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Nitrogen uptake kinetics in the Ross Sea, Antarctica

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Abstract

The first estimates of uptake kinetic parameters for NH_4^+ , NO_3^- , and urea in the Ross Sea, Antarctica were measured on three cruises during austral late winter–early spring 1996 (pre-bloom), late spring 1997 (bloom development), and summer 1997 (bloom decline). Nitrogen (N) uptake experiments were conducted with water collected at the 50% light penetration depth using trace-metal clean protocols and ^{15}N tracer techniques. At all sites, ambient NO_3^- concentrations ranged from 5.8 to 30.5 $\mu\text{g-at N l}^{-1}$ and silicic acid concentrations were greater than 62.0 $\mu\text{g-at Si l}^{-1}$. The following trends were observed. First, based on maximum uptake rates (V_{max}), apparent N utilization followed the order $\text{NO}_3^- > \text{NH}_4^+ > \text{urea}$ during the pre-bloom and bloom development cruises. During the summer cruise, as the bloom was declining, the apparent order of utilization was $\text{NH}_4^+ > \text{NO}_3^- > \text{urea}$. Second, evidence for possible repression of NO_3^- uptake by elevated NH_4^+ concentrations was only observed at one site. Third, the kinetic parameters of NH_4^+ uptake rates corrected for isotope dilution were compared with the kinetic parameters determined from uncorrected rates. In this comparison, the measure of substrate affinity, α ($\alpha = V_{\text{max}}/K_s$) increased by an average of 4.6-fold when rates were corrected for isotope dilution, but values of V_{max} remained unchanged. Fourth, using bacterial production data, the magnitude of bacterial N uptake was estimated. Assuming that all bacterial N demands were met with NH_4^+ , the estimated bacterial portion of NH_4^+ uptake ranged from <1%, when the ratio of bacteria to autotrophic biomass was low, to 35%, when bacterial abundance and biomass were highest. Finally, dramatic changes in NH_4^+ uptake capacity were observed at one station (Stn. O), where kinetic parameters were measured during all three cruises. We hypothesize that a mutualistic relationship exists between phytoplankton and heterotrophic bacteria, and that the creation of microzones of high NH_4^+ concentrations contributed to the changes seen at this station. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

It is well known that the growth of marine phytoplankton is directly dependent on their ability to utilize nutrients and photosynthesize in environments where neither nutrients nor light are necessarily optimal for these processes. Nitrogen (N) is the macro-nutrient thought to limit marine primary productivity in most coastal (e.g., Ryther and Dunstan, 1971) and oceanic areas (e.g., Goldman et al., 1979). Field studies in temperate and tropical environments have usually confirmed the early models, which characterized the uptake of N as a hyperbolic function of nutrient concentration (e.g., MacIsaac and Dugdale, 1969) or photosynthetic photon flux density (e.g., MacIsaac and Dugdale, 1972) using the Michaelis–Menten equation for enzyme kinetics. However, large portions of the world's ocean, including much of the Southern Ocean, are characterized as high nutrient, low chlorophyll regions (HNLC; Minas et al., 1986), where phytoplankton growth and nitrate (NO_3^-) uptake rates are modest despite the high concentrations of NO_3^- available for planktonic use. In these areas, other environmental factors, including the availability of essential trace metals (most notably iron; e.g., Martin et al., 1990, 1991) or inhibition by elevated concentrations of ammonium (NH_4^+) (e.g., Owens et al., 1991) have been suggested to reduce the efficiency of NO_3^- uptake and phytoplankton growth. Although these open ocean areas of the Southern Ocean have captured the attention of oceanographers, due to their potential role(s) as a sink or source of carbon dioxide (CO_2), there are vast expanses of the Southern Ocean that do not fit this description. These other regions are characterized by relatively high phytoplankton biomass and measurable declines in macro-nutrient concentration, even to levels of depletion during blooms in coastal (Holm-Hansen et al., 1989; Kocmur et al., 1990; Holm-Hansen and Mitchell, 1991), under-ice (platelets: Smetacek et al., 1992), and ice-edge environments (Nelson and Smith, 1986). One of the largest of these productive regions is the broad continental shelf of the Ross Sea—a major study area of the US JGOFS Antarctic Environment and Southern Ocean Process Study (AESOPS) and the focus of this study of N uptake kinetics.

Satellite composite images of pigments identify the broad continental shelf and coastal region of the Ross Sea as the largest area of consistently elevated pigment concentrations in the Southern Ocean (Comiso et al., 1993). Large phytoplankton blooms are highly predictable in this area, and form within the Ross Sea polynya early in the austral spring; maximal biomass and productivity are reached by mid- to late-December, soon after the ice melts and/or is advected northward (El-Sayed et al., 1983; Smith and Nelson, 1985a; Wilson et al., 1986; Smith and Gordon, 1997; Smith et al., 2000b). The bloom decreases in extent and magnitude throughout January, and by February biomass is relatively low (chlorophyll *a* (chl *a*) = $1 \mu\text{g l}^{-1}$ or less; Arrigo and McClain, 1994; Smith et al., 1996, 2000b).

Initiation and termination of blooms in the Ross Sea, and other coastal and continental shelf regions, have been linked to the seasonal advance and retreat of sea ice, and form part of a well-defined region known as the Antarctic marginal ice zone (MIZ). Earlier studies have suggested that such blooms resulted from vertical stability imparted by the melting ice, thus providing more optimal light conditions for phytoplankton growth and biomass accumulation (e.g., Smith and Nelson, 1985b; Sullivan et al., 1988). More detailed studies, however, suggest that the variability in biomass and productivity seen in the Ross Sea cannot be attributed to differences in vertical stability alone (Comiso et al., 1993; Smith et al., 1996), and that the availability of micro-nutrients

such as Fe are also important in controlling the location of phytoplankton blooms in this area (e.g., Fitzwater et al., 2000). In a detailed seasonal study of the southern Ross Sea, Sweeney et al. (2000) used seasonal deficits in macro-nutrient and CO₂ concentrations in the upper water column to divide the Ross Sea into three distinct biogeochemical regimes. Their regimes are characterized by different dominant species, such as the diatom-dominated ice-edge blooms in the southwestern region (Region I), blooms of the colonial prymnesiophyte *Phaeocystis antarctica* in the center of the deep-mixed Ross Sea polynya (Region II), and a region of low productivity in the shallow-mixed eastern and northern regions (Region III). In Region III, the winter ice coverage lasts much longer than Regions I and II, and the area is characterized by low productivity and biomass presumably from trace-metal limitation.

In general, the main source of N supporting high phytoplankton productivity in Antarctic MIZs is NO₃⁻ (Nelson and Smith, 1986; Smith and Nelson, 1990; Bury et al., 1995). However, as Goeyens et al. (1991a) have demonstrated in the MIZ region of the Scotia–Weddell Confluence area, the nitrogenous nutrition can be highly temporal, with a shift from phytoplankton growth on new (NO₃⁻) to regenerated (NH₄⁺ and urea) N in only a matter of weeks. They suggested that this shift in nutrition was due to increasing availability of NH₄⁺ in the water column, and a transition from a diatom- to a flagellate-dominated community. A recent study suggested that the preferential utilization of NH₄⁺ over NO₃⁻ (due to elevated concentrations and/or lack of Fe) may also explain the spatial variability in phytoplankton community composition in the Ross Sea (Arrigo et al., 1999), although direct N uptake measurements were not conducted.

Despite the importance of quantifying N nutrition as a means to explain phytoplankton dynamics and export flux (Eppley, 1981), there have been no previous studies of the kinetics of N utilization in either neritic or oceanic Antarctic waters. The present study provides the first kinetic parameters of regenerated N (NH₄⁺ and urea) use by Antarctic phytoplankton, and demonstrates the variability in NH₄⁺ uptake affinity both spatially and temporally in the Ross Sea.

2. Materials and methods

2.1. General

Sampling was conducted during three oceanographic cruises in 1996 and 1997 to the Ross Sea, Antarctica aboard the R.V.I.B. *Nathaniel B. Palmer* during the US Southern Ocean JGOFS AESOPS Process Cruises NBP 96-4A (Process I; 10/02/96–11/09/96; austral late winter–early spring, pre-bloom, complete ice coverage), NBP 97-1 (Process II; 01/13/97–02/11/97; austral summer, late bloom, ice-free) and NBP 97-8 (Process IV; 11/05/97–12/16/97; late spring, early bloom, partial ice coverage). The last cruise was conducted during the subsequent austral growing season between the periods bracketed by the first and second cruise, thus permitting observation of the complete progression of the bloom. Most sampling was concentrated along a 76.5°S transect in the southern Ross Sea (Stations Orca, O and E), although samples were also collected within the pack ice (Stn. Pack Ice) near Victoria Land, and adjacent to the Ross Ice Shelf (Stn. Emperor) during cruise NBP 97-1 (Fig. 1). Environmental conditions for each station are given in Table 1 and the distribution of seasonal ice coverage for the study area is shown graphically in Smith et al. (2000a).

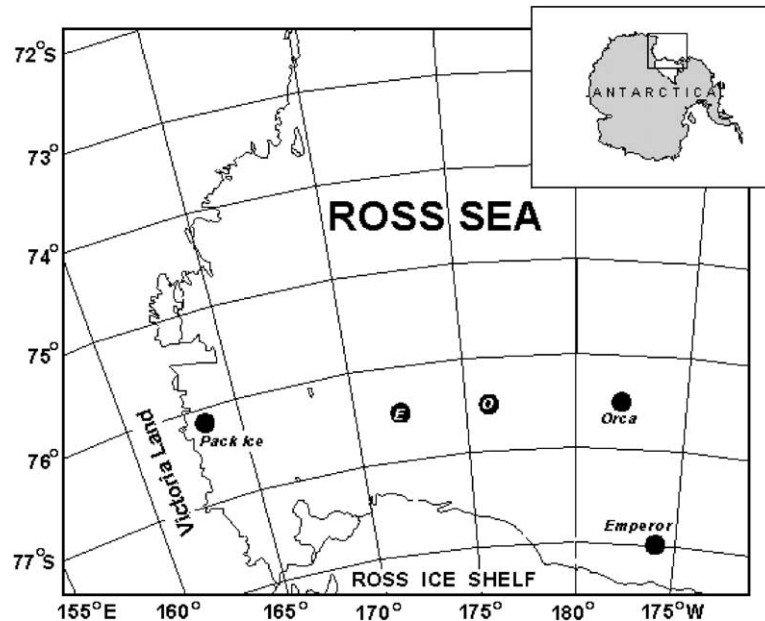


Fig. 1. Map of the study area and station locations in the Ross Sea where N uptake kinetic experiments were performed during 1996 and 1997.

Table 1

Initial environmental conditions of seawater collected for nitrogen uptake vs. concentration experiments in the Ross Sea during AESOPS process (P) cruises in early spring (NBP96-4A; P I), late spring (NBP97-8; P IV), and summer (NBP97-1; P II)

Station name, No. and Cruise.	Latitude, Longitude	Sampling date	Depth (m) ^a	Temp. (°C)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	Ambient conc. ($\mu\text{g-at l}^{-1}$)			
						NH_4^+	NO_3^-	Urea	Si(OH)_4
Stn. O, No. 20, P I	76.4623S, 176.0150E	11/05/1996	1.9	-1.79	0.72	0.0	30.47	Nd	76.1
Stn. O, No. 17, P II	76.4455S, 176.2131E	01/31/1997	3.9	-0.27	4.10	0.0	11.19	0.09	73.9
Stn. O, No. 28, P IV	76.4761S, 176.0942E	12/07/1997	4.9	-1.23	1.61	0.10	25.47	0.03	68.8
Stn. Emperor, No. 23, P II	77.9921S, 176.0498W	02/04/1997	5.3	-0.36	1.12	0.13	11.78	0.13	64.7
Stn. Pack Ice, No. 27, P II	76.1552S, 163.3735E	02/07/1997	4.1	-1.07	1.03	0.71	5.84	0.38	62.2
Stn. Orca, No. 8, P II	76.4833S, 178.0438W	01/21/1997	4.0	+0.27	2.39	0.01	14.34	0.09	65.0
Stn. Orca, No. 16, P IV	76.5629S, 178.1629W	11/29/1997	14.7	-1.79	0.44	0.02	30.08	0.0	75.3
Stn. E, No. 27, P IV	76.5012S, 172.5942E	12/05/1997	4.7	-1.44	7.93	0.16	23.17	0.12	Nd

^a Depth is actually recorded as pressure measured in decibars.

Nd, not determined.

0.0, below detection limits of 0.02 and 0.04 $\mu\text{g-at N l}^{-1}$ for NH_4^+ and urea analyses, respectively.

Discrete samples were collected from the upper mixed layer, at the depth of 50% subsurface photosynthetic photon flux density (PPFD), using 30-l trace-metal free Go-Flo bottles mounted on a trace-metal clean, instrumented rosette (Hunter et al., 1996); when not deployed, the bottles were covered with plastic to reduce airborne contamination by trace metals. The N uptake

samples were transferred to acid-washed, trace-metal clean 1.2-l polycarbonate bottles while wearing plastic gloves. All subsequent sample manipulations were conducted within a laminar-flow hood (HEPA) using trace-metal clean techniques (e.g., Fitzwater et al., 1982).

2.2. Analytical methods

Samples for determination of ambient concentrations of NO_3^- and silicic acid ($\text{Si}(\text{OH})_4$) were stored in 30-ml polyethylene bottles, and analyzed fresh with a Technicon AutoAnalyzer II following the procedures of Wood et al. (1967) and Armstrong et al. (1967), respectively, as outlined in Gordon et al. (1993). Duplicate or triplicate 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 (Degobbis, 1973). The remaining reagents were added and the samples manually analyzed within 24 h using a spectrophotometer equipped with a 10-cm cell (Solorzano, 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 digestion time (30 min.) and lower digestion temperature (85°C). Although others have found that freezing may decrease urea concentration (Mulvenna and Savidge, 1992), tests conducted with seawater standards of known concentrations of urea demonstrated no such losses, in agreement with Price (1987). Samples for 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 Turner Designs 10-AU fluorometer (Parsons et al., 1984) calibrated at the beginning of each cruise. Samples for the enumeration of phototrophic and heterotrophic nanoplankton and microplankton were preserved and stored for epifluorescence microscopy following the protocols outlined in Dennett et al. (2001). Biomass estimates were determined from microscopic measurements of cell dimensions and using volume equations of appropriate geometric shapes; these estimates were subsequently converted to carbon concentrations using published conversion factors listed in Dennett et al. (2001).

2.3. Nitrogen uptake experiments

Within 0.5–1 h of collection, N uptake experiments were conducted in duplicate after inoculation with ^{15}N -ammonium chloride (98.85 at.%; Cambridge Isotopes) or ^{15}N -urea (98.2 at.%) at a range of initial substrate concentrations (0.05, 0.1, 0.2, 0.4, 0.75, 0.99, 1.97, 4.93 and $9.86 \mu\text{g-at N l}^{-1}$). All ^{15}N -labeled substrates were prepared using trace metal clean techniques. Inoculated bottles were hermetically heat-sealed within polyethylene bags, and incubated for either 24 h (NBP 96-4A, early spring), ~ 12 h (NBP 97-8, late spring) or 6–9 h (NBP 97-1, summer) in clear Plexiglas[®] deck incubators at *in situ* temperature and 50% surface irradiance. Duplicate samples also were inoculated with ^{15}N -sodium nitrate or ^{15}N -urea (98.25 and 98.2 at.%, respectively; Cambridge Isotopes) at ‘saturating’ concentrations ($< 10\%$ ambient concentration for NO_3^- and $10 \mu\text{g-at N l}^{-1}$ for urea) to estimate maximal uptake rates for these N substrates. Samples for ^{15}N analysis were collected by filtration (< 80 mm Hg) onto precombusted Whatman[®] GF/F filters (2.5 cm; 4 h at 450°C), and frozen in polypropylene cryovials until mass

spectrometric analysis on shore. Analysis by epifluorescence microscopy of samples stained with acridine orange (Hobbie et al., 1977) from incubated, and GF/F-filtered incubated samples showed that GF/F filters retain an average of $81.0 \pm 10.2\%$ ($n = 12$ pairs) of bacteria during the early spring and summer periods (Cochlan and Ducklow, unpubl. data).

Based on the isotopic enrichment and concentration of the ^{15}N -substrate added, and the at.% excess and concentration of the particulate N (PN) collected at the end of the incubations, it was calculated that on average (± 1 SD) $3.3 \pm 3.5\%$ ($n = 16$), $5.2 \pm 4.5\%$ ($n = 54$), and $13.8 \pm 15.6\%$ ($n = 72$) of the ^{15}N -substrate was used during the $^{15}\text{NH}_4^+$ and urea kinetic experiments conducted during Process I (early spring), Process II (late summer) and Process III (late spring), respectively. Even with the smallest $^{15}\text{NH}_4^+$ enrichments ($0.05 \mu\text{g-at N l}^{-1}$), only an average of $19.9 \pm 18.3\%$ ($n = 16$) of the isotope was used during these kinetic experiments, and this percentage never exceeded 60% despite low ambient concentrations of NH_4^+ and relatively long incubation periods. In the urea and NO_3^- experiments, where saturated concentrations were used, isotope usage during the incubations never exceeded ~ 1 –2%. Substrate depletion was, therefore, not considered a problem in any of the N-uptake experiments conducted during this study.

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), prior to mass spectrometric analysis.

All ^{15}N enrichments and PN concentrations were measured on a Europa Tracermass mass spectrometer. Nitrogen specific uptake rates were calculated using Eq. (5) of Dugdale and Wilkerson (1986) and corrected for isotopic dilution according to (Glibert et al., 1982a) using the average aqueous enrichment term (Laws, 1984).

2.4. Kinetic parameters

Curve fitting was completed using a computerized, iterative non-linear least-squares technique (Kaleidograph[®]; Abelbeck Software), which utilizes the Levenberg–Marquardt algorithm (Press et al., 1992). Data initially were linearized and plotted using a double reciprocal method (Hanes–Woolfe; Dowd and Riggs, 1965), and these derived values were entered into the curve-fitting package. The N kinetics data were fitted directly to the Michaelis–Menten formulation:

$$V = \frac{V_{\max} S}{(K_s + S)}, \quad (1)$$

where V is the PN specific uptake rate (h^{-1}), V_{\max} is the maximal specific uptake rate, S is the substrate concentration ($\mu\text{g-at N l}^{-1}$), and K_s is the half-saturation constant for the N substrate ($\mu\text{g-at N l}^{-1}$). In this paper, V_{\max} values for NH_4^+ refer to the maximum predicted value of V derived from the curve fit. The NO_3^- and urea uptake rates (except Stn. O sampled on 12/07/97) were determined at concentrations considered saturating for N uptake ($\geq 10 \mu\text{g-at N l}^{-1}$). The substrate affinity at low concentrations (i.e., $S < K_s$) was determined from the initial slope (α) of the Michaelis–Menten plot, and was calculated as $\alpha = V_{\max}/K_s$; the derivative of Eq. (1), with respect to S , as S approaches zero (Button, 1978; Healey, 1980; Cochlan and Harrison, 1991a).

3. Results and discussion

Here, we describe the chemical and biological characteristics of the experimental sites, and present results from the first N kinetic experiments conducted in Antarctic coastal waters. These results are used to estimate f -ratios (the ratio of NO_3^- uptake to total N uptake) at ambient N concentrations, and are discussed with respect to the importance of including urea uptake rates in estimates of regenerated production, the effect of NH_4^+ inhibition of NO_3^- uptake rates, the importance of correcting NH_4^+ uptake rates for isotope dilution, and the potential impact of the variable incubation times used on the different cruises. Our results demonstrate a revised view of phytoplankton N nutrition in the Ross Sea, describe the potential importance of heterotrophic bacterial N uptake, particularly during low ambient concentrations, and present evidence supportive of a mutualistic relationship between phytoplankton and heterotrophic bacteria in this region.

3.1. General chemical and biological characteristics

During cruises in the austral summer (Process II) and late spring (Process IV), conditions were favorable for the growth of phytoplankton and a substantial accumulation of biomass (Table 1). However, during the late winter–early spring (Process I cruise), ice cover was still complete, and phytoplankton growth was minimal. Ambient concentrations of NO_3^- in the surface waters were greatest ($> 30 \mu\text{g-at N l}^{-1}$) in early November at Stn. O and during late November at Stn. Orca, whereas the lowest NO_3^- concentrations were observed in close proximity to the Ross Ice Shelf ($11.8 \mu\text{g-at N l}^{-1}$; Stn. Emperor) and within the pack-ice nearest Victoria Land ($5.8 \mu\text{g-at N l}^{-1}$; Stn. Pack Ice) during summer (Table 1). However, at all stations and during all sampling periods, the ambient concentrations of NO_3^- are considered saturating for phytoplankton uptake, whereas ambient NH_4^+ concentrations were only elevated beneath the pack ice ($0.71 \mu\text{g-at N l}^{-1}$). Although not tested here, based on N uptake kinetic studies conducted in other high NO_3^- environments (e.g., Kanda et al., 1985; Kristiansen et al., 1994), it is likely that the ambient NO_3^- concentrations observed in the Ross Sea are saturating ($> 5\text{--}30 \mu\text{g-at N l}^{-1}$; Table 1). Surface (0–15 m) concentrations of NH_4^+ at all other stations sampled were very low ($< 0.2 \mu\text{g-at N l}^{-1}$) and generally at or below our methodological limit of detection ($0.02 \mu\text{g-at N l}^{-1}$); conditions were thus optimal for conducting NH_4^+ uptake kinetic experiments in the field (e.g., Harrison et al., 1989). Surface concentrations of urea were similarly low, except beneath the pack-ice ($0.38 \mu\text{g-at N l}^{-1}$). Ambient silicic acid was high ($> 60 \mu\text{g-at Si l}^{-1}$) at all stations sampled during this study (Table 1).

The species composition of the phytoplankton community, at the 50% light depth, varied considerably both temporally and spatially during this study (Fig. 2; Dennett et al., 2001). During Process I, in late winter–early spring, the assemblage at Stn. O was dominated by the colonial prymnesiophyte, *Phaeocystis antarctica* and autotrophic nanoflagellates (composed primarily [93%] of the motile, unicellular *P. antarctica*; S. Mathot, pers. comm.). As the growing season progressed and the bloom began to decline, numbers of colonial *P. antarctica* increased 8-fold, increasing from 64% to 76% of the relative abundance of cells, and from 60% to 70% of community biomass (Process II, Stn. O, 1/31/97). The remainder of the assemblage at this station was composed primarily of heterotrophic dinoflagellates and nanoflagellates, and the numerical

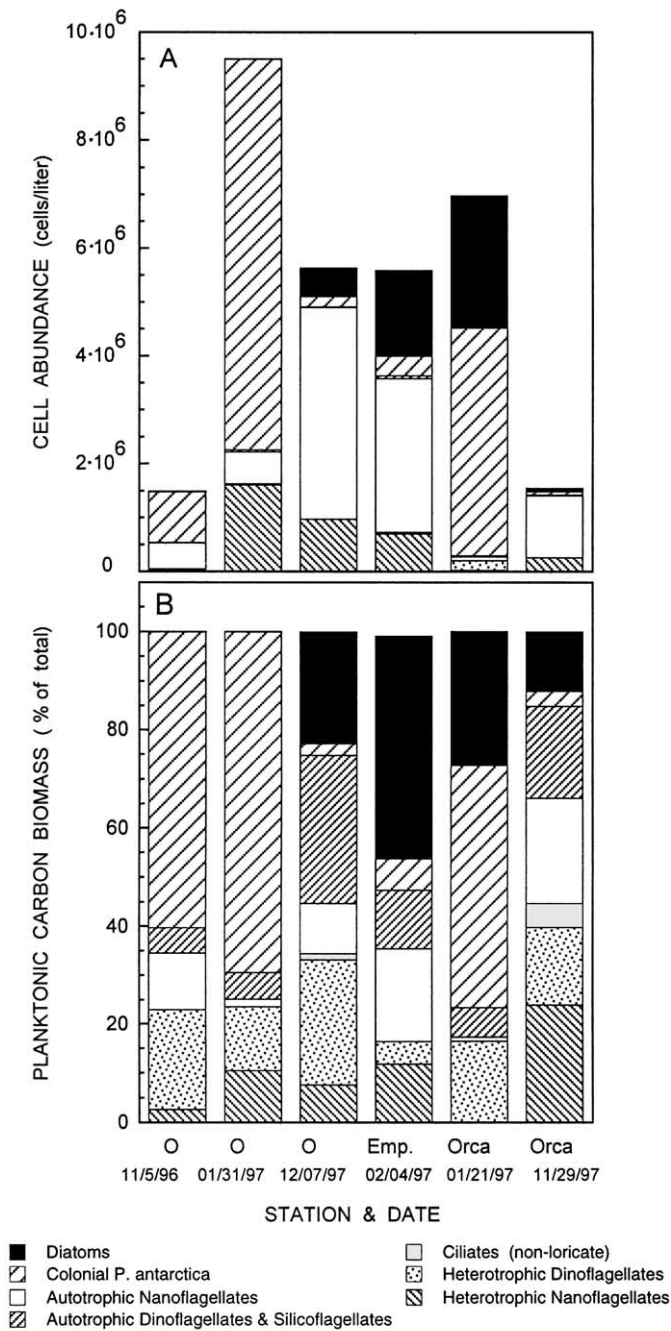


Fig. 2. Composition of the phytoplankton community in the Ross Sea. (A) Cell abundance (cells l^{-1}). (B) Planktonic carbon biomass as a percentage of total biomass. Samples were not enumerated for Stn. Pack Ice during austral summer (Process II) or Stn. E during spring (Process IV).

abundance of unicellular *P. antarctica* increased by <20%. Similar dominance of the Ross Sea polynya (categorized as Region II by Sweeney et al., 2000), by *P. antarctica* was observed by Arrigo et al. (1999) during a cruise in the same area conducted between Process I and II.

The following spring, during Process IV, diatoms (almost exclusively of the pennate form) were observed at Stn. O (10% of abundance), but the community was dominated by heterotrophic (17%) and autotrophic (70%) nanoflagellates, and relatively few colonial *P. antarctica* cells (3%). Unicellular motile forms were a minor component (<3%) of the autotrophic nanoflagellates (M. Dennett, pers. comm.) during this new season of growth, and carbon biomass of the assemblage was composed primarily of autotrophic (40%) and heterotrophic dinoflagellates and nanoflagellates (33%), and diatoms (23%). Pennate diatoms were most numerous adjacent to the Ross Ice Shelf (Stn. Emperor; 28% of abundance, 45% of biomass), and at the eastern most sampling station (Stn. Orca).

During Process II, in the summer, the phytoplankton assemblage at the 50% light depth at Stn. Orca was composed predominantly of pennate diatoms (35%) and colonial *P. antarctica* (61%). The following spring, diatoms were still present (4% of abundance), but the community was composed primarily of heterotrophic (16%) and autotrophic (75%) nanoflagellates; only 2% of the latter were unicellular *P. antarctica*, similar to the low proportions seen at Stn. O during this time (M. Dennett, pers. comm.). The phytoplankton composition was not determined for the Pack Ice station; however, this southwestern region of the Ross Sea (termed Region I; Sweeney et al., 2000) is generally dominated by diatoms, or they are present in large quantities during this time (e.g., Arrigo et al., 1999; Sweeney et al., 2000, Dennett et al., 2001).

3.2. Nitrogen uptake kinetics

The uptake of NH_4^+ and urea by the natural assemblages of phytoplankton in the Ross Sea can be related to substrate concentrations following Michaelis–Menten kinetics (Fig. 3); the estimated kinetic parameters derived from iterative fits of NH_4^+ and urea uptake rates to the Michaelis–Menten hyperbola are presented in Table 2. At all stations, elevated ambient NO_3^- concentrations precluded the possibility of determining NO_3^- uptake kinetics, and the reported V_{max} values for NO_3^- and urea (except Stn. O 12/07/97) were not derived from the Michaelis–Menten equation, but are the observed specific uptake rates determined at concentrations considered saturating ($\geq 10 \mu\text{g-at N l}^{-1}$) for their uptake by phytoplankton. In the high NO_3^- environment of the Ross Sea, such rates are actually realized by the phytoplankton, and the $^{15}\text{NO}_3^-$ inocula were true ‘tracer-level’ enrichments (<10% of the ambient concentration, Dugdale and Wilkerson, 1986), whereas V_{max} values for urea and NH_4^+ are potential rates, not normally observed in the Southern Ocean. Although the effects of greater additions ($> 10 \mu\text{g-at N l}^{-1}$) of urea and NH_4^+ on their uptake were not conducted in this study, previous investigations have determined that these reduced N forms generally saturate uptake at $< 10 \mu\text{g-at N l}^{-1}$ for both polar (see Table 3) and temperate phytoplankton assemblages (e.g., Eppley et al., 1969; Kanda et al., 1985; Kudela and Cochlan, 2000). However, based on reported half-saturation constants, there are some phytoplankton species (e.g., *Thalassiosira pseudonana*, Dortch et al., 1991) and natural assemblages (e.g., Kanda et al., 1985; Muggli and Smith, 1993) that neither maximize their N uptake of reduced N forms nor NO_3^- until higher concentrations are realized than were observed in this study.

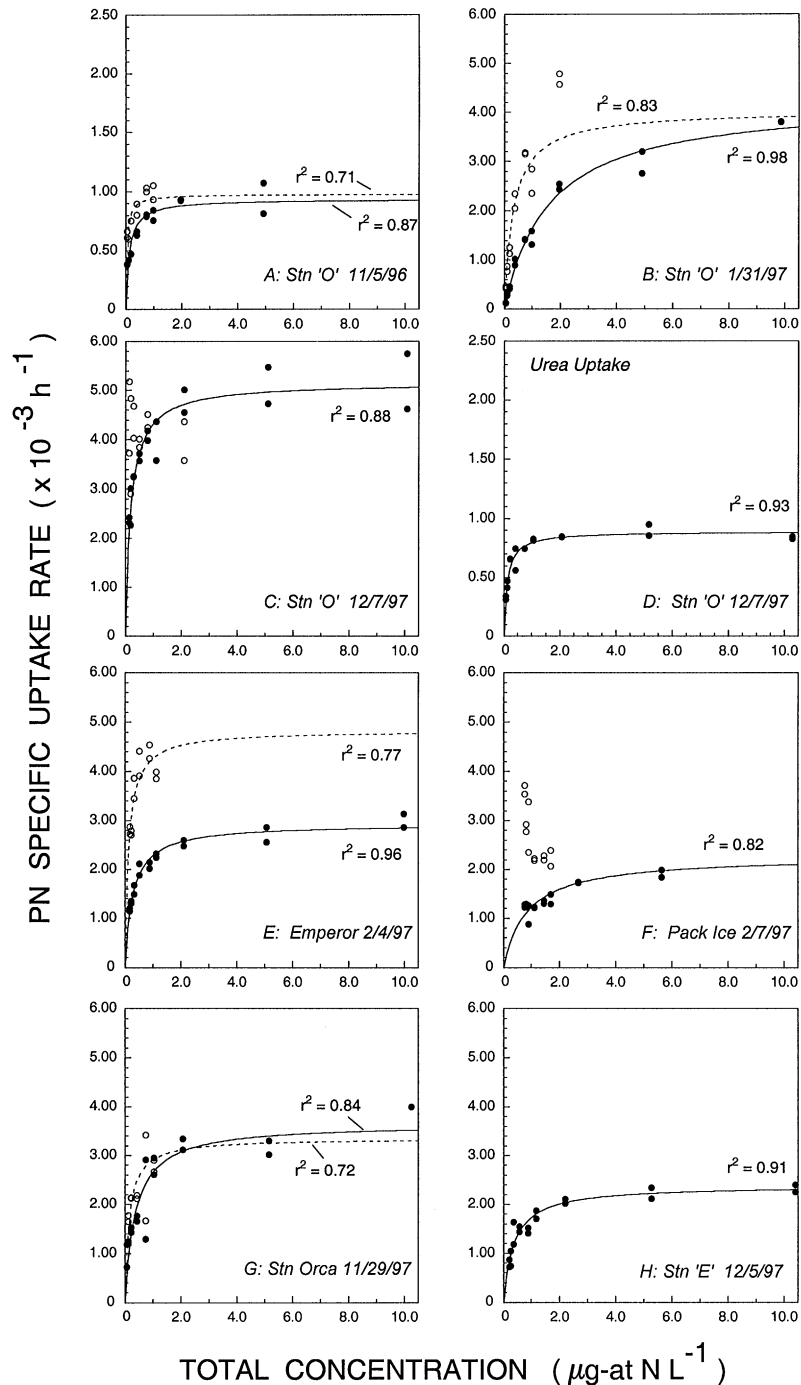


Fig. 3. Specific uptake rates (h^{-1}) of NH_4^+ and urea (panel D) by natural assemblages of phytoplankton plotted versus total NH_4^+ or urea concentration. Uncorrected (\bullet) and isotope-dilution corrected (\circ) rate estimates are fitted directly to the Michaelis–Menten equation and denoted as solid (—) and dashed (---) lines, respectively. Note that uncorrected rates determined at the elevated concentrations ($> 2 \mu\text{g-at N L}^{-1}$) were used together with the corrected rates from lower concentrations to produce the corrected (dashed) rectangular hyperbola. At Stn. O (panel C) and Stn. Pack-Ice (panel F), the corrected rates could not be fitted to the Michaelis–Menten equation.

Table 2

Kinetic parameters for N uptake by Ross Sea phytoplankton. Rates of NH_4^+ , NO_3^- and urea uptake, and NH_4^+ uptake rates corrected for isotopic dilution are reported in units of $\times 10^{-3} \text{h}^{-1}$. Half-saturation constants (K_s) are reported in $\mu\text{g-at N l}^{-1}$ and PN concentrations are reported as $\mu\text{g-at N l}^{-1}$, and are the average of all samples used in NH_4^+ uptake kinetic experiments after incubation. V_{max} values, designated with an asterisk, are uptake rates realized at concentrations considered saturating for uptake (5–10 $\mu\text{g-at N l}^{-1}$). Substrate affinity ($\alpha = V_{\text{max}}/K_s$) is reported in units of $\times 10^{-3} \text{h}^{-1}/(\mu\text{g-at N l}^{-1})$. Estimated standard error (SE) values of parameters and rates are given in parentheses

Station, date	PN (mean)	NH_4^+			Corrected NH_4^+			Urea			NO_3^-	
		V_{max}	K_s	α	V_{max}	K_s	α	V_{max}	K_s	α	V_{max}	
Stn. O, 11/05/96	0.64	0.936 (0.040)	0.124 (0.025)	7.6	0.977 (0.035)	0.040 (0.010)	24.4	—	—	—	14.001* (0.178)	
Stn. O, 01/31/97	3.57	4.256 (0.157)	1.597 (0.163)	2.7	4.036 (0.299)	0.333 (0.098)	12.1	1.376* (0.212)	—	—	2.222* (0.246)	
Stn. O, 12/07/97	3.34	5.154 (0.162)	0.193 (0.027)	26.7	—	—	—	0.889 (0.022)	0.120 (0.015)	7.41	8.472* (2.89)	
Stn. Emperor, 02/04/97	2.72	2.929 (0.063)	0.284 (0.025)	10.3	4.826 (0.288)	0.131 (0.032)	36.8	0.971* (0.040)	—	—	1.096* (0.103)	
Stn. Pack Ice, 02/07/97	4.30	2.275 (0.117)	0.861 (0.141)	2.6	—	—	—	0.308* (0.041)	—	—	1.187* (0.028)	
Stn. Orca, 01/21/97	5.64	3.887* (0.215)	—	—	—	—	—	1.885* (0.086)	—	—	3.104* (0.156)	
Stn. Orca, 11/29/97	0.63	3.652 (0.248)	0.378 (0.093)	9.7	3.352 (0.201)	0.152 (0.043)	22.0	0.841* (0.209)	—	—	14.458* (1.496)	
Stn. E, 12/05/97	6.89	2.383 (0.087)	0.378 (0.051)	6.3	—	—	—	—	—	—	8.949* (0.290)	

Table 3

Summary of half-saturation constants (K_s) determined for natural phytoplankton assemblages from polar regions. Isotope-dilution corrected NH_4^+ uptake rates were used in the Ross Sea study to determine K_s ; all other sources report uncorrected rates

Region	$K_s\text{-NH}_4^+$	$K_s\text{-NO}_3^-$	$K_s\text{-Urea}$	Source
Barents Sea				
$[\text{NO}_3^-] \geq 1 \mu\text{g-at N l}^{-1}$	1.3	1.8	0.2	Kristiansen et al. (1994)
$[\text{NO}_3^-] \leq 1 \mu\text{g-at N l}^{-1}$	0.1–0.2	0–2.2	0.0–0.1	Kristiansen and Farbrot (1991) Kristiansen and Lund (1989)
East Greenland Sea ^a	2.24	0.29, 2.24	—	Muggli and Smith (1993)
Barrow Strait, NWT (<i>ice algae</i>)	1.6	<4.0	0.9	Harrison et al. (1990)
Eastern Canadian Arctic	0.17	0.87	0.30	Smith and Harrison (1991)
$[\text{NO}_3^-] < 1 \mu\text{g-at N l}^{-1}$				
Toolik Lake, Alaska	0.05–0.49	0.05–0.30	—	Whalen and Alexander (1986)
$[\text{NO}_3^-] < 1 \mu\text{g-at N l}^{-1}$				
Western Ross Sea	0.04–0.33, 0.12–1.60 ^b	^c	0.12	This study
$[\text{NO}_3^-] > 5 \mu\text{g-at N l}^{-1}$				

^a *Phaeocystis pouchetii* bloom.

^b Uncorrected values.

^c Ambient NO_3^- concentration too high to determine K_s .

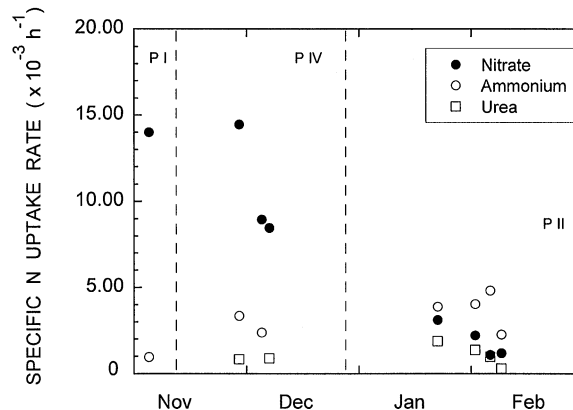


Fig. 4. Maximum specific uptake rates (V_{\max}) of NO_3^- (●), NH_4^+ (○) and urea (□) (from Table 2) by natural assemblages of phytoplankton as a function of time during the austral growing season. P I (early spring, 1996), P II (late summer, 1996) and P IV (late spring, 1997).

The V_{\max} values for NO_3^- ($V_{\max}\text{-NO}_3^-$) were consistently greater than the corresponding $V_{\max}\text{-NH}_4^+$ values early in the growing season (November–December), but less than $V_{\max}\text{-NH}_4^+$ observed later in the season (January–February; Table 2 and Fig. 4). Maximum specific uptakes of urea ($V_{\max}\text{-urea}$) were always less than $V_{\max}\text{-NO}_3^-$, and averaged 4-fold less than $V_{\max}\text{-NH}_4^+$ (range 2.1–7.4-fold less). There were no significant relationships observed between ambient concentrations of NH_4^+ and urea, and the maximal uptake rates of the three N forms measured; this would be expected based on the very low ambient concentrations of the reduced N forms.

Except for the pack ice station, there were no obvious inhibitory effects of NH_4^+ on NO_3^- uptake previously noted elsewhere (e.g., see review by Dortch, 1990) and suggested for the Southern Ocean by temporal and spatial correlation studies of low NO_3^- uptake rates associated with elevated NH_4^+ concentrations (e.g., Smith and Harrison, 1991; Owens et al., 1991). At the Pack Ice station the relatively low rate of NO_3^- uptake, despite the still elevated NO_3^- concentration ($> 5 \mu\text{g-at N l}^{-1}$), may be due to preferential utilization of NH_4^+ and/or inhibition of NO_3^- uptake by NH_4^+ . The magnitude of this inhibitory effect can be estimated by extrapolating the NO_3^- uptake rate data presented in Fig. 5 to zero NH_4^+ concentration using the exponential fit of these data. The intercept of $1.68 \times 10^{-3} \text{ h}^{-1}$ is 40% greater than the rate measured in situ suggesting that the ambient NH_4^+ concentration beneath the pack ice ($0.71 \mu\text{g-at N l}^{-1}$) could reduce NO_3^- uptake rates by as much as 30%. However, any repressive effects of NH_4^+ on NO_3^- uptake in the Ross Sea are variable; high concentrations of NH_4^+ present beneath the pack ice likely contributed to the lowered rates of NO_3^- utilization at this station, but some of the fastest NO_3^- uptake rates of the study were observed at Stn. E in the presence of relatively high NH_4^+ concentration ($0.16 \mu\text{g-at N l}^{-1}$). Clearly, NH_4^+ inhibitory effects are but one of many factors including community species composition and the availability of light and micronutrients that may influence the magnitude of NO_3^- uptake rate in the Ross Sea.

Maximal uptake rates of NO_3^- were positively correlated with the ambient concentration of NO_3^- ($P < 0.01$), which can be adequately described by either a linear or exponential relationship (Fig. 6). No significant relationships were observed between ambient NO_3^- concentration and or $V_{\text{max-NH}_4^+}$ or $V_{\text{max-urea}}$. Previous authors have suggested that the relationship between $V_{\text{max-NH}_4^+}$ and ambient NO_3^- concentration is a linear function (Dugdale and Wilkerson, 1991), and that the intercept at $\sim 6 \mu\text{g-at N l}^{-1}$ was hypothesized to represent a threshold concentration below which a phytoplankton bloom could not develop. Interestingly, the intercept of the linear plot of our lower rates of maximal specific NO_3^- uptake versus ambient NO_3^- demonstrates a

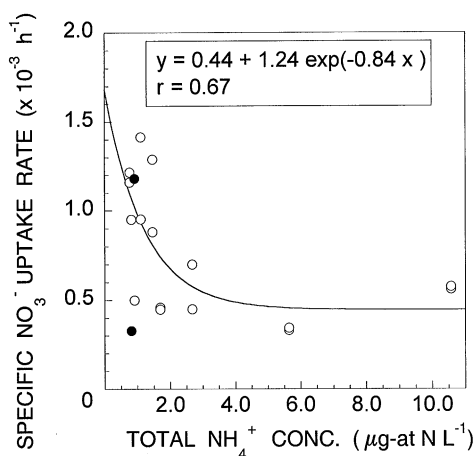


Fig. 5. Inhibition of NO_3^- uptake rate by NH_4^+ at Station Pack Ice during austral late summer. Specific NO_3^- uptake rates (○) were measured at increasing NH_4^+ concentrations for natural planktonic assemblages during 9.5-h incubation periods following NH_4^+ addition. Data were fit to the 3-parameter exponential model of Varela and Harrison (1999), with two values excluded (●). The ambient concentration of NH_4^+ was $0.71 \mu\text{g-at N l}^{-1}$, and NO_3^- was $5.84 \mu\text{g-at N l}^{-1}$.

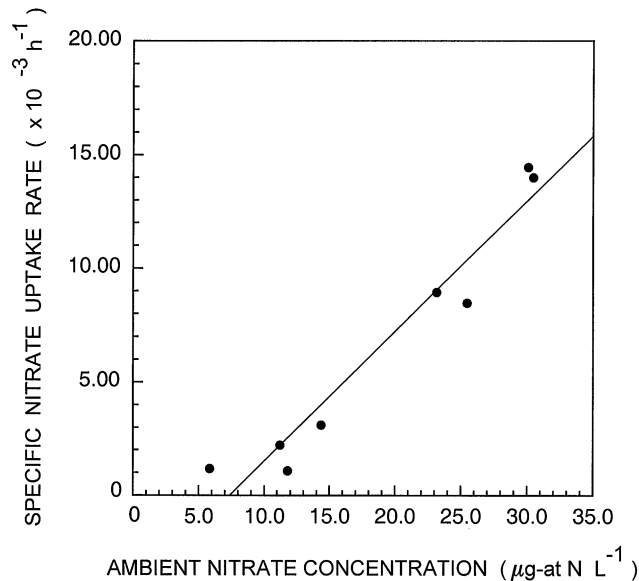


Fig. 6. The relationship between specific NO_3^- uptake rates by natural assemblages of phytoplankton and ambient NO_3^- concentrations in the Ross Sea during 1996–97. The solid line denotes a least-squares, linear regression line, $r^2 = 0.94$ ($P < 0.001$).

similar intercept at $\sim 7 \mu\text{g-at N l}^{-1}$, which may represent a value below which the phytoplankton community is supported primarily by regenerated N forms such as NH_4^+ and urea.

Based on the V_{max} values, apparent N utilization follows the order: $\text{NO}_3^- > \text{NH}_4^+ > \text{urea}$ during the pre-bloom and bloom development period (November–January), and the order: $\text{NH}_4^+ > \text{NO}_3^- > \text{urea}$ during late January–February as the bloom declines (Fig. 4). As mentioned earlier, these V_{max} values are not indicative of actual environmental in situ rates, but signify uptake rates that can be used to indicate potential N preference (Dortch, 1990). The reader is cautioned not to confuse this preference with the relative preference index (RPI, McCarthy et al., 1977), which compares the relative utilization of a particular N substrate to the relative availability of that N substrate in the water. As pointed out by others (e.g., Dortch, 1990; Stolte and Riegman, 1996), the RPI has several problems that limit its ecological relevance and contribute to its misinterpretation; it cannot be used for potential rates realized at substrate concentrations considered saturating for their uptake by phytoplankton.

The variability in the magnitude of the V_{max} values for NO_3^- cannot be ascribed solely to phytoplankton species composition, although higher rates usually were observed in those stations where diatoms were a substantial proportion of the community. The NO_3^- uptake rates also may reflect the ambient concentrations of macro- and micro-nutrients, which can either repress (i.e., NH_4^+) or enhance (i.e., Fe) NO_3^- uptake by phytoplankton. As discussed earlier, substantial NH_4^+ inhibition of NO_3^- uptake was only readily apparent at the pack ice station, but previous studies in the Ross Sea have provided evidence for Fe limitation (e.g., Martin et al., 1991; Sedwick and DiTullio, 1997; Fitzwater et al., 2000; Cochlan et al., 2001) throughout the study area. Although the sources of Fe in the Ross Sea are not well known, it appears that re-suspended shelf sediment,

glacial ice melt, and melting sea ice all may contribute various amounts of Fe to the Ross Sea. Since phytoplankton growing on NO_3^- require more Fe than those growing on NH_4^+ (e.g., Raven, 1990; Maldonado and Price, 1996) it is possible that the higher NO_3^- uptake rates realized at the beginning of the austral growing season in November (Stn. O, 11/05/96; Stn. Orca, 11/29/97) and the subsequent decline in NO_3^- uptake rates may be partially due to increasing Fe-deficiency in the Ross Sea over time and the preferential uptake of NH_4^+ over NO_3^- as an more energetically efficient strategy for obtaining N (Fig. 4).

3.3. Variables to consider when calculating kinetic parameters and estimating f -ratios

The ratio of ‘new’ to total production (*sensu* Dugdale and Goering, 1967), the so-called f -ratio (Eppley and Peterson, 1979) was estimated as $f = V_{\text{NO}_3^-} / [V_{\text{NO}_3^-} + V_{\text{NH}_4^+}]$. At ambient N concentrations, the f -ratio ranged from 0.83 to 0.92 (mean $f = 0.938$) at Stns. O, Orca, and E; the lower values were observed later in the growth season. The apparent strong reliance on ‘new N’ (i.e., NO_3^-) was not observed adjacent to the Ross Ice Shelf (Stn. Emperor: $f = 0.31$) or within the pack-ice (Stn. Pack Ice: $f = 0.54$). These f -ratio estimates were calculated using the Michaelis–Menten equation and the kinetics parameters from Table 2, and assuming ambient NH_4^+ concentrations were $0.02 \mu\text{g-at N l}^{-1}$ when below our limit of detection. There were five points we considered when evaluating these N uptake rates and kinetics parameters: the inclusion of urea in f -ratio calculations, NH_4^+ inhibition of NO_3^- uptake, correcting for isotope dilution, differences in incubation times, and potential effects of dissolved organic nitrogen (DON) release. Each is discussed below.

First, if one assumes that the f -ratios are overestimated by 24% because of non-inclusion of urea uptake (mean value for polar regions reported by Wafar et al. 1995), then corrected f -ratios range from 0.63 to 0.74 (mean = 0.71). However, the single urea kinetic experiment conducted during this study indicates a much smaller correction; f -ratio for Stn. O declines from 0.83 to 0.81 with the inclusion of urea. Recently, Sambrotto and Mace (2000) also found a minor impact of urea uptake on the f -ratio south of the Antarctic Polar Front (APF). They report an average decrease of only 9% when urea uptake is included, but a much greater impact (22% reduction) with its inclusion in the f -ratio north of the APF. Second, although not previously reported for field assemblages, inhibition of NH_4^+ uptake by NO_3^- has been reported in the laboratory for a marine diatom, *Thalassiosira pseudonana*, under high light conditions (Yin et al., 1998), and such an effect may contribute to the depressed rates of NH_4^+ uptake seen in the Ross Sea.

Third, Glibert et al. (1982a), and others, have shown unequivocally that the ^{15}N enrichment of the aqueous pool during $^{15}\text{NH}_4^+$ uptake experiments is not necessarily constant because of its progressive dilution through remineralization of ^{14}N during the incubation period. Using a forward-difference computer model, Garside (1984) demonstrated that by ignoring the effects of this remineralization, it is possible to obtain results from uptake kinetic experiments that are plausible in terms of Michaelis–Menten kinetics, but are actually entirely artifactual. Since the effects of isotope dilution diminish at higher initial enrichment concentrations of $^{15}\text{NH}_4^+$, the maximal uptake rates (V_{max}) determined in the present study without isotope-dilution corrections do not differ significantly from corrected values (2-tailed paired t -test, $P > 0.05$), although uncorrected K_s estimates can be seriously overestimated (1-tailed paired t -test, $P < 0.01$). Consequently, the α parameter ($\alpha = V_{\text{max}}/K_s$), which is considered a more robust indicator for

substrate affinity when substrate concentrations are low ($< K_s$) or when interspecific competition is likely (Healey, 1980; Harrison et al., 1989; Cochlan and Harrison, 1991a), increases by an average of 4.6-fold (range 2.3–9.4) after correction for isotope-dilution. The corrected parameters of affinity demonstrate that Ross Sea phytoplankton are considerably more suited for acquiring the low ambient concentrations of NH_4^+ than would be otherwise indicated, or even expected, in these high NO_3^- waters.

During late spring 1997 (Process IV) at Stn. O, and within the pack ice during late summer (Process II), PN specific uptake rates, adjusted for the effects of isotope dilution, plotted versus total NH_4^+ concentration could not be described by the Michaelis–Menten rectangular hyperbola. This is not surprising for the latter given the elevated ambient concentration of NH_4^+ present beneath the ice ($0.71 \mu\text{g-at N l}^{-1}$). At Stn. O, however, the ambient concentrations of NH_4^+ were not elevated ($0.10 \mu\text{g-at N l}^{-1}$), and the distribution of data in the plot of corrected uptake rates versus concentration for this station (Fig. 3C) is similar to those observed previously for kinetic data obtained when ambient NH_4^+ concentrations have been overestimated (Eppley et al., 1977; Fisher et al., 1981). Thus it is likely that NH_4^+ contamination, either during pre-incubation inoculation or post-incubation concentration and/or isolation of the aqueous fraction, contributed to the discrepancy in the isotope-adjusted rates. Consequently at these two stations and Stn. E, where isotope-dilution measurements were not made, uncorrected rates were used to estimate kinetic parameters.

Our results clearly demonstrate the need to correct NH_4^+ uptake kinetic data for isotope dilution to ensure accurate estimates of N affinity, particularly in regions where substantial N regeneration is expected and/or areas where long incubations are necessary to ensure measurable ^{15}N enrichment of particulate material during the experiment. These more realistic parameters demonstrate the potential importance of NH_4^+ for the N nutrition of the Ross Sea phytoplankton, and should prove valuable in future modeling efforts to describe the nutrient acquisition strategies of phytoplankton as the season progresses and heterotrophic remineralization contributes to increased ambient concentrations of NH_4^+ (e.g. Goeyens et al., 1991a, b).

Fourth, comparison of the kinetic parameters determined during the three cruises is complicated by the necessity of using different incubation periods during the year. During low biomass periods (late winter and spring) long incubations (11–24 h) were required to ensure there was adequate ^{15}N enrichment in the collected PN measurable by mass spectrometry, whereas during the summer period, shorter (6–9 h) incubations were necessary to avoid complete utilization of added NH_4^+ during the kinetic experiments, particularly for the lower $^{15}\text{NH}_4^+$ enrichments. Deviations from linearity for the uptake rates of all three N substrates determined during these longer incubations periods also can be expected due to the effects of diel periodicity on planktonic N uptake (e.g., Cochlan et al., 1991; Vincent, 1992). Although the magnitude of diel and diurnal effects is presently unknown for Antarctic phytoplankton assemblages, one should consider the hourly rates determined during long incubation rates as conservative, and the rates determined during shorter incubation periods as potentially overestimated since the latter were conducted only during daytime periods. However, non-linearity in short-term N uptake responses (e.g., ‘surge uptake’: Conway et al., 1976; McCarthy and Goldman, 1979; Cochlan and Harrison, 1991b) is unlikely due to the nutrient sufficiency expected of phytoplankton in these high NO_3^- waters. Estimates of PN-specific uptake rates also can be biased by the presence of detrital particulate matter in the water. Artifactual increases in PN-specific uptake rates due to the

dilution effect of detrital N (e.g., Garside, 1991) may affect our estimates of V_{\max} ; however, the specific uptake rates for all three N substrates and enrichment levels would be uniformly biased within an experiment, and estimates of K_s will remain unchanged.

Fifth, another caveat to consider with regards to determining uptake rates and estimating kinetic parameters is the potential for significant dissolved organic N (DON) release. DON release can impact calculated uptake rates because the ^{15}N label that is taken up by the cell passes into the DON pool, and is therefore, no longer in the PN pool to be measured as uptake (Bronk et al., 1994). Ward and Bronk (in press) found that rates of DON release were positively correlated with rates of NH_4^+ regeneration in both the Southern California Bight and Monterey Bay, California ($r^2 \geq 0.73$; $P < 0.001$ for both sites combined). The correlation between rates of DON release and NH_4^+ regeneration is likely due to the importance of grazing in both processes. During the kinetic experiments presented here, rates of NH_4^+ regeneration were highest during summer 1997 (Process II; Bronk and Cochlan, unpubl. data). Hu and Smith (1998) measured DON release rates, as a result of NO_3^- uptake, in the Ross Sea using ^{15}N tracer techniques. During two cruises, they found that the percentage of NO_3^- released as DON, during 24-h incubations, was greater later in the growing season ($8.0 \pm 3.8\%$ in early spring 1994 versus $19.0 \pm 13.9\%$ in late spring 1995/1996). Based on our measured rates of NH_4^+ regeneration (Bronk and Cochlan, unpubl. data) and the results of Hu and Smith (1998), we suspect that the difference between gross and net N uptake, as a result of DON release, was likely greater later in the growing season.

3.4. Insights into the N cycle of the Ross Sea

Despite some possible limitations of our experiments, these derived kinetic parameters are the first reported for Antarctic phytoplankton assemblages. Since they have been corrected for the effects of isotopic dilution, they should accurately reflect the N uptake capabilities of phytoplankton in the Ross Sea. Our results demonstrate a number of new observations regarding the N nutrition of the phytoplankton in this region. With the exception of the pack ice station, the affinity for NH_4^+ is consistently high compared to other marine systems, as demonstrated by low K_s values $< 0.40 \mu\text{g-at N l}^{-1}$ and isotope-corrected α values that range from 12.1 to $36.8 \times 10^{-3} \text{ h}^{-1}/(\mu\text{g-at N l}^{-1})$. The affinity for low concentrations of NH_4^+ only declines substantially [$K_s = 0.86 \mu\text{g-at N l}^{-1}$, $\alpha = 2.6 \times 10^{-3} \text{ h}^{-1}/(\mu\text{g-at N l}^{-1})$] within the high NH_4^+ waters beneath the pack ice. Although previous N uptake kinetics have not been determined for Antarctic phytoplankton, lower affinities for NH_4^+ have been reported for natural assemblages in Arctic waters (Table 3). In these comparably high- NO_3^- waters, the values for $K_s\text{-NH}_4^+$ range from < 0.1 to $2.2 \mu\text{g-at N l}^{-1}$, and are similar to those values reported for temperate neritic diatoms and flagellates ($1.82 \pm 0.09 \mu\text{g-at N l}^{-1}$; Eppley et al., 1969) and natural assemblages of coastal phytoplankton (~ 0.5 to $2.0 \mu\text{g-at N l}^{-1}$; Kudela and Cochlan, 2000). However, the lower K_s values presented here for the open Ross Sea ($0.04\text{--} < 0.4 \mu\text{g-at N l}^{-1}$) are more similar to those reported for oceanic phytoplankton species ($0.1\text{--}0.5 \mu\text{g-at N l}^{-1}$; Eppley et al., 1969) and oligotrophic oceanic regions ($0.03\text{--}0.6 \mu\text{g-at N l}^{-1}$; MacIsaac and Dugdale, 1969; Kudela and Cochlan, 2000).

The estimated half-saturation constant for urea ($K_s = 0.12 \mu\text{g-at N l}^{-1}$) from Stn. O (composed primarily of diatoms and nanoflagellates) is similar to those reported for the Barents Sea ($0\text{--}0.20 \mu\text{g-at N l}^{-1}$; Kristiansen and Farbot, 1991; Kristiansen et al., 1994). The K_s values from

both regions are lower than the few values reported for cultures of neritic marine diatoms ($0.4\text{--}2.0\ \mu\text{g-at N l}^{-1}$, McCarthy, 1972; Rees and Syrett, 1979) and the picoflagellate *Micromonas pusilla* ($0.4\ \mu\text{g-at N l}^{-1}$, Cochlan and Harrison, 1991a), but very similar to the range reported for natural assemblages in non-polar, oceanic areas ($0.02\text{--}0.27\ \mu\text{g-at N l}^{-1}$, Kudela and Cochlan, 2000). In summary, it appears that although the Ross Sea is a NO_3^- -rich region populated by relatively large phytoplankton, the half-saturation constants are more characteristic of oligotrophic waters dominated by picoplankton assemblages. In this respect the Ross Sea phytoplankton communities are similar to oceanic phytoplankton in that they possess a relatively efficient means of acquiring these reduced N forms, even though they are normally available in very low concentrations in the euphotic zone during the growing season.

3.5. Potential N uptake by heterotrophic bacteria

It is well known that heterotrophic bacteria are capable of utilizing a significant, but highly variable ($<5\text{--}90\%$), fraction of the total dissolved N taken up in estuarine and marine systems (e.g., review by Kirchman, 1994, Kirchman et al., 1994, Hoch and Kirchman, 1995; Middelburg and Nieuwenhuize, 2000). In general, bacteria preferentially utilize NH_4^+ before NO_3^- or urea, and likely gain most of their N requirement from dissolved free amino acids in coastal environments (e.g., Billen and Fontigny, 1987; Kirchman, 1994; Hoch and Kirchman, 1995), although recent studies of the inner reaches of the turbid estuaries of western Europe have shown a strong preference for NH_4^+ and NO_3^- (Middelburg and Nieuwenhuize, 2000). In Antarctica, bacterial uptake of NO_3^- was first hypothesized by Glibert et al. (1982b) based on observations of anomalously high rates of specific NO_3^- uptake at low light levels in the MIZ of the Scotia Sea, and 24 h dark NO_3^- uptake rates, which approximated rates determined under normal light/dark cycles. The importance of heterotrophic N uptake in the Scotia Sea also was suggested by Rönner et al. (1983) based on similar 24 h experiments where they found that the percentage of total uptake independent of light increased with depth such that dark NO_3^- uptake equaled light uptake at the 1% isolume. Others, however, have reported that nighttime NO_3^- uptake either ceased (Olson, 1980) or was very low (10–30%) compared to daytime uptake rates (Koike et al., 1986), or argue that phytoplankton are adapted to utilize NO_3^- at low light depths (Nelson and Smith, 1986).

Direct measurements of N uptake by heterotrophic bacteria in the Southern Ocean have only been conducted in the coastal waters of the northern Gerlache Strait region of the Antarctic Peninsula (Tupas et al., 1990, 1994). They determined that bacteria ($<0.80\ \mu\text{m}$ filtrates incubated in the dark) were responsible for 8–25% (mean = 17%) of the total community $^{15}\text{NH}_4^+$ uptake as measured using GF/F filters, even with low bacterial abundance during a rich phytoplankton bloom. In the present study, we did not directly measure uptake of ^{15}N -labeled N forms by heterotrophic bacteria, but have estimated bacterial N demand from estimates of incorporation rates of ^3H -thymidine (or ^3H -leucine when not available) determined using the microcentrifugation method of Smith and Azam (1992) and reported in detail by Ducklow et al. (2000). To do these calculations, the following assumptions were made. First, to compare the biomass of autotrophic phytoplankton and heterotrophic bacteria at the stations where N kinetic experiments were conducted, heterotrophic bacterial carbon biomass was calculated using a conversion factor of $106\ \text{fg C}\ \mu\text{m}^{-3}$ (Carlson et al., 1999) from bacterial abundance and biovolume

estimates (Ducklow et al., unpublished data). Autotrophic biomass was estimated using the equations of Smayda (1978) and Eppley et al. (1970) for volumes determined from geometric shapes (Smith et al., unpublished data). Second, the ratio of bacterial biomass to phytoplankton biomass was corrected for the mean efficiency of 81% for bacterial capture on combusted GF/F filters (described in methods above). Third, bacterial N demand was determined using 8.6×10^{17} cells mol^{-1} thymidine incorporated (Ducklow et al., 1999) and 5.6 fg N cell^{-1} (Lee and Fuhrman, 1987). At stations where only leucine incorporation rates were available, we used the conversion factor of 1.5 kg C mol^{-1} leucine incorporated (Ducklow et al., 2000) and a bacterial C : N ratio of 3.7 (Lee and Fuhrman, 1987).

Assuming that all the N demands are met by NH_4^+ alone, the bacterial portion of total NH_4^+ uptake, during trace-level enrichment experiments, was relatively low and variable (mean = 13, range: <1–26%) when the ratio of bacterial to autotrophic biomass in the seawater was low (<5%; Table 4). When bacterial abundance and biomass were highest (Stn. O, during the bloom of *P. antarctica* in January), the bacterial portion of total NH_4^+ uptake was maximal (35%), and greater than when diatoms were a significant portion of the assemblage (26%; Stn. Orca). Total NH_4^+ uptake determined using saturating (5–10 $\mu\text{g-at N l}^{-1}$) enrichments of $^{15}\text{NH}_4^+$ indicates a much lower bacterial contribution to total uptake in the Ross Sea (mean = 8, range: <1–15%). Although this study does not attempt to estimate the extent to which Antarctic bacteria compete

Table 4

Nitrogen uptake by heterotrophic bacteria and phytoplankton in the Ross Sea, Antarctica Ratio of bacterial carbon biomass to phytoplankton biomass collected on glass fiber filters used in the N kinetic experiments. Bacterial N demand calculated from the incorporation of ^3H -thymidine using the microcentrifugation method of Smith and Azam (1992). The potential contribution of bacterial N uptake to total community NH_4^+ and NO_3^- uptake (as measured on GF/F filters) is shown as percentages in parentheses

Station, date	Bact/Phyto on GFF ^a (%)	Bacterial N demand ^b (ng-at $\text{N l}^{-1} \text{h}^{-1}$)	Total NH_4^+ uptake (ng-at $\text{N l}^{-1} \text{h}^{-1}$)		Total NO_3^- uptake (ng-at $\text{N l}^{-1} \text{h}^{-1}$)
			Trace ^c	Saturated ^d	Trace ^e
Stn. O, 11/05/96	3.3	0.100	0.398 [20.3]	0.637 [12.7]	8.51 [0.9]
Stn. O, 01/31/97	17.1	1.423	3.25 [35.5]	10.55 [10.9]	9.59 [12.0]
Stn. O, 12/07/97	3.2	0.068*	12.44 [0.4]	17.27 [0.3]	19.17 [0.3]
Stn. Emperor, 02/04/97	4.6	1.499	7.27 [16.7]	8.34 [14.6]	3.34 [36.4]
Stn. Orca, 01/21/97	3.9	1.709	5.23 [26.4]	22.13 [6.3]	16.28 [8.5]
Stn. Orca, 11/29/97	4.5	0.041	1.09 [3.0]	2.28 [1.4]	6.43 [0.5]
Stn. E, 12/05/97	—	—	5.01	17.0	57.2

^a Ratio of bacterial biomass to phytoplankton biomass corrected for the mean efficiency of 81% of bacterial capture on combusted GF/F filters. Conversion factors for estimating heterotrophic and autotrophic biomass are presented in the text.

^b Bacterial N demand determined using 8.6×10^{17} cells mol^{-1} thymidine incorporated (Ducklow et al., 1999) and 5.6 fg N per cell (Lee and Fuhrman, 1987), except * where leucine incorporation rates were used (1.5 kg C mol^{-1} leucine incorporated, and C : N ratio of 3.7).

^c Trace NH_4^+ uptake rates determined from 0.10 $\mu\text{g-at N l}^{-1}$ enrichments of $^{15}\text{NH}_4^+$.

^d Saturated NH_4^+ uptake rates determined from 5–10 $\mu\text{g-at N l}^{-1}$ enrichments of $^{15}\text{NH}_4^+$.

^e Trace NO_3^- uptake rates determined from $^{15}\text{NO}_3^-$ enrichments <10% of ambient NO_3^- concentration.

with phytoplankton for NH_4^+ , it is likely that the ambient concentration of NH_4^+ influenced the partitioning of NH_4^+ into bacteria and phytoplankton, and that by increasing the NH_4^+ concentration, relatively more of the NH_4^+ goes into the larger plankton (Suttle et al., 1990). Therefore one can expect that the magnitude of the community half-saturation parameters (K_s) will reflect more of the contribution of bacterial NH_4^+ uptake than the maximal uptake rates (V_{\max}) since bacteria did not acquire a significant portion of the total NH_4^+ utilized when it was available at the higher concentrations necessary to support V_{\max} rates.

If one assumes that all the N demands are met by NO_3^- alone, rather than NH_4^+ , then the bacterial portion of total NO_3^- uptake is similar to those obtained with trace-level NH_4^+ uptake rates and range from < 1% to 36% (mean = 10%), which is substantially lower than the 5–60% (mean = 32%) recently reported for the high NO_3^- waters of the sub-arctic Pacific (Kirchman and Wheeler, 1998). Clearly heterotrophic bacteria contribute to the measured uptake rates and kinetic parameters determined in our study; however, because bacteria are generally a minor component of the planktonic biomass in these waters, the kinetic parameters determined in this study are more likely attributable to the natural phytoplankton assemblages rather than heterotrophic bacteria. Therefore, in the Ross Sea heterotrophic bacteria likely play a more important role as remineralizers rather than competitors for dissolved inorganic N. Similarly, bacteria have been shown to produce more NH_4^+ than they take up in the Gerlache Strait region of the Antarctic Peninsula (Tupas et al., 1994).

3.6. Potential mutualistic relationship between phytoplankton and bacteria

Nitrogen uptake by microorganisms is influenced by a number of factors including the size, shape, mobility and intracellular processes of planktonic cells, in addition to the physical and chemical characteristics of the external environment. It is difficult to compare the N kinetics obtained in this study from station to station due to the changing species composition of the natural assemblages, but there may be changes in the chemical composition of the external environment at any one station that are also missed in the present analyses. Specifically, we have compared the kinetic parameters at Stn. O during the first growing season (Process I and II). Although there were only very slight differences in ambient NH_4^+ concentrations observed between the cruises, NH_4^+ uptake capacity varied substantially from the beginning (11/05/96) to the end (1/31/97) of the growing period. We hypothesize that bacteria–phytoplankton mutualism and the creation of microzones of high NH_4^+ concentrations from intense remineralization (directly by bacteria and indirectly due to predation of bacteria by heterotrophic nanoflagellates; Azam and Smith, 1991) can explain the dramatic change in phytoplankton NH_4^+ uptake capability at Stn. O over the growing period, despite any measurable change in ambient NH_4^+ concentration.

Our rationale for this hypothesis is as follows. First, Stn. O was initially dominated by colonial cells of *P. antarctica*, and autotrophic nanoflagellates, which were >90% unicellular motile *P. antarctica*. Second, the maximal uptake rate of NH_4^+ at that time was the lowest recorded in the study, but the ability to utilize low concentrations of NH_4^+ was relatively high (i.e., low K_s , high α). Twelve weeks later, the V_{\max} increased 4-fold, but the estimated affinity was the lowest value obtained in this study (K_s increased 8-fold, α was halved). Although the community was still dominated by colonial *P. antarctica* (8-fold increase in abundance), N remineralizers had

increased dramatically in number—heterotrophic bacteria increased 24-fold (Ducklow et al., unpublished data) and heterotrophic nanoflagellates increased 51-fold, with <20% increase in abundance of unicellular, *P. antarctica*. We suggest that the polysaccharide mucus secreted by *P. antarctica* could both promote the growth, and harbor these heterotrophic microorganisms (in close association with the colonies) and produce microzones of high NH_4^+ from remineralization, which are not necessarily measurable by traditional bulk water sampling. Thus the high V_{max} value would enable the phytoplankton to exploit these microzones of high concentrations, and also maintain their competitive kinetic advantage over bacteria for NH_4^+ , despite the appearance of low ambient concentrations. In reality, maximum uptake rates may be even greater than those reported at the end of the bloom period due to the effects of dilution by high concentrations of heterotrophic bacteria (both viable and non-viable) on PN-specific uptake rates. Finally, the increased abundance of larger colonial cells over smaller motile, unicellular *P. antarctica* may contribute to the decreased ability to utilize low concentrations of NH_4^+ on the basis of reduced surface area per volume. Although our study provides no direct evidence of bacteria–phytoplankton mutualism, or even the competitive abilities of bacteria in Antarctica, it demonstrates that knowledge of the uptake capabilities and interactions of both heterotrophic and autotrophic microorganisms are necessary for a mechanistic understanding of N dynamics in this and other marine systems.

4. Conclusions

During the three cruises for which kinetics results are presented for microplankton assemblages in the Ross Sea, ambient concentrations of NO_3^- and silicic acid were at saturating levels, and the concentrations of the reduced N forms were low and variable. Maximum NO_3^- uptake rates were positively correlated to ambient NO_3^- concentrations, but there were no relationships between ambient concentrations of NH_4^+ or urea, and the V_{max} values of NH_4^+ , urea, or NO_3^- . Based on measured V_{max} values, apparent N utilization followed the order $\text{NO}_3^- > \text{NH}_4^+ > \text{urea}$ during the pre-bloom and bloom development conditions of early spring (1996) and late spring (1997), but changed to $\text{NH}_4^+ > \text{NO}_3^- > \text{urea}$ as the bloom was declining in the summer (1997). This change in potential N preference reflects either the decreasing ability of the phytoplankton to utilize NO_3^- as the season progresses, possibly due to the effects of Fe limitation, or an increasing ability to use NH_4^+ when available in elevated concentrations, or both. With respect to kinetic parameters, we found that: inclusion of urea in the calculation of f -ratios resulted in a smaller correction than anticipated; evidence for inhibition of NO_3^- uptake by NH_4^+ was rarely observed; failure to account for NH_4^+ isotope dilution resulted in a 4.6-fold average underestimate of α ; and that underestimates in N uptake rates due to DON release was likely most significant during the summer (Process II) cruise. Other novel findings were that the microplankton community consistently showed a high affinity for NH_4^+ , relative to other marine systems, and that estimates of bacterial NH_4^+ uptake ranged from <1%, when the ratio of bacteria to autotrophic biomass was low, to 35%, when bacterial abundance and biomass was highest. Finally, experiments performed at Stn. O during all three cruises indicate the presence of plankton communities with very different NH_4^+ uptake capabilities, despite the fact that ambient NH_4^+ concentrations were virtually unchanged. We suggest that these results may be due to a mutualistic relationship

between phytoplankton and bacteria, and the creation of microzones of high NH_4^+ concentration, which remain undetected by traditional sampling methods, but contribute to the observed kinetic variability in N utilization by Ross Sea planktonic assemblages.

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