THE MASS SPECTROMETRIC DETERMINATION OF NITROGEN UPTAKE BY FRESHWATER PHYTOPLANKTON — THE EFFECT OF CHLORINE AND CHLORAMINE

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PREFACE

The objective of this study was to use quantitative mass spectrometry to examine nitrate and ammonia uptake by phytoplankton and the effects of chlorine and chloramine.

Dr. Louis P. Varga served as major adviser. The other members of the advisory committee included Drs. D. W. Toetz, H. L. Gearhart, and E. J. Eisenbraun. I am grateful for the guidance of these and many other faculty members and colleagues. Special gratitude is extended to Dr. E. M. Hodnett for his donation of a Toepler pump. Dr. S. E. Scheppele and Mr. N. Perreira of the mass spectrometry laboratory and Mr. W. Adkins of the glass shop exceeded their responsibilities in kindly providing assistance and advice. A note of thanks is extended to Dr. R. J. Ryba and Mr. J. D. Caplinger for their stimulating discussions and kind assistance.

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TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION AND LITERATURE REVIEW.	1
	Primary Production and the Kinetics of Nitrogen Uptake.	1
	Pathways of Nitrogen Uptake and Assimilation.	3
	^{15}N and the Michaelis-Menten Model.	9
	Chlorine	16
	The Chlorine-Water-Ammonia Equilibria	20
	The Breakpoint Phenomenon	23
II.	EXPERIMENTAL ACTIVITY	27
	Study Area	27
	General Experimental Procedure	27
	Experiments During 1973.	30
	Gas Collection	31
	Mass Spectrometric Analysis.	32
	Mass Spectrometer Output - Electronic Filtration	39
	Calculation of Uptake Rate and Error Propagation.	42
	A_f, the Atom Percent ^{15}N in the Sample.	42
	A_i, the Atom Percent ^{15}N in the Solution.	43
	Experiments During 1974.	46
	Experiments During 1975.	47
III.	RESULTS AND DISCUSSION.	48
	Experiments During 1973.	48
	A One Compartment Model.	59
	Experiments During 1974.	74
	Experiments During 1975.	86
	Procedural and Instrumental Modifications.	97
IV.	SUMMARY	103
BIBLIOGRAPHY		109
APPENDIX A - THE DERIVATION OF THE MICHAELIS-MENTEN EQUATION . . .		117
APPENDIX B - THE COMPUTER PROGRAM FOR THE CALCULATION AND PLOTTING OF THE MICHAELIS-MENTEN CONSTANTS		120

LIST OF TABLES

Table		Page
I.	Mass Spectrometers Which Have Been Used in ^{15}N Uptake Studies.	17
II.	The Standard Potentials for Reaction of Chlorine in Solution	21
III.	The Absolute Error in the Natural Abundance of ^{15}N as a Function of the Errors in Measuring the Peak Heights of m/e 29 in Millimeters.	36
IV.	The Absolute and Relative Error at Various Enrichment Levels of ^{15}N as a Function of a Constant Error (1 mm) in Measuring the Peak Height of m/e 29	37
V.	Temperature and Irradiance Data in Lake Carl Blackwell During 1973	49
VI.	The Concentrations of Nitrate, Nitrite, Ammonia, Chlorophyll *a*, and Particulate Nitrogen Found in Lake Carl Blackwell During 1973	50
VII.	Experimentally Derived Values of V_{max} and K_s and Their Standard Deviations for Nitrate Uptake at 0.8 Meters During 1973	51
VIII.	The Atom Percent ^{15}N and Their Variance Based on Peak Height Measurement for Controls and Enriched Samples for the Experiment on June 6, 1973.	53
IX.	Computer Calculations of A_f, $\sigma^2_{A_f}$, A_i and $\sigma^2_{A_f}$ for the Experiment on June 6, 1973.	55
X.	Computer Calculations of the Relative A_f, Relative A_i, $V_{NO_3^-}$, $\sigma_{V_{NO_3}}$, V_{max}, $\sigma_{V_{max}}$, K_s, and σ_{K_s} for the Experiment on June 6, 1963.	56
XI.	Temperature and Irradiance Data in Lake Carl Blackwell During 1974	75
XII.	Concentrations of Nitrate, Nitrite, Ammonia, Phosphate, Chlorophyll *a*, Particulate Nitrogen, and pH Found in Lake Carl Blackwell During 1974.	76

LIST OF TABLES (CONTINUED)

Table		Page
XIII.	Experimentally Derived Values of V_{max} and K_s, Their Standard Deviations and Regression Coefficients From the Woolf Plot During 1974	77
XIV.	A Comparison of Nitrogen Flux for the Controlled and Perturbed $^{15}NO_3^-$ Uptake Experiments During 1974.	79
XV.	The Effect of Adding 100 µg PO_4^{-3}-P/L on NO_3^- and NH_3 Uptake During 1974.	81
XVI.	A Summary of the Initial and Equilibrium Conditions and the Response for the Chlorine-Chloramine Experiment on July 16, 1974.	83
XVII.	Temperature and Irradiance Data for Experiments During 1975.	88
XVIII.	Concentrations of Nitrate, Nitrite, Ammonia, Phosphate, Chlorophyll a, and Particulate Nitrogen Found in Lake Carl Blackwell During 1975	89
XIX.	A Summary of the Initial and Equilibrium Conditions and the Response for the Chlorine-Chloramine Experiment During 1975	90
XX.	Initial Conditions for the Experiment of $V_{NO_3^-}$ vs. pH and Orthophosphate on August 29, 1975	95
XXI.	A Summary of the Ge Perturbed Experiments During 1975	96
XXII.	A Summary of the Mass Spectrometric Analyses of Control Samples From Lake Carl Blackwell During 1973, 1974, and 1975.	102

LIST OF FIGURES

Figure		Page
1.	Simplified Nitrogen Cycle Showing Main Molecular Transformations	4
2.	Proposed Pathways of Nitrogen Incorporation	5
3.	The Hyperbolic Michaelis-Menten Plot.	12
4.	The Lineweaver-Burk Plot.	12
5.	The Woolf Plot.	13
6.	The Hofstee Plot.	13
7.	The Relationship of Ammonia-Nitrogen and Chlorine - the Breakpoint Phenomenon	25
8.	Shoreline Map of Lake Carl Blackwell at Spillway Level. "X" Indicates Location of Sampling Station.	28
9.	The Vacuum System Used for the Collection of Nitrogen Gas	34
10.	Circuit Diagram of the Filtering and Amplifying Network	41
11.	The Michaelis-Menten Plot of $V_{NO_3^-}$ vs. Nitrate Concentration for the Experiment on June 6, 1973.	52
12.	A Measure of the Water Level of Lake Carl Blackwell During 1973.	57
13.	A Plot of $V_{NO_3^-}$ vs. Nitrate Concentration for Replicate Samples for the Experiment on September 29, 1973.	58
14.	The Ambient Nitrate Ion Concentration in Lake Carl Blackwell in 1973	62
15.	The Ambient Ammonia Concentration in Lake Carl Blackwell in 1973	63
16.	A Plot of Equation 68, the Ammonia Correction Factor for Nitrate Uptake by Algae	64

LIST OF FIGURES (CONTINUED)

Figure		Page
17.	A Plot of the Temperature Factor of 2.0 for Nitrate Uptake by Algae at 20°C	65
18.	This Curve Shows the Behavior of Three Functions.	66
19.	V_{max}, the Maximum Specific Nitrate Uptake Rate for Algae as Experimentally Determined From the $^{15}NO_3^-$ Uptake.	67
20.	V_{max} for Nitrate Uptake by Algae Corrected for the Effects of Non-Optimum Light, Temperature, and the Effect of Ammonia on Nitrate Uptake	68
21.	K_s, the Michaelis-Menten Half-Saturation Constant for the Uptake of Nitrate by Algae as Experimentally Determined From the $^{15}NO_3^-$ Uptake Studies	69
22.	The Flux of Nitrate into the Phytoplankton Compartment Calculated by Equations 65 or	70
23.	The Concentration of Chlorophyll *a* in Lake Carl Blackwell in 1973	71
24.	A Three-Dimensional Calcomp Plot of $V_{NO_3^-}$ vs. Free and Bound Chlorine From 1974 Data	85
25.	A Plot of the Predicted Surface for $V_{NO_3^-}$ vs. Chlorine and Chloramine Concentrations	87
26.	A Three-Dimensional Calcomp Plot of $V_{NO_3^-}$ vs. Free and Bound Chlorine From 1975 Data	92
27.	A Three-Dimensional Calcomp Plot of $V_{NO_3^-}$ vs. Free and Bound Chlorine From 1975 Data (Second View)	93
28.	A Typical Oscillographic Trace Prior to the Electronic Filtration Modification	99
29.	A Typical Oscillographic Trace After the Electronic Filtration Modification	100

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Primary Production and the Kinetics of Nitrogen Uptake

Primary production is the rate at which radiant energy is stored by photosynthetic and chemosynthetic activity of producer organisms in the form of organic substances which can be used as food materials (Odum, 1971). The ideal manner to measure primary production is to measure the rate at which energy flows into a system. Since almost all production in nature results in new protoplasm, an equation of productivity might be written as follows:

$$1{,}300{,}000 \text{ cal (radiant energy)} + 106\ CO_2 + 90\ H_2O + 16\ NO_3^- + 1\ PO_4^{-3} + \text{mineral elements} = 13{,}000 \text{ cal potential energy in } 3{,}258 \text{ g protoplasm (106 C, 180 H, 46), 16 N, 1 P, 815 g mineral ash)} + 154\ O_2 + 1{,}287{,}000 \text{ cal heat energy dispersed (99\%).} \quad [1]$$

Since there is no practical method in which to measure this energy, several different methods have been used to estimate primary production indirectly. The harvest method is straightforward for producers such as agricultural crops over time periods of months or more. Obviously, this method is not applicable to phytoplankton production during short

periods of time. Other methods include monitoring the rate of appearance of a metabolite such as oxygen, chlorophyll, etc., or disappearance of a nutrient such as carbon dioxide, ammonia, nitrate, phosphate, etc. In general, these chemical methods have been found to have insufficient sensitivity and precision for measuring rates of assimilation (Uphaus, Flaumenhaft and Katz, 1967).

In 1952, Steemann-Nielson introduced a procedure using ^{14}C-carbon dioxide. This radioactive tracer method has maintained popularity due to its superior sensitivity. Other radioactive tracer procedures have not been as successful as that of ^{14}C. One essential requirement of such methods is that the half-life of the element be of sufficient period to allow the experiment and analysis to be performed during that period ($t_{1/2}$ of ^{14}C = 5720 years) (Friedlander, Kennedy and Miller, 1964). Unfortunately, the longest half-life of the radioactive isotopes of nitrogen is very short ($t_{1/2}$ of ^{13}N = 10.0 minutes) (Friedlander, et al., 1964). Burris (1941) points out that in 1940, other investigators, Ruben, Hassid and Kamen (1940), arrived at erroneous conclusions regarding N_2 fixation of barley while using ^{13}N. Radioactive phosphorus, ^{32}P, has not proved very satisfactory in short term productivity studies, because it is readily adsorbed by sediments and particulate matter and is thus not available for adsorption into living cells.

Estimations of primary productivity based upon the uptake rate of nitrogen is a more logical choice than that for carbon or phosphorus (Dugdale and Goering, 1967). It has been suggested that carbon uptake occurs only during photosynthesis. Nitrogen assimilation is a continuous process only indirectly related to photosynthesis thereby

maintaining an average C to N ratio. Nitrogen is a major structural component of cells. Carbon and phosphorus are not only structural components but are also continuously turned over in the energetic processes of organisms where as nitrogen is not.

Pathways of Nitrogen Uptake and Assimilation

Chemical elements tend to flow in characteristic cyclic paths in the biosphere. Of the three major nutrients (carbon, nitrogen and phosphorus), nitrogen has the most complex cycle, see Figure 1, and is quantitatively understood the least. This may be due to the fact that it is involved in many biochemical transformations (Brezonik, 1972). Mechanisms of nitrogen transports and transformations in phytoplankton are not well established. While this investigation does not strive to elucidate the exact pathways of nitrogen incorporation into cellular organic nitrogen, it does measure the kinetics of uptake. It is therefore felt that a brief discussion of the present views of the systems in question is appropriate. Figure 2 is a summary of this discussion.

Uptake and assimilation of a nutrient are two separate and distinct processes. For the purpose of this manuscript, uptake is defined as the transfer of a nutrient from the media or environment into the cell. In phytoplankton nitrogen uptake is believed to occur at specific sites on the cell wall rather than by an osmotic process. Assimilation is defined as the transformation of a nutrient into organic cellular material. In some cases, phytoplankton are known to accumulate a nutrient against a negative concentration gradient. This indicates the presence of one or more active transport processes rather than

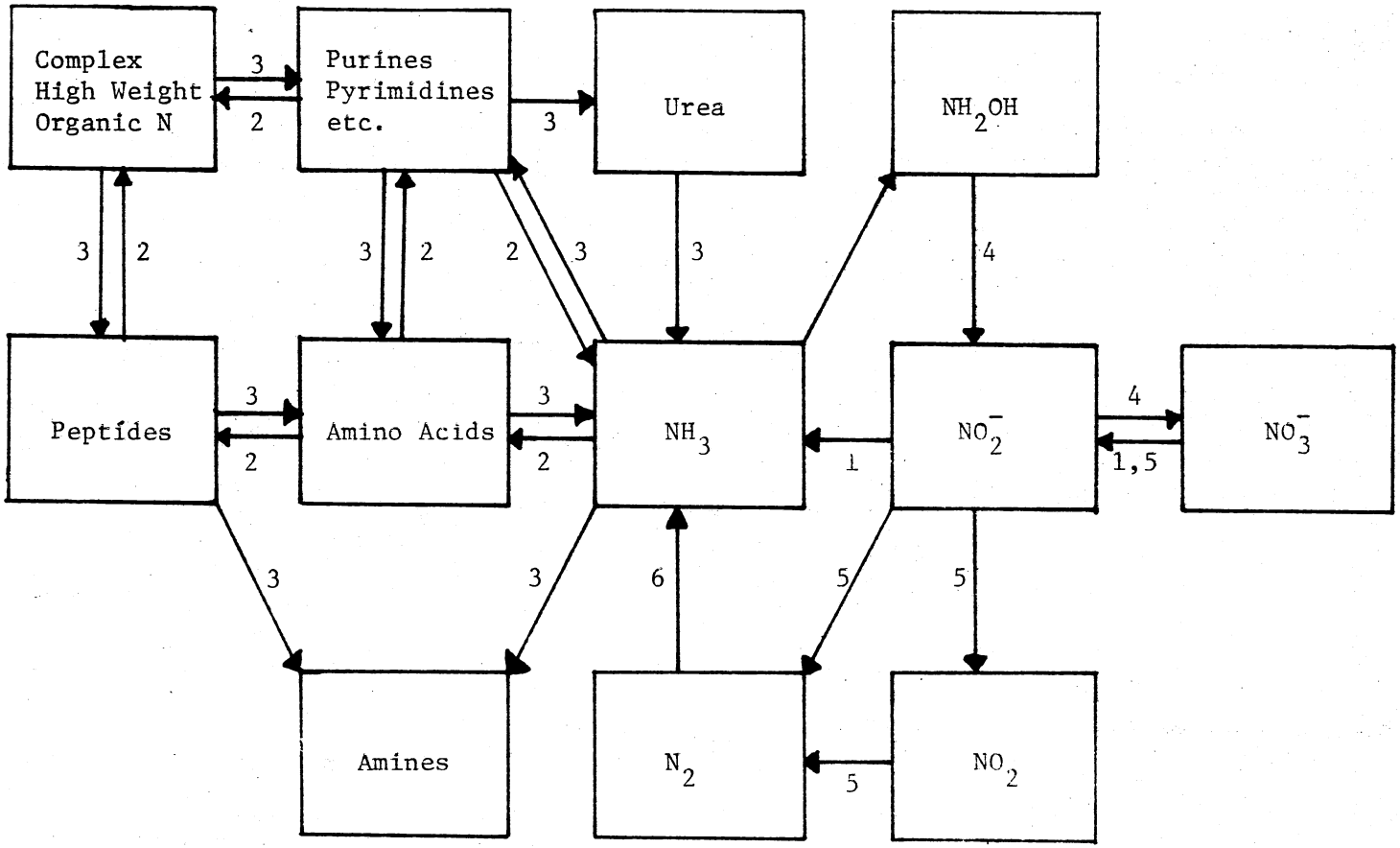


Figure 1. Simplified Nitrogen Cycle Showing Main Molecular Transformations: 1. Nitrate Assimilation, 2. Ammonia Assimilation, 3. Ammonification, 4. Nitrification, 5. Denitrification, 6. Nitrogen Fixation (Brezonik, 1972)

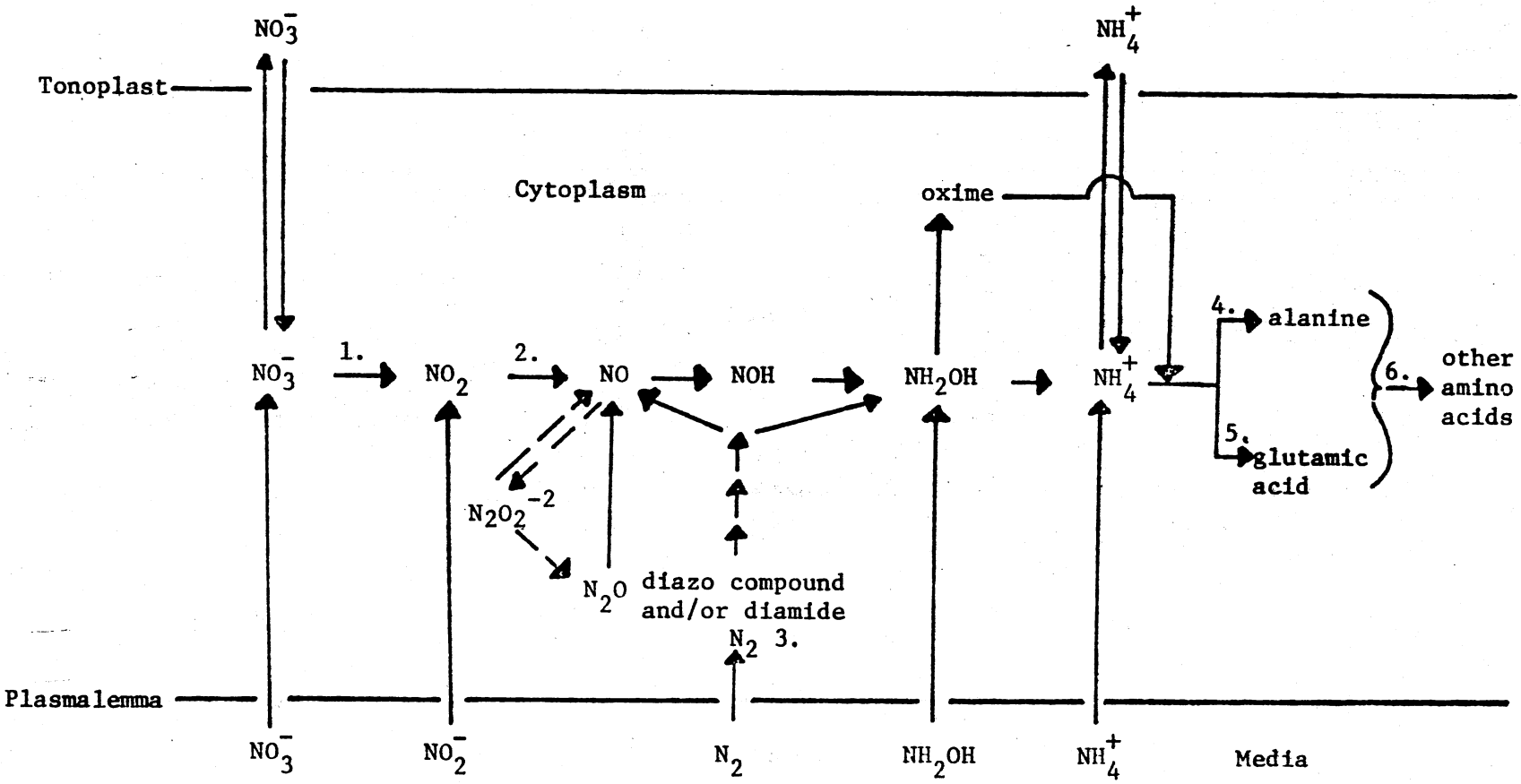


Figure 2. Proposed Pathways of Nitrogen Incorporation. Some Enzymes Associated in Reactions are 1. Nitrate:NADH-oxidoreductase, 2. Nitrite Reductase, 3. Nitrogenase, 4. and 5. Glutamic Dehydrogenase (4. Pyruvate, 5. α-Ketoglutamate), and 6. Transaminase Reactions (Eppley and Rogers, 1970)

mere diffusion. Falkowski (1975) has presented data which suggests the presence of a transport system enzyme, NO_3^-, Cl^- activated ATPase, located in the plasmalemma.

Nitrogen compounds may be present in natural waters as cellular constituents, nonliving particulate matter, soluble organic compounds and inorganic ions in solution.

Naturally occurring organic nitrogen is primarily in the form of amino and amide (proteinaceous) compounds. Phytoplankton are through to have limited capability to assimilate organic nitrogen (primarily urea and some amino acids) (Brezonik, 1972). Gardner and Lee (1975) found that the concentrations of dissolved free amino acids in Lake Mendota were controlled by bacteria. Using the reagent fluorescamine, about half of the primary amines found in California coastal water were found to be rapidly taken up by phytoplankton (North, 1975). Those amines not taken up may occur as peptides. Hobbie and Crawford (1968) found that the total amino acid flux represented 1 to 10 percent of the daily photosynthetic carbon fixation in the York River. Droop (1968, 1961) has demonstrated the need for vitamin B_{12} in Monochrysis Lutheri. Urea-nitrogen was found to be an important source of nitrogen for plankton in the northern Pacific Ocean (Eppley, Rogers and McCarthy, 1969) while plankton in the western Sargasso Sea were barely able to use this nutrient (Carpenter and McCarthy, 1975). Eppley, et al. (1971) have compared the growth rate of phytoplankton grown on three sources of nitrogen: urea, nitrate and ammonia.

Inorganic nitrogen in natural water exists primarily as nitrate, ammonia, nitrite and nitrogen gas. Nitrite uptake occurs in many

phytoplankton (Lui and Roels, 1972); however, nitrite is an intermediate in nitrate assimilation. It is therefore not shown in Figure 1. Ammonia and nitrate assimilation are responsible for the greatest influx of inorganic nitrogen into organisms (Brezonik, 1972).

The ultimate source of nitrogen is atmospheric nitrogen. Those plants which can convert nitrogen gas to ammonia are called nitrogen fixers. The multienzyme complex, nitrogenase, pyruvate as an electron donor, adenine triphosphate, and a divalent metal ion are necessary for nitrogen fixation (Mahler and Cordes, 1971). An "electron carrying protein", ferredoxin (or flavodoxin in the absence of sufficient iron), is involved in the transfer of electrons from pyruvate to nitrogenase. The mechanistic pathway is not completely clear. Several intermediates which have not been found but are theorized include diimide, hydrazine and a diazo-organic compound. Nitrogen fixation is of undoubted importance in blue-green algae (Painter, 1970). (Other nitrogen fixers include photosynthetic, faculative, and various aerobic bacteria, legume root nodules, non-legume root-nodulated plants, and the Alder tree.) However, fixation rates have been considered to be too low and sporadic to be of significance in the overall nitrogen budgets of most lakes (Brezonik, 1972). This should be reassessed as further studies show fixation potentials (Toetz, 1972) and high variability of algal behavior (Vanderhoef, et al., 1975).

Nitrate is the most abundant inorganic nitrogen form in surface waters and can be used by most plants (Brezonik, 1972). After nitrate uptake has occurred, nitrate may be stored in vacuoles, which often are a very large portion of the cell volume, or may be reduced to

nitrite by the enzyme nitrate: NADH-oxidoreductase (Eppley and Thomas, 1969). This enzyme is said to be inducible in that it is present in phytoplankton cells only while the cells are assimilating nitrate. Nitrate reductase, a nicotinamide nucleotide-linked molybdoflavoprotein, is employed as an initial electron donor; the electron transfer has been elucidated in Neurospora (Mahler and Cordes, 1971). Equation 2 is not a reaction but elucidates the sequence in which the electron transfer agent

$$\text{ferredoxin} \xrightarrow{\text{flavoprotein}} \text{NADH} \rightarrow \text{FAD} \rightarrow \text{Mo}^{+5} \rightarrow \text{NO}_3^- \qquad [2]$$

precipitate in the overall transfer. The oxidation steps of molybdenum involved appear to be +5 and +6 (Lui and Roels, 1972).

The reduction of nitrite is accomplished by nitrite reductase, which is usually a nicotinamide nucleotide-linked metalloflavoprotein. At the present time there is no evidence to indicate that nitrite is accumulated in cell vacuoles. The final product of nitrite reduction is ammonia, but whether this reaction is direct or proceeds in a multi-step manner is not clear (Hattori, 1962). Substantial evidence exists for the formation of nitric oxide. Evidence for other intermediates such as hyponitrite, nitroxyl, nitrous oxide, nitramide and dihydroxy ammonia is scanty (Painter, 1970). Some studies (Grant and Turner, 1969; Morris and Ahmed, 1969) have shown that light stimulates nitrate and nitrite assimilation by photosynthetically reducing flavoproteins, ferredoxins, etc.

Ammonia is the preferred form for planktonic assimilation because it is already at the reduction level of organic nitrogen. Both hydroxylamine and ammonia can be taken directly into the cell, and the latter can be stored in vacuoles of many phytoplankton. Hydroxylamine

can react with α-keto-acids to form oximes which are catalytically reduced to the corresponding amino acids by L-glutamate:NAD oxido-reductase (Painter, 1970). Ammonia is believed to react with pyruvate and α-keto-glutarate to form alanine and glutamic acid; other amino acids are thought to be formed from these by transaminase reactions. Furthermore, ammonia assimilation is believed to suppress synthesis of nitrate reductase thereby inhibiting assimilation of nitrate (Syrett and Morris, 1963).

Finally, some species of phytoplankton are adaptive toward using different forms of nitrogen (Hattori, 1962) and to the range of concentrations to which they are accustomed (Carpenter and Guillard, 1971).

^{15}N and the Michaelis-Menten Model

Until 1941, experiments testing nitrogen fixation were based on relatively unreliable changes in the total nitrogen analyzed by procedures such as the Kjeldahl method. Burris, et al. (Burris and Miller, 1941; Burris, 1941; Burris and Eppling, 1943) first used the stable isotope of nitrogen, ^{15}N, in nitrogen fixations studies. These investigators were able to show data proving, and in some cases disproving, conclusions of other investigators. However, for very small changes in total cellular nitrogen, very highly enriched samples of ^{15}N were needed. Highly enriched compounds of ^{15}N did not become commercially available until later.

In the late 1950's and early 1960's, Dugdale and coworkers (Neess, et al., 1962) began using the ^{15}N technique in nitrogen fixation studies in lakes of Pennsylvania and Wisconsin (Dugdale and Dugdale,

1965; Dugdale, Dugdale, Nees, and Goering, 1959; Dugdale and Dugdale, 1962) and in the Sargasso Sea near Bermuda (Dugdale, Menzel and Ryther, 1961). Diurnal variations in the uptake of ammonia and nitrate were followed in the Sargasso Sea a few years later (Goering, Dugdale and Menzel, 1964).

In 1965, the uptake of three forms of nitrogen, $^{15}NO_3^-$, $^{15}NH_4^+$, and $^{15}N_2$ were easily characterized into three seasonal stages: 1.) a spring bloom when NH_4^+, NO_3^-, and N_2 are assimilated strongly in that order of importance, 2.) a midsummer period when weak assimilation of NH_4^+ and N_2, but not of NO_3^-, occurred, and 3.) a fall bloom with intense nitrogen fixation and some NH_4^+ uptake, but characterized by low NO_3^- activity. These variations are attributed to the seasonal changes in the phytoplankton community.

Dugdale and Goering (1967) examined primary production in several oceanic locations using ^{15}N and ^{14}C labeled compounds. Uptake of nitrate as a fraction of nitrate plus ammonia uptake was characterized as having values typical of subtropical regions or northern temperate coastal or inland water regions. Vertical profiles of ^{15}N labeled nitrate and ammonia uptake and ^{14}C uptake were found to exhibit very similar patterns.

Early quantitative observations resulted in the determination of two relationships. Uptake rate is a function of nutrient in the environment of the phytoplankton. Total phytoplankton yield is proportional to initial nutrient concentration, not a function of growth rate.

In 1967, two investigators, Dugdale (1967) and Caperon (1967, 1969), reported that a hyperbolic relationship between the growth

rate-limiting nutrient concentration and the uptake rate of that nutrient can often describe phytoplankton behavior. Monod (1942), had shown this to be the case for bacteria populations. When this is true, the Michaelis-Menten theory of enzyme kinetics can be used to describe the hyperbola, Equation 3. (The derivation of the Michaelis-Menten Equation is shown in Appendix A (Segal, 1968.)

$$v = \frac{V_m \times S}{K_s + S} \qquad [3]$$

where v is the rate of nutrient uptake, V_m is the maximum rate of nutrient uptake, K_s is the nutrient concentration at which $v = V_m/2$, and S is the concentration of nutrient or substrate.

The simplest plot of Equation 3, v vs. S, is hyperbolic, Figure 3. Mathematical transformations of this equation yield three forms from which linear plots can be made. The first transformation,

$$1/v = K_s/V_m \times 1/S + 1/V_m \ , \qquad [4]$$

is the Lineweaver-Burk plot of 1/v vs. 1/S, Figure 4. In this case the x-intercept is $-1/K_s$, the y-intercept is $1/V_m$, and the slope is K_s/V_m. The next transformation,

$$S/v = 1/V_m \times S + K_s/V_m \ , \qquad [5]$$

is the Woolf plot of S/v vs. S, Figure 5. Here the x-intercept is $-K_s$, the y-intercept is again K_s/V_m, and the slope is $1/V_m$. Finally, the last transformation,

$$v = -K_s \times v/S + V_m \ , \qquad [6]$$

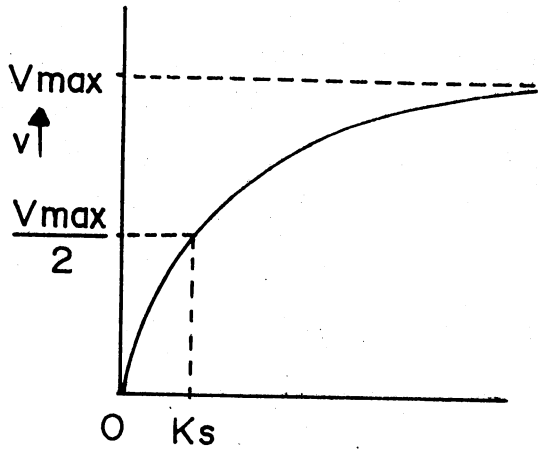


Figure 3. The Hyperbolic Michaelis-Menten Plot

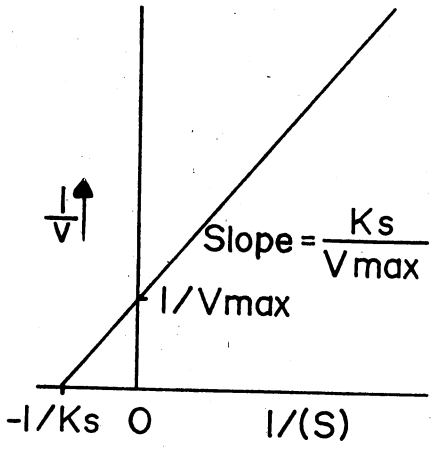


Figure 4. The Lineweaver-Burk Plot

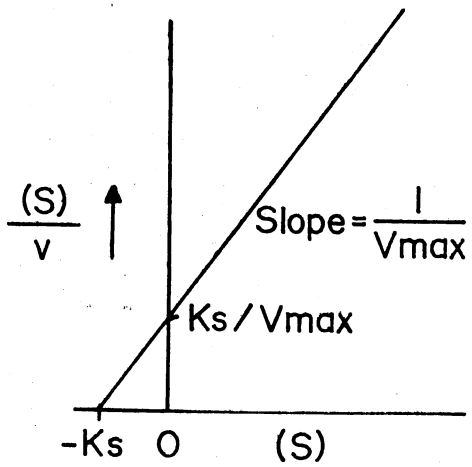


Figure 5. The Woolf Plot

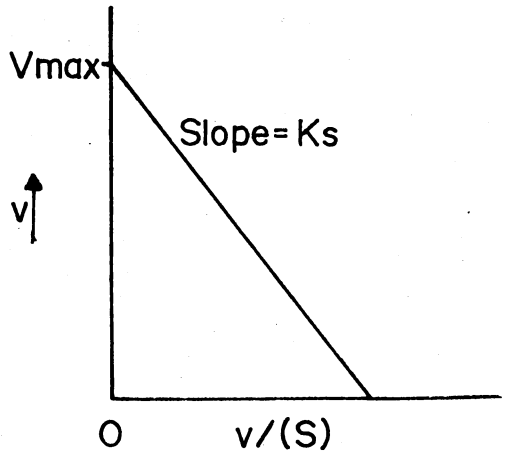


Figure 6. The Hofstee Plot

is the Hofstee plot of v vs. v/S, Figure 6. The y-intercept is V_m and the slope is $-K_s$.

Normally v and S are controlled or measured and V_m and K_s are subsequently calculated. Since Equations 4 and 5 are identical to Equation 6, they all should yield the same numerical values of V_m and K_s. This would be the case if v and S contained no error. When error is present and the curves are fitted by eye or an unweighted least squares regression analysis, the three transformations give estimates of the parameters with varying degrees of accuracy. Small values of v tend to have the greatest percentage error; thus, those plots involving an axis containing v^{-1} tend to emphasize points containing the most uncertainty. Plots containing the same variable in the terms of both axes have some unfounded correlation.

Dowd and Riggs (1965) statistically compared the results of these three transformations using a computer to generate data for values of v each of which had a normal distribution around its mean; S was assumed to contain no error. Estimates of K_s and V_m from the Lineweaver-Burke transformation were by far the least reliable. Estimates from the Woolf and Hofstee transformations differed only slightly depending upon the type of error assumed.

The constants K_s and V_m allow a mathematical basis on which to compare different bodies of water and different types of phytoplankton. This may prove to explain phytoplankton competition and succession.

For example, Dugdale (1967) has shown hypothetical curves for two species of algae in which the alga with the higher V_m also has a higher K_s than the other alga. This enables the second alga to have higher uptake rates than the first at low nutrient concentrations.

Such a situation may explain such phenomena as diatom dominance in nutrient rich areas.

Finally, these constants may be used to control the phytoplankton community by controlling the concentration of nutrients for such applications as fish farms and the elimination of harmful or noxious algae such as Microcystis aeruginosa Kutzing, which produces a diarrhea toxin in rats (Aziz, 1974).

The use of nitrogen enriched with the stable isotope, ^{15}N, to quantitatively measure the rate of nitrogen uptake immediately poses the question of isotopic effect. The isotope ratio of $^{15}N/^{14}N$ in biological material does not in general differ strikingly or consistently in the composition of non-biological material (Neess, et al., 1962). Hoering and Ford (1960) studied the fixation rates of $^{15}N^{14}N$ and $^{14}N^{14}N$ in Azotobacter and found the ratio of these two rates to be 1.000 ± 0.001. In their study an "overall" isotope effect in this fixation process is not observed.

Uphaus, Flaumenhaft and Katz (1967) have grown the alga, Chlorella vulgaris, on three stable isotopes of elements which are biologically important, carbon (^{13}C), oxygen (^{18}O) and nitrogen (^{15}N). Effects in highly substituted cells such as larger cell size and changes in the quantity and distribution of cellular components (nucleic acids, carbohydrates and proteins) were noted. However, this study verified previous findings of other investigators (Neess, et al., 1962; Uphaus, et al., 1967) that ^{15}N has a very small biochemical kinetic effect and can be expected to produce cellular changes of a much smaller magnitude than ^{18}O or ^{13}C.

Since the concept of quantitating nutrient uptake rate with the constants of Michaelis-Menten kinetics was first demonstrated for nitrate and ammonia in phytoplankton, K_s and V_m have been measured for natural populations (Eppley, Rogers and McCarthy, 1969; MacIsaac and Dugdale, 1969; MacIsaac and Dugdale, 1972; Toetz, Varga and Loughran, 1973) and specific species (Eppley and Renger, 1974; Carpenter and McCarthy; 1975; Eppley and Coatsworth, 1968; Caperon and Meyer, 1972). Lehman, Botkins and Likens (1975) have compiled from the literature the K_s values for 21 species and the V_m for seven species of phytoplankton for nitrate and/or ammonia uptake. The K_s values listed have a range of 0.1 to 70 uM nitrate and 0.1 to 7.5 µM ammonia. Typical values for V_m for nitrate and ammonia are 10^{-10} to 10^{-6} µmoles hr^{-1}.

Many different types of mass spectrometers have been used to study ^{15}N uptake. These types include such instruments as the Bendix Time of Flight (Dugdale and Goering, 1967; MacIsaac and Dugdale, 1967), single beam Nier sector (McCarthy and Eppley, 1972), and isotope ratio (Dugdale, et al., 1959; Dugdale, et al., 1961; Nees, et al., 1962) mass spectrometers, Table I. In general, the isotope ratio mass spectrometers are capable of more accurate and precise measurements. However, they have two disadvantages: sample sizes of several milligrams are required and samples must be very pure if accurate results are to be obtained (Caprioli, 1973).

Chlorine

The use of chlorine in water systems is extensive. It is used for general disinfection, organism control in swimming pools, sterilization in fish hatchery water, fly control at wastewater facilities,

TABLE I

MASS SPECTROMETERS WHICH HAVE BEEN USED IN ^{15}N UPTAKE STUDIES

Type of Mass Spectrometer	Sample	Sample Value Atom % ^{15}N	Number of Samples	Sample Standard Deviation	Mean Standard Deviation	Other Error Expression	Error Value	Ref.
Single Beam Nier Sector	---	0.364	42	0.0098	---	---	---	a
Consolidated Nier Isotope Ratio	Air	0.359	--	---	---	---	---	b
	Lake Water	0.365	--	---	---	---	---	
Consolidated Nier Isotope Ratio	$(NH_4)_2SO_4 \rightarrow N_2$	0.361	--	---	---	95% Confidence Interval Reproducibility	0.003 0.001	c
Consolidated Model 21-201 Isotope Ratio	$(NH_4)_2SO_4 \rightarrow N_2$	0.3668	17	0.00347	0.00084	---	---	d
	Lake Water Samples	0.3693	13	0.00670	0.00186			
	Lake Mendota Samples	0.3694	13	0.00379	0.00010	---	---	
	Lake Wingra Samples	0.3680	15	0.00633	0.0016	---	---	
	Tank N_2	0.3508	19	0.0018	0.00024	---	---	

TABLE I (CONTINUED)

Type of Mass Spectrometer	Sample	Sample Value Atom % ^{15}N	Number of Samples	Sample Standard Deviation	Mean Standard Deviation	Other Error Expression	Error Value	Ref.
	$(NH_4)_2SO_4 \rightarrow N_2$	0.3528	8	0.00085	0.00030			
	Lake Water Samples	0.3531	11	0.00052	0.00016	---	---	
Time of Flight	Ocean Samples	0.370	2	---	---	Precision	0.01	e
MI 1305 Made in USSR		---	---	---	---	Relative Error Reproducibility	0.5% 0.006	f
MI 1305 Made in USSR	$NH_4Cl \rightarrow N_2$	---	---	---	---	Coefficient of Variation of $^{15}N/^{14}N$	0.5 - 0.7%	g
Single Beam Nier Sector	Enriched Seawater Samples	0.448	13	0.0124	---	---	---	h
AEI Model MS-10	Marine Samples	---	--	---	---	Corrections for temperature, background, and ion pump	---	i

[a] Burris, et al., 1943
[b] Dugdale, et al., 1959
[c] Dugdale, et al., 1961
[d] Neess, et al., 1962
[e] Dugdale and Goering, 1967
[f] Kralova, 1967
[g] Prochazkova, et al., 1970
[h] McCarthy and Eppley, 1972
[i] Pavlou, et al., 1974

reduction in BOD, clarification, and taste and odor control (White, 1972). The use of chlorine has provided major protection of public health. Furthermore chlorine has proven to be an efficient and cheap method of removing biological slimes on the heat exchange surfaces necessary in many industries and power plants (Draley, 1972).

The impact on man and his environment of the extensive use of chlorine has not yet been fully evaluated. An estimated 1000 tons of chlorinated organic compounds are discharged annually into this nation's waterways as a result of chlorination of wastewater. Carlson (1974) and Jolley (1973) have presented excellent studies on the characterization of the chlorinated organic compounds from wastewater.

Brungs (1973) has reviewed the effect of residual chlorine on aquatic life. Most of the studies have focused on the effect of chlorine on fish. Tsai (1971) found that 100 ppb residual chlorine reduced the species diversity index by 50% for fish. Sprague and Drury (1969) have concluded that 10 ppb residual chlorine and 12 days of exposure are fatal to trout.

Some investigators have focused on the effect of chlorine on primary productivity. It has been estimated that power plants alone will use twice the annual fresh-water runoff of the nation by the year 2000. This presents the synergistic effect of heat plus residual chlorine affecting primary production (Sorge, 1969; Hamilton, et al., 1970; Carpenter, Peck and Anderson, 1972). Nevertheless, chlorine residuals are believed to be the primary cause of a reduction in photosynthesis (Brook and Baker, 1972; Carpenter, et al., 1972; Hamilton, et al., 1970). One investigator has shown how a theoretical 15% depression of photosynthesis for one day in the

effluent on an electric power generating station with a consumption of 500,000 gal/min. could result in a loss of 424 tons of dried tissue in one growing season (Morgan and Stross, 1969). Other investigators (Hirayama and Hirano, 1970) have shown that while some algae are killed by ten minute exposures of 1.5 to 2.3 ppm Cl, other algae are not irreversibly damaged by twenty minute exposures of 20 ppm Cl. Specific damage to some algae have been shown to include changes in cell color, damage to cell walls, leaching of cellular content, and cellular deformation (Betzer and Knott, 1969).

The Chlorine-Water-Ammonia Equilibria

Chlorine gas is only slightly soluble in water as shown by the equilibrium constant, K = 0.062. Nine half-cell reactions with their standard potentials aid in defining the potential chlorine species in aqueous solution, Table II (Cotton and Wilkinson, 1967). Equation 12 coupled with the half-cell of the oxidation of water, Equation 16, demonstrates the oxidation strength of chlorine:

$$O_2 + 4H^+ + 4e^- = 2H_2O, \quad E^\circ = 1.23 \text{ v.} \qquad [16]$$

Although the oxidation of water is thermodynamically favorable, Equation 17, the activation energy is so high that disproportionation of chlorine into hypochlorous and hydrochloric acid occurs instead, Equation 18:

$$Cl_2 + H_2O = 2H^+ + 2Cl^- + 1/2O_2, \quad E^\circ = 0.13 \text{ v,} \qquad [17]$$

$$Cl_2 + H_2O = HClO + H^+ + Cl^-, \quad K = 4.2 \times 10^{-4}. \qquad [18]$$

TABLE II

THE STANDARD POTENTIALS FOR REACTION OF CHLORINE IN SOLUTION*

Half-Cell Reactions	E° (Volts)	
$H^+ + HOCl + e^- = 1/2Cl_2 + H_2O$	1.63	[7]
$3H^+ + HClO_2 + 3e^- = 1/2Cl_2 + 2H_2O$	1.64	[8]
$6H^+ + ClO_3^- + 5e^- = 1/2Cl_2 + 3H_2O$	1.47	[9]
$8H^+ + ClO_4^- = 7e^- = 1/2Cl_2 + 4H_2O$	1.42	[10]
$1/2Cl_2\ e^- = Cl^-$	1.36	[11]
$ClO^- + H_2O + 2e^- = Cl^- + 2OH^-$	0.89	[12]
$ClO_2^- + 2H_2O + 4e^- = Cl^- + 4OH^-$	0.78	[13]
$ClO_3^- + 3H_2O + 6e^- = Cl^- + 6OH^-$	0.63	[14]
$ClO_4^- + 4H_2O + 8\ e^- = Cl^- + 8OH^-$	0.56	[15]

*(Cotton and Wilkinson, 1967)

A saturated solution of chlorine would contain 0.061 M Cl_2, and 0.030 M H^+, Cl^-, and HOCl. Hypochlorous acid is a weak acid with a dissociation constant of 3.4×10^{-8}. Thus, hypochlorous acid and hypochlorite ion concentrations will be equal at a pH of 7.5.

In basic solution, where hypochlorite ion formation is favored, a further disproportionation has a favorable equilibrium constant:

$$3ClO^- = 2Cl^- + ClO_3^-, \; K = 10^{27} \; . \qquad [19]$$

However, this reaction is slow at room temperature and below, such that fairly pure solutions of HOCl and OCl^- can be produced in cold solutions. In hot solutions, 75°C, a good yield of ClO_3^- can be obtained. Disproportionation of hypochlorous acid into chlorous and hydrochloric acids is quite unfavorable and does not take place to an appreciable extent:

$$2HClO = Cl^- + H^+ + HClO_2, \; K = 10^{-5} \; . \qquad [20]$$

Similar disproportionation of hypochlorite ions to chloride and chlorite ions is favorable, Equation 21, but is very slow and must compete with Equation 19 and is therefore not observed.

$$2ClO^- = Cl^- + ClO_2^-, \; K = 10^{7} \qquad [21]$$

Disproportionation of chlorate into chloride and perchlorate is once again thermodynamically very favorable, Equation 22, but very slow even in solutions near 100°C.

$$4ClO_3^- = 3ClO_4^- + Cl^-, \; K = 10^{29} \qquad [22]$$

Ammonia exists in aqueous solution as free ammonia, NH_3, and as the ammonium ion, NH_4^+, in the relative proportion described by Equation 23:

$$NH_3 + H_2O = NH_4^+ + OH^-, \quad K = 1.81 \times 10^{-5}. \qquad [23]$$

Hypochlorous acid reacts rapidly with ammonia to form monochloramine, dichloramine, and trichloramine:

$$NH_3 + HOCl = NH_2Cl + H_2O, \quad K = 3.6 \times 10^9, \qquad [24]$$

$$NH_2Cl + HOCl = NHCl_2 + H_2O, \quad K = 1.33 \times 10^6, \qquad [25]$$

$$NHCl_2 + HOCl = NCl_3 + H_2O, \quad K \text{ is unavailable.} \qquad [26]$$

The reaction products are dependent upon pH and reactant concentrations. Distribution of mono- and dichloramine as a function of pH and ammonium ion concentration can be seen by combining Equations 23, 24 and 25:

$$2NH_2Cl + H^+ = NH_4^+ + NHCl_2, \quad K = 6.7 \times 10^5. \qquad [27]$$

Trichloramine is in significant concentrations below a pH of 4. Dichloramine predominates between a pH of 5 and 6.5 and chloramine is the major specie at pH values greater than 7.5.

The Breakpoint Phenomenon

When chlorination of near neutral solutions occurs at a chlorine to ammonia ratio of less than 5 to 1 (by weight), the practice is called the chloramine or the chlorine-ammonia process and Equation 24

describes the principal reaction and product, chloramine. However, as the chlorine to ammonia ratio increases from 5 to 1, to 10 to 1, a decrease in chlorine residuals (chloramine, hypochlorous acid, etc.) and a decrease in ammonia concentration is observed. After a ratio of chlorine to ammonia (initial) reaches approximately 10 to 1, further chlorine additions yield free and combined residuals of chlorine in a linear fashion. These observations are graphically presented in Figure 7 and were first published by Griffin (1941). Attempts to explain Griffin's findings prompted many scientific investigations. However, the breakpoint phenomenon is only partially understood today.

The importance of the breakpoint phenomenon is in its relation to tastes and odors and in the germicidal efficiency of the species present. It has been shown that between the maxima and minima of Figure 7, the dichloramine concentration is significantly enhanced at the expense of monochloramine. Dichloramine has a disagreeable chlorinous taste while monochloramine does not. Finally, the germicidal efficiency to the right of the minima is 25 times or more greater than that on the left (White, 1972).

On a pilot plant scale, Barnes, Atkins, and Scherger (1972) have found that up to 98% of ammonia-nitrogen can be removed from raw sewage as a result of the breakpoint reactions. Pressley, et al. (1973) found laboratory breakpoint reaction results were in agreement with pilot plant studies. Both of these investigations considered the effect of pH and temperature.

The exact pathway(s) of the breakpoint phenomenon reactions have not been quantitatively determined. However, three steps are generally believed to occur:

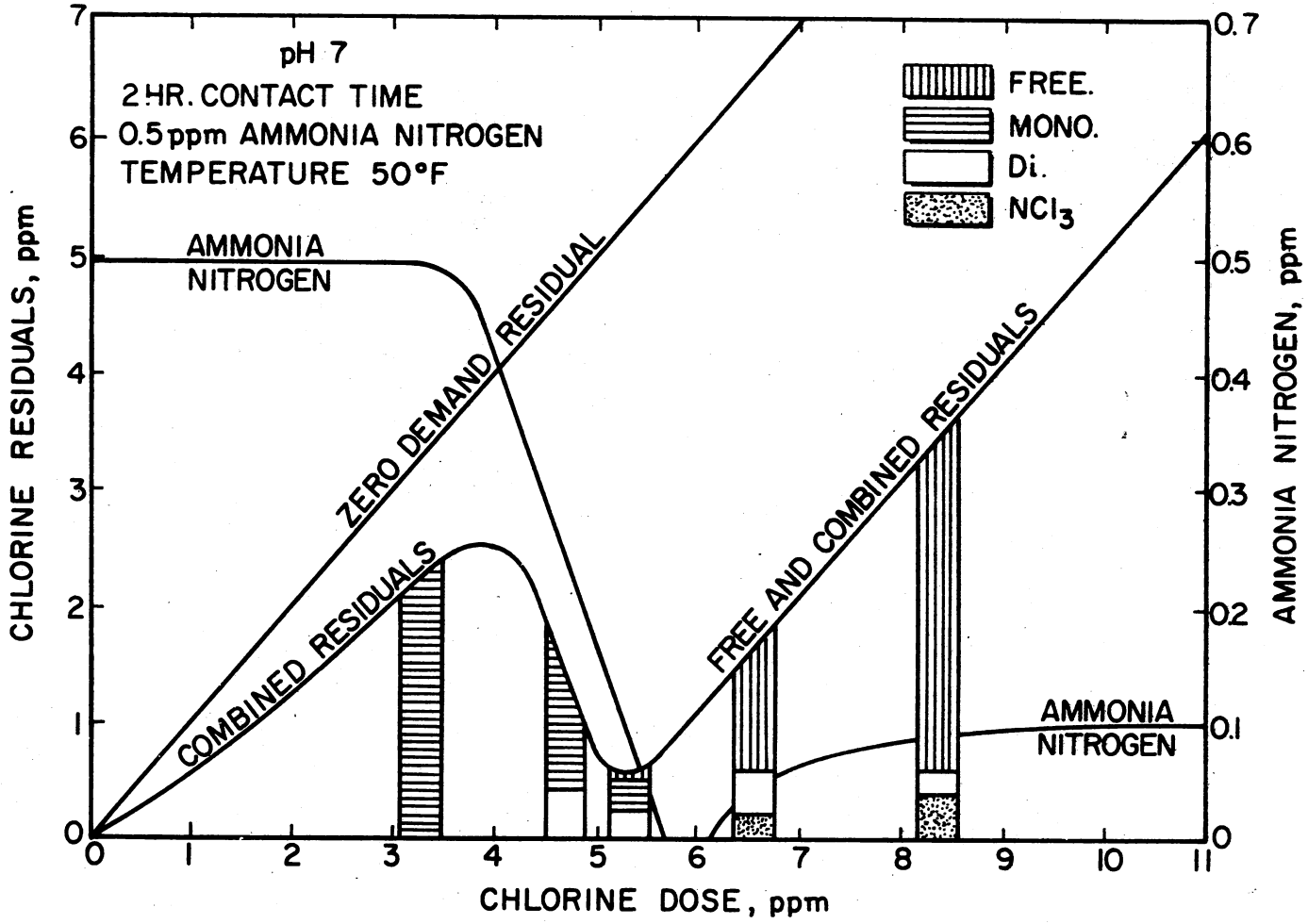


Figure 7. The Relationship of Ammonia-Nitrogen and Chlorine - the Breakpoint Phenomenon (White, 1972)

A. the disproportionation of monochloramine,

$$NH_2Cl + H_2O \rightleftarrows HOCl + NH_3, \quad [28]$$

$$NH_2Cl + HOCl \rightleftarrows NHCl_2 + H_2O, \quad [29]$$

$$\text{or, } NH_2Cl + \text{acid} \rightleftarrows (NH_2Cl\cdot\text{acid}), \quad [30]$$

$$(NH_2Cl\cdot\text{acid}) + NH_2Cl \rightleftarrows NHCl_2 + NH_3 + \text{acid}, \quad [31]$$

B. the decomposition of dichloramine,

$$NHCl_2 \rightarrow H^+ + NCl_2^-, \quad [32]$$

$$NCl_2^- \xrightarrow{(OH^-)} NCl + Cl^-, \quad [33]$$

$$NCl + OH^- \rightarrow NOH + Cl^-, \quad [34]$$

$$\text{or, } NCl_2^- + OH^- \rightarrow NCl(OH)^- + Cl^- + H^+, \quad [35]$$

$$NCl(OH)^- \rightarrow NOH + Cl^-, \quad [36]$$

and, C. the decomposition of the nitroxyl radical intermediate,

$$2NOH \rightarrow H_2N_2O_2 \rightarrow N_2O + H_2O, \quad [37]$$

$$NOH + NH_2Cl \rightarrow N_2 + H_2O + H^+ + Cl^-, \quad [38]$$

$$NOH + NHCl_2 \rightarrow N_2 + HOCl + H^+ + Cl^-, \quad [39]$$

$$NOH + 2HOCl \rightarrow NO_3^- + 3H^+ + Cl^- \ . \quad [40]$$

CHAPTER II

EXPERIMENTAL ACTIVITY

Study Area

The experiments described herein were performed in Lake Carl Blackwell, Figure 8. This lake is located in north-central Oklahoma in Payne County and is used for recreation and as a water supply for the city of Stillwater, which is 11 km east of the lake (Cole, 1975). The lake was built in 1938, and reached spillway level in 1945. It is an impoundment of Stillwater Creek; major water inflow is from water drainage of pastured grassland and wheat farmland. The lake has a surface area of approximately 15 square kilometers at spillway level, 283.2 m m.s.l.

Due to increased municipal demand and low rainfall, the surface area decreased to less than 10 square kilometers by 1961 (Norton, 1968). The lake again rose to spillway level in 1973. It has fluctuated near this level since that time. The lake stratifies in early summer, and turbidity and chemical distribution indicate the epilimnion is wind circulated (Rice, 1972).

General Experimental Procedure

Each experiment was initiated by the collection of water at a depth of approximately 60 cm. Both water collection and incubation

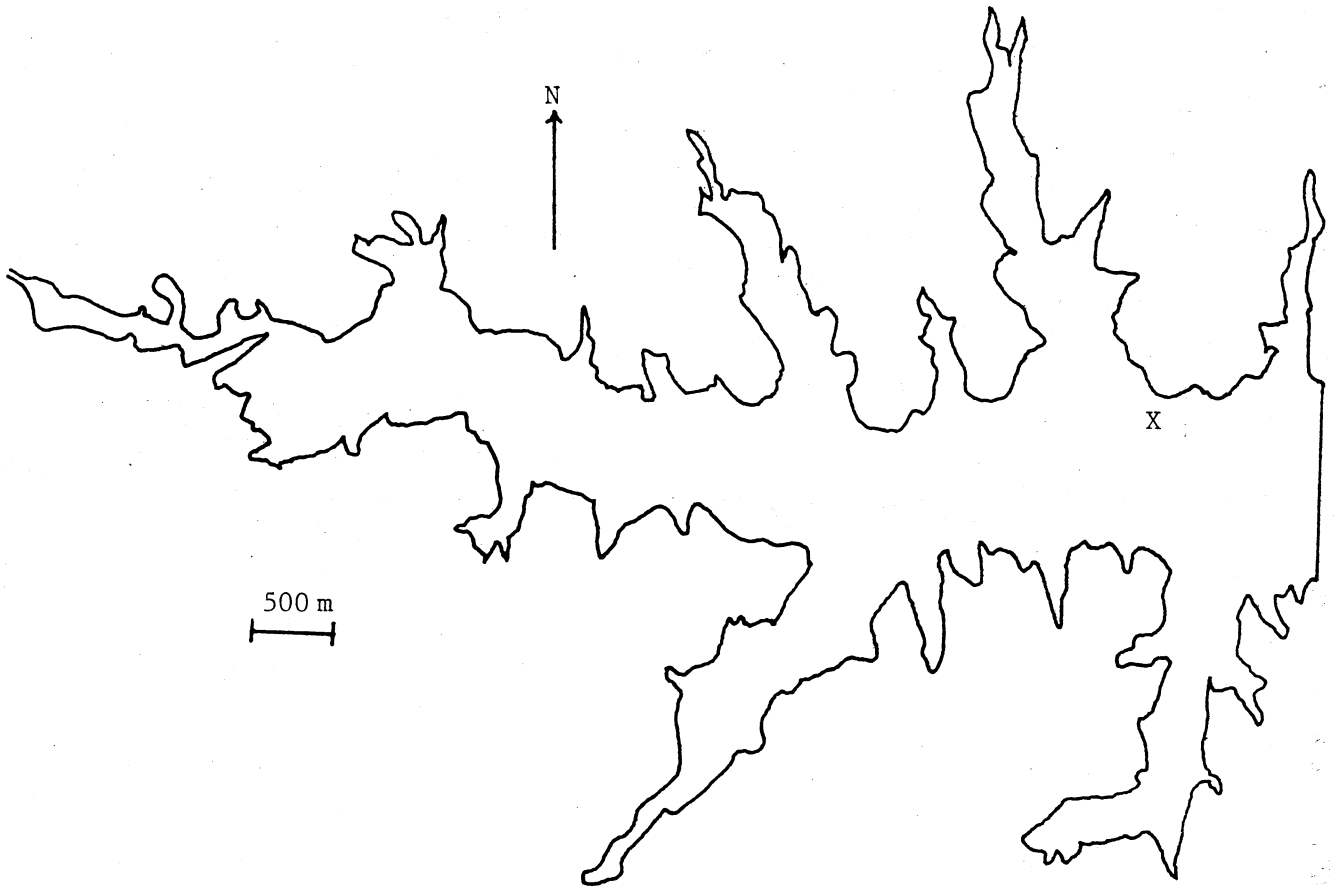


Figure 8. Shoreline Map of Lake Carl Blackwell at Spillway Level. "X" Indicates Location of Sampling Station

occurred at the station shown in Figure 8. The sample was filtered through a 0.202 mm net to remove the larger zooplankton. The sample was then transported to shore in a 20 l carboy.

Water samples were preserved and later the species and density were determined by the method of McNabb (1960). Aliquots of the lake water sample were used for the determinations of particulate nitrogen and chloraphyll a by the spectrophotometric methods of Parsons and Strickland (1963) and Holm-Hansen (1968), respectively. A third portion of water was filtered through 0.45 μ membrane filter, stored on ice, and transported to Stillwater for chemical analyses. Laboratory procedure followed the phenolhydrochlorite method of Soloranzo (1969) for the determination of ambient ammonia concentrations. The Azo method of Strickland and Parsons (1968) was used to determine ambient nitrate and nitrite concentrations. Ambient phosphate concentrations were determined using the phosphomolybdate blue method (Strickland and Parsons, 1968).

The incubation quart bottles were filled with lake water and spiked with the perturbing chemicals of interest and rigorously shaken to ensure mixing. These bottles were placed in a steel frame in linear order; a bottle of unaltered lake water was placed at each end. They were immediately transported to the incubation station and suspended from a float into the lake. A black cloth was used to cover the bottles during transportation to minimize exposure to direct sunlight. Incubation periods were approximately 24 hours in length to avoid complication of diel periodicity. Results are therefore expressed as mean rates for the period of incubation. Upon

removal of the bottles from the lake, a 10 ml portion of Lugol's solution was added to each bottle to terminate the experiment. The bottles were transported to Stillwater where their contents were filtered onto muffled Reever Angel 984 H ultra glass filters. These filters were dried and stored in a desiccator until later conversion to nitrogen gas.

Lake water temperature was monitored at the beginning and end of each experiment. A pyranometer (Weather Measure Corporation) was used to continuously monitor solar radiation during the experiment. A planimeter was used to integrate the area under the curve for the incubation interval. A submarine photometer (G. M. Manufacturing Co., Brooklyn, N. Y.) was used to obtain the extinction coefficient for the lake water as a measure of turbidity.

Conversion of the nitrogen compounds retained by the glass filters to N_2 gas followed Barsdate and Dugdale (1965). A Coleman Nitrogen Analyzer, Model 29 A, pyrolyzed the sample over a copper oxide catalyst. The gases were swept through nitrogen cold traps and pumped into pyrex breakseal ampoules. The gas was then introduced into a mass spectrometer (CEC 21-110B) for isotope analysis. Data was oscillographically recorded on strip charts, manually read, and coded for processing in an IBM 360 computer.

Experiments During 1973

From late in April, 1973, to the middle of October, 1973, a total of 27 experiments were performed on 15 experimental dates. Labeled nitrate uptake was the focus during this entire period. In general, these experiments involved six concentrations of $^{15}NO_3^-$ incubated at

the surface or at 0.8 m subsurface. However, four experiments were designed in triplicate to check the reproducibility of the final data. Between June 6 and September 9, twelve experiments were performed at approximately weekly intervals measuring the nitrate uptake rates. Incubation was accomplished by suspending the bottles from the float at just under the water surface. The goal of these experiments was to follow the change in K_s and V_{max} through the summer of the lake. Eight experiments were performed by incubation at 0.8 m subsurface. A separate study evolved from these experiments measuring the vertical variation in nitrate uptake by phytoplankton as a function of light attenuation (Cole, 1975). Finally, three experiments were designed as a pilot study to indicate the feasibility of measuring the effect of chlorine on nitrate uptake. Two of these experiments were designed to observe the effect on nitrate uptake while holding total chlorine constant and varying the nitrate concentrations. The final experiments held nitrate concentration constant at 10% above the ambient and varied the total chlorine.

Gas Collection

After analysis of the experiments and results for 1973, procedures and experimental designs were reevaluated. Combustion of the individual samples, manual switching of the Toepler pump, and sealing of the ampoules were found to be time consuming procedural steps. Manipulation of the Toepler pump was also found to be the step in which samples were frequently lost due to operator error.

Three changes were made in an attempt to improve this phase of sample processing. The vacuum system was redesigned to have a minimal

volume thus allowing a more efficient transfer of gas. A compact semiautomatic Toepler pump, see Figure 9, of the design of Urry and Urry (1956) was donated by Dr. E. M. Hodnett. A controller was built from a 4PDT latching relay (Potter and Brumfield, KB17AY) and two solenoid valves (ASCO, Ca. NO. 82602). Finally, the liquid nitrogen cold trap used for trapping carbon dioxide and water was redesigned from a tube within a tube to radiator coils. This allowed a minimal volume, maximum surface area and therefore more efficient trapping.

Mass Spectrometric Analysis

The mass spectrometric determinations of the atom percent ^{15}N were made on a CEC Model 110B high resolution instrument (single detector). Masses corresponding to $^{14}N_2$ and ^{14}N-^{15}N were automatically and alternately scanned. The signal from the electron multiplier was fed through five galvanometers and oscillographically recorded as five lines (peaks on a photosensitive strip chart). The galvanometers were calibrated with a reference voltage and five precision resistors to correspond to amplifications of 1, 3, 10, 30 and 100 to within one percent.

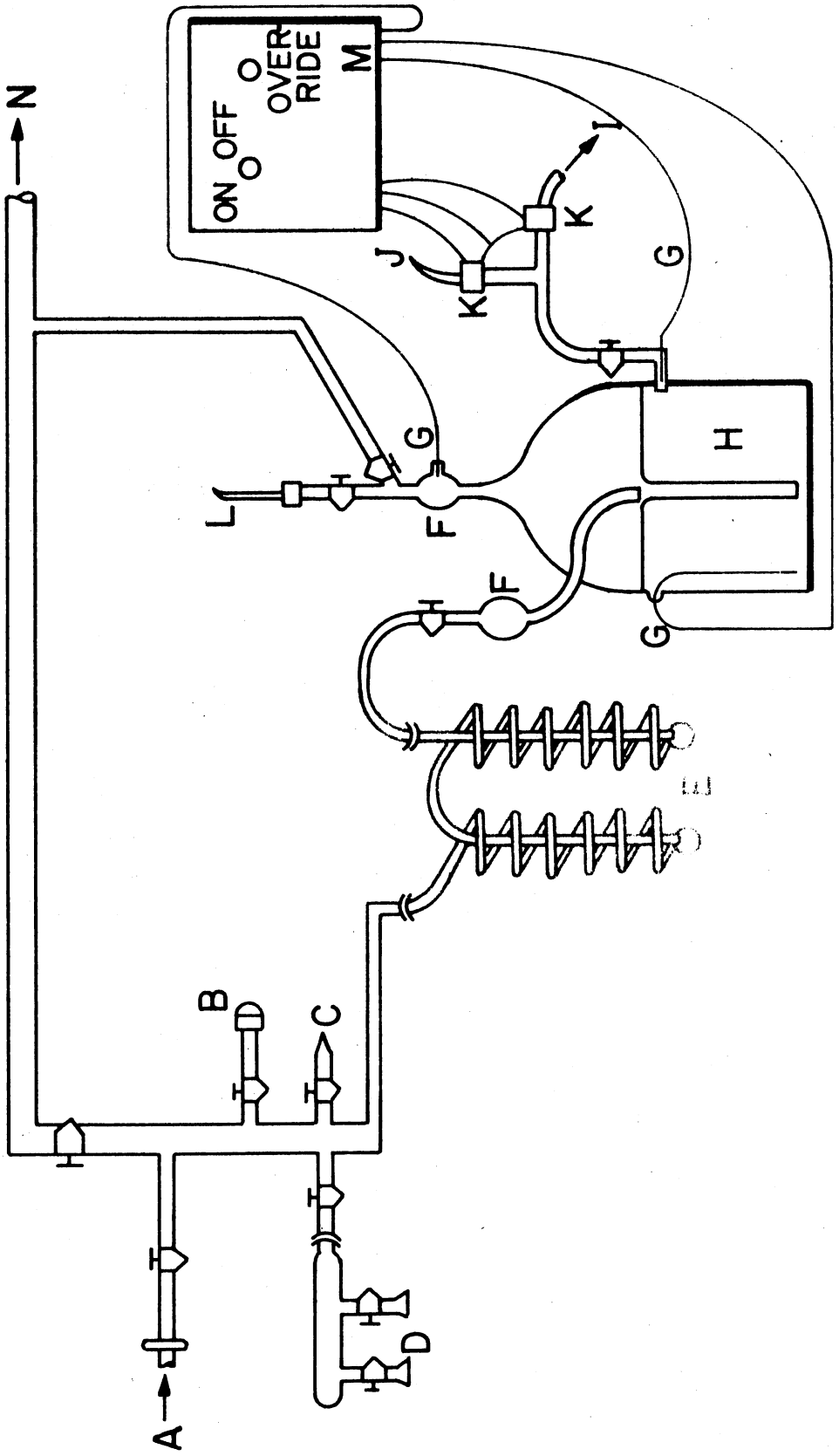
The atom percent ^{15}N in a sample of nitrogen gas is given by Equation 41:

$$\text{atom \% } ^{15}N = \frac{(^{14}N^{15}N) + 2(^{15}N_2)}{2((^{14}N_2) + (^{14}N^{15}N) + (^{15}N_2))} \times 100. \qquad [41]$$

The equilibrium constant for the reaction

$$^{14}N_2 + {}^{15}N_2 = 2\,^{14}N^{15}N \qquad [42]$$

Figure 9. The Vacuum System Used for the Collection of Nitrogen Gas. A. Coleman Analyzer; B. Thermal Couple of the Hastings Pressure Gauge; C. Vent to Atmosphere; D. Hyprobromite Conversion Manifold; E. Two Liquid Nitrogen Radiator Coil Traps; F. Mercury Float Valves; G. Mercury Contact Wires; H. Toepler Pump; I. Rough Pump; J. Vent to Atmosphere; K. Solenoid Valves; L. Breakseal Ampoule; M. Toepler Controller; N. Oil Diffusion Pump


N
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A

is 4.000 at 25°C and is given by Equation 43 (Beynon, 1960):

$$K = \frac{(^{14}N^{15}N)^2}{(^{14}N_2)\ (^{15}N_2)} \quad . \qquad [43]$$

Solving Equation 43 for $^{15}N_2$ and substituting into Equation 41 yields Equation 44, from which the ^{15}N abundance can be calculated (Bremner, 1965).

$$\text{Atom } \%^{15}N = \frac{100}{2R + 1} \quad , \qquad [44]$$

where R is the ratio of the ion current at m/e 28 to that at m/e 29. This equation is advantageous when the ion currents at m/e 28 and 29 are much larger than that of 30 or when dual detectors are used. In the latter case R can be obtained directly from a voltage divider.

In this investigation the atom percents ^{15}N for all samples were below 40% and typically 0.5 to 10%. Thus, the oscillographic traces were separated by one or more of the stated amplifications. The error of manually determining the peak heights was responsible for a much larger error in the signal from the m/e 29 than that of m/e 28. The total error in the atom percent ^{15}N as a function of errors in the peak heights can be found by the derivative of Equation 44, which is Equation 45.

$$d(\text{atom } \%\ ^{15}N) = 200(2xy^{-1} + 1)^{-2}\ (x\ dy\ y^{-2} - dx\ y^{-1}), \qquad [45]$$

Table III was constructed to show the error in measuring P_{29} at different amplifications as a function of the error in the atom percent ^{15}N at natural abundance. It was assumed that $P_{28} = 150$ mm on the galvanometer with an amplification of unity and no error existed in measuring P_{28}.

TABLE III

THE ABSOLUTE ERROR IN THE NATURAL ABUNDANCE OF ^{15}N AS A FUNCTION OF THE ERRORS IN MEASURING THE PEAK HEIGHTS OF m/e 29 IN MILLIMETERS

Absolute Error in Atom % ^{15}N	Amplification				
	1	3	10	30	100
1.0				9.1	3.0
0.5		>10.0	>10.0	4.5	1.5
0.1		9.1	3.0	0.9	0.3
0.05	>10.0	4.5	1.5	0.5	0.2
0.1	3.0	0.9	0.3	0.1	<0.1
0 005	1.5	0.5	0.2	<0.1	
0.001	0.3	0.1	<0.1		

Table IV was constructed to show the effect of a constant error in measuring P_{29} at different levels of enrichment. The following assumptions were made in calculating the values in this Table: P_{28} = 150 mm on the galvanometer with an amplification of unity, the amplification of P_{29} and the sample enrichment were such that P_{29} = 110.2 mm on its resulting galvanometer, and the error in measuring P_{29} = 1 mm. This table shows that as the enrichment of ^{15}N increases, the percent relative error in the atom percent ^{15}N, decreases as a result of the constant error in reading the P_{29}.

TABLE IV

THE ABSOLUTE AND RELATIVE ERROR AT VARIOUS ENRICHMENT LEVELS OF ^{15}N AS A FUNCTION OF A CONSTANT ERROR (1 mm) IN MEASURING THE PEAK HEIGHT OF m/e 29

Enrichment Level in Atom % ^{15}N	Absolute Error in Atom % ^{15}N	% Relative Error in Atom % ^{15}N
0.366	0.003	0.90
1.090	0.01	0.90
3.543	0.03	0.88
9.926	0.08	0.82
26.865	0.18	0.66

These Tables emphasize the importance of accurately determining the peak height corresponding to the m/e 29. This was done when the sample size was maximized and a "practical" maximum amount of the sample was used in the analysis.

All of a sample could not be used in the analysis. A maximum amount of sample was present in the ion source shortly after sample introduction; from that moment, the sample amount (and therefore the m/e 28 and 29 signals) was decreasing. A finite amount of time was required to scan each mass of interest such that when m/e 28 is scanned the amount of $^{14}N_2$ in the ion source was not the same as when the previous or next scan of m/e 29 was made. Two techniques are

commonly used to compensate for this. A very small leak line between the sample and ion source allows the change of sample amount to be smaller than other errors in the analysis. Therefore, no correction or time averaging need be considered; however, many scans of each mass (1,000 or more) are necessary to use an appreciable amount of the sample in the analysis. The second method involves analysis of a large amount of sample by use of a larger leak line and requires a correction or time average to compensate for the decrease in the sample pressure of the ion source. This method requires that the leak rate be linear or known.

A measure of the leak rate of the capillary used in our line was made. A typical sample was loaded and introduced into the mass spectrometer. The pressure of the ion source was found to decrease only a few percent during a one hour period. Several capillaries having approximately ten times the previous leak rate were made and installed. The new leak was found to give much greater signals while the signals decreased only a few percent during each analysis.

Until this time, three scans of m/e 28 and 29 were taken for analysis. After modification of the leak rate, procedure was modified to include time averaging: i.e. six scans of m/e 29 and five scans of m/e 28 were collected for analysis.

The use of larger amounts of sample allowed a reduction in the applied voltage per stage of the electron multiplier. This gave the further advantageous result of decreasing the noise per unit deflection on the strip chart. Tank nitrogen samples were processed at this new and higher range of ion source pressures to test for the possibility of "space charging" occurring in the

ion source. No mass discrimination as a function of source pressure was detected.

Mass Spectrometer Output - Electronic Filtration

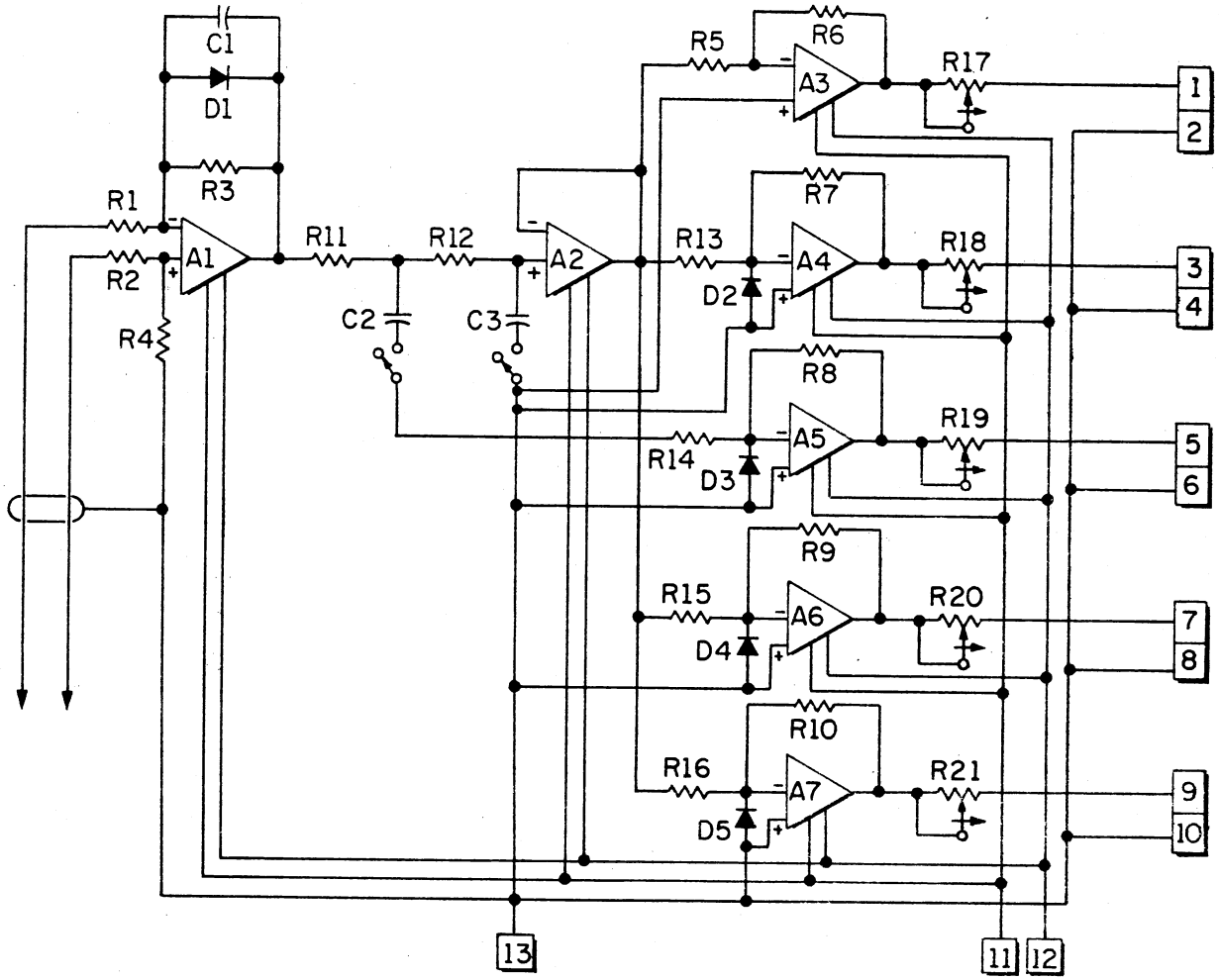
The CEC Model 110B instrument was originally designed without the unamplified galvanometer signal having been filtered. Four of the galvanometers received signals which had passed through two RC filters in series, and four separate voltage following operational amplifiers with the previous stated gains of 3, 10, 30, and 100.

In determining the atom percent ^{15}N it was found that the best results could be obtained when the signal corresponding to m/e 29 was maximized. This made use of the galvanometer with amplification of unity highly desirable for measurement of the signal corresponding to m/e 28. The unfiltered signal had a very low signal to noise ratio as compared to the filtered and amplified signals. It was therefore decided to modify the circuit such that all the galvanometers would receive signals which had been filtered.

The output of the operational amplifier, A2, was directly fed into the appropriate galvanometer. This resulted in a very large current load with irreproducible atom percent ^{15}N data as a function of ion source pressure.

Figure 10 is a schematic of the final circuit modification. The signal from A2 was fed into a voltage following operational amplifier, A3, set at a gain of one with 30 K ohms resistors. This gave sufficient input impedance to the corresponding galvanometer.

Figure 10. Circuit Diagram of the Filtering and Amplifying Network. All Resistors are 1% and 1/2 W: R1 Through R8 = 30 K, R9 Through R10 = 60.4 K, R11 = 3.9 K, R12 = 12 K, R13 = 9.090 K, R14 = 3K, R15 = 1.8 K, R16 = 604, and R17 Through R21 = 1K. Diodes: D1 = 1N457A, D2 Through D5 = 1N2070. Capacitors: C1 = 0.005 MFD 600 V, C2 = 1.5 MFD 200 V, and C3 = 0.47 MFD 100 V. Operational Amplifiers: A1 Through A7 are CEC Part Number C-142110. 1-2, 3-4, 5-6, 7-8, and 9-10 = Outputs. 11 = D.C. Biasing Voltage at +15 V. 12 = D.C. Biasing Voltage at -15 V. 13 = Ground.


C1
D1
R3
R1
R2
R4
A1
R11
R12
A2
C2
C3
R5
R6
A3
R17
R7
R13
A4
R18
D2
R8
R14
A5
R19
D3
R9
R15
A6
R20
D4
R10
R16
A7
R21
D5
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Calculation of Uptake Rate and Error Propagation

The flux of nitrogen which is taken into phytoplankton, N_f, from its surroundings is given by

$$N_f = v_s \cdot N_p, \qquad [46]$$

where v_s is the uptake rate of the substrate, and N_p is a measure of the planktonic biomass per unit volume (in this case chosen to be particulate nitrogen).

$$v_s = \frac{N_f}{N_p} = \frac{A_f}{A_i} 100, \qquad [47]$$

where A_i is the atom percent excess ^{15}N in the enriched substrate supplied at the beginning of the experiment, and A_f is the atom percent excess ^{15}N in the particulate nitrogen.

A_f, the Atom Percent ^{15}N in the Sample

Equation 48 was used to calculate the atom percent ^{15}N in the sample:

$$\text{Atom \% } ^{15}N = \frac{100}{2R + 1} . \qquad [48]$$

A measure of the error in this quantity, its variance, can be directly found from the variance of the signal intensities, I, corresponding to m/e 28 and 29:

$$\sigma_M^2 = \frac{\sum_i (I_M - \bar{I}_M)^2}{N - 1} , \qquad [49]$$

where M corresponds to m/e 28 or 29, N is the number of scans of each m/e (three for both m/e 28 and 29 in 1973 and five for m/e 28 and six for m/e 29 after 1973), and $\bar{I}$ is the average signal intensity.

$$\sigma_R^2 = R^2(\sigma_{28}^2/I_{28}^2 + \sigma_{29}^2/I_{29}^2) \ . \qquad [50]$$

Finally,

$$\sigma^2_{\text{Atom \% } ^{15}N} = (-200/(2R + 1)^2)^2\sigma_R^2 \ . \qquad [51]$$

The atom percent excess ^{15}N, A_f, is found by subtracting the blank values from the sample values:

$$A_f = \text{Atom \% } ^{15}N_{\text{sample}} - \text{Atom \% } ^{15}N_{\text{blank}} \ . \qquad [52]$$

Similarly, an estimate of the error in A_f is given by

$$\sigma^2_{A_f} = (dA_f/d\text{Atom \% } ^{15}N_{\text{sample}})^2\sigma^2_{\text{sample}} + (dA_f/d\text{Atom \% } ^{15}N_{\text{blank}})^2\sigma^2_{\text{blank}}. \qquad [53]$$

Thus, $\sigma^2_{A_f}$ was an estimate of the error attributed to the mass spectrometric analysis of each uptake rate at the corresponding substrate level.

A_i, the Atom Percent ^{15}N in the Solution

The preparation and addition of a solution of ^{15}N labeled $NaNO_3$ or NH_4Cl and the determination of the ambient concentration of substrate were required to determine the percent excess ^{15}N in the incubation solutions at the onset of the experiments, A_i. The salts used to prepare the solutions were obtained from Isomet Corporation, Palisades Park, New Jersey with their atom percent ^{15}N given; no

error was assigned to these values or to the weighing procedure. Increasingly rigorous procedures can be used to minimize the errors mentioned above such that their contribution to the experiment would be relatively insignificant. However, the determination of the ambient concentration of substrate does contain errors which were included in the analyses.

Spectrophotometric methods were chosen for these determinations and Beer's Law type calibration curves were constructed by dilution. A least squares analysis of these dilutions gave a straight line function:

$$A = aC + b, \qquad [54]$$

where A is the measured absorbance, a and b are constants (The curve deviated from Beer's Law since it does not intersect the origin, due to the reagent blanks.) and C, the concentration, is the number of millimeters of stock solution. Based on photometric theory, Equation 55 gives an estimate of the standard error of the absorbance, σ_A, as a function of the photometric error, ΔT (approximately 0.4 for the Beckman DU), and measured absorbance, A.

$$\sigma_A = (0.43429)\ (\Delta T)\ (\text{antilog } A) \qquad [55]$$

It follows from Equation 54 that

$$C = \frac{A - b}{a} \qquad [56]$$

and

$$\sigma_C^2 = (dC/da)^2\sigma_a^2 + (dC/dA)^2\sigma_A^2 + (dC/db)^2\sigma_b^2 \ . \qquad [57]$$

Furthermore,

$$dC/da = -(a - b)/a^2 \quad . \qquad [58]$$

$$dC/dA = 1/a, \qquad [59]$$

and,

$$dC/db = -1/a \quad . \qquad [60]$$

By substituting Equations 58, 59 and 60 into Equation 56, one obtains Equation 61:

$$\sigma_C^2 = \frac{a^2\sigma_A^2 + a^2\sigma_b^2 + (A^2 - 2Ab + b^2)\sigma_a^2}{a^4} \quad . \qquad [61]$$

Thus σ_C^2 is an estimate of the error in the substrate concentration when C ml of ^{15}N solution are added to the lake water; the variance of A_i was readily calculated by Equation 62:

$$\sigma_{A_i}^2 = \frac{A_i}{\text{Total N}} \sigma_c^2 \quad . \qquad [62]$$

The total error of the uptake rate, σ_v^2, was therefore viewed as the multiplicative sum of the relative errors:

$$\sigma_{V_s}^2 = V^2((\sigma_{A_f}^2/A_f^2) + (\sigma_{A_i}^2/A_i^2)). \qquad [63]$$

In this manner the contributions to the error from the two major phases of each experiment were directly compared. In 1973, approximately 90% of the relative error was attributed to the mass

spectrometric analysis. This emphasized the need to improve the mass spectrometric analyses.

Appendix B is a listing of the computer program which was used to calculate the Michaelis-Menten constants as well as the accompanying error propogation.

Experiments During 1974

The preliminary investigation of the effect of chlorine on nitrate uptake rate was successful and the work in 1974 was therefore focused on the effect of chlorine and chloramine on nitrate and ammonia uptake. A total of 17 experiments were performed in 1974. Three $^{15}NH_4^+$ and three $^{15}NO_3^-$ uptake experiments were used for comparison to chlorine perturbed experiments which included the following designs: variable $^{15}NO_3^-$ with constant free chlorine, variable $^{15}NO_3^-$ with constant chloramine, variable $^{15}NH_4^+$ with constant total chlorine, and constant $^{15}NH_4^+$ with variable total chlorine.

Realizing that additions of free chlorine to different solutions would be followed by the dynamic equilibria previously discussed, one other type of experimental design was implemented. Experiments of this design were termed "chlorine block experiments". A constant amount of $^{15}NO_3^-$ was added to variable amounts of ammonia and free chlorine. Concentrations were kept below breakpoint stoichiometry. In this manner, a surface of $V_{NO_3^-}$ vs free chlorine and bound chlorine was generated from which the trends of the two independent variables could be analyzed.

Experiments During 1975

Results of the chlorine block experiment of 1974 were to a small extent unpredicted. This was hypothesized to be due to the effect of the breakpoint phenomenon. Although the experiment was designed to be below literature values of breakpoint stoichiometry, the observed trends were believed to be a result of the breakpoint reactions. A hypothetical surface bracketing the breakpoint was therefore predicted and tested during 1975.

During the previous years it was noted that diatoms constituted a major portion of the population densities of Lake Carl Blackwell. The literature (Lewin, 1966; Azam, Hemmingsen, and Volcani, 1974; Azam and Volcani, 1974) indicates that at high Ge/Si ratios, germanium competitively inhibits silicon uptake and diatom growth. It was hoped that germanium might also decrease the uptake of nitrate and/or ammonia by diatoms and in so doing minimize their contribution to the overall observed nitrate and ammonia uptake rates. Two experiments were therefore designed and performed in an effort to test the above hypothesis.

A 4 x 4 matrix design was also used in an attempt to investigate the effect of orthophosphate and pH on $V_{NO_3^-}$ during this year.

CHAPTER III

RESULTS AND DISCUSSION

Experiments During 1973

The overall objective, for 1973, was to determine the concentration of nitrogen nutrients, light, temperature, and K_s and V_{max} for nitrate uptake by the phytoplankton. Table V shows temperature and irradiance data; Table VI shows the concentrations of the three nitrogen nutrients as well as the "standing crop" of the phytoplankton in terms of chlorophyll _a_ and particulate nitrogen. Greater consistency and a more complete data set of values of K_s and V_{max} was obtained for experiments incubated at 0.8 m in contrast to those incubated at the surface. These values and their standard deviations calculated by a linear regression analysis of the Woolf Plot are shown in Table VII.

Enumeration of each calculation for the values shown in Table VII would be redundant. Therefore, a representative experiment, June 6, was chosen and a summary of the computer calculations are shown in the following figure and tables. Figure 11 is the Michaelis-Menten plot of nine points of $V_{NO_3^-}$ vs total substrate concentration. Other than some typical scatter which often occurs at low substrate concentrations, the plot shows the normal hyperbolic enzyme kinetic response. Table VIII shows the results of calculations of the atom

TABLE V

TEMPERATURE AND IRRADIANCE DATA IN LAKE CARL BLACKWELL DURING 1973

	Surface			0.8 Meters		
	Average	Temperature °C		Average	Temperature °C	
Date	ly/min	Start	End	ly/min	Start	End
6/6	0.6815	22.4	---	0.1322	22.4	---
6/15	0.4953	26.5	26.8	0.0462	26.5	26.8
6/20	0.5852	25.4	26.0	0.586	25.1	26.0
6/27	0.7370	----	27.0	0.1513	----	27.0
7/5	0.6829	28.0	29.2	0.0950	27.8	28.8
7/12	0.5323	28.6	29.2	0.0600	29.0	28.9
7/19	0.6757	28.0	28.6	0.1692	27.9	28.3
7/26	0.7262	29.2	31.2	0.1747	29.0	30.2
8/1	0.6103	28.0	29.0	0.0677	28.0	28.2
8/23	0.4202	27.5	29.2	0.0286	27.5	28.9
8/30	0.3774	27.5	27.5	0.0229	27.5	27.0
9/6	0.1879	24.8	25.0	0.0031	24.8	25.0
9/28	0.3375	23.0	23.0	------	----	----

TABLE VI

THE CONCENTRATIONS OF NITRATE, NITRITE, AMMONIA, CHLOROPHYLL *a*, AND PARTICULATE NITROGEN FOUND IN LAKE CARL BLACKWELL DURING 1973

Date	µg/l				Particulate Nitrogen µmoles/l	
	NO_3^--N	NO_2^--N	NH_3-N	Chlorophyll *a*	Surface	0.8 M
4/26	4.17	---	---	14.10	---	---
6/6	29.07	0.00	7.03	5.01	5.95	---
6/15	7.83	0.26	5.27	14.71	7.92	---
6/20	14.45	0.34	0.66	13.83	8.57	---
6/27	8.59	0.11	3.71	17.87	8.69	8.69
7/5	4.18	0.46	2.88	14.69	7.50	7.50
7/12	3.58	0.28	2.71	20.55	9.90	9.90
7/19	1.29	0.01	1.01	11.69	10.21	10.21
7/26	0.95	0.11	2.33	8.69	10.15	10.15
8/1	2.68	0.17	2.37	15.77	10.22	10.22
8/23	0.99	0.06	---	6.02	7.44	---
8/30	3.89	0.00	7.04	7.23	7.94	7.94
9/6	4.83	0.83	3.33	9.70	---	8.15
9/28	2.12	0.00	3.33	9.44	---	---

TABLE VII

EXPERIMENTALLY DERIVED VALUES OF V_{max} AND K_s AND THEIR STANDARD DEVIATIONS FOR NITRATE UPTAKE AT 0.8 METERS DURING 1973

Date	V_{max} ($\pm \sigma_{V_{max}}$) (x 10^3 $hour^{-1}$)	K_s ($\pm \sigma_{K_s}$) (µg at N/1)
4/26*	3.0	642
6/6*	5.68 (± 1.06)	89.9 (± 31.0)
6/27	7.28 (± 0.23)	3.27 (± 0.92)
6/27 with 0.101 mg Cl_2/1	5.47 (± 0.94)	32.6 (± 14.6)
7/5	3.88 (± 0.72)	12.0 (± 9.1)
7/5 with 0.013 mg Cl_2/1	6.24 (± 1.25)	24.7 (± 14.2)
7/12	10.2 (± 1.2)	41.6 (± 13.5)
7/19	7.49 (± 1.93)	24.3 (± 13.1)
7/26	10.5 (± 3.8)	30.2 (± 17.5)
8/1	10.6 (± 1.7)	10.2 (± 5.1)
8.23	2.83 (± 0.84)	2.77 (± 2.64)
8/30	4.85 (± 1.16)	27.1 (± 15.2)
9/6	0.577 (± 0.139)	14.5 (± 8.1)

*Samples were incubated at the surface only on these dates.

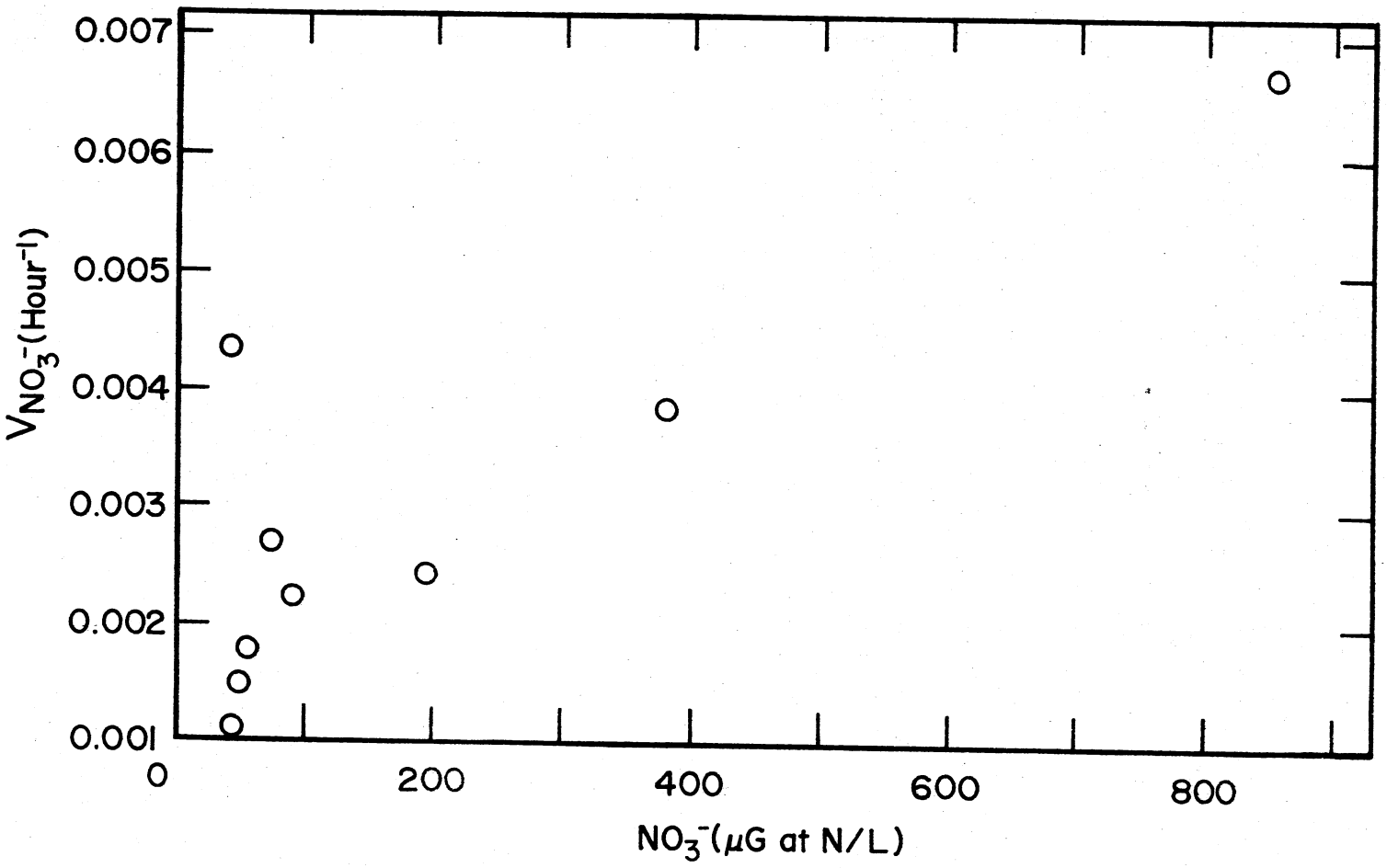

Figure 11. The Michaelis-Menten Plot of $V_{NO_3^-}$ vs. Nitrate Concentration for the Experiment on June 6, 1973

TABLE VIII

THE ATOM PERCENT ^{15}N AND THEIR VARIANCE BASED ON PEAK HEIGHT MEASUREMENT FOR CONTROLS AND ENRICHED SAMPLES FOR THE EXPERIMENT ON JUNE 6, 1973

SAMPLE	R	AT. % ^{15}N	$\sigma_{AT.\ \%\ ^{15}N}$
Control 0944	133.7839	0.3723	0.0073
Control 0946	129.3284	0.3851	0.0105
Control 0948	120.4134	0.4135	0.0063
Control 0949	122.2464	0.4073	0.0110
Averages	126.4429	0.3946	0.0147
Experiment 0156	102.9866	0.4832	0.0394
Experiment 0157	106.6247	0.4667	0.0088
Experiment 0158	99.2676	0.5012	0.0185
Experiment 0159	70.5785	0.7034	0.0064
Experiment 0163	29.3831	1.6732	0.0377
Experiment 0164	27.3554	1.7950	0.0109
Experiment 0165	13.7335	3.5129	0.1289
Experiment 0167	7.9436	5.9216	0.0387

percent ^{15}N and its variance based on the measurement of the peak heights of the mass spectrometric oscillographic traces for both controls and enriched samples. Table IX shows the results of computer calculations of A_f, $\sigma^2_{A_f}$, A_i, and $\sigma^2_{A_i}$. Finally, Table X shows the results of calculations of relative A_f, relative A_i, $V_{NO_3^-}$, $\sigma_{V_{NO_3^-}}$, V_{max}, $\sigma_{V_{max}}$, K_s, and σ_{K_s}. Note that the principle error in determining $V_{NO_3^-}$ was found to be in the relative A_f rather than the relative A_i in all but one experimental point, #0164.

1973 was not a typical year for algal behavior in Lake Carl Blackwell. Figure 12 shows the changes in the lake level during 1973. This "flood year" prevented the normal algal bloom and therefore its accompanying decrease in nitrate concentration. The nutrient conditions from mid-March throughout the summer and into the fall during this year were more typical of a new lake. Consequentially the normal competition and seasonal succession of algal species may not have occurred.

Three experiments were performed during 1973 in which replicate spikes of $^{15}NO_3^-$ were added to identical incubation bottles. Figure 13 shows the results of one such experiment performed on September 28. The scatter of points within replicates was 5.8% for the first three points, 4.3% for the second three points (two points are superimposed), and 9.2% for the last two points. In this particular experiment Michaelis-Menten behavior was not observed. Rather inhibition occurred such that values for V_{max} and K_s could not be calculated. Similar behavior also occurred during the experiments of June 15 and June 20. Although the reason for this inhibition is unknown, the most plausible explanation of this behavior might include contamination of the spike

TABLE IX

COMPUTER CALCULATIONS OF A_f, $\sigma^2_{A_f}$, A_i, AND $\sigma^2_{A_i}$ FOR THE EXPERIMENT ON JUNE 6, 1973

Experiment Number	A_f	$\sigma^2_{A_f}$	A_i	$\sigma^2_{A_i}$
0156	0.00958	2.068×10^{-5}	2.23	0.0133
0157	0.00780	0.345×10^{-5}	5.38	0.0728
0158	0.0115	0.654×10^{-5}	10.21	0.2357
0159	0.0334	0.302×10^{-5}	18.51	0.6371
0163	0.138	1.912×10^{-5}	52.99	1.7098
0164	0.151	0.393×10^{-5}	69.06	1.2335
0166	0.199	2.459×10^{-5}	84.43	0.4408
0165	0.337	19.601×10^{-5}	91.19	0.1500
0167	0.598	2.006×10^{-5}	95.80	0.0292

TABLE X

COMPUTER CALCULATIONS OF THE RELATIVE A_f, RELATIVE A_i, $V_{NO_3^-}$, $\sigma_{V_{NO_3}}$, V_{max}, $\sigma_{V_{max}}$, K_s, AND σ_{K_s} FOR THE EXPERIMENT ON JUNE 6, 1973

Experiment Number	Relative $A_f \times 10^2$	Relative $A_i \times 10^2$	$V_{NO_3^-} \times 10^3$	$\sigma_{V_{NO_3^-}} \times 10^3$
0156	47.5	5.18	4.30	2.055
0157	23.8	5.01	1.45	0.352
0158	22.2	4.76	1.13	0.256
0159	5.21	2.31	1.80	0.122
0163	3.16	2.47	2.61	0.105
0164	1.31	1.61	2.19	0.045
0166	2.49	0.786	2.36	0.062
0165	4.15	0.425	3.70	0.154
0167	0.750	0.179	6.24	0.048

$V_{max} = 5.69 \times 10^{-3}$ $\quad$ $\sigma_{V_{max}} = 1.07 \times 10^{-3}$

$K_s = 89.9$ $\quad$ $\sigma_{K_s} = 31.0$

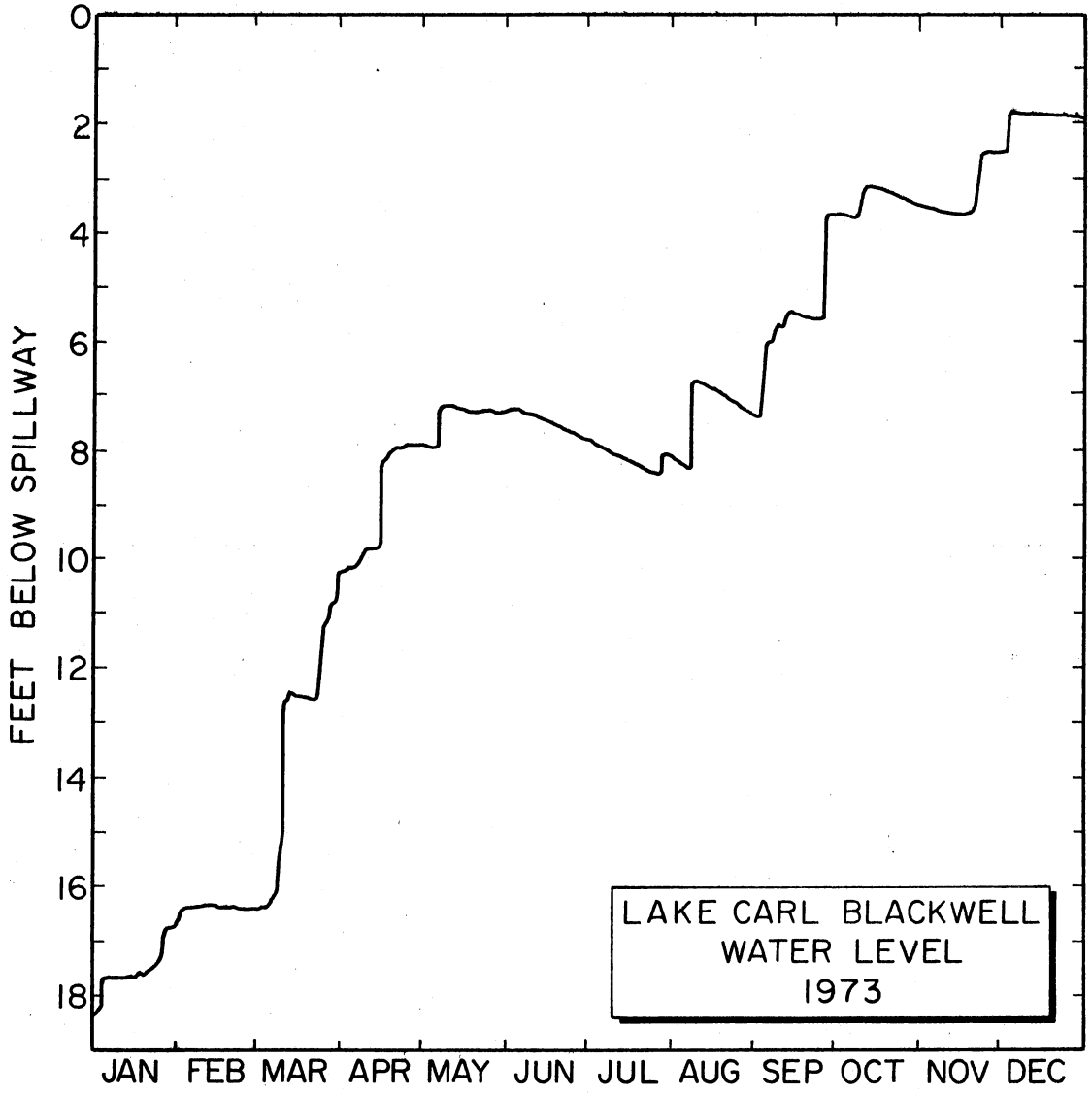

Figure 12. A Measure of the Water Level of Lake Carl Blackwell During 1973 (Varga and Toetz, 1974)

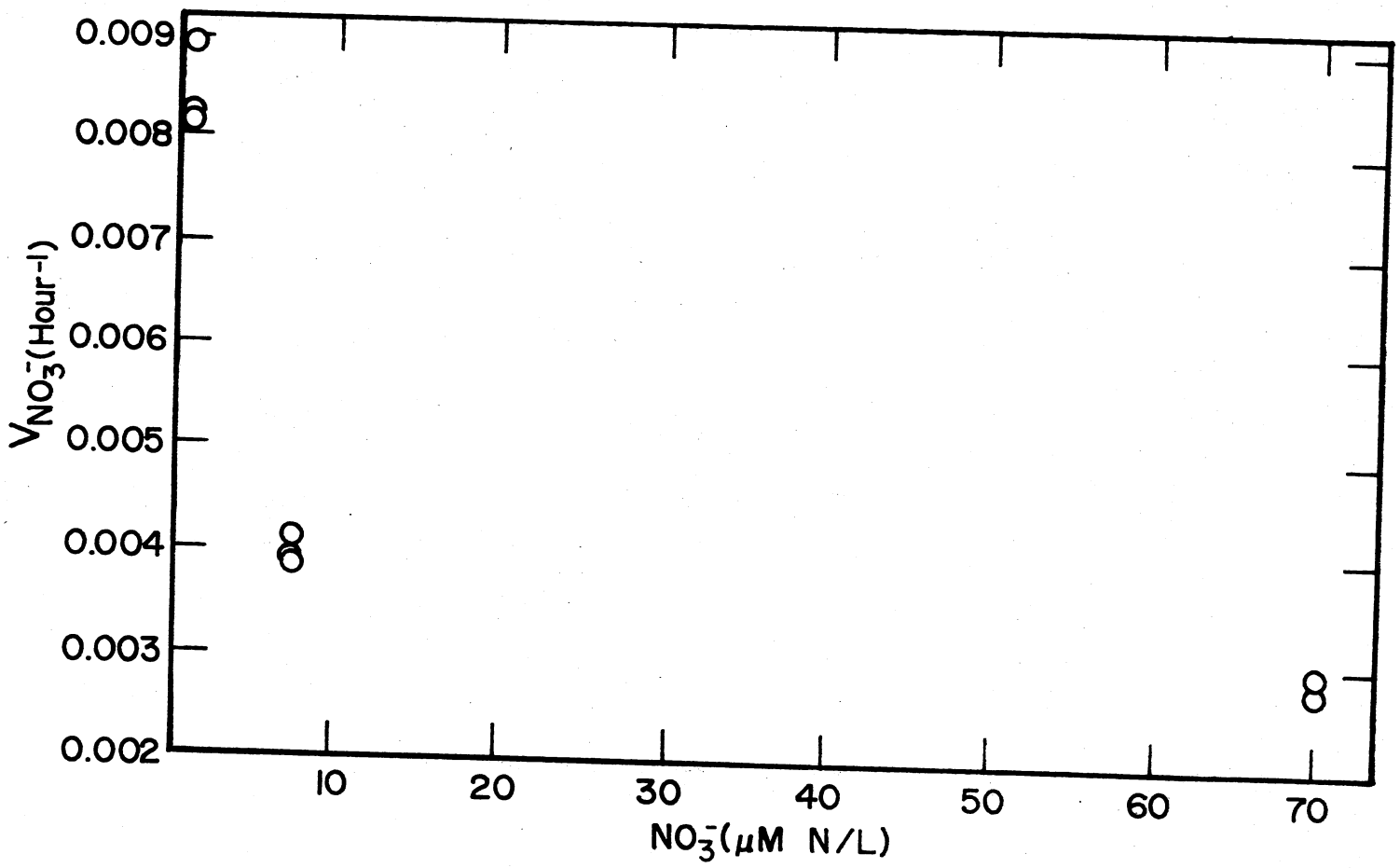

Figure 13. A Plot of $V_{NO_3^-}$ vs. Nitrate Concentration for Replicate Samples for the Experiment on September 29, 1973

with ammonia or other substances. Despite the inhibition, the variation between replicates was felt to be acceptable.

The effect of additions of a constant amount of chlorine to each incubation bottle had a dynamic result on K_s as can be seen in Table VII. Additions of 0.101 mg Cl_2/1 increased K_s tenfold on June 27. On July 5, additions of 0.013 mg Cl_2/1 doubled the value of K_s.

These results were rather surprising in view of the sparse literature values of the effect of chlorine on phytoplankton. For example, Brook and Baker (1972) have reported that a concentration of 0.320 mg Cl_2/1 depressed respiration and photosynthesis of a mixed population of freshwater algae by 50% during chlorination in a power plant. The combined effects of thermal and mechanical shock plus exposure to the toxicant should be greater than that of the toxicant alone.

Further effect of chlorine was demonstrated on July 12, by incubation at a constant $^{15}NO_3^-$ level 10% of ambient substrate concentration. Chlorine was added in concentrations varying from 0.01 to 3.00 mg Cl_2/1 resulting in an LC-50 value of 0.028 mg Cl_2/1 (Varga and Toetz, 1974).

A One Compartment Model

The simplest model possible for nitrogen uptake is specified by Equation 64:

$$\frac{dN_c}{dt} = (V_{NO_3^-})N_c = \frac{V_{max}S}{K_s + S}, \qquad [64]$$

where dN_c/dt is the rate of change (increase) or flux of phytoplankton or cellular nitrogen, N_c. Thus the nitrogen flux is a constant fraction of the instantaneous cell nitrogen level (Dugdale and Goering, 1967). From experimental values of V_{max}, K_s, and S as function of time, the value of N_c, the concentration of living nitrogen, can be calculated for any moment in time. This was accomplished by simple integration of Equation 64 using IBM's software "Continuous Systems Modeling Program (CSMP)" (IBM, 1968). This model assumed negligible grazing and sinking of algae during the time period in question. These assumptions were probably not valid, even during a flood year such as 1973.

The experimental value of V_{max} for nitrate incorporated the effects of the ambient values of the forcing parameters, light flux and temperature, and the perturbation parameter, ammonia concentration. The specific effects of these parameters must be known before a corrected maximum uptake rate, V_c, can be obtained.

The one-compartment model, which incorporates the effects of light flux, temperature, and ammonia concentration, may be written

$$dN_c/dt = \frac{V_c S}{K_s + S} \frac{R_j Q_3}{F} N_c \qquad [65]$$

so that

$$V_c = \frac{V_{max} F}{R_j Q_3} \quad . \qquad [66]$$

R_j is a function of the experimental light flux which gives the decrease in nitrogen uptake due to non-optimum light conditions (Di Toro, Connor, and Thomson, 1971).

$$Q_3 = 2\exp\left(\frac{T - 20}{10}\right) , \qquad [67]$$

which gives a temperature factor of 2 at 20°C.

$$F = 2.21 - 62.03\ (NH_3) + 724.2\ (NH_3)^2 - 2896\ (NH_3)^3 , \qquad [68]$$

which is a relation determined empirically (Prochazkova, Blazk, and Kralova, 1970) to represent the effect on nitrate uptake of ammonia expressed in mg N/1.

The data through August in Table VII was modeled in this manner and the results were computer plotted for assistance in visual correlation, Figures 14-23.

Figures 14 and 15 show the ambient nitrate and ammonia concentrations (µg-at N/1), respectively. Figure 16 shows a plot of Equation 68, the ammonia correction factor, and Figure 17 shows a plot of Equation 67, the temperature effect on nitrate uptake as a function of time. Figure 18 shows the experimental light flux (langleys/min) at the depth of incubation, while simultaneously giving Di Toro's, et al. (1971) light attenuation factor, R_j, by a simple change in ordinate scale. Figure 18 also gives an empirical estimate of V_{max} for nitrate uptake based on least-squares linear regression analyses of correlations between V_{max} and light flux observed by Varga and Toetz (1974) over several years. The relation is

$$V_{max}\ (hr^{-1}) = 0.2054\ (\text{langleys/min}) + 0.04958\ (\text{langleys/min})^2 . \qquad [69]$$

Figure 19 shows the experimentally observed values of V_{max} in units of the fractional uptake per day. The transformation of units

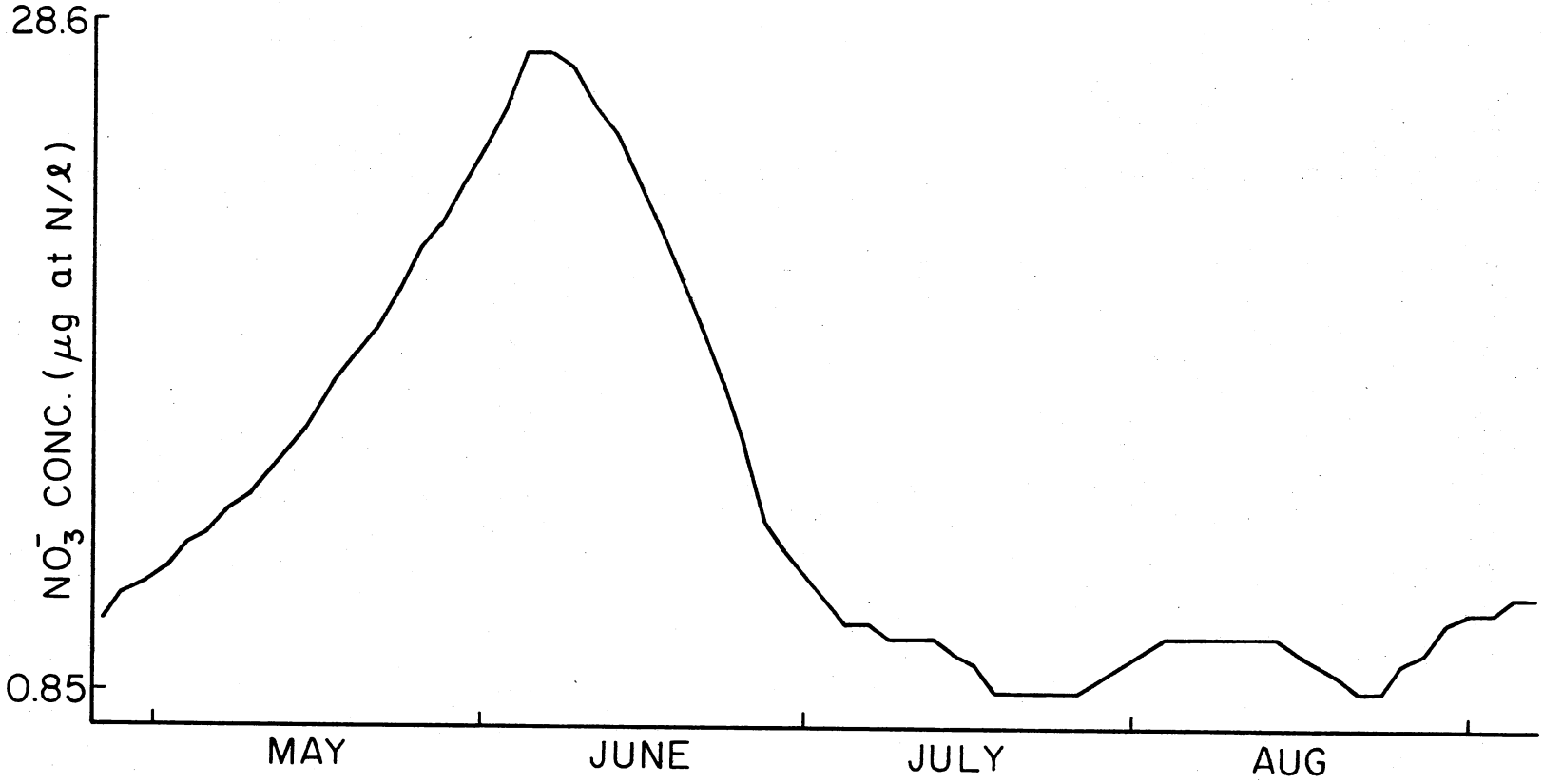

Figure 14. The Ambient Nitrate Ion Concentration in Lake Carl Blackwell in 1973

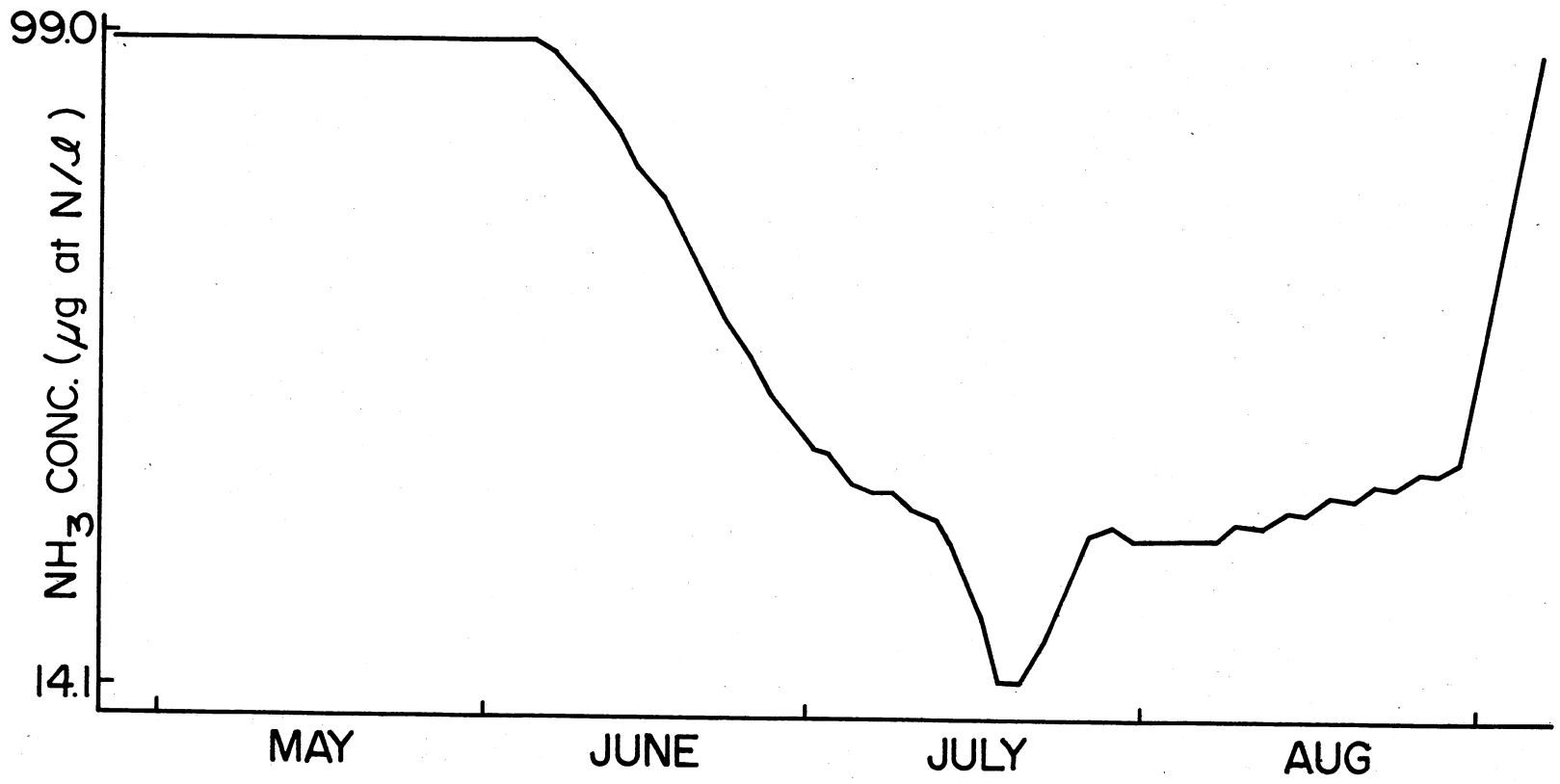

Figure 15. The Ambient Ammonia Concentration in Lake Carl Blackwell in 1973

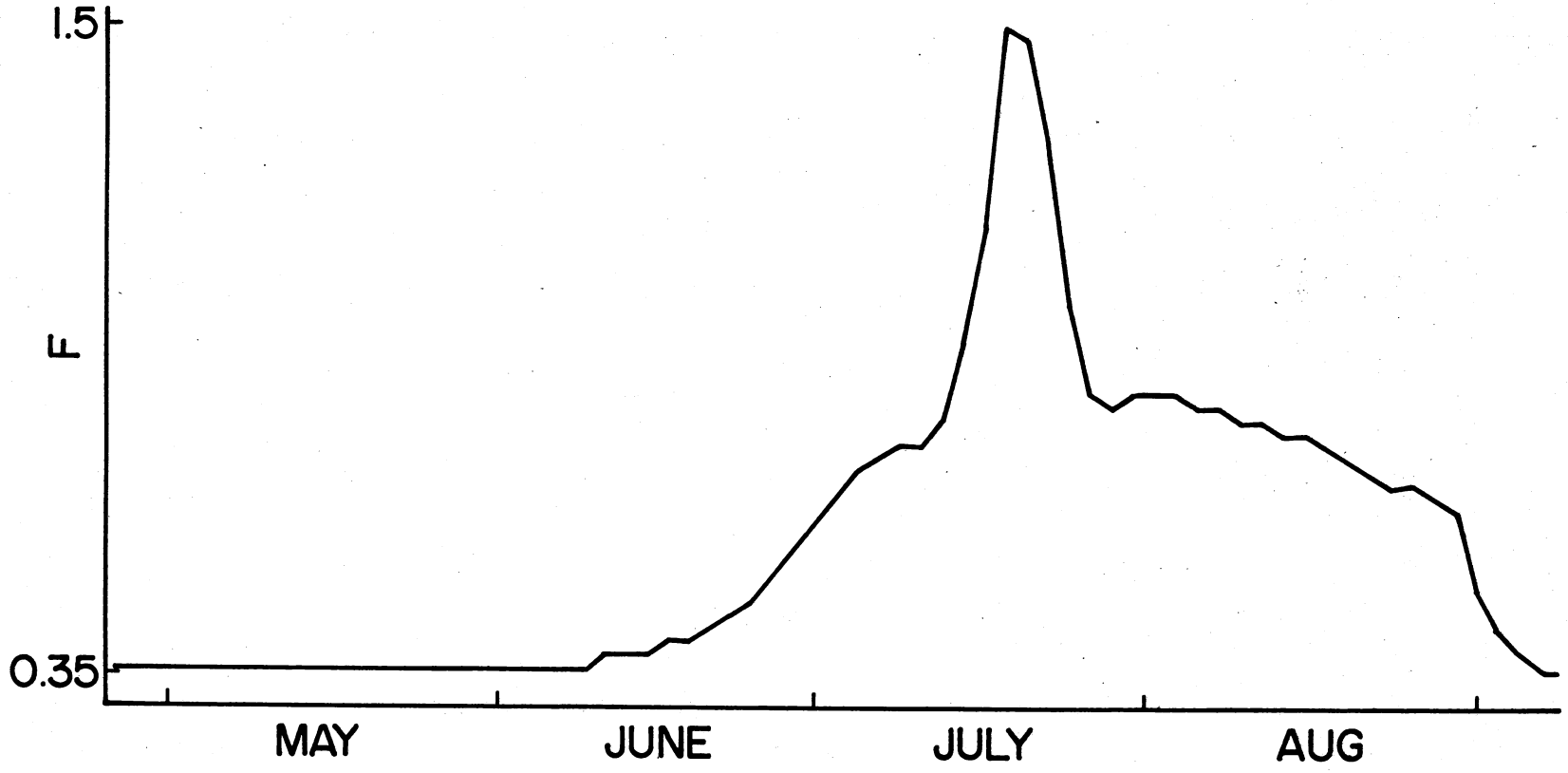

Figure 16. A Plot of Equation 68, the Ammonia Correction Factor for Nitrate Uptake by Algae

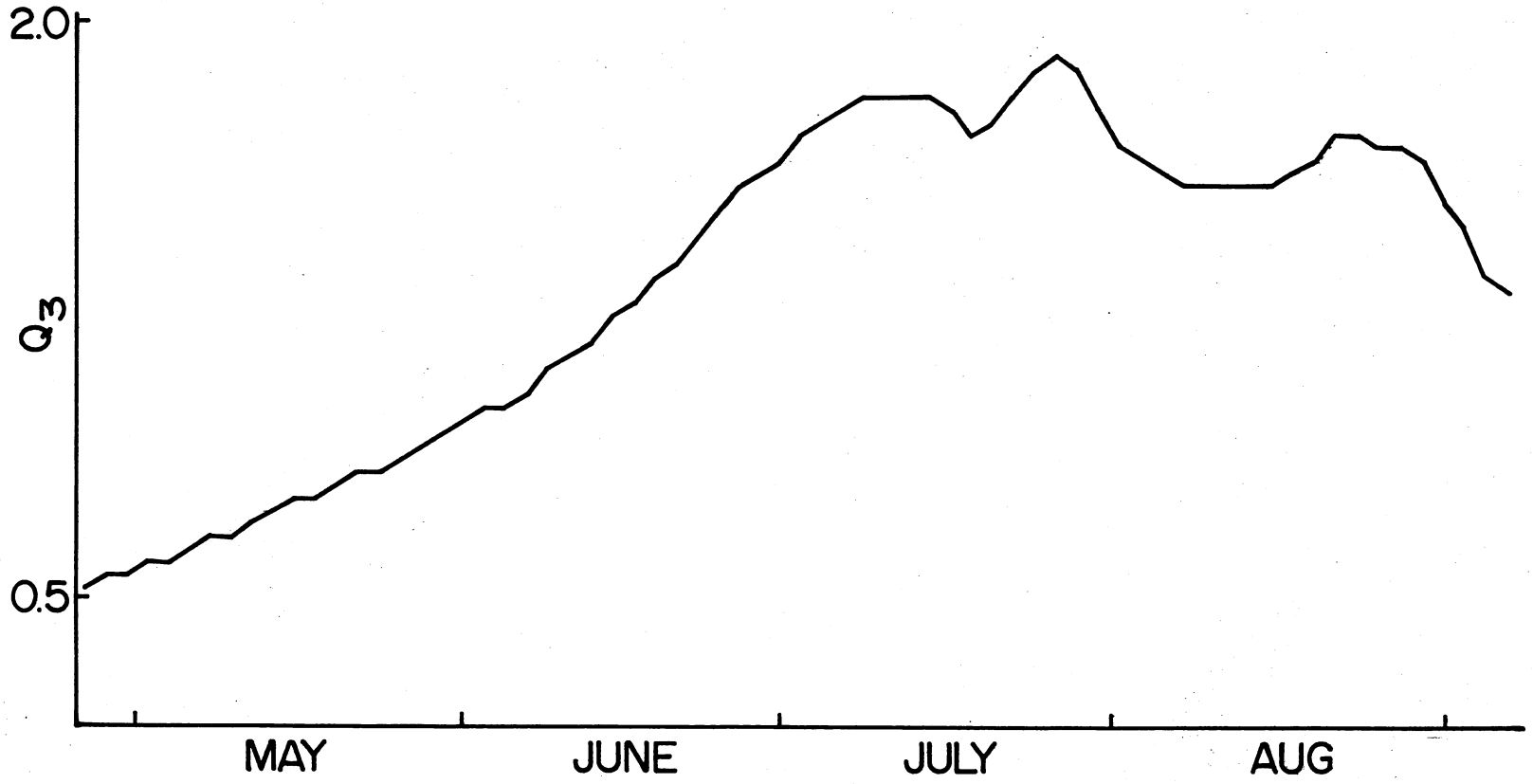

Figure 17. A Plot of the Temperature Factor of 2.0 for Nitrate Uptake by Algae at 20°C

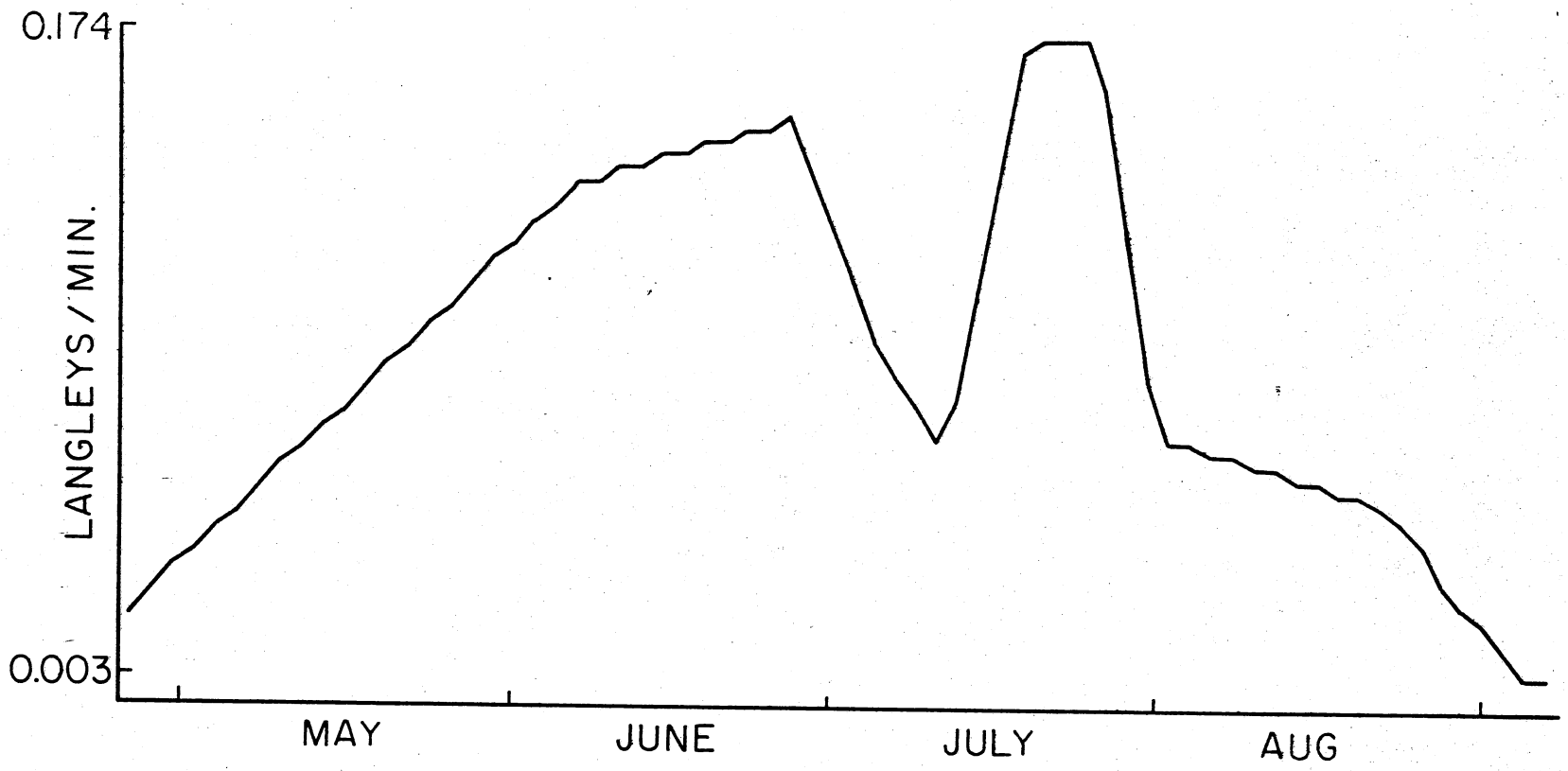

Figure 18. This Curve Shows the Behavior of Three Functions: the Experimental Value of the Irradiance, I, in Langleys/Min Averaged Over the Incubation Periods During Nitrate Uptake Studies, and Two Other Functions, R_j and V_{max} Which are Functions of I. The Range of R_j, the Correction Factor for Non-Optimum Light Flux at the Depth of Incubation, is from 0.0184 to 0.7210. The Range for V_{max} in Hr^{-1} is From 6.6 (10^{-4}) to 3.72 (10^{-2})

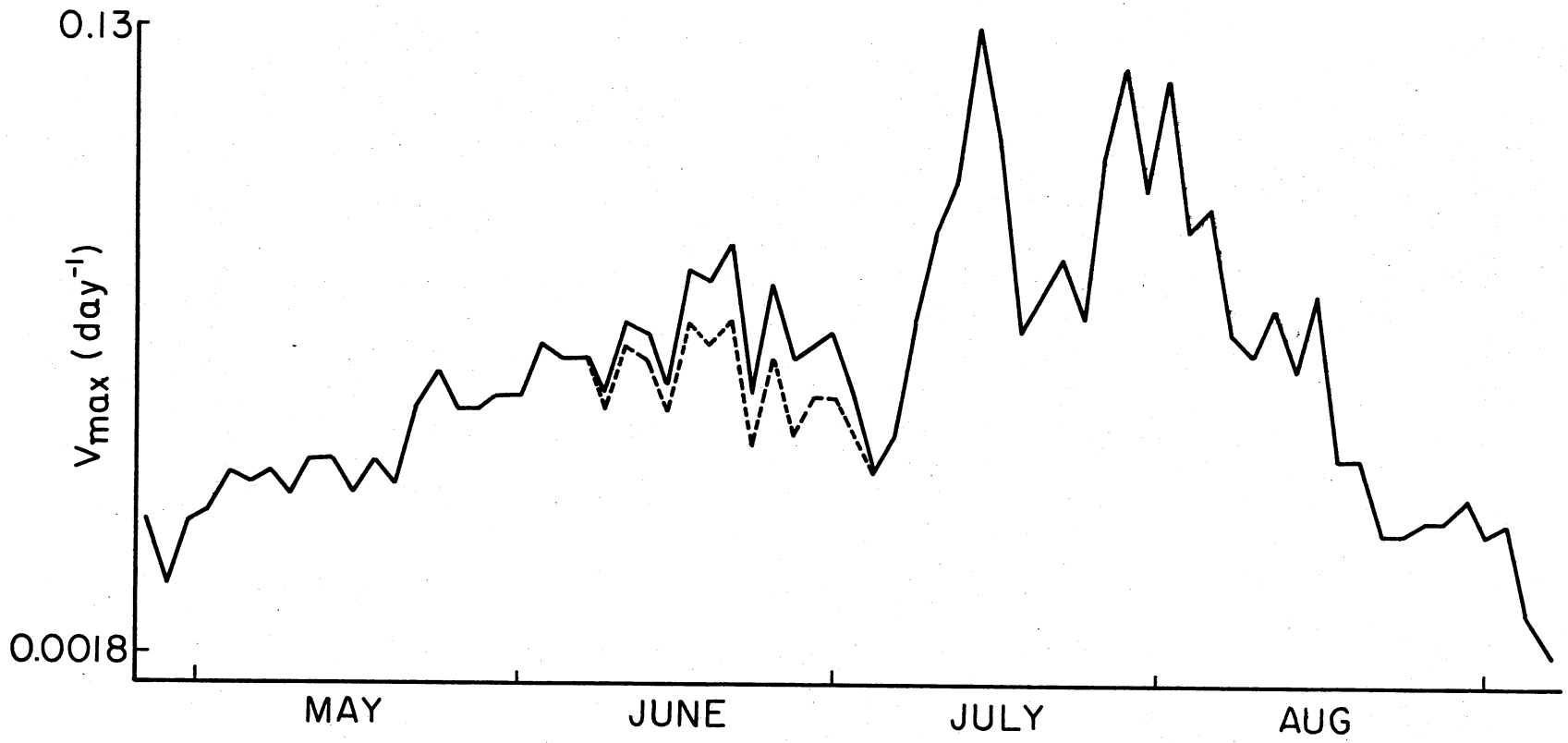

Figure 19. V_{max}, the Maximum Specific Nitrate Uptake Rate for Algae as Experimentally Determined From the $^{15}NO_3^-$ Uptake Studies and Then Transformed to the Units (Day^{-1}). The Lower Dotted Curve Shows the Effect of Chlorine

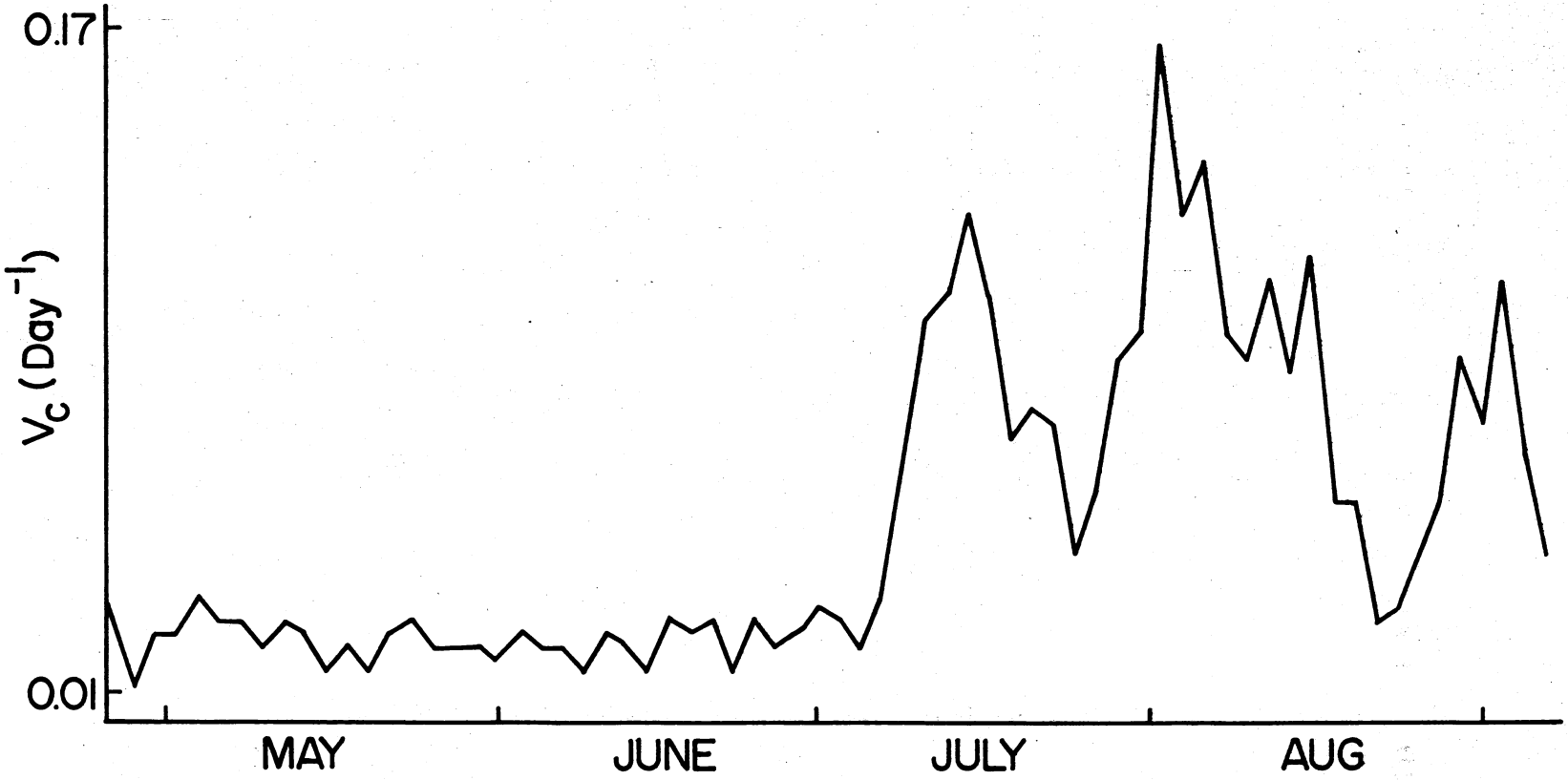

Figure 20. V_{max} for Nitrate Uptake by Algae Corrected for the Effects of Non-Optimum Light, Temperature and the Effect of Ammonia on Nitrate Uptake

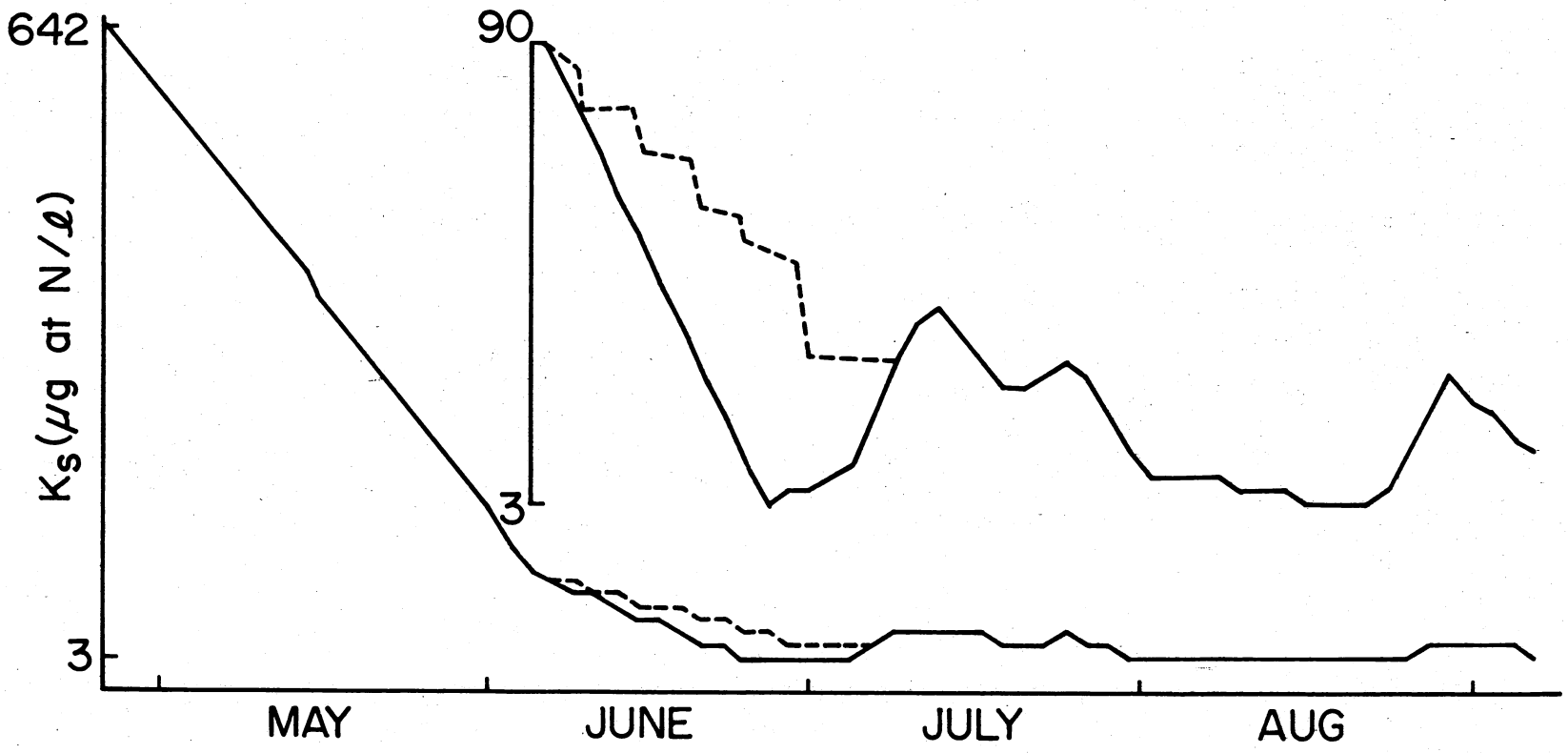

Figure 21. K_s, the Michaelis-Menten Half-Saturation Constant for the Uptake of Nitrate by Algae as Experimentally Determined From the $^{15}NO_3^-$ Uptake Studies. The Dotted Portion of the Curves Show the Effect of Chlorine

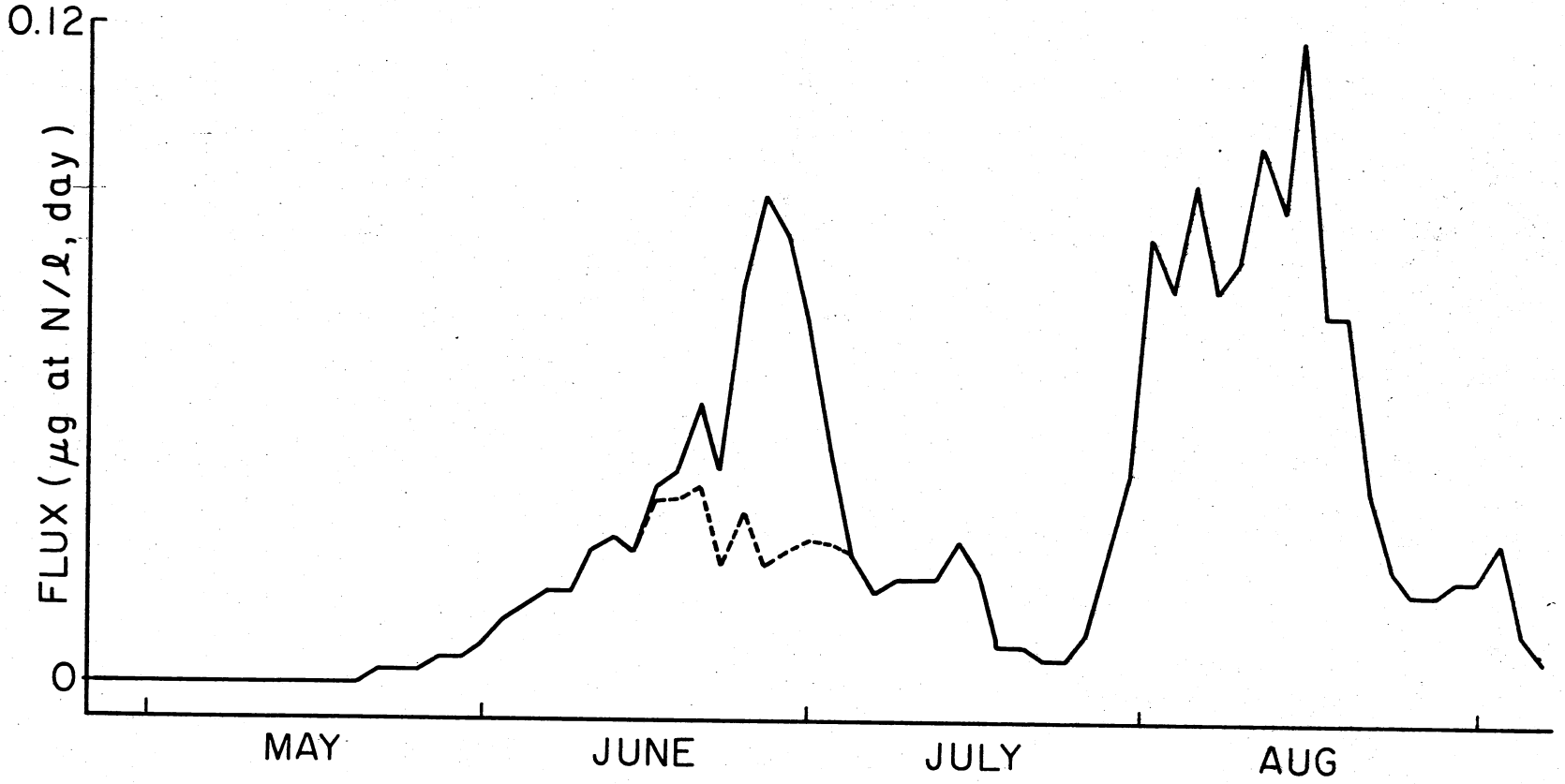

Figure 22. The Flux of Nitrate into the Phytoplankton Compartment Calculated by Equation 65. The Lower Dotted Curve Shows the Effect of Chlorine

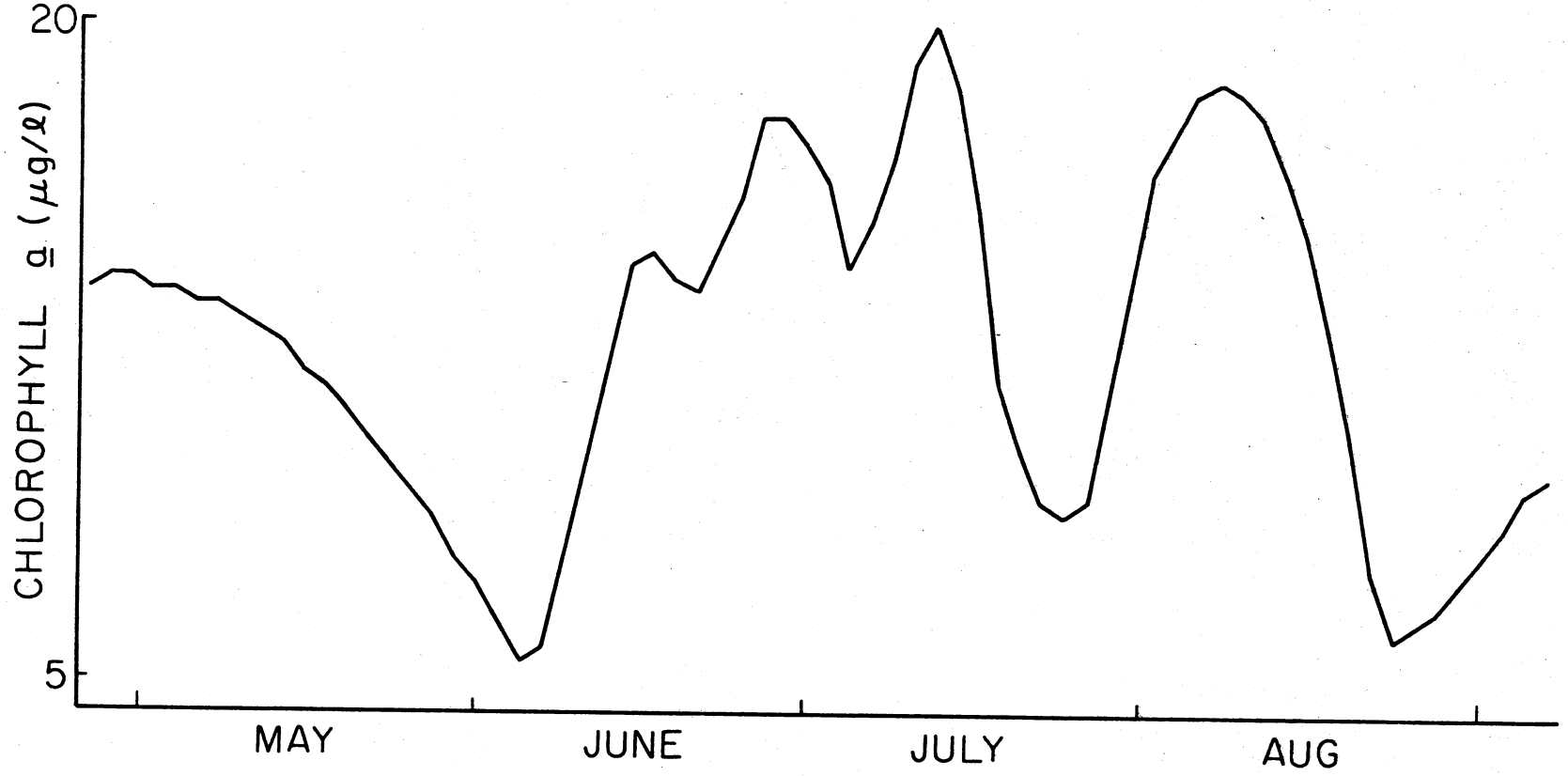

Figure 23. The Concentration of Chlorophyll a in Lake Carl Blackwell in 1973

from hr^{-1}, as given in Table VII, to day^{-1} involved multiplication by the photo period or effective number of hours per day that would sustain the observed V_{max} per hour. At this point a statistical factor of ± 15% was imposed since variable cloud cover and turbidity conditions at the sampling station during the time interval between the weekly experiments was not measured. The relation used was

$$\text{photo period (hrs/day)} = (3 + 4 \times (1 + \text{SIN}(0.0172 \times \text{Date}))) \pm 15\% \qquad [70]$$

where Date is the number of days from March 21, the vernal equinox.

The values of V_{max} derived empirically from light flux data (Equation 69) and observed values of V_{max} showed a good correlation by inspection. The empirical data for V_{max} (Figure 18) showed a steady rise in the magnitude of the parameter through May and most of June followed by a decline in early July and a subsequent increase to a maximum in late July. The experimental data on V_{max} (Figure 19) showed the same initial trend, except that high values of V_{max} were sustained into early August.

However, when one factors out the effects of non-optimum light, temperature, and ammonia concentrations, as shown in Figure 20, the rise in V_{max} in May and June is completely eliminated, leaving a fluctuating pattern for saturated nitrate uptake by algae in mid-summer which appears to be species dependent.

The actual nitrate uptake rate for algae is dependent upon K_s as well as V_{max}, and the inverse relationship expected between K_s and the NO_3^--N flux, according to Equation 65, is well corroborated upon inspection of K_s in Figure 21 and nitrate flux in Figure 22. The minima is K_s toward the end of June and all of August are matched by

high nitrate fluxes at those times. The principal forcing functions for the appreciable nitrate flux in early and mid-June, as shown in Figure 22, were the high nitrate levels (Figure 14) and the high light flux (Figure 18) which were in effect at this time. These values were able to overcome, to some degree, the relatively high value of K_s in early June.

Two experiments were performed in late June and early July in which chlorine was added to the bottles at the beginning of the incubation period. One set of incubation bottles used on June 27 was spiked with 0.101 mg Cl_2/1, and a second set on July 5 received 0.013 mg Cl_2/1. The effect of these additions of free chlorine on V_{max} is shown in Figure 19.

The lower curve gives the perturbed values of V_{max} simulated by interpolation and showing the presence of Cl_2 for a full month. The corresponding values of K_s, in the presence of these Cl_2 concentrations, is shown by the curve in Figure 21, and the seomwhat remarkable effect of this chlorine on the nitrate flux is shown in Figure 22. These results indicate that these levels of Cl_2 could prevent a summer algal pulse, without destroying the population.

Comparison of the nitrate uptake kinetics as summarized by the nitrate flux data of Figure 22 with algal biomass in terms of cell counts or chlorophyll concentration or particulate nitrogen concentration is a necessary step in model validation.

The chlorophyll *a* data is presented in Figure 23 in units of µg/1. In spite of the difference in units a direct comparison with the data for nitrate flux into the phytoplankton compartment, Figure 22, shows a reasonable correlation except for the data taken

on one date, July 12, 1973. The value for chlorophyll a on that date was over 20 µg/l. If the real value were approximately half this value, the whole peak in mid-July would disappear and excellent correlation with the late June maxima in nitrate flux of Figure 22 would result. The agreement between the chlorophyll a maxima and the nitrate flux maxima in August was excellent.

Experiments During 1974

The temperature and irradiance data for 1974, are shown in Table XI. Table XII shows the concentrations of three forms of nitrogen and the "standing crop" of the lake in terms of chlorophyll a and particulate nitrogen. Both of these tables are basically of the same design as those of 1973 with a few exceptions. Incubation was not at the same depth. The ambient concentration of phosphate is included since the effect of phosphate concentration was concurrently investigated on each experimental date.

The major focus of 1974 was on the effect of chlorine on nitrate and ammonia uptake. It was hoped that K_s and V_{max} might provide a convenient quantification of these effects. Table XIII is a summary of the uptake experiments during this year.

For the three control experiments, V_{max} and K_s for nitrate uptake was of the same order of magnitude as previously determined values. Chlorine, at a concentration of 0.1 mg Cl_2/1, decreased V_{max} significantly on July 2 and August 6. However, the regression coefficient for the experiment on September 10 of chlorine perturbation is 0.21; thus, V_{max} and K_s for the experiment on this date cannot be safely considered significant. All values of K_s for the experiments performed on July 2 were negative. Negative K_s values

TABLE XI

TEMPERATURE AND IRRADIANCE DATA IN LAKE CARL BLACKWELL DURING 1974

Date	Depth in Meters	Duration Total	(Hrs) In Light	Percent Incident Irradiance	Average ly/min	Temperature °C Start	End
7/2	0.05	23.75	14.63	25.45	0.21	27.0	28.0
7/16	0.37	24.00	14.00	46.84	0.28	27.5	27.5
7/23	0.37	23.50	13.50	45.06	0.29	28.0	27.5
8/6	0.37	24.00	13.25	43.24	0.27	23.2	23.2
9/10	0.25	25.50	12.50	22.07	0.16	20.0	20.0

TABLE XII

CONCENTRATIONS OF NITRATE, NITRITE, AMMONIA, PHOSPHATE, CHLOROPHYLL *a*, PARTICULATE NITROGEN, AND pH FOUND IN LAKE CARL BLACKWELL DURING 1974

	µmoles/l				µg/l		
Date	NO_3^--N	NO_2^--N	NH_3-N	PO_4^{-3}-P	Chlorophyll *a*	Particulate Nitrogen	pH
7/2	5.47	0.34	3.86	0.13	5.47	62.90	8.30
7/16	1.68	0.16	0.61	0.25	5.28	56.66	8.54
7/23	0.79	0.03	1.14	0.11	8.88	65.54	8.60
8/6	1.40	0.04	3.05	0.19	4.72	73.23	8.22
9/10	6.53	0.04	0.70	0.24	4.44	-----	8.25

TABLE XIII

EXPERIMENTALLY DERIVED VALUES OF V_{max} AND K_s, THEIR STANDARD DEVIATIONS AND REGRESSION COEFFICIENTS FROM THE WOOLF PLOT DURING 1974

Date	Nutrient	Perturbation	$V_{max} \pm \sigma_{V_{max}}$ x 10^3 $hour^{-1}$	$K_s \pm \sigma K_s$ µM N/1	RC
7/2	$^{15}NO_3^-$	Control	2.75 ± 0.49	-1.47 ± 2.55	0.94
7/2	$^{15}NO_3^-$	0.1 mg Cl_2/1	0.31 ± 0.05	-22.6 ± 9.22	0.96
7/2	$^{15}NO_3^-$	0.1 mg NH_2Cl/1	0.10 ± 0.02	-11.2 ± 12.1	0.50
7/2	$^{15}NH_3$	Control	7.27 ± 0.64	2.78 ± 0.85	0.98
7/2	$^{15}NH_3$	0.1 mg Cl_2/1	0.12 ± 0.01	-2.80 ± 0.85	0.78
8/6	$^{15}NO_3^-$	Control	0.95 ± 0.1	0.99 ± 0.33	0.99
8/6	$^{15}NO_3^-$	0.1 mg Cl_2/1	0.26 ± 0.005	2.96 ± 0.29	0.99
8/6	$^{15}NO_3^-$	0.1 mg NH_2Cl/1	0.01 ± 0.0004	-1.65 ± 0.31	-0.17
8/6	$^{15}NH_3$	Control	Inhibition		
8/6	$^{15}NH_3$	0.1 mg Cl_2/1	Inhibition		
9/10	$^{15}NO_3^-$	Control	5.00 ± 0.12	4.34 ± 1.55	0.99
9/10	$^{15}NO_3^-$	0.1 mg Cl_2/1	5.31 ± 1.08	11.5 ± 17.4	0.21
9/10	$^{15}NO_3^-$	0.1 mg NH_2Cl/1	*	-169. ± 298.	0.05
9/10	$^{15}NH_3$	Control	11.6 ± 0.2	9.38 ± 0.29	0.94
9/10	$^{15}NH_3$	0.1 mg Cl_2/1	16.4 ± 1.3	20.5 ± 1.8	0.52

*Not calculated.

have no biological meaning; however, the K_s value for the control experiment of July 2 was within an order of magnitude agreement with observations of previous years. Only the experiments of August 6 adequately show the debilitating effect of chlorine by decreasing V_{max} and increasing K_s.

The three experiments showing the effect of chloramine had regression coefficients of 0.50, -0.17, and 0.05. The atom percent excess ^{15}N was below 0.14 for 15 out of a total of 18 samples for the three experiments. Thus a comparison of V_{max} and K_s between the control and chloramine perturbed experiments is not meaningful. However, a comparison of nitrogen flux for the three types of experiments, Table XIV, does indicate that chloramine was more toxic than chlorine during two of the experimental dates, August 6 and September 10.

Very little can be concluded from the similar ammonia uptake experiments during 1974. The ammonia control experiments on July 2 and September 10 gave reasonable values of V_{max} and K_s and derived regression coefficients of 0.98 and 0.94, respectively. The perturbation of ammonia uptake with chlorine on July 2 results in low ^{15}N flux or a small atom percent excess ^{15}N in the samples. Thus, V_{max} and K_s for this experiment could not be considered useful. The perturbation of ammonia uptake with chlorine on September 10 resulted in an increase in K_s as compared to the control of this date. This would be expected at an appropriate level of inhibition. However, the regression coefficient was 0.52. It is therefore difficult to consider this V_{max} and K_s significant. On August 6 uptake decreased with increasing ammonia concentration rather than

TABLE XIV

A COMPARISON OF NITROGEN FLUX FOR THE CONTROLLED AND PERTURBED $^{15}NO_3^-$ UPTAKE EXPERIMENTS DURING 1974

		μM NO_3^--N Taken Up/μM Particulate Nitrogen-Hour		
Date	% ^{15}N Added	Control	0.1 mg Cl_2/1	0.1 mg NH_2Cl/1
8/6	36	0.79	0.19	0.03
8/6	53	0.66	0.14	0.03
8/6	80	0.82	0.19	0.01
9/10	11	2.04	2.13	0.26
9/10	70	4.43	0.44	0.18
9/10	84	4.51	0.13	0.16

producing a hyperbolic response for both the control and perturbed experiments. Despite the failure to obtain meaningful values of V_{max} and K_s for ammonia uptake inhibited with chlorine, it is apparent that a debilitating response was present.

The decrease in ammonia uptake was best demonstrated by the experiment of July 23. Bottles containing lake water were spiked with a constant amount of $^{15}NH_3$ at 5.46 percent of ambient NH_3-N and varying amounts of chlorine from 0.0 to 0.5 mg Cl_2 resulting in a LC-50 value of 0.101 mg Cl_2/1 (Varga and Toetz, 1975). During the previous year it was determined that 0.028 mg Cl_2/1 depressed nitrate uptake to 50% of the control rate (Varga and Toetz, 1974). Although these experiments are not directly comparable since they were performed during different years, it appears that additions of chlorine at low levels has a greater effect on nitrate uptake than on ammonia uptake.

Nitrate and ammonia uptake can be attenuated if phosphate as a nutrient is limiting growth. As a test for this, another type of experiment was performed on each experimental date. One bottle of the ^{15}N labeled control series for nitrate and for ammonia uptake were duplicated. To one of these duplicates, 100 µg PO_4^{-3}-P/1 were added. If conditions of phosphate limited growth were present the uptake of ^{15}N in each duplicate bottle would have been much greater than the corresponding bottle in the control series. Table XV is a summary of these experiments. The nitrate control was lost for the experiment of July 2. However, the nitrate flux for this concentration was calculated for the corresponding uptake rate given by the regression analysis. Although this value implied the highest percent increase in

TABLE XV

THE EFFECT OF ADDING 100 µg PO_4^{-3}-P/L ON NO_3^- AND NH_3 UPTAKE DURING 1974

Date	Ambient PO_4^{-3}-P (µg/l)	Flux (nM N/nM Particulate Nitrogen-Hour) NO_3^--N Control	NO_3^--N Plus PO_4^{-3}	NH_3-N Control	NH_3-N Plus PO_4^{-3}
7/2	4.32	3.2*	4.8	4.8	0.9
7/16	8.03	ND	ND	ND	ND
7/23	3.62	ND	ND	10.9	10.6
8.6	5.85	0.8	0.9	33.9	31.7
9/10	7.64	20.3	19.6	7.4	6.2

*The control sample was lost. The reported value was calculated using the results of the regression analysis of the Woolf transformation.

ND = No Data

nitrogen flux, the value 3.2, is fairly close to the flux when phosphate was added, 4.8. The conclusion drawn from Table XV is that phosphate was not limiting to growth. This was probably due to the relatively high concentration of phosphate already in the water. The reason for the apparent decrease in the flux of nitrogen when phsophate was added, which was often observed, is not clear.

On July 16 a constant amount of $^{15}NO_3^-$ and varying amounts of chlorine and chloramine were added to lake water. In this manner, samples were treated giving a total of 16 conditions. This experiment was run in duplicate such that 32 bottles were incubated.

Using the pertinent equilibrium constants, known concentrations of total chlorine, total ammonia and the pH, the equilibrium concentrations for all chemical species can be calculated. Subsequent to the experiment, these calculations were made using an iterative computer program written by Bard and King (1965) and modified by Whitfield (1975). The concentrations of those species corresponding to bound chlorine and free chlorine were then summed. Subsequent laboratory and computer analysis gave the mean nitrate uptake calculation. Table XVI is a summary of the experimental conditions and calculated results.

Using the Stistical Analysis Systems computer program developed by Goodnight (1972), a regression analysis of the nitrate uptake rates, v, as a function of the concentrations at equilibrium of free chlorine, x, and bound chlorine, y, yielded Equation 71 as the best fit:

$$v = 2.45\,(10^{-4}) - 3.61\,(10^{-3})\,y + 1.13\,(10^{-2})\,y^2 + 8.56\,x\,y\ , \qquad [71]$$

where x is the concentration of free chlorine and y is the concentration of bound chlorine in ppm. The multiple correlation coefficient of

TABLE XVI

A SUMMARY OF THE INITIAL AND EQUILIBRIUM CONDITIONS AND THE RESPONSE FOR THE CHLORINE-CHLORAMINE EXPERIMENT ON JULY 16, 1974

Element Number	Cl_2 Added, (ppm)	Chloramine Added, (ppm)	Equilibrium Free Cl_2, (ppm) (Cl_2,$HOCl$,OCl^-)	Equilibrium Bond Cl_2, (ppm) (NH_2Cl,$NHCl_2$)	$V_{NO_3^-} \times 10^3$, (hr^{-1})	
					Bottle 1	Bottle 2
1,1	0.00	0.00	0.00	0.00	3.976	1.503
1,2	0.00	0.025	8.46×10^{-4}	1.17×10^{-2}	2.276	2.197
1,3	0.00	0.050	5.46×10^{-3}	1.95×10^{-2}	2.740	0.577
1,4	0.00	0.125	3.88×10^{-2}	2.37×10^{-2}	1.640	1.689
2,1	0.025	0.00	7.89×10^{-4}	2.42×10^{-2}	1.140	1.146
2,2	0.025	0.025	2.20×10^{-3}	3.53×10^{-2}	1.274	0.894
2,3	0.025	0.050	6.82×10^{-3}	4.32×10^{-2}	1.344	1.682
2,4	0.025	0.125	3.69×10^{-2}	5.06×10^{-2}	2.141	2.178
3,1	0.05	0.00	1.53×10^{-3}	4.85×10^{-2}	0.551	0.557
3,2	0.050	0.025	3.29×10^{-3}	5.93×10^{-2}	0.430	0.435
3,3	0.050	0.050	7.82×10^{-3}	6.72×10^{-2}	0.449	-----
3,4	0.050	0.125	3.51×10^{-2}	7.74×10^{-2}	0.506	0.620
4,1	0.125	0.00	3.32×10^{-3}	1.22×10^{-1}	0.227	0.317
4,2	0.125	0.025	5.50×10^{-3}	1.32×10^{-1}	0.249	0.633
4,3	0.125	0.050	9.44×10^{-3}	1.41×10^{-1}	0.172	0.168
4,4	0.125	0.125	3.08×10^{-2}	1.57×10^{-1}	0.084	0.127

this equation and the three variables was 0.79. A three-dimensional plot of this equation was made using a computer program written by Oines and is shown in Figure 24. It can be seen that $V_{NO_3^-}$ decreases rapidly with increasing concentration of bound chlorine.

The response of $V_{NO_3^-}$ to increasing concentrations of free chlorine was not demonstrated with a high degree of certainty by this experiment. This was due to several factors. The highest equilibrium concentration of bound chlorine used in this experiment, 0.157 mg Cl/l, was approximately five times that of the free chlorine, 0.0308 mg Cl/l. The distribution of the concentrations at equilibrium, also shown in Figure 24, slightly favors measurement of the response as a function of bound chlorine rather than free chlorine concentration. These factors coupled with the uncertainty in the equation of the surface as calculated by the SAS program does not allow a rigorous interpretation of the response of $V_{NO_3^-}$ as a function of free chlorine. Nevertheless, it is apparent that at equal concentrations chloramine (bound chlorine) decreased $V_{NO_3^-}$ to a greater extent than free chlorine within the range of data in this experiment. It should be noted that the experiment was designed to be below literature values of "breakpoint" stoichiometry. The ambient-ammonia nitrogen level for this experiment was 0.0086 ppm. The largest addition of chlorine was 0.10 ppm. The "breakpoint" minima was exceeded by only one set of samples with a ratio of 11.6:1.

The smaller response of $V_{NO_3^-}$ as a function of increasing free chlorine concentration may be due to the breakpoint reaction. If this is the case, as total chlorine concentration increases one might expect an initial decrease in $V_{NO_3^-}$ followed by an increase in $V_{NO_3^-}$ as

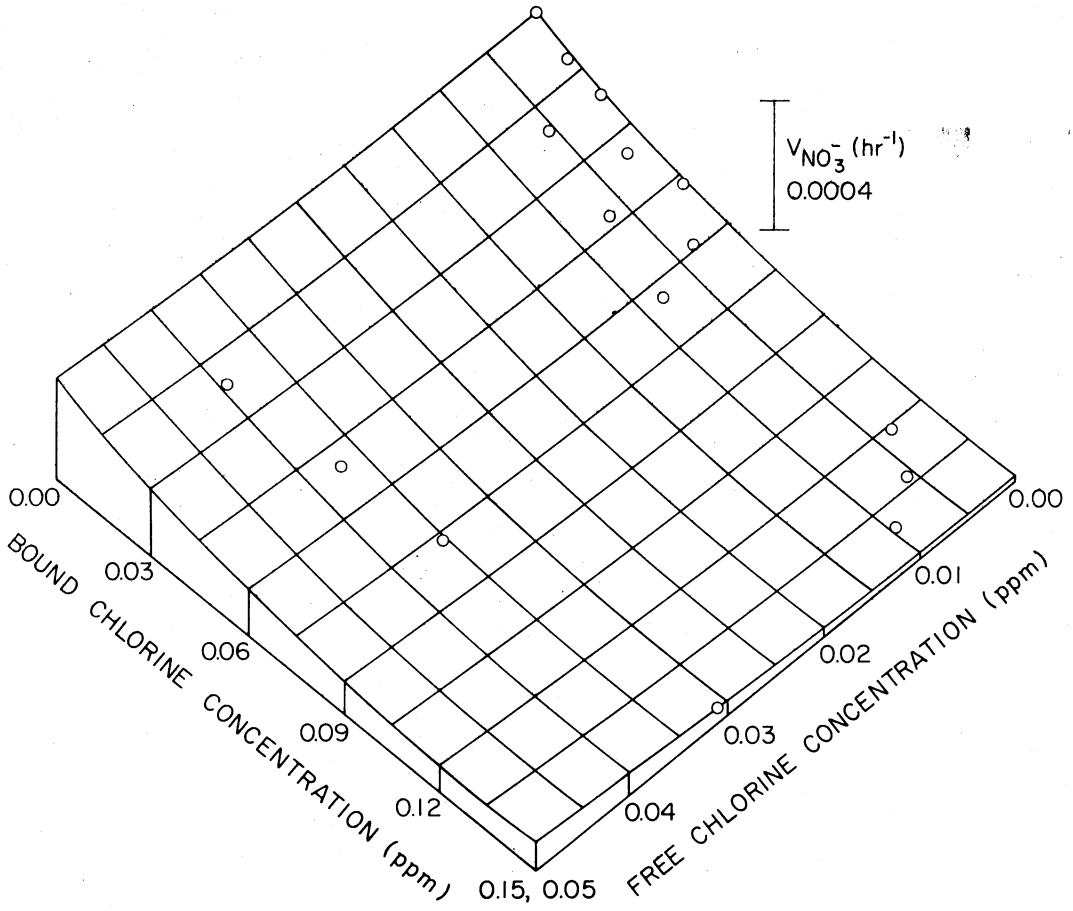

Figure 24. A Three-Dimensional Calcomp Plot of $V_{NO_3^-}$ vs. Free and Bound Chlorine From 1974 Data

toxic species are removed by the oxidation-reduction reactions of the "breakpoint" phenomenon. Further additions of chlorine which are not reduced should be accompanied by a decrease in $V_{NO_3^-}$. Thus a plot of $V_{NO_3^-}$ vs amount of chlorine added might be the inverse of Figure 6. A topographical plot of an extrapolation of the data is presented in Figure 25.

Experiments During 1975

The following data are presented in Tables XVII and XVIII: temperature, irradiance, concentration of three forms of nitrogen and phosphate and the standing crop of the lake in terms of particulate nitrogen and chlorophyll a.

The primary objective of 1975 was to test the response of $V_{NO_3^-}$ as a function of free and bound chlorine in the theoretical equilibrium concentration ranges bracketing the "breakpoint". This was done in experiments performed on June 16 and August 1.

The program of Bard and King (1965) was again modified by Whitfield (1975) to calculate the amounts of chlorine and ammonia necessary to produce specific concentrations of free and bound chlorine at equilibrium. This allowed a design of a square 4 x 4 matrix of 16 duplicated experimental conditions or 32 incuabtion bottles, Table XIX. The samples were processed in the usual manner to obtain uptake rates.

The regression analysis of the data by the SAS program gave Equation 72 as the best fit with a multiple correlation coefficient of 0.94:

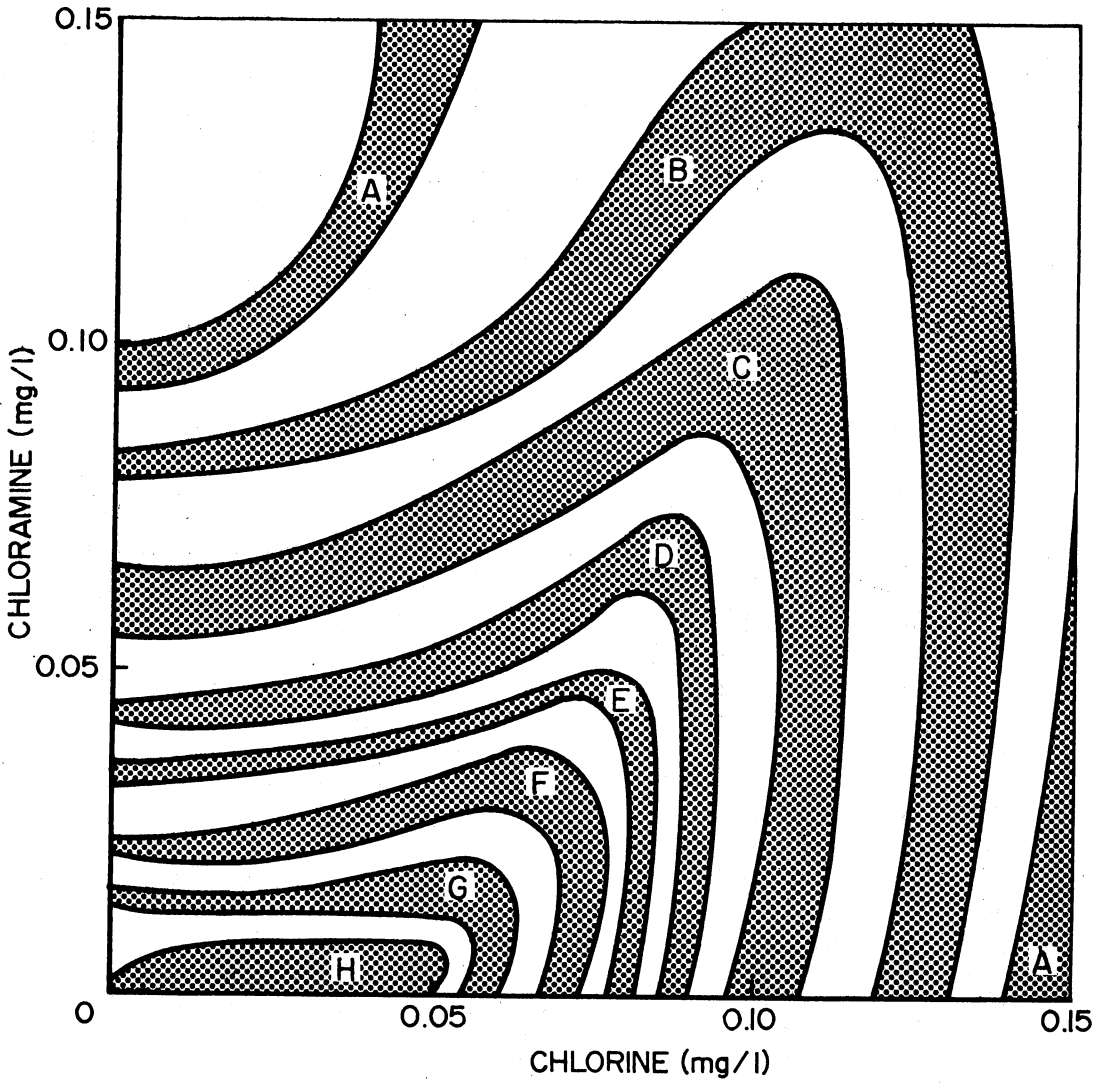

Figure 25. A Plot of the Predicted Surface for $V_{NO_3^-}$ vs. Chlorine and Chloramine Concentrations. $V_{NO_3^-}$ = (A=1, B=4, C=7, D=10, E=13, F=16, G=19, H=22) 10^{-4}. Actual Data for 1974 was 0 to 0.0388 ppm Chlorine and 0 to 0.157 ppm Chloramine

TABLE XVII

TEMPERATURE AND IRRADIANCE DATA FOR EXPERIMENTS DURING 1975

Date	Percent Incident Irradiance	Average ly/min	Temperature °C Start	End
6/12	9.33	0.350	23.3	ND*
6/18	11.60	0.233	ND	ND
7/2	21.0	0.340	ND	ND
7/14	21.0	ND	23.9	30.0
7/71	21.0	0.318	23.3	26.7
8.29	30.8	0.318	23.3	30.0

*No Data.

TABLE XVIII

CONCENTRATIONS OF NITRATE, NITRITE, AMMONIA, PHOSPHATE, CHLOROPHYLL a, AND PARTICULATE NITROGEN FOUND IN LAKE CARL BLACKWELL DURING 1975

	µM/l			µg/l		
Date	NO_3^--N	NO_2^--N	NH_3-N	Chlorophyll a	Particulate N	PO_4^{-3}-P
6/12	6.1378	0.0940	1.6274	1.51	87.0	0.68
6/18	4.3021	0.2210	0.7730	3.879	72.6	0.40
7/16*	0.4459	0.2183	4.0087	8.60	142.6	0.41
7/18*	0.4459	0.2183	4.0087	9.04	74.3	0.77
8/1	0.4946	0.3092	1.6864	4.29	70.3	0.75
8/29	1.0038	0.5789	2.7220	8.29	97.7	5.60

*Experiment and data for Ham's Lake.

TABLE XIX

A SUMMARY OF THE INITIAL AND EQUILIBRIUM CONDITIONS AND THE RESPONSE FOR THE CHLORINE-CHLORAMINE EXPERIMENT DURING 1975

Initial Chlorine Added	Initial Added	Equilibrium Free Chlorine	Equilibrium Bound Chlorine	$V_{NO_3^-} \times 10^3$ hour^{-1}	
				Bottle #1	Bottle #2
2.001 (10^{-1})	6.02 (10^{-1})	5 (10^{-5})	0.10	0.052	0.043
3.000 (10^{-1})	8.856 (10^{-3})	0.05	0.10	1.140	1.105
4.000 (10^{-1})	5.242 (10^{-3})	0.10	0.10	1.790	1.586
5.000 (10^{-1})	3.309 (10^{-3})	0.15	0.10	0.640	1.272
4.001 (10^{-1})	1.241	5 (10^{-5})	0.20	0.125	0.041
5.000 (10^{-1})	4.071 (10^{-2})	0.05	0.20	0.545	0.579
6.000 (10^{-1})	3.348 (10^{-2})	0.10	0.20	0.870	-----
7.000 (10^{-1})	2.962 (10^{-2})	0.15	0.20	0.658	0.885
6.001 (10^{-1})	1.873	5 (10^{-5})	0.30	0.111	0.014
7.000 (10^{-1})	7.257 (10^{-2})	0.05	0.30	0.184	-----
8.000 (10^{-1})	6.172 (10^{-2})	0.10	0.30	0.299	0.378
9.000 (10^{-1})	5.593 (10^{-2})	0.15	0.30	0.521	0.479
9.001 (10^{-1})	2.822	5 (10^{-5})	0.45	0.001	0.000
1.000	1.204 (10^{-1})	0.05	0.45	0.061	0.014
1.100	1.041 (10^{-1})	0.10	0.45	0.111	-----
1.200	9.539 (10^{-2})	0.15	0.45	0.196	0.172

$$V_{NO_3^-} = 0.033 + 3.64\ (10^{-2})x - 9.13\ (10^{-2})xy - 1.89\ (10^{-1})x^2 + 5.04\ (10^{-1})x^2y \quad [72]$$

where x is the concentration of free chlorine in ppm and y is the concentration of bound chlorine in ppm.

Figures 26 and 27 are two three-dimensional views of the surface given by this equation. The experimental conditions were 5×10^{-5} to 0.15 ppm free chlorine and 0.10 to 0.45 ppm bound chlorine. The plots however range only to 0.4 ppm bound chlorine. This is due to the fact that the equation predicts near zero negative values of $V_{NO_3^-}$ at 0.45 ppm bound chlorine. As a result the four data points at this concentration are not shown in these figures.

This surface is in agreement with the general characteristics of the surface predicted in 1974. Both equations or surfaces generated by the data in 1974 and 1975 are considered valid in their applied range of toxin concentrations. As free chlorine concentration increases at a constant concentration of bound chlorine, $V_{NO_3^-}$ initially decreases, increases forming a hump or ridge and then decreases to zero at approximately 0.2 ppm free chlorine. A decrease of $V_{NO_3^-}$ to zero cocurs at a concentration of about 0.5 ppm bound chlorine.

In an attempt to measure the effect of pH and orthophosphate on nitrate uptake the square 4 x 4 matrix design of 15 duplicated conditions was used on August 29. Phosphate was added in amounts of 0, 20, 100 or 200 ppm; the pH was adjusted to 6.6, 7.6, 8.1 or 9.2 with dilute solutions of hydrochloric acid and/or sodium hydroxide;

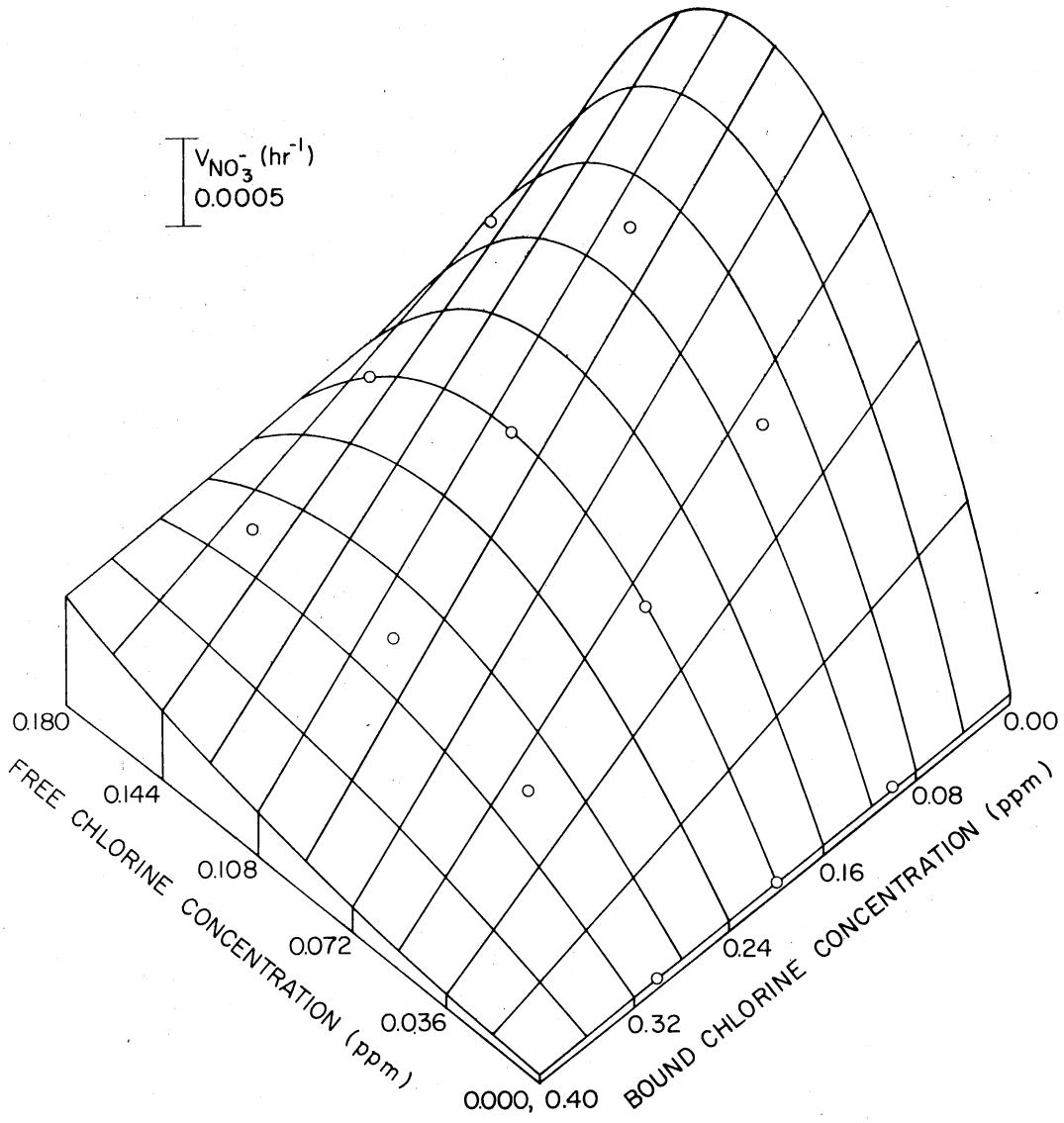

Figure 26. A Three-Dimensional Calcomp Plot of $V_{NO_3^-}$ vs. Free and Bound Chlorine From 1975 Data

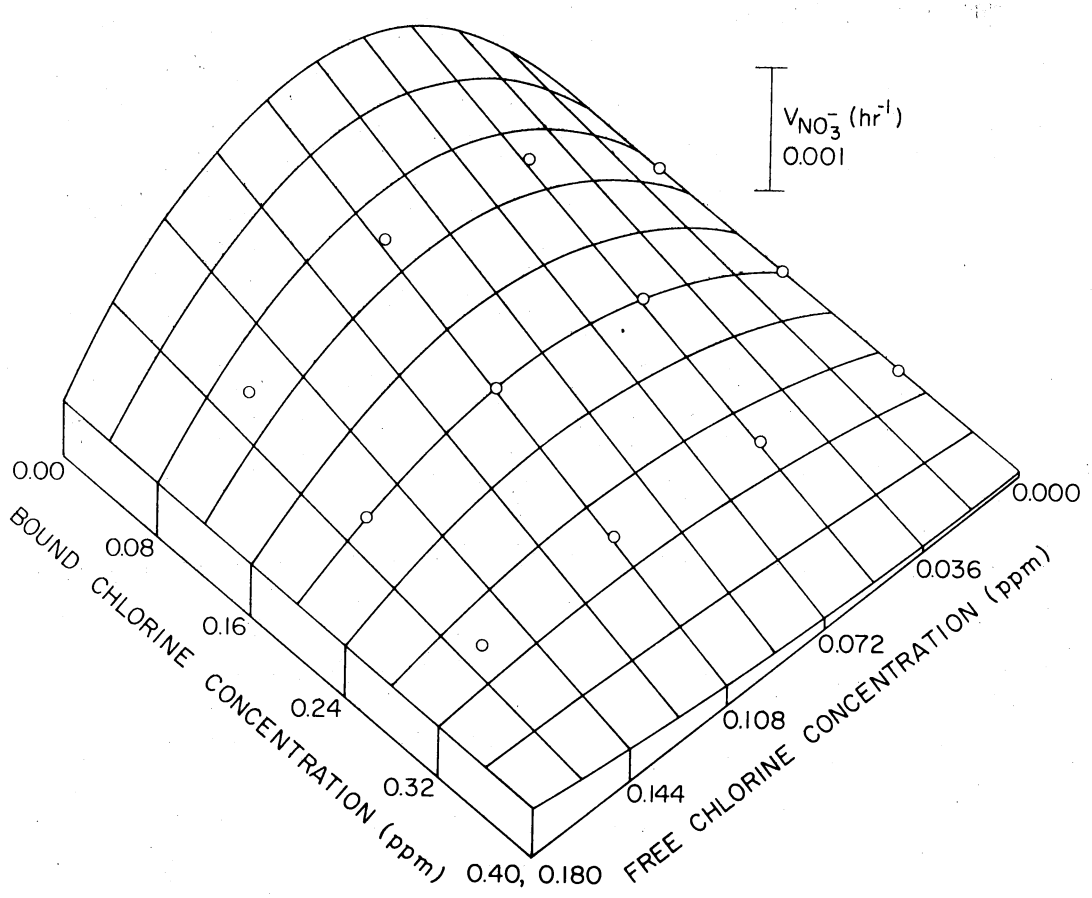

Figure 27. A Three-Dimensional Calcomp Plot of $V_{NO_3^-}$ vs. Free and Bound Chlorine From 1975 Data (Second View)

and, the ^{15}N-nitrate was added at 50% above the ambient nitrate concentration. The matrix design and calculated uptake rates in a range of 0.66×10^{-3} to 1.78×10^{-3} (hr^{-1}) are shown in Table XX. The variability of uptake rates within duplicates was approximately the same as the variability of uptake rates between experimental conditions. For this reason a meaningful regression analysis could not be performed.

The lack in response of $V_{NO_3^-}$ as a function of phosphate concentration was anticipated. Experiments during 1974 showed little response in $V_{NO_3^-}$ to additions of phosphate. However, the lack of response in $V_{NO_3^-}$ to a change in pH was predicted to be dramatic based on the germanium experiment and experiments reported in the literature (Sharma and Kumar, 1975).

The experiments of July 15 and 17 were performed to examine the effect of germanium on nitrate and ammonia uptake reflected by a change in the overall $V_{NO_3^-}$. On these experimental dates the ambient silicon concentration was found to be approximately 4 mg SiO_2-Si/l using the method of Strickland and Parsons (1968). Germanium oxide was dissolved in 0.1 N NaOH and added to lake water at an amount of 19 mg GeO_2/l. This resulted in a change in the pH of the lakewater. ^{15}N-nitrate and ammonia spikes were at 20% of ambient concentrations. In order to differentiate the effect of germanium from that of the increased pH, various controls were simultaneously incubated. Table XXI is a summary of the experiments on these dates. No significant difference can be seen in samples spiked with germanium and ^{15}N-nitrate or ^{15}N-ammonia in contrast to samples spiked with NaOH and ^{15}N-nitrate or ^{15}N-ammonia.

TABLE XX

INITIAL CONDITIONS FOR THE EXPERIMENT OF $V_{NO_3^-}$ VS pH AND ORTHOPHOSPHATE ON AUGUST 29, 1975

pH	µg PO_4^{-3}-P Added/l	$V_{NO_3^-}$ (hour^{-1})	
		Bottle #1	Bottle #2
6.6	0	1.381	0.744
6.6	20	1.780	1.719
6.6	100	0.824	1.514
6.6	200	1.410	1.501
7.6	0	1.047	1.555
7.6	20	1.548	1.456
7.6	100	1.083	1.468
7.6	200	1.112	1.488
8.1	0	0.810	0.763
8.1	20	-----	-----
8.1	100	1.212	1.342
8.1	200	1.132	1.318
9.2	0	0.657	1.079
9.2	20	1.425	1.434
9.2	100	1.056	1.594
9.2	200	1.187	1.353

TABLE XXI

A SUMMARY OF THE Ge PERTURBED EXPERIMENTS DURING 1975

Date	Perturbation	pH	# of Samples	Mean AT % ^{15}N	Standard Deviation	Mean $V_{NO_3^-}$	Standard Deviation
7/15	NaOH	8.9	8	0.3620	0.0060		
7/15	Ge	9.1	2	0.3630	0.0030		
7/15	NaOH + $^{15}NO_3^-$	9.0	3	0.5407	0.0633	4.43×10^{-4}	1.59×10^{-4}
7/15	Ge + $^{15}NO_3^-$	9.1	3	0.5792	0.0074	5.40×10^{-4}	1.8×10^{-5}
7/15	NaOH + $^{15}NH_3$	9.0	3	2.9762	0.9146	6.140×10^{-3}	2.15×10^{-3}
7/15	Ge + $^{15}NH_3$	9.1	1	3.5973	0.0439*	7.599×10^{-3}	---
7/17	Unaltered	8.2	3	0.3620	0.0061		
7/17	NaOH	9.1	3	0.3609	0.0007		
7/17	Ge	9.0	2	0.3609	0.0018		
7/17	$^{15}NO_3^-$	8.2	3	0.7620	0.0733	9.76×10^{-4}	1.79×10^{-4}
7/17	NaOH + $^{15}NO_3^-$	9.0	2	0.4641	0.0054	2.46×10^{-4}	1.3×10^{-5}
7/17	Ge + $^{15}NO_3^-$	9.0	1	0.4756	0.0080*	2.74×10^{-4}	---
7/17	$^{15}NH_3$	8.2	2	3.3786	0.0930	6.932×10^{-3}	2.14×10^{-4}
7/17	NaOH + $^{15}NH_3$	9.0	2	1.3003	0.5202	2.153×10^{-3}	1.196×10^{-3}
7/17	Ge + $^{15}NH_3$	9.1	2	3.6353	0.1928	7.522×10^{-3}	4.43×10^{-4}

*Two of three samples were lost during processing. The standard deviations of the Atom percent ^{15}N are based on the peak height measurements of the remaining sample.

Several explanations of the lack of differential responses are possible. Germanium may not have sufficiently inhibited silicon uptake to cause a change in nitrogen uptake. The diatoms may have been using an intracellular pool of silicon. The response may require a longer incubation period and/or a higher level of spike for the change to become measurable. Alternatively, diatoms may be contributing to the observed $V_{NO_3^-}$ to only a negligible extent.

The effect of pH on nitrate uptake can be seen in the data of July 17. Raising the pH to 9.0 reduced $V_{NO_3^-}$ to one third its value at a pH of 8.2.

Procedural and Instrumental Modifications

All modifications were made with two objectives in mind. The first and most important objective was to decrease the uncertainty of the mass spectrometric analysis. Many steps were required in sample preparation before meaningful data was obtained. Therefore, a reduction in the time required to process each sample and a reduction in procedural errors became significant.

Implementation of the semi-automatic Toepler pump was a significant improvement in processing the samples. Two mercury float valves of the pump and two solenoid valves eliminated four stopcocks, five stopcock manipulations per cycle of the pump, and approximately fifty manipulations per sample. This resulted in a more complete transfer of each sample and fewer samples lost from operator error.

The need for maximizing the sample size has been shown in Chapter II. Minimizing the volume of the vacuum system and optimizing the leak

rate into the ion source of the mass spectrometer allowed use of the unattenuated galvanometer. Filtering of this galvanometer was accomplished. Figures 28 and 29 are sketches of the oscillographic traces of samples prior to and after modification of the appropriate circuit (Figure 10).

The high resolution mass spectrometer is most commonly used for the purpose of measuring the relative abundances and masses of fragment ions of organic molecules. Due to the accumulation on the surfaces of the ion source of compounds with high boiling points the instrument normally has a high background which could interfere with the determination of the atom percent ^{15}N in a nitrogen gas sample. It was imperative that the ion source be removed and cleaned prior to nitrogen analysis. It was also found that turning off the ion source pressure gauge reduced the background.

Periodically high resolution mass spectrometric fragmentation patterns (10 to 1000 amu) were made on samples to verify that the gas ampoule contained 99+ % of nitrogen gas, i.e. a check of complete combustion of algae and adequate trapping of CO_2, H_2O, etc.

The mass of $^{14}N_2$ is 28.00646 amu and the mass of $^{12}C\text{-}^{16}O$ is 27.994914 amu. Carbon dioxide which will fragment to carbon monoxide is by far the most abundant contaminant from atmospheric leaks into the instrument and from inadequate trapping after sample combustion. For this reason the high resolution capabilities of the instrument were used to resolve these masses. This requires a resolution of 1:2493 at equal constituent contribution. The isotopes of ^{13}C and ^{17}O result in masses of carbon monoxide that are too close to that of $^{14}N\text{-}^{15}N$ to be resolved with the instrument. However,

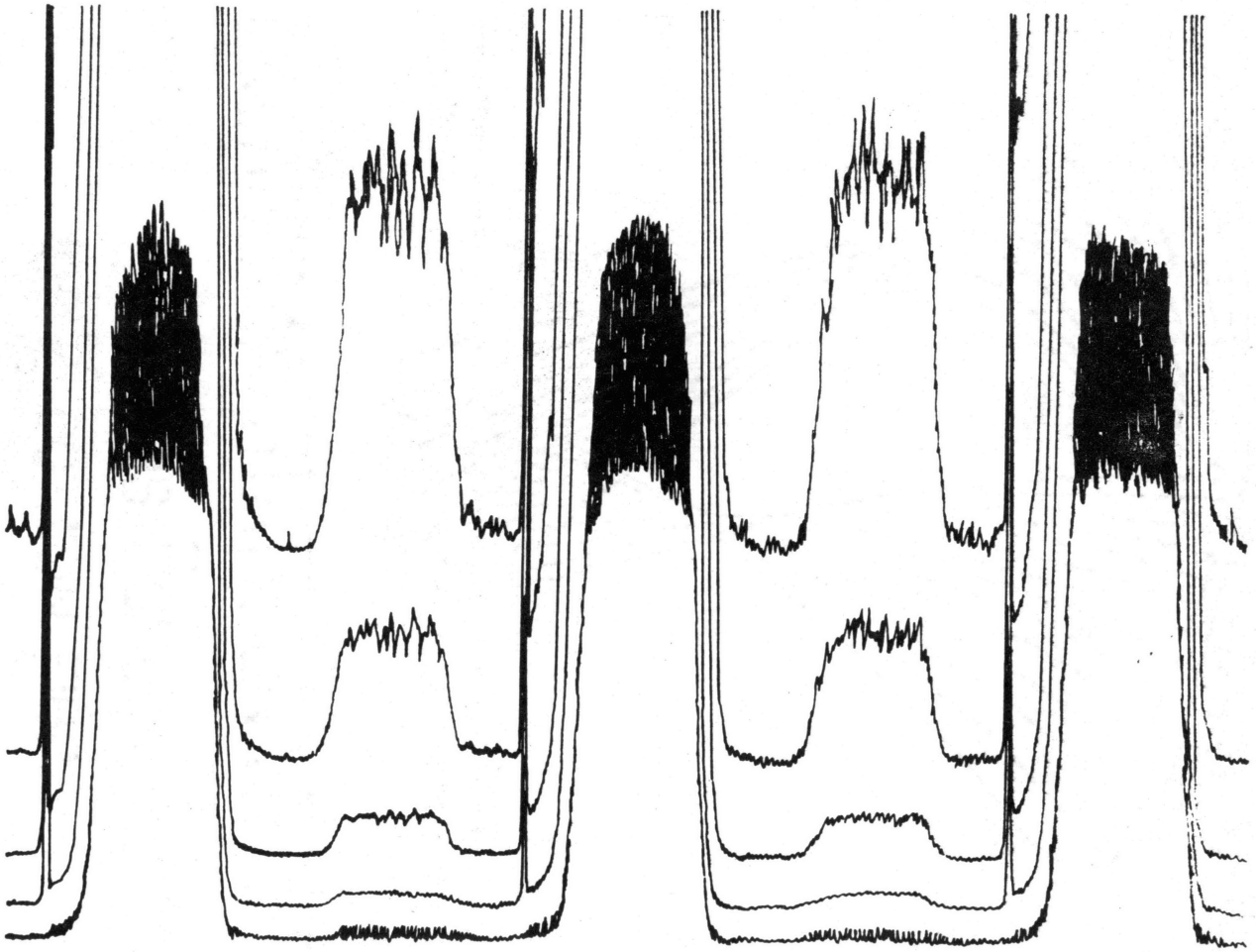

Figure 28. A Typical Oscillographic Trace Prior to the Electronic Filtration Modification

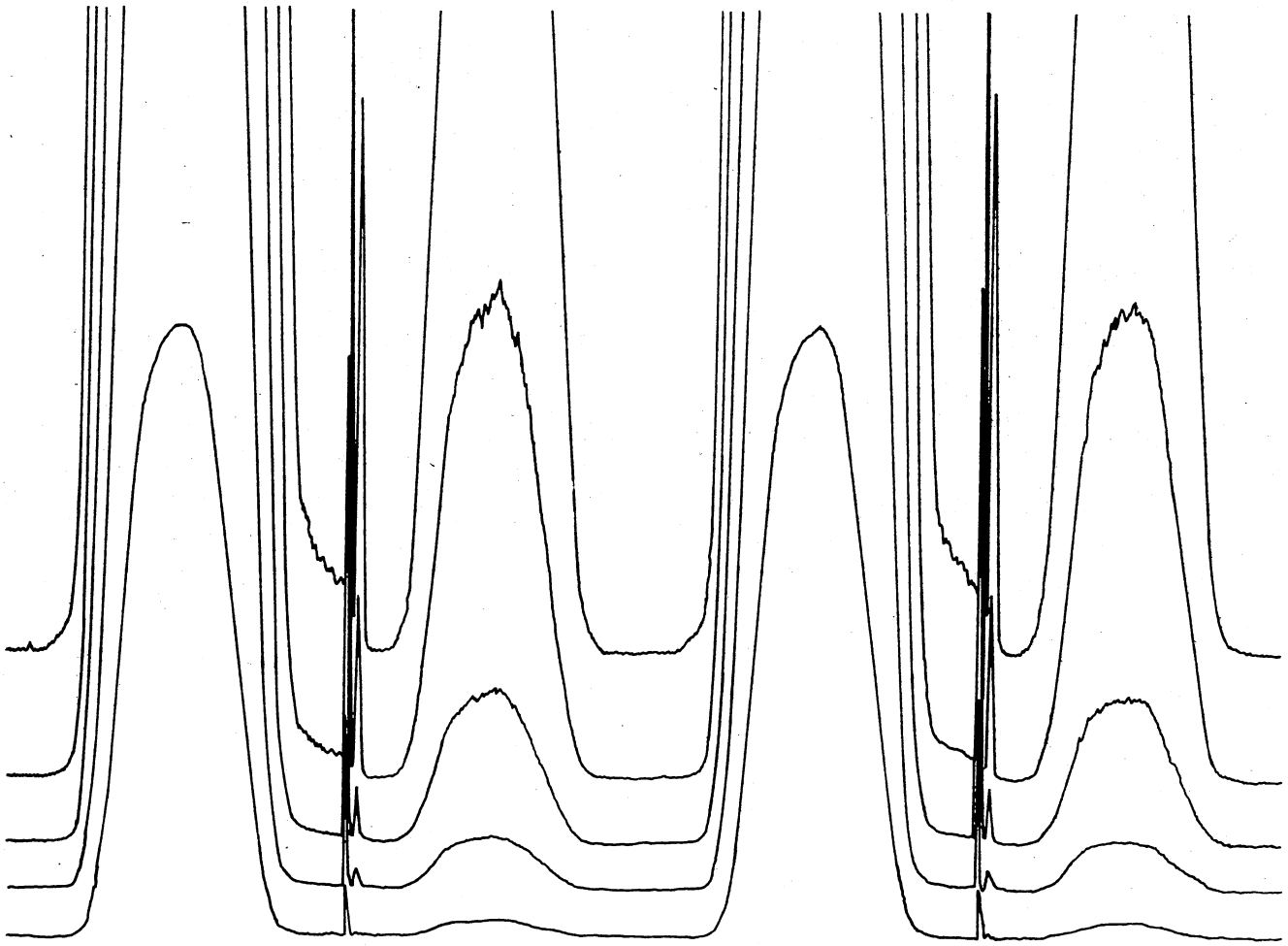

Figure 29. A Typical Oscillographic Trace After the Electronic Filtration Modification

their natural abundances, 1.107 atom percent and 0.0374 atom percent, respectively, make their contributions negligible.

It is impossible to quantitate the improvement in analyses from each modification. However, the net result of the changes made during each year can be seen in Table XXII. The value of the atom percent ^{15}N as compared to natural abundance, 0.366, is not an absolute criteria of the quality of data since one can consistently measure the heights of the oscillographic traces incorrectly. The standard deviations and confidence limits are important since they reflect consistency and improvement in the data.

TABLE XXII

A SUMMARY OF THE MASS SPECTROMETRIC ANALYSES
OF CONTROL SAMPLES FROM LAKE CARL BLACKWELL
DURING 1973, 1974, AND 1975

Year	Number of Samples	Average At % ^{15}N	Standard Deviation	Minimum At % ^{15}N	Maximum At % ^{15}N	Confidence Intervals Around Mean Values 90%	95%	99%
1973	45	0.3557	0.0417	0.2630	0.4777	± 0.0686	± 0.0818	± 0.1075
1974	20	0.3622	0.0169	0.3207	0.3919	± 0.0278	± 0.0332	± 0.0436
1975	25	0.3638	0.0081	0.3534	0.3860	± 0.0133	± 0.0157	± 0.0208

CHAPTER IV

SUMMARY

The following parameters in Lake Carl Blackwell were determined on a weekly basis during the summer of 1973 and periodically during 1974 and 1975: light intensity; temperature; ambient concentrations of nitrate, nitrite, and ammonia; and standing crop of phytoplankton in terms of chlorophyll a and particulate nitrogen. A consistent set of values of K_s and V_{max} for nitrate uptake at 0.8 m depth was found using the ^{15}N incubation technique followed by mass spectrometric analyses and regression analyses based on the Woolf transformation of the Michaelis-Menten equation. Triplicated experiments showed variability in $V_{NO_3^-}$ at less than 10%.

The effect of chlorine on $V_{NO_3^-}$ was shown in three experiments. Additions of 0.101 mg Cl_2/1 caused an increase in K_s from 3.27 to 32.6 µg at N/1. Additions of 0.013 mg Cl_2/1 increased K_s from 12.0 to 24.7 µg at N/1. Increasing concentrations of chlorine from 0.1 to 3.00 mg Cl_2/1 at an $^{15}NO_3^-$ level 10% above ambient nitrate concentration gave an LC-50 value of 0.028 mg Cl_2/1.

A one compartment model of nitrate uptake was applied to the data of 1973. The model considered effects of light flux, temperature, and ammonia concentrations. Factoring out these effects gave a fluctuating pattern for saturated nitrate uptake by algae in mid-summer which may have been species dependent. The effect of chlorine on V_{max} and K_s

from the experiments in late June and early July were used to simulate by interpolation the presence of chlorine for a full month. The resultant nitrate flux was significant in implying that these concentrations of chlorine could reduce algal density significantly.

During 1974 the effect of chlorine and chloramine on the uptake of nitrate and ammonia was investigated. Comparison of nitrate flux data for two experimental dates indicated that chloramine inhibited nitrate uptake to a greater extent than does chlorine at low concentrations. Additions of 0.1 mg chloramine/l were found to inhibit uptake of nitrate to a greater extent than additions of chlorine at the same concentration. Varying concentrations of chlorine from 0.0 to 0.5 mg Cl_2/l while adding $^{15}NH_3$ at 5.46% of ambient gave an LC-50 value of 0.101 mg Cl_2/l for ammonia uptake.

The uptake rate of nitrate as a function of free and bound chlorine was found by simultaneously varying their concentrations. The equation of best fit (correlation coefficient = 0.79) describing the response at stoichiometry below the "breakpoint" phenomenon was found to be

$$V_{NO_3^-} = 2.45\ (10^{-4}) - 3.61\ (10^{-3})\ y + 1.13\ (10^{-2})\ y^2 + 8.56\ x\ y$$

where x is the concentration of free chlorine in ppm and y is the concentration of bound chlorine in ppm. A three dimensional plot of this equation was shown in Figure 24. It was felt that this equation more accurately described the response of $V_{NO_3^-}$ to concentrations of bound chlorine than free chlorine.

The data describing the trends of response were extrapolated beyond the experimental conditions of 1974 to show nitrate uptake rates above the breakpoint stoichiometry. The resulting surface, Figure 25, was tested during 1975. Various theoretical concentrations of free and bound chlorine bracketing the breakpoint stoichiometry gave data which described $V_{NO_3^-}$ by the following equation of best fit (correlation coefficient = 0.94):

$$V_{NO_3^-} = 0.033 + 3.64\ (10^{-2})\ x - 9.13\ (10^{-2})\ xy - 1.89\ (10^{-1})\ x^2 + 5.04\ (10^{-1})\ x^2\ y,$$

where x and y are the concentrations in ppm of free and bound chlorine, respectively. Three dimensional plots of the equation, Figures 26 and 27, have the general characteristics of the surface predicted in 1974, Figure 25.

Combining the information from 1974 and 1975, it was concluded that $V_{NO_3^-}$ initially increases as the breakpoint reaction lowers the concentrations of the toxic species. Above the breakpoint stoichiometry $V_{NO_3^-}$ again decreases with increasing concentration of free chlorine. The uptake rate of nitrate is completely inhibited at concentrations of approximately 0.2 ppm free chlorine and/or 0.5 ppm bound chlorine.

During 1974, additions of 100 mg PO_4^{-3}-P/1 were made to duplicated incubation bottles. Nitrate uptake was not significantly effected and it was therefore concluded that phosphate was not limiting to phytoplankton growth in Lake Carl Blackwell at concentrations observed.

A three dimensional analysis of $V_{NO_3^-}$ as a function of pH and orthophosphate was attempted during 1975. Results were found to be erratic and non-reproducible.

Germanium at high Ge/Si ratios inhibits silicone uptake and diatom growth. In an attempt to measure the contribution of nitrate and ammonia uptake by diatoms to the overall community uptake rates, germanium was dissolved in NaOH and added to incubation bottles of lake water during two experiments. Various controls were simultaneously incubated. No measureable difference could be detected between samples incubated with ^{15}N plus NaOH and ^{15}N plus NaOH and germanium for nitrate or ammonium uptake experiments. However, a decrease in pH was found to decrease nitrate uptake significantly. Samples incubated with only $^{15}NO_3^-$ (pH = 8.2) had uptake rates of approximately 9.76×10^{-4} hr^{-1}; samples incubated with $^{15}NO_3^-$ plus NaOH (pH = 9.1) had uptake rates of approximately 2.46×10^{-4} hr^{-1}.

An error propagation involving the determination of the atom percent excess ^{15}N in the initial incubation solution and in the final particulate sample was developed. This allowed an estimate of the precision of the Michaelis-Menten constants as well as the quantitative determination of the uncertainty in each experimental phase. This analysis showed that the mass spectrometric determinations were the major source of error for most samples.

Various instrumental and procedural modifications were made to improve the overall procedure and reduce the uncertainty in the mass spectrometric analyses.

A semi-automatic Toepler pump with mercury float valves was built and installed to be used in the collection of nitrogen gas after sample

pyrolysis. This eliminated approximately 50 stopcock manipulations per sample. The vacuum system was redesigned to have a minimal volume. Nitrogen cold traps of the tube within a tube design were replaced with radiator coil traps which minimized the volume to surface area ratio thus increasing the efficiency of trapping undesired gases such as water vapor, CO_2, etc. These modifications resulted in a more complete transfer and collection of purer samples in a shorter time with less chance of sample loss due to operator error.

The leak rate between the sample chamber and the ion source of the mass spectrometer was optimized for the sample sizes obtained from typical incubation experiments. More of the sample was used in the analyses which was reflected by an increase in signal intensities without increasing the absolute uncertainty in quantifying these signals.

Time averaging of the signals was incorporated into the procedure to ensure that the decay of the sample in the ion source would not give erroneous isotope ratios. Six scans of m/e 29 and five scans of m/e 28 of each sample were collected for analysis.

The use of larger amounts of sample allowed a reduction in the applied voltage per stage of the electron multiplier. This gave the further advantageous result of decreasing the noise per unit deflection on the strip chart. Tank nitrogen samples were processed at this new and higher range of ion source pressures to test for the possibility of space charging occurring in the ion source. No mass discrimination as a function of source pressure was detected.

Increased sample sizes made desirable the use of the unamplified m/e 28 signal. The appropriate circuit was modified so that the

existing filtration network would be used by this galvanometer. The decrease in the signal to noise ratio greatly decreased the uncertainty in measuring the signal with the unamplified galvanometer.

A significant procedural change was made in the mass spectrometric analysis. The mass of $^{14}N_2$ is 28.00646 amu and the mass of $^{12}C\text{-}^{16}O$ is 27.994914 amu. Carbon dioxide which will fragment to carbon monoxide is by far the most abundant contaminant from atmospheric leaks into the instrument and potentially from inadequate trapping after sample combustion. The high resolution capabilities of the instrument were used to resolve these masses. This required a resolution of 1:2493 at equal constituent contribution.

The decrease in error of the mass spectrometric analyses from each procedural or instrumental modification is impossible to quantitate. However, the overall improvement can be seen in the standard deviations of control samples during the three years. The standard deviation was decreased from 0.0417 atom percent ^{15}N (for 45 samples) in 1973, to 0.0169 (for 20 samples) in 1974, and finally to 0.0081 (for 25 samples) in 1975. The values represent a reduction of over 80 percent in the variability of the uptake rates due to the mass spectrometric analyses. Many of the conclusions from 1974 and 1975 experiments would not have been possible without this improvement.

BIBLIOGRAPHY

Azam, F., B. B. Hemmingsen, and B. E. Volcani, "Role of Silicon in Diatom Metabolism: V. Silicic Acid Transport and Metabolism in Heterotrophic Diatom Nitzschia alba.," Arch. Microbiol., 97, 103 (1974).

Azam, F., and B. E. Volcani, "Role of Silicon in Diatom Metabolism: VI. Active Transport of Germanic Acid in Heterotrophic Diatom Nitzschia alba.," Arch. Microbiol., 101, 1 (1974).

Aziz, K. M. S., "Diarrhea Toxin Obtained from a Waterbloom-Producing Species, Microcystis aeruginosa Kutzing," Science, 183, 1206 (1974).

Bard, A. J., and D. M. King, "General Digital Computer Program for Chemical Equilibrium Calculations," J. Chem. Ed., 42, 127 (1965).

Barnes, R. A., P. A. Atkins, and D. A. Scherger, "Ammonia Removal In a Physical-Chemical Wastewater Treatment Process," Environmental Protection Technology Series EPA-R2-72-123, U. S. Environmental Protection Agency, Washington, D. C., November, 1972.

Barsdate, R. J., and R. C. Dugdale, "Rapid Conversion of Organic Nitrogen to N_2 for Mass Spectrometry: an Automated Dumas Procedure," Anal. Biochem., 13, 1 (1965).

Betzer, N., and Y. Kott, "Effect of Halogens on Algae - II. Cladophora Sp.," Water Res., 3, 257 (1969).

Beynon, J. H., Mass Spectrometry and Its Applications to Organic Chemistry, Elsevier Publishing Co., N. Y., 1960.

Bremner, J. M., "Isotope Ratio Analysis of Nitrogen in Nitrogen-15 Tracer Analysis," in Methods of Soil Analysis. Part 2, C. A. Black, ed., Am. Soc. Agronomy, Madison, Wisc., 1965, pp. 1256-1288.

Brezonik, P. L., Chapter I, "Nitrogen: Sources and Transformations," in Nutrients in Natural Waters, H. E. Allen and J. R. Krammer, eds., John Wiley and Sons, N. Y., 1972, pp. 1-50.

Brook, A. J., and A. L. Baker, "Chlorination at Power Plants: Impact on Phytoplankton Productivity," Science, 176, 1414 (1972).

Brungs, W. A., "Effects of Residual Chlorine on Aquatic Life," JWPCF, 45, 2180 (1973).

Burris, R. H., "Failure of Barley to Fix Molecular N^{15}," Science, 94, 238 (1941).

Burris, R. H., F. J. Eppling, H. B. Wahlin, and P. W. Wilson, "Detection of Nitrogen Fixation with Isotopic Nitrogen," J. Biol. Chem., 148, 349 (1943).

Burris, R. H., and C. E. Miller, "Application of N^{15} to the Study of Biological Nitrogen Fixation," Science, 93, 114 (1941).

Caperon, J., "Population Growth in Micro-organisms Limited by Food Supply," Ecology, 48, 715 (1967).

Caperon, J., "Population Growth Response of Isochrysis galbana to Nitrate Variation at Limiting Concentrations," Ecology, 49, 866 (1969).

Caperon, J., and J. Meyer, "Nitrogen-Limited Growth of Marine Phytoplankton - I. Changes in Population Characteristics with Steady-State Growth Rate," Deep Sea Res., 19, 601 (1972).

Caprioli, R. M., "Determination of Abundance and Application of Stable Isotopes," Finnigan Spectra, 3, 1 (1973).

Carlson, R. M., Organic Compounds Produced During Wastewater Chlorination, The Symposium on the Identification and Transformation of Aquatic Pollutants, Athens, Georgia, April 8, 1974.

Carpenter, E. J., and R. R. L. Guillard, "Intraspecific Differences in Nitrate Half-Saturation Constants for Three Species of Marine Phytoplankton," Ecology, 52, 183 (1971).

Carpenter, E. J., and J. J. McCarthy, "Nitrogen Fixation and Uptake of Combined Nitrogeneous Nutrients by Oscillatoria (Trichodesmium) thiebautii in the Western Sargasso Sea," Limnol. and Oceanogr., 20, 389 (1975).

Carpenter, E. J., B. B. Peck and S. J. Anderson, "Cooling Water Chlorination and Productivity of Entrained Phytoplankton," Marine Biology, 16, 37 (1972).

Cole, B. P., "Vertical Variation in Nitrate Uptake by Natural Populations of Reservoir Phytoplankton," M.S. Thesis, Oklahoma State University, December, 1975.

Cotton, F. A., and G. Wilkinson, Advanced Inorganic Chemistry, A Comprehensive Text, 2nd Edition, John Wiley and Sons, N. Y., 1967.

Di Toro, D. M., Donnor, D. J., and Thoman, R. V., "A Dynamic Model of the Phytoplankton Population in the Sacramento-San Joaquine Delta," Adv. Chem. Ser., 106, 131 (1971).

Dowd, J. D., and D. S. Riggs, "A Comparison of Estimates of Michaelis-Menten Kinetic Constants From Various Linear Transformations," J. Biol. Chem., 240, 863 (1965).

Draley, J. E., "The Treatment of Cooling Waters with Chlorine," U. S. Atomic Energy Commission ANL-ES-12, February, 1972.

Droop, M. R., "Vitamin B_{12} and Marine Ecology: the Response of Monochrysis Lutheri," J. Mar. Biol. Ass. U. K., 41, 69 (1961).

Droop, M. R., "Vitamin B_{12} and Marine Ecology. IV. The Kinetics of Uptake, Growth and Inhibition in Monochrysis Lutheri," J. Mar. Biol. Ass. U. K., 48, 689 (1968).

Dugdale, R. C., "Nutrient Limitation in the Sea; Dynamics Identification and Significance," Limnol. Oceanogr., 12, 685 (1967).

Dugdale, V. A., and R. C. Dugdale, "Nitrogen Metabolism in Lakes. II. Role of Nitrogen Fixstion in Sanctuary Lake, Pennsylvania," Limnol. Oceanogr., 7, 170 (1962).

Dugdale, V. A., and R. C. Dugdale, "Nitrogen Metabolism in Lakes. III. Tracer Studies of the Assimilation of Inorganic Nitrogen Sources," Limnol. Oceanogr., 10, 53 (1965).

Dugdale, R., V. Dugdale, J. Neess, and J. Goering, "Reports: Nitrogen Fixation on Lakes," Science, 130, 859 (1959).

Dugdale, R. C., and J. J. Goering, "Uptake of New and Regenerated Forms of Nitrogen in Primary Productivity," Limnol. Oceanogra., 12, 196 (1967).

Dugdale, R. C., D. W. Menzel, and J. H. Ryther, "Nitrogen Fixation in the Sargasso Sea," Deep Sea Res., 7, 297 (1961).

Eppley, R. W., A. F. Carlucci, O. Holm-Hansen, D. Kiefer, J. J. McCarthy, E. Venrick, and P. M. Williams, "Phytoplankton Growth and Composition in Shipboard Cultures Supplied with Nitrate, Ammonium or Urea as the Nitrogen Source," Limnol. Oceanol., 16, 741 (1971).

Eppley, R. W., and J. L. Coatsworth, "Uptake of Nitrate and Nitrite by Ditylum brightwellii-Kinetics and Mechanisms," J. Phycol., 4, 151 (1968).

Eppley, R. W., and E. H. Renger, "Nitrogen Assimilation of an Oceanic Diatom in Nitrogen-Limited Continuous Culture," J. Phycol., 10, 15 (1974).

Eppley, R. W., E. H. Renger, E. L. Venrick, and M. M. Mullin, "A Study of Plankton Dynamics and Nutrient Cycling in the Central Gyre of the North Pacific Ocean," Limnol. Oceanogr., 18, 534 (1973).

Eppley, R. W., J. N. Rogers, and J. McCarthy, "Inorganic Nitrogen Assimilation of Ditylum brightwellii, a Marine Diatom," J. Phycol., 6, 344 (1970).

Eppley, R., J. Rogers, and J. McCarthy, "Half-Saturation Constants for Uptake of Nitrate and Ammonium by Marine Phytoplankton," Limnol. Oceanogr., 14, 912 (1969).

Eppley, R. W., and W. H. Thomas, "Comparison of Half-Saturation Constants for Growth and Nitrate Uptake of Marine Phytoplankton," J. Phycol., 5, 375 (1969).

Falkowski, P. G., "Nitrate Uptake in Marine Phytoplankton: Comparison of Half-Saturation Constants from Seven Species," Limnol. Oceanogr., 20, 412 (1975).

Friedlander, G., J. W. Kennedy, and J. M. Miller, Nuclear and Radiochemistry, 2nd Edition, John Wiley and Sons, N. Y., 1964.

Gardner, W. S., and G. F. Lee, "The Role of Amino Acids in the Nitrogen Cycle of Lake Mendota," Limnol. Oceanogr., 20, 379 (1975).

Goering, J., R. Dugdale and D. Menzel, "Cyclic Diurnal Variation in Uptake of Ammonia and Nitrate by Photosynthetic Organisms in the Sargasso Sea," Limnol. Oceanogr., 9, 448 (1964).

Goodnight, J. H., A User's Guide to the Stistical Analysis System, Student Supply Stores, Raleigh, N. C., 1972.

Grant, B. R., and I. M. Turner, "Light-Stimulated Nitrate and Nitrite Assimilation in Several Species of Algae," Comp. Biochem. Physiol., 29, 995 (1969).

Griffin, A. G., and N. S. Chamberlin, "Some Chemical Aspects of Breakpoint Chlorination," J. NEWWA, 55, 371 (1941).

Hamilton, D. H., Jr., D. A. Flemer, C. W. Keefe, J. A. Mihursky, "Power Plants: Effects of Chlorination on Estuarine Primary Production," Science, 169, 197 (1970).

Hattori, A., "Adaptive Formation of Nitrate Reducing System in Anabaena cylindrica," Plant and Cell Physiol., 3, 371 (1962).

Hirayama, K., and R. Hirano, "Influences of High Temperature and Residual Chlorine on Marine Phytoplankton," Marine Biology, 7, 205 (1970).

Hobbie, J. E., and C. C. Crawford, "Amino Acid Flux in an Estuary," Science, 195, 1463 (1968).

Hoering, T. C., and H. T. Ford, "The Isotope Effect in the Fixation of Nitrogen by Azoto bacter," J. Amer. Chem. Soc., 82, 376 (1960).

Holm-Hansen, O., "Determination of Particulate Organic Nitrogen," Limnol. Oceanogr., 13, 175 (1968).

IBM, System/360, Continuous System Modeling Program (360A-CS-16X), Users Manual, Edition H20-03672, 1968.

Jolley, R. L., Chlorination Effects on Organic Constituents in Effluents from Domestic Sanitary Sewage Treatment Plants, ORWL-TM-4290, Oak Ridge National Laboratory, 1973.

Kralova, M., "The Investigation of ^{15}N in Plant Material Mass Spectrometric Analysis," Rostlinna Vyroba, 13, 427 (1967).

Lehman, J. T., D. B. Botkin, and G. E. Likens, "The Assumptions and Rationales of a Computer Model of Phytoplankton Population Dynamics," Limnol. Oceanogr., 20, 343 (1975).

Lewin, J., "Silicon Metabolism in Diatoms: V. Germanium Dioxide, a Specific Inhibitor of Diatom Growth," Phycol., 6, 1 (1966).

Lui, N. S. T. and O. A. Roels, "Nitrogen Metabolsim of Aquatic Organisms. II. The Assimilation of Nitrate, Nitrite and Ammonia by Biddulphia aurita," J. Phycol., 8, 259 (1972).

MacIsaac, J., and R. C. Dugdale, "The Kinetics of Nitrate and Ammonia Uptake by Natural Populations of Marine Phytoplankton," Deep Sea Research, 16, 45 (1969).

MacIsaac, J. J., and R. C. Dugdale, "Interactions of Light and Inorganic Nitrogen in Controlling Nitrogen Uptake in the Sea," Deep Sea Research, 19, 209 (1972).

Mahler, H. R., and E. H. Corder, "Chapter 17: Metabolism of the Amino Acids," Biological Chemistry, 2nd Edition, Harper and Row, N. Y., pp. 756-762, 1971.

McCarthy, J. J., and R. W. Eppley, "A Comparison of Chemical Isotopic and Enzymatic Methods for Measuring Nitrogen Assimilation of Marine Phytoplankton," Limnol. Oceanogr., 17, 371 (1972).

McNabb, C. D., "Enumeration of Fresh Water Phytoplankton Collected on the Membrane Filter," Limnol. Oceanogr., 5, 57 (1960).

Monod, J., "La Croissance des Cultures Bacteriennes," Herman, Paris, 210 pp., 1942.

Morgan, R. P., and R. G. Stross, "Destruction of Phytoplankton in the Cooling Water Supply of a Steam Electric Station," Chesapeake Sci., 10, 165 (1969).

Morris, I., and J. Ahmed, "The Effect of Light on Nitrate and Nitrite Assimilation by Chlorella and Ankistrodesmus," Physiologpia Plantarum, 22, 1166 (1969).

Neess, J. C., R. C. Dugdale, V. A. Dugdale, and J. J. Goering, "Nitrogen Metabolism in Lakes. I. Measurement of Nitrogen Fixation with ^{15}N," Limnol. Oceanogr., 7, 1630 (1962).

North, B. B., "Primary Amines in California Coastal Waters: Utilization by Phytoplankton," Limnol. Oceanogr., 20, 20 (1975).

Norton, J. L., "The Distribution, Character and Abundance of Sediments in a 3,000-Acre Impoundment in Payne County, Oklahoma," M.S. Thesis, Oklahoma State University, 1968.

Odum, E. P., Fundamentals of Ecology, 2rd. ed., W. B. Saunders Co., Philadelphia, Pa., pp. 43-77, 1971.

Oines, R. K., PLOTZ (a Fortran IV Subroutine which is compatible with the IBM Calcomp 565 digital plotter), Research Foundation, Oklahoma State University.

Painter, H. A., "A Review of Literature on Inorganic Nitrogen Metabolism in Microorganisms," Water Research, 4, 393 (1970).

Parsons, T. R., and J. D. H. Strickland, "Duscission of Spectrophotometric Determination of Marine-Plant Pigments, with Revised Equations for Ascertaining Chlorophylls and Carotenoids," Marine Research, 21, 155 (1963).

Pavlou, S. P., G. E. Friedrich, and J. J. MacIsaac, "Quantitative Determination of Total Organic Nitrogen and Isotope Enrichment in Marine Phytoplankton," Anal. Biochem., 61, 16 (1974).

Pressley, T. A., D. F. Bishop, A. P. Pinto, and A. F. Cassel, "Ammonia-Nitrogen Removal by Breakpoint Chlorination," Environmental Protection Technology Series EPA-670/2-73-058, U. S. Environmental Protection Agency, Washington, D. C., September, 1973.

Procházková, L., P. Blazka, and M. Králová, "Chemical Changes Involving Nitrogen Metabolism in Water and Particulate Matter During Primary Production Estimates," Limnol. Oceanogr., 15, 797 (1970).

Rice, G. K., "Continuous Physicochemical Monitoring and Modeling of an Aquatic Ecosystem," Ph.D. Dissertation, Oklahoma State University, 1972.

Ruben, S., W. Z. Hassid, and M. D. Kamen, "Radioactive Nitrogen in the Study of N_2 Fixation by Nonleguminous Plants," Science, 91, 578 (1940).

Segel, I. H., "Appendix: Enzyme Kinetics III," Biochemical Calculations, John Wiley and Sons, Inc., N. Y., pp. 366, 1968.

Sharma, V. K., and H. D. Kumar, "Influence of pH and Concentration of Phosphate Ions on Growth and Nitrogen Fixation in a Blue-Green Alga Cylindrospermum majus," Experimentia, 31, 182 (1975).

Solorzano, L., "Determination of Ammonia in Natural Waters by the Phenolhypochlorite Method," Limnol. Oceanogr., 14, 799 (1969).

Sorge, E. V., "The Status of Thermal Discharges East of the Mississippi River," Chesapeake Sci., 10, 131 (1969).

Sprague, H. A., and H. W. Divary, "The Chemical Composition of Chlorella, Effects of Environmental Conditions," Plant Physiol., 24, 120 (1969).

Steemann-Nielsen, E. J., "The Use of Radioactive Carbon (^{14}C) for Measuring Organic Production in the Sea," J. Conseil, Conseil Perm. Inter. Exploration Mer., 18, 177 (1952).

Strickland, J. D. H., and T. R. Parsons, A Practical Handbook of Seawater Analysis, Bulletin 167, Fisheries Research Board, Ottawa, Canada, 1968.

Syrett, P. J., and I. Morris, "The Inhibition of Nitrate Assimilation by Ammonium in Chlorella," Biochem. Biophys. Acta, 67, 566 (1963).

Toetz, D. W., "The Limnology of Nitrogen in an Oklahoma Reservoir: Nitrogenase Activity and Related Limnological Factors," American Midland Naturalist, 89, 369 (1972).

Toetz, D. W., L. Varga, and D. Loughran, "Half-Saturation Constants for Uptake of Nitrate and Ammonia by Reservoir Plankton," Ecology, 54, 903 (1973).

Tsai, C., Water Quality and Fish Life Below Sewage Outfalls, Progress Report, National Resources Institute, Univ. of Maryland, College Park, Md., 1971.

Uphaus, R. A., E. Flaumenhaft, and J. J. Katz, "Isotope (D, 15-N, 13-C, and 18-O) Effects in Alga Chlorella Vulgarus," Biochim. Biophys. Acta., 141, 625 (1967).

Urry, G., and W. H. Urray, "Automatic Toepler Pump of Improved Design," The Review of Scientific Instruments, 27, 819 (1956).

Vanderhoef, L. N., P. J. Leibson, R. J. Musil, C. Y. Huang, R. E. Fiehweg, J. W. Williams, D. L. Wackwitz, and K. T. Mason, "Diurnal Variation in Algal Acetylene Reduction (Nitrogen Fixation) in Situ," Plant Physiol., 55, 273 (1975).

Varga, L. P., and D. W. Toetz, Biogeochemistry of a Reservoir Ecosystem, Report No. 5, U. S. Atomic Energy Commission, ORO-4254-16, 1974.

Varga, L. P., and D. W. Toetz, Biogeochemistry of a Reservoir Ecosystem, Report No. 6, U. S. Atomic Energy Commission, ORO-4254-25, 1975.

Varga, L. P., and F. C. Veatch, "Nature of Hafnium-Chloranilic Acid Metallochrome by Matrix Rank, Contour Mapping and Iterative Analysis of Absorption Spectra," Anal. Chem., 39, 1101 (1967).

White, G. C., Handbook of Chlorination, Van Nostrand Reinhold Co., N. Y., pp. 190-224, 1972.

Whitfield, P. D., "Analysis of Chlorine and Chloramine in a Natural Water System," M.S. Thesis, Oklahoma State University, 1975.

APPENDIX A

THE DERIVATION OF THE MICHAELIS-MENTEN EQUATION

Assume that the sequence of events in the overall enzyme-catalyzed reaction is the reversible combination of the enzyme, E, and substrate, S, to form an ES complex which then reversibly decomposes to form free enzyme and product, P. This is summarized by Equation 1 in which k_1, k_2, k_3, and k_4 are first order rate constants.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E \qquad [1]$$

Also assume a steady-state situation such that

$$k_1 [E] [S] + k_4 [E] [P] = k_2 [ES] = k_3 [ES] \qquad [2]$$

By simplification,

$$[E] (k_1 [S] + k_4 [P]) = [ES] (k_2 + k_3) . \qquad [3]$$

$$[ES]/[E] = \frac{k_1 [S] + k_4 [P]}{k_2 + k_3} . \qquad [4]$$

$$[ES]/[E] = \frac{k_1 [S]}{k_2 + k_3} + \frac{k_4 [P]}{k_2 + k_3} . \qquad [5]$$

Assume that at time = 0, [P] = 0. Therefore,

$$[E[/[ES] = \frac{k_2 + k_3}{k_1 [S]} . \qquad [6]$$

Let $(k_2 + k_3)/k_1 = K_s$. Therefore, $[E]/[ES] = K_s/[S]$. Let $[E]_T$ = total enzyme concentration. Therefore,

$$[E]_T = [E] + [ES] \qquad [7]$$

or,

$$[E] = [E]_T - [ES] \ . \qquad [8]$$

Dividing by [ES],

$$[E]/[ES] = \frac{[E]_T - [ES]}{[ES]} = \frac{[E]_T}{[ES]} - 1. \qquad [9]$$

Therefore,

$$\frac{[E]_T}{[ES]} - 1 = \frac{K_s}{[S]} \ . \qquad [10]$$

The maximum initial velocity, V_{max}, is proportional to the total enzyme present and the velocity at any given substrate concentration is proportional to the amount of enzyme present as the ES complex. Therefore, one can substitute V_{max}/v for $[E]_T/[ES]$:

$$V_{max}/v = \frac{K_s}{[S]} + 1 \ . \qquad [11]$$

Thus, the basic equation can be written as

$$v = \frac{V_{max}[S]}{K_s + [S]} \ . \qquad [12]$$

APPENDIX B

THE COMPUTER PROGRAM FOR THE CALCULATION AND PLOTTING OF THE MICHAELIS-MENTEN CONSTANTS

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      C     THIS PROGRAM WAS WRITTEN BY L P VARGA, OKLA. S. UNIV., JUNE, 1973
      C     AND MODIFIED BY JAMES MICHAEL PIERCE, SPRING, 1975,
      C     FOR THE PROJECT 'BIOGEOCHEMISTRY OF A RESERVOIR ECOSYSTEM'
      C     U S ATOMIC ENERGY COMMISSION CONTRACT AT-(40-1)-4254
0001        DIMENSION PKHT28(5),          PKHT29(6),          R(35),PRCN15(35)
           1,SN15(35),CP28(5),CP29(6),RAVG(35),PNAVG(35),E(10,35),EA(35),AVG(6
           1),OOV(35), OOS(35),CAV28(35),CAV29(35),DEL28(5),DEL29(6),DELSQ8(5)
           1,DELSQ9(6),CAV8SQ(35),CAV9SQ(35),SUMSQ8(35),SUMSQ9(35),VAR28(35)
           1,VAR29(35),VARR(35),VARN15(35),EAV28(35),EAV29(35),SMSQ8E(35),
           1 SMSQ9E(35),PCN15E(35),VAN15E(35),B(8),SIG(8),AB(4),DEL2(4),SE(35)
           1,VSTN15(35),AF(35),AI(35),VARAF(35),VARAI(35),VNO3(35),VVNO3(35)
           1,SIGOOV(35),X(35),Y(35),Z(1),RELEAF(35),RELEAI(35),SIGV(35), S(35)
           1,SOV(35),SIGSOV(35),VOS(35)
C002        DATA IE/'E'/,IC/'C'/
0003      2 FORMAT('1')
0004        AVGRC = 0.0
0005        AVGNC = 0.0
0006        AVVNC = 0.0
0007        NCTRL = 0
0008        READ(5,102) NPLOT, NH3
0009      102 FORMAT(6X,I1,6X,I1)
      C     NPLOT=1, WOOLF PLOT -- NPLOT=2, LINEWEAVER-BURK PLOT
      C     NPLOT=3, HOFSTEE PLOT -- NPLOT=4, TRY ALL THREE
0010        NSCAN8 = 5
0011        NSCAN9 = 6
0012      3 READ(5, 1,END=99)KODE,ID,ATT28,ATT29,VSTD15,(PKHT28(I),I=1,NSCAN8)
           1,(PKHT29(I),I=1,NSCAN9),BKGD28,BKGD29
0013      1 FORMAT(A1,A4,1X,F3.0,1X,F2.0,F3.0,5(1X,F4.1),6(1X,F4.1),2X,F4.1,
           12X,F2.1)
0014        IF(KODE.EQ.IE) GO TO 10
0015       22 NCTRL = NCTRL + 1
0016        CAV28(NCTRL)=0.0
0017        CAV29(NCTRL)=0.0
0018        SUMSQ8(NCTRL)=0.0
0019        SUMSQ9(NCTRL)=0.0
0020        RAVG(NCTRL) = 0.0
0021        PNAVG(NCTRL) = 0.0
0022        DO 5 I = 1,NSCAN8
0023        CP28(I) = PKHT28(I)*ATT28 - BKGD28
0024      5 CAV28(NCTRL)=CAV28(NCTRL)+CP28(I)
0025        DO 6 I = 1,NSCAN9
0026        CP29(I) = PKHT29(I)*ATT29 - BKGD29
0027      6 CAV29(NCTRL)=CAV29(NCTRL)+CP29(I)
0028        CAV28(NCTRL)=CAV28(NCTRL)/FLOAT(NSCAN8)
C029        CAV29(NCTRL)=CAV29(NCTRL)/FLOAT(NSCAN9)
0030        CAV8SQ(NCTRL)=CAV28(NCTRL)*CAV28(NCTRL)
0031        CAV9SQ(NCTRL)=CAV29(NCTRL)*CAV29(NCTRL)
0032        DO 27 I=1,NSCAN8
0033        DEL28(I)=CAV28(NCTRL)-CP28(I)
0034        DELSQ8(I)=DEL28(I)*DEL28(I)
0035       27 SUMSQ8(NCTRL)=SUMSQ8(NCTRL)+DELSQ8(I)
0036        DO149 I = 1,NSCAN9
0037        DEL29(I)=CAV29(NCTRL)-CP29(I)
0038        DELSQ9(I)=DEL29(I)*DEL29(I)
0039      149 SUMSQ9(NCTRL)=SUMSQ9(NCTRL)+DELSQ9(I)
0040        VAR28(NCTRL)=SUMSQ8(NCTRL)/FLOAT(NSCAN8-1)
0041        VAR29(NCTRL)=SUMSQ9(NCTRL)/FLOAT(NSCAN9-1)
0042        R(NCTRL) = CAV28(NCTRL)/CAV29(NCTRL)
```

```
0043            VARR(NCTRL)=R(NCTRL)*R(NCTRL)*(VAR28(NCTRL)/(CAV8SQ(NCTRL)) +
                1           VAR29(NCTRL)/(CAV9SQ(NCTRL)))
0044            PRCN15(NCTRL) = 100./(2.*R(NCTRL)+1.)
0045            VARN15(NCTRL)=(-200./((2.*R(NCTRL)+1.)**2))**2*VARR(NCTRL)
0046            SN15(NCTRL) = SQRT(VARN15(NCTRL))
0047            AVGRC = R(NCTRL) + AVGRC
0048            AVGNC = PRCN15(NCTRL) + AVGNC
0049            AVVNC = VARN15(NCTRL) + AVVNC
0050            IF(NCTRL .GT. 1) GO TO 104
0051            WRITE(6,4)
0052        4   FORMAT(1H-,T5,'CONTROLS', /,T20,'R',T39,'AT %',T58,'SIG AT %',/)
0053            WRITE(6,7)
0054      104   WRITE(6,9) ID
0055        9   FORMAT(1H0,T5,'CONTROL',T13,A4)
0056            WRITE(6,66) R(NCTRL), PRCN15(NCTRL), SN15(NCTRL)
0057       66   FORMAT(T16,F9.4,T37,F8.4,T58,F8.4)
0058            GO TO 3
0059       10   WRITE(6,7)
0060        7   FORMAT(1H+,T17,'________', T38,'_______',T59,'_______')
0061            AVGRC = AVGRC/FLOAT(NCTRL)
0062            AVGNC = AVGNC/FLOAT(NCTRL)
0063            AVVNC = AVVNC/FLOAT(NCTRL)
0064            SMSQ = 0.0
0065            DO 70 I=1,NCTRL
0066            DELTA2 = (AVGNC-PRCN15(NCTRL))**2
0067       70   SMSQ = SMSQ + DELTA2
0068            IF(NCTRL .EQ. 1) GO TO 71
0069            AVVNC = SMSQ/FLOAT(NCTRL-1)
0070       71   SIGCTL = SQRT(AVVNC)
0071       72   WRITE(6,8) AVGRC, AVGNC, SIGCTL
0072        8   FORMAT(T5,'AVERAGES',T16,F9.4,T37,F8.4,T58,F8.4)
0073            NEXP = 0
0074            GO TO 15
0075       11   READ(5, 1,END=99)KODE,ID,ATT28,ATT29,VSTD15,(PKHT28(I),I=1,NSCAN8)
                1,(PKHT29(I),I=1,NSCAN9),BKGD28,BKGD29
0076       15   IF(KODE.EQ.IC) GO TO 12
0077            NEXP = NEXP + 1
0078            VSTN15(NEXP) = VSTD15
0079            EAV28(NEXP) = 0.0
0080            EAV29(NEXP) = 0.0
0081            SMSQ8E(NEXP) = 0.0
0082            SMSQ9E(NEXP) = 0.0
0083            DO 13 I = 1,NSCAN8
0084            CP28(I) = PKHT28(I)*ATT28 - BKGD28
0085       13   EAV28(NEXP) = EAV28(NEXP) + CP28(I)
0086            DO148 I = 1,NSCAN9
0087            CP29(I) = PKHT29(I)*ATT29 - BKGD29
0088      148   EAV29(NEXP) = EAV29(NEXP) + CP29(I)
0089            EAV28(NEXP) = EAV28(NEXP)/FLOAT(NSCAN8)
0090            EAV29(NEXP) = EAV29(NEXP)/FLOAT(NSCAN9)
0091            CAV8SQ(NEXP)= EAV28(NEXP)*EAV28(NEXP)
0092            CAV9SQ(NEXP)= EAV29(NEXP)*EAV29(NEXP)
0093            DO 26 I=1,NSCAN8
0094            DEL28(I) = EAV28(NEXP)-CP28(I)
0095            DELSQ8(I)=DEL28(I)*DEL28(I)
0096       26   SMSQ8E(NEXP) = SMSQ8E(NEXP) + DELSQ8(I)
0097            DO147 I= 1,NSCAN9
0098            DEL29(I) = EAV29(NEXP)-CP29(I)
```

```
C099              DELSQ9(I)=DEL29(I)*DEL29(I)
0100        147   SMSQ9E(NEXP) = SMSQ9E(NEXP) + DELSQ9(I)
0101              VAR28(NEXP) = SMSQ8E(NEXP)/FLOAT(NSCAN8-1)
C102              VAR29(NEXP) = SMSQ9E(NEXP)/FLOAT(NSCAN9-1)
0103              R(NEXP) = EAV28(NEXP)/EAV29(NEXP)
0104              VARR(NEXP) = R(NEXP)*R(NEXP)*(VAR28(NEXP)/CAV8SQ(NEXP) +
                 1 VAR29(NEXP)/CAV9SQ(NEXP))
0105              PCN15E(NEXP) = 100./(2.*R(NEXP)+1.)
0106              VAN15E(NEXP) = (-200./((2.*R(NEXP)+1.)**2))**2*VARR(NEXP)
0107              SE(NEXP) = SQRT(VAN15E(NEXP))
0108              IF(NEXP .GT. 1) GO TO 100
0109              WRITE(6,103)
0110        103   FORMAT(1H-,T2,'EXPERIMENTS',/,T20,'R',T39,'AT %',T58,'SIG AT %',/)
0111              WRITE(6,7)
C112        100   WRITE(6,101) ID
0113        101   FORMAT(1HO,T2,'EXPERIMENT',T13,A4)
0114              WRITE(6, 66) R(NEXP), PCN15E(NEXP),      SE(NEXP)
C115              GO TO 11
0116        12    WRITE(6,39) NEXP
0117        39    FORMAT(1HO,'NEXP = ',I3)
C118              DO 19 I=1,NEXP
0119              OOS(I) = 0.0
0120              OOV(I) = 0.0
0121              AF(I) = PCN15E(I) - AVGNC
0122        19    VARAF(I) = VAN15E(I) + AVVNC
0123              CALL LSQFIT (OOS,OOV ,SIGOOV,B,SIG,MM,NSAMP,NO2,1,0)
0124              READ(5,14) CNO3ST, VOLSAM,(AB(I),I=1,NSAMP)
            C   CNO3ST = NO3 STANDARD USED TO CONSTRUCT BEERS PLOT (UG NO3-N / ML).
            C     VOLSAM = NUMBER OF ML OF WATER SAMPLE.
            C     AB = ABSORBANCE UNITS.
0125        14    FORMAT(8F10.4)
0126              AVABS = 0.0
0127              SMSQ = 0.0
0128              DO 23 I=1,NSAMP
0129        23    AVABS = AVABS + AB(I)
0130              AVABS = AVABS/FLOAT(NSAMP)
0131              DO 16 I=1,NSAMP
0132              DEL2(I) = (AVABS-AB(I))**2
0133        16    SMSQ = SMSQ + DEL2(I)
0134              VARA = SMSQ/FLOAT(NSAMP-1)
0135              VARAP = (0.43429*0.004*10.**(AVABS))**2
0136              IF(VARA .GT. VARAP) GO TO 17
0137              VA = VARAP
0138              GO TO 20
0139        17    VA = VARA
0140        20    CML = (AVABS-B(1))/B(2)
0141              CNO3   = CML*CNO3ST*1.E3/(VOLSAM*14.0067)
0142              VCML=(B(2)*B(2)*(VA+SIG(1)*SIG(1)) + SIG(2)*SIG(2)*
                 1   (AVABS*AVABS*B(1)*B(1)))/B(2)**4
0143              VACNO3 = (CNO3  *SQRT(VCML)/CML)**2
0144              CNO2UL = 0.0
0145              VACNO2 = 0.0
0146              IF(NO2 .EQ. 0) GO TO 45
0147              CALL LSQFIT (OOS,OOV ,SIGOOV,B,SIG,MM,NSAMP,NO2,1,0)
0148              READ(5,14) CNO2ST, VOLSAM,(AB(I),I=1,NSAMP)
0149              AVABS = 0.0
C150              SMSQ = 0.0
0151              DO 40 I=1,NSAMP
```

```
0152        40    AVABS = AVABS + AB(I)
0153              AVABS = AVABS/FLOAT(NSAMP)
0154              DO 41 I=1,NSAMP
0155              DEL2(I) = (AVABS-AB(I))**2
0156        41    SMSQ = SMSQ + DEL2(I)
0157              VARA = SMSQ/FLOAT(NSAMP-1)
0158              VARAP = (0.43429*0.004*10.**(AVABS))**2
0159              IF(VARA .GT. VARAP) GO TO 42
0160              VA = VARAP
0161              GO TO 43
0162        42    VA = VARA
0163        43    CML = (AVABS-B(1))/B(2)
0164              CNO2UL = CML*CNO2ST*1.E3/(VOLSAM*14.0067)
0165              VCML=(B(2)*B(2)*(VA+SIG(1)*SIG(1)) + SIG(2)*SIG(2)*
                 1   (AVABS*AVABS+B(1)*B(1)))/B(2)**4
0166              VACNO2 = (CNO2UL*SQRT(VCML)/CML)**2
0167        45    CNO3UL = CNO3 - 0.95*CNO2UL
            C     CNO3UL IN UNITS OF UMNO3-N/LITER.
0168              IF(NH3.GT.0) GO TO 78
0169              GO TO 80
0170        78    WRITE(6,79) CNO3UL
0171        79    FORMAT(1HO,10X,'CONC OF NH3-N IN SAMPLE =',F10.4,' UM/L')
0172              GO TO 86
0173        80    WRITE(6,21) CNO3UL
0174        21    FORMAT(1HO,10X,'CONC OF NO3-N IN SAMPLE =',F10.4,' UM/L')
0175              WRITE(6,44) CNO2UL
0176        44    FORMAT(1HO,10X,'CONC OF NO2-N IN SAMPLE =',F10.4,' UM/L')
0177        86    VANO3C = VACNO3 + (-.95)*(-.95)*VACNO2
0178              READ(5,46) FRAN15, GNANO3, STKVOL, DILUTN, SPKVOL, TIME
0179           46 FORMAT(6F10.4)
            C     FRAN15 = FRACTIONAL COMP OF N AS N-15.
            C     GNANO3 = GRAMS NANO3 WEIGHED.
            C     STKVOL = ML OF STOCK SOLUTION MADE.
            C     DILUTN = ML OF FINAL DILUTION/ ML STOCK.
            C     SPKVOL = NO. LITERS OF WATER SAMPLE SPIKED.
            C     DURATION OF INCUBATION IN HOURS.
            C      VSTN15 = ML OF STANDARD NO3-N-15 USED PER SPIKE.
0180              WRITE(6,18)
0181        18    FORMAT(1HO,T20,'AF(HR-1)',T42,'VARAF',T62,'AI',T82,'VARAI')
0182              IF(NH3.GT.0) GO TO 81
0183              FWMNO3=22.9898+47.9982+FRAN15*15.0001+(1.0-FRAN15)*14.003
            C     MNO3 = NANO3.
0184              CSTN15=GNANO3*1.E6/(FWMNO3*STKVOL*DILUTN*SPKVOL)
0185              GO TO 82
0186        81    FWNH3 = 35.453 + 4.032 + FRAN15*15.0001 +(1.0-FRAN15)*14.003
            C     NH3 = NH4CL
0187              CSTN15 = GNANO3*1.E6/(FWNH3*STKVOL*DILUTN*SPKVOL)
0188        82    DO 29 I=1,NEXP
0189              AF(I) = AF(I)/TIME
0190              VARAF(I) = VARAF(I)/(TIME*TIME)
0191              SPK = CSTN15*VSTN15(I)
0192              S(I) = SPK + CNO3UL
            C     S(I) = SPK
0193              AI(I) = ((SPK*FRAN15/S(I)) - 0.003622)*100.0
            C     AI (I) =  SPK*(FRAN15-0.00366)*100./S(I)
0194              VARAI(I) = (AI(I)/S(I))**2*VANO3C
            C     VARAI(I) = 0.0
0195              WRITE(6,24) AF(I), VARAF(I), AI(I), VARAI(I)
```

```
0196        24    FORMAT(1H ,T18,F10.7,T40,F10.8,T58,F10.6,T80,F10.7)
0197              VNO3(I) = AF(I)/AI(I)
            C     VVNO3(I) = VNO3(I)*VNO3(I)*(VARAF(I)/(AF(I)*AF(I)) + VARAI(I)/
            C    1 (AI(I)*AI(I)))
            C     SIGV(I) = SQRT(VVNO3(I))
0198              SIGV(I) = 1./S(I)
0199              VVNO3(I) = SIGV(I)*SIGV(I)
0200              RELEAF(I) = SQRT(VARAF(I))/AF(I)
0201              RELEAI(I) = SQRT(VARAI(I))/AI(I)
0202              SOV   (I) = S(I)/VNO3(I)
0203              OOS(I) = 1.0/S(I)
0204              OOV(I) = 1.0/VNO3(I)
0205              VOS(I)=1./SOV(I)
0206              SIGSOV(I) = SOV(I)*SOV(I)*(VANO3C/(CNO3UL*CNO3UL) +
                 1 VVNO3(I)/(VNO3(I)*VNO3(I)))
0207              SIGSOV(I) = SQRT(SIGSOV(I))
0208        29    SIGOOV(I) = SIGV(I)/(VNO3(I)*VNO3(I))
0209              IF(NH3.GT.0) GO TO 83
0210              WRITE(6,30)
0211        30    FORMAT(1HO,T23,'VNO3',T41,'SIGVNO3',T60,'RELER AF',T79,'RELER AI')
0212              GO TO 84
0213        83    WRITE(6,85)
0214        85    FORMAT(1HO,T23,'VNH3',T41,'SIGNH3',T60,'RELER AF',T79,'RELER AI')
0215        84    DO 31 I=1,NEXP
0216        31    WRITE(6,32) VNO3(I),  SIGV(I), RELEAF(I), RELEAI(I)
0217        32    FORMAT(1H ,T20,F10.6,T39,F10.6,T58,F10.6,T77,F10.6)
            C     CALCULATE YMIN, YMAX FOR THE VNO3 VS. S PLOT.
0218              YMIN = VNO3(1)
0219              DO 33 J=2,NEXP
0220              IF(YMIN.LT.VNO3(J)) GO TO 33
0221              YMIN = VNO3(J)
0222        33    CONTINUE
0223              YMAX = VNO3(1)
0224              DO 34 J=2,NEXP
0225              IF(YMAX.GT.VNO3(J)) GO TO 34
0226              YMAX = VNO3(J)
0227        34    CONTINUE
0228              YMAX = YMAX + 0.1*(YMAX-YMIN)
0229              DO 35 K=1,NEXP
0230              X(K) = S(K)
0231        35    Y(K) = VNO3(K)
0232              Z(1) = 1.0
            C     CALCULATE XMIN,XMAX FOR PLOT.
0233              XMIN = X(1)
0234              DO 36 J=2,NEXP
0235              IF(XMIN.LT.X(J)) GO TO 36
0236              XMIN = X(J)
0237        36    CONTINUE
0238              XMAX = X(1)
0239              DO 37 J=2,NEXP
0240              IF(XMAX.GT.X(J)) GO TO 37
0241              XMAX = X(J)
0242        37    CONTINUE
0243              XMAX = XMAX + 0.1*(XMAX-XMIN)
0244              CALL PLOT(X, 0.0,XMAX,0,Y,YMIN,YMAX,0,Z,.0,.0,0,NEXP,1,1,3,2)
            C     REGRESSION COEFFICIENT: REMINGTON & SCHORK,"STATISTICS WITH
            C     APPLICATIONS TO BIO & HEALTH SCIENCES",PRENTICE-HALL,1970,PP160-75
0245              NCOUNT=1
```

```
0246          55 SXI=0.0
0247             SYI=0.0
0248             SXI2=0.0
0249             SYI2=0.0
0250             SXIYI=0.0
0251             DO 50 I=1,NEXP
0252             GO TO(47,48,49,47),NPLOT
0253          47 GO TO(58,48,49),NCOUNT
0254          58 SXI=SXI+  S(I)
0255             SYI=SYI+SOV(I)
0256             SXIYI=SXIYI+  S(I)*SOV(I)
0257             SXI2=SXI2+  S(I)*  S(I)
0258             SYI2=SYI2+SOV(I)*SOV(I)
0259             GO TO 50
0260          48 SXI=SXI+OOS(I)
0261             SYI=SYI+OOV(I)
0262             SXIYI=SXIYI+OOS(I)*OOV(I)
0263             SXI2=SXI2+OOS(I)*OOS(I)
0264             SYI2=SYI2+OOV(I)*OOV(I)
0265             GO TO 50
0266          49 SXI=SXI+VOS(I)
0267             SYI=SYI+VNO3(I)
0268             SXIYI=SXIYI+VOS(I)*VNO3(I)
0269             SXI2=SXI2+VOS(I)*VOS(I)
0270             SYI2=SYI2+VNO3(I)*VNO3(I)
0271          50 CONTINUE
0272             PLXY=FLOAT(NEXP)*SXIYI-SXI*SYI
0273             PLXX=FLOAT(NEXP)*SXI2-SXI*SXI
0274             PLYY=FLOAT(NEXP)*SYI2-SYI*SYI
0275             RCOEF=PLXY/SQRT(PLXX*PLYY)
0276             GO TO(51,52,53,51),NPLOT
0277          51 GO TO(56,52,53),NCOUNT
           C     LSQFIT TO WOOLF PLOT ,  S/V VS. S, IF NEXT CARD IS USED.
0278          56 CALL LSQFIT(  S,SOV ,SIGSOV,B,SIG,NEXP,NSAMP,NO2,2,NCOUNT)
0279             VMAX = 1./B(2)
0280             SIGVM = SIG(2)/(B(2)*B(2))
0281             CKS  =   B(1)/B(2)
0282             SIGKS=SQRT(CKS*CKS*(SIG(1)*SIG(1)/(B(1)*B(1)) + SIG(2)*SIG(2)/
                1 (B(2)*B(2))))
0283             GO TO 54
           C     LSQFIT TO LINEWEAVER-BURK PLOT, 1/V VS. 1/S, IF NEXT CARD IS USED.
0284          52 CALL LSQFIT(OOS,OOV ,SIGOOV,B,SIG,NEXP,NSAMP,NO2,2,NCOUNT)
0285             VMAX = 1./B(1)
0286             SIGVM = SIG(1)/(B(1)*B(1))
0287             CKS  =   B(2)/B(1)
0288             SIGKS=SQRT(CKS*CKS*(SIG(1)*SIG(1)/(B(1)*B(1)) + SIG(2)*SIG(2)/
                1 (B(2)*B(2))))
0289             GO TO 54
           C     LSQFIT TO HOFSTEE PLOT, V VS. V/S, IF NEXT CARD IS USED.
0290          53 CALL LSQFIT(VOS,VNO3,SIGV  ,B,SIG,NEXP,NSAMP,NO2,2,NCOUNT)
0291             VMAX=B(1)
0292             SIGVM=SIG(1)
0293             CKS=-B(2)
0294             SIGKS=SIG(2)
0295          54 CONTINUE
0296             WRITE(6,132)RCOEF
0297         132 FORMAT(1HO,5X, 9H RCOEF = ,F8.5)
0298             WRITE(6,38) VMAX, SIGVM, CKS, SIGKS
```

```
0299        38    FORMAT(1H0,'VMAX = ',1PE12.4,'   SIGVMAX = ',1PE12.4,'   KS =
                 1',1PE12.4,'   SIGKS = ',1PE12.4)
0300              AVGRC = 0.0
0301              AVGNC = 0.0
0302              AVVNC = 0.0
0303              NCTRL = 0
0304              IF(NPLOT.LT.4)GO TO 3
0305              NCOUNT=NCOUNT+1
0306              IF(NCOUNT.GT.3)GO TO 3
0307              GO TO 55
0308        99    WRITE(6,2)
0309              STOP
0310              END
```

```
0001            SUBROUTINE LSQFIT(XX,Y,P,B,SIG,MM,NSAMP,NO2,INDEX,ITRANS)
                C     LEAST SQUARES ROUTINE          FORTRAN LISTING FOR IBM /360
                C     L.P.VARGA AND E.L.BUTLER OSU75.001 OKLA STATE UNIV COMPUTER CENTER
                C     CALCULATE THE SET OF POSITIVE BETA VALUES, B(I), FOR ALL 127
                C     POSSIBLE COMBINATIONS OF I FROM 1 TO 7 IN THE POLYNOMIAL
                C     Y=B(0)+B(1)X+B(2)X**2+B(3)X**3+B(4)X**4+B(5)X**5+B(6)X**6+B(7)X**7
                C     (EXCLUDING Y=B(0)).  B(I)-STD DEV OF B(I) MUST BE POSITIVE AND
                C     SMIN/ DF    MUST BE 1.5 OR LESS.  THE MAXIMUM SIZE OF THE MODEL
                C     MAY BE DECREASED AND THE BETAS MAY BE GIVEN FIXED VALUES.
0002                  INTEGER CONTL,SIZE
                C     DIMENSION OF YHAT, DEV, AND S SHOULD BE GREATER THAN OR EQUAL
                C     TO NO OF DATA POINTS
0003                  DOUBLE PRECISION DX
0004                  DIMENSION X(8,9),      DX(8,9),BETA(8),CONTL(8),B(8),SIG(8),VAR(8)
                     1,YHAT(35),DEV(35),S(35) ,V(13,35),Z(8,9), XX(35), Y(35), P(35)
                     2,XPLOT(90),YPLOT(90),ZPLOT(1)
0005                  COMMON DX,N,M
                C     DATA FORMAT.  INDEPENDENT VARIABLE READ IN AS XX
0006                1 FORMAT(2E10.3,F6.2)
0007                2 FORMAT(1H ,1P9E12.4)
                C     CONTROL AND NO OF POINTS FORMAT.
                C     OUTPUT FORMAT
0008                3 FORMAT(8I2)
0009                4 FORMAT(1H0,8I2)
0010               22 FORMAT(1H1,8I2)
                C     COLUMN HEADING FORMAT.  CHANGE AS DESIRED
0011                5 FORMAT(6X,1HX,9X,3HPHI,7X,10H    H     ,13H    ERROR    ,5X,1HY,
                     X11X,1HW,8X,6HCALC Y,5X,8HY-CALC Y,17H   W(Y-CALC Y)**2)              99
0012                7 FORMAT(2H  )
                C     GOODNESS OF FIT FORMAT
0013                8 FORMAT(16X,19H SMIN/DEG. FREEDOM=,1PE10.3)
                C     FIXED BETA FORMAT
0014               33 FORMAT(8E10.4)
                C     INTERMEDIATE RANGE GOODNESS OF FIT FORMAT
0015               34 FORMAT(13H SMIN/(DF-1)=,F5.2,5H FOR ,8I5/)
                C     BETA AND STD DEV FORMAT
0016               35 FORMAT(1H ,1P2E15.4/)
0017               36 FORMAT(7X,4HBETA,10X,7HSTD DEV)
0018              101 FORMAT(1H ,1P2E10.2)
0019              102 FORMAT(1H ,1P3E10.2)
0020              103 FORMAT(1H ,1P4E10.2)
0021              104 FORMAT(1H ,1P5E10.2)
0022              105 FORMAT(1H ,1P6E10.2)
0023              106 FORMAT(1H ,1P7E10.2)
0024              107 FORMAT(1H ,1P8E10.2)
0025              108 FORMAT(1H ,1P9E10.2)
0026              133 KCONTL= 0
0027                  DO6 I=1,8
0028                  DO6 J=1,9
0029                6 X(I,J)=0.0
                C     READ MAX MODEL SIZE AND NO OF POINTS
0030                  SIZE = 2
0031                  NCONTL = 1
0032                  IF(INDEX.EQ.2) GO TO109
0033                  READ(5,3) MM,NSAMP, NO2
                C     BETA MAY BE FIXED OTHERWISE READ IN ZERO
0034                  READ(5,33)(BETA(I),I=1,8)
                C     READ IN DATA AND CALCULATE Y.  INDEPENDENT VARIABLE READ IN AS XX
```

```
0035          109 DO12 K=1,MM
C036              IF(INDEX.EQ.2) GO TO 99
0037              READ(5,1) XX(K),Y(K),P(K)
                C     CALCULATE WEIGHT OF Y.  W=1/STD. DEV. OF Y SQUARED
0038           99 IF(P(K).EQ.0.0) GO TO 14
0039              W = 1./(P(K)*P(K))
0040              GO TO 19
0041           14 W= 1.
                C     CALCULATE POWERS OF X AND STORE IN V
0042           19 V(1,K)=1.0
C043              V(2,K)=XX(K)
C044              V(3,K)=XX(K)*XX(K)
0045              DO9I=4,8
0046              L=I-1
0047            9 V(I,K)=XX(K)**L
                C     STORE Y,W AND DATA IN V
0048              V(9,K)=Y(K)
0049              V(10,K)=W
C050              V(11,K)=0.0
0051              V(12,K)=0.0
0052              V(13,K)=P(K)
                C     BUILD MATRIX CONTAINING ELEMENTS OF NORMAL EQUATIONS
0053           10 DO12 I=1,8
0054              PIVOT=V(I,K)
0055           11 DO12 J=1,9
0056           12 X(I,J)=X(I,J)+     W*PIVOT*V(J,K)
0057           13 IF(INDEX.EQ.2)GO TO 1111
                C     READ CONTROL CARD
0058              READ(5,3          )(CONTL(I),I=1,8)
0059         1111 KCONTL = KCONTL+1
                C     SHRINK MATRIX ACCORDING TO SIZE
0060              II=1
0061              DO16 I=1,SIZE
0062              JJ=1
0063              DO15 J=1,SIZE
0064              IF(CONTL(I).EQ.1)GOTO16
0065              IF(CONTL(J).EQ.1)GOTO15
0066              DX(II,JJ)=X(I,J)
0067              JJ=JJ+1
0068           15 CONTINUE
0069              II=II+1
C070           16 CONTINUE
                C     SHRINK MATRIX ACCORDING TO FIXED BETA
0071              N=II-1
0072              M=II
0073              II=1
0074               DO17 I=1,SIZE
0075              IF(CONTL(I).EQ.1)GOTO17
0076              DX(II,M)=X(I,9      )-BETA(1)*X(I,1)-BETA(2)*X(I,2)-BETA(3)*X(I,3)-
                 1BETA(4)*X(I,4)-BETA(5)*X(I,5)-BETA(6)*X(I,6)-BETA(7)*X(I,7)-
                 2BETA(8)*X(I,8)
0077              II=II+1
0078           17 CONTINUE
0079              IF(INDEX .EQ. 2) GO TO 20
                C     PRINT CONTROL PARAMETERS
C080              WRITE(6,4)(CONTL(I),I=1,SIZE)
0081              GO TO 18
C082           20 WRITE(6,22)(CONTL(I),I=1,SIZE)
```

```
0083          18 WRITE(6,7)
              C     INVERT MATRIX
0084              CALL INVERT
0085              DO25 I=1,N
0086              DO25 J=1,M
0087           25 Z(I,J)=DX(I,J)
0088              KK=1
0089              DO185 K=1,SIZE
0090              IF(CONTL(K).EQ.1)GOTO184
              C     STORE CALCULATED BETA FROM MATRIX OR FIXED BETA
0091              B(K)=DX(KK,M)
0092              VAR(K)=DX(KK,KK)
0093              KK=KK+1
0094              GOTO185
0095          184 B(K)=BETA(K)
0096          185 CONTINUE
0097              DO186 K=1,SIZE
              C     IF BETA LESS THAN ZERO, TRY NEXT MODEL
              C     IF(B(K).LT.0.0)GOTO13
0098          186 CONTINUE
              C     CALCULATE DEGREES OF FREEDOM
0099              DF=MM-N
0100              SMIN=0.0
              C     DETERMINE SET OF CALCULATED Y'S
0101              DO21 K=1,MM
0102              YBAR=0.0
0103              DO32 I=1,SIZE
0104           32 YBAR=YBAR+B(I)*V(I,K)
0105              YHAT(K)=YBAR
              C     CALCULATE DEVIATION
0106              DEV(K)=V(9,K)-YHAT(K)
              C     CALCULATE WEIGHTED SQUARE OF DEVIATION
0107              S(K)=V(10,K)*DEV(K)*DEV(K)
              C     CALCULATE SMIN /(DEG. OF FREEDOM), THE GOODNESS OF FIT PARAMETER
0108           21 SMIN=SMIN+S(K)
0109              SMIN=SMIN/DF
0110               DO188 K=1,SIZE
0111              IF(CONTL(K).EQ.1)GOTO187
              C     CALCULATE STD DEV OF BETA
0112              SIG(K)=SQRT(VAR(K)*SMIN)
              C     IF BETA-STD DEV IS NEGATIVE, TRY NEXT MODEL
              C     IF(SIG(K)-B(K).GE.0.0)GOTO13
0113              GOTO188
0114          187 SIG(K)=0.0
0115          188 CONTINUE
              C     IF GOODNESS OF FIT GREATER THAN 6, TRY NEXT MODEL
              C     IF(SMIN.GT.6.0)GOTO13
              C     IF GOODNESS OF FIT BETWEEN 1.5 AND 6, PRINT GOODNESS PARAMETER AND CONTROL
              C     CARD AND THEN TRY NEXT MODEL
              C     IF(SMIN.GT.1.5)WRITE(6,34)SMIN,(CONTL(I),I=1,SIZE)
              C     IF(SMIN.GT.1.5)GO TO 13
0116              GO TO(121,122,123,124,125,126,127,128),N
0117          121 WRITE(6, 101) ((Z(I,J),J=1,M),I=1,N)
0118              GOTO 110
0119          122 WRITE(6, 102) ((Z(I,J),J=1,M),I=1,N)
0120              GOTO 110
0121          123 WRITE(6, 103) ((Z(I,J),J=1,M),I=1,N)
0122              GOTO 110
```

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0123          124 WRITE(6, 104) ((Z(I,J),J=1,M),I=1,N)
0124              GOTO 110
0125          125 WRITE(6, 105) ((Z(I,J),J=1,M),I=1,N)
0126              GOTO 110
0127          126 WRITE(6, 106) ((Z(I,J),J=1,M),I=1,N)
0128              GOTO 110
0129          127 WRITE(6, 107) ((Z(I,J),J=1,M),I=1,N)
0130              GOTO 110
0131          128 WRITE(6, 108) ((Z(I,J),J=1,M),I=1,N)
0132          110 WRITE(6,36)
              C     PRINT BETA AND STD DEV
0133                WRITE(6,35)(B(K),SIG(K),K=1,SIZE)
              C     PRINT 4 COLUMNS OF INPUT DATA, Y, W, CALCULATED Y, DEVIATION, WEIGHTED
              C     SQUARE OF DEVIATION
0134                WRITE(6,5)
0135                DO23 K=1,MM
0136           23 WRITE(6,2) V(2,K),V(11,K),V(12,K),V(13,K),V(9,K),V(10,K),
                  1 YHAT(K),DEV(K),S(K)
              C     PRINT GOODNESS OF FIT
0137                WRITE(6,7)
0138                WRITE(6,8)SMIN
0139                IF(ITRANS.EQ.0)GO TO 24
0140                DO 38 K=1,MM
0141                XPLOT(K)=V(2,K)
0142           38 YPLOT(K)=V(9,K)
0143                DO 26 K=1,MM
0144                J=K+MM
0145                XPLOT(J)=V(2,K)
0146           26 YPLOT(J)=YHAT(K)
0147                NPT=MM*2
0148                XMIN=XPLOT(1)
0149                DO 27 K=2,MM
0150                IF(XMIN.LT.XPLOT(K))GO TO 27
0151                XMIN=XPLOT(K)
0152           27 CONTINUE
0153                XMAX=XPLOT(1)
0154                DO 28 K=2,MM
0155                IF(XMAX.GT.XPLOT(K))GO TO 28
0156                XMAX=XPLOT(K)
0157           28 CONTINUE
0158                YMIN=YPLOT(1)
0159                DO 29 J=2,NPT
0160                IF(YMIN.LT.YPLOT(J))GO TO 29
0161                YMIN=YPLOT(J)
0162           29 CONTINUE
0163                YMAX=YPLOT(1)
0164                DO 30 J=2,NPT
0165                IF(YMAX.GT.YPLOT(J))GO TO 30
0166                YMAX=YPLOT(J)
0167           30 CONTINUE
0168                GO TO(56,52,53),ITRANS
0169           56 WRITE(6,57)
0170           57 FORMAT(1H1,20X,'WOOLF PLOT')
0171                GO TO 31
0172           52 WRITE(6,60)
0173           60 FORMAT(1H1,20X,'LINEWEAVER-BURK PLOT')
0174                GO TO 31
0175           53 WRITE(6,59)
```

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0176            59 FORMAT(1H1,20X,'HOFSTEE PLOT')
0177            31 CALL PLOT(XPLOT,XMIN,XMAX,0,YPLOT,YMIN,YMAX,0,ZPLOT,0.,0.,0,
                  1NPT,2,1,2,2)
0178            24 IF(KCONTL .EQ. NCONTL) GO TO 111
                C     TRY NEXT MODEL
0179               GOTO13
0180           111 RETURN
0181               END
```

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FORTRAN IV G LEVEL 21          INVERT          DATE = 76169      14/30/49          PAGE 0001

0001                SUBROUTINE INVERT
                C   MATRIX INVERSION ROUTINE
0002                DOUBLE PRECISION DX,PIVOT1,PIVOT2
0003                DIMENSION DX(8,9)
0004                COMMON DX,N,M
0005                DO 30 I=1,N
0006                PIVOT1=1.D0/DX(I,I)
0007                DX(I,I)=PIVOT1
0008                DO 10 J=1,M
0009                IF(J.EQ.I) GO TO 10
0010                DX(I,J)=PIVOT1*DX(I,J)
0011             10 CONTINUE
0012                DO 25 K=1,N
0013                IF(K.EQ.I) GO TO 25
0014                PIVOT2=DX(K,I)
0015                 DX(K,I)=-PIVOT2*PIVOT1
0016                DO 20 L=1,M
0017                IF(L.EQ.I) GO TO 20
0018                DX(K,L)=DX(K,L)-PIVOT2*DX(I,L)
0019             20 CONTINUE
0020             25 CONTINUE
0021             30 CONTINUE
0022                RETURN
0023                END
```

```
0001            SUBROUTINEPLOT(X,XMIN,XMAX,LX,Y,YMIN,YMAX,LY,Z,ZMIN,ZMAX,LZ,NPT,
               1NPLOT,NCOPY,NCD,NDIM)
              C  THIS GENERAL PLOTTING SUBROUTINE WAS WRITTEN BY E. J. KOBETICH
              C  DEPT OF PHYSICS, KANSAS STATE UNIV, MANHATTAN, KANSAS.
              C  X, Y, AND Z ARE SINGLE SUBSCRIPTED VARIABLES IDENTIFYING THE
              C  COORDINATES OF THE POINTS TO BE PLOTTED.   XMIN, YMIN, AND ZMIN ARE
              C                THE MINIMUM VALUES, AND XMAX, YMAX, AND ZMAX CORRESPOND
              C  TO THE MAXIMUM VALUES ON THE X, Y, AND Z AXES.   LX, LY, AND LZ
              C  DEFINE THE TYPE OF SCALE USED ALONG THE X, Y, AND Z AXES AS FOLLOWS—
              C  0%LINEAR<, 1%ONE CYCLE LOG<, 2%TWO CYCLE LOG<, ETC.
              C  NPT IS THE TOTAL NUMBER OF POINTS TO BE PLOTTED.   NPLOT IS THE NO OF
              C  TWO DIMENSIONAL RELATIONSHIPS %CURVES< WHICH ARE TO BE PLOTTED, THE
              C  MAXIMUM VALUE IS 40.   NCOPY IS THE NO OF PLOTS TO BE PRINTED.
              C  NCD DETERMINES THE NO OF INFORMATION CARDS TO BE READ BY THIS SUB-
              C  PROGRAM-- 0%NO CARDS READ<, 1%CARD ONE IS READ<, 2%CARD TWO IS READ<,
              C  3%BOTH CARD ONE AND CARD TWO ARE READ<.   NDIM IS THE DIMENSIONALITY
              C  OF THE FUNCTION TO BE PLOTTED.  IF NDIM#3 TOPOGRAPHIC MAPPING OF A
              C  3-DIMENSIONAL SURFACE ONTO THE X-Y PLANE OCCURS.
0002            DIMENSION X(1),Y(1),Z(1),SX(13),TITLE(20),L(134),NCH(41),MOP(18),
               1TAB1(18)
0003            JREAD5=5
0004            JRITE6=6
0005           1 FORMAT(20A4)
0006           2 FORMAT(80A1)
0007           3 FORMAT(1H1,26X,20A4)
0008           4 FORMAT(1H ,A1,1PE9.2 ,121A1)
0009           5 FORMAT(132A1)
0010           6 FORMAT(8X,1PE9.2,11(1X,1PE9.2))
0011           7 FORMAT(1PE17.2,E116.2)
0012           8 FORMAT(1PE17.2,E61.2,E55.2)
0013           9 FORMAT(1PE17.2,2E40.2,E36.2)
0014          10 FORMAT(1PE17.2,3E30.2,E26.2)
0015          11 FORMAT(1PE17.2,4E24.2,E20.2)
0016          12 FORMAT(1HK,62X,18A1)
0017             LLX=LX+1
0018             NDD=NCD+1
0019             GO TO(15,13,14,13),NDD
0020          13 READ(JREAD5,1) (TITLE(I), I=1,20)
0021             WRITE(JRITE6,3) (TITLE(I), I=1,20)
0022          14 IF(NDD.GE.3)READ(JREAD5,2) (MOP(I), I=1,18), (NCH(I), I=1,40),
                1(TAB1(I), I=1,18),ND,NP,NM,NB
0023          15 NCH(41)=NB
0024             NPN=NPT/NPLOT
0025             IF(LX.GT.0)  GO TO 17
0026         1717 CX=120./(XMAX-XMIN)
0027             SX(1)=XMIN
0028             SX(13)=XMAX
0029             U=XMIN
0030             DO 16 K=2,12
0031             U=(XMAX-XMIN)/12.+U
0032          16 SX(K)=U
0033             GO TO 19
0034          17 XLX=LX
0035             CX=120./XLX
0036             NX=ALOG10(XMIN)
0037             DO 18 K=1,LLX
0038          18 SX(K)=10.**(NX+K-1)
0039          19 CALLPOT(X,XMIN,LX,NPT,0,120.,CX)
```

```
0040            IF(LY.GT.0)  GO TO 20
0041         2020 CY=50./(YMAX-YMIN)
0042            GO TO 21
0043           20 YLY=LY
0044            CY=50./YLY
0045            KY=CY
0046            NY=ALOG10(YMIN)
0047           21 CALLPOT(Y,YMIN,LY,NPT,1,50.,CY)
0048            IF(NDIM.LT.3)  GO TO 24
0049         2424 IF(LZ.GT.0)  GO TO 22
0050         2222 CZ=40./(ZMAX-ZMIN)
0051            GO TO 23
0052           22 ZLZ=LZ
0053            CZ=40./ZLZ
0054           23 CALLPOT(Z,ZMIN,LZ,NPT,0,40.,CZ)
0055           24 DO 50 NN=1,NCOPY
0056            M1=1
0057            T1=33.
0058            LYY=LY
0059            TT=50.
0060            DO 43 KK=1,51
0061            N=1
0062            NNN=NPN
0063            JED=1
0064            T=51-KK
0065            DO 25 J=1,133
0066           25 L(J)=NB
0067            L(133)=ND
0068            IF(LY.GT.0)  GO TO 26
0069         2626 L(13)=NP
0070            IF(T.GT.TT)  GO TO 30
0071         3030 SCALE=T/CY+YMIN
0072            L(133)=NP
0073            N=0
0074            TT=TT-5.
0075            IF(T.LE.0.) SCALE=YMIN
0076          303 GO TO 30
0077           26 GO TO(27,27,28,28,27,28),LY
0078           27 SS=KY*LYY
0079            GO TO 29
0080           28 SS=KY*LYY+1
0081           29 L(13)=ND
0082            IF(T.GT.SS)  GO TO 30
0083         2929 SCALE=10.**(NY+LYY)
0084            N=0
0085            LYY=LYY-1
0086            L(13)=NP
0087            L(133)=NP
0088           30 IF(50..EQ.T)  GO TO 31
0089          313 IF(0..NE.T)  GO TO 37
0090           31 DO 32 J=14,133
0091           32 L(J)=NM
0092            IF(LX.GT.0)  GO TO 34
0093          444 DO 33 J=13,133,10
0094           33 L(J)=NP
0095            GO TO 36
0096           34 KX=120/LX
0097            DO 35 J=13,133,KX
```

```
0098          35 L(J)=NP
0099          36 IF(50..EQ.T) L(133)=ND
0100          37 DO 40 LM=1,NPLOT
0101             DO 39 I=JED,NNN
0102             IF(Y(I).NE.T)  GO TO 39
0103        9393 J=X(I)
0104             IF(NDIM.NE.3)  GO TO 38
0105        8383 IZ=Z(I)
0106             L(J+13)=NCH(IZ+1)
0107             GO TO 39
0108          38 L(J+13)=NCH(LM)
0109          39 CONTINUE
0110             JED=NNN+1
0111             NNN=NNN+NPN
0112          40 CONTINUE
0113             IF(T1.NE.T)  GO TO 41
0114         411 IF(15..GE.T)  GO TO 41
0115         412 L(2)=MOP(M1)
0116             M1=M1+1
0117             T1=T1-1.
0118          41 IF(N.EQ.1)  GO TO 42
0119        2420 WRITE(JRITE6,4) L(2),SCALE,(L(J), J=13,133)
0120             GO TO 43
0121          42 WRITE(JRITE6,5) (L(J), J=1,2),(L(I), I=4,133)
0122          43 CONTINUE
0123             GO TO(44,45,46,47,48,49,44),LLX
0124          44 WRITE(JRITE6,6) (SX(K), K=1,12)
0125             GO TO 50
0126          45 WRITE(JRITE6,7) (SX(K), K=1,LLX)
0127             GO TO 50
0128          46 WRITE(JRITE6,8) (SX(K), K=1,LLX)
0129             GO TO 50
0130          47 WRITE(JRITE6,9) (SX(K), K=1,LLX)
0131             GO TO 50
0132          48 WRITE(JRITE6,10) (SX(K), K=1,LLX)
0133             GO TO 50
0134          49 WRITE(JRITE6,11) (SX(K), K=1,LLX)
0135          50 WRITE(JRITE6,12) (TAB1(I), I=1,18)
0136             RETURN
0137             END
```

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FORTRAN IV G LEVEL  21                  POT                 DATE = 76169        14/30/49            PAGE 0001

 0001                SUBROUTINEPOT(V,VMIN,LV,NP,J,VC,C)
 0002                DIMENSION V(1)
 0003                IF(LV.GT.0)  GO TO 2
 0004              22 DO 1 I=1,NP
 0005               1 V(I)=FLOAT(IFIX(C*(V(I)-VMIN)+.5))
 0006                GO TO 4
 0007               2 DO 3 I=1,NP
 0008               3 V(I)=FLOAT(IFIX(C*(ALOG(V(I)/VMIN)/2.302585)+.5))
 0009               4 IF(J.GT.0)  GO TO 7
 0010              77 DO 6 I=1,NP
 0011                IF(V(I).LT.0.)  GO TO 5
 0012              55 IF(V(I).LE.VC)  GO TO 6
 0013               5 V(I)=VC+1.
 0014               6 CONTINUE
 0015               7 RETURN
 0016                END
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VITA 2

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Candidate for the Degree of

Doctor of Philosophy

Thesis: THE MASS SPECTROMETRIC DETERMINATION OF NITROGEN UPTAKE BY FRESHWATER PHYTOPLANKTON — THE EFFECT OF CHLORINE AND CHLORAMINE

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