients are well balanced either due to remineralisation (Del Amo *et al.*, 1997), either due to Atlantic waters inflows.

All together, these elements indicate that *Rhizosolenia* spp. are able to grow and reach high biomass on low nutrients concentrations provided their are equilibrated. Rather than a $Si(OH)_4$ limitation, some PO_4 limitation or/and $Si(OH)_4$ and PO_4 co-limitation could control the diatom growth. The potential PO_4 limitation of diatoms could also well explain the non-limiting $Si(OH)_4$ concentrations (3 to 5 µM) recorded during the *Phaeocystis* spring bloom in the Belgian coastal waters (chapter 5). This would also indicate that the diatoms of the *Rhizosolenia* spp.-dominated community are better competitors than *Phaeocystis* colonies if N, P and Si are well balanced. This is not contradictory with the observation that the relative proportion of diatom in the phytoplankton community is related to Si availability (Egge & Asknes, 1992), provided that the other nutrients are not limiting.

A better light adaptation of *Rhizosolenia* spp. to the turbid waters close to the estuary could however not be excluded.

The nutrient behaviour of Phaeocystis

The relationship observed between the average *Phaeocystis* colonial cells C-biomass and the average salinities prevailing during spring (1988-1999) indicates a preferential *Phaeocystis* colonies in high salinity water masses (Fig. 2). This observation, the very low PO_4 concentrations recorded during the *Phaeocystis* colonies bloom and the excess of NO_3 left over by the diatoms (chapter 4-7) would indicate that Phaeocystis is able to grow NO_3 excess to grow in PO_4 deficient waters masses (chapter 5). The magnitude and extent of *Phaeocystis* colony bloom would rely then not only on new sources of NO_3 but also on regeneration processes of ungrazed diatom-derived organic matter for their PO_4 requirements (chapters 5 & 7). *Phaeocystis* would therefore be characterized by a "mixed" nutritional behaviour, growing on new sources of NO_3 and partially remineralised PO_4. A carbon budget established for the spring bloom period in the Belgian coastal waters shows most of diatom production underwent lysis and was remineralised by bacteria (chapter 7). On the other hand, the ability of

Phaeocystis to grow on organic source of phosphorus has been established in cultures (Veldhuis *et al.*, 1987; van Boekel & Veldhuis, 1990; van Boekel, 1991).

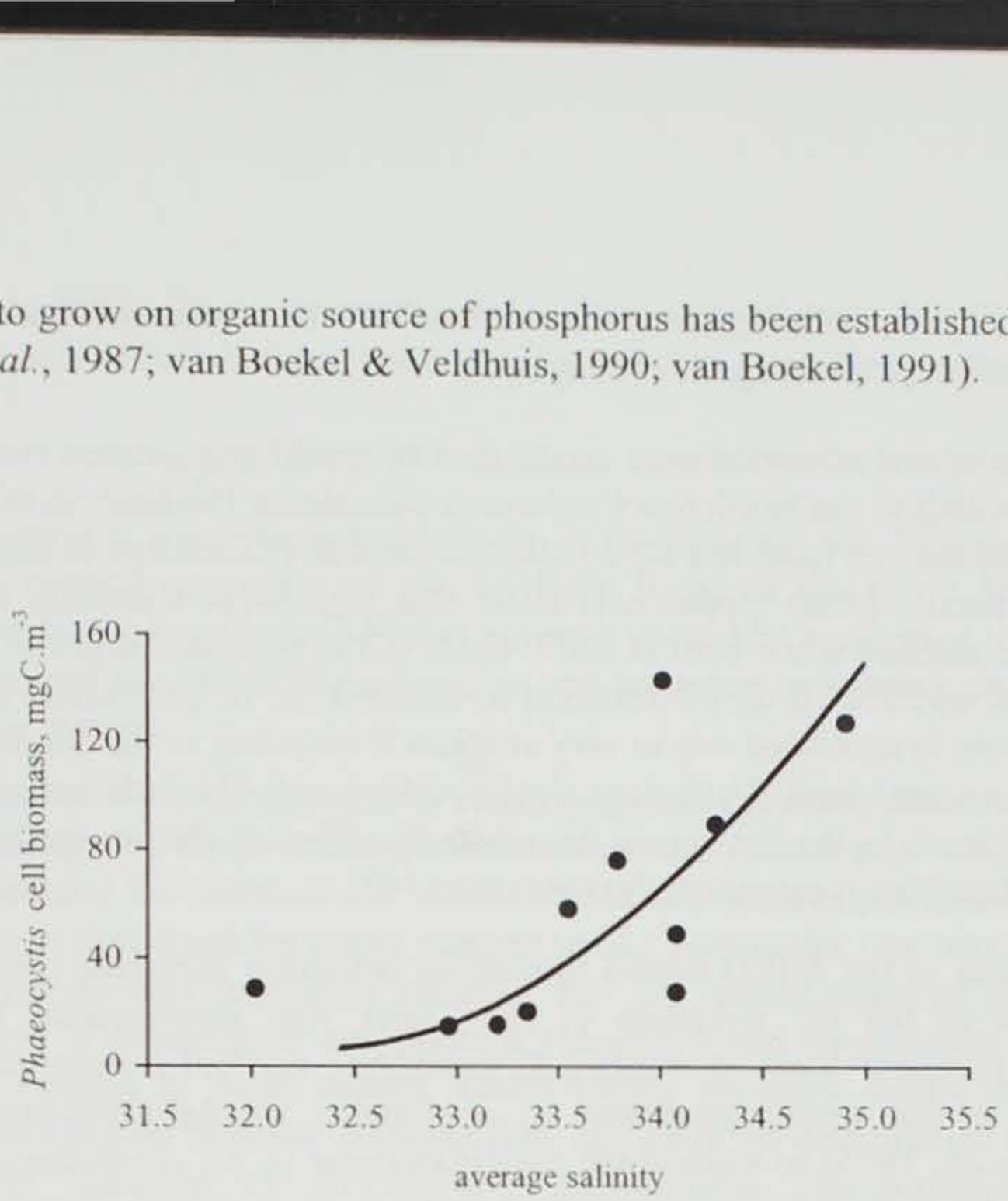

Figure 2 : Relationship between average spring *Phaeocystis* colonial cell C-biomass and the average salinity prevailing during their growth (data 1988-1999).

This has been recently shown in the field where a positive correlation between alkaline phosphatase activity and *Phaeocystis* colony biomass (S. Becquevort; pers. comm.) would indicate the ability of *Phaeocystis* to hydrolyze organically bound P.

The conclusions of the above discussion suggest that the reduction of PO_4 loads would therefore have little impact on *Phaeocystis* colony bloom while reducing diatom flowering. On the contrary, a reduction of the NO_3 loads should be required for efficient lowering of *Phaeocystis* bloom intensity.

Nutrient reduction scenarios

Substantial reduction of harmful *Phaeocystis* blooms in the continental coastal waters of the North Sea is nowadays the main concern of Belgian and international public authorities. This occurs through the formulation of regulations on sewage treatment facilities, land use change and farming practices aiming at the reduction of riverine nutrient delivery to the coastal sea.

At the Second International Conference on the Protection of the North Sea (London, November 1987), the participating ministers of environment decided, as a preventive approach, to reduce by 50 % the N and P loads into the North Sea over the period 1985-1995. In parallel, the EC guideline of May 1991 on urban waste water treatment for sensitive areas, recommend strong nutrient removal from point sources, *i.e.* 90 % phosphate removal and/or 75 % denitrification. The response of the MIRO model to

reduction scenarios of riverine nutrient delivery of the coastal zone consequent to the two directives was investigated. (Chapter 8)

Nutrient reduction scenarios were established by modifying nutrient riverine inputs of 1985, according to the North Sea Conference Ministerial Declaration of 1987 *i.e.* 50% of P load on the one hand and 50% reduction of N & P discharge to the coastal sea on the other hand. MIRO model runs show that significant reduction of *Phaeocystis* blooms are reached when both N and P loads to the Southern Bight of the North Sea are reduced by 50%. If only P removal is effective, no or little effect on *Phaeocystis* colony bloom is simulated due to very efficient P recycling in the coastal environment and its consequent limited effect on algal development. The 50% reduction of both N and N & P loads to the coastal sea are primarily affecting diatom spring development reducing their bloom magnitude by more than 30%.

The response of the MIRO model to nutrient reduction scenarios consequent to the application of the EC guideline of May 1991 was investigated. These nutrient reduction scenarios were established by modifying nutrient riverine inputs of 1985, according to the results generated by application of the RIVERSTRAHLER model (Garnier *et al.*, 1995) to watersheds similar to those of the rivers Seine, Scheldt and Rhine and for various scenarios of urban waste water treatment. Severe reductions of *Phaeocystis* blooms colonies are predicted after 90 % P removal. On the contrary, 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 P removal and denitrification treatment of urban waste waters is not required for obtaining the largest reduction of *Phaeocystis* blooms in the coastal North Sea.

These exploratory nutrient reduction scenarios clearly show that the proposed measures by the European Commission don't achieve the 50% reduction of N and P load to the coastal North Sea needed to substantially reduce *Phaeocystis* blooms in the eutrophicated Southern Bight of the North Sea. This is due to the complex interactions between the continental and coastal marine system. Only an integrated land-coastal sea modeling approach would be able to provide guidance to better select the available control actions on the watershed in order to reduce the development of *Phaeocystis* colony blooms.

Historical data analysis show that the occurrence of *Phaeocystis* blooms has been reported at the end of the century in the Dutch (Cadée & Hegeman, 1991) and French (Grossel, 1985) coastal waters. Through a retrospective modelling approach, Billen (1993) showed that these waves of *Phaeocystis* colony blooms were positively correlated to the fluctuations of nitrate delivery to the sea following increasing urbanization, industrialization and agriculture intensification in the watershed.

The consequences of the measures taken in the eighties for reduce riverine nutrient have already been recorded in the coastal waters of the Southern Bight of the North Sea. A downward trend in PO_4 loads was indeed recorded in the Belgian (Chapter 5), Dutch (de Vries *et al.*, 1998) and German (Hickel *et al.*, 1993) coastal waters in response to measures taken on the watershed for reducing riverine P loads. As a consequence of the N:P increase, an extension of *Phaeocystis* colony bloom duration towards summer was observed in the Dutch coastal waters (Cadée & Hegeman, 1991;

Riegman *et al.*, 1992). These observations constitute a direct evidence of the response of the *Phaeocystis*-dominated ecosystem to modifications of nutrient loads.

Perspectives

This thesis provides some semi-quantitative conclusions on the functioning of the eutrophicated coastal ecosystem of the Southern Bight of the North Sea. It also identifies gaps in our knowledge. Future research should be performed to provide a deeper insight into the underlying principles and processes regulating phytoplankton growth and succession.

The determination of maximum growth rates (μ_{max}) and the comparative study of the nutrient physiology of the 3 diatoms communities and *Phaeocystis* colonies would allow to understand the mechanisms behind succession and competition. Chemostat studies aiming to determine the major nutrient uptake parameters (the maximal uptake rate Vmax and the half saturation constant Ks) are indeed required for a proper characterisation of *Phaeocystis* and diatoms succession and competition. Also, PO_4 and $Si(OH)_4$ cycling in these coastal waters should be more deeply investigated. Further research should be deserved to not only inorganic but also organic nutrient. In particular, the capacity of co-occurring *Rhizosolenia* spp.-dominated diatom community and *Phaeocystis* to utilize organic sources of PO_4.

The specific photo-physiology of these key-groups, and particularly their adaptation to turbid coastal waters is another field of research for a deeper understanding of phytoplankton dynamics and more particularly their different distribution.

Palatability difference for the prevailing mesozooplankton and selective grazing constitute an alternative explanation for the varying dominance of each of the taxa and should be further investigated.

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