Effects of iron enrichment on phytoplankton in the Southern Ocean during late summer: active fluorescence and flow cytometric analyses

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Abstract

Eight shipboard iron-enrichment experiments were carried out during the late summers of 1997 and 1998 in the Ross Sea and the Polar Front, respectively, as part of the US JGOFS Southern Ocean program. Using active fluorescence techniques (pump-during-probe flow cytometry/microfluorometry and fast repetition rate fluorometry) and flow cytometry, we examined responses of phytoplankton to iron enrichment over time scales of days. Results of both individual cell and bulk water measurements suggest that physiological iron limitation was widespread in the Ross Sea gyre in the late summer, but that in the region just south of the Polar Front other factors were limiting phytoplankton growth. In the five experiments in which responses to enrichment occurred, all the phytoplankton groups we examined, with the exception of cryptophytes, responded to iron enrichment by increasing normalized variable fluorescence (F_v/F_m) over several days. Normalized variable fluorescence of cryptophyte cells was typically higher than that of other cells and often near the maximum observed. Significant correlations were observed between ambient iron concentrations and normalized variable fluorescence at the beginning of each experiment, and also between ambient iron and the response of normalized variable fluorescence to enrichment. These relationships, which have not been previously documented, support the use of ambient active fluorescence measurements to predict iron-limiting conditions without conducting incubations. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The biomass and productivity of phytoplankton in Antarctic pelagic waters have long been noted as lower than expected in view of the high concentrations of major inorganic nutrients, even in the summer when light and water column stability appear to provide favorable environmental conditions for phytoplankton growth. It was suggested early on that trace nutrients might limit phytoplankton growth (Gran, 1931; Hart, 1934), and with the development of low-level trace metal measurement techniques it was found that iron concentrations can be very low in offshore regions of the Southern Ocean (Martin et al., 1988). Shipboard incubation experiments in which iron-enriched samples were compared with controls often showed that phytoplankton biomass, chlorophyll concentration or activity was limited by iron (de Baar et al., 1990; Martin et al., 1990; Buma et al., 1991; Helbling et al., 1991; van Leeuwe et al., 1997; Timmermans et al., 1998). In addition, a recent in situ enrichment experiment has provided even stronger evidence for the importance of iron availability in regulating primary productivity and phytoplankton biomass in the Southern Ocean (Boyd et al., 1999).

Although in situ enrichment experiments are preferable to shipboard incubations because they involve fewer manipulations, it is not likely that in situ experiments will become common because they are expensive and logistically difficult. Shipboard incubations, which are relatively easy and inexpensive, ought to allow more widespread and controlled investigations into selected aspects of phenomena such as iron limitation. Shipboard experiments have been questioned because of "bottle effects", such as the exclusion of large grazers that occurs when a small seawater sample is isolated. These types of bottle effects can be minimized by assaying the physiological state of phytoplankton cells, which should not be affected by grazing, rather than measuring biomass or chlorophyll accumulation.

We report here results of shipboard iron enrichment experiments carried out as part of the US JGOFS Southern Ocean program in the Ross Sea and the Polar Front in the late summers of 1997 and 1998, respectively. Our objective, in addition to documenting the extent and distribution of iron limitation in the Southern Ocean, was to investigate taxonomic diversity in responses to iron enrichment. To this end we examined properties of phytoplankton (including variability among size classes, taxa, and individual cells) from enriched and control bottles, using active fluorescence techniques that have been shown to be diagnostic for iron limitation, and flow cytometry.

1.1. Active fluorescence assays for phytoplankton iron limitation

Iron deprivation has been shown to cause structural changes in photosystem 2 (PS2) reaction centers such that they become non-functional, and to slow the photosynthetic electron transport which enables reaction centers to "turn over" (Greene et al., 1992; Falkowski et al., 1992; Falkowski and Kolber, 1995). When dark-adapted phytoplankton are illuminated, all of the functional reaction centers are "open", ready to receive an exciton, and their fluorescence yield is low (F_0) since maximal excitation energy can be efficiently used for photosynthesis. When a given

reaction center has received an exciton, it is "closed" and the associated fluorescence yield increases. When all the reaction centers are closed (or non-functional), fluorescence is maximal (F_m). For a phytoplankton cell in which all the reaction centers are functional, the maximum change in fluorescence yield is about 3-fold, while a cell with no functional reaction centers shows no change in fluorescence since its fluorescence yield is high from the beginning of the measurement. Variable fluorescence ($F_v = F_m - F_0$), when normalized to F_m, is thus an indicator of the proportion of PS2 reaction centers that are functional; F_v/F_m has been empirically determined to be about 0.65 for cultures of phytoplankton growing under optimal conditions (e.g., Kolber et al., 1988). The fact that F_v/F_m is affected by nitrogen as well as iron limitation was not a source of ambiguity in this work because there were high concentrations of available nitrogen at all the stations we examined (nitrate $> 10\,\mu M$). Silicate was also present at relatively high concentrations ($> 10\,\mu M$ at all experimental stations), so growth of diatoms should not have been limited by macronutrients. An overview of surface nutrient distributions is given in Smith et al. (2000).

The fast repetition rate (FRR) fluorometry approach has been used for monitoring iron-enrichment bottle experiments (Greene et al., 1994) and in situ iron-enrichment experiments (Kolber et al., 1994; Behrenfeld et al., 1996) to detect iron-induced changes in photosynthetic parameters in natural phytoplankton assemblages. These measurements were of bulk water samples, so their results represent weighted average properties of all the fluorescent particles present in a sample. Since cells of different types or with different histories may respond to a given environment in different ways, we have expanded these active fluorescence techniques to examine individual cells. These individual cell measurements should allow us to examine the occurrence and consequences of differences in iron requirements as well as the importance of potential artifacts caused by fluorescence from non-photosynthetically active material (dead cells, detritus, fecal material, grazers with ingested phytoplankton cells). For example, in a bulk measurement, an increasing proportion of detrital fluorescence and an increasing degree of N-limitation would both appear as a decrease in photosynthetic performance; measurements made on a cell-by-cell basis would be unaffected by fluorescence from detrital material.

Conventional flow cytometry also has been used to investigate the responses of phytoplankton in natural populations to iron-enrichment (Zettler et al., 1995; Cavender-Bares et al., 1999). These measurements of individual cell optical properties can detect changes in cell size and pigment content, as well as in the abundance of several broad groups of cell types.

In this paper we will describe results of several iron-enrichment experiments, first addressing bulk water (or average cell) properties and then group- and genus-specific characteristics. Since we did not have bulk active fluorescence measurements (i.e., FRR fluorometry) on the Ross Sea cruise, we will present data from the Polar Front cruise in which bulk water and single cell measurements were performed on the same samples; these data confirm that the two approaches lead to similar conclusions about iron limitation. Finally, we will discuss the relationships between measured ambient iron concentrations, the observed active fluorescence characteristics of the phytoplankton, and the responses of the phytoplankton to iron-enrichment.

2. Methods

The work described here was carried out as part of the US JGOFS Southern Ocean program, in the Ross Sea (R/V *Nathaniel B. Palmer* Process II cruise NBP97-1) and south of the Polar Front (R/V *Roger Revelle* Process II cruise KIWI-09) in the summers of 1997 (January–February) and 1998 (February–March), respectively. In the Ross Sea we used a pump-during-probe (PDP) flow cytometer to assay populations of phytoplankton cells distinguishable on the basis of cell size (from side scattering) and fluorescence, and a microscope-based PDP microfluorometer to analyze individual cells of selected taxa. Details of these instruments and their operation are described elsewhere (Olson et al., 1999) so they will be reviewed only briefly here. In both instruments, a dark-adapted cell is initially detected using infrared light (which is not absorbed by photosynthetic pigments). The cell is then illuminated by a 150-μs flash of light whose intensity is such that all the photosystem II reaction centers are filled by photons within 30–100 μs. The time course of fluorescence induction is measured and fitted to a model of photosynthetic electron transport to estimate the photosynthetic parameters (Olson et al., 1996). Since the induction curves from individual small cells are noisy, we typically measure each cell 50 times (at 2 Hz) in the microfluorometer. In the flow cytometer, it is not possible to do this, so the data for small cells must be analyzed by combining signals from populations of cells with similar light scattering and fluorescence characteristics (chosen by the operator). Populations of cells as small as 2 μm diameter can be measured with the PDP flow cytometer and as small as 5 μm with the PDP microfluorometer. During PDP microfluorometry we used the IR-illuminated video images to identify cells to the genus level (Tomas, 1997), so in this paper only genus names will be used. Both PDP instruments were equipped with sample cooling systems (− 2°C water jacketed).

The PDP flow cytometer was modified between the Ross Sea and Polar Front cruises. Measurements of forward light scattering were added for the Polar Front cruise, which means we were able to distinguish pennate diatoms from other cells (Olson et al., 1989). In addition, the PDP laser modulator was placed at the output of the laser for the Polar Front cruise (rather than after a beamsplitter as in Olson et al., 1999) to increase the extinction ratio of the PDP beam and thus decrease the background excitation level. This change also caused an increase in the duration of the PDP induction curve collected from about 120 to 150 μs.

In addition to the single cell instruments, on the Polar Front cruise we also used a FAST[tracka] FRR fluorometer (Chelsea Instruments, Ltd.) to monitor bulk water samples. The FRR and PDP measurements of active fluorescence utilize similar excitation time scales and are thus expected to yield comparable results for variable fluorescence, and on the cruise in the Polar Front, where we used both methods, we found good agreement between them (see below). The FRR fluorometer was set up in the laboratory with a custom glass cuvette positioned in one chamber of the instrument. The instrument was covered with black optical cloth to block room light during the measurements and background signals (measured on CTD casts in deep waters where fluorescence was below detection) were subtracted from all fluorescence measurements. Excitation conditions were set to provide 100 flashlets (∼ 1 μs dura-
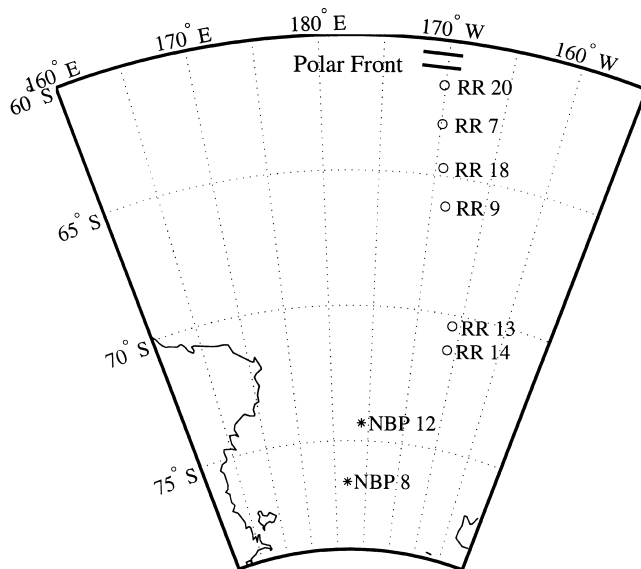
Fig. 1. Locations of stations at which enrichment experiments were carried out. Asterisks = Ross Sea cruise, January–February 1997; circles = Polar Front cruise, February–March 1998. The approximate location of the Polar Front during the 1998 cruise is indicated.

tion) spaced approximately 2.8 µs apart, followed by 20 additional flashlets 50 µs apart. The latter set of flashlets are designed to assay the decay of fluorescence yield back to F_0 (which is used to calculate reaction center turnover time); these results are not discussed here. On most samples, this sequence was repeated 320 times at a rate of $1 \, \text{s}^{-1}$ and the results were averaged. This signal averaging increased the signal : noise ratio, but was not absolutely necessary. Examination of individual flash sequences yielded results similar to those of the averaged results, and no trends in fluorescence parameters were observed during the measurements. To correct for variations in detector response during the 120 flashlet sequence, all fluorescence yields (signals divided by reference measurements of the excitation source) were scaled such that the yield for each flashlet was the same when analyzing chlorophyll extracted in methanol. Induction curve parameters (including F_v/F_m) were determined based on the model of Kolber et al. (1998) using software provided by Chelsea Instruments (FRS v. 1.4).

In the Ross Sea we monitored two iron-enrichment experiments, at stations 8 and 12 (Fig. 1). These experiments were carried out by the Moss Landing group, who also measured dissolved ($< 0.4 \, \mu\text{m}$) iron and zinc concentrations in the initial samples according to the methods described by Johnson et al. (1997). The experiments were incubations of 20-l samples (obtained using trace-metal-clean Go-Flo bottles; Fitzwater et al., 1982) from 20-m depth, consisting of two controls and six treatments: iron (added as $FeCl_3$) at 4 levels ranging from 0.2 to 2.5 nM, Zn (5 nM) and Zn + Fe (5 and 2.5 nM, respectively). The samples in polycarbonate carboys were cooled with flowing surface seawater and exposed to natural sunlight in a clear Plexiglas-covered incubator (which represents a significant increase in light intensity over in situ

conditions at 20m). Each carboy was sampled every 48 h using filtered pressurized gas to expel samples from the carboys.

On the Polar Front cruise we carried out six iron-enrichment experiments, using samples from the 50% light depth (10–30 m) collected with a "trace metal clean" plastic-coated Go-Flo rosette. Dissolved (< 0.22 μm) iron and zinc concentrations were determined by C. Measures (Measures et al., 1995). Trace-metal cleaned polycarbonate sample bottles (250 ml) were incubated under simulated in situ conditions using plastic window screen to attenuate surface sunlight. To avoid contaminating the bottles by subsampling during the incubations, we prepared for each experiment a number of individual control and iron-enriched (4 nM $FeCl_3$) bottles; at each sampling point a set of control and enriched bottles (typically two replicates of each) were sacrificed. Dark adapted (>15 min) water samples from the incubation experiments were analyzed directly on the PDP flow cytometer and in the FRR fluorometer, but were concentrated (300 to 5 ml) by gravity over 5 μm Nitex before analysis on the microfluorometer. Samples were kept on ice in dim light (< 0.1 μmol photon $m^{-2} s^{-1}$) during manipulations.

Five milliliter samples were preserved with 0.1% glutaraldehyde and stored in liquid nitrogen for post-cruise analysis of the abundance and size distribution of phytoplankton by conventional flow cytometry (Olson et al., 1993). These samples were analyzed using a modified Coulter EPICS flow cytometer in which each optical signal was split and measured by two photomultipliers at different gain settings, which increased the dynamic range of the measurements (allowing cells ranging in size from approximately 1 to 40 μm to be analyzed simultaneously). Forward light scattering (FLS) was used to estimate cell size through an empirical relationship between FLS and cell volume for a number of marine phytoplankton cultures (Shalapyonok et al., 2000). Cell volume of pennate diatoms (for which forward light scattering in our instrument severely underestimates cell volume; Olson et al., 1989) was estimated from chlorophyll fluorescence of the cells, assuming the same relationship between fluorescence and volume as in other phytoplankton cells in the same sample. While we recognize that this adjustment is subject to uncertainty, we note that the adjusted data (converted to carbon according to a modified Strathman relationship as in Shalapyonok et al. (2000)) were well correlated with estimates of pennate diatom carbon from microscopic counts for the Ross Sea cruise (data from W. Smith) (r^2 for the log transformed data = 0.90, slope = 1.3).

3. Results and discussion

3.1. Ross Sea cruise: responses to enrichment

Both iron-enrichment experiments in the Ross Sea indicated that the phytoplankton were released from iron limitation by additions of various levels of iron. At station 8, F_v/F_m of the small cell population (∼ 2–5 μm, estimated from flow cytometric light scattering) in the iron addition bottles (as well as control #2) increased gradually for at least the first 6 days, while control #1 and the zinc-enriched sample did not change (Fig. 2). The time course of F_v/F_m in control #2 was similar to those of
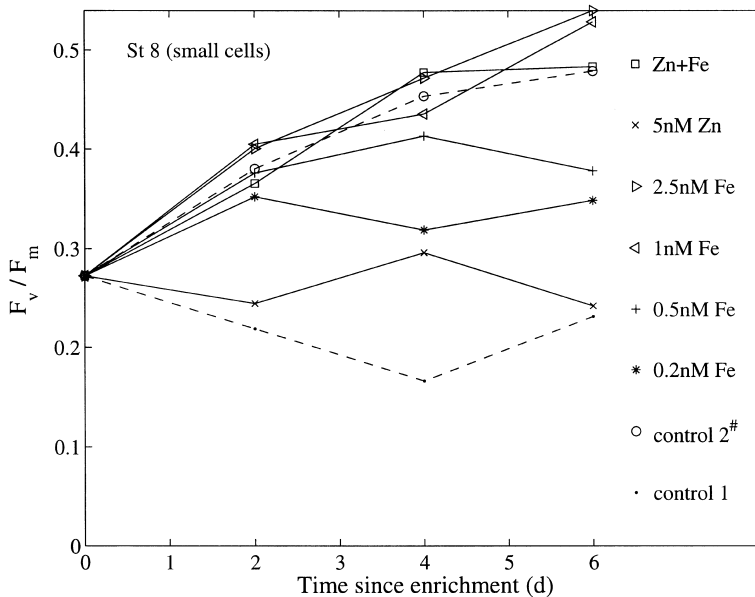
Fig. 2. Effects of iron enrichment on F_v/F_m of small cells at station 8 in the Ross Sea, as analyzed by PDP flow cytometry. Each of eight incubation bottles was sampled every 2 days. The bottle identified as "control $2^\#$" was apparently contaminated with iron (see text).

the higher-level iron-enrichments, suggesting that this bottle had been contaminated. This suggestion is supported by the fact that the initial measured dissolved iron concentration in control #2 was 0.15 nM, while in control #1 and the Zn bottle the initial iron concentration was 0.03 nM (S. Fitzwater, pers. comm.). F_v/F_m values in the bottles with the highest levels of iron addition (1 and 2.5 nm Fe and 2.5 nm Fe + Zn) were higher than those of the lower enrichments (0.2 and 0.5 nM), and these in turn were large compared to control #1 and the zinc treatment, which remained low throughout the experiment (both comparisons significant; t-test; $p < 0.01$). This pattern was generally apparent by the first sampling point at day 2, and was magnified at later times. It is interesting to note that the response of control #2 (whose measured iron concentration, though elevated, was lower than any of the intentional iron additions) was similar to those of the highest level additions. This suggests a difference in availability between the iron inadvertently introduced to this bottle and that intentionally added as $FeCl_3$ to the other bottles.

The second enrichment experiment (at station 12) also suggested iron-limiting conditions (Fig. 3), although the responses were not as dramatic as at station 8. The initial level of F_v/F_m was slightly higher than at station 8, and although F_v/F_m in the iron-enriched bottles reached levels similar to those of the first experiment, the change took longer, and F_v/F_m values in the lowest iron-enrichments (0.2 and 0.5 nM) were not significantly higher than in the controls ($p = 0.11$). In addition, although F_v/F_m did not increase in either of the control bottles in this experiment, the zinc-only addition did respond (in contrast to the experiment at Station 8). However, this
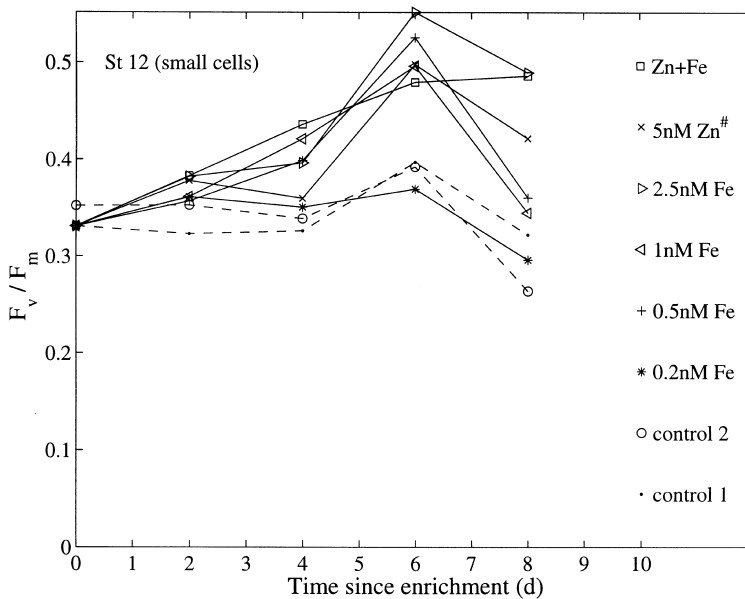
Fig. 3. As for Fig. 2, but for station 12. The bottle identified as "5 nM Zn#" was apparently contaminated with iron (see text).

probably reflects contamination rather than a co-limitation by iron and zinc, since the measured initial iron concentration in the Zn bottle was 0.12 nM (compared to 0.03 and 0.04 for controls 1 and 2, respectively; S. Fitzwater, pers. comm.).

Responses to enrichment also were observed in optical properties of the entire phytoplankton community as measured by conventional flow cytometry (though these were not apparent as early as the patterns in active fluorescence), and eventually in cell abundances (Figs. 4 and 5). At both stations the mean cell size of the phytoplankton increased in the bottles enriched to more than 0.2 nm Fe, while remaining nearly constant in the other bottles (again excepting the contaminated control #2 at station 8 and the contaminated Zn bottle at station 12). Mean chlorophyll fluorescence of both enriched and control cells initially declined at station 8, but the decline was smaller in the enriched bottles (> 0.2 nm Fe) than in the controls, and by the sixth day mean cell fluorescence was higher in the enriched bottles than in control bottle 1 and in the zinc-enriched bottle (chlorophyll fluorescence per cell in control bottle 2 was similar to that in the high-enrichment iron bottles). As with F_v/F_m, the degree of increase in fluorescence per cell was positively correlated with the enrichment level. The initial decrease in fluorescence likely reflects photoacclimation, since the original sample was probably acclimated to a lower light intensity than provided in the on-deck incubator. At station 12 fluorescence per cell increased more in the enriched bottles than in the controls, but there was no systematic trend with enrichment level.

At both stations 8 and 12, cell concentration increased in both control and enriched samples (possibly due to exclusion of grazers from the bottles), although at station 12
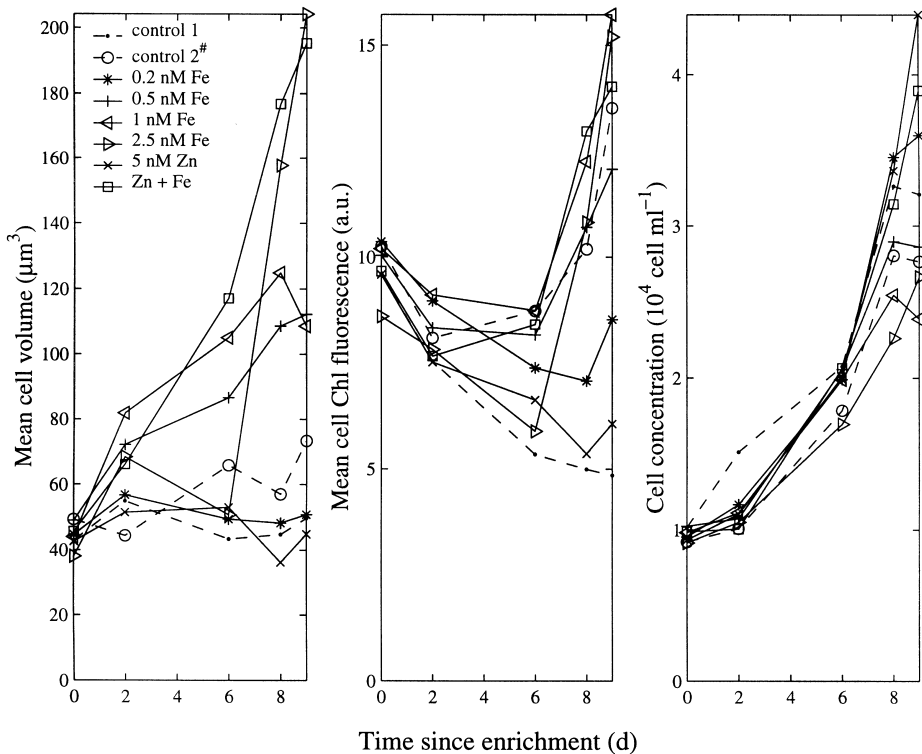
Fig. 4. Mean cell volume, mean cell chlorophyll fluorescence (relative to standard beads), and cell concentration of samples from the enrichment experiment at station 8 in the Ross Sea, as obtained by conventional flow cytometry of 5-ml frozen samples. The bottle identified as "control $2^\#$" was apparently contaminated with iron (see text).

the increase did not begin for several days. When comparing total numbers of cells, controls and enriched bottles were similar until late in the experiments, but the size distributions diverged sooner. For example, in the experiment at station 8 (Fig. 6), the size distribution of the phytoplankton in control bottle 1 did not change by very much (though the number of cells increased), while the enriched cells' size distribution began to change by day 2 and was dramatically skewed toward larger cells by day 6 (indicating a shift in community composition).

3.2. Polar Front Zone cruise: responses to enrichment

Active fluorescence in iron-enrichment experiments during the Polar Front zone cruise were analyzed both with bulk (FRR) and single-cell (PDP) methods, at stations ranging from latitude 62 to 71 S (see Fig. 1). The F_v/F_m results obtained by the two instruments were well correlated (Fig. 7), which increases our confidence in the results (especially for those of the earlier Ross Sea experiments, for which only PDP measurements were available). It also suggests that fluorescence from dead cells or detritus did not affect the results of bulk measurements of physiological state. Of the
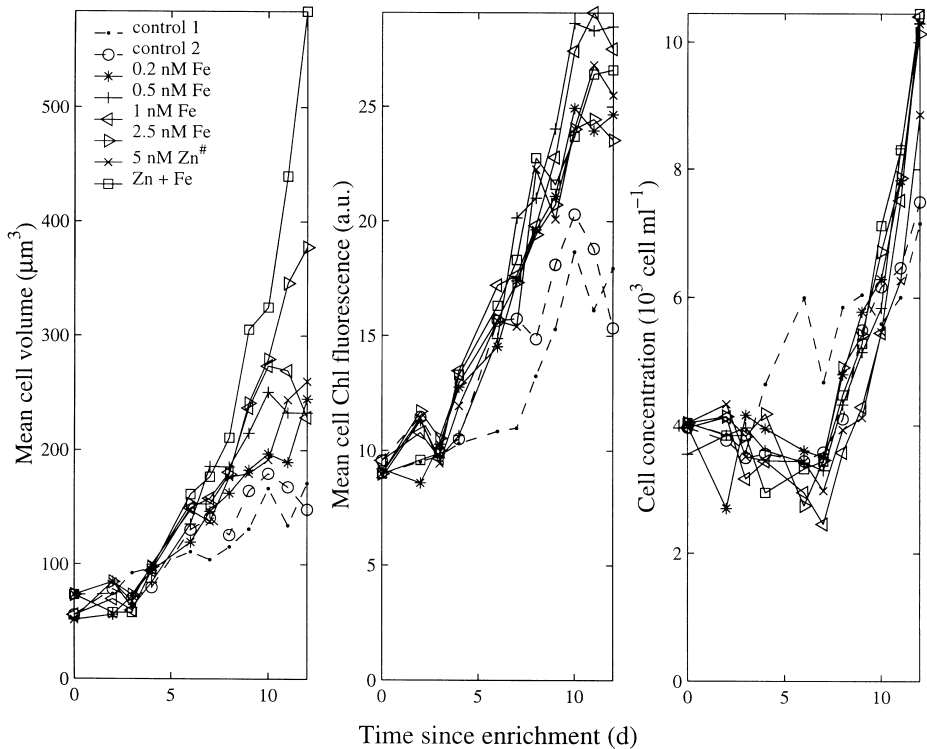
Fig. 5. As for Fig. 4, but for station 12. The bottle identified as "5 nM Zn$^{\#}$" was apparently contaminated with iron (see text).

six enrichment experiments, three (stations 9, 13 and 14) responded to iron-enrichment similar to those in the Ross Sea, with significant increases in F_v/F_m in enriched bottles relative to controls by day 2 (*t*-test, $p < 0.005$), and small or no increases in the controls (Fig. 8). In all three experiments the increase in F_v/F_m was maximal by day 4; in two of the three, F_v/F_m then declined.

In the other three experiments, F_v/F_m in iron-enriched bottles was similar to that in control bottles. In one experiment (station 7, just south of the Polar Front), both control and iron-enriched bottles showed increases in F_v/F_m. This suggests a relatively high iron concentration in the initial water, and may have been due to contamination, although it could have been natural (note that the iron concentration measured at this station was the highest of any experimental station, 0.22 nM). In the two experiments in which no response was seen (stations 18 and 20), relatively little rise in F_v/F_m of the controls was observed (Fig. 8).

Conventional flow cytometry revealed a similar trend in responses among stations, although the results were much less obvious (Fig. 9). Cell size and chlorophyll fluorescence per cell both appeared to increase in iron-enriched bottles relative to controls at stations 9, 13 and 14 (but not at stations 7, 18 and 20). The only statistically significant difference between enriched and control bottles, however, was in fluorescence per cell for the combined data from stations 9, 13 and 14 (*t*-test, $p < 0.05$). Cell
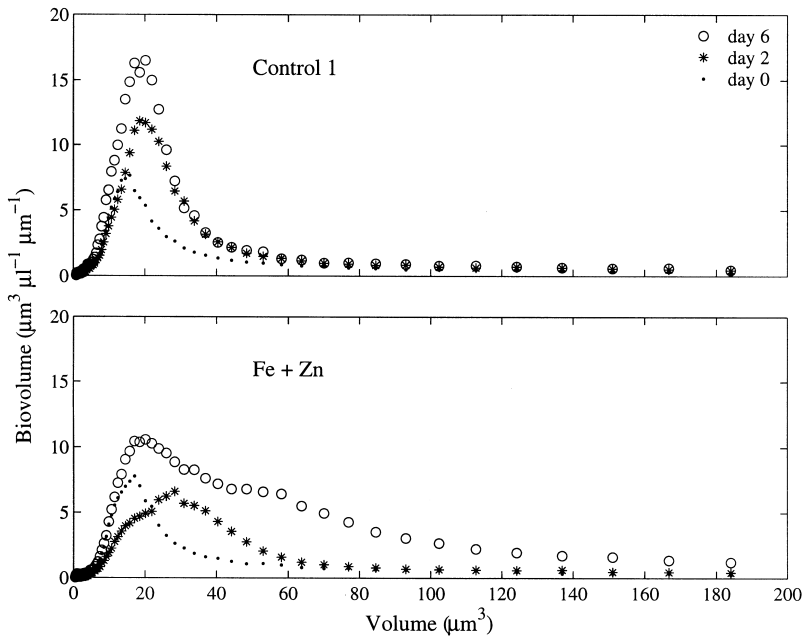
Fig. 6. Size distributions of phytoplankton in control bottle 1 and the Fe + Zn bottle during the enrichment experiment at station 8 in the Ross Sea.

numbers did not change systematically in response to enrichment during these 6-day experiments. Previous workers in the Southern Ocean also observed little effect of iron-enrichment in flow cytometric measurements of short-term incubations (Timmermans et al., 1998).

Both mean cell size and chlorophyll fluorescence were high at the stations where responses to enrichment were observed (Fig. 9), suggesting that a difference in phytoplankton community composition may have been related to the iron conditions. The absolute abundance of cells, however, does not follow this pattern completely. At the two southernmost stations (13 and 14), which exhibited large responses to enrichment, cell abundances were high, but at station 9 (farther north), a large response also was observed even though cell abundance was similar to the stations where little response was seen. The surface mixed layer tended to extend deeper near the Polar Front than farther south (data not shown), so we might speculate on light limitation as a factor regulating biomass accumulation. For these six stations, however, there was no significant relationship between mixed-layer depth and either biomass or response to enrichment.

3.3. Responses of different phytoplankton size classes and taxa to iron-enrichment

In addition to determining the extent of iron-limiting conditions in the Southern Ocean, we hoped to learn something about effects of such conditions on phytoplankton taxonomic composition through single cell measurements of active fluorescence.
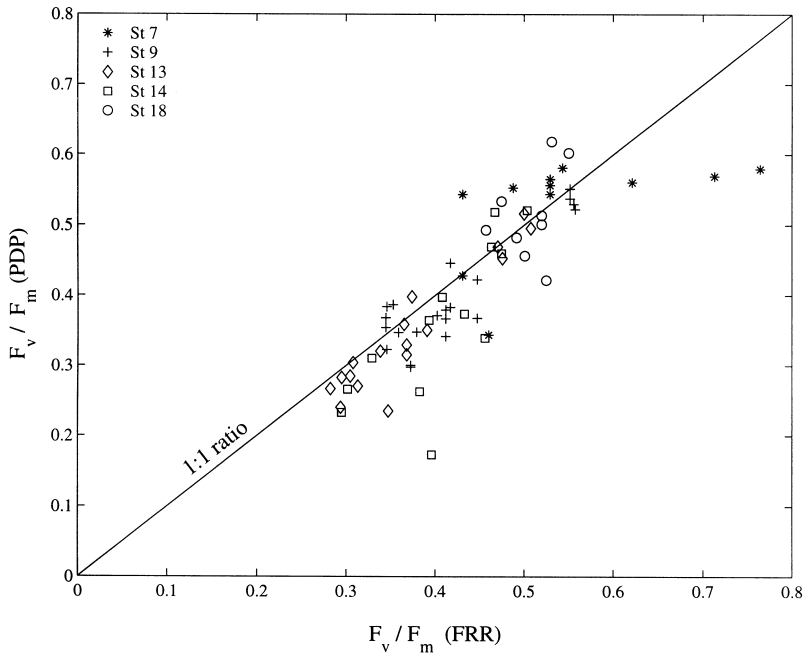
Fig. 7. Comparison of normalized variable fluorescence estimates from FRR and PDP flow cytometry (considering all the phytoplankton as a single population), from analyses of 5 enrichment experiments in which both instruments were used ($n = 72$, $r^2 = 0.85$).

These measurements consisted of PDP flow cytometric comparisons of different groups distinguished by light scattering and fluorescence signatures and PDP micro-fluorometry of visually identified individual cells.

3.3.1. Analysis of phytoplankton groups by PDP flow cytometry

The comparison of small ($\sim$ 2–5 μm) and large cells by the PDP flow cytometer was hampered by an apparent artifact in the measurement of the large cells. Inspection of fluorescence induction curves from individual large phytoplankton cells (as identified from side scattering measurements) revealed that fluorescence from some cells continued to increase throughout the measurement period and never saturated. Although it is conceivable that such signals could come from cells with very low absorption cross sections, the fact that in extreme cases the signals increased by more than 3-fold suggests that these signals are artifacts, possibly related to incorrect normalization to the excitation time course caused by difference in sizes (or velocities in the flow cell) of cells and the beads used as reference particles. We attempted to eliminate the artifactual signals by inspection of each individual cell curve (looking for signals which never saturated). We believe this was successful for the Polar Front data, since the total population F_v/F_m results from the PDP flow cytometry were well correlated with the bulk measurements of the FRR fluorometer (see Figs. 7 and 8). During the Ross Sea cruise, however, we collected shorter PDP induction curves than during the Polar Front cruise (120 vs. 150 μs), which made the identification of
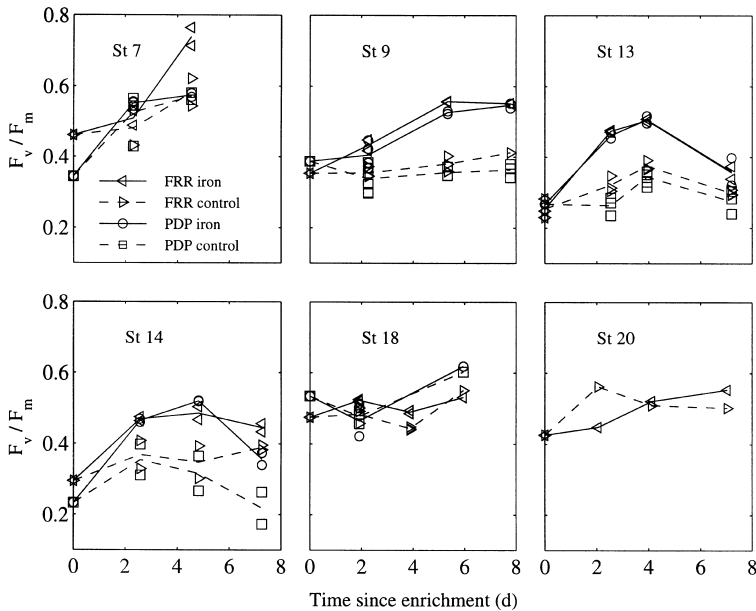
Fig. 8. Normalized variable fluorescence of iron enrichment experiments at 6 stations during the Polar Front cruise, measured with both FRR fluorometry (bulk samples) and PDP flow cytometry (considering all cells as a single population). When available, results from replicate bottles are indicated as separate symbols with lines connecting the mean values.

artifactual signals more difficult, and we therefore have reported only the data for small cells from the Ross Sea cruise.

When flow cytometric signatures were analyzed after subdivision into populations of pennate diatoms, cryptophytes, other small cells (approximately 2–5 μm diameter), and larger cells, only the cryptophytes consistently differed in fluorescence induction characteristics (Fig. 10; Olson et al., 1999). Cryptophytes almost always had high initial F_v/F_m (> 0.5), and showed only minor responses to iron addition. We do not know why these cells were so different from all the others. Is it just a coincidence that cryptophytes are also unique in that they use phycoerythrin as a light harvesting pigment? Since absorption of 488 nm light by phycoerythrin is less efficient than by the accessory pigments of most other phytoplankton, the functional absorption cross section of cryptophytes appears lower than that of other kinds of cells in our assays. This causes the rise in fluorescence yield to be slower in these cells, but should not affect the measurement of variable fluorescence as long as saturation is reached during the measurement, as it typically was (e.g., see Fig. 11 in Olson et al., 1999). Perhaps a more likely explanation for the apparent lack of iron limitation in the cryptophytes is their ability to carry out phagotrophy, which could serve as a means of obtaining iron when dissolved iron concentrations are low (Maranger et al., 1998). Although some other phytoplankton groups also can capture particles, any effect of these cells (which have "typical" pigments) on average F_v/F_m would be diluted by all the other (non-phagotrophic) cells grouped together during our analysis; cryptophytes with their unique pigment signature can be examined independently.
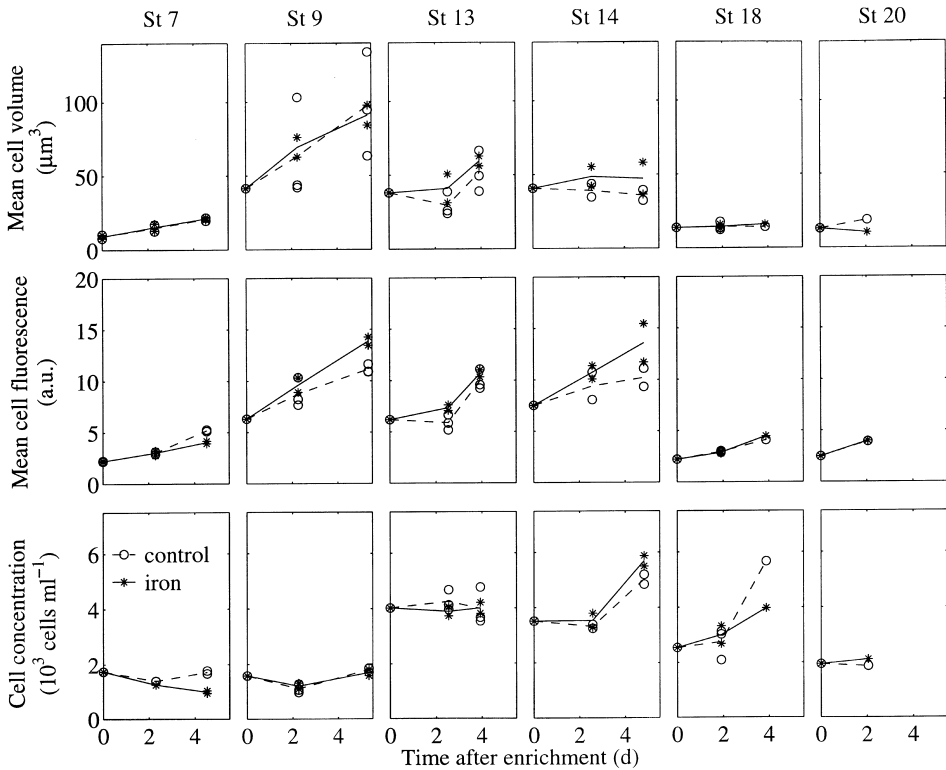
Fig. 9. Mean cell volume, mean cell chlorophyll fluorescence (relative to standard beads), and cell concentration of phytoplankton from enrichment experiments on the Polar Front Cruise, as obtained by conventional flow cytometry of 5-ml frozen samples. When available, results from replicate bottles are indicated as separate symbols with lines connecting the mean values. Data are shown only for about the first 4 days of the experiment, but most features were apparent by then.

3.3.2. Analysis of individual cells by PDP microfluorometry

In the Ross Sea enrichment experiments, measurements of individual cells larger than 5 μm showed the same qualitative trends as the flow cytometric analyses of small cells (Fig. 11). When all cells measured in the station 8 experiment were considered, the mean F_v/F_m for the 2.5 nM Fe enrichment was significantly higher than for control bottle 1 (t-test, $p < 0.01$). Similar results were seen in the station 12 experiment when all cells were considered. In the station 8 experiment, we were able to measure sufficient numbers of the small prymnesiophyte *Phaeocystis* and the large diatom *Corethron* to evaluate the response of these taxa to enrichment (Fig. 12). In both cases the response was significant ($p < 0.01$), although the wide distributions of F_v/F_m values for *Phaeocystis* compared to *Corethron* suggest that these cells may indeed be regulated differently. In particular, the presence of control *Phaeocystis* cells with high F_v/F_m is noteworthy.

In the Polar Front enrichment experiments at stations 9 and 14 several taxa of diatoms were observed in high enough numbers to make comparisons (Fig. 13). When
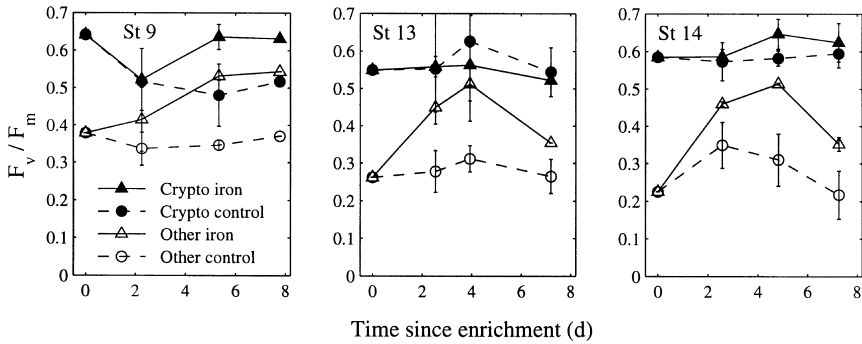
Fig. 10. Normalized variable fluorescence for populations of cryptophytes, and of all other cells considered as a single population, from the Polar Front cruise experiments in which responses to iron enrichment were observed, as measured by PDP flow cytometry. Error bars represent one SD above and below the mean of replicate bottles (the initial and sometimes the final points were single bottles, without error bars).
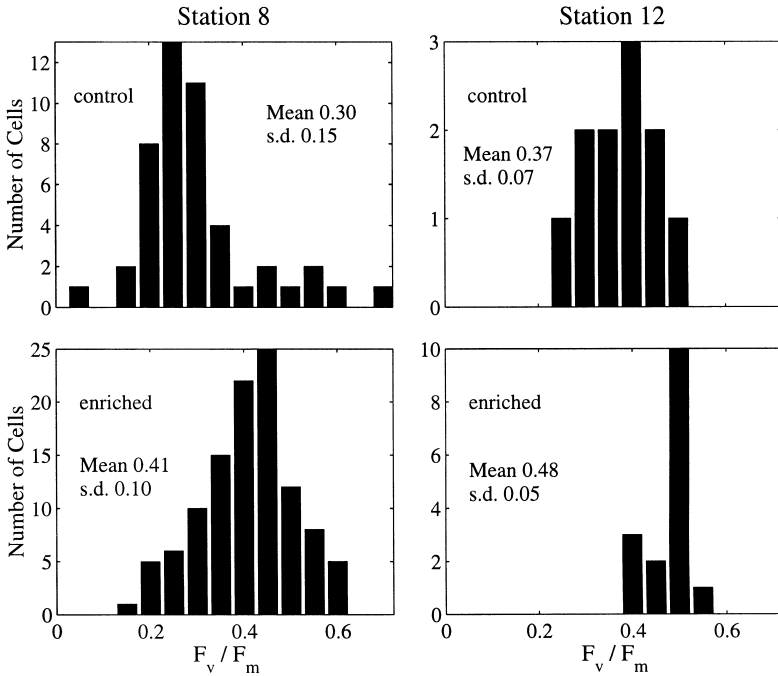

Fig. 11. Distributions of F_v/F_m for phytoplankton cells larger than $\sim 5\,\mu m$ in control bottle 1 and the Fe + Zn enriched bottles from the experiments at stations 8 and 12, as measured by PDP micro-fluorometry.

all cells were considered, the mean F_v/F_m of iron-enriched and control cells were significantly different (*t*-test, $p < 0.01$) in both experiments. At station 14, no differences could be seen between the three taxa of diatoms observed. At station 9, however, *Fragilariopsis* in the control appeared to be more severely affected than at least some other groups. Very few *Fragilariopsis* cells were found in the control sample, and those
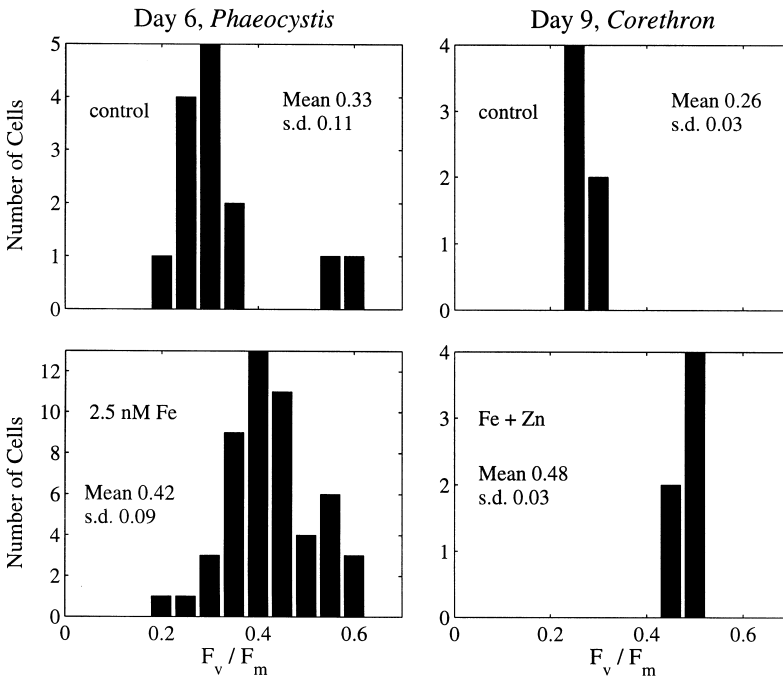
Fig. 12. Distributions of F_v/F_m for *Phaeocystis* cells in control bottle 1 and in the 2.5 nM Fe bottle on day 6 (left panels), and for *Corethron* cells in control bottle 1 and in the Fe + Zn enriched bottle on day 9 of the experiment at station 8, as measured by PDP microfluorometry.

observed had lower F_v/F_m than the other taxa (significantly so for *Dactyliosolen* and *Chaetoceros*; *t*-test, $p < 0.01$). These results may indicate that *Fragilariopsis* is more sensitive to iron stress than other phytoplankton.

Although these data and laboratory culture work (e.g., Hutchins et al., 1999) point to species differences in iron nutrition and uptake strategies as potentially important in determining phytoplankton community composition, many more measurements are needed at different stages of the growing season before we can draw firm conclusions.

3.4. Relationships between response to enrichment and ambient iron concentration

All three experiments in which no clear response was observed were located in the northern part of the study area, and the experiments in which a clear response to enrichment occurred were located in the southern part, suggesting a latitudinal gradient in iron stress. Such a gradient is consistent with the observation in the Atlantic sector of the Southern Ocean that dissolved iron concentrations were higher in the Polar Front than to either side (de Baar et al., 1995). Measurements of iron concentrations by other workers in the AESOPS program also support this interpretation (Ross Sea and Polar Front data were provided by S. Johnson/K. Coale and C. Measures, respectively). We observed a significant ($p < 0.05$) relationship between F_v/F_m at the beginning of each experiment and the measured ambient iron concentration, both in the Polar Front data set alone and when both cruises were considered together (Fig. 14A).
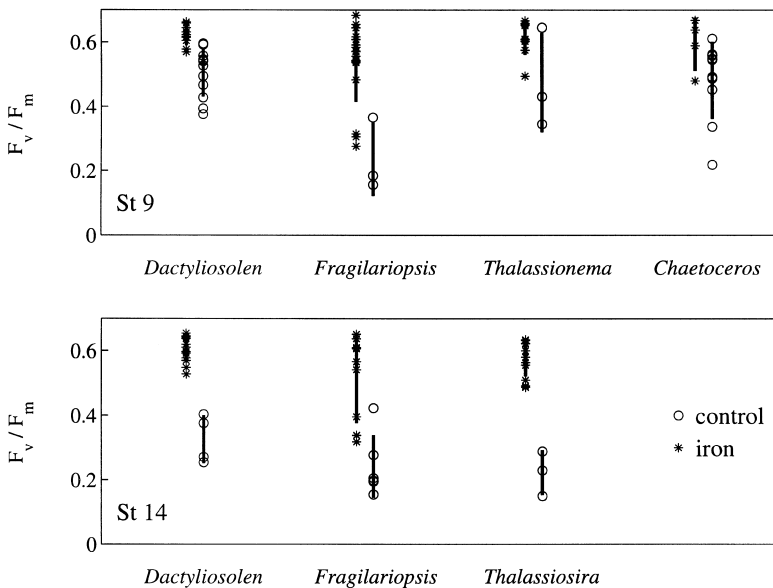
Fig. 13. Distributions of F_v/F_m in four species of diatoms from control and enriched bottles on day 4 of the experiment at station 9, and in three species of diatoms from control and enriched bottles on day 2 of the experiment at station 14, as measured by PDP microfluorometry. Solid vertical lines indicate one SD on each side of the mean.

We quantified the response to iron-enrichment in each experiment by comparing control and enriched F_v/F_m values using the following index:

$$[(F_v/F_m)_{+Fe} - (F_v/F_m)_{control}]/[0.65 - (F_v/F_m)_{control}],$$

where 0.65 represents the maximum potential value of F_v/F_m. We used both FRR and PDP results and averaged results over all time points after enrichment, and observed a significant ($p < 0.05$) inverse relationship between the ambient iron concentration at each station and the response to iron-enrichment (Fig. 14B). When the ambient iron concentration exceeded ~ 0.15 nM, there was no response of F_v/F_m to iron additions.

As expected, we also found an inverse relationship between the response to iron-enrichment and the initial F_v/F_m (Fig. 14C); dramatic differences between control and iron enriched bottles occurred only when F_v/F_m at the station was less than ~ 0.4. Because F_v/F_m in control as well as enriched bottles sometimes increased to higher values during the incubations, this suggests that in some regions a factor other than iron was initially limiting photosynthetic electron transport, or that in these waters an initially unusable source of iron became available to phytoplankton during the incubation. (Alternatively, these results could simply reflect contamination, but the coincidence of these results with the higher measured iron concentrations argues against this.)

Even though the relationship between iron and response to enrichment was significant, the amount of variation in response to enrichment explained by measured iron concentration was only 45%. It is difficult to say how much of the unexplained variation is due to experimental error or measurement uncertainty as opposed to
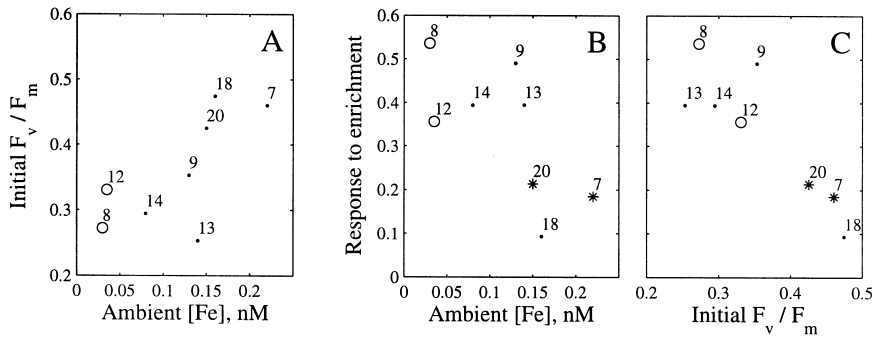
Fig. 14. Relationships between ambient iron concentration and phytoplankton properties and responses to enrichment at each station at which experiments were conducted on the Polar Front (dots) and Ross Sea (open circles) cruises. Station numbers are indicated for each datum. (A) Normalized variable fluorescence (initial F_v/F_m) at the beginning of the experiment as a function of ambient iron concentration; (B) relative response to iron enrichment as a function of ambient iron concentration; (C) relative response to iron enrichment as a function of initial F_v/F_m. The calculation of response to iron enrichment included both FRR and PDP results for all time points after enrichment. Asterisks mark two experiments in which an FRR data point was omitted from the calculation of response to enrichment. These two samples (the last time point in the experiment at station 7 and the second time point at station 20; see Fig. 8) were the smallest signals in the data set, only slightly higher than the blank, which apparently led to overestimation of F_v/F_m; in the case of the station 7 data, the values were higher than the "theoretical" maximum of 0.65.

limitation of F_v/F_m by factors other than iron. Potential complicating factors in the interpretation of these data include uncertainties in comparing the iron measurements on the two cruises (due to methodological differences that may involve the chemical forms of iron assayed; C. Measures, in prep.), the fact that iron stress might be expected to be a function of the rate of supply rather than the absolute concentration of iron, and the recent recognition that iron availability may be affected by a variety of organic chelators (Hutchins et al., 1999).

An additional factor, irradiance, must also be considered in the interpretation of in situ F_v/F_m data (through photoinhibitory depression of F_v/F_m in near-surface samples). For the Polar Front cruise data set irradiance was low, but midday depression of F_v/F_m was observed and thus may have contributed to variance not explained by iron (Sosik et al., in prep.). The suggestion that iron availability can be predicted from observations of ambient F_v/F_m is thus strongly supported by our results, although further work is required to evaluate its generality.

4. Conclusions

Iron-enrichment in late summer caused stimulation of phytoplankton in the Ross Sea and in waters to the north that are part of the Ross Sea gyre, but not in the Antarctic Circumpolar Current seasonal ice zone (61–65°S). Both photosynthetic capacity as measured by three active fluorescence techniques (F_v/F_m) and optical properties as measured by flow cytometry revealed cellular differences between

enriched samples and controls. Measurements of individual cells and populations indicated that most kinds of cells appeared to be limited by iron and responded similarly to enrichment (although cell-to-cell variations in physiological state were sometimes observed). One notable exception were the cryptophytes, which often had F_v/F_m values much higher than other phytoplankton in the same water samples, and which did not respond to enrichment. The seasonal timing and number of measurements were too restricted for us to make firm conclusions about species differences in iron limitation in these waters.

This is the first work to show a relationship between measurements of ambient Fe and F_v/F_m in the ocean. Furthermore, the relative response to enrichment was inversely related to the initial iron concentration, and to the initial value of F_v/F_m. Obvious responses to enrichment were observed only at stations where the iron concentration was less than 0.15 nM, and where F_v/F_m was less than 0.4. These observations indicate that it should be possible to predict the degree of iron limitation in different regions of the Southern Ocean from ambient values of iron concentration and F_v/F_m.

Acknowledgements

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