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GROWTH RESPONSE OF ANTARCTIC PHYTOPLANKTON TO IRON ENRICHMENT

Shigenobu TAKEDA[1] and Kentaro WATANABE[2]

[1]*Central Research Institute of Electric Power Industry, 1646 Abiko, Chiba 270–11*
[2]*National Institute of Polar Research, 9–10, Kaga 1-chome, Itabashi-ku, Tokyo 173*

Abstract: Laboratory culture experiments were conducted with marine phytoplankton isolated from the Southern Ocean to determine the growth responses to iron enrichment. Batch cultures of ten diatom clones and two *Phaeocystis* clones were grown under various total iron concentrations (0.1–10.1 nM) using trace metal clean techniques. These Antarctic phytoplankton clones responded to iron enrichment by increasing their growth rates. When growth rates are plotted against the total iron concentration on a semilogarithmic graph, the growth curves may be grouped into three patterns. *Odontella weissflogii* showed a linear increase with total iron. *Chaetoceros dichaeta* (clone AA-B-40 and C-21), *Chaetoceros hendeyi, Nitzschia* sp. 1 and sp. 4, *Phaeocystis* sp. 1 and sp. 2 showed hyperbolic increase with total iron from 0.1 nM. *Corethron criophilum, Nitzschia* sp. 2, sp. 3, and sp. 5 showed hyperbolic increase with total iron from 0.2 nM, or a sigmoid pattern. There was no clear difference in their growth response to iron enrichment between isolates from the open ocean and marginal ice waters. The dependence of growth rates on cell volume size was also insignificant. Our results suggest that Antarctic phytoplankton may respond differentially and significantly to changes of iron level even within the natural fluctuation in the Southern Ocean.

1. Introduction

The Southern Ocean has high nutrient levels in surface waters while only low standing stocks of phytoplankton are found (EL-SAYED, 1984; HOLM-HANSEN, 1985). The phytoplankton biomass in the most productive ice-edge regions remains relatively modest even during austral spring (SULLIVAN *et al.*, 1988). Nutrient levels are still above depletion after phytoplankton blooms (HAYES *et al.*, 1984; SAKSHAUG and HOLM-HANSEN, 1984). A number of factors have been hypothesized to control phytoplankton productivity in the Southern Ocean (CULLEN, 1991): Nutrient effects (*e.g.*, ammonia, silica, iron and other trace metals), physical limitation (via irradiance, temperature, turbulent mixing and ice cover), and biological interactions (loss process such as herbivorous grazing and sinking). The relative importance of each factor undoubtedly varies with time and space in the vast Southern Ocean, and the reason for the high-nutrient, low-chlorophyll (HNLC) conditions remains unresolved.

Recently, MARTIN *et al.* (1989) hypothesized that iron deficiency might limit the growth of phytoplankton in a number of HNLC waters, including the Southern Ocean. There are several biochemical functions of iron in marine phytoplankton.

Iron-containing proteins are essential for photosynthetic and respiratory electron transport, nitrate and nitrite reduction, chlorophyll synthesis, and a number of biosynthetic and degradative reactions (GEIDER and LA ROCHE, 1994). Therefore, many cellular processes are likely to be affected simultaneously by iron-limitation. Although the idea of iron limitation in the Southern Ocean was suggested by GRAN (1931), the topic suffered from analytical problems for about half a century (DE BAAR, 1994). Accurate measurements of dissolved iron concentration in Antarctic surface waters, obtained by trace metal clean procedures, have demonstrated that in some oceanic habitats iron concentrations (0.1–0.2 nM) are unlikely to support high phytoplankton biomass (MARTIN *et al.*, 1990b; DE BAAR *et al.*, 1995, FITZWATER *et al.*, 1996). Reliable iron enrichment experiments with clean techniques also indicated that iron indeed stimulated growth of phytoplankton in Antarctic waters (DE BAAR *et al.*, 1990; MARTIN *et al.*, 1990a, 1991). Furthermore, it was found that the species composition of the plankton community changed in favor of diatoms when iron was added (BUMA *et al.*, 1991). However, phytoplankton growth in unenriched controls was also extensive, and the role of iron as a factor limiting phytoplankton production remains uncertain (DE BAAR *et al.*, 1990; BUMA *et al.*, 1991).

Broad differences among phytoplankton in their adaptations to iron availability have been identified by laboratory studies. BRAND *et al.* (1983) and BRAND (1991a) observed that growth rates of oceanic phytoplankton are less limited by low concentrations of iron than those of coastal species. However, cellular iron uptake rates are similar among the neritic and oceanic species when rates are normalized to cell surface area, and the ability of the oceanic species to outgrow its neritic congener at low iron concentrations is due almost entirely to its low cellular iron requirement (SUNDA *et al.*, 1991; SUNDA and HUNTSMAN, 1995). Laboratory studies have also shown that sub-optimum iron levels probably restrict the development of large phytoplankton cells more than small cells (HUDSON and MOREL, 1990). These differences indicate that iron will become an important environmental factor in those places or times when supply rates are below the level of iron demand by the plankton community. Therefore, changes in the rate of iron input and recycling in nutrient-replete regions of the Southern Ocean may result in species and cell-size shifts in phytoplankton assemblage. However, data on iron demand by Antarctic phytoplankton species are lacking. In addition to the importance of studying representative species of the Antarctic phytoplankton community, it is important to use local isolates because of the genetic differences among populations (BRAND, 1991b).

In the present study, we isolated clonal cultures of diatoms and other phytoplankton species from the Southern Ocean, and investigated the differences among species in their growth rates as a function of dissolved iron concentration.

2. Materials and Methods

Growth rates were measured as a function of dissolved iron concentrations for 12 clones of phytoplankton isolated from the Southern Ocean (Table 1). *Nitzschia* sp. 3 and sp. 4 were isolated from the permanent open ocean zone (55°S, absence of sea ice

Table 1. Origins of experimental cultures and mean volume per cell.

Species name	Clone	Location		Date	Cell volume* (μm^3 $cell^{-1}$)
Centric diatoms					
Corethron criophilum	C-1	63°00′S	90°07′E	6 Mar. 1993	210000
Odontella weissflogii	C-15	63°00′S	90°07′E	6 Mar. 1993	14000
Chaetoceros dichaeta	AA-B-40	62°47′S	112°05′E	21 Mar. 1992	6700
Chaetoceros dichaeta	C-21	63°00′S	90°07′E	6 Mar. 1993	4000
Chaetoceros hendeyi	C-17	63°00′S	90°07′E	6 Mar. 1993	400
Pennate diatoms					
Nitzschia sp. 1 (section Fragilariopsis)	C-19	63°00′S	90°07′E	6 Mar. 1993	1300
Nitzschia sp. 2 (section Fragilariopsis)	C-20	63°00′S	90°07′E	6 Mar. 1993	630
Nitzschia sp. 3	A-27	55°00′S	107°26′E	8 Dec. 1992	610
Nitzschia sp. 4 (section Pseudonitzschia)	A-28	55°00′S	107°26′E	8 Dec. 1992	72
Nitzschia sp. 5	B-35	64°41′S	38°24′E	17 Feb. 1993	49
Prymnesiophytes					
Phaeocystis sp. 1	AA-A-8	68°00′S	75°29′E	24 Feb. 1992	120
Phaeocystis sp. 2	SJ-H-34	64°49′S	150°04′E	12 Mar. 1992	84

*Cell volume was calculated from microscopic measurements of cell width and length. The mean cell volume was estimated from measurement of 50 sells.

cover), while other clones were from the marginal ice zone (63°–68°S). Although sterile techniques were used and bacteria were never apparent in the cultures, most were probably not axenic.

The experimental culture media were prepared from filtered (0.03 μm pore-size polyethylene hollow fiber membrane filter) Indian Ocean surface water enriched with nutrients (30 μM $NaNO_3$, 1 μM NaH_2PO_4, 30 μM Na_2SiO_3), trace metals (10 nM $ZnCl_2$, 10 nM $MnCl_2$, 1 nM $(NH_4)_6Mo_7O_{24}$, 1 nM $CoCl_2$, 1 nM $CuCl_2$), and vitamins (0.04 nM Vitamin B_{12}, 0.2 nM Biotin and 30 nM Thiamin HCl). The major nutrient stock solutions were treated with Chelex-100 resin to remove trace metal contaminants (MOREL *et al.*, 1979; PRICE *et al.*, 1988/1989). Background iron concentration in the medium before Fe enrichment was 0.1 nM, measured by chelating resin concentration and chemiluminescence detection methods (OBATA *et al.*, 1993). The media also received addition of various levels of FeEDTA: none, 0.1, 1, 10 nM. Total iron concentration (0.1, 0.2, 1.1 and 10.1 nM) in the media was computed from the sum of the concentration of added FeEDTA and the background iron concentration. Na_2EDTA was added at a final concentration of 100 nM to buffer the free ion concentration of iron and other trace metal nutrients. Although iron uptake by phytoplankton is typically correlated with free hydrated Fe^{3+} in seawater containing synthetic chelators, it is the labile hydrolysis species comprising Fe(III)′ and Fe(II)′ that actually control uptake rates (WELLS *et al.*, 1995). However, recent findings (RUE and BRULAND, 1995) suggesting >99% organic complexation of iron in natural systems imply that photochemical or other redox mechanisms are necessary to provide a constant supply of iron. The media were sterilized by filtration using an acid-washed 0.2 μm Teflon filter. Cells were grown in acid-washed 25×90-mm

polycarbonate tubes with screw caps at 1±0.5°C under continuous light (33 $\mu E/m^2/s$). Experimental cultures were grown in batch culture and monitored by measuring *in vivo* fluorescence with a Turner 10-AU-005 fluorometer (BRAND *et al.*, 1983). Transfer of an inoculum from an exponentially growing batch culture to a new batch culture was repeated ~6 times well before the culture population depleted the nutrients in the medium. The exponential doubling rate of each sequential replicate was determined by the least-squares method of linear regression on the logarithmically transformed data. The maintenance of batch cultures in a constant condition would lead to steady state phytoplankton growth rate with sufficient acclimation time, although reproduction may not be completely constant (BRAND *et al.*, 1981).

3. Results

The growth rates of Antarctic phytoplankton were enhanced with iron enrichment (Figs. 1–3). Cultures with 1.1 nM total Fe showed growth rates 2.5–8.5 times higher than those with 0.1 nM Fe. The growth curves may be grouped into three patterns. *Odontella weissflogii* showed a linear increase with total iron, although the growth rate was not significantly different between 0.1 and 0.2 nM. *Chaetoceros dichaeta* (clone AA-B-40 and C-21), *Chaetoceros hendeyi, Nitzschia* sp. 1 and sp. 4, *Phaeocystis* sp. 1 and sp. 2 showed hyperbolic increase with total iron from 0.1 nM. *Corethron criophilum, Nitzschia* sp. 2, sp. 3, and sp. 5 showed hyperbolic increase with total iron from 0.2 nM, or a sigmoid pattern. The second and third groups consist of cells with various sizes from different habitats (Table 1).

Nitzschia sp. 3 and sp. 4 were isolated from the permanent open ocean zone, where dissolved iron concentration is commonly at subnanomolar level because of the low supply rate of iron from the terrestrial source. These oceanic isolates might be adapted to a low iron environment and be expected to grow faster than other isolates at low iron concentrations. However, the growth rate of *Nitzschia* sp. 3 was near zero at 0.1 nM total Fe and did not show significant response to an increase in total Fe from 0.1 to 0.2 nM. Total iron concentration of 10.1 nM Fe only supported low maximum growth rates of *Nitzschia* sp. 3 of 0.19±0.03 doubling d^{-1} (n=5). On the other hand, *Nitzschia* sp. 4 exhibited the highest growth rate among clones examined at total iron from 0.1 to 10.1 nM. The maximum rate observed at 1.1 nM Fe was 0.65±0.05 doubling d^{-1} (n=6). This rate was 72% of the maximum doubling rate at 1°C calculated with EPPLEY's (1972) formula. The growth rates of other clones isolated from the marginal ice zone fell between those of *Nitzschia* sp. 3 and sp. 4.

At high total iron concentrations of 1.1 and 10.1 nM (Fig. 4), the growth rate tends to increase with decrease in cell volume. However, the result of regression analysis on this inverse relationship is not significant, probably due to relatively low growth rates of large size *Odontella weissflogii* and medium size *Nitzschia* sp. 2 and sp. 3 as compared to other clones. In contrast, no consistent trend in effects of cell volume on growth rate was observed at total iron concentrations of 0.1 and 0.2 nM.

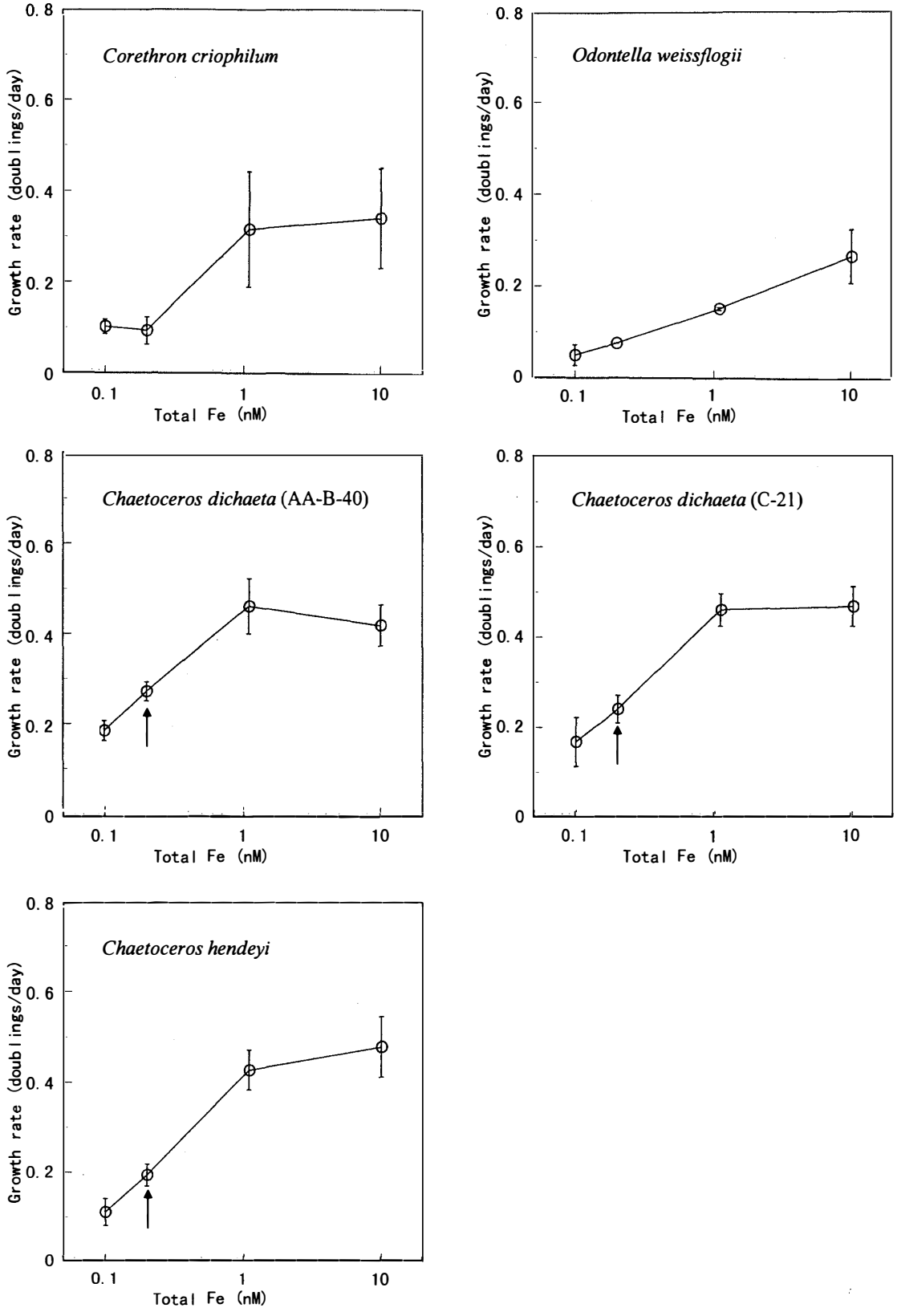

Fig. 1. Mean (±SD) growth rates of centric diatoms as a function of total dissolved iron concentration in culture media. Cases which showed significant increase from 0.1 to 0.2 nM Fe are indicated by arrows (t-test, $P<0.05$).

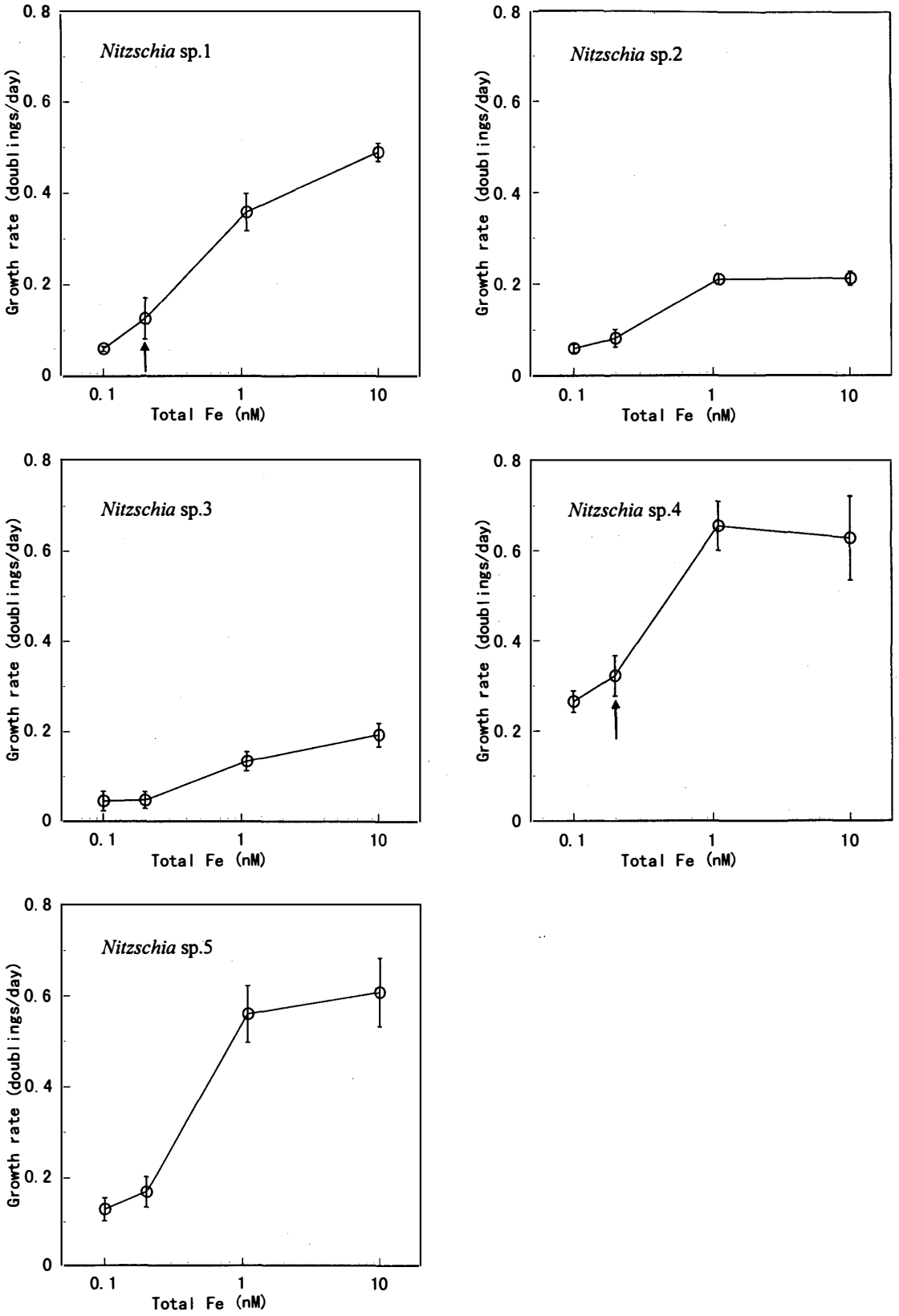

Fig. 2. Same as Fig. 1 but for pennate diatoms.

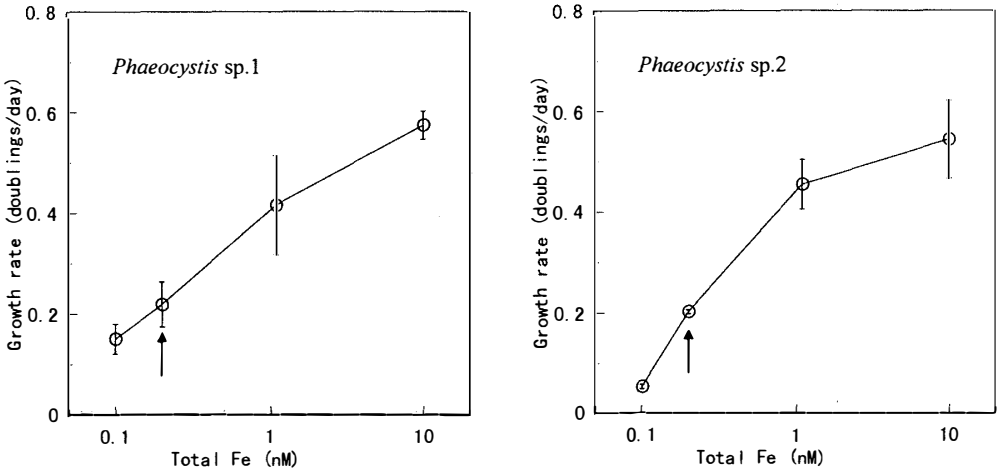

Fig. 3. Same as Fig. 1 but for prymnesiophytes.

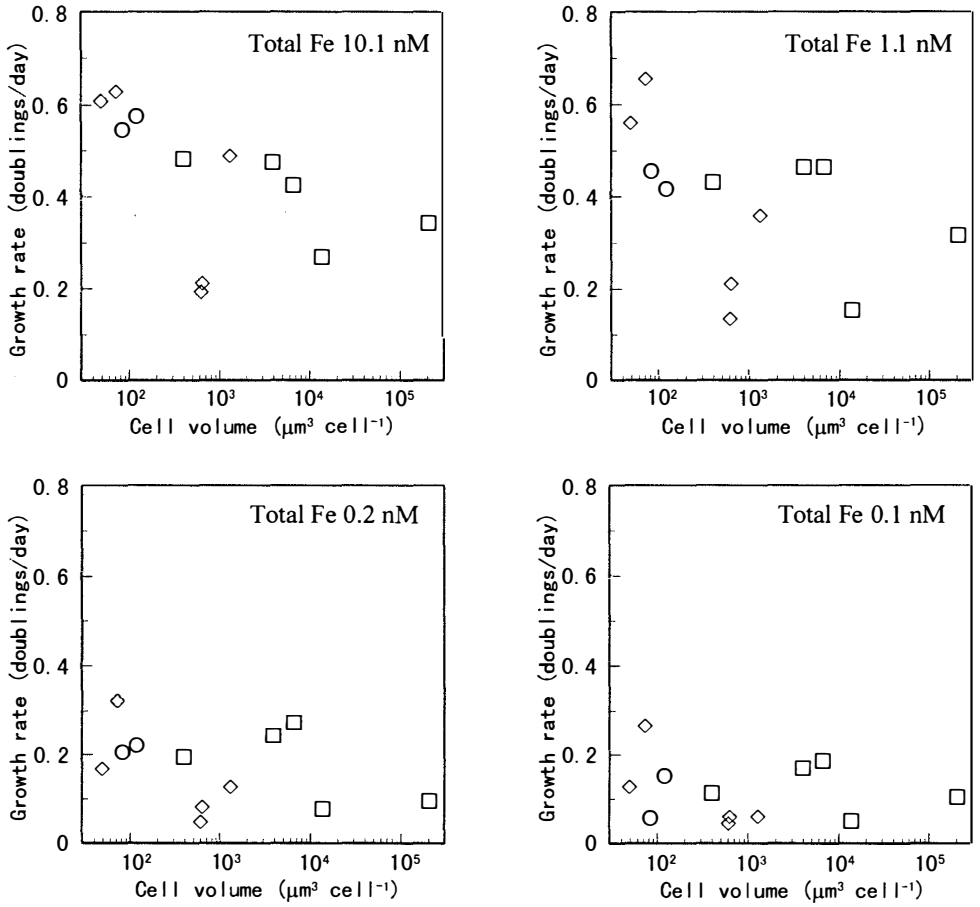

Fig. 4. Relationships between mean phytoplankton growth rate in culture media with various total concentrations of Fe and mean cell volume for centric diatoms (□), pennate diatoms (◇), and prymnesiophytes (○). Data for total concentration of 10.1 nM Fe (upper left), 1.1 nM Fe (upper right), 0.2 nM Fe (lower left), and 0.1 nM Fe (lower right).

4. Discussion

Antarctic phytoplankton clones, in their growth response to iron enrichment, show three different patterns of growth curves. First, linear increase with total Fe was observed for large size *Odontella weissflogii*, indicating that this clone needs rather high concentration of iron (>10 nM) for maximum growth. Second, hyperbolic increase with total Fe from 0.1 nM was observed for three *Chaetoceros* clones, *Nitzschia* sp. 1 and sp. 4, and two *Phaeocystis* clones. A hyperbolic curve is a usual growth response of phytoplankton to iron enrichment as observed in a previous study (SUNDA and HUNTSMAN, 1995). These clones have the capability to respond to increase in total iron from 0.1 to 0.2 nM, although there are no comparable experimental data on growth response at such extremely low iron concentrations. Neither habitat trend nor cell size could explain this group, because it includes a wide range of small and large size cells (84–6700 μm^3 $cell^{-1}$) from oceanic and marginal ice waters. Third, hyperbolic increase with total Fe from 0.2 nM or sigmoid pattern was observed for *Corethron criophilum, Nitzschia* sp. 2, sp. 3 and sp. 5. The feature of habitat trend and cell size in this group is as varied as in the above second group. Species in this group failed to increase their growth rate at 0.2 nM Fe, suggesting that these clones are less sensitive to subnanomolar increase in iron concentration than the other groups. In the opposite sense, these clones were still able to grow at 0.1 nM Fe with rates similar to those found at 0.2 nM. SUNDA and HUNTSMAN (1995) observed the sigmoid shape in the growth curve for *Prorocentrum minimum* under iron deficient condition. They considered the sigmoid growth pattern to result from the enhanced iron uptake via the release of siderophores (iron-specific organic ligands). However, little is known about diatoms concerning the production and use of siderophores. Other possibilities include the ability to reduce their metabolic iron requirement at 0.1 nM Fe. Under iron-stress conditions, for example, the iron-sulfur protein ferredoxin is replaced by non-metalloprotein flavodoxin in diatoms (LA ROCHE *et al.*, 1993). Therefore, the observed difference in the growth pattern could possibly be related to variations in the uptake mechanism and in the metabolic iron requirement. The effect of the taxonomic difference on growth patterns is not clear, because of the small number of species used in our experiments.

To understand the competitive interactions in a mixed phytoplankton assemblage, it is worthwhile to compare the growth rate as well as the growth curve pattern. At low iron levels where growth rates of neritic phytoplankton species decrease to near zero, oceanic species are still able to grow at near maximum rates (BRAND *et al.* 1983; BRAND, 1991a; SUNDA *et al.*, 1991; SUNDA and HUNTSMAN, 1995). Consistently, the oceanic clone *Nitzschia* sp. 4 showed the highest growth rate among all clones examined at 0.1–10.1 nM Fe. When 12 clones examined in this study compete for iron under the same mortality rate (by advection, diffusion, sinking and grazing), the fast growing *Nitzschia* sp. 4 is predicted to competitively displace all other clones at equilibrium. On the other hand, another oceanic clone, *Nitzschia* sp. 3, exhibited the lowest rates. The growth rates of other clones from marginal ice zone ranged between those of the above two oceanic clones. Thus, the habitat-related trend in the capability to grow at low iron levels is not clear in our experiment comparing isolates

from the open ocean zone and others from the marginal ice zone. The marginal ice zone is one of the highly productive regions in the Southern Ocean during the austral spring (SULLIVAN *et al.*, 1988). Melting of sea ice cover, which accumulates iron input from aerosol, might also contribute to the development of marginal ice zone blooms (DE BAAR *et al.*, 1995). Nevertheless, poor growth dependence on habitat in our experiments indicates that the seasonal iron input from sea ice may not have influenced the evolution of phytoplankton nutritional adaptations and the distribution of neritic and oceanic species.

The cell size is also closely related to the growth rate, because the growth rate is the ratio of the uptake rate to the cellular iron quota. The cellular iron quota increases or decreases as the cube of the cell radius, whereas the membrane area available for uptake changes as the square of the radius. An obvious way for phytoplankton to grow at extremely low iron concentration is to become smaller (HUDSON and MOREL, 1990; SUNDA and HUNTSMAN, 1995). However, the growth rates tend to depend on cell size only at iron concentrations of 1.1 and 10.1 nM (Fig. 4), possibly because of the variations both in the uptake mechanism and the metabolic iron requirement at lower iron concentrations. The relatively low growth rates of *Odontella weissflogii* and *Nitzschia* sp. 2 and sp. 3 compared to other similar size clones imply the presence of co-limitation by iron and other factors. SOMMER (1986) reported high half-saturation constants of silicate-limited growth for five Antarctic diatoms ranging from 6 to 89 μM. For example, the half-saturation constant for *Corethron criophilum* at 0°C (60 μM) is higher than the initial silicate concentration in our experimental seawater (30 μM). Therefore, some diatoms, at least *Corethron criophilum*, most likely experienced simultaneous effects of Fe and Si in our culture medium.

Recent measurements have shown that concentrations of dissolved iron in the Southern Ocean surface waters are in the range of 0.08–8 nM (Table 2). Although the biological availability of operationally defined dissolved (<0.2 or 0.4 μm) fraction to phytoplankton is not clearly elucidated (WELLS *et al.*, 1995), our results suggest that Antarctic phytoplankton may respond differentially and significantly to the changes of iron level within the above natural fluctuation. Iron limitation in the surface waters is thought to play an important role in reducing phytoplankton growth in regions where iron levels are extremely low, like the Ross Sea (FITZWATER *et al.*, 1996).

Table 2. Dissolved iron concentration in surface waters of the Southern Ocean.

Location	Fe (nM)	Reference
Ross Sea	0.08~0.12	FITZWATER *et al.*, 1996
Drake Passage	0.16	MARTIN *et al.*, 1990b
Southern ACC* (51~58°S, 6°W)	0.17~0.6	DE BAAR *et al.*, 1995
Antarctic divergence (64~65°S, 141°E)	0.17~0.8	TAKEDA, 1996
Polar front (47~50°S, 6°W)	1~3.5	DE BAAR *et al.*, 1995
Weddell–Scotia Sea	2~8	NOLTING *et al.*, 1991
Gerlache Strait	4.7~7.4	MARTIN *et al.*, 1990b

*ACC: Antarctic circumpolar current.

However, it is difficult to extrapolate results of the laboratory culture experiments to *in situ*. Both the variety of iron speciation in seawater and the diversity of iron uptake strategies may contribute to the difficulty. A more precise understanding of these two factors is necessary before we can quantify the role of iron in phytoplankton dynamics. In addition, the interactions between iron and other limiting factors remain as unresolved issues in understanding phytoplankton growth response to iron in a natural ecosystem.

In conclusion, the difference among Antarctic phytoplankton clones in their growth response to iron enrichment is identified as linear, hyperbolic and sigmoid patterns of growth curves. The difference in shape of growth curves is attributable to factors other than the habitat trend and the cell size. The habitat-related trend in the growth rate is not clear between isolates from the open ocean zone and others from the marginal ice zone. The dependence of growth rate on cell volume size is also not significant, particularly at low iron concentration. Natural fluctuations of iron level in Antarctic surface water would induce definite changes in phytoplankton growth.

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