



Effects of light on nitrogen and carbon uptake during a *Prorocentrum minimum* bloom

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Abstract

During a 4-week period in late spring 1998 an extensive *Prorocentrum minimum* (Pavillard) Schiller bloom developed in several tributaries of the Chesapeake Bay. Experiments were carried out in one of these tributaries using ¹³C and ¹⁵N isotopic techniques to characterize C and N uptake as a function of irradiance during the course of this bloom. Uptake rates of N substrates (NO₃⁻, NH₄⁺, urea, and an amino acid mixture) and C substrates (bicarbonate and urea) were measured. For each N substrate, short-term uptake rates (0.5 h) were not substantially different over the irradiance range measured, suggesting that N uptake of this dinoflagellate was not strongly light-dependent over this time scale. Dark uptake rates of all N substrates ranged between 35 and 113% of light uptake rates. Over the duration of the *P. minimum* bloom, however, total ambient N uptake rates increased with increasing natural irradiance. Uptake of bicarbonate showed typical light-dependent photosynthetic characteristics and the measured photosynthetic parameters suggested that at least on the short time scale (0.5 h), *P. minimum* cells were adapted to high light. Rates of C uptake from the substrate urea were minimal, <1% of total C uptake from photosynthesis, but doubled over the course of the bloom, and like N uptake, were not strongly light-dependent on the short time scale (0.5 h). Significant N dark uptake by *P. minimum* was likely to have been important by providing N sources over the daily scale to sustain the bloom.

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1. Introduction

Prorocentrum minimum is a common bloom-forming photosynthetic dinoflagellate species in Chesapeake Bay, USA. Blooms of *P. minimum* in the Chesapeake Bay typically occur in the spring and summer (Tyler and Seliger, 1978; Glibert et al., 2001) and have been related to landward transport beneath

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the pycnocline from the southern to the northern Bay (Tyler and Seliger, 1978, 1981; Harding et al., 1983). Migrations from nutrient-poor surface water to the higher nutrient pycnocline region may assist this species in obtaining its nutrient requirement (Tyler and Seliger, 1981). In the tributaries of the Bay, where such blooms also are observed, nutrients from runoff may stimulate and maintain these blooms (Glibert et al., 2001; Fan et al., 2003). In the late spring of 1998, a massive *P. minimum* bloom was observed in several tributaries of the Chesapeake Bay region. Uptake of C and N were characterized as a function of irradiance on both short (0.5 h) and longer (days) time scales in one of the impacted tributaries, the Choptank River. The contribution of dark uptake of N, as well as the potential contribution of organic substrates to both the N and the C requirements of the blooms, was assessed.

Regulation of N uptake by light in dinoflagellates is quite variable. Paasche et al. (1984), in comparing the day/night responses of many dinoflagellates to N, found that *P. minimum* could utilize NO_3^- and NH_4^+ under both light and dark conditions, but many other species could not. Similarly, Kudela and Cochlan (2000) showed that the bloom-forming dinoflagellate *Lingulodinium polyedrum* (Stein) Dodge (formerly *Gonyaulax polyedra*) could take up the substrates NO_3^- , NH_4^+ , and urea in both light and dark, and that dark uptake accounted for about 50% of the light saturated values. Elevated dark-uptake values are often associated with N deficiency (Harrison, 1976) but MacIsaac (1978) also showed significant NO_3^- uptake values in light and dark environments when *L. polyedrum* (reported as *G. polyedra*) was NO_3^- sufficient.

The potential for organic substrates to supplement the N, as well as the C, requirements of bloom-forming dinoflagellates has been increasingly recognized (Granéli et al., 1989; Carlsson et al., 1999; Glibert et al., 2001; Mulholland et al., 2003). Dissolved organic N (DON) comprises a substantial portion of the total N available in estuarine environments (e.g. Correll and Ford, 1982; Sharp, 1983), and the increasing use of organic fertilizers in agricultural regions (Constant and Sheldrick, 1992; Glibert et al., 2004) may be leading to an increase in availability of organic nutrients in some estuaries and embayments. This high DON input may play a

role in the regulation of phytoplankton succession by fueling blooms of those species able to utilize it (Granéli et al., 1989; Glibert et al., 2001). The ability of dinoflagellates to use these sources and how they are regulated by light still remains largely unknown.

Dinoflagellate blooms are a worldwide phenomenon often associated with adverse environmental effects such as the depletion of oxygen, mortality of fish, and in some cases toxicity of fish and shellfish (Anderson, 1989; Smayda, 1990, 1997; Hallegraeff, 1993). While in some cases adverse conditions such as toxicity can develop when cell numbers remain in relatively low concentrations, in many other cases, blooms with high cell densities can lead to visual accumulations of biomass. These latter conditions result in unique stresses for the phytoplankton, both in terms of demand for nutrients and in the reduction in light availability due to developing self-shading. The effects of light on N and C dynamics during a massive *Prorocentrum minimum* (Pavillard) Schiller bloom are the focus of this study.

2. Materials and methods

2.1. Sampling and environmental parameters

In May 1998, the extensive bloom of *P. minimum* that developed in several tributaries of the Chesapeake Bay represented roughly 95% of the total phytoplankton cells (Glibert et al., 2001; Fan et al., 2003). Experiments were conducted on May 7, 11, 14, 18, and 21, covering the duration of this bloom. On each date samples were collected off the dock of the Horn Point Laboratory, University of Maryland Center for Environmental Science, located on the bank of the Choptank River (Fan et al., 2003). Surface whole water samples were collected using an acid-washed bucket, transferred into clean 20 L carboys (rinsed three times with sample water), and immediately transported to the laboratory for experimentation. All samples were collected at approximately 1300 local time. In situ irradiance was measured with a biospherical instruments QSL-100 light meter and temperature, pH, and salinity were measured with a YSI 85 handheld meter. Ambient nutrient samples, filtered through precombusted (2 h at 400 °C)

Whatman GF/F filters (nominal pore size = 0.7 μm), were stored frozen for further analysis as described below. Chlorophyll *a* and particulate N (PN) and particulate C (PC) samples (50 mL) were obtained by filtration onto precombusted GF/F filters. Cell counts were measured using a coulter counter (Coulter Multisizer II) in glutaraldehyde (1% final) preserved water samples. Samples were also examined microscopically.

2.2. N and C uptake versus irradiance experiments

Water samples were dispensed into 50 mL clear polycarbonate flasks within 15 min after transfer of the sample water to the laboratory. Experiments were then initiated by the addition of labeled ^{15}N and ^{13}C substrates. Four N substrates (NO_3^- , NH_4^+ , urea, and an amino acid mixture) were used to characterize N uptake as a function of irradiance. The amino acid mixture consisted of alanine (7.3%), arginine (6.8%), aspartic acid (9.5%), glutamic acid (10.4%), glycine (6.2%), histidine (1.9%), isoleucine (4.0%), leucine (10.6%), lysine (13.7%), methylamine (1.0%), phenylalanine (4.5%), proline (6.5%), serine (4.1%), threonine (4.6%), tyrosine (3.9%), and valine (5.1%). Each ^{15}N substrate (Cambridge Isotope Laboratories, all 99 atom % of ^{15}N) was added at saturating concentrations (20 μg atom $\text{N}\cdot\text{L}^{-1}$).

Two sources of C were used to characterize C uptake as a function of irradiance. Bicarbonate (HCO_3^-), labeled with ^{13}C (Cambridge Isotope Laboratories, 99 atom% enriched), was added to the same polycarbonate flasks that were enriched with ^{15}N substrates. The final concentration of $\text{H}^{13}\text{CO}_3^-$ addition was 300 μg atom $\text{C}\cdot\text{L}^{-1}$. In addition, another set of flasks was enriched with ^{13}C -urea (Cambridge Isotope Laboratories, 99 atom% enriched) with a final concentration of 20 μg atom $\text{C}\cdot\text{L}^{-1}$.

After the ^{15}N and ^{13}C substrate additions, the flasks were placed in a flowing water incubator. Neutral density screening was used to simulate the following light levels: 44, 22.4, 13, 5.7, 2.3, and 1.2% of natural irradiance. In addition, one set of flasks was kept unwrapped to yield 100% of natural irradiance and another set was wrapped with several layers of aluminum foil to yield a dark treatment. The incubations were 0.5 h, and terminated when the samples were returned to the laboratory and filtered

onto pre-combusted GF/F filters. Filters were placed in a drying oven (50 °C, 24 h) then processed at a later time by mass spectrometry (Finnigan Mass Spectrometer Model 251, Finnigan MAT Inc.).

2.3. Analytical methods

Ambient concentrations of inorganic nutrients were determined using a Technicon AutoAnalyzer (Lane et al., 2000) and urea was analyzed manually using the urease method (Parsons et al., 1984). Concentrations of dissolved free amino acids (DFAA) were measured using the technique of Lindroth and Mopper (1979). Concentrations of PN and PC were determined using a control equipment CHN analyzer using acetanilide as a standard. Concentrations of dissolved inorganic carbon (DIC) were measured using the gas-stripping/GC method of Stainton (1973). Samples for chlorophyll *a* were ground in 90% acetone on ice and concentrations were determined fluorometrically on a Turner designs model 10 fluorometer calibrated against an HPLC measured chlorophyll standard (Van Heukelem et al., 1994).

2.4. Uptake versus irradiance parameters

Specific uptake rates of N (V , h^{-1}) were calculated according the equation of Dugdale and Goering (1967). Absolute uptake rates (ρ) were calculated by multiplying V by the PN values and uptake rates were further normalized to cell-absolute uptake rate by dividing by cell density. Comparable calculations were made for C uptake.

Several mathematical models, ranging from simple equations to multi-parameter formulations, have been developed to describe photosynthesis as a function of irradiance (Platt and Gallegos, 1980; Cullen, 1990; Frenette et al., 1993). In this study, photosynthetic parameters were calculated by using the Platt and Gallegos (1980) equation:

$$P = P_{\max} \left(1 - \exp\left(\frac{-\alpha E}{P_{\max}}\right) \right) \left(\exp\left(\frac{-\beta E}{P_{\max}}\right) \right) \quad (1)$$

where P_{\max} is the estimate of photosynthetic capacity, E the irradiance, and α and β are the respective light-limited and light-inhibited slopes of the uptake versus irradiance curve. Values of α and β are expressed in

the unit of $(\text{pg atom-C cell}^{-1} \text{h}^{-1})/(\mu\text{mol photons m}^{-2} \text{s}^{-1})$. The conventional index of light adaptation (E_k) is determined as P_{max}/α and has the units of $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

To calculate the parameters of N uptake versus irradiance, a modification of Eq. (1) was used:

$$\rho = \rho_{\text{dark}} + \rho_{\text{max}} \left(1 - \exp\left(\frac{-\alpha E}{\rho_{\text{max}}}\right) \right) \times \left(\exp\left(\frac{-\beta E}{\rho_{\text{max}}}\right) \right) \quad (2)$$

A positive y-intercept (ρ_{dark}) was added, the parameter P was replaced by ρ , and P_{max} was replaced by ρ_{max} . In this case, values of α and β are expressed in the unit of $(\text{fg atom-N cell}^{-1} \text{h}^{-1})/(\mu\text{mol photons m}^{-2} \text{s}^{-1})$. A software package (Jandel Scientific SigmaPlot) was used for the computerized, iterative calculation for the fitted parameters.

3. Results

3.1. Ambient environmental conditions

The 1998 bloom of *P. minimum* in the Chesapeake Bay and its tributaries was unusually large (Fan et al., 2003). During the 3-week study period, the cell density of *P. minimum* reached $1.16 \times 10^8 \text{ cells L}^{-1}$ (Table 1), representing ~95% of the total cell count. Chlorophyll *a* concentrations were sustained above $\sim 50 \mu\text{g L}^{-1}$, and on one date concentrations of chlorophyll *a* reached $498 \mu\text{g L}^{-1}$ (Table 1; Fan et al., 2003). At this high chlorophyll *a* concentration physical concentrating mechanisms likely contributed to the high biomass accumulation.

Over the bloom duration, ambient nutrient concentrations were relatively low. The concentrations of NO_3^- ranged from 0.31 to $3.14 \mu\text{g atom-N L}^{-1}$, NH_4^+ and urea ranged from 0.64 to $1.08 \mu\text{g atom-N L}^{-1}$ and 0.16 to $0.53 \mu\text{g atom-N L}^{-1}$, respectively, and DFAA varied from 0.12 to $0.17 \mu\text{g atom-N L}^{-1}$ (Table 1). Concentrations of PO_4^{3-} were $< 0.3 \mu\text{g atom-P L}^{-1}$ (Table 1). Weather conditions during the bloom period were mostly calm (Chesapeake Bay Observing System, <http://www.cbos.org>) and the incident irradiance varied from 863 to $1875 \mu\text{mol}$

$\text{photons m}^{-2} \text{s}^{-1}$. Surface water temperatures increased from 15.7 to 23°C (Table 1).

3.2. N uptake as a function of irradiance and bloom duration

Relationships between N uptake rates and irradiance varied both with substrate and date (Fig. 1). Only 9 out of 20 N uptake versus irradiance experiments, representing results from only three of the five sampling dates, could be fitted to Eq. (2) with an $r^2 > 0.5$ (Table 2). For all experiments and substrates, rates of dark uptake ranged from 35 to 113% of ρ_{max} , which suggests that N uptake by this assemblage was not strongly light-dependent on the short time scale (0.5 h). For the three sampling dates for which parameters for the nitrogen uptake versus irradiance relationship were calculated (Table 2), the parameter α , the initial slope of the uptake versus irradiance response, was highest for the uptake of NH_4^+ and amino acids and lowest for NO_3^- . The values of β (light-inhibited slope of the N uptake) were about one order of magnitude less than α , suggesting a very small photo-inhibition effect. The values of E_k for all the N substrates ranged from 11 to $138 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 1.2 to 16.6% of the average incident irradiance.

The values of ρ_{max} for NH_4^+ , urea, and the amino acid mixture increased from the first two sampling dates to the third and fourth sampling date, but declined by the fifth sampling date (Fig. 1). In contrast, the values of ρ_{max} for NO_3^- did not change significantly with bloom duration. The overall means of ρ_{max} of NH_4^+ and the amino acid mixture for all dates combined were not significantly different ($P = 0.76$, *t*-test), averaging $362.3 \pm 178.4 \text{ fg atom-N cell}^{-1} \text{h}^{-1}$ ($n = 24$) and $374.4 \pm 188.1 \text{ fg atom-N cell}^{-1} \text{h}^{-1}$ ($n = 16$), respectively. In all experiments, rates of ρ_{max} of urea were lower than those of NH_4^+ and the amino acid mixture. The lowest uptake rates at all times were observed for NO_3^- at $35.6 \pm 20.9 \text{ fg atom-N cell}^{-1} \text{h}^{-1}$ ($n = 32$).

The N uptake rates for this population at ambient substrate levels have previously been estimated based on uptake kinetics (Fan et al., 2003). Rates of total N uptake at ambient concentrations (as the sum of uptake of NH_4^+ , NO_3^- , urea, and amino acids) were found to increase over the course of the bloom (Fig. 2A). They

Table 1
Summary of environmental data for the dates indicated in the 1998 study of *P. minimum*

Dates	NO ₃ ⁻ (μg atom-N L ⁻¹)	NH ₄ ⁺ (μg atom-N L ⁻¹)	Urea (μg atom-N L ⁻¹)	Amino acids (μg atom-N L ⁻¹)	PO ₄ ³⁻ (μmol-P L ⁻¹)	PN (μmol-N L ⁻¹)	PC (μmol-C L ⁻¹)	pH	Temp (°C)	Chl <i>a</i> (μg L ⁻¹)	Cell count (cells L ⁻¹)	Light photons m ⁻² s ⁻¹)
7 May	0.31	0.64	0.16	0.15	0.29	264	2255	9.0	15.7	498	1.16E ⁺⁸	863
11 May	3.14	1.03	0.50	0.12	0.17	77.9	685	9.0	16.8	49.2	1.53E ⁺⁷	913
14 May	2.24	0.95	0.44	0.12	0.15	65.0	607	9.0	19.5	48.7	1.59E ⁺⁷	1278
18 May	1.91	1.08	0.53	0.12	0.17	70.1	601	9.5	21.7	73.1	1.64E ⁺⁷	1875
21 May	2.10	0.98	0.44	0.17	0.28	76.4	668	9.0	23.0	97.2	1.83E ⁺⁷	1743

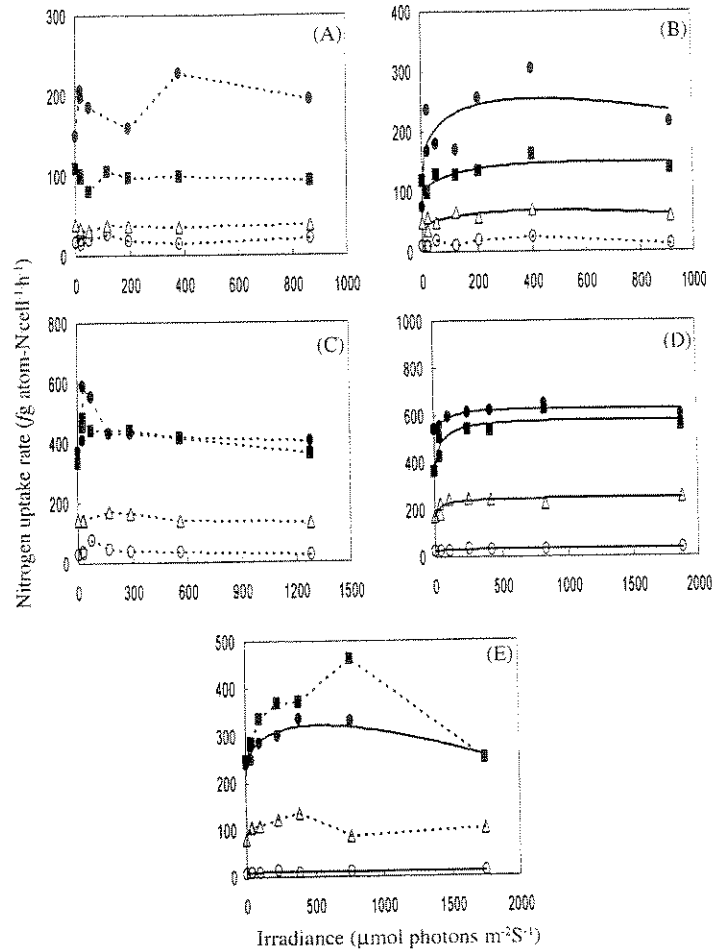


Fig. 1. Rates of uptake of NO_3^- (○), NH_4^+ (●), urea (△), and amino acids (■) under N-saturated conditions, as a function of irradiance, for the natural *Proocentrum minimum* bloom studied. Each panel represents one date: (A) 7 May, (B) 11 May, (C) 14 May, (D) 18 May, and (E) 21 May. The results that could be fit to Eq. (2) with an $r^2 > 0.5$ are shown in solid lines; other results are connected with dashed lines.

varied with average ambient irradiance levels that were relatively low ($\sim 500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) at the beginning of the bloom and increased over the course of the bloom (Fig. 2B). Thus, while on the short time scale (0.5 h) of the incubation experiments reported here uptake rates were not strongly light-dependent, over the time scale of the 3-week bloom uptake rates increased with light intensity.

3.3. C uptake as a function of irradiance and bloom duration

In contrast with N uptake, short-term inorganic C uptake rates were light-dependent during the bloom,

and with the exception of the results of 11 May, could be fit to Eq. (1) with an $r^2 \geq 0.88$ (Fig. 3 and Table 3). On 11 May, there was no evidence of uptake saturation within the irradiance range tested. The difference between these rates and those traditionally determined in photosynthetic experiments is that these measurements were made in the presence of N enrichments. There was no significant difference in the rates of C uptake for the *P. minimum* bloom enriched with any of the N substrates, with one exception. On the fourth sampling date, C uptake rates in the NH_4^+ treatment were significantly lower than the rates of the other N enrichment treatments ($P < 0.01$, $N = 16$; t -test. Fig. 3). Excluding 11 May data, values of P_{max} (for all

Table 2

Calculated parameters for N (NO_3^- , NH_4^+ , urea, and a mixed amino acids substrate) uptake vs. irradiance for the 1998 study of *Prorocentrum minimum*

Date and N enrichment	ρ_{dark} (fg atom-N cell ⁻¹ h ⁻¹)	ρ_{max} (fg atom-N cell ⁻¹ h ⁻¹)	α (fg atom-N cell ⁻¹ h ⁻¹ /μmol photon m ⁻² s ⁻¹)	β (fg atom-N cell ⁻¹ h ⁻¹ /μmol photon m ⁻² s ⁻¹)	E_k (μmol photon m ⁻² s ⁻¹)	r^2
11 May						
NH ₄ ⁺	76 (54.4)	226 (59.7)	13.5 (7.7)	1.01	11	0.56
Urea	45 (6.8)	64 (8.4)	0.2 (0.1)	0.02	94	0.51
Amino acids	105 (9.7)	149 (13.6)	0.3 (0.1)	0.02	138	0.69
18 May						
NH ₄ ⁺	534 (14.1)	625 (15.9)	0.7 (0.5)	0.05	123	0.87
NO ₃ ⁻	25 (2.1)	33 (2.4)	0.1 (0.1)	ND ^a	125	0.76
Urea	171 (17.4)	245 (19.1)	1.3 (0.8)	0.01	57	0.76
amino acids	366 (38.1)	567 (41.8)	3.1 (1.9)	0.04	61	0.83
21 May						
NH ₄ ⁺	233 (28.3)	305 (31.2)	0.8 (0.5)	0.07	91	0.53
NO ₃ ⁻	7 (1.0)	11 (1.1)	0.1 (0.1)	ND ^a	83	0.72

Only those data that could be fit to Eq. (2) with a coefficient of determination $r^2 > 0.5$ are shown. Standard errors are given in parentheses.

^a ND means β is < 0.01 .

treatments) increased from a mean of 3.40 pg atom-C cell⁻¹ h⁻¹ 7 May to 8.82 pg atom-C cell⁻¹ h⁻¹ on 18 May, but then decreased to 6.51 pg atom-C cell⁻¹ h⁻¹ on 21 May (Table 3). Values of α ranged from 0.014 to 0.099 pg atom-C cell⁻¹ h⁻¹ during the course of this bloom. The decline in P_{max} was correlated with a decline in α (Fig. 4). That chlorophyll per cell likely changed as the bloom

aged is indicated in the comparison on the trajectory of rate of change in the relationship between α and P_{max} , when these parameters are expressed on a volume basis (Fig. 4A) and on a cellular basis (Fig. 4B). Values of β were very low compared to those of α , suggesting little photoinhibition. Values of E_k ranged from 86 to 341 μmol photons m⁻² s⁻¹ and represented 7 to 26% of incident irradiance (Table 3).

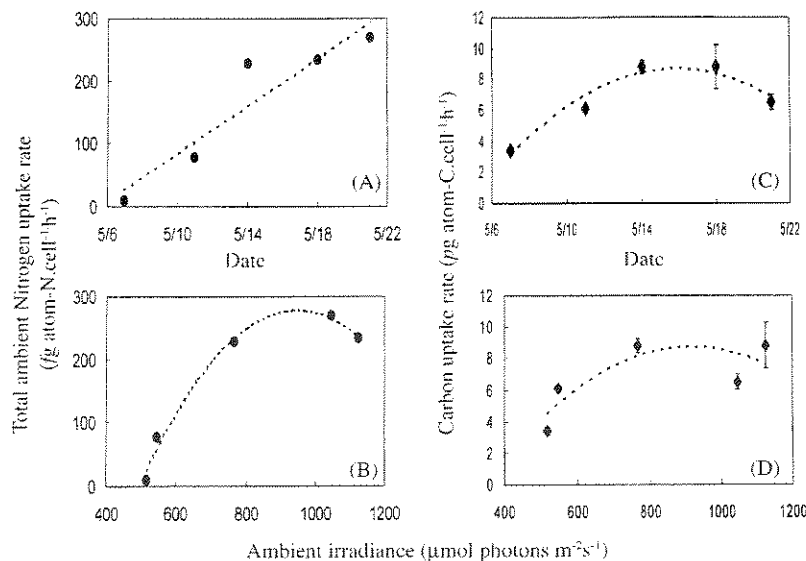


Fig. 2. Total ambient N uptake rates under natural irradiance (panels A–B) and HCO_3^- uptake rates (panels C–D) as function of the duration of the bloom and irradiance for the natural *Prorocentrum minimum* bloom studied. Error bars represent standard error.

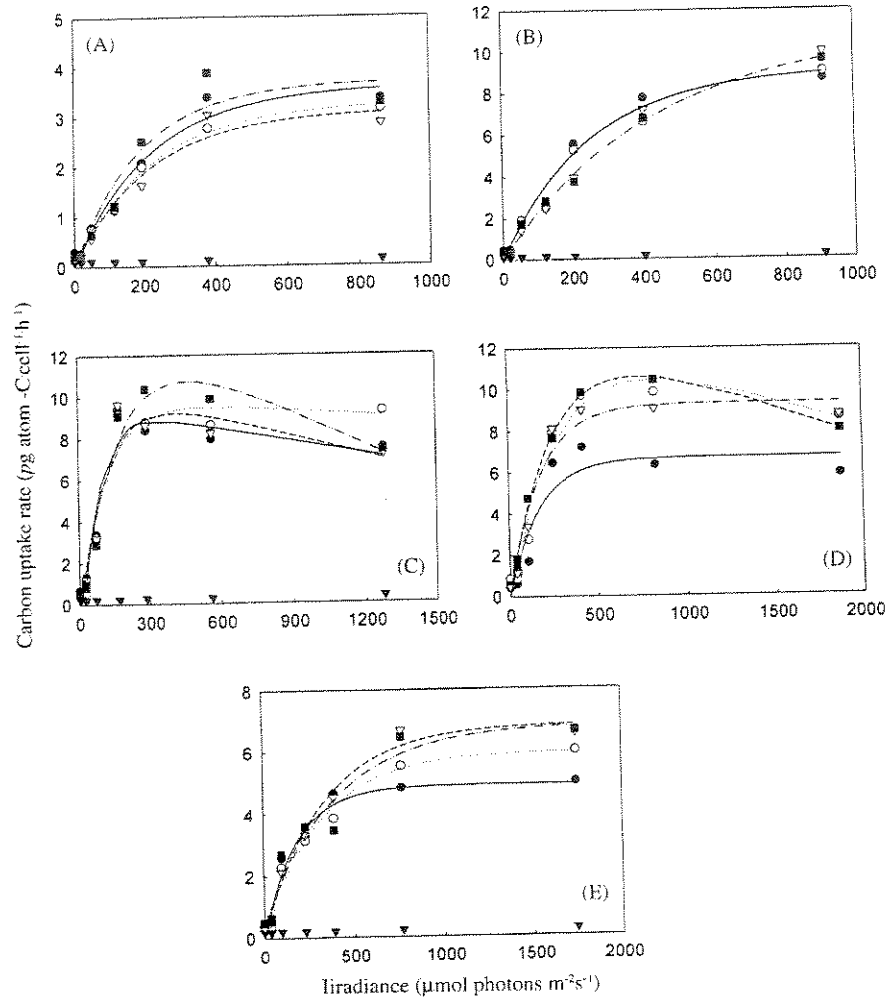


Fig. 3. Rates of uptake of C (as HCO_3^-) as a function of irradiance for the natural *Prorocentrum minimum* bloom studied. Rates were determined in the presence of saturating levels of N: NO_3^- (○), NH_4^+ (●), urea (▽), and amino acids (■). Rates of uptake of C-urea as a function of irradiance are also given (▼). Each panel represents one date of sampling: (A) 7-May, (B) 11-May, (C) 14-May, (D) 18-May, and (E) 21-May.

Organic C uptake from urea increased at the highest test irradiances from an average of $0.12 \text{ pg atom-C cell}^{-1} \text{ h}^{-1}$ on 7 May to a maximum of $0.28 \text{ pg atom-C cell}^{-1} \text{ h}^{-1}$ on 14 May, before declining to $0.22 \text{ pg atom-C cell}^{-1} \text{ h}^{-1}$ on 21 May. Like the relationship between N uptake and irradiance, urea-C uptake on a short-term scale did not follow a light-dependent pattern as there was uptake in the dark. Nevertheless, on all dates, urea-C accounted for <1% of HCO_3^- uptake at the high light levels tested (Fig. 3).

4. Discussion

Species that form high biomass blooms under natural condition raise many important physiological questions, particularly with respect to light adaptation. The ability of *P. minimum* to sustain itself in low light has been well studied (e.g. Harding et al., 1983; Coats and Harding, 1988; Harding, 1988). Results from this study further explored the relationships between C uptake and light for this species and have provided the first examination of the

Table 3
Calculated parameters for C (as HCO_3^-) uptake vs. irradiance for the 1998 study of *Prorocentrum minimum*

Date	P_{\max} (pg atom-C cell $^{-1}$ h $^{-1}$)	α (pg atom-C cell $^{-1}$ h $^{-1}$ /μmol photons m $^{-2}$ s $^{-1}$)	β (pg atom-C cell $^{-1}$ h $^{-1}$ /μmol photons m $^{-2}$ s $^{-1}$)	E_k (μmol photons m $^{-2}$ s $^{-1}$)	r^2
7 May					
NH $_4^+$	3.47 (0.24)	0.019 (0.003)	5.31E-4	190	0.98
NO $_3^-$	3.28 (0.15)	0.015 (0.002)	4.45E-4	219	0.99
Urea	3.12 (0.26)	0.014 (0.003)	5.09E-4	215	0.96
Amino acids	3.72 (0.41)	0.019 (0.005)	5.55E-4	191	0.94
14 May					
NH $_4^+$	8.58 (0.82)	0.090 (0.029)	1.09E-3	96	0.89
Urea	8.54 (0.89)	0.099 (0.037)	8.79E-4	86	0.89
Amino acids	9.32 (1.09)	0.068 (0.029)	1.17E-3	137	0.90
18 May					
NH $_4^+$	6.68 (0.73)	0.041 (0.013)	9.37E-4	161	0.88
NO $_3^-$	9.74 (0.82)	0.051 (0.013)	8.33E-4	192	0.93
Urea	9.59 (0.60)	0.062 (0.012)	1.06E-3	156	0.95
Amino acids	9.27 (0.53)	0.057 (0.010)	8.96E-4	161	0.97
21 May					
NO $_3^-$	5.97 (0.33)	0.019 (0.003)	3.70E-4	313	0.98
Urea	6.84 (0.31)	0.021 (0.003)	4.03E-4	323	0.98
Amino acids	6.71 (0.64)	0.020 (0.005)	3.11E-4	341	0.94

Uptake rates were measured in the presence of the N substrate indicated, as described in text. Standard errors are given in parentheses. Data for the 11 May sampling period were not saturated, and could not be fitted to the model.

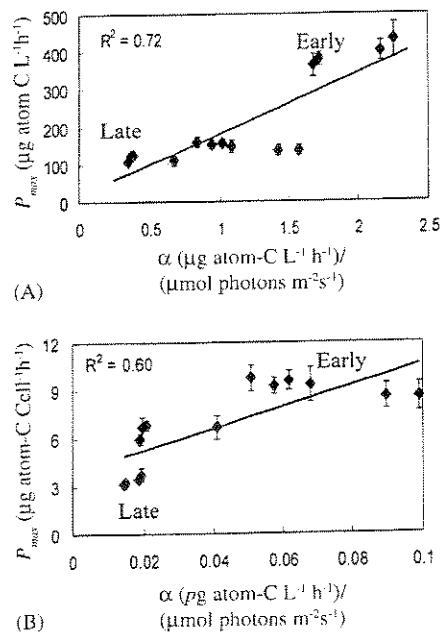


Fig. 4. Relationship between the carbon uptake parameters P_{\max} vs. α for the *Prorocentrum minimum* bloom studied. Panel (A) is expressed in volumetric units and panel (B) in cell-specific units. 'Early' and 'Late' refer to stages in bloom development. Error bars represent standard error.

relationships between N uptake and light under bloom conditions.

4.1. Significant dark N uptake

The ability of phytoplankton to take up N in darkness is species dependent and is also a function of the physiological state of the cell (Eppley and Harrison, 1975; Harrison, 1976). Dark uptake of N by cells migrating vertically to nutrient rich waters at the pycnocline has been suggested as an important mechanism for sustaining blooms. Dark uptake of N has been observed in some dinoflagellate-dominated populations in field studies (Dortch and Maske, 1982; Kudela and Cochlan, 2000) but findings depend on the state of the cells at the time of sampling. For example, MacIsaac (1978) found that the uptake of NO_3^- and NH_4^+ in a natural population of *Lingulodinium polyedrum* (reported as *Gonyaulax polyedra*) off the coast of California was strongly dependent on light while Kudela and Cochlan (2000) found little light dependence in a natural bloom of the same species. Other studies have shown significant differences between species. Paasche et al. (1984) found a range of responses to light by seven dinoflagellate species

Table 4

Comparison of the parameters for C (as HCO_3^-) uptake vs. irradiance for the natural bloom of *Prorocentrum minimum* from this study with the same species from other studies

	P_{\max} ($\mu\text{g atom cell}^{-1} \text{h}^{-1}$)	α ($\mu\text{g atom cell}^{-1} \text{h}^{-1} / \mu\text{mol photons m}^{-2} \text{s}^{-1}$)	E_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
Field data (this study)			
7 May	3.39 (0.26)	0.017 (0.002)	196 (22.8)
14 May	8.81 (0.44)	0.086 (0.016)	106 (19.1)
18 May	8.82 (1.44)	0.053 (0.009)	167 (14.4)
21 May	6.51 (0.47)	0.020 (0.001)	325 (14.8)
Field studies (Harding and Coats, 1988)			
Surface mixed layer	12.2 (2.8)	0.152 (0.043)	238 (45)
Subpycnocline	16.3 (5.9)	0.193 (0.046)	90 (12)
Laboratory time-course studies (Harding, 1988)			
Highlight	NA ^a	0.059 (0.026)	136 (22)
Low light	NA ^a	0.116 (0.041)	68 (4)

Data are the averages for all treatments for each sampling date; standard errors are given in parentheses.

^a P_{\max} data for laboratory time-course studies (Harding, 1988) were not available, however, based on the P-I figure plotted as α vs. P_{\max} , the P_{\max} values were in the same range those reported here.

grown under laboratory conditions. Of those species, *P. minimum* and *Karenia mikimotoi* (reported as *Gyrodinium aureolum*) displayed extreme responses: *P. minimum* continued to take up N in the dark under all physiological states while *K. mikimotoi* had nearly an absolute light requirement for N uptake (Paasche et al., 1984).

Consistent with the laboratory study by Paasche et al. (1984), these results demonstrated that uptake of N in the dark was sustained at high rates by the *P. minimum* population over short-term incubation periods. There were significant dark uptakes for all N substrates tested and dark uptake accounted for 35–113% of light saturated uptake rates in this study. It is important to note, however, that short-term (0.5 h) uptake may not necessarily reflect nocturnal uptake rates as long-term (hours) exposure to darkness may alter physiological status.

During this *P. minimum* bloom, ambient N concentrations in near surface waters were not sufficient to support even a doubling of biomass (Table 1), indicating that some additional sources of nutrients must have been available. The ambient uptake rates previously measured for this bloom (Fan et al., 2003) demonstrated that NH_4^+ was the primary nitrogen source supporting the bloom, that the respective contributions of urea and NO_3^- represented 2–10% and <20% of the nitrogen taken up, and that the contribution of NO_3^- decreased and that of NH_4^+

increased as the bloom progressed. One such source to support the high demand for NH_4^+ may have been high rates of N regeneration from the sediments. A study in the same area showed that NH_4^+ regeneration from sediments approximated $7 \mu\text{g atom-N L}^{-1} \text{d}^{-1}$ (Kemp et al., 1990). This value is about 40% of ambient NH_4^+ uptake during this bloom. Rates of water column regeneration were not measured in this study but likely also served as a source of nutrients.

In this study, except for the last set of experiments, N uptake by *P. minimum* for all tested substrates showed no obvious photo-inhibition on a short-term basis and C uptake showed only modest photo-inhibition on one sampling date. Lomas et al. (2000), in a study exploring rates of N uptake and release following a rapid shift from low light to highlight, showed that *P. minimum* sustained net NH_4^+ uptake even after the rapid shift in irradiance.

Table 5

Comparison of the molar particulate C:N ratio and the molar ratio of the uptake rate of C:N at ambient substrate concentrations during the 1998 natural *Prorocentrum minimum* bloom studied

Date of sampling	PC:PN	C:N uptake ratio
7 May	8.55	33.4
11 May	8.80	9.6
14 May	9.33	4.7
18 May	8.57	4.4
21 May	8.73	2.3

4.2. C uptake versus irradiance

Inorganic C uptake by *P. minimum* was strongly light-dependent as would be expected for photosynthesis. Photosynthetic parameters can be compared with other results on *P. minimum* studies from the Chesapeake Bay (e.g. Harding and Coats, 1988; Table 4). The previous studies demonstrated that *P. minimum* adjusts its photosynthetic apparatus to increase photosynthetic performance within several days of exposure to low light and α increases as much as three to four-fold within the same period. Values of α values can be viewed as an index of the physiological adaptation to low light conditions. In the present study, α values ($\text{pg atom cell}^{-1} \text{h}^{-1} / \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for C uptake were variable (from 0.017 to 0.086) but were in the range of previously reported values (Table 4). Furthermore, α values for this study were comparable to highlight adapted laboratory culture values (Harding, 1988) but were lower than previously reported low light adapted field and laboratory populations (Harding, 1988; Harding and Coats, 1988). Thus, despite the high cell density of the bloom, cells appeared to be adapted to high light for most of the bloom duration, at least for the mid-day period when experiments were conducted.

An important distinction must be made between these studies and previous measurements of C uptake by natural *P. minimum* populations. In these studies, rates of C uptake were measured with added N substrates. The effect of NH_4^+ pulsing on C uptake has previously been examined in laboratory cultures (Goldman et al., 1981; Turpin, 1983; Elrif and Turpin, 1985; Goldman and Dennett, 1985) but rarely in the field. While under some conditions for some clones, suppression of C uptake, up to 50% in 15 min incubations, was observed (e.g. Turpin, 1983); most species that have been examined did not show any response to added N (Goldman and Dennett, 1985). A significant suppression in the NH_4^+ enriched treatment was observed in these experiments on 18 May, while mild suppression (but not statistically significant) was also observed on 21 May (Fig. 3D,E). Elrif and Turpin (1985) suggested that photosynthetic suppression upon NH_4^+ addition would be greatest at low relative growth rate. Our observations would be consistent as we only observed this phenomenon late in the bloom.

The kinetics of N uptake have been previously reported for this *P. minimum* bloom (Fan et al., 2003)

so it is possible to calculate the ambient C:N uptake ratio under natural irradiance over the course of the bloom. While the *P. minimum* particulate C:N ratios remained relatively consistent over the bloom period (8.8 ± 0.32) and within $\sim 30\%$ of the Redfield ratio (6.6), the C:N uptake ratio decreased as the bloom progressed, from 33.4 to 2.34 (Table 5). The highest C:N uptake ratio was observed during the first sampling date when ambient irradiance was low and when ambient N concentrations were also at their lowest level during the study. The decrease in the C:N uptake ratio with bloom duration was mainly a function of the increase in nitrogen uptake rates over the course of the bloom. Even though the carbon uptake rate under in situ irradiance increased ca. two-fold, the ambient nitrogen uptake increased from ca. 10 to 270 $\text{fg atom-N cell}^{-1} \text{h}^{-1}$ (Fig. 2). As these ratios are based on short-term uptake rates, measurements as a function of time of day would be required to determine daily integrated C:N uptake ratios. It appears that to balance the high C:N uptake ratios of the early bloom period, another N source is required, such as dark uptake, while during the latter stages of the bloom, another C source would be necessary.

4.3. Organic C and N uptake

The role of organic nutrients in sustaining some HABs has been recognized (Granéli et al., 1989; Kondo et al., 1990; Berg et al., 1997; Lomas et al., 2001; Anderson et al., 2002; Glibert et al., 2004), and the evidence is quite strong that this is the case for *P. minimum* (Heil et al., 2004). In the field Granéli et al. (1989) showed that dinoflagellate populations, including *P. minimum*, were stimulated by inorganic N only when added in combination with humic acids and Stonik (1995) observed growth in *P. minimum* in the Japan sea following the discharge of organic material. Carlsson et al. (1999) found that humic additions could stimulate the growth of *P. minimum* and Heil (2004) demonstrated that stimulations in growth of *P. minimum* by humic and fulvic acids were significant but dependent on the initial growth conditions of the *P. minimum*. Stoecker et al. (1997) have also documented mixotrophic feeding in *P. minimum* populations in Chesapeake Bay.

The direct measurements of the uptake of organic substrates urea and amino acids reported here show

that DON was used during this bloom. While the ρ_{\max} of amino acids was one of the highest of the N substrates measured, it contributed only a relatively small proportion of total uptake because of the low ambient concentration of amino acids. Urea contributed from 2 to 10% of total N uptake during the bloom (Fan et al., 2003) but sharply elevated levels of urea were noted immediately preceding the bloom (Glibert et al., 2001).

The role of DOC in sustaining this bloom is difficult to assess. During the latter part of this bloom, pH values in the range of 9–9.5 were observed (Table 1), suggesting that HCO_3^- limitation was potentially developing. While ^{13}C -urea uptake was <1% of C uptake from HCO_3^- , there is some evidence that it was a source of carbon to the cells. First, the uptake of ^{13}C -urea was sustained in the dark while that of HCO_3^- was obviously not. Furthermore, on the scale of the evolution of the bloom, C uptake from urea increased over two-fold as the bloom progressed. Lastly, the molar ratio of C:N uptake from urea, based on the ^{13}C and ^{15}N uptake rates, averaged 2.3 indicating greater retention of C than N. Other potential sources of DOC were not estimated but it is unlikely that urea was the only DOC source being used.

In summary, results from this and a previous study (Fan et al., 2003) have yielded insight into the environmental parameters influencing growth of *P. minimum*. N uptake was not strongly light-dependent on the short-term scale for this *P. minimum*-dominated bloom but was related to light on over the duration of the bloom. Significant dark N uptake and uptake of organic sources by *P. minimum* were the likely mechanisms providing the N and C substrates required to sustain the bloom.

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