EVALUATION OF ALGAL PRODUCTIVITY IN GREEN LAKE, WISCONSIN WITH BIOASSAY TECHNIQUES

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TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF PREVIOUS WORK

Limnological Study of Green Lake

Green Lake, located in Green Lake County (Latitude 42°48' N, Longitude 89°00'W) is the deepest lake (69.8m) in Wisconsin. Its outline is narrowly oval with a length of 11.9 km and a maximum width of 3.2 km with a northeast to southeast orientation (Bumby, 1972). It has a water acreage of 7,325 acres and the shoreline measures 27.3 miles (43.9 km) (Department of Natural Resources, 1967). The lake has a preglacial origin (Fenneman, 1910), being carved out of sandstone by streams prior to the advent of glacial ice; the lake itself was formed by the impoundment of water following the deposition of a moranial dam across the preglacial valley at the west end of the present lake.

Silver Creek is the largest stream entering the lake. Additional water comes from springs or five smaller and intermittent streams (see Figure 1). Scientific study of Green Lake began in the latter part of the 19th century (by C. Dwight Marsh of Ripon College) and includes:

Marsh (1891, 1892, 1894, 1898, 1903): deep water crustacea, annual distribution of limnetic crustacea, notes on the depth and temperature of the lake.

Marsh (1898b): hydrographic map of Green Lake.

Birge and Juday (1911, 1922): dissolved gases (oxygen, carbon dioxide, nitrogen, methane, and hydrogen sulfide) and their significance; mechanisms and types of stratification over a 2 year period; nature and quantity of plankton.

Juday (1924); Juday and Birge (1941): chemical nature of bottom deposits; water chemistry.

Domogalla et. al. (1925): forms of nitrogen and concentration in Green Lake.

Rickett (1924): qualitative/quantitative analysis of macrophyte population.

Mackenthun (1947): fish species survey.

Lueschow et. al. (1970): determined trophic level of Green Lake and 11 other Wisconsin lakes; observations made during 1966.

Hacker (1961, 1964): lake trout gill net studies 1956-1964.

Hasler (1967): fairy shrimp (Mysis relicta) population in Green Lake; chemical and physical data presented.

Department of Natural Resources (1969, 1970): water chemistry of selected sites during the past six years.

Lueschow (1963): infrequent nutrient sample analysis of Green Lake.

Lueschow (1970): nutrient survey of Green Lake and 11 other Wisconsin lakes--conducted in 1966.

Bumby (1972): changes in submerged macrophytes from 1924 to 1971.

Although the previous studies have varied in site and analyses performed, the following general table can be prepared:

	concentration (mg/l)				
	Domogalla et.al. 1925	Lueschow 1963	Hasler 1967	DNR 1969-70	Lueschow 1970
Nitrate	.03	.05-.06	.20.5	.0-.3	---
Organic nitrogen	.42	.34-3.67	.58-.84	.38-.82	---
Total phosphorus	---	.08-.64	.07-.17	-----	.028-.12

In addition to these published studies many symptomsof deteriorating water quality have been qualitatively detected in Green Lake in recent years. A heavy algal bloom was recorded in 1963. Students at Ripon College (Litton and Reinecke, 1971) during the summer of 1971, making limited checks of the lake water, noted the following:

1) the filamentous green alga Cladophora grew to lengths of three feet or more in Norwegian Bay of Green Lake.

2) various aquatic rooted vascular plants (macrophytes) were also common (ex: Myriophyllum) and, at several points, abundant.

3) the presence of Microcystis in the Sunnyside Acres area was accompanied by a high bacterial count.

4) plankton samples revealed "blooms" of Aphanizomenon flos-squae, Microcystis aeruginosa, Gleotrichia achinura, Anabaena spiroides, the desmid Staurastrum, and the diatom Fragilaria.

During the period of June 1, 1972 through August 22, 1972 data on biological, chemical, and physical parameters of Green Lake and Lake Winnebago was collected by a Student Originated Studies team (NSF Grant GY-9650) at Ripon College. This systematic survey established baseline data for Green Lake from which trends or changes in eutrophication can be noted in the future. In addition, it indicated that the lake will take a long time to pollute, and a long time to flush out the pollutants by itself. Most pollution abatement schemes would require considerable time to produce any visible change if the lake's natural means of flushing are the sole methods used, unless there is some nutrient for which a critical concentration has just been passed. If this is so, and it is indicated by the fact that the incidence of the more noticeable signs of eutrophication is only 10 or so years old, then it might require only a slight lowering in the level of the nutrient to return conditions to normal (e.g., lowered plant productivity).

Upon completion of the summer, 1972 SOS study of the eutrophication of Green Lake the following recommendations were made:

1) a continued examination of nutrient input (at all possible entry points) and phytoplankton populations on Green Lake, September 1972-May 1972. This baseline survey information will give a year-round indication of assimilable nutrients in the lake proper; most importantly it will correct one deficiency of the summer study: sampling during the spring runoff period from the 65,000 acre watershed. This recommendation was implemented by a grant from the Green Lake Property Owners' Association to the SOS team and the results are reported herein.

2) an intensive investigation of the nutrient sources of Green Lake, using carefully placed transects should be completed during the summer of 1973; this study's primary purpose will be to determine the exact entry points of nutrients and their relative importance to the total assimilable plant nutrient concentration in Green Lake.

3) completion of an intensive investigation of the algal population of Green Lake; this will include: enumeration of the algal population, in situ bioassay measurements of algal productivity, and in vitro examination of algal growth under varying chemical conditions. These methods would be utilized in an effort to determine the limiting nutrient for the noxious "bloom" producing algae.

This recommendation was implemented by the study reported herein.

Biological Assays in the Determination of Algal Productivity

Objective evaluation of the quality of water is possible only by the analyses of its physical, chemical, and biological properties. Chemical analyses can give a picture of the composition of water but cannot show its overall quality, for the individual factors can affect each other, e.g., a result of the antagonism of ions. The complex influence of individual factors can be determined only by means of a complex analysis.

Besides the physical and chemical analyses, which may present individual values related to water quality at the moment of sampling, biological methods for water-quality determination become increasingly important. These procedures include:

1) Direct or ecological procedures (descriptive-analytical), which classify water according to the occurrence and abundance of organisms and to their community structure.
2) Indirect or physiological procedures (experimental biological tests), which also include procedures for the determination of toxicity. Two main procedures are involved:
   (a) exposure of natural populations to the same water or to different dilutions of toxic substances, and
   (b) exposure of suitable test organisms to the water under examination, after removal of the original population.

Biological methods for water-quality determination are numerous. A complete review of bioassay subject methods is found in Matulova, 1970.

The Provisional Algal Assay Procedures (1969) presented suggestions for standardized algal growth tests. These initial provisional algal bioassay procedures as well as their modification (Porcella et.al., 1970; AAP Bottle Test, 1971, and Toerien et.al., 1971) served as the basis for the study of algal productivity in Green Lake. The bioassays recognized three fundamentally different test procedures:

1) A Bottle Test: Candidate test algae are added to the sample in the laboratory, and growth is determined.
2) A Continuous Flow Chemostat Test: Candidate test algae are added to a continuous - sample flow system. An attempt is made to keep the environment constant, and the objective is to develop a steady state of algal production. The test result is a growth rate.
3) In Situ Test: Part of the water body is isolated and incubated in place within a transparent container. The test result is an instantaneous growth rate for the con-

fined portion of the body of water in question. Nutrients may be added to the isolated sample as a bioassay *in situ*.

METHODOLOGY

The project methodology will be reported in three phases:

(1) Water Analysis

(2) Phytoplankton Enumeration

(3) Bioassay Experiments

(1) WATER ANALYSIS

(A) STATIONS:

Water samples were removed from eighteen sites in Green Lake in the summer grant period. These complemented samples were taken during a period of September 1, 1972 - May 15, 1973, under grant from the Green Lake Property Owners' Association. Water samples collected were analyzed directly as well as used in completing the algal bioassay experiments. Figure 1 shows sampling sites (and depths of each station sampled) for Green Lake.

Below are listed the procedures employed in analyzing the various parameters studied:

(B) PHYSICAL:

Transparency

Transparency was measured with a 20 cm. Secchi disk.

Conductivity

Conductivity was measured on surface samples with a conductivity bridge (Yellow Springs Instruments); results were plotted by computer to show lines of isoconductivity.

Temperature/Dissolved Oxygen

Temperature and dissolved oxygen were measured with a Galvanic Cell Oxygen Analyzer with thermistor (Precision Scientific Co.); electronic temperature readings were checked with surface sample

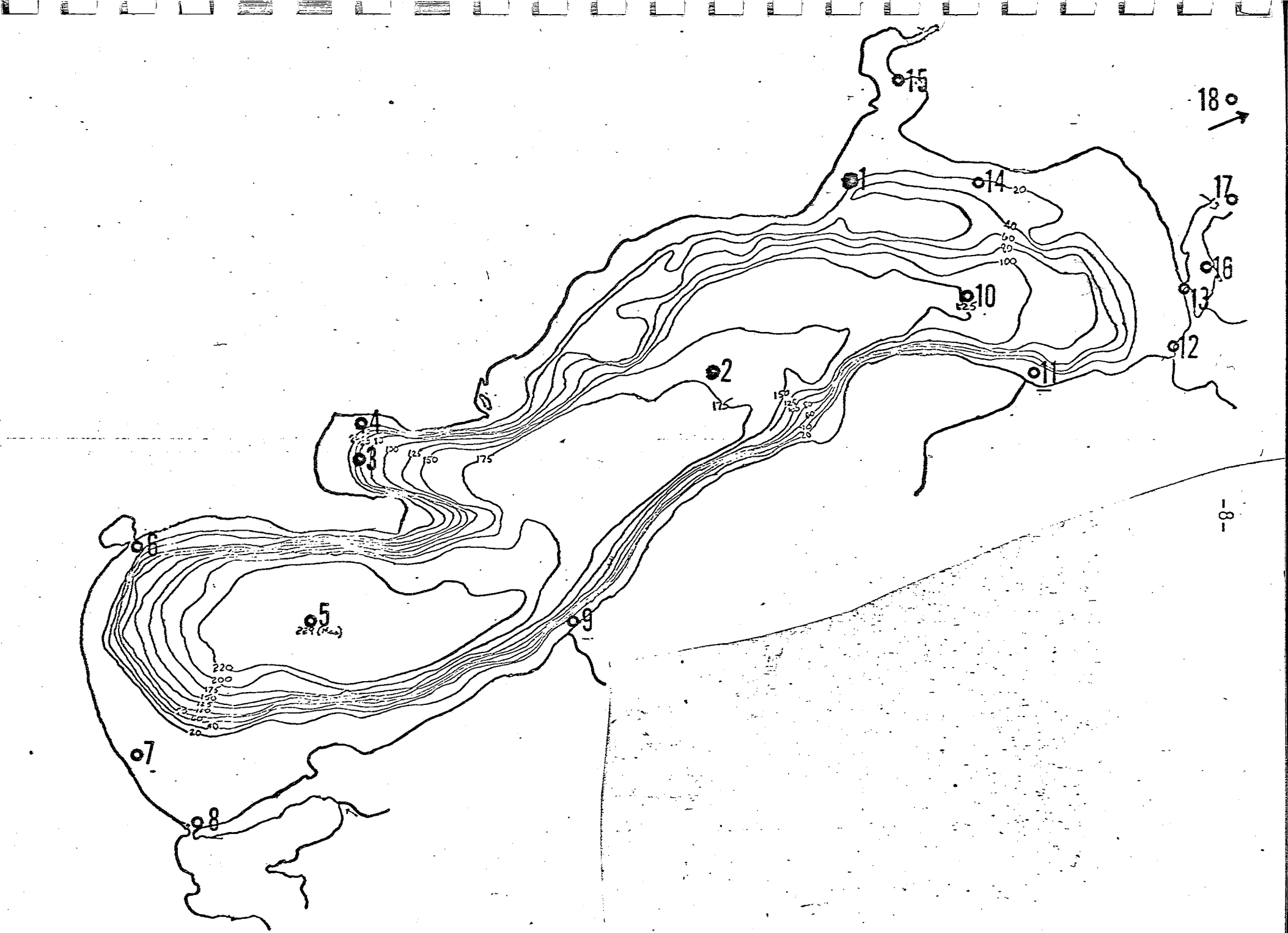


FIGURE 1. Green Lake Sampling Stations.

temperature and dissolved oxygen were measured with a Galvanic Cell Oxygen Analyzer with thermistor (Precision Scientific Co.); electronic temperature readings were checked with surface sample temperatures with a -10° to 110°C range mercury thermometer; dissolved oxygen was also measured with the Azide modification of the Winkler method (Standard Methods, 1971).

(C) CHEMICAL:

Water samples were collected with a 4.1 liter Van Dorn bottle (Wildlife Supply Co.) from a platform pontoon boat, placed in two 2 liter polyethylene jugs, cooled on ice, and removed to the lab within three hours. Samples were maintained in a light-tight refrigeration room at near freezing temperatures (4°C). No acid or germicide was added since analyses of sampled commenced immediately upon arrival and were completed within the allotted 72 hours (EPA, 1971). Each analytical procedure was checked for accuracy by comparison with reference samples (unknowns) from the Environmental Protection Agency, Analytical Quality Control Lab, Cincinnati, Ohio.

Alkalinity

Alkalinity was determined potentiometrically by titration with .02 N Sulfuric Acid to an endpoint of pH 4.5. Minimum detection was 2-3 mg/l (Standard Methods, 1971).

Chloride

Chloride is measured by liberating thiocyanate ion (SCN) from mercuric thiocyanate through sequestration of mercury by chloride ion to form unionized mercuric chloride. In the presence of ferric ion, the liberated SCN forms highly colored ferric thiocyanate, in concentration proportional to the original chloride concentration.

Ammonia Nitrogen

Free ammonia nitrogen (NH_3) is measured by the phenate method. An intensely blue compound, idophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by manganous salt. (Standard Methods, 1971).

Nitrate Nitrogen

Nitrate (NO_3-) was determined by replacing a 2,4-xylenol with 2,6-xylenol in a common procedure based on the nitration of phenols when a high concentration of sulfuric acid is present (Andrews, 1964). Chloride interference was prevented by the addition of 0.1% mercuric sulfate in sulphamic acid. Nitrate interference was prevented by adding sulphamic acid.

Organic Nitrogen

Organic or Kjeldahl nitrogen determination was completed using the standard Kjeldahl technique (Standard Methods, 1971) modified to the Fischer model 21-150 improved Kemmerer-Hallet type micro-nitrogen distillation apparatus and 21-15L digestion-distillation flask.

Soluble Phosphorus

Soluble phosphorus was determined by the ascorbic acid method (Standard Methods, 1971). Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Total Phosphorus

Total phosphorus is determined by converting polyphosphates and some organic phosphorus compounds to orthophosphate forms by manual sulfuric acid hydrolysis. Organic phosphorus compounds may be converted

to the orthophosphate form by manual persulfate digestion (Standard Methods, 1971). The orthophosphate is then measured as above.

Trace Metals (Ca, K, Na, Fe)

The trace metals calcium, potassium, sodium and iron were determined by using atomic absorption spectroscopy and flame emission spectroscopy. A Beckman model 440 Integral atomic absorption spectrophometer was utilized. Calcium and Iron were measured by atomic absorption while Potassium and Sodium were measured by flame emission (Standard Methods, 1971; EPA, 1971).

(2) PHYTOPLANKTON ENUMERATION

The procedure utilized in the analysis of the water samples approximated the millipore filter technique outlined by McNabb(1958) for phytoplankton enumeration. Several aspects of the method were modified to adapt the procedure to the research on Green Lake.

Following weekly collections on the lakes, as outlined in Methodology Section 1C, the water samples were stored, without the application of preservatives, in a cold room regulated to maintain a constant $4^{o}C$ environment. Since examination of the water was performed within twenty-four hours after arrival at the lab, it was deemed unnecessary to employ fixatives to retard deterioration of the samples. Refraining from the utilization of preservatives, "fresh" specimens would, thus, be available for the verification of the identity of an organism crushed on the surface of the membrane filter.

Prior to removal from the bottle, the sample material was thoroughly agitated to obtain a uniform suspension of particulate matter. An aliquot was then transferred into the tube of a filter apparatus designed to accommodate a one-inch diameter membrane.

Plain, white type HA Millipore .45m pore filters, a product of the Millipore Corporation, Bedford, Massachusetts, were used in this study. The sample was then drawn through the filter by a vaccum pump adjusted to exert a constant eight pounds of pressure per square inch. The pressure was maintained at this level to insure that the organisms were not mutilated beyond recognition upon impact with the membrane filter. Subsequently, the filter was removed from the suction apparatus and placed on an appropriately numbered glass slide. Following a four hour period in which the filter was set aside to dry at room temperature, several drops of highly viscous immersion oil were placed on the membrane. Within a few seconds the oil had replaced the water in the interstices of the filter rendering it transparent. When the investigator was ready to examine the prepared slide, a coverslip was placed over the transparent filter and the slide was put into position on the mechanical stage of a microscope. All samples were analyzed for species present and, in those instances where the densities conformed to the criterion discussed below, quantified.

Several procedural difficulties arose at the outset of this study. The suction pressure of the filtration pump, though maintained at eight pounds per square inch, nevertheless, tended to mangle a few algal forms as they came in contact with the membrane surface. Particularly susceptible to mutilation were the unicellular green algae (i.e., Pandorina and Chlorella) and various flagellated forms (i.e, Ceratium and Volvox). The problem encountered in identifying these crushed species was alleviated by occasionally scanning "wet mounts" of fresh samples and comparing the similar features of homologous organisms.

Further complicating our investigation was the determination of the volume of sample to be filtered. The size of the aliquot withdrawn was dependent upon both the algal concentration and the quantity of nonorganic debris present in the water. It was essential to deposit the optimum quantity of planktonic material on the filter, while, at the same time, insure that the organisms would not accumulate on top of each other making visual differentiation prohibitive. Conversely, if the amount of algae were too small, the distance of separation between organisms would become so great as to make the quantification process cumbersome, if not impossible. The relative scarcity of significant numbers of organisms in Green Lake amplified this difficulty.

Although the amount of extraneous matter in the samples was low, the large volume of water needed to "plate" a quantifiable layer of algae on the filter generally resulted in an accumulation of silt sufficient to prohibit the identification and enumeration of the organisms present. It was determined, therefore, to filter only that amount of sample which the siltation level would permit. As a result, the algae adhering to the filter were often of an insufficient number to allow the investigator to effect a statistically accurate count with a Whipple micrometer. According to the guidelines drafted by McNabb (1958), the sampling field chosen should be of such a size that it would contain individuals of the most abundant species present approximately 80% of the time. However, in a majority of the samples from Green Lake the only field which would fulfill this requirement was the entire area of the filter. Since there were no delineations on the Whipple disc large enough to accommodate this size field, it was necessary to resort to a direct count method of quanti-

fication on the vast majority of Green Lake samples. This involved systematically scanning the entire area of the filter and recording each individual present on the field. With the variety of species present and the substantial number of samples to examine, it was considered neither practical nor necessary to count each organism encountered in the lake samples. It was felt, therefore, that an arbitrary reference point of quantification should be established in an effort to determine the minimal value of organisms per milliliter necessitating a direct count.

One of the fundamental aspects of this study involved the recognition and observation of an abnormal planktonic growth. It was, thus, decided that any effort at algal enumeration should be directed toward those growths which could be considered "blooms." While Lackey (1945) defines a bloom as 500 organisms per milliliter, Palmer (1959) contends that any concentrated growth dense enough to be readily evident to the eye could be regarded as a bloom. Due to the fact that few organisms were present in preliminary surveys executed on Green Lake, Lackey's figure appeared too high to be useful for our work. Through several initial studies on laboratory cultures of unicellular phytoplankton, it was ascertained that a density of approximately fifty organisms per milliliter was necessary before the algae became readily visible in the culture medium. The "cutoff" value for plankton quantification in this study was, therefore, defined as 50 organisms per milliliter. In other words, a count would be taken, if, after a preliminary scanning of the filter, it was decided that the quantity of a species of organism exceeded 50 organisms in which the density did not exceed the minimum level. It should be noted that 50 organisms per milliliter is a purely arbitrary value and does not represent the ultimate criterion of bloom determination.

On several Green Lake samples sufficient quantities of algae were present to facilitate enumeration with a Whipple micrometer. In these instances, strict adherence was made to the techniques described by McNabb (1958) for a statistical determination of the quantities of algae existent in the water.

(D) ANALYSIS OF DATA

The determination of critical nutrients in a lake can possibly be determined by placing the lake under survey for one calendar year and employing analysis for critical nutrients. In this way it should be possible to ascertain which nutrients are actually critical in the body of water (Sawyer, 1968). Figure 2 shows typical seasonal variations in the concentration of a critical element and of a noncritical element in relation to primary productivity. This sort of variation in concentration on a yearly basis on Green Lake could be related to instances of increased or decreased biological productivity.

Water chemistry data was analyzed by use of a computer program (see appendix 6) to determine if significant correlations between the various parameter studied existed. As the data available was in two formats, continuous (all chemical data) and discontinuous (algal specie counts--either present or absent) it was necessary to use three different types of tests. The first test, for continuous-continuous correlation, was the coefficient of correlation, R:

$$R = \frac{N\Sigma(X_i Y) - (\Sigma X)(\Sigma Y)}{[N\Sigma(X^2) - (\Sigma X)^2]\,[N\Sigma(Y^2) - (\Sigma Y)^2]^{1/2}}$$

$$a = \frac{(\Sigma X^2)(\Sigma U) - (\Sigma(X.Y))(\Sigma X)}{N(\Sigma X^2) - (\Sigma X)^2}$$

$$b = \frac{N(\Sigma(X.Y)) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2}$$

$$z = 1/2\,[(\log_e(1+r) - \log_e(1-r)]$$

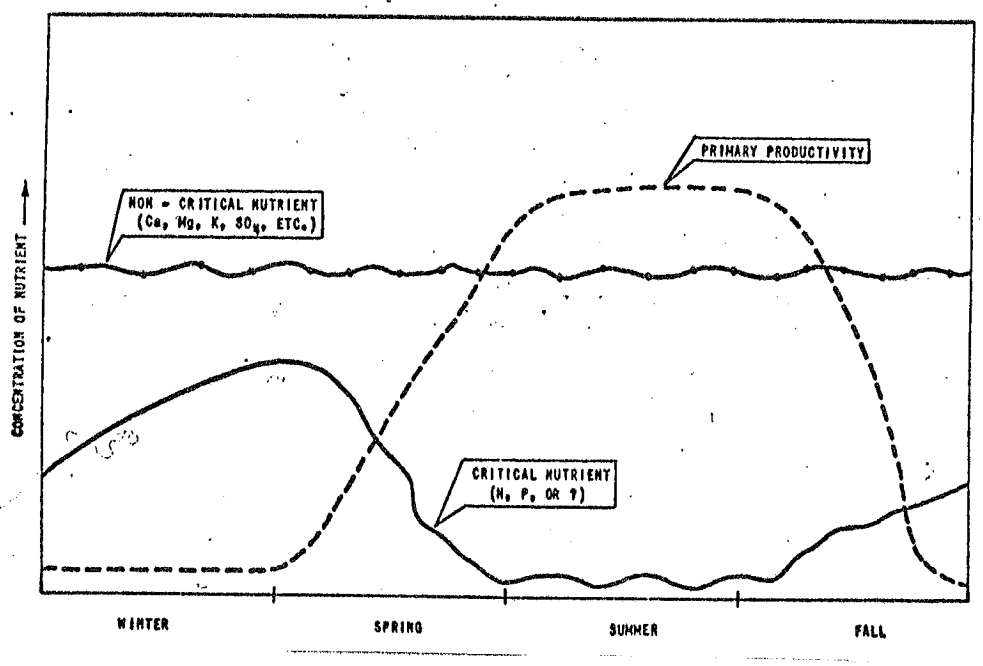


FIGURE 2. Relationship of critical nutrient concentration in lake waters to primary productivity. (from Sawyer, 1968)

$$\sigma_2 = \pm\sqrt{\frac{1}{n-3}}$$

$$P = \frac{s}{\frac{1}{\sqrt{n-3}}} = s\sqrt{n-3}$$

R has a value of 1 for a perfect correlation and 0 (zero) for no correlation. This is converted to s and then to p, which represents the probability of such a value of R arising by chance. It is in standard deviation; a value of p=2 is approximately equal to 95% confidence.

The second test for continuous-discontinuous correlations, is the standard error of the mean. Values of the continuous variable are divided into 2 groups, 0 and 1, depending on the value of the discontinuous variable. The standard deviation for each group is then determined.

$$\sigma p = \sqrt{\frac{\sigma_o^2}{n_o} + \frac{\sigma_1^2}{n_1}} \qquad \sigma = \sqrt{\frac{\Sigma(x^2)}{n-1} - \frac{(\Sigma x)^2}{n(n-1)}}$$

$$p = \frac{/\,\sigma_1 - \sigma_o\,/}{\sigma p}$$

p has the same meaning as for continuous-continuous.

The final method for comparison of two dichotomous parameters is a chi-square test. Based on the percentage of each parameter which is 1, theoretical percentages for occurences of (0,0), (0,1), (1,0), (1,1) are calculated and used in a chi-square test.

$$X^2 = \sum\left[\frac{(f_o - f)^2}{f}\right]$$

f = theoretical frequency

f_o = observed frequency

The computer program may be divided into two sections: a control section, which determines the sequences and types of data comparison, and a mathematics section, which handles the calculations.

At the most basic level, there are three types of comparisons: parameter-parameter (pp), in which 2 parameters, such as alkalinity and phosphates, are compared; parameter time (PT), in which one variable is analyzed as a function of time; and parameter-sample (PS) in which the comparison is between a parameter and some function of the sample.

In parameter-parameter calculations, corresponding values in the parameter are compared. For example, the values of week 5, sample 16 are compared.

In parameter time calculations the comparison is between a parameter and a reference file. As an example, one reference file contained rainfalls for the weeks of sampling. Here all the samples taken on week 1 are compared to value 1, then week 2 to value 2, etc.

In parameter sitecorrelations, the comparison is again to a reference file. Here, one example is a reference file containing the depth of the sample. Thus, all values for sample 1 are compared to the depth of sample 1, etc.

Correlations Completed

All of the parameters were checked against every other one. The programscontains internal checks for lack of data, and to set for the correct type of calculation. Additional correlation of all parameters versus time function were made precipitation day before sampling, precipitation day of sam-

pling, total weekly precipitation, weekly average maximum temperatures, weekly average temperature plus 20 sin function, of the form: $\sin (2\pi x(\frac{T+n}{360}))$ where t=the day of the year on which samples were prepared, and n=0, 18, 36, . . . 342. All parameters were correlated against sample depth also.

Finally, in an effort to locate possible nutrient sinks or sources, a special type was done. A grid was established on the lake, and each sampling point given a set of co-ordinates. Then, a thousand points were picked, evenly distributed over the lake. The distances from the samples to one of these points were then correlated against the various sampling parameters, for an additional 25,000 correlations.

In the interpretation of the data, two cautions must be kept in mind. First, that attention be paid to the number of values in the calculation: (a small number is not very reliable), and second that statistical tests are intended for parameters with normal distribution. The fact that the distributions of many of the parameters are far from normal throws in uncertainty; and the values should be interpreted as indications, not conclusive evidence.

Printout

The format of the printout depends on the type of calculator; continuous-continuous, contenuous-discontinuous, etc.

For c-c, it appears as follows:

ALKALINITY vs. NITRATE NITROGEN

y= (Ø.Ø1Ø42) x + -1.46255 N=232 R=Ø.23399 P=3.6Ø77Ø

The heading is self-explanatory. The next line, Y=.01042 x + -1.46255, is the equation of the best possible line to fit the points. N=232 means that 232 points were used in the calculator, R is the regression coefficient, and P is the probability, in standard deviation, that this is a significant difference from 0(zero).

For C-D.

ORTHO-PHOSPHATES vs. CERATIUM

XØ MEAN = Ø.Ø7572 X1 MEAN = Ø.31097 P=1.04853

NØ = Ø68 N1 = 1Ø3

XØ is the mean for phosphates when Ceratium was absent, X1 the mean when it is present; P as before. NØ is the number of points used in which Ceratium was absent, N1 when it is present.

For D-D:

CHLORELLA vs. ANABAENA

CHI SQUARE = 36.2Ø32 ØØ = 017 01 = 058 10 = 001

11 = 016

Chi square is self-explanatory. It can be converted to a probability with any table of chi square with the number of degrees of freedom=3. ØØ = 017 means there were 17 cases when both algae were absent, 01 = 058 means 58 cases where Chlorella was absent and Anabaena present, etc.

(3) BIOASSAY EXPERIMENTS

(A) THEORY AND RATIONALE

(i) General Theory of Growth of Microorganisms

The following sections are modified from the Final Report - Provisional Algal Assay Procedures (Toerien et. al., 1971). Symbols used in the development of kinetic equations in the following pages are based upon the unified concept of fundamental symbols suggested by the "Second International Symposium on Continuous Cultivation of Microorganisms" held in Prague, 1962.

Growth of Unicellular Organisms

In the biological sense, growth is an orderly increase of all the components of an organism, not merely of some of its constituents. In all cellular organisms, cell multiplication is a consequence of growth. In unicellular bacterial and algal growth, cell multiplication leads to an increase in the number as well as mass of cells.

Generation Time and Growth Rate

Because growth represents an increase in the amount of living matter, it leads to an increase in self-duplicating material. The more living substance that is formed, the greater becomes the amount of matter that can grow and reproduce itself. Consequently, under ideal conditions for growth and reporduction, the amount of living matter increases not in direct proportion to time, but it multiplies itself by a constant factor in each successive unit of time. The geometric progression with time is illustrated most dramatically

by the growth of cultures of most unicellular bacteria and algae. Each individual cell grows, and after reaching a certain size, divides to form two or more complete individuals. In cultures the time of division is randomized, but the time required for each successive doubling of the total population under optimum conditions remains constant. This period of time is called the generation time (g).

Because the number of cells doubles during each generation, the total population increases as the exponent of 2, and such growth is referred to as exponential growth and can be algebraically described as:

$$X = X_o 2^N \qquad (1)$$

where

X = concentration of microorganisms at time t, M/V,

X_o = concentration of microorganisms at time 0, M/V, and

N ==number of cell divisions

The following equation

$$\frac{t}{N} = g \qquad (2)$$

where

t = time, t,

N = number of cell divisions in time interval t

g = generation time, t

therefore expresses the average generation time or doubling time for the culture as a whole. A rearranged form of Equation 2 can be substituted in Equation 1 and one obtains

$$X = (X_o)\ \ 2^{t/g} \qquad (3)$$

Taking logarithms and rearranging

$$g = \frac{(\ln 2)\ (t)}{\ln (X) - \ln (X_o)} \qquad (4)$$

and solving for X,

$$\ln (X) = t\ \frac{(\ln 2)}{g} + \ln (X_o) \qquad (5)$$

When the expression (ln 2)/g reaches its maximum value for a specific organism under specific growth conditions, the maximum specific growth rate, $\hat{\mu}$, can be substituted for (ln 2)/g as follows:

$$\ln (X) = \hat{\mu}t + \ln (X_o) \quad , \qquad (6)$$

or

$$X = (X_o)\ e^{\hat{\mu}t} \qquad (7)$$

The equation in this form is recognized as the general growth equation. Taking the derivative with respect to time, the following relationship is obtained

$$\frac{dX}{dt} = \mu X \qquad (8)$$

The gross specific growth rate is less than the maximum growth rate when either the substrate is limiting or when conditions are not optimum. The net growth rate is obtained when both the specific growth rate (μ) and organism decay rate (k_d) (i.e., the decrease in cellular death with subsequent lysis) are considered:

$$\frac{dX}{dt} = (\mu - k_d)\ X \qquad (9)$$

Specific Growth Rate as a Function of Limiting Nutrient Concentration

The specific growth rate, μ, is specific for organism, environment, and culture medium. It depends on the growth capacity of the microorganism and the environment in which it grows. The specific growth rate is a function of the growth rate limiting nutrient concentration when all other factors are in excess. Then

$$\mu = f(S) \tag{10}$$

where

S = growth rate limiting nutrient concentration, M/V.

This function is generally expressed in such a way that at low nutrient concentration the specific growth rate is first order with respect to nutrient concentration, whereas at high nutrient concentration, the specific growth rate is zero order with respect to nutrient concentration and it approaches its maximum value, $\hat{\mu}$.

Monod Model (Michaelis-Menten Equation)

The rectangular hyperbola is the model widely accepted in expressing the relationship between specific growth rate, μ, and the rate-limiting concentration. This relationship is based on the Michaelis-Menten equation which was developed to describe the rate of an enzyme reaction as a function of substrate concentration and which was proposed empirically by Monod to describe the relationship between bacterial growth and substrate concentration:

$$\mu = \hat{\mu} \left(\frac{S}{K_s + S} \right) \tag{11}$$

where

$\hat{\mu}$ = maximum specific growth rate, $time^{-1}$

μ = specific growth rate, $time^{-1}$

S = concentration of the growth rate limiting nutrient, M/V

K_s = half saturation constant, numerically equal to the nutrient concentration at which the specific growth rate is one-half the maximum growth rate, i.e., $\mu = \hat{\mu}/2$.

The Michaelis-Menten (monod) kinetic model for describing the relationship between the growth rate and nutrient concentration is shown in Figure 3, and this relationship has been widely used by microbiologists and engineers working with continuous culture systems. In sanitary engineering, several workers have been successful in using this model for descriptions of activated sludge kinetics, anaerobic fermentation system kinetics, and also algal growth kinetics. Others have used this kinetic model to describe growth and nutrient uptake of marine phytoplankton.

(ii) Algal Growth Kinetics in Bottle (Batch) Bioassay

The basic concern with algae in eutrophication is their multiplication or growth. The dynamics of multiplication, as well as of other functions related ot it, are the most important aspects of algal growth. Figure 4 represents an ideal batch growth curve in which cell concentration-time, and nutrient concentration-time profiles are superimposed. The algae begin multiplication in a surplus of nutrients, which gradually decreases; however, the single components often are utilized at different rates.

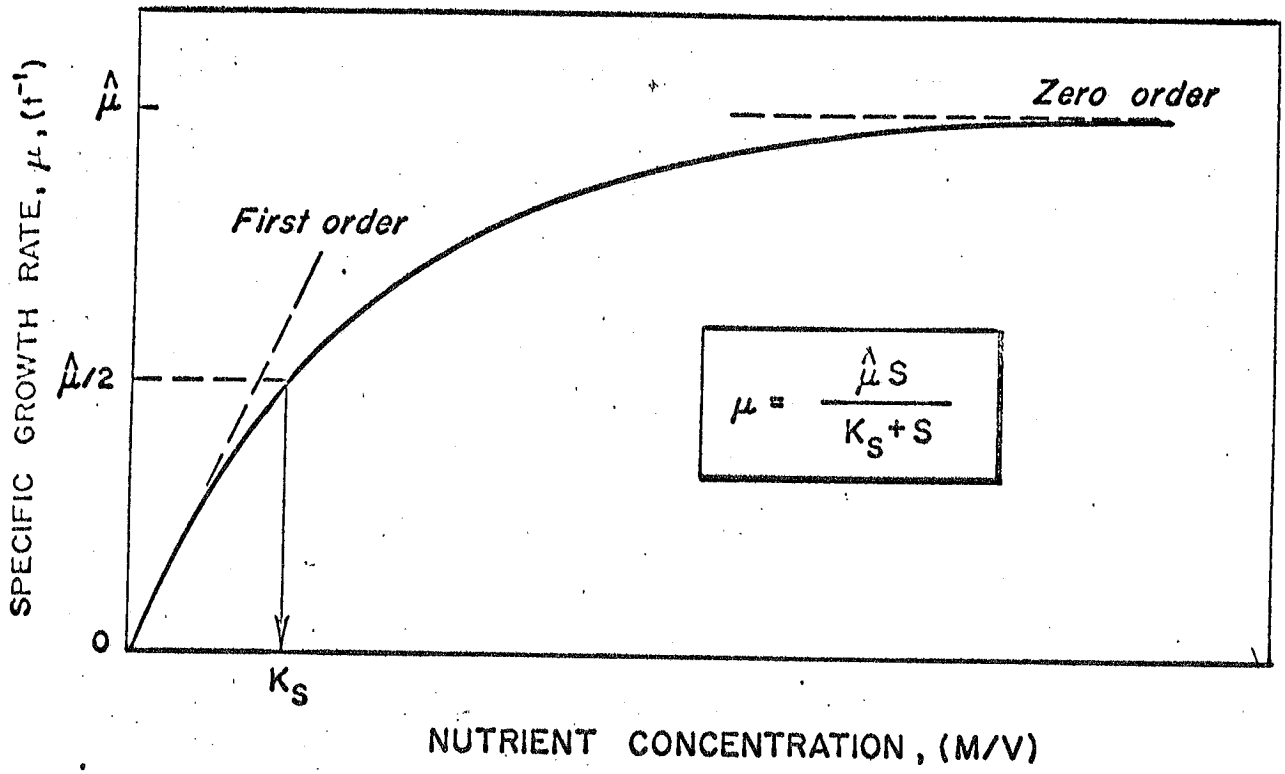


FIGURE 3. Michaelis-Menten (Monod) Kinetic Model.

After inoculation of the nutrient medium, some time elapses before the number of algae beings to increase. This period is termed the "lag phase" (Zone 1, Figure 4) and the length of time before cell division depends on the type and age of inoculum (phase of growth of the culture from which the inoculum originated) and the characteristics of the medium. This dependency on the age of the inoculum reflects the wide physiological variation between different growth phases in an ordinary algal batch culture toward the end of the lag phase. The mass of algal cells present in the culture increases considerably without cell division occurring. The cells are preparing for cell division and multiplication. This stage is therefore called the "physiological youth" of the culture, a description which must be taken figuratively, as it is not possible to consider the culture as a single organism. The next stage represents the beginning of multiplication and of the exponential or logarithmic part of the growth curve (Zone 2, Figure 4) in which the algae are multiplying uniformly with an approximately constant growth rate. The rate of growth during this phase is characteristic for any given alga under the particular condition of cultivation and represents the maximal reproductive capacity of that alga in the specific environment. The environmental factors that govern the rate of growth include the nature and concentration of the nutrients, pH, temperature, light, and other physical and chemical variables. The length of the exponential phase differs according to the available amounts of some essential nutrient. However, in a batch culture the period in which

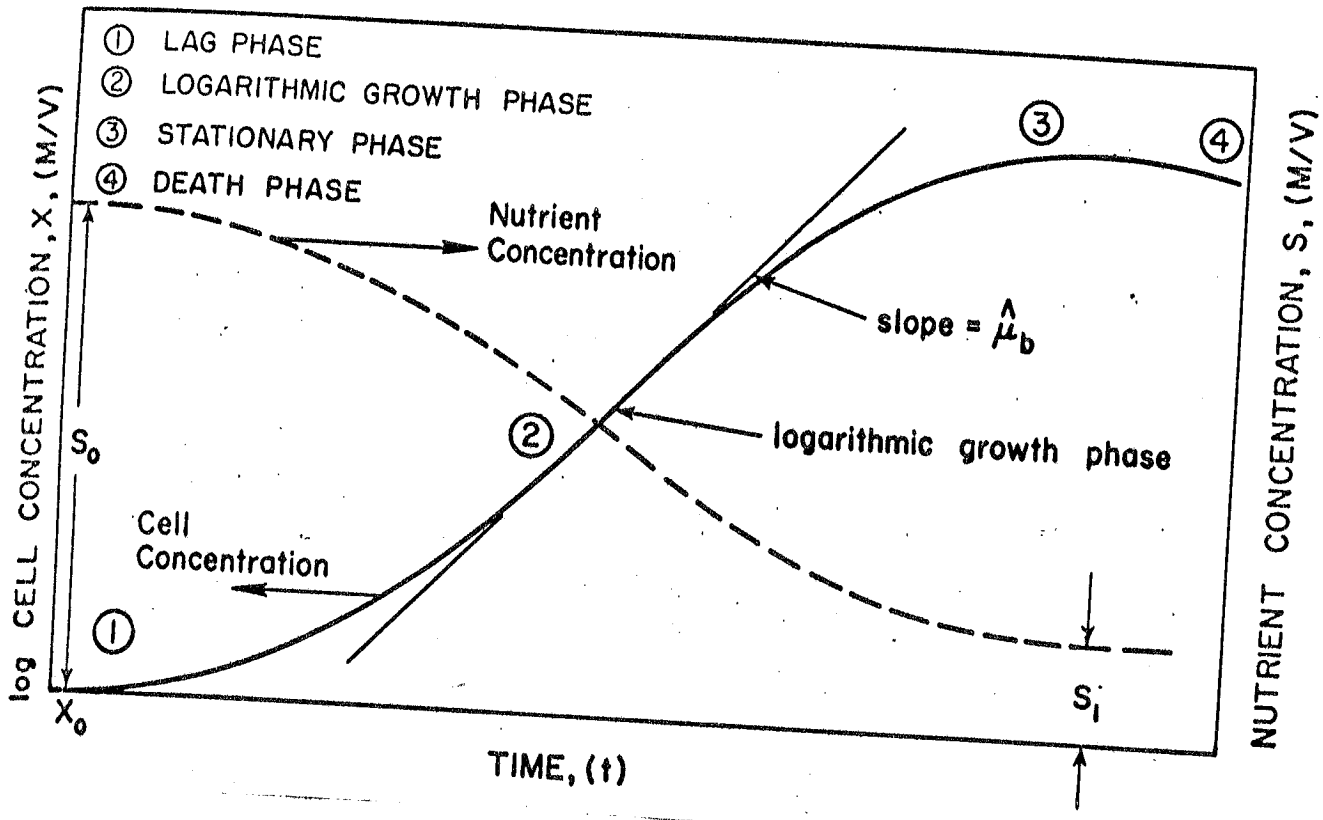


FIGURE 4. Ideal Batch Growth Curve.

the living biophase does not change its properties and grow in a dynamic steady state is generally short and often steady state does not occur at all but is replaced by continuous transition states. When the algae are sufficiently numerous, their activities begin to have an appreciable effect on the medium. Eventually, some nutrient becomes exhausted, the environment becomes less favorable to growth, and the growth rate decreases to a stationary phase (Zone 3, Figure 4). The stationary phase of some algal cultures passes into a subsequent stage (Zone 4, Figure 4) where the growth curve bends downward as death and lysis of algae in the culture begins to predominate over growth.

The form of the growth curve which is based on the number or the mass of algae does not express completely all changes occurring in such cultures. The algae of one stage of the growth cycle appear to differ physiologically from those of another stage. Therefore, growth in a batch culture is considered to be "unbalanced," i.e., cell concentrations, nutrient concentrations, surface to volume ratios, etc. are changing continually. It has been suggested that the characteristic form of the growth curve and the maximum concentration of cells attainable in a given culture medium reflect the basic natural properties of the algae and their naturel population growth characteristics, and on this basis the use of batch culture methods to study these properties has been justified. However, such a concept is erroneous because the basic properties of the algae are affected by the conditions of the culture method. Therefore, the exclusive use of batch cultures

over other methods was not completed because of their "unbalanced" growth characteristics.

Evaluation of Batch Growth Kinetics. Mathematical expressions for the kinetics of growth in batch cultures have been presented in the section on the general theory of growth of microorganisms. The growth parameter of batch assays of water samples traditionally is expressed most often as the maximum cell concentration, $\hat{X}$. It is presumed that $\hat{X}$ allows differentiation between samples based on initial nutrient concentration. However, due to difficulties in interpreting what actually limits $\hat{X}$ and in the application of $\hat{X}$ values to practical problems, attention has been directed to the use of other growth parameters, such as maximum specific growth rate batch, u_b; half saturation constants, K_s, for each rate limiting nutrient; and yield coefficient, Y, for each nutrient.

Maximum Cell Concentration, $\hat{X}$. The $\hat{X}$ from batch cultures can be described using the following equation,

$$\hat{X} = X_o \, e^{\mu_b t_e} \qquad (7)$$

where

t_e = length of time needed to reach zero growth rate

and is measured experimentally as the final cell concentration at the stationary stage of batch growth.

As shown in Equation 7, maximum cell yield varies with initial inoculum, maximum specific growth rate batch, and time to reach zero growth rate. The important concept to remember is that the maximum specific growth rate batch, $\hat{\mu}_b$, is a

single function of the growth rate limiting nutrient concentration (Equation 10), while the time needed to reach zero growth is a complex function of all nutrient concentrations and possibly other factors.

Maximum Cell Concentration Limiting Conditions. Batch algal growth in rich "unrestricted" nutrient environments (i.e., all nutrients are considerably in excess) is characterized by the situation where the $\hat{\mu}_b$ approaches $\hat{\mu}$ (largely determined by the environmental conditions of the test) and hence, none of the nutrient species is the growth rate limiting nutrient. This condition is shown schematically in Figure 5. Due to the difficulties of identifying the presumed growth rate limiting nutrient and the possibilities of multiple growth rate limiting conditions, there is no rational mechanism to describe the relationship between maximum cell production and the concentration of each nutrient species. However, theoretically a maximum cell concentration limiting nutrient is defined as the nutrient species which is exhausted first during the batch growth and presumably has the most significant effect on maximum cell concentration.

Maximum Cell Concentration and Growth Rate Limiting Conditions. If the algae grow under a specific growth rate limiting condition (i.e., only one specific nutrient limits the growth rate while the other nutrients, growth substances, and growth factors are in excess), the only meaningful growth parameter related to the limiting nutrient concentration is the specific growth rate as indicated in Figure 6. Although the rate-limiting nutrient limits the maximum cell concentration as well,

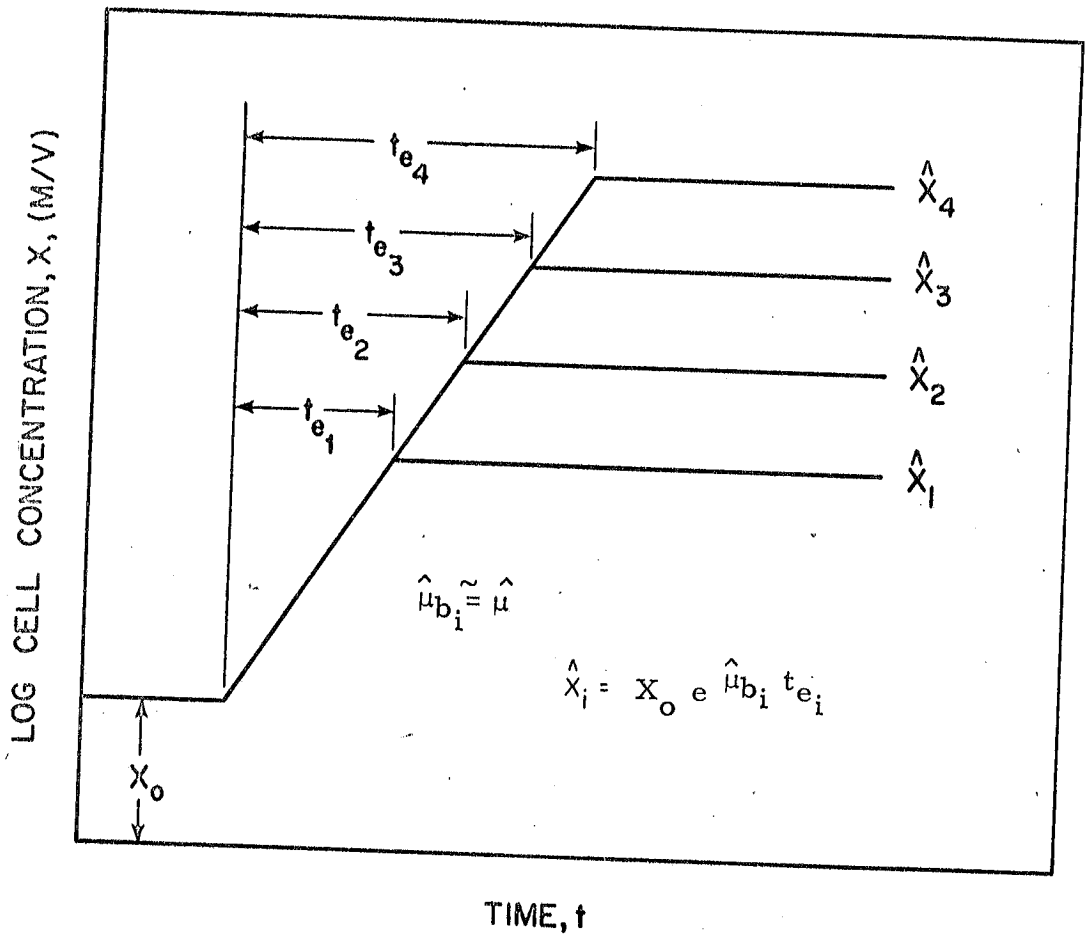


FIGURE 5. Batch Algal Growth In Unrestricted Nutrient Environments.

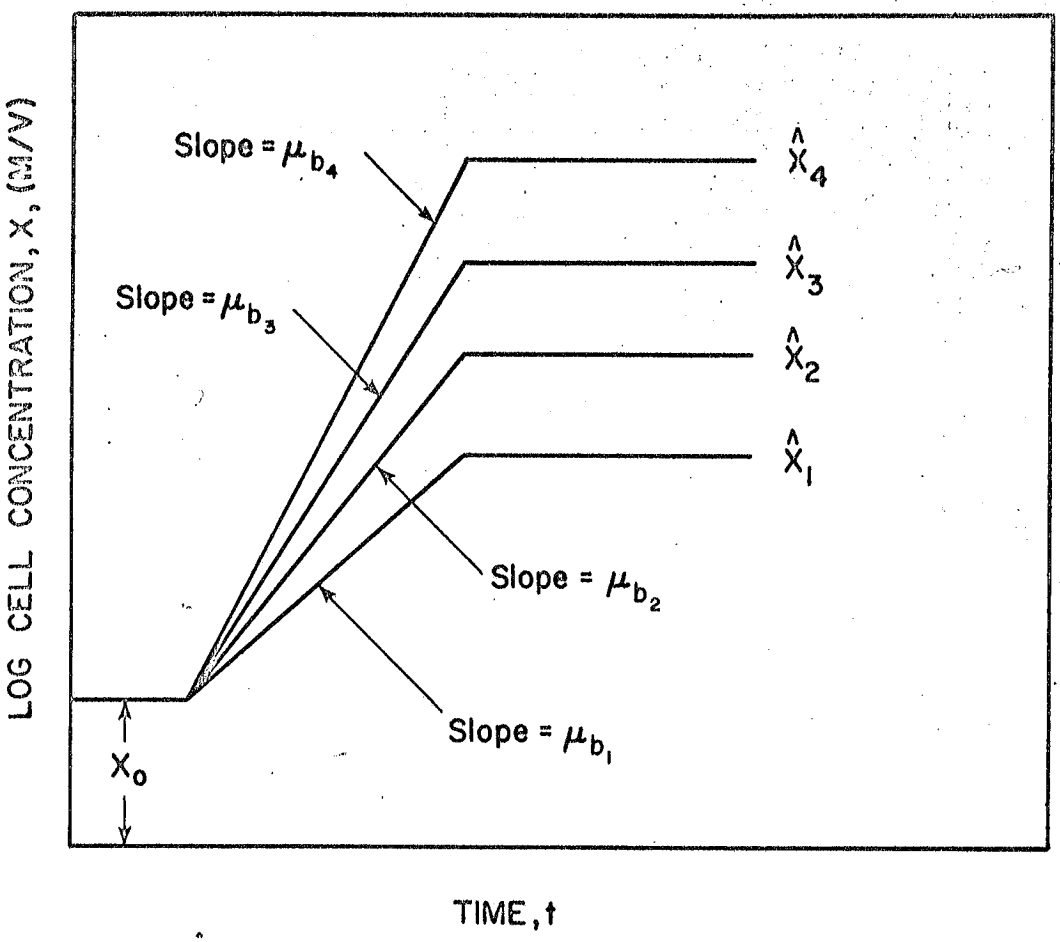


FIGURE 6. Batch Algal Growth Under Specific Growth Rate Limiting Conditions.

the maximum cell concentration is also related to the length of time needed to reach zero growth rate. This time period cannot be defined because of its variable character depending upon the characteristics of the sample, the condition of the test alga, and the test method.

Maximum Specific Growth Rate, $\hat{\mu}_b$. To determine the value of the u_b, Equation 6 can be rearranged as follows:

$$\hat{\mu}_b = \frac{\ln (X_{n+1}) - \ln (X_n)}{t} \qquad (12)$$

where

X_{n+1} = cell mass at time n+1

Xn = cell mass at time n

t = time interval between exponential growth at time n and n+1.

As stated previously, the $\hat{\mu}_b$ is affected by the environmental conditions of the test and is limited by the concentration of the rate-limiting nutrient (Equation 11)

$$\hat{\mu}_b = \hat{\mu} \left(\frac{S}{K_s + S} \right) \qquad (11)$$

The specific growth rate batch is equal to the maximum growth rate multiplied by the fraction $S/(K_s + S)$. As K_s is always greater than zero, the value of the fraction is smaller than one and $\hat{\mu}_b$ approaches u asymptotically as S is increased (i.e., $S \gg K_s$).

To predict the effects of addition of nutrients to and/or removal of nutrients from a water, one must be able to relate the biostimulatory response to the concentration of the specific nutrient. If it is determined that sufficient nutrients

are present in the sample to allow $\hat{\mu}_b$ to approach $\hat{\mu}$, it can be concluded that the nutrients are in excess in the natural situation and some other factor(s) (light, CO_2, temperature, predation, etc.) is limiting the algal population.
On the other hand, if $\hat{\mu}_b \ll \hat{\mu}$, some nutrient is likely limiting growth in the batch culture and may be controlling growth in nature. Consequently, $\hat{\mu}_b$ should be the most interpretable biostimulatory growth response parameter from batch cultivation for specific growth rate-limiting conditions.

(iii) Algal Growth Kinetics in Chemostat (Continuous Culture) Systems

Continuous flow (chemostat) culture is characterized by the continuous addition of fresh medium (or sample) to the culture, complete mixing of culture and medium, and a continuous outflow of part of the culture at a rate identical to the inflow rate of fresh medium, ensuring a constant culture volume. As long as the chemostat operates, an open dynamic system results in which a steady state is attained. The algal cells in the growth vessel continue to grow at the expense of the fresh nutrients and the total number of algal cells in the chemostat remains constant. Furthermore, the growth rate of the cells can be controlled at will because the slower the fresh medium is delivered to the growth vessel, the slower the cell growth rate. Chemostats provide a method for keeping an algal culture growing for an indefinite period of time, at a constant population size, a controlled and constant rate of growth, a specific, constant physiological state, and constant environmental conditions. It is obvious that the "physiological state" reflects the sum of the biochemical, morphological,

and especially physiological features and activities characteristic of the most important factors operative for growth and metabolic activities of the organisms. It was noted in the description of the growth curve for batch cultures that the physiological state often changes rapidly under different environmental conditions and is the most important characteristic of the state of the culture. However, with the continuous flow (chemostat) culture, culture conditions and the physiological state are maintained essentially constant and at a steady state.

Continuous homogenous cultures can be characterized as complex open systems of constant volume, being as a whole in a dynamic steady state with essentially constant concentrations of all nutrients and of cell biomass. During continuous flow cultivation the biophase permits determination of the kinetics of growth and of growth parameters which can be used in both theoretical analyses as well as for application to practical problems, as will be shown later.

Basic Assumptions. To develop rationally a mathematical model for a continuous flow (chemostat) system, three basic assumptions are made: 1) A constant proportion of the organisms are viable; 2) The (gross) specific growth rate of organisms in a chemostat is some function of the growth rate-limiting nutrient concentration, thus

$$\mu = \frac{1}{X} \frac{dX}{dt} = f(s) \quad ; \qquad (12)$$

and 3) The specific growth rate of the organisms varies with

the rate of consumption of the growth rate limiting nutrient,

$$\frac{dX}{dt} = Y \frac{-dS}{dt} \qquad (13)$$

Y = cell yield coefficient.

It is generally assumed that the yield coefficient, Y, is a growth constant with respect to any nutrient. However, this is only true when the energy requirement for endogenous respiration for the organism does not affect the constant endogenous metabolism of the organisms at different growth rates, and/or when there is no "excess" uptake or storage of the growth rate-limiting nutrient.

Growth Kinetics in Chemostat with a Constant Yield Coefficient. In developing the growth kinetics of a continuous flow culture system with no cell return and with a constant yield coefficient, the continuity of biomass and nutrients in steady state or the materials balances for biomass and nutrients are developed.

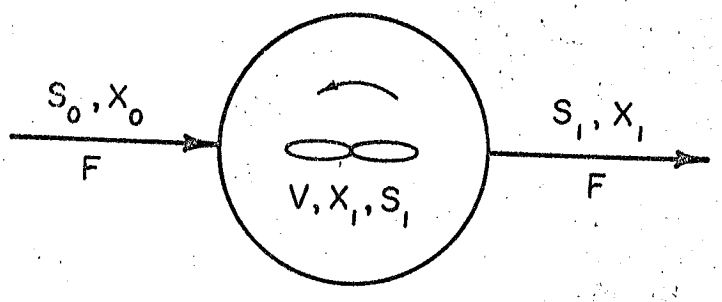
Materials Balance for Cell Biomass. For a CFSTR (Figure 7) a materials balance for the reactor is expressed by the following equation:

$$\left[\begin{array}{c}\text{rate of change of}\\ \text{cell biomass in}\\ \text{the reactor}\end{array}\right] = \left[\begin{array}{c}\text{rate of input}\\ \text{of cells}\end{array}\right] - \left[\begin{array}{c}\text{rate of output}\\ \text{of cells}\end{array}\right] + \left[\begin{array}{c}\text{growth rate}\\ \text{of cells}\end{array}\right] - \left[\begin{array}{c}\text{decay rate}\\ \text{of cells}\end{array}\right]$$

where

$$V\left(\frac{dX_1}{dt}\right) = F X_o - F X_1 + \mu X_1 V - k_d X_1 V \qquad (14)$$

F = flow rate, volume/time
V = volume of reactor.



Symbols
- F = Flow Rate
- X = Cell Mass Concentration
- S = Limiting Nutrient Concentration
- V = Reactor Volume

Subscripts
- o = Influent
- 1 = Effluent or Reactor

FIGURE 7. Continuous Flow, Stirred Tank Reactor System (CFSTR).

At steady state (that is dX/dt = 0) in a reactor with no cell recycling and receiving no cells in the input, the above equation becomes

$$\mu - k_d = \frac{F}{V} = \frac{1}{\theta} = \frac{1}{\theta_c} \qquad (15)$$

where

θ = hydraulic residence time, t

θ_c = mean cell age in the reactor, t.

Materials Balance for Nutrient. The materials balance of the rate-limiting nutrient through a noncell recycling reactor can be described as follows:

$$\begin{bmatrix}\text{rate of change of growth rate limiting nutrient in reactor}\end{bmatrix} = \begin{bmatrix}\text{rate of input of rate-limiting nutrient}\end{bmatrix} - \begin{bmatrix}\text{rate of output of rate-limiting nutrient}\end{bmatrix} - \begin{bmatrix}\text{rate of uptake of rate-limiting nutrient}\end{bmatrix}$$

$$V \frac{dS_1}{dt} = F S_o - F S_1 - \text{uptake}$$

The term "uptake" can be defined in terms of the yield coefficient, Y, and the derived form of the natural growth equation (Equation 13). Thus,

$$\frac{dS}{dt} = \frac{dS}{dX} \cdot \frac{dX}{dt} = \frac{1}{Y} \mu X \qquad (16)$$

and "uptake" = $\mu X_1 V/Y$.

Therefore,

$$V \frac{dS_1}{dt} = F S_o - F S_1 - \frac{\mu X_1 V}{Y} \qquad (17)$$

At steady state

$$\frac{dS}{dt} = 0$$

$$F\ (S_o - S_1) = \frac{\mu X_1}{Y}.\ V$$

or

$$X_1 = \frac{F}{V}\ \frac{(S_o - S_1)\ Y}{\mu} \quad , \quad \text{and}$$

$$X_1 = \frac{(S_o - S_1)\ Y}{\mu\theta} \quad . \qquad (18)$$

It must be noted that the net yield coefficient ($Y_n = X_1/(S_o - S_1)$) is related to the yield coefficient as follows:

$$Y = \left(\frac{X_1}{S_o - S_1}\right)\mu\theta = Y_n\ (1 + \theta_x k_d) \qquad (19)$$

It can also be shown that the specific growth rate is a function of the rate-limiting nutrient removal velocity, q, which in turn is a function of the rate-limiting nutrient concentration.

$$\mu = \frac{S_o - S_1}{X_1\ \theta}\ Y = Yq \qquad (20)$$

$$q = \frac{S_o - S_1}{X_1\ \theta}$$

Substituting Equation 20 into Equation 15 one obtains the cell continuity equation:

$$\frac{1}{\theta} = \frac{1}{\theta_c} = Yq - k_d = \mu - k_d \qquad (21)$$

It should be noted that $1/\theta = 1/\theta$ is only true for nonrecycle reactors. Substituting Equation 11 into Equation 21 and solving for S_1,

$$S_1 = \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} \quad . \qquad (22)$$

Equations 18 and 22 describe steady state values of cell concentration and limiting nutrient concentration, respectively. Inspection of Equation 22 reveals that for nonrecycle reactors where $1/\theta = 1/\theta_c$, once the residence time, θ is selected, the reactor or effluent concentration S_1 has a single value, that is, it is fixed by the residence time as all other terms in the equation are constants. By substituting the value of S_1 from Equation 22 into Equation 18 one obtains the following:

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \qquad (23)$$

It should be emphasized that at steady state and for a given residence time θ, and organism, the cell concentration X_1 is a function only of the concentration of nutrient (S_o) in the feed stream.

Again it should be noted that for a given residence time, the effluent concentration S_1, is fixed and independent of the feed concentration S_o. However, the reactor cell concentration (X_1, X_2, and X_3) is determined by feed concentration S_{o_1}, S_{o_2}, and S_{o_3} for that residence time. There is a critical value of residence time below which the algal concentration, X_1, decreases exponentially and finally approaches zero because the residence time is insufficient for the algae to reproduce and maintain the population. Then the nutrient con-

centration in the chemostat and in the effluent, S_1, increases until it reaches the influent nutrient concentration, S_o, because there is no uptake and no algae present. This critical value is referred to as "washout." Using Equations 15 and 21, when Θ approaches "washout" (Θ_w), u approaches its maximum value, $\hat{\mu}$, then

$$\frac{1}{\Theta_w} = \hat{\mu} - k_d \qquad (24)$$

where

Θ_w = residence time at which "washout" occurs.

Evaluation of Chemostat Growth Kinetic Constants. The general biostimulatory growth parameters of major concern in the chemostat are: steady state cell concentration, X_1; maximum specific growth rate, $\hat{\mu}$; the half saturation constant, K_s; the nutrient utilization constants, K_A and K_B; and the decay rate of the organism, k_d.

Steady State Cell Concentration in Chemostat, X_1. Analogous to X of batch cultures, X_1 is the steady state cell concentration attainable in a chemostat for a specific residence time and nutrient concentration in the feed stream. The steady state cell concentration reflects directly the biological available nutrient concentration as shown by Equation 23. For a given residence time, the cell concentration X_1 is only dependent on the nutrient concentration, S_o, in the feed. However, the kinetic description for X_1 in terms of S_o has different parameters for the case of "excess" nutrient uptake as compared to no "excess" uptake.

Maximum Specific Growth Rate, $\hat{\mu}$. The $\hat{\mu}$ of a specific algal species determined by chemostat assays is a growth constant in

the M-M, M equation (Equation 11) and is closely related to "washout" residence time, θ_w, (Equation 24). This value represents the maximum growth rate of an algal culture and corresponds to the $\hat{\mu}_b$ value of a batch culture under unrestricted conditions, i.e., under conditions where no nutrient or factor is limiting growth. The maximum specific growth rates of algal species are important growth parameters affecting the characteristics and composition of natural biological systems (e.g., they determine which species will grow in a given system as well as the distribution of species based on the competitive ability of particular species for the rate-limiting nutrient in the environment).

Half Saturation Constant, K_s. The half saturation constant is defined as the growth rate limiting nutrient concentration which can support algal growth at one-half the maximum specific growth rate ($\hat{\mu}/2$) and it is specific for each organism and growth rate limiting condition (Figure 3). It is the most important growth parameter in growth kinetics as well as in the application of growth constants to practical problems. The practical significance of K_s will be discussed in a subsequent section.

Decay Rate, k_d. The decay constant, k_d, for an organism reflects the rate of decrease in cellular mass due to endogenous respiration and cellular death with subsequent lysis. The magnitude of k_d might be expected to vary from about zero to values as high as 0.05 day^{-1} (i.e., $0 \leq k_d \leq 0.05$ day $^{-1}$). Frequently the decay rate is ignored or neglected regardless of its possible significant effect on the net cell growth rate.

The significance of the decay rate, k_d, is at present not known and because of its small magnitude many investigators apparently choose to neglect it in kinetic analyses. However, from a theoretical and complete analytical standpoint, the decay rate must be included in the materials balance equations. Only by its inclusion and laboratory evaluation of its magnitude will it be possible to understand its role and significance in algal culture systems.

(B) Bottle Bioassay Test

(i) Procedure

The bottle test procedure is a modification of PAAP, 1964. In the bottle test, the test alga Selenastrum capricornutum is added tothe sample and growth is determined by assaying the algal crop on a daily schedule. The immediate test result is a measure of the algal mass at any given time. From this provisional growth rate can be derived. Growth was determined by the following methods: (a) gravimetric and (b) absorbance.

Apparatus

1. Constant temperature incubator: Percival Model I-30-L.
2. Illumination "cool white" fluroscent lighting in incubator to provide an intensity of both 100 ft⁻c (1076 lux) and 400 ft⁻c (4304 lux) at midpoint (from top to bottom) of c culture test vessel.
3. Bausch and Lomb Spectrophotometer Model 20. for use at 600 mμ.
4. Balance with precision of ± o.1 mg.
5. Oven, dry heat with temperature set at 120°C.
6. Millipore filter apparatus for use with 47 mm pre-filter pads and 0.45μ parosity membrane filters.
7. Culture vessels--Erlemmeyer flasks 500 ml; washed only in Fisher FL-70 2% v/v solution.
8. Culture vessel closures 150 ml low form Griffin beakers.

9. Algal Nutrient Medium

Macronutrients - Following salts, Reagent grade, in mg per liter of glass distilled water.

$NaNO_3$	85.00
K_2HPO_4	3.48
$MgCl_2$	19.00
$MgSO_4.7H_2O$	49.00
$CaCl_2.2H_2O$	14.70
Na_2CO_3	50.00
$FeCl_3$	0.32

Note: K_2HPO_4 should be added last to avoid iron precipitation. Stock solutions of individual salts may be made up in 1000 times the final concentration.

Trace Metals - Following salts, Biological or Reagent grade, in micrograms per liter of glass distilled water.

H_3BO_3	618.40
$MnCl_2$	880.88
$ZnCl_2$	109.03
$CoCl_2$	2.60
$CuCl_2$	0.03
$Na_2MoO_4 \cdot 2H_2O$	24.20
$Na_2EDTA \cdot 2H_2O$	7.44 mg/l

Note: The trace metals and EDTA can be combined in a single stock mix in 1000 times the final concentration.

Concentrations of essential constituents* in Algal Nutrient Medium.

N	14.00 mg/l	Ca	4.01 mg/l
P	0.62 mg/l	K	1.56 mg/l
Mg	9.68 mg/l	Fe	0.11 mg/l
S	6.37 mg/l	Mn	0.38 mg/l
B	0.11 mg/l	Zn	0.05 mg/l
Co	1.18 µg/l	Cu	0.01 µg/l
Mo	9.60 µg/l		

*Does not take into consideration N available in Na_2EDTA

Experimental Procedure

The test procedure was stated as soon as possible, but did not exceed two days after collection. Each sample was tested in triplicate, but for statistical purposes the sample was divided into three alequats before filtration and thereafter treated as a separate sample. Sample water was filtered through a 0.45µ porosity membrane filter and added to the test flask.

Cultures, one to three weeks old, of Selenastrum Capricornutum (Printz) were used as a source of inoculum. This culture was obtained from Dr. Tamotsu Shiroyama, Office of Research and Monitoring, National Eutrophication Research Center, Corvallis, Oregon. The stock culture is centrifuged and the supernatant discarded. The sedimented cells (pellet) are resuspended in enough filtered sample water which, when added volumetrically, will result in a concentration of 10^6 cells/ml in the test flask.

Flasks are then incubated under the following conditions:

Temperatures - 24°C ± 1°C

Illumination - continuous "cool white" fluorescent lighting at 400 ft~c (4304 lux).

Shaking - swirled once daily.

Flasks are observed daily:

Gravimetric Measurements - dry weight of algal mass is measured initially and after each 24 hours as follows:

1. Dry glass fiber filters for several hours at 90°C in an oven. The filters were placed in tared aluminum cups upon which the weights and codes may be written.

2. Cool filters in a desiccator containing desiccant.

3. Filter a suitable measured aliquot of the culture under a vacuum of 0.5 atmosphere.

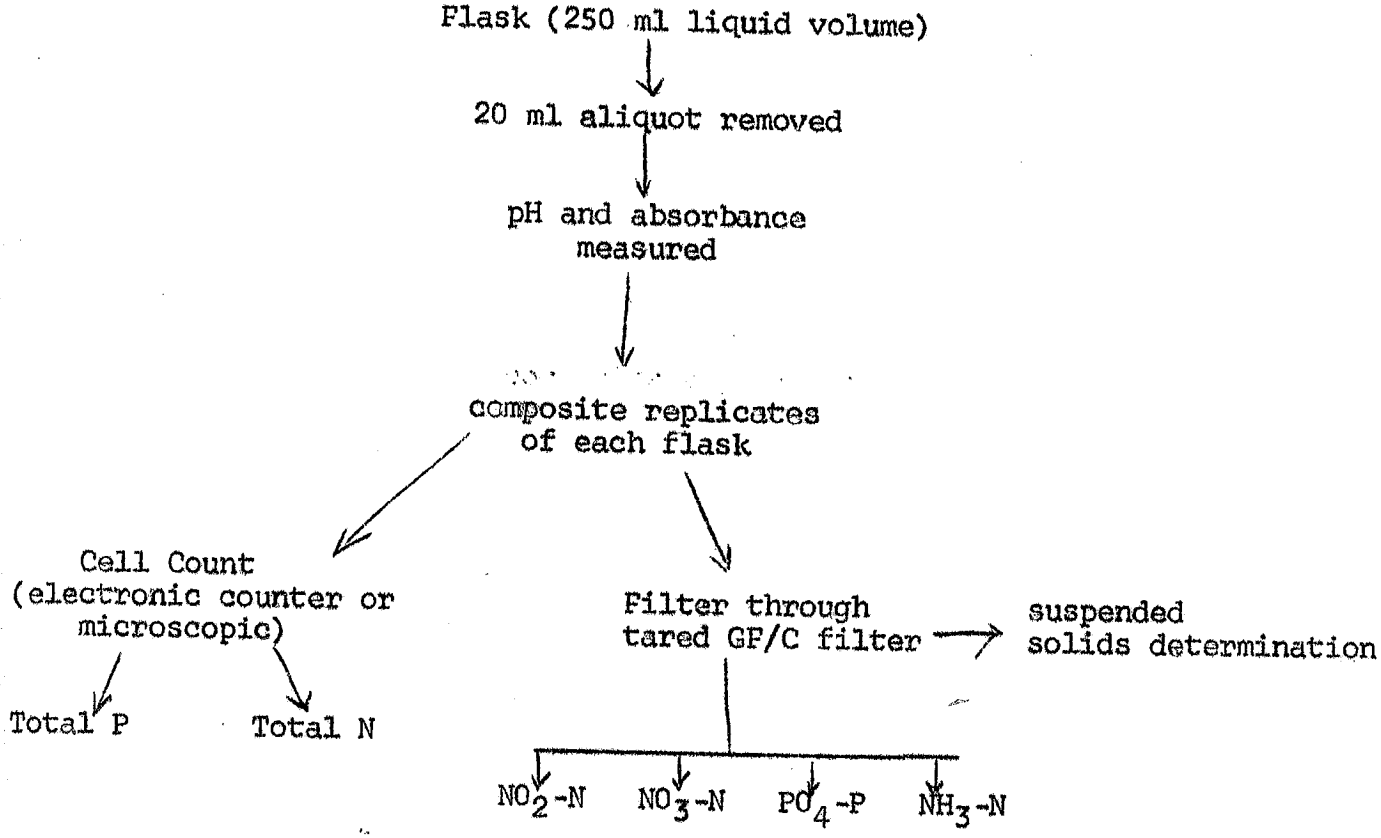
4. Rinse the filter funnel with 50ml distilled water using a wash bottle and allow the rinsings to pass through the filter. This serves to transfer all of the algae to the filter and to wash the nutrient salts from the filter.

5. Dry the filter in a tared aluminum cup at 90°C, cool in desiccator, and weigh.

6. To correct for loss of weight of filters during washing, wash two blank filters with 50 ml of distilled water, pouring it through slowly under reduced vaccum. Dry and weigh filters and record weight loss.

Absorbance Measurements - Absorbance is measured initially and after 24 hours with a B & L "Spec 20."

The daily analysis of each flask follows the outlined scheme:



(ii) Experimental Designs

Stations noted in each experiment correspond to Figure . map.

Bottle Bioassay Experiment #1

Objective: To determine maximum cellular growth in the measured natural waters.

Length of Experiment: 15 days

Test Alga: Selenastrum capricornutum (Printz)

Bottles in replicates of 3 (30 bottles for test)

Analysis: Daily

Natural water sources:

1. 2^{o} effluent from Ripon Sewage Plant (station 18).
2. Silver Creek Water before entrance into sewage plant--bridge off Union Street.

3. Outlet at dam to Puchyan River (station 15).
4. GL station 3-2
5. GL station 5-2
6. GL station 6-2
7. GL station 8-2
8. GL station 9-2
9. GL station 10-2
10. GL station 13-2

Note that these are all surface samples.

Bottle Bioassay Experiment #2

Objective: To determine if N or P limits growth (in terms of cell production) in the samples collected; to determine the biologically available concentration of the nutrients; to determine if the sample is sensitive to changes in the concentration of the limiting nutrient.

ASSUMPTION: that N and P are the limiting nutrients.

Length of Experiment: 13 days.

Test alga: Selenastrum capricornutum (Printz)

Bottles in replicates of 3 (= 69 bottles for test)

Analysis: Biomass--daily
Chemistry--natural water samples (not from bottle)
days 4,8,12

Treatment:	No. Flasks
1. Lake Water Samples	
2^{o} effluent from Ripon Sewage Plant (station 18)	3
Puchyan River (dam outlet in Green Lake)	3
Site 5-2 (deepest point on lake)	3
Quimby Cove (center of cove)	3
Station 13-2 (Silver Creek inlet area)	8

ALL SPIKES BELOW WILL USE WATER FROM SITE 5-2

2. Phosphorus Spikes	3
Lake Water + .005 mg P/1	3
Lake Water + .015 mg P/1	3
Lake Water + .050 mg P/1	3
3. Nitrogen Spikes	
Lake Water + .075 mg N/1	3
Lake Water + .225 mg N/1	3
Lake Water + .750 mg N/1	3
4. Combined Spikes	
Lake Water + .005 mg P/1 + .075 mg N/1	3
Lake Water + .015 mg P/1 + .225 mg N/1	3
Lake Water + .050 mg P/1 + .750 mg N/1	3
5. Growth References--Phosphorus	
(Medium* - P)	3
(Medium - P) + .005 mg P/1	3
(Medium - P) + .015 mg P/1	3
(Medium - P) + .050 mg P/1	3

Bottle Bioassay Experiment #3

Objective: To determine 1) linearity of response and the precision of maximum cell concentration (X) in the water samples, 2) the effect of dilution on the growth rate (ub), 3) the effect and linearity of response of and addition of secondary effluent addition to samples, and 4) the identification of the growth-limiting nutrient in the samples.

Length of experiment: 13 days

Test alga: Selanastrum capricornutum Printz

Bottles in replicates of 3 (= 63 bottles in test)

Analysis: Biomass-daily
Chemistry-1st day complete analysis of water samples
days 4,8,12 analysis of aliquots from assay flasks.

TREATMENTS:	No. Flasks
1. Lake Water samples:	
Site 18-2	3
Site 13-2	3

Site 6A (Quimby Cove Center)	3
Site 9	3
Site 8	3
Site 15	3
Site 4	3
2. Enrichment (Spike) of Site GL 5-2:	
None	3
N[a]	3
P[b]	3
Fe[c]	3
N,P	3
N,Fe	3
P,Fe	3
N,P,Fe	3
N,P,Fe, Micronutrients[d]	3
30% PAAP medium[e]	3
Secondary effluent (inside pipe)[f]	3
Control: 30% PAAP[g]	3
TOTAL	63

[a]Spiked with NaNO3, final concentration after spike 2.5 mg N/l + (N) of sample

[b]Spiked with KH_2PO_4, final concentration after spike 200 ug P/l + (P) of sample

[c]Spiked with $FeCL_3$, final concentration after spike 20 ug Fe/l + (Fe) of sample

[d]Micronutrients spike identical in composition and concentration to that of 30% PAAP medium

[e]Final concentration of nutrients after spike equal to 30% PAAP medium

[f]Secondary effluent from sewage discharge pipe of Ripon Sewage Plant

[g]Control was 30% PAAP medium prepared as usual

(c) Chemostat Bioassay

Procedures used in the chemostat bioassay are found in PAAP, 1969. Modifications made in this study are primarily in the structural design of the chemostat unit.

(i) Procedure

Apparatus/Experimental Procedure

1. Continuous culture apparatus--see Figures 8,9 &10 for details and modifications.

2. Preparation of chemostats--the chemostats are prepared in duplicate, by using the test medium as the as the diluent and adding designated concentrations of nutrients to be tested to the chemostat before inoculation. A feed reservoir of one gallon capacity containing the nutrient medium of specified concentration is maintained in a dark refrigerator at $4^{o}C$ and connected to the chemostat system.

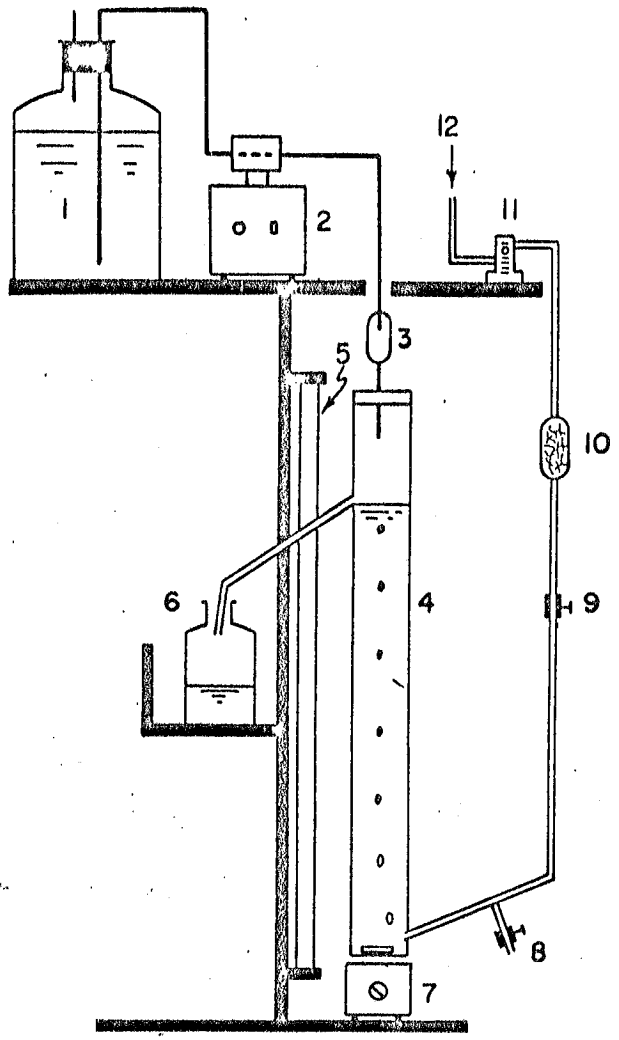
3. Continuous mixing is provided by a magnetic stirring bar and magnetic mixer and an air treatment/distribution system. See Figure 4 for details of air treatment/distribution system. Stirring is such that no vortex is formed at the surface of the water.

4. Individual chemostats were inoculated with 10^6 cells as in the bottle test procedure.

5. Biomass, physical, and chemical measurements were completed in an identical manner to the bottle test procedure.

6. Chemostats were not autoclaved. They were washed with a Fisher FL-70 2% V/V solution and flushed with double distilled deionized water and steam.

7. Effluent volume--the effluent volume from the chemostat was measured daily and if the fluctuation in flow was greater than



1.	FEED BOTTLE	7	MAGNETIC STIRRER
2	POLYSTALTIC PUMP	8	SAMPLING POINT
3	SIPHON BREAK	9	AIR FLOW RATE CONTROL
4	CHEMOSTAT REACTOR	10	COTTON WOOL AIR FILTER
5	FLUORESCENT LIGHT	11	ROTAMETER
6	EFFLUENT BOTTLE	12	AIR - CO_2 INPUT

FIGURE 8. Diagram of Individual Chemostat Assay Unit. (from Toerien et. al., 1971).

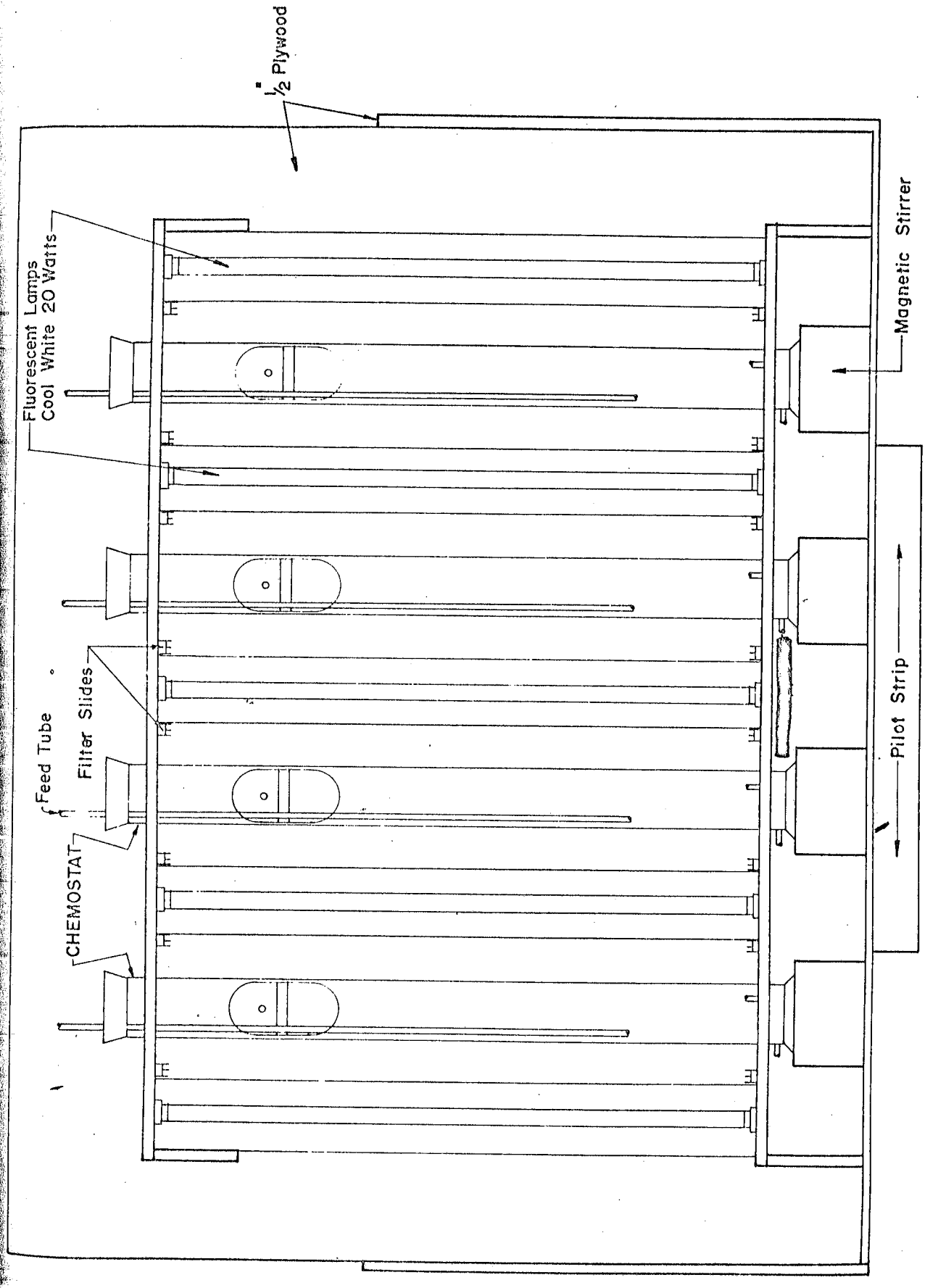
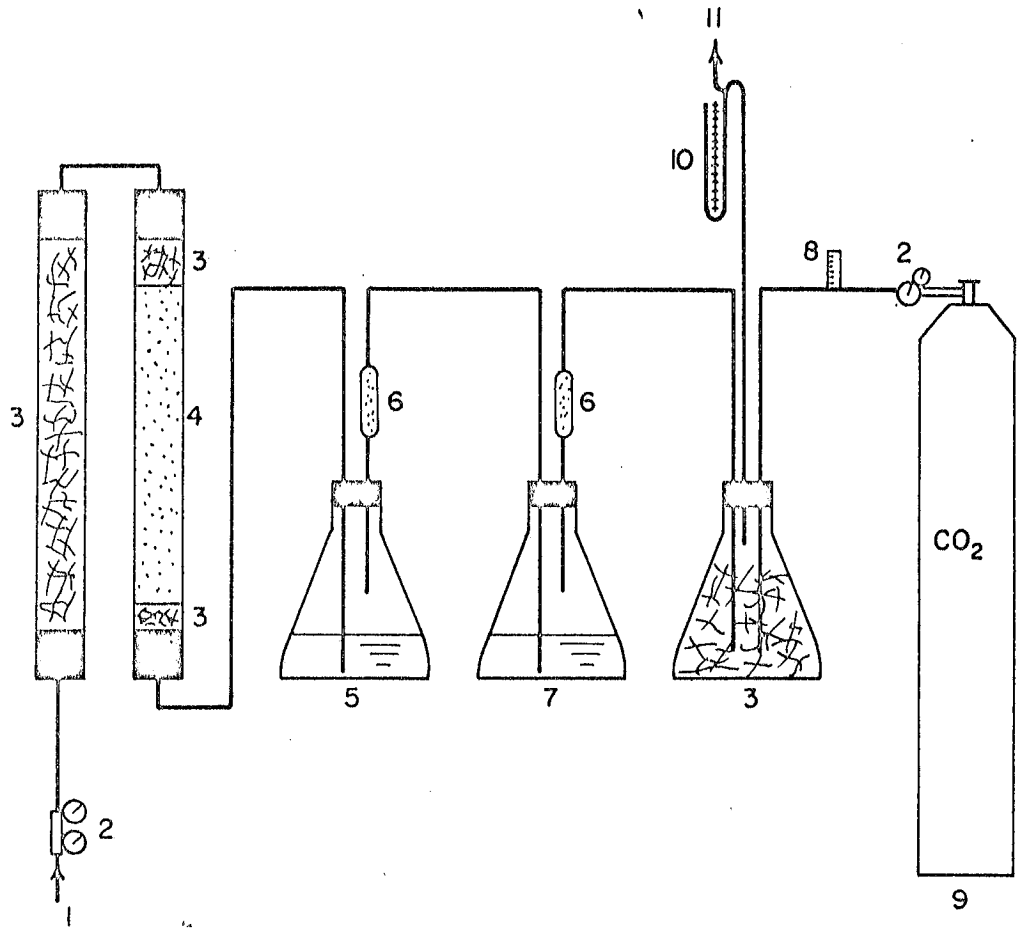


FIGURE 9. Diagram Shwoing Arrangement of A Four-Unit Chemostat System (after PAAP , 1969).



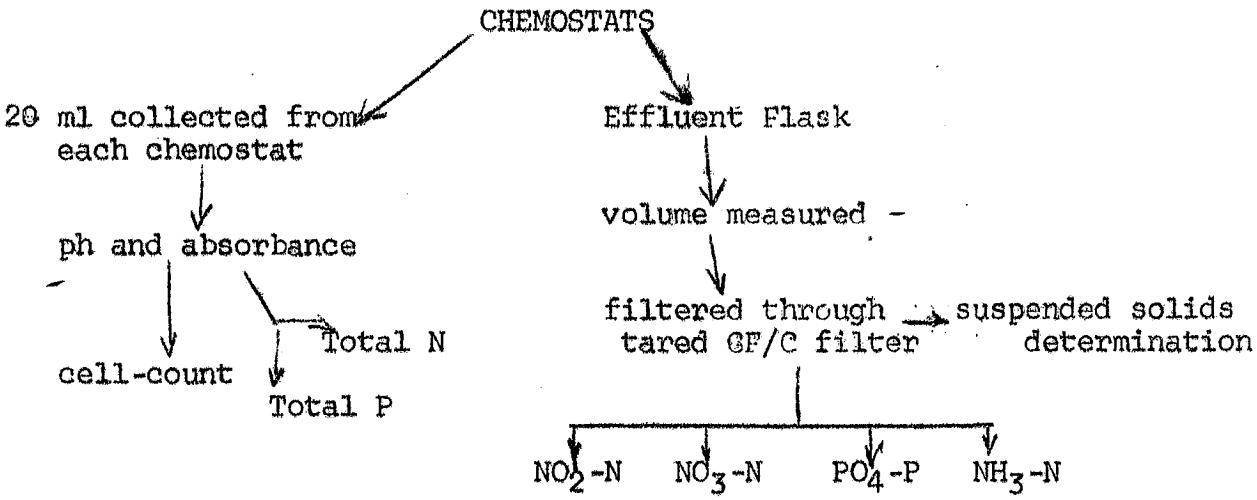
1	AIR SUPPLY	6	GLASS BEAD TRAP
2	REGULATORS	7	DISTILLED H_2O
3	GLASS WOOL	8	ROTAMETER
4	ACTIVATED CARBON	9	CO_2 SUPPLY
5	0.1 N H_2SO_4	10	MANOMETER

11 TO GROWTH UNITS

FIGURE 10. Diagram of Air Treatment and Distribution System for Chemostat (after Toerien et. a., 1971).

10% from the flow rate as determined for the appropriate hydraulic residence time, the flow rate is adjusted.

8. Analysis scheme for chemostat:



(ii) Experimental Designs

Chemostat Bioassay Experiment #1

Objective: To determine linearity of steady state cell concentrations response.

Length of Experiment: 3 hydraulic residents times (about 15 days).

Test alga: Selenastrum capricornutum Printz.

Analysis: daily.

Chemostat Constituents:

1. Control: 30% PAAP medium.
2. 100% Green Lake Water Site 13-2.
3. 50% Green Lake Water Site 13-2.
4. 100% sewage effluent from Wastewater Plant City of Ripon.

Chemical Analyses Schedule: same as 10 samples taken for bioassay bottle test.

Chemostat Bioassay Experiment #2

Objective: To determine the maximum cell concentrations supported in Green Lake and sewage plant effluent; to determine the major nutrients removed from the water during maximum cellular growth.

Length of Experiment: 3 hydraulic residents times (10+ days)

Test alga: Selenastrum capricornutum Printz

Analysis: Biomass-daily

Chemistry 3 times following steady state (after 5-6 days.

Chemostat Constituents:

1. Control: 30% PAAP medium.
2. 100% Green Lake Water Site 13-2.
3. 50% Green Lake Water Site 13-2.
4. 100% sewage effluent from Wastewater Plant City of Ripon.

Chemostat Bioassay Experiment #3

Objective: to identify the growth-limiting nutrient by enrichment (spiking) procedures.

Length of experiment: probably about 15-17 days, dependent upon achievement of steady state conditions (assume about 6 days)

Test alga: Selenastrum capricornutum Printz

Analysis: Biomass-daily
Chemistry-complete analysis at achievement of steady state and at termination of experiments; also complete analysis of water anytime before achievement of steady state.

Chemostate Constituents:
1, 2, 3 will all be Green Lake 5-2 water
4 will be the Control: 30% PAAP medium

Experiment schedule: this assumes 7 days necessary for the achievement of steady state; date selections represent the appropriate spike that is to be added--spikes for each day added after removal of samples for analysis.

Day 8 -- Macronutrients[2]

Day 9 -- Macronutrients

Day 10 -- Macronutrients + Fe

Day 11 -- Macronutrients + Fe

Day 12 -- Macronutrients + Fe + P

Day 13 -- Macronutrients + Fe + P

Day 14 -- Macronutrients + Fe + P + N

Day 15 -- Macronutrients + Fe + P + N

Day 16 -- Macronutrients + Fe + P + N + micronutrients

Day 17 -- Macronutrients + Fe + P + N + micronutrients[f]

[a]Final concentration of spiked nutrients = 30% PAAP medium

[c]Control chemostat operated on 30% PAAP medium; received no enrichment

[e]Macronutrients Mg, Ca, K, S, and Cl

[f]Micronutrients spike identical in composition and concentration to that of 30% PAAP medium.

(D) In-Situ Bioassay.

The in situ procedure is a short-term "Bottle Test" to measure the primary productivity of Green Lake. In this procedure sample water was collected at two sites on Green Lake from various depths, distributed into suitable containers, $NaH^{14}CO_3$ added, and the containers returned to the depth of collections for incubation. Thus, this procedure allows for maintenance of a natural phytoplankton population at the prevailing conditions of light and temperature with dark controls to estimate non-photosynthetic carbon fixation and absorption during the incubation period.

(i) Procedure

Primary productivity was measured using the methods of Schindler and Holmgren (1971). The number of minor modifications necessary in the procedure was great enough to warrant inclusion of the summary field and laboratory procedures.

Field

1. Upon reaching site of measurement complete a reading of light intensity using a light meter--hold light meter one meter above water level.
2. Remove water sample with Van Dorn bottle from prescribed depth; fill two light and two dark bottles, using a tygon tube connected to the Van Dorn bottle, starting at the bottom of the bottle, slowly letting the water rise in the bottle and then letting the bottle overflow for several seconds.
3. Inoculate each bottle with 2μCi(= 0.2 ml) of $NaH^{14}CO_3$ with a hypodermic syringe; deliver the radioactive material as far down in the bottle as possible. "Bag" dark bottles in opaque plastic containers immediately.

4. Incubate in situ for four hours; record time of entrance of bottles in water and removal of bottles from water.
5. Upon removel of bottles from water add 10 ml of 25% formalin to each bottle to stop activity. MAKE ALL BOTTLES LIGHT TIGHT FROM THIS POINT ON.
6. Refrigerate bottles in dark at $4^{o}C$;; remove bottles to lab for analysis.

LABORATORY

1. Place a .2 ml of radioactive source in scintillator vial, add distilled water soaked Millipore filter, and add scintillation cocktail (toluene based PPO + POPOP). This will serve as a measure of complete activity; prepare a second bottle with filtered lake water- this will serve as a measure of background radiation.
2. Record time between end of incubation and filtration.
3. a) Filter a 100 ml aliquot of sample bottle through Millipore filter (o.045μ) at a vacuum of 4-5 inches of mercury onto pre-numbered Millipore filters.
   b) Wash sides of filter with distilled water.
   c) Suck filters dry; then add to scintillation vials with a Millipore forceps, curling the filter so that the number facing inward bears the phytoplankton, and gently lower into scintillation vials--use two forceps for this operation.
   d) Place in dessicator over silica gel for 24-36 hours to dry.
   e) After drying fill vials with toluene based cocktail (PPO + POPOP).
4. Place in scintillation counter.
5. Measure counts per minute for ten replicates; also measure time for a preset number of counts.

(ii) Experimental Design

Weekly measurements of primary productivity in Quimby Cove (Station 6-A) and Slidingrocks (Station 9) were made. The following station depths were used:

Station 6-A:

1 meter

2 meter

Station 9:

1 meter

3 meter

5 meter

10 meter

15 meter

20 meter

III. RESULTS.

(A) Water Analysis

A complete wet of results of the water analyses can be found in Appendix 5. The Table 1 series which follows includes correlations which were considered minimally statistically significant. A complete printout of the correlation program results is found in Appendix 7.

(B) Phytoplankton Enumeration

Data collected on the algal population of Green Lake is presented in Tables 2, 3, and 4. Green Lake has a great variety of algal species with blue-green and green algae being present in almost equal percentages during the summer. Very similar results were noted in summer, 1972 phytoplankton studies (Litton et.al., 1972). Cosmarium, Pediastrum, Scenedesmus, Staurastrum, and Fragilaria were present throughout the summer in relatively stable amounts. The blue green algae, Anabaena, Aphanizomenon, and Gleotrichia, replaced the green algae as the predominant class in late July and early August. Blooms of Anabaena and Scenedesmus were noted on Green Lake during the twelve week period. The Anabaena bloom of June 26 was ubiquitous but dispersed by July 3. Fragilaria and Scenedesmus remained relatively abundant throughout the summer, Fragilaria being concentrated in early June with Scenedesmus having its highest concentration in late July.

(C) Bioassay Experiments

(1) Bottle Test Bioassays

Results of the three Bottle experiments are reported in the following Table 5 series. Effects of selected nutrient additions/deletions to Green Lake water and PAAP medium are shown in Figure 11.

(2) Chemostat Bioassays

Kinetic parameters for the chemostat bioassay are not reported owing to a) insufficient chemostat runs to be statistically valid, and 6) the inability to complete runs at several different hydraulic residence times due to lack of time. However, the data gathered is presented in Figure 12 series and was analyzed visually. Results of these analyses appears in the discussion section.

(3) In-Situ Bioassay

Tables 6 and 7 indicate the primary productivity measurements made during the twelve week study period at Stations 6 and 9 respectively. Productivity at station 6 was appreciably higher (Quimby Cove) than that of station 9 (Sliding Rocks) during the twelve week period. 1 meter measurements at sites 6 and 9 ranged from 0.890-.373 mg $C/M^3hr.$ and from 2.736-6.377 mg $C/M^3hr.$ These represent statistically distinct rates of productivity as measured with a student's t-test (.05 confidence level).

TABLE 1 SERIES

(SERIES OF COMPUTER PRINT-OUT SHEETS)

Table 2. Algae present in Green Lake during Summer, 1973.

Blue-green algae	Unicellular and colonial green algae	Filamentous green algae	Pigmented flagellate algae	Diatoms
Anabaena	Actinastrum	Cladophora	Ceratium	Asterionella
Aphanizomenon	Ankistrodesmus	Microspora	Dinobryon	Fragilaria
Gloeotrichia	Chlorella	Mougeotia	Pandorina	
Microcystis	Closterium	Spirogyra	Volvox	
Nostoc	Cosmarium	Zygnema		
Oscillatoria	Pediastrum			
Rivularia	Scenedesmus			
	Staurastrum			

Table 3. Occurrence of Algal Species in Green Lake, Summer, 1973.

Algal genus	June 13	June 19	June 26	July 3	July 10	July 17	July 25	July 31
Anabena	X	X	X	X	X	X	X	X
Ankistrodesmus	X	X					X	X
Aphanizomenon							X	X
Aphanothece	X							X
Asterionella	X	X	X	X		X	X	X
Ceratium	X	X	X	X	X	X	X	X
Chlorella	X	X	X	X	X	X	X	X
Closterium	X		X	X	X	X	X	X
Cosmarium	X	X	X	X	X	X	X	X
Crucigenia							X	X
Cyclotella	X		X	X	X	X	X	X
Dinobryon		X						
Gonium				X				
Gloetrichia		X	X	X	X			
Fragillaria	X	X	X	X	X	X	X	X
Microcystis	X	X	X			X	X	X
Microspora	X		X					
Mougeotia	X							
Oscillatoria	X							
Pandorina	X	X	X	X	X	X	X	X
Pediastrum	X	X	X	X	X	X	X	X
Scenedesmus	X	X	X	X	X	X	X	X
Spirogyra	X	X	X		X			
Staurastrum	X	X	X	X	X	X	X	X
Volvox	X	X	X	X				
Zygnema	X							

Table 4. Direct counts of algae in Green Lake (Summer, 1973).

Date	Algal genus	Station	Count (organisms/ml)
June 13	Scenedesmus	13	100.8
June 26	Anabena	3	73.6
		5	37.3
		10	368.0
		13	257.6
		15	145.6
	Asterionella	6	73.6
	Scenedesmus	6	106.6
July 3	Anabena	3	14.9
	Pediastrum	13	72.0
	Scenedesmus	6	81.06
		8	211.2
		13	433.6
July 10	Anabena	3	7.46
		9	23.46
	Scenedesmus	6	110.4
		8	286.4
		13	384.0
July 17	Scenedesmus	6	42.66
		8	368.0
		13	572.8
July 25	Scenedesmus	6	121.6
		8	646.4
		13	867.2
July 31	Scenedesmus	6	88.53
		8	646.4
		13	867.2

TABLE 5 SERIES

RESULTS OF BOTTLE BIOASSAY EXPERIMENTS 1, 2, AND 3: DATA, SPECIFIC GROWTH RATES, MAXIMUM CELL PRODUCTION

BOTTLE BIOASSAY # 1

BOTTLE CODE	DESCRIPTIVE CODE	T1	T2	X1*10	X2*10	SPEC.GWTH. RATE(DAYS-1)	MAX.ST. CROP
RSPT1	RIPSEWPLANT	2	12	.02	3.3	.5105946	461
RSPT2	RIPSEWPLANT	2	15	.02	4.9	.4231737	461
RSPT3	RIPSEWPLANT	2	16	.03	4.15	.352119	461
				AVERAGE GROWTH RATE		.4286291	
UNST1	UNIONSTRT	2	15	.03	2.7	.3461392	248.5
UNST2	UNIONSTRT	2	12	.02	2.18	.4691348	248.5
UNST2	UNIONSTRT	2	9	.03	1.68	.5750502	248.5
				AVERAGE GROWTH RATE		.4634414	
GL15T1	GRLKST15	2	7	.02	.11	.3409496	13.7
GL15T2	GRLKST15	2	8	.01	.25	.5364793	13.7
GL15T3	GRLKST15	2	7	.01	.27	.6591674	13.7
				AVERAGE GROWTH RATE		.5121988	
GL32T1	GRLKST3=2	1	5	.01	.12	.6212267	.019
GL32T2	GRLKST3=2	1	5	.01	.09	.5493062	.019
GL32T3	GRLKST3=2	1	5	.01	.07	.4864775	.019
				AVERAGE GROWTH RATE		.5523368	
GL52T1	GRLKST5=2	1	6	.01	.13	.5129099	.19
GL52T2	GRLKST5=2	1	5	.01	.07	.4864775	.19
GL52T3	GRLKST5=2	1	5	.01	.09	.5493062	.19
				AVERAGE GROWTH RATE		.5162579	
GL62T1	GRLKST6=2	1	5	.01	.07	.4864775	.06
GL62T2	GRLKST6=2	1	5	.01	.05	.4023595	.06
GL62T3	GRLKST6=2	1	5	.01	.09	.5493062	.06
				AVERAGE GROWTH RATE		.4793811	
GL82T1	GRLKST8=2	2	7	.01	.11	.4795791	2.12
GL82T2	GRLKST8=2	2	7	.01	.12	.4969813	2.12
GL82T3	GRLKST8=2	2	7	.01	.15	.54161	2.12
				AVERAGE GROWTH RATE		.5060568	
GL92T1	GRLKST9=2	2	8	.01	.15	.4513417	5.02
GL92T2	GRLKST9=2	2	8	.01	.16	.4620981	5.02
GL92T3	GRLKST9=2	2	8	.01	.18	.4817286	5.02
				AVERAGE GROWTH RATE		.4650562	
GL10T1	GRLKST10=2	2	7	.01	.2	.5991465	6.96
GL10T2	GRLKST10=2	2	5	.01	.08	.6931472	6.96
GL10T3	GRLKST10=2	1	5	.01	.15	.6770126	6.96
				AVERAGE GROWTH RATE		.6564354	
GL13T1	GRLKST13=2	4	8	.05	.51	.5805969	36.9
GL13T2	GRLKST13=2	2	9	.01	.5	.5588604	36.9
GL13T3	GRLKST13=2	2	8	.01	.51	.6553043	36.9
				AVERAGE GROWTH RATE		.6982539	

BOTTLE BIOASSAY # 2

BOTTLE CODE	DESCRIPTIVE CODE	T1	T2	X1*10	X2*10	SPEC.GWTH. RATE(DAYS-1)	MAX.ST. CROP
RSPT1	RIPSEWPLANT	1	10	.05	2	.4098755	180.87
RSPT2	RIPSEWPLANT	1	10	.05	1.7	.3916178	180.87
RSPT3	RIPSEWPLANT	1	10	.04	1.25	.3824466	180.87
				AVERAGE GROWTH RATE		.3947133	
PUCT1	PUCHYANOUTLE	1	3	.01	.15	1.354025	2.13
PUTCT2	PUCHYANOUTLE	1	3	.01	.15	1.354025	2.13
PUCT3	PUCHYANOUTLE	1	3	.01	.15	1.354025	2.13
				AVERAGE GROWTH RATE		1.354025	
GL52T1	GRLKST5-2	1	3	.01	.12	1.242453	.19
GL52T2	GRLKST5-2	1	3	.01	.12	1.242453	.19
QCT1	QUIMBY COVE	1	3	.01	.12	1.242453	.19
				AVERAGE GROWTH RATE		1.242453	
QCT2	QUIMBY COVE	1	5	.01	.35	.888837	21.45
QCT3	QUIMBY COVE	1	5	.01	.24	.7945135	21.45
QCT1	QUIMBY COVE	1	5	.01	.12	.6212267	.19
				AVERAGE GROWTH RATE		.7681924	
GL13T1	GRLKST13-2	1	7	.06	.28	.2567408	14.69
GL13T2	GRLKST13-2	1	7	.01	.22	.5151737	14.69
GL13T3	GRLKST13-2	2	8	.02	.25	.4209548	14.69
				AVERAGE GROWTH RATE		.3976231	
005PT1	.005MGP/L	1	3	.01	.12	1.242453	5.02
005PT2	.005MGP/L	1	3	.01	.15	1.354025	5.02
005PT3	.005MGP/L	1	3	.01	.18	1.445186	5.02
				AVERAGE GROWTH RATE		1.347221	
015PT1	.015MGP/L	1	2	.02	.38	2.944439	24.35
015PT2	.015MGP/L	1	3	.08	.22	.5058005	24.35
015PT3	.012MGP/L	2	3	.1	.22	.7884574	24.35
				AVERAGE GROWTH RATE		1.412899	
075NT1	.075MGN/L	5	7	.01	.1	1.151293	2.13
075NT2	.075MGN/L	5	7	.01	.1	1.151293	2.13
075NT3	.075MGN/L	5	6	.01	.15	2.70805	2.13
				AVERAGE GROWTH RATE		1.670212	
225NT1	.225MGN/L	5	12	.01	.15	.3868643	6.96
225NT2	.225MGN/L	5	11	.01	.18	.4817286	6.96
225NT3	.225MGN/L	5	12	.01	.2	.4279618	6.96
				AVERAGE GROWTH RATE		.4321849	
750NT1	.750MGN/L	1	3	.01	.15	1.354025	2.13
750NT2	.750MGN/L	1	3	.01	.15	1.354025	2.13
750NT3	.750MGN/L	1	3	.01	.15	1.354025	2.13
				AVERAGE GROWTH RATE		1.354025	
5PNT1	.005P/.075N	1	9	.1	2.8	.4165256	14.69
5PNT1	.005P/.075N	1	12	.01	.18	.2627611	14.69
5PNT3	.005P/.075N	1	11	.01	.2	.2995732	14.69
				AVERAGE GROWTH RATE		.3262866	
15PNT1	.015P/.225N	2	9	.05	.19	.1907144	5.99
15PNT2	.015P/.225N	2	9	.06	.15	.1308987	5.99
15PNT3	.015P/.225N	2	9	.05	.12	.125067	5.99
				AVERAGE GROWTH RATE		.1488934	
50PNT1	.050P/.75N	1	9	.45	.71	.05700218	60.1
50PNT2	.050P/.75N	2	9	.35	.75	.1088771	60.1
50PNT3	.050P/.75N	2	10	.3	.6	.0866434	60.1
				AVERAGE GROWTH RATE		.08417424	

BOTTLE BIOASSAY # 2

BOTTLE CODE	DESCRIPTIVE CODE	T1	T2	X1*10	X2*10	SPEC. GWTH. RATE(DAYS-1)	MAX. ST. CROP
MD-PT1	PAAP-P	2	3	.01	.15	2.70805	5.02
MD-PT2	PAAP-P	2	3	.01	.18	2.890372	5.02
MD-PT3	PAAP-P	2	3	.01	.18	2.890372	5.02
				AVERAGE GROWTH RATE		2.829598	
MD5PT1	PAAP-P/.005P	2	9	.01	.1	.3289407	6.96
MD5PT2	PAAP-P/.005P	2	9	.03	.09	.1569446	6.96
MD5PT3	PAAP-P/.005P	1	3	.01	.2	1.497866	6.96
				AVERAGE GROWTH RATE		.6612505	
M15PT1	PAAP-P/.015P	1	3	.01	.14	1.319529	6.96
M15PT2	PAAP-P/.015P	1	3	.01	.2	1.497866	6.96
M15PT3	PAAP-P/.015P	1	3	.01	.2	1.497866	6.96
				AVERAGE GROWTH RATE		1.43842	
M50PT1	PAAP-P/.50P	1	11	.01	.8	.4382027	93.91
M50PT2	PAAP-P/.05P	2	12	.05	1.1	.3091042	93.91
M50PT3	PAAP-P/.05P	1	12	.01	.8	.3983661	93.91
				AVERAGE GROWTH RATE		.381891	
MD-NT1	PAAP-N	1	3	.01	.18	1.445186	6.96
MD-NT1	PAAP-N	1	3	.01	.18	1.445186	6.96
MD-NT3	PAAP-N	1	3	.01	.2	1.497866	6.96
				AVERAGE GROWTH RATE		1.462746	
M75NT1	PAAP-N/.075N	1	3	.01	.15	1.354025	5.02
M75NT2	PAAP-N/.075N	1	3	.01	.18	1.445186	5.02
M75NT3	PAAP-N/.075N	2	3	.01	.18	2.890372	5.02
				AVERAGE GROWTH RATE		1.896528	
225NT1	PAAP-N/.225N	1	3	.01	.18	1.445186	5.02
225NT2	PAAP-N/.225N	1	3	.01	.15	1.354025	5.02
225NT3	PAAP-N/.225N	2	3	.01	.18	2.890372	5.02
				AVERAGE GROWTH RATE		1.896528	
750NT1	PAAP-N/.750N	1	4	.01	.21	1.014841	7.92
750NT2	PAAP-N/.750N	1	3	.01	.2	1.497866	7.92
750NT3	PAAP-N/.750N	2	3	.01	.18	2.890372	7.92
				AVERAGE GROWTH RATE		1.801026	
MT1	PAAP	1	12	.01	.28	.3029277	14.69
MT2	PAAP	1	12	.02	.28	.2399143	14.69
MT3	PAAP	1	12	.01	.25	.2926251	14.69
				AVERAGE GROWTH RATE		.278489	
015PT1	.015MGP/L	1	3	.02	.38	1.47222	24.35
015PT2	.015MGP/L	1	3	.08	.22	.5058005	24.35
015PT3	.015MGP/L	2	3	.1	.22	.7884574	24.35
				AVERAGE GROWTH RATE		.9221591	

BOTTLE BIOASSAY # 3

BOTTLE CODE	DESCRIPTIVE CODE	T1	T2	X1*10	X2*10	SPEC.GWTH. RATE(DAYS-1)	MAX.ST. CROP
G182T1	GRLKST18-2	3	11	.02	.83	.4657117	83.28
G182T2	GRLKST18-2	3	12	.02	.96	.4301335	83.28
G182T3	GRLKST18-2	3	12	.02	.99	.4335525	83.28
				AVERAGE GROWTH RATE		.4431326	
G132T1	GRLKST13-2	3	6	.02	.28	.8796858	16.62
G132T2	GRLKST13-2	3	7	.02	.28	.6597643	16.62
G132T2	GRLKST13-2	2	7	.02	.3	.54161	16.62
				AVERAGE GROWTH RATE		.6936867	
QCT1	QUIMBY COVE	2	8	.01	.35	.592558	21.44
QCT2	QUIMBY COVE	2	8	.01	.32	.5776227	21.44
QCT3	QUIMBY COVE	2	8	.01	.32	.5776227	21.44
				AVERAGE GROWTH RATE		.5826011	
G19T1	GRLKST9	1	4	.01	.25	1.072959	11.79
G19T2	GRLKST9	1	4	.02	.25	.8419096	11.79
G19T3	GRLKST9	1	4	.01	.23	1.045165	11.79
				AVERAGE GROWTH RATE		.9866776	
GL8T1	GRLKST8	3	4	.02	.28	2.639057	14.69
GL8T2	GRLKST8	3	4	.01	.28	3.332205	14.69
GL8T3	GRLKST8	3	4	.01	.28	3.332205	14.69
				AVERAGE GROWTH RATE		3.101155	
G15T1	GRLKST15	2	4	.01	.28	1.666102	16.62
G15T2	GRLKST15	2	4	.01	.21	1.522261	16.62
G15T3	GRLKST15	2	4	.01	.21	1.522261	16.62
				AVERAGE GROWTH RATE		1.570208	
G4T1	GRLKST4	2	4	.01	.22	1.545521	8.88
G4T2	GRLKST4	2	4	.03	.21	.9729551	8.88
G4T3	.000GRLKST4	2	4	.01	.21	1.522261	8.88
				AVERAGE GROWTH RATE		1.346913	
NT1	N SPIKE	1	12	.01	1.8	.472087	161.55
NT2	N SPIKE	2	11	.01	1.44	.5522015	161.55
NT3	N SPIKE	2	12	.01	1.28	.485203	161.45
				AVERAGE GROWTH RATE		.5031638	
PT1	P STIKE	2	4	.01	.24	1.589027	10.82
PT2	P SPKIE	2	4	.01	.22	1.545521	10.82
PT3	P SPKIE	2	4	.01	.18	1.445186	10.82
				AVERAGE GROWTH RATE		1.526578	
FET1	FE SPIKE	2	4	.01	.15	1.354025	7.92
FET2	FE SPIKE	2	4	.01	.19	1.47222	7.92
FET3	FE SPIKE	2	4	.01	.21	1.522261	7.92
				AVERAGE GROWTH RATE		1.449502	
NPT1	NPSPIKE	2	12	.01	2.28	.5429346	207.92
NPT2	NPSPIKE	2	12	.01	2.19	.5389072	207.92
NPT3	NPSPIKE	1	11	.01	1.89	.5241747	207.92
				AVERAGE GROWTH RATE		.5353388	
NFET1	NFESPIKE	2	12	.02	1.74	.4465908	155.75
NFET2	NFESPIKE	3	12	.06	1.61	.3655161	155.75
NFET3	NFESPIKE	3	12	.03	1.59	.4411435	155.75
				AVERAGE GROWTH RATE		.4177502	
PFET1	PFESPIKE	2	4	.01	.25	1.609438	16.62
PFET2	PFESPIKE	2	4	.01	.31	1.716994	16.62
PFET3	PFESPIKE	3	4	.02	.3	2.70805	16.62
				AVERAGE GROWTH RATE		2.011494	

BOTTLE BIOASSAY #-3

BOTTLE CODE	DESCRIPTIVE CODE	T1	T2	X1*10	X2*10	SPEC.GWTH. RATE(DAYS-1)	MAX.ST. CROP
FPFETI	NPFESPIKE	2	12	.01	1.78	.5181784	253.33
NPFET2	NPFESPIKE	2	12	.02	2.75	.4923624	253.33
NPFET3	NPFESPIKE	2	12	.05	2.22	.379324	253.33
				AVERAGE GROWTH RATE		.4632882	
NPFTT1	NPFMICSPIKE	2	11	.01	1.59	.5632116	141.26
NPFTT2	NPFMICSPIKE	2	12	.01	1.58	.5062595	141.26
NPFTT3	NPFMICSPIKE	2	11	.01	1.55	.5603806	141.26
				AVERAGE GROWTH RATE		.5432839	
30MT1	30%PAAPSPIKE	2	12	.02	1.22	.4110874	11.3
30MT2	30%PAAPSPIKE	2	12	.01	1.18	.4770685	111.3
30MT3	30%PAAPSPIKE	2	12	.02	1.28	.4158883	111.3
				AVERAGE GROWTH RATE		.4346814	
RSPT1	RIPSEWPLANT	8	12	.01	1.31	1.218799	114.2
RSPT2	RIPSEWPLANT	6	11	.05	1.08	.6145387	114.2
RSPT3	RIPSEWPLANT	8	11	.08	.62	.6825643	114.2
				AVERAGE GROWTH RATE		.8386341	
30MT1	PAAPCONTROL	2	12	.01	1	.460517	94.88
30MT2	PAAPCONTROL	3	12	.01	1.01	.5127912	94.88
30MT3	PAAPCONTROL	3	12	.01	1.11	.5232811	94.88
				AVERAGE GROWTH RATE		.4988631	

TABLE 6. In Situ Bioassay Measurements: Station Six.

Primary Productivity in 10^{-3} mg Carbon/m^3/hour

Date	depth 1 meter	3 meters	5 meters	10 meters	15 meters	20 meters
6/6	0.890	1.753	0.373	0.262	0.054	0.006
6/13	0.346	0.323	0.179	0.593	0.365	0.253
6/19	0.937	0.898	0.272	0.011	0.009	0.130
6/26	1.92	0.212	0.040	0.184	0.057	-----
7/3	0.763	1.690	0.548	0.164	0.386	0.022
7/10	-----	-----	-----	-----	-----	-----
7/17	1.253	0.906	0.134	0.025	0.021	0.041
7/25	3.296	3.627	1.121	0.206	0.033	0.033

TABLE 7. In Situ Bioassay Measurements: Station Nine.

Primary Productivity in 10^{-3} mg Carbon/m^3/hour

Date	depth 1 meter	2 meters
6/6	2.769	0.750
6/13	6.377	2.008
6/19	-----	-----
6/26	2.280	-----
7/3	3.186	1.686
7/10	2.736	1.881
7/17	5.821	3.307
7/25	4.803	0.517

DISCUSSION

(A) WATER ANALYSIS CORRELATIONS

Results of a correlation analysis of all collected water chemistry data, meterological data, and selected phytoplankton data is in Appendix 7. Table 1 indicates those correlations which were considered statistically significant at the level p greater than 1.0. Table 8 is a listing of selected values from Table 1 chosen for their practical significance.

The following points are made concerning the correlations:

1) No consistently significant correlation between a particular station on Green Lake and a chemical species concentration or algal presence was found. Only sporadic instances of increased nutrient concentration and a point source were noted; this notation is difficult to verify and only leads to speculation as to year round nutrient loading at this point.
2) As would be expected, the bloom producers Microcystis and Fragilaria abundance was linked to nitrogen species concentration. The at times ubiquitous Anabaena was not linked to a Nitrogen species concentration. This is expected owing to its documented nitrogen fixing ability.
3) Total nitrogen and total phosphorus concentrations are linked together. P and N species concentrations rise and fall together in Green Lake. This may

TABLE 8. SELECTED WATER ANALYSIS CORRELATIONS

Note: Selected statistics are at p 1.00.
(p of 1 = 32% probability of error;
p of z = 4% probability of error;
p of 3 = 0.2% probability of error).

Parameters	p value
Nitrate Nitrogen vs. Total Nitrogen	1.07
Ammonia Nitrogen vs. Fragilaria	3.09
Ammonia Nitrogen vs. Microcystis	3.09
Total Nitrogen vs. Total Phosphates	1.43
Orthophosphates vs. Fragilaria	1.01
Orthophosphates vs. Microcystics	1.01
Precipitation Day before sampling vs. Orthophosphates	3.00
Precipitation Day before sampling vs. Total Nitrogen	3.08
Sample Depth vs. Ammonia Nitrogen	1.09
Sample Depth vs. Total Phosphates	1.48
[Note: ManyCyclic Times vs. Forms of N and P]	2

indicate that P and N enter the lake from similar or identical points. This points to a series of rather diffuse sources and not a point nutrient source of P or N.

4) Precipitation enhances the measurable concentration of orthophosphates and Total Nitrogen. This extremely positive correlation ($p = 3.08$) supports the concept of diffuse sources of N and P in the watershed entering the lake. Point (on the shoreline or Silver Creek) sources would not be expected to increase contributions of P or N as significantly with precipatation. The ability of increased precipitation, however, to change combined or organic N and P species to measurable/available species remains unclear. Hence, the above interpretation is subject to considerable question.

5) Ammonia Nitrogen and Total Phosphorus concentrations increase with depth. Maintenance of higher concentrations of N or P at lower depths is not unusual (Hutchinson, 1957). While these high concentrations are probably not available for algal growth in the epilimnion during summer months, they do become available in the spring and fall during the periods of complete circulation and, hence, are of some consequence to algal growth.

6) The many significant correlations of cyclic time (yearly time) with species of N and P indicates the seasonal fluctuations noted in these species. These fluctuations are well documented in the literature (Hutchinson, 1957) and are related to biological and physical factors.

(B) PHYTOPLANKTON DISCUSSION.

In contrast to a decidedly eutrophic lake Green Lake has a great variety of algal species. The blue-green and green algae were found in almost equal percentages. According to Wessenberg-Lund (1904), the normal situation in respect to phytoplankton is that green algal dominate in early to mid-summer, while the blue-green algae are concentrated in the late summer. As was noted in last summer's student, this appears to be the case for Green Lake. Unicellular and colonial green algae, such as Ankistrodesmus, Cosmarium, Pediastrum, and Staurastrum, were present throughout the summer in relatively stable amounts, but others including Chlorella and Closterium and filamentous green algae, such as Microspora, Mougeotia, Spirogyra, and Zygnema, dominated from June to July. The blue-greens Anabaena, Aphanizomenon, and Gleotrichia replaced the green algae as the predominant class in late July and early August.

This periodicity can, as in last year's study, be partially explained through the growth responses of the green and blue-green algae in regard to nitrogen and phosphorous species concentrations. Many species of algae, including the green algae, diatoms, flagellates, and blue-green algae, are able to grow in the presence of high concentrations of nitrogen and phosphorus. If there is competition, it is the green algae which tend to become abundant, while the blue-green algae and diatoms remain small in numbers. When the available nitrogen and phosphorus have been reduced by early summer growths, the blue-green algae may then become dominant

(Palmer, 1967). Average phosphate levels for Green Lake followed a pattern of higher concentrations in early June, which decreased by half in late July and early August, thus indicating that possibly such competition had occurred. Also higher water temperatures in the latter part of the summer are more condusive to blue-green algae growth.

On several occasions during this and last summer, certain algal species, specifically Anabaena, Aphanizomenon, Fragilaria, and Scenedesmus, appeared in abundance in Green Lake. These are among the most common species capable of massive growths, and hence are considered bloom producers (Palmer, 1967). Whether or not it is justifiable to consider noted increased growths as blooms is questionable due to the lack of a strict definition of the term Quantitatively. Lackey (1945) defined a bloom as 50 organisms /ml. Counts were taken on the occurrence of increased growths (Table 4), but these are hardly valuable when making comparisons between unicellular green algae and filamentous blue-green algae. In this situation it would be more practical to use a qualitative definition such as Palmer's (1959), who considers a concentrate growth of plankton sufficiently dense to be readily visible as determination of a bloom. Such a situation was observed for Anabaena during both summers on June 26.

No single environmental factor controls the quality and quantity of alga flora, though often times a single factor may be primarily responsible for a sudden change in the nature of its growth. Important among these are temperature, light, turbidity, nitrogen and phosphorus species levels, and pH;

each particular species having individual requirements for optimum growth.

Anabaena, the ordinary form of the "bloom" in deep water lakes (Marsh, 1903), was found in varying degrees of concentration on every sampling date. Its greatest abundance occurred on June 26 when environmental conditions were favorable for such a bloom. Anabaena was a tolerance of high water temperatures, blooms of which appear when the temperature is 14°C and upwards (Hammer, 1964). At each station, which had the greatest concentrations of Anabaena, the temperature ranged between 20-23°C. The nitrogen levels for those same areas ranged between .3-.5 mg/l. Nitrogen levels of less than 1.0 mg/l provide the impetus for blooms of blue-green algae, such as Anabaena (Palmer, 1967). This is due to the fact that Anabaena is able to fix nitrogen and therefore predominate at times when shortages of nitrogen limits other algal growth.

(C) BIOASSAY EXPERIMENTS

(1) Bottle

Results shown in the Table 5 series and highlighted by Figure 11 indicate that the test alga, Selenastrum capricornutum, responded to variations in P and N species in natural and synthetic water supply. Due to lack of sufficient number of replicates a clear estimate of the variance could not be made. However, the following major inferences were made on the basis of the computed specific growth rate and maximum standing crop for each assay:

1. Inoculum from the Ripon Sewage Plant produced the greatest increase in algal biomass in all bottle bioassays (max = 461 mg/l). Chemical analyses of these assays indicates the utilization of both N and P forms available in the water to produce the increased biomass. Inoculum from Silver Creek at Union Street produced the third largest increase in biomass (248.5 mg/l). While the Ripon Sewage Plant effluent is a significant nutrient source, it should be carefully noted that Silver Creek tributary water can support an algal population about one as large as the RSP effluent. This indicates watershed nutrient contributions to the water not attributable to the sewage plant effluent. Therefore the station 18-2 sample's (Silver Creek inlet) ability to support a heightened algal growth is a result of nutrient input from both the sewage pland AND the Silver Creek watershed.

TABLE 9. BOTTLE BIOASSAY MAXIMUM STANDING CROP VALUES ARRANGED IN DECREASING MAGNITUDE

ASSAY DESCRIPTION	MAXIMUM STANDING CROP VALUE (mg/l)
Ripon Sewage Plant	461, 180.87, 114.2
N + P + Fe Spike	253.33
Union Street	248.5
N + P Spike	207.92
N Spike	161.55
N + Fe Spike	155.75
N + P + Fe + Micronutrients Spike	141.26
30% PAAP Medium	111.3
PAAP Control	94.88
(PAAP-P) = .05 P	93.91
GL 18-2	83.28
.05P + .75N Spike	60.1
GL 13-2	36.9, 14.69, 1662
.015 P Spike	24.35
Quimby Cove	21.45, 21.44
P + Fe Spike) PAAP) GL-8)	14.69
GL-15	13.7
GL-9	11.79
P Spike	10.82
GL-4	11.79
(PAAP-N) + .750N) Fe Spike)	7.92
225N Spike) PAAP-P) .005 P Spike) PAAP-N) 10-2)	6.96
GL 9-2) PAAP-P) .005 P Spike) (PAAP-N) + .075N) (PAAP-N) + .225N)	5.02
Puchyan) .075 N Spike) .750 N Spike)	2.13
GL 8-2	2.13
GL 5-2	.19, .19
GL 6-2	.06
GL 3-2	.019

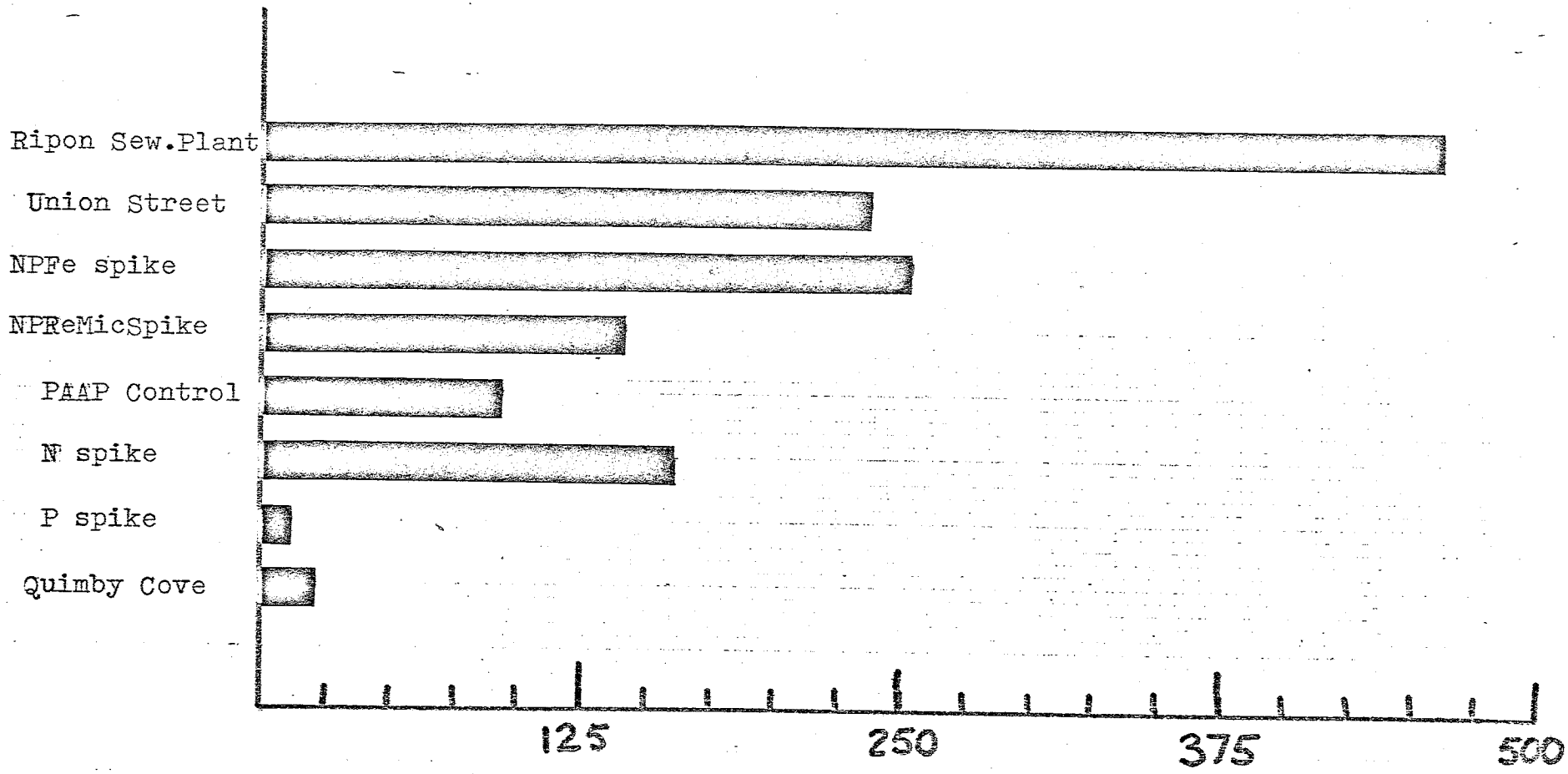


FIGURE 11. Selected Bottle Test Maxium Cell Productions.

2. Algal productivity was enhanced most when a natural water sample was spiked with N, P and Fe (MSC = 253.33 mg/l). Elimination of the Fe from this spike depressed biomass production to MSC = 207.92. The role of Fe is hypothesized as catalytic. Iron probably enhances N and P species transport across the cell membrane. It is not believed to be the critical nutrient in Green Lake's case.
3. Nitrogen spikes of natural water samples produced increased biomass production (MSC = 161.55 mg/l) while P spikes did not appreciably do so (MSC - 10.82 mg/l). In the case of Selenastrum a form of nitrogen is limiting in Green Lake water. Additional input of P and Fe seems to allow for a more efficient utilization of nutrients for biomass productions. Therefore decreased biomass production would require both a decrease in N and P forms.
4. The addition of micronutrients to a P + N + Fe spike resulted in a decrease in biomass production from that of the P + N $\pm$ Fe spike alone. Therefore, no micronutrient can be labeled as limiting in Green Lake. The micronutrient solution's toxicity has been examined in the literature and not found to be a significant problem (PAAP, 1970, 1971).
5. Inoculums of natural water samples from on Green Lake stations, with the exception of Quimby Cove, resulted in very little algal growth. Quimby Cove water, with

its slightly higher concentrations of N and P, produced MSCs of 21.45 mg/l and 21.44 mg/l in two replicates; this value is statistically distinct from the other on lake MSC values.

(2) Chemostat (Continuous Culture)

No kinetic data was calculated from the collected chemostat data owing to its statistical "unreliability" (e.g., insufficient runs to provide valid kinetic factors). However, visual inspection of this data in the Figure 12 series indicates the 1. sustained high biomass production with Ripon Sewage Plant effluent, and 2. the low level response of on lake water samples. Attempts to produce sharp increases in biomass in response to series spikes in the third bioassay were unsuccessful. This non-response might have been eliminated had the spikes been maintained for longer periods (e.g., 2-3 days).

(3) In-Situ (Primary Productivity)

The carbon-14 uptake study completed at two stations on Green Lake indicated two distinctly different rate of productivity. The disparity noted in carbon fixation at site six (Quimby Cove) and site nine (Sliding Rocks) accentuates the same observation in the summer, 1972 (Litton, et.al, 1972). Again it indicates that the large increase in rate in Quimby Cove may be due to prior dredging and subsequent release of available nutrients on an "on-going" basis in this area. Lee, et. al, and Lee (1970) consider the release of orthophosphate and nitrate as from sediment and dredged marshes as nutrient sources for enhanced plant productivity.

FIGURE 12 SERIES.

RESULTS OF CONTINUOUS FLOW

(CHEMOSTAT) EXPERIMENTS.

CHEMOSTAT BIOASSAY EXPT. NO. 1

COLUMN NO. 2 CONSTITUENT: 100% GL site 13-2

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO3-N	NO2-N	NH3-N	total N
1	8.75	.028	2.7						
2	9.10	.002	5.4						
3	8.75	.000	2.4						
4	8.30	.002	3.0						
5	8.40	.000	2.4	.105	.054	1.08	.070	.044	---
6	8.85	.000	2.4						
7	8.65	.002	4.0						
8	8.75	.005	3.3	.037	.017	0.63	.083	.000	1.570
9	8.68	.002	3.7						
10	8.55	.003	0.4						

CHEMOSTAT BIOASSAY EXPT. NO. 1

COLUMN NO. 3 CONSTITUENT: 50% GL site 13-2

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO3-N	NO2-N	NH3-N	total N
1	8.82	.003	4.4						
2	9.08	.000	12.7						
3	8.70	.001	3.7						
4	8.18	.002	3.4						
5	8.75	.002	1.7	.105	.054	1.23	.108	.000	---
6	8.90	.000	2.7						
7	9.00	.000	2.7						
8	8.95	.005	4.0	.052	.025	1.20	.217	.332	1.60
9	8.90	.002	4.0						
10	8.85	.001	0.4						

CHEMOSTAT BIOASSAY EXPT. NO. 1

COLUMN NO. 4 CONSTITUENT: 100% 2^0 effluent RSP

TIME (in days)	pH	absorbance	tot. dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	8.25	.128	3.8						
2	8.35	.038	0.4						
3	7.92	.135	12.0						
4	7.78	.001	7.0						
5	7.70	.001	3.7	3.74	3.99	0.05	0.152	.220	---
6	8.00	.003	4.0						
7	7.90	.010	5.0						
8	7.80	.008	3.0	.780	.661	0.88	0.166	.080	1.60
9	7.70	.008	0.0						
10	7.70	.008	1.7						

CHEMOSTAT BIOASSAY EXPT. NO. 2

COLUMN NO. 1 CONSTITUENT: 30% PAAP - Control

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	7.83	.005	3.7						
2	7.82	.010	4.0						
3	7.77	.032	2.7						
4	7.73	.028	7.4						
5	7.75	.055	5.4						
6	8.02	.061	4.4						
7	8.08	.115	4.4						
8	8.32	.149	8.7	1,123	3.05	1.82	0.319	1.25	6.40
9	8.22	.175	6.4						
10	8.21	.170	9.7			0.18	.000	.000	1.60
11	8.80	.165	8.3						
12	8.64	.210	13.4	2.79	2.82	1.82	---	.000	.550

CHEMOSTAT BIOASSAY EXPT. NO. 2

COLUMN NO. 2 CONSTITUENT: 100% GL site 13-2

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	8.83	.005	3.7						
2	8.57	.015	2.7						
3	8.73	.028	3.0						
4	8.73	.010	5.7						
5	8.63	.022	3.7						
6	8.55	.012	1.7						
7	8.47	.015	11.4						
8	8.46	.010	3.0	.456	.523	0.53	.000	1.12	4.00
9	8.63	.031	0.0						
10	8.38	.005	3.0			0.23	.000	.000	0.120
11	8.60	.008	3.7						
12	8.50	.005	7.0	.110	.187	0.15	---	.000	.064

CHEMOSTAT BIOASSAY EXPT. NO. 2

COLUMN NO. 3 CONSTITUENT: 50% GL site 13-2

TIME (in days)	pH	absorbance	tot.dis solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	8.48	.001	3.7						
2	8.35	.008	4.4						
3	8.47	.025	2.4						
4	8.57	.005	5.0						
5	8.45	.018	4.9						
6	8.40	.005	0.7						
7	8.27	.010	3.4						
8	8.18	.003	2.7	.456	.507	0.12	.000	.047	4.00
9	8.45	.002	0.4						
10	8.19	.000	3.4			0.10	.000	.000	.070
11	8.42	.000	3.4						
12	8.30	.002	8.0	.123	.164	0.13	---	.000	0.110

CHEMOSTAT BIOASSAY EXPT. NO. 2

COLUMN NO. 4 CONSTITUENT: 100% 2°effluent RSP

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	8.88	.018	4.4						
2	8.83	.020	2.7						
3	8.93	.042	5.7						
4	8.97	.040	6.0						
5	8.87	.042	6.4						
6	8.80	.016	2.7						
7	8.60	.022	6.4						
8	8.70	.025	3.4	.484	.804	0.60	0.258	1.60	3.20
9	8.55	.010	0.4						
10	8.70	.045	4.7			0.23	.033	.262	0.430
11	8.50	.045	5.0						
12	8.41	.082	89.7	.216	.247	0.23	---	.008	0.460

CHEMOSTAT BIOASSAY EXPT. NO. 3

COLUMN NO. 1 CONSTITUENT: 30% PAAP

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	4.35	.005	1.7						
2	8.73	.005	1.0						
3	7.85	.002	1.4	.104	.133	4.28	.057	.000	.000
4	7.95	.021	1.4						
5	7.83	.005	2.7						
6	7.90	.001	3.0						
7	7.42	.012	4.4						
8	7.95	.025	3.4						
9	8.15	.040	10.4						
10	8.53	.038	6.0	---	---	2.80	.009	.000	---
11	8.01	.045	5.7						
12	8.37	.035	3.4						
13	---	---	---						
14	7.20	.029	5.0						
15	7.60	.020	4.0						
16	7.12	.015	0.0						
17	7.20	.015	5.4						
18	7.10	.015	9.7						

CHEMOSTAT BIOASSAY EXPT. NO. 3

COLUMN NO. 2 CONSTITUENT: GL Site 5

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	8.49	.005	3.4						
2	8.75	.009	.0						
3	8.83	.005	1.4	.104	.133	0.49	.000	.000	.467
4	8.65	,025	1.4						
5	8.90	.008	1.7						
6	8.50	.000	2.0						
7	8.35	.003	4.4						
8	8.55	.005	3.0						
9	8.43	.002	2.4						
10	8.77	.002	4.7	---	---	.020	.000	.000	---
11	8.30	.006	3.0						
12	8.55	.002	3.4						
13	---	---	---						
14	8.38	.000	3.4						
15	8.00	.002	4.4						
16	8.10	.002	3.4						
17	7.80	.001	3.4						
18	8.00	.001	1.7						

CHEMOSTAT BIOASSAY EXPT. NO. 3

COLUMN NO. 3 CONSTITUENT: GL Site 5

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3-N	NO_2-N	NH_3-N	total N
1	8.63	.005	3.0						
2	8.88	.000	0.4						
3	8.85	.005	2.4	.099	.120	0.45	.000	.000	1.332
4	8.80	.025	2.4						
5	8.85	.000	3.4						
6	8.60	.000	2.4						
7	8.57	.001	3.0						
8	8.57	.001	.8						
9	8.50	.000	2.0						
10	8.75	.005	4.0	---	---	0.18	.000	.000	---
11	8.49	.005	.0						
12	8.67	.000	3.4						
13	---	---	---						
14	8.40	.001	3.0						
15	8.25	.000	8.4						
16	8.30	.001	3.0						
17	8.10	.002	4.0						
18	8.05	.001	1.7						

CHEMOSTAT BIOASSAY EXPT. NO. 3

COLUMN NO. 4 CONSTITUENT: CL Site 5

TIME (in days)	pH	absorbance	tot.dis. solids	PO_4-P	total P	NO_3ON	NO_2-N	NH_3-N	total N
1	8.63	.009	4.7						
2	8.65	.008	.00						
3	8.60	.010	2.4	.160	.205	0.39	.000	1.382	1.332
4	8.71	.029	1.7						
5	8.67	.000	3.4						
6	8.45	.001	2.4						
7	8.48	.008	3.4						
8	8.53	.008	2.7						
9	8.37	.005	2.7						
10	8.85	.005	3.4	---	---	.000	.000	.000	---
11	8.55	.009	3.4						
12	8.53	.005	3.4						
13	---	---	---						
14	8.28	.002	3.4						
15	8.10	.001	4.7						
16	8.30	.005	3.0						
17	8.05	.002	3.0						
18	8.20	.001	3.0						

RECOMMENDATIONS

The discussion indicated that forms nitrogen and phosphorus have stimulatory effects on algal growth in Green Lake. Bottle bioassay information points to nitrogen as the critical nutrient and phosphorus as enhancing nitrogen's effect. Therefore we recommend the following in an effort to limit the impact of these nutrients on Green Lake plant productivity.

While the obvious source of phosphorus for Green Lake is the Ripon Sewage Plant, several other urban and rural sources exist. Based on present P and N concentrations in Green Lake at the present time, these sources must also be controlled to maintain and/or decrease plant productivity. Unfortunately, the other sources of phosphorus for Green Lake are diffuse sources which are not as readily controllable as domestic wastewaters. In order to further reduce the frequency and severity of excessive algae growth in Green Lake it will be necessary for each individual living in the Green Lake watershed to conduct his activities in such a manner as to reduce and, where possible, eliminate the transport of phosphorus to the lake.

The following recommendations, based on SOS research, references from scientific literature, and research completed at the University of Wisconsin, would reduce the phosphorus input to the lake (recommendations directly from Lee, 1970):

1) All fertilization of lawns and gardens by rural and urban homeowners should be done in such a manner as to apply only the minimum amounts of fertilizer needed to maintain a healthy lawn. All fertilizer application should be made directly to the lawn

and care should be exercised to prevent fertilizers from being applied to sidewalks, driveways and other paved areas. No fertilizer application should be practiced in the very late fall, the winter, or very early spring; fertilizer application should be made only during that time of the year when there is at least moderate growth of the grass. Application of fertilizers in late fall when the grass has turned brown or in the early spring before it has turned green will result in a large portion of the fertilizer being washed into a nearby drainage ditch or urban storm sewer, eventually carrying the fertilizer into one of Madison's lakes. Under no circumstances should fertilizers be applied in the winter when the ground is still frozen.

2) All grass clippings, leaves and other debris normally raked up in the yard should be worked into the soil of one's own property or placed in impervious containers such as metal trash cans, plastic bags, etc., to be collected by the city or municipal trash collection service. Under no circumstances should the leaves, clippings or other debris be piled in the streets, gutters or at some other location in the yard where water could run through the pile and reach a nearby street or drainage ditch.

Every effort must be made to handle leaves and other normal yard debris in such a manner as to keep rain water and water from yard and lawn irrigation from leeching the phosphorus from these materials and carrying the phosphorus to the lakes. The practices of burning leaves in the gutter should not be allowed.

3) All use of water for home and rural irrigation should be done in a manner as to prevent any runoff from this water to the streets or any other drainage courses. Application of water to a

lawn at a rate which is faster than the rate it can be soaked into the lawn will result in the transport of phosphorus to a nearby street and eventually to the lakes. This means that the watering of a lawn on the side of a hill has to be done at a considerably slower rate than a flat lawn in order to avoid run-off from the lawn.

4) Steps should be taken immediately to prevent anyone from spreading manure on land in Green Lake's drainage basin during the period of the year when the ground is frozen. Current efforts to find a satisfactory method for storage of manure over the winter period should be greatly accelerated. This storage must be conducted in a manner which does not lead to ground water contamination with nitrogen compounds and to highly offensive odors in the spring.

5) All fertilizer application in the Green Lake watershed for agricultural purposes should be given very close scrutiny in order to ascertain if any of the common fertilizing processes tend to increase the runoff of phosphorus from the land to the streams/creeks tributary to Green Lake. Particular attention should be given to any fertilizer application which is not immediately worked into the soil, especially during the fall of the year.

6) The residents of the Green Lake watershed should work toward seeing that all new high density urban developments within this area utilize a central waste water sewage system where the wastewaters are transported via pipe to one or two central wastewater treatment plants. These treatment plants should provide adequate wastewater treatment to maintain at least existing water

quality in the receiving waters of the wastewater effluent. High density urban developments utilizing septic tank disposal systems should be discouraged.

Since further urban development of the type conventionally found today and further intensified agricultural practices, especially those involving the housing of large numbers of animals, in the vicinity will cause water quality deterioration in Green Lake, all new developments of these types should be required to be developed with the latest techniques available to minimize the transport of phosphorus from the development or farm to Green Lake. Serious consideration should be given to the installation and use of holding tanks instead of present planned septic tank/leeching fields in all lake front residences located on soil considered severely limited (refer to map prepared by USDA Soil Service-Green Lake County). The tanks could be emptied periodically by a tank cleaning operator and the waste transported by truck to a municipal treatment system.

7) Often, after storms, large amounts of debris will accumulate along the shoreline. This debris will consist of algae and rooted aquatic plants which have been torn loose by the wind and waves, dead fish and other trash. The governmental units responsible for the shoreline of Green Lake should provide sufficient funds to have one or more crews of individuals who will collect all of this debris within a few days after accumulation. Such a pick-up would help remove some nutrients and greatly increase the utility and aesthetic quality.

8) The various governmental units responsible for control of land use within the Green Lake watershed should appropriate suf-

ficient funds to provide for a long-term monitoring program on groundwater and surface water quality in the county. In general, at the present time, there is inadequate data to assess the changes that have taken place in the waters of the county over the years. It is impossible to determine with any degree of reliability how various events in the county have affected the lakes. This is due to the fact that there has been no systematic monitoring of water quality in the lakes. A carefully designed and executed monitoring program on Green Lake would provide the types of data necessary to assess further deterioration in the lake ahead of the time that this would generally be noticeable to the public. Such information could be used effectively to initiate corrective steps.

9) Since the drainage of marshes can result in a potentially significant release of nitrogen and especially phosphorus, further drainage of marshes in the watershed should be minimized and, if possible, prohibited.

10) Efforts should be made to greatly reduce the amount of erosion of soils that occurs in the watershed which will eventually reach Green Lake. Particular attention should be given to erosion control in new urban developments and in various types of agricultural activities. While the soil and sand particles transported to the lake generally do not add large amounts of nutrients to the lake, these materials tend to accumulate along the shore near their point of entry thereby causing a reduction in water depth. This reduction tends to promote the growth of aquatic weeds and attached algae. Such growths interfere with swimming, boating, fishing and other recreational uses.

11) Residents of the watershed should do everything reasonably possible to support the harvesting of excessive growths of aquatic weeds in those areas of Green Lake where the weeds would interfere with the recreational activities of the public. Excessive weed harvesting should not be practiced since some weeds are necessary for fisheries' habitat and also the growth of weeds tends to minimize the growth of algae due to competition for available nutrients.

The residents of the watershed, and particularly the shoreline residents, should work toward an effective means of translating these recommendations and technical information generated by the SOS team into meaningful action programs designed to at least maintain and, where possible, enhance Green Lake's water quality.

ACKNOWLEDGEMENTS

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Executive Board and membership, Green Lake Property Owners' Association.

Green Lake Sanitary District

Mr. Jack Mason, Bureau of Research, Department of Natural Resources.

Dr. Jonathon A. Winter, Chief, Analytical Control Laboratory, Environmental Protection Agency, Cincinnati, Ohio.

Dr. Tamotsu Shiroyama, Office of Research and Monitoring, Natural Eutrophication Research Center, Corvallis, Oregon, Environmental Protection Agency.

United States Department of Agriculture-Soil Service, Green Lake, Wisconsin.

Dr. Paul Uttormark, Eutrophication Research Program, University of Wisconsin-Madison.

Dr. Arthur D. Hasler, Laboratory of Limnology, University of Wisconsin-Madison.

Dr. G. Fred Lee, Water Chemistry Program, University of Wisconsin-Madison.

Mr. Mark Conrad, Mayor, and Mr. Claude Lee, Director of Public Works, City of Ripon, Wisconsin.

Miss Mary Jane Bumby, Green Lake, Wisconsin.

Special acknowledgement and thanks is made to Dr. William Bowen and Dr. Earle Scott, project advisors, and to the members of the faculties of the Biology and Chemistry Departments, Ripon College, for their assistance prior to, during, and after the summer project.

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APPENDIX 1
PROJECT PARTICIPANTS

Name	Academic Class/ Major	Segment of Project Participated In
GUBBINS, Earl	Junior Biology & Chemistry	construction and operation of chemostat congrols; construction of electronic particle counter; computer operations; electronic instruments maintenance.
JAEGER, Dorothy	Senior Biology & Chemistry	Analytical Chemistry Lab
KOBRIGER, Kim	Junior, Biology (Non-Stipend)	graphical analysis of collected chemical data.
LITTON, James	Junior Biology & Bio-Chem.	Student Project Director; assisted in primary productivity measurements, chemostat construction.
MARINAC, Patricia	Senior, Biology (Lawrence Univ.)	Phytoplankton enumeration and field collections; scintillation counting.
MILIKOWSKI, Martha	Junior, Biology (Non-Stipend)	Bottle & chemostat bioassay measurements, Analytical Chemistry, reprint collection.
MIZEN, Michael	Senior, Biology	Construction & operation of chemostat, culture of algae and preparation media, field collections, Analytical Chemistry.
NAKAGAWA, Alan	Junior, Chemistry (Grinnell College)	Analytical Chemistry Lab, trace metals analysis.
SMITH, Christopher	Junior, Biology & Psycho-Bio.	directed all field operations, scintillation counting, photography.
SNYDER, Michael	Junior, Physics & Mathematics	construction of electronic particle counter, computer operations, electronic instrument maintenance.

APPENDIX 2

PLANNED PUBLICATIONS

The following papers will be submitted to the Wisconsin Academy of Science, Arts, and Letters and/or another scientific journal for possible publication:

I. "Measurements of Primary Productivity in Green Lake, Green Lake County, Wisconsin."

II. Evaluation of Algal Productivity in Green Lake, Wisconsin with Bioassay Techniques.

These papers will be updated and revised on the basis of this study:

I. "Limnological Measurements of Green Lake, Green Lake County, Wisconsin during Summer, 1972" (entire 1972 SOS team).

II. "Quantitative Analysis of Macro-Benthic Invertebrates in Green Lake, Wisconsin" (Robert Andritsch, Mark Moffler).

APPENDIX 3

RIPON COLLEGE

RIPON, WISCONSIN 54971

STUDENT PROJECT DIRECTOR'S EVALUATION

The problem of cultural eutrophication is and will continue to be of growing public concern. It is scrutinized increasingly not only by the scientist but by an environmentally aware public and government. There are many facets to the problem, only one of which is the environmental requirements of algae. This problem area, however, cries out for research attention. This project was initiated to provide more information on the specific factors related to algal bloom production in Green Lake, Wisconsin. Without specific information on algal growth it is doubtful whether the tools of ecological control now available can give any relief from the ravages of algal blooms.

While the study fell short of its original goal of providing definitive information on the critical (limiting) algal nutrients and their specific concentrations, it did gather sufficient information to give a direction to corrective measures. Taken as a whole the recommendations of the study, if implemented, will provide for a sound water quality management program. Too little is completely understood in lake ecosystem dynamics to truly have definitive answers at this point in time.

As with the previous summer's SOS study of Green Lake the team experienced many of the "technique" difficulties associated with such a massive research effort. It was both encouraging and puzzling to utilize some of the latest research methods available--the Provisional Algal Assay Procedure (PAAP). It provided an opportunity to make modifications and question the rationale of our techniques. Many of these problems were overcome with the willing and able assistance of Dr. William Bowen and Dr. Earle Scott, and members of the Biology and Chemistry department faculty. Special assistance was also provided by the Environmental Protection Agency, Corvalles, Oregon. I should also mention many problems were solved owing to the flexibility and organization of the Student Originated Studies program. The student projector and many team participants laud the National Science Foundation staff for the opportunities and experiences made possible via the Student Originated Studies program. We sincerely recommend its continuance and strengthening in years to come.

James R. Litton Jr.

January 10, 1974

James R. Litton, Jr.
Student Project Director

APPENDIX 4

RIPON COLLEGE

RIPON, WISCONSIN 54971

Biology Department
1 November 1973

FACULTY ADVISOR'S REPORT

In a continuation of a previous (1972) SOS effort, the 1973 SOS project at Ripon College was devoted to an evaluation of the potential for the water quality of Green Lake to under further deterioration in the future. Such an effort at "prediction" obviously is very difficult, especially in light of our poor understanding of nutrient interaction in causing algal growth in lakes.

The SOS team's efforts in this avenue of research are to be commended. They did not lose sight of their original objectives in spite of a series of unforeseen problems at the very onset of their work; the problems included late or non-delivery of supplies such as electronic parts and an initial indifference of a physical plant to certain aspects of the project. Moreover, the probability of accomplishing all of the original objectives in just one summer were indeed slight when one realizes that years of research has gone into the development of our limited knowledge regarding the interaction of nutrients and algal growth. Thus, in final analysis, the SOS team did not obtain all of the results that comprised their original goal but it must be emphasized that this failing, if indeed it is that, cannot be judged the fault of either the SOS program, the project, or the team. Without question, the members of this student group have gained new insight into the "trials and tribulations" of research. The end result is, of course, a successful learning experience. Again, as a student endeavour, this project was exceptional in its planning, execution and management.

There is yet another aspect to the SOS work at Ripon College. The Green Lake Property Owner's Association (GLPOA) has long been concerned about the ecological status of Green Lake. Their concern now, thanks to the efforts of these students, has turned to action! The GLPOA now only supported a continuation of the initial SOS efforts through the rest of the academic year, but is now in the process of financing a comprehensive land-use study as a guide to, and hopefully the control of, the future development of Green Lake.

William R. Bowen
Faculty Advisor

APPENDIX 5: Water Chemistry Data

The following pages contain water chemistry data collected by the SOS team from June, 1972 through July, 1973. Data for each parameter has been grouped together in blocks. The following code for dates and points is used in the following computer "print-outs":

Date	Computer Code Week	Green Lake Station	Computer Code Point
6 - 5 - 72	1	1-G- 2	1
6 - 12 - 72	2	2-G- 2	2
6 - 19 - 72	3	2-G-18	3
6 - 26 - 72	4	2-G-36	4
7 - 3 - 72	5	2-G-54	5
7 - 10 - 72	6	3-G- 2	6
7 - 17 - 72	7	3-G-21	7
7 - 24 - 72	8	3-G-42	8
7 - 31 - 72	9	4-G- 2	9
8 - 7 - 72	10	5-G- 2	10
9 - 22 - 72	11	5-G-18	11
10 - 27 - 72	12	5-G-36	12
12 - 1 - 72	13	5-G-55	13
1 - 19 - 73	14	5-G-70	14
2 - 16 - 73	15	6-G- 2	15
3 - 9 - 73	16	7-G- 2	16
4 - 6 - 73	17	8-G- 2	17
5 - 9 - 73	18	9-G- 2	18
5 - 31 - 73	19	10-G- 2	19

Date	Computer Code Week	Green Lake Station	Computer Code Point
6 - 6 - 73	20	10-G-18	20
6 - 13 - 73	21	10-G-36	21
6 - 19 - 73	22	11-G- 2	22
6 - 26 - 73	23	12-G- 2	23
7 - 3 - 73	24	13-G- 2	24
7 - 10 - 73	25	14-G- 5	25
7 - 17 - 73	26	15-G- 5	26
7 - 25 - 73	27	16-G- 5	27
7 - 31 - 73	28	17-G-5	28
		18-G- 5	29
		1-W- 1	30
		1-W- 6	31
		1-W-12	32
		1-W-18	33
		SC-1	34
		SC-2	35
		SC-3	36
		SC-4	37
		SC-5	38
		SC-6	39