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NUTRIENT LIMITATION IN THE NORTH SEA: A BIOASSAY APPROACH

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ABSTRACT

A differential nutrient enrichment bioassay with *Skeletonema costatum* showed that phytoplankton in the continental Dutch coastal waters was potentially phosphorus and silicon limited in 1988. In the central North Sea, potential N limitation predominated. The bioassay results depended on the ratio of the dissolved nutrients in the test water and on the optimum atomic ratio for the test diatom species. The nutrient concentrations were often so low that actual limitations of nutrient uptake rates were possible. In the stratified region of the North Sea dinoflagellates and μ-flagellates were dominant, while diatoms were absent in June and July in that area. In the mixed parts of the study area both diatoms and flagellates were present. The absence of diatoms during the summer stratification in the central North Sea cannot be explained by Si limitation. It seems more probable that it is caused by a combination of N depletion and losses from sedimentation.

1. INTRODUCTION

In the last thirty years the riverine phosphorus and nitrogen loads into the North Sea have increased considerably (VAN BENNEKOM *et al.*, 1975; POSTMA, 1985). Large-scale oxygen deficiencies in the German Bight (RACHOR & ALBRECHT, 1983; GERLACH, 1984) and the increase in phytoplankton and shifts in its species composition (CADÉE & HEGEMAN, 1986; RADACH & BERG, 1986) have been attributed to eutrophication. This prompted the decision of the Second North Sea Conference to halve the anthropogenic load of phosphorus and nitrogen by 1995. To be able to evaluate the effect of reduction measures, it is required that the distribution in space and time of nutrients that have the potential to limit phytoplankton development is known.

A nutrient limits potential phytoplankton biomass formation when its availability decreases to a critical level at which it prevents the algal population from increasing further, even though other nutrients may still be available in sufficient amounts. REDFIELD (1958) contended that phosphorus limits net primary production in the oceans. He argued that biological fixation of nitrogen could meet any nitrogen deficit. Phosphorus would then be the primary factor limiting phytoplankton development. In the 1970's however, marine biologists widely accepted the contention put forward by RYTHER & DUNSTAN (1971) that nitrogen, rather than phosphorus, is the main limiting factor for phytoplankton growth in marine waters. This contention was based on nutrient enrichment bioassays conducted on polluted estuaries and coastal waters along Long Island and the New York Bight. However, it is now clear that nitrogen is not always limiting in the marine environment and that the proportion in which nutrients are loaded to a system has to be taken into account (HECKY & KILHAM, 1988; HOWARTH, 1988; SMITH, 1984). In fact, no *a priori* statements about which nutrient is limiting phytoplankton in oligotrophic — eutrophic or coastal — non-coastal environments can be made. STEPHEN BISHOP *et al.* (1984) and GRANÉLI (1987) found nitrogen limitation in non-oligotrophic coastal waters. CHIAUDANI & VIGHI (1982) and GRANÉLI & SUNDBÄCK (1985) found phosphorus limitation in coastal waters influenced by river discharge and by the discharge of tertiary treated sewage, respectively. BERLAND *et al.* (1978) state phosphorus as the primary limiting nutrient in the oligotrophic (west) Mediterranean, while this is nitrogen in the oligotrophic east (IGNATIADES & MOSCHOPOULOU, 1988). The *Phaeocystis* spring bloom in the North Sea is nitrogen limited off the Belgian coast (LANCELOT *et al.*, 1986) but is phosphorus limited off the Dutch coast (VELDHUIS, 1987). This may be due to differences in N/P ratios for the river Scheldt, influencing the Belgian coast: N/P=8 (WOLLAST, 1988), and the river Rhine, influencing the Dutch coast: N/P=17-23 (POSTMA, 1978; KRAMER & DUINKER, 1988). In Norwegian waters a high N/P ratio in fresh water and a low N/P ratio in seawater causes phosphorus limitation in fresh and brackish waters, and a balance or nitrogen limitation in marine waters (SAKSHAUG & OLSEN, 1986). Furthermore there may also be a seasonal switch from nitrogen to phosphorous limitation (HOWARTH, 1988), simultaneous limitation (DODDS *et al.*, 1988; HOWARTH, 1988), zooplankton influencing the degree of limitation (ELSER *et al.*, 1988) and limitation by other nutrients such as silicon (FRANSZ & VERHAGEN, 1985), trace metals (SAKSHAUG & MYKLESTAD, 1973) or vitamins (CORREDOR, 1979).

The nutrient enrichment bioassay has been criticized for its inability to indicate nutrient limitation of *in situ* phytoplankton communities (VINCENT, 1981; ZEVENBOOM *et al.*, 1982; HECKY & KILHAM, 1988). However, it is the only method that allows one to ascertain which nutrient is potentially the first to become limiting in algal biomass formation or to rank the other nutrients (including vitamins and trace metals) in the order of their likelihood of becoming limiting (MAESTRINI *et al.*, 1984a). When light is not limiting, the total amount of the first limiting nutrient will determine the maximum attainable standing stock and productivity of the phytoplankton. This can be important information for the implementation of nutrient reduction programmes. Physiological assays may not indicate *in situ* nutrient limitation when there is a strong nutrient turnover (GOLDMAN, 1986), while there is (of course) a potentially limiting nutrient. Such situations may be common in the North Sea in summer, when zooplankton grazing can be intense (FRANSZ & GIESKES, 1984).

Eutrophication causes a decline in the ratio of silicon to other nutrients such as N and P, which in turn can bring about a relative decline in diatoms (OFFICER & RYTHER, 1980). If the role of silicon in the eutrophication of the North Sea is to be evaluated, silicon has to be included in an assay, but this precludes the use of natural phytoplankton. An assay with natural water in which diatoms are absent cannot indicate Si depletion, even if a low concentration of silicon did cause the absence of the diatoms. Therefore a diatom has been chosen as test organism. Moreover, it is easier to compare potential nutrient limitation in very different areas and periods if the same test organism is used (MAESTRINI *et al.*, 1984a), than with natural samples having widely differing species compositions.

In this paper we describe a bioassay with *Skeletonema costatum* cultured in small tubes (CLAESSON & FORSBERG, 1978) in which the bioassay culture is monitored by measuring the *in vivo* fluorescence (BRAND *et al.*, 1981).

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2. MATERIALS AND METHODS

2.1. SAMPLING

Surface samples were taken along two salinity gradients, the Terschelling (Ts) and Noordwijk (Nw) transects, (Fig. 1) in the North Sea in weeks 15 (mid-April), 22 (late May), 26 (end of June), 33 (mid-August) and 43/44 (late October/early November) in 1988. The former transect crosses the completely mixed zones of the continental coastal area and the Dogger Bank, as well as the areas north and south of the Dogger Bank, where temperature stratification occurs in summer. The Frisian Front, the boundary between the mixed coastal and stratified waters, runs through the North Sea in a broad zone around 54°N. The Noordwijk transect lies completely within the mixed zone of the Southern Bight.

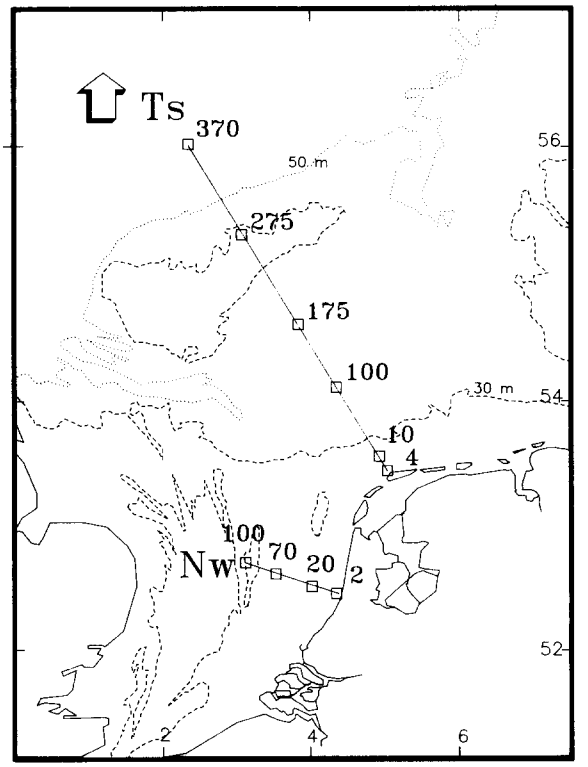

Fig. 1. Map of the study area and sampling stations (Nw=Noordwijk transect; Ts=Terschelling transect). Numbers indicate distance (km) offshore.

Single water samples were taken in the first two cruises and duplicate samples during the rest of the year. Surface samples were filtered through Whatman GF/C filters and stored at −20°C in polyethylene bottles. Samples were thawed one day before analysis to allow silicon to repolymerize (MACDONALD *et al.*, 1986).

2.2. PHYTOPLANKTON COUNTS

One-dm^3 samples were fixed with Lugol, and after sedimentation their phytoplankton was studied through an inverted microscope. Species abundance was recorded in four classes 0 (absent), 1 (< 10% of total numbers), 2 (10-50% of total numbers) and 3 (> 50% of total numbers).

2.3. NUTRIENTS, CELL VOLUME AND NUMBERS, AND CHLOROPHYLL *A*

Nutrients were determined according to GRASSHOFF *et al.*, 1983. Detection limits are: nitrate = 0.14 μM, nitrite = 0.07 μM, ammonium = 0.29 μM, ortho-phosphate = 0.06 μM, dissolved silicate = 0.36 μM. Cell volume and numbers were determined with a Coulter Counter (Coulter Electronics, Hialea, Fl).

Chlorophyll *a* was extracted according to GIESKES & KRAAY (1984) and analysed by a HPLC method with a 85-100% aceton/water-water gradient, using a reversed phase RP18 Novopack column (Waters) in a Spectra Physics Chromatography station. Chlorophyll *a* was detected with a Perkin Elmer LS-2B fluorimeter (excitation: 410-430 nm; emission: >530 nm). Calibration was achieved using a standard chlorophyll *a* solution.

2.4. BIOASSAY

In this work most of the recommendations made by MAESTRINI *et al.*, (1984 a, b) were followed. The media were prepared in polyethylene containers. Polycarbonate tubes (13 mm ∅, Nalgene) that fitted in a Turner Designs model-10 Fluorimeter were used for the assay. The stoppers were of silicone rubber. The culture vessels, flasks and tubes, were soaked overnight in 0.3 M HCl, rinsed three times with tap water and de-ionized water and dried in an oven before use.

Ten different nutrient enrichment combinations were made (no addition, + N (as nitrate), + P, + Si, + trace metals and vitamins (TMV), all, all −N, all −P, all −Si, all −TMV) from 4 stock solutions containing nitrate, ortho-phosphate, silicate and a mixture of vitamins and trace metals. Because a complete factorial design using all trace metals and vitamins separately would have been impracticable, the trace metals and vitamins were included in one mix. Reagent grade chemicals were used for the stock solutions. Concentrations were chosen in such a way that the separate addition of 50 mm^3 to a 5 cm^3 assay sample resulted in an increase of 50 μM N, 3.5 μM P, 25 μM Si, 2.5 mM C (HCO_3^-), 1 μM Fe and 0.5 nM vitamin B12. The concentrations of the other trace metals and vitamins were in the same ratio as in f-media (MCLACHLAN, 1973), except that the EDTA concentration was doubled. Bicarbonate was added to prevent possible CO_2 depletion, because tubes were stoppered air-tight. Tests showed that no such depletion occurred, not even at high nutrient concentrations. Macronutrient additions were doubled when sample concentration tended to exceed them.

Skeletonema costatum is very suited for this test because it is small, *i.e.* convenient to handle, and easy to culture. Moreover, it is a widespread and very common diatom in the coastal zone of the North Sea (DREBES, 1974; LEEWIS, 1985; CADÉE, 1986). The strain used was isolated from the Oosterschelde.

Stock cultures of the test species were maintained in f/2-medium (N:P:Si = 24:1:3) at 15°C, an irradiance of 20 W m^{-2} (measured with a calibrated Photodyne 88 XLA photometer) and a light-dark cycle of 16-8 hours. Assay experiments were carried out under the same conditions, except that irradiance was 60 $W \cdot m^{-2}$.

To obtain nutrient-depleted inoculums, stock cultures at the end of the exponential phase were diluted five times with seawater poor in nutrients, until a cell concentration of 1 to $5 \cdot 10^6 \cdot dm^{-3}$ was reached. These cultures were incubated for 2 days under experimental conditions. Depletion periods longer than 2 days led to failing inoculations. It was calculated that the amount of nutrients in the inoculum could be about 100% of the lowest total inorganic N concentration (~0.5 μM), 30% of the lowest ortho-phosphate concentration (~ 0.06 μM = detection limit) and 40% of the lowest Si concentration (~0.5 μM) found during this study. The amount of nutrients in the inoculum may therefore diminish the sensitivity of discrimination of the limiting nutrients at low nutrient concentration because the inoculums are added to every tube. The amount of depleted cells, added to assay tubes, was adapted to ensure that the initial concentration was $5 \cdot 10 \cdot dm^{-3}$.

Daily biomass increase was measured as *in vivo* fluorescence (IVF) with a Turner Designs, model-10, fluorimeter equipped with a 10-045 blue lamp, a 5-60 excitation, a 70 and 16 emission and a 3-66 reference filter. Before *in vivo* fluorescence was measured, cells were acclimatized to dim (7 $W \cdot m^{-2}$) light for 15 min (BRAND *et al.*, 1981) to obtain a constant fluorescence reading. Fluorescence was measured daily at approximately the same time of the day. The measurements were terminated when the stationary phase fluorescence declined by more than 10%. The maximum fluorescence achieved was designated IVF_{max}. The fluorimeter was calibrated by measuring the fluorescence of dilution series of dense algal cultures (see also: THOMAS *et al.*, 1974; BRAND *et al.*, 1981). Plots of fluorescence (IVF) *vs.* chlorophyll *a* concentration were linear (*Skeletonema costatum*: IVF=140.8 [chl-a] + 2567, r^2=0.990, chl-a:2-572 μg dm^{-3}, IVF:400- 81000).

The effect of the type of nutrient limitation on fluorescence and other cell properties was investigated by culturing *S. costatum* in media in which P, N or Si were limiting. At regular intervals, subsamples were taken to ascertain *in vivo* fluorescence, chlorophyll *a* concentration and cell numbers. The effect of limitation by N, P or Si media on the maxima of the chlorophyll *a* concentration, the cell concentration, the *in vivo* fluorescence and the fluorescence-

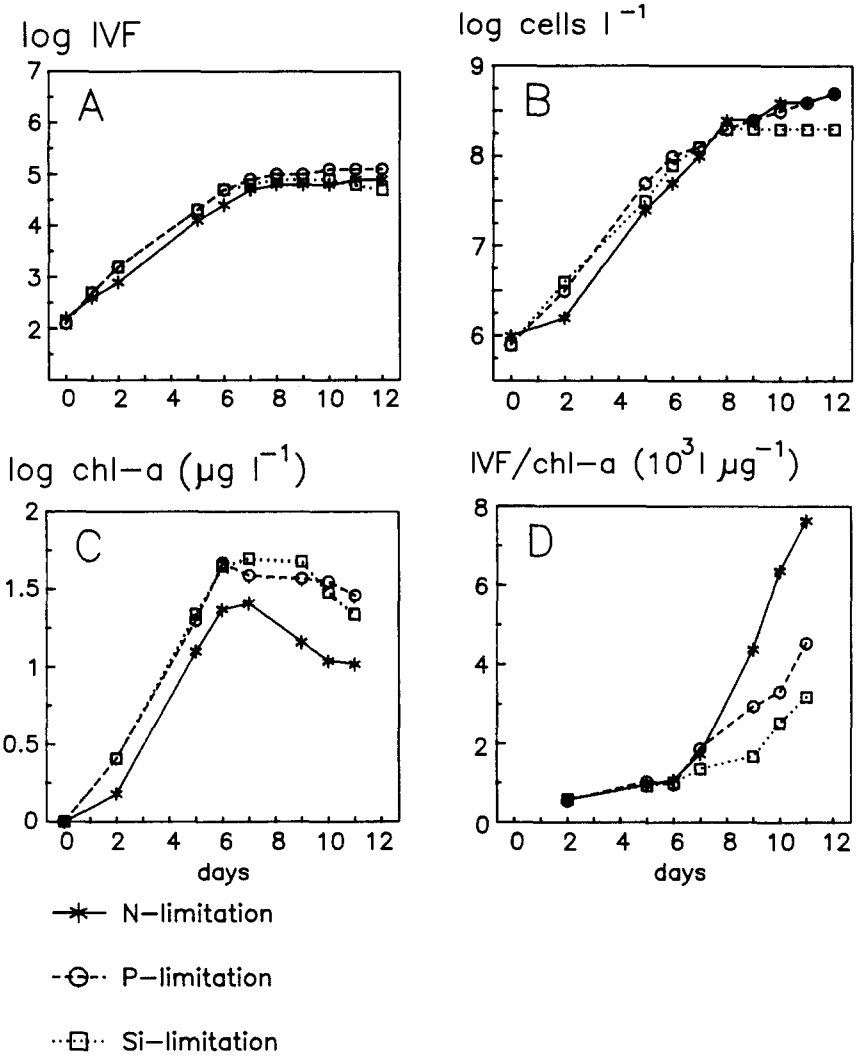

Fig. 2. The effects of the type of nutrient limited medium on (A): log *in vivo* fluorescence (IVF), (B): log cell concentration (numbers per dm^3, (C): log chlorophyll *a* concentration (µg per dm^3) and (D): IVF/chlorophyll *a* ratio. Solid line: N limitation; dashed line: P limitation; dotted line: Si limitation.

chlorophyll *a* ratio is shown in Fig. 2. In general, chlorophyll reaches its maximum level earlier and declines sooner than cell concentration and IVF, resulting in an increase of the IVF/chl ratio with time, which indicates an increase in severity of nutrient limitation (KIEFER, 1973). The N limited culture shows a stronger decline of the amount of chlorophyll after the seventh day than the P and Si limited cultures. The IVF/chl ratio of the N limited culture in the stationary phase is therefore much higher than those of the P and Si limited cultures. In the stationary phase, the IVF/cell volume ratios (not shown) with N, P or Si limitation were not significantly different. The IVF_{max} seems a fair indicator of the attainment of the maximal biomass and concomitant nutrient depletion, nearly as good as cell number and better than chlorophyll *a* concentration.

Maximum algal biomass in the assay should be linearly related to the concentration of the limiting nutrient. To test this relationship, *in vivo* fluorescence

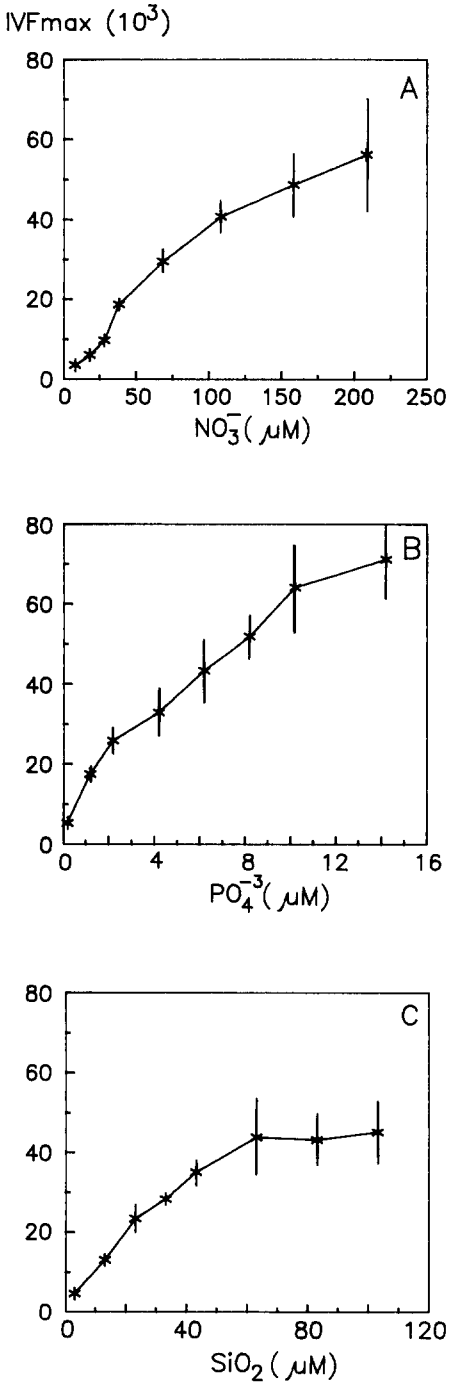

Fig. 3. Relationship between maximum *in vivo* fluorescence (IVF_{max}) and concentration of limiting nutrients in defined media (see text) (A): nitrate; (B): phosphate; (C): silicate. Bars indicate standard deviation (n=4).

was followed in cultures grown in the media where the chemical compounds were made limiting. The relationship between mean IVF_{max} and the limiting concentrations of N, P and Si were curvilinear (Figs 3a, b and c). These relationships, however, remained approximately linear within the concentration ranges found in this study. This was also found by MAESTRINI *et al.*, (1984a). Earlier determinations of this relationship with this *S. costatum* strain were linear over the entire range of concentrations.

The optimum atomic ratio of N:P:Si estimated from these relationships (n=4) is 16:1:7. The optimum atomic ratio defines the cellular nutrient ratio at the transition point between limitation by one nutrient to another (TERRY, 1980; HECKY & KILHAM, 1988). Differences between these ratios and those of the dissolved nutrients can indicate potential limitations. Curves relating trace metal concentration to IVF_{max} (not shown) suggest that the inoculum may not have fully depleted these compounds; this might also have been the case with vitamins. There was, however, always a significant difference between the tubes containing N+P+Si+TMV and tubes containing N+P+Si. Yet it has to be concluded that the present assay can only be used to detect potential limitation of the nutrients N, P and Si.

The effect of the type of N source on the fluorescence maximum was measured in media containing nitrate, nitrite and ammonium. Nitrogen had to be administered as nitrate, since ammonium concentrations above 25 μM become toxic (results not shown; J. Rijstenbil, pers. comm.).

2.6. DATA ANALYSIS

A hypothesis for the first and second potentially limiting nutrient was formed on 10%-IVF_{max} differences between treatments. This hypothesis was tested by a multiple comparisons test (Bonferroni approach; WILKINSON, 1988) for duplicate samples only.

3. RESULTS

Concentrations of inorganic nutrients were found to be generally higher in the Noordwijk transect than in the Terschelling transect (Fig. 4). Coastal stations had higher concentrations than the offshore stations except for inorganic phosphorus and silicate in week-15 on the Terschelling transect. The concentrations in week-15 were already low compared with the winter concentrations, due to the spring bloom (see also GIESKES & KRAAY, 1975). In particular, inorganic phosphorus was often very low (lower than the detection limit) in the offshore areas in weeks-22 and -26 (Fig. 4b). This was also true, to a lesser extent, of silicate in week-15 (Figs 4c and f). For the purpose of comparison, the ranges of half-saturation (K_s) values

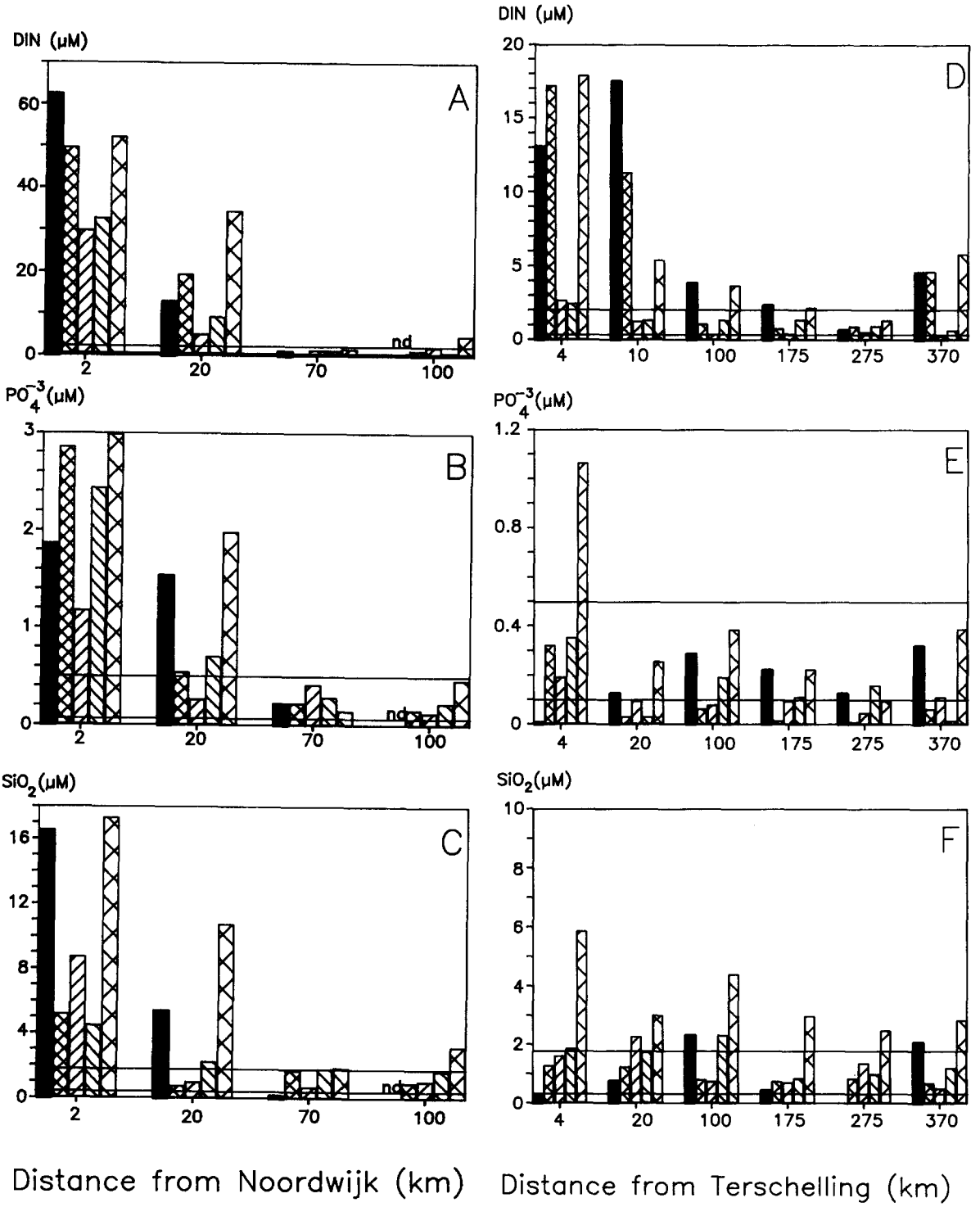

Fig. 4 Concentrations of nutrients on the Noordwijk and Terschelling transects in 1988; (A-C): Noordwijk transect, (D-F): Terschelling transect; (A+D): dissolved inorganic nitrogen (nitrate + nitrite + ammonium), (B+E): orthophosphorus, (C+F): silicate. The groups of bars per sampling station indicate from left to right the weeks-15, -22, -26, -33 and -43/44. Ranges of K_s values for nutrient uptake from the literature are indicated. nd=not determined.

for N, P and Si uptake rates found in the literature are presented in Fig. 4. The selected ranges of the K_s values are: phosphate ~0.1 to 0.5 μM (NALEWAJKO & LEAN, 1980) and 0.1 to 2.5 μM for *P. pouchetii* (VELDHUIS, 1987), nitrate and ammonium ~0.3 to 2.0 μM (GOLDMAN & GLIBERT, 1983), silicate ~0.3 to 1.8 μM for *S. costatum* and 0.3 to 5.0 μM for other marine species (PAASCHE, 1980; OFFICER & RYTHER, 1980; DUGDALE *et al.*, 1981). The choice of the K_s value for nutrient uptake was made because the $K\mu$ for growth is mostly much lower than our detection limits.

Fig. 5 shows the spatial and temporal distribution of the nutrients that are potentially the first to limit *S. costatum*. The number of significant ($p < 0.05$) determinations of the potentially first limiting nutrient was 23 out of 31; 8 hypotheses were non-significant and 1 yielded contradictory results. The results indicate that in a wide coastal zone phosphorus and silicon were in most cases the first potentially limiting nutrients, but that nitrogen was the first potentially limiting nutrient in the central North Sea. The transition zone between the two areas roughly coincides with the S=34.5 isohaline. Exceptions were the occurrences of P limitation in week-22 west of this salinity boundary and of N limitation east of it in week-43. Potential silicon limitation mainly occurred along the coast.

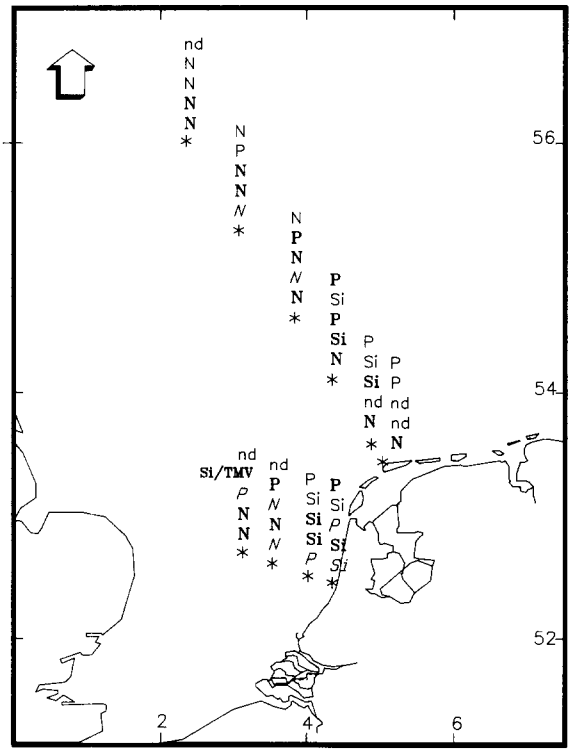

Fig. 5. Distribution of first potentially limiting nutrients in the study area in 1988. Sample periods are from top to bottom: weeks-15, -22, -26, -33 and -43/44. Bold=sample in duplicate, contrast significant; italics=sample in duplicate, contrast not significant; standard=single sample, nd=not determined.

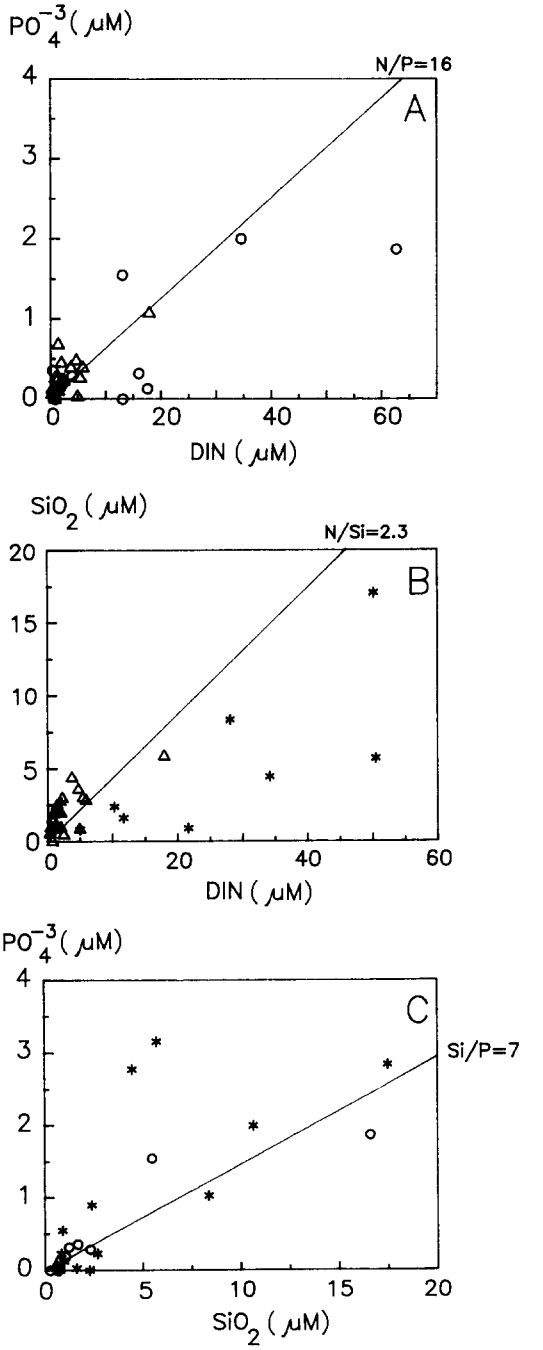

Fig. 6. The relationships between nutrient ratios and the first potentially limiting nutrients; (A): total inorganic N *vs.* ortho- phosphate, (B): total inorganic N *vs.* silicate, (C): silicate *vs.* orthophosphate. Δ=potential N limitation; ○=potential P limitation; * =potential Si limitation.

In Figs 6a, b and c the bioassay results (all points) are shown in plots of inorganic N *versus* dissolved inorganic P, Si *versus* inorganic N and Si *versus* dissolved inorganic P. The optimum atomic ratios of N:P:Si = 16:1:7, calculated from the regression coefficients of 4 calibration experiments (Fig. 3), are shown. The ratio of inorganic nutrient concentrations and the optimum atomic ratio reasonably predict the outcome of a bioassay experiment. Most exceptions are found when the ratio of the inorganic nutrients approaches the optimum atomic ratio, which of course occurs more often at low concentrations. This may be caused by experimental error, but uptake of organic P or N can also play a role. Dissolved organic phosphorus and dissolved organic nitrogen (not shown) were always present in measurable amounts.

Estimates of the abundance of various phytoplankton groups in the study area are presented in Table 1. Diatoms were present at the coastal stations of the Terschelling transect Ts (4, 10) and all stations of the Noordwijk transect almost throughout the season. Diatoms were practically absent, however, in weeks -22, -26 and -33 north of Dogger Bank and in weeks-22 and -26 at the other stations of the stratified part of the North Sea. Storms in the period between weeks-26 and -33 caused diatoms to become common again from the Dogger Bank to the island of Terschelling in week-33. The Frisian Front shifted from about halfway between stations Ts-10 and Ts-100 in week-26 to halfway between Ts-100 and Ts-175 in week-33. At the times of our cruises *S. costatum* was scarce, probably because it has its peak development in the coastal waters and estuaries of the North Sea in spring. Dinoflagellates were found in most samples; they were absent at some coastal stations [week-22: Nw (-2, -20, -70) and Ts-4; week-43: Nw-20 and weeks-15 and -22: Ts-370. *Phaeocystis pouchetii* was abundant in week-15 at the coastal stations of the Nw and Ts transects. Later, we only found small numbers of this species and it was absent in many samples. Other

TABLE 1

Occurrence of phytoplankton groups in the study area in 1988. nd=not determined; 0=not found; 1= <10% of total numbers; 2= 10-50 % of total numbers; 3= >50 % of total numbers.

Period	*Stations*									
	NW				*TS*					
Phytoplankton group	*2*	*20*	*70*	*100*	*4*	*10*	*100*	*175*	*275*	*370*
week-15										
Diatoms	nd	1	2/3	nd	2	2	1	1	2/3	1
Dinoflagellates	nd	1	1	nd	1	1	1	1	1	0
μ-flagellates	nd	0	1	nd	1	1	0	1	0	0
Phaeocystis	nd	3	2/3	nd	3	2	0	0	1	0
week-22										
Diatoms	2/3	2/3	1	1	1	0	0	0	0	0
Dinoflagellates	0	0	0	1	1	0	1	1	2	0
μ-flagellates	1	1	2	1	1	1	1	2	2	1
Phaeocystis	0	0	1	0	1	0	0	0	0	0
week-26										
Diatoms	1	2	2	2	2	nd	0	1	0	0
Dinoflagellates	1	1	2	1	1	nd	2	1	1	1
μ-flagellates	1	1	1	1	2	nd	1	2	2	2
Phaeocystis	0	0	0	1	1	nd	0	0	0	0
week-33										
Diatoms	2	1	1	1	1	1	1/2	1/2	1	0
Dinoflagellates	1	1	2	2	1	1	2/3	2	1	2
μ-flagellates	2	2	1	1	1	1	1	1	1	1
Phaeocystis	1	0	1	1	1	0	0	0	0	0
week-43/44										
Diatoms	1	1	1	1	1	1	1	1	1	1
Dinoflagelates	1	0	1	1	1	1	1	1	2	2
μ-flagellates	1	1	1	1	1	1	1	1	1	0
Phaeocystis	0	0	0	0	0	0	0	0	0	0

TABLE 2

List of dominant phytoplankton species in 1988. Dominant is interpreted as occurring in class-2 or -3.

Period	*Station*	*Phytoplankton species*
week-15	NW-20	*Phaeocystis pouchetii*
	NW-70	*P. pouchetii, Rhizosolenia stolterfothii*
	TS-4	*Biddulphia sinensis, P. pouchetii*
	TS-10	*Biddulphia sinensis, P. pouchetii*
	TS-275	*Rh. hebetata, Nitzschia seriata*
week-22	NW-2	*Rh. stolterfothii*
	NW-20	*Rh. stolterfothii*
	TS-275	*Ceratium furca, C. longipes, C. fusus, C. horridum*
week-26	NW-20	*Rh. shrubsolei*
	NW-70	*Chaetoceros densus, Nitzschia sp., C. fusus*
	NW-100	*Nitzschia* sp.
	TS-4	*Rh. shrubsolei*
	TS-100	*C. fusus, C. tripos, C. furca*
	TS-175	*Chroomonas* sp. and other cryptophytes
	TS-275	*Chroomonas* sp. and other cryptophytes
	TS-370	*Chroomonas* sp. and other cryptophytes
week-33	NW-2	several diatom species in small numbers
	NW-20	cryptophytes a.o. μ-flagellates
	NW-70	*C. fusus*
	NW-100	*Prorocentrum micans, Exuviaella baltica, C. fusus, C. furca*
	TS-100	*Rh. styliformis, C. horridum, C. fusus, Dinophysis rotundata*
	TS-175	*Rh. stolterfothii, C. fusus, C. furca*
	TS-275	*C. fusus, C. furca*
	TS-370	*C. fusus, C. furca*
week-43	TS-275	*C. fusus, C. furca*
	TS-370	*C. fusus, C. furca*

microflagellates were present in nearly all samples. Table 2 lists the dominant species. *Rhizosolenia* was a prominent diatom genus; among the dinoflagellates the genus *Ceratium* is dominant.

4. DISCUSSION

This new variant of the bioassay technique, using small culture tubes fitting a fluorimeter, can in principle be used in a wide range of nutrient conditions. To obtain reliable results it is necessary that duplicate samples are taken to test the significance of measured contrasts between the treatments. Non-significant contrasts may be caused by experimental error but also by nutrient concentration ratios that are around the optimal nutrient ratios. The experimental error might be reduced by using more replicates, by using larger tubes, by measuring more frequently and using the optimum atomic ratio of *S. costatum* for the medium of the preculture. These measures, however, partially conflict with the aim of a labour-extensive technique.

The geographical pattern of potential limitations found in this study (Fig. 5), depends on the relative distribution of nutrients and on the nutrient requirements of *S. costatum* (Figs 6a and b). Rhine water, which strongly influences the continental coastal zone, has a high N:P ratio, whereas water of Atlantic origin has a much lower N:P ratio (JOHNSTON, 1973; POSTMA, 1978, 1985). Therefore P tends to be limiting in the coastal areas and N in the central North Sea. Other authors also report P limitation in areas influenced by rivers: for the continental coastal water mass in the Southern Bight (VAN BENNEKOM *et al.*, 1975; VELDHUIS, 1987), for the Norwegian coastal waters (SAKSHAUG & OLSEN, 1986) and for the northern Adriatic (CHIAUDANI & VIGHI, 1982). However, LANCELOT'S (1983) data on *Phaeocystis* suggest N limitation off the Belgian coast. Using a nutrient-uptake kinetics technique RIEGMAN *et al.* (1990) mainly found N limitation in the summer of 1988 around the Dogger Bank. They found N, P and Si limitation, sometimes simultaneously, near the Dutch Wadden Sea coast. SAKSHAUG *et al.* (1983) also found N limitation off the west Norwegian coast. In most plumes of rivers that are not affected by eutrophication, the amount of Si far exceeds those of N and P. In rivers and lakes highly loaded with N and P Si concentrations decline (SCHELSKE & STOERMER, 1971; ELSTER, 1974; VAN BENNEKOM & SALOMONS, 1981). The eutrophic coastal waters are therefore often silicon depleted (VAN BENNEKOM *et al.*, 1975). Si limitation was found in spring for the Dutch coast (FRANSZ & VERHAGEN, 1985).

Nutrient requirements, expressed as optimum atomic ratios, are species-specific (SAKSHAUG *et al.*, 1983; HECKY & KILHAM, 1988) and, according to TURPIN (1986), also depend on growth rate. The latter might be one of the reasons why our N/P ratios, determined when growth stopped, are higher than those reported by other authors (HARRISON *et al.*, 1976, 1977; SAKSHAUG *et al.*, 1983; SAKSHAUG & OLSEN, 1986). They found N/P ratios of 8.8 to 12. The former group worked with continuous cultures, the latter group studied naturally growing populations of *S. costatum*. HARRISON *et al.* (1976, 1977) found a N/Si ratio of 1.9 in nutrient-saturated cultures, a value which agrees reasonably well with our ratio of 2.3. GILLBRICHT (1988) reports an optimum N:P:Si atomic ratio of 16:1:16 for diatoms. Other authors report still higher relative contributions of Si to the optimum atomic ratio (DE VRIES *et al.*, 1984).

Species with very different optimum atomic ratios will also show different patterns of potential nutrient limitation in space and time. Such species may be potentially limited by different nutrients and still co-exist (*e.g.* TILMAN, 1977). There is some information on this for marine, Antarctic algae (SOMMER, 1988).

A major advantage of this new variant of the nutrient-enrichment bioassay is that it requires very little time on board ship and that in the laboratory too it saves time because no subsampling is required. Nutrient-enrichment assays using natural plankton would have been impractible in this investigation because the duration of our cruises was too short, it would require too much space (large samples would be required in the central parts of the North Sea) and the labour of subsampling would only allow a very limited number of samples to be processed. A basically different approach is the measurement of nutrient-uptake kinetics. It allows the nutrients that actually limit the uptake rate of natural phytoplankton to be identified in a few hours (ZEVENBOOM, 1980, 1986; RIEGMAN, 1985). However, it requires the concentration of large sample volumes in areas where phytoplankton density is low (RIEGMAN *et al.*, 1990). This makes it difficult to concentrate the small-sized fractions. To determine all parameters of the uptake kinetics a large number of samples have to be processed (ZEVENBOOM, 1980, 1986).

A distinct advantage of the one-species bioassay over one with natural phytoplankton and the measurement of nutrient-uptake kinetics is that it can detect (potential) nutrient limitation of species absent in the natural sample. A major disadvantage of the one-species assay is that it can only identify potentially limiting nutrients for that particular species. Only indirect statements about the nutrient status of the natural phytoplankton community can be made. For that reason we included *Rhodomonas* sp. in our 1989 programme.

As a simple and limited method to explore the possible existence of *in situ* limitation of nutrient-uptake rate, the measured concentrations of the potentially

first limiting nutrient, as determined with the bioassay, were compared with published K_s values (Fig. 4). The range of published K_s values was used as a criterion for the determination of possible limitation of nutrient uptake rate. The results of this comparison (Table 3) show that at most stations nutrient concentrations could have been moderately limiting the uptake rate (the concentration of the potentially limiting nutrient was within the range of reported K_s values). At some stations the concentrations of the potentially limiting nutrient were lower than the reported range of K_s values: *e.g.* Ts-4, week-15 for P and in some instances limitation of the uptake rate seemed improbable like at most stations in week-43/44. At the coastal stations Nw 2 and Nw 20, P uptake rate would not have been limiting for diatoms in week-15 if the range of K_s values of NALEWAJKO & LEAN (1980) had been used as a criterion. However, the P uptake rate might have been limited for th colony-form of *P. pouchetii* abundant at that time, for which VELDHUIS (1987) found a K_s value of 2.5 μM.

The concentration of silicates relative to other nutrients can determine the abundance of diatoms relative to other groups (OFFICER & RYTHER, 1980; HECKY & KILHAM, 1988; VERITY *et al.*, 1988; DOERING *et al.*, 1989). A salient feature is the presence of diatoms during the whole study period in the Noordwijk transect and the coastal part of the Terschelling transect, whereas they are hardly present in the stratified part of the latter transect in the weeks 22 and 26. GIESKES & KRAAY (1984) did not find diatoms in the Oyster Ground area in summer 1981 either. The low abundance of diatoms during stratification was not accompanied by potential Si limitation of the *S. costatum* assay, except at station Ts 100 in week-22. On the other hand, potential Si limitation and low Si concentrations did coincide with the presence of diatoms in the coastal areas and the central Southern Bight. Diatoms seem to be able to maintain themselves at low Si concentrations, especially when there is sufficient turbulent mixing.

The absence of diatoms in surface samples north of the Frisian Front in weeks-22 and -26 might be explained by a combination of nutrient limitation, (not Si), and of population loss processes, such as sedimentation. Diatoms are vulnerable to sedimentation when the water column becomes stratified (REYNOLDS, 1984). Nutrient depletion (N, P and Si) in the surface layer of stratified areas caused by sedimentation of faecal pellets and cells, might have exacerbated the effect of sedimentation on diatom abundance. In week-33, after a stormy period, dissolved inorganic P, N and Si concentrations were higher at station Ts-100 than in the preceding sampling periods (Fig. 4) This coincided with the recurrence or increase of diatoms at this station, and also at Ts-175 and Ts-275 (Table 1). The storms caused the Frisian Front to shift from about halfway between stations Ts-100 and Ts-10, to halfway between Ts-100 and Ts-175. The storms must have caused an increase of nutrients and 'reseeded' the area with diatoms. 'Reseeding' might consist of transport from other areas but will mainly be caused by mixing up of sedimented, still living diatom populations (SMETACEK, 1985). Riegman (pers. comm.) observed large numbers of planktonic diatoms a few meters above the seabed in the Dogger Bank area in July 1988. The continuous presence of dinoflagellates and μ-flagellates in the whole area indicates that these groups are better adapted to stratifying conditions than diatoms (no losses from sinking, ability to

TABLE 3

Possibility of nutrient uptake limitation. Concentrations of potentially limiting nutrients (Figs 4 and 5) have been compared with literature values of K_s for nutrient uptake (see text).

Criteria for limitation of nutrient uptake. Limitation is probable when: [potentially limiting nutrient] < lowest reported K_s value; limitation is possible when: lowest reported K_s value < [potentially limiting nutrient] < highest reported K_s value; limitation is improbable when: [potentially limiting nutrient] > highest reported K_s value. * Here the K_s values for P uptake of *P. pouchetii* were used (VELDHUIS, 1987). For other species P limitation would have been improbable in this situation according to the criteria defined above. nd= not determined.

	PERIOD				
Station	*week-15*	*week-22*	*week-26*	*week-33*	*week-43/44*
Nw-2	P pos.*	Si impr.	P impr.	Si pos.	Si impr.
20	P pos.*	Si pos.	Si pos.	Si pos.	P impr.
70	nd	P pos.	N pos.	N pos.	N pos.
100	nd	Si pos.	N impr.	N pos.	N impr.
Ts-4	P prob.	P pos.	nd	nd	N impr.
10	P pos.	Si pos.	Si pos.	nd	N impr.
100	P pos.	Si pos.	P prob.	Si pos.	N impr.
175	N impr.	P prob.	N pos.	N pos.	N impr.
275	N pos.	P prob.	N pos.	N pos.	N pos.
370	nd	N impr.	N prob.	N pos.	N impr.

exploit nutrients in or under the thermocline).

The outcome of a one-species bioassay experiment will depend on the optimum atomic ratio of the test species and the ratios of the inorganic nutrients. This should be kept in mind when judging potential nutrient limitation determined with a mono-species bioassay in any waterbody. A large-scale reduction of the effective nitrogen and phosphorus loads into the North Sea would probably have different effects in different regions. In the coastal zone a reduction in phosphorus load may lead to P becoming actually limiting phytoplankton biomass more often than now and the peaks of blooms might become lower. Especially species which require a relatively large amount of P, such as *Phaeocystis pouchetii* (JAHNKE, 1989), might be affected. An effective reduction of the N load might not lead to an increase of N limitation of the phytoplankton in the near-coastal zone, when judged with the results of the *Skeletonema* assay alone. In the transition zone between the coastal zone and the central parts of the Southern Bight reduction in N load may lead to a decrease of phytoplankton development. The resulting increase of respectively the Si/P or Si/N ratios might favour diatoms in these areas. In the central North Sea physical factors, notably stratification, play an important role while actual nitrogen depletion is already taking place quite frequently. Nutrient reduction therefore is not likely to have a marked influence on phytoplankton biomass or species here.

5. REFERENCES

BENNEKOM, A.J. VAN, W.W.C. GIESKES & S.B. TIJSSEN, 1975. Eutrophication of Dutch coastal waters.—Proc. R. Soc. Lond. **B189:** 359-374.

BENNEKOM, A.J. VAN & W. SALOMONS, 1981. Pathways of nutrients and organic matter from land to ocean through rivers. SCOR Working Group 46. River Inputs to Ocean Systems. Rome, 1979: 33-51.

BERLAND, B.R., D.J. BONIN & S.Y. MAESTRINI, 1978. Facteurs limitant la production primaire des eaux oligotrophes d'une aire côtière méditerranéenne (Calanque d'En-Vau, Marseilles).—Int. Revue Ges. Hydrobiol. **63:** 501-531.

BRAND, L.E., R.R.L. GUILLARD & L.S. MURPHY, 1981. A method for the rapid and precise determination of acclimated phytoplankton reproduction rates.—J. Plankt. Res. **3:** 193-201.

CADÉE, G.C., 1986. Recurrent and changing seasonal patterns in phytoplankton of the westernmost inlet of the Dutch Wadden Sea from 1969 to 1985.—Mar. Biol. **93:** 281-289.

CADÉE, G.C. & J. HEGEMAN, 1986. Seasonal and annual variation in *Phaeocystis pouchetii* (Haptophyceae) in the westernmost inlet of the Wadden Sea during the 1973 to 1985 period.—Neth. J. Sea Res. **20:** 29-36.

CHIAUDANI, G. & M. VIGHI, 1982. Multistep approach to identification of limiting nutrients in Northern Adriatic eutrophied coastal waters.—Water Res. **16:** 1161-1166.

CLAESSON, A. & A. FORSBERG, 1978. Algal assay procedure with one or five species. Minitest.—Mitt. Internat. Verein. Limnol. **21:** 21-30.

CORREDOR, J. E., 1979. Phytoplankton response to low level nutrient enrichment through upwelling in the Colombian Caribbean Basin.—Deep-Sea Res. **26A:** 731-741.

DODDS, W.K. , K.R. JOHNSON & J.C. PRISCU, 1988. Simultaneous nitrogen and phosphorous deficiency in phytoplankton: Theory, empirical evidence, and implication for lake management. North American Lake Management Soc. Arlington, VA (USA): 44.

DOERING, P.H., C.A. OVIATT, L.L. BEATTY, V.F. BANZON, R. RICE, S.P. KELLY, B.K. SULLIVAN & J.B. FRITHSEN, 1989. Structure and function in a model coastal ecosystem: silicon, the benthos and eutrophication.—Mar. Biol. Progr. Ser. **52:** 287-299.

DREBES, G., 1974. Marines phytoplankton. Thieme Verlag, Stuttgart: 1-186.

DUGDALE, R.C., B.H. JONES JR & J.J. MACISAAC, 1981. Adaptation of nutrient assimilation.—Can. Bull. Fish. Aquat. Sc. **210:** 234-250.

ELSER, J.J., M.M. ELSER, N.A. MACKAY & S.R. CARPENTER, 1988. Zooplankton-mediated transitions between N- and P-limited algal growth.—Limnol. Oceanogr. **33:** 1-14.

ELSTER, H.J., 1974. Wechselwirkungen zwischen Chemie und Biologie bei der Eutrophierung des Bodensees.—Vom Wasser **43:** 1-14.

FRANSZ, H.G. & W.W.C. GIESKES, 1984. The unbalance of phytoplankton and copepods in the North Sea.—Rapp. P.-v. Réun. Cons. Int. Explor. Mer **183:** 218-225.

FRANSZ, H.G. & J.H.G. VERHAGEN, 1985. Modelling research on the production cycle of phytoplankton in the Southern Bight of the North Sea in relation to riverborne nutrient loads.—Neth. J. Sea Res. **19:** 241-250.

GERLACH, S.A., 1984. Oxygen depletion 1980-1983 in coastal waters of the Federal Republic of Germany.—Ber. Inst. Meeresk. **130:** 1-87.

GIESKES, W.W.C. & G.W. KRAAY, 1975. The phytoplankton spring bloom in Dutch coastal waters of the North Sea.—Neth. J. Sea Res. **9:** 166-196.

——, 1984. Phytoplankton, its pigments, and primary production at a central North Sea station in May, July and September 1981.—Neth. J. Sea Res. **18:** 51-70.

GILLBRICHT, M., 1988. Phytoplankton and nutrients in the Helgoland region.—Helgoländer Meeresunters. **42:** 435-467.

GOLDMAN, J.C., 1986. On phytoplankton growth rates and particulate C:N:P ratios at low light.—Limnol. Oceanogr. **31:** 1358-1363.

GOLDMAN, J.C. & P.M. GLIBERT, 1983. Kinetics of inorganic nitrogen uptake by phytoplankton. In: E.J. CARPENTER & D.G. CAPONE. Nitrogen in the marine environment. Academic Press, New York: 233-274.

GRANÉLI, E., 1987. Nutrient limitation of phytoplankton biomass in a brackish water bay highly influenced by river discharge.—Estuar. Coast. Shelf Sci. **25:** 555-565.

GRANÉLI, E. & K. SUNDBÄCK, 1985. The response of planktonic and microbenthic algal assemblages to nutrient enrichment in shallow coastal waters, southwest Sweden.—J. Exp. Mar. Biol. Ecol. **85:** 253-268.

GRASSHOFF, K., M. EHRHARDT & K. KREMLING, 1983. Methods of seawater analysis. Verlag Chemie, Weinheim: 1-419.

HARRISON, P.J., H.L. CONWAY & R.C. DUGDALE, 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady-state growth kinetics of *Skeletonema costatum*.—Mar. Biol. **35:** 177-186.

HARRISON, P.J., H.L. CONWAY, R.W. HOLMES & C.O. DAVIS, 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira gravida*.—Mar. Biol. **43:** 19-31.

HECKY, R.E. & P. KILHAM, 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment.—Limnol. Oceanogr. **33:** 796-822.

HOWARTH, R.W., 1988. Nutrient limitation of net primary production in marine ecosystems.—Am. Rev. Ecol. **19:** 89-110.

IGNATIADES, L. & N. MOSCHOPOULOU, 1988. Nitrogen as a factor affecting algal growth potential of an oligotrophic coastal environment of eastern Mediterranean Sea.—Int. Revue ges. Hydrobiol. **73:** 457-464.

JAHNKE, J., 1989. The light and temperature dependence of growth rate and elemental composition of *Phaeocystis globosa* Scherffel and *P. pouchetii* (Har.) Lagerh. in batch cultures.—Neth. J. Sea Res. **23:** 15-21.

JOHNSTON, R., 1973. Nutrients and metals in the North Sea. In: E.D. GOLDBERG. North Sea Science. MIT Press: 326-334.

KIEFER, D.A., 1973. Chlorophyl *a* fluorescence in marine centric diatoms: responses of chloroplasts to light and nutrient stress.—Mar. Biol. **23:** 39-46.

KRAMER, K.J.M. & J.C. DUINKER, 1988. The Rhine/Meuse Estuary. In: W. SALOMONS, B.L. BAYNE, E.K. DUURSMA & U. FÖRSTNER. Pollution of the North Sea. An assessment. Springer Verlag, Berlin: 194-212.

LANCELOT, C., 1983. Factors affectin phytoplankton extracellular release in the Southern Bight of the North Sea.—Mar. Ecol. Prog. Ser. **12:** 115-121.

LANCELOT, C. , S. MATHOT & N.J.P. OWEN, 1986. Modelling protein synthesis, a step to an accurate estimate of net primary production: *Phaeocystis pouchetii* colonies in Belgian coastal waters.—Mar. Ecol. Progr. Ser. **32:** 193-202.

LEEWIS, R.J., 1985. Phytoplankton off the Dutch coast. Thesis. University of Nijmegen: 1-147.

MACDONALD, R.W., F.A. MCLAUGHLIN & C.S. WONG, 1986. The storage of reactive silicate samples by freezing.—Limnol. Oceanogr. **31:** 1139-1142.

MAESTRINI, S.Y., D.J. BONIN & M.R. DROOP, 1984a. Phytoplankton as indicators of sea water quality: Bioassay approaches and protocols. In: L.E. SHUBERT. Algae as ecological indicators. Academic Press, London: 71-132.

MAESTRINI, S.Y., M.R. DROOP, & D.J. BONIN, 1984b. Test algae as indicators of sea water quality: Prospects. In: L.E. SHUBERT. Algae as ecological indicators. Academic Press, London: 135-188.

MCLACHLAN, J., 1973. Growth media-marine. In: J.R. STEIN. Handbook of phycological methods. Cambridge University Press, Cambridge: 25-51.

NALEWAJKO, C. & D.R.S. LEAN, 1980. Phosphorus. In: I. MORRIS. The physiological ecology of phytoplankton. Blackwell, Oxford: 235-258.

OFFICER, C.B. & J.H. RYTHER, 1980. The possible importance of silicon in marine eutrophication.—Mar. Ecol. Progr. Ser. **3:** 83-91.

PAASCHE, E., 1980. Silicon. In: I. MORRIS. The physiological ecology of phytoplankton. Blackwell, Oxford: 259-284.

POSTMA, H., 1978. The nutrient contents of North Sea water: changes in recent years, particularly in the Southern Bight.—Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer **172:** 350-357.

——, 1985. Eutrophication of Dutch coastal waters.—Neth. J. Zool. **35:** 348-359.

RACHOR, E. & H. ALBRECHT, 1983. Sauerstoff-Mangel im Bodenwasser der Deutschen Bucht.—Veröff. Inst. Meeresforsch. Bremerh. **19:** 209-227.

RADACH, G. & J. BERG, 1986. Trends in der Konzentrationen der Nährstoffe und des Phytoplanktons in der Helgoländer Bucht (Helgoland Reede Daten).—Ber. Biol. Anst. Helgoland **2:** 1-63.

REDFIELD, A.C., 1958. The biological control of chemical factors in the environment.—Am. Sci. **46:** 205-222.

REYNOLDS, C.S., 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge: 1-384.

RIEGMAN, R., 1985. Phosphate-phytoplankton interactions. Thesis. University of Amsterdam: 1-143.

RIEGMAN, R., F. COLIJN, J.F.P. MALSCHAERT, H.T. KLOOSTERHUIS & G.C. CADÉE, 1990. Assessment of growth rate limiting nutrients in the North Sea by the use of nutrient-uptake kinetics.—Neth. J. Sea Res. **26:** 61-73.

RYTHER, H.J. & W.M. DUNSTAN, 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment.—Science **171:** 1008-1113.

SAKSHAUG, E., K. ANDRESEN, S. MYKLESTAD & Y. OLSEN, 1983. Nutrient status of phytoplankton communities in Norwegian waters (marine, brackish, and fresh) as revealed by their chemical composition.—J. Plankt. Res. **5:** 175-196.

SAKSHAUG, E. & S. MYKLESTAD, 1973. Studies on the phytoplankton ecology of the Trondheimsfjord. III. Dynamics of the phytoplankton blooms in relation to environmental factors, bioassay experiments and parameters for the physiological state of the populations.—J. Exp. Mar. Biol. Ecol. **11:** 157-188.

SAKSHAUG, E. & Y. OLSEN, 1986. Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition.—Can. J. Fish. Aquat. Sc. **43:** 389-396.

SCHELSKE, C.L. & E.F. STOERMER, 1971. Eutrophication, silica, and predicted changes in algal quality in Lake Michigan.—Science **173:** 423-424.

SMETACEK, V.S., 1985. Role of sinking in diatom life-history: Ecological, evolutionary and geological significance.—Mar.Biol. **84:** 239-251.

SMITH, S.V., 1984. Phosphorus *versus* nitrogen limitation in the marine environment.—Limnol. Oceanogr. **29:** 1149-1160.

SOMMER, U., 1988. The species composition of Antarctic phytoplankton interpreted in terms of Tilman's competition theory.—Oecologia **77:** 464-467.

STEPHEN BISHOP, S., K.A. EMMANUELE & J.A. JODER, 1984. Nutrient limitation of phytoplankton growth in Georgia nearshore waters.—Estuaries **7:** 506-512.

TERRY, K.L., 1980. Nitrogen and phosphorus requirements of *Pavlova lutheri* in continuous culture.—Bot. Mar. **23:** 757-764.

THOMAS, W.H., D.L.R. SEIBERT & A.N. DODSON, 1974. Phytoplankton enrichment experiments and bioassays in natural coastal sea water and in sewage outfall receiving waters off southern California.—Estuar. Coast. Mar. Sci. **2:** 191-206.

TILMAN, D., 1977. Resource competition between planktonic algae: An experimental and theoretical approach.—Ecology **58:** 8-348.

TURPIN, D.H., 1986. Growth rate dependent optimum ratios in *Selenastrum minutum* (Chlorophyta): Implications for competition, coexistence and stability in phytoplankton communities.—J. Phycol. **22:** 94-102.

VELDHUIS, M.J.W., 1987. The eco-physiology of the colonial alga *Phaeocystis pouchetii*. Thesis. University of Groningen: 1-127.

VERITY, P.G., T.A. VILLAREAL & T.J. SMAYDA, 1988. Ecological investigations of blooms of colonial *Phaeocystis pouchetii*. I. Abundance, biochemical composition, and metabolic rates.—J. Plankt. Res. **10:** 219-248.

VINCENT, W.F., 1981. Rapid physiological assays for nutrient demand by the plankton. I. Nitrogen.—J. Plankt. Res. **3:** 685-696.

VRIES, I. DE, C.F. HOPSTAKEN & M. VEUL, 1984. The influence of nutrient availability on the ecosystem behaviour of Lake Grevelingen. Report Wabasim (R 1310-11), Delfts Hydraulics Laboratory, Delft: 1-54.

WILKINSON, L., 1988. Systat: the system for statistics. - Systat Inc., Evanston: 1-822.

WOLLAST, R., 1988. The Scheldt estuary. In: W. SALOMONS, B.L. BAYNE, E.K. DUURSMA & U. FÖRSTNER. POLLUTION OF THE NORTH SEA. AN ASSESSMENT. SPRINGER VERLAG, BERLIN: 183-193.

ZEVENBOOM, W., 1980. Growth and nutrient uptake kinetics of *Oscillatoria agardhii*. Thesis. University of Amsterdam: 1-178.

——, 1986. Ecophysiology of nutrient uptake, photosynthesis, and growth.—Can. Bull. Fish. Aquat. Sc. **214:** 391-422.

ZEVENBOOM, W., A. BIJ DE VAATE & L.R. MUR, 1982. Assessment of factors limiting growth rate of *Oscillatoria agardhii* in hypertrophic Lake Wolderwijd, 1978, by use of physiological indicators.—Limnol. Oceanogr. **27:** 39-52.