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Effects of irradiance on nitrogen uptake by phytoplankton: comparison of frontal and stratified communities

W. P. Cochlan[1,2,*], N. M. Price[1,3], P. J. Harrison[1]

[1] Department of Oceanography, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5
[2] Marine Biology Research Division 0202, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA
[3] R. M. Parsons Laboratory, Department of Civil Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

ABSTRACT: Rates of NO_3^- and urea uptake by phytoplankton in the shallow and deep chlorophyll layers of the Strait of Georgia, British Columbia (Canada) were measured, over a gradient of photosynthetic photon flux densities (PPFD), using the ^{15}N tracer technique. The results of these experiments could be fitted with a hyperbolic function similar to the Michaelis-Menten equation and included a term for dark uptake. Half-saturation constants (K_{LT}) for light-dependent uptake of urea and NO_3^- ranged from 0 to 14 % of the surface PPFD, and dark uptake of both urea and NO_3^- ranged from 0 to 58 % of the uptake at saturating PPFD. Although the importance of dark uptake increased with increased N limitation, the dramatic difference in phytoplankton community composition between the N-replete frontal waters and the N-depleted stratified waters precludes a simple explanation of PPFD effect(s) on N uptake based solely on phytoplankton N status. The results of this study are compared to those reported for other aquatic systems.

INTRODUCTION

In most marine and freshwater systems, the uptake of nitrogenous nutrients by phytoplankton is related to the availability of the nutrients (e.g. MacIsaac & Dugdale 1969, Probyn 1985) and to photosynthetic photon flux density (PPFD) (e.g. MacIsaac & Dugdale 1972, Priscu 1984). The dependence of nitrogen uptake upon PPFD has been described by a rectangular hyperbola similar to the Michaelis-Menten formulation in many marine (e.g. MacIsaac & Dugdale 1972, Slawyk et al. 1976) and freshwater (e.g. Priscu 1984, Whalen & Alexander 1984) communities. Although nitrogen uptake and assimilation by phytoplankton are dependent upon PPFD as an energy source, either directly or indirectly through photosynthesis, the exact biochemical mechanism(s) by which light regulates nitrogen metabolism remains unresolved (e.g. see review by Syrett 1981). The presence of NO_3^--activated ATPase, apparently located within the cell membranes of a number of marine phytoplankters (Falkowski 1975a,b), provides a physiological basis for the coupling between light and NO_3^- uptake, and specific ATPases probably exist for the uptake of NH_4^+ and urea as well. The energy (ATP) generated by photophosphorylation is ultimately required for the functioning of these uptake enzymes (permeases) and may also drive the reactions of NH_4^+ (GS/GOGAT) and urea (UAL-ase) assimilation. In addition, the photogeneration of reductants NAD(P)H and reduced ferredoxin will drive the reduction of NO_3^-, NO_2^- and the GOGAT reaction of NH4+ assimilation. Other possible interactions of light with inorganic nitrogen metabolism of phytoplankton are discussed in detail by Syrett (1981).

Numerous culture studies have demonstrated that N-deprived phytoplankton have greater dark uptake rates of N than do N-replete phytoplankton (e.g. Syrett 1962, Eppley & Coatsworth 1968, Thacker & Syrett

* Addressee for correspondence: W. P. Cochlan in La Jolla, California

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1972, Rees & Syrett 1979), suggesting a lesser light dependence on N uptake during N stress. This, together with field studies which show that deep-living phytoplankton sustain substantial N uptake velocities with little or no light (e.g. Conway & Whitledge 1979, Nelson & Conway 1979, Priscu 1984), suggests that both light exposure and nutritional history of phytoplankton may be important in determining their ability to sequester nitrogen, and that these controlling factors may differ for the various forms of nitrogen.

Shallow sea fronts, located at the boundary between stratified and vertically mixed regimes (see reviews by Denman & Powell 1984, LeFèvre 1986), are generally areas of high primary productivity (e.g. Pingree et al. 1975, Parsons et al. 1981, 1983, Holligan et al. 1984). These regions are characterized by having surface water with high phytoplankton biomass and measurable concentrations of nitrate, and a shallow pycnocline which extends to the surface at the frontal boundary (e.g. Simpson & Pingree 1978).

A surface transect normal to a frontal boundary progresses from high concentrations of dissolved NO_3^- on the well-mixed side to N-depleted, stratified waters, and thus represents a gradient of both nitrogen and light availability and consequently of phytoplankton physiological states. Moreover, the nitrogenous nutrition of the phytoplankton would likely differ along such a transect. In the N-impoverished waters, the N demands of phytoplankton are supplied by reduced N forms such as NH_4^+ and urea from regenerative processes, whereas in N-rich areas, nitrogen compounds are generally utilized at rates proportional to their availability (e.g. Dugdale & Goering 1967, McCarthy et al. 1977).

The experiments presented in this study were conducted in the Strait of Georgia, a partially enclosed coastal basin on the west coast of Canada (see reviews by Harrison et al. 1983, LeBlond 1983), where several tidally induced frontal regions have been previously described (Parsons et al. 1981, Price et al. 1985). The influence of PPFD on the uptake of NO_3^- and urea by phytoplankton from nitrate-replete frontal water and nitrate-depleted stratified water was examined, and the dependence of N uptake on PPFD by the phytoplankton from the subsurface chlorophyll maximum of these 2 distinct areas was compared. Simulated in situ experimental conditions were attempted in order to obtain a better understanding of the true NO_3^- uptake response to PPFD in these physically and chemically distinct environments. Most previous studies of the effect(s) of PPFD on N uptake by phytoplankton have employed saturating enrichments of isotopically labelled N forms (e.g. MacIsaac & Dugdale 1972, Priscu 1984, Mitamura 1986), and reported uptake rates may reflect the effects of both PPFD and N concentration.

MATERIALS AND METHODS

General. Nitrogen uptake experiments were conducted in the Strait of Georgia, B.C., Canada, aboard the CSS 'Vector' during July and August 1984; station locations and names are shown in Fig. 1. Between 14:00 and 15:00 h PDT (Pacific Daylight Time), water samples were collected using 5 l PVC Niskin bottles, from just below the sea surface (0 to 1 m) and from depths corresponding to the deep chlorophyll maximum (DCM). The shallow sampling depth represents a light environment of ca 80 to 100 % surface PPFD (I_o) for each station, whereas the DCM depth corresponds to ca 8, 3 and 2 % I_o for Stations T14, A5 and T8, respectively. Samples were shielded from direct sunlight during transfer to 10 l Nalgene® carboys and taken into the ship's laboratory. Subsamples for nutrient analyses were removed with an acid-washed syringe and gently filtered through combusted (460 °C for 4 h) Whatman GF/F filters (mounted in 25 mm Millipore Swinex® filter holders) into acid-washed polyethylene bottles. Nitrate plus nitrite ($NO_3^- + NO_2^-$) and ammonium (NH_4^+) were measured immediately with a Technicon AutoAnalyzer® II, following the procedures outlined in Wood et al. (1967) and Slawyk & MacIsaac (1972), respectively. Urea was determined by the diacetyl monoxime thiosemicarbizide technique described by Price & Harrison (1987). Samples for chlorophyll *a* (chl *a*) [coefficient of variation (CV) = 4.4 ± 4.1 %; 5 sample pairs] were collected on Whatman GF/F filters and stored frozen in a desiccator. Chl *a* was extracted in 90% acetone overnight and analyzed by in vitro fluorometry (Strickland & Parsons 1972) using a Turner Designs Model 10 fluorometer. Particulate organic carbon (POC) and nitrogen (PON) (CV = 5.2 ± 4.8 % and 3.8 ± 4.1 % respectively; 7 sample pairs), collected on combusted Whatman GF/F filters, were stored similarly and analyzed later after drying (24 h at <60 °C) with a Perkin Elmer Model 240 elemental analyzer, using the dry combustion method described by Sharp (1974).

At each station continuous vertical profiles (0 to 20 m) of temperature, salinity, fluorescence and $NO_3^- + NO_2^-$ were run prior to the bottle casts. Temperature and salinity were determined with an InterOcean 514A CSTD system; in vivo fluorescence and $NO_3^- + NO_2^-$ concentrations were obtained from pumped samples (mRoy FR162-144 diaphragm pump, flow rate ca 1 l min^{-1}) and measured with a Turner Model 111 fluorometer (equipped with a flow-through cell) and a Technicon AutoAnalyzer® II, respectively. These data were logged onto a computer and plotted in real-time using a custom software programme which compensates for time lags in pumping and machine analyses (Jones et al. in press). Incident solar irradiance (PAR,

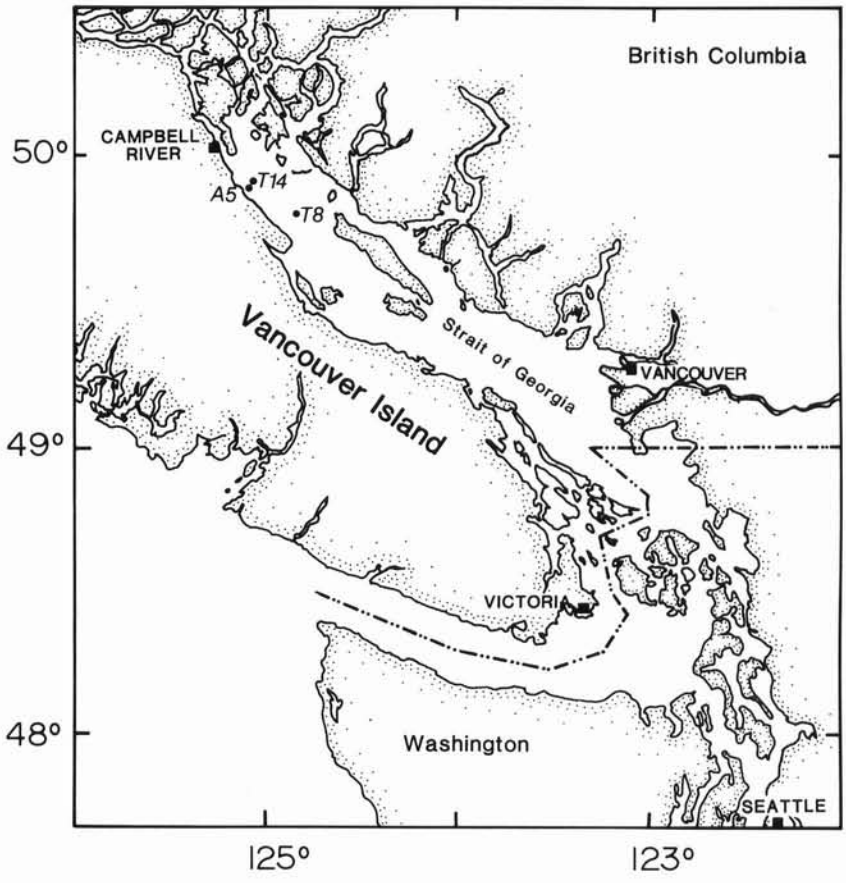

Fig. 1. Station locations for nitrogen uptake experiments. Frontal (T14), shallow stratified (A5) and deeply stratified (T8) stations in the Strait of Georgia, British Columbia

400 to 700 nm) was monitored continuously with a Lambda Instruments LI-185 light meter that was equipped with a LI-190SB Surface Quantum Sensor and connected to a chart recorder. Subsurface irradiances were measured with a LI-185B light meter equipped with a LI-192S Underwater Quantum Sensor (2π).

Phytoplankton samples (250 ml) were preserved in acid Lugol's solution (Parsons et al. 1984) and stored in the dark until counting. Subsamples (10 ml) were settled (24 h) and counted on a Wild inverted microscope following Utermöhl (1958).

Experimental. Within 1 h of collection, water samples from each depth were transferred under reduced-light conditions to 500 ml Wheaton glass bottles with teflon-lined caps. Nitrate and urea uptake rates were measured using the stable isotope ^{15}N (Kor Isotopes) as a tracer (Dugdale & Goering 1967). For the urea experiments, $CO(^{15}NH_2)_2$ (99 atom %) was added to bring the final ^{15}N concentration to either 2 or 4 µg-at. $N\,l^{-1}$. In the nitrate experiments, $Na^{15}NO_3$ (99 atom %) was added in concentrations of either 0.05 µg-at. $N\,l^{-1}$ or <10 % of the ambient $NO_3^- + NO_2^-$ concentration. These enrichments were not always true tracer additions (usually defined as ≤ 10 % of ambient), but the term 'tracer' will be used here to distinguish the low $^{15}NO_3^-$ enrichments from the saturating enrichments associated with the urea uptake experiments. Following enrichment, bottles were immediately mixed and placed within neutral-density screening to simulate the following PPFDs: 95, 55, 31, 10, 3.4, 1.1 and 0 % I_o. The screen material used in the incubators was calibrated with a Biospherical Instruments QSL-100 4π sensor placed within an incubation bottle. The 0 % PPFD was achieved by multiple wrappings of the bottle with black tape. Incubations were conducted at in situ temperatures ($\pm$ 1.5 °C) under natural light in clear, Plexiglas® deck incubators. Clear, cloudless skies prevailed throughout the cruise, resulting in virtually identical ambient light conditions during each experiment. Samples from the surface waters were cooled with flowing surface seawater, while deeper samples were incubated in a separate, temperature-controlled incubator. Incubations were terminated after 2 to 4 h by filtration (pressure differential <125 mm Hg) onto combusted Whatman GF/F filters, placed into plastic petri dishes, and stored frozen in a desiccator. Based on the ambient dissolved nitrogen concentration, the initial particulate nitrogen concentration, and the final ^{15}N atom percentage in the particulate fraction, it was calculated that a mean ($\pm$ SD) of 24.1 $\pm$ 15.3 % and 8.5 $\pm$ 5.2 % of the NO_3^- and urea, respectively, in solution was incorporated into particulate material during

the incubation period. Substrate exhaustion was therefore not considered a problem in the experiments of this study.

Nitrogen in the particulate samples was converted to dinitrogen gas (N_2) by the micro-Dumas dry combustion technique (La Roche 1983) and then analyzed for ^{15}N enrichment with a JASCO Model N-150 emission spectrometer (Fiedler & Proksch 1975). Generally, each sample was scanned 6 times (minimum of 3 times), and the average $^{15}N/^{14}N$ peak height ratio was used in the calculation of the atom percent ^{15}N (specific activity) in the particulate material. Automatic selection of peak heights during scans, and isotopic ratio calculations, were performed utilizing in-house software (Jones unpubl.) on an IBM-compatible PC interfaced with the spectrometer. The emission spectrometer was routinely calibrated with a series of pure N_2 gas standards supplied by JASCO of known ^{15}N enrichment.

Nitrogen uptake rates were calculated using Equation 7 of Dugdale & Wilkerson (1986) (equivalent to Equation 5 of Collos 1987), which corrects for changes in PON during the incubation period. Corrections were not made for isotopic dilution from remineralization of ^{14}N-urea during the incubation (Hansell & Goering 1989), as this correction would probably be negligible given the large amount of ^{15}N-labelled urea added to the bottles. Specific rates of nitrogen transport were calculated by dividing the volumetric rates by the phaeophytin-corrected chl *a* concentration at the beginning of the experiments. Although chl *a* per cell may vary with depth due to PPFD differences, it was chosen as the normalization parameter because it absorbs the light necessary to fuel cellular transport mechanisms. Use of chl *a* specific uptake rates also facilitates comparison with previously published studies on chl *a* normalized nitrogen and carbon uptake vs irradiance.

Kinetic parameters of uptake. The kinetic constants for NO_3^- and urea uptake with respect to irradiance were obtained by a direct fit of the data to a modified Michaelis-Menten hyperbola using a computerized, iterative, non-linear least-squares technique (Labtec Notebook Curvefit®, Laboratories Technologies Corp.). The Michaelis-Menten equation, modified to account for dark uptake, describes uptake over the hyperbolic part of the curve (MacIsaac & Dugdale 1972) and is as follows:

$$V = V_D + V'_{max}\left[\frac{I}{K_{LT} + I}\right] \quad (1)$$

where V = total uptake of N per unit of chlorophyll; V_D = dark value of V; I = integrated average PPFD during the incubation period; V'_{max} = maximum N uptake per unit chlorophyll at saturating PPFD; and K_{LT} (the half-saturation constant for light) = PPFD at 0.5 V'_{max}. The assumption is made that dark uptake is a constant at all light levels. Only data obtained from non-photoinhibitory PPFDs were used in this analysis.

RESULTS AND DISCUSSION

General description of stations

The vertical profiles of temperature, salinity, relative in vivo chl *a* fluorescence and $NO_3^- + NO_2^-$ concentration, for the 3 stations at which N uptake vs PPFD experiments were conducted, are presented in Fig. 2. The diagnostic features of the frontal water (Station T14) included a weak thermocline and halocline which extended from the surface to ca 9 m, a subsurface fluorescence maximum layer (ca 5 to 8 m), a nitracline which extended to the surface, and relatively high $NO_3^- + NO_2^-$ concentrations throughout the water column.

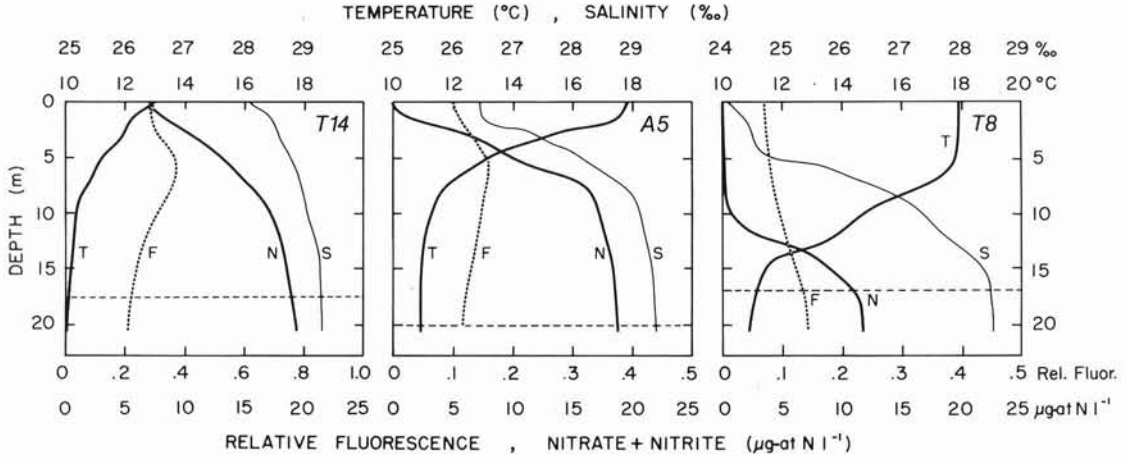

Fig. 2. Depth profiles of temperature (T), salinity (S), in vivo fluorescence (F) and nitrate plus nitrite concentration (N) for the 3 stations sampled (T14: frontal; A5: shallow stratified; T8: deeply stratified). The horizontal dashed line denotes the depth of 1% surface PPFD (photosynthetic photon flux density)

Table 1. Initial environmental conditions of seawater collected for N-uptake vs irradiance experiments. PDT: Pacific Daylight Time; Chl *a*: chlorophyll *a*; PON: particulate organic nitrogen; POC: particulate organic carbon; (–): not determined

Station and location		Description	Date (1984)	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. (NO_3^-)	Urea (μg-at. $N\,l^{-1}$)	NH_4^{+a}	Chl *a* ($\mu g\,l^{-1}$)	PON (μg-at. $N\,l^{-1}$)	POC (μg-at. $C\,l^{-1}$)
T14	49°53′24″N 125°05′06″W	Frontal	27 Jul	15:30 h	0	6.02	–	0.23	1.29	5.28	43.1
					8	15.05	–	0.21	2.28	6.96	40.6
A5	49°53′02″N 125°05′48″W	Shallow stratified	30 Jul	14:30 h	0	<.05	0.63	0.16	0.33	2.57	22.9
					15	20.89	0.72	0.32	0.67	2.01	14.5
T8	49°48′36″N 124°50′39″W	Deep stratified	1 Aug	15:00 h	0	<.05	0.82	0.17	0.35	2.90	24.1
					15	7.54	0.17	0.40	0.99	3.83	24.1

[a] NH_4^+ concentrations from separate bottle casts

In the deeply stratified station (T8), fluorescence increased slightly with depth; the nitracline occurred at ca 12 m, and the upper 10 m was devoid of measurable $NO_3^- + NO_2^-$. A strong thermocline and halocline at 5 to 15 m separated the deep NO_3^--replete water from the NO_3^--depleted mixed surface water. Similar conditions were observed at the shallow stratified station (A5), but the halocline, thermocline, and nitracline all developed within the upper 5 m of the water column. The initial biomass data and environmental conditions for each station are given in Table 1.

The species composition of the phytoplankton community in the frontal and stratified waters varied considerably (Table 2). In the frontal waters, large, chain-forming diatoms were the most common phytoplankton at both the surface and the DCM layer. *Chaetoceros socialis* was the dominant species, followed in abundance by *Skeletonema costatum* and other diatoms of the genus *Chaetoceros*, including *C. debilis*. Small pigmented flagellates (<5 μm) were the most abundant phytoplankton in the surface waters of both stratified stations (A5 and T8); dominant diatoms were still *S. costatum* and *Chaetoceros* spp., although *Thalassiosira* spp. and pennate diatoms belonging to the genera *Navicula* and *Nitzschia* appeared in small numbers.

Table 2. Phytoplankton community composition in frontal and stratified water in the Strait of Georgia, B.C., Canada. Phytoplankton density given × 10^6 cells l^{-1}

Station	Depth (m)	Phytoplankton Diatoms	Flagellates[a]
Frontal T14	0	2.3	0.96
	8	2.2	0.76
Shallow stratified A5	0	0.23	1.9
	15	0.73	0.79
Deeply stratified T8	0	0.026	1.5
	15	0.15	1.7

[a] <5 % of flagellates were dinoflagellates

Dinoflagellates were always a small numerical fraction (<5 %) of the total flagellates present and were almost exclusively *Gymnodinium* or *Amphidinium* spp. The DCM communities of the 2 stratified stations differed in the relative abundance of flagellates and diatoms, but the species composition was similar.

Effect of irradiance on nitrogen uptake rates

MacIsaac & Dugdale (1972) first showed that the uptake of nitrate and ammonium by natural phytoplankton assemblages could be related to PPFD by a rectangular hyperbola; PPFD may be treated as a substrate, following Michaelis-Menten kinetics, under conditions of no nutrient stress. Such a model assumes that there is no N uptake at 0 PPFD (i.e. the PPFD response curve passes through the origin). They suggested that the consequences of not subtracting dark uptake from light uptake, when uptake in the dark is greater than ca 15 % of uptake at saturating PPFD, can be significant; linear transformations of such kinetic data are distorted beyond usefulness and thus the values of derived parameters are questionable. For situations in which dark uptake is a substantial portion (>10 to 15 % of PPFD – saturated uptake), they proposed a slightly modified equation, employed in the present study, which takes into account a constant dark uptake rate and describes N uptake over the hyperbolic portion of the PPFD response curve, but not photoinhibition. Photoinhibition problems can be overcome by using either an equation developed by Parker (1974) or a modification of the equation of Platt et al. (1980), originally developed for the light response of photosynthesis (Lewis & Levine 1984, Dodds & Priscu 1989, Priscu 1989). Numerous studies in both marine (MacIsaac & Dugdale 1972, MacIsaac et al. 1974, Nelson & Conway 1979, Slawyk 1979, Kanda et al. 1989) and freshwater (Priscu 1984, Whalen & Alexander 1984, Mitamura 1986) natural communities have

demonstrated that the response in uptake of NO_3^- and NH_4^+ can be described by the Michaelis-Menten formulation.

In the present study, nitrate and urea uptake were dependent on PPFD at both depths sampled in stratified and frontal waters of the Strait of Georgia. Experiments in which the natural phytoplankton communities from the surface and DCM layers were exposed to a gradient in PPFD yielded data which could be adequately described by the Michaelis-Menten formulation, up to inhibiting PPFD levels (Figs. 3 and 4). Photoinhibition occurred between 55 and 95 % of I_o and was only observed for samples collected from the DCM layers. Photoinhibition of N uptake cannot be adequately discussed in this study due to the paucity of data at high PPFD, but suffice it to say that it is probably not a problem for the surface samples, which are naturally exposed to high PPFD; phytoplankton collected near the bottom of the euphotic zone are effectively excluded from the high PPFD in the mixed surface waters by the pycnocline and are not likely to encounter such high PPFDs naturally.

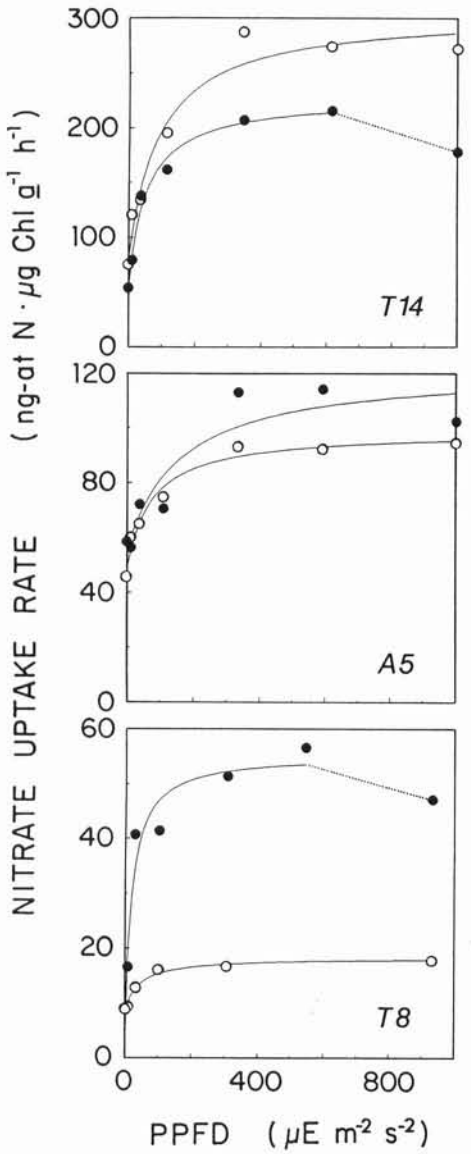

Fig. 3. Nitrate uptake of the surface (○) and DCM (●) phytoplankton communities of the Strait of Georgia, British Columbia. The curved plots are fitted directly to the Michaelis-Menten equation; the linear (dotted line) PPFD-inhibited portions were not included in the calculations. Stations are T14: frontal; A5: shallow stratified; and T8: deeply stratified

Kinetic parameters of nitrogen uptake

Dark uptake, the half-saturation constant (K_{LT}), and maximum nitrogen uptake velocity (V'_{max}) for light-dependent urea and nitrate uptake are summarized in Table 3. The K_{LT} values in Table 3 are those representing the PPFD at which 0.5 V'_{max} occurs. However, it is important to remember that these Michaelis-Menten parameters only represent uptake data from the hyperbolic (or light) portion of the PPFD response curve and do not include the substantial dark N uptake observed. Some investigators (e.g. Priscu 1984) have ignored dark uptake in the linear transformation of their kinetic data and forced their PPFD response curves to pass through the origin even though dark uptake was substantial (ca 50 % of total N uptake). Half-saturation constants derived in this manner are not an accurate

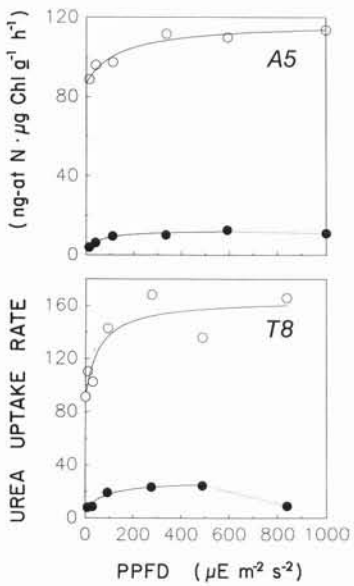

Fig. 4. Urea uptake of surface (○) and DCM (●) phytoplankton communities of the Strait of Georgia. The curved plots are fitted directly to the Michaelis-Menten equation; the linear (dotted line) PPFD-inhibited portions were not included in the calculations. A5: shallow stratified station; T8: deeply stratified station

Table 3. Parameters describing the characteristics of nitrogen uptake, as a function of PPFD (photosynthetic photon flux density), for phytoplankton assemblages in the Strait of Georgia, B.C., Canada. Stations are T14: frontal; A5: shallow stratified; and T8: deeply stratified. Definitions of V_D, V'_{max} and K_{LT} are given in the 'Materials and Methods'; estimated standard errors of parameters are shown in parentheses

Station	Nitrogen substrate	Depth (m)	V_D [ng-at. N (μg chl *a*)$^{-1}$ h^{-1}]		V'_{max} [ng-at. N (μg chl *a*)$^{-1}$ h^{-1}]		K_{LT} μE m^{-2} s^{-1}		K_{LT} % I_o	
T14	NO_3^-	0	80	(16.8)	225	(22.3)	91	(40.1)	8.2	(3.6)
		8	53	(9.4)	174	(12.5)	53	(15.7)	4.8	(1.4)
A5	NO_3^-	0	48	(3.2)	50	(4.0)	74	(25.9)	6.7	(2.3)
		15	55	(8.8)	67	(15.3)	156	(151)	14	(14)
	Urea	0	87	(4.2)	31	(4.3)	140	(103)	13	(9.3)
		15	1.8	(2.3)	11	(1.9)	54	(40.7)	4.9	(3.7)
T8	NO_3^-	0	8.5	(0.79)	9.8	(1.02)	45	(18.7)	4.6	(1.9)
		15	0		55	(4.2)	18	(6.6)	1.8	(0.7)
	Urea	0	92	(14.1)	73	(17.6)	59	(63.4)	6.7	(7.2)
		15	4.0	(3.9)	24	(3.6)	72	(56.2)	8.2	(6.4)

measure of the PPFD at which $V = V'_{max}/2$ and should be interpreted with caution, particularly as an indicator of the phytoplankton communities' abilities to assimilate specific N substrates at low PPFD. A better estimate of the PPFD at which one-half the total maximal N uptake (light + dark) of the phytoplankton community is achieved (K_{LT}') can be calculated by a simple rearrangement of the Michaelis-Menten equation employed in the present study:

$$K_{LT}' = (V - V_D) \times K_{LT}/(V'_{max} - V + V_D) \quad (2)$$

where $V = (V'_{max} + V_D)/2$; V'_{max} = maximum uptake described by the rectangular hyperbola; K_{LT} = its half-saturation constant; V_D = dark uptake rate. Alternatively, another half-saturation constant, K_{LT}'', can be calculated by substituting V for one-half the velocity of total N uptake (light + dark) at saturating PPFD, according to Equation 1. Both of these derived half-saturation constants will generate values that are more realistic measures of the PPFD at one-half the actual maximum N uptake taking place in the phytoplankton community, since they include dark uptake (Table 4).

The values of the half-saturation constant for NO_3^- uptake in the present study, with or without the correction for dark uptake, range from 0 to 14 % I_o (0 to 156 μE m^{-2} s^{-1}), which is consistent with previously published values for marine and freshwater natural phytoplankton assemblages (Table 5). The half-saturation values for urea uptake are similar, ranging from 0 to 13 % I_o (0 to 140 μE m^{-2} s^{-1}). Previously published kinetic studies for urea are few. Webb & Haas (1976) report a K_{LT} of ca 0.01 langley min^{-1} (35 μE m^{-2} s^{-1}) for phytoplankton from the York River estuary in Virginia, USA, during the summer, although values in the autumn

Table 4. Indices of N uptake dependency on PPFD (photosynthetic photon flux density) for phytoplankton in the Strait of Georgia: ratio of dark to light-saturated total uptake rate (V_D:V_L); the PPFD at which half of total N uptake occurs (K_{LT}', K_{LT}'' – see definitions in 'Results and Discussion: Kinetic parameters of nitrogen uptake'); and ratio of uptake under 1 % I_o (surface PPFD) to that under 55 % I_o ($V_{1\%}$:$V_{55\%}$). The K_{LT} values are expressed as PPFD values (μE m^{-2} s^{-1}) and as a percentage of I_o (shown in parentheses)

Station	Nitrogen substrate	Depth (m)	V_D:V_L	K_{LT}'		K_{LT}''		$V_{1\%}$:$V_{55\%}$
T14	NO_3^-	0	0.28	36	(3.2)	43	(3.9)	0.38
		8	0.25	24	(2.1)	28	(2.5)	0.40
A5	NO_3^-	0	0.51	0	–	1.6	(0.1)	0.60
		15	0.49	3.0	(0.3)	15	(1.3)	0.56
	Urea	0	0.77	0	–	0	–	0.80
		15	0.15	33	(3.0)	39	(3.5)	0.33
T8	NO_3^-	0	0.48	1.9	(0.2)	3.1	(0.3)	0.60
		15	0	0	–	0	–	0.37
	Urea	0	0.58	0	–	0	–	0.65
		15	0.16	39	(4.5)	52	(5.8)	0.28

ranged from 0.02 to 0.12 langley min^{-1} (69 to 418 μE m^{-2} s^{-1}). A similar summer K_{LT} value was reported by Mitamura (1986) for urea uptake by phytoplankton from oligotrophic Lake Biwa in Japan (2.44 Kilolux = 39 μE m^{-2} s^{-1}). Mitamura also reported a similar K_{LT} value for NH_4^+ uptake (28 μE m^{-2} s^{-1}) and a greater K_{LT} value for nitrate uptake (67 μE m^{-2} s^{-1}). Interpretation of the small differences in the half-saturation constants of the present study, either between N substrates or between the communities taken from different depths, is rather difficult. However, this kinetic parameter has been included for purposes of literature comparison. (Previously published values were converted to μE m^{-2} s^{-1}, according to the conversions in the footnotes to Table 5. The reader is cautioned that conversions between units of illumination and energy to quanta are complicated and, due to the varying nature of these measurements, imprecise.)

A simpler and more straightforward index for assessing the effect of PPFD on N uptake can be determined by comparing total N uptake, calculated according to Equation 1, at low (1 % I_o) and at saturating (55 % I_o) PPFD (Conway & Whitledge 1979); lower percentages represent greater PPFD dependency. At the frontal station (T14) both surface and DCM communities had the same PPFD dependency for NO_3^- uptake (38 to 40 %), which probably reflects the similarity in both species composition and physiological state of these 2 N-replete communities. At the shallow stratified station (A5), the 2 phytoplankton communities were very similar with respect to NO_3^- uptake response (60 and 56 %), but there was a substantial difference between the surface and DCM urea uptake dependency (80 and 33 %, respectively). Similar large differences were found for both NO_3^- (60 and 37 %) and urea (65 and 28 %) uptake response in the two communities of the deeply stratified station (T8). It appears that uptake of the regenerated N source, urea, has a greater dependency on PPFD in the NO_3^--replete DCM community, which was effectively isolated from the well-lit surface layers by the strong pycnocline present and normally received only ca 1 to 3 % I_o. The lesser PPFD dependency of the surface phytoplankton may be a consequence of their N-depleted physiological state, which could explain the decrease in PPFD dependency of NO_3^- uptake in the surface populations of the stratified waters (60 %) relative to those of the frontal waters (38 %). Alternatively, their decreased PPFD depen-

Table 5. Comparison of half-saturation constants (K_{LT}) for inorganic nitrate transport in various aquatic ecosystems. I_o: surface photosynthetic photon flux density

Region	Area	Depth (% I_o)	K_{LT} (NO_3^-) % Surface light range (mean)	K_{LT} (NO_3^-) μE m^{-2} s^{-1} range (mean)	Source
Oceanic	E Tropical Pacific	25	14.0	–	MacIsaac & Dugdale (1972)
Upwelling	Peru	100	0.9–12.7 (5.4)	14–108 (63)[a]	MacIsaac & Dugdale (1972)
		10	0.9–13.3 (8.9)	7–199 (122)[a]	
Upwelling	Peru	50	–	<170–630 (ca 460)[a]	MacIsaac et al. (1985)
		1	–	140	
Upwelling	NW Africa	50	1.5– 7.0 (5.4)	–	MacIsaac et al. (1974)
Upwelling	NW Africa	50–0.1	5.5– 6.2 (5.9)	–	Nelson & Conway (1979)
Upwelling	Baja Calif., Mexico	50–3	3.3–32.4 (16.1)	–	Nelson & Conway (1979)
Upwelling	Antarctic Sea	50–25	1.1– 2.3 (1.7)	2.3–4.4 (3.3)[b]	Slawyk (1979)
		7	1.3	2.8[b]	
Coastal	Peru	100	4.4	45	MacIsaac & Dugdale (1972)
		10	1.0	14	
Coastal	Delaware Estuary, USA	100–50	–	60–569 (258)	Pennock (1987)
Coastal	Strait of Georgia	100–80	4.6– 8.2 (6.5)	45– 91 (70)	Present study
		ca 8–2	1.8–14 (6.9)	18–156 (76)	
Coastal	Auke Bay, Alaska, USA	–	–	23.9–261 (92.4)	Kanda et al. (1989)
Freshwater	Toolik L., Alaska, USA	ca 15–10	6 –31 (15)	7–29 (16)	Whalen & Alexander (1984)
Freshwater	L. Kinneret, Israel	–	–	77	Berman et al. (1984)
Freshwater	L. Biwa, Japan	100	4.29	70.8[c]	Mitamura (1986)
Freshwater	Castle L., Calif., USA	ca 50	2.6– 2.7 (2.65)	15.1–16.2 (15.7)[d]	Priscu (1984)
		ca 1	0.6– 3.7 (1.55)	4.6–25.5 (10.7)[d]	
Freshwater	L. Fryxell, Antarctica	0.4	0.08	0.38[e]	Priscu (1989)

[a] Values calculated by converting from langleys min^{-1} using 1 langley min^{-1} = 3485 μE m^{-2} s^{-1} (Richardson et al. 1983)
[b] Values calculated by converting from quanta m^{-2} h^{-1} using 1 quanta m^{-2} h^{-1} = 4.614 × 10^{-22} μE m^{-2} s^{-1} (Lüning 1981)
[c] Values calculated by converting from Kilolux using 1 Kilolux = 16.5 μE m^{-2} s^{-1} (Richardson et al. 1983)
[d] Values calculated from total PPFD during incubation periods (ca 12 h)
[e] Corrected value (Priscu pers. comm.)

dency may be attributed merely to an accumulation of stored energy and C skeletons produced during photosynthesis. One cannot directly compare the uptake responses of urea and nitrate in the surface waters, due to differences in ^{15}N enrichment (saturating vs trace). At the DCM, however, saturating concentrations of NO_3^- were present during all the uptake experiments, and it appears that the phytoplankton in the stratified DCM communities had a similar degree of PPFD dependency for both saturated NO_3^- and urea uptake.

Dark nitrogen uptake

Nitrate and urea uptake occurred in the dark in both frontal and stratified waters (Table 3). In the stratified surface waters (A5, T8), the relative contribution of dark NO_3^- uptake to total NO_3^- uptake under saturating PPFD was ca 50 %, while in the surface waters of the frontal station (T14) the dark uptake contribution was only 28 % (Table 4). At the deeply stratified station (T8), there was no dark NO_3^- uptake by the DCM population, whereas the relative dark NO_3^- uptake at the shallow stratified (A5) and frontal (T14) DCM communities was similar to that of their respective surface phytoplankton communities (49 and 25 %). Dark uptake of urea was also a substantial portion of total urea uptake, averaging 16 and 68 % for the DCM and surface communities, respectively.

Dark N uptake by phytoplankton is not uncommon; a summary of literature values for the ratio of dark:light (total) N uptake rates (V_D:V_L; dark incubation bottles: clear incubation bottles) for natural phytoplankton assemblages is shown in Table 6. It is important to realize that dark uptake by phytoplankton is a function of incubation time; the rate of dark uptake will likely decrease, relative to light uptake, as incubation time increases and stored energy reserves are depleted. Nevertheless, a review of the literature permits 2 generalizations to be made concerning dependence of light for N uptake: (1) in N-impoverished waters, the V_D:V_L ratio is greater (approaching unity) than in N-replete waters, suggesting the enhancement of dark uptake by nutrient stress; (2) V_D:V_L ratio is generally greater (closer to unity) in samples collected from and incubated under lower PPFD, suggesting a lesser dependence of light for N uptake with increasing depth in the euphotic zone. The first suggestion is not new, as many laboratory experiments have shown that N deprivation enhances the uptake of N to a greater degree in the dark than in the light (e.g. Syrett 1962, Thacker & Syrett 1972, Harrison 1976, Rees & Syrett 1979, Paasche et al. 1984). The ability to take up nitrogen in the dark, however, may be species dependent; for example, Eppley et al. (1971) showed that although a somewhat N-depleted tropical oceanic coccolithophorid (*Emiliana huxleyi*) took up nitrate in the dark, a similarly N-depleted coastal diatom (*Skeletonema costatum*) did not. Also, whether or not a species is able to take up a significant amount of nitrogen at night or in the dark may depend on its degree of N depletion. This has been suggested for nitrate-limited continuous cultures of *Chaetoceros* spp. (Malone et al. 1975) and *Micromonas pusilla* (Cochlan 1989); at the lower dilution rates, nitrate uptake was continuous and independent of the natural light/dark cycle, but there was diel periodicity in nitrate uptake at the higher dilution rates. It should be noted that the dark uptake rates reported in the present study and used in the ratios of Table 6 were determined during daytime and may not necessarily reflect the uptake rates observed during the night.

During 24-h time-course experiments conducted in frontal waters similar to those referred to in the present study, Price et al. (1985) observed a constancy in V_D:V_L for NH_4^+, although NH_4^+ uptake rates of phytoplankton exposed to the natural light/dark cycle were periodic, suggesting that NH_4^+ uptake in frontal waters was circadian; in absence of the light/dark cycle the rhythm is free-running (see Chisholm 1981) . The conclusion of Price et al. (1985) is supported by Goering et al. (1964), who found rhythmic variation in both potential NH_4^+ and NO_3^- uptake rates by surface phytoplankton communities of the Sargasso Sea under continuous illumination. However, in the stratified waters of the Strait of Georgia, Price et al. (1985) found that V_D:V_L for NO_3^- and urea demonstrated both diel and diurnal variability. Diurnal (daytime) variability in the V_D:V_L of NH_4^+ uptake by freshwater phytoplankton assemblages of Lake Calado (Fisher et al. 1988) and the South River estuary (Fisher et al. 1982) has also been observed. In the present study, all the experiments were conducted at approximately the same time of day, thereby compensating for any diurnal variability in N uptake (either independent of or dependent on the daily light cycle) and thus permitting comparisons between stations.

An unknown portion of the dark uptake in the present experiments may also be attributed to marine heterotrophic bacteria. Horstmann & Hoppe (1981), using the ammonium analogue methylamine ($^{14}CH_3NH_2$), first demonstrated competitive NH_4^+ uptake by natural communities of bacteria and phytoplankton in the Baltic Sea. They found that bacteria (>0.2 µm) could assimilate up to half the quantity of methylamine as could phytoplankton (>3 µm) and that decreasing PPFD increased the methylamine uptake by surface communities of bacteria relative to phytoplankton. By comparing the nitrogen uptake rate of $^{15}NH_4^+$ with the rate of $^{14}CO_2$ incorporation into protein, Laws et al. (1985) concluded that heterotrophic N

Table 6. Summary of literature values of dark:light (dark incubation bottles:clear incubation bottles) specific (V_D/V_L) or absolute (ρ_D/ρ_L) nitrogen uptake rates, determined during daytime, in natural phytoplankton communities (values given are ranges, with means in parentheses)

Area	Ambient NO_3^- conc. (μg-at. N l^{-1})	ρ_D/ρ_L or V_D/V_L NO_3	NH_4	Urea	Source
Oceanic					
N Atlantic Gyre (Sargasso Sea)	–	– (0.30)	– (0.59)	–	Dugdale & Goering (1967)[a]
N Pacific Central Gyre					
50° N, 155° W	>10	0.0–0.63 (0.30)	0.38–2.0 (0.83)	–	Hattori & Wada (1972)
40° N, 155° W	0–1.0	0.92	0.78–1.5 (1.2)		
N Pacific Ocean					
Northern (J1–J7)	>2	0.0–0.097 (0.024)	0.057–0.27 (0.17)	0.0–0.19 (0.093)	Kanda et al. (1985)
Tropical/subtropical (J9–J23)	<0.1	0.075–0.32 (0.22)	0.17–0.53 (0.34)	0.12–0.50 (0.26)	
NE Pacific Ocean	12	0.0–0.02 (0.09)[g]	–	–	Cochlan (1989)
Upwelling					
NW Africa	>10	0.00–0.02 (0.07)	0.05–0.57 (0.36)	–	Nelson & Conway (1979)
Baja Calif. (Mexico)	>10	0.01–0.67 (0.16)	0.10–1.46 (0.49)	–	Nelson & Conway (1979)
Baja Calif. (Mexico)*	–	0.02	0.34	–	MacIsaac (1978)[b]
Polar					
Scotia Sea	>20	0.88–1.2 (1.0)	0.30–0.47 (0.38)	–	Glibert et al. (1982)[c,g]
Scotia Sea	>20	0.27–1.0	13.0–75.0	–	Rönner et al. (1983)[c]
Barents Sea	0–1.5	–	–	0.3–0.5	Kristiansen & Lund (1989)[c]
Coastal					
Oslofjord (Norway)	>2	0.06–0.57 (0.17)	–	–	Paasche & Erga (1988)[a]
	<1	0.18–1.7 (0.47)	–	–	
New York Bight (USA)	ca 0.1	0.2–1.0 (0.7)	0.4–1.3 (0.7)	–	Conway & Whitledge (1979)
Gulf of Maine (USA)	ca 1–2	0.0–0.2 (0.10)	0.0–0.4 (0.26)	–	Dugdale & Goering (1967)[a,b]
Peru*	0.09	0.60–0.86 (0.73)	–	–	Dortch & Maske (1982)
Strait of Georgia, B.C.					
Frontal	3.0–4.6	0.00–0.08 (0.03)	0.37–0.39 (0.38)	0.00–0.81 (0.36)	Price et al. (1985)
Stratified	<0.05	0.00–0.18 (0.09)	0.52–0.58 (0.55)	0.06–0.66 (0.36)	
Strait of Georgia, B.C.					
Frontal	6–15	0.25–0.28 (0.27)	–	–	Present study
Surface stratified	<0.05	0.48–0.51 (0.50)	–	0.58–0.77 (0.68)	
Bottom stratified	7–20	0.00–0.49 (0.25)	–	0.15–0.16 (0.16)	
Washington coast (USA)	–	– (0.21)	– (0.43)	– (0.38)	Dortch & Postel (1989)
Auke Bay, Alaska	>1.0	0.0–0.057 (0.005)	0.086–0.096 (0.091)	–	Kanda et al. (1989)[i]
	<1.0	0.14–0.47 (0.27)	0.41–0.55 (0.48)	–	
Western Irish Sea					
Surface stratified	ca 2.5	–	–	0.47–1.1 (0.67)	Turley (1985)[h]
Mixed & bottom strat.	ca 4.5	–	–	0.37–1.3 (0.72)	

Table 6 (continued)

Area	Ambient NO_3^- conc. (μg-at. N l^{-1})	ρ_D/ρ_L or V_D/V_L NO_3	NH_4	Urea	Source
Estuarine					
Pamlico River (N.C., USA)		0.25–0.36	0.71–0.82	–	Fisher et al. (1982)
South River (N.C., USA)		–	0.18–1.01 (0.57)	–	
Neuse River (N.C., USA)		–	0.04–0.95 (0.61)	–	
Newport River (N.C., USA)		–	0.02–0.11 (0.06)	–	
Delaware Bay (USA)		0.00–0.09	0.06–1.02	–	
Chesapeake Bay (USA)		–	0.26	–	
Freshwater					
L. Kinneret (Israel)	ca 0.10 0.2–0.6	0.40–0.91 (0.56) 0.16–0.33 (0.22)	0.29–1.0 (0.60) 0.53 (0.53)	0.13–0.67 (0.34) 0.33–0.43 (0.38)	McCarthy et al. (1982)[a]
L. Kinneret (Israel)	<0.05	0.32	0.59	–	Berman et al. (1984)
L. Nakanuma (Japan)	–	–	0.21–1.1 (0.57)	–	Miyazaki et al. (1985)[d]
L. Biwa (Japan)	–	0.26	0.78	0.51	Mitamura & Saijo (1986)
L. Kasumigaura (Japan)**	≤ 0.07	0.18–0.27	0.60–0.90	0.71	Takamura et al. (1987)
Shagawa L. (Minnesota, USA)	–	–	0.27–2.3 (1.0)	–	Toetz & Cole (1980)[a,e]
Toolik L. (Alaska, USA)	ca 1.0	0.05–0.31 (0.15)	0.27–0.57 (0.41)	–	Whalen & Alexander (1984)
L. Vanda (Antarctica)	0.0–0.7				Priscu (1989)[f]
Surface pop.		0.41	0.47	–	
Deep-chlorophyll pop.		0.23	0.83	–	
Czechoslavakian reservoirs	>35	0.05–0.38 (0.11)	–	–	Procházková et al. (1970)
L. Calado (Brazil)	ca <0.1	0.00–0.32 (0.16)	0.11–1.0 (0.49)	–	Fisher et al. (1988)
Amazon River (Brazil)	11.1	0.20	0.54	–	Fisher et al. (1988)
Castle L. (California, USA)	0–ca 2.5	– (0.50)	– (0.55)	–	Priscu (1984)[c]
Flathead L. (Montana, USA)	–	0.0–0.48 (0.21)	0.44–0.82 (0.64)	–	Dodds & Priscu (1989)[f]
L. Ontario (Canada)	0–15	0.02–0.30 (0.14)	0.30–0.60 (0.40)	–	Liao & Lean (1978)[g]

[a] Values calculated as $1/(V_L/V_D)$ or $1/(\rho_L/\rho_D)$ from reported values of V_L/V_D or ρ_L/ρ_D
[b] Values estimated from figures
[c] Values reported in text, no data available
[d] Values calculated from turnover times
[e] Light rates determined at ambient N conc., dark rates determined at saturating N conc.
[f] Values are ρ_d/N_m^b, where N_m^b is the chlorophyll-specific transport rate at optimal PPFD
[g] Experiments utilized 24 h incubations over natural light/dark cycle
[h] Average values reported
[i] Values calculated as $V_D/(V_D + V'_{max})$
* Dinoflagellate bloom
** Microcystis bloom

uptake accounted for at least 50 to 75 % of total microbial N uptake in the waters near the Hawaiian Islands. Similarly, Wheeler & Kirchman (1986), utilizing metabolic inhibitors, size-fractionation and ^{15}N methodology, estimated that 78 ± 22 % of ammonium uptake in the <1 µm size fraction of the surface waters off Georgia was due to bacteria. Brown et al. (1975) reported both NO_3^- uptake and reduction, and NH_4^+ uptake, by batch cultures of a marine pseudomonad, and Remsen et al. (1972) have demonstrated competition for urea among both bacteria and phytoplankton of the estuaries/coastal waters of Georgia.

In our experiments, Whatman GF/F filters were used to collect particulate material after incubation with ^{15}N-labelled urea and NO_3^-; these filters do not discriminate completely between bacteria and phytoplankton and can capture 42 to 56 % of the bacteria in marine systems (Lee & Fuhrman 1987). In the present study, the proportion of inorganic- and organic-N uptake which may be attributed to bacteria is unknown; previous studies in shallow sea frontal systems have reported both greater bacterial biomass and relative heterotrophic activity (as determined by glucose uptake) on the stratified side of a front in Saanich Inlet (Parsons et al. 1983), Liverpool Bay (Floodgate et al. 1981), and the Irish Sea (Egan & Floodgate 1985, Lochte 1985).

SUMMARY

The N uptake response to PPFD of the phytoplankton in the frontal and stratified communities of the Strait of Georgia could be described by a modified Michaelis-Menten formulation which included a dark uptake term. Dark uptake of nitrate and urea was a substantial portion of total uptake in these phytoplankton communities, and should be considered in PPFD-dependent uptake models. In the frontal waters, dependency on PPFD for NO_3^- uptake was similar for both surface and DCM communities, whereas in the stratified waters, surface phytoplankton exhibited less PPFD dependency than those from the DCM, particularly for urea uptake. The dramatic difference in species composition of the phytoplankton communities – from those dominated by large, chain-forming diatoms in the N-replete frontal waters to those composed primarily of microflagellates in the N-depleted stratified waters – probably contributed to the observed variability in their PPFD response and precludes a simple explanation of PPFD effect(s) on N uptake based merely on phytoplankton N status. Clearly, more detailed studies on the response of N uptake to PPFD in unialgal (and axenic) phytoplankton cultures, at various degrees of N deficiency, need to be conducted before the effect(s) of N limitation on the N uptake response to PPFD can be adequately explained.

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