



PERGAMON

Deep-Sea Research II 49 (2002) 3365–3390

DEEP-SEA RESEARCH
PART II

www.elsevier.com/locate/dsr2

Trace metals and nitrogenous nutrition of Antarctic phytoplankton: experimental observations in the Ross Sea

William P. Cochlan^{a,*}, Deborah A. Bronk^b, Kenneth H. Coale^c

^a *Romberg Tiburon Center for Environmental Studies, San Francisco State University, 3152 Paradise Drive, Tiburon, CA 94920-1205, USA*

^b *Virginia Institute of Marine Science, College of William and Mary, P.O. Box 1346, Gloucester Point, VA 23062, USA*

^c *Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039, USA*

Abstract

As part of the US JGOFS Antarctic Environment Southern Ocean Process Study (AESOPS), shipboard incubation experiments were conducted to investigate the effects of iron and zinc addition on ammonium (NH_4^+) and nitrate (NO_3^-) uptake by natural assemblages of Antarctic phytoplankton. Samples were collected from the Ross Sea during austral summer (January–February, 1997) within the mixed layer at two stations: a continental shelf station—Orca, and a high NO_3^- , low chlorophyll (HNLC) station located further offshore—Blue. Twenty-liter bottle incubations were sampled repeatedly during an 8-day period at Orca, and a 13-day period at Blue for short-term ^{15}N -tracer uptake experiments. Ambient concentrations of NO_3^- were elevated at both locations. Biomass, measured as chlorophyll *a* (chl *a*), was relatively high at Orca (chl *a* = $2.31 \mu\text{g l}^{-1}$), whereas true HNLC conditions were observed at Blue (chl *a* = $0.52 \mu\text{g l}^{-1}$). All Fe enrichments produced an acceleration in NO_3^- decline, and accumulation in biomass. Chl *a* increased 2–3-fold at Orca and 4–5-fold at Blue; these increases were directly correlated with increasing Fe enrichment. Unambiguous responses to zinc addition were not evident at Blue, whereas increased total biomass accumulation and nitrate drawdown were observed at Orca.

Rates of biomass (particulate nitrogen) specific NO_3^- -uptake in the Fe-enriched samples were up to 2-fold greater than un-enriched controls at both sites. There were no significant changes in specific uptake rates of NH_4^+ at the HNLC site, and only a 30–40% increase at Orca with the highest Fe enrichments. These results clearly indicate that Fe additions resulted in faster rates of NO_3^- consumption per unit phytoplankton biomass at both sites. The N-uptake response to zinc enrichment was not as evident as with Fe, presumably due in part to the relatively high dissolved concentrations of zinc (ambient concentrations = ca. 2 nM). However, during the later sampling periods, zinc addition resulted in a 40% increase in the specific uptake rates of NO_3^- but not NH_4^+ , whereas the specific uptake rates of NO_3^- and NH_4^+ increased by 4–16% and 18–49%, respectively, at Orca. Absolute uptake rates of NO_3^- and NH_4^+ (corrected for isotopic dilution) were 4–5 and 2–3 times greater, respectively, than the controls at Blue, whereas at Orca both NO_3^- - and NH_4^+ -uptake rates doubled as a result of Fe enrichment. Post-incubation, size-fractionation uptake experiments demonstrated that the higher rates of nitrogen uptake were primarily due to larger phytoplankton ($> 5 \mu\text{m}$). The *f*-ratio [$f = \text{NO}_3^-$ -uptake / (NO_3^- + NH_4^+ -uptake)] increased from ca. 0.7 to 0.8 as a result of Fe enrichment at the HNLC site, but declined from an average of 0.84 to 0.52–0.69 in both the controls and the Fe-amended samples collected further onshore at Orca. This decrease may be due to the inhibition of NO_3^- -uptake by elevated NH_4^+ concentrations resulting from increased heterotrophic remineralization within the carboys over time. Based on the apparent half-saturation constant (K_s) of 0.09 nM Fe estimated for community planktonic NO_3^- -uptake, the availability of dissolved Fe (ambient

*Corresponding author. Tel.: +1-415-338-3541; fax: +1-415-435-7120.

E-mail address: cochlan@sfsu.edu (W.P. Cochlan).

concentration = 0.03–0.04 nM) limits the uptake of NO_3^- by phytoplankton at the HNLC and continental shelf regions of the western Ross Sea during austral summer. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Much of the open, Southern Ocean is classified as a high-nutrient low-chlorophyll (HNLC) region (Minas et al., 1986). These regions are characterized by uniformly low phytoplankton biomass and non-limiting concentrations of macronutrients, specifically nitrate (NO_3^-), phosphate (PO_4^-), and silicate (SiO_4^{4-}), a condition shared by over 20% of the world's ocean, including the equatorial and North Pacific Oceans. The exact mechanisms responsible for the relatively low biomass and lack of NO_3^- depletion in HNLC regions are unknown, but explanations generally have been divided into either 'top-down' (ecological) or 'bottom-up' (physiological) controls. Top-down controls prevent the accumulation (i.e., yield) of phytoplankton biomass, and include processes such as intense grazing (e.g., Banse, 1991; Frost, 1991; Miller et al., 1991). Conversely, an inadequate supply of an essential micronutrient, most notably iron (Fe) (e.g., Martin and Gordon, 1988) may control productivity from the bottom-up by controlling rates of specific physiological functions. Several factors have been suggested as responsible for the physiological regulation of phytoplankton growth in HNLC regions, including light limitation due to mixing below critical depths (Mitchell et al., 1991; Nelson and Smith, 1991), low intrinsic (biomass-specific) rates of NO_3^- -uptake (Dugdale and Wilkerson, 1991), diffusion-limited carbon (C) assimilation (Reibersell et al., 1993), and low input and availability of Fe (e.g., Martin and Fitzwater, 1988; Martin et al., 1991). Iron limitation may affect many phytoplankton physiological processes, including photosynthetic energy conversion efficiency (e.g., Greene et al., 1992, 1994), NO_3^- assimilation (e.g., Raven, 1990), and the synthesis of chlorophyll (Chereskin and Castelfranco, 1982).

In the Southern Ocean, low concentrations of dissolved Fe have been reported both offshore

(e.g., Martin et al., 1990; Scharek et al., 1997) and onshore, including the marginal ice zone (MIZ) shelf waters of the Ross Sea during summer (Fitzwater et al., 1996, 2000; Sedwick et al., 1997; Sedwick and DiTullio, 1997). Shipboard Fe-enrichment experiments (grow-out experiments), conducted in the MIZ and the open waters of the Southern Ocean, indicate that Fe addition will lead to increased phytoplankton biomass and greatly reduced concentrations of macronutrients (NO_3^- and PO_4^-), and provide strong support for the idea of Fe limitation of phytoplankton growth in the Southern Ocean (de Baar et al., 1990; Helbling et al., 1991; Martin et al., 1990, 1991; Sedwick and DiTullio, 1997). It is clear, however, that Fe-deficiency is but one of the many factors that regulate the temporal and spatial variations in phytoplankton productivity and biomass, particularly in regions such as the Ross Sea, where massive phytoplankton blooms occur predictably each season and are thought to be controlled, in part, by the increased vertical stability of the upper water column from melting sea ice (e.g., Smith et al., 1996, 2000). Multiple nutrient interactions in the Southern Ocean, of both macronutrients and micronutrients, also may influence the ecosystem composition and rates of phytoplankton growth in the Southern Ocean. For example, ammonium (NH_4^+) inhibition of NO_3^- utilization has long been considered an important factor preventing greater rates of NO_3^- -uptake in both Southern Ocean regions (Koike et al., 1986; Goeyens et al., 1991a, 1998a; Semeneh et al., 1998) and other non-productive HNLC regions (Price et al., 1994; Wheeler and Kokkinakis, 1990).

Recent experiments in the Southern Ocean have implicated zinc as a co-limiting nutrient, primarily through the regulation of silicic acid uptake by diatoms (Franck et al., 2000). Fe and Zn limitation also have been linked to increased Si:N depletion ratios and increased silicification of diatoms (Hutchins and Bruland, 1998; Takeda, 1998),

which are disproportionately represented in previous Fe-enriched blooms in both *in vitro* (e.g., Martin et al., 1989, 1994) and *in situ* experiments (e.g., Coale et al., 1996; Boyd et al., 2000). Although phytoplankton do not have an absolute zinc requirement during NO_3^- and NH_4^+ -uptake and assimilation, zinc is required for SiO_4^{4-} -uptake (Rueter and Morel, 1981; De La Rocha et al., 2000), and for the activities of the metallo-enzymes, carbonic anhydrase and alkaline phosphatase (e.g., Anderson et al., 1978). However, under Zn limitation, other elements may substitute for Zn in these enzymes both *in vivo* and *in vitro* (Price and Morel, 1990; Morel et al., 1994; Lee and Morel, 1995).

The crucial role of Fe in the bioenergetics of C and nitrogen (N) metabolism is well recognized (e.g., Morel et al., 1991; Sunda, 1989). Substantial amounts of Fe are required in both photosynthetic and respiratory electron transport chains (e.g., Raven, 1988), the synthesis of chlorophyll (Chereskin and Castelfranco, 1982), and the assimilation of NO_3^- . Theoretical calculations based on Fe-use efficiencies and cellular metabolic Fe demands predict that phytoplankton growing on NO_3^- require 60% more Fe than those growing on NH_4^+ (Raven, 1988, 1990). Recently, these greater cellular Fe requirements for NO_3^- growth have been demonstrated for laboratory cultures of diatoms (Maldonado and Price, 1996). The extra Fe is needed to reduce NO_3^- to NH_4^+ before it can be incorporated into amino acids. This process requires the assimilatory enzymes NO_3^- reductase (requires one atom of Fe) and nitrite reductase (requires five atoms of Fe), and either ferredoxin (an Fe-containing e^- donor) or flavodoxin (a non-ferrous e^- donor; La Roche et al., 1993; Doucette et al., 1996), plus the need for greater quantities of reducing power (8 mol e^- /mol N) derived from Fe-dependent photosynthetic redox reactions. Thus the preferential uptake of NH_4^+ over NO_3^- observed in presumably Fe-limited HNLC regions can be assumed to be a more energetically efficient strategy for obtaining N. Only a few studies have directly investigated the effects of Fe on both NO_3^- and NH_4^+ -uptake by natural assemblages. In earlier studies using waters

considered replete with NO_3^- , but deficient in Fe, the addition of Fe not only enhanced net phytoplankton growth and biomass, but also increased biomass-specific uptake rates of NO_3^- with no significant change in specific NH_4^+ -uptake rates (equatorial Pacific Ocean: Coale et al., 1996; Price et al., 1991, 1994; Cochlan and Kudela, 1996; subarctic Pacific Ocean: Boyd et al., 1996; Pacific region of the Southern Ocean: Timmermans et al., 1998a).

The nitrogenous nutrition of phytoplankton blooms in the MIZ of the Southern Ocean are generally considered to be dominated (at least initially during bloom development) by NO_3^- (Nelson and Smith, 1986; Smith and Nelson, 1990; Kristiansen et al., 1992; Bury et al., 1995) with a seasonal increase in the use of regenerated N (NH_4^+ and urea) as the austral summer progresses (e.g., Goeyens et al., 1991a, 1998a). This seasonal transition in nitrogenous utilization has been suggested to be the result of a number of factors including the increasing availability of NH_4^+ in the water column, the change from diatom- to flagellate-dominated assemblages, and the increased deficiency in biologically available Fe (e.g., Arrigo et al., 1999).

The purpose of the present study was to investigate the NO_3^- and NH_4^+ -uptake response to variable additions of Fe and Zn in two contrasting regions of the Ross Sea—a typical HNLC region located far offshore in deep waters where blooms do not normally develop (Blue), and a region located closer to the Ross Ice Shelf where massive phytoplankton growth is expected each austral spring (Orca; Smith et al., 1996, 2000; Smith and Gordon, 1997). Unlike most previous studies, which have relied on the decline of ambient NO_3^- within experimental bottles to estimate N-uptake by phytoplankton, the experiments described in the present study employ ^{15}N tracer techniques to provide a direct measure of NO_3^- and NH_4^+ utilization by the natural planktonic communities as a function of trace metal (TM) enrichment over time. These Ross Sea studies were conducted as part of the US JGOFS Antarctic Environment and Southern Ocean Process Study (AESOPS) during austral summer 1997.

2. Materials and methods

2.1. Sample collection

Sampling was conducted in the Ross Sea, Antarctica aboard the R.V.I.B. *Nathaniel B. Palmer* during the US JGOFS AESOPS Process Cruise NBP 97-1 (01/12/97–02/11/97; austral summer, late bloom period, ice-free). Two sites were sampled for TM enrichment experiments: an offshore HNLC station—Blue (74.3°S, 176°W) and a continental shelf station—Orca (76.5°S, 178°W; Fig. 1). Ammonium inhibition and uptake experiments were conducted at a third station, Emperor (77.99°S, 176.05°E). Environmental conditions for each station are given in Table 1. Discrete samples were collected from the mid mixed-layer depth (25 m) using the ultra-clean water collection system of the Moss Landing Marine Laboratories: 30-l Teflon[®]-coated Go-Flo bottles (General Oceanics, Miami, FL) deployed on a Kevlar[®] hydroline, and tripped with a Teflon messenger. Go-Flo samples were transferred to well-rinsed, acid-cleaned 20-l polycarbonate carboys (Nalgene[®]) within a filtered-air,

class-100 clean van; this and all subsequent manipulations were conducted using TM clean techniques (Fitzwater et al., 1982). Handling of the 20-l carboys is described in Coale (1991).

2.2. Experimental procedures

Iron (FeCl_3) was added as small-volume injections (ca. 200–250 μl) to the carboys to obtain final concentrations of 0.16, 0.32, and 1.50 nM Fe (duplicate controls=no additions); Zn as ZnCl_2 was added alone (Orca: 4.47 nM and Blue: 6.31 nM) and with Fe (Orca: 6.20 nM Zn + 1.25 nM Fe; Blue: 6.53 nM Zn + 1.64 nM Fe). The carboys were placed in an on-deck incubator maintained at a photosynthetic photon flux density (PPFD) of ca. 40% of incident flux and the approximate in situ temperature using running surface seawater. The incubations were conducted at optimal light irradiance (40% surface irradiance; Hu and Smith, 1998) to distinguish a clear response to TM enrichment. N-uptake experiments were conducted on days 2.7, 5.7, 9.8, and 12.7 at Blue, and days 2.0, 5.8, and 8.0 at Orca after the initial TM enrichments. At each sampling

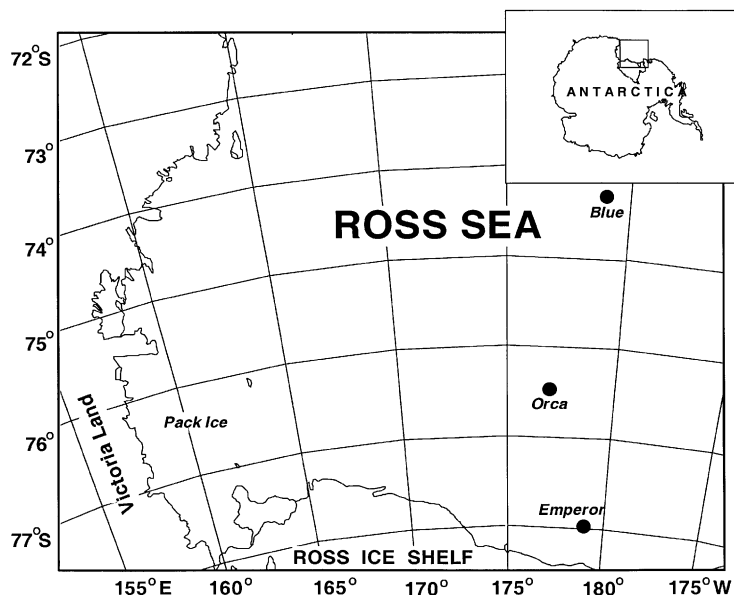


Fig. 1. Map of the study area and station locations in the western Ross Sea where N-uptake experiments were performed in January–February, 1997.

Table 1

Initial environmental conditions of seawater collected for trace-metal enrichment experiments at Orca (inshore) and Blue (HNLC; offshore), and the NH_4^+ inhibition experiment at Emperor in the Ross Sea during the AESOPS austral summer cruise NBP97-1. Metals are reported as dissolved concentrations ($<0.4 \mu\text{M}$)

Station name and number	Latitude Longitude (°)	Sampling date	Depth (m)	Temp. (°C)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	PC:PN (atomic)	Fe (nM)	Zn (nM)	Ambient concentration (μM)		
									NH_4^+	NO_3^-	SiO_4^{4-}
Stn. Orca No. 8	76.502S 178.008W	01/20/1997	25	-0.9	2.31	7.8	0.04	2.06	0.02 ^a	21.6	71.3
Stn. Blue No. 12	74.3247S 175.9877W	01/26/1997	25	-0.8	0.52	6.5	0.03	2.15	0.14 ^a	26.9	63.6
Stn. Emperor No. 23	77.9921S 176.0498W	02/04/1997	5.3	-0.4	1.12	—	0.04 ^b	0.43 ^b	0.13	11.8	64.7

^a Determined from sampling the control carboys at the beginning of the first ^{15}N -uptake experimental period (day 2.0 for Orca; day 2.7 for Blue).

^b Determined from a separate cast at 20 m.

point, samples were transferred into acid-washed, TM-clean, 1.2-l polycarbonate bottles, and $^{15}\text{N-NH}_4\text{Cl}$ (98.85 atom %; Cambridge Isotopes) and $^{15}\text{N-NaNO}_3$ (98.25 atom %) were added to separate incubation bottles. The isotopes were added at tracer levels ($<10\%$ of the ambient concentration) for NO_3^- and $0.10 \mu\text{M}$ for NH_4^+ . Enrichments were done within a HEPA laminar-flow hood. Inoculated bottles were hermetically heat-sealed within duplicate polyethylene bags, and incubated for 6–8 h in a Plexiglas[®] deck incubator at in situ temperature, with PPFD attenuated to ca. 50% of incident flux using neutral density film (Cinemills).

The potential suppressive effects of NH_4^+ on the rate of NO_3^- -uptake were determined at Emperor. Although this station was located closer to the Ross Ice Shelf than either Orca or Blue, ambient biomass, nutrient concentrations, and planktonic species composition were very similar to those observed at Orca. Duplicate 1.21 bottles from Emperor were inoculated with un-enriched NH_4Cl at a range of initial substrate concentrations (0.05, 0.10, 0.20, 0.40, 0.75, 0.99, 1.97, 4.93 and $9.86 \mu\text{M}$), in addition to tracer-level concentrations of $^{15}\text{NO}_3^-$, and incubated for 11 h as noted above. Ammonium-uptake kinetic experiments were conducted concurrently with similar concentrations of

$^{15}\text{N-NH}_4\text{Cl}$, and are described in Cochlan and Bronk (2001).

2.3. Analytical methods

Samples for determination of ambient concentrations of NO_3^- and SiO_4^{4-} were stored in 30-ml polyethylene bottles, and analyzed fresh with a Technicon AutoAnalyzer II as described in Wood et al. (1967) and Armstrong et al. (1967), respectively. Duplicate NH_4^+ samples, were collected directly into 60-ml polypropylene centrifuge tubes (Corning[®]) and stored refrigerated after addition of the phenolic reagent; the addition of the phenolic reagent binds NH_4^+ and eliminates the need to freeze samples. Within 24 h the remaining reagents were added, and the samples manually analyzed using a spectrophotometer equipped with a 10-cm cell (Solórzano, 1969). Duplicate urea samples were collected as above, initially frozen at -80°C , and subsequently thawed at room temperature before manual analysis using the diacetyl monoxime thiosemicarbazide technique (Price and Harrison, 1987), modified to account for a longer (30 min.) and lower digestion temperature (85°C). Although others have found that freezing may cause decreases in urea concentration (Mulvenna and Savidge, 1992), tests

conducted with seawater standards of known concentrations demonstrated no such losses in agreement with Price (1987). Samples for chlorophyll *a* (chl *a*) were filtered onto Whatman® GF/F filters, extracted for ca. 24 h in 7 ml of 90% acetone (–20°C), and analyzed for chl *a* and phaeopigments by *in vitro* fluorometry using a recently calibrated Turner Designs 10-AU fluorometer (Parsons et al., 1984).

Samples for ^{15}N analysis were collected by filtration (<80 mm Hg) onto pre-combusted Whatman® GF/F filters (2.5 cm; 4 h at 450°C), and frozen in polypropylene cryovials until analysis ashore with a Europa Scientific Robo-Prep Tracer mass spectrometer. To correct for isotopic dilution of $^{15}\text{NH}_4^+$ by $^{14}\text{NH}_4^+$, GF/F filtrates of the $^{15}\text{NH}_4^+$ -uptake experiments were analyzed for NH_4^+ concentration and isotopic enrichment at the end of all NH_4^+ incubations. Solid-phase extraction (spe) reverse-phase columns (Supelco Supelclean LC-18) were used to concentrate the NH_4^+ in the aqueous fraction using a modification of the techniques used by Selmer and Sorensson (1986) and Brzezinski (1987).

Phytoplankton biomass values (as carbon) were calculated from cell counts and dimensions following the protocol outlined by Chavez et al. (1991). Samples were preserved with 1% glutaraldehyde, stained with 4,6-diamidino-2-phenylindole (DAPI), and filtered onto black, 0.8- μm polycarbonate filters. Filters were mounted on glass slides with immersion oil, and frozen until analysis ashore. At least 2000 cells were enumerated at 1000 \times magnification for each sample using an Olympus BH-2 epifluorescent microscope. Biovolumes estimates were determined from microscopic measurements of cellular linear dimensions and using volume equations of appropriate geometric shapes; published conversion factors were used to convert these estimates to carbon biomass (Booth, 1987; Chavez et al., 1991).

2.4. Calculations and statistics

Absolute and biomass specific (N taken up per unit particulate N) uptake rates were estimated from ^{15}N accumulation in the particulate N (PN), and calculated using Eqs. (3) and (5), respectively,

of Dugdale and Wilkerson (1986). These N-uptake rates have been corrected for isotopic dilution according to Glibert et al. (1982a) using the average aqueous enrichment term (Laws, 1984). Although dissolved organic N (DON) release has been shown to impact measured rates of N-uptake (Bronk et al., 1994), insufficient sample volume prevented measurement of DON concentrations and DON atom % enrichment. Therefore, these uptake rates should be considered net uptake rates in that they have not been corrected for potential losses of ^{15}N to the DON pool (Bronk et al., 1994). Due to the limited water available for ^{15}N experiments, N-uptake rates were not determined in duplicate. However, during the same cruise and area of study, duplicate N-uptake rates were determined for both NO_3^- and NH_4^+ at the same level of isotopic enrichment during N-uptake kinetic experiments (Cochlan and Bronk, 2001). The precision of N-uptake rates, as estimated from the coefficient of variation (CV) of duplicate samples (CV = standard deviation as a percentage of the mean), averaged 5.8% (range 0.05–15.6%, $n = 10$ pairs) and 11.7% (range 0.8–21.8%, $n = 14$ pairs) for specific and absolute N-uptake rates, respectively. If one assumes this level of precision for the N-uptake rates determined during the TM experiments, then duplicate rates displayed graphically in Figs. 3–6 would generally be within the size of the symbols employed.

In the present study the NH_4^+ -uptake rates may be overestimated due to the concentration-dependent effects of the 0.10 μM inoculum of ^{15}N - NH_4^+ used during each incubation experiment. However, there were generally little or no difference in the ambient NH_4^+ concentrations between control and TM-amended samples during each time period, so any observable differences in uptake rates are still be attributable to the effects of TM addition. It is possible to evaluate the degree of overestimation due to substrate enrichment by using kinetic parameters calculated for NH_4^+ -uptake ($V_{\text{max}} = 4.826 \times 10^{-3} \text{ h}^{-1}$, $K_s = 0.13 \mu\text{M}$; Cochlan and Bronk, 2001) determined at a similar station (Emperor) in the western Ross Sea during the same period. During the initial sampling of Orca, when NH_4^+ concentrations were $\leq 0.01 \mu\text{M}$, 2–3-fold artifactual enhancements of NH_4^+ -uptake

rates can be expected; however, enhancements averaged only 50% and 22% during days 5.8 and 8.0, when the average NH_4^+ concentrations for the control and TM-amended samples were 0.09 and 0.17 μM , respectively. At Blue, where initial NH_4^+ concentrations were elevated (0.15 μM), the enhancements due to substrate enrichment are relatively minor (22%), but averaged 115% (range: 72–183%) during the remainder of the grow-out experiment due to the low concentrations of NH_4^+ present in the control and TM-amended samples.

Statistical analyses were conducted using Minitab[®] 12 statistical software (Minitab Inc., Copyright, 1997), and were carried out using either two-way ANOVA for balanced data (equal representation of observations) or General Linear Model procedures for unbalanced data. All data were tested for adherence to the assumptions of parametric analysis (normality of distribution and homogeneity of variances) prior to their analyses. Michaelis–Menten parameters: half-saturation constants (K_s) and maximum uptake velocities (V_{\max}) were estimated from a direct, non-linear curve-fitting model (Kalidograph[®], Abelbeck Software) to the Michaelis–Menten equation for enzyme kinetics, $V = V_{\max}S/(K_s + S)$, where S is the ‘total’ dissolved Fe concentration (ambient + added Fe) measured at the initiation of the TM enrichment experiments. Therefore, these parameters should be termed ‘apparent’ due to the uncertainty in the concentration of Fe present when the actual N-uptake experiments were conducted. Ammonium inhibition of NO_3^- -uptake was modeled with a 3-parameter exponential equation according to Varela and Harrison (1999).

3. Results and discussion

On-deck incubation experiments have been used previously in the Southern Ocean to study the response of neritic (e.g., Helbling et al., 1991; Martin et al., 1990; Sedgewick and DiTullio, 1997) and oceanic (e.g., de Baar et al., 1990, 1995; Scharek et al., 1997; van Leeuwe et al., 1997) phytoplankton to TM enrichment. A common feature of these and the present experiments is the considerable growth of phytoplankton that occurs

in both the TM-amended bottles and the un-enriched controls, following a lag phase of minimal phytoplankton growth (neither significant NO_3^- decline nor biomass accumulation), which can vary significantly in duration depending on the initial biomass and physiological status of the phytoplankton assemblages. The contrasting conditions of the two study sites sampled in the Ross Sea during late austral summer enable us to examine the role of Fe in the planktonic nitrogenous nutrition in two fundamentally different oceanographic regimes; an oligotrophic deep oceanic site with very low planktonic biomass, and a coastal site in the southern Ross Sea where phytoplankton blooms are a regular, seasonal occurrence (e.g., El Sayed et al., 1983; Arrigo and McClain, 1994; Arrigo et al., 1999; Smith and Nelson, 1985; Smith et al., 1996, 2000).

Growth rates, determined from the depletion of NO_3^- , PO_4^- and SiO_4^{4-} , as well as the accumulation of particulate matter (chl *a*, and particulate C and N) over time, can be used to estimate the phytoplankton response to the various TM treatments, and will be presented elsewhere (Coale et al., in prep). Here we wish to focus on the effects of TM enrichments on the utilization of NO_3^- and NH_4^+ , the latter of which cannot be estimated from net disappearance due to its simultaneous uptake and regeneration, which occurs within the incubation carboys over these periods. During the ^{15}N -uptake experiments described below, the effects of simultaneous uptake and regeneration of NH_4^+ have been determined, and the resultant uptake rate estimates have been corrected for the effects of heterotrophic regeneration of NH_4^+ .

3.1. Biomass and dissolved inorganic N changes

Blue is located in deep waters (>1500 m), 570 km east of Victoria Land (Fig. 1). The surface waters at Blue are a considerable distance from both the bottom sediments and ice/land-Fe sources (Fig. 1). The low initial chl *a* concentration (0.52 mg m^{-3}) and high ambient NO_3^- concentrations (close to the maximal values observed in Southern Ocean surface waters; US JGOFS data base) are indicative of a HNLC region. There was a substantial lag phase (ca. 4–5 days) before

differences in the phytoplankton response between the TM-enriched and control treatments were observed (Fig. 2A and C). However, by the end of the 13-day experiment phytoplankton biomass (chl *a*) in those carboys enriched with the largest Fe concentrations (1.50 nM Fe and 1.64 nM Fe + 6.53 nM Zn) were 4–5-fold greater than the controls (Fig. 2C). Similarly, ambient NO_3^- concentrations only declined by $5 \mu\text{M}$ (<20%) in the control, but decreased by ca. 18–20 μM (ca. 70%) at the greatest Fe enrichments (Fig. 2A). At Blue, the initial NH_4^+ concentrations were elevated (mean: $0.15 \mu\text{M}$; range: 0.14 – $0.17 \mu\text{M}$) and declined to an average concentration of $0.06 \mu\text{M}$ (range: 0.04 – $0.08 \mu\text{M}$) on day 5.7, and $0.03 \mu\text{M}$ (range: 0.01 – $0.04 \mu\text{M}$) by day 9.7. During the 3-day interval before the final sampling, NH_4^+ concentrations rose ca. $0.06 \mu\text{M}$ resulting in an

average concentration of $0.09 \mu\text{M}$ (range: 0.03 – $0.14 \mu\text{M}$) on day 12.8.

Station Orca was considerably shallower (600 m) and closer to the Ross Ice Shelf (240 km closer than Blue; Fig. 1). The lag phase was shorter at Orca (<2 days), but the differences in the phytoplankton response between the TM-enriched samples and control treatments were not as large as those observed at Blue. The maximal chl *a* concentrations were only 3-fold greater than the control on the eighth day of the experiment in those bottles enriched with the largest Fe concentrations (1.50 nM Fe and 1.25 nM Fe + 6.20 nM Zn; see Fig. 2D). The experiment was subsequently terminated due to exhaustion of NO_3^- in those bottle treatments (Fig. 2B). Initial NH_4^+ concentrations (day 2.0) were very low at Orca (generally at or below our methodological limit of detection

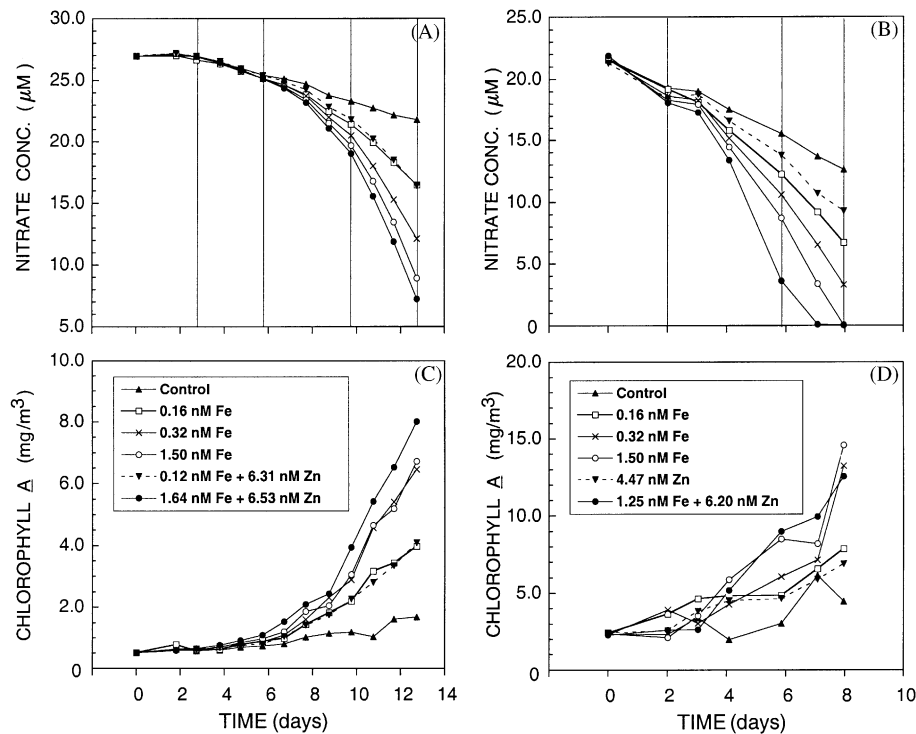


Fig. 2. Nitrate (A and B) and chl *a* (C and D) concentrations as a function of time in the TM-enrichment experiments conducted at the HNL site (Blue; A and C) and the coastal continental shelf site (Orca; B and D) in the western Ross Sea. Note the exhaustion of NO_3^- by days 7–8 for the higher Fe enrichments at Orca. Vertical lines in A and B indicate when short-term N-uptake experiment were conducted.

(0.02 μM) in both control and TM-amended samples. Average concentrations increased to 0.09 μM (range: 0.06–0.16 μM) on day 5.8, and to 0.17 μM (range 0.11–0.23 μM) on the final sampling (day 8.0).

Although only trace concentrations of dissolved Fe were measured at both stations at the initiation of these experiments (0.03–0.04 nM Fe), clearly there was adequate Fe to support previous growth of phytoplankton at Orca, as evidenced by the elevated biomass (chl *a* = 2.3 mg m^{-3}) and lower ambient NO_3^- concentration (21.6 μM) found in these surface waters (Table 1). This is not surprising given the shallowness of the region, and its closer proximity to the Ross Ice Shelf, an area where katabatic winds prevail.

3.2. Absolute uptake rates of NO_3^- and NH_4^+

The N-uptake rate data, calculated from ^{15}N accumulation in the particulate fractions, can be used to estimate both absolute (transport) rates and biomass (PN) specific uptake rates of N. Since absolute uptake rates are a function of both total cellular biomass and the physiological rate processes of the cells (Dugdale and Wilkerson, 1986), these N-uptake rates must be normalized to biomass before they can be used as indicators of physiological response to Fe. Although others have advocated the use of chl *a* as the biomass normalization parameter of choice (Garside, 1991; Dickson and Wheeler, 1995), we feel that PN is more appropriate due to the known effects of Fe limitation on chl *a* content per cell (e.g., Sunda and Huntsman, 1995), and thus our specific N-uptake rates are reported relative to PN. However, the trends observed in response to Fe enrichment were readily apparent regardless of the normalization parameter employed.

At Blue, the absolute NO_3^- -uptake rates do not differ substantially in the Fe-treatment carboys from the rates observed in the controls early in the experiment; (i.e., during the first 5.7 days; the NO_3^- -uptake rates measured in the 1.50 nM Fe treatments were only 1.4-fold greater than the control, Fig. 3). However, during the later two sampling periods, NO_3^- -uptake rates were 3.1 and 4.8-fold greater than the controls in the 1.50 nM

Fe treatment. Rates of NO_3^- -uptake were significantly correlated to the total dissolved Fe concentrations measured at the start of these enrichment experiments ($P < 0.10$, and $P < 0.05$ if 1.64 nM Fe + 6.53 nM Zn treatments are included in the correlation analyses). Although absolute NO_3^- -uptake rates increased 4-fold in the control bottles over the 10-day sampling period, the increase from day 2.7 to day 12.7 was considerably greater in the Fe-enriched bottles (e.g., 14-fold increase in the 1.50 nM Fe treatment).

All NH_4^+ -uptake rates have been corrected for isotope dilution as a result of NH_4^+ regeneration during the incubation (Glibert et al., 1982a). Previous studies to evaluate the effects of Fe enrichment on planktonic nitrogenous nutrition have failed to correct for this source of error, thereby potentially underestimating the use of NH_4^+ by phytoplankton in HNLC regions, including the equatorial (Price et al., 1991, 1994) and subarctic Pacific (Boyd et al., 1996), and the Pacific region of the Southern Ocean (Timmermans et al., 1998a). As reported for NO_3^- -uptake rates, the NH_4^+ -uptake rates increased in both the control and Fe-enriched samples over time, but the increase was greater in those bottles enriched with Fe (e.g., NH_4^+ -uptake rates increased 9.4-fold in the 1.50 nM Fe treatment, compared to a 1.2-fold increase in the control over time; Fig. 3). However, absolute NH_4^+ -uptake rates increased less than NO_3^- rates, and only minimal increases were observed due to Fe-treatment until the final day of sampling when the NH_4^+ -uptake rate measured in the 1.50 nM Fe treatment was 3-fold greater than the rate measured in the control. Absolute rates of NH_4^+ -uptake were never significantly ($P > 0.05$) correlated to the total dissolved Fe concentrations measured at the start of the experiments (Fig. 3).

At Orca, absolute rates of both NO_3^- and NH_4^+ were consistently greater than those observed at Blue, due in part to the > 4-fold higher concentrations of phytoplankton (Table 1). As seen at Blue, there were also lag periods before any substantial increases were observed in the absolute uptake rates of both NO_3^- and NH_4^+ , relative to their corresponding controls (Fig. 3). However, compared to Blue, these increases were observed earlier

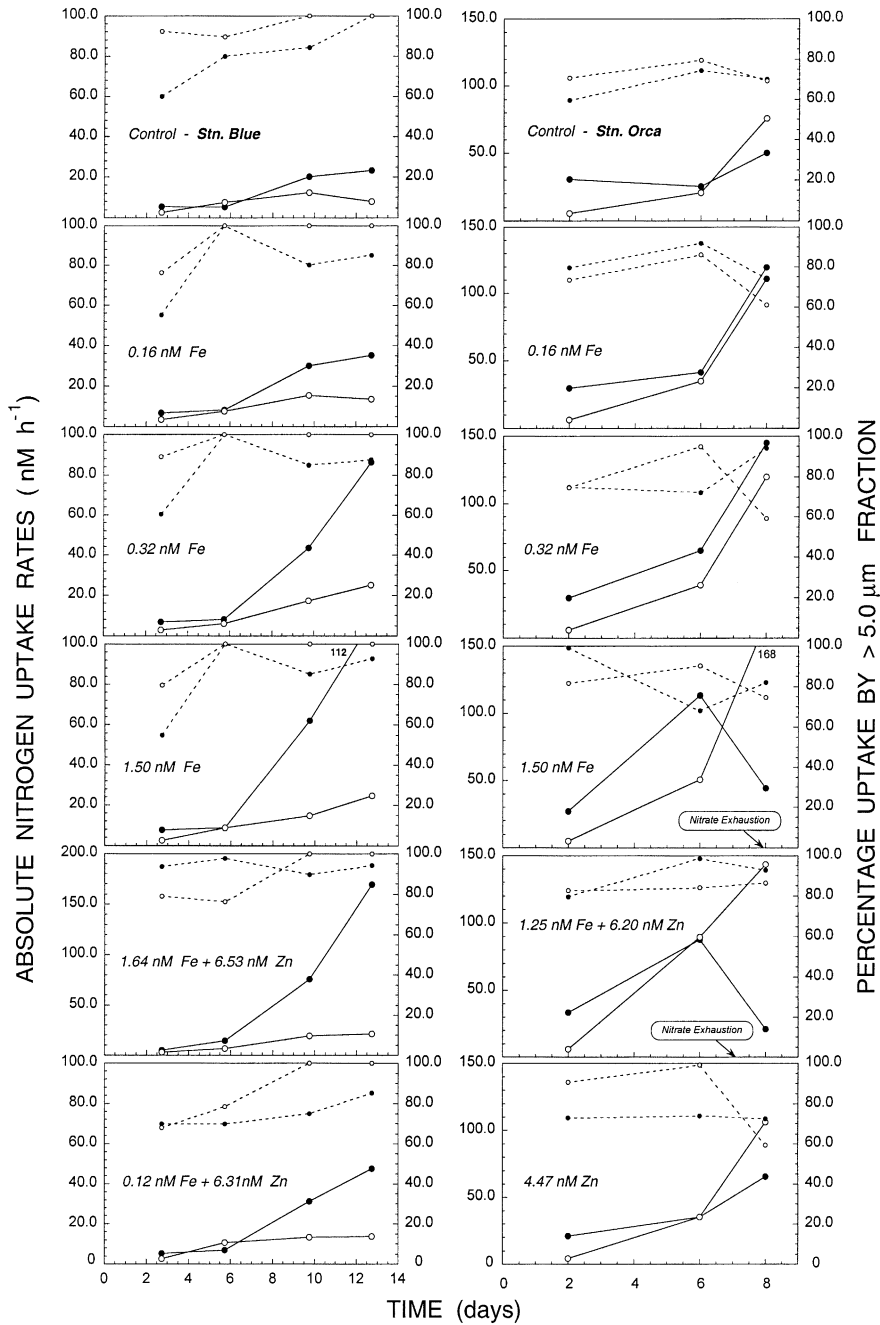


Fig. 3. Absolute uptake rates of NO₃⁻ (●) and NH₄⁺ (○) as a function of time at the HNLc site (Blue; left column) and the continental shelf site (Orca; right column) for the control and TM-amended experiments, initially enriched at time zero. Dashed lines indicate the percentage N-uptake by the > 5.0 μm phytoplankton for each N substrate.

(day 5.8 versus day 8.0). By day 5.8, the absolute uptake rate of NO_3^- measured in 1.50 nM Fe treatment was 4.5-fold greater than in the control. Absolute NO_3^- -uptake rates were significantly correlated to the total dissolved Fe concentrations measured at the start of the enrichment experiments for both of the latter sampling periods ($0.05 < P < 0.10$; Fig. 5).

In contrast to Blue, absolute NH_4^+ -uptake rates were also significantly correlated to Fe enrichment level ($P < 0.10$) during both of the latter sampling periods, and maximal uptake rates observed for the 1.5 nM Fe-treatments averaged 2.3-fold greater than control samples (Fig. 6). Both NO_3^- - and NH_4^+ -uptake rates increased as a function of time, and whereas this increase was relatively modest for NO_3^- -uptake (1.6-fold increase for controls from day 2.0 to day 8.0), the NH_4^+ -uptake rates increased 14-fold in the controls at Orca, and a total of 24-fold at the highest Fe treatment (Fig. 6). Ambient NH_4^+ concentrations in both control and TM treatments also increased over the duration of the incubation experiment from an average concentration of $< 0.01 \mu\text{M}$ on day 2.0, to $0.17 \mu\text{M}$ on day 8.0, and may have contributed to the increase in the rates of NH_4^+ -uptake realized, and/or the inhibition of NO_3^- -uptake (see discussion below). At Blue systematic increases in ambient NH_4^+ concentrations were not observed in either control or TM treatments, but concentrations did increase during the final 3-day sampling interval by an average of $0.06 \mu\text{M}$ (range 0.02– $0.10 \mu\text{M}$), resulting in an average NH_4^+ concentration of $0.09 \mu\text{M}$ on day 12.8.

3.3. Nitrogen regeneration

Rates of NH_4^+ regeneration increased through time at Orca with rates on day 8.0 being 9–23 times higher than on day 2.0. On days 5.8 and 8.0 there was also a slight trend of NH_4^+ regeneration rates increasing with an increase in Fe enrichment; on day 8.0 the rates in the control were 78.04 nM h^{-1} , but 231.8 nM h^{-1} in the 6.20 nM Zn + 1.25 nM Fe treatment. At Blue, rates of NH_4^+ regeneration increased from day 2.7 to 5.7, but then decreased from day 5.7 to 12.7, and there was no consistent trend for NH_4^+ regeneration and Fe enrichment.

Another important form of regenerated N is DON. It has been shown that during the course of ^{15}N -uptake incubations, a fraction of the ^{15}N label can be released as DON (e.g., Bronk and Glibert, 1991). This release results in an underestimate in the total amount of N taken up by the cell (Bronk et al., 1994). In the present study, DON release rates were not quantified due to severe limitations in sample volume. The uptake rates presented in this paper therefore should be considered net uptake rates in that they are a measure of the amount of N taken up by cells that remains within the cell at the end of the incubation. These are in contrast to gross uptake rates, which are a measure of the N taken up by the cell, corrected for any loss of N to the DON pool (Bronk et al., 1994).

Although we did not measure DON release directly, three lines of evidence suggest that DON release likely occurred in our incubations. First, DON release has been shown to correlate closely with rates of NH_4^+ regeneration; presumably because both processes are impacted by grazing. During size-fractionation experiments off California, DON release was found to be approximately half that of NH_4^+ regeneration in Monterey Bay and in the Southern California Bight ($r^2 = 0.80$, $n = 29$; Ward and Bronk, 2001); however, during another study in the Southern California Bight, DON release was approximately 10% of NH_4^+ -regeneration ($r^2 = 0.73$, $n = 18$; Bronk and Ward, unpublished data). In the present study, the increase in NH_4^+ regeneration rates through time at Orca suggests that DON release, and therefore gross N-uptake, increased through time as well. Likewise, the lack of a consistent trend in rates of NH_4^+ regeneration and Fe enrichment at both Orca and Blue is circumstantial evidence that DON release also did not respond to Fe enrichment.

Second, ^{15}N mass balances can provide clues to whether DON release was substantial. If there is a loss of ^{15}N during the course of an experiment it suggests that label was transferred to an unmeasured pool—potentially DON. Based on ^{15}N mass balances, there was more ^{15}N unaccounted for at the end of the incubations when NH_4^+ was the measured substrate (29–55% missing at Orca; 32–75% missing at Blue) relative to when NO_3^-

was the measured substrate (0–16% missing at Orca; 2–10% missing at Blue). The large amount of ‘missing ^{15}N ’ in the NH_4^+ incubations is likely due to the incorporation of label by bacteria, (Bronk and Glibert, 1994) which pass through combusted GF/F filters that retain an average of 81% of the heterotrophic bacteria found within the euphotic zone of the Ross Sea (Cochlan and Bronk, 2001). More importantly, however, there was no clear trend between the amount of missing ^{15}N and trace-metal addition.

Third, one can also compare the decrease in dissolved inorganic N concentrations with the increase in the concentration of PN during an incubation period as circumstantial evidence for DON release. Similar to the ^{15}N mass-balance approach, if the decrease in NO_3^- through time is greater than the increase in PN, it suggests that the NO_3^- taken up was converted to some other form—such as DON; this procedure is not applicable for NH_4^+ because ambient NH_4^+ concentrations are very low and NH_4^+ regeneration masks net changes over time. At Orca, a mean of 26–54% of the NO_3^- decrease went into a N pool other than PN from day 2.0 to 5.8; this decreased to 0–20% from day 5.8 to 8.0. At Blue, the amount of NO_3^- transferred to an unmeasured pool averages 21, 51, and 37 on days 5.7, 9.8 and 12.7, respectively. However, there was no trend between the percentage of NO_3^- going into another N pool and TM enrichment at either station.

3.4. Specific uptake rates of NO_3^- and NH_4

The specific uptake rates of NO_3^- reported for both Blue and Orca prior to Fe enrichment are very slow, and similar in magnitude to the initial pre-enrichment rates reported for the HNLC regions of subarctic Pacific (ca. 0.005 h^{-1} ; Boyd et al., 1996), equatorial Pacific (ca. $<0.010\text{ h}^{-1}$; Price et al., 1994), and Southern Ocean regions (e.g., $0.001\text{--}0.005\text{ h}^{-1}$, van Leeuwe et al., 1997; Goeyens et al., 1998a; Timmermans et al., 1998a) as well as uptake rates determined during kinetic experiments conducted at the same time in the Ross Sea (Cochlan and Bronk, 2001). These low NO_3^- -uptake rates confirm the observation of Dugdale and Wilkerson (1991), in their analysis

of pre-1991 N-uptake studies, that low specific uptake rates of NO_3^- are a common phenomenon in HNLC waters, and indicate that coastal Southern Ocean MIZ regions are also characterized by these lower specific rates, at least temporally, during non-bloom periods when phytoplankton growth appears to be limited by Fe availability.

The specific NO_3^- -uptake rates, at the HNLC site Blue and the coastal bloom site Orca, are clearly enhanced by Fe enrichment relative to control samples (Figs. 4–6). At Blue, initially the specific NO_3^- -uptake rates for the Fe-treatments did not differ substantially from the uptake rates observed in the controls (Fig. 5C). It is not until the later sampling periods (days 9.8 and 12.7), that the specific NO_3^- -uptake rates measured in the 1.50 nM Fe treatment were >1.9 -fold greater than the rates measured in the controls. However, during these later two sampling periods, NO_3^- -uptake rates also were significantly correlated to the total dissolved Fe concentrations measured at the start of these enrichment experiments ($P<0.10$, and $P<0.02$ if 1.64 nM Fe + 6.53 nM Zn treatments are included in the correlation analyses; Fig. 5C). Specific NO_3^- -uptake rates were variable in the control bottles, but they did not systematically increase with incubation time. Similar results were observed at Orca, with no evidence of Fe enhancement of NO_3^- -uptake rates until the latter sampling periods. By day 5.8, the specific NO_3^- -uptake rate measured in the 1.50 nM Fe treatment was >2.6 -fold greater than the rate measured in the control sample, and NO_3^- -uptake rates were significantly correlated to the total dissolved Fe concentrations measured at the start of the experiments ($P<0.05$, and $P<0.02$ if 1.25 nM Fe + 6.20 nM Zn treatments are included in the correlation analyses; Fig. 6C).

These results of enhanced specific NO_3^- -uptake rates are in direct contrast with the conclusion reached by Dugdale and Wilkerson (1990, 1991) in their analyses of NO_3^- -depletion data for similar deck incubation experiments conducted in the Ross Sea during January–February, 1990 by Martin et al. (1990), but are supported by the more recent results of other $^{15}\text{NO}_3^-$ -Fe-enrichment studies conducted in the low Fe concentrations oceanic waters of the Antarctic Circumpolar

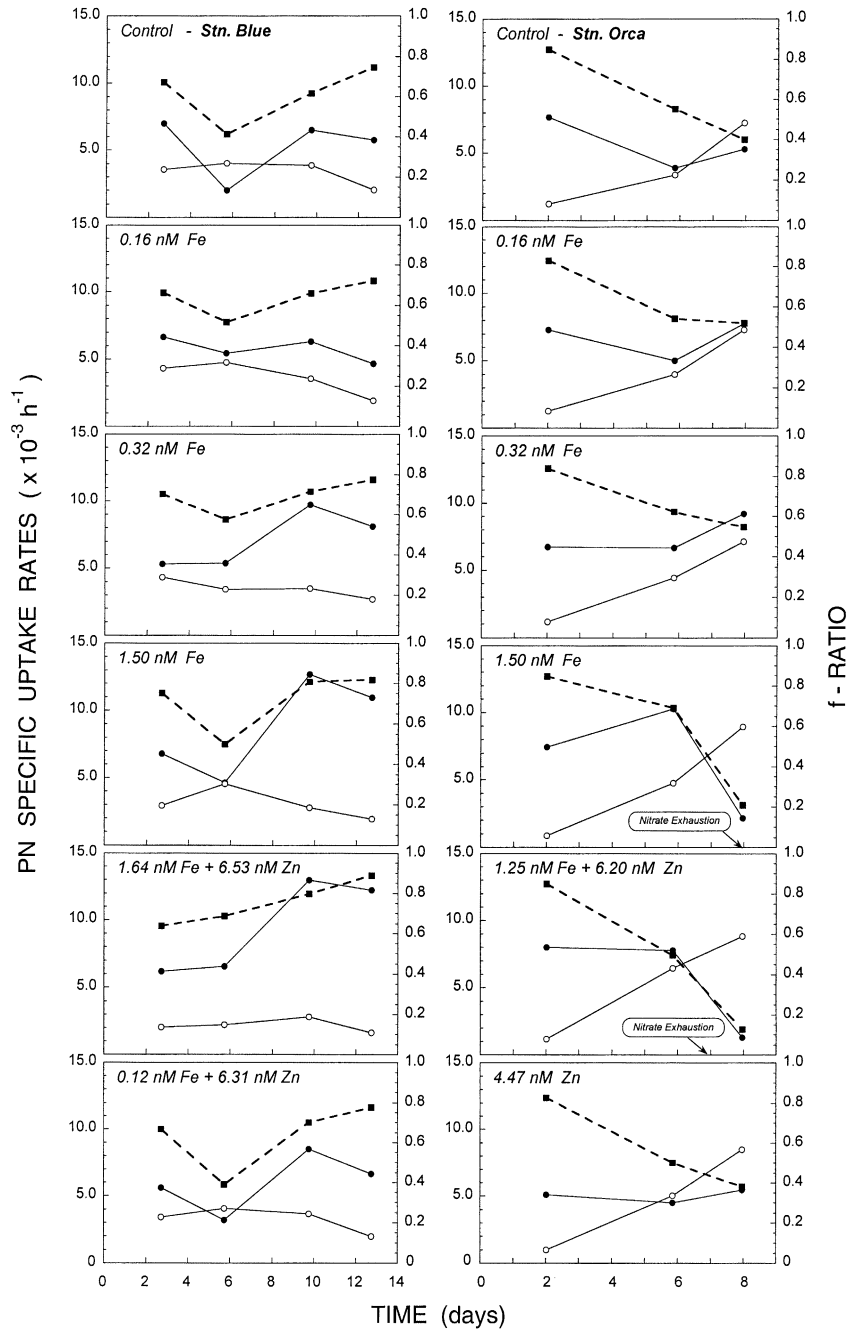


Fig. 4. Nitrogen-specific uptake rates of NO₃⁻ (●) and NH₄⁺ (○) as a function of time at the HNLC site (Blue; left column) and the continental shelf site (Orca; right column) for the control and TM-amended experiments, initially enriched at time zero. Dashed line indicates the daytime *f*-ratio value determined for the total planktonic community from absolute N-uptake rates.

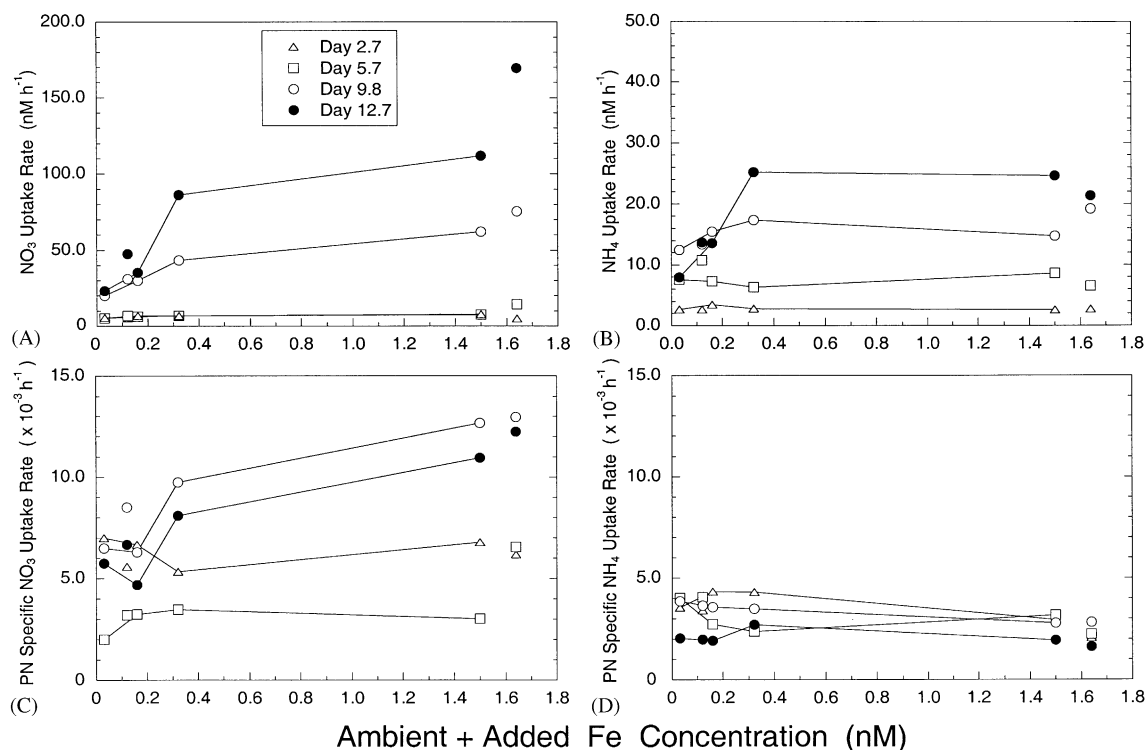


Fig. 5. Nitrogen uptake by natural assemblages of phytoplankton plotted as a function of total (ambient plus added) Fe concentration conducted on four separate occasions during the long-term incubation experiment at the HNLC station (Blue). (A) Absolute uptake rates of NO_3^- , (B) absolute uptake rates of NH_4^+ , (C) specific uptake rates (h^{-1}) of NO_3^- , and (D) specific uptake rates (h^{-1}) of NH_4^+ during short-term daytime incubations. Symbols unconnected by lines indicate zinc-enriched samples.

Current and the Weddell Sea (van Leeuwe et al., 1997), and HNLC waters north and south of the Polar Front in the Pacific sector of the Southern Ocean (Timmermans et al., 1998a). Similar enhancement of specific NO_3^- -uptake rates by Fe has also been shown for other HNLC regions including the equatorial (Price et al., 1991, 1994; Coale et al., 1996) and subarctic Pacific (Boyd et al., 1996, 1998).

Previously, the uptake rates of Fe (Morel et al., 1991; Price et al., 1994) and community growth rates (Fitzwater et al., 1996) of oceanic phytoplankton have been shown to follow Michaelis–Menten type kinetics. Comparison of the specific NO_3^- -uptake rates determined in the present study also can be described as a hyperbolic function of total dissolved Fe concentration, where the specific NO_3^- -uptake rate is proportional to the substrate (Fe) concentration at low concentrations, and

reaches a maximum (V_{\max}) at substrate concentrations that exceed the half-saturation constant (K_s). Least-squares estimates of the Michaelis–Menten equation by non-linear curve fitting results in V_{\max} estimates of 0.012 and 0.0092 h^{-1} for Blue and Orca, respectively, and an apparent half-saturation constant of 0.09 nM Fe for both regions (Fig. 7). These kinetic parameters represent the first estimates of NO_3^- -uptake as a function of Fe concentration for natural assemblages of phytoplankton in the Southern Ocean. They also demonstrate that since the ambient Fe concentrations in the surface waters of the western Ross Sea are considerably less than the half-saturation constant, the phytoplankton communities' ability to effectively utilize NO_3^- , which is found in abundance throughout the Southern Ocean, should be considered limited by the availability of Fe during austral summer.

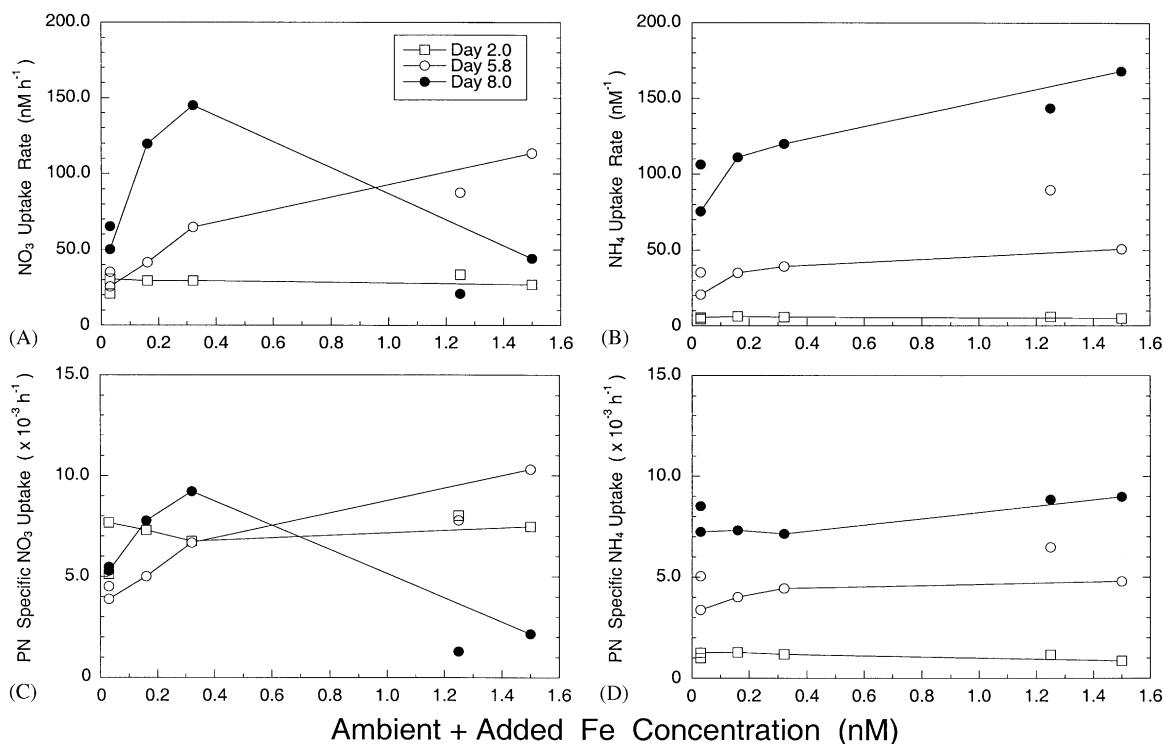


Fig. 6. As Fig. 5, except at the continental shelf station (Orca), and the N-uptake experiments were conducted on three separate occasions during the long-term incubation experiment.

The specific rates of NH_4^+ -uptake, unlike NO_3^- , did not increase with Fe enrichment or time at Blue, but generally declined (relative to the control bottles) with increasing Fe addition (Fig. 5D), despite sometimes slightly higher NH_4^+ concentrations in the TM-amended bottles. These results indicating no positive effect of Fe on specific rates of NH_4^+ , including a significant negative correlation ($P < 0.05$) with total dissolved Fe concentrations on day 9.8, are in agreement with the sole Fe-enrichment study to have measured $^{15}\text{NH}_4^+$ -uptake previously in the Southern Ocean (Timmermans et al., 1998a). They found that Fe had no effect on the specific uptake rates of NH_4^+ for samples collected south of the Polar Front during short-term (2–6 h) uptake experiments of samples enriched with 2 nM Fe for 27–29 h, but significant increases were observed for specific NO_3^- -uptake rates, averaging 30% (range 25–45%). Similarly, Fe enrichment has been reported to have no stimulatory effect on specific NH_4^+ -

uptake rates during deck incubation experiments conducted in HNLC regions of the equatorial (Price et al., 1991, 1994; Cochlan and Kudela, 1996) and subarctic Pacific (Boyd et al., 1996).

In contrast to Blue and previous HNLC studies described above, the specific rates of NH_4^+ increased with both Fe enrichment and time at Orca (Figs. 4 and 6D). The enhancement of specific NH_4^+ -uptake rates by Fe was not apparent until the two later sampling periods (days 5.8 and 8.0), where rates measured in 1.50 nM Fe treatments were on average 30% greater than rates measured in the controls. Additionally, during the final day NH_4^+ -uptake rates were significantly correlated to the total dissolved Fe concentrations measured at the start of these enrichment experiments ($P < 0.05$). The specific NH_4^+ -uptake rates for all treatments, including the controls, increased with incubation time and are likely the result of increasing NH_4^+ concentration over time in the 20-l carboys. The ambient NH_4^+ concentration

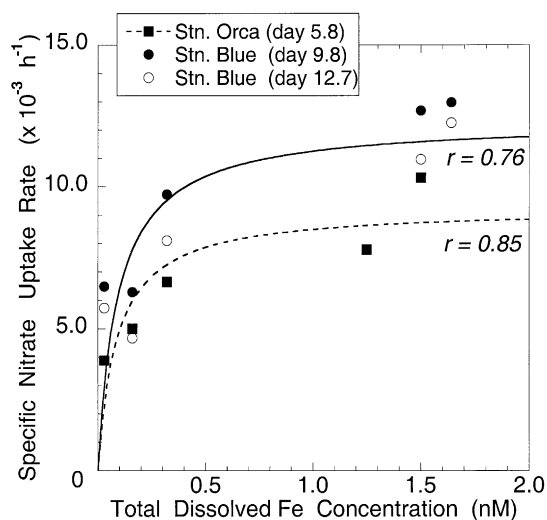


Fig. 7. Phytoplankton community NO_3^- -uptake rates (PN specific) as a function of dissolved Fe concentration demonstrating Michaelis–Menten type kinetics. A non-linear curve fit results in a V_{\max} of 0.012 and 0.0092 h^{-1} for stations Blue (HNLC site) and Orca (continental shelf site), respectively, and an average half-saturation (K_s) of 0.09 nM Fe for both stations. Zinc addition treatments were not included in the calculations, and rates from sampling days 9.8 (●) and 12.7 (○) were used in the estimation of kinetic parameters for Blue, but only rates from day 5.8 (■) were used for Orca due to NO_3^- exhaustion in some samples.

increased by an average of $0.16\text{ }\mu\text{M}$ (range $0.09\text{--}0.22\text{ }\mu\text{M}$) above the detection levels measured on day 2.0 to the final sampling on day 8.0 for TM-amended and control carboys (data not shown). Although such increases may seem modest, NH_4^+ -uptake kinetic experiments conducted during the same time in the Ross Sea by Cochlan and Bronk (2001) demonstrate that such an enrichment in ambient NH_4^+ could increase specific NH_4^+ -uptake rates by up to 10-fold. Such increases in ambient NH_4^+ , presumably from heterotrophic remineralization, grazing activity and cell lysis, have been reported previously during long-term incubation experiments (e.g., Boyd et al., 1996), and demonstrate a potentially serious limitation of this type of experiment where artifactual changes in one N substrate may mask the magnitude of a planktonic N-uptake response to TM enrichment.

Within 1 day of the start of the TM experiments at Orca, specific NH_4^+ -uptake rates also were

determined at a concentration ($10\text{ }\mu\text{M}$ of $^{15}\text{NH}_4^+$) considered saturating for its uptake. These rates, determined during similar short-term incubations conducted immediately after initial sampling from the Ross Sea, may not be directly comparable to the reported rates from the TM experiments, which were determined after multiple days of ship-board incubation. However, the saturated uptake rate (mean = $3.89 \times 10^{-3}\text{ h}^{-1}$) is less than those rates realized with Fe enrichment on days 5.8 and 8.0 when elevated ambient NH_4^+ concentrations were found in the bottles, suggesting that Fe stimulates NH_4^+ -uptake in excess of the purely substrate concentration effects, notwithstanding any potential changes in the species composition of the enclosed planktonic assemblage over time.

The experimental results made it possible to investigate the influence of total dissolved Fe concentration (Fe enrichment plus ambient Fe concentration) and time (days after Fe enrichment) on absolute and specific N-uptake rates by ‘Two-Way Analysis of Variance’ (Sokal and Rohlf, 1995); a procedure for investigating the influence of several experimentally controlled factors (Fe concentration and days after enrichment) and their interaction (synergistic effect) on a response variable (N-uptake rate). To fulfill the requirements and assumptions underlying this analysis, the following adjustments and tests were made. Owing to a lack of replication within the combinations of days and Fe concentrations, the data were first pooled over several day and Fe concentration treatments in order to evaluate the interactions of day \times Fe concentrations. For example, the earlier sampling periods (days 2.7 and 5.7 for Blue, and days 2.0 and 5.8 for Orca) were coded as 1, and the later days (days 9.8 and 12.7 for Blue, and day 8.0 for Orca) were coded as 2. Fe treatments were likewise coded: 0.03 and 0.16 nM Fe concentrations coded as 1, and 0.32 and 1.5 nM Fe concentrations coded as 2 for both stations. Statistical analyses were then carried out using either two-way ANOVA for balanced data (equal representation of observations within each combination of the two treatments) or General Linear Model procedures for unbalanced data (Minitab[®] 12 statistical software). The data were tested for adherence to the assumptions of parametric

Table 2

Summary of two-way analysis of the interaction between [Fe] treatment and the time (days) since the initial treatment for absolute and PN-specific uptake rates of nitrate and ammonium at Blue (HNLC region) and Orca (coastal region). See text for complete explanation of treatment codes. Normality of data distribution determined using the Anderson–Darling and/or Ryan–Joiner tests; homogeneity of variance with both Barlett's and Levene's tests

	Absolute uptake rates		Specific uptake rates		Analysis
	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	
<i>Blue</i>					
[Fe] treatment	NS	NS	NS	NS	ANOVA ^a
Days	0.006	0.002	0.006	0.016	"
Coded factors					
Coded days ^b	0.006	0.049	0.031	NS	"
Coded Fe treatment ^c	<0.001	<0.001	0.003	NS	"
Interaction (code 1 × code 2)	0.009*	0.041	0.027	NS	"
<i>Orca</i>					
[Fe] treatment	NS	NS	NS	NS	GLM ^d
Days	0.044	<0.001	NS	<0.001	"
Coded factors					
Coded days ^b	NS	NS	SIG ^e	NS	"
Coded Fe treatment ^c	0.016	<0.001	NS	<0.001	"

^a ANOVA = two-way analysis of variance.

^b Early vs. later in experiment.

^c Lower vs. higher [Fe].

^d GLM = general linear model.

^e SIG: 0.05 < *P* < 0.10.

* = non-normal distribution.

NS = not significant (*P* > 0.05).

analysis (normality of distribution and homogeneity of variances), and no serious violations were detected. As shown in Table 2, sufficient replication was obtained for the pooled data from Blue experiments to detect significant interactions between days and Fe treatment for both absolute uptake rates of NO₃⁻ and NH₄⁺, but only specific uptake rates of NO₃⁻. This indicates that the differences between Fe treatment averages for particular combinations of days (day code 1 versus day code 2) vary depending on the Fe treatment employed during these days and vice versa. That is, the main effects (days or Fe treatments) should not be interpreted independently because of their synergistic effects (Sokal and Rohlf, 1995). Lack of replication precluded determinations of interactions for N-uptake results from Orca.

3.5. *f*-Ratios

At Blue, Fe addition modestly increased the proportion of NO₃⁻-uptake to the total inorganic

N-uptake (NO₃⁻ and NH₄⁺) measured throughout the experiment. This uptake ratio, termed the *f*-ratio (Eppley and Peterson, 1979), is used to estimate 'new' and 'regenerated' production (*sensu* Dugdale and Goering, 1967) in natural marine systems, and is used here merely to express relative N-uptake rates. The *f*-ratios determined for the 0.32 and 1.50 nM Fe treatments were greater than the control, by an average of 12% and 17%, respectively. However, *f*-ratios also increased as a function of time by ca. 10% in the control samples from 0.67 (day 2.7) to 0.75 (day 12.7), which is similar to the enhancement observed at the highest Fe-addition (1.5 nM [Fe]: *f* = 0.75–0.85) during the same period. Such a temporal increase in the *f*-ratio of control samples has been seen previously (Boyd et al., 1996), and is likely an experimental artifact of bottle confinement experiments relative to the in situ response of planktonic assemblages. The relatively modest increase in the *f*-ratio ascribed to Fe addition is similar to the 5% and 50% increases in *f*-ratio (compared to controls)

reported by Timmermans et al. (1998a) for two HNLC stations sampled south of the Polar Front in the Southern Ocean (Pacific sector), and with the $\leq 5\%$ increase in f for Fe-amended phytoplankton samples (both 0.7–5 and $> 5\mu\text{m}$ size fractions) from northeast Pacific HNLC waters (estimated from Fig. 5 of Boyd et al., 1996). However, our results contrast with the 2 to 3-fold increase in f -ratios reported by Price et al. (1991) for Fe-enriched equatorial Pacific samples. The absolute values of f in our experiments at Blue are greater than those reported from Fe-enrichment studies conducted in other HNLC regions. For example, the f -ratio, determined for the first sampling period from the control bottle at Blue is 0.67, compared to control f -ratios of 0.14 and 0.41 for the equatorial Pacific (Price et al., 1991 their Table 2), and 0.10 and 0.22 reported previously for the Southern Ocean (Timmermans et al., 1998a), although even higher f -ratios of ~ 0.85 – 0.90 have been reported for the $> 5\mu\text{m}$ ‘control’ phytoplankton from the subarctic Pacific (Boyd et al., 1996; estimated from their Fig. 5B and D). Enhanced NO_3^- utilization is generally ascribed to larger-sized phytoplankton cells (see review by Chisholm, 1992), which dominated the planktonic assemblages of both the present and previous HNLC in vitro and in situ enrichment experiments following Fe addition (see discussion below).

At Orca, the proportion of total inorganic N-uptake due to NO_3^- is initially greater than measured at Blue. The f -ratios of both control and Fe-amended samples averaged 0.84 at the start of the experiment (day 2.0), but decreased by 50% to 0.40 on day 8.0 in the control carboy, and by an average of 25% in the Fe-amended samples (prior to NO_3^- exhaustion). The percentage decrease in relative NO_3^- utilization was greatest at the lower Fe enrichments (i.e., the final f -ratios, before NO_3^- exhaustion, were 0.52 for 0.16 nM [Fe], 0.55 for 0.3 nM [Fe] and 0.69 for 1.50 nM [Fe]); however, the consistently higher f -ratios observed in Fe-amended samples (compared to control samples) demonstrate an enhancement of NO_3^- -uptake relative to NH_4^+ , prior to NO_3^- exhaustion. Similar differences of 31% (range 8.3–47%) in the f -ratios between control and Fe-amended samples have

been reported during short-term ^{15}N -uptake experiments conducted in the North Sea after extensive blooms of *Emiliania huxley* (Timmermans et al., 1998b). This region is not representative of an HNLC region, but like Orca it also had lower ambient concentrations of Fe and NO_3^- , and elevated NH_4^+ concentrations compared to pre-bloom conditions. The general decline of f -ratios over time observed in our experiments is due to increasing NH_4^+ -uptake, and possibly NH_4^+ inhibition of NO_3^- -uptake from slightly elevated concentrations of NH_4^+ found within the incubation bottles over time, a situation not generally observed at Blue.

3.6. Phytoplankton species composition and size-fractionated N uptake

Planktonic biomass, as determined from microscopic species counts (not shown) of the main phytoplankton taxa, indicated that diatoms dominated the assemblages at the initiation of the experiments at Blue and Orca, and that their numbers increased dramatically in both control and Fe- and/or Zn-amended samples (Fig. 8). The diatoms at Blue, equally represented by centric and pennate forms, were initially 59% of the total C biomass and increased by 5- and 8-fold, respectively, to form upto 95% of the total biomass at the end of the experiment (Fig. 8A). In contrast to the control sample, all of the TM-enriched samples at Blue saw 5–9-fold increases in the biomass of the prymnesiophytes, namely *Phaeocystis antarctica*, but their relative proportion to the total community biomass remained constant at 22 to 33%, whereas it decreased in the control bottle from 28 to 2% of the total biomass (Fig. 8A). Increases in the biomass of dinoflagellates were not observed in either controls or enriched samples, and their relative C biomass was only ca. 3% of the total phytoplankton community.

The centric and pennate diatoms $> 5\mu\text{m}$ in length, which dominated the planktonic assemblages at Blue, were also responsible for the majority of NO_3^- and NH_4^+ -uptake determined from parallel post-incubation filtrations on 0.7- μm (considered total community uptake) and 5- μm

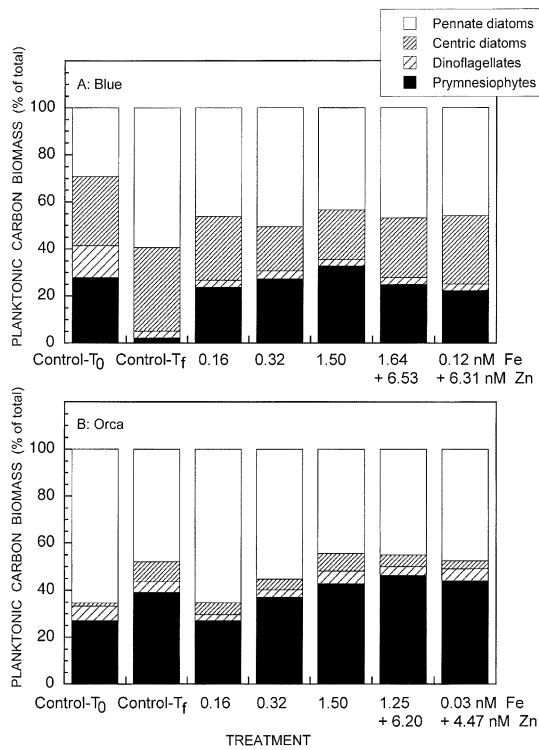


Fig. 8. Composition of the phytoplankton community at the beginning and end of the trace-metal enrichment incubation experiments in the Ross Sea shown as a percentage of total biomass for the major phytoplankton taxa at Blue (A) and Orca (B).

pore-sized filters (Fig. 3). By day 5.7, the N-uptake attributed to this larger fraction had increased from an average of 65 and 83% for NO_3^- and NH_4^+ , respectively, to consistently greater than 85% in both control and Fe-enriched samples for both N substrates (Fig. 3). Previous HNLC studies also have seen an increase in NO_3^- -uptake attributable to larger phytoplankton on alleviation of Fe limitation (Price et al., 1994; Coale et al., 1996; Boyd et al., 1996); however, these previous studies were initially dominated by picoplankton, not the larger microplankton observed in the western Ross Sea during austral summer (Dennett et al., 2001). In this respect, the Ross Sea represents a uniquely different HNLC region composed primarily of larger phytoplankton whose growth is predominately fueled by NO_3^- , not reduced N substrates

(NH_4^+ , urea), despite the very low ambient concentrations of dissolved Fe available for NO_3^- reduction. Consequently, the Fe-mediated floristic shifts observed in the Ross Sea HNLC region are not as dramatic as seen during previous *in vitro* and *in vivo* Fe-enrichment experiments in the equatorial Pacific (Price et al., 1991, 1994; Fryxell and Kaczmarska, 1994; Coale et al., 1996; Landry et al., 2000), northeast Pacific (Martin et al., 1989; Boyd et al., 1996, 1998), and other open areas of the Southern Ocean (Buma et al., 1991; Boyd et al., 2000).

Diatoms also dominated the phytoplankton biomass at Orca. Diatoms were 67% of the initial biomass, and were almost exclusively pennates at the beginning of the enrichment experiments (98%, Fig. 8B). Diatom biomass increased substantially in Fe- and/or Zn-amended carboys by almost 5-fold, but these levels were only 1.5 greater than the diatom biomass measured in the control carboy, and represented 50–70% of the total C biomass at the end of the experiment as similarly observed at Blue. In contrast to Blue, both the control and TM-amended carboys saw 7–15-fold increases in the biomass of the pymnesiophytes, but there was no difference between control and enriched carboys, and their relative proportion to the total community biomass only increased from 27% to ca. 40%. Dinoflagellate biomass increased by 4–6-fold in both control and amended carboys, but their relative C biomass was only 3–5% of the total phytoplankton community. As seen at Blue and other HNLC studies, pennate diatoms dominate the community biomass following Fe-enrichment (45–65%). The pennate diatoms were 98% of the diatom biomass and these larger cells ($>5\mu\text{m}$ in length) were also responsible for the majority of NO_3^- and NH_4^+ -uptake determined over time (Fig. 3). In the control carboys, the N-uptake due to this larger fraction was originally 60% and 71% of total NO_3^- and NH_4^+ , respectively, but Fe enrichment increased their relative uptake to 80–90% for both NO_3^- and NH_4^+ . Nitrogen uptake by the $>5\mu\text{m}$ phytoplankton in the control carboys was somewhat lower during the experiment, and averaged 68% and 73% of the total community uptake for NO_3^- and NH_4^+ , respectively.

3.7. NH_4^+ inhibition of NO_3^- -uptake

In the Southern Ocean, a number of temporal and spatial correlation studies have shown lower f -values with elevated ambient NH_4^+ concentrations (e.g., Olson, 1980; Owens et al., 1991; Mengesha et al., 1998; Sambrotto and Mace, 2000), where the reduction in f may be due to increased NH_4^+ -uptake and/or reduced NO_3^- -uptake rates. However, few inhibition (suppression) studies, where NH_4^+ concentrations are artificially increased to evaluate the effects on NO_3^- -uptake, have actually been conducted in the Southern Ocean. In one such seasonal study of the western Ross Sea, (Cochlan and Bronk, in press; Cochlan et al., 2002) report that rates of NO_3^- -uptake generally decrease exponentially with increasing NH_4^+ concentrations. They report that the maximal reduction of NO_3^- -uptake, as a percentage of NO_3^- -uptake in the absence of NH_4^+ , averaged 63% (range 40–83%) with the greatest reductions seen later in the growing season. However, even using saturating concentrations of NH_4^+ (5–10 μM) the uptake of NO_3^- was never completely inhibited, and residual NO_3^- -uptake averaged 37% of the theoretical maximal uptake at zero NH_4^+ concentration. A similar study conducted in the Indian Sector of the Southern Ocean demonstrated an 88% reduction in NO_3^- -uptake in the spring, but minimal reduction in the summer due to elevated ambient NH_4^+ concentrations (Mengesha et al., 1998). The only other inhibitory study in the Southern Ocean was conducted at two stations in the Scotia Sea by Glibert et al. (1982b), and they saw an inhibitory effect of ca. 30% with a 5 μM NH_4^+ addition at only one station.

During the present study, NH_4^+ inhibition experiments were not conducted with Fe-amended samples, but an inhibition study was conducted at Emperor located south of Orca during the same study period. The species composition of the phytoplankton assemblage at this station was very similar to our study stations with pennate diatoms dominating the biomass (45%), followed by prymnesiophytes (25%) and dinoflagellates (17%; Cochlan and Bronk, 2001). The suppressive effect of increasing NH_4^+ concentrations on resulting

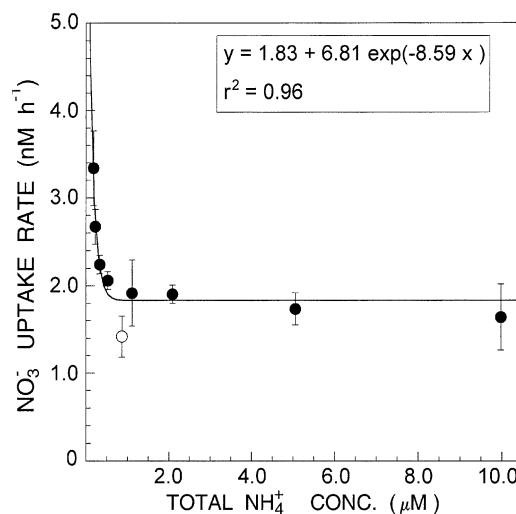


Fig. 9. Nitrate-uptake rates measured at increasing NH_4^+ concentrations for natural planktonic assemblages collected at Station Emperor. Each symbol (●) represents the mean \pm range of duplicate samples; no error bars indicates the errors are smaller than width of the symbol. Data were fit to the 3-parameter exponential model of Varela and Harrison (1999), with one value excluded (○). The ambient concentration of NH_4^+ was 0.13 μM , and NO_3^- was 11.78 μM .

NO_3^- -uptake rates at Emperor can be effectively described by a simple, 3-parameter exponential equation (Fig. 9; Varela and Harrison, 1999). Assuming that the NO_3^- -uptake capability of the phytoplankton in our grow-out experiments respond similarly to NH_4^+ , then the inhibitory effects of NH_4^+ may explain the decline in f -values observed within control and Fe-amended carboys at Orca. The ambient NH_4^+ concentration increase of 0.09 μM between days 2.0 and 5.8, and the additional increase of 0.08 μM from days 5.8 to 8.0 within these carboys would result in corresponding reductions in rates of NO_3^- -uptake by 38% and 31%. At Blue such net increases in ambient NH_4^+ concentrations were rare, and generally NH_4^+ declined in both control and TM-amended carboys as the experiment progressed, so NH_4^+ inhibitory effects were probably relatively minor or non-existent at this station.

It is unclear if increased NH_4^+ availability contributes to greater total N-uptake, or merely shifts N utilization from one form to another. In either case, the resultant f -values at Orca would

decline substantially because of these net increases in NH_4^+ . In this respect, the grow-out experiment at Orca appears to mimic, on a much shorter time scale, the in situ response observed in the surface waters of the Ross Sea (Nelson and Smith, 1986; Cochlan and Bronk, 2001, in press) and the open-ocean areas of the Southern Ocean (e.g., Goeyens et al., 1991b, 1998a; Semeneh et al., 1998), where rates of heterotrophic ammonification presumably exceed autotrophic assimilation as the growing season progresses. The results of our grow-out experiments also suggest that the absolute concentrations of both macronutrients, and micronutrients can potentially affect the planktonic N-uptake response, and therefore caution should be exercised when extrapolating such experimental results as representative of an in situ response.

3.8. Zinc effects

Low surface concentrations of Zn have been previously measured in the Southern Ocean, including the Ross Sea (e.g., Martin et al., 1990; Fitzwater et al., 2000). However, due to the relatively high concentrations of dissolved Zn measured at the initiation of the present experiments (Blue: 2.15 nM; Orca: 2.06 nM), evidence for any alleviation of potential Zn deficiency is not observed until the end of the grow-out periods (see for example the final biomass yields of the Zn + Fe carboys in Fig. 2). To ensure that the effects of Zn, not Fe addition, are reflected in the N-uptake response at Blue, the intended 'zinc only' carboy is compared with the 0.16 nM Fe carboy because of concurrent Fe contamination of 0.12 nM Fe with the addition of Zn (0.12 nM Fe + 6.31 nM Zn carboy). The effects of Zn addition are not seen until later in the experiment (days 9.8 and 12.7), when the specific uptake rates of NO_3^- , but not NH_4^+ , are enhanced by ca. 40% at Blue (Figs. 4 and 5). In the intended 'Zn + Fe' carboys, Fe concentration was increased to 1.64 nM, so it is difficult to discern any effects of Zn on N-uptake separate from those resulting from increased Fe concentrations. However, a comparison of the results obtained from the 1.50 nM Fe carboy with the 1.64 nM Fe + 6.53 nM Zn carboy demonstrates an average 10% increase in the specific uptake

rates of NO_3^- , but not NH_4^+ , during the last two sampling periods (Fig. 5C and D).

At Orca, the 'zinc only' carboys were not contaminated with Fe, and a comparison of these results with the control carboy shows a 4–16% increase in the specific uptake rates of NO_3^- and an 18–49% increase in NH_4^+ -uptake rates during the last two sampling periods (Figs. 6C and D). In the 'Zn + Fe' carboy, Fe concentration was not as high as in the highest Fe treatment, but specific NO_3^- -uptake rates did not increase in these apparently Zn-replete cells throughout the experiment. Specific uptake rates of NH_4^+ were enhanced during the first two sampling periods by ca. 35%, although it is unlikely that Zn would have been limiting for planktonic growth that early in the experiment (Fig. 6D). Since there is no specific Zn requirement for N utilization by phytoplankton, our results of enhanced N-uptake at either station resulting from Zn addition is likely due to physiologically 'healthy' phytoplankton in the carboys, in particular diatoms, where Zn availability may alter the efficiency of SiO_4^{4-} -uptake and silicification (De La Rocha et al., 2000).

4. Conclusions

All Fe enrichments resulted in the acceleration in the decline of NO_3^- concentrations over time, and the increased accumulation of biomass (as chl *a*) during these shipboard incubation experiments. Increasing the concentrations of dissolved Fe above ambient levels (0.3–0.4 nM) clearly resulted in enhanced absolute and PN-specific NO_3^- -uptake rates by phytoplankton from both the HNLC (Blue) and continental shelf (Orca) waters of the western Ross Sea. Fitting these data to Michaelis–Menten equations by non-linear curve fitting results in the first estimates of NO_3^- -uptake rates as a function of Fe concentration for plankton communities in the Southern Ocean: V_{\max} estimates were 0.012 and 0.0092 h^{-1} for Blue and Orca, respectively, and an apparent half-saturation constant of 0.09 nM Fe was calculated for both regions. These kinetic parameters demonstrate that since the ambient Fe concentrations in the surface waters of the western Ross Sea are

considerably less than the apparent half-saturation constant, the phytoplankton communities' ability to effectively utilize NO_3^- , which is found in abundance throughout the Southern Ocean, should be considered limited by the availability of Fe during austral summer. Due to the initially elevated concentrations of dissolved zinc (ca. 2 nM), the N-uptake responses to zinc enrichment were not as evident, and enhanced PN-specific NO_3^- -uptake rates were only observed towards the end of the incubation experiments.

Alleviation of Fe deficiency in the Ross Sea clearly enhances the growth of larger phytoplankton and their utilization of NO_3^- , but this area differs from most open-ocean regions of the Southern Ocean and other HNLC regions, in that prior to Fe enrichment the phytoplankton community is already composed primarily of diatoms and large colonies of *P. antarctica* whose growth is fueled mostly by 'new', not 'regenerated' N substrates. Consequently, Fe enrichment of samples collected in the Ross Sea demonstrate neither the dramatic floristic nor nitrogenous nutritional shifts from picoplankton growing on 'regenerated' N to microplankton growing on 'new' N as seen in other HNLC or Fe-deficient areas.

Acknowledgements

This study was supported by NSF Grants OPP 9530716 and 9896268 to WPC, OPP 9530732 to DAB, and OCE 9530762 to KHC as part of the US JGOFS Southern Ocean Process Study. These experiments would not have been possible without the excellent collaborative efforts of S. Fitzwater (MBARI) and M. Gordon (MLML) for their collection of TM-clean water samples, and their supervision of the overall design and conduction of the trace-metal experiments. We thank the Captain, officers and crew of the R.V.I.B. *Nathaniel B. Palmer*, and the shipboard personnel of Antarctica Support Associates for their support at sea. We acknowledge the assistance of J. Herndon (USC/SFSU) during shipboard experiments, and B.R. Wheeler (UGA/UD) and S. Tanner (MLML) for their excellent analyses ashore. Nitrate concentrations were determined by the US JGOFS

Management Office. Finally, we thank Dr. R.M. Kudela (UCSC) and M.P. Sanderson (VIMS) for mass spectrometry assistance, Dr. S. Obrebski (SFSU) for statistical help, and S. Polk (VIMS) for preparation of Fig. 1.

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