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In vitro iron enrichment experiments at iron-rich and -poor sites in the NE subarctic Pacific

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Abstract

Despite evidence of Fe limitation of phytoplankton biomass in large areas of the ocean, few studies have assessed the relationships between algal stocks and Fe supply. An E–W survey transected the coastal and the open NE subarctic Pacific in May 1995, and revealed low nitrate (<1 μM) inshore, which increased westwards to ca. 8 μM. Over this transect, dissolved Fe fell from >3 nM kg^{-1} inshore to <0.5 nM kg^{-1} offshore. Fluorescence indices of photosystem II quantum efficiency (F_v/F_m, measured using DCMU) increased with distance offshore, but were always submaximal, indicating physiological constraints on photosynthetic capabilities, likely nitrogen inshore and Fe offshore. Six day in vitro Fe enrichments were performed at stations that were 500 (P12, Fe-rich) and 1000 km offshore (P26, Fe-poor). At P26, there were marked increases in NO_3 uptake and in chlorophyll *a* in both the control and Fe enrichment (3.5 nM Fe). Changes in nitrate reductase (NR) activity paralleled changes in NO_3 uptake, indicating true physiological responses. Measurements showed that controls had been contaminated with 1.8 nM Fe. However, transient increases in F_v/F_m were observed only in Fe treatments and not in contaminated controls. This may have been due to the form in which the Fe was added (chelated Fe in treatments versus probably non-chelated Fe in ''controls''). The time scale for the rise and fall of F_v/F_m is similar to that reported in the Ironex experiments. At P12, Fe-enrichment did not increase NO_3 uptake or NR activity, compared to the controls. Reasons for the differences in the results of enrichments at P26 and P12 are unclear. Neither Fe nor NO_3 was limiting at P12, yet F_v/F_m remained sub-maximal, suggesting that non-physiological factors (e.g. grazing) were not responsible. Unlike P26, at P12, there was a relatively small ''seed'' population of diatoms; factors controlling diatom abundances under ambient conditions may be crucial in determining the outcome of Fe enrichments.

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1. Introduction

High nitrate low chlorophyll (HNLC) regions are oceanic provinces that have low phytoplankton standing stocks despite high levels of macronutrients, such as nitrate (Chisholm and Morel, 1991). It is hypothesized that limitation by micronutrients such as Fe result in reduced levels of new production and, hence, in less efficient sequestration of biogenic carbon to the deep ocean (Chisholm and Morel, 1991). The potential significance of iron limitation to the oceanic carbon cycle is considerable, since these regions may occupy as much as 30% of the World Ocean. Examination of the datasets from HNLC regions indicates poor spatial and temporal coverage (Banse, 1991a; Cullen, 1991) and, thus, it is necessary to better constrain the areal extent of these regions (La Roche et al., 1996).

Recent studies have indicated that oceanic regions described as HNLC are not homogeneous with respect to dissolved iron (DFe) and that such heterogeneity may cause spatial variations in the magnitude of phytoplankton stocks within each region. Phytoplankton within a downstream plume to the W of the Galapagos Islands in the equatorial Pacific are unlikely to be Fe-limited and elevated phytoplankton biomass has been observed in this region (Martin et al., 1994). In the S. Ocean, de Baar et al. (1995) reported an inverse relationship between DFe and phytoplankton biomass and production, particularly in frontal regions, supporting the hypothesis that iron is limiting in this region. In the NE subarctic Pacific, the majority of Fe-enrichment studies have been performed at the former site of Ocean Station Papa (P26) (Martin et al., 1989; Coale, 1991; Boyd et al., 1996). However, it is now known that this region is characterized by an E–W gradient in DFe, with an order of magnitude decrease from the coastal to the open ocean, but there appears to be little change in the magnitude of phytoplankton biomass in response to this gradient (La Roche et al., 1996).

Techniques that provide quasi-instantaneous assessment of phytoplankton physiological status are being used increasingly in iron enrichment studies. Biophysical techniques have provided convincing evidence of iron limitation of photosynthesis during the Ironex I (Kolber et al., 1994) and Ironex II (Behrenfeld et al., 1996) in situ enrichments in the equatorial Pacific. Biophysical and biochemical techniques may also be used in conjunction with in vitro iron enrichment. Photosynthetic capacity can be indexed by fluorescence techniques that measure the quantum yield of fluorescence of photosystem II (F_v/F_m and, thus, the proportion of photosynthetically active reaction centres) using photosynthetic inhibitors such as DCMU (Cullen and Renger, 1979; Geider et al., 1993), or intense, rapid flashes of light (Kolber and Falkowski, 1993). Biochemical techniques available for the in situ assessment of algal physiological status include an assay for NR, a key enzyme in the pathway of nitrate incorporation, which is strongly correlated with nitrate incorporation in several phytoplankton species (Berges and Harrison, 1995a,b), and can be assayed in field situations (Berges et al., 1995). NR activity reflects acclimation of phytoplankton to in situ conditions on a scale of hours, and is thus relatively insensitive to any incubation artifacts.

In the present study, biophysical measurements (F_v/F_m) were performed along an E–W transect in the NE subarctic Pacific in order to map the region and ascertain the physiological status of the resident phytoplankton under ambient conditions. Both F_v/F_m and NR activity were used to assess the effect of Fe enrichment at stations in Fe-poor (sub-nanomolar) and Fe-rich (supra-nanomolar) waters, in order to assess the physiological effect of iron supply on phytoplankton stocks in the NE subarctic Pacific.

2. Materials and methods

2.1. Water column measurements

In May 1995, stations were occupied along a transect from P4 (referred to as coastal) in the coastal ocean (ca. 1000 m depth) to P26 in the open ocean (ca. 4200 m depth) (Fig. 1). Water samples were taken, within 2 h of local noon, from the mixed layer (ca. 35 m at all stations) at each station using clean 30 L Go-Flo samplers after Boyd et al. (1996). Samples were analysed for nitrate, ammonium, particulate nitrogen (PN), chlorophyll *a*, DFe, size-fractionated chlorophyll *a* (0.2–5 μm, 5–18 μm and >18 μm fractions) using procedures described in Boyd et al. (1996). Surface salinity readings

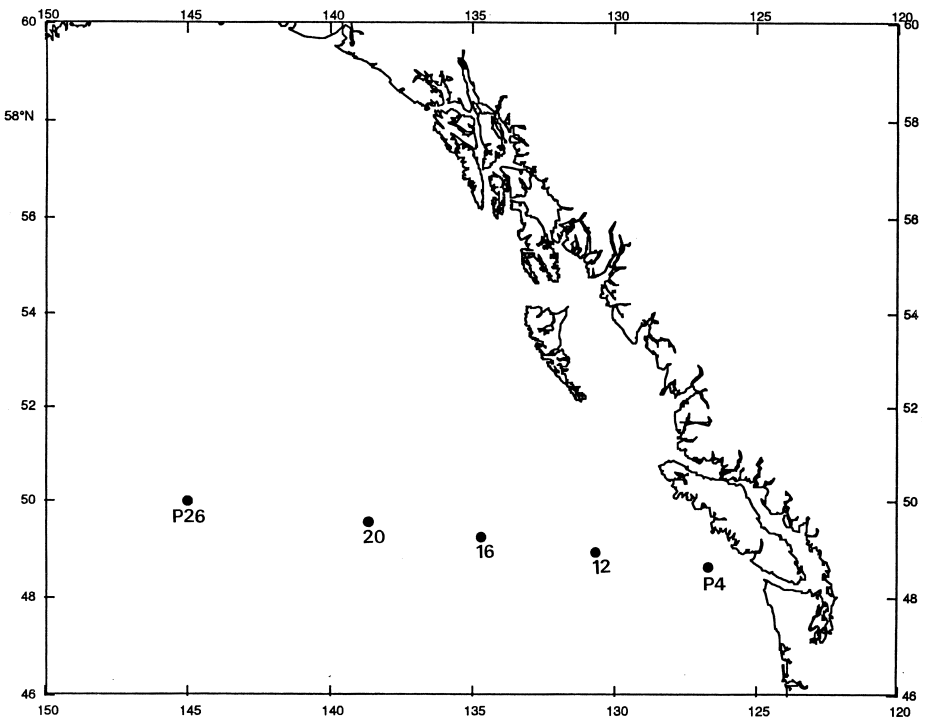

Fig. 1. Map of the main stations sampled along a ca. E–W transect (from 48°16′N to 50°N) in the NE subarctic Pacific. P4 is the coastal, P12 the intermediate, and P26 the offshore site.

were obtained using a Guildline CTD (Smith Falls, ON, Canada) calibrated with discrete salinity values. Phosphate and silicate were measured using the autoanalyser methods outlined in Boyd et al. (1995).

2.2. Quantum yield of fluorescence

F_v/F_m values from triplicate samples were measured in a Turner Designs model 10 fluorometer, with a water-jacketed cell that was cooled to in situ temperature. F_v/F_m was calculated as described in Geider et al. (1993), where the variable fluorescence $F_v = (F_m - F_o)$, F_o is the fluorescence in samples that were dark-acclimated for at least 5 min at in situ temperature, and F_m is the fluorescence after the addition of 10 μM DCMU. Preliminary experiments showed that 30 min and 1 h dark acclimation did not decrease F_o, but did decrease F_v/F_m, indicating the detrimental effects of maintaining samples for longer periods before making measurements. Accordingly, our measurements are based on 5 min acclimations. Under these conditions, both F_o and F_m showed no evidence of change over the course of the measurements, i.e. time-dependent increases in F_o, indicating closure of reaction centres or a rise in F_m, due to the time taken for DCMU to diffuse into cells. Background fluorescence (defined as the fluorescence of the < 0.2 μm filtrate) was subtracted from both F_v and F_o. Fluorescence backgrounds were of the order of 10% higher than distilled water blanks, and were unaffected by the addition of DCMU. The trends in F_v/F_m were identical regardless of whether or not background was subtracted, and were very similar, though more variable, if normalized to extracted chlorophyll *a*.

2.3. In vitro iron enrichments

At stations P12 (referred to as intermediate) and P26 (referred to as offshore) (Fig. 1), Fe-enrichment experiments were performed. Water sampling (with respect to the use of clean techniques), transfer to carboys under clean conditions, carboy size and incubation conditions were as previously described (Boyd et al., 1996). DFe was added as a 1:1.5 mol Fe–EDTA solution to give a final DFe concentration of 2 nM. One experiment was conducted at P12 and two at P26. Water samples were taken from control (C) and iron-enriched (Fe) carboys after 12 (day one), 36 (day two), 60 (day three), 84 (day four), 108 (day five) and 132 h (day six), sampling at as close to 1100 h local time as possible. Samples were analysed for nitrate, silicate, chlorophyll *a*, size-fractionated chlorophyll *a*, and samples were preserved using acid Lugol's solution for later phytoplankton counts. The initial DFe levels in the carboys were measured on 0.2 μm filtered samples following the method of Yang (1993). Size fractionated F_v/F_m was also measured for both experiments at the offshore site. Water samples were divided into whole water, < 2 μm, < 5 μm and > 18 μm fractions. The < 2 μm and < 5 μm fractions were obtained by gravity filtration in parallel through 47 mm diameter polycarbonate filters. The > 18 μm fraction was collected by reverse differentiation filtration under gravity. A 200-ml volume was fractionated until ca. 30 ml of sample remained, resulting in ca. seven-fold concentration of large cells. In all cases, the fractions were collected rapidly (average < 1 min) in 10 ml glass cuvettes (previously cooled to seawater ambient temperature)

located within a 500-ml beaker containing seawater. Upon collection, samples were placed in the dark at ambient temperature. For all enrichment experiments, samples for particulate nitrogen (750 ml or less) were filtered onto 25 mm GF/F filters, frozen at −20°C, and later analysed using a Carlo Erba CNS analyser. NR activity was assayed in 1 l samples that had been filtered onto 47 mm diameter GF/F filters, including additions of flavin adenine dinucleotide (FAD) and measuring nitrite production (Berges and Harrison, 1995a; Berges et al., 1995).

3. Results

3.1. Physical, chemical and biological transect data

Salinity measurements along the transect indicated progressive increases from the coastal station westwards for 90 km, and for a further 80 km west to the intermediate station (Fig. 2A). Westwards of the intermediate station, there was relatively little change along the remainder of the transect. Nitrate concentrations were <0.5 μM from the coastal station westwards for 400 km and, thereafter, progressively increased to 8 μM at the offshore station (Fig. 2B). DFe levels decreased slightly from >3 nM kg^{-1} at the coastal station to ca. 2 nM kg^{-1} at the intermediate site, and then markedly to <0.3 nM kg^{-1} at the offshore site in May 1995 (Boyd, unpublished data; La Roche et al., 1996). Despite these changes in macro- and micro-nutrient levels, chlorophyll *a* was nearly constant, at ca. 0.35 μg l^{-1}, west of the coastal station (Fig. 2C). F_v/F_m was low (ca. 0.2) at the coastal site, increased to 0.35 at the intermediate station, but rose no higher than 0.45 at stations westwards of the latter site (Fig. 2C). Phytoplankton taxonomic data (Table 1) indicates that biomass is dominated by small autotrophic nanoflagellates and, to a lesser extent, by dinoflagellates at the intermediate station. This trend is also observed at the coastal station (data not shown). At the offshore site (Table 1) and east of this site (P20) (data not shown), autotrophic biomass is dominated by small autotrophic nanoflagellates and, to a lesser extent, by diatoms.

3.2. In vitro iron enrichment (iron-rich site)

In the enrichment experiment at the intermediate station, there were only slight decreases in NO_3 over the six days, no systematic changes in ammonium or silicate, and no marked differences between nitrate or silicate utilisation between C or Fe carboys (Fig. 3). These trends were not consistent with the increases in chlorophyll *a* levels observed towards the end of the experiment (Fig. 3). This discrepancy between the magnitude of macronutrient depletion and increases in phytoplankton biomass may have been due to the alleviation of grazing pressure towards the end of the experiment; as chlorophyll *a* is a proxy for biomass (growth minus grazing), if there was an alleviation in grazing pressure, then chlorophyll would increase (growth minus less grazing) whereas the uptake of nutrients would remain the same in each instance. The slight decreases in macronutrient levels were reflected in NR activity, dPN and dNO_3^- (changes

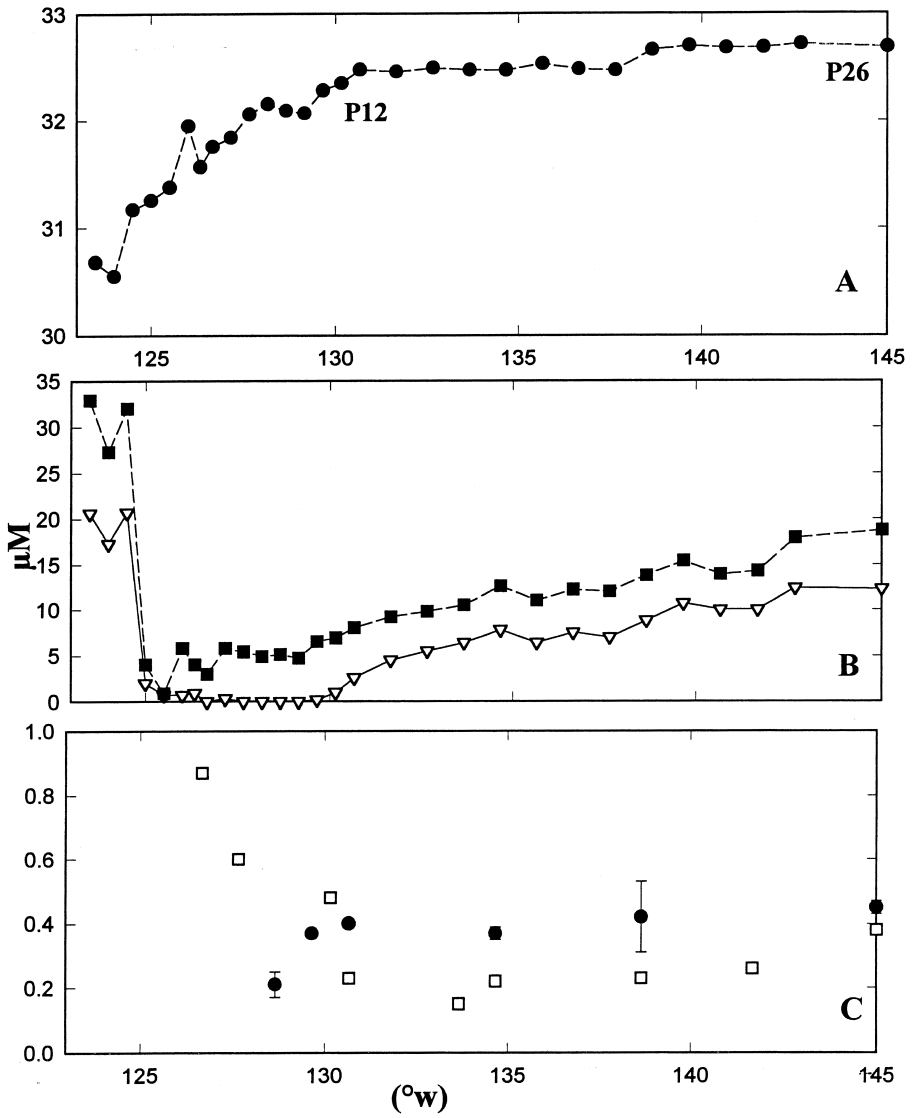

Fig. 2. Changes in surface properties: (A) Salinity (dimensionless); (B) silicate (closed symbols), nitrate (open symbols) and (C) chlorophyll *a* concentration ($\mu g\ l^{-1}$) (open symbols), and quantum yield of fluorescence for photosystem II (F_v/F_m) (closed symbols) along the E–W transect. The positions of the intermediate and offshore stations are shown. There are no replicates for salinity, nitrate, silicate or chlorophyll *a*. The error bars for F_v/F_m represent the standard error of the mean ($n = 3$); some error bars are smaller than the symbol size.

in particulate nitrogen and nitrate levels over the preceding 24 h period, respectively, see Fig. 4). NR levels were low and were highly variable in all cases. NR was not different from nitrate incorporation rates predicted by dPN and dNO_3^-. Due to technical difficulties, no F_v/F_m data were obtained during this experiment.

Table 1
Comparison of taxonomic data from water samples, obtained at ca. 10 m at the intermediate (P12) and offshore (P26) stations, for diatoms, dinoflagellates and nanoflagellates

Abundance (cells l^{-1})			
Station	Diatoms	Nanoflagellates	Dinoflagellates
Intermediate	$< 1.0 \cdot 10^3$	$1.2 \cdot 10^5$	$3.1 \cdot 10^3$
Offshore	$7.9 \cdot 10^5$	$13.0 \cdot 10^5$	$1.6 \cdot 10^3$

Diatoms at the offshore site were mainly < 10 μm pennates

3.3. In vitro iron enrichment (iron-poor site)

In contrast to the intermediate site, nitrate and silicate levels decreased markedly in both experiments conducted at the offshore site (Fig. 5). Ammonium levels were

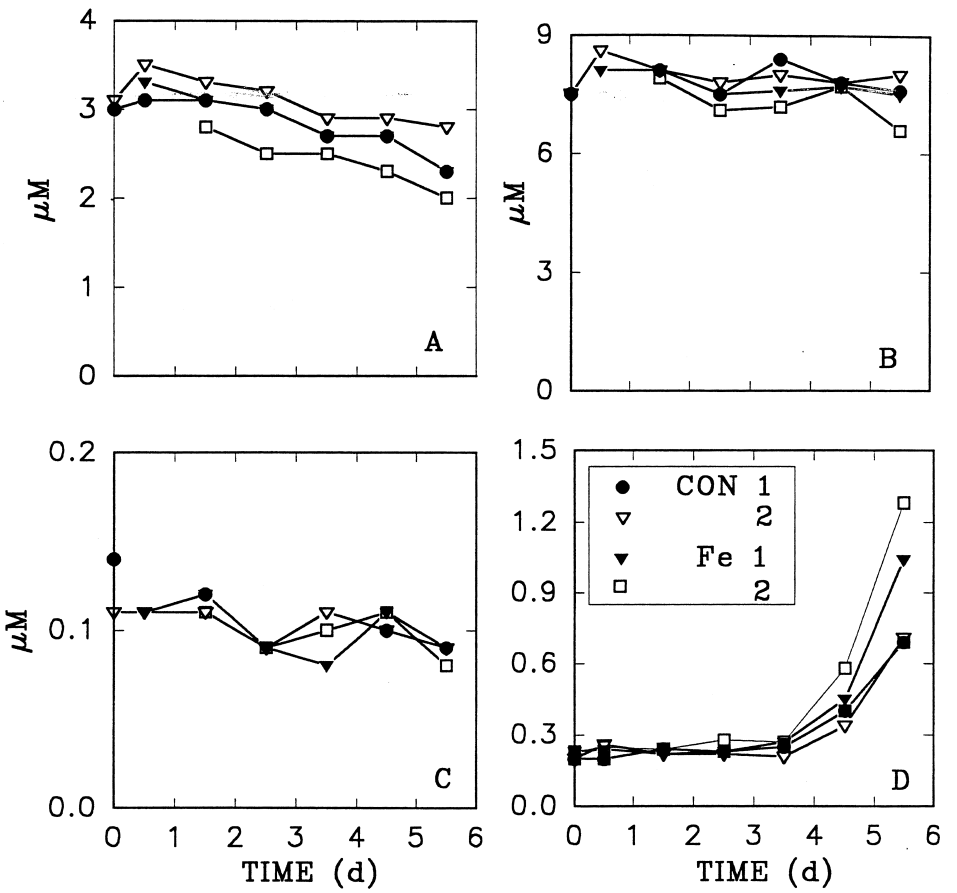

Fig. 3. Fe enrichment experiment at the intermediate station. Changes in (A) nitrate, (B) silicate, (C) ammonium and (D) chlorophyll *a* (μg l^{-1}) are shown. The were no replicates.

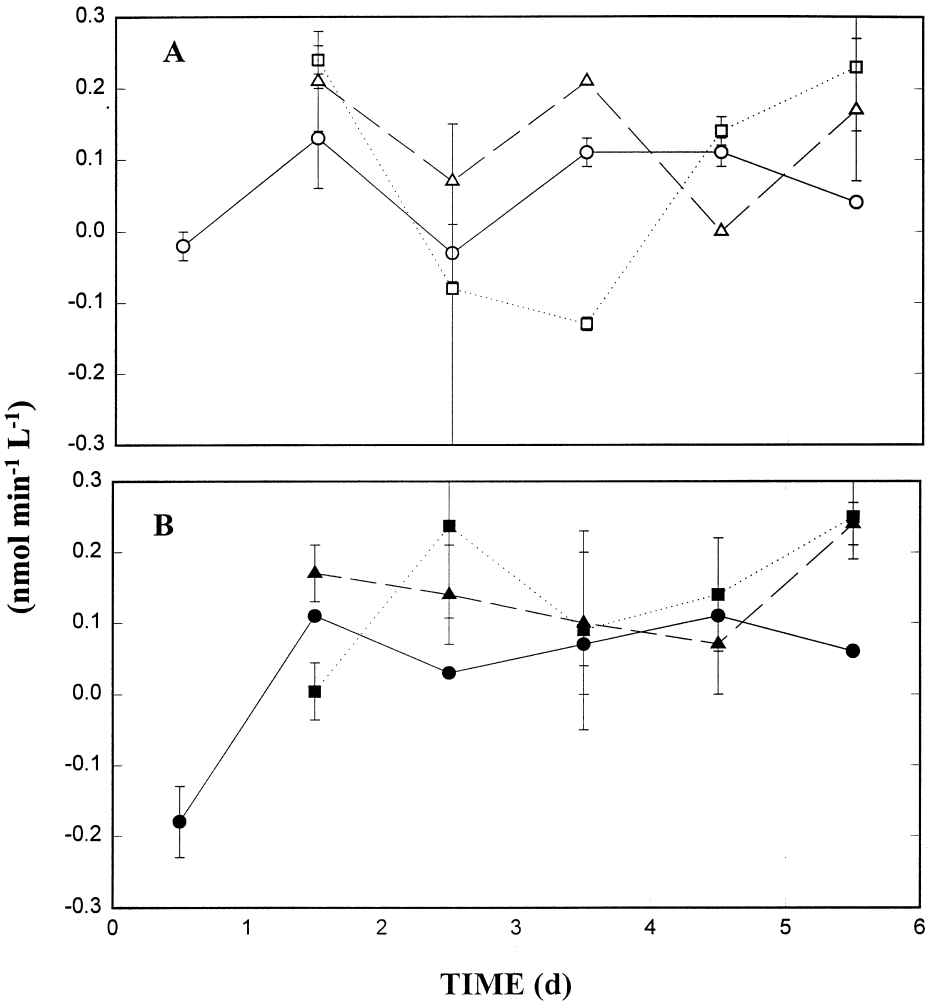

Fig. 4. Fe enrichment experiment at the intermediate station. (A) Control experiment (open symbols): NR (○), δNO_3^- (□) and δPN (△); (B) Fe enrichment (closed symbols): NR (●), δNO_3^- (■) and δPN (▲). The latter two were calculated based on changes in NO_3^- and PN over the previous 24 h of the experiment. The error bars represent the standard error of the mean ($n = 3$); some error bars are smaller than the symbol size.

relatively constant until 108 h had elapsed and, thereafter, showed up to twofold changes. No differences in macronutrient levels were found between C and Fe treatments. This was later attributed to inadvertent contamination (likely by shipboard dust); DFe levels in the C (now referred to as 1.8 nM Fe treatment) and Fe (now referred to as the 3.5 nM Fe treatment) carboys at the start of the experiment were 1.8 and 3.5 nM, respectively. Chlorophyll *a* increased by nearly tenfold in both the 1.8 and 3.5 nM Fe treatments (Fig. 5). dPN and dNO_3^- followed the trends observed for chlorophyll *a*

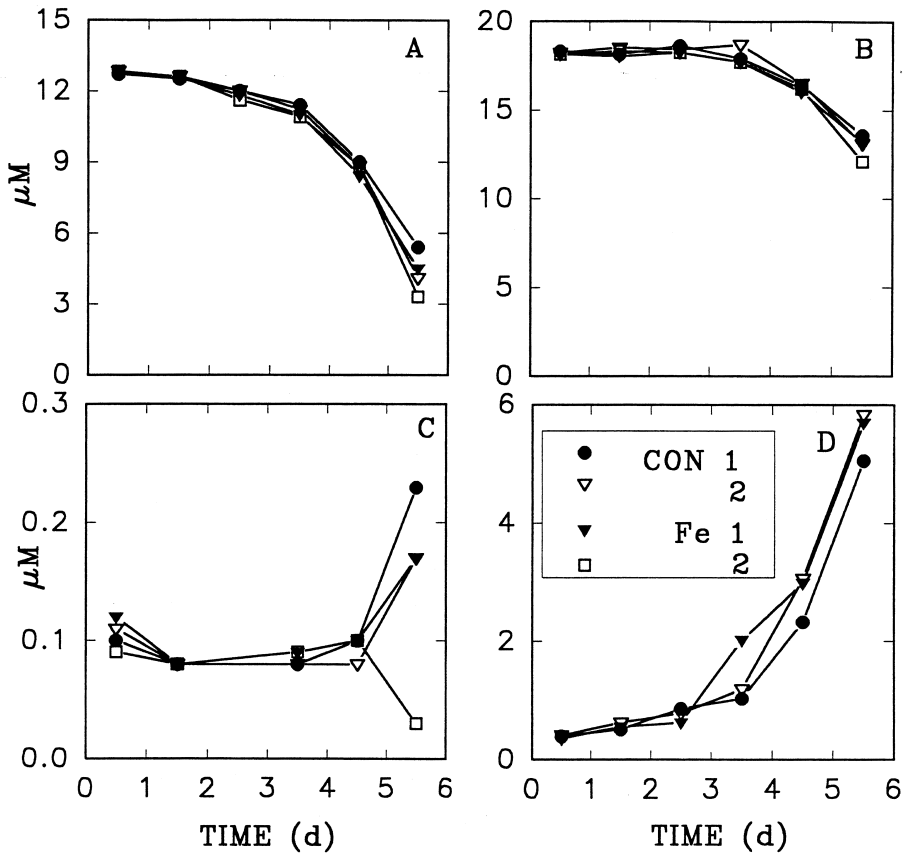

Fig. 5. Fe enrichment experiment at the offshore site. (A) nitrate, (B) ammonium, (C) silicate and (D) chlorophyll *a* ($\mu g\ l^{-1}$). There were no replicates.

(Fig. 6). NR activity followed patterns in dPN and dNO_3^-, but expressed in the same units, NR activity was usually significantly greater than dPN or dNO_3^-.

In the first enrichment experiment at the offshore site, there were distinct differences between fractionated F_v/F_m in 1.8 and 3.5 nM Fe treatments (Fig. 7). In the 1.8 nM Fe treatment, there was little change in F_v/F_m during the experiment. Values remained low (ca. 0.4) and there were no differences between fractions. In whole samples from the 3.5 nM Fe treatment, F_v/F_m increased after 36 h to nearly 0.8 and declined thereafter to the level observed for cells in the 1.8 nM Fe treatment. Changes in F_v/F_m in the 3.5 nM treatment differed between fractions; the >18 μm fraction reflected the pattern of the whole community, while only slight increases in F_v/F_m were observed in <5 and <2 μm fractions (Fig. 7). In the second enrichment at the offshore site, there was little change in F_v/F_m in whole samples or >18 μm fractions from the 1.8 nM Fe enrichment, but transient increases after 36 h in the <5 and <2 μm fractions (Fig. 8). In the 3.5 nM Fe enrichment, whole samples showed a pronounced and transient

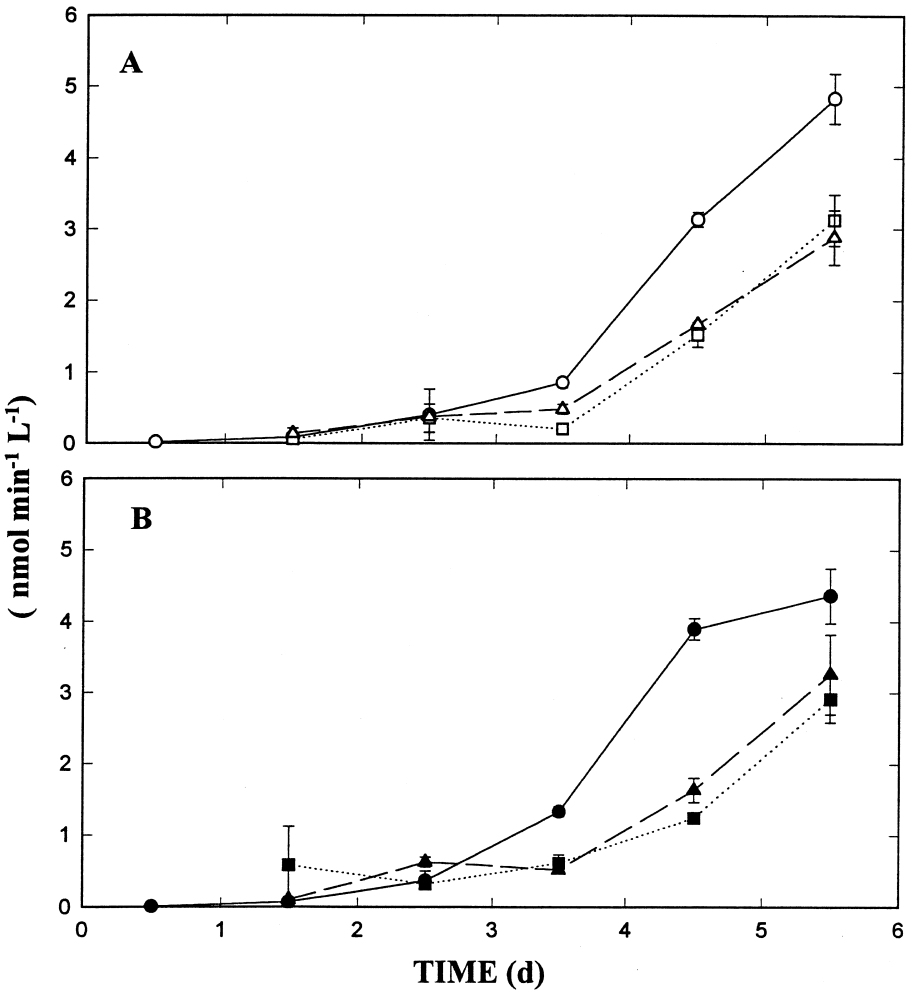

Fig. 6. Fe enrichment experiment 1 at the offshore site. (A) Control experiment (open symbols): NR (○), δNO_3^- (□) and δPN (△); (B) Fe enrichment (closed symbols): NR (●), δNO_3^-, (■) and δPN (▲). (δNO_3^- and δPN were calculated as for Fig. 5). The error bars represent the standard error of the mean ($n = 3$); some error bars are smaller than the symbol size.

increase in F_v/F_m at 36 h, as in the first enrichment, but, in contrast, the increase was observed in all size fractions.

4. Discussion

4.1. Evidence of heterogeneity in physical and chemical properties

There was evidence of marked changes in salinity from E–W in the NE subarctic

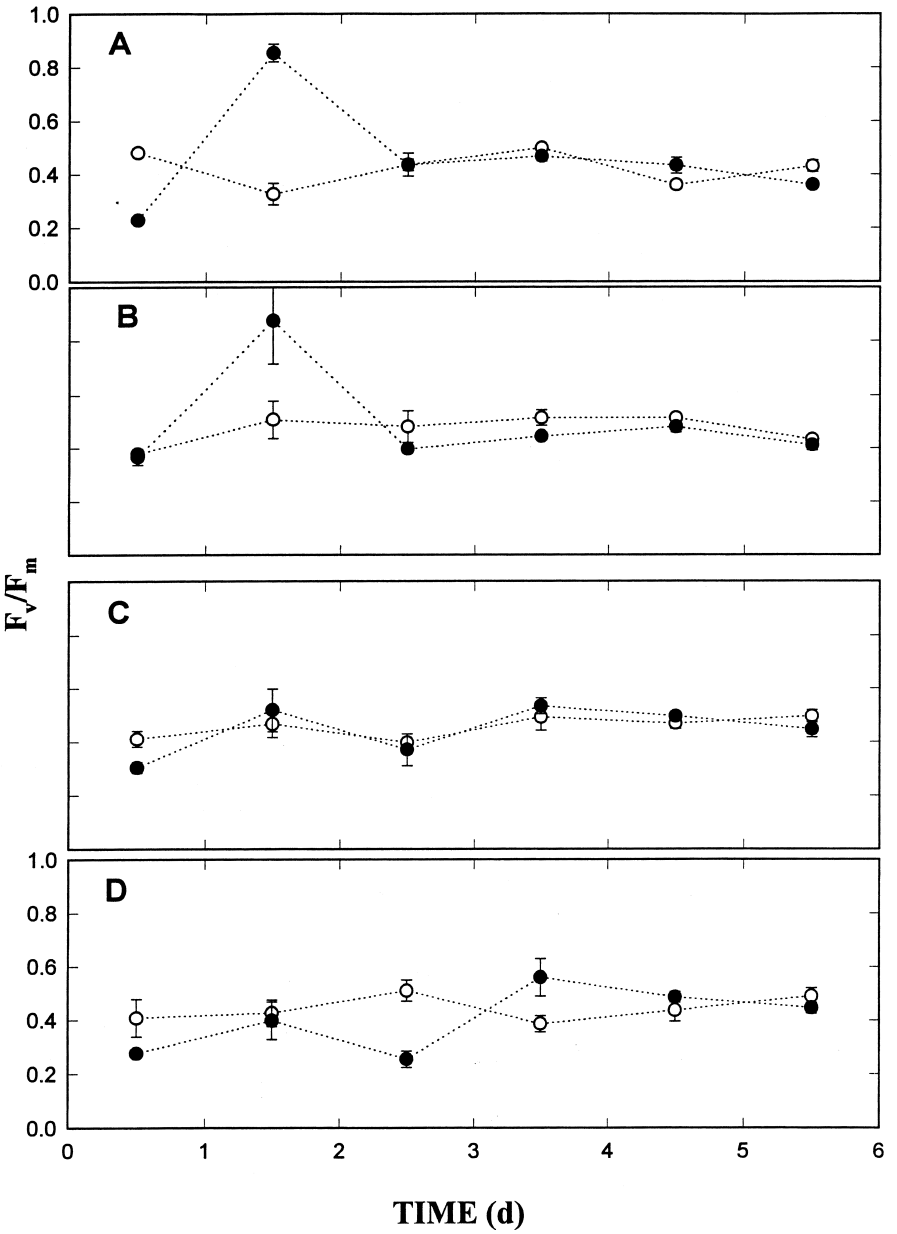

Fig. 7. Fe enrichment experiment 1 at the offshore site. Controls are represented by open symbols and Fe carboys by closed symbols. Quantum yield for photosystem II (F_v/F_m) for (A) whole community (>0.2 μm), (B) >18 μm, (C) <5 μm and (D) <2 μm). The error bars represent the standard error of the mean ($n = 3$); some error bars are smaller than the symbol size.

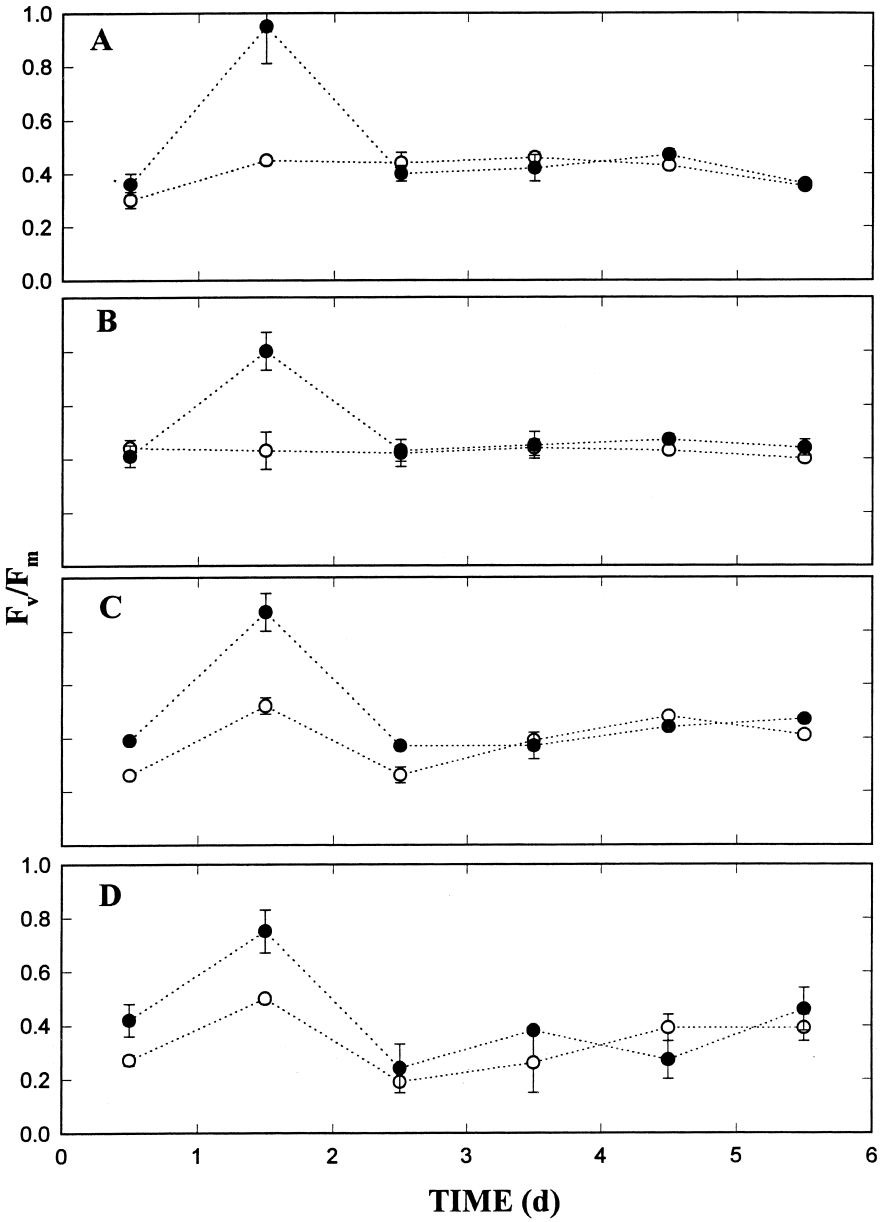

Fig. 8. Fe enrichment experiment 2 at the offshore site. Controls are represented by open symbols and Fe carboys by closed symbols. Quantum yield for photosystem II (F_v/F_m) for (A) whole community (>0.2 μm), (B) >18 μm, (C) <5 μm and (D) <2 μm). The error bars represent the standard error of the mean ($n = 3$); some error bars are smaller than the symbol size.

Pacific, likely due to the offshore movement of coastally influenced lower salinity waters (F. Whitney, pers. comm.). In addition, there appeared to be a relationship between salinity and nitrate, such that nitrate in the lower salinity water was <0.5 μM (i.e. at limiting concentrations in the absence of other N sources; Eppley et al., 1969). This low salinity region had relatively high DFe levels (2–3 nM kg^{-1}), again these were probably associated with the coastally influenced waters. Higher levels of chlorophyll *a* (ca. 1 μg l^{-1}) were observed in late winter in this region (Boyd, unpublished data) and are presumably supported by the availability of both macronutrients and DFe earlier in the year.

The intermediate site marks a transition from less saline low nitrate waters, to higher salinity waters that change little further west. Nitrate levels at the intermediate station are ca. 3 μM. La Roche et al. (1996) reported that DFe was ca. 2 nM kg^{-1} and, using a diagnostic biochemical marker for iron limitation (flavodoxin), they found that diatoms do not display signs of Fe-limitation at this station. Despite marked changes in physical and chemical properties, chlorophyll *a* remains relatively constant over the transect. It could be hypothesized that this is the result of the interaction of two limiting factors; nitrate to the E of the intermediate site, and DFe in the waters up to 250 km E of the offshore site (La Roche et al., 1996). However, in the region between the intermediate site and the waters 250 km to the west, cells appear to have sufficient macronutrients and DFe, thus, the reasons for the constancy in phytoplankton biomass is unclear. Autotrophic nanoflagellates are the dominant phytoplankton group along the transect. They may be ubiquitous in waters characterized by low DFe levels as they appear to have lower Fe requirements than larger cells, such as diatoms (Muggli and Harrison, 1996), and do not appear to respond to Fe-enrichment at the offshore site (Boyd et al., 1996). In addition, their dominance of phytoplankton biomass in the coastal ocean in late spring may result from these small cells preferentially utilising regenerated nitrogen (Boyd et al., 1996).

4.2. Fe-enrichment experiments at the iron-rich vs. iron-poor sites

The results of the Fe-enrichments at the offshore site were similar to those reported by others (Martin et al., 1989; Coale, 1991; Boyd et al., 1996): Fe enrichment led to increases in chlorophyll *a* and nitrate depletion. Fe contamination of C carboys in May 1995 was an unfortunate occurrence, but data from experiments (of identical design to the present study) in May 1993, May 1994 (Boyd et al., 1996) and May 1996 (Boyd, unpublished data) at this site provides repeated evidence of only minor decreases in nitrate and minor increases in chlorophyll *a* in control carboys over a six-day period. In the present study, increases in chlorophyll *a* in all carboys were predominantly due to tenfold increases in the large diatoms *Chaetoceros* spp. and *Pseudonitzschia* spp. in the first enrichment experiment and to sevenfold increases in pennate diatom species (<10 μm length) in the second. Floristic changes involving diatoms have been observed in previous Fe-enrichment studies at this offshore site (see Martin et al., 1989; Boyd et al., 1996).

It is likely that the endemic community is Fe-replete under ambient conditions at the intermediate site (La Roche et al., 1996). However, given the availability of nitrate (ca. 3

μM) at this station, the lack of response to Fe enrichment is puzzling. Buma et al. (1991) observed pronounced uptake of macronutrients by phytoplankton from a high DFe region of the HNLC Southern Ocean during an Fe enrichment. It might be hypothesized that the observed low phytoplankton biomass at the intermediate site is due to control by grazing. However, if this were the only factor, it does not explain the low F_v/F_m values that clearly suggest a physiological limitation of the population (see later). Examination of the phytoplankton species data (Table 1) suggests an alternative explanation. Diatoms, rather than autotrophic nanoflagellates and dinoflagellates, have been shown to be iron-limited in the NE subarctic Pacific (Martin et al., 1989; Boyd et al., 1996) and are thus the most likely group to respond to enrichment. At the intermediate site, there is a relatively low ''seed population'' of diatoms (Table 1), such that, even if growing at iron-induced elevated rates (1 division day^{-1}, see Boyd et al., 1996), more than five days would be required before populations equal to ambient levels at the offshore site were achieved. A limited seed source of bloom-forming diatoms has been suggested by Chavez et al. (1991) to influence the observed distribution of phytoplankton biomass in the Equatorial Pacific.

4.3. Photosynthetic efficiency of PSII photochemistry

The F_v/F_m values obtained along the E–W transect in the subarctic Pacific (Fig. 2C) are markedly lower than those typically found in nutrient-replete cells in either laboratory or natural populations (0.65 or higher, see Geider et al., 1993). Geider et al. (1993) found that diatoms starved of nitrogen or iron had F_v/F_m values of ca. 0.2 and 0.3, respectively. These are close to the values of F_v/F_m found at the coastal site (a nitrate-depleted region) and in the Fe-poor waters west of the intermediate site. While there is evidence that cells at stations 250 km west of the intermediate site are nitrogen-replete (Fig. 2) and Fe-limited (see La Roche et al., 1996), thus explaining the observed range of F_v/F_m, it is puzzling why cells at the intermediate site and the waters >200 km to the west, where neither nitrate nor DFe should be limiting, exhibit sub-maximal values of F_v/F_m. Although Coale (1991) did observe that the in vitro addition of manganese to phytoplankton at P26 led to increases in the magnitude of algal stocks, the distribution of manganese in N Pacific surface waters, albeit at lower latitudes, indicates that levels are >1 nM kg^{-1} to the east of the 130°W meridian (Orians and Bruland, 1986), the approximate longitude of the intermediate station. In addition, while there is little other micronutrient data available for this NE region, McKelvie and Orians (1993) reported a decrease in hafnium and zirconium levels (elements that have similar distributions to zinc, cobalt and copper; K. Orians, pers. comm.) with distance from the coast, a trend similar to that observed by La Roche et al. (1996) for DFe. It may be that the nanophytoplankton, which dominate the assemblage at the intermediate station, may be limited by physiological factors other than micronutrients, resulting in sub-maximal F_v/F_m and NR activities. At present, the nature of these factors is unclear.

The maximum value of F_v/F_m was higher in the present study than noted in other reports (Kolber and Falkowski, 1993; Kolber et al., 1994; Behrenfeld et al., 1996). The precise values of F_v/F_m obtained using the Turner fluorometer and DCMU may differ

numerically from those obtained using techniques such as the pump and probe method (with DCMU often giving higher results), but the results obtained using the two methods are normally highly correlated (see Geider et al., 1993; Berges et al., 1996). In laboratory cultures, the F_v/F_m value for diatoms by the DCMU method is often >0.72 (Berges et al., 1996).

In both of the in vitro Fe-enrichments at the offshore station, increases in F_v/F_m occurred transiently at 36 h. As the transient responses were consistent across two independent experiments and between replicates, it is unlikely to be a methodological error. These transient responses are similar to the pattern observed by Kolber et al. (1994) and Behrenfeld et al. (1996) during Ironex I and II in situ fertilisations in the equatorial Pacific, respectively. The elevated F_v/F_m values in the present study were not due to other factors such as high variability in background fluorescence; the raw background values obtained at this time were 2.20 ± 0.16 (±1 S.D.; $n=4$). The rapid decline in F_v/F_m over time in the enrichments at the offshore site was not observed in the equatorial Pacific by Kolber et al. (1994), who reported a gradual decrease from >0.6 to 0.45 over five days during the in situ enrichment. The reason for these different trends is not known. At the offshore station, this sharp decline suggests that the cells may be rapidly driven back into Fe limitation, despite evidence from other studies in this region (Boyd et al., 1996) that cells may have been iron-replete up until day four of such an enrichment. Indeed, during Ironex II, after an initial iron-mediated increase in F_v/F_m, subsequent Fe additions were needed to prevent a decrease in F_v/F_m (Behrenfeld et al., 1996).

In the in vitro Fe-enrichments at the offshore site, increases in F_v/F_m appear to reflect shifts in the phytoplankton community towards diatoms. Values of F_v/F_m for different sized fractions suggest that all cells, including those in the <2 or <5 μm size classes, grow sub-maximally offshore. This concurs with the observations of Kolber et al. (1994) from the equatorial Pacific, but appears to be at odds with the reports from Booth et al. (1988), Booth et al. (1993) and Boyd et al. (1996), that, based on a proxy for phytoplankton division rates, the small dominant phytoplankton are growing at close to maximal rates [based on Banse's curve (Banse, 1991b)] and are thought to be grazer-limited. Grazer limitation of small phytoplankton cells has also been demonstrated using conventional techniques in the Equatorial Pacific (Price et al., 1994). Interpretations of F_v/F_m may be problematic in some groups of algae of small size; Berges et al. (1996) and Kolber et al. (1994) noted that nutrient-replete cyanobacteria growing at maximal rates rarely have F_v/F_m values greater than 0.5. While Graziano et al. (1996) have reported on the non-linear functional relationship between F_v/F_m and algal growth rate in the N Atlantic under ambient conditions; clearly more work is required to compare phytoplankton growth rates and F_v/F_m under ambient and iron-enriched conditions.

It is interesting to note that different patterns of F_v/F_m were noted in the 1.8 and 3.5 nM Fe treatments, despite similar levels of chlorophyll *a* accumulation and macronutrient depletion. F_v/F_m may be responding to more subtle differences, such as DFe levels of 1.8 nM in C versus ca. 3.5 nM in Fe carboys, yet either level should be sufficient to alleviate Fe limitation (Coale et al., 1996; La Roche et al., 1996). One difference between the 1.8 and 3.5 Fe treatments might be the form in which the Fe was added or the use of EDTA; it is unlikely that the iron that contaminated the C carboys was

chelated, as in Fe carboys. However, at the onset of Ironex I, Fe added in a non-chelated form produced changes in F_v/F_m (Kolber et al., 1994). At present, we have no satisfying explanation as to why such differences in F_v/F_m values should be observed between treatments.

4.4. Fe supply, NR and NO_3 utilisation

In both the intermediate and offshore site enrichment experiments, NR activity and nitrate incorporation rates (as judged by disappearance from the medium, or increases in particulate nitrogen) follow each other closely. This suggest that Fe availability and nitrogen metabolism are strongly coordinated. This control could be mediated in several ways. Direct effects on the enzymes of nitrogen metabolism could occur, because both NR and the downstream enzyme, nitrite reductase (NiR), contain iron. Price et al. (1991) and Milligan and Harrison (1997) demonstrated in the equatorial Pacific and in laboratory cultures of *Thalassiosira weissflogii*, respectively, that NiR appeared to be more strongly affected than NR under iron-deplete conditions, in accordance with it requiring more Fe for synthesis. Indeed, the observation that NR activity exceeds nitrate incorporation rates (Fig. 6) might argue that it is the downstream enzyme (NiR) that is affected: cells might be reducing nitrate to nitrite but are unable to incorporate it, leading potentially to nitrite excretion. However, the absence of significant nitrite in samples (data not shown), and the fact that nitrate uptake (disappearance from the medium) corresponded to particulate nitrogen increases, suggests that this was not the case.

A second possibility is that Fe affects photosynthesis primarily, and nitrogen metabolism only secondarily. The photosynthetic electron transport system contains markedly more Fe than do the enzymes of nitrogen metabolism (Geider and La Roche, 1994). Fe effects on carbon would likely be reflected in nitrogen metabolism, due to the close regulation between C and N metabolism (Berges and Harrison, 1995a). There may also be a species-specific component to Fe effects on nitrogen metabolism. There is evidence that NR in *Emiliania huxleyi* is not so strongly affected by Fe deficiency (Timmermans et al., 1994; Muggli and Berges, unpublished).

In general, NR activity consistently exceeded incorporation rates during the enrichment experiments in the NE subarctic Pacific, despite quantitative relationships between these variables for certain diatom species (Berges and Harrison, 1995a). A diel pattern has been noted that appears to be related to the addition of FAD in NR assays. In diel studies conducted with Monterey Bay diatom assemblages, FAD apparently increased NR activity compared to dPN or dNO_3^- by up to twofold during midday periods, but not in early morning (Berges et al., 1996; Cochlan and Berges, unpublished). Sampling in the present study was performed near midday in all cases. In future, NR assays performed on samples collected over a diel cycle should carefully evaluate the effects of FAD.

4.5. Areal extent of NE subarctic Pacific HNLC waters

The findings of the present study indicate that the waters to the east of 130°W

meridian are not HNLC, and that the relatively low nitrate levels probably result in phytoplankton cells with low values of F_v/F_m during late spring. Other studies (La Roche et al., 1996) indicate that this eastern boundary for HNLC waters may be as far west as the 135°W meridian. Survey data (Frost, 1991; Boyd, unpublished data) to the W of the offshore site suggest that while there is up to twofold variability in phytoplankton stocks, this region is likely to be HNLC. Analysis of the Coastal Zone Color Scanner archive for this region (English et al., 1996) indicate that although the record is incomplete, due to considerable cloud cover, there appears to be low spatial and temporal variability in phytoplankton biomass in the area to the west of the offshore site. From data available on the distributions of surface nitrate along 50°N (Anderson et al., 1969), relatively high nitrate concentrations are observed to the west of 145°W until at least 160°W, the most westerly extent of the dataset. With regard to the N and S boundaries of HNLC waters, Martin et al. (1989) conducted measurements of macronutrients and micronutrients along a transect from 60–35°N. They observed that nitrate concentrations were >1 μM in the region between 55 and 42°N (see their Figure 2) and that DFe was <0.5 nM in the upper 500 m of the water column to the south of 48°N. It therefore appears possible to define the N, S and E boundaries of the NE subarctic Pacific HNLC region at 48°N, 42°N and 134°W, respectively. This probably represents a smaller region than may have been included in global estimates of the areal extent of HNLC regions.

5. Conclusions

The results of this study show that the relationship between DFe and phytoplankton stocks in the NE subarctic Pacific may be more complex than that reported for the S. Ocean (de Baar et al., 1995). The NE Pacific may be divided into three regions (i) from the coast westwards for ca. 500 km, where there appears to be sufficient micronutrients to permit the utilization of virtually all macronutrients early in the season. In May, the cells in this region have low values of F_v/F_m, presumably due to nitrate-limitation, (ii) a region at least 200 km to the west of the 130°W meridian in which there appears to be sufficient macronutrients and in which DFe is not limiting. Puzzlingly, the cells in this region have sub-maximal values of F_v/F_m. The reason for this is not known. (iii) To the west of the 134°W meridian, cells exhibit values of F_v/F_m of ca. 0.3, which is probably due to Fe-limitation. Coale et al. (1996) have also observed algal populations limited by either DFe or nitrate along a meridional transect in the equatorial Pacific. The areal extent of the HNLC waters of the NE subarctic Pacific are less than the size of the basin. Thus, care must be taken in the extrapolation of results and trends from individual stations in HNLC regions. A comparison of values for F_v/F_m derived from iron enrichment studies and from ambient conditions suggest that all cells in NE subpolar HNLC waters may be growing sub-optimally. This concurs with similar observations made in the equatorial Pacific, but is at odds with conventional measurements which indicate that small cells are growing at close to maximum rates in both regions and are grazer-limited. More effort is needed to resolve conventional growth rate estimates with quantum yield of fluorescence approaches.

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