UPTAKE OF AMMONIUM AND NITRATE BY PHYTOPLANKTON IN LAKES OF THE EXPERIMENTAL LAKES AREA OF NORTHWESTERN ONTARIO

A Thesis

Submitted to

the Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfilment

of the Requirements for the Degree

Master of Science

by

Andrew Gregory Dwilow

July, 1977.

UPTAKE OF AMMONIUM AND NITRATE BY PHYTOPLANKTON IN LAKES OF THE EXPERIMENTAL LAKES AREA OF NORTHWESTERN ONTARIO

BY

ANDREW GREGORY DWILOW

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

The author wishes to express sincere thanks to Dr. N.E.R. Campbell, Professor of the Department of Microbiology, University of Manitoba, for his support and assistance in the preparation of this manuscript, Dr. D.W. Schindler, adjunct Professor, University of Manitoba (Fisheries Research Board of Canada) for the use of the facilities of the Experimental Lakes Area, and Dr. R.J. Flett, post-doctoral fellow of McGill University, for his invaluable knowledge of ^{15}N isotope methodology.

Many thanks are given to Mr. Michael Turner for preparing the computer programs used for data analysis.

Mr. J. Prokopowich provided access to ELA water chemistry data and Mr. D. Findlay performed all phytoplankton identification and counting.

A sincere debt of thanks is given to my wife and parents, the persons to whom this project mattered the most.

ABSTRACT

A ^{15}N isotope enrichment method was used to determine rates of ammonium and nitrate uptake by phytoplankton of two lakes in the Canadian Precambrian Shield. Half-saturation constants for nitrogen uptake were highly variable between sampling periods but indicated a definite preference for NH_4^+ over NO_3^- as a phytoplankton N-source. Rates of NH_4^+ and NO_3^- uptake by phytoplankton of a hypolimnetic bloom were found to be extremely low.

To My Father

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLE PAGE

FIGURE

FIGURE PAGE

INTRODUCTION

INTRODUCTION

Eutrophication is a naturally occurring process whereby a lake gradually fills with sediment eventually becoming a bog. In recent years however, man's activities have accelerated this process to the extent that in 1974 the incidence of man-made eutrophic lakes was exponentially increasing (84). This man-made (cultural) eutrophication is a world-wide problem (26) resulting primarily from excessive loading of inorganic nitrogen and phosphorous in the form of human sewage, agricultural fertilizers, and industrial wastes. The results are numerous and objectionable; desirable plant and fish species are succeeded by nuisance species, recreational value is lost due to the foul smell and appearance of the water, fish kills occur due to oxygen depletion following the crash of algal and macrophyte blooms, and engineering problems, such as clogging of water supply filters by excessive plant material, often arise; to list only a few, and are well documented (19,26,62,84).

In 1967 the Canada Department of the Environment's Freshwater Institute at Winnipeg, Manitoba established the Experimental Lakes Area to provide the facility for performing whole lake experiments dealing with such aspects of eutrophication as cycling and persistence of nutrient pollutants, overall effects, and possible remedial measures (47). The Experimental Lakes Area (ELA) is located in the Canadian Precambrian shield approximately 35 miles east southeast of Kenora,

Ontario. The lakes are for the most part small, have relatively low conductivity water, and are subject to thermal stratification during the ice-free season. Lake basins are composed primarily of igneious rock such as Precambrian acid-granite and receive little or no influence from ground water thus facilitating determination of complete nutrient budgets (8).

The lakes used in the present study were Lake 302 (Fig. 1) and Lake 226 (Fig. 2). Lake 302 is a double basin lake 23.7 hectares in area divided approximately in half by a sea curtain which extends from lake surface to lake bottom across the narrow channel separating the two basins. The south basin receives no nutrient supplementation whereas the north basin has received 0.54 $g.m.^{-2}$ P as phosphoric acid, 27.9 $g.m.^{-2}$ N as ammonium chloride, and 3.73 $g.m.^{-2}$ C as sucrose annually since 1972. In order to test a management hypothesis the nutrients were added to the lake at a depth of 8m., i.e. well below the thermocline. It was felt that nutrient uptake by hypolimnetic microplankton followed by sedimentation would avert development of a summer epilimnetic algal bloom; which ultimately proved to be true (69).

Lake 226 is also a double basin lake, 16.1 hectares in area divided approximately in half by a sea curtain. The southwest basin receives 3.2 $g.m.^{-2}$ N as ammonium chloride and 6.1 $g.m.^{-2}$ C as sucrose annually since 1973. The northeast basin receives the same amounts of N and C and as well receives 0.6 $g.m.^{-2}$ P as phosphoric acid annually. The results of this practice provided for a massive annual phytoplankton bloom only in the phosphorous-supplemented basin indicating that P is the key limiting nutrient in lakes of the Canadian Precambrian shield (32,67,69).

The object of the present study was to determine the kinetic parameters involved in uptake (assimilation) of NH_4^+ and NO_3^- by phytoplankton of Lakes 302 and 226. It should be noted that the terms uptake and assimilation can be defined respectively as i) the gross removal of nutrient from the medium and ii) the net incorporation of the nutrient into cellular material. In the present text the terms are used synonymously and refer to gross uptake of nutrient.

Fig. 1. Bathymetric map of Lake 302.

(from J. Fish, Res. Bd. Can. 28:2, 1971)

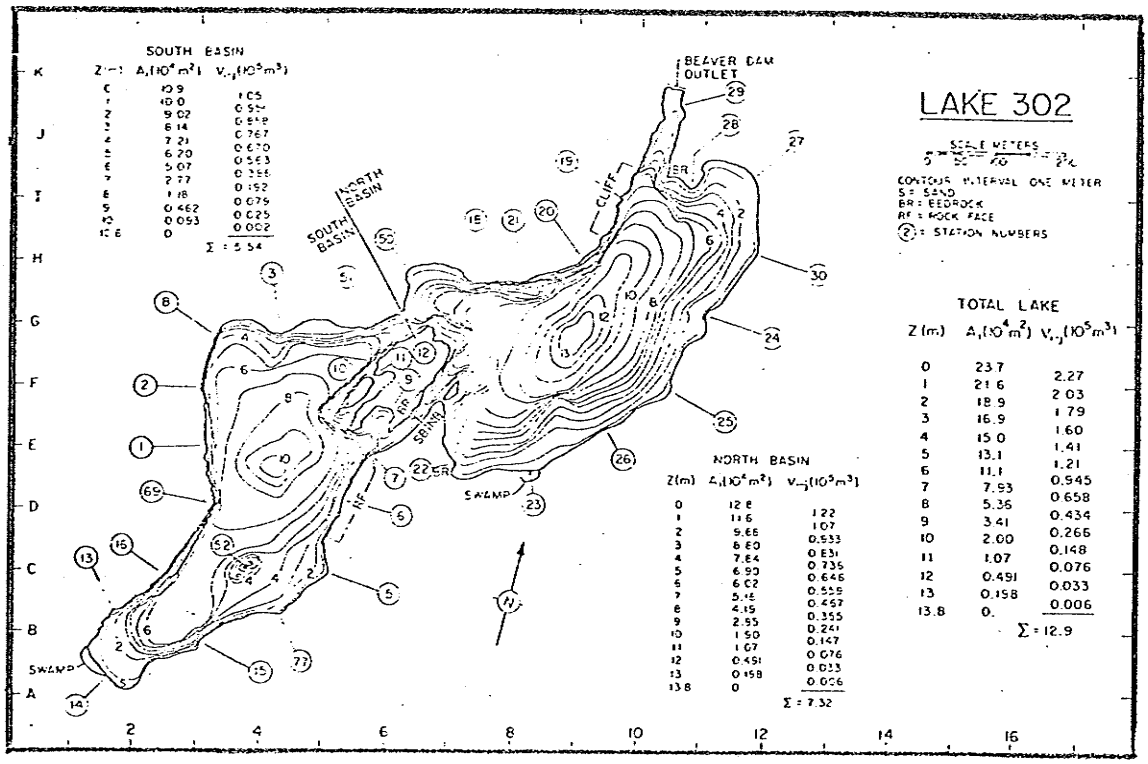
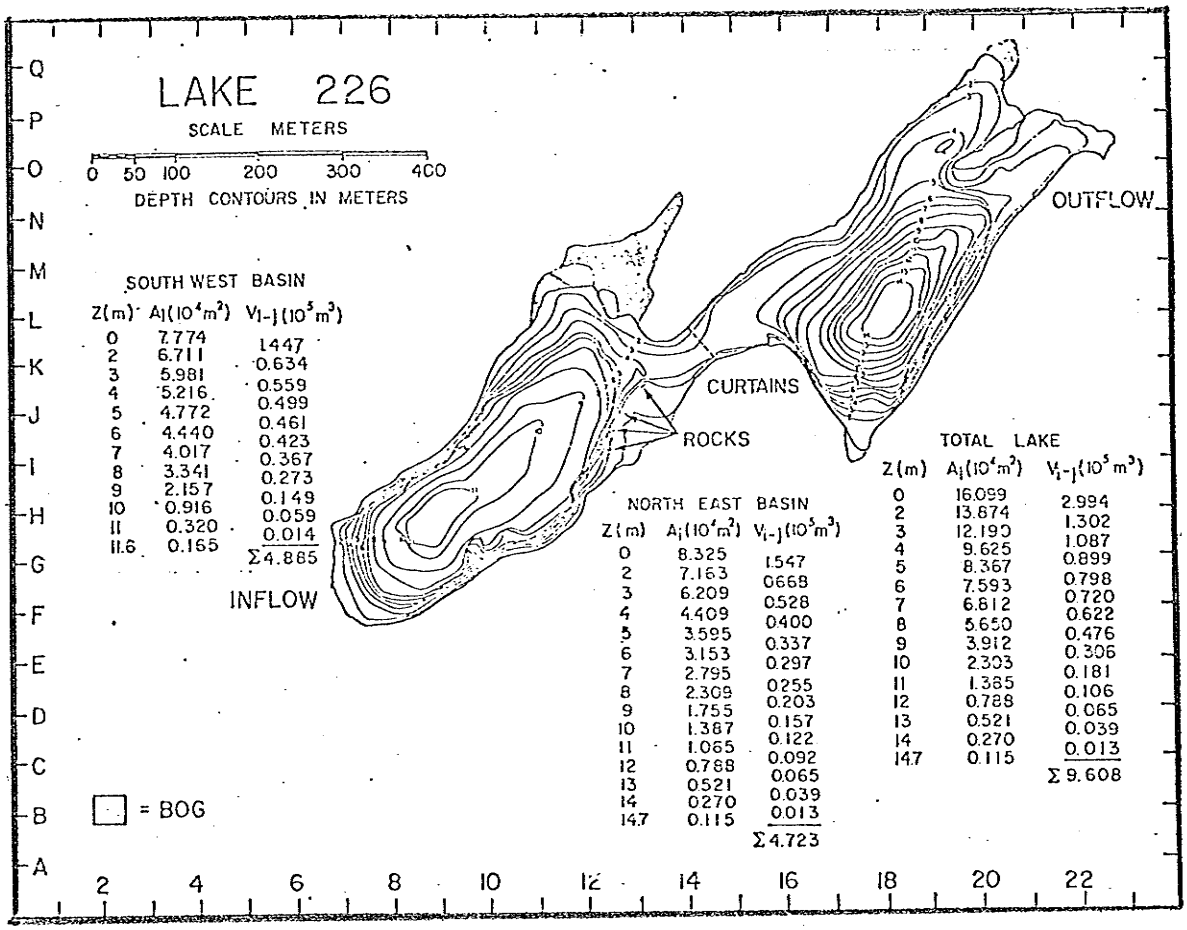

LAKE 302
SOUTH BASIN
NORTH BASIN
BEAVER DAM OUTLET
CLIFF
SPRING
SWAMP
SCALE METERS
CONTOUR INTERVAL ONE METER
S = SAND
BR = BEDROCK
RF = ROCK FACE
(2) = STATION NUMBERS
TOTAL LAKE
Z (m) A_i(10^4 m^2) V_i-j(10^5 m^3)
0 23.7
1 21.6 2.27
2 18.9 2.03
3 16.9 1.79
4 15.0 1.60
5 13.1 1.41
6 11.1 1.21
7 7.93 0.945
8 5.36 0.658
9 3.41 0.434
10 2.00 0.266
11 1.07 0.148
12 0.491 0.076
13 0.158 0.033
13.8 0. 0.006
Σ = 12.9
NORTH BASIN
Z(m) A_i(10^4 m^2) V_i-j(10^5 m^3)
0 12.8 1.22
1 11.6 1.07
2 9.66 0.933
3 8.60 0.831
4 7.64 0.735
5 6.90 0.646
6 6.02 0.555
7 5.16 0.467
8 4.15 0.355
9 2.95 0.241
10 1.50 0.147
11 1.07 0.076
12 0.491 0.033
13 0.158 0.006
13.8 0
Σ = 7.32

Fig. 2. Bathymetric map of Lake 226.

(courtesy Mr. I. Davies, Freshwater Institute)


LAKE 226
SCALE METERS
0 50 100 200 300 400
DEPTH CONTOURS IN METERS
OUTFLOW
CURTAINS
ROCKS
INFLOW
= BOG
SOUTH WEST BASIN
Z(m) Ai(10⁴m²) Vi-j(10⁵m³)
0 7.774 1.447
2 6.711 0.634
3 5.981 0.559
4 5.216 0.499
5 4.772 0.461
6 4.440 0.423
7 4.017 0.367
8 3.341 0.273
9 2.157 0.149
10 0.916 0.059
11 0.320 0.014
11.6 0.165 Σ4.885
NORTH EAST BASIN
Z(m) Ai(10⁴m²) Vi-j(10⁵m³)
0 8.325 1.547
2 7.163 0668
3 6.209 0528
4 4.409 0400
5 3.595 0.337
6 3.153 0.297
7 2.795 0255
8 2.309 0.203
9 1.755 0.157
10 1.387 0.122
11 1.065 0.092
12 0.788 0.065
13 0521 0.039
14 0270 0.013
14.7 0.115 Σ4.723
TOTAL LAKE
Z(m) Ai(10⁴m²) Vi-j(10⁵m³)
0 16.099 2.994
2 13.874 1.302
3 12.190 1.087
4 9.625 0.899
5 8.367 0.798
6 7.593 0.720
7 6.812 0.622
8 5.650 0.476
9 3.912 0.306
10 2.303 0.181
11 1.385 0.106
12 0.788 0.065
13 0.521 0.039
14 0.270 0.013
14.7 0.115 Σ 9.608
A B C D E F G H I J K L M N O P Q
2 4 6 8 10 12 14 16 18 20 22

HISTORICAL

HISTORICAL

It has now become generally accepted that phytoplankton production in aquatic ecosystems, both freshwater and marine, is most often limited by the availability of N and P (16, 26, 31, 48, 50, 66, 67, 69). Nitrogen cycling, unlike that of phosphorous, in such systems can be considered as open, in that an essentially limitless reservoir is available in the form of atmospheric N_2. The dominance of N_2 fixing species of Cyanophyceae in N-depleted systems is well documented (32, 49, 76, 82) and excretion of fixed N_2 in forms utilizable by non-fixing species has been observed (31, 75, 88). The forms, amounts, and distribution of dissolved nitrogen compounds present in aquatic systems are mediated by numerous physical and biological processes.

Common species of dissolved organic nitrogen compounds found in water include amino acids, peptides, nucleic acids, nucleotides, urea, uric acid, as well as pigment molecule moieties and complex organic aggregates.

Wheeler et al (1974) found glycine and serine to be the most abundant amino acids present in sea water, with concentrations ranging from 0.2 - 2.0 µg. at $NL.^{-1}$. Alanine, aspartate, threonine, valine, glutamate, and ornithine were found to be 10 - 30% as concentrated as glycine and serine. Axenic cultures of marine phytoplankton were able to sustain growth utilizing these low ambient levels of amino-N with doubling times similar to those estimated for natural marine populations, i.e., 4 - 5 days.

Gardner and Lee (1975) found dissolved free amino acids in the range of <0.01μM in Lake Mendota, Wisconsin, serine and alanine being the most prevalent species. Levels of combined amino acids were approximately an order of magnitude greater than those of dissolved free amino acids, and showed little variation throughout the year suggesting that they were not utilizable by the phytoplankton.

Phytoplankton utilization of amino acids has also been reported by North (1975) and Schell (1974). Goering (1972) proposes that glycine is the most "readily assimilable" amino acid but that overall, organic N is generally inferior to inorganic N as a phytoplankton N-source.

Amino acids and peptides in natural waters originate from zooplankton and fish excretion, autolysis and bacterial degradation of organic matter, and, in populated areas, from sewage discharge.

The presence of urea in both freshwater and marine systems and its utlization as an N-source by phytoplankton are documented. Concentrations of 0.1-5.0 μg. at. $NL.^{-1}$ (89) for ocean surface waters and 80-150 μg. $NL.^{-1}$ (3) for Lake Kinneret have been reported. The presence of urea in the epilimnion of Lake Kinneret was found to be sporadic and corresponded to zooplankton blooms. Healey (1977) has reported half-saturation constants for urea uptake by chemostat cultures of Scenedesmus quadricauda and Pseudoanabaena catenata as 0.4-2.0 μg. at. $NL.^{-1}$ and 0.4 - 1.4 μg. $at.NL.^{-1}$ respectively. Eppley et al. (1971) have also demonstrated assimilation of urea by cultures of marine phytoplankton.

Utilization of xanthine, guanine, and uric acid (6,77) and non-utilization of allantoin and creatinine (6) as sole N-sources are reported for algal cultures: the significance of these compounds as N-sources in natural systems is questionable.

The most important forms of combined nitrogen utilized by phytoplankton are inorganic, i.e., NH_4^+, NH_2OH, NO_2^-, and NO_3^-.

Mineralization (ammonification) of proteinaceous material by heterotrophic bacteria in sediments is responsible for the large quantities of ammonium present in sediments (NH_4^+ being the most abundant N-form in both marine and freshwater sediments (34)). Leaching of ammonium from sediment interstitial water into the water column of stratified systems results in the high concentrations of NH_4^+ (several $mg.L.^{-1}$) commonly found in hypolimnetic waters (49). During periods of overturn the formation of an oxidized microzone at the sediment surface may adsorb substantial amounts of NH_4^+ released from the interstitial water (36). Ammonium levels in epilimnetic waters are generally low and originate from decomposition of epilimnetic plankton, zooplankton and fish excretion, rainfall, run-off, efflux from littoral sediments, and the largest amounts originating from the hypolimnion during overturn (46, 76).

Factors leading to the depletion of NH_4^+ from water include oxidation to NO_3^- under aerobic conditions by nitrifying bacteria (13, 49), uptake (immobilization) by phytoplankton, and volatilization to the atmosphere. This last point is of limited significance in that ammonia in aqueous solution is present mainly as NH_4^+ and NH_4OH, the relative proportions depending on the pH. At neutrality the NH_4^+: NH_4OH ratio is approximately 300 and only under extremely alkaline conditions (such as in some eutrophic lakes where pH values may reach as high as 10 due to high photosynthetic activity) would there be significant loss

of NH_4^+ due to volatilization (34, 46). Ammonia can also be bound to colloidal particles, particularly in neutral or alkaline brown humic water.

Nitrate (NO_3^-) concentrations in stratified systems are often low in both the epilimnion and hypolimnion due to uptake by phytoplankton and denitrification to molecular N_2 (12) respectively. Metalimnetic concentration maxima for NO_3^- are often observed and are possibly the result of nitrifying bacteria. During periods of overturn this nitrifying activity may be extended to oxygenated sediments resulting in NO_3^- efflux into the water column. As with NH_4^+, substantial amounts of NO_3^- are introduced into aquatic systems via rainfall and agricultural run-off.

Direct photochemical oxidation or reduction of NH_4^+ or NO_3^- has been shown to be of little significance (37).

Although being a major intermediate in both the nitrification and denitrification processes, nitrite (NO_2^-) is generally found at very low levels in both freshwater and seawater. Keeney (1972) reports concentrations of 0-10 µg. $NL.^{-1}$ for Wisconsin lakes. Levels found in the Experimental Lakes Area are usually undetectable occasionally reaching metalimnetic concentrations of 10 µg.$NL.^{-1}$ in early spring. Phytoplankton utilization of NO_2^- as a sole N-source is documented and Eppley et al. report half-saturation values of 1-4 µM for NO_2^- uptake (21, 22).

Hydroxylamine (NH_2OH) is seldom found in lakes and then only in extremely low concentrations (2).

Classical considerations on the productivity of aquatic systems were based mainly on the measurement of absolutes such as changes in biomass, chlorophyll, species composition, etc. Recent work however has assumed a much more mechanistic approach concerning actual rates of production, transformation, and utilization of essential nutrients. This latter type of approach has resulted in the adaptation of one of biochemistry's fundamental concepts, Michaelis-Menten saturation kinetics, for the purpose of determining actual rates of nturient flux. The rationale is outlined by Wright and Hobbie (1966); "when uptake of a solute is mediated by a transport system on or in the cell membrane, the rate of uptake will be described in terms of the Michaelis-Menten equation:"

$$v = \frac{V(S)}{Km + S}$$

where v is the uptake velocity at a given substrate concentration S, V is the maximum uptake velocity attainable under the given conditions, and Km being the Michaelis constant and represents the substrate concentration when $v = \frac{V}{2}$. For the remainder of the present manuscript the term Kt, not Km, will be used to represent the Michaelis constant for nutrient uptake by transport.

Determination of the values Kt and V is achieved by linearization of the hyperbola described by the Michaelis-Menten equation. This is achieved by one of several linear transformations (15, 63) perhaps of which the most widely used is the Lineweaver-Burk equation:

$$\frac{S}{v} = \frac{S}{V} + \frac{Kt}{V}$$

The best estimates of V values for phytoplankton nutrient uptake are likely those obtained from in situ experiments (78) since V is variable with irradiance, day length, temperature, and population density (24, 59). However, for these same reasons, values of V are generally given little credence.

A much more useful parameter for construction of nutrient cycling models and establishment of management practices for nutrient loading capabilities of lakes is the Kt value since it is dependent only on the physiological state of the population rather than the variables outlined above (21, 24, 34, 59, 80).

A simple calculation allows determination of the turnover (residence) time of the nutrient by the system:

$$Tt = \frac{Kt + Sn}{V}$$

where Tt is the turnover time and Sn is the naturally occurring concentration of substrate.

An additional calculation:

$$G = \frac{n}{N\ (t)}$$

has been used to determine a specific growth rate G in terms of a specific nutrient where n is the mass of nutrient taken up, N is the mass of nutrient in the particulate form, and t is the incubation time (11, 55).

The validity and application of Michaelis-Menten kinetics to uptake of C, N, and P by aquatic microbial populations are covered extensively in the literature (9, 27, 34, 38, 39, 45, 60, 78, 79, 80, 91).

Numerous estimations of the kinetics involved in uptake of inorganic nitrogen by marine phytoplankton have been reported.

Dugdale and Goering (1967) working with mixed populations obtained from the North Pacific and North Atlantic concluded that phytoplankton of these regions depended primarily on NH_4^+ as a N-source. Dark uptake of NH_4^+ (as a percentage of light uptake) ranged from 25% for temperate regions to 50% for tropical regions. Dark uptake of NO_3^- ranged from 10 - 30% for the corresponding regions.

Several published values of half-saturation constants for uptake of NO_3^- and NO_2^- by marine phytoplankton are presented by Eppley and Coatsworth (1968): Anabaena cylindrica $Kt_{NO_3^-}$ = 70 µM, $Kt_{NO_2^-}$ = 40 µM; Ditylum brightwellii $Kt_{NO_3^-}$ = 2 µM, $Kt_{NO_2^-}$ = 4 µM; Phaeodactylum trichornutum $Kt_{NO_3^-}$ = 10 µM; Dunaliella tertiolecta $Kt_{NO_3^-}$ = 10 µM; Chaetoceros gracilis $Kt_{NO_3^-}$ = 10 µM; and Chlorella pyrenoidosa $Kt_{NO_2^-}$ = 25 µM.

Eppley et al. (1969) found $Kt_{NO_3^-}$ values of 0.1-10.3 µM and $Kt_{NH_4^+}$ values of 0.1 - 2.4 µM for sixteen species of marine phytoplankton. Ammonium half-saturation constants were generally found to be lower than those for NO_3^- for the corresponding species.

MacIsaac and Dugdale (1969) suggest that marine phytoplankton from oligotrophic regions are better suited to survival at low nutrient

concentrations than those from eutrophic regions based on their estimations of $Kt_{NO_3^-}$ values of <0.2 µg. at.L.$^{-1}$ and >1.0 µg. at.L.$^{-1}$ for the two respective regions. This suggestion is substantiated by the work of Carpenter and Guillard (1971) which estimated $Kt_{NO_3^-}$ values of 0.7 µM for species from nutrient depleted regions, 0.1 - 1.2 µM for a more productive shelf region, and 1.5 µM for the same species from an estuarine region.

$Kt_{NO_3^-}$ values of 0.7 - 1.3 µM and 0.1 - 0.3 µM for Asterionella japonica and Chaetoceros gracilis respectively have been reported by Eppley and Thomas (1969).

Caperon and Meyers (1972) found NO_3^- uptake by marine phytoplankton to be partially inhibited by simultaneous addition of NH_4^+. Considerable variation was found in Kt values, those for NH_4^+ being significantly lower than those for NO_3^-: Dunaliella $Kt_{NH_4^+}$ = 0.17 µg. at.L.$^{-1}$, $Kt_{NO_3^-}$ = 0.21 µg. at.L.$^{-1}$; Monochrysis $Kt_{NH_4^+}$ = 0.29 µg. at.L.$^{-1}$, $Kt_{NO_3^-}$ = 0.42 µg. at.L.$^{-1}$; Coccochloris $Kt_{NO_3^-}$ = 0.31 µg. at.L.$^{-1}$; and Cyclotella $Kt_{NO_3^-}$ = 0.35 µg. at.L.$^{-1}$.

Lui and Roels (1972) observed preferential use of NH_4^+ by a culture of the marine centric diatom Biddulphia aurita, NH_4^+ being depleted from the medium 2 - 3 times faster than NO_3^- or NO_2^-.

Carpenter and McCarthy (1975), using a ^{15}N enrichment technique,

report a $Kt_{NH_4}^+$ value of 6.7 µg. at.NL.$^{-1}$ for Oscillatoria thiebautii.

Axenic cultures of Skeletonema costatum and an unidentified marine chlorophyte (Bates, 1976) displayed a definite preference for NH_4^+ over NO_3^-. Dark uptake of NH_4^+ was very high (i.e., approximately 90% of light uptake) whereas dark uptake of NO_3^- ranged from 25 - 75% of light values. At all light intensities NH_4^+ depressed NO_3^- uptake.

Bhovichitra and Swift (1977), working with the N-depleted oceanic dinoflagellates Pyrocystis noctiluca, Pyrocystis fusiformis, and Dissodinium lunula found $Kt_{NH_4}^+$ values of 4.4, 1.4, and 3.8 µM and $Kt_{NO_3}^-$ values of 5.0, 3.0, and 1.7 µM respectively. NH_4^+ and NO_3^- uptake were taken up at essentially equal rates both day and night, dark uptake being approximately 80% of light uptake.

Far fewer values of Kt and V values for N uptake by freshwater phytoplankton have been published.

Dugdale and Dugdale (1965) found simultaneous utilization of NO_3^-, NH_4^+, and N_2 in Sanctuary Lake, Penn. However, a definite seasonal variation in N uptake was observed, NH_4^+ being the dominant source in the spring being replaced by N_2 as the major N-source in the fall. Uptake of NH_4^+ in the dark was much less depressed than that of NO_3^- or N_2.

Toetz et al. (1972), using a ^{15}N tracer technique, observed that the phytoplankton population of NO_3^- depleted Lake Carl Blackwell, Okla. possessed lower $Kt_{NO_3}^-$ values and higher $V_{NO_3}^-$ values than phytoplankton of NO_3^- replete Lake Keystone, Okla. Lake Carl Blackwell was dominated

primarily by blue-greens and diatoms and $Kt_{NO_3^-}$ values varied with depth being 18.4, 43.0, and 16.1 µg. $NL.^{-1}$ at depths of 0, 0.5-1.0, and 2.0 m. respectively. The Lake Keystone bloom was comprised mainly of phytoflagellates, diatoms, and small green coccoid forms and showed $Kt_{NO_3^-}$ values of 204.0 and 2434.0 µg. $NL.^{-1}$ for the 0 and 0.5 m. depths respectively.

Vanderhoef et al. (1974) found Microcystis to be the dominant phytoplankter in a nutrient-rich region of Green Bay, Lake Michigan. However, as NH_4^+ and NO_3^- were depleted, both as a function of time and as a function of distance from the nutrient-laden Fox River mouth, N_2 fixing Aphanizomenon became dominant. The overall standing crop of Aphanizomenon decreased as P concentrations decreased as a function of distance from the river mouth.

Wollen and Cartier (1975) have determined NO_3^- half-saturation constants of 14.9 and 148.0 µM for cultures of Navicula pelliculosa and Chlamydomonas reinhardtii respectively.

$Kt_{NH_4^+}$ values of 0.1-0.3 µg. at.$NL.^{-1}$ for cultures of Scenedesmus quadricauda and Pseudoanabaena catenata have been reported (Healey, 1977). An unexplained pH dependence for NH_4^+ uptake was observed, S. quadricauda being favoured at low pH (i.e. 6-7) and P. catenata being favoured at high pH (i.e. >9). Uptake of both NH_4^+ and urea was found to be dependent on the presence of a chelator which was present in eutrophic Lake Winnipeg water but not in oligotrophic water from Lake 239 of the ELA. The nature and function of the natural

chelation are unknown but addition of EDTA to the culture medium facilitated N uptake.

The actual mechanisms of phytoplankton uptake of dissolved inorganic N are poorly understood. The major enzymes responsible for NH_3^+ incorporation into amino acids are glutamate dehydrogenase and alanine dehydrogenase. These enzymes mediate the amination of ∝-ketoglutarate and pyruvate to glutamate and alanine respectively (22, 55, 57, 77).

Incorporation of NO_3^- - N into amino -N is mediated by nitrate reductase. It is suggested that NO_3^- is reduced by nitrate reductase to NO_2^- which is in turn reduced by nitrite reductase to NH_4^+ which is incorporated into amino acids as outlined above (55). Light induction and NH_4^+ repression of the nitrate reductase system is well documented (5, 22, 42, 55, 77). Healey (1973) suggests three possible mechanisms for the influence of light on NO_3^- uptake: i, direct photoreduction of the NO_3^- by a reductant produced during photosynthetic electron transport, ii, a requirement for ATP energy for reduction but not actual uptake of NO_3^-, iii, a need for photosynthetically produced C skeletons which act as receptors for the acquired N. Complete inhibition of NO_3^- uptake by NH_4^+ at levels of 0.5 – 1.0 μg. at.L.$^{-1}$ (5, 22), 0.5 μg. at.L.$^{-1}$ (55), and 160 μg. N.L.$^{-1}$ (61) have been reported. High levels of NADH have been found to inactivate the nitrate reductase system (44) and it is suggested that NH_4^+ may somehow be influencing the oxidation - reduction status of the enzyme itself.

It should be noted that the enzyme systems outlined above are all found intracellularly and are responsible for the ultimate incorporation of the N-source into amino-N. Little is known of the transport systems involved in the actual uptake of the solute from the medium. Falkowski (1975) has reported the presence of a NO_3^--Cl^--ATPase as being responsible for the active transport of NO_3^- across the plasmalemma of several species of marine phytoplankton.

MATERIALS AND METHODS

Basic Experimental Design

All experiments concerned with phytoplankton uptake of inorganic N were performed in situ in Lakes 302 and 226 of the Experimental Lakes Area. A large carboy was filled with lake water sampled from a desired depth by means of a portable peristaltic pump. Once full, aliquots of the lake water were delivered from the carboy to 125 ml. incubation bottles which were then stoppered, enriched with the desired amount of nutrient, and incubated in situ at the corresponding depth. Incubation was terminated by injection of either $HgCl_2$ or Lugol's acetic acid solution into the incubation bottles. The stoppered samples were then transported to the field laboratory where they were prepared for chemical and/or isotopic analysis.

SAMPLING PROCEDURE

All water sampling and sample handling was performed in a small boat secured to an anchored buoy in the center of the lake in question.

Lake water was obtained with an internal battery-operated Masterflex peristaltic pump* via a calibrated 0.5 cm. I.D. tygon tube**. The pump outflow tube was inserted into an acid-washed 9.0 litre nalgene carboy which was rinsed and then filled with lake water from the desired depth.

*Cole Parmer Instrument Co. Chicago, Ill., U.S.A.
**Norton Plastics and Synthetics. Akron, Ohio, U.S.A.

During the entire sampling procedure the carboy was covered with a heavy sheet of dark green plastic in order to minimize exposure of the phytoplankton to high surface light intensities (86). Once full, the carboy was shaken to provide an even distribution of phytoplankton throughout and then used to rinse and fill acid-washed 125 ml. Pyrex reagent bottles* via a spigot and length of tygon tubing at the bottom of the carbouy. Immediately after filling, the bottles were stoppered with a no. 2 rubber stopper whose center had been bored half through in order to facilitate penetration by a hypodermic needle. In order to ensure a tight seal and expulsion of air bubbles from the sample a 1.5 in. 18G. hypodermic needle was pushed through the stopper as it was inserted into the bottle.

NUTRIENT ENRICHMENT

Once filled with lake water, duplicate bottles were injected with desired concentrations of either $^{14}NH_4Cl$, $K^{14}NO_3$ **, $^{15}NH_4Cl$ (95 atom %), or $K^{15}NO_3$ (95 atom %)*** by means of a 1.0 ml. glass syringe fitted with a 1.5 in. 18G. hypodermic needle. In all cases, a series of stock solutions of the nutrient to be used was prepared so that an injection of 1.0 ml. of each stock solution would yield the desired concentration

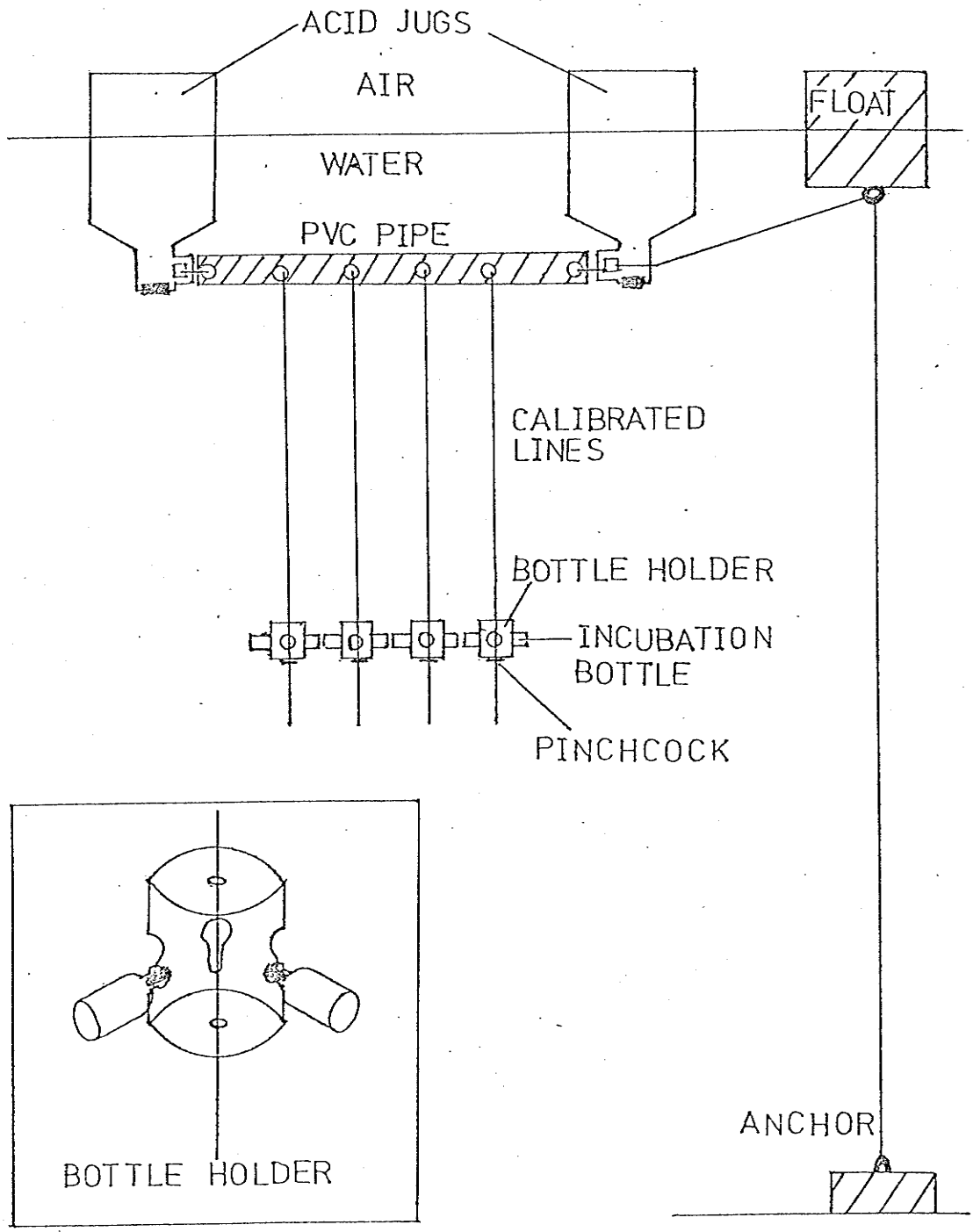
* Corning Glass Works, Medfield, Mass., U.S.A.
** Fisher Scientific Co. Fair Lawn, N.J., U.S.A.
*** ICN Isotope and Nuclear Div. Cleveland, Ohio, U.S.A.

of inorganic nitrogen in the respective 125 ml. incubation bottles. This procedure was adopted so that dilution of the samples upon introducing various concentrations of nutrient would be consistent for all concentrations (i.e. 0.008). Nutrient supplementation with NH_4^+ or NO_3^- was in the range of 1-200 µg. $N.L.^{-1}$, occasionally being as high as 1.0 mg. $N.L.^{-1}$. Concentrations used are listed for each experiment in the Results and Discussion section. Filling, stoppering, and nutrient injection of the incubation bottles was performed in a closed heavy canvas packsack in order to minimize surface light exposure, however, sample manipulation necessitated periodic opening of the pack for brief periods of time.

INCUBATION

Following nutrient injection the incubation bottles were removed from the pack, secured to plexiglass bottle holders (70), and incubated in situ at the appropriate sample depths. Two stoppered commercial acid jugs connected by a 1.0 m. length of pvc plumbing pipe were used to suspend four bottle holders, each on its own calibrated polypropylene line, in the water column (Fig. 3). Incubation depth was determined by positioning of a screw-type pinchcock on each calibrated line. The entire procedure outlined above, from filling of the carbouy to the placing of the twelve nitrogen-supplemented bottles at the desired depth in the water column required approximately 30 minutes.

Fig. 3. In Situ Incubation Apparatus


ACID JUGS
AIR
WATER
FLOAT
PVC PIPE
CALIBRATED LINES
BOTTLE HOLDER
INCUBATION BOTTLE
PINCHCOCK
ANCHOR
BOTTLE HOLDER

INCUBATION TIME

In order to determine an appropriate incubation time for epilimnetic samples a time-course study utilizing two concentrations of $^{14}NH_4Cl$ as nitrogenous nutrient was performed on Lake 302N epilimnetic water (i.e. 2.5m.).

One set of samples was supplemented with 40 µg.NH_4^+-NL.$^{-1}$ and the second set with 160 µg. NH_4^+-NL.$^{-1}$ followed by in situ incubation for periods of time ranging from 0-4.0 hours. Incubation was terminated by injection of $HgCl_2$ followed by colourimetric determination of NH_4^+. From the results illustrated in Fig. 4 it was decided that an incubation time of 1.5 hr. would be used in order to avoid nutrient depletion or any lag phase associated with uptake of nutrients at low concentrations.

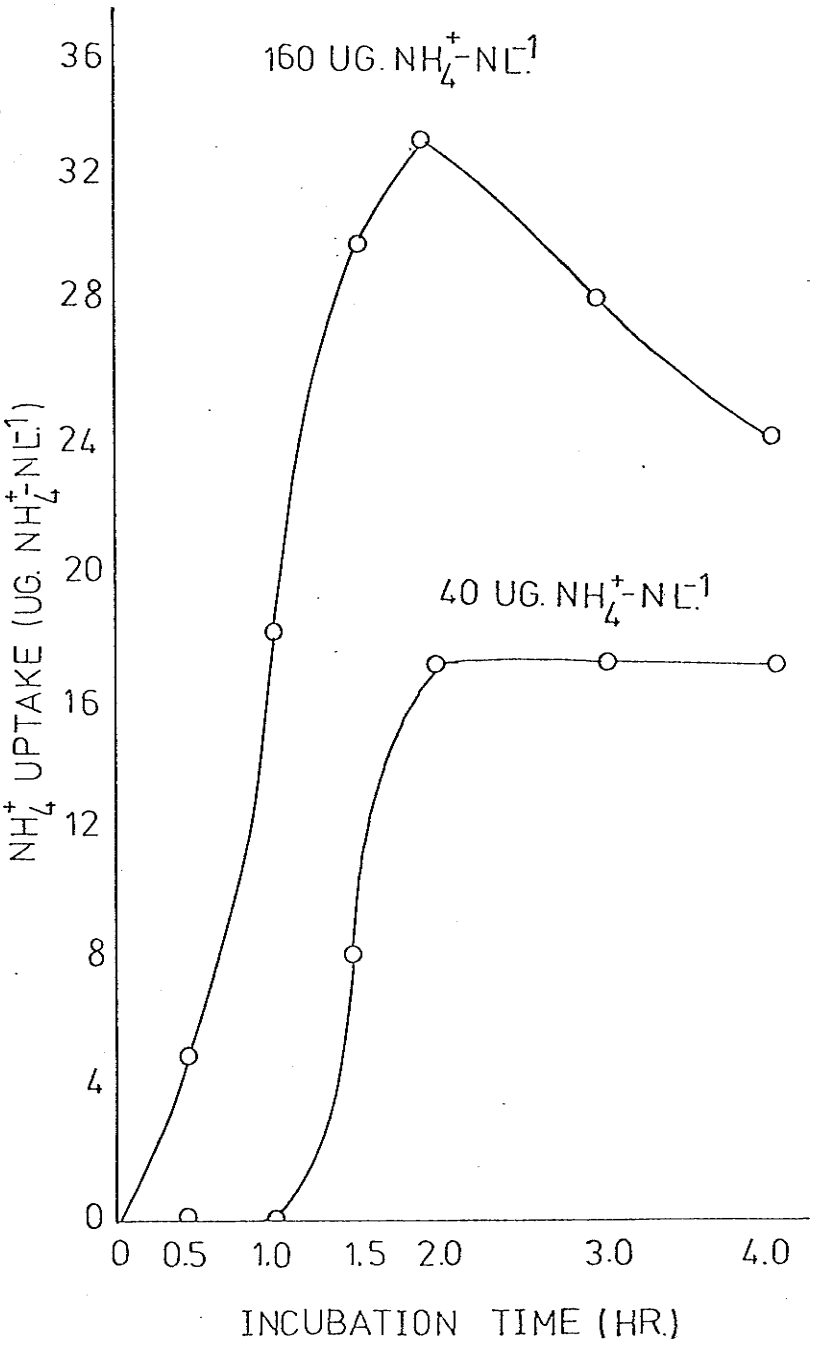
For hypolimnetic samples incubation time ranged from 2.5-4.5 hr., depending on the algal cell density and also on the degree of cloud cover.

The relatively short inclubation times used minimized deleterious bottle effects and also avoided problems associated with substrate depletion.

POST-INCUBATION TREATMENT

At the end of the desired incubation time the bottled samples were brought to the surface and injected with $HgCl_2$ to a final concentration of 20 ppm Hg. This practice was abandoned in favour of injection of

Fig. 4. NH_4^+ Uptake versus Incubation Time


160 UG. NH_4^+-N L^{-1}
40 UG. NH_4^+-N L^{-1}
NH_4^+ UPTAKE (UG. NH_4^+-N L^{-1})
0
4
8
12
16
20
24
28
32
36
0
0.5
1.0
1.5
2.0
3.0
4.0
INCUBATION TIME (HR.)

Lugol's acetic acid solution (80) when it was found (51) that physiological processes such as motility and CO_2 fixation ceased immediately upon exposure to Lugol's solution. A volume of 0.2 ml. of Lugol's was injected into each incubation bottle resulting in the desired "weak tea" final colour.

COLOURIMETRY

The killed samples were transported to the field laboratory, requiring a time of 15 min. for Lake 302 samples and 25 min. for Lake 226 samples. Samples supplemented with $^{14}NO_3^-$ were immediately filtered at 127 mm. Hg pressure through Whatman GFC* glass fibre filters (mean pore size, 0.8 µm.). The filtrate was then assayed colourimetrically for NO_3^- after hydrazine reduction to NO_2^- utilizing the diazo coupling method of Sawicki and Scaringelli (64). The absorbance of the red diazo complex formed was directly proportional to the NO_3^- - N concentration in the range of 0-600 µg.NO_3^--NL.$^{-1}$ (Fig. 5). Each experimental sample was measured against a glass distilled water blank and a 400 µg. NO_3^--NL.$^{-1}$ standard using a Klett-Summerson photoelectric colourimeter** equipped with a green filter

* Whatman Inc. Clifton, N.J., U.S.A.

** Klett Manufacturing Co. Ltd. New York, N.Y., U.S.A.

(500-570 µm.) and a 1.0 cm. path length glass cuvette.

Samples supplemented with $^{14}NH_4^+$ were not filtered but were immediately assayed colourimetrically for residual NH_4^+ using the phenol-hypochlorite method of Stainton et al. (74). The relationship between the absorbance of the green colour complex formed and the concentration of NH_4^+ was found to be linear between 0-1000 µg. NH_4^+-NL.$^{-1}$ (Fig.6). Each sample was measured against a glass distilled water blank and a 200 µg.NH_4^+-NL.$^{-1}$ standard with the Klett colourimeter equipped with ared filter (640-700µm.) and a 1.0 cm. path length glass cuvette.

Occasionally samples were analyzed for the presence of NO_2^- (64) (Fig. 7) but concentrations of this nitrogen species were always undetectable.

Fig. 5. Nitrate Standard Curve

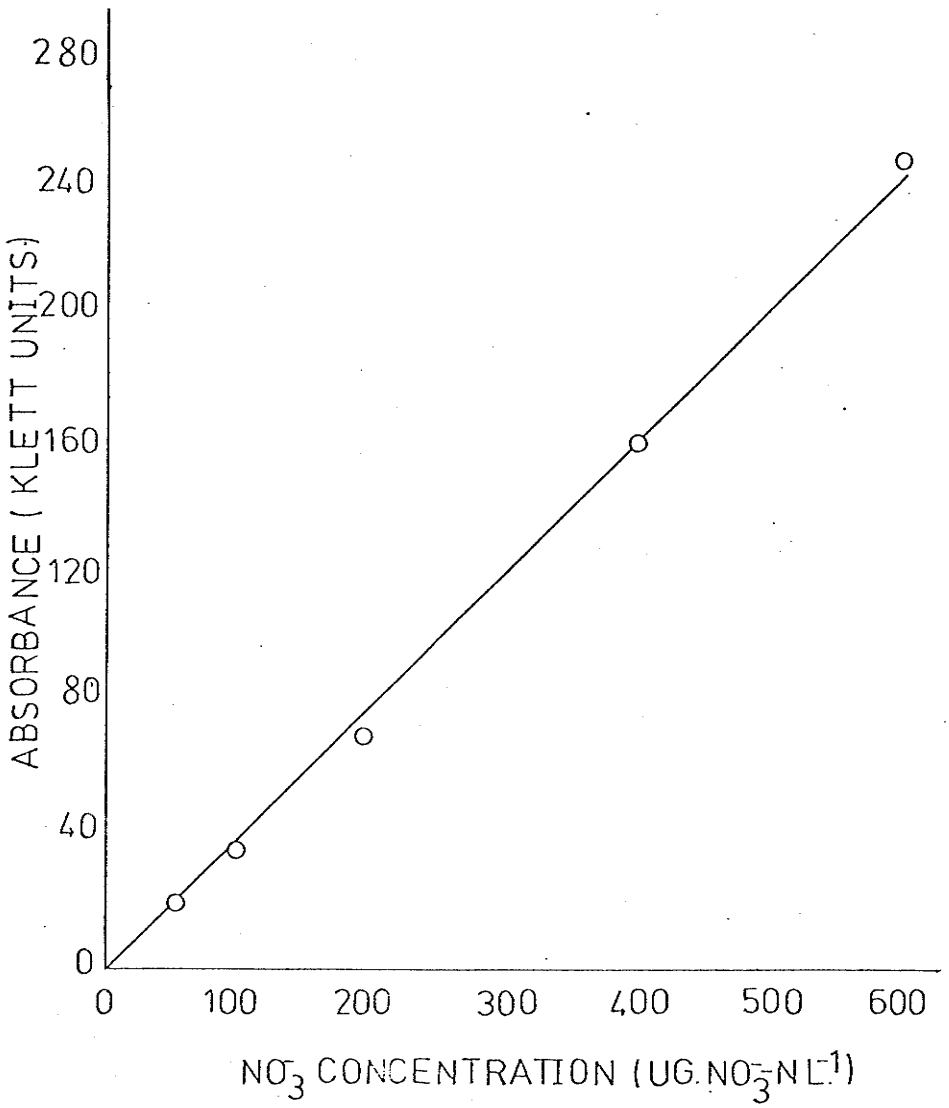

280
240
200
160
120
80
40
0
ABSORBANCE (KLETT UNITS)
0
100
200
300
400
500
600
NO3 CONCENTRATION (UG.NO3-N L-1)

Fig. 6. Ammonium Standard Curve

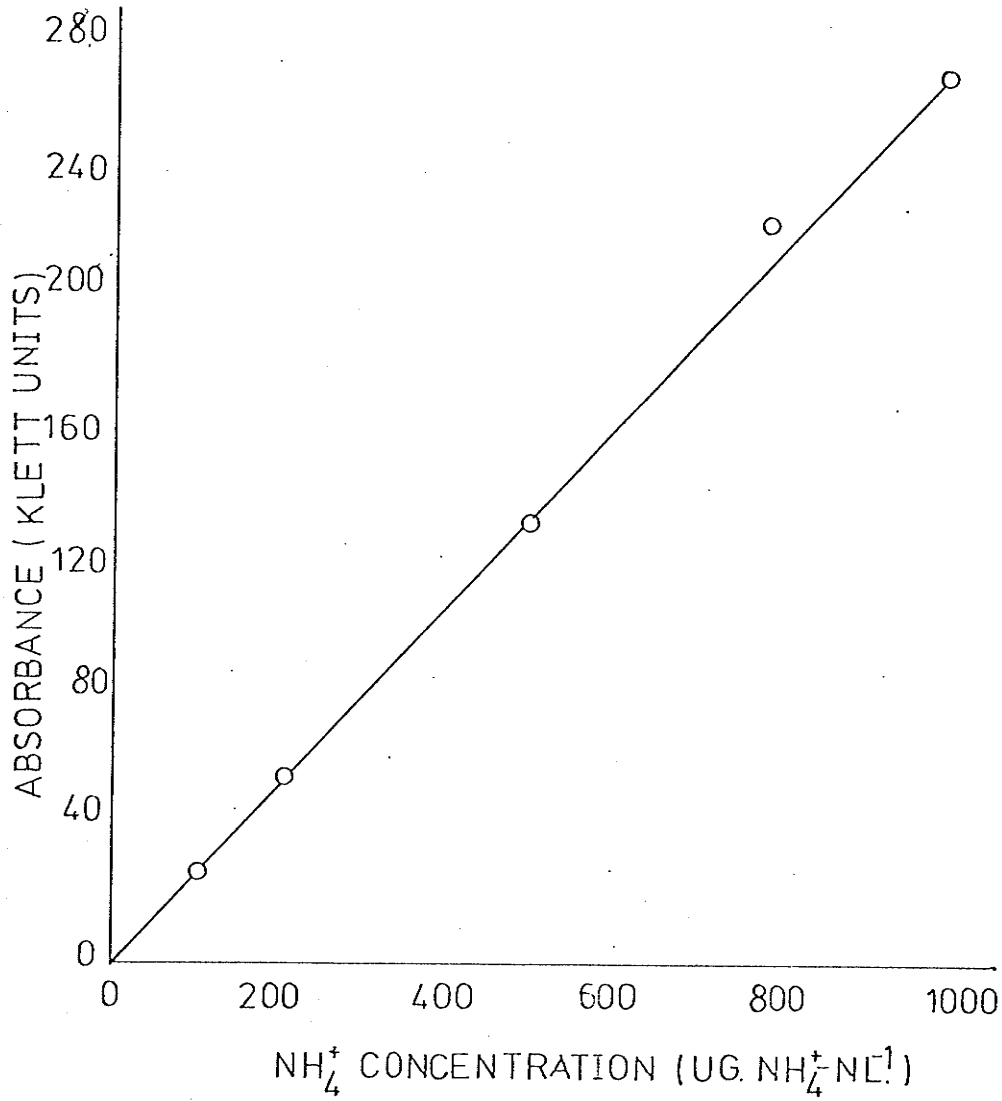
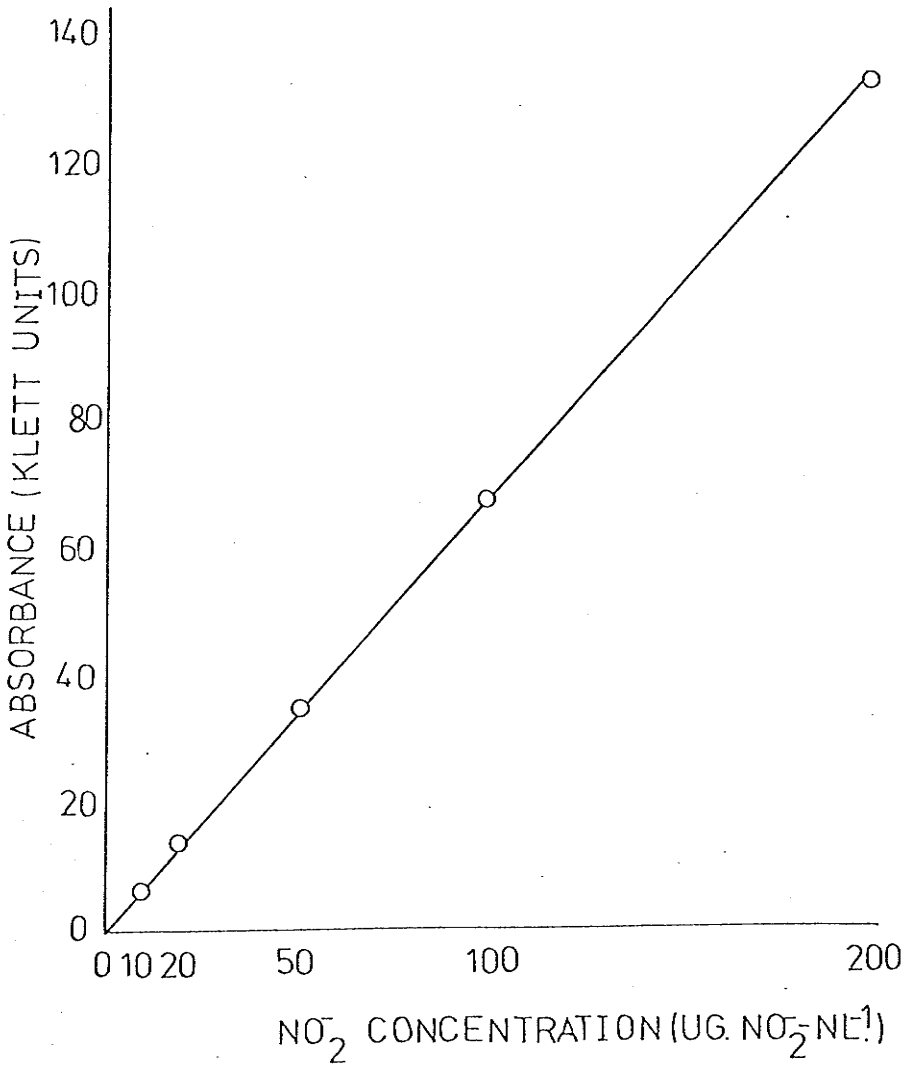

280
240
200
160
120
80
40
0
ABSORBANCE (KLETT UNITS)
0
200
400
600
800
1000
NH4+ CONCENTRATION (UG. NH4+-NL-1)

Fig. 7. Nitrite Standard Curve


140
120
100
80
60
40
20
0
ABSORBANCE (KLETT UNITS)
0 10 20
50
100
200
NO2 CONCENTRATION (UG. NO2- NL-1)

^{15}N METHODOLOGY

Samples supplemented with either $^{15}NO_3^-$ or $^{15}NH_4^+$ were filtered at 127.00 mm. Hg. pressure through 2.0 cm. diameter Reeve Angel 984 H Ultra* glass fibre filters (mean pore size of 0.5 µm. (73)). These filters have been shown to have a low and consistent nitrogen content (17) and are thus ideally suited to the task. The volume filtered was dependent on the phytoplankton density of the sample since a minimum of 5.0 µg. particulate N was required for ^{15}N enrichment analysis with the method used (32). Generally filtration of 30-50 ml. of sample (requiring a filtration time of approximately 30 min.) provided sufficient nitrogen for analysis.

The filter was then gently rinsed with 5.0 ml. of glass distilled water, removed from the filtration candle with clean forceps, quickly dried over a heat gun**, and stored individually in plastic Petri dishes which were kept frozen (72,80) pending analysis for ^{15}N enrichment at the Freshwater Institute, Winnipeg. The filtrates were immediately analyzed colourimetrically for NO_3^- and/or NH_4^+ by the methods outlined above in order to obtain a direct comparison of the colourimetric and isotope enrichment methods for determining nitrogen uptake.

* Whatman Inc. Clifton, N.J., U.S.A.

**Master Appliance Corp. Racine, Wisc., U.S.A.

The frozen filters were removed from the freezer 2 days prior to analysis and placed in a dessicator jar containing anhydrous silica gel in order to ensure complete drying. Samples were then prepared according to the method of Flett (32) and analyzed for ^{15}N enrichment with a Statron NOI-5 emission spectrometer*.

The basic principles in preparation and analysis of the samples are as follows. The dried filter-phytoplankton sample is placed with clean metal forceps into a metal combustion tube which is then sealed, evacuated, and rinsed and filled with ultra-pure O_2** to a pressure of approximately 2 lb. $in.^{-2}$. The combustion tube containing the sample in a pure O_2 atmosphere is now placed in an $800^{o}C$ furnace for 5 min. in order to combust the particulate material to gas. The now gaseous sample is vented into a pre-evacuated glass manifold where i) CO_2 and H_2O vapour are frozen out by passage through a liquid N_2 trap, ii) nitrogen oxides are reduced to N_2 by passage through a column of heated copper filings, and iii) any residual CO_2 and H_2O vapour is removed by passage through a second liquid N_2 trap. The pressure of N_2 gas now in the manifold is measured by a two station Pyrani guage*** and the amount of particulate nitrogen originally present in the sample calculated by interpolation of a pressure versus particulate N standard curve (Fig.8). This standard curve was prepared using disodium EDTA

* P.C.H. Statron, Ehrenfried-Jopp-Str. 59, G.D.R.

** Welders' Supply Ltd. Winnipeg, Man., Canada.

*** Kinney Vacum Co. Boston, Mass., U.S.A.

as the N-source in order to provide a situation analogous to the actual composition of a phytoplankton sample (i.e. carbon, hydrogen, and oxygen, as well as nitrogen). The N_2 gas sample is now vented from the glass manifold to a Uviol glass discharge tube positioned in the ^{15}N analyzer.

The three isotopic species of N_2 gas, ^{14}N-^{14}N, ^{14}N-^{15}N, and ^{15}N-^{15}N, present in the discharge tube display characteristic electron transition vibration band heads which when stimulated by the high frequency generator (i.e. 27,12 MHz. pulse modulated at 180 Hz.) display characteristic light emissions in the UV region; ^{14}N-^{14}N at 297.68 nm., ^{14}N-^{15}N at 298.89 nm., and ^{15}N-^{15}N at 298.86 nm. These UV emissions are focussed by a quartz condenser lens onto a 56^{o} NaCl prism which separates the incoming spectrum. The separated spectrum falls upon the photomultiplier tube (gain setting of 1000-1400 volts, depending on the amount of nitrogen in the sample) producing proportional electric signals which are recorded with a Hewlett Packard model 7127A chart recorder. A standard curve of the stated ^{15}N atom % of commercial $^{15}NH_4Cl$ standards* versus the ^{15}N atom % as determined by the ^{15}N analyzer showed the calculated values to be from 82-85% of the ^{15}N atom % values stated on the standards (Fig.9). For this reason all ^{15}N atom % determinations were corrected by a factor of 1.19 in order to obtain the actual values.

A more rigorous discussion of the sample preparation unit and the Statron NOI-5 analyzer is presented by Flett (1976).

* Veb Berlin-Chemie

Fig. 8. N_2 Gas Pressure versus Particulate Nitrogen Standard Curve

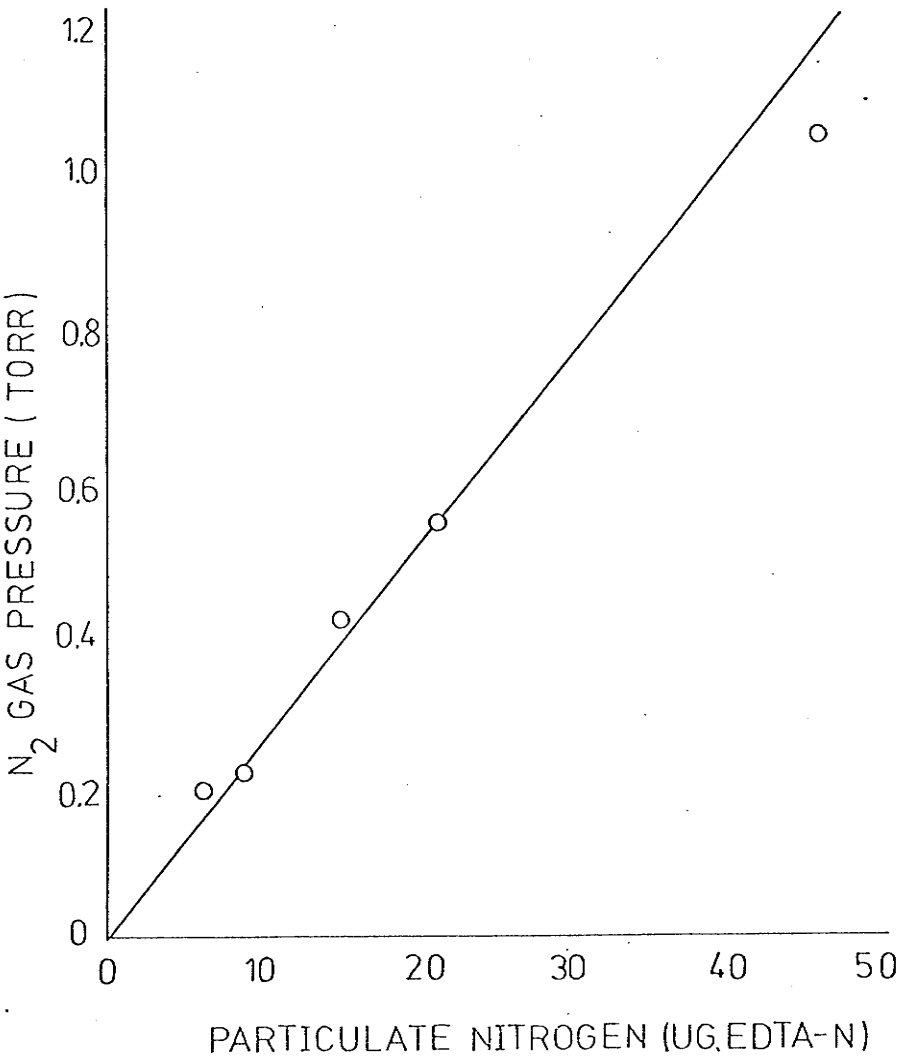
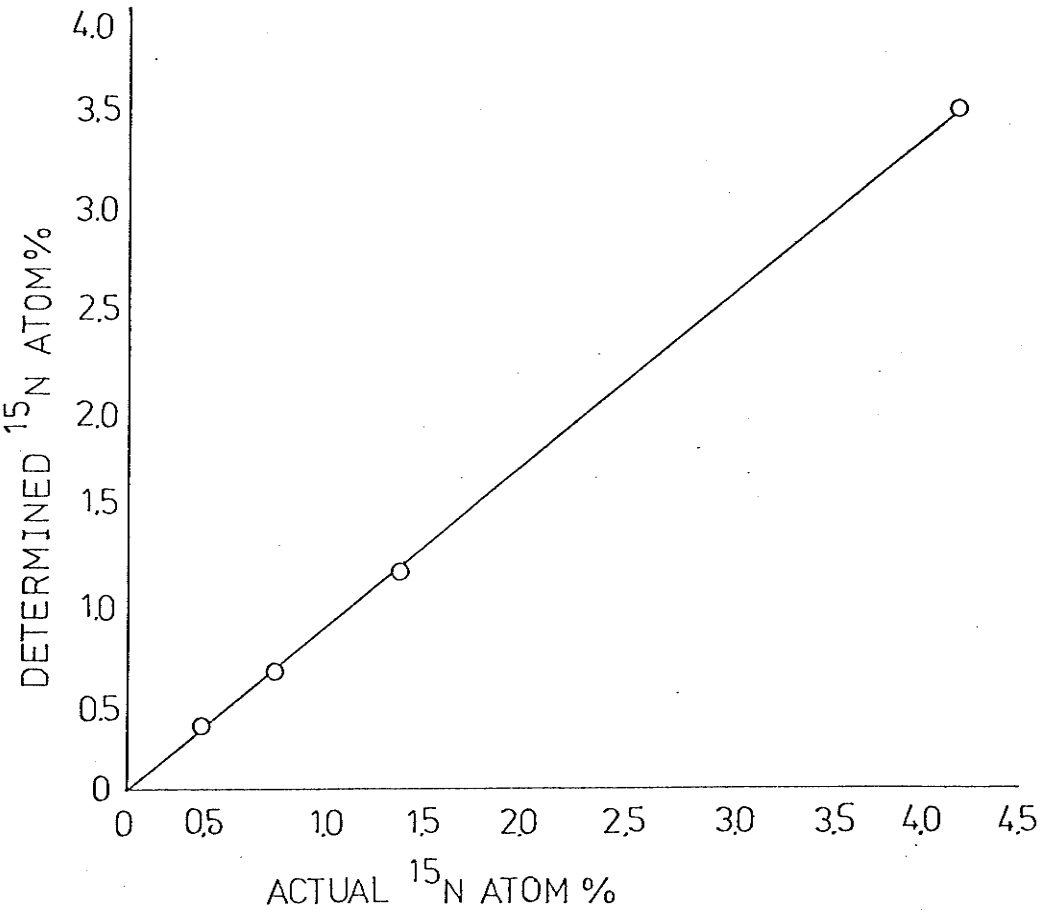

1.2
1.0
0.8
0.6
0.4
0.2
0
N_2 GAS PRESSURE (TORR)
0
10
20
30
40
50
PARTICULATE NITROGEN (UG.EDTA-N)

Fig. 9. Actual ^{15}N atom % versus Determined ^{15}N atom % Standard Curve


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
DETERMINED ^{15}N ATOM%
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
ACTUAL ^{15}N ATOM %

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

A chart recording of a ^{15}N natural abundance spectrum is illustrated in Fig.10. ^{14}N-^{14}N and ^{14}N-^{15}N peak heights were determined from visual fitting of the dashed lines shown in the figure and ^{15}N enrichment was calculated according to the methods of Ferraris and Proksch (1972) and Meyer et al. (1974):

$$R = \frac{a \times 10{,}000}{b \times 300}$$

$$^{15}N \text{ atom } \% = \frac{100 \times 1.19}{2R + 1}$$

$$\Delta\ ^{15}N \text{ atom } \% = {}^{15}N \text{ atom } \%_s = {}^{15}N \text{ atom } \%_{nat}$$

$$N \text{ uptake} = \frac{\Delta\ ^{15}N \text{ atom } \% \times N_2 \text{ pressure}}{^{15}N \text{ atom } \%^{*}_{i} \times 0.024}$$

N.B. s= sample, nat= natural abundance, and i= supplemented N.

* Isotope dilution of the supplemented N(95.0 atom % ^{15}N) by naturally occurring nitrogenous nutrient of the same species was corrected for as follows:

$$^{15}N \text{ atom } \%_i = \frac{\text{ambient N } (\mu g.L.^{-1}) \times {}^{15}N \text{ atom } \%_{nat} + \text{supplemented N}(\mu g.L.^{-1}) \times 0.95}{\text{total N } (\mu g.L.^{-1})}$$

Natural abundance values for Lake 302 and Lake 226 phytoplankton samples ranged from 0.353 - 0.401 ^{15}N atom % with an average value of 0.382 ^{15}N atom %. Although variations have been reported (30), this upward deviation from the generally accepted ^{15}N natural abundance value of 0.367 atom % (7, 41) was felt to be significant and was likely due to contamination of the unammended water samples used for ^{15}N natural abundance determinations from the filtration apparatus and/or the combustion tube. Therefore, for each experiment, an unammended water sample was used for determination of a ^{15}N natural abundance value which was then subtracted from the ^{15}N determination for each sample of the corresponding experiment.

The calculated ^{15}N uptake data were plotted graphically by two separate methods using a Hewlett Packard 9830 computer equipped with a Hewlett Packard 9862A calculator plotter. Values of kt and V were estimated by a Michaelis-Menten type program which plotted data as uptake velocity versus N concentration. The basic rationale of the program consisted of the minimization of the deviance (S^2) between an observed uptake velocity (Vo) and an estimated uptake velocity (Ve);

$$S^2 = \Sigma \frac{Ve \times S}{Kt + S} - Vo^2$$

by partial derivative analysis. A complete description of the Michaelis-Menten program is given by Turner (1977).

Data were also plotted as the reciprocal of uptake velocity versus the reciprocal of N concentration (i.e. Lineweaver-Burk transformation). A polynomial regression program which first transformed velocity and concentration values to corresponding reciprocal values

plotted the data as a least squares fit of the first degree polynomial. Values of Kt and V were calculated from the y interept (1/v) and the slope of the plot (Kt/V) respectively. It has been shown (15,63) that a least squares fit should not be used for a Lineweaver-Burk plot if the data are unweighted since the most unrealiable points (i.e. the lowest concentrations and velocities being the points which yield the highest corresponding reciprocal values) exert the most influence on the plotted line. Since the regression program used did not weight the data the data pair representing the lowest N concentration and its corresponding uptake velocity was omitted from the operation.

The Michaelis-Menten and Lineweaver-Burk plots obtained are illustrated in Figs. 11-23. A summary of Kt and V values obtained by the two plotting methods is given in Table I.

Fig. 10. ^{15}N Natural Abundance Emission Spectrum

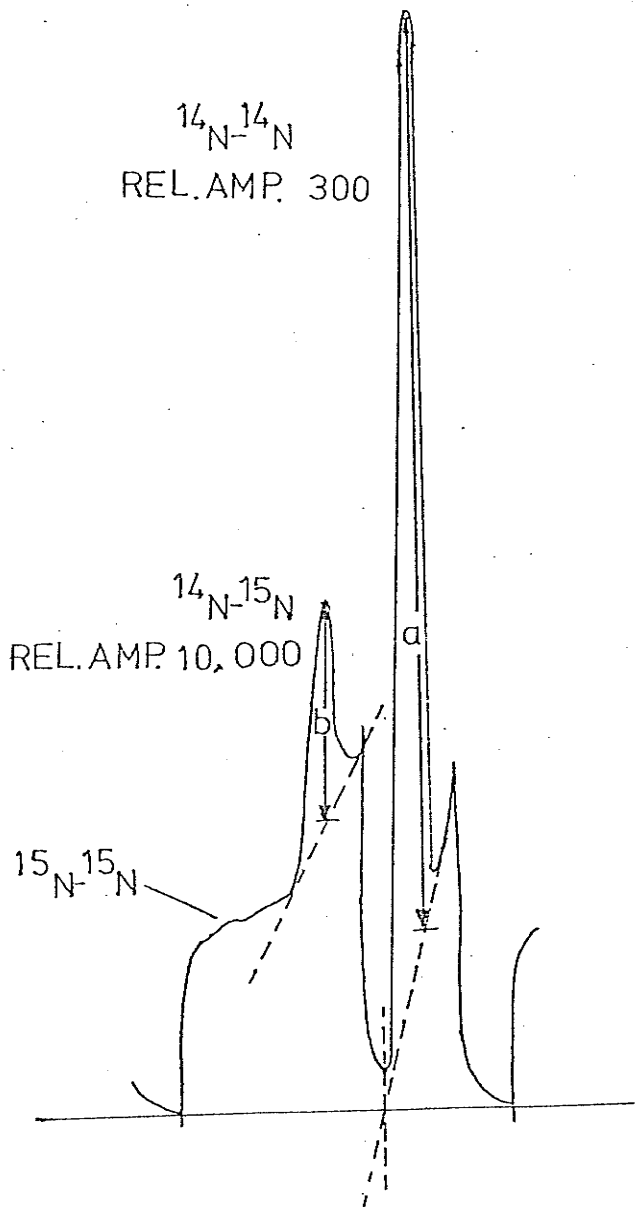

^{14}N-^{14}N
REL. AMP. 300
^{14}N-^{15}N
REL. AMP. 10, 000
^{15}N-^{15}N
a
b

Fig. 11. Lake 226 NE and SW basins, 2.5 m. July 12, 1976.

1-50 µg. $^{15}NH_4^+$ - $NL.^{-1}$

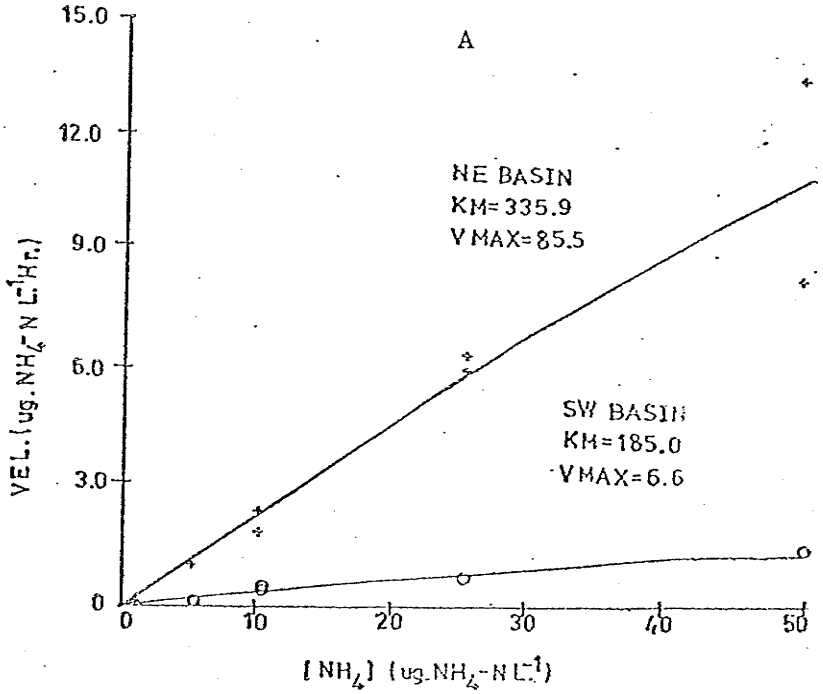
NE: ambient NH_4^+ = 0 $\mu g.NL.^{-1}$, ambient NO_3^- = 115 $\mu g.NL.^{-1}$

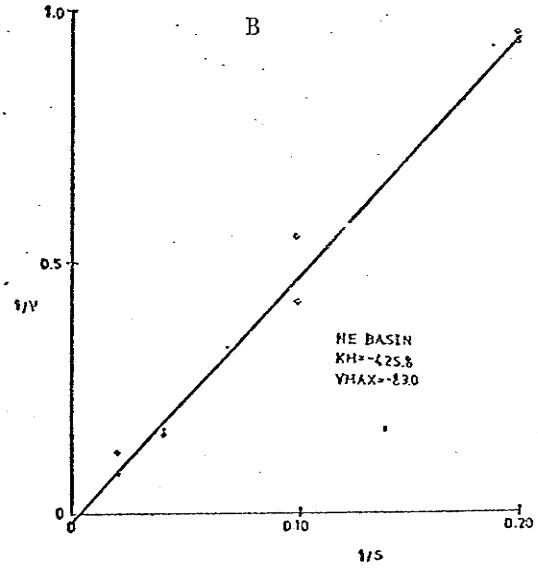
SW: ambient NH_4^+ = 0 $\mu g.NL.^{-1}$, ambient NO_3^- = 110 $\mu g.NL.^{-1}$

A. Michaelis-Menten plot

B. Lineweaver-Burk plot; NE Basin

C. Lineweaver-Burk plot; SW Basin.


A
15.0
12.0
9.0
6.0
3.0
0
VEL. (ug.NH4-N L-1Hr.)
NE BASIN
KM=335.9
VMAX=85.5
SW BASIN
KM=185.0
VMAX=6.6
0
10
20
30
40
50
[NH4] (ug.NH4-N L-1)


B
1.0
0.5
0
1/V
NE BASIN
KM=425.8
VMAX=83.0
0
0.10
0.20
1/S

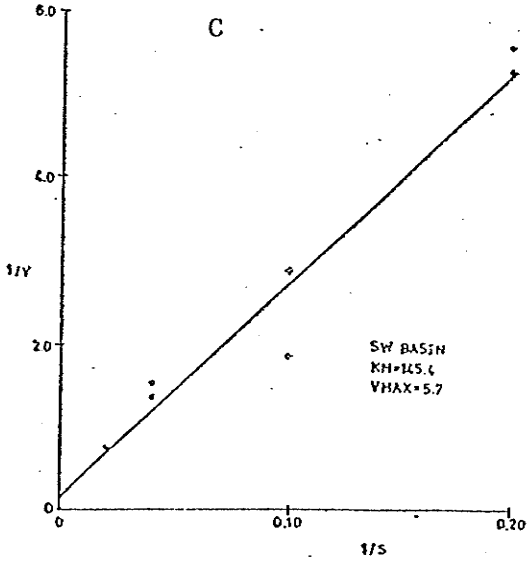

C
6.0
4.0
2.0
0
1/V
SW BASIN
KM=145.4
VMAX=5.7
0
0.10
0.20
1/S

Fig. 12. Lake 302 N and S basins, 2.5 m. July 20, 1976.

1 - 50 µg. $^{15}NH_4^+$ - N.L.$^{-1}$

N: ambient NH_4^+ = 0 µg. NL.$^{-1}$

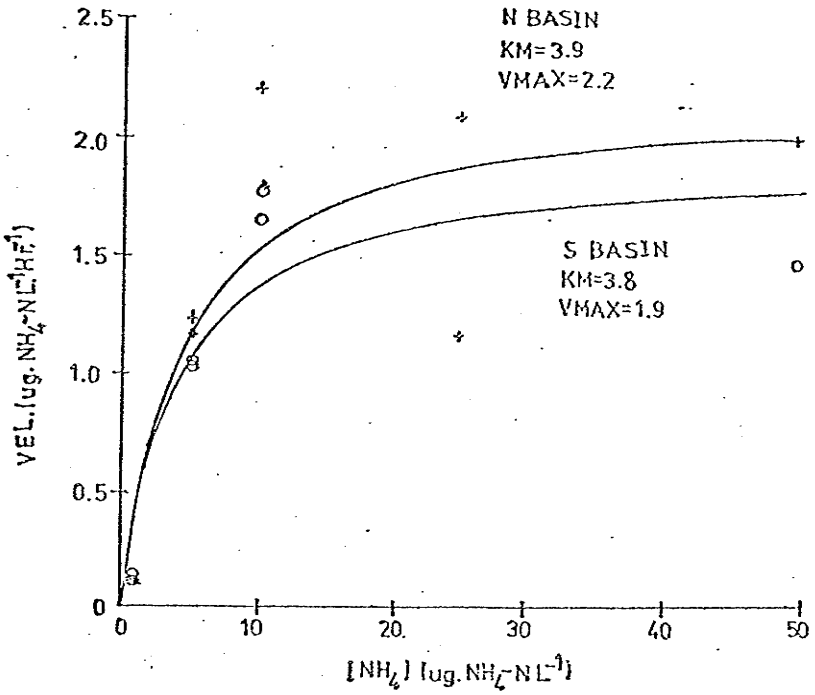
S: ambient NH_4^+ = 0 µg. NL.$^{-1}$

A. Michaelis-Menten plot

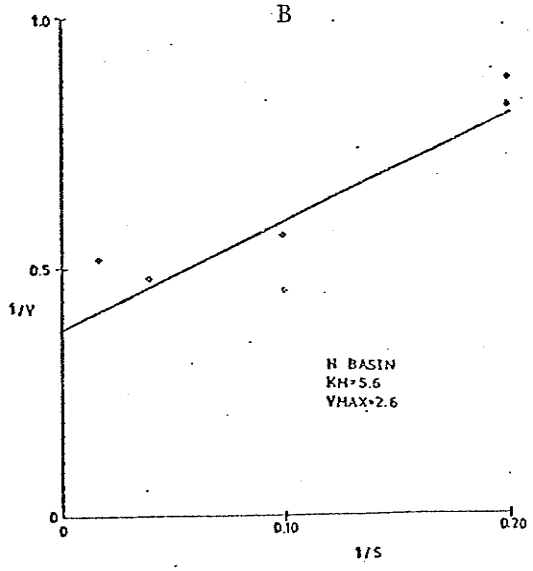
B. Lineweaver-Burk plot; N Basin

C. Lineweaver-Burk plot; S Basin.

A


N BASIN
KM=3.9
VMAX=2.2
S BASIN
KM=3.8
VMAX=1.9
VEL.(ug.NH4-N L-1 Hr-1)
[NH4] (ug.NH4-N L-1)
2.5
2.0
1.5
1.0
0.5
0
0
10
20
30
40
50

B


N BASIN
KM=5.6
VMAX=2.6
1/V
1/S
1.0
0.5
0
0
0.10
0.20

C

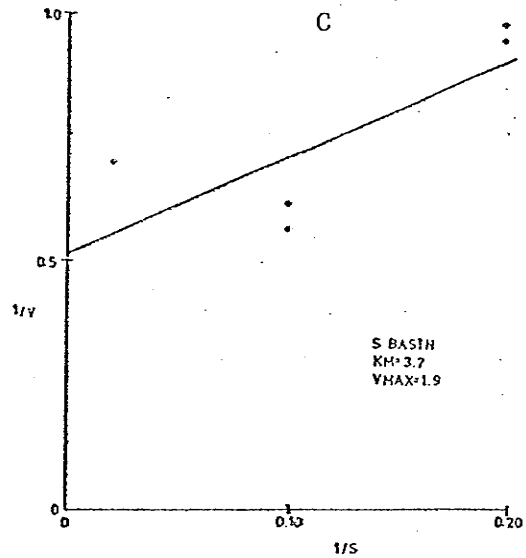

S BASIN
KM=3.7
VMAX=1.9
1/V
1/S
1.0
0.5
0
0
0.10
0.20

Fig. 13. Lake 302 N and S basins, 2.5 m. July 27, 1976.

1-50 µg. $^{15}NH_4^+$-NL.$^{-1}$

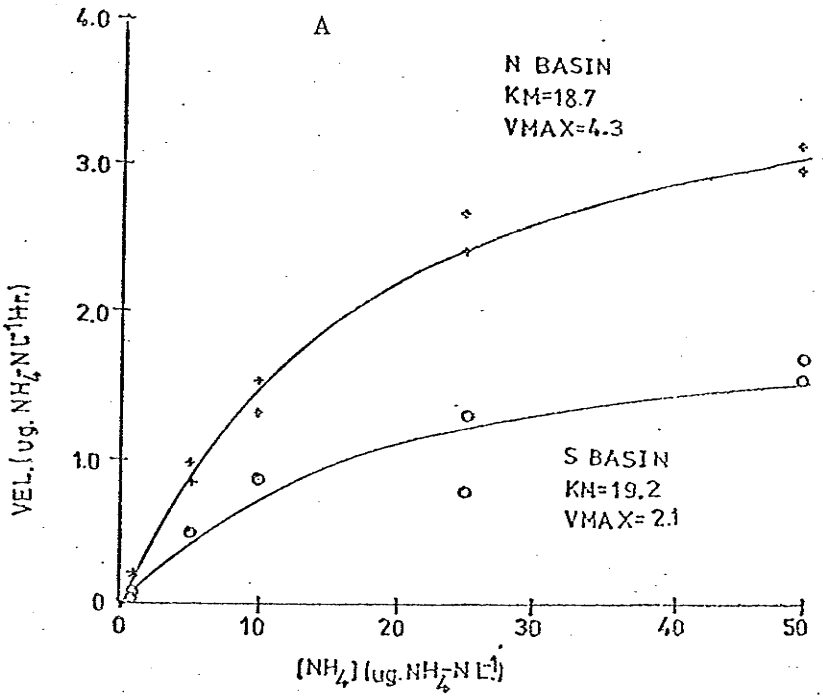
N: ambient NH_4^+ = 0 µg.NL.$^{-1}$

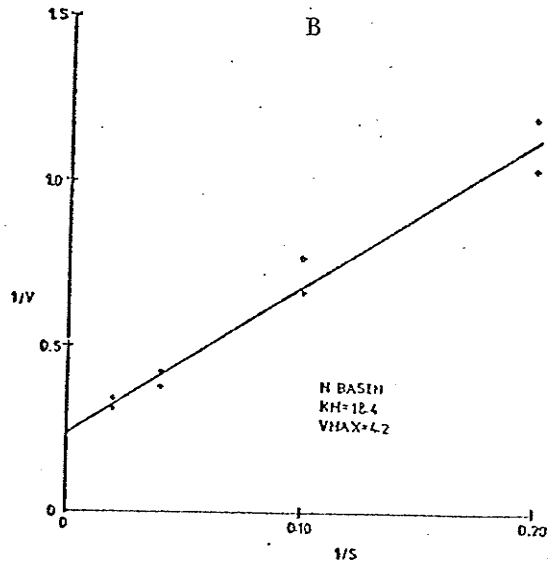
S: ambient NH_4^+ = 0 µg.NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; N Basin.

C. Lineweaver-Burk plot; S Basin.


A
N BASIN
KM=18.7
VMAX=4.3
S BASIN
KM=19.2
VMAX=2.1
VEL. (ug. NH4 N L-1 Hr.)
(NH4) (ug. NH4-N L-1)
4.0
3.0
2.0
1.0
0
0
10
20
30
40
50


B
N BASIN
KM=12.4
VMAX=4.2
1/V
1/S
1.5
1.0
0.5
0
0
0.10
0.20

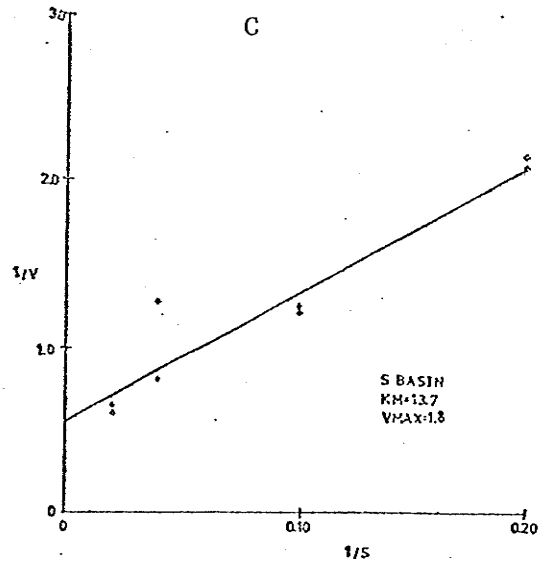
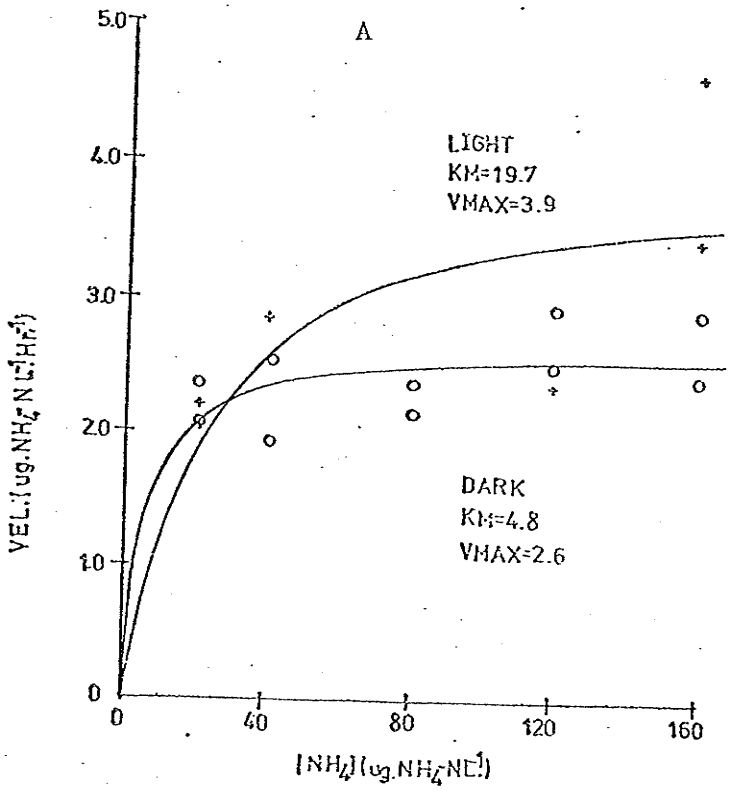

C
S BASIN
KM=13.7
VMAX=1.8
1/V
1/S
3.0
2.0
1.0
0
0
0.10
0.20

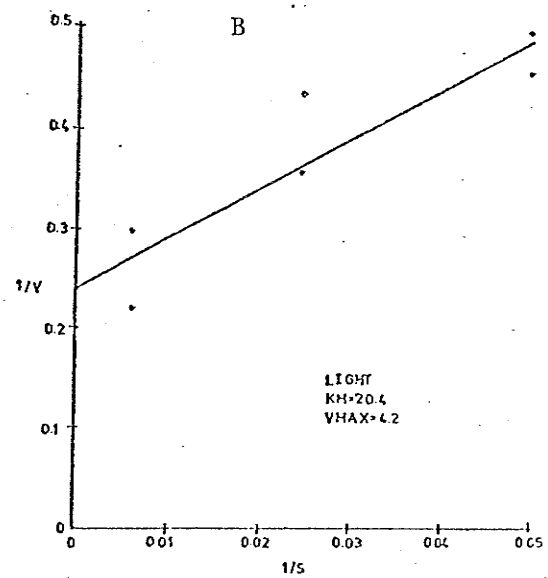
Fig. 14. Lake 302N basin, Light versus Dark, 2.5m. Aug. 11, 1976.
20-160 µg. $^{15}NH_4^+$-NL.$^{-1}$
ambient NH_4^+ = Q µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; Light.

C. Lineweaver-Burk plot; Dark.


A
LIGHT
KM=19.7
VMAX=3.9
DARK
KM=4.8
VMAX=2.6
5.0
4.0
3.0
2.0
1.0
0
0
40
80
120
160
[NH4] (ug.NH4-NL-1)


B
LIGHT
KM=20.4
VMAX=4.2
1/V
1/S
0.5
0.4
0.3
0.2
0.1
0
0.01
0.02
0.03
0.04
0.05

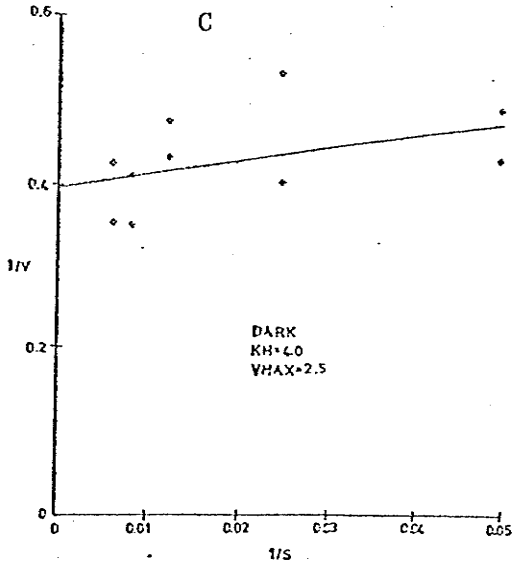

C
DARK
KM=4.0
VMAX=2.5
1/V
1/S
0.6
0.4
0.2
0
0.01
0.02
0.03
0.04
0.05

Fig. 15. Lake 226 NE and SW basins, 2.5 m. Aug. 25, 1976

20-200 µg. $^{15}NH_4^+$ - NL.$^{-1}$

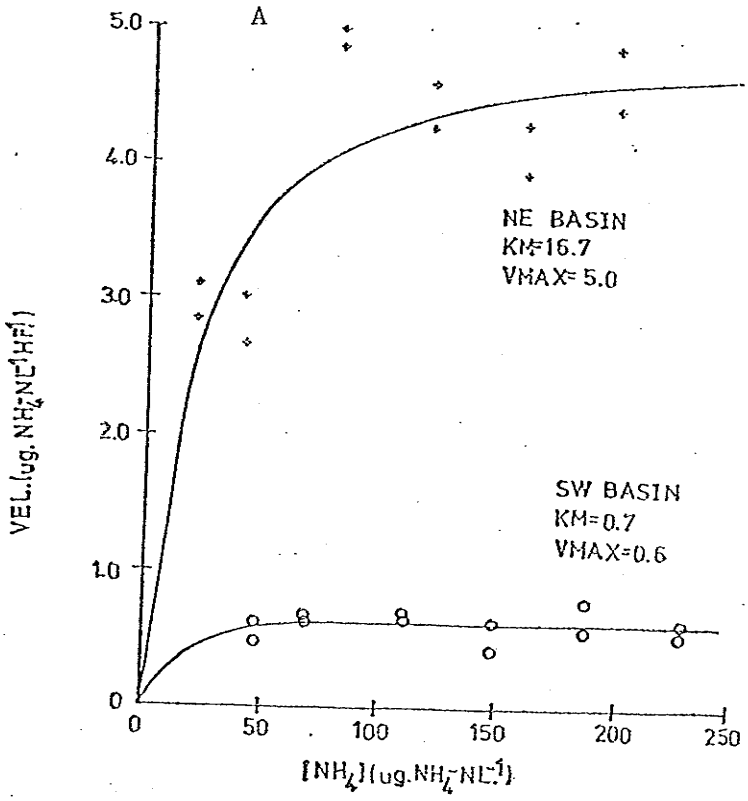
NE: ambient NH_4^+ = 0 µg. NL.$^{-1}$

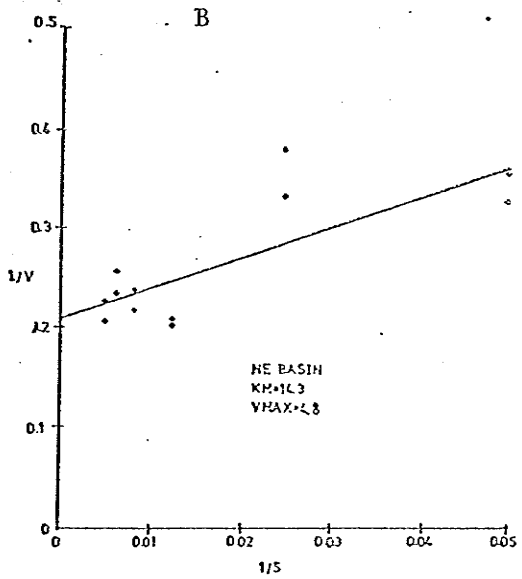
SW: ambient NH_4^+ = 0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; NE Basin.

C. Lineweaver-Burk plot; SW Basin.


A
5.0
4.0
3.0
2.0
1.0
0
VEL.(ug.NH4-NL-1HF-1)
NE BASIN
KM=16.7
VMAX=5.0
SW BASIN
KM=0.7
VMAX=0.6
0
50
100
150
200
250
[NH4](ug.NH4-NL-1)


B
0.5
0.4
0.3
1/V
0.2
0.1
0
NE BASIN
KM=1C.3
VMAX=4.8
0
0.01
0.02
0.03
0.04
0.05
1/S

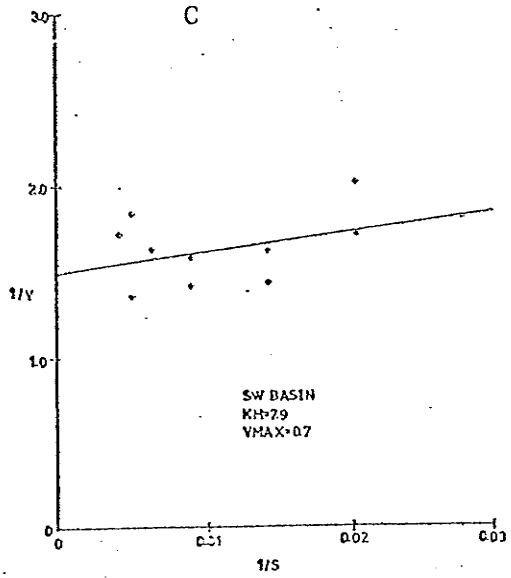

C
3.0
2.0
1/V
1.0
0
SW BASIN
KM=2.9
VMAX=0.7
0
0.01
0.02
0.03
1/S

Fig. 16. Lake 226 NE and SW basins, 2.5 m. Spet. 1, 1976.

10 - 160 µg. $^{15}NO_3^-$ - NL.$^{-1}$

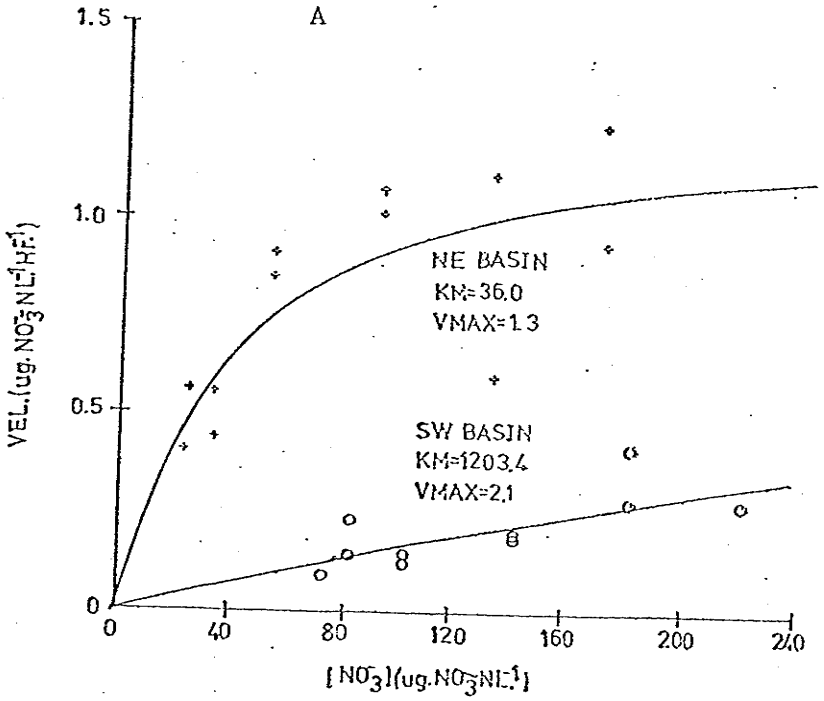
NE: ambient NO_3^- = 12.8 µg. NL.$^{-1}$

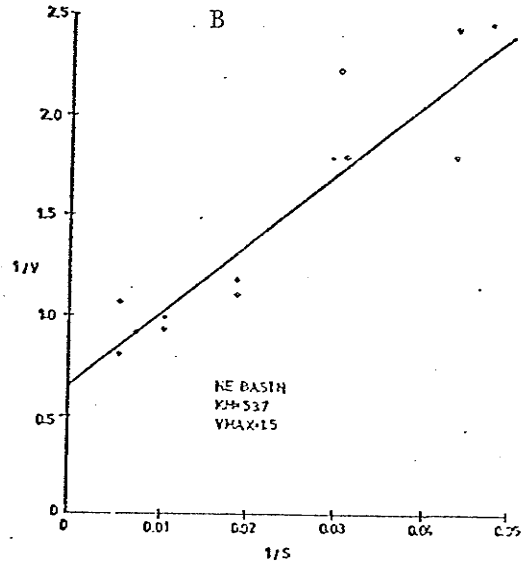
SW: ambient NO_3^- = 61.4 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; NE Basin.

C. Lineweaver-Burk plot; SW Basin.


A
1.5
1.0
0.5
0
VEL.(ug.NO3NL-1HR-1)
NE BASIN
KM=36.0
VMAX=1.3
SW BASIN
KM=1203.4
VMAX=2.1
0
40
80
120
160
200
240
[NO3](ug.NO3NL-1)


B
2.5
2.0
1.5
1.0
0.5
0
1/V
NE BASIN
KM=537
VMAX=1.5
0
0.01
0.02
0.03
0.04
0.05
1/S

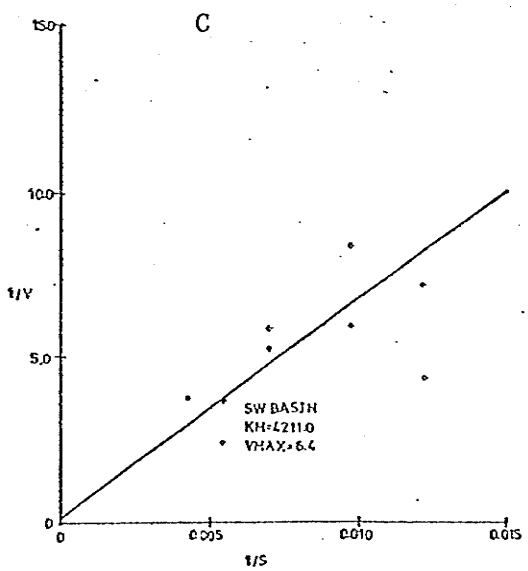

C
15.0
10.0
5.0
0
1/V
SW BASIN
KM=4211.0
VMAX=6.4
0
0.005
0.010
0.015
1/S

Fig. 17. Lake 226 NE and SW basins, 2.5 m. Sept. 8, 1976

10 - 160 µg. $^{15}NO_3^-$ - NL.$^{-1}$

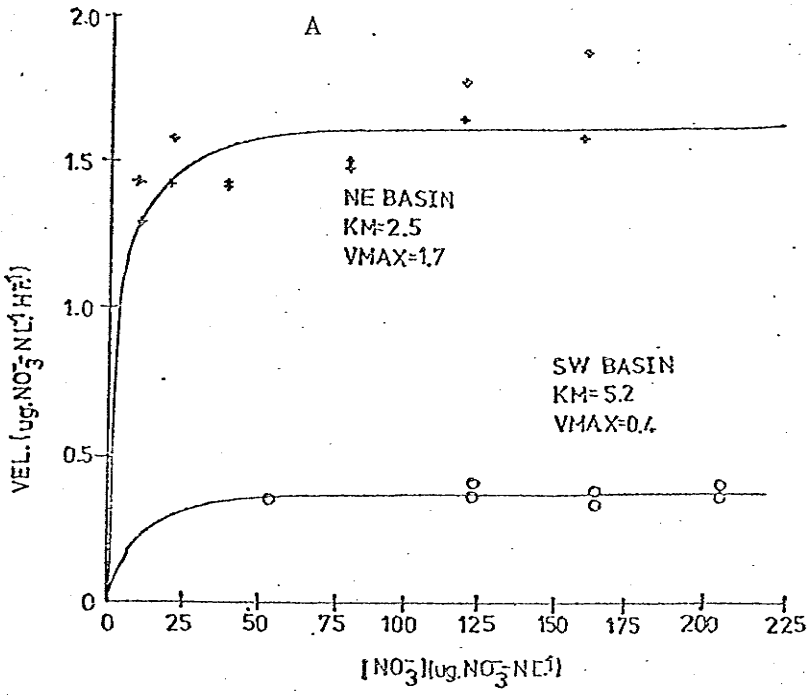
NE: ambient NO_3^- = 0 µg. NL.$^{-1}$

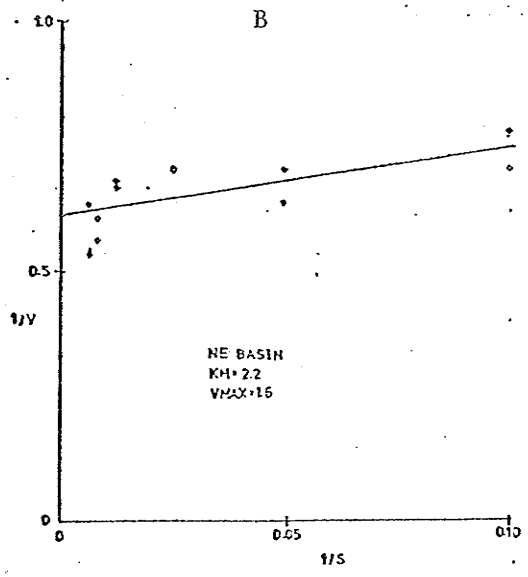
SW: ambient NO_3^- = 44.2 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; NE Basin.

C. Lineweaver-Burk plot; SW Basin.


A
NE BASIN
KM=2.5
VMAX=1.7
SW BASIN
KM=5.2
VMAX=0.4
VEL. (ug.NO3-N L-1 HR-1)
[NO3] (ug.NO3-N L-1)
2.0
1.5
1.0
0.5
0
0
25
50
75
100
125
150
175
200
225


B
NE BASIN
KM= 2.2
VMAX=1.6
1/V
1/S
1.0
0.5
0
0.05
0.10

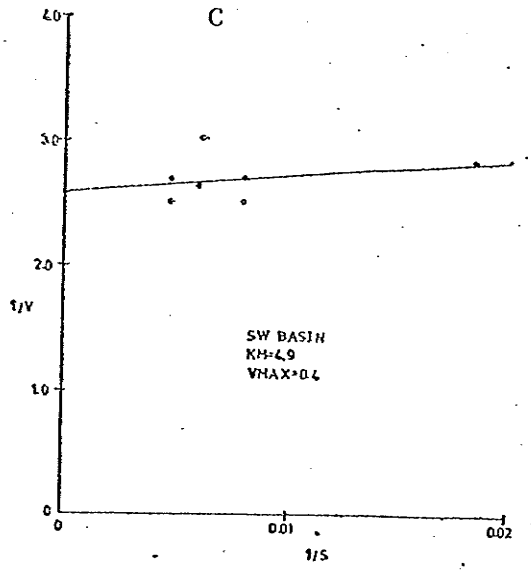

C
SW BASIN
KM=4.9
VMAX=0.4
1/V
1/S
4.0
3.0
2.0
1.0
0
0.01
0.02

Fig. 18. Lake 226 NE basin, $^{15}NO_3^- \pm {}^{14}NH_4^+$, 2.5m. Sept. 9, 1976.

20-200 µg. $^{15}NO_3^-$ - NL.$^{-1}$ ± 80 µg. $^{14}NH_4^+$ - NL.$^{-1}$

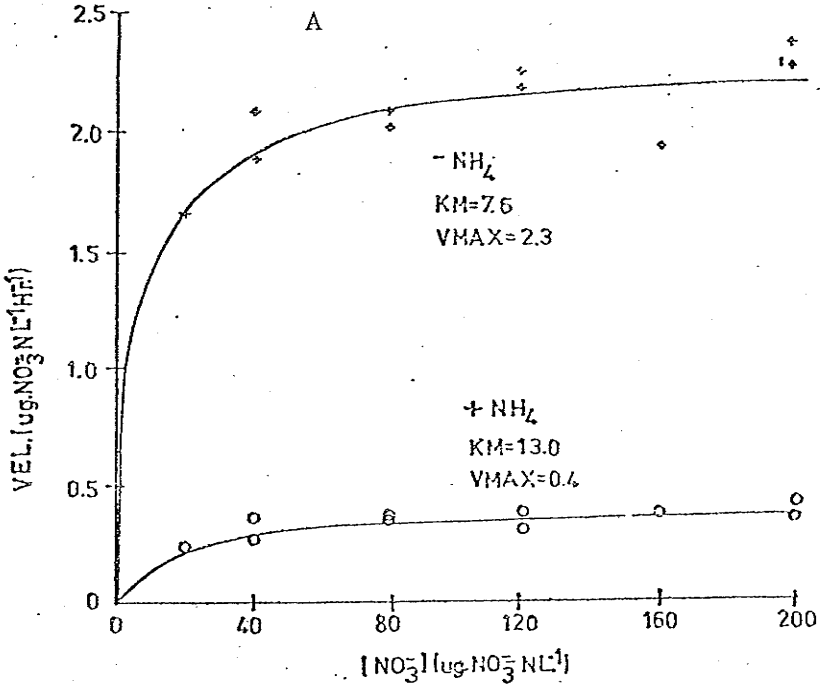
ambient NO_3^- = 0 µg. NL.$^{-1}$

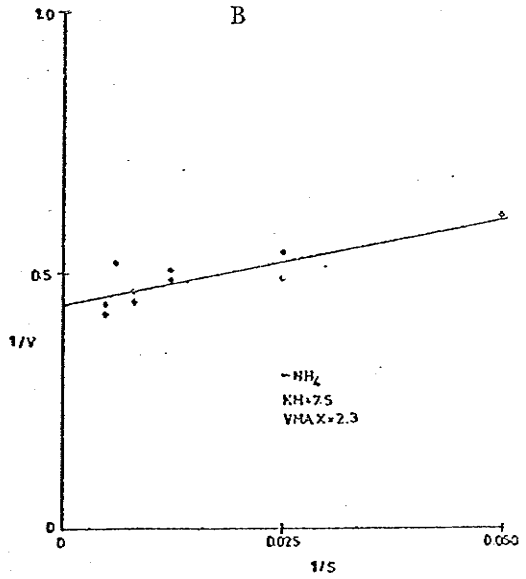
ambient NH_4^+ = 0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; NO_3^- uptake ($-NH_4^+$).

C. Lineweaver-Burk plot; NO_3^- uptake ($+NH_4^+$).


A
2.5
2.0
1.5
1.0
0.5
0
VEL. [ug.NO3 NL-1HR-1]
- NH4
KM=7.6
VMAX=2.3
+ NH4
KM=13.0
VMAX=0.4
0
40
80
120
160
200
[NO3] [ug.NO3 NL-1]


B
1.0
0.5
0
1/V
- NH4
KM=7.5
VMAX=2.3
0
0.025
0.050
1/S

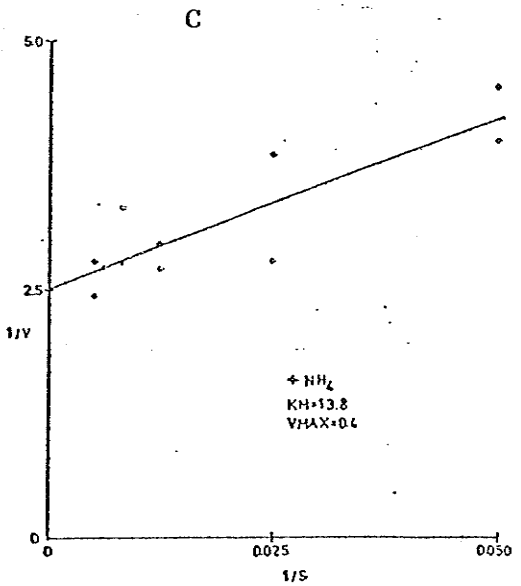

C
50
25
0
1/V
+ NH4
KM=13.8
VMAX=0.4
0
0.025
0.050
1/S

Fig. 19. Lake 226 NE basin, $^{15}NO_3^- + {}^{14}NH_4^+$, 2.5 m. Sept. 14, 1976

20 - 200 µg. $^{15}NO_3^-$ - NL.$^{-1}$ ± 80 µg. $^{14}NH_4^+$ - NL.$^{-1}$

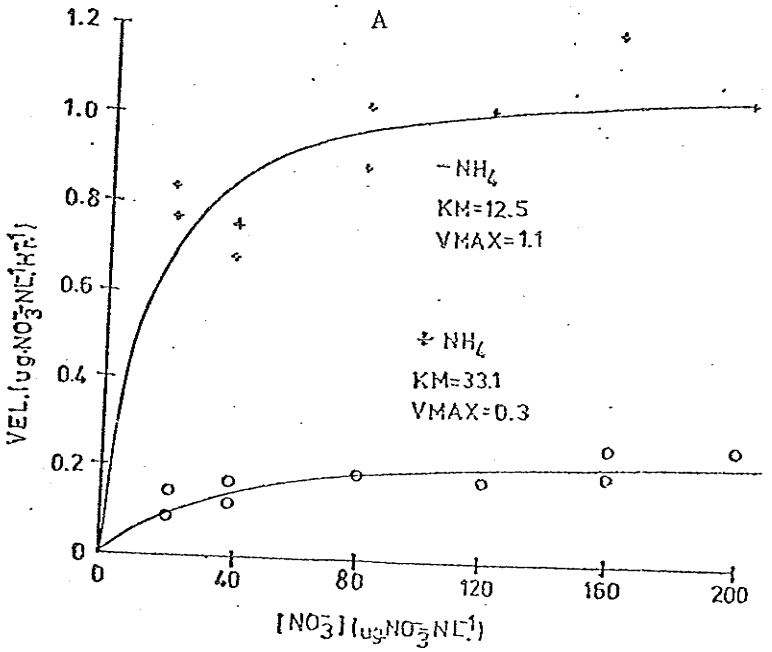
ambient NO_3^- = 0 µg. NL.$^{-1}$

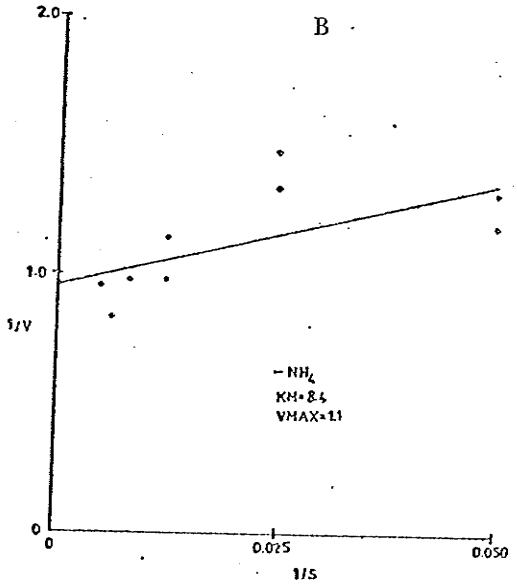
ambient NH_4^+ = 0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot; NO_3^- uptake ($-NH_4^+$).

C. Lineweaver-Burk plot; NO_3^- uptake ($+NH_4^+$).


A
1.2
1.0
0.8
0.6
0.4
0.2
0
VEL.(ug NO3 NL-1 HR-1)
- NH4
KM=12.5
VMAX=1.1
+ NH4
KM=33.1
VMAX=0.3
0
40
80
120
160
200
[NO3] (ug NO3 NL-1)


B
2.0
1.0
0
1/V
- NH4
KM=8.4
VMAX=1.1
0
0.025
0.050
1/S

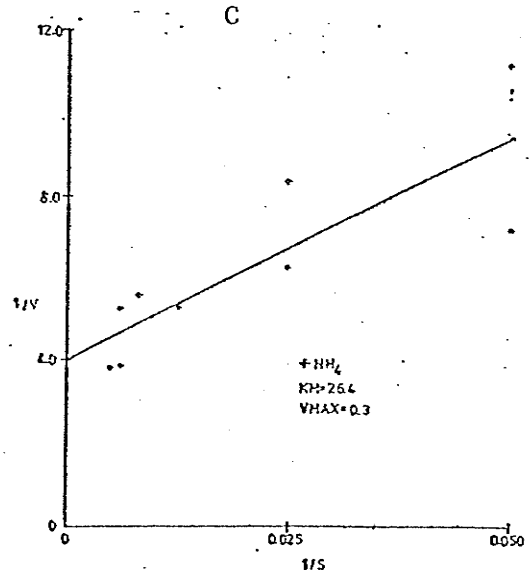

C
12.0
8.0
4.0
0
1/V
+ NH4
KM=26.4
VMAX=0.3
0
0.025
0.050
1/S

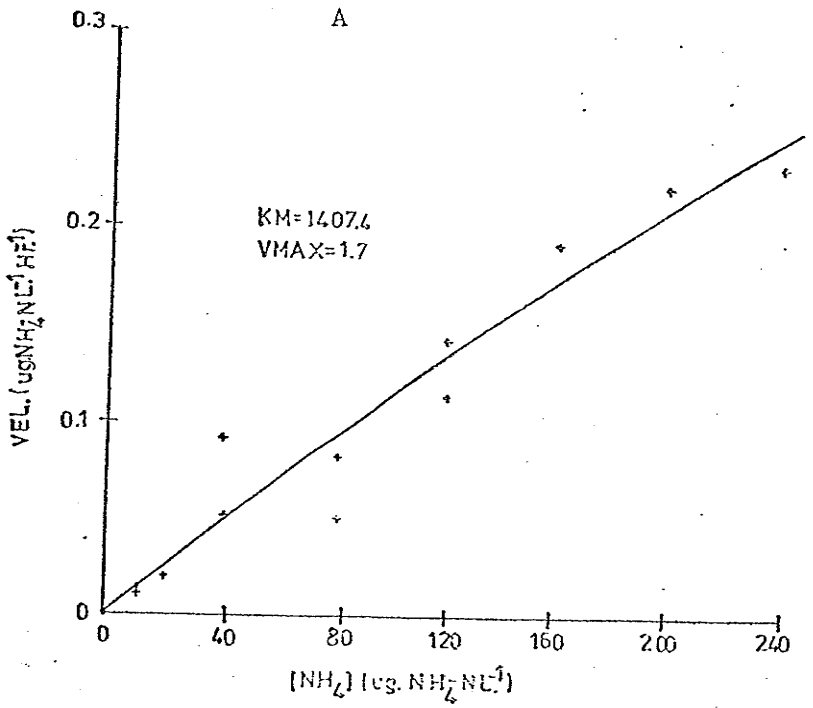
Fig. 20. Lake 226 SW basin, hypolimnion, 6.5 m. Sept. 16, 1976.

10 - 240 µg. $^{15}NH_4^+$ - NL.$^{-1}$

ambient NH_4^+ = 0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot.


A
0.3
0.2
0.1
0
KM=1407.4
VMAX=1.7
0
40
80
120
160
200
240
[NH4] (ug. NH4-NL-1)

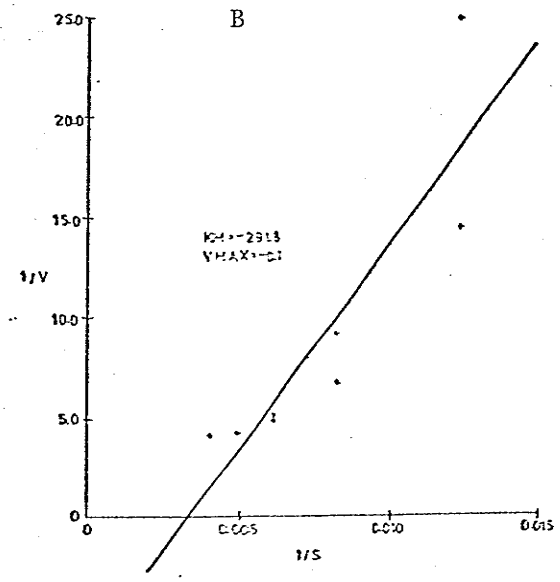

B
25.0
20.0
15.0
10.0
5.0
0
1/V
0.005
0.010
0.015
1/S

Fig. 21. Lake 226 SW basin, hypolimnion, 6.8 m. Sept. 22, 1976.

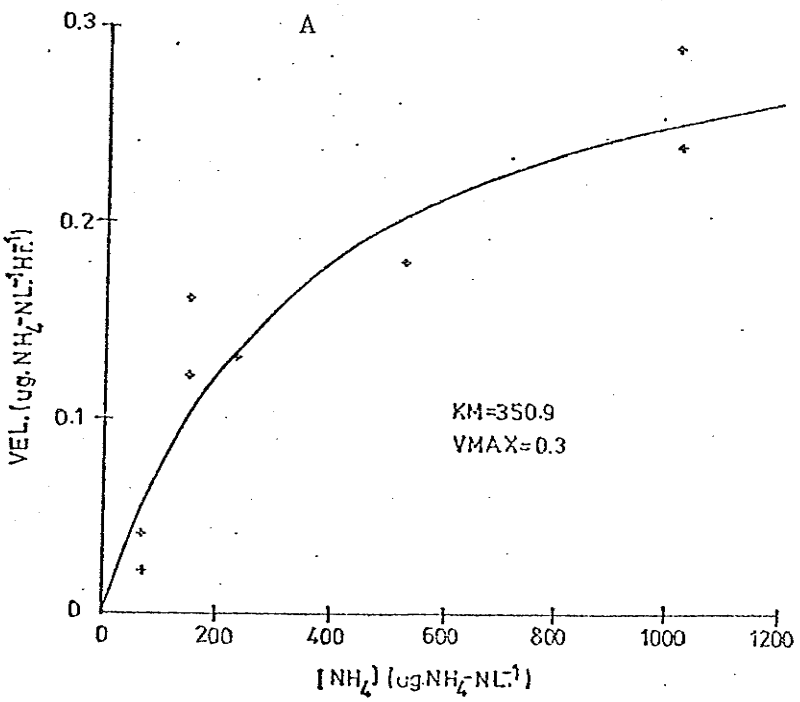
40 - 1000 µg. $^{15}NH_4^+$ - NL.$^{-1}$

ambient NH_4^+ = 29.0 µg. NL.$^{-1}$

ambient NO_3^- = 22.0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot.


A
0.3
0.2
0.1
0
VEL. (ug.NH4-NL-1HF-1)
KM=350.9
VMAX=0.3
0
200
400
600
800
1000
1200
[NH4] (ug.NH4-NL-1)

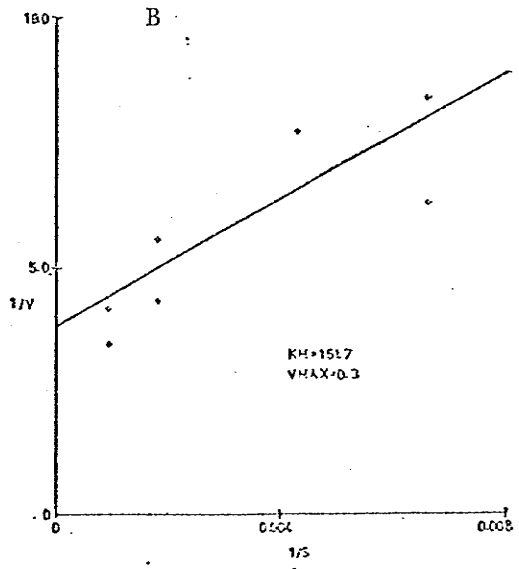

B
180
5.0
1/V
KM=151.7
VMAX=0.3
0
0
0.008
1/S

Fig. 22. Lake 226 NE basin, 2.5 m. Sept. 28, 1976.

40 - 1000 µg. $^{15}NH_4^+$ - NL.$^{-1}$

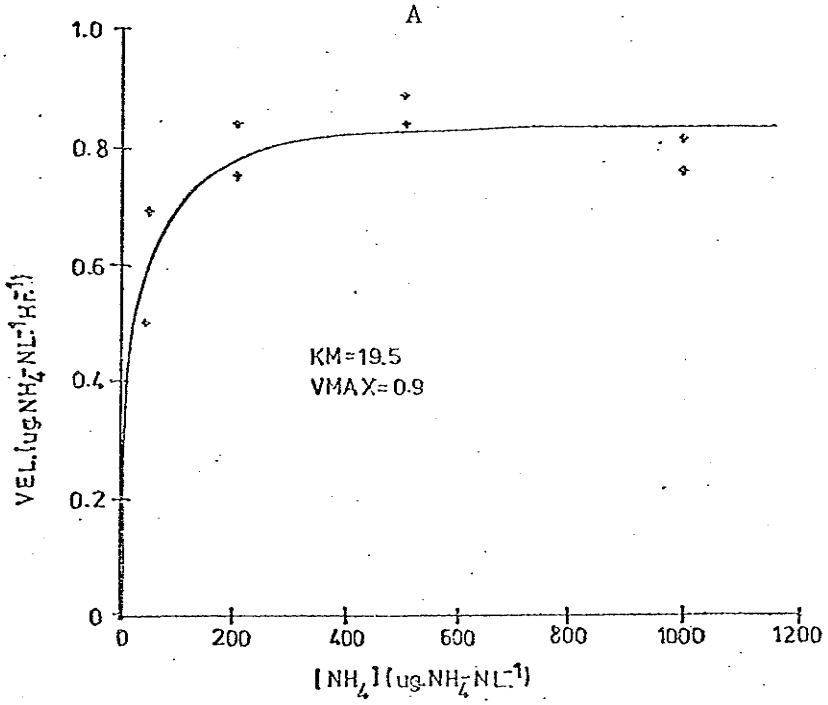
ambient NH_4^+ = 7.0 µg. NL.$^{-1}$

ambient NO_3^- = 2.0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot.

A


1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
KM=19.5
VMAX=0.9
[NH4] (ug.NH4-NL-1)

B

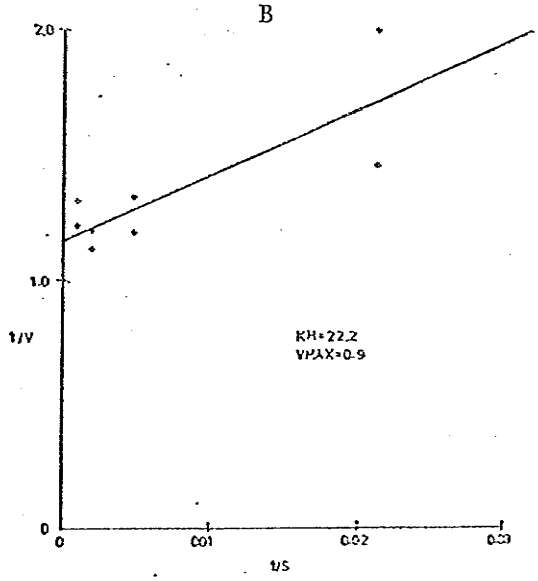

2.0
1.0
0
1/V
KM=22.2
VMAX=0.9
0
0.01
0.02
0.03
1/S

Fig. 23. Lake 226 NE basin, 2.5 m. Sept. 28, 1976.

10 - 160 µg. $^{15}NO_3^-$ - NL.$^{-1}$

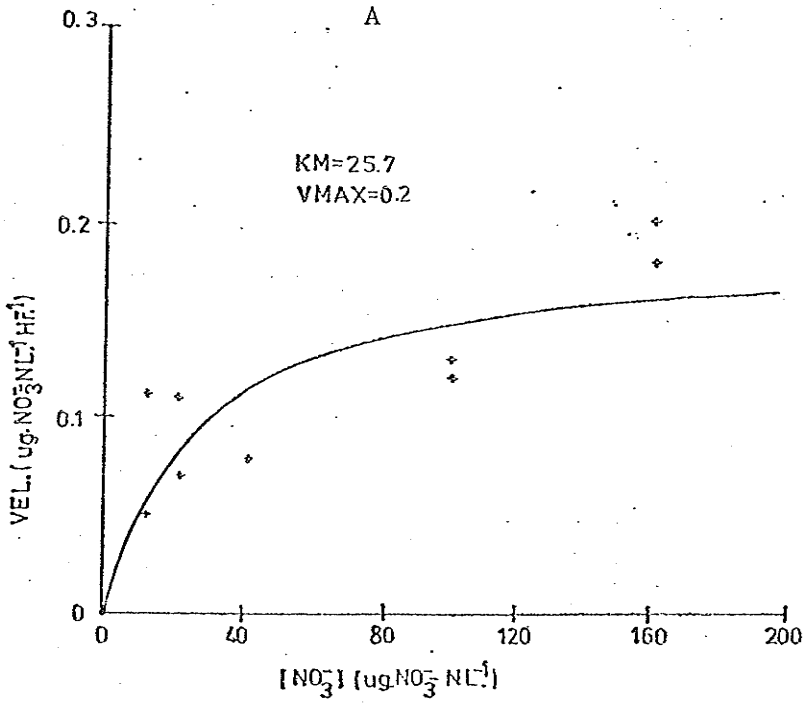
ambient NO_3^- = 2.0 µg. NL.$^{-1}$

ambient NH_4^+ = 7.0 µg. NL.$^{-1}$

A. Michaelis-Menten plot.

B. Lineweaver-Burk plot.

A


KM=25.7
VMAX=0.2
0.3
0.2
0.1
0
0
40
80
120
160
200
VEL. (ug.NO3NL-1 HR-1)
[NO3-] (ug.NO3- NL-1)

B

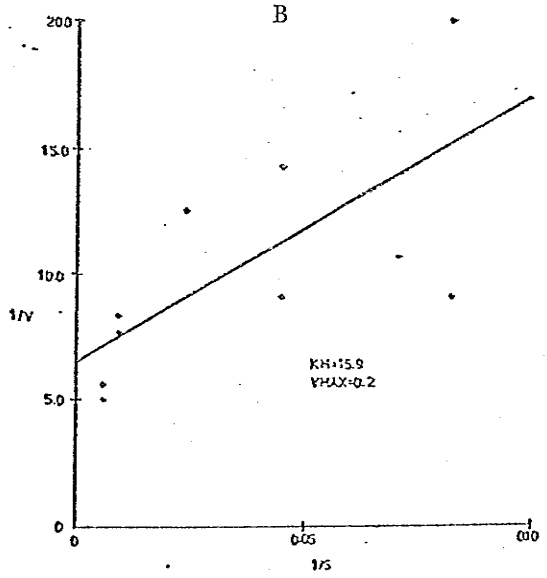

200
150
100
50
0
1/V
KM=15.9
VMAX=0.2
0
0.05
0.10
1/S

Table I. Kinetics of $^{15}NH_4^+$ and $^{15}NO_3^-$ Uptake by Phytoplankton of Lake 302 and Lake 226.

TABLE I

DATE	LAKE	AMBIENT $[NO_3^-]$ ($\mu g.NL.^{-1}$)	AMBIENT $[NH_4^+]$ ($\mu g.NL.^{-1}$)	SUPPLEMENTED N SOURCE	Kt(MM)	Kt(LB)	V(MM)	V(LB)	Tt(HRS.)
July12/76	226NE	115	0	$^{15}NH_4^+$	335.9	-425.8	85.5	-89.0	---
"	226SW	110	0	"	185.0	145.4	6.6	5.7	--
July20/76	302N	-	0	$^{15}NH_4^+$	3.9	5.6	2.2	2.6	1.8
"	302S	-	0	"	3.8	3.6	1.9	1.9	2.0
July27/76	302N	-	0	$^{15}NH_4^+$	18.7	18.4	4.3	4.2	4.3
"	302S	-	0	"	19.2	13.7	2.1	1.8	9.2
Aug.11/76	302N	-	0	$^{15}NH_4^+$ Light	19.7	20.4	3.9	4.2	5.0
"	"	-	0	$^{15}NH_4^+$ Dark	4.8	3.9	2.6	2.5	1.8
Aug.25/76	226NE	-	0	$^{15}NH_4^+$	16.7	14.3	5.0	4.8	3.0
"	226SW	-	0	"	-0.7	7.9	0.6	0.7	51.9
Sept1/76	226NE	12.8	-	$^{15}NO_3^-$	36.0	53.7	1.3	1.5	37.5
"	226SW	61.4	-	"	1203.4	4211.0	2.1	6.4	602.3
Sept8/76	226NE	0	-	$^{15}NO_3^-$	2.5	2.2	1.7	1.6	1.5
"	226SW	44.2	-	"	5.2	4.9	0.4	0.3	123.5
Sept9/76	226NE	0	0	$^{15}NO_3^- - ^{14}NH_4^+$	7.6	7.5	2.3	2.3	3.3
"	"	0	0	$^{15}NO_3^- + ^{14}NH_4^+$	13.0	13.8	0.4	0.4	32.5

...cont'd

Table I (Cont'd)

DATE	LAKE	AMBIENT $[NO_3^-]$ ($\mu g.NL.^{-1}$)	AMBIENT $[NH_4^+]$ ($\mu g.NL.^{-1}$)	SUPPLEMENTED N SOURCE	Kt(MM)	KT(LB)	V(MM)	V(LB)	Tt(HRS.)
Sept14/76	226NE	0	0	$^{15}NO_3^-$ $-^{14}NH_4^+$	12.5	8.4	1.1	1.1	11.4
"	"	0	0	$^{15}NH_4^+$	33.1	26.4	0.3	0.3	110.3
Sept16/76	226NE hypolimnion	20	0	$^{15}NH_4^+$	1407.4	-291.8	1.7	-0.1	--
Sept22/76	226SW hypolimnion	22	29	$^{15}NH_4^+$	350.9	161.7	0.3	0.3	1266.0
"	"	"	"	$^{15}NO_3^-$	below analytical detection limits				
Sept28/76	226NE	2	7	$^{15}NH_4^+$	19.5	22.2	0.9	0.9	29.4
"	"	"	"	$^{15}NO_3^-$	25.7	24.5	0.2	0.2	138.5

* (MM) - Michaelis-Menten plot

** (LB) - Lineweaver-Burk plot

*** - - no determination made

DISCUSSION

The results of the $^{15}NH_4^+$ uptake experiment of July 12, 1976 are illustrated in Fig. 11. The concentration range of NH_4^+ enrichment used was 1-50 µg. NH_4^+-NL.$^{-1}$ and as can be seen from the figure, these levels were insufficient to saturate uptake by either the NE or SW basins of Lake 226. Since saturation was not achieved (especially in the case of the NE basin whose Lineweaver-Burk plot yields a negative intercept) computer estimates of Kt and V are likely invalid if considered as absolute values. However, relative comparison of the plots of Fig.11 reveal that the V in the NE (i.e. P supplemented) basin is at least 4-5 times that for the SW basin. It also appears that the Kt value for the NE basin is greater than that for the SW basin. Even though ambient NH_4^+ concentrations for both basins were below detection limits, NO_3^- concentrations were greater than 100 µg. NL.$^{-1}$ for both basins, and in conjunction with the rapid uptake of $^{15}NH_4^+$ observed it appears that NH_4^+ is the preferred N-source. Any evaluation of NH_4^+ turnover times for the experiment in question is difficult due to the unreliability of the Kt and V estimations.

Fig. 12 illustrates the results of the $^{15}NH_4^+$ uptake experiment of July 20, 1976 performed on Lake 302. Nutrient supplementation was in the range of 1-50 µg. $^{15}NH_4^+$-NL.$^{-1}$ for both basins and, as can be seen, there was little difference between the kinetic parameters of the two basins. Ambient NH_4^+ concentrations for both basins were zero and NH_4^+ turnover time values were 1.8 and 2.0 hr. for the N and S basins respectively.

The experiment of July 27, 1976 (Fig. 13) was a repeat of the previous experiment. Nutrient supplementation was in the range of 1-50 µg. $^{15}NH_4^+$-NL.$^{-1}$ and ambient NH_4^+ concentrations were zero for both basins. Phytoplankton of both basins consisted primarily of Chrysophytes including Mallomonas spp., Dinobryon spp., Tabellaria spp., and Chrysochromulina parva, with some Chlorophytes such as Chlamydomonas and Monoraphidium setiforme, and Kt values (which have been suggested as a measure of a population's affinity for a nutrient (91)), were not significantly different for the two basins, being 18.7 and 19.2 µg. NH_4^+-NL.$^{-1}$ for the N and S basins respectively. Respective V values for the N and S basins were 4.3 and 2.1 µg. NL.$^{-1}$ hr.$^{-1}$ resulting in calculated turnover times of 4.3 and 9.2 hr. This two-fold difference in Tt values cannot be simply ascribed to an increased phytoplankton biomass in the N basin. Although a two fold difference in biomass (mg. dry wt. m.$^{-3}$) did exist (i.e. 3900 for the N basin and 1600 for the S basin) on July 27, this difference was already present on July 20 when Tt values for both basins were essentially the same.

The experiment of Aug. 11, 1976 (Fig. 14) was performed on Lake 302N in order to determine differences in light and dark uptake of $^{15}NH_4^+$ by phytoplankton. Nitrogen supplementation was from 20-160 µg. $^{15}NH_4^+$-NL.$^{-1}$ in order to ensure uptake saturation. As expected, the V value for dark uptake was less than that for light uptake, 2.6 and 3.9 µg. NH_4^+-NL.$^{-1}$hr.$^{-1}$. However, the dark samples also displayed a decreased Kt value, 4.8 µg.NL.$^{-1}$ as compared to 19.7 µg.NL.$^{-1}$ for

the light samples, resulting in calculated turnover times of 1.8 hr. for dark uptake and 5.0 hr. for light uptake. The light NH_4^+ Tt value agrees well with that calculated for the previous NH_4^+ uptake experiment performed on Lake 302. No attempt will be made to substantiate the decreased Kt value (indicative of increased efficiency) observed in the dark. Since all data points for the dark experiment occurred in the uptake saturation region the computer estimate of Kt may be inaccurate.

Fig. 15 illustrates the results of a $^{15}NH_4^+$ uptake experiment performed on Lake 226 on Aug. 25, 1976. $^{15}NH_4^+$ supplementation was in the range of 20-200 µg. $^{15}NH_4^+$-NL.$^{-1}$ and ambient NH_4^+ concentrations were 0 and 28.4 µg.NL.$^{-1}$ for the NE and SW basins respectively. Uptake saturation (i.e. V) for the SW basin was 0.6 µg.$^{15}NH_4^+$-NL.$^{-1}$ and all experimental points were situated on this plateau. For this reason the computer estimate of Kt from the Michaelis-Menten plot assumes a negative value (-0.7 µg.NL.$^{-1}$) and hence may not be used for the estimation of a Tt value. Turnover times of 3.0 and 51.9 hr. for the NE and SW basins respectively are calculated from the Lineweaver-Burk plots and in conjunction with the relatively high ambient NH_4^+ concentration in the SW basin as compared to the NE basin indicate severe N depletion in the NE basin of Lake 226.

Fig. 16 illustrates the results of a $^{15}NO_3^-$ uptake experiment performed on Lake 226 on Sept. 1, 1976. Ambient NO_3^- concentrations were 12.8 and 61.4 µg.NL.$^{-1}$ for the NE and SW basins respectively and $^{15}NO_3^-$ additions were from 10-160 µg. $^{15}NO_3^-$-NL.$^{-1}$. NO_3^- uptake was far greater in the NE basin than in the SW basin with respective turn-

over times of 37.5 and 602.3 hr. These Tt values are approximately an order of magnitude greater than those calculated for $^{15}NH_4^+$ uptake by Lake 226 phytoplankton on Aug. 25, with the NE basin again showing a much more rapid utilization of N than the SW basin. The observation that appreciable amounts of NO_3^- were present in the epilimnion of both basins and that turnover times were much longer for NO_3^- than for NH_4^+ point to NH_4^+ as the preferred N-source of Lake 226 phytoplankton.

The $^{15}NO_3^-$ uptake experiment was repeated on Sept. 8, 1976 (Fig. 17). Turnover time remained more rapid for the NE basin than the SW basin (i.e. 1.5 and 123.5 hr. respectively), however, NO_3^- Tt for the NE basin was now only 1/25 of the value calculated one week previously and for the SW basin was only 1/5 of the previous week's value. It appears that both basins were adapting to NO_3^- as a N-source, the NE basin to a greater degree than the SW basin. The ambient NO_3^- concentration in the NE basin was now 0 whereas it was still high (i.e. 44.2 $\mu g.NL.^{-1}$) in the SW basin.

To further test the hypothesis that NH_4^+ is preferred over NO_3^- as a N-source an experiment utilizing $^{15}NO_3^-$ (20-200 $\mu g.^{15}NO_3^-\text{-}NL.^{-1}$) with and without addition of 80 $\mu g.^{14}NH_4^+\text{-}NL.^{-1}$ was performed on Lake 226 NE (Fig. 18). NH_4^+ inhibition of $^{15}NO_3^-$ uptake was manifest as an increased Kt value and a decreased V value resulting in NO_3^- turnover time of 3.3 hr. in the absence of NH_4^+ and 32.5 hr. in the presence of NH_4^+.

The experiment of Sept. 14, 1976 (Fig. 19) was a repeat of the $^{15}NO_3^- + ^{14}NH_4^+$ experiment outlined above. Again it was observed that NH_4^+ caused a decrease in the V value and an increase in the Kt value

for NO_3^-. Tt for NO_3^- in the absence of NH_4^+ was 11.4 hr. and in the presence of NH_4^+ was 110.3 hr.

A $^{15}NH_4^+$ uptake experiment (Fig. 20) was performed on the hypolimnetic chlorophyll peak of Lake 226 SW (28) on Sept. 16, 1976. This peak was present at a depth of 6.5 m. and sample bottles were filled from the outlet of the fluorometer used to detect the peak. Uptake values were very low (i.e. V = 1.7 $\mu g.NL.^{-1}hr.^{-1}$ and Kt = 1407.4 $\mu g.NL.^{-1}$) being not much greater than passive adsorption samples (duplicate samples killed with Lugol's solution followed by supplementation with 120 $\mu g.\ ^{15}NH_4^+\text{-}NL.^{-1}$) which had an average uptake velocity of 3.2×10^{-2} $\mu g.\ NH_4^+\text{-}NL.^{-1}hr.^{-1}$. In spite of NH_4^+ uptake rates being very low they did not approach saturation in the concentration range of 10-240 $\mu g.\ ^{15}NH_4^+\text{-}NL.^{-1}$, therefore the Lineweaver-Burk transformation of the almost linear Michaelis-Menten plot yielded a negative intercept.

Fig. 21 illustrates the results of a second $^{15}NH_4^+$ uptake experiment performed on the hypolimnetic chlorophyll peak of Lake 226 SW. As in the experiment outlined above rates of uptake were very low. However, NH_4^+ uptake was now more close to being saturated since a range of 40 - 1000 $\mu g.\ ^{15}NH_4^+\text{-}NL.^{-1}$ was used. Values of Kt and V were 350.9 $\mu g.NL.^{-1}$ and 0.3 $\mu g.NL.^{-1}hr.^{-1}$ respectively estimated a turnover time of greater than 1200 hr. Although this hypolimnetic peak represents a major component of the lake's phytoplankton biomass its rate of NH_4^+ uptake is extremely low, likely due to light and/or P limitation (29).

In addition, a set of hypolimnetic samples was supplemented with $^{15}NO_3$ (10-160 µg.NL.$^{-1}$), however, uptake was so low (i.e. 2 x 10^{-3}-9 x 10^{-2} µg. $^{15}NO_3^-$-NL.$^{-1}$hr.$^{-1}$) and erratic as to prohibit plotting of the data.

Figs. 22 and 23 illustrate $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake experiments performed on the NE basin of Lake 226 on Sept. 29, 1976. From the Kt and V values given in the figures it can again be seen that NH_4^+ is the preferred N-source with Tt values of 29.4 and 138.5 hr. for NH_4^+ and NO_3^- respectively.

CONCLUSIONS

Although the kinetic parameters discussed throughout the text show considerable variation throughout consecutive sampling dates it appears that NH_4^+ is the preferred inorganic N-source for phytoplankton of Lakes 302 and 226 of the Experimental Lakes Area. Half-saturation values for NH_4^+ uptake are lower than those for NO_3^- uptake and NH_4^+ inhibition of NO_3^- uptake was manifest as both a decreased V and increased Kt.

The observation that diatoms are frequently the dominant phytoplankton populations in oliotrophic systems (16, 90) and the suggestion that this is due to their ability to utilize low nutrient concentrations (i.e. low Kt values) are substantiated by the work performed on Lake 226. The phytoplankton of the SW basin was composed mainly of large and small Chrysophyceae such as Mallomonas spp., Dinobryon spp., Cyclotella, and Tabellaria spp. from July through

September. Half-saturation values for both NH_4^+ and NO_3^- uptake by the SW basin were consistently lower than those for the NE basin which supported large numbers of Anabaena solitaria and Oscillatoria spp. as well as Ankistrodesmus, Chlamydomonas, and Staurastrum.

The hypolimnetic phytoplankton peak of Lake 226 SW, which was comprised primarily of large and small Chrysophytes such as Synura, Dinobryon spp., and Chrysochromulina parva, showed extremely low rates of NH_4^+ and NO_3^- uptake and does not appear to be N-limited.

As stated previously, colourimetric analysis of residual NH_4^+ and NO_3^- was performed in conjunction with isotopic analysis in order to compare the two methods. Data obtained by the colourimetric techniques were erratic and were omitted from the present manuscript for the following reasons. Uptake rates of NH_4^+ as determined by colourimetry were extremely high, being directly proportional to the supplemented NH_4^+ concentration, showing no saturation even at NH_4^+ concentrations as high as 1.0 mg.NH_4^+-NL.$^{-1}$. In the experiments where the effects of $^{14}NH_4^+$ on $^{15}NO_3^-$ uptake were investigated colourimetric determination of NO_3^- revealed that NO_3^- uptake was not at all affected by addition of 80µg.NH_4^+-NL.$^{-1}$. Colourimetric estimations of NO_3^- uptake were much higher than estimations made by the ^{15}N isotope enrichment method. Degobbis (1973) discusses the many contradictory reports present on the storage of seawater for NH_4^+ analysis. Included are phenomena such as increases in NH_4^+ concentration due to cell lysis and excretion, decreases in NH_4^+

concentration due to adsorption onto suspended particulate material and bottle wall material, and chloroform inhibition of indophenol formation. Since experiments performed with standard solutions of inorganic N prepared with deionized water showed no decrease in NH_4^+ concentration due to rigorous filtration and/or addition of Lugol's solution it seems likely that a physical adsorption process involving the incubation bottle material or some filterable entity present in the samples was responsible for rendering the supplemented NH_4^+ (and to a somewhat lesser extent NO_3^-) as undetectable by colourimetric analysis.

The results of the ^{15}N isotope enrichment method used are felt to represent gross uptake of nutrient since such short incubation times were used (17,61).

Numerous factors must be considered before kinetic data from enrichment studies are incorporated into systems models. Harrison (1973) suggests that phytoplankton blooms are likely not controlled by simply one nutrient but rather by the integrated stimulii of several factors including temperature and salinity, as well as actual nutrient concentrations. This view is supported by MacIsaac and Dugdale (1969) who suggest that "other limited phenomena" are possible, being that at some point below a calculated V value for a specific nutrient some other nutrient may become limiting thereby underestimating both the V and Kt values.

High ambient nutrient concentrations may also lead to erroneous uptake data since, short of starvation, experimental nutrient supplementation cannot be made in the range where uptake velocity is concentration-dependent.

Preconditioning nutrient and light histories (4, 17, 31, 43, 81) are likely to affect kinetic constants obtained in enrichment studies by exerting their effects on levels of cellular energy reserves and by controlling induction or repression of inducible enzyme systems.

Eppley et al. (20, 22, 24) have demonstrated diel periodicity in phytoplankton uptake of NH_4^+ and NO_3^-.

Differences between half-saturation values for nitrogenous nutrient uptake and those for actual cellular incorporation of the acquired N have been reported and concern expressed regarding extrapolation of uptake data to growth estimations (22, 25, 27).

The present study utilized Reeve Angel 984H Ultra glass fibre filters having a mean pore size of 0.5 µm. (73) which was assumed to retain all algal cells and most bacterial cells. This assumption is likely valid for the most part, however, much recent debate concerning bacteria smaller than 0.5 µm., phytoplankton smaller than 0.5 µm, and bacterial colonization of algal cell surfaces and large detrital particles indicates yet another variable involved in nutrient uptake studies.

As stated earlier, future studies concerning aquatic productivity and nutrient dynamics will necessitate determination of pertinent kinetic parameters. In situ experimentation and careful consideration

of the factors outlined above will prove invaluable in such studies.

REFERENCES

References Cited

1. Bates, S.S. 1976. Effects of light and ammonium on NO_3^- uptake by two estuarine phytoplankton species. Limnol. Oceanogr. 21:212-218.

2. Baxter, R.M., Wood, R.B., and Prosser, M.V. 1973. The probable occurrence of hydroxylamine in the water of an Ethiopian lake. Limnol. Oceanogr. 18:470-472.

3. Berman, T. 1974. Urea in the waters of Lake Kinneret (Sea of Galilee). Limnol. Oceanogr. 19:77-979.

4. Bhovichitra, M. and Swift, E. 1977. Light and dark uptake of nitrate and ammonium by large oceanic dinoflagellates: Pyrocystis noctiluca, Pyrocystis fusiformis, and Dissodinium lunula. Limnol. Oceanogr. 22:73-83.

5. Bienfang, P.K. 1975. Steady state analysis of nitrate-ammonium assimilation by phytoplankton. Limnol. Oceanogr. 20:402-411.

6. Birdsey, E.C. and Lynch, V.H. 1962. Utilization of nitrogen compounds by unicellular algae. Science. 137:763-764.

7. Bremner, J.M. 1965. Isotope-ratio analysis of nitrogen in nitrogen-15 tracer investigations. ed. C.A. Black. Methods of soil analysis. American Society of Agronomy, Inc., Publisher. Madison, Wisconsin, U.S.A.

8. Brunskill, G.J. and Schindler, D.W. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Bd. Can. 28:139-155.

9. Caperon, J. and Meyer, J. 1972. Nitrogen-limited growth of marine phytoplankton - II. Uptake kinetics and their role in nutrient limited growth of phytoplankton. Deep-Sea Res. 19:619-632.

10. Carpenter, E.J. and Guillard, R.R.L. 1971. Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton. Ecology. 52:183-185.

11. Carpenter, E.J. and McCarthy, J.J. 1975. Nitrogen fixation and uptake of combined nitrogenous nutrients by Oscillatoria (Trichodesmium) thiebautii in the western Sargasso Sea. Limnol. Oceanogr. 20:389-401.

12. Chan, Y.K. 1977. Denitrification and Phytoplankton Assimilation of Nitrate in Lake 227 During Summer Stratification. PhD. Thesis. University of Manitoba. 188 pages.

13. Chen, R.L., Keeney, D.R., and Konrad, J.G., 1972. Nitrification in sediments of selected Wisconsin Lakes. J. Environ. Qual. 1:151-154.

14. Degobbis, D. 1973. On the storage of seawater samples for ammonia determination. Limnol. Oceanogr. 18:146-150.

15. Dowd, J.E. and Riggs, D.S. 1965. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. J. Biol. Chem. 240:863-869.

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

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

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

19. Edmondson, W.T. 1970. Phosphorous, nitrogen, and algae in Lake Washington after diversion of sewage. Science. 169: 690-691.

20. Eppley, R.W., Carlucci, A.F., Holm-Hansen, O., Kiefer, D., McCarthy, J.J., Venrick, E., and Williams, P.M. 1971. Phytoplankton growth and composition in shipboard cultures supplied with nitrate, ammonium, or urea as the nitrogen source. Limnol. Oceanogr. 16:741-751.

21. Eppley, R.W. and Coatsworth, J.L. 1968. Uptake of nitrate and nitrite by Ditylum brightwellii - Kinetics and mechanisms. J. Phycol. 4:151-156.

22. Eppley, R.W. and Rogers, J.N. 1970. Inorganic nitrogen assimilation of Ditylum brightwellii, a marine plankton diatom. J. Phycol. 6:344-351.

23. Eppley, R.W., Rogers, J.N., and McCarthy, J.J. 1969. Half-saturation constants for uptake of NO_3^- and NH_4^+ by marine phytoplankton. Limnol. Oceanogr. 14:912-920.

24. Eppley, R.W., Rogers, J.N., and McCarthy, J.J. 1971. Light/dark periodicity in nitrogen assimilation of the marine phytoplankters Skeletonema costatum and Coccolithus huxleyi in N-limited chemostat culture. J. Phycol. 7:150-154.

25. Eppley, R.W. and Thomas, W.H. 1969. Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. J. Phycol. 5:375-379.

26. Eutrophication: Causes, Consequences, Correctives. Nat. Acad. of Sciences. Washington D.C. 1969.

27. Falkowski, P.G. 1975. Nitrate uptake in marine phytoplankton: Comparison of half-saturation constants from seven species. Limnol. Oceanogr. 20:412-417.

28. Fee, E.J. 1976. The vertical seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, northwestern Ontario. Implications for primary production estimates. Limnol. Oceanogr. 21:767-783.

29. Fee, E.J. personal communication.

30. Ferraris, M.M., and Proksch, G. 1972. Calibration methods and instrumentation for optical ^{15}N determinations with electrodeless discharge tubes. Anal. Chim. Acta. 59: 177-185.

31. Fitzgerald, G.P. 1969. Field and laboratory evaluations of bioassays for nitrogen and phosphorous with algae and aquatic weeds. Limnol. Oceanogr. 14:206-212.

32. Flett, R.J. 1976. Nitrogen Fixation in Canadian Precambrian Shield Lakes. Ph.D. Thesis. University of Manitoba.. 197 pages.

33. Gardner, W.S. and Lee, G.F. 1975. The role of amino acids in the nitrogen cycle of Lake Mendota. Limnol. Oceanogr. 20:379-388.

34. Goering, J.J. 1972. The role of nitrogen in eutrophic processes. ed. R. Mitchell. Water Pollution Microbiology. Wiley-Interscience.

35. Golterman, H.L. H_2O, seventh annual, 7 and 8, 134-137 and 152-155.

36. Graetz, D.A., Keeney, D.R., and Aspiras, R.B. 1973. Eh status of lake sediment-water systems in relation to N transformations. Limnol. Oceanogr. 18:908-917.

37. Hamilton, R.D. 1964. Photochemical processes in the inorganic nitrogen cycle of the sea. Limnol Oceanogr. 9:109-111.

38. Hamilton, R.D. and Austyn, K.E. 1967. Assay of relative heterotrophic potential in the sea: the use of specifically labelled glucose. Can. J. Microbiol. 13:1165-1173.

39. Hamilton, R.D. and Preslan, J.E. 1970. Observations on heterotrophic activity in the eastern tropical Pacific. Limnol. Oceanogr. 15:395-401.

40. Harrison, W.G. 1973. NO_3^- reductase activity during a dinoflagellate bloom. Limnol. Oceanogr. 18:457-465.

41. Hauck, R.D. 1973. Nitrogen tracers in nitrogen cycle studies - past use and future needs. J. Environ. Qual. 2:317-327.

42. Healey, F.P. 1973. Inorganic nutrient uptake and deficiency in algae. CRC Crit. Rev. Microbiol. 3:69-113.

43. Healey, F.P. 1977. Ammonium and urea uptake by some freshwater algae. Can. J. Bot. 55:61-69.

44. Herrera, J., Paneque, A., Maldonado, J.M., Barea, J.L., and Losada, M. 1972. Regulation by ammonia of nitrate reductase synthesis and activity in Chlamydomonas reinhardi. Biochem. Biophys. Res. Commun. 48:996-1003.

45. Hollman, M. and Stiller, M. 1974. Turnover and uptake of dissolved phosphate in freshwater. A study in Lake Kinneret. Limnol. Oceanogr.19:774-783.

46. Hutchinson, G.E. A Treatise on Limnology Vol I. John Wiley and Sons Inc. London. Chapman and Hall Ltd. 1957.

47. Johnson, W.E. and Vallentyne, J.R. 1971. Rationale, background, and development of experimental lake studies in northwestern Ontario. J. Fish. Res. Bd. Can. 28:123-128.

48. Kalff, J. 1971. Nutrient limiting factors in an arctic tundra pond. Ecology. 52:655-659.

49. Keeney, D.R. 1972. The Fate of Nitrogen in Aquatic Ecosystems. Literature review. The University of Wisconsin, Water Resources Center. Eutrophication Information Program.

50. Keeney, D.R. 1973. The nitrogen cycle in sediment-water systems. J. Environ. Qual. 2:15-29.

51. Knoechels, R. personal communication.

52. Landner, L. and Larsen, T. 19 . Indications of disturbances in the nitrification process in a heavily nitrogen-polluted water body. Ambio. 2:154-157.

53. Lui, N.S.T. and Roels, O.A. 1972. Nitrogen metabolism of aquatic organisms II. The assimilation of nitrate, nitrite, and ammonia by Biddulphia aurita. J. Phycol. 8:259-264.

54. 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.

55. McCarthy, J.J. and Eppley, R.W. 1972. A comparison of chemical, isotopic, and enzymatic methods for measuring nitrogen assimilation of marine phytoplankton. Limnol. Oceanogr. 17:371-382.

56. Meyer, G.W., McCaslin, B.D., and Gast, R.G. 1974. Sample preparation and ^{15}N analysis using a Statron NOI-5 optical analyzer. Soil Sci. 117:378-385.

57. Neilson, A.H. and Doudoroff, M. 1973. Ammonia assimilation in blue-green algae. Arch. für Mikrobiol. 89:15-22.

58. North, B.B. 1975. Primary amines in California coastal waters: utilization by phytoplankton. Limnol. Oceanogr. 20:20-27.

59. Packard, T.T. 1973. The light dependence of NO_3^- reductase in marine phytoplankton. Limnol. Oceanogr. 18:466-469.

60. Parsons, T.R. and Strickland, J.D.H. 1962. On the production of particulate organic carbon by heterotrophic processes in seawater. Deep-Sea Res. 8:211-222.

61. Procházková, L., Blažka, P., and Králová, M. 1970. Chemical changes involving nitrogen metabolism in water and particulate matter during primary production experiments. Limnol. Oceanogr. 15:797-807.

62. Reuss, H.S., Edmondson, W.T., and Vallentyne, J.R. 1970. Eutrophication - key elements. Science 170:1153-1154.

63. Riggs, D.S. The Mathematical Approach to Physiological Problems. M.I.T. Press. Cambridge, Mass., U.S.A. 1963.

64. Sawicki, C.R. and Scaringelli, F.P. 1971. Colormetric determination of nitrate after hydrazine reduction to nitrite. Microchem. Journ. 16:657-672.

65. Schell, D.M. 1974. Uptake and regeneration of free amino acids in marine waters of southeast Alaska. Limnol. Oceanogr. 19:260-270.

66. Schindler, D.W. 1971. Carbon, nitrogen, and phosphorous and the eutrophication of freshwater lakes. J. Phycol. 7:321-329.

67. Schindler, D.W. 1973. Evolution of phosphorous limitation in lakes. Science. 195:260-262.

68. Schindler, D.W. 1973. Experimental approaches to limnology - an overview. J. Fish. Res. Bd. Can. 30:1409-1413.

69. Schindler, D.W., and Fee, E.J. 1974. Experimental lakes area: whole-lake experiments in eutrophication. J. Fish. Res. Bd. Can. 31:937-953.

70. Schindler, D.W., and Holmgren, S.K. 1971. Primary production and phytoplankton in the experimental lakes area, north-western Ontario, and other low-carbonate waters, and a liquid scintillation method for determining ^{14}C activity in photosynthesis. J. Fish. Res. Bd. Can. 28:189-201.

71. Shapiro, J. 1973. Blue-green algae: why they become dominant. Science. 179:382-384.

72. Sharp, J.H. Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr. 19:984-988.

73. Sheldon, R.W. 1972. Size separation of marine seston by membrane and glass-fiber filters. Limnol. Oceanogr. 17:494-498.

74. Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1974. The Chemical Analysis of Fresh Water. Miscellaneous Special Publication No. 25. Research and Development Directorate. Freshwater Institute, Winnipeg, Manitoba, Canada.

75. Stewart, W.D.P. 1963. Liberation of extracellular nitrogen by two nitrogen fixing blue-green algae. Nature. 200:1020-1021.

76. Stewart, W.D.P. ed. D.F. Jackson. Algae, Man, and the Environment. Proc. Int. Symp. Syracuse Univ. June, 1967. Syracuse Univ. Press. Syracuse, N.Y. 1968.

77. Syrett, P.J. ed. R.A. Lewin. Physiology and Biochemistry of Algae. Academic Press. London and New York. 1962.

78. Thomas, W.H. 1970. Effect of ammonium and nitrate concentration on chlorophyll increases in natural tropical Pacific phytoplankton populations. Limnol. Oceanogr. 15:386-394.

79. Thompson, B.M. and Hamilton, R.D. 1973. Heterotrophic utilization of sucrose in an artifically enriched lake. J. Fish. Res. Bd. Can. 30:1547-1552.

80. Toetz, D.W., Varga, L.P., and Loughran, E.D. 1972. Half-saturation constants for uptake of nitrate and ammonia by reservoir plankton. Ecology. 54:903-908.

81. Topinka, J.A. and Robbins, J.V. 1976. Effects of nitrate and ammonium enrichment on growth and nitrogen physiology in Fucus psiralis. Limnol. Oceanogr. 21:659-664.

82. Torrey, M.S. and Lee, G.F. 1976. N_2 fixation in Lake Mendota, Madison, Wisconsin. Limnol. Oceanogr. 21:365-378.

83. Turner, M. personal communication.

84. Vallentyne, J.R. The Algal Bowl Lakes and Man. Miscellaneous Special Publication 22. Department of the Environment. Fisheries and Marine Service. Ottawa, Ontario, Canada. 1974.

85. Vanderhoef, L.N., Huang, C.Y., Musil, R., and Williams, J. 1974. Nitrogen fixation (acetylene reduction) by phytoplankton in Green Bay, Lake Michigan, in relation to nutrient concentrations. Limnol. Oceanogr. 19:119-125.

86. Vollenweider, R.A. (ed.). A Manual on Methods for Measuring Primary Production in Aquatic Environments. IBP handbook no. 12. F.A. Davis Co. Philadelphia, Pa. 1969.

87. Wada, E. and Hattori, A. 1971. Nitrite metabolism in the euphotic layer of the central north Pacific Ocean. Limnol. Oceanogr. 16:766-772.

88. Webb, K.L., DuPaul, W.D., Wiebe, W., Sottile, W., and Johannes, R.E. 1975. Enewetak Atoll: aspects of the nitrogen cycle on a coral reef. Limnol. Oceanogr. 20:198-210.

89. Wheeler, P.A., North, B.B., and Stephens, G.C. 1974. Amino acid uptake by phytoplankters. Limnol. Oceanogr. 19: 249-259.

90. Wollen, D.G. and Cartier, L.D. 1975. Molybdenum dependence, nitrate uptake, and photosynthesis of freshwater plankton algae. J. Phycol. 11:345-349.

91. Wright, R.T. and Hobbie, J.E. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology. 47:447-464.