

Temperature Dependence of Inorganic Nitrogen Uptake: Reduced Affinity for Nitrate at Suboptimal Temperatures in Both Algae and Bacteria

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Nitrate utilization and ammonium utilization were studied by using three algal isolates, six bacterial isolates, and a range of temperatures in chemostat and batch cultures. We quantified affinities for both substrates by determining specific affinities (specific affinity = maximum growth rate/half-saturation constant) based on estimates of kinetic parameters obtained from chemostat experiments. At suboptimal temperatures, the residual concentrations of nitrate in batch cultures and the steady-state concentrations of nitrate in chemostat cultures both increased. The specific affinity for nitrate was strongly dependent on temperature ($Q_{10} \approx 3$, where Q_{10} is the proportional change with a 10°C temperature increase) and consistently decreased at temperatures below the optimum temperature. In contrast, the steady-state concentrations of ammonium remained relatively constant over the same temperature range, and the specific affinity for ammonium exhibited no clear temperature dependence. This is the first time that a consistent effect of low temperature on affinity for nitrate has been identified for psychrophilic, mesophilic, and thermophilic bacteria and algae. The different responses of nitrate uptake and ammonium uptake to temperature imply that there is increasing dependence on ammonium as an inorganic nitrogen source at low temperatures.

The forms of inorganic nitrogen used most commonly by bacteria and algae are nitrate and ammonium (82). In many estuarine and marine systems the nitrogen concentration is limiting (14, 23), and nitrate concentrations are generally much higher than ammonium concentrations, although ammonium is invariably the preferred nitrogen source when it is available (46, 82). Despite the relatively low concentrations of ammonium, the great importance of ammonium to the global nitrogen cycle has been increasingly recognized (60, 61). Information concerning the mechanisms of uptake of the two forms of nitrogen is limited (19), and most data have been derived from measurements of uptake systems in higher plants (12, 42, 43) rather than measurements of uptake systems in algae and bacteria.

Previous work has shown that the affinity of bacteria for organic substrates can be highly temperature sensitive (51) and that a low temperature exacerbates any effect of nutrient limitation by making it increasingly difficult to sequester substrates (83). It has been pointed out that decreased affinity for inorganic substrates, such as nitrate, at low temperatures could have profound effects on the productivity of low-temperature environments, such as the Southern Ocean (51). Relatively little is known about the temperature sensitivity of inorganic nitrogen uptake by microorganisms, but it has been suggested that there is a difference between the temperature dependence of the uptake system for nitrate and the temperature dependence of the uptake system for ammonium (13).

A low affinity for inorganic nutrients, as indicated by a high half-saturation constant (K_s) for uptake of silicate and nitrate, has been reported previously for Southern Ocean phytoplankton at low temperatures (36, 69). However, no consistent trend

of changing affinity for inorganic nitrogen with temperature has been identified previously by using K_s values alone. The use of K_s to measure affinity can be misleading as this parameter does not necessarily reveal changes in substrate affinity at low concentrations (9). On the other hand, specific affinity (a_A°) is a more robust measure of substrate affinity. This parameter is the initial slope of the rectangular hyperbola (Michaelis-Menten or Monod) function relating growth rate (μ) to substrate concentration and is given by $a_A^\circ = \mu_{\max}/K_s$, where μ_{\max} is the maximum growth rate and K_s is the concentration at which $\mu = 0.5\mu_{\max}$. a_A° is the slope of the hyperbola at zero concentration and thus provides an unambiguous measure of the ability of cells to accumulate substrate and grow at very low concentrations, and this parameter is independent of the uptake mechanism (7, 8, 30). Because a_A° for growth is a rate divided by a concentration, it has the dimensions $\text{time}^{-1} \cdot (\text{mass} \cdot \text{liter}^{-3})^{-1}$ and in our calculations has the units liters per micromole per hour. Such a measurement of affinity is related to growth through the cell yield.

In this study we investigated the influence of temperature on affinity for nitrate and ammonium in a range of algae and bacteria by using a_A° to describe changes in the affinity of an organism for inorganic nitrogen. Note that ammonium is used below to indicate both ammonia (NH_3) and ammonium (NH_4^+), except where a distinction between the two is required, when the appropriate chemical formulae are used.

MATERIALS AND METHODS

Bacterial and algal isolates were chosen so that a wide range of physiological and taxonomic types was represented (Table 1). All cultures were monospecific and axenic and were checked regularly for contamination (6).

The μ_{\max} values for both bacterial and algal isolates were determined by using a temperature gradient block incubator (51, 77). The temperature range was adjusted so that it was suitable for each organism (Table 1). For algal isolates a temperature gradient block with illuminated wells was used. Illumination was provided by a bank of Triton fluorescent tubes (38 W; 400 to 710 nm, with peaks at 450, 550, and 620 nm; Interpet Ltd., Dorking, England.) placed immediately below the temperature gradient block (well illumination, 200 μmol of quanta ·

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TABLE 1. Isolates used in this study and incubation temperature ranges used for batch culture experiments

Taxon	Strain ^a	Source	Incubation temp range		Person who isolated or reference
			Minimum temp (°C)	Maximum temp (°C)	
<i>Vibrio logei</i>	NCIMB 1143	Seawater, Puget Sound, Southern Ocean	0.4	23	R. R. Colwell
<i>Hydrogenophaga pseudoflava</i>	NCIMB 13125	Lake sediment, Signy Island, Antarctica	1.7	26.5	51
<i>Brevibacterium</i> sp.	NCIMB 13126	Lake sediment, Signy Island, Antarctica	1.7	26.5	51
<i>Klebsiella oxytoca</i> ^b		Freshwater sample, Colne River Estuary, England	2.4	27	52
<i>Escherichia coli</i>	NCIMB 09001	Unknown	15	35	Unknown
<i>Bacillus stearothermophilus</i>	NCIMB 11401	Hot water supply, Porton Down, Wiltshire, England	12.9	46.1	M. J. Cromer
<i>Chaetoceros</i> sp. ^c	CCAP 1010/10	Seawater sample, Southern Ocean	2	13.6	Unknown
<i>Chaetoceros curvisetum</i> ^{b,c}		Seawater sample, North Sea	10.1	22.9	A. Clarke
<i>Dinahiella tertiolecta</i> ^c	CCAP 19/6B	Seawater sample, Oslo Fjord, Norway	3.3	23.9	6

^a NCIMB, National Collections of Industrial and Marine Bacteria Ltd., Aberdeen, Scotland; CCAP, Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, Oban, Scotland.

^b The isolate was not obtained from a culture collection.

^c Microalgal isolate.

$m^{-2} \cdot s^{-1}$). Optically standardized test tubes containing 10 ml of sterile FC2 medium for bacterial isolates (51, 52) or modified f/2 medium for algae (27) were prepared with either 80 μM NH_4Cl or 80 μM $NaNO_3$ as the nitrogen source. Two diatoms, *Chaetoceros curvisetum* and *Chaetoceros* sp., were not grown on NH_4Cl in batch cultures. Preliminary experiments showed that at a concentration of 80 μM , nitrogen was the first nutrient to be depleted, which induced the stationary phase. Subsequent aseptic addition of either a sterile nitrate solution or a sterile ammonium solution resulted in a further increase in the optical density, which confirmed that N limitation occurred. Tubes were placed in the wells of the temperature gradient block, and each tube was aerated continuously; the airstream was humidified to prevent evaporation. After all of the tubes had become equilibrated to the temperature in the block, each tube was inoculated with 0.2 ml of an exponential-phase culture grown on the same medium at the optimum growth temperature of the organism.

After inoculation, growth at each temperature was monitored by periodic measurement of turbidity with a nephelometer (model EEL Unigalvo DS29; Diffusion Systems, London, United Kingdom). The μ_{max} at each temperature was obtained from a first-order linear regression analysis which determined the slope of the linear part of the semilogarithmic plot of optical density versus time.

Batch cultures were regarded as being in the stationary phase when the variation (standard error) in the optical densities at four successive times over a 24-h period was <2% of the mean optical density for the same period. The residual concentration of the limiting nutrient (nitrate or ammonium) during the stationary phase in batch cultures reflected the affinity of the uptake system for the substrate (55). Changes in the residual substrate concentration with temperature provide an indication of changes in affinity (51), although K_s values cannot be calculated directly. When cultures reached the stationary phase, they were removed from the temperature gradient block and centrifuged at $6,000 \times g$ for 15 min. Supernatant (triplicate 1-ml samples) was then removed and analyzed to determine either the residual nitrate content or the residual ammonium content. Nitrate contents were determined colorimetrically (72). The method used was linear for concentrations ranging from 1 to 20 μM , and the limit of detection was 0.25 μM . Ammonium contents were analyzed by the indophenol blue method (29), which was modified by substituting sodium dichloroisocyanuric acid for the original unstable chlorine donor, hypochlorite (37). The ammonium values were linear for concentrations between 1 and 40 μM , and the limit of detection was 0.5 μM . Samples were diluted when necessary in order to obtain concentrations within the detection ranges of the colorimetric methods used.

Chemostat cultures were used to measure μ_{max} independent of batch cultures for all isolates. The use of chemostats also allowed us to measure the K_s and a_A^0 values over a range of temperatures. The chemostat incubation temperatures were set to give a range up to and including the optimum temperature for each isolate. Bacterial (FC2) and algal (f/2) media containing either 80 μM NH_4Cl or 80 μM $NaNO_3$ were used for all chemostat experiments so that nitrogen was the growth rate-limiting nutrient. Dilution rates were set at 0.018 h^{-1} for all chemostats, which were continuously mixed and aerated. All algal cultures were also continuously illuminated (200 μmol of quanta $\cdot m^{-2} \cdot s^{-1}$) with twin banks of Triton fluorescent tubes.

Each chemostat was inoculated with 1 ml of an exponential-phase culture grown on the same medium at the optimum growth temperature for the isolate. After inoculation, the growth of each isolate to the steady state was monitored by periodically aseptically removing a 1-ml subsample, whose optical density at 550 nm (OD_{550}) was then determined with a spectrophotometer. A chemostat was considered to be in a steady state when the variation in the standard error for at least six optical densities determined over a period of at least 60 h was <2% of the mean OD_{550} (51). Nitrogen limitation was confirmed by aseptically adding to a chemostat either 5 ml of a sterile 10 mM nitrate solution or 5 ml of a sterile 10 mM ammonium solution. A subsequent increase in the optical density confirmed that N limitation occurred under steady-state conditions.

Under steady-state conditions K_s values were determined by removing 5-ml subsamples from the chemostats. Each subsample was filtered through a Whatman cellulose acetate filter (nominal particle retention size, 0.2 μm), and residual nitrate or ammonium content in the filtrate was measured. K_s values were calculated by using the following equation: $K_s = s(\mu_{max} - D)/D$, where s is the residual substrate concentration and D is the dilution rate (per hour) (55, 69). Note that the K_s values derived in this way were K_s values for growth and thus are not always equivalent to K_s values determined for uptake (25).

μ_{max} values in the chemostats were determined by increasing the dilution rate to values that were higher than the critical dilution rate in order to induce washout. μ_{max} was calculated from the slope of a plot of $\ln OD_{550}$ versus time during washout. a_A^0 values were then calculated by using μ_{max} values determined for the same chemostat cultures rather than μ_{max} values determined for batch cultures.

Data were analyzed by performing box plots, F tests, one-way analyses of variance (ANOVAs), and first-order linear regression analyses (LRAs) (21). Statistical analysis and data plotting were performed by using the data analysis packages supplied in Systat version 5.04 (Systat Inc.), Excel version 7.0 (Microsoft), and SigmaPlot version 3.0. (Jandel Scientific).

RESULTS

Batch cultures. The bacterial and algal isolates used grew at a wide range of temperatures and, consequently, there was a wide range of optimum incubation temperatures (Table 2). The residual concentrations of nitrate and ammonium at the stationary phase in batch cultures exhibited a similar trend with all of the bacterial and algal species investigated (Fig. 1). At or near the optimum temperatures for growth the residual concentrations of either nitrate or ammonium were below the sensitivity of the analyses. As the temperature deviated from the optimum temperature, the residual concentration of either nitrate or ammonium generally increased, although the residual nitrate concentrations tended to increase more than the

TABLE 2. Temperature ranges at which isolates grew and optimum temperatures for growth

Taxon	Growth temp range (°C)	μ_{max} range (h^{-1})	Optimum incubation temp (°C)
<i>Vibrio logei</i>	0.4–23	0.031–0.077	15
<i>Brevibacterium</i> sp.	1.7–26.5	0.055–0.11	20
<i>Hydrogenophaga pseudoflava</i>	1.7–26.5	0.037–0.074	24
<i>Klebsiella oxytoca</i>	8.2–27	0.033–0.21	27
<i>Escherichia coli</i>	15–35	0.04–0.21	35
<i>Bacillus stearothermophilus</i>	18.4–46.1	0.023–0.161	46.1
<i>Chaetoceros</i> sp. ^a	2–9.2	0.017–0.028	6
<i>Chaetoceros curvisetum</i> ^a	10.1–22.9	0.003–0.02	22.9
<i>Dinahiella tertiolecta</i> ^a	9.1–23.9	0.05–0.104	23.9

^a Microalgal isolate.

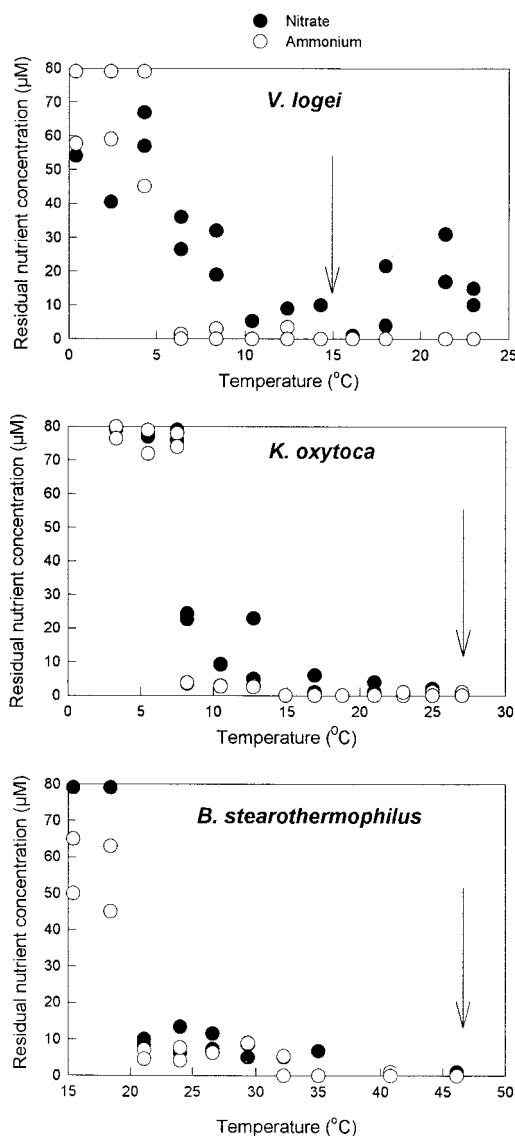


FIG. 1. Residual concentrations of nitrate (●) and ammonium (○) in batch cultures of three bacterial isolates grown at a range of temperatures. The arrows indicate the optimum growth temperature for each of the isolates.

residual ammonium concentrations increased. As the incubation temperature approached the minimum growth temperature of an organism, the residual concentrations of both N sources increased rapidly. The maximum concentrations (80 µM, equivalent to the starting N concentration in the medium) were observed at temperatures at which growth ceased.

μ_{\max} values exhibited considerable interspecies variation. Psychrotolerant bacteria (*Hydrogenophaga pseudoflava*, *Brevibacterium* sp., *Vibrio logei*) had lower μ_{\max} values over their growth temperature ranges than meso- and thermophilic bacteria (*Klebsiella oxytoca*, *Escherichia coli*, *Bacillus stearotherophilus*) had. The flagellate alga *Dunaliella tertiolecta* had μ_{\max} values that were significantly higher than the μ_{\max} values obtained for the diatoms *C. curvisetum* and *Chaetoceros* sp. The μ_{\max} values for all of the bacterial and algal isolates were significantly dependent on temperature ($P < 0.05$, as determined by LRA). We observed no significant difference ($P > 0.2$, as determined by ANOVA) between the μ_{\max} values for

nitrate-limited and ammonium-limited batch cultures of the isolates grown on both substrates.

Chemostat cultures. There were no significant differences between the μ_{\max} values measured in batch cultures and the μ_{\max} values measured in chemostat cultures for any of the bacterial or algal cultures, as determined by ANOVA ($P > 0.5$). The steady-state nitrate concentrations exhibited a significant negative correlation with temperature for all of the isolates (Tables 3 and 4), which reflected the substantial increases in the residual nitrate concentrations that occurred with decreasing temperature in batch cultures. The half-saturation constant for nitrate (K_{nit}) increased as the incubation temperature decreased for most of the bacterial and algal isolates, although no consistent trend was identified for *E. coli* or *B. stearotherophilus*. The half-saturation constant for ammonium (K_{amm}) did not consistently increase as the temperature decreased for any isolate.

The specific affinity for nitrate (a_{nit}°) values calculated for the temperatures by using the relevant μ_{\max} and K_s values consistently decreased as the temperature decreased. An LRA showed that there was a significant linear relationship between a_{nit}° and temperature for all isolates ($P < 0.05$, as determined by LRA), although there were large interspecies variations among a_{nit}° values. The response of a_{nit}° to temperature change was greater in the psychrotolerant species (whose Q_{10} values ranged from 3 to 5, where Q_{10} is the proportional change with a 10°C temperature increase) than in the thermotolerant species (whose Q_{10} values ranged from 1.8 to 2.5). The specific affinity for ammonium (a_{amm}°) values also varied greatly among species but generally showed little temperature dependence (the Q_{10} values for individual species ranged from 0.75 to 2.1). Only one isolate (a *Brevibacterium* sp. isolate) showed any significant dependence on temperature ($P < 0.05$, as determined by LRA; $Q_{10} \approx 2$). It must be noted that in some cases steady-state concentrations of ammonium approached the limit of detection, and the specific affinity values in such cases must be regarded as lower limits.

To illustrate the consistent response of a_A° to temperature change, a_A° data for nitrate and ammonium were normalized to express the a_A° value at a given temperature as a percentage of the value at 15°C (Fig. 2). (The psychrophilic diatom *Chaetoceros* sp. and the thermophilic bacterium *B. stearotherophilus* did not grow at 15°C, so these organisms were omitted from this comparison.) The normalized plot showed that there was significant dependence of a_{nit}° on temperature ($P < 0.001$, as determined by LRA) but no significant temperature dependence of a_{amm}° ($P = 0.73$, as determined by LRA). The Q_{10} values for normalized a_A° were 2.9 ± 0.43 (mean \pm standard error; $n = 21$) and 0.98 ± 0.6 ($n = 25$) for nitrate and ammonium, respectively. These normalized data again indicated that the temperature response of affinity for nitrate was consistently greater than the temperature response of affinity for ammonium in all of the bacterial and algal isolates examined.

DISCUSSION

Relationship between affinity and temperature. The mechanisms that limit growth at high temperatures (protein denaturation, etc.) are well-documented (16, 64), but the mechanisms responsible for limiting μ at low temperatures are still a source of contention. Our data provide consistent evidence that growth of both bacteria and marine algae under N-limited conditions at low temperatures is restricted by the reduced ability of the organisms to sequester inorganic nitrogen. (A similar decreased affinity for uptake of organic substrates at low temperatures has been reported previously [51].) Although

TABLE 3. Growth kinetics for bacterial isolates grown to steady state in nitrogen-limited chemostat cultures

Taxon	Temp (°C)	Steady-state NO ₃ ⁻ concn (μM)	μ _{max} with NO ₃ ⁻ (h ⁻¹)	K _{nit} (μM)	a _{nit} ^o (liter · μmol ⁻¹ · h ⁻¹)	Steady-state NH ₄ ⁺ concn (μM)	μ _{max} with NH ₄ ⁺ (h ⁻¹)	K _{amm} (μM)	a _{amm} ^o (liter · μmol ⁻¹ · h ⁻¹)
<i>Vibrio loei</i>	15	2.5 (0.1) ^a	0.0677 (0.0003)	6.9	0.0098	9.3 (0.1)	0.0614 (0.002)	22.4	0.0027
	10	8.5 (0.1)	0.053 (0.001)	16.5	0.0032	10.8 (0.5)	0.053 (0.003)	20.8	0.0025
	8	8.5 (0.3)	0.0375 (0.005)	9.2	0.0041	9.1 (0.01)	0.048 (0.001)	15.1	0.0032
	6	11.0 (0.1)	0.0373 (0.0001)	11.8	0.0032	4.3 (0.2)	0.049 (0.005)	7.4	0.0066
	4	13.5 (0.1)	0.0381 (0.001)	15.1	0.0025	11.4 (0.2)	0.0391 (0.002)	13.4	0.0029
<i>Hydrogenophaga pseudoflava</i>	15	NG ^b	NG	NG	NG	1.6 (0.02)	0.074 (0.001)	5.0	0.0148
	10	NG	NG	NG	NG	4.5 (0.4)	0.06 (0.009)	10.5	0.0057
	5	NG	NG	NG	NG	1.6 (0.03)	0.06 (0.002)	3.7	0.016
<i>Brevibacterium</i> sp.	15	NG	NG	NG	NG	2.7 (0.7)	0.12 (0.02)	13.2	0.0091
	10	NG	NG	NG	NG	4.5 (0.04)	0.08 (0.01)	15.5	0.0052
	5	NG	NG	NG	NG	6.4 (0.1)	0.055 (0.004)	15.4	0.0036
<i>Klebsiella oxytoca</i>	20	2.5 (0.1)	0.143 (0.005)	17.3	0.0083	2.7 (0.02)	0.128 (0.008)	16.5	0.0078
	15	5.0 (0.1)	0.116 (0.007)	27.2	0.0043	2.8 (0.03)	0.105 (0.002)	13.6	0.0077
	10	9.5 (0.5)	0.087 (0.01)	36.6	0.0024	3.1 (0.03)	0.092 (0.003)	12.8	0.0072
<i>Escherichia coli</i>	35	11 (0.05)	0.212 (0.004)	118.6	0.0018	8.7 (0.1)	0.172 (0.0001)	74.4	0.0023
	25	12 (1)	0.15 (0.009)	88.0	0.0017	6.2 (0.04)	0.064 (0.001)	15.8	0.0041
	20	14.5 (0.1)	0.17 (0.011)	122.4	0.0014	NM ^c	NM	NM	NM
	15	26 (0.3)	0.08 (0.0001)	89.6	0.0009	8.1 (0.2)	0.037 (0.001)	8.6	0.0043
<i>Bacillus stearothermophilus</i>	45	5.5 (0.1)	0.144 (0.007)	38.4	0.0038	5.5 (0.1)	0.144 (0.01)	38.4	0.0038
	40	6.0 (0.2)	0.098 (0.007)	26.6	0.0037	7.1 (0.06)	0.096 (0.005)	30.7	0.0031
	35	8.5 (0.02)	0.071 (0.012)	24.9	0.0029	3.4 (0.04)	0.078 (0.007)	11.3	0.0069
	30	13.5 (0.1)	0.058 (0.003)	29.9	0.0019	6.3 (0.3)	0.067 (0.0001)	17.0	0.0039
	25	NG	NG	NG	NG	5.3 (0.05)	0.058 (0.005)	11.61	0.0050

^a The values in parentheses are standard errors of the means ($n = 3$).

^b NG, no growth.

^c NM, not measured.

inhibition of membrane transport at low temperatures that leads to growth limitation has been suggested before (35, 49), consistent evidence which supports the hypothesis has not been presented previously.

In the N-limited batch cultures that were grown at or near the optimum growth temperatures of species, the residual concentrations of both nitrate and ammonium were below the analytical limits, indicating that utilization of both N sources is most effective at these temperatures. However, at suboptimal temperatures the increases in the residual concentrations of nitrate and ammonium during the stationary phase illustrated that bacteria and algae became less able to take up inorganic

nitrogen as the temperature decreased, whether the isolate was psychrophilic, mesophilic, or thermophilic. This implied that the efficiency of the uptake system for both nitrate and ammonium decreased with temperature and so reduced the ability of the organisms to sequester inorganic nitrogen from the surrounding medium (55). However, the greater effect of suboptimal temperatures on residual nitrate concentrations than on residual ammonium concentrations indicated that low temperatures had different effects on nitrate utilization and ammonium utilization.

The μ_{max} values varied with temperature, as reported previously for N-limited cultures (62, 73). The generally lower

TABLE 4. Growth kinetics for microalgal isolates grown to steady state in nitrogen-limited chemostat cultures

Taxon	Temp (°C)	Steady-state NO ₃ ⁻ concn (μM)	μ _{max} with NO ₃ ⁻ (h ⁻¹)	K _{nit} (μM)	a _{nit} ^o (liter · μmol ⁻¹ · h ⁻¹)	Steady-state NH ₄ ⁺ concn (μM)	μ _{max} with NH ₄ ⁺ (h ⁻¹)	K _{amm} (μM)	a _{amm} ^o (liter · μmol ⁻¹ · h ⁻¹)
<i>Chaetoceros</i> sp.	5	3 (0.1) ^a	0.033 (0.001)	2.5	0.0132	3 (0.02)	0.033 (0.002)	2.5	0.0132
	3	14 (0.02)	0.029 (0.005)	8.2	0.0035	7 (0.02)	0.029 (0.001)	4.2	0.0069
	1	21.5 (0.2)	0.022 (0.0001)	4.7	0.0047	NM ^b	0.022 (0.004)	NM	NM
<i>Chaetoceros curvisetum</i>	22	3 (0.1)	0.028 (0.0001)	1.7	0.0165	28.7 (0.1)	0.0284 (0.009)	16.5	0.0017
	17	6.5 (0.01)	0.027 (0.003)	3.3	0.0082	38.6 (0.3)	0.0252 (0.001)	14	0.0018
	12	42.4 (0.3)	0.02 (0.001)	5.7	0.0035	47.4 (0.5)	0.019 (0.001)	5.3	0.0036
<i>Dunaliella tertiolecta</i>	25	5.5 (0.04)	0.078 (0.01)	19.1	0.0041	9 (0.05)	0.068 (0.007)	25	0.0027
	20	14 (0.05)	0.1 (0.001)	63.4	0.0016	7.8 (0.3)	0.087 (0.001)	30	0.0029
	15	18 (0.2)	0.095 (0.001)	77	0.0012	8 (0.4)	0.059 (0.0001)	18	0.0033
	10	47 (0.1)	0.045 (0.005)	71	0.0006	13.4 (0.4)	0.058 (0.002)	30	0.0019

^a The values in parentheses are standard errors of the means ($n = 3$).

^b NM, not measured.

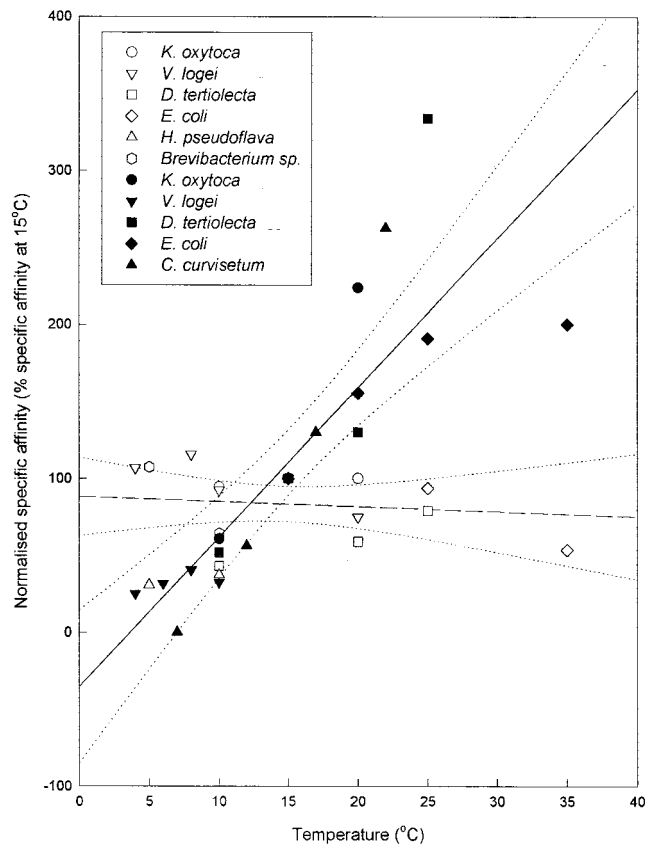


FIG. 2. Plot of normalized (to 15°C) a_{amm}° and a_{nit}° values versus incubation temperature for a range bacteria and microalgae. The dashed line is the first-order linear regression line through normalized ammonium data ($n = 25$; $r^2 = 0.01$; $P = 0.73$). The solid line is the first-order linear regression line through normalized nitrate data ($n = 21$; $r^2 = 0.71$; $P < 0.001$). The dotted lines are the 95% confidence limits for the regression lines.

μ_{max} values for the psychrotrophic and psychrophilic bacteria (*H. pseudoflava*, *Brevibacterium* sp.) than for mesotrophic isolates (e.g., *K. oxytoca* and *E. coli*) and the greater μ_{max} values for the microflagellate alga *D. tertiolecta* than for the two diatoms are both previously identified trends (7).

Nitrate uptake and temperature. An examination of the measured K_s values indicated that affinity for nitrate was reduced to a greater extent by low temperature than affinity for ammonium was, but when K_s was used alone as a measure of affinity, this trend was by no means consistent. Such inconsistency is common when K_s alone is used as a measure of substrate affinity (15, 48). Such use of K_s has been criticized as it does not adequately reflect changes in the ability of cells to sequester substrate at low concentrations (8), which depends on both K_s and μ_{max} . Alternatively, a_{A}° , approximated by μ_{max}/K_s , has been employed (9, 25, 30, 40, 51). a_{A}° provides an unambiguous measure of the ability of an organism to sequester substrate at low concentrations and is independent of uptake mechanism (26). Measurements of a_{A}° , therefore, allow examination of the effect of temperature on affinity for a substrate and identify temperature effects on uptake alone (8). Using a_{A}° , we demonstrated for the first time that a temperature below the optimum growth temperature consistently resulted in decreased affinity for nitrate in a number of bacteria and marine algae. This trend was consistent for psychrophilic, mesophilic, and thermophilic species despite the different

growth temperature ranges of the organisms. These findings suggest that there is a common mechanism that is responsible for the decrease in affinity for nitrate that occurs as the temperature decreases.

Our K_s values for the algae were high compared to the few other values reported previously for algal chemostat cultures (7). Many of the K_s values reported previously for algal growth were determined with fed-batch cultures (17, 44, 81) and cannot be compared with our data because they are K_s values for uptake rather than K_s values for growth and do not represent steady-state conditions (25). It must also be asked whether K_s values by themselves provide any useful information about affinity for a substrate, which might be why coherent trends of a_{A}° with temperature are not seen with K_s alone. The a_{nit}° values that we determined for algae are similar to the few other values that are available in the literature with comparable units (we have not been able to find any such values for bacteria). For example, our values for a_{nit}° near the optimal growth temperatures for *D. tertiolecta* ($0.004 \mu\text{mol liter}^{-1} \text{h}^{-1}$ at 25°C), *C. curvisetum* ($0.0165 \mu\text{mol liter}^{-1} \text{h}^{-1}$ at 22°C), and *Chaetoceros* sp. ($0.013 \mu\text{mol liter}^{-1} \text{h}^{-1}$ at 5°C) are similar to the values reported for *Scenedesmus* sp. ($0.02 \mu\text{mol liter}^{-1} \text{h}^{-1}$ at 20°C [62]) and *Chaetoceros neglectum* ($0.015 \mu\text{mol}^{-1} \text{liter}^{-1} \text{h}^{-1}$ at 0°C [69]).

Despite the interspecies variations in actual a_{nit}° values, the Q_{10} values were similar (mean, 3.1; standard error, ± 0.48 ; $n = 8$), and this finding corroborated the idea that the temperature responses for nitrate uptake by algae and bacteria are similar. Although no previously published Q_{10} values for a_{nit}° were found, our Q_{10} value for a_{nit}° normalized to 15°C agrees well with the relatively high Q_{10} values reported for nitrate uptake by microorganisms (39, 59, 74, 75), a feature which is characteristic of active, carrier-mediated transport systems (39). An important consequence of the decrease in affinity for nitrate that occurs as the temperature decreases is the fact that at a low temperature there is an increase in the growth rate-limiting concentration of nitrate (55) and therefore an increased degree of nitrate limitation imposed by the low temperature. In other words, at a low temperature there is exacerbation of nitrate limitation for both algae and bacteria. A corollary of this is the hypothesis that addition of more nitrate should reverse the nitrate limitation imposed by low affinity at a low temperature, as demonstrated for organic substrates by Wiebe et al. (83). This implies that nutrient limitation bioassays must be carried out at the in situ temperature or the effective availability of external substrate pools may change.

The temperature dependence of nitrate use agrees with what is known about the nitrate uptake system. Nitrate is apparently taken up by an active transport system in bacteria, algae, and higher plants, and this transport system appears to be ATP driven rather than directly dependent on an electrochemical gradient (12, 18, 71, 79). There is additional evidence that active nitrate uptake is strictly Na^+ dependent (39), is highly sensitive to metabolic inhibitors (78), and may be strongly inhibited by the presence of ammonium (13, 39). Such active uptake systems can be very responsive to temperature changes (66). Although the temperature dependence of nitrate uptake has been demonstrated most clearly in higher plants (22, 42), it seems increasingly apparent from our studies and other studies (39, 41, 74) that nitrate uptake by bacteria and microalgae is also highly temperature dependent. Furthermore, uptake of other inorganic algal nutrients which are primarily sequestered by active transport is also likely to be adversely affected by low temperatures because of decreased affinity.

The data in the literature for μ_{max} and K_s values in chemostats at different temperatures is extremely restricted, but the

data which is available tends to support our paradigm. Uptake of nitrate at different temperatures in nitrate-limited cultures of *Scenedesmus* sp. (62), uptake of phosphate in phosphate-limited cultures of *Scenedesmus* sp. (1), and silicate uptake in silicate-limited cultures of the ice alga *Pseudonitzschia seriata* (70) all indicate that decreases in affinity occur at temperatures below the optimum temperature when affinity is measured by determining a_A^0 . It has been pointed out (5) that phosphate, a nutrient primarily acquired through active uptake (4), is not utilized efficiently in cold high-latitude waters.

Ammonium uptake, N preference, and temperature. The more constant concentrations of ammonium than of nitrate in steady-state chemostats at a range of incubation temperatures indicated that ammonium uptake is less temperature dependent than nitrate uptake is. The fact that low temperature has a greater inhibitory effect on nitrate uptake than on ammonium uptake has been documented previously in higher plant roots by several workers (42, 43), but until now this difference has not been established for phytoplankton and bacteria (53). The fact that the response of affinity for ammonium to decreased temperature is consistently less than the response of affinity for nitrate in bacteria, algae, and higher plants suggests that there are fundamental differences in the ammonium and nitrate uptake mechanisms across a broad phylogenetic range. This is not surprising since the differing biochemical requirements for assimilation of nitrate compared with that of ammonium apply to all organisms and are therefore likely to be evolutionarily highly conserved.

The low Q_{10} value (≈ 1) of normalized a_{amm}^0 agrees with data from previous studies of ammonium uptake in higher plants (42, 43). However, higher Q_{10} values have been reported for ammonium uptake by phytoplankton in the field (56, 68). The low Q_{10} values found for a_{amm}^0 are characteristic of channel-mediated ion fluxes (66), but the exact mechanisms involved in ammonium uptake and control of uptake are poorly understood. In the last 20 years several uptake pathways, including pathways for both active and passive uptake of ammonium, have been suggested (2, 12, 34, 38, 79, 80). Ammonia (NH_3) can diffuse freely through cell membranes (32), but at neutral pH >99% of NH_3 is protonated as NH_4^+ (38). In slightly alkaline environments, such as marine systems, the proportion of NH_3 may increase to >10% of the total ammonium (NH_3 and NH_4^+ combined) (67). The concentration gradient of NH_3 across a cell membrane may be maintained by NH_3 protonation within the cell and by equilibration between ammonium and NH_3 outside the cell (31). Passive uptake of NH_3 may therefore make a significant contribution to the N requirements of bacteria and algae (particularly organisms with large surface area/volume ratios). Several studies have confirmed that there is a preference for, or selection by, small phytoplankton for ammonium (28, 41, 57). In a comparison of ammonium preference in diatoms, dinoflagellates, cyanobacteria, chlorophytes, and other organisms (13), it was found that the greatest contrast was between the diatoms and the other organism category, which consisted mainly of small flagellates. The microflagellate preference for ammonium was much greater than the preference in diatoms, while the larger diatoms showed greater preference for nitrate (45, 54).

Low temperature probably affects nutrient uptake by causing alterations in physical characteristics of the cell membrane. Such changes associated with low temperature may control active nutrient uptake across cell membranes in several ways (10, 58). Low temperature may hinder conformational changes in membrane transport proteins and thus prevent solute molecules from combining with their carrier proteins, or it may result in reduction in the substrate supply to a transport pro-

tein and inactivation of carrier proteins. Low temperature may also result in a reduction in membrane fluidity; it is known that reductions in membrane fluidity decrease the activity of transporter and respiratory proteins (3, 20, 76) embedded in the membrane phospholipids, and indeed the embedded proteins may reciprocally influence the membrane fluidity (47). Thus, while different species may adapt their membranes to be functional over different ranges of temperature by changing the ratios of saturated, unsaturated, or branched-chain membrane lipids (see references 63 and 65 for reviews), we hypothesize that within the range of temperature for each species there is decreased affinity for substrates taken up by active transport as the temperature decreases below the optimum temperature for growth (50). As passive uptake is less affected by temperature than active uptake is (38), any significant passive component should make overall ammonium sequestration less dependent on temperature than active nitrate uptake is.

Nitrogen nutrition at low temperature and its ecological implications. We demonstrated that a reduction in temperature results in reduced affinity for nitrate and decreased utilization in several algal and bacterial isolates representing a wide range of physiological types. Ammonium uptake and affinity in the same isolates did not appear to be affected by a reduction in temperature to the same extent. This suggests that in low-temperature aquatic plankton ecosystems, microbial nitrogen utilization tends to be biased away from nitrate and that ammonium is a more important substrate.

In the Southern Ocean, surface water temperatures reach as low as -1.8°C , and the maximum summer temperature is around 4.0°C (33). In such a low-temperature environment, we would expect that nitrate uptake by phytoplankton would be reduced and that ammonium would be increasingly important as a nitrogen source. This hypothesis is consistent with the generally low f ratios, 0.2 to 0.6 (13, 53), reported for the Southern Ocean (the f ratio is the uptake of nitrate expressed as a proportion of the total inorganic nitrogen uptake). The low f ratios occur despite nitrate concentrations which are often more than 40 times the ammonium concentrations (11). The importance of ammonium may be further increased by the competitive effects of ammonium and nitrate; even relatively low concentrations of ammonium may inhibit nitrate uptake by microorganisms (13, 24, 82).

The temperature dependence of nitrogen preference in microbial plankton, especially in marine phytoplankton, could have far-reaching implications for biogeochemical nutrient cycling on a global scale. Several large regions of the World Ocean have been characterized as high-nutrient–low-chlorophyll regions, where annual primary production is too low to exhaust the supply of inorganic nutrients. Any changes in the environmental controls which result in suboptimal use of nitrate in these regions could result in major alterations in the pattern of primary production and thus accumulation of organic material.

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