# VERTICAL VARIATION IN NITRATE UPTAKE BY NATURAL POPULATIONS OF RESERVOIR PHYTOPLANKTON

By

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POPULATIONS OF RESERVOIR PHYTOPLANKTON

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ABSTRACT

Variation in the vertical profiles of $NO_3^-$ uptake in Lake Carl Blackwell was the result of the interaction between light intensity and $NO_3^-$ concentration. At high ambient $NO_3^-$ levels the profile of $NO_3^-$ uptake was controlled by light intensity, and uptake was not light saturated at the highest subsurface light intensities at which observations were made. At low ambient $NO_3^-$ concentrations, uptake rates were $NO_3^-$ limited in the upper part of the euphotic zone and were light limited below the depth to which 10% of incident light penetrated. Michaelis-Menten kinetics were observed in two of the three experiments, and $K_s$ values were similar to those obtained for eutrophic regions of the sea.

# INTRODUCTION

The $^{14}C$ method is used widely as a measure of primary production in freshwater and marine ecosystems (Steeman-Nielsen 1952). Any other major element in the phytoplankton could be used similarly. A logical choice would be nitrogen, since cell growth consists largely of protein and nucleic acid synthesis (Dugdale and MacIsaac 1971). In algae, the ratio of nitrogen (N) to carbon (C) to phosphorus (P) is relatively constant. However, since C and P are constantly being turned over in energetic processes, growth measurements with C and P might be expected to show more variability than with N (Dugdale and Goering 1967). A stable isotope ($^{15}N$) of N is readily available in a variety of compounds and can be detected by mass spectroscopy after conversion to $N_2$. Thus, N appears to be an excellent element to use in measuring primary production.

Several workers have studied the uptake of $^{15}N$- labeled nitrate ($NO_3^-$) and ammonium ($NH_4^+$) by phytoplankton. With the exception of the pioneer studies in lakes (Dugdale and Dugdale 1965) and recent work in reservoirs (Toetz et al. 1973), almost all N uptake work has been in the sea. No work has been done on N productivity where <u>in situ</u> incubation was used at ambient nutrient levels. Instead, the emphasis has been on the kinetics of uptake as a function of substrate concentration.

Dugdale (1967) suggested that the uptake of inorganic N could be described by the Michaelis-Menten equation:

$$v = \frac{V_{max} \times S}{K_s + S} \qquad (1)$$

Where v = uptake velocity

$V_{max}$ = maximum uptake velocity

S = concentration of substrate

$K_s$ = S at $1/2 \cdot V_{max}$

Thus, the relationship between v and S is hyperbolic, and v can be predicted if S, $K_s$ (the half-saturation constant), and $V_{max}$ are known. Michaelis-Menten kinetics for $NO_3^-$ and $NH_4^+$ uptake were shown for a number of marine algae in culture (Eppley, Rogers, and McCarthy 1969) and similar kinetics were observed for natural assemblages of marine phytoplankton (MacIsaac and Dugdale 1969, Eppley et al. 1973) and reservoir plankton (Toetz et al. 1973).

Since the $^{15}N$ tracer method involves the addition of labeled nutrients, it is not possible to measure v at ambient nutrient levels. In the past, in situ uptake rates have been approximated by limiting N enrichment to 10% or less of the ambient concentration (Dugdale and Goering 1967, MacIsaac and Dugdale 1972). At times it is necessary to use greater enrichment to increase the $^{15}N$ content of the particulate fraction to levels detectable with mass spectroscopy. Consequently, uptake velocities reported in the literature are overestimates of the in situ rates, because v increases as S increases. However, Equation 1 can be used to back-calculate v at ambient nutrient levels if S, $V_{max}$, and $K_s$ are known. Thus, to get an accurate assessment of N productivity at ambient nutrient levels, $K_s$, S and $V_{max}$ must be measured concurrently with an in situ vertical profile of N uptake rates.

No data on the uptake of N in the euphotic zone exist for lakes.

Dugdale and Goering (1967) compared the vertical profile of $^{15}N\text{-}NO_3^-$ uptake with $^{14}C\text{-}CO_2$ assimilation in the sea and concluded that uptake of inorganic N was closely coupled to photosynthesis. Although Dugdale and Goering drew samples from several depths in the euphotic zone, all were incubated under either constant artificial or natural surface radiation. This incubation procedure did not approximate the quantity or quality of light normally encountered by algae in the euphotic zone. Samples were enriched to levels 5-20% above ambient $NO_3^-$ concentrations, and no in situ uptake rates were calculated.

MacIsaac and Dugdale (1972) studied the interactions of light and inorganic N concentration in controlling N uptake in eutrophic and oligotrophic regions of the sea. They took water samples at depths to which 100, 50, 25, 10 and 1% of the surface light penetrated and uptake rates were measured for both $^{15}N\text{-}NO_3^-$ and $^{15}N\text{-}NH_4^+$. Incubation was conducted on-board ship, and neutral density filters were used to approximate the light intensity at the depth from which the samples were drawn. It is certain that these filters did not duplicate the wavelength-selective absorption of light that occurs in water (Ruttner 1972). Wallen and Geen (1971 a, b, c) demonstrated that changes in light quality significantly affect the rate and end products of photosynthesis and N metabolism. MacIsaac and Dugdale back-calculated in situ uptake rates from enhanced rates, but the $K_s$ values used in the calculations had often been measured on another date at other stations.

Dugdale (1967) proposed a mathematical model for N limitation in the sea that was used by MacIsaac and Dugdale (1972) in describing the interaction of light, N concentration, and N uptake. Dugdale divided the euphotic zone into an upper region, which has ample light but is

nutrient limited, and a lower region which receives a sufficient supply of N from the rich waters below but is light limited. This model was based on data derived from subtropical and tropical seas where the euphotic zone often extends to 100 m. However, in lakes, particularly turbid Great Plains impoundments, the euphotic zone is much narrower and rarely exceeds 4 m. Since the thermocline is usually deeper than 4 m in reservoirs, the model of a two-layered system does not apply. Thus, another model is necessary to describe adequately the relationship between nutrients, light, and phytoplankton in lakes.

The purpose of the present study is to describe the vertical profile of $NO_3^-$ uptake using in situ incubation to estimate uptake rates at ambient nutrient levels. Light intensity, nutrient concentrations, and biomass were determined at the depths of incubation to identify the environmental factors controlling uptake.

## MATERIALS AND METHODS

Three experiments were conducted at Lake Carl Blackwell on 30 July and 14 and 21 August, 1974. Depths corresponding to 90, 50, 25, 10 and 1% of incident surface radiation were determined with a submersible photometer the day prior to the experiment and after incubation had begun on the day of the experiment.

Fifteen liters of water were collected from each depth using a non-toxic Kemmerer bottle. Water from each depth was poured through 0.202 mm nylon mesh into polypropylene carboys to remove the large zooplankton. The carboys were taken to a lakeside laboratory where lake water was prepared for incubation. The excess water was used for the determination of $NO_3^-$, $NO_2^-$, $NH_4^+$, particulate nitrogen (PN), orthophosphate ($PO_4^{+3}$), algal density, and biomass of chlorophyll *a*.

An unlabeled control, two levels of $^{15}N$-$NO_3^-$ enrichment (treatments 1 and 2), and a dark bottle were prepared for each depth. The control consisted of a 1 ℓ bottle containing approximately 800 ml of unlabeled lake water. The average $^{15}N$:$^{14}N$ ratio of the five controls was used as an estimate of the natural abundance of $^{15}N$ in the seston. Treatment 1 consisted of duplicate water samples enriched with 99% $^{15}N$-$KNO_3$ to a level not more than 10% above the ambient $NO_3^-$ concentration as determined the day prior to the experiment. Treatment 2 consisted of duplicate water samples enriched with 99% $^{15}N$-$KNO_3$ to a level approximating 1000% of the ambient $NO_3^-$ concentration. The dark bottle contained 800 ml of lake water enriched with 99% $^{15}N$-$KNO_3$ to a level approximating

1000% of the ambient concentration. Each incubation bottle for treatments 1 and 2 was prepared by adding a measured volume of lake water from each depth to a brown 4 ℓ bottle containing the appropriate amount of $^{15}N\text{-}KNO_3$. The brown 4 ℓ bottle was agitated to achieve complete mixing and approximately 800 ml was poured into each of the duplicate incubation bottles.

An additional series of four bottles was incubated at the depth corresponding to 50% of surface radiation. These bottles contained 800 ml of lakewater which was spiked with $^{15}N\text{-}KNO_3$ at concentrations 20, 30, 100 and 300% above the ambient $NO_3^-$ concentration. Thus, a sufficient number of $NO_3^-$ concentrations were available at this depth to determine whether uptake followed Michaelis-Menten kinetics.

After all bottles were capped and wired into steel incubation frames, the bottles were covered with a black shroud to prevent exposure to direct sunlight. The incubation frames were suspended by chains from a 2.5 $m^2$ plywood and styrofoam raft. The chains were hung from two boards extending 1 m beyond the raft to prevent shading by the raft. The bottles corresponding to light intensities of 50, 10 and 1% were suspended from the south side of the raft, and the remainder of the bottles were suspended from the north side.

After incubation for 24 h, the racks were removed from the water, and all bottles were poisoned with 10 ml of Lugol's solution to terminate the experiment. The bottles were returned to the laboratory and stored for not more than 48 h before the contents of each bottle were filtered. The seston in each bottle was retained on Reeve Angel 984 H Ultra Glass Fiber Filters which had been muffled at 450° C for 2 h. Filters were dried and placed in a desiccator.

A Coleman Nitrogen Analyzer II was used to convert the combined N on the filters to $N_2$ gas, which was stored in pyrex breakseal tubes to await mass spectroscopy (Barsdate and Dugdale 1965). A high resolution mass spectrometer was used to determine the $^{15}N:^{14}N$ ratio of the samples. Calculation of uptake followed Neess et al. (1962) and Dugdale and Goering (1967).

Particulate nitrogen in the seston was determined in triplicate at each depth by passing measured aliquots of unlabled lake water from each carboy through Reeve Angel 984 H Ultra glass fiber filters which had been muffled at $450^{o}$ C for 2 h. The filters were dried and stored in a desiccator prior to digestion according to Holm-Hansen (1968). The filtrate resulting from seston removal was passed through a 0.45 μ membrane filter and stored on ice in a polyethylene bottle for chemical analysis. Nitrate, $NO_2^-$, and $PO_4^{+3}$ were determined in triplicate according to Strickland and Parsons (1968). Ammonium was determined in triplicate by the method of Solórzano (1969). Ammonium and $PO_4^{+3}$ were analyzed within 4 h after filtration. Nitrate and $NO_2^-$ were analyzed within 24 h. A measured aliquot of unlabeled lake water from each depth was filtered onto Reeve Angel 984 Ultra glass fiber filters for determination of chlorophyll *a* (Strickland and Parsons 1968). The modified equations of Parsons and Strickland (1963) were used to calculate pigment concentrations. A sample of water from each depth was preserved with Lugol's solution and phytoplankton were enumerated using the method of McNabb (1960).

## AREA DESCRIPTION

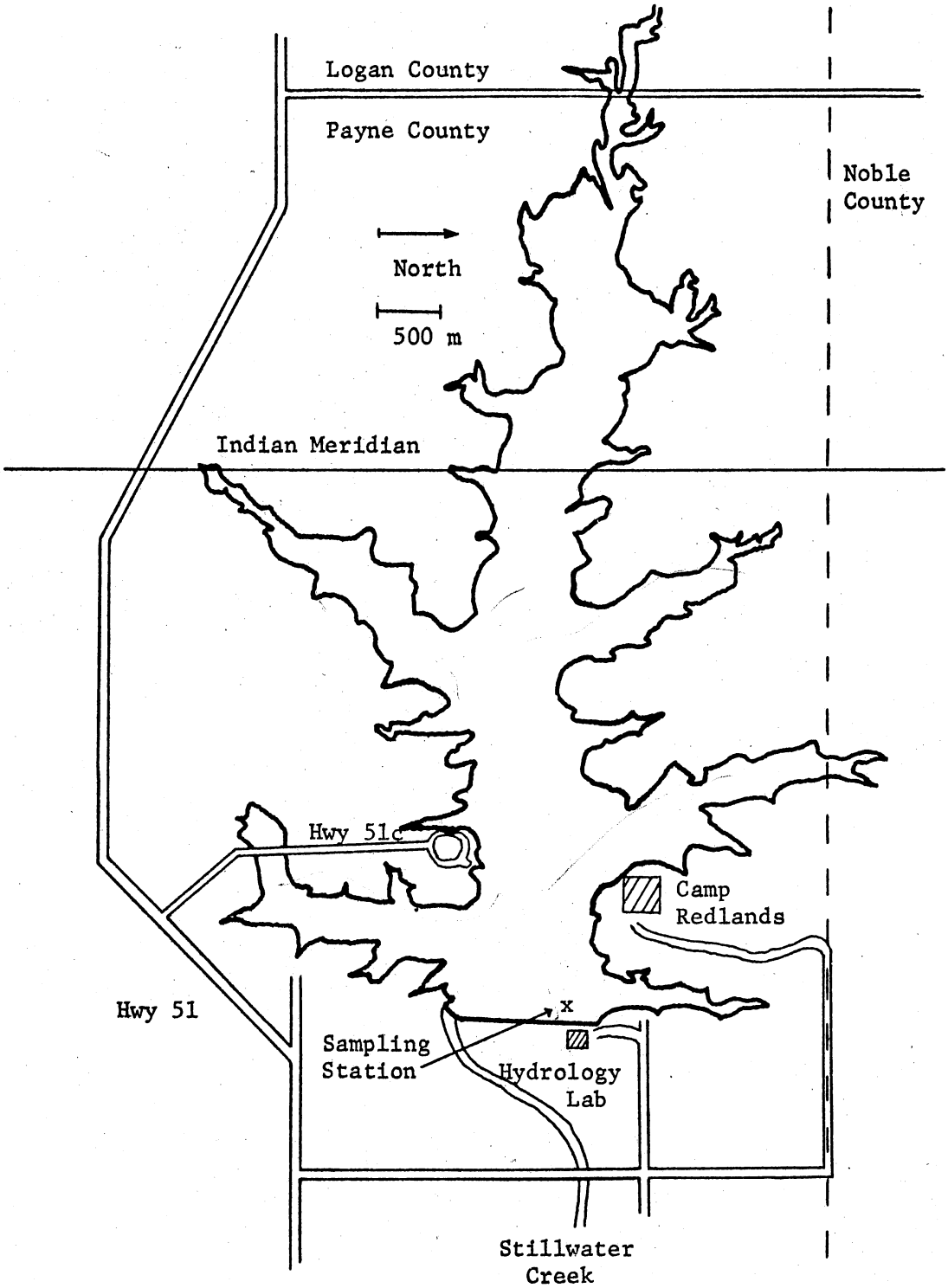
Lake Carl Blackwell (Figure 1) is an impoundment on Stillwater Creek, located 11 km west of Stillwater, Payne County, Oklahoma. Construction began in 1936 as a Works Progress Administration project, and the earth and rock dam was completed in 1938. Maximum surface area of approximately 1486 ha with a volume of 75 million $m^3$ was reached in 1945 at a spillway elevation of 228.4 m, msl. Fear that the dam might wash out prompted reconstruction and enlargement of the spillway in 1948. The spillway elevation was lowered to 287.7 m, msl, reducing surface area to 1355 ha and volume to 68 million $m^3$. Originally constructed for recreation, the reservoir has also served as the domestic water source for Stillwater since 1950.

Water level in the reservoir had been receding since 1961 due to low rainfall and increased municipal demand; it dropped to 283 m, msl in 1968. Only minor fluctuations occurred until 1973, when heavy rainfall returned the lake to spillway elevation. Wind-induced wave action suspends particles of montimorillonite clay, keeping the water turbid most of the time. Since the rise in lake level in 1973, turbidity has been lower than usual.

The main body of the lake lies on an east-west axis with several broad arms extending north and south from the old stream channel. The gently rolling hills surrounding the lake provide little shelter from the prevailing winds. Consequently, thermal stratification occurs only sporadically during the summer, when wind velocity is low and air

temperature is high. The weak thermocline which develops is easily destroyed by wind action, and the lake mixes from surface to bottom.

Figure 1. Lake Carl Blackwell, Payne County, Oklahoma.

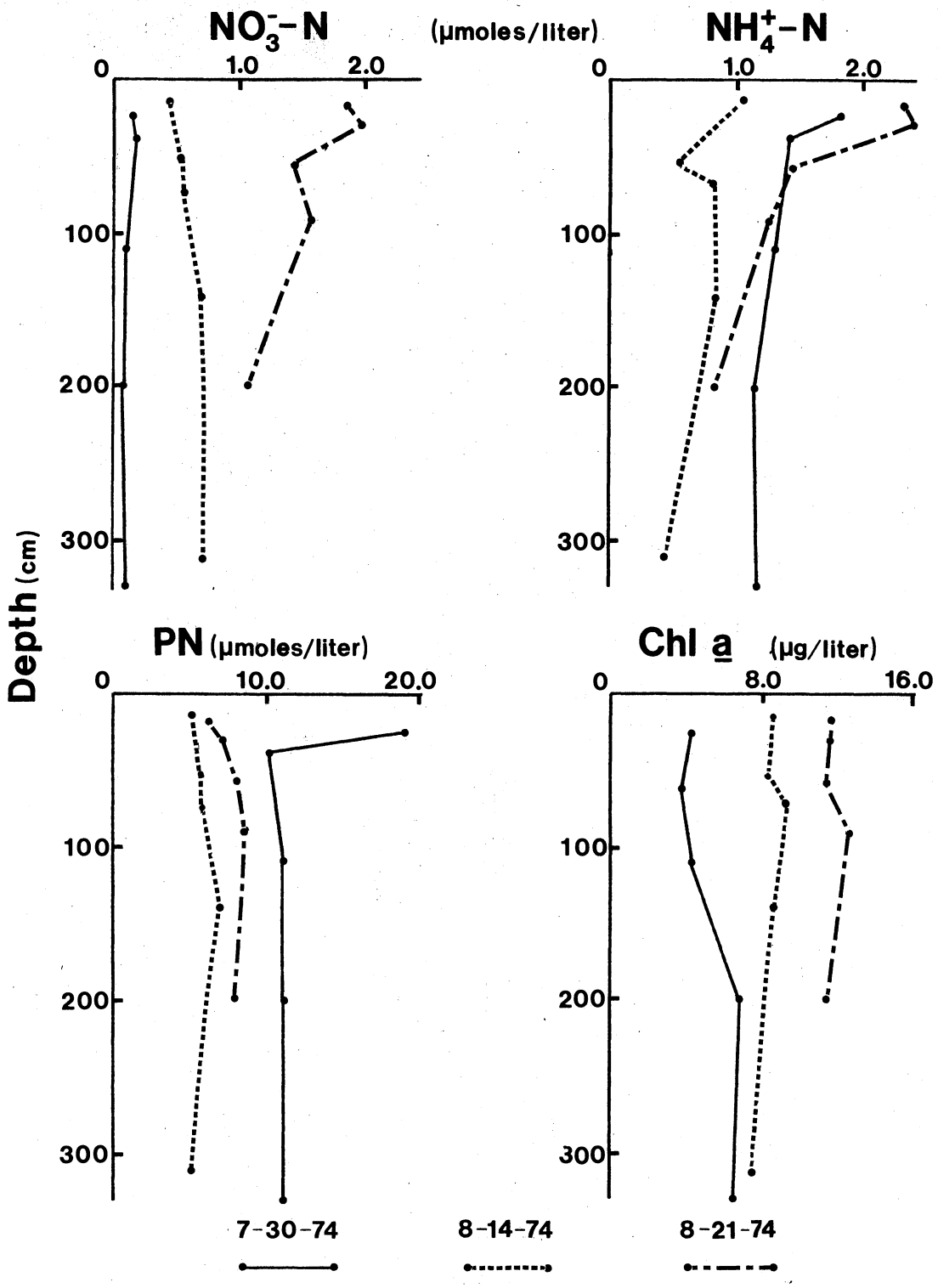

Logan County
Payne County
Noble County
North
500 m
Indian Meridian
Hwy 51c
Camp Redlands
Hwy 51
x
Sampling Station
Hydrology Lab
Stillwater Creek

## RESULTS

The vertical profiles of $NO_3^-$, $NH_4^+$, PN, and chlorophyll *a* are illustrated in Figure 2. The mean (n = 15, averaged over depth) PN concentrations among experiments were significantly different (p = 0.05). The greatest PN concentrations were observed during the first experiment and the least during the second experiment. No significant vertical differences (p = 0.05) were found in the mean (n = 3) PN concentration during the second and third experiments. The mean PN concentration (19.1 μmoles N/liter) observed at a depth of 25 cm during the first experiment was significantly greater than at other depths on that date and was probably due to floating detritus trapped in the sampling device.

The greatest algal biomass was observed during the third and the least during the first experiment, as indicated by chlorophyll *a* concentrations and algal density. Replicate determinations of chlorophyll *a* were not made during the first experiment and an AOV was not possible. However, visual inspection of the vertical profile (Figure 2) illustrates an orthograde distribution. The lack of coincidence in minimum and maximum biomass of PN and chlorophyll *a* indicates a variable detritus component in the PN. The centric diatoms *Melosira* sp. and *Coscinodiscus* sp. were the dominant algae in each experiment. The uniform vertical distribution of PN and chlorophyll *a* implied the presence of a single well-mixed phytoplankton population, and vertical differences in phytoplankton biomass are of no value in explaining

Figure 2. Vertical profiles of nitrate ($NO_3^-$-N), ammonium ($NH_4^+$-N), particulate nitrogen (PN), and chlorophyll *a* (Chl *a*) concentrations on the dates of the three experiments in Lake Carl Blackwell.


$NO_3^-$–N (µmoles/liter)
0
1.0
2.0
$NH_4^+$–N
0
1.0
2.0
Depth (cm)
100
200
300
PN (µmoles/liter)
0
10.0
20.0
Chl a (µg/liter)
0
8.0
16.0
7-30-74
8-14-74
8-21-74

changes in uptake with depth.

The mean (n = 15, averaged over depth) $NO_3^-$ concentration was significantly different (p = 0.05) among experiments. The ambient $NO_3^-$ concentrations increased during the study with mean (n = 15, averaged over depth) concentrations of $0.12 \pm 0.03$ (SD), $0.53 \pm 0.04$, and $1.57 \pm 0.06$ µmoles $NO_3^-$-N/liter from the first to the third experiment. The only significant vertical differences (p = 0.05) in the mean (n = 3) $NO_3^-$ concentration were found in the third experiment. The mean (n = 15, averaged over depth) $NH_4^+$ concentration was significantly different between the second and third experiments. In the third experiment the mean (n = 6) $NH_4^+$ concentration at the depths corresponding to 90% and 50% of incident light was significantly (p = 0.05) greater than the mean (n = 9) $NH_4^+$ concentration at the depths corresponding to 25, 10 and 1% of incident light. No other significant vertical differences were found in $NH_4^+$ concentrations. Detectable levels of $NO_2^-$ and $PO_4^{+3}$ were observed only during the third study with mean (n = 15) concentrations of $0.17 \pm 0.05$ (SD) and $0.25 \pm 0.04$ µmoles/liter, respectively. No significant vertical differences (p = 0.05) were found in the mean (n = 3) concentrations of $NO_2^-$ and $PO_4^{+3}$. Thus, vertical variation in uptake rates cannot be explained by vertical differences in nutrient concentrations.

Temperature and dissolved oxygen profiles indicate that the euphotic zone was not stratified during the experiments. The thermocline was located 0.5 m below the deepest incubation depth in the first experiment and approximately 2 m below in the other two experiments.

The greatest transparency of the water was observed in the first experiment and the least in the third study (Figure 3). The maximum

Figure 3. Vertical profiles of light penetration on the dates of the three 1974 experiments in Lake Carl Blackwell. Data points represent incubation depths.

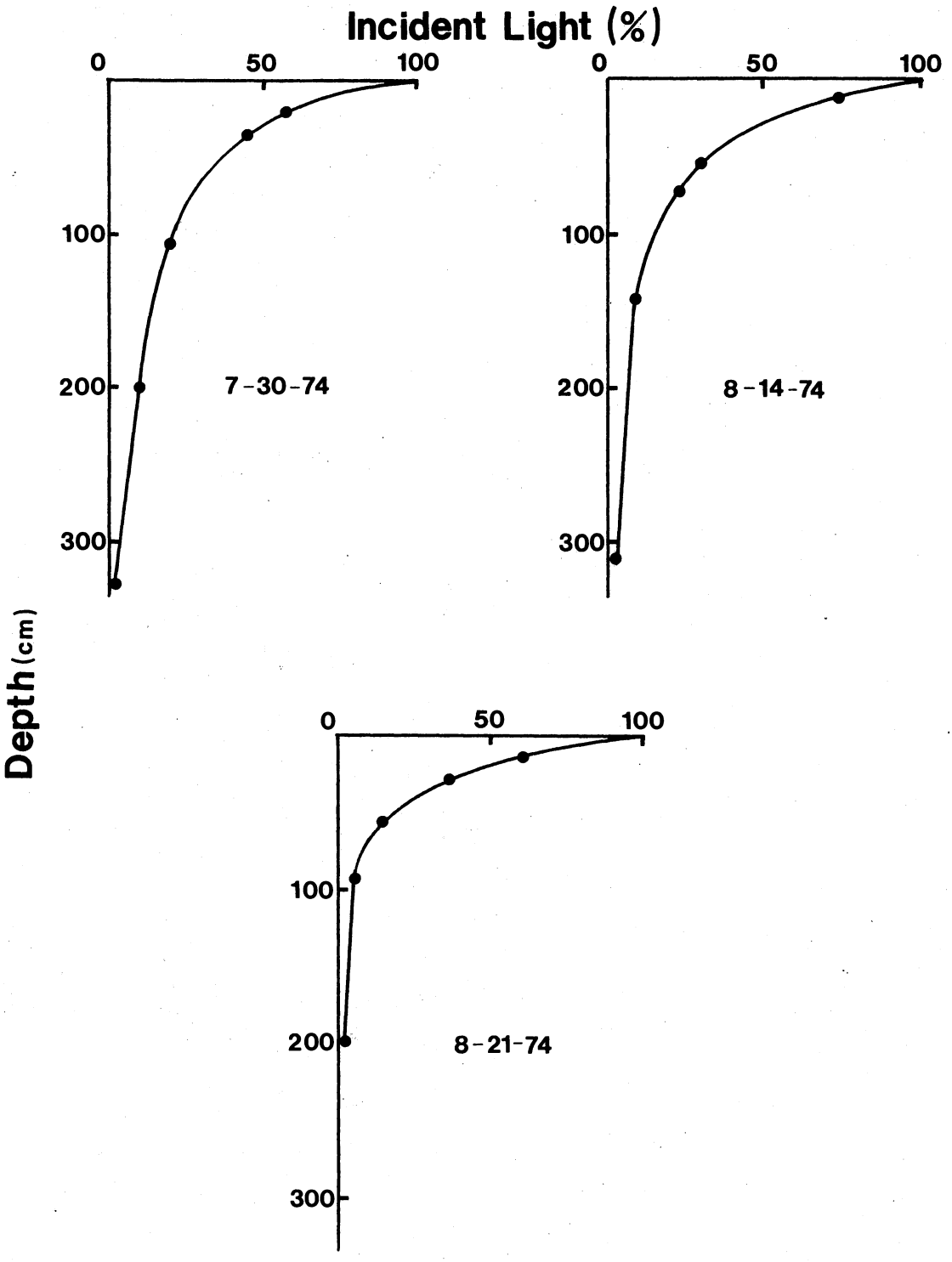
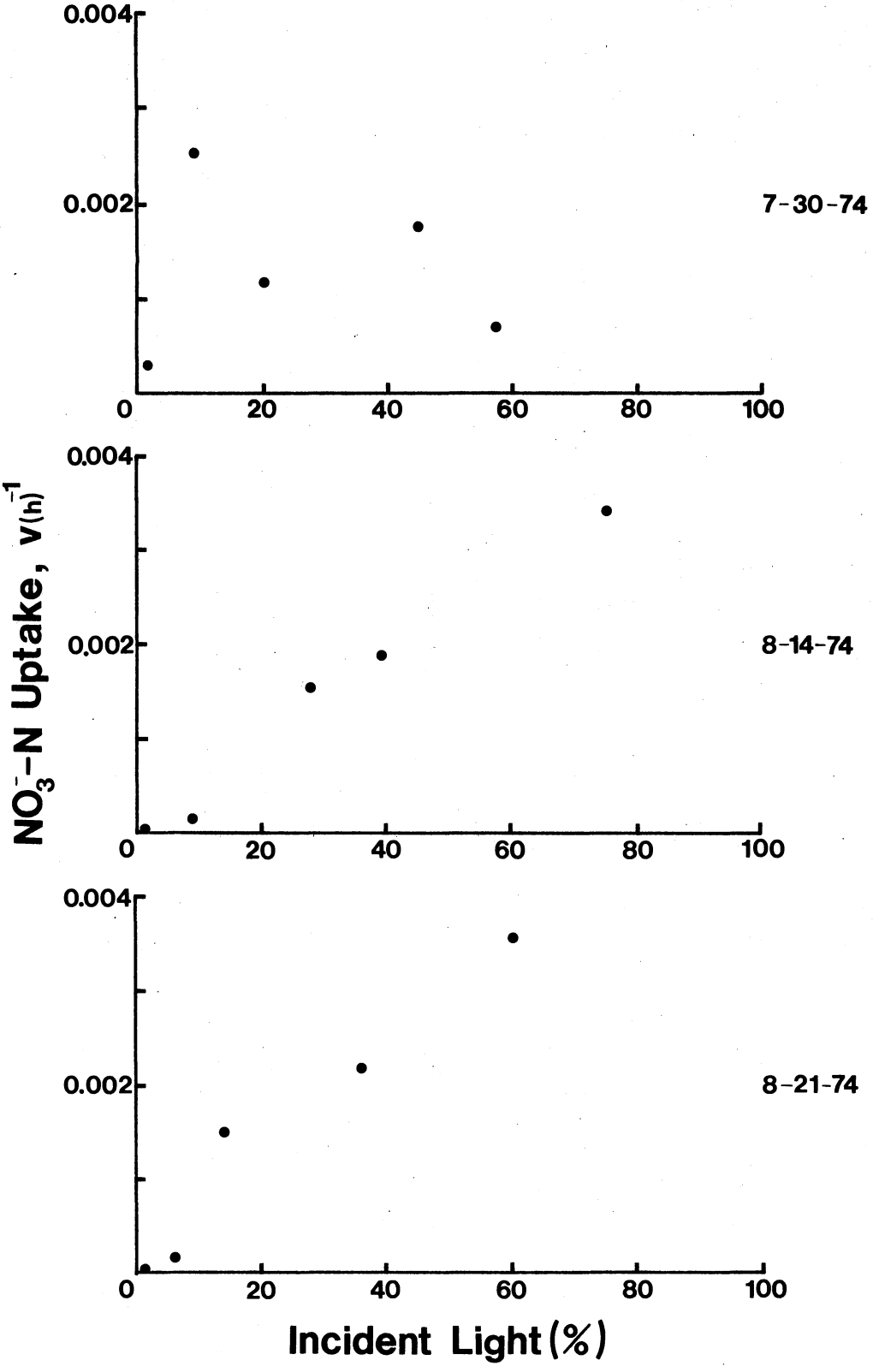

Incident Light (%)
0
50
100
Depth (cm)
100
200
300
7-30-74
8-14-74
8-21-74

Figure 4. Nitrate ($NO_3^-$-N) uptake rates (v) at saturating $NO_3^-$ concentrations as a function of light intensity on the dates of three 1974 experiments in Lake Carl Blackwell.

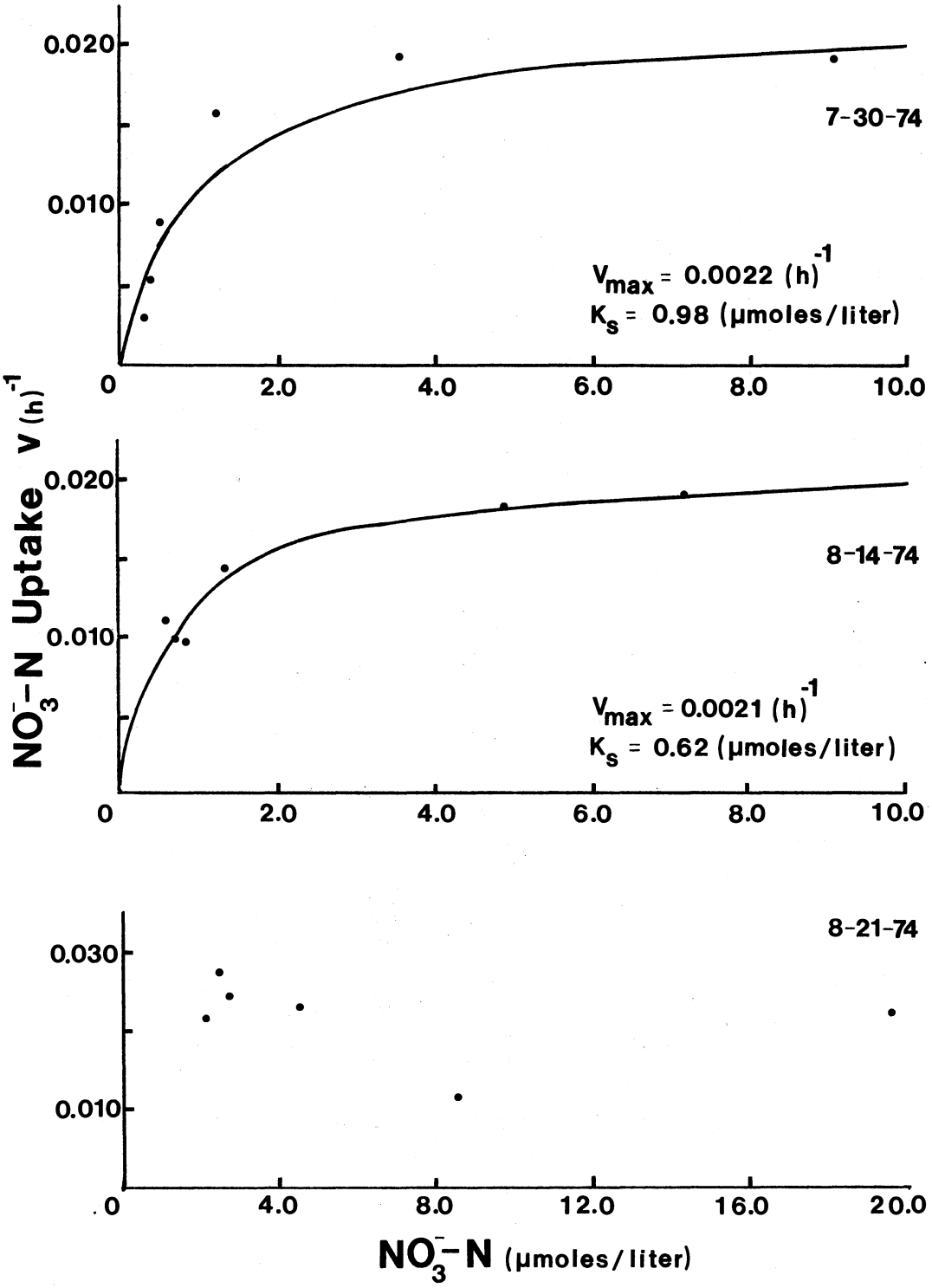

0.004
0.002
7-30-74
0
20
40
60
80
100
0.004
0.002
8-14-74
0
20
40
60
80
100
0.004
0.002
8-21-74
0
20
40
60
80
100
$NO_3^-$-N Uptake, $v_{(h)}^{-1}$
Incident Light (%)

chlorophyll a biomass coincided with the minimum transparency, and the maximum transparency occurred during the first experiment when chlorophyll a concentrations were lowest (Figures 2 and 3). Thus, light penetration was inversely correlated to chlorophyll a in Lake Carl Blackwell during the course of the study.

The effect of light intensity on $NO_3^-$ uptake rate at non-limiting $NO_3^-$ concentrations is shown in Figure 4. Uptake rates obtained in the dark bottles were always less than rates obtained in the deepest (1% of incident radiation) light bottles, and uptake rates reported in the present paper have not been corrected for dark uptake. Uptake did not become light saturated even at the highest intensities encountered (75% of incident radiation). MacIsaac and Dugdale (1972) demonstrated that $NO_3^-$ and $NH_4^+$ uptake rates in the sea showed a hyperbola-shaped response to light intensity. They used the Michaelis-Menten expression (Equation 1) to describe these hyperbolae by substituting light intensity for substrate concentration. Half-saturation constants ($K_{LT}$) for light ranged from 1 to 14% of incident radiation in the sea. In the present study linear transformations (Dowd and Riggs 1963) of the data in Figure 4 showed that the relationship between v and light intensity was not hyperbolic. Light saturation of uptake would probably not have occurred at subsurface light intensities in Lake Carl Blackwell.

The relationship between $NO_3^-$ uptake rate and $NO_3^-$ concentration was examined using the linear transformations reviewed by Dowd and Riggs (1963). The Wolfe plot (S/v vs S) yielded the highest correlation in each experiment and was used to estimate $K_s$ and $V_{max}$ by the least squares method. Figure 5 illustrates plots of v vs S, the fitted hyperbolae, and the estimated kinetic constants. The relationship

Figure 5. Nitrate ($NO_3^-$-N) uptake rates as a function on $NO_3^-$ concentration on the dates of the three 1974 experiments in Lake Carl Blackwell. Fitted curves and kinetic constants are shown for dates on which Michaelis-Menten kinetics were observed.


0.020
0.010
7-30-74
$V_{max}$ = 0.0022 $(h)^{-1}$
$K_s$ = 0.98 (µmoles/liter)
0
2.0
4.0
6.0
8.0
10.0
$NO_3^-$-N Uptake $V_{(h)}^{-1}$
0.020
0.010
8-14-74
$V_{max}$ = 0.0021 $(h)^{-1}$
$K_s$ = 0.62 (µmoles/liter)
0
2.0
4.0
6.0
8.0
10.0
8-21-74
0.030
0.010
0
4.0
8.0
12.0
16.0
20.0
$NO_3^-$-N (µmoles/liter)

between v and S was clearly hyperbolic in the first two experiments, but in the third, the model does not appear to fit the data adequately and $K_s$ and $V_{max}$ cannot be reported.

According to the theory proposed by Dugdale (1967), the hyperbolae relating v and S may be used to determine if uptake rates are limited by the ambient nutrient concentration. Dugdale showed that points along the plateau of the hyperbolae represent nutrient concentrations which do not limit uptake, while points along the slope of the curve may be interpreted to mean that the nutrient is limiting in the Liebig sense. In the first two experiments on Lake Carl Blackwell, the ambient $NO_3^-$ concentration fell along the slope of the fitted hyperbolae (Figure 5); thus the ambient concentration of $NO_3^-$ was limiting the $NO_3^-$ uptake rate. The lack of Michaelis-Menten kinetics in the third study precludes a similar analysis for that date.

Figure 6 illustrates the vertical profiles of $NO_3^-$ uptake for <u>in situ</u> $NO_3^-$ levels, for enrichments approximating 10% of the ambient $NO_3^-$ concentration, and for saturating nutrient levels. Confidence intervals (p=0.05) about the mean (n=2 or 3) uptake rate at each depth indicate that v at high enrichment was significantly greater than v at low enrichment during the first experiment at depths of 25, 38, and 200 cm (57, 45, and 9% of incident light, respectively). Thus, the ambient $NO_3^-$ concentration was clearly limiting uptake rates at these depths. In addition, at a depth of 110 cm (20% light intensity) in the first experiment at depths of 14 and 54 cm (75 and 39% incident light) in the second experiment, the mean (n=2 or 3) uptake rate at high enrichment was 2 to 4 times larger than at low enrichment, although the differences were not statistically significant (Figure 6).

Figure 6. Vertical profiles of nitrate ($NO_3^-$-N) uptake rates (v) determined at low and high enrichments and at ambient (in situ) $NO_3^-$ concentrations on the dates of three 1974 experiments in Lake Carl Blackwell.

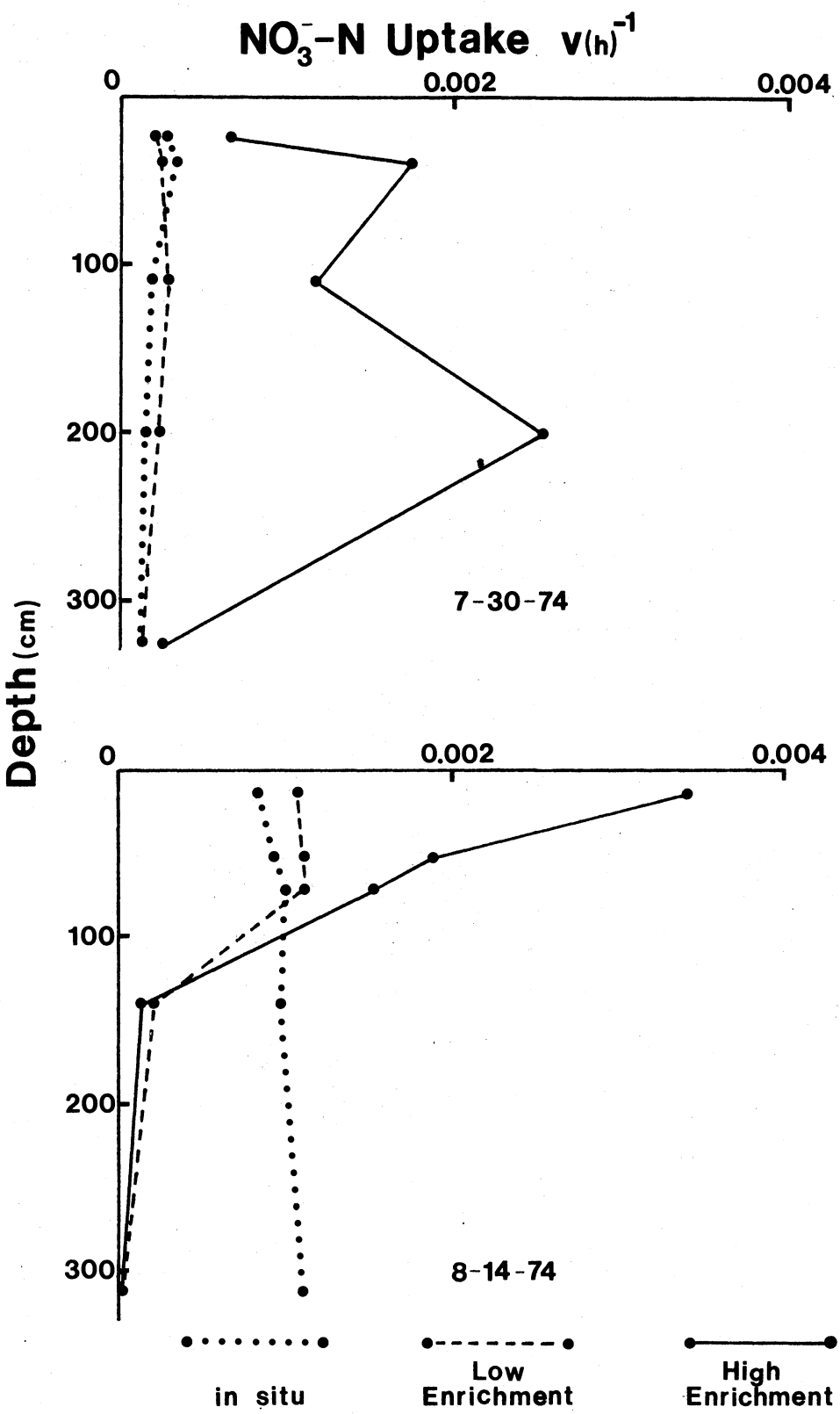
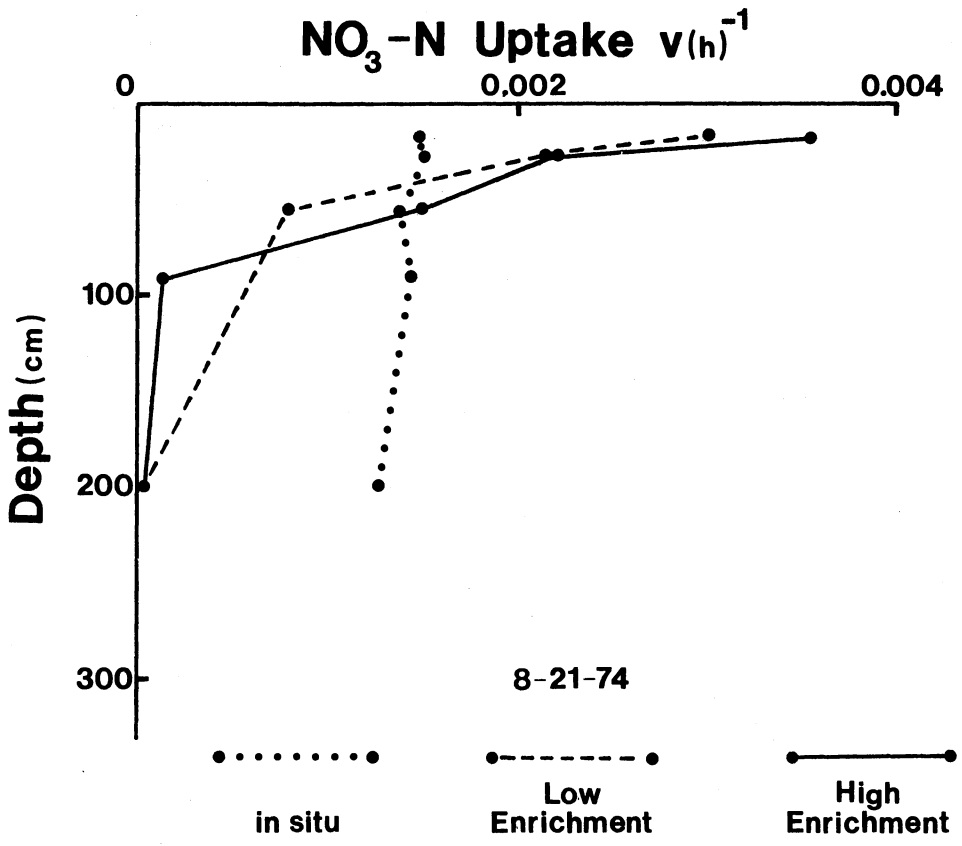

$NO_3^-$-N Uptake $v(h)^{-1}$
0
0.002
0.004
100
200
300
Depth (cm)
7-30-74
8-14-74
in situ
Low Enrichment
High Enrichment

Figure 6. (Continued)


NO3-N Uptake v(h)-1
0
0.002
0.004
100
200
300
Depth (cm)
8-21-74
in situ
Low Enrichment
High Enrichment

The lack of significant difference is attributed to small sample size, and enhancement of uptake with $NO_3^-$ additions occurred at all depths just described.

Thus, a condition of $NO_3^-$ limitation existed at all but the 2% depth in the first experiment and at the 75 and 39% depth in the second experiment, as evidenced by enhancement of uptake with $NO_3^-$ enrichment. At greater depths in the first two experiments and at all depths in the third study, uptake was not enhanced by $NO_3^-$ additions, and some other factor was limiting the uptake of $NO_3^-$. The shape of the uptake profile at high $NO_3^-$ levels in the second and third experiments closely resembles the light extinction curves (Figure 3), and light was almost certainly limiting uptake at non-limiting $NO_3^-$ levels. Thus, at ambient $NO_3^-$ levels, light was limiting $NO_3^-$ uptake at the bottom of the euphotic zone in the first two experiments and at all depths in the third experiment, when $NO_3^-$ was at saturating concentrations.

In the first experiment the uptake profile at saturating $NO_3^-$ concentrations did not appear to be light limited at depths above 200 cm (9% of surface light). This is attributed to the phenomenon termed luxury uptake which often occurs when N depleted cells are exposed to fresh supplies of a nitrogen and assimilate it at rates much higher than required for growth. The lack of coupling between uptake and growth accounts for the lack of coupling between uptake and light. Nitrogen depletion of the cells is suspected in the first experiment because of the high PN biomass and the low ambient $NO_3^-$ concentration.

In situ uptake rates were calculated from equation (1) using values for $K_s$ and $V_{max}$ determined by the Michaelis-Menten expression on each date and the concentration of nitrate (S) at each depth. The

*in situ* rates showed excellent agreement with rates measured at low enrichment at depths where $NO_3^-$ was limiting uptake. However, in the second and third experiments, the calculated *in situ* rates were higher below the 23% light-penetration depth than rates measured at either low or high enrichment and were obviously unrealistic. Uptake at these depths was controlled by light, a variable not accounted for in back-calculation.

## DISCUSSION

The uptake profiles for the first two experiments in Lake Carl Blackwell were similar to the $NO_3^-$ profiles described by MacIsaac and Dugdale (1972) for eutrophic regions of the sea. MacIsaac and Dugdale encountered *in situ* nutrient limitation in the upper euphotic zone and light limitation below depths corresponding to 10-25% of incident light. They described similar results for $NH_4^+$ uptake.

However, MacIsaac and Dugdale (1972) reported that plots of v vs percent incident light described hyperbolae, indicating that uptake was light saturated at light intensities ranging from 25 to 60% of incident light. In the present study, light saturation did not occur at the highest light levels used for incubation (75%). Since incubation was not physically possible at light intensities closely approaching surface irradiance in the present study, it is conceivable that rapid saturation or even photoinhibition would have occurred at intensities above 75% of surface radiation. However, that does not seem likely considering the pattern of the data (Figure 4). Another model is required to describe the relationship between v and light intensity in turbid reservoirs. An exponential model should be considered, since the uptake profiles at saturating nutrient levels resemble the exponential light extinction profiles (Figure 3).

Perhaps the hyperbolic response obtained by MacIsaac and Dugdale was an artifact of the incubation system, since they used neutral density filters to simulate *in situ* light intensities. The increased

proportion of blue and green light found with increasing depth in the euphotic zone has been shown to enhance photosynthesis and protein production in cultures and natural populations of marine phytoplankton, when compared to white light of the same intensity (Wallen and Green 1971 a, b, c). Neutral density filters transmit white light.

In oligotrophic regions of the sea the euphotic zone often extends to depths greater than 100 m and the volume of water per unit surface area which receives non-limiting light levels is much greater than in reservoirs. It is possible that reservoir phytoplankton are acclimated or adapted to the narrow euphotic zone typical of turbid impoundments. In a well-mixed euphotic zone typical of Great Plains impoundments, it would be advantageous for algae to make maximum use of the highest light levels encountered, because of their infrequent exposure to adequate light.

MacIsaac and Dugdale (1969) found $K_s$ values for $NO_3^-$ uptake by natural populations of phytoplankton to range from 0.01 to 0.04 µmoles/liter in oligotrophic regions of the sea and from 0.7 to 4.2 µmoles/liter in eutrophic regions. Values of $V_{max}$ for the respective trophic states had ranges of 0.001 to 0.007 $(h)^{-1}$ and 0.016 to 0.036 $(h)^{-1}$. While the $K_s$ values obtained for Lake Carl Blackwell were characteristic of eutrophic regions of the sea, the $V_{max}$ values fell in the oligotrophic range (Figure 5). This suggests the possibility that $V_{max}$ was limited by some factor other than $NO_3^-$. Phosphorous is a likely possibility since $PO_4^{+3}$-P was undetectable in the first two experiments. In the third experiment the mean (n = 3) $PO_4^{+3}$-P concentration at the Michaelis-Menten incubation depth was 0.25 µmoles/liter, and the ratio of total inorganic N to orthophosphorous was 8.3 (cellular ratio is approximately 12:1). Thus, phosphorous was probably not limiting uptake during the third experiment.

Eppley et al. (1969) examined $K_s$ for $NO_3^-$ as a function of cell size for a large number of marine phytoplankton in unialgal cultures. They found that small celled species typical of the open ocean, where extremely low $NO_3^-$ concentrations are encountered, had low $K_s$ values ranging from 0.1 to 0.3 µmoles/liter. Large, neritic diatoms, which encountered high $NO_3^-$ concentrations, had higher $K_s$ values ranging from 0.4 to 5.1 µmoles/liter. The large centric diatoms (Melosira sp. and Concinodiscus sp.) which dominated the flora in the present study may account for the high $K_s$ values observed for Lake Carl Blackwell (Figure 5).

MacIsaac and Dugdale (1969, 1972) observed that $NH_4^+$-N concentrations greater than approximately 1 µmoles/liter resulted in uniterpretable uptake data or in actual inhibition of uptake. In the present study, $NH_4^+$-N concentrations greater than 1 µmole/liter were encountered in the first and third experiments. No inhibition was observed in the first experiment, when the mean (n = 3) $NH_4^+$ concentration was 1.4 µmoles/liter. However, the mean (n=3) $NH_4^+$-N concentration in the water used for the Michaelis-Menten study from the third experiment was 2.38 µmoles/liter and may account in part for the response observed on that date (Figure 5).

## SUMMARY

Using the stable isotope of nitrogen ($^{15}N$), vertical profiles of nitrate ($NO_3^-$) uptake rates were determined during three experiments in Lake Carl Blackwell. Variation in the vertical profiles was the result of the interaction between light intensity and $NO_3^-$ concentration. At high ambient $NO_3^-$ levels the profile of $NO_3^-$ uptake was controlled by light intensity, and uptake was not light saturated at the highest subsurface light intensities at which observations were made. At low ambient $NO_3^-$ concentrations, uptake rates were $NO_3^-$ limited in the upper part of the euphotic zones and were light limited below the depth to which 10% of incident light penetrated. Michaelis-Menten kinetics were observed in two of the three experiments, and $K_s$ values were similar to those obtained for eutrophic regions of the sea.

## REFERENCES


Barsdate, R. J., and Dugdale, R. C. 1965. Rapid conversion of organic nitrogen to $N_2$ for mass spectroscopy: an automated Dumas procedure. Anal. Biochem. 13:1-5.

Dowd, J. D., and Riggs, O. S. 1963. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. J. Biol. Chem. 240:863-869.

Dugdale, R. C. 1967. Nutrient limitation in the sea: dynamics, identification, significance. Limnol. Oceanogr. 12:685-695.

Dugdale, V. A., and Dugdale, R. C. 1962. Nitrogen metabolism in lakes. II. Role of nitrogen fixation in Sanctuary Lake, Pennsylvania. Limnol. Oceanogr. 7:170-177.

Dugdale, V. A., and Dugdale, R. C. 1965. Nitrogen metabolism in lakes. III. Tracer studies of the assimilation of inorganic nitrogen sources. Limnol. Oceanogr. 10:53-57.

Dugdale, R. C., and Goering, J. J. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12: 196-206.

Dugdale, R. C., and MacIsaac, J. J. 1971. A computation model for the uptake of nitrate in the Peru upwelling region. Invest. Pesq. 35: 299-308.

Eppley, R. W., Rogers, J. N., and McCarthy, J. J. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14:912-920.

Eppley, R. W., Renger, E. H., Venrick, E. L., and Mullin, M. M. 1973. A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific Ocean. Limnol. Oceanogr. 18:534-551.

Holm-Hansen, O. 1968. Determination of particulate organic nitrogen. Limnol. Oceanogr. 13:175-178.

MacIsaac, J. J., and Dugdale, R. C. 1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep Sea Res. 16:45-57.

MacIsaac, J. J., and Dugdale, R. C. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep Sea Res. 19:209-232.

McNabb, C. D. 1960. Enumeration of freshwater phytoplankton concentrated on the membrane filter. Limnol. Oceanogr. 5:57-61.

Neess, J. C., Dugdale, R. C., Dugdale, V. A., and Goering, J. J. 1962. Nitrogen metabolism in lakes I. Measurement of nitrogen fixation with $^{15}N$. Limnol. Oceanogr. 7:163-169.

Parsons, T. R., and Strickland, J. D. H. 1963. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Mar. Res. 21:155-163.

Ruttner, F. 1972. Fundamentals of limnology. University of Toronto Press, 295 pp.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14:799-801.

Steemann-Nielsen, E. 1952. The use of radioactive carbon ($^{14}C$) for measuring organic production in the sea. J. Conseil, Conseil Perm. Inter. Exploration Mer. 18:117-140.

Strickland, J. D. H., and Parsons, T. R. 1968. A practical handbook of seawater analysis. Fish. Res. Bd. of Canada, Bull. 167, 311 pp.

Toetz, D. W., Varga, L. P., and Loughran, E. D. 1973. Half-saturation constants for uptake of nitrate and ammonia by reservoir plankton. Ecology 54:903-908.

Wallen, D. G., and Geen, G. H. 1971a. Light quality in relation to growth, photosynthetic rates, and carbon metabolism in two species of marine plankton algae. Mar. Biol. 10:34-43.

Wallen, D. G., and Geen, G. H. 1971b. Light quality and concentration of proteins, RNA, DNA, and photosynthetic pigments in two species of marine plankton algae. Mar. Biol. 10:44-51.

Wallen, D. G., and Geen, G. H. 1971c. The nature of the photosynthate in natural phytoplankton populations in relation to light quality. Mar. Biol. 10:157-168.

# APPENDIXES

Table 1. Vertical profiles of light intensities, sample depths, mean nutrient and biomass concentrations, and mean uptake velocities during the first experiment on Lake Carl Blackwell (number of samples comprising means are indicated in parentheses).

| Light Intensity at Sample Depth (% incident light) | Sample Depth (cm) | Light Intensity (ly/da) | Nitrogen (µmoles/liter) | | | | $PO_4^{+3}$-P (µmoles/liter) | Chl a (µg/liter) | Uptake Velocity $(h)^{-1}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $NO_3^-$ | $NH_4^+$ | $NO_2^-$ | PN | | | $^{15}N$-$NO_3^-$ Enrichment | | Dark Bottles |
| | | | | | | | | | Low | High | |
| 57 | 25 | 354 | 0.15 (3) | 1.56 (3) | 0.01 (3) | 19.12 (3) | * (3) | 4.3 (1) | 0.00022 (3) | 0.00068 (3) | 0.00001 (1) |
| 45 | 38 | 279 | 0.18 (3) | 1.39 (3) | 0.01 (3) | 10.22 (3) | * (3) | 3.8 (1) | 0.00026 (3) | 0.00176 (2) | 0.00006 (1) |
| 20 | 110 | 124 | 0.11 (3) | 1.29 (3) | 0.03 (3) | 11.14 (3) | * (3) | 4.4 (1) | 0.00029 (3) | 0.00119 (3) | 0.00005 (1) |
| 9 | 200 | 56 | 0.08 (3) | 1.12 (3) | 0.02 (3) | 11.39 (3) | * (3) | 6.8 (1) | 0.00024 (3) | 0.00255 (3) | 0.00004 (1) |
| 2 | 330 | 12 | 0.08 (3) | 1.14 (3) | 0.02 (3) | 11.45 (3) | * (3) | 6.5 (1) | 0.00015 (3) | 0.00028 (3) | 0.00006 (1) |

*: Concentration equal to or less than zero as determined by least squares fit of standard curve

**: Uptake velocities based on entire 24 h incubation period

Table 2. Vertical profiles of light intensities, sample depths, mean nutrient and biomass concentrations, and mean uptake velocities during the second experiment on Lake Carl Blackwell (number of samples comprising means are indicated in parentheses).

| Light Intensity at Sample Depth (% incident light) | Sample Depth (cm) | Light Intensity (ly/da) | Nitrogen (µmoles/liter) | | | | $PO_4^{+3}$-P (µmoles/liter) | Chl a (µg/liter) | Uptake Velocity $(h)^{-1}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $NO_3^-$ | $NH_4^+$ | $NO_2^-$ | PN | | | $^{15}N$-$NO_3^-$ Enrichment | | Dark Bottles |
| | | | | | | | | | Low | High | |
| 75 | 14 | 128 | 0.42 (3) | 1.03 (3) | * (3) | 5.02 (3) | * (3) | 8.5 (2) | 0.00107 (2) | 0.00342 (2) | 0.00003 (1) |
| 39 | 54 | 67 | 0.51 (3) | 0.54 (3) | * (3) | 5.52 (3) | * (3) | 8.2 (2) | 0.00112 (2) | 0.00190 (1) | 0.00006 (1) |
| 28 | 74 | 39 | 0.70 (3) | 0.81 (3) | * (3) | 5.59 (3) | * (3) | 9.3 (2) | 0.00121 (2) | 0.00154 (2) | 0.00006 (1) |
| 9 | 141 | 15 | 0.55 (3) | 0.81 (3) | * (3) | 6.59 (3) | * (3) | 8.5 (2) | 0.000271 (2) | 0.00015 (2) | 0.00003 (1) |
| 1 | 311 | 2 | 0.70 (3) | 0.42 (3) | * (3) | 5.29 (3) | 0.16 (3) | 7.4 (2) | 0.00003 (1) | 0.00004 (2) | 0.00001 (1) |

*: Concentration equal to or less than zero as determined by least squares fit of standard curve

**: Uptake velocities based on entire 24 h incubation period

Table 3. Vertical profiles of light intensities, sample depths, mean nutrient and biomass concentrations, and mean uptake velocities during the third experiment on Lake Carl Blackwell (number of samples comprising means are indicated in parentheses).

| Light Intensity at Sample Depth (% incident light) | Sample Depth (cm) | Light Intensity (ly/da) | Nitrogen (µmoles/liter) | | | | $PO_4^{+3}$-P (µmoles/liter) | Chl a (µg/liter) | Uptake Velocity $(h)^{-1}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $NO_3^-$ | $NH_4^+$ | $NO_2^-$ | PN | | | $^{15}N$-$NO_3^-$ Enrichment | | Dark Bottles |
| | | | | | | | | | Low | High | |
| 60 | 17 | 355 | 1.84 (3) | 2.30 (3) | 0.19 (3) | 6.27 (3) | 0.23 (3) | 11.6 (2) | 0.00301 (1) | 0.00354 (2) | 0.00003 (1) |
| 36 | 29 | 213 | 1.96 (3) | 2.38 (3) | 0.18 (3) | 7.22 (3) | 0.25 (3) | 11.4 (2) | 0.00217 (2) | 0.00218 (2) | 0.00004 (1) |
| 14 | 56 | 83 | 1.43 (3) | 1.40 (3) | 0.18 (3) | 8.16 (3) | 0.23 (3) | 11.3 (2) | 0.00081 (2) | 0.00150 (2) | 0.00002 (1) |
| 6 | 91 | 36 | 1.53 (3) | 1.22 (3) | 0.15 (3) | 8.73 (3) | 0.25 (3) | 12.6 (2) | ___ | 0.00015 (2) | ___ |
| 1 | 200 | 6 | 1.06 (3) | 0.80 (3) | 0.15 (3) | 7.94 (3) | 0.26 (3) | 11.3 (2) | 0.00009 (2) | 0.00004 (2) | 0.00002 (1) |

**: Uptake velocities based on entire 24 h incubation period

Table 4. Uptake velocities as a function of total (ambient + enrichment) $NO_3^-$ concentration during the three experiments on Lake Carl Blackwell.

| Experiment #1 | | Experiment #2 | | Experiment #3 | |
|---|---|---|---|---|---|
| Total $NO_3^-$-N (μmoles/liter) | $v(h)^{-1}$ | Total $NO_3^-$-N (μmoles/liter) | $v(h)^{-1}$ | Total $NO_3^-$-N (μmoles/liter) | $v(h)^{-1}$ |
| 0.25 | 0.00026 | 0.58 | 0.00112 | 2.17 | 0.00217 |
| 0.39 | 0.00054 | 0.68 | 0.00106 | 2.48 | 0.00279 |
| 0.50 | 0.00090 | 0.74 | 0.00101 | 2.69 | 0.00244 |
| 1.22 | 0.00160 | 1.31 | 0.00143 | 4.50 | 0.00232 |
| 3.52 | 0.00194 | 3.85 | 0.00181 | 8.62 | 0.00108 |
| 9.05 | 0.00192 | 7.17 | 0.00190 | 19.62 | 0.00218 |

# VITA

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