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Responses of marine phytoplankton in iron enrichment experiments in the northern North Sea and northeast Atlantic Ocean

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Abstract

Short-term iron enrichment experiments were carried out with samples collected in areas with different phytoplankton activity in the northern North Sea and northeast Atlantic Ocean in the summer of 1993. The research area was dominated by high numbers of pico-phytoplankton, up to 70,000 ml^{-1}. Maximum chlorophyll *a* concentrations varied from about 1.0 $\mu g\ l^{-1}$ in a high-reflectance zone (caused by loose coccoliths, remnants from a bloom of *Emiliania huxleyi*) and about 3.5 $\mu g\ l^{-1}$ in a zone in which the phytoplankton were growing, to about 0.5 $\mu g\ l^{-1}$ in the northeast Atlantic Ocean. From the high-reflectance zone to the northeast Atlantic Ocean, nitrate concentrations increased from 0.5 μM to 6.0 μM. Concentrations of reactive iron in surface water showed an opposite trend and decreased from about 2.6 nM in the high-reflectance zone to < 1.0 nM in the northeast Atlantic Ocean. In the research area, no signs of true iron deficiency were found, but iron enrichments in the high-reflectance zone, numerically dominated by *Synechococcus* sp., resulted in increased nitrate uptake. Ammonium uptake was hardly affected. Strong support for the effect of Fe on cell physiology is given by the increase in the *f*-ratio. Net growth rates of the phytoplankton (changes in cell numbers over 24 h) were almost unchanged. Phytoplankton collected from the northeast Atlantic Ocean, did not show changes in the nitrogen metabolism upon addition of iron. Net growth rates in these incubations were low or negative, with only slightly higher values with additional iron.

Keywords: Iron enrichment experiments; Marine phytoplankton; North Sea

1. Introduction

The complex interactions between trace metals and phytoplankton demand the use of state of the art of chemical and biological methodologies (Bruland et al., 1991; Bruland and Wells, 1995). The distribution and speciation of trace elements has a distinct influence on phytoplankton primary production, species composition and trophic structure. In return, phytoplankton activity may affect the distribution and speciation of trace metals.

Of all bioactive trace metals, iron has received

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substantial attention in recent years (de Baar et al., 1990; Buma et al., 1991; Price et al., 1991; Morel et al., 1991; Martin, 1992; Sunda and Huntsman, 1995; Takeda and Obata, 1995; Boyd et al., 1996; Coale et al., 1996). Iron is the most important essential trace element for plankton growth, and per unit biomass the plant cell needs more Fe than other trace constituents Zn, Mn and Co. Yet, surface water concentrations of Fe are low, and for Fe the supply is relatively much less than the demand, when compared to the other metals Zn, Mn and Co (Sedwick et al., 1997). Research on interactions between iron and phytoplankton started in the 1930s (Gran, 1931), but until recently was frustrated due to contamination problems (de Baar, 1994). Iron has been related to several biological phenomena. Studies have ranged from its effect on occurrences of phytoplankton blooms (Glover, 1978) to differentiation between oceanic and neritic phytoplankton species (Brand, 1991). The debate on the exact role of iron in the marine ecosystem continues to the present day (Bruland and Wells, 1995).

The effects of iron additions on phytoplankton (de Baar et al., 1990; Buma et al., 1991; Timmermans et al., 1994; van Leeuwe et al., 1997; Scharek et al., 1997), the distribution of iron and other trace elements in the water column (Saager et al., 1989; Nolting et al., 1991; de Baar et al., 1995; Löscher et al., 1997) and the chemical speciation of iron in seawater (Gledhill and van den Berg, 1995; Nolting et al., in press) are central themes in our work. Here, we report on the results of iron enrichment experiments conducted in areas with different phytoplankton activity in the northern North Sea and the adjacent northeast (NE) Atlantic Ocean in the summer of 1993. Similar experiments, performed in the past, lasted several days up to 1 week (Martin and Fitzwater, 1988; de Baar et al., 1990; Buma et al., 1991). Over this long time period enclosure artefacts are conceivable. In general, larger mesozooplanton grazers are excluded from the bottles, also the light regime differs from the in situ light spectrum and intensity, and notably the dissolved Fe may be lost due to adsorption to the bottle wall. Thus, while obtaining significant Fe effects on bulk parameters, like Chl *a* and nutrient inventories, after several days (Martin and Fitzwater, 1988; de Baar et al., 1990), major shifts in the plankton community towards predominance of small protozoa and large diatoms have been observed (Buma et al., 1991). Moreover, a general lack of response of bulk parameters within the first 48 h has previously been noted (de Baar et al., 1990; their Figs. 8, 9, 10). The various so-called 'bottle effects' have been subject of debate and it has been suggested that an in situ Fe enrichment experiment would be the only way to obtain firm proof of Fe limitation of the local community (Watson et al., 1991). On the other hand it is known that physiological indicators of Fe stress such as fluorescence response (Greene et al., 1991), nitrate reductase (Price et al., 1991, 1994; Timmermans et al., 1994) and ^{15}N labelled nitrate uptake rates, are much more sensitive then the bulk parameters, thus more amenable for short-term assays. This approach has here been chosen, as it allows observation of an immediate, or at least short-term, response within 24–36 h while the plankton community remains relatively uniform.

The effects of iron on the nitrogen metabolism are probably the best documented (Verstreate et al., 1980; Rueter and Ades, 1987; Doucette and Harrison, 1991; Price et al., 1991, 1994; Timmermans et al., 1994; de Baar et al., 1997). An adverse effect on nitrate reduction is commonly observed when iron is deficient. We therefore hypothesised that if any effects were to be expected upon addition of iron, they would most likely be on nitrate assimilation (cf. Price et al., 1991). In the time span of these experiments no effects of iron enrichments were expected on ammonium uptake.

Using a combination of biological and (bio)chemical observations, we describe the effects of iron additions on natural phytoplankton assemblages under different nutritional conditions. The effect of iron on phytoplankton nitrogen uptake was followed in separate experiments from the uptake of ^{15}N labelled nitrate and ^{15}N labelled ammonium. Furthermore, with changes in the nutrient composition, the following parameters were assessed: taxonomic composition, cell numbers of selected species, as well as cellular characteristics like size and chlorophyll fluorescence.

2. Material and methods

The cruise (BLOOM 93) lasted from 28 June until 13 July 1993. Temperatures of the surface water

ranged from 9–14°C. The depth of the mixed layer ranged from 25–35 m. In the central sector of the research area (Fig. 1), the surface water was milky white, caused by the presence of loose coccoliths (numbers up to 300,000 ml^{-1}), indicating that a bloom of *Emiliania huxleyi* had been present. Nutrient concentrations were low (Fig. 2A). This zone will be called the high-reflectance zone. A narrow band of approximately 40 nautical miles wide, with high chlorophyll *a* (Chl *a*) and nitrate concentrations, was encountered further to the north west. Flowcytometry and HPLC pigment analyses indicated that next to phycoerythrin containing *Synechococcus* sp., large numbers ($\sim$ 50,000 ml^{-1}) of pico-eukaryotes (2–3 μm size) were present, together with *E. huxleyi* and a mixture of larger algae ($>$ 5 μm). This zone was called the growing phytoplankton zone. Further to the north, a NE Atlantic zone was discerned, with high nitrate and phosphate concentrations, and low Chl *a* concentrations (Fig. 2B). More information on the research area is given by van der Wal et al. (1995) and Buitenhuis et al. (1996).

2.1. Clean techniques

Clean techniques were used throughout. Trace metal contamination caused by the ship was avoided by immediately sampling upon arrival at the station, by lowering the CTD frame to at least 60 m and closing of the bottles during the upward cast. All further manipulations were performed inside the temperature-controlled, clean air container. Samples from the incubation bottles were collected after additional gentle mixing. All materials with which the seawater had contact had been rigorously cleaned with 6 N HCl and Milli-Q water before the incubations started.

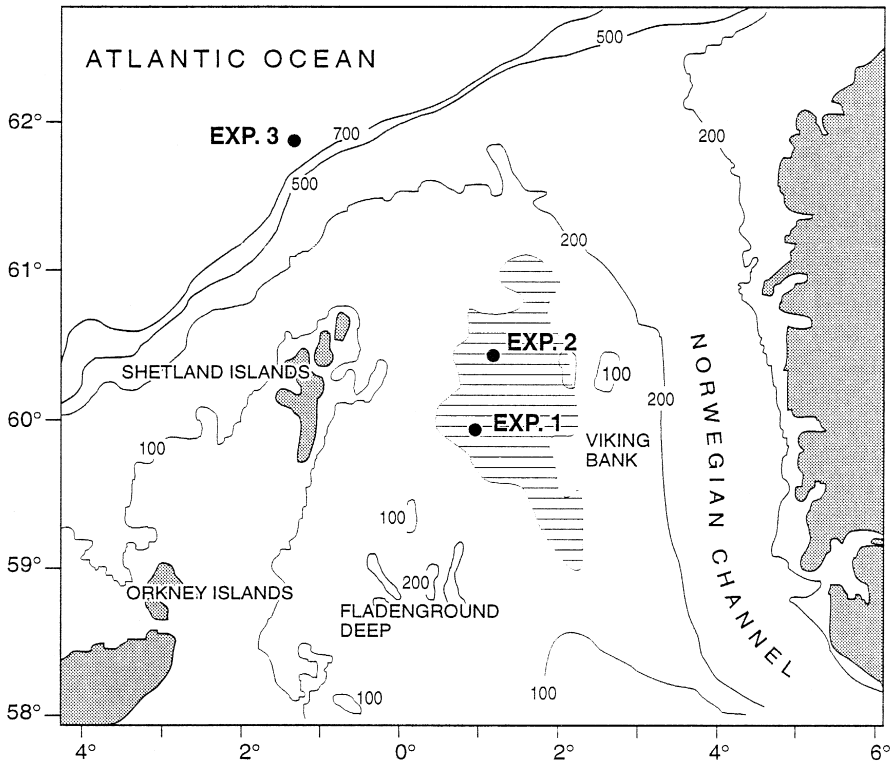

Fig. 1. Overview of the research area with the locations of the iron enrichment experiments. The zone with the high-reflectance (post-bloom of *E. huxleyi*) is hatched. For exact positions, see also Table 1. The depths are in meters.

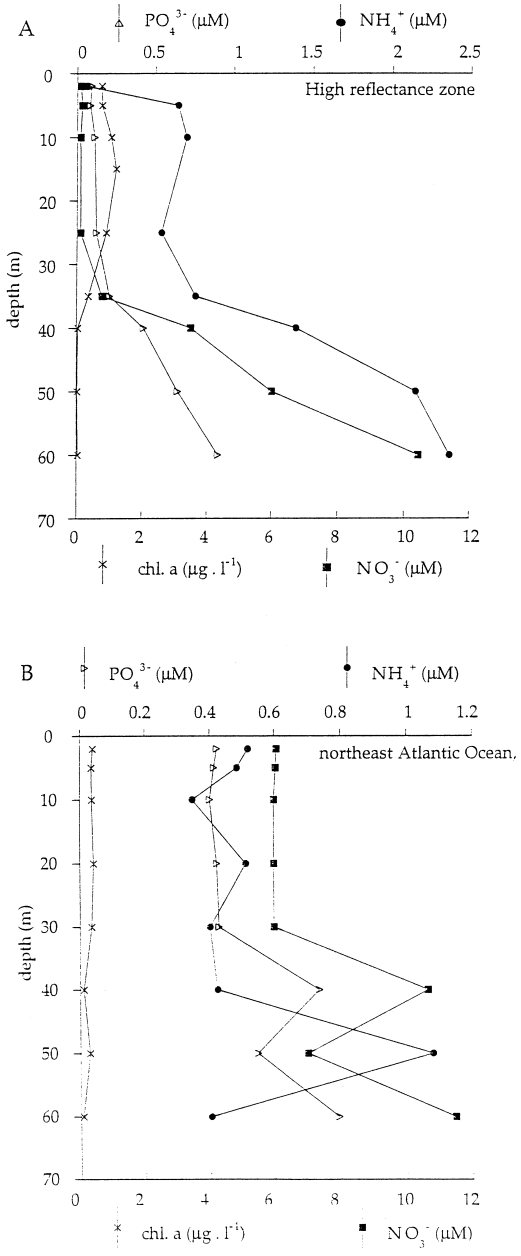

Fig. 2. Concentrations of nitrate, ammonium, phosphate (μM) and Chl *a* (μg l^{-1}) along depth profiles in the high-reflectance zone (A) and the NE Atlantic (B).

2.2. Iron enrichment experiments

Three experiments will be discussed: two in the high-reflectance zone and one in the NE Atlantic (Table 1, Fig. 1). Sampling was performed at 10.00 h GMT with six 12-l Go-Flo bottles mounted on a Teflon-coated CTD rosette frame. Once filled with water at a depth of 10 m, the bottles were mounted outside a clean, temperature-controlled container. Water was led through the wall of the container using Teflon tubing and dispensed into the incubation bottles. Each incubation bottle was filled with water from an individual Go-Flo bottle.

In every experiment, four 20 l pre-cleaned Polycarbonate incubation bottles were filled, without filtration or screening, with 10 l seawater each. Light conditions were chosen to simulate the ambient light climate (i.e., 100 μEinstein m^{-2} s^{-1} in a 18:6 light dark regime, using white fluorescent tubes) and at ambient temperature (11°C). Gentle revolving of the incubation bottles minimized sedimentation of particles.

At $t = 0$ h sampling for the initial conditions was performed in control and enriched (2.5 nM Fe as Fe(III) in a chloride solution) bottles. At 4 h intervals, 50 ml subsamples were taken for analyses of cell abundance and composition. At $t = 24$ h ^{15}N labelled nitrate and ^{15}N labelled ammonium additions were made (see below). At $t = 27$ and 29 h, filtrations for the determination of ^{15}N incorporation were performed.

2.3. Biological and chemical analyses

The taxonomic composition of the phytoplankton, the abundance, the size and the fluorescence characteristics of selected species were determined by applying shipboard flowcytometry (Veldhuis and Kraay, 1993). The following species were observed: phycoerythrin-containing *Synechococcus* sp. (~ 1 μm in diameter); unidentified pico-eukaryotes (2–3 μm in diameter); *E. huxleyi* (cell diameter 5–7 μm); and clusters with species within the 3–7 μm diameter range. The presence of these species was checked microscopically and with HPLC analysis (data not shown). Net growth rates were calculated over the first 24 h. Cell volumes were calculated assuming spherical dimensions of the cells. Concentrations of Chl *a* were measured according to Holm-Hansen et al. (1965), using GF/F filters.

Concentrations of dissolved nitrate, nitrite, phosphate and ammonium were determined on shipboard

Table 1
General (A) and specific (B) information on the sampling stations where water for the iron enrichment experiments was collected

(A) Experiment	Day of the year	Latitude	Longitude
1	181–182	59.57.80 N	01.00.39 E
2	189–190	60.28.89 N	01.20.52 E
3	190–191	61.47.82 N	01.02.76 W

(B) Depth	Experiment 1			Experiment 2			Experiment 3[a]		
	A	B	C	A	B	C	A	B	C
0 m	1.7	1.7	–	5.3	1.27	2.1	7.1	1.11	1.8
5 m	–	–	–	–	–	–	–	–	–
10 m	2.2	2.6	–	2.1	0.98	6.1	1.5	0.82	1.9
20 m	–	–	–	0.6	0.92	2.4	2.1	0.59	2.3
25 m	4.2	1.4	–	–	–	–	–	–	–
30 m	–	–	–	1.5	1.12	5.9	2.9	0.46	2.6
40 m	–	–	–	–	–	–	1.6	0.55	2.1
50 m	–	–	–	1.7	2.37	4.5	1.5	0.76	3.2
70 m	–	–	–	3.0	2.14	5.2	–	–	–

See also Fig. 1 for locations.
All samples for Cathodic Stripping Voltammetry (CSV) and the 0 m samples for Graphite Furnace Atomic Absorption Spectrometry (GFAAS) measurements were unfiltered.
Other samples for the GFAAS analyses were filtered.
A, dissolved Fe (nM) measured with GFAAS.
B, reactive Fe (nM) measured with CSV.
C, total Fe (nM) measured with CSV.
–, not analysed/sampled.
[a] Iron analyses from position 61.42.80 N 1.10.3 W.

using Traacs Auto Analysers (cf. Grasshoff, 1983). Except for $t = 0$ h in experiment 1, all nutrient samples were filtered through 0.2 μm cellulose acetate filters (Acrodisc, Gelman) prior to analysis, as unfiltered samples resulted in irregular increases and decreases especially in the nitrate data (Table 2A). Limits of detection were as follows: nitrate 0.02 μM, nitrite 0.02 μM, phosphate 0.02 μM and ammonium 0.1 μM.

Concentrations of reactive iron, defined as that Fe which was freely complexed by 20 μM nitroso-naphtol at pH 6.9, were determined in unfiltered samples on shipboard using cathodic stripping voltammetry (Gledhill and van den Berg, 1995; Gledhill et al., in press). Total Fe was measured in the Oceanography Laboratories at the University of Liverpool (Gledhill et al., in press), using a comparable protocol as for the reactive Fe analyses. In addition to analyses for iron in the incubation bottles, field samples were collected for analyses of trace metal distribution and iron speciation in relation to phytoplankton activity in the research area. Dissolved (0.4 μm filtered) concentrations of Fe, Cu, Cd, Zn, Ni and Pb were measured after pre-concentration by solvent extraction and subsequently measurement by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (Nolting et al., submitted).

2.4. ^{15}N uptake

As a rule, ^{15}N labelled nitrate or ammonium was added at a concentration equal to about 10% of ambient levels (cf. Dugdale and Goering, 1967). An exception was made for the incubations performed in the high-reflectance zone. Here very low ambient nitrate concentrations were present, and the addition of ^{15}N labelled nitrate resulted in a doubling of the overall concentration (see Table 2A, experiments 1 and 3), as very low nitrate concentrations were pre-

Table 2

(A) Concentrations of ammonium, nitrate, phosphate and nitrite (all average ± S.D., in μM) during the experiments. Except where indicated, four replicates were taken shortly before the addition of iron (begin, $t = 0$ h) and shortly before the addition of ^{15}N spike ($t = 24$ h)

Experiment	NH_4^+	NO_3^-	PO_4^{3-}	NO_2^-
1 begin	0.63 ± 0.08 ($n = 3$)	0.04 ± 0.02 ($n = 3$)	0.13 ± 0.02	0.02 ± 0.01
$t = 24$ h	0.59 ± 0.06	0.09 ± 0.03	0.08 ± 0.01	0.02 ± 0.00
2 begin	0.52 ± 0.02 ($n = 3$)	0.13 ± 0.06 ($n = 3$)	0.09 ± 0.01 ($n = 3$)	0.02 ± 0.01 ($n = 3$)
$t = 24$ h	0.48 ± 0.03 ($n = 3$)	0.12 ± 0.02 ($n = 3$)	0.07 ± 0.01 ($n = 3$)	0.02 ± 0.00 ($n = 3$)
3 begin	0.62 ± 0.05	5.99 ± 0.04	0.44 ± 0.01	0.17 ± 0.01
$t = 24$ h	0.43 ± 0.03	5.92 ± 0.01	0.37 ± 0.01	0.18 ± 0.01

(B) Concentrations of reactive iron (nM) during the iron enrichment experiments at the beginning of the experiment and after 24 h (shortly before addition of ^{15}N spike)

	experiment 1	experiment 2	experiment 3
Control begin	–	1.20	–
$t = 24$ h	0.55	0.60	1.05
+ Fe begin	1.91	–	1.35
$t = 24$ h	1.52	2.10	1.48

–, denotes not analysed/sampled

sent. Particulate matter was collected by gentle filtration on small (11 mm diameter), pre-combusted glass fibre filters (GF/F, Whatman, nominal pore size 0.7 μm). Filters were stored at −20°C until analysis. In the home laboratory, the calcium carbonate was removed by acidification, then the particulate nitrogen was converted to gaseous N_2 with a Fisons Element Analyser, and the ^{15}N contents determined with a coupled Fisons Mass Spectrometer. These analyses also provided POC and PON concentrations. Average absolute uptake rates of nitrate and ammonium (ρ in μmol N l^{-1} d^{-1}) were calculated (Dugdale and Goering, 1967) for $t = 3$ and $t = 5$ h after addition of the ^{15}N labelled nitrate or ammonium. Given the short incubation time, no correction for isotope dilution was made for the calculation of ammonium uptake rates (Glibert et al., 1992). The *f*-ratios were calculated as

$$\frac{(\rho\ \text{nitrate})}{(\rho\ \text{nitrate} + \rho\ \text{ammonium})} \times 100$$

in paired control and Fe enriched incubations.

3. Results

The abundance of Chl *a* was about 1 μg l^{-1} in surface waters of the high-reflectance zone, and only about 0.5 μg l^{-1} in the NE Atlantic. The nutrients showed an opposite trend, and were highest in the NE Atlantic (Fig. 2). The concentrations of dissolved iron were lowest in the NE Atlantic and higher at the continental stations of the North Sea. The concentrations of Fe were generally very low in the surface waters where Chl *a* was at maximum, then tended to increase at intermediate depth and in the bottom waters (Table 1, Fig. 2).

3.1. Experiment 1

3.1.1. Dissolved nutrients and iron

Concentrations of nutrients and reactive iron were low in the incubation bottles during this experiment (Table 2B). These low concentrations made it difficult to discern differences in nutrient uptake in the incubations in the course of the experiment. The low reactive iron concentration (0.55 nM) at $t = 24$ h in

the controls, is an indication for confidence in the clean sampling and handling procedures. The added Fe was not fully recovered: reactive Fe concentrations were below 2.0 nM (Table 2B).

3.1.2. Species composition, cellular characteristics and net growth rates

A high abundance of phycoerythrin-containing *Synechococcus* sp. with initial numbers of approximately 40,000 cells ml^{-1} was observed. Lower abundances of small pico-eukaryotes (7000 cells ml^{-1}, ~ 2 μm in diameter) and a mixture of algae in the 2 to 5 μm diameter range were observed.

No differences in cellular characteristics or net growth rates of the three groups of phytoplankton studied were observed (Table 3). Net growth rates in the control bottles (0.1 d^{-1}) were comparable to those calculated in the field (0.08 d^{-1}), which were determined during in situ production measurements using ^{14}C labelled bicarbonate (van der Wal et al., 1995, van der Wal, NIOZ, Texel, pers. comm.). The fact that the average chlorophyll fluorescence signal per cell did not change over the incubation period (data not shown), indicated that the light regime was close to the prevailing light intensity the cells experienced in the field. However, the phycoerythrin signal of *Synechococcus* sp. gradually decreased to one-third of the start value. The small numbers of cells larger than 2 μm totally dominated the rest of the phytoplankton (Table 3) in terms of cell volume.

3.1.3. ^{15}N uptake and elemental composition of particulate material

The results from the ^{15}N uptake experiments show that two aspects demand further consideration. Firstly, addition of iron positively affected nitrate uptake (Fig. 3A), whereas ammonium uptake was not affected by iron addition. Therefore the calculated *f*-ratio (Table 4) showed a consistent trend of higher values in the incubations with iron. Secondly,

Table 3
Net growth rates (A, in d^{-1}) and biovolumes (B, in $\mu m^3\ l^{-1}$) of phytoplankton in the incubation bottles

	Cyanobacteria	Eukaryotes						
	Synechococcus sp.	Pico (< 2 μm)		2–5 μm				
	A	B	A	B	A	B		
Experiment 1								
Control $^{15}N–NO_3^-$	0.20	$5.1*10^3$	0.14	$6.3*10^4$	−0.16	$2.5*10^7$		
Control $^{15}N–NH_4^+$	0.06	$4.5*10^3$	0.10	$5.8*10^4$	0.03	$1.8*10^7$		
Fe $^{15}N–NO_3^-$	0.19	$4.5*10^3$	0.19	$5.2*10^4$	0.01	$2.7*10^7$		
Fe $^{15}N–NH_4^+$	0.02	$4.7*10^3$	0.06	$5.6*10^4$	−0.16	$2.1*10^7$		
Experiment 2	*Synechococcus* sp.	Pico		2–5 μm				
	A	B	A	B	A	B		
Control $^{15}N–NO_3^-$	0.54	$3.2*10^3$	0.35	$3.2*10^4$	0.02	$8.9*10^6$		
Control $^{15}N–NH_4^+$	0.37	$3.2*10^3$	0.40	$3.0*10^4$	0.03	$8.7*10^6$		
Fe $^{15}N–NO_3^-$	0.39	$3.5*10^3$	0.28	$3.5*10^4$	−0.07	$9.8*10^6$		
Fe $^{15}N–NH_4^+$	0.51	$3.4*10^3$	0.32	$3.2*10^4$	−0.19	$10.6*10^6$		
Experiment 3	*Synechococcus* sp.	Pico 1[a]		Pico 2[b]		2–5 μm		
	A	B	A	B	A	B	A	B
Control $^{15}N–NO_3^-$	−0.43	$7.6*10^3$	−0.25	$3.1*10^5$	−1.29	$3.1*10^4$	−0.96	$2.9*10^6$
Control $^{15}N–NH_4^+$	−0.29	$5.7*10^3$	−0.16	$2.4*10^5$	−0.09	$2.1*10^4$	−0.52	$2.1*10^6$
Fe $^{15}N–NO_3^-$	−0.15	$5.1*10^3$	0.54	$2.1*10^5$	−0.91	$1.9*10^4$	−0.58	$2.1*10^6$
Fe $^{15}N–NH_4^+$	−0.18	$5.0*10^3$	−0.17	$2.2*10^5$	−0.37	$1.1*10^4$	−0.76	$1.8*10^6$

The net growth rates are calculated over 24 h for the specific groups of phytoplankton and biovolumes are calculated for $t = 0$ h.
[a]Pico 1: ~ 2 μm in diameter, low in red fluorescence.
[b]Pico 2: < 2 μm in diameter.

ammonium uptake was substantially larger than nitrate uptake during this experiment. In these waters regenerated production was therefore found to be more important than new production. This is not surprising, considering that ambient nitrate concentrations were low at this station. The C:N ratios of particulate material in experiment 1 were well above the average Redfield ratio of approximately 6.5 (Table 4), indicative of nitrogen limitation.

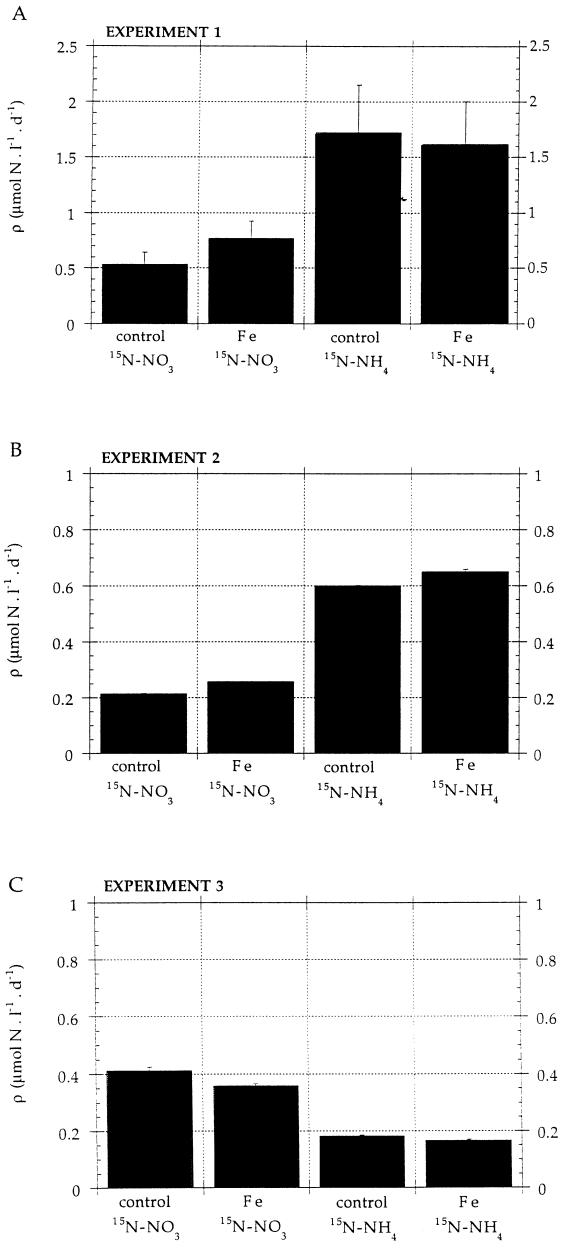

Fig. 3. (A–C) Absolute ^{15}N uptake rates (average $\pm$ S.D., $n = 3$) in μmol N l^{-1} d^{-1}, in iron enrichment experiments 1–3. Note the differences in scales.

Table 4
C:N ratios in particulate material and *f*-ratios during the incubation experiments

	C:N ratio		*f*-ratio	
	$t = 27$ h	$t = 29$ h	$t = 27$ h	$t = 29$ h
Experiment 1				
Control ^{15}N–NO_3^-	10.3	9.0	–	–
Control ^{15}N–NH_4^+	9.6	8.0	24.7	23.0
Fe ^{15}N–NO_3^-	9.5	9.6	–	–
Fe ^{15}N–NH_4^+	3.7	9.0	33.0	31.6
Experiment 2				
Control ^{15}N–NO_3^-	6.8	6.0	–	–
Control ^{15}N–NH_4^+	7.1	7.9	26.4	26.0
Fe ^{15}N–NO_3^-	7.2	7.4	–	–
Fe ^{15}N–NH_4^+	7.1	6.6	28.6	38.1
Experiment 3				
Control ^{15}N–NO_3^-	6.5	5.8	–	–
Control ^{15}N–NH_4^+	6.2	6.2	69.2	69.6
Fe ^{15}N–NO_3^-	6.0	6.0	–	–
Fe ^{15}N–NH_4^+	6.0	6.4	67.3	67.6

3.2. Experiment 2

3.2.1. Dissolved nutrients and iron

The results from this experiment and experiment 1, both performed in the high-reflectance zone, are similar (Table 2A). During the experiment, no consistent trends in bulk nutrient changes could be observed in the incubation bottles. The low reactive iron concentrations (0.6–1.2 nM) again illustrated the effectiveness of clean procedures. As in experiment 1, the reactive Fe was lower than the nominal 2.5 nM added (+background $\sim$ 1 nM) iron (Table 2B), suggesting either firm irreversible uptake in the phytoplankton or some adsorption to the wall of the bottles or a combination of both processes.

3.2.2. Species composition, cellular characteristics and net growth rates

Species abundance, cell size and fluorescence characteristics and their changes were largely comparable with those found in experiment 1, with the exception that numbers were about twofold lower.

The net growth rates, however, were substantially higher than in experiment 1 (Table 3). No trends in relation to the differences in additions in incubations can be observed. The cells larger than 2 μm diameter had lowest numbers, but nevertheless were dominant in terms of cell volume (Table 3).

3.2.3. ^{15}N uptake and elemental composition of particulate material

The uptake rates of both nitrate and ammonium were slightly enhanced upon addition of iron (Fig. 3B). Ammonium uptake rates were approximately three times higher than nitrate uptake rates. The predominance of regenerated production is reflected in the *f*-ratios (Table 4). Upon addition of iron, *f*-ratios increased both at $t = 27$ and 29 h, indicative of enhanced nitrate uptake. The elemental composition of the particulate material demonstrated a relatively high C:N ratio (Table 4), most ratios being well above 6.5.

3.3. Experiment 3

3.3.1. Dissolved nutrients and iron

The seawater for experiment 3 was characterised by high nitrate and phosphate and relatively low reactive iron concentrations (Fig. 2B). The concentrations of reactive iron ($\sim$ 1.1 nM) in the controls of experiment 3 were slightly higher than observed in the controls of the high-reflectance zone ($\sim$ 0.6 nM). Concentrations of Chl *a* were the lowest encountered during the cruise. The addition of iron did not affect uptake rates of nitrate or ammonium (Table 2A).

3.3.2. Species composition and net growth rates

As in the previous incubations, *Synechococcus* sp. was numerically the dominant species with initial values of $\sim$ 50,000 cells ml^{-1}. Two types of picoeukaryotes co-occurred, both in high numbers. The first group was only slightly larger than the cyanobacteria ($\sim$ 1.5 μm in diameter) and was present with $\sim$ 30,000 cells ml^{-1}, the second group ($\sim$ 2 μm) numbered about 8000 ml^{-1}. Larger phytoplankton species (2–5 μm diameter) were present only in low numbers ($<$ 1000 cells ml^{-1}), but dominated in cell volume (Table 3). Negative net growth rates were seen in most incubations. The negative net growth rates in the control bottles corresponded to the net growth rate measured in the field (-0.05 d^{-1}) (van der Wal, pers. comm.).

3.3.3. ^{15}N uptake and elemental composition of particulate material

In contrast to experiments 1 and 2, the observed nitrate uptake was larger in experiment 3 (Fig. 3C, Table 4). As can be seen from the high *f*-ratios (Table 4), nitrate uptake is most important, i.e., new production is taking place. Addition of iron led to a decrease of the uptake rates of nitrate and ammonium.

4. Discussion

Major parts of the oceans have been described as high-nutrient, low-chlorophyll regions (Chisholm and Morel, 1991; Minas and Minas, 1992; Bruland and Wells, 1995). Among these regions are the Antarctic Ocean (de Baar et al., 1990, 1995, 1997; Buma et al., 1991; van Leeuwe et al., 1997; Scharek et al., 1997), the Equatorial Pacific Ocean (Martin, 1992; Price et al., 1991, 1994; Coale et al., 1996) and the subarctic Pacific Ocean (Boyd et al., 1996). Iron limitation has been put forward as the explanation for the phenomenon that nutrients, especially nitrate, are not depleted in surface waters (Martin and Fitzwater, 1988). In the equatorial Pacific region, a recent in situ Fe enrichment experiment has provided convincing evidence of Fe limitation of the local plankton community (Behrenfeld et al., 1996; Coale et al., 1996). The immediate response of fluorescence is the most convincing evidence in support of in situ Fe stress of the whole phytoplankton assemblage, likely by impairment of photosystem II (Behrenfeld et al., 1996). On the other hand, the effect on bulk variables such as biomass accumulation (Chl *a*), nutrient inventories and CO_2 contents took several days to fully develop, where major changes in community composition were observed. This in retrospect confirmed the validity of the long term bottle incubations (Martin and Fitzwater, 1988; Buma et al., 1991). Apparently these type of perturbation experiments either in bottles or in situ in the field, provide the most convincing evidence after several days. Then, quite ironically, the population in the

bottle or in the enriched patch of water deviates more and more from the natural system. In retrospect there is not so much a bottle effect, but more an ecosystem drift over prolonged time. For proper understanding it appears advisable to make experimental observations over the whole range of responses from immediate (Behrenfeld et al., 1996) up to 1 week (Martin and Fitzwater, 1988; Buma et al., 1991; Coale et al., 1996).

The goal of our study was modest though, and we had chosen to investigate only the short-term responses of the phytoplankton community to Fe addition over intermediate time intervals of 24–36 h. This was accomplished by relying on more sensitive physiological rate variables (^{15}N uptake rates) which are deemed to be more relevant than state variables such as biomass yield (Banse, 1991).

In the northern North Sea, no signs of overriding iron deficiency were found, but it was demonstrated that iron additions had some effects on phytoplankton physiology, notably the nitrate uptake, as discussed below. During the expedition, conditions ranged from low-nutrient, low-Chl *a* concentrations in the high-reflectance zone to high-nutrient, low-chlorophyll *a* concentrations in the NE Atlantic. Previous observations in the research area have demonstrated ample capacity for phytoplankton growth. Several reports mention frequent occurrence of (coccolithophorid) blooms (Holligan et al., 1989; Westbroek et al., 1993). Based on the analyses along depth profiles (Gledhill et al., in press), sufficient iron appeared to be present for synthesis of chlorophyll and reduction of nitrate.

The effect of iron on algal physiology was most clearly demonstrated in experiments in the high-reflectance zone. Under low-iron conditions, addition of iron led to enhancement of nitrate incorporation (^{15}N nitrate uptake/*f*-ratio). As in the control bottles phytoplankton also grew, it was highly unlikely that iron acted as the single limiting factor for phytoplankton activity. In these aspects, our findings are consistent with the results of Martin et al. (1993), studying the effects of iron additions during a bloom in the North Atlantic Ocean. With an ambient iron concentration in surface water of 0.07 nmol kg^{-1}, Martin et al. (1993) reported only weak evidence for iron deficiency in the phytoplankton. But addition of 2 nmol kg^{-1} iron did increase CO_2 uptake and POC formation by a factor 1.3–1.7. Based on these observations, the authors had concluded that iron acted as a phytoplankton growth enhancer in the North Atlantic Ocean.

In the NE Atlantic, ample iron was present in the control incubations. As a result only minor effects on cell growth or nitrogen uptake were noted upon addition of iron.

4.1. Nitrogen metabolism

Iron–nitrogen interactions have been found to be very sensitive, useful indicators of iron stress in the field (Price et al., 1991, 1994; Doucette and Harrison, 1991). Therefore, these interactions were used to investigate effects of iron addition on the N-metabolism of the phytoplankton in the northern North Sea and the NE Atlantic. In the high-reflectance zone, according to expectations, addition of iron resulted in higher nitrate uptake rates. Ammonium uptake either decreased or was not affected.

The shift to enhanced nitrate uptake (new production) upon iron addition is clearly illustrated in the *f*-ratios. Upon addition of iron, the *f*-ratios increased in experiments 1 and 2. The *f*-ratio may be especially well suited for comparisons of effects of iron additions, as it is independent of the effect of detrital nitrogen. Price et al. (1991) observed in Equatorial Pacific phytoplankton a doubling of the *f*-ratios approximately 60 h after addition of iron. In our experiment, differences were already apparent after 27–29 h. The apparent opposite trend in C:N ratio (decreasing with increasing Fe) appears to counter the increased efficiency of N-assimilation.

The results of the ^{15}N uptake experiments clearly demonstrated the N-source in use by the phytoplankton. In the high-reflectance zone, with low nitrate concentrations, ammonium was mainly used, in spite of its relatively low concentrations. As Wheeler and Kokkinakis (1990) reported, low ammonium concentrations do not imply that regenerated production is insignificant. In the NE Atlantic, use of nitrate, i.e., new production, dominated.

In experiment 3, the phytoplankton did use some of the available nitrate or ammonium. Growth rates were negative, N-uptake rates and nitrate reductase activity (Timmermans et al., 1994) were low and Fe seemed to have a negative effect on nitrate and

ammonium uptake. Clearly, the experiment 3 is different from 1 and 2. Fe limitation is highly unlikely here given the prevalent new production and the lack of effect of Fe addition on overall and specific biochemical parameters in the phytoplankton.

4.2. Dissolved nutrients

The differences in distribution of nitrate and ammonium in relation to phytoplankton activity were typical given the generally established preference of phytoplankton for the reduced forms of nitrogen. Generally spoken, ammonium is considered as the preferred form of nitrogen (Dugdale and Goering, 1967; Eppley et al., 1977), even at low levels (Wheeler and Kokkinakis, 1990). Transport of nutrient-rich ocean water into the northern North Sea will, in addition to use of nutrients by phytoplankton, be responsible for the observed increasing gradient in nutrient concentrations from south to north. In addition, the shortness of the growing season, combined with the depth of mixing may be invoked as explanations for the incomplete utilisation of nitrate (Taylor et al., 1993).

4.3. Iron concentrations

Clean sampling is an absolute prerequisite for iron enrichment experiments. If possible, a Zodiac is to be preferred for sampling away from the ship. The present data from the incubation experiments demonstrated that good results also can be obtained while sampling at 10 m depth from the research vessel. With exception of some surface values, low iron concentrations were observed. This is surprising given the fact that iron input from the underlying continental shelf sediment, as well as nearby land sources could be expected. Low concentrations of dissolved iron in the North Atlantic Ocean have been measured by Martin et al. (1993), ranging from 0.07 nmol kg^{-1} at 10 m depth to 0.76 nmol kg^{-1} at 2400 m depth. We observed low dissolved (and reactive) iron concentrations in the NE Atlantic. Recent measurements of iron in the northern North Sea are scarce. Danielsson et al. (1985) observed a trend of decreasing iron concentrations from the North Sea to the Atlantic Ocean, but their values were substantially higher (from south to north ranging from >10 nM to 2–5 nM) than ours. A similar decreasing gradient in iron concentrations, however, was observed in the present study. The lowest values (less than 1.0 nM reactive iron) were measured in the NE Atlantic, whereas higher values (2–3 nM reactive iron) were measured inside the high-reflectance zone. The shallower water depth as well as the closer proximity to land are consistent with the increase of concentrations towards the south. This trend in elevated surface water iron concentrations in the research area was not reflected in the iron measurements in the incubation bottles. Clearly, the very surface samples are more prone to having received some aeolian Fe input than those collected in subsurface waters.

The added Fe was not fully recovered in the incubation bottles, even not immediately after addition. This is not surprising given the fact that 2.5 nM total Fe was added and reactive Fe was measured. Further, wall adsorption and, more general, adsorption and uptake, may have reduced Fe concentrations in the incubation bottles, especially after 24 h.

4.4. Species composition and net growth rates

The availability of nitrate or ammonium was probably responsible for the differences in cell dynamics between experiments 1, 2 and 3. Nutrient shortage (experiments 1 and 2) might have restricted the cell division to only a small portion of the population, thereby reducing the overall phytoplankton growth rates (Table 3). Although high nitrogen concentrations as measured during experiment 3 allowed high growth rates, with nitrate as the principal nitrogen source (as shown by the high *f*-ratio), the ultimate increase in phytoplankton biomass was negligible (Table 3). The reasons for these curious observations remain unclear: Fe limitation can however be ruled out as no effects of Fe addition were observed.

Calculation of the overall net growth rates from cell counts provided little useful additional information (Table 3). Effects of iron addition were often not observed. The conclusion derived from these observations may be that 24 h was too short for the determination of effects of iron addition on growth rates, especially as no assessment of the zooplankton grazing activity could be made. Short-term rate vari-

ables as nitrogen uptake (this study) and enzyme activity (Timmermans et al., 1994) showed more pronounced effects.

On the other hand, specific information on cell numbers of the different taxonomic entities did show some differences. In most cases not the dominant *Synechococcus* sp. species, but the less frequently occurring algae determined the ultimate growth rate response. This is to be expected, as the dominant species would be well adapted to the ambient conditions. The species composition may further have influenced the experiments. Without exception, small phytoplankton dominated in cell numbers. Probyn and Painting (1985) and Price et al. (1991, 1994) observed that small cells predominantly use ammonium as the N-source. As a result, it is possible that these cells will suffer less from iron limitation. Larger cells using nitrate, on the other hand, may be expected to suffer from iron limitation. For cyanobacteria, the reported effects of iron deficiency are slightly conflicting. Rueter and Unsworth (1991) concluded that clear physiological changes could be observed in relation to iron limitation in *Synechococcus* sp. However, Brand (1991) reported relatively high Fe: P molar ratios in cyanobacteria, indicative of a high iron requirement. On the other hand, cyanobacteria may be less vulnerable to iron limitation, due to their small size and corresponding favourable high specific surface area for uptake, and the ability to synthesise and utilize siderophores. DiTullio et al. (1993) postulated that prokaryotes were hardly affected by iron and nutrients, whereas diatoms were affected. These authors give size advantage and synthesis of siderophores as explanations. It seems that in oligotrophic waters prokaryotic picoplankton can outcompete eukaryotic phytoplankton for recycled nutrients such as ammonium and iron. Judging from the discrepancies of the various observations in the published literature, more research on the response of marine cyanobacteria to iron deficiency would be welcome.

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