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Growth rates, half-saturation constants, and silicate, nitrate, and phosphate depletion in relation to iron availability of four large, open-ocean diatoms from the Southern Ocean

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Abstract

Four large, open-ocean diatoms from the Southern Ocean (*Actinocyclus* sp., *Thalassiosira* sp., *Fragilariopsis kerguelensis,* and *Corethron pennatum*) were grown in natural (low iron) Southern ocean seawater with increasing Fe concentrations. With increasing dissolved iron (Fe_{diss}) concentrations, the growth rates increased three- to sixfold. The species with the smallest cells had the highest growth rates. The half-saturation constants (K_m) for growth were low (0.19–1.14 nmol L^{-1} Fe_{diss}), and close to the ambient Fe_{diss} concentrations of 0.2 nmol L^{-1}. The range in K_m with respect to Fe_{diss} also varied with the size of the diatoms: the smallest species had the lowest K_m and the largest species had the highest K_m. As Fe_{diss} concentrations decreased, silicate consumption per cell increased, but nitrate consumption per cell decreased. Phosphate consumption per cell varied without clear relation to the dissolved iron concentrations. The differences in nutrient consumption per cell resulted in marked differences in elemental depletion ratios in relation to Fe_{diss} concentrations, with the depletion ratios being most affected by iron limitation in the largest cells. These experimental findings are in agreement with previous laboratory and field studies, showing the relatively high requirements of large diatoms for Fe. The size-dependent response of the diatoms with respect to nutrient depletion is a good illustration of the effects of Fe on silicate, nitrate, and phosphate metabolism.

While the understanding of the temporal and spatial patterns in the environmental control of phytoplankton in the Southern Ocean has increased over the last years (Boyd 2002), species-specific studies on key species of open Southern Ocean phytoplankton are scarce. An understanding of the physiological response of large Southern Ocean diatoms at the species level to environmental stress factors such as iron and light limitation and the concomitant effects on major nutrient uptake is virtually absent. At the community level, more information is available for Southern Ocean diatoms (Boyd et al. 2000; Blain et al. 2002; Coale et al. 2003), but the relevance of maximum growth rates (μ_{max}) and half-saturation constants (K_m for growth or K_s for nutrient uptake) for assemblages of species or even genera is limited. Strictly speaking, μ_{max} and K_m or K_s are only applicable to single species and are unique physiological characteristics of a species, not a community. Even small changes in species composition or changes in dominance of species during an algal bloom will have a major influence on these physiological parameters.

The large diatoms, albeit low in numbers, are important primary producers (Goldman 1993) and play a prominent role in biogeochemical cycles in the Southern Ocean (Boyd et al. 2000). In the field (De Baar et al. 1995), in bottle Fe-enrichment experiments (Timmermans et al. 1998; Coale et al. 2003) as well as in mesoscale Fe-enrichment experiments in this high-nutrient, low-chlorophyll (HNLC) region (Boyd et al. 2000), it has been shown that large diatoms have the strongest response (reviewed in De Baar and Boyd 2000). On a global scale, it is estimated that diatoms account for a major contribution of the global primary production in the ocean (Nelson et al. 1995). Moreover, diatoms are important exporters of organic carbon and silicate to the seafloor (Smetacek 1999). As such, the remains of diatoms could be used as tracers for paleoproductivity, if it were not for the fact that their elemental composition is strongly influenced by environmental factors. It has been demonstrated for several diatom species that their elemental composition is affected by availability of both light (Claquin et al. 2002) and iron (Bucciarelli et al. pers. comm.; Takeda 1998). Such experiments under natural, yet fully controlled, conditions with recently isolated open-ocean Antarctic diatom species are limited (Timmermans et al. 2001). Yet species-specific response will enable us to predict, to some extent, which species will dominate depending on environmental conditions (Huisman and Weissing 2002). Further, detailed knowledge on elemental composition of the diatoms, especially the large species responsible for a significant portion of the new production (Goldman 1993), is essential, as only with that information can better global biogeochemical models be derived. Finally, it will lead to a better understanding and prediction of responses of HNLC areas to iron input (natural or artificial) and the effects of global climate change.

Here, we present and discuss Fe-limitation experiments

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with four large diatoms isolated from the open Southern Ocean: *Actinocyclus* sp., *Thalassiosira* sp., *Fragilariopsis kerguelensis,* and *Corethron pennatum.* The experiments were performed in natural, iron-poor HNLC water from the Southern Ocean without artificial complexing agents. Specific growth rates; half-saturation constants for growth; silicate, nitrate, and phosphate consumption per cell; and elemental depletion ratios in relation to iron availability are presented. Physiological explanations for the observed effects are given, including relations with cell volume, cell surface area, and cell surface-to-cell volume ratio and biogeochemical consequences are discussed.

Material and methods

Diatoms—Four species of diatoms (Bacillariophyceae) were tested for their response to Fe availability. Three species belong to the Centrales: *Actinocyclus* sp., disk-shaped single cells with a diameter of 140 μm (cf. *A. ehrenbergii*); *Thalassiosira* sp., disk-shaped cells with a diameter of 70 μm, forming chains via thin silica rods (cf. *T. polychorda*); and *Corethron pennatum,* elongated single cells cylinder-shaped with a length of 120 μm and a diameter of 20 μm. The fourth species belongs to the Pennales: *Fragilariopsis kerguelensis,* chain-forming cells (60 μm long, 20 μm wide), with individual cells diamond-shaped. These kind of large diatoms are heavily affected by availability of iron under field conditions (De Baar et al. 1995; Timmermans et al. 2001; Boyd 2002).

Medium and culture conditions—Filtered (0.2 μm) natural surface seawater, collected south of the Polar Front during the ANT 18/2 expedition with the RV *Polarstern* (November 2000), was used as growth medium. This seawater can be regarded as typical HNLC water. Clean sampling and handling techniques (De Jong et al. 1998) were used throughout. During the experiments performed in the home laboratory, initial dissolved nutrient concentrations (as measured on board and verified in the home laboratory) were, for silicate ($Si(OH)_4$), 25.4 μmol L^{-1}; for nitrate (NO_3^-), 26.7 μmol L^{-1}; and for phosphate (PO_4^{3-}), 1.8 μmol L^{-1}. Background Fe_{diss} concentrations (as measured on board and verified in the home laboratory) during the experiments were 0.21 $\pm$ 0.14 nmol L^{-1} (average $\pm$ SD, $n = 5$). The diatoms were maintained in culture in this seawater before the experiments started. Shortly before the laboratory experiments started, the seawater was filtered a second time, using a Sartobran 0.45-μm prefilter with a 0.2-μm endfilter, rendering a filter-sterilized growth medium. All precautions were taken to prevent trace metal contamination and this was checked in randomly selected culture bottles. Similarly, abundance of bacteria in the culture media was checked before and during the experiments, verifying the marginal role of bacteria in our experiments. Rinsing and subsequent handling of all materials coming in contact with the growth medium was done under trace-metal–clean conditions. Prior to use, the culturing bottles were cleaned for at least 24 h in 1 mol L^{-1} HCl, followed by triple rinsing with Milli-Q water. Finally, the bottles were sterilized using boiling Milli-Q water. This procedure resulted in trace-metal–clean, sterile bottles. All subsequent manipulations of the phytoplankton cultures were done in a Class 100 laminar flow bench. Only in the experiment with *F. kerguelensis* was 35 μmol L^{-1} silicate added. Further, no nutrients or iron complexing agents were added to the medium, thus rendering a natural iron-poor but silicate-, nitrate- and phosphate-rich seawater.

The experiments were performed in 125 ml polycarbonate square bottles at 2–4°C, in a 16 : 8 h light : dark (LD) cycle. Irradiance was 60 μmol quanta m^{-2} s^{-1}, provided by cool white fluorescence tubes (Phillips, TLD 36W/54). The bottles were gently shaken on a daily basis, keeping the cells in solution, and preventing diatoms from adhering to the bottle wall. Sampling for nutrients and cell densities was also performed on a daily basis in a flow bench, which also allowed exchange of headspace gas with the atmosphere.

The following incubations were done: 0.2 (blank), 0.4, 0.6, 1.0, 1.8, 3.4, and 10.2 nmol L^{-1} Fe (as $FeCl_3$), except for the experiments with *F. kerguelensis,* for which the following incubations were done: 0.1 (blank), 0.25, 0.40, 0.68, 1.25, 2.40, 4.58, and 10.2 nmol L^{-1} Fe, using a different batch of HNLC Southern Ocean water, originating from another carboy of seawater collected in the same geographical area but at a different time.

Initial cell abundances at the start of each experiment were ~1 cell ml^{-1} for *Actinocyclus* sp., ~30 cells ml^{-1} for *C. pennatum* and *Thalassiosira* sp., and ~200 cells ml^{-1} for *F. kerguelensis.*

The length of the experiments differed for each species and experimental condition. Incubations with the lowest iron concentrations lasted up to 4 weeks, due to the iron stress imposed on these cultures. Silicate, nitrate, and phosphate concentrations were never depleted to more than approximately 30% of their initial concentrations to prevent artifacts introduced by extreme changes in medium composition or unnaturally high cell densities, except for the experiments with *F. kerguelensis,* where silicate was depleted at the end of the incubations. In the calculation of nutrient-depletion ratios, these latter data points were omitted. The incubations were repeated as a series several times until series obtained reproducible results. The data from the first incubation series was routinely discarded due to the time needed by the diatoms to adapt to the change in Fe_{diss} concentrations. All results are presented in relation to the total amount of Fe_{diss} (nmol L^{-1}), as it is still unclear which chemical form(s) of iron are accessed by the algae. With 99.9% of the iron bound to organic complexes, Fe^{3+} as well as Fe′ (all inorganic Fe species) can be calculated from these Fe_{diss} concentrations (Timmermans et al. 2001).

Parameters

Specific growth rates—Changes in cell numbers of the diatoms were monitored in 5-ml settling chambers using a Zeiss Axiovert 25 inverted microscope. Average growth rates were calculated from cell counts during exponential growth. Results were fitted with the nonlinear Monod equation. Maximum growth rates and half-saturation values (and their 95% confidence limits) for growth were calculated via nonlinear regression with average growth rates of at least six different growth conditions.

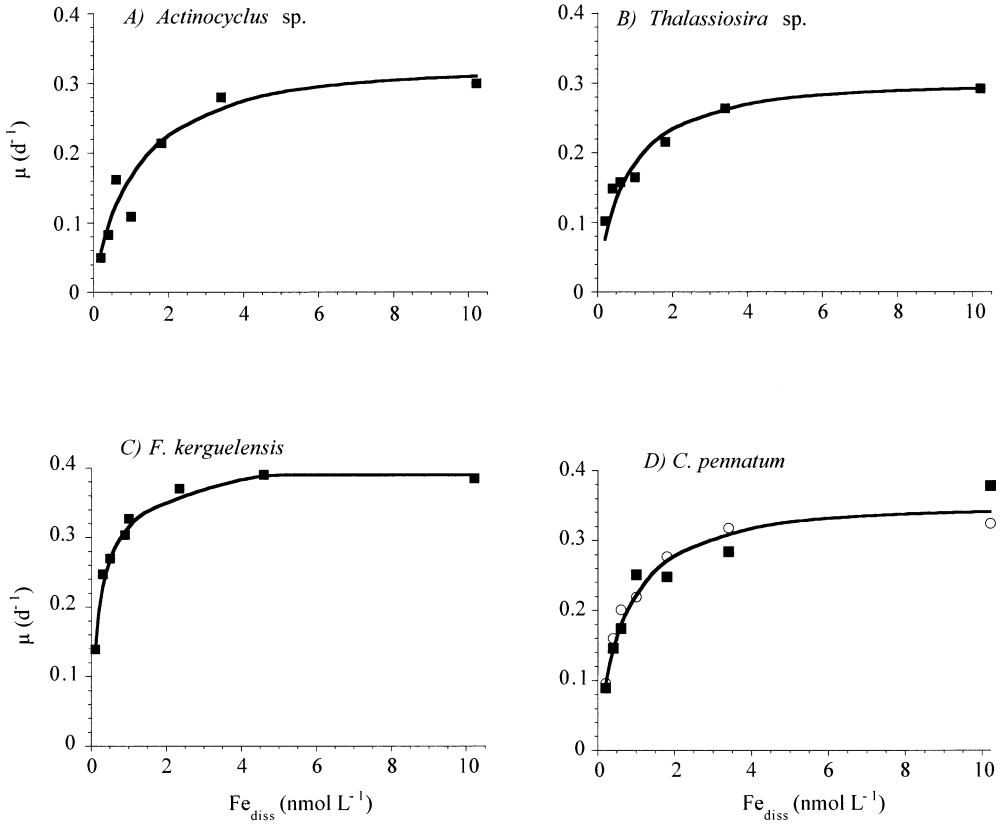

Fig. 1. Growth rates (d^{-1}) and nonlinear Monod fit (line) in cultures of (A) *Actinocyclus* sp., (B) *Thalassiosira* sp., (C) *F. kerguelensis*, and (D) *C. pennatum* in relation to Fe_{diss} concentrations (nmol L^{-1}). For cultures of *C. pennatum*, results of duplicate incubations are given with different symbols.

Nutrients—Dissolved silicate, nitrate (and nitrite and ammonium), and phosphate concentrations were measured using a Skalar Autoanalyser during the experiments in filtered (0.2-μm) growth medium. Nitrite and ammonium concentrations were low during the experiments, below 1% of the nitrate values, and therefore only nitrate will be considered. In order to prevent analytical off-set due to day-to-day measurements, filtered nutrient samples were collected, stored at 4°C (NO_3^- and PO_4^{3-}) or frozen (−20°C for $Si(OH)_4$) and analyzed at the end of each experimental period. The samples were collected with the necessary precautions, i.e., moderate pressure, rinsing of all equipment, etc. The depletion in dissolved nutrient concentrations in relation to Fe_{diss} concentrations, together with the increase in cell numbers, were used to calculate the nutrients consumed per cell. Furthermore, nutrient-depletion ratios were calculated by linear regression (Model II) of one nutrient against another in relation to Fe_{diss} concentrations. The latter approach ensured the most straightforward assessment of the effects of Fe on nutrient depletion. For comparisons of depletion ratios between the species, relative depletion ratios were calculated, defined as depletion ratios under Fe-deplete conditions (=below $K_m(Fe_{diss})$ value)/depletion ratios under Fe-replete conditions (=above $K_m(Fe_{diss})$ value).

Surface area and cell volume—Surface area (A, μm^2) and cell volume (V, μm^3) were calculated for the centric diatoms using measurements of living algae under the microscope, i.e., $2\pi rh + 2\pi r^2$ for cell surface area and $\pi r^2 h$ for cell volume. For the diamond-shaped (pennate) *F. kerguelensis*, cell surface area was calculated using the formula $2 \times (L \times W) + 4 \times ((2/3)L \times H)$ and cell volume was calculated using $(2/3)L \times W \times H$, where L = length, H = height, and W = width.

Results

Growth rates and K_m *in relation to* Fe_{diss} *concentrations*—The growth of the diatoms was strongly influenced by the amounts of iron added, albeit not always following a simple function of Fe_{diss} concentrations. Generally, the incubations with the highest amounts of iron added had the highest cell numbers. At high iron concentrations, growth rates were threefold (*Thalassiosira* sp., *F. kerguelensis*), fourfold (*C. pennatum*), and sixfold (*Actinocyclus* sp.) (Fig. 1A–D, Table 1) higher than growth rates at the lowest Fe_{diss} concentrations. The reproducibility of the experimental procedure is illustrated for cultures of *C. pennatum*, where duplicate incubations were performed (Fig. 1D). Growth rates at a given iron concentration varied by 10–15%. The values of μ_{max} did not differ much among the species tested and ranged between 0.31 d^{-1} (*Thalassiosira* sp.) and 0.39 d^{-1} (*C. pennatum*) (Table 1). Given the ranges in 95% confidence limits, no significant difference in μ_{max} between the four species

Table 1. Maximum growth rates (μ_{max}), half-saturation values (K_m) with respect to $Fe_{diss,}$ with their 95% upper and lower confidence limits (in parentheses) and corresponding R^2, growth temperatures (T), surface area (*A*), cellular volumes (*V*), and surface to volume ratios (*A*/*V*) in cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis, C. pennatum,* compared with results from other iron studies with diatoms.

	μ_{max} (d^{-1})	K_m (nmol L^{-1})		T (°C)	*A* (μm^2)	*V* (μm^3)	*A*/*V* (μm^{-1})
Actinocyclus sp.	0.34 (0.24–0.45)	1.14 (0.50–2.64)	$R^2 = 0.91$	4	48,000	620,000	0.08
Thalassiosira sp.	0.31 (0.27–0.36)	0.62 (0.27–0.92)	$R^2 = 0.93$	4	12,000	77,000	0.16
C. pennatum	0.36 (0.33–0.39)	0.57 (0.44–0.73)	$R^2 = 0.96$	4	8,200	38,000	0.22
F. kerguelensis	0.39 (0.38–0.41)	0.19 (0.16–0.26)	$R^2 = 0.98$	4	6,000	16,000	0.38
*Chaetoceros dichaeta**	0.62	1.12		4	11,500	75,000	0.15
*C. brevis**	0.39	0.0006		4	61	50	1.22
Actinocyclus sp.†	0.02	ND		16	ND	3,080–2,530	ND
Actinocyclus sp.‡	0.019	ND		16	ND	4,100–4,460	ND
Cylindrotheca fusiformis§	1.89	0.020		20	480–92	342–102	ND
Thalassiosira pseudonana§	2.85	0.21		20	49–165	45–103	ND
T. pseudonana‖	1.80	0.08		20	50	32	1.56
T. oceanica‖	1.58	0.04		20	95	87	1.09

ND, not determined.
* Timmermans et al. (2001).
† Muggli and Harrison (1997): range of *V* from Fe deficient to Fe sufficient.
† Muggli et al. (1996) range of *V* from Fe deficient to Fe sufficient in cultures grown with NO_3^- as N source.
§ Bucciarelli et al. (pers. comm.), range of *V* from Fe deficient to Fe sufficient.
‖ Sunda and Huntsmann (1995).

appears to be present. In comparison with previous studies on (small) diatoms, originating from temperate waters, the specific growth rates as reported here were low (Table 1). In this overview, the range in maximum growth rates is considerable, which is not really surprising considering the large variety in locations of origin, size of the cells (cell surface area ranging from 50 to 48,000 μm^2, cell volume ranging from 32 to 620,000 μm^3), light and temperature regime and, of course, the extent of the Fe limitation during the experiments.

Relationships between maximum growth rates and cell volume (Fig. 2A) were weak: smaller cells tended to have higher growth rates, but the results for *Actinocyclus* sp. deviated from this trend. Similar observations were made for the relationship between μ_{max} and surface-to-volume (*A*/*V*) ratio: cells with a high *A*/*V* ratio had the highest μ_{max} (Fig. 2B). The relative effects of iron limitation on growth rates, expressed as $\mu_{Fe\ deplete}/\mu_{max}$, had weak relationships with the cell volume (Fig. 3B) and *A*/*V* ratio (Fig. 3B) in the four species tested, but confirmed the general trend that the most prominent effects of Fe_{diss} concentrations on growth were seen in the largest cells.

The ranges in 95% confidence limits suggest that the $K_m(Fe_{diss})$ of *F. kerguelensis* is significantly lower than those of the other three species (Table 1). The smallest (in cell volume and cell surface area) of the four diatom species, *F. kerguelensis,* had the lowest $K_m(Fe_{diss})$ and the large *Actinocyclus* sp. did have the highest $K_m(Fe_{diss})$ (Table 1). Consistent with trends presented above, *A*/*V* ratios were negatively correlated with $K_m(Fe_{diss})$ values (Table 1): Cells with the highest *A*/*V* ratio (*F. kerguelensis*) had the lowest $K_m(Fe_{diss})$. Finally, the $K_m(Fe_{diss})$ values from this study and those reported in other studies with marine diatoms ranged over three orders of magnitude (Table 1).

Nutrient consumption per cell in relation to Fe_{diss} concentrations—The silicate consumption per cell varied between the species tested and per incubation (Table 2). Generally, silicate consumption was relatively high at low Fe_{diss} concentrations and relatively low at high Fe_{diss} concentrations. Only in experiments with *F. kerguelensis,* did $Si(OH)_4$ consumption per cell vary without a clear relationship with the Fe_{diss} concentrations.

In all cultures, nitrate consumption per cell increased with increasing Fe concentrations (Table 2): From Fe deplete to Fe replete, the nitrate consumption per cell roughly doubled. In three of the species tested, phosphate consumption per cell remained fairly constant in the incubations in response to Fe concentration (Table 2).

Elemental depletion ratios in relation to Fe_{diss} concentrations—The silicate to nitrate depletion ratios increased with decreasing Fe_{diss} concentrations (Table 3), caused by the combination of an increase in silicate depletion and a decrease in nitrate depletion at low Fe_{diss} concentration. The difference in ratio was from 4.7 (Fe deplete) to 2.0 (Fe replete) in *Actinocyclus* sp., but sometimes more extreme (18 to 3, in cultures of *Thalassiosira* sp.) or virtually absent (1.2 to 1.4, *C. pennatum*). The silicate-to-nitrate depletion ratio deviated considerably from the average silicate-to-nitrate ratio for diatoms of 0.8 ± 0.3 (Brzezinski 1985).

The silicate-to-phosphate depletion ratios were high (up

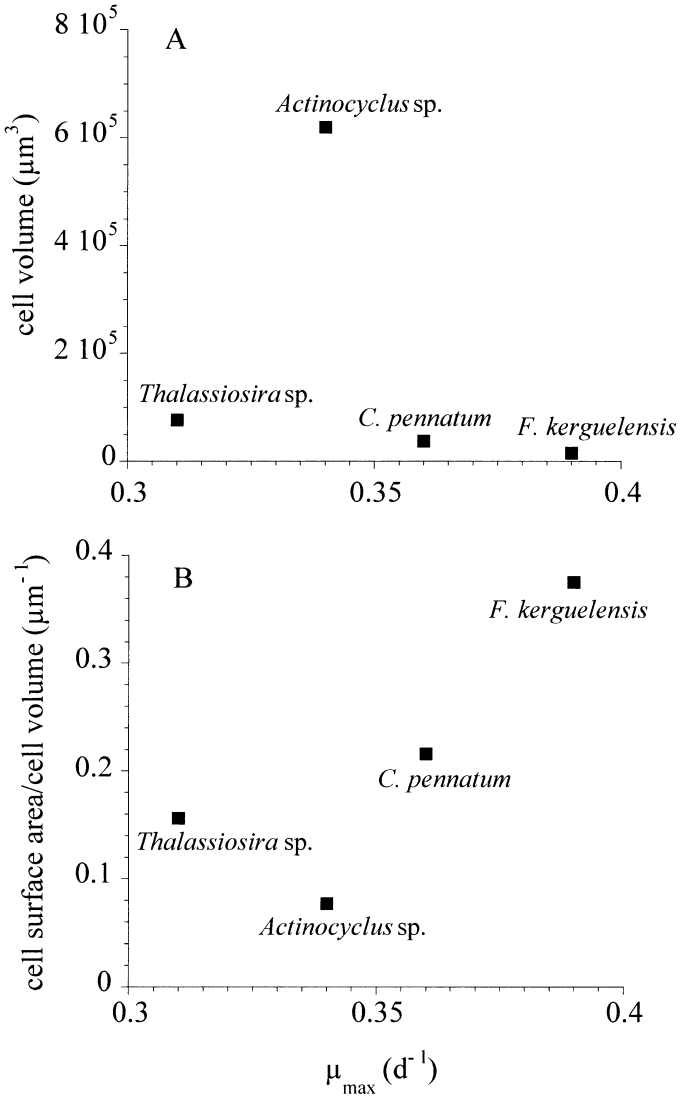

Fig. 2. Maximum growth rates (d^{-1}) in relation to (A) cell volume (μm^3) and (B) cell surface area/cell volume (μm^{-1}) in cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis,* and *C. pennatum.*

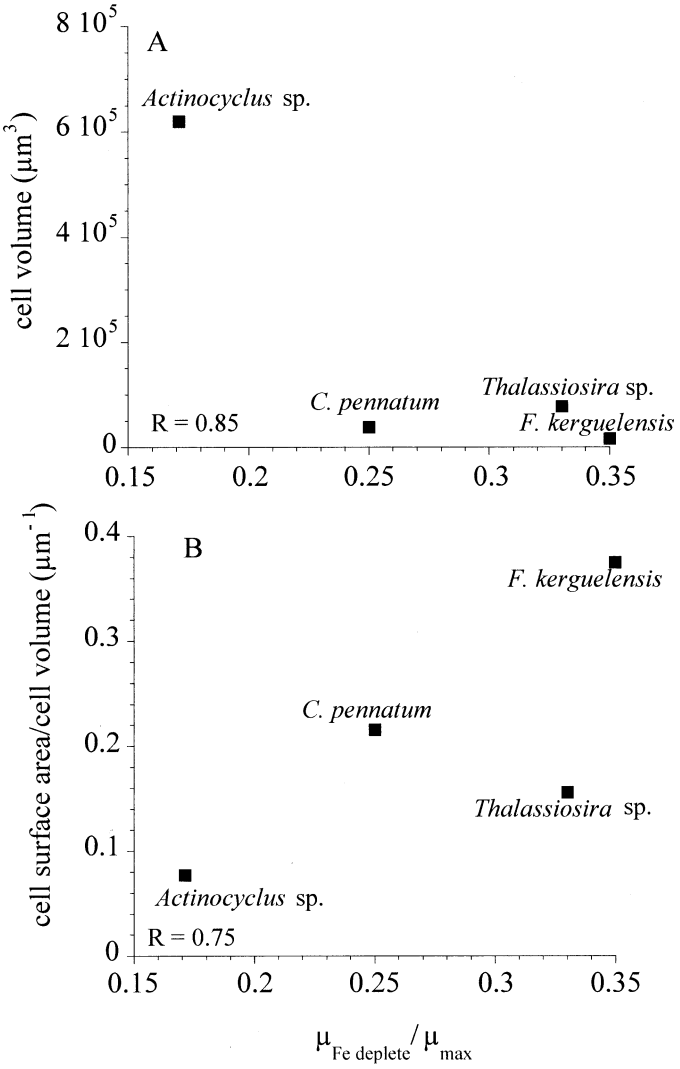

Fig. 3. The ratio of (A) $\mu_{Fe\ deplete}/\mu_{max}$ versus cell volume (μm^3) and (B) cell surface area /cell volume (μm^{-1}) in cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis,* and *C. pennatum.* The $\mu_{Fe\ deplete}$ is the growth rate at the lowest Fe_{diss} concentration, μ_{max} was determined from the nonlinear Monod fit. *R* is the correlation coefficient.

to 39) and tended to decrease slightly with increasing Fe_{diss} concentrations (Table 3). Only for cultures of *Actinocyclus* sp., was a marked decrease observed (Table 3). For the other three species (Table 3), these differences were smaller. The silicate-to-phosphate depletion ratios were, with the exception of those for *Thalassiosira* sp. (Table 3), well above the average silicate-to-phosphate ratio of 5.9 ± 1.3 for diatoms (Sarthou et al. in press).

The nitrate-to-phosphate depletion ratios tended to increase with increasing Fe_{diss} concentrations (Table 3). Given the stable phosphate depletion, this difference could be attributed to an increase in nitrate depletion at higher Fe_{diss} concentrations. The nitrate-to-phosphate depletion ratios in cultures of *Thalassiosira* sp. and *F. kerguelensis* increased by twofold or more from Fe-deplete to Fe-replete conditions. Whereas in cultures of *Actinocyclus* sp., only a slight increase was observed; in cultures of *C. pennatum,* nitrate-to-phosphate ratios remained unaffected by changes in Fe_{diss} concentrations (Table 3).

Relative changes in nutrient-depletion ratios in relation to relative changes in growth rates in Fe-deplete versus Fe-replete cultures—The differences in nutrient consumption per cell, as well as the growth rates of the four species tested, varied in relation to Fe_{diss} concentrations. For the sake of a proper comparison between the four species tested, the relative changes in nutrient depletion (e.g., $silicate_{Fe\ deplete}$: $nitrate_{Fe\ deplete}/silicate_{Fe\ replete}$: $nitrate_{Fe\ replete}$) were related to relative changes in growth rates ($\mu_{Fe\ deplete}/\mu_{max}$) (Fig. 4).

The relative silicate-to-nitrate (Fig. 4A) depletion ratios increased with the changes in relative growth rates, but the relation was weak. The smallest cells (*F. kerguelensis*) were the least affected by Fe limitation. The relative silicate-to-

Table 2. Consumption per cell of $Si(OH)_4$, NO_3^- and PO_4^{3-} during the Fe_{diss} addition experiments with cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis,* and *C. pennatum.* ND denotes not determined.

Fe_{diss} (nmol L^{-1})	$Si(OH)_4$ (pmol $cell^{-1}$)	NO_3^- (pmol $cell^{-1}$)	PO_4^{3-} (pmol $cell^{-1}$)
Actinocyclus sp.			
0.2	1,436	150	34
0.4	1,657	220	41
0.6	1,225	289	40
1.0	704	250	32
1.8	941	267	43
3.4	784	316	36
10.2	624	361	43
Thalassiosira sp.			
0.2	58	36	14
0.4	43	33	7
0.6	54	32	14
1.0	34	37	11
1.8	38	42	22
3.4	29	50	10
10.2	23	51	8
F. kerguelensis			
0.11	6.7	0.7	0.22
0.25	5.5	0.8	0.14
0.40	4.2	1.5	1.16
0.68	4.9	1.0	0.16
1.25	4.0	1.2	0.12
2.40	3.8	3.5	0.36
4.69	3.2	1.2	0.12
9.17	4.2	1.1	0.17
C. pennatum			
0.2	216	97	13
0.4	267	179	26
0.6	184	136	21
1.0	186	139	27
1.8	248	193	32
3.4	256	225	27
10.2	ND	ND	ND

phosphate (Fig. 4B) and especially the relative nitrate-to-phosphate (Fig. 4C) depletion ratios showed systematic relations with the changes in relative growth rates. For the silicate-to-phosphate depletion ratios, this was a negative relation; for the nitrate-to-phosphate depletion ratios, this was a positive relationship. In both cases, the largest cells (*Actinocyclus* sp.) were the most affected by iron limitation.

Discussion

There is a general consensus that marine phytoplankton strongly influence oceanic distribution, cycling, and chemical speciation of trace elements and vice versa (Morel and Price 2003). Yet the mode(s) of environmental control of key phytoplankton species at the level of relevant functional groups or species is virtually absent (Boyd et al. 2002). Among environmental control factors, iron has received more attention than other trace elements because 40% of the world ocean is thought to be Fe limited (Moore et al. 2002). Iron limitation regulates rates of carbon and nitrogen cycling (Morel and Price 2003). From a global biogeochemical modeling as well as fundamental scientific perspective, it is of great importance to understand the physiological responses of representative phytoplankton species to iron limitation. Over the years, numerous marine phytoplankton species have been subjected experimentally to Fe limitation. These studies yielded valuable information on the response of phytoplankton to Fe limitation, for example, in terms of effects on growth rates and nutrient consumption. Extrapolation of these results to field conditions may be difficult given the obvious artificial conditions. Artificial seawater, surplus of nutrients (100 times the natural concentrations), trace-metal chelating agents (μmol L^{-1} of EDTA), 24-h light periods, etc., will affect the outcome of the studies. With the possibilities of growing phytoplankton under natural conditions, there now is a need to make progress with such experimental studies and to work with ecologically relevant species.

In our present study, the diatoms were cultured under realistic conditions, i.e., natural HNLC Southern Ocean water without significant changes in the chemical composition of the growth medium (Gerringa et al. 2000; Timmermans et al. 2001). The choice of working with natural seawater originating from HNLC conditions was a deliberate one, but not without significant logistical problems. The common practice in previous studies has been to stabilize the culture medium with artificial chelators such as EDTA, but it is obvious that this grossly disturbs the chemical equilibria and kinetics in the medium (Gerringa et al. 2000). Given the conditions under which this study was performed, addition of EDTA was not necessary because of the well-controlled low Fe concentrations in the medium. We assume that the response of the diatoms represents the response under natural field conditions. Further, the diatoms that were used have been demonstrated to be important bloom-forming primary producers in the Southern Ocean (De Baar et al. 1997).

Effects of Fe_{diss} concentrations on growth rates—The differences in maximum growth rates were small for the four species tested in this study. The larger species (*Actinocyclus* sp.) had a lower maximum growth rate than the smallest species, *F. kerguelensis.* This is in agreement with the general trend of lower growth rates in larger diatom species (Sarthou et al. in press). Comparison with maximum growth rates in other laboratory Fe-limitation studies (Table 1) revealed that small diatom species from temperate waters can have maximum growth rates up to nine times higher than those reported here for the large Antarctic diatoms, likely due to differences in water temperature (Eppley 1972). The relation of A/V to maximum growth rate for the four species tested (Fig. 2B, Table 1) showed a clear indication of allometric effects. Including the A/V data from the small diatoms used in other studies would confirm this trend (Sunda and Huntsman 1995; Bucciarelli et al. pers. comm.). The relative changes in growth rates from Fe-limited to Fe-replete cultures indicated that the largest diatom species are most affected by iron limitation (Fig. 3). This corresponds very well with observations in the field (bottle and in situ experiments), where the largest diatom species have the most marked response to iron addition (De Baar and Boyd 2000).

Table 3. Dependence on Fe_{diss} concentrations for $Si(OH)_4 : NO_3^-$, $Si(OH)_4 : PO_4^{3-}$, and $NO_3^- : PO_4^{3-}$ molar depletion ratios, calculated from Model II linear regressions of one nutrient against another, correlation coefficient (*R*), and number of observations (*n*), in cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis*, and *C. pennatum.*

Fe_{diss} (nmol L^{-1})	$Si(OH)_4 : NO_3^-$			$Si(OH)_4 : PO_4^{3-}$			$NO_3^- : PO_4^{3-}$		
	Ratio	*R*	*n*	Ratio	*R*	*n*	Ratio	*R*	*n*
Actinocyclus sp.									
0.2	4.73	0.79	4	39.58	0.95	5	4.07	0.93	4
0.4	5.68	0.93	3	38.54	0.97	4	1.81	0.71	3
0.6	3.70	0.93	5	30.18	0.98	5	6.63	0.98	5
1.0	2.62	0.96	5	21.83	0.99	5	7.36	0.99	5
1.8	2.40	0.97	5	21.72	0.99	5	8.36	0.99	5
3.4	2.92	0.96	5	22.47	0.99	5	6.81	0.99	5
10.2	2.02	0.84	4	27.19	0.97	5	5.67	0.93	4
Thalassiosira sp.									
0.2	18.33	0.94	4	7.22	0.99	4	0.28	0.95	4
0.4	7.82	0.97	4	6.80	0.99	4	0.78	0.98	4
0.6	5.49	0.97	4	5.11	0.98	4	0.81	0.98	4
1.0	4.52	0.98	4	5.65	0.99	4	1.07	0.97	4
1.8	5.56	0.99	4	9.47	0.99	4	1.67	0.98	4
3.4	3.67	0.99	4	7.39	0.99	4	1.87	0.98	4
10.2	2.84	0.99	4	7.85	0.99	4	2.57	0.98	4
C. pennatum sp.									
0.2	1.20	0.99	5	14.74	0.99	6	12.11	0.98	5
0.4	1.29	0.99	5	14.97	0.99	6	11.58	0.98	5
0.6	1.44	0.95	5	9.37	0.93	6	6.89	0.96	5
1.0	1.34	0.99	5	14.99	0.98	6	10.83	0.98	5
1.8	1.18	0.92	5	8.36	0.91	6	5.26	0.87	5
3.4	1.21	0.96	5	10.99	0.95	6	8.47	0.96	5
10.2	1.12	0.97	5	12.17	0.98	6	9.99	0.98	5
F. kerguelensis sp.									
0.1	3.69	0.97	6	30.71	0.99	6	7.81	0.99	6
0.25	6.95	0.99	6	38.35	0.99	6	5.43	0.99	6
0.4	0.64	0.71	6	10.09	0.86	6	7.99	0.94	6
0.68	1.75	0.75	6	28.77	0.98	6	4.58	0.87	6
1.25	3.04	0.99	6	32.87	0.99	6	10.68	0.99	6
2.4	2.30	0.99	6	30.35	0.99	6	13.07	0.99	6
4.58	2.21	0.99	6	31.07	0.99	6	13.67	0.98	6
9.17	2.23	0.99	6	29.93	0.99	6	12.70	0.98	6

Similarly, these findings corroborate the conclusion that the diffusion limitation of Fe becomes disproportionately more important as the cell diameter increases (Sunda and Huntsman 1995; Timmermans et al. 2001). Obviously, this also holds true for diatom species with different shapes (Pahlow et al. 1997). Comparison with field data shows further good agreement with the data from our present study: community μ_{max} of 0.2–0.6 d^{-1} (Coale et al. 2003) or 0.15–0.40 d^{-1} (Blain et al. 2002) were reported for Antarctic phytoplankton assemblages, and a μ_{max} of 0.2–0.3 d^{-1} was reported for field populations of *F. kerguelensis* (Coale et al. 2003).

Half-saturation values with respect to Fe_{diss} concentrations—In cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis*, and *C. pennatum*, the $K_m(Fe_{diss})$ values varied considerably. These differences in affinity for iron can be attributed to differences in cell size parameters: the smallest cells, either in cell surface or cell volume, with the highest surface-to-volume ratio had the lowest $K_m(Fe_{diss})$ values, thus a higher affinity for Fe_{diss}. As with the effects on growth rates, this was irrespective of the differences in morphology of the species tested. In our study, the size of the diatoms did not vary with the dissolved iron concentrations.

Comparison with other $K_m(Fe_{diss})$ values from laboratory studies shows differences of three orders of magnitude (Table 1). It is hard to judge whether these differences are caused by physiological differences between the species or just by different methods used to calculate the concentration of Fe_{diss}. But in general, it confirms the trend that small species have the lowest $K_m(Fe_{diss})$ values. This is confirmed by results from other small diatom species (Sunda and Huntsman 1995; Bucciarelli et al. pers. comm.). For the four Antarctic species, the $K_m(Fe_{diss})$ values were at or above the ambient concentrations of Fe_{diss}. The species with the lowest $K_m(Fe_{diss})$ in this study, *F. kerguelensis*, was typically one of the more dominant species encountered in the field when the seawater in which we performed the experiments was collected (U. Freyer and P. Assmy, AWI, Bremerhaven, Germany, pers. comm.). During the SOIREE (Southern Ocean Iron Enrichment Experiment), *F. kerguelensis* was the

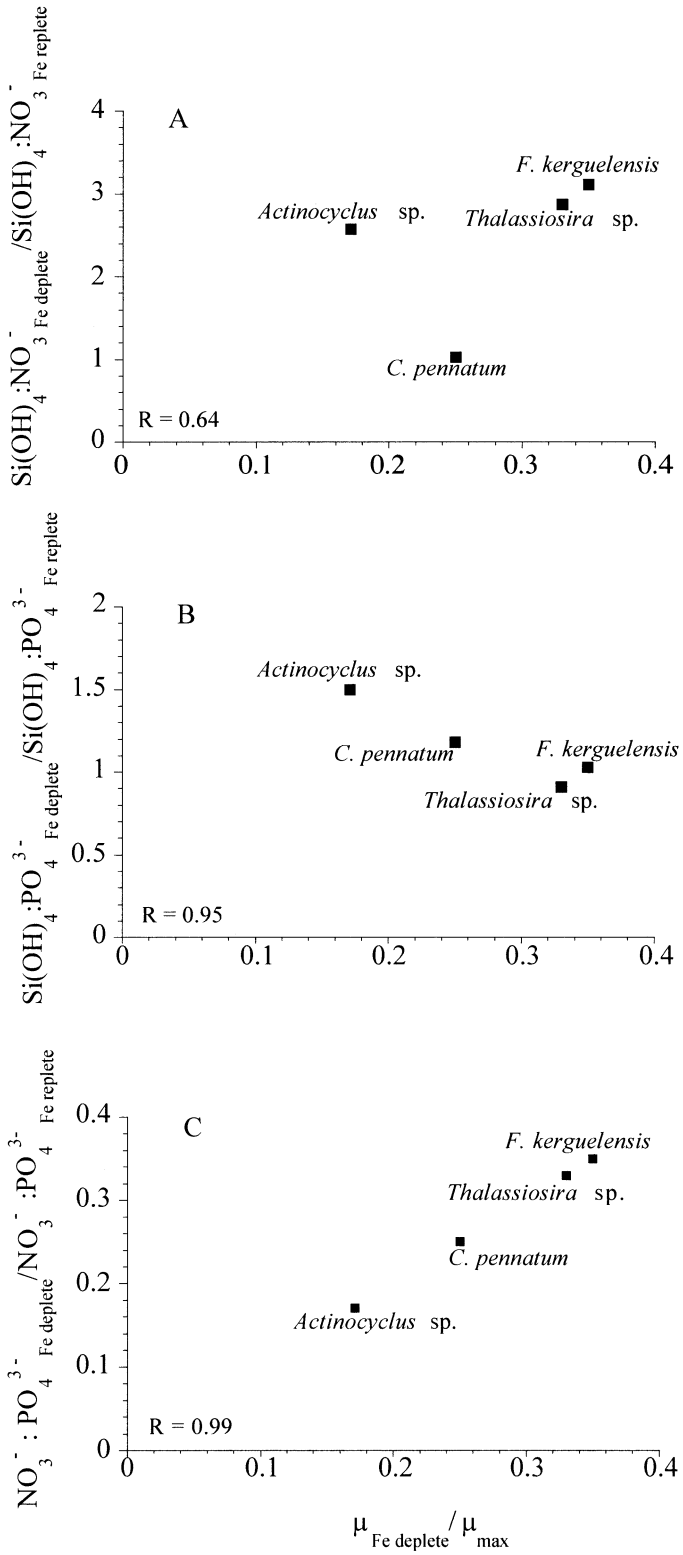

Fig. 4. The depletion ratio of (A) silicate to nitrate, (B) silicate to phosphate, and (C) nitrate to phosphate in Fe-deplete and Fe-replete incubations in relation to $\mu_{\text{Fe deplete}}/\mu_{\text{max}}$ in cultures of *Actinocyclus* sp., *Thalassiosira* sp., *F. kerguelensis*, and *C. pennatum*. The relative nutrient ratios were calculated for the incubations with the lowest and the highest Fe concentration. The $\mu_{\text{Fe deplete}}$ is the growth rate at the lowest Fe concentration, μ_{max} was derived from the nonlinear Monod fit, and the correlation coefficients (R) are indicated.

bloom-forming species in response to the Fe addition (Boyd et al. 2000). This is in good agreement with the reported relatively low $K_m(Fe_{diss})$ values. The other species tested in this study clearly had higher $K_m(Fe_{diss})$ values, with concomitant limitations in their growth. These findings further strengthen the picture derived from field observations, bottle experiments, and in situ Fe-enrichment experiments that small cells are less affected by iron limitation. When compared with community $K_m(Fe_{diss})$ values, our single-species experimental data are in fair agreement with the $K_m(Fe_{diss})$ of 0.15–0.40 nmol L^{-1} reported for Antarctic phytoplankton (Blain et al. 2002). Similarly, a $K_m(Fe_{diss})$ of 0.1 nmol L^{-1} for *F. kerguelensis* was reported (Coale et al. 2003), close to the value of 0.19 nmol L^{-1} that we report for this species in the present study.

With the knowledge of the responses of Antarctic diatoms on growth rates and half-saturation values with respect to Fe_{diss} concentrations, it should be realized that the response in the field also depends on the presence of enough seeding cells. In SOIREE, *F. kerguelensis* became dominant (Boyd et al. 2000); in the EisenEx study, *Pseudonitschia* spp. was the most abundant species (Smetacek et al. 2001); whereas in the SEEDS (Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study) experiment, *Chaetoceros debilis* became dominant (Tsuda et al. 2003). This exemplifies the chaotic fluctuations, characteristic of competition between multiple phytoplankton species for multiple resources (Huisman and Weissing 2002) and makes it clear that other parameters such as aggregation, grazing, and export fluxes have to be taken into consideration.

Effects of Fe_{diss} concentrations on nutrient consumption per cell and nutrient-depletion ratios—In three (*Actinocyclus* sp., *Thalassiosira* sp., *C. pennatum*) of the four species tested in this study, $Si(OH)_4$ consumption per cell increased under iron limitation. Maximum silicate depletion rates increased with added Fe in the Southern Ocean, the upwelling center off central California, and the eastern tropical Pacific (Franck et al. 2003) and in cultures of *Thalassiosira weisflogii* (De La Rocha et al. 2000), cultures of *Chaetoceros dichaeta* (Takeda 1998), and cultures of *T. pseudonana* and *Cylindrotheca fusiformis* (Bucciarelli et al. pers. comm.). Silicate metabolism is regarded as a relatively energy-cheap metabolic process (Raven 1983), and silicate uptake may continue in specific phases of the cell cycle that are prolonged under iron limitation (Claquin et al. 2002), both effects leading to higher silicate uptake per cell under Fe limitation. These processes do not necessarily have to lead to more highly silicified cells, as the size of the cells may change under Fe limitation (Takeda 1998). In our study, however, no changes in cell sizes were observed, and here increased silicate consumption per cell must have resulted in more highly silicified cells. The ecological reason(s) for the highly silicified cells remain unclear, perhaps a thicker Si wall makes division possible even under low silicate conditions (Claquin et al. 2002) or offers a better protection against grazing (Hamm et al. 2003).

In all four diatoms species tested, nitrate consumption per cell increased with increasing Fe concentrations. In general, the effects of iron limitation on nitrogen uptake, more spe-

cifically nitrate uptake, are well documented: Nitrate uptake and metabolism requires energy in the form of reducing power from the photosynthetic and respiratory electron transport systems (Timmermans et al. 1994; Franck et al. 2003). The stimulation of nitrate uptake by iron has been demonstrated in situ (De Baar et al. 1997) and in several bottle experiments under HNLC conditions (Timmermans et al. 1998). Most recently, it was demonstrated that Fe directly regulates maximum uptake rates of NO_3^- in HNLC regions (Franck et al. 2003).

Reports on phosphate consumption per cell in relation to iron availability are, to the best of our knowledge, absent. Therefore, our findings for phosphate are unique. We showed that, for all four species, phosphate consumption per cell was variable and not clearly influenced by dissolved iron concentrations.

With the changes in consumption of silicate and nitrate under Fe-deplete and Fe-replete conditions as described above, it is not surprising that the nutrient-depletion ratios will also change with the changing dissolved iron availability.

The $Si(OH)_4$-to-NO_3^- ratio was high under Fe-deplete conditions and decreased with higher Fe concentrations in all species tested in this study, except for *C. pennatum.* Similar findings of increased silicate-to-nitrate ratios at decreased iron concentrations were reported for low Fe concentrations in a coastal upwelling regime (Franck et al. 2003), in the Southern Ocean (Takeda 1998; Franck et al. 2003), in the North Pacific Ocean (Takeda 1998), in the eastern tropical Pacific (Takeda 1998), and under controlled laboratory conditions (Takeda 1998; Bucciarelli et al. pers. comm.). The change in $Si(OH)_4$-to-NO_3^- depletion ratio is most likely the consequence of a combined increased $Si(OH)_4$ depletion and a decreased NO_3^- depletion under Fe-limiting conditions, as described above. The result of this decoupling of $Si(OH)_4$ and NO_3^- depletion under Fe-limiting conditions will lead to alterations in the silicate and nitrate biogeochemical cycles. The growth of heavy, more silicified diatoms under Fe-limiting conditions will result in a more efficient draw down of silicate (silicate pump; Dugdale et al. 1995), leading to low iron, low silicate, high nitrate, but low chlorophyll waters (De La Rocha et al. 2000).

The silicate-to-nitrate depletion ratios for two Antarctic diatoms (*Chaetoceros dichaeta* and *Nitzschia* sp.) were described to decrease from 1.9 to 0.7 and from 2.1 to 1.2, respectively, from low- to high-Fe conditions (Takeda 1998). In phytoplankton communities in the Southern Ocean, the Subarctic Pacific, and the Equatorial Pacific, similar reductions in $Si(OH)_4$-to-NO_3^- depletion ratios in response to iron addition were described (Takeda 1998). Silicate-to-nitrate depletion ratios decreased from 3.0 to 0.5 (*T. pseudonana*) and from 0.6 to 0.2 (*Cylindrotheca fusiformis*) in laboratory experiments (Bucciarelli et al. pers. comm.). During SOIREE, the silicate : nitrate depletion ratios were about two outside and about one inside the fertilized patch (Boyd et al. 2000). Our experimental findings for cultures of *Actinocyclus* sp., *Thalassiosira* sp., and *F. kerguelensis* agree well with the previously reported observations (Table 3). These three species showed a marked decrease in $Si(OH)_4$-to-NO_3^- depletion ratio in response to increased Fe availability. The ratios for these species are more extreme than the previously published data on the $Si(OH)_4$-to-NO_3^- depletion ratios for single species. Also, the $Si(OH)_4$-to-NO_3^- depletion ratios in cultures of *Actinocyclus* sp., *Thalassiosira* sp., and *F. kerguelensis* are much higher than those reported for mixed field populations. Obviously, the species that we used were larger and more silicified. It is clear that the most likely explanation for the shifts in the silicate-to-nitrate depletion ratios is caused by the increased silicate and the decreased nitrate consumption under Fe limitation. It should be noted that the changes in $Si(OH)_4 : NO_3^-$ ratio are not observed in experiments with cultures of *C. pennatum.* De La Rocha (2000) described a similar phenomenon and explained this by the extent of iron limitation of the phytoplankton; when there is a variability in Fe stress in cells, this can result in a variability in physiological state and thus in considerable differences in elemental ratios.

The $Si(OH)_4$-to-PO_4^{3-} depletion ratio in the four Antarctic diatoms used in this study was slightly increased in incubations with less than 1 nmol L^{-1} Fe added. Above 1 nmol L^{-1} Fe added, the $Si(OH)_4 : PO_4^{3-}$ depletion ratios were more or less constant, but the value of the ratio varied considerably (30 in *F. kerguelensis* to 10 in *Thalassiosira* sp.) (Table 3). This again emphasizes the importance of species-specific studies. A more pronounced effect on the silicate-to-phosphate depletion ratios in response to Fe limitation is described in both the field and in single-species experiments (*Chaetoceros dichaeta* and *Nitzschia* sp.; Takeda 1998). In field populations of phytoplankton in the Southern Ocean, the $Si(OH)_4$-to-PO_4^{3-} depletion ratios decreased from 2.3 to 0.95; in the subarctic Pacific, from 2.6 to 1.2; and in the Equatorial Pacific, from 1.3 to 0.45 (Takeda 1998). Similarly, in experiments with cultures of *Chaetoceros dichaeta,* the silicate : phosphate depletion ratios decreased from 16 to 7.1; and in *Nitzschia* sp., from 42 to 21 (Takeda 1998). From Takeda's findings as well as our own results, it is, as with the $Si(OH)_4 : NO_3^-$ ratios, clear that depletion ratios measured in the field are markedly lower than those from single-species experiments. Given the fact that, in our experiment, we did not observe a systematic effect of Fe availability on phosphate consumption per cell, it is likely that the differences in $Si(OH)_4$-to-PO_4^{3-} depletion ratio are mainly caused by the increased silicate consumption at low dissolved iron concentrations.

The nitrate-to-phosphate depletion ratios doubled in two of the four species in this study, when going from Fe-deplete to Fe-replete conditions (Table 3). In cultures of *C. pennatum,* $NO_3^- : PO_4^{3-}$ depletion ratios remained unaltered. The changes in nitrate : phosphate ratio are in good agreement with those previously reported (Takeda 1998), although in both field and experimental data, the differences in NO_3^--to-PO_4^{3-} ratio in Fe-deplete and Fe-replete conditions were relatively small. The NO_3^--to-PO_4^{3-} ratio in SOIREE (enriched) was 10, and no data were reported for NO_3^--to-PO_4^{3-} ratios in phytoplankton outside the Fe-enriched patch (Boyd et al. 2000).

Relative changes in nutrient ratios and growth rates—Species-specific studies clearly have the advantage of providing exact physiological information on the reactions of a

single species to changing environmental conditions. However, not all species react the same. Absolute effects will differ from species to species, thereby making it difficult to discern general patterns. Therefore, the responses of the four diatoms on Fe limitation were studied based on relative changes (Fe deplete/Fe replete) in both growth rates and nutrient-depletion ratios. This resulted in some striking general patterns (Fig. 4). For both the relative changes in $Si(OH)_4$: PO_4^{3-} and the $NO_3^- : PO_4^{3-}$ depletion ratios, the largest cells (*Actinocyclus* sp.) were the most heavily affected by iron limitation. The smallest species, *F. kerguelensis*, were the least affected by Fe limitation. For the relative changes in silicate-to-nitrate depletion ratios, the response was less clear, but also here there was a trend that the larger cells were the most affected by iron limitation. In addition to the relationship between μ_{max} and K_m and the volume of the diatoms (Table 1), the relative changes in silicate-to-phosphate and nitrate-to-phosphate depletion ratios are the most convincing experimental evidence for the size-dependent response of phytoplankton to Fe addition.

The variability in nutrient consumption per cell and (relative) nutrient depletion observed in the present study and other studies make it clear that caution must be used when estimating paleoproductivity from, for example, silicate deposition rates. Moreover, because the carbon-to-silicate uptake ratio can vary more than twofold, calculations of modern or ancient air–sea exchange of CO_2 are very uncertain.

The supply to and utilization of iron by diatoms in surface waters are major factors in global change and climate. Our results show that Fe not only has marked effects on growth rates, it significantly affects nutrient consumption per cell and thus nutrient-depletion ratios. Moreover, most of these effects are volume dependent, with the largest cells showing the most pronounced responses. The species that we have used in our study typically belong to the larger size class, seldom used in experimental laboratory studies with natural HNLC water, but potentially important bloom formers with a substantial contribution to new production, as demonstrated in several in situ iron-enrichment experiments. With this knowledge, it will be possible to improve biogeochemical modeling and estimates of primary (paleo)productivity and carbon export.

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