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## Differential response of equatorial Pacific phytoplankton to iron fertilization

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### *Abstract*

Recent unenclosed iron-fertilization experiments in the equatorial Pacific Ocean have shown that phytoplankton biomass can be increased substantially by the addition of iron. Analyses of size-fractionated chlorophyll indicate that much of the increase during the most recent fertilization experiment, IronEx II, occurred in the >10- $\mu\text{m}$  size fraction. We used flow cytometry, combined with taxon-specific pigment measurements by high-performance liquid chromatography (HPLC), to analyze the responses of five different groups of phytoplankton: *Prochlorococcus*, *Synechococcus*, ultraplankton, nanoplankton, and pennate diatoms. These results are unique in the suite of measurements from the IronEx studies in that they simultaneously examine individual cell properties, which are grazer independent, and population dynamics, which reflect the net result of growth and grazing. Our results show that the overall increase of chlorophyll *a* (Chl *a*) in the patch was due in part to increases in chlorophyll content per cell and in part to increases in cell numbers of specific groups. Cellular fluorescence was stimulated by iron addition in all five groups to a qualitatively similar degree and was correlated with taxon-specific changes in cellular pigments. In terms of net cell growth, however, these groups responded very differently. The groups that dominated the community before the addition of iron increased at most twofold in cell number; *Prochlorococcus* actually decreased. In contrast, the initially rare pennate diatoms increased 15-fold in number by the peak of the iron-induced bloom. Within 1 week, this differential response led to a dramatic change in the phytoplankton community structure, from one dominated by picoplankton to one dominated by large diatoms. It is not known whether this shift would be sustained over extended periods of fertilization, a response that would ultimately change the structure of the food web.

Oceanographers have long been puzzled by the simultaneous abundance of macronutrients, such as nitrate and

phosphate, and the paucity of phytoplankton biomass in the three high-nutrient, low-chlorophyll (HNLC) regions of the world ocean: the equatorial Pacific, the subarctic Pacific, and the Southern Ocean. Early oceanographers noted these anomalies (Gran 1931; Hart 1934), and many hypotheses have been offered and debated over the years (*see* Chisholm and Morel 1991), the most compelling of which are the “iron hypothesis” (Martin 1990), the “grazing hypothesis” (Walsh 1976; Frost 1991), and the unifying “ecumenical hypothesis” (Morel et al. 1991a; Price et al. 1994). The debate on the iron hypothesis, based largely on iron-enriched “grow out” experiments (e.g., Martin and Fitzwater 1988; Buma et al. 1991; Coale 1991; Takeda and Obata 1995; Boyd et al.

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1996; Coale et al. 1996a; Zettler et al. 1996), culminated with two open-ocean in situ iron-fertilization experiments (IronEx I and II; Martin et al. 1994; Coale et al. 1996b).

The biological response to added iron during IronEx II, as indicated by changes in Chl *a*, exceeded the observed increase in IronEx I by nearly one order of magnitude (Martin et al. 1994; Coale et al. 1996b). A large portion of the response to iron fertilization was due to a bloom of initially rare diatoms, whose biomass increased 85-fold inside the iron-fertilized patch (Coale et al. 1996b). Concomitant with this massive increase in phytoplankton biomass was a draw-down of roughly 5  $\mu\text{M}$  nitrate, attributed almost completely to cells  $>5 \mu\text{m}$  (Coale et al. 1996b). Other changes of biogeochemical significance included a decrease in  $\text{CO}_2$  fugacity of nearly 100  $\mu\text{atm}$  (Cooper et al. 1996), a 3.5-fold increase in dimethyl sulfide production (DMS; Turner et al. 1996), and a 7‰ enrichment in the  $\delta^{13}\text{C}$  of phytoplankton (Bidigare et al. submitted).

There is evidence that the smaller, initially dominant phytoplankton were also stimulated by the iron addition. Using epifluorescence microscopy, Coale et al. (1996b) found modest increases in cell biomass for the smaller, nondiatom taxonomic groups. This analysis did not include the ubiquitous picoplankton *Prochlorococcus*, which cannot be resolved easily using this method. Photochemical quantum efficiency, the ratio of the quantum yield of variable fluorescence ( $F_v$ ) to that of the light-saturated fluorescence yield ( $F_m$ ), increased dramatically in both experiments hours after the addition of iron (Kolber et al. 1994; Behrenfeld et al. 1996). Behrenfeld et al. (1996) contend that since  $F_v/F_m$  increased prior to a shift in species composition, all phytoplankton taxa were physiologically limited by iron—reinforcing the finding of significant increases in  $F_v/F_m$  for all size fractions during IronEx I (Kolber et al. 1994). In addition, results from bottle dilution experiments (Landry and Hassett 1982) indicate that the net autotrophic growth rate of the phytoplankton community  $<5 \mu\text{m}$  in size more than doubled inside the enriched patch while the microzooplankton grazing rates increased threefold (Coale et al. 1996b). Higher grazing rates inside the patch and only a modest increase in small phytoplankton biomass suggest that all phytoplankton were stimulated by the addition of iron, but only the initially rare diatoms escaped grazing pressure and bloomed.

Most of the reports to date use bulk measurements, which do not distinguish between subgroups, to investigate phytoplankton dynamics inside the iron-fertilized patch. We used analyses of size-fractionated chlorophyll to monitor changes between large ( $>10 \mu\text{m}$ ) and small ( $<10 \mu\text{m}$ ) size classes following enrichment. To follow the response of specific groups of phytoplankton to iron enrichment, we used flow cytometry, combined with taxon-specific pigment measurements. This allowed us to distinguish between changes in cell number and cellular chlorophyll since a change in either would affect bulk chlorophyll measurements. Moreover, flow cytometric analyses provide a measure of cell-specific responses to iron enrichment, independent of any changes, or lack thereof, in population numbers. Finally, this is the first report documenting the response to in situ iron fertilization of the picophytoplankton *Prochlorococcus*,

which is typically a large contributor to standing stocks in the equatorial Pacific (e.g., Binder et al. 1996).

## Methods

*Iron patch and sampling*—IronEx II was conducted between 29 May and 15 June 1995 starting at 4°S, 105°W in a region with high (ca. 10  $\mu\text{M}$ ) nitrate concentrations (Coale et al. 1996b). Iron, added as Fe(II) in seawater at pH 2, was injected into surface waters three times over a 7-d period, initially forming a 72-km<sup>2</sup> patch. The target iron concentration for the initial fertilization was 2 nM—a 40-fold increase from the 0.05-nM ambient concentration; the second and third injections were designed to add another 1 nM Fe each (Coale et al. 1996b). A 1,000-km<sup>2</sup> survey of the general study site was conducted prior to the first iron infusion to ensure against possible patch subduction, as occurred in IronEx I (Martin et al. 1994), and to verify that a representative site was chosen. Stations were occupied daily inside and outside the patch, although in some cases, only one of these was possible. All data presented here are from stations sampled at local dawn to eliminate complications arising from diel patterns.

*Pigments*—We used size-fractionated Chl *a* concentrations to compare broad changes in phytoplankton community structure in and out of the patch. The fractions were analyzed in duplicate by parallel filter fractionation, using polycarbonate 10- $\mu\text{m}$  pore-size filters (Poretics) and Whatman GF/F filters for the total. After extraction in 90% acetone for 24 h, Chl *a* was assayed fluorometrically (Parsons et al. 1984) on a 10-AU fluorometer (Turner Designs), which was calibrated using extracts of spinach Chl *a* (Sigma-Aldrich). Chl *a* in the  $>10\text{-}\mu\text{m}$  fraction equaled the material retained on the 10- $\mu\text{m}$  filter, whereas the  $<10\text{-}\mu\text{m}$  fraction was the difference between the total and that retained on the 10- $\mu\text{m}$  filter.

Taxon-specific pigments were monitored in whole-water (bulk) samples to complement the flow cytometry data (*see below*). Seawater samples were filtered onto Whatman GF/F filters, and acetone extracts were then analyzed by HPLC for chlorophyll and carotenoid quantification (Goericke and Repeta 1993). Divinyl chlorophyll *a* (Chl *a*<sub>2</sub>) and fucoxanthin were used as chemotaxonomic markers for *Prochlorococcus* spp. and diatoms, respectively. The sum of 19'-hexanoyloxyfucoxanthin, 19'-butanoyloxyfucoxanthin, and peridinin (HBP) was used as a marker for the group consisting of pelagophytes, prymnesiophytes, and dinoflagellates (Vesk and Jeffrey 1987; Wright and Jeffrey 1987; Simon et al. 1994; Andersen et al. 1996). It has been shown that prymnesiophytes and dinoflagellates contribute significantly to the nanophytoplankton (2–20  $\mu\text{m}$ ) biomass of the equatorial Pacific (Chavez et al. 1990; Coale et al. 1996b), and it is likely that the pelagophytes are in the group of eukaryotic picoplankton ( $<2 \mu\text{m}$ ) termed the “red fluorescing picoplankton” (Chavez et al. 1990; Coale et al. 1996b). Thus, for the sake of comparison, we have used the HBP sum to represent the pigment biomass of the ultra- and nanophytoplankton groups combined (*see below*). Note that chemotaxonomic markers for *Synechococcus* were not measured.

**Flow cytometry**—A modified Coulter EPICS V flow cytometer (Cavender-Bares et al. 1998) was used to characterize the phytoplankton community in terms of autofluorescence per cell, which is related to pigment concentration (Sosik et al. 1989; Li et al. 1993; Jonker et al. 1995), and forward angle light scatter (FALS) per cell, which is correlated with cell size (Van de Hulst 1957; Ackelson and Spinrad 1988). A 40.5-mm spherical lens was used to focus the 488-nm laser beam (Innova 90 at 800 mW; Coherent), either alone for picoplankton analyses or in combination with a 150-mm cylindrical lens, which was focused on the back focal plane of the spherical lens, for analyses of larger cells. These configurations produced a 17- $\mu\text{m}$  circular and a 17  $\times$  400- $\mu\text{m}$  elliptical laser spot, respectively, for sample illumination within the quartz flow cell. Chlorophyll or red fluorescence (Red FL) signals passed first through a 488-nm long-pass filter, then were reflected by a 630-nm short-pass dichroic filter, and finally passed through a 680-nm band-pass filter (40-nm bandwidth; Omega Optical). Phycoerythrin or orange fluorescence (Orange FL) signals passed through the 630-nm short-pass dichroic and also through a 515-nm long-pass filter. FALS signals, which passed beyond a wide obscuration bar and then through a vertical polarization filter, were measured using a PMT (photo multiplier tube) detector. Log-integrated values were collected for all three signals. When the range of fluorescence values exceeded the nominal three-decade range of the instrument, we employed an extra electronic circuit to expand the Red FL scale. A subtraction module can be used to subtract electronically one signal from a second and is typically used to separate spillover of one signal into a second. Only partial subtraction of one signal from the other is possible with a maximum of 50% for those modules associated with the flow cytometer. Two modules were used in series, and the Red FL signal from the PMT was split and input as two separate signals, resulting in a new signal that equaled 25% of the original Red FL signal. This “reduced” Red FL signal was used to characterize those cells that were offscale on the standard Red FL scale.

We are able to differentiate five separate groups of photosynthetic cells based on their distinct fluorescence and light scatter signals as represented in Fig. 1. The two cyanobacteria *Prochlorococcus* and *Synechococcus* are easily differentiated from each other since only *Synechococcus* exhibits Orange FL (Chisholm et al. 1988; Olson et al. 1990). Both cyanobacteria have relatively low FALS signals, which separates them from the ultra- and nanoplankton. The smallest ultraplankton are also distinguishable from *Synechococcus* because of their lack of Orange FL. The ultra- and nanophytoplankton are identified based on their size and fluorescence characteristics (Zettler et al. 1996), and pennate diatoms can be identified because they scatter less light than would be expected for their size because of their long, slender shape (Olson et al. 1989; Zettler et al. 1996). Fluorescence and light scatter values are reported relative to the value of calibration microspheres added to each sample (Polysciences; 0.474 and 2.02  $\mu\text{m}$  [Fig. 1A,B and C, respectively]). Note that although red fluorescence values are shown in Fig. 1 for *Synechococcus*, they are not reported in our

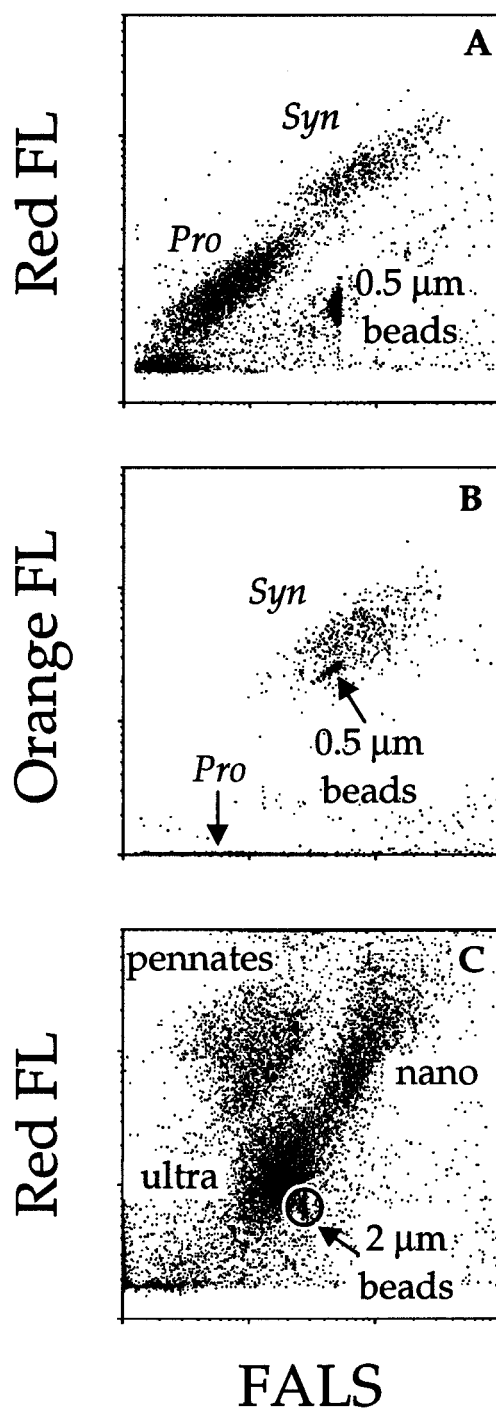


Fig. 1. (A) Flow cytometric scattergram of *Prochlorococcus* and *Synechococcus*, with Red FL plotted against FALS. (B) Orange FL vs. FALS scattergram showing how *Synechococcus* are separated from *Prochlorococcus*, since the latter do not exhibit Orange FL. (C) Scattergram showing the larger cells in the ultra- and nanoplankton and pennate diatom groups. Each dot denotes an individual cell for a 10-m sample taken on day 7 inside the iron patch. Calibration microspheres (beads) of different sizes were added to each sample as internal standards: 0.47  $\mu\text{m}$  for A,B; and 2.02  $\mu\text{m}$  circled in C.

results since a portion of the orange fluorescence from phycoerythrin may also have been detected as red fluorescence.

To put bounds on the sizes of the flow cytometrically determined ultra- and nanoplankton groups, we analyzed material that passed through differently sized Poretics polycarbonate membrane filters. On day 5 inside the patch, 75% of the ultraplankton passed through a 2- $\mu\text{m}$  filter, while 95% passed through a 5- $\mu\text{m}$  filter. On the same day, 50% of the nanoplankton passed through a 2- $\mu\text{m}$  filter, and >95% passed through a 10- $\mu\text{m}$  filter. These flow cytometric populations do not conform well to the “pico” (<2  $\mu\text{m}$ ) and “nano” (2–20  $\mu\text{m}$ ) size ranges as originally defined (Sieburth et al. 1978); therefore, our use of “ultra” and “nano” modifiers in this paper is operational. Determining that 60% of the pennates passed through a 10- $\mu\text{m}$  filter reveals little about their dimensions because of their long slender shape and uncertainty as to how they would pass through a filter. However, Zettler et al. (1996) found that this flow cytometric group consisted mainly of cells up to 50  $\mu\text{m}$  in length, in agreement with microscopic observations during IronEx II, which found this group included cells up to roughly 60  $\mu\text{m}$  in length (Tanner pers. comm.).

There is good evidence that flow cytometrically derived fluorescence per cell is related to pigment per cell for individual species (e.g., Sosik et al. 1989; Li et al. 1993; Jonker et al. 1995; Moore et al. 1995; Moore and Chisholm in press), and we demonstrate this directly for *Prochlorococcus* in this study (see below). This relationship need not be linear, however, and can be confounded by variations in accessory pigments and the “package effect” (Sosik et al. 1989). Thus, particularly for larger cells, equivalent increases in pigment per cell may not result in proportional increases in Red FL per cell, thereby limiting quantitative interspecific comparisons of cellular fluorescence. The relationship between cellular fluorescence and absolute pigment concentration may also be influenced by changes in fluorescence yield (Falkowski and Kiefer 1985; Falkowski and Kolber 1995). Since the addition of DCMU [3-(3, 4-dichlorophenyl)-1,1-dimethylurea] does not affect flow cytometrically derived fluorescence signals except at excitation intensities well below those used in this study (Xu et al. 1990; Furuya and Li 1992), we do not consider variable fluorescence yield to be an issue in interpreting our data.

## Results and discussion

*Size-fractionated Chl a*—Total Chl *a*, a proxy for phytoplankton biomass, averaged 0.2  $\mu\text{g liter}^{-1}$  in surface waters outside the iron-enriched patch (Fig. 2A), a typical value for the equatorial Pacific (Chavez et al. 1990). After 2 d of iron enrichment, total Chl *a* had doubled, and by day 6, it had increased 12-fold to 2.6  $\mu\text{g liter}^{-1}$  (Fig. 2A,B). Initially, the phytoplankton community was dominated by cells <10  $\mu\text{m}$ , which accounted for 90% of the total. By day 6, total chlorophyll in the <10- and >10- $\mu\text{m}$  fractions was roughly equal inside the patch and had increased 7- and 60-fold, respectively (Fig. 2A,B). The >10- $\mu\text{m}$  fraction rose from 10 to 60% of the total, representing a major shift in community composition from small to large cells (Fig. 2C). This shift

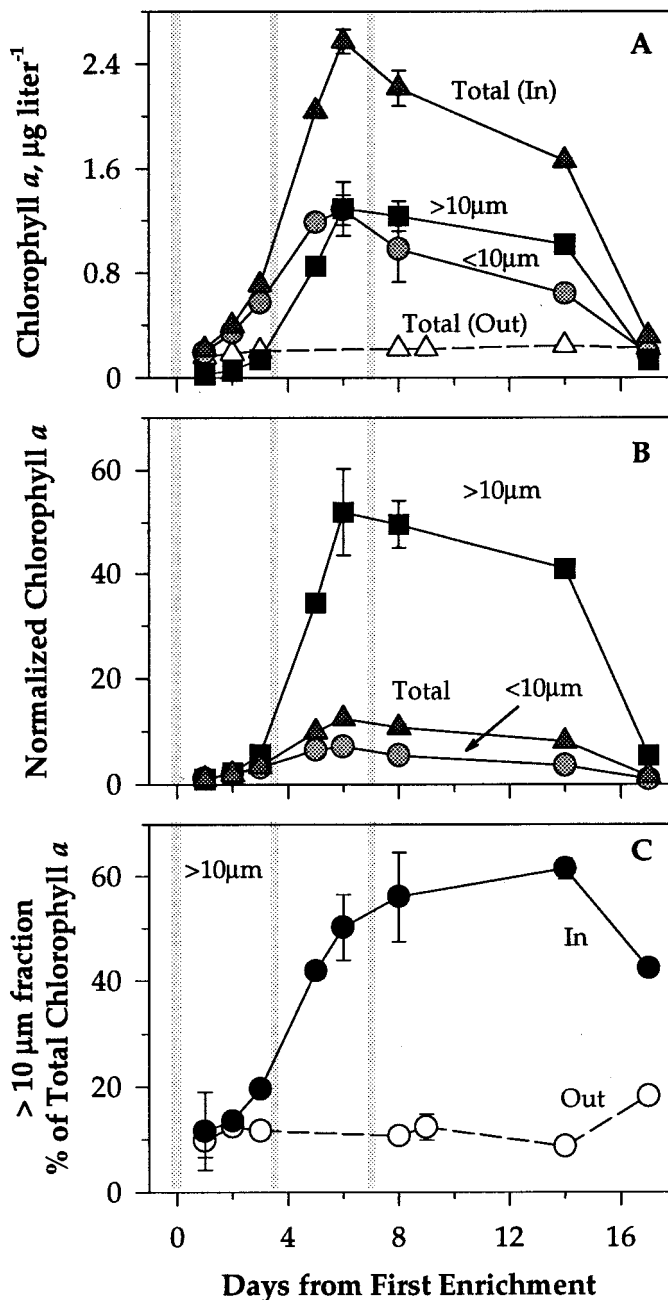


Fig. 2. Size-fractionated Chl *a*  $\pm$  1 SD at 15 m as a function of time over the course of the iron-enrichment experiment. (A) Concentrations inside the iron-enriched patch (solid symbols and lines) and outside the patch (open symbols, dashed line). (B) Concentrations measured inside the patch normalized to the average for each fraction outside the patch. (C) The >10- $\mu\text{m}$  fraction is shown as a percentage of the total Chl *a* inside and outside the patch (symbols and lines as in A). Days of iron addition are marked with vertical bands.

is consistent with observations that high total Chl *a* is accompanied by an abundance of large cells (Raimbault et al. 1988; Chavez et al. 1996). Judging from the analysis of material passing through filters (discussed in Methods), all of the flow cytometrically defined groups contributed to the

<10- $\mu\text{m}$  fraction, while a significant portion of the pennate diatoms were caught on 10- $\mu\text{m}$  filters, thereby contributing to the >10- $\mu\text{m}$  fraction.

**Flow cytometric pigments**—Chlorophyll fluorescence (or in the case of *Synechococcus*, phycoerythrin fluorescence) per cell increased in all groups over the first 7 d of the experiment (Fig. 3A). The simplest interpretation of these increases, supported reasonably well by the taxon-specific pigment data presented below, is that they reflect an increase in cellular pigment concentration for all groups in response to iron enrichment, which indicates that all of these groups were iron limited at the start of the experiment. Such an interpretation is consistent with what is known about the physiological responses of phytoplankton when released from nutrient limitation, since increased cellular Chl *a* concentration is a standard response when phytoplankton are shifted from nutrient-limited to nutrient-replete conditions (e.g., Eppley and Renger 1974; Perry 1976). This pattern has held true in a range of iron-limited phytoplankton cultures (Geider et al. 1993; Trick et al. 1995; Wilhelm and Trick 1995; Kudo and Harrison 1997; McKay et al. 1997; Sunda and Huntsman 1997) and can be indicative of the degree of iron limitation. However, because of the potentially complex relationship between flow cytometrically derived cellular fluorescence and pigment per cell, we cannot interpret the relative magnitude of the fluorescence response between the groups as a measure of the degree of iron limitation. Further, a change in species composition within a group over time might influence its mean cellular fluorescence—a nuance that would be missed by the process of averaging. That is, it is impossible to distinguish between either an entire group, or just a subset of that group responding to iron, if there is no change in shape of the flow cytometric signature.

**Taxonomic marker pigments by HPLC**—Pigments that are indicative of different classes of phytoplankton were measured over the course of the experiment using HPLC. By normalizing these pigment concentrations to the cell numbers in the different groups defined flow cytometrically, we used these data to interpret Red FL  $\text{cell}^{-1}$  values and to help judge how well the pennate diatoms detected by flow cytometry represented the larger diatom community as a whole. Cellular Red FL values are expected to covary with xanthophyll concentrations for the ultra- and nanoplankton and pennate diatom groups, since they absorb light at the excitation wavelength used in the flow cytometer (488 nm) and pass energy on to Chl *a* (Bidigare et al. 1990). In contrast, Chl  $a_2$  was directly compared to cellular Red FL for *Prochlorococcus*, as it is unique to this group. When plotted on a relative scale, we found that variations in cell-specific pigment concentrations determined for *Prochlorococcus* and the ultra- and nanophytoplankton combined (see *Methods*) followed the same patterns as those observed for the corresponding cellular Red FL values (Fig. 4A,B). Calculated cellular Chl  $a_2$   $\text{cell}^{-1}$  for *Prochlorococcus* ranged from 0.2 to 0.9 fg  $\text{cell}^{-1}$ , which is consistent with values measured in laboratory cultures (Moore et al. 1995; Moore and Chisholm in press). Because *Prochlorococcus* uniquely contains Chl

$a_2$ , the significant correlation shown in Fig. 4D indicates that when Chl  $a_2$   $\text{cell}^{-1}$  doubled, so did cellular Red FL (model II regression; Sokal and Rohlf 1995). For the ultra- and nanoplankton, when HBP  $\text{cell}^{-1}$  doubled, so did Red FL  $\text{cell}^{-1}$  (Fig. 4E). Yet, without knowing the relationship between cellular HBP and Chl *a*, a strong conclusion about the relationship between Red FL  $\text{cell}^{-1}$  and Chl *a*  $\text{cell}^{-1}$  cannot be made for this group. A varying cellular HBP:Chl *a* ratio would, for instance, likely cause variation in the relationship between cellular Red FL and Chl *a* (Sosik et al. 1989).

To estimate the degree to which the total diatom community can be represented by our flow cytometric pennate diatom group, we compared the relative changes in the bulk concentration of the diatom-specific pigment fucoxanthin with the relative changes in total pennate fluorescence measured per liter (Fig. 4C,F). Diatoms as a group were represented by more than the pennates over the course of the experiment (Coale et al. 1996b). The largest diatoms, particularly the chain-forming types, would have been poorly sampled by flow cytometry. In addition, a second pennate diatom population, common to iron-enrichment studies (Zettler et al. 1996), was detected on days 6–8 only. This population was offscale on the y-axis above the pennate population depicted in Fig. 1C and had a low cell abundance (maximum = 450 cells  $\text{ml}^{-1}$ ), but it had a 10-fold higher mean Red FL  $\text{cell}^{-1}$  than the more numerous pennate population. This ephemeral population was not represented in Fig. 3 because the cells were undetectable outside the patch, but we do include it in the bulk Red FL data for Fig. 4. The difference between the dashed and solid half-tone lines in Fig. 4C indicates the importance of this population to the total Red FL per milliliter. By day 8, this second pennate population had waned, and our subsequent flow cytometric analyses clearly missed a large component of the diatom community, as is evidenced by the disparity between the bulk concentrations of Red FL and fucoxanthin (Fig. 4C). For this reason, data from day 8 were excluded from the regression in Fig. 4F. However, the agreement between pigment and fluorescence concentrations prior to day 8 (Fig. 4C) confirms that the pennate diatoms we identified by flow cytometry were the major contributors to the total diatom assemblage up to that point.

**Individual cell size**—Mean FALS per cell, an indicator of cell size (Van de Hulst 1957; Ackelson and Spinrad 1988), increased over the first 7 d of the experiment for all groups, roughly paralleling changes in fluorescence per cell (Fig. 3B). Mean FALS per cell increased more than twofold for *Prochlorococcus* and *Synechococcus*, which corresponds approximately to a 1.5-fold increase in cell volume for these species. Similar increases for the ultra- and nanophytoplankton over this same period reflect roughly a twofold increase in mean cell volume (data not shown; see DuRand 1995; Dusenberry 1995). The mean FALS per cell for the pennate diatoms, which could not be calibrated because of the geometry of the cells (Olson et al. 1989), showed an increase similar to that of the other populations, but not as pronounced.

Given the simultaneous increase in cellular FALS and FL in all populations, it is unclear if the response to iron en-

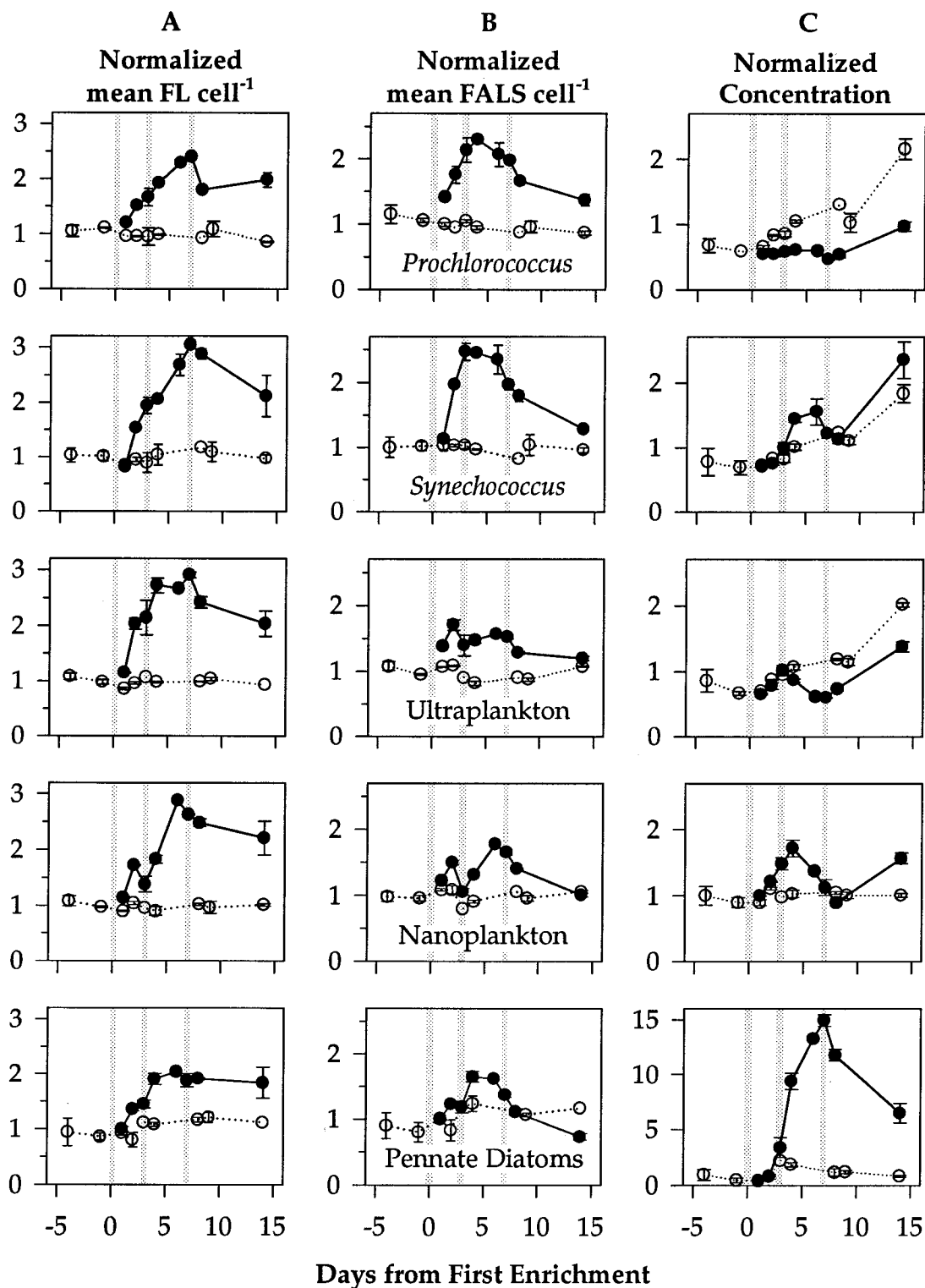


Fig. 3 Response of different phytoplankton groups to iron enrichment in the patch (solid symbols and lines) relative to outside the patch (open symbols, dashed lines) as measured by flow cytometric analysis of individual cells. (A) Normalized mean pigment fluorescence per cell: Orange FL for *Synechococcus* and Red FL for the other groups; (B) normalized mean FALS per cell; and (C) normalized cell concentration. All values reported normalized to the average values outside the patch; data points typically represent average mixed-layer values (3, 10, and 15 m  $\pm$  1 SD). Average cell concentrations outside the patch were  $1.4 \times 10^5$ ,  $7.6 \times 10^3$ ,  $1.1 \times 10^4$ ,  $2.0 \times 10^3$ , and  $1.4 \times 10^2$  cells  $\text{ml}^{-1}$  for *Prochlorococcus*, *Synechococcus*, ultraplankton, nanoplankton, and pennate diatoms, respectively. Note change of scale in normalized concentration for the pennate diatoms. Days of iron addition are marked by vertical bands.

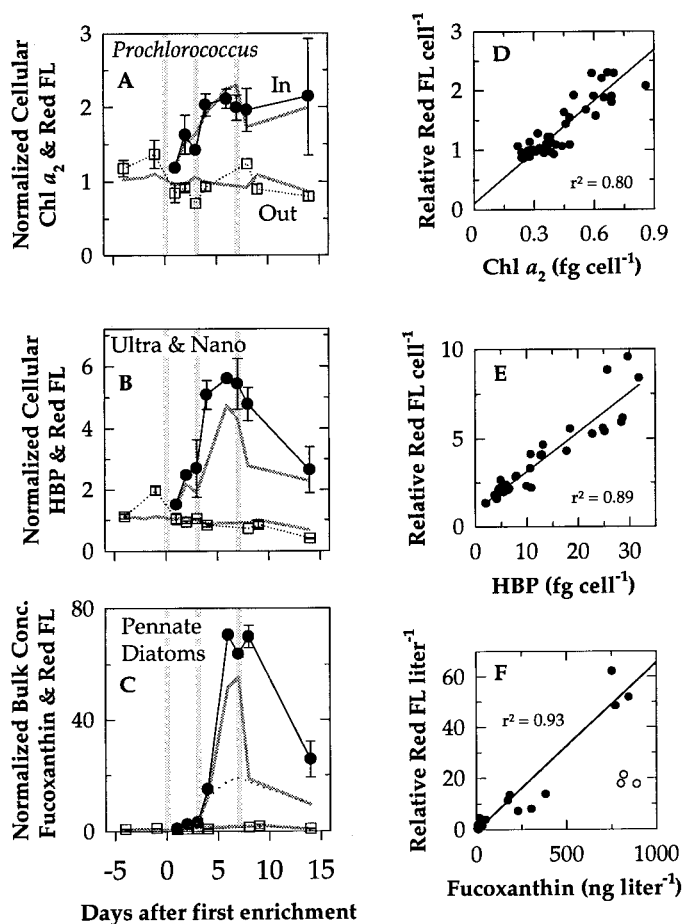


Fig. 4 (A–C) Phytoplankton pigments in the patch (solid circles and lines) and outside the patch (open squares, dashed lines) as measured by HPLC, with corresponding flow cytometry data from Fig. 3 shown for reference (half-tone lines). (A) Normalized cell-specific Chl  $a_2$  concentration and Red FL for *Prochlorococcus*. (B) Normalized cell-specific HBP concentration and Red FL for the ultra- and nanoplankton combined. In A and B, bulk pigment concentrations representative of each of the groups of phytoplankton were divided by the flow cytometrically derived cell abundances to yield pigment per cell; they were then normalized to the average value of 3-, 10-, and 15-m samples outside of the patch ( $\pm 1$  SD), as in Fig. 3. (C) Normalized bulk concentrations of fucoxanthin and red fluorescence (Red FL  $\text{cell}^{-1} \times \text{cells liter}^{-1}$ ); a second dashed half-tone line added to show the contribution of the numerically dominant pennate population, whereas the solid half-tone line combines red fluorescence values from this and the far less abundant, but highly pigmented, pennate population that appeared on days 6–8 (see text). (D–F) Relationship between HPLC and flow cytometry data using all of the non-normalized data from (A–C), respectively, including Model II regression lines (reduced major axis; Sokal and Rohlf 1995). Note that the data from day 8 (open circles) were excluded from the regression in F.

richment involved an increase in the volumetric concentration of pigment or whether pigment simply increased in proportion to increases in cellular volume. For all the groups except *Prochlorococcus*, the uncertainties involved in converting FL to Chl  $a$  and FALS to volume are too large to distinguish between these two possibilities. Based on culture

studies (Moore et al. 1995; Moore and Chisholm in press) and the data reported here, we know that the 2.5-fold increase in cellular Red FL for *Prochlorococcus* over the first 4 d of the experiment (Fig. 3A) corresponded to a 2.5-fold increase in Chl  $a_2$   $\text{cell}^{-1}$  (Fig. 4D). Using an empirical calibration of FALS to volume for this species (data not shown) and evidence from synchronized populations in cultures and in the field, where the drop in the FALS signal as cells divide reflects a halving of the cell volume (DuRand 1995; Dusenberry 1995; Binder et al. 1996), we find that over the first 4 d, the 2.3-fold increase in mean FALS per cell for *Prochlorococcus* (Fig. 3B) was associated with a 1.6-fold increase in mean cell volume. Thus, these data suggest that Chl  $a_2$ : volume for *Prochlorococcus* increased approximately 1.5-fold inside the patch. That is, *Prochlorococcus* increased Chl  $a_2$  beyond that which would have been necessary simply to match increases in cell volume. An elevated Chl  $a_2$ : volume is consistent with a positive correlation between Chl  $a$ :C and iron concentration observed in iron-limited cultures of coastal eukaryotic phytoplankton (Sunda and Huntsman 1997) since cell volume and cell C are positively correlated (Strathman 1967; Lee and Fuhrman 1987; Simon and Azam 1989; Verity et al. 1992). We are currently working to constrain the uncertainty in estimates of Chl  $a$ : volume for the other phytoplankton groups based on these flow cytometric data.

*Cell number response*—In terms of cellular Red FL and FALS, all of the groups responded to iron enrichment in a qualitatively similar manner. However, when changes in cell numbers are considered, dramatic differences between the groups are apparent (Fig. 3C). The pennate diatoms, which averaged 140 cells  $\text{ml}^{-1}$  outside the patch, reached 2,100 cells  $\text{ml}^{-1}$  inside the patch. Over the first 4 d, their net growth (reflecting  $\mu$ , the rate of cell division, minus cell losses) was exponential, and they had a net growth rate ( $\mu_{\text{net}}$ ) of 1.0  $\text{d}^{-1}$ . There was a distinct increase in ultraplankton cell numbers inside the patch, but a similar increase was observed outside the patch; thus, the former cannot necessarily be attributed to iron addition. The nanoplankton, however, displayed increases in cell number inside the patch by day 3, more than doubled in number by day 4, and then returned to out-of-patch levels. In contrast, the increase in cell numbers of the smaller phytoplankton inside the enriched patch was modest at best. Relative to unfertilized waters, *Prochlorococcus* cell numbers actually decreased inside the patch. Using cell cycle analysis, Mann and Chisholm (submitted) found that  $\mu$  for *Prochlorococcus* increased approximately 60% inside the patch, which suggests that the decrease in *Prochlorococcus* cells inside the patch was due to increased grazing rates.

*Synechococcus* cell numbers increased twofold inside the patch by day 7, but they then quickly receded to match out-of-patch populations. The majority of this increase occurred between days 3 and 4, suggesting a rapid decrease in grazing pressure, an increase in  $\mu$ , or both. Rue and Bruland (1997) found increased production of an organic ligand capable of binding iron shortly following iron enrichment. *Synechococcus* is known to produce such ligands in culture, apparently to maintain high growth rates at very low iron concentrations

(Wilhelm et al. 1996). Production of siderophores by *Synechococcus* would increase their access to the iron pool and could therefore be viewed as a signal that *Synechococcus* responded to the iron addition much as if it were an aeolian deposition event. Speculating one step further, if the growth rate of *Synechococcus* were limited by iron, sequestration of added iron by newly produced siderophores would enhance the availability of iron to *Synechococcus*, leading to an increase in  $\mu$ .

These data indicate that even though some of the smaller phytoplankton groups increased twofold in concentration, only the initially rare pennate diatoms broke free from grazer control. This is in agreement with evidence that microzooplankton exert strong control over the typically dominant phytoplankton of the equatorial Pacific (Price et al. 1994; Landry et al. 1995, 1997) and with evidence suggesting that an increased  $\mu$  of the phytoplankton community was matched by heightened microzooplankton grazing pressure during IronEx II (Coale et al. 1996b). Assuming that cell losses were dominated by the rate of grazing ( $g$ ) during the early stages of the iron-enriched patch (i.e.,  $\mu_{\text{net}} \approx \mu - g$ ), then a simple explanation for the bloom of pennates is that iron fertilization caused a substantial elevation in  $\mu$  relative to  $g$  for these populations (Cullen 1995), resulting in the measured  $\mu_{\text{net}}$  of  $1.0 \text{ d}^{-1}$ . This is in accord with the limited grazing impact of the mesozooplankton measured inside the patch (Coale et al. 1996b). After this growth burst, cell numbers declined, even though there had been a third addition of iron on day 7 and mean fluorescence per cell remained elevated over out-of-patch levels (Fig. 3A).

The decline in pennate cell numbers could have resulted from a combination of events, including increased removal rates, such as grazing and or sinking (Bidigare et al. submitted), or reduction in growth rate because of limitation by another factor.  $^{234}\text{Th}$  inventories measured during IronEx II indicate that the particulate organic carbon (POC) flux increased sevenfold between days 7 and 14 following initial enrichment (Bidigare et al. submitted). Kudela and Chavez (1996) suggest that the decreased transparency of patch waters may have induced light limitation of phytoplankton growth. As argued by Erdner (1997), incomplete relief from iron limitation was also a possibility. She showed that there was no detectable switch from flavodoxin to ferredoxin in the phytoplankton community throughout IronEx II, as would be expected upon the relief of iron limitation. In addition, silica, which is essential for diatom growth, has recently been argued as the ultimate factor producing HNLC conditions in the equatorial Pacific (Dugdale et al. 1995; Dugdale and Wilkerson 1998). As nitrate dropped from ca.  $10 \mu\text{M}$  outside the patch to  $7 \mu\text{M}$  inside on day 7 (Coale et al. 1996b), silicate dropped from ca.  $4 \mu\text{M}$  to  $1 \mu\text{M}$  (Coale pers. comm.). Dugdale and Wilkerson (1998) argue that at silicate concentrations below roughly  $2 \mu\text{M}$ , nitrate uptake by diatoms is curtailed, which could help explain the decrease in net growth seen for the pennate diatoms after day 7 (Fig. 3C).

## Conclusions

These results document clear differential changes within the phytoplankton community over the course of the iron-

enriched bloom during IronEx II. Using fractionated Chl *a*, we measured a dramatic size-based shift as biomass in the  $>10\text{-}\mu\text{m}$  fraction increased 60-fold. The overall increase in Chl *a* resulted in part from increases in cellular pigment concentration, which were amplified to varying extents by increases in cell numbers across the groups studied. The pennate diatoms, which increased  $>10\text{-fold}$ , were the only cells identified in this study that bloomed following iron enrichment. These findings are consistent with the recent equatorial Pacific synopsis (Landry et al. 1997), as well with as the "ecumenical iron hypothesis" (Morel et al. 1991b; Price et al. 1994; Cullen 1995), which allows for the coregulation of phytoplankton in this region by iron limitation and grazing, the latter playing a particularly significant role in regulating the small, dominant cells.

In our view, the most significant conclusion from the IronEx experiments and related work (e.g., Martin and Fitzwater 1988; Coale 1991; Takeda and Obata 1995; Boyd et al. 1996; Coale et al. 1996a; Zettler et al. 1996) is that phytoplankton community structure changes dramatically when productivity is stimulated by nutrient enrichment. As pointed out by Howarth (1988), the concept of "nutrient limitation" must include both the limitation of extant dominant species and the limitation of the potential rate of net primary production, which can only be realized upon the supply of the limiting nutrient together with an accompanying shift in community structure. It is important to keep in mind that the response of the phytoplankton community seen in IronEx II was transient. We do not know what the structure of the food web would look like if iron were supplied continuously over time scales much longer than the generation times of the relevant organisms. Modeling studies (e.g., Sarmiento and Orr 1991; Frost and Franzen 1992; Armstrong 1994), which take into account the potential for changes in community structure, will be important for projecting hypothetical outcomes to fertilization scenarios. Only through carefully designed long-term experiments, however, will we be able to predict the ecological consequences of, and changes in export carbon resulting from, ocean fertilization. These will be important for developing policies to regulate commercial ocean fertilization (Markels 1995; Sørensen 1995; Jones 1996; Jones and Young 1997).

## References

- ACKELSON, S. G., AND R. W. SPINRAD. 1988. Size and refractive index of individual marine particulates: A flow cytometric approach. *Appl. Opt.* **27**: 1270–1277.
- ANDERSEN, R. A., R. R. BIDIGARE, M. D. KELLER, AND M. LATASA. 1996. A comparison of HPLC pigment signatures and electron microscopic observations for oligotrophic waters of the North Atlantic and Pacific Oceans. *Deep-Sea Res.* **43**: 517–537.
- ARMSTRONG, R. A. 1994. Grazing limitation and nutrient limitation in marine ecosystems: Steady state solutions of an ecosystem model with multiple food chains. *Limnol. Oceanogr.* **39**: 597–608.
- BEHRENFELD, M. J., A. J. BALE, A. S. KOLBER, J. AIKEN, AND P. G. FALKOWSKI. 1996. Confirmation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. *Nature* **383**: 508–511.
- BIDIGARE, R. R., M. E. ONDRUSEK, J. H. MORROW, AND D. A.

- KIEFER. 1990. *In vivo* absorption properties of algal pigments. Proc. SPIE Ocean Opt. **X** 1302: 290–302.
- BINDER, B. J., S. W. CHISHOLM, R. J. OLSON, S. L. FRANKEL, AND A. Z. WORDEN. 1996. Dynamics of picophytoplankton, ultraphytoplankton and bacteria in the central equatorial Pacific. Deep-Sea Res. **43**: 907–931.
- BOYD, P. W., D. L. MUGGLI, D. E. VARELA, R. H. GOLDBLATT, R. CHRETIEN, K. J. ORIAN, AND P. J. HARRISON. 1996. *In vitro* iron enrichment experiments in the NE subarctic Pacific. Mar. Ecol. Prog. Ser. **136**: 179–193.
- BUMA, A. G. J., H. J. W. DE BAAR, R. F. NOLTING, AND A. J. VAN BENNEKOM. 1991. Metal enrichment experiments in the Weddell-Scotia Seas: Effects of iron and manganese on various plankton communities. Limnol. Oceanogr. **36**: 1865–1878.
- CAVENDER-BARES, K. K., S. L. FRANKEL, AND S. W. CHISHOLM. 1998. A dual sheath flow cytometer for shipboard analyses of phytoplankton communities from the oligotrophic oceans. Limnol. Oceanogr. **43**: 1383–1388.
- CHAVEZ, F. P., K. R. BUCK, AND R. T. BARBER. 1990. Phytoplankton taxa in relation to primary production in the equatorial Pacific. Deep-Sea Res. **37**: 1733–1752.
- , ———, S. K. SERVICE, J. NEWTON, AND R. T. BARBER. 1996. Phytoplankton variability in the central and eastern tropical Pacific. Deep-Sea Res. **43**: 835–870.
- CHISHOLM, S. W., AND F. M. M. MOREL [EDS.]. 1991. What controls phytoplankton production in nutrient-rich areas of the open sea? Limnol. Oceanogr. **36**: 1507–1965.
- , R. J. OLSON, E. R. ZETTLER, R. GOERICKE, J. B. WATERBURY, AND N. A. WELSCHMEYER. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **334**: 340–343.
- COALE, K. H. 1991. Effects of iron, manganese, copper, and zinc enrichments on productivity and biomass in the subarctic Pacific. Limnol. Oceanogr. **36**: 1851–1864.
- , S. E. FITZWATER, R. M. GORDON, K. S. JOHNSON, AND R. T. BARBER. 1996a. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. Nature **379**: 621–624.
- , AND OTHERS. 1996b. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature **383**: 495–501.
- COOPER, D. J., A. J. WATSON, AND P. D. NIGHTINGALE. 1996. Large decrease in ocean-surface CO<sub>2</sub> fugacity in response to *in situ* iron fertilization. Nature **383**: 511–513.
- CULLEN, J. J. 1995. Status of the iron hypothesis after the Open-Ocean Enrichment Experiment. Limnol. Oceanogr. **40**: 1336–1343.
- DUGDALE, R. C., AND F. P. WILKERSON. 1998. Silicate regulation of new production in the equatorial Pacific upwelling. Nature **391**: 270–273.
- , ———, AND H. J. MINAS. 1995. The role of a silicate pump in driving new production. Deep-Sea Res. **42**: 697–719.
- DURAND, M. D. 1995. Phytoplankton growth and diel variations in beam attenuation through individual cell analysis. Ph.D. thesis, M.I.T. and W.H.O.I.
- DUSENBERRY, J. A. 1995. Picophytoplankton photoacclimation and mixing in the surface oceans. Ph.D. thesis, M.I.T. and W.H.O.I.
- EPPLEY, R. W., AND E. H. RENGER. 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol. **10**: 15–23.
- ERDNER, D. L. 1997. Characterization of ferredoxin and flavodoxin as molecular indicators of iron limitation in marine eukaryotic phytoplankton. Ph.D. thesis, M.I.T. and W.H.O.I.
- FALKOWSKI, P., AND D. A. KIEFER. 1985. Chlorophyll *a* fluorescence in phytoplankton: Relationship to photosynthesis and biomass. J. Plankton Res. **7**: 715–731.
- FALKOWSKI, P. G., AND Z. KOLBER. 1995. Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans. Aust. J. Plant Physiol. **22**: 341–355.
- FROST, B. W. 1991. The role of grazing in nutrient-rich areas of the open sea. Limnol. Oceanogr. **36**: 1616–1630.
- , AND N. C. FRANZEN. 1992. Grazing and iron limitation in the control of phytoplankton stock and nutrient concentration: A chemostat analogue of the Pacific equatorial upwelling zone. Mar. Ecol. Prog. Ser. **83**: 291–303.
- FURUYA, K., AND W. K. W. LI. 1992. Evaluation of photosynthetic capacity in phytoplankton by flow cytometric analysis of DCMU-enhanced chlorophyll fluorescence. Mar. Ecol. Prog. Ser. **88**: 279–287.
- GEIDER, R. J., J. LA ROCHE, R. M. GREENE, AND M. OLAIZOLA. 1993. Response of the photosynthetic apparatus of *Phaeodactylum tricorutum* (Bacillariophyceae) to nitrate, phosphate, or iron starvation. J. Phycol. **29**: 755–766.
- GOERICKE, R., AND D. J. REPETA. 1993. Chlorophylls *a* and *b* and divinyl chlorophylls *a* and *b* in the open subtropical North Atlantic Ocean. Mar. Ecol. Prog. Ser. **101**: 307–313.
- GRAN, H. H. 1931. On the conditions for the production of plankton in the sea. Rapp. P-V. Reun. Cons. Int. Explor. Mer. **75**: 37–46.
- HART, T. J. 1934. On the phytoplankton of the south-west Atlantic and the Bellingshausen Sea, 1929–31. Discovery Rep. **8**: 1–268.
- HOWARTH, R. W. 1988. Nutrient limitation of net primary production in marine ecosystems. Annu. Rev. Ecol. **19**: 89–110.
- JONES, I. S. F. 1996. Enhanced carbon dioxide uptake by the world's oceans. Energy Convers. Manage. **37**: 1049–1052.
- , AND H. E. YOUNG. 1997. Engineering a large sustainable world fishery. Environ. Conserv. **24**: 99–104.
- JONKER, R. R., J. T. MEULEMANS, G. B. J. DUBELAAR, M. F. WILKINS, AND J. RINGELBERG. 1995. Flow cytometry: A powerful tool in analysis of biomass distributions in phytoplankton. Water Sci. Technol. **32**: 177–182.
- KOLBER, Z. S., AND OTHERS. 1994. Iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. Nature **371**: 145–149.
- KUDELA, R. M., AND F. P. CHAVEZ. 1996. Bio-optical properties in relation to an algal bloom caused by iron enrichment in the equatorial Pacific. Geophys. Res. Lett. **23**: 3751–3754.
- KUDO, I., AND P. J. HARRISON. 1997. Effect of iron nutrition on the marine cyanobacterium *Synechococcus* grown on different N sources and irradiances. J. Phycol. **33**: 232–240.
- LANDRY, M. R., AND OTHERS. 1997. Iron and grazing constraints on primary production in the central equatorial Pacific: An EqPac synthesis. Limnol. Oceanogr. **42**: 405–418.
- , J. CONSTANTINOU, AND J. KIRSHTEIN. 1995. Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. Deep-Sea Res. **42**: 657–671.
- , AND R. P. HASSETT. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. **67**: 283–288.
- LEE, S., AND J. A. FUHRMAN. 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. **53**: 1298–1303.
- LI, W. K. W., T. ZOHARY, Y. Z. YACOBI, AND A. M. WOOD. 1993. Ultraphytoplankton in the eastern Mediterranean Sea: Towards deriving phytoplankton biomass from flow cytometric measurements of abundance, fluorescence and light scatter. Mar. Ecol. Prog. Ser. **102**: 79–87.
- MARKELS, M. 1995. Fishing for markets. Regulation **3**: 73–79.
- MARTIN, J. H. 1990. Glacial-interglacial CO<sub>2</sub> change: The iron hypothesis. Paleoceanography **5**: 1–13.
- , AND OTHERS. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature **371**: 123–129.

- , AND S. E. FITZWATER. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* **331**: 341–343.
- McKAY, R. M. L., R. J. GEIDER, AND J. LAROCHE. 1997. Physiological and biochemical response of the photosynthetic apparatus of two marine diatoms to Fe stress. *Plant Physiol.* **114**: 615–622.
- MOORE, L. R., AND S. W. CHISHOLM. Photophysiology of the marine cyanobacterium *Prochlorococcus*: Ecotypic differences among cultured isolates. *Limnol. Oceanogr.* In press.
- , R. GOERICKE, AND S. W. CHISHOLM. 1995. Comparative physiology of *Synechococcus* and *Prochlorococcus*: Influence of light and temperature on growth, pigments, fluorescence and absorptive properties. *Mar. Ecol. Prog. Ser.* **116**: 259–275.
- MOREL, F. M. M., R. J. HUDSON, AND N. M. PRICE. 1991a. Limitation of productivity by trace metals in the sea. *Limnol. Oceanogr.* **36**: 1742–1755.
- , J. G. RUETER, AND N. M. PRICE. 1991b. Iron nutrition of phytoplankton and its possible importance in the ecology of ocean regions with high nutrient and low biomass. *Oceanography* **4**: 56–61.
- OLSON, R. J., S. W. CHISHOLM, E. R. ZETTLER, M. A. ALTABET, AND J. A. DUSENBERRY. 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep-Sea Res.* **37**: 1033–1051.
- , E. R. ZETTLER, AND O. K. ANDERSON. 1989. Discrimination of eukaryotic phytoplankton cell types from light scatter and autofluorescence properties measured by flow cytometry. *Cytometry* **10**: 636–643.
- PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.
- PERRY, M. J. 1976. Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in oligotrophic waters of the central North Pacific. *Limnol. Oceanogr.* **21**: 88–107.
- PRICE, N. M., B. A. AHNER, AND F. M. M. MOREL. 1994. The equatorial Pacific Ocean: Grazer-controlled phytoplankton populations in an iron-limited ecosystem. *Limnol. Oceanogr.* **39**: 520–534.
- RAIMBAULT, P., M. RODIER, AND I. TAUPIER-LETAGE. 1988. Size fraction of phytoplankton in the Ligurian Sea and the Algerian Basin (Mediterranean Sea): Size distribution versus total concentration. *Mar. Microb. Food Webs* **3**: 1–7.
- RUE, E. L., AND K. W. BRULAND. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. *Limnol. Oceanogr.* **42**: 901–910.
- SARMIENTO, J. L., AND J. C. ORR. 1991. Three-dimensional simulations of the impact of Southern Ocean nutrient depletion on atmospheric CO<sub>2</sub> and ocean chemistry. *Limnol. Oceanogr.* **36**: 1928–1950.
- SIEBURTH, J. MCN., V. SMETACEK, AND J. LENZ. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.* **23**: 1256–1263.
- SIMON, M., AND F. AZAM. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* **51**: 201–213.
- SIMON, N., R. G. BARLOW, D. MARIE, F. PARTENSKY, AND D. VAULOT. 1994. Characterization of oceanic photosynthetic picoeukaryotes by flow cytometry. *J. Phycol.* **30**: 922–935.
- SOKAL, R. R., AND F. J. ROHLF. 1995. *Biometry*, 3rd ed. W. H. Freeman.
- SØRENSEN, R. 1995. Maricult-planting the seeds of a blue revolution. *Tellus* **2**: 4–7.
- SOSIK, H. M., S. W. CHISHOLM, AND R. J. OLSON. 1989. Chlorophyll fluorescence from single cells: Interpretation of flow cytometric signals. *Limnol. Oceanogr.* **34**: 1749–1761.
- STRATHMAN, R. R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.* **12**: 411–418.
- SUNDA, W. G., AND S. A. HUNTSMAN. 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature* **390**: 389–392.
- TAKEDA, S., AND H. OBATA. 1995. Response of equatorial Pacific phytoplankton to subnanomolar Fe enrichment. *Mar. Chem.* **50**: 219–227.
- TRICK, C. G., S. W. WILHELM, AND C. M. BROWN. 1995. Alterations in cell pigmentation, protein expression, and photosynthetic capacity of the cyanobacterium *Oscillatoria tenuis* grown under low iron conditions. *Can. J. Microbiol.* **41**: 1117–1123.
- TURNER, S. M., P. D. NIGHTINGALE, L. J. SPOKES, M. I. LIDDICOAT, AND P. S. LISS. 1996. Increased dimethyl sulphide concentrations in sea water from *in situ* iron enrichment. *Nature* **383**: 513–517.
- VAN DE HULST, H. C. 1957. Light scattering by small particles. Wiley.
- VERITY, P. G., C. Y. ROBERTSON, C. R. TRONZO, M. G. ANDREWS, J. R. NELSON, AND M. E. SIERACKI. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* **37**: 1434–1446.
- VESK, M., AND S. W. JEFFREY. 1987. Ultrastructure and pigments of two strains of the picoplanktonic alga *Pelagococcus subviridis* (Chrysophyceae). *J. Phycol.* **23**: 322–336.
- WALSH, J. J. 1976. Herbivory as a factor in patterns of nutrient utilization in the sea. *Limnol. Oceanogr.* **21**: 1–13.
- WILHELM, S. W., D. P. MAXWELL, AND C. G. TRICK. 1996. Growth, iron requirements, and siderophore production in iron-limited *Synechococcus* PCC 7002. *Limnol. Oceanogr.* **41**: 89–97.
- , AND C. G. TRICK. 1995. Physiological profiles of *Synechococcus* (Cyanophyceae) in iron-limiting continuous cultures. *J. Phycol.* **31**: 79–85.
- WRIGHT, S. W., AND S. W. JEFFREY. 1987. Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. *Mar. Ecol. Prog. Ser.* **38**: 259–266.
- XU, C., J. AUGER, AND GOVINDJEE. 1990. Chlorophyll *a* fluorescence measurements of isolated spinach thylakoids obtained by using single-laser-based flow cytometry. *Cytometry* **11**: 349–358.
- ZETTLER, E. R., R. J. OLSON, B. J. BINDER, S. W. CHISHOLM, S. E. FITZWATER, AND M. R. GORDON. 1996. Iron-enrichment bottle experiments in the equatorial Pacific: Responses of individual phytoplankton cells. *Deep-Sea Res.* **43**: 1017–1029.

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