Tidal Waters Division

Nutrients and light as factors controlling phytoplankton biomass on the Dutch Continental Shelf (North Sea) in 1988-1990

Authors: J.C.H. Peeters
         H.A. Haas
         L. Peperzak
         I. de Vries

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Samenvatting

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In de discussie over de reduktie van de nutriëntenbelasting op de Noordzee is de vraag gesteld of alleen fosfor of fosfor èn stikstof gesaneerd moeten worden. Om deze vraag te kunnen beantwoorden moet bekend zijn welke factoren, waar en wanneer, beperkend zijn voor het fytoplankton.

De maximale fytoplanktonbiomassa wordt bepaald door verschillende limiterende factoren. In deze studie is de mate van lichtbeperking geschat m.b.v. P/P_{max}, een ratio van de fotosynthese bij de gemiddelde lichtintensiteit over de diepte en de dag en de maximale fotosynthese. Er zijn verscheidene methoden gebruikt om de aard en de mate van nutriëntenbeperking vast te stellen. Met de ratio's van opgeloste anorganische nutriënten (P, N en Si) en met bioassays zijn de potentieel eerst limiterende nutriënten per periode en gebied vastgesteld. De mate van nutriëntenbeperking werd berekend met V/V_{max}, de ratio van de nutriëntenopnamesnelheid en de maximale nutriëntenopname-snelheid. De berekeningen werden gebaseerd op gepubliceerde opnameconstanten en op gemeten waarden van opgeloste anorganische nutriënten. Andere methoden om de mate van nutriëntenlimitatie vast te stellen zijn de ratio Particulair Organisch Stikstof/Particulair Fosfaat (PON/PP), de ratio van intracellulair glutamine/glutamaat (GLN/GLU) en actueel gemeten nutriëntenopnamesnelheden.

Aangetoond werd dat in de winter licht op alle locaties beperkend is voor fytoplankton. In het algemeen neemt de over diepte en tijd gemiddelde lichtintensiteit toe van de kust naar volle zee. In het voorjaar neemt het fytoplankton toe door de toename van de lichtintensiteit tot een niveau waar nutriënten beperkend worden.

De ratio's van anorganische opgeloste nutriënten en de bioassays geven aan dat in het voorjaar P en in de zomer N potentieel limiterend zijn in de hele Noordzee. In alle seizoenen bestaat er een gradiënt van kust naar volle zee waarbij de potentiële limitatie van P afneemt en die van N toeneemt. De voorjaarsbloei van *Phaeocystis* in 1990 werd actueel beperkt door N, ook op locaties zeer dicht aan de kust. Si is beperkend voor diatomeeën in de hele Noordzee in het late voorjaar en zomer. De resultaten van sommige nutriëntenopname-metingen suggereren gelijktijdige beperking van het fytoplankton door verschillende nutriënten. Een verklaring is dat individuele soorten sterk verschillende nutriëntenbehoeften kunnen hebben. Twee soorten, die in deze studie gebruikt zijn, verschilden aanmerkelijk in hun fosfor- en stikstofbehoefte. Als gevolg daarvan was de ene soort P-beperkt en de andere N-beperkt, in dezelfde omgeving. De patronen van nutriëntenbeperking, nutriëntentoevoer en interne processen van het kustwaterecosysteem geven aan dat zowel P als N gesaneerd moeten worden om een betrouwbare afname van algenbloeien in voorjaar en zomer te bereiken. Een strategie, die alleen op een reduktie van de P-belasting is gebaseerd, geeft 's zomers mogelijk toch nog hoge

P-concentraties te zien. Wanneer de P-concentraties wel zouden dalen, zal het dan ontstane N-surplus naar N-beperkte gebieden worden getransporteerd, waardoor het fytoplankton zou kunnen toenemen. Theoretisch bestaat het risico dat een eenzijdige en extreme beperking van de P-belasting zou kunnen leiden tot de ongewenste dominantie van een beperkt aantal planktonsoorten.

Summary

In the discussion in the Netherlands on the sanitation of the nutrient loads discharged into the North Sea the question has been raised of whether only the load of phosphorus or the loads of both nitrogen and phosphorus should be reduced. To answer this question it should be known which factor(s) limit(s) phytoplankton where and when, and how fast the marine ecosystems react to load reductions.

The maximum phytoplankton biomass depends on several limiting factors. In this study the degree of light limitation is determined by P/P_{max}, a ratio of photosynthesis at a light intensity averaged over day and depth, and the maximal photosynthesis. First potentially limiting nutrients (P, N, Si) are identified from the ratios of dissolved inorganic nutrients and with bioassays. The degree of limitation is calculated by V/V_{max}, the ratio of the rate of nutrient uptake and the maximum rate of nutrient uptake. The calculations are based on dissolved inorganic nutrient concentrations and published values of uptake constants. Other methods to identify a degree of limitation are the ratio of Particulate Organic Nitrogen/Particulate Phosphate (PON/PP), the ratio of the intracellular glutamine/glutamate (GLN/GLU) and actually measured rates of nutrient uptake.

It is shown that in winter, light strongly limits phytoplankton at all locations. In spring, light increases and simultaneously phytoplankton increase to a level where nutrients can become limiting and light also. In general, the average daily irradiance in the water column increases from coast to offshore areas.

There is a seasonal trend in the ratios of inorganic nutrients and bioassays, indicating that P is the first potentially limiting nutrient in spring at the locations investigated. The decrease in N concentrations, relative to P concentrations, in the course of the year and all over the North Sea often leads to potential N limitation in summer. There is a spatial tendency for P to be potentially the main limiting factor in the coastal zone and for N to be in the offshore areas. There also is a trend that N-limitation increase from spring to summer.
Nutrient uptake rates measured at the end of the spring bloom of 1990 indicate that N is actually limiting also at coastal stations. At that time *Phaeocystis pouchetii* was the predominant species. Silicon is limiting for diatoms at all stations during late spring and summer.

The nutrient uptake rates measured sometimes suggest simultaneous limitation by several nutrients. An explanation is that individual species of the phytoplankton have widely differing nutrient requirements. Two bioassay species used in this study did differ considerably in their phosphorus and nitrogen requirements. As a consequence in the same environment, P limited one species and N the other.
The pattern of nutrient limitation, data on nutrient discharges and internal processes of the ecosystem indicate that both P and N loads

need to be reduced to ensure that algal blooms in spring and summer will decline. N reduction will probably lead to N limitation predominating in the coastal zone of the North Sea. A strategy based solely on P reduction has the drawback that in summer benthic phosphorus reserves are released which may retard the reduction of the P concentrations. The resulting N surplus in the coastal zone might be transported to areas which are predominantly N-limited, where it could cause an increase of phytoplankton blooms. Theoretically, there is a risk that an extreme reduction of the P-load will lead to the unwanted dominance of a few phytoplankton species.

1 Introduction

In the last fifty years the riverine phosphorus and nitrogen loads discharged into the North Sea have increased considerably (van Bennekom et al., 1975; Postma, 1985). Extensive oxygen deficiencies in the German Bight (Rachor & Albrecht, 1983; Gerlach, 1984; Colijn, 1992) and the increase in phytoplankton (Cadée & Hegeman, 1986) and shifts in its species composition (Radach & Berg, 1986) have been attributed to eutrophication. This prompted the decision of the Second (1987) and the Third International Ministers Conference on the Protection of the North Sea (1990) to halve the anthropogenic load of phosphorus and nitrogen by 1995. The phosphorus load of the Rhine has been declining since about 1980 (Klein & Van Buuren, 1992). This decline is accompanied by low river discharges.

From 1988-1990 the effects of eutrophication on the oxygen economy, primary production, phytoplankton species composition, biomass and the principal limiting factors throughout the North Sea were studied in the EUZOUT project (Peeters & Peperzak, 1990; Peeters et al., 1991; Peperzak & Peeters, 1991; Peperzak, in prep). The data generated in this project were used for this report.

The main question in the Dutch nutrient sanitation programme is whether only the load of phosphorus or the load of nitrogen plus phosphorus should be reduced. The sanitation of phosphorus can be justified on the grounds of the eutrophication problems in Dutch freshwater lakes, canals and other bodies of water. Any arguments for the sanitation of nitrogen have to come from the marine environment, as N fixation by cyanobacteria can counteract N limitation (Schindler, 1977). In addition, in most marine environments N fixation is unimportant (Howarth et al., 1988). An other question is if the nutrient input to the North Sea should be reduced by 50% or more.

An abatement measure aimed at a particular nutrient is justified only if that nutrient limits or potentially limits phytoplankton from developing in the growing season. Therefore the nutrients and physical factors that have the potential to limit phytoplankton in the North Sea must be known. Ratios of nutrient concentrations, indicating potential limitations, have been published by Bauernfeind et al. (1990) and Brockmann et al. (1989, 1990). There are few studies on phytoplankton reactions on nutrient concentrations. Bioassays have been used by Peeters & Peperzak (1990); the kinetics of nutrient uptake were studied by Riegman et al. (1990).

This report describes the results of applying the above-mentioned methods and several others for the detection of nutrient stress to samples taken from the coastal and central North Sea in 1988, 1989 and 1990 during the EUZOUT project.

Acknowledgements

2 Description of the North Sea

2.1 Hydrography

The North Sea is a shallow shelf sea which widens and deepens northwards (Fig. 1). This causes a concomitant decrease in the maximum tidal current velocities. In the Southern Bight tidal currents keep the water column well mixed throughout the year, but during summer a temperature stratification develops in the central and northern North Sea. Frontal zones form the boundaries between the stratified and the mixed areas (Pingree et al., 1978; Otto et al., 1990). Near the river outflows a temporary salinity stratification sometimes develops (Nelissen & Stefels, 1988; Otto et al., 1990). The water budget is quantitatively dominated by the North Atlantic in- and outflow. This inflow and outflow of ocean water proceeds counterclockwise. It causes the water from most British rivers to be mixed with sea water and it restricts the northward spread of the continental coastal current. This in turn causes a strong riverine influence to occur along the south-eastern continental coast. The ocean water is high saline and has low nutrient concentrations (at least in the surface layer), N/P ratios close to or less than the Redfield ratio, and low concentrations of suspended matter and yellow substances. The influence of the rivers is shown by low salinities and high nutrient concentrations whose N/P ratio is larger than the Redfield ratio, and high concentrations of suspended matter and yellow substances. In the coastal regions the concentration of suspended matter has been increased by resuspension of bottom sediment and a coastwards undercurrent (van der Giessen et al., 1990).

2.2 Eutrophication state

2.2.1 Nutrient chemistry
Phytoplankton need very small quantities of inorganic nutrients (micronutrients), much larger amounts of other inorganic nutrients (macronutrients) and organic growth factors such as vitamins. The research reported here is restricted to the macronutrients phosphorus (P), nitrogen (N) and silicon (Si). Phosphorus compounds can be classified into the following main groups: dissolved inorganic P (DIP, mostly ortho- and polyphosphate), dissolved organic P (DOP), particulate inorganic P (PIP) and particulate organic P (POP). No distinction between POP and PIP is made so the fraktion is called particulate phosphate (PP). Phytoplankton prefer ortho-phosphate. Many species can use organic phosphorus when DIP concentration is low (Veldhuis, 1987). In winter a large fraction of the total P concentration consists of DIP, while in summer large fractions of P are in the organic form, either DOP or POP (Gillbricht, 1988). Fixed forms of P are released quickly as dissolved organic and inorganic phosphorus when organisms die (Officer & Ryther, 1980). P can be stored in large amounts in sediments, e.g. by reversible adsorption. This can cause large annual fluctuations in the concentration of DIP in shallow areas (Kelderman, 1984; Wetsteyn et al., 1990).

Figure 1
Bathymetry of the North Sea

Legend
waterdepth in meters below MSL

	0 - 10
	10 - 20
	20 - 30
	30 - 40
	40 - 50
	50 - 100
	100 - 200
	200 - 500
	≥ 500

source: Hydrographic Service, 1990;
Hydrographer of the Navy, 1989;
Royal Danish Hydrogr. Office,1989

0   100   200 km
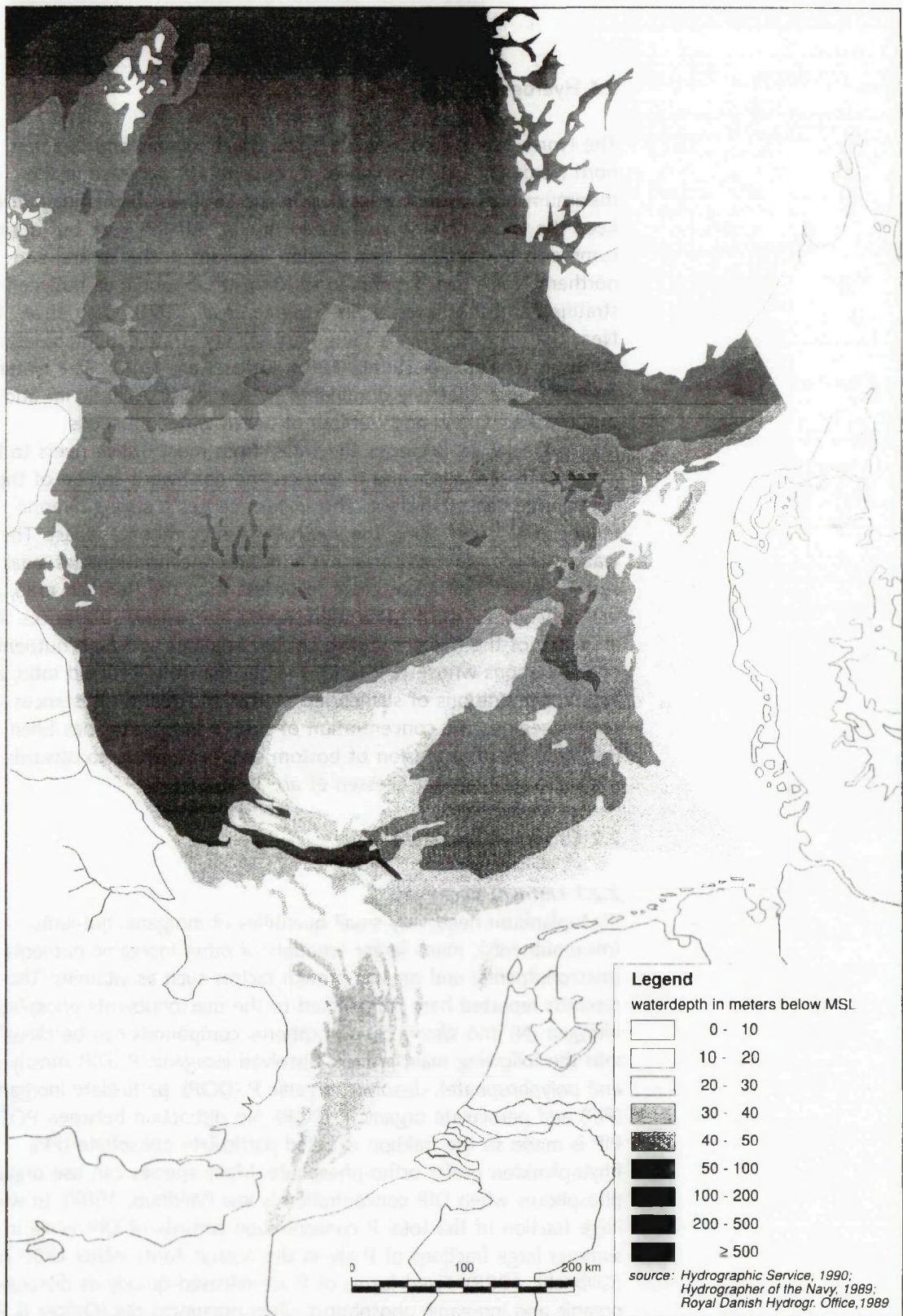
Nitrogen is present in sea water as ammonium, nitrite, nitrate, urea, amino acids and many other organic compounds. Phytoplankton prefer N in the order: ammonium, urea, nitrite, nitrate (Postma *et al.*, 1984). Some species can also use organic N (Admiraal *et al.*, 1984). It seems that the release of N compounds from dead phytoplankton and their subsequent remineralization are slower than the release and remineralization of P (Officer & Ryther, 1980; Gillbricht, 1988). This may be caused by a combination of reversible adsorption of P in sediments (Kelderman, 1984) and of denitrification as suggested by Postma et. al. (1984). The N metabolism of aquatic ecosystems is more complicated than the metabolism of most other nutrients. Apart from remineralization, nitrification, denitrification and nitrogen fixation can occur. The latter two processes represent potentially important sink and source terms respectively in the aquatic nitrogen budget.

Dissolved Si exists as $H_4S_iO_4$. Particulate forms (biogenic and mineral) show large differences in solubility. Dissolved Si regenerates slowly from particulate Si (Officer & Ryther, 1980; Gillbricht, 1988). Some authors (De Vries & Hopstaken, 1984), however, concluded from model studies that diatom frustules dissolve in sea water as fast as nitrogen mineralizes. Diatoms are the only important phytoplankton group that require Si.

2.2.2 External sources and sinks of nutrients

Nutrients enter the North Sea via the Channel, the North Atlantic, rivers, the anthropogenic discharges and the atmosphere. The Atlantic water entering the shallow North Sea in summer is nutrient-depleted surface water. Nutrient concentrations in the deep water of the North Atlantic are much higher than those of the surface layer (Hoppema & De Baar, 1992). Nutrient concentrations in most of the North Sea are lower than in the Atlantic Ocean, both in summer and winter (Nelissen & Stefels, 1988; Brockmann *et al.*, 1990). For the North Sea as a whole the North Atlantic inflow contributes 70-75% of the N and 81-82% of the P loads. The Channel contributes 5-8% of the total N load and 5-6% of the total P load. The rivers contribute 12-17% of the total N load and 8-11% of the total P load (Nelissen & Stefels, 1988; Brockmann *et al.*, 1990). Sydow *et al.* (1990) estimate the contribution of the Channel to be twice as high, for both N and P. These proportions shift towards a dominance of the influence of rivers and the Channel inflow in the mass of continental coastal water. The Si load of the North Sea is made up of a large contribution from the oceanic inflows and from the rivers. The oceanic inflow contributes to the Si load of the North Sea by a large water transport and moderate concentrations, whereas the rivers do so by high (natural) Si concentrations, but a small water transport.

The contribution of the atmosphere to the nutrient load of the North Sea is thought to be small for most nutrients (Nelissen & Stefels, 1988; Brockmann *et al.*, 1989) except for nitrogen, for which it is significant (Warmenhoven *et al.*, 1989; van Boxtel *et al.*, 1991). Nutrient budgets based on model calculations indicate atmospheric inputs as fraction of total inputs ranging from 1% in the northern North Sea, 2.5% in the coastal area of the southern Bight to 5% in the central part of the southern Bight (Anonymous, 1992).

The nutrient loads of the rivers vary during the season (Waterkwaliteitsplan, 1985; van der Meyden, 1992). The phosphorus load fluctuates the least, the silicon load the most and the nitrogen load is intermediate. The minimum/maximum loads of total N and total P vary over the year in the order of 1:6 and 1:3 respectively (Klein & van Buuren, 1992). The low silicon loads in spring and summer are caused by the low river discharges in that period and the uptake of Si by freshwater diatoms. The latter are able to deplete this element since N and P concentrations are very high in the river basins of Rhine and other rivers (Schelske & Stoermer, 1971; van Bennekom & Salomons, 1981; Smayda, 1990; Admiraal & Van Der Vlugt, 1990).

The anthropogenic contribution to the riverine and atmospheric inputs of nutrients into the North Sea is larger than the natural contribution (Nelissen & Stefels, 1988; van Boxtel et al., 1991). As a consequence of the larger proportion of river water along the Dutch coast and in the German Bight the anthropogenic fractions of N and P concentrations are very high there (>50%) (Waterkwaliteitsplan, 1985; Nelissen & Stefels, 1988). These fractions decrease to ±10% in the central part of the Southern Bight and to very small values in the northern North Sea (Anonymous, 1992). The abundance of Si relative to N and P has decreased in eutrophic parts of the North Sea (van Bennekom & Salomons, 1981; Laane, 1992). The ratio of total N load/total P load has changed over time, due to eutrophication. In pristine conditions this ratio would have been 20-30 (Laane, 1992). More recently the ratio of total N to total P varied from 18-30 at the end of the seventies to 20-40 in 1990 (Klein & Van Buuren, 1992; van der Meyden, 1991).

2.2.3 Internal ecosystem processes affecting nutrient concentrations
Other processes in addition to the import and export of nutrients in aquatic ecosystems affect nutrient concentrations. In winter and early spring nutrient concentrations are high and for a large part are inorganic. In the growing season the uptake by phytoplankton is the main process causing nutrient concentrations to decline. A substantial fraction of the nutrients bound in phytoplankton settles and can be stored in the benthic pool. An other part of the phytoplankton dies in the water column, is grazed by zooplankton or zoobenthos and is more or less remineralized. This remineralization promotes the production of new phytoplankton. For detailed reviews of nutrient cycling in marine ecosystems see Nelissen & Stefels (1988) and Brockmann et al. (1990).

Nitrate can be used by bacteria as an oxygen source in conditions where oxygen is scarce. This leads to denitrification, a process in which gaseous nitrogen compounds are produced (mainly as N_2, a small fraction as N_2O). In ecosystems with well oxygenated water columns denitrification is almost entirely restricted to the sediment. The rate of this process is dependent on local sediment characteristics and nitrate concentrations (Law & Owens, 1990; van Raaphorst et al., 1992). The estimated N loss through denitrification in the North Sea is substantial. The loss via this process is estimated at 10-20% of the annual load (Brockmann et al., 1989; Law & Owens, 1990). From model calculations (Anonymous, 1992) the total loss of nitrogen in this case

including formation of refractory organic forms, can be estimated at more than 50% (67%) of the total input into the North Sea. In other marine ecosystems (estuaries and the Baltic Sea) denitrification is in the order of 40 - 50% of the N input (Seitzinger, 1988).

Large amounts of phosphorus are stored in sediments (Rutgers van der Loeff, 1980; de Jonge et al., 1992). Part of this amount ($\pm$16-50 %) was found to be biologically available in sediments of the western Wadden Sea (de Jonge et al., 1992). This fraction seems to accumulate in the sediment in winter, but when temperatures rise in spring it is mobilized as adsorption capacity decreases. This leads to a rise of the dissolved P concentration in the summer season, not only in the saline Lake Grevelingen (Kelderman, 1984), but also in inlets like the Oosterschelde (Wetsteyn et al., 1990). According to Brockmann et al. (1989) and Rutgers van der Loeff (1980) large fractions of nutrients taken up by the phytoplankton originate from the North Sea sediments. In contrast van Raaphorst et al. (1990) found a much smaller contribution of the sediments. They attributed the disparity to much smaller coefficients of apparent nutrient diffusion. Much less phosphorus than nitrogen is lost or retained; model calculations (Anonymous, 1992) yield an estimate of 15% of the total input into the North Sea.

Figure 2
Map of the study area and the
sampling stations (Nw-Noordwijk
transect; Ts = Terschelling transect).
Numbers indicate distance (km)
offshore.
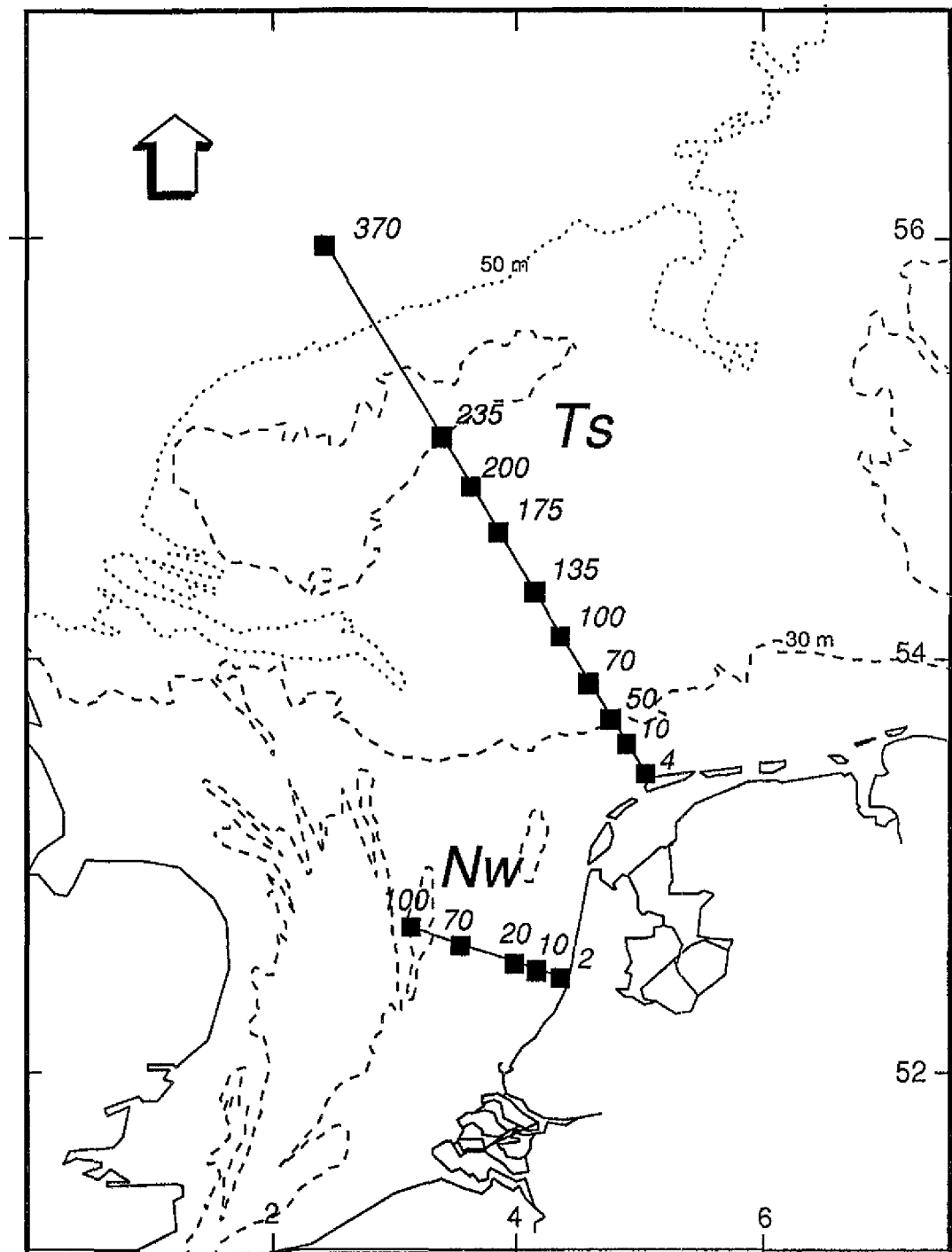
3 Material and Methods

3.1 Sampling area and period

Surface samples were taken along two transects, the Terschelling (Ts) and Noordwijk (Nw) transect in the North Sea during 1988-1990 (Fig. 2). The Terschelling transect crosses the completely mixed zones of the continental coastal area and of the Dogger Bank, as well as the areas north and south of the Dogger Bank, where temperature stratification occurs in summer. The Noordwijk transect lies completely within the mixed zone of the Southern Bight. A survey of the cruises is presented in Table 1.

Table 1

Survey of the North sea cruises in 1988, 1989 and 1990 of the EUZOUT project and the routine monitoring programme (MONO)

	1988		1989		1990	
	Cruise	Week	Cruise	Week	Cruise	Week
1.	MONO	2	MONO	2	MONO	1
2.	MONO	4	MONO	8	MONO	11
3.	MONO	8	EUZOUT	10	EUZOUT	15
4.	MONO	12	MONO	11	MONO	16
5.	EUZOUT	15	EUZOUT	14	EUZOUT	17
6.	MONO	16	MONO	15	MONO	20
7.	MONO	20	EUZOUT	16	EUZOUT	23
8.	EUZOUT	22	EUZOUT	19	EUZOUT	27
9.	MONO	25	MONO	21	MONO	29
10.	EUZOUT	26	EUZOUT	23	EUZOUT	31
11.	MONO	29	MONO	25	MONO	34
12.	EUZOUT	33	MONO	29	EUZOUT	36
13.	MONO	34	EUZOUT	30	MONO	43
14.	MONO	38	MONO	33	MONO	47
15.	MONO	42	EUZOUT	34	MONO	51
16.	EUZOUT	43/44	MONO	38		
17.	MONO	45	MONO	43		
18.	MONO	50	EUZOUT	44		
19.			MONO	48		

3.2 Light

Irradiance data from 'De Kooy' weather station at Den Helder, transformed to PAR (Photosynthetic Available Radiation), were used. Underwater light attenuation was measured according to Peeters et al. (1991).

3.3 Nutrients and chlorophyll-a

After filtration on GF/C filters on board, the filters and filtrates were frozen until analysis. Nutrients were determined according to Grasshoff et al. (1983). Nutrient samples were thawed one day before analysis to allow silicon to repolymerize (MacDonald et al., 1986). 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. Chlorophyll-a was extracted on GF/C filters according to Gieskes & Kraay (1984) and

analyzed by a HPLC method with a 85-100% acetone/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). A standard chlorophyll-a solution was used for calibration.

3.4 Bioassays

Bioassay experiments were performed according to Peeters & Peperzak (1990). The diatom *Skeletonema costatum* was used in 1988, 1989 and 1990, and the cryptophyte *Rhodomonas sp.* in 1990. The data on *S. costatum* came from ten stations and 13 cruises and that for *Rhodomonas sp.* came from 9 cruises. First, potentially limiting nutrients were judged according to Peeters & Peperzak (1990). Additional bioassays were done on coastal phytoplankton in 1990. Samples were spiked with $N-NH_4^+$, $P-PO_4^{3-}$ and SiO_3^{2-} (15, 2 and 10 μM resp.) and incubated at 35 W m^{-2} and near *in situ* temperatures and *in situ* light/dark cycles. After 3 days chlorophyll-a was measured as described above.

3.5 Primary production

The photosynthetic characteristics of phytoplankton were measured on board in an incubator and data were processed according to Peeters *et al.* (1991).

3.6 Kinetics of nutrient uptake

In 1990 surface samples were taken at several stations along the Noordwijk and Terschelling transects in weeks 15, 17, 23, 27, 31 and 36. Samples were concentrated using a 10 μm nylon plankton net. Part of this concentrate was used to determine chlorophyll-a concentration. The other part, 250 ml, was incubated at approximate *in situ* temperatures in polycarbonate Erlenmeyer flasks. The flasks were illuminated at 55 W.m^{-2} by fluorescent tubes at the bottom of the incubator. This was ca. 40% of the mean optimum irradiance as determined by six P/I curves at Nw2 and Nw100 in 1989. The samples were spiked with NH_4^+, NO_3^-, PO_4^{3-} and SiO_3^{2-} to yield concentrations of 15, 15, 2 and 10 μM respectively above the *in situ* concentration. Subsamples were taken for nutrient analysis at 0, 30, 60, 90, 120 and 180 minutes after the start of the experiment. They consisted of ca. 8 ml of water filtered through Whatman GF/C filters. The filters were stored immediately at - 18°C until analysis. All materials, except the filters, were made of polythene. After week 17, the nutrient concentrations sometimes behaved erratically, possibly by zooplankton grazing. To rule out zooplankton interference and to save larger phytoplankton, in weeks 31 and 36 samples were sieved through 250 μm gauze. This removed the larger copepods and the heterotrophic dinoflagellate *Noctiluca scintillans.*

Data were analyzed by a linear regression of nutrient concentration versus time. The slope of the regression line and the chlorophyll-a concentrations were used to calculate nutrient uptake rates

($nmol.h^{-1}.\mu g^{-1}$ Chl-a). Non-linear nutrient uptake was observed, but generally it could be modelled in a linear way, thereby underestimating the true, initial, uptake rate. Uptake rates were compared with published data. Values above V_{NH4}^+ and $V_{NO3}^- = 30$, $V_{Si} = 15$ and $V_{PO4}^{3-} = 3$ $nmol.h^{-1}.\mu g^{-1}$ Chl-a were assumed to indicate nutrient stress (Riegman et al., 1990). Lower rates may not be interpreted as indicating a nutrient surplus.

3.7 GLN/GLU ratios

A 1-5 l sample was concentrated through GF/C filters and frozen immediately at -18°C. Amino acids were extracted and analyzed as described by Rijstenbil (1989). The concentration of glutamine (nmol/l) was dirided by the concentration of glutamate (nmol/l).

4 The concept of limiting factors

The wax and wane of any biological population is controlled by the population growth and loss rates (Reynolds, 1984):

$$dN/dt = N(\mu-\delta) \tag{1}$$

where:
N = population number
μ = specific growth rate of population
δ = specific loss rate of population

The specific population growth rate depends on light (e.g. the Monod model) or nutrients (the Monod or Droop models). Indirectly the specific growth rate can be dependent on the nutrient uptake rate (Zevenboom, 1986; Kilham & Hecky, 1988). The maximum cell number that can be reached in a certain situation depends on the amount of the least available factor and the minimum amount of that factor a cell requires. So, a factor limits phytoplankton growth when its availability controls the growth rate (μN) and/or size of the algal population (N).

To formulate effective sanitation measures it must be known which nutrient(s) need(s) to be reduced in order to abate eutrophication. It is therefore necessary to know which factor(s) currently limit(s) phyto-plankton development, because it is more efficient to reduce a nutrient which is already limiting phytoplankton biomass. In the case of eutrophication there are two factors directly controllable by sanitation measures: the loads of phosphorus and of nitrogen. Other factors (Si, light), however, do influence phytoplankton and must also be considered for a good judgment of the situation. We used a restricted number of methods to indicate the type and degree of nutrient limitation. One group of methods is based on relative rates (of photosynthesis or nutrient uptake). Another group is directly or indirectly based on the ratio of nutrient concentrations.

4.1 Light limitation

The underwater light climate for phytoplankton depends on surface irradiance, total and mixing depth and light attenuation coefficient (k_d). The daily surface irradiance varies with time of year and latitude. The magnitude of k_d depends on the amount of suspended matter, particulate organic matter, chlorophyll-a and yellow substance (Jerlov, 1976; DiToro, 1978; Stronkhorst, 1988). The highest concentrations of these substances occur in coastal areas. As a consequence the k_d decreases going from coastal to offshore waters. In winter and spring k_d is especially high (up to 3 m^{-1}) near the Dutch coast because winter storms resuspend sediments and there are large river discharges with large amounts of suspended matter and yellow substance. Finally, the phytoplankton spring bloom can considerably contribute to k_d in transparent waters (Colijn, 1982).

For a comparison of irradiance as a limiting factor in space and time it is practical to use the column-averaged irradiance:

$$I_{av} = I_0 (1 - \exp\text{-}(k_d z_m))/k_d z_m \tag{2}$$

where:
I_0 = average daily surface irradiance $[W.m^{-2}.d^{-1}]$
k_d = light attenuation coefficient $[m^{-1}]$
z_m = depth of mixing in the water column $[m]$

Equation (2) implies that the average irradiance in the surface mixed layer (SML) roughly doubles when the water column of the central North Sea stratifies (assuming a thermocline about halfway down the water column). Accordingly, the bottom mixed layer (BML) gets a very low average irradiance. In this study estimates of I_{av} are presented for the whole water column in mixed situations and for the surface mixed layer (SML) of stratified waters. It is assumed that both in well-mixed and in stratified areas the photosynthetic characteristics of the phytoplankton are the result of the local light climate expressed as I_{av}.
We use the characteristic (I_k) of the P/I curve (Eilers & Peeters, 1988) to indicate the degree of light limitation and the daily average irradiance in the water column (I_{av}) to calculate the rate of photosynthesis. A simplified P/I curve yields a theoretical maximum rate of photosynthesis:

$$P/P_{max} = I_{av}/(I_k + I_{av}) \tag{3}$$

It is assumed that the I_k of the curve in which photoinhibition is included does differ greatly from the I_k of a curve without inhibition.

4.2 Nutrient limitation

We used several indicators of nutrient limitation, such as nutrient concentrations and ratios, nutrient enrichment bioassays and nutrient uptake kinetics.

4.2.1 Ratios of dissolved inorganic nutrients
Redfield et al. (1963) introduced a ratio by which phytoplankton assimilate nutrients (C:N:P = 106:16:1). Deviations from this ratio are widely used as indicators of potential nutrient limitation. It is assumed that phytoplankton are limited by a nutrient whose supply or concentration is proportionally less than the other nutrients. These ratios can therefore only indicate potential nutrient limitation, as other theory predicts that the absolute concentration and the species involved are important factors for limitation of growth or other processes as well (Zevenboom, 1986; Kilham & Hecky, 1988). It is important to realize that individual species do differ considerably in their nutrient requirements (Tilman, 1977; Kilham & Kilham, 1984). This implies that in a certain sample species with very different nutrient requirements can be limited by different nutrients (Tilman, 1977; Riegman et al., 1990; this report). In general, for phytoplankton communities, with mixtures of species whose nutrient requirements differ, a Redfield ratio around 16 for DIN/DIP may roughly indicate the kind of nutrient limitation, with DIN/DIP > 16 indicating P limitation and < 16 indicating N limitation.

4.2.2. V/V_{max}

To be able to interpret ratios of dissolved inorganic nutrients the concentration of the potential limiting nutrient itself must concomitantly lead to nutrient stress. In other words, when an N/P ratio suggests a potential P limitation, but P concentration is so high that the phytoplankton could multiply several times, there is no nutrient limitation. In this report we scale nutrient concentrations with published K_{su} values for nutrient uptake as a measure of nutrient stress, according to Zevenboom (1986):

$$V/V_{max} = S/(S + K_{su}) \tag{4}$$

where:

$V, V_{max} =$ rate of nutrient uptake, rate of maximum nutrient uptake
$S \quad\quad =$ concentrations of dissolved nutrients
$K_{su} \quad =$ half saturation constant for nutrient uptake

In a similar way K_{su} values for nutrient uptake were used to interpret bioassay results (Peeters & Peperzak, 1990).

4.2.3 Ratios of particulate nutrients

When phytoplankton cultures are manipulated to obtain extreme limitation of a certain nutrient, their internal concentration of the limiting nutrient will also become low relative to the other nutrients (Hecky & Kilham, 1988). So, ratios of particulate nutrients (in living cells) may indicate actual nutrient limitation. Healy & Hendzel (1980) used ratios of particulate material to get indications of nutrient stress in freshwater lakes.

4.2.4 Nutrient enrichment bioassays

A nutrient enrichment bioassay is a test to find which nutrient is potentially limiting for natural phytoplankton or a one-species culture of phytoplankton. Bioassays on natural phytoplankton often indicate which species will profit most from enrichment. Mono-specific bioassays allow the limitation of a particular species to be investigated (Maestrini et al., 1984; Peeters & Peperzak, 1990). It has been found that two species can be potentially limited by different nutrients in the same sample if they have different nutrient requirements (Peperzak & Peeters, 1991).

4.2.5 Kinetics of nutrient uptake

In this technique the rate of nutrient uptake is measured. Phytoplankton cells that are nutrient deficient are more able to take up nutrients. Phytoplankton cells that are not nutrient limited show a low constant rate of nutrient uptake after nutrients have been supplied. This uptake rate is proportional to their growth rate. Phytoplankton under nutrient stress develop an enhanced capacity for nutrient uptake; their uptake rate is initially high and declines with time (Zevenboom, 1986). The technique of nutrient uptake kinetics is one of the few that indicates actual physiological adaptations to nutrient stress in situ. A practical drawback for most marine environments is the need to concentrate samples before applying the test. This may distort the sample, e.g. by damaging delicate organisms, allowing small species to escape and

concentrating the zooplankton. The latter may graze the phytoplankton, thereby disturbing the measurement by taking up and excreting of nutrients.

4.2.6 GLN/GLU ratios

The molar ratio of intracellular glutamine/glutamate (GLN/GLU) can be regarded as an indicator of nitrogen limitation in phytoplankton (Flynn, 1990). GLN/GLU >0.5 indicates no N limitation, GLN/GLU <0.3: N deprivation, <0.1: prolonged N deprivation (Flynn et al., 1989).

4.3 Shift in species composition

The species composition of the phytoplankton in the sea at any given moment is the result of growth and loss processes. Both processes can be influenced by abiotic and biotic factors, the important ones being underwater light climate, nutrient concentrations and a species ability to compete for nutrients and light. Short-term changes in abiotic variables allow several phytoplankton to co-exist (Sommer, 1985). Long-term seasonal changes, for instance in water temperature and light availability, induce transitions in the phytoplankton community as a whole. Long-term stress may unbalance the system and may lead to an increase in the biomass of opportunistic species. Increased amounts of P and N relative to Si, for instance, lead to a shift from diatom dominance to a phytoplankton community dominated by flagellates, as observed e.g. in the Marsdiep coastal zone (Cadée & Hegeman, 1986) and the North Sea (Radach & Berg, 1986). A recent hypothesis also links changes in NH_4/NO_3 and DIN/DIP ratios to an increase in blooms of colonial Phaeocystis (Riegman et al., 1992). The intricate system of constantly changing biotic and abiotic variables which determine species composition is poorly understood. Therefore, in this report only chlorophyll-a will be used as a measure of total phytoplankton biomass. Furthermore, only the effect of the abiotic variables light and nutrients on the phytoplankton standing stock will be dealt with.
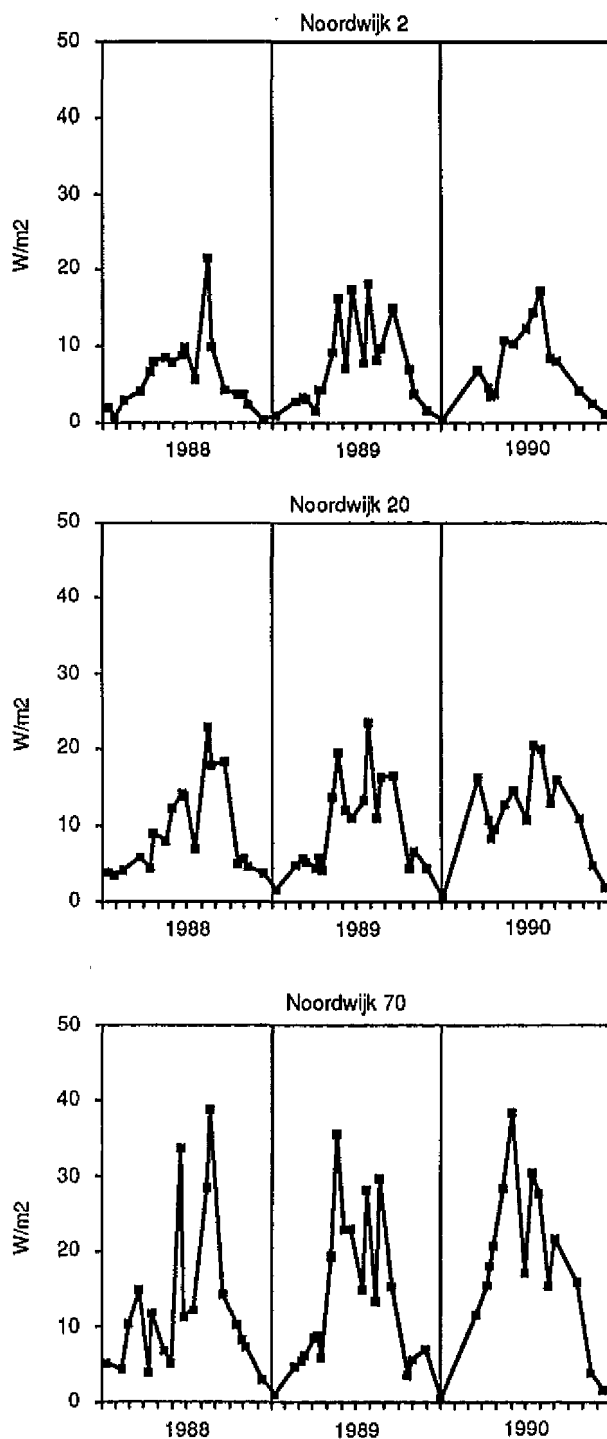
5 Results

5.1 Light climate

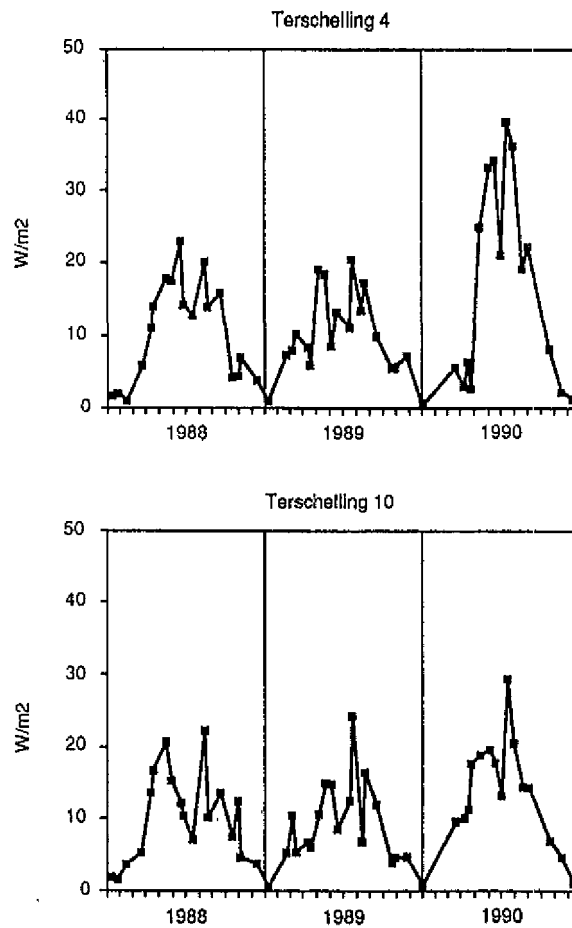The seasonal courses of the average column irradiance (I_{av}) are presented in Figs. 3, 4 and 5.

Figure 3
The average daily irradiance (I_{av}) of the water column at Noordwijk 2, 20 and 70.

Figure 4

The average daily irradiance (I_{av}) in the water column at Terschelling 4 and 10.

Terschelling 4

Terschelling 10

Figure 5

The average daily irradiance (I_{av}) in the water column and the surface layer during stratification at Terschelling 100 and 175.

Terschelling 100

Terschelling 175

Nutrients and light as factors controlling phytoplankton biomass

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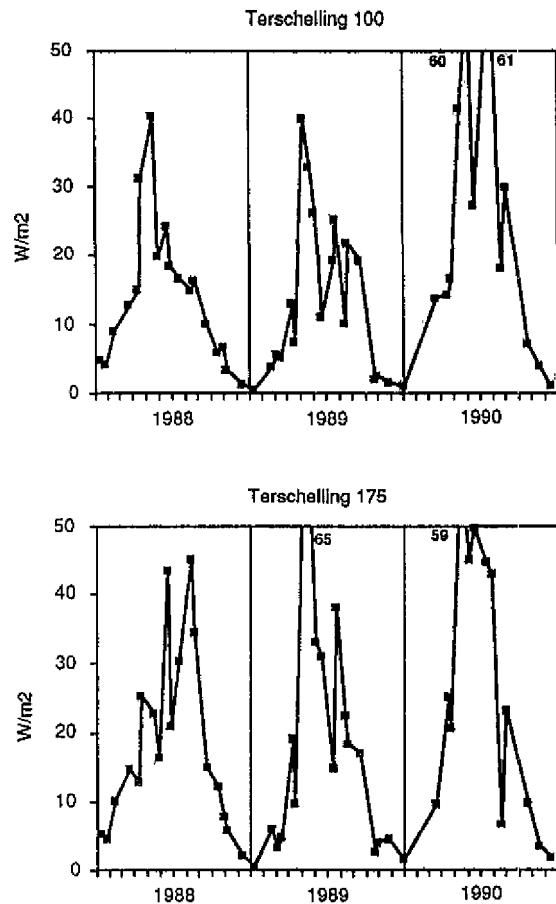
The values for I_{av} increase from coast to offshore. The light attenuation coefficient (not shown, Peeters et al., 1991) has steeper gradients than I_{av}, but its effects are mitigated by deeper water offshore. Occasionally a higher average irradiance is measured at the most inshore station, e.g. Ts4 compared with Ts10 (Fig. 4). When stratification occurs, as at Ts100 and Ts175, the relatively small value of the multiplication of mixing depth and $*k_d$ results in high average irradiance in the surface mixed layer during stratification (Fig. 5).

5.2 Nutrients

The seasonal variation and the concentration of a nutrient differ per nutrient, year and location. At Nw2, where the influence of the Rhine is relatively large, N, P and Si concentrations are high (Fig. 6).
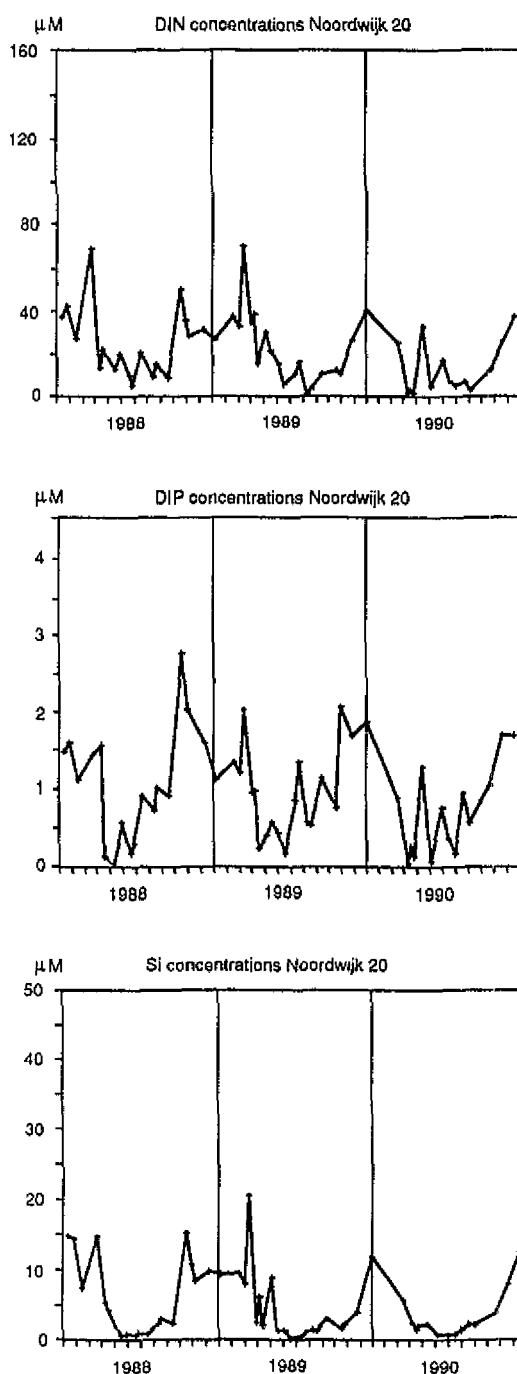
Figure 6
Surface concentrations (μM) of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP) and silicate (Si) at Noordwijk 2.
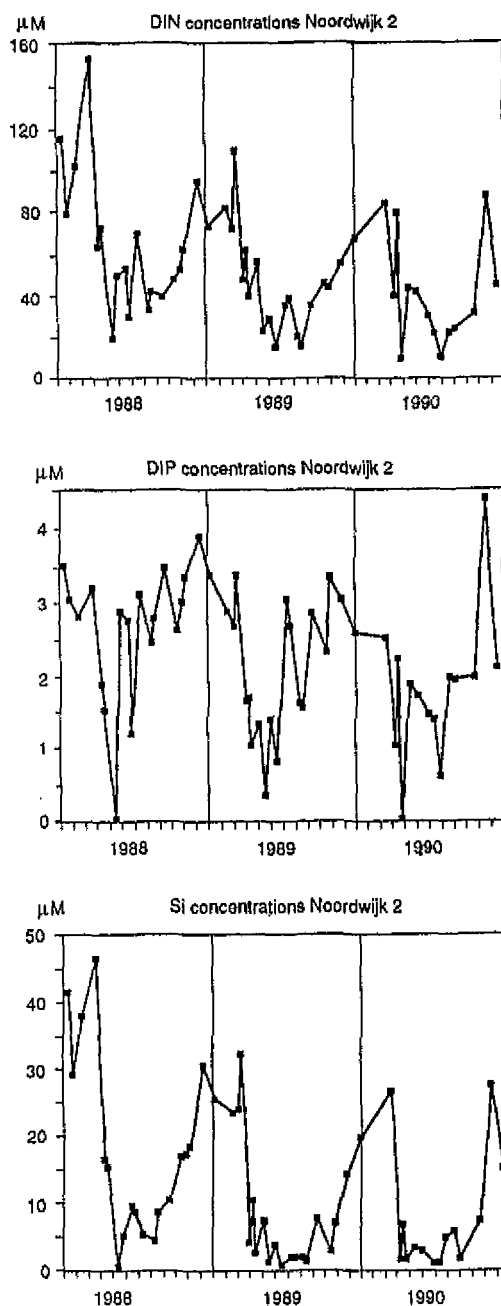
The ratio of winter maximum/summer minimum for DIN is about 4. In 1988 and 1989 the DIP concentrations are about equal in winter and summer, with short troughs caused by the spring bloom. In 1990 the spring and summer troughs lasted longer. By comparison with the DIN and DIP concentrations the Si concentrations show very pronounced differences between summer and winter. This is caused by a combination of a decrease of the Si river discharge in summer and the uptake by diatoms in spring and summer at sea. At Nw20 the average concentrations of all nutrients are smaller by a factor of about 2 (Fig. 7), and the period of lowered DIP concentrations lasted longer than at Nw2. At Nw70 (Fig. 8) the concentrations are much lower than at Nw20.
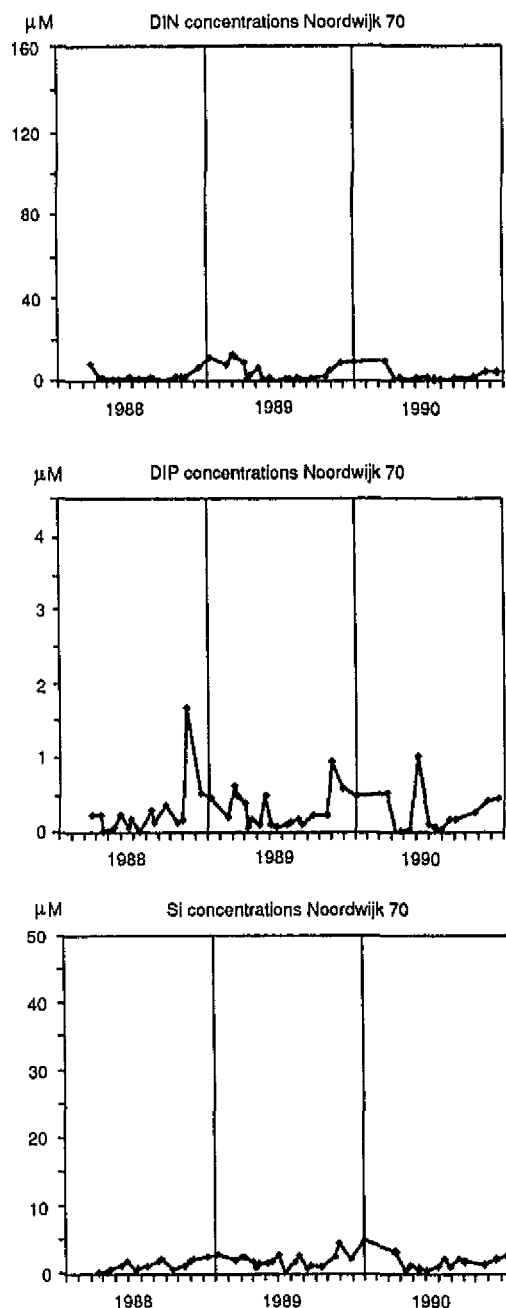
Figure 7
Surface concentrations (µM) of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP) and silicate (Si) at Noordwijk 20.

Figure 8

Surface concentrations (μM) of
dissolved inorganic nitrogen (DIN),
dissolved inorganic phosphate (DIP)
and silicate (Si) at Noordwijk 70.

DIN concentrations at Nw70 are in the order of 10 μM in winter and
1 μM in summer. Compared with Nw20 the reduction of the
concentrations of DIP at this station is less than for DIN. The Si
concentrations are low all year and their relative fluctuations are much
smaller than at Nw2 and Nw20.

The general level and the characteristics of the trends in the nutrient
concentrations at Ts4 and Ts10 (Figs. 9 and 10) are very similar to
those of Nw20 (Fig. 7). The main difference between stations Ts100
and Ts175 (Figs. 11 and 12) and the two coastal stations of that
transect (Ts4, Ts10) is that winter concentrations are lower. For DIN
this is a factor of about 5 (Ts100) to about 12 (Ts175), for DIP it is in
the order of 2.5 for both Ts100 and Ts175 and for dissolved Si it
ranges from 2 to 4 (depending also on the year; 1990 has low winter
concentrations in the coastal zone).

Thus the spatial gradients perpendicular to the coast are steeper for N than P in both transects. This indicates the large relative importance of riverine inputs and biochemical processes and sinks in the nitrogen cycle compared with the phosphorus cycle.
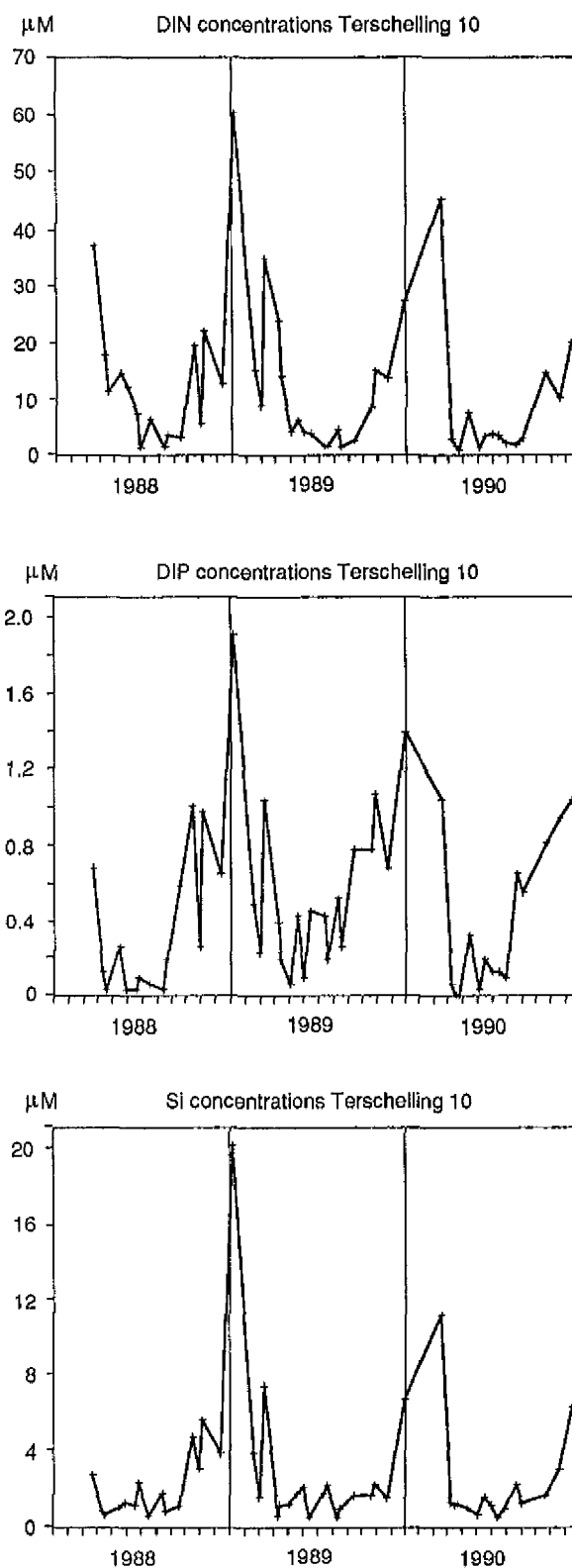
Figure 9

Surface concentrations (μM) of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP) and silicate (Si) at Terschelling 4.
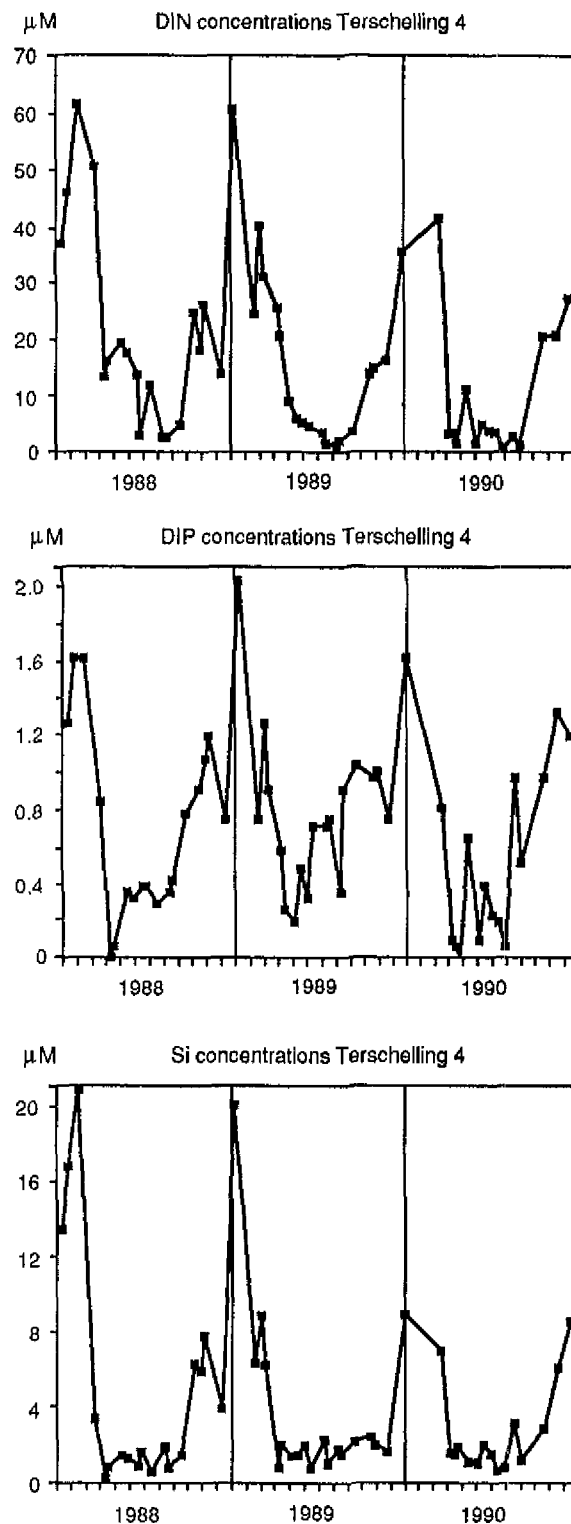
Figure 10

Surface concentrations (μM) of
dissolved inorganic nitrogen (DIN),
dissolved inorganic phosphate (DIP)
and silicate (Si) at Terschelling 10.

DIN concentrations Terschelling 10

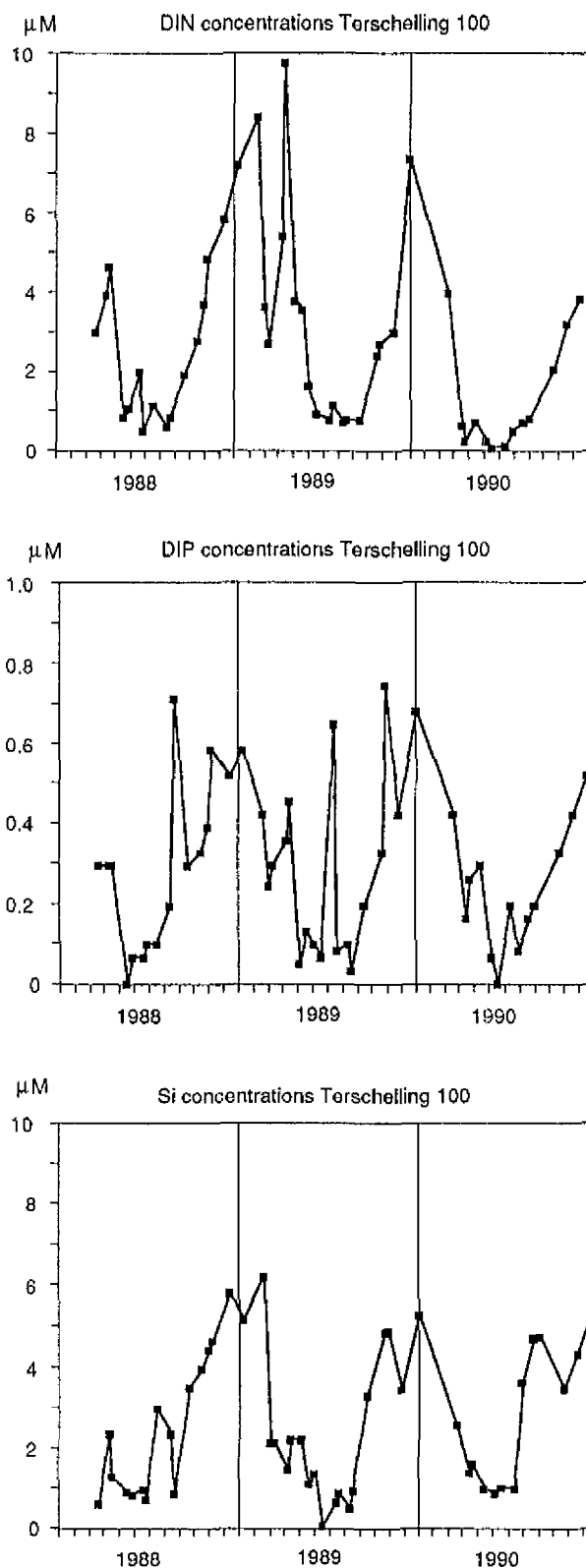DIP concentrations Terschelling 10

Si concentrations Terschelling 10

Figure 11

Surface concentrations (μM) of
dissolved inorganic nitrogen (DIN),
dissolved inorganic phosphate (DIP)
and silicate (Si) at Terschelling 100.

. . . .
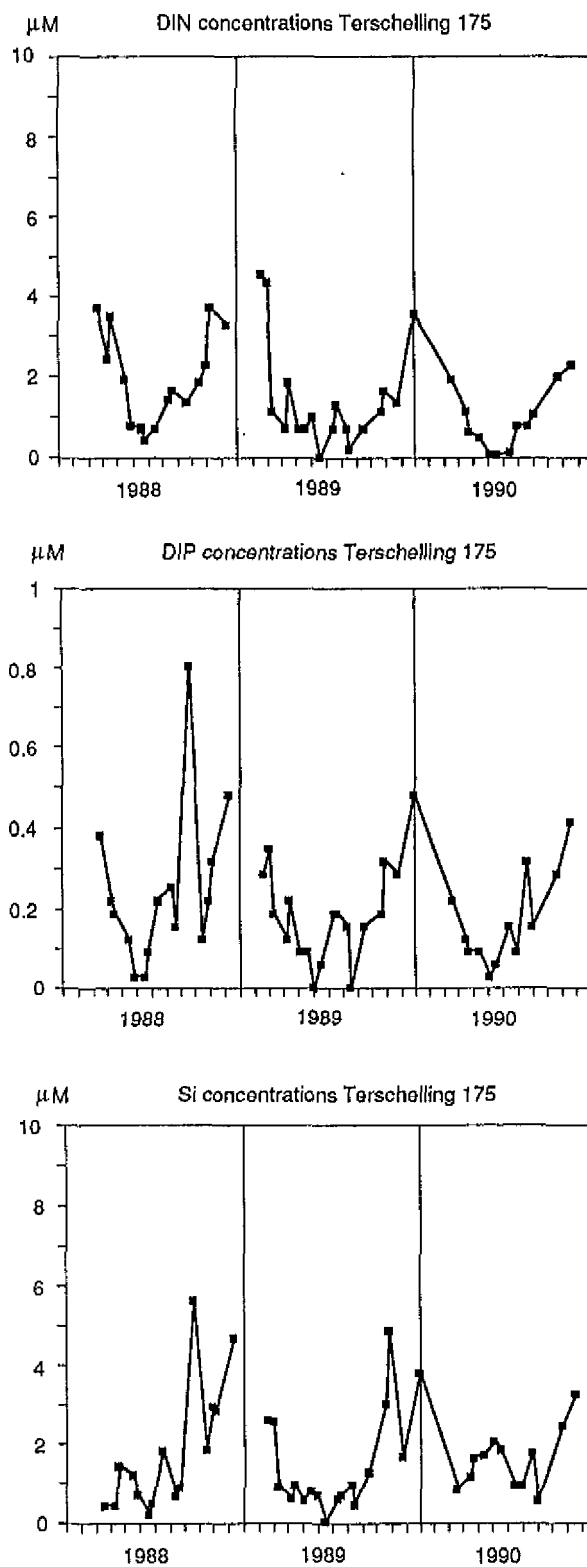
Figure 12

Surface concentrations (μM) of
dissolved inorganic nitrogen (DIN),
dissolved inorganic phosphate (DIP)
and silicate (Si) at Terschelling 175.

Nutrients and light as factors
controlling phytoplankton biomass

5.3 Phytoplankton biomass

The spatial and temporal distributions of chlorophyll-a are shown in Figs. 13, 14 and 15. The general patterns of chlorophyll-a concentrations at the coastal stations of the Noordwijk transect are similar. A spring bloom is usually followed by chlorophyll-a concentrations in summer which are about half as high as the spring peak. The concentrations are roughly the same in Nw2, Nw20 and Ts4. The chlorophyll-a concentration at Nw70 is relatively low. Concentrations are always below 10 μg.l^{-1} and even mostly below 5 μg.l^{-1}. Sometimes summer peaks are of a similar height as spring peaks (Ts 4, 1988; Ts10, 1989; Fig. 14). The concentrations at Ts10, especially those of the spring bloom, are clearly lower than at the other coastal stations. Chlorophyll-a concentrations at Ts100 and Ts175 (Fig. 15) are even lower than those of Nw70; they rarely exceed 5 μg.l^{-1}.
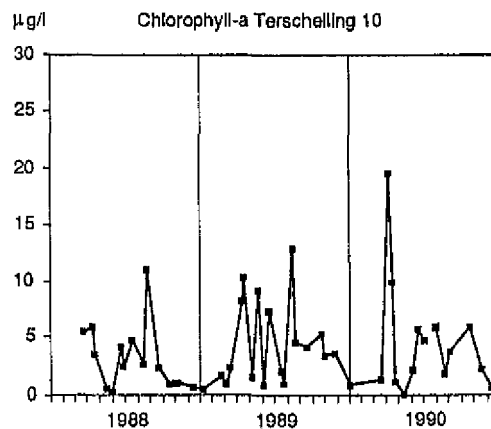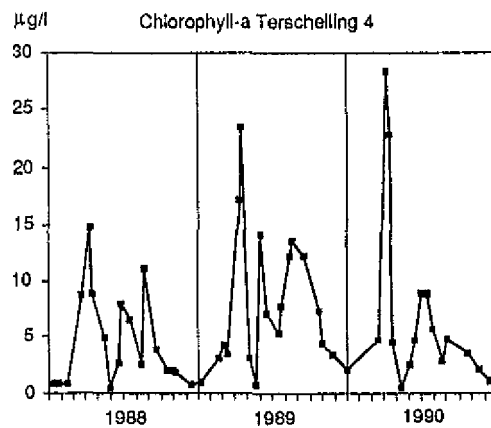
Figure 13

Surface chlorophyll-a concentrations (μg.l^{-1}) at the non-stratified stations Noordwijk 2, 20 en 70.

Chlorophyll-a Noordwijk 2

Chlorophyll-a Noordwijk 20

Chlorophyll-a Noordwijk 70
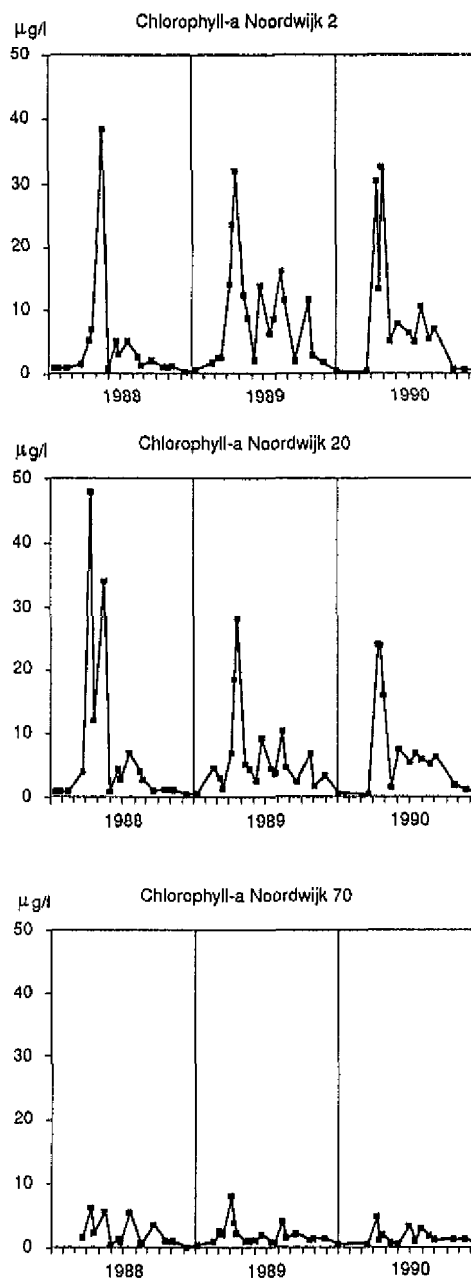
Figure 14

Surface chlorophyll-a concentrations (μg.l⁻¹) at the non-stratified stations Terschelling 4 and 10.

Chlorophyll-a Terschelling 4

Chlorophyll-a Terschelling 10

Figure 15

Surface chlorophyll-a concentrations (μg.l⁻¹) at the stratified stations Terschelling 100 and 175.

Chlorophyll-a Terschelling 100

Chlorophyll-a Terschelling 175

Nutrients and light as factors controlling phytoplankton biomass
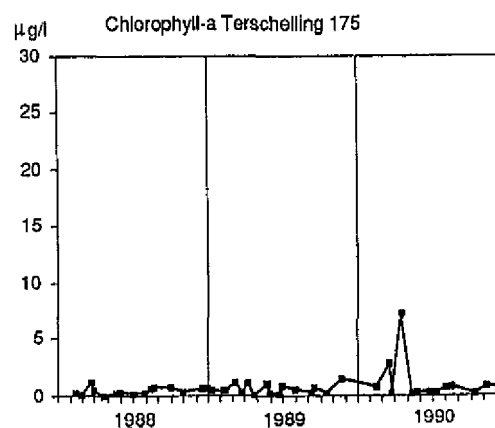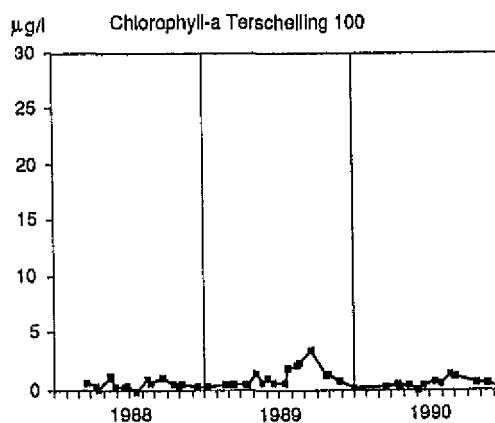
5.4 Identification of limiting factors

5.4.1 Ratios of dissolved inorganic nutrients

At Nw 2 the ratio of DIN/DIP shows a strong seasonal trend; in spring this ratio is larger than the Redfield ratio of 16 for N/P (Fig. 16). In summer this ratio declines to below 16. This indicates a potential N limitation of the summer phytoplankton. The DIN/Si ratio (Fig. 16) is more than 2.3 throughout the year, with very high values in the growing season. The value of 2.3 is the optimum atomic DIN/Si ratio found for *S. costatum* by Peeters & Peperzak (1990); Gillbricht (1988) used an optimum atomic N/Si ratio of 1 for diatoms.

Figure16
Inorganic nutrient ratios; DIN/DIP, Si/DIP and DIN/Si at Noordwijk 2. Lines indicate DIN/DIP = 16, Si/DIP = 7 and DIN/Si = 2.3.
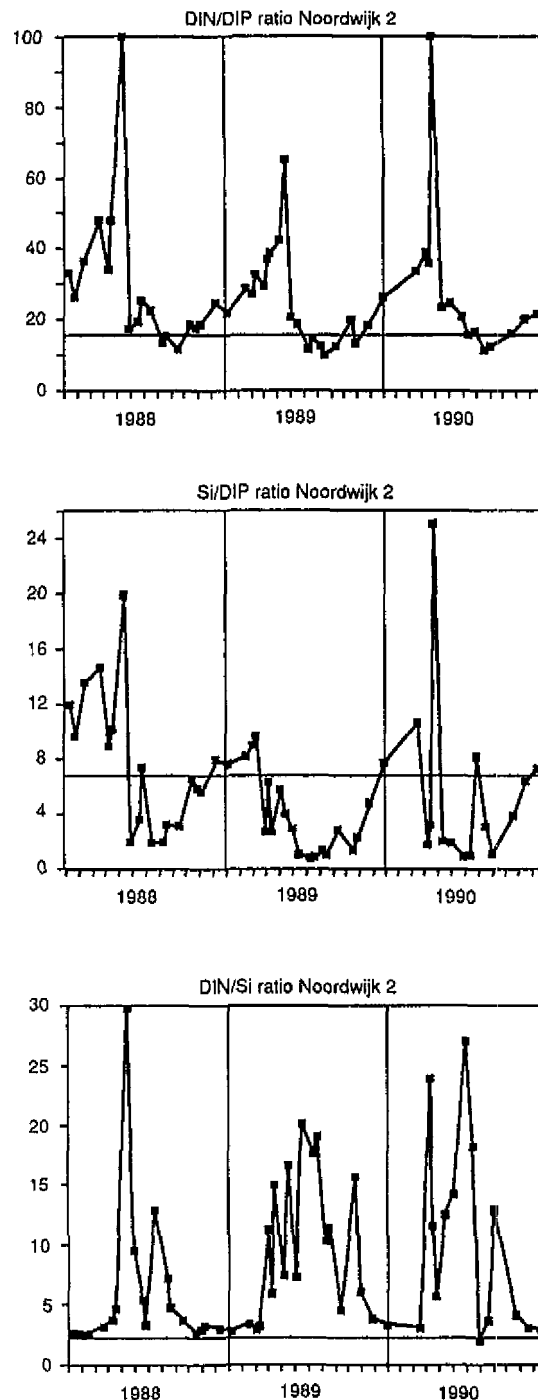
The Si/DIP ratio (Fig. 16) is relatively high in spring and low in summer, when compared to the optimum atomic Si/P ratio of 7 (Peeters & Peperzak, 1990). Given the optimum atomic Si/P ratio of 16, used by Gillbricht (1988), our results show that Si should be potentially limited all over the year.

The pattern of these ratios is very similar at Nw20 (Fig. 17). The periods with DIN/DIP ratios below 16 are somewhat longer and the minimum values are less than 10. The general levels of the Si/DIP ratio and of the DIN/Si ratio are very similar to those at Nw2. The peaks of the latter ratio last for a shorter period. It can be concluded that at Nw2 and Nw20 the potential limitation for diatoms, averaged over the year, increases in the order DIN, DIP and Si.

Figure 17
Inorganic nutrient ratios; DIN/DIP,
Si/DIP and DIN/Si at Noordwijk 20.
Lines indicate DIN/DIP = 16,
Si/DIP = 7 and DIN/Si = 2.3.
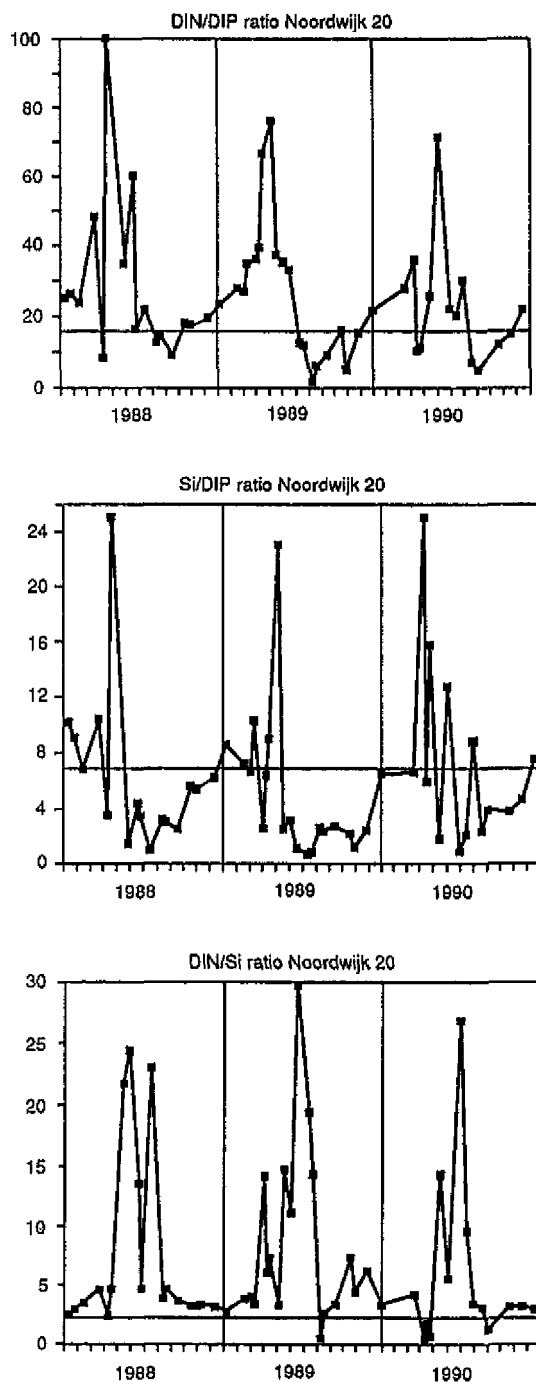
At Nw70 the nutrient ratios do differ considerably from those at Nw2 and Nw20 (Fig. 18). Compared with Nw2 and Nw20, at Nw70 the DIN/DIP ratio exceeds 16 for shorter periods in spring and falls below 16 for longer periods in summer. On average, the Si/DIP ratio at Nw70 is larger and fluctuates more than at Nw2 and Nw20. The DIN/Si ratio at Nw70 is completely different from that at Nw2 and Nw20. Apart from one outlier this ratio rarely exceeds 5 and is even less than 2.5 for long periods. The conclusion is that at Nw70 the potential nutrient limitation increases in the order: DIP, Si and DIN.

Figure 18
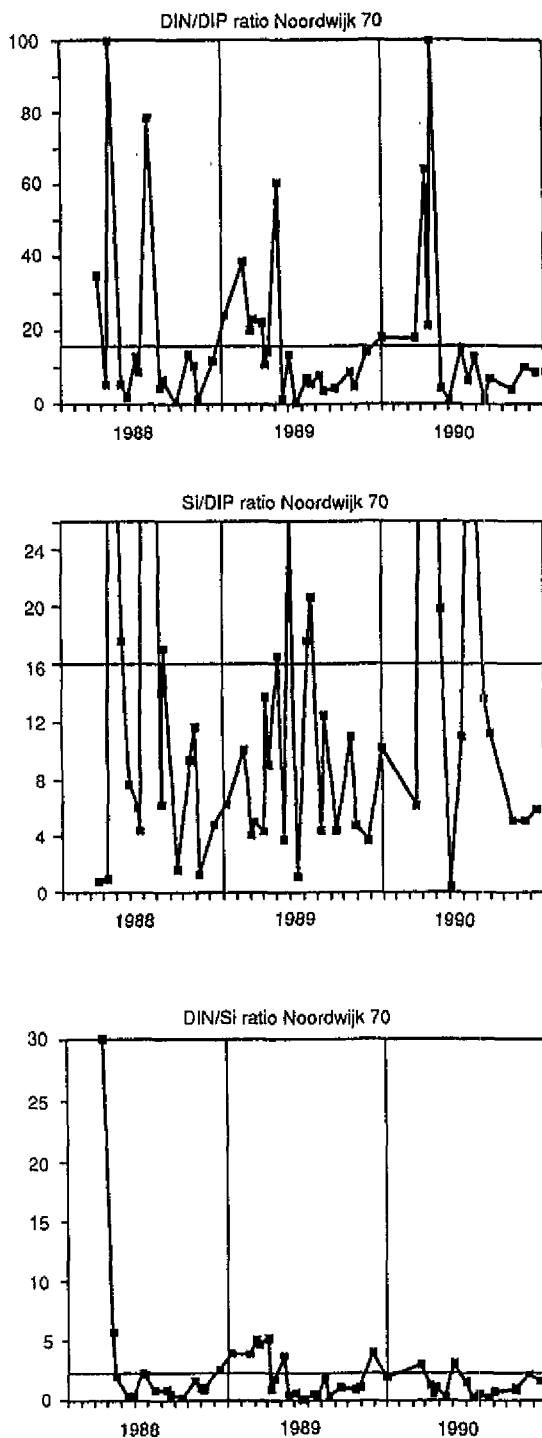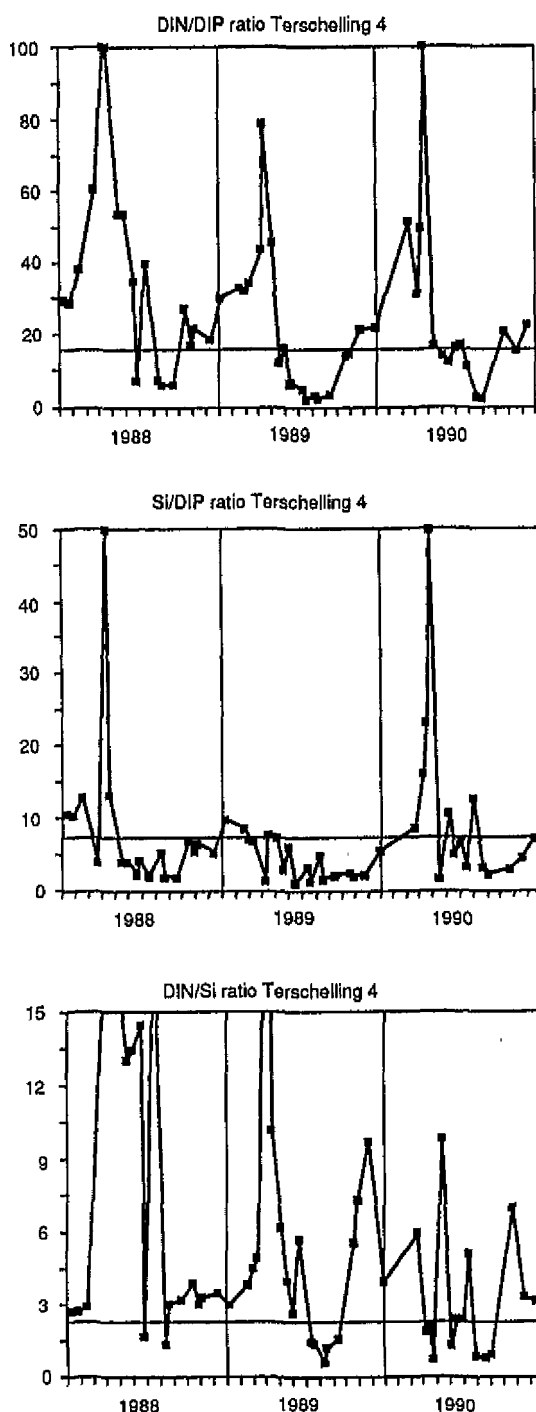Inorganic nutrient ratios; DIN/DIP, Si/DIP and DIN/Si at Noordwijk 70. Lines indicate DIN/DIP = 16, Si/DIP = 7 and DIN/Si = 2.3.

The nutrient ratios at Ts4 and Ts10 (Figs. 19 and 20) are comparable to each other and to Nw20 (Fig. 17). At the coastal Ts stations the minimum values of the DIN/DIP ratio are slightly lower than at Nw20 and the DIN/Si ratio is in general lower (more often less than 2.5). The potential nutrient limitation for diatoms increases in the order DIN, DIP to Si. The ratios of DIN/DIP at Ts 100 and Ts175 are less than 16 almost throughout the year (Figs. 21 and 22). At Ts100 there is a slight tendency for higher values in spring, but not at Ts 175. The ratio of Si/DIP fluctuates around 7 and the ratio of DIN/Si is less than 2.5. The order of increasing potential nutrient limitation for diatoms at these stations is: DIP, Si and DIN. The difference between P and Si is not large and depends on the Si/DIP ratio considered.

Figure 19
Inorganic nutrient ratios; DIN/DIP, Si/DIP and DIN/Si at Terschelling 4. Lines indicate DIN/DIP = 16, Si/DIP = 7 and DIN/Si = 2.3.

Figure 20

Inorganic nutrient ratios; DIN/DIP,
Si/DIP and DIN/Si at Terschelling 10.
Lines indicate DIN/DIP = 16,
Si/DIP = 7 and DIN/Si = 2 3.

DIN/DIP ratio Terschelling 10

Si/DIP ratio Terschelling 10

DIN/Si ratio Terschelling 10
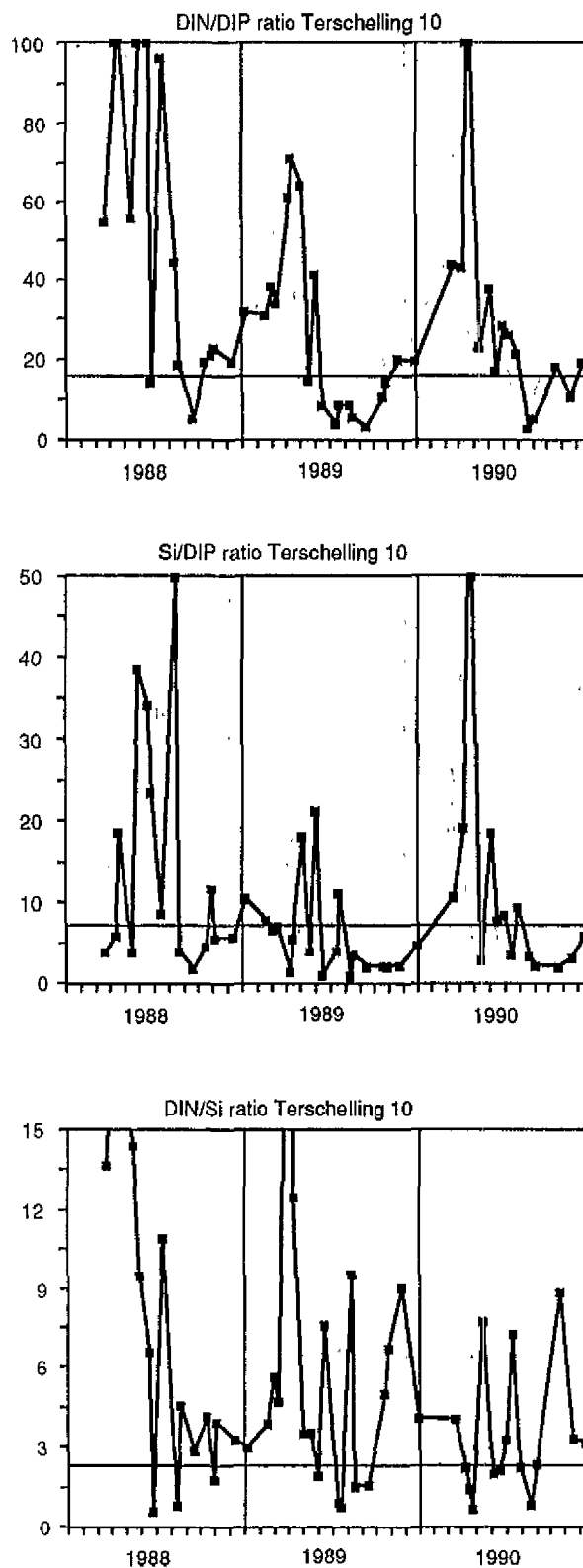
Figure 21

Inorganic nutrient ratios; DIN/DIP,
Si/DIP and DIN/Si in the surface
mixed layer at Terschelling 100.
Lines indicate DIN/DIP = 16,
Si/DIP = 7 and DIN/Si = 2.3.

DIN/DIP ratio Terschelling 100

Si/DIP ratio Terschelling 100
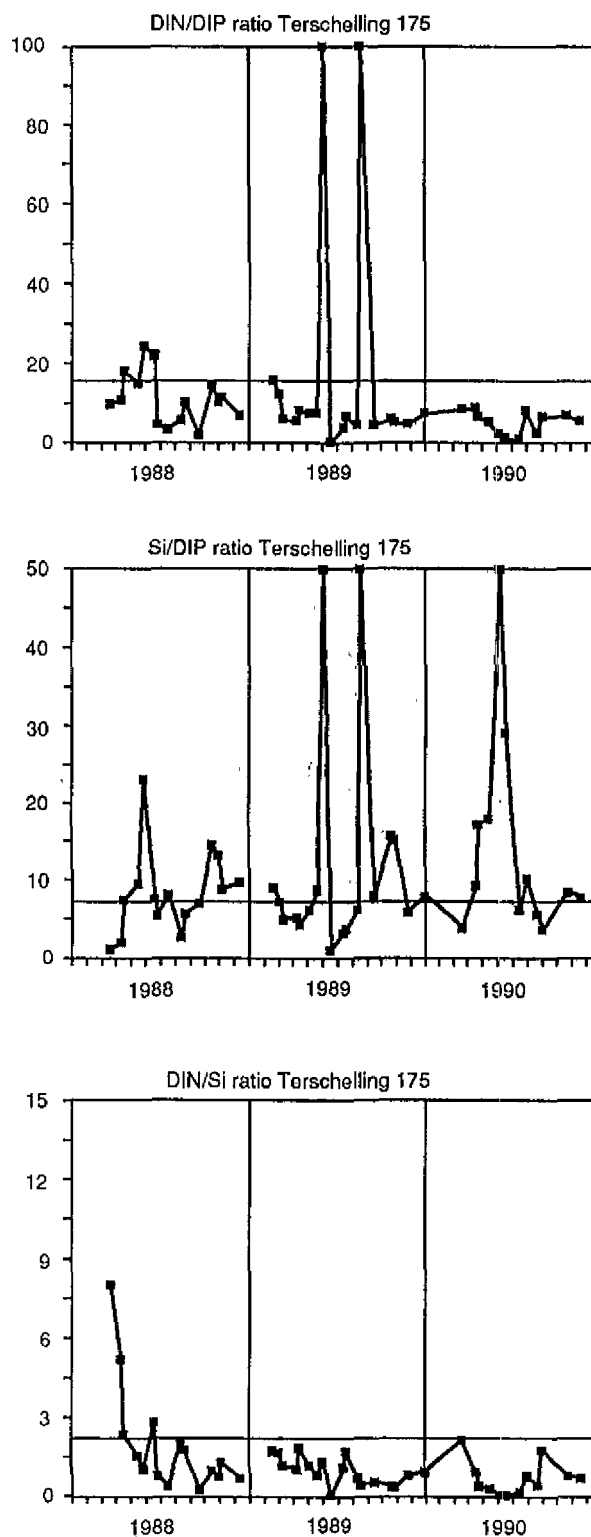
DIN/Si ratio Terschelling 100
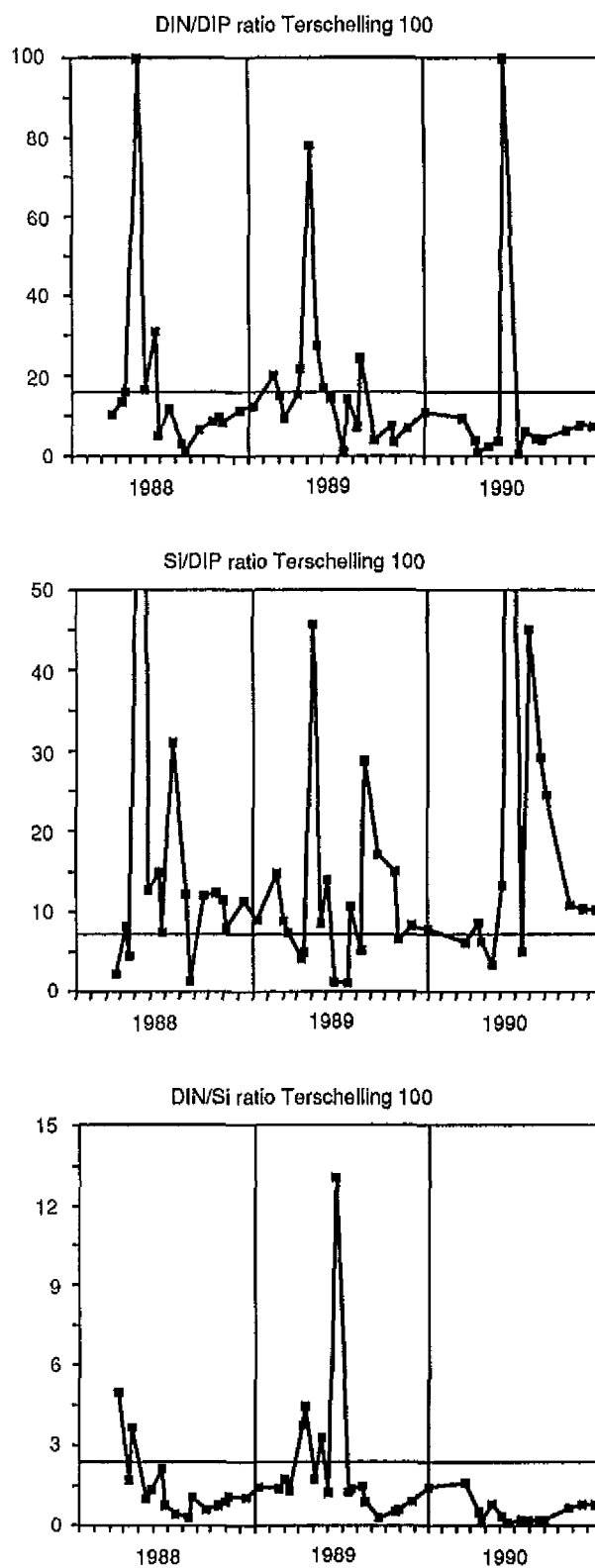
Figure 22

Inorganic nutrient ratios; DIN/DIP,
Si/DIP and DIN/Si in the surface
mixed layer at Terschelling 175
Lines indicate DIN/DIP = 16,
Si/DIP = 7 and DIN/Si = 2.3.

DIN/DIP ratio Terschelling 175

Si/DIP ratio Terschelling 175

DIN/Si ratio Terschelling 175

5.4.2 Ratios of particulate nutrients, V/V_{max} and P/P_{max}

In Figs. 23 to 29 the DIN/DIP, PON/PP ratios and the results of the calculations of relative nutrient uptake rate (V/V_{max}) and relative photosynthetic rate (P/P_{max}) are summarized. The PON/PP ratios (of the total suspended matter) do not co-vary with the DIN/DIP ratios at any of the stations shown and the latter ratios generally exceed 16 and have greater variance In the more oligotrophic regions.

The lower panels of Figs. 23 to 29 show the results of the calculations of V/V_{max} for dissolved Si, PO_4^3 (representing for DIP), $NO_3^- + NO_2^- + NH_4^+$ (representing for DIN) and of P/P_{max}. The bars at the top of the panel indicate periods in which V/V_{max} is less than 0.5 and periods in which P/P_{max} falls in the categories 0-0.1, 0.1-0.5 and >0.5. The size of the peaks (only presented for nutrients) and their duration indicate the severity of the limitation.

At Nw2 (Fig. 23) there is severe irradiance limitation during winter ($P/P_{max} > 0.1$) and during summer there is a moderate limitation. This means that irradiance limits phytoplankton biomass in winter. Although nutrient concentrations are generally very high at this station, there are a few periods of low V/V_{max}, because of low DIP and Si. Peaks in V/V_{max} for DIP and Si correspond with the Si/DIP ratio (see also Fig. 16). Based on the criteria of inorganic nutrient ratios and V/V_{max}, N limitation does not occur at this station. At Nw20 (Fig. 24) the periods of low V/V_{max} for DIP and Si occur more often and last longer than at Nw2. In addition, short periods with low V/V_{max} for DIN occur in 1989 and 1990. The periods of low values of P/P_{max} (<0.1) are shorter than at Nw2 and in spring and summer 1990 periods of $P/P_{max} > 0.5$ do occur. At Nw70 (Fig. 25) periods of low P/P_{max} in winter are very short and P/P_{max} is high during summer. The low concentration of DIN, DIP and Si causes long periods of $V/V_{max} < 0.5$ to occur.

This approach applied to the stations of the Terschelling transect (Figs. 26 to 29) shows that in general the limitation of nutrient uptake increases from Ts4 to Ts175 and that the limitation by DIN increases particularly in the same direction. The values of P/P_{max} at Ts4 and Ts10 are similar to those at Nw20.
At Ts100 P/P_{max} is low for moderately long periods in winter. The high values of P/P_{max} in the summer of 1990 are attributable to the combination of stratification and clear water. At Ts175 stratification results in long periods in summer with high values of P/P_{max}; in winter, periods of low P/P_{max} are shorter than at Ts100. The spatial and temporal gradients and trends in V/V_{max} are consistent with the potential limitations indicated by ratios of inorganic nutrient concentrations. However, there is a trend of an increasing light limitation of the photosynthetic rate from offshore to the coastal areas and a trend in the opposite direction of nutrients limiting phytoplankton biomass.

Figures 23-24

The upper panel shows ratios of DIN/DIP (curve) and of particulate organic N/ particulate P (PON/PP; solid squares) of the surface layer at Noordwijk 2 en 20. Horizontal lines indicate the range of two standard deviations of the PON/PP ratio. In the lower panel V/V_{max} ratios for P,N and Si are depicted. Nutrient limitation is assumed when $V/V_{max} < 0.5$.
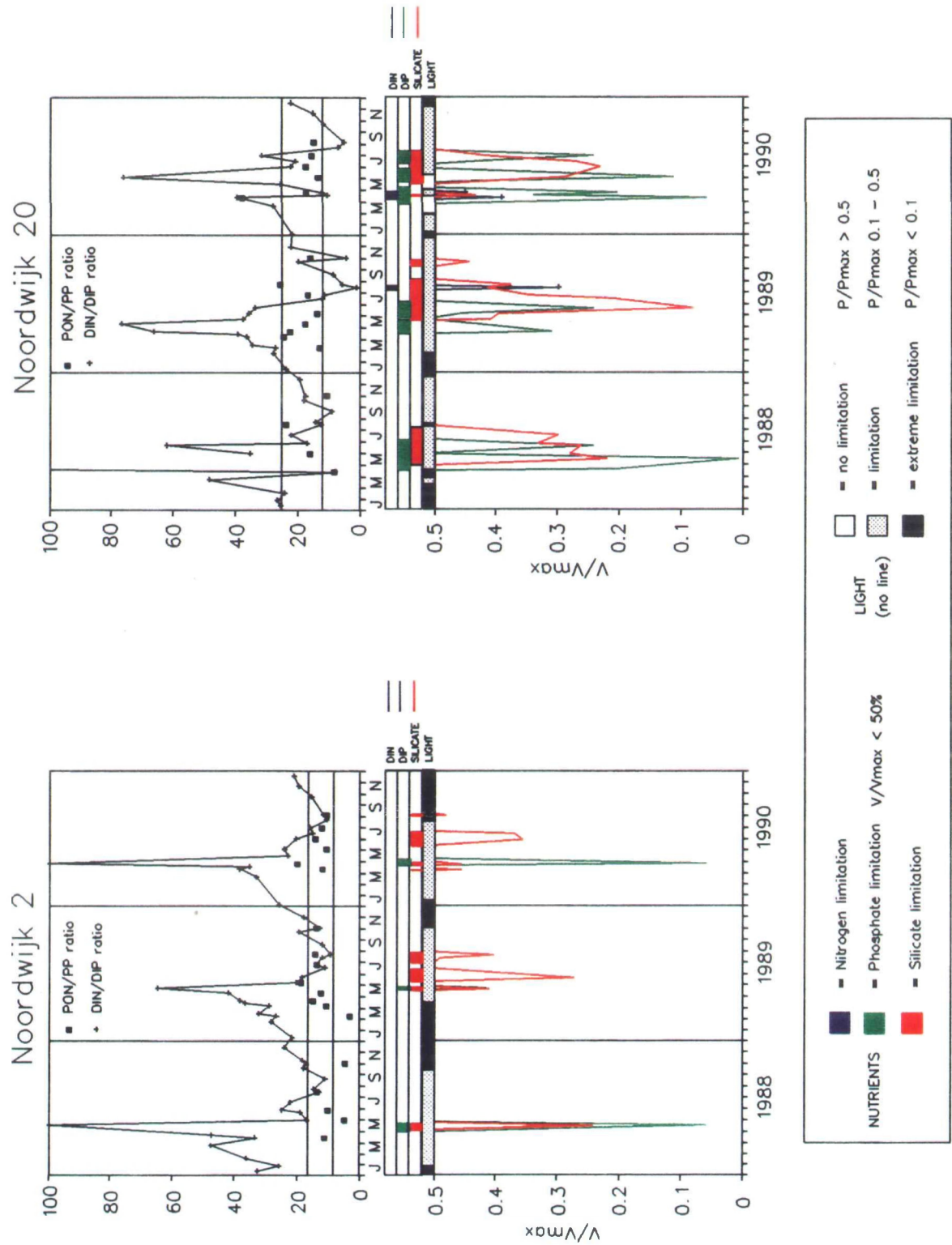
Figure 25

The upper panel shows ratios of DIN/DIP (curve) and of particulate organic N/ particulate P (PON/PP; solid squares) of the surface layer at Noordwijk 70. Horizontal lines indicate the range of two standard deviations of the PON/PP ratio. In the lower panel V/V$_{max}$ ratios for P,N and Si are depicted. Nutrient limitation is assumed when V/V$_{max}$ < 0.5.
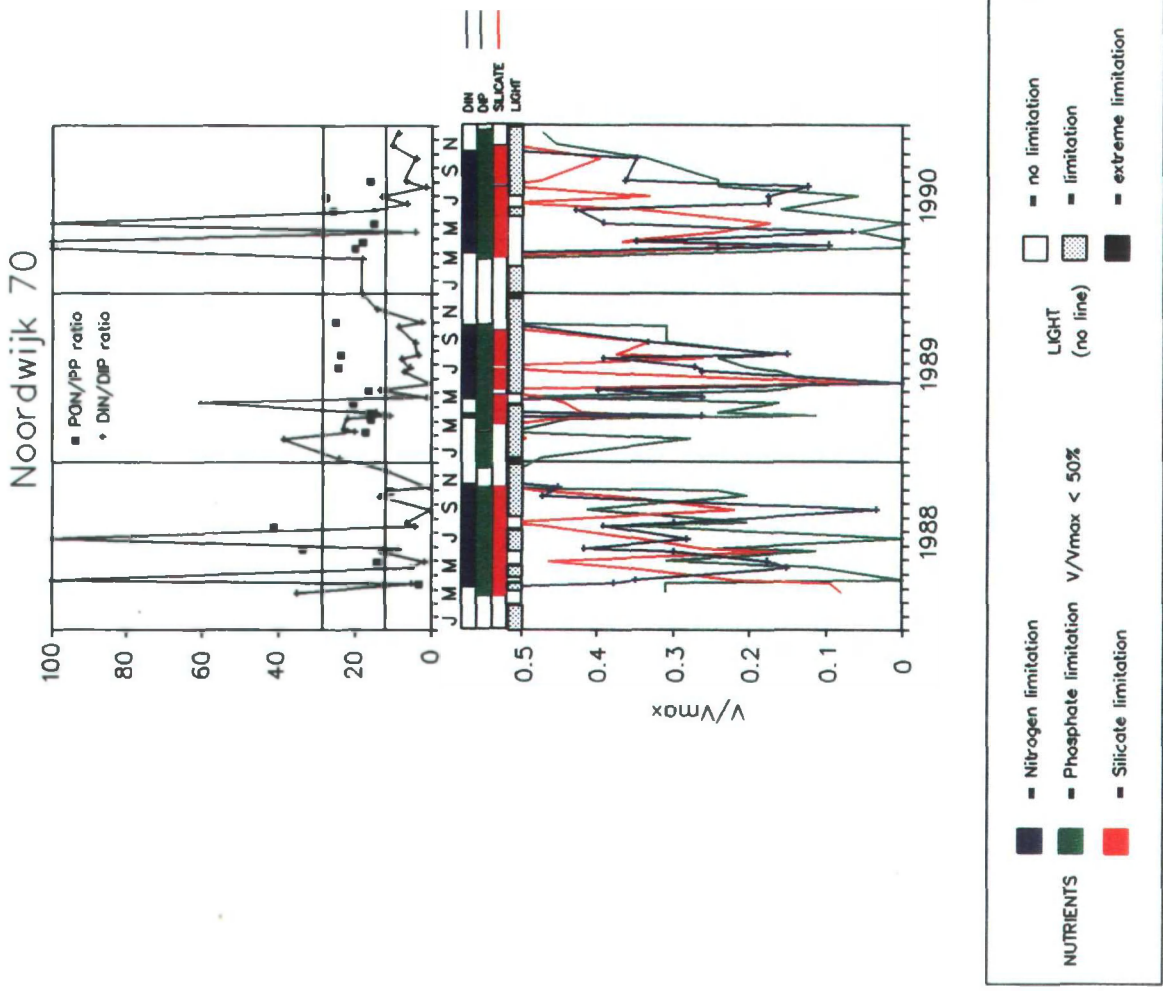
Figures 26-27

The upper panel shows ratios of DIN/DIP (curve) and of particulate organic N/ particulate P (PON/PP; solid squares) of the surface layer at Terschelling 4 en 10. Horizontal lines indicate the range of two standard deviations of the PON/PP ratio. In the lower panel V/V$_{max}$ ratios for P,N and Si are depicted. Nutrient limitation is assumed when V/V$_{max}$ < 0.5.

Figurer 28-29

The upper panel shows ratios of DIN/DIP (curve) and of particulate organic N/ particulate P (PON/PP; solid squares) of the surface layer at Terschelling 100 en 175. Horizontal lines indicate the range of two standard deviations of the PON/PP ratio. In the lower panel V/V_{max} ratios for P,N and Si are depicted. Nutrient limitation is assumed when $V/V_{max} < 0.5$.
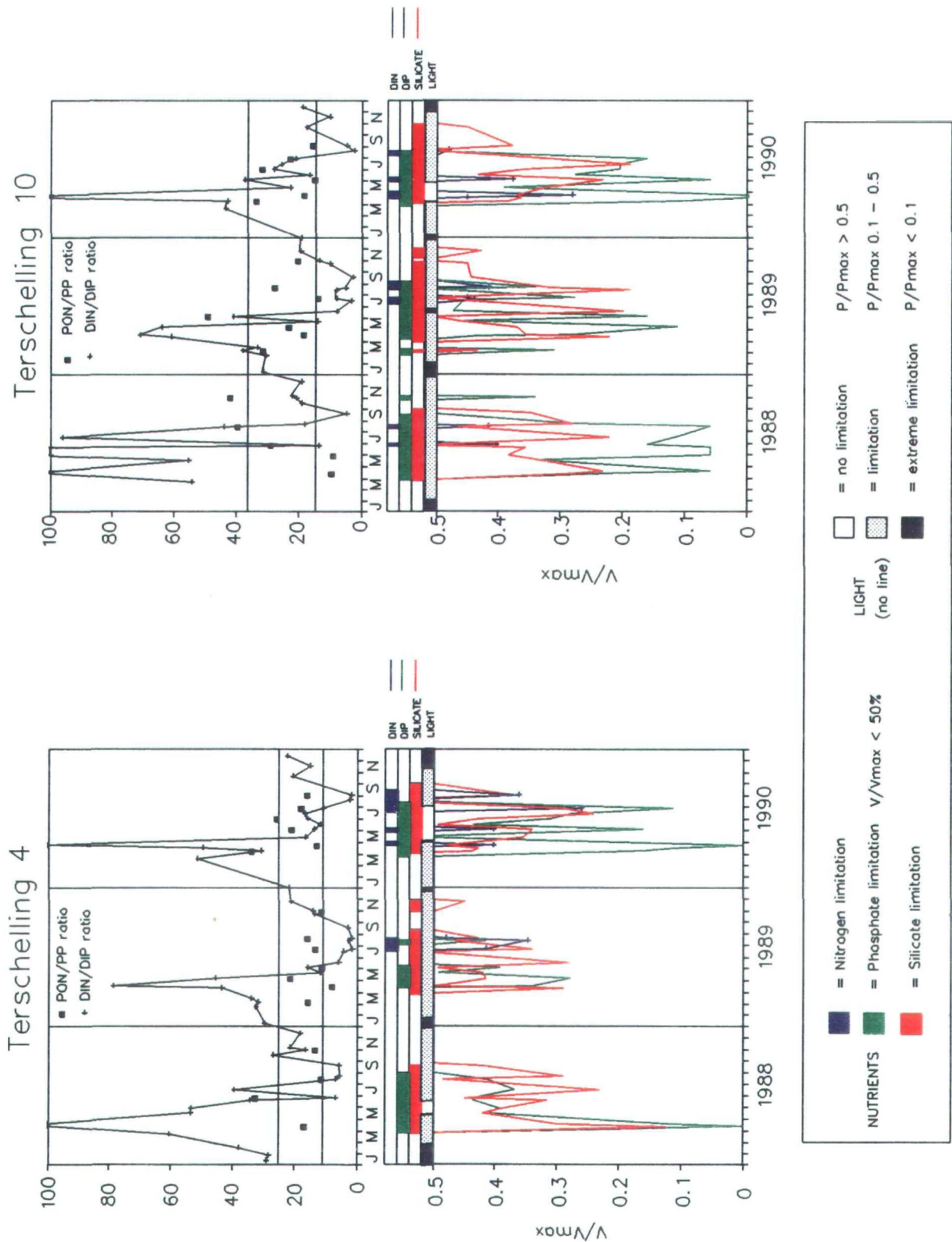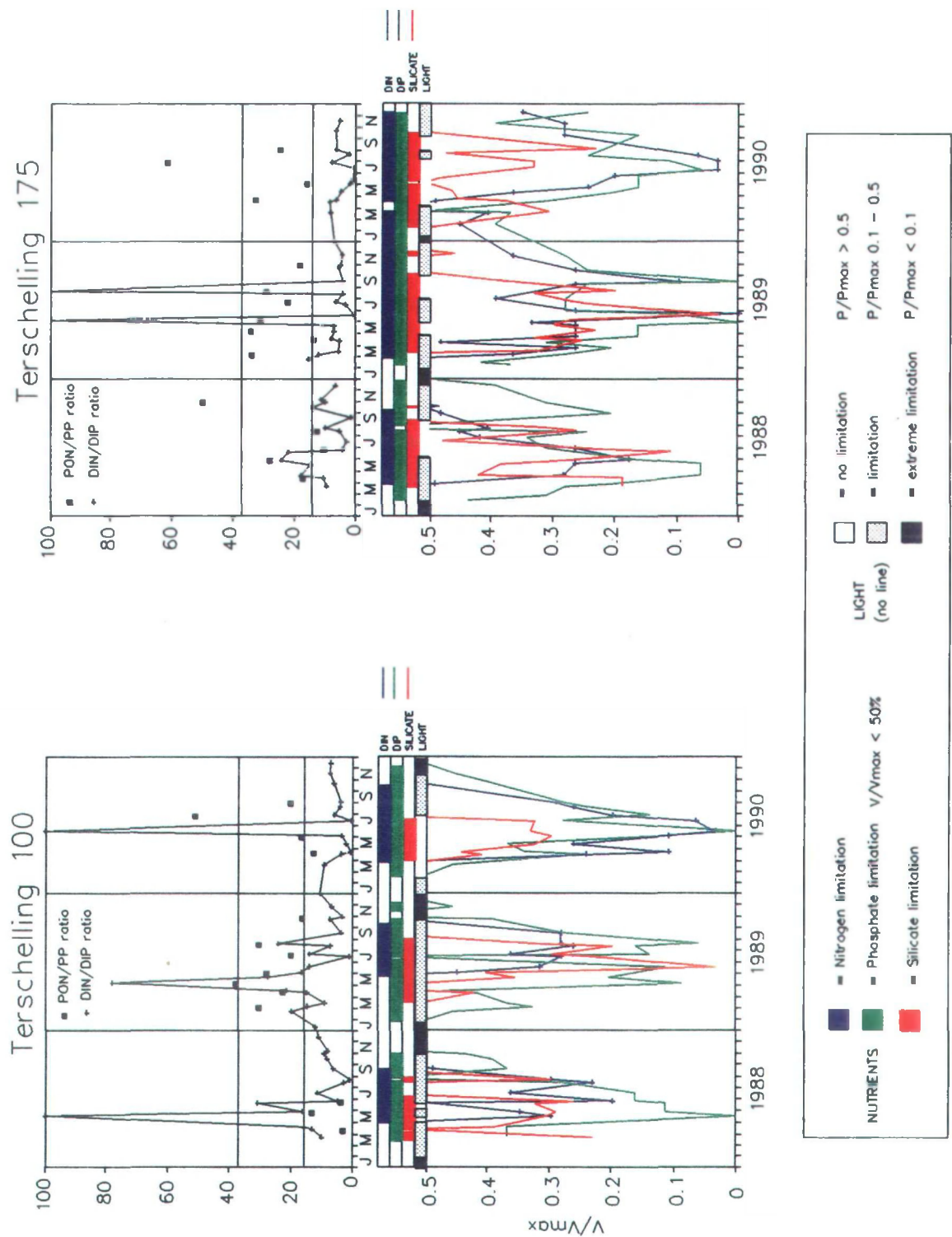
5.4.3 Bioassays

Skeletonema costatum	1988 and 1989 (P:Si:N)/n	Rhodomonas 1989 (P·N)/n
All stations.		
- Spring	(21:35:11)/8	(7:34/5
- Summer	(5:20:23)/5	(9:10)/2
- All year	(26:55:33)/13	(16:44)/7
Coastal stations:		
- N2, N20, T4, T10 Central North Sea N70, N100, Ts100,	(12:33:3)/13	(7:15)/7
- Ts175, Ts275, Ts370	(14:22:30)/13	(9:29)/7

n = number of cruises.

Table 2 shows the frequency of occurrence of the first potentially limiting nutrients as determined from bioassays in 1988 and 1989 in the area of the North Sea studied. There is a clear difference in frequency of potential P and N limitation for *S. costatum* and *Rhodomonas sp. S. costatum* has a tendency to be P limited in situations where *Rhodomonas* is N limited. This is because of the higher N requirement of the latter species (optimum atomic N/P ratio of 25; *S. costatum* has a optimum atomic ratio of 16 for N/P (Peeters & Peperzak, 1990)). In the first half of the year (January to June) P tends to be the first limiting nutrient for *S. costatum* at all stations more often than N. In the second half of the year (July to December) the opposite is true. Potential N limitation does not predominate in the *Rhodomonas* samples from July to December, because for some reason most of the samples from one cruise were P limited. At the coastal stations potential P limitation predominates throughout the year, whereas the stations in the central part of the North Sea are N limited twice as often as they are P limited (*S. costatum*). In both regions *Rhodomonas* is more often N than P limited.

Station	week	first potential (p<0,05)
Ts4	15	none*
Nw10	17	none*
Nw10	23	P
Nw10	27	P
Nw10	31	P
Nw10	36	N

* incubation period too short to measure significant differences between treatments

The results of the coastal phytoplankton multispecies bioassay (Table 3) show a trend similar to that of the DIN/DIP ratio at Nw2, Nw20 and Ts4 (Figs. 23, 24 and 26). In 1990 this ratio reaches its yearly minimum at the end of August, early September (week 36), when the bioassay indicates N as the first potentially limiting nutrient. The results are also consistent with the relatively decreasing role of P as the first potentially limiting nutrient that was found with the *S. costatum* bioassay (Table 3).

5.4.4. Kinetics of nutrient uptake

The results of the experiments in weeks 15, 17, 23, 27, 31 and 36 (1990) are presented in Figs. 30a and 30b. In weeks 15 and 17 chlorophyll-a concentrations were large enough to allow experiments on moderately concentrated samples and presumably zooplankton grazing was negligible. After the spring bloom the concentration factors had to be increased. This resulted in an unfavourable zooplankton to phytoplankton biomass ratio, in some cases leading to a rise in nutrient concentrations rather than a decline. The chlorophyll-a contents of concentrated surface samples from the Oyster Ground were so low that uptake rates could not be measured with the chemical methods employed.

Figure 30a

Results of experiments on kinetics of nutrient uptake in weeks 15, 17, 23, 27, 31 and 36 in 1990 at Noordwijk. (V in nmol/μg Chl-a×h)

n.d. = not determined
< : [Chl-a] too low for accurate determinations (<10µg/l)

NO3   NH4   P   Si
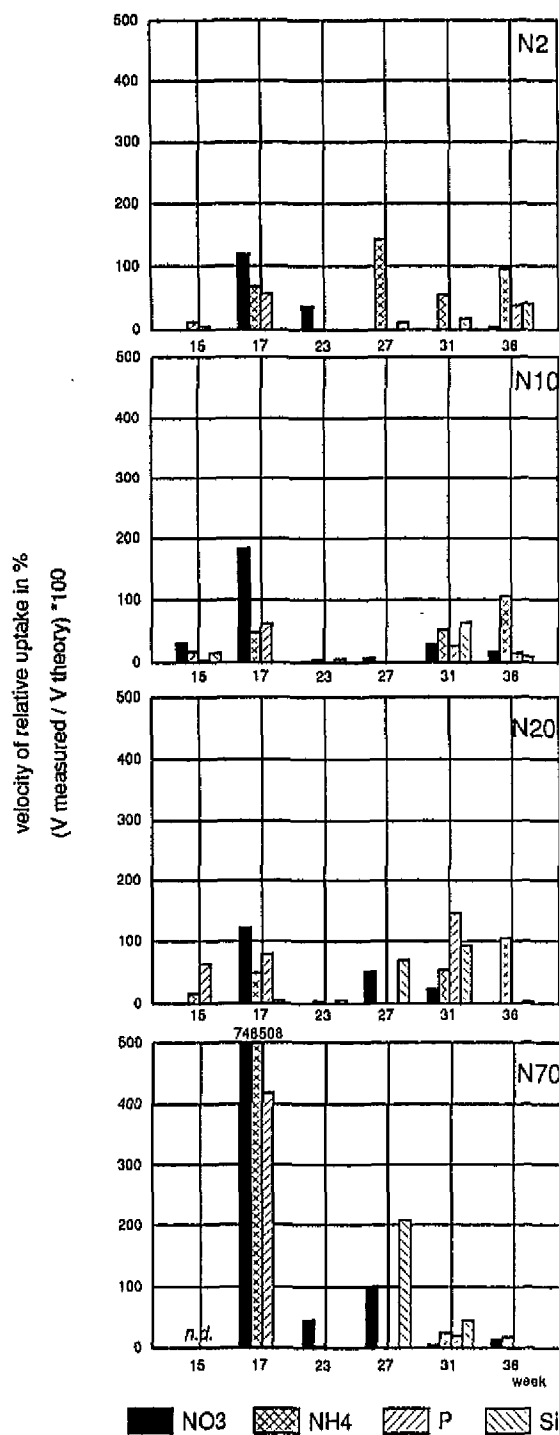
In week 36 very high uptake rates occurred but, surprisingly, the decline in nutrient concentrations was linear (cf. Riegman et al., 1990). In week 15 none of the nutrient species was limiting according to the nutrient uptake kinetics at the stations examined. In week 17 however, nitrate and/or ammonium were limiting at all stations with measurable uptake rates. P limitation was confined to Nw70 and the stations of the Terschelling transect, and there was Si limitation at most stations.

Figure 30b

Results of experiments on kinetics of
nutrient uptake in weeks 15, 17,
23, 27, 31 and 36 in 1990 at
Terschelling.
(V in nmol/μg Chl-a x h)

NO3   NH4   P   SI

n.d. = not determined
< : [Chl-a] too low for accurate determinations (<10μg/l)
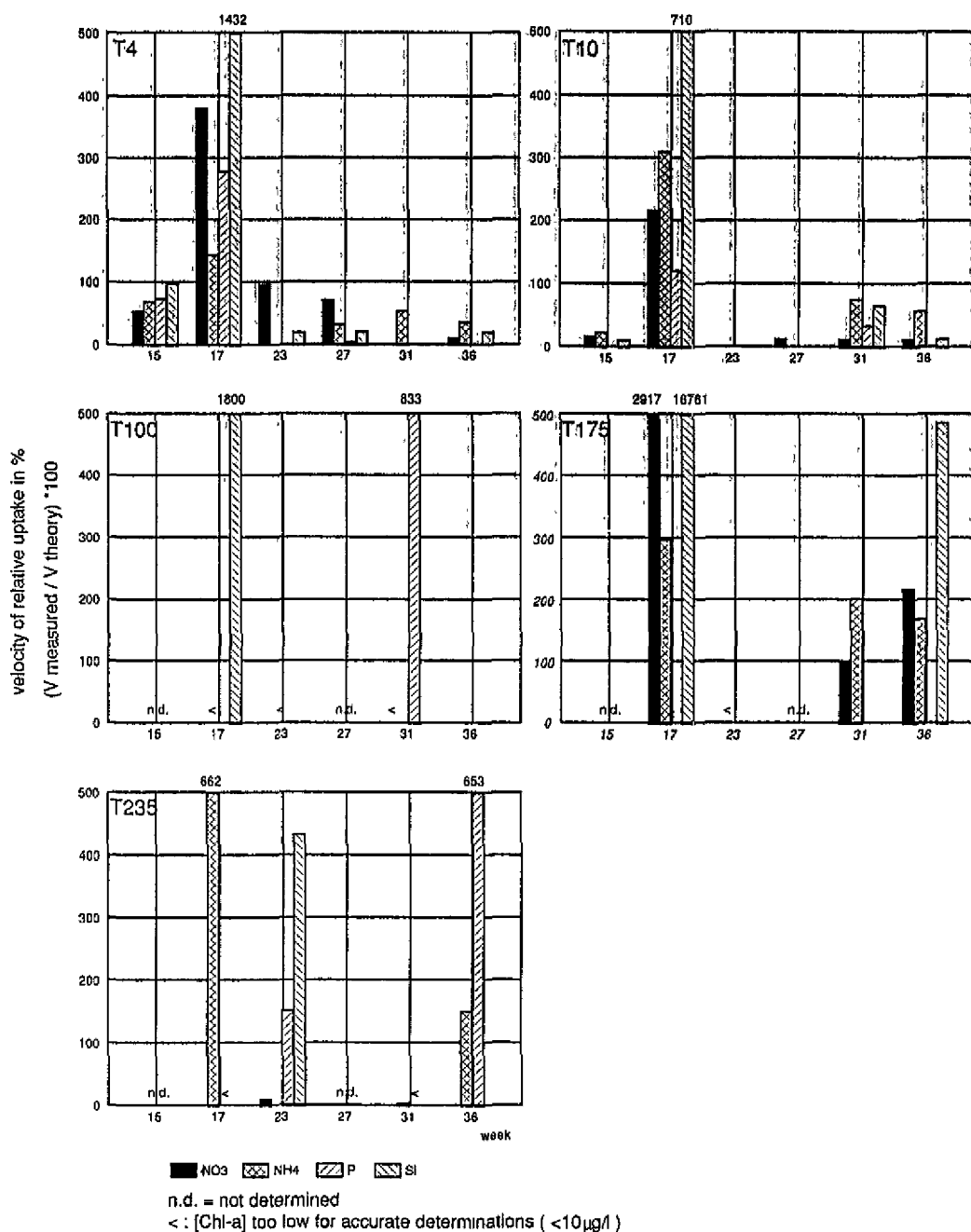
Figure 31a

Contour plot of GLN/GLU ratio as a function of DIN/DIP ratio and DIN concentrations.
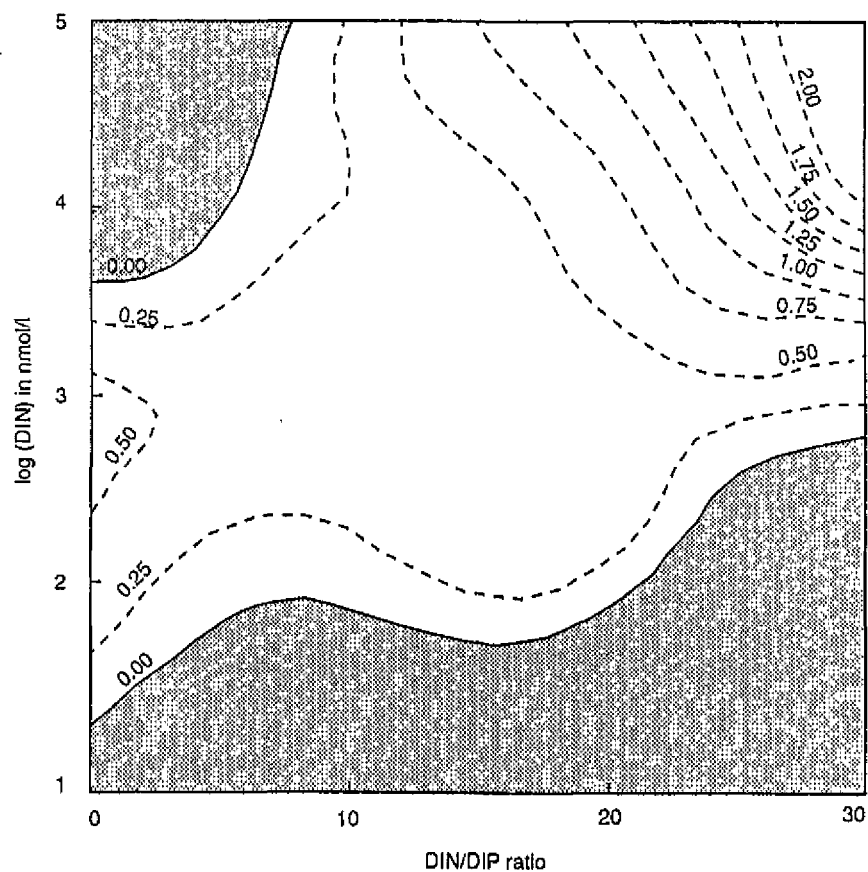
Figure 31b

Contour plot of the model results of GLN/GLU ratios as a function of DIN/DIP ratio and DIN concentrations.
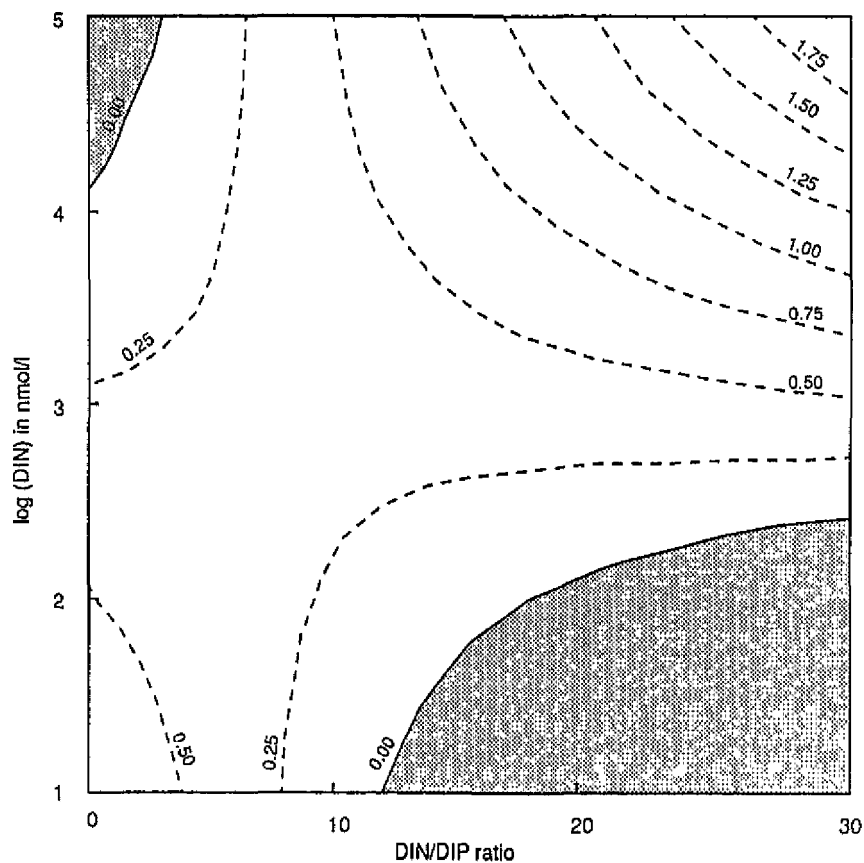
5.4.5 GLN/GLU ratios

Amino acids were determined in samples from cruises made in weeks 23, 27, 31 and 36 (1990). Therefore, there are few sample stations with high nutrient concentrations and high DIN/DIP ratios. Fig. 31a shows the ratio of the amino acids glutamine and glutamate in a contour plot as a function of DIN/DIP ratio and DIN concentration. Shaded areas indicate ratios < 0. We fitted the following empirical model to the data:

$$GLN/GLU = 1.00 - 0.25logDIN - 0.1DIN/DIP + 0.04logDIN \times DIN/DIP$$

$(r^2 = 0.61)$

and used this model to calculate GLN/GLU ratios in the same range of DIN concentrations and DIN:DIP ratios (Fig. 31b). The model has not been validated and shows a flaw at very low DIN/DIP ratios and DIN concentrations, where it unrealistically predicts increasing GLN/GLU ratios. However, we tentatively concluded that no N stress (GLN/GLU ratio ≥ 0.5) is detectable at DIN > 1 μM and DIN/DIP > 10.

5.4.6 Relations between nutrient concentrations and chlorophyll-a

The concentrations of DIN and DIP just before the spring bloom correlate with spring peak concentrations of chlorophyll-a (Fig. 32). In Fig. 32 DIP and DIN scales are presented in the proportion 1:16. The slopes of the regression lines, when transformed in this proportion, are statistically different ($p < 0.003$, $r^2 = 0.805$).

Figure 32

Relationship between February concentrations of DIN and DIP and the peak spring bloom concentrations of chlorophyll-a at Noordwijk 2, 20 and Terschelling 4, 10.
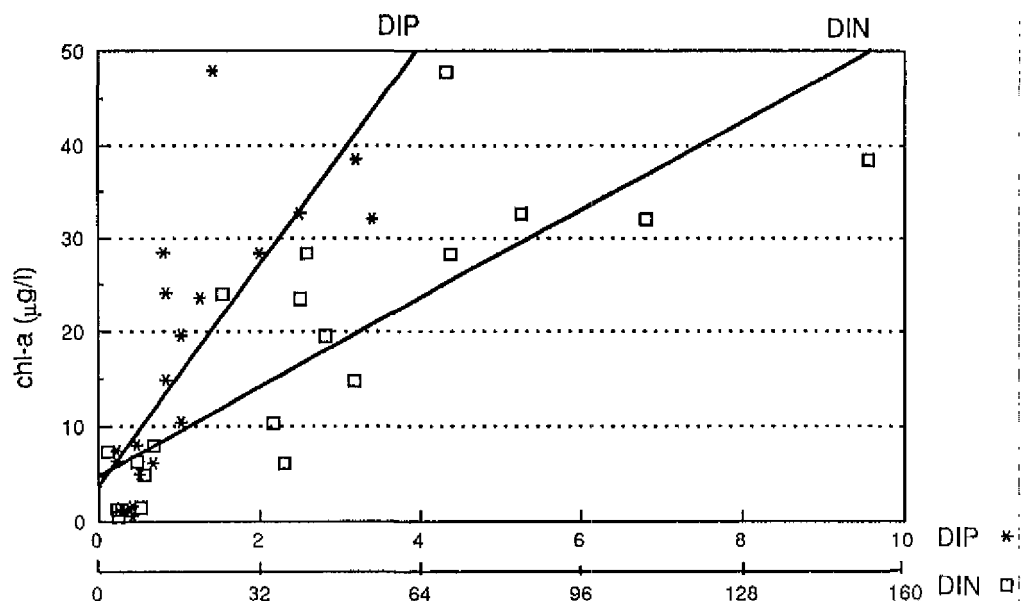
6 Discussion

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According to the P/P_{max} ratio, light is the dominating limiting factor in winter at all locations, and actually prevents phytoplankton development. In spring and summer it still more or less limits the rate of photosynthesis, but it does allow phytoplankton to develop. In fact, the rates of photosynthesis and of nutrient uptake can be limited simultaneously. Related to P/P_{max}, light is more limiting in the coastal areas than in offshore areas. Gieskes & Kraay (1975) found that the spring bloom in the Southern Bight begins earlier offshore because irradiance is greater there than near the coast. Peeters et al. (1991) found rather weak gradients of decreasing integral rates of carbon fixation from coast to offshore areas in spite of the strong gradients in nutrients and chlorophyll-a.

The different methods of identifying nutrient limitation yield different kinds of results. The ratios of inorganic nutrients and the different bioassays indicate potential limitation. It is possible to identify the first potentially limiting nutrient and its spatial and temporal occurrence with these methods (Maestrini et al., 1984; Peeters & Peperzak, 1990). The picture that emerges from the study of inorganic nutrients is that P (and Si for diatoms) tend is to be the first potentially limiting nutrient(s) in spring and that N is so in summer. In the offshore areas N is more often potentially limiting than in the coastal zone.

Monospecific bioassays indicate that there are large differences in relative requirements for nutrients. Rhodomonas sp. needs more N than S. costatum and therefore is more often potentially N limited. It is well known that species do differ considerably in their nutrient requirements (see e.g. Hecky & Kilham, 1988). This partly explains why two or more nutrients can simultaneously limit natural phytoplankton (Eppley et al., 1973; Riegman et al., 1990; this report). The difference in nutrient requirement of phytoplankton is important in species succession (Tilman, 1977; Kilham & Kilham, 1984; Hecky & Kilham, 1988). Apart from monospecific differences in nutrient requirements, our bioassay studies yield spatial and temporal trends similar to those of the ratios of inorganic nutrients. The bioassays on natural plankton also give results consistent with the ratios of inorganic nutrients.

Contrary to the findings of Healy & Hendzel (1980) the ratio of PON/PP did not reveal severe actual nutrient limitation. The method is probably insensitive because dead organic and particulate inorganic material is incorporated in the ratio. PON includes living and dead organic material; PP includes particulate inorganic material as well (e.g. P adsorbed on clay, apatite). The variation caused by these factors probably masks any possible effects of nutrient limitation on this ratio.

'Actual' nutrient limitation as estimated with V/V_{max}, calculated from literature data (Figs. 23-29), is restricted to spring and summer.

Spatially, nutrient limitation is most severe in offshore areas and concentrations of N, P and Si are often limiting simultaneously. Near the coast the limitation occurs for shorter periods, mostly by P and Si and to a lesser degree by N, than offshore. In general P limitation of spring blooms predominates in the coastal zone and to a lesser degree offshore, whereas N tends to limit phytoplankton during summer offshore and in the coastal zone. In this respect the interpretation of ratios of inorganic nutrients and of V/V_{max} lead to the same conclusions. In contrast with the ratios of inorganic nutrients, calculation of V/V_{max} ratios can suggest simultaneous limitation of two or more nutrients. This means that the concentrations are more or less limiting. For example the level of the K_{su} for nitrate from literature is in the order of 1-2 μM, which agrees well with the results of the GLU/GLN determination.

The measured kinetics of nutrient uptake indicate simultaneous P, N and Si limitation (Figs. 30-31). This result agrees with the findings of Riegman et al. (1990). At the end of the spring bloom nitrate is limiting (has an increased uptake rate) at Nw2 where concentrations are still rather high (Fig. 5), but this is not surprising given that in this period the colonial flagellate Phaeocystis cf. pouchetii is dominant. That species has much higher K_{su} for P uptake in the colonial form than in the flagellate form (Veldhuis, 1987). It seems probable that the same is true for nitrogen uptake. In fact, Lancelot et. al. (1986) found a half saturation constant for protein synthesis of 4 μM N (ammonium + nitrate). These high K_{su} values were excluded when calculating the V/V_{max} ratios shown in Figs. 23-29. Thus the picture of nutrient limitation emerging from the analysis of inorganic concentration and bioassays may underestimate N and P limitation in the presence of Phaeocystis.

The regression of DIP and DIN concentrations at the end of the winter on the chlorophyll-a of the spring bloom peak suggests that P rather than N determines the maximum bloom size in that period. However, because of the time lag between winter concentration of inorganic nutrients and the spring peak of chlorophyll-a, other processes influencing the concentrations have to be considered too. DIN-consuming processes such as denitrification and the slow remineralization of organic N may be especially important in reducing DIN concentrations. Therefore, these regressions only indicate that the reduction of winter concentrations of nutrients will lead to lower peaks of the spring blooms.

The overall conclusion is that phytoplankton development and biomass are predominantly limited by:

* *light* during winter at all stations;
* *phosphorus* at the end of the spring at all stations and during summer in the coastal area;
* *nitrogen* at offshore stations and to a lesser degree in the coastal zone at the end of the spring bloom and during summer;
* *silicate* at all stations during spring and summer (diatoms only);
* several factors can be *simultaneously limiting* for the phytoplankton community.

7 Implications for nutrient reduction strategies

7.1 Possible effects of reduction measures

Limitations of light, P, N and Si alternate in space and time, and occur in combination or simultaneously. Light cannot be controlled and Si only indirectly controlled by man. So, to control phytoplankton abundance, both N and P should be reduced. The probable environmental pros and cons of different sanitation strategies with respect to phytoplankton are discussed below.

There are two potentially feasible strategies: reducing P or reducing both P and N; (the alternative of only N-reduction is unrealistic because in freshwater it is often not limiting and when N is scarce relative to P, N fixation by cyanobacteria is promoted (Schindler, 1977).
It is easier and cheaper to reduce P alone than to reduce both P and N. Reducing P might cause an increased P limitation in spring which occurs earlier in the year and results in lower peaks of the spring blooms. A second possible advantage of reducing phosphorus discharges is the possibility that silica levels in the rivers will increase and that in turn diatoms will become relatively more abundant than other groups in coastal waters.

It is not clear, however, whether this advantage of drastic P reduction would occur soon. The P cycle includes processes with differing time scales, of which the uptake of P by phytoplankton, the remineralization of organic matter and reversible adsorption to and desorption from sediments are important. Rates of remineralization (Officer & Ryther, 1980) and desorption (Kelderman, 1984; Wetsteyn et al., 1990) increase as temperature rises. Apparently as a consequence of these processes and the large benthic P reserves, DIP increases in the coastal zone in summer (Fig. 6). Over longer time scales P desorption in marine areas can be an important source of P, as has been shown in lake Grevelingen (Stronkhorst, 1989) and in the Oosterschelde (Wetsteyn et al., 1990; Wetsteyn & Bakker, 1991). So it is uncertain whether a strategy based solely on P reduction will lead to a quick decline of DIP and in any case the variability in river loads (Klein & Van Buuren, 1992) will more or less mask any trend in the decline of the loads (De Vries, unpublished).

A second potential drawback is that reducing P only will lead to a surplus of DIN. It is likely that this surplus will diffuse towards areas with predominant N limitation, where it can lead to an increase in phytoplankton.

A third drawback of P reduction only might be that extreme shifts in the relative availability of a nutrient might lead to the dominance of one or a few species. Competition experiments with phytoplankton ended with dominance of certain species which depended on the nutrient ratios (Tilman, 1977; Kilham & Kilham, 1984).

Apparently species which can efficiently utilize nutrients present in small amounts, are competitively superior. It is uncertain whether an extreme reduction of P would lead to the dominance of such a restricted number of species, or whether such a shift would favour the competitiveness of undesirable species. The increase of nitrogen in N limited areas could also lead to such shifts.

Two advantages can be expected if both the N and P loads are reduced. Initially, a reduction of the N load will immediately lead to a reduction of the N availability because, contrary to P, there are no large reserves of N to buffer the decline in concentration. The tendency for N to decrease more sharply than P concentration has recently been shown in the Oosterschelde. A reduction of 64% of the freshwater load on the Oosterschelde caused a sharp decline of N and Si concentrations, but those of P remained unchanged (Wetsteyn & Bakker, 1991). Reduction of N and P will lead to actual limitation of both nutrients earlier in the year and therefore to smaller spring and summer blooms in coastal and offshore areas.

Secondly, a combination of P and N reduction will affect species with relatively high requirements of P and species with high requirements of N. This combined reduction of P and N will with more certainty lead to a reduction of phytoplankton than a reduction of the P load only. Moreover, it will not give rise to extreme changes in nutrient ratios and therefore to unforeseen changes of the species composition.

A major practical disadvantage of N reduction is that most N comes from diffuse sources, like agricultural runoff, which are difficult to control.

7.2 Other reasons justifying nutrient reduction

The current pursuit of a sustainable economy is also justification for reducing anthropogenic nutrient loads. Nutrient reserves are either limited (P) or, as is the case with N fertilizers, require large amounts of fossil fuel to transform N_2 into ammonium or nitrates. Giving global population increase, the loss of agricultural soils and the fact that the world food reserves no longer increase, it Is sensible to tackle eutrophication by recycling of nutrients.

Denitrification yields N_2 and nitrous oxide (N_2O). The latter gas can react with ozone and it is more effective as a greenhouse gas (on a molar basis) than CO_2 (Hoppema & de Baar, 1991). The percentage of the total nitrate N that is converted into N_2O varies between about 0.01-6%. This implies that the popular denitrification method should not be used to remove N because it would greatly increase the total amount of N_2O formed if the nitrogen load was reduced substantially, given that about 10 % of the nitrogen load in the North Sea is denitrified (Brockmann et al., 1989; Laws & Owens, 1990). A more appropriate method is one that removes ammonia by selective ion exchange on zeolites and in which the ammonia and zeolite are reusable (Farrell, 1969; Mercer & Ames, 1970).

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