

Growth rates of large and small Southern Ocean diatoms in relation to availability of iron in natural seawater

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Abstract

Blooms of large diatoms dominate the CO₂ drawdown and silicon cycle of the Southern Ocean in both the past and present. The growth of these Antarctic diatoms is limited by availability of iron (and light). Here we report the first assessment of growth rates in relation to iron availability of two truly oceanic Antarctic diatom species, the large, chain-forming diatom *Chaetoceros dichaeta* and the small, unicellular diatom *C. brevis*. In filtered natural, untreated Southern Ocean water, a maximum specific growth rate of $0.62 \pm 0.09 \text{ d}^{-1}$ and a K_m for growth of $1.12 \times 10^{-9} \text{ M}$ dissolved iron was calculated for *C. dichaeta*. This response could only be seen during a long-day light period. *C. brevis* maintained growth rates of $0.39 \pm 0.09 \text{ d}^{-1}$ with and without iron addition, even under short-day light conditions, and could only be forced into iron limitation by adding the siderophore desferri-ferrioxamine B (DFB), an iron immobilizing agent. Using this approach, the low K_m value for growth of $0.59 \times 10^{-12} \text{ M}$ dissolved Fe was calculated for this species. The size-class dependent growth response to iron (and light) confirms the key role of these parameters in structuring Southern Ocean ecosystems and thus the CO₂ dynamics and the silicon cycle.

The Southern Ocean is the largest upwelling region of the globe, comprising 20% of the world oceans. Equilibration of the excess CO₂ (potential pCO₂ = ~500 μatm in upper circumpolar deep water, Hoppema et al. 1999) from the upwelling deep waters with the atmosphere would cause outgassing of CO₂ to the atmosphere. However, the concomitant upwelling of major nutrients (N, P, Si) is adequate to support significant CO₂ fixation by phytoplankton, thereby minimizing or preventing the outgassing of CO₂. Remarkably, the majority of the upwelling major nutrients are not fully used due to missing growth factors in this largest high-nutrient, low-chlorophyll (HNLC) region of the world. The hypothesis of Fe limitation (Gran 1931) was tested in the Southern Ocean by shipboard incubations (de Baar et al. 1990; Martin et al. 1990; Buma et al. 1991; Timmermans et al. 1998), by

direct field observations in the iron-rich polar frontal jet (Fe > $1 \times 10^{-9} \text{ M}$; de Baar et al. 1995), and by in situ Fe enrichment (Boyd et al. 2000). In all HNLC regions, the addition of iron has always led to growth enhancement (de Baar et al. 1990; Martin et al. 1990; Buma et al. 1991; Timmermans et al. 1998), if not blooms (de Baar et al. 1997; Boyd et al. 2000), of the largest size class of phytoplankton, in particular chain-forming diatoms (de Baar and Boyd 2000). Owing to their large size, these 20–100-μm diameter algae have a high growth requirement for dissolved iron (Sunda and Huntsman 1995). An interesting feature of these big diatoms of taxa such as *Chaetoceros* spp. (Bathmann et al. 1997), *Corethron* spp. (Crawford et al. 1997), *Fragilaria kerguelensis* (de Baar et al. 1997), and *Actinocyclus* sp. (Muggli and Harrison 1997) is that they are characterized by very robust opal frustules (silicon oxide cell walls). These frustules are the major components of the biogenic silica sedimentation, with some 75–90% depositing in the silica belt underlying the Antarctic polar front. Massive deposition events (Wefer and Fischer 1991) have been recorded in moored time series sediment traps in all three major HNLC regions. Determining the controlling factors (e.g., iron and light) and the fate of Antarctic blooms of large diatoms is crucial for unraveling the cause–effect relations of the various glacial/interglacial global climate shifts (Petit et al. 1999; Moore et al. 2000).

Current research on the iron–phytoplankton interactions has to deal with some major gaps in knowledge. Until recently, the growth response in relation to availability of iron, here expressed as K_m in equation

$$\frac{\mu}{\mu_{\max}} = \frac{[\text{Fe}]}{(K_m + [\text{Fe}])} \quad (1)$$

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(after Monod 1950) of marine algae has been assessed in laboratory cultures containing 10^{-4} M amounts of ethylenediaminetetraacetate (EDTA) (Sunda and Huntsman 1995), in which the biological availability of Fe is controlled by concentrations of inorganic iron (Fe'). Conversely, this has led to the current paradigm that the inorganic Fe (Fe') pool is controlling the availability for biological uptake and growth. However, the EDTA affects the equilibria and kinetics of the various natural chemical forms of Fe (Gerringa et al. 2000). Upon finding that 99% or more of dissolved iron (Fe_{diss}) is complexed by organic ligands in natural seawater (Gledhill and van den Berg 1994; Rue and Bruland 1995), it was immediately realized (Wells et al. 1994) that the corresponding calculated inorganic iron would by far be too low for sustaining growth of even the small phytoplankton species. Inevitably, some of the organic complexes leave Fe available for uptake by phytoplankton (Hutchins et al. 1999). A tentative hypothesis can therefore be that right now it is not known yet which form(s) of Fe in natural waters are actually taken up by phytoplankton. Furthermore, there have hardly been any studies on the relation between growth and availability of iron in unialgal cultures of the large size class of oceanic diatoms (Muggli and Harrison 1997). These species have proven to be hard to maintain in cultures, due to their fragile chains and/or spines. In spite of their importance for primary production in the Southern Ocean, the largest HNLC region on earth, growth responses in relation to availability of iron have not been assessed for truly oceanic Antarctic (large or small) diatom species.

Here we use an alternative approach, addressing some of the above raised gaps in knowledge. Unialgal cultures of truly oceanic Antarctic diatoms (large and small) served as the ultimate indicators of biological availability of Fe in natural Southern Ocean waters. Estimates of the maximum specific growth rates (μ_{max}) and half-saturation values (K_m) for growth in response to Fe are reported. Given the uncertainties on the chemical form(s) accessed by the algae, we deliberately choose Fe_{diss} as master variable, not because we assume that (all) Fe_{diss} is actually taken up, but as the sum of all possible available iron in seawater. For the sake of comparison with existing literature data, Fe^{3+} values are also given. With the Fe^{3+} concentrations, a further comparison to Fe' (all inorganic Fe species) concentrations is possible by using the inorganic side reaction coefficient α_{inorg} , 10^{10} ($[\text{Fe}'] = 10^{10} \times [\text{Fe}^{3+}]$) (Millero 1998).

Material and methods

Antarctic seawater was taken directly from a tubing inlet on a "torpedo" (de Jong et al. 1998). The torpedo was towed adjacent to the research vessel *Polarstern* during expedition ANT XVI/3 (18 March–10 May 1999). The water pumped from the torpedo was led through an in-line filter cartridge (nominal size cut off 0.2 μm) into 2-liter polycarbonate bottles, mixed, and then distributed over the incubation vessels (250- or 100-ml polycarbonate square bottles). All handling of the experimental samples was done inside a clean (Class 100), temperature-controlled container. Time series incubations were set up of the large (60–80

μm per cell) chain-forming *C. dictyota* and the small (4–6 μm) unicellular diatom *C. brevis*. Using cool white fluorescence TL tubes 12:12 (*C. brevis*, intensity: 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) or 20:4 h light:dark (L:D) cycles (*C. dictyota*, intensity, 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) were maintained. The temperature was maintained between 0 and 3°C. *C. dictyota* cells were counted under the microscope using 5-ml settling chambers. Samples from *C. brevis* cultures were analyzed using an Epics XL flowcytometer, providing cell counts, cell size, and cellular autofluorescence. A suite of increasing Fe_{diss} concentrations was made from the natural 0.16×10^{-9} M background (no addition) to a maximum addition of 8×10^{-9} M. To determine the responses of *C. brevis* to decreased iron availability, experiments were conducted in seawater to which serial additions of desferri-ferrioxamine B (DFB) had been added. This fungal siderophore strongly complexes iron and thereby decreases (when in excess of the dissolved iron) the concentration of biologically available dissolved ferric hydrolysis species (Fe'), as well as probably the Fe bound to natural ligands (Wells 1999). From the time series, the period of exponential growth was used to calculate the daily growth rates at given Fe concentrations. The specific maximum growth rates and the half-saturation value for growth in relation to the Fe_{diss} (and Fe^{3+} , and thus Fe') concentrations were estimated by a SYSTAT nonlinear fitting using a least-square fit with the simplex algorithm (Wilkinson et al. 1992). For *C. dictyota*, these calculations were done using the measured background Fe_{diss} values. Using the measurements of the natural organic Fe-binding ligands, the concentrations of Fe' and Fe^{3+} were calculated. For *C. brevis*, this calculation was more complicated because not only the natural organic ligands had to be considered, but also the additions of the DFB. Taking into account the concentrations and conditional stability constants of the natural organic ligands and DFB and the concentration of actually measured Fe_{diss} , the response of *C. brevis* was first related to Fe^{3+} concentrations. This was then recalculated to reconstructed Fe_{diss} concentrations by ignoring DFB, taking into account only the abundance and affinity of natural ligands. In practice the following equations were used:

$$[\text{Fe}^{3+}] = \frac{[\text{Fe}_{\text{diss}}]}{\left(1 + \sum_{j=1}^2 K'_j [\text{L}_{\text{inorg}}^-]^a + K' [\text{L}_{\text{org}}^-] + K' [\text{L}_{\text{DFOB}}^-]\right)} \quad (2)$$

[recalculated Fe_{diss}]

$$= [\text{Fe}^{3+}] \times \left(1 + \sum_{j=1}^2 K'_j [\text{L}_{\text{inorg}}^-]^a + K' [\text{L}_{\text{org}}^-]\right) \quad (3)$$

in which $j = 2$ and a is 1 or 2 for $\text{Fe}(\text{OH})^{2+}$ and $\text{Fe}(\text{OH})_2^+$, respectively (Kuma et al. 1996; Millero 1998). The product of K' and the ligand concentration not bound to Fe is called α . The term $\text{Fe}^{3+} (1 + \sum K'_j [\text{L}_{\text{inorg}}^-])$ constitutes Fe' , which is the sum of all inorganic dissolved forms of Fe(III) in seawater, that is the free Fe^{3+} and the hydrolyzed species of $\text{Fe}(\text{OH})^{2+}$ and $\text{Fe}(\text{OH})_2^+$. The Fe^{3+} (in Eq. 2) was calculated using Newton's algorithm, then, Eq. 3 was used to estimate the Fe_{diss} concentration, assuming the presence of only nat-

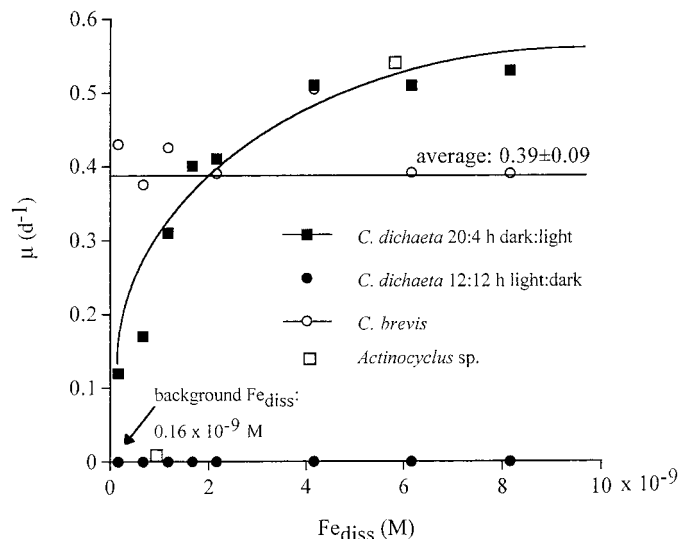


Fig. 1. Fe_{diss} ($\times 10^{-9}$ M) versus growth rates (d^{-1}) of *C. dichaeata* and *C. brevis*. Filtered water from station 174 ($69^{\circ}50'S$, $6^{\circ}40'E$), 50 m depth. Fe_{diss} , 0.16×10^{-9} M; NO_3 , 22.9×10^{-6} M; SiO_3 , 49.1×10^{-6} M; PO_4 , 1.44×10^{-6} M. *Actinocyclus* sp. was grown in natural Pacific ocean water after addition of 5×10^{-9} M Fe and at background Fe concentration ($\sim 1 \times 10^{-9}$ M) (Muggli and Harrison 1997).

ural organic ligands (ignoring the presence of DFB). For calculation of $K'[L_{\text{DFB}}^-]: L^-$ was derived from the known additions, K'_{DFB} of $10^{24.5}$ was used (E. Rue, UCSC, unpubl. data). The α_{org} of the natural ligands was derived according to speciation measurements during the cruise. More specifically, results from Sta. 182 ($70^{\circ}13'S$, $06^{\circ}07'W$, 60 m depth) were used for the calculation, i.e., $\log K = 22.1$, excess ligands of 0.5×10^{-9} M, leading to a $\log \alpha_{\text{org}} = 12.8$. For the inorganic hydrolysis species, the $\log \alpha_{\text{inorg}}$ of 10 was used (Kuma et al. 1996; Millero 1998). In practice, this means that 99.84% ($[\alpha_{\text{org}}/(\alpha_{\text{org}} + \alpha_{\text{inorg}})] \times 100$) of the iron is organically complexed, enabling calculation of Fe' (and thus Fe^{3+}) from Fe_{diss} and vice versa. Fe(II) , either by biologically mediated surface reduction or by photoreduction, was ignored throughout.

This calculation ultimately enabled comparison of the findings in DFB-treated seawater (*C. brevis*) with the $K_m(\text{Fe}_{\text{diss}})$ of *C. dichaeata* in natural seawater, and with literature results from EDTA-controlled cultures.

Results

The Monod curve of *C. dichaeata* yielded a $K_m(\text{Fe}_{\text{diss}})$ of 1.12×10^{-9} M (Fig. 1). The K_m with respect to Fe' was obtained via the measured $K_m(\text{Fe}_{\text{diss}})$. In the natural situation 99.84% of the dissolved iron was organically complexed, thus 0.16% was Fe' . Owing to the Fe additions, the Fe concentration at the $K_m(\text{Fe}_{\text{diss}})$ value has exceeded the organic ligand concentration (0.66×10^{-9} M). This leads to a calculation of a $K_m(\text{Fe}')$ of $(1.12 - 0.66 \times 10^{-9}) = 0.46 \times 10^{-9}$ M ($= K_m[\text{Fe}^{3+}]$ of 0.46×10^{-19} M). The "titration" of the natural organic ligands, by addition of increasing amount of Fe, caused differences in the calculated maximum growth

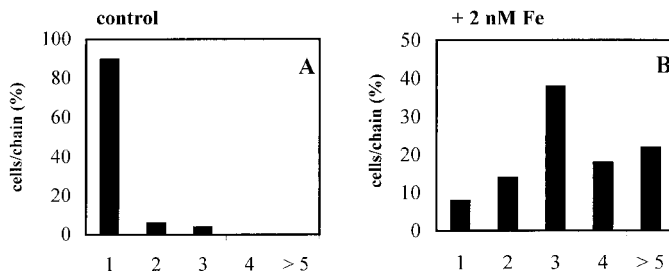


Fig. 2. Percentage of chains of *C. dichaeata* with 1, 2, 3, 4, or 5 or more cells per chain in (A) control and (B) 2×10^{-9} M iron addition determined after 8 d of growth (note the differences in scales). Filtered water from station 161 ($49^{\circ}57'S$, $19^{\circ}03'E$), 60 m depth. Fe_{diss} , 0.47×10^{-9} M; NO_3 , 23.9×10^{-6} M; SiO_3 , 15.8×10^{-6} M; PO_4 , 1.51×10^{-6} M.

rates in relation to Fe_{diss} or $\text{Fe}^{3+}/\text{Fe}'$. For Fe_{diss} a specific μ_{max} of $0.62 \pm 0.09 \text{ d}^{-1}$, for $\text{Fe}^{3+}/\text{Fe}'$ a specific μ_{max} of $0.53 \pm 0.16 \text{ d}^{-1}$ was calculated. In addition to iron, light played a major role. *C. dichaeata* grew only under long-day light conditions (20:4 h L:D period, $30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Growth of *C. dichaeata* was stopped at $80 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the 12:12 h L:D regime (Fig. 1). Changes in the iron concentration also caused remarkable changes in cell morphology of *C. dichaeata*. In incubations replete with iron (with water from a different station than the other experiments), the number of cells per chain was ~ 3 to ~ 5 , whereas there were generally single cells in the control experiments without added Fe (Fig. 2).

The small diatom *C. brevis* maintained a specific growth rate of $0.39 \pm 0.09 \text{ d}^{-1}$ and was not stimulated in growth upon addition of Fe. Even in seawater with the lowest, ambient Fe_{diss} concentration of 0.16×10^{-9} M growth was unaffected (Fig. 1). The treatment with DFB resulted in progressively lower growth rates in the *C. brevis* cultures (Fig. 3). The response to DFB was fully reversible by addition of excess Fe, verifying that the treatment only affected the biological availability of Fe (Fig. 3).

Using nonlinear fit of the data from the incubations in

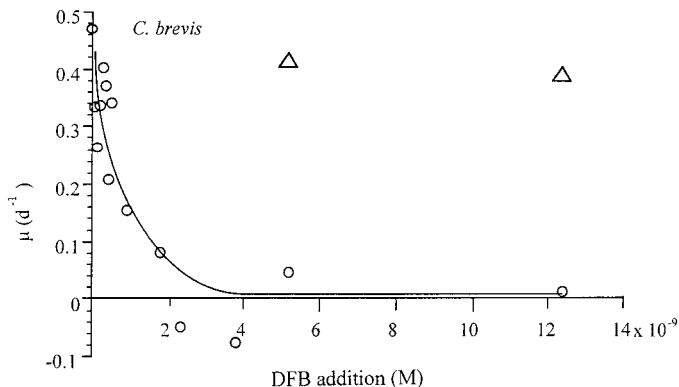


Fig. 3. DFB ($\times 10^{-9}$ M) additions versus growth rates of *C. brevis*. Filtered water from station 174 (see above for characteristics). The triangles indicate restoration of growth rate (within 72 h) when 7×10^{-9} M and 14×10^{-9} M Fe were added to the 5.8×10^{-9} M and 12.2×10^{-9} M DFB cultures, respectively.

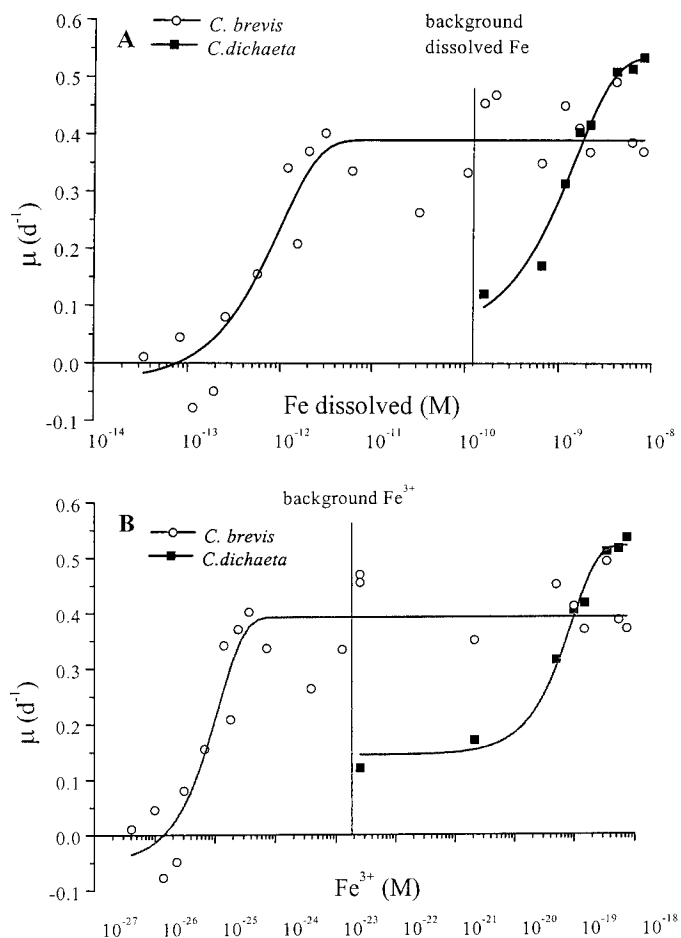


Fig. 4. Growth rates of *C. dichaeata* and *C. brevis* versus (A) Fe_{diss} and (B) Fe^{3+} . When calculating Fe^{3+} for the lowest Fe additions for *C. dichaeata* and *C. brevis*, the “titration” of the natural ligands by increasing amounts of Fe causes a relatively slow increase in Fe^{3+} as compared to the increase of Fe_{diss} . This results in a different nonlinear fit of the Monod curve to the data and thus different μ_{max} and K_m values with respect to Fe_{diss} and Fe^{3+} for *C. dichaeata*, but not for *C. brevis*. Filtered water from station 174 (see above for characteristics). The ambient Fe_{diss} concentration in the untreated, filtered seawater is indicated.

natural seawater with iron additions and those in natural seawater with DFB addition resulted in the calculation of a $K_m(\text{Fe}')$ value of $0.94 \times 10^{-15} \text{ M}$ ($= 0.94 \times 10^{-25} \text{ M Fe}^{3+}$) for *C. brevis*. Using the method of recalculating to Fe_{diss} (as explained in the Materials and Methods section, Eq. 3), a $K_m(\text{Fe}_{\text{diss}})$ of $0.59 \times 10^{-12} \text{ M}$ was deduced. With the recalculation of the *C. brevis* responses to Fe_{diss} and Fe^{3+} ($= \text{Fe}' \times 10^{-10}$), the data of both species can be compared (Fig. 4A,B), showing a 3 to 6 orders of magnitude difference in K_m values between *C. brevis* and *C. dichaeata*.

Discussion

The findings of *C. dichaeata* justify the choices of the parameters in the model simulations of the observed spring blooms of large diatoms at the Polar Front (de Baar et al. 1997) in an ecosystem model driven primarily by light con-

Table 1. Comparison of $K_m(\text{Fe}')$ and $K_m(\text{Fe}_{\text{diss}})$ of *C. brevis* and *C. dichaeata* with temperate diatoms as reported by Sunda and Huntsman (1995).

	$K_m(\text{Fe}')$	$K_m(\text{Fe}_{\text{diss}})$
<i>T. oceanica</i>	$4 \times 10^{-12} \text{ M}$	$2.5 \times 10^{-9} \text{ M}^*$
<i>T. pseudonana</i>	$81 \times 10^{-12} \text{ M}$	$50.6 \times 10^{-9} \text{ M}^*$
<i>T. weissflogii</i>	$68 \times 10^{-12} \text{ M}$	$42.5 \times 10^{-9} \text{ M}^*$
<i>C. dichaeata</i>	$0.46 \times 10^{-9} \text{ M}$	$1.12 \times 10^{-9} \text{ M}$ (this study)
<i>C. brevis</i>	$0.94 \times 10^{-15} \text{ M}$	$0.59 \times 10^{-12} \text{ M}$ (this study)

* Fe_{diss} concentrations were calculated assuming 99.84% organic complexation of Fe (as measured in this study).

ditions, as well as by four parallel nutrients (N, P, Si, Fe) (Lancelot et al. 2000). In this model, the big diatoms were found to be colimited by both light and iron ($K_m[\text{Fe}_{\text{diss}}] = \sim 1.2 \times 10^{-9} \text{ M}$), as opposed to the small phytoplankton, which was limited by light and grazing (Lancelot et al. 2000). Muggli and Harrison (1997) observed a similar μ_{max} upon addition of $5 \times 10^{-9} \text{ M}$ dissolved Fe to otherwise natural Pacific Ocean seawater in the large centric diatom *Actinocyclus* sp. Similarly, the assumed role of light is confirmed. *C. dichaeata* did only grow under typical long-day conditions (20 : 4 h L : D cycle) of the austral Antarctic summer. When comparing $K_m(\text{Fe}')$ and $K_m(\text{Fe}_{\text{diss}})$ values of *C. dichaeata* with those of temperate diatoms (Sunda and Huntsman 1995), it is clear that *C. dichaeata* has comparable $K_m(\text{Fe}_{\text{diss}})$ for growth as those for *T. oceanica*, but distinctly lower values than for *T. pseudonana* and *T. weissflogii* (Table 1). With respect to the $K_m(\text{Fe}')$, *C. dichaeata* has a considerably higher value than the temperate diatoms. Obviously, this large, truly oceanic diatom has a higher Fe requirement for growth than the other, relatively small, diatoms. The calculated $K_m(\text{Fe}')$ and $K_m(\text{Fe}_{\text{diss}})$ values for growth of *C. brevis* are about three to four orders of magnitude lower than those found for the other diatom species (Table 1), and even 6 orders of magnitude different with respect to the $K_m(\text{Fe}')$ of *C. dichaeata*. The Fe' concentrations (and K_m value deduced from this) as calculated here may be lower than the actual concentrations due to for example bioreductive dissociation of the Fe-DFB complex (Maldonado and Price 1999). Enhanced Fe' concentrations due to photoreductive dissociation is not likely as it has been reported that Fe-DFB to be stable to photochemical degradation (Wells 1999). The derived $K_m(\text{Fe}_{\text{diss}})$ of $0.59 \times 10^{-12} \text{ M}$ for *C. brevis* is at least one order of magnitude below the range of the lowest Southern Ocean Fe_{diss} surface water values ($30\text{--}500 \times 10^{-12} \text{ M}$) reported thus far (de Baar et al. 1999), and also below to the K_m of $\sim 30 \times 10^{-12} \text{ M Fe}_{\text{diss}}$ for other oceanic nanophytoplankton (Price et al. 1994; Gordon et al. 1998; Lancelot et al. 2000). It should be realized that the K_m value of *C. brevis* is strongly dependent on the affinity of DFB for Fe (here: K'_{DFB} of $10^{24.5}$). With other choices of the $\log K'_{\text{DFB}}$, the K_m values will show a proportional decrease or increase. From the growth response of *C. brevis*, however, it is clear that the $\log K'_{\text{DFB}}$ must be larger than 22.1, the value measured for the Fe-binding natural organic ligands in the experiments. Otherwise no strong growth response, even with relatively low DFB concentrations, would have been observed. In spite of uncertainties on the exact values of the

Table 2. Diffusive transport of chemical Fe-species to *C. dictyota* and *C. brevis*. The maximum diffusion rate (ρ) was calculated according to $4\pi rD[\text{Fe}]$, where D is the molecular diffusion coefficient: $D_{\text{Fe}^{3+}} = 2.8 \times 10^{-8} \text{ dm}^2 \text{ s}^{-1}$, $D_{\text{Fe}' } = 4.1 \times 10^{-8} \text{ dm}^2 \text{ s}^{-1}$, $D_{\text{Fe}_{\text{diss}} } = 0.5 \times 10^{-8} \text{ dm}^2 \text{ s}^{-1}$, all taken or derived from (Li and Gregory 1974; Völker and Wolf-Gladrow 1999), and extrapolated to 0°C according to the Stokes-Einstein relation. The radius of *C. dictyota* was $4 \times 10^{-4} \text{ dm}$, of *C. brevis* $2 \times 10^{-5} \text{ dm}$.

	K_m concentrations (M)		ρ (M d ⁻¹)		Potential diffusive supply atoms Fe d ⁻¹		Fe atoms needed d ⁻¹ *		Ratio supply : need	
	<i>C. dictyota</i>	<i>C. brevis</i>	<i>C. dictyota</i>	<i>C. brevis</i>	<i>C. dictyota</i>	<i>C. brevis</i>	<i>C. dictyota</i>	<i>C. brevis</i>	<i>C. dictyota</i>	<i>C. brevis</i>
Fe ³⁺	0.46×10^{-19}	0.94×10^{-25}	5.6×10^{-25}	5.7×10^{-32}	3.4×10^{-1}	3.4×10^{-8}	2.7×10^9	1.1×10^5	1.3×10^{-10}	3.1×10^{-13}
Fe'	0.46×10^{-9}	0.94×10^{-15}	8.2×10^{-15}	8.4×10^{-22}	4.9×10^9	5.1×10^2	2.7×10^9	1.1×10^5	1.8	4.4×10^{-3}
Fe _{diss}	1.12×10^{-9}	0.59×10^{-12}	2.4×10^{-15}	6.4×10^{-20}	1.4×10^9	3.8×10^4	2.7×10^9	1.1×10^5	5.4×10^{-1}	3.4×10^{-1}

* *C. brevis*: cell volume $50 \mu\text{m}^3$, half $\mu_{\text{max}} = 0.20 \text{ d}^{-1}$, $19 \times 10^{-6} \text{ M Fe}$ (liter cell volume)⁻¹ (Sunda and Huntsman 1995), and *C. dictyota*: cell volume $75,000 \mu\text{m}^3$, half $\mu_{\text{max}} = 0.30 \text{ d}^{-1}$, $200 \times 10^{-6} \text{ M Fe}$ (liter cell volume)⁻¹ (Sunda and Huntsman 1995).

Fe' and Fe³⁺ and the actual K'_{DFB} , it is obvious that *C. brevis* has an extremely low requirement for Fe and is not likely to be limited by iron under true oceanic conditions. Clearly this species would never experience abiotic growth-limiting factors of importance in the Southern Ocean. Light was a not significant parameter, as *C. brevis* grew even under typical short-day conditions (12:12 h L:D).

The above findings and internal consistency of various calculations suggest that excess of exogenous chelators DFB and EDTA do complex Fe in a consistent manner, such that the bound Fe is no longer available for uptake by the cell. This is encouraging to know, but in untreated natural seawater we still do not know which form(s) of Fe are taken up by the plant cell and/or are determining the rate of overall Fe uptake by the cell. Alternatively, the gradual response of *C. brevis* (Fig. 3) to addition of DFB can be interpreted as evidence that, as long as DFB is not in excess, some (species of) Fe is still available for growth. These observations support the findings by Hutchins et al. (1999), who demonstrated that, with molar ratios of Fe to DFB of 1:5 to 1:10, some Fe was available for *T. weissflogii*. With higher molar Fe:DFB ratios, as used in this study (Fig. 3), the Fe obviously becomes unavailable, thereby shutting *C. brevis* growth down. Some further insights in uptake of Fe might be gained by comparing the various above calculated species with the diffusive uptake rates by the cell necessary to support its growth (Table 2). Based on the ratio of ρ (the maximum diffusion rate) and the need for Fe, it is clear that only Fe' is enough to sustain growth of *C. dictyota* (Table 2). Fe species would not sustain growth of *C. brevis* or *C. dictyota* in any of the other cases (Table 2). It should be realized that these calculations were done for concentrations of Fe³⁺, Fe', and Fe_{diss}, at which μ is $(1/2)\mu_{\text{max}}$ and with different cellular iron requirements for the large and the small diatom. Taking a Fe_{diss} of $0.16 \times 10^{-9} \text{ M}$ (the measured background Fe concentration) shows that this is by far not enough to diffuse enough Fe to *C. dictyota* (potential diffusive supply 0.2×10^9 atoms Fe d⁻¹, need 0.9×10^9 atoms Fe d⁻¹). Still, *C. dictyota* is capable of slow growth at this Fe_{diss} concentration (Fig. 1), indicative of the fact that the assumed $200 \times 10^{-6} \text{ M Fe}$ liter cell volume actually may be an overestimate. In accordance with the growth of *C. brevis* at ambient Fe_{diss}, the diffusive supply (10.5×10^6 atoms Fe d⁻¹) is more than the need (0.2×10^6 atoms Fe d⁻¹).

The results of our study fit well in the size-class dependent

response of marine phytoplankton to iron limitation (Price et al. 1994). All intentional iron enrichment experiments in the world oceans worldwide, in situ or in bottles, have demonstrated a strikingly similar response, with the largest size classes of diatoms blooming (de Baar and Boyd 2000, and references therein). The availability of iron directly influences the structure of the ecosystem (exemplified in Figs. 1 and 4). Under low iron conditions, the food web is comprised of small diatoms (and other nanophytoplankton) growing at maximum growth rates. Biomass is controlled from the top down by zooplankton grazers. Upon episodic increased iron availability and under long-day light conditions of austral summer, the large oceanic diatoms (which have higher maximum growth rates and are less affected by zooplankton grazers) will dominate, increasing new production and export of fixed carbon (Lancelot et al. 2000). The differences in response to availability of iron can explain these phenomena and expand on the preceding explanation in terms of coastal (high Fe requirement) and oceanic (low Fe requirement) (Sunda et al. 1991; Sunda and Huntsman 1995). Large diatoms with high Fe requirements are significantly present in oceanic waters, and they are capable of pronounced population growth (Muggli and Harrison 1997; Scharek et al. 1999; Smetacek 1999). The low requirement of *C. brevis* for Fe can be seen as an adaptation to a low iron environment. Based on volume, *C. dictyota* is 1,500 times larger than *C. brevis* (estimated spherical dimensions, *C. dictyota* $75,000 \mu\text{m}^3$, *C. brevis* $50 \mu\text{m}^3$), and based on surface area *C. dictyota* is 100 times larger than *C. brevis*. These allometric considerations can explain part of the about 3 to 6 orders of magnitude differences of K_m values between *C. brevis* and *C. dictyota*. The remainder of the difference is unknown. It can be hypothesized that *C. brevis* has a more efficient uptake system, for example using surface reductases (Völker and Wolf Gladrow 1999), or that smaller cells need disproportionately less Fe in comparison with larger cells. The fact that *C. dictyota* is chain-forming and *C. brevis* is a single cell species is not considered as important: Pahlow et al. (1997) concluded that chain-forming diatom species with spaces between the cells (as is the case for *C. dictyota*) can obtain equal or even higher nutrient uptakes than solitary cells.

Important as the ecophysiological considerations and formalisms (Monod 1950) are, it should be kept in mind that life cycle strategies are as important. Success of a species is

in terms of biomass dominance in ecology, but also in terms of effective gene transfer in the evolutionary sense. Transfer of genetic information to the next generation can be achieved in a foodweb as well as in a once per year big bloom. The two diatoms species examined in this study represent separate, but equally successful evolutionary strategies: *C. brevis* is adapted to grow at slow growth rates at the low iron concentrations that normally exist in the HNLC regions of the Southern Ocean, whereas *C. dictyota* is adapted to bloom episodically following occasional high iron inputs (e.g., from pulsed atmospheric events). Both strategies appear to be equally successful in terms of species procreation and continuance through time. All this can be deduced without knowing the chemical form(s) of Fe taken up. The unialgal cultures thus accomplish a task as indicators of biologically available Fe, which presently is not feasible using analytical chemical techniques, given the low concentrations and the unknown kinetics.

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