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Inorganic nitrogen metabolism by Antarctic phytoplankton with special reference to ammonium cycling

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ABSTRACT: Nitrogen metabolism of planktonic populations in coastal waters of the southern Scotia Sea was studied in February-March 1981 aboard R/V *Melville*. In spite of the high concentration of nitrate present in these waters (21 to 29 μM), up to 93 % of phytoplankton nitrogen was assimilated in the form of ammonium, with the overall mean being 78 %. Time course experiments showed that uptake of nitrate and ammonium during nighttime amounted to approximately 15 and 50 %, respectively, of daytime values. This light dependency of nitrate, and – to a lesser extent – ammonium, is reflected in the depth profiles of nitrate and ammonium assimilation. Over the shallow waters of the Scotia Arc close to Elephant Island, most of the ammonium uptake was associated with nanoplankton (< 20 μm size). Estimating the flux of ammonium through the food web from the biomass and metabolic activities of bacteria, phytoplankton and zooplankton suggests that heterotrophic microbial organisms are responsible for most of the ammonium which is regenerated and assimilated by phytoplankton. Data from isotope dilution experiments confirmed that micrograzers do account for most of the regenerated ammonium. The high concentrations of ammonium in these coastal waters (average of 2.1 μM) are discussed relative to seasonal events and to zooplankton activity.

INTRODUCTION

All waters south of the Antarctic Polar Front are characterized by low temperature (approximately + 4.5 to − 1.8 °C) and high nutrient concentrations, which are maintained by large-scale upwelling and turbulent mixing in the upper water column. The relative instability of the water column often results in deep 'mixed layers' which influence the distribution of planktonic organisms and enhances the supply of nutrients to surface waters. The Antarctic marine ecosystem is thus unique in the world's oceans, as it is quite different from Arctic regions where there is generally depletion of nutrients in surface waters (Nemoto & Harrison 1981). It shares some similarities with the upwelling systems off the west coasts of South America and Africa, but these are relatively much warmer and constitute a much smaller area.

Antarctic waters also differ markedly from the upwelling areas off Peru and South Africa in regard to primary production and nutrient characteristics of the water. Although the Antarctic was historically considered to be very rich in phytoplankton productivity (Hart 1934, Murphy 1962), this view apparently was based on the assumption that the high standing stocks of krill, seals, and whales required a high rate of primary production. Extensive studies of recent years, however, have indicated that primary production in Antarctic waters is moderate to low as compared to other oceanic areas and, when converted to yearly rates, is comparable to the oligotrophic tropical gyres (El-Sayed 1967, Holm-Hansen et al. 1977, Bröckel 1981).

Ammonium seems to play an important role in the nutrition of Antarctic phytoplankton and is generally in substantial amounts throughout the euphotic zone. Concentrations increase from ~ 0.1 μM in late winter-early spring (Olson 1980) to over 1.0 μM in late summer and fall (Glibert et al. 1982, Rönner et al. 1983). As ammonium is generally not detectable in deep waters, the presence of ammonium in the euphotic zone is

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related primarily to biological processes occurring in the upper water column. Primary production which utilizes ammonium as the nitrogen source is termed 'regenerated production' (Dugdale & Goering 1967) and is intimately related to rates of mineralization of organic matter by biological and chemical processes in the euphotic zone. Nitrate-supported production, on the other hand, is termed 'new production' because supply of nitrate is maintained by advection and turbulence from deeper waters. Thus, if a steady state is maintained between biological activities and physical mixing processes in the euphotic layer, several interesting features of organic matter transformations, including residence time of organic matter in the euphotic layer and removal of organic matter from surface layers, can be estimated by measuring the nitrate and ammonium uptakes rates in the euphotic layer (Eppley & Peterson 1979). Although phytoplankton production in Antarctic waters is not limited by any nutrient deficiency (Hayes et al. 1984), the study of nitrogen metabolism is important in order to quantify rates of organic matter transformations and to furnish information on the dynamics of the microbial food web.

Previous investigations have been mostly in offshore waters (Slawyk 1979, Olson 1980, Glibert et al. 1982, Rönner et al. 1983) and have shown that Antarctic phytoplankton generally derive at least 50 % of their nitrogen from ammonium. Primary production measurements, however, have shown that waters over the continental shelf in the Antarctic are generally much more productive than offshore waters (Fogg 1977, El-Sayed et al. 1983), and hence it is of interest to assess the dynamics of nutrient assimilation and mineralization in inshore waters. In this paper we report on experiments carried out mainly in coastal waters during late summer in the area of the Antarctic Peninsula. In addition to documenting the relative uptake rates of ammonium and nitrate as a function of light intensity, our studies were concerned with differential uptake rates of nitrate and ammonium in regard to cell size and rates of regeneration of ammonium. The flux of ammonium, as indicated by our uptake data, is discussed in regard to the biomass and metabolic activities of bacteria, phytoplankton, and zooplankton populatons.

MATERIALS AND METHODS

Experimental data were collected on the Vulcan Expedition (Holm-Hansen & Foster 1981) of R/V *Melville* (Leg 7, February 25 to March 27, 1981). The cruise track covered portions of the Scotia Sea, Weddell Sea, and Bransfield Strait (Fig. 1). Continuous profiles of temperature and salinity were obtained at each station

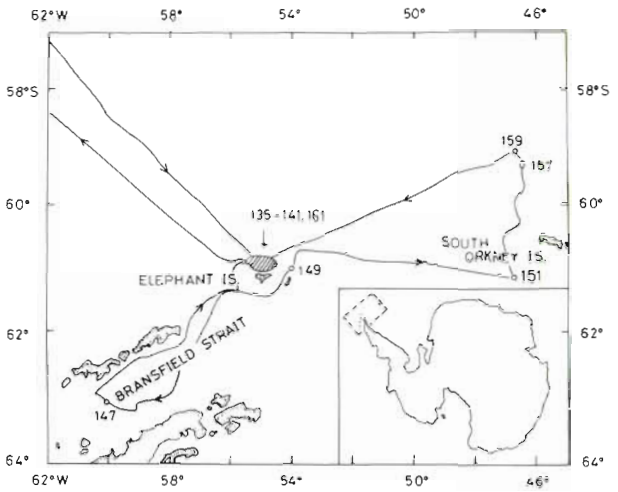

Fig. 1. Cruise track and station locations for R/V *Melville* cruise in the Scotia Sea, February 25 to March 27, 1981

using a Neil Brown Mark III CTD. The vertical profile of solar irradiance was measured with a submersible quantum scalar irradiance meter (Booth 1976). Occasionally, a Secchi disk was also used to determine the sampling depths for ^{15}N-assimilation experiments. Water samples were obtained with PVC 30 l Niskin bottles and used for the measurements listed below.

^{15}N uptake experiments. Immediately after sampling, water was transferred to 4 l polycarbonate bottles or to 1 l borosilicate glass bottles and enriched by addition of either $(NH_4)_2SO_4$ (99.8 % ^{15}N) to a final concentration of 0.2 µmol $^{15}N\ l^{-1}$ or KNO_3 (99.8 % ^{15}N) to a final concentration of 4.0 µmol $^{15}N\ l^{-1}$. These additions caused maximum increases of 18 % (ammonium) and 16 % (nitrate) in inorganic nitrogen concentrations because of the high ambient concentrations in the study area (Table 1). $Hg(Cl)_2$ was added to other water samples (final concentration 10 µM) before addition of ^{15}N-substrates and served as controls.

Incubations were done under simulated *in situ* conditions on deck. Temperature was maintained by pumping surface sea water through the incubator, and the intensity of solar radiation to the samples was adjusted by using neutral filters. Light intensity inside the experimental bottles was occasionally measured by insertion of the light probe of a scalar irradiance quantum meter (BSI model QSL-100) into the bottles. Except for time course experiments, all samples were incubated for 24 h. At the end of incubation, 1.0 l aliquots were filtered through combusted glass fiber filters (GF/F, 47 mm or 24 mm) and the residue on the filter was rinsed with small amounts of filtered seawater (ca 30 ml). The filter was dried in a vacuum desiccator over silica gel and transported in the dry state back to Tokyo for ^{15}N analysis.

Table 1. Inorganic nitrogen uptake rates in Antarctic surface waters. Cultures were incubated for 24 h at ~46 % of incident solar radiation

Station	Sample depth (m)	Depth to bottom (m)	Nitrate (μM)	Ammonium (μM)	Chl *a* (μg l^{-1})	PON (μgN l^{-1})	Total uptake ($V_{NH_4^+}+V_{NO_3^-}$) (nmol N l^{-1} d^{-1})	Total uptake (nmol N [μg chl *a*]$^{-1}$ d^{-1})	$\frac{V_{NH_4^+}}{V_{NH_4^+}+V_{NO_3^-}}$ (%)
Off Elephant Island									
135	5	549	24.8	1.1	0.93	16.6	50	54	73
138	2	258	23.9	2.1	0.69	18.7	65	94	72
139	5	575	25.5	2.1	0.46	11.4	38	83	65
141	5	587	28.8	2.1	0.78	20.0	119	153	77
161	5	152	22.2	1.2	0.59	9.8	41	69	73
									$\bar{X}$ ± SD: 72 ± 4.4
Elsewhere									
147	5	870	21.0	4.5	0.72	13.5	48	67	78
149	5	367	26.9	2.3	1.05	10.0	39	37	85
151	5	292	23.6	2.8	1.04	17.5	78	75	93
157	1	1495	28.0	1.0	0.40	13.3	58	145	82
159	1	1050	27.8	0.9	0.29	6.5	46	159	83
									$\bar{X}$ ± SD: 84 ± 5.5

Size fractionation experiments were carried out at Stns 135 and 138. At the end of the incubation at these stations, 1.0 l aliquots from the 4 l bottles were filtered through a nylon mesh net (either 20 or 10 μm) and the filtrate subsequently filtered through the glass fiber filter as described above.

The $^{15}N/^{14}N$ ratio of particulate organic matter (PON) on the filter was measured by mass spectrometry with the method of Wada et al. (1977). PON was first converted to nitrogen gas by direct combustion in the presence of copper oxide, and the nitrogen gas then was introduced into a Hitachi RMU-6 mass spectrometer equipped with a double collector for ratiometry. The overall precision of the mass spectrometry was 0.002 atomic % in ^{15}N content. Uptake rates for nitrate or ammonium were calculated on the basis of the PON concentration and its $^{15}N/^{14}N$ ratio following the equation given by Dugdale & Goering (1967). To calculate the *in situ* nutrient uptake rates, we ignored any possible effect of nutrient increase caused by the addition of ^{15}N nutrients as the nutrient additions never increased ambient concentrations by more than 18 %.

For ammonium isotope dilution experiments, 4.0 l of seawater were enriched with ^{15}N-$(NH_4)_2SO_4$ to a final concentration of 0.4 μmol ^{15}N l^{-1} and incubated as described above. At intervals between 24 to 48 h, 1 l aliquots of water were filtered through glass fiber filters (GF/F, 47 mm) and the residue of the filter was preserved for ^{15}N measurements. ^{15}N ammonium in the filtrate was extracted and concentrated following the method of Hattori et al. (1980). The $^{15}N/^{14}N$ ratios of particulate organic matter and of ammonium obtained from these dilution experiments were measured by mass spectrometry with the method of Grunseich et al. (1980).

Chemical analyses. Chlorophyll *a* concentrations in the sample water were determined by measurement of fluorescence as described by Holm-Hansen et al. (1965) after extraction of the phytoplankton residue on a glass fiber filter (Whatman GF/C, 24 mm) in absolute methanol (Holm-Hansen & Riemann 1978).

Samples for particulate organic carbon and nitrogen were filtered onto a combusted glass fiber filter (Whatman GF/C, 24 mm) and measured in a Hewlett Packard CHN gas analyser (Sharp 1974).

Nitrate, nitrite, and ammonium concentrations in the sample waters were measured on board ship, using a Technicon AA-II Auto Analyzer, following methods detailed by Biggs et al. (1982). The determination of nitrate is based on the method described by Armstrong et al. (1967), while that for ammonium is based on the method described by Solórzano (1969). Near-surface concentrations of nitrate and ammonium are shown in Table 1; the concentration of nitrite was generally less than 0.3 μM throughout the region (see Biggs et al. 1982) and hence negligible compared to the nitrate concentration.

Samples for determination of adenosine triphosphate (ATP) concentration were filtered through glass fiber filters (Whatman GF/F, 25 mm), extracted in boiling TRIS buffer, and frozen until analysis on board ship (Holm-Hansen & Booth 1966). ATP was measured in an ATP-Photometer (SAI, Inc., model #2000) by the bioluminescent reaction (Holm-Hansen 1973).

RESULTS

Nitrogen assimilation rates in near surface waters at all 10 stations investigated are shown in Table 1, together with data on ambient concentrations of nitrate, ammonium, chl *a*, and PON. Total nitrogen assimilation (nitrate and ammonium) rates varied only from 38 to 119 nmol N l^{-1} d^{-1}. However, if total inorganic nitrogen uptake is normalized by chl *a* concentrations, assuming that the phytoplankton are mainly responsible for the inorganic nitrogen uptake, the range of specific uptake rates of inorganic nitrogen increased to values between 37 and 159. Very high specific uptake rates were found at one station near Elephant Island (Stn 141) and the 2 stations (Stns 157 and 159) north of South Orkney Islands.

Ammonium accounted for most of the total daily nitrogen requirement of the phytoplankton, ranging from 65 to 77 % off Elephant Island and from 78 to 93 % elsewhere. One reason for grouping the stations located in the shelf waters north of Elephant Island is that this was the area where bioacoustic data (Macaulay et al. 1984) detected a very large swarm of krill (approximately 2.1 million metric tons over an area of 450 km^2). The density of krill within the swarm (200 to 500 g m^{-3}) could be expected to have pronounced effects on nutrient concentrations and phytoplankton biomass as compared to the other areas

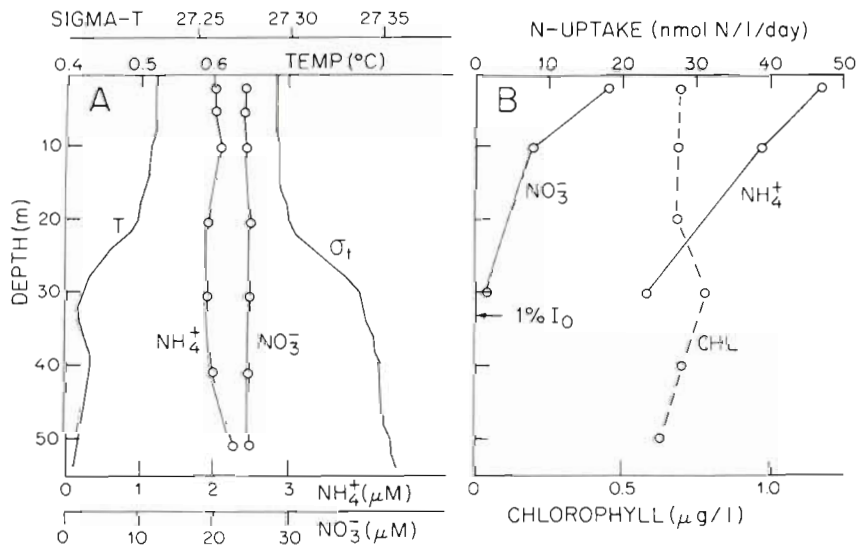

Fig. 2. Depth profiles of physical, nutritional and biological properties at a coastal station (Stn 138) in the Antarctic. See text for details of inorganic nitrogen uptake. Depth of euphotic zone is indicated by the arrow. Note that ranges for temperature and sigma-t are greatly expanded to show changes with depth

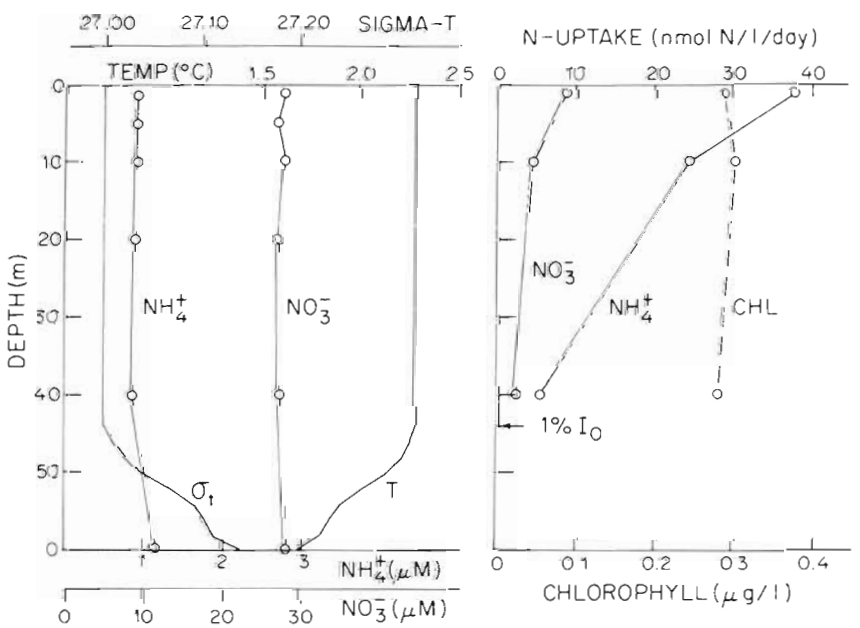

Fig. 3. Depth profiles of physical, nutritional and biological properties at an offshore station (Stn 159) in the Antarctic. See text for details of inorganic nitrogen uptake rates. Depth of euphotic zone is indicated by the arrow. Note that ranges for temperature and sigma-t are greatly expanded to show changes with depth

studied, where krill were sparse or not detected acoustically.

Profiles of nitrogen uptake rates, together with profiles of nutrients and water density (σ_t), are shown at a coastal station (Stn 138) and an offshore station (Stn 159) in Fig. 2 and 3, respectively. At both stations, the upper 50 m of the water column was quite homogeneous with respect to the physical and biological parameters. High nitrate and ammonium concentrations in the euphotic zone were found at both stations, although coastal waters had much more ammonium as compared to offshore waters. Nitrate concentrations at both stations were ca 25 µM and showed little regional variation (Table 1).

Uptake rates of ammonium and nitrate both decreased with depth, suggesting that light intensity

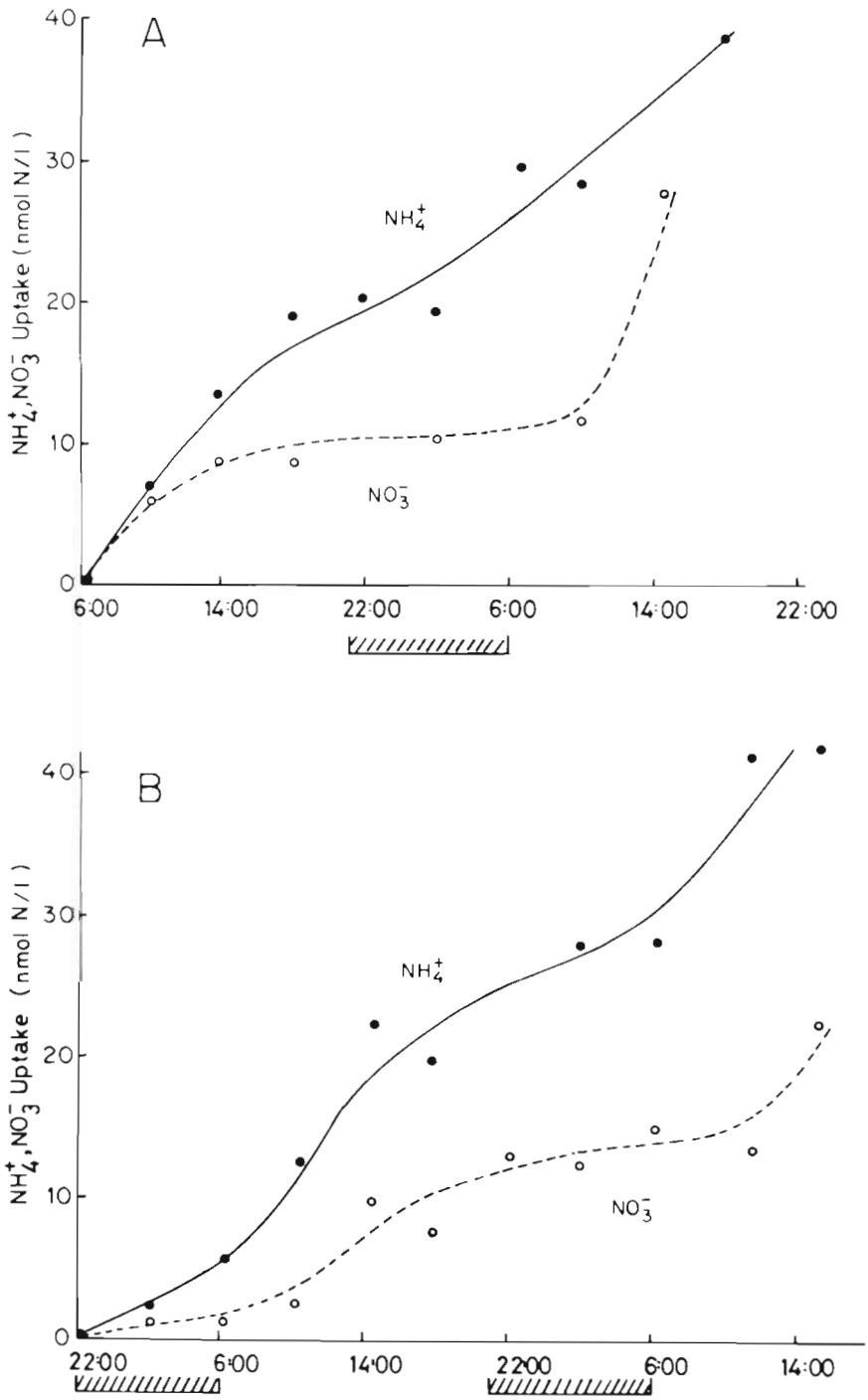

Fig. 4. Time course of nitrate (○) and ammonium (●) uptake in Antarctic surface waters. Unit of incubation period is local time, and the shade represents dark period. (A) Stn 139, 5 m depth, ambient nitrate, 25.5 µM, ammonium, 2.13 µM. (B) Stn 161, 5 m depth, ambient nitrate, 22.2 µM, ammonium, 1.21 µM

in the water column primarily controlled the *in situ* uptake of inorganic nitrogen. The rate of ammonium uptake was much faster than that of nitrate uptake at all depths and increasingly so in the deeper samples, in spite of nitrate being present in concentrations which are over 10 times as high as the ammonium concentrations. Even though nitrogen uptake rates decrease with depth in a manner similar to the attenuation of light in the water column, it is seen that this is not reflected in the concentrations of nitrate or ammonium throughout the euphotic zone. These nutrients, as well as chl *a* concentrations, are quite uniform in the upper 30 m of the water column, suggesting that physical mixing processes are relatively rapid.

The time courses of ammonium and nitrate uptake were examined using surface water collected near Elephant Island (Fig. 4). Both nitrate and ammonium uptake rates decreased during the night period, but the nitrate uptake was reduced much more (10 to 30 % of day time value) as compared to ammonium uptake (ca 50 % of daytime value). This result was consistent with the depth profiles of nitrate and ammonium uptake rates in the same area, where the phytoplankton populations were distributed rather homogeneously throughout the euphotic zone (0 to 30 m). Nitrate uptake at ca 1 % light intensity was less than 10 % of that at 45 % light intensity, while ammonium uptake at 1 % light intensity was about half that at 45 % light intensity (Fig. 2). Olson (1980) and Glibert et al. (1982) reported similar trends of inorganic nitrogen uptake as functions of light intensity and depth for phytoplankton populations also collected from the western region of the Scotia Sea in early spring and summer of 1978–79.

Differential response of phytoplankton (phylogenetic group, cell size, etc.) to varying concentrations of nitrate and ammonium may be of some importance in regard to species composition of phytoplankton crops in Antarctic waters. The size dependency of phytoplankton cells in regard to nitrogen uptake rates was studied at 2 stations. At Stn 135 (Table 2) about one-half the chl *a* was found in the nanoplankton (< 20 µm diameter cells) fraction. The uptake rates of nitrate were roughly proportional to the chlorophyll distribution. The ammonium uptake, however, was almost totally attributed to the nanoplankton population. A similar trend was observed at Stn 138, where 10 µm size net was used (Table 3). More than one-half the ammonium uptake was accounted for by the plankton population less than 10 µm in size while only one-third of the chl *a* was found in this size fraction. Nitrate uptake at Stn 138 was proportional to chl *a* concentrations, except at 30 m depth. These results clearly demonstrate the importance of small size planktonic populations for inorganic nitrogen metabolism in this region because ammonium uptake accounted for over 75 % of their total inorganic nitrogen assimilation, and

Table 2. Size fractionation of ammonium and nitrate uptake by phytoplankton in Antarctic waters (Stn 135)

Parameter	Control (without fractionation)		< 20 µm	
Depth 5 m, light 46 % of I_0				
Chlorophyll *a* (µg l^{-1})	0.93	(100 %)	0.45	(48 %)
NH_4^+ uptake (nmol N l^{-1} d^{-1})	37	(100 %)	38	(103 %)
NO_3^- uptake (nmol N l^{-1} d^{-1})	13	(100 %)	6.2	(48 %)
Depth 10 m, light 8.2 % of I_0				
Chlorophyll *a* (µg l^{-1})	1.07	(100 %)	0.52	(49 %)
NH_4^+ uptake (nmol N l^{-1} d^{-1})	40	(100 %)	39	(98 %)
NO_3^- uptake (nmol N l^{-1} d^{-1})	29	(100 %)	17	(58 %)

Table 3. Size fractionation of ammonium and nitrate uptake by phytoplankton in Antarctic waters (Stn 138)

Parameter	Control (without fractionation)		< 10 µm	
Depth 2 m, light 46 % of I_0				
Chlorophyll *a* (µg l^{-1})	0.69	(100 %)	0.26	(38 %)
NH_4^+ uptake (nmol N l^{-1} d^{-1})	47	(100 %)	25	(55 %)
NO_3^- uptake (nmol N l^{-1} d^{-1})	18	(100 %)	7.0	(38 %)
Depth 10 m, light 8.2 % of I_0				
Chlorophyll *a* (µg l^{-1})	0.68	(100 %)	0.27	(40 %)
NH_4^+ uptake (nmol N l^{-1} d^{-1})	38	(100 %)	30	(79 %)
NO_3^- uptake (nmol N l^{-1} d^{-1})	7.4	(100 %)	2.8	(38 %)
Depth 30 m, light 1.3 % of I_0				
Chlorophyll *a* (µg l^{-1})	0.77	(100 %)	0.26	(34 %)
NH_4^+ uptake (nmol N l^{-1} d^{-1})	22	(100 %)	16	(73 %)
NO_3^- uptake (nmol N l^{-1} d^{-1})	1.4	(100 %)	1.2	(86 %)

their ammonium uptake per unit chlorophyll was much higher than in the microplankton.

Table 4 summarizes the rates of ammonium uptake and production in surface waters at 3 stations as measured by the ammonium isotope dilution method of Hattori et al. (1980) on water pre-screened through 370 μm mesh. Because incubation times for these experiments ranged from 2 to 6 d, we recognize that absolute rates might thus be different from those of 24 h incubations for ammonium uptake (Tables 1 to 3). Nonetheless, it is clear that significant amounts of ammonium (15 to 90 % of ammonium uptake) can be regenerated by planktonic organisms smaller than 370 μm in size.

Table 4. Rates of ammonium uptake and production in the surface waters of the Scotia Sea. Biological and chemical data on the particulate material (< 200 μm) in the water samples are also shown for interpretive reasons

Station	Depth (m)	NH_4^+ uptake[1] (nmol N l^{-1} d^{-1})	NH_4^+ production[1] (nmol N l^{-1} d^{-1})	Chl (μg l^{-1})	ATP (μg l^{-1})	Chl/ATP	POC (μg l^{-1})	Phyto-C[2] (μg C l^{-1})	Phyto-C as % of POC	Phyto. composition (% of total) Diatoms	Dino-flagellates	Monads and flagellates
138	10	310	270	0.62	.223	2.78	115	14.6	12.7	50.4	38.8	10.7
145	5	230	34	3.15	.456	6.91	232	76.3	33.	78.3	8.0	13.5
152	5	310	190	0.78	.241	3.24	69	19.5	28.	16.5	8.4	75.1

[1] Estimates from ^{15}N-isotope dilution method of Hattori et al. (1980)

[2] Organic carbon of phytoplankton as determined by cell counts and volume measurements of all phytoplankton cells using inverted microscope techniques

DISCUSSION

Uptake of nitrogenous nutrients by marine phytoplankton

Preferential uptake of ammonium over oxidized forms of inorganic nitrogen by phytoplankton has been reported in various marine environments (Dugdale & Goering 1967, McCarthy et al. 1977) and is well known from laboratory investigations. The Southern Ocean is no exception, in spite of the fact that nutrients in surface waters there occur in very high concentrations, with nitrate frequently in excess of 20 μM. Even in early spring, when the ammonium concentration in surface waters was low (0.1 to 0.4 μM), phytoplankton in the Scotia Sea assimilated approximately half their nitrogen as ammonium and half as nitrate (Olson 1980). In late summer, when surface ammonium concentrations in the same areas studied by Olson had increased to values of approximately 1.0 μM, the phytoplankton obtained more than 60 % of their inorganic nitrogen demand in the form of ammonium (Glibert et al. 1982). Our observations complement and extend the above results, showing that ammonium uptake ranged from 65 to 93 % of the inorganic nitrogen assimilated by phytoplankton in the area of the Scotia Arc in late summer 1981. Our values are also in close agreement with those of Rönner et al. (1983), who showed that ammonium accounted for an average of 78 % of the nitrogen assimilated by phytoplankton in the oceanic areas of the Scotia Sea earlier in the same austral summer.

The size dependency of ammonium and nitrate uptake has been little studied, however, in Southern Ocean waters. We found a remarkable difference in the assimilation of ammonium and nitrate between nanoplankton (< 20 μm) and microplankton (> 20 μm in cell diameter). Nanoplankton actively assimilated both ammonium and nitrate, while the microplankton assimilated mostly nitrate. In fact, almost all the

ammonium uptake was attributed to nanoplankton (and more than half this was by the phytoplankters less than 10 μm in size), though 50 % of the phytoplankton cells were larger than 20 μm in size and about 65 % were larger than 10 μm in size. Moreover, the total uptake rate of inorganic nitrogen by nanoplankton (103 μmol N (μg chl *a*)$^{-1}$ d^{-1}; average of 2 depths at Stn 135) was several times higher than that of the microplankton (19 μmol N (μg chl *a*)$^{-1}$ d^{-1}). These rates should reflect differences in growth rates between nano- and microplankton. Bröckel (1981) has reported that nanoplankton account for about 70 % of the phytoplankton biomass in the Scotia Sea during summer and for about 90 % of total primary production. Thus, both carbon and nitrogen assimilation data are consistent in indicating the importance of nanoplankton as primary producers in the Scotia Sea, and as the dominant organisms involved with ammonium assimilation.

Pool sizes and fluxes of nitrogen in the euphotic zone

The finding that ammonium is the main nitrogen substrate to support primary production in Antarctic waters strongly suggests that ammonium arises by mineralization processes associated with the food web in the upper water column. It is thus of interest to view the flux of ammonium in the euphotic zone relative to the concentrations of fixed nitrogen. Data in Table 5 summarize estimates of such pool sizes for the euphotic zone of Antarctic waters near Elephant Island. To estimate amounts of phytoplankton-nitrogen, we have used the average chl *a* concentration (0.76 μg l^{-1}; n = 30) and converted to nitrogen by multiplying by a factor of 10. This factor is based on analyses of both cultures and natural populations of Antarctic phytoplankton which show an average chl *a*/N ratio of 0.10 by weight (Sakshaug & Holm-Hansen 1986). Nitrogen contents in bacterioplankton and micro-zooplankton were estimated on the basis of cell numbers and volumes (pers. comms from F. Azam and J. R. Beers). Net zooplankton-nitrogen was estimated by the average wet volume of net zooplankton collected by Bongo nets (333 μm mesh) in a series of consecutive oblique tows from 150 m to the surface and converted to nitrogen (Brinton et al. 1981). This estimate of net zooplankton does not include krill swarm data and thus represents a generalized 'background number' for non-aggregated macrozooplankton. The nitrogen content of krill was estimated on samples collected by Bongo nets (Brinton et al. 1981) and calculated as per Holm-Hansen & Huntley (1984); it should be noted that this value represents 'non-swarm' areas. In the superswarm described by Macaulay et al. (1984) in the area north of Elephant Island, the krill density was 200 to 500 g wet wt m^{-3}, which equates to approximately 4 to 10 × 10^3 μg N l^{-1}. Detritus-nitrogen was calculated by subtracting phytoplankton, bacteria, and microzooplankton-nitrogen from total PON; the value so obtained (2.8 μg N l^{-1}) is close to the estimate of 3.0 μg N l^{-1} of detritus obtained by Sakshaug & Holm-Hansen (1986) by linear regressions of chlorophyll, POC, and PON.

Table 5. Estimates of standing stock of fixed nitrogen (mg N l^{-1}) in the euphotic zone of Antarctic coastal waters. See text for derivation of these estimates

Particulate nitrogen:			
Phytoplankton		7.6	
Bacteria		3.1	
Detritus		2.8	
Krill		2.0	
Net zooplankton		1.4	
Microzooplankton		0.7	
	Subtotal		17.6
Dissolved nitrogen:			
Nitrate (include nitrite)		350	
Ammonium		29	
Dissolved organic-N		77	
	Subtotal		456
	TOTAL		474

The partitioning of nitrogen standing stocks in Antarctic surface water has several interesting features compared to the other oceanic regions. First of all, total nitrogen in the water was ca 34 μmol N l^{-1}, or 5.5 times higher than the global average for surface oceanic water, 0 to 100 m (Sharp 1983). About 73 % of this total nitrogen in Antarctic surface waters was in the form of nitrate. Particulate organic nitrogen, of which about 84 % was found in living organisms, represented less than 4 % of total nitrogen. Total microbial biomass (< 200 μm) estimated independently by ATP measurements (av. 177 ng ATP l^{-1}; n = 13, or about 0.63 μmol N l^{-1}) agreed quite well with the estimations shown in Table 5 (0.81 μmol N l^{-1}).

Bacterial biomass represented about 27 % of the total microbial biomass. While this is in the upper range of previous observations in coastal water in temperate regions (3 to 25 %, Ferguson & Rublee 1976, Fuhrman et al. 1980), such standing stocks are in agreement with previous observations on the importance of bacterial metabolism in Antarctic waters (Azam et al. 1981).

Microzooplankton-nitrogen was ca 6 % of the total microbial biomass-N (9 % of phytoplankton-N), or somewhat lower than the previous observations by Bröckel (1981), who reported that microzooplankton standing stock averaged 16 % of phytoplankton biomass in this area at the same time of year. Exclud-

ing krill swarms, macro-zooplankton represented ca 23 % of total biomass-N.

The presence of large amounts of inorganic nitrogen, including ammonium, relative to particulate nitrogen, indicates that the supply of nitrogenous nutrients does not limit the growth rate of phytoplankton in this area. In fact, the ambient ammonium-nitrogen standing stock alone should support over 4 generations of exponential phytoplankton growth.

Table 6 summarizes estimates of the rates of ammonium consumption and production associated with various components of food webs in surface waters. To estimate production, we used data between 0 to 30 m depth. In this depth range, assimilation of ammonium by phytoplankton populations accounts for most biological ammonium consumption. Since nitrification, which is another pathway to remove ammonium, is inhibited by light, it is expected to be low in the surface waters (Olson 1981). To estimate ammonium excretion by net-zooplankton, we used the biomass of zooplankton shown in Table 5 and the rate of ammonium excretion per unit biomass of zooplankton, obtained during this and previous cruises to the Antarctic (El-Sayed et al. 1978, Biggs 1982,). Excretion by non-swarm krill was estimated by using a factor of 30 µg NH_3 excreted per gm dry weight per hour, which is intermediate between the values published by Biggs (1982) and Ikeda (1981). Unfortunately, information on ammonium excretion by microzooplankton in Antarctic waters is not available. However, we used the specific NH_3 excretion rate (200 µg N (mg dry wt)$^{-1}$ d^{-1}), estimated by Harrison (1980) in temperate/tropical seas and corrected the temperature effect by using a Q_{10} of 2.0. It should be noted, however, that our 'microzooplankton' data does not include heterotrophic nanoplankton (mostly flagellates), which undoubtedly are of high significance with regard to nutrient regeneration. Evidence for this is provided by the data of Hewes et al. (1985), who found that heterotrophic organisms make up approximately 30 % of the total nanoplankton biomass in Antarctic surface waters. The heterotrophic eucaryotic organisms in the picoplankton and nanoplankton may thus be the dominant source of ammonium in Antarctic waters. The significance of bacterioplankton on ammonium concentrations in Antarctic waters is not known, as they can both compete with phytoplankton for assimilation of ammonium (Azam et al. 1983) and also produce ammonium from nitrogenous substrates.

Table 6. Ammonium consumption and production (nmol N l^{-1} d^{-1}) by major food web components in the euphotic zone of Antarctic coastal waters

Ammonium consumption	
Phytoplankton	30 – 40
Nitrifying bacteria	?
Ammonium production	
Microzooplankton	20
Net zooplankton	~3
Krill	~1
Bacteria	?

Impact of micrograzers on nitrogen transformations

That microzooplankton are important producers of ammonium in Southern Ocean surface waters is consistent with our observations that nanoplankton were the primary consumers of ammonium. Since nanoplankton represent the major source of primary production, they will, in turn, serve as food for micrograzers. The importance of such micrograzers (including those in the nanoplankton fraction) for ammonium production is also indicated by our results of ammonium production and uptake as measured by the ^{15}N ammonium isotope dilution method. In 2 (Stns 138 and 152) of the 3 stations shown in Table 4, ammonium production by planktonic assemblages of less than 370 µm roughly balanced the ammonium uptake by the phytoplankton in the same fraction. At the third station (Stn 145), however, ammonium production was but 15 % of the ammonium assimilated as measured by the method of Hattori et al. (1980). The accessory data shown in Table 4 suggest an explanation for this marked difference between the stations. It is seen that at Stn 145 the chl *a* concentration was very high (3.15 µg l^{-1}), the phytoplankton carbon content (76 µg C l^{-1}) represented the highest percentage of the POC, and the chl/ATP ratio was very high (6.91), which suggests an autotrophic crop with relatively few heterotrophic organisms. This is also borne out by the floristic composition of the crop, which contained over 78 % diatoms (by organic carbon content), all of which are easily recognized as autotrophic cells. Such a 'bloom' of photoautotrophic cells would be expected to show relatively little regeneration of ammonium, which agrees with the data in the Table. Stn 138, which showed the highest rate of ammonium regeneration, also apparently had the highest proportion of heterotrophic to autotrophic biomass. This is suggested by the low chlorophyll concentration, the much lower chl/ATP ratio (2.78), the high percentage of detrital organic carbon, and the high percentage of dinoflagellates, a group which includes many heterotrophic species (note: after preservation with formalin and storage for a few months, it is not possible to distinguish autotrophic from heterotrophic cells with any degree of certainty). Stn 152 was intermediate between

the other 2 stations, in regard both to rate of ammonium regeneration and to the apparent proportion of heterotrophic to autotrophic cells (in this case, the heterotrophic cells would be found in the flagellates and monads, which constituted 75 % of the measured biomass).

Phytoplankton crops similar to that seen at Stn 145 are not very common, as the mean chl *a* concentration in Antarctic waters is close to 0.6 $\mu g\ l^{-1}$. Stns 138 and 152 may thus be considered to represent the more general situation in the southern ocean, which suggests that more of the ammonium assimilated by phytoplankton is produced *in situ* by microbial cells than by either macrozooplankton or by advection, melting ice, etc. This is strong evidence for a dynamic 'food web' in Antarctic waters, in contrast to the relatively simple 'food chain' (diatoms to krill) mentioned by many researchers. This same conclusion has been reached previously on the basis of microscopic analysis of the variety and nutritional mode of the microbial fraction (Hewes et al. 1985).

Seasonal considerations

Although the data in Tables 4 and 6 indicate that the bulk of ammonium originates from mineralization reactions catalyzed by microbial cells, we stress that there are important scales of time-space on which net zooplankton and krill will contribute significant amounts of ammonium. In spring and early summer the phytoplankton tend to 'bloom', which is related to low grazing pressure and hence low ammonium production. The impact by adult krill and their developmental stages can be expected to be greatest in the period February to April. Krill swarms, in particular, will markedly increase the ammonium concentration, as has been documented by Johnson et al. (1984). There should thus be a seasonal trend showing a relatively high ratio of autotrophic to heterotrophic microbial biomass in spring to a much lower ratio in summer and fall coinciding with the development of the macrozooplankton populations. This increasing heterotrophic biomass should be reflected in increasing ammonium concentrations throughout the euphotic zone. Ammonium measurements at different times of the year in this area do indicate such increasing concentrations of ammonium. In early spring of 1978 ammonium concentrations in this area were reported to be 0.2 to 0.4 μM (Olson 1980). About 5 mo after these observations, 1.8 μM of ammonium was found at a station in the same area (Glibert et al. 1982), quite close to our observation in March 1981 (2.1 μM).

Reports of high ammonium concentrations in summertime in near-surface arctic seas (Saino et al. 1983) hint that low temperatures may also favor summertime ammonium accumulation in near-surface Antarctic waters. Neori & Holm-Hansen (1982) concluded that the rate of phytoplankton growth in Antarctic surface waters during summer is limited by thermodynamic effects on metabolic reactions, in spite of high nutrients and saturating light intensity. For example, even in active nanoplankton, a doubling time of 5.3 d was calculated in terms of nitrogen, based on the data in Table 2. This is slightly lower than the growth rates indicated by the data of Neori & Holm-Hansen (1982) and Rönner et al. (1983). It is, however, in agreement with photosynthetic assimilation numbers for phytoplankton in the South Scotia Sea and Bransfield Strait described by Tilzer et al. (1985). We do not know why phytoplankton growth rates should be lower in these coastal areas, but it may be related to effects of 'seeding' by ice flora as well as reflecting the physiological characteristics of nanoplankton populations which 'survive' krill predation. Temperature has a similar effect on the metabolism of both phytoplankton and zooplankton, i.e. a slowing down of metabolic activity, with a Q_{10} of approximately 2.0. However, only slight differences in Q_{10} values between autotrophic and heterotrophic organisms should be adequate to create accumulation of ammonium, on a seasonal basis, in cold waters. More specifically, metabolic activity of bacteria in Antarctic waters is rather comparable to that in temperature waters, suggesting that the Q_{10} for bacterial metabolism is much less than 2.0 (F. Azam, pers. comm.).

Strong seasonality is one of the characteristic features of Polar ecosystems. In terms of fluxes and transformations of nitrogen, in spring there is usually a phytoplankton bloom, composed mainly of large diatoms which assimilate both nitrate and ammonium. If fueled primarily by nitrate, 'new' nitrogen will dominate the particulate nitrogen pool. However, as springtime progresses, the particulate nitrogen pool will become more and more based on ammonium through zooplankton grazing and bacterial degradation of organic substrates. If, at the same time, local ammonium production becomes greater than ammonium consumption by surface phytoplankton, a gradual accumulation of ammonium in the upper water column will take place. This accumulation of ammonium, in turn, will result in a decrease of 'new' production. Thus, by summer, about 80 % of primary production is supported by ammonium, and the composition of the phytoplankton crop has changed to a nanoplankton-dominated population. Over the fall and winter, most of the ammonium in the water column apparently disappears, either by advective processes or by assimilatory or oxidative processes. Thus, when austral spring once again arrives, the 'cycle' of

ammonia production and utilization begins anew, with fluxes and transformations of nitrogen once again linked to the species composition and to the metabolism of microbial populations.

NOTE ADDED IN PROOF

A recent note (Probyn, T. A., Painting, S. J. (1985). Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. Limnol. Oceanogr. 30: 1327–1332) also provides evidence for nitrogen resource partitioning between the alga of different size classes at 3 of 5 stations in oceanic water off the Enderby Coast of Antarctica. Picoplankton and nanoplankton took up mostly reduced nitrogen (ammonium, urea), while net-plankton took up higher proportions of nitrate.

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