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The elemental stoichiometry and composition of an iron-limited diatom

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Abstract

We grew *Thalassiosira weissflogii* to steady state over a range of Fe-limiting conditions with nitrate or ammonium as the N source. Nitrate-dependent cells had faster Fe-uptake rates, contained significantly higher intracellular Fe quotas, and grew faster than cells cultivated with NH_4^+ when Fe was most limiting. Under these conditions, carbon (C) : chlorophyll *a* ratios and the minimum fluorescence yield per chlorophyll *a* increased, but N source had no effect on either parameter. The ratio of variable to maximum fluorescence ($F_v F_m^{-1}$) declined little with Fe limitation even when *T. weissflogii* was grown at 25% of its maximum rate (μ_{max}). C : N ratios were higher in nitrate than in ammonium-grown cells and were constant at all Fe levels. Protein was independent of Fe and N, and amino acids were lowest in cells using NO_3^-. The P content of *T. weissflogii* (mol P L^{-1} cell volume) increased by 1.5 times as Fe became most limiting to growth, causing N : P and C : P ratios to decline significantly. The elemental stoichiometry for Fe-limited new production of *T. weissflogii* ($0.25\mu_{max}$) was thus 70C : 10N : 5.9Si : 1P : 0.00074Fe (by mols) compared with 97C : 14N : 4.7Si : 1P : 0.029Fe for nutrient-replete conditions. Uptake rate ratios of $NO_3^- : PO_4^{3-}$ showed the same dependence on Fe as the cellular N : P quotas, decreasing as [Fe] decreased. Iron limitation influenced the elemental composition of this marine diatom and will alter the assimilation ratios of C, N, and P in the high nitrate, low chlorophyll regions of the sea.

The results of numerous field experiments leave little doubt that phytoplankton in high-nitrate, low-chlorophyll (HNLC) regions of the sea are strongly Fe limited. Addition of small quantities of Fe to Fe-poor surface waters triggers rapidly a dramatic increase in photosynthetic C fixation and alters the biomass structure and dominance of the primary producers (Tsuda et al. 2003). These ecosystem-level changes affect energy flow (Hall and Safi 2001), particulate organic C export (Bishop et al. 2004), and the biogeochemical cycling of other important major and minor nutrients, such as Cd, Zn, N, and Si (Hutchins and Bruland 1998; Takeda 1998; Cullen et al. 2003).

From a physiological perspective, we have a fairly good understanding of the Fe-induced changes to phytoplankton following Fe enrichment. Laboratory studies have provided detailed information on the effects of Fe limitation on selected species and on their mechanisms of recovery. These studies show characteristic responses to low Fe, primarily in the structure and function of the photosynthetic apparatus (Greene et al. 1991; Geider et al. 1993; Moseley et al. 2002), in the amounts of cellular Fe required for growth (Sunda and Huntsman 1995; Maldonado and Price 1996), and in the pathways of Fe acquisition (Harrison and Morel 1986; Maldonado and Price 2000, 2001). Changes in macromolecular composition are also common features of Fe-limited phytoplankton. Indeed, one of the most noticeable effects is a decrease in cellular chlorophyll *a* (Chl *a*) concentration (Glover 1977). Protein (Glover 1977; Rueter and Ades 1987; Doucette and Harrison 1991), lipid, and carbohydrate (Milligan and Harrison 2000; Van Oijen et al. 2004) content as well are observed to vary with Fe nutritional state. Because these constituents are major reservoirs of N and C, variations in their concentrations could affect the elemental composition of Fe-limited phytoplankton and their requirements for these elements for growth. Such changes in phytoplankton requirements are predicted to affect the biogeochemical cycles of both major and minor nutrients (Peers and Price 2004).

The majority of information on the elemental stoichiometry of phytoplankton has been obtained under nutrient sufficiency and during limitation and starvation by N, P, and Si (Geider and La Roche 2002). Much less is known about the effects of Fe, considering its widespread shortage and importance in the sea. A few studies have shown that Fe availability has no influence on the C : N : P ratio of phytoplankton (the Redfield ratio) (Greene et al. 1991; La Roche et al. 1993; Takeda 1998). Yet a number of other studies have shown that, compared with nutrient-replete cultures, algal C : N, N : P, and C : P may increase (Muggli and Harrison 1996; De La Rocha et al. 2000; Berman-Frank et al. 2001) or decrease (Doucette and Harrison 1991; Maldonado and Price 1996; Sakshaug and Holm-Hansen 1977) when Fe is limiting. Growth conditions (i.e., steady-state compared with non-steady–state limitation) and species differences may be responsible for these contrasting results.

Evidence for a role for Fe in affecting nutrient consumption ratios and elemental composition of phytoplankton comes from incubation experiments and measurements of dissolved $NO_3^- : PO_4^{3-}$ in the Southern Ocean (Fanning 1992; De Baar et al. 1997). Anomalously low C : P and N : P ratios in particulate matter have been reported in this region (Copin-Montegut and Copin-Montegut 1978), but their cause is unknown. Faster rates of NO_3^- draw down relative to PO_4^{3-} were observed in Fe-amended samples by Martin and col-

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leagues (Martin et al. 1989), and subsequent experiments have confirmed these results, e.g., Hutchins et al. (2002). Thus, the relative requirements of phytoplankton for major nutrients could be influenced by their Fe nutritional state.

One important effect of Fe limitation on the elemental composition of phytoplankton is an increase in Si content of diatoms (Hutchins and Bruland 1998; Takeda 1998). This increase alters diatom Si : N consumption ratios, which affects the relative distribution of NO_3^- and SiO_3^{2-} in the sea. Because Fe inputs to HNLC regions vary naturally over times scales of days to millennia, the relative rates of Si and N cycling are predicted to fluctuate and the composition of the particles sinking from the sea surface to be variable (Takeda 1998). Any Fe-induced changes in the Redfield proportions of phytoplankton should affect in a similar way the biogeochemical cycling of C, N, and P.

In the context of oceanic Fe limitation, diatoms are an important group of phytoplankton to study because they are strongly Fe limited and dominate the fluxes of dissolved resources and energy following Fe enrichment in HNLC waters. Understanding how Fe limitation affects their elemental stoichiometry and composition is important for predicting their impact on the cycling of nutrients and C both in the modern day and in the past. Diatoms are estimated to account for roughly 25% of global production (Nelson et al. 1995) and directly or indirectly compose the bulk of the particles sinking to the interior of the sea (Ducklow et al. 2001). Like other phytoplankton in Fe-limited waters, they rely primarily on regenerated N for growth and only consume NO_3^- in large quantities once Fe replete (Price et al. 1991, 1994). Because N source affects C : N ratio, iron quota, growth rate, and photon yield of some phytoplankton (Raven et al. 1992; Wood and Flynn 1995; Maldonado and Price 1996), changes in the composition of natural populations may occur independently of changes in Fe nutritional state. The present study examines how the elemental and chemical composition of a well-studied diatom, *Thalassiosira weissflogii*, varies with N source and Fe limitation.

Materials and methods

T. weissflogii (clone Actin) was obtained from the Provasoli Guillard Center of Culture of Marine Phytoplankton (CCMP 1336) and maintained in axenic culture in Aquil medium (Price et al. 1988/89). All cultures were grown at 20°C under a continuous photon flux density of 170 μmol quanta m^{-2} s^{-1} supplied by cool-white fluorescent lights. Irradiance was measured with a QSL-100 4π quantum scalar irradiance sensor, Biospherical Instruments.

The culture medium consisted of synthetic ocean water (SOW) at pH 8.1–8.2 enriched with 10 μmol L^{-1} PO_4^{3-}, 100 μmol L^{-1} SiO_3^{2-}, and either 50 μmol L^{-1} NO_3^- or 50 μmol L^{-1} NH_4^+. Vitamins and trace metals (Cu, Mn, Zn, Co, Se, and Mo) complexed with 100 μmol L^{-1} edetic acid (EDTA) were filter sterilized using acid-washed, 0.2-μm filters (Acrodisc) and added to microwave-sterilized medium. Iron was added filter sterilized as an Fe-EDTA complex (1 : 1.05 molar ratio) at seven different concentrations: 8,410, 127, 50, 30, 15, 12.9, and 10 nmol L^{-1}. These total-Fe concentrations corresponded to free ferric ion concentrations of $10^{-18.2}$, 10^{-20}, $10^{-20.5}$, $10^{-20.6}$, $10^{-20.9}$, 10^{-21}, and $10^{-21.1}$ mol L^{-1}, respectively, determined using the chemical equilibrium program MINEQL (Westall et al. 1976). Inorganic Fe concentrations [Fe′] were calculated using an empirically derived relationship relating the concentration of Fe′ and Fe-EDTA in seawater at 175 μmol quanta m^{-2} s^{-1} (Sunda and Huntsman 1995). These values equaled 18, 0.27, 0.11, 0.063, 0.032, 0.027, and 0.021 nmol L^{-1} Fe′, respectively. Direct measurement of Fe′ in medium containing 12.9 nmol L^{-1} Fe was in excellent agreement with the calculated value (Maldonado and Price 2001) (33 vs. 27 pmol L^{-1}). In the high Fe medium, the [Fe′] calculation may be in error because of the possible precipitation of Fe hydroxides (Sunda and Huntsman 1995). For this reason, the total concentration of Fe added to the medium was used as the independent variable during data analysis and to graphically represent the results.

Once the medium was enriched with Fe, it was divided equally into two acid-cleaned polycarbonate bottles: one was enriched with NO_3^- and the other was enriched with NH_4^+. Background concentrations in the media of NO_3^- plus NO_2^- and NH_4^+ were <0.1 μmol L^{-1}, as determined by colorimetric analysis (Parsons et al. 1984). Stock solutions of both N substrates were prepared from analytical reagent-grade salts and chelexed to remove trace metal impurities (Price et al. 1988/89). The efficiency of Fe removal from these stocks was determined experimentally to be 100% using ^{55}Fe as a tracer.

Stock cultures of *T. weissflogii* were maintained in semicontinuous batch cultures in 28-ml polycarbonate centrifuge tubes (Oakridge) at each Fe concentration with either NO_3^- or NH_4^+ as N source. These cultures were derived from a single culture grown with full Fe and NO_3^-. They were acclimated for at least two transfers (20 cell divisions) in each medium or until growth rates of successive transfers varied by less than 10%. The acclimated cultures were constantly maintained in exponential growth by serial dilution and were used to inoculate 1- or 2-liter cultures for the experiments described following.

Growth rates of acclimated cells were determined from daily or twice-daily measurements of in vivo Chl *a* fluorescence with a Turner Designs model 10-AU fluorometer. Specific growth rates (d^{-1}) were calculated from linear regression analysis of ln(fluorescence) versus time during the exponential phase of growth. Exponential cultures were grown to midexponential phase (~40,000 cells ml^{-1}) and harvested by filtration for chemical and elemental analysis. Triplicate subsamples were obtained from each culture for analysis of Chl *a* (Parsons et al. 1984) and particulate C, N, and P. These sample replicates were averaged and the mean value was reported for each culture. The particulates collected by filtration were rinsed with nutrient-free SOW before drying. Precombusted GF/F glass fiber filters (Whatman) were used for samples of C, N, and Chl *a* and 1-μm polycarbonate filters (Poretics) were used for samples of P. Carbon and N were analyzed with a Fisons Instruments EA 1108, using acetanilide as standard and the appropriate filter blanks. Particulate P was analyzed using the persulfate oxidation method (Hansen and Koroleff 1999). Field samples

for C, N, and P were collected by filtration and analyzed similarly.

Single samples were taken from each culture for measurement of cellular protein and amino acids. These were analyzed using the Lowry and fluorescamine methods described by Dortch et al. (1984). Bovine serum albumin and glutamate were used as standards for the protein and amino acid analyses, respectively. The ratio of variable to maximum Chl fluorescence (F_v F_m^{-1}) was determined in dark-adapted samples using 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) to block noncyclic electron flow (Parkhill et al. 2001). One sample was obtained from each culture during exponential growth. Dissolved nutrients were measured in triplicate by standard methods (Parsons et al. 1984) with an Alpken RFA autoanalyzer. Cell densities were determined by Coulter Counter analysis (Model TA II) and by counting with a microscope (see following).

Subsamples of the acclimated cultures were also transferred to media supplemented with ^{55}Fe to determined Fe quotas (Maldonado and Price 1996). Triplicate cultures were grown at each Fe concentration with each N substrate. Filtered samples were rinsed with the Ti-citrate EDTA reagent (Hudson and Morel 1989) and analyzed by liquid scintillation counting with a Packard 2100 TR. The efficiency of sample counting was determined using the instrument's external standard. The radioactivity retained by blank filters was subtracted from the samples to correct for nonspecific adsorption of ^{55}Fe. These blanks were insignificant. Cell densities were determined by counting by microscopy using a Palmer Maloney chamber and cell dimensions were measured by a calibrated ocular micrometer. A minimum of 200 cells was counted for each sample to estimate density and 50 cells were sized for volume. Cell volumes were calculated assuming a cylindrical shape, using the formula $V = \pi r^2 l$, where r was the value radius and l the pervalvar length. Statistical analysis was performed with Systat 10 and Microsoft Excel. Relative growth rates were arcsine transformed for regression analysis.

Results

Growth rate of *T. weissflogii* increased with increasing Fe concentration and was well described by the Michealis–Menten equation (Fig. 1). The results reported here summarize measurements from 11 separate experiments conducted over a 2-yr period. In total, more than 160 independent growth-rate estimates were obtained for cells growing in NO_3^-- and NH_4^+-enriched media. These rates were for fully acclimated cultures in steady state. Equations describing the growth rate of *T. weissflogii* as a function of total [Fe] differed significantly depending on the N substrate (ANCOVA, $p < 0.001$, $t = 5.19$, $df = 330$). At the two lowest Fe concentrations tested, corresponding to 21 and 27 pmol L^{-1} Fe′, growth rates were significantly faster with NO_3^- than NH_4^+ (*t*-test, $p < 0.001$; $t = 7.20$, $df = 12$, $p < 0.001$; $t = 6.77$, $df = 63$, respectively). Nitrate-grown cells were thus less Fe-limited than ammonium-grown cells at these very low Fe concentrations. In relative terms (μ μ_{max}^{-1}), the cells using NO_3^- grew about 25% faster than those using

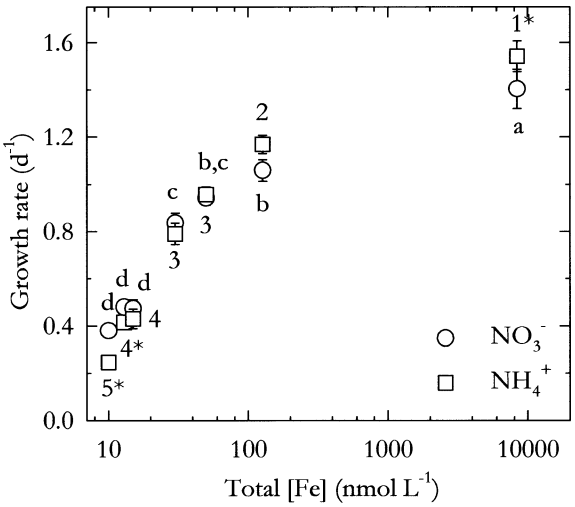

Fig. 1. Specific growth rate (d^{-1}) of *T. weissflogii* in NO_3^-- and NH_4^+-amended media as a function of total iron concentration ([Fe]). Equilibrium concentrations of the free Fe(III) ion and of the Fe(III) inorganic species are reported in the Materials and methods. The equations describing the relationships between growth rate (μ) and [Fe] are: NO_3^--based medium, $\mu = (1.38 \times [Fe])/(0.0027 + [Fe])$, $n = 166$, $r^2 = 0.8509$; NH_4^+-based medium, $\mu = (1.55 \times [Fe])/(0.0019 + [Fe])$, $n = 164$, $r^2 = 0.7604$. The numbers of replicates of the nitrate- and ammonium-grown cultures at each [Fe] were [Fe] = 10 nmol L^{-1}: 13, 13; [Fe] = 12.9 nmol L^{-1}, 66, 64; [Fe] = 15 nmol L^{-1}: 15, 15; [Fe] = 30 nmol L^{-1}: 14, 14; [Fe] = 50 nmol L^{-1}: 17, 17; [Fe] = 127 nmol L^{-1}: 15, 15; [Fe] = 8,410 nmol L^{-1}: 25, 25, respectively. Error bars indicate ±1 SE and, if absent, were smaller than the width of the symbol. Within each N treatment, growth rates marked with different letters (NO_3^-) or numbers (NH_4^+) were significantly different from one another (NO_3^-, ANOVA, $F_{6,161} = 92.42$, $p < 0.0001$; Bonferroni *t*-test method, $p < 0.05$; NH_4^+, ANOVA, $F_{6,159} = 169.7$, $p < 0.0001$; Bonferroni *t*-test method, $p < 0.05$). Growth rates of *T. weissflogii* in NO_3^-- and NH_4^+-amended media were compared at each [Fe] using a paired *t*-test with Bonferroni correction. Those rates that were significantly different from one another are marked with an asterisk.

NH_4^+. At the highest Fe concentration, ammonium-grown cells maintained the fastest rates of growth (NH_4^+, 1.54 ± 0.34 d^{-1}; NO_3^-, 1.40 ± 0.42 d^{-1}) (*t*-test, $p < 0.05$, $t = 3.52$, $df = 25$).

Iron quotas of the nitrate-grown cells were higher than the ammonium-grown cells in the low-Fe medium (Fig. 2). Statistically, the effect was significant for cellular Fe content (attomol Fe $cell^{-1}$) at [Fe′] = 63, 32, and 27 pmol L^{-1}. In fully Fe-replete medium, the opposite was true: ammonium-grown cells contained significantly more Fe than the nitrate-grown cells. Regression equations describing the dependence of Fe quota on the Fe concentration differed significantly whether Fe quota was expressed per cell or per unit biomass (μmol Fe mol^{-1} C) (see Fig. 2 legend). Steady-state uptake rates (ρ^{ss}) calculated from the product of growth rate (μ) and quota (Q) (viz. $\rho^{ss} = \mu Q$) were consequently faster in the nitrate than the ammonium-grown cells at low Fe, but the reverse was true at high Fe (Fig. 3). Biomass normalized rates showed the same general pattern. Thus, nitrate-grown cells maintained faster steady-state uptake rates under severe Fe deficiency, accumulated greater intracellular quotas, and

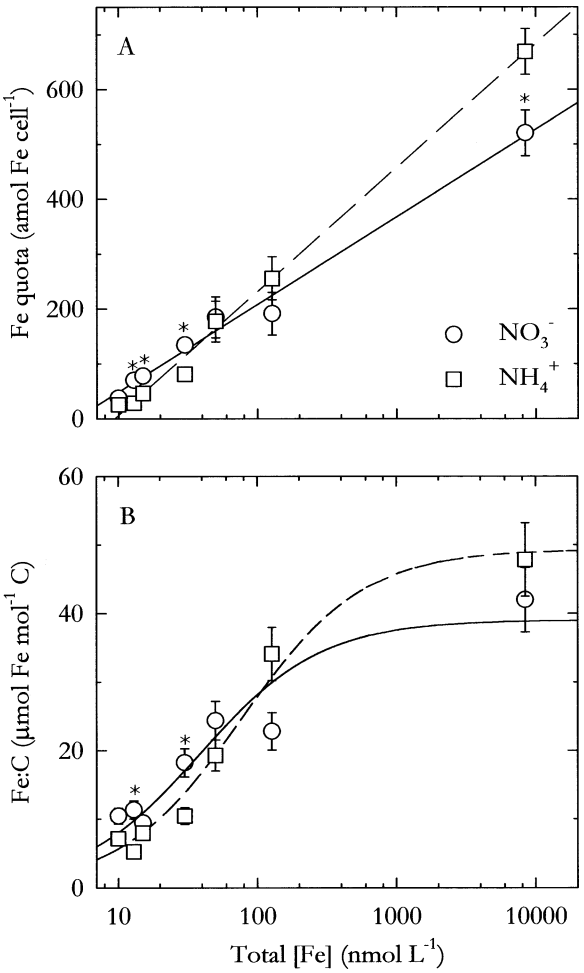

Fig. 2. Intracellular Fe content measured as (A) Fe quota (attomol Fe cell^{-1}), and (B) Fe:C ratio (μmol Fe mol^{-1} C) of *T. weissflogii* grown in NO_3^-- and NH_4^+-amended media as a function of total iron concentration ([Fe]). The solid and dashed lines through the mean data points were obtained using the least-squares fitting software of SigmaPlot 10. For statistical analyses, the data were transformed to obtain straight-line relationships of the form (A) Fe quota = a + b × (log[Fe]), and (B) 1/Fe:C = 1/[Fe] × (Ks/Fe:C_{max}) + 1/Fe:C_{max}. Regression equations of the nitrate- and ammonium-grown cells differed significantly (ANCOVA, Fe cell^{-1}: $p < 0.001$, $t = 6.87$, $df = 38$; Fe:C: $p < 0.001$, $t = 4.74$, $df = 38$). The effect of N substrate on the intracellular Fe content was evaluated at each [Fe] using a *t*-test with Bonferroni correction. Those rates that differed from one another are marked with an asterisk ($p < 0.05$).

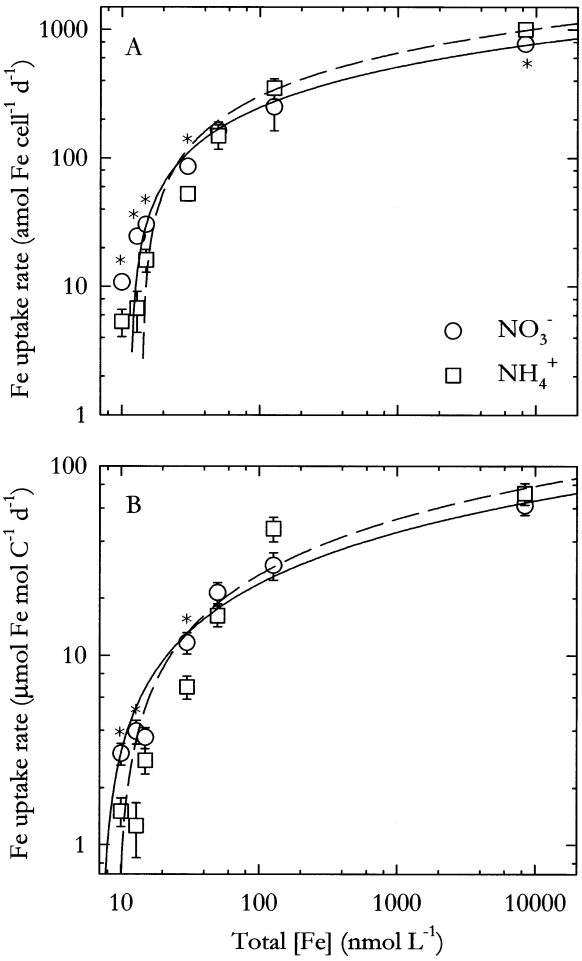

Fig. 3. Steady-state Fe-uptake rates of *T. weissflogii* grown in NO_3^-- and NH_4^+-amended media as a function of total iron concentration ([Fe]). (A) Cellular Fe-uptake rate (attomol Fe cell^{-1} d^{-1}). (B) Fe-uptake rate normalized to cell C (μmol Fe mol C^{-1} d^{-1}). The solid and dashed lines were obtained by fitting the mean data to a logarithmic equation of the form $y = y_0 + a \times (\log[Fe])$ using the least-squares fitting software of SigmaPlot 10. Statistical analysis of linear regression equations of the Fe-uptake rates of the nitrate- and ammonium-grown cells showed significant differences (ANCOVA, attomol Fe cell^{-1} h^{-1}: $p < 0.001$, $t = 7.17$, $df = 45$; μmol Fe mol C^{-1} h^{-1}: $p < 0.005$, $t = 3.31$, $df = 45$). The effect of N substrate on the rate of Fe uptake was evaluated at each [Fe] using a *t*-test with Bonferroni correction. Those rates that differed from one another are marked with an asterisk ($p < 0.05$).

grew faster than cells cultivated with ammonium. The opposite result was true at the highest [Fe].

Specific growth rate of *T. weissflogii* was a hyperbolic function of cellular Fe quota and statistically indistinguishable between cells grown in NO_3^-- and NH_4^+-amended media (Fig. 4). Because the nitrate-grown cells were on average smaller under Fe-limitation than the ammonium-grown cells, they contained more Fe:C at the same slow growth rate. The greater requirement of Fe for the nitrate-dependent cells was only apparent at low Fe, where Fe:C ratios were less than 25 μmol Fe mol^{-1} C (Fig. 4).

Chemical composition of the nitrate- and ammonium-grown cells was examined over the range of Fe-limited growth rates. In these analyses, the data were expressed as a function of relative growth rate ($\mu\ \mu_{max}^{-1}$) to isolate the effect of N source on the dependent parameter and as a function of Fe concentration to determine its affect on cell physiology. Maximum growth rates (μ_{max}) used to calculate relative growth rates of the nitrate and ammonium-dependent cells were measured in Fe-replete medium and were significantly different, as reported above ($n = 25$, NO_3^- and NH_4^+).

Cellular concentrations of Chl *a* (Chl *a* cell^{-1}) decreased as Fe became more limiting. To account for variations in cell size, the Chl *a* data reported here were expressed as C:Chl

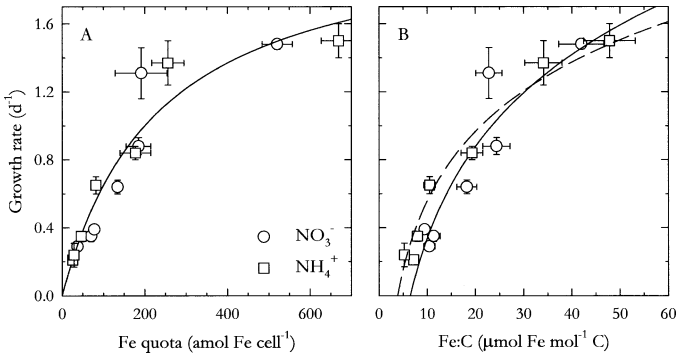

Fig. 4. Relationships between growth rate of *T. weissflogii* and iron quota expressed as (A) attomol Fe cell^{-1} and (B) Fe : C (μmol Fe mol^{-1} C). Linearized regression equations describing growth rate of nitrate- and ammonium-amended cells as a function of Fe cell^{-1} were not significantly different (ANCOVA, $p > 0.05$, $t = 1.79$, $df = 38$) but differed significantly when Fe quota was normalized to cell C ($p < 0.05$, $t = 2.04$, $df = 38$). The curves through the data points were obtained using the least-squares fitting software of SigmaPlot 10.

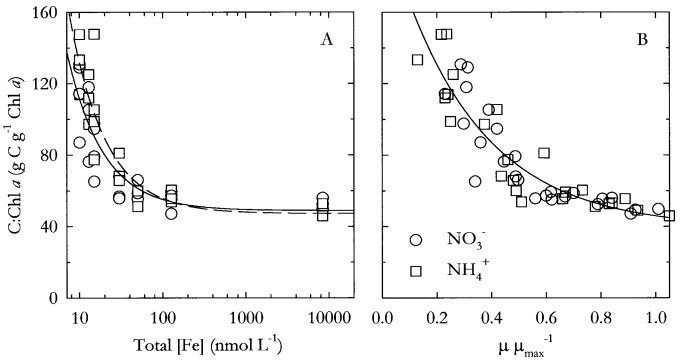

Fig. 5. C : Chl *a* ratios (g g^{-1}) of *T. weissflogii* grown in NO_3^-- and NH_4^+-amended media as a function of (A) total Fe concentration ([Fe]) and (B) relative growth rate (μ μ_{max}^{-1}). The lines describing the data were fit by a least-squares fitting software of SigmaPlot 10 using equations of the form (A) C : Chl a = a/[Fe] + b and (B) C : Chl a = y_o + a × exp(b × μ μ_{max}^{-1}).

a. The C : Chl *a* ratio of the most Fe-limited cultures was roughly 2.5 times higher than that of the Fe-replete cultures and was dependent on N source (Fig. 5). Statistical analysis showed that the regression equations describing the NO_3^- and NH_4^+ data as a function of Fe concentration were significantly different (ANCOVA, $p < 0.05$, $t = 2.31$, $df = 45$). At the lowest Fe levels examined, C : Chl *a* was higher in the ammonium- than in the nitrate-grown cells because they contained lower Chl *a* concentrations. There were no significant differences in the C : Chl *a* ratio measured in two separate experiments conducted 6 months apart (data not shown). When the data were reanalyzed as a function of relative growth, the results demonstrated that N source had no direct effect on cellular composition (ANCOVA, $p > 0.05$, $t = 0.153$, $df = 45$) (Fig. 5B). Thus, nitrate- and ammonium-dependent cells growing at the same relative degree of Fe limitation contained the same C : Chl *a*. Subsequent comparisons of phytoplankton physiology and cellular composition were made as function of relative growth rate because of differences in the maximum growth rates of cells in the NO_3^-- and NH_4^+-enriched cultures.

Dark-adapted fluorescence yield per Chl *a* increased dramatically as Fe became more limiting to growth (i.e., as the relative growth rate decreased) (Fig. 6). Nitrate- and ammonium-grown cells showed an identical response (ANCOVA, $p > 0.05$, $t = 0.061$, $df = 45$). The efficiency of PSII (F_v F_m^{-1}) was relatively constant across the range of Fe-limited growth rates and not dramatically affected by N source (Fig. 6B). In these experiments, as in all others, Fe-limited cells were maintained under steady-state growth conditions. When the phytoplankton entered stationary phase in the Fe-limiting medium, F_v F_m^{-1} decreased dramatically to ~0.1 (Fig. 6). Statistically, steady-state F_v F_m^{-1} was independent of relative growth rate of *T. weissflogii* in NH_4^+ (ANOVA, $p > 0.05$, $F_{1,5} = 0.101$), but not in NO_3^--amended media (ANOVA, $p < 0.05$, $F_{1,5} = 11.52$).

Elemental composition was expressed per unit cell volume to eliminate the effect of variation in cell size. Cellular C

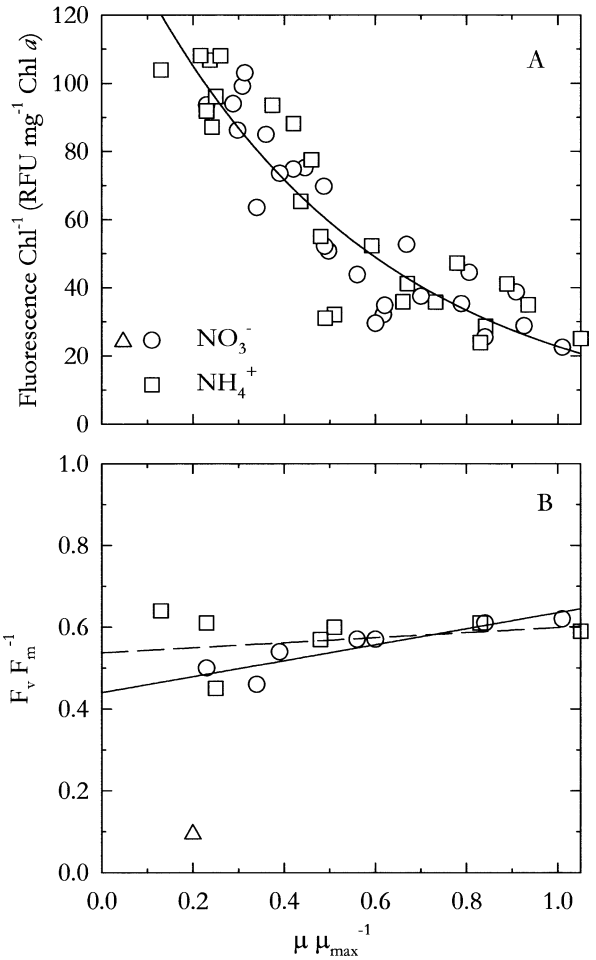

Fig. 6. (A) Minimum fluorescence yield (F_0) of dark-adapted *T. weissflogii* normalized to extracted Chl *a* and (B) quantum yield of PS II (F_v F_m^{-1}) as a function of Fe-limited relative growth rate. Fluorescence yield data were fit to an exponential decay equation and linearized for statistical analysis. Quantum yield was also measured in a Fe-limited NO_3^--dependent culture that entered stationary phase (open triangle).

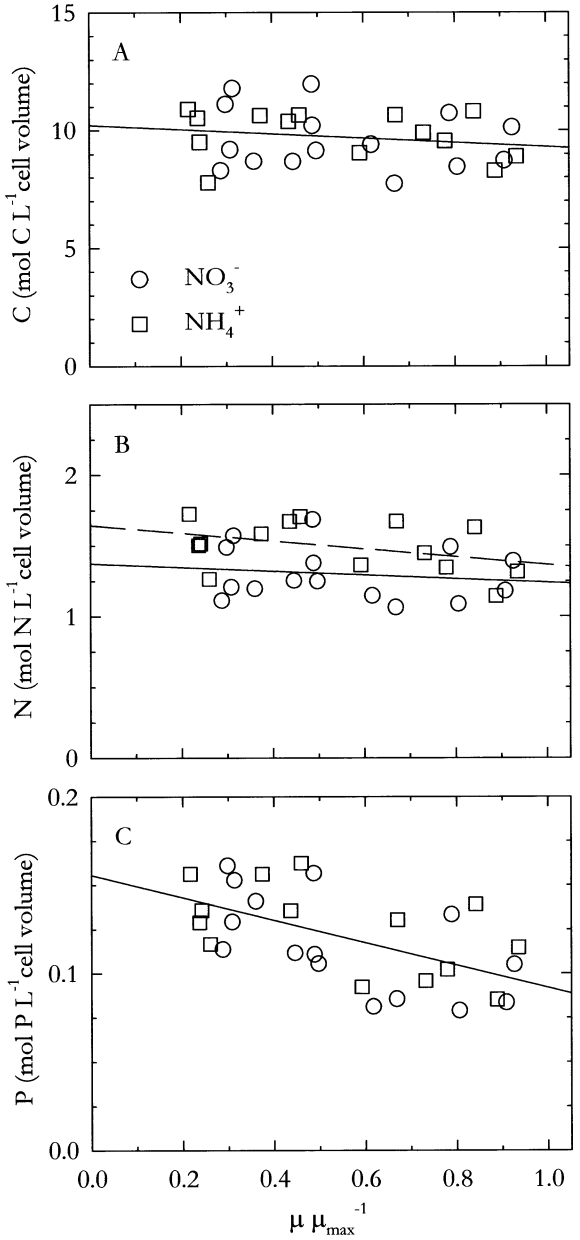

Fig. 7. (A) Carbon, (B) nitrogen, and (C) phosphorus content (mol L^{-1} cell volume) of *T. weissflogii* as a function of Fe-limited relative growth rate in NO_3^-- and NH_4^+-amended media. Lines drawn through the data represent linear regressions fitted using the least-squares procedure of SigmaPlot 10. There were no statistically significant variations in C (ANOVA, NH_4^+: $p > 0.05$, $F_{1,13} = 0.807$, NO_3^-: $p > 0.05$, $F_{1,14} = 0.349$) or N (NH_4^+: $p > 0.05$, $F_{1,13} = 2.76$, NO_3^-: $p > 0.05$, $F_{1,14} = 0.290$) content with relative growth rate. Phosphorus content of the nitrate- and ammonium-grown cells increased significantly with decreasing relative growth rate (NH_4^+: $p < 0.05$, $F_{1,13} = 5.24$, NO_3^-: $p < 0.05$, $F_{1,14} = 7.34$).

content was not affected by N substrate (*t*-test, $p > 0.05$, $t = 0.461$, $df = 27$) or Fe-limited growth rate (Fig. 7). The mean value of cellular C was 9.7 ± 1.15 mol C L^{-1} cell volume. Nitrogen per cell volume was independent of Fe-limited growth rate, but was significantly higher in ammonium- than in nitrate-grown *T. weissflogii* (*t*-test, $p < 0.05$, $t = 2.77$, $df = 27$). Phosphorus content was inversely related to relative growth rate (see Fig. 7 legend) and identical for both N substrates (*t*-test, $p > 0.05$, $t = 0.318$, $df = 27$). Thus, strongly Fe-limited cells ($\mu\ \mu_{max}^{-1} = 0.25$) contained roughly 1.5 times more cellular P than Fe-sufficient cells.

Proteins and amino acids were measured to evaluate whether variations in their concentrations could explain the differences in total cellular N of the nitrate- and ammonium-grown cells (Fig. 8). Two separate experiments were performed for protein analysis, but because these yielded slightly different results (*t*-test, $p < 0.05$, $t = 1.69$, $df = 45$) the data are reported separately in the figure. In both experiments, protein content normalized per N and per liter cell volume was independent of Fe limitation and N substrate. The mean protein content as a percentage of total cellular N, calculated according to Dortch et al. (1984), was 56.4 ± 5.6 and 49.2 ± 9.6% in the two independent experiments. Amino acid concentration was not significantly affected by Fe limitation, but ammonium-grown cells contained significantly higher levels (*t*-test, amino acid N L^{-1} cell volume: $p < 0.001$, $t = 6.87$, $df = 18$; mol amino acid N mol^{-1} cellular N: $p < 0.001$, $t = 4.83$, $df = 18$). These levels of amino acids were almost twice those per unit volume of the nitrate-grown cells (0.22 ± 0.04 mol N L^{-1} cell volume compared with 0.12 ± 0.02 mol N L^{-1} cell volume, respectively).

The ratios of the major elements in *T. weissflogii* showed deviations from ideal Redfield proportions (106C : 16N : 1P) (Geider and La Roche 2002). Carbon : nitrogen composition was independent of relative growth rate and significantly higher in the nitrate- than the ammonium-amended cultures (*t*-test, $p < 0.001$, $t = 7.04$, $df = 43$) (Fig. 9). Mean C : N values were 7.64 ± 0.44 and 6.72 ± 0.44 mol mol^{-1}, respectively. Nitrogen substrate had no statistically significant effect on N : P and C : P molar ratios, despite its effect on the amount of N per liter cell volume. Under nutrient-replete conditions, N : P and C : P were 14 and 97, respectively. Both N : P and C : P ratios decreased significantly as Fe-limited growth rate declined (ANOVA, $p < 0.001$, $F_{1,27} = 17.09$; $p < 0.001$, $F_{1,27} = 17.16$, respectively). The decrease in N : P and C : P was caused by an increase in the volumetric P content of the cells (Fig. 7C).

Systematic variation with relative growth rate in the steady-state N : P and C : P composition of *T. weissflogii* implied that the relative rates of uptake of these elements were affected by Fe nutritional state. Disappearance of NO_3^- and PO_4^{3-} from the media was thus monitored during growth of Fe-limited and -sufficient cultures and uptake rate ratios were calculated (Table 1). Silicate disappearance was also measured. Growth rates of duplicate cultures used in this experiment were similar and typical of Fe-deficient and Fe-sufficient cells. The absolute rates of cellular uptake were reduced by Fe limitation as expected (data not shown) because growth rates were slower in the Fe-depleted cells. The N : P uptake-rate ratios calculated during the exponential phase of growth of the phytoplankton were 11.8 ± 2.6 and 8.1 ± 0.2 mol N mol^{-1} P (mean ± range of duplicates) in the high- and low-Fe media, respectively. These consumption ratios agreed well with the steady-state N : P composition of Fe-sufficient and -deficient *T. weissflogii* (Fig. 9). The NO_3^- disappearance rates used in these calculations were cor-

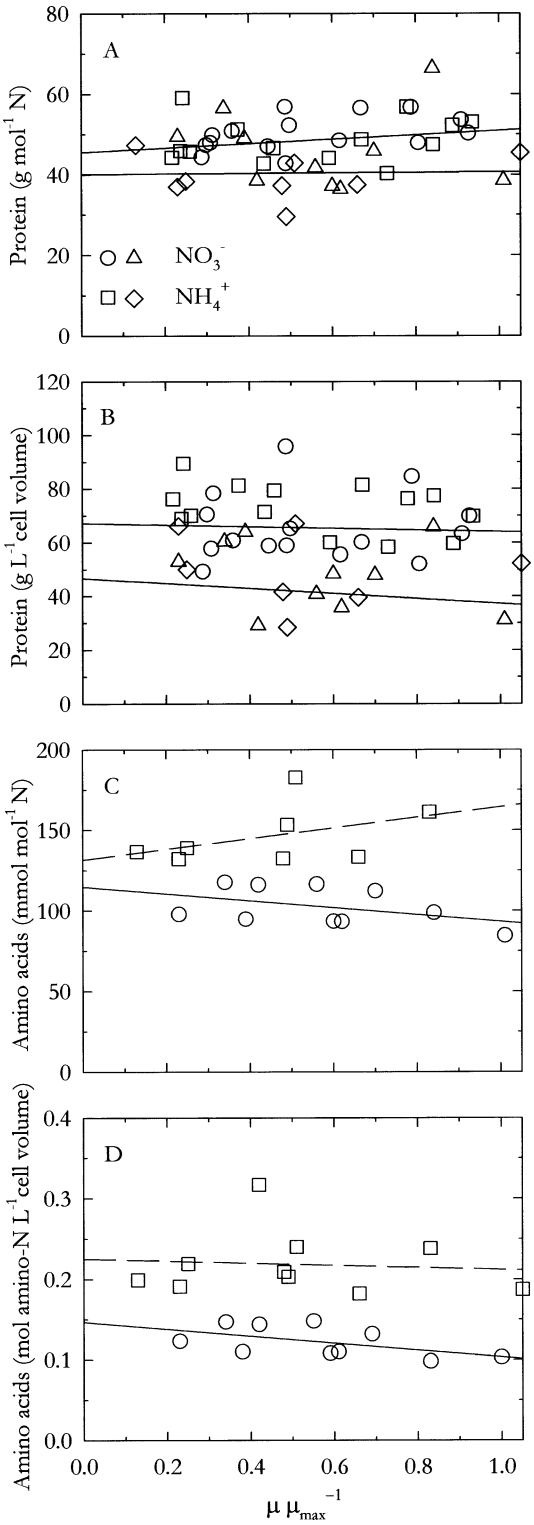

Fig. 8. Protein and amino acid content of nitrate and ammonium-grown *T. weissflogii* as a function of Fe-limited relative growth rate. Protein concentration was expressed (A) per mol of cellular N and (B) normalized per liter cell volume. The results of two separate experiments are reported: these are distinguished by circles and squares (experiment 1) and by triangles and diamonds (experiment 2). The solid lines represent least-squares linear regressions fit to the protein data for the two separate experiments. Statistical analyses showed no relationship between protein content and relative growth rate for either experiment or N source (ANOVA, $p > 0.05$). The results for each N substrate compared between experiments differed significantly and were thus not combined. The protein content of the nitrate- and ammonium-grown cells was indistinguishable in both experiments (*t*-test, experiment 1 [protein/N]: $p > 0.05$, $t = 2.05$, $df = 27$; [protein/CV]: $p > 0.05$, $t = 0.93$, $df = 27$, experiment 2 [protein/N]: $p > 0.05$, $t = 2.12$, $df = 16$; [protein/CV]: $p > 0.05$, $t = 1.37$, $df = 15$). Amino acid (AA) concentrations were measured in one experiment and (C) normalized per cell N (mmol mol^{-1} N), and (D) cell volume (mol amino-N L^{-1} cell volume). Amino acid concentration was independent of Fe-limited relative growth rate (ANOVA, AA/N: NH_4^+, $p > 0.05$, $F_{1,9} = 0.20$; NO_3^-, $p > 0.05$, $F_{1,9} = 2.65$; AA/CV: NH_4^+, $p > 0.05$, $F_{1,9} = 0.20$; NO_3^-, $p > 0.05$, $F_{1,9} = 3.34$). The solid and dashed lines represent least-squares linear regressions fit to the NO_3^- and NH_4^+ data.

rected for NO_2^- excretion by the phytoplankton during growth and represent net rates of N consumption. Considerably more NO_2^- accumulated in the low- than in the high-Fe cultures during the end of exponential growth (maximum values of 1.2 ± 0.3 compared with 0.2 ± 0.01 μmol NO_2^- L^{-1}, respectively), but all of it was consumed once the cells entered stationary phase. Silicate : nitrate uptake-rate ratios were much higher in the Fe-limited than in the Fe-sufficient cells (0.56 compared with 0.34 mol Si mol^{-1} N, respectively).

An extended Redfield ratio of C : N : Si : P : Fe calculated from the quota measurements and disappearance ratios reported above differed according to Fe nutritional status. Under nutrient-replete conditions the composition of *T. weissflogii* was 97C : 14N : 4.7Si : 1P : 0.029Fe on a mol basis, whereas Fe-limited cells growing on NO_3^- at 0.25 μ_{max} contained 70C : 10N : 5.8Si : 1P : 0.0074Fe.

Discussion

Culture conditions—The vast majority of experimental work on Fe limitation in phytoplankton has been conducted under non-steady–state conditions. Iron deficiency has typically been induced by growing cells to high densities to deplete Fe from the medium or by transferring cells of uncertain Fe status to medium containing no added Fe. Both approaches have been successful in depriving phytoplankton of Fe and for studying their physiological responses to its absence. Generating Fe deficiency in these ways has provided insights to how phytoplankton may respond to fluctuations in resource availability they encounter in their environment. Non-steady–state methods, however, are generally difficult to replicate, provide little or no opportunity for the phytoplankton to adapt, and cannot be used to study a range of Fe nutritional states. Observations that the biomass and productivity of HNLC ecosystems are relatively stable (Miller et al. 1991) suggest that Fe-limited regions of the sea exist in a quasi-steady–state, regardless of any fine-scale changes in Fe supply they may experience. The natural variations in biological properties of HNLC habitats are certainly small compared with the rapid and massive transient changes to the phytoplankton community following Fe fertilization. In this report, a marine diatom was grown under well-defined,

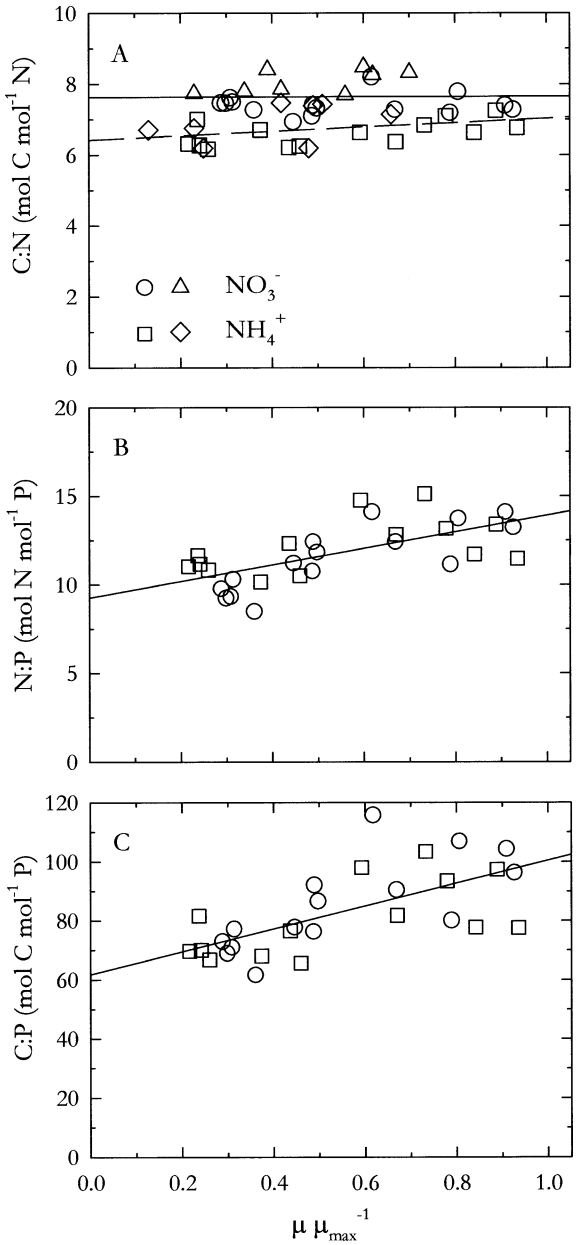

Fig. 9. Elemental ratios (by atoms) of *T. weissflogii* as a function of Fe-limited relative growth rate. (A) C : N, (B) N : P, and (C) C : P. Two separate experiments were conducted for the analysis of C : N ratio (experiment 1: circles and squares; experiment 2: triangles and diamonds).

steady-state conditions to allow complete adaptation. As discussed below, some of the differences reported here in composition and physiology compared with published reports may be caused by the methodology used to achieve Fe-limited growth.

Nitrate- versus ammonium-based growth—The N dependence of Fe requirements of phytoplankton is well established, both theoretically (Raven 1988) and experimentally (Maldonado and Price 1996). Growth on NO_3^- increases the Fe : C ratio of diatoms by 1.8 times compared with NH_4^+, reflecting the costs associated with the assimilation of a more oxidized form of N. The results for *T. weissflogii* extend these earlier measurements to a wide range of Fe concentrations. They show that Fe : C ratios of nitrate-grown cells were higher on average than ammonium-grown cells at similar Fe-limited rates of growth (Fig. 4). At rates faster than 0.7 d^{-1}, Fe : C ratios were the same regardless of N source and, at μ_{max}, they were highest in ammonium-grown cells. The Fe-use efficiencies calculated for nitrate- and ammonium-dependent growth (0.34 ± 0.07 and $0.51 \pm 0.16 \times 10^5$ mol C mol^{-1} Fe d^{-1}, respectively) agree well with published values (Sunda and Huntsman 1995; Maldonado and Price 1996) and establish that the cultures were fully acclimated with respect to Fe and N.

An important difference between cells using NO_3^- and NH_4^+ was their ability to acquire Fe at low, limiting concentrations. The higher Fe quotas of nitrate-grown cells were a result of their faster rates of Fe transport (Fig. 3). Maldonado and Price (2000) attributed this phenomenon to an inducible Fe (III) reductase in nitrate-grown cells that increased the bioavailable pool of Fe for transport. The faster rates in *T. weissflogii* were only observed when growth rates were less than 60–70% of μ_{max}, consistent with the inducible nature of such an Fe-acquisition system. Judging from the growth rate results, the nitrate-dependent cells were more than able to acquire the extra Fe needed for metabolism. Indeed, they grew more quickly than the ammonium-dependent cells at the lowest Fe concentrations tested (Fig. 1). Maldonado and Price (1996, 2000) made similar observations for the growth of a related species, *T. oceanica.* They found that only under conditions of extreme Fe limitation, when no Fe was added to the culture medium, was the growth rate of nitrate-dependent cells less than that of the ammonium-dependent cells. As described below, the results reported here show no other obvious changes in chemical composition that could account for the greater fitness of the nitrate-grown cells at low Fe. They do show, however, that Fe limitation causes a significant change in diatom elemental composition that is independent of N source and consistent with nutrient distributions and particulate ratios measured in HNLC regions of the sea.

C : Chl a composition and cellular fluorescence—The requirement for Fe in chlorophyll biosynthesis is related to key enzymatic steps catalyzing the conversion of Mg protoporphyrin to protochlorophyllide and to production of pigment-binding proteins and redox carriers (Davey and Geider 2001). Thus, low Fe decreases chlorophyll concentrations in lab and field populations, as illustrated in *T. weissflogii.* The typical result is a two- to three-fold reduction in cellular chlorophyll or increase in C : Chl *a* ratio at low Fe. Nitrogen source is reported to have no effect on the chlorophyll content of diatoms (Thompson et al. 1989), so the result of higher C : Chl *a* ratios at low Fe in the ammonium-amended cultures is surprising. Differences in the C : Chl *a* ratios of nitrate and ammonium-grown *T. weissflogii* (Fig. 5) are consistent, however, with the higher Fe quotas of the nitrate-metabolizing cells (Fig. 4) and not a consequence of N substrate per se. The simplest explanation for this result is that greater Fe quotas of the nitrate-grown cells allowed greater

Table 1. Nutrient consumption ratios (ΔN : ΔP and ΔSi : ΔN) of replicate cultures of *T. weissflogii* calculated from changes in concentrations of nitrate plus nitrite (ΔN), phosphate (ΔP), and silicate (ΔSi) during exponential growth in Fe-replete and Fe-deplete media. Fe-replete media contained 8,410 nmol L^{-1} Fe and Fe-deplete media contained 12.9 nmol L^{-1} Fe. Growth rates (μ) were determined from the log-linear portion of the growth curve. Initial concentrations of the nutrients were 93.8 ± 0.7 μmol L^{-1} SiO_3^{2-}; 54.8 ± 0.7 μmol L^{-1} NO_3^-; 0.18 ± 0.01 μmol L^{-1} NO_2^-; 11.0 ± 0.13 μmol L^{-1} PO_4^{3-}; 0.11 ± 0.03 μmol L^{-1} NH_4^+.

Growth medium	Replicate	μ (d^{-1})	ΔN (μmol L^{-1})	ΔP (μmol L^{-1})	ΔSi (μmol L^{-1})	ΔN : ΔP (mol mol^{-1})	ΔSi : ΔN (mol mol^{-1})
Fe replete	A	1.61	19.6	1.49	6.63	13.1	0.34
	B	1.75	21.7	2.07	7.56	10.5	0.35
Fe deplete	A	0.67	38.7	4.69	23.4	8.2	0.61
	B	0.62	43.0	5.34	22.4	8.0	0.52

production of chlorophyll at low Fe and hence decreased C : Chl *a* ratios compared with ammonium-grown cells. Because the nitrate- and ammonium-grown cells achieved different maximum rates of growth, their relative degree of Fe limitation at each Fe concentration was not the same. Normalizing growth rate at each [Fe] to the maximum, nutrient-replete rate provided a means to assess the physiological response of the phytoplankton to N substrate at the same degree of Fe limitation. Statistical analysis showed that C : Chl *a* ratio as a function of Fe limitation was independent of N source. Thus, nitrate and ammonium-grown cells contained the same C : Chl *a* when they were equally Fe limited.

Nitrogen source had no dramatic effect on the minimum fluorescence yield per Chl *a* (Fig. 6). In Fe-stressed phytoplankton, a substantial increase in Chl *a*-specific absorption coefficient is caused by a decrease in cellular Chl *a* and self-shading of the chlorophyll (Davey and Geider 2001) and is likely responsible for the increase in fluorescence seen here. The ratio of variable to maximum fluorescence, $F_v F_m^{-1}$, used to estimate the maximum quantum yield of PSII, showed little change during steady-state Fe limitation. At relative growth rates as low as 0.25 μ_{max}, $F_v F_m^{-1}$ was similar to that measured in healthy, nutrient-replete cultures. Statistically, NO_3^-- and NH_4^+-enriched cultures showed different responses, but further replication is required to validate this result, considering the small sample size and the variability observed (Fig. 5). The relative constancy of $F_v F_m^{-1}$ in *T. weissflogii* differs radically from the expected result (see, e.g., Greene et al. 1991; Vassiliev et al. 1995). Indeed, a survey of the literature shows that $F_v F_m^{-1}$ in low-Fe phytoplankton typically decreases to 0.2–0.35 compared with 0.65 in non-limited cultures. In these published reports, however, Fe stress was induced transiently. Measurements obtained in other studies from Fe-limited cultures in steady-state show the same general response as in *T. weissflogii*. For example, McKay et al. (1997) observed that $F_v F_m^{-1}$ equaled 0.51 and 0.62 in Fe-limited diatom cultures growing at 0.72 and 0.85 μ_{max} and Peers and Price (2004) recorded a $F_v F_m^{-1}$ of 0.6 in a *T. weissflogii* culture growing at 0.73 μ_{max}. Doucette and Harrison (1990) also found that $F_v F_m^{-1}$ in *Gymnodinium sanguineum* decreased little over a range of Fe-limited growth rates. Thus, steady-state growth may allow phytoplankton to acclimate to Fe limitation and adjust the balance between light harvesting and electron transport in such a way as to maximize efficiency. Note that Fe starvation during the stationary phase of growth induced a dramatic reduction in $F_v F_m^{-1}$ (~0.1) in *T. weissflogii* consistent with the results obtained in other transient experiments. Parkhill et al. (2001) reported a similar effect of growth conditions on the fluorescence properties of N-limited diatoms and provided a thorough discussion of growth acclimation in phytoplankton.

C, N, and P quotas—Changes in the elemental stoichiometry of phytoplankton induced by resource limitation are well known to arise from phenotypic plasticity and luxury consumption of nonlimiting resources (Geider and La Roche 2002). The most predictable of these are increases in cellular C : N and decreases in N : P or increases in cellular C : P and N : P as N or P become limiting to growth. Deviations from ideal Redfield proportions (106C : 16N : 1P) are generally indicative of slow relative growth rates of phytoplankton (Goldman et al. 1979) and have been used to argue for and against nutrient limitation in the sea. As shown here in *T. weissflogii*, Fe nutritional state influences C : N : P composition. Fe limitation elicits a distinct pattern in elemental stoichiometry compared with other types of deficiencies, decreasing C : P and N : P, but having no effect on C : N. The important point from an oceanographic perspective is not the absolute values of C : N : P in *T. weissflogii*, but the direction and magnitude of the change in C : N : P composition induced by Fe limitation. These results are in contrast with previous reports that observed no difference in the elemental composition of phytoplankton grown in Fe-deficient and Fe-sufficient seawater (Greene et al. 1991; La Roche et al. 1993; Takeda 1998).

Phytoplankton C : N is typically not affected by Fe limitation, although there are a few exceptions. Among these, the response under transient conditions is an increase in C : N in low-Fe medium (La Roche et al. 1993; Muggli and Harrison 1996; De La Rocha et al. 2000), with no consensus on whether the increase is driven by changes in C, N, or both. Even under steady-state, however, non-Redfield C : N ratios may be observed, implying that species may respond differently to low Fe. Maldonado and Price (1996), for example, found that C : N of *T. pseudonana* decreased under Fe limitation, but it was the only one of six diatoms examined to show such a response. A constant C : N ratio as a function of Fe-limited growth implies that C and N assimilatory pathways remain coupled and may share a common Fe-dependent rate-limiting step, possibly in the provision of energy or biochemical intermediates. Indeed, energy limitation has been invoked by others (Sakshaug and Holm-Hansen 1977;

Muggli and Harrison 1996; Milligan and Harrison 2000) to explain the biochemical consequences of Fe limitation in phytoplankton. Energy limitation, however, which is equivalent to light limitation in photoautotrophs, tends to increase elemental content relative to C because it decreases photosynthetic C acquisition and presumably the build-up of C stores. A number of studies with diatoms, including *T. weissflogii*, show that C : N ratio decreases as light decreases (Laws and Bannister 1980; Goldman 1986; Thompson et al. 1989), which differs from the stoichiometric pattern seen here and suggests that energy and Fe limitations may not be synonymous in this diatom.

The lower N quotas of the NO_3^--grown cells means that Fe addition to field samples might increase the C : N ratio of plankton by increasing new compared with regenerated production. This effect, however, should be rather small because the average difference in N quotas of the NO_3^-- and NH_4^+-grown cell was only 0.19 mmol N L^{-1} cell volume, roughly 10% of the total N quota. Interestingly, the greater N content of the NH_4^+-grown cells coincided with a higher amino acid concentration compared with NO_3^--grown cells. If we assume that N substrate has little effect on the types of amino acids that phytoplankton contain (see, e.g., Wood and Flynn 1995) and that amino acid concentrations (measured as glutamate equivalents) can be expressed in terms of N (Dortch et al. 1984), then the difference in concentrations between NO_3^-- and NH_4^+-grown cells (0.12 mmol N L^{-1} cell volume) is roughly equivalent to the difference in their N quotas.

Accumulation of P under low-Fe conditions was caused by a more rapid decline in growth than in steady-state uptake rate. Under the most Fe-limiting conditions, *T. weissflogii* accumulated 1.5 times more P per liter cell volume than when nutrient replete. Whether this extra P serves a specific metabolic function or represents luxury consumption is presently unknown, although we have some evidence for the latter (Tremblay and Price, unpubl. data). Consumption of P in excess of requirements frequently occurs in phytoplankton when concentrations of PO_4^{3-} are high and when other resources are limiting to growth. Given that PO_4^{3-} in our medium was 10 μmol P L^{-1}, the question naturally arises whether the P accumulation observed here is an artifact of the culturing conditions. Some of it may be, but because all media contained the same quantities of macronutrients, the effect of Fe on P accumulation is real. Ambient concentrations of PO_4^{3-} in the surface waters of the HNLC ocean are in the low micromolar range, roughly one order of magnitude less than our media. Are such levels sufficient to allow Fe-limited phytoplankton to accumulate P in excess of optimal growth requirements? As discussed below, three of lines of evidence suggest, yes, phytoplankton accumulate greater amounts of P relative to N at low P and do so in Fe-limited parts of the sea.

Field observations—Summarized in Table 2 are measurements of the elemental composition of seston from surface waters throughout the world ocean (Web Appendix 1 at http://www.aslo.org/lo/toc/vol_50/issue_4/1159a1.pdf). They include new data, measured here, from two sites in the California upwelling zone. The global average, which includes most of the data in Table 2, is C : N = 7.3 ± 1.7 and C : P = 114 ± 45 mol mol^{-1} (Geider and La Roche 2002). These values are in good agreement with canonical values (C : N = 6.6 and C : P = 106) and those derived from deep-water measurements of NO_3^-, PO_4^{3-}, and TCO_2, the so-called remineralization ratios (C : N = 7.3 and C : P = 117) (Anderson and Sarmiento 1994). Averaging the composition of all the particulate measurements, however, obscures significant variations among different ocean provinces that may tell us something about their ecology and biogeochemistry. In the Southern Ocean, for example, C : N of seston ranges between 5.6 and 7.4 and C : P and N : P are between 46 and 72 and 7 and 11, respectively. These values are substantially lower than in other parts of the sea. The equatorial regions of the Atlantic and Pacific Oceans as well contain particulates with low N : P and C : P ratios. Some upwelling zones, including those that are known to be Fe limited, also appear to have lower C : P and N : P than typical oceanic and coastal waters. In the northwestern African region, for example, particulates are enriched in P and have an elemental composition similar to that of Fe limited *T. weissflogii*. Samples obtained from Fe-replete and Fe-deplete sections of the California upwelling (Hutchins and Bruland 1998) differ significantly in their composition (Table 2). Specifically, Fe-deplete samples have lower C : P and N : P ratios than Fe-replete samples, as predicted from the lab data. Because phytoplankton biomass at both sites (measured as Chl *a*) was high and the species were predominantly diatoms, the difference in composition may be related to the Fe nutritional state of the communities. Dissolved macronutrients were plentiful at both sites. One confounding issue in all these comparisons is the relative contribution of detritus and living phytoplankton to the elemental signals measured in the field. Detritus may be in various states of remineralization so that its composition could be quite different from the phytoplankton from which it originated. Redfield ratios derived from regressions of C and N against P attempt to remove the detrital signal that may overwhelm the elemental composition determined from individual measurements. In the California upwelling, the detrital contribution is likely to be small given that the Chl *a* concentrations were 4–6 μg L^{-1} and the algae actively growing. Collectively, the data suggest that particulate matter in low-Fe waters contains lower C : P and N : P ratios than in other parts of the sea: Fe appears to influence the elemental stoichiometry of phytoplankton in the field.

Changes in the $NO_3^- : PO_4^{3-}$ draw-down ratios in response to Fe addition are now well documented in HNLC regions: as much as a 1.6-fold stimulation in NO_3^- consumption relative to PO_4^{3-} is observed. Part of this effect may be due to differences in the amount of recycling of NH_4^+ and other reduced N sources and their contributions to phytoplankton growth in the control and Fe-treated samples. This could be important because Fe-limited communities rely primarily on regenerated N for growth (Price et al. 1991). This would act to reduce the $NO_3^- : PO_4^{3-}$ draw-down ratio, although it would be difficult to sustain net biomass production under such a scenario because [NH_4^+] is so low. Shifts in phytoplankton community composition could also affect the nutrient draw-down ratios. Cyanobacteria, for example, have low P requirements relative to N and C compared with eukaryotic algae (Bertilsson et al. 2003), whereas some diatoms have

Table 2. Elemental ratios of marine seston (mol mol^{-1}) in surface samples collected from the mixed layer or depths of less than 100 m. Values in parentheses represent the number of samples analyzed. Data sources are listed in Web Appendix 1 (http://www.aslo.org/lo/toc/vol_50/issue_4/1159a1.pdf).

Region	Latitude	Longitude	C : N	C : P	N : P	Data source
Oceanic						
Southern Ocean	40°–70°E	45°–56°S	5.6 (62)	46 (34)	6.8(37)	8*
	57°06′W	60°55′S	6.2 (1)	29.5(1)	4.8(1)	9†
	63°–83°E	50°–66°S	7.4 (34)	72.3(34)	11.3(34)	19*
	176°W	54°S	6.9 (3)	105 (3)	15.4(3)	17†
Atlantic Ocean	8°51′W	2°47′N	7.9 (3)	158 (3)	20 (3)	2†§
	18°–45°W	27°N–36°S	10.7 (8)	179.5(8)	16.5(8)	16*
	4°W	0°	6.0 (100)	56 (100)	8.8(108)	23*
Indian Ocean	57°–77°E	23°–45°S	5.5 (82)	101 (83)	18.6(91)	8*
	50°–67°E	25°N–20°S	5.4 (3)	81.6(3)	15.3(3)	4†‖
	65°E	18°N	5.9 (1)	141 (1)	24 (1)	11†¶
Pacific Ocean	155°W	30°N	8.8 (1)	152 (1)	18 (1)	12†
	158°W	22°45′N	6.9	133	19	14†‡
	123°W	34°50′N	6.5 (4)	170 (4)	26.6(4)	17†
	160°W	0°56′N	5.4 (2)	80.9(2)	14.7(2)	20†
	153°–172°W	10°–22°N	12.1 (3)	242 (3)	38.5(3)	20†
	158°W	21°30′N	7.3 (4)	131 (4)	18.3(4)	22†
Upwelling regions						
Cap Blanc	17°5′E	20°45′N	7.6	82.5	10.9	5†
Gulf of Guinea	7.5°E	1.5°N	8.1 (6)	121 (7)	15 (7)	5†
NW Africa	10°E	31°N	5.6 (60)	78 (18)	13.9(22)	1*
California	122°14′W	37°N	7.02(5)	130 (5)	18.5(5)	This study†
	121°35′W	35°58′N	6.81(5)	95.8(5)	14.0(5)	This study†
Nearshore and inland seas						
Ligurian Sea	9°E	43°50′N	6.19(296)	134 (62)	22.5(60)	1*
Mediterranean Sea	20°E	36°N	6.23(12)	140 (10)	23.2(13)	1*#
N. Indian Ocean	59°–67°E	21°–23°N	5.6 (4)	170 (4)	30 (4)	11†
Panama Basin	86°10′W	0°45′N	6.1 (3)	196 (3)	32 (3)	3†§
	82°W	5°N	6.8 (5)	139 (5)	20.3(5)	7†
Santa Catalina Basin	118°40′W	33°18.5′N	6.3 (3)	88 (3)	14 (3)	6†
W. North Pacific	128°E	34°N	7.7 (30)	366 (30)	47.6(30)	18*
Bay of Biscay	2°W	45°30′N	6.5 (32)	217 (32)	33.5(32)	21†
W. North Atlantic	65°W	40°–42°31′N	5.3 (33)	91 (29)	17.5	13**
	66°W	45°N	7.6 (3)	140 (3)	19.4(3)	10†
Bransfield Strait	60°–63°W	62°–63.5°S	8.7 (16)	165 (14)	19.8(14)	15†

* Ratios derived from the slope of regression analyses of C versus N, C versus P, and N versus P.
† Mean of elemental ratios of individual samples.
‡ Average values determined from 9 years of biweekly measurements.
§ Ratios measured in particles of 1–53 μm.
‖ Samples collected at depth with >10% of sea-surface irradiance.
¶ Samples collected by sediment trap.
# Samples from May cruise.
** Ratios derived from the slope of regression analyses of P versus C and N versus C.

high P requirements relative to N and C (Quigg et al. 2003). As shown in *T. weissflogii*, NO_3^- : PO_4^{3-} draw-down ratios are also Fe dependent, like the N : P and C : P quotas, so changes in Fe nutritional status of natural populations is expected to affect nutrient consumption ratios.

High concentrations of particulate P have been measured in surface waters in the southeast Pacific quadrat of the Southern Ocean (Correll 1965). In the small size fraction (<20 μm), particulate P is roughly two times higher in low-Fe waters south of the Antarctic Convergence compared with samples collected between 45° and 55°S. Although this result could be caused by differences in plankton biomass, the high- and low-latitude samples appear to contain similar levels of macronutrients and chlorophyll (Hardy et al. 1996). Correll (1965) also measured speciation of P in the particulates using chemical fractionation methods and observed that the southern samples contained a much greater proportion of orthophosphate relative to RNA and polyphosphate than those from the north (1.64 ± 0.42 vs. 0.62 ± 0.9). Such observations suggest that these plankton communities differ in P metabolism, possibly because of differences in Fe availability in their environment.

Oceanographic relevance—The results presented here offer an alternative explanation for the anomalous utilization ratios of C, N, and P in surface waters of the Southern

Ocean. The current view proposes that differences in taxonomic composition of the phytoplankton community are largely responsible for the non-Redfield N : P and C : P drawdown ratios and is strongly supported by observational data (Arrigo et al. 1999; Sweeney et al. 2000; Smith and Asper 2001). Although species-specific requirements for nutrient resources are well known (Quigg et al. 2003) and undoubtedly important for certain elements (Si, C), a role for Fe in modifying major nutrient consumption cannot be ruled out at present. Indeed, Fe concentrations vary widely in the Southern Ocean and are limiting to phytoplankton growth. It could be argued that taxonomic composition of phytoplankton communities in this region is controlled by Fe availability, so that Fe indirectly affects nutrient draw down (Fitzwater et al. 2000). A direct effect of Fe can also be proposed because nutrient consumption ratios are Fe dependent. Simply put, the results presented here suggest that the low N : P and C : P ratios measured in the Southern Ocean (and perhaps elsewhere?) are a result of excess P accumulation during Fe-limited phytoplankton growth. One form of this proposal has already been advanced by De Baar and colleagues (1997), who hypothesized that Fe limitation impeded N assimilation and hence the relative rates of N : P (and, by inference, N : C) consumption. However, the constant C : N ratio in *T. weissflogii* over a wide range of Fe concentrations seems to rule out Fe limitation of NO_3^- assimilation per se, otherwise an increase in C : N ratio would be expected as Fe becomes more limiting. The fact that P per liter cell volume increases with Fe limitation provides direct proof that changes in P content are responsible for the anomalous N : P and C : P relationships. Iron limitation may thus increase the relative utilization of P in the sea while at the same time decreasing C production. Changes in the Redfield composition of sinking phytoplankton debris would thus be predicted as Fe inputs to the Southern Ocean wax and wane over geologic time scales. Such changes could influence the interpretations of the paleo-oceanographic records of nutrient utilization and calculations of productivity from P.

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