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COMPARATIVE KINETICS OF NITRATE

UPTAKE BY FRESHWATER ALGAE

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CHAPTER I

INTRODUCTION

Nitrogen is important in the biosphere because it is a primary constituent of protein and nucleic acids, which are major components of living material. Nitrogen is particularly significant in aquatic communities, because it is one of the factors limiting the growth rate of phytoplankton (Rhodhe 1948), especially in marine environments (Thomas 1966 and 1967). Nitrogen is also one of the elements necessary for the production of chlorophyll (Rabinowich 1945). Rhodhe (1948) showed that upon exhaustion of nitrate (NO_3^-) from algal culture media, chlorophyll production by the algae quickly ceased and soon thereafter the concentration of chlorophyll within the cells began to decrease. Gerking (1962) argued that protein synthesis is the most characteristic feature of animal growth, and Dugdale and Goering (1967) maintained that measurements of algal population growth using nitrogen may show less scatter than using carbon or phosphorus because the latter two elements are not only structural components, but are turned over in the energetics processes . Emphasis, therefore, should be placed on the study of production processes in terms of nitrogen.

The metabolic processes directly relating nutrients to algal production are uptake, assimilation and growth. Mechanisms and rates, as well as the influence of environmental conditions, have been examined. Algal ecologists are particularly interested in growth, the ultimate

measure of competition and succession. In part, growth is a function of nutrient utilization. However, the exact relationship between nutrient uptake and growth is not clearly understood. Nutrients first pass through the cell membrane, afterwhich they are either stored or used for maintenance and growth. Growth is partially dependent upon the rates, determined by genetic and environmental factors, by which nutrients are taken across the cell membrane and assimilated.

The study presented here is a determination of the rates of NO_3^- uptake by 18 species of freshwater algae. It is difficult to compare the absolute nutrient uptake rates of different species of algae; however, comparisons of uptake kinetics provide a means of a quantitative comparison of competitive abilities of algae (Dugdale 1967).

Nutrient uptake kinetics resemble Michaelis-Menten enzyme kinetics; i.e., when uptake rate is plotted against nutrient concentration, the resulting curve is hyperbolic. The concentration of nutrient at which the uptake rate equals one half of the maximum possible uptake (V_m) is the half-saturation constant (K_s), and is considered to be a measure of the ability of a species of algae to take up nutrients occurring at low concentrations (Dugdale 1967).

Although considerable attention has been directed to studies of the uptake kinetics of NO_3^- by marine algae (Dugdale 1967, Dugdale and Goering 1967, Eppley and Coatsworth 1968, Eppley et al. 1969a, Eppley and Renger 1974, Falkowski 1975, Lehman et al. 1975 and Underhill 1977), there has been little work on similar uptake kinetics by freshwater algae. Toetz (1973), Toetz et al. (1973), Toetz (1976), Toetz et al. (1977), and Cole (1977) studied uptake kinetics of mixed natural populations of algae in reservoirs. They found K_s to be about 3 μm NO_3^--N/l.

Hatori (1962), studying uptake by monocultures of Anabaena cylindrica, estimated the K_s to be 70 μm/l. Little is known about the NO_3^- uptake capability of freshwater algae, especially for individual species as determined by studies on monocultures.

The data obtained from the work described here could be of use in predicting the outcome of competition between algae species when NO_3^- is limiting. Furthermore, the K_s and V_m values presented here could be compared to the uptake constants of cultures perturbed by pollutants, thus serving as a possible diagnostic tool for evaluating a physiological impact of pollution. Data on K_s and V_m for NO_3^- could also be of use in developing mathematical ecosystem models.

CHAPTER II

LITERATURE REVIEW

Forms of Nitrogen

Nitrogen (N) exists in many forms in aquatic ecosystems. Nitrogen compounds are found as cellular constituents, nonliving particulate matter in the form of organic compounds, and inorganic ions. All these forms of N are related by the complex of reactions known as the nitrogen cycle (Figure 1).

Inorganic dinitrogen (N_2) can be fixed by some members of the Phylum Cyanophyta (Wolk 1973, and the references therein, Tyagi 1975). The rest of the Cyanophyta, as well as all other algae, must use inorganic N as either nitrate (NO_3^-), nitrite (NO_2^-) or ammonia (NH_4^+) (Syrett 1962). Some algae can also use organic N, i.e., urea, amino acids, amides, uric acid and xanthine.

The processes involving the incorporation of inorganic N into phytoplankton are examined here. These processes fall into two categories, uptake and assimilation.

Uptake and Assimilation

Uptake is the transfer of a nutrient from the environment into a cell. The uptake of nutrients by phytoplankton often occurs against a concentration gradient, precluding simple diffusion as the mechanism by

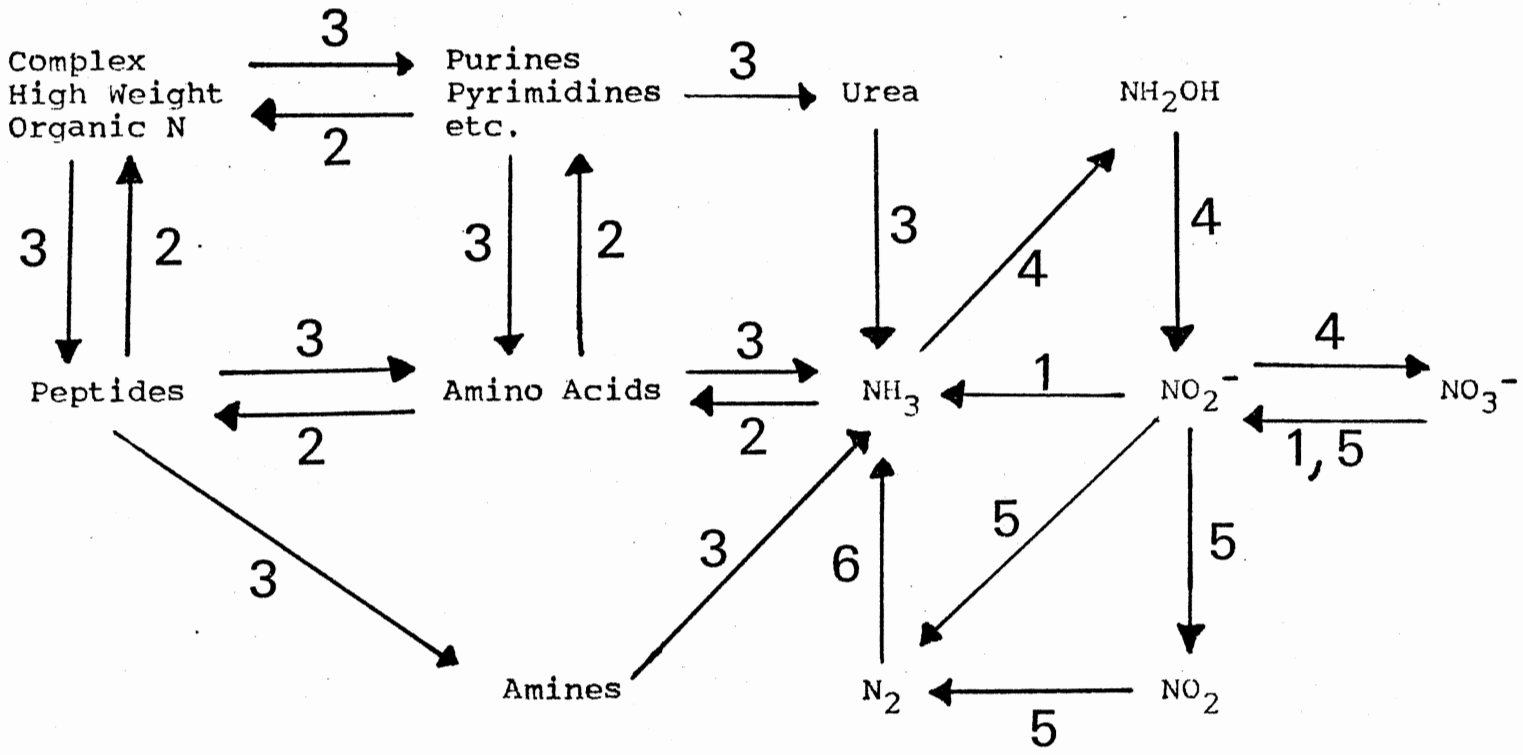

Figure 1. The Nitrogen Cycle. 1. Nitrate Assimilation, 2. Ammonia Assimilation, 3. Ammonification, 4. Nitrification, 5. Denitrification, 6. Nitrogen Fixation (Brezonik 1972)

which these substances cross the plasmalema. The exact mechanisms of the active transport processes for inorganic ions, including N compounds, are not completely understood. A carrier-ion complex as described by Epstein (1973) appears to be the most widely accepted model for transfer across the membrane. The complex, formed at the outer surface of the membrane, traverses the membrane or undergoes some spatial rearrangement within it, and the substrate ion is brought through the membrane. In his monograph, Hodges (1973) presented a model, based on the evidence of many workers, describing the transport of inorganic anions and cations across the plasma membrane into root cells. The model contains two separate types of carriers, one for cations and one for anions. Hodges also reviewed considerable evidence indicating that ATP, or in some tissues, electron transfer reactions, serve as the energy source for ion-transport.

Histochemical studies by Hall (1969) and Hodges and Leonard (1973) have shown that isolated cell membranes of oat roots possess ion-stimulated ATPase. Falkowski (1975) reported a membrane-bound anion (NO_3^- and Cl^-) activated ATPase which appears to be responsible for NO_3^- transport across the plasmalema of the marine diatom Skeletonema costatum. MacRobbie (1970), however, summarized findings that indicate ATP is the source for cation transport (K^+), but not for anion transport (Cl^-).

Assimilation refers to the series of reactions by which nutrients are transformed into organic cellular material. Ammonia is the form of inorganic N which enters into assimilatory synthetic reactions. Before NO_3^- or NO_2^- can enter assimilatory pathways they must be reduced to NH_4^+. Figure 2 shows a simplified version of the processes of inorganic N metabolism for Ditylum brightwelli presented by Eppley and Rogers

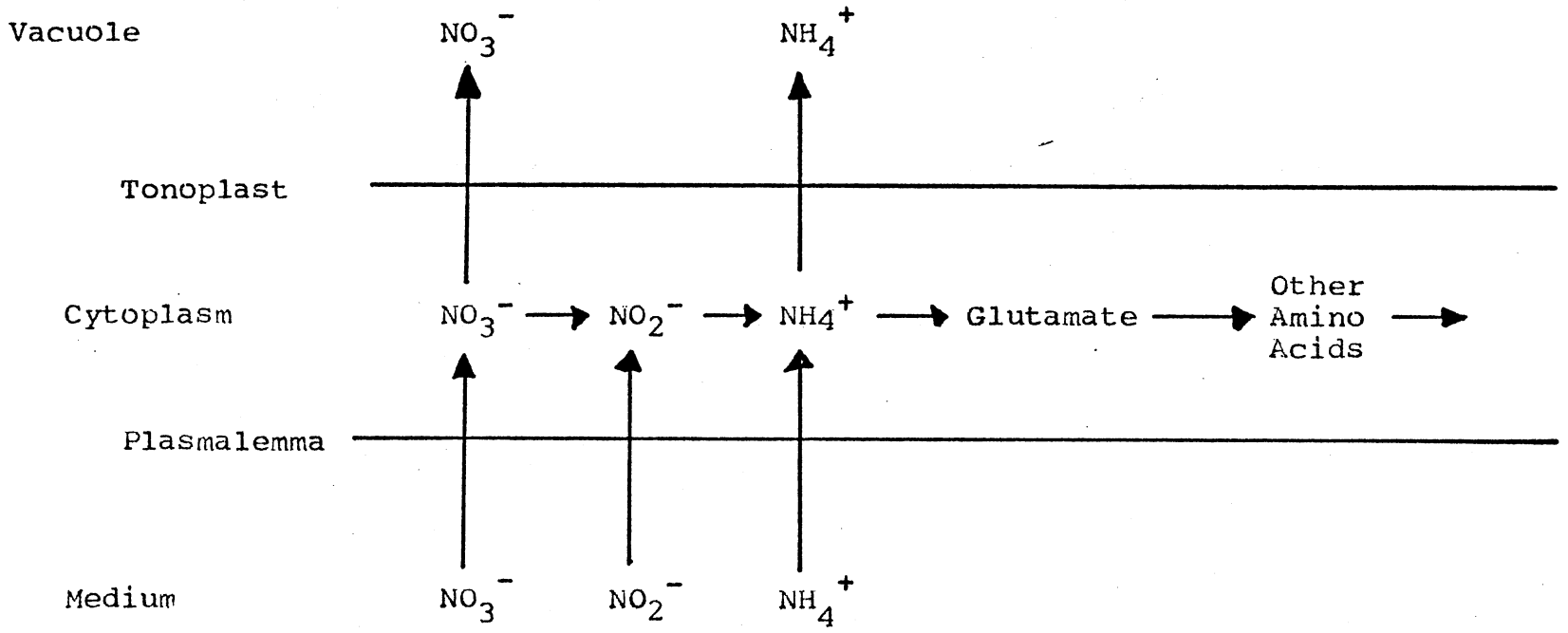

Figure 2. Proposed Pathways of Nitrogen Incorporation in Alagae (Eppley and Rogers 1970)

(1970). Nitrate is reduced to NO_2^- by the inducible enzyme NADH-nitrate reductase (NR). Reduction to NO_2^- is the rate-limiting step in the assimilation of NO_3^- in higher plants (Beevers and Hageman 1972). As shown in Figure 2, NO_3^- is accumulated in the cell, either in the cytoplasm or in the vacuole. Eppley and Thomas (1970) compared the rates of NO_3^- uptake and reduction, and concluded that the intra-cellular pool of NO_3^- serves as the substrate for reduction.

Solomonson and Spehar (1977) presented a model in which CO_2 fixation and NO_3^- assimilation in algae are coordinately controlled by the intra-cellular ratio of the concentration of O_2 and CO_2. A central feature of the model is that the assimilation of NO_3^- can be initiated via the activation of NR by cyanide.

Nitrite taken into cells is not stored in the vacuole, but is reduced to NH_4^+ by the inducible enzyme nitrite reductase (NiR) in both higher plants (Hewitt et al. 1968) and in algae (Eppley and Rogers 1970).

Ammonia assimilation appears to take place by way of several different routes. In many organisms NH_4^+ is assimilated by the enzyme glutamate dehydrogenase (GDH) which catalyzes the reductive amination of α-ketoglutarate (or 2-oxoglutarate) to form glutamic acid (Bassham and Kirk 1964). Other amino acids are formed by analagous reactions, but they are considered secondary to the glutamic dehydrogenase pathways. For blue-green algae, however, no GDH activity (Hoare et al. 1967), or very low levels of GDH (Pearce et al. 1969) have been reported. The action of glutamine synthetase, catalyzing the synthesis of glutamine, appears to mediate most of the initial metabolism of NH_4^+ in blue-green algae (Meeks et al. 1977). They report that in Anabaena

cylindrica the principal initial product of ^{13}N-NH_4^+ metabolism, grown with either N_2 or NH_4^+ as the N source, was amide-labeled glutamine.

It is generally believed that phytoplankton are limited in their capabilitiy to utilize organic N (Brezonik 1972). Algae in the Sargasso Sea could assimilate only small quantities of urea-N (Carpenter and McCarthy 1975). Eppley et al. (1971), however, showed that mixed natural populations of marine algae off the coast of southern California could grow in N-depleted cultures with urea added as the sole N-source. Growth and increase in chlorophyll a concentration, however, was not as rapid for urea-grown cells as with NO_3^- and NH_4^+-grown cells.

The primary source of N for algae is inorganic N; NO_3^-, NH_4^+, and to a much lesser extent, NO_2^- (Round 1965). Eppley and Coatsworth (1968) demonstrated that NO_3^- inhibited NO_2^- uptake, presumably by competition for cofactors or enzyme sites involved in intracellular reduction. Uptake of NO_3^- on the other hand, has been shown to be inhibited by NH_4^+ in the culture medium. Morris and Syrett (1972) observed a very rapid decrease in NO_3^- uptake when NH_4^+ was added to algal cultures. Nitrate assimilation is also repressed by the presence of NH_4^+. Eppley et al. (1969b), using several species of marine phytoplankton, observed that when the concentration of NH_4^+ and NO_3^- in culture media were both 5 to 15 µm/l, NH_4^+ was preferentially assimilated. It was only when the concentration of NH_4^+ decreased to 0.5 to 1.0 µm/l that NO_3^- assimilation began. It was also observed by Eppley et al. (1969b) that the presence of NH_4^+ inhibited the synthesis of NR.

Kinetics

The absorption rates of ions by roots of higher plants increase

as the external ion concentration increases until saturation occurs. When ion uptake rate is plotted against ion concentration, a hyperbolic curve results (see reviews by Hodges 1973 and Epstein 1973). Epstein and Hagen (1952) applied Michaelis-Menten enzyme kinetics analysis to quantitatively describe ion absorption. They likened the carrier-ion complex, thought to be involved in uptake, to an enzyme-substrate complex.

The relationship between ion concentration and uptake by algae has been shown to be hyperbolic, i.e., following saturation or Michaelis-Menten kinetics. This relationship has been demonstrated for unialgal cultures of marine phytoplankton (Dugdale 1967, Eppley and Coatsworth 1968, Carpenter and Guillard 1971), mixed populations of marine algae (MacIsaac and Dugdale 1972), unialgal cultures of freshwater algae (Hatori 1962), and mixed natural populations of freshwater algae (Toetz et al. 1973).

The relationship between NO_3^- uptake rate (v) and NO_3^- concentration (S) can be described by the Michaelis-Menten equation:

$$v = \frac{V_m S}{K_s + S} \qquad (1)$$

where V_m is the maximum uptake rate and K_s is the half-saturation constant for uptake and equals S when v is ½ of V_m (Figure 3).

The value of K_s for a given nutrient provides a means by which quantitative comparisons might be made of the ability of different species of phytoplankton to use low levels of nutrients. The lower the K_s, the greater the efficiency of uptake (Eppley and Coatsworth 1968); that is, the lower the K_s, the lower are the concentrations of NO_3^- which

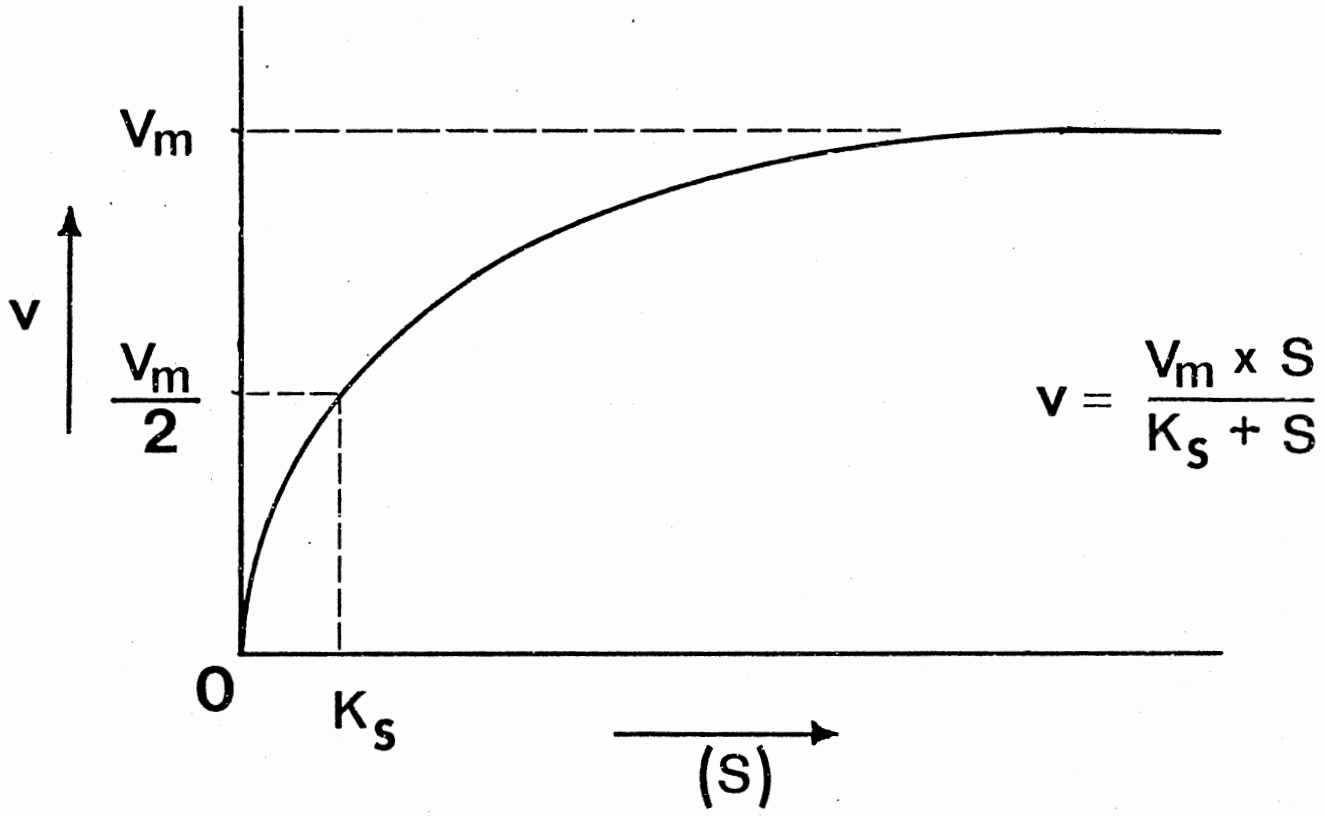

Figure 3. Hyperbolic Uptake Curve for an Algal Nutrient with Half-Saturation Constant (K_s) and Maximum Uptake Velocity (V_m) Illustrated

saturate the carriers responsible for uptake.

There is ecological significance in K_s values for different species of algae. Margalef (1956) observed that the decline in concentration of nitrogenous nutrients accompanied the seasonal succession of algal species; therefore, utilization of low levels of nutrients appears to be a significant factor regulating species succession. Menzel et al. (1963) drew similar conclusions from enrichment experiments with Sargasso Sea water, stating that the ability of algal species to utilize nutrients in limited supply reflects competitive ability. Eppley and Coatsworth (1968) and Eppley et al. (1969a) have agreed that there is ecological significance in K_s since low or high values appear to determine the succession of algal species when NO_3^- is limiting.

Eppley and Renger (1974) suggested that for K_s to be a factor in competition between species it must be related to growth rate. Dugdale (1967) proposed a model for the dynamics of nutrient-limited productivity. The essential element of the model is that the rate of uptake of nutrients by phytoplankton follows Michaelis-Menten kinetics, and this rate of uptake governs the rate of population growth. The model was based on an expression utilizing both uptake rate and growth rate, mainly:

$$V_{N_4} = N_4 \cdot \frac{V_m}{K_{s_g} + N_4} \qquad (2)$$

where V_{N_4} is the specific growth rate of the phytoplankton in terms of the limiting nutrient, N_4 is the concentration of the limiting nutrient, V_m is the maximum uptake velocity and K_{s_g} is the half-saturation constant for growth, that is, the substrate concentration at which V_{N_4} is

equal to ½ V_m. The equation for growth (μ) is:

$$\mu = \frac{U_m S}{K_{s_g} + S} \qquad (3)$$

in which μ is the specific growth rate, U_m is the maximum specific growth rate, K_{s_g} is the half-saturation constant for growth, and S is the nutrient concentration. Equation 3 does not incorporate uptake rates and therefore differs from equation 2. A hyperbolic response of growth rate to nutrient concentration has been observed in batch cultures (Caperon 1967, Eppley and Thomas 1969) and chemostats (Caperon 1968). However, the relationship between growth and uptake of NO_3^- is not well understood. In some cases K_s for uptake is found to be an order of magnitude higher than the K_s for growth (Droop 1968, Caperon and Meyer 1972, Rhee 1973), although Eppley and Thomas (1969) have obtained good agreement between both constants. Several workers consider that the specific growth rate is due to the nutrient content of cells rather than to the external concentration of nutrient (Droop 1968, Caperon 1968, Caperon and Meyer 1972, Fuhs 1969, Rhee 1973).

Dugdale (1967) related competitive ability to uptake characteristics and growth rates at high and low NO_3^- concentrations. Curves (hypothetical) were presented for NO_3^- uptake vs NO_3^- concentration, incorporating V_m for growth calculated from data presented by Riley (1963). The effect of a low K_s value in compensating for low maximum growth rates, under conditions of low NO_3^- concentration, was shown for Chaetoceros socialis (V_m for growth, 0.68/hr; K_s, 2.6 µm/l0 and Rhizosolenia alata (V_m for growth, 0.034/hr; K_s, 0.25 µm/l). Rhizoselenia, with a maximum growth rate half of Chaetoceros, would show

higher instantaneous growth rates at concentrations of NO_3^- less than 2.0 µm/l.

Eppley et al. (1969a) reported an apparent relationship between cell size (diameter) and K_s. They observed that large-celled species had high K_s values and small-celled species had low K_s values. Parsons and Takahashi (1973) discussed the relationship between cell size, K_s and available nutrient levels. They concluded that in areas of low nutrient concentrations the predominant species have small cells and low K_s values. Conversely, large species with high K_s values predominate in areas of higher nutrient concentrations.

Knowledge of nutrient uptake and growth constants alone are not sufficient to accurately predict the outcome of competition between different species of algae. Eppley et al. (1969a) predicted the competitive advantage of one species over another by calculating growth rates as a function of nutrient concentration for species with known growth responses to light, temperature and photoperiod. Their results showed that competitive advantage shifts from species to species as light and nutrient concentrations change.

Computer simulation of phytoplankton competition based on formulae incorporating loss rates (O'Brien 1974) demonstrated the importance of zooplankton grazing and algal cell sinking in determining the outcome of phytoplankton competition. The influence of variable death rates may be very important in the seasonal succession of phytoplankton, possibly more important than light and temperature.

It is important to realize that the studies discussed above (relating competition and growth to environmental influences) do not discount the importance of uptake constants in determining competitive

ability. They demonstrate that various environmental parameters such as temperature, light and loss rates are coupled with physiological constants such as K_s and V_m to determine growth rates in a rather complex manner.

CHAPTER III

MATERIALS AND METHODS

Half-saturation and V_m values were determined for 17 species of algae. Eighteen species were tested; they are listed by source below.

U. S. Environmental Protection Agency, Corvalis, Oregon

Selenastrum capricornutum

Microcystis aeruginosa

U. S. Environmental Protection Agency, Athens, Georgia. Isolated from the Black Warrior River, Alabama by J. O'Kelly and T. Deason.

Carteria sp

Golenkiniopsis sp

Monoraphidium sp

Actinastrum sp

Koliella sp

Nitzschia w-31

Nitzschia w-32

Richard Starr Algal Collection, University of Texas

Chlorella vulgaris 262*

Chlorella pyrenoidosa 26

Scenedesmus obliquus 393

Chlamydomonas reinhardi 90

Chlorococcum hypnosporum

* strain number

Gloeocapsa alpicola 589

Navicula pelicullosa

Hantzschia amphioxis

Anabaena A7214

Algal Culture Methods

Unialgal cultures were grown in Woods Hole MBL liquid medium (Stein 1969) with 25 μm NO_2^-/l as the N source instead of of NO_3^- (Table I). The algae were cultured at 20 C ±0.5 C at light saturation (Table II). Light was provided by Westinghouse 30 W cool white flourescent lamps. Light levels were regulated by Powerstat variable autotransformers. Continuous illumination was utilized throughout all uptake experiments to avoid the complications of periodicity in assimilation (Eppley et al. 1971), light induced NR activity (Hageman et al. 1961, Beevers et al. 1965, Shibata et al. 1969) and periodicity in phytoplankton growth (Tamiya 1966, Pirson and Lorenzen 1966). The cultures were stirred and aerated continuously with NH_4^+-free air obtained by bubbling the air through dilute H_2SO_4 and KOH.

Experimental Procedures

It was beyond the scope of the study to determine the growth rates of the algae cultured for the experiments discussed here. Experimental evidence gathered on several species, however, verified that under the conditions described above, the cells were undergoing exponential growth when the NO_2^- was depleted from the media. After the NO_2^- was depleted (when it was no longer detected in the culture medium), 1 μm NO_3^- per liter of culture medium was added to the cultures. If NO_3^-

TABLE I

THE FORMULATION OF WOODS HOLE MBL CULTURE MEDIUM WITH 25μM NITRITE REPLACING NITRATE

a.	Macronutrients	
	$CaCl_2.2H_2O$	36.76 g/l
	$MgSO_4.7H_2O$	36.97
	$NaHCO_3$	12.60
	K_2HPO_4	8.71
	KNO_2	0.02
	$Na_2SiO_3.9H_2O$	28.42
b.	Micronutrients	
	Na_2.EDTA	4.36
	$FeCl_3.6H_2O$	3.15
	$CuSO_4.5H_2O$	0.01
	$ZnSO_4.7H_2O$	0.022
	$CoCl_2.6H_2O$	0.01
	$MnCl_2.4H_2O$	0.18
	$Na_2MoO_4.2H_2O$	0.006
c.	Vitamins	
	Thiamine. HCl	0.1 mg/l
	Biotin	0.5 μg/l
	Cyanocobalamin	0.5 μg/l
d.	Tris- 2ml/l	
	Tris(hydroxymethyl)-aminomethane	50 g/200 ml

TABLE II

LIGHT LEVELS USED FOR THE GROWTH OF THE ALGAE CULTURES

CHLOROPHYTA	foot-candles
Carteria sp	500
Chlamydomonas reinhardi	500
Chlorococcum hypnosporum	500
Monoraphidium sp	500
Chlorella vulgaris	250
Chlorella pyrenoidosa	500
Selenastrum capricornutum	400
Golinkiniopsis sp	500
Actinastrum sp	500
Scenedesmus obliquus	500
Koliella sp	500
CYANOPHYTA	
Gloeocapsa alpicola	500
Microcystis aeruginosa	200
Anabaena A7214	400
CHRYSOPHYTA	
Navicula pelicullosa	500
Hantzschia amphioxis	500
Nitzschia w-31	500
Nitzschia w-32	500

was not added immediately, the cells became N-starved and exhibited non-linear uptake (with time), quite uncharacteristic of cells in exponential growth. Uptake experiments were carried out 2 to 3 hr after the addition of the 1 μM NO_3^-/l. This preincubation eliminated the lag in NO_3^- uptake, which results in an inaccurate determination of K_s and V_m values (Eppley and Thomas 1968). After preincubation the cultures were subdivided into 90 ml aliquots, each of which was enriched with 10 ml of NO_3^-, giving final concentrations ranging from 1 to 20 μm NO_3^--N/l.

Immediately after each culture was enriched, one half (50 ml) was filtered through a 0.45 μ membrane filter under vacuum (0.3 atmosphere). The cell-free filtrates were analyzed for NO_3^-. The values obtained represented the experimentally determined NO_3^- concentrations at the beginning of the uptake experiments. The remaining cells were then incubated at 20 C for 30 to 120 minutes, at the same light intensity as that used for growth. The flasks were shaken by hand every 5 minutes to insure complete distribution of cells within the culture medium. At the end of the uptake period the cells were harvested by filtration and the filtrate was analyzed for NO_3^-. Uptake was calculated as the difference between initial and final NO_3^- concentration divided by the time interval used. Nitrate was determined as NO_2^- after reduction by passage through a copper-cadmium column (Strickland and Parsons 1968). All determinations were made in duplicate.

Observations were made twice with Scenedesmus obliquus to determine the effects of temperature on K_s and V_m. Here all experimental conditions were the same as above, but the temperature was 15.5 C (instead of 20.0 C) during growth of the cultures and during measurement of uptake.

At the time of each experiment the cells were measured using a "Whipple" grid occular micrometer which had been calibrated with an American Optical stage micrometer. The diameters of spherical cells and the diameters and lengths of cylindrical cells were used to calculate the surface areas of the cells. Cell concentration in each culture was measured with an American Optical "Brightline" hemacytometer. The pH of each culture was measured to 0.1 pH unit with a Corning pH meter.

Calculation of K_s and V_m

The Woolfe plot, a linear transform of the Michaelis-Menten equation, was used to calculate K_s and V_m. In this case S is plotted against (S/v) as shown in Figure 4. Equation 4 describes the curve.

$$S = V_m (S/v) - K_s \tag{4}$$

The K_s values were calculated by first determining the slope and Y-intercept, and then determining the X-intercept by equation 5. The negative X-intercept is K_s.

$$\text{X-intercept} = \frac{-(\text{Y-intercept})}{\text{Slope}} \tag{5}$$

The confidence intervals on K_s were calculated by the method described in Ott (1977), giving confidence intervals for the value of x for a given value of y. This method was necessary because an estimation of the confidence interval of an independent variable corresponding to a measured value of the dependent variable was needed. The values of V_m were determined by equation 6.

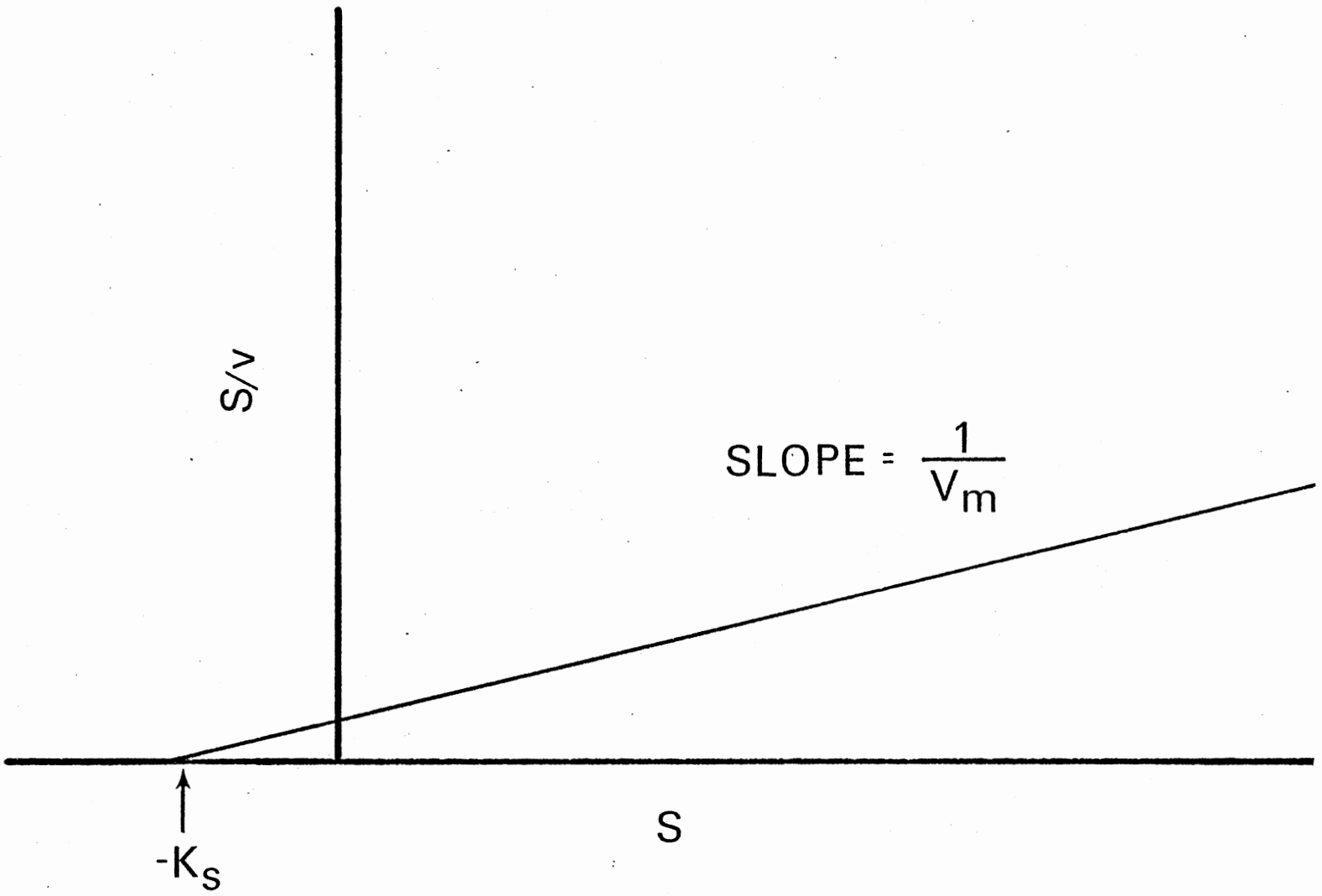

Figure 4. Linear Transform of Uptake (v) vs Concentration (S) Using the Woolfe Plot Showing the Relationship of K_s and V_m to the Regression

$$\text{Slope} = \frac{1}{V_m} \qquad (6)$$

The popular Lineweaver-Burke (double reciprocal) plot was not used for the reasons of Dowd and Riggs (1965).

CHAPTER IV

RESULTS

Early attempts to determine K_s and V_m failed because the cells became N-starved and exponential growth was not maintained. Upon the addition of NO_3^-, under N-starved conditions, uptake is non-linear. Uptake must be linear, i.e., the uptake rate must remain constant throughout the experiment in order to obtain the reproducible, species-specific values necessary for determining meaningful uptake constants.

Figure 5 shows the non-linear rate of disappearance of NO_3^- when it is added to a culture of N-starved cells. The rates of NO_3^- uptake so determined were useless in determining K_s. Figure 6 shows the disappearance of NO_3^- from a culture which had depleted the original N in the growth medium, but had not been allowed to become N-starved. The latter was characteristic of uptake by cells during the experiments by which K_s and V_m were determined here, and resemble optimal conditions suggested by Eppley et al. (1969a) and Carpenter and Guillard (1971) for uptake experiments.

With the exception of Actinastrum (Figure 7) uptake was hyperbolic when graphed against NO_3^- concentration. Figures 8 and 9 show typical hyperbolae for the algae tested. Superimposed on the same graphs are the Woolfe plot linear transforms by which K_s and V_m were derived. Uptake rates at each concentration of NO_3^- for all species, from which K_s and V_m determinations were made, are listed in the Appendix. A corre-

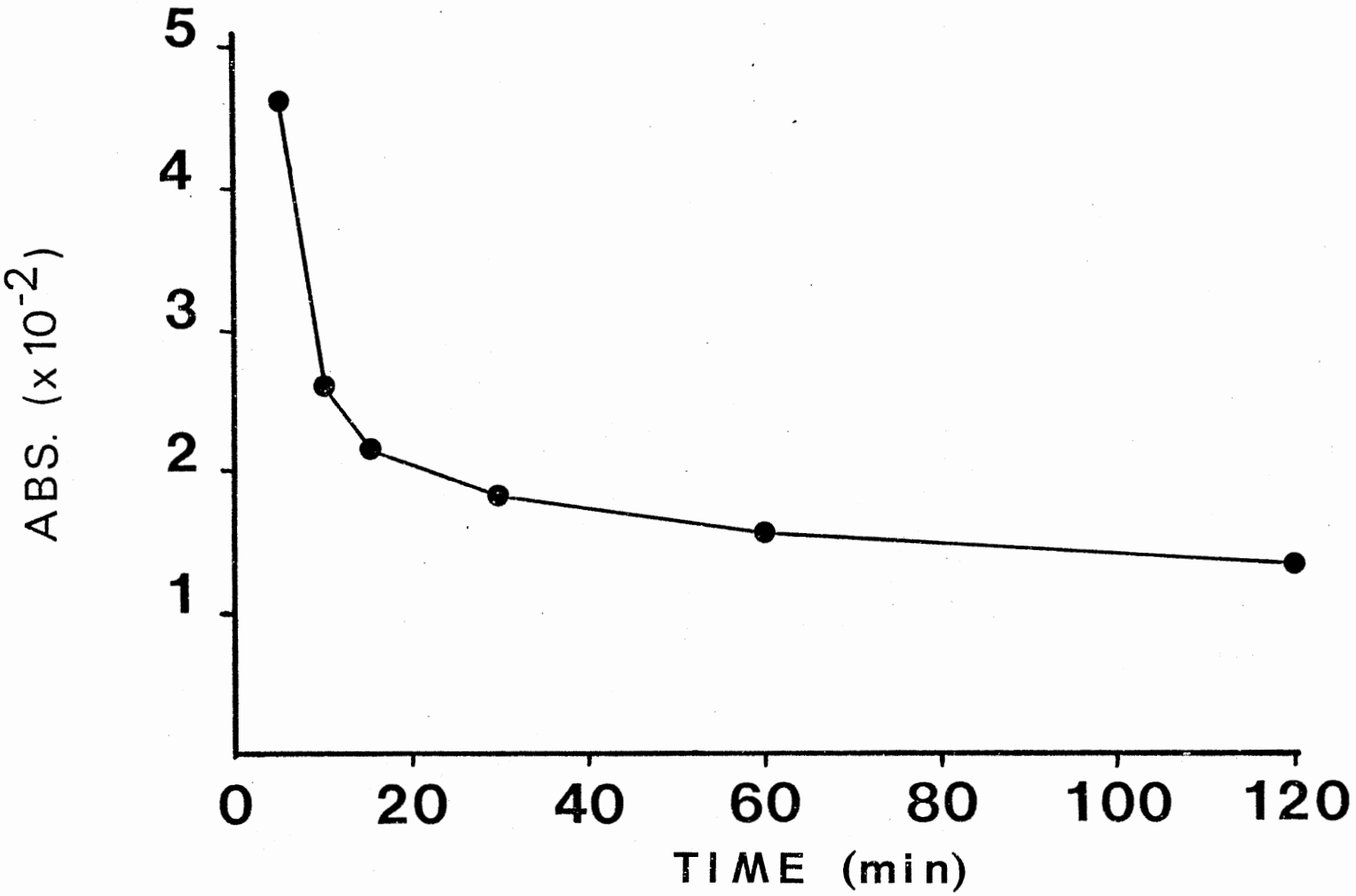

Figure 5. Uptake of Nitrate by a Culture of Nitrate-Starved Cells. ABS is the Absorbance by Azo Dye, the Intensity of Which is Directly Proportional to Nitrate Concentration in the Medium

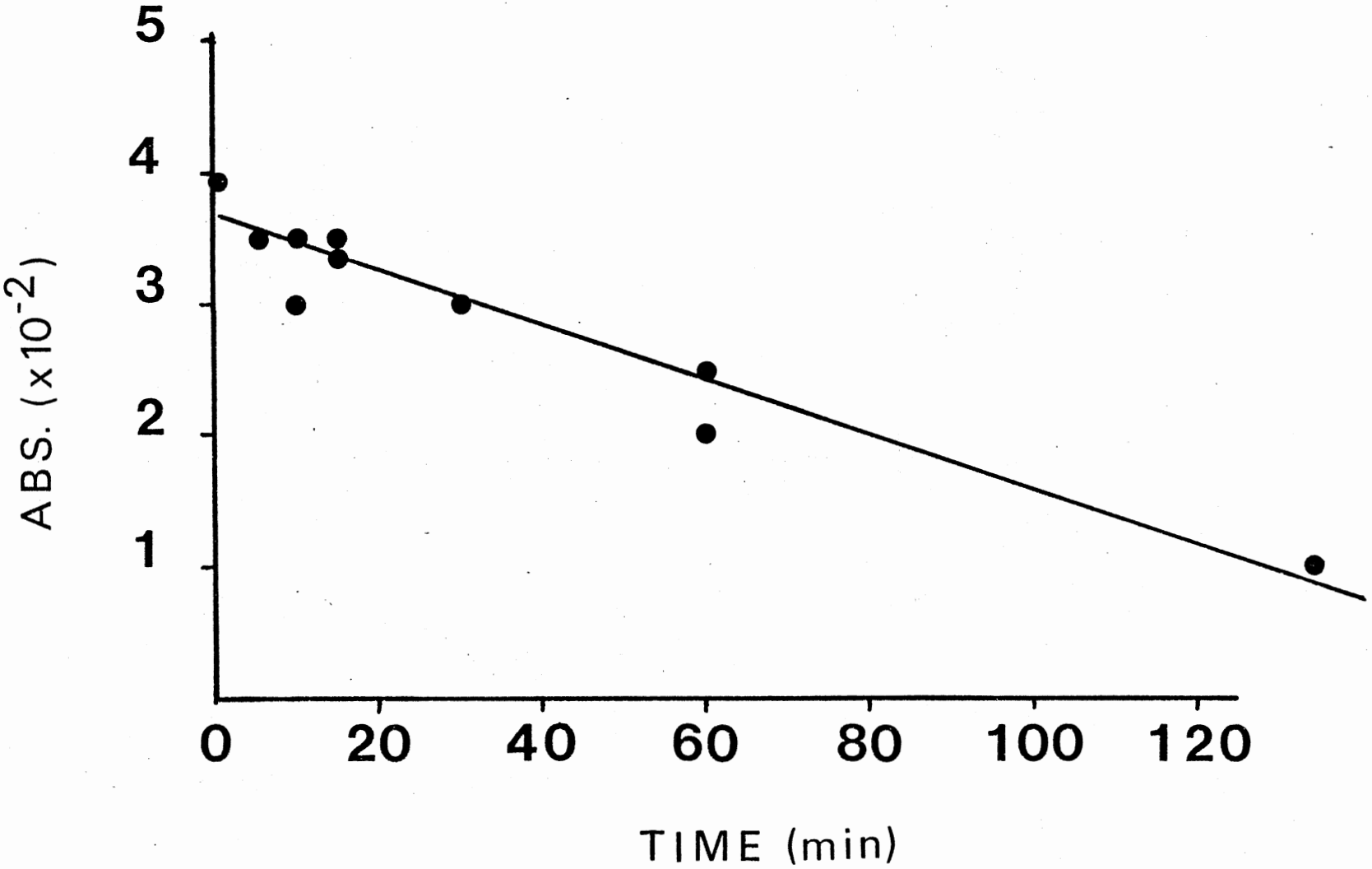

Figure 6. Uptake of Nitrate by Cells in a Nitrate-Depleted Culture in Which the Cells are not Nitrate-Starved. ABS is the Absorbance, by Azo Dye, the Intensity of which is Directly Proportional to Nitrate Concentration in the Medium

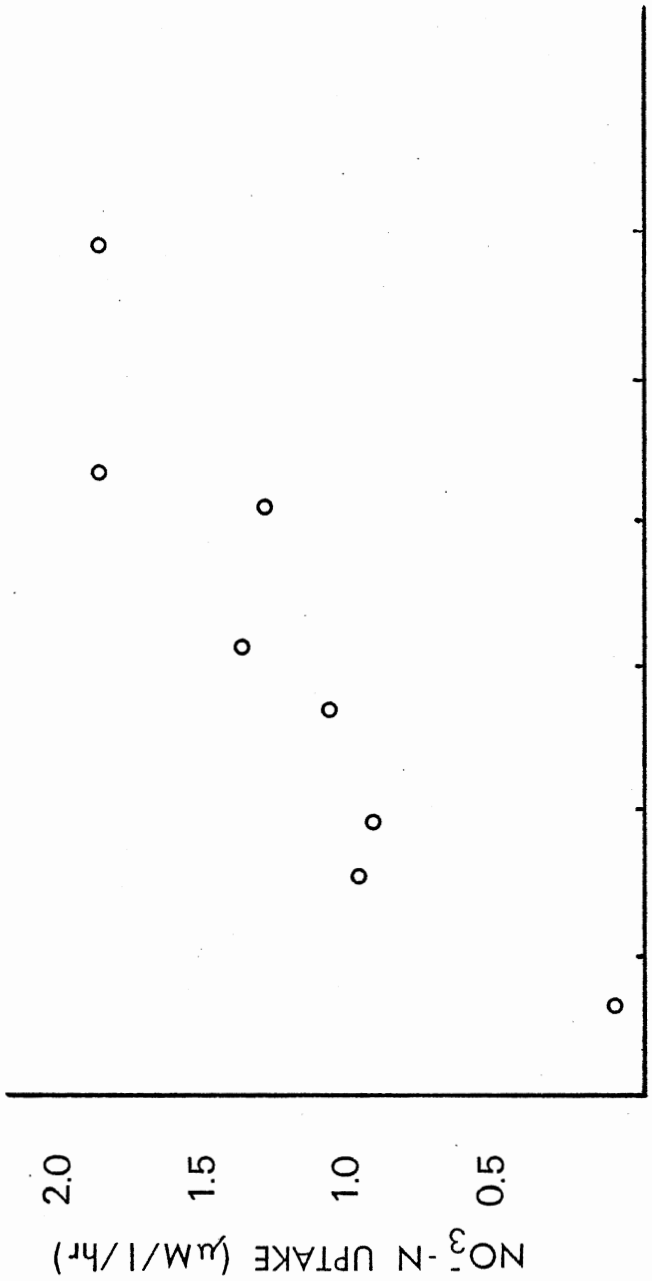

Figure 7. Nitrate Uptake by Actinastrum

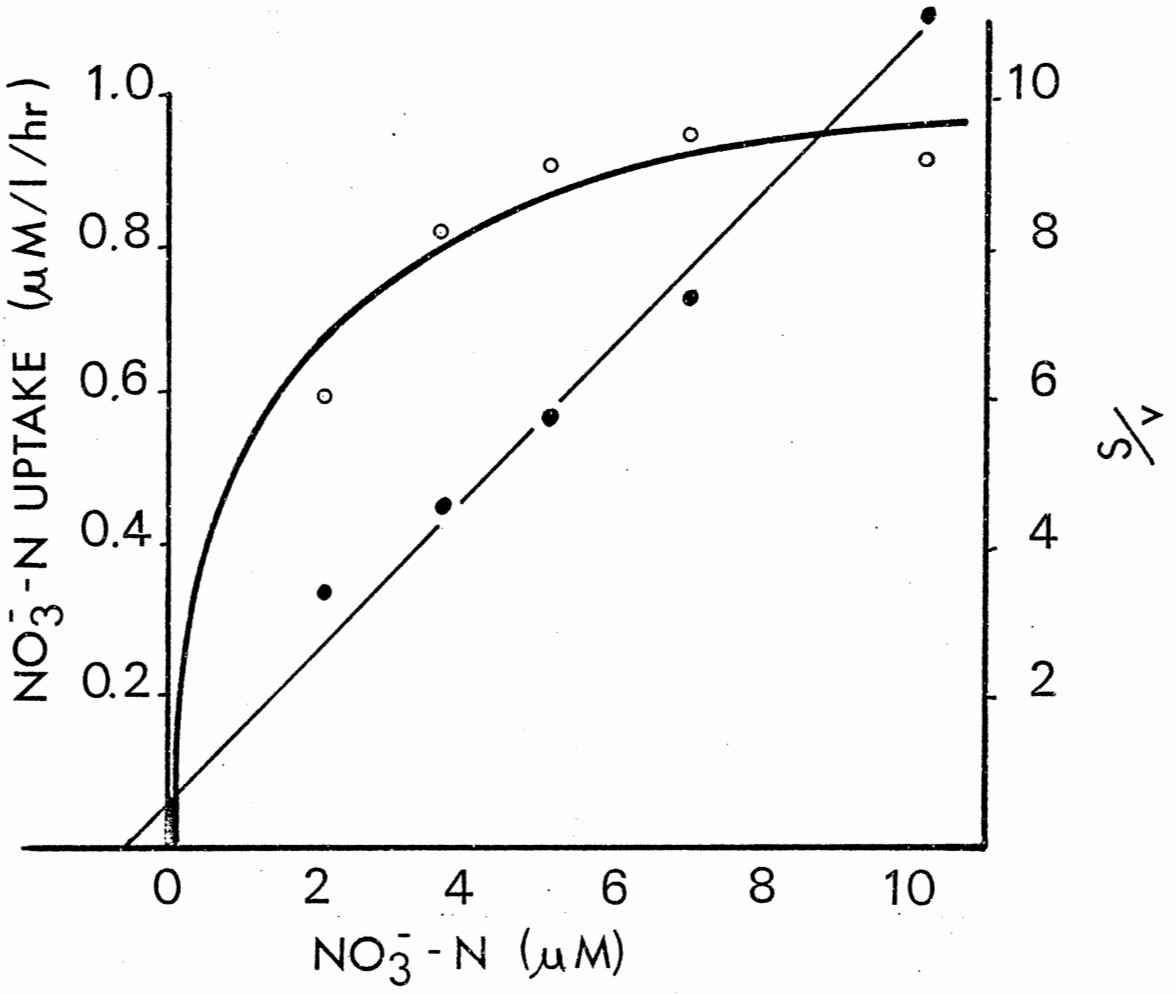

Figure 8. Nitrate Uptake by Monoraphidium. The Straight Line is the S/v vs NO_3^- Concentration plot

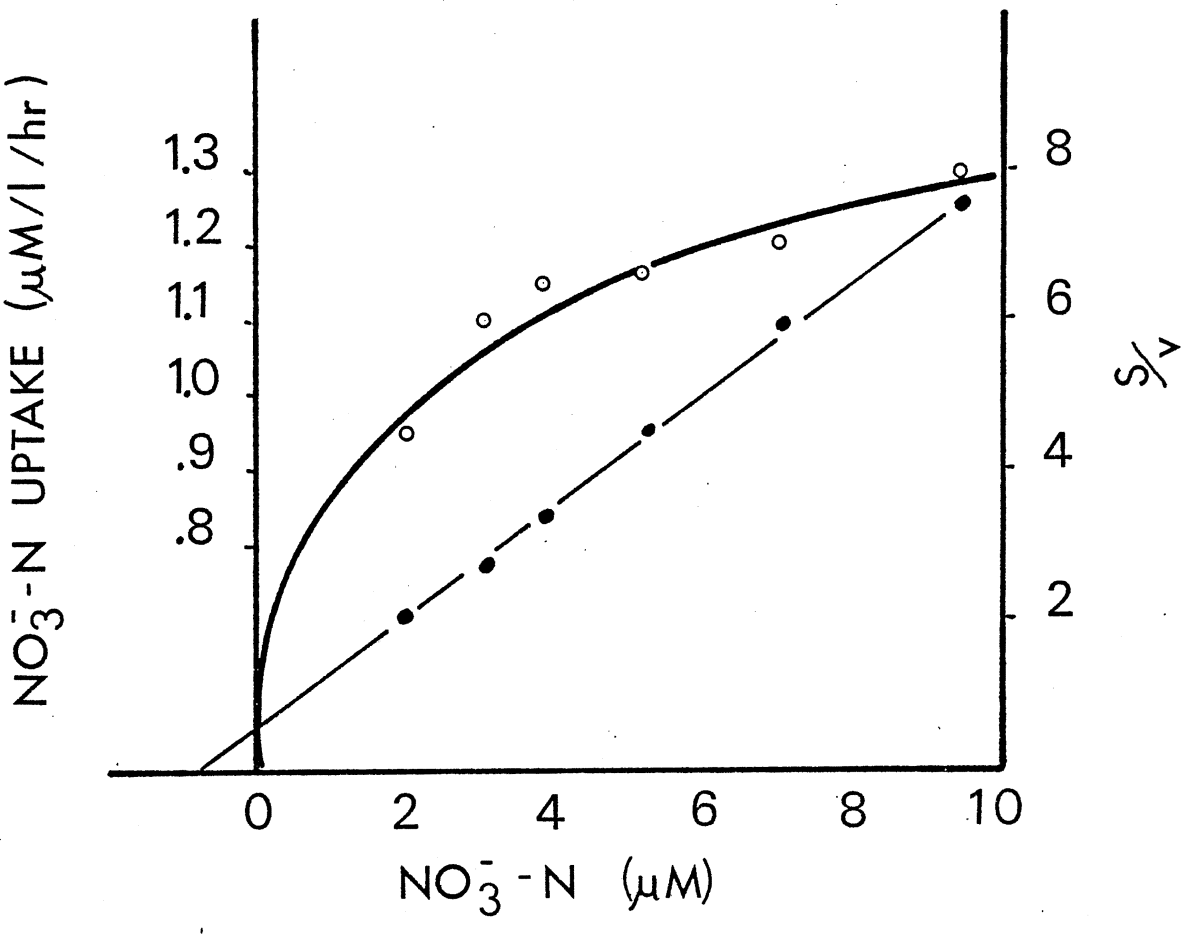

Figure 9. Nitrate Uptake by Chlorella vulgaris. The Straight Line is the S/v vs NO_3^- Concentration Plot

correlation coeficient for the transform of 0.95 or more was used to determine if the regression fit the data.

For species with low K_s values most of the points on the uptake vs concentration plot approach V_m; with no points at concentrations less than K_s. Eppley et al. (1969a) and MacIsaac and Dugdale (1969) also encountered this problem. Therefore, the extrapolation of K_s depends entirely on the assumed linearity of the S vs (S/v) regression line.

Table III presents K_s and V_m values determined during this study. The values of V_m are normalized (100,000 cells) so that comparisons can be made between species. Half-saturation constants for the Chlorophyta range from 0.1 µm NO_3^--N/l for Scenedesmus obliquus to 4.4 µm NO_3^--N/l for Chlorococcum hypnosporum. The values for V_m for the same phylum range from 0.1 µm NO_3^--N/l/hr for Koliella sp to 13.9 µm NO_3^--N/l/hr for Carteria sp. Negative values for K_s were obtained with two of the algae, Koliella sp and Selenastrum capricornutum. No biological meaning can be attributed to these negative values.

Half-saturation and V_m values were determined for 4 algae of the Phylum Chrysophyta. Values for K_s range from 1.3 µm NO_3^--N/l for Nitzschia w-32 to 7.0 µm NO_3^--N/l for Navicula pelicullosa. The values of V_m range from 1.1 µm NO_3^--N/l/hr for Nitzschia w-31 to 4.4 µm NO_3^--N/l/hr for Nitzschia w-32.

Three algae of the Phylum Cyanophyta were used for uptake determinations; Anabaena, a filamentous algae capable of N-fixation as well as Gloeocapsa alpicola and Microcystis aeruginosa, both incapable of N-fixation. Table III shows the K_s and V_m values determined for these three species.

The effects of temperature on K_s and V_m were determined for

TABLE III

VALUES OF HALF-SATURATION CONSTANTS, CONFIDENCE LIMITS, AND MAXIMUM UPTAKE VELOCITIES FOR THE UPTAKE OF NITRATE

CHLOROPHYTA	K_s (μm NO_3^--N/l) *			V_m (μm NO_3^--N/l/hr)**
Carteria sp	(0.4)	1.4	(2.5)	13.9
Chlamydomonas reinhardi	-(1.2)	0.6	(2.8)	1.1
Chlorococcum hypnosporum	(1.9)	4.4	(8.0)	-
Monoraphidium sp	-(0.1)	1.1	(2.5)	0.4
Chlorella vulgaris	(0.4)	0.8	(1.2)	0.2
Chlorella pyrenoidosa	-(0.6)	0.3	(1.3)	0.3
Selenastrum capricornutum	-(1.4)	-0.2	(1.2)	0.2
Golenkiniopsis sp	(2.4)	4.4	(7.2)	1.8
Actinastrum sp***				
Scenedesmus obliquus	-(1.9)	0.1	(2.6)	0.6
Scenedesmus obliquus	(1.5)	4.0	(6.9)	0.6
Koliella sp	-(1.9)	-0.1	(2.0)	0.1
MEAN *****		1.6		1.9
CYANOPHYTA				
Gloeocapsa alpicola	(2.3)	6.2	(12.6)	0.4
Microcystis aeruginosa	-(0.4)	0.8	(2.3)	0.4
Anabaena A7214	-(5.7)	-2.1	(2.4)	0.1
MEAN *****		3.5		0.3

* upper and lower confidence limits in parenthesis
** data normalized to 100,000 cells
*** hyperbolic uptake not observed
**** run at 15.5 C, all others at 20 C
***** negative K_s values not included in the mean

TABLE III, (Continued)

CHRYSOPHYTA	K_s (μm NO_3^--N/l)			V_m (μm NO_3-N/l/hr)
Navicula pelicullosa	(5.6)	7.0	(8.6)	1.7
Hantzschia amphioxis	(1.2)	4.3	(10.2)	2.5
Nitzschia w-31	(1.9)	2.8	(3.7)	1.1
Nitzschia w-32	-(1.0)	1.3	(4.4)	4.4
MEAN		3.8		2.4

Scenedesmus obliquus. Experiments were run at 20 C and 15.5 C. The lower temperature appeared to cause K_s to increase (lowered the efficiency of uptake) from 0.1 µm NO_3^--N/l to 4.0 µm NO_3^--N/l. The value of V_m, however, was 0.6 µm NO_3^--N/l/hr at both 20 and 15.5 C. Since the confidence intervals on the values of K_s for the different temperatures overlap slightly, the differences cannot be considered highly significant. However, Carter and Lathwell (1967) observed similar results in studies on the uptake of low levels of phosphorus (P) by corn roots. Studies at 20 and 30 C resulted in K_s values of 3.56 and 6.09 µm P/l respectively, with the same V_m value for both temperatures. However, at high P concentrations, increased temperatures resulted in an increase in both K_s and V_m.

The Appendix summarizes the cell sizes (surface area) of the cells in each culture during the uptake experiments. Figure 10 shows the relationship between cell surface area and K_s values. No correlation was seen between K_s and surface area (Figure 10) or cell diameter (not shown).

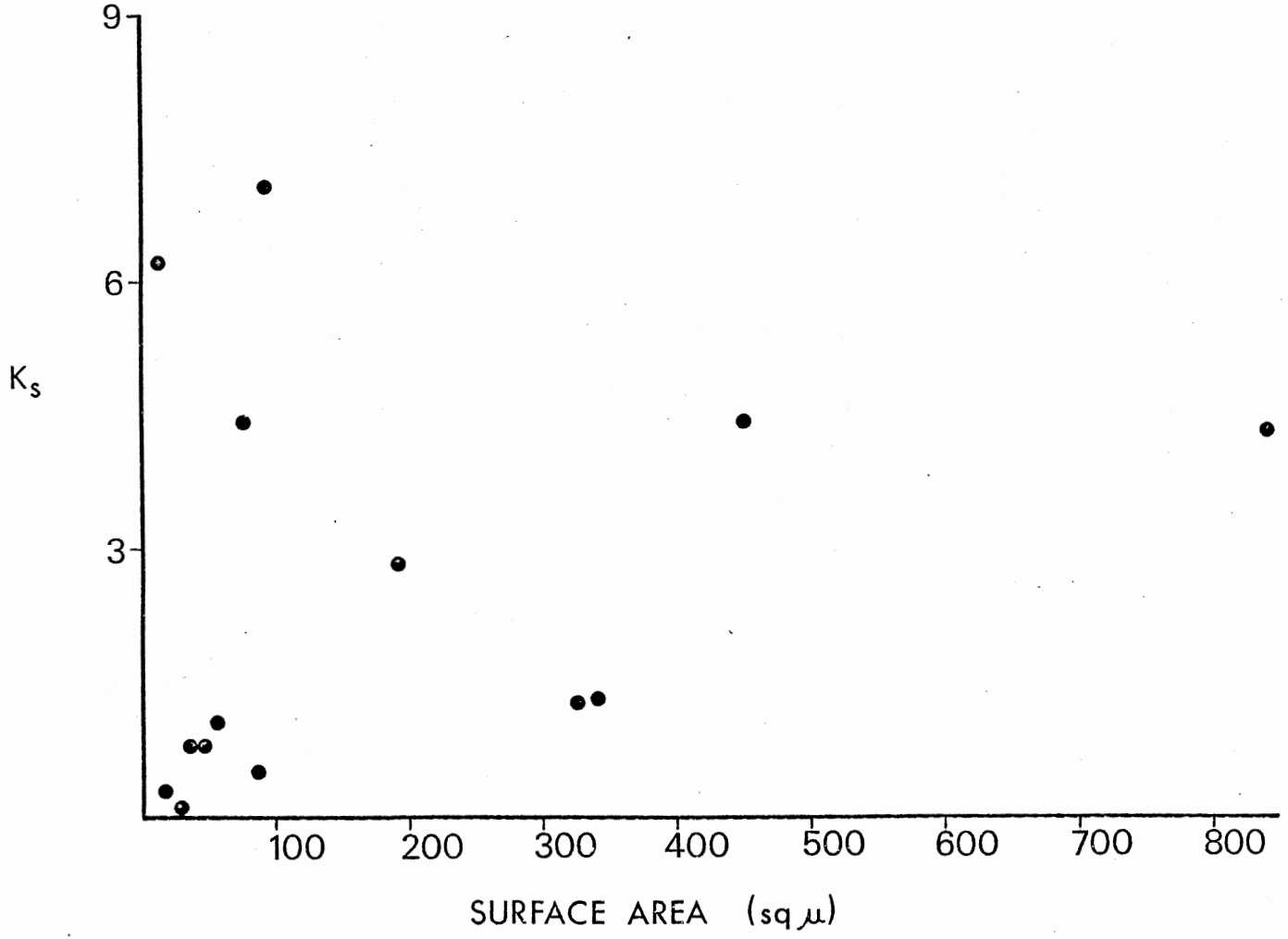

Figure 10. Half-Saturation Constant vs Surface Area for all Algae Tested

CHAPTER V

DISCUSSION

Freshwater algae vary greatly in their ability to take up NO_3^-. These differences reflect variations in the evolution of mechanisms by which algae compete for a nutrient, NO_3^-, when it is limiting growth. Although P is generally believed to limit algal growth in freshwaters, there are times when N is the limiting nutrient (Keeney 1974). However, since it has been demonstrated that NH_4^+ inhibits NO_3^- uptake and assimilation (Eppley et al. 1969b, Morris and Syrett 1972), the data given here would apply to conditions under which a) N is limiting growth and b) NH_4^+ is at extremely low concentrations (or not present) and NO_3^- is present. In many lakes the concentration of NH_4^+ is generally higher than that of NO_3^-. However, the reverse can be true in surface waters of summer-stratified lakes. Hutchinson (1975) presented data in which NO_3^--N was about 1.4 µm/l while NH_4^+ -N was about 0.8 µm/l. Faust (1973) reported summer NO_3^--N concentrations ranging from undetectable to 4 µm/l in an Oklahoma reservoir. Although no NH_4^+ concentrations were reported, the results of NO_3^- enrichment algal assays led him to believe NO_3^--N was limiting the growth of some species of algae. In shallow streams NH_4^+ can be oxidized to NO_3^- by nitrifying bacteria on the surface of the substrate and vegetation (Tuffey et al. 1974). Lopez-Bernal et al. (1974), Tuffey et al. (1974) and Ruane and Krenkal (1978) have reported complete oxidation of NH_4^+ to NO_3^- in streams.

Under conditions in which NO_3^- could be limiting growth, it could be predicted that the succession of algae parallels changes in NO_3^- concentration. In part this would be the result of the variation in competitive abilities caused by differences in K_s and V_m for NO_3^- uptake. These arguments are supported by the observation that almost all of the values of K_s and V_m reported here fall into the range of NO_3^- concentrations present during those times when it is most likely to be limiting algal growth.

The values of K_s and V_m are taken as measures of uptake and are most useful in making quantitative comparisons of competitive ability. However, it must be noted that a "high" or "low" value for a species is relative, having little ecological meaning unless that species is compared to another species. In the absence of confounding responses to light, temperature or loss rates, predictions can be made as to the outcome of competition at various NO_3^- concentrations. Such predictions can be made based on the data by comparing uptake values listed in Table III. Therefore, a discussion about which species would win in competition will not appear here. Instead, the patterns of uptake kinetics that occur in the data are discussed, as well as some thoughts concerning the possible evolution of those patterns. Secondly, the trends that occur with regard to uptake capabilities of the major taxonomic groups are illustrated.

High and low values of K_s and V_m are relative in terms of predicting the outcome of competition. It would be arbitrary and artificial to group the values into high, low or intermediate categories, especially in light of the large confidence intervals of the K_s values of some of the species. However, since the values of K_s and V_m are proposed as a

means to compare the competitive abilities evolved by each species, it is necessary to consider certain patterns apparent in the data.

The species discussed here fall into a continuum of combinations of low to high values of both K_s and V_m. That is, there is a range from species with low K_s and V_m values to species with high values for both K_s and V_m. Rather than impose static limitations to the continuum by grouping the species, the extremes are discussed as being representative of the evolutionary strategies to be outlined.

First, several species are characterized by a low K_s and a low V_m value. Examples of this combination are Scenedesmus obliquus (K_s= 0.1 µm/l and V_m= 0.6 µm/l/hr) and Chlorella pyrenoidosa (K_s= 0.3 µm/l and V_m= 0.3 µm/l/hr). These species have apparently evolved an efficient mechanism for uptake when NO_3^- is in low concentrations, but at the expense of the ability to take up NO_3^- rapidly when it occurs at high concentrations. It could therefore be predicted that these species would be dominant in waters in which NO_3^- concentration is very low.

Second, at the other extreme are those species which have evolved mechanisms by which they can take up NO_3^- very efficiently (rapidly) at high concentrations, but at the expense of rapid uptake at low concentrations. These species are characterized by high V_m and K_s values. Hantzschia amphioxis with a K_s of 4.3 µm/l and a V_m of 2.5 µm/l/hr is a good example. It could be predicted that species in this category would be dominant in waters with high NO_3^- concentrations.

Finally, several of those species studied fall into a group characterized by high K_s values (poor uptake at low concentrations) and low V_m values (poor uptake at high concentrations). They have not evolved efficient mechanisms for NO_3^- uptake at high or low concentrations.

Gloeocapsa alpicola with a K_s of 6.2 µm/l and V_m of 0.4 µm/l/hr is characteristic of this third category. These species have possibly evolved under conditions in which NO_3^- was not limiting. Therefore, there would have been no selective pressure for the evolution of efficient NO_3^- uptake mechanisms. Studies on their growth responses to light and temperature, however, might reveal secondary mechanisms by which they could successfully compete.

When considering the means of the K_s and V_m values for the three phyla studied another trend is evident. The green algae and the blue-green algae have lower K_s and V_m values than do the diatoms (Table III). This indicates the possibility that the greens and blue-greens would be better at taking up NO_3^- at low concentrations, but are not able to take it up as efficiently as diatoms when NO_3^- is present at high concentrations. The diatoms, with higher K_s and V_m values would not be as efficient as the other two groups at taking up NO_3^- at low concentrations, but are adapted to take it up more rapidly at high concentrations.

These findings agree with the observation that diatoms are often dominant in lakes in April and May, after "turnover", when NO_3^- is abundant, and are replaced by green and blue-green algae after nutrient concentrations decrease (Fogg 1965). The presence of diatoms in nutrient-rich waters in marine environments was suggested by Dugdale (1969) to be due to a high V_m, evolved at the cost of a correspondingly high K_s value. It appears that diatoms in both freshwater and marine environments have evolved similar strategies for uptake of NO_3^-.

The study on the effect of temperature on uptake kinetics indicated a possible increase in the value of K_s with a decrease of temperature. The 95% confidence interval on the two K_s values overlapped, however,

so the difference is not interpreted as being highly significant. Others, however, have seen variations in uptake constants as a function of temperature (Carter and Lathwell 1967). Further studies must be conducted before the relationship between temperature and nutrient uptake capabilities (as indicated by K_s and V_m) can be fully described.

If temperature, or any variable, affects the uptake capabilities of algae, it is important that studies be conducted in which environmental factors are coupled to physiological factors when attempting to determine the ability of algae to compete for nutrients. It is likely that the factors involved in species succession are more complex than just differences in K_s and V_m values. It is, however, beyond the scope of this study to couple observed uptake kinetics with growth responses to light, temperature, loss rates or any other environmental factors. This area, which has been studied with some marine algae (Eppley et al. 1969a, Lehman et al. 1975), has not been studied with freshwater forms and is a fertile field for further study.

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APPENDIX

TECHNICAL DATA FOR UPTAKE EXPERIMENTS

	S ($\mu m\ NO_3^-$-N/l)	v ($\mu m\ NO_3^-$-N/l/hr)
Carteria sp	1.1	0.7
	2.5	1.3
r* =0.99	3.5	1.3
pH= 7.5	5.0	1.5
cell size= 346 sq μ	8.1	1.5
cell density=13,000/ml	10.3	1.6
bacterial contamination- slight		
Chlamydomonas reinhardi	3.5	2.3
	4.5	1.6
r= 0.98	4.3	1.8
pH= 7.3	5.0	1.5
cell size=86.8 sq μ	13.2	2.0
cell density= 195,556/ml	16.3	2.0
bacterial contamination- not observed		
Chlorococcum hypnosporum	2.2	0.8
	3.4	0.9
r= 0.95	4.5	0.9
pH= not recorded	5.9	1.2
cell size= 452.4 sq μ	7.0	1.1
cell density= not observed	12.5	1.5
bacterial contamination- none		
Monoraphidium sp	2.1	0.6
	3.7	0.8
r= 0.99	5.2	0.9
pH= 7.5	7.0	1.0
cell size= 58.8 sq μ	10.2	0.9
cell density= 268,333/ml		
bacterial contamination- none		

* correlation coefficient of the S vs (S/v) regression

	S	v
Chlorella vulgaris	2.0	1.0
	3.1	1.1
r= 0.99	3.9	1.2
pH= 7.3	5.2	1.2
cell size= 36.3 sq μ	7.0	1.2
cell density= 685,000/ml	9.5	1.3
bacterial contamination- none		
Chlorella pyrenoidosa	2.4	1.2
	3.1	1.1
r= 0.99	4.0	1.1
pH= 7.2	5.8	1.3
cell size= 18.9 sq μ	8.2	1.2
cell density= 492,500/ml		
bacterial contamination- none		
Selenastrum capricornutum	1.4	0.7
	2.7	1.2
r= 0.98	3.0	1.2
pH= not recorded	7.5	1.5
cell size= 58.4 sq μ	8.2	1.4
cell density= 508,333/ml		
bacterial contamination- not observed		
Golenkiniopsis sp	5.1	1.3
	5.8	1.4
r= 0.97	6.8	1.6
pH= 7.4	8.6	1.7
cell size= 75.4 sq μ	9.8	1.7
cell density= 141,667/ml		
bacterial contamination- not observed		
Actinastrum sp	1.2	0.1
	3.0	1.0
r= non-hyperbolic uptake	3.7	1.0
pH= 7.7	5.3	1.1
cell size= 63.2 sq μ	6.2	1.4
cell density= 466,667/ml	8.1	1.3
bacterial contamination- none	8.6	1.9
	11.7	1.9

	S	v
Scenedesmus obliquus (15.5 C)	1.1	0.3
	3.0	0.7
r= 0.98	4.0	0.9
pH= 7.9	5.5	0.8
cell size= 30.7 sq μ	7.4	0.9
cell density= 250,000/ml	12.4	1.2
bacterial contamination- slight	16.2	1.2
Scenedesmus obliquus (20 C)	2.5	1.0
	3.4	1.2
r= 0.98	4.6	1.1
pH= 7.2	6.1	1.4
cell size= 30 sq μ	10.5	1.3
cell density= 231,666/ml	15.7	1.4
bacterial contamination- not observed		
Koliella sp	1.1	0.5
	4.3	0.8
r= 0.99	6.2	0.8
pH= 7.3	11.0	0.8
cell size= 26.5 sq μ	15.0	0.7
cell density= 616,667/ml		
bacterial contamination- slight		
Gloeocapsa alpicola	4.1	0.6
	7.5	0.7
r= 0.95	9.3	0.8
pH= 7.1	11.2	0.9
cell size= 11 sq μ	15.5	1.0
cell density= 318,333/ml		
bacterial contamination- none		
Microcystis aeruginosa	1.3	0.5
	2.4	0.9
r= 0.98	2.9	0.8
pH= 7.5	4.4	0.9
cell size= 46.6 sq μ	9.9	1.1
cell density= 240,000/ml		
bacterial contamination- not observed		

	S	v
Anabaena A7214	2.6	0.6
	6.2	0.6
r= 0.97	9.8	0.6
pH= 7.3	19.2	0.4
cell size= 33.2 sq μ		
cell density= 697,500/ml		
bacterial contamination-		
not observed		
Navicula pelicullosa	3.0	0.8
	5.0	1.1
r= 0.99	6.9	1.3
pH= 7.2	9.3	1.5
cell size= 91.3 sq μ	15.2	1.8
cell density= 155,000/ml	19.9	2.0
bacterial contamination-		
none		
Hantzschia amphioxis	2.8	0.3
	3.9	0.4
r= 0.91	5.5	0.6
pH= 7.2	7.0	0.5
cell size= 841.4 sq μ	9.0	0.6
cell density= 31,667/ml		
bacterial contamination-		
moderate		
Nitzschia w-31	1.3	1.1
	4.2	2.0
r= 0.99	5.2	2.2
pH= not recorded	8.1	2.3
cell size= 192.3 sq μ	9.9	2.6
cell density= 290,000/ml	12.4	2.7
bacterial contamination-	15.5	2.8
very slight		
Nitzschia w-32	4.1	2.9
	5.3	3.2
r= 0.97	7.1	3.8
pH= 7.2	9.1	4.6
cell size= 324 sq μ	12.4	4.1
cell density= 103,333/ml	15.7	4.0
bacterial contamination-		
very slight		

VITA

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Candidate for the Degree of

Master of Science

Thesis: COMPARATIVE KINETICS OF NITRATE UPTAKE BY FRESHWATER ALGAE

Major Field: Zoology

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