Highly Specialized Nitrogen Metabolism in a Freshwater Phytoplankter, *Chrysochromulina breviturrita*

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A field and laboratory culture study was carried out on the nitrogen metabolism of isolates of the freshwater phytoplankter *Chrysochromulina breviturrita* Nich. (Prymnesiophyceae). These were isolated from two different softwater lakes, one believed to be influenced by acidic precipitation (Cinder Lake) and another which was experimentally acidified with H_2SO_4 (Lake 302-South). The alga was able to utilize only NH_4^+ as an inorganic N source. A range of irradiances and molybdenum concentrations failed to induce NO_3^- utilization. Among 17 organic N compounds including amino acids, purines, and other amines, only urea plus Ni^{2+} as a cofactor would serve as the sole N source for this species. Nonetheless, growth rates in media supplied with urea were significantly less than with NH_4^+. Field data from Lake 302-S indicate that a predominance of NH_4^+ versus NO_3^- as the major inorganic N species may have favored the development of a *Chrysochromulina*-dominated community during August 1984. A detailed depth profile also indicated that a metalimnetic peak ($>20 \times 10^6$ cells/L) of this alga coincided with a distinct NH_4^+ depletion, which occurred at no other time during the year. Experiments with isolates of *C. breviturrita* and a *Nannochloris* sp. (Chlorophyceae) (~1 μm in diameter) from this community indicated that the former alga possessed a highly specialized N metabolism much like the Cinder Lake isolate. The *Nannochloris* sp. from the same environment grew on NO_3^- and NH_4^+ equally well. It is suggested that the specialized NH_4^+ utilization by *C. breviturrita* may itself influence the pH regime of poorly buffered waters through selective NH_4^+ uptake and H^+ generation.

Les auteurs ont étudié sur le terrain et en laboratoire le métabolisme de l'azote chez des isolats de l'organisme phytoplanctonique d'eau douce *Chrysochromulina breviturrita* Nich. (prymnésiophycées). Les organismes ont été prélevés dans deux lacs d'eau douce différents. Le premier, le lac Cinder, semble subir les effets des précipitations acides. Le second, le lac 302-sud, a été expérimentalement acidifié au H_2SO_4. Les algues ne pouvaient utiliser que le NH_4^+ comme source d'azote inorganique. L'utilisation d'une gamme variée d'irradiations et de diverses concentrations de molybdène n'a pas permis d'induire l'utilisation du NO_3^-. Des 17 composés azotés organiques utilisés, notamment des acides aminés, des purines et d'autres amines, seule l'urée (avec du Ni^{+2} comme co-facteur) était utilisée comme source d'azote par cette espèce. Les taux de croissance obtenus en milieux enrichis à l'urée étaient cependant significativement inférieurs à ceux obtenus en milieux enrichis au NH_4^+. Les données obtenues sur le terrain au lac 302-S indiquaient que la prédominance du NH_4^+ par rapport au NO_3^- comme principale substance azotée inorganique avait pu favoriser l'expansion, en août 1984, d'une communauté dominée par les *Chrysochromulina*. Un profil des profondeurs détaillé indiquait aussi l'existence d'un pic métalimnétique ($>20 \times 10^6$ cellules/L) de cette algue qui coïncidait avec une nette carence de NH_4^+, phénomène qui ne s'est produit à aucun autre moment de l'année. Des essais portant sur des isolats de *C. breviturrita* et de *Nannochloris* sp. (chlorophycées) d'environ 1 μm de diamètre prélevés de cette communauté ont montré que la première algue possédait un métabolisme fortement spécialisé de l'azote qui ressemblait beaucoup à celui noté pour l'isolat du lac Cinder. Les *Nannochloris* sp., qui provenaient du même environnement, utilisaient aussi bien le NO_3^- que le NH_4^+. Les auteurs émettent l'hypothèse que l'utilisation spécialisée du NH_4^+ par *C. breviturrita* peut en elle-même influer sur le régime du pH des eaux mal tamponnées de par une assimilation sélective du NH_4^+ et la production de H^+.

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While phosphorus is widely recognized as the nutrient which most commonly regulates algal productivity in lakes (Schindler 1977; Wetzel 1983), nitrogen may influence algal biomass in some systems and may also affect species composition (Kalff 1971; Liao and Lean 1978). Phytoplankton growth in Castle Lake, a mesooligotrophic lake in California, is limited at least during part of the year by N availability and regeneration (Axler et al. 1982). Several forms of N are usually present and are recycled in freshwaters. The cycling of ammonium (NH_4^+) is particularly rapid (a few hours), which indicates a close coupling between regeneration and assimilation (Brezonik 1972; Axler et al. 1981).

In oligotrophic, granitic basins, NH_4^+ inputs are frequently derived in large part from atmospheric sources such as gaseous forms and precipitation (Likens et al. 1977). In such systems, which are also influenced by acidic precipitation, inputs of NH_4^+ are retained much more strongly than nitrate (NO_3^-) (Galloway et al. 1983). This could be significant, particularly as NH_4^+ represents on average the second most abundant

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cation in acidic precipitation (Gorham et al. 1984). Further, it has been shown in whole-lake experiments that NH_4^+ utilization by phytoplankton may further acidify poorly buffered waters (Schindler et al. 1985).

Culture studies have indicated that nearly all algae can utilize either NO_3^-, NO_2^-, or NH_4^+ as a N source. The latter form is utilized most efficiently, as more oxidized forms must be reduced before being incorporated into organic N compounds (Healey 1973; Syrett 1981). Our interest in N metabolism arose while developing a dilute, defined culture medium for the freshwater phytoplankter *Chrysochromulina breviturrita* Nich. (Prymnesiophyceae) (Wehr et al. 1985). In nature, this organism "blooms" in softwater lakes influenced by acidic precipitation and is associated with characteristic sour or rotten cabbage odors (Nicholls et al. 1982). In preliminary culture experiments, we found that the alga could use NH_4^+, but apparently not NO_3^- or urea, as the sole N source (Wehr et al. 1985). In addition, cells grown over the short term (~ 10 d) in a NO_3^--based medium had significantly less chlorophyll *a* per cell than NH_4^+-grown cells. Utilization of NH_4^+, as shown previously (e.g. Pratt and Fong 1940; Brewer and Goldman 1976), also led to the acidification of the growth medium unless a pH buffer was used (Wehr et al. 1986).

Relatively few freshwater algal species have been shown to have distinct preferences for certain forms of N. The most widely studied group are the euglenoids, which typically occur in acidic waters. Many isolates will not utilize NO_3^-, but all can apparently substitute a wide variety of organic N compounds (especially amino acids) for NH_4^+ (Cramer and Myers 1952; Birdsey and Lynch 1962; Cook 1968; Kempner and Miller 1972). One marine cryptomonad, *Hemiselmis virescens*, has been shown to possess a similarly limited N metabolism (Antia et al. 1975). *Cyanidium caldarium*, which occurs in acidic hot springs, is also somewhat specialized, with some isolates which can and others which cannot use NO_3^- as a N source (Rigano et al. 1975), although a number of organic compounds will suffice. An unusual example of NO_3^- preference has also been described in two *Haematococcus* spp. (Proctor 1957), which was shown to be related to pH interactions with NH_4^+ toxicity (Stross 1963). This might be expected, particularly with the excessive N concentrations used in these early studies (millimolar range).

Although many algal species apparently "prefer" NH_4^+ over NO_3^- or organic N (Syrett 1981), we know of no studies in which neither NO_3^- nor an array of organic N compounds can supply the N needs of an alga. In the present study, we demonstrate in detail that *C. breviturrita* possesses an exceptionally specialized N metabolism and that this has particular importance when blooms are formed in acid-sensitive lakes.

Materials and Methods

Culture Experiments

Chrysochromulina breviturrita was originally collected in July 1982 using surface water (1 m depth) grab samples from Cinder Lake (78°51′W, 45°04′N), a moderately acidic, oligotrophic lake in south-central Ontario. It was isolated into unialgal, axenic culture and has been maintained since early 1984 in a chemically defined medium which approximates the chemistry of softwater lakes. Exhaustive electron microscope studies of this isolate (BT130) indicated that *C. breviturrita* was the only species present in culture. Complete details of isolation and the culture medium have been given previously (Wehr et al. 1985). Nearly all culture experiments were conducted using this isolate. In one experiment, two other phytoplankters were used. *Chrysochromulina breviturrita* (isolate BT138: identity determined by TEM) and *Nannochloris* sp. (isolate LRGT-1: ~1 μm in diameter) were both isolated from a sample collected from Lake 302-South in the Experimental Lakes Area (northwestern Ontario) in August 1984. Details of this site are given in the field program below. Both were rendered unialgal and axenic by methods described previously (Wehr et al. 1985). The plus-N medium routinely used for maintenance of cultures (Muskoka No. 112) is identical to that used in previous studies (No. 42: Wehr and Brown 1985; Wehr et al. 1985) except that half the N and P (50 μ*M* $(NH_4)H_2PO_4$) and only two vitamins (thiamine, B_{12}) were added. The water used for media was glass distilled and then passed through a cation-exchange column (Biorad AG50-8W, H^+ form) to remove trace NH_4^+-N. Experimental cultures were grown in acid-washed (10% HCl) 125-mL Erlenmeyer flasks containing 50 mL of medium. They received an irradiance of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ on a 12:12 LD photoperiod ("cool-white" fluorescent lights) at 20°C, unless stated otherwise. In one experiment a range of irradiances (10–200 $\mu E \cdot m^{-2} \cdot s^{-1}$) were also tested. When nitrate (as $Ca(NO_3)_2$) or other N compounds were supplied, P was added as NaH_2PO_4. Organic N compounds (and NH_4^+ controls) were added after autoclaving the minus-N basal medium via filter sterilization (0.2-μm pore size). All amino acids (except glycine) used were in the L form. Experimental treatments were run in triplicate. Prior to a specific N treatment, cells were grown in a NH_4^+-N based medium (Muskoka No. 112) up to late exponential phase (~7–9 d). Approximately 80–90% of the medium (minus cells) was then aseptically aspirated off and replaced with an equivalent volume of N-deficient medium. Cells remained in this regime for approximately 3–4 d prior to inoculation into experimental conditions. This "starvation" was, however, probably insufficient to completely eliminate traces of NH_4^+-N being carried over with the inoculum. Cultures were inoculated to produce an initial density of between 2×10^3 and 5×10^3 cells/mL. Cells were counted using a Bio/Physics (model 6300A) laser-based particle counter which was calibrated daily with hemacytometer counts.

Field Program

In August 1984, a bloom with odor of *C. breviturrita* (plus *C. laurentiana* Kling: identified by TEM) occurred in the south basin of Lake 302 in the Experimental Lakes Area. Details of geography and bathymetry of this lake are given in Brunskill and Schindler (1971). The lake was divided in half in 1981 using a vinyl curtain and experimental acidification of the south basin with concentrated H_2SO_4 (electrolyte grade) began in 1982. Acidification procedures were similar to that described for Lake 223 (Schindler et al. 1980). Most of the HCO_3^- in the epilimnion of Lake 302-S was destroyed during the first year, while in subsequent years further acid was added to decrease the pH by about 0.3 units/yr (Schindler 1985). In 1984, annual pH averages in both the epilimnion and the whole lake were 5.6. Average total alkalinities in these two regimes were 16 and 4.5 μEq/L, respectively (M. A. Turner, Freshwater Institute, Winnipeg, Man., pers comm.).

Nitrogen chemistry and phytoplankton abundance in Lake

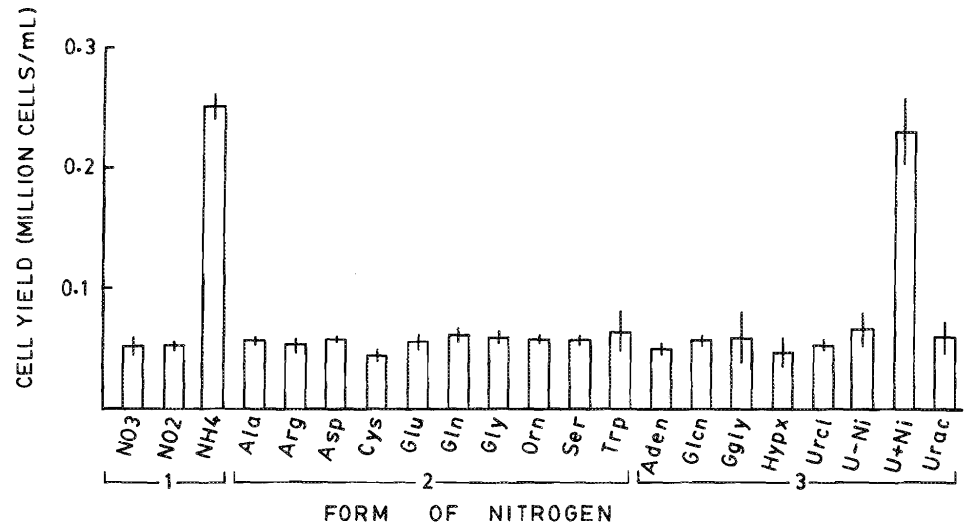

FIG. 1. Comparison of growth (expressed as cell yield) of *C.breviturrita* when supplied with 21 different N compounds as the sole N source. All N sources (group 1 = inorganic, group 2 = amino acids, group 3 = other organic forms) were supplied at a concentration of 50 μM. Results represent growth in experiments based on the first subculture only (n = 3 per treatment, error bars represent ± 1 SD). Ala = alanine, Arg = arginine, Asp = aspartic acid, Cys = cysteine, Glu = glutamate, Gln = glutamine, Gly = glycine, Orn = ornithine, Ser = serine, Trp = tryptophan, Aden = adenine, Glcn = glucosamine, Ggly = glycylglycine, Hypx = hypoxanthine, Urcl = uracil, U − Ni = urea minus Ni^{2+}, U + Ni = urea plus Ni^{2+}, Urac = uric acid.

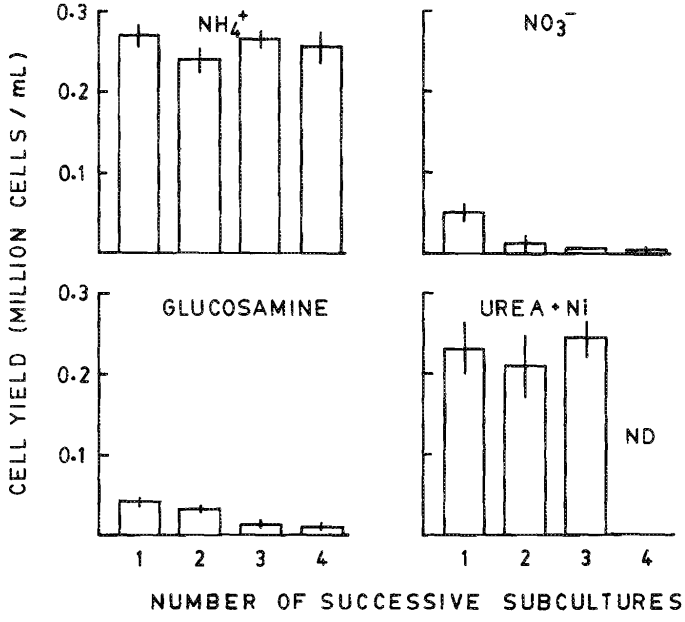

FIG. 2. Long-term growth and maintenance of *C. breviturrita* in media supplied with either NH_4^+, NO_3^-, glucosamine, or urea plus 1 μM) Ni^{2+}. N was supplied at a concentration of 50 μM. Each culture (n = 3) was sequentially transferred to an identical medium upon reaching the late exponential phase (~7 d for NH_4^+, NO_3^-, glucosamine; ~12–14 d for urea).

302-S were compared during the development of the bloom. In 1984, water was sampled at monthly intervals for chemical analysis at 1-, 4-, 6-, 7-, 8-, 9-, and 10-m depths using a peristaltic pump with polyethylene tubing at a station near the deepest part of the lake. The epilimnion was sampled weekly. Methods used for the analysis of NO_3^-, NH_4^+, and organic N follow those described in Stainton et al. (1977) and Linsey et al. (1985). Phytoplankton were also collected by an integrating sampler (Shearer 1978) where depths were divided into three strata: epilimnion, metalimnion, and hypolimnion. The depth of each stratum was based on temperature (epi-metalimnion) and light (meta-hypolimnion) profiles on a given date (J. A. Shearer, Freshwater Institute, Winnipeg, Man., pers. comm.). Samples were preserved using Lugol's iodine. Identification of *Chrysochromulina* spp. was carried out by D. L. Findlay (Freshwater Institute, Winnipeg, Man.) using light microscopy. Hence, *C. parva* was counted separately, but *C. breviturrita* and *C. laurentiana* were counted together as a single taxon. Details of routine preservation, counting, and enumeration methods as applied to Lake 223 samples (identical to 302-S procedures) are given in Findlay and Saesura (1980). Subsequent electron microscope (TEM) studies of plankton samples from Lake 302-S revealed the presence of both *C. breviturrita* and *C. laurentiana*.

Results

A comparison of *C. breviturrita* growth (expressed as final cell yield) in a NH_4^+-N medium with that observed with media containing 19 other inorganic and organic N compounds as the sole N source (Fig. 1) revealed a highly specialized N metabolism. All were supplied at 50 μM N concentrations. Only urea, when supplied with Ni^{2+} as a cofactor (1 μM), proved to support growth which was statistically equivalent to NH_4^+ controls (ANOVA, $p < 0.05$). When Ni^{2+} was not supplied, growth was not stimulated. Lower Ni^{2+} concentrations which have been tested (e.g. 0.1 μM) have resulted in intermediate yields. All other forms of N tested yielded significantly lower ($p < 0.05$) yields (approximately 20% that of controls on the first subculture into these treatments). The acidification of the

TABLE 1. Influence of irradiance (PAR: photosynthetically active radiation) and Mo concentration on cell yields of *C. breviturrita* (after 21 d) in media supplied with NO_3^--N, compared with NH_4^+-N controls. All N sources supplied at 50 μM (n = 3); ND = no data available.

Treatment	Cell yields (10^5 cells/mL) at irradiance ($\mu E \cdot m^{-2} \cdot s^{-1}$): 10	60	100	200
NO_3^- + 50 nM Mo	0.48±0.05	0.71±0.15	0.53±0.17	0.55±0.06
NO_3^- + 100 nM	ND	0.63±0.04	ND	0.65±0.01
NO_3^- + 1000 nM Mo	ND	0.64±0.08	ND	0.47±0.08
NH_4^+	0.73±0.26	2.03±0.16	2.10±0.29	1.69±0.14

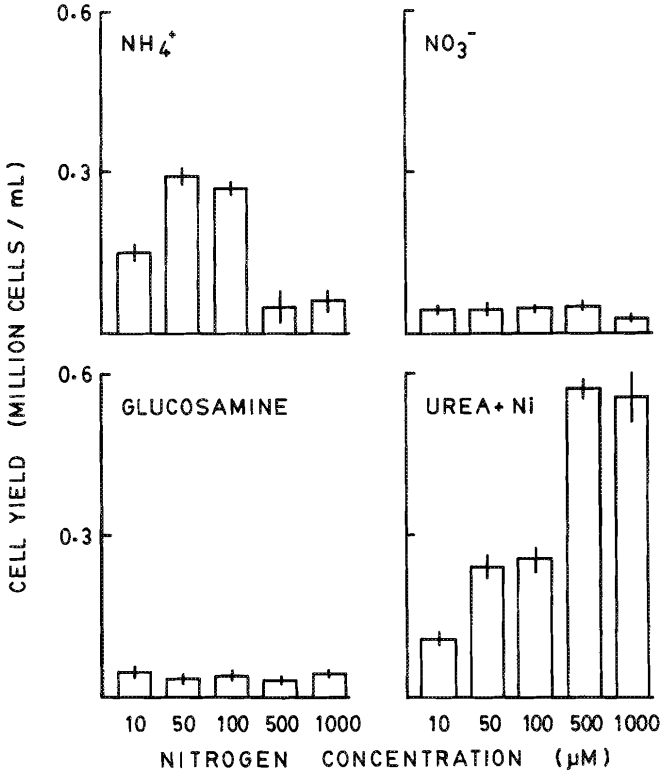

FIG. 3. Influence of N concentration on final cell yields of *C. breviturrita* with N supplied with either NH_4^+, NO_3^-, glucosamine, or urea (plus 1 μM Ni^{2+}) as the sole N source (n = 3, error bars represent ± 1 SD). Cultures were subcultured every 7 d (NH_4^+, NO_3^-, glucosamine) or 10–12 d (urea).

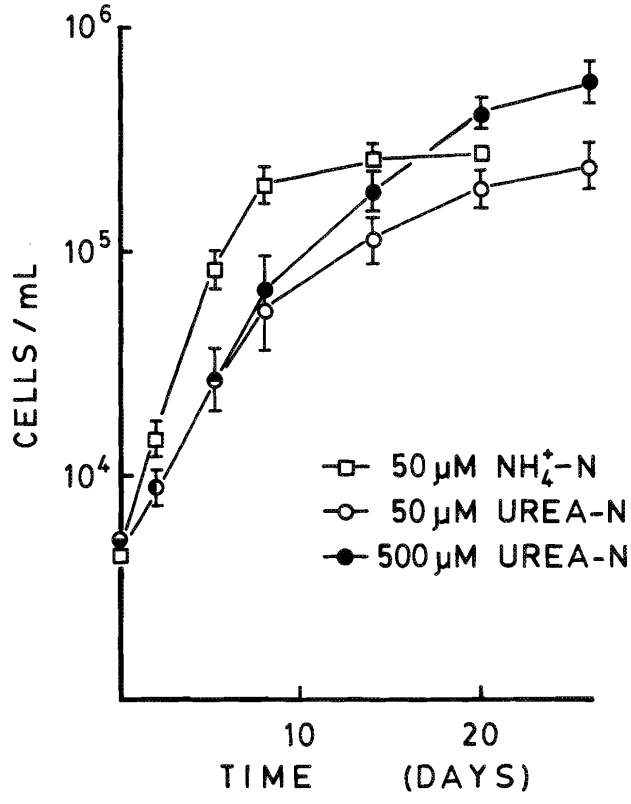

FIG. 4. Growth curves of *C.breviturrita* when supplied with 50 μM NH_4^+, 50 μM urea (plus Ni^{2+}), or 500 μM urea (plus Ni^{2+}). Each treatment was replicated three times (SD bars less than symbol size not shown).

medium was considerably less when *C. breviturrita* was grown on urea-N (pH 6.0–5.30) than on NH_4^+-N (pH 6.0–4.5). A decline in pH to 5.30 was not significantly different from that measured in a NO_3^--N based medium (to pH 5.26), where negligible growth was observed.

Long-term growth of *C. breviturrita*, when supplied with different N sources (Fig. 2), revealed that the low yields initially observed with NO_3^--N and glucosamine-N could not be maintained. After growth had reached late exponential phase, cells were transferred to fresh media containing the same N source. No evidence was found to indicate that the alga was capable of adapting over time (~ 40 d) to either NO_3^- or glucosamine utilization. Maximum yield declined to levels less than 5% of controls. In contrast, urea proved to be capable of supporting growth upon the first subculture and maintaining similar yields over successive transfers.

An attempt was made to induce NO_3^- utilization by increasing the irradiance (stimulation of nitrate reductase) and Mo concentrations (enzyme cofactor) (Table 1). No increase in cell yield was observed in the NO_3^- medium at 60–200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Lower light resulted in reduced yield, which was also observed with controls. Greater Mo concentrations also failed to induce NO_3^- utilization, even in combination with greater irradiance.

The same N compounds (NO_3^-, NH_4^+, urea, glucosamine) which were tested for long-term effects (at 50 μM) were also compared over a broad concentration range (Fig. 3), from 10 to 1000 μM. No significant increases were observed in the growth of *C. breviturrita* ($p > 0.05$) for either NO_3^- or glucosamine-N. At 1000 μM NO_3^--N, yield decreased significantly (~40%). The maximum yield in the NH_4^+-N treatments

TABLE 2. Changes in densities of *Chrysochromulina* spp. (*C. breviturrita* and *C. laurentiana*) in Lake 302-S in epilimnion ("epi") and metalimnion ("meta") strata after the addition of H_2SO_4 in 1982.

		Cell density (10^6 cells/L)							
		n		$\bar{x}$		SD		Maximum	
Treatment	Year	epi	meta	epi	meta	epi	meta	epi	meta
Pre-acid	1981	16	7	0.10	0.13	0.07	0.17	0.28	0.46
$+H_2SO_4$	1982	14	10	0.24	0.12	0.25	0.18	0.91	0.64
	1983	13	8	0.44	0.44	0.57	0.75	1.6	2.1
	1984	15	9	2.4	8.6	3.1	6.5	8.9	20.4

was observed between 50 and 100 μM, with an apparent toxicity at concentrations >500 μM. In contrast, yields more than doubled (2.47×10^5 vs. 5.73×10^5 cells/mL) in response to increased urea concentrations between 100 and 500 μM. These yields are the greatest so far achieved in culture for *C. breviturrita*. However, consideration of the entire growth curves for urea treatments (Fig. 4) clearly shows that maximum growth rates of this alga in urea-based media (e.g. 50 μM: 0.46 ± 0.067 divisions/d) are significantly less ($p < 0.05$) than in NH_4^+-N (50 μM: 0.78 ± 0.066 divisions/d). Maximum cell numbers are reached only after about 25 d, as compared with about 8–10 d with NH_4^+-N.

The development of greater populations of *C. breviturrita* (plus *C. laurentiana*) in Lake 302-S was observed following the addition of H_2SO_4 (Table 2). Maximum densities in the epilimnion and metalimnion prior to the addition of acid were 0.28×10^6 and 0.46×10^6 cells/L, respectively. Although standard deviations of annual averages frequently reached or exceeded 100% of the mean, these average densities also followed an increasing trend over this period. By 1984, populations in each stratum had increased more than 30-fold, resulting in the symptomatic bloom with "sour" or "rotten" cabbage odors in August. During the entire 1984 sampling period, concentrations of NH_4^+-N were nearly always greater than NO_3^--N, regardless of depth (G. A. Linsey, Freshwater Institute, Winnipeg, Man., unpubl. data). Densities of *Chrysochromulina* spp. during 1984 increased markedly after the spring turnover (late March – early April); epilimnion densities increased by a factor of five, while in the metalimnion, cell densities increased 20-fold. A detailed comparison of late spring and late summer populations with the N regime (Fig. 5) indicates that with this metalimnetic algal maximum there was a distinct depletion of NH_4^+-N. NO_3^--N concentrations, however, followed no obvious pattern in relation to depth or algal densities.

As a follow-up to these observations, *C. breviturrita* was isolated from a sample collected from Lake 302-S during the bloom. *Nannochloris* sp. (Chlorophyceae) was also isolated and is considered here for comparison. The *Nannochloris* sp. was confirmed to be a member of the Chlorophyceae by chlorophyll and carotenoid analyses and electron microscopy of sectioned material (unpublished data). Both axenic isolates were tested for growth in media that had either NO_3^- or NH_4^+ (50 μM) as the sole N source. Like the Cinder Lake isolate, *C. breviturrita* from L302-S was apparently unable to utilize NO_3^-. Upon first subculture the cell yield of this isolate was $0.33 (\pm 0.19) \times 10^5$ cells/mL versus $1.92 (\pm 0.10) \times 10^5$ cells/mL in a NH_4^+-based medium. Further subcultures in this medium have proven that this isolate also cannot be maintained with NO_3^--N only, substantiating earlier results (Fig. 2). In contrast, the *Nannochloris* sp. isolated from the same environment on the same date grew equally well on either form of N. The yield of this picoplankter, when using NO_3^--N, was $1.11 (\pm 0.13) \times 10^7$ cells/mL and with NH_4^+ was $1.21 (\pm 0.32) \times 10^7$ cells/mL (values not significantly different, $p < 0.05$).

Discussion

The present evidence from culture experiments (Table 1; Fig. 1–3) confirms earlier suggestions that *C. breviturrita* has an extremely specialized N metabolism. The fact that this alga may occur in massive numbers ("blooms") as the dominant alga in certain softwater lakes (Nicholls et al. 1982) is all the more remarkable and suggests that it is still a highly successful competitor. A restricted N metabolism has been demonstrated for euglenoids and other algal taxa from highly acidic environments (pH < 4.0) (Cook 1968; Rigano et al. 1975). However, none of these are as specific in their requirements as *C. breviturrita* and none are known from pelagic, planktonic communities in lakes. A few softwater phytoplankton species, such as the desmid *Arthrodesmus*, have recently been shown to utilize NO_3^-, urea, and in some cases one or more amino acids equally well (Vieira and Klaveness 1985). At present, little or nothing is known of the N requirements of other freshwater *Chrysochromulina* spp. (e.g. *C. parva*), although several marine species grow equally well on NO_3^- and NH_4^+, and can utilize at least some amino acids (Pintner and Provasoli 1968; Carpenter et al. 1972). The inability of *C. breviturrita* to grow successfully at NH_4^+ concentrations greater than 100 μM (Fig. 3) may suggest an extreme sensitivity to ammonia toxicity (i.e. NH_4^+), which would be present at less than 0.05% (25 nM), given the pH, ionic strength, and temperature regime in our experiments (Messer et al. 1984).

It is clear that NO_3^- will not serve as a N source for *C. breviturrita*. Light stimulation and Mo, factors well established to stimulate assimilation (Syrett 1981), had no effect (Table 1). Further, there was no evidence that the alga could adapt its metabolism if given sufficient time (Fig. 2). Some experimental evidence suggests that NO_3^- limitation may occur in marine phytoplankton due to unfavorable SO_4^{2-} to MoO_4^{2-} ratios (a competitive inhibition) exceeding $\sim 1 \times 10^5$ (Howarth and Cole 1985). In our experiments, control treatments had a ratio of $\sim 3 \times 10^3$ (Wehr et al. 1985), and elevated Mo concentrations (S:Mo ratios as low as 1.6×10^2) had no positive effect. The only form other than NH_4^+ that was utilized was urea and only in conjunction with Ni^{2+}. Such results suggest that the enzyme urease is present (Rees and

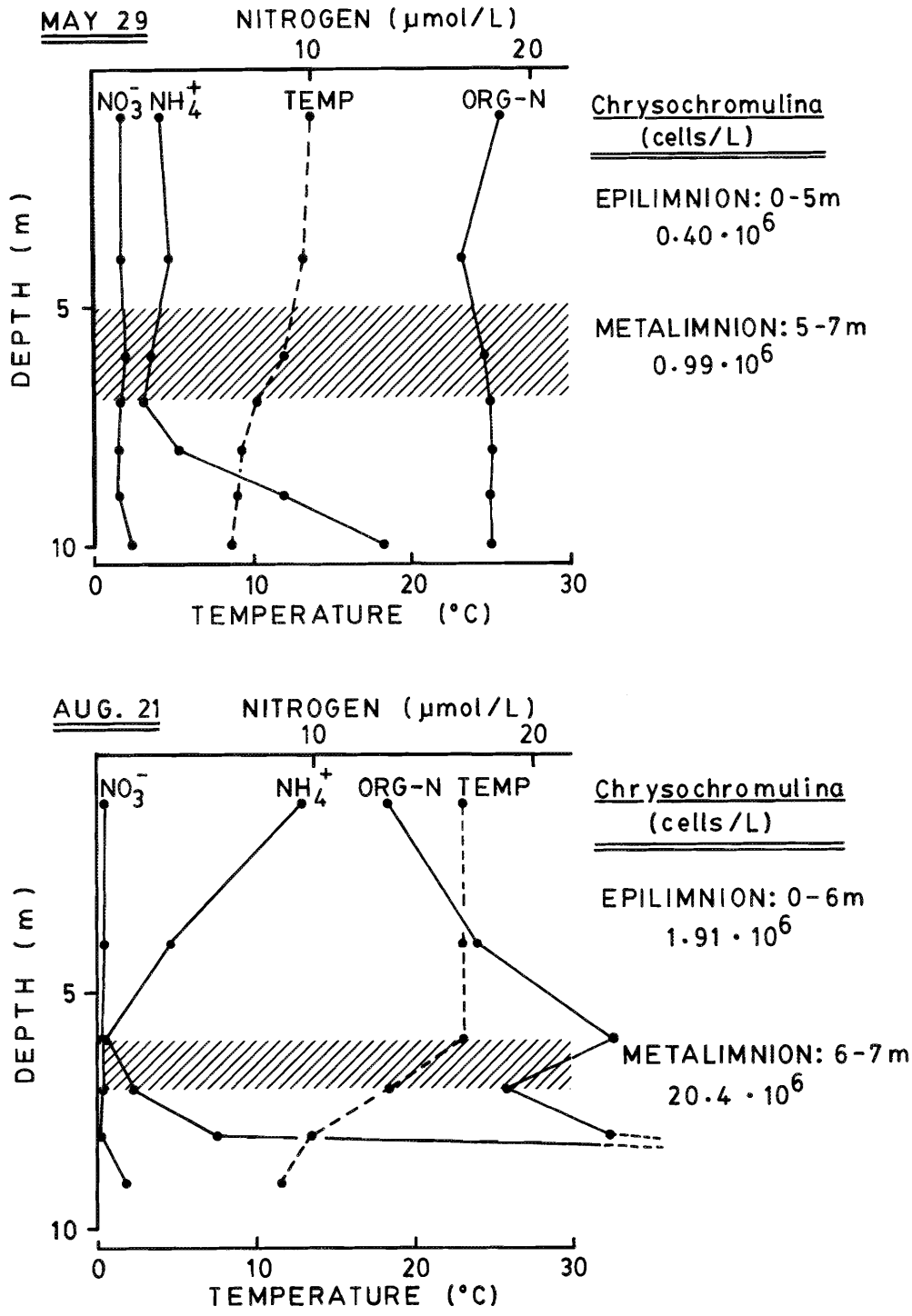

FIG. 5. Vertical profiles of filtrable NO_3^-, NH_4^+, and organic N concentrations as well as temperature in Lake 302-S during May 29 and August 21, 1984, before and during a *Chrysochromulina* spp. bloom, respectively. Algal densities indicated for two integrated strata were measured immediately prior to the dates of the water chemistries.

Bekheet 1982; Eskew et al. 1983). However, this N source, while capable of supporting a considerable biomass, is apparently much less efficiently utilized than NH_4^+, judging from the significantly lower exponential growth rates (Fig. 4). Although specific urea concentrations in Precambrian Shield lakes subject to acidic precipitation are lacking, it would seem unlikely that this form of N is of importance in the ecology of *C. breviturrita*.

The predominance of NH_4^+-N over NO_3^--N and NO_2^--N in experimentally acidified Lake 302-S may have been one factor which favored the marked increase of *C. breviturrita* in 1984. In the first 2 yr of acidification (1982–83), NO_3^- tended to be the predominant inorganic N species in the euphotic zone (~0–7 m) during spring and summer (Linsey et al. 1985). By 1984, when *C. breviturrita* increased markedly, the N regime changed and NH_4^+ dominated throughout most of the year,

including May through August (see also Fig. 5). By late August 1984, a dense metalimnetic maximum of cells coincided with the depletion of NH_4^+ between 6 and 7 m in depth.

Considering the ability of *C. breviturrita* to develop (often abrupt) blooms (Nicholls et al. 1982), it may perhaps be expected that selective NH_4^+ utilization in a specific localized stratum (e.g. metalimnetic peak) could lead to short-term H^+ generation and further acidification. Broad surveys of water chemistry data (Linsey et al. 1985; G. A. Linsey, pers. comm.), however, indicate only slight pH differences with depth during periods of maximum cell numbers. It remains to be seen, however, possibly by the use of in situ experiments, whether such organism-mediated changes may occur.

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