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Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California

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ABSTRACT: The kinetics of nitrogen (nitrate, ammonium, urea) and carbon uptake by a red tide bloom consisting almost exclusively of the dinoflagellate *Lingulodinium polyedrum* (Stein) Dodge were determined with ^{15}N- and ^{13}C-tracer techniques, as a function of substrate concentration (for nitrogen) and irradiance (for both carbon and nitrogen). Samples were collected from Newport Beach, California, in late March 1995, during a massive red tide bloom which occurred off the California coast. At the collection site, surface concentrations of *L. polyedrum* reached 1.1×10^6 cells l^{-1}, with chlorophyll *a* = 125 µg l^{-1}. Maximal uptake rates of urea-N were approximately twice the maximal rates for either ammonium or nitrate during both the uptake versus substrate and uptake versus irradiance experiments, and the affinity for nitrate was much greater than previously demonstrated: half-saturation constant (K_s) = 0.47 µg-at N l^{-1}. Carbon and nitrogen uptake rates as a function of irradiance were well described by a 3-parameter P versus E relationship (photosynthesis vs irradiance) proposed by Platt & Gallegos (1980), although dark-uptake of nitrogen compounds accounted for ca 50% of V_{max}. These results demonstrate that *L. polyedrum* is capable of utilizing a broad range of both nitrogen concentrations and light fluences, and that urea could potentially provide a large percentage of the nitrogen demand at ambient urea concentrations and across the entire spectrum of light fluences. These data represent a more complete quantification of the N uptake dynamics of this bloom-forming species and contrast markedly compared to previous studies of *L. polyedrum*.

KEY WORDS: *Lingulodinium polyedrum* · Carbon · Nitrogen · Urea · Irradiance · Uptake kinetics

INTRODUCTION

During the late winter and early spring of 1995, a massive red tide bloom composed primarily of the dinoflagellate *Lingulodinium polyedrum* (Stein) Dodge (basionym *Gonyaulax polyedra*) occurred off the coast of California. Although blooms of this organism are relatively common in these waters, this particular episode was unusual in both its spatial extent and its temporal occurrence. The 1995 bloom extended from the upper Baja peninsula in Mexico to Monterey Bay, California, and as far offshore as San Clemente Island. At Newport Beach, where our samples were collected, chlorophyll *a* (chl *a*) concentrations were 125 µg l^{-1} while cell counts were in excess of 1.1×10^6 cells l^{-1}; even greater values were reported at La Jolla, where chl *a* concentrations reached 519 µg l^{-1} and cell counts exceeded 2×10^6 l^{-1} (Hayward et al. 1995). This represents the largest and most widespread red tide off California since 1902 (Torrey 1902). Typically, red tide blooms occur in southern California during the late spring and early summer, and the 1995 bloom was also the earliest known occurrence of a red tide for this region. Fortunately, *L. polyedrum* has rarely been reported to have direct toxic effects on other marine organisms, although a toxin similar to that which causes paralytic shellfish poisoning was first isolated

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from this species by Schradie & Bliss (1962). Since that report, *L. polyedrum* has regularly been included in tables of algal toxicity and screened with variable results (see review by Lewis & Hallett 1997). It appears, however, that marine fauna mortality associated with bloom concentrations of *L. polyedrum* is likely the result of deoxygenation and not direct toxicity.

Although *Lingulodinium polyedrum* has been extensively studied in both field (e.g. Holmes et al. 1967, Walsh et al. 1974, Eppley & Harrison 1975, MacIsaac 1978) and laboratory (e.g. Harrison 1976, Prézelin & Sweeney 1979, Heaney & Eppley 1981, Prézelin & Matlick 1983, Balch 1985) conditions, relatively little is known about the nitrogenous nutrition of this species. These data represent the first study to simultaneously examine the utilization of nitrate, ammonium and urea as a function of concentration, and to determine the effects of irradiance on N uptake. Previous studies have suggested that *L. polyedrum* must vertically migrate through the nutricline to obtain sufficient nitrogen to support observed growth rates (MacIsaac 1978, Heaney & Eppley 1981). This was predicated on the elevated dark-uptake values and the abnormally high half-saturation (K_s) values for NO_3^- in this species. We demonstrate that at the time of our study, *L. polyedrum* was capable of meeting its nitrogen demands from regenerated nutrients exclusively, and that the K_s values for all N substrates are much lower than previously reported.

MATERIALS AND METHODS

Sampling. This opportunistic 2 d study was conducted with samples collected aboard the RV 'Marda' during a cruise off Newport Beach, California (33° 34.58' N, 117° 53.24' W), on March 30, 1995, at approximately 10:30 h Pacific Daylight Time (PDT). Whole water was collected from the near surface using a clean plastic bucket, and stored in 2 acid-cleaned polyethylene carboys (rinsed 3× with sample water). The carboys were transported in dim light to the laboratory where they were placed in a walk-in culture room at low light (25 µmol photons m^{-2} s^{-1}), constant temperature (14°C), and with a bubbling air source. Vertical profiles of pH, temperature, salinity, and dissolved oxygen were conducted using a Seabird SBE19-03 CTD profiler equipped with an O_2 sensor. Light was measured with a Biospherical Instruments profiling 4π PAR meter with a matching deck unit. Discrete nutrient and pigment samples were collected using 5 l Niskin bottles (equipped with silicon springs) deployed on a hydrowire. Nutrient and pigment samples were stored (frozen) on dry ice until analysis at the laboratory, and samples for the determination of species composition were collected and stored after preservation with acid Lugol's solution. Additional samples for nutrients, pigments, and species composition were collected again immediately before the initiation of laboratory experiments.

Tracer uptake experiments. Water was dispensed into 280 ml polycarbonate incubation bottles on March 31, 1995, ca 24 h after initial collection. Kinetic parameters of uptake for NO_3^-, NH_4^+, and $CO(NH_2)_2$ were measured by adding varying concentrations (10 substrate levels ranging from 0 to 36 µg-at ^{15}N l^{-1} (Cambridge Isotope Laboratories; all 99 atom% ^{15}N) to duplicate sample bottles. Uptake versus irradiance parameters were determined by adding saturating concentrations (final concentration = 8.92 µg-at ^{15}N l^{-1}) of the isotopes to duplicate sample bottles (except urea, for which no replicates were conducted). The $^{15}NH_4^+$-labeled bottles were also inoculated (final concentration = 178.6 µg-at ^{13}C l^{-1}) with $NaH^{13}CO_3$ (Cambridge Isotope Laboratories; 99.9 atom% ^{13}C) for determination of carbon uptake rates.

The uptake kinetics experiments were conducted from 11:15 to 12:30 h PDT, while the photosynthesis versus irradiance (P vs E) and N uptake velocity versus irradiance (V vs E) experiments were conducted from 14:30 to 15:35 h PDT. All incubations were conducted in clear Plexiglas® incubators on the roof of the Allen Hancock building at the University of Southern California (ambient sunlight, ca 2300 µmol photons m^{-2} s^{-1}). Ambient water temperature was maintained at 14 ± 1°C. The kinetics experiments were conducted at ca 50% of the average incident irradiance (E_o; µmol photons m^{-2} s^{-1}) while the P versus E and V versus E sample bottles were placed in clear Plexiglas® tubes wrapped with neutral-density film (Courtaulds Performance Films) to simulate the following light levels: 75, 43, 26, 18, 15, 11.5, 8.5, 5, 3, 1, and 0.27% E_o. The 100% (unscreened) and 0% (wrapped in aluminum foil) bottles were not placed in Plexiglas® tubes, but were in the same incubator as the tubes.

All incubations were terminated by filtration (pressure differential <150 mm Hg) onto pre-combusted (450°C, 4 h) Whatman GF/F filters, and the filters were immediately placed in a drying oven (<60°C, >24 h). The filters labeled with ^{13}C were acidified with 3 drops of H_2SO_3. Time-zero samples were taken to correct for cellular adsorption of the isotopes, and additional samples were taken to ensure that the addition of H_2SO_3 did not affect the ^{15}N analysis. No dark bottle correction was made (e.g. dark bottles were not subtracted from light bottles) in the calculation of uptake rates (e.g. Li et al. 1993). Samples were prepared and analyzed for POC, PN and isotopic enrichment using a Europa Tracermass mass spectrometer.

Absolute uptake rates were calculated for both nitrogen and carbon using Eq. (3) in Dugdale & Wilkerson (1986). For calculation of the carbon uptake rates, the

ambient TCO_2 concentration was assumed to be 2000 µg-at C (Slawyk 1979). This value is well within the error estimate of the TCO_2 value estimated from pH, salinity, and assumed total alkalinity (2008 µg-at C) using the calculations of Lewis & Wallace (1997). Uptake rates for ammonium and urea were not corrected for isotope dilution (Glibert et al. 1982), and therefore should be considered conservative estimates of uptake to the extent that isotope regeneration may have occurred at the relatively high ambient pre-inoculation nutrient concentrations during the short experimental periods utilized.

Analytical methods. Determinations of $NO_3^- + NO_2^-$ (hereafter referred to as NO_3^-) and $Si(OH)_4^{4-}$ were carried out using a Technicon AutoAnalyzer II following the procedures outlined in Wood et al. (1967) and Armstrong et al. (1967), respectively. Ammonium samples, collected directly into the polypropylene reaction containers and stored refrigerated after addition of the phenolic reagent, were manually analyzed (within 48 h) using a spectrophotometer equipped with a 10 cm cell according to Sólorzano (1969). Urea samples (stored frozen) were also manually analyzed according to Price & Harrison (1987) and modified to account for a longer (30 min) and lower (80 to 85°C) digestion temperature. Pigment samples (chls *a*, *b*, *c*, carotenoids and phaeopigments) were collected on combusted Whatman GF/F filters and stored frozen prior to extraction in 90% acetone for 24 h at −20°C. Analysis for pigments was conducted using a 1 or 10 cm cell following the spectrophotometric method described by Parsons et al. (1984) using the extinction coefficients provided therein. All nutrient and pigment samples were collected in duplicate; reported values are means of the replicates. Phytoplankton species samples were preserved in acid Lugol's solution (Parsons et al. 1984) and stored in the dark until enumeration following Utermöhl procedures (Utermöhl 1958).

Curve parameters. All curve fitting was completed using a computerized, iterative non-linear least-squares technique (Deltagraph, Deltapoint Inc.) which utilizes the Levenberg-Marquardt algorithm (Press et al. 1992). The nitrogen kinetics data were fitted to the Michaelis-Menten formulation, after removing 1 extraneous point (see below):

$$V = \frac{V_{max} \times S}{K_s + S} \quad (1)$$

where V is the specific uptake rate (pg-at S cell^{-1} h^{-1}), V_{max} is the maximal specific uptake rate, S is the substrate concentration (µg-at S l^{-1}), and K_s is the half-saturation constant for the substrate (µg-at S l^{-1}). Uptake versus irradiance data were fitted to the 3-parameter P versus E model proposed by Platt & Gallegos (1980; hereafter referred to as the Platt model) for both carbon and nitrogen substrates. The original equation was modified to account for dark uptake by the inclusion of a positive y-intercept (as described by Cochlan et al. 1991b):

$$V = V_s\left(1 - e^{\frac{-\alpha \times E}{V_s}}\right)\left(e^{\frac{-\beta \times E}{V_s}}\right) + V_D \quad (2)$$

where V_s is the maximal uptake rate in the absence of photo-inhibition, α and β are the respective light-limited and light-inhibited slopes of the uptake versus irradiance curve defined by the equation with units of (pg-at S cell^{-1} h^{-1}) (µmol photons m^{-2} s^{-1})$^{-1}$, and E represents irradiance between 400 and 700 nm (µmol photons m^{-2} s^{-1}). For convenience we have not standardized the units for α and β (i.e. converted to either h^{-1} or s^{-1}), but have left the units in the same form as was used for the V and E values. The V_D term represents the positive intercept in the presence of dark-uptake, and is excluded from the carbon curves. Note that V_{max} and V_s are functionally equivalent if no photo-inhibition occurs, where V_{max} represents the maximal uptake rate observed, and V_s represents the maximal uptake rate in the absence of inhibition. The conventional index of light adaptation (E_k) is determined as the initial slope of the V versus E curve (V_{max}/α), and has units of µmol photons m^{-2} s^{-1}. For clarity, we have utilized nitrogen symbols; the same equation can be parameterized for carbon by substituting the symbol P^B (biomass-specific carbon uptake) for V (biomass-specific nitrogen uptake). Curve fits were completed using all available points; for ease of interpretation, the graphical representations provide the mean and error bars (±1 SD) for points where replicates were conducted.

As demonstrated by Frenette et al. (1993), intercomparison of various curve-fitting methods may lead to markedly different fitted parameters. All of these data demonstrated some degree of photo-inhibition, which is not accounted for by the Michaelis-Menten formulation. Furthermore, the fitted parameters (V_{max}, E_k, α) were similar using either the Platt model or the Michaelis-Menten hyperbola (modified to allow for dark-uptake) when the photo-inhibited samples were removed from the analyses (data not shown). Therefore, although most of the (few) previous studies of nitrogen uptake versus irradiance have used the Michaelis-Menten formulation, we present these data using the Platt model (except where noted). All curve fits (uptake vs irradiance, and uptake kinetics) are normalized to cell number. Rates are given in units of pg-at S cell^{-1} h^{-1} which is calculated by dividing the absolute uptake rate (ρ; pg-at S h^{-1} l^{-1} where S is carbon or nitrogen) by the cell abundance (cell l^{-1}). This rate is proportional to the biomass or PN-specific rate (V; h^{-1}). Statistical analyses for goodness-of-fit were

determined using the variance approximation techniques described by Zimmerman et al. (1987).

RESULTS

General hydrographic characteristics

In the spring of 1995, a massive algal bloom composed of the dinoflagellate *Lingulodinium polyedrum* occurred off the west coast of North America, extending from the northern Baja peninsula to Monterey Bay, California. In Newport Beach, California, surface concentrations of *L. polyedrum* were 1.1×10^6 cells l^{-1} and chl *a* concentrations were in excess of 125 mg m^{-3}. Biomass maxima (as determined from photosynthetic pigments) were found at both the surface and subsurface (6 m). The deepest collection depth for this study (10 m) exhibited chl *a* concentrations in excess of 60 mg m^{-3}, suggesting that the total biomass was substantially higher than the 835 mg m^{-2} calculated from trapezoidal integration of the upper 10 m. The predominant pigment was chl *a*, although secondary pigments (primarily carotenoids) accounted for ca 40% of the total at all depths (Fig. 1). Phaeopigments were negligible at all depths, indicating that at the time of collection, the bloom was composed of active cells rather than degradation products. The diffuse attenuation coefficient (K_{PAR}) for these waters was 1.075 m^{-1}. Floristic analyses of the samples indicated that *L. polyedrum* accounted for ca 97% of the autotrophic biovolume; co-occurrence of high concentrations of the heterotrophic dinoflagellate *Noctiluca scintillans* resulted in ca 42 and 56% of the total biovolume being attributed to *L. polyedrum* and *N. scintillans*, respectively. The *N. scintillans* population was not accounted for in the present analysis due to previous results indicating this species to be an apochlorotic, obligate phagotrophic dinoflagellate (Buskey 1995). During the approximately 24 h that the samples were maintained in our culture facility, cell numbers of *L. polyedrum* approximately doubled while chl *a* declined from 125 to 61 µg l^{-1}. No changes in the phaeopigment or accessory pigment to chl *a* ratios were detected, suggesting a physiologically mediated decrease in chlorophyll per cell.

Although previous dinoflagellate blooms off the California coast have often been associated with upwelling events (e.g. Dugdale 1979), the first 4 mo of 1995 for the southern California coast were characterized by positive sea surface temperature anomalies and negative upwelling index anomalies when compared to the long-term harmonic mean, and were accompanied by unusually heavy rainfall with comparably heavy coastal runoff throughout California (Hayward et al. 1995). Previous red tide blooms for this area have been reported from May to September (Hayward et al. 1995), which also makes this bloom the earliest known occurrence for this location. Vertical profiles indicated a relatively warm (15.0°C) and fresh (32.95 PSS) strongly stratified upper water column with cooler (12.9°C, 20 m), more saline (33.45 PSS, 20 m) waters below the pycnocline located at approximately 12 m depth. Dissolved oxygen concentrations were supersaturated throughout the mixed layer.

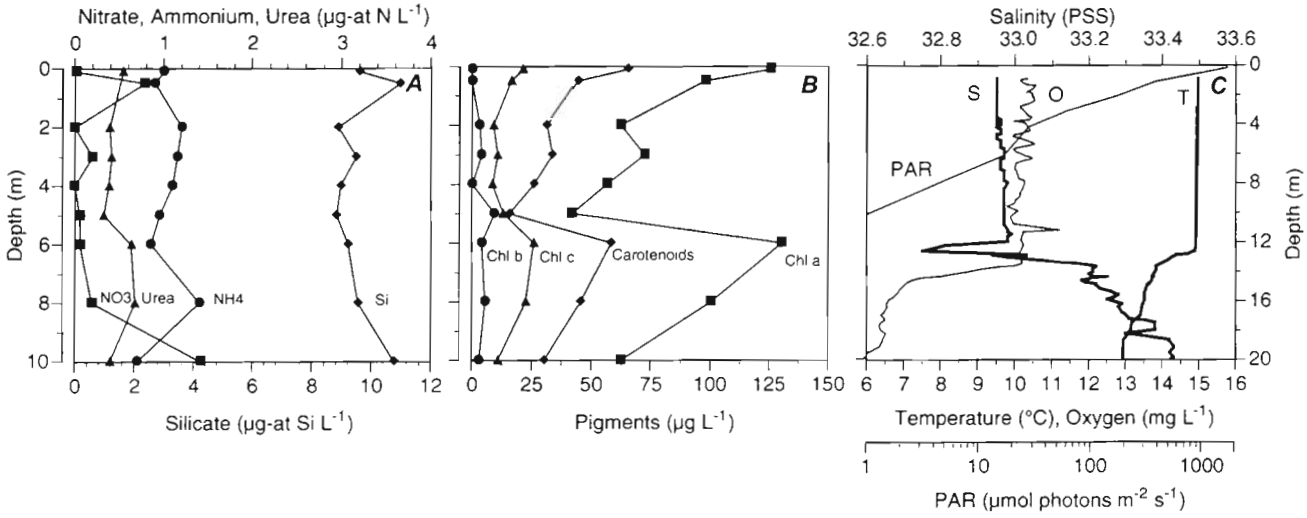

Fig. 1. Ambient conditions for the water column of Newport Beach, California, during sampling (March 30, 1995). (A) Depth profiles of $Si(OH)_4^{4-}$ (◆), NO_3^- (■), NH_4^+ (●), and urea (▲) concentration within the mixed layer (water column depth = 127 m). (B) Depth profile of plant pigment concentration (■: chl *a*; ●: chl *b*; ▲: chl *c*; and ◆: carotenoids) within the mixed layer. (C) Temperature (°C), salinity (PSS) oxygen (mg l^{-1}) and photosynthetically available radiation (PAR; µmol photons m^{-2} s^{-1}) at the study site. Note the change in depth (*y*-axis) scale from 10 to 20 m in (C)

Nutrient concentrations at the study site were typical of a red tide bloom (Eppley & Harrison 1975), with elevated $Si(OH)_4^{4-}$ concentrations and depleted N levels overlying a shallow thermocline and a deeper supply of NO_3^-. The anomalous near-surface peak at 0.5 m in NO_3^- and $Si(OH)_4^{4-}$ could be caused by freshwater runoff (Hayward et al. 1995; but note the lack of a corresponding salinity signal) aeolian deposition, or a mislabeled nutrient sample. Ammonium concentrations were elevated (ca 1 µg-at N l^{-1}) throughout the water column, with a maximum occurring at 8 m depth, below the subsurface chl *a* maximum (6 m). Approaching the thermocline, NO_3^- concentrations increased while NO_4^+ concentrations decreased. Measured urea concentrations (ca 0.5 µg-at N l^{-1}) were considerably lower than the NO_4^+ concentrations; urea exhibited a similar profile to NO_4^+, with a subsurface maximum at ca 6 to 8 m (Fig. 1).

Nitrogen kinetics

The estimated kinetic parameters for the uptake of nitrate and urea are presented in Table 1, and plotted in Fig. 2. It was not possible to determine the kinetics of ammonium using the iterative fitting procedure, due to elevated ambient concentrations present in the collected water (0.75 µg-at N l^{-1}). Fitting the data after linearizing with the Hanes-Woolf transformation (Dowd & Riggs 1965) provided values of 0.586 µg-at N l^{-1} and 1.01 pg-at $cell^{-1}$ h^{-1} for the K_s and V_{max}, respectively (Fig. 2). However, these values rely on the assumed linearity of the data and should only be considered as approximations. At the highest nutrient inoculation (36 µg-at NO_3^- l^{-1}), NO_3^- uptake was depressed (0.231 ± 0.063 pg-at N $cell^{-1}$ h^{-1}) to less than half of our reported V_{max}. We are not aware of any inhibitory or toxic effects in *Lingulodinium polyedrum* at these relatively low NO_3^- concentrations. It is unclear what the mechanism for this suppression was,

Table 1. Calculated kinetics parameters for uptake of NO_3^-, urea, and NH_4^+. Values for NO_3^- and urea were fitted using the Michaelis-Menten formulation; NH_4^+ values were derived from linearization (Hanes-Woolf) of the available data, and are presented for comparison only. The r^2 column provides the coefficient of determination and the sample size (n). Units for α_N are (pg-at N $cell^{-1}$ h^{-1}) (µg-at N l^{-1})$^{-1}$. Cell abundance was 2.076 × 10^6 cells l^{-1}, PN was 44.5 µg-at N l^{-1} at the beginning of the experiment

	V_{max} (pg-at N $cell^{-1}$ h^{-1})	K_s (µg-at N l^{-1})	α_N	r^2
NO_3^-	0.480	0.467	1.03	0.563
(SD)	(0.034)	(0.180)	(0.051)	(18)
Urea	1.321	0.989	1.34	0.729
(SD)	(0.119)	(0.316)	(0.111)	(16)
NH_4^+	1.01	0.586	1.72	0.922
(SD)	(0.069)	(0.627)	(0.397)	(20)

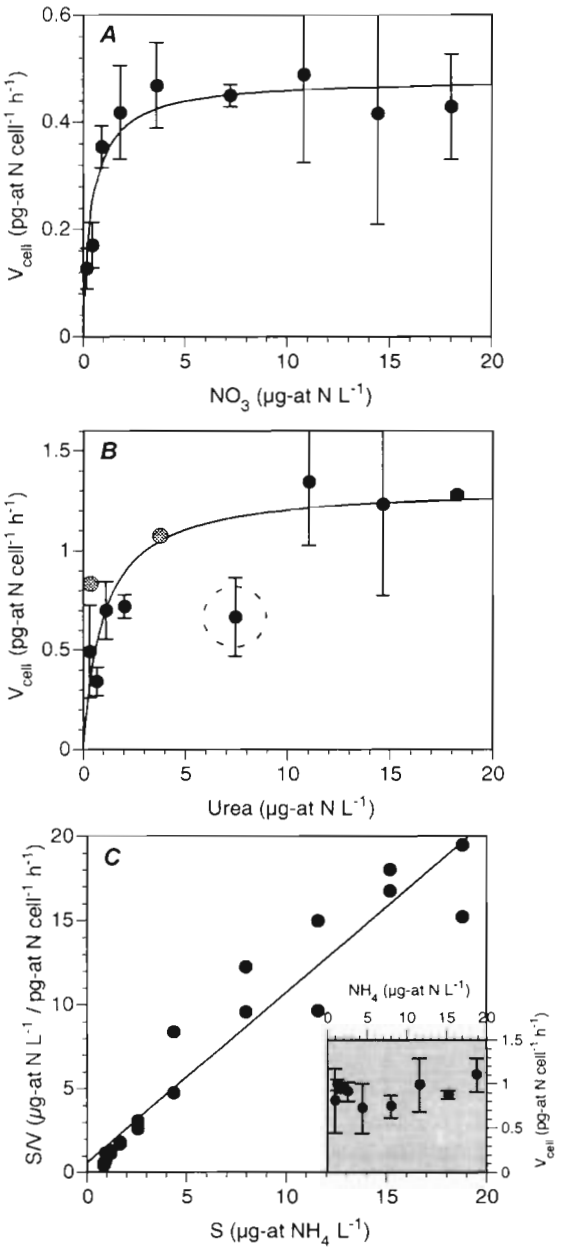

Fig. 2. Nitrogen uptake as a function of substrate concentration (A: NO_3^-; B: urea; C: NH_4^+) for a natural assemblage of *Lingulodinium polyedrum* off Newport Beach, California. The curved plots are fitted directly to the Michaelis-Menten formulation. Error bars indicate ±1 SD of duplicate samples. Data in dashed circle were not included in the curve fit or in the estimation of kinetic parameters. ◍: non-replicate data. Note that for NH_4^+ (C), a Hanes-Woolf linearization of the data is plotted, with the non-linearized data presented as an inset graph

and these points were not used in our analyses. A comparison of maximal uptake rates (V_{max}, pg-at N cell^{-1} h^{-1}) indicated that uptake of urea was greatest, followed by uptake of NH_4^+ and NO_3^-, with values of 1.32, 1.01 and 0.480 pg-at N cell^{-1} h^{-1}, respectively. The K_s for urea was also greater than that for both NH_4^+ and NO_3^-, indicating that *L. polyedrum* could utilize lower concentrations of NH_4^+ and NO_3^- compared to urea. Because of the effects of V_{max} on K_s, these values are not necessarily a reliable indicator of preference at low nutrient concentrations (Healey 1980). The α parameter, referred to here as α_N ($\alpha_N = V_{max}/K_s$; [pg-at S cell^{-1} h^{-1}][μg-at S l^{-1}]$^{-1}$) to differentiate from the P versus E symbol, provides a more robust indicator for substrate affinity when substrate concentrations are low ($<K_s$), and substrate or inter-species competition is likely to occur (Healey 1980, Harrison et al. 1989, Cochlan & Harrison 1991). The α_N parameters (NO_3^-, NH_4^+, urea) were within a factor of 2.

Uptake versus irradiance

The estimated parameters for the uptake versus irradiance data are summarized in Table 2 for all substrates examined (NO_3^-, NH_4^+, urea and carbon) and plotted in Fig. 3. Lower maximal uptake rates (V_{max}) were obtained for the nitrogen substrates in the uptake versus irradiance experiments compared to the kinetics experiments (using V_{max} values from Michaelis-Menten curve fits for both data sets). There are several possibilities for this discrepancy, including the use of what we later determined to be less than saturating isotope enrichments (based on the kinetics experiments) or diurnal periodicity (e.g. MacIsaac 1978, Miyazaki et al. 1987, Pettersson & Sahlsten 1990, Cochlan et al. 1991a, Glibert & Garside 1992).

Although it was possible to fit the Platt model to all of these data, the N uptake data are not strictly light-dependent. Dark-uptake rates account for a significant proportion of the total uptake in these experiments, ranging from 37 to 52% of V_{max}. In contrast, dark carbon uptake was ca 1.1% of V_{max} for carbon. Both carbon and NO_3^- uptake versus irradiance data exhibited low E_k values and correspondingly high values for the initial slope of the uptake versus irradiance curves (α). The α value for carbon (after converting to chl-specific uptake rates) is proportional to 98% of the theoretical maximal value proposed by Platt & Jassby (1976) for coastal marine phytoplankton. All of the N substrates exhibited similar affinities for uptake at low light levels, with α values ranging from 2.04 to 2.41 × 10^{-3} (pg-at N cell^{-1} h^{-1}) (μmol photons m^{-2} s^{-1})$^{-1}$. Although the nitrogen substrate curves demonstrated pronounced uptake in the dark, the E_k value for carbon was 2 to 10 times lower than for the N substrates (25.8 μmol photons m^{-2} s^{-1} vs 64.1 to 253 μmol photons m^{-2} s^{-1} for carbon and nitrogen, respectively). These values, which represent the light intensity at the inflection point between light-limited and light-saturated uptake, translate into ca 1.2% of the average incident surface irradiance during the mid-afternoon incubations for carbon, and between 3 and 12% for nitrogen.

DISCUSSION

Lingulodinium polyedrum has been an oft-studied organism due to its frequent presence in red tide blooms off the southern California and Baja California coasts (review by Lewis & Hallett 1997), including examination of both nutrient (e.g. Eppley & Harrison 1975, Harrison 1976, MacIsaac 1978, Balch 1985) and light (e.g. Prézelin & Sweeney 1979, Heaney & Eppley

Table 2. Calculated uptake versus irradiance parameters for NO_3^-, NH_4^+, urea and carbon. All data were fitted to the Platt & Gallegos (1980) model modified for dark uptake, and error analyses were determined using the methods described by Zimmerman et al. (1987). *S*: various substrates being fitted; the Optimal % E_o column represents E_k as a percentage of near-surface irradiance (E_o). The r^2 column provides the coefficient of determination and the sample size (n). Units for α and β were omitted to conserve space; the units are (pg-at *S* cell^{-1} h^{-1})/(μmol photons m^{-2} s^{-1}) for both

	V_{max} (pg-at *S* cell^{-1} h^{-1})	V_{dark} (pg-at *S* cell^{-1} h^{-1})	E_k (μmol photons m^{-2} s^{-1})	α	β	Optimal % E_o	r^2 (n)
NO_3^-	0.321	0.166	64.1	2.41E^{-3}	2.05E^{-5}	3.0	0.769
(SD)	(0.026)	(0.002)		(1.04E^{-3})	(2.86E^{-5})		(26)
NH_4^+	0.488	0.182	149	2.05E^{-3}	7.41E^{-5}	7.0	0.801
(SD)	(0.126)	(0.018)		(9.48E^{-4})	(2.14E^{-5})		(23)
Urea	0.898	0.381	253	2.04E^{-3}	1.81E^{-4}	12.0	0.773
(SD)	(0.019)	(ND)		(6.67E^{-5})	(1.87E^{-5})		(12)
CO_2	5.25	0.059	25.7	2.04E^{-1}	2.15E^{-3}	1.2	0.838
(SD)	(0.404)	(1.31)		(1.39E^{-1})	(5.23E^{-4})		(23)

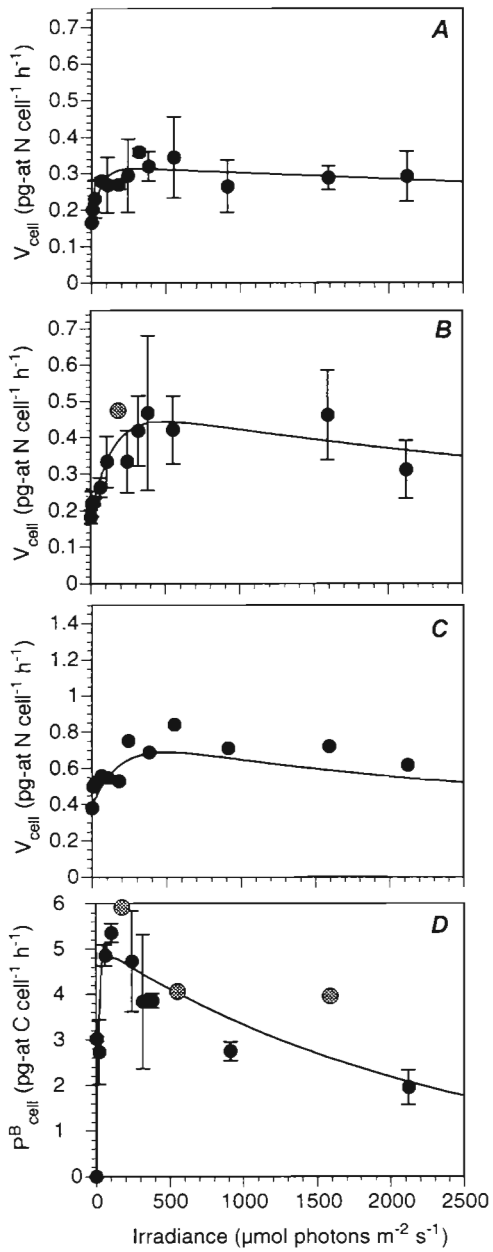

Fig. 3. Nitrogen (A: NO_3^-; B: NH_4^+; C: urea) and (D) carbon uptake as a function of irradiance for a natural assemblage of *Lingulodinium polyedrum* off Newport Beach, California. The curved plots are fitted directly to the 3-parameter P versus E curve (Platt & Gallegos, 1980) modified to account for dark uptake (Cochlan et al. 1991). Error bars indicate ±1 SD of replicate samples, except for urea (C) for which there were no replicates. ●: data were not included in the curve fit or in the estimation of kinetic parameters. ◍: non-replicate data

1981, Meeson & Sweeney 1982, Prézelin & Matlick 1983) physiological responses in this species. To date, however, no study has examined the nitrogen kinetics and photosynthetic response of this species simultaneously. Our reported values also represent one of the few descriptions of the dynamics of urea uptake for a dinoflagellate under natural bloom conditions.

Previous field observations coupled with studies in the laboratory have led to the speculation that *Lingulodinium polyedrum* achieves maximal biomass concentrations in nutrient-depleted waters by vertically migrating through the nutricline at night (MacIsaac 1978, Heaney & Eppley 1981). By doing so, *L. polyedrum* could maximize its ability to utilize NO_3^- in the dark, which could account for 50 to 100% of its nitrogenous nutrition (Harrison 1976), while photosynthesizing during the daylight hours. Our results confirm that *L. polyedrum* is capable of maintaining significant uptake in the dark. It is important to note, however, that the culture room lighting (25 µmol photons m^{-2} s^{-1}) was equal to the E_k value for carbon uptake by this species (25.7 µmol photons m^{-2} s^{-1}), suggesting that significant amounts of stored photosynthate may have been present before the initiation of the experiment. Although elevated dark-uptake values are often associated with N deficiency (e.g. Harrison 1976, Paasche et al. 1984), this interpretation is confounded by the often high rates of dark-N uptake found in *L. polyedrum*, even under N-sufficient conditions (Eppley & Harrison 1975, Harrison 1976, MacIsaac 1978, Heaney & Eppley 1981). Harrison (1976) showed that in culture, dark NO_3^- uptake was enhanced from ca 20 to 40% of the light assimilation value when the culture was nitrogen starved, and that dark-uptake was more affected by starvation than was light uptake. Heaney & Eppley (1981) also showed that *L. polyedrum* exhibits complex behavior when nutrient limited, including avoidance of high light levels and cessation of vertical migration. In their data, this was accompanied by a rapid (ca 1 d) drop in the atomic C:N ratio from 8.5 to 6.5 with the addition of nitrogen. For our experiments, dark uptake accounted for ca 45% of total N uptake and the mean C:N ratio from the ^{13}C samples was 9.1 ± 0.20 (±SE). Based on the references cited above, this suggests that the assemblage was approaching nitrogen limitation. This remains speculative, however, given the wide range of dark-uptake capacities and C:N ratios in this and other algal species.

Heterotrophic uptake (especially by bacteria) of dissolved N compounds could also contribute to our measured dark-uptake rates. However, the elevated concentrations of *Lingulodinium polyedrum* would make the contribution of other potential competitors negligible in the measured uptake rates. Bacteria are known to preferentially utilize ammonium before nitrate or urea (e.g. Kirchman 1994, Kirchman et al. 1994, Kirchman & Wheeler 1998), and may gain most of their nitrogen requirement from dissolved free amino acids in coastal environments (Billen & Fontigny 1987, Kirchman 1994, but see Kirchman & Wheeler 1998). Auto-

trophic organisms have also been shown to outcompete heterotrophs at elevated substrate concentrations (Suttle et al. 1990) such as were measured at our study site. We are therefore confident that our dark uptake rates are not representative of heterotrophic uptake given the elevated ambient concentrations of ammonium and the use of near-saturating additions of N in our uptake versus irradiance experiments.

Based on V_{max} values, apparent N utilization followed the order: urea, NH_4^+, NO_3^-. Calculation of the f-ratio [$f = V_{NO_3}/(V_{NO_3} + V_{NH_4} + V_{urea})$] at ambient nutrient concentrations also demonstrates a reliance on regenerated forms of nitrogen, with an estimated value of 0.01. This value was calculated using the Michaelis-Menten equation and the kinetics parameters from Table 1. Even if saturating concentrations of NO_3^- were present (for example if the population were to vertically migrate below the nutricline), simple calculations demonstrate that the f-ratio would only rise to 0.29. This value is based on the measured uptake rates and kinetics parameters (Table 1) when the ambient NH_4^+ and urea concentrations are held constant and the NO_3^- concentration is increased to 12 µg-at N l^{-1}, stoichiometrically equal to the ambient $Si(OH)_4^{4-}$ concentration. Calculation of the percent urea uptake (% $Uptake_{urea} = [V_{urea}/(V_{urea} + V_{NH_4} + V_{NO_3}) \times 100]$, determined using kinetics parameters from Table 1 and average nutrient concentrations (in the upper 10 m), provides a relative urea utilization of 33.8%. This value falls well within previously reported literature values for other natural assemblages in oceanic, coastal, polar, upwelling, and freshwater systems (Table 3).

It is possible that the NO_3^- uptake rates, but apparently not the urea uptake rates, were inhibited by the relatively high ambient NH_4^+ concentrations (e.g. McCarthy 1981) or were diurnally fluctuating. However, Harrison (1976) reported that *Lingulodinium polyedrum* exhibited no NH_4^+ inhibition at 50 µg-at NH_4^+ l^{-1} for short periods. He also reported maximal NO_3^- uptake and nitrate reductase (NR) activity at midday in both N-saturated and N-deficient cultures. Similarly, Packard & Blasco (1974) reported high NR activity in low-NO_3^- waters. Regardless, the instantaneous nitrogen demand for this assemblage could easily be met by a combination of NH_4^+ and urea assimilation (assuming 7:1 C:N assimilation ratio, a sustained V_{max} for C uptake, and saturating irradiances). It is important to note, however, that sustained uptake at V_{max} could deplete the ambient nutrient pools in about 4 h, requiring an equally rapid regeneration rate to maintain this growth rate.

MacIsaac (1978) in a study of a naturally occurring bloom off the Baja peninsula argued that *Lingulodinium polyedrum* required both sufficient (and simultaneous) surface light and NO_3^- to achieve red tide concentrations, and that there was no evidence for the vertical migration and dark uptake of NO_3^-. Although our E_k values (3 to 12% of near-surface light) are similar to the values MacIsaac (1978) reported (ca 4 and 10% for NO_3^- and NH_4^+ respectively), MacIsaac also reported insignificant dark-uptake of NO_3^-. Prézelin & Matlick (1983) demonstrated that under nutrient-limiting conditions, *L. polyedrum* is more susceptible to photo-damage, while Heaney & Eppley (1981) reported that under nutrient-limiting conditions, *L. polyedrum* will delay or even cease vertical migration into higher light. These behavioral adaptations likely provide both prolonged exposure to higher subsurface NO_3^- levels, and avoid photodamage associated with the near surface.

In this study, uptake rates for all of the tested substrates demonstrated at least some photoinhibition (β values; Table 2). The carbon data were more pronounced in this inhibition, which is not unexpected given the extremely low E_k value and the direct dependence on light for carbon assimilation. Nitrogen uptake is potentially buffered by the ability to utilize indirect sources of reducing energy, and so would not be expected to demonstrate the same degree of dependency on irradiance. Notably, however, uptake rates at even the highest irradiance (>2100 µmol photons m^{-2} s^{-1}) were not appreciably depressed, with N uptake rates at 63 to 90% of V_{max}. Given the elevated dark-uptake of N, this lack of suppression may be partly explained by the lack of dependence on photon fluences for N uptake. However, examination of the dark-corrected V_{max} values (V'_{max}; calculated as $V'_{max} = V_{max} - V_{dark}$) indicates that N uptake for the highest light levels remained at >50% of V'_{max} while the light-inhibited carbon uptake rates were ca 37% of V_{max}.

Despite the low E_k values, this assemblage was capable of maintaining uptake rates of all N substrates at 50% of V_{max} or better, even at the highest irradiance (2119 µmol photons m^{-2} s^{-1}). This irradiance is unlikely to be encountered for any length of time in ecologically relevant conditions, given that it was achieved at noon in a Plexiglas® incubator. These data, together with the dark-uptake values which demonstrated that *Lingulodinium polyedrum* could maintain 37 to 52% of V_{max} in darkness, indicate that this dinoflagellate is capable of maintaining high uptake rates of nitrogenous substrates at all light levels. Similarly, photosynthetic carbon assimilation reached 50% of V_{max} at the extremely low level of 1.2% of surface irradiance, and was still at 37% of V_{max} at the highest light levels (Fig. 3D). It is possible to calculate the apparent demand for nitrogen at varying light levels and ambient nutrient concentrations based on our reported uptake versus irradiance and uptake versus substrate concentration kinetic

Table 3. Summary of literature values of urea uptake relative to total uptake, % $Uptake_{urea}$ = $[Uptake_{urea}/(Uptake_{NO_3^-} + Uptake_{NH_4^+} + Uptake_{urea})] \times 100$ determined in natural assemblages from absolute[a] or specific[b] uptake rates conducted under natural conditions. Values reported are means with ranges given in parentheses. Depths sampled are reported in meters or percentage of surface irradiance (I_0)

Area	% Urea	Depth (m or % I_0)	Incubation period (h)	Isotope dilution correction	Comments	Source
Oceanic						
NE Subarctic Pacific Ocean						
Winter	12.2[a] (8.2–19.3)	100–1%	24	No	Stns P20, OSP	Varela & Harrison (1999)
Spring	30.0[a] (7.0–56.0)	100–1%	24	No	(integrated rates and	
Late summer	19.2[a] (22.5–30.1)	100–1%	24	No	high $[NO_3^-]$)	
Central North Pacific Gyre	42[a] (27–57)	81–1%	24	No	Integrated rates	Eppley et al. (1973)
Subtropical (low $[NO_3^-]$)					November	
Central North Pacific Gyre						Eppley et al. (1977)
Winter (subtropical)	30[a,d] (20–45)	0–85 m	24	No	Integrated rates	
Summer (subtropical)	25[a,d] (13–39)	0–80 m	24	No	Low $[NO_3^-]$	
Central North Pacific Gyre	32[a]	100–1%	3–4	Yes	Integrated rates	Sahlsten (1987)
Subtropical (low $[NO_3^-]$)					August–September	
North Pacific						
Northern (J1–J7)	12[a,f] (8–22)	Surface	3	No	Maximum rates	Kanda et al. (1985)
Tropical/Subtropical (J9–J23)	31[a,f] (26–36)	Surface	3	No	(V vs S expt)	
Eastern Equatorial Pacific	5.6[a,d] (3.8–7.3)	15 m	6–8	No	Iron Ex II (controls and pre-fertilization)	Cochlan & Kudela (unpubl.)
Gulf Stream warm core rings	12[a]	100–3%	4	Yes	Integrated rates	McCarthy & Nevins (1986)
South Atlantic (off Brazil)	13.1[a]	50 and 1%	~1	Yes		Metzler et al. (1997)
South Atlantic (off S. Africa)	13.1[a] (0–17)[c]	Surface	4–6	No	Low $[NO_3^-]$	Probyn (1985)
NW Indian Ocean	28[a] (0–64)	100–1%	24	No	Integrated rates (inter-monsoon)	Watts & Owens (1999)
Coastal						
Baltic Sea (low $[NO_3^-]$)	31[a] (24–44)	0–15 m	4–6	No	Drogue study of cyanobacteria bloom *Nodularia spumigena*	Sörensson & Sahlsten (1987)
Oslofjord (Norway)	19 (0–53)	0–2 m	3–5	No	April–October	Kristiansen (1983)
Eastern Skagerrak (Sweden)	Daytime %				Nighttime %	Pettersson (1991)
Autumn	13[a] (10–20)	0.5–4.0 m (50%)	~3	Yes	10[a] (8–12)	
Spring	7[a] (1–13)	0.5–4.0 m (50%)	~3	Yes	13[a] (6–22)	
Summer	14[a] (10–17)	0.5–4.0 m (50%)	~3	Yes	17[a] (14–20)	
Open Skagerrak (Sweden)					High $[NO_3]$ during May diel study	Pettersson & Sahlsten (1990),
North	37[a] (22–53)	4–13 m	3–5	–		Rosenberg et al. (1990)
South	28[a] (19–61)	4–15 m	3–5	–		
Western English Channel						L'Helguen et al. (1996)
Winter	17[a,c] (8–29)	1.5–6 m (50%)	4	Yes		
Spring	10[a,c] (4–12)	1.5–6 m (50%)	4	Yes		
Summer	13[a,c] (12–14)	1.5–6 m (50%)	4	Yes		
Autumn	29[a,c] (10–47)	1.5–6 m (50%)	4	Yes		
Western Irish Sea	48[a]	Surface (4 m)	4.5	No	Daily rates used (night and daytime)	Turley (1985)
Lower Narragansett Bay, RI (USA)					Furnas (1983)	
Winter/spring	15[b] (0–44)	Surface	3.5–9	No	Generally substrate	
Summer	23[b] (3–64)	Surface	3.5–9	No	depletion (>80% used)	

(Table 3 continued on next page)

Table 3 (continued)

Area	% Urea	Depth (m or % I_0)	Incubation period (h)	Isotope dilution correction	Comments	Source
Strait of Georgia (Canada)						Price et al. (1985)
Frontal	23[a] (14–34)	2 m (50%)	6	No	4, serial 6 h incubations	
Stratified	32[a] (26–38)	3 m (50%)	6	No		
British Columbia coast (Canada)						Varela & Harrison (1999)
Winter	25.9[a] (8.4–45.7)	100–1%	24	No	Stns P4, P12, P16 (integrated rates)	
Spring	28.6[a] (12.9–45.7)	100–1%	24	No		
Late summer	14.0[a] (3.4–22.7)	100–1%	24	No		
Washington coast (USA)					Possible [urea] errors;	Dortch & Postel (1989)
Shelf (fall-winter/spring/summer)	13.9/26.3/34[a]	100–1%	4–28	No	seasonal averages,	
Shelf break	15/26.8/30[a]	100–1%	4–28	No	using integrated rates	
Offshore	20/23/21.9[a]	100–1%	4–28	No		
Oregon & Washington (USA)	20.6[a] (7.5–36.9)	15 m	2–4	No	Low nitrate stns (serial 0.5–1.0 h incubations)	Kokkinakis & Wheeler (1987)
Southern California (La Jolla, USA)	25[b] (6–47)	87–1%	24	No	Integrated rates	McCarthy et al. (1977)
Southern California (Newport Beach, USA)	33.8[b]	surface	~1	No	*Lingulodinium polyedrum* bloom	This study
Brazilian coast	18.0[a]	50 and 1 %	~1	Yes		Metzler et al. (1997)
Tasman Sea (Westland, NZ)						
Summer inshore	34.1[a] (5.1–79.4)	10 m	4–6	Yes	Artificial light	Chang et al. (1995)
Summer offshore	24.2[a] (15.9–31.4)	10 m	4–6	Yes	Artificial light	
Winter inshore	24.5[a] (10.9–38.7)	10 m	4	No	Potential (max) rates	Chang et al. (1989)
Winter offshore	25.4[a] (18.4–33.3)	10 m	4	No	Potential (max) rates	
Kaneohe Bay, HI (USA)	53.5[b] (7.2–100.0)	3 m	3–4	No	Time-series expt (every 0.5–0.75 h)	Harvey & Caperon (1976)
Upwelling						
Oregon & Washington coast (USA)	3.8[a] (2.0–7.2)	15 m	2–4	No	High nitrate stns (serial 0.5–1.0 h incubations)	Kokkinakis & Wheeler (1987)
Southern Benguela (S. Africa)						Probyn (1985)
Inshore	13.5[a] (0–19)[c]	Surface	4–6	No	High [NO_3^-]	
Shelf	13.7[a] (0–24)[c]	Surface	4–6	No	Low [NO_3^-]	
Namibian Coast	20[a] (8–44)	100–1%	4–6	Yes	Integrated rates	Probyn (1988)
Tasman Sea (New Zealand)						
Inshore	6.6[a] (1.8–12.0)	5–15 m	4–6	No	Mid-winter	Chang et al. (1992)
Offshore	104[a] (4.1–22.3)	5–15 m	4–6	No	(after upwelling)	
Estuarine						
Carmans River, NY (USA)	11.3[a] (8.4–13.7)	Surface	~2	No		Carpenter & Dunham (1985)
Great South Bay, NY (USA)						
Spring	53[a]	0–1 m	2–3	No	Shallow lagoon	Kaufman et al. (1983)
Summer	53[a]	0–1 m	2–3	No		
York River, VA (USA)					Serial, daytime	Webb & Haas (1976)
Summer	14–45[h]	1 and 4 m	2	No	incubations every 2 h	
Autumn	~5[h]	0.5 and 2 m	2	No	using ^{14}C-urea	

Table 3 (continued)

Chesapeake Bay (USA)						
Spring	37.2[a,e]	Near-surface	0.5	Yes		Bronk et al. (1998)
Summer	28.2[a,e]	Near-surface	0.5	Yes		
Autumn	11.8[a,e]	Near-surface	0.5	Yes	11.1% including nitrite	
Chesapeake Bay (USA)					Drogue study	Glibert et al. (1991)
Winter	34[a,c] (22–57)	Near-surface	1	Yes	February	
Spring	12[a,c] (6–19)	Near-surface	1	Yes	April	
Summer	33[a,c] (11–62)	Near-surface	1	Yes	June and August	
Chesapeake Bay (USA)	20.3[a,e]	75 and 50%	2–4	No	Annual mid-day rates	McCarthy et al. (1977)
Gulf of Bothnia (Sweden)						Cochlan & Wikner (1993)
Northern basin (high $[NO_3^-]$)	29.0[a] (24.9–33.1)	100–1%	6	No	Summer, daytime	
Southern basin (low $[NO_3^-]$)	62.8[a] (50.5–84.3)	100–1%	6	No	integrated rates	
Polar						
Northeast Water Polynya	21[a,g]	100–0.1%	24	No	Spring/summer integrated rates	Smith et al. (1997)
Northern Baffin Bay (Canada)	32[a] (7–53)	100–1%	24	No	Summer integrated rates	Harrison et al. (1985)
Barents Sea					Autumn/winter rates	Kristiansen & Lund (1989)
Large biomass layer	11[a] (SD = 9)	5–60 m	6–8	No	Fluorescent light used	
Small biomass layer	27[a] (SD = 25)	5–60 m	6–8	No	Fluorescent light used	
Barents Sea						Kristiansen & Farbrot (1991)
Ice algae	2–3[a]	–	6–8	No	Spring/summer rates	
Marginal Ice Zone (MIZ)	3[a] (SE = 1)	Subsurface chl max	6–8	No	Fluorescent light used	
South of MIZ	7[a] (0–20)	26–30 m	6–8	No	Fluorescent light used	
Barents Sea	2–13[a]	0.25–15 m	6–12	No	Average rates from 1984–1988	Kristiansen et al. (1994)
Barrow Strait (NWT, Canada)	26.7[a] (13.3–43.8)	Sea-ice; (bottom 3–5 cm)	1	No	Spring (blue-light incubations)	Harrison et al. (1990)
Bellingshausen Sea					Marginal Ice Zone	Bury et al. (1995)
Brown ice	58[a] (39–76)	–	24	No	Spring/summer	
Clear ice	7[a]	–	24	No	Spring/summer	
Water under ice	91[a]	–	24	No	Spring/summer	
Open water	14[a] (5–24)	2 m	24	No	Spring/summer	
Drake Passage	52[a]	100–0.1%	24	Yes	Spring/summer	Waldron et al. (1995)
Bellingshausen Sea MIZ	21[a]	100–0.1%	24	Yes	Regions 2 and 3	
Western Bellingshausen	16[a] (5–38)	100–0.1%	24	Yes	Region 4	
Gerlache Strait, Antarctica	3.5[a,d] (1.2–5.9)	0–50 m	24	No	Austral winter integrated rates	Cochlan et al. (1993)
Between Cape Ann & Mawson	27[a] (11–54)	1 m	5–9	Yes	Austral autumn	Probyn & Painting (1985)
Freshwater						
Lake Kinneret (Israel)	10[a] (3–37)	0–5 m	2–4	No	2 yr period *Peridinium* bloom	McCarthy et al. (1982)
Lake Biwa (Japan)	31.0[a] (21.2–43.5)	Surface	–	No	Artificial light	Mitamura (1986)
Lake Kasumigaura (Japan)	~20[b,f]	Surface	0.7–1	No	V vs S and V vs I experiments	Takamura et al. (1987)

[a]Absolute uptake rates
[b]Specific uptake rates
[c]Estimated from figures
[d]Conservative rates utilized in calculations of percentage of urea uptake
[e]Nitrite and/or DFAA uptake included in calculations
[f]Maximal uptake rates used in calculations
[g]Average values for study used in calculations
[h]Urea contribution based on Redfield C:N ratio of 6.6:1

parameters, and the reported atomic C:N ratio of 9:1 (Fig. 4). Fig. 4A provides the potential uptake of nitrogen at ambient concentrations of substrate (using the average concentration in the upper 10 m of the water column) versus irradiance. To determine these rates, we used Eq. (1) to calculate an ambient V value based on the kinetics parameters reported in Table 1 and the average nutrient concentration. We then used the calculated ambient V in Eq. (2) as V_s to determine ambient uptake versus irradiance (Fig. 4A). In Fig. 4B, the same data are presented as C:N assimilation ratios individually and for varying combinations of nutrient uptake, again as a function of irradiance.

Interpretation of these plots is somewhat difficult since the measurements were conducted at a single time point and we are assuming that the nutrient kinetics and uptake versus irradiance responses are both independent and representative of the population as a whole. However, several important points can be made. First, the low ambient concentrations of NO_3^- make this substrate the least utilizable form of nitrogen for this assemblage, while the ability to efficiently utilize urea and NH_4^+ at ambient concentrations and all light levels suggests a greater reliance on these reduced forms of nitrogen for growth. Second, if the assemblage were to maintain its atomic C:N ratio at 9:1, all of the nitrogen requirement could be met by urea assimilation alone. If the desired C:N ratio is closer to Redfield proportions (6.6:1), nitrogen demand could be met by utilizing urea subsidized with either NH_4^+ or NO_3^-. Third, exclusive reliance on NO_3^- assimilation could only meet the N demand at either extremely low or extremely high irradiance levels, while NH_4^+ utilization capacity falls between NO_3^- and urea. Reliance on urea or NH_4^+ of course requires a continuous supply of these nutrients, since at the cell abundance and nutrient concentrations measured the population could strip the water column of all the N in less than a day.

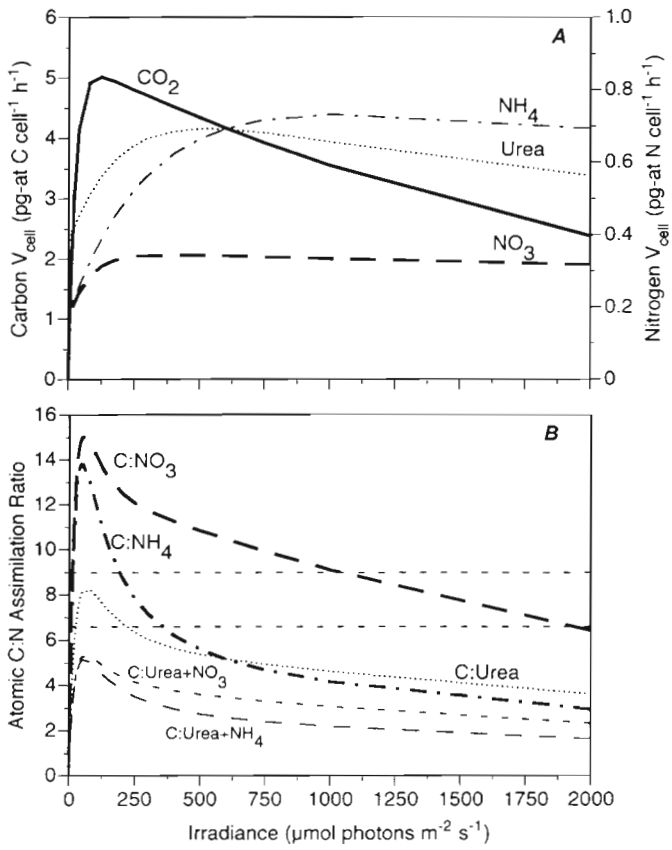

Fig. 4. (A) Uptake versus irradiance plots for all substrates are plotted using the calculated uptake values at ambient nutrient concentrations as described in the text. Note that N uptake parameters are on a separate y-axis. (B) Provides the same data plotted as C:N assimilation ratios versus irradiance for differing combinations of nitrogen. The dashed horizontal lines represent the measured atomic C:N ratio for the assemblage (9:1) and the Redfield ratio (6.6:1)

We observed an approximate doubling of cell number during the ca 24 h in which the samples were held in the laboratory, with an approximately 4-fold decrease in chlorophyll per cell (from 125 to 61 µg l^{-1} $cell^{-1}$; the relative proportion of phaeopigments did not change). Conditions in the culture room (25 µmol photons m^{-2} s^{-1}) were equivalent to considerably less than 5 m depth at noon for our collection site, using a zenith solar irradiance of 2300 µmol photons m^{-2} s^{-1}, 15% loss at the air/water interface (Kirk 1994), and the measured K_{PAR} value of 1.075 m^{-1}. Before and after the solar zenith, this depth would be proportionately shallower. Possible mechanisms for the decrease in cell pigmentation include photo-adaptation (since mean light levels in the culture room were constant and possibly higher than the average mixed-layer depth) and/or catabolization of the pigments as a nitrogen source to support growth. Final cell chl *a* levels (ca 35 pg chl *a* $cell^{-1}$) are consistent with the results reported by Prézelin & Sweeney (1979) for high- and low-light cultures (44 and 77 pg chl *a* $cell^{-1}$, respectively) and Prézelin & Matlick (1983) for N-limited (35 to 54 pg chl *a* $cell^{-1}$) and N-saturated (increase to ca 72 pg-at chl *a* $cell^{-1}$) cultures. In southern California, reported values for natural assemblages range from 22 to 38 pg chl *a* $cell^{-1}$ (Holmes et al. 1967), 114 pg $cell^{-1}$ at our study site, and with the presumed pigment concentrations of 260 pg chl *a* $cell^{-1}$ ($>2 \times 10^6$ cells l^{-1}, 519 µg chl *a* l^{-1}; Hayward et al. 1995). The growth rate suggests that the phytoplankton assemblage was not yet severely nutrient limited (typical growth rates range from 0.4 to 1.0 doublings d^{-1} in natural samples, ca 0.2 doublings d^{-1} in culture; Walsh et al. 1974, Eppley & Harrison 1975, Harrison 1976, Prézelin & Sweeney 1979). Unfortunately we do not have a direct estimate of the regeneration rates during this experiment. The presence of >50% of the plank-

tonic biomass as *Noctiluca scintillans*, and the extremely high total biomass combined with the potential for the catabolization of the chl *a* pool and the lack of inorganic nitrogen in the carboys suggest that all of the N demand *in vitro* was met by recycled nitrogen and/or a decrease in N per cell. Whether a similar mechanism occurred *in situ* is impossible to determine from these data, and we have no evidence for or against the *Lingulodinium polyedrum* population in this study augmenting their N nutrition by vertically migrating. However, based on the measured *f*-ratio and the ability of *L. polyedrum* to meet essentially all of its nitrogen requirements from regenerated sources, we believe that it is not necessary to invoke vertical migration from a nutritional standpoint.

Another trend reported in the literature that we could not corroborate in our study were the high K_s values for NO_3^- and NH_4^+ (Table 4), which are about 1 order of magnitude higher than the values reported here (Table 1). Harrison (1976) reported a K_s value for NO_3^- uptake in cultures that was similar to ours (ca 1 µg-at N), but went on to conclude that this 'low value' was likely due to poor growth rates in culture (0.2 d^{-1}). Because K_s values themselves are often misleading, we have also presented α_N values. Comparisons of our measured α_N values are similar to the historical estimates for NO_3^-, but are at the upper range of previously reported values. Since our α_N value for NO_3^- is the lowest of the 3 substrates tested, we again conclude that natural assemblages of *Lingulodinium polyedrum* can exhibit higher than expected (from literature values) affinities for reduced nitrogen. It is apparent that under stratified conditions where diatoms fare poorly (e.g. Margalef 1978), *L. polyedrum* is extremely competitive for both light and nitrogen.

Table 4. Kinetic parameters for nitrate uptake of *Lingulodinium polyedrum* from culture and natural assemblages. There were no literature values for ammonium and urea uptake (except NH_4^+ K_s = 5.5; Eppley et al. 1969). The V_{max} value for Dugdale (1979) is in units of h^{-1}, since no cell counts were provided. Units for the other parameters are the same as Table 1. Parameters from Harrison (1976) were estimated from his Fig. 6

V_{max}	K_s	α_N	Source
–	9.50	–	Eppley et al. (1969)
0.14	0.50	0.280	Dugdale (1979)
7.82	10.5	0.745	Harrison (1976)[a]
3.20	3.46	0.945	Harrison (1976)[a]
1.13	7.20	0.157	Harrison (1976)
4.38	15.1	0.290	Harrison (1976)
0.13–0.50	0.5–2.0	0.250–0.260	Harrison (1976)[b]
2.24	2.40	0.933	Balch (1987)
0.480	0.467	1.03	This study

[a]Values from Eppley et al. (1969) and Eppley (pers. comm.) as reported by Harrison (1976)
[b]Values from low growth rate (0.25 d^{-1}) cultures

Our K_s values for NO_3^-, NH_4^+ and urea suggest that *Lingulodinium polyedrum* is capable of competing with typical coastal phytoplankton such as diatoms. Those studies which have simultaneously examined uptake kinetics for urea, NO_3^- and NH_4^+ (Table 5) demonstrate that urea utilization is consistently in the same range as for NO_3^- and NH_4^+ in natural assemblages. Our study is no exception, and it is clear that there is no *a priori* reason for believing that *L. polyedrum* cannot compete (from a physiological perspective) with other coastal assemblages. The reported values are more in range with previously reported values for neritic diatoms and flagellates, with K_s (SD) = 1.66 (±0.07) and 1.82 (±0.09) µg-at N l^{-1} for NO_3^- and NH_4^+ respectively (Eppley et al. 1969). Our estimated half-saturation constants for urea are the first reported for *L. polyedrum* and are similar to the few values reported for cultures of marine neritic diatoms (0.4 to 2.0 µg-at N l^{-1}; McCarthy 1972, Rees & Syrett 1979), freshwater chlorophytes (generally 0.2 to 1.8 µg-at N l^{-1}; Healey 1977, Kirk & Kirk 1978), the freshwater cyanobacterium *Pseudoanabaena catenata* (0.8 µg-at N l^{-1}; Healey 1977), the picoflagellate *Micromonas pusilla* (0.4 µg-at N l^{-1}; Cochlan & Harrison 1991), and the neritic marine bacterium *Deleya venusta* (2.8 µg-at N l^{-1}; Jahns 1992). In contrast, much higher half-saturation constants have also been reported for cultures of the inshore diatom *Skeletonema costatum* (8.5 µg-at N l^{-1}; Carpenter et al. 1972), some freshwater chlorophytes (5 to 30 µg-at N l^{-1}; Syrett & Bekheet 1977, Williams & Hodson 1977, Kirk & Kirk 1978, Bekheet & Syrett 1979) and the lacustrine diatom *Melosira italica* (0.13 to 22 µg-at N l^{-1}; Cimbleris & Cáceres 1991).

Hayward et al. (1995) reported that the *Lingulodinium polyedrum* population was present in southern California coastal waters prior to the beginning of the heavy rainfall and associated runoff beginning in January 1995. They speculated that the peak biomass associated with our sampling period in March was likely triggered by the heavy rains just prior to this time, which, combined with the intense solar heating, caused strong stratification and isolation of the anthropogenic nutrients in the surface waters. Urea is a likely contaminant in heavily urbanized regions such as our study site (e.g. Antia et al. 1991) and was found at elevated ambient concentrations despite the demonstrated capacity for uptake by the dominant autotrophic species, *L. polyedrum*.

This opportunistic study clearly cannot evaluate the physiological condition and nutritional status of this bloom during either the formational period or the subsequent crash. Therefore, our results are more representative of a physiological 'snapshot' during a red

Table 5. Summary of the average nitrogen kinetic parameters — half-saturation constant (K_s) and maximum specific uptake rate (V_{max}) — determined for natural phytoplankton assemblages when urea uptake rates were determined. Range of study values are in parentheses

Area	Nitrate K_s (µg-at N l^{-1})	V_{max} (h^{-1})	Ammonium K_s (µg-at N l^{-1})	V_{max} (h^{-1})	Urea K_s (µg-at N l^{-1})	V_{max} (h^{-1})	Source
Oceanic							
Central North Pacific Gyre Subtropical (low [NO_3])	0.03	0.003	0.03	0.016	0.02	0.016	Sahlsten (1987)
Central North Pacific Gyre	0.24 (0.02–0.14)	–	0.055 (–0.14–0.13)	–	–0.017 (–0.35–0.20)	–	Eppley et al. (1977)
North Pacific							
Northern (J1–J7)	1.97[a]	1.11[b]	0.20[a]	2.79[b]	0.06[a]	0.60[b]	Kanda et al. (1985)
Tropical/Subtropical (J9–J23)	0.08[a]	0.58[b]	0.09[a]	3.48[b]	0.07[a]	1.82[b]	
Benguela Upwelling region	0.93	0.0082	0.10	0.0098	0.17	0.0041	Probyn (1985)
North Sea	–	–	–	–	0.27	0.002	Kristiansen (1983)
Coastal							
Oslofjord (Norway)	0.64 (0.1–1.0)	–	1.03 (0.1–2.0)	–	0.80 (0.2–1.8)	–	Paasche & Kristiansen (1982) Kristiansen (1983)
Southwest Finland	–	–	–	–	0.069–0.13[a]	0 037–0.094	Tamminen & Irmisch (1996)
Washington coast	0.05	0.0058	0.71	0.00682	0.78	0.0046	Dortch & Postel (1989)
Southern California							
Lingulodinium polyedrum bloom	0.467	0.0224	0.586[d]	0.0471	0.989	0.0616	This study
Western New Zealand							Chang et al. (1995)
Inshore	1.1	0.0138	0.5	0.0207	0.4–0.6	0 011–0.013	
Offshore	0.4	0.0104	0.4	0.0165	–	–	
Baltic Sea							
Nodularia spumigena bloom	0.22	–	0.02	–	0.27[c]	0.002	Sörensson & Sahlsten (1987)
Polar							
Barents Sea							Kristiansen et al. (1994);
[NO_3] ≥ 1 µg-at N^{-1}	1.8		1.3		0.2		Kristiansen & Farbrot (1991)
[NO_3] ≤ 1 µg-at N^{-1}	0.2		0.1		0.1		
Barrow Strait NWT Ice algae	–		1.60		0.94		Harrison et al. (1990)
Freshwater							
Lake Biwa (Japan)	2.59	0.0011	0.79	0.0094	0.73	0.0063	Mitamura (1986)
0.25 µm fraction during light	(0.82–6.87)	($4.0E^{-4}$ –$1.4E^{-3}$)	(0.20–1.81)	(0.0063–0.0124)	(0.10–2.01)	(0.003–0.0117)	
Lake Kasumigaura (Japan)							Takamura et al. (1987)
Microcystis bloom	3.90 (0.93–8.6)	0.033 (0.025–0.046)	9.34 (5.53–13.2)	0.15–0.17	0.75	0.040	

[a]$[K_s + S]$ values used when ambient $[S]$ is unknown, or known with poor precision
[b]Absolute rates used (ng-at N l^{-1} h^{-1})
[c]Value from North Sea used in this study
[d]Presented for comparison only, ambient $[S]$ too high for accurate determination of K_s

tide event. However, we are confident that these data clearly demonstrate that *Lingulodinium polyedrum* exhibits K_s values typical of other coastal species, and that this species is capable of utilizing a broad range of both nitrogen concentrations and light fluences. It is not necessary to invoke vertical migration accompanied by dark-N uptake to meet the nutritional demands of this assemblage. This study also demonstrates that *L. polyedrum* can readily utilize urea as a nitrogen source. Although this is not surprising, it nevertheless remains one of the few studies demonstrating that dinoflagellates and other red tide organisms make use of this potentially anthropogenic source of nitrogen for growth.

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