



# Phytoplankton composition in Dutch coastal waters responds to changes in riverine nutrient loads

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## ABSTRACT

The Southern Bight of the North Sea is a shallow shelf sea, strongly influenced by river-borne nutrient loads. Eutrophication symptoms manifest themselves as high levels of chlorophyll-*a* and long-lasting, extensive blooms of *Phaeocystis globosa*, especially in the waters along the continental coast. As a consequence of measures to reduce eutrophication, riverine phosphorus loads have decreased more than 50% in the last two decades, and nitrogen loads show a decrease of ca 30%. While decreases in riverine N and P loads are observed, an increase in summer river-borne loads of silica occurred.

Since 1990, The Netherlands has carried out a routine monitoring program in the North Sea, including analysis of phytoplankton composition and carbon biomass. An analysis of these data for the period 1990–2007 shows a trend in phytoplankton composition, toward an increase in diatom biomass, increased bloom frequency and maximum bloom cell numbers of several diatom species, in particular *Chaetoceros socialis*, in the coastal waters. These changes coincide with increases in riverine Si loadings and increased Si concentrations in coastal waters as a consequence of changed river loads.

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## 1. Introduction

The Southern Bight of the North Sea is a shallow shelf sea that is strongly influenced by Channel water and by riverine discharges. In particular the south-eastern coastal waters of the North Sea, along the continental coast from Belgium to Denmark, can be considered a Region of Freshwater Influence (ROFI). A number of continental rivers (Scheldt, Meuse, Rhine, Ems, Weser, Elbe), draining watersheds with a total surface of ca 430,000 km<sup>2</sup> and a total population of ca 81 million inhabitants (Tockner et al., 2009; Wollast 2003), discharge into the southern North Sea. The nutrient loads from these rivers are a major source of nutrients and fuel phytoplankton blooms in these coastal waters (De Vries et al., 1998; McQuatters-Gollop et al., 2009). Dutch coastal waters are predominantly affected by the rivers Scheldt, Meuse and Rhine. Of these three rivers, the river Rhine contributes by far the largest fraction (>70%). Changes in riverine nutrient loads, as a consequence of increases in nutrient concentrations in the freshwater systems after 1970 (Billen et al., 2005; Colijn et al., 2002; De Vries et al., 1998; Soetaert et al., 2006) and decreases due to measures to reduce eutrophication in the last two decades, can be expected to be reflected in the nutrient concentrations in Dutch coastal waters. Indeed, it was shown by De Vries et al. (1998) that there is a strong relation between

riverine nutrient loads and nutrient concentrations in Dutch coastal waters of the North Sea.

Phosphorus concentrations have decreased in many European rivers since the early 1990's, and this reduction is also apparent in coastal waters, whereas the reduction in nitrogen concentrations in freshwater systems and coastal waters is much smaller (Ærtjeberg et al., 2001; Clausen et al., 2009; Lenhart et al., 2010; McQuatters-Gollop et al., 2007). It is generally assumed that the elevated levels of N and P in coastal waters not only resulted in elevated levels of phytoplankton biomass, but may also have caused shifts in phytoplankton composition. The surplus of nitrogen relative to silicon is assumed to have led to increased silicon-limitation of diatoms and a competitive advantage for non-silicon using phytoplankton species (Egge and Aksnes, 1992; Officer and Ryther, 1980). The high levels of blooms of *Phaeocystis globosa* in the southern North Sea, for example, have been associated with the surplus of nitrogen left at the end of the diatom spring bloom, and the capacity of this species to compete under low phosphorus concentrations (Gypens et al., 2007).

It has been proposed that eutrophication in the freshwater systems and the associated elevated levels of phytoplankton blooms also affect silicon retention in the freshwater ecosystems, and may lead to decreased silicon loads to coastal waters. Thus, freshwater eutrophication may not only have caused increased N and P loads to coastal waters, but also decreased Si loads, enhancing the effect of increased riverine N and P loads on changes in coastal phytoplankton composition (Admiraal et al., 1990).

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Decreases in N and P loads occurring during the last decades, as a consequence of measures to reduce eutrophication, could be expected to reverse the system to conditions favoring diatom growth.

Along the Dutch coast the freshwater influence extends in a relatively narrow band of up to 20–50 km wide (Baretta-Bekker et al., 2009; De Vries et al., 1998). This coastal area and the offshore waters in the Dutch part of the North Sea are part of an extensive Dutch monitoring program. In this program detailed information on phytoplankton community composition is obtained by quantitative microscopical analysis. In a first analysis of these data distinguishing four functional groups of phytoplankton (diatoms, auto- and mixotrophic dinoflagellates, *P. globosa* and other flagellates), Baretta-Bekker et al. (2009) showed that the overall picture was an increase in diatom and dinoflagellate biomass over the period 1990–2005. The authors did not try to identify the factors causing these changes.

Here we present the results of a more elaborate analysis of the trends in phytoplankton composition during the period 1990–2007, at eight monitoring stations in Dutch coastal waters near the discharge points of the main rivers and at two offshore stations in the southern North Sea. At the coastal stations effects of changes in riverine nutrient loads can be expected to have the strongest effects on phytoplankton biomass and composition. To study the changes in phytoplankton composition and biomass, we distinguished the same phytoplankton groups as in the earlier analysis by Baretta-Bekker et al. (2009), viz. diatoms, dinoflagellates, *P. globosa* and other flagellates. In addition to having a data series for a longer period, changes in phytoplankton abundance and biomass were studied in more detail by looking at the development of a selection of bloom-forming species. Changes in riverine discharges were analyzed to study the relationships between changes in phytoplankton

abundance and composition and potentially causal factors like nutrient loads.

## 2. Material and methods

### 2.1. Sampling stations

The North Sea sampling stations that were selected for this analysis are shown in Fig. 1. These stations form part of the extensive Dutch national monitoring program ([www.waterbase.nl](http://www.waterbase.nl)). The sampling stations comprise three transects perpendicular to the coast.

The Walcheren transect is located in the southwest of The Netherlands, near the mouth of the Westerschelde estuary (the outflow of the river Scheldt), and has three stations at 2, 20 and 70 km distance from the coast (indicated as W02, W20 and W70, respectively). The station G06 is situated at 6 km off the coast in the southwest, near the mouth of the Haringvliet where a mixture of water from the rivers Meuse and Rhine is discharged. The stations were sampled at regular intervals with a monthly frequency.

The Noordwijk transect is located on the central western coast of The Netherlands, north of the discharge points Haringvliet and Maassluis which are the outflows of the rivers Rhine and Meuse. The transect has four stations at 2, 10, 20 and 70 km off the coast (indicated as N02, N10, N20 and N70, respectively). The Terschelling transect is located in the northwest of The Netherlands. Two stations, at 4 and 10 km off the coast of the barrier islands of the Wadden Sea, were included in this analysis (indicated as T04 and T10, respectively). The stations at the Noordwijk and Terschelling transect were sampled at least bi-weekly. Stations at the Terschelling transect further offshore, with summer

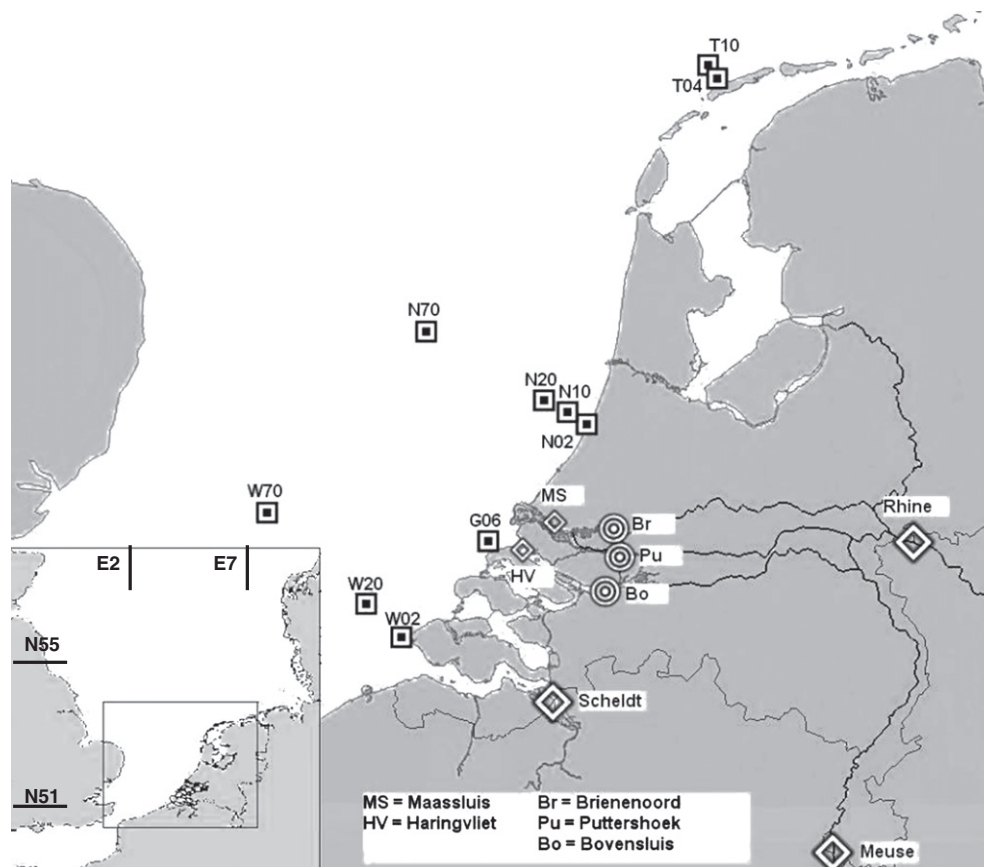


Fig. 1. Map of the research area showing the sampling stations in the North Sea (squares), the main river discharge points (diamonds), the sampling stations in the tributaries of Rhine and Meuse (circles), in the Rhine at the Dutch/German border and in the Meuse at the Dutch/Belgian border (diamonds).

thermal stratification, were not included in this study. A more detailed description of the stations is given in Baretta-Bekker et al. (2009).

The salinities at the stations (Fig. 2) give an indication of the freshwater influence at the stations. Stations W70 and N70 are hardly influenced by river outflows. The stations near the outflow of the river Rhine have the lowest salinities (N02, N10, G06), other stations with a relatively high freshwater influence are W02, N20 and T04.

At none of the stations used in this study summer stratification occurs although short-term thermal and haline stratification can occur at the near shore stations, in particular at the Noordwijk transect (De Ruijter et al., 1997).

## 2.2. Sampling methods

Water samples were collected at 1 m below the water surface. One liter of phytoplankton samples was preserved with 4 ml acid Lugol's iodine and stored in brown glass bottles at 4 °C. Phytoplankton cells were identified and enumerated using inverted microscopy (Utermöhl sedimentation technique). To optimize the counting process, the Lugol-preserved samples were checked for high densities of particles (phytoplankton or silt), and appropriate dilutions or concentrations were prepared for use with the 2 ml sedimentation chambers. If sample concentration was needed, samples were left for at least 1 week in a vibration-free place before the supernatant was siphoned off, resulting in a 10–20× concentration of the sample. In total, a volume of 2–30 ml of the (undiluted) sample was investigated for analysis, depending on the amount of phytoplankton and/or silt in the sample.

The quantitative determination was performed in several steps, with the aim to achieve an appropriate number of observations for each different taxon. Usually 4–5 different sub fractions were counted in sedimentation chambers on an inverted microscope (Olympus IMT-2). Initially five to ten fields of view were counted at high magnification (600×), to account for the most abundant and/or smallest algae. In the following steps a larger number of fields of view were counted at high magnification for less abundant species, ending with a scan of the whole sedimentation chamber at a lower magnification (200×). The target in each counting step was to count at least 15 cells of the most abundant taxa. Auxiliary analysis with UV epifluorescence microscopy was applied for the determination of, for instance, thecate dinoflagellates. The detection limit differs per taxon and is related to the subsample size. Furthermore it is strongly affected by the presence of silt and other particulate matter in the samples, which is mainly site-dependent. Near-shore stations in ROFI's such as G06, W02, N02 and N10, generally have relatively high

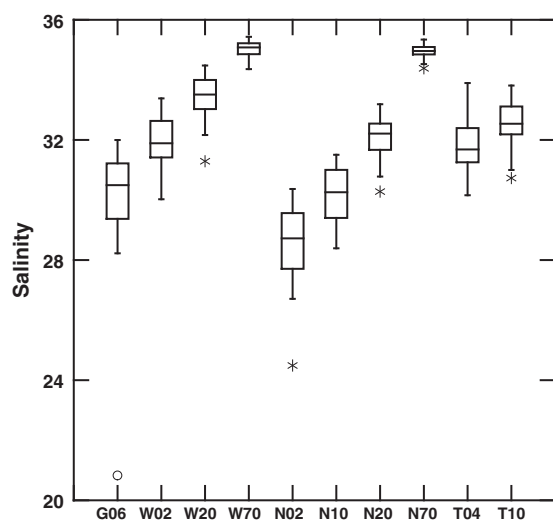


Fig. 2. Box-plots of winter means (Nov–Feb) of salinity at the 10 monitoring stations. Data for the years 1990–2007 are presented.

particulate matter content. To enhance the comparability of results over time an annotated species list has been maintained since the beginning of the monitoring program.

The Dutch monitoring program built up a large number of detailed phytoplankton composition records over a period of almost two decades of which we here analyzed the results from 1990 to 2007, for the months March–September.

## 2.3. Data pre-processing and analysis

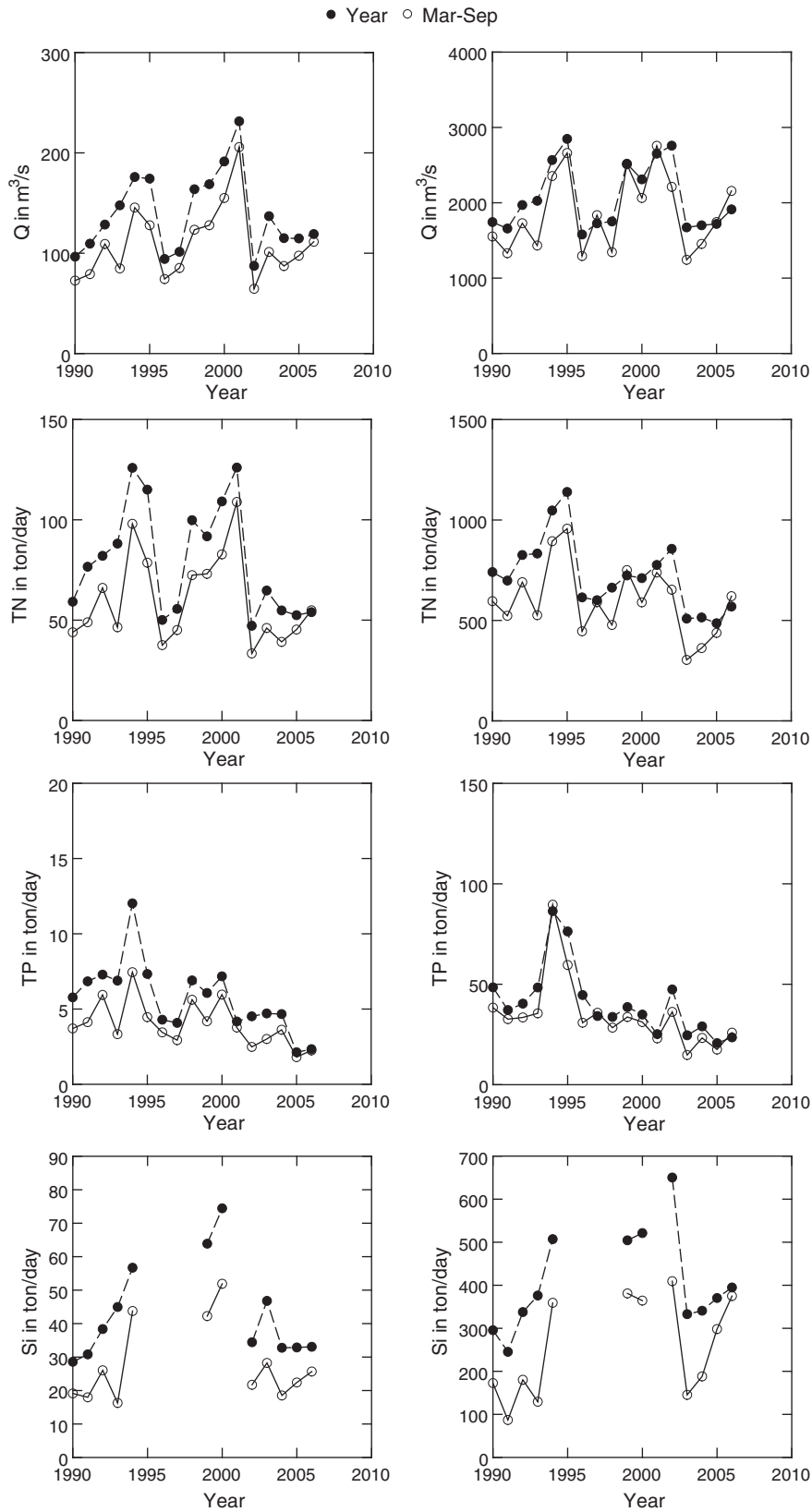
It is commonly accepted to use chlorophyll-a as proxy for total phytoplankton biomass. Cell counts per (functional) group give information about the relative abundances of the various entities. However, using cell numbers as variable for studying changes in ecosystem functioning over time, ignores the differences in cell sizes between taxa and so disregards differences in biomass. Therefore a conversion from cell counts to carbon biomass has been applied taking into account species-specific cell volumes. Menden-Deuer and Lessard (2000) developed algorithms for the conversion from cell counts into carbon biomass. These algorithms are widely used for the conversion of cell counts to carbon biomass (see e.g. Breton et al., 2006; Bratbak et al., 2011; Frigstad et al., 2011; Rousseau et al., 2002). The equations to calculate biomass from cell volume are:  $\text{pgC/cell} = 0.288 \times \text{volume}^{0.811}$  for diatoms and  $\text{pgC/cell} = 0.216 \times \text{volume}^{0.939}$  for flagellates, including dinoflagellates. Cell volumes were derived from a table of species specific cell volumes (Koeman, 2004). Values for higher taxonomic groups are also included in the list. Dependent on the morphological properties of each taxon, two different formulas were applied in this table to calculate cell volumes of filiform and disciform taxa. For all other geometric shapes different equations were applied as there are many cell shapes that expand equally in all directions, like a sphere. Each equation incorporates a linear dimensional variable which characterizes a taxon and could be measured reliably under all circumstances. Biovolumes have been calculated as a function of this dimension. All species have been categorized into autotrophs or mixotrophs. The autotrophic and mixotrophic species have been grouped together into three functional groups: diatoms, flagellates and dinoflagellates, or a fourth group that was formed by the genus *Phaeocystis*, which is presumably the species *P. globosa* (Lancelot et al., 2009). Due to homogenization and preservation with acid Lugol's solution, any *Phaeocystis* colonies usually disintegrated into separate cells which could be counted as individual cells.

The phytoplankton data analysis in this study has been limited to the main phytoplankton growing season, i.e., the months of March up to and including September. About 150 different diatom species, 50 dinoflagellate species and 10 other species have been identified in the samples from the 10 monitoring stations during the study period. Additionally, the database contains counts of groups that were identified at the genus level or at a higher taxonomic level. In some cases, a further distinction is made between e.g. size-classes within these groups. The biomass of all observations was aggregated into the four different functional groups, viz. diatoms, *Phaeocystis*, dinoflagellates, and other flagellates.

Observed trends over the period 1990–2007 were analyzed using the Mann–Kendall test. The Mann–Kendall test is a non-parametric test to evaluate whether values show a monotonic increasing or decreasing trend (Helsel and Hirsch, 2002), and is a robust test that does not require a linear trend and a normal distribution of the residuals. All other data analysis was carried out with Systat 11.

## 2.4. Potential observer effects in the phytoplankton data

The microscopical analysis of the phytoplankton samples from the routine monitoring program has been carried out by different laboratories during the 1990–2007 period. Changes between labs occurred in 1993, in 1999 and in 2000, but basically the same technicians have been involved in the analysis during 1990–1999 (M. Rademaker, pers. comm.). The analytical procedures have been subject to standard



**Fig. 3.** Average river discharges ( $Q$  in  $m^3/s$ ), and riverine loads of TP, TN and Si (ton/day) from the river Scheldt (left panels) and from Haringvliet and Nieuwe Waterweg (combined; right panels) into the Dutch North Sea. Averages for the entire year (full circles, dashed line) and the months March–September (open circles, full line).

quality procedures, but an intercalibration between the labs was not carried out. The lack of intercalibration implies that there is a risk that temporal trends observed in the data set are caused by observer effects,

rather than reflecting changes in phytoplankton composition. This observer effect could be expected in 2000 in particular, when also a change in technicians occurred.

**Table 1**

Percentual change in concentrations of chlorophyll-a and silica over the period 1990–2007, calculated from the median slope estimate, with the result of the Mann-Kendall trend test, and the Pearson's correlation coefficient ( $r$ ) for the linear correlation between growing season average Chl-a and Si concentrations. For location of sampling stations, see Fig. 1.

Trend 1990–2007				
Site	Mar–Sep	Mar–Sep	Dec–Feb	Mar–Sep
	Chl-a	Si	Si	Si-Chl
Meuse (Dutch–Belgian border, Eijsden)	–67% <sup>**</sup>	83% <sup>**</sup>	8%	–0.85 <sup>***</sup>
Rhine (Dutch–German border, Lobith)	–61% <sup>*</sup>	76% <sup>***</sup>	16% <sup>**</sup>	–0.90 <sup>***</sup>
Brienoord	–61% <sup>*</sup>	86% <sup>**</sup>	11%	–0.94 <sup>***</sup>
Puttershoek	–57% <sup>**</sup>	88% <sup>**</sup>	5%	–0.93 <sup>***</sup>
Bovensluis	–54% <sup>***</sup>	150% <sup>***</sup>	12%	–0.85 <sup>**</sup>
Maassluis	–59% <sup>*</sup>	102% <sup>**</sup>	10%	–0.69 <sup>**</sup>
Haringvliet	–34%	152% <sup>***</sup>	23%	–0.63 <sup>**</sup>
Scheldt (Dutch–Belgian border, Doel)	6%	4%	1%	–0.05

\*  $p < 0.050$ .

\*\*  $p < 0.010$ .

\*\*\*  $p < 0.001$ .

In an earlier analysis of the data from the Dutch phytoplankton monitoring program Zuur et al. (2009) used data from all 31 Dutch sampling stations. An elaborate statistical analysis of the observed changes in concentrations over time was made, using cell counts for the aggregated group of diatoms with size  $< 1000 \mu\text{m}^3$ . They included a factor to account for lab effects. Zuur et al. (2009) concluded that, although the analysis indicated the existence of a lab effect, this effect could not be distinguished from a naturally occurring trend and the lab pattern did not appear to be of a structural kind.

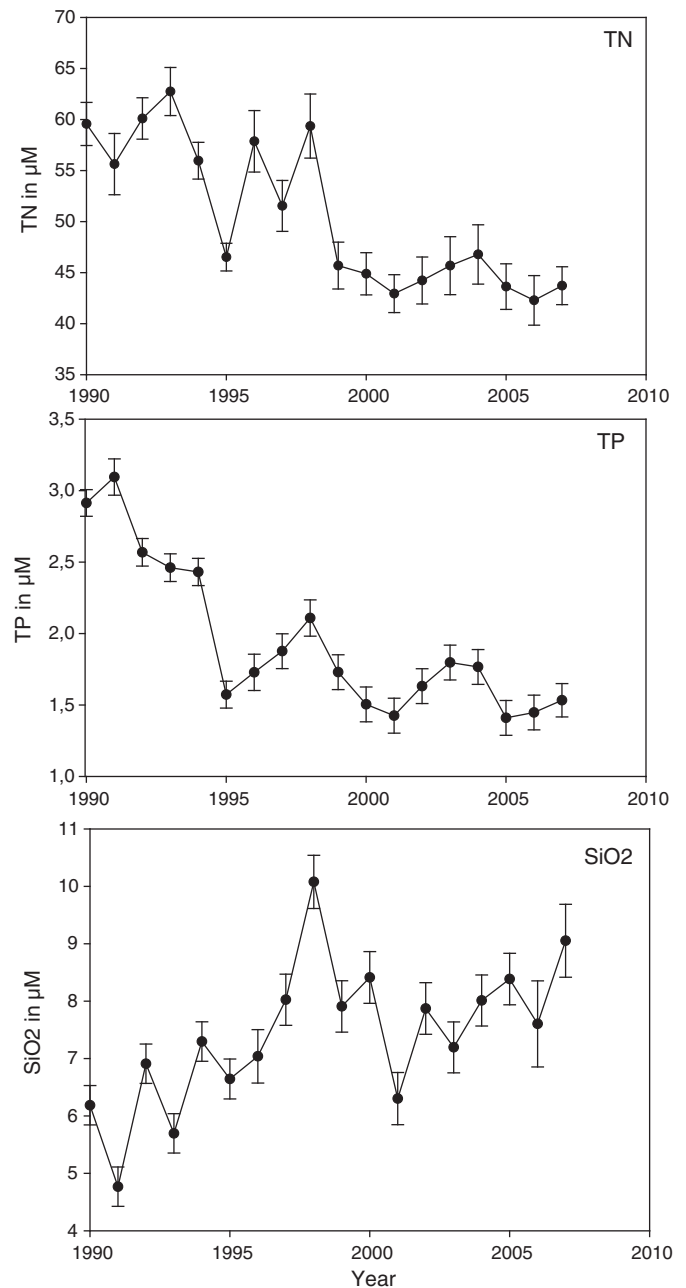
Peperzak (2010) also looked at the effects of changes in laboratories, using the data from the station N02 over the years 1990–2005. A multivariate ordination of annual phytoplankton community composition revealed three clusters of years, viz. 1990–1993, 1994–1999 and 2000–2005. The period 2000–2005 is related to one of the laboratories, but the distinction between the clusters 1990–1993 and 1994–1999 does not coincide with a shift in laboratories. Peperzak (2010) concluded that an observer effect is mainly responsible for the observed shifts in community composition between years. This observer effect is caused by a 'learning' effect, resulting in an increasing number of species identified over the years by a laboratory. Peperzak (2010) argued that, without quality control that includes intercalibration between laboratories, temporal changes in phytoplankton diversity in long-term datasets should be interpreted with care. To remove the observer effect, he proposed a procedure that results in the selection of a limited number of species that can easily be identified at the species level and occur frequently in the samples.

The phytoplankton data were analyzed to check for the influence of an observer effect on phytoplankton biomass. We looked at the differences between labs in the level of detail in the identification of species. The biomass changes between years of the four functional groups were analyzed and tested for a lab effect.

### 3. Results

#### 3.1. Analysis of potential observer effect

The database for the 10 stations used in this study contained approximately 200 species of diatoms, dinoflagellates and other flagellates. Inspection of the data showed that in the years 1990–1999 annually 88–114 species were identified, while in the years 2000–2007 114–132 species were identified. The higher number in



**Fig. 4.** Regression estimates of total nitrogen, total phosphorus and dissolved silica ( $\pm$  standard error) at salinity 30, based on linear regression of growing season (March–September) averages of nutrient concentrations on salinity.

the years 2000–2007 was caused by higher numbers of species of both diatoms and dinoflagellates. About 30% of the species within the groups diatoms, dinoflagellates and other flagellates were observed by only one of the laboratories and many of these species were observed in less than 5 samples (17% of all diatom species and 28% of all dinoflagellate species).

Additionally, the database contained about 110 differently identified "entities", sometimes identified as genus, family or a higher taxonomic level (e.g. *Gymnodinium*, Peridiniaceae), sometimes as a morphological group (e.g. pennate diatoms) that was further described in size classes where necessary. To account for differences in descriptions used by the various laboratories several corrections to the database were made by aggregating species to the same level



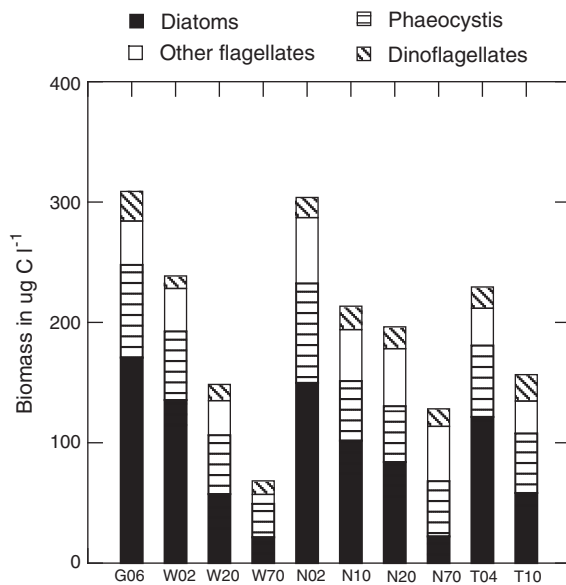


Fig. 5. Growing season phytoplankton biomass (diatoms, *Phaeocystis*, other flagellates, dinoflagellates), averaged for the entire period 1990–2007.

(e.g. the dinoflagellate *Gyrodinium sp.* was identified as one type in the years 1990–1999, whereas in the years 2000–2007 five different morphological types were distinguished). After this correction approximately 100 different “entities” remained. The annual number observed varied between 26 and 40 in the years 1990–1999, and between 48 and 59 in the years 2000–2007. The higher numbers in the years 2000–2007 were mainly caused by a higher number of different types of nanoflagellates that were distinguished.

All species and groups that were identified by one laboratory only are considered rare species in this study. To check to what extent total biomass levels were influenced by differences in species identification, the contribution of these rare species to the average biomass was calculated. Growing season average diatom biomass with and without rare species was highly correlated ( $r^2 > 0.94$  for all stations), and the average contribution of the rare species to diatom biomass was approximately 1%. In 2005 at the stations W70, T04 and T10 a higher contribution of rare species (12–35%) to total diatom biomass was observed, due to a large contribution of *Pseudo-nitzschia fraudulenta* at stations T04 and T10 and of *Dactyliosolen phuketensis* at station W70. For dinoflagellates, the contribution of rare species to growing season average biomass was approximately 19% in the years 1990–1999, and 1% in the years 2000/2007. Yet, the correlation between biomass with and without the rare species was high for all stations ( $r^2 > 0.87$ ). For flagellates, the contribution of rare species to growing season average biomass was 1%, with the exception of station G06 in 1995 due to an exceptional bloom of Euglenophyceae.

For an additional check on potential observer effects, the coincidence of changes in laboratory with large relative changes in biomass of diatoms, dinoflagellates, *Phaeocystis* and other flagellates in the time series was analyzed. The relative increase or decrease in biomass between years was calculated by dividing the absolute change with the average (1990–2007) biomass for each station. Subsequently a two-way ANOVA was carried out. The results showed that for diatoms, both the years 2000 and 2001 showed much larger relative increases in biomass than the other years. For dinoflagellates, the year 2001 showed much larger relative increases than the other years. For *Phaeocystis* and the group of other flagellates there was no clear pattern.

### 3.2. Changes in riverine nutrient loads

Nutrient loads to the southern North Sea by the rivers Rhine and Meuse through the discharge points at Haringvliet (mixture of water from Meuse and Rhine) and Maassluis (predominantly water from Rhine), and by the river Scheldt through the Westerschelde estuary (Fig. 1), have changed steadily from 1990 onwards. Data assembled by Pätzsch and Lenhart (2004, 2008) show that annual freshwater discharge volumes have hardly changed. Due to changes in freshwater nutrient concentrations, however, river nutrient loads did change.

In the outflow of Rhine and Meuse (loads of Haringvliet and Maassluis combined), a major decrease of more than 50% in annual TP loads since 1990 has occurred, as well as a decrease of approximately 20–40% in the TN loads. Annual loads of silica showed a slight increase of approximately 10%. However, more pronounced increases in Si-loads were observed during the phytoplankton growing season March–September. Si-loads during this period were approximately 40% of the total annual loads. Over the period 1990–2006 Si-loads from Rhine and Meuse during March–September increased with approximately 50% since 1990 (Fig. 3). Unfortunately, for a number of years (1995–1998, 2001) data on Si loads were missing.

TP loads from the river Scheldt decreased significantly as well, but TN and Si loads from the Scheldt showed no significant trend.

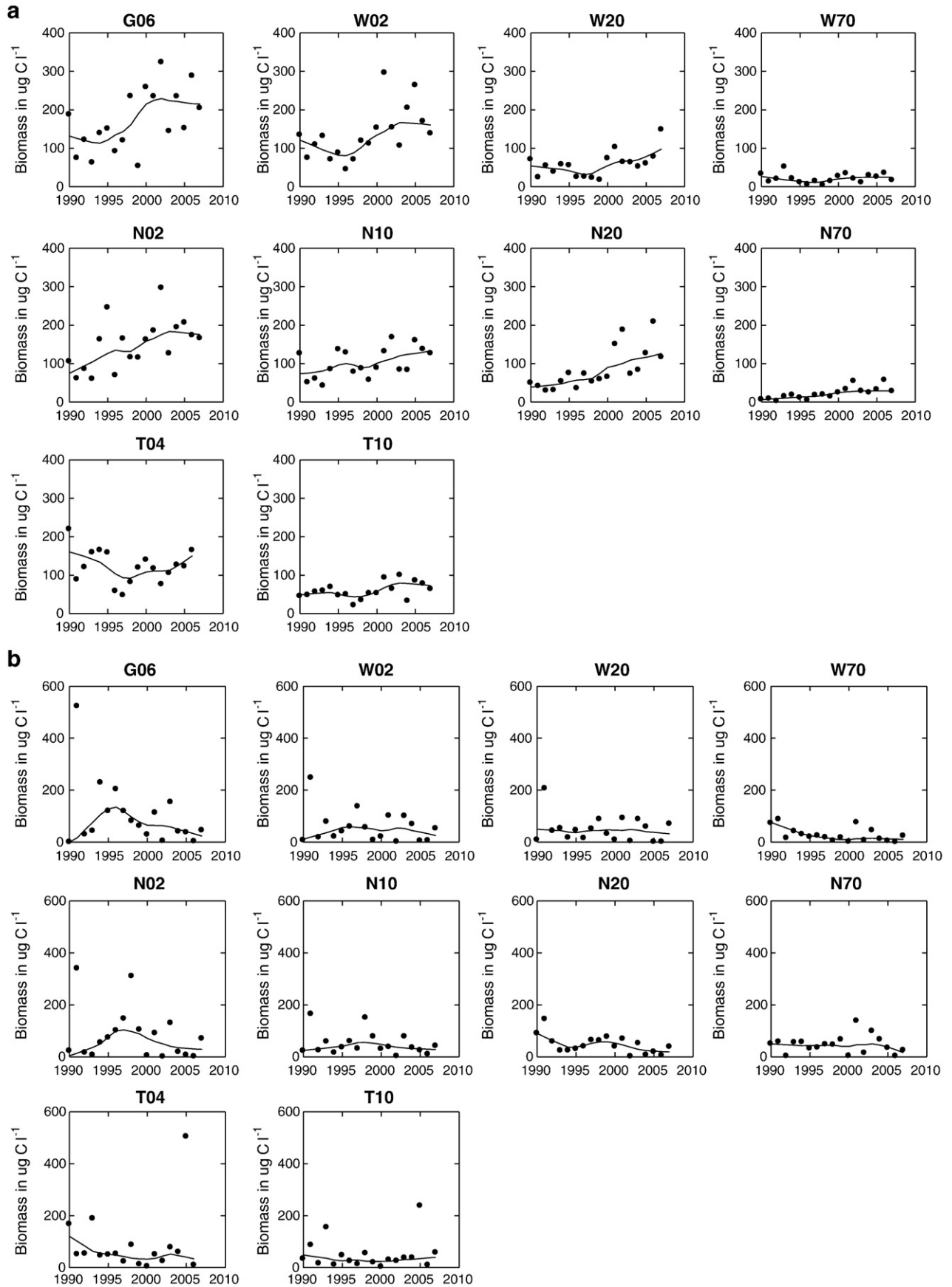
For a range of sampling stations in the rivers Rhine, Meuse and Scheldt water quality data were analyzed for the period 1990–2007 (Table 1). Trend analysis (Mann–Kendall test; Helsel and Hirsch, 2002) showed a significant decrease in chlorophyll-a and an increase in silica concentrations during the growing season March–September at all upstream sampling stations (Fig. 1) in the main river branches of Rhine and Meuse. The decrease in chlorophyll-a concentrations during March–September, estimated from the median slope estimate (Helsel and Hirsch, 2002), ranged between approximately 50–70%, while silica concentrations increased with approximately 80–150%. A significant negative linear correlation between chlorophyll-a concentrations and silica concentrations during the growing season was found for all stations in the Rhine–Meuse system. Silica concentrations in the winter period December–February showed a significant increase only at the sampling station in the Rhine at the Dutch–German border.

In the river Scheldt no significant changes in summer chlorophyll-a or silica concentrations were observed during 1990–2007.

### 3.3. Changes in nutrient and chlorophyll-a concentrations in coastal waters

In the entire coastal area, the reductions in riverine loads of TN and TP have resulted in decreased winter concentrations of DIN and DIP and decreased concentrations of TN and TP.

Mixing diagrams of nutrient concentrations in Dutch coastal waters show highly significant linear correlations with salinity (De Vries et al., 1998). These linear relations were used to calculate winter and growing season average concentrations of TN, TP and Si at a reference salinity of 30 (Fig. 4). Normalizing to a standard salinity is done to ensure that long-term trends in nutrient concentrations are not influenced by interannual differences in salinity at fixed monitoring stations. A trend analysis (Mann–Kendall test) showed significant decreases in winter average concentrations of TN ( $p < 0.001$ ) and TP ( $p < 0.010$ ), as well as in summer average concentrations of TN ( $p < 0.001$ ) and TP ( $p < 0.001$ ). The estimated trend showed a decrease of 40–50% in TP and 30% in TN concentrations over the period 1990–2007. Concentrations of silica in winter did not show a significant trend, but growing season averages of silica concentrations increased significantly ( $p < 0.010$ ) with approximately 40%.



**Fig. 6.** a. Diatom biomass at the North Sea sampling stations. Curves are fitted by LOESS smoothing. b. *Phaeocystis* biomass at the North Sea sampling stations. Curves are fitted by LOESS smoothing. c. Biomass of other flagellates at the North Sea sampling stations. Curves are fitted by LOESS smoothing. d. Dinoflagellate biomass at the North Sea sampling stations. Curves are fitted by LOESS smoothing.

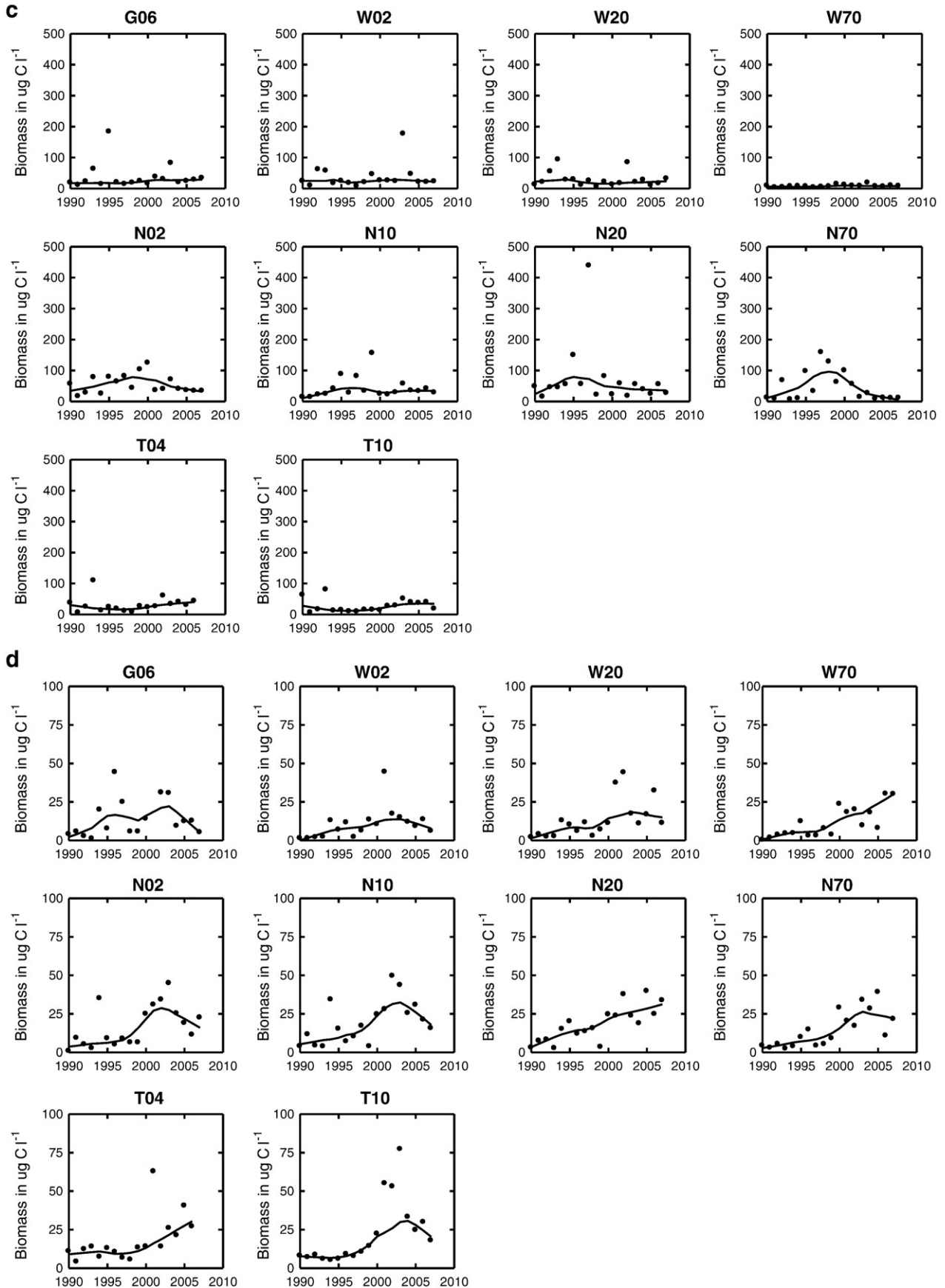


Fig. 6 (continued).



Chlorophyll-a concentrations showed a large interannual variation, and a general spatial trend of decreasing concentrations with distance from the coastline. No significant trends in chlorophyll-a concentrations during the period 1990–2007 were observed (not shown).

### 3.4. Changes in phytoplankton biomass and composition

Diatoms made up approximately 30–40% of the average phytoplankton biomass at the offshore stations W70 and N70, and 50% at the other stations (Fig. 5). *Phaeocystis* biomass contributed about 20–40% to the average biomass at all stations. Flagellates (mainly *Chrysoomonadales*, *Cryptophyceae* and *Euglenophyceae*) had a relatively high biomass at the Noordwijk transect. Dinoflagellates form 10–15% of average biomass, and there was a tendency toward relatively higher biomass at the offshore stations.

Fig. 6a, b, c and d shows the growing season average biomass of diatoms, *Phaeocystis*, other flagellates and dinoflagellates, respectively, for all 10 stations during 1990–2007. A regression line was calculated by LOESS smoothing (Cleveland and Devlin, 1988). The monitoring stations at W02, G06 and N02, N20 and N70 at the Noordwijk transect showed significant increases in diatom biomass (Fig. 6a, Table 2). *Phaeocystis* biomass showed a significant decrease at station W70 (Fig. 6b, Table 2), while the biomass of other flagellates did not show a clear trend (Fig. 6c, Table 2). Dinoflagellate biomass increased significantly at all stations except G06 (Fig. 6d, Table 2), mainly due to increases in relatively large-sized species ( $10^4$ – $10^6 \mu\text{m}^3$ ). Total phytoplankton biomass did not show significant changes (Table 2).

### 3.5. Blooms of species

The occurrence of blooms of species was analyzed by looking at blooms above the level of  $10^6$  cells/l. This level of  $10^6$  cells/l is an arbitrary threshold but has been used earlier to describe the duration of *Phaeocystis* blooms in the western Dutch Wadden Sea (Cadée and Hegeman, 1986, 2002) and is also applied in ecological assessments (Carletti and Heiskanen, 2009). Dense blooms of *Phaeocystis*, with maximum cell numbers up to  $>100 \times 10^6$  cells/l, were observed nearly every year. Several diatom species also exceeded the threshold of  $10^6$  cells/l. Bloom frequency of *Phaeocystis* and of diatom species was calculated as the percentage of the period March–September when observed cell concentrations were higher than  $10^6$  cells/l.

**Table 2**

Mann–Kendall test statistic  $z$  and significance levels for trends in growing season (March–September) averaged biomass of diatoms, *Phaeocystis globosa*, other flagellates and dinoflagellates. A positive value of  $z$  indicates an increase, a negative value a decrease. For the location of sampling stations, see Fig. 1.

	Diatoms	<i>Phaeocystis</i>	Other flagellates	Dino-flagellates	Total biomass
W02	2.20*	−0.68	0.30	2.42*	1.59
W20	1.59	−0.53	−0.45	3.03**	0.38
W70	0.45	−2.35*	1.59	3.56***	0.00
G06	2.05*	−1.36	1.67	1.36	−0.15
N02	2.58*	−0.83	−0.23	2.27*	0.08
N10	1.89	−0.38	1.59	2.27*	1.21
N20	3.56***	−1.82	−0.30	3.64***	1.44
N70	3.71***	−0.38	−0.61	3.41***	1.52
T04	−0.37	−0.78	1.61	3.01**	0.78
T10	1.74	0.23	1.52	3.26**	1.29

\*  $p < 0.050$  ( $n = 18$ ).

\*\*  $p < 0.010$  ( $n = 18$ ).

\*\*\*  $p < 0.001$  ( $n = 18$ ).

Maximum cell numbers of *Phaeocystis* (Fig. 7a) showed a significant decrease at one station only (N20), and the bloom frequency of *Phaeocystis* showed no changes in the period 1990–2007 (Table 3).

In the entire data set, 28 diatom species were identified for which a bloom level of  $> 10^6$  cells/l was observed at least once. Of these 28 species the chain-forming *Chaetoceros socialis* showed the highest number of blooms. This species sometimes bloomed in spring, coinciding with the bloom of *Phaeocystis*, but it was also observed in dense blooms during summer and early autumn (July–September). While *Phaeocystis* blooms with  $> 10^6$  cells/l were observed in almost all years, blooms of *C. socialis* occurred less frequently. In general, blooms were more frequent and reached higher concentrations at stations close to the coast and on the Noordwijk transect. Maximum cell numbers of *C. socialis* showed a significant increase at all stations during 1990–2007 (Fig. 7b, Table 3). Blooms of *C. socialis* were observed in 4% of the samples during 1990–1994, in 34% of the samples in 1995–1999 and in 60% of the samples in 2000–2007. A significant increase in bloom frequency of *C. socialis* was observed at station N02.

There were 6 other diatom species that exceeded the level of  $10^6$  cells/l in more than 1 year during 1990–2007 (*Rhizosolenia delicatula*, *Skeletonema costatum*, *Pseudonitzschia delicatissima*, *Leptocylindrus danicus*, *Leptocylindrus minimus*, *Chaetoceros debilis*). The number of diatom blooms showed an increase; blooms were observed in 28% of the samples in 1990–1994, 56% in 1995–1999, and 75% from 2000 onwards. Blooms were more frequent and reached higher maximum concentrations at the stations close to the coast (Table 3). Maximum concentrations showed an increase at W02 and the Noordwijk transect, and the bloom frequency of diatoms increased at several of these stations (W20, N02, N10, N20, T10).

## 4. Discussion

### 4.1. Changes in freshwater eutrophication and riverine nutrient loads

This phytoplankton monitoring program started in 1990, when anthropogenic eutrophication of Dutch coastal waters was high (De Vries et al., 1998). Since 1990, riverine loads to the North Sea of nitrogen and, in particular, phosphorus have decreased substantially as a consequence of various sanitation measures (Colijn et al., 2002; McQuatters-Gollop et al., 2007; Soetaert et al., 2006). These measures have resulted in a significant decrease of freshwater eutrophication, as is illustrated by decreased levels of phytoplankton blooms in many lakes in NW Europe (Jeppesen et al., 2005).

In general, studies on the effect of eutrophication focus on the importance of phosphorus in freshwater ecosystems and on nitrogen in coastal waters, with P generally being the limiting element in freshwaters and N being the limiting element in coastal and marine waters (Caraco et al., 1990; Howarth, 1988; Howarth and Marino, 2006). In a model approach simulating general conditions in western European river systems, it was shown by Billen and Garnier (1997) that human activities in the river basins not only have an impact on riverine N and P discharges to coastal waters, but also have effects on Si loads. The natural source of dissolved silica is rock weathering. Hence, the source of riverine silica should remain constant in the long-term, and seasonal variations in the loadings to the sea are expected due to seasonal variability in river runoff and biological processes (Carbonnel et al., 2009). Domestic sources may be recorded in urban wastewater since silica is used in detergents (Verbanck et al., 1989; Garnier et al., 2006), but the anthropogenic contribution to total silica loads is estimated to be negligible (Soetaert et al., 2006; Van Dokkum et al., 2004).

In the past, eutrophication has been indicated as the most likely cause for Si depletion in European rivers (Friedrich and Müller, 1984; Wollast and De Broeu, 1971). Other causes are the damming of rivers for flood regulation and man-made sedimentation areas, which increase sedimentation and retention of biogenic silica (Admiraal et al., 1990; Humborg et al., 2000). Since the 1960s a decrease in riverine silica loadings to the Black Sea and the Baltic Sea has been observed due to

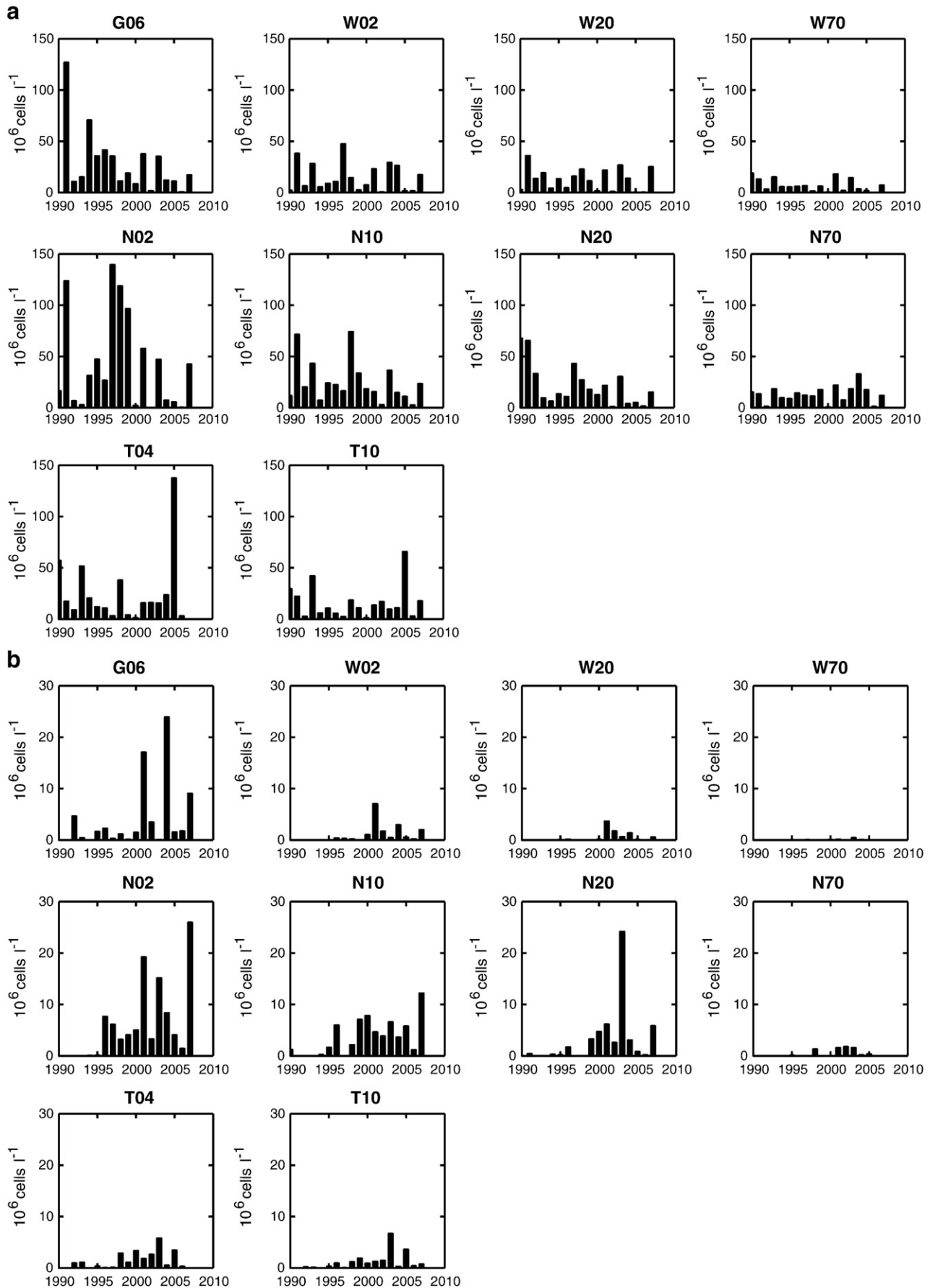


Fig. 7. a. Annual maximum cell numbers (in  $10^6$  cells/l) of *Phaeocystis globosa*. b. Annual maximum cell numbers (in  $10^6$  cells/l) of *Chaetoceros socialis*.

**Table 3**

Average (a) and maximum (m) over the period 1990–2007, of the annual maximum cell numbers and bloom frequency of *Phaeocystis globosa* and *C. socialis* blooms, and all diatom blooms. The results of the Mann–Kendall trend test (t) are indicated. All significant trends are increases, except for significant decrease indicated by d\*.

	<i>Phaeocystis globosa</i> blooms						<i>Chaetoceros socialis</i> blooms						Diatom blooms					
	Annual max. concentration (10 <sup>6</sup> cells/l)			Bloom frequency (%)			Annual max. concentration (10 <sup>6</sup> cells/l)			Bloom frequency			Annual max. concentration (10 <sup>6</sup> cells/l)			Bloom frequency		
	a	m	t	a	m	t	a	m	t	a	m	t	a	m	T	a	m	t
W02	15	47		27	60		1	7	***	6	43		1	7	*	10	57	
W20	13	36		20	50		0	4	**	3	29		1	4		6	29	*
W70	7	19		22	50		0	0	*	No bloom			0	3		2	14	
G06	27	127		25	57		4	24	*	12	29		4	24		19	43	
N02	43	139		25	50		6	26	**	14	43	**	6	26	**	25	62	**
N10	25	74		27	43		4	12	*	14	57		4	12	*	23	61	*
N20	21	68	d*	31	57		3	24	*	9	52		4	24	**	14	52	*
N70	13	33		28	43		0	2	*	3	21		1	2	*	3	21	
T04	25	137		35	63		1	6	*	6	25		3	7		23	69	
T10	16	66		24	43		1	7	**	4	25		2	7		8	38	*

\* p<0.050 decrease when marked d.

\*\* p<0.010.

\*\*\* p<0.001.

damming and eutrophication in the watersheds (Humborg et al., 2000). In an analysis of data from the river Rhine Admiraal et al. (1990) showed that diatom blooms are partly responsible for the retention of silica in river systems, and speculated that freshwater eutrophication may have increased retention of silica in river systems, thus enhancing silica depletion in the coastal zone. Indeed, an increase in dissolved silica concentrations in the river Rhine and its tributaries is observed following the recovery from eutrophication (Hartmann et al., 2007). In the last decade an increase in dissolved silica concentrations in the German part of the river Rhine of approximately 70% is observed (Hartmann et al., 2010), and this points to the importance of retention due to biological processes in the rivers, for Si discharges to the sea (Hartmann et al., 2007). In this study, we showed that an increase in dissolved silica concentrations in the rivers Rhine and Meuse occurred, with the most pronounced increase during March–September. Silica concentrations showed a highly significant negative correlation with chlorophyll-a concentrations during the March–September period. This implies that reduced retention of silica in the rivers, following the reduction of algal blooms in freshwater systems, leads to increased silica loads to the coastal waters, especially in the growing season when Si limitation of diatoms is more likely to occur (e.g. Loebel et al., 2009). It should be realized, however, that our data only refer to dissolved silica. Changes in dissolved silica concentrations in the rivers may be partly counteracted by the contribution of biogenic Si (silica stored in freshwater phytoplankton biomass) to riverine loads to the sea (Conley, 1997).

Another downstream tributary of the river Rhine discharges in the shallow Lake IJssel and eventually the water from this lake is discharged into the western Dutch Wadden Sea. The discharges from this lake may differ in N:P:Si ratio from the main discharge of the river near Maassluis, as a consequence of the long residence time of the water in Lake IJssel leading to higher retention of nutrients in the lake (Van Raaphorst and De Jonge, 2004). Nevertheless, loads of total-N, total-P and silica from lake IJssel to the western Dutch Wadden Sea showed strong decreases in TP loads, smaller decreases in TN loads and increases in Si loads (Philippart et al., 2007). The pattern is comparable to the changes in loads from Rhine and Meuse through the main river discharges at Haringvliet and Maassluis.

The observations in the lower tributaries of Rhine and Meuse indicate that, particularly in summer, chlorophyll-a concentrations have decreased and silica concentrations have increased since 1990, illustrating the effect of reduced freshwater eutrophication. Although there are some gaps in Si-load data, the data showed an increase in Si loads from Rhine and Meuse to the coastal zone, and slight increases in summer Si concentrations in the coastal waters were observed (Fig. 4). In contrast to Rhine and Meuse,

these effects of reduced eutrophication were not clearly visible in the river Scheldt. The river Scheldt is recovering from hypereutrophication. It has always acted as a net sink for Si (Soetaert et al., 2006) and recent data indicate that chlorophyll-a concentrations are increasing since 2000 (Cox et al., 2009), thus increasing the retention of Si in the river.

#### 4.2. Observer effects in the phytoplankton data

As was argued by Peperzak (2010), the correct identification and enumeration of phytoplankton cells depend on individual skills and experience, and he concluded that observer effects caused by changes in laboratories in 1994 and 2000 are the main cause for the shifts in phytoplankton composition observed in the dataset. However, the distinction of clusters for the years 1990–1993 and 1994–1999 does not coincide with changes in laboratories as erroneously assumed by Peperzak (2010). Some of the observer effects in the analysis by Peperzak (2010) may have been due to inaccuracies in the database, but even after correction of these errors we found a higher number of species in the period 2000–2007, coinciding with one of the laboratories doing the analysis. This supports the conclusion of Peperzak (2010) that, without additional data handling, the dataset is not suitable for the study of changes in phytoplankton species diversity. In our analysis, we aggregated the data to biomass data for the four groups' diatoms, dinoflagellates, *P. globosa* and other flagellates. These four groups are well-defined, and it is unlikely that errors in species identification result in allocation of biomass to the wrong group. Indeed, our analysis showed that species that were observed by one laboratory only and that may have been subject to errors in species identification, generally form a small fraction of total biomass. The spatial and temporal trends in biomass were not influenced by this group of 'rare' species. Moreover, significant year-to-year changes in biomass did not coincide mainly with a change in laboratories (years 1994, 1999 and 2000), which was to be expected if an observer effect would have been the main cause for changes in laboratory. Diatoms showed large changes in the year 2000 (coinciding with a change in laboratories), but also in 2001. Dinoflagellates showed the largest change in 2001. In the time series of *Phaeocystis* and other flagellates relatively large year-to-year changes were observed in many years and those changes did not coincide with changes in laboratories. We conclude therefore that the observed temporal patterns in biomass were not caused by an observer effect.

Several of the diatom species that were observed as blooms (including *C. socialis*) are mentioned in Table 1 in Peperzak (2010), where after several selection steps he retained a number of well-defined species

that could reliably be used for the study of changes in the phytoplankton community. With the exception of one species (*Cylindrotheca closterium*), all other bloom species that were observed in this study are mentioned in Drebes (1974), which was one of the selection criteria used by Peperzak (2010). We conclude that the diatom species for which blooms were observed are well-described species and errors in species identification are unlikely.

#### 4.3. Phytoplankton blooms in coastal waters

The data showed trends with significant increases in diatom biomass in the period 1990–2007 at several stations near the coast (W02, G06, N02, N20) and significant but smaller increases at the offshore station N70. In addition to this overall trend in diatom biomass, a remarkable increase in the magnitude of blooms of the chain forming diatom *C. socialis* was observed, and an increase in the magnitude and frequency of diatom blooms in general, at several of the coastal stations (in particular at N02, N10, N20). Both the pattern of increase in total diatom biomass and the increase in blooms of *C. socialis* and other diatoms reflect the spatial pattern of river influence. The highest increases in diatom biomass and the highest blooms of *C. socialis* were observed at the stations closest to the main river discharges, with the exception of G06. In mesocosm experiments and field observations, *C. socialis* appears to be a typical species for spring bloom conditions with relatively high availability of nutrients (Escaravage and Prins, 2002; Lancelot and Mathot, 1987). Philippart et al. (2000) observed changes in phytoplankton composition and size distribution in the western Dutch Wadden Sea during the period 1974–1994, and showed that blooms of *C. socialis* had a major contribution to the total diatom biomass in the western Wadden Sea during this eutrophic period. A comparison of the patterns observed in the coastal waters of the North Sea with observations in the western Dutch Wadden Sea is not straightforward, as there is a relatively strong top-down control on the phytoplankton by bivalve suspension feeders in the western Dutch Wadden Sea (Philippart et al., 2007). However, the observations in both Dutch coastal waters of the North Sea (this study) and the western Dutch Wadden Sea (Philippart et al., 2000, 2007) point at a relation between nutrient loads and the abundance of *C. socialis*.

The observed increasing trend in magnitude and frequency of diatom blooms in general, coincides with increased Si loads and suggests a positive effect of increased riverine Si discharges on diatom blooms in the coastal zone.

Station G06 showed a somewhat deviating pattern. Although this station lies relatively close to the outflow of Rhine and Meuse through Haringvliet and Nieuwe Waterweg, salinities are higher than those at the station N02, showing that the river influence is smaller. At G06 diatom biomass was high and increased during 1990–2007. Dense *C. socialis* blooms occurred, but no trend in blooms of diatoms was observed, which differs from the pattern at the nearby stations W02, N02 and N10. Discharges at the Haringvliet outflow are discontinuous and more variable than those through the Nieuwe Waterweg. Possibly this higher variability affected the detection of trends in the occurrence of blooms.

Dinoflagellates showed significant increases in the period 1990–2007 at nearly all stations. The observed spatial pattern does not show a decrease in biomass with increasing distance from the shore. We conclude that there is no clear relation between inorganic nutrient levels and dinoflagellate biomass, and the increase in dinoflagellate biomass must be related to other environmental factors. The increase in seawater temperature may have been an important factor, as both phenology and abundance of dinoflagellate blooms is positively correlated to sea surface temperatures (Edwards and Richardson, 2004; Edwards et al., 2006).

A remarkable phenomenon is the lack of trends in *Phaeocystis* biomass, and a limited decrease in maximum cell numbers of *Phaeocystis*. A decrease in *Phaeocystis* blooms might have been expected as a

response to the decreased N-loadings to coastal waters (Gypens et al., 2007). *P. globosa* in its colony form is assumed to have a competitive advantage when PO<sub>4</sub> is low (Veldhuis and Admiraal, 1987), also due to its ability to grow on dissolved organic phosphorus (Schoemann et al., 2005; Veldhuis et al., 1991). *Phaeocystis* also seems to be a better competitor for NH<sub>4</sub> than diatoms (Tungaraza et al., 2003). The colony matrix gives an additional competitive advantage as it may act as an energy and nutrient reservoir, and, as most of the biomass in spring is dominated by large colonies, *P. globosa* escapes top-down control by grazing (Schoemann et al., 2005) and can dominate diatoms at the end of the spring bloom in April/May, when phosphorus and silica are depleted (De Vries et al., 1998) and become potentially limiting (Loebl et al., 2009). It is assumed that the level of *P. globosa* blooms in the southern Bight is ultimately controlled by the surplus of nitrogen left at the end of the spring diatom bloom (Breton et al., 2006; Gypens et al., 2007). Consequently, present conditions where riverine P-loads have decreased much stronger than riverine N-loads and N:P ratios have increased, should be favorable for *Phaeocystis* growth.

The increased diatom biomass in response to the increased riverine Si loads during the growing season apparently does not result in a reduction of *Phaeocystis* growth through competition for resources.

#### 4.4. Concluding remarks

The decreases in phosphorus and nitrogen concentrations in freshwater systems have resulted in decreased eutrophication symptoms like algal blooms in rivers and lakes. As a consequence, retention of silica in the river systems decreased, especially during the growing season, resulting in increased Si loads to Dutch coastal waters. The increased Si loads coincide with increases in Si concentrations, increased diatom biomass and increased occurrence of dense diatom blooms in Dutch coastal waters in the North Sea. The observed increase in total diatom biomass, and in bloom frequency and maximum bloom levels of the colony-forming *C. socialis*, indicate that the present, partial solution of the eutrophication problem, leads to complex responses in the entire watershed. While the measures have been beneficial for freshwater ecosystems, they seem to result in growth conditions in coastal waters that are, on the one hand, still favorable for the nuisance alga *P. globosa* due to elevated nitrogen concentrations, and on the other hand may now enhance dense diatom blooms due to higher Si availability. At present, we have no information on the ecological effects of this increase in diatom blooms in the coastal waters of the North Sea. Some of the diatom species that have been observed as blooms are considered potentially harmful species (in particular *Pseudo-nitzschia* spp.) and the gelatinous colonies of *C. socialis* may not be easily grazed by zooplankton. Whether the increase in diatom biomass and the occurrence of dense blooms of diatom species have harmful effects, or should be considered beneficial by supporting a better transfer of energy to higher trophic levels in the food web, remains uncertain.

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