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Peter G. Murphy

Major professor

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FACTORS INFLUENCING SPECIFIC GROWTH RATES AND SEASONAL ABUNDANCE OF EUTROPHIC LAKE PHYTOPLANKTON

By

Gary F. Marx

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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Department of Botany and Plant Pathology

1979

ABSTRACT

FACTORS INFLUENCING
SPECIFIC GROWTH RATES AND SEASONAL ABUNDANCE
OF EUTROPHIC LAKE PHYTOPLANKTON

By

Gary F. Marx

The seasonal succession of dominant phytoplankton populations and the daily concentrations of major nutrients in a central Michigan waste treatment basin were monitored over the 1977 growing season in order to study the factors controlling the growth of the various species. A short bloom of a centric diatom, Cyclotella sp., immediately after ice break was followed by a bloom of a small flagellated green alga, Chlamydomonas sp. These dominant species were followed by the coccoid green alga, Scenedesmus communis which produced the largest standing crop of the year. Two small blooms of Pandorina morum, a flagellated green alga, followed and a large growth of the nitrogen fixing blue-green alga Anabaenopsis elenkinii subsequently dominated from mid-July through late August. Three of the dominant species, S. communis, P. morum, and A. elenkinii were isolated and cultured for growth kinetics studies related to carbon and nitrogen, both of which were suspected of being important causal factors in the observed species succession.

Presumptive evidence indicates that S. communis declined because of an interaction of limiting quantities of nitrate and free CO_2. Early growth of S. communis was probably limited by low irradiance due to

shading by earlier populations. The upper limit of A. elenkinii growth was determined by low levels of free CO_2, while the week to week fluctuations were related to light attenuation (primarily due to self-shading). The onset of A. elenkinii growth seemed to be delayed by low temperature. Data suggested that two minor blooms of P. morum may have been due to mixotrophic growth, possibly using as a substrate dissolved organic matter released from stationary or declining populations of the other two species. In this phosphorus-rich hypereutrophic lake, carbon and light attenuation due to large algal standing crops, as well as the availability of dissolved organic substances, seem to be the major factors controlling annual algal productivity.

While a simple, kinetics based model may not be able to accurately predict the weekly specific growth rates of the various species, predictions of approximate periods of growth may be quite accurate, making such a model of considerable value for practical use.

This work is dedicated to Debby,
who was always there to help
smooth the rough edges.

ACKNOWLEDGMENTS

I want to extend my appreciation to my committee members, Drs. P. G. Murphy, C. D. McNabb, R. G. Wetzel, and D. L. King for their critical evaluation of this manuscript and helpful suggestions. In particular, Dr. Murphy provided outstanding guidance throughout my doctoral program. I would like to thank him for his encouragement, and for always being an accessable source of helpful counsel. Also, many discussions with Dr. King helped greatly to clarify and refine my thoughts related to this project.

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Introduction

Many factors that may control the seasonal rise and fall of phytoplankton populations have been studied. A review paper by Lund (1965) concludes that the general seasonal succession of species can be related to the interaction of light and temperature, but indicates that other factors are also important. Lund considers the influence of nutrients, particularly phosphorus, nitrogen, silicates and carbon (under enriched conditions) potentially important. Also discussed is the importance of a number of micronutrients, including magnesium, potassium, iron, manganese, cobalt, molybdenum, zinc, sodium, calcium and chlorine. Other potentially influential factors include extracellular organic substances released by algae and higher plants, and the effects of grazing and parasitism.

More recent studies have provided additional evidence that phytoplankton seasonality may be primarily a result of nutrient dynamics. Studies of algal distributions in the Great Lakes (Schelske and Stoermer, 1971, 1972) indicate that the phytoplankton are primarily phosphorus limited. The increasing phosphorus inputs associated with domestic pollution increases the growth of the dominant diatom populations causing the depletion of the silica reserves in the water. As silica levels decrease, the diatoms (with a high silica requirement) are gradually replaced by green and blue-green algae, which require little silica.

Intense growth in highly enriched lakes can result in reduction of

nitrate to undetectable levels. Only the nitrogen fixing blue-green algae have the capability to grow well photosynthetically under these conditions and often form dense surface blooms after the nitrate-requiring species have declined (Moss, 1972; Fogg et al. 1973; Schindler, 1977).

Moss (1972, 1973a, 1973b, 1973c) investigated the distributions of a large number of algal species which he divided into an oligotrophic group and a eutrophic group by their tolerances to varying levels of different nutrients and pH. The eutrophic group grew well above a pH of 8.85 while those in the oligotrophic group did not. Moss suggested that the oligotrophic species were limited at high pH by lack of sufficient free CO_2. King (1972) provided evidence implicating free CO_2 limitation in an observed seasonal periodicity in a sewage lagoon. It was shown that the green algae (more oligotrophic) were limited at a much higher level of free CO_2 than were some blue-green algae, so that as the dominant green algae grew rapidly early in the season, free CO_2 was decreased to self-limiting levels and blue-green algae rapidly attained dominance.

A number of studies have used measurements of species specific population growth and uptake kinetics values as a means of obtaining more specific information of the growth responses of individual species to varying levels of limiting factors (Caperon and Smith, 1978; Goldman and McCarthy, 1978; Goldman, 1974, 1977; Eppley and Thomas, 1969; Droop, 1974; Tilman and Kilham, 1976; MacIsaac and Dugdale, 1969). The specific growth rate is assumed to follow the Michaelis-Menten or Monod (1949) expression for enzyme kinetics:

$$\mu = \mu_{max} S/K_s + S \quad (1)$$

where μ is the specific growth rate, μ_{max} is the maximum growth rate, S is the concentration of the single limiting nutrient, and K_s is the half saturation value, that is, the concentration of S at which $\mu = \frac{1}{2}\mu_{max}$.

Dugdale (1967) suggested that the various algal species may have different values of μ_{max} and K_s for the different potential limiting factors and these may be the basis for their differential distributions. For example, it would be advantageous for a species which occurred in nutrient deficient areas to have a high efficiency at extracting the limiting nutrient from very low ambient levels (low K_s), wheras in enriched areas a higher K_s would suffice, and a high growth rate (high μ_{max}) would be necessary to compete with other fast growing species which would be favored by the abundance of nutrients.

Evidence for this proposition was produced in a series of studies by Tilman in which most outcomes of competition between two species of planktonic diatoms with experimentally determined uptake efficiencies could be predicted using this information (Titman, 1976) when concentrations of limiting nutrients were experimentally varied. Using this information, 70 percent of the variance in the distribution of the two species of diatoms along a natural phosphate-silicate gradient in Lake Michigan could be explained (Tilman, 1977).

Using data from the literature, King (1970) determined efficiency values for free CO_2 uptake for a number of common planktonic species and, using these data, was able to explain the seasonal distribution of various green and blue-green algae. Other studies have determined these rate values for different species and used this information to simulate

or explain species distribution with various degrees of success (Eppley et al. 1969; Droop, 1968; Kilham, S., 1975: Kilham, P., 1971; Lehman et al. 1975a, 1975b).

The majority of these studies have focused on a single limiting factor as the sole agent controlling specific growth rates. Droop (1974) has shown that under steady state conditions only one nutrient can be limiting at any one time. There is no evidence, however, to show conclusively that population growth in the natural environment responds to the rapidly changing conditions there in the same fashion as to steady state conditions in the laboratory. In addition, several studies have shown a strong interaction between light and various commonly limiting nutrients (King and King, 1974; Davis, 1976; Senft, 1978) demonstrating that some limiting factors may in fact interact to control growth in natural systems.

An important aspect of phytoplankton ecology not often considered in these studies is the specific sinking rate from the euphotic zone of the different species populations in relation to stress conditions. Eppley et al. (1967) observed an inverse relationship between growth rate and sinking rate for several species. Eppley et al. (1967) and Smayda (1974) provide evidence that sinking rate can be directly related to physiological stress, such as that due to nutrient deficiency. While increased sinking rate may increase uptake rates of limiting nutrients and thus increase specific growth rate (Titman and Kilham, 1976), such increases do not compensate for the increased loss rates caused by the accelerated rate of sinking (Canelli and Fuhs, 1976). Studies in fully light containers (e.g. continuous cultures) provide estimates of the maximum physiologically attainable uptake efficiencies

of the test species since, even when the cells have become stressed and sink to the bottom, they continue to photosynthesize until their physiological limits are reached. King and Hill (1978) have shown that the inclusion of sinking can substantially increase the K_s values and the lower tolerance limits obtained in kinetics studies, providing a more accurate estimate of the ecological efficiency, that is, the uptake efficiency attainable under natural conditions.

This study utilized batch cultures to determine kinetic efficiency values of three species of phytoplanktonic algae to determine if a nutrient based model could provide an explanation for their occurrence in a seasonal sequence of populations that occurred in a waste treatment basin during the 1977 growing season. The application of the Monod model was modified to allow an examination of possible simultaneous limitation by two limiting nutreints and the effects of specific sinking rates on algal growth to determine the importance of these factors in influencing the observed periodicity. The effects of algal heterotrophy and variable water transparency were also examined in an attempt to assess the possible influence of these factors on the ability of the nutrient based model to predict population dynamics.

Methods and Materials

The System

The study was conducted on Lake 3 of the Michigan State University Water Quality Management Project located at T4N, R1, 2W, Secs. 1, 6, 31, and 36, Ingham County, Michigan on the MSU campus. The project is designed to improve the water quality of secondary sewage effluent from the East Lansing sewage treatment plant. It consists of four man-made lakes with a total surface area of 16 ha. and a mean depth of 1.8 meters connected in sequence by gravity flow lines (Figure 1). Effluent is pumped 7.25 kilometers from the treatment plant to the site where it is discharged into Lake 1. The water then moves by gravity flow, the rate of flow dependent on the discharge rate, through Lakes 2, 3, and 4 and is finally discharged into the local watershed (Institute of Water Research, 1976).

Levels of inorganic phosphate and nitrate decrease substantially through the system due to the intense growth of phytoplankton and aquatic macrophytes, with a decrease in total phosphorus from ca. 0.75 to 0.07 mg/l and nitrate-N from ca. 5.0 to 0.2 mg/l in the final discharge from the initial effluent (Institute of Water Research, 1976). Lake 3 was chosen for this study as it was the only one of the four which was phytoplankton dominated throughout the majority of the growing season, the others being primarily macrophyte dominated. This algal dominance reduced the need to consider competition from macrophytes as

Figure 1. Diagram of MSU Water Quality Management Project indicating water flow patterns. Drawn to scale. X indicates sampling area. (Modified from IWR, 1976).

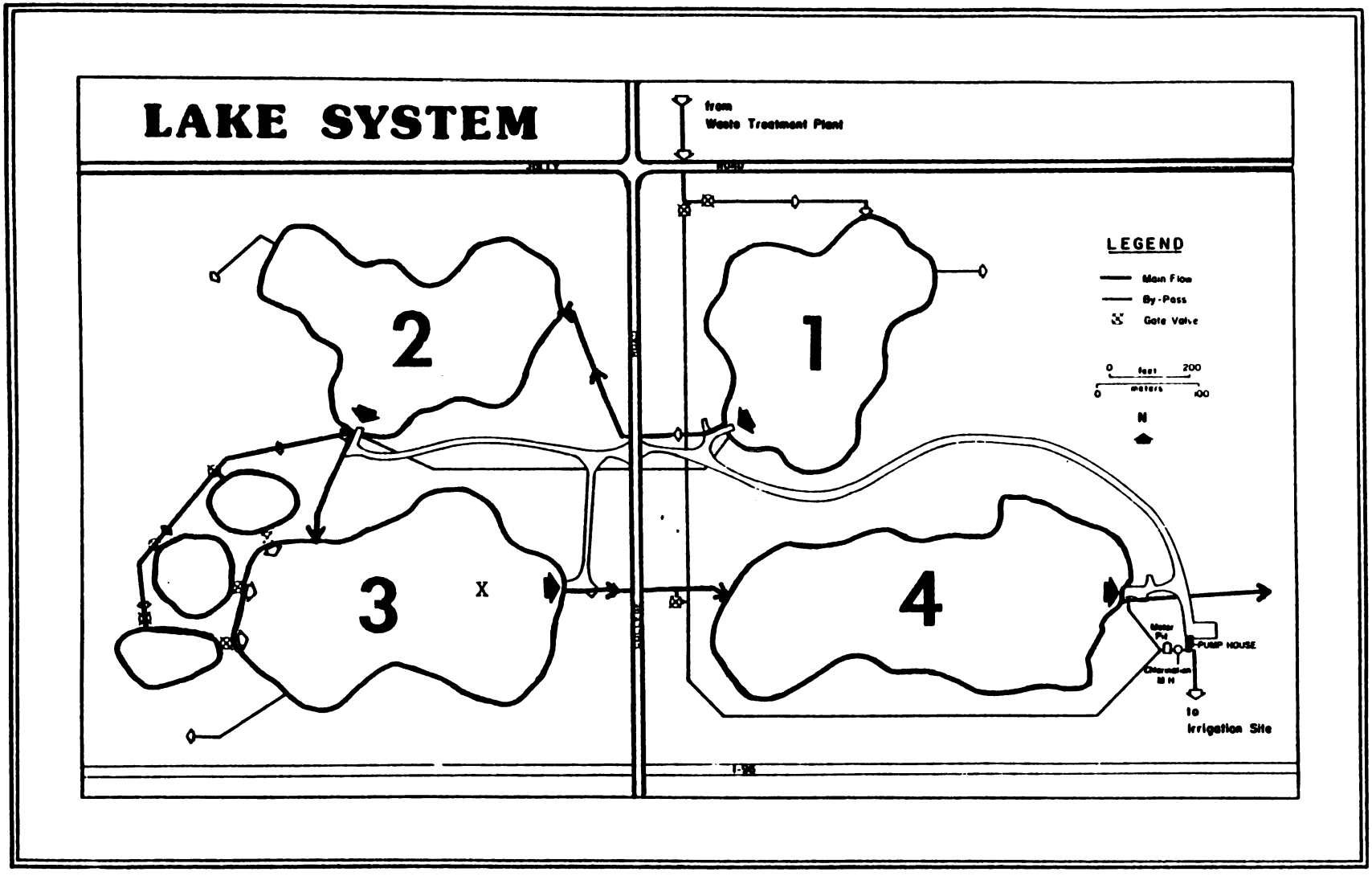

LAKE SYSTEM
from
Waste Treatment Plant
LEGEND
Main Flow
By-Pass
Gate Valve
0 feet 200
0 meters 100
N
1
2
3
4
X
PUMP HOUSE
to
Irrigation Site

Figure 1.

a major factor influencing algal growth. Although macrophytes occurred in substantial amounts at one point in the summer, they were subsequently harvested and greatly reduced.

Field Study

Water samples were collected weekly from a small area (Figure 1) of Lake 3 of the project from March 29 through October 30, 1977. Three samples were taken on each date at a depth of 0.5 meters using a non-metallic Kemmerer sampler. Measurements of the temperature at depth intervals of 0.3 meters and Secchi disc transparency were also made during this period. Samples were transported to the laboratory in a darkened container and immediately fixed with a solution of iodine, potassium iodide, and glacial acetic acid (Prescott, 1970). After standing for one hour, measured amounts of the samples (depending on algal density) were filtered onto 0.45 μm pore size Millipore membrane filters which were allowed to air dry. The filters were mounted on large (2" x 3") microslides using Type "A" immersion oil, and covered with a large coverglass for storage until counting (Amer. Public Health Assoc., 1976).

Cell densities for each species in the samples were determined by cell counts (McNabb, 1960; Amer. Public Health Assoc., 1976). Twenty fields at high power (950X) were counted, the mean number per field multiplied by a factor to obtain total cells per filter and divided by the volume of sample filtered to obtain cells per milliliter for each species in the samples. Cell volume per ml was calculated by determining a mean volume per cell by first measuring cell dimensions of 100 cells of each species, calculating their cell volumes and determining a mean value. Cells per ml was then multiplied by cell volume per cell

to obtain cell volume per ml (Amer. Public Health Assoc., 1976). Daily measurements of various water chemistry parameters (Appendis 1) were provided by the MSU Institute of Water Research.

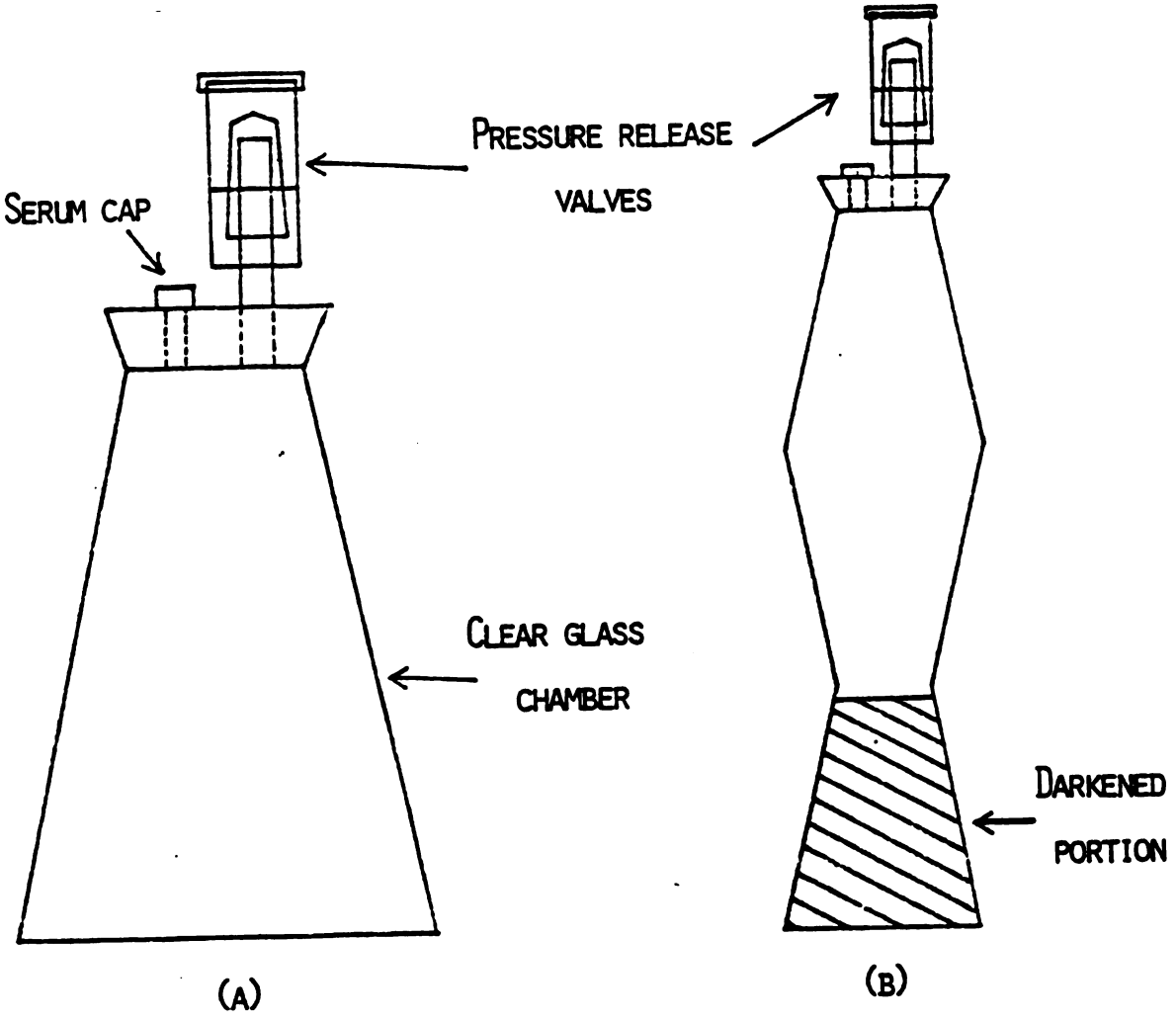
Kinetics Experiments

Three of the species that dominated the phytoplankton of Lake 3 for various periods during the 1977 growing season Scenedesmus communis Hegewald (formerly S. quadricauda), Anabaenopsis elenkinii Miller, and Pandorina morum (Muell.)Bory., were isolated and grown in unialgal cultures in the laboratory. By examination and multiple regression studies of the field data it was determined that nitrate and free CO_2 were the most likely factors limiting to the growth of the dominant species. The kinetics experiments were designed to test the effects of varying nitrate and free CO_2 concentrations on the growth of these dominant species.

The experiments were conducted using batch cultures in specially designed culture chambers (King and Hill, 1978), using the methods of Young and King (1973). Each treatment consisted of paired containers, a light chamber and a light-dark (LD) chamber. The light chamber (Figure 2a) consisted of a 1000 ml Erlenmeyer flask sealed with a rubber stopper with two holes. Into one hole was fitted a serum cap through which samples could be extracted; the other accomodated an air lock to minimize gas exchange with the air to reduce recarbonation of the medium and to maintain atmospheric pressure in the chambers. In this chamber, the maximum physiological growth efficiency of the species was measured.

The LD chamber was constructed from three 500 ml Erlenmeyer flasks fused as shown in Figure 2b. The bottom section of the container was painted black and covered with aluminum foil to create a darkened zone,

Figure 2. (A) Light chamber, (B) light-dark chamber, and (C) experimental arrangement used in kinetics experiments.


PRESSURE RELEASE
VALVES
SERUM CAP
CLEAR GLASS
CHAMBER
DARKENED
PORTION
(A)
(B)

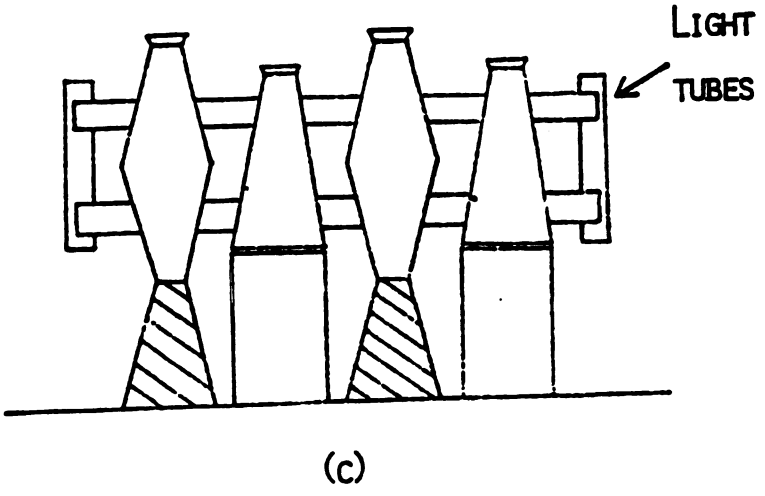

LIGHT
TUBES
(C)

Figure 2.

into which sinking cells would fall and be prevented from photosynthesizing. This chamber enabled the measurement of kinetic values in a situation more similar to field conditions than was possible in the light chambers.

Illumination of the chambers was by two horizontal 35-watt cool white flourescent light tubes, which provided a constant intensity of 3200 lux. The cultures were lighted from the side to minimize light penetration into the dark portion of the LD chambers. Light chambers were raised on platforms to compensate for the greater height of the LD chambers (Figure 2c). The lights were oriented so that all eight chambers could be simultaneously illuminated. Light intensity was measured using a Weston 756 footcandle meter.

Growth Medium

The growth medium used (Appendix 2) was modified from Bold's basic medium (James, 1974) to include bicarbonate for a carbon source and to be dominated by monovalent cations to reduce the possibility of carbonate precipitation during photosynthesis. All nutrients were assumed to be well in excess of need except for nitrate and CO_2 which varied according to treatment.

The medium was autoclaved at 121°C, 1.05 kg/cm^2, for 15 minutes, cooled overnight and bubbled with air for four hours to return it to atmospheric equilibrium. Alkalinity was then measured using the potentiometric method (Amer. Public Health Assoc., 1976) and adjusted to 2.00 meq/l with autoclaved $NaHCO_3$ to simplify calculations.

The algae used to seed the cultures were from cultures of the three species isolated from Lake 3 and maintained in the laboratory in identical medium and under similar light conditions to those used in the

experimental cultures. Prior to each experiment, the algae were subcultured in fresh, nitrate free medium and allowed to grow until the pH of the medium had increased 1-2 units (3-5 days). These cultures were then well mixed and, for each treatment, equal amounts were concentrated by centrifugation, decanted, and resuspended in fresh medium. This procedure was performed twice for each treatment. In this manner, equal amounts of inoculum were added to each experimental chamber.

pH Measurements

The progress of growth in the cultures was monitored through daily measurement of pH. The pH was measured to the nearest 0.02 unit using a Horizon Model 5996 pH meter calibrated frequently with standard buffer solutions. Samples for measurement were removed from the chambers with a hypodermic needle inserted through the serum cap in the top of each chamber. The samples were then deposited, slowly to minimize recarbonation, into small containers into which the probe was lowered.

Treatments

The population growth rates of the three species were measured in relation to varying concentrations of free CO_2 and nitrate. The treatments consisted of four initial nitrate concentrations (10.0, 1.0, 0.1, and 0.01 mg/l-N) with free CO_2 being allowed to vary over the course of the experiment. Instantaneous growth rates were calculated daily as CO_2 declined. In this manner, an examination of the effects of the interaction of these two factors on the growth of these species was accomplished.

Calculations

The calculation of specific growth rates followed the methods of King and King (1974) and King and Hill (1978), the kinetic variables

(μ_{max}, K_s) being a function of free CO_2. Total carbon (ΣCO_2) and free CO_2 were calculated using carbonate-bicarbonate equilibrium equations (King and Novak, 1974) in conjunction with measurements of pH, alkalinity, and temperature. Carbon fixation for photosynthesis was assumed to draw exclusively from the carbonate-bicarbonate alkalinity and all carbon removed from the alkalinity was assumed to be due to fixation by the algae.

Total carbon and free CO_2 were calculated using the daily pH measurements, alkalinity (due to the sealed container) and temperature were constant. Algal growth was assumed to follow a first order growth equation:

$$M_t = M_o \, e^{\mu g t} \tag{2}$$

where M_t is the mass at time t, M_o is the initial mass, and μ_g is the specific growth rate. μ_g was calculated by dividing the change in ΣCO_2 per day by the average biomass (carbon fixed) over the period of measurement.

The specific growth rate was assumed to follow the Michaelis-Menten or Monod model, modified to include a minimum required concentration or threshold level of free CO_2:

$$\mu_g = \mu_{max} \frac{\{CO_2\} - C_o}{(K_c - C_o) + (\{CO_2\} - C_o)}$$

where μ_{max} is the maximum specific growth rate, $\{CO_2\}$ is the concentration of free CO_2, K_c is the concentration of CO_2 at which $\mu_g = \frac{1}{2} \mu_{max}$, and C_o is the minimum concentration of CO_2 required for growth. K_c and μ_{max} were calculated using a linear regression of a $\mu_g/\{CO_2\}$ vs. μ_g

transformation of the specific growth rate equation (Dowd and Riggs, 1965). C_o was the concentration of free CO_2 at which growth ceases in culture.

While in the light chambers, sinking cells fell to the bottom and continued to photosynthesize, in the LD chambers sinking cells were removed from the growing biomass of the population. Because of this removal, the accrual of active biomass was slower in these than in the light chambers and the rate of this accrual is calculated somewhat differently. Since both chambers were incubated under identical conditions, it was assumed that μ_g in the lighted zone was the same in both chambers at the same free CO_2 levels. To calculate accrued biomass (ab), the change in total carbon per day was divided by the μ_g that occurred at that particular CO_2 concentration in the light chamber. The rate of biomass accrual (μ_{ab}) was calculated then as before by dividing the change in accrued biomass per day by the mean accrued biomass for that period. This value may be positive or negative depending on whether there was an increase or decrease in ab over that period.

The specific growth rate (μ_g) represents the total growth rate when effects from sinking are prevented in the light chamber. The biomass accrual rate (μ_{ab}) represents the specific growth rate minus the specific sinking rate (μ_s). Therefore μ_s can be calculated by subtracting μ_{ab} from μ_g.

Organic Carbon Experiments

The field data provided evidence to suggest that the two growth periods of P. morum were in response to increased amounts of organic matter which stimulated its growth through either heterotrophy or mixotrophy. Two experiments were designed to test this possibility.

In the first, the response of P. morum to additions of acetate (a simple organic substrate) at high and low nitrate levels was measured.

Only light chambers were used in this experiment, with culture medium and illumination as before. The four treatments were as follows: high nitrate (10 mg/l-N) with acetate (10 mg/l); high nitrate without acetate; low nitrate (0.01 mg/l-N) with acetate; and low nitrate without acetate. A replicate of each of these treatments was maintained in complete darkness. Cultures were inoculated as before and pH was measured daily. To account for the possibility of heterotrophic growth which would not predictably affect pH, cell counts were made approximately every other day using a Sedgewick-Rafter counting cell (Amer. Public Health Assoc., 1976). Values for cell volume per ml were calculated as before.

In the second experiment, the response of P. morum to growth in medium filtered from growing cultures of S. communis and A. elenkinii was compared to sterile medium of similar initial pH. The purpose of this experiment was to test the possibility that the organic substrate which stimulated the growth of P. morum in the lake was that released from the populations of the other species during their log growth phases.

One culture of S. communis and one of A. elenkinii, inoculated and cultured as before were allowed to grow until the pH of the nitrate free medium reached 9.0. The cultures were then filtered through Reeve Angel 984 H glass fiber filters (0.5 μm porosity)to remove the algae, and bubbled with CO_2 gas until the pH equalled that in the sterile medium (approximately 7.5). All three were inoculated with P. morum as before, with daily pH measurements and cell counts every two days.

Results

Phytoplankton

Populations of five algal species dominated Lake 3 for various periods during the 1977 growing season (Figure 3a). The dominant phytoplanktonic alga at the beginning of the sampling period (3/29) was Cyclotella sp., a centric diatom, which peaked in early April at a cell volume of 238 mm^3/l. Cyclotella declined rapidly and was immediately replaced by a volvocalean green alga, Chlamydomonas sp., which reached its maximum development the next week at 205 mm^3/l. Soon after the subsequent rapid decline of Chlamydomonas from dominance, a large bloom of Scenedesmus communis, a common chlorococcalean alga, occurred and reached its peak in early May with a cell volume of 297 mm^3/l, the largest of the season. This population then decreased precipitously to 27 mm^3/l by 24 May.

A volvocalean green alga, Pandorina morum, reached its highest value that week at 116 mm^3/l, its major growth period occurring while S. communis was still dominant. P. morum exhibited a second peak of 113 mm^3/l in early July during a small bloom of Anabaenopsis elenkinii which later dominated the phytoplankton. This pattern of growth, with major increases occurring during periods of reduced light due to large populations of other species, suggested that P. morum growth may have been augmented by heterotrophic uptake of organic carbon.

A nitrogen fixing blue green alga, Anabaenopsis elenkinii (Nostocales)

Figure 3. 1977 growing season time course measurements of (a) phyto-plankton populations, (b) Secchi disc measurements, (c) mean temperature, (d) dept-time temperature profile, (e) phosphate, (f) nitrate and ammonium nitrogen, and (g) free CO_2.

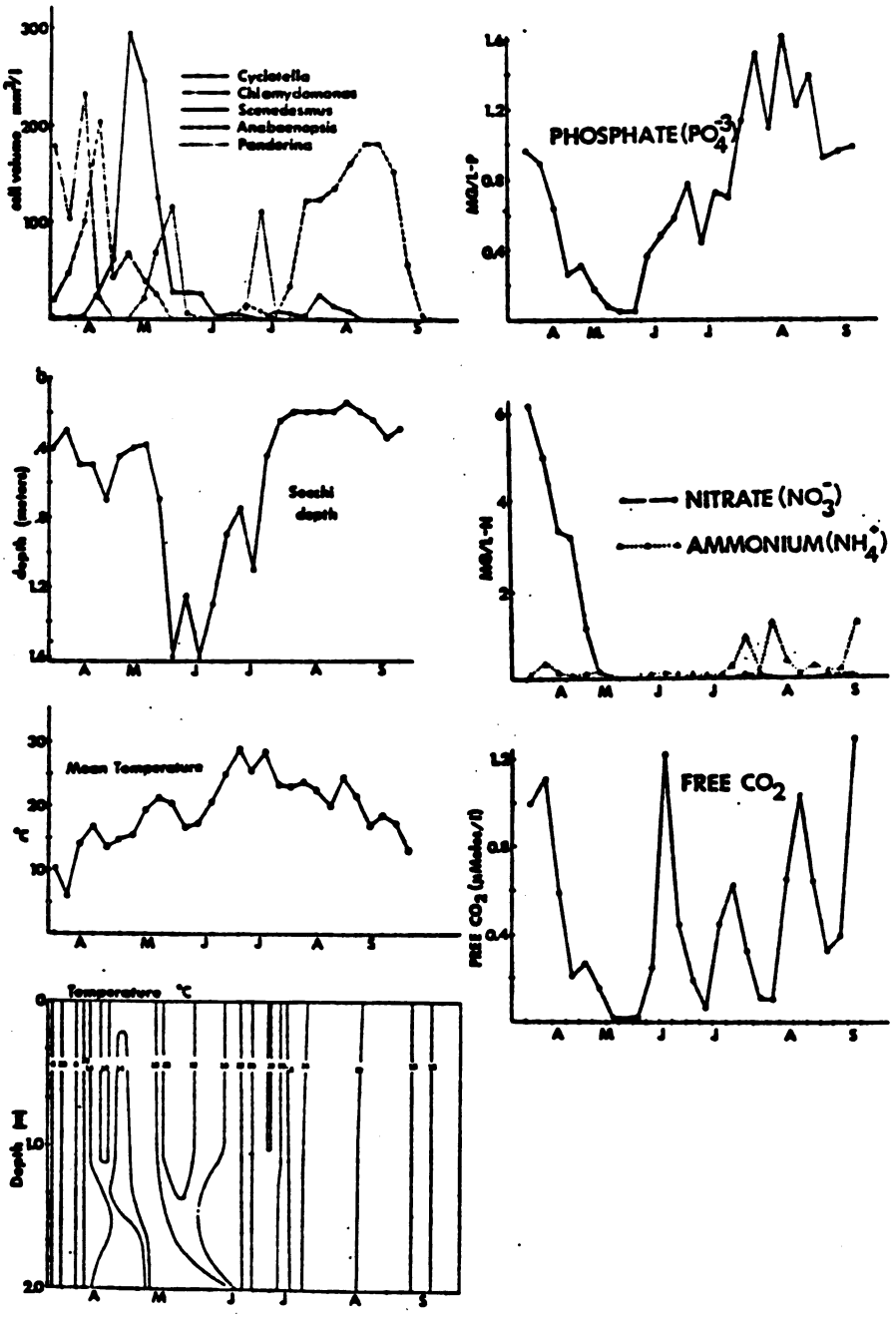

Cyclotella
Chlamydomonas
Scenedesmus
Anabaenopsis
Pandorina
PHOSPHATE (PO_4^{-3})
Secchi depth
NITRATE (NO_3^-)
AMMONIUM (NH_4^+)
Mean Temperature
FREE CO_2
Temperature °C
A M J J A S

Figure 3.

showed a small peak of 15 mm^3/l at the end of June, then increased through early July to plateau at 123 mm^3/l, then increased to a maximum of 181 mm^3/l in late August. By late September, A. elenkinii had declined to undetectable levels.

Secchi Disc Transparency

Measurements of Secchi disc transparency (Figure 3b) correlated well with measurements of total phytoplankton cell volume per liter ($r = 0.77$). The transparency was very low early in the season when populations of Cyclotella, Chlamydomonas, Scenedesmus, and Pandorina were abundant, varying from 0.3 to 0.7 meters during this period. The transparency increased greatly with the decline of these populations towards the end of May, to 1.6 meters. With the onset of the second P. morum bloom and subsequent A. elenkinii growth, the measurement decreased the depth of transparency to about half of that which occurred during the earlier blooms. This decrease in transparency may have resulted from the blue-green algae being highly concentrated at the surface due to the presence of gas vacoules in the cells allowing flotation.

Temperature

Mean water temperature increased gradually from a low of $6^{o}C$ in early April to a maximum of $29^{o}C$ in early July (Figure 3c). The shallow nature of the lake caused lake temperature to reflect changes in air temperature causing the observed weekly fluctuations throughout the season. Depth profiles of temperature (Figure 3d) show the absence of stratification for the major part of the growing season. Only two periods of slight stratification occurred, one lasting two weeks in mid-April when a maximum difference of $5^{o}C$ between surface and bottom water

occurred, and the other in late May, lasting for three weeks, with a maximum difference of 12°C. The absence of continuous stratification reflects the almost continual mixing of the entire water column of Lake 3.

Chemical Parameters

Thirteen parameters in Lake 3 were monitored daily by the Institute of Water Research at MSU. Orthophosphate, nitrate nitrogen, and free CO_2 were examined in detail as these are most often the nutrients implicated in studies of nutrient limitation of photosynthesis in eutrophic fresh waters. Micronutrients were assumed to be in abundance as these lakes are supplied with secondary effluent from treated domestic wastewater.

Orthophosphate (PO_4^{-3}) underwent considerable seasonal variation but at no time decreased to levels limiting to algal growth (Figure 3e). In early spring, the phosphate concentration was approximately 1 mg/l but declined rapidly during the Cyclotella, Chlamydomonas, and Scenedesmus blooms to a low of 49 µg/l in late May, a level in the range of the minimal concentrations necessary for optimal growth in most algal species including S. communis (Hutchinson, 1957; Wetzel, 1975). The levels subsequently increased to a high of 1.6 mg/l in mid-August and declined somewhat to the end of the sampling period. Due to the adequate levels of orthophosphate in the lake throughout the growing season it was decided that, under these conditions of heavy artificial enrichment, phosphorus was not likely to be an important factor influencing the periodicity of algal populations observed, and was not considered further in laboratory experiments.

Nitrate nitrogen fluctuated considerably less than phosphate

(Figure 3f), declining from an early high concentration of 6.0 mg/l to undetectable levels by mid-May. Nitrate was not detected again until the end of July when a small concentration (0.1 mg/l-N) appeared for two weeks. The early decline coincided with the growth of the three early species suggesting that the decline was due to uptake by these populations. Ammonia nitrogen was very low throughout the sampling period except for two peaks of about 1 mg/l which occurred in late July and early August. Since there seemed to be insufficient nitrogen as nitrate or ammonium to allow the large population growth of A. elenkinii, it was assumed that elemental nitrogen, N_2, was used as a nitrogen source by the heterocystous blue green alga, species of which have been shown to have this ability (Fogg, 1974; Watanabe, 1951). The ammonium was presumably either taken up by the algae or was released into the air as ammonia (NH_3) which occurs to an increasing extent when pH increases past the pK (9.2) for the dissociation of NH_4^+ into ammonia gas (Institute of Water Research, 1976) a level that was surpassed during most of this period.

Free CO_2 exhibited fluctuations within a narrowly restricted range, occurring outside the range of 0.1 to 1.3 μM/l on only two occasions (Figure 3g). For the most part, the trends seemed to be explicable on the basis of phytoplankton growth and decomposition in the lake. The decline early in the season corresponds, as with nitrogen and phosphorus, to the large increases of Cyclotella sp., Chlamydomonas sp., and S. communis as well as P. morum during that period. The increase in early June is likely due to the decomposition of those previous blooms, but the cause of the subsequent rapid decline of CO_2 is unclear, but may have been due to uptake by macrophytes. The decline of CO_2 in late

July corresponded with the first major growth phase of A. elenkinii, while the late August decline corresponded somewhat less precisely to the second blue-green growth period.

On the basis of these considerations, it was determined that nitrate and free CO_2, singly or in combination, were most likely the two most important nutrient factors influencing the population dynamics of the three dominant species which were studied in the laboratory. The studies of population growth kinetics were based on these apparent correlations.

Laboratory Experiments

Scenedesmus

Daily measurements of pH were taken from each of the four culture treatments in both light and LD chambers and plotted against time. The extent to which S. communis could increase the pH diminished with decreasing initial nitrate concentration (Figure 4). The maximum pH values were lower for each treatment in the LD chamber than in the light, except for the 10 mg/l-N LD treatment, where attached growth on the chamber walls apparently caused a pH increase equalling that in the light chamber (see below). Kinetic values for μ_{max}, K_c, and C_o were calculated (Table 1) using specific growth rate (μ_g) vs. free CO_2 concentration data and curves were calculated and plotted for the light chambers (Figure 5). It is evident that μ_{max} (the maximum growth rate) decreases ca. 20 percent over the range of decreasing initial nitrate concentrations. C_o values (the minimum CO_2 concentration at which growth can occur) increased by almost two orders of magnitude, from 0.0015 to 0.104 µM/l, as did the K_c values (half saturation value) increasing from 0.033 µM/l at an initial nitrate concentration of 10 mg/l-N to 0.795 µM/l at 0.01 mg/l-N.

Figure 4. Daily pH measurements of S. communis growth experiments at four initial nitrate concentrations in light and light-dark chambers.

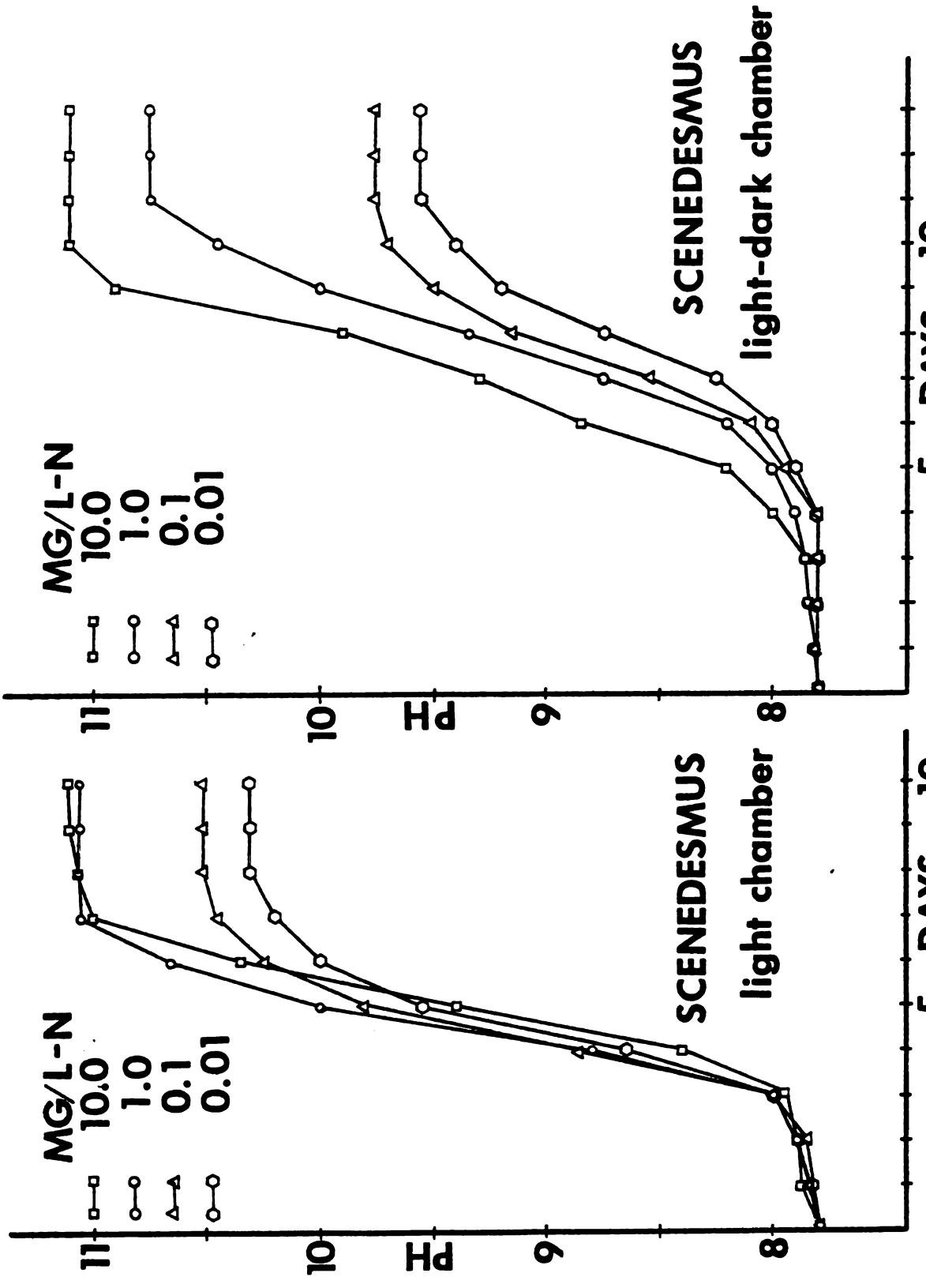

MG/L-N
10.0
1.0
0.1
0.01
SCENEDESMUS
light chamber
light-dark chamber
PH
11
10
9
8
5
DAYS
10

Figure 4.

Table 1. Kinetic values for S. communis growth related to free CO_2 and initial nitrate concentration obtained (a) by calculation using linear regression of transformed data and (b) from graphs fitted to data by visual approximation. (a) correlation coefficients indicate fit of linear equation to transformed data.

(a)	Light Chamber			
Initial-N (mg/l-N)	μ_{max} (day^{-1})	K_c (μM/l)	C_o (μM/l)	r
10.0	1.19	0.0329	0.0015	0.75
1.0	1.11	0.0404	0.0018	0.76
0.10	1.08	0.4082	0.0369	0.70
0.01	0.96	0.7950	0.1048	0.65

(b)	Light-dark Chamber	
10.0	0.036	0.0015
1.0	0.050	0.011
0.10	2.30	1.010
0.01	5.90	3.00

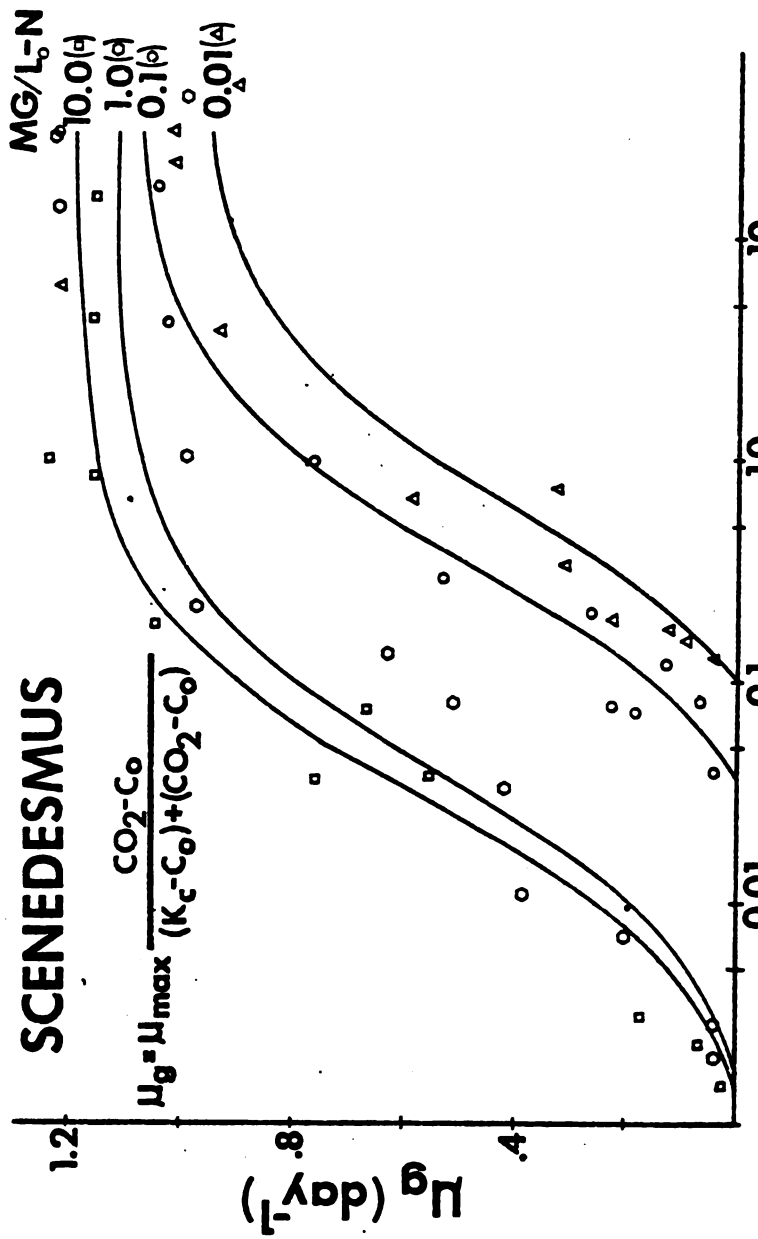


Figure 5. Specific growth rate (μ_g) of S. communis over free CO_2 levels at various initial nitrate concentrations.

When data from the LD chambers (μ_{ab}) are plotted for NO_{3i}^{-} = 0.10 mg/l-N (Figure 6)(the mean lake concentration the week when S. communis began to decline) it is clear that the inclusion of sinking as a factor further increases the values of C_o (from 0.035 to 1.01 μM/l) and K_c (from 0.408 to 2.30 μM/l). The concentration of free CO_2 in the lake during the time when S. communis first began to decline was 0.15 μM/l, a value which fell between the C_o values of the μ_g and μ_{ab} curves, provided evidence that the experimentally determined values may be extremes within which one could expect the field values to fall, when carbon and nitrate are the controlling factors.

This relationship was tested for the remainder of the growth period by determining upper and lower estimates of specific growth rate in the field using the mean weekly concentrations of nitrate and free CO_2 in Lake 3 in the following equation:

$$\mu_g = \mu_{max_i} \frac{\{CO_2\} - C_{oi}}{(K_{ci}-C_{oi})+(\{CO_2\}-C_{oi})} \qquad (4)$$

where μ_{max}, K_{ci}, and C_{oi} are the values of these variables that would be expected at the mean nitrate concentration of the i^{th} week. K_c and C_o values (in units of $\mu M\text{-}CO_{2f}/l$) from both light and LD chambers were plotted against initial nitrate concentration, linear regression equations were calculated (Figure 7), and values were calculated from these equations for incorporation in equation (4). As previously mentioned, the C_o value for the 10 mg/l-N LD treatment is probably inaccurate due to the attached growth on the walls of the chamber. However, the elimination of that point does not significantly change the prediction equation or the calculated C_o values used in equation (4). Values for

Figure 6. Relationship of μ_g, μ_{ab}, and μ_s of S. communis at an initial nitrate concentration of 0.10 mg/l.

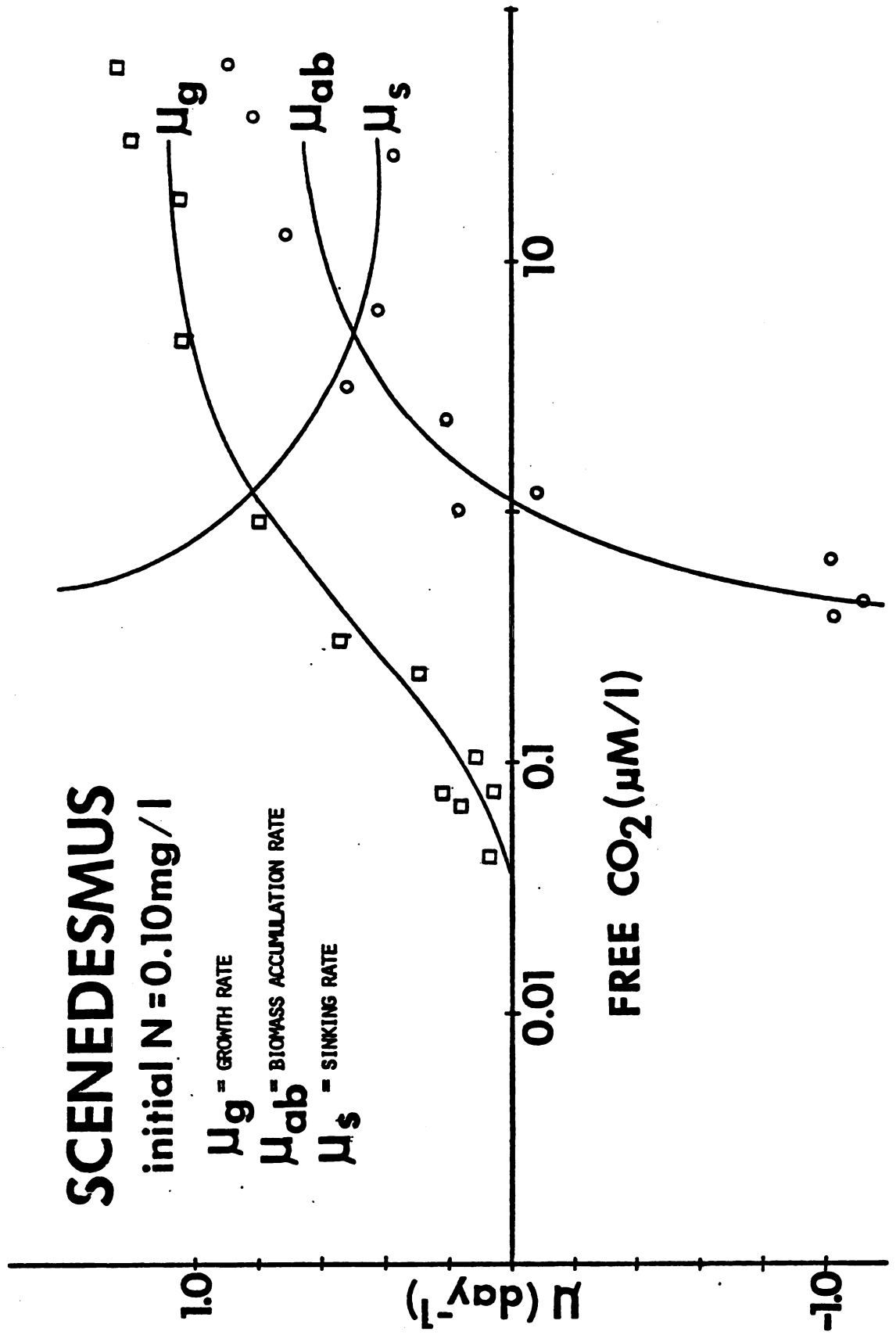

SCENEDESMUS
initial N = 0.10mg/l
μ_g = GROWTH RATE
μ_{ab} = BIOMASS ACCUMULATION RATE
μ_s = SINKING RATE
μ_g
μ_{ab}
μ_s
1.0
-1.0
μ (day⁻¹)
0.01
0.1
10
FREE CO_2 (μM/l)

Figure 6.

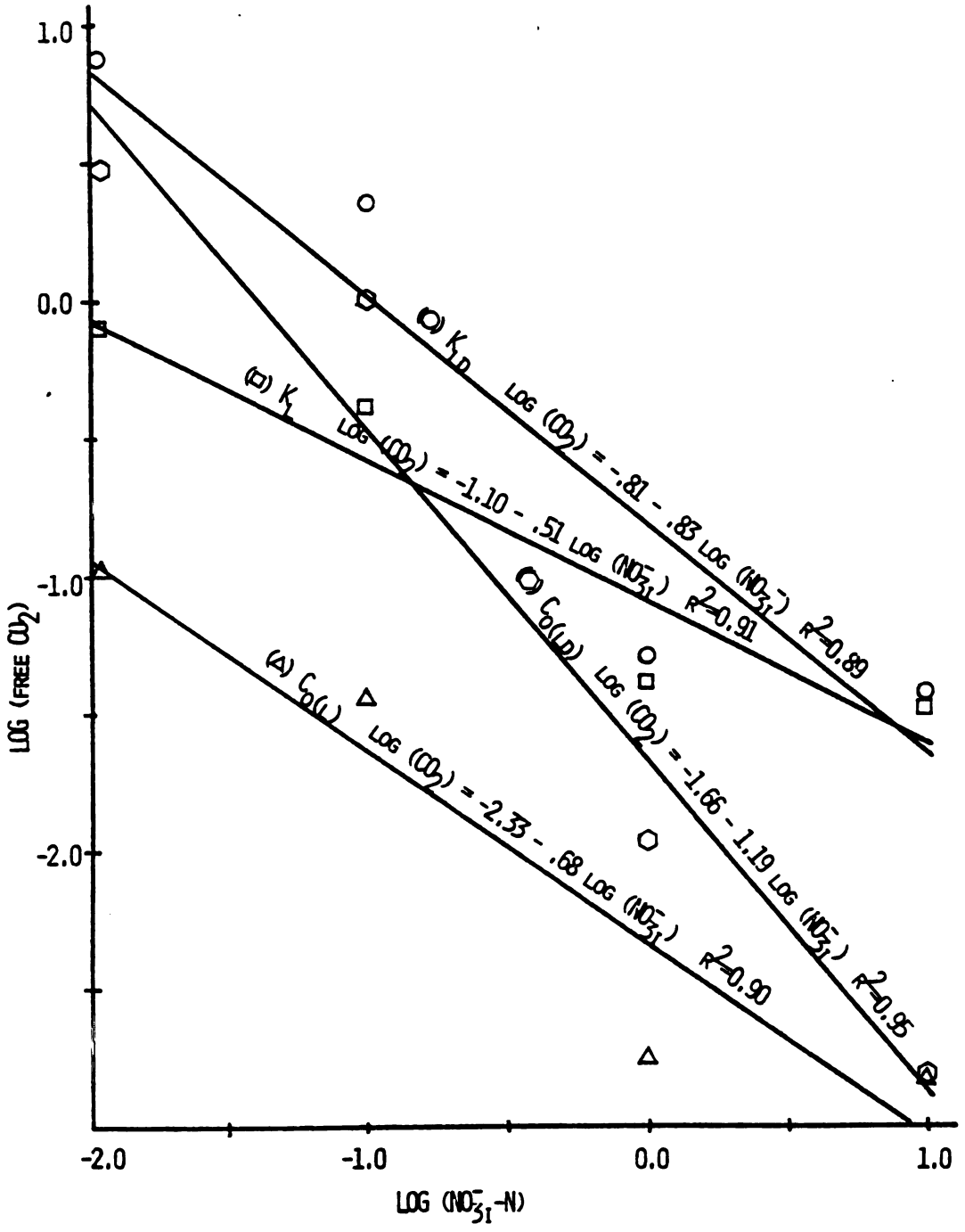


Figure 7. Regression equations and lines of K_c and C_o values from light and LD chambers at different initial nitrate concentrations.

μ_{max} were obtained from similar plots.

The actual growth rate of S. communis in Lake 3 was calculated as follows (Fogg, 1975):

$$\mu_{f_i} = \frac{(\ln N_{n+1}) - (\ln N_n)}{7 \text{ days}} \quad (5)$$

where μ_{f_i} is the specific growth rate of S. communis in Lake 3 during week i, N_n is its cell volume on the sampling day previous to week i, and N_{n+1} is the cell volume on the following sampling date. μ_g (physiological efficiency), and μ_{ab} (ecological efficiency), were corrected for day length, and with μ_f were then plotted over the period of growth (Figure 8).

The period from the initial S. communis appearance until its major decline at the end of May was included. During weeks 1-3 of this period, while S. communis was a minor component of the algal community, its measured field growth rate fell below the predicted range. In the following four weeks, during which it was the dominant species, the field values fell between the estimates calculated from laboratory data.

Similar plots were constructed holding values of K_c and C_o constant to determine if the field variations in free CO_2 alone could better account for the changes in μ_f. When the kinetic values obtained at NO_3^--N = 0.01 mg/l, μ_f was similar to the μ_g curve but actually exceeded it for the portion of the growth period when the nitrate concentrations in the field were well above 0.01 mg/l. These results demonstrate the better fit attainable using both factors.

A problem with this analysis is the likelihood that the values of K_c and C_o calculated using the methods described may be artificially

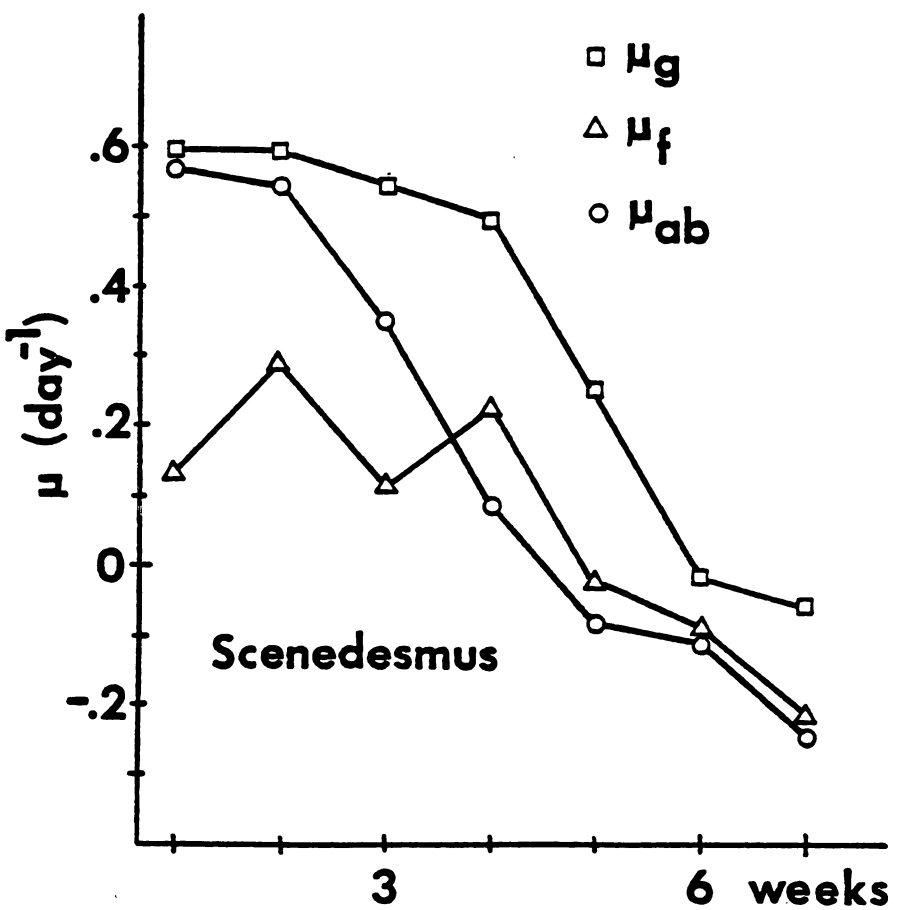


Figure 8. Comparison of field growth rates (μ_f) of S. communis to estimates (corrected for day length) calculated with data from light (μ_g) and LD (μ_{ab}) chambers.

high. This error occurred because nitrate concentration was not kept constant in the chambers, and decreased over time due to uptake by the algae. Since the values of K_c and C_o come from data obtained near the end of the culture period when nitrate was considerably lower than initial levels, it is likely that the algae would grow more poorly, producing values that were higher (less efficient) than if nitrate maintained its initial concentration. These higher values would produce low values of μ_g and μ_{ab} in the above analysis, possibly causing less agreement with field values. However, it is unlikely that a more accurate determination of the K_c and C_o values would substantially change the trends exhibited by the μ_g and μ_{ab} curves and it is the agreement of μ_f with these trends, rather than whether or not it falls between the two curves, that is most significant.

Anabaenopsis

Initial nitrate concentration had little effect on the rate or extent of pH increase of A. elenkinii in the light chambers (Figure 9). When kinetic values of μ_{max}, K_c and C_o were calculated (Table 2) it was notable that the lowest μ_{max} value, 0.65 day $^{-1}$, was obtained at the highest initial nitrate concentration, 10 mg/l-N, indicating possible growth inhibition by high levels of nitrate (Figure 10). In the LD chambers, a substantial decrease in the highest pH attained was found at the two lowest nitrate concentrations, 0.10 and 0.01 mg/l-N, and the values attained in the LD chambers were substantially less than those in the light chambers.

When μ_{ab} was calculated from the LD chamber data with $NO_{3i}^- = 0.01$ mg/l-N (the approximate concentration of nitrate during the period of A. elenkinii growth) the C_o and K_c values were over two orders of magnitude

Figure 9. Daily pH measurements of A. elenkinii growth experiments at four initial nitrate concentrations in light and light-dark chambers.

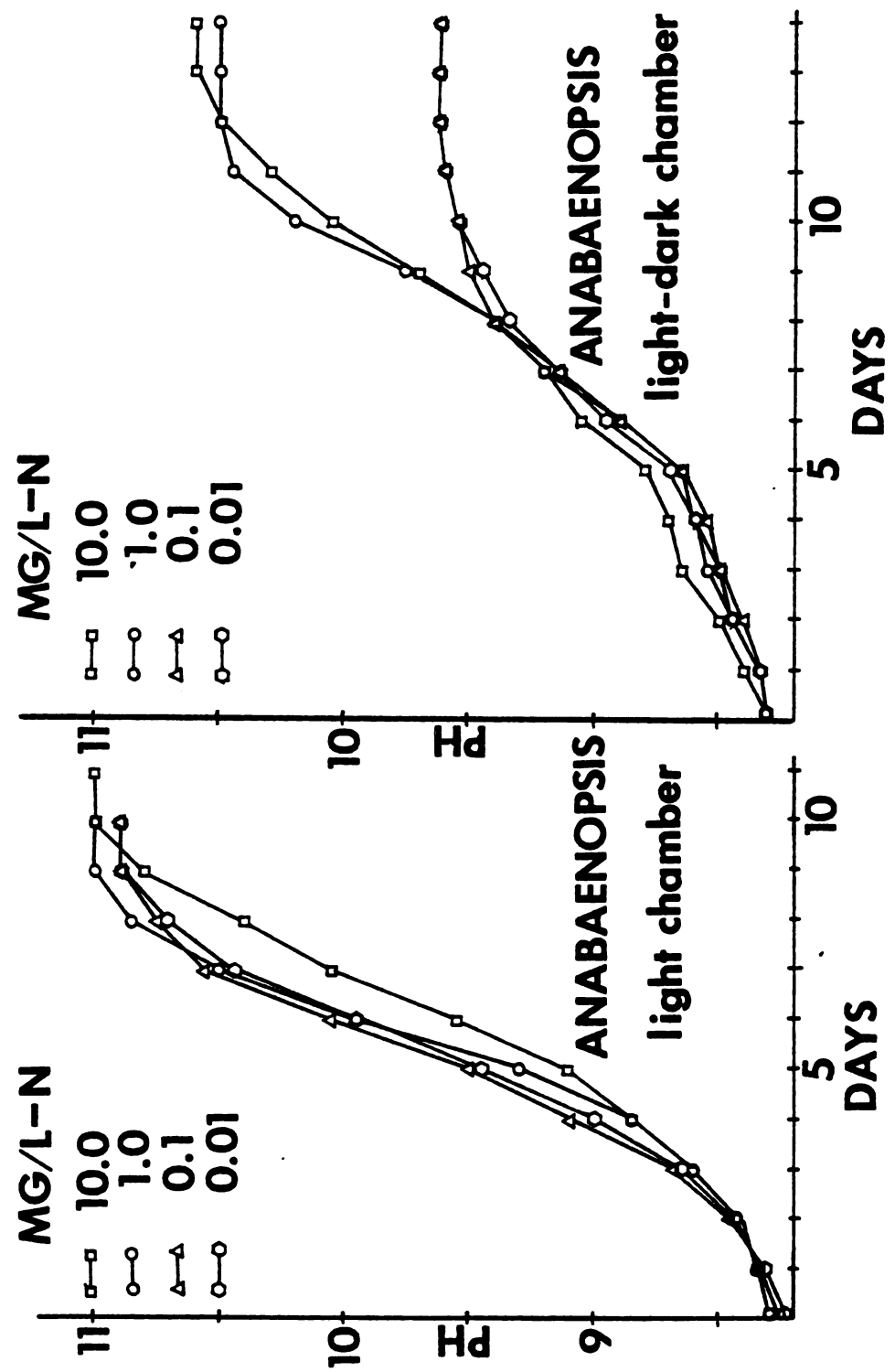

MG/L-N
10.0
1.0
0.1
0.01
11
10
9
pH
ANABAENOPSIS
light chamber
5
10
DAYS
ANABAENOPSIS
light-dark chamber

Figure 9.

Table 2. Kinetic values for A. elenkinii growth related to free CO_2 and initial nitrate concentration obtained (a) by calculation using regression of transformed data and (b) from graphs fitted to data by visual approximation. (a) correlation coefficients indicate fit of linear equation to transformed data.

(a)	Light Chamber			
Initial-N (mg/l-N)	μ_{max} (day^{-1})	K_c (μM/l)	C_o (μM/l)	r
10.0	0.646	0.0166	0.003	0.78
1.0	0.902	0.0306	0.003	0.78
0.10	0.757	0.0455	0.0048	0.84
0.01	0.725	0.0369	0.0048	0.85
(b)	Light-dark Chamber			
10.0		0.40	0.14	
1.0		0.70	0.23	
0.10		3.70	1.80	
0.01		3.50	1.80	

Figure 10. Specific growth rate (μ_g) of A. elenkinii over free CO_2 levels at various initial nitrate concentrations.

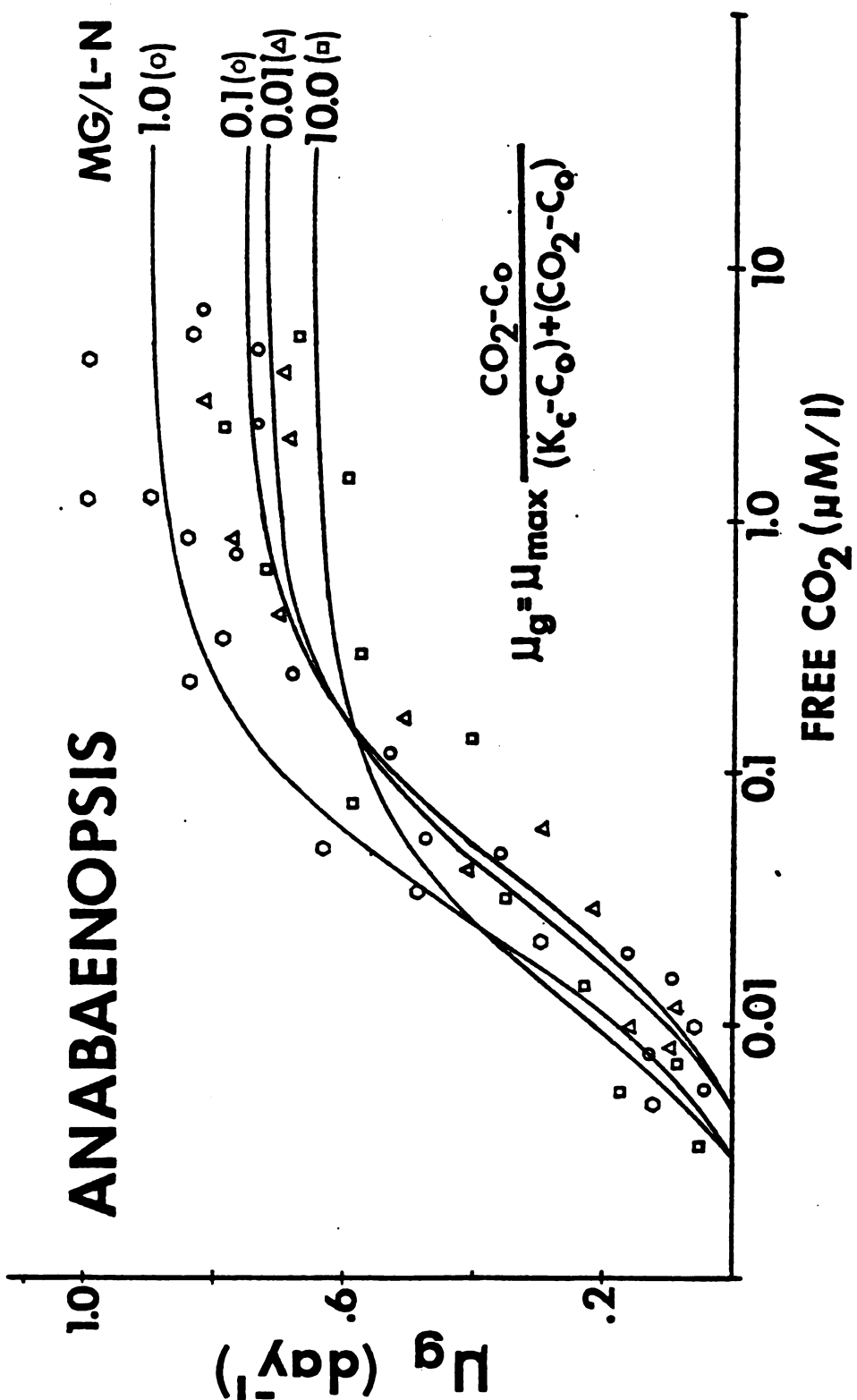

ANABAENOPSIS
MG/L-N
1.0(○)
0.1(○)
0.01(△)
10.0(□)
$\mu_g = \mu_{max} \frac{CO_2 - C_0}{(K_c - C_0) + (CO_2 - C_0)}$
μ_g (day^{-1})
1.0
.9
.2
0.01
0.1
1.0
10
FREE CO_2 (μM/l)

Figure 10.

greater than those found in the light chambers (Figure 11). C_o increased from 0.005 to 0.8 μM/l and K_c increased from 0.037 to 3.00 μM/l. The free CO_2 concentration in the lake during the entire period of A. elenkinii domination fell within the range of the two experimentally determined C_o values.

Using values of μ_{max}, K_c and C_o determined in light and LD chambers in equation (4), along with mean weekly values of free CO_2 and nitrate (mostly less than 0.01 mg/l-N) determined from Lake 3, upper and lower estimates (based on physiological and ecological efficiencies) of A. elenkinii growth were made as with S. communis. Measurement of the actual growth rate were made using equation (5) and all three growth rate values (μ_g and μ_{ab} corrected for day length) were plotted against time (Figure 12).

The actual growth rate (μ_f) fell between the high (μ_g) and low (μ_{ab}) estimates on all but two of the thirteen weeks A. elenkinii was present in the lake. The range provided by the estimates was rather large and μ_f underwent some fluctuations between them, presumably due to other factors not included in the model, but the overall agreement of μ_f with the calculated values provided some indication of CO_2 limitation (since nitrogen was presumably plentiful as N_2).

Pandorina

The pattern of growth of P. morum was similar in most respects to that of S. communis (Figure 13). Calculated values of μ_{max} in light chambers decreased with decreasing initial nitrate concentration to a greater extent than in S. communis. Minimum C_o values (at high NO_3^-) were higher in P. morum while at low NO_3^- the values were similar. With $NO_{3i}^- = 0.01$ mg/l, $\mu_{max} = 0.64$ day^{-1}, the lowest value found under any

Figure 11. Relationship of μ_g, μ_{ab}, and μ_s of A. elenkinii at an initial nitrate concentration of 0.01 mg/l-N.

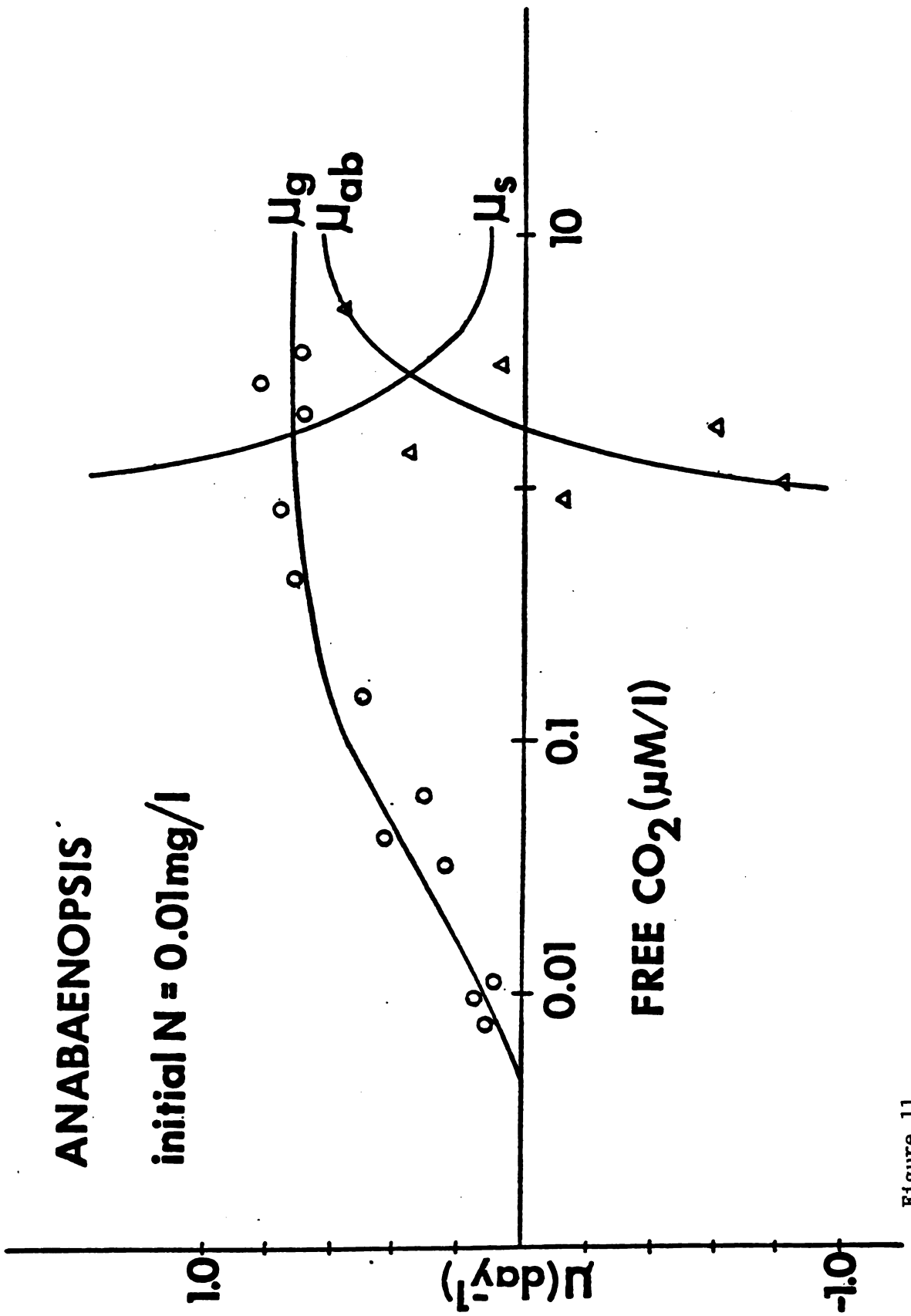

ANABAENOPSIS
initial N = 0.01mg/l
μ(day⁻¹)
1.0
-1.0
0.01
0.1
10
FREE CO_2 (μM/l)
μ_g
μ_{ab}
μ_s

Figure 11.

Figure 12. Comparison of field growth rates (μ_f) of A. elenkinii to estimates (corrected for day length) calculated with data from light (μ_g) and LD (μ_{ab}) chambers.

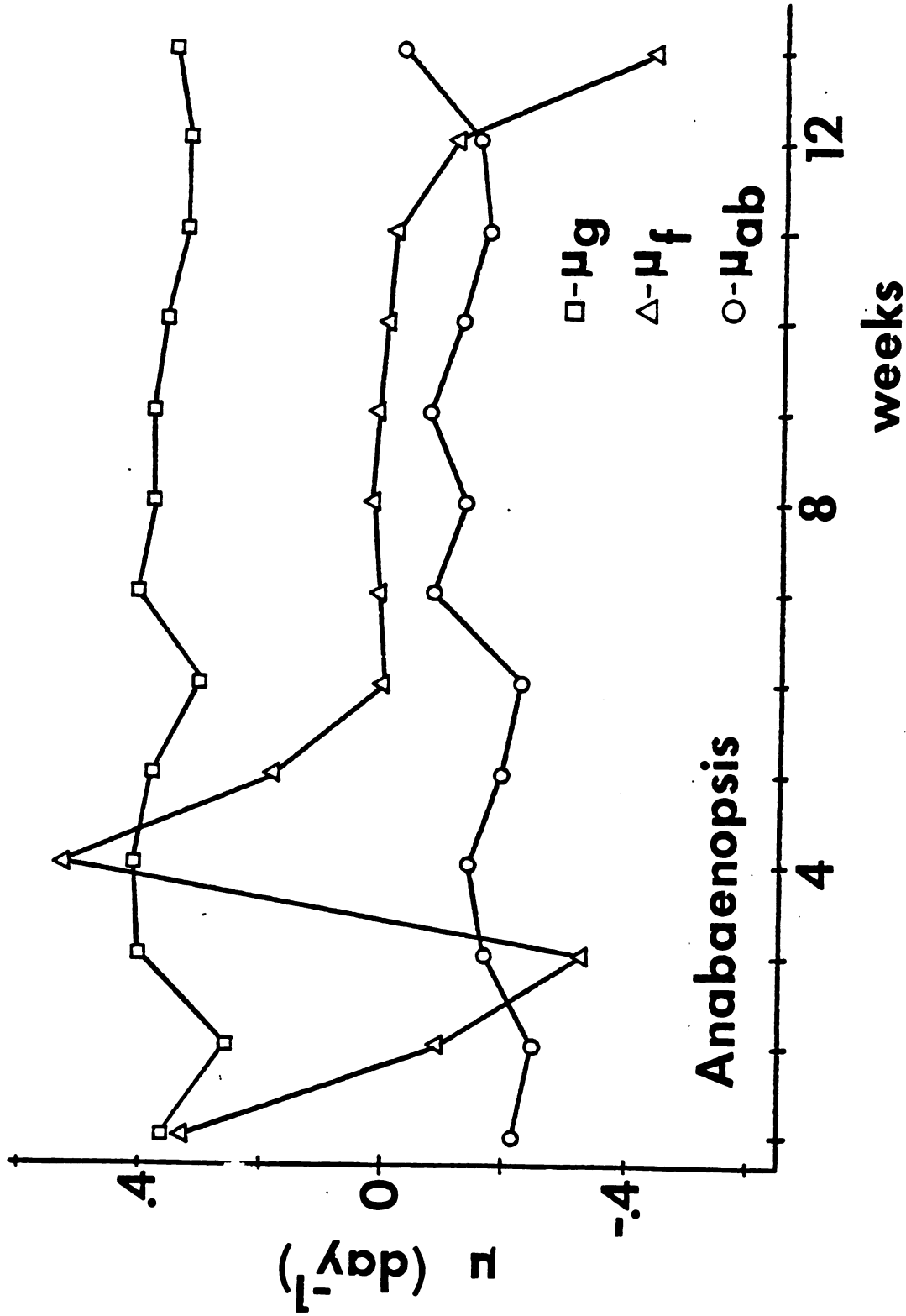


Figure 12.

Figure 13. Specific growth rate (μ_g) of P. morum over free CO_2 concentration at various initial nitrate concentrations.

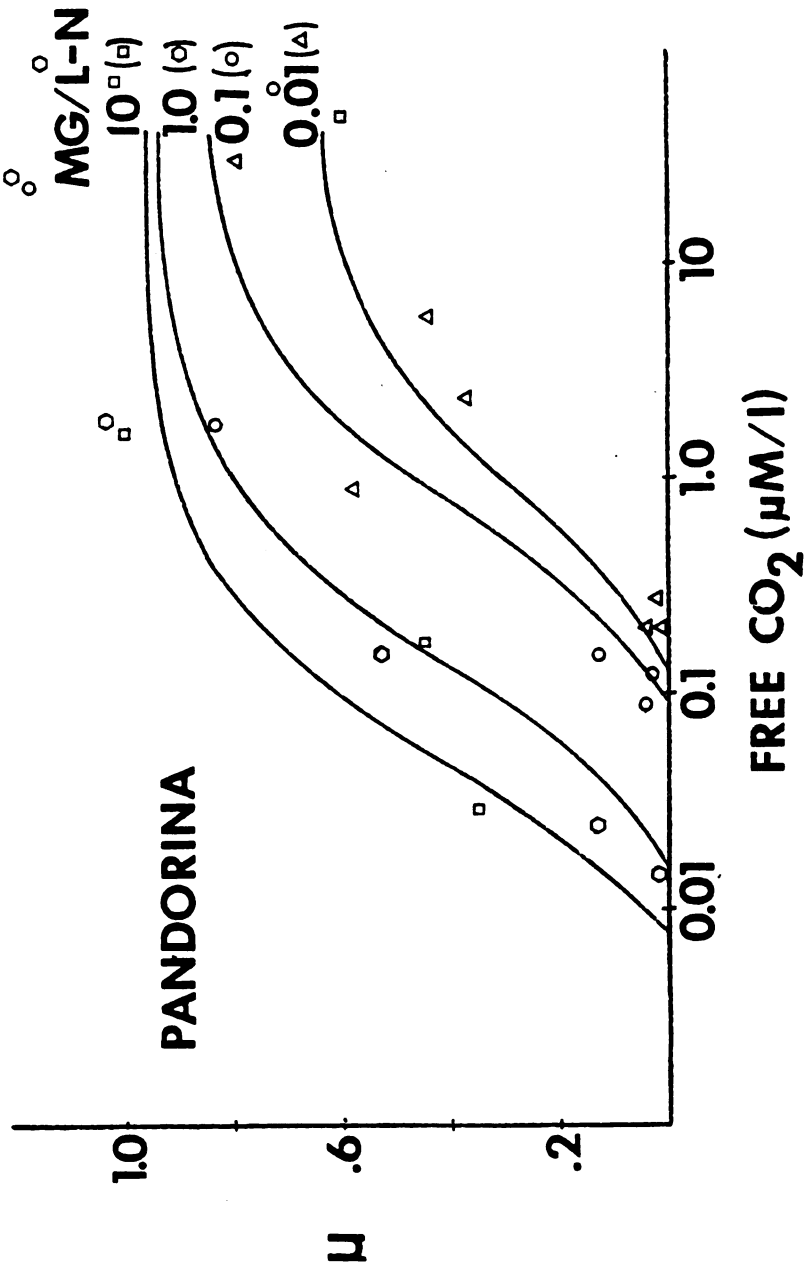


Figure 13.

conditions for the three species and C_o = 0.125 μM/l, a relatively high value compared to those of S. communis. C_o of the μ_{ab} curve was less than an order of magnitude greater at 0.58 μM/l (Figure 14).

These calculated values did not account for the growth of P. morum in Lake 3. During its first growth period in early May when ambient nitrate values dropped from 0.1 to less than 0.01 mg/l-N, the mean weekly free CO_2 concentration was well below that level which was observed to be required to grow P. morum under the culture conditions that were used. According to laboratory results, the expected C_o value under those nitrate levels would have varied between 0.09 and 0.125 μM/l. The mean ambient CO_2 concentration during that period was approximately 0.06 μM/l, well below that need for growth if it were responding only to the factors considered in the model.

Upper and lower calculated estimates of specific growth rate were determined as described earlier. Limits were calculated for the twelve weeks that P. morum occurred in Lake 3, and on only five dates did the actual field (μ_f) value fall between the predicted limits. Even this small amount of agreement between the model and P. morum's growth was probably fortuitous. The two major growth episodes both occurred when the model, using ambient levels of CO_2 and nitrate, predicted the growth rate should be negative. These results suggested the possibility of heterotrophic growth, which was considered in further experiments.

Heterotrophy Experiments

Cultures of P. morum grew substantially more rapidly in treatments with acetate added in initial concentrations of 10 mg/l than without acetate (Figure 15). The most rapid initial growth occurred with acetate and low nitrate (0.01 mg/l-N) concentration, but the greatest

Figure 14. Relationship of μ_g, μ_{ab}, and μ_s for P. morum at an initial nitrate concentration of 0.01 mg/l-N.

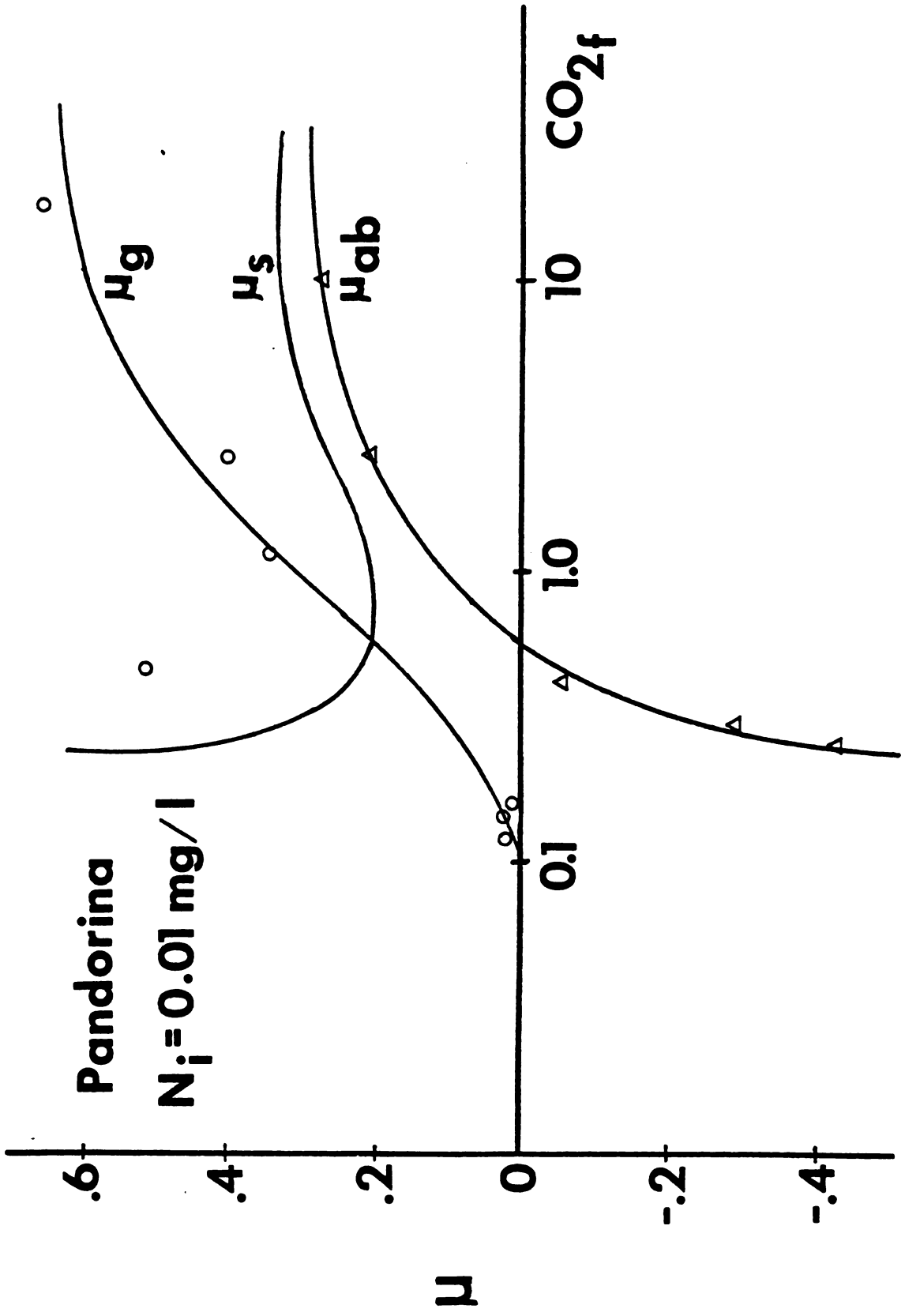


Figure 14.

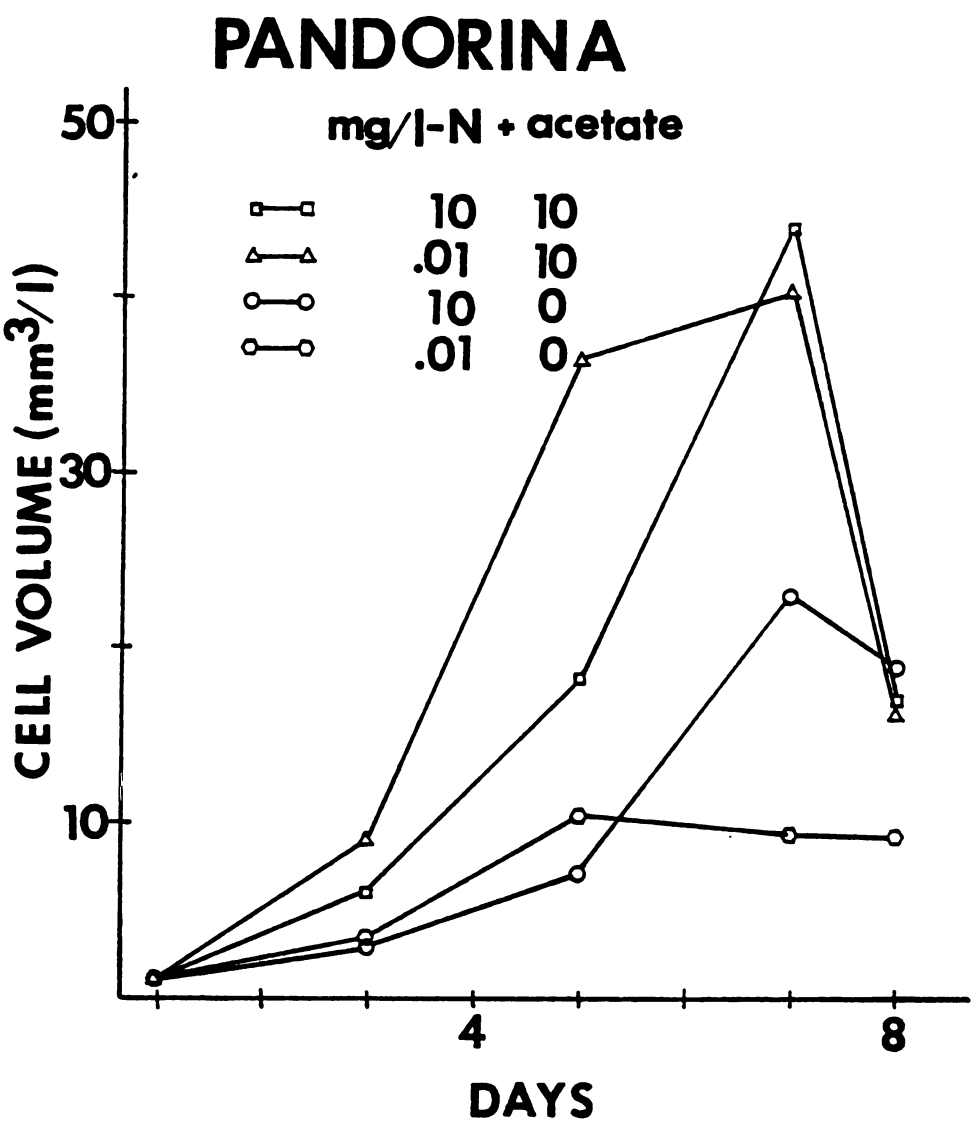


Figure 15. Response of populations of P. morum in culture to the addition or non-addition of acetate at high and low nitrate concentrations.

cell volume was reached with acetate and 10.0 mg/l-N. With acetate added, the cell volume reached with high nitrate was double that reached without acetate, and at low nitrate, the addition of acetate nearly quadrupled the maximum cell volume reached. Substantial increases in pH occurred in all treatments.

All four cultures in the dark exhibited rapid decline of the inoculated populations as indicated by cell counts. This decline indicated that this strain of P. morum was incapable of dark heterotrophic growth.

In the third experiment, P. morum growth did not differ significantly between the culture with sterile medium and either culture using medium filtered from actively growing cultures of S. communis and A. elenkinii. This experiment provided no evidence that organic compounds were released from log growth phases of S. communis and A. elenkinii that stimulated the growth of P. morum.

Discussion

General Discussion

The model used here to simulate the growth of planktonic algae in Lake 3 is a modified form of the Monod (1949) equation for nutrient limited growth (equation 3). The model has been modified to include a minimum or threshold concentration (S_o) of the limiting nutrient necessary for net growth to occur. Numerous other workers have included an S_o term to obtain the best fit with their data. Caperon and Smith (1978) included such a term in their study of carbon limited growth of three species of marine phytoplankton. Others include Caperon and Meyer (1972) with nitrate limitation, Paache (1973) with silicate limitation, and King and Hill (1978) with carbon limited growth.

The model is manipulated in a fashion that allows the possible interaction of two simultaneous limiting factors to be considered in the calculation of predicted growth rates. Droop (1974) has shown that under the steady state conditions of the chemostat, the growth of a species is determined solely by the internal concentration of the single most limiting nutrient. Tilman (1977) demonstrated that much of the distributional variability of two species of diatoms in Lake Michigan could be explained using kinetic values which were applied assuming that a species is only limited by a single nutrient at a time, thus supporting Droop's findings. However, in a study by Goldman and McCarthy (1978), growth rate of a marine diatom limited by ammonium did not fit the model of

Droop. Their study demonstrated that, while Droop's (1968) expression (used in the 1974 study) is applicable for limiting nutrients such as phosphorus and vitamin B_{12}, which comprise a very small percentage of cell biomass, it is less so for nitrogen and silica which constitute a greater percentage, and completely invalid for inorganic carbon. They indicated that, in general, the validity of the model decreased as the limiting nutrient:cell weight ratio increases.

Evidence for an interactive limit in my study came from the laboratory experiments. The dramatic changes in K_c and C_o values in the kinetics experiments with S. communis with changing nitrate concentration clearly indicated that the specific growth rate was controlled in culture by the interaction of limiting quantities of free CO_2 and nitrate. A plot of free CO_2 vs. initial nitrate at various μ_g values (Figure 16) serves to illustrate this interaction. If rate of growth responded only to the single most limiting of the two factors, a plot showing threshold changes from one limiting factor to the other would be expected (Droop, 1974). Instead, the figure shows a reduction in CO_2 requirement at higher nitrate concentrations, and conversely, that more CO_2 is required to maintain μ_g at lower nitrate concentrations. A similar plot of C_o values (Figure 17) from μ_g and μ_{ab} data for S. communis further illustrates that the minimum CO_2 concentration necessary for growth is strongly dependent on nitrate concentration, providing further evidence for an interactive limit. These considerations and the laboratory results of this study make the validity of Droop's single limit model in this case unlikely.

A problem inherent in the methods used in this study concerns the use of initial nitrate-nitrogen concentrations with the measurements of

Figure 16. Plot of various levels of specific growth rate (μ_g) for S. communis showing relationship to various combinations of free CO_2 and initial nitrate concentrations.

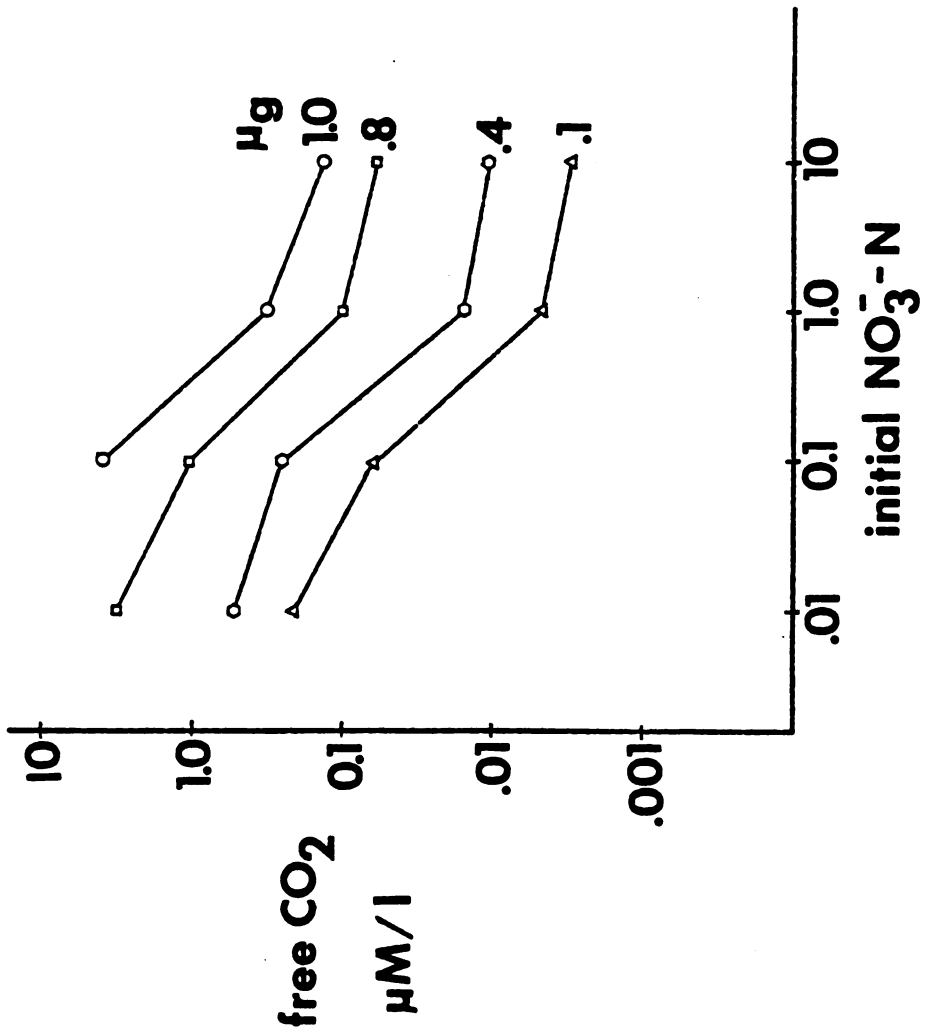


Figure 16.

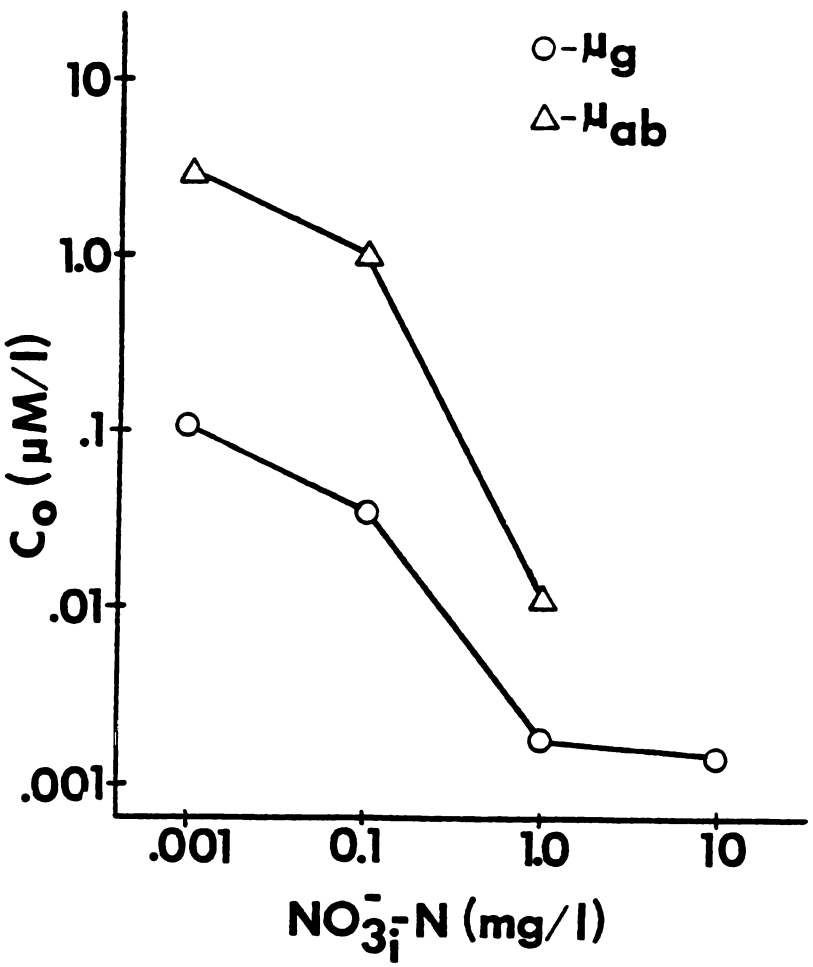


Figure 17. C_o values (minimum CO_2 required for growth) for S. communis from light (μ_g) and LD (μ_{ab}) chambers showing relationship to initial nitrate concentration.

carbon kinetics. A more precise analysis of growth related to interactions of free carbon dioxide and nitrate could be made if nitrate concentration had been monitored continuously for each treatment along with pH. Nitrate decreased over the course of the experiments and this must have caused some error in the calculation of the carbon kinetic values. The inclusion of nitrate measurements would help to increase the precision of the patterns detected in this study, and allow a more accurate appraisal of the importance of this interaction.

The model used external concentration (concentration in the medium) of the limiting nutrients to predict specific growth rates instead of internal cell concentration or cell quota which is widely used. Since cell enzyme systems respond only to the conditions with which they are in direct contact, it follows that specific growth rate is likely to be a function of internal rather than external concentrations. This relationship has been found to be the case in a number of studies (e.g. Droop 1974; Goldman, 1978; Tilman, 1977). However, it also follows that the cell quota of a limiting nutrient depends on the external concentration of that nutrient (Brown and Harris, 1978). The substance must first diffuse or be transported into the cell before it can be used for growth. The problem this causes in predicting growth rates is that it can produce a time lag between nutrient uptake and the growth response to that uptake, especially when cells take up nutrients in excess of present need and store them for use in later growth (luxury consumption)(Fogg, 1975). This time lag causes predictions to be less precise that if cell quota were measured, as growth response may depend as much on past as on present nutrient levels.

Measurement of external concentrations is, however, more feasable to

accomplish in the field than is the determination of cell quotas of the various species, making it more practical for routine sampling programs. It would be beneficial then to determine whether reasonably precise modeling of algal population dynamics can be accomplished through the use of external nutrient concentrations, which was an objective of this study.

Laboratory Kinetic Data

The value of a model is in its ability to accurately predict phenomena in the field. Therefore, to test the validity of this model and applicability of the kinetic data, they were applied to the chemical measurements from the field to determine the population changes the model would predict under the prevailing conditions in Lake 3 in 1977. Since kinetic data were obtained for only three species, predictions were make assuming only those three were in the system.

To determine how the growth characteristics of the three species would compare under varying combinations of carbon and nitrogen concentrations, μ_g curves for the three species at each initial nitrogen concentration were poltted together (Figures 18 a-d). It is clear from these figures that at all nitrate and carbon dioxide concentrations considered, μ_g for P. morum is substantially lower than that for one or both of the other two species. This result suggests that there is no combination of CO_2 and nitrate concentrations within the ranges used under which P. morum would have a greater μ_g, and therefore a competitive advantage, over the other two species. The model predicts, then, that this species would at no time be able to compete with the other species, and so would not become dominant at any time. Since P. morum clearly did dominate in two instances during the season, the implicit assumption

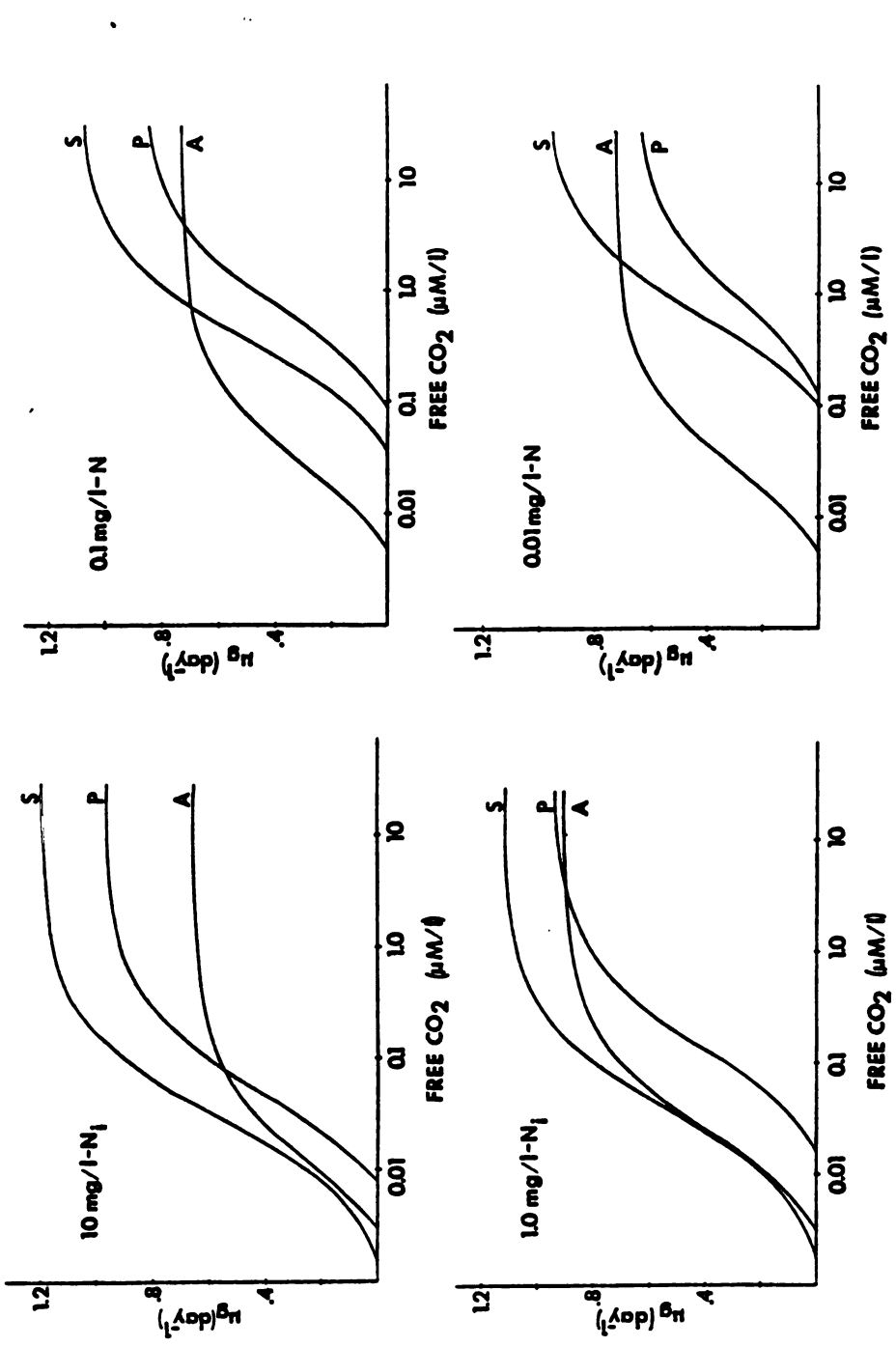


Figure 18. Specific growth rate (μg) of S. communis (S), A. elenkinii (A), and P. morum (P) compared over free CO_2 concentration at four initial nitrate concentrations.

in the model of strictly autotrophic growth must be questioned, since in the model P. morum cannot compete on this basis with the other species. This disparity suggested some form of heterotrophic augmentation of growth, discussed in more detail in a subsequent section, or the involvement of other nutrients not investigated.

According to the model then, only S. communis and A. elenkinii should be competitive under the conditions of CO_2 and nitrate found in the lake and imposed in the laboratory. If figures 18 a-d are examined, it can be seen that at the two higher initial nitrate concentrations (10 and 1.0 mg/l-N), S. communis has the higher μ_g over all levels of CO_2 and therefore would be expected to dominate over A. elenkinii at those nitrate levels regardless of available free CO_2. However, at the two lower nitrate levels (0.1 and 0.01 mg/l-N) it can be seen that while S. communis has the greater μ_g at higher CO_2 levels, the curves for the two species cross so that at low nitrate levels S. communis would dominate while CO_2 remained high (greater than 0.7 to 1.9 μM/l, depending on the nitrate-nitrogen concentration), but when CO_2 dropped below the cross-over point, A. elenkinii would be expected to have a greater μ_g and to attain dominance.

To demonstrate this relationship in a different manner, the physiological maximum growth rates (μ_{max}) for the two species calculated at the four initial nitrate levels were plotted (Figure 19a). This figure clearly shows that with unlimited amounts of available CO_2 , S. communis has a greater growth rate regardless of initial nitrate concentration and thus would be expected to dominate over the blue-green when CO_2 is present in abundance. The advantage of A. elenkinii becomes apparent when growth threshold values (C_o) are plotted for the two species (Figure

Figure 19. (a) μ_{max} values for S. communis (S) and A. elenkinii (A) showing relationship to initial nitrate concentration. (b) C_o values for S. communis (S) and A. elenkinii (A) showing relationship to initial nitrate concentration.

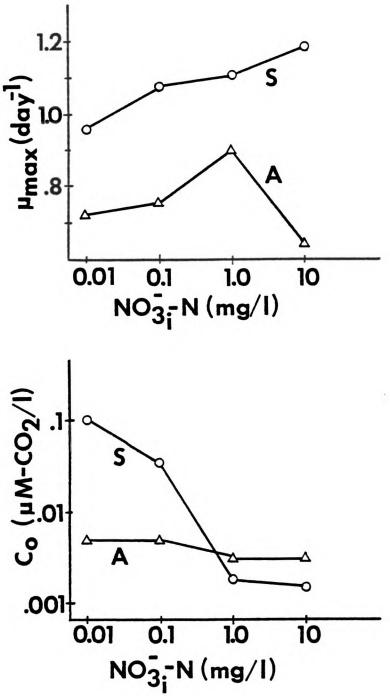

1.2
1.0
.8
μ_{max} (day^{-1})
S
A
0.01
0.1
1.0
10
$NO_{3_i}^-$-N (mg/l)
.1
.01
.001
C_o (μM-CO_2/l)
S
A
0.01
0.1
1.0
10
$NO_{3_i}^-$-N (mg/l)

Figure 19.

19b). While S. communis has the advantage at nitrate levels greater than 0.56 mg/l-N due to its ability to extract CO_2 to somewhat lower levels than the blue-green in that nitrogen range, A. elenkinii has a clear advantage over the green algae when nitrate nitrogen drops below the crossover point. At the lowest nitrate level, the blue-green algae can extract CO_2 to levels more than an order of magnitude lower than can the green, allowing it to grow well under conditions in which S. communis cannot survive.

To summarize, the laboratory kinetic data for S. communis and A. elenkinii indicate that when nitrate is present in abundance, S. communis will dominate over all levels of CO_2. When nitrate decreases to levels below the 0.1 to 1.0 mg/l-N range, the green algae will be expected to dominate when CO_2 is abundant, but when it drops to levels below the 1.0 μM/l range, the blue-green algae should attain dominance.

When these data were applied to the chemical trends in the field, the model predicted a clear pattern of growth of the two species. The early high nitrate and moderate CO_2 concentrations indicated rapid growth of S. communis for the first five weeks and a decline thereafter since the mean NO_3^--CO_2 point for week 6 fell below the physiological C_o for the species. After the projected decline of the green algae, nitrate became undetectable in the water and CO_2 fluctuated at low to moderate levels (0.1 to 1.0 μM/l). Under these conditions, an immediate rapid increase of A. elenkinii to high levels was predicted. Since nitrate remained low for the remainder of the growing season and CO_2 maintained its fluctuations between ca. 0.1 and 1.0 μM/l, the blue-green algae were expected to maintain dominance throughout that period. A short period of increased ammonium to ca. 1.0 mg/l-N in late July and early August

was predicted to temporarily give a competitive edge to S. communis (since ammonium can be used as an alternate nitrogen source) but because the pulse of nitrogen was relatively short and the large standing crop of the blue-green created a poor light climate because of intense shading, the S. communis was expected to be small. As previously mentioned, P. morum was not expected to appear under the assumptions of the model.

Predicted vs. Actual Trends

When these predicted population trends were compared with those which occurred in the field, substantial agreement was found. Growth of S. communis was restricted to the first five weeks of the growing season and one week in early August as predicted, and a major and long lived bloom of A. elenkinii occurred after the demise of S. communis, as predicted.

A further demonstration of the agreement found between the predicted and actual growth trends is shown in Figure 20. Points representing combinations of mean CO_2 and nitrate concentrations of weeks during which growth of the various species occurred were plotted to illustrate that each species grew under chemical conditions consistent with the findings of the kinetics studies. The points indicating S. communis growth are clustered in the region of high nitrate and low to moderate CO_2 concentrations, A. elenkinii growth points are primarily below 1.0 mg/l-N and within the same CO_2 range, and the P. morum points occur where both nitrate and CO_2 are low (where none of the species would be expected to grow). The figure illustrates a clear segregation of S. communis and A. elenkinii over nitrate concentration, indicating, as the kinetic data suggest, that nitrogen is the most important factor in

Figure 20. Points representing combinations of mean CO_2 and nitrate concentrations of weeks during which growth of S. communis (S. c.), A. elenkinii (A. e.), and P. morum (P. m.) occurred. Two points connected indicate weeks when two species both increased.

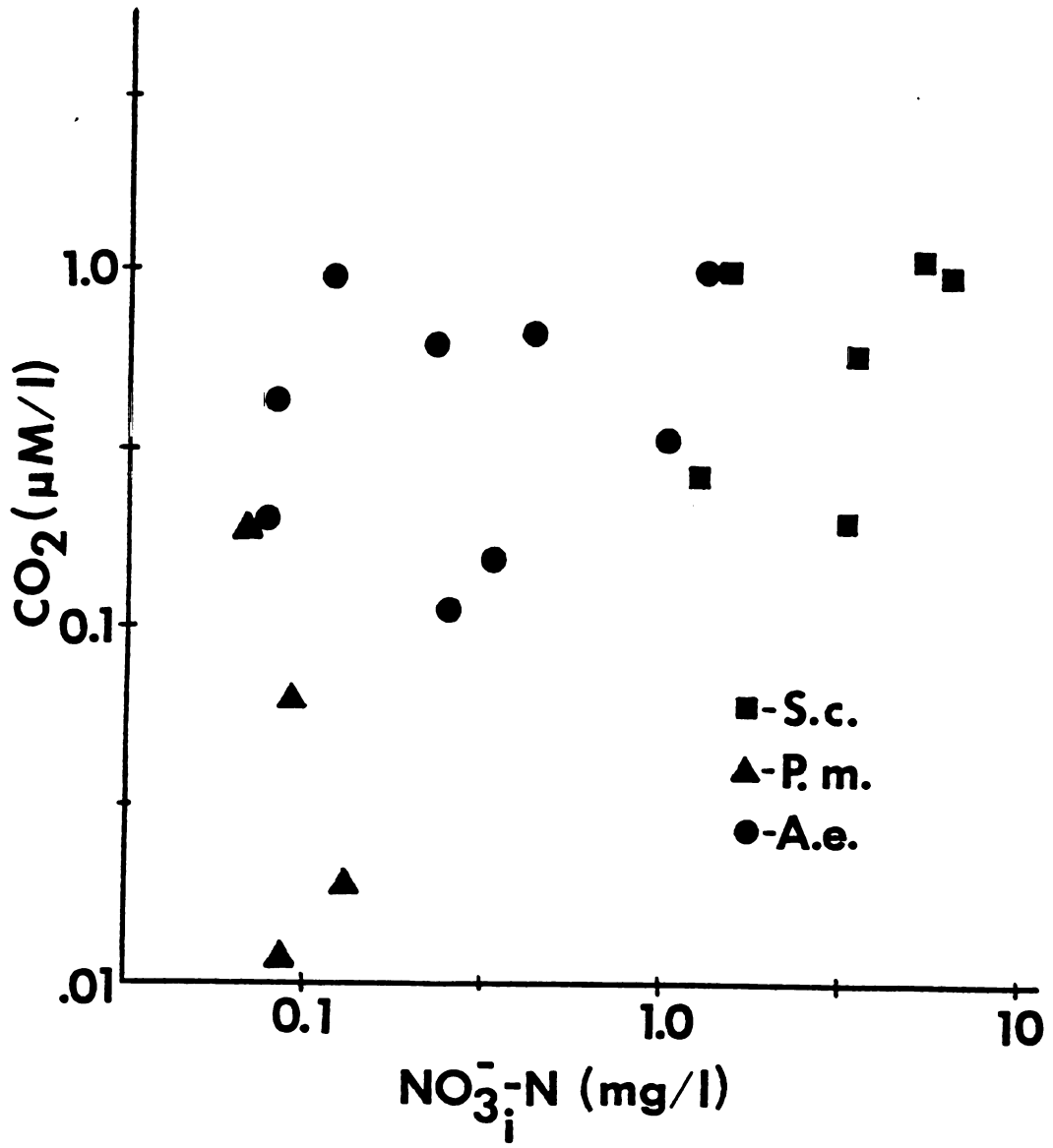


Figure 20.

determining when one or the other will attain dominance in this eutrophic lake situation. the segregation of these points into predictable combinations of conditions further illustrates the agreement of the field growth of these species to the predictions (except for P. morum). However, the predictions did not precisely mirror the rates and times of growth found in the field.

Scenedesmus

While S. communis did undergo its major growth episode during the first five weeks as predicted, its rate of growth during the first three weeks was lower than predicted (Figure 8). Examination of the field data suggests two factors not included in the model that may have been responsible for this lower than predicted rate. During these first weeks the large populations of the early dominants (Cyclotella sp. and Chlamydomonas sp.) inhibited light penetration as indicated by Secchi transparencies of only 0.3 to 0.5 meters, presumably causing a decrease in the growth rate that would have been attainable with those nutrient conditions had light been at saturating intensities. When the Chlamydomonas sp. bloom collapsed, the intensity of light increased to the point where it was presumably no longer limiting, and the growth rate increased to a rate determined by the ambient concentrations of free CO_2 and nitrate, as assumed in the model. The low temperature in the lake, which did not reach 15^{o} C until the fourth week of measurement, may also have contributed to the low growth rate recorded in the first three weeks.

This close agreement between the predicted and actual growth of S. communis strongly supports the contention that its growth was largely limited by interacting limits of nitrate and carbon dioxide. If μ_g is

calculated for the sixth week (when S. communis began to delcine), an increase in either free CO_2 or nitrate caused the population to increase in both cases, further indicating an interactive limit.

Anabaenopsis

A. elenkinii underwent a rapid period of growth after the decline of S. communis and dominated for an extended period from July through September as predicted. However the onset of growth did not occur until a considerable period of time (ca. 4 weeks) had elapsed after the major decline of the green algae. The cause for this delay is uncertain but may be attributable to the low temperatures occurring in the lake. The temperature decreased following the decline of S. communis and growth of A. elenkinii began during the first week that the temperature exceeded 20^{o} C after that decline. This growth continued as the temperature increased to a maximum of 29^{o} C and ceased when it again decreased to 20^{o} C. This evidence is circumstantial, but in general, blue-green algae are known to have temperature optima in the $30-35^{o}$ C range (Fogg et al., 1973) suggesting that the temperature may have been an important factor in delaying the onset of A. elenkinii growth.

That the growth of the blue-green was in agreement with the predictions of the model is further demonstrated in Figure 12, where μ_f falls between calculated values of μ_g and μ_{ab} on all but 3 of 12 dates. Examination of the field data suggests light limitation may have been responsible for the unpredicted major fluctuation of μ_f in the first 5 weeks of growth.

When growth rate of A. elenkinii (μ_f) is compared with Secchi disc transparency over the eleven week period of its growth, a close correspondence is found (Figure 21). The decrease in μ_f during the second and

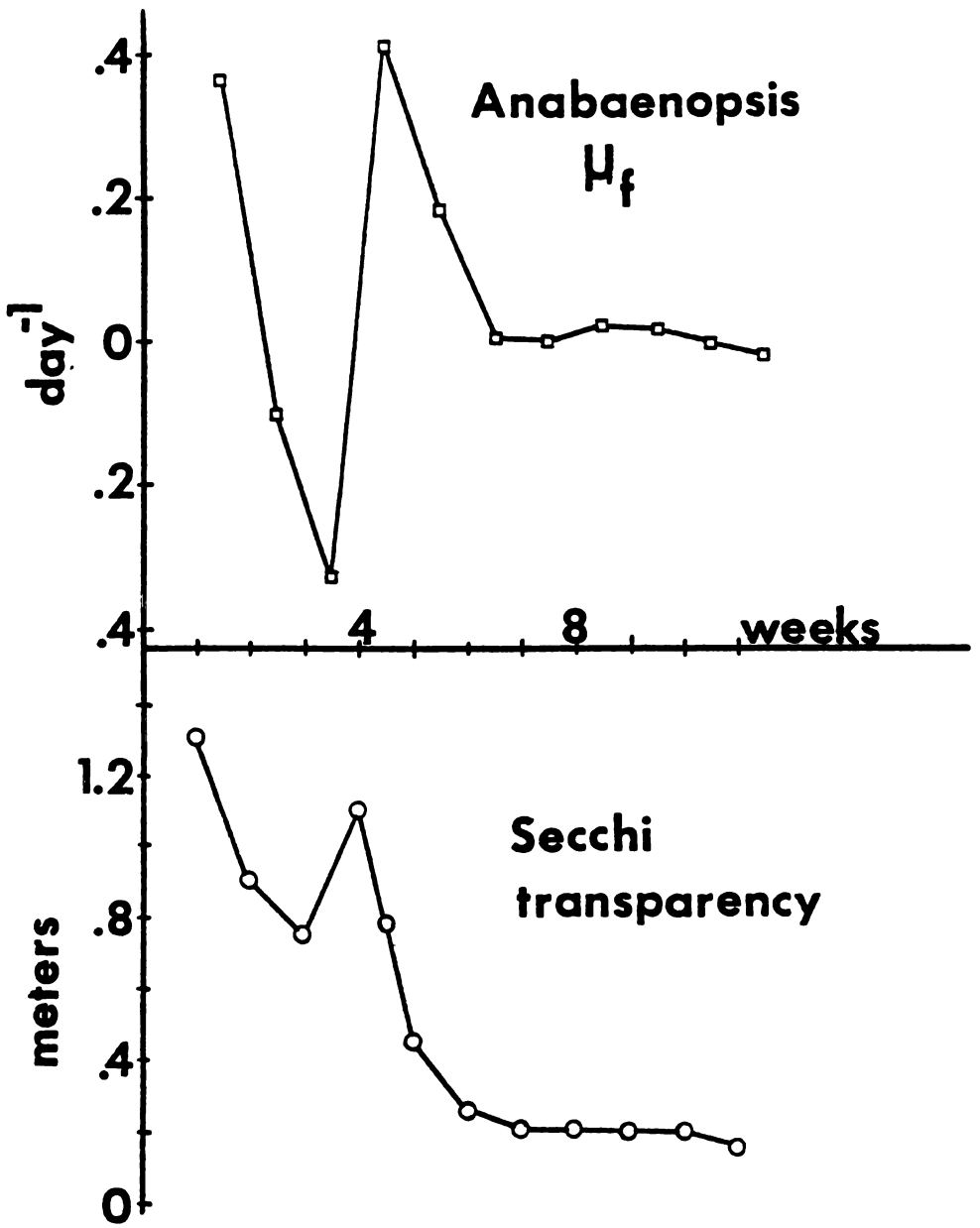


Figure 21. Comparison of field growth rate (μ_f) of A. elenkinii with Secchi transparency showing similar patterns of variation.

third weeks of growth correspond to a dip in transparency coinciding with the second bloom of P. morum. When that population declined with a concommitant increase in light penetration, μ_f for the blue-green increased dramatically. The two weeks of subsequent A. elenkinii growth decreased the transparency to its lowest levels of the summer, and μ_f decreased to correspondingly low levels and remained there, presumably because transparency did not improve.

These correlations suggested that a limiting factor of the growth of the blue-green algae during its major bloom was a lack of light caused by shading from P. morum or by self-shading. Since light decreases exponentially as a function of cell concentration and depth (Fogg, 1975), the growth habit of A. elenkinii of becoming concentrated near the surface due to the formation of gas vacuoles would tend to exacerbate the latter condition. Jewson (1976, 1977) and Jones (1977a, 1977b) domonstrated the influence of various light related phenomena including total daily irradiance as related to day length, and light attenuation caused by self-shading by dense populations. Jones provided evidence of limitation of photosynthesis because of intense self-shading by a blue-green bloom. The near zero growth rate of A. elenkinii measured during weeks 6-11 of its growth was probably the net result of growth by those cells with sufficient light for net photosynthesis, and the sinking and death of cells with insufficient light. That the sinking of cells may be an important factor in keeping μ_f low (nearer to μ_{ab} then μ_g) during this period is indicated by the high sinking rate (μ_s) of the species under conditions of low CO_2 and nitrate (Figure 11).

The source of carbon for this "stationary growth" was most likely diffusion of CO_2 into the water from the atmosphere. Schindler (1971)

and Emerson (1975) have shown that sufficient CO_2 can diffuse into the water to support large phytoplankton populations. The CO_2 measured probably represents this source as well as respiratory CO_2 circulated up from the bottom and from decomposing algal cells.

Evidence implicating a particular factor in the final decline of A. elenkinii in September is inadequate. As mentioned earlier, the decline coincides with a drop in temperature to below 20^{o} C. The concentration of such a large population at the water surface may have resulted in the accumulation of a self-produced autotoxin to levels fatal to the blue-green (Fogg, 1975). Concentration of blue-greens at the surface often results in high light intensity photoinhibition which can cause the demise of such a population. This may occur when photosynthesizing cells become over-vacuolated because of light limitation during a period of some turbulence, and are, in effect, trapped at the surface when becalmed (Fogg et al. 1973).

Pandorina

Predictions from the kinetic data indicated no growth of P. morum because under all conditions where it had the capability of autotrophic growth, S. communis or A. elenkinii had a greater μ_g and thus were expected to dominate P. morum under all such conditions. However, this species did produce substantial populations on two occasions, both at times when nitrate and free CO_2 conditions were such that the kinetic data would predict no growth.

These results suggested that a different factor was involved in the stimulation of this growth, possibly heterotrophic utilization of dissolved organic material present in the water column.

Palmer and Starr (1971) have shown that mixotrophic growth of P.

morum in axenic culture is stimulated by a variety of organic compounds including a number of metabolic intermediates and amino acids, as well as other compounds, including acetate. Approximately a third of the strains they studied were able to grow heterotrophically in the dark using acetate as a substrate. Another third were only stimulated by acetate in the light. Similar experiments for the strain isolated from Lake 3 showed stimulation of growth by acetate in the light but no growth in the dark. These results supported the possibility of photoheterotrophy (light enhanced heterotrophic growth) or mixotrophy (organic substrate stimulation of growth in the light over and above the autotrophic growth possible at that light intensity (Palmer and Starr, 1971)). A large increase in pH during acetate stimulated growth in culture indicated autotrophic uptake of CO_2, providing preliminary evidence for for mixotrophy as the mode of nutrition of P. morum under these conditions. Since these cultures were not axenic and 10 mg/l of acetate is orders of magnitude greater than concentrations typically found in the field, further experiments are necessary to provide more convincing evidence for this.

Possible sources of substrates for this growth are organic compounds released from dead cell autolysis, excreted from decomposing material circulated up from the bottom. Experiments conducted here to determine if excreted organic matter from log phase cultures of S. communis and A. elenkinii would stimulate growth of P. morum gave negative results. Sharpe (1977) has shown that there is little evidence to suggest that natural populations of phytoplankton release significant amounts of organic matter during their active growth phase.

The growth of P. morum did occur primarily, however, during the

decline of earlier populations. This result suggests that the number of stationary phase and dying cells sinking through the water column would be great at that time. These would provide ample substrate for growth stimulated by organic matter as excretion from stationary phase cells is well documented (Sharpe, 1977). The large number of dead cells that could be expected under these conditions (Jassby and Goldman, 1974) would undergo decomposition during sinking and release much of their cell contents to the water column (Otsuki and Hanya, 1972). Recirculation of organic matter from the bottom sediments is also a possibility due to the frequent turnover of Lake 3 during the period of study.

Based on the limited experimental evidence obtained and the above considerations, it seems possible that growth of P. morum in Lake 3 was associated with mixotrophic nutrition. Further experimentation is necessary to reach a more definite conclusion.

Conclusions

A number of studies have shown that phytoplankton population fluctuations are influenced primarily by the dynamics of inorganic nutrients. This study provides further supportive evidence for that as well as evidence suggesting that other factors may at times be of overriding importance. The occurrence of S. communis and A. elenkinii during specific periods of the growing season was shown to be largely due to the interaction of limiting levels of free CO_2 and nitrogen, while limited evidence suggested P. morum may have utilized organic matter to stimulate its autotrophic growth, allowing it to grow under conditions that would otherwise be unfavorable.

Further evidence was presented to suggest that while inorganic nutrients are of primary importance in regulating phytoplankton population growth, temperature and light also play critical roles. Different groups of algae have different ranges of temperature tolerance and this may influence growth patterns within the constraints set by nutrient considerations. In this case, low temperatures may have been responsible for delaying the onset of A. elenkinii growth which could have occurred up to a month earlier had nutrients been the only controlling factors. The attenuation of light by dense phytoplankton blooms was implicated as a factor controlling growth rates of two of the populations. Evidence suggested that both S. communis and A. elenkinii were light limited during a portion of their growth due to this shading.

The results of the study do not provide evidence to enable a clear evaluation of the role of variable sinking rate in seasonal succession. Data from the LD chambers provide strong evidence that under stagnant conditions, sinking rate can significantly affect the ability of a species to survive under nutrient deficient conditions. However, due to the frequent turbulence of Lake 3, the effects of sinking are difficult to evaluate. While in only one case did the field growth rates ($\tilde{\mu}_f$) for S. communis and A. elenkinii exceed the μ_g values calculated, it is not clear that the difference between the two is due entirely to sinking. It would be expected, however, that under more quiescent conditions the effects of sinking could be substantial. Under these conditions, kinetics values determined from continuous cultures, which fail to provide a darkened zone, would not be expected to accurately estimate field values.

This study suggests that while depletion of nitrate and ammonia may cause a shift in species populations from green to nitrogen-fixing blue-green algae, large standing crops can still develop, suggesting that nitrogen is not a significant factor limiting the annual productivity of this system. Since phosphorus was always likely present in quantities adequate for optimum growth, it seems probable that light and carbon are the most important factors limiting the annual phytoplanktonic productivity of this system.

Thes work has shown that while a simple, kinetics based model may not satisfactorily reproduce the week to week specific growth rates of the dominant species of planktonic algae, predictions of approximate periods of growth can be quite accurate. As more kinetic data become available on the more common dominant algal species of freshwater systems,

such a model has potential to predict species changes that would result from perturbations of existing nutrient conditions of lakes. Such a capability could be very useful in the analysis of the biological effects of various water resources related projects.

More precise predictions of actual growth rates require the inclusion of more factors in a more complex polynomial model such as that proposed by Droop (1973). Such a model would likely provide more precise predictive capabilities and increase our knowledge of the precise factors that influence algal growth and seasonal abundance patterns.

LITERATURE CITED

Literature Cited

American Public Health Association. 1976. Standard Methods for the Examination of Water and Wastewater. 14th ed., Washington, D.C. American Public Health Association. 1193 pp.

Brown, E.J. and R.F. Harris, 1978. Kinetics of algal transient phosphate uptake and the cell quota concept. Limnol. Oceanogr. 23: 35-40.

Canelli, E. and G. W. Fuhs. 1976. Effect of the sinking rate of two diatoms (Thalassiosira spp.) on uptake from low concentrations of phosphate. J. Phycol. 12: 93-99.

Caperon, J., and J. Meyer. 1972. Nitrogen-limited growth of marine phytoplankton. 1. Changes in population characteristics with steady state growth rate. Deep Sea Res. 19: 601-618.

Caperon, J. and D. F. Smith. 1978. Photosynthetic rates of marine algae as a function of inorganic carbon concentration. Limnol. Oceanogr., 23: 704-708.

Davis, C. O. 1976. Continuous culture of marine diatoms under silicate limitation. II. Effect of light intensity on growth and nutrient uptake of Skeletonema costatum. J. Phycol. 12: 291-300.

Dowd, J. E. and D. S. Riggs. 1965. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. Jour. Biological Chem., 20: 863-869.

Droop, M. R. 1968. Vitamin B_{12} and marine ecology. 4. The kinetics of uptake, growth and inhibition in Monochrysis lutheri. J. Mar. Biol. Assoc. U. K. 48: 689-733.

Droop, M. R. 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Assoc. U. K. 54: 825-855.

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

Emerson, Steve. 1975. Chemically enhanced CO_2 gas exchange in a eutrophic lake: A general model. Limnol. Oceanogr. 20: 743-753.

Eppley, R. W. and W. H. Thomas. L969. Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. J. Phycol. 5: 365-369.

Eppley, R. W., R. W. Holmes, and J. D. H. Strickland. 1967. Sinking rates of marine phytoplankton measured with a flourometer. J. Exp. Mar. Biol. Ecol. 1: 191-208.

Eppley, R. W., J. N. Rogers, and J. J. McCarthy. 1969. Half saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 15: 912-920.

Fogg, F. E. 1974. Nitrogen fixation. In: W. D. P. Stewart, ed. Algal Physiology and Biochemistry. Berkeley, Univ. of California Press, pp. 560-582.

Fogg, G. E. 1975. Algal Cultures and Phytoplankton Ecology. 2nd ed. Madison, Univ. of Wisconsin Press, 175 pp.

Fogg, G. E., W. D. P. Stewart, P. Fay, and A. E. Walsby. 1973. The Blue-Green Algae. New York, Academic Press, 459 pp.

Goldman, J. C., W. J. Oswald, and D. Jenkins. 1974. The kinetics of inorganic carbon limited algal growth. J. Water Pollut. Control Fed. 46: 554-574,

Goldman, J. C., Steady state growth of phytoplankton in continuous culture: comparison of internal and external nutrient equations. J. Phycol. 13: 251-258.

Goldman, J. C., and E. J. Carpenter. 1974. A kinetic approach to the effect of temperature on algal growth. Limnol. Oceanogr. 19: 756-766.

Goldman, J. C., and J. H. McCarthy. 1978. Steady state growth and ammonium uptake of a fast-growing diatom. Limnol. Oceanogr. 23: 695-703.

Hegewald, E. 1977. Scenedesmus communis Hegewald, a new species and its relation to Scenedesmus quadricauda (Turp.)Breb. Algological Studies. 19: 142-155.

Institute of Water Research, Michigan State University. 1976. Utilization of natural ecosystems for wastewater renovation. Final report for EPA Project No. Y005065.

Jassby, A. D., and C. R. Goldman. 1975. Loss rates from a phytoplankton community. Limnol. Oceanogr. 19: 618-627.

Jewson, D. H. 1976. The interaction of components controlling net phytoplankton photosynthesis in a well mixed lake (Lough Neagh, Northern Ireland). Freshwater Biology. 6: 551-576.

Jewson, D. H. 1977. Light penetration in relation to phytoplankton content of the euphotic zone of Lough Neagh, N. Ireland. Oikos. 28: 74-83.

Jones, Roger I. 1977. Factors controlling phytoplankton production and succession in a highly eutrophic lake (Kinnego Bay, Lough Neagh). I. The phytoplankton community and its environment. J. Ecol. 65: 547-559.

Jones, R. I. 1977. Factors controlling phytoplankton production and succession in a highly eutrophic lake (Kinnego Bay, Lough Neagh). II. Phytoplankton production and its chief determinants. J. Ecol. 65: 561-577.

Jones, R. I. 1977. Factors controlling phytoplankton production and succession in a highly eutrophic lake (Kinnego Bay, Lough Neagh). III. Interspecific competition in relation to irradiance and temperature. J. Ecol. 65: 579-586.

Kilham, P. 1971. A hypothesis concerning silica and the freshwater planktonic diatoms. Limnol. Oceanogr. 16: 10-18.

Kilham, S. S. 1975. Kinetics of silicon limited growth in the freshwater diatom Asterionella formosa. J. Phycol. 11: 396-399.

King, D. L. 1970. The role of carbon in eutrophication. Jour. Water Poll. Control Fed. 42: 2035-2051.

King, D. L. 1972. Carbon limitation in sewage lagoons. In Nutrients and eutrophication, Special Symposium, Amer. Soc. Limnol. Oceanogr. 1: 98-105.

King, D. L. and M. T. Hill. 1978. Interacting environmental factors which control the sinking rate of planktonic algae. OWRT Project Number A090Mich.

King, D. L. and R. E. King. 1974. Interaction between light and carbon dioxide availabilities as a control of algal species succession. A report to the Missouri Water Resources Res. Center, University of Missouri, Columbia, Mo.

King, D. L. and J. T. Novak. 1974. The kinetics of inorganic carbon-limited algal growth. Jour. Water Poll. Control Fed. 46: 1812-1816.

Lehman, J. T., D. B. Botkin, and G. E. Likens. 1975a. Lake eutrophication and the limiting CO_2 concept: a simulation study. Verh. Int. Ver. Limnol. 19: 300-307.

Lehman, J. T., D. B. Botkin, and G. E. Likens. 1975b. The assumptions and rationales of a computer model of phytoplankton population dynamics. Limnol. Oceanogr. 20: 343-364.

Lund, J. W. G. 1965. The ecology of the freshwater phytoplankton. Biol. Rev. 40: 231-293.

MacIsaac, J. J., and R. C. Dugdale. 1969. The kinetics of nitrate and ammonium uptake by natural populations of marine phytoplankton. Deep Sea Res. 16: 445-457.

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

Monod, J. 1949. The growth of bacterial cultures. Annual Rev. Microbiology. 3: 371-399.

Moss, B. 1972a. Studies on Gull Lake, Michigan. I. Seasonal and depth distribution of phytoplankton. Freshwat. Biol. 2: 289-307.

Moss, B. 1972b. The influence of environmental factors on the distribution of freshwater algae: An experimental study. I. Introduction and the influence of calcium concentration. J. Ecol., 60: 917-932.

Moss, B. 1973a. The influence of environmental factors on the distribution of freshwater algae: An experiemental study. II. The role of pH and the carbon dioxide-bicarbonate system. J. Ecol. 61: 157-177.

Moss, B. 1973b. The influence of environmental factors on the distribution of freshwater algae: An experimental study. III. Effects of temperature, vitamin requirements and inorganic nitrogen compounds on growth. J. Ecol. 61: 179-192.

Moss, B. 1973c. The influence of environmental factors on the distribution of freshwater algae: An experimental study. IV. Growth of test species in natural lake waters, and conclusion. J. Ecol. 61: 193-211.

Otsuki, A., and T. Hanya. 1972. Production of dissolved organic matter from dead green algal cells. I. Aerobic microbial decomposition. Limnol. Oceanogr., 17: 248-257.

Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms. 1. Thalassiosira pseudonana (Cyclotella nana) grown in a chemostat with silicate as the limiting nutrient. 2. Silicate uptake kinetics in five diatom species. Mar. Biol. 19: 117-126, 262-269.

Palmer, E. G. and R. C. Starr. 1971. Nutrition of Pandorina morum. J. Phycol. 7: 85-89.

Prescott, G. W. 1970. How to Know the Freshwater Algae. Dubuque, Iowa. W. C. Brown Co. 348 pp.

Schelske, D. L. and E. F. Stoermer. 1971. Eutrophication, silica depletion, and predicted changes in algal quality in Lake Michigan. Science, 173: 423-424.

Schelske, C. L., and E. F. Stoermer. 1972. Phosphorus, silica, and eutrophication of Lake Michigan. In Nutrients and eutrophication, Special Symposium, Amer. Soc. Limnol. Oceanogr. 1: 98-105.

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

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science. 195: 260-262.

Senft, W. H. 1978. Dependence of light saturated rates of algal photosynthesis on intracellular concentrations of phosphorus. Limnol. Oceanogr., 23: 709-718.

Sharpe, J. H. 1977. Excretion of organic matter by marine phytoplankton: Do healthy cells do it? Limnol. Oceanogr. 22: 381-399.

Smayda, T. J. 1974. Some experiments on sinking characteristics of two freshwater diatoms. Limnol. Oceanogr. 19: 628-635.

Tilman, D. 1977. Resource competition between planktonic algae: An experimental and theoretical approach. Ecology 38: 338-348.

Tilman, D. and S. S. Kilham. Phosphate and silicate growth and uptake kinetics of the diatoms Asterionella formosa and Cyclotella meneghiniana in batch and semicontinuous culture. J. Phycol. 12: 375-383.

Titman, D. 1976. Ecological competition between algae: Experimental confirmation of resource based competition theory. Science. 192: 463-465.

Titman, D. and P. Kilham. 1976. Sinking in freshwater phytoplankton: Some ecological implications of cell nutrient status and physical mixing process. Limnol. Oceanogr. 21: 409-417.

Watanabe, A. 1951. Production in cultural solution of some amino acids by the atmospheric nitrogen-fixing blue-green algae. Archs Biochem. Biophys. 34: 50-55.

Wetzel, R. G. 1975 Limnology. Philadelphia. W. B. Saunders Co. 743 pp.

Young, T. C. and D. L. King. 1973. A rapid method of quantifying algal carbon uptake kinetics. Limnol. Oceanogr. 18: 978-981.

APPENDICES

Appendix 1. Water chemistry parameters measured by MSU Institute of Water Research on Lake 3 using grab samples over 1977 growing season.

Parameter	Units
Total Alkalinity	mg/l $CaCO_3$
Hardness	mg/l $CaCO_3$
Chloride	mg/l Cl
Dissolved Oxygen	mg/l
Ammonium Nitrogen	mg/l-N
Nitrate Nitrogen	mg/l-N
Nitrite Nitrogen	mg/l-N
Total Kjeldahl Nitrogen	mg/l-N
pH	pH
Total Phosphorus	mg/l-P
Orthophosphorus	mg/l-P
Specific Conductivity	µmhos/cm
Temperature	^{o}C

Appendix 2. Composition of culture medium used in algal growth kinetics experiments.

Compound	Concentration
$MgSO_4 \cdot 7H_2O$	50 mg/l
K_2HPO_4	100 mg/l
$CaCl_2$	25 mg/l
NaCl	25 mg/l
$NaHCO_3$	100 mg/l
$NaNO_3$	variable
EDTA	50 mg/l
KOH	31 mg/l
$FeSO_4 \cdot 7H_2O$	5 mg/l
H_2BO_3	11.4 mg/l
$ZnSO_4$	8.8 mg/l
MoO_4	0.71 mg/l
$CoCl_2$	0.49 mg/l
$MnCl_2$	1.44 mg/l
$CuSO_4 \cdot 5H_2O$	1.57 mg/l

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