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Effect of high iron concentration enrichment on the phytoplankton in the Prydz Bay

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Abstract Shipboard iron enrichment phytoplankton incubations were carried out in the Prydz Bay, Antarctic, in January through to March 2002. Waters for the three incubations (Exp 1, 2 and 3) were collected from 20 m depth in three stations (St. iv-1, -1 and -5), respectively. Although the nutrient concentrations in the surface waters of the three stations were consistently high, the Chl *a* concentrations varied considerably. Chl *a* concentrations in the 20 m depth of St. iv-1 and -1 were 0.13-0.17 $\mu g \cdot dm^{-3}$ and 0.20-0.26 $\mu g \cdot dm^{-3}$, respectively, while this figure was 2.35-2.65 for St. -5. There were six levels of enriched iron concentrations (control 5, 10, 20, 40 and 80 nM) in Exp 1 (6-29th, January) while three enriched iron levels (control 10 and 40 nM) were arranged in Exp 2 and 3 (both were from 20th February to 4th March). The iron enrichments stimulated the phytoplankton growth and nutrient drawdown in Exp 1 and Exp 2. In Exp 3, phytoplankton growth and nutrient drawdown were at nearly the same rate in the control and iron enriched bottles. In Exp 1, Chl *a* concentrations in the bottles with 20, 40 and 80 nM iron enrichments grew exponentially to 40-43 $\mu g \cdot dm^{-3}$ on the 17th, 17th and 19th day, respectively, with a growth rate of 0.36-0.38 d^{-1}. Chl *a* concentration in the bottle enriched with 10 nM iron reached its peak (19.35 $\mu g \cdot dm^{-3}$) on the 23rd day (growth rate 0.27 d^{-1}). Phytoplankton growth rates in the control bottle and the bottle enriched with 5 nM iron were 0.13 and 0.16 d^{-1}, respectively. In Exp 2, the Chl *a* growth rates were 0.13, 0.32 and 0.40 d^{-1} in the control bottle and bottles with 10 and 40 nM iron enrichments, respectively. It seems that 10 nM iron enrichment was not enough to stimulate the phytoplankton to reach their maximum growth rate. The result that the phytoplankton < 10 μm bloomed in Exp 1 and 2 was controversial to the "Ecumenical Iron Hypothesis" of Morel *et al*. (1991) that upon enrichment of iron, phytoplankton > 10 μm would grow faster than phytoplankton < 10 μm.

Key words iron, enrichment incubation, Prydz Bay

1 Introduction

For the past 15 years, much attention of oceanographers has been focused on the role of iron in limiting phytoplankton productivity in the high-nutrient, low-chlorophyll (HNLC) regions. On board ship iron enrichment incubations have been carried out in the three well known HNLC regions: notably equatorial Pacific Ocean, subarctic Pacific Ocean and Southern Ocean (see references in Table 1). The *in situ* iron enrichment experiments have been accomplished in the equatorial Pacific (IronEx1 in 1993 (Wells 1994) and IronEx2 in 1996 (Coale *et al*. 1996)) and the Southern Ocean (SOIREE (Chisholm 2000)). These studies showed that several nM iron addition could stimulate phytoplankton growth and deplete macronutrients in the HNLC regions.

Table 1 Iron enrichment incubation carried out in the HNLC regions

References	Study sites	Enriched iron / nM
Martin and Fitzwater 1988	North-east Pacific subarctic	1, 5, 10
Coale 1991	Subarctic Pacific	0.89
Boyd *et al*. 1996	NE subarctic Pacific	2, 4
Schmidt and Hutchins 1999	NE Pacific (6-8 daylight hours)	1
Maldonado and Price 1999	Subarctic Pacific (25 hours)	2, 20
DiTullio *et al*. 1993	North Pacific Ocean	1
Zettler *et al*. 1996	Equatorial Pacific	2.5
Price *et al*. 1994	Equatorial Pacific	1
Price *et al*. 1991	Equatorial Pacific	1
Fitzwater *et al*. 1996	Equatorial Pacific	0.25, 2.5, 10, 1000
De Baar *et al*. 1990 and Buma *et al*. 1991	Weddell-Scotia Sea	10, 20
Timmermans *et al*. 1998	Pacific region of the Southern Sea (approximately along 90 °W)	2
Van Leeuwe *et al*. 1997 and Scharek *et al*. 1997	Atlantic region (6°W)	2
Takeda 1998	64.2 °S, 140.7 °E	1.2
Olsen *et al*. 2000	Pacific region of the Southern Ocean 160°-180°W	0.2, 0.5, 1, 2.5
Martin *et al*. 1991 and Martin *et al*. 1990	Ross Sea	5

In the aforementioned iron enrichment incubations, the enriched iron concentrations were usually lower than 10 nM (Table 1). The study of La Roche *et al*. (1995) showed that flavodoxin, which is indicative of iron stress in diatoms endemic in the HNLC water, was high in concentration in a diatom (*Phaeodactylum tricornutum*) batch cultures enriched with > 10 nM iron. A considerable concentration happened in the cultures with iron concentration larger than 100 nM. The growth rate of this batch culture did not reach its peak when the enriched iron concentration was 100 nM. (Fig. 2 in La Roche *et al*. 1995). It is plausible to ask if the natural phytoplankton assemblage achieve higher growth rate when enriched with > 10 nM Fe?

Among iron limitation studies in the Southern Ocean, those in the Indian region of the Southern Ocean have received comparatively little attention (Table 1). In this paper, we report the high iron concentration enrichment experiments in the Indian region of the Southern Ocean.

2 Materials and methods

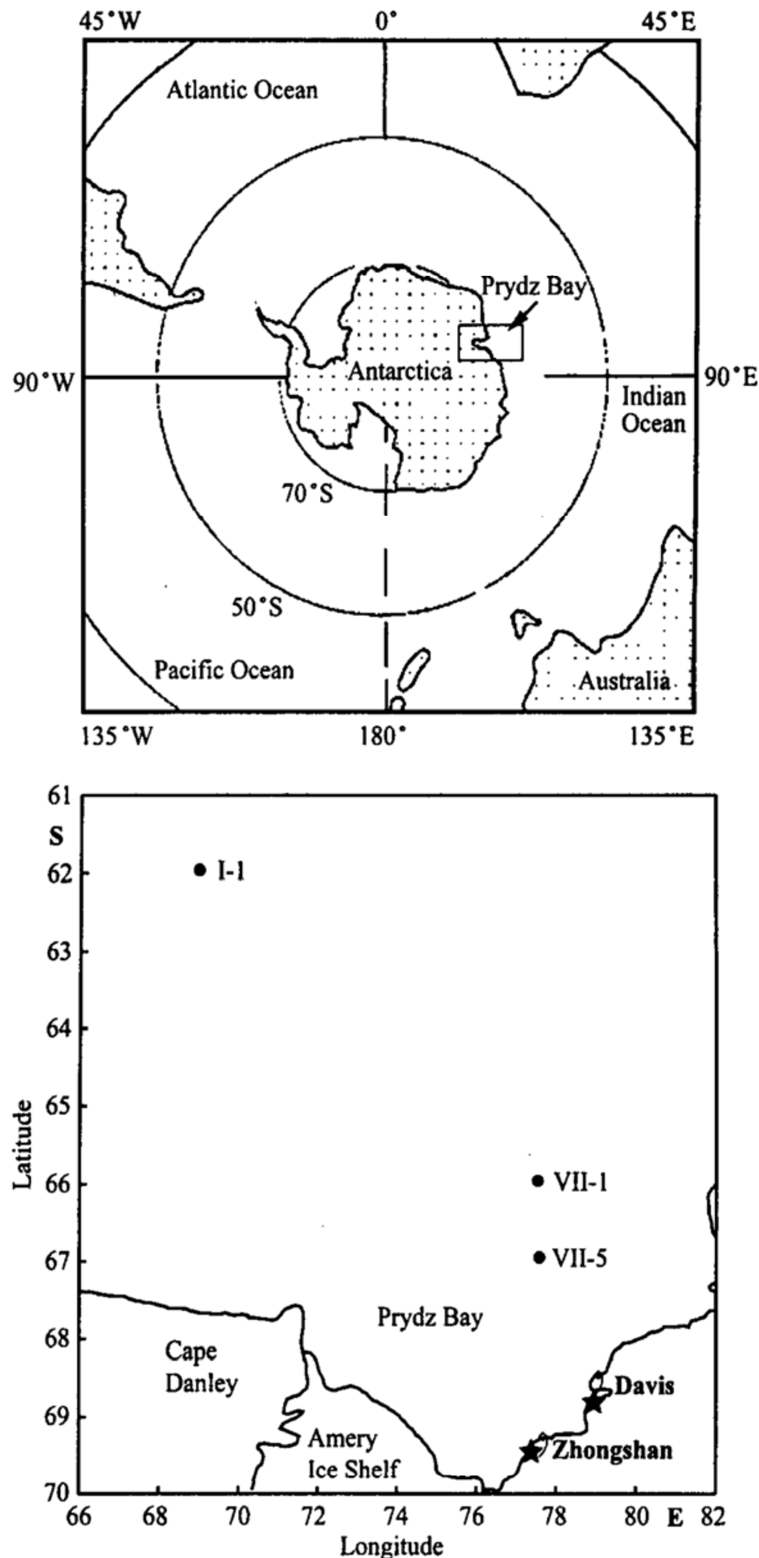

Fig. 1. The positions of the stations in the Prydz Bay.

The iron-enrichment incubations were carried out on board R. V. “Xuelong” during the 18th Chinese Antarctic Expedition (December, 2001 to April, 2002). Three iron enrichment experiments (Exp 1, 2 and 3) were carried out at three stations (St. iv-1, St. -1 and St. -5), respectively. The positions of the stations were shown in Fig. 1. St. iv-1 (68°30′E, 62°S) was located at the pelagic zone. There was sea ice cover in the sea south of the longitude 65 °S before 10th February. After the sea ice broke and open water appeared, the incubations at St. -1 and -5 were carried out. St. -1 (76°45′E, 65° 59′S) was at the outer part of Prydz Bay while St. -5 (76°45′E, 66°56′S) was at the inner part of the Prydz Bay.

At every station, waters for the incubation were collected using pre-cleaned 10-L Niskin bottles at the depth of 20 m. The underwater part of the ship is 9 m in depth. Six (Exp 1) or three (Exp 2 and 3) 18-L polycarbonate bottles, which were socked in HCl (1∶5 v/v) for 6 h, were rinsed using large amount of sea-water. The waters were directly poured into the bottles in a Class-100 bench to minimize contamination. A very small volume (0.1-1.4 cm^3) of $FeSO_4$ solution was injected into the bottles by pipetter. The final iron concentration series (the original iron concentrations in the seawater were not considered) are as listed in Table 2. Every bottle was covered by three plastic zip bags to avoid contamination. Surface water flowing system was used to keep the temperature.

Table 2 Initial conditions of the three iron enrichment incubations

Exp No.	Station	Depth /(m)	Beginning time (2002)	End time (2002)	Iron Enriched (nM)* •	Temperature/(°C) at 20 m depth	Salinity At 20 m depth
Exp 1	iv-1	4600	6 Jan.	29 Jan.	0, 5, 10, 20, 40, 80	− 0.12	33.79
Exp 2	-1	2650	20 Feb.	4 Mar.	0, 10, 40	− 0.07	33.66
Exp 3	-5	900	20 Feb.	4 Mar.	0, 10, 40	− 0.37	33.72

* The original iron concentrations in the seawater were not considered

Water samples (About 300 cm^3) were taken from every bottle at the beginning and every 2-3 days later on. Samples of 250 cm^3 or 150 cm^3 were filtered through GF/F filters. The filters and filtrates were used to measure Chlorophyll a (Chl *a*) and nutrients concentrations, respectively. The filters were extracted in 90% acetone and kept in − 20 ℃ in dark. Chl *a* concentrations were determined according to the fluorescence reading using a Turner designed Fluorometer. The filtrates were fixed by $HgCl_2$ and taken back to laboratory. The nutrients concentrations were measured using Automatic Nutrients Analyzer.

The size-fractioned Chl *a* concentrations were determined at the beginning and end of the three incubations and the last several samplings of Exp 1. In this case, the samples were filtered first through a nylon mesh (mesh size 20 μm), and then through the GF/F filters. The mesh and filters were used to determine the concentrations of Micro Chl *a* and Nano+ Pico Chl *a*, respectively, as above.

When the incubation finished, one liter water from each bottles were fixed with Lugol’s solution (final concentration 1%). The phytoplankton assemblages were examined and photographed using an Olympus microscope in laboratory.

3 Results

3.1 The growth of Chl a and decline of nutrients

The initial conditions of the three experiments were listed in Table 2 and 3. There was high nutrient concentrations and low Chl *a* concentrations (< 0.3 μg•dm^{-3}), a typical HNLC (High Nutrients, Low Chl *a*) condition, in St. iv-1 and -1. Although nutrient concentrations in St. -5 were also high, the Chl *a* concentration was much higher (> 2.3 μg•dm^{-3}).

In Exp 1, Chl *a* concentration in the control and the 5 nM iron enrichment bottles changed very little (0.6 and 1.7 μg•dm^{-3} respectively). Nutrients showed no obvious drop in these bottles. The Chl *a* concentrations in the bottles with 20, 40 and 80 nM iron enrichment reached 40-43 μg•dm^{-3} on the 17th, 17th and 19th day, respectively. The concentrations of nitrate, nitrite and phosphate dropped to nearly zero at the same time. Chl *a* in the bottle of 10 nM iron enrichment grew slowly, and reached its peak of 19.4 μg•dm^{-3} at the end of incubation. Correspondingly, the nutrients concentrations in the 10 nM iron enrichment bottle fell slowly, and drop to near zero at the end (Fig. 2).

In Exp 2, Chl *a* concentrations in the iron-added bottles increased rapidly. Bottles with higher concentration iron enrichment showed higher Chl *a* growth rate. The final Chl *a* concentrations were 0.6, 6.1 and 13.1 μg•dm^{-3} in the control 10 nM and 40 nM iron enrichment bottles, respectively. The nutrients in the three bottles showed a slight growth on the 3 rd day. After that, the phosphorous and nitrate concentration in the iron-added bottles dropped rapidly. In the control bottle, phosphate and nitrate concentration remained unchanged or raised slightly and dropped at the last sampling. Silicate concentration showed no change during the incubation period. Nitrite in the 40 nM iron enriched bottle began to drop at the 8th day, while nitrate in the 10 nM iron bottle began to drop at the 10th day (Fig. 3).

In Exp 3, Chl *a* grew at about the same rate. The iron enriched bottles had a high Chl *a* concentration at the end of the incubation. Phosphate and nitrate concentration declined at about the same rate, while the high iron addition bottles witnessed a comparatively large decline. The silicate concentration dropped slower than in the experiment of St. I-1, which had a sharp decline in silicate concentration. The nitrite concentration increased firstly and then declined at the 8th or 10th day. The biggest drop of nitrite concentration happened in the control bottle (Fig. 4).

3.2 The size fractionated Chl a

In Exp 1, the ratios of Micro: Nano+ pico Chl *a* were determined at the beginning and after the 9 th day. This ratio was consistently low (lower than 1) in the 20, 40 and 80 nM iron enrichment bottles. The highest value (0.91) appeared on the 11th day in the bottle of 20 nM iron enrichment. From then on, ratios lower than 0.4 were observed in these three bottles (Fig. 5).

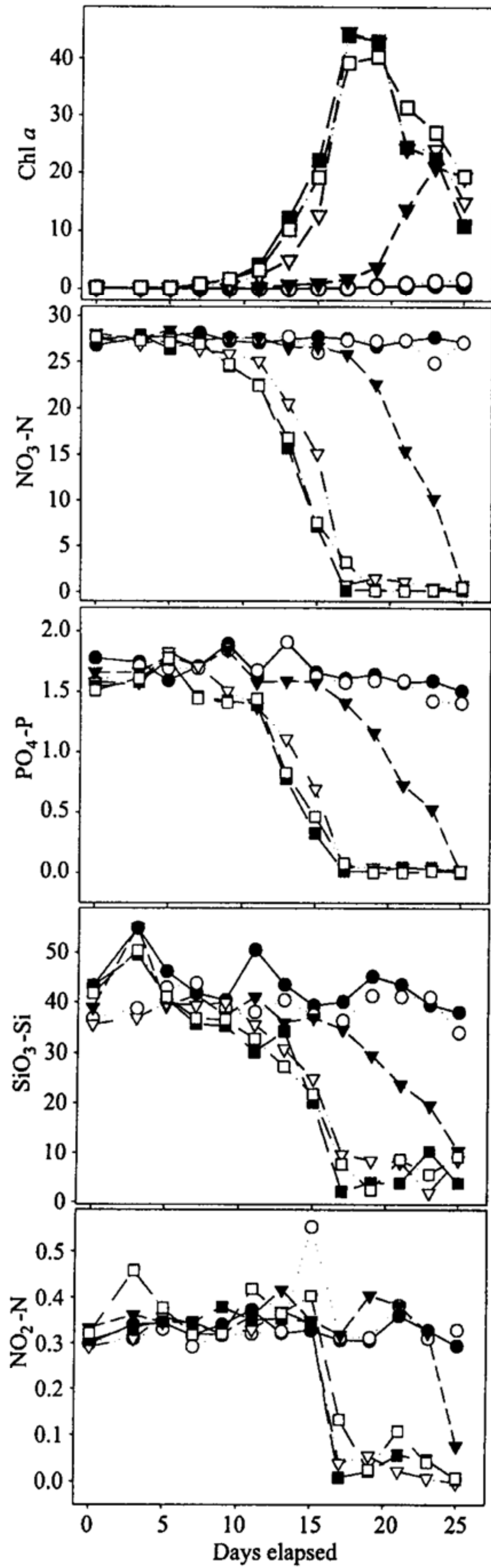

Fig. 2. The Chl *a* (μg·dm^{-3}) growth and nutrients (μM) drawdown of Exp 1 (●: control; ○: 5 nM enriched; ▼10 nM Fe enriched; •: 20 nM Fe enriched; ■: 40 nM Fe enriched; □: 80 nM Fe enriched).

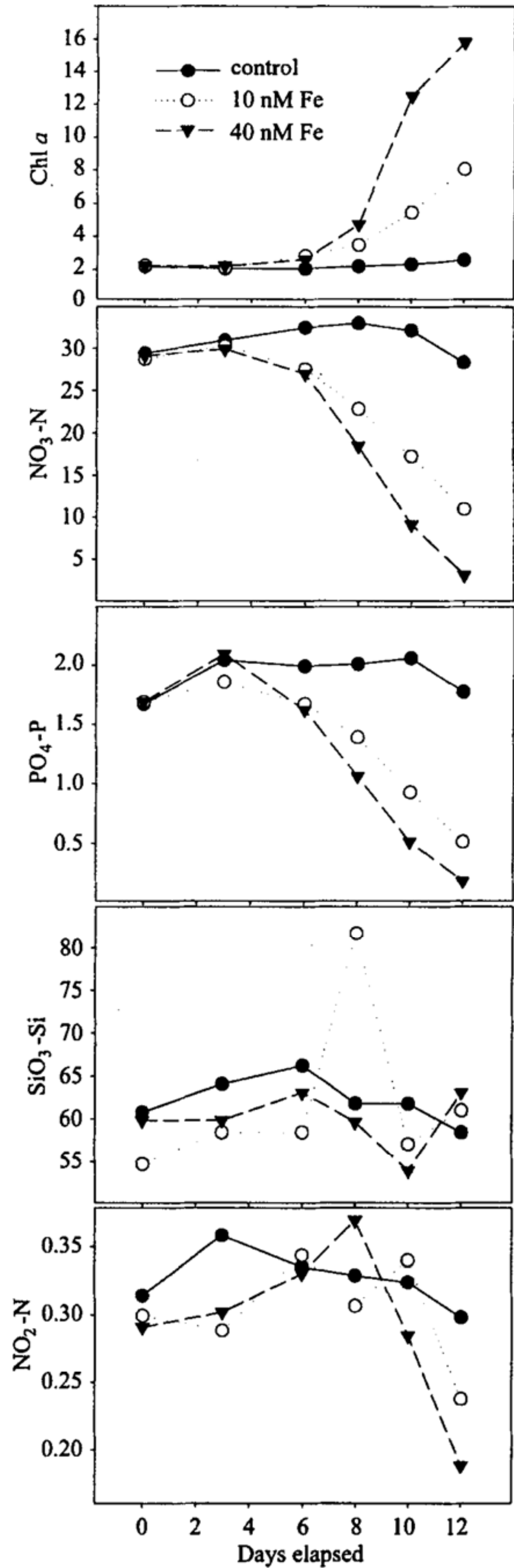

Fig. 3. The Chl *a* (μg·dm^{-3}) growth and nutrients (μm) drawdown of Exp 2.

Table 3. Chl *a* concentration ($\mu g \cdot dm^{-3}$) and nutrient concentrations (μM) in the incubation bottles at the beginning and end of the iron enrichment incubation (Exp 1, 2 and 3). The chl *a* growth rates (K) were calculated using exponential regression

	Iron added	—Initial Chl *a* and nutrient concentration—					—Chl *a* and nutrient concentrations at the end—					Chl *a* growth rate		—Nutrients absorbed—				Nutrient uptake ratio	
		Chl *a*	NO_3^-	PO_4^{3+}	SiO_4^{2+}	NO_2^-	Chl *a*	NO_3^-	PO_4^{3+}	SiO_4^{2+}	NO_2^-	K(d^{-1})	R^2	NO_3^-	PO_4^{3+}	SiO_4^{2+}	NO_2^-	Si: N	Si: P
Exp1	Control	0.14	26.79	1.78	43.30	0.30	0.63	27.17	1.51	38.05	0.29	0.13	0.61	0	0.27	5.25	0.01	infinite	19.44
	5	0.17	27.66	1.58	36.76	0.30	1.69	27.12	1.41	13.99	0.33	0.16	0.71	0.54	0.17	2.77	-0.33	5.13	16.29
	10	0.13	28.05	1.66	38.94	0.33	19.35	0.84	0.01	10.43	0.08	0.27	0.90	27.2	1.65	28.51	0.25	1.05	17.28
	20	0.14	27.50	1.58	35.57	0.29	44.64*	0.81*	0.05*	9.63*	0.04*	0.36	0.94	26.69	1.53	25.94	0.25	0.97	16.95
	40	0.15	27.34	1.54	43.30	0.31	44.08*	0.13*	0.01*	2.11*	0.01*	0.38	0.95	27.21	1.53	41.19	0.30	1.51	26.92
	80	0.15	27.66	1.51	41.81	0.32	40.35ζ	0.14ζ	0.00ζ	2.41ζ	0.02ζ	0.37	0.95	27.52	1.51	39.40	0.30	1.43	26.09
Exp2	control	0.21	29.39	1.68	60.82	0.31	0.59	28.36	1.78	58.45	0.30	0.13	0.40	1.03	-0.10	2.38	0.01	2.31	-23.80
	10	0.25	28.73	1.69	54.68	0.30	6.08	10.96	0.51	61.62	0.24	0.32	0.84	17.77	1.18	-6.34	-0.06	-0.36	-5.37
	40	0.23	29.10	1.69	59.83	0.29	13.82	3.14	0.18	63.10	0.19	0.40	0.90	25.96	1.51	-3.27	0.10	-0.13	-2.17
Exp3	control	2.65	23.19	1.18	41.22	0.25	29.35#	5.58	0.23	30.13	0.13	0.30	0.80	17.61	0.95	11.09	0.12	0.63	11.67
	10	2.35	22.85	1.21	49.24	0.21	48.08#	3.29	0.13	23.89	0.20	0.346	0.85	19.56	1.08	25.35	0.01	1.3	13.47
	40	2.41	24.29	1.24	45.28	0.21	43.63#	2.86	0.13	33.99	0.19	0.354	0.76	21.43	1.11	11.29	0.02	0.53	10.17

#: the 10th day; * the 17 th day; ζ: the 19th day.

The Micro: Nano+ pico Chl *a* in the 5 nM iron enrichment bottle was much higher (> 1. 1) than in other bottles, and showed peak (5. 6) on the 13th day. This ratio in the 10 nM iron enriched bottle and control bottle showed the same trend as in the 5 nM iron enrichment bottle, but with lower peaks (3. 6 and 2. 8 on the 13th day, respectively) .

The ratios of Micro: Nano+ pico Chl *a* in Exp 2 and 3 were determined at the beginning and the end of the experiments as shown in Table 4. At the beginning of Exp 2, Micro-Chl *a* concentration was only about 1/ 10 of the Nano+ Pico Chl *a* concentration. This ratio increased slightly in the iron enriched bottles at the end of the incubation. Micro-Chl *a* concentration in the control bottle was about the same with Nano+ Pico Chl *a* at the end of Exp 2.

At the beginning of Exp 3, Micro-phytoplankton slightly dominated the phytoplankton community with the Micro: Nano+ Pico Chl *a* ratios between 1. 27 and 1. 64. This dominance increased at the end of the incubation. The Micro: Nano+ Pico Chl *a* ratios were 3. 17, 4. 11 and 5. 19 in the three bottles, higher ratio for the higher iron enriched bottles.

Table 4. The ratios of Micro: Nano+ pico Chl *a* at the beginning and the end of Exp 2 and 3

Bottles	Exp 2		Exp 3	
	Beginning	End	Beginning	End
Control	0. 12	1. 01	1. 30	3. 17
10 nM Fe enrichment	0. 13	0. 16	1. 27	4. 11
40 nM Fe enrichment	0. 08	0. 18	1. 64	5. 19

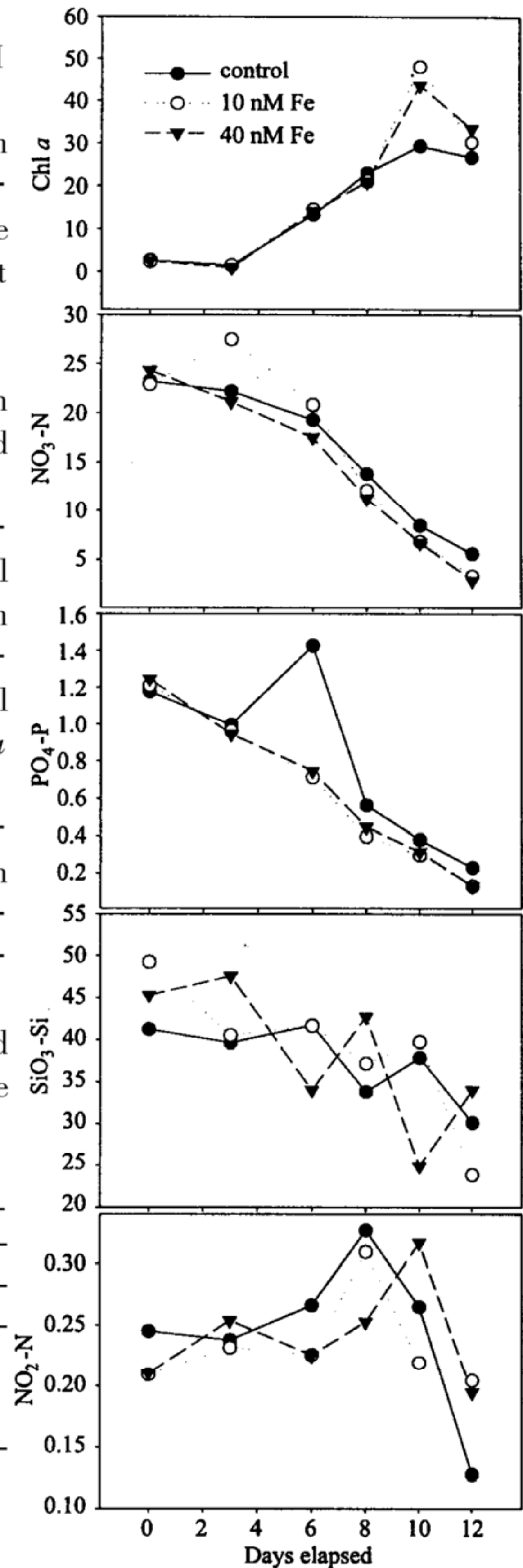

Fig. 4. The Chl *a* (μg·dm^{-3}) growth and nutrients (μM) drawdown of Exp 3.

3. 3 The dominant phytoplankton species

Microscopic examination of the water samples from each bottle at the end of every incubation showed that different phytoplankton species bloomed in the three experiments. In Exp 1, the large dominant species in the Micro

phytoplankton was *Fragilaria striatula* and *Corehron criophilum*. The small Nano phytoplanktons were diatom *Nitzschia curta* (4-8 μm in length and 2 (m in width) and a flagellate. A small dinoflagellate *Gymnodinium baccatum* (7-9 μm in diameter) dominated absolutely in Exp 2. Large diatoms bloomed in Exp 3 with *Chaetoceros* sp and *C. criophilum* being the dominant species.

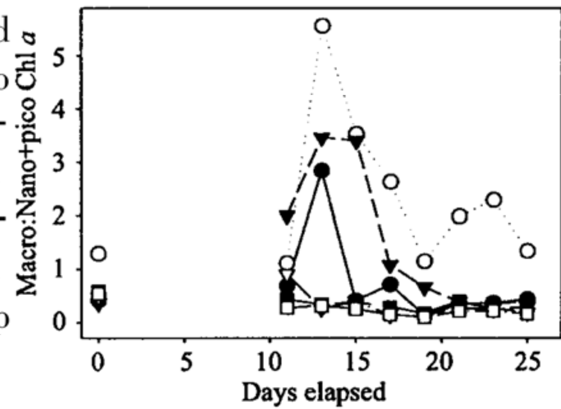

Fig. 5. The ratio of Micro: Nano+ pico Chl *a* in Exp 1
(●: control; ○: 5 nM Fe enriched; ▼: 10 nM Fe enriched; • : 20 nM Fe enriched; ■: 40 nM Fe enriched; □: 80 nM Fe enriched).

4 Discussion

4.1 *Effects of contamination and adsorption on the iron concentration*

In this paper, the iron concentration in the incubation bottles was not measured. This adds uncertainty to the explanation of the experimental results. Contamination and adsorption on the bottle wall were two aspects that affect the iron concentrations (Timmermans *et al*. 1998). In Exp1 and 2, the control bottles showed no obvious Chl *a* growth. It seems that iron contamination did not occur in these experiments.

Adsorption of iron on the bottle wall might occur in our experiment. In the experiment by previous authors (see Table 1 for references), only small amount of iron (such as 1 nM) was enriched. Five nM would be a very high concentration. In Exp 1, Chl *a* in the 5 nM iron-enriched bottle did not grow substantially as in other authors' work. Two explanations may be accountable. The first one is the possible heterogeneous sampling of the initial phytoplankton community. We can see that the Micro: Nano+ Pico Chl *a* is high in the 5 nM iron bottle than others at the beginning of Exp 1. The second one may be adsorption of iron onto the bottle walls. As a result, concentrations of available iron to the phytoplankton were lower than added.

4.2 *Phytoplankton growth and nutrient drawdown*

The results of Exp 1 and Exp 2 showed that iron enrichments stimulated the phytoplankton growth and nutrient drawdown, but it seems that 10 nM iron enrichment was not enough to stimulate the phytoplankton to reach their maximum growth rate. In Exp 1, the Chl *a* concentrations in the bottles with 20, 40 and 80 nM iron enrichments increased exponentially to 40-43 $\mu g \cdot dm^{-3}$ on the 17 th day, with a growth rate of 0.36-0.38 d^{-1}. Chl *a* concentration in the bottle enriched with 10 nM iron reached its peak (19.35 $\mu g \cdot dm^{-3}$) on the 23 rd day (growth rate 0.27 d^{-1}). Phytoplankton growth rates in the control bottle and the bottle enriched with 5 nM iron were 0.13 and 0.16 d^{-1}, respectively. In Exp 2, the Chl *a* growth rates were 0.13, 0.32 and 0.40 d^{-1} in the control bottle and bottles with 10 and 40 nM iron enrichments, respectively.

In the 10 nM Fe enriched bottle in the Exp 1, the nutrients were depleted to a level as low as those in the 20, 40 and 80 nM Fe enriched bottles. But the Chl *a* concentration in the 10 nM Fe enriched bottle was about three-fold lower than those in the bottles with 20, 40 and 80 nM Fe enrichment. That may be because the iron in the bottle was limited for the synthesis of Chl *a* (Fe is required for the synthesis of Chl *a* (Martin and Fitzwater 1988)), but still enough to deplete the macronutrients at a longer time scale.

In the former iron enrichment incubations, either onboard or in situ, the enriched iron concentrations were lower than 20 nM (Hutchins and Bruland 1998, Wells 1994, Coale *et al*. 1996 Chisholm 2000 and references in Table 1), most of them lower than 10 nM. Few of the experiments witnessed the phytoplankton peak.

4.3 The difference between St. -1 and -5

Although St. -1 and -5 are very near in distance, the phytoplankton communities were different in these two stations. As shown in Table 4. There are more microphytoplankton in St. -5. The response of phytoplankton to iron enrichment in the two stations showed significant difference. Iron enrichment promoted the phytoplankton in Exp 2, while the phytoplankton in the control bottles showed little growth. In St. -5, phytoplankton in both the control and iron-added bottles showed similar growth rate, with very little variation.

Because the added iron concentration is 10 and 40 nM in Exp 3, the iron concentration at St. -5 might be very high, maybe higher than 10 nM as indicated in Exp 1 and 2. Such a high level of dissolved Fe was found in former reports. For example, De Baar *et al*. (1990) reported 5-7 nM iron concentration in the Weddell-Scotia Sea Confluence and Weddell Sea, while 60 nM iron was found for a station on the shelf of the South Orkney Island (Nolting and De Baar 1990). The iron maybe released from the sea ice and icebergs. Before February 13, 2002, the Prydz Bay was covered by sea ice thicker than 1.1 m, dotted with huge icebergs. At the bottom of the sea ice and the icebergs were dirt withered material which had been believed to be rich in Al and Fe (De Baar *et al*. 1990).

4.4 The "Ecumenical Iron Hypothesis"

The "Ecumenical Iron Hypothesis" of Morel *et al*. (1991) suggested that small phytoplankton (< 10 μm) were less affected by low iron concentrations while the rare, larger (> 10 μm) phytoplankton were more severely Fe limited. Upon enrichment of iron, phytoplankton > 10 μm would grow faster than phytoplankton < 10 μm. Large diatoms bloomed in the iron enrichment incubations of Cavender-Bars *et al*. (1999) and Gall *et al*. (2001) Martin and Fitzwater (1988), DiTullio *et al*. (1993) Buma *et al*. (1991).

The phytoplankton bloomed in the iron enriched bottles in Exp 1 and 2 were smaller than 10 μm. In Exp 2, non-diatom species bloomed in the incubations. This result is inconsistent with the "Ecumenical Hypothesis". Other authors also have reported negative evidence against this Hypothesis. For example, Zettler *et al*. (1996) suggested that most

phytoplankton cells (Synechococcus, ultraphytoplankton, nanophytoplankton, pennate diatoms and coccolithophorids) were physiologically affected by the low iron concentration in the equatorial Pacific. Hutchins and Bruland (1998) found that large cells dominated in both control and Fe-enriched incubations in their Big Sur experiments.

4.5 The Si∶N and Si∶P uptake ratio

There were reports on the Fe limitation effects on diatom Si∶N and Si∶P uptake ratio. In the iron-enrichment incubation of Hutchins and Bruland (1998), the control and iron-enrichment bottles had differential NO_3^- drawdown but similar H_2SiO_3 utilization. As a result, the molar ratio of Si∶N consumed in the Fe-enriched incubations was close to 1.0 (typical of rapidly growing diatoms under nutrient replete conditions) while this ratio was two to three times higher in Fe-limited controls. Takeda (1998) reported that addition of iron to phytoplankton assemb lages in the incubation bottles halved the silicate: nitrate and silicate: phosphate consumption ratios, in spite of the preferential growth of diatoms. In response to the iron limitation, diatom cellular silicon will increase while cellular nitrogen and phosphorus decrease. Both the authors have pointed out that these estimates of Fe limitation effects on diatom Si∶N uptake ratios are conservative, because other algal taxa also assimilate NO_3^-.

In this paper, phytoplankton assemblages in the controls of Exp 1 and 2 were obviously iron-limited. In Exp1, the control and 5 nM Fe-enriched bottles had higher Si∶N consumption ratio than those with higher concentration Fe-enriched bottles, a phenomenon consistent with above report of Hutchins and Bruland (1998) and Takeda (1998). However, the Si∶P uptake ratio did not decrease in the iron enriched bottles.

In Exp 2, a non-diatom algae that do not require silicon bloomed in the iron enriched bottles. As a result, the silicate concentration did not change much (and in fact increased) despite of the large decline of nitrate and phosphate concentrations in the iron enrich bottles.

5 Conclusion

Our results showed that 10 nM iron enrichment did not stimulate the phytoplankton in the Prydz Bay to grow at the maximum rate. The result that the phytoplankton < 10 μm bloomed in Exp 1 and 2 was controversial to the "Ecumenical Iron Hypothesis" of Morel *et al*. (1991) that upon enrichment of iron, phytoplankton > 10 μm would grow faster than phytoplankton < 10 μm.

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