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Co-limitation of phytoplankton growth by light and Fe during winter in the NE subarctic Pacific Ocean

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Abstract

Phytoplankton acclimate to low irradiance by increasing their cellular demand for Fe, to allow synthesis of additional light-harvesting pigments and Fe-containing redox proteins involved in photosynthesis. In the open NE subarctic Pacific, Fe concentrations limit primary productivity and irradiances may be suboptimal, particularly during winter. Phytoplankton thus may be unable to fulfill their extra Fe requirements for growth under these low-light conditions and become effectively co-limited. We tested this hypothesis by manipulating Fe and light in in vitro experiments at OSP (Ocean Station PAPA, 50°N 145°W) during winter 1997. The results show that metabolic rates, growth, and photosynthetic parameters of phytoplankton are enhanced in winter by increasing either irradiance or Fe. The greatest response occurs when Fe and light are amended concomitantly, confirming that the community is indeed co-limited by both resources. Analysis of environmental conditions (i.e. incident irradiance, mixed layer depth and Fe concentrations) in winter at OSP reveals that they are similar to those observed in the austral spring and fall at three sites in the Southern Ocean. Extrapolating our experimental field results to the Southern Ocean illustrates that co-limitation by light and Fe also may play an important role in regulating phytoplankton growth in this region.

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1. Introduction

Theoretical calculations (Raven, 1990) and culture studies (Sunda and Huntsman, 1997) illustrate the high Fe requirements for growth of phytoplankton at low light. Acclimation of these photoautotrophs to low irradiance involves an increase in the concentration of light-harvesting pigments and change in the abundance and stoichiometry of Fe-containing components of the photosynthetic electron transport chain (Falkowski and LaRoche, 1991). Because most of the Fe is involved in photosynthesis (Raven, 1988,1990), a decrease in irradiance is predicted to increase the cellular Fe requirement of photoautotrophs. This extra cellular Fe allows synthesis of the photosynthetic units (PSU) needed for low light adaptation (Falkowski et al., 1981; Raven, 1990). Measurements of lab-cultured marine phytoplankton confirm this increase and show that their Fe requirements quadruple with a 10-fold decline in irradiance (500–50 μmol quanta m^{-2} s^{-1}) (Sunda and Huntsman, 1997).

While growth rates of light-limited phytoplankton are slow, Fe uptake is kept at the maximum rate allowed by diffusion and the rate of water loss from inorganic Fe species (Sunda and Huntsman, 1997). This latter rate ultimately limits the rate of complexation of Fe with the transporters on the cell surface of phytoplankton (Hudson and Morel, 1990). At steady state, Fe uptake rate ($\rho^{ss}\text{Fe} = Q_{\text{Fe}}{*}\mu$) is determined by the intracellular Fe concentration (Fe : C ratio) and the growth rate. If rates of Fe uptake are kept at a maximum, a decrease in algal growth rate at low light results in an increase in Fe quota (Fe : C ratio), allowing synthesis of the extra PSUS. If the concentration of dissolved Fe is too low; however, light-limited phytoplankton cannot achieve maximum rates of Fe uptake needed to support their growth requirements. Low light levels in Fe-poor waters may thus impose serious constraints on phytoplankton growth and result in an effective co-limitation by both resources (Sunda and Huntsman, 1997). Although Fe is known to limit phytoplankton growth in certain regions of the sea (Behrenfeld et al., 1996), light limitation also has been suggested to control primary production in open-water areas (i.e. Southern Ocean, Nelson and Smith, 1991; Mitchell et al., 1991), including those that are Fe-poor. The co-limitation hypothesis suggests that both light and Fe could simultaneously be limiting because of the biochemical dependence of photosynthesis on Fe. Field tests of this co-limitation hypothesis, however, are lacking.

Phytoplankton in the open NE subarctic Pacific are Fe-limited during spring and summer (LaRoche et al., 1996) and are stimulated to grow in response to Fe additions (Martin and Fitzwater, 1988; Boyd et al., 1996). Yet, in winter a previous 5-day in vitro experiment at OSP showed that Fe-enrichment did not enhance phytoplankton growth (Boyd et al., 1995a). Because the winter irradiances are considerably less than those required to maintain maximum rates of production (see Fig. 1, Welschmeyer et al., 1993), phytoplankton grow relatively slowly (cf. spring) so their net biomass might not have increased significantly 5 days after the addition of Fe.

During February 1997, we reexamined this issue of phytoplankton growth co-limitation at OSP by conducting an in vitro experiment in which we manipulated the levels of both Fe and light. Phytoplankton biomass, metabolic rates, and photosynthetic parameters were measured over a period of 7 days. The results of this experiment are reported here.

2. Methods

2.1. Seawater collection and experimental set up

Using trace-metal-clean techniques, seawater was collected at 15 m at OSP in mid-February 1997 (Boyd et al., 1996). The water was transferred directly to six pre-cleaned 25-l polycarbonate carboys within a portable clean room. Three carboys were amended with low Fe (2 nM Fe : 50 μM EDTA, see below), while the other three were enriched with high Fe concentrations (20 nM Fe : 0.5 μM EDTA). The lids of all the carboys were sealed with Parafilm, and the carboys were triple-bagged and covered by a series of neutral density screen to obtain a range of irradiances corresponding to 40, 12, and 3% I_0 (as percentage of incident irradiance). The carboys were then placed in deck incubators maintained within 1°C of ambient temperature (≈ 6°C) by flowing seawater. Seawater samples also were collected at the start of the experiment to ascertain the initial biological and chemical characteristics of the water. Algal community growth rates at time zero were estimated from the water column ^{14}C uptake divided by integrated chl *a* after Boyd and Harrison (1999).

2.2. Uptake rates and Fe : C ratios determinations

Iron : carbon ratios, as well as Fe and C uptake rates, were measured simultaneously using ^{55}Fe (20 mCi mg^{-1}, DuPont) and $H^{14}CO_3^-$ (8.4 Ci mol^{-1}, DuPont) (Tortell et al., 1996; Maldonado and Price, 1999). Seawater was dispensed into 4-l polycarbonate bottles, which were treated in the same way as the 25-l carboys, except that seawater was enriched with ^{55}Fe (2 nM Fe : 50 μM EDTA for the low Fe addition or 20 nM Fe : 0.5 μM EDTA for the enriched Fe addition) and $H^{14}CO_3^-$ (2.4 μM). Samples were collected at 24, 48 and 68 h after the start of the experiment on 1 and 3 μm porosity Poretics polycarbonate filters, and rinsed with Ti(III) citrate EDTA reagent (Hudson and Morel, 1989). A series of experiments verified that the Ti(III) rinse did not cause any C loss due to cell breakage (Maldonado and Price, 1999). Volumetric uptake rates were calculated from linear regression of particulate C or Fe per ml against time, after correcting for abiotic uptake.

Iron was added complexed to the synthetic chelator EDTA. The ^{55}Fe stock and FeEDTA solutions were prepared as described in Maldonado and Price (1999). In the low-Fe treatment, the concentration of EDTA (50 μM) was greatly in excess of that of ^{55}Fe (2 nM), and buffered a remarkably low inorganic Fe concentration (20 pM, measured in non-illuminated Aquil samples using the sulfoxine method, Hudson et al., 1992). This inorganic Fe concentration is below that required to support maximum growth rates of oceanic phytoplankton (100 pM for *Thalassiosira oceanica*, Sunda and Huntsman, 1995), and thus should maintain in situ Fe-limited phytoplankton growth rates, but allow accurate measurement of Fe quotas and uptake rates. The ambient dissolved Fe concentration at OSP (50 pM, Martin et al., 1989) is much lower than the added ^{55}Fe (2 nM) and has a negligible effect on the specific activity of ^{55}Fe. Thus, relatively high additions of ^{55}Fe and excess EDTA allow estimates of in situ Fe uptake rates and quotas, despite lack of data for ambient dissolved Fe concentration.

2.3. Photosynthetic parameters (P^{b}_{max} and alpha)

Assimilation number (P^{b}_{max}, maximum rate of photosynthesis) and alpha (initial slope of the photosynthesis–irradiance curve) were measured by sub-sampling the 25-l carboys at 2-day intervals and spiking the seawater with 10 μCi ^{14}C in 70-ml polycarbonate bottles. Samples were incubated at 18 irradiances (2–1200 μmol quanta m^{-2} s^{-1}) for 4 h. The lamp was W–halogen, and water temperature was maintained by flowing surface seawater. Carbon fixation rates were corrected for dark ^{14}C uptake, and normalized to chl *a* biomass, μg C (μg chl *a*)$^{-1}$ h^{-1}. Where data were available, the rates also were normalized to particulate organic carbon (POC), μg C (μg POC)$^{-1}$ h^{-1}. Particulate organic carbon was determined using procedures of Parsons et al. (1984). The relationship between photosynthetic rate and irradiance was obtained from curve-fitting, based on a hyperbolic tangential equation (Smith, 1936), and using the fitting routine of Lederman and Tett (1988). In order to avoid the effects of diel periodicity on photosynthetic characteristics, only two experiments were performed each day around noon. No pronounced photoinhibition was observed.

2.4. Underwater light regime

The estimated integrated irradiance received by an algal cell in situ as it circulates through the water column was calculated using a mixed-layer model constrained by a basal density gradient (Denman and Gargett, 1983), in conjunction with irradiance data and an estimate of the mixed-layer depth. Ten-minute averaged incident irradiance (PAR) data were obtained from a calibrated Licor 2-PI PAR sensor located aft on the vessel in order to ensure no shading by the ship's superstructure. The mixed-layer depth (ca. 80 m) was estimated from calibrated temperature profiles obtained using a Guildline CTD. The attenuation coefficient (0.04 m^{-1}) of the water column was calculated from a vertical PAR profile – using a calibrated 4-PI sensor (Biospherical Instruments). The mixing time for a cell over the surface mixed layer was computed using the Denman and Gargett model (1983), the mixed-layer depth, and wind-stress data (mean = 25 knots from ship's log). The mean estimate of in situ irradiance received by a cell traversing the water column was 0.91 mol quanta m^{-2} d^{-1} over the 7-day duration of the experiment. This value is within the range of irradiance levels received by cells in the experimental carboys: 3.65, 1.09, and 0.27 mol quanta m^{-2} d^{-1} for the 40, 12, and 3% I_0 treatments, respectively.

3. Results and discussion

3.1. Physical, biological and chemical parameters at OSP

The depth of the surface mixed layer was 80 m, and mean incident PAR (I_0) was 9.12 mol quanta m^{-2} d^{-1} over the 7-day incubation. Macronutrient concentrations were high ($[NO_3^-]$ = 13.2 μM, $[PO_4^{3-}]$ = 1.24 μM and $[SiO_3^{2-}]$ = 19.3 μM), phytoplankton biomass was low (ca. 0.3 μg chl *a* l^{-1}), and > 70% of the cells were < 5 μm in diameter. The phytoplankton community growth rate was 0.30 d^{-1} (Boyd and Harrison, 1999). Flavodoxin levels in the phytoplankton community (LaRoche et al., 1996) in winter 1996 were comparable to those observed in spring and summer, indicating that the algal community was indeed Fe-stressed at this time of the year (R.M. McKay, pers. comm.). No measurements of flavodoxin were available for winter 1997.

3.2. Fe requirements of phytoplankton growth at different light levels

In winter at OSP, when irradiances are typically $\frac{1}{3}$ of those measured in spring (see Fig. 1 in Welschmeyer et al., 1993; Boyd, unpublished data), phytoplankton should have a high Fe content, reflecting their Fe requirements for photo-acclimation (Raven, 1990; Sunda and Huntsman, 1997). In situ Fe quotas (μmol Fe : mol C) were measured on samples collected at 15 m, amended with ^{14}C and low ^{55}Fe concentrations, and incubated for a week on deck at three light levels: 3, 12, and 40% I_0. The mean estimated irradiance received by cells in situ was comparable to the two lowest light treatments (see methods/Table 1). The low-Fe addition (2 nM) was complexed to EDTA (50 μM EDTA), buffering a low inorganic Fe concentration (20 pM, see methods) that did not greatly stimulate phytoplankton growth (Table 1). Iron to carbon ratios declined in the large autotrophs (> 3 μm) as light increased from 3 to 40% I_0 [μmol Fe (mol C)$^{-1}$ = 4.32 (3% I_0), 2.56 (12% I_0), and 1.19 (40% I_0)], in accordance with the higher amount of Fe required for growth at low light. A similar trend was observed for the 1–3 μm phytoplankton, even though the Fe : C ratios at 12 and 3% I_0 were similar and higher than those at 40% I_0 (mean Fe : C ratio at 12 and 3% I_0 was 7 ± 1 vs. 2.5 μmol Fe (mol C)$^{-1}$ at 40% I_0).

3.3. Effects of increasing light levels and/or Fe concentrations

3.3.1. On phytoplankton growth

The higher Fe requirement elicited by low light acclimation might further impede growth of phytoplankton in low-Fe waters. If cells at OSP are indeed co-limited by light and Fe, increasing either of these two resources should enhance algal growth, and the greatest effect should be observed when both light and Fe are increased concomitantly. Our findings support these predictions (Table 1). By day 7, changes in chl *a* and macronutrient concentrations were greatest in samples that were enriched with Fe (20 nM Fe : 0.5 μM EDTA) and exposed to the highest irradiance (40% I_0) and least in the samples that received low additions of Fe at the lowest

Table 1
Size-fractionated chl *a* concentrations ($\mu g\,l^{-1}$) on day 3 after Fe addition, and day 7 (last day of the experiment) in the experimental 25-l carboys subjected to a variety of irradiance levels (40, 12 and 3% I_0; $I_0 = 9.12$ mol quanta $m^{-2}\,d^{-1}$, $n = 7$) and Fe conditions (low Fe, 2 nM Fe : 50 μM EDTA; enriched Fe, 20 nM Fe : 0.5 μM EDTA). Initial chl *a* concentration = 0.3 μg chl $a\,l^{-1}$ (< 1 μm = 0.002, 1–3 μm = 0.102, and > 3 μm = 0.159 μg chl $a\,l^{-1}$). In parentheses: nitrate concentrations (μM) in the carboys on the last day of the experiment. Initial [NO_3^-] = 13.2 μM (macronutrient data from F.A. Whitney)

Irradiance	Size fraction	Low Fe		Enriched Fe	
		Day 3	Day 7	Day 3	Day 7
40% I_0	< 1 μm	0.065	0.234	0.06	0.438
	1–3 μm	0.143	0.427	0.142	0.387
	> 3 μm	0.23	1.44	0.282	3.706
			(12.5)		(10.9)
12% I_0	< 1 μm	0	0.003	0.073	0.313
	1–3 μm	0.008	0.008	0.148	0.537
	> 3 μm	0.008	0.021	0.211	1.664
			(13.7)		(11.0)
3% I_0	< 1 μm	0.061	0.221	0.056	0.204
	1–3 μm	0.088	0.189	0.129	0.223
	> 3 μm	0.184	0.578	0.195	0.74
			(13.5)		(12.2)

irradiance (3% I_0) (Table 1). At all light levels, the greatest increase in chl *a* in the high iron treatments occurred in the > 3 μm size-fraction. Thus, large phytoplankton appear to be the most Fe-limited, as expected from their relatively low surface area to volume ratio (Morel et al., 1991; Sunda and Huntsman, 1997). Note that the chl *a* values for the 12% I_0/low Fe treatment are consistently low and therefore suspect; the 25-l carboy might inadvertently have contained a large zooplankter (i.e. a copepod).

3.3.2. On metabolic rates

Since neither chl *a* nor macronutrient concentrations changed dramatically during the first 3 days of the experiment (Table 1), uptake rates could be compared among treatments in the 4-l carboys using the volumetric rates (mol $ml^{-1}\,h^{-1}$, Fig. 1).

Carbon fixation rates. At low light and Fe, carbon uptake rates were slow (0.64 ± 0.18 pmol C $ml^{-1}\,h^{-1}$). When irradiance was increased ca. 13-fold to 40% I_0, C fixation rates were enhanced 20 times by day 3, demonstrating that light was limiting production (Fig. 1). This and all other increases in C fixation resulted primarily from a physiological response since biomass (chl *a*) increased at most by 1.7 times relative to ambient chl *a* concentrations (Table 1). Moreover, identical results were obtained one day after beginning the experiment (Fig. 1), when no changes in biomass were evident among treatments. Iron addition to the low irradiance treatments (12 and 3% I_0) had a similar outcome, increasing C fixation by 5–15 times relative to the low Fe addition. Simultaneously augmenting light and Fe in the

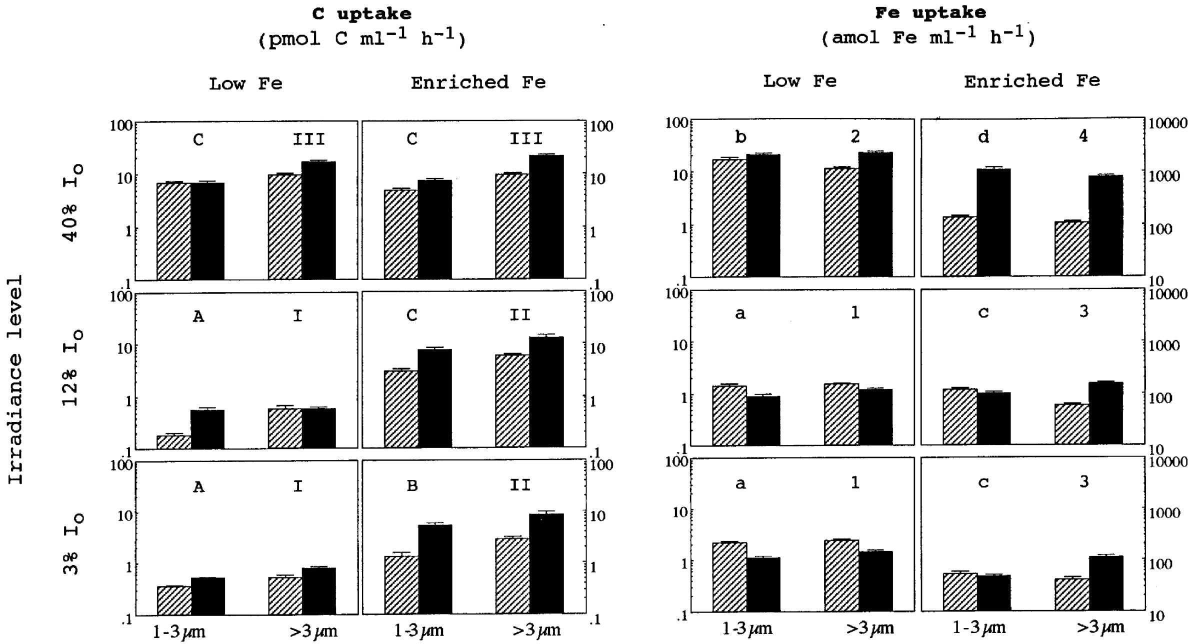

Fig. 1. In vitro size-fractionated Fe (amol Fe ml^{-1} h^{-1}) and C uptake rates (pmol C ml^{-1} h^{-1}) of phytoplankton subjected to a variety of irradiance levels and Fe conditions (4-l carboys, see methods and legend Table 1). Uptake rates were measured 24 (dashed bars) and 70 h (shaded bars) after ^{55}Fe and ^{14}C additions, and are the mean of duplicates with error bars representing the range around the mean. Note the different scale of the Fe-enriched Fe uptake rates. (pmol = 10^{-12} mol, amol = 10^{-18} mol). Uptake rates (day 3) with the same letter or number above the bars were not significantly different from one another (paired t-test, $p < 0.05$). Statistical analysis using Pairwise comparison adjusted with a sequential Bonferroni correction showed that the following uptake rates were significantly different from one another: 1–3 μm C uptake rates [low Fe/40% I_0 vs. low Fe/12% I_0 ($p < 0.0001$), and vs. /3% I_0 ($p < 0.0001$); high Fe/40% I_0 vs. low Fe/12% I_0 ($p < 0.0003$), and vs. /3% I_0 ($p < 0.0004$)].

1–3 μm Fe uptake rates [low Fe/40% I_0 vs. low Fe/12% I_0 ($p < 0.0001$), and vs. /3% I_0 ($p < 0.0001$); high Fe/3% I_0 vs. low Fe/40% I_0 ($p < 0.0013$), vs. /12% I_0 ($p < 0.0003$), and vs. /3% I_0 ($p < 0.0003$)].

> 3 μm C uptake rates [low Fe/40% I_0 vs. low Fe/12% I_0 ($p < 0.0012$), and vs. /3% I_0 ($p < 0.0013$); high Fe/40% I_0 vs. low Fe/12% I_0 ($p < 0.0015$), and vs. /3% I_0 ($p < 0.0013$); high Fe/40% I_0 vs. high Fe/3% I_0 ($p < 0.0049$)].

> 3 μm Fe uptake rates [low Fe/40% I_0 vs. low Fe/12% I_0 ($p < 0.0026$), and vs. /3% I_0 ($p < 0.0029$)].

samples produced the greatest response. As the large phytoplankton showed the most significant increase in biomass at the end of the experiment, we believe that the populations in this size fraction were co-limited by both resources. Taxon-specific responses to light and Fe, however, cannot be established at present.

Light-mediated enhancement of C uptake in the Fe-enriched samples was modest (2.5 times) compared to that observed at low Fe (Fig. 1). Under the Fe-enriched condition, phytoplankton were apparently able to increase the number of PSUs and hence to photosynthesize optimally at low irradiance. The demand for Fe was reduced at high light, as C-fixation rates increased only slightly in the high-light, high-Fe treatment.

Fe transport rates. Algal transport rates of Fe were concentration-dependent and influenced by irradiance levels (Fig. 1). While the sulfoxine reactive Fe concentration was 1000 times higher in the 'enriched' samples (20 nM) compared to the low addition (20 pM), Fe uptake rates were only 100-times faster. Thus, either the Fe addition in the 'enriched' treatment was sufficient to saturate phytoplankton transport systems or to exert a feedback inhibition on the enzymes involved in Fe acquisition (Harrison and Morel, 1986). At both Fe levels, Fe uptake rates were 10-times faster at the highest light level than at the two lowest irradiances. In the low-Fe treatment, the faster Fe uptake rates at high light were either due to a greater concentration of bioavailable Fe as a result of photoreduction, or to faster photosynthetic rates allowing greater allocation of energy for Fe acquisition.

3.3.3. *On photosynthetic parameters*

Short-term measurements of photosynthetic parameters confirmed the results from the in vitro incubations. An increase in irradiance from in situ levels to 40% I_0, elicited a 40% increase in P^{b}_{max} (assimilation number: maximum rate of photosynthesis normalized to chl *a* biomass, μg (C μg chl *a*)$^{-1}$ h^{-1}) by day 2 compared to the initial rate (Table 2). At all light levels, the effects of high-Fe relative to low-Fe addition on P^{b}_{max} were less apparent, possibly because chl *a* was used as a biomass proxy to normalize the rates. In this case, chl *a*, which is synthesized by an Fe-dependent pathway, may have increased in response to Fe addition and masked the increase in photosynthetic rate. However, using the available C biomass data (POC) to normalize P^{b}_{max}, we also observed increases in P^{b}_{max} at the highest irradiance, and a suggestion of a slightly greater effect in the enriched-Fe treatment [low Fe $P^{b}_{max} = 0.016$ and enriched-Fe $P^{b}_{max} = 0.017$ μg C (μg C)$^{-1}$ h^{-1} on day 2] relative to the initial rate (0.012 μg C (μg C)$^{-1}$ h^{-1}). Thus, both Fe and irradiance were apparently limiting phytoplankton C fixation rates. These results are consistent with those obtained from Fe-limited lab cultures, which show that phytoplankton have fewer PSUs and are correspondingly less efficient in light absorption and excitation energy transfer than Fe-sufficient cultures (Green et al., 1991). Our results suggest that under winter irradiance levels at OSP, photosynthesis is impaired unless Fe bioavailability is increased significantly. As previously demonstrated in lab studies (Kolber et al., 1988; Green et al., 1991), alpha (initial slope of the photosynthesis–irradiance curve, μg C (μg chl *a*)$^{-1}$ h^{-1}/(μmol quanta m^{-2} s^{-1})) was unaffected by Fe or irradiance levels, and thus nearly identical for all treatments and $t = 0$ (data not shown).

Table 2
Phytoplankton community assimilation number – P^{b}_{max}, maximum rate of photosynthesis normalized to chl *a* biomass, μg C (μg chl *a*)$^{-1}$ h^{-1} – of phytoplankton for a range of irradiance levels, and Fe conditions (in 25-l carboys, see methods/Table 1). Æ Day of the experiment when the photosynthetic parameters were determined

Irradiance	Time[Æ]	Low Fe	Enriched Fe
40% I_0	$t = 2$	0.91 ± 0.07	0.90 ± 0.05
12% I_0	$t = 6$	0.82 ± 0.07	0.91 ± 0.07
3% I_0	$t = 4$	0.77 ± 0.07	0.79 ± 0.07
Initial	$t = 0$	0.61 ± 0.04	

Table 3
Mean irradiance levels received by cells traversing the mixed layer (mol quanta m^{-2} d^{-1}), and the mixed layer depth (m) of three locations in the Southern Ocean during austral spring/fall, and Ocean Station PAPA (winter) in the NE subarctic Pacific

Location	Coordinates	Irradiance (mol quanta m^{-2} d^{-1})	Mixed layer depth (m)
Bellingshausen Sea[a] (November 1992)	68.5°S 85°W	0.85 (2 d mean)	60
Subantarctic Pacific[b] (April 1997)	46°S 178.5°E	1.18 (3 d mean)	85
Subantarctic Pacific[b] (October 1997)	46.5°S 178.5°E	2.3 (4 d mean)	90
Ocean Station PAPA[c] (February 1997)	50°N 145°W	0.91 (7 d mean)	80

[a]Boyd et al. (1995b).
[b](Boyd unpubl.).
[c]This study.

3.3.4. Co-limitation of phytoplankton by light and Fe

At OSP, the calculated average irradiance received by an algal cell within the mixed layer (80 m) is ca. 1.0 mol quanta m^{-2} d^{-1}. In spite of differences in spectral quality (Kirk, 1989) and irradiance fluctuations (Dera and Gordon, 1968) experienced by phytoplankton in vitro (the carboys) and in situ (see methods/Table 1), the low light and limiting Fe conditions that characterize the winter environment at OSP are comparable to the low-Fe treatment exposed to the lowest light treatments (3–12% I_0). Under these conditions, phytoplankton had the highest Fe demand (highest Fe : C ratios) as required for photo-adaptation, but had insufficient Fe supply (the slowest Fe uptake rates), resulting in the slowest C fixation rates and the lowest P^{b}_{max}. Increasing irradiance 4-fold increased chl *a* levels in the low-Fe treatment 7-fold relative to ambient chl *a* levels. The greatest response by phytoplankton was observed in the high light/enriched Fe samples, where chl *a* levels increased by more than 10 times over the

7 d experiment. These results demonstrate that in winter at OSP, phytoplankton are co-limited by light and Fe.

Other Fe-poor regions, such as the open Southern Ocean, where the surface mixed layer may extend below the euphotic zone (Nelson and Smith, 1991; Mitchell et al., 1991), have similar environmental conditions to those observed at OSP during winter (Table 3). At three locations in the Southern Ocean (north and south of the polar front) during the austral spring/fall, irradiance levels are low (1.44 ± 0.62 mol quanta $m^{-2}\,d^{-1}$), mixed layers are deep (78 ± 16 m), and Fe concentrations are limiting (Martin and Fitzwater, 1988; Martin et al., 1990; Sedwick et al., 1997; de Baar et al., 1995). Thus, our results provide experimental evidence that suggests that co-limitation of phytoplankton by light and Fe might be an important characteristic over much of the annual cycle in the Southern Ocean.

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References

de Baar, H.J.W., de Jong, J.T.M., Bakker, D.C.E., Loscher, B.M., Veth, C., Bathmann, U., Smetacek, V., 1995. Importance of iron for plankton blooms and carbon dioxide drawdown in the Sourthern Ocean. Nature 373, 412–415.

Behrenfeld, M.J., Bale, A.J., Kolber, Z.S., Aiken, J., Falkowski, P.G., 1996. Confirmation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific. Nature 383, 508–511.

Boyd, P.W., Harrison, P.J., 1999. Phytoplankton dynamics in the NE subarctic Pacific. Deep-Sea Research II 46, 2405–2432.

Boyd, P.W., Muggli, D.L., Varela, D.E., Goldblatt, R.H., Chretien, R., Orians, K.J., Harrison, P.J., 1996. *In vitro* iron enrichment experiments in the NE subarctic Pacific. Marine Ecology Progress Series 136, 179–193.

Boyd, P.W., Whitney, F.A., Harrison, P.J., Wong, C.S., 1995a. The NE subarctic Pacific in winter: II. Biological rate processes. Marine Ecology Progress Series 128, 25–34.

Boyd, P.W., Robinson, C., Savidge, G., leB. Williams, P.J., 1995b. Water column and sea ice production during austral spring in the Bellinghausen sea. Deep-Sea Research II 42, 1177–1200.

Denman, K., Gargett, A., 1983. Time and space scales of vertical mixing and advection of phytoplankton in the upper ocean. Limnology and Oceanography 28, 801–815.

Dera, J., Gordon, H., 1968. Light field fluctuations in the photic zone. Limnology and Oceanography 13, 697–699.

Falkowski, P.G., LaRoche, J., 1991. Molecular biology in the study of ocean processes. International Review in Cytology 128, 261–303.

Falkowski, P.G., Owens, T.G., Ley, A.C., Mauzerall, D.C., 1981. Effects of growth irradiance levels on the ratio of reaction centers in two species of marine phytoplankton. Plant Physiology 68, 969–973.

Green, R.M., Geider, R.J., Falkowski, P.G., 1991. Effect of iron limitation on photosynthesis in a marine diatom. Limnology and Oceanography 36, 1772–1782.

Harrison, G.I., Morel, F.M.M., 1986. Response of the marine diatom *Thalassiosira weissflogii* to iron stress. Limnology and Oceanography 31, 989–997.

Hudson, R.J.M., Covault, D.T., Morel, F.M.M., 1992. Investigating of iron coordination and redox reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes. Marine Chemistry 38, 209–235.

Hudson, R.J.M., Morel, F.M.M., 1989. Distinguishing between extra- and intracellular iron in marine phytoplankton. Limnology and Oceanography 34, 1113–1120.

Hudson, R.J.M., Morel, F.M.M., 1990. Iron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions. Limnology and Oceanography 35, 1002–1020.

Kirk, J.T.O., 1989. The upwelling light stream in natural waters. Limnology and Oceanography 34, 1410–1425.

Kolber, Z., Zehr, J., Falkowski, P., 1988. Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. Plant Physiology 88, 923–929.

LaRoche, J., Boyd, P.W., McKay, R.M.L., Geider, R.J., 1996. Flavodoxin as an *in situ* marker for iron stress in phytoplankton. Nature 382, 802–805.

Lederman, T.C., Tett, P., 1988. Problems in modelling the photosynthesis–irradiance relationship for phytoplankton. Botanica Marina 24, 124–134.

Maldonado, M.T., Price, N.M., 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. Deep-Sea Research II 46, 2447–2473.

Martin, J.H., Fitzwater, S.E., 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature 331, 341–343.

Martin, J.H., Fitzwater, S.E., Gordon, R.M., 1990. Iron deficiency limits phytoplankton growth in Antarctic waters. Global Biogeochemical Cycles 4, 5–12.

Martin, J.H., Gordon, R.M., Fitzwater, S.E., Broenkow, W.W., 1989. VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Research 36, 649–680.

Mitchell, B.G., Brody, E.A., Holm-Hansen, O., McClain, C., Bishop, J., 1991. Light limitation of phytoplankton biomass and macronutrient utilization in the Southern Ocean. Limnology and Oceanography 36, 1662–1677.

Morel, F.M.M., Hudson, R.J.M., Price, N.M., 1991. Limitation of productivity by trace metals in the sea. Limnology and Oceanography 36, 1742–1755.

Nelson, D.M., Smith, W.O., 1991. Sverdrup revisited: Critical depths, maximum chlorophyll levels, and the control of Southern Ocean productivity by the irradiance-mixing regime. Limnology and Oceanography 36, 1650–1661.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A manual of chemical and biological methods for seawater analysis.. Pergamon Press, Oxford, UK.

Raven, J.A., 1988. The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources. New Phytologist 109, 279–287.

Raven, J.A., 1990. Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and C assimilation pathway. New Phytologist 116, 1–17.

Sedwick, P.N., Edwards, P.R., Mackey, D.J., Griffiths, F.B., Parslow, J.S., 1997. Fe and Mn in the surface waters of the Australian subantarctic region. Deep-Sea Research I 44, 1239–1253.

Smith, E.L., 1936. Photosynthesis in relation to light and carbon dioxide. Proceedings of the National Academy of Science USA 22, 504–511.

Sunda, W.G., Huntsman, S.A., 1995. Iron uptake and growth limitation in oceanic and coastal phytoplankton. Marine Chemistry 50, 189–206.

Sunda, W.G., Huntsman, S.A., 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. Nature 390, 389–392.

Tortell, P.D., Maldonado, M.T., Price, N.M., 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. Nature 383, 330–332.

Welschmeyer, N.A., Strom, S., Goericke, R., DiTuillio, G., Belvin, M., Peterson, W., 1993. Primary production in the subarctic Pacific Ocean: Project SUPER. Progress in Oceanography 32, 101–136.