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Spatial variation in phytoplankton dynamics in the Belgian coastal zone of the North Sea studied by microscopy, HPLC-CHEMTAX and underway fluorescence recordings

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Abstract

Spatial variation in the succession of phytoplankton in the Belgian Coastal Zone (BCZ) was investigated by monitoring phytoplankton biomass and community composition using microscopical cell counts, HPLC pigment analyses and in vivo fluorescence recordings. Monthly monitoring of phytoplankton community composition at five stations revealed a succession of three distinct diatom communities. The succession of these three communities was the same at each site, but the succession from the winter-spring to the summer community occurred one month earlier and the succession from the summer to the autumn community one month later at the SW than at the NE stations of the BCZ. Monthly monitoring of chlorophyll *a* at ten fixed sites and inspection of in vivo fluorescence recordings during various cruises of RV 'Zeeleeuw' indicated that the spring bloom started about one month earlier in the SW part of the BCZ than in the NE part. The spatial difference in the onset of the spring bloom was ascribed to the higher water column turbidity at the NE coast compared to the SW coast. Although a *Phaeocystis* bloom occurred at all monitoring stations, a clear spatial variation in the magnitude of such blooms was observed, with more intense blooms at the NE coast than at the SW coast. A close relation was observed between the intensity of the *Phaeocystis* bloom and the availability of inorganic nutrients (N and P) before the onset of the bloom. Comparison of microscopical cell counts and CHEMTAX analysis of accessory pigment data indicated that HPLC analysis may be a useful tool for monitoring *Phaeocystis* in the North Sea. The presence of chlorophyll c_3 containing diatoms, however, probably resulted in the detection of small quantities of *Phaeocystis* by HPLC-CHEMTAX analysis when microscopical analyses showed that the species was absent.

Keywords: Phytoplankton; Diatoms; *Phaeocystis*; HPCL; CHEMTAX; Southern Bight of North Sea; Scheldt or Schelde estuary

1. Introduction

Anthropogenic inputs of N and P to coastal waters have resulted in an increase in the N/Si and P/Si ratios in coastal waters (e.g. Billen et al., 2001). These altered

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nutrient ratios have resulted in a shift from N and/or P limitation to Si limitation of the diatom spring bloom in coastal ecosystems (Egge and Aksnes, 1992). As a result of Si limitation of the diatom bloom, relatively large amounts of N and P have become available to non-diatom algae after the termination of the spring bloom. These non-diatom algae are often flagellates that are a nuisance to the ecosystem rather than being the base of the coastal food web (Conley et al., 1993; Humborg et al., 2000).

In the Southern Bight of the North Sea, the nuisance alga *Phaeocystis globosa* tends to form blooms in regions receiving high inputs of inorganic nutrients (e.g. Cadée and Hegeman, 2002). *Phaeocystis* blooms tend to be initiated when a certain irradiance threshold is exceeded (Peperzak et al., 1998). Bloom formation in *Phaeocystis* is related to the transition from solitary cells to colonies at high irradiances (Peperzak, 1993; Riegman and Van Boekel, 1996). In contrast to solitary *Phaeocystis* cells, *Phaeocystis* colonies escape predation by zooplankton because they are surrounded by a tough skin (Hamm et al., 1999). It is this resistance to grazing that allows *Phaeocystis* to form massive blooms (Lancelot, 1995).

The Belgian Coastal Zone (BCZ) of the North Sea is an area that is strongly influenced by the eutrophic rivers Scheldt, Rhine and Meuse and where *Phaeocystis* blooms occur annually (Lancelot et al., 1987). Phytoplankton in the BCZ has been monitored continuously since 1988 but this monitoring was carried out mainly at a single sampling station (station '330'; Lancelot et al., 2005). As a result, relatively little information is available on the spatial dynamics of the phytoplankton bloom in the BCZ. Despite the limited area of the BCZ, spatial differences in the distribution of phytoplankton can be expected given the existence of a pronounced gradient in environmental conditions in this area. The influence of the estuaries of the Scheldt, Rhine and Meuse in the NE part of the BCZ results in lower salinities and higher nutrient and suspended matter concentrations than in the SW (Van Bennekom and Wetsteijn, 1990; Van Raaphorst et al., 1998; De Galan et al., 2004; Lacroix et al., 2004).

Analysis of phytoplankton pigments by High Performance Liquid Chromatography (HPLC) and subsequent processing of pigment data using the software CHEMTAX has been proposed as an alternative to relatively time-consuming microscopic cell counts to study phytoplankton community composition (Mackey et al., 1996). Theoretically, this technique could be useful for monitoring the succession from diatoms to *Phaeocystis* in coastal ecosystems, as Prymnesiophytes such as *Phaeocystis* possess a unique pigment, 19′–hexanoyloxyfucoxanthin, that distinguishes them from diatoms (Bjørnland et al., 1988; Llewellynn and Gibb, 2000). 19′–hexanoyloxyfucoxanthin concentrations in *Phaeocystis* strains, however, tend to be highly variable (Zapata et al., 2004). Recent studies show that *Phaeocystis* strains from the North Sea do not contain 19′–hexanoyloxyfucoxanthin at all (Breton et al., 2000; Antajan et al., 2004). Therefore, chlorophyll c_3 rather than hex-fuco has been proposed as an indicator of *Phaeocystis* in North Sea waters. Chlorophyll c_3, however, also occurs in diatoms, albeit only in relatively few taxa (Stauber and Jeffrey, 1988). Nevertheless, Breton et al. (2000) and Antajan et al. (2004) found a good agreement between chlorophyll c_3 concentrations and *Phaeocystis* biomass in North Sea samples.

The first aim of this study was to investigate to what extent environmental gradients influence the succession of phytoplankton in the BCZ of the North Sea, especially during the spring bloom. Therefore, we monitored phytoplankton biomass and community composition along off-shore and along-shore transects in the BCZ at monthly intervals. In addition, the spatial distribution of phytoplankton was studied at a higher spatial and temporal resolution by means of continuous in vivo fluorometric chlorophyll *a* recordings during various cruises of RV 'Zeeleeuw'. A second aim was to evaluate whether HPLC-CHEMTAX analysis can be used to distinguish *Phaeocystis* from diatoms. Therefore, phytoplankton community composition was analysed both using pigment analysis and microscopy.

2. Materials and methods

2.1. Study area

The BCZ of the North Sea is characterised by relatively shallow waters (<30 m depth). The shallow depth of the water column in combination with strong semi-diurnal tidal currents (up to about 1 m s^{-1}) and frequent strong winds result in a vertically homogeneous water column (Lee, 1980) with relatively high concentrations of suspended particulate matter (SPM) (Van Raaphorst et al., 1998). The BCZ is strongly influenced by the English Channel and the residual water current is directed to the NE (Nihoul and Ronday, 1975; Otto et al., 1990). The area is characterised by a complex system of sand banks which follow the residual current and are therefore orientated parallel to the coast. Mainly due to freshwater inputs by the Rhine and Meuse rivers, salinity increases from N to S and is higher off-shore than near-shore (Lacroix et al., 2004). The BCZ

receives high inputs from eutrophic rivers, resulting in relatively high nutrient concentrations (Van Bennekom and Wetsteijn, 1990; De Galan et al., 2004).

2.2. Sampling

Sampling was carried out with RV 'Zeeleeuw' at monthly intervals in 2003. Samples were collected at 10 stations located within 25 km of the Belgian coast (51° 11′ to 51° 28′ N, 2° 30′ to 3° 18′ E, Fig. 1). The stations formed three short transects perpendicular to the coastline, in the NE (stations 700, B07, 710, 780), central (stations 130, 230, 330) and SW part (stations 120, 215, ZG02) of the BCZ. No samples were collected in October and November due to maintenance of the ship. At each station, subsurface water was collected at 1 m depth with a Niskin bottle. A 250 ml subsample was fixed with Lugol's solution for microscopical analysis. A 500-1000 ml subsample was filtered over a GF/F filter for analysis of phytoplankton pigments. The filters were wrapped in aluminum foil and immediately stored in a deep freezer (−20 °C) available on board. After the cruise, the filters were transported to the laboratory where they were stored at −80 °C until analysis by HPLC. Subsamples for nutrient analyses were filtered over GF/F (for phosphorus) or Nuclepore polycarbonate filters (for nitrogen and silicate) and stored frozen until analysis. SPM was collected on pre-weighted Nuclepore polycarbonate filters for gravimetrical analysis. At each station, Secchi depth was measured with a black and white disk. Temperature and salinity were recorded using a Seabird Thermosalinograph SBE21. During the monthly cruises as well as during other cruises of RV 'Zeeleeuw' in the BCZ, continuous in vivo chlorophyll fluorescence data were recorded using a Chelsea Minitracka III fluorometer.

2.3. Sample analysis

In samples collected at stations 120, 215, 330, 700 and B07, phytoplankton was identified and enumerated using an inverted microscope. A minimum of 250 cells or colonies were enumerated in 50 ml subsamples at 200 to 400 × magnification. Some taxa such as certain *Thalassiosira*, *Chaetoceros* or *Rhizosolenia* spp. could not be identified to species level by this technique and were grouped in multispecies taxa. As all *Phaeocystis* colonies had disintegrated during sample storage, individual cells or colony fragments were counted.

Pigments were extracted in 90% acetone using sonication (tip sonicator at 40 W for 30 s). Pigments in the extracts were immediately analysed by means of reverse phase HPLC following the method of Wright and Jeffrey (1997) with some modifications. This method uses a gradient of three solvents: (1) methanol 80%–ammonium acetate 20%, (2) acetonitrile 90% and (3) ethyl acetate. Three detectors were connected to a Gilson HPLC system: an Applied Biosystems 785A Programmable Absorbance Detector to measure absorbance at 785 nm, a Gilson model 121 fluorometer to measure fluorescence of chlorophylls and their derivates and a Gilson 170 diode array detector to measure

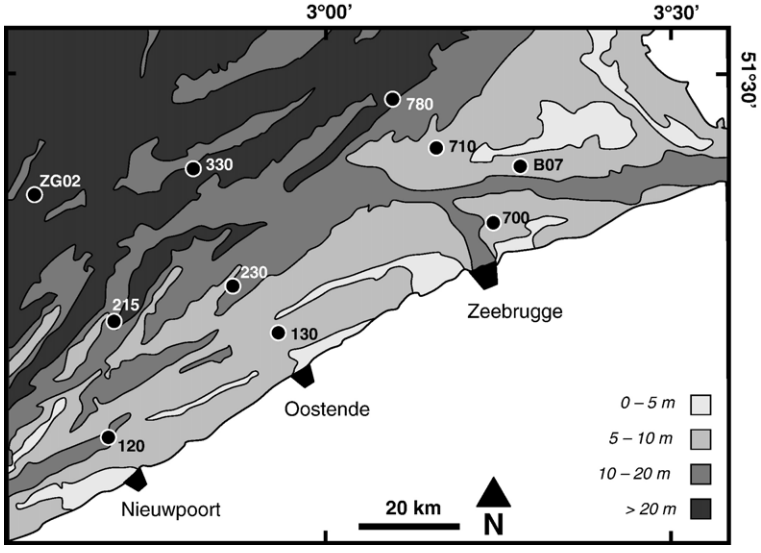


Fig. 1. Map of the Belgian Coastal Zone indicating the position of the sampling sites. Isobaths refer to the depth at average low water during spring tide.

absorbance spectra for individual pigment peaks. Pigments were identified by comparison of retention times and absorption spectra with pure pigment standards (supplied by DHI, Denmark) and with pigment composition of pure cultures of diatoms and *Phaeocystis globosa* isolated from the North Sea. The method used was not capable of fully separating the three main chlorophyll *c*'s; only chlorophyll c_1+c_2 and chlorophyll c_3 were resolved. Retention times of the HPLC system were very reliable within batches of samples (<5 sec) but often differed between batches (up to 2 min). The detection limit of chlorophyll *a* was 0.02 μg l^{-1} (for a sample volume of 500 ml and an extraction volume of 5 ml) and the difference between two replicate measurements was usually <2%.

Dissolved silicate, phosphate, nitrate, nitrite and ammonium were measured following the methods described in Grasshoff et al. (1983). Ammonium was measured with the indophenol blue technique according to Koroleff (1969). Nitrate, nitrite and ammonium were summed to yield dissolved inorganic nitrogen (DIN).

2.4. Data analyses

Spatio-temporal variation in community composition of diatoms —the most species-rich phytoplankton group in the BCZ- was analysed in detail by correspondence analysis (CA). A preliminary detrended correspondence analysis revealed that the gradient length of the first ordination axes exceeded 2 standard deviation units, indicating a predominance of unimodal species response curves. Therefore, weighted averaging-based CA was chosen over linear regression-based principal component analysis. The analyses were carried out using the CANOCO software (Ter Braak and Smilauer, 1998). Species abundances were log(x+1) transformed to reduce the influence of the most abundant taxa on the analysis.

The CHEMTAX software was used to calculate the contribution of different algal groups to total chlorophyll *a* using concentrations of accessory pigments. This software package was developed specifically for the analysis of phytoplankton pigment data (Mackey et al., 1996). The CHEMTAX software uses three matrices: (1) a matrix containing concentrations of all marker pigments in the samples, (2) an initial matrix containing marker pigment to chlorophyll *a* ratios for all algal groups and (3) a ratio limit matrix defining limits on the theoretical marker pigment to chlorophyll *a* ratios. The CHEMTAX program optimises the contribution of different algal groups to total chlorophyll *a* based on measured pigment concentrations (matrix 1), using the pigment ratio matrix (matrix 2) as a starting point and allowing pigment ratios to vary according to constraints defined in the limit matrix (matrix 3). The initial pigment ratio matrix was based on published accessory pigment to chlorophyll *a* ratios (Schlüter et al., 2000; Antajan et al., 2004).

3. Results

Annual mean salinity and Secchi depth at the sampling sites increased from the NE to the SW transect (Table 1). Within each transect, salinity and Secchi depth increased with distance from the coast. Annual mean SPM and nutrient concentrations were inversely related to salinity and Secchi depth and decreased in SW direction and with distance from the coast. During spring, nutrient concentrations declined rapidly from their maximum winter concentrations to low levels (Fig. 2). The timing of this decline differed between nutrients and between the sampling stations. DSi concentrations declined strongly (>50%) between February and March in all stations of the SW transect and in the most offshore stations of the other transects (stations 330, 710 and 780). The decline in DSi between February and

Table 1
General description of the sampling sites, including geographical coordinates, depth, and annual means (n=10) for salinity, Secchi depth and SPM and nutrient concentrations

Transect	Station	Latitude	Longitude	Salinity (psu)	Secchi (cm)	SPM (mg l^{-1})	PO_4 (μM)	DIN (μM)	Si (μM)
SW	120	51°11′ N	2°42′ E	32.8	228	10.9	0.58	13.8	8.9
	215	51°17′ N	2°37′ E	33.2	424	3.3	0.46	10.64	6.0
	ZG02	51°20′ N	2°30′ E	33.9	563	2.4	0.33	7.43	3.8
Central	130	51°16′ N	2°54′ E	31.6	166	47.3	1.01	23.65	22.4
	230	51°19′ N	2°51′ E	32.1	198	26.6	0.94	18.14	12.8
	330	51°26′ N	2°49′ E	33.2	494	2.9	0.48	10.61	5.7
NE	700	51°23′ N	3°13′ E	31	74	48.2	1.15	23.13	16.7
	B07	51°26′ N	3°18′ E	31.7	83	34.3	0.94	15.67	13.9
	710	51°27′ N	3°08′ E	32.2	146	26.6	0.74	12.76	10.2
	780	51°28′ N	3°04′ E	32.4	258	12.9	0.71	18.08	9.4

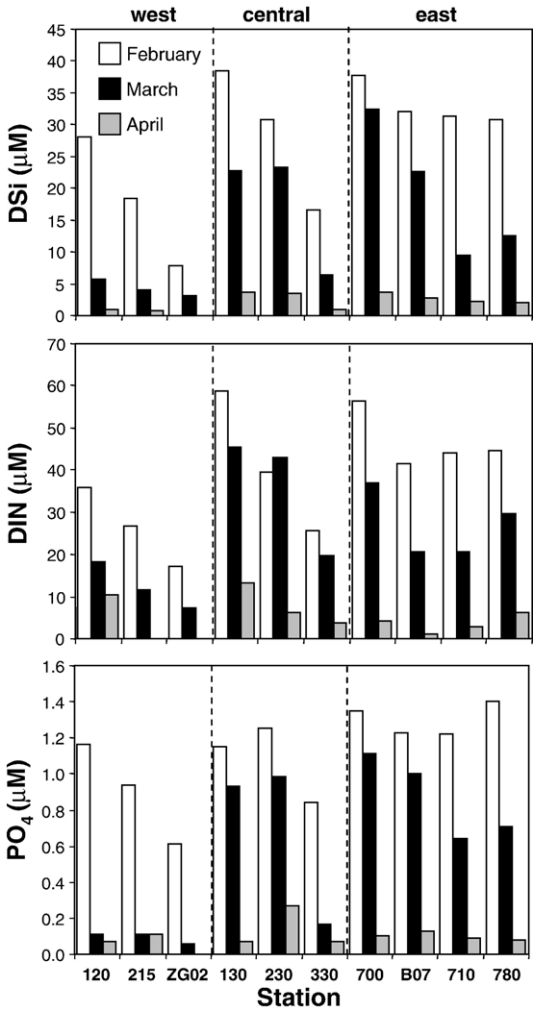


Fig. 2. Dissolved inorganic nutrient concentrations at the sampling stations during the development of the phytoplankton spring bloom in February to April.

March was less pronounced (<50%) at the other stations. Between March and April, DSi had declined to levels below 5 μM at all stations. DIN concentrations declined by approximately 50% between February and March at the stations of the SW transect and the two most offshore stations of the NE transect. Between March and April, DIN declined further at all stations. PO_4 concentrations declined to below 0.2 μM between February and March at the stations of the SW transect and at station 330. At the other stations, PO_4 concentrations were still >50% of the February concentrations. Between March and April, PO_4 concentrations declined at all sites below 0.2 μM. More detailed information on the nutrient data collected during this study is presented in Van der Zee and Chou (2005).

Chlorophyll *a* concentration displayed a pronounced spring maximum at all stations (Fig. 3). The maximum chlorophyll *a* concentration reached during the spring bloom tended to decrease from the NE to the SW transects and decreased in each transect from the near-shore to the off-shore stations. In the stations of the SW transect, chlorophyll *a* concentrations were at their maximum or close to their maximum already in March. At the other stations, maximum chlorophyll *a* concentrations were reached one month later, in April. An exception to this general pattern was station 130, where no clear spring bloom occurred. We presume that this was due to a problem during HPLC analysis because in vivo fluorometric recordings (see further) as well as independent chlorophyll *a* determination at the same site by Van der Zee and Chou (2005) showed a chlorophyll *a* peak in April at this site.

Continuous fluorescence readings during various cruises by RV 'Zeeleeuw' provided a more detailed view of the spatial and temporal development of the spring bloom in the BCZ. In vivo fluorescence measurements were closely correlated with chlorophyll *a* concentrations measured by HPLC (Spearman correlation coefficient 0.78, $n=43$, $p<0.001$; Fig. 4). As the fluorometer was set for maximal sensitivity at low chlorophyll *a* concentrations, in vivo fluorescence measurements could not discriminate between sites with high chlorophyll *a* concentrations (>20 μg l^{-1}). Maps of in vivo fluorescence intensity along the ship tracks showed that in the second half of February, phytoplankton biomass was low throughout the BCZ (Fig. 5). Slightly elevated fluorescence was observed only near-shore in the SW part of the BCZ. From the beginning of March, a bloom had clearly developed in the SW part of the BCZ, with fluorescence readings being higher near-shore than off-shore. Although in March fewer cruises were conducted in the NE than in the SW part of the BCZ, fluorescence was always low during the cruises in the NE part, indicating that a bloom had not yet developed at that time. Compared to the end of February, however, fluorescence had slightly increased in the NE part of the BCZ, indicating limited phytoplankton development. In the NE part of the BCZ high fluorescence readings were recorded only from the beginning of April onwards. During April, fluorescence was high throughout the monitored part of the BCZ, but due to the lack of resolution of the fluorometer at high chlorophyll *a* concentrations, the data do not provide information on spatial differences in maximal phytoplankton biomass at the height of the bloom.

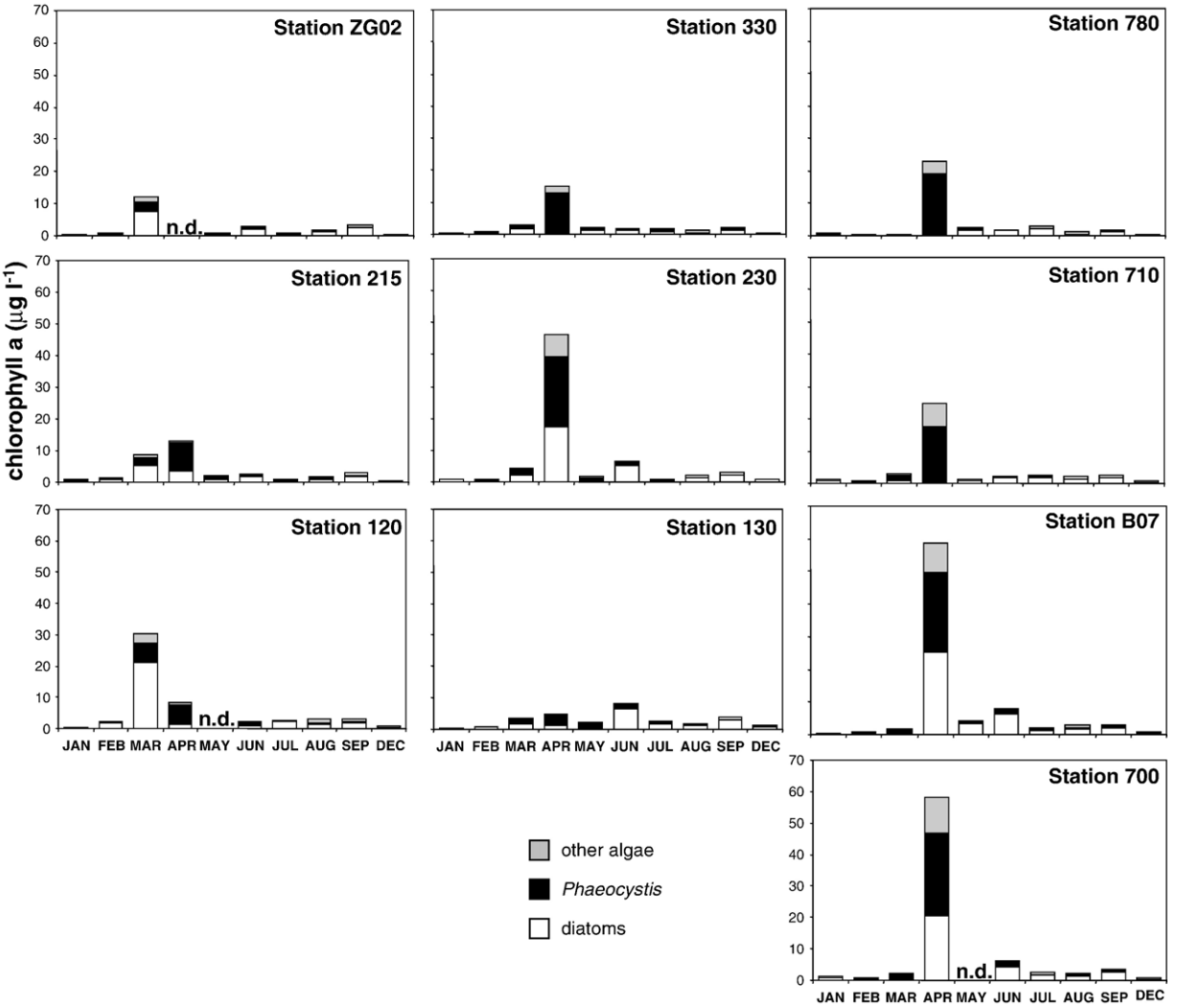


Fig. 3. Chlorophyll *a* concentration and phytoplankton community composition (as assessed by CHEMTAX processing of pigment data) at the sampling stations. When no data were available, this is indicated as 'n.d.'.

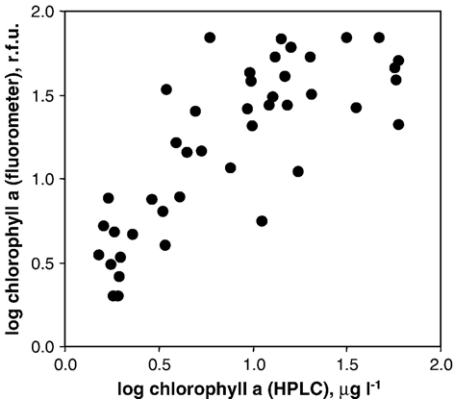
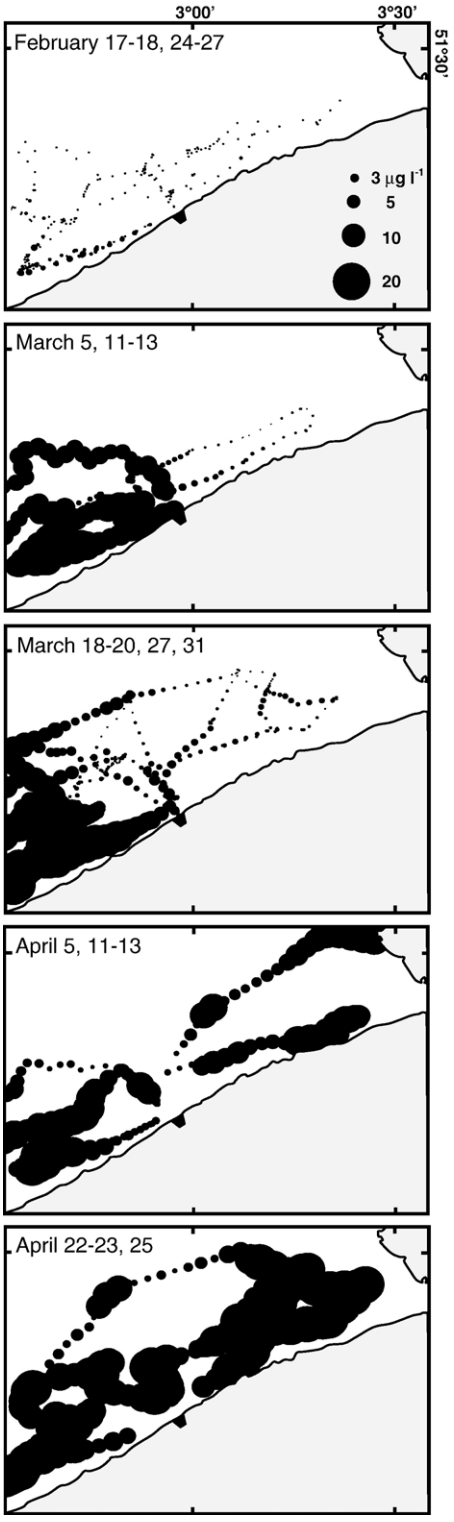


Fig. 4. Correlation between chlorophyll *a* concentration measured by means of HPLC analysis and in vivo fluorescence readings on board the RV Zeeleeuw.

Microscopical analyses of samples collected at five selected stations showed a dominance of the phytoplankton community by diatoms and *Phaeocystis*. Although 11 dinoflagellate species were identified in the samples, these contributed >5% of total cell numbers in only four out of 50 samples. *Hillea fusiformis*, a cryptophyte-like flagellate, was identified in the samples but this species contributed >5% of total cell numbers in only two samples. Cyanobacteria, chlorophytes or euglenophytes were never observed during the microscopical analyses. Seasonal variation in total cell numbers of *Phaeocystis* is indicated in Fig. 6. *Phaeocystis* abundance displayed a peak at station 120 in March and at the other stations in April. *Phaeocystis* was only observed in these two months. *Phaeocystis* abundance reached 10^7 cells l^{-1} at stations B07 and 700.

At stations 330 and 215, maximum *Phaeocystis* abundance was lower and at station 120, *Phaeocystis* abundance never exceeded 10^6 cells l^{-1}.



Diatoms were the most diverse phytoplankton group in the samples with 42 taxa. The seasonal succession of the diatom community was therefore investigated in more detail using multivariate analysis. Fig. 7 shows the first two axes of a CA ordination of the diatom community data. Axes 1 and 2 had eigenvalues of 0.236 and 0.147, respectively, and together explained 26% of the variation in the diatom data. The ordination revealed the presence of three relatively distinct diatom communities. Community 1 was characterised by two types of diatoms: taxa with a bentho-pelagic life-style (*Actinoptychus senarius, Paralia sulcata*, *Plagiogrammopsis vanheurckii*, *Rhaphoneis amphiceros*, *Odontella aurita*) and relatively small pelagic diatoms (*Thalassiosira* spp. <20 µm and >20 µm, *Thalassionema nitzschioides*). Community 2 was characterised by species from the genus *Chaetoceros* (unidentified *Chaetoceros* spp. and *C. danicus*) as well as *Lithodesmium undulatum*, *Leptocylindricus danicus* and *Skeletonema costatum*. Community 3 was characterised by species from the genus *Rhizosolenia* or related genera (*R. hebetata*, *Guinardia flaccida*, *G. delicatula*, *G. striata*, *Dactyliosolen fragilissima*) as well as *Pseudonitzschia* spp. These three communities were observed in the same order (1 – 2 – 3 – 2 – 1) at all sites but the timing of their appearance and disappearance in the plankton differed between the sites. At stations 120, 215 and 330, community 1 was observed in January and February and returned in the plankton from August to December (Fig. 6). At stations 700 and B07, community 1 was dominant from January to March and already returned in the plankton from July to December. Community 3 replaced community 1 from late spring to summer at all sites. Community 2 was never dominant but had its maximum contribution to total diatom abundance during the transition from the community 1 to community 3 in spring and, vice versa, during the transition from community 3 to community 1 in late summer.

The following accessory pigments were observed during the HPLC analyses: chlorophyll c_{1+2}, chlorophyll c_3, peridinin, fucoxanthin, diadinoxanthin, diatoxanthin, lutein, zeaxanthin and chlorophyll *b*. No alloxanthin was detected with certainty, indicating an absence or at least a minimal biomass of cryptophytes

Fig. 5. Chlorophyll *a* measured by means of in vivo fluorescence along various cruises of the RV Zeeleeuw during the period February to April 2003. Circles indicate points of measurements and circle size is related to fluorescence intensity at that point. The scale for the size of the points and latitude and longitude are only shown in the upper graph but are identical for all other graphs.

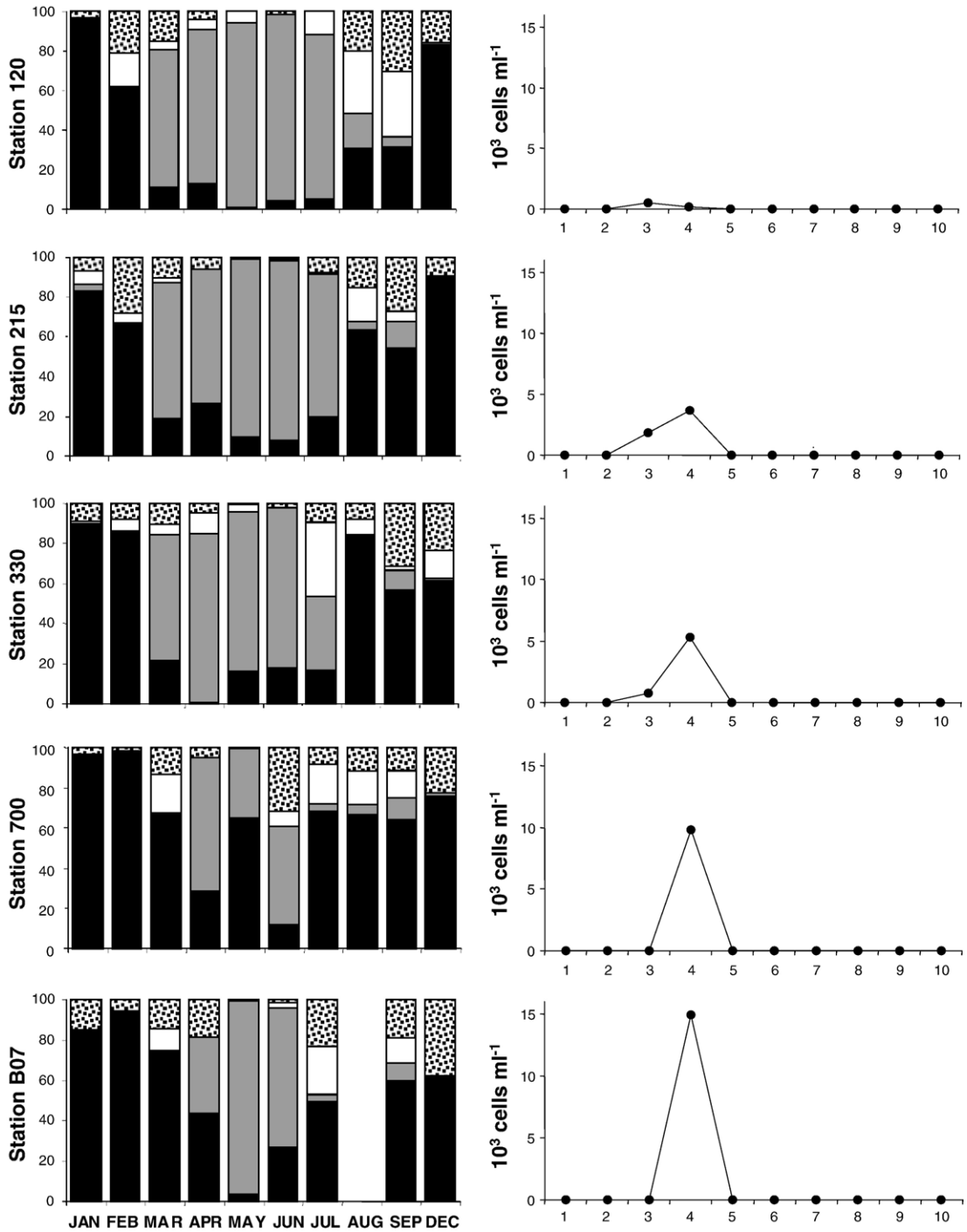


Fig. 6. Left: contribution of taxa belonging to each of the 3 diatom communities delineated by means of CA analysis to total diatom abundance (black: community 1, white: community 2, grey: community 3, speckled: other taxa); for an overview of the taxa belonging to the different communities, see text. Right: abundance of *Phaeocystis* at the same sites.

(or taxa with a pigment signature such as cryptophytes, e.g. *Hillea fusiformis*) in the samples. Small quantities of 19′–hexanoyloxyfucoxanthin and 19′–butanoyloxyfucoxanthin were detected throughout the year but no peak was observed during the *Phaeocystis* bloom in April, confirming previous observations by Breton et al.

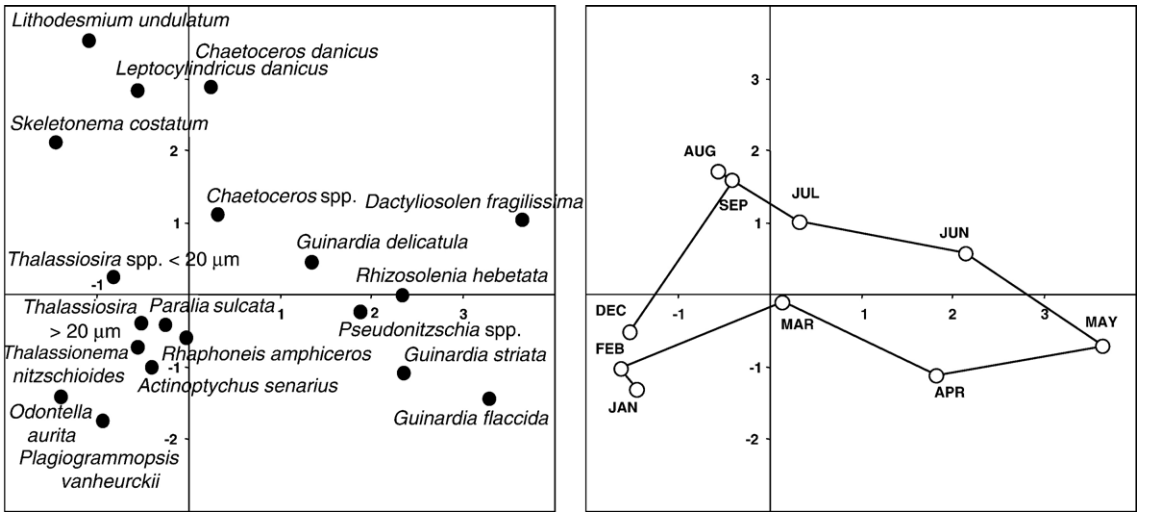


Fig. 7. Results of a canonical correspondence analysis of diatom communities at stations 120, 215, 330, 700 and B07. In both figures the first (horizontal) and second (vertical) ordination axes are presented. On the left, the points represent the position of the taxa; only taxa of which ≥20 % of the variation was explained are shown in the ordination diagram. On the right, the mean of the sample scores of all stations is presented for each month.

(2000) and Antajan et al. (2004) that these pigments are not a useful marker for *Phaeocystis* in the North Sea.

The following algal groups were included in the CHEMTAX analysis: diatoms, *Phaeocystis*, dinoflagellates, chlorophytes, euglenophytes and cyanobacteria. As proposed by Antajan et al. (2004), we used chlorophyll c_3 as an indicator pigment for *Phaeocystis* in CHEMTAX. The final matrix of accessory pigment to chlorophyll *a* ratios for the different algal groups obtained after CHEMTAX analysis is shown in Table 2. CHEMTAX analysis identified diatoms and *Phaeocystis* as the two dominant algal groups at all stations, contributing 44 and 40%, respectively, of total chlorophyll *a* averaged over all samples and over the year. In general, a good agreement was found between *Phaeocystis* equivalent units of chlorophyll *a* estimated by CHEMTAX and microscopically determined *Phaeocystis* cell abundance (Pearson correlation coefficient $r=0.93$, $n=10$, $p<0.001$; Fig. 8). It should be noted that in samples collected in the months prior to or after the *Phaeocystis* bloom, no *Phaeocystis* cells had been detected but CHEMTAX nevertheless indicated the presence of a small quantity of *Phaeocystis*. Although no chlorophytes were detected during the cell counts, chlorophytes were identified as the third dominant algal group in the samples, contributing on average 13% of chlorophyll *a*. Dinoflagellates, euglenophytes and cyanobacteria were relatively rare in the samples and contributed together on average only 3% of total chlorophyll *a*. The contribution of diatoms, *Phaeocystis* and other algae (mainly chlorophytes) to total chlorophyll *a* is shown in Fig. 3. According to the CHEMTAX analyses, diatoms dominated chlorophyll *a* throughout the year except in March at the stations of the SW and central transect and in February and March at the stations of the NE transect, when *Phaeocystis* dominated chlorophyll *a*. Chlorophytes tended to have a higher contribution to total chlorophyll *a* at the stations of the NE transect (on average 18%) than at the stations of the central and SW transects (on average 11%).

Table 2
Accessory pigment to chlorophyll *a* ratios in the different algal groups produced by an analysis of pigment data of all samples by means of CHEMTAX

	Peridinin	Fucoxanthin	Chlorophyll c_3	Diatoxanthin + diadinoxanthin	Lutein	Zaxantin	Chlorophyll *b*
Chlorophytes	–	–	–	–	0.058	0.034	0.240
Cyanobacteria	–	–	–	–	–	0.232	–
Diatoms	–	0.378	–	0.043	–	–	–
Dinoflagellates	0.366	–	–	0.152	–	–	–
Euglenophytes	–	–	–	0.137	–	–	0.277
Phaeocystis	–	0.387	0.075	0.013	–	–	–

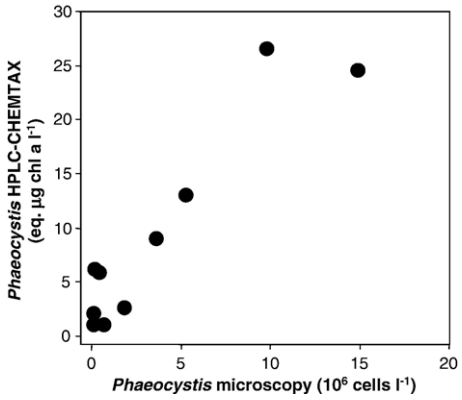


Fig. 8. Comparison of *Phaeocystis* abundance estimated by means of microscopical cell counts and using CHEMTAX analysis of HPLC pigment data in April.

To investigate the role of nutrients in regulating the magnitude of the *Phaeocystis* bloom in the BCZ, we related biomass of *Phaeocystis* at the maximum of the bloom (in April) estimated using HPLC-CHEMTAX with DIN and phosphate concentrations one month prior to the bloom (Fig. 9). Data from station 130 were not included in this analysis due a presumed problem with HPLC analysis of the sample (see above). In general, a good agreement was found between phosphate and DIN concentrations in March and *Phaeocystis* chlorophyll *a* equivalents in April. We compared inorganic nutrient concentrations in March with published limiting levels for colonial *Phaeocystis*, which are about 0.7 µM for phosphate and 4 µM for DIN (Schoemann et al., 2005). This analysis showed that phosphate concentrations in March were much closer to the limiting level for *Phaeocystis* than DIN concentrations.

4. Discussion

We attempted to estimate the contribution of major algal groups to total chlorophyll *a* using CHEMTAX analysis of HPLC pigment data. The main goal of the CHEMTAX analysis was to distinguish between diatoms and *Phaeocystis* using chlorophyll c_3 as an indicator pigment for *Phaeocystis* (cf. Antajan et al., 2004). The final pigment ratio matrix produced by CHEMTAX was in good agreement with the initial pigment ratio matrix, indicating that published pigment ratios could be used to reconstruct the contribution of algal groups in our samples. *Phaeocystis* biomass in equivalent units of chlorophyll *a* as estimated using CHEMTAX was closely related to *Phaeocystis* cell abundance determined microscopically. The detection of low concentrations of *Phaeocystis* by CHEMTAX in samples where no *Phaeocystis* was observed microscopically may be due to the presence of chlorophyll c_3 containing diatoms. Although chlorophyll c_3 is relatively rare in diatoms, some diatom species that occurred in our samples (*Thalassionema nitzschioides* and *Rhizosolenia setigera*) contain this pigment (e.g. Stauber and Jeffrey, 1988) and CHEMTAX is not capable of distinguishing these chlorophyll c_3 containing diatoms from *Phaeocystis*. Especially *T. nitzschioides* contributed substantially to total diatom abundance (up to 40%) just before and during the *Phaeocystis* bloom. The contribution of this species to total diatom biomass, however, was probably much less than its contribution to abundance as *Thalassionema* is a relatively small diatom (biovolume 650 µm^3 cell^{-1}). Nevertheless, its presence in the plankton may have led to an overestimation of *Phaeocystis* in the CHEMTAX analyses prior to and during the bloom.

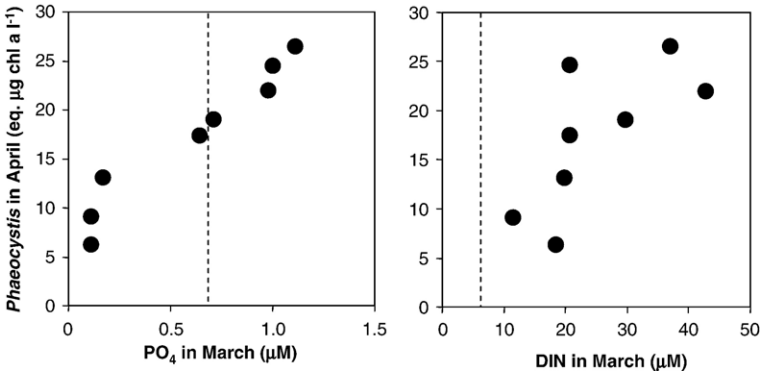


Fig. 9. Relation between dissolved phosphate and DIN concentrations in the water column in March and *Phaeocystis* biomass attained at the same stations one month later in April. *Phaeocystis* biomass, expressed in equivalent chlorophyll *a* concentration, was estimated using CHEMTAX analysis of HPLC derived pigment data. The vertical broken line corresponds to the half-saturation constant of colonial *Phaeocystis* for uptake of phosphate and nitrate (Schoemann et al., 2005).

In agreement with the microscopical analyses, the HPLC-CHEMTAX method did not identify cyanobacteria, euglenophytes, cryptophytes (or cryptophyte-like flagellates such as *Hillea fusiformis*) or dinoflagellates as an important component of the phytoplankton community of the BCZ. The HPLC-CHEMTAX approach, however, did identify chlorophytes as an important component of the phytoplankton community, while no chlorophytes were detected during the microscopical analyses. CHEMTAX assigned part of the chlorophyll *a* to chlorophytes due to the presence of lutein and chlorophyll *b* in the samples. Possibly, these pigments were not contained in living phytoplankton but were associated with phytoplankton detritus. This phytoplankton detritus may be imported into the BCZ through the Scheldt estuary, where chlorophytes are a major component of the phytoplankton community (Muylaert et al., 2000). This is supported by the fact that chlorophytes were more prominent at the NE stations close to the mouth of the Scheldt estuary than at the SW stations. The detection by the HPLC-CHEMTAX method of phytoplankton groups that were not or rarely observed during microscopical analyses may also be related to the presence of picoplanktonic algae. Picoplankton algae are generally not detected by light microscopy but may contribute significantly to total phytoplankton biomass, even in relatively nutrient-rich coastal waters (e.g. Ansotegui et al., 2003).

Microscopical analysis showed a succession of three distinct diatom communities in the BCZ. These communities were comparable to the three communities described by Rousseau et al. (2002), except for the presence of *Pseudonitzschia* spp. in the third community. Similar diatom communities have also been observed in Dutch coastal waters (e.g. Philippart et al., 2000). The succession of these three communities was comparable at all stations but the timing of the succession differed between the NE and SW stations (see below). The first community was composed of bentho-pelagic taxa and small pelagic diatom species and was present in winter and late summer to autumn. The second community was dominated by *Chaetoceros* spp. and appeared only briefly in spring. The brief appearance of this second community is in agreement with previous observations (Rousseau et al., 2002). The third community was dominated by *Rhizosolenia* spp. or species from related genera (*Guinardia* and *Dactyliosolen*) and lasted most of the summer. In late summer, the succession of these three communities was reversed. Rousseau et al. (2002) suggested that the replacement of community 1 by communities 2 and 3 is related to the depletion of dissolved silicate, as diatoms from communities 2 and 3 are less silicified than diatoms from community 1. The reappearance of community 1 in late summer and autumn may be related to increasing dissolved silicate levels. The fact that community 1 returned one month earlier at stations 700 and B07 may be explained by the fact that dissolved silicate concentrations at those stations increased earlier than at the other stations, possible due to silicate inputs from the Scheldt estuary (Van der Zee and Chou, 2005). Diatom community composition in our samples often shifted radically between successive months. Therefore, a higher sampling frequency would probably have been more suitable for studying phytoplankton succession in the BCZ.

In the SW part of the BCZ, chlorophyll *a* concentrations already exceeded 5 μg l^{-1} in March while, in the NE stations, chlorophyll *a* concentrations exceeded 5 μg l^{-1} only one month later. In vivo fluorometric chlorophyll *a* recordings made during various RV 'Zeeleeuw' cruises in between the monthly sampling campaigns confirmed this spatial difference in the onset of the spring bloom. Chlorophyll *a* maps presented in Borges and Frankignoulle (2002) also indicated an earlier development of the phytoplankton spring bloom in the SW part of the BCZ compared to the NE part. Not only the spring bloom but also the succession in the diatom community started one month earlier in the SW stations than in the NE stations. The observed spatial differences in the onset of the spring bloom and diatom succession is probably related to the lower turbidity in the SW compared to the NE coast. In the shallow, turbid waters of the southern North Sea, light is an important factor regulating phytoplankton development (Gieskes and Kraay, 1975; Tett and Walne, 1995; Colijn and Cadée, 2003). In the SE English Channel, for instance, the spring phytoplankton bloom was also observed to start earlier in the shallow and clear waters north of the Bay of Somme than in the deep waters close to the Seine estuary (Brunet et al., 1996). Analysis of a long-term time-series of phytoplankton succession at station 330 of the BCZ also indicated that the onset of the spring succession was related to underwater light levels (Lancelot et al., 2005).

Apart from differences in the onset of the spring bloom between the SW and NE part of the BCZ, there was also a spatial difference in the intensity of the bloom. Maximum chlorophyll *a* concentrations were generally higher in the NE than at the SW stations. Both microscopical cell counts and CHEMTAX

analysis of pigment data indicated that the *Phaeocystis* bloom was more intense at the NE stations. This can probably be ascribed to higher nutrient concentrations in the NE of the BCZ due to inputs from the rivers Scheldt, Rhine and Meuse. The intensity of the *Phaeocystis* bloom was indeed related to the availability of inorganic nutrients (DIN and phosphate) one month before the bloom. Phosphate concentrations before the onset of the bloom were much closer to the limiting level for *Phaeocystis* than DIN concentrations (Schoemann et al., 2005). Van der Zee and Chou (2005) already found very high nitrogen to phosphorus ratios during the *Phaeocystis* bloom in the BCZ in 2003. This suggests that the magnitude of the *Phaeocystis* spring bloom in 2003 was regulated by phosphorus rather than nitrogen. There is much debate on whether nitrogen or phosphorus controls phytoplankton blooms in the Southern Bight of the North Sea. Several authors have concluded that *Phaeocystis* blooms or phytoplankton blooms generally occur in nitrogen-enriched waters (Riegman et al., 1992; Lancelot, 1995; Hydes et al., 1999). A recently developed ecosystem model which incorporated *Phaeocystis* blooms in the BCZ, however, predicted depletion of phosphorus before depletion of nitrogen (Lancelot et al., 2005). Different conclusions regarding the relative importance of nitrogen and phosphorus in controlling *Phaeocystis* blooms in the Southern Bight of the North Sea may be related to long-term shifts in the relative inputs of these nutrients in coastal waters (Philippart et al., 2000).

5. Conclusions

Comparison of the CHEMTAX analysis of HPLC pigment data with microscopical cell counts indicates that HPLC-CHEMTAX may be a useful tool for monitoring *Phaeocystis* blooms. However, CHEMTAX results should always be interpreted with caution due to the confounding effect of chlorophyll c_3 containing diatoms. Our monitoring data revealed clear spatial differences in the timing, community composition and intensity of the spring bloom in the BCZ in 2003. Probably due to spatial differences in turbidity, the spring bloom and diatom succession started one month earlier in the SW part of the BCZ than in the NE part. The magnitude of the *Phaeocystis* bloom was related to inorganic nutrient concentrations and was higher in the NE than in the SW. Low phosphate concentrations relative to DIN concentrations suggest that the *Phaeocystis* bloom was regulated by phosphorus rather than nitrogen.

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