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Regulation by low temperature of phytoplankton growth and nutrient uptake in the Southern Ocean

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ABSTRACT: During oceanographic cruises in 1996 and 1998, phytoplankton from 15 stations in the southwest Atlantic sector of the Southern Ocean were incubated at ambient temperature and 2 elevated temperatures (ambient plus 3°C, and ambient plus 6°C). Rates of growth, nutrient depletion, ^{15}N-nitrate uptake and nutrient interaction were all studied. Microalgal growth rate showed a strong positive relationship to temperature elevation, indicating ambient temperatures were sub-optimal for the phytoplanktonic community as a whole. Ratios of silicate uptake to chlorophyll *a* increase were high at ambient temperatures and showed a strong negative relationship with temperature elevation. Nitrate uptake rates, measured by ^{15}N-nitrate incorporation, showed a consistent trend of increased uptake rate at elevated temperature. Specific nitrate-depletion rates, ^{15}N-nitrate uptake rates, and the *f* ratio all showed an inverse relationship to increasing ammonium concentration. The results of this study imply that ambient temperature, in addition to direct iron limitation, is important in the maintenance of the high nutrient, low chlorophyll conditions common to the Southern Ocean.

KEY WORDS: Nitrate · Ammonium · Iron · Limitation · Inhibition · HNLC regions

INTRODUCTION

Over the past 30 yr interest has increased in oceanic areas which exhibit the apparent paradox of year-round abundant macronutrients (e.g. nitrate, phosphate and silicate), but low chlorophyll and low primary productivity (e.g. Dugdale & Goering 1967, Barber et al. 1971, DeBaar 1990, Wheeler & Kokkinakis 1990, Chisholm & Morel 1991, Cullen 1991). These regions are commonly denoted 'high nutrient, low chlorophyll' (HNLC) areas (Minas et al. 1986), and potentially represent areas of increased primary production, and enhanced CO_2 drawdown, if changed environmental conditions favour increased phytoplankton growth (Sarmiento & Orr 1991, Priddle et al. 1992). The Southern Ocean is the largest and potentially the most important of these regions. Understanding the factors which produce and maintain its status as an HNLC region is crucial when constructing a global carbon budget and predicting the effects of environmental change (Joos et al. 1991).

The importance of iron limitation in the Southern Ocean HNLC area was first suggested by Hart (1934), and put into a more global context by Martin et al. (1990). More recent iron addition experiments in the equatorial and sub-Arctic Pacific HNLC regions have underlined the potential for increased primary production and CO_2 drawdown in HNLC areas (e.g. Price et al. 1991, Behrenfeld et al. 1996, Frost 1996, Turner et al. 1996). Iron addition experiments in the Southern Ocean HNLC region have produced similar results

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(e.g. Scharek et al. 1997, VanLeeuwe et al. 1997, Sedwick et al. 1999). Although in some areas iron concentrations appear to be above limiting levels (Buma et al. 1991, Basterretxea & Aristegui 1999), the importance of iron limitation in the Southern Ocean as a whole is clear (DeBaar et al. 1995). However, this should not distract from the existence of other factors contributing to the maintenance of HNLC conditions in the Southern Ocean (see Chisholm & Morel 1991, Boyd et al. 1999, Bracher et al. 1999). As well as iron limitation, environmental temperature may also play an important role in limiting nutrient drawdown and net primary production in this region (Tilzer et al. 1986, Priscu et al. 1989). Such a role may be particularly important when considered in the context of subtle changes in temperature arising from global warming.

In the Southern Ocean, surface water temperatures reach as low as –1.8°C, with a maximum summer temperature of around 4.0°C (Herbert & Bhakoo 1979). It is increasingly accepted that the majority of oceanic phytoplankton in the Southern Ocean are psychrotolerant, rather than psychrophilic, with temperature optima for growth and photosynthesis far in excess of any environmental temperature encountered (Tilzer et al. 1986, Smith & Harrison 1991). Reduced photosynthetic capacity in phytoplankton and reduced affinity for organic substrates by bacteria at low temperatures have already been demonstrated (Li et al. 1984, Li 1985, Nedwell & Rutter 1994). In low-temperature environments, active uptake of inorganic nutrients such as nitrate, silicate and, in particular iron, may also be reduced.

Several studies have reported a strong temperature-dependence of nitrate reductase activity and hence nitrate assimilation in algae (e.g. Tischner & Lorenzen 1981, Gao et al. 1993). Limitation of nitrate transport in the freshwater cyanobacterium *Synechococcus* sp. by low temperature has also been well documented (Sakamoto & Bryant 1997, 1998, 1999). Similarly, in a previous laboratory study, we have demonstrated that the specific affinity of a range of bacteria and marine microalgae for nitrate is significantly reduced at suboptimal temperatures (Reay et al. 1999). Indeed, low affinity for inorganic nutrients, indicated by a high half-saturation constant (K_s) for uptake of both silicate and nitrate, has already been reported for Southern Ocean phytoplankton at low temperatures (Jacques 1983, Sommer 1986). The exact mechanism responsible for, and temperature dependence of, iron uptake in marine algae and bacteria remains undefined. However, work on iron reduction and assimilation in *Saccharomyces cerevisiae* indicates an active, NADPH-dependent process (Lesuisse et al. 1991, 1998, Anderson et al. 1992), which might also be expected to exhibit temperature-dependence.

Here, we investigate the effects of elevated temperature on growth and inorganic nutrient uptake by natural populations of Southern Ocean phytoplankton collected around the Subantarctic island of South Georgia.

MATERIALS AND METHODS

The study was carried out on British Antarctic Survey Cruises JR11 and JR38 aboard the RRS 'James Clark Ross' in January-February 1996 and December-January 1998/99 respectively. The study area comprised both deep-sea and shelf waters to the north of South Georgia, a large Subantarctic island in the northern Scotia Sea. South Georgia is located between 54 and 55° S latitude, and is situated south and east of the Polar Front, as the Antarctic Circumpolar Current trends to the northwest of the island before resuming an easterly course. Samples were taken at a total of 15 stations encompassing an area running from north of the Polar Front (51° S) down to the shelf waters of South Georgia (Fig. 1). Three of the sampling stations (1, 5 and A) were situated north of the Polar Front. More detailed information on the study area can be found in Priddle et al. (1997).

Sampling protocol, nutrient and chlorophyll analyses. Bulk water samples (20 l) were collected from 30 to 40 m depth using a General Oceanics water-bottle rosette system mounted on a Neil Brown Mark 3 CTD.

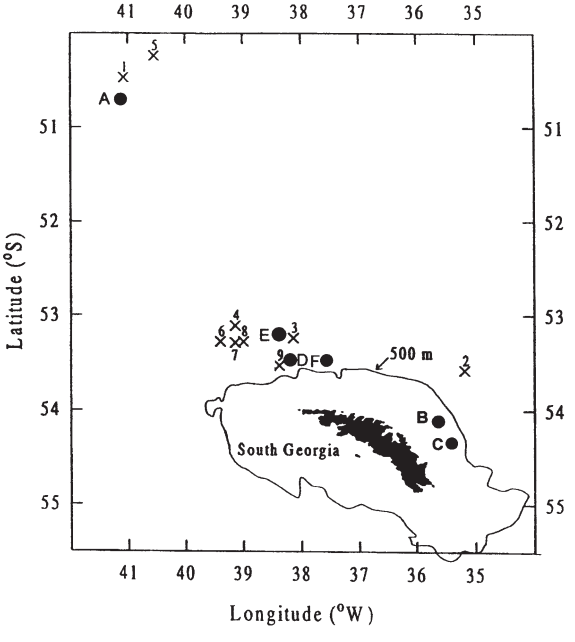

Fig. 1. Site map of sample stations around Georgia. ×: stations on Cruise JR11 (1996). ●: stations on Cruise JR38 (1998/99)

Triplicate sub-samples (30 ml) were taken from the 20 l bulk sample. These time-zero sub-samples were filtered (Whatman GF/F: nominal particle retention 0.7 µm) and analysed colorimetrically for dissolved nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{2-}) and silicate ($Si(OH)_4$), using a segmented flow auto-analyser. Precision was better than ± 1% for all analyses, and nutrient concentrations did not fall below minimum detection levels (Whitehouse & Woodley 1987). On 3 occasions, control samples of ultra high-purity water (UHP) were also filtered and analysed to check for any contamination caused by filtration and sample processing.

For nutrient depletion experiments on Cruise JR11, duplicate sub-samples of 100 to 400 ml were taken from the initial 20 l water sample and filtered (Whatman GF/F). Filters were extracted in 90% v:v acetone:water (HPLC grade: Rathburn Chemicals Ltd, Walkerburn, UK) in the dark at approximately 2°C for 24 h for chlorophyll *a* (chl *a*) and phaeopigment analysis. Fluorescence was measured before and after acidification using a Sequoia-Turner Model 112 benchtop fluorometer. Chl *a* extracted from the cyanobacterium *Anacystis nidulans* (Sigma Chemical Company) provided a standard for calibration of the fluorometer, and the standard solution itself was calibrated spectrophotometrically (Parsons et al. 1984).

The temperatures of 3 large water baths containing roller beds were set to give incubation temperatures of 3 and 6°C above ambient, together with a control at ambient temperature. Incubation temperatures were controlled by thermo-circulators (Conair-Churchill Ltd, Uxbridge, Middlesex, England) which gave control to ±0.1°C. Nine clear-glass Duran bottles (620 ml) were prepared for each incubator, 3 bottles being covered in black plastic to exclude light. Samples were incubated under constant illumination (200 µmol m^{-2} s^{-1}) and at a constant rotation rate (70 rpm) for 72 h to maintain plankton in suspension. Illumination was provided by a bank of fluorescent tubes (Triton 38 W, 400 to 710 nm [Peaks 450, 550 and 620 nm], Interpet Ltd, Dorking, UK) suspended directly above each incubator. Temperature and light levels in each incubator were recorded daily. Sub-samples (30 ml) were removed from each bottle at 12 h intervals, filtered (GF/F), and analyzed immediately for nutrients. After a 72 h incubation period, final sub-samples were removed for nutrient analyses and a 100 to 300 ml sample was taken from each bottle and filtered (GF/F). These filters were then extracted for chl *a* analysis as described previously.

Experimental protocol for nutrient-depletion experiments in the second part of Cruise JR11 (Stns 4, 5 and 6) was identical to that described above. However, filtered water samples for nutrient analysis were preserved with $HgCl_2$ (20 µg ml^{-1}) and stored at 2°C until analysis in the UK (Kirkwood 1996). Chl *a* samples were collected by the previously described method, but filters were then frozen at –20°C until acetone extraction and fluorometric measurement of chl *a* concentration was undertaken in the UK. At Stn 6, the above protocol was used (Stn 6a), together with a parallel set of incubations where sufficient NH_4Cl was added to give an increase of approximately 1 mmol m^{-3} above the ambient concentration (Stn 6b).

^{15}N-labeled nitrate uptake experiments. Methods for ^{15}N-nitrate uptake experiments were identical on both cruises unless otherwise noted. A 20 l water sample was collected as described previously, and sub-samples were taken immediately for nutrient and chl *a* analysis. An addition of $Na^{15}NO_3$ (99.6 at.%) was made to the water sample, to give approximately 10% enrichment above ambient concentrations, and further sub-samples for nutrient analyses were taken. The temperatures of the 3 water baths were again set to give incubation temperatures of 3 and 6°C above ambient, together with an ambient control. For Cruise JR11 each water bath contained four 620 ml bottles, 3 clear and 1 blacked out, and these were filled completely with the ^{15}N-nitrate-labeled sample water. For incubations on Cruise JR38, sets of triplicate bottles were also spiked with either an ammonium addition equivalent to an elevation of 1 mmol m^{-3}, or $FeSO_4$ equivalent to a 10 µmol m^{-3} elevation, a putative saturating concentration.

Bottles were incubated under constant illumination for 24 h, after which a triplicate sub-sample of 100 to 200 ml was taken from each bottle. Sub-samples were filtered onto aluminium oxide filters (Whatman Anodisc 25: nominal particle retention 0.2 µm) either directly, or after pre-screening through a cellulose acetate filter (Whatman Grade 4: nominal particle retention 20 µm). Filters were then frozen at –20°C and later dried at 50°C for analysis. Isotope and total nitrogen analysis of filters were undertaken in the UK using a Roboprep-CN elemental analyzer coupled to a Tracermass mass spectrometer (Barrie & Prosser 1996) at the NERC stable-isotope facility, ITE Merlewood, Cumbria, by Mr Chris Quarmby.

Iron analysis. A limited number of water samples were also taken both immediately after collection (n = 4) at Stns A and D on Cruise JR38, and after 48 h under standard incubation conditions (n = 4) for subsequent iron analysis. These water samples were collected into acid-washed low-density polyethylene bottles under a laminar flow hood to prevent particulate iron contamination. Iron analyses were carried out in the UK at the Southampton Oceanography Centre. Dissolved iron in the samples was separated and preconcentrated from the seawater using a chelation and

Table 1. Site characteristics of stations sampled on Cruises JR11 and JR38. nm: not measured; 1: nutrient depletion experiments; 2: ammonium addition (1 mmol m^{-3}); 3: ^{15}N-nitrate incorporation experiment; 4: iron addition (10 µmol m^{-3}) experiment

Stn	Procedure 1	2	3	4	Location (Latitude, Longitude)	Date	Depth (m)	Temp. (°C)	Chl *a* (mg m^{-3})	NO_3^- (mmol m^{-3})	NH_4^+ (mmol m^{-3})	SiO_3^- (mmol m^{-3})	PO_4^{2-} (mmol m^{-3})	NO_2^- (mmol m^{-3})
1	√				50° 47′ S, 41° 08′ W	7 Jan 1996	40	5.5	0.5	nm	nm	nm	nm	nm
2	√				53° 58′ S, 35° 19′ W	13 Jan 1996	30	2.0	0.3	20.5	1.25	30.12	1.98	0.24
3	√				53° 24′ S, 38° 14′ W	19 Jan 1996	30	3.0	2.9	17.7	0.5	12.0	1.21	0.31
4	√				53° 11′ S, 39° 14′ W	25 Jan 1996	30	2.5	1.3	23.4	0.55	18.5	1.58	0.34
5	√				50° 24′ S, 40° 55′ W	13 Jan 1996	30	5.5	1.2	16.5	1.15	nm	2.02	0.29
6	√	√			53° 28′ S, 39° 40′ W	24 Jan 1996	35	3.5	2.5	17.6	0.33	21.12	1.44	0.33
7			√		53° 29′ S, 39° 14′ W	20 Jan 1996	30	3.6	2.7	19.9	0.4	12.2	1.49	0.34
8			√		53° 28′ S, 39° 00′ W	21 Feb 1996	35	3.5	1.2	21.7	1.1	21.5	1.58	0.31
9			√		53° 53′ S, 38° 38′ W	22 Feb 1996	30	3.5	3.1	18.0	1.2	29.42	1.78	0.35
A			√	√	50° 47′ S, 41° 09′ W	22 Dec 1998	30	5.5	0.5	23.4	0.26	10.2	1.45	nm
B		√	√		54° 15′ S, 35° 43′ W	29 Dec 1998	30	2.5	5.4	24.0	0.29	16.1	1.36	nm
C		√	√		54° 23′ S, 35° 33′ W	30 Dec 1998	30	2.5	5.7	22.6	0.55	16.6	1.37	nm
D		√	√	√	53° 47′ S, 38° 35′ W	4 Jan 1999	30	2.5	4.0	21.6	1.85	11.5	1.31	nm
E		√	√		53° 23′ S, 38° 23′ W	5 Jan 1999	40	2.5	2.7	19.0	1.5	7.2	1.20	nm
F		√	√		53° 51′ S, 37° 35′ W	7 Jan 1999	30	2.9	15.5	15.3	0.08	8.2	1.11	nm

solvent-extracted procedure based on established procedures (Bruland et al. 1979, Statham 1985). Dissolved metals in samples were complexed with a mixed dithiocarbonate reagent and the complexes extracted from the seawater into chloroform. The chloroform extracts were reduced to dryness in a clean bench, and the residue was oxidized with concentrated nitric acid. The oxidized residue was dissolved in a small volume of dilute nitric acid and the metals in this final extract were determined by graphite furnace atomic-absorption spectrophotometry. Analytical quality-control procedures used analysis of a certified reference material (CRM, NASS-2, CRCC), which showed good agreement between measured and quoted CRM values. Iron analyses represent minimum concentrations, as any iron adsorbed into bottle walls was excluded, although the use of acid-washed Go-Flo bottles should have minimized such losses.

Data analysis. Relationships between data were examined by 1-factor and 2-factor analysis of variance (ANOVA), with post-hoc Tukey tests where appropriate, by using linear least-squares regression analysis (LRA), and by Spearman's rank correlation (SRC). Data were log-transformed where assumptions of normality and variance homogeneity were not met. Statistical analysis and plots of data were undertaken using the data analysis packages supplied in Systat, Version 7.0 (SPSS Inc., Richmond, USA) and in Sigma Plot, Version 5.0 (SPSS).

RESULTS

Temperature, chlorophyll and nutrient concentrations

Mean surface water temperatures (30–40 m) ranged from 2 to 5.5°C over the course of the 2 cruises (Table 1) and concentrations of chl *a* were relatively low at all stations. Only Stn F on the second cruise (JR38) showed chl *a* concentrations approximating to bloom conditions, although chl *a* concentrations at Stns B, C and D were also high for the Scotia Sea. No significant relationship (LRA, $p > 0.1$) between *in situ* temperature and chl *a* or nutrient concentration was evident. Nitrate and phosphate concentrations at all stations were high for oceanic waters, but concentrations of all nutrients were in the range reported previously for this region (Whitehouse et al. 1996). Ambient nutrient concentrations generally showed an inverse relationship with concentrations of chl *a*. Concentrations of all measured nutrients were considerably lower at Stn F compared to all others, with ammonium concentrations bordering on undetectable (<0.01 mmol m^{-3}). Where examined (Stns 7, 8 and 9), the phytoplanktonic assemblage was

dominated by the <20 µm size fraction, although microscopic examination showed the haptophyte *Phaeocystis* sp. to be very common at Stns 4, 5 and 6. Nitrite concentrations were low and invariable throughout the study, and are not considered further here.

Growth rate and nutrient uptake

Microalgal growth, as calculated from increases in chl *a* over 72 h, demonstrated a difference in both growth rate and its degree of response to temperature elevation between stations. Temperature increases of both 3 and 6°C above ambient had a highly significant (ANOVA, Tukey $p < 0.01$) positive effect on growth rate compared to that at ambient temperature across Stns 1 to 6 (Fig. 2), the growth rate doubling or even tripling at elevated temperatures. Little or no net algal growth was observed in blacked-out bottles, chl *a* concentrations generally falling slightly over the course of experiments (Table 2). Previous studies (e.g. Geider et al. 1997) have indicated that the ratio of chl *a* to biomass tends to increase with elevated temperature. Such an effect may therefore overestimate the actual increase in growth rate brought about by temperature elevation. The possibility of uncoupling of chl *a* synthesis with biomass accumulation was examined by plotting the increase in chl *a* against the decrease in inorganic nitrogen (Fig. 3). Data for the ammonium addition experiment at Stn 6 are also included (Stn 6b). The ratio of chl *a* produced to nitrogen utilized (g:mol), when all data were considered, was 0.9 ± 0.11 (±SE,

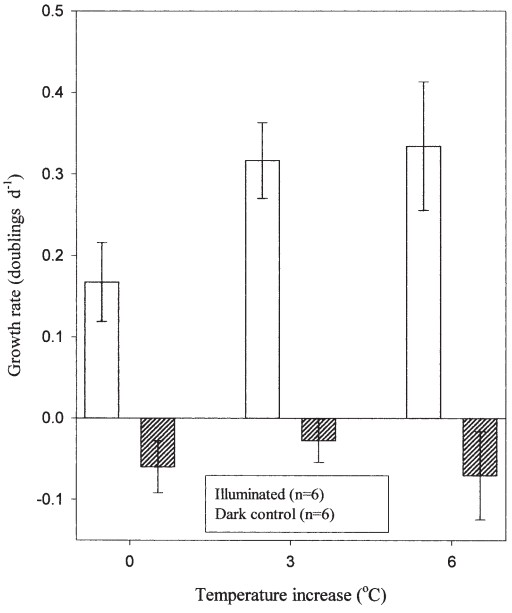

Fig. 2. Changes in microalga growth rate with increasing temperature. Data are mean (±SE) growth rates from 6 stations on Cruise JR11

Table 2. Mean (±SE) changes in chlorophyll *a* (chl *a*) concentration, growth rate and uptake ratios with temperature elevation and station on Cruise JR11 in light (n = 6) and dark (n = 3). All data are derived from 72 h incubations. Si:chl *a* = Si: silicic acid used: chl *a* produced; Si:N = silicic acid used:nitrate used; na = not available; nN = no net nitrate depletion

Stn	Temp. (°C)	Chl *a* (mg m^{-3}) Light	Chl *a* (mg m^{-3}) Dark	Growth rate (d^{-1}) Light	Growth rate (d^{-1}) Dark	Si:chl *a* (mol:g)	Si:N (mol:mol)
1	5.5	1.79 ± 0.05	0.32 ± 0.05	0.86 ± 0.024	−0.12 ± 0.019	na	na
	8.5	2.2 ± 0.11	0.39 ± 0.01	1.13 ± 0.057	−0.07 ± 0.002	na	na
	11.5	2.2 ± 0.12	0.24 ± 0.03	1.13 ± 0.062	−0.17 ± 0.022	na	na
2	2	0.79 ± 0.04	0.29 ± 0.04	0.54 ± 0.028	−0.01 ± 0.002	1.5	nN
	5	1.36 ± 0.06	0.29 ± 0.03	1.18 ± 0.052	−0.01 ± 0.001	0.68	nN
	8	2.65 ± 0.29	0.36 ± 0.04	2.61 ± 0.286	0.07 ± 0.007	0.49	2.3
3	3	4.15 ± 0.41	2.68 ± 0.51	0.14 ± 0.014	−0.03 ± 0.005	5.8	1.3
	6	7.55 ± 0.75	2.39 ± 0.10	0.53 ± 0.053	−0.06 ± 0.002	1.4	1.1
	9	8.86 ± 0.60	2.51 ± 0.61	0.69 ± 0.046	−0.04 ± 0.011	1.1	1.0
4	2.5	1.7 ± 0.1	1.2 ± 0.1	0.10 ± 0.006	−0.03 ± 0.002	10.4	0.7
	5.5	3.8 ± 0.2	1.5 ± 0.1	0.64 ± 0.034	0.05 ± 0.003	1.7	0.8
	8.5	3.4 ± 0.37	1.2 ± 0.1	0.54 ± 0.059	−0.03 ± 0.002	1.7	0.7
5	5.5	1.8 ± 0.05	0.11 ± 0.01	0.17 ± 0.005	−0.30 ± 0.028	na	na
	8.5	2.44 ± 0.10	0.52 ± 0.39	0.34 ± 0.014	−0.19 ± 0.141	na	na
	11.5	2.45 ± 0.16	0.11 ± 0.01	0.35 ± 0.023	−0.30 ± 0.028	na	na
6a	3.5	3.7 ± 0.09	2.5	0.16 ± 0.003	0.00	na	na
	6.5	5.1 ± 0.75	2.4	0.35 ± 0.051	−0.01	0.75	1.10
	9.5	2.8 ± 0.3	2.5	0.04 ± 0.004	0.00	na	na
6b (+NH_4)	3.5	2.6 ± 0.15	2.5	0.01 ± 0.001	0.00	11.18	1.33
	6.5	3.8 ± 0.86	2.5	0.17 ± 0.039	0.00	0.87	1.00
	9.5	4.8 ± 0.67	2.7	0.31 ± 0.043	0.03	1.00	0.39

n = 82). This value equates well with the Redfield ratio (C:N of 6.625:1)-based value of 0.8, assuming a carbon to chl *a* ratio of 60.

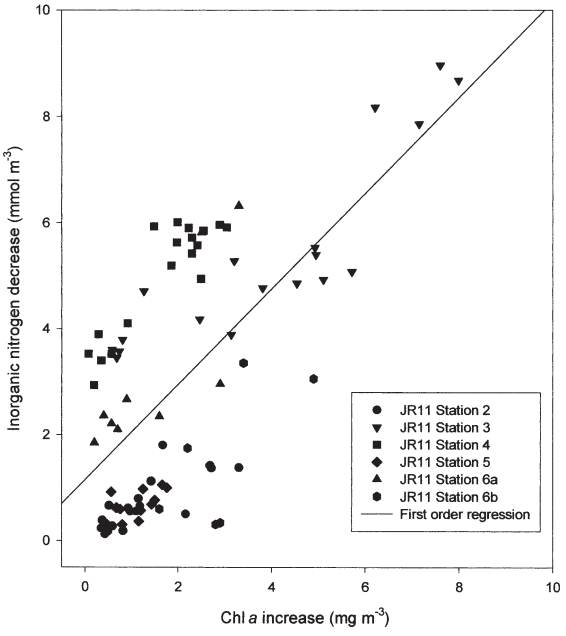

Fig. 3. Relationship between decrease in inorganic nitrogen (nitrate + ammonium) and simultaneous increase in chlorophyll *a* on Cruise JR11. Data from elevated temperature incubations are included. Stns 6a and 6b represent control and ammonium addition (1 mmol m^{-3}) incubations respectively; line represents first-order linear regression through all data (slope = 0.9, r^2 = 0,45, n = 82, p < 0,001)

We calculated both specific and absolute nutrient-depletion rates for Stns 2 to 6 (Table 3). Absolute depletion rates indicate the changes in nutrient chemistry occurring in each incubation bottle with time. However, it is biomass-specific nutrient depletion which is most valuable in assessing the effect of temperature elevation on nutrient transport rates. Specific rates of silicate depletion tended to be inversely related to temperature elevation, with highest rates occurring at *in situ* temperatures (Table 3). No clear dependence of phosphate depletion rate on temperature was evident, with both negative and positive effects apparent across the 5 stations considered. The relationship between temperature elevation and specific nitrate depletion was also highly variable between stations, with low depletion rates associated with stations of high (>1 mmol m^{-3}) *in situ* ammonium concentration. Indeed, ammonium addition resulted in reduced specific nitrate uptake rate at Stn 6 with a 6°C temperature elevation. Specific ammonium-depletion rates tended to be highest where *in situ* ammonium concentrations were also high, with ammonium addition resulting in an increase in net ammonium uptake at Stn 6. Again, no clear relationship with temperature elevation was apparent at this station. In dark incubations (data not shown), ammonium concentrations generally showed a net increase over 72 h; however, it is worth noting that ammonium production was inversely related to ammonium concentration in these incubations. Rates of specific nutrient depletion are unavailable for Stn 1 on Cruise JR11

Table 3. Mean ± SE (n = 6) specific (µmol chl *a* h^{-1}) and absolute (nmol h^{-1}) nutrient-depletion rates and *f* ratio as a function of temperature elevation and station on Cruise JR11. na = not available

Stn	Temp. (°C)	Silicate		Phosphate		Nitrate		Ammonium		*f* ratio
		Specific	Absolute	Specific	Absolute	Specific	Absolute	Specific	Absolute	(light)
2	2	12.6 ± 3.7	6.3 ± 2.0	14 ± 1	0.6 ± 0.5	−3.7 ± 2.6	−1.7 ± 1.3	4.0 ± 0.9	1.9 ± 0.4	0.14
	5	7.1 ± 2.0	5.9 ± 1.6	2.0 ± 0.3	1.7 ± 0.3	−0.2 ± 0.7	−0.2 ± 0.5	5.1 ± 0.6	4.3 ± 0.6	0.08
	8	5.8 ± 1.2	8.6 ± 1.0	1.6 ± 0.0	2.6 ± 0.2	2.8 ± 0.8	4.0 ± 0.7	4.7 ± 0.7	7.6 ± 1.0	0.35
3	3	18.3 ± 0.8	46.3 ± 2.6	0.7 ± 0.1	1.7 ± 0.2	13.8 ± 0.4	35.1 ± 2.6	0.1 ± 0.3	0.2 ± 0.8	0.98
	6	11.1 ± 0.5	51.4 ± 4.3	0.4 ± 0.1	1.7 ± 0.5	10.0 ± 0.6	47.3 ± 6.6	0.7 ± 0.1	3.1 ± 0.3	0.93
	9	10.1 ± 0.2	55.3 ± 4.1	0.4 ± 0.1	2.3 ± 0.6	9.9 ± 0.5	54.9 ± 6.6	0.5 ± 0.1	2.7 ± 0.3	0.95
4	2.5	18.5 ± 1.2	18.9 ± 0.7	2.5 ± 0.1	2.6 ± 0.1	25.3 ± 1.5	25.9 ± 1.1	4.7 ± 0.3	4.8 ± 0.3	0.84
	5.5	15.3 ± 0.4	35.6 ± 0.8	1.6 ± 0.1	3.7 ± 0.2	19.5 ± 0.7	45.3 ± 0.7	1.9 ± 0.1	4.5 ± 0.2	0.91
	8.5	14.1 ± 0.9	28.9 ± 2.0	2.0 ± 0.2	4.0 ± 0.3	21.2 ± 1.2	43.6 ± 2.4	2.2 ± 0.5	4.3 ± 1.0	0.91
5	5.5	na	na	17.9 ± 5.4	19.9 ± 6.0	8.7 ± 4.9	9.7 ± 5.5	0.5 ± 11.4	0.6 ± 12.7	0.79
	8.5	na	na	14.1 ± 4.0	21.4 ± 6.0	2.8 ± 1.3	4.2 ± 2.0	−4.1 ± 13.0	−6.3 ± 19.7	0.53
	11.5	na	na	14.2 ± 3.8	21.6 ± 5.7	3.1 ± 1.6	4.7 ± 2.4	7.8 ± 4.3	11.8 ± 6.6	0.71
6a	3.5	−1.7 ± 1.4	−3.8 ± 3.1	0.8 ± 0.0	1.9 ± 0.1	9.7 ± 0.8	23.0 ± 2.0	−0.6 ± 0.4	−1.34 ± 0.9	1.00
	6.5	8.9 ± 6.5	24.9 ± 16.2	1.8 ± 0.2	5.7 ± 0.2	10.2 ± 2.0	32.4 ± 4.6	0.2 ± 0.4	0.6 ± 1.1	0.99
	9.5	−6.1 ± 7.5	−11.9 ± 14.1	4.4 ± 1.3	7.4 ± 1.4	28.3 ± 0.8	47.6 ± 2.1	−1.7 ± 2.5	−3.5 ± 5.4	0.99
6b	3.5	10.5 ± 0.8	17.1 ± 1.7	1.4 ± 0.0	3.8 ± 0.2	10.0 ± 0.3	20.7 ± 0.5	3.4 ± 0.6	6.7 ± 0.9	0.75
	6.5	4.2 ± 1.0	8.7 ± 1.3	1.8 ± 0.5	6.0 ± 0.3	8.1 ± 2.8	17.8 ± 5.4	2.0 ± 1.4	4.6 ± 3.8	0.84
	9.5	5.4 ± 0.8	15.5 ± 0.2	1.8 ± 0.2	8.4 ± 0.4	13.9 ± 1.8	36.9 ± 3.5	2.3 ± 0.6	6.0 ± 1.2	0.86

due to incorrect storage of these samples prior to chemical analysis.

Little or no net depletion of the measured nutrients was observed in dark controls over the 72 h incubation period, indicating that heterotrophic nutrient depletion was relatively insignificant at the 5 stations examined. The lack of significant heterotrophic inorganic nitrogen utilization at Stns 2 to 6 was also indicated by the consistent, positive relationship between chl *a* increase and inorganic nitrogen decrease (Fig. 3). However, variations in this relationship within and between stations may represent some degree of heterotrophic utilization.

Ratios of silicic acid (Si) depletion to chl *a* increase (Si:chl *a*) were examined wherever possible (Table 2), as high ratios can reflect the degree of nutrient stress in the diatom population of each sample (Hutchins & Bruland 1998). High ratios occur when phytoplanktonic growth rates are lowered by nutrient stress, but silicate uptake rates remain constant. These ratios are not available for Stns 1 and 5 on Cruise JR11 because of lack of Si analysis at these stations. Similarly, such ratios are not available for incubations where no net silicate depletion was observed (e.g. Stn 6a). The highest Si:chl *a* ratios occurred at ambient temperatures, the ratio decreasing to around 1 with elevated temperature at all stations.

Low *f* ratios, as calculated from the proportion of nitrate depletion constituting total nitrate and ammonium depletion, were associated with stations with highest *in situ* ammonium concentrations (Table 3). The *f* ratio showed a highly significant negative correlation with *in situ* ammonium concentration (SRC, $p < 0.001$). This relationship was particularly conspicuous in the ammonium addition experiment (Stn 6), where the *f* ratio showed clear variations between the 2 treatments. In the control incubations, uptake was nitrate dominated (*f* ratio close to 1) at all 3 incubation temperatures. However, in incubations with elevated ammonium (plus 1 mmol m^{-3}), *f* ratios were lower and were positively related to temperature increase.

A typical time course of nitrate and ammonium concentration is presented for one of the stations in Fig. 4. This shows the commonly observed pattern of inorganic nitrogen use during the study. During the first 24 h ammonium was rapidly reduced; after this time, ammonium depletion slowed considerably, with concentrations staying relatively constant for the rest of the incubation. Nitrate depletion, on the other hand, showed a more consistent rate throughout the incubation, the depletion rate staying constant for all but the last 12 to 24 h, when concentrations tended to stabilize.

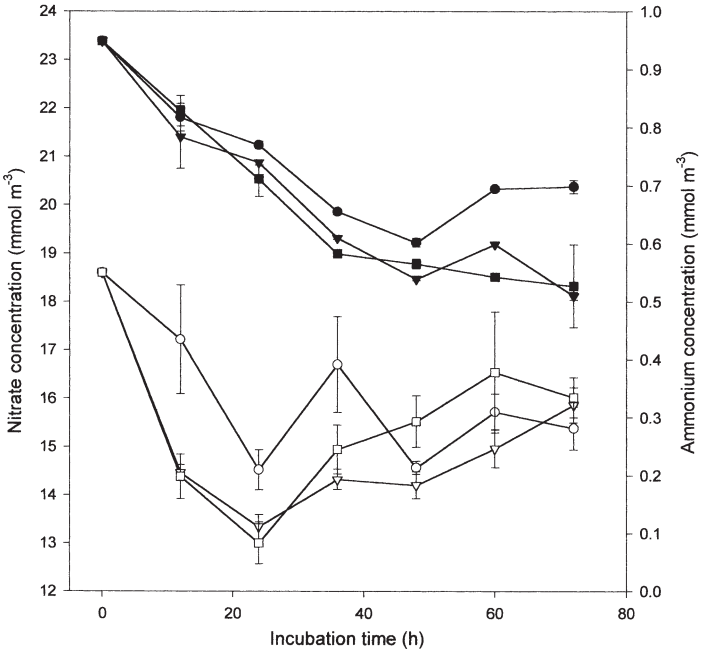

Fig. 4. Change in nitrate (black symbols) and ammonium (open symbols) concentrations with time and incubation temperature for Stn 4. (●, ○) ambient temperature, (▲, △) ambient plus 3°C elevated temperature data, (■, □) ambient plus 6°C elevated temperature data. Error bars = SE (n = 6)

^{15}N utilization

Specific nitrate uptake, measured as ^{15}N-nitrate incorporation, showed a positive relationship with temperature elevation in all 3 ^{15}N experiments from Cruise JR11 and in 5 out of 6 ^{15}N-nitrate experiments from Cruise JR38 (Fig. 5). A highly significant increase (ANOVA, Tukey $p < 0.01$) in specific nitrate uptake rate was seen with a temperature elevation of 6°C at Stn B, and with both a 3 and 6°C temperature elevation at Stn E. However, a temperature elevation of 6°C at Stn A resulted in a significant decrease (ANOVA, Tukey $p < 0.05$) in specific nitrate uptake rate. Specific nitrate uptake rates were similar across the 2 cruises, with highest rates occurring on Cruise JR38 at Stn A.

In the 3 experiments from Cruise JR11, the biomass-specific nitrate uptake rate by the whole phytoplankton assemblage was generally greater than that of the <20 µm fraction, although nitrate uptake by the <20 µm fraction followed a trend with temperature elevation similar to that seen for the whole assemblage. Dark nitrate uptake was low or negligible where measured, aside from a peak in uptake rate at Stn 9 at the highest temperature elevation (ambient plus 6°C). Specific nitrate uptake rates for the 3 nitrate incorporation experiments on Cruise JR11 each represent an analysis of bulked triplicate samples due to problems of detection using individual samples.

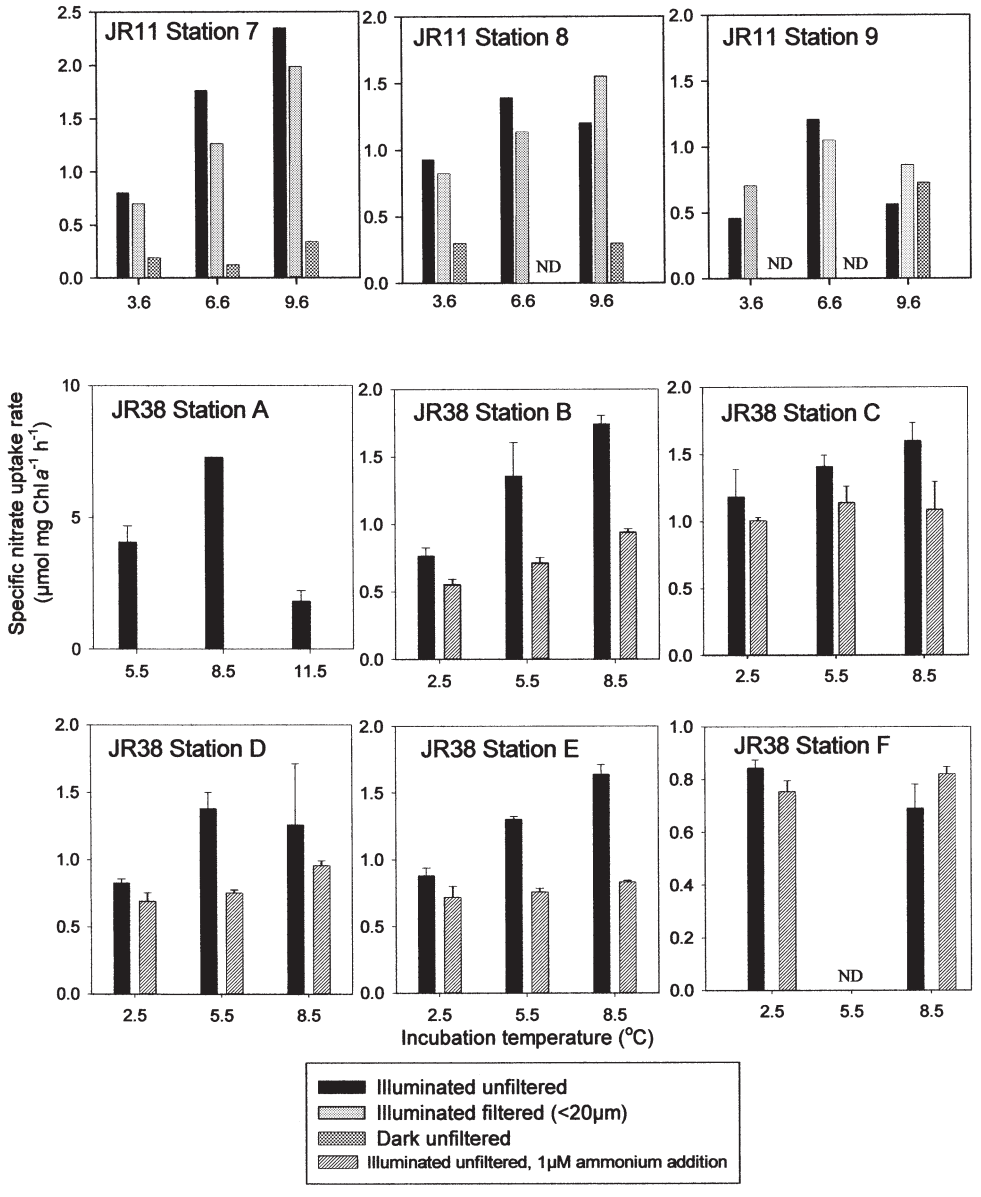

Fig. 5. Effect of temperature elevation and ammonium addition (1 mmol m^{-3}) on specific ^{15}N-nitrate at 9 stations on Cruises JR11 and JR38. Error bars represent SE (n = 3). ND: insufficent biomass for detection of ^{15}N-nitrate

The lowest rates of ^{15}N-nitrate uptake from both cruises tended to coincide with the highest ambient ammonium concentrations. Similarly, the 1 mmol m^{-3} supplementation of ammonium in experiments from JR38 consistently reduced specific nitrate uptake rate at both ambient and elevated temperatures. Despite the reduction in specific nitrate uptake rate caused by ammonium addition, a significant increase (ANOVA, Tukey $p < 0.05$) in uptake rate was still observed with elevated temperatures at Stns B and D. Aside from Stn F, the negative effect of ammonium addition on biomass-specific nitrate uptake rate was significant (ANOVA, $p < 0.05$) at all stations where ammonium additions were made. However, the addition of ammonium appeared to have the greatest effect at elevated temperatures. No data were available for the first temperature elevation (ambient plus 3°C) at Stn F on Cruise JR38 due to failure of the thermocirculator.

Iron addition experiments were made at Stns A and D. A significant (ANOVA, $p < 0.05$) positive effect of iron addition on biomass specific nitrate uptake rate was observed at Stn A, although this effect was apparent at elevated rather than ambient temperature (Table 4). Iron addition to Stn A incubations at a temperature elevation of ambient plus 3°C resulted in more than a 3-fold increase in specific nitrate uptake

Table 4. Effect of iron addition (10 µmol m^{-3}) on ^{15}N-nitrate uptake rate (mean ± SE, n = 3; µmol mg^{-1} chl *a* h^{-1})at 2 stations on Cruise JR38. nd = insufficient biomass for detection of ^{15}N-nitrate

Stn	Incubation temperature (°C)	Ambient iron	10 µmol m^{-3} iron addition
A	5.5	4.1 ± 0.61	2.3 ± 1.74
	8.5	7.3	23.9
	11.5	1.8 ± 0.41	nd
D	3.5	1.07 ± 0.02	1.31 ± 0.18
	9.5	1.07 ± 0.16	1.76

rate over that seen in ambient iron controls. No significant effect (ANOVA, $p = 0.06$) of iron addition on specific nitrate uptake was seen at Stn D; however, the low number of usable replicates in this experiment made any statistical analysis problematic. Where no specific nitrate uptake rate is given, levels of ^{15}N-nitrate were below detectable levels due to insufficient biomass on the sample collection filters.

Concentrations of ambient iron were below detection levels (<0.25 µmol m^{-3}) at both Stns A and D, with no evidence for any iron contamination during Go-Flo bottle sampling. Subsequent analysis of iron concentrations in water samples incubated under standard conditions for 48 h showed a slight increase in iron concentration to between 0.6 and 1.8 µmol m^{-3}, indicating some small introduction of Fe during experimentation, possibly by leaching from incubation bottles.

DISCUSSION

Physicochemical environment and the phytoplankton community

Water temperatures (2 to 5.5°C) encountered during the 2 cruises are consistent with those previously recorded for this part of the Southern Ocean during summer (e.g. Whitehouse et al. 1996). The consistently high nitrate and phosphate concentrations, together with generally low chl *a* levels are also commonly observed features of the Southern Ocean (Dugdale & Wilkerson 1991). The lack of any significant relationship between *in situ* temperature and chl *a* or nutrient concentrations is not surprising, given the large heterogeneity of this region and the consequently wide-ranging nutrient histories of the experimental stations. Instead, it is more valuable to examine temperature-change effects where all other factors are equal, as attempted in this study.

The large representation of small (<20 µm) phytoplankton identified in the algal assemblage at Stns 7, 8 and 9 is not uncommon for open-sea areas of the Southern Ocean (Hewes et al. 1985). Areas dominated by larger (>20 µm) phytoplankton are often encountered at ice-edge and inshore stations (Kang & Lee 1995), although such phytoplankton may also be prevalent in open waters (Whitehouse et al. 1996).

Growth rates

Growth rates of the phytoplankton incubated at ambient temperatures were in the range measured for Southern Ocean algae in previous studies (e.g. Fiala & Oriol 1990, Cota et al. 1992). Similarly, the clear importance of temperature to phytoplankton growth in this region concurs with the findings of Bracher et al. (1999). Our study indicates that the dominant phytoplankton in the area around South Georgia are not adapted for maximal growth at the low ambient temperatures encountered, and that either directly or indirectly these low temperatures may limit potential primary production and nutrient drawdown. This agrees with the findings of several previous studies of oceanic phytoplankton in the Southern Ocean (e.g. Neori and Holm-Hansen 1982, Jacques 1983, Tilzer et al. 1986). Given the apparent regulation of phytoplankton growth by low temperature, one may wonder why psychrophillic algal species are not more dominant in these waters. However, it seems that the costs of adaptation to specialized low-temperature growth commonly balance the benefits in this environment. Indeed, as stated by Russell (1990) 'it is not necessary for a microbe to function at optimal rates as long as it can compete effectively in its particular environment'.

Although we recognize that the degree of uncoupling between chl *a* increase and biomass production may increase at elevated temperature, our study indicates that, even with this taken into account, temperature elevation resulted in greatly increased algal growth rates. The observed increases in chl *a*, particularly at elevated temperatures, are likely to be conservative estimates, as no account was taken of possible losses due to grazing within experiments. However, the lack of any significant change in chl *a* concentration in blacked-out bottles indicates that grazing losses, if any, were relatively small over the duration of these experiments.

The positive relationship between inorganic nitrogen uptake and chl *a* production evident at all stations indicates that the increases in chl *a* observed at elevated temperature did indeed represent an increase in growth rate. However, the variation in the strength of this relationship between stations indicates that a certain degree of uncoupling between chl *a* increase and biomass accumulation did occur, particularly at Stns 5, 6a and 6b.

The Si depletion to chl *a* increase ratios (Si:chl *a*) of around 1 generally seen at elevated temperatures, indicated rapid diatom growth under nutrient-replete conditions. However, the higher Si:chl *a* ratios observed at ambient temperature (particularly at Stns 3 and 4) indicated a more heavily silicified diatom biomass growing under nutrient stress. Such heavy silicification has previously been attributed to iron limitation, where the slowing of growth rate by iron limitation is combined with a constant silicate uptake rate and so excess silicate loading per cell (Hutchins & Bruland 1998). Measurements of *in situ* iron concentrations in this area on the second cruise (JR38) add some credence to this theory, iron concentrations being undetectable (<0.25 µmol m^{-3}) at the 2 stations where it was measured.

If iron limitation was indeed responsible for high silicification at ambient temperatures in this study, then an alleviation of iron limitation by temperature elevation seems evident. However, an increased Si:chl *a* ratio at low temperature may also result from nitrogen limitation, differential growth response of diatoms and non-diatoms to temperature change, or the fact that the enzymes responsible for synthesis of most organic components of diatom cell walls have a higher Q_{10} than Si crystallization (where Q_{10} represents the ratio of the rate constants for a reaction at 2 temperatures 10°C apart).

Specific nutrient depletion

The inverse relationship generally seen between specific silicate depletion and temperature supports the idea of heavier silicification of diatoms at ambient temperature due to nutrient stress, as identified through Si:chl *a* ratios. However, assuming that elevated temperature increases the availability of iron, the increase in dominance of diatoms which is often seen with iron addition (e.g. Coale et al. 1996) may counteract this trend given sufficient time (>5 d).

The variability in response of specific nitrate depletion to temperature elevation may be a result of the interaction between *in situ* ammonium concentration and nitrate uptake. Certainly, the previously reported trends of low temperature limitation of nitrate transport (Sakamoto & Bryant 1998) and affinity (Reay et al. 1999) involved laboratory studies where nitrate was the sole N source and where any significant ammonium effect was unlikely.

Several studies have identified a negative relationship between nitrate uptake and ammonium concentrations in the range encountered in this study (e.g. Dortch 1990, Flynn et al. 1997, Lomas & Glibert 1999a, Page et al. 1999), with studies in the Southern Ocean also supporting this relationship (Glibert et al. 1982, Owens et al. 1991, Mengesha et al. 1998). A recent model proposed the co-limitation of nitrate and phytoplankton growth in HNLC areas by iron, light and ammonium (Armstrong 1999). Certainly, the coincidence of highest nitrate depletion with stations of lowest ambient ammonium concentration agrees with the hypothesis of inhibition by, or preference for, ammonium where ammonium concentrations are high (>1 mmol m^{-3}). Similarly, the trend of low depletion/net increase of nitrate at stations with high *in situ* ammonium concentrations agrees well with such an ammonium effect.

The positive relationship generally observed between ambient ammonium concentration and specific ammonium uptake also reflects the often observed preference for ammonium over nitrate by phytoplankton when ammonium concentrations are high (Olson 1980, Dortch 1990). The absence of any clear temperature dependence of specific ammonium uptake agrees with our previous study, which indicated little temperature-dependence of affinity for ammonium in a range of algae and bacteria (Reay et al. 1999). However, Priscu et al. (1989) identified a temperature dependence of both nitrate and ammonium uptake in sea-ice microalgae.

The lack of significant nutrient depletion via heterotrophic uptake agrees with other studies in this area which have reported bacterial impact in surface waters to be relatively small compared to that of the phytoplankton (e.g. Bird & Karl 1999). However, under certain conditions heterotrophic nutrient depletion can be very significant, particularly in coastal/ice-edge areas or in post-bloom conditions (e.g. Robinson et al. 1999, Vanucci & Bruni 1999).

The negative correlation between ammonium concentration and the *f* ratio seen in this study can be attributed to both the inhibition of nitrate uptake by high ammonium concentrations and to the increase in ammonium depletion rate associated with high ammonium concentrations. Reported *f* ratios for the Southern Ocean vary widely, and reflect both the heterogeneous nature of this area and the seasonality of nitrogen preference. Ammonium concentrations are generally low early in the spring and nitrogen uptake tends to be dominated by nitrate use, with consequently high *f* ratios. However, despite the fact that nitrate concentrations are rarely depleted to what would be considered limiting concentrations in temperate oceanic areas, ammonium utilization becomes increasingly important during the course of the season, with a shift from predominantly new to regenerated production (Mengesha et al. 1998). Increasing evidence suggests that the reduced forms of N, such as ammonium and urea, may be less temperature-dependent than nitrate, so further increasing their importance as N sources at low temperatures. Reay et al.

(1999) showed specific affinity for ammonium in a range of microalgae and bacteria to be much less dependent on temperature than affinity for nitrate. Likewise, Sakamoto & Bryant (1998) showed that while cells of the unicellular cyanobacterium *Synechococcus* sp. became chlorotic and grew arithmetically at low temperature when grown solely on nitrate, they grew exponentially, with no symptoms of chlorosis, with urea as their nitrogen source.

The representative time course of nitrate and ammonium depletion highlights the large changes in nitrogen utilization over the course of the 72 h incubations. Rather than the microalgal community simultaneously using both nitrate and ammonium throughout the incubation, it appears that ammonium is quickly depleted to a point where little further net depletion can occur. Nitrate, on the other hand, shows a more uniform pattern of depletion with time, becoming increasingly favoured as the ammonium concentration falls. It appears that the microalgal community is able to rapidly switch between nitrogen sources depending on their relative concentrations, an important ability where inorganic nitrogen supply may be episodic and confined to small patches.

^{15}N uptake

The positive relationship between temperature and ^{15}N-nitrate incorporation, seen at 8 of the 9 stations where ^{15}N-nitrate uptake was measured, is in accord with previous studies of nitrate uptake by marine phytoplankton (Olson 1980, Le Bouteiller 1986, Priscu et al. 1989, Glibert & Garside 1992, Dauchez et al. 1996). This trend is also consistent with studies of the temperature-dependence of nitrate assimilation and affinity (Gao et al. 1993, Reay et al. 1999) and with studies of nitrate transport in the freshwater cyanobacterium *Synechococcus* sp. (Sakamoto & Bryant 1997, 1998, 1999). However, the opposite trend has been reported for cool-water diatoms in Chesapeake and Delaware Bays (Lomas & Glibert 1999b). In our study, the clear dependence of nitrate uptake on temperature is further demonstrated by the trend of increasing specific nitrate uptake even where ammonium concentrations are elevated. Within the range of ammonium concentrations encountered in this region, temperature plays a part in the control of nitrate use.

The trend of greater specific nitrate uptake rate in the whole phytoplanktonic assemblage compared to that in the <20 µm fraction is consistent with a preference of small phytoplankton for ammonium, compared to large phytoplankton which tend to rely more on nitrate as an N source (Dortch 1990, Owens et al. 1991). We have suggested previously (Reay et al. 1999) that a reliance of phytoplankton on passive NH_3 uptake may favour nano- and picoplankton over larger plankton. Similarly, passive uptake of urea could favour phytoplankton with a large surface area to volume ratio.

The negative effect of high ammonium concentrations on nitrate utilization is underscored by the ^{15}N-nitrate incorporation experiments. It is clear that, irrespective of temperature, ammonium concentrations may have a significant effect on specific nitrate depletion in the area studied. However, the absolute values of ammonium at which nitrate use becomes limited are highly contentious, and several workers have reported no inhibition even at concentrations >1 mmol m^{-3} (Dortch 1990). It is likely that the effect of ammonium concentration on nitrate utilization is modified by a variety of factors (Armstrong 1999), such as community composition and previous nitrogenous nutrition of the microalgal assemblage. The importance of ammonium inhibition of nitrate uptake to the maintenance of HNLC conditions in the Subarctic northeastern Pacific has already been suggested (Wheeler & Kokkinakis 1990, Varela and Harrison 1999). Our study now provides further evidence that this factor may also be important in the maintenance of the Southern Ocean HNLC region. The additive effect of low temperature and elevated ammonium in limiting nitrate uptake again shows the importance of considering more than just one factor when attempting to parameterize nitrate use in this area.

Some divergence in the rate and trend of nitrate uptake measured with ^{15}N-nitrate incorporation versus that measured in the simple nutrient-depletion experiments was evident during this study. This divergence is likely to have arisen as a result of the greater incubation time of the simple depletion experiments, with a consequently increased likelihood of ammonium regeneration and more complex nitrate uptake dynamics.

The very low *in situ* iron concentrations encountered at the 2 stations where iron was measured agree with values reported previously for this region (Löscher et al. 1997). The lack of a significant increase in specific nitrate uptake rate with iron addition is slightly surprising considering these very low *in situ* concentrations. However, low concentrations of a nutrient do not necessarily translate to limiting concentrations, factors such as low temperature may be as important as direct iron limitation of nitrate use at *in situ* temperatures in the Southern Ocean. The increase in specific nitrate uptake rate seen at elevated temperature with iron addition, relative to incubations without iron addition, argues that it is the low *in situ* temperature that limits nitrate use in this instance. Some interaction between temperature and iron limitation seems evident in such a case, with low temperature limitation of iron uptake being a likely component.

Ecological implications for the Southern Ocean HNLC region

The importance of temperature to algal growth is now well established in many aquatic environments (Eppley 1972, Raven & Geider 1988). Our results suggest that under optimal conditions, increased environmental temperature in the Southern Ocean of only 1 to 2°C could result in significantly higher rates of nitrate utilization and microalgal growth. Obviously, the effect of oceanic warming on net primary production is, in practice, a highly complex one, with factors such as grazing, nutrient recycling and water-column stability all being sensitive to temperature change. However, this study provides valuable information both on the potential importance of temperature in the maintenance of the Southern Ocean as an HNLC region and the effects that temperature change may have.

When the inhibitory effect of high ammonium concentration (≈1 mmol m^{-3}) on nitrate use is coupled with the trend of increased ammonium-depletion rates with increased ammonium concentrations, the significance of ammonium to Southern Ocean microalgae becomes apparent. Ammonium is typically found at much lower concentrations than nitrate, and is supplied by recycling as the result of microbial activity and zooplankton grazing in the euphotic zone (Priddle et al. 1997) and remineralization by land-based endotherms (Whitehouse et al. 1999). However, under Southern Ocean conditions, phytoplankton often cannot utilize fully the larger pool of nitrate present in the water. This study indicates that the combined effects of reduced affinity for nitrate at low temperature and preferential assimilation of ammonium where ammonium concentrations exceed ≈1 mmol m^{-3} may contribute to this incomplete utilization of nitrate. Phytoplankton production is therefore potentially regulated in part by the rate of remineralisation and ammonium production (Priddle et al. 1997).

Although it seems likely that iron is often present in the Southern Ocean at limiting concentrations, our study of the effects of temperature and ammonium on nitrate uptake and phytoplankton growth suggest that enhanced iron concentration alone may not always enable phytoplankton to utilize the nitrate pool fully. This may be due to the lower affinity for nitrate at low temperature (Reay et al. 1999) and the additional energetic cost of nitrate uptake and assimilation compared to that for ammonium. Furthermore, under low temperature conditions, requirements for iron in both the electron transport system and in nitrate- and nitrite-reductases (Dugdale & Wilkerson 1991, Price et al. 1991) could be elevated if the algae were to respond by increasing the number of enzyme-active sites (Maldonado & Price 1996). A situation could therefore be conceived whereby nitrate in the Southern Ocean is not fully utilized due to insufficient iron availability, an insufficiency which itself is exacerbated by low temperature. Obviously the temperature-dependence of iron utilization needs to be established for such an indirect effect of low temperature on nitrate utilization and algal growth. However, as indicated earlier, the active nature of iron uptake so far identified for microorganisms (Lesuisse et al. 1991) certainly presages temperature dependence.

There have been many suggestions to explain HNLC conditions in both the Southern Ocean and elsewhere (see Cullen 1991). Limitation of nutrient drawdown and primary production by low concentrations of trace elements, especially iron (e.g. Martin & Fitzwater 1988), seems of obvious importance, but should not be taken as the sole and independent determinant. The heterogeneous nature of HNLC regions, coupled with interactions between limiting factors, means that co-limitation of nitrate use and phytoplankton growth in the Southern Ocean is the more likely large-scale scenario, rather than direct limitation by a single factor. This study supports the idea outlined by Armstrong (1999) that the various factors cited as responsible for HNLC conditions should be viewed not as alternatives, but as part of a whole.

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