

## 3.2 Biological and Physiological Responses

### Zooplankton responses during SEEDS

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During the Subarctic Pacific Iron Experiment for the Ecosystem Dynamics Study (SEEDS), chlorophyll *a* concentration in the iron patch reached near maximum in 9 days (D9) after the iron fertilization and after that remained more or less constant. In spite of the stability of chlorophyll *a*, primary production was higher than 1.4–2.0 g C m<sup>-2</sup> d<sup>-1</sup> during D9–D13. As most of the carbon synthesized by phytoplankton after the iron fertilization (IF) was suspended in the water column (Tsuda *et al.*, 2003), these results indicated that loss by grazing, and other factors in the surface mixed layer during D9–D13, was as high as 3–4 mg chlorophyll *a* m<sup>-3</sup> d<sup>-1</sup>, assuming a C:Chl ratio of 30 and the mixed-layer depth of 15 m.

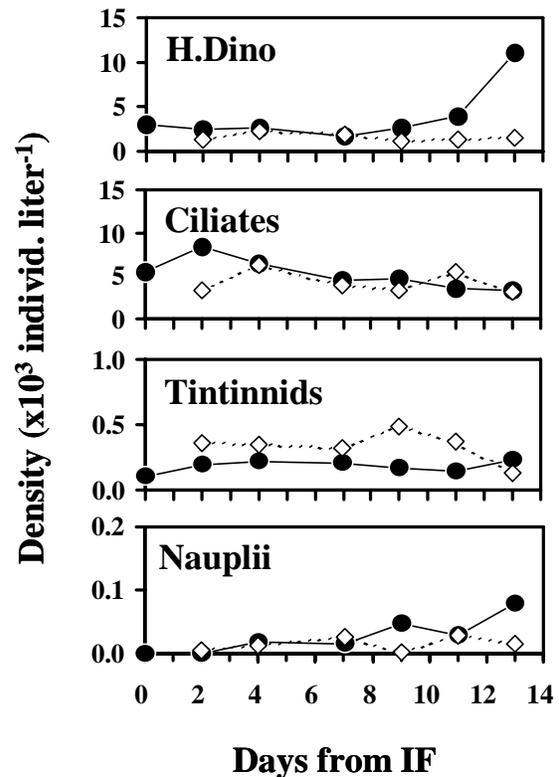
During the SEEDS experiment, heterotrophic nanoflagellates (HNF), microzooplankton, and macrozooplankton grazing rates were examined to understand the impact of zooplankton grazing pressure on phytoplankton dynamics and the carbon budget. Grazing rates of HNF and microzooplankton were examined by dilution experiments. Macrozooplankton grazing was measured by gut fluorescence method.

In the experimental site (in-patch), copepods *Neocalanus cristatus*, *N. plumchrus*, *Eucalanus bungii*, and *Metridia pacifica* were the dominant macrosized grazers. Gut pigment contents of copepods in-patch increased from D7–D8 and were 4 to 18 times higher than those outside of the iron patch (out-patch) during the peak bloom period (Tsuda *et al.*, 2005). The community grazing rates in-patch were in the range between 2.4–10.2% of the daily primary production.

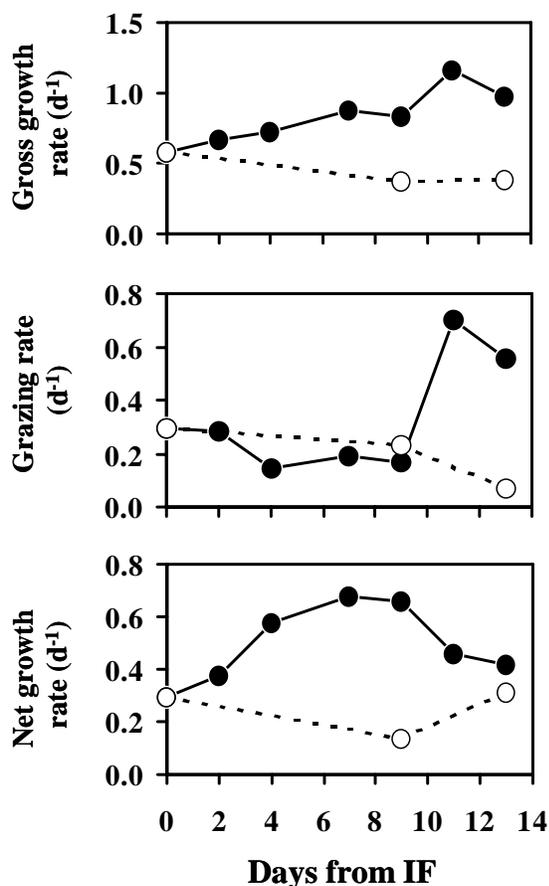
Prior to the IF, aloricate ciliates were the most dominant microzooplankton followed by heterotrophic dinoflagellates (Fig. 1). Tintinnids and copepod nauplii were relatively minor components. Aloricate ciliates gradually decreased

after the IF. Heterotrophic dinoflagellates, dominated by *Gyrodinium* spp., increased rapidly after D9 and were the most dominant in the microzooplankton assemblage at the end of the experiment. The net growth rate reached 0.51 d<sup>-1</sup> during D11–D13.

Grazing rates of microzooplankton decreased with the development of the diatom bloom until D9 (Fig. 2). On D11, the grazing rate abruptly increased. The net growth rates (gross grazing rate – grazing rate) of phytoplankton decreased after D11.



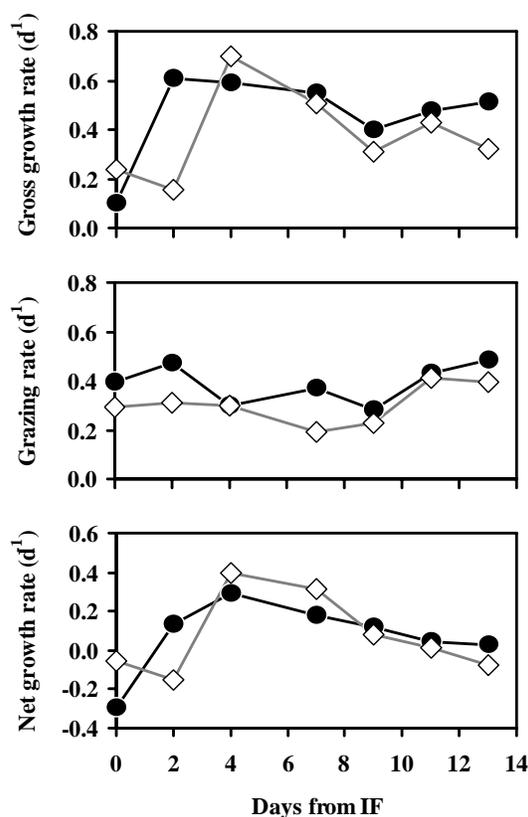
**Fig. 1** Temporal change in abundance of microzooplankton (>10 μm) in the top 10 m of the water column. Filled and open symbols mean in-patch and out-patch, respectively.



**Fig. 2** Results of dilution experiments for total phytoplankton. Filled and open circles mean in-patch and out-patch, respectively.

In phytoplankton smaller than *ca.* 5  $\mu\text{m}$  ESD (equivalent spherical diameter), determined by flow cytometry analysis, eukaryotic ultraphytoplankton (EUKU) and cyanobacteria *Synechococcus* (SYN) predominated. Before the IF, gross growth rates of EUKU and SYN were  $0.10\text{ d}^{-1}$  and  $0.24\text{ d}^{-1}$ , respectively (Fig. 3). Gross growth rates of EUKU and SYN increased on D2 and D4, respectively, then gradually decreased. The grazing rates on EUKU and SYN decreased slightly with time, and increased again after D11. Net growth rates of EUKU and SYN increased after the IF and gradually decreased with time. At the end of the SEEDS experiment, growth and grazing mortality rates of ultraphytoplankton balanced each other.

Present results show that all the grazers increased their feeding activity after the IF but responses were variable. Increase in gross growth rate of



**Fig. 3** Gross growth rates, grazing rate and net growth rates of eukaryotic ultraphytoplankton (filled circles) and cyanobacteria *Synechococcus* (open diamonds).

ultraphytoplankton was the first biological response to the IF during SEEDS. The grazing rate of HNF on ultraphytoplankton did not increase rapidly and excess growth was observed. However, the relationship between growth and grazing returned to the original balanced one within 6–8 days. Results show that the relationship between growth rates and grazing mortality rates of ultraphytoplankton had a relatively strong convergent potential to balance each other against environmental perturbation.

Grazing by heterotrophic dinoflagellates was an important loss factor of phytoplankton at the later part of SEEDS, and they might have played an important role in keeping chlorophyll *a* concentration steady after D11. In general, chain-forming diatoms like *Pseudonitzschia pungens* and *Chaetoceros debilis* are too large to be prey for microzooplankton. However, it is known

that some heterotrophic dinoflagellates can feed on prey larger than themselves (e.g., Jacobson and Anderson, 1986). Light and scanning electron microscope studies showed that *Gyrodinium* spp. contained diatom cells. The biology and ecology of *Gyrodinium* spp. in the subarctic Pacific (e.g., distribution, feeding rate, feeding behavior, growth rate, fecal pellet production rate, elemental composition of feces, etc.) are not well understood. Further studies are needed to understand the role of *Gyrodinium* spp. on phytoplankton dynamics and the fate of carbon synthesized during the diatom bloom induced by IF.

Although the response of copepods to the increase in prey phytoplankton was quite obvious, their grazing influenced only slightly the phytoplankton dynamics within the 13-day SEEDS experiment. This was due to the slow growth rate of copepods compared to microzooplankton and HNF. Saito *et al.* (2002) showed that the copepod grazing influence was insignificant at the peak phase of the diatom bloom in the Oyashio region, but it was an important loss factor of phytoplankton after nutrient depletion. This suggests that the fate of carbon synthesized during the IF-induced bloom would be influenced by copepod grazing after nutrient depletion as well as by *Gyrodinium* grazing.

One of the important pieces of information obtained by SEEDS is that relatively minor components in the food-web functional group prior to the IF became key players after the IF.

*Chaetoceros debilis* was a negligible component in the phytoplankton assemblage prior to the IF but increased abruptly after the IF and was the most dominant. The most significant response of grazers to the outburst of *C. debilis* was by a relatively minor component in zooplankton, *Gyrodinium* spp. These results have shown that prediction of the ecosystem response to anthropogenic or natural perturbation is still a challenging issue, and further studies on the ecosystem structure and the functions of its components are needed.

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## Phytoplankton community response to iron and temperature gradient in the NW and NE subarctic Pacific Ocean

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During the 2001 SEEDS (Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study) and 2002 SERIES (Subarctic Ecosystem Response to Iron Enrichment Study) experiments, on-board bottle incubation experiments were carried out by adding different amounts of iron to elucidate phytoplankton community responses to iron concentrations. Temperature gradients from 5 to 18°C were also applied to the incubation experiments because temperature affects growth rate and metabolic functions, such as enzyme reactions, in phytoplankton cells. At the same iron

concentration, specific chlorophyll *a* increase rates (growth rates) for micro-sized phytoplankton (>10 µm) were the highest, between 9 and 13°C, almost doubled from 5 to 9°C. The surface mixed layer temperature was 9°C at the beginning of iron fertilization, but was 5°C just two weeks before. We believe that this drastic increase in growth rate with temperature was a reason for the highest chlorophyll *a* increase in SEEDS among all the mesoscale iron enrichment experiments conducted in the HNLC regions.

## SERIES: Copepod grazing on diatoms

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During the Subarctic Ecosystem Response to Iron Enrichment Study (SERIES), the phytoplankton population depleted dissolved silicate, bringing an end to a bloom which had increased diatom biomass in the patch by more than 10-fold over the 4-week study. Silicate has been implicated as an important ballasting material that adds density to sinking particles and increases carbon flux out of the upper ocean. Therefore, processes which enhance either the recycling or export of silicon (Si) are crucial in understanding carbon fluxes.

Phytoplankton biomass increases through the uptake of silicate, along with carbon and other macro (N and P) and micro (*e.g.*, Fe) nutrients. The increased biomass is either recycled in the upper ocean, or is “exported” as increased animal biomass or as sinking detritus (Fig. 1). Open ocean ecosystems export ~10% of primary production, the balance being remineralized in the surface layer. Any incorporation of organic matter into other trophic levels does not include silicon, causing its enrichment in detrital materials. Thus, particles sinking out of the subarctic surface layer are commonly enriched in Si compared with C or N.

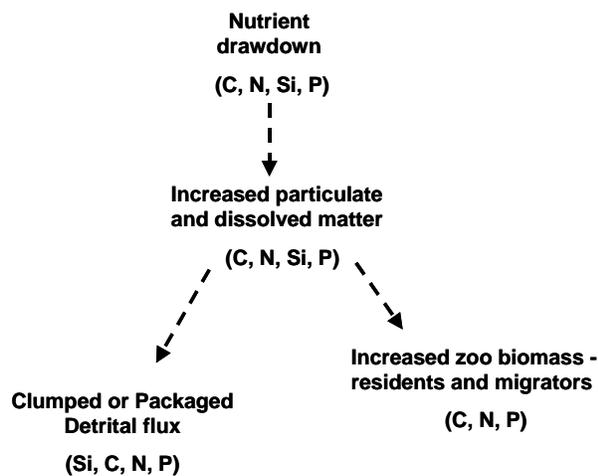


Fig. 1 Elemental cycling in the upper ocean.

At Ocean Station Papa (OSP), silicate is used by diatoms predominantly in May and June (Whitney and Freeland, 1999). This is the time of year that the major copepod species, *Neocalanus plumchrus*, is actively grazing (Mackas *et al.*, 1998). May–June is also the period that sees the highest fluxes of detrital material into the interior of the ocean at OSP (Wong *et al.*, 1999). Since the SERIES experiment was conducted in July of a warm, strongly stratified year, it is likely that *N. plumchrus* had entered diapause before iron fertilization took place. Thus a major grazer would not have been active to crop the diatom bloom that iron enrichment stimulated. To look at the effects of grazers on the diatom community in SERIES, major copepod species (the dominant macro-zooplankton) in both the water column and from sediment traps were analyzed for Si content. Ten to 20 copepods were picked from samples then desiccated and digested with 1%  $\text{Na}_2\text{CO}_3$  solution in an 85°C bath for 2 to 4 h. Samples were then analyzed for silicate using standard Autoanalyzer procedures.

Silicon dynamics for SERIES is summarized in Figure 2. Silicate was rapidly drawn down between July 23 and 28, to the point where it was likely limiting diatom growth (along with iron). The diatom bloom resulted in an accumulation of particulate Si in the upper 20 m by July 26 and a subsequent export of this material to sediment traps at 50 m depth. The copepod community showed some response to phytoplankton growth (*e.g.*, Fig. 3), their numbers increasing during the bloom. However, an increase in numbers is likely the result of the patch collecting or attracting grazers as it drifts. Copepod reproduction is not rapid enough to account for increased numbers.

Copepods attracted into sediment traps (Fig. 4) show an increased activity in the latter part of the study. The dominant (>1 mm) zooplankton collected by traps were the copepods *Eucalanus bungii* and *Neocalanus cristatus*. Collections of over 150 copepods per trap cylinder per day, largely

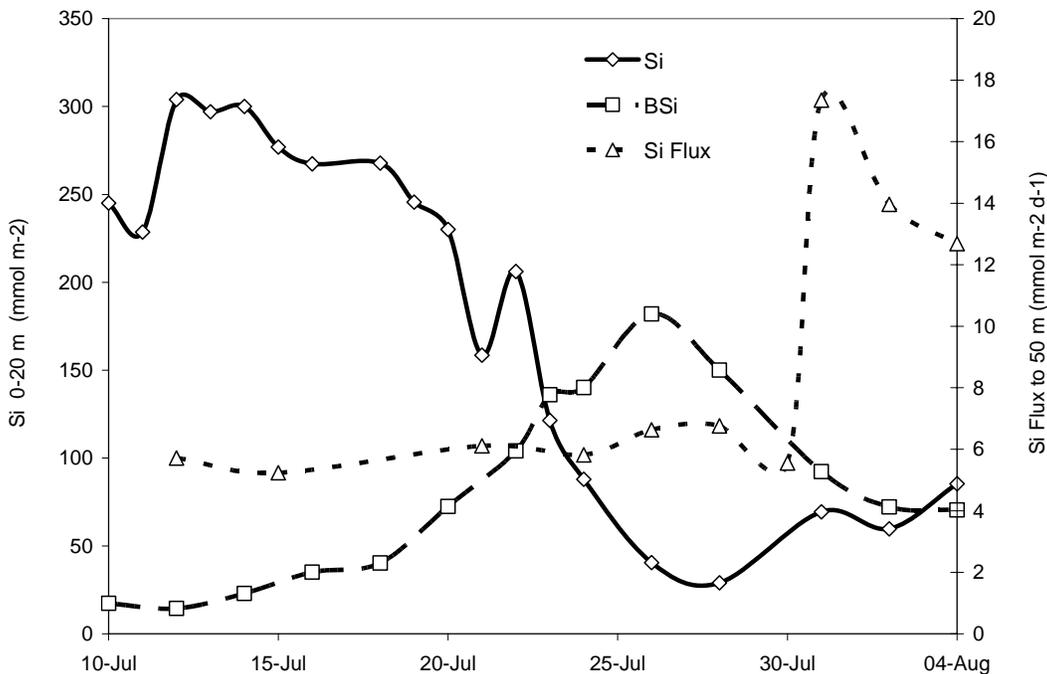
*E. bungii*, are much higher than actual numbers of copepods collected in net tows (tows = 3,000 m<sup>-2</sup>, traps = 26,000 m<sup>-2</sup>). Also, the 100- and 125-m traps, which were imbedded in the pycnocline rather than the mixed layer, collected more *E. bungii* and *N. cristatus* in the final trap deployments.

In Figure 5, we show our results for Si content in copepods collected by net tows or in sediment traps. The data set is incomplete in late experiment, but provides information on grazing by various species. The smaller copepods, *Pseudocalanus*, *Oithona* and *Metridia* may graze on diatoms, but because of their size contain little Si. Most interesting is the trend in *E. bungii* showing increased Si content at the time of the Si export event. These copepods appear to have increased their feeding on diatoms in the last several days of SERIES.

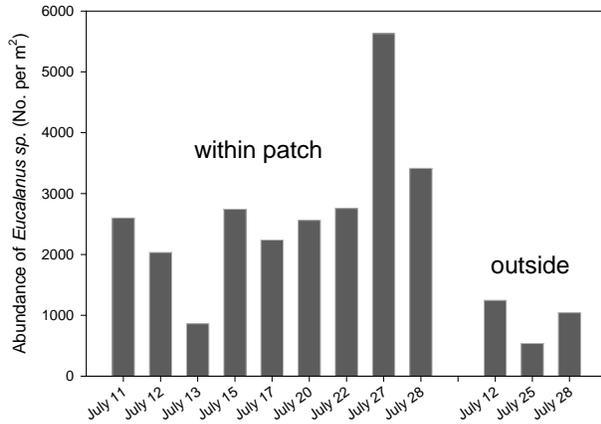
To consider whether a species such as *E. bungii* could have contributed to the export of Si from the surface layer during SERIES, we construct a budget based on the number of these copepods

collected by net hauls and on their Si content from traps. The budget shows that 3,000 copepods containing ~5 nmol Si copepod<sup>-1</sup> (0.15 µg copepod<sup>-1</sup> in Fig. 5) yields 15 µmol m<sup>-2</sup> in gut content, compared with 5 to 15 mmol m<sup>-2</sup> d<sup>-1</sup> Si flux to 50 m depth. Even if copepods were producing fecal pellets containing this amount of Si every 30 minutes throughout the day, they would only contribute 1 to 2% of the Si flux observed during SERIES.

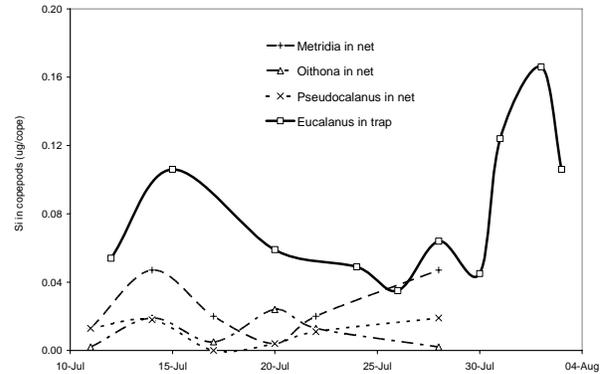
These results should be considered preliminary. Further analyses of increases in copepod body size will provide independent evidence on the importance of grazers as a sink for organic material. At the present, we can only speculate that copepod grazing did not contribute significantly to export from the patch. However, a cautionary note is that patch net tows were carried out during daylight hours. Goldblatt *et al.* (1999) showed that migrating species such as *N. plumchrus* are more abundant in surface waters at night than during the day in spring.



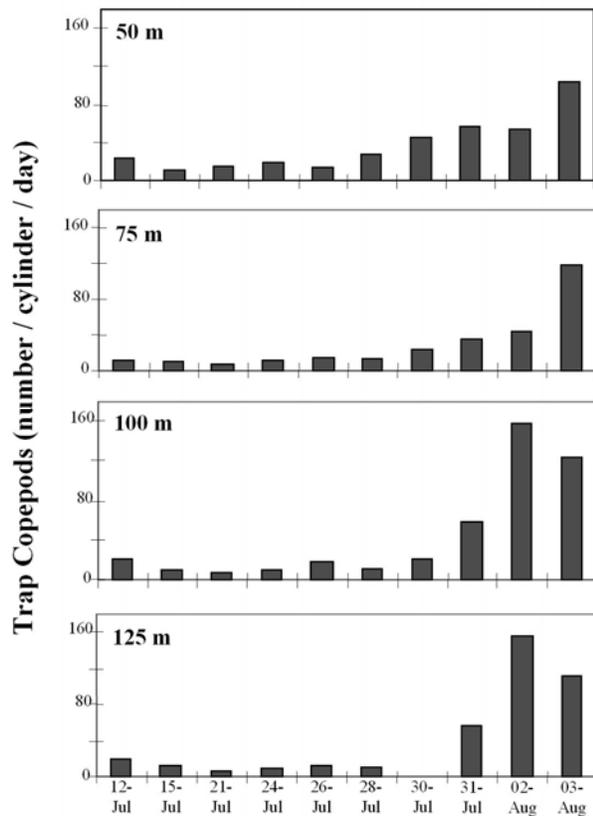
**Fig. 2** Silicon dynamics in the SERIES patch and transport to a sediment trap at 50 m below the patch.



**Fig. 3** Abundance of *Eucalanus bungii* inside and outside the patch from 150-m tows collected in daylight hours.



**Fig. 5** Si content of copepods collected either by net haul or by removal as swimmers from sediment traps.



**Fig. 4** Number of swimming copepods (>1 mm) removed from sediment trap materials during SERIES. The copepods *Eucalanus bungii*, followed by *Neocalanus cristatus*, were the most dominant.

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# The Southern Ocean Iron Enrichment Experiment: The nitrogen uptake response

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## Introduction

Over the last decade considerable effort has been directed toward understanding the relationship between phytoplankton productivity and the availability of iron, particularly as a cause for the lack of significant autotrophic growth in high nitrate, low chlorophyll (HNLC) regions of the open ocean. The results of iron-enrichment shipboard incubation (grow-out) experiments and *in situ* iron-enrichment experiments conducted in the subarctic North Pacific, equatorial Pacific, and the Southern Ocean strongly support the idea that phytoplankton growth rates and biomass accumulation in HNLC areas are limited, at least in part, by the availability of iron. Despite the abundance of nitrate in the surface waters of these regions, phytoplankton are supported primarily by regenerated N forms (*i.e.*, ammonium and urea), presumably because of the lower energetic costs needed to utilize these reduced substrates compared to nitrate when iron is limiting (*e.g.*, Raven, 1990). In this current study, the planktonic nitrogen uptake response to iron addition is described in the largest of these HNLC regions — the Southern Ocean during the Southern Ocean Iron Experiment (SOFeX) conducted in the austral summer 2002 (Coale *et al.*, 2004). This is the first report documenting the nitrogenous uptake response of natural phytoplankton communities to *in situ* iron fertilization of the Southern Ocean, although two previous open-ocean iron-enrichment experiments have been conducted in the Southern Ocean, one in the Australian sector [Southern Ocean Iron RElease Experiment (SOIREE, Boyd *et al.*, 2000)] and the other in the Atlantic sector [Eisen (iron) Experiment (EisenEx, Gervais *et al.*, 2002)]. The present study describes two nitrogen processes affected by the alleviation of iron limitation in the southern patch of SOFeX: 1) the nitrogenous nutrition (nitrate, ammonium, nitrite and urea) of the natural phytoplankton communities, and 2) the potential inhibitory effects of ammonium on the observed uptake rates of nitrate.

## Materials and methods

### General

The SOFeX study was conducted at two sites both north and south of the Antarctic Polar Front Zone (APFZ), in low- and high-silicate waters, respectively. We focus here on the ‘southern patch’ which was characterized by high nitrate (~28  $\mu\text{M}$ ) and silicic acid (~60  $\mu\text{M}$ ) concentrations, an average mixed layer (ML) temperature of  $-0.5^\circ\text{C}$  and ML depth of 45 m. Iron (as acidic iron sulphate) and the inert tracer sulfur hexafluoride ( $\text{SF}_6$ ) were added to a 15 by 15 km patch in the vicinity of  $65^\circ\text{S}$ ,  $172^\circ\text{W}$  during four separate injections beginning on January 24, 2002 (day 0). The patch was then tracked and monitored by three research vessels in a Lagrangian fashion for one month. Details of the experimental methodology of the SOFeX, patch dilution and mixing rates, biological and chemical responses, and export flux are presented elsewhere (Bishop *et al.*, 2004; Buesseler *et al.*, 2004; Coale *et al.*, 2004).

### Sampling and N-uptake experiments

Samples for nitrogen uptake experiments were collected from within and outside (control waters) of the iron-enriched patch using 30-L trace-metal free Go-Flo bottles mounted on a trace-metal clean, instrumented rosette (Hunter *et al.*, 1996); all subsequent sub-sampling and manipulations were conducted within a laminar-flow hood (HEPA) using trace-metal clean techniques (*e.g.*, Fitzwater *et al.*, 1982). Chlorophyll *a* concentration was measured by *in vitro* fluorometry (Parsons *et al.*, 1984) and nitrogen uptake rates were determined using the  $^{15}\text{N}$  isotope technique (Dugdale and Wilkerson, 1986) and on-deck simulated *in situ* incubations of tracer-level enriched samples. The ammonium uptake rates reported have not been corrected for the effects of isotopic dilution (Glibert *et al.*, 1982), and thus should be considered as

conservative estimates. The inhibitory effects of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  uptake were determined by inoculating 1.2-L or 280-mL polycarbonate bottles with a tracer-level ( $< 10\%$  of ambient  $\text{NO}_3^-$ ) concentration of  $^{15}\text{N}$ -labeled sodium nitrate (98.25 atom %; Cambridge Isotope Laboratories) and a series of 9 to 10 concentrations (conducted in duplicate) of unlabeled ammonium sulfate ranging from 0.05 to 10  $\mu\text{M}$ . Samples were incubated on-deck in clear, spectrally corrected (blue) Plexiglas<sup>®</sup> deck incubators at the ambient *in situ* temperature with photosynthetic photon flux density (PPFD) attenuated to the light depth of collection (47% of the incident surface flux). Incubations were terminated by filtration ( $< 80$  mm Hg) after 8.5–9 h onto precombusted Whatman<sup>®</sup> GF/F filters (2.5 cm; 4 h at 450°C), and frozen in polypropylene cryovials until mass spectrometric analysis ashore.

#### *Inhibition parameters*

The inhibition of  $\text{NO}_3^-$  uptake by  $\text{NH}_4^+$  was determined by fitting the absolute  $\text{NO}_3^-$  uptake rate versus  $\text{NH}_4^+$  concentration data to two different functions: a simple 3-parameter exponential model (Varela and Harrison, 1999) and a modification of the inverse Michaelis-Menten equation (Harrison *et al.*, 1996). The curve fitting was completed using a computerized, iterative non-linear least-squares technique (Kaleidograph<sup>®</sup>; Abelbeck Software) which utilizes the Levenberg-Marquardt algorithm (Press *et al.*, 1992). Details and the actual equations employed are presented in Cochlan and Bronk (2003).

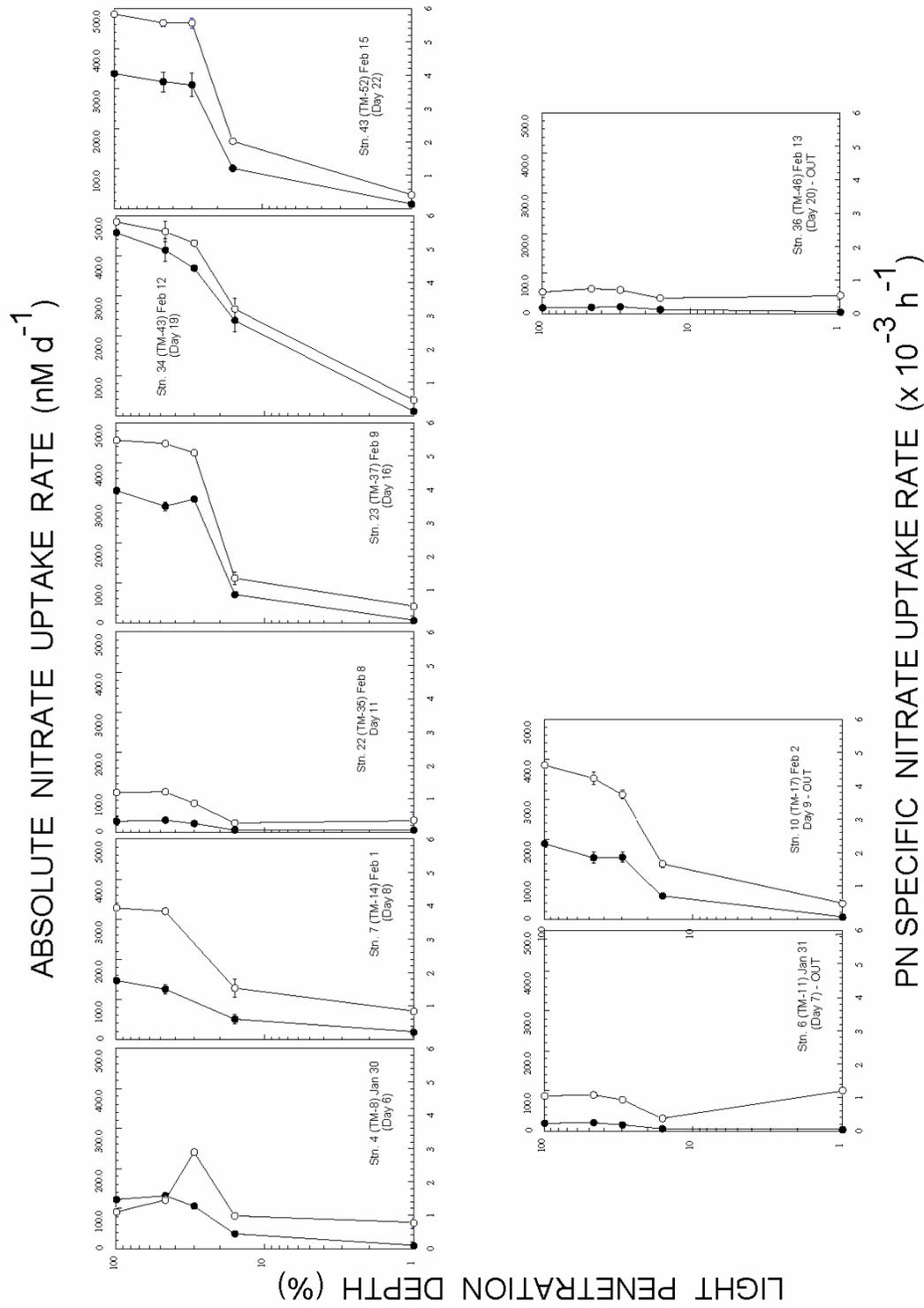
#### **Results and discussion**

The fertilization of a patch of the surface waters of the Southern Ocean with small quantities of iron resulted in a massive bloom of phytoplankton (*ca.* 20-fold increase), and a measurable draw-down of nitrate (*ca.* 2  $\mu\text{M}$ ; Coale *et al.*, 2004). Coincident with the increase in phytoplankton biomass were dramatic increases in nitrate utilization rates by phytoplankton in the upper mixed layer of the south patch. Absolute (transport) uptake rates increased by a factor of *ca.* 25 in the south patch relative to outside (control) regions (Fig. 1). Addition of iron also greatly increased biomass (particulate nitrogen) specific  $\text{NO}_3^-$  uptake rates by *ca.* 10-fold, indicative of faster rates of  $\text{NO}_3^-$  consumption per unit phytoplankton biomass, a result similar to

those reported for iron-amended bottle experiments conducted previously in HNLC regions of the Southern Ocean (Timmermans *et al.*, 1998; Cochlan *et al.*, 2002).

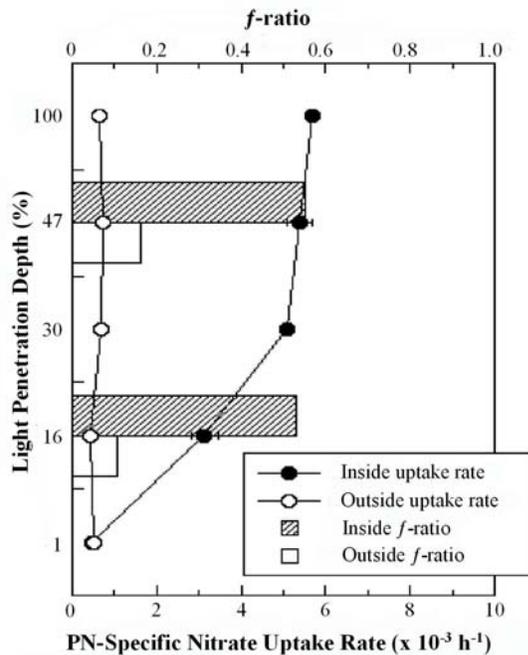
The cycling of nitrogen is dramatically affected by iron availability. The extent to which iron enrichment allows for the utilization of new forms of nitrogen may reflect a source of community growth, and at steady state, the subsequent flux which is unrealized in an iron-deficient system. Throughout the 28-day monitoring period of SOFeX, the proportion of  $\text{NO}_3^-$  uptake to the total N ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and urea) uptake was measured and can be used to estimate ‘new’ production – the amount of total production that can be exported from the system, without depleting the system (Dugdale and Goering, 1967). This uptake ratio, termed the *f*-ratio (Eppley and Peterson, 1979), increased from 0.1–0.2 to 0.5–0.6 in the iron-enriched waters of the southern patch within 3 weeks of the initial Fe injection (Fig. 2). The increased *f*-ratio indicates that alleviation of iron limitation allows for greater relative utilization of the ambient nitrate reserves that would otherwise be underutilized for growth in the surface waters south of the APFZ, and confirms the role of iron limitation in the creation of HNLC conditions in the open waters of the Southern Ocean.

In order to assess the potential inhibitory effects of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  uptake rates, short-term incubation experiments were conducted with increased  $\text{NH}_4^+$  availability on surface samples collected from both the outside waters (day 13) and inside the southern patch at the beginning (day 12) and end (day 22) of the monitoring period from the 47% light penetration depth. The  $\text{NO}_3^-$  uptake versus  $\text{NH}_4^+$  concentration data are well described by both the three-parameter exponential model (Varela and Harrison, 1999) and the modified inverse Michaelis-Menten model (Harrison *et al.*, 1996). Using the exponential model, the values for  $\rho\text{N}_{\text{max}}$  (theoretical maximum  $\text{NO}_3^-$  uptake rate) were estimated for ‘zero’  $\text{NH}_4^+$  concentration at each station, and the extrapolated value subsequently used in a Michaelis-Menten function. Selected kinetic parameters derived from the two models are shown graphically in Figure 3 and presented in Table 1. These measurements allow for an assessment of the relative  $\text{NH}_4^+$  inhibition of  $\text{NO}_3^-$  uptake rates as the iron-induced phytoplankton bloom developed. The  $\rho\text{N}_{\text{max}}$  values increased

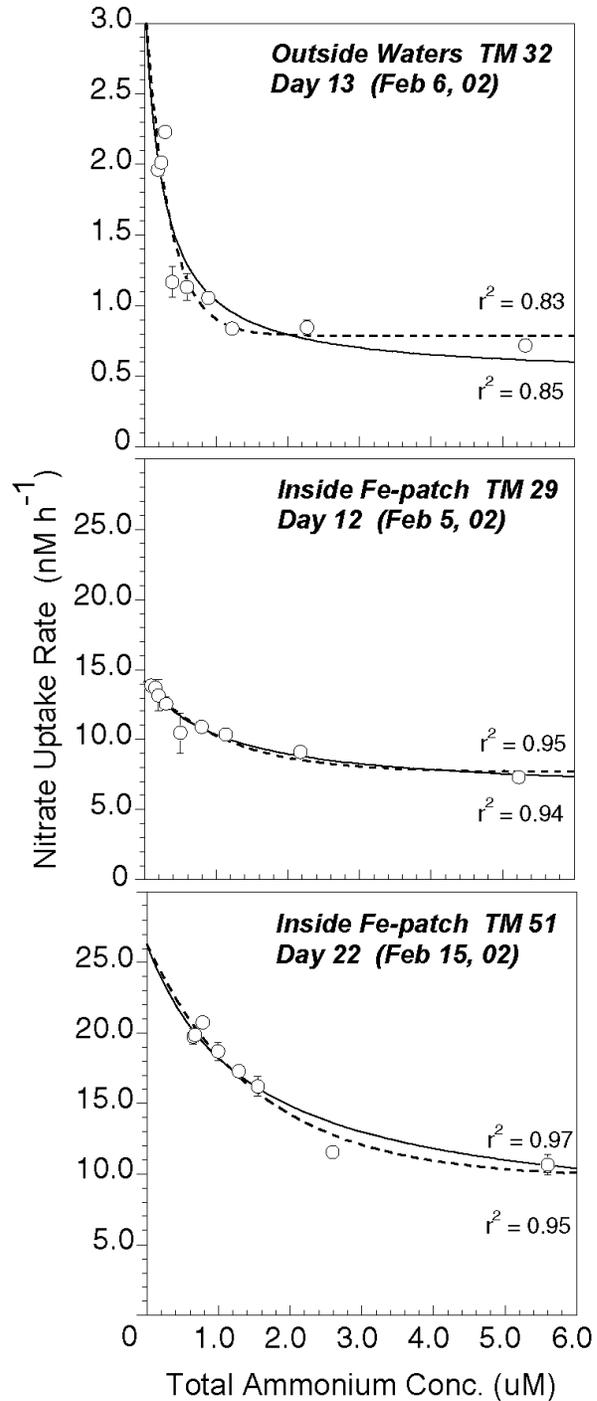


**Fig. 1** Depth profiles of absolute (●) and PN-specific (○) uptake rates of nitrate in the southern iron-fertilized patch and outside (control) waters during SOFeX. Error bars represent the range of duplicate samples (n = 2); when not visible they are smaller than the symbol size.

dramatically following alleviation of iron limitation and are 4- to 8-fold greater than the  $\rho N_{\max}$  values determined in the outside waters. While some of the increase in this theoretical uptake rate can be ascribed to the greater biomass present following iron enrichment, the particulate nitrogen (PN)-specific uptake rates (estimated by dividing these rates by the particulate nitrogen concentrations) in the iron patch also increased and are 2- and 3-fold greater on days 12 and 22, respectively, than the outside rate. The maximal realized inhibition ( $I_{\max}$ ) value was greatest for the outside (control) waters (84%) and decreased to 57 and 75% on days 12 and 22, respectively. In other words, the maximum possible reduction in  $\text{NO}_3^-$  uptake rates due to  $\text{NH}_4^+$  was lessened due to alleviation of iron limitation. The half-saturation constants of inhibition ( $K_I$ ) were very low in the un-enriched waters (0.26  $\mu\text{M}$ ), but increased with time in the iron patch (1.09 and 1.45  $\mu\text{M}$ ) suggesting that greater concentrations of  $\text{NH}_4^+$  are necessary to reduce the  $\text{NO}_3^-$  uptake rate by 50% following iron enrichment. Using these derived inhibition parameters and the ambient  $\text{NH}_4^+$  concentrations for the outside waters (0.18  $\mu\text{M}$ ),



**Fig. 2** Depth profiles of PN-specific nitrate uptake rates for samples collected within (iron-enriched) and outside (control waters) of the iron-enriched patch south of the APFZ. The  $f$ -ratios were determined at the 47 and 16% light depths, and are not corrected for the effects of isotopic dilution. Error bars represent the range of duplicate samples ( $n = 2$ ).



**Fig. 3** Absolute  $\text{NO}_3^-$  uptake rates ( $\text{nM h}^{-1}$ ) by natural assemblages of phytoplankton as a function of total (added + ambient)  $\text{NH}_4^+$  concentration. Rates estimates are fitted directly to the 3-parameter exponential and the inverse Michaelis-Menten functions, and denoted as dashed (---) and solid (—) lines, respectively.

**Table 1** Summary of the kinetic parameters describing the inhibitory effects of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  uptake by Southern Ocean phytoplankton during SOFeX.

Station Name, Cast and Day	$I_{\max}$	Ambient $\text{NH}_4^+$ conc. ( $\mu\text{M}$ )	$K_I$	$N_{\text{inhib}}$
Outside, TM 32	0.84	0.18	0.26	2.3
Day 13	(0.067)		(0.07)	(0.67)
Fe-Patch, TM 29	0.57	0.09	1.09	9.8
Day 12	(0.061)		(0.29)	(2.6)
Fe-patch, TM 51	0.75	0.57	1.45	13.1
Day 22	(0.045)		(0.23)	(2.1)

The potential maximal inhibition ( $I_{\max}$ ) estimates and half-saturation constants of inhibition ( $K_I$ ) were determined from the inverse Michaelis-Menten equation. The values of  $N_{\text{inhib}}$ , the  $\text{NH}_4^+$  concentration above which no further  $\text{NO}_3^-$  uptake occurs, were approximated as  $9 \times K_I$ . Standard errors (SE) values of parameters are reported in parentheses.

one can compare the relative inhibitory effects of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  uptake rates at one low ammonium concentration for all stations. Based on such an analysis, this relatively low  $\text{NH}_4^+$  concentration would decrease  $\rho N_{\max}$  values for outside, and iron-patch samples from days 12 and 22, by 31, 7 and 3%, respectively. These results suggest that the potential inhibitory effects of  $\text{NH}_4^+$  on  $\text{NO}_3^-$  utilization are diminished (or dis-inhibited) by the alleviation of iron limitation in the Southern Ocean.

## Conclusions

The alleviation of iron limitation in the HNLC waters south of APFZ contributes to enhanced nitrate utilization by phytoplankton; absolute uptake rates increased *ca.* 25-fold (relative to un-enriched waters) and PN-specific rates increased by *ca.* 10-fold. This enhancement of new production by iron fertilization is reflected in an increase in *f*-ratio from 0.1–0.2 to 0.5–0.6 in surface phytoplankton communities. Ammonium inhibition experiments, conducted on-deck, suggest that iron-replete communities are less sensitive to the potential inhibitory effects of ammonium on nitrate utilization.

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