January 1975

Biostimulation and Nutrient Assessment

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BIOSTIMULATION AND NUTRIENT ASSESSMENT

Proceedings of a
Workshop held at Utah State University
Logan, Utah
September 10-12, 1975

Edited by

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Sponsored by the

Eutrophication and Lake Restoration Branch
Pacific N.W. Environmental Research Laboratory
U.S. Environmental Protection Agency
Corvallis, Oregon

and the

Division of Environmental Engineering
and
Utah Water Research Laboratory
Utah State University
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November 1975 **PRWG168-1**

Acknowledgments

This publication and the Workshop were partially supported by Grant Number R-903523 01 received from the Water Pollution Control Research, Development, and Improvement Program of the U.S. Environmental Protection Agency, and the Eutrophication and Lake Restoration Branch, Pacific N.W. Environmental Research Laboratory, EPA, Corvallis, Oregon.

The great expenditure of time and effort made by the Workshop participants and authors of the papers is greatfully acknowledged. Without such willingness to share knowledge and experiences, meetings such as this would be impossible.

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Introduction

In June, 1969, our research program co-sponsored a workshop with the Sanitary Engineering Laboratory of the University of California at Berkeley entitled "Eutrophication-Biostimulation Assessment," in which some of you participated. From the experience of that conference we have determined that this type of a meeting is more productive with fewer attendees so that everyone has the opportunity of participating, and so that topics under discussion are more specific. During this workshop we will concentrate on biostimulation and nutrient assessment. We hope this will be a most productive endeavor and that you will actively participate.

A meeting of this nature requires a great deal of "behind the scenes" effort in order to ensure its success. In addition to Professor Middlebrooks, who has been responsible for arranging the meeting, special acknowledgment is due to Kathy Bayn, Vanette Durtschi, and the Utah Water Research Laboratory staff of Utah State University.

Thomas E. Maloney, Chief
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Application of the Algal Assay Procedure in Biostimulation and Toxicity Testing

A. G. Payne*

INTRODUCTION

Our research in Procter & Gamble has demonstrated, over the past several years, the versatility of the Algal Assay Procedure (AAP): Bottle Test (U.S. EPA, 1971) in algal stimulation studies in natural waters. The sensitivity of this algal assay to low levels of phosphorus (1-2 μg P/1) in highly oligotrophic waters compares favorably with the sensitivity of chemical analyses for phosphorus (Payne, 1975). The AAP has been applied successfully to the assessment of possible eutrophication effects of detergent candidate materials such as trisodium nitrilotriacetate (NTA) (Sturm and Payne, 1973) and sodium citrate (Payne, 1973).

In a number of studies, the AAP has been used to assess the effects of nutrient enrichment of natural waters with sewage effluent (Payne, 1975; Sturm and Payne, 1973). Studies with the AAP have demonstrated that nutrient removal by tertiary treatment greatly reduces the stimulatory properties of sewage. Growth of test algae in the presence of tertiary sewage was comparable to growth in control lake waters without added sewage.

The AAP also has been shown to be useful in the determination of growth-limiting nutrients and in the comparison of the algal growth potential of natural waters to that of defined media (Payne, 1975).

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CORRELATION WITH PHYSICAL/CHEMICAL MEASUREMENTS

More recent studies have demonstrated the usefulness of the AAP to increase our knowledge of lake conditions when used in conjunction with other measurements. Hypereutrophic Sylvan Lake (630 acres) is located in north-east Indiana in Noble County and for many years has received municipal sewage from the town of Kendallville (population, 6,675), approximately 4 miles upstream, and septic tank drainage from hundreds of cottages at Rome City which is located along the shoreline of the lake. Laboratory algal assays with *Selenastrum capricornutum* on a December 1974 sample of Sylvan Lake water indicated a high algal growth potential of the lake for the coming growing season (see Figure 1). The oxygen profile measured in May 1975 confirmed the predicted eutrophic state by showing supersaturation of oxygen in the epilimnion and oxygen depletion in the hypolimnion due to readily apparent excessive algal growth.

A similar situation exists in eutrophic Long Lake in Steuben County, Indiana. This 92 acre lake receives sewage discharge from the town of Angola (population 4,725) approximately 4 miles up stream, and, in addition, receives drainage from marshes and agricultural muck land. Again, the December 1974 algal assays of epilimnetic waters indicate a high algal growth potential, and the May 1975 oxygen profile confirms a depletion of oxygen below the thermocline in the lower 5 meters of this lake which has a maximum depth of only 9 meters (Figure 2).

Lake Wawasee, on the other hand, has fairly good water quality for Indiana lakes which range, generally, from mesotrophic to eutrophic. Wawasee, located in Kosciusko County, covers 3,060 acres, and is Indiana's largest lake. This mesotrophic lake receives no municipal sewage, but is ringed by hundreds of homes with septic tanks. The assimilative capacity of this large lake apparently has been sufficient to absorb these loadings without undue visible deterioration. Last December's algal assays showed the upper waters to be both nutrient and chelator-limited, and this spring the oxygen profile was that of a reasonably healthy lake with approximately 5 mg/l dissolved oxygen at the bottom of the 23 meter water column (Figure 3).

In Figure 4, December 1974 phosphorus levels (as total and soluble ortho-P) and algal assays for a number of Indiana lakes are compared with May 1975 measurements of chlorophyll *a*, dry weight, and Secchi readings. These lakes range in water quality from mesotrophic (Wawasee) to hypereutrophic (Sylvan). The algal growth potential of these waters as determined by winter algal assays is a reasonably good prediction of the algal biomass found in these waters the following spring.

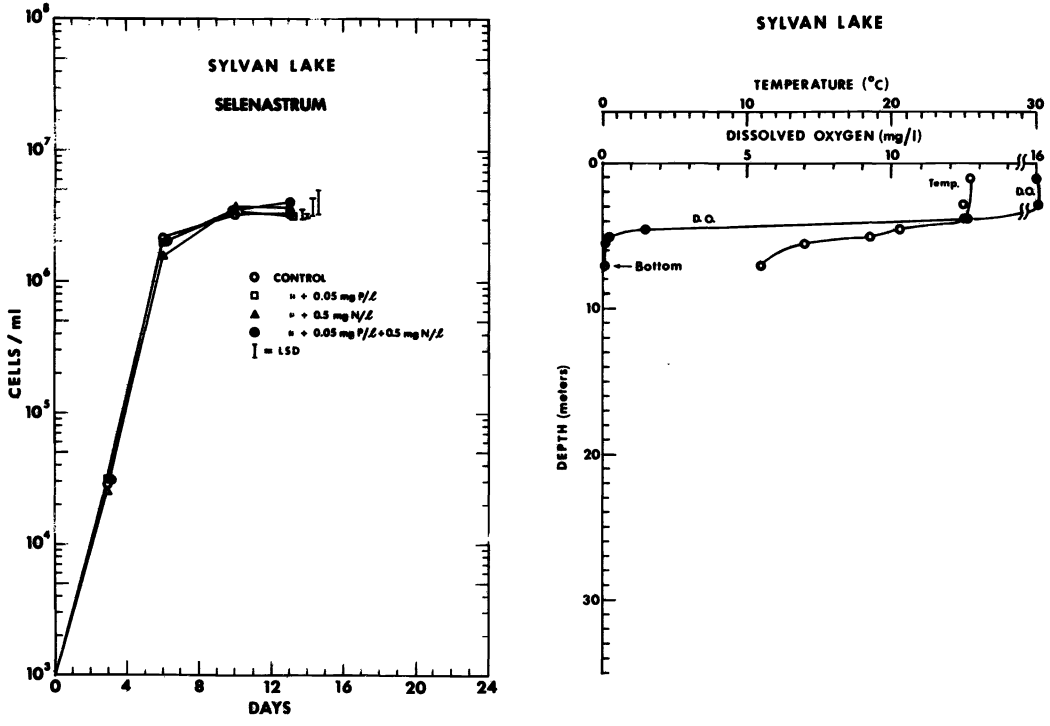

Figure 1. December 1974 algal assays and May 1975 dissolved oxygen and temperature profiles of Sylvan Lake water, Indiana.

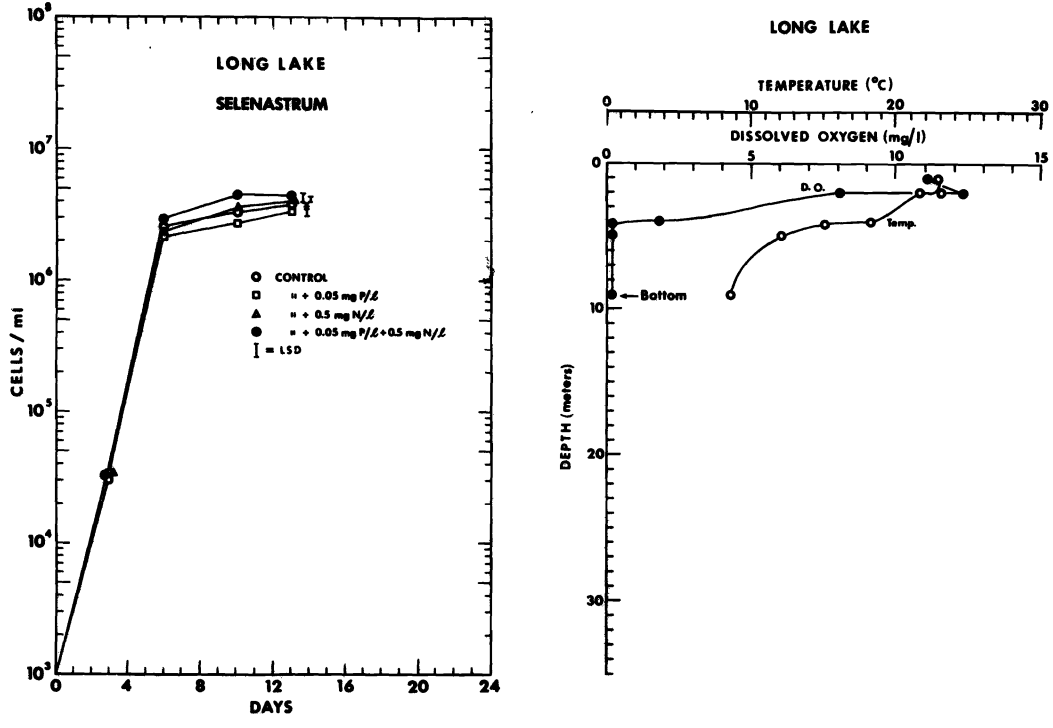

Figure 2. December 1974 algal assays and May 1975 dissolved oxygen and temperature profiles of Long Lake water, Indiana.

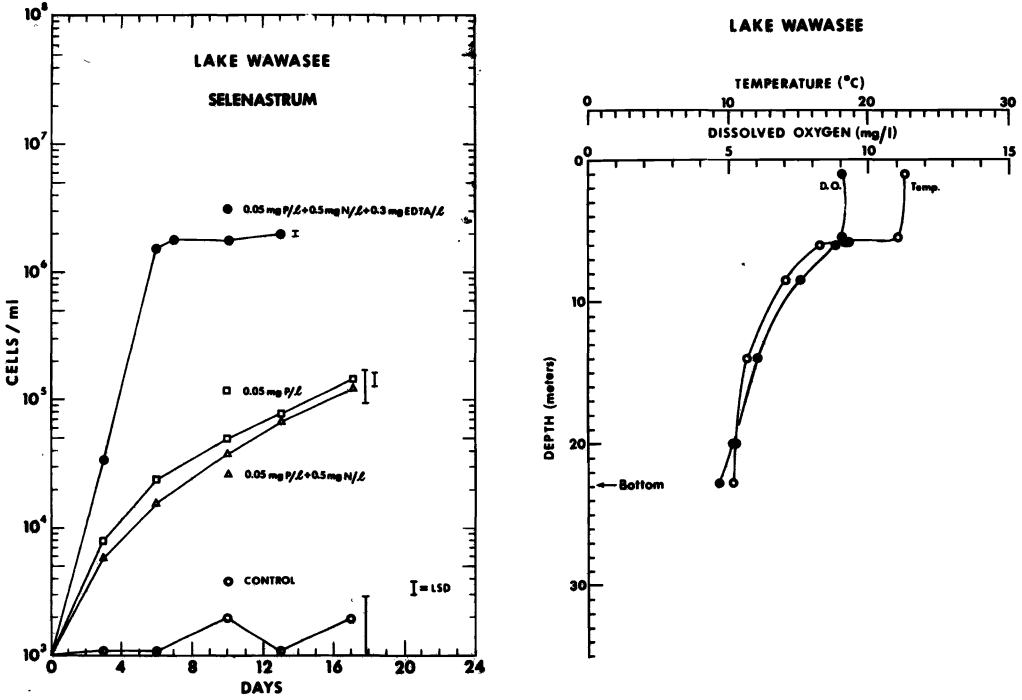

Figure 3. December 1974 algal assays and May 1975 dissolved oxygen and temperature profiles of Lake Wawasee water, Indiana.

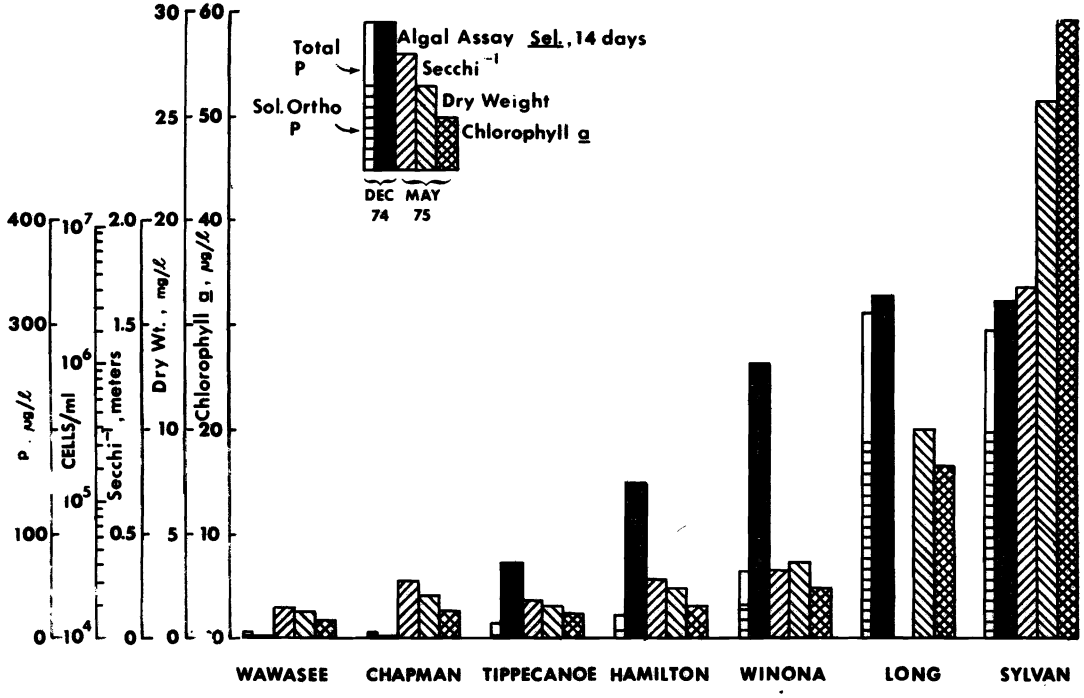

Figure 4. Comparison of December 1974 algal assays and phosphorus levels with May 1975 measurements of chlorophyll *a*, dry weight, and Secchi readings for seven N.E. Indiana lakes.

AAP TESTING OF ELY, MINNESOTA, AREA WATERS

Ely, Minnesota, is a study site for an EPA lake restoration project (Brice and Powers, 1969). Water from oligotrophic Burntside Lake flows through Burntside River into Shagawa Lake (Figure 5). Shawaga Lake has been receiving sewage effluent from the small town of Ely (population 5,000) since 1903 and, unlike the other good water quality lakes in this area, has been experiencing heavy algal blooms. An EPA-sponsored full-scale tertiary treatment sewage plant went into operation at Ely in January 1973 in this first United States attempt at lake restoration by nutrient removal from municipal sewage. With the cooperation of Environmental Protection Agency personnel at Ely, we have used waters from that area in the development of algal assay techniques and to test for possible eutrophication effects of detergent candidate materials.

The results of our survey over a 6-year period show that, under AAP test conditions, no substantial growth of any test algae was supported in oligotrophic Burntside Lake water alone, and some die-offs of *Microcystis aeruginosa* occurred (Figure 6). Only slightly higher growth was supported by Burntside River water alone (Figure 7). Eutrophic Shawaga Lake water, on the other hand, supported substantial growth of the three algae, especially in its nutrient-rich waters sampled during late fall to early spring (Figure 8). The growth of *Selenastrum,* in particular was found to be clearly dependent upon nutrient levels in Shawaga Lake water, confirming the findings of Miller and Maloney (1971).

The response of the blue-greens, especially *Microcystis,* was complicated by trace metal/chelator interactions. This is illustrated in Figure 9, which shows trace nutrient-limited growth of *Microcystis* in upstream Burntside Lake water sampled through the ice in February 1970. This sensitivity to trace metal effects was not observed with *Selenastrum* and *Anabaena,* and was one of the earliest indications to us that *Microcystis* might serve as a good test organism for these effects. We later used Burntside Lake water (enriched with 1 percent secondary sewage and 0.3 mg N/l) and *Microcystis* to study the relative chelation effects of two synthetic chelators, NTA and sodium citrate, along with a natural chelator, fulvic acid (Lange, 1970). Figure 10 shows the maximum standing crop of *Microcystis* after 20 days in test when increasing levels of these chelators were added to increasing levels of the AAP trace metal mixture. The chelation capacity of the natural fulvic acid is comparable to that of the two synthetic chelators at the lower, expected environmental levels of these trace metals.

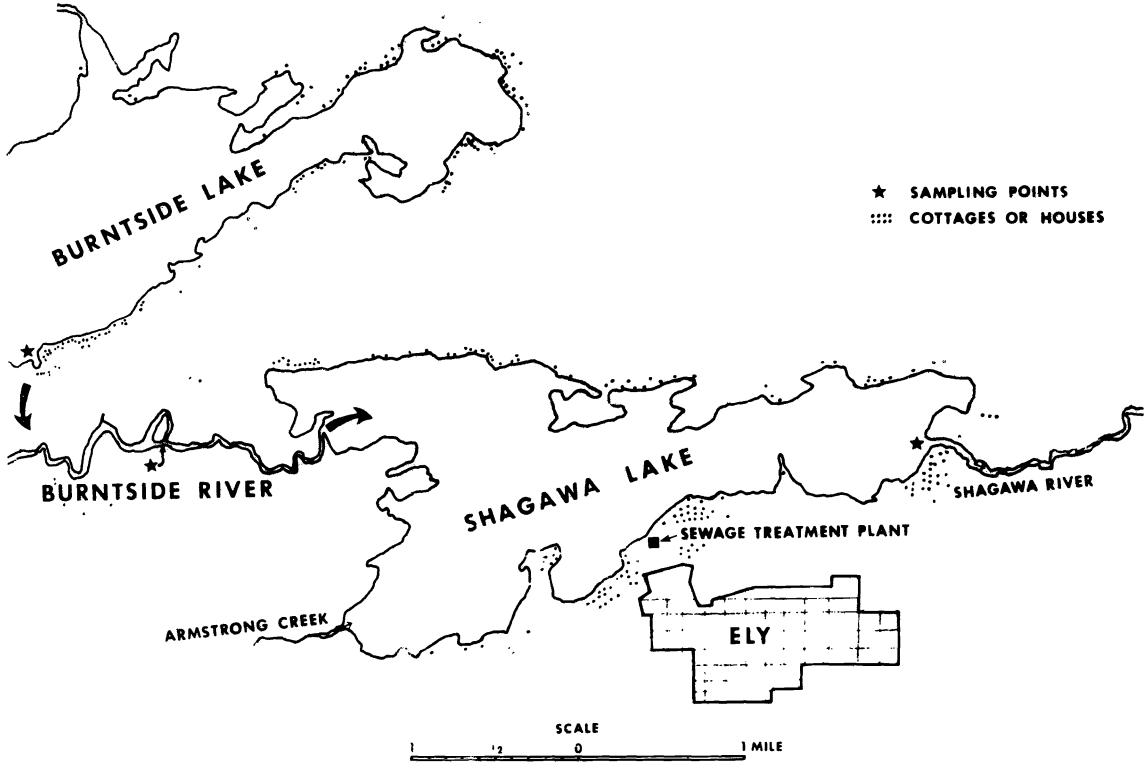

Figure 5. Shagawa Lake, Burntside River, and Burntside Lake, Ely, Minnesota.

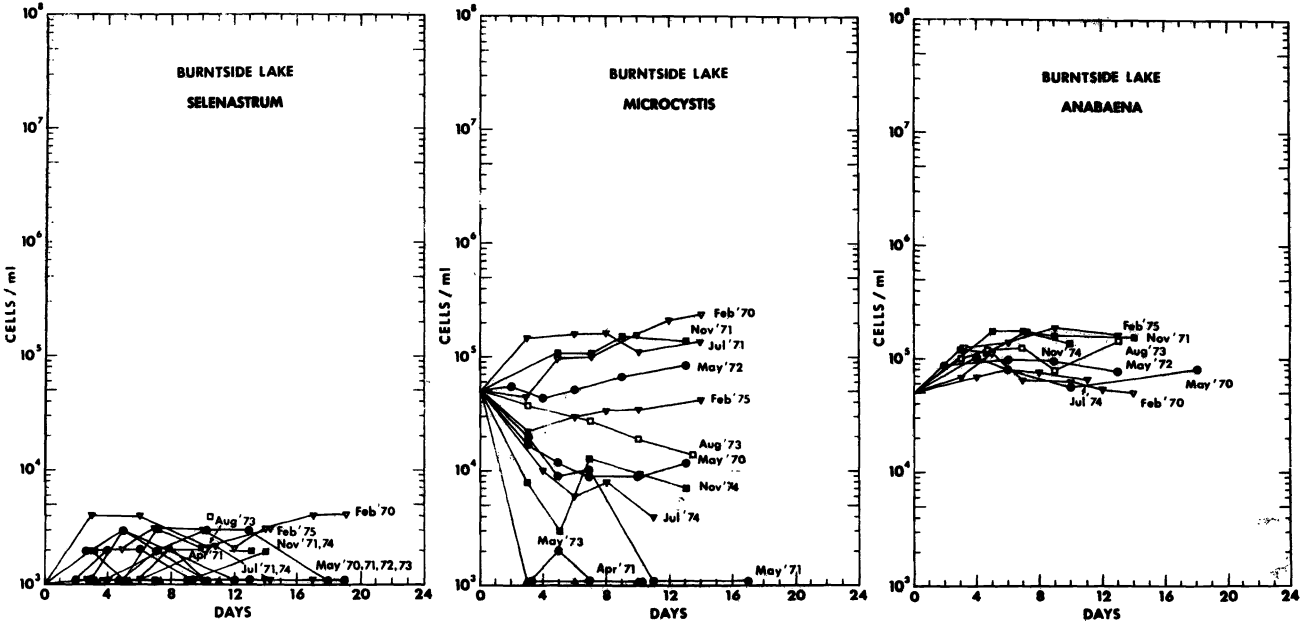

Figure 6. Response of AAP test algae in filtered (0.45 u) Burntside Lake water, Ely, Minnesota.

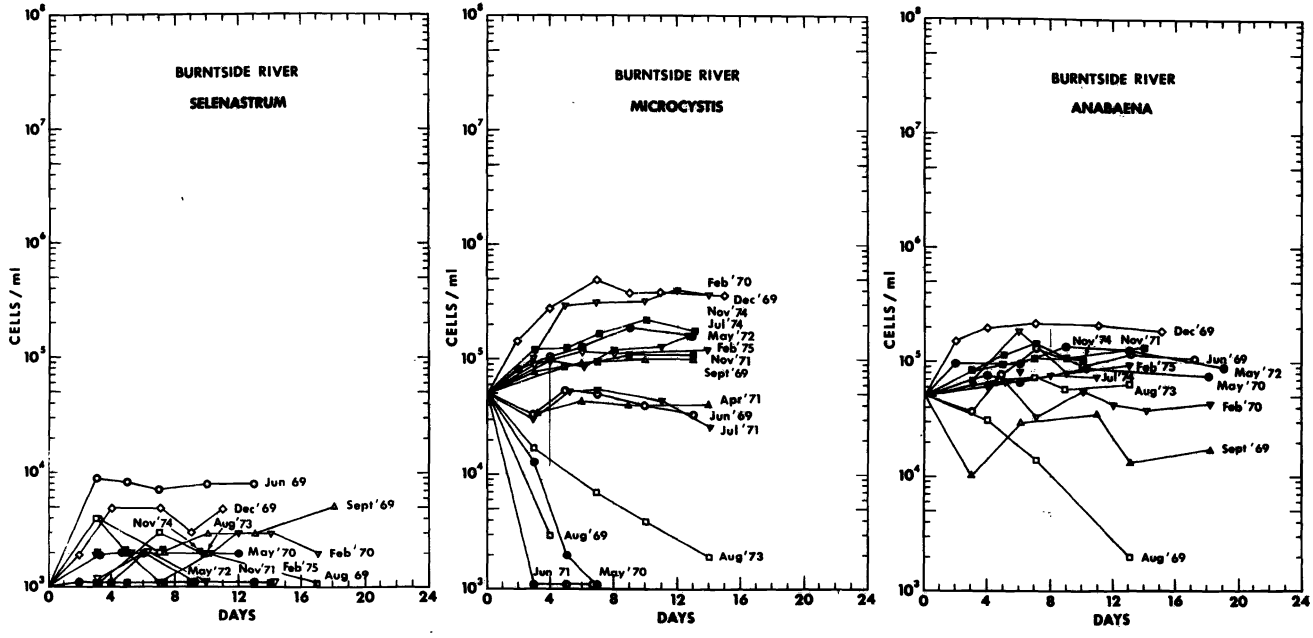

Figure 7. Response of AAP test algae in filtered (0.45 u) Burntside River water, Ely, Minnesota.

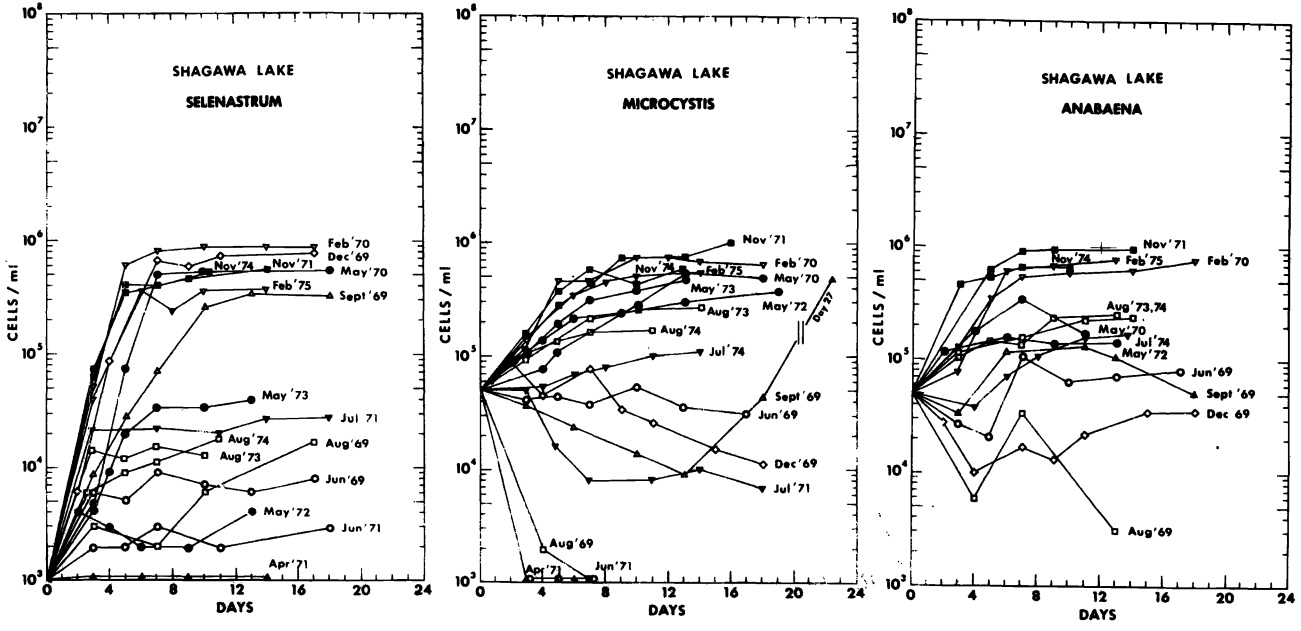

Figure 8. Response of AAP test algae in filtered (0.45 u) Shagawa Lake water, Ely, Minnesota.

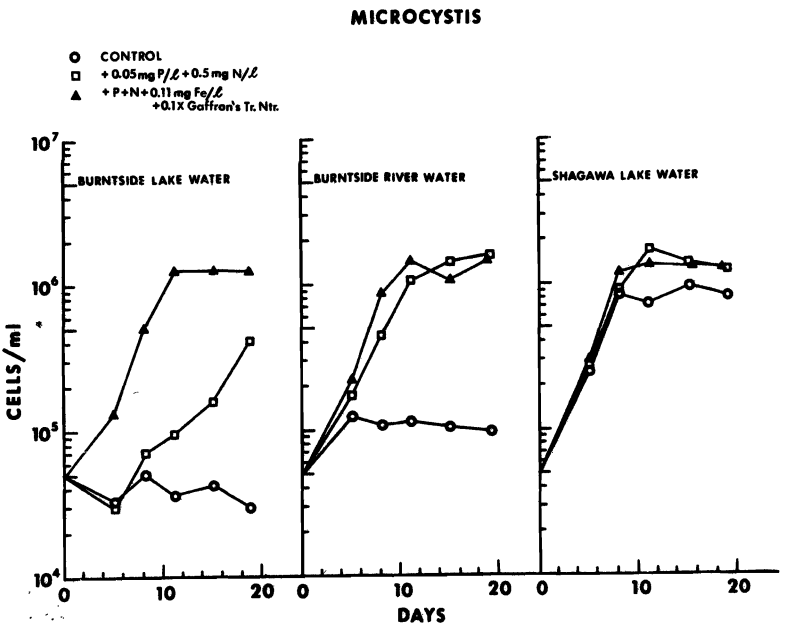

Figure 9. Effect of iron and trace nutrient additions on growth rate of *Microcystis aeruginosa* in Ely, Minnesota area waters.

TRACE METAL/CHELATOR BIOASSAYS

In order to develop data on the relative chelating ability of detergent builder candidates in defined systems, we alter the trace metal/chelator system of the AAP media. The media normally contains EDTA (300 μg/l, 8.0 x 10^{-7} M), Fe^{+++} (33 μg/l, 5.9 x 10^{-7} M) and other trace metals (Mn Zn, Cu, Co, Mo). We would like to be able to add only one metal and one chelator to the media, but our testing has shown that iron, in a chelated form, is required for normal growth of *Microcystis* and cannot be deleted. We also have established that the iron must be added to the chelator solution prior to addition to the growth media in order to avoid formation of insoluble hydroxides. The following media changes, therefore, have been made for use in chelation studies:

a) A stock solution of 96 mg/l Fe^{+++} (as $FeCl_3$) and 220 mg/l EDTA (1:1 M) replaces the normal EDTA-trace metal stock. A 1 ml addition of this stock provides the normal AAP level of iron in a chelated form. There is no "extra" EDTA.

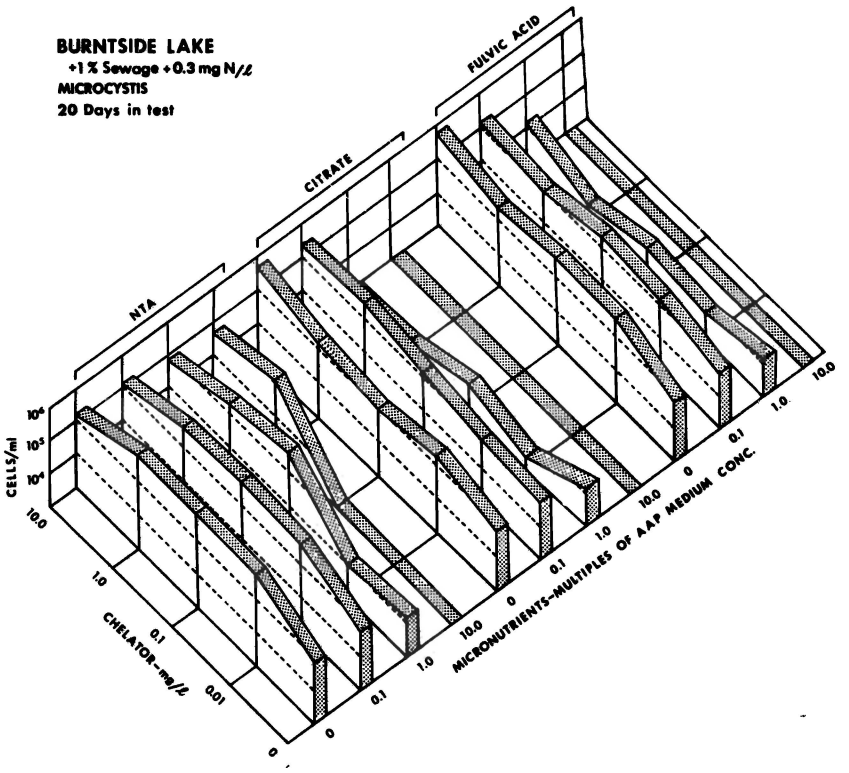

Figure 10. Effect of levels of three chelators added to levels of AAP trace metals on maximum standing crop of *Microcystis aeruginosa* in enriched Burntside Lake water, Ely, Minnesota.

b) The trace metals are eliminated from the media and only the metal of interest (e.g., Zn^{++}) is added at the desired levels.

This allows normal control growth and permits us to add specific chelators at desired levels and assess their ability to protect against toxicity. The result is a "comparative chelating capacity" test which gives some measure of the expected effects with detergent builders in natural waters.

We have compared the effects of trace nutrients and EDTA on the growth of three test algae, *Microcystis, Selenastrum,* and *Navicula seminulum. Navicula* is not sensitive to changes in the metal/chelator

levels (Figure 11). *Selenastrum* exhibits reduced growth rates only when all the metals are included and EDTA is omitted (Figure 12). The laboratory strain of *Microcystis*, however, is very sensitive to changes in the metal/chelator balance. Figure 13 shows that both EDTA and metals (mainly iron) are required for *Microcystis* growth, and that if the normal AAP trace metals are not chelated with EDTA, the cells die-off.

In Figure 14, a toxic level of Zn, 45 μg/l, is added to the test media. Increasing levels of EDTA, from 33 μg/l to 1 mg/l, show increasing protection against Zn toxicity. This type of assay has been done successfully with several synthetic chelators and in natural water samples, resulting in a quantitation of the relative chelating capacity of the synthetic compounds and the natural waters. Natural chelators in the form of humic compounds or "yellow organic acids," seem to occur at some levels in most natural waters, formed mainly by microbial degradation of organic matter. The chelation effect of sewage also must be considered wherever waste discharges impact natural waters. We currently are involved with a chemical characterization of the metal/chelator interactions which are present in AAP media and plan to publish the results along with a detailed description of the bioassay methods.

Incidentally, the 15 μg/l Zn level in normal AAP media with 300 μg/l EDTA is close to the toxicity level for *Microcystis* (Hall, 1974). This may well account for the difficulty which is often encountered by some laboratories in maintaining healthy *Microcystis* cultures.

ALGAL TOXICITY TESTING

In our evaluation of raw materials considered for use in consumer and industrial products, we have expanded the generally accepted application of the AAP to include an assessment of algal toxicity. The goal of this method development was to provide a toxicity test that was both operationally simple and environmentally meaningful. Typical algal toxic responses are shown in Figure 15. It became apparent that measurement of the algistatic response, i.e., no net cell production, would meet our two test criteria. The basis for selection of this method over other methods is shown graphically in Figure 16. Reduction of 50 percent in standing crop does not seem to be a meaningful toxic response for algae. This parameter (EC_{50}) often depends on the point in time during a 14 day growth period that measurements are made. A reduction of 50 percent in the growth rate (TL_{50}) appears meaningful but the requirement for at least daily estimations of biomass makes this measurement somewhat difficult and time consuming.

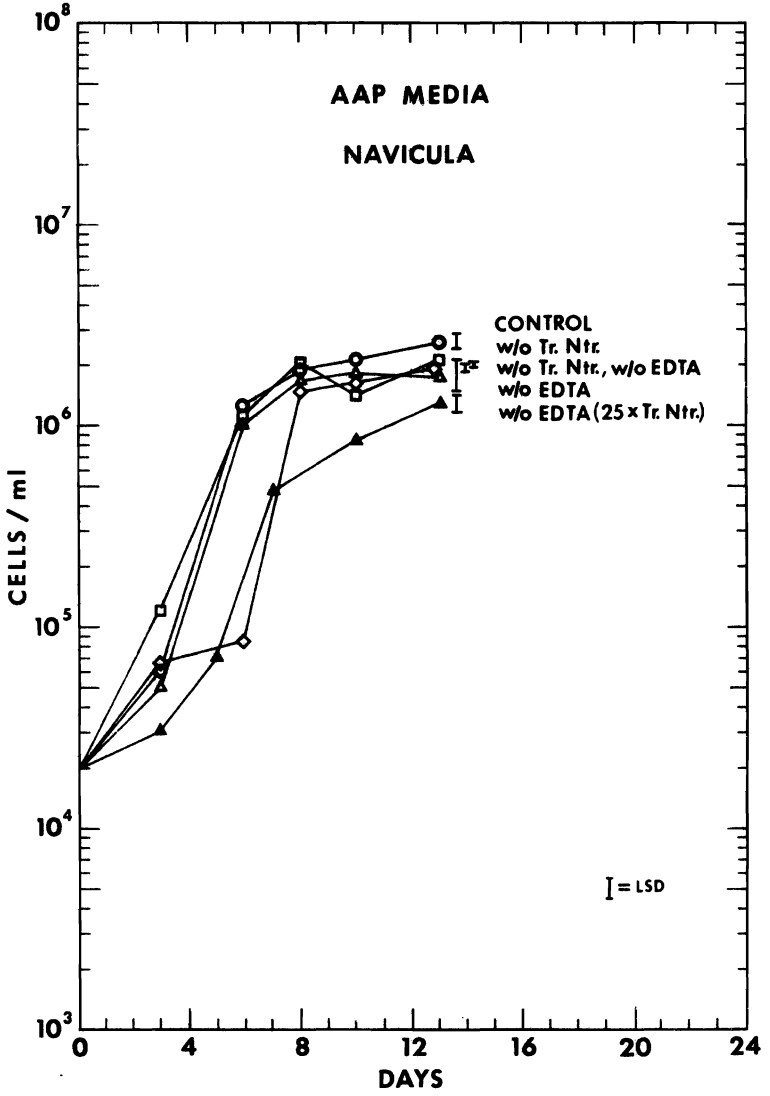

Figure 11. Effect of trace nutrients and EDTA on growth of *Navicula seminulum* in AAP media.

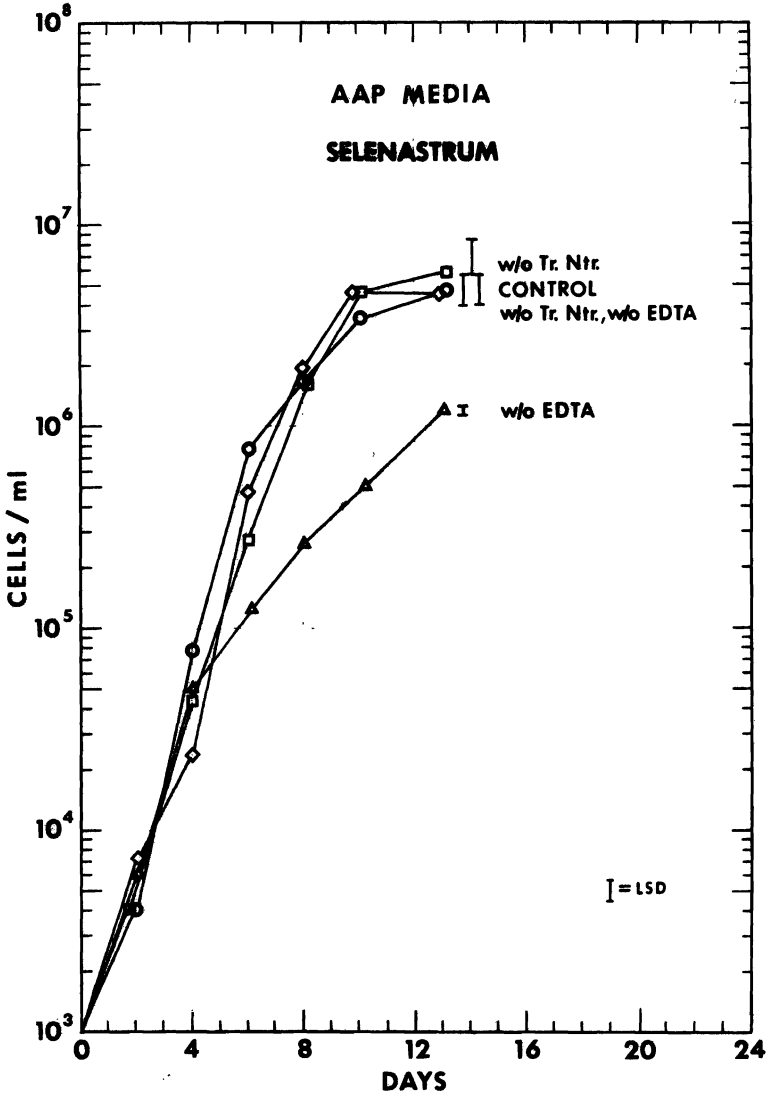

Figure 12. Effect of trace nutrients and EDTA on growth of *Selenastrum capricornutum* in AAP media.

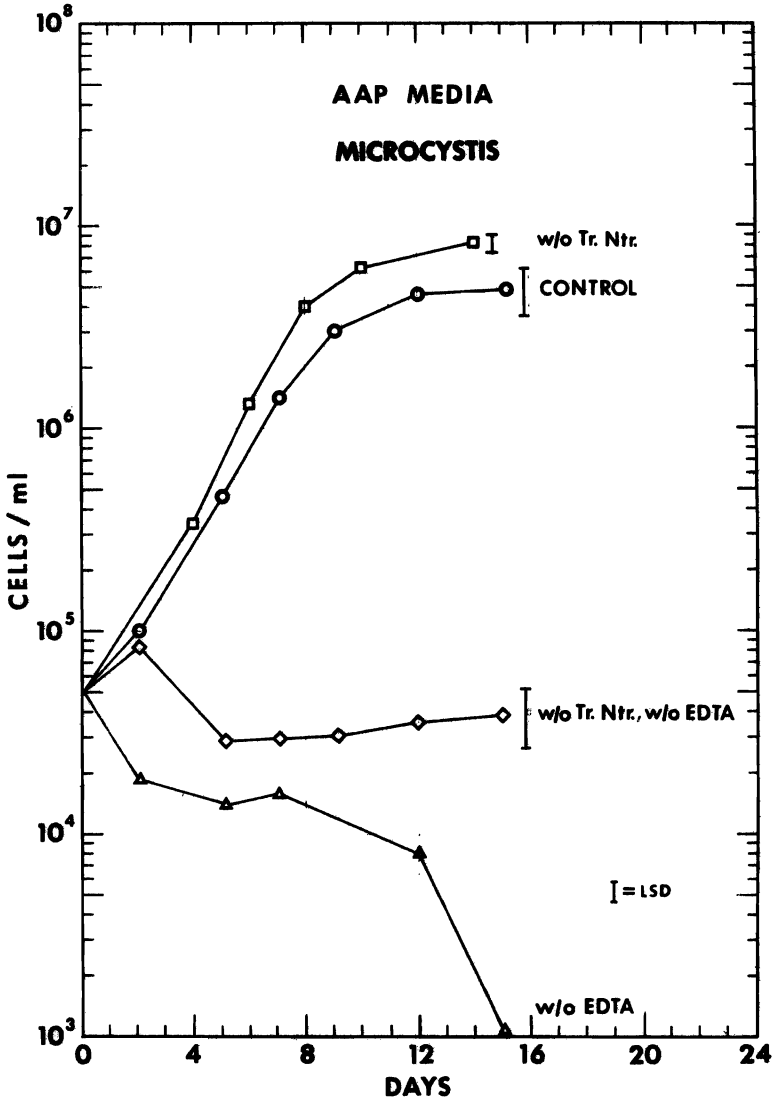

Figure 13. Effect of trace nutrients and EDTA on growth of *Microcystis aeruginosa* in AAP media.

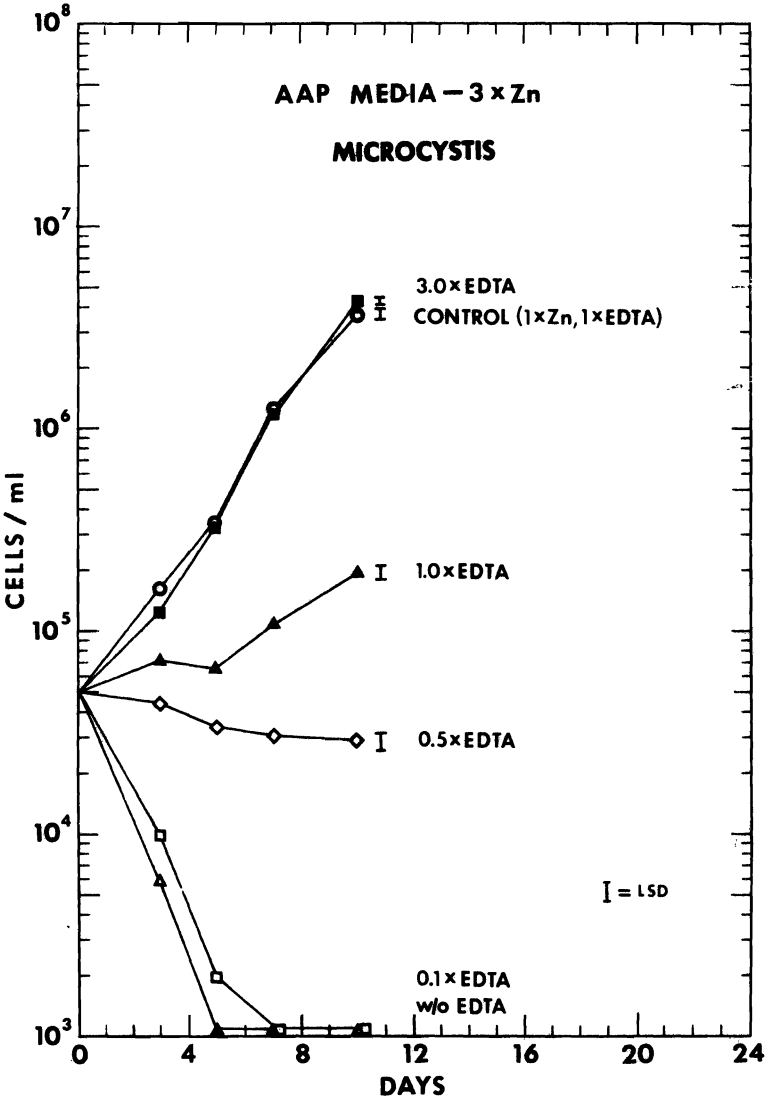

Figure 14. Effect of levels of EDTA on growth of *Microcystis aeruginosa* in AAP media containing a toxic level (45 ug/l) of Zn (as $ZnCl_2$).

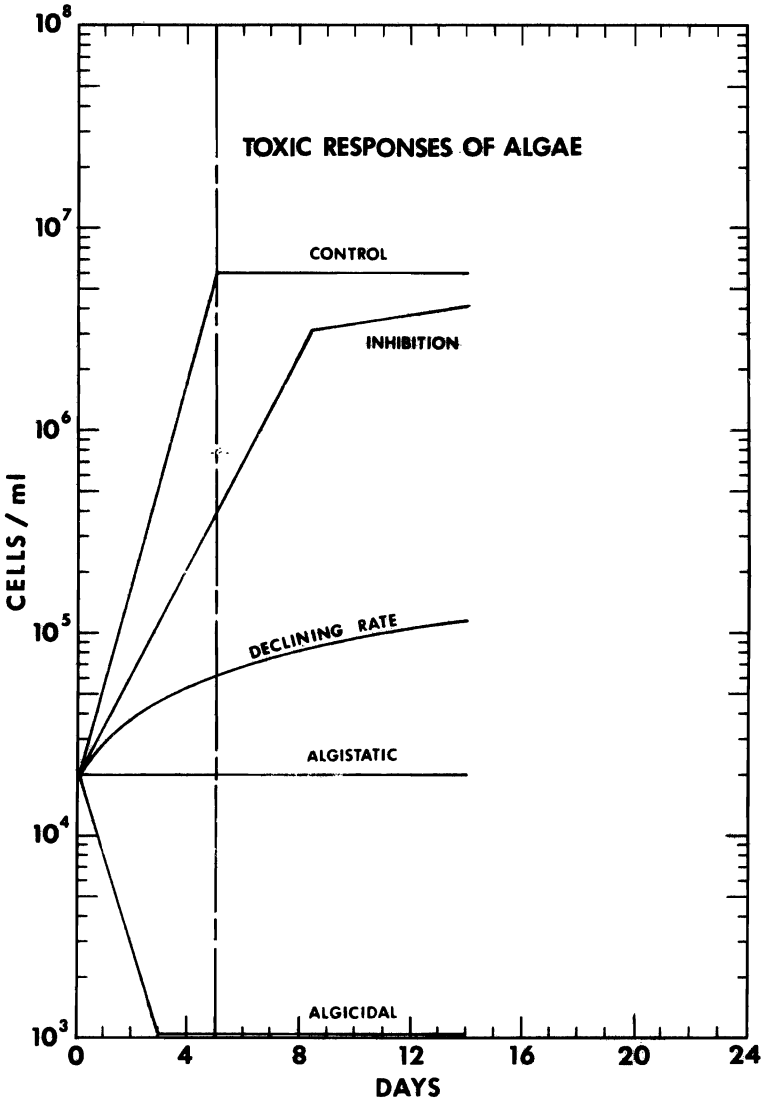

Figure 15. Typical effects of toxic materials on growth of algae.

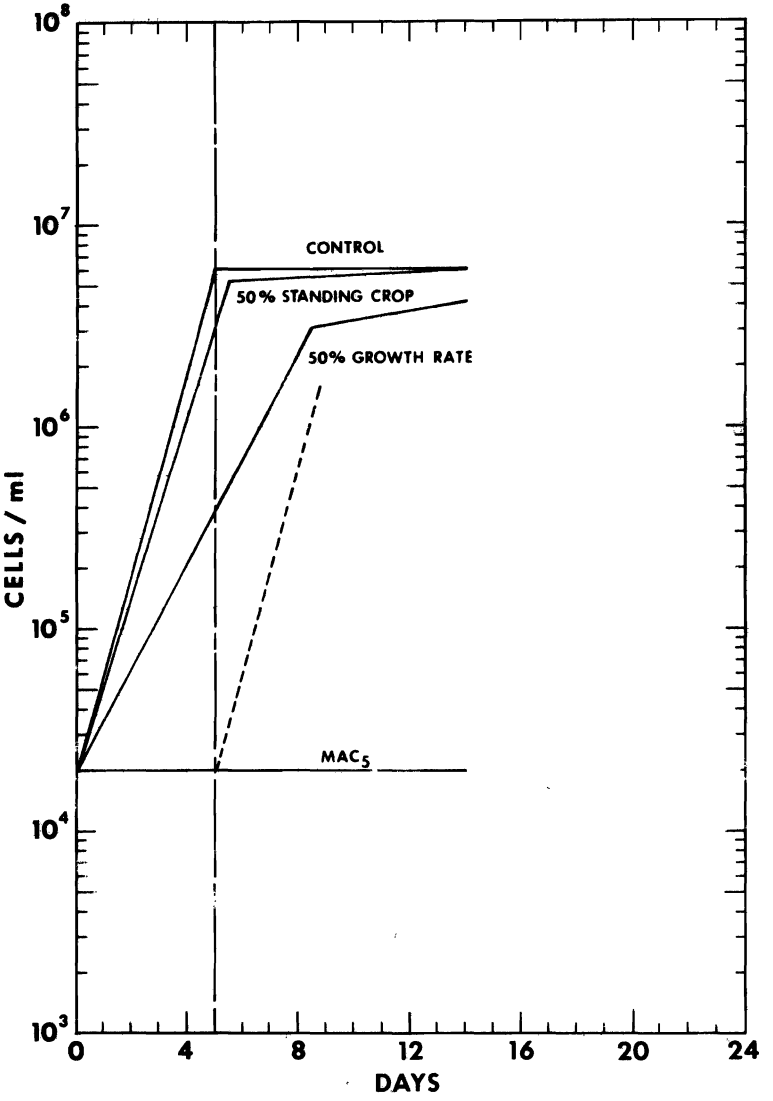

Figure 16. Three different measurements of algal toxicity.

The test result sought in our method is an algistatic response during the normal log growth phase of 5 days but from which the cells recover when removed from the presence of the test material and resuspended in fresh AAP medium. The lowest concentration of test material which causes this response has been termed the Minimum Algistatic Concentration after 5 days, or MAC-5 day.

Measurement of MAC-5 day can be accomplished by either cell counts or *in vivo* fluorescence measurements (Figure 17). Our experience, however, indicates that cell count is the preferred method because some materials appear to inhibit cell division but allow growth and chlorophyll *a* production to continue for at least a few days. In its simplest form, the method requires cell counts only on two days, day 5 and day 14, plus a transfer of cultures which show an algistatic response on day 5.

Three test species are included routinely in our toxicity testing (Figure 18). The green (*Selenastrum*), blue-green (*Microcystis*), and diatom (*Navicula*) species show striking differences in toxic response to many test materials, in this example, to a nonionic surfactant, linear alkylethoxylate (LAE).

By further increasing the test material concentration, the algicidal level of a test material can also be determined as earlier reported by Fitzgerald (1971). Our method, therefore, can be applied to both the assessment of environmental hazard of materials to planktonic algae and to the screening of chemicals designed as potential algicides.

This method also has been applied successfully to marine algal toxicity testing with the green flagellate *Dunaliella* in artificial seawater media as defined in the Marine Algal Assay Procedure (EPA, 1974).

SUMMARY

The algal assay procedure, used in conjunction with physical/chemical measurements of natural waters has been successfully applied to the assessment of the algal growth potential of those waters.

In testing nutrient conditions of natural waters, the AAP showed logical differences among waters and by seasonal patterns. Samples from oligotrophic waters, when filtered, inoculated and maintained under laboratory culture conditions, supported low standing crops of algae throughout the year. Natural populations in these waters also remained low throughout the year. Eutrophic waters during the winter months were rich in nutrients and supported high maximum standing crops of test algae in AAP tests. The following spring, these lakes, as predicted, supported high natural population levels.

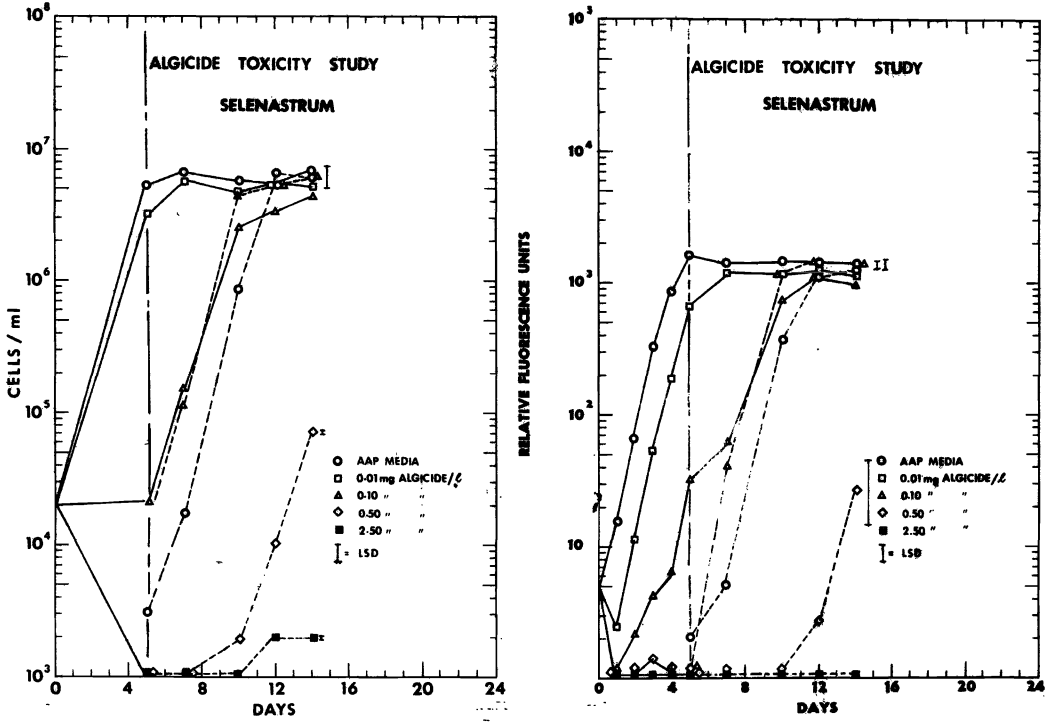

Figure 17. Comparison of cell number and *in vivo* fluorescence measurements in algal toxicity testing.

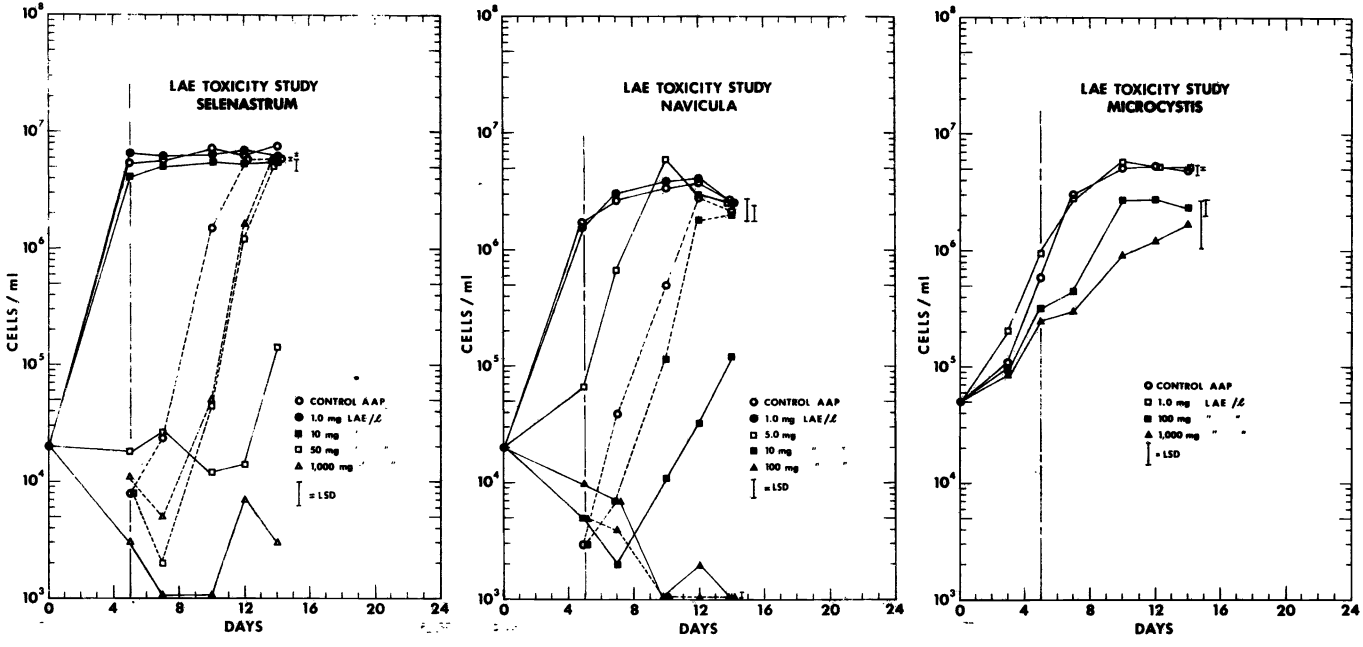

Figure 18. Comparison of three test algae responses to levels of nonionic surfactant, linear alkyl ethoxylate (LAE).

The AAP can provide useful information on the relative chelating capacity of synthetic compounds, sewage effluent, and natural chelators in the aquatic environment. The sensitivity of laboratory-cultured *Microcystis aeruginosa* to low levels of free metals makes this a useful test organism for trace metal/chelator studies. The level of Zn in AAP media is close to the toxicity level for *Microcystis* and may need to be lowered for optimization of *Microcystis* growth.

The application of the AAP has been expanded to include an assessment of algal toxicity of test materials. A toxicity test is described which is both operationally simple and environmentally meaningful. The Minimum Algistatic Concentration after 5 days represents that level of a test material which causes no net change in the population of the test algae after chronic exposure. Inclusion of a recovery period following this contact allows interpretation of the MAC-5 day to be the level of the test material which causes primary inhibitory effects at the cellular level but does not cause permanent or irreversible damage to the test culture.

ACKNOWLEDGMENTS

I gratefully acknowledge the assistance of R. H. Hall for his generous contributions to the sections on chelation and toxicity. The technical assistance of J. W. Williams in carrying out the studies and in the preparation of the figures also is greatly appreciated.

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Field Evaluation of the Algal Assay Procedure on Surface Waters of North Carolina

C. M. Weiss*

INTRODUCTION

The problem of man induced eutrophication in the United States has led to the development of bioassay procedures for establishing both quantitative and qualitative response to the major algal nutrients. It has been particularly important that these determinations be based upon a reference procedure so that effective regional comparisons could be carried out. With this in mind a joint industry government task force on eutrophication drafted a provisional algal assay procedure one of whose objectives was to "encourage and promote the development of algae and other assay procedures for use in predicting the impact of change of levels of nutrient elements in natural water environment as well as of the addition of new nutrient or nutrient-synergistic compounds to the water environments" (Joint Industry/Government Task Force on Eutrophication, 1969).

An intensive coordinated program of development and evaluation followed involving university and industrial laboratories. Both batch or bottle test and continuous culture algal bioassays were evaluated as to their applicability for establishing limiting nutrient levels in surface waters (U.S. EPA, 1971). Following detailed definition of the growth constraints for the batch test, eight laboratories participated in a joint evaluation of the assay bottle procedure is reported by Weiss and Helms (1970). The same water samples were distributed to each of the eight laboratories and batch tests were carried out using a standardized assay protocol. This

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interlaboratory comparison established that whereas there was relatively a low level of precision between the results of the participating laboratories, variation being over 30 percent, within any one laboratory the precision was considerably better. Unknown systematic errors were variables in establishing the exactitude of the individual laboratory conditions. These contributed to the magnitude of difference in biomass grown at the several laboratories.

It has been noted in the original Industry/Government Task Force on the algal assay procedure that "even when this developmental phase is completed an extensive field evaluation was to be undertaken." Without this correlation to the conditions in nature, the true relationship of the results obtained from the assay procedure would still remain uncertain. One of the immediate and very useful applications of the algal assay has been to establish the extent of response of batch cultures to environmental safety evaluations of raw materials that might be considered for use in consumer products, Sturm and Payne (1973), Payne (1973), Mitchell and Buzzell (1971). Examples of algal response in natural systems using the batch assay to establish trophic levels and identification of growth limiting nutrients have been used by Toerien and Steyn (1973), Francisco and Weiss (1973), Steyn et al. (1974), Payne (1975), and Doemel and Brooks (1975).

One of the basic problems inherent in the batch algal assay procedure is the necessary pretreatment required of the water sample, either filtration through a 0.45μ membrane filter or autoclaving followed by filtration to remove particulate and insoluble materials. Either pretreatment is essential in order to remove or destroy living algal cells so that reseeding with the test alga is possible. Using filtration, the assumption is made that all nutrient components in solution would be available to algal growth and would be representative of the quantity supporting the existing natural biomass in the original body of water. In the use of autoclaving, which also solubilizes nutrient materials, growth in such a pretreated sample would reflect the total nutrient potential for algal growth assuming temperature, light, and other growth factors were not limiting. In either case if the assay is meaningful the magnitude of growth of the reseeded sample should be related to the trophic state of the original water sample. To establish the relationship between the batch assay results and the existing trophic or nutrient level of a body of water, algal assays were used to evaluate these levels in 44 different bodies of water, sampled at several locations, in many instances as well as several frequencies, for a total of 345 assays. The location and type of the sampled waters is shown in Figure 1. The name, identification code, and size, with approximate level of pollution entering each body of water, is presented in Table 1.

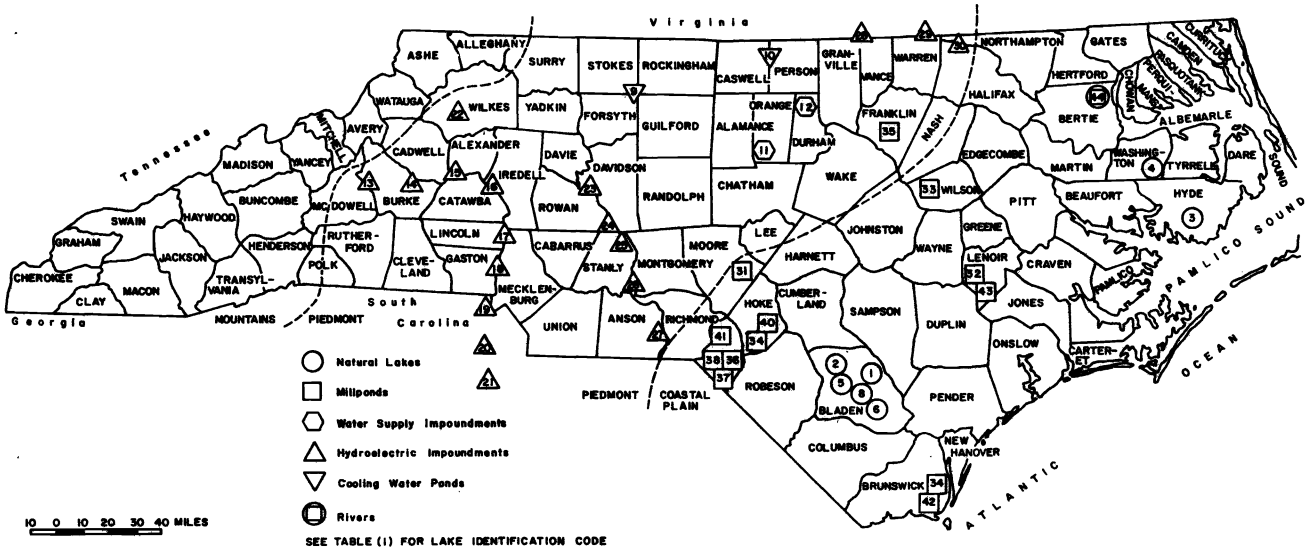

Figure 1. Location and nature of surface waters sampled in North Carolina and adjacent areas.

Table 1. Surface waters of North Carolina evaluated by algal assay.

Type and Name	Surface Area (Acres)	Mean Depth-ft.	Stations Sampled	Pollution Sources	
				Direct Point Discharge	Inflowing River[b]
Natural Lakes					
1 Black	1,418	6[a]	2	No	No
2 Jones	224	6[a]	2	No	No
3 Mattamuskeet	30,000	4[a]	1	No	No
4 Phelps	16,000	6[a]	1	No	No
5 Salters	315	6[a]	2	No	No
6 Singletary	572	6[a]	2	No	No
7 Waccamaw	8,938	6[a]	2	No	No
8 White	1,068	8[a]	2	No	No
Impounded Cooling Lakes					
9 Belews	3,700	50	2	No	No
10 Hyco	3,750	21	2	No	No
Water Supply Impoundments					
11 University	200	9	1	No	No
12 Michie	507	25	1	No	No
Hydroelectric Impoundments					
Catawba River					
13 James	6,510	46	4	Yes	No
14 Rhodhiss	3,515	21	6	Yes	No
15 Hickory	4,110	31	3	Yes	No
16 Lookout Shoals	1,270	24	3	No	No
17 Norman	32,510	34	6	No	No
18 Mt. Island	3,235	18	3	No	No
19 Wylie	12,455	22	8	Yes	No
20 Fishing Creek	3,370	17	2	No	Yes
21 Wateree	13,710	23	5	No	Yes
Yadkin River					
22 W.Kerr Scott (Flood Control)	3,980	38	3	No	No
23 High Rock	15,180	16	3	No	Yes
24 Tuckertown	2,529	17	3	No	No
25 Badin	5,973	24	3	Yes	No
26 Tillery	5,000	34	7	No	Yes
27 Blewett Falls	2,500	36	3	Yes	No
Roanoke River					
28 John H. Kerr	83,000	34	11	Yes	Yes
29 Gaston	22,000	18	3	No	No
30 Roanoke Rapids	4,900	16	3	No	No
Old Millponds					
31 Crystal (1885)	100	6[a]	1	No	No
32 Davies (1850)	60	6[a]	1	No	No
33 Finches (1875)	20	6[a]	1	No	No
34 Hodgins (1871)	100	5[a]	1	No	No
35 Jackson (1885)	75	10[a]	1	No	No
36 Johns (1840)	125	8[a]	1	No	No
37 Jones (1810)	75	3[a]	1	No	No
38 Lytches (1870)	325	7[a]	1	No	No
39 McKensie (1860)	50	7[a]	1	No	No
40 McNeils (1870)	100	4[a]	1	No	No
41 Monroe (1825)	70	5[a]	1	No	No
42 Orton (1810)	500	8[a]	1	No	No
43 Tull (1875)	180	6[a]	1	No	No
River Segments					
44 Chowan (U.S. 13 to Albemarle Sound)	-		6	Yes	-

[a]Estimated.

[b]Yes if river carries pollution discharges from upstream communities with no intervening impoundments.

METHODS

In order to accommodate the considerable number of samples being processed over the several years of this study and to stay within the recommended procedures of the "bottle test" the growth responses of the reseeded samples were determined only at the end of a fixed period of incubation. It has been shown in the developmental steps of the assay procedure that biomass achieves a level of growth proportionate to available nutrients in 10 to 14 days with occasional samples, depending on the particular character of the water, requiring upwards of 21 days to achieve the growth plateau (U.S. EPA, 1971). For this field evaluation the assay protocol was standardized as follows:

1. Each raw water sample was split into two portions—one for autoclaving and one for filtration (0.45 μ filter) as pretreatment procedures.

2. Each of the samples following pretreatment was divided into subsets of three portions, each 60 ml in 250 ml Erlenmeyer flasks, and spiked with 5 and 50 μg of phosphorus, 75 and 750 μg of nitrogen, 5 μgP plus 75 μgN, and 50 μgP plus 750 μgN. In addition another set of three flasks was used as a nonspiked control.

3. Each flask was seeded with 1,000 cells/ml of *Selenastrum capricornutum* previously washed and resuspended in distilled water to adjust to the inoculation concentration. The inoculum was cell culture grown 10-21 days in NAAM (U.S. EPA, 1971).

4. The seeded flasks were placed in an incubator at 24°C±2.0°C under 400 ft. candles of "daylight" fluorescent illumination.

5. Incubation was for 14 days with a daily swirling of the culture flasks.

6. With each incubation set, which would total several hundred flasks, the capacity of the incubator, the standard control media NAAM, in a replicate set of flasks was also seeded with the same cell concentration of the test organism and grown for the same period under similar temperature and illumination conditions.

7. At the end of the growth period the optical density of the cell suspension was determined using a Beckman Spectronic 20 colorimeter at a wavelength of 640 mμ. In addition the total biomass grown in the NAAM control samples was optically measured, filtered, and weighed, and used to determine the unit absorbance for the particular growth conditions of that incubation set.

8. The unit of absorbance of the NAAM control for each incubation set was used to convert the unit absorbance of the spiked samples and controls to biomass.

Since the requirement of maintaining extensive records over a relatively long period of time on the algal assays and systematizing the procedures for conversion of data, data record forms were designed to accommodate the basic information of each water sample from which data cards were punched, programs written for data analysis, and a good deal of the computational work processed by computer.

At the time of collection of the original water sample on which the algal assay was to be carried out, a broad spectrum of limnological parameters were also determined. From these the original trophic state could be established for comparison to the nutrient characteristics as determined by the algal assay.

All analyses of the several nitrogen and phosphorus species were made using Technicon Autoanalyzers. For the purposes of this investigation the nitrogen and phosphorus forms are defined or were determined as follows:

NH_3-N	Ammonia nitrogen.
NO_2+NO_3-N	One analysis which includes oxidation of NO_2 to NO_3 and the total determined.
Inorganic-N	The sum of NH_3-N and NO_2+NO_3-N.
Kjel-N	Kjeldahl nitrogen.
Total-N	NO_2+NO_3-N plus Kjel-N.
PO_4-P	Orthophosphate phosphorus, soluble reactive phosphorus.
T-Sol P	Total soluble phosphorus. The total fraction that passes the 0.45 micron filter and is persulfate digested.
T-Particulate P	Total-P minus total soluble P.
T-P	Total phosphorus. The total raw sample persulfate digested.

Phytoplankton cell counts were determined on live samples, concentrated by centrifugation with a clinical centrifuge. The resuspended concentrate was transferred to a microscope slide and sealed under a 22x22 mm cover glass. Transects were counted at 500 X and specific identifications made at 1250 X. Cell density was calculated from the cover glass area, area of transects counted and original volume concentrated.

The chlorophyll *a* determinations were made on a Turner photo-fluorometer equipped with a photo detector that enhanced its sensitivity to chlorophyll fluorescence. Comparison of the values determined with a Turner photo-fluorometer and the standard acetone extraction procedure showed a high level of correlation. However, since the conversion factor appeared to vary somewhat from lake to lake the Turner scale unit has been used in this report as the chlorophyll unit. An approximate conversion to chlorophyll *a* mg/l can be made by multiplying Turner units by 0.44.

To provide a direct comparative procedure for the determination of productivity, $mgC/m^3/hr$, all water samples for this purpose were returned to the laboratory, stored in the dark at room temperature overnight and then exposed in a light/dark bottle set at 24°C, 400 f.c. for 6 hours. The rate of carbon fixed per hour was computed from the net oxygen production.

RESULTS

Nutrient Characterization of North Carolina Lakes

The results of this investigation fall into two parts. In the first the quality of the original lake waters are defined in order that the results of the algal assay may be compared to the original nutrient levels. For the former purpose the limnological parameters of the lake samples used in the algal assay have been compiled in Table 2. In this tabulation all determinations taken in the period April through November or December through March have each been averaged to show seasonal effects. The experience of sampling North Carolina waters has shown that these periods generally define a growing season and winter season. However, the difference between the two seasons, with respect to algal growth, is often small with light probably being the limiting element rather than temperature or other growth controlling factors. If more than one sample has been taken, the values shown are averages. In some instances, such as a major body of water where more detailed studies have been carried out,

Table 2. Mean values of limnological parameters of lake samples used in algal assays.

	Season	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.
	Lake	(3) Mattamuskeet		(4) Phelps		(1) Black		(2) Jones (JO)	
No. of Samples		1	None	1	None	1	1	1	1
Water Temp. °C		26.1	-	25.3	-	25.3	15.0	25.0	15.2
Secchi Depth-m		0.61	-	2.13	-	0.305	0.24	1.22	0.92
NH_3-N mg/m^3		20	-	10	-	35	90	20	40
NO_2+NO_3-N		-	-	87	-	513	600	50	45
Inorg-N		-	-	97	-	548	690	70	85
Kjel-N		350	-	260	-	700	920	250	280
Total-N		-	-	347	-	1213	1520	300	325
PO_4-P		5	-	5	-	50	195	5	5
T-Soluble P		7	-	5	-	165	205	5	12
T-Particulate P		36	-	17	-	50	35	8	8
Total P		43	-	22	-	215	240	13	20
TN/TP		-	-	15.7	-	5.6	6.3	23.1	16.3
Phyto. Cell Density no./ml		16068	-	1279	-	1365	7944	454	2363
Chlor *a* Turner Units		44	-	23	-	71	80	20	27
Productivity mgC/m^3/hr.		-	-	-		-	-	-	-
		(5) Salters		(6) Singletary		(7) Waccamaw		(8) White	
No. of Samples		1	1	1	1	1	1	1	1
Water Temp. °C		25.5	15.0	26.0	15.1	25.1	15.2	26.5	15.1
Secchi Depth-m		0.61	0.92	0.92	1.22	2.13	1.22	>2.74	>3.05
NH_3-N mg/ m^3		15	35	10	50	15	45	55	30
NO_2+NO_3-N		24	10	31	40	7	10	13	15
Inorg-N		39	45	41	90	22	55	68	45
Kjel-N		350	290	250	240	380	320	150	110
Total-N		374	30	281	280	387	330	163	125
PO_4-P		5	5	5	5	5	5	7	5
T-Soluble P		11	14	10	13	5	9	30	9
T-Particulate P		6	1	14	2	12	11	-	1
Total P		17	15	24	15	17	20	13	10
TN/TP		22	20.0	11.7	18.6	22.7	16.5	12.5	12.5
Phyto. Cell Density no./ml		2335	3152	430	231	348	650	777	62
Chlor *a* Turner Units		27	37	36	13	29	18	13	8
Productivity mgC/m^3/hr.		-	-	-	-	-	-		-
		(9) Belews 1116		(9) Belews 1906		(10) Hyco 1116		(10) Hyco 1906	
No. of Samples		9	5	9	5	5	2	6	2
Water Temp. °C		21.9	8.4	18.9	8.6	20.5	11.6	22.6	13.5
Secchi Depth-m		2.0	1.9	2.3	2.1	0.7	0.7	1.5	0.7
NH_3-N mg/m^3		20	131	38	125	44	55	56	45
NO_2+NO_3-N		36	98	52	80	67	115	100	128
Inorg-N		56	226	90	205	111	170	156	173
Kjel-N		277	360	296	220	416	225	193	185
Total-N		313	458	348	300	483	340	293	313
PO_4-P		6.7	6.6	6.2	54	6.0	7.5	6.6	7.5
T-Soluble P		11.7	8.3	7.7	11.6	10.2	15.0	10.8	15.0
T-Particulate P		13.5	11.0	13.6	1.0	9.0	250	9.8	70.0
Total P		24.6	19.6	20.4	13.6	19.2	40.0	18.8	85.0
TN/TP		20.5	25.9	28.8	23.8	28.4	8.6	16.0	6.2
Phyto. Cell Density no./ml		4237(7)	698	1435(6)	734	1564	2571	2058	2029
Chlor *a* Turner Units		14(4)	10(1)	14(4)	9(1)	18(2)	23	19(4)	23
Productivity mgC/m^3/hr		13(5)	12(2)	9(5)	9(2)	21(3)	23	18(4)	18
		(11) University		(12) Michie					
No. of Samples		12	7	12	5				
Water Temp. °C		23.3	8.4	23.3	7.9				
Secchi Depth-m		1.0	0.5	1.0	0.4				
NH_3-N mg/m^3		86	106	60	110				
NO_2+NO_3-N		58	297	178	398				
Inorg-N		144	403	238	508				
Kjel-N		541	510	540	397				
Total-N		600	820	7.18	795				
PO_4-P		10.2	17.1	9.0	14.0				
T-Soluble P		14.4	32.1	17.3	24.0				
T-Particulate P		21.7	35.0	10.9	47.0				
Total P		35.4	67.1	27.5	71.0				
TN/TP		22.0	10.6	27.5	14.9				
Phyto. Cell Density no./ml		3980(8)	906(4)	7531(8)	496				
Chlor *a* Turner Units		-	-	-	-				
Productivity mgC/m^3/hr		44(7)	25(5)	22(6)	5				

Table 2. Continued.

Season	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.
	(22) Kerr Scott		(23) High Rock		(24) Tuckertown		(25) Badin	
No. of Samples	3	None	3	-	3	-	2	-
Water Temp. °C	13.7	-	14.9	-	14.0	-	13.7	-
Secchi Depth-m	0.8	-	0.3	-	0.25	-	0.3	-
NH_3-N mg/m³	62	-	248	-	242	-	245	-
NO_2+NO_3-N	220	-	503	-	500	-	505	
Inorg-N	282	-	751	-	742	-	750	-
Kjel-N	200	-	343	-	470	-	345	
Total-N	420	-	846	-	970	-	850	
PO_4-P	5.0	-	11.0	-	5.0	-	20.0	
T-Soluble P	21.6	-	60.0	-	38.3	-	40.0	
T-Particulate P	25.0	-	55.0	-	81.7	-	62.5	-
Total P	46.6	-	115	-	120	-	102	-
TN/TP	9.3	-	7.4	-	8.1	-	8.8	-
Phyto. Cell Density no./ml	399	-	426	-	559	-	1929	-
Chlor *a* Turner Units	19	-	26.3	-	28.7	-	29.0	-
Productivity mgC/m³/hr.	-	-	-	-		-	-	-
	(26) Tillery		(27) Blewett Falls					
No. of Samples	3	-	3	-				
Water Temp. °C	15.4	-	13.5	-				
Secchi Depth-m	0.3	-	0.3	-				
NH_3-N mg/m³	45.8	-	15.3	-				
NO_2+NO_3-N	501	-	380	-				
Inorg-N	546	-	395	-				
Kjel-N	337	-	206	-				
Total-N	838		670	-				
PO_4-P	42.5	-	7.0	-				
T-Soluble P	84.2	-	26.0	-				
T-Particulate P	36.3	-	37.3	-				
Total P	88.0	-	63.3	-				
TN/TP	7.4	-	10.6	-				
Phyto. Cell Density no./ml	1015	-	921	-				
Chlor *a* Turner Units	21.3	-	17.6	-				
Productivity mgC/m³/hr.	-	-	-	-				

	(31) Crystal	(32) Davies	(33) Finches	(34) Hodgins	(35) Jackson	(36) Johns	(37) Jones (JP)
No. of Samples	1	1	1	1	1	1	1
Water Temp. °C	13.1	14.4	12.7	14.5	11.5	16.1	17.1
Secchi Depth-m	0.61	0.92	1.22	0.92	0.92	1.22	0.92
NH_3-N mg/m³	400	510	170	55	520	45	305
NO_2+NO_3-N	500	400	350	45	435	125	940
Inorg-N	900	910	520	100	955	170	1245
Kjel-N	550	900	340	360	950	350	530
Total-N	1050	1300	690	405	1385	475	1470
PO_4-P	250	235	15	5	50	165	5
T-Soluble P	390	240	35	18	65	195	10
T-Particulate P	20	75	15	7	60	55	10
Total P	410	315	50	25	125	250	20
TN/TP	2.5	4.1	13.8	16.2	11.1	1.9	73.5
Phyto. Cell Density no./ml	1018	1393	496	292	744	463	781
Chlor *a* Turner Units	56	27	24	44	24	26	31
Productivity mgC/m³/hr.	-	-	-	-	-	-	-

	(38) Lytches	(39) McKensie	(40) McNeils	(41) Monroe	(42) Orton	(43) Tull
No. of Samples	1	1	1	1	1	1
Water Temp. °C	14.8	16.0	13.5	12.7	17.0	14.7
Secchi Depth-m	1.22	0.61	1.22	0.92	0.61	0.92
NH_3-N mg/m³	80	30	45	40	45	55
NO_2+NO_3-N	230	40	270	25	60	195
Inorg-N	310	70	315	65	105	250
Kjel-N	280	360	640	260	270	410
Total-N	510	400	910	285	330	605
PO_4-P	30	5	20	5	5	5
T-Soluble P	45	10	35	10	10	20
T-Particulate P	5	15	35	10	20	30
Total P	50	25	70	20	30	50
TN/TP	10.2	16.0	13.0	14.2	11.0	12.1
Phyto. Cell Density no./ml	99	374	300	100	457	8815
Chlor *a* Turner Units	19	37	29	16	34	43
Productivity mgC/m³/hr.		-	-	-	-	-

Table 2. Continued

Season	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.
	(28) Kerr 19, 20, 24		(28) Kerr 2, 8, 14		(28) Kerr 118, 1308		(28) Kerr 114, 111	
No. of Samples	8	9	8	11	5	6	5	3
Water Temp. °C	24.8	9.3	22.8	10.0	26.3	10.5	23.2	9.8
Secchi Depth-m	0.9	0.7	1.9	1.0	0.7	0.6	1.1	0.8
NH_3-N mg/m^3	29	65	61	62.3	54	245	57	65
NO_2+NO_3-N	139	228	66	236	88	134	23	183
Inorg-N	168	293	127	299	142	380	80	248
Kjel-N	356	224	224	237	650	1006	336	443
Total-N	495	453	290	473	738	1140	359	626
PO_4-P	6.9	17.6	7.2	9.0	24.0	51.0	7.0	6.7
T-Soluble P	14.4	30.4	16.2	23.1	36.8	80.0	7.0	11.7
T-Particulate P	21.0	38.4	3.4	15.4	71.6	103	12.4	26.7
Total P	35.4	68.9	19.6	38.4	108	191	23.4	38.3
TN/TP	15.1	8.4	14.6	13.4	7.7	6.4	15.7	16.2
Phyto. Cell Density no./ml	8904	1872	2936	2508	29653	25209	9520	11799
Chlor *a* Turner Units	43	33	23	27(6)	100	138	41	85
Productivity mgC/m^3/hr.	67	78	31	28(9)	109	178	47	102
				Rapids	13, 17, CO1, SW1			

	(28) Kerr 108, 103		(29) Gaston		(30) Roanoke Rapids		(44) Chowan 13, 17, CO1, SW1	
No. of Samples	7	6	1	3	None	3	16	None
Water Temp. °C	23.8	9.8	26.0	10.8	-	10.7	25.8	-
Secchi Depth-m	1.6	1.2	-	0.9	-	1.0	0.7	-
NH_3-N mg/m^3	34	52	40	75	-	38	63	-
NO_2+NO_3-N	45	194	20	280	-	265	137	-
Inorg-N	79	241	60	355	-	303	200	-
Kjel-N	285	288	390	217	-	190	677	-
Total-N	331	482	410	497	-	455	814	-
PO_4-P	7.1	6.7	16.0	8.3	-	5.0	15.0	
T-Soluble P	9.3	13.0	-	22.0	-	19.0	35.6	-
T-Particulate P	9.4	13.7	-	11.5	-	15.0	44.4	
Total P	18.7	26.7	25.0	20.0	-	34.0	80.0	-
TN/TP	19.4	21.8	16.4	18.4	-	13.4	11.4	-
Phyto. Cell Density no./ml	3426	3340	-	5590	-	4109	3969	
Chlor *a* Turner Units	29	36	-	29(2)	-	35	60	-
Productivity mgC/m^3/hr.	33	43	-	-	-	-	-	-

	(44) Chowan MS1, RO45, AL37		(13) James		(14) Rhodhiss		(15) Hickory (16) Lookout Shoals	
No. of Samples	9	None	6	None	5	None	9	8
Water Temp. °C	21.4	-	15.7	-	14.4	-	15.8	15.8
Secchi Depth-m	1.1	-	1.86	-	0.9	-	1.1	1.1
NH_3-N mg/m^3	49	-	28	-	120	-	62	51
NO_2 +NO_3-N	81	-	59	-	73	-	115	114
Inorg N	130	-	87	-	148	-	177	166
Kjel-N	465	-	205	-	350	-	296	327
Total-N	546	-	256	-	401	-	387	411
PO_4-P	8.3	-	6.2	-	38.6	-	7.5	7.9
T-Soluble P	20.0	-	7.0	-	45.0	-	12.5	13.6
T-Particulate P	22.2	-	12.0	-	26.7	-	25.0	24.3
Total P	42.2	-	17.5	-	68.7	-	38.3	38.7
TN/TP	13.9	-	21.4	-	10.6	-	16.6	18.6
Phyto. Cell Density no./ml	1846	-	1620(3)	-	2033(2)	-	5014(4)	5678(3)
Chlor *a* Turner Units	28	-	20.6(3)	-	20(2)	-	28(4)	30(3)
Productivity mgC/m^3/hr.	-	-	-	-	-	-	-	-

Table 2. Continued

Season	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.	Apr.-Nov.	Dec.-Mar.
	(17) Norman		(18) Mt. Island		(19) Wylie 789, 831		(19) Wylie 681, 708, AC22	
No. of Samples	15	5	12	3	7	1	9	2
Water Temp. °C	22.1	9.1	24.1	10.1	23.3	151	23.3	12.3
Secchi Depth-m	1.7	1.1	1.2	0.9	1.0	0.8	1.5	0.6
NH_3-N mg/m^3	58	37	35	28	76	140	74	85
NO_2+NO_3-N	81	309	102	320	130	395	84	390
Inorg-N	139	346	137	348	206	535	158	475
Kjel-N	215	156	231	113	250	220	267	205
Total-N	297	465	334	433	380	615	351	595
PO_4-P	5.5	5.0	6.0	5.0	13.6	30.0	8.1	22.5
T-Soluble P	6.4	6.6	6.5	6.6	25.1	24.0	13.2	31.0
T-Particulate P	3.9	7.8	5.3	6.6	6.3	31.0	7.9	34.0
Total P	10.5	14.4	12.2	13.3	31.4	65.0	21.1	65.0
TN/TP	29.5	33.1	27.6	32.6	13.5	9.4	16.7	9.1
Phyto. Cell Density no./ml	2267(13)	841	1348(9)	945	1854(6)	621	1849	341
Chlor *a* Turner Units	27(13)	15	27(9)	17	26(6)	9	28	15
Productivity mgC/m^3/hr.	-	-	-	-	-	-	-	-
	(19) Wylie SF 30		(20) Fishing Creek		(21) Wateree			
No. of Samples	3	1	3	None	11	3		
Water Temp. °C	25.1	13.2	21.7	-	23.5	11.3		
Secchi Depth-m	0.7	0.6	0.8	-	0.9	0.4		
NH_3-N mg/m^3	58	180	83	-	88	220		
NO_2+NO_3-N	148	420	366	-	129	441		
Inorg-N	206	600	450	-	217	661		
Kjel-N	380	250	500	-	419	350		
Total-N	528	670	867	-	548	741		
PO_4-P	23.3	50	81	-	14.8	45.0		
T-Soluble P	33.6	60	120	-	25.4	70.0		
T-Particulate P	44.6	45	30	-	25.6	45.0		
Total P	78.3	105	135	-	48.9	115		
TN/TP	6.7	6.3	6.4	-	13.4	6.9		
Phyto. Cell Density no./ml	3272	823	-	-	11112(9)	795		
Chlor *a* Turner Units	102	15	-	-	54(9)	15		
Productivity mgC/m^3/hr.	-	-	-	-	-	-		

sets of stations within the lake may be used to subdivide the data particularly where flow-through gradients have caused changes in the factors contributing to the trophic state. In other cases the data from adjacent lakes in a series along an impounded river or adjacent lakes in geographical proximity may be combined in the determination of mean values. Generally lakes of similar nature or location are arranged in Table 2 on the same page in order to facilitate comparison.

To provide a profile of the North Carolina waters that have been used in the algal assays, it was deemed essential to describe the range of values of both nutrient levels and biological parameters within the two growing seasons and the associated physical and chemical determinations. This was readily accomplished through a sorting procedure since the limnological data for each lake had been card punched. As an example a first step would be to arrange the entire set of nearly 350 data cards in rank order for one of the nutrient components e.g. inorganic nitrogen. This quickly established that the lowest value of the entire series was 20 mg/m^3 and the largest 1245 mg/m^3. The entire range of inorganic

nitrogen values was then divided into four segments with the lowest range, 20-120 mg/m^3 the next 130-245 mg/m^3 then 250-400 mg/m^3 and the highest 500-1245 mg/m^3, Table 3. A computer program then selected out all lake samples in each of the five ranges as defined by the quantity inorganic nitrogen and calculated mean values for all other variables within that set. And as can be seen in the April-November growing season, the 68 samples which occurred in the range of 20-120 mg/m^3 inorganic nitrogen had an average water temperature of 25.2°C, a Secchi depth of 1.5 m, and ammonia nitrogen concentration of 35.5 mg/m^3, etc. If for some reason the data set was incomplete for a particular parameter the actual number of samples used in the average is identified in a parenthesis following the value such as shown for chlorophyll *a* where the mean value is 37.1 Turner units was defined by averaging 53 samples rather than the total number of 68 for that set. This method of data analysis shows for example that for the five ranges of inorganic nitrogen the total soluble phosphorus increased from 11.8 mg/m^3, the mean value of those samples in the range of 20-120 mg/m^3 of inorganic nitrogen, to 54.4 mg/m^3 of total soluble phosphorus for the highest range of inorganic nitrogen.

Of most significance to the ultimate concern of this study, the relationship of the algal assay to the trophic state, the raw waters, would be the three biological determinations in the list of parameters, cell density, chlorophyll *a*, and productivity and their relationship to the ranges of the nutrient levels. As an example the cell density, in Table 3, decreased systematically with increase in inorganic nitrogen concentration. By contrast chlorophyll *a* remained relatively constant throughout the range of concentrations and productivity appeared to reach a peak in midrange decreasing towards either end. These changes were all in samples taken in the April-November growing season. Such comparisons can be made with other parameters and other related components and provide an opportunity to see how the mean values of various parameters relate to each other.

This sorting procedure has been carried out for total nitrogen, Table 4; total soluble phosphorus, Table 5; total phosporus, Table 6; and the ratio of total nitrogen to total phosphorus, Table 7. In every instance the particular values used to define the upper and lower limit of a range was an actual determination and not an arbitrary division of the total rank of values. Thus there might be a gap between upper and lower limits of ranges since these values never existed in the total range of values.

A similar sorting procedure was carried out for the three biological determinations, cell density, chlorophyll *a*, and productivity, to determine the mean values of nutrients that were associated with the ranges selected for averaging. The data of Table 8 examine the total range of cell density from a low of 62 cells per ml to the maximum value of 82,671, in five

Table 3. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter inorganic nitrogen ($NH_3+NO_2NO_3$) mg/m^3.

Sample Period	April - November				December - March			
Range of Values mg/m^3	20-120	130-245	250-400	500-1245	20-120	130-245	250-480	500-1245
N	68	65	50	17	12	19	46	17
Water Temp. °C	25.2	19.1	19.4	15.3	13.9	11.5	9.6(35)	11.2
Secchi Depth-m	1.5	1.2	0.8(44)	0.4	1.2	1.1	0.8	0.6
NH_3-N mg/m^3	35.5	59.2	56.4	199.1	44.5	72.8	63.8	272
NO_2 NO_3	30.8	119.7	238.5	443.4	92.0	119.2	260.9	426
Inorganic-N	64.1	182.1	327.8	671.3	69.9	190.3	338.5	730
Kjel-N	341.7	318.9	387.0	450.0	287.0	301.5	265.2	598
Total-N	361.2	405.8	585.1	912.2	335.3	420.8	537.4	1035
PO_4-P	7.3	11.5	17.4	21.9	6.6	17.6	10.6	69.1
T-Soluble P	11.8	18.7	32.4	54.4	14.6	25.3	22.8	93.4
T-Particulate P	15.0	15.8	21.3	49.7	12.5	31.2	19.9	44.4
Total-P	26.5	35.5	50.1	105.3	29.4	57.1	41.2	137.4
TN/TP	17.6	18.5	16.0	12.4	15.1	15.4	16.9	12.5
Cell Density no./ml	6444	3411	3181	1190	3864	5320(16)	2353(33)	2559
Chlor *a* Turner Units	37.1(53)	32.5(40)	33.8(24)	30.1(14)	39	53.3(13)	24.5(30)	36.5(13)
Productivity mgC/m^3/hr.	36.3(26)	54.8(16)	42.0(4)	26.5(2)	144(1)	445(14)	41.1(15)	78.4(5)

Table 4. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter total nitrogen - mg/m^3.

Sample Period	April - November				December - March			
Range of Values mg/m^3	120-260	270-495	510-830	910-1580	120-260	270-495	510-830	910-1580
N	36	90	57	17	3	44	35	12
Water Temp. °C	21.2	21.3	20.7	19.7	14.4	11.3	9.6(24)	11.5
Secchi Depth-m	1.6	1.3	0.8	0.5	2.1	1.0	0.6	0.6
NH_3-N mg/m^3	51.2	49.2	58.9	166	30.0	58.4	93.7	296
NO_2 NO_3	50.1	155	208	310	155	168	295	372
Inorganic-N	91.9	178	282	506	71.6	235	381	714
Kjel-N	193	285	456	654	135	224	323	837
Total-N	212	371	665	983	191	399	621	1204
PO_4-P	10.5	11.5	12.1	22.5	6.6	9.6	17.6	84.5
T-Soluble P	12.8	18.9	26.3	50.0	13.3	16.5	32.9	113
T-Particulate P	7.4	14.9	25.7	52.1	11.3	16.5	29.7	50.8
Total-P	22.7	31.6	51.9	103	28.3	32.4	62.8	163
TN/TP	17.0	17.9	16.1	15.8	14.5	18.9	11.8	14.5
Cell Density no./ml	2206(30)	4234(62)	4347(42)	8965	2534	2940(39)	2360(23)	6579(10)
Chlor *a* Turner Units	24(27)	30(56)	43(38)	55(10)	16	29(36)	29(19)	72(9)
Productivity mgC/m^3 /hr.	20(8)	36(21)	51(14)	83(5)	23(1)	45(17)	32(12)	114(5)

Table 5. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter total soluble-P - mg/m^3.

Sample Period	April - November				December - March			
Range of Values mg/m^3	5-19	20-40	45-80	90-390	5-19	20-40	45-80	90-390
N	102	57	17	6	42	33	9	6
Water Temp. °C	22.0	19.3	17.7	17.1	11.8	9.3	12.2	11.7
Secchi Depth-m	1.4	0.9	0.6	0.6	1.0	0.8	0.5	0.7
NH_3 -N mg/m^3	50.0	77	105	87.5	56	86	204	340
$NO_2 NO_3$	89.2	193	347	318	191	271	336	344
Inorganic-N	146	269	408	356	260	333	540	685
Kjel-N	290	395	453	444	236	336	435	886
Total-N	378	577	682	646	440	589	771	1230
PO_4 -P	6.4	10.4	31.0	77.2	5.3	13.1	42.0	187
T-Soluble P	8.4	25.5	60.1	130	10.4	27.1	65.5	213
T-Particulate P	13.1	25.1	41.8	27.5	13.6	31.1	48.3	52.5
Total-P	22.7	51.5	100	145	24.0	59.1	113	265
TN/TP	21.2	13.6	8.1	7.1	21.6	11.6	7.3	5.1
Cell Density no./ml	4260(86)	3492(47)	9940(12)	1327(4)	2844(36)	2793(25)	5107(7)	5299
Chlor *a* Turner Units	30(77)	39(39)	47(2)	30(3)	28(37)	36(16)	49(7)	58
Productivity mgC/m^3 /hr.	34(34)	51(11)	149(12)	-	31(16)	50(16)	179(1)	138(2)

Table 6. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter total-P - mg/m^3.

Sample Period	April - November				December - March			
Range of Values mg/m^3	5-18	20-40	43-80	85-410	5-18	20-40	43-80	85-410
N	53	81	36	27	16	35	27	17
Water Temp. °C	22.2	22.1	19.5	17.5	10.7	10.9	11.4	10.5
Secchi Depth-m	1.7	1.1	0.8	0.5	1.3	0.9	0.6	0.6
NH_3-N mg/m^3	38	60	60	113	58	71	82	241
NO_2NO_3	69	108	194	341	179	197	260	342
Inorganic-N	122	188	269	428	249	285	326	584
Kjel-N	224	382	422	489	180	280	333	588
Total-N	298	470	575	725	375	485	589	930
PO_4-P	5.6	8.0	13.8	34.6	4.9	6.5	13.7	82.4
T-Soluble P	7.4	14.7	28.9	63.2	7.7	14.8	28.0	109
T-Particulate P	4.5	13.6	31.4	52.6	5.3	13.9	28.9	62.6
Total-P	11.8	27.5	60.2	118.0	12.5	28.2	57.8	172
TN/TP	24.7	17.8	11.1	8.3	29.5	18.1	10.4	6.1
Cell Density no./ml	2113(44)	5038(59)	5070(26)	7249(18)	1084(13)	3482(27)	3459(18)	4337
Chlor *a* Turner Units	24(41)	32(49)	50(23)	44(17)	18(13)	31(27)	37(14)	56(12)
Productivity $mgC/m^3/hr.$	13(12)	43(26)	56(6)	124(3)	21(3)	33(16)	69(10)	77(6)

Table 7. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter TN/P.

Sample Period	April - November					December - March				
Range of Values Ratio	1.9-6.8	7.0-10.8	11.0-15.7	15.0-21.5	22.0-87.0	1.9-6.8	7.0-10.8	11.0-15.7	16.0-21.5	22.0-87.0
N	14	42	46	44	50	11	25	26	13	18
Water Temp. °C	20.5	20.1	20.2	21.9	21.9	13.3	10.1	10.9	11.1	10.5
Secchi Depth-m	0.8	0.7	1.2	1.3	1.4	0.6	0.6	0.9	0.9	1.2
NH_3-N mg/m^3	73	71	61	55	52	185	144	85	66	79
NO_2 NO_3	164	240	122	84	123	330	243	225	156	263
Inorganic-N	271	301	202	152	187	516	367	299	264	335
Kjel-N	486	364	302	357	360	472	357	327	317	238
Total-N	648	545	402	435	465	802	609	545	508	499
PO_4-P	34.7	16.1	11.3	7.2	8.0	101	17.2	10.7	6.9	5.1
T-Soluble P	61.9	36.9	21.0	11.7	9.3	130	34.4	23.9	14.1	9.4
T-Particulate P	55.5	35.6	13.5	12.5	6.4	60.9	37.5	19.0	13.5	7.2
Total-P	113	64.6	36.5	24.3	19.4	191	70.7	43.5	26.8	16.6
TN/TP	5.1	8.5	13.5	17.9	30.6	4.7	8.7	13.0	17.9	32.7
Cell Density no./ml	12306(10)	3350(28)	3818(35)	4583(40)	3048(33)	4963	4039	3266(20)	2669	1130(13)
Chlor *a* Turner Units	72(8)	39(27)	28(32)	36(33)	24(27)	56(9)	40(16)	32(16)	32(11)	19(14)
Productivity mgC/m^3/hr.	124(3)	57(6)	32(14)	49(12)	22(13)	230(2)	44(4)	26(8)	54(7)	18(4)

Table 8. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter cell density No./ml.

Sample Period	April - November					December - March				
Range of Values no./ml	62-599	604-1183	1210-2366	2449-9926	13085-82671	62-599	604-1183	1210-2386	2449-9926	11308-82671
N	23	26	41	46	14	22	22	7	23	7
Water Temp. °C	15.8	17.3	22.0	22.9	27.2	10.9	10.5	11.5	11.5	11.8
Secchi Depth-m	0.8	1.1	1.3	1.2	1.0	1.0	0.9	0.8	0.9	0.7
NH_3-N mg/m^3	133	75	67	55	35	75	116	189	74	151
NO_2NO_3	232	180	132	92	99	224	275	284	221	93
Inorganic-N	345	275	204	147	134	299	392	473	295	203
Kjel-N	339	295	305	354	496	311	275	429	318	786
Total-N	559	488	432	447	595	535	551	713	540	806
PO_4-P	14.9	7.7	10.0	9.1	13.2	17.6	27.2	49.3	18.0	29.1
T-Soluble P	32.9	18.5	18.7	15.9	20.3	28.6	41.6	56.1	27.0	45.4
T-Particulate P	32.0	15.6	16.9	18.0	33.3	22.4	27.9	30.3	22.0	79.3
Total-P	64.2	33.6	35.8	33.9	53.7	51.1	69.5	86.4	49.0	144
TN/TP	14.9	21.5	16.0	17.5	14.7	16.8	16.3	15.5	13.9	8.4
Cell Density no./ml	410	905	1801	4803	23326	305	902	1712	5090	25397
Chlor *a* Turner Units	21	18(22)	33(37)	42(40)	67(11)	20(18)	20(19)	31(5)	37	138
Productivity mgC/m^3/hr.	-	15(7)	31(10)	44(18)	80(9)	5(9)	27(9)	40(5)	64(15)	176

ranges. In the growing season the soluble components of nitrogen show a clearly indicated inverse relationship between cell number and nutrient. However, the phosphorus mean values are less systematic in their relationship to cell number except that the overall values in the winter period seem to be somewhat higher than comparable values in the April-November growing season.

A similar sorting for chlorophyll *a*, Table 9, in four ranges of values, shows a strong positive relationship to cell density and productivity, as would be expected, and to Kjeldahl nitrogen another measure of biological materials. Nevertheless, the phosphorus relationship continues to be ambiguous with higher mean values at the extreme ends of the ranges than found in the midranges.

The sorting of the dependent variable productivity, Table 10, describes for those samples taken in the growing season a parallel increase in organic nitrogen and soluble phosphorus components with an increase in productivity. The mean values of the other biological determinations, cell density and chlorophyll *a*, show a similar parallelism. Some of the anomalies may be explained in part by the fact that cell density may not be as precise a measure of total biological activity as cell volume which probably is more nearly reflected in the measurements of chlorophyll *a* and productivity. An important relationship of nutrients, which appears to be strikingly linked to the biological activity is that of the ratio between total nitrogen and total phosphorus. In the case of both chlorophyll *a* and productivity these parameters increase as this ratio systematically decreases.

Algal Assay

One aspect of the algal assay which was clearly established by the "interlaboratory precision test" was the degree variability between laboratories even though within a laboratory the degree of precision could be maintained at a reasonable level (Weiss and Helms, 1971). One approach to extablishing the systematics of reliability in the algal assay is the use of the NAAM control media with each of the spiked sampled series. This was essential to the determination of biomass in the spike samples which was based on the actual dry weight determination of biomass grown in NAAM under the same conditions as that of the specific spiked series. To provide some indication of the variability of the assay system over the several years of this investigation, all of the NAAM samples used in conjunction with each of the specific lake samples have been arranged in Table 11. This describes the actual measurement of biomass, maximum and minimum and mean values, standard deviation, the unit absorbance for these same samples, and the mean values and standard deviation for unit absorbance.

Table 9. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter chlorophyll *a* Turner Units.

Sample Period	December - March				April - November			
Range of Values Units	8-14	16-30	31-60	64-139	8-14	16-30	31-60	64-139
N	15	64	40	12	17	24	19	6
Water Temp. °C	18.6	19.5	23.5	25.8	10.1	12.0	11.7	11.7
Secchi Depth-m	1.6	1.2	1.1	0.7	1.2	0.8	0.9	0.6
NH_3-N mg/m^3	82	86	59	36	94	104	102	160
NO_2NO_3	71	174	111	148	270	248	240	162
Inorganic-N	148	260	174	183	364	352	342	322
Kjel-N	216	288	333	617	215	309	353	738
Total-N	278	462	445	765	486	556	593	900
PO_4-P	19.6	7.2	9.1	19.5	16.7	28.1	23.7	54.0
T-Soluble P	26.9	17.9	15.3	43.4	24.2	38.4	40.5	77.1
T-Particulate P	5.1	21.2	17.2	51.6	15.9	23.9	19.0	58.3
Total P	27.5	39.1	33.3	95.0	40.1	62.4	59.6	135
TN/TP	18.9	17.2	16.4	10.0	21.3	14.8	17.2	8.0
Cell Density no./ml	1164	2030	5863	14879	644	1505	4330	18048
Chlor *a* Turner Units	11	23	41	98	12	22	39	132
Productivity mgC/m^3/hr.	7(5)	26(13)	63(13)	106(5)	9(6)	24(7)	81(9)	156(4)

Table 10. Mean values of physical, chemical and biological parameters of lake samples used for algal assays as determined within value ranges of indicated parameter productivity - mgC/m^3/hr.

Sample Period	April - November			December - March		
Range of Values mgC/m^3/hr.	1-27	30-57	60-281	1-27	30-57	50-281
N	17	19	11	15	8	8
Water Temp. °C	23.3	23.9	23.5	9.0	9.7	9.8
Secchi Depth-m	2.1	1.2	0.8	1.1	1.0	0.6
NH_3-N mg/m^3	42	46	76	85	41	219
NO_2NO_3	56	95	116	166	214	178
Inorganic-N	100	167	192	251	256	297
Kjel-N	285	359	533	249	249	710
Total-N	343	472	649	415	463	888
PO_4-P	6.1	7.2	15.9	9.0	6.2	28.0
T-Soluble P	8.9	13.6	24.4	16.3	14.5	53.7
T-Particulate P	5.1	16.3	41.9	16.4	21.5	54.4
Total-P	13.9	29.2	66.3	32.7	36.0	108
TN/TP	25.2	15.5	14.1	16.9	14.9	10.4
Cell Density no./ml	2422	9446(17)	14124	1493	4211	13314
Chlor *a* Turner Units	18(12)	36(14)	71	16(11)	37	110
Productivity mgC/m^3/hr.	14	40	95	13	44	150

Table 11. Algal assay, growth of *Selenastrum capricornutum* in NAAM (14 days, 24°C, 400 f.c.) NAAM absorbance and biomass used to determine unit absorbance for conversion of spiked samples to biomass.

Lake(s)	Time Span of Assays	Number of NAAM Samples Used	Biomass in NAAM, mg/l				Unit Absorbance			
			Max.	Min.	Mean	S.D.	Max.	Min.	Mean	S.D.
University, Michie	Oct. 12, 1971-Sept. 11, 1973	13	148.8	110.9	125.0	10.6	307.1	225.9	259.1	23.5
Belews, Hyco	Oct. 8, 1971-Aug. 9, 1974	19	144.3	88.4	119.3	12.6	403.1	211.1	263.7	45.8
Kerr, Gaston, Roanoke Rapids	Sept. 20, 1971-Jan. 7, 1975	20	136.9	85.1	114.9	13.1	275.1	182.0	240.2	33.9
Chowan River	Sept. 7, 1972-Aug. 10, 1973	4	131.8	117.5	125.1	6.1	262.0	232.1	247.5	14.5
Kerr Scott, Yadkin, High Rock, Tuckertown, Badin, Tillery, Blewett Falls	Dec. 6, 1971-Jan. 7, 1975	7	123.2	92.2	107.7	13.8	259.4	207.6	230.2	17.4
James, Rhodhiss, Hickory, Lookout Shoals	Nov. 15, 1971-Sept. 24, 1974	8	135.9	67.8	112.1	20.4	367.9	178.8	252.7	53.1
Norman, Mt. Island, Wylie, Fishing Creek, Wateree	Nov. 15, 1971-Sept. 24, 1974	7	113.4	101.8	119.2	11.6	367.9	235.0	268.6	46.3
Norman, Mt. Island, Wylie, South Fork, Wateree	Sept. 24, 1973-June 25, 1974	16	123.2	83.9	110.0	10.0	309.0	200.9	266.7	28.3
Black, Clear, Davies, Finches, Hodgins, Johns, Jackson, Jones, Lytches, Mattamuskeet, McNeils, McKensie, Monroe, Orton, Phelps, Salters, Singletary, Tull, Waccamaw, White	Oct. 27, 1971-Jan. 21, 1975	6	114.1	85.1	99.7	11.1	367.9	182.0	242.5	65.0

It is clear that over a period of several years variability is inherently built into a bioassay simply because of changes in personnel, changes in procedures which creep into an assay protocol, changes in quality of controls and perhaps even changes in the growth response of the specific alga used in the assay. However, the biomass determinations, as grown in NAAM, do indicate that on the average the values over the several years are comparable. The standard deviation of the sets indicate a variation on the order of 10-20 percent of the mean. Growth of the test alga in media or waters of low nutrient concentrations has shown a good deal greater variability and it is such variation that must be kept in mind in any evaluation of an assay. In this particular study it has been instrumental in determining the approach used in the final evaluation of the results.

Assay for Limiting Nutrients

The basic procedure for establishing limiting nutrients in a surface water is to determine the growth response of a seeded sample, following pretreatment either by autoclaving or filtration, to the addition of phosphorus and nitrogen nutrient supplements or spikes. For the summary of the extensive series of algal assays on the surface waters of North Carolina, the response of spike samples has been assembled in a series of tables which describe by the response at each specific spike and the magnitude of the response, under the standard incubator growth conditions, as to whether there was indication of growth limitation by phosphorus, nitrogen or both, Tables 12-22. Since the mean growth of the control as well as standard deviation is indicated, the significance of any specific response can be readily estimated.

For purposes of this nutrient limiting evaluation, the growth ranges of biomass developed by specific spikes were defined as 0-5, 5.1-15, 15.1-30, and 30 mg/l. This is growth in excess of that found in the control, the biomass that grew in the unspiked sample. The control growth itself defines a magnitude of nutrient level since from sample to sample and lake to lake the control growth describes the ambient nutrient levels. The data are arranged either by samples taken from one lake, samples taken from a set of stations in a particular body of water and compared to another set of stations in the same lake or in still other instances where the number of samples taken from any one lake was small, assay results from ponds that showed comparable limnological characteristics were compiled into one set. As will be noted in examination of these data, the growth in the range 0-5 mg/l was generally indicative of no significant response particularly when examined in relationship to the variation indicated by size of the standard deviation. Growth at higher ranges, 5.1 mg/l and above, generally described a significant response to the spike and is indicative that this nutrient was probably limiting to growth.

Table 12. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
University	N=20	9.2		10.7					3.1		3.6				
	05P		8.2	7.3	18	2	-	-		4.0	3.3	20	-	-	-
	50P		11.1	0.3	14	5	1	-		10.0	7.8	13	3	4	-
July 1971-	75N		8.0	6.7	17	3	-	-		3.6	2.7	20	-	-	-
July 1973	750N		10.5	10.4	17	1	2	-		3.5	3.0	20	-	-	-
	5P+75N		9.2	7.3	18	2	-	-		4.2	2.7	18	2	-	-
	50P+750N		27.9	12.3	3	2	13	2		23.1	12.5	4	2	8	6
Michie	N=17	4.4		3.9					2.8		4.2				
	05P		5.1	3.9	17	-	-	-		3.2	2.2	16	1	-	-
	50P		9.5	7.4	10	6	1	-		10.8	8.6	7	6	4	-
July 1971-	75N		4.2	3.2	17	-	-	-		2.7	2.1	17	-	-	-
July 1973	750N		5.7	6.6	16	-	1	-		2.6	2.0	17	-	-	-
	5P+75N		5.5	3.7	16	1	-	-		3.6	2.4	16	1	-	-
	50P+750N		23.9	12.5	2	4	7	4		24.0	11.7	3	2	7	5
Chowan	N=17	4.3		3.1					1.9		2.3				
13,17,CO1,	05P		5.6	5.3	15	2	-	-		2.6	2.9	15	2	-	-
SW1	50P		7.2	6.2	13	4	-	-		5.7	4.8	12	5	-	-
Sept. 1972-	75N		7.9	6.6	13	4	-	-		1.9	3.2	15	-	-	-
Aug. 1973	750N		8.6	5.5	10	6	1	-		3.9	7.6	17	1	-	
	5P+75N		8.2	6.7	12	5	-	-		2.7	3.1	17	-	-	-
	50P+750N		26.9	13.6	3	1	8	5		19.7	9.4	2	3	12	-
Chowan	N=8	2.3		2.0					1.1		1.5				
Al, RO 45	05P		2.5	2.5	8	-	-	-		1.1	1.3	8	-	-	-
	50P		3.5	2.6	8	-	-	-		3.3	3.9	7	1	-	-
Sept. 1972-	75N		3.3	2.5	7	1	-	-		1.0	0.8	8	-	-	-
Aug. 1973	750N		3.9	2.9	7	1	-	-		4.2	8.5	7		1	-
	5P+75N		4.6	2.9	6	2	-	-		1.7	0.9	8	-	-	-
	50P+750N		24.0	9.7	1	1	5	1		17.1	10.1	1	3	3	1

Table 13. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Belews 1906	N=15	1.8		1.8					1.2		1.1				
	05P		2.7	1.8	15	-	-	-		2.1	1.2	15	-	-	-
August 1971-	50P		5.1	3.9	10	5	-	-		5.6	3.6	10	5	-	-
April 1974	75N		2.0	1.8	15	-	-	-		1.1	0.8	15	-	-	-
	750N		2.0	2.2	15	-	-	-		1.2	1.2	15	-	-	-
	5P+75N		2.9	2.0	15	-	-	-		2.0	2.0	15	-	-	-
	50P+750N		19.5	10.6	1	7	4	3		18.0	12.2	2	6	5	2
Belews 1116	N=15	2.6		1.7					1.3		1.2				
	05P		3.0	1.9	15	-	-	-		1.7	1.2	15	-	-	-
Aug. 1971-	50P		5.6	3.5	11	4	-	-		4.4	4.4	12	3	-	-
April 1974	75N		2.9	1.7	15	-	-	-		1.5	1.2	15	-	-	-
	750N		2.5	1.9	15	-	-	-		1.2	0.9	15	-	-	-
	5P+75N		3.1	1.6	15	-	-	-		2.0	1.9	14	1	-	-
	50P+750N		19.1	9.7	2	3	8	2		14.3	14.0	7	2	4	2
Hyco 1906	N=9	5.1		3.2					1.4		1.8				
	05P		3.6	3.3	9	-	-	-		2.8	2.6	9	-	-	-
Oct. 1972-	50P		4.5	3.0	9	-	-	-		4.9	4.3	6	3	-	-
July 1974	75N		5.0	3.6	9	-	-	-		1.8	2.0	9	-	-	-
	750N		5.3	4.5	6	3	-	-		1.6	2.1	9	-	-	-
	5P+75N		7.3	5.6	7	2	-	-		3.5	2.5	9	-	-	-
	50P+750N		30.0	7.5	-	1	6	2		28.9	9.5	-	1	5	3
Hyco 1116	N=7	3.7		2.4					2.8		3.6				
	05P		3.8	2.9	7	-	-	-		2.6	1.8	7	-	-	-
Oct. 1972-	50P		5.8	4.5	6	1	-	-		5.9	3.8	4	3	-	-
July 1974	75N		3.8	3.0	7	-	-	-		2.0	2.0	7	-	-	-
	750N		6.3	8.8	6	-	1	-		4.9	8.8	6	-	1	-
	5P+75N		5.2	3.2	7	-	-	-		2.7	2.7	7	-	-	-
	50P+75N		27.9	13.4	1	-	4	2		30.5	9.2	-	1	3	3

Table 14. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
James 201,	N=7	2.0		2.2					0.6		0.5				
206,210,212	05P		2.9	2.2	7	-	-	-		1.1	1.0	7	-	-	-
July 1971-	50P		3.2	1.8	7	-	-	-		1.3	1.3	7	-	-	-
Nov. 1973	75N		2.7	3.1	7	-	-	-		0.9	0.9	7	-	-	-
	750N		7.0	11.3	6		1	-		3.9	7.2	6	-	1	-
	5P+75N		3.8	2.3	7	-	-	-		1.9	1.2	7	-	-	-
	50P+750N		15.1	10.8	2	2	3	-		9.5	8.5	3	2	2	-
Rhodhiss	N=14	9.8		6.2					5.4		3.9				
3,7,9,13,	05P		9.6	6.4	13	1	-	-		6.4	4.8	13	1	-	-
1724, 1778,	50P		10.0	6.1	13	1	-	-		7.8	5.9	11	3	-	-
1836	75N		11.1	7.2	13	1	-	-		6.8	5.0	12	2	-	-
Nov. 1971-	750N		25.5	18.8	5	1	6	2		13.6	13.4	9	2	2	1
Nov. 1973	5P+75N		12.1	6.2	12	2	-	-		7.0	5.0	13	1	-	-
	50P+750N		35.1	14.1	-	2	9	3		28.9	16.2	2	2	7	3
Hickory 1542,	N=5	9.9		6.2					0.8		1.2				
1632,1489	05P		6.8	4.2	5	-	-	-		1.2	1.3	5	-	-	-
	50P		8.9	3.1	5	-	-	-		5.9	4.9	3	2	-	-
Aug. 1971-	75N		9.0	5.6	5	-	-	-		0.4	0.5	5	-	-	-
Nov. 1973	750N		15.1	12.9	3	1	1	-		0.4	0.7	5	-	-	-
	5P+75N		11.3	5.7	5	-	-	-		1.9	1.5	5	-	-	-
	50P+750N		35.3	4.8	-	-	5	-		21.1	10.7	-	1	3	1
Lookout	N=4	8.5		9.5											
Shoals 1466,	05P		8.1	10.5	4	-	-	-	2.8		3.3	4	-	-	-
1498, 1438	50P		10.6	7.0	3	1	-	-		3.6	4.8	1	3	-	-
April 1972-	75N		7.2	9.2	4	-	-	-		9.3	2.4	4	-	-	-
Nov. 1973	750N		5.9	7.3	4	-	-	-		3.3	4.1	4	-	-	-
	5P+75N		10.3	9.6	3	1	-	-		3.3	4.3	3	1	-	-
	50P+750N		37.8	7.7	-	-	2	2		4.6	5.7	-	1	1	2

Table 15. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Norman 109,	N=11	1.3		1.6					0.5		1.2				
116,126,	05P		2.8	2.9	10	1	-	-		1.1	1.4	11	-	-	-
1302,DC26,	50P		6.5	4.5	5	6	-	-		6.4	3.7	3	8	-	-
RM10	75N		1.4	1.8	11	-	-	-		0.7	1.1	11	-	-	-
Aug. 1971-	750N		1.2	1.7	11	-	-	-		0.7	1.2	11	-	-	-
Nov. 1973	5P+75N		3.2	2.2	11	-	-	-		1.3	1.3	11	-	-	-
	50P+750N		19.3	10.5	-	5	5	1		18.3	9.5	1	4	5	1
Mt. Island	N=12	0.4		0.5					0.1		0.1				
941,960,977	05P		1.3	1.4	12	-	-	-		0.6	1.0	12	-	-	-
	50P		4.1	2.9	8	4	-	-		4.5	2.6	7	5	-	-
July 1973-	75N		1.1	1.5	12	-	-	-		0.5	0.7	12	-	-	-
Jan. 1974	750N		1.3	1.5	12	-	-	-		0.3	0.4	12	-	-	-
	5P+750N		3.7	4.4	10	2	-	-		2.5	4.7	11	-	1	-
	50P+750N		15.9	3.4	-	5	7	-		14.5	6.0	1	5	6	-
Wylie 789,	N=9	5.9		4.8					1.0		2.0				
83, 831	05P		4.5	3.7	9	-	-	-		2.2	2.3	8	1	-	-
	50P		4.9	3.3	9	-	-	-		3.9	2.2	8	1	-	-
July 1973-	75N		5.5	4.6	9	-	-	-		1.6	3.0	9	-	-	-
Jan. 1974	750N		13.2	10.5	5	2	2	-		4.1	8.1	7	-	1	1
	5P+75N		6.2	4.6	9	-	-	-		2.5	2.3	9	-	-	-
	50P+750N		22.2	7.5	-	2	7	-		18.6	7.3	1	1	7	-
Wylie 681,	N=13	5.8							2.3		3.8				
708,74,	05P		5.7	4.6	12	1	-	-		3.0	4.1	13	-	-	-
AC22	50P		5.5	4.4	13	-	-	-		4.7	4.5	11	2	-	-
July 1973-	75N		5.8	4.2	13	-	-	-		2.7	3.9	13	-	-	-
Jan. 1974	750N		9.1	7.5	10	3	-	-		4.2	5.8	12	1	-	-
	5P+75N		6.7	4.5	13	-	-	-		3.5	4.0	12	1	-	-
	50P+750N		21.6	7.3	-	7	6	-		17.6	9.0	1	7	5	-

Table 16. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station (s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Wylie	N=4	10.2		4.3					8.9		9.5				
SF 30	05P		8.6	3.9	4	-	-	-		9.7	9.3	4	-	-	-
	50P		9.7	3.2	4	-	-	-		11.0	8.3	4	-	-	-
July 1973-	75N		10.0	5.4	4	-	-	-		13.7	16.6	3	-	1	-
Jan. 1974	750N		27.4	8.1	-	1	3	-		15.6	17.5	3	-	1	-
	5P+75N		12.1	5.7	3	1	-	-		10.3	8.6	4	-	-	-
	50P+750N		32.6	6.4	-	-	4	-		34.4	13.1	-	-	4	-
Fishing	N=3	28.9		4.6					20.1		5.6				
Creek 27,31	05P		26.6	8.7	3	-	-	-		16.5	8.8	3	-	-	-
	50P		24.9	6.4	3	-	-	-		15.1	10.5	3	-	-	-
Aug. 1971-	75N		29.1	7.8	3	-	-	-		17.9	11.1	3	-	-	-
April 1972	750N		60.0	8.5	-	-	1	2		36.6	23.5	-	1	2	-
	5P+75N		28.9	6.9	3	-	-	-		18.7	9.0	3	-	-	-
	50P+750N		60.6	6.5	-	-	1	2		46.8	8.3	-	-	3	-
Wateree 2,	N=15	11.1		9.5					5.6		7.0				
58,100,104,	05P		7.8	7.5	15	-	-	-		4.5	4.9	15	-	-	-
157	50P		7.9	7.3	15	-	-	-		6.5	4.8	12	3	-	-
Aug. 1971-	75N		9.7	8.1	15	-	-	-		5.8	6.4	14	-	1	-
Jan. 1974	750N		21.6	15.9	8	2	5	-		11.7	14.4	10	2	3	-
	5P+75N		11.0	8.1	15	-	-	-		7.0	5.8	15	-	-	-
	50P+750N		31.0	9.8	1	2	11	1		30.1	8.5	1	1	9	4

Table 17. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Kerr-Roanoke	N=8	12.1		11.5					4.2		5.9				
19,24	05P		8.9	6.7	8	-	-	-		5.3	5.6	7	1	-	-
	50P		8.6	6.0	8	-	-	-		7.2	7.4	6	2	-	-
Dec. 1971-	75N		12.2	7.8	8	-	-	-		5.0	7.3	8	-	-	-
March 1974	750N		26.4	11.8	2	2	3	1		13.7	16.3	5	-	2	1
	5P+75N		12.7	8.5	8	-	-	-		6.5	6.7	8	-	-	-
	50P+750N		33.5	10.2	-	3	4	1		28.3	12.5	1	-	5	2
Kerr-Nutbush	N=11	12.4		13.1					4.7		9.9				
118, 1308	05P		10.8	12.0	10	1	-	-		4.0	10.5	11	-	-	-
	50P		13.2	12.6	9	2	-	-		4.3	9.7	11	-	-	-
Jan. 1973-	75N		12.9	12.3	10	1	-	-		5.6	11.3	11	-	-	-
May 1974	750N		32.7	17.0	2	1	6	2		14.6	18.6	5	3	3	-
	5P+75N		13.0	11.4	9	2	-	-		5.6	10.9	11	-	-	-
	50P+750N		35.9	16.8	1	1	6	3		22.6	18.9	2	3	4	2
Kerr-Nutbush	N=8	3.0		2.7					0.7		0.6				
111, 114	05P		3.3	3.0	8	-	-	-		8.0	0.8	8	-	-	-
	50P		5.9	6.3	6	2	-	-		1.8	1.6	8	-	-	-
Jan. 1973-	75N		3.6	4.0	8	-	-	-		0.9	0.9	8	-	-	-
May 1974	750N		8.6	12.9	6	1	-	1		4.6	8.9	7	-	1	-
	5P+75N		5.5	3.5	7	1	-	-		1.0	0.9	8	-	-	-
	50P+750N		28.9	6.7	-	-	6	2		19.9	13.8	-	2	2	4
Kerr-Nutbush	N=13	2.0		2.9					1.6		3.6				
103, 108	05P		1.7	1.9	13	-	-	-		2.0	3.5	13	-	-	-
	50P		6.0	3.4	9	4	-	-		7.3	7.2	7	5	1	-
Sept. 1972-	75N		2.3	4.4	12	1	-	-		1.7	4.0	13	-	-	-
May 1974	750N		4.3	9.3	11	1	1	-		5.3	12.5	11	-	1	1
	5P+75N		3.5	3.9	13	-	-	-		3.6	5.3	12	1	-	-
	50P+750N		25.9	8.1	-	1	9	3		23.8	10.6	1	1	8	3

Table 18. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	51.1-30	>30 mg/l
Kerr-Roanoke	N=19	5.8		6.0					1.4		1.8				
1, 2, 8	05P		6.8	6.8	16	3	-	-		2.4	3.0	18	1	-	-
	50P		9.7	7.2	13	5	1	-		5.6	6.4	13	4	2	-
July 1971-	75N		6.2	6.4	18	1	-	-		1.8	2.4	19	-	-	-
May 1974	750N		8.0	9.6	17	1	1	-		5.6	13.3	17	-	-	2
	5P+75N		7.9	6.7	16	3	-	-		2.4	2.3	19	-	-	-
	50P+750N		28.1	12.6	-	2	14	3		20.8	14.3	4	3	6	6
Gaston	N=5	7.5		4.9					1.2		1.6				
82,166,324	05P		8.9	5.2	4	1	-	-		1.9	2.1	5	-	-	-
	50P		12.9	5.5	3	2	-	-		8.5	7.3	2	2	1	-
July 1971-	75N		6.9	5.0	5	-	-	-		1.4	1.6	5	-	-	-
March 1974	750N		6.1	4.3	5	-	-	-		1.3	1.6	5	-	-	-
	5P+75N		8.4	4.7	5	-	-	-		1.8	1.9	5	-	-	-
	50P+750N		34.9	14.6	-	1	2	2		19.1	16.4	-	3	1	1
Roanoke	N=3	6.0		2.3					0.2		0.4				
Rapids 2,	05P		4.8	3.4	3	-	-	-		0.5	0.5	3	-	-	-
28, 56	50P		11.3	3.3	2	1	-	-		8.2	2.0	-	3	-	-
Mar. 15, 1974	75N		3.1	2.7	3	-	-	-		0.1	0.2	3	-	-	-
	750N		2.7	2.9	3	-	-	-		0.1	0.1	3	-	-	-
	5P+75N		4.9	2.7	3	-	-	-		0.3	0.4	3	-	-	-
	50P+750N		33.2	1.4	-	-	3	-		12.4	12.9	-	1	2	-

Table 19. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Kerr Scott	N=6	3.0		1.5					0.3		0.3				
3 Stations	05P		3.8	1.6	6	-	-	-		0.4	0.3	6	-	-	-
	50P		5.1	1.3	6	-	-	-		2.2	0.4	6	-	-	-
April 1974	75N		1.9	0.5	6	-	-	-		0.3	0.3	6	-	-	-
	750N		2.5	0.2	6	-	-	-		0.5	0.4	6	-	-	-
	5P+75N		3.1	0.5	6	-	-	-		0.6	0.5	6	-	-	-
	50P+750N		20.0	6.1	-	2	4	-		4.7	3.1	2	4	-	-
High Rock	N=6	11.8		9.0					4.0		5.8				
594,654,750	05P		7.9	7.2	6	-	-	-		6.1	7.2	6	-	-	-
	50P		9.5	5.2	4	2	-	-		15.3	2.2	-	4	2	-
April 4,1974	75N		10.7	9.4	4	2				6.4	8.1	4	2		
	750N		25.4	12.9	-	4	2	-		6.2	8.5	4	2	-	-
	5P+75N		13.7	8.6	4	2	-	-		4.9	6.0	6	-	-	-
	50P+750N		35.5	8.8	-	-	6	-		16.0	12.4	2	-	4	-
Tuckertown	N=6	16.1		9.8					0.1		-				
516,548,580	05P		16.4	8.0	6	-	-	-		0.1	-	6	-	-	-
	50P		20.7	2.9	4	2	-	-		15.0	1.2	-	4	2	-
	75N		14.9	10.5	6	-	-	-		0.1	-	6	-	-	-
	750N		15.8	10.0	6	-	-	-		0.3	0.4	6	-	-	-
	5P+75N		15.0	6.9	6	-	-	-		0.8	1.1	6	-	-	-
	50P+750N		37.3	6.7	-	-	6	-		13.1	10.8	2	2	2	-

Table 20. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Table 20

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Badin 438,	N=6	9.8		5.2					6.0		0.4				
458, 490	05P		12.5	2.6	4	2	-	-		1.0	3.2	6	-	-	-
	50P		18.7	4.1	2	2	2	-		18.2	1.5	-	-	6	-
April 5, 1974	75N		13.3	3.0	4	2	-	-		0.4	0.4	6	-	-	-
	750N		16.2	5.2	2	4	-	-		0.4	0.6	6	-	-	-
	5P+75N		11.0	1.2	4	2	-	-		0.4	0.6	6	-	-	-
	50P+750N		37.8	2.5	-	-	4	2		29.4	3.0	-	-	4	2
Tillery	N=14	13.1		9.5					1.9		1.6				
	05P		14.2	7.9	12	2	-	-		3.0	1.2	12	2	-	-
Nov. 1971-	50P		19.6	10.5	6	8	-	-		10.4	9.5	8	2	4	
April 1974	75N		13.0	9.8	12	2	-	-		2.0	1.7	14	-	-	-
	750N		14.5	11.9	12	2	-	-		2.0	1.7	14	-	-	-
	5P+75N		14.4	9.2	12	2	-	-		2.6	1.3	14	-	-	-
	50P+750N		30.3	15.5	2	2	10	-		13.6	13.5	8	-	4	2
Blewett	N=6	10.7		2.6					0.8		1.0				
2, 26, 56	05P		14.4	3.2	4	2	-	-		2.4	1.8	6	-	-	-
	50P		18.5	0.2	2	4	-	-		16.8	2.2	-	2	4	-
April 9, 1974	75N		13.6	1.1	6	-	-	-		1.1	1.5	6	-	-	-
	750N		13.5	5.5	4	2	-	-		1.1	1.1	6	-	-	-
	5P+75N		13.0	5.8	4	2	-	-		1.5	1.4	6	-	-	-
	50P+750N		33.3	2.9	-	-	6	-		31.5	1.8	-	-	2	4

Table 21. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth mg/l		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Tull, Davies,	N=4	0.6		1.2					0.2		0.2				
McKensie,	05P		4.1	7.4	3	-	1	-		0.6	0.6	4	-	-	-
Orton	50P		6.1	9.2	3	-	1	-		1.0	0.9	4	-	-	-
	75N		4.6	7.0	3	-	1	-		1.5	2.4	4	-	-	-
	750N		3.3	4.2	3	-	1	-		0.4	0.3	4	-	-	-
	5P+75N		3.8	3.9	3	-	1			0.8	1.2	4	-	-	-
	50P+750N		14.3	3.8	-	3	1	-		13.0	4.2	-	2	2	-
Jackson,	N=3	21.8		3.9					16.6		6.3				
Black	05P		18.6	5.7	3	-	-	-		14.4	4.7	3	-	-	-
	50P		19.1	5.4	3	-	-	-		15.8	5.5	3	-	-	-
	75N		20.3	6.1	3	-	-	-		16.5	4.8	3	-	-	-
	750N		34.9	8.3	-	3	-	-		24.8	2.9	-	3	-	-
	5P+75N		20.4	6.9	3	-	-	-		16.8	6.0	3	-	-	-
	50P+750N		34.8	8.0	-	2	1	-		28.9	0.6	-	2	1	-
Finches,	N=7	2.9		2.7					1.2		1.3				
Hodgins,	05P		2.5	1.9	7	-	-	-		1.9	2.4	7	-	-	-
McNeils,	50P		3.1	2.4	7	-	-	-		3.3	2.3	7	-	-	-
Lytches,	75N		3.8	3.8	7	-	-	-		1.3	2.2	7	-	-	-
Maccamaw	750N		7.1	7.5	5	2	-	-		2.2	4.1	6	1	-	-
	5P+75N		6.9	9.8	6	-	1	-		5.3	8.1	5	1	1	-
	50P+750N		22.1	12.2	1	2	2	2		16.1	11.1	2	1	4	-
Monroe,	N=5	0.3		0.4					0.3		0.4				
Jones (JO),	05P		0.6	0.4	5	-	-	-		0.5	0.5	5	-	-	-
Salters,	50P		1.3	1.1	5	-	-	-		0.9	0.8	5	-	-	-
Singletary,	75N		1.0	1.6	5	-	-	-		0.4	0.4	5	-	-	-
White	750N		0.6	0.4	5	-	-	-		0.5	0.4	5	-	-	-
	5P+75N		0.7	0.5	5	-	-	-		0.5	0.5	5	-	-	-
	50P+750N		5.8	8.6	4	-	1	-		2.0	1.5	5	-	-	-

Table 22. Growth response of *Selenastrum capricornutum* in lake samples with nutrient spikes of nitrogen and phosphorus.

Lake Station(s) Sample Period	Spike	Autoclaved							Filtered						
		Mean Growth mg/l		No. Responding, Growth Ranges					Mean Growth		No. Responding, Growth Ranges				
		Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l	Control	Spiked	S.D.	0-5	5.1-15	15.1-30	>30 mg/l
Jones (JP)	N=1	0.4		-					0.0		-				
	05P		15.3	-	-		1	-		0.0	-	1	-	-	-
	50P		59.6	-		-	-	1		45.7	-	-	-	-	1
	75N		6.8	-	-	1	-	-		1.0	-	1	-	-	-
	750N		11.5	-	-	1	-	-		0.2	-	1	-	-	-
	5P+75N		5.1	-	-	1	-	-		0.2	-	1	-	-	-
	50P+750N		65.3	-	-	-	-	1		32.9	-	-	-	-	1
Johns	N=1	4.1							0.9						
	05P		3.6	-	1	-	-	-		0.0	-	1	-	-	-
	50P		2.9	-	1	-	-	-		1.2	-	1	-	-	-
	75N		8.9	-	1		-	-		4.2	-	1	-	-	-
	750N		48.2	-	-	-	-	1		35.1	-	-	-	-	1
	5P+75N		5.5	-	1	-	-	-		4.2	-	1	-	-	-
	50P+750N		43.7	-	-	-	-	1		34.5	-		-	-	1
Crystal	N=1	53.6		-					30.6		-				
	05P		50.0	-	1	-	-	-		24.1	-	1	-	-	-
	50P		51.7	-	1	-	-	-		19.5	-	1	-	-	-
	75N		53.2	-	1		-	-		28.6	-	1	-	-	-
	750N		96.0		1	-	-	1		66.7	-	-		-	1
	5P+75N		59.0	-	1	-	-	-		24.9	-	1	-	-	-
	50P+750N		88.4	-	-	-	-	1		56.1	-	-	-	1	-
Mattamuskeet,	N=2	0.1		-					0.1		-				
Phelps	05P		0.45	0.49	2	-	-	-		0.1	-	2	-	-	-
	50P		2.8	1.3	2	-	-	-		6.9	3.8	1	1	-	-
	75N		0.1	-	2	-		-		0.1	-	2	-	-	-
	750N		0.15	0.1	2	-	-	-		0.1	-	-	-	-	-
	5P+75N		0.2	0.1	2		-	-		0.1	-	-	-	-	-
	50P+750N		15.5	16.5	1	-	1			6.6	6.6	1	1	-	-

From the growth response frequencies, the number of samples found in each of the growth ranges, a judgment can be made as to whether the particular body of water was phosphorus or nitrogen limited, or both. In reviewing this information two things need to be kept in mind. For each set of samples the number of samples (N) will, of course, differ and it is this number which defines the total number that can respond in the several growth ranges. In addition the mean growth of the control and its standard deviation describes a baseline for referencing the magnitude of growth found in the various spiked samples.

Control Growth and Pretreatment Nutrient Levels

The data developed in Tables 12-22 provide the opportunity to examine two questions of the algal assay procedure. One is the relationship of the control growth, nonspiked sample, to the nutrient levels resulting from the two pretreatment procedures, autoclaving and filtration. Does the response of the seeding with the test alga indicate, by the relationship of growth and nutrient concentration, some degree of correlation with the original quantities present? The second is the relationship of the indicated nutrient limitation and the original raw water quality.

To examine the first question the various sets of samples from the series of lakes or subsets of stations within lakes were assembled in Tables 23-26. Each of the tables has been arranged in some geographical or hydrologic relationship. For instance those lakes in Table 23 represents the entire series on the Catawba River from Lake James downstream to Wateree Lake a distance of over 200 river miles. The data in the tables include the mean control growth of the samples tested, their standard deviation for each of the pretreatment sets and correlation coefficients, significant at the 95 percent level, between the control growth and the quantities of soluble nutrients found in the sample after pretreatment.

The use of autoclaving as a pretreatment and the resulting solubilization produces higher nutrient levels and the inevitable greater control biomass. There is no instance in which the autoclaved growth was less than that of samples filtered as pretreatment, although in one or two instances it was approximately the same due to very marginal growth in both. Only where the coefficient of correlation was significant at 95 percent or greater is the actual (r) value shown. Thus, as shown in Table 23 for Lake James, of the seven samples tested when related to the nutrient levels, following pretreatment, no significant correlations were found. However, in Lake Rhodhiss it is evident that good correlations are shown for both autoclaved and filtered samples for the phosphorus component of the water and in the filtered sample also for the quantity of ammonia that

Table 23. Correlation of seeded growth control and nutrient concentrations following pretreatments.

Lake and Stations	Samples Tested	Treat-ment	Control Growth[a]	S.D.	Corr. Coef. (r) P ≥ 0.05			
					NH_3	NO_2NO_3	PO_4-P	Total-P
James	7	A	2.0	2.2	-	-	-	-
201,206,210,212		F	0.6	0.5	-	-	-	-
Rhodhiss	14	A	9.8	6.3	-	-	0.7169	0.6770
3, 7, 9, 13, 1724, 1778, 1836		F	5.4	3.9	0.5334	-	-	0.6080
Hickory	5	A	9.9	6.2	-	-	-	-
1542, 1632, 1689		F	0.8	1.2	-	-	-	-
Lookout Shoals	4	A	8.4	9.5	-	-	-	0.9802
1466, 1498, 1538		F	2.8	3.3	-	-	0.9719	-
Norman	11	A	1.3	1.6	-	-	-	-
109, 116, 126, 1302, DC26, RM10		F	0.6	1.2	-	-	-	-
Mt.Island	12	A	0.4	0.5	-	-	-	0.6356
960, 977, 941		F	0.1	0.1	-	-	-	-
Wylie	9	A	5.9	4.8	-	0.9479	0.6891	-
83, 831, 789		F	1.0	2.0	-	-	0.7910	0.9331
681, 708, 74, AC22	13	A	5.8	4.6	-	0.7934	0.8767	0.7190
		F	2.3	3.8	-	-	0.7388	0.8451
SF 30	4	A	10.2	4.3	-	-	-	-
		F	8.9	9.5	-	-	0.9335	0.9504
Fishing Creek	3	A	28.9	4.6	-	-	-	-
27, 31		F	20.1	5.6	-	-	-	0.9899 (P≥.10)
Wateree	15	A	11.1	9.5	-	0.9177	0.8359	0.6691
2, 58, 100, 104, 157		F	5.7	7.1	0.8012	0.8454	0.8004	0.6703

[a]Average of all samples, mg/l.

Table 24. Correlations of seeded control growth and nutrient concentrations following pretreatments.

Lake and Stations	Samples Tested	Treat-ment	Control Growth[a]	S.D.	Corr. Coef (r) ≥ 0.05			
					NH_3	NO_2NO_3	PO_4-P	Total-P
Kerr-Roanoke	8	A	12.2	11.5	-	-	-	-
19, 24		F	4.2	5.9	-	-	0.6617	0.7455
8, 2, 1	19	A	5.8	6.0	-	-	-	-
		F	1.4	1.8	-	0.5517	-	-
Kerr-Nutbush	11	A	12.4	13.1	0.7347	0.7791	-	-
118, 1308		F	4.7	9.9	-	0.6049	0.6548	0.6313
114, 11	8	A	3.0	2.7	-	0.8633	-	0.7833
		F	0.7	0.6	-	-	0.6579 (P ≥ 0.10)	-
108, 103	13	A	2.0	2.9	-	-	0.7561	0.7138
		F	1.6	3.6	-	-	0.9641	0.9604
Gaston	5	A	7.5	4.9	-	-	-	-
82, 166, 324		F	1.2	1.6	-	-	-	-
Roanoke Rapids	4	A	6.0	2.3	-	-	-	-0.9566
		F	0.2	0.4	-	-	-	-
Chowan	17	A	4.3	3.1	-	-	-	-
13, 17, CO1, SW1		F	1.9	2.3	-	-	-	-
A1, RO45	8	A	2.3	2.0	-	-	0.7052	-
		F	1.1	1.5	0.7755	-	-	-

[a]Average of all samples, mg/l.

Table 25. Correlation of seeded control growth and nutrient concentrations following pretreatments.

Lake and Stations	Samples Tested	Treatment	Control Growth[a]	S.D.	Corr. Coef. (r) ≥ 0.05			
					NH_3	NO_2NO_3	PO_4-P	Total-P
Kerr Scott	6	A	3.0	1.6	-	-	0.9294	-
2566, 2610, 2628		F	0.3	0.3	0.8910	-	-	-
High Rock	6	A	11.8	9.0	-	0.8632	-	-
594, 654, 750		F	4.0	5.8	-	-	0.9607	0.9017
Tuckertown	6	A	16.1	9.8	-0.8411	0.9075	-	-0.8705
516, 548, 580		F	0.1	-	-	-	-	-
Badin	6	A	9.8	5.2	-	-	-	-
438, 458, 490		F	0.5	0.4	-0.9026	0.8660	-0.8660	-0.8660
Tillery	14	A	13.2	9.4	-	-	0.6214	0.5786
3, 11, 18, 22, 268, 300, 344		F	1.9	1.6	-	0.9519	0.5699	-
Blewett Falls	6	A	10.7	2.6	0.8130	-	0.9281	0.8744
2, 26, 56		F	0.8	1.0	-	0.8537	-	-

[a]Average of all samples, mg/l.

Table 26. Correlation of seeded control growth and nutrient concentrations following pretreatments.

Lake and Stations	Samples Tested	Treatment	Control Growth[a]	S.D.	Corr. Coef. (r) ≥ 0.05			
					NH_3	NO_2NO_3	PO_4-P	Total-P
Jackson, Black	3	A	21.8	3.9	-	-	-	-0.9958
		F	16.6	6.3	-	-	-	-
Mattamuskeet, Phelps	2	A	0.1	0.0	-	-	-	-
		F	0.1	0.0	-	-	-	-
Tull, Davies, McKensie, Orton	4	A	0.6	1.1	-	-	-	-
		F	0.2	0.2	-	-	-	-
Finches, Hodgins, McNeils,	7	A	2.9	2.7	-	-	0.9389	-
Lytches, Waccamaw		F	1.2	1.3	-	0.7642	0.8876	-
Monroe, Jones (JO), Salters,	5	A	0.3	0.4	-	-	0.9593	-
Singletary, White		F	0.3	0.4	-	-	-	-
Jones (JP)	1	A	0.4	-	-	-	-	-
		F	0.2	-	-	-	-	-
Johns	1	A	4.1	-	-	-	-	-
		F	0.9	-	-	-	-	-
Crystal	1	A	53.6	-	-	-	-	-
		F	30.6	-	-	-	-	-
University 1	20	A	9.2	10.7	-	-	0.7663	0.7555
		F	3.1	3.6	-	-	0.7911	-
Michie 1	17	A	4.4	3.9	-	-	-	-
		F	2.8	2.4	-	0.7911	-	-
Belews 1906	15	A	1.8	1.8	-	-	-	-
		F	1.2	1.1	-	-	-	-
1116	16	A	2.6	1.7	-	-	-	-
		F	1.2	1.2	-	-	-	-
Hyco 1906	9	A	5.1	3.2	-	-	-	-
		F	1.4	1.8	-	0.7132	-	0.7173
1116	7	A	3.7	2.4	-	-	-	0.8708
		F	2.8	3.6	-	-	-	-

[a]Average of all samples, mg/l.

was present. In this particular series of lakes of the Catawba River, it is apparent that the considerable enrichment from municipal and industrial wastes entering Lake Wylie and further downstream results in higher levels of correlation in each sample as well as correlations with different nutrient species. It would appear that the correlation between growth of the seeded alga and the quantity of nutrient improves as the nutrient levels increase.

This was confirmed in the sets of data from the waters of the two arms of the John H. Kerr Reservoir, Table 24. Strong correlations between control growth and nutrients were evident in the section of the lake noted as Kerr-Nutbush with an indication of a shift from good correlations to nitrogen and phosphorus at the upper end of Kerr-Nutbush, Stations 118 and 1308, to a solely phosphorus correlated response at Stations 108 and 103, paralleling the downstream decrease in nutrient concentrations.

Occasionally such as the waters from Roanoke Rapids, Table 24, and Badin and Tuckerton, Table 25, negative correlations were found which were at the 95 percent significance level. The meaning of these negative relationships is not clear. Omissions of correlations from these tables may mean too few samples were taken to carry out a correlation analysis.

Nitrogen and Phosphorus Limitation

From the data of Tables 12-22 an estimate was made as to whether the particular set of waters was phosphorus limited, nitrogen limited, or limited by both nutrients. This was determined for both autoclaved or filtered samples. The possibility, therefore, was one of three conditions of nutrient limitation following the two pretreatment procedures. Thus the results of all algal assays for all bodies of water, or stations within a particular lake, were arranged in a set of tables for each specific nutrient limitation and pretreatment, Tables 27-32. The information assembled in these data sets includes mean control growth, the correlation coefficients to mean nuturient levels in the treated samples, the original mean lake quality with reference to inorganic nitrogen, PO_4-P, soluable-P and total-P and the ratio or inorganic-N to soluble-P. Thus in Table 27, 104 water samples taken over a period of time from 11 different lakes or stations have been shown to be phosphorus limited. The control growth in these samples, mean values, ranges from as low as 0.1 mg/l to as high as 16.1 mg/l. Where control growth was reasonably high the correlation coefficient was positive to the nutrient levels in the pretreated water and showed in four instances to be significant to total-P, in two instances to PO_4-P, and, in one instance to NO_2NO_3 and nitrogen ammonia. In one case the correlation to ammonia was high but negative.

Table 27. Phosphorus limited waters based on response to N and P spikes autoclaved pretreatment control growth and nutrients of non-spiked water.

Lake or Lake Station	No. of Samples Assayed	Control Growth[a]	Corr. Coef. (r) ⩾ 0.05				Mean Values Original Lake Quality mg/m³				Ratio
			NH_3	NO_2NO_3	PO_4-P	Sol-P	Inorg-N	PO_4-P	Sol-P	T-P	Inorg-N/Sol-P
Belews 1906	15	1.8	-	-	-	-	56	7	12	25	4.6
Belews 1116	15	2.6	-	-	-	-	90	6	8	20	11.2
Norman	11	1.3	-	-	-	-	139	6	6	11	23.1
Mt. Island	12	0.4	-	-	-	0.6356	137	6	7	12	19.6
Gaston	5	7.5	-	-	-	-	281	10	22	28	12.8
Michie	17	4.4	-	-	-	-	238	9	17	28	14.0
Tuckertown	6	16.1	-0.8411	0.9075	-	0.8705	742	5	38	120	19.5
Tillery	14	13.2	-	-	0.6214	0.5786	546	43	84	88	6.5
Blewett Falls	6	10.7	0.8130	-	0.9281	0.8744	395	7	26	63	15.2
Jones (JP)	1	0.4	-	-	-	-	1245	5	10	20	124.5
Mattamuskeet, Phelps	2	0.1	-	-	-	-	97	5	6	33	16.2

[a]Biomass, mg/l 14 days growth of S. capricornutum in non-spiked water.

Table 28. Phosphorus limited waters based on response to N and P spikes filtered pretreatment control growth and nutrients of non-spiked water.

Lake or Lake Station	No. of Samples Assayed	Control Growth[a]	Corr. Coef. (r) $P \geq 0.05$				Mean Values Original Lake Quality mg/m³				Ratio
			NH_3	NO_2NO_3	PO_4-P	Sol-P	Inorg-N	PO_4-P	Sol-P	T-P	Inorg-N/Sol-P
Belews 1906	15	1.2	-	-	-	-	56	7	12	25	4.6
1116	15	1.2	-	-	-	-	90	6	8	20	11.2
Hyco 1906	9	1.4	-	0.7132	-	0.7173	156	7	11	19	14.2
1116	7	2.8	-	-	-	-	111	6	10	19	11.1
Hickory	5	0.8	-	-	-	-	177	8	13	38	13.6
Lookout Shoals	4	2.8	-	-	0.9719	-	177	8	13	38	13.6
Norman	11	0.6	-	-	-	-	139	6	6	11	23.1
Mt. Island	12	0.1	-	-	-	-	137	6	7	12	19.6
Kerr (1, 2, 8)	19	1.4	-	0.5517	-	-	127	7	16	20	7.9
Gaston	5	1.2	-	-	-	-	281	10	22	28	12.8
Roanoke Rapids	4	0.2	-	-	-	-	303	5	19	34	15.9
University	20	3.1	-	-	0.7911	-	144	10	14	35	10.3
Michie	17	2.8	-	0.7911	-	-	238	9	17	28	14.0
Chowan (13, 17, CO1, SW1)	17	1.9	-	-	-	-	200	15	36	80	5.6
High Rock	6	4.0	-	-	0.9607	0.9017	751	11	60	115	12.5
Tuckertown	6	0.1	-	-	-	-	742	5	38	120	19.5
Badin	6	0.5	-0.9026	0.8660	0.8660	-0.8660	750	20	40	102	18.7
Tillery	14	1.9	-	0.9519	0.5699	-	546	43	84	88	6.5
Blewett Falls	6	0.8	-	0.8537	-	-	395	7	26	63	15.2
Jones (JP)	1	0.0	-	-	-	-	1245	5	10	20	124.5
Mattamuskeet, Phelps	2	0.1	-	-	-	-	97	5	6	33	16.2

[a]Biomass, mg/l 14 days growth of S. capricornutum in non-spiked water.

Table 29. Nitrogen limited waters based on response to N and P spikes autoclaved pretreatment control growth and nutrients of non-spiked water.

Lake or Lake Station	No. of Samples Assayed	Control Growth[a]	Corr. Coef. (4) P ≥ 0.05				Mean Values Original Lake Quality mg/m³				Ratio
			NH_3	NO_2NO_3	PO_4-P	Sol-P	Inorg-N	PO_4-P	Sol-P	T-P	Inorg-N/Sol-P
Hyco 1906	9	5.1	-	-	-	-	156	7	11	19	14.2
Rhodhiss	14	9.8	-	-	0.7169	0.6770	148	39	45	69	3.3
Hickory	5	9.9	-	-	-	-	177	8	13	38	13.6
Wylie (789, 83, 831)	9	5.9	-	0.9479	0.6891	-	206	14	25	31	8.2
Wylie (681, 708, 74, AC22)	13	5.8	-	0.7934	0.8767	0.7190	158	8	13	21	12.1
Wylie (SF30)	4	10.2	-	-	-	-	206	23	34	78	6.1
Fishing Creek (27, 31)	3	28.9	-	-	-	-	450	81	120	135	3.7
Wateree (2,58,100,104,157)	15	11.1	-	0.9177	0.8359	0.6691	217	15	25	49	8.7
Kerr (19, 24)	8	12.2	-	-	-	-	168	7	14	35	12.0
Kerr (118, 1308)	11	12.4	0.7347	0.7791	-	-	142	24	37	108	3.8
Chowan (13,17,CO1,SW1)	17	4.3	-	-	-	-	200	15	36	80	5.6
(Al, RO45)	8	2.3	-	-	0.7052	-	130	8	20	42	6.5
High Rock	6	9.0	-	0.8632	-	-	751	11	60	115	12.5
Johns	1	4.1	-	-	-	-	170	165	195	250	0.9
Jackson, Black	3	21.8	-	-	-	-0.9958	752	50	115	170	6.5
Monroe, Jones (JO)	5	0.3	-	-	0.9593	-	57	5	13	17	4.3
Singletary, Salters, White Crystal	1	53.6	-	-	-	-	900	250	390	410	2.3

[a]Biomass, mg/l 14 days growth of S. capricornutum in non-spiked water.

Table 30. Nitrogen limited waters based on response to N and P spikes filtered pretreatment control growth and nutrients of non-spiked water.

Lake or Lake Station	No. of Samples Assayed	Control Growth[a]	Corr. Coef. (r) P ⩾ 0.05				Mean Values Original Lake Quality mg/m³				Ratio
			NH_3	NO_2NO_3	PO_4-P	Sol-P	Inorg-N	PO_4-P	Sol-P	T-P	Inorg-N/Sol-P
Rhodhiss	14	5.4	0.5334	-	-	0.6080	148	39	45	69	3.3
Wylie (789, 83, 831)	9	1.0	-	-	0.7910	0.9331	206	14	25	31	8.2
Wylie (SF30)	4	8.9	-	-	0.9335	0.9504	206	23	34	78	6.1
Fishing Creek	3	20.1	-	-	-	0.9899 (P⩾0.10)	450	81	120	135	3.7
Wateree	15	5.7	0.8012	0.8454	0.8004	0.6703	217	15	25	49	8.7
Kerr (19, 24)	8	4.2	-	-	0.6617	0.7455	168	7	14	35	12.0
Kerr (118, 1308)	11	4.7	-	0.6049	0.6548	0.6313	142	24	37	108	3.8
Johns	1	0.9	-	-	-	-	170	165	195	250	0.9
Jackson, Black	3	16.6	-	-	-	-	752	50	115	170	6.5
Crystal	1	30.6	-	-	-	-	900	250	390	410	2.3

[a]Biomass, mg/l 14 days growth of S. capricornutum in non-spiked water.

Table 31. Phosphorus and nitrogen limited waters based on response to N and P spikes autoclaved pretreatment control growth and nutrients of non-spiked water.

Lake or Lake Station	No. of Samples Assayed	Control Growth[a]	Corr. Coef. (r) P ⩾ 0.05				Mean Values Original Lake Quality mg/m³				Ratio
			NH_3	NO_2NO_3	PO_4-P	Sol-P	Inorg-N	PO_4-P	Sol-P	T-P	Inorg-N/Sol-P
Hyco 1116	7	3.7	-	-	-	0.8708	111	6	10	19	11.1
James	7	2.0	-	-	-	-	87	6	7	18	12.4
Lookout Shoals	4	8.4	-	-	-	0.9802	177	8	13	38	13.6
Kerr (111, 114)	8	3.0	-	0.8633	-	0.7833	80	7	7	23	11.4
Kerr (103, 108)	13	2.0	-	-	0.7561	0.7138	79	7	9	19	8.8
Kerr (1, 2, 8)	19	5.8	-	-	-	-	127	7	16	20	7.9
Roanoke Rapids (2, 28, 56)	4	6.0	-	-	-	-0.9566	303	5	19	34	15.9
University	20	9.2	-	-	0.7663	0.7555	144	10	14	35	10.3
Kerr Scott	6	3.0	-	-	0.9294	-	282	5	22	47	12.8
Badin	6	9.8	-	-	-	-	750	20	40	102	18.7
Tull, Davies, Orton, McKensie	4	0.6	-	-	-	-	334	63	70	105	4.8
Finches, Hodgins, McNeils, Lytches, Waccamaw	7	2.9	-	-	0.9389	-	253	15	28	42	9.0

[a]Biomass. mg/l 14 days growth of S. capricornutum in non-spiked water.

Table 32. Phosphorus and nitrogen limited waters based on response to N and P spikes filtered treatment control growth and nutrients of non-spiked water.

Table 32.

Lake or Lake Station	No. of Samples Assayed	Control Growth[a]	Corr. Coef. (r) P ≥ 0.05				Mean Values Original Lake Quality mg/m³				Ratio
			NH_3	NO_2NO_3	PO_4-P	Sol-P	Inorg-N	PO_4-P	Sol-P	T-P	Inorg-N/Sol-P
James	7	0.6	-	-	-	-	87	6	7	18	12.4
Wylie (681,708,74,AC22)	13	2.3	-	-	0.7388	0.8451	158	8	13	21	12.1
Kerr (111, 114)	8	0.7	-	-	0.6579	-	80	7	7	23	11.4
Kerr (103, 108)	13	1.6	-	-	0.9641	0.9604	79	7	9	19	8.8
Chowan (Al, RO45)	8	1.1	0.7755	-	-	-	130	8	20	42	6.5
Kerr Scott	6	0.3	0.8910	-	-	-	282	5	22	47	12.8
Tull, Davies, Orton, McKensie	4	0.2	-	-	-	-	334	63	70	105	4.8
Finches, Hodgins, McNeils, Lytches, Waccamaw	7	1.2	-	0.7642	0.8876	-	253	15	28	42	9.0
Monroe, Jones (JO) Singletary, Salters, White	5	0.3	-	-	-	-	57	5	13	17	4.4

[a]Biomass, mg/l 14 days growth of S. capricornutum in non-spiked water.

The original lake quality showed a wide range of nutrient levels. Inorganic nitrogen was as low as 56 mg/m^3 and as high as 1245 mg/m^3. Soluble phosphorus ranged from 6 mg/m^3 to 84 mg/m^3. Following filtration pretreatment, Table 28, a considerably larger number of assayed samples were found to be phosphorus limited, in this instance 191 from 21 different lakes or stations. Fewer autoclaved samples showed phosphorus limitation than the same samples following the pretreatment by filtration. It might be concluded that autoclaving released sufficient quantity of phosphorus so that phosphorus limitation on algal growth was masked. Of the 191 samples that showed phosphorus limitation following filtering pretreatment, there was no clear indication that the correlation of growth and nutrient levels in the non-spiked waters was predominately to phosphorus or nitrogen. The evidence would appear to be evenly divided.

The waters found to be nitrogen limited after autoclaving pretreatment showed that out of 132 samples from 17 sets of lakes or lake stations, six were highly correlated to the nitrogen level in the treated sample whereas nine were positively phosphorus correlated and one negatively correlated to phosphorus (Table 29). When the pretreatment consisted of filtration only 69 assays were nitrogen limited distributed in 10 sample sets. Of these four were correlated to the soluble nitrogen component of the filtered sample and 12 were highly correlated at a significant level to the soluble phosphorus components (Table 30). Of 105 samples in 12 sets which showed both phosphorus and nitrogen limitation following autoclaving only one showed a significant correlation to soluble nitrogen, nine were correlated to soluble phosphorus, and one was negatively correlated to soluble phosphorus (Table 31). In the samples pretreated by filtration, 71 in nine sets were phosphorus and nitrogen limited with three correlated to the soluble nitrogen components and six to the soluble phosphorus components (Table 32).

The totality of these results can be summarized in two ways. In one, shown in Table 33, the mean control growth of each of the set of samples that were phosphorus, nitrogen or phosphorus and nitrogen limited following the two pretreatment procedures has been correlated to the original lake nutrient quality, specifically the soluble nitrogen and phosphorus components and the ratio of these two. In this presentation the actual (r) value derived from the correlation determinations is shown without noting a level of significance. The values which are less than .5 are generally assumed to be of little or no significance. It is evident that autoclaving as a pretreatment resulted in control growths in the phosphorus limited series that was highly correlated to the original soluble phosphorus level, and (r) value of .7928. All other correlations were at nonsignificant levels. In contrast the nitrogen limited samples following both autoclaved and filtered pretreatment showed unusually high

Table 33. Relationship of control growth, seeded *Selenastrum capricornutum* in autoclaved and filtered waters and nutrient levels of original raw water.

Nutrient Limitation Based on Response to Spikes	Corr. Coef. (r)			
	N	Inorg-N	Soluble P	Ratio N/P
Phosphorus				
Autoclaved	11	0.3151	0.7928	-0.2992
Filtered	45	-0.3844	-0.2380	-0.3565
Nitrogen				
Autoclaved	17	0.7653	0.8270	-0.2932
Filtered	10	0.9103	0.7439	0.4720
Phosphorus and Nitrogen				
Autoclaved	12	0.4172	-0.1344	-0.0067
Filtered	9	-0.2331	-0.3693	0.3042

correlations to both inorganic nitrogen as well as soluble phosphorus of the original lake quality. Samples limited in both phosphorus and nitrogen, whether autoclaved or filtered as pretreatment, showed correlations with neither of the soluble nitrogen or phosphorus components of the original lake water. It would thus appear that this particular analysis did not descriminate in any consistent manner between the two pretreatment procedures to indicate whether one or the other was preferable in relating the response of the algal assay growth to the quality of the original lake water.

Nutrient Limitation and Original Lake Quality

In the second summary, as shown in Table 34, the mean value for all autoclaved and filtered samples for each of the limitation series, phosphorus, nitrogen, and phosphorus and nitrogen are compared to the mean values of the original lake water nutrient concentrations. A clearer relationship emerges regardless of what nutrient might be limiting. Greater growth is the consistent result of the autoclaved pretreated samples. This would be expected. It also shows that those samples that were nitrogen limited whether autoclaved or filtered had an average growth which was greater than either the phosphorus limited or phosphorus and nitrogen limited samples. When these summary data are compared to the mean values of the original lake water nutrient levels, it is clear that the phosphorus limited samples had at least soluble phosphorus component of about 23 mg/m with a mean ratio of nitrogen/phosphorus of about 14. Nitrogen limited samples averaged considerably higher in

Table 34. Indicated nutrient limitation of algal assayed samples and original lake nutrient quality.

Nutrient Limitation	Pre-Treatment	No. of Assays	Mean Control Biomass, mg/l	Mean Values Original Lake Nutrients mg/m³				Mean Ratios Inorg-N/Sol-P
				Inorg-N	PO_4-P	Sol-P	T-P	
Phosphorus	Autoclaved	104	5.31 (5.81)[a]	360 (272)	9.9 (10.4)	21.4 (22.6)	40.7 (42.8)	24.3 (14.3)
	Filtered	191	1.37 (1.37)	326 (281)	9.8 (10.1)	22.3 (22.9)	45.1 (46.4)	18.6 (13.3)
Nitrogen	Autoclaved	132	12.15	293	42.9	68.5	98.1	7.3
	Filtered	69	9.81	335	66.8	100.0	133.5	5.5
Phosphorus and Nitrogen	Autoclaved	105	4.70	227	13.3	21.3	41.8	11.4
	Filtered	71	0.92	162	13.8	21.0	37.1	9.1

[a]( ) mean values with Jones Pond (JP) deleted because of unusual nitrogen concentration.

mean soluble phosphorus, 3-5 times, depending on whether the sample was autoclaved or filtered but the nitrogen/phosphorus ratio was lower in the range of 5-7 for the two pretreatment procedures. Phosphorus and nitrogen limited samples showed soluble phosphorus about the same as for the phosphorus limited but nitrogen was considerably the lowest on the average and the ratio of soluble nitrogen to soluble phosphorus was in the range of 9-11.

SUMMARY

The implication of these findings may be summarized as follows:

1. The pretreatment procedure of the algal assay bottle test, autoclaving or filtration, provides a varied quantity of algal nutrients that will allow growth of an algal biomass that may be interpreted as reflecting total growth potential and immediate available growth potential respectively. These quantities are of use in describing the relative trophic state of a body of water.

2. The consistent pattern of the ratio of the soluble nitrogen and phosphorus components to the indicated limitation of either one, the other, or both of these algal nutrients suggest that this ratio can be used to define which nutrient is limiting for the particular body of water.

CONCLUSIONS

The growth of the reseeded test alga, *Selenastrum capricornutum*, in water samples follows pretreatment either by autoclaving or filtration provides a good indication of the total growth potential by the first procedure and ambient growth potential by the second. The additional growth response of samples, to which spikes of phosporus and nitrogen have been added to establish the nature of the limiting nutrient describe with reference to original nutrient levels clearly indicated ranges of nitrogen to phosphorus ratio. The determination of the N/P ratio would appear to provide the same information derived from the more complicated assay spiking procedure.

RECOMMENDATIONS

The standard assay procedure could be modified to limit the determination to biomass grown in reseeded samples following autoclaving and filtration. The determination of whether the waters are limited in their growth potential by the relative quantity of phosphorus or nitrogen can be satisfactorily determined from the ratio of the total soluble nitrogen and phosphorus components.

ACKNOWLEDGMENTS

This extended investigation carried out over a period of several years involved the efforts of many staff and students of the Department of Environmental Sciences and Engineering. The samples collected for the field evaluation were the particular responsibility of Terry Anderson, Mark Mason, and Chris Knud-Hansen. These algal assays were carried out by Sandy Hoffman, Pat Appeldorn, and Chris Knud-Hansen. Dr. Peter H. Campbell did the phytoplankton counts.

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Application of Algal Assays to Define the Effects of Wastewater Effluents Upon Algal Growth in Multiple Use River Systems

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INTRODUCTION

The establishment of realistic water quality criteria for the control or prevention of eutrophication within multiple use river systems is complicated by chemical, physical, and biological interactions. Recently, attention has centered upon the role of algal assays in defining or predicting the eutrophication potential of selected lakes, streams, and impoundments (Miller et al., 1974, Greene et al., 1975a). These assays have led to studying the response of algal growth to stress under controlled environmental conditions (Greene et al., 1975). Stress to biological populations in multiple use river systems is usually exerted by complex wastes of organic and inorganic origin. However, the interactions of these wastes on the growth of planktonic algae in multiple use river systems has not been defined. As a result, proposed municipal and industrial wastewater treatment measures to control the eutrophication process are often met with considerable debate.

Federal, state, and municipal enforcement agencies need practical methodology to help them deal with water quality management problems including: (1) the effects of municipal, industrial, and agricultural waste discharges upon aquatic productivity; (2) the degree to which wastes must

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be controlled to enhance water quality; and (3) the effect on algal productivity when inhibitory or toxic substances are removed from wastewaters and nutrients are not.

The research described herein is concerned with the design and interpretation of laboratory algal assay experiments to define some of the effects of complex wastewater effluents upon growth in multiple use river systems.

METHODS

The algal assay procedure bottle test (U.S. EPA, 1971a) was used in the study with the green alga, *Selenastrum capricornutum,* as the test organism. The standard procedure was modified in order to examine trace metal deficiency and toxicity. Modifications included: (1) assays were carried out in 500 ml polycarbonate Erlenmeyer flasks; (2) laboratory glassware used for test preparation and algal culture was leached of heavy metal contaminants with a 10 percent solution of nitric acid; and (3) the selected test waters were pretreated prior to assay by adding 1.00 mg Na_2 EDTA/l (Miller et al., 1975). Chemical analysis of the water samples was in accordance with EPA methods (U.S. EPA, 1971b).

ASSESSMENT OF NUTRIENT STATUS

The Eutrophication and Lake Restoration Branch of the U.S. Environmental Protection Agency has conducted several investigations relating to the application of algal assays to define nutrient limitation of algal growth (Miller and Maloney, 1971; Miller et al., 1974). Results of these studies indicated: (1) Algal assays can be used to determine and predict the effects of wastewater effluents upon algal growth in natural waters; (2) a high degree of correlation exists between the reported trophic state of lakes and their algal assay productivity response; (3) in general, the increase of calcium, magnesium, alkalinity and carbon content does not correlate to maximum yield of *S. capricornutum* in natural waters; and (4) phosphorus limitation decreased as the trophic (productivity) classification of the lake waters increased.

The high degree of correlation between the nitrogen and phosphorus content of natural waters and algal assay growth response led to the definition of phosphorus and nitrogen yield relationships for *S. capricornutum* grown in inorganic culture medium (Shiroyama, Miller, and Greene, 1975). Factors for converting orthophosphorus and total soluble inorganic nitrogen (TSIN = $NO_2 + NO_3 + NH_3$) content of a test water into maximum yield of the test alga have also been determined. Waters containing $\geqslant$ 0.010 mg/l ortho-P will yield 0.43 milligrams dry weight of the alga per 1.00 μg P/l. Similarly each μg/l TSIN will yield

0.038 milligrams dry weight of the test alga. Actual yield is considered statistically significant within ± 20 percent of the predicted yield. Recent application of these nitrogen and phosphorus yield factors to predict the productivity of *S. capricornutum* in waters collected from the Snake River system have been evaluated.

Based on the conversion factor for ortho-P content (1.0 μg P/l) the Snake River sampled at Tilden Bridge (Figure 1) should yield < 0.10 mg dry weight/l of test alga. The sample contained sufficient TSIN (0.16 mg

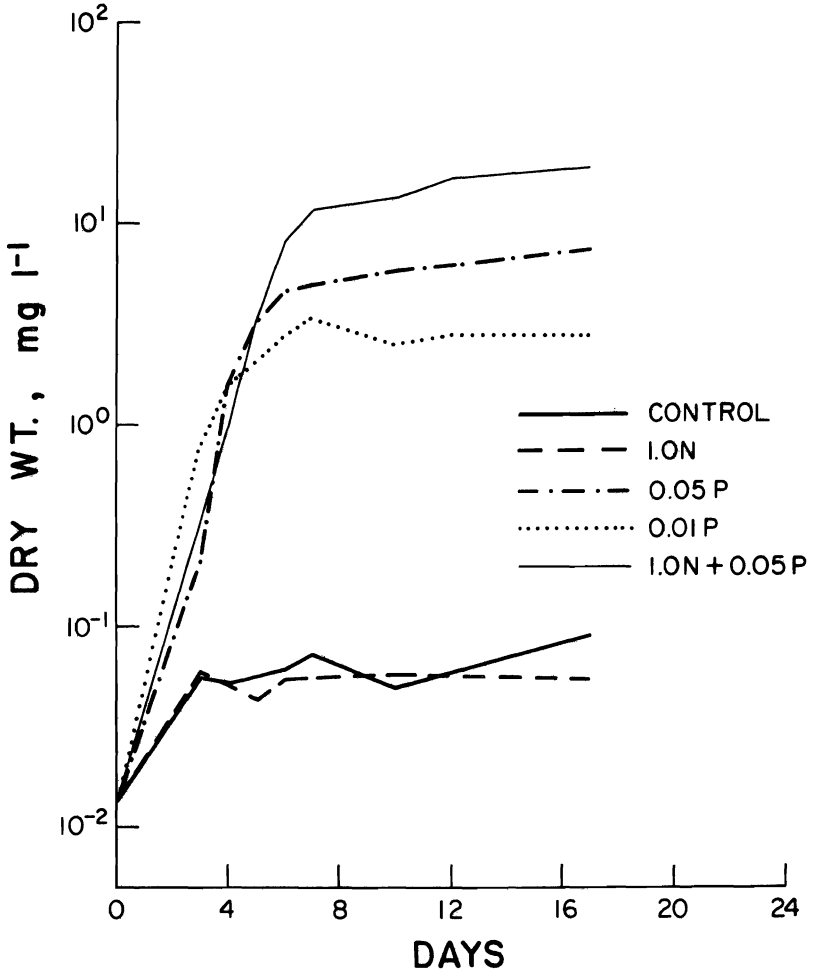

Figure 1. Effect of various nutrient additions to Snake River water, Tildon Bridge, on the growth of *S. capricornutum*.

N/l) to yield 6.2 mg dry weight/l if sufficient phosphorus was added to the sample. Adding 0.05 mg P/l stimulated growth to 6.3 mg dry weight/l, indicating phosphorus as the primary limiting nutrient. The addition of both N and P should have supported 23.6 mg dry weight/l of algal growth, but the actual yield was 26.6 mg dry weight/l. This indicates that sufficient nutrients other than N and P were present in Tilden Bridge water and that algal growth toxicants were absent.

The Palouse River water (Figure 2) did not significantly respond to the addition of 0.05 mg P/l. However, adding 1.00 mg N/l to the control water increased the maximum yield 177 fold over that produced in the control. This response confirmed the predicted yield based on the ortho-P (0.04 mg P/l) and TSIN content (0.005 mg N/l) of the test water.

SIGNIFICANCE OF NITROGEN AND PHOSPHORUS RATIOS

The ratio of the TSIN to ortho-P yield factors (0.038/0.43) indicates an optimum N:P ratio of 11.3 for the support of *S. capricornutum.* This ratio compares favorably to that of 10 to 1 reported by Chiaudani and Vighi (1974). The N:P ratio can be used to predict nutrient limitation in most natural waters. That is, waters containing greater than 11.3:1 N:P content may be considered phosphorus limited, while those containing N:P ratios less than 11.3:1 can be considered nitrogen limited for algal growth.

The N:P ratio of the Tilden Bridge water sample was 32.6:1. This, in addition to the yield factor prediction, pointed to phosphorus deficiency in the test water. The N:P ratio of the Palouse River sample of 8:1 indicated nitrogen limitation. Algal assay analysis confirmed the N:P ratio prediction of nutrient limitation in these waters.

Placement into a nitrogen or phosphorus limitation category without actual assay analysis can be hazardous. A case in point is Waldo Lake, Oregon. Waldo Lake water (Figure 3) did not support algal growth beyond 0.06 mg dry weight/l. This water had an average N:P ratio of 16:1, suggesting phosphorus limitation for algal growth. The addition of 0.05 mg P/l singularly and in combination with 1.00 mg N/l failed to stimulate growth. This indicates that other constituents were growth limiting.

Calculated yields for TSIN and ortho-P can be considered more reliable than N:P ratios as indicators of algal productivity. However, the application value of N:P ratio interpretation relates to the designing of assay experiments. When faced with both economic and manpower constraints, the number of assays to define the nutrient status of a test water can be reduced by applying N:P ratio criteria. For example, only

controls and controls plus phosphorus need be run on waters having a N:P ratio $\geqslant$ 11.3. Similarly only controls and controls plus TSIN would be assayed on waters with a N:P ratio < 11.3. Assay outliers could then be rerun with the addition of both TSIN and P singularly and in combination to determine their nutrient status.

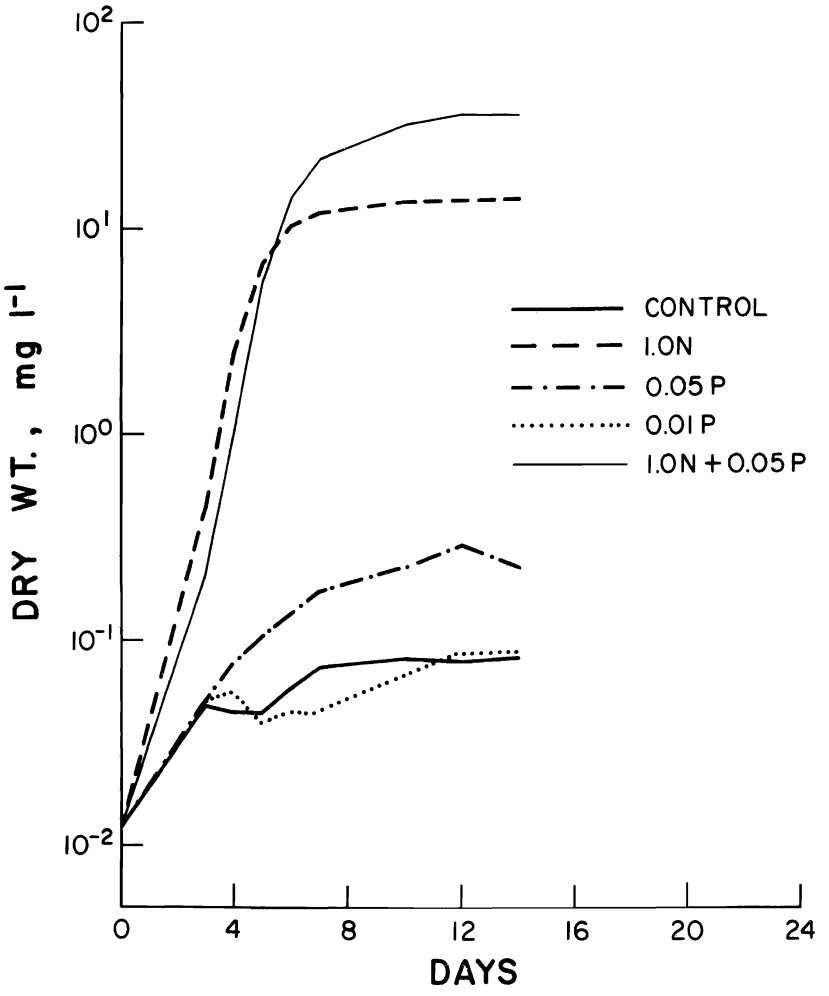

Figure 2. Effect of various nutrient additions to Palouse River water, on the growth of *S. capricornutum*.

The interpretation of actual algal assay results, relative to predicted values based upon nitrogen to phosphorus ratios and yield factors, depends on the reliability of the test procedure. To be effective an assay experiment should be designed to include builtin checks and balances of known growth responses, such as the standard addition of nitrogen and phosphorus singularly and in combination. The growth results obtained can be used to verify both the chemical analysis for nitrogen and

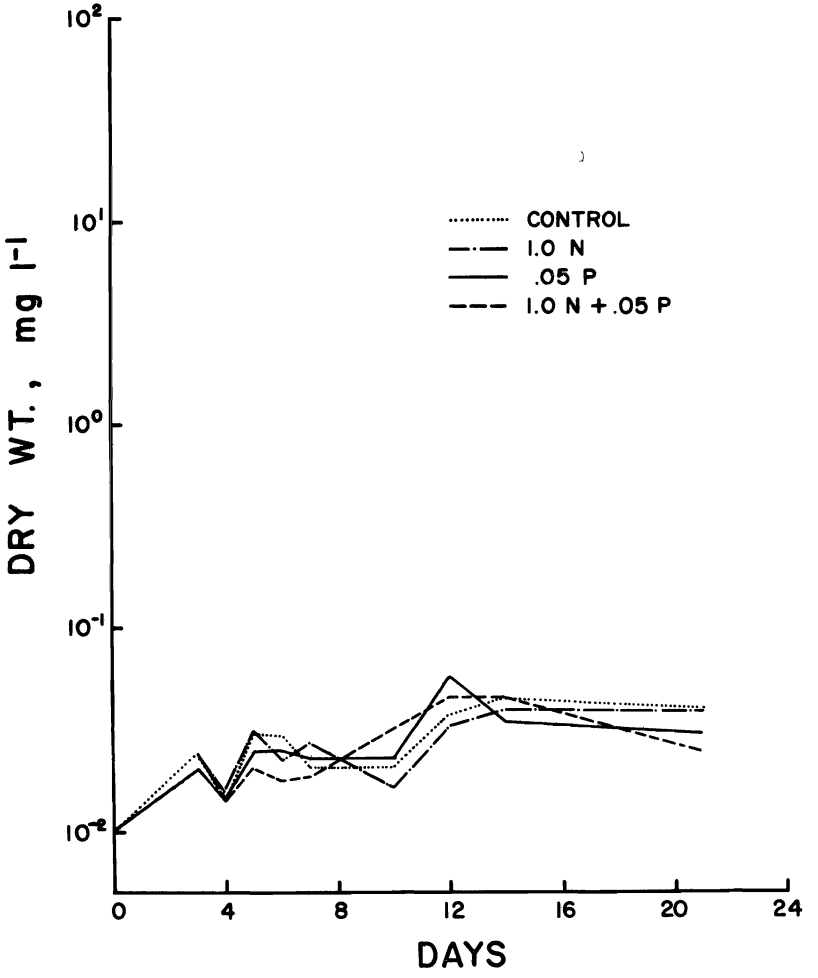

Figure 3. Effect of various nutrient additions to Waldo Lake water, on the growth of *S. capricornutum*.

phosphorus and the precision and accuracy of the assay response. For example, the predicted maximum yield of *S. capricornutum* for the TSIN and ortho-P content of the Snake River sampled at Swan Valley (Figure 4) is 2.1 and 0.10 mg dry weight/l respectively, indicating phosphorus limitation for algal growth in this water. The addition of 0.01 mg P/l should support growth to the extent of nitrogen availability (2.1 mg dry weight/l) in the test water. Actual yield obtained with this phosphorus

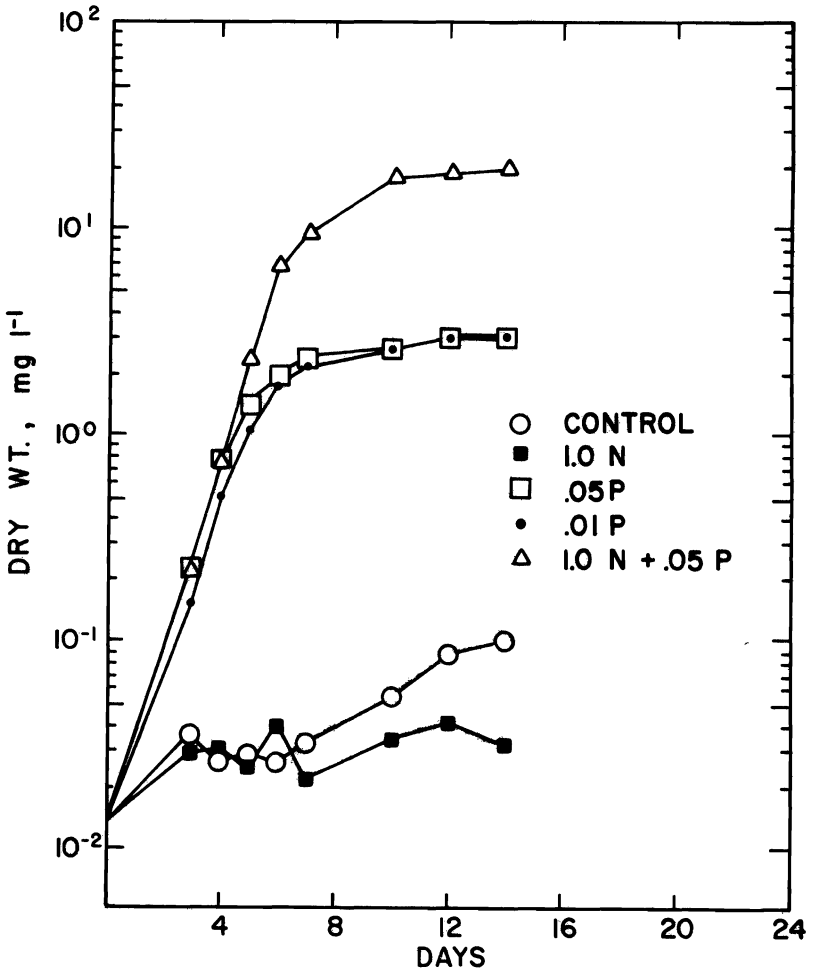

Figure 4. Effect of various nutrient additions to Snake River water, Swan Valley, on the growth of *S. capricornutum*.

addition was 2.9 mg dry weight/l, verifying that the TSIN content of the test water was limiting algal growth. Under conditions of phosphorus limitation the control and the control plus nitrogen yields should closely agree. Actual yields were 0.10 and 0.04 mg dry weight/l, respectively. Similar interpretation can be used with yields for control and controls plus phosphorus to confirm nitrogen limitation.

The singular addition of phosphorus in this case verified phosphorus limitation. However, many test waters may be limited for algal growth by constituents other than nitrogen and phosphorus. The combined addition of 1.00 mg N/l (38.0 mg dry weight/l) and 0.05 mg P/l (21.5 mg dry weight/l) can be used to indicate nutrient deficiency as well as toxicity. This addition of nitrogen and phosphorus to the sample of Snake River water taken at Swan Valley supported growth of 19.7 mg dry weight/l. This weight was within ± 20 percent of that predicted from the phosphorus content, thus confirming both the low level of ortho-P in the control test water and the absence of other growth limiting constituents.

The biological availability of nitrogen and phosphorus can be calculated by dividing the maximum assay yield by either the TSIN or ortho-P yield factors. This calculation can also be used to determine the accuracy of chemical analysis for reflecting the biologically available concentration of nitrogen and phosphorus within the test waters. The maximum yield obtained with phosphorus addition should be used to calculate TSIN availability. Conversely, the yield obtained with TSIN addition should be used to determine available phosphorus. Validity of these nitrogen and phosphorus availability calculations depends on the absence of other growth limiting nutrients or inhibitory constituents. Failure of a test water to attain the predicted yield or nutrient limitation status may be attributed to any one or a combination of such causes as: (1) the absence of other growth limiting nutrients; (2) the presence of toxicants; or (3) unreliable chemical analysis for ortho-P and TSIN.

TRACE-ELEMENT DEFICIENCY

The use of synthetic organic ligands such as ethylene diamine tetra acetic acid (EDTA) is a common practice to insure the availability of trace elements for algal growth in defined culture media. Phytoplankton have also been identified as producers of organic ligands capable of complexing trace-elements (Barber, 1974). Recognition of the growth enhancement qualities of organic ligands led to the addition of EDTA to natural test waters before assay to define trace-element availability.

Columbia River water collected at Rock Island Dam contained 0.012 mg P/l, and 0.109 mg TSIN/l. Theoretical yield of the test alga in this water based on TSIN content would be 4.14 mg dry weight. The nitrogen

to phosphorus ratio of this water was 9:1, indicating potential nitrogen limitation. This water did not support the predicted yield in the control or control plus nitrogen and phosphorus added singularly or in combintion. The addition (Figure 5) of 1.00 mg/l Na_2 EDTA, however, stimulated growth to 5.4 mg dry weight. Similar growth enrichment was obtained by adding EDTA to Columbia River water collected near Bridgeport, Washington (Figure 6).

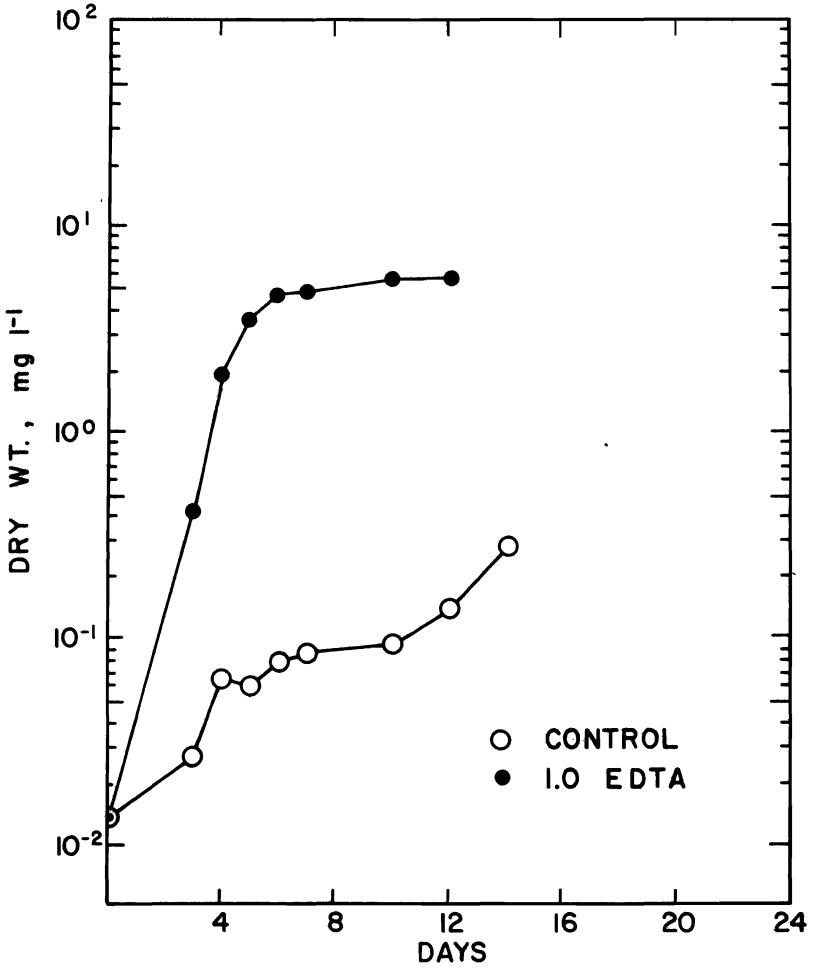

Figure 5. Effect of Na_2 EDTA addition to Columbia River water, Rock Island Dam, on the growth of *S. capricornutum*.

Silker (1964) reported seasonal variations of trace-elements within the Columbia River near Hanford, Washington. With the exception of iron, with concentrations ranging from a low of 6 μg/l to a high of 101 μg/l, no other trace-element appeared lacking for the support of algal growth. The addition of EDTA to the Columbia River samples may have increased iron availability, thus stimulating growth. Lewin and Chen (1973) reported the maintenance of iron availability for algal growth by

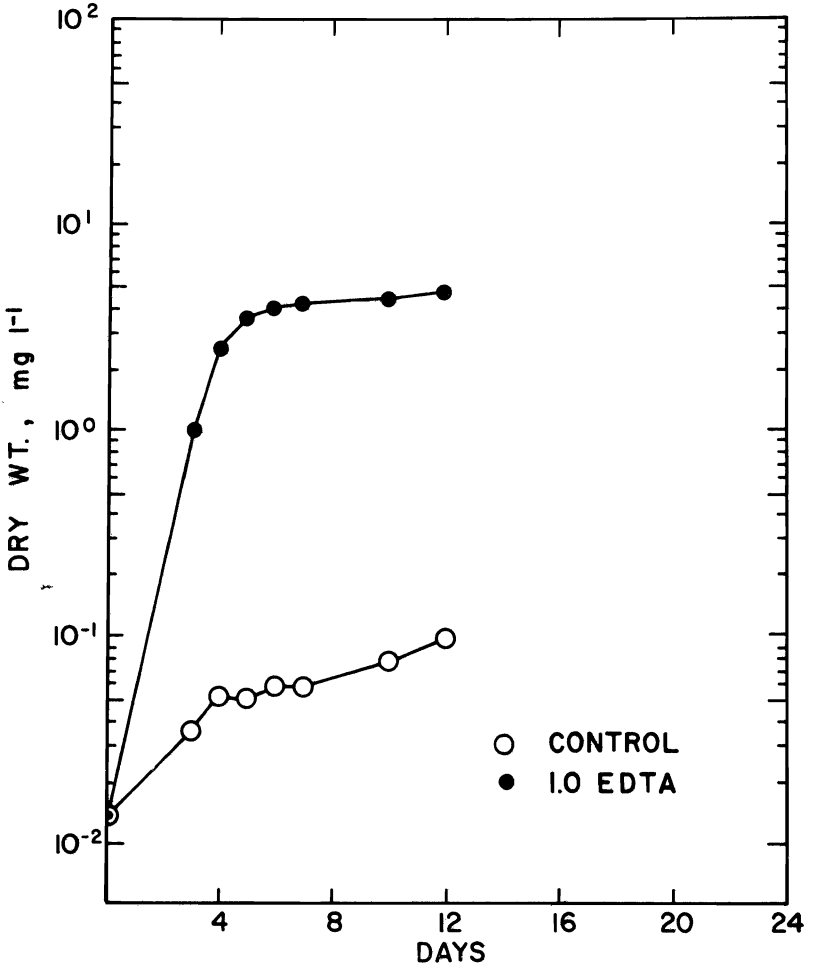

Figure 6. Effect of Na_2 EDTA addition to Columbia River water, Bridgeport, Washington, on the growth of *S. capricornutum*.

addition of EDTA in media containing 6.5 μg Fe/l. Adding EDTA as a qualitative indicator of trace-element deficiency is necessary prior to assessing algal growth stimulation by the addition of nitrogen or phosphorus into a receiving water lacking in available trace-elements. Without this information, one cannot judge which might be the limiting element.

IDENTIFICATION OF HEAVY METAL TOXICITY

Recent attention has focused on the biological response to heavy metal stress, rather than strict reliance on specific concentration of the heavy metal contained in wastewater effluents. This approach is necessitated, in part, by the fact that ligands and particles bond heavy metals in varying degrees. Therefore, absolute concentration of a metal does not necessarily reflect the degree to which it affects biological organisms. Direct evaluation of heavy metal toxicity through biological assay involving the use of living organisms, is one way to obtain basic heavy metal stress information. The response of an organism to a heavy metal pollutant depends on several basic factors: (1) solubility or ion exchange equilibria; (2) ionic strength; (3) metal concentration; (4) contact time; (5) environmental characteristics of the test; and (6) physiological condition of the test organism.

Greene et al. (1975b) evaluated the use of the algal assay to identify zinc toxicity in defined inorganic culture medium under controlled environmental conditions. Those results indicated that the sensitivity of *S. capricornutum* to zinc is inversely proportional to the ionic strength of the test substrate. Furthermore, a 20 fold increase in the phosphorus content of the test medium did not affect zinc toxicity. An understanding of the relationship between ionic strength and phosphorus interaction upon zinc toxicity led to the study of the effects of waste discharges upon algal growth within the Spokane River basin (Figure 7).

Assessing the effectiveness of wastewater treatment practices to control nutrient enrichment within the Spokane River system was complicated by the occurrence of heavy metals (predominantly zinc) in the upper reaches of the Coeur d' Alene Lake drainage basin. Zinc concentrations ranged from < 20 μg/l at Mullan and Long Lake Dam to 7,500 μg/l at Smelterville. The mean zinc concentration from Post Falls to Bowl and Pitcher State Park was 112 μg/l.

The maximum 14-day algal yield obtained in the (autoclaved and filtered) Spokane River water sample collected at Seven Mile Road Bridge was 0.11 mg dry weight/l of *S. capricornutum* (Figure 8). This was only 0.3 percent of the yield expected (36.6 mg dry weight/l) from the 85 μg P/l present in the sample. The predicted yield for this phosphorus level was

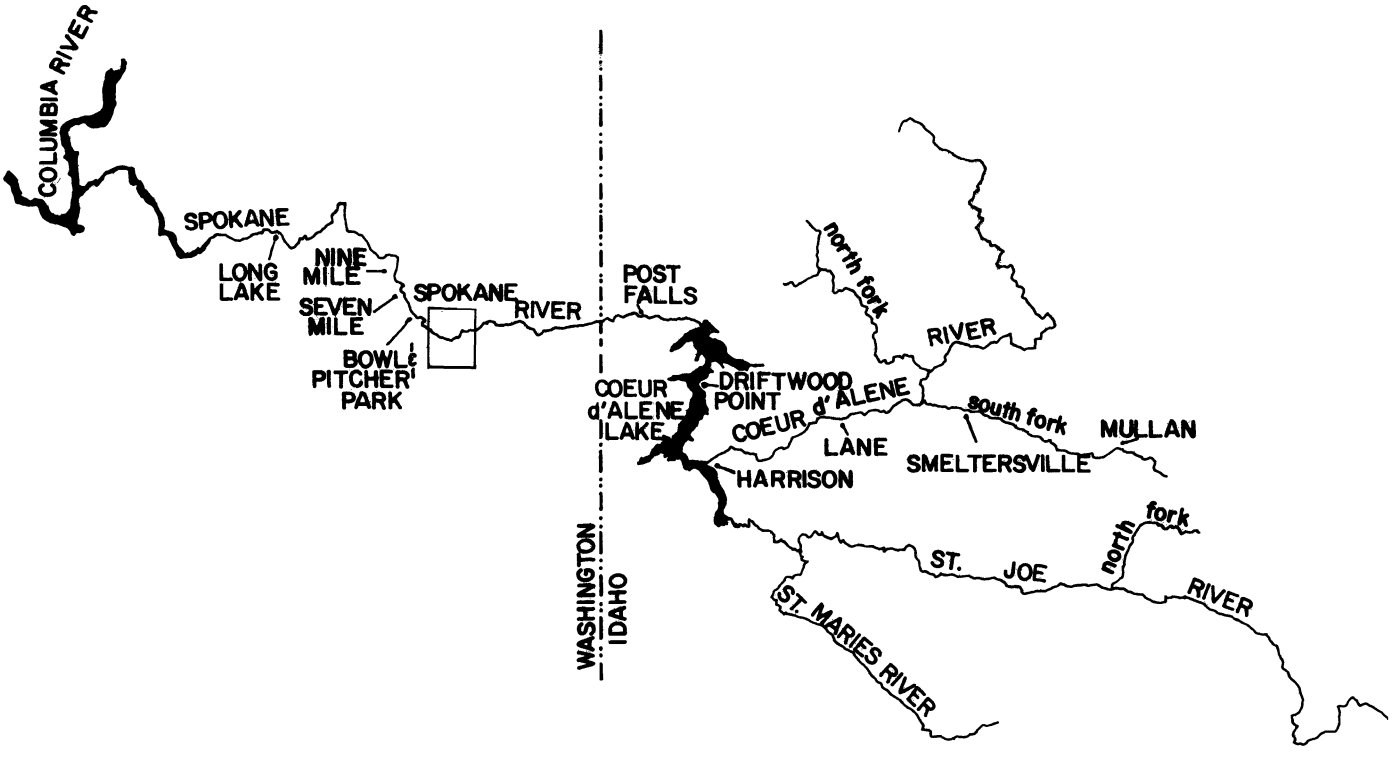

Figure 7. Spokane River Basin sampling sites.

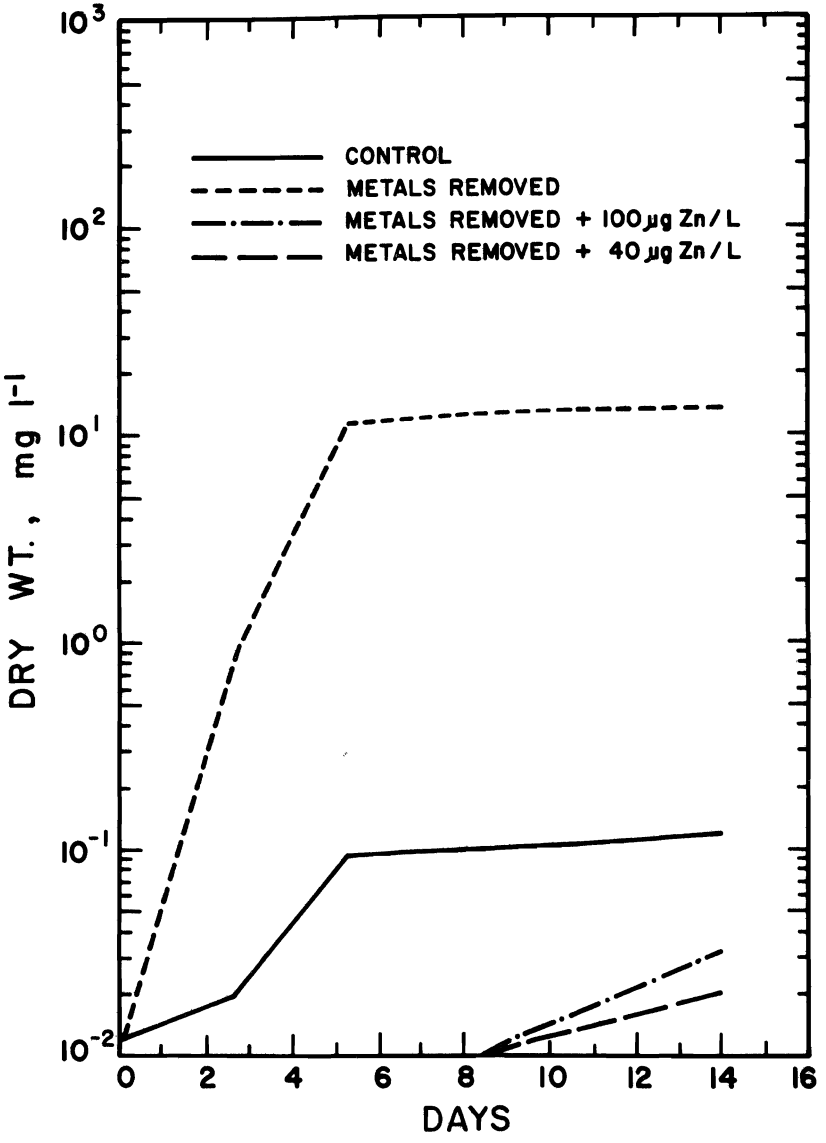

Figure 8. Effect of zinc removal from Seven Mile Road Bridge water on the growth of *S. capricornutum.*

valid when either no toxicant was present in the water or when other nutrients were not growth limiting. The Seven Mile Road Bridge sample contained 75 μg/l of dissolved zinc. The inability of the test water to support algal growth relative to its nutrient status was attributed to zinc toxicity.

The effect of zinc toxicity was evaluated by adding 1.0 mg EDTA/l to an autoclaved and filtered Bowl and Pitcher State Park water sample containing 115 μg P/l, 509 μg TSIN/l, and 125 μg Zn/l. The untreated sample yielded 0.12 mg dry weight/l *S. capricornutum.* This represented less than 1 percent of the 19.4 mg dry weight/l expected yield based on the growth limiting TSIN content of the water (Figure 9). After chelation with 1.0 mg EDTA/l this test water supported 21.7 mg dry weight/l of the test alga indicating that the addition of EDTA can be an effective method to suppress heavy metal toxicity. These results also illustrate the value of algal assays to assess nutrient enrichment problems in multiple use river systems receiving heavy metal wastes.

SUMMARY AND CONCLUSIONS

Algal assay has been demonstrated as a useful technique to define the effects of wastewater discharges upon algal growth in multiple use river systems. The design and interpretation of assay experiments have been presented to identify the effects of domestic and industrial waste discharges upon algal growth within the Spokane River system. Algal response was reported for condition of nitrogen and phosphorus limitation, trace-element deficiency, and heavy metal toxicity in natural waters. The study led to these conclusions:

1. The critical N:P ratio necessary for maximum yield of *S. capricornutum* in a test water was 11.3 μg N:1 μg P.

2. The N:P ratio was useful in preliminary assessment of algal growth limitation in natural waters. Waters containing N:P ratios < 10 may be considered nitrogen limiting while those waters with N:P ratios > 10 may be phosphorus limiting for algal growth.

3. The interpretation of actual algal assay results to predicted yields based upon N:P ratios depended on the reliability of the test procedure.

4. The biological availability (concentration) of TSIN and ortho-P in a test water can be calculated by dividing the maximum assay yield by either the TSIN or ortho-P yield coefficient.

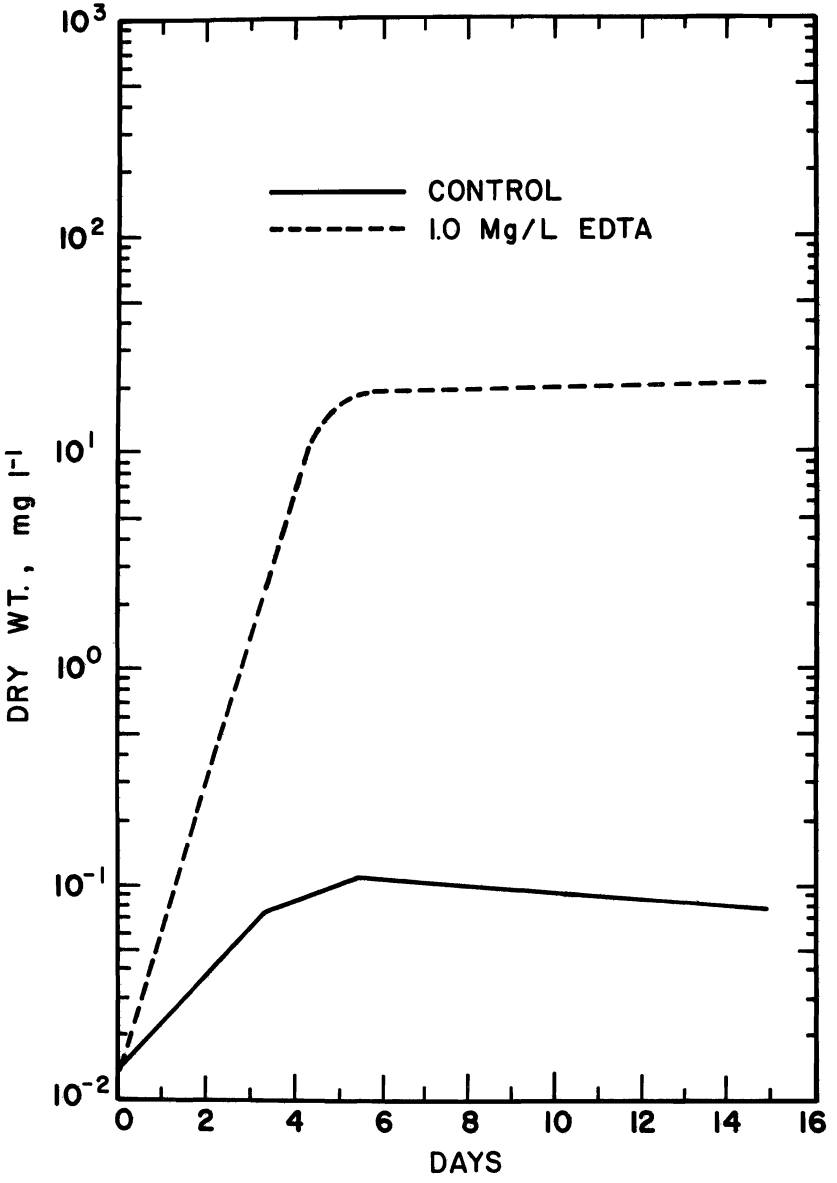

Figure 9. Effect of Na_2 EDTA addition to Spokane River water, Bowl and Pitcher State Park, Washington, on the growth of *S. capricornutum*.

5. Failure of a test water to attain the predicted assay yield or nutrient limitation status may be attributed to growth limiting nutrients other than nitrogen and phosphorus, presence of toxicants, or unreliable chemical analysis for ortho-P and TSIN.

6. The addition of EDTA prior to assay of test waters can be used to assess nutrient enrichment problems in multiple use river systems receiving heavy metal wastes.

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The Relationship of Laboratory Algal Assays to Measurements of Indigenous Phytoplankton in Long Lake, Washington

J. C. Greene, R. A. Soltero, W. E. Miller, A. F. Gasperino, and T. Shiroyama*

INTRODUCTION

Long Lake Reservoir, an impoundment of the Spokane River, is in an advanced state of eutrophication (Soltero, Gasperino, and Graham, 1973, and 1974; Soltero et al., 1975). Repeated blooms of algae cloud its depths in the late summer. Large masses of water plants, primarily *Nymphoides peltatum,* are rooted in the shallower waters along the shoreline. There have been complaints, particularly in the past 10 years, about the deterioration of water quality. In fact, the State of Washington Department of Ecology (Cunningham and Pine, 1969; Bishop and Lee, 1972) and Region X of the U.S. Environmental Protection Agency, (Schmidt and Kreizenbeck, 1971) have defined nutrient over enrichment and heavy metals toxicity as major water pollution problems within the Spokane-Coeur d'Alene River drainage basin.

The effluent of a primary sewage treatment plant, located at river kilometer 102.8 and serving the 180,000 residents of Spokane, has been identified as a major source of nutrient input influencing the trophic status of Long Lake Reservoir. During this study, millions of visitors attended the 1974 World's Fair, EXPO '74, in Spokane, and the average daily discharge of primary sewage emptying into the Spokane River

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increased to 113,500 m^3 (Williams, 1975). Correspondingly, ambient total nitrogen and phosphate loads increased by 4.52 and 1.61 metric tons day^{-1}, respectively. That effluent comprised about 0.7 percent of the river's flow below the discharge point, while about 96 percent of that total net inflow to the reservoir came from the Spokane River.

Bioassay assessment of the nutrient enrichment problem in the Spokane River Basin is complicated by the occurrence of heavy metals (predominantly zinc) originating in the upper Coeur d'Alene Lake drainage basin. A lead smelter, an electrolytic zinc plant and a cadmium refinery located at Smelterville, Idaho, reduce ore and concentrate it to refined marketable metals. Discharges from these operations enter the South Fork of the Coeur d'Alene River and affect the algal growth potential in the Spokane River, the effluent stream for Lake Coeur d'Alene.

Algal assay has been used to manage water quality and to evaluate the fertility of waters. Because of its success in these investigations, undoubtedly a standardized bioassay such as the Algal Assay Procedure: Bottle Test (U.S. EPA, 1971) will have widespread use in evaluating the potential fertility of water. An important question still remains unanswered: How closely do algal assays performed in the laboratory on natural waters reflect the conditions found in the environment?

The objectives of this study were: (1) To determine the algal growth potential of autoclaved and filtered Long Lake water utilizing the algal assay procedure bottle test (U.S. EPA, 1971) and *Selenastrum capricornutum* Printz as the laboratory test alga; (2) to determine if the maximum yield (mg dry weight l^{-1}) of a monospecific laboratory algal assay reflects field conditions (i.e., indigenous phytoplankton standing crop and/or chlorophyll *a*); and (3) to determine how autoclaving and filtering the test waters affects the correlation between laboratory algal assay and field results of the test water.

Algal assays were performed at the Pacific Northwest Environmental Research Laboratory on test waters from Shagawa Lake, Minnesota, and Long Lake, Washington. When compared with indigenous phytoplankton standing crop, results indicated a potential similarity to assay maximum yields. These observations led to this hypothesis: "A monospecific laboratory algal assay utilizing *S. capricornutum* Printz cultured under optimal environmental conditions will produce a maximum yield (mg dry weight l^{-1}) analogous to the indigenous phytoplankton standing crop found in natural waters—at least under bloom conditions and if zooplankton grazing is not a significant influence."

DESCRIPTION OF THE STUDY AREA

In 1915 the construction of Long Lake Dam at river kilometer 54.5 formed Long Lake Reservoir, an impoundment of the Spokane River located approximately 24 km northwest of Spokane, Washington. The reservoir has a storage capacity of 305 x 10^6 m^3 with 180 x 10^6 m^3 as active storage, and a surface area of 21 x 10^6 m^2 at maximum capacity. Table 1 gives morphometric data for the reservoir.

Figure 1 shows the five sampling stations established at approximately 8 km intervals. Station 0 was located just behind the dam at river kilometer 54.5, and station 4 was 32 km up the reservoir.

METHODS AND MATERIALS

During the study all water samples and *in situ* measurements were taken at each station on Long Lake during 12 biweekly cruises from June 8 to September 30, 1974, and then monthly until the study terminated in December.

Field Investigations

Water Chemistry. Water samples collected with a one-liter Kemmerer sampler were measured in the field for pH, temperature, and conductivity (at 25°C) using a Hydro-lab Surveyor^R (Model 6). Analyses

Table 1. Morphometric data for Long Lake at maximum capacity (elevation 468.2 m).

Maximum Length	35.4 km (22.0 mi)
Maximum Effective Length	5.8 km (3.6 mi)
Maximum Width	1.1 km (0.7 mi)
Maximum Effective Width	1.1 km (0.7 mi)
Mean Width	571.8 m (1,875.9 ft)
Maximum Depth	54.7 m (180.0 ft)
Mean Depth	14.7 m (48.1 ft)
Area	208 x 10^5 m^2 (5,148.8 acres)
Volume	304 x 10^6 m^3 (247,934 acre-ft)
Shoreline Length	74.3 km (46.2 mi)
Shoreline Development	4.6%
Slope of Basin	0.15%

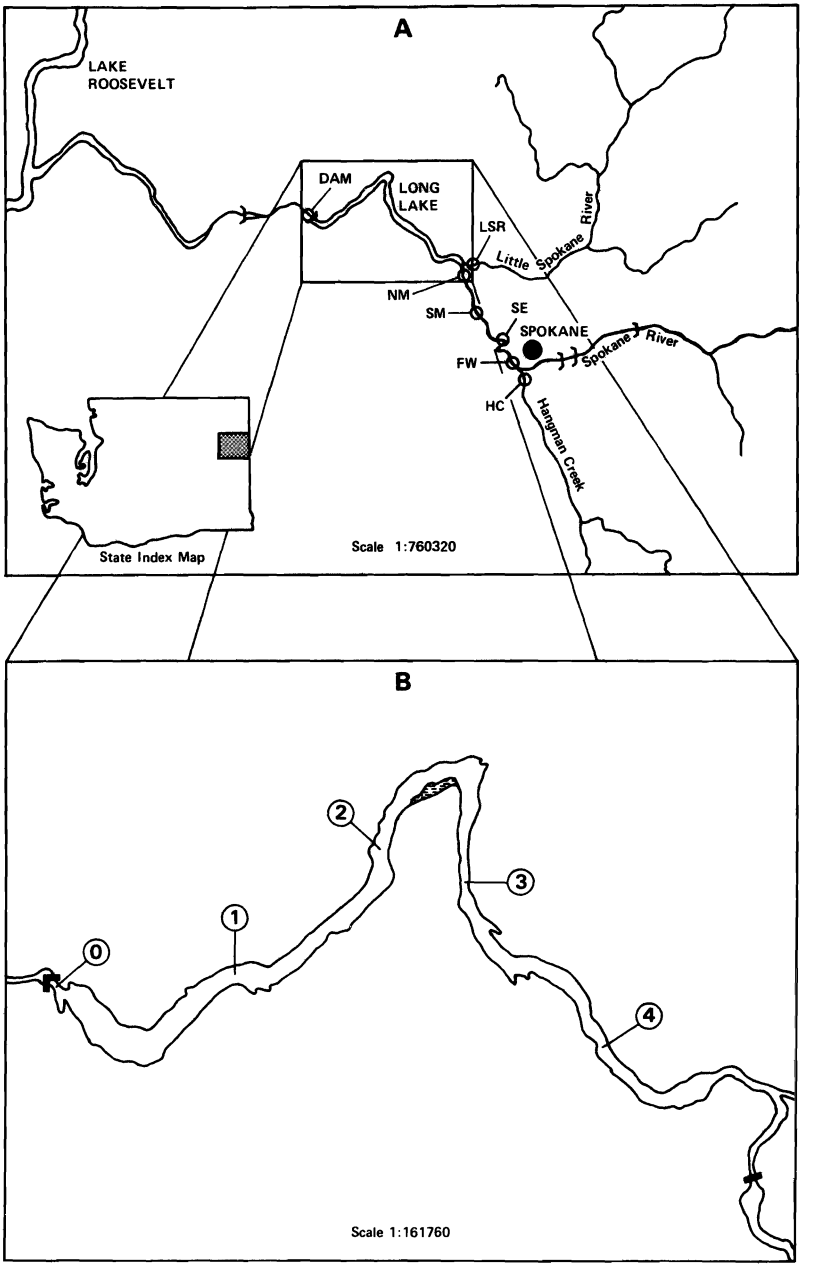

Figure 1. Map of the lower Spokane River system detailing the study area.

for nitrate, nitrite, ammonia, orthophosphate (Table 2) and silica (Figure 2) were conducted as described by the American Public Health Association (1971). All analyses were made within the suggested time limits (APHA, 1971).

Light. Mean solar radiation data were obtained from the records of the National Weather Service at Spokane International Airport (Figure 2).

Phytoplankton Standing Crop and Chlorophyll. Water samples were collected at 2 meter intervals from the surface to the lower limit of the euphotic zone. Equal volumes of the euphotic zone samples were composited and used for chlorophyll determinations, phytoplankton volume determinations, and counts by species. A 250 ml sample of the euphotic zone composite was preserved with Lugol's solution. Cell volumes and counts per unit volume of water were determined for each taxon in the phytoplankton community (Figure 2) utilizing the sedimentation method described by Schwoerbel (1970). Lund, Kipling, and LeCren (1958) have discussed the statistical validity of such direct count methods. Phytoplankton were identified to species using the taxonomic keys of Hustedt (1930), Smith (1950), Prescott (1962), and Patrick and Reimer (1966).

Table 2. Average chemical content of the five Long Lake sampling stations (mg l^{-1}) and their corresponding N:P ratios.

Date	A&F	Filtered Only		
	PO_4-P	PO_4-P	TSIN	N:P[a] Ratio
6- 8	0.001	0.010	0.092	9.2
6-25	0.005	0.010	0.082	8.2
7- 9	0.006	0.007	0.232	33.1
7-22	0.008	0.003	0.083	27.7
8- 5	0.015	0.014	0.482	34.4
8-19	0.006	0.007	0.082	11.7
9- 3	0.014	0.016	0.381	23.8
9-16	0.003	0.007	0.291	41.6
9-30	0.007	0.023	0.431	18.7
10-21	0.025	0.026	0.544	20.9
11-25	0.036	0.049	0.658	13.4
12-16	0.027	0.068	0.867	12.8

[a]For filtered only samples.

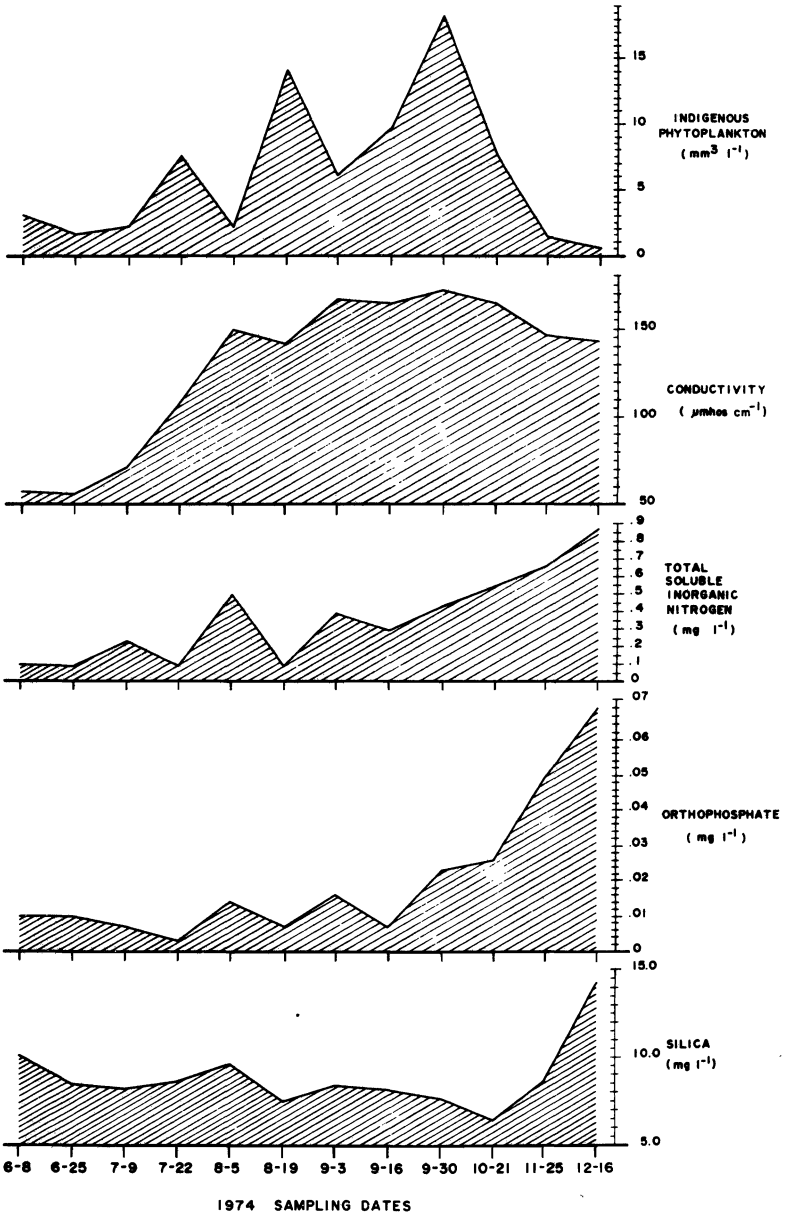

Figure 2. Limnological and chemical profiles of Long Lake during 1974.

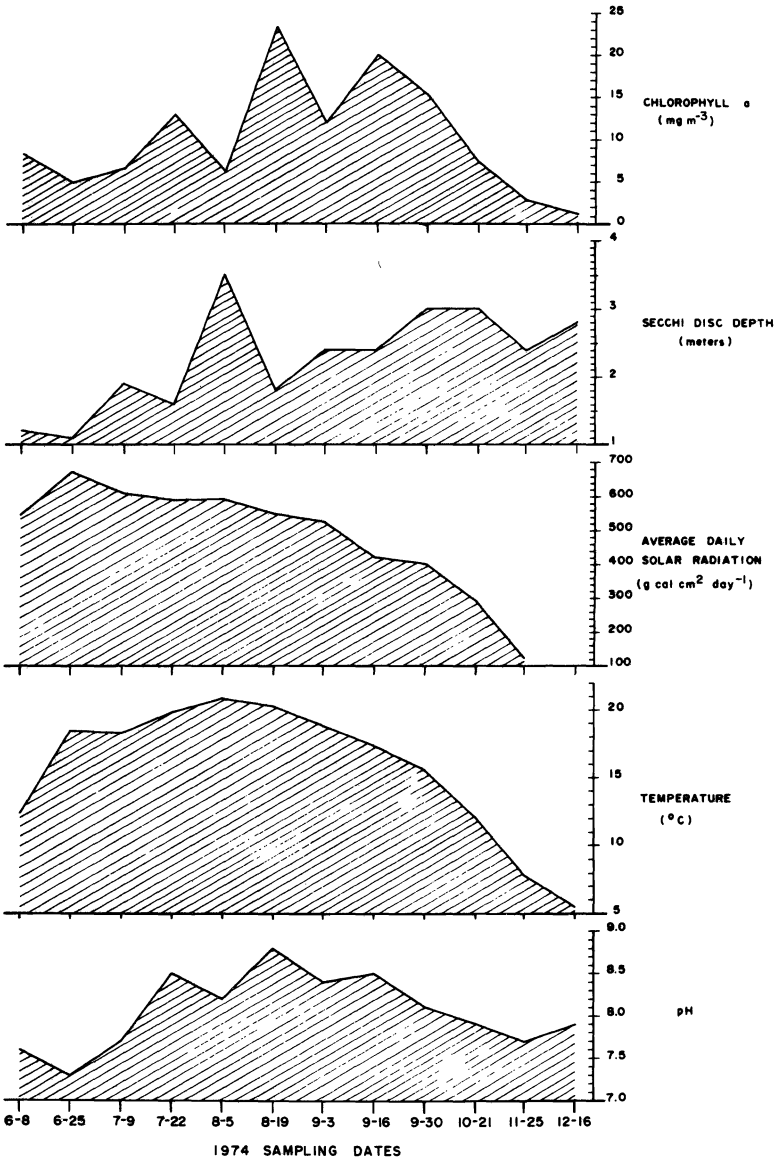

Figure 2. Continued.

Chlorophyll *a* concentrations were determined by filtering (0.45 micron Millipore filters) a known volume (usually 500 ml) of the euphotic zone composite water. Acetone (90 percent) was used as the extraction solvent and the chlorophyll concentrations (mg m^{-3}) were determined as outlined by the American Public Health Association (1971) (Figure 2).

Hydrology. Stage, net inflow, total discharge, and storage records for the reservoir were supplied by the Washington Water Power Company. Water exchange rates for the reservoir were computed by dividing the mean storage for a month by the mean daily outflow for that month (see Table 3).

Laboratory Investigations

Algal Assays. The algal assay procedure bottle test (U.S. EPA, 1971) was used in this study. The water collected for the algal assay was a euphotic zone composite of equal volumes from each of the five stations sampled during each of the 12 cruises. These samples were composited in 1 gallon polypropylene bottles (washed in 10 percent HCL) and autoclaved for 15 minutes at 121°C at the Eastern Washington State College, Department of Biology. Upon cooling, the bottles were sealed and shipped to the Pacific Northwest Environmental Research Laboratory, Eutrophication and Lake Restoration Branch, for completion of preparation and assay.

Upon arrival, the test waters were carbonated with a mixture of 1 percent CO_2 in air until the original pH was obtained. The sample was then filtered through a 0.45 micron porosity membrane filter to remove

Table 3. Water exchange rates (days) in Long Lake (June through December, 1974).

Month	Mean Storage[a] Per Month	Mean Daily[a] Outflow	Exchange Rate (Days)
June	29.72	6.72	4.42
July	30.23	1.82	16.61
August	30.24	0.64	47.25
September	30.23	0.67	45.12
October	30.27	0.71	42.63
November	30.26	0.85	35.60
December	30.34	0.82	37.00

[a]Times 10^7 m^3.

particulate matter which would interfere with an electronic particle counter (Coulter Model ZBI).

The assay was carried out in 500 ml Erlenmeyer flasks containing 100 ml of total sample. Lake waters without nutrient or chelator additions served as controls. The first three tests (6/8, 6/25, and 7/9/74) consisted of a control series and a series spiked with 1.00 mg EDTA l^{-1}. Data from these tests indicated a need for further assessment of nutrient limitation. Beginning with the test water collected on 7/22/74, a 0.02 mg P l^{-1} spike was added. As the test on the water collected on 9/3/74 began, an additional spike of 1.00 mg EDTA l^{-1} plus 0.02 mg P l^{-1} was added to assess the combined effect of nutrient limitation and metal toxicity (Table 4).

All assays were conducted in triplicate and each flask was inoculated to give a final concentration of 1 x 10^3 cells ml^{-1} from a 7-day old culture of *S. capricornutum* Printz. All flasks were incubated for 14 days or until the linear phase of growth was attained.

Water Chemistry. Orthophosphate analyses were made according to the colorimetric procedure outlined by the U.S. Geological Survey (1960).

Table 4. Growth potential of algal assays and indigenous phytoplankton standing crop measurement in Long Lake, Washington.

Date	Algal Assay Maximum Yields[a]				Indigenous Phytoplankton	
	Control	1.00 mg EDTA l^{-1}	0.02 mg P l^{-1}	P+EDTA	(mm^3 l^{-1}) Fresh Weight	(mg m^{-3}) Chlorophyll *a*
6- 8	0.21	0.24	----	----	2.97	8.19
6-25	1.04	0.86	----	----	1.42	4.95
7- 9	2.77	2.27	----	----	2.08	6.68
7-22	2.97	3.42	2.96	----	7.45	12.89
8- 5	8.53	9.26	8.74	----	2.06	6.10
8-19	3.08	2.65	2.73	----	13.75	23.24
9- 3	8.61	12.59	12.67	12.35	6.03	11.92
9-16	10.33	10.08	10.09	10.24	9.61	19.72
9-30	0.08	0.13	6.76	6.38	18.11	15.18
10-21	0.08	0.08	6.25	5.42	7.61	8.31
11-25	0.09	2.34	0.21	7.92	1.31	2.72
12-16	0.09	0.70	1.92	8.43	0.50	1.27

[a]mg dry weight *Selenastrum capricornutum* l^{-1}.

A standard addition of 0.05 mg P l^{-1} was added to each sample before analysis to place the orthophosphate concentration into a more precise analytical range. All determinations were made on a Hitachi Perkin-Elmer Model 139 spectrophotometer (Table 2).

RESULTS AND DISCUSSION

Algal Assay Results

Only controls and EDTA spiked samples were to be run on each water sample collected from Long Lake, a decision based on information from previous studies of Long Lake and the Spokane-Coeur d'Alene drainage basin. The three major pieces of information were: (1) Effluent from the Spokane primary sewage treatment plant had been shown to be the major source of nutrients effecting the enrichment of Long Lake (Soltero et al., 1973 and 1974). (2) Nitrogen trends in the Spokane River system indicated increasing nitrate concentrations from groundwater accretions. The industries, Spokane sewage treatment plant, and tributaries confluent with the Spokane River were relatively insignificant sources of nitrate nitrogen compared to the groundwater (Schmidt and Kreizenbeck, 1973). And (3) zinc concentrations averaging 0.112 mgl^{-1} at the Spokane sewage treatment plant were reduced to less than 0.020 mgl^{-1} (in November 1972 Long Lake water) by natural decomposition and/or complexing of zinc by organic compounds or some other zinc removal mechanism downstream from the Spokane sewage treatment plant (Miller et al., 1975). The Coeur d'Alene Lake watershed is the main contributor of zinc to the Spokane River system—83 percent of the total monthly load during September 1972 (Schmidt and Kreizenbeck, 1973). Climatological conditions in the Spokane-Coeur d'Alene River basin would, therefore, have a major effect on the concentrations of zinc found in Long Lake.

Algal assay data for the period from June 8 to August 19 (Table 4) indicate that biomass could not be increased, relative to the control biomass, with the addition of phosphorus or EDTA. The only exception was the August 5 sample that indicated the presence of a metal toxicant. Algal yield was increased over that found in the control by 8.5 percent with the addition of EDTA.

Water samples collected on September 3 produced approximately 46 percent more biomass than the control with the addition of phosphorus or EDTA, either singly or in combination (Table 4). The factor for converting the orthophosphate content (Table 2) of the test substrate (430 x mg PO_4-P l^{-1}) into maximum yield of the test alga (Shiroyama, Miller, and Greene, 1975) indicated that the chemical analysis for orthophosphate in the September 3 sample was somewhat low. The conversion factor of Shiroyama et al., (1975) for converting the total soluble inorganic

nitrogen (TSIN = NH_3 $\pm NO_3$ $\pm NO_2$) content of the test substrate into maximum yield of the test alga indicated that 14.50 mg dry weight *S. capricornutum* 1^{-1} (±20% = 11.60 to 17.40 mg dry weight 1^{-1}) would be produced if nitrogen was the secondary limiting nutrient. Figure 3 shows that the addition of phosphorus or EDTA, singly or in combination, produced a biomass yield of 12.35 to 12.67 mg dry weight *S. capricornutum* 1^{-1}. These data indicate that the single addition of phosphorus acted almost as efficiently as the EDTA spike to produce greater yields in the spiked flasks than in the control flasks. Leckie and James (1974) reported that phosphorus can act as an inorganic ligand. However, Greene et al., (1975) could not produce a biomass increase in laboratory cultures of *S. capricornutum* grown in the presence of sublethal levels of zinc ($ZnCl_2$) by adding as much as 0.930 mg P 1^{-1}.

During the period of September 16 to 30, fall overturn occurred in Long Lake. Concurrent with this occurrence, water samples taken for algal assays (September 30 and October 21) did not produce significant increased biomass of *S. capricornutum* (Figures 4 and 5) in control or EDTA spiked flasks. However, the phosphorus and phosphorus plus EDTA spiked samples produced biomass increases of approximately 7980-fold and 6780-fold, respectively, over that in the control flasks. Chemical analysis for orthophosphate indicated a sufficient quantity of this element to produce a large biomass. However, algal assay results (Figures 4 and 5) indicated that the phosphorus present was not biologically available. The addition of 0.020 mg P 1^{-1} to the September 30 and October 21 test waters, although greatly increasing yield, did not achieve the predicted biomass that the nutrient addition alone should have yielded (8.60 mg dry weight 1^{-1} ±20%). Chemical analysis for TSIN also indicated that sufficient nitrogen was present to produce biomass in excess of the levels achieved. Although phosphorus was the primary limiting nutrient in both of these samples, heavy metal stress was obvious—particularly in the October 21 sample. The phosphorus plus EDTA spiked October 21 sample achieved a maximum yield of 5.42 mg dry weight *S. capricornutum* 1^{-1} in 6 days. The sample spiked only with phosphorus achieved an almost identical yield of 5.54 mg dry weight *S. capricornutum* 1^{-1}. However, 24 days of culturing were required to reach this level of growth (Figure 5). A possible explanation for this growth lag recovery is that extracellular substances produced by the test alga formed chemical complexes with the growth inhibiting substance. Fogg and Westlake (1955) demonstrated that *Anabaena* produces a polypeptide which forms a nontoxic complex with copper, iron, and phosphorus. Other freshwater algae form other polypeptides.

Determination of the limiting nutrient for each sample was calculated from the TSIN and orthophosphate ratios for these elements in the filtered water samples (Table 2). The ratio of the TSIN to orthophosphate yield

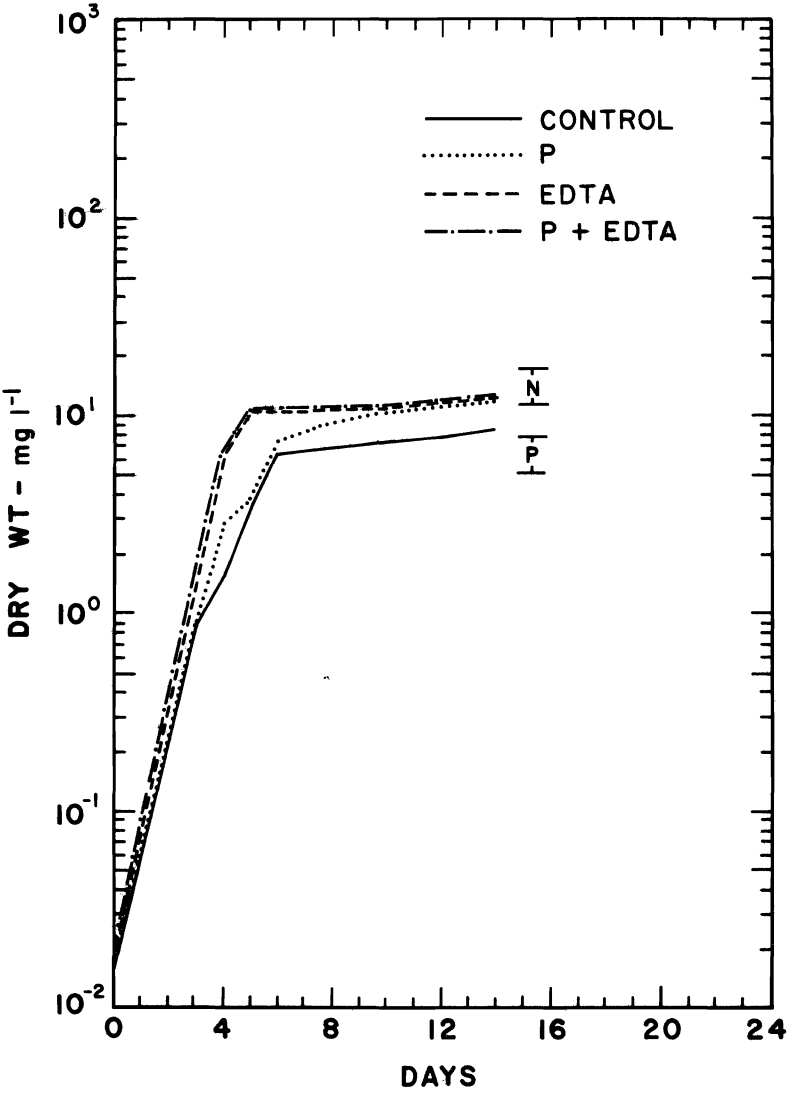

Figure 3. Growth response of *S. capricornutum* to the addition of phosphorus and EDTA, singly and in combination, in Long Lake euphotic zone composite water (autoclaved and filtered) collected on September 3, 1974.

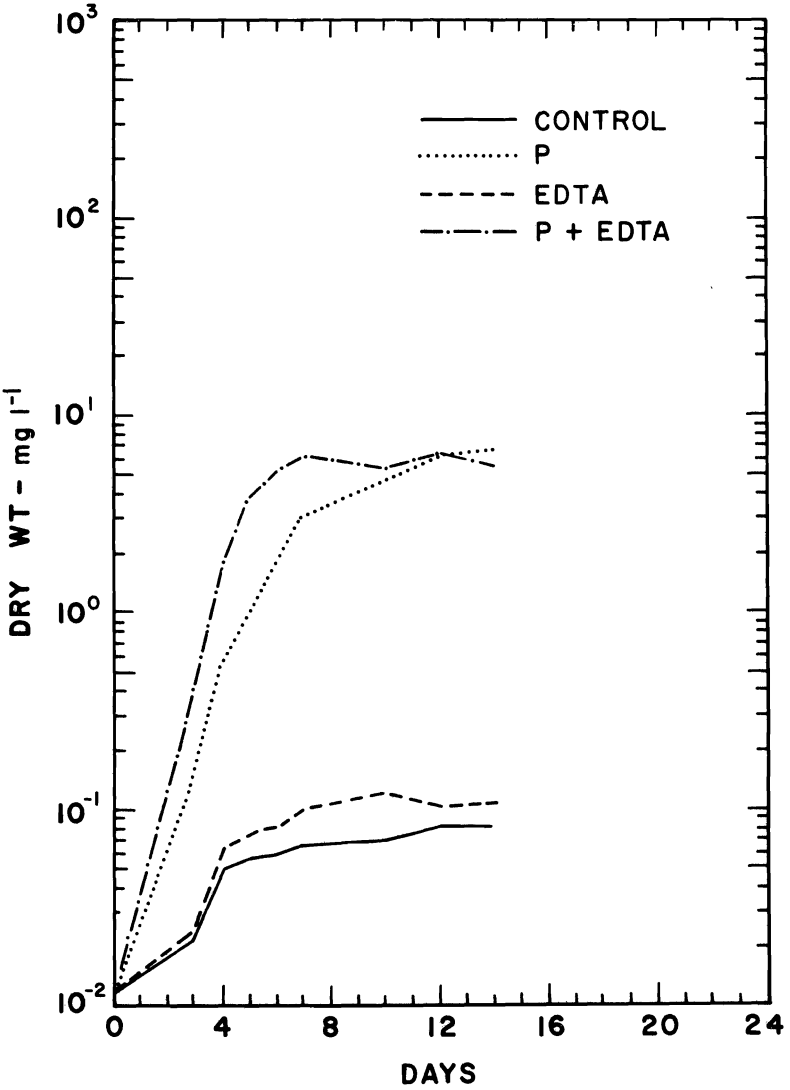

Figure 4. Growth response of *S. capricornutum* to the addition of phosphorus EDTA, singly and in combination, in Long Lake euphotic zone composite water (autoclaved and filtered) collected on September 30, 1974.

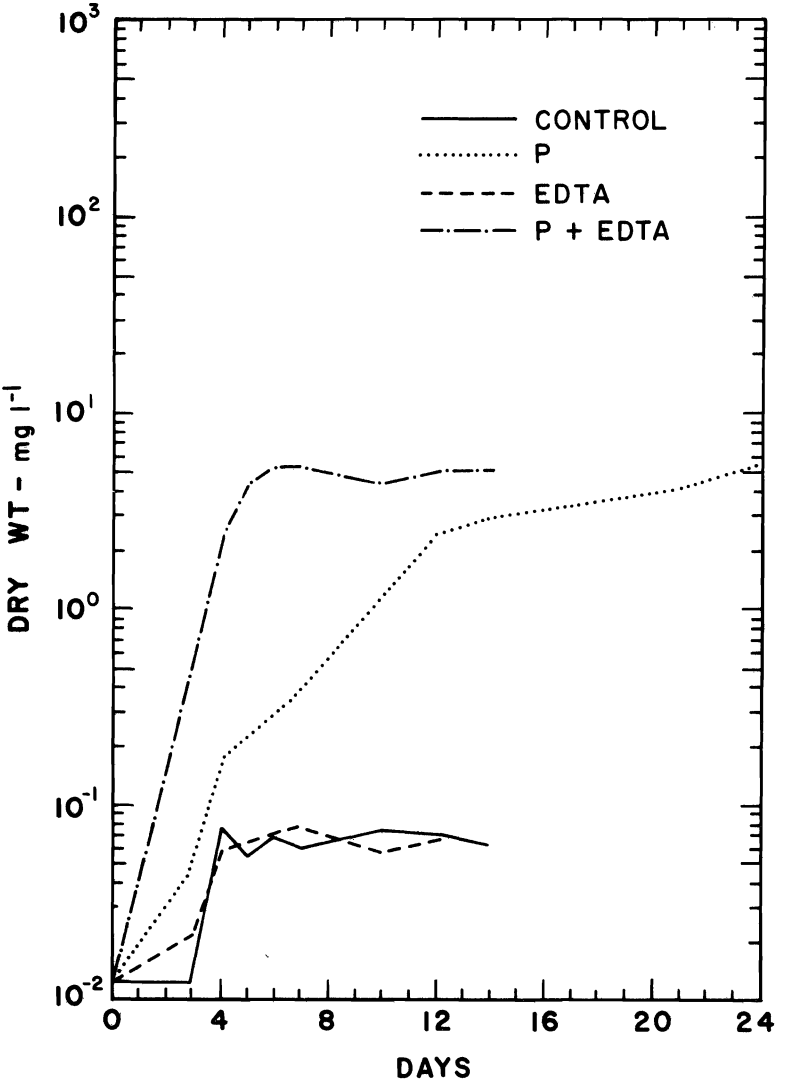

Figure 5. Growth response of *S. capricornutum* to the addition of phosphorus and EDTA, singly and in combination in Long Lake euphotic zone composite water (autoclaved and filtered) collected on October 21, 1974.

factors (38/430) indicated an optimum N:P ratio of 11.3 for the support of *S. capricornutum* grown in inorganic nutrient medium (Shiroyama et al., 1975). This ratio compared favorably to that of 10 to 1 reported by Chiaudani and Vighi (1974) for *S. capricornutum* cultured in filtered waters from 26 temperate lakes in northern Italy.

The N:P ratio in natural waters can theoretically indicate which of these elements may be limiting algal growth. Waters containing greater than 11.3 N:P content may be considered phosphorus limited, while those containing N:P ratios less than 11.3 can be considered nitrogen limited for algal growth.

The TSIN and orthophosphate content of the filtered water samples from each of the five stations on Long Lake were averaged to relate to the euphotic zone composite samples used for algal assay. The average N:P ratios for these samples (Table 2) indicate that phosphorus was the primary limiting nutrient in Long Lake during the study period. The samples collected on June 8 and 25 contained N:P ratios of 9.2 and 8.2, respectively (Table 2), coinciding with the period of peak inflow to Long Lake (Table 3). The limiting nutrient determinations showed a high degree of correlation with indigenous phytoplankton volumes (based solely on chemical analysis of filtered waters). See Figures 6 and 7.

Phosphorus as the primary limiting nutrient in Long Lake was further substantiated by the responses of *S. capricornutum* to both the nutrient and chelator additions in laboratory algal assays, as well as by predicted maximum yields for the alga. Those yields were computed from the orthophosphate and TSIN content of both filtered and autoclaved and filtered (PO_4-P only) test waters—TSIN was not analyzed in the autoclaved and filtered waters. Both the assay and yield results produced similar limiting nutrient relationships in 9 of the 12 test samples (Table 6). The algal assay for the June 25 and July 22 samples (Table 5 and Figure 8) indicated that the primary limiting nutrient could not be determined. The growth response (Figure 8) indicates nearly simultaneous growth limitations by both nitrogen and phosphorus.

Although Long Lake is highly eutrophic, major blue-green algal blooms did not occur. The data in Figure 9 suggest that the reservoir might need a period of primary nitrogen limitation before blue-green alga can become a major part of the indigenous phytoplankton standing crop. Field data indicate that Long Lake was primarily nitrogen limited only on June 8 and 25. The only appearance of blue-green alga occurred on those dates, as well as on July 9.

Long Lake was highly eutrophic during the summer growth period (Table 4). Miller, Maloney and Greene (1974) defined four productivity

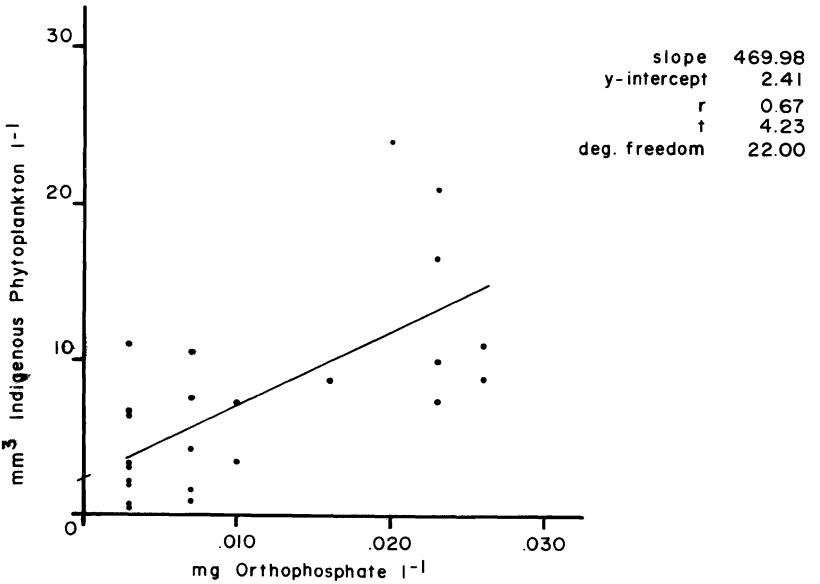

Figure 6. Linear regression analysis of orthophosphate (mg l^{-1}) and indigenous phytoplankton (mm^3 l^{-1}) in phosphorus limited Long Lake euphotic zone composited and filtered waters.

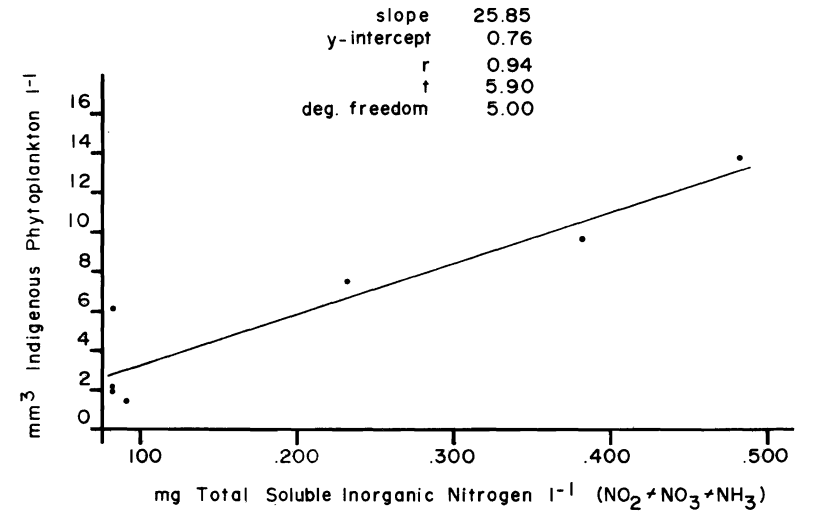

Figure 7. Linear regression analysis of total soluble inorganic nitrogen (mg l^{-1}) and indigenous phytoplankton (mm^3 l^{-1}) in nitrogen limited Long Lake euphotic zone composited and filtered waters.

Table 5. The order of primary nutrient limitation in waters collected from Long Lake, Washington (1974).

Date	Limiting Factors	
	Indig. Phyto.	Algal Assay
6- 8	N / P[a]	P/N
6-25	N / P	P ≠ N
7- 9	P/N	P/N
7-22	P/N	N ≠ P
8- 5	P/N	P/N
8-19	P ≠ N	P ≠ N
9- 3	P/N	P/N
9-16	P/N	P/N
9-30	P/N	P/X
10-21	P/N	P& T/X
11-25	P/N	P/N
12-16	P/N	P/N

[a]The letters in each column indicate the: (1) primary limiting nutrient, and (2) the secondary limiting nutrient, i.e., P/N. T indicates the presence of a heavy metal toxicant.

groups which help define the trophic status of the test water. Within this productivity grouping, algal assay yields equal to or greater than 6.10 mg dry weight *S. capricornutum* l^{-1} indicate a high productivity test water. The September 3 Long Lake water sample produced almost twice that yield (Table 4).

Phytoplankton Standing Crop and Chlorophyll *a*

Water quality studies routinely determine chlorophyll *a* concentration in water as a measure of algal biomass. However, chlorophyll *a* measurements are subject to two major sources of error—mechanical problems with the analytical procedure and inherent biological conditions. For example, Glooshenko et al. (1974) and Munawar and Nauwerck (1971) reported diel changes in chlorophyll *a* content of the seston, attributing them to differences of chlorophyll to biomass concentrations or changes in species composition.

Linear regression analysis indicated a high degree of correlation ($r = 0.80$) between chlorophyll *a* (mg m^{-3}) and indigenous phytoplankton ($mm^3\ l^{-1}$) standing crop measurements made during the study. Figure 10 illustrates the regression line derived from statistical analysis of the 60 euphotic zone samples. Although correlation between the two parameters

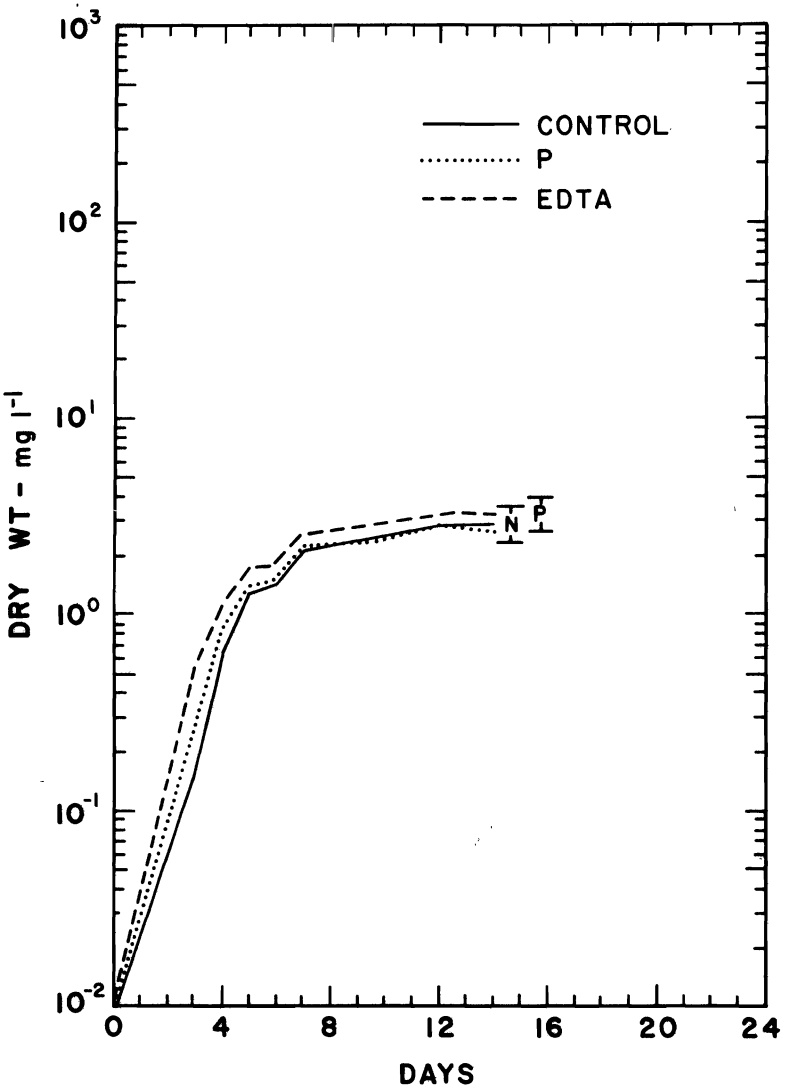

Figure 8. Growth responses of *S. capricornutum* to singular additions of phosphorus and EDTA in Long Lake euphotic zone composited water (autoclaved and filtered) collected on July 22, 1974.

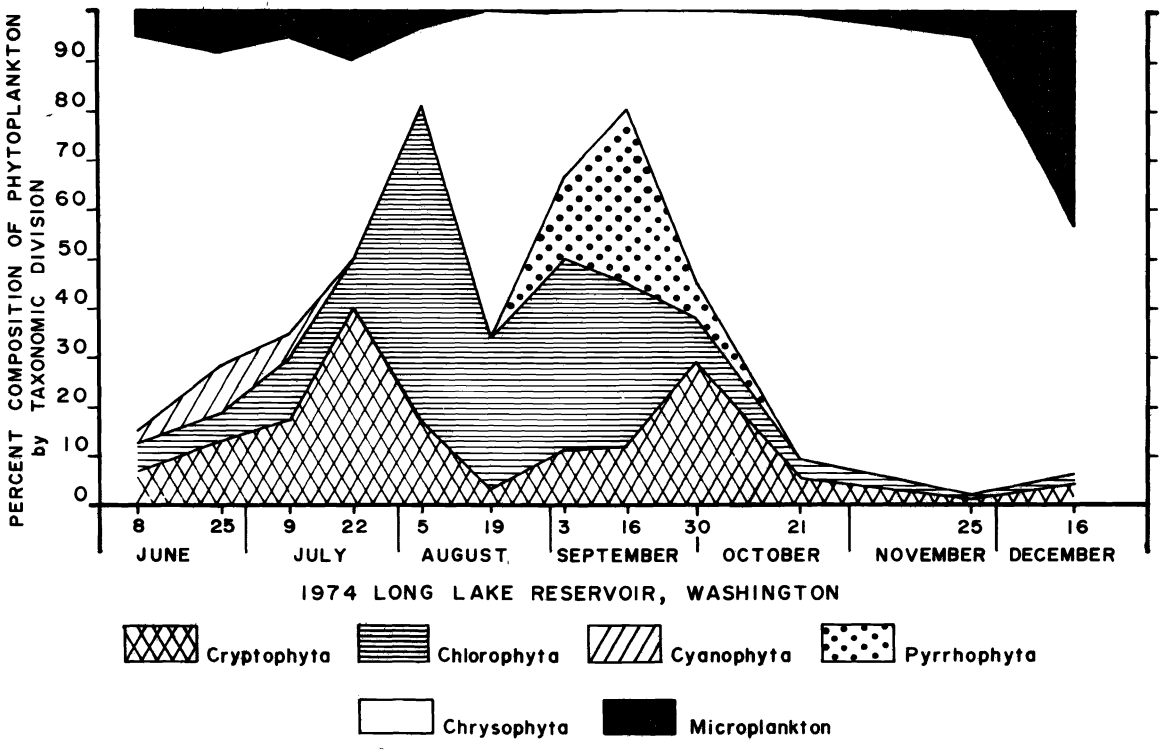

Figure 9. Percent composition of indigenous phytoplankton in Long Lake, Washington.

is very good, a scatter diagram of the chlorophyll *a* and phytoplankton standing crop means of the euphotic zone composites clearly indicates that the samples collected on September 30 and October 21 contain approximately one-half of the chlorophyll *a* as found in equal volumes of phytoplankton in other samples collected during the study. A linear regression analysis excluding these two deviant samples resulted in a greatly improved correlation (r = 0.98).

Prior to the collection of the September 30 euphotic zone water sample, fall turnover caused a substantial vertical mixing in the reservoir. The lower temperature (greater density) of the inflowing river resulted in a bottom density flow as shown in Figure 11 by the distinct cells of higher conductive water intersecting the bottom at stations 0, 1 and 2. This underflow initiated fall homothermy.

Soltero et al. (1975) suspected the presence of parahopeite ($Zn^3(PO_4) \cdot 4H_2O$) in sediment core samples collected during this study. The resuspension of this zinc phosphate complex could be one possible cause

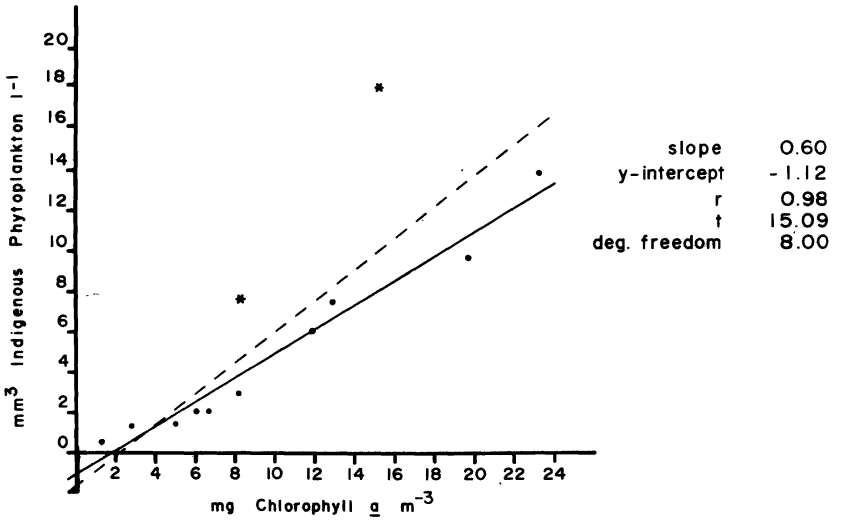

Figure 10. Linear regression analysis of indigenous phytoplankton chlorophyll *a* content (mg m^{-3}) and cell-volumes (mm^3 l^{-1}) in filtered Long Lake euphotic zone composited waters. Asterisks indicate data points for samples collected on September 30 and October 21, 1974. The broken line indicates the relationship between the parameter when these two outliers are included in the linear regression analysis (r = 0.80).

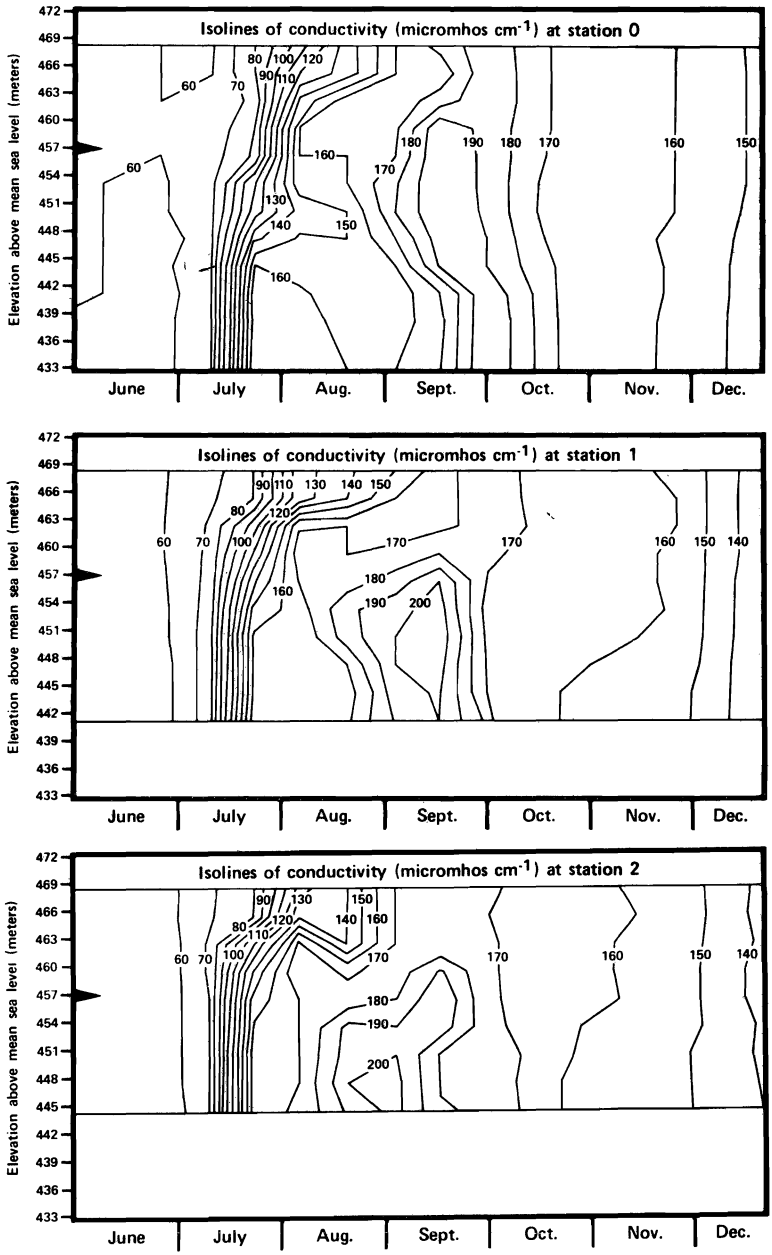

Figure 11. Isolines of conductivity (micromhos cm^{-1}) at stations 0, 1, and 2, Long Lake (1974).

of the reduced chlorophyll *a* content of the indigenous phytoplankton at this time. Laboratory algal assays performed on these two water samples also produced aberrant results previously discussed in the section of algal assays.

Watson, Carpenter and Munawar (1975) demonstrated seasonal differences between the surface chlorophyll content of Lake Ontario and estimates of phytoplankton biomass derived from counts and volume conversions. Chlorophyll *a* and estimated phytoplankton biomass corresponded fairly closely during the spring months when diatoms were predominant in the samples. On the summer cruises, chlorophyll *a* estimates were low while phytoplankton biomass was maximal. At that time the species composition was different, with large numbers of green and blue-green algae present.

Seasonal data for Long Lake indicate that the Bacillariophyceae dominated the phytoplankton throughout this study (Figure 12). *Fragilaria crotonensis, Melosira italica, M. granulata, Cryptomonas sp.* and *Sphaerocystis schroeteri* were the major contributors to the algal community. *Fragilaria crotonensis* contributed the greatest biomass (37 percent) to the total algal standing crop (Figure 13) and occurred in 68 percent of the samples. *Cryptomonas sp.* and *S. schroeteri* composed 27 percent of the standing crop and were present over half of the time.

During the period of August 8 to September 16, the Chlorophycea composed from 30.5 to 64.1 percent of the phytoplankton standing crop. Chlorophyll *a* estimates relative to phytoplankton biomass were consistent throughout the study when diatoms or green algae were dominant (Figures 9 and 10).

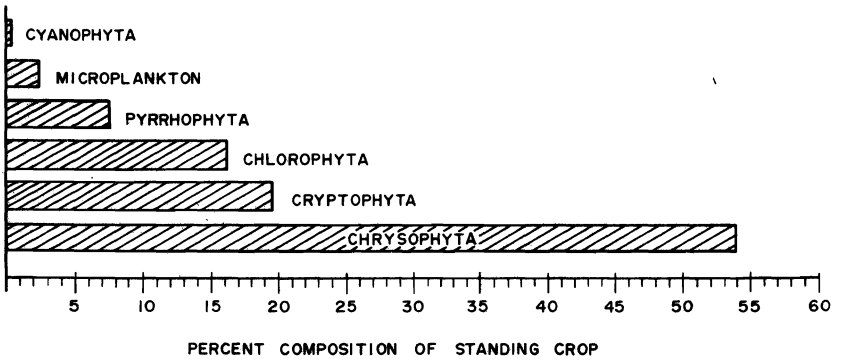

Figure 12. Average percent of indigenous phytoplankton standing crop composition (by taxonomic division) for entire 1974 study.

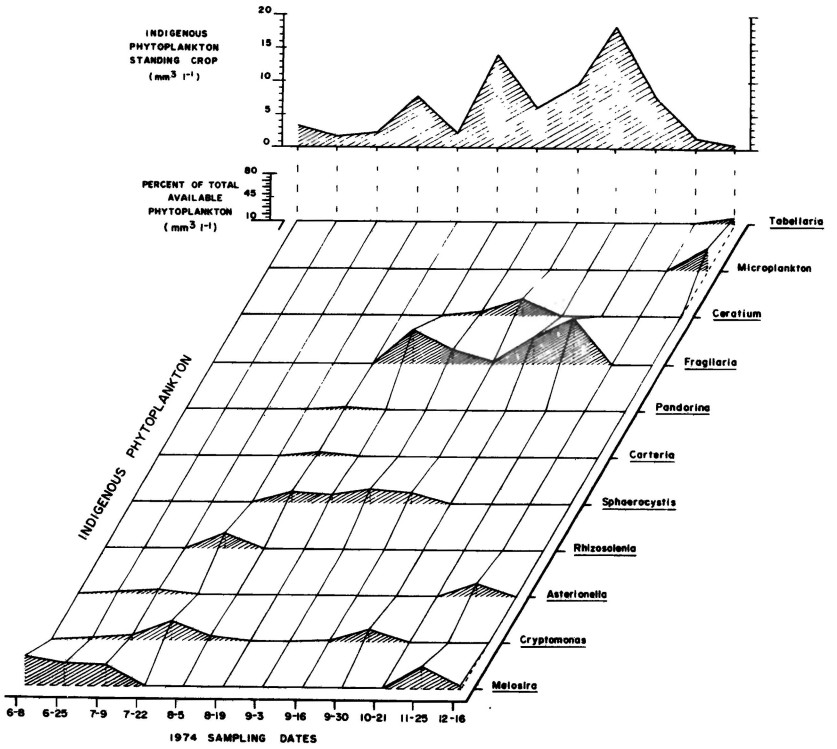

Figure 13. Average percent of indigenous phytoplankton genera composing greater than 10 percent of the standing crop in each Long Lake euphotic zone composite and total indigenous phytoplankton standing crop ($mm^3 l^{-1}$).

Indigenous Phytoplankton Standing Crops and Algal Assay Maximum Yields

Aquatic biologists generally understand that plankton move passively with the water mass and do not move away from perturbed conditions. Thus their community structure tends to reflect the immediate past history of the water mass from which the plankton are sampled. In general, species changes are the result of environmental modifications originating both from the community itself and from independent sources.

Figure 13 shows the 10 genera that comprised the major portion of the indigenous phytoplankton standing crop during this study. Seven of

the genera alternated between a position of dominance and mere presence. For example, *Melosira sp.* composed 54, 44, 43, and 45 percent of the standing crop of June 8 and 25, July 9, and November 25, respectively. *Fragilaria sp.* constituted 61, 32, 14, 50 and 78 percent of the phytoplankton standing crop of samples collected on August 19, September 3, 16, and 30, and October 21, respectively. The green alga, *Sphaerocystis schroeteri,* produced 27, 24, 31 and 27 percent of the standing crop of the August 5 and 18, and September 3 and 16 samples, respectively.

Under natural conditions, phytoplankton possess a host of characters probably essential to their survival in the natural habitat. The assemblage of regulatory mechanisms they have evolved enable the phytoplankton specie best suited to the particular environmental conditions surrounding it to produce biomass to the limits of the nutrient concentrations present. Brandt (1899) was perhaps the first to suggest that the availability of nutrients present in a body of water limits the energy fixed by the phytoplankton in that body of water. Inorganic nutrients are necessary for biochemical synthesis of cell material. Consequently, the life processes depend on the quantity, nature, and rate of addition of nutrients—particularly nitrates and phosphates.

Figure 14 illustrates the biomass relationships between the indigenous phytoplankton standing crop ($mm^3\ l^{-1}$) and mg dry weight l^{-1} of the laboratory test alga, *S. capricornutum*. At first glance an inverse relationship seemed obvious between these two parameters. However, results derived from a linear regression analysis performed on these data revealed a very low correlation ($r = 0.34$).

Further examination of the data determined that what appeared to be an inverse relationship was actually a two week shift of comparable data caused by a combined effect of two events: (1) The autoclaving and filtration of the test water, and (2) the time period required for the indigenous phytoplankton to convert available nutrients into biomass. For example, chemical anaysis of the filtered euphotic zone composite water sample collected on July 9 indicated the presence of .007 mg PO_4-P l^{-1}. The sample collected two weeks later (July 22) contained a biomass of 7.44 mm^3 indigenous phytoplankton l^{-1}. The orthophosphate content of the autoclaved and filtered July 22 sample was, therefore, nearly identical to that level of orthophosphate determined to be present in the filtered July 9 sample. Figure 6 illustrates data derived from a linear regression analysis of indigenous phytoplankton biomass and chemical analysis for PO_4-P of filtered water collected two weeks prior to the samples containing the indigenous biomass. The 24 samples determined to be primarily phosphorus limited produced a correlation with biomass of $r = 0.67$ at the $P = 0.001$ confidence level.

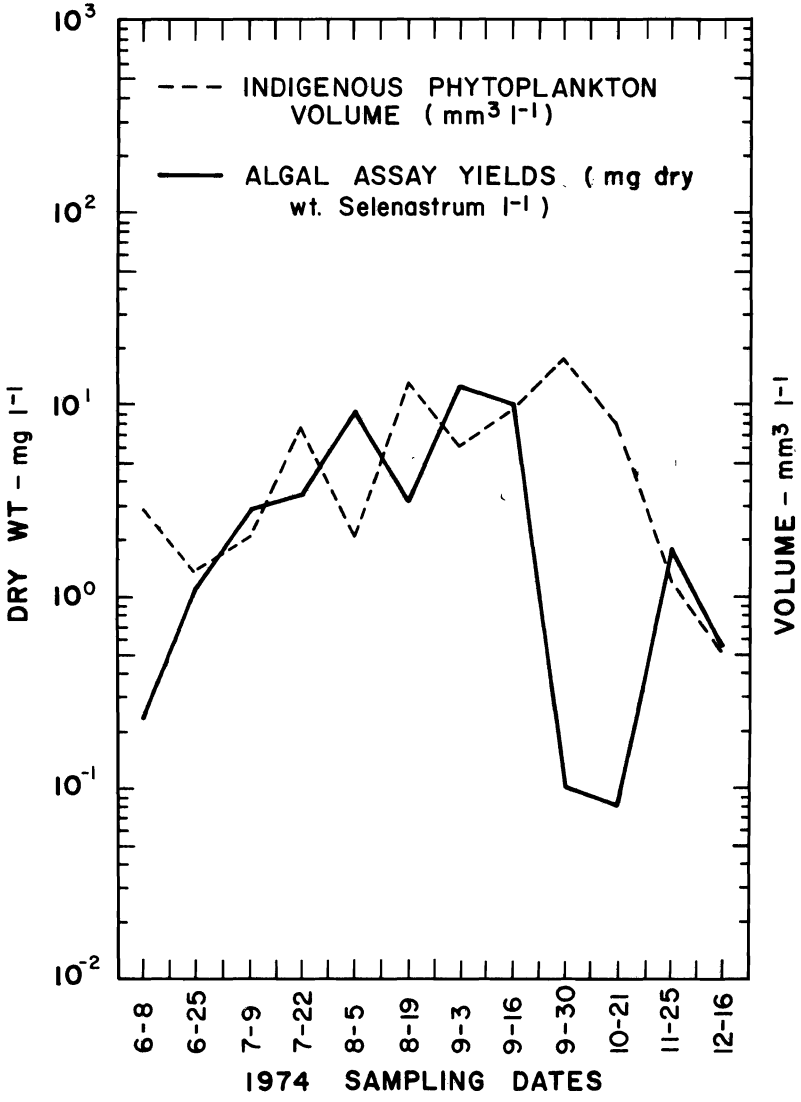

Figure 14. Relationship between indigenous phytoplankton standing crop ($mm^3\ l^{-1}$) and laboratory algal assay maximum yields (mg dry weight l^{-1}).

Skulberg (1966) stated that the concentrations of essential nutrients in natural waters are determined by a dynamic state of balance. Taking a water sample to the laboratory and using it for the algal assay experiment completely alters this condition. Some nutrient alteration probably did occur in the Long Lake samples which had been autoclaved and filtered. However, no serious discrepancies between laboratory and indigenous phytoplankton biomass could be attributed to this cause.

When the September 30 and October 21 samples were rejected as outliers (on the evidence presented previously), a linear regression analysis of the relationship between indigenous phytoplankton and maximum yields of *S. capricornutum* (Figure 15) indicated a high degree of correlation (r = 0.95). However, some data manipulation was necessary to achieve this high correlation (Table 6). The samples collected on June 8, September 16, November 25, and December 16 were entered into the linear regression program with the algal assay yields relative to the indigenous phytoplankton standing crop at the same time the sample was

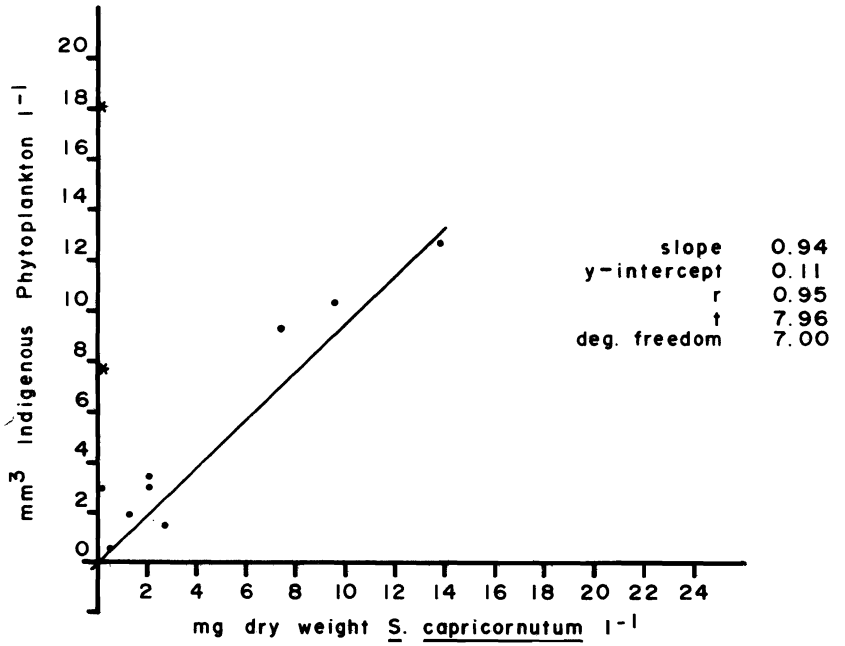

Figure 15. Linear regression analysis of *S. capricornutum* maximum yields (mg dry weight l^{-1}) and the indigenous phytoplankton standing crop ($mm^3 l^{-1}$).

Table 6. The relationship between field and laboratory biomass yields as entered into the program for the linear regression anaysis shown in Figure 15.

Date	Algal Assay[b] Maximum Yield	Indigenous[c] Phytoplankton	Date
6- 8	0.24	2.97	6- 8
7- 9	2.77	1.42	6-25
7-22	3.42	2.08	7- 9
8- 5	9.26	7.44	7-22
8-19	3.08	2.06	8- 5
9- 3	12.59	13.75	8-19
9-16	10.33	9.62	9-16
9-30	0.10[a]	18.11[a]	9-30
10-21	0.08[a]	7.61[a]	10-21
11-25	1.92	1.31	11-25
12-16	0.54	0.50	12-16

[a]Data rejected as outliers. Asterisks are also used to indicate these data points on Figure 15.

[b]Dependent variable (X_1) in mg dry weight l^{-1}.

[c]Independent variable (Y_1) in mm^3 l^{-1}.

collected. The samples collected from July 9 through September 13 were entered with the laboratory yields relative to the indigenous phytoplankton standing crop two weeks prior to the sampling dates. At first this anomaly in the data was believed to be caused by the effect of physical conditions (temperature and/or light) upon the indigenous phytoplankton. Further investigation determined that the shift of some data relative to indigenous phytoplankton biomass and *S. capricornutum* maximum yields correlated when the reservoir was chemically and thermally stratified (Figure 16).

The linear regression analysis of the relationship between indigenous phytoplankton volume and *S. capricornutum* maximum yields (Table 6 and Figure 15) resulted in an equation that can be used to predict the average indigenous phytoplankton standing crop in Long Lake Reservoir. This information can be calculated from the expression:

$$\begin{pmatrix}\text{Indigenous Phytoplankton} \\ (mm^3\ l^{-1})\end{pmatrix} = 0.94 \begin{pmatrix}\textit{S. capricornutum} \\ \text{Maximum Yield} \\ (\text{mg dry weight } l^{-1})\end{pmatrix} + 0.11$$

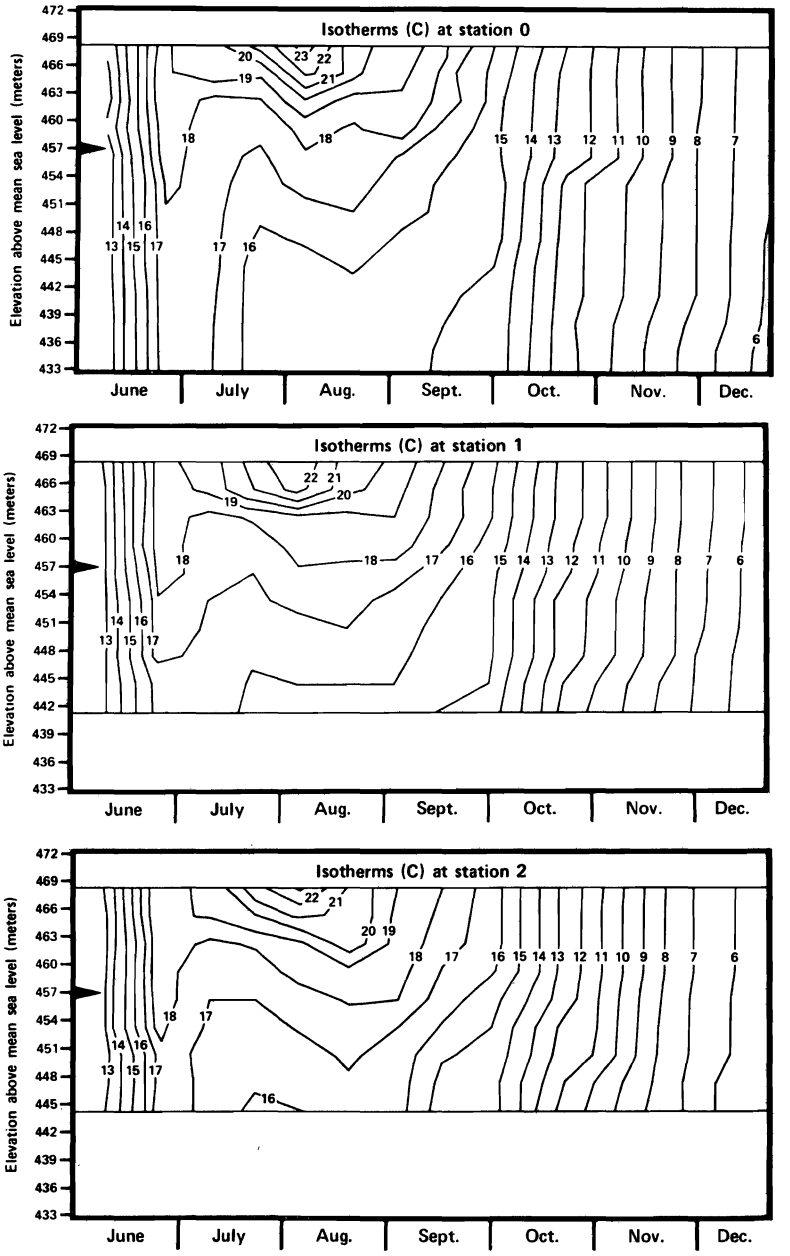

Figure 16. Isotherms (c) at stations 0, 1 and 2, Long Lake (1974).

If the reservoir is homothermal, the equation can be used to calculate the approximate indigenous phytoplankton standing crop at the time of sampling. If the reservoir is stratified, the results from the equation will indicate the phytoplankton biomass that was present approximately two weeks prior to the sampling date.

Indigenous Phytoplankton Chlorophyll *a* and Algal Assay Maximum Yields

Although chlorophyll *a* measurements are subject to error from analytical procedure and inherent biological conditions, it is a most useful parameter in studies of phytoplankton ecology. This simple technique consumes much less time than cell-volume biomass assessments.

A linear regression analysis was done on the chlorophyll *a* content of indigenous phytoplankton (mg m^{-3}) and mg dry weight *S. capricornutum* 1^{-1} (Figure 17). The data were manipulated in the same manner as the analysis of indigenous phytoplankton volume and *S. capricornutum* yields, depending on whether Long Lake was stratified or homothermal (Table 7). The linear regression analysis of the relationship between chlorophyll *a* (mg m^{-3}) in the indigenous phytoplankton populations and *S. capricornutum* maximum yields (mg dry weight 1^{-1}) resulted in an equation that can be used to predict the mean chlorophyll *a* content of Long Lake Reservoir. This information can be calculated from the equation:

$$\text{Chlorophyll } a \ (\text{mg m}^{-3}) = 1.54 \left(\begin{matrix} \textit{S. capricornutum} \\ \text{Maximum Yield} \\ (\text{mg dry weight } 1^{-1}) \end{matrix} \right) + 1.96$$

Zooplankton

Zooplankton were not collected during this study. Nevertheless they are an important element influencing the standing crop of certain algal species within the reservoir (Graham, 1975). Porter (1973) has shown that zooplankton can produce an effect similar to physical and chemical factors and can determine which algae comprise a phytoplankton community. Her controlled field experiments demonstrated that heavy grazing pressure increased the abundance of *Sphaerocystis schroeteri* in the community. In previous Long Lake investigations, Graham (1975) found that an increase in biomass and abundance of *S. schroeteri* coincided with an increase in the biomass of the reservoir's major herbivore, *Daphnia retrocurva.*

SUMMARY AND CONCLUSIONS

The Spokane primary sewage treatment plant is the major source of nutrient input influencing the trophic status of Long Lake. An average of 113,500 m^3 of primary sewage was discharged into the Spokane River during the study period increasing loads of total nitrogen and phosphate by 4.52 and 1.61 metric tons day^{-1}, respectively.

Increased yields of *S. capricornutum* in EDTA spiked test waters indicated the continued presence of toxic levels of heavy metal(s).

Test water collected on September 3, 1974, from the highly eutrophic Long Lake produced a maximum yield of 12.67 mg dry weight of *S.*

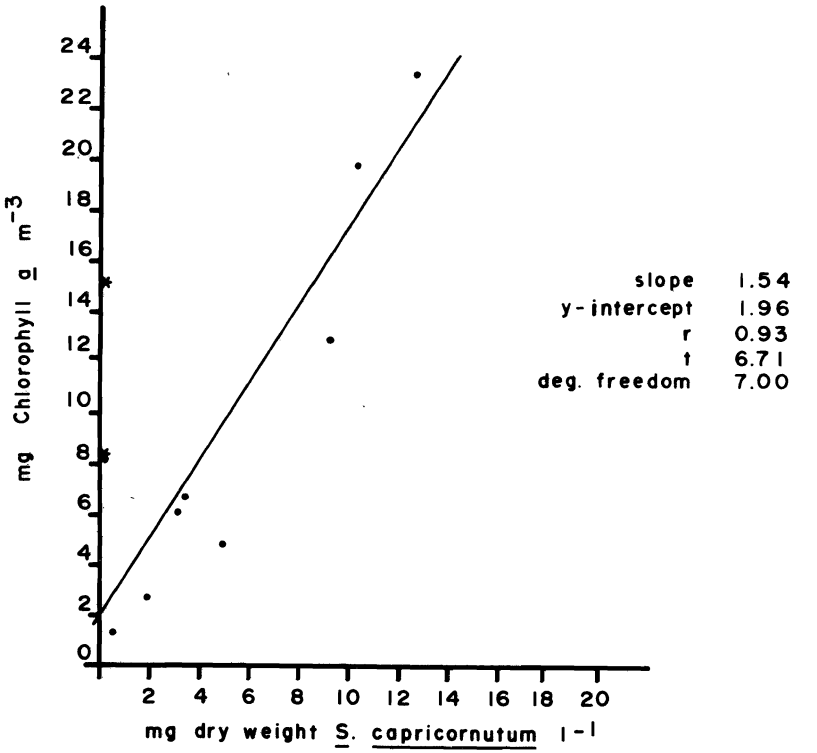

Figure 17. Linear regression analysis of *S. capricornutum* maximum yields (mg dry weight l^{-1}) and the chlorophyll *a* content (mg m^{-3}) of the indigenous phytoplankton.

capricornutum 1^{-1}. This yield is more than twice the minimum biomass level indicative of highly eutrophic water.

Algal assays indicated that Long Lake composite samples were primarily phosphorus limited. On three occasions the reservoir appeared to be limited so closely by both nitrogen and phosphorus that the primary limiting nutrient could not be determined.

Correlation coefficients of 0.67 and 0.94 were achieved between the filtered water PO_4-P and TSIN content, respectively, and the indigenous phytoplankton standing crop in the samples collected two weeks after those analyzed chemically.

Between September 16 and 30, 1975, cooler temperatures and higher conductance of the influent Spokane River caused an underflow in the reservoir that eventually broke down thermal and chemical stratification and induced extensive vertical mixing. Water samples collected after this occurrence could not support growth of *S. capricornutum* in control or EDTA spiked samples. Concomitantly, indigenous phytoplankton

Table 7. The relationship between indigenous phytoplankton chlorophyll *a* and maximum yields of *S. capricornutum* as entered into the program for the linear regression analysis shown in Figure 17.

Date	Algal Assay[b] Maximum Yield	Extracted[c] Chlorophyll *a*	Date
6- 8	0.24	8.19	6- 8
7- 9	2.77	4.95	6-25
7-22	3.42	6.68	7- 9
8- 5	9.26	12.89	7-22
8-19	3.08	6.10	8- 5
9- 3	12.59	23.24	8-19
9-16	10.33	19.72	9-16
9-30	0.10[a]	15.18[a]	9-30
10-21	0.08[a]	8.31	10-21
11-25	1.92	2.72	11-25
12-16	0.54	1.27	12-16

[a]Data rejected as outliers. Asterisks are used to indicate these data points on Figure 17.

[b]Dependent variable (X_1) in mg dry weight 1^{-1}.

[c]Independent variable (Y_1) in mg m^{-3}.

contained approximately one-half of the chlorophyll *a* concentration per unit volume found in all other samples collected during the study. Resuspension of a possible zinc phosphate complex or other materials from the sediments may have caused these anomalous results.

Correlation between the chlorophyll *a* content and cell volume measurements of indigenous phytoplankton in the Long Lake euphotic zone composited waters had a coefficient of 0.98 when the September 30 and October 21 data were discarded as outliers.

A high correlation ($r = 0.95$) between mm^3 indigenous phytoplankton l^{-1} and mg dry weight *S. capricornutum* l^{-1} was achieved when consideration was given to whether the reservoir was stratified or homothermal. An equation was developed which can be used to predict the mean indigenous phytoplankton standing crop in Long Lake Reservoir (mm^3 indigenous phytoplankton $l^{-1} = 0.94$ x mg dry weight *S. capricornutum* $l^{-1} + 0.11$). If the reservoir is homothermal, the equation can be used to calculate the approximate indigenous phytoplankton standing crop at the time of sampling. If stratified, the answer to the equation will indicate the phytoplankton standing crop that had been present approximately two weeks prior to the sampling date.

An equation also was developed for predicting chlorophyll *a* concentrations in Long Lake. The guidelines for the application of the standing crop biomass equation also apply to this procedure. The equation to predict chlorophyll *a* was: Chlorophyll *a* (mg m^3) = 1.54 x mg dry weight *S. capricornutum* $l^{-1} + 1.96$ ($r = 0.93$).

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Comparison of the Algal Growth Responses of *Selenastrum capricornutum* Printz and *Anabaena flos-aquae* (Lyngb.) De Brebisson in Waters Collected from Shagawa Lake, Minnesota

T. Shiroyama, W. E. Miller, and J. C. Greene*

INTRODUCTION

Since 1900, a variety of algal assays have been used to determine the fertility of lakes and streams (Skulberg, 1966). Variation in assay procedures, however, made it virtually impossible to compare data among different investigators. Therefore, in 1969, a Joint Industry/Government Task Force on Eutrophication was established for the purpose of developing a standard, reproducible algal assay procedure. This effort culminated in publication of the Algal Assay Procedure: Bottle Test (AAP: BT) in 1971 (U.S. EPA, 1971).

Selenastrum capricornutum Printz, a green alga, was selected as the "white laboratory mouse" for the development of the AAP: BT. Although not indigenous to this hemisphere, the organism possesses several qualities making it a desirable laboratory organism. Among these are its ease of culture, identification and minimum morphological changes during growth phase. The organsim is single celled, thus facilitating ease of counting with an electronic particle counter. In its natural habitat *S. capricornutum* is distributed widely in both eutrophic and oligotrophic waters (Skulberg, 1966).

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Reliability of the AAP: BT has been demonstrated by its repeated ability to accurately predict the growth potential of wastewater enriched lakes and streams (Miller and Maloney, 1971; Maloney, Miller, and Blind, 1973; Greene et al., 1975a) and by its ability to determine the limiting nutrients in natural waters (Maloney, Miller, and Shiroyama, 1972; Miller, Maloney, and Greene, 1974). One of the conclusions derived from these studies was that the assay is more appropriate for determining the availability of nutrients than the standard chemical analysis for nitrogen, phosphorus, and other growth-promoting elements. Good agreement in predicting potential productivity was also reported between *S.* capricornutum cultured under laboratory conditions in autoclaved-filtered water samples and the indigenous phytoplankton in the natural environment (Mahoney, 1973; Greene et al., 1975b).

The study of *Anabaena flos-aquae* (Lyngb.) De Brebisson, a filamentous nitrogen-fixing blue-green alga, was initiated in 1972 to determine the reliability of this organism as a test alga and to compare its growth response with *S. capricornutum* in natural waters. This paper describes the results of that work.

METHODS

The water samples were collected bi-weekly from the East End Deep Hole at 0.1 and 10.5 meters (Figure 1). The samples were divided into filtered (0.45 μm membrane filter) and unfiltered portions, then shipped to the Environmental Protection Agency (EPA) laboratory in Corvallis, Oregon. Upon receipt of the water samples, the laboratory autoclaved (1.1 kg/cm^2 @ 121°C for 10 min/1 of sample) the unfiltered portion to solubilize the nutrients associated with indigenous matter. The samples were cooled to room temperature and equilibrated with a mixture of 1 percent carbon dioxide and air and then filtered through a 0.45 μm membrane filter. Total soluble phosphorus, orthophosphorus, total soluble nitrogen, nitrate-nitrogen, nitrite-nitrogen, and ammonia-nitrogen (U.S. EPA, 1974) were determined prior to assay.

The AAP: BT was used exclusively throughout this study, except that the initial inoculum of *A. flos-aquae* was reduced from the recommended 50,000 cells/ml to 10,000 cells/ml.

For each depth, the control was assayed, as well as three nutrient additions: (1) 1.00 mg/l N, (2) 0.02 mg/l P, and (3) 1.00 mg/l N + 0.02 mg/l P.

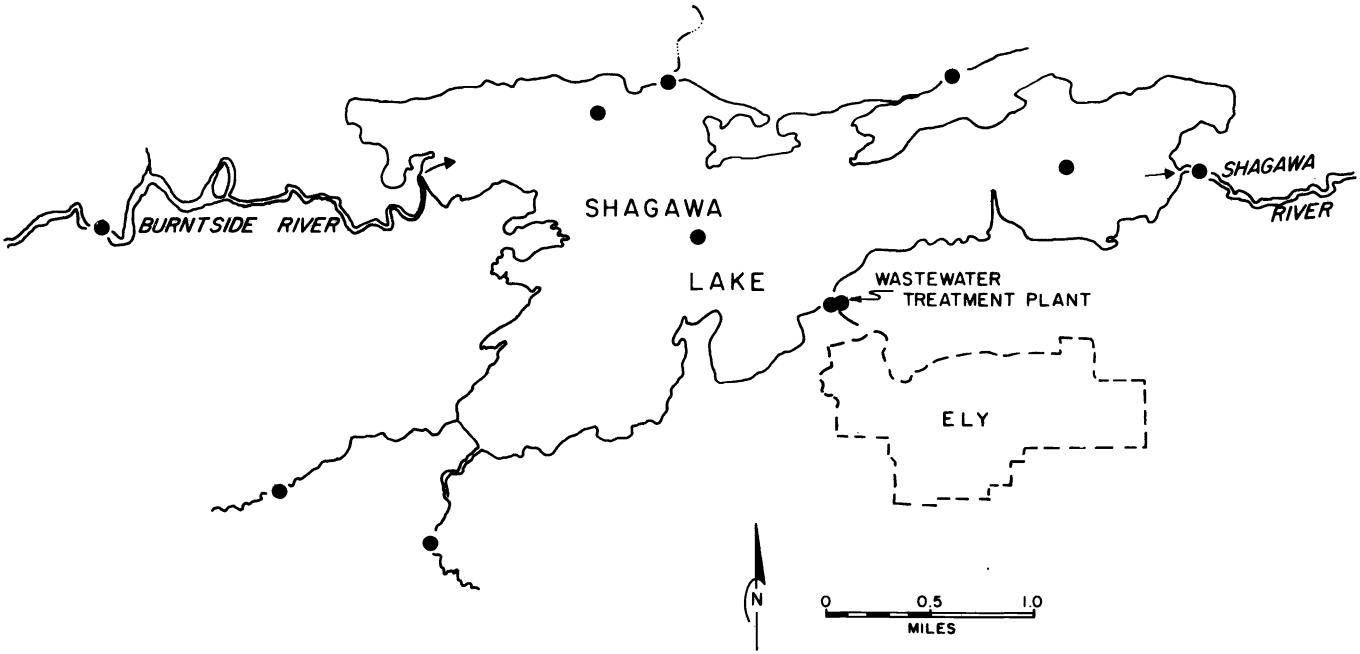

Figure 1. Sampling locations shown as • The East End Deep Hole is located near the outlet, Shagaway River (Malueg et al., 1973).

RESULTS AND DISCUSSIONS

Development of Blue-Green Assay Technique

The most common technique for algal cell enumeration has been the direct counting procedure utilizing either the Petroff-Hauser Counting Chamber or other hemacytometer. This procedure is tedious, time-consuming, and of questionable accuracy. Also, the method is not conducive to comparing the growth response of unicellular green algae and filamentous blue-green algae because of their phylogenetic and physiological differences. The blue-green filaments are frequently nonrandom, instead lined in variable numbers (Burnham, Stetak, and Boulger, 1973) and this produces irregularities in the counts.

To simplify the counting procedure (by use of an electronic particle counter) the filaments had to be broken into fragments small enough to avoid clogging the orifice and to insure recording of the mean cell volume. The AAP: BT lists several methods for breaking filaments. While these techniques are not without their drawbacks, two of the methods—sonication and blending—were evaluated. A sonifier Cell Disrupter equipped with a micro-tip was used to sonicate *A. flos-aquae* filaments for various periods. This sonifier has a ten-position calibrated energy output switch and an energy meter to monitor the output for experimental determination and reproducibility. The two sonication treatments discussed in this report are position 2 for 15 seconds and position 4 for 10 seconds.

Blending, as a method of fragmenting the filaments, was evaluated using a Sorvall Omni-Mixer Homogenizer. The filaments satisfactorily separated at a speed of approximately 2500 RPM for 30 seconds.

The growth response curve of *A. flos-aquae* (Figure 2) shows that the sonication treatment (max. 1,100,000 fragments/ml) fragmented more filaments than from the blending treatment (max. 650,000 fragments/ml). *In vivo* chlorophyll *a* analysis (relative fluorescence units = RFU) for the sonicated cells also indicated a higher growth response (Figure 3). These data suggest sonication to be most efficient in breaking up the filaments. However, the RFU readings of the filtrates from each of the respective treatments (Figure 4) show that a significant part of the RFU reading for the sonicated samples was contained in the filtrates (38.5 percent and 54.0 percent). The RFU readings for the filtrates from the blended and control samples were not significantly different (5.80 percent and 4.70 percent). The higher RFU in the filtrates of the sonicated samples suggests the possibility of cell disruptions. Microscopic examination of the sonicated cells did show evidence of cell damage; however, damaged cells were not as evident in the blended samples. Even though sonication breaks up

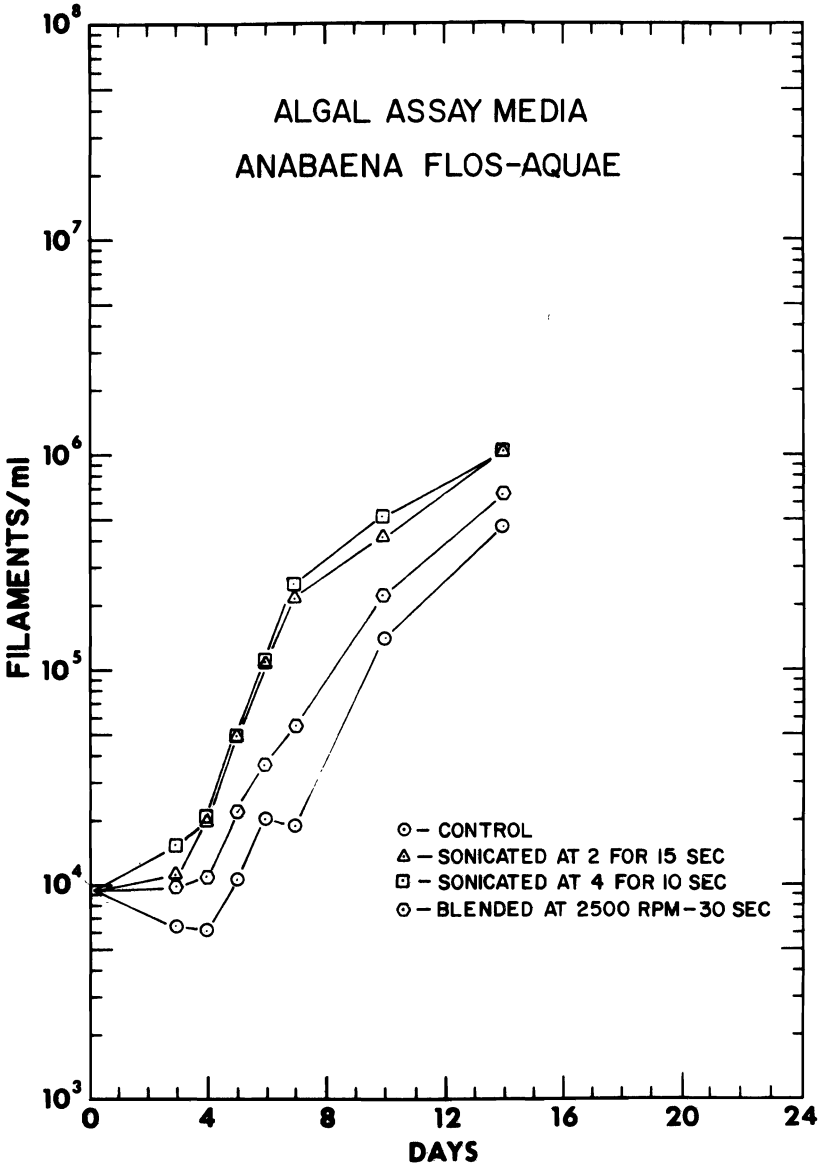

Figure 2. Comparison of the different methods in fragmenting the filaments into small segments.

filaments prior to counting more efficiently than blending, sonication cannot be used to prepare inoculum because of its tendency to damage the

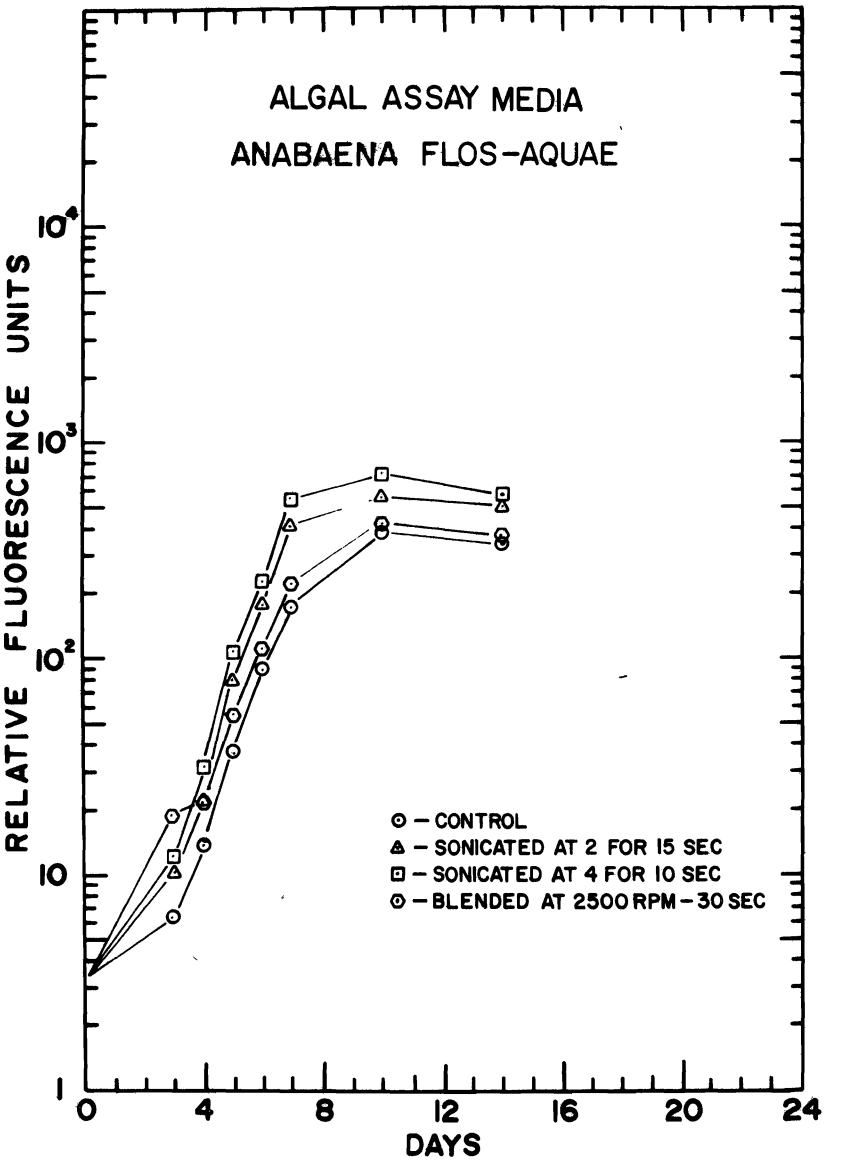

Figure 3. Comparison of *in vivo* chlorophyll *a* readings from the different fragmentation methods.

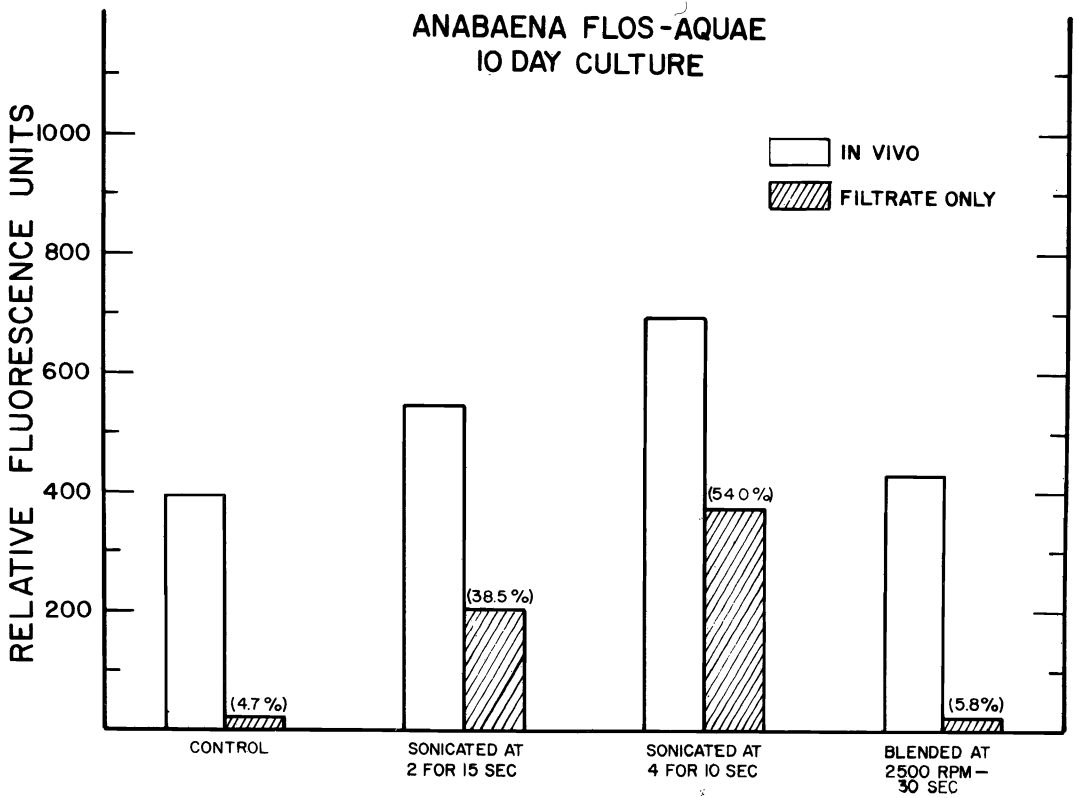

Figure 4. Chlorophyll *a* content expressed as relative flourescence units in both *in vivo* cell suspensions and their correspond' ing filtrates.

cells. Therefore, all results from *A. flos-aquae* reported hereafter in this paper are based on the blending treatment prior to counting.

The initial cell count for each test was established by hemacytometer cell counts of a drop of blended *A. flos-aquae* culture. Microscopic counts were made for total number of filaments and total number of individual cells in each filament. A cell count factor for the inoculum was derived from these parameters (average number of cells per filament).

Figure 5 shows the growth response obtained with four different levels of inoculum of *A. flos-aquae* cultured in the algal assay medium (AAM). T-test analysis showed no significant differences in the maximum yield (mg/l dry wt.) obtained after 10 days for the inoculum levels of 10,000, 25,000, and 50,000 cells/ml. Since growth response for this study is expressed in maximum yield (mg/l dry wt.), and not the rate of nutrient uptake or maximum growth rate, 10,000 cells/ml (final concentration), was chosen as the inoculum level for *A. flos-aquae* rather than the 50,000 cells/ml recommended in the AAP: BT. The lower level of inoculum also minimized the potential nutrient carry-over from the inoculum into the test substrate.

Figure 6 compares the growth response between *S. capricornutum* and *A. flos-aquae* : cultured in AAM. The similar maximum yields obtained for both test algae encouraged initiation of a study comparing the algal species growth response in natural water.

Assessment of Growth Response in Natural Waters

For the past several years, the Eutrophication and Lake Restoration Branch of the EPA has monitored limnological characteristics of Shagawa Lake in northeastern Minnesota, to assess how wastewater discharges from the city of Ely impact the biological productivity of the lake. Field and laboratory experiments indicated that high levels of phosphorus discharged by Ely's secondary treatment plant resulted in obnoxious blooms of blue-green algae (*Aphanizomenon* and *Anabaena* species) and subsequent water quality deterioration (Brice and Powers, 1969; Miller and Maloney, 1971; Malueg et al., 1975). A 6-year study (1967-1972) showed that 80 percent of the total phosphorus and 24 percent of the total nitrogen entering Shagawa Lake was discharged from Ely's secondary sewage treatment plant (Malueg et al., 1975).

Shiroyama, Miller, and Greene (1975) demonstrated that the maximum yield for *S. capricornutum* in AAM is predictable if the nitrogen and phosphorus concentrations are known, other essential nutrients are in adequate supply, and toxicants are absent. Waters containing greater than 0.010 mg/l ortho-P can yield 0.43 mg/l dry weight

of the alga per 0.001 mg/l phosphorus. Similarly, each 0.001 mg/l total soluble inorganic nitrogen (TSIN = NO_2 + NO_3 + NH_3) can yield 0.038 mg/l dry weight of the alga. Actual yield is considered statistically significant within ± 20 percent of the predicted yield. A similar ortho-P

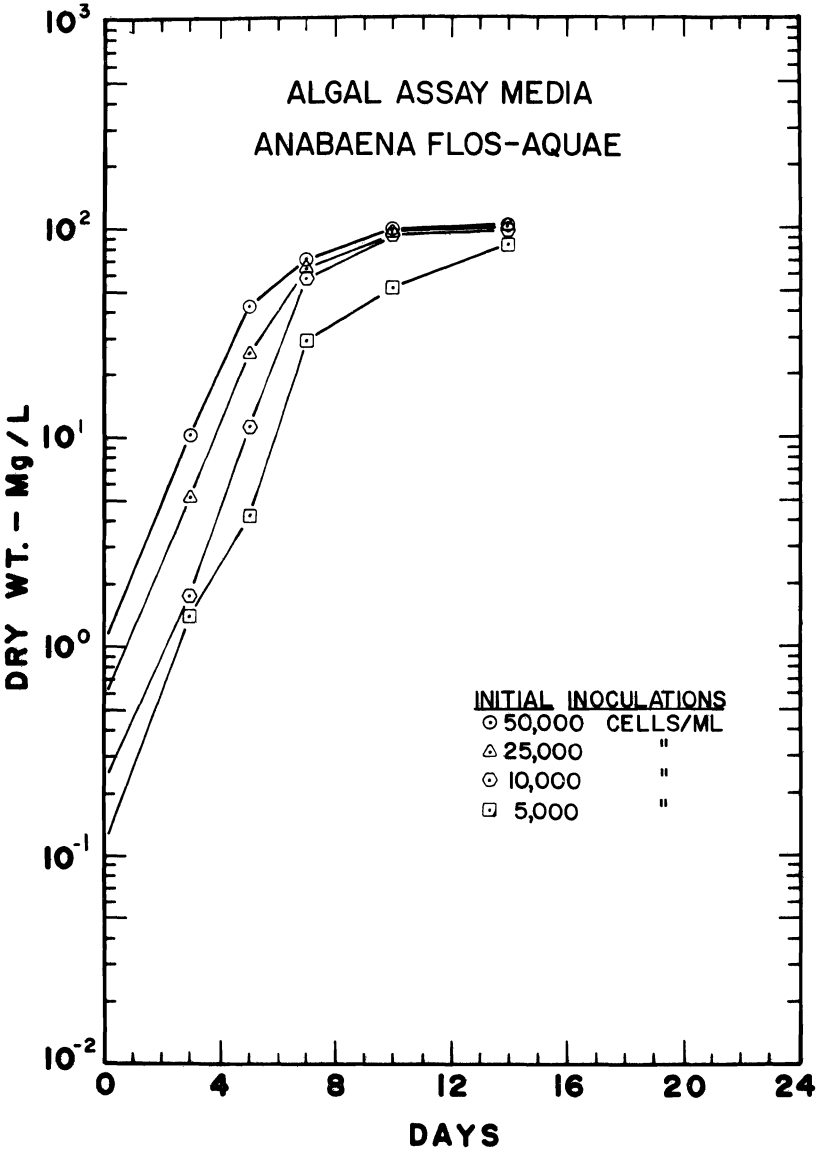

Figure 5. Growth curves of the initial inoculum concentrations.

yield factor has been determined for *A. flos-aquae*. Waters containing greater than 0.010 mg/l ortho-P can yield 0.45 mg/l dry weight of the alga per 0.001 mg/l phosphorus. Statistically, this yield factor for *A. flos-aquae* is not significantly different from the yield factor obtained for

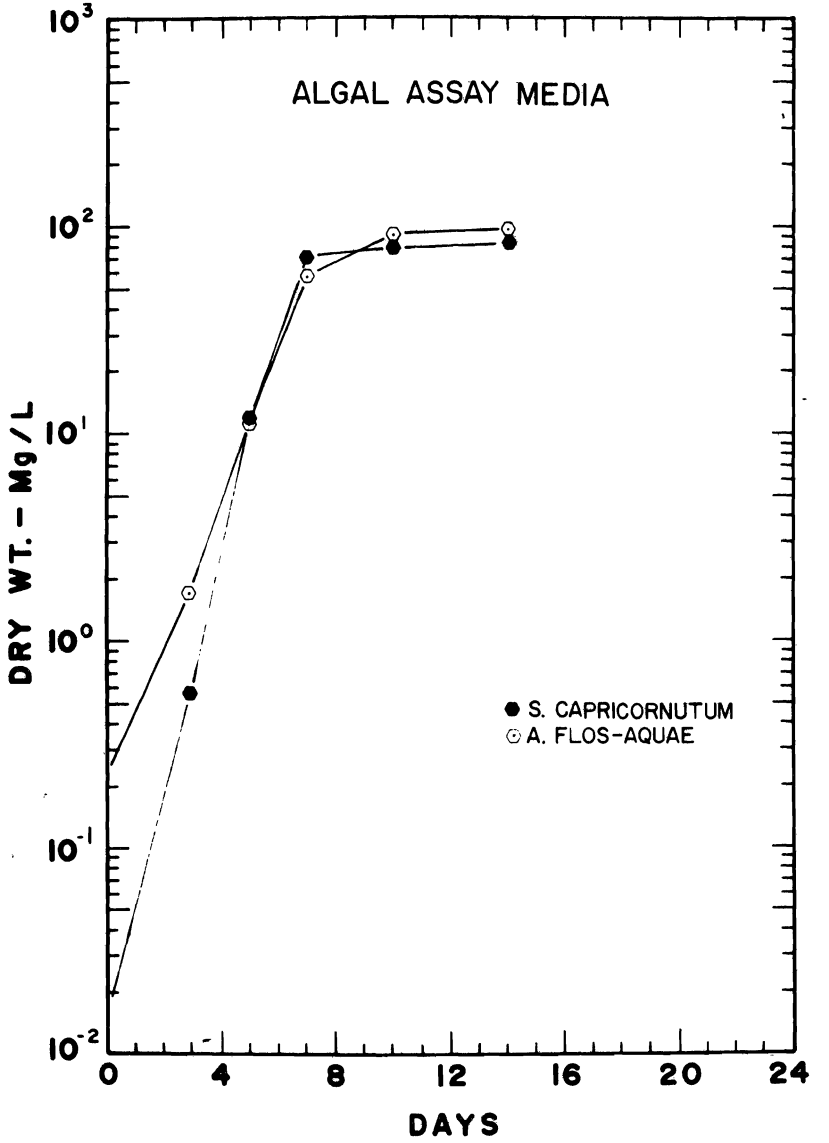

Figure 6. Comparative growth curve between *S. capricornutum* **and** *A. flos-aquae*.

S. capricornutum. The correlation coefficients between maximum yield and ortho-P concentration for *S. capricornutum* and for *A. flos-aquae* are 0.998 and 0.930, respectively. Since *A. flos-aquae* is a nitrogen-fixing blue-green alga, inorganic nitrogen requirements cannot be determined under conditions of free atmospheric gas exchange (standard condition for AAP: BT).

The magnesium, potassium, and sulfur requirements for both algae have also been defined. In the presence of the essential nutrients in AAM, concentrations of 0.040 and 0.100 mg/l magnesium; 0.350 and 0.460 mg/l potassium; and 0.320 and 0.100 mg/l sulfur supported maximum yield of *S. capricornutum* and *A. flos-aquae,* respectively (77-86 mg/l dry wt.). The low levels of magnesium, potassium, and sulfur indicate that these elements will seldom be growth-limiting in natural water (Hem, 1959). Preliminary results also indicate that only trace levels of calcium are necessary to support the two algae.

Figures 7 and 8 show the effects of nitrogen, phosphorus, and nitrogen plus phosphorus additions, compared with control samples on the growth response of *S. capricornutum* and *A. flos-aquae* in filtered and autoclaved-filtered Shagawa Lake samples.

Response of *S. capricornutum*

In all of the filtered and autoclaved-filtered water samples collected from 0.1 and 10.5 meters, the addition of 0.02 mg/l phosphorus failed to stimulate additional growth of *S. capricornutum* (Table 1). The filtered control samples produced an average maximum yield of 3.08 mg/l dry weight and the autoclaved-filtered control samples yielded an average of 6.01 mg/l dry weight. Employing the yield factor for ortho-P (ORTHO-P x 430) with the addition of 0.02 mg/l phosphorus and assuming that other essential nutrients are available, the yield of the alga should have been at least 8.60 mg/l (.02 x 430). The chemical analysis (Table 2) shows an average ortho-P content of 0.018 mg/l for the filtered samples and .042 mg/l for the autoclaved-filtered samples. The theoretical maximum yield of *S. capricornutum* for these phosphorus levels are 7.70 ±1.54 and 17.90 ±3.50 mg/l dry weight, respectively (Table 1).

To determine the significance of the phosphorus addition, a t-test analysis was performed on control and control plus phosphorus spiked samples for both filtered and autoclaved-filtered waters. Basically no significant differences in maximum yields were obtained between the control and phosphorus spiked samples.

The average TSIN in the filtered and autoclaved-filtered water samples is 0.123 and 0.177 mg/l, respectively (Table 2). Using the TSIN

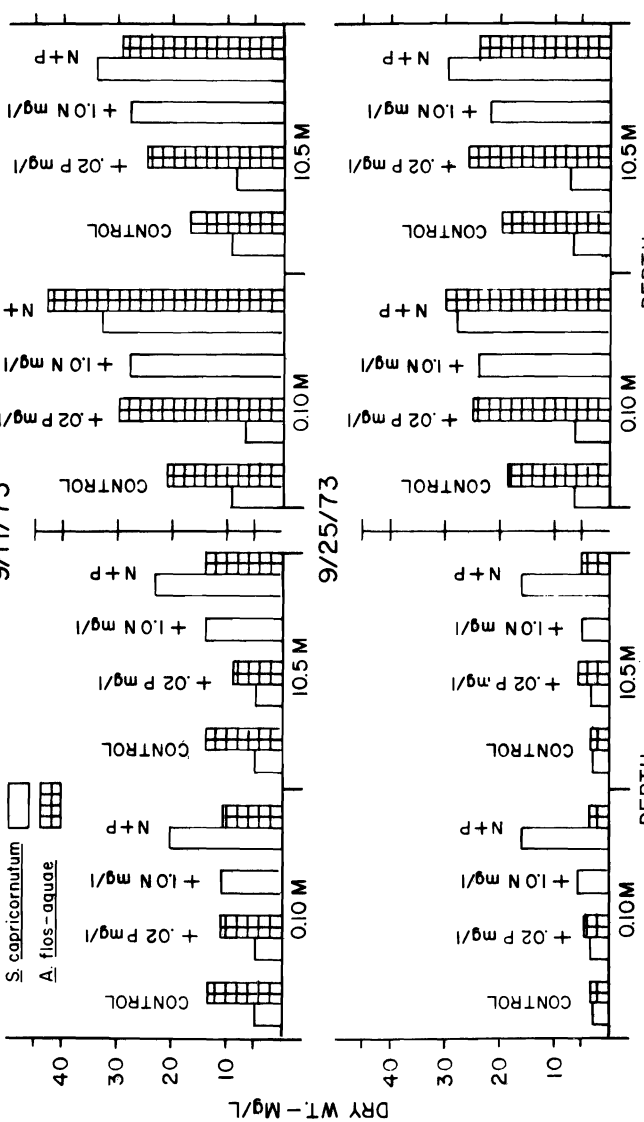

Figure 7. The effects of nitrogen, phosphorus, and nitrogen plus phosphorus additions as compared with control samples on the growth response of *S. capricornutum* and *A. flos-aquae* in filtered and autoclaved-filtered Shagawa Lake samples.

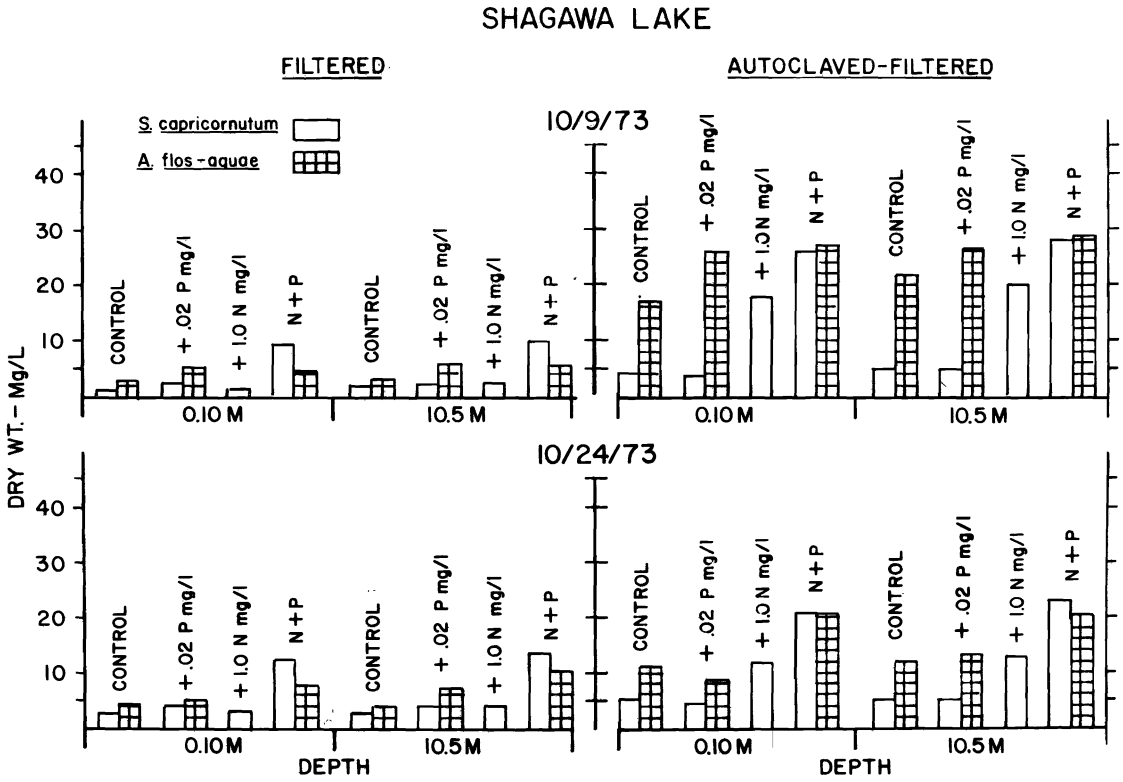

Figure 8. The effects of nitrogen, phosphorus, and nitrogen plus phosphorus additions as compared with control samples on the growth response of *S. capricornutum* and *A. flos-aquae* in filtered and autoclaved-filtered Shagawa Lake samples.

Table 1. Theoretical yields (mg/l dry wt.) based on chemical analysis and actual yields (mg/l dry wt.) of *S. capricornutum*.

Table 1.

DATE	DEPTH	THEORETICAL YIELD (mg/l)[a]		ACTUAL YIELD (mg/l)			
		ORTHO-P	TSIN	CONTROL	1.0 N	0.02 P	N+P
			FILTERED				
9/11/73	0.10M	12.47(11.61)	5.17(4.94)	4.79	11.20	5.07	21.20
	10.5 M	15.48(14.19)	6.68(4.75)	4.73	14.10	4.80	23.30
9/25/73	0.10M	8.60 (6.88)	3.12(2.81)	2.76	5.86	2.72	16.60
	10.5 M	3.44 (6.02)	3.76(2.58)	2.43	5.06	2.71	16.00
10/9/73	0.10M	3.87 (0.869)	4.98(2.01)	1.44	1.64	2.57	9.22
	10.5 M	6.45 (1.72)	4.26(2.20)	2.02	2.50	2.40	9.94
10/24/73	0.10M	3.10 (3.87)	3.27(3.53)	2.90	3.50	4.17	13.00
	10.5 M	8.17 (4.30)	6.12(3.95)	2.54	4.20	4.35	13.80
AVERAGES:	Combined	7.70 (6.18)	4.67(3.34)	3.08	6.01	3.60	15.38
	0.10M	6.99 (5.80)	4.14(3.31)	2.17	5.55	3.63	15.01
	10.5 M	8.39 (6.55)	5.21(3.34)	3.18	6.47	3.57	15.76
			AUTOCLAVED-FILTERED				
9/11/73	0.10M	24.08(26.23)	6.88(8.32)	8.97	28.20	7.65	33.10
	10.5 M	28.38(26.66)	8.82(8.55)	8.63	28.20	8.50	34.00
9/25/73	0.10M	15.05(21.50)	5.43(5.97)	6.15	23.90	5.76	27.50
	10.5 M	16.34(21.07)	6.04(6.50)	6.36	21.90	6.64	28.80
10/9/73	0.10M	15.05(17.63)	6.31(3.95)	4.11	18.00	3.76	26.10
	10.5 M	18.90(19.35)	7.71(4.71)	4.75	19.70	4.70	27.60
10/24/73	0.10M	12.47(12.04)	6.12(4.75)	4.90	11.90	4.62	20.80
	10.5 M	12.90(13.33)	6.35(4.53)	4.53	13.10	4.48	22.80
AVERAGES:	Combined	17.90(19.73)	6.73(5.89)	6.05	20.60	5.76	27.60
	0.10M	16.66(19.35)	6.19(5.74)	6.03	20.50	5.43	26.88
	10.5 M	19.13(20.10)	7.23(6.08)	6.07	20.73	6.08	28.30

[a]Theoretical yield (mg/l) is calculated from the chemistry (ORTHO-P & TSIN) given in Table 2:
Theoretical yield (mg/l dry wt.) = ORTHO-P (mg/l) X 430
Theoretical yield (mg/l dry wt.) = TSIN (mg/l) X 38

Table 2. Comparison of ORTHO-P and TSIN concentrations derived from chemical analysis and assay yields of *S. capricornutum* shown in Table 1.

DATE	DEPTH	ORTHO-P (mg/l)		TSIN (mg/l)	
		FILTERED	A-F[a]	FILTERED	A-F
9/11/73	0.10M	0.029(0.027)	0.056(0.061)	0.136(0.130)	0.181(0.219)
	10.5 M	0.036(0.033)	0.066(0.062)	0.176(0.125)	0.232(0.225)
9/25/73	0.10M	0.020(0.016)	0.035(0.050)	0.082(0.074)	0.143(0.157)
	10.5 M	0.008(0.014)	0.038(0.049)	0.099(0.068)	0.159(0.171)
10/9/73	0.10M	0.009(0.002)	0.035(0.041)	0.131(0.053)	0.166(0.104)
	10.5 M	0.015(0.004)	0.044(0.045)	0.112(0.058)	0.203(0.124)
10/24/73	0.10M	0.007(0.009)	0.029(0.028)	0.086(0.093)	0.161(0.125)
	10.5 M	0.019(0.011)	0.030(0.031)	0.161(0.104)	0.167(0.119)
AVERAGES:	Combined	0.018*0.015)	0.042(0.046)	0.123(0.088)	0.177(0.155)
	0.10M	0.016(0.014)	0.039(0.045)	0.109(0.087)	0.163(0.151)
	10.5 M	0.020(0.015)	0.045(0.047)	0.137(0.088)	0.137(0.160)

[a]A-F = Autoclaved and Filtered
Numbers in ( ) = ORTHO-P & TSIN analysis obtained by back calculating the max. yield of S. capricornutum. The equations used are:

$$\text{ORTHO-P (mg/l)} = \frac{[\text{Max. yield from 1.0 N} + 0.02\text{ P} - (430 \times 0.02\text{P})] + (\text{Max. Yield from 1.0 N})}{(430)\,(2)}$$

$$\text{TSIN (mg/l)} = \frac{(\text{Max. yield from Control}) + (\text{Max. yield from 0.02 P})}{(38)\,(2)}$$

The maximum yields were taken from Table 1.

conversion factor (TSIN x 38), the average theoretical yield for the two sets of samples should be 4.67 ± 0.94 and 6.73 ± 1.35 mg/l dry weight (Table 1). The actual yields for the same samples were 3.08 and 6.05 mg/l. The addition of 1.00 mg/l nitrogen to the September and October autoclaved-filtered water samples increased the average maximum yield of *S. capricornutum* to 20.60 mg/l dry weight, or 3.4 times greater than the control sample. Nitrogen addition also stimulated algal growth in the filtered samples collected in September (9.07 mg/l dry weight). These two actual assay yields directly correlate with the theoretical yields based on the ortho-P content of both the autoclaved-filtered and filtered (September only) Shagawa Lake water samples (17.90 ±3.50 and 10.00 ± 2.00 mg/l dry wt., respectively). This suggests nitrogen is the primary nutrient limiting *S. capricornutum* growth in these samples.

The singular addition of nitrogen and phosphorus to the surface (0.1 M) and bottom (10.5 M) filtered October samples did not generate additional growth response of *S. capricornutum* over that obtained in the controls. The relative closeness of these maximum yields indicates that the biologically available concentrations of both ortho-P and TSIN did not change with depth of collection. Theoretical maximum yields for these samples, based on their chemically determined ortho-P content (Table 1), indicate that the bottom samples should have supported an average yield 2.6 times greater than the surface samples. The TSIN and ortho-P yield factors may be used to calculate the biological availability of nitrogen or phosphorus in the test waters.

Biologically available phosphorus can be determined by dividing either the nitrogen or nitrogen plus phosphorus yields (or both) by 430. For example, the October 24 filtered bottom sample supported 4.20 and 13.80 mg/l dry weight of the test alga with the addition of nitrogen and nitrogen plus phosphorus, respectively. The calculated available phosphorus to produce these yields would be 0.010 mg/l (4.20 ÷ 430) and 0.012 mg/l [(13.80 ÷ 430) - 0.02 mg/l P], respectively. The excellent agreement of these calculations indicates that the chemical analysis for ortho-P (0.031 mg/l P) in this sample (filtered 10/24/74 at 10.5 M) is 1.7 times greater than it should be and that other constituents are not limiting algal growth. This substantiates the reason for the average theoretical and actual assay yield differences reported in Table 1 for the October filtered surface and bottom samples.

Growth Response of *A. flos-aquae* **to Nutrient Additions**

The combined average growth response of *A. flos-aquae* to the 0.02 mg/l phosphorus addition (Table 3) when compared to the control sample (6.74 versus 6.19 mg/l dry weight, respectively), indicates that

constituents other than phosphorus and nitrogen may be limiting its growth in all of the filtered Shagawa Lake waters. The same phosphorus addition to the autoclaved water samples averages a 40 percent increase in growth.

Using the yield factor for ortho-P (ORTHO-P x 450) for *A. flos-aquae* and assuming the other essential nutrients or toxicants are not growth-limiting, the addition of 0.02 mg/l phosphorus should yield at least 9.0 ± 1.80 mg/l dry weight. Failure of *A. flos-aquae* to respond to the added phosphorus in the filtered lake samples may have been due to: (1) biologically produced toxins; (2) trace element deficiency; or (3) an imbalance of nutrients.

Biologically produced inhibitory substances can be important factors in the development and persistence of unialgal blooms of various species (Boyd, 1973). Toxins attributed to the collapse of an *Aphanizomenon* bloom have been reported to inhibit *Anabaena* and not affect *Selenastrum* (Lange, 1971). According to Tassigny and Lefevre (1971), a number of algae grew poorly in media prepared from water that had contained a large population of *Aphanizomenon gracile.* Figure 9 shows that prior to the September and October samplings, a large blue-green bloom did exist in Shagawa Lake (Schults et al., 1975) dominated at its maximum by

Table 3. Theoretical yields (mg/l dry wt.) based on chemical analysis and actual yields (mg/l dry wt.) of *A. flos-aquae*.

DATE	DEPTH	THEORETICAL YIELD (mg/l)[a]		ACTUAL YIELD (mg/l)	
		ORTHO-P	CONTROL	0.02 P	N + P
		FILTERED			
9/11/73	0.10M	13.05(12.15)	13.60	10.90	10.80
	10.5 M	16.20(14.85)	14.00	8.93	14.30
9/25/73	0.10M	9.00 (7.20)	3.20	4.49	3.63
	10.5 M	3.60 (6.30)	3.64	5.57	5.00
10/9/73	0.10M	4.05 (0.90)	2.96	5.42	5.76
	10.5 M	6.75 (1.80)	3.44	5.87	5.76
10/24/73	0.10M	3.15 (4.05)	4.47	5.40	8.20
	10.5 M	8.55 (4.95)	4.23	7.34	10.60
AVERAGES: Combined		8.04 (6.52)	6.19	6.74	7.88
	0.10M	7.32 (6.07)	6.06	6.55	6.85
	10.5 M	8.77 (6.98)	6.33	6.93	8.92
		AUTOCLAVED-FILTERED			
9/11/73	0.10M	25.20(27.45)	21.40	29.50	43.00*
	10.5 M	29.70(27.90)	16.70	24.50	29.50
9/25/73	0.10M	15.75(22.50)	18.50	25.00	30.20
	10.5 M	17.10(22.05)	19.90	26.20	23.80
10/9/73	0.10M	15.75(18.45)	17.20	25.80	27.00
	10.5 M	19.80(20.05)	21.80	26.70	27.80
10/24/73	0.10M	13.05(12.60)	11.60	9.27*	20.90
	10.5 M	13.50(13.95)	12.10	13.40	21.50
AVERAGES: Combined		18.73(20.64)	17.40	24.44	25.81
	0.10M	17.44(20.25)	17.18	26.77	26.03
	10.5 M	20.03(21.04)	17.60	22.70	25.65

[a]Theoretical yield is based on the chemistry for ORTHO-P given in Table 2.
Theoretical yield (mg/l dry wt.) = ORTHO-P (mg/l) X 450.
* = Only one replication–not used in calculating the averages.

Aphanizomenon. The failure of *A. flos-aquae* to grow in the filtered water samples may be due to the presence of a toxin (or toxins) produced by *Aphanizomenon*. Trace element deficiency can also be an important growth-limiting factor (Lund, 1965). Deficiency of an element may not necessarily be an absolute deficiency but an indication of an imbalance of nutrient ratios. Growth-retarding effects may stem from such nutrient imbalance (Provasoli, McLaughlin, and Pinter, 1954).

The combined average growth response of *A. flos-aquae* in the control autoclaved-filtered Shagawa Lake water samples (Table 3) indicates the absence of growth-limiting constituents other than phosphorus. The addition of 0.02 mg/l phosphorus supported an additional 9.00 ± 1.80 mg/l dry weight. This suggests phosphorus to be the primary limiting nutrient for the growth of *A. flos-aquae* in these waters. The addition of a combined nitrogen plus phosphorus spike to these samples further verified phosphorus limitation. Table 3 reports yields of 24.44 and 25.81 mg/l dry weight for the singular addition of phosphorus and nitrogen plus phosphorus, respectively—yields that reinforce an assumption that neither trace elements nor nutrient imbalance limited the growth of *A. flos-aquae* in the autoclaved-filtered samples. This strongly suggests the presence of a biologically produced toxin which may have been responsible for limiting growth in the filtered samples. If the toxin is of biological origin, it would have been denatured by the heat of autoclaving. Future studies will have to be conducted to determine the presence or absence of toxin (or toxins) from the growth or decompositon of blue-green algae in natural waters.

Comparative Growth Response of
S. capricornutum **and** *A. flos-aquae*

During September and October 1973, *S. capricornutum* production was nitrogen limited in filtered and autoclaved-filtered Shagawa Lake water samples from both the surface and the bottom depths.

The growth response of *A. flos-aquae* indicates that constituents other than nitrogen and phosphorus limited its growth in the September and October filtered Shagawa Lake water samples. However, phosphorus added to autoclaved-filtered samples collected during this study stimulated growth up to the maximum predicted values.

Table 4 gives comparative summary for these test algae. Both test algae are sensitive to the phosphorus content of the test waters. In the presence of adequate nitrogen, similar combined average maximum yields were obtained for both test algae. A linear regression analysis (Figure 10) of the relationship between phosphorus and maximum yields in the autoclaved-filtered samples shows a high degree of correlation ($R = 0.947$ and $R = 0.921$, respectively) for *S. capricornutum* and *A. flos-aquae*.

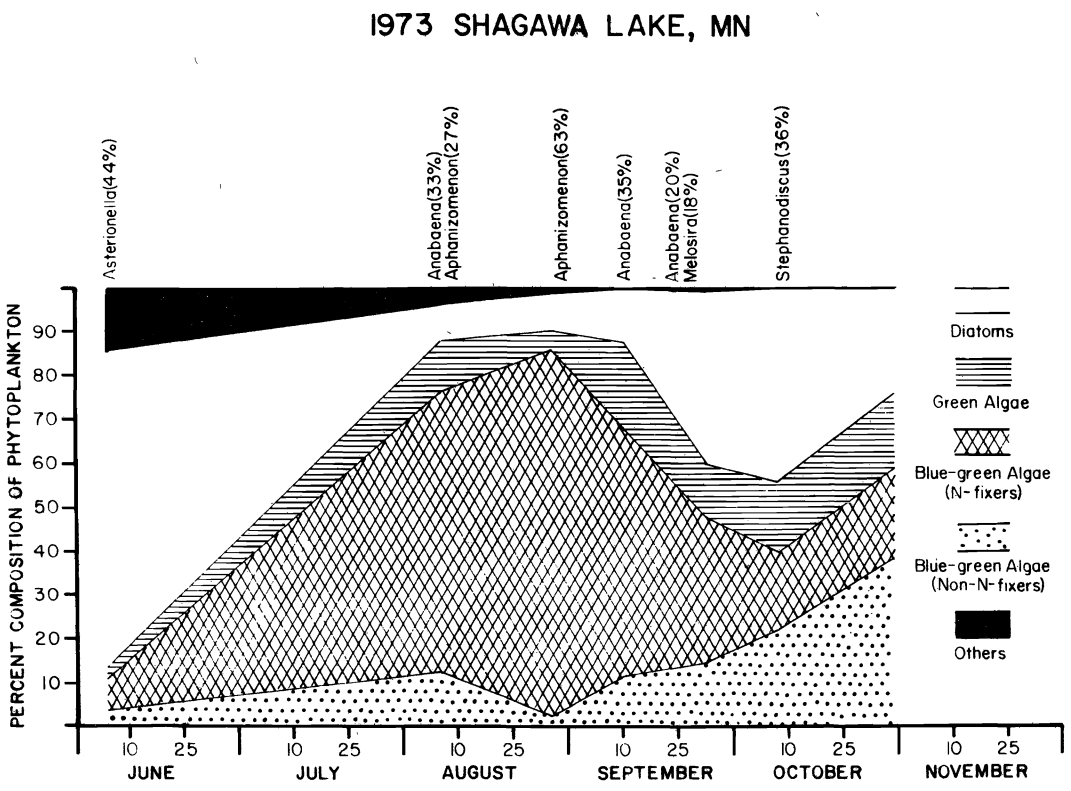

Figure 9. Percent composition of the indigenous phytoplankton.

As expected, the addition of nitrogen maximized growth of *S. capricornutum* in the filtered and autoclaved-filtered samples, but did not alter the growth of *A. flos-aquae*.

The combined addition of nitrogen and phosphorus produced greater growth of *S. capricornutum* than with the singular addition of nitrogen. This growth increase was proportional to the added phosphorus. Similar addition of nitrogen and phosphorus failed to stimulate the growth of *A. flos-aquae* in the October filtered water samples, a failure attributed to the probable presence of a biologically produced toxin. Autoclaving the water samples increased the growth of *A. flos-aquae* in proportion to the phosphorus content of the samples.

The comparative analysis of the growth response of these algae suggests the need to use more than one test species to determine the growth potential or nutritional status of a test water. The analysis also shows the need to assess both filtered and autoclaved-filtered samples if the presence of a biologically produced toxin (or toxins) is suspected.

SUMMARY AND CONCLUSIONS

Anabaena flos-aquae can be used as a test organism to define the growth potential and nutrient status of natural lake waters. The design of

Table 4. Comparative summary of *S.capricornutum* and *A. flos-aquae*, using the same parameters for each alga.

PARAMETERS	*SELENASTRUM CAPRICORNUTUM*	*ANABAENA FLOS-AQUAE*
Taxonomic	1. Unicellular green alga. 2. Non-nitrogen-fixer.	1. Filamentous blue-green alga. 2. Nitrogen-fixer.
P Addition	3. Displayed a linear utilization of P as a function of concentration (Figure 12). 4. No additional growth response in both the FILT and A-F samples.	3. Similar response (Figure 12). 4. No additional growth response in the FILT only. Good correlation with the predicted yield in A-F samples.
N Addition	5. Stimulated max. growth in both FILT and A-F samples.	5. No change in growth response in both FILT and A-F samples.
N + P Addition	6. Stimulated greater growth than that obtained by N addition only. The max. yield obtained was equivalent to the P content, indicating other essential constituents were not growth-limiting.	6. No significant response in the FILT samples. Similar response to that obtained by P addition in the A-F samples.
Combined max. yields relative to P conc. in the control sample	7. With N addition (Table 1), 6.01 mg/l in the FILT samples and 20.6 mg/l in the A-F samples.	7. The control samples yielded (Table 3) 6.19 mg/l in the FILT samples and 17.40 mg/l in the A-F samples.
Mg/l of Mg, K, S, & Ca to achieve max. yield in AAM	8. (a) Mg @ 0.040 mg/l. (b) K @0.350 mg/l. (c) S @0.320 mg/l. (d) Ca trace amt.	8. (a) Mg @0.100 mg/l. (b) K @0.460 mg/l. (c) S @0.100 mg/l. (d) Ca trace amt.
Yield coefficient for max. yield with P and N	9. 0.001 mg/l ORTHO-P will yield 0.430 mg/l dry wt. of the alga. 10. 0.001 mg/l N will yield 0.038 mg/l dry wt. of the alga.	9. 0.001 mg/l ORTHO-P will yield 0.450 mg/l dry wt. of the alga. 10. N yield coefficient undeterminable under conditions of free atmospheric gas exchange.

Note: FILT denotes filtered samples
A-F denotes autoclaved filtered samples

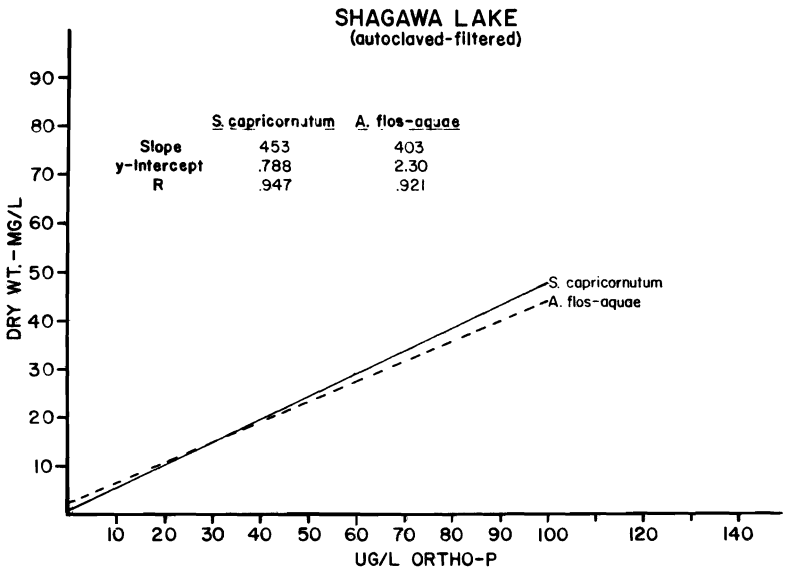

Figure 10. Linear regression curve of the relationship between phosphorus and maximum yield in the autoclaved-filtered samples for *S. capricornutum* and *A. flos-aquae*.

experiments to establish comparative growth response parameters between *S. capricornutum* and *A. flos-aquae* has been presented for September and October (1973) filtered and autoclaved-filtered Shagawa Lake waters. This study reported algal response to conditions of nitrogen and phosphorus limitation, as well as apparent biologically induced toxicity in Shagawa Lake waters. The following conclusions were drawn from the study:

1. Sonication is more efficient than blending to fragment *A. flos-aquae* filaments for counting, but its tendency to damage the cells precludes its use to prepare inoculum. Blending is recommended prior to inoculation or counting of the test alga in assay studies.

2. An ortho-P yield factor has been determined for *A. flos-aquae*. Waters containing greater than 0.010 mg/l ortho-P will yield 0.45 mg/l dry weight of the alga per 0.001 mg/l phosphorus. Statistically, this yield factor is not significantly different from the yield factor obtained for *S. capricornutum* .

3. The theoretical maximum yield of both *A. flos-aquae* and *S. capricornutum* based on chemically determined ortho-P content of the

October 24 filtered Shagawa Lake bottom sample, was approximately three times greater than that obtained by actual assay. Back calculation of the biologically available ortho-P content of this test water from the yields obtained with both test algae suggests that the chemical analysis for ortho-P was in error.

4. During the study period the filtered and autoclaved-filtered Shagawa Lake water samples were primarily nitrogen limited for support of *S. capricornutum*. Growth response of *A. flos-aquae* indicated that constituents other than nitrogen and phosphorus limited its growth in filtered Shagawa Lake water samples. However, phosphorus addition stimulated its growth to the maximum predicted values in autoclaved-filtered samples collected during this study.

5. The comparative analysis of the growth response of both algae strongly suggest the use of more than one test species to determine the growth potential or nutritional status of a test water if the presence of a biologically produced toxin is suspected.

6. Both filtered and autoclaved-filtered samples must be assayed to substantiate the presence of a biologically produced growth-inhibitor.

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Seasonal Variation of Algal Biomass Production Potential and Nutrient Limitation in Yaquina Bay, Oregon

D. T. Specht*

INTRODUCTION

Most investigators have characterized coastal marine and estuarine waters as nitrogen limited for algal growth (Harvey, 1947; Ryther, 1954; Redfield, 1958; Ketchum, 1969; Ryther and Dunstan, 1971; Goldman et al., 1974). Thayer (1974) and Specht (1974) have shown that estuarine systems can be phosphorus limited for at least part of the yearly seasonal cycle. Disposal of domestic and industrial wastes can aggravate eutrophic tendencies in highly productive coastal and estuarine waters that are nitrogen limited (Ryther and Dunstan, 1971; Goldman, Tenore, and Stanley, 1974). However, removal of nitrogen alone, as suggested by Goldman et al. (1974) and Ryther and Dunstan (1971), should not be viewed as a comprehensive solution to nutrient input problems related to pollution, especially in brackish or saline waters that are potentially phosporus limited.

This report discusses research performed to delineate the characteristics of Yaquina Bay, Oregon (Figure 1), that change its potential nutrient limitation. The Marine Algal Assay Procedure (U.S. EPA, 1974a) was used to determine the nutrient limitation in this estuary.

Yaquina Estuary Physical Characteristics

The Yaquina estuary has a surface area of 1582 hectares at the mean high water line (19-year average) of which 61 percent (1207 hectares) is

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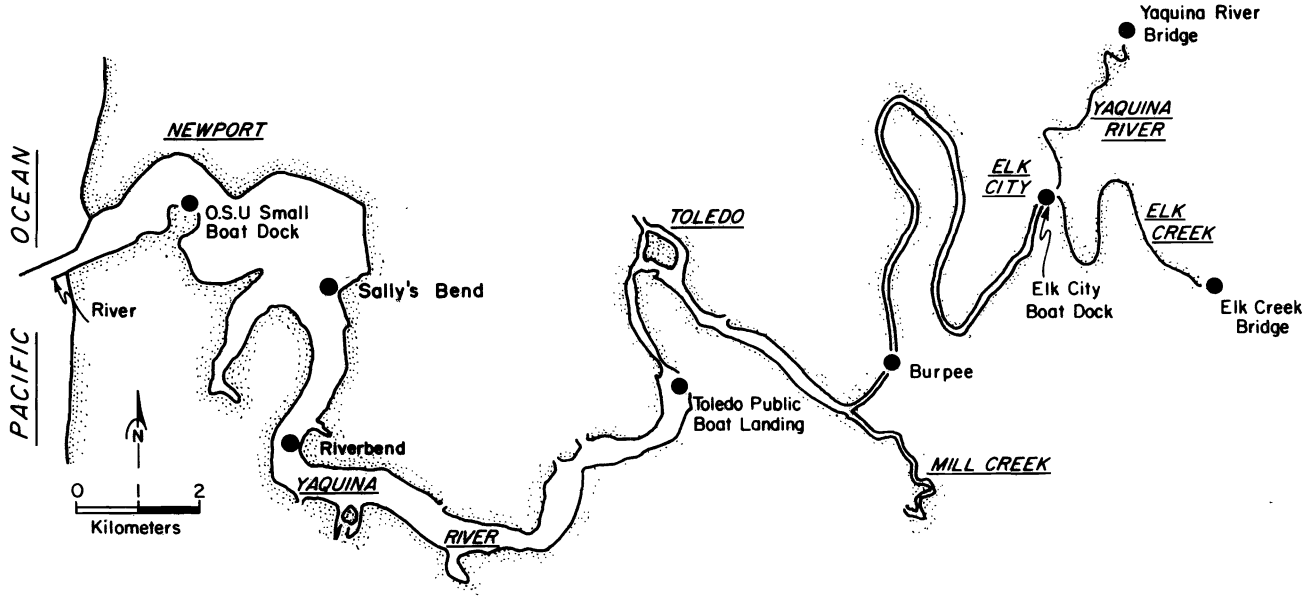

Figure 1. Marine algal assay field sampling sites, Yaquina Estuary, Newport, Oregon.

affected by tidal action. The normal flow of the Yaquina River is estimated at 30.5 m^3/sec, with summer flow estimated at about 0.93 m^3/sec. The mean tide range is 1.8 m, with a diurnal range of 2.4 m and extreme range of 3.5 m. The tidal prism during the tidal range is 2.41 x 10^7 m^3 with a diurnal range of 3.26 x 10^7 m^3. Annual rainfall averages from 152 cm at the coast to 254-279 cm in the eastern portions of the drainage basin, contributing an annual average runoff of 9.6 x 10^8 m^3 of fresh water. The drainage basin covers 655 km^2 of which 87 percent is forested, 4 percent is cropland, 2 percent is rangeland, and 7 percent is classified miscellaneous (Percy et al., 1973).

Pollution Sources

The City of Newport operates a secondary sewage treatment plant (SSTP) discharging wastewater through an outfall to the Pacific Ocean at an average rate ranging from 3.4 x 10^3 m^3/day during dry weather to 11.4-18.9 x 10^3 m^3/day in rainy weather. The City of Toledo operates a SSTP which discharges 1.83-1.96 x 10^3 m^3/day directly to the estuary. The Georgia-Pacific Corporation at Toledo operates a SSTP for its Kraft paper mill, discharging the more potent wastes year-around to a pipeline outfall (separate from the Newport outfall) off Yaquina Head in the Pacific Ocean. The mill discharges bleaching solutions to the estuary during the winter high flows and to the ocean outfall during the summer low flows. Accordingly, the main pollution source for the Yaquina estuary is at Toledo (data from Percy et al., 1973).

The Yaquina estuary is considered a well-mixed system from February through June, depending on seasonal flows, and partly mixed from August through January (Burt and McAlister, 1959).

METHODS AND MATERIALS

Bioassays were run according to procedures outlined in the Marine Algal Assay Procedure Bottle Test (MAAP) (U.S. EPA, 1974a), and the Algal Assay Procedure Test (AAP) (U.S. EPA, 1971). Chemical analyses were performed according to "Methods for Chemical Analysis of Water and Wastes (U.S. EPA, 1974b). Field measurements of salinity, conductivity, and temperature were made with a Beckman RS5-3 portable induction-type salinometer.

Samples have been taken in the Yaquina estuary on an irregular basis since August 1972 for analysis with the MAAP using the green unicellular alga, *Dunaliella tertiolecta* Butcher (DUN clone); the diatom, *Thalassiosira pseudonana* Hasle & Heimdal (CN clone) (*Cyclotella nana* Hustedt); and the freshwater AAP test alga, *Selenastrum capricornutum* Printz.

All samples were collected on the incoming tide at or near high water. Quarterly samples, in 1973-1974, also were collected at or near the following or preceding low water. Samples were collected at up to six stations (going up river in Figure 1): Oregon State University (OSU) Dock, Sally's Bend, Riverbend, Toledo, Burpee, and Elk City, the head of tidewater.

Surface grab samples, either from near shore or from floating docks, were collected in acid washed and distilled water rinsed polyethylene cubitainers. Samples were kept in iceboxes, transported to the laboratory, and stored at 4°C in the dark until they could be filtered (usually within less than 24 hours). Subsamples were taken before and after 0.45 μm membrane filtration and were analyzed for these parameters: Conductivity, total organic carbon, alkalinity, nitrite-N, nitrate-N, ammonia-N, total P, ortho-P, sulfate, reactive silica, and, at times, various metals such as copper, iron, manganese, zinc, cadmium, lead, and mercury. The filtered samples were then stored in the dark at 4°C until bioassayed.

RESULTS

Although samples were taken on 12 dates over a period of nearly 3 years, most of the samples were quarterly, on 1 December 1973, 9 February 1974, 12 May 1974, and 4 August 1974. For the quarterly series, samples were taken at high and low water at the same six stations. Samples taken on most other early dates did not cover all six stations.

High water samples from the OSU Dock station (at river kilometer 2.8, measured from the seaward end of the south jetty) averaged a maximum final yield of 15.9 mg/l dry weight in control flasks and 62.9 mg/l for the +P +N spiked samples. If one corrects using the difference between the chemical analysis of the filtered and unfiltered subsamples for nutrients bound in indigenous organisms lost upon filtering, the control yield average can be adjusted to 16.9 mg/l (Tables 1a, and 2 through 13c). The correction is computed by determining the yield coefficient for that particular sample (control) and calculating the additional yield that could be derived from that amount of P or N, whichever is growth limiting. The amount is determined by subtracting the amount of that element found in the filtered sample from that in the unfiltered sample. This correction would approximate the total potential standing phytoplankton crop.

OSU Dock low water samples averaged 20.2 mg/l dry weight in control flasks (corrected for filtration loss to 28.7 mg/l) and 74.9 mg/l for the +P +N spiked samples. Samples from the OSU Dock station were potentially P-limited on only two of 12 dates, on 12/1/73 at both high and low water (after several days of torrential rains and flooding) and at low water on 2/9/74.

Table 1a. Average MAAP biomass yields, Yaquina Estuary, August 1972 through July 1975 for *Dunaliella tertiolecta*.

Station	Average max final yield control, mg/l	range	Avg max yield +P +N, mg/l	range	Station	Average max final yield control, mg/l	range	Avg max yield +P +N, mg/l	range
OSU Dock - actual high water	15.9 ± 8.2	7.3 -33.0	62.9 ±20.2	19.8 -96.1	OSU Dock - actual low water	20.2 ±12.5	7.1 -32.8	74.9 ± 3.2	71.5 -77.7
filtration corrected (see text)	16.9 ± 9.5	7.3 -37.4			filtration corrected	28.7 ±18.6	7.5 -47.4		
Sally's Bend- actual high water	12.3 ± 5.0	5.1 -19.2	62.1 ±18.3	35.6 -76.9	Sally's Bend- actual low water	10.8 ± 7.5	5.1 -21.8	70.5 ± 5.8	65.3 -76.7
filtration corrected	13.2 ± 5.4	5.1 -19.2			filtration corrected	13.3 ± 8.2	6.2 -24.9		
Riverbend- actual high water	12.7 ± 8.3	7.9 -27.5	55.2 ±26.7	25.0 -84.4	Riverbend - actual low water	7.8 ± 4.6	1.67 -12.6	66.7 ± 9.4	58.4 -76.9
filtration corrected	14.7 ± 8.0	9.6 -28.7			filtration corrected	9.6 ± 7.1	1.67 -18.9		
Toledo - actual high water	9.6 ± 6.2	4.9 -18.6	48.4 ±12.4	36.1 -65.2	Toledo - actual low water	0.79 ± 0.9	0.07 - 1.8	33.1 ±56.8	1.08 -98.7
filtration corrected	13.9 ± 5.4	6.5 -18.9							
Burpee - actual high water	6.0 ± 5.7	1.1 -13.5	29.3 ±18.2	3.4 -45.9	Burpee - actual low water	0.3		5.7	
filtration corrected	10.6 ± 7.3	6.4 21.6							
Elk City - actual high water	0.67	0.02 - 0.7	19.1 ±24.9	1.59 -36.8	Elk City - actual low water	0.11 ± 0.13	0.02 - 0.2	27.9 ±36.5	2.1 -53.7
filtration corrected	3.2 ±2.4								

Samples at Sally's Bend (river km 6.1), taken at high water on six dates, averaged 12.3 mg/l dry weight in control (corrected to 13.2 mg/l) and 62.1 mg/l in the +P +N spiked samples. Sally's Bend low water samples averaged 10.8 mg/l dry weight (corrected to 13.3 mg/l) and 70.5 mg/l for the +P +N spiked samples. Samples from the Sally's Bend station were potentially P-limited only on those same two dates and tides as were the OSU Dock station samples, and N-limited on all other sampling dates (see Table 1a, and 8 -13c).

Samples at Riverbend (river km 9.3), taken at high water on six dates, averaged 12.7 mg/l dry weight in control (corrected to 14.7 mg/l) and 55.2 mg/l in the +P +N spiked samples. The samples taken at low water averaged 7.8 mg/l dry weight (corrected to 9.6 mg/l) in control and 66.7 mg/l in the +P +N spiked samples. Samples at Riverbend were potentially P-limited at high and low water on 12/1/73 and 2/9/74, simultaneously limited by P and N and at low water on 5/12/74, (see Table 10) but N-limited at all other sampling dates (see Tables 1a, and 8 -13c).

Samples from the Toledo Boat Landing station (river km 18.5) taken on nine dates averaged 9.6 mg/l dry weight (corrected to 13.9 mg/l) and an average of 48.4 mg/l dry weight in the +P +N spiked samples. The samples were potentially P-limited on 11/1/72, 6/28/73, at low water on 2/9/74, high and low water on 5/12/74 and on 4/23/75. Tests with *Dunaliella* gave no response at high and low water on 12/1/73 and high water 2/9/74. At low water on 8/4/74, N and P apparently were simultaneously limiting (see Table 1b), while the samples were N-limited on 8/8/72, 8/4/74 high water and 7/9/75. (See Tables 1a, and 2, 3, 5, 8 -13c.)

Samples at the Burpee station (river km 25.9) taken on nine dates averaged 6.0 mg/l dry weight (corrected to 10.6 mg/l) in the control and 29.3 mg/l in the + P + N spiked samples. P was potentially limiting at all dates except 8/8/72 and 7/9/75, when N was limiting. The alga did not respond to samples from 12/1/73, 2/9/74, 5/12/74, 4/23/75, and 7/9/75. The reason appears to be low salinity and not toxicity, as the 4/23/75 samples were rerun with *Selenastrum* yielding statistically significant results. (See Discussion section and Tables 1a, and 2, 3, 5, 8 -13f.)

In samples at the Elk City station (river km 26.1) sampled on seven dates, control samples averaged 0.67 mg/l (corrected to 3.2 mg/l) and 19.1 mg/l in the +P +N spiked samples. P appeared to be limiting on all dates. Again, no response was generated in the high and low water samples on 12/1/73, 2/9/74, high water on 5/12/74, 4/23/75, and 7/9/75. Low salinity was again suspected, as subsamples using

Table 1b. Algal biomass in mg dry weight/l, day 10 or 12, with indication of growth limiting nutrient (N = nitrogen, P = phosphorus, ? = not identified). Values are means of triplicate flasks. H or L after date indicates high (H) or low (L) tide at time of collection.

		8/ 8/72 High	11/ 1/72 High	5/24/73 High	6/28/73 High	7/25/73 High	10/30/73 High	12/ 1/73 High	12/ 1/73 Low	2/ 9/74 High	2/ 9/74 Low	5/12/74 High	5/12/74 Low	8/ 4/74 High	8/ 4/74 Low	4/23/75 High	7/ 9/75 High
OSU DOCK	Dunaliella control	16.2	16.3	14.7	10.1	9.3	13.3	33.0	32.8	16.8	28.9	9.0	12.1	28.7	7.1	7.3	18.6
	Dunaliella +P or +N	50.7 N	52.6 N	19.6 N	32.3 N	35.2 N	33.7 P	36.1 P	37.3 N	38.8 P	37.3 N	30.6 N	22.2 N	81.6 N	38.3 N	27.1 N	58.3 N
	Dunaliella +P +N	45.9	68.6	19.8	65.1	60.2	54.1	73.1	77.7	---	---	72.1	71.5	96.1	75.6	73.9	83.2
SALLY'S BEND	Dunaliella control							12.8	8.6	19.2	21.8	11.6	7.6	5.1	5.2	13.1	23.2
	Dunaliella +P or +N							45.7 P	44.8 P	36.4 N	46.2 P	33.3 N	13.2 N	43.4 N	28.9 N	33.4 N	61.9 N
	Dunaliella +P +N							65.3	43.6	---	---	70.7	69.5	80.7	76.7	84.4	79.2
RIVERBEND	Dunaliella control							7.9	12.6	27.5	7.4	8.9	9.4	9.3	1.7	9.7	12.1
	Dunaliella +P or +N							45.4 P	47.2 P	34.7 P	43.8 P	28.3 N	P&N	42.2 N	14.0 N	26.6 N	48.4 N
	Dunaliella +P +N							41.9	58.4	---	---	69.5	64.9	84.4	76.9	25.0	75.8
TOLEDO	Dunaliella control	6.5	18.7		8.3			no gr	no gr	no gr	0.07	4.3	0.5	6.0	1.8	5.0	8.5
	Dunaliella +P or +N	23.9 N	N&P		9.3 P			no gr	no gr	no gr	0.08 P	13.2 P	0.64 P	20.4 N	N&P	34.2 P	39.6 N
	Dunaliella +P +N	36.1	49.2		43.0			no gr	no gr	no gr	---	70.2	0.6	99.6	98.7	65.2	72.3
BURPEE	Dunaliella control	7.6	13.5		2.0			no gr	no gr	no gr	no gr	no gr	no gr	5.3	0.3	0.4	5.6
	Dunaliella +P or +N	10.6 N	20.3 P		4.9 P			no gr	no gr	no gr	no gr	no gr	no gr	6.8 P	0.6 P	?	7.4 P
	Dunaliella +P +N	32.2	45.9		3.4			no gr	no gr	no gr	no gr	no gr	no gr	98.1	5.7	0.07	58.1
	Selenastrum control															1.1	0.39
	Selenastrum +P or+N															16.6 P	0.37 P?
	Selenastrum +P +N															35.6	0.06 ?
ELK CITY	Dunaliella control				0.7			no gr	no gr	no gr	no gr	no gr	0.02	0.2	0.2	0.06	0.18
	Dunaliella +P or +N				6.8 P			no gr	no gr	no gr	no gr	no gr	24.2 P	1.9 P	2.7 P	--- ?	--- ?
	Dunaliella +P +N				1.6			no gr	no gr	no gr	no gr	no gr	53.7	17.2	2.4	0.12	0.1
	Selenastrum control															0.7	6.3
	Selenastrum +P or+N															16.0 P	8.5 P
	Selenastrum +P +N															36.8	28.3

Selenastrum with the 4/23/75 and 7/9/75 samples were successfully rerun with statistically significant results (see Tables 1a, and 8 - 13f).

DISCUSSION

All samples were assayed with the green alga, *Dunaliella.* Six of the twelve samples collected at the OSU Dock station were assayed with the diatom, *Thalassiosira*. *Selenastrum*, the freshwater test species, was used with samples from the two uppermost stations on 23 April 1975 and 9 July 1975. Although euryhaline in nature, *Dunaliella* and *Thalassiosira* failed to produce substantial growth in these essentially freshwater samples and in samples from those stations and the Toledo station at previous times in the preceding year (see Table 1b). The *Selenastrum* assays demonstrated (see Table 1b, and 12a, 13a,c) that, in fact, the waters were not algicidal or algistatic because of a toxic substance, but instead lacked only the necessary salinity for *Dunaliella* or *Thalassiosira* to grow effectively for accurate measurement (Specht and Miller, 1974).

Table 2. Algal assay growth response and associated water chemistry from samples collected 8/8/72, (surface, membrane filt.).

Dunaliella	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee	Elk City
Control dry wt., day10, mg/L ‡	16.237			6.530	7.575	
+ 0.05 mg P/L ‡	15 854			5.272	8.181	
+ 1.0 mg N/L ‡	50.666*			23.863*	10.639*	
+ 0.05 mg P & + 1.0 mg N/L ‡	45.871*			36.071*	32.231*	
Limiting nutrient	N			N	N	
salinity, °/oo	33.2			25.9	18.6	
ortho-P, mg/L	0.047			0.013	0.009	
combined N (NO_3 + NO_2 + NH_3), mg/L	0.191			0.031	0.075	
mg dry wt/ ug P	0.345			0.502	0.841	
mg dry wt/ ug N	0.085			0.210	0.101	
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032			NH_3 0.077 NO_3 0.033	NH_3 0.078 NO_3 0.033	

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	250.4	4.36	0.956*	8.704*	7
N (of N limited samples)	64.02	3.82	0.961*	9.199*	7
Salinity, °/oo	0.59	-5.25	0.792	3.438	7

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

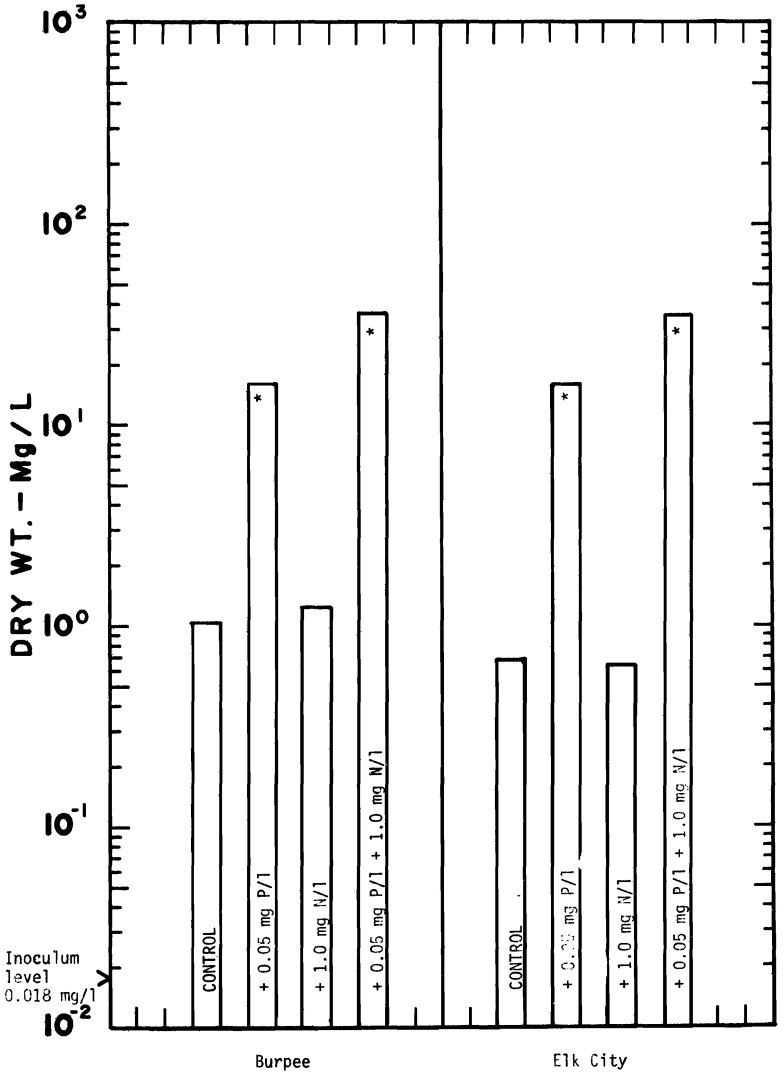

Figure 2. Day 10 dry weights of *Selenastrum capricornutum* Printz grown in 4/23/75 samples at Burpee (0.9°/oo) and Elk City (0.06°/oo).

Tallqvist (1973) reported the ability of *Selenastrum* to grow in brackish waters, finding that salinity below 4(°/oo) (°/oo = parts per thousand salinity) did not affect its growth.

In tests using 23 April 1975 samples, filtered water from the Burpee station (0.9°/oo) was mixed 50/50 with water from four downstream stations (which supported growth of *Dunaliella,* but not of *Selenastrum*): Toledo (10.67°/oo, yielding a mix salinity of 5.8°/oo), Riverbend (29.2°/oo, yielding a mix salinity of 15.1°/oo), Sally's Bend (30.8°/oo, yielding a mix salinity of 15.9°/oo), and OSU Dock (32.1°/oo, yielding a mix salinity of 16.5°/oo. These test waters (controls and controls plus nutrient spikes of 1.0 P/l, 1.0 mg N/l, and 1.0 mg P with 1.0 mg N/l) were inoculated to give a final concentration of 1,000 cells/ml of *Selenastrum*. As in the undiluted Elk City and Burpee samples of the same date (Figure 2), *Selenastrum* responded in a statistically significant manner in the Toledo/Burpee mixed sample (5.8°/oo), showing potential P-limitation (t-statistic, 12.615, 4 df, significant at the 0.1 percent level). Although *Selenastrum* in all flasks increased threefold over the inoculum in the Riverbend/Burpee sample (15.1°/oo), there was no significant difference between the control

Table 3. Algal assay growth response and associated water chemistry from samples collected 11/1/72, (surface, membrane filt.).

Dunaliella	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee	Elk City
Control dry wt., day10, mg/L ‡	16.33			18.66	13.46	
+ 0.05 mg P/L ‡	15.74			20.94	20.32*	
+ 1.0 mg N/L ‡	52.60*			20.18	14.32	
+ 0.05 mg P & + 1.0 mg N/L ‡	68.55*			49.20*	45.92*	
Limiting nutrient	N			N&P	P	
salinity, °/oo	33.83			24.03	17.02	
ortho-P, mg/L	0.038			0.022	0.015	
combined N (NO_3 + NO_2 + NH_3), mg/L	0.177			0.320	0.367	
mg dry wt/ ug P	1.384			0.971	1.354	
mg dry wt/ ug N	0.058			0.037	0.033	
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032			NH_3 0.077 NO_3 0.033	0.99	

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	0.98	-1.76	0.846*	3.175*	4
N (of N limited samples)	0.02	12.55	0.525	1.234	4
Salinity, °/oo	0.125	13.65	0.262	0.72	7

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

and any of the spikes. In the Sally's Bend/Burpee (15.9°/$_{oo}$) and OSU Dock/Burpee (16.5°/$_{oo}$) mixtures, *Selenastrum* showed no significant increase over the inoculum level (see Table 12c).

In similar tests using 9 July 1975 samples, *Dunaliella* grew in mixed samples (Burpee, low yield, and Elk City, no growth mixed with downstream stations that supported adequate growth; see Tables 13d and 13e). Results indicated a low probability that toxicity was responsible for the low or no growth of the alga in the upper river samples. The Burpee/Toledo (15.83°/$_{oo}$), Elk City/Toledo (9.34°/$_{oo}$) and Elk City/Burpee (5.83°/$_{oo}$) also were inoculated with *Selenastrum.* The Burpee/Toledo and Elk City/Toledo samples did not support its growth, but *Selenastrum* responded in a significant manner to the Elk City/Burpee mixture, indicating potential P-limitation (see Table 13f).

It appears reasonable, therefore, to use *Selenastrum* to qualitatively bioassay estuarine water of salinities of up to at least 6°/$_{oo}$, but not to exceed 9°/$_{oo}$. This provides some overlap with the use of *Dunaliella,* which

Table 4. Algal assay growth response and associated water chemistry from samples collected 5/30/73, (surface, membrane filt.).

Dunaliella	OSU Dock
Control dry wt., day10, mg/L ‡	14.68
+ 0.05 mg P/L ‡	14.58
+ 1.0 mg N/L ‡	19.63*
+ 0.05 mg P & + 1.0 mg N/L ‡	19.81*
Limiting nutrient	N
salinity, °/oo	28.71
ortho-P, mg/L	0.041
combined N (NO_3 + NO_2 + NH_3), mg/L	0.224
mg dry wt/ ug P	0.358
mg dry wt/ ug N	0.065
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)					
N (of N limited samples)					
Salinity, °/oo					

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

responds reliably to the addition of limiting nutrients at salinities as low as 5°/oo (McLachlan, 1960; Specht, 1974; Specht and Miller, 1974).

Although samples from the OSU Dock station were nitrogen limited on all but two occasions (12/1/73 and 2/9/74), the algal assay showed that, for the most part, the estuary is wholly nitrogen limited for algal growth only in the generally precipitation free late summer months (July, August, and September) and is potentially phosphorus limited during the rainy season. However, the Yaquina estuary is probably light-limited a significant proportion of the rainy season. Qualitatively, the indication of nitrogen or phosphorus limitation was identical regardless of whether *Dunaliella, Thalassiosira* (see, for instance, Tables 12a and 12b), or *Selenastrum* was used, although the levels of biomass produced differed considerably at a given sample point and time. The maximum yields and control yields varied considerably according to the season (Figure 3 and Table 1b), but depended principally upon the amount of nutrient present (Skulberg, 1967; Specht, 1974; and Specht and Miller, 1974). In P-limited samples, the dry weight to P concentration correlation coefficient was 0.791; in N-limited samples, the N concentration correlation coefficient

Table 5. Algal assay growth response and associated water chemistry from samples collected 6/28/73, (surface, membrane filt.).

Dunaliella	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee	Elk City
Control dry wt., day10, mg/L ‡	10.10			8.32	2.04	0.68
+ 0.05 mg P/L ‡	11.51			9.34*	4.85*	6.81*
+ 1.0 mg N/L ‡	32.33*			7.65	2.06	0.68
+ 0.05 mg P & + 1.0 mg N/L ‡	65.14*			42.96*	3.37*	1.59*
Limiting nutrient	N			P	P	P
salinity, °/oo	30.76			14.14	2.88	0.03
ortho-P, mg/L	0.030			0.013	0.011	0.009
combined N (NO_3 + NO_2 + NH_3), mg/L	0.120			0.128	0.416	0.498
mg dry wt/ ug P	1.077			0.718	0.440	0.756
mg dry wt/ ug N	0.058			0.038	0.002	0.00]
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032			0.862	0.557	0.557

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	1.896	-17.14	0.931*	6.789*	7
N (of N limited samples)	---	---	---	---	---
Salinity, °/oo	0.312	1.61	0.924*	7.66*	10

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

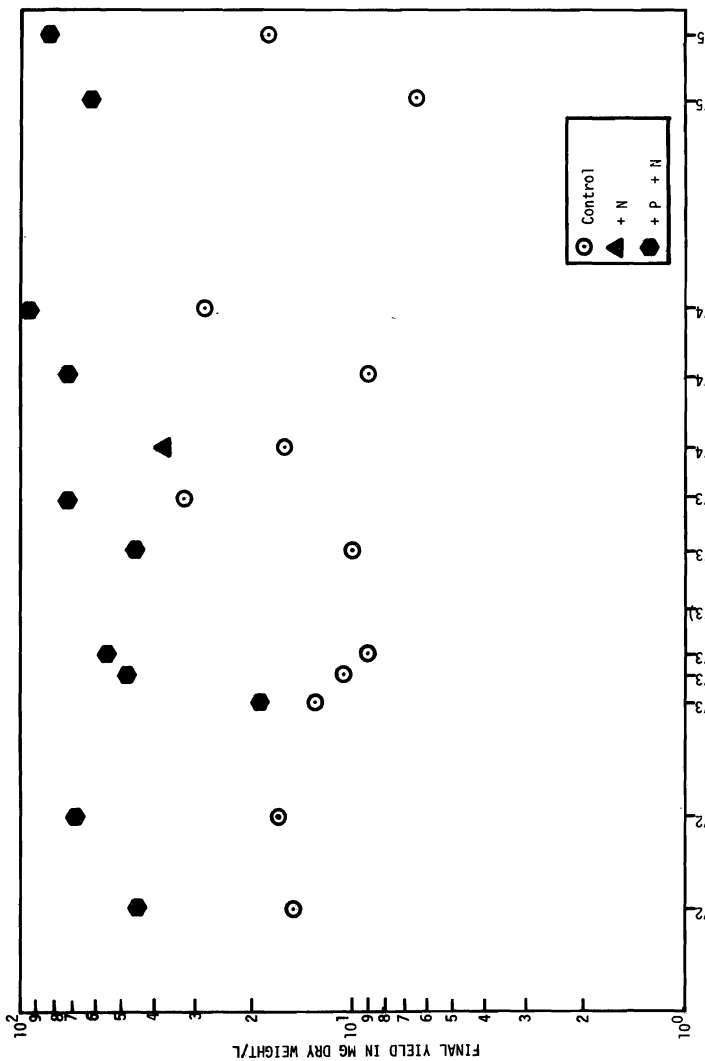

Figure 3. Dry weight biomass produced by *Dunaliella tertiolecta* at day 10 or 12, control and spikes, from samples taken at the OSU Dock station, from August, 1972, to July, 1975.

was 0.804. Both were statistically significant at the 0.1 percent level. Dry weight production depended secondarily upon the salinity of the sample.

McIntire (1975) states that the primary factor for benthic diatom speciation in the Yaquina estuary is mean salinity, and, to a lesser degree, mean salinity range, temperature, and isolation. These attached diatom populations are alternately flooded (within a range determined by the seasonal freshwater tributary outflow) with P-limited freshwater tributary outflow that is relatively N-rich and P-poor and the N-limited tidal plug saline water inflow that is relatively P-rich and N-poor. Thus, benthic algal populations so situated should rarely be nutrient limited. More probably, they are limited by light, salinity, temperature, and desiccation. Planktonic algae, which stay in and move with the ebb and flow of the tidal plug, are more likely to become nutrient limited, at least during the late spring, summer, and early fall months.

Table 6. Algal assay growth response and associated water chemistry from samples collected 7/30/73, (surface, membrane filt.).

Dunaliella	OSU Dock					
Control dry wt., day10, mg/L ‡	9.33					
+ 0.05 mg P/L ‡	9.67					
+ 1.0 mg N/L ‡	35.23*					
+ 0.05 mg P & + 1.0 mg N/L ‡	60.23*					
Limiting nutrient	N					
salinity, °/oo	32.88					
ortho-P, mg/L	0.03					
combined N (NO_3 + NO_2 + NH_3), mg/L	0.24					
mg dry wt/ ug P	1.467					
mg dry wt/ ug N	0.028					
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032					
Linear regression of parameter vs dry weight		Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)						
N (of N limited samples)						
Salinity, °/oo						

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

A significant response (54 mg/l, +P +N spike) was generated from the 5/12/74 Elk City low water sample (see Table 1b), indicating that some contaminant inhibitory to *Dunaliella* may have been released at Toledo and carried upstream by the tide (see Burt and Marriage, 1957, for hydrological detail), affecting the high water sample, but not the low water sample. Salinity was lower at Elk City at low water and higher at high water, eliminating low salinity as a possible cause of no growth in the high water sample. Chemical analysis revealed no significant differences in other parameters.

A seasonal trend can be seen in the filtered water samples indicating that the amount of nutrients available for biomass production at a given time partially reflect the amount of nutrients bound in the indigenous biomass present in the unfiltered sample. Nutrient (N or P) spiking allows one to determine the amount of biomass producible from both the major

Table 7. Algal assay growth response and associated water chemistry from samples collected 10/30/73 (surface, membrane filt.).

Dunaliella	OSU Dock
Control dry wt., day12, mg/L ‡	13.34
+ 0.05 mg P/L ‡	12.38
+ 1.0 mg N/L ‡	33.69*
+ 0.05 mg P & + 1.0 mg N/L ‡	54.12*
Limiting nutrient	N
salinity, °/oo	29.45
ortho-P, mg/L	0.052
combined N (NO_3 + NO_2 + NH_3), mg/L	0.172
mg dry wt/ ug P	0.647
mg dry wt/ ug N	0.046
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.32

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)					
N (of N limited samples)					
Salinity, °/oo					

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

nutrients (N and P) in the sample. For instance, if N is growth limiting, the final yield in the control indicates the potential for the N present in the sample, but not the P. Accordingly, adding an excess amount of N allows the maximum biomass attributable to the sample level of P to be determined (assuming another element does not become limiting first).

With reference to McIntire's (1975) statement about speciation and the influence of salinity, one should note here that *Dunaliella* and *Thalassiosira* were both chosen because of their euryhaline character. Although this allows them to grow in a wide range of salinities, the dry weight that they can produce outside their optimal salinity can in no way be used to accurately predict the maximum yield producible by an alga whose optimum lies at that sample salinity. Apparently the qualitative indications are identical, but the quantitative levels are not necessarily so. The levels so attained should be regarded as perhaps the minimum potential level of biomass that could be stimulated by that nutrient addition under ideal physical conditions. Young and Barber (1973), working in the New York Bight, showed that the biomass response to

Table 8. Algal assay growth response and associated water chemistry from samples collected 12/1/73, (surface, membrane filt.).

Dunaliella	OSU Dock low water	OSU Dock high water	Sally's Bend low water	Sally's Bend high water	Riverbend low water	Riverbend high water
Control dry wt., day10, mg/L ‡	32.8	33.0	8.57	12.8	12.6	7.89
+ 0.05 mg P/L ‡	37.3*	36.1	44.8*	45.7*	47.2*	45.4*
+ 1.0 mg N/L ‡	31.5	32.8	7.31	14.1	10.6	5.72
+ 0.05 mg P & + 1.0 mg N/L ‡	77.7*	73.1*	43.6*	65.3*	58.4*	41.9*
Limiting nutrient	P	(P)	P	P	P	P
salinity, °/oo	13.2	13.0	6.5	7.4	8.1	7.2
ortho-P, mg/L	0.035	0.030	0.024	0.022	0.016	0.014
combined N (NO_3 + NO_2 + NH_3), mg/L	0.674	0.690	0.921	0.918	0.912	0.977
mg dry wt/ ug P	0.911	1.100	0.357	0.582	0.785	0.563
mg dry wt/ ug N	0.048	0.047	0.009	0.013	0.013	0.008
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	0.836	0.831	0.623	0.654	0.659	0.629

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	1150.	-6.69	0.897*	8.13*	16
N (of N limited samples)	-87.7	92.7	-0.964*	-14.7*	16
Salinity, °/oo	3.77	-16.6	0.959*	13.6*	16

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

natural samples by an indigenous algal inoculum was virtually the same as a pure culture inoculum of the dominant bloom organism obtained from that sample. However, it does not necessarily follow that a pure culture of any euryhaline alga can respond to the same degree. The chief advantage of the use of the MAAP is standardization and ease of enumeration of unialgal cultures whose physiological background in a wide latitude of parameters has been established (Tarzwell, 1971; Fitzgerald, 1972).

Analysis of the quarterly series of samples in 1974 suggests as the season progresses from December through August, that the amount of nutrients available for algal growth declines except at the OSU Dock station (see Figure 4). The potential productivity, estimated from the filtration-corrected control series, appears to show a trend to maximize in the spring, reflecting both the high nutrient content of the winter runoff, and the low indigenous biomass levels due to light limitation. The exception to this is the influence at the mouth of the estuary of the upwelling caused by offshore winds during the summer months, for instance, the 8/4/74 high tide sample at the OSU Dock station as opposed to the low tide sample; see Figure 4.

Table 9. Algal assay growth response and associated water chemistry from samples collected 2/9/74, (surface, membrane filt.).

Dunaliella	OSU Dock low water	OSU Dock high water	Sally's Bend low water	Sally's Bend high water	Riverbend low water	Riverbend high water
Control dry wt., day10, mg/L ‡	28.9	16.8	21.8	19.2	7.35	27.5
+ 0.05 mg P/L ‡	37.3*	17.1	46.2*	17.3	43.8*	34.7*
+ 1.0 mg N/L ‡	30.5	38.8*	21.6	36.4*	8.1	31.3*
+ 0.05 mg P & + 1.0 mg N/L ‡	---	---	---	---	---	---
Limiting nutrient	P	N	P	N	P	P(N)
salinity, °/oo	19.0	29.0	13.8	28.2	6.7	22.8
ortho-P, mg/L	0.024	0.027	0.021	0.027	0.014	0.024
combined N (NO_3 + NO_2 + NH_3), mg/L	0.530	0.234	0.685	0.263	0.818	0.478
mg dry wt/ ug P	1.20	0.621	1.04	0.710	0.524	1.15
mg dry wt/ ug N	0.054	0.071	0.031	0.072	0.008	0.057
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	1.11	0.077	0.810	0.076	0.614	1.16

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	2090.	-21.9	0.996*	35.5*	10
N (of N limited samples)	42.1	7.9	0.990*	19.2	7
Salinity, °/oo	0.359	13.1	0.392	1.71	16

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

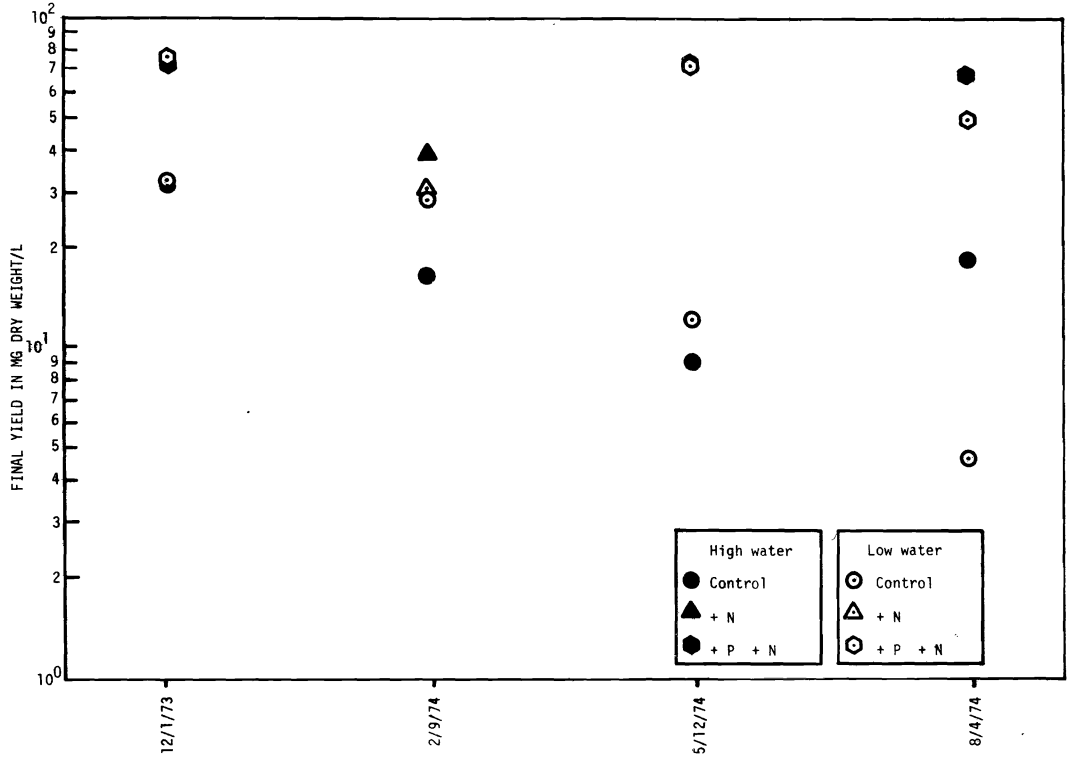

Figure 4. Dry weight biomass by *Dunaliella tertiolecta* at day 10, control and spikes, from samples taken at consecutive high and low tides at the OSU Dock station on 12/1/73 through 8/4/74.

Specht (1974) described the formation of a boundary in the Yaquina estuary separating N-limited seaward water and P-limited tributary water. Apparently the boundary moves back and forth with the tidal plug and its relative range changes seasonally according to relative tributary flow (see Table 1 and, especially 8 and 9). Bioassay of subsequent samples (5/12/74, 8/4/74, 4/23/75, and 7/9/75) has verified the existence of this mechanism (see Tables 1b, and 10 to 13a). The data in Table 1 show that the N-limited - P-limited boundary moves fairly consistently with the season, so that the estuary is potentially wholly N-limited only in precipitation-free periods (June through October, generally), potentially wholly P-limited only during periods of prolonged precipitation and runoff (November through April) and partitioned the remainder of the time.

SUMMARY

The use of the MAAP nutrient bioassay has shown that the potential nutrient limitation and biomass potential in the Yaquina estuary, Oregon, changes with hydrological and precipitation changes associated with seasonal cycles. The maximum sensitivity of the estuary to the addition of nutritive wastes appears to be in the late spring and summer months. This

Table 10. Algal assay growth response and associated water chemistry from samples collected 5/12/74, (surface, membrane filt.).

Dunaliella	OSU Dock low water	OSU Dock high water	Sally's Bend low water	Sally's Bend high water	Riverbend low water	Riverbend high water
Control dry wt., day10, mg/L ‡	12.1	8.95	7.62	11.6	9.44	8.91
+ 0.05 mg P/L ‡	9.14	7.52	7.25	11.2	9.90	8.10
+ 1.0 mg N/L ‡	22.2*	30.6*	13.2*	33.3*	9.76	28.3*
+ 0.05 mg P & + 1.0 mg N/L ‡	71.5*	72.1*	69.5*	70.7*	64.9*	69.5*
Limiting nutrient	N	N	N	N	P&N	N
salinity, °/oo	28.5	28.5	20.8	29.0	16.4	26.0
ortho-P, mg/L	0.018	0.018	0.008	0.024	0.012	0.020
combined N (NO_3 + NO_2 + NH_3), mg/L	0.155	0.126	0.121	0.180	0.19	0.149
mg dry wt/ ug P	1.233	1.700	1.650	1.387	0.825	1.415
mg dry wt/ ug N	0.061	0.064	0.061	0.059	0.054	0.060
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.033	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.031 P 0.930	NH_3 0.077 NO_3 0.033

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	217.6	6.24	0.360	1.395	13
N (of N limited samples)	55.1	1.31	0.730*	3.71*	12
Salinity, °/oo	0.194	5.137	0.309	1.302	16

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

Table 11. Algal assay growth response and associated water chemistry from samples collected 8/4/74, (surface, membrane filt.).

Dunaliella	OSU Dock low water	OSU Dock high water	Sally's Bend low water	Sally's Bend high water	Riverbend low water	Riverbend high water
Control dry wt., day 12, mg/L ‡	7.10	28.7	5.15	5.10	1.67	9.32
+ 0.05 mg P/L ‡	7.59	24.7	4.51	5.10	1.30	8.97
+ 1.0 mg N/L ‡	38.3*	81.6*	28.9*	43.4*	14.0*	42.2*
+ 0.05 mg P & + 1.0 mg N/L ‡	75.6*	96.1*	76.7*	80.7*	76.9*	84.4*
Limiting nutrient	N	N	N	N	N	N
salinity, °/oo	28.0	30.2	28.0	30.2	23.4	29.9
ortho-P, mg/L	0.025	0.055	0.02	0.035	0.03	0.005
combined N (NO_3 + NO_2 + NH_3), mg/L	0.064	0.244	0.044	0.033	0.008	0.059
mg dry wt/ ug P	1.53	1.48	1.45	1.24	0.466	8.44
mg dry wt/ ug N	0.071	0.077	0.073	0.078	0.076	0.079
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.033	NH_3 0.077 NO_3 0.032

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of N limited samples)	0.515	-5.09	0.586	2.803	15
N (of N limited samples)	0.132	0.000	0.826*	5.686*	15
Salinity, °/oo	2.275	-53.9	0.431	1.850	15

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

Table 12a. Algal assay growth response and associated water chemistry from samples collected 4/23/75, (surface, membrane filt.).

Dunaliella**	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee**	Elk City**
Control dry wt., day12, mg/L ‡	7.30	13.1	9.66	4.98	1.05	0.66
+ 0.05 mg P/L ‡	6.47	15.6	10.5	34.2*	16.6*	16.0*
+ 1.0 mg N/L ‡	27.1*	33.4*	26.6*	6.59	1.25	0.64
+ 0.05 mg P & + 1.0 mg N/L ‡	73.9*	84.4*	25.0*	65.2*	35.6*	36.8*
Limiting nutrient	N	N	N	P	P	P
salinity, °/oo	32.10	33.20	29.20	10.67	1.10	0.06
ortho-P, mg/L	0.020	0.032	0.022	0.006	0.004	0.002
combined N (NO_3 + NO_2 + NH_3), mg/L	0.086	0.197	0.131	0.437	0.630	0.347
mg dry wt/ ug P	1.36	1.04	1.21	1.16	0.659	0.707
mg dry wt/ ug N	0.068	0.070	0.066	0.023	0.026	0.046
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.032	NH_3 0.077 NO_3 0.032	0.760	n.a.	n.a.

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	1230.	-3.11	0.888*	5.116*	7
N (of N limited samples)	51.5	2.96	0.980*	13.09*	7
Salinity, °/oo	0.309	0.442	0.930*	10.166*	16

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

** *Selenastrum capricornutum* used(see text, Methods and Discussion sections)

Table 12b. Algal assay growth response and associated water chemistry from samples collected 4/23/75, (surface, membrane filt.).

Thalassiosira	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee	Elk City
Control dry wt., day10, mg/L ‡	9.22	24.3	13.1	18.2	4.20	0.64
+ 0.05 mg P/L ‡	10.4	19.4	11.1	45.3*	2.83	0.21
+ 1.0 mg N/L ‡	35.1*	38.9*	21.4*	16.9	4.81	0.15
+ 0.05 mg P & + 1.0 mg N/L ‡	29.2*	75.3*	22.8*	58.6*	4.06	0.06
Limiting nutrient	N	N	N	P	?	?
salinity, °/oo	32.10	33.2	29.2	10.67	1.10	0.06
ortho-P, mg/L	0.020	0.032	0.022	0.006	0.004	0.002
combined N (NO_3 + NO_2 + NH_3), mg/L	0.086	0.197	0.131	0.437	0.630	0.347
mg dry wt/ ug P	1.76	1.22	0.972	1.046	1.02	0.32
mg dry wt/ ug N	0.026	0.062	0.020	0.130	0.007	0.001
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	n.a.	n.a.	n.a.	n.a.	n.a	n.a.

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	---	---	---	---	---
N (of N limited samples)	138.6	-3.49	0.975*	11.71*	7
Salinity, °/oo	0.316	6.68	0.566	2.57	14

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

Table 12c. Algal assay growth response and associated water chemistry from samples collected 4/23/75, (surface, membrane filt.).

	Burpee/ Riverbend Dunaliella	Burpee/ Toledo Bt L Dunaliella	Burpee/ Riverbend Thalassiosira	Burpee/ Toledo Bt L Thalassiosira	Burpee/ Riverbend Selenastrum	Burpee/ Toledo Bt L Selenastrum
Control dry wt., day10, mg/L ‡	36.8	3.58	47.3	6.1	0.054	6.02
+ 0.05 mg P/L ‡	50.2*	36.6*	48.7	13.1*	0.11	19.16*
+ 1.0 mg N/L ‡	25.3	3.5	44.9	1.3	0.24	2.78
+ 0.05 mg P & + 1.0 mg N/L ‡	119.0*	41.7*	43.7	10.3*	0.055	28.8*
Limiting nutrient	P	P	(P)	P	?	?
salinity, °/oo	16.4	7.1	16.4	7.1	16.4	7.1
ortho-P, mg/L	0.013	0.005	0.013	0.005	0.013	0.005
combined N (NO_3 + NO_2 + NH_3), mg/L	0.380	0.533	0.380	0.533	0.380	0.533
mg dry wt/ ug P	2.83	0.72	3.64	1.22	0.004	1.20
mg dry wt/ ug N	0.097	0.007	0.126	0.016	0.0001	0.011
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	n.a.	n.a.	n.a.	n.a.	n.a	n.a.

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)					
N (of N limited samples)					
Salinity, °/oo					

‡ mean of triplicate flasks

* signifies significantly different from control, determined by t-test

Table 13a. Algal assay growth response and associated water chemistry from samples collected 7/9/75, (surface, membrane filt.).

Dunaliella	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee	Elk City
Control dry wt., day12, mg/L ‡	18.6	23.2	12.1	8.47	5.65	0.18
+ 0.05 mg P/L ‡	18.5	24.2	13.5	7.63	7.43*	0.03
+ 1.0 mg N/L ‡	58.3*	61.9*	48.4*	39.6*	5.54	0.07
+ 0.05 mg P & + 1.0 mg N/L ‡	83.2*	79.2*	75.8*	72.3*	58.1*	0.10
Limiting nutrient	N	N	N	N	P	?
salinity, °/oo	32.18	31.82	30.26	20.25	11.63	0.04
ortho-P, mg/L	0.029	0.048	0.035	0.019	0.008	0.008
combined N (NO_3 + NO_2 + NH_3), mg/L	0.198	0.283	0.179	0.100	0.124	0.253
mg dry wt/ ug P	0.64	0.48	0.35	0.45	0.71	0.023
mg dry wt/ ug N	0.094	0.082	0.068	0.085	0.045	0.0007
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.076 NO_3 0.031	NH_3 0.076 NO_3 0.031	NH_3 0.076 NO_3 0.032	NH_3 0.076 NO_3 0.033	0.74	

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	---	---	---	---	---
N (of N limited samples)	83.3	-0.74	0.952*	4.38*	10
Salinity, °/oo	0.69	-3.69	0.858*	2.89*	13

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

Table 13b. Algal assay growth response and associated water chemistry from samples collected 7/9/75, (surface, membrane filt.).

Selenastrum					Burpee	Elk City
Control dry wt., day12, mg/L ‡					0.39	6.33
+ 0.05 mg P/L ‡					0.37	8.53*
+ 1.0 mg N/L ‡					0.18	3.71
+ 0.05 mg P & + 1.0 mg N/L ‡					0.06	28.3*
Limiting nutrient					?	P
salinity, °/oo					11.63	0.04
ortho-P, mg/L					0.008	0.008
combined N (NO_3 + NO_2 + NH_3), mg/L					0.124	0.253
mg dry wt/ ug P					0.049	0.796
mg dry wt/ ug N					0.003	0.025
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)					n.a.	n.a.

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)					
N (of N limited samples)					
Salinity, °/oo					

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

Table 13c. Algal assay growth response and associated water chemistry from samples collected 7/9/75, (surface, membrane filt.).

Thalassiosira	OSU Dock	Sally's Bend	Riverbend	Toledo Bt L	Burpee	Elk City
Control dry wt., day10, mg/L ‡	27.9	17.0	22.0	14.5	10.4	0.52
+ 0.05 mg P/L ‡	29.1	36.8	22.0	13.4	13.8	0.10
+ 1.0 mg N/L ‡	115.2*	52.4*	119.9*	51.0*	18.9*	0.76
+ 0.05 mg P & + 1.0 mg N/L ‡	87.5*	88.9*	136.2*	63.8*	104.1*	0.09
Limiting nutrient	N	N	N	N	N	?
salinity, °/oo	32.18	31.82	30.26	20.25	11.63	0.04
ortho-P, mg/L	0.029	0.048	0.035	0.019	0.008	0.008
combined N (NO_3 + NO_2 + NH_3), mg/L	0.198	0.283	0.179	0.100	0.124	0.253
mg dry wt/ ug P	0.962	0.345	0.629	0.763	1.30	0.065
mg dry wt/ ug N	0.141	0.06	0.123	0.145	0.084	0.008
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	---	---	---	---	---
N (of N limited samples)	38.3	11.5	0.403	0.76	13
Salinity, °/oo	0.62	2.68	0.828*	2.55*	13

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

Table 13d. Algal assay growth response and associated water chemistry from samples collected 7/9/75, (surface, membrane filt.).

Dunaliella	Elk City/ OSU Dock	Elk City/ Sally's Bend	Elk City/ Riverbend	Elk City/ Toledo Bt L	Elk City/ Burpee
Control dry wt., day12, mg/L ‡	16.0	22.5	15.5	8.6	2.5
+ 0.05 mg P/L ‡	18 5	25.7	16.6	11.4*	9.5*
+ 1.0 mg N/L ‡	25.5*	32.1*	23.7*	9.1	2.6
+ 0.05 mg P & + 1.0 mg N/L ‡	83.2*	79.4*	81.5*	62.0*	55.9*
Limiting nutrient	N	N	N	P	P
salinity, °/oo	16.11	15.93	15.15	9.34	5.83
ortho-P, mg/L	0.019	0.028	0.022	0.014	0.008
combined N (NO_3 + NO_2 + NH_3), mg/L	0.226	0.268	0.216	0.177	0.189
mg dry wt/ ug P	0.842	0.804	0.686	0.614	0.313
mg dry wt/ ug N	0.071	0.084	0.070	0.049	0.013
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.084 NO_3 0.031	NH_3 0.084 NO_3 0.031	NH_3 0.084 NO_3 0.033	0.703	0.56

Linear regression of parameter vs dry weight	Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)	---	---	---	--	---
N (of N limited samples)	176.1	-24.6	0.793*	3.45*	7
Salinity, °/oo	1.48	-5.92	0.853*	5.90*	13

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

Table 13e. Algal assay growth response and associated water chemistry from samples collected 7/9/75, (surface, membrane filt.).

Dunaliella	Burpee/ OSU Dock	Burpee/ Sally's Bend	Burpee/ Riverbend	Burpee/ Toledo Bt L		
Control dry wt., day12, mg/L ‡	13.1	15.9	11.9	8.1		
+ 0.05 mg P/L ‡	14.9	18.2	13.0	9.4		
+ 1.0 mg N/L ‡	29.7*	30.3*	28.7*	13.2*		
+ 0.05 mg P & + 1.0 mg N/L ‡	83.8*	80.6*	82.9*	78.3*		
Limiting nutrient	N	N	N	N		
salinity, °/oo	21.91	21.73	20.94	15.83		
ortho-P, mg/L	0.019	0.028	0.022	0.014		
combined N (NO_3 + NO_2 + NH_3), mg/L	0.161	0.204	0.152	0.112		
mg dry wt/ ug P	0.689	0.568	0.541	0.579		
mg dry wt/ ug N	0.081	0.078	0.078	0.072		
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	NH_3 0.077 NO_3 0.033	NH_3 0.077 NO_3 0.033	NH_3 0.077 NO_3 0.033	NH_3 0.084 NO_3 0.031		
Linear regression of parameter vs dry weight		Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)		---	---	---	---	---
N (of N limited samples)		75.15	0.65	0.902*	6.62	10
Salinity, °/oo		0.83	-4.2	0.760*	3.69*	10

‡ mean of triplicate flasks * signifies significantly different from control, determined by t-test

Table 13f. Algal assay growth response and associated water chemistry from samples collected 7/9/75, (surface, membrane filt.).

Selenastrum	Burpee/ Toledo Bt L	Elk City/ Toledo Bt L	Elk City/ Burpee			
Control dry wt., day12, mg/L ‡	0.27	0.48	0.46			
+ 0.05 mg P/L ‡	0.11	0.93	4.20*			
+ 1.0 mg N/L ‡	0.04	0.24	0.50			
+ 0.05 mg P & + 1.0 mg N/L ‡	0.10	0.05	21.6*			
Limiting nutrient	?	?	P			
salinity, °/oo	15.83	9.34	5.83			
ortho-P, mg/L	0.014	0.014	0.008			
combined N (NO_3 + NO_2 + NH_3), mg/L	0.112	0.177	0.189			
mg dry wt/ ug P			0.058			
mg dry wt/ ug N			0.002			
mg dry wt/ ug of limiting nutrient in ASW (Specht & Miller, 1974)	n.a.	n.a.	n.a.			
Linear regression of parameter vs dry weight		Slope	Intercept	Correlation coefficient (r)	T-test	# degrees of freedom
P (of P limited samples)						
N (of N limited samples)						
Salinity, °/oo						

‡ mean of triplicate flasks * signifies significantly different from control. determined by t-test

is at a time when the light and temperature potential are highest, permitting the maximum exploitation of the nutrients by algae. Nutrient bioassays show that the addition of either phosphorus or nitrogen or both can stimulate algal growth depending on the point of introduction and time of season or day. Because of this, serious consideration should be made for the removal of both nutrients from sewage effluents or other pollutant sources before disposal into estuaries or their tributaries.

The utility of *Selenastrum capricornutum* Printz, the AAP freshwater test species, is described as an assay organism for low salinity brackish waters.

(Note: Tables 2-13f allow side-by-side comparison of bioassay results, the potential growth-limiting nutrient, and their associated sample water chemistry. Calculations have been made to show the per unit biomass yield for the limiting nutrient and the yield that could be expected from that alga's growth in artificial sea-water (ASW), adjusted for salinity. Correlation coefficients have been computed to show the dependence of dry weight production on the amount of limiting nutrient present as opposed to other factors.)

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Algal Nutritional Bioassays of Lake Wylie, North Carolina

R. M. Gerhold*

INTRODUCTION

Lake Wylie is a multiple use recreational, hydroelectric, and cooling water lake located 14 miles southwest of Charlotte, North Carolina. The lake was created by the Southern Company in 1904 with the erection of a dam on the Catawba River for hydroelectric production. The original impoundment area was increased in 1924 and 1925 by Duke Power Company when the dam was raised 50 feet and a new hydroelectric plant was constructed. In addition to the operation of this 60 megawatt hydroelectric facility, the lake serves as a cooling water source for the Allen Steam Station and will supply makeup water for the proposed Catawba Nuclear Station.

Lake Wylie exhibits the thermal properties of a warm monomictic lake (Hutchinson, 1957), which is characterized by winter water temperatures always exceeding 4°C, summer thermal stratification, and mixing during winter. Retention time under average flow is 32 days. The intensity of stratification and the time of turnover are complicated in Lake Wylie by the operation of both the Wylie and Mountain Island Hydroelectric Stations. Most of the watershed for Lake Wylie overlies igneous and metamorphic bedrock in the southern Piedmount physiographic provience. The Catawba River and the South Fork streamflow contributions to Lake Wylie calculated for the study period were about 71 percent and 18 percent respectively. The balance (11 percent) was derived from drainages contiguous to Lake Wylie itself. The water is soft (10-15

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mg/l - $CaCO_3$ during the study), slightly buffered, with a mean pH of 6.5. Most of the total dissolved solids in Lake Wylie water come from weathering of the bedrock. Large quantities of free iron oxide and iron hydroxide give the surrounding soils a red color. The dominant clay mineral is kaolinite.

In light of both present and proposed use of Lake Wylie for electrical power generation purposes, Duke Power Company commissioned Industrial BIO-TEST Laboratories, Inc. (now Nalco-Environmental Sciences) to conduct a comprehensive study of the lake in August 1973. **Environmental information acquired from the study will be utilized by** the utility as a management tool for long term planning.

Lake Wylie, historically, has been characterized by excellent water quality and freedom from problems of eutrophication. However, development of the shoreline areas of Lake Wylie, such as on the eastern shores opposite the Catawba Nuclear Station site, is continuing at a rapid pace. Urbanization of lake shores and drainage basins has contributed to serious problems of cultural eutrophication and excessive aquatic growths in many other areas of the world, and has been recognized as a national water quality problem for several years in the United States (National Academy of Sciences, 1969; and American Society of Limnology and Oceanography, Inc. 1972).

This study was undertaken to better understand the nutrient relationships in Lake Wylie waters as related to problems of cultural eutrophication, seasonal changes in the algal growth potential, limiting nutrients, biological availability of the limiting nutrients, the water quality characteristics that are important in controlling the aquatic biota, and as a baseline for future detection of significant changes if they occur.

METHODS

Limiting Nutrient Bioassays

Sample Collection. Water samples for the nutritional bioassays were collected with a Van Dorn sampler on a quarterly basis in conjunction with a separate water chemistry study. Sampling locations are shown in Figure 1. All casts were simultaneous; one person collected separate water for an A composite sample and another person collected water for a B composite sample. Subsamples of composite A were employed for bioassays. Subsamples of both A and B were subjected to water chemistry analyses.

The samples were returned by air freight to the laboratory in 1 gallon polyethylene containers where they were stored under refrigeration in the dark until processing was initiated.

Sampling Pretreatment. For the limiting nutrient bioassays, approximately 3 liters of the well-mixed sample was placed in a 4-liter pyrex Erlenmeyer flask with a stirring bar and autoclaved using an

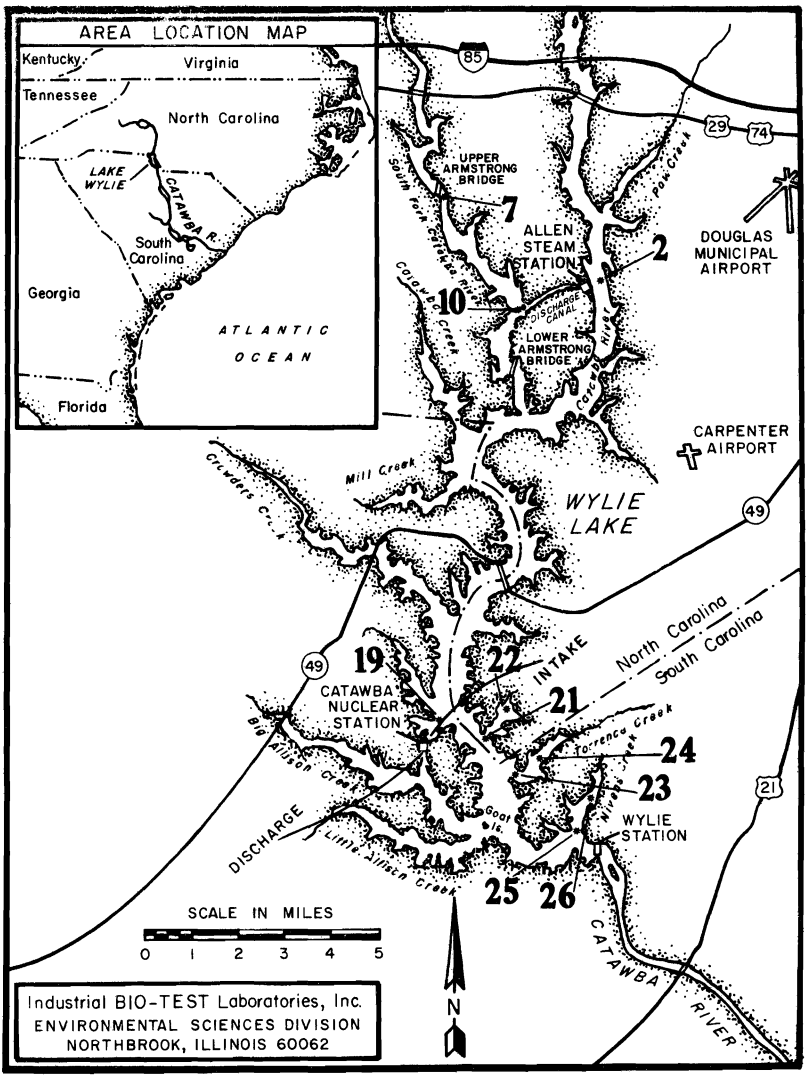

Figure 1. Sampling locations for aquatic nutrient bioassays, October 1973, January, April, and July 1974.

aluminum foil closure at 121°C for 15-20 minutes. Glass siphons were autoclaved separately.

The nutritional bioassays were conducted using 40 ml algal suspension volumes in 125 ml Erlenmeyer flasks as an option suggested by the algal assay procedure bottle test (AAP) (U.S. EPA, 1974). Closures were made of loosely fitted aluminum foil.

Prior to use in bioassays, all galss and lab ware contacting algae were cleaned in the following sequence: alkaline laboratory detergent solution soak for not less than 24 hours, brushed, 4-5 hot tap-water rinses, 1:1 hydrochloric acid rinse, six deionized water rinses, and two glass distilled water rinses.

Experimental Set-up Procedure. The pretreated sample was equilibrated to room temperature with magnetic stirring for 10-30 minutes and sterile CO_2-sparging to redissolve any materials that precipitated during autoclaving. A portion of the sample was then employed to rinse each flask prior to set-up. To increase statistical confidence in comparisons between blank and enriched flasks, five blank flasks were routinely employed. All other treatments had three flask replicates.

The agal cells for inoculation (*Selenastrum capricornutum*) were prepared from 4-7 day old AAP stock cultures. Cells were washed in bicarbonate solution and recovered by centrifugation according to AAP. The inoculation was delivered to the unsupplemented sample reservoir so as to avoid the time-consuming task of inoculating each flask individually.

Incubation. Illumination of all *Selenastrum* cultures was accomplished by means of General Electric F48T12CW high output cool white fluorescent lamps to provide roughly 400 foot candles (4300 lux).

Ambient fluid temperatures on the incubation shelves were estimated to 0.1C, five to seven times per week, with two or three mercury thermometers, the bulb of each immersed in separate 125 ml Erlenmeyer flasks.

These bioassay incubation temperatures were:

	Low	High	Mean
First Quarter Samples, Collected 8, 9 October 1973	22.5	29.6	26.0
Second Quarter Samples, Collected 7, 8 January 1974	19.4	25.8	23.4
Third Quarter Samples, Collected 8, 9 April 1974	24.3	27.7	25.7
Fourth Quarter Samples, Collected 8, 9 July 1974	25.1	28.3	27.4

Air over the flasks was circulated with a fan. Ambient air temperatures were recorded continuously on a circular chart to provide a check on general conditions.

Flasks were placed on the incubation shelves in a (non-formal) random fashion. On at least 5 (often 7) days per week, each flask was swirled to resuspend settled cells and the flask's shelf-position was changed to ensure that during incubation all flasks were exposed to nearly uniform temperatures and illumination.

Growth Monitoring. Absorbance of a sample from each flask at 750 nm in a 1-inch tube was determined as the index of biomass. On the 12th or 13th day of incubation, a few flasks representing the highest nutrient levels were selected for monitoring. These flasks were monitored again the following day to assure that the 24-hour growth increment was not greater than 5 percent and that peak cell production was attained. Absorbance readings were then taken on all flasks.

A composite was made of the five replicate flasks from the unsupplemented lake water (blanks). This composite was then measured for absorbance and for overnight oven dry weight (103 ± 2C) by an aluminum dish method. To ensure constant weights, deionized water was repeatedly evaporated from the aluminum dishes at oven temperature prior to use.

The relationship of absorbance to biomass was computed by correcting the final absorbances by the absorbance at day zero and correcting the final (composite) dry weight by the initial dry weight of the sample. The ratio, mg/l net composite dry weight divided by net absorbance due to algal growth, was applied to the final individual flask absorbances (corrected for day zero absorbance) to give individual estimated biomass values.

Biomass in the reference medium (AAP) cultures was estimated by applying an average factor determined for the lake water blanks by uncorrected absorbance values.

Data Interpretation. All data were processed by computer to give plots showing the algal biomass versus the treatment or location sampled. Bars were plotted around the data points to indicate the mean values and the 95 percent confidence range on the means. Examples of these computer plots are shown in Figure 2. When the biomass supported in the nitrogen (N) or phosphorus (P)-supplemented flasks was greater than the biomass supported in the unsupplemented (blank) flasks, N, P or Iron was identified as the limiting nutrient. Combined N and P supplements, in some cases, led to significant responses when little or no response to N or P

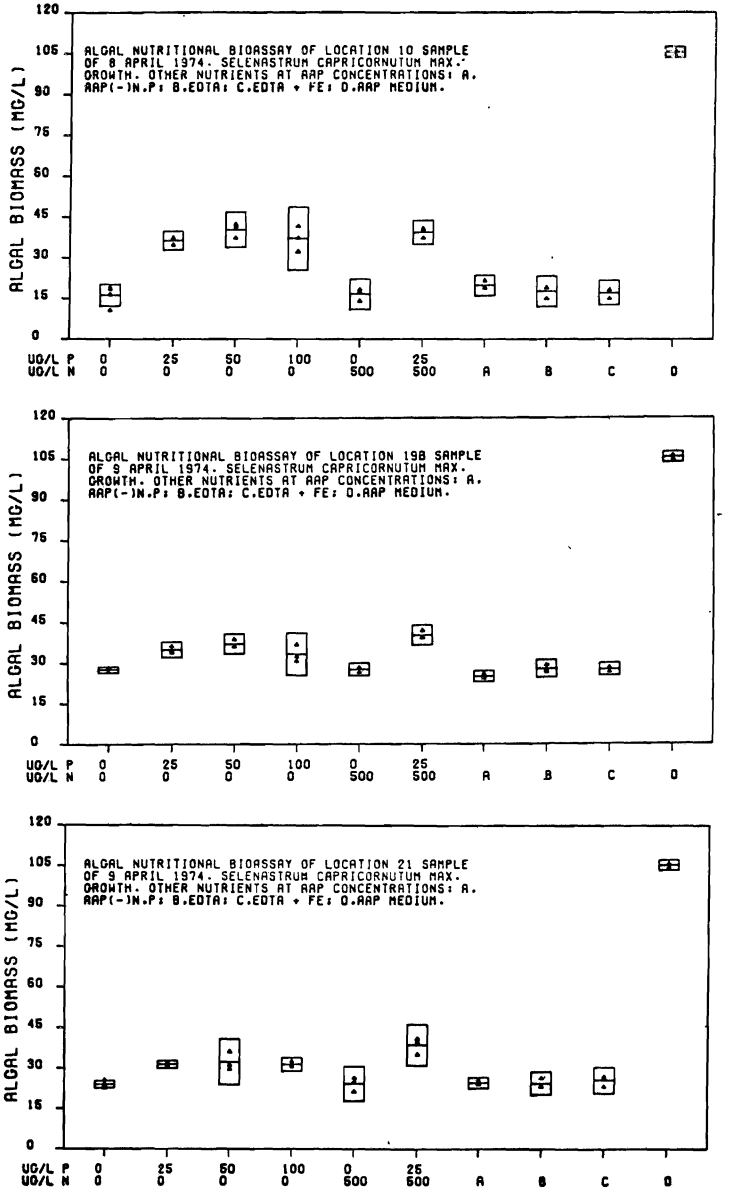

Figure 2. Examples of computer plots used in data interpretation.

was found. In this case, both N and P together were identified as limiting. Synthetic medium reference response curves were also plotted as a function of the concentration of the limiting nutrient, for both phosphorus and nitrogen. The computer-plotter also indicated the slope (both least squares and via the origin, if appropriate), intercept, and the standard error of estimate. Example nitrogen and phosphorus response curves are shown in Figure 3. One section of the program's plotter also was employed to compare the biomass determined for the unenriched sample to show the relative algal growth supported. Further, the computer program separately printed out the raw data in tabular form with sample location and bioassay treatments indicated. In all cases, the least squares slope was reported, but when the fitted curve did not pass through the origin (despite the absence of growth in the zero-P or zero-N spiked reference medium) a new slope was computed to force the fitted curve through the origin and the reference medium equivalent of available nutrient was computed.

In some cases, a positive intercept was found suggesting the presence of background or carryover of N or P and this intercept was employed in the calculations.

When nitrogen (N), phosphorus (P) or a combination of the two was identified as the limiting nutrient in the sample, the available concentration of N or P in the sample was related to the amount of growth achieved by the same culture in the N- or P-limited reference medium, and a reference medium equivalent amount of N or P was computed.

As an indication of goodness of fit of the reference medium curve the standard error of estimate (mg algae) was computed. Confidence limits on the means were computed at (a) 0.05.

Experimental Design and Controls. The enrichment routine employed for most of the bioassays is illustrated on the bottom scale of Figure 2. Phosphorus (P) was added as K_2HPO_4 and nitrogen (N) as $NaNO_3$. Treatment A was the addition of AAP nutrients (except N and P) to determine if any other essential element was limiting. Treatment B was the addition of ethylene diamine tetraacetic acid (EDTA) at the AAP concentration. This chelant, in artificial cultures, is employed to control heavy metal toxicity (example copper) and to keep essential metals, such as iron, available to growing cultures; and is employed in AAP to simulate natural chelants important to algal growth. Treatment C was the addition of EDTA and iron as $FeCl_3$ at AAP concentrations. Treatment D was the AAP reference standard culture medium.

Ancillary Experiments

The main objective of the ancillary laboratory experiments was to determine the effect of pH on the early growth responses of blue-green

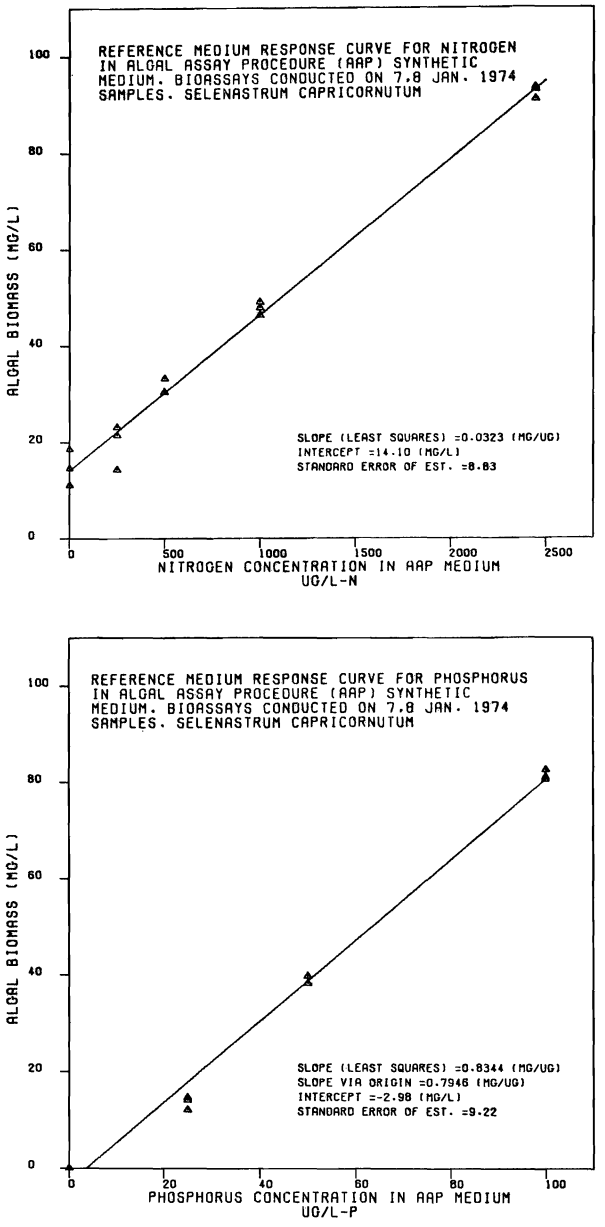

Figure 3. Example nitrogen and phosphorus response curves.

algae, especially the potential nuisance species, *Microcystis aeruginosa.* The two AAP test organisms, *Selenastrum capricornutum* and *Anabaena flos-aquae,* were compared in the third experiment and *Selenastrum* was compared with *Microcystis* in the second experiment. The basic methods employed are shown in Table 1. Further details follow.

First Experiment. A pellicle from an old *Microcystis aeruginosa* culture was mag-stirred in its overlying medium for several hours in an attempt to break loose and suspend individual cells. This culture was apparently nonviable as judged by its lack of growth in previous AAP transfers. A clear suspension from this preparation was employed as the inoculum for seven pH-adjusted AAP medium subsamples and each subsample was again divided among three replicate flasks for incubation. The inoculation resulted in 1000-2000 *Microcystis* cells per ml.

Second Experiment. Using Lake Wylie water, both inoculum cell density and initial pH were varied using *Microcystis.* Since the experiment was considered an exploratory test, no flask replication was employed. *Selenastrum* was similarly compared, but with this green algae species only pH was varied. The pH was adjusted upward from the natural composite pH, 6.8. The bicarbonate-washed inoculum for both *Microcystis* and *Selenastrum* was made from 6 day AAP cultures. Following inoculation, the flasks were stored at 5°C in darkness until the incubation was started 3 days later. Growth of the cultures was followed daily by the *in vivo* fluorescence of chlorophyll *a* (Lorenzen, 1966). Uninoculated controls consisting of light and dark-incubated flasks were employed to correct for the natural background fluorescence of the untreated Lake Wylie composite sample. Non-destructive sampling was employed. The objective was to determine the early growth responses of the cultures, especially the lag phase, long before visible greening occurred, and before the growing cultures could markedly affect their own culture (pH) environment, especially the CO_2 - carbonate equilibria.

Third Experiment. The third experiment, also conducted using Lake Wylie water, focused primarily on the growth rate (μ). A comparison was made between *Selenastrum* inoculated at 1,000 cells per ml, and *Microcystis* and *Anabaena flos-aquae,* each inoculated at 50,000 cells per ml from a 7 day old AAP culture. The higher cell densities for the blue-green species were employed to overcome lag. *Anabaena* is a nitrogen-fixing blue-green alga. For this experiment neither cell washing nor continuous skaking was employed. Uninoculated light and dark control flasks at the natural initial pH were again employed to correct for background Lake Wylie composite sample relative-fluorescence values. Three replicate flasks were employed for each sample treatment. The incubation was continued with daily observations for only 7 days, but beyond the maximum specific log growth rate (μ max) phase, and before visible

Table 1. Comparison of basic experimental methods employed in the ancillary experiments.

Methodology	Experiment No. 1	Experiment No. 2	Experiment No. 3	Experiment No. 4
Culture flasks - volume - type - brand	125 ml Erlenmeyer Kimax	125 ml Erlenmeyer Kimax	125 ml Erlenmeyer Kimax	125 ml Erlenmeyer Kimax
Culture volume	40 ml	40 ml	40 ml	40 ml
Flask closures	Aluminum foil	Aluminum foil	Aluminum foil	Aluminum foil
Illumination quality - type - manufacturer - other	Fluorescent General Electric Cool white	Fluorescent General Electric Cool white	Fluorescent Duro-Test Optima Rapid start	Fluorescent General Electric Cool white
Illumination intensity - lux - foot candles	2150 200	2150 200	2150 200	2150 200
Culture medium	Algal Assay Procedure Medium	Equal-volume composite, 10 Lake Wylie samples of 7, 8 January 1974	Equal-volume composite, 10 Lake Wylie samples of 7, 8 January 1974	Equal-volume composite, 11 Lake Wylie samples of 8, 9 April 1974
pH adjustment	HCl, NaOH	HCl	Sat. Sod. Carbonate Soln.	Near-Sat. Sod. Carbonate Soln.
Incubation culture mixing	Occasional cell resuspension	Continuous, 136 rpm, Gyrotary shaker	Daily resuspension of cells	Continuous, 136 rpm, Gyrotary shaker
Inoculum treatment	None	Washed, resuspended in Sod. bicarbonate soln.	None	Washed, resuspended in Sod. bicarbonate soln.
Inoculation timing	Immediate	Friday to Monday with 5 C dark storage	Immediate	Immediate
Growth monitoring	Gross observation plus microscopic	Fluorescence of chlorophyll *a* and microscopic	Fluorescence of chlorophyll *a* and microscopic	Fluorescence of chlorophyll *a*, Dry Wt., microscopic
Dates	16 July to 21 August 1973	22 February to 15 Mar 1974	8 April to 15 April 1974	9 July to 24 July 1974
Incubation - mean temperature - temperature range	24 C 23.7-24.5 C	22.4 C 18.8-26.7 C	21.7 C 19.3-23.7 C	26.4 C 24.8-27.7 C

greening occurred. The specific growth rate (μ) was calculated as the difference between the natural logarithms of the initial and final relative-fluorescence of chlorophyll *a* divided by the time interval.

Fourth Experiment. In the fourth experiment, Lake Wylie water at pH 7.1 was split into two subsamples. One subsample was adjusted to pH 10 and both were each then split into three parts which were (a) left untreated, (b) supplemented with single-strength AAP nutrients, and (c) supplemented with double-strength AAP nutrients. In this experiment the inoculum was calculated to result in 25,000 *Microcystis* cells per ml. Observations were recorded daily.

Water Quality Analyses and Field Biology/Taxonomy

The methods employed for the collection, preservation, enumeration, and identification of periphytic and planktonic algae reported in conjunction with this study as well as field and laboratory chemical analytical methods are published elsewhere in detail (Industrial BIO-TEST Laboratories, Inc., 1974).

RESULTS AND DISCUSSION

Limiting Nutrients

Overall, Lake Wylie waters were phosphorus-limited. Of the 40 samples in which limiting nutrients were identified, phosphorus was involved in 38 of them (95 percent). In 29 of these samples (72.5 percent) phosphorus alone was identified as limiting. No identification of a limiting nutrient was possible at one location because of rainfall and turbidity problems resulting in high background absorbances which either interfered with or masked algal growth (location number 7, South Fork, 8 July 1974). At four of the nine locations where multiple or combined limiting nutrients were identified, nitrogen was involved and nitrogen alone was identified as the limiting nutrient in the January 1974 sample at the single bottom station (19B) included in the series. The need for chelation of metals as indicated by a response to EDTA was critical in the location 19, 9 April 1974 surface sample, and iron as a multiple or combined limiting nutrient was apparently critical at six of ten 7-8 January 1974 stations studied (Table 2). The basic growth response data are presented in Tables 3-6. New research suggesting that increased iron levels may induce shifts from green to blue-green algal dominance (Morton and Lee, 1974) combined with the finding in this study that iron was critical in a few of the samples, indicates that the biological and chemical role of iron in Lake Wylie waters is worthy of more than cursory attention.

Table 2. Summary of nutrients identified as limiting in Lake Wylie waters.

Date of Sample Collection	Location										
	2	7	10	19	19B	21	22	23	24	25	26
8, 9 October 1973	P	a	P&N	P	P	a	P	P	P	P	P
7, 8 January 1974	P	a	P	P,Fe	N	P,(Fe?)	Fe,(N&P)	P,Fe	P,Fe	P&N(Fe?)	P
8, 9 April 1974	P	P,N	P	P,Chel. Ability	P	P	P	P	P	P	P
8, 9 July 1974	P	?	P(?)	P	P	P	P	P	P	P	P

[a]Sample not collected.

Table 3. Biomass determined for each bioassay flask in bioassay of Lake Wylie samples collected 8-9 October 1973 in milligrams per liter. Treatment A, algal assay procedure (AAP) medium nutrients except nitrogen (N) and phosphorus (P); B, AAP medium.

Treatment	Location								
	1	10	19	19B	22	23	24	25	26
0 μg/l P + 0 μg/l N	1.1	22.0	7.8	6.4	1.2	4.3	3.9	0.8	5.3
	0.9	23.3	6.0	4.3	1.6	5.2	2.5	8.0	2.0
	-0.3	22.9	7.5	5.7	1.9	4.8	3.6	8.8	2.7
	0.3	16.0	6.0	5.4	1.9	4.5	3.6	8.8	4.0
	0.9	26.3	6.0	6.4	2.4	5.2	3.9	0.8	2.7
50 μg/l P + 0 μg/l N	3.1	29.4	15.3	20.7	6.8	11.0	15.0	32.0	21.3
	5.4	22.9	12.6	18.6	5.9	11.2	12.8	37.6	32.7
	5.6	22.5	13.5	20.4	6.9	13.8	12.2	36.0	27.3
0 μg/l P + 500 μg/l N	0.9	22.0	5.1	6.4	1.5	4.8	2.5	8.8	3.3
	0.4	15.1	6.3	4.3	1.6	2.6	1.7	10.4	2.7
	1.1	22.0	6.6	4.3	1.7	3.8	2.2	8.8	3.3
50 μg/l P + 500 μg/l N	13.1	47.1	21.6	34.6	11.1	25.5	22.5	65.6	46.0
	11.0	51.4	15.0	35.0	11.1	21.4	20.6	64.0	56.0
	12.3	49.2	16.8	35.0	10.0	22.9	17.2	67.2	46.0
A	1.7	25.5	5.1	6.1	1.1	5.2	1.4	10.4	2.7
	1.1	23.3	6.6	7.9	1.6	3.8	2.2	8.0	3.3
	0.9	22.5	6.9	6.8	1.7	3.8	1.9	8.8	2.7
B				145.1					
				141.3					
				145.8					

Table 4. Biomass determined for each bioassay flask in bioassay of Lake Wylie samples collected 7-8 January 1974 in milligrams per liter. Treatment A, algal assay procedure medium (AAP) nutrients except nitrogen (N) and phosphorus (P); ethylene diamine tetraacetic acid (EDTA); C, EDTA plus iron; D, AAP medium.

Treatment	Location									
	2	10	19	19B	21	22	23	24	25	26
0 µg/l P + 0 µg/l N	3.0	16.3	19.2	27.9	24.8	26.6	16.4	21.3	26.7	17.4
	2.3	17.3	21.2	25.5	18.8	25.8	19.3	21.6	23.3	20.4
	3.8	16.5	25.9	26.0	24.3	25.2	20.6	25.5	24.5	18.3
	2.7	18.7	18.9	27.0	24.3	24.2	14.8	24.2	26.7	20.4
	3.4	16.7	20.4	29.1	26.6	20.4	19.1	22.9	24.0	20.4
50 µg/l P + 0 µg/l N	30.5	29.3	32.1	35.2	30.8	38.4	22.8	33.4	32.9	21.6
	27.4	31.8	31.1	33.0	31.3	35.2	22.2	33.9	25.0	26.9
	44.6	31.8	27.9	29.1	30.3	30.3	27.1	28.1	28.1	25.6
100 µg/l P + 0 µg/l N	73.2	43.4	39.3	37.6	37.6	25.2	22.8	32.1	35.8	32.1
	28.6	36.5	38.3	44.2	30.3	31.2	23.4	32.1	31.5	30.7
	35.4	47.9	39.3	27.9	31.3	30.3	31.9	39.2	31.5	37.4
150 µg/l P + 0 µg/l N	34.3	39.1	42.1	31.3	31.1	45.9	25.3	32.6	21.1	26.9
	24.8	34.2	49.3	31.6	35.2	30.6	28.6	33.7	19.5	24.6
	46.9	38.7	37.8	36.9	29.8	29.5	26.9	33.1	24.5	26.9
0 µg/l P + 500 µg/l N	3.0	15.7	25.4	35.2	28.2	31.2	21.2	26.8	28.1	22.9
	3.8	15.7	22.9	31.3	30.0	31.7	17.7	30.0	29.3	20.8
	2.3	14.1	20.2	32.8	21.4	22.3	17.7	28.7	34.6	21.9
50 µg/l P + 500 µg/l N	43.8	42.0	41.1	42.5	47.5	47.3	31.0	40.5	47.3	35.3
	28.6	39.3	42.1	47.8	43.3	44.6	28.0	40.5	45.4	33.8
	47.3	34.2	38.3	41.3	38.1	38.4	27.4	42.9	43.2	35.9
A	3.8	4.1	30.9	32.8	29.2	29.8	22.2	32.1	30.0	20.8
	2.7	4.1	29.1	30.4	30.8	29.8	22.0	32.9	33.4	20.4
	3.8	5.1	31.6	28.4	31.3	37.9	22.2	32.6	32.7	19.8
B	4.6	7.7	19.7	27.4	28.4	29.3	17.1	24.2	28.1	20.8
	3.8	5.1	25.4	30.4	23.5	32.0	18.9	25.5	29.8	21.0
	3.0	9.6	20.2	33.0	23.5	22.8	19.7	26.0	24.5	20.8
C	3.0	2.4	31.1	41.0	37.3	37.3	26.1	34.7	28.3	18.9
	3.8	5.7	34.6	29.9	32.6	36.5	26.7	36.5	33.9	20.6
	3.8	7.7	33.9	32.1	29.5	35.7	27.1	32.1	35.3	20.8
D				114.4						
				116.2						
				115.7						

The experimental design employed for bioassay of the January, April, and July samples of 1974 included the use of multiple phosphorus additions. This design permitted not only the identification of phosphorus as a limiting nutrient, but also suggests the point at which a further increase in available phosphorus concentration will no longer result in increased algal growth.

This is also considered as the phosphorus concentration above which growth becomes limited by another nutrient, or similarly, the phosphorus-demand of the test algae. In the January 1974 experimental series, phosphorus additions of 50, 100, and 150 µg/l phosphorus were employed. The growth responses to these additions suggest that, overall, the

phosphorus demand was satisfied at 50 μg/l or less added phosphorus. In the April and July experimental series, the added phosphorus quantities were reduced to better define the concentration range of the phosphorus limitation. Accordingly, 25, 50, and 100 μg/l phosphorus additions were employed. In both the final series, and with only few exceptions, the data suggest that the phosphorus demand was satisfied in Lake Wylie waters at or below 25 μg/l added available phosphorus. The growth responses suggest that if this mean increase in available phosphorus level is ever achieved in Lake Wylie, nitrogen would doubtless become the nutrient of primary critical concern. On average, this would require an approximate doubling of the mean annual available phosphorus concentration. Such increased phosphorus levels are not anticipated and probably will never

Table 5. Biomass determined for each bioassay flask in bioassay of Lake Wylie samples collected 8-9 April 1974 in milligrams per liter. Treatment A, Algal Assay Procedure medium (AAP) nutrients except nitrogen (N) phosphorus (P); B, ethylene diamine tetraacetic acid (EDTA); C, EDTA plus iron; D, AAP medium.

Treatment	Location										
	2	7	10	19	19B	21	22	23	24	25	26
0 μg/l P + 0 μg/l N	3.3	26.2	16.4	21.8	28.2	22.8	20.6	27.3	24.1	26.6	23.5
	3.5	30.6	16.7	19.5	26.6	24.2	21.1	27.3	22.8	26.6	18.9
	2.8	29.9	19.0	22.4	28.2	25.6	21.4	29.0	22.8	25.6	18.3
	3.2	26.8	10.7	22.4	28.2	23.6	20.3	27.3	23.6	25.0	19.8
	3.0	26.8	18.4	23.9	26.6	23.6	21.7	27.3	22.0	27.5	19.2
25 μg/l P + 0 μg/l N	10.0	32.0	36.7	32.0	36.2	32.1	28.8	30.7	33.5	35.1	27.8
	10.1	30.6	37.4	32.6	34.0	31.2	32.1	40.9	30.8	33.5	29.8
	9.5	32.5	34.7	36.1	34.6	31.0	30.2	37.9	29.5	31.3	28.4
50 μg/l P + 0 μg/l N	11.3	33.9	37.4	44.2	36.2	36.3	30.2	35.3	30.0	39.2	27.8
	11.3	31.6	41.4	39.9	38.8	31.2	33.0	30.0	34.9	38.6	28.4
	12.9	32.5	42.4	39.3	36.2	29.8	32.1	34.1	28.2	37.7	31.2
100 μg/l P + 0 μg/l N	14.4	30.4	41.7	31.1	36.9	32.7	28.0	35.8	26.6	38.6	32.1
	14.4	32.7	32.4	31.1	32.4	31.2	31.6	30.0	29 5	34.5	31.2
	14.2	33.5	37.4	36.1	30.8	30.7	30.2	32.4	29.5	40.8	34.1
0 μg/l P + 500 μg/l N	3.3	32.7	17.4	25.3	28.2	25.1	19.2	30.0	20.1	24.7	22.1
	3.1	29.5	14.0	27.4	26.6	21.4	20.6	27.3	25.8	27.5	20.6
	3.2	32.0	18.4	25.3	28.2	26.5	20.9	28.3	22.0	28.2	21.2
25 μg/l P + 500 μg/l N	10.8	34.6	40.1	39.9	39.4	41.1	35.7	37.5	32.2	45.3	41.3
	11.0	37.9	40.7	38.4	39.4	35.2	34.3	45.4	34.3	42.4	36.1
	10.8	34.6	37.4	39.0	42.0	39.7	35.7	42.7	31.7	45.6	37.0
A	3.4	27.9	19.0	25.3	24.4	25.6	22.0	26.3	23.1	27.5	21.2
	2.9	26.2	19.0	26.8	26.0	24.2	20.3	28.3	24.1	28.2	19.8
	3.3	46.7	21.7	26.2	25.0	24.2	22.0	25.6	22.0	28.2	21.2
B	2.8	23.7	19.0	29.7	29.2	23.4	22.5	30.0	24.1	27.2	22.1
	3.4	24.3	15.0	26.8	26.6	26.5	21.7	27.3	22.3	24.4	21.8
	3.4	24.7	19.0	28.2	27.6	23.6	20.9	23.9	24.1	27.2	21.2
C	3.0	27.9	15.0	25.3	26.6	23.4	22.0	30.0	25.5	30.7	22.1
	2.8	24.7	18.0	25.3	28.2	27.0	23.1	29.7	23.3	26.3	21.2
	3.2	27.9	18.4	26.8	28.2	26.5	20.3	31.4	23.6	27.8	22.6
D					106.0						
					104.6						
					106.0						

occur without some unforeseen catastrophic event in the Lake Wylie watershed, such as the introduction of increased sewage or industrial waste effluents in untreated form. Thus, any future nutrient-input control program could safely focus on phosphorus alone as the critical nutrient.

Biological Availability of Limiting Nutrients

The biologically available quantities of phosphorus, when it was identified as the limiting nutrient, are presented in Table 7 and the statistics employed in computation of the available concentrations are presented in Table 8. Examination of Table 7 reveals that with the

Table 6. Biomass determined for each bioassay flask in bioassay of Lake Wylie samples collected 8-9 July 1974 in milligrams per liter. Treatment A, algal assay procedure medium (AAP) nutrients except nitrogen (N) and phosphorus (P); B, ethylene diamine tetraacetic acid (EDTA); C, EDTA plus iron; D, AAP medium.

Treatment	Location										
	2	7	10	19	19B	21	22	23	24	25	26
0 µg/l P + 0 µg/l N	14.7		6.8	13.9	10.6	10.7	15.9	10.3	28.4	6.2	7.6
	17.4		5.7	13.5	11.1	10.3	15.9	9.4	22.1	15.5	6.0
	16.2	12.4	10.9	12.2	10.6	16.7	13.8	9.4	28.8	21.7	5.2
	16.8			13.5	10.8	17.5	9.5	7.5		9.3	5.2
	15.9	4.1		10.0	10.1	16.7	18.0	9.4		27.9	6.4
25 µg/l P + 0 µg/l N	26.7			29.2	15.8	32.1	23.3	16.0	15.8	31.0	16.4
	28.5		14.6	31.8	15.0	30.6	21.2	12.2	16.6	15.5	18.0
	18.3		4.2	34.0	17.4	26.6	23.3	16.9	16.2	21.7	28.0
50 µg/l P + 0 µg/l N	22.8			45.7	19.4	34.1	24.4	16.9	38.3	34.1	26.4
	24.3		4.2	40.5	18.6	34.9	17.0	38.5	31.6	74.4	29.2
	34.2		30.2	29.6	15.3	35.7	26.5	16.9	33.9	89.9	30.8
100 µg/l P + 0 µg/l N	34.8		1.6	40.1	18.9	33.3	28.6	18.8	39.5	58.9	36.0
	27.9		14.6	29.6	16.6	30.2	25.4	43.2	31.6	58.9	27.2
	11.4		19.8	38.3	18.6	26.2	23.3	59.2	27.6	145.7	26.0
0 µg/l P + 500 µg/l N	18.0			12.2	8.0	8.3	12.7	9.4	15.0	3.1	4.0
	17.7			11.3	9.3	5.6	9.5	5.6	11.8	0.0	8.4
	17.4			12.2	9.6	7.5	10.6	5.6	15.0	3.1	2.4
25 µg/l P + 500 µg/l N	38.1		25.0	42.7	21.0	36.5	23.3	19.7	48.2	15.5	37.2
	34.5	4.1	33.8	37.0	19.7	37.3	20.1	31.0	40.7	21.7	33.2
	36.0	53.9		40.5	19.9	40.1	26.5	19.7	45.8	83.7	36.4
A	16.5			14.4	10.6	10.3	7.4	10.3	12.6	0.0	6.4
	14.7			11.3	9.9	9.5	11.7	9.4	14.2	9.3	20.4
	13.2			11.3	9.6	12.3	7.4	9.4	11.8	15.5	7.2
B	16.8			7.8	9.9	6.3	11.7	15.0	16.6	0.0	5.2
	14.7			9.1	9.8	7.5	10.6	15.0	13.0	15.5	5.2
	16.2			5.7	9.0	7.5	14.8	21.6	15.8	6.2	5.2
C	17.7			7.8	10.6	13.9	9.5	9.4	13.8	0.0	4.4
	13.8			7.8	11.7	8.3	12.7	12.2	12.6	-3.0	5.6
	14.1			10.0	10.9	8.7	17.0	11.3	15.8	3.1	6.4
D					275.6						
					278.7						
					284.9						

Table 7. Biologically available concentrations of phosphorus as reference medium equivalent concentrations at those locations where phosphorus was identified as limiting (g/l).

Date of Sample Collection	Location										
	2	7	10	19	19B	21	22	23	24	25	25
8, 9 October 1973	9	a	40	18	16	a	11	15	13	16	13
7, 8 January 1974	4	a	22	27	c	30	31	23	30	31	24
8, 9 April 1974	4	35	20	27	34	30	26	34	28	32	25
8, 9 July 1974	8	b	4	6	5	7	7	4	13	8	3

[a]Sample not collected.

[b]Extreme background turbidity interfered with bioassay.

[c]Phosphorus not among nutrients identified as limiting.

Table 8. Statistics derived for AAP reference response curves for phosphorus and nitrogen.

Sample Date	Phosphorus Response Curves				Nitrogen Response Curves			
	Slope (mg/μg)		Intercept (mg/l)	Standard Error of Estimate	Slope (mg/μg)		Intercept (mg/l)	Standard Error of Estimate
	Least Squares	Via Origin			Least Squares	Via Origin		
8, 9 October 1973	0 .886[a]	N.C.[b]	(-)7.34	66.15	0.040	N.C.	(+) 4.64	7.40
7, 8 January 1974	0.834	0.795	(-)2.98	9.22	0.032	N.C.	(+)14.1	8.83
8, 9 April 1974	0.797	0.809	(+)0.90	1.57	0.036	N.C.	(+)22.72	6.61
8, 9 July 1974	2.048	2.054	(+)0.47	17.54	0.087	N.C.	(+)67.24	78.16

[a]Underlined slope values were employed in calculation of available quantities (Table 7).

[b]N.C. = Not calculated (not applicable).

exception of location 2, the only sample site on the Catawba River, the higher available phosphorus concentrations occurred during the high lake flow months of January and April 1974. The data suggest that higher flows, probably resulting from greater runoff may have diluted the available phosphorus at location 2 in the Catawba River water studied. The clearest (least turbid) samples throughout the study were those from location 2, the most turbid from the South Fork location 7 samples.

Since much less effort is involved in the chemical analytical determinations of phosphorus than in determination of the biologically available phosphorus, the biological and chemical availability data were compared to determine which chemical form of phosphorus, if any, was the least biased (slope = 1) estimator of the available portion in the Lake Wylie waters. These comparisons are presented in Table 9 as percentages. The corresponding chemically determined nutrient concentrations are tabulated by sample date and location in Tables 10-13. The soluble orthophosphate determination was the least biased of all sampling periods and of all three phosphorus determinations in the 7-8 January sample-set, but extreme bias was exhibited in all other comparisons. It is not surprising that the total phosphorus determination grossly overestimated the available portion since the severe digestion in this method is required to recover the bound phosphorus in organic and insoluble forms. With several exceptions, the soluble forms of phosphorus underestimated the available portion. This relationship is explained logically in that the algae normally derive their phosphorus requirements from the readily soluble forms and from organic phosphorus recycled from other organisms and from inorganic phosphorus fractions of particulate matter (Lean, 1973).

It has been demonstrated that algae are capable of deriving phosphorus as sole source from some of the most insoluble compounds known to man (Gerhold and Thompson, 1969; Fitzgerald, 1970). It has also been demonstrated that similar principles also apply to the availability of nitrogen forms when nitrogen is the limiting nutrient (Gerhold, 1974).

To determine which chemical determination was the best estimator of the biologically available portion without regard to bias (slope $\neq$ 1), a correlation analysis was performed comparing the biologically available concentration with the three different phosphorus analyses. First, all the data without regard to season were compared and indicated that soluble orthophosphate correlated best (correlation coefficient 0.814). However, when the four sampling periods were considered separately the best correlated (underlined) estimators of the biologically available portion were as follows:

Table 9. Biologically available phosphorus concentrations in Lake Wylie waters as a percent of three chemically determined phosphorus forms (biological quantity x 100 ÷ chemical quantity).

Day Month Year	Chemical Phosphorus Form	Location										
		2	7	10	19	19B	21	22	23	24	25	26
8, 9	Total	30	a	57	45	32	a	28	30	26	27	26
October	Total soluble	128	a	154	45	61	a	122	115	81	145	162
1973	Soluble ortho	128	a	210	133	400	a	550	375	433	350	650
7, 8	Total	12	a	34	33	d	47	53	34	50	56	52
January	Total soluble	19	a	65	79	d	70	74	55	68	67	83
1974	Soluble ortho	100	a	95	87	d	100	100	100	100	97	100
8, 9	Total	9	25	29	32	21	21	14	26	23	32	25
April	Total soluble	57	194	142	90	126	130	100	104	112	114	109
1974	Soluble ortho	133	350	333	169	179	214	144	154	127	114	250
8, 9	Total	24	b	6[c]	19	4	39	4	4	12	8	7
July	Total soluble	158	b	32[c]	87	37	100	142	90	256	130	38
1974	Soluble ortho	198	b	54[c]	203	173	350	1100	225	427	390	100

[a]Sample not collected.

[b]Extreme turbidity interfered with bioassay.

[c]Data may be questionable because of high turbidity component of the sample from South Fork influence.

[d]Nitrogen was limiting.

Table 10. Baseline aquatic nutrient levels measured in Lake Wylie water samples collected 8-9 October 1973.

Parameters	Rep	Sampling Locations									
		2 TOP	10 TOP	19 TOP	19 BOT	21 TOP	22 TOP	23 TOP	24 TOP	25 TOP	26 TOP
Ammonia	A	0.06	0.09	0.12	0.12	0.11	0.11	0.11	0.10	0.10	0.08
(mg/l-N)	B	0.07	0.08	0.12	0.12	0.11	0.10	0.10	0.10	0.11	0.08
Nitrate	A	0.03	0.15	0.03	0.05	0.06	0.05	0.06	0.06	0.03	0.02
(mg/l-N)	B	0.03	0.13	0.04	0.06	0.07	0.08	0.08	0.06	0.04	0.06
Nitrite	A	0.0023	0.0068	0.0029	0.0030	0.0034	0.0032	0.0038	0.0030	0.0030	0.0028
(mg/l-N)	B	0.0026	0.0068	0.0028	0.0028	0.0032	0.0034	0.0036	0.0028	0.0030	0.0027
Organic nitrogen, total	A	0.33	0.39	0.34	0.15	0.36	0.35	0.31	0.33	0.25	0.33
(mg/l-N)	B	0.33	0.33	0.16	0.23	0.33	0.36	0.33	0.29	0.28	0.30
Orthophosphate, soluble	A	0.007	0.019	0.012	0.004	0.003	0.002	0.004	0.003	0.004	0.002
(mg/l-P)	B	0.007	0.019	0.011	0.004	0.003	0.002	0.004	0.003	0.004	0.002
Phosphorus, total	A	0.03	0.07	0.04	0.05	0.07	0.04	0.05	0.05	0.06	0.05
(mg/l-P)	B	0.04	0.06	0.03	0.04	0.04	0.03	0.07	0.03	0.07	0.04
Phosphorus, total soluble	A	0.007	0.026	0.012	0.026	0.017	0.009	0.013	0.016	0.011	0.008
(mg/l-P)	B	0.014	0.026	0.011	0.033	0.012	0.014	0.021	0.008	0.027	0.006
Silica, soluble	A	8.6	9.4	9.6	9.6	9.7	9.4	9.6	9.5	9.5	9.4
(mg/l-SiO_2)	B	8.7	9.5	9.6	9.5	9.6	9.4	9.6	9.5	9.4	9.4
Alkalinity, total	A	13	14	13	14	14	12	13	12	13	12
(mg/l-Ca CO_3)	B	14	14	14	15	14	12	13	12	13	11
pH		6.2	6.3	6.5	6.3	6.3	6.2	6.3	6.2	6.3	6.4

Table 11. Baseline aquatic nutrient levels measured in Lake Wylie water samples collected 7-8 January 1974.

Parameters	Rep	Sampling Locations									
		2 TOP	10 TOP	19 TOP	19 BOT	21 TOP	22 TOP	23 TOP	24 TOP	25 TOP	26 TOP
Ammonia	A	0.03	0.07	0.13	0.17	0.14	0.14	0.14	0.18	0.14	0.12
(mg/l-N)	B	0.03	0.07	-	0.17	0.14	0.14	0.14	0.20	0.13	0.12
Nitrate	A	0.23	0.37	0.30	0.26	0.29	0.24	0.29	0.30	0.25	0.18
(mg/l-N)	B	0.22	0.41	0.33	0.27	0.29	0.25	0.27	0.27	0.26	0.15
Nitrite	A	0.0029	0.0038	0.013	0.013	0.013	0.015	0.014	0.014	0.016	0.014
(mg/l-N)	B	0.0035	0.0045	0.014	0.014	0.014	0.014	0.015	0.015	0.016	0.013
Inorganic nitrogen[a]	A	0.263	0.444	0.443	0.443	0.443	0.395	0.444	0.494	0.406	0.314
(mg/l-N)	B	0.254	0.484	-	0.454	0.444	0.404	0.425	0.485	0.406	0.283
Organic nitrogen, total	A	0.35	0.26	0.20	0.22	0.20	0.17	0.20	0.21	0.15	0.21
(mg/l-N)	B	0.31	0.20	0.23	0.21	0.20	0.20	0.18	0.19	0.18	0.21
Orthophosphate, soluble	A	0.004	0.022	0.030	0.024	0.021	0.029	0.023	0.020	0.032	0.021
(mg/l-P)	B	0.007	0.022	0.029	0.024	0.022	0.026	0.023	0.017	0.039	0.025
Phosphorus, total	A	0.033	0.061	0.078	0.071	0.064	0.058	0.067	0.060	0.055	0.046
(mg/l-P)	B	0.031	0.062	0.078	0.058	0.066	0.064	0.067	0.058	0.055	0.042
Phosphorus, total soluble	A	0.021	0.034	0.034	0.039	0.043	0.042	0.042	0.044	0.046	0.029
(mg/l-P)	B	0.017	0.034	0.042	0.042	0.039	0.039	0.040	0.045	0.044	0.024
Silica, soluble	A	7.4	12	15	14	14	14	14	14	14	15
(mg/l-SiO_2)	B	12	12	12	14	14	14	14	14	11	15
Alkalinity, total	A	13	13	13	14	13	13	13	13	14	14
(mg/l-$CaCO_3$)	B	13	14	12	13	13	13	13	14	14	14
pH		6.5	6.3	6.5	6.5	6.5	6.5	6.5	6.5	6.6	6.5

[a]Inorganic nitrogen is the sum of the ammonia, nitrate and nitrite forms.

Table 12. Baseline aquatic nutrient levels measured in Lake Wylie water samples collected 8-9 April 1974.

Parameters	Rep	Sampling Locations										
		2 TOP	7 TOP	10 TOP	19 TOP	19 BOT	21 TOP	22 TOP	23 TOP	24 TOP	25 TOP	26 TOP
Ammonia	A	0.03	0.06	0.04	0.08	0.09	0.09	0.07	0.08	0.08	0.07	0.07
(mg/l-N)	B	0.03	0.06	0.04	0.08	0.09	0.09	0.07	0.08	0.08	0.07	0.06
Nitrate	A	0.27	0.41	0.27	0.46	0.51	0.42	0.47	0.42	0.44	0.36	0.33
(mg/l-N)	B	0.22	0.40	0.29	0.48	0.37	0.45	0.38	0.47	0.43	0.44	0.33
Nitrite	A	0.0034	0.011	0.0040	0.0075	0.0083	0.0085	0.0080	0.0089	0.0083	0.0080	0.0063
(mg/l-N)	B	0.0032	0.012	0.0060	0.0083	0.0085	0.0089	0.0080	0.0083	0.0081	0.0080	0.0065
Inorganic nitrogen[a]	A	0.303	0.481	0.314	0.548	0.608	0.518	0.548	0.509	0.528	0.438	0.406
(mg/l-N)	B	0.253	0.472	0.336	0.568	0.468	0.549	0.458	0.558	0.518	0.518	0.396
Organic nitrogen,	A	0.22	0.39	0.26	0.26	0.28	0.24	0.24	0.24	0.21	0.20	0.29
total (mg/l-N)	B	0.19	0.39	0.33	0.27	0.28	0.26	0.25	0.25	0.20	0.20	0.29
Orthophosphate,	A	0.003	0.010	0.006	0.016	0.019	0.014	0.018	0.022	0.022	0.028	0.010
soluble (mg/l-P)	B	0.003	0.010	0.005	0.015	0.019	0.016	0.017	0.021	0.022	0.021	0.012
Phosphorus, total	A	0.044	0.14	0.068	0.084	0.16	0.14	0.12	0.13	0.12	0.10	0.10
(mg/l-P)	B	0.040	0.14	0.072	0.084	0.16	0.14	0.12	0.14	0.12	0.11	0.11
Phosphorus, total	A	0.007	0.018	0.014	0.030	0.027	0.023	0.026	0.027	0.025	0.028	0.023
soluble (mg/l-P)	B	0.005	0.014	0.011	0.028	0.025	0.027	0.028	0.025	0.026	0.031	0.019
Silica, soluble	A	7.6	8.4	7.1	8.2	9.2	7.4	7.6	9.0	11	9.4	8.2
(mg/l-SiO_2)	B	7.4	9.0	6.8	8.2	10	8.2	9.0	8.6	11	9.0	9.2
Alkalinity,	A	9	10	9	11	10	11	11	11	11	11	11
total (mg/l-$CaCO_3$)	B	9	10	9	12	11	11	11	11	11	12	12
pH		6.4	6.5	6.2	6.21	6.2	6.2	6.3	6.3	6.3	6.3	6.4

[a]Inorganic nitrogen is the sum of the ammonia, nitrate and nitrite forms.

Table 13. Baseline aquatic nutrient levels measured in Lake Wylie water samples collected 8-9 July 1974.

Parameters	Rep	Sampling Locations										
		2 TOP	7 TOP	10 TOP	19 TOP	19 TOP	21 TOP	22 TOP	23 TOP	24 TOP	25 TOP	26 TOP
Ammonia	A	0.02	0.04	0.04	0.02	0.13	0.01	<0.01	<0.01	<0.01	0.01	<0.01
(mg/l-N)	B	0.02	0.03	0.03	0.01	0.12	0.02	<0.01	<0.01	<0.01	0.01	<0.01
Nitrate	A	0.06	0.45	0.24	0.23	0.26	0.16	0.21	0.21	0.24	0.13	0.09
(mg/l-N)	B	0.09	0.43	0.26	0.26	0.29	0.13	0.19	0.22	0.24	0.11	0.08
Nitrite	A	0.0034	0.0068	0.0031	0.0019	0.0013	0.0022	0.0023	0.0016	0.0015	0.0007	<0.0001
(mg/l-N)	B	0.0045	0.0063	0.0035	0.0023	0.0012	0.0030	0.0014	0.0016	0.0016	0.0009	<0.0001
Inorganic Nitrogen[a]	A	0.083	0.497	0.283	0.252	0.391	0.172	<0.222	<0.222	<0.252	0.141	<0.100
(mg/l-N)	B	0.115	0.466	0.294	0.272	0.411	0.153	<0.201	<0.232	<0.252	0.121	<0.090
Organic nitrogen,	A	0.37	0.88	0.39	0.40	0.22	0.34	0.33	0.34	0.27	0.31	0.31
total (mg/l-N)	B	0.37	0.71	0.48	0.39	0.22	0.32	0.33	0.33	0.29	0.31	0.36
Orthophosphate,	A	0.004	0.016	0.007	0.003	0.003	0.002	0.002	0.002	0.003	0.002	0.003
soluble (mg/l-P)	B	0.003	0.006	0.006	0.002	0.001	0.002	0.002	0.002	0.003	0.003	0.004
Phosphorus, total	A	0.033	0.29	0.062	0.032	0.15	0.018	0.17	0.13	0.11	0.10	0.043
(mg/l-P)	B	0.029	0.31	0.072	0.037	0.18	0.020	0.13	0.11	0.12	0.11	0.056
Phosphorus, total	A	0.005	0.029	0.012	0.007	0.014	0.007	0.005	0.005	0.005	0.006	0.008
soluble (mg/l-P)	B	0.006	0.026	0.007	0.007	0.014	0.006	0.005	0.005	0.005	0.004	0.008
Silica, soluble	A	9.8	11	8.9	12	10	10	9.9	10	10	11	11
(mg/l-SiO_2)	B	9.9	12	8.7	11	11	10	11	10	10	11	11
Alkalinity, total	A	11	10	10	12	13	12	11	12	11	12	11
(mg/l-$CaCO_3$)	B	10	10	11	13	12	13	11	12	11	12	12
pH		6.4	6.4	6.4	7.4	6.4	6.8	6.6	6.6	6.6	6.5	6.6

[a]Inorganic nitrogen is the sum of the ammonia, nitrate, and nitrite forms.

Dates	Correlation Coefficients Total	Total Soluble	Soluble Ortho
8, 9 October 1973	0.772	0.701	0.862
7, 8 January 1974	0.657	0.859	0.858
8, 9 April 1974	0.683	0.769	0.477
8, 9 July 1974	(-) 0.097	(-) 0.487	(-) 0.215
Overall (39 comparisons)	0.317	0.760	0.814

The unexpected poor correlation for the July samples is dramatic. A stepwise multiple regression analysis (not shown above) indicated that, over all, both soluble phosphorus forms taken together were better estimators of the available portion than either chemical determination by itself.

It is not surprising that seasonal influences play a large role in determining which chemical analytical determination is a better estimator of the biologically available portion since rainfall, turbidity, sorption reactions, and biological growth, death, and decay all play variable and constantly changing roles in nutrient relationships.

Although a systematic statistical examination of correlations between all three phosphorus chemical forms and the biologically available portion and the possible effects of autoclaving has not yet been undertaken, several workers have made various comparisons and concluded that in most situations soluble orthophosphorus is a good estimator of the biologically available portion (Fitzgerald et al., 1973; Lee, 1973). If a reliable chemical method exists, its use would facilitate broader eutrophication-control assessment programs because less time is presumably required to perform the chemical tests than the bioassays which generally require from 1-2 days (short-term sorption and extraction tests) to weeks (long-term growth tests) to conduct.

Very little work along these lines has been done with nitrogen but several reports are available comparing orthophosphate phosphorus to biologically available phosphorus. Fitzgerald et al. (1973) have reported work suggesting that the standard molybdate, stannous chloride test on filtered samples is the best estimator of biologically available phosphorus whereas the work of Chamberlain and Shapiro (1969) indicate that the organic extraction method (isobutanol) is a good procedure. However, the best chemical test to use is a controversial subject (Rigler, 1968) and it appears that the best correlation with bioassays may depend on several factors including the specific level of orthophosphate phosphorus in the natural water system (Lee, 1970; Lee and Veith, 1971; and Chamberlain

and Shapiro, 1969), the method of sample pretreatment used (e.g. membrane filtration vs. autoclaving, U.S. EPA, 1971) as well as on the type of bioassay employed. Data from the Fitzgerald et al. study (1973) suggest that average absolute differences between the orthophosphate chemical test and biologically available phosphorus are least when a short-term biological exposure (field collected, phosphorus-starved *Cladophora.*) followed by hot-water extraction and chemical measurement of the sorbed PO_4 is employed.

The best method to use remains a subject for further research and the value of the present study lies in a demonstration of the importance of seasonal influences on the possible correlations, and it serves further to illustrate the continuing need to perform the biological assays.

Assessment of Allen Station Effects

The bioassay data do not indicate any influence of the Allen Station on the ability of the Lake Wylie study area waters to support algal growth as a function of nutrient content. The mean maximum biomass of unsupplemented (blank) lake waters is presented in Table 14 and these values are presented graphically and compared in Figures 4-7.

Only Catawba River (location 2) and discharge waters (location 10) were collected in October and January. The October and January bioassay data suggested that the Allen Station had an effect of increasing the algal growth potential from six to twenty-two fold (compare locations 2 and 10, Table 14). To the uninitiated, these data might suggest that passage of the water through the condensers resulted in a greater algal growth potential. However, these results can be explained on the basis that the nutrient content of location 10 water was influenced more by the South Fork nutrient load than by the Catawba River nutrients passing through the Allen Station. Both were surface samples and the hotter water leaving the plant remains largely at the surface. However, some mixing had already occurred, as revealed by a careful examination of the water chemistry data for locations 1, 2, 3, 7, 8, 10, and 12. (See Tables 10-13.)

Effects of Urbanization

No measurable effects of urbanization were encountered in this study. Only the northernmost of the three developing eastern embayments is (apparently) served by septic tank disposal systems. The homesites in the two southernmost eastern embayment areas are sewered in a development known as Tega Cay. If the developing urbanization is an important factor in eutrophication of the southern end of Lake Wylie, several comparisons and observations should confirm the trend.

Table 14. Comparison of maximum mean algal biomass supported in nutritional bioassay flasks containing unsupplemented blank water samples from the Lake Wylie study area (mg/l).

Dates Samples Collected	Location										
	Above the Confluence			Near Catawba Intake		Embayments at Southeastern End of Lake Wylie					
						North		Middle		South	
	Catawba River	South Fork	Comb. (Discharge)	Surface	Bottom	Outer	Inner	Outer	Inner	Outer	Inner
	2	7	10	19	19B	21	22	23	24	25	26
8, 9 October 1973	1	a	22	7	6	a	2	5	4	5	3
7, 8 January 1974	3	a	17	21	27	24	25	18	24	25	19
8, 9 April 1974	3	28	16	22	28	24	21	28	23	26	20
8, 9 July 1974	16	b	8	13	11	14	15	9	26	16	6

[a]Sample not collected.

[b]Confidence in this number doubtful. Extreme turbidity in sample.

First, the sewered embayments would be expected to have fewer evidences of eutrophication than the areas served by septic tank systems. The assumptions here are that the sewage is collected and provided some sort of waste-treatment that removes most of the nutrients, and that nutrients from the septic system effluents may enter the embayments and ultimately the lake proper. A reverse situation could also be postulated, i.e. poor sewage treatment with large amounts of nutrients entering the lake from a point source as compared to well-designed septic systems with soils permitting no breakthrough of nutrients to the lake. Examination of the data summarized in Table 14 and detailed in Figures 4 through 7 does not reveal any consistent pattern that would characterize significant differences between the sewered and septic tank-served areas.

Second, if it can be assumed that the embayments are protected somewhat from internal circulation and mixing with the mainstem waters, and that the overall water movement is from the inner portion of the embayment to the outer portion, then urbanization-derived nutrients should be expected to result in differences in algal growth potentials between the inner and outer portion of the embayments. No such differences in algal growth potentials were consistently found during the study.

Finally, if urbanization at the lower end of Lake Wylie is contributing significantly to eutrophication of the lake, then greater algal growths should be expected at the southern stations than at the northern stations. Careful examination of the data presented in Figures 4 through 7 reveals no such trend.

Probably of more concern than the developments at the lower end of the lake are the older (and more extensive) home developments in the

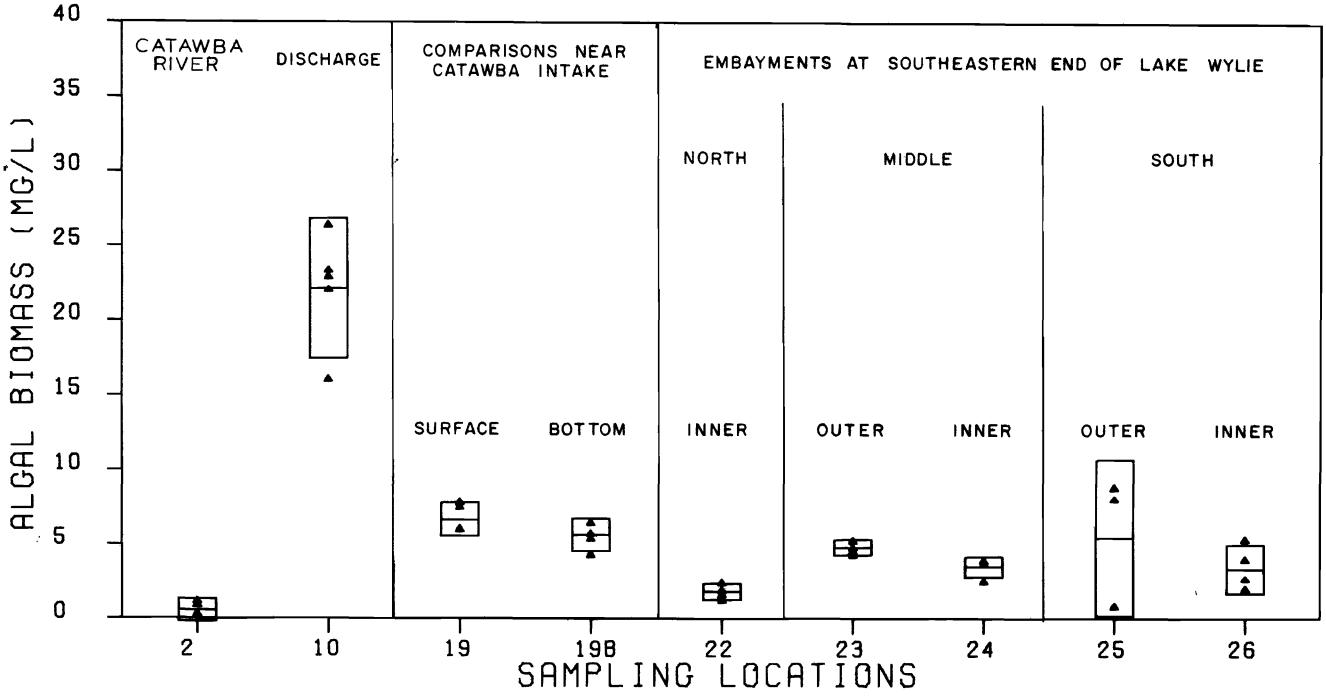

Figure 4. Comparison of algal biomass supported in nutritional bioassay flasks containing unsupplemented blank lake-water samples from 9 Catawba River System locations on 8-9 Oct. 1974.

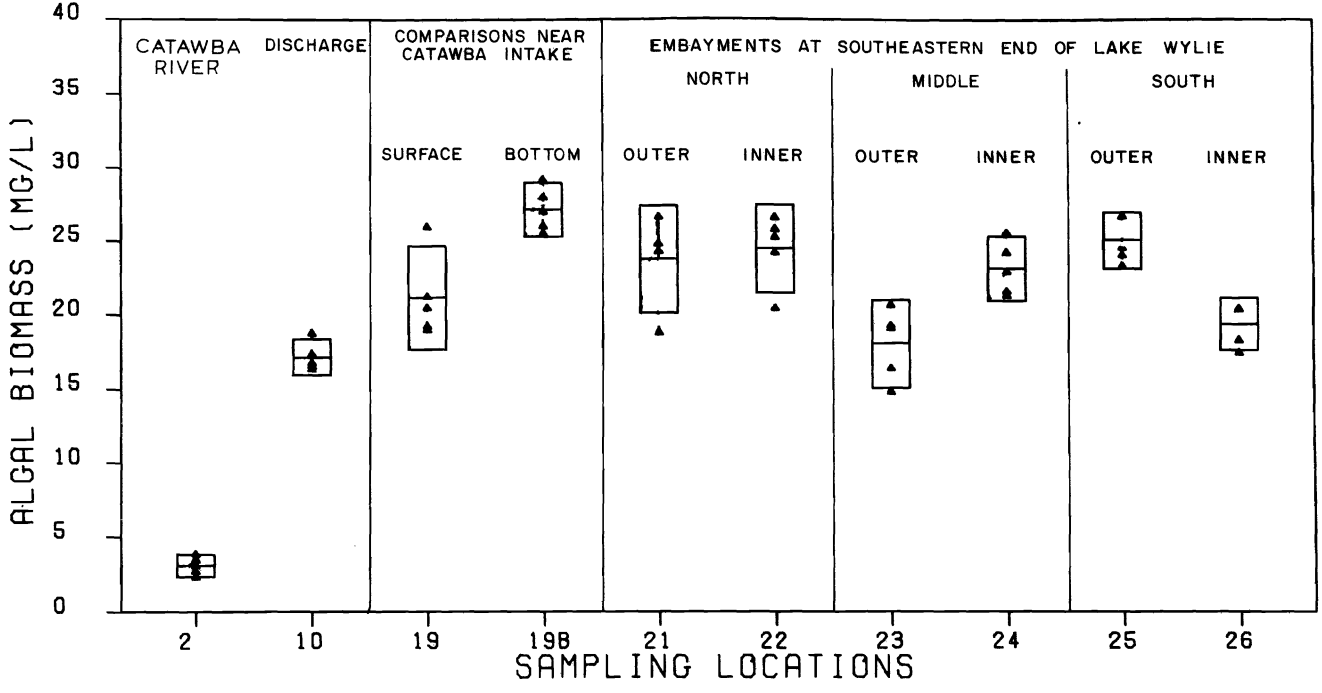

Figure 5. Comparison of algal biomass supported in nutritional bioassay flasks containing unsupplemented blank lake-water samples from 10 Catawba River System locations on 7-8 Jan. 1974.

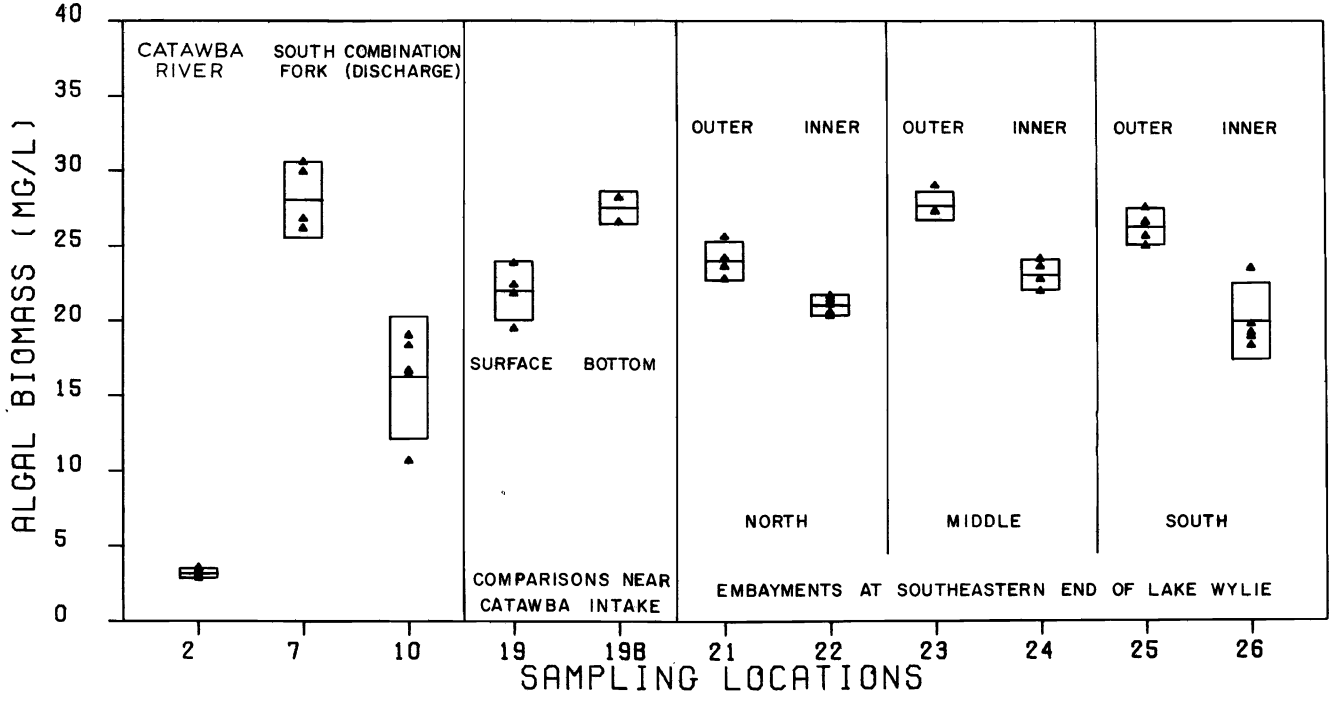

Figure 6. Comparison of algal biomass supported in nutritional bioassay flasks containing unsupplemented blank lake-water samples from 11 Catawba River System locations on 8-9 April, 1974.

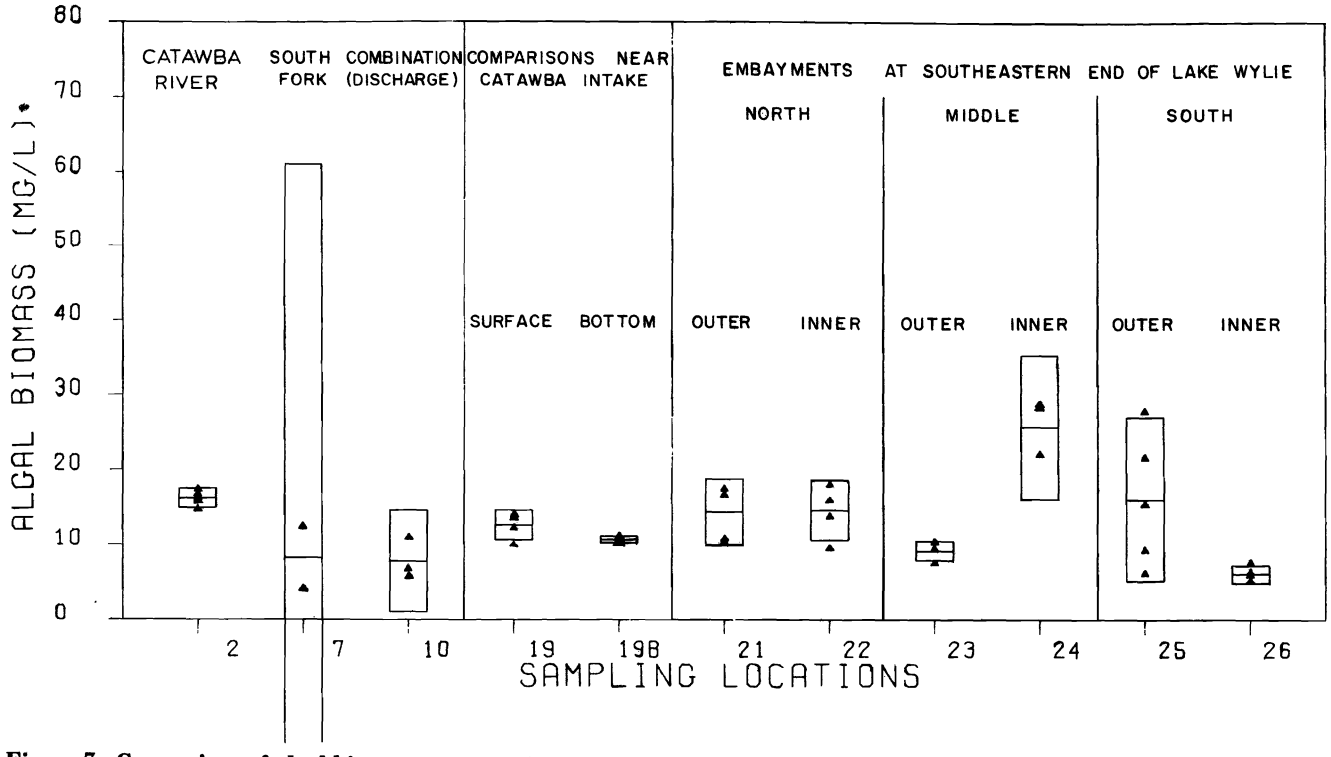

Figure 7. Comparison of algal biomass supported in nutritional bioassay flasks containing unsupplemented blank lake-water samples from 11 Catawba River System locations on 8-9 July, 1974.

middle and upper reaches of Lake Wylie and the embayment represented by sampling locations 21 and 22 that are not sewered and therefore are presumably served only by aging septic systems of unknown design. These systems do conceivably pose a long range threat of eutrophication.

One of the best literature sources on potential nutrient contamination of lakes from septic tank disposal systems may be that of Dudley and Stephenson (1973) which confirms the generally recognized belief that nitrates from septic systems are more often a threat than phosphorus, and that under certain conditions phosphorus too may enter lakes from these systems.

Ancillary Laboratory Experiments

First Experiment. A relatively high pH favored the recovery of apparently nonviable *Microcystis* cells (Table 15). After 16 days, all three of the flasks initially set at pH 9.5 were visibly colored and microscopic examination revealed viable and growing *Microcystis* cultures. Five days later all three of the flasks initially set at pH 10.1 were blue-green with *Microcystis*. *Microcystis*, up to this point, did not recover in any flasks initially set at pH's below 9.5

Second Experiment. *Microcystis* did not grow at the lower inoculum cell densities nor at the lower pH's, including the natural Lake Wylie sample pH (Table 16). Highly sensitive growth-measurement techniques are essential to determine early growth responses of microbial cultures before the growth itself changes the culture environment (Droop, 1973). *In vivo* fluorescence is an ideal technique for this purpose using algae. The monitoring technique did not permit asepsis, and a contaminating flagellate grew in some cultures starting as early as day 4. In the 1000 cells/ml-pH 9.5 cultures, *Microcystis* finally initiated growth after a lag of 10-12 days; in the 10,000 cell/ml-pH 8.6 flask after 8-9 days; in the 25,000 cells/ml-pH 8.6 and 9.5 flasks after 2-3 days; and in the 50,000 cells/ml-pH 8.6 flasks after 1-2 days. *Selenastrum* at 1,000 cells/ml initiated growth without lag at the three pH's tested. Increased inoculum cell density was a factor in overcoming lag, and is a technique often employed by microbiologists for this purpose (Lamanna and Mallette, 1965).

Third Experiment. Higher initial pH's produced higher specific growth rates in *Microcystis*, but lower growth rates for *Anabaena* and for *Selenastrum* (Figure 8). Table 17 is a summary (mean values) taken from plots of the growth curves and shows that maximum relative fluorescence achieved within the 7 day culture period for *Microcystis* was also a function of the initial culture pH. Furthermore, between days 5 and 7 at the natural Lake Wylie composite pH (6.8), *Microcystis* fell into a sharp

phase of decline. For *Anabaena,* however, maximum fluorescence was achieved in flasks at the lower initial pH's.

Not reflected in the data of Table 17 is the fact that mean fluorescence in *Anabaena* pH 6.8-8.9 flasks declined sharply after day 4 or 5 whereas fluorescence remained relatively stable in the pH 9.8 flasks. Maximum fluorescence was not achieved in *Selenastrum* flasks prior to the 7 day period.

The actual pH's that influenced the results in these experiments are probably not those pH's reported as the "initial" pH's. The hydrogen

Table 15. Recovery of *Microcystis aeruginosa* cultures in algal assay procedure (AAP) medium as a function of initial pH. Ancillary experiment No. 1.

Initial pH 7/16/73	Flask	Appearance on 8/1/73	Appearance on 8/6/73
	a	No growth	Slightly green[a]
10.1	b	No growth	Slightly green[a]
	c	No growth	Slightly green[a]
	a	Blue green[b]	Blue green
9.5	b	Blue green[b]	Blue green
	c	Blue green[b]	Blue green
	a	No growth	No growth
9.0	b	No growth	No growth
	c	No growth	No growth
	a	No growth	No growth
8.1	b	No growth	No growth
	c	No growth	No growth
	a	No growth	No growth
7.5	b	No growth	No growth
	c	No growth	No growth
	a	No growth	No growth
7.0	b	No growth	No growth
	c	Green[c]	Green[c]
	a	No growth	No growth
6.3	b	No growth	No growth
	c	No growth	No growth

[a,b]Microscopic examination revealed an apparently unispecific culture of Microcystis aeruginosa.

[c]Microscopic examination on 8/21/73 indicated an apparently unispecific culture of Selenastrum, probably a chance contaminant.

Table 16. Effect of initial pH and inoculum cell density on lag phase growth of *Microcystis aeruginosa* comparing *Selenastrum capricornutum* at standard inoculum density. Ancillary experiment No. 2.

Organism	Inoculum (cells/ml)	Initial pH	Time Lag in Growth (Days)
Microcystis aeruginosa	1,000	6.8 (natural pH, not adjusted)	No growth (exper. continued 15 days)
	1,000	7.5	No growth (flagellates noted, day 12)
	1,000	8.6	No growth
	1,000	9.5	10-12 (some flagellates by day 15)
	10,000	6.8 (natural pH, not adjusted)	No growth
	10,000	7.5	No growth
	10,000	8.6	8-9
	10,000	9.5	No growth (flagellates started by day 9)
	25,000	6.8 (natural pH, not adjusted)	No growth (flagellates started by day 7)
	25,000	7.5	No growth (flagellates started by day 9)
	25,000	8.6	2-3
	25,000	9.5	2-3
	50,000	6.8 (natural pH, not adjusted)	2-3
	50,000	7.5	2-3
	50,000	8.6	1-2
	50,000	9.5	(flagellates started by day 4)
Selenastrum capricornutum	1,000	7.5	0
	1,000	8.6	0
	1,000	9.5	0

ion concentration is difficult to control in naturally buffered waters in batch cultures without the use of strong inorganic or organic buffering systems that could have other effects on algae unrealted to their influence

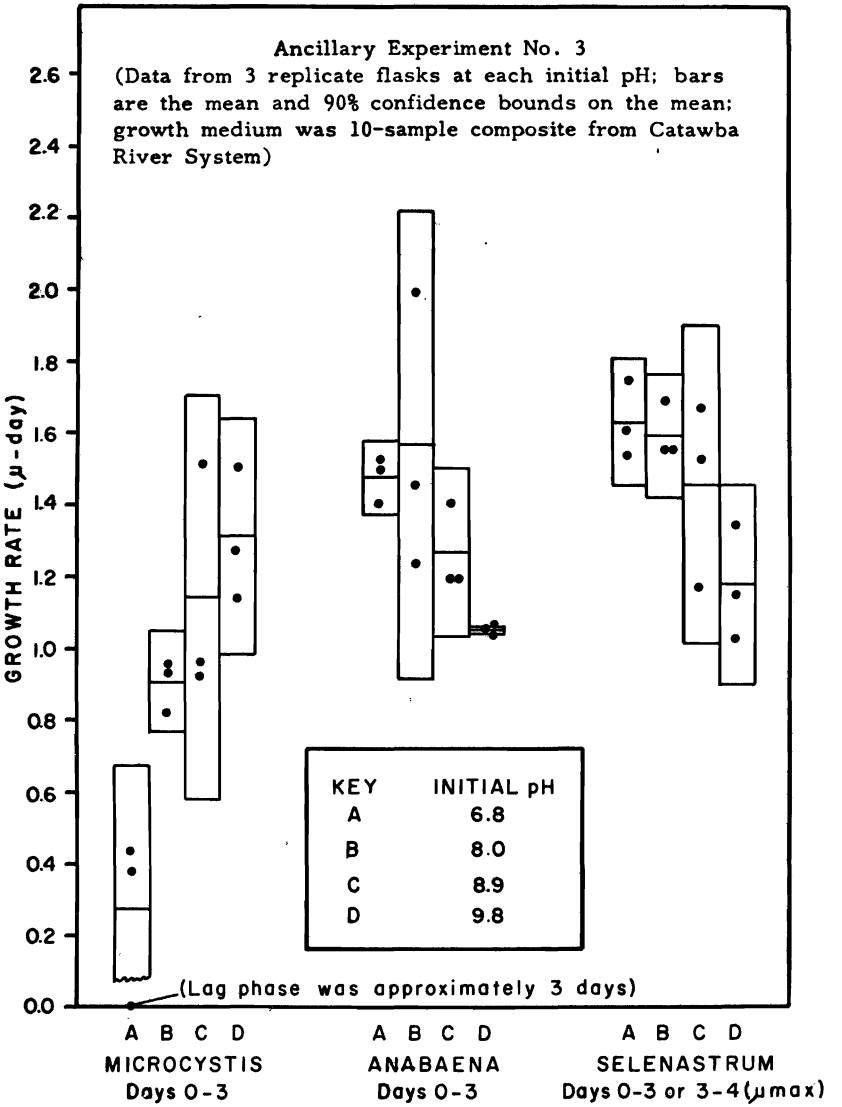

Figure 8. Influence of initial pH on the growth rate of *Microcystis aeruginosa*, *Anabaena floss-aquae*, and *Selenastrum capricornutum* in subsamples of a Catawba River system 10-sample composite. Ancillary experiment No. 3.

Table 17. Maximum *in-vivo* fluorescence and day of incubation on which the maximum fluorescence was achieved in composited Lake Wylie water samples collected 7-8 January 1974. Ancillary experiment No. 3.

Initial pH	*Microcystis* Fluorescence (Relative Intensity Units)	Day	*Anabaena* Fluorescence (Relative Intensity Units)	Day	*Selenastrum* Fluorescence (Relative Intensity Units)	Day
6.8 (Natural pH, not adjusted)	0.09	5	0.98	4	6.9	>7[a]
8.0	0.23	7	0.83	4	2.0	>7
8.9	0.43	7	0.88	5	2.2	>7
9.8	0.48	6	0.53	5	1.6	>7

[a]Experiment concluded on day 7, but Selenastrum was still growing.

on hydrogen ion concentration. The pH in single flasks from each treatment in the third experiment at day 5 is indicated in Table 18. By day 5 all the pH's in the pH adjusted flasks had dropped by a difference of 0.8 to 1.7 pH units from the initial values. However, a proper relationship from low to high pH remained in each series suggesting that despite influential pH's being lower than initial pH's an effect of pH can still be reported.

Fourth Experiment. The results of the fourth experiment suggest that the total amount of algal growth in Lake Wylie waters will be a function of total nutrient loading (Table 19); that *Microcystis* can grow well in laboratory incubations in Lake Wylie waters (Figure 9); and that no statistically significant differences occurred in the maximum specific growth rate (μ-max) of *Microcystis aeruginosa* as a function of initial pH or nutrient loading (Figure 10). However, adjustment of the initial *Microcystis* cultures to pH 10 led to an increased overall growth rate; and in the 15 day incubation period the achievement of maximum fluorescence of chlorophyll *a* was reduced by 6 to 7 days from the time required for full nutrient utilization under the influence of the sample's natural pH (Figure 9).

It is obvious that the full influence of the initial pH cannot persist throughout this type of experiment (Table 19). The initial pH's are immediately influenced by the growing cultures and by the normal CO_2/carbonate/bicarbonate equilibria. In this experiment, the inoculum was calculated to result in 25,000 *Microcystis* cells per ml. It is hypothesized, however, that the initial pH persisted with only moderate change at least for several days. It is obvious that the addition of the AAP nutrients altered pH significantly and the final pH's suggest that pH differences existed throughout the incubation (Table 19). From an ecological point of view, the most significant feature of this experiment is the reduced overall growth rate of *Microcystis* at the natural (laboratory)

Table 18. Hydrogen ion concentration of algal cultures after a 5 day incubation-growth period. Ancillary experiment No. 3.

Initial pH	Day 5 pH		
	Microcystis	*Anabaena*	*Selenastrum*
6.8[a]	6.7	7.1	7.0
8.0	7.2	7.3	7.2
8.9	7.4	7.5	7.4
9.8	8.1	8.1	8.1

[a]Natural pH, not adjusted.

pH of the samples. Examination of Table 12 indicates an average pH of 6.3 for 8, 9 April samples at the time of collection. Thus, autoclaving and storage of the samples led to a pH increase of 0.8 units. Presumably, the lower *in situ* natural pH reduces still further the growth rates of this potential nuisance alga in Lake Wylie

Meaning of the Ancillary Experiments. The experiments reported here support the hypothesis that Lake Wylie waters are not conducive to the overabundant growth of the potential nuisance blue-green alga *Microcystis aeruginosa.* These data also support the findings of others indicating that low pH waters have a negative influence on the growth of blue-green algae (Brock, 1973) and that hydrogen ion concentration is in fact an important factor in algal ecology and species succession (Shaprio, 1973; and Goldman and Shapiro, 1973). A growing body of evidence supports the theory that many green algae growing autotrophically can use primarily aqueous CO_2 as their carbon source, while others, notably blue-green algae, dominate in higher pH environments and may utilize HCO_3 and even CO_3 ion directly (Goldman et al., 1971). Furthermore, these data appear to correlate with recent reports in the literature attempting to explain the ecological succession of the blue-green algae in natural waters that are becoming more eutrophic (Goldman and Shapiro, 1973).

Field Observations and Taxonomy

Field observations on Lake Wylie lend support to the conviction that Lake Wylie will not support a nuisance algal population. Only near the Allen Station discharge canal were there any visible signs of a blue-green algae growth. These tiny scattered colonies were doubtless influenced by the heated water discharge and are generally overlooked by most casual observers. They occur only in quiet embayments just at the water's edge and often on soils only at the water's edge. Interviews with native residents

Table 19. Maximum growth of *Microcystis aeruginosa* as a function of initial pH and nutrient loading in a Lake Wylie eleven-station composite of samples collected 8-9 April 1974. Ancillary experiment No. 4.

(Except where indicated, data represent one of three replicate flasks at day 15 of experiment)	Initial pH	Final pH[a]	Cells/ml (in millions)	Mean Relative[b] Fluorescence	Day of Incubation on Which Peak Fluorescence Occurred	Biomass[c] (mg/l Algal Dry Wt.)
Without nutrient supplement	7.1	7.1	1.3	0.63	15	5
	10	8.3	1.5	0.69	8-9	15
Single strength AAP nutrient added	7.8	8.2	13.0	4.64	13	181
	10	8.9	14.4	4.74	13	213
2 x AAP nutrients added	8.2	9.7	26.8	7.83	13	250
	10	9.7	23.9	9.47	13	231

[a]Average of three replicate flasks. Average final pH's in uninoculated flasks: Initial pH 7.1-final pH 7.0; initial pH 10-final pH 8.3.

[b]Corrected by fluorescence of uninoculated blanks. In this case the mean of three replicate flasks is reported.

[c]Corrected by dry weight of uninoculated flasks.

from the area suggested that, historically, nuisance algae blooms have never occurred on Lake Wylie.

Tables 20 and 21 summarize the blue-green algae among the periphyton and phytoplankton collected in the monthly sampling

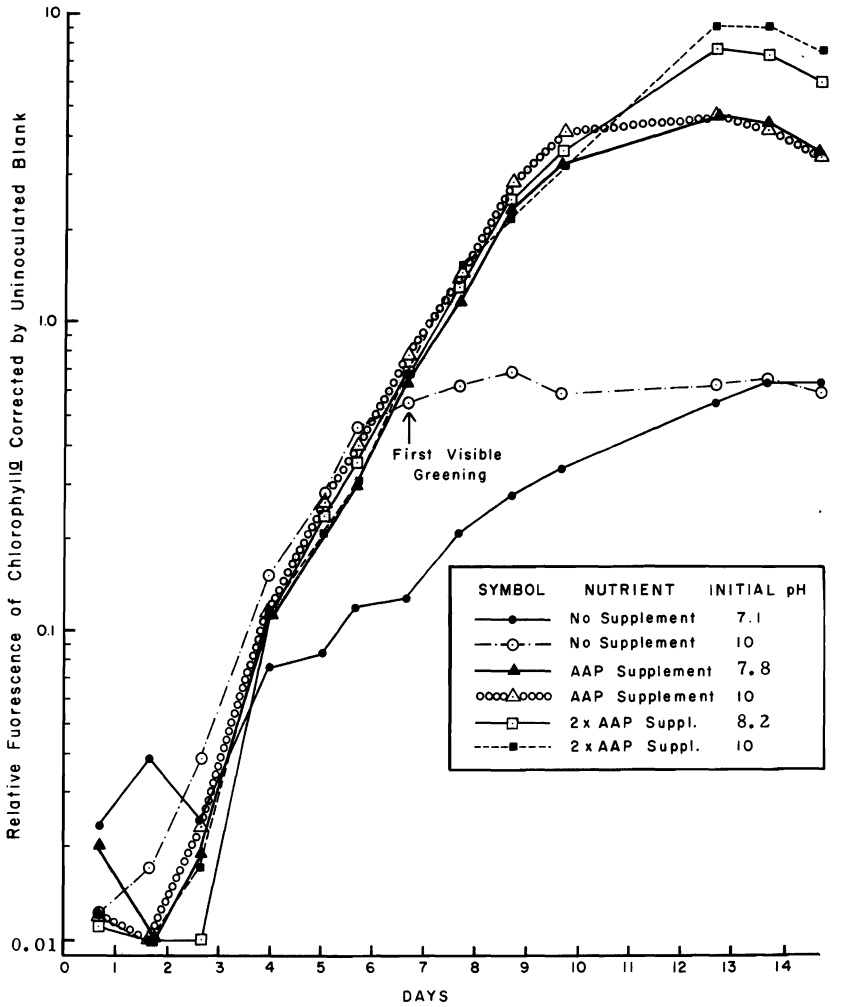

Figure 9. Growth of *Microcystis aeruginosa* in an 8-9 April Lake Wylie composite sample as a function of initial pH and nutrient level. Each data point is the mean of three replicate flasks. Visible greening occurred only in flasks above the arrow. Ancillary experiment No. 4.

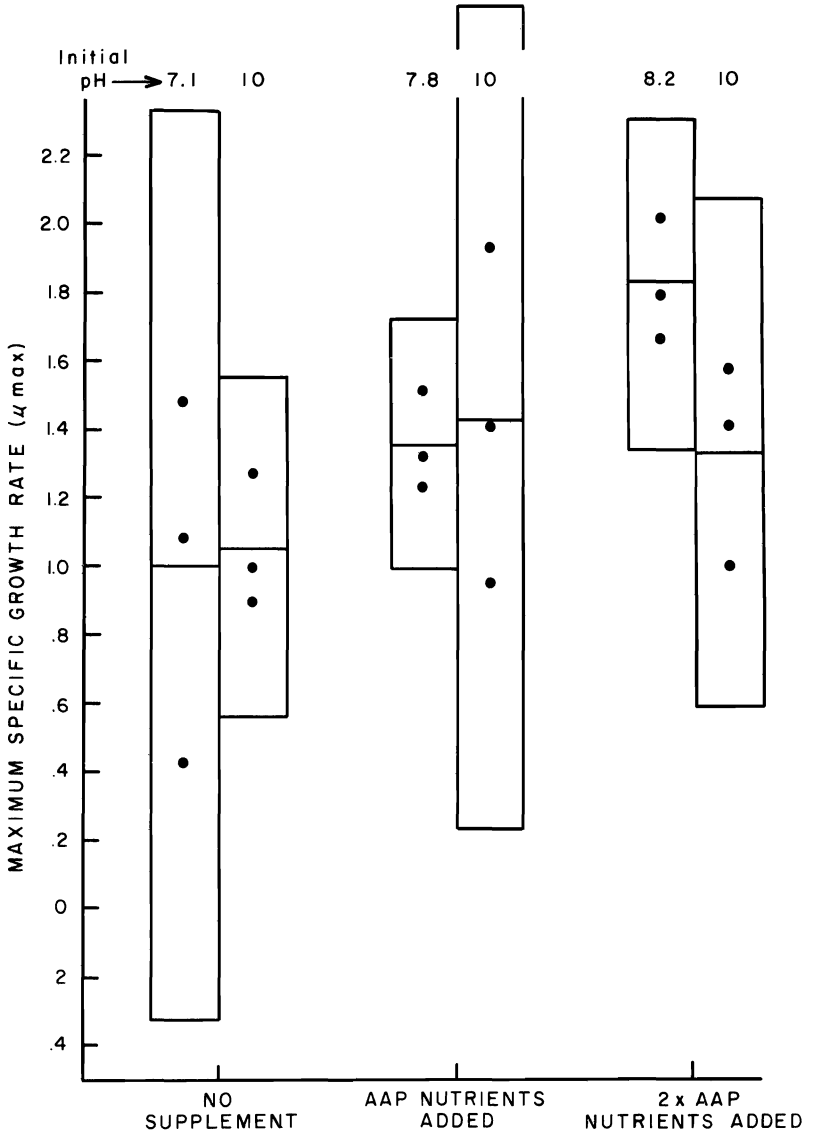

Figure 10. Maximum specific growth rates of *Microcystis aeruginosa* as a function of initial pH and nutrient levels in a Lake Wylie composite of 8-9 April samples. Each point represents growth in a single shake-flask culture. Bars are the mean and 95 percent confidence limits on the mean (μ max) growth rates measured by the relative fluorescence of chlorophyll *a*. Ancillary experiment No. 4.

Table 20. Summary of available data on blue-green algae in monthly periphyton samples from Lake Wylie reported as percent of total estimated biovolume. Parenthetical values are percent contributed by *Oscillatoria curviceps*.

Sampling Date	Locations					
	Catawba River Near Allen Intake	South Fork		Below Confluence		
		Discharge	Below Discharge			
	3	10	12	16	19	31
11 October 1973	-[a]	67.6 (66.8)	33.4 (30.7)	5.2	0.5	0.6
2 November 1973	0.2	63.2 (56.4)	2.0	5.2	0.3	0.4
5 December 1973	5.9	32.8 (31.5)	0.3	3.6	1.5	0.1
9 January 1974	19.3 (14.6)	20.6 (16)	3.6	5.7	0	0.5
6 February 1974	0.3	0.4	0.2	1.8	1.2	0
13 March 1974	0.1	9.6	0.2	<0.1	<0.1	<0.1
11 April 1974	<0.1	0.2	<0.1	<0.1	<0.1	<0.1
16 May 1974	2.3	15.9 (15.1)	0.8	<0.1	0.1	0.6
6 June 1974	0.3	70.6 (67.2)	0.3	0.5	-[a]	0.4
11 July 1974	<0.1	5.9[b]	<0.1	<0.1	<0.1	<0.1
8 August 1974	5.6	91.5[c]	6.2	2.1	0.5[d]	-[a]

[a]Samples lost.

[b]5.8 percent Oscillatoria curviceps.

[c]89 percent Oscillatoria limosa. Oscillatoria curviceps did not appear in sample.

[d]Location "19A" (Beaver Dam Creek).

programs (Industrial BIO-TEST Laboratories, Inc., 1974) and are reported as percent of total algal biovolume.The parenthetical values in both tables denote the percent biovolume of those species where the blue-green algae comprised 10 percent or more of the total biovolume.

Among the periphyton collected (Table 20), *Oscillatoria curviceps* was the dominant blue-green whenever blue-green algae formed a significant portion of the total algal biovolume. Except for the colder water months of February, March, and April (and also July), the warmer waters at the Allen Station discharge (location 10) have supported a prominent blue-green periphyton population. Blue-green periphytic algae have formed a relatively insignificant portion of the total periphyton population at locations not under the sphere of influence of the hot water discharge from the Allen Station.

The blue-green algae in 238 phytoplankton samples are summarized in Table 21. Differences in the biovolume and species between the final three months and previous months were dramatic. Several observations which suggest that seasonal influences are important in favoring the blue-greens in Lake Wylie are worthy of comment:

First, in 22 of the 60 samples analyzed for the last 3 months shown (June, July, and August), blue-green algae comprised more than 10 percent of the total phytoplankton algal biovolume. In the preceding months, none of the 119 samples contained as much as 10 percent blue-greens. In fact, the highest percent blue-green biovolume during this period was 4.7 percent (location 32, surface, 11 February 1974).

Second, the incidence of blue-green algal occurrence greater than 10 percent biovolume occurred in the Catawba River, South Fork, and below the confluence in both surface and bottom samples. It appears, therefore, that these incidences occurred randomly with respect to sampling location. *Oscillatoria geminata* was the dominant species at five locations within the southern half of Lake Wylie during June. Although this organism appeared in previous collections during 1973 and 1974 and in July, it was never dominant nor even very prominent with respect to percent biovolume. Why, then, did this particular blue-green species become dominant at several locations in lower Lake Wylie only in the spring? The answer to this question could be important in fully understanding the obvious freedom from blue-green algal blooms that has characterized the history of Lake Wylie. It is also interesting that with no exceptions among periphyton and with one exception among the phytoplankton, in those instances where blue-gree algae were dominant, the dominant genus among them was *Oscillatoria.* The single exception was *Microcystis aeruginosa* which comprised more than 52 percent of the biovolume in the location 14 bottom sample collected 8 October 1973.

Table 21. Summary of data on blue-green algae in monthly phytoplankton samples from Lake Wylie reported as percent of total estimated biovolume; parenthetical values are percentages contributed by *Anabaena circinalis* (Ac); *Anabaena wisconsinense* (Aw); *Raphidiopsis curvata* (Rc); *Microcystis aeruginosa* (Ma); *Oscillatoria tenuis* (Ot); *Oscillatoria geminata* (Og); *Anabaena scheremetievi* (As); *Anabaena spiroides* (Asp); *Anabaena spiroides* var. *crassa* (Av); *Arthrospira jenneri* (Aj).

Sampling Date	Site in Water Column	Sampling Locations: North Fork 1	North Fork 2	South Fork 7	South Fork 8	South Fork 10	South Fork 12	Confluence 13	Confluence 14	Below Confluence 15	Below Confluence 17	Below Confluence 19	Below Confluence 27	Below Confluence 28	Below Confluence 32
5 September 1973	Surface	-	3.4	6.0	0.8	18.5 (Ma 16.9)	4.6	3.4	-	1.8	1.2	0.4	2.7	1.1	0.4
	Bottom	-	12.3[a]	-	12.6 (Ot 9.6)	8.7	-	12.2 (Ma 9.6)	-	6.4	1.4	10.8 (Ma 8.2)	-	-	2.0
8 October 1973	Surface	-	19.2 (Ma 15.9)	42.6 (Ot 41.1)	0.01	2.8	0.3	-	0.2	0.7	0.3	0.5	0.4	0.3	1.8
	Bottom	-	2.2	-	4.6	2.6	-	-	56.0 (Ma 52.5)	3.5	1.0	0.8	-	-	6.4
9, 10 November 1973	Surface	-	17.0 (Ma 13.6, Aw 3.1)	-	0.1	1.4	0	-	1.4	0.4	0.2	0.01	0.4	0.04	0.21
	Bottom	-	2.9	-	4.8	0.2	-	-	1.8	0.8	0.2	0.1	-	-	0.5
10 December 1973	Surface	-	8.3	2.4	0.3	4.1	0.6	-	2.0	2.3	3.7	0.1	0.9	0.2	0.1
	Bottom	-	4.4	-	0.7	0.5	-	-	0.1	0.6	0.9	0.02	-	-	0.2
8 January 1974	Surface	0.2	0.3	1.0	0.6	1.2	0.3	-	-	0.1	0.9	1.2	1.1	-	0.2
	Bottom	0.04	0.03	-	0.2	0	-	-	-	0.01	0.6	0.9	-	-	0.4
11 February 1974	Surface	0.10	0.27	1.47	1.20	0.37	0.32	-	-	0.19	0.02	0.02	0	0.01	4.73
	Bottom	0.26	2.79	-	0.25	2.43	-	-	-	0.54	0.02	0.03	-	-	0.41
11, 12 March 1974	Surface	0.01	0.01	0.39	0.53	0.10	0.06	-	-	0.07	0.33	0.02	<0.01	0.11	<0.01
	Bottom	0.13	0.02	-	0.70	0.12	-	-	-	0.24	0.01	3.35	-	-	0.45
8, 9 April 1974	Surface	0.09	0.01	0.69	0.23	0.15	0.81	-	-	2.03	0.34	0.01	0.21	0.49	1.30
	Bottom	0.02	0.11	-	0.54	0.12	-	-	-	0.15	0.77	0.34	-	-	0.02

Table 21. Continued.

Sampling Date	Site in Water Column	Sampling Locations: North Fork 1	North Fork 2	South Fork 7	South Fork 8	South Fork 10	South Fork 12	Confluence 13	Confluence 14	Below Confluence 15	Below Confluence 17	Below Confluence 19	Below Confluence 27	Below Confluence 28	Below Confluence 32
13, 14 May 1974	Surface	4.14	2.54	0.04	0.22	0.46	0.17	-	-	0.20	2.52	2.30	0.24	2.04	4.71
	Bottom	3.00	4.52	-	0.22	3.43	-	-	-	2.85	0.41	0.43	-	-	1.93
10, 11 June 1974	Surface	16.81 (As 9.3, Og 6.4)	6.17	0.39	1.47	2.17	8.32	-	-	12.37 (Og 11.2)	50.00 (Og 45.3 As 2.4)	77.42[b]	84.97[c]	76.72[d]	77.42 (Og 57.1, Aw 18.3)
	Bottom	8.58	5.43	-	1.06	1.17	-	-	-	2.14	4.96	38.92 (Og 38.3)	-	-	24.92 (Og 19.2, Aw 5.3)
8, 9 July 1974	Surface	15.01 (Aw 10.4)	1.67	2.45	23.74 (Ma 21.4)	4.07	0.52	-	-	0.38	0.65	0.52	2.81	0.46	0.57
	Bottom	13.91 (Aw 8.1, Ma 3.7)	4.88	-	12.41 (Aw 5.6, As 5.6)	10.13[e]	-	-	-	15.95 (Aw 2.3, Aj 8.4)	3.85	8.03	-	-	4.27
12, 13 August	Surface	26.72 (Aw 18.7, As 5.7)	9.77	13.27 (Aw 6.9, Aj 3.9)	16.70 (Aw 14)	6.42	1.63	-	-	3.05	0.96	0.56	1.00	0.85	1.03
	Bottom	15.81 (Aw 13.7)	15.28 (Aw 9.5, As 2.8)	-	22.72 (Aw 7.3, Asp 10.1)	6.29	-	-	-	14.23[f]	0.00	1.11	-	-	1.85

[a] (Ac 3.8%; Aw 3.8%; Rc 4.9% and Ma 2.2%). [b] (Og 69.9%; As 2.5%; Aw 4.6%). [c] (Og 75.9%; Aw 6%; Asp 2.5%). [d] (Og 65.9%; Aw 6%; Av 3.8%).
[e] (Aw 1.8%; Ma 2%; As 2.4%; Ot 2.7%). [f] (Aw 2.1; Aj 5.3; Av 1.2; Ot 4.6).

Various *Oscillatoria* species have been known for years as indicators of serious eutrophication problems (Fogg et al., 1973; Whitton, 1973). Although they are poised as a threat, it is postulated that water quality conditons in Lake Wylie (pH, nutrient loadings, organic matter, dissolved oxygen, temperature) would have to change dramatically before either *Oscillatoria* or *Microcystis* will become a threat in fact.

SUMMARY AND CONCLUSIONS

The key findings from the bioassay studies, ancillary laboratory experiments, and the field observations are summarized below:

A. Phosphorus was identified as a limiting nutrient in Lake Wylie waters.

B. Overall, it appears that an approximate doubling of the mean annual available phosphorus concentration in the Lake Wylie study area would have to occur before another nutrient would become limiting.

C. The bioassays did not indicate any influence of the Allen Steam Station on the ability of the Lake Wylie study area waters to support algal growth as a function of nutrient content.

D. No measurable effects of urbanization were encountered in the study.

E. Ancillary laboratory experiments and field observations support the hypothesis that the Lake Wylie study area waters are not conducive to the overabundant growth of potential nuisance blue-green algae, and that the low hydrogen ion concentration may be prominent among the factors responsible.

F. Field observations suggest that the genus *Oscillatoria* was the most prominently represented blue-green algae during the study period.

ACKNOWLEDGMENTS

The water chemistry analyses were supervised by Kerstin E. Katnik and Julie V. Dacone, Assistant Chemists. Collection and analysis of phytoplankton was performed by Edward W. Wilde and Timothy A Paulishen; and of periphyton by Ann Jackson. Overall project supervision was the responsibility of Drs. David W. Anderson (Charlotte, North Carolina Laboratory) and Gerald W. Wadley (Northbrook, Illinois Laboratory). The helpful assistance of Dr. Arnold Gnilka, Duke Power Company is gratefully acknowledged.

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Nutrient Assessments as a Basis for Lake Management Priorities

P. D. Uttormark and J. P. Wall*

INTRODUCTION

In the contiguous 48 states there are roughly 100,000 lakes, give or take thousands depending on the definition of "lake." Of these, about 15,000 are larger than 40 ha (100 acres)—a size capable of supporting sufficient recreational use to give these lakes a degree of regional importance. The immense value of these natural resources is recognized, as evidenced by large-scale efforts to combat the effects of eutrophication. Recent examples include state and local bans on phosphate detergents (with consideration of eliminating the use of phosphate detergents nationally) and the 1972 Amendment to the Federal Water Pollution Control Act which authorizes 300 million dollars for lake renovation and protection. Considering the cost and social impact of these actions, it would be extremely beneficial if more information were available which characterizes the lake resource and provides guidelines for appropriate management options. Quantified information is needed so that alternatives can be evaluated realistically and management priorities, consistent with the resource base and public needs, can be established.

As an initial step, it would be desirable to know the number, size and location of lakes which comprise the following three groups:

1. Those lakes which are presently of satisfactory quality and are not likely to degrade seriously in the future.

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2. Those lakes which are presently in satisfactory condition, but are susceptible to degradation.

3. Those lakes which have degraded to the point that renovation would be desirable.

This knowledge would provide a much-improved perspective of large-scale lake management needs and would provide a basis for establishing management objectives and priorities. Unfortunately, it is not possible to accomplish the above grouping with any significant degree of certainty—the necessary information is simply not available for the vast majority of lakes. This paper deals with one approach for assessing broad-scale management options. It is based on a combinaton of lake classification techniques and estimates of phosphorus loadings of lakes.

It is assumed that trophic condition is a continuous function of specific P-loading rates. Thus, if loading rates are plotted against lake condition, a graph similar to that shown in Figure 1 would be expected where different lakes are represented by each of the data points.

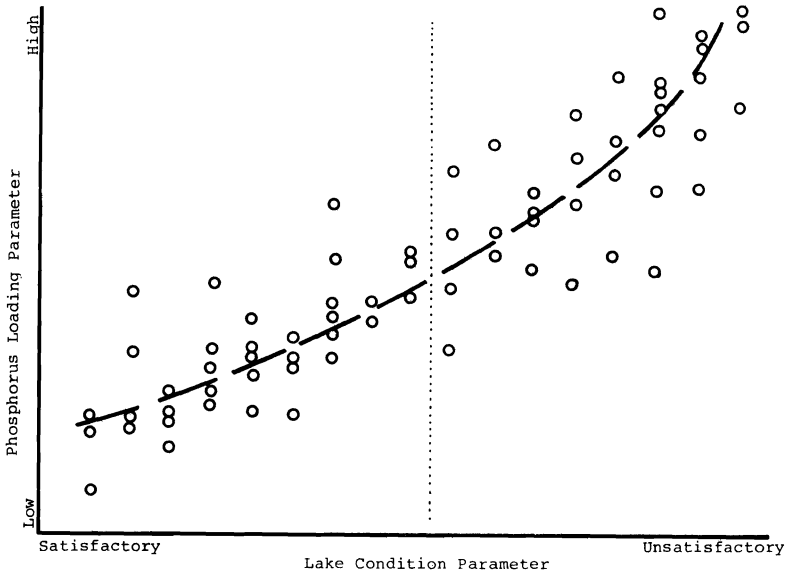

Figure 1. Relationships between phosphorus loading and lake condition (hypothetical data).

(Hypothetical data are shown in Figure 1.) The dashed line represents the stable or equilibrium relationship between P-loading and lake condition. Obviously, the process of eutrophication is far too complex to be expressed uniquely by these two parameters, and thus some degree of data scatter would be expected. However, if the data are restricted to a fairly small geographical area, i.e., a single state as opposed to the entire United States, data scatter would be minimized and it should be possible to define a reasonable relationship between loading rate and lake condition. The advantages of developing such a relationship are outlined as follows.

Some insight into possible management options can be obtained from a plot of the type shown in Figure 1 if it is viewed as being divided into four "quadrants" fromed by the intersection of the dashed line with a vertical line (small dots) that separates lakes of satisfactory conditon from those whose condition is unsatisfactory. Thus, those points on the left side of the plot represent lakes of satisfactory condition; those above the dashed line have "high" loadings with respect to their present condition. This subdivision of the plot results in four sets of lakes to which general management may be assigned.

Set	Phosphorus Loading	Lake Condition	General Water Quality Management Grouping
a	low	satisfactory	No present danger
b	low	unsatisfactory	Renewal desirable; long-term benefits may be possible without extensive nutrient abatement
c	high	satisfactory	Prompt protection needed; degradation may be imminent
d	high	unsatisfactory	Problem lakes; renovation desirable but lasting improvement may require extensive nutrient abatement

Set a. These lakes of high quality would be identified as unendangered. The management approach for these lakes might be to maintain the status quo, i.e., protection to avoid degradation. These lakes would be good candidates for inclusion in monitoring programs designed to measure "background levels" of chemical constituents. Also, by the

addition of information relating to public access, shoreline development, public ownership, etc., some of these lakes could be selected for special purpose management, such as "wilderness" recreation area.

Set b. Lakes in this group would be prime candidates for lake renewal efforts because of the possibility for lasting improvement. In-lake renewal techniques, such as aeration, sediment manipulation, etc., could yield long-term benefits in this type of situation because the influx of nutrients from external sources is small. This might be a particularly advantageous approach if there is reason to believe that prior actions (such as the improvement of upstream waste treatment facilities) have reduced present levels of nutrient influx below levels which occurred previously.

Set c. The condition of lakes in this group would be expected to be progressing toward further degradation. Based on the general consensus that eutrophication prevention is better understood than eutrophication reversal, and that preventive management may be more economical than restorative measures, these lakes require protective action with some degree of urgency. Nutrient removal from wastewaters in the drainage basin could be a high priority consideration.

Set d. The lakes in this group are not only fertile, but also receive high inputs of nutrients. Extensive nutrient abatement may be required before long-lasting lake rehabilitation could be anticipated. Perhaps the immediate focus of management for these lakes should be to ease the symptoms of excess fertility and to direct use toward those activities compatible with fertile waters until renewal techniques are more refined and related costs and benefits are better defined. Another option would be to manage the lake and shorelands as fertile areas, emphasizing environmental diversity and high productivity as positive attributes.

By noting the number of lakes in each set, their size, and perhaps auxiliary parameters, such as the proximity to population centers, some general management priorities can be ascertained. For example, if the majority of a state's lakes fall into sets c and d, a concerted program of nutrient abatement would be of high priority. If the majority of lakes fall into group b, the development and refinement of in-lake renewal techniques might be a high priority objective. Of course, a clean line of distinction between "high and low" and "satisfactory and unsatisfactory" is difficult to establish, and selected levels would be influenced by personal preferences. Nevertheless, the approach does place the various management options in an improved perspective and requires that preference levels be quantified. Both aspects are advantageous for sound resource management.

LAKE CONDITION

To accomplish the assessment of lake management options described above, it is necessary that lake conditions be quantified. A number of schemes for classifying lakes numerically according to trophic status have been reported in the literature. A summary of these is given in Table 1. This summary illustrates some of the difficulties related to developing these systems. There presently exists no generally-accepted definition of the term, "trophic status." This is pointed out quite clearly by the different parameters which were selected as indicators, and the different ways in which these parameters were combined in the various systems. The number of input parameters ranged from 4 to 16 and, in some cases, single measurements were sufficient while repetitive determinations were required in others.

Lack of a precise definition of "trophic status" also makes it difficult to assess the "accuracy" of different systems. At best, one can evaluate the results on a subjective basis to see if they appear to be reasonable. All of the techniques used were judged by the investigators to yield reasonable results. In most cases it was felt that the technique might have broader application, and it was suggested that the systems be applied with caution elsewhere.

The techniques reported by the U.S. EPA (1974), Feuillade (1972), Lueschow et al. (1970), McColl (1972) and Sheldon (1972) are all relative systems in which lakes are classified only with respect to each other and not to some independent scale. As a consequence, none of these techniques is applicable for developing nutrient loading-lake condition relationships because the index values do not relate directly to trophic character. The index values have no absolute meaning; they are simply indicative of a lake's position within a group of lakes which have been "sorted" by the classification process.

It has been demonstrated that the system devised by Shannon and Brezonik (1972) is useful for determining lake loading-condition relationships, and the technique reported by Newton and Fetterolf (1966) may also be applicable. However, lack of data prevents the use of these systems for most lakes in Wisconsin. Because of this, a classification technique was developed which is based on some of the more readily observable indicators of eutrophication (Uttormark and Wall, 1975). "Penalty points" were assigned to lakes depending upon the degree to which they exhibited undesirable symptoms of water quality. Four input parameters were selected, and ranges of values for each parameter were specified to depict lake conditions ranging from desirable to undesirable. The parameters used and the range of possible points assigned are listed in Table 2.

The parameters were treated independently, and composite lake ratings were determined by summing the number of points assigned in each of the four categories. The sum was termed a "Lake Condition Index" (LCI). Thus, if a lake exhibited none of the specified undesirable

Table 1. Summary of systems to classify lakes according to trophic status.

Investigators	Lakes Classified	Group	Rank	Independent	Relative	Parameters Used
EPA	209-USA		X		X	DO, tot-P, sol-P, inorg-N, Secchi, chlor-*a*
Feuillade	2-France	X			X	Temp, DO, cond, NH_3-N, NO_3-N, NO_2-N, Ca, Mg, Cl, SO_4, Si, sol-P, K, Na, hardness, alk
Lueschow et al.	12-Wisconsin		X		X	DO, plankton wt, Secchi, org-N, inorg-N (avg monthly values)
McColl	7-N. Zealand	X			X	DO, Secchi, alk, chlor-*a*, pigment, tot-P, sol-P, NO_3-N, NH_3-N
Newton and Fetterolf	10-Michigan		X	X		Sol-P, COD, org-N, NH_3-N
Shannon and Brezonik	55-Florida		X	X		Secchi, cond, org-N, tot-P, prim. prod, chlor-*a*, Ca, Mg, Na, K (avg of 3-6 seasonal values)
Sheldon	121-N. America	X			X	pH, trans, alk, temp, depth, DO
Sheldon	15-Sweden	X			X	pH, trans, color, $KMnO_4$ demand, cond

Table 2. Point system for lake condition index.

Parameter	Points
Hypolimnetic dissolved oxygen	0-6
Transparency	0-4
Fishkills	0-4
Use impairment (extent of macrophyte or algal growths)	0-9
Total	0-23

symptoms of eutrophication, it received no points (LCI = 0). Conversely, for a lake to have an LCI of 23 it would have had to have all the undesirable characteristics in the most severe degree. Details of the classification methodology are given by Uttormark and Wall (1975).

After the classification procedure was subjected to a number of tests to establish the "reasonableness" of results, it was used to calculate an LCI value for all lakes in Wisconsin with surface areas larger than 40 ha (100 acres). Summary results based on 1129 LCI determinations are presented in Figures 2 through 4.

A frequency distribution based on the numbers of lakes having specific LCI values is shown in Figure 2. The distribution is very much skewed to the left, with more than 50 percent of the lakes having an LCI of 6 or less. A frequency distribution was also plotted as a function of surface area as shown in Figure 3. Data for Lake Winnebago (LCI = 13, area = 137,708 acres) are not included in this plot because the lake is so large. However, even without the inclusion of this data, there is a shift in the distribution toward the higher LCI values. Whereas only 20 percent of the lakes had LCI values of 10 or greater, these lakes included 31 percent of the total area (43 percent if Winnebago is included). Similar results are shown in Figure 4 in which average lake size is plotted against LCI. As shown on this plot, average lake sizes are much larger at the higher LCI values.

Although LCI values are not necessarily synonymous with trophic status—two equally productive lakes could have different LCI values, depending on the manner in which this productivity influences the oxygen regimen, transparency, fish survival and recreational uses of the lakes—additional perception of the condition of Wisconsin's lakes is

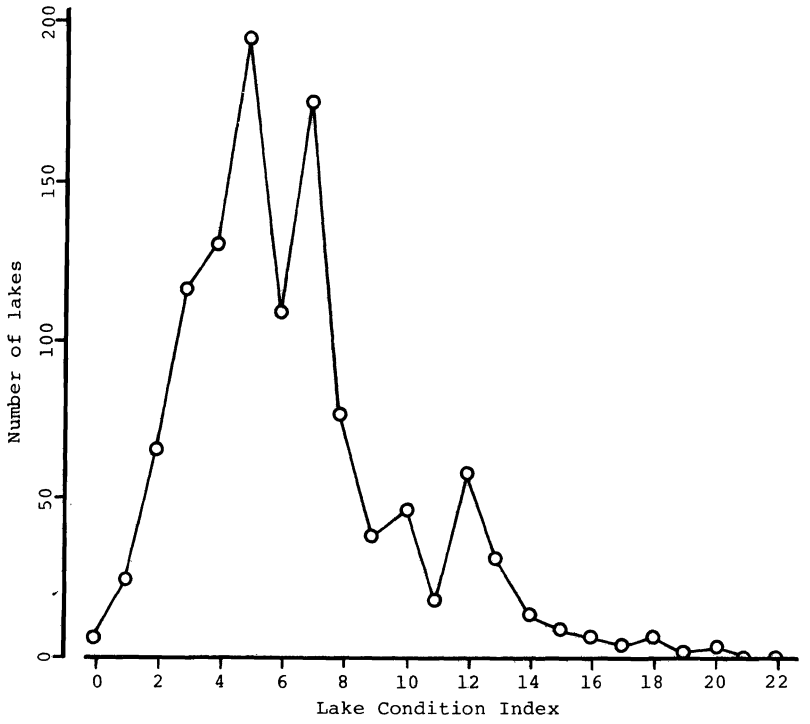

Figure 2. Frequency distribution of Wisconsin lakes according to condition index.

attained by relating these values to the traditional limnological classifications. With qualification, the following comparisons apply:

LCI	Trophic classification	Number of lakes
0-1	Very oligotrophic	28
2-4	Oligotrophic	308
5-9	Mesotrophic	586
10-12	Eutrophic	125
13-	Very eutrophic	82
		1129

Thus, approximately 30 percent of the lakes might be considered oligotrophic; 50 percent, mesotrophic; and 20 percent, eutrophic.

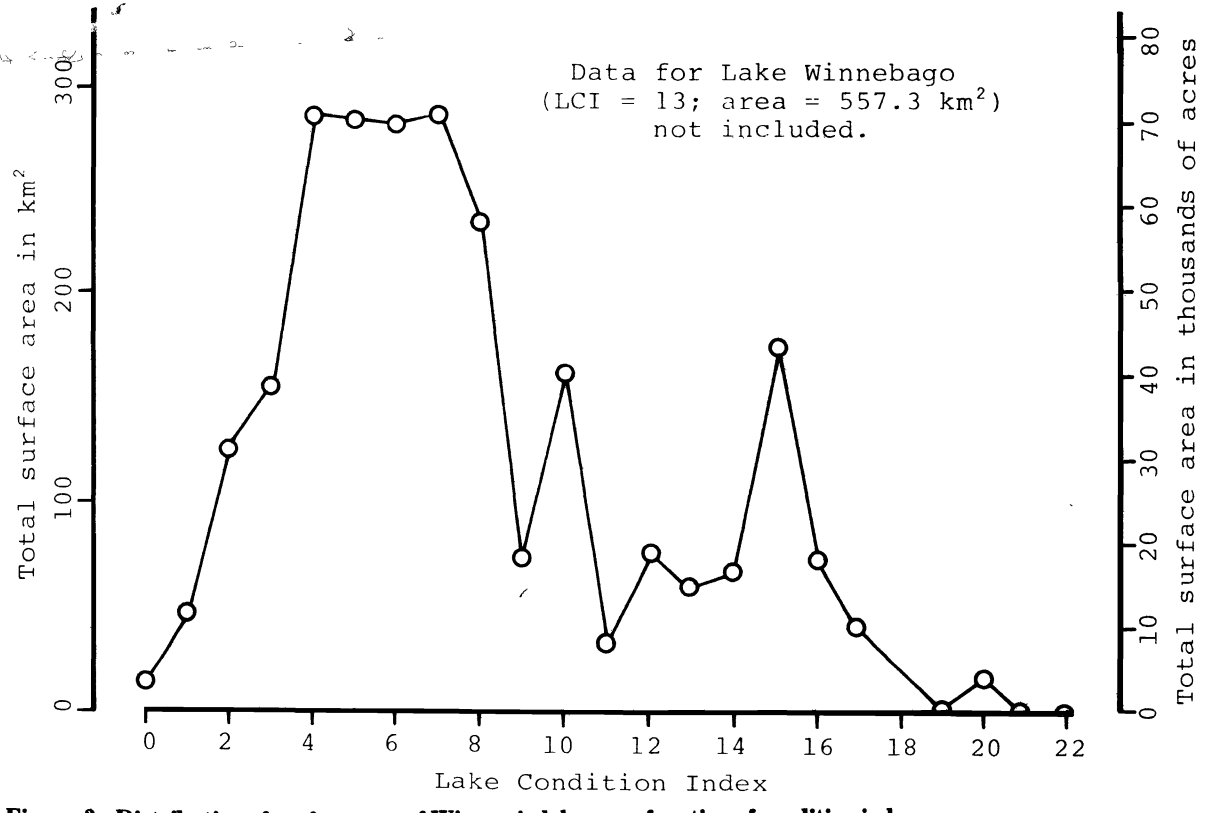

Figure 3. Distribution of surface area of Wisconsin lakes as a function of condition index.

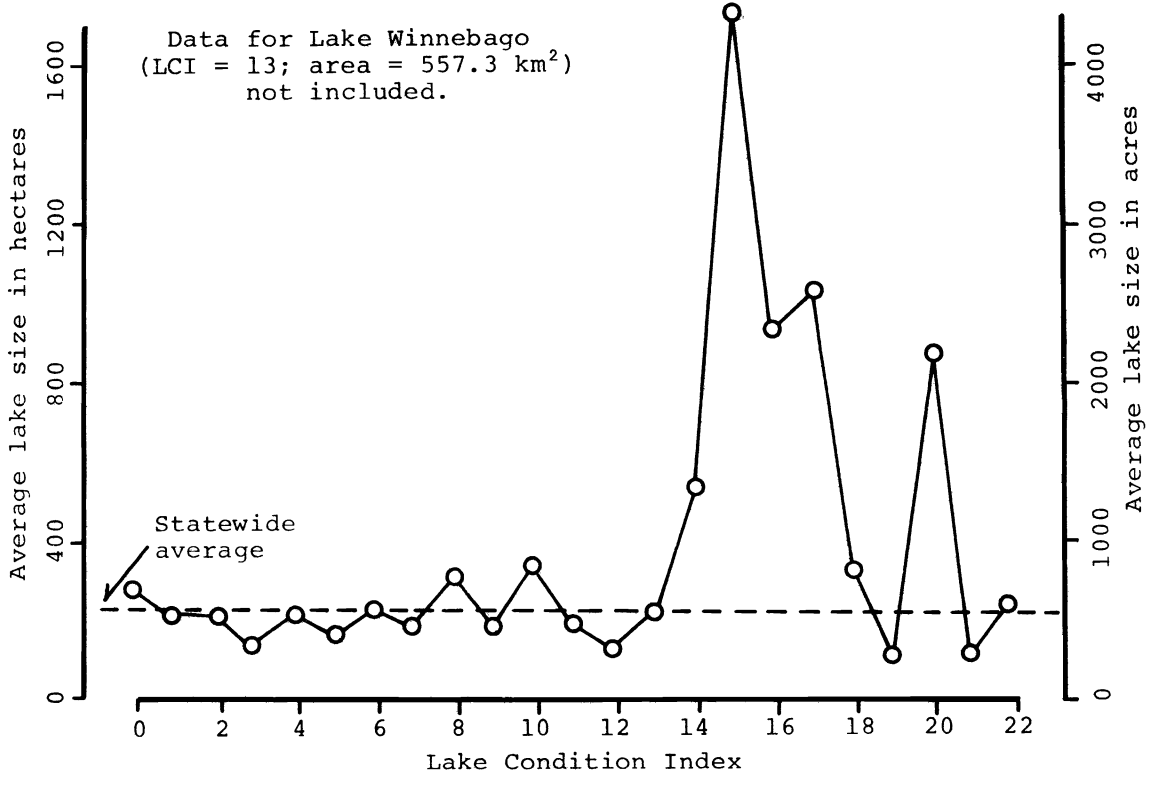

Figure 4. Average surface area of Wisconsin lakes as a function of condition index.

Assessing the adequacy of classification systems is a difficult task because universal definitions for these trophic categories are lacking, and there are no quantified baselines for comparison. In view of this, the LCI approach was subjected to a number of retrospective tests to check the validity of the technique. The results of two checks are given in the following.

For one check, the data set was divided into subsets according to lake "type" as shown in Figure 5. The warm/cold water lakes, i.e., lakes which support a cold water fishery in the hypolimnion, spanned the LCI range from 0 to 8, with the maximum number occurring at LCI = 4. Warm water lakes spanned virtually the entire LCI range, but the majority had LCI values between 2 and 9. LCI values for reservoirs (warm water fisheries, also) covered most of the total range, as well. These distributions were consistent with observed conditions in Wisconsin.

Average LCI values for various types of lakes in Wisconsin are given below:

Lake type	Number of lakes	Avg LCI
Warm/cold water fishery	173	3.8
Warm water fishery	739	6.6
Reservoirs	191	7.8
Bog lakes	8	8.0
Marsh lakes	18	12.5
Total	1129	6.5

As part of the test conducted early in the project, it was estimated that LCI values were reproducible to within ±2 units when different sources of input data were used to classify the same lakes. After all the lakes were classified, the results were then submitted to area managers in the Wisconsin Department of Natural Resources for their assessments of the extent to which computed LCI values portrayed the actual water quality characteristics of Wisconsin lakes. Of the 1129 lakes classified, 303 lakes were reviewed in detail by the area resource managers. A summary of their critiques is given in Table 3.

It was found that 202 (66 percent) of the LCI values reviewed were left unchanged; 60 scores (20 percent) were changed by 2 or fewer points; and only 41 scores (14 percent) were considered to be in error by 3 or more points. It was concluded that the LCI values gave a reasonably good perspective of water quality conditions for the more than 1100 lakes in the study set, and that these index values could be used to quantify lake conditions in Wisconsin.

Table 3. Summary of classification system review by Wisconsin DNR area managers.

Area Number	Total Lakes	LCI Number Unchanged	LCI Number Changed by 2 or less	LCI Number Changed by 3 or more
1	9	2	6	1
2	42	32	4	6
3	32	20	6	6
4	62	52	7	3
5	84	63	11	10
6	16	11	4	1
7	21	5	10	6
8	23	8	9	6
9	14	9	3	2
Totals	303(100%)	202 (66%)	60(20%)	41(14%)

NUTRIENT LOADINGS

Whereas information relating to water quality conditions in lakes is sparse, nutrient loading data are almost nonexistent. As a substitute, heavy reliance is placed on estimated loading rates which are based on the quantities of nutrients which are expected to be carried from watershed areas by runoff and streamflow. The nutrient flux data are typically given as average annual export per unit area for various types of land use.

Table 4 gives a summary of nutrient flux coefficients for runoff from watersheds. The values listed are based on reported studies which were conducted at sites scattered throughout the United States, and include a few studies from foreign countries as well. The values listed were obtained by averaging and comparing available coefficients without regard to geographical location. Thus, the specification of high or low values is relative to the other numbers in the data set, and the average values do not necessarily apply to all portions of the country.

Unfortunately, drainage basin characteristics are not known for most lakes in Wisconsin, and therefore it is difficult to make reasonable estimates of P-loadings. Most drainage basins have not been delineated, topographic quadrangle maps have not been prepared for all parts of the state, and land use information is fragmentary. To test the validity of the general approach for assessing lake management options in Wisconsin,

Table 4. Typical values of nutrient runoff coefficients[a].

	NO_3 -N+NH_4 -N kg/ha/yr			Total-N kg/ha/yr		
Land Use	High	Low	Ave	High	Low	Ave
Urban	5.0	1.0	2.0	10.0	2.5	5.0
Forests	3.0	0.5	1.6	5.0	1.0	2.5
Agricultural	10.0	1.0	5.0	10.0	2.0	5.0
	Diss inorg-P kg/ha/yr			Total-P kg/ha/yr		
	High	Low	Ave	High	Low	Ave
Urban	2.0	0.5	1.0	5.0	1.0	1.5
Forests	0.1	0.01	0.05	0.8	0.05	0.2
Agricultural	0.5	0.05	0.1	1.0	0.1	0.3

[a]From Uttormark, Chapin, and Green (1974).

estimates were made of the P-loading rates for a subset of selected lakes. The following criteria were used in selecting the subset:

1. Lakes should have both an inlet and an outlet to eliminate lakes dominated by groundwater flows (Born et al., 1974).

2. Lakes should be classified as "natural" or "natural with a level control" marsh lakes, bog lakes, and flow-through reservoirs were excluded.

3. Lakes should exceed 8m (25') in depth to eliminate lakes which do not retain seasonal stratification. (Four lakes were included which did not meet this criterion.)

4. Lakes should not have known point sources of nutrients (as listed by Wall et al., 1973).

In addition, it was required that the selected lakes lie in regions for which topographic maps were available, and an attempt was made to include lakes of differing sizes and LCI values. These criteria were stipulated to maximize the possibility that surface water inflows provide the primary mode of nutrient transport to the lakes and to minimize the effect of hydraulic flushing rates (Vollenweider, 1975; Dillon, 1975).

Based on these criteria, 34 lakes were selected for study. Drainage basin boundaries were delineated on U.S. Geological Survey maps (1:24000 or 1:48000 scale), and the areas devoted to various land use categories were calculated using information obtained from aerial photos of the watersheds. The basins were subdivided using the following land use categories:

1. Urban—municipal areas drained by storm sewer systems
2. Agricultural—crop lands and pasture
3. Forest—all wooded lands
4. Open—cleared, rural lands not presently devoted to agriculture
5. Wetlands—marshes and swamps with emergent vegetation

Estimated P-loadings were calculated using the runoff coefficients shown in Table 5.

Somewhat higher coefficients were used for watersheds in southern Wisconsin to reflect the more intense urbanization and agricultural activity of that portion of the state. Contribution from cottages (septic tanks) was increased to reflect the higher proportion of lakeshore dwellings which are occupied year-round.

A plot of estimated P-loadings versus LCI values for the selected subset of Wisconsin lakes is given in Figure 5. As shown in that figure, considerable data scatter resulted and, consequently, no attempt was

Table 5. Phosphorus runoff coefficients in kg/ha/yr.

Land Use	Northern Wisconsin	Southern Wisconsin
Urban	1.0	1.5
Agriculture	0.3	0.4
Forest	0.2	0.2
Open	0.2	0.2
Wetlands	0.0	0.0
Cottages	1 kg/yr	2.5 kg/yr

made to define a mathematical relationship between estimated loading rates and LCI values. Therefore, it is not possible to designate the four water quality management subsets in the manner described previously. As a substitute, Figure 5 is divided into four quadrants by the lines, P-loading = 0.13 g/m^2/yr and LCI = 9. Vollenweider (1968) suggested that P-loading rates in excess of 0.13 g/m^2/yr were "dangerous," i.e., they tended to produce eutrophic conditions in lakes with mean depths of 5m (16′) or less. The line LCI = 9 is the approximate point of division between mesotrophic and eutrophic lakes. Thus, the four quadrants shown provide an approximation of the desired management categories, but they do not incorporate loading criteria of known validity to Wisconsin lakes.

It may be noted from Figure 5 that several lakes with relatively low LCI values have estimated P-loading rates which are characteristic of

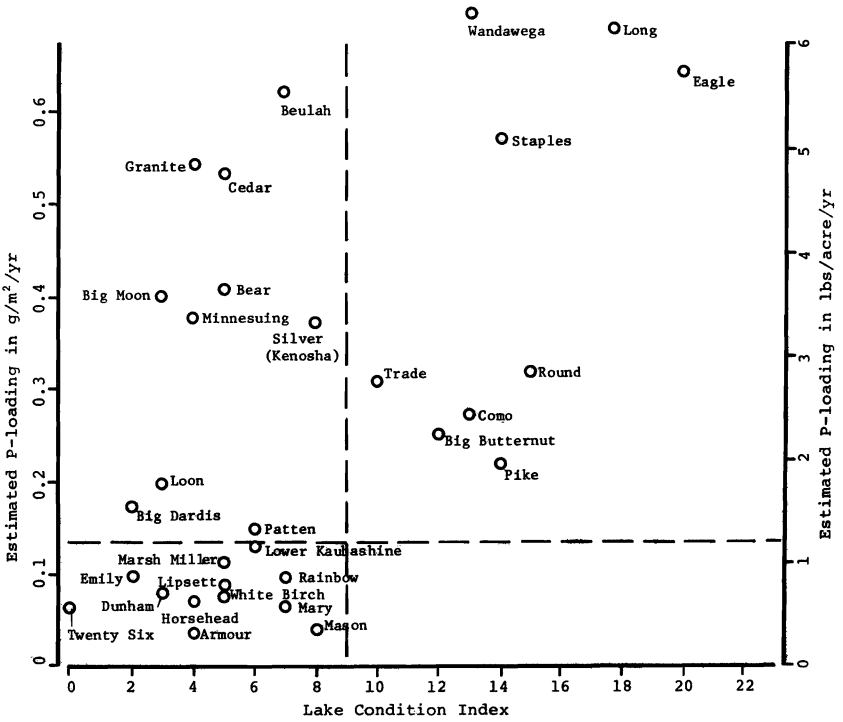

Figure 5. Estimated P-loading versus LCI values for selected Wisconsin lakes.

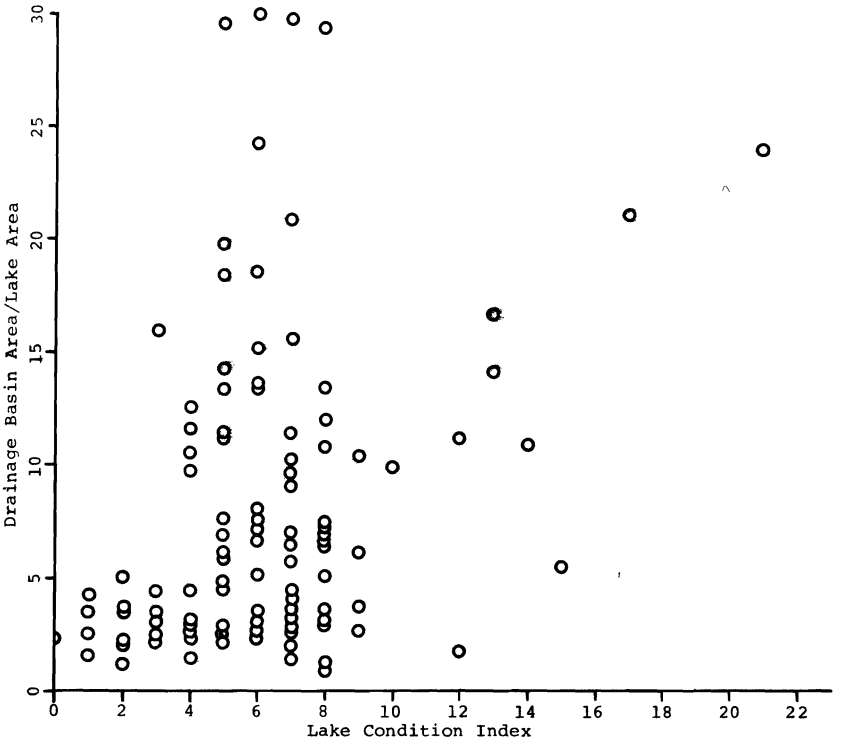

Figure 6. Relative drainage area versus lake condition for selected lakes in Wisconsin.

eutrophic lakes. Similar findings also resulted from preliminary studies which compared drainage basin area/lake area ratios to LCI values for selected lakes. If it is assumed that point source contributions of P are negligible, that surface water inflows provide the primary mode of P transport to the lakes, and that the same mean P runoff coefficient applies to each lake watershed in question, then the ratio of drainage area/lake area is proportional to the specific P-loading rate. By using area ratios as a rough approximation of loading rates, it was possible to consider a larger number of lakes, so an analysis was undertaken which utilized drainage basin data obtained from the Wisconsin Department of Natural Resources. A set of lakes was selected using the selection criteria outlined above, and the area ratios were plotted against LCI as shown in Figure 6. Again, it was found that many lakes with low LCI values had high apparent loading rates. Several factors could account for this: (1) The results could be due to errors in the technique, however, the opposite condition—high LCI and low apparent loadings—was far less common;

(2) the lakes may be receiving excessive loadings and are in the process of degradation; (3) nutrient contributions from some watersheds may be far less than anticipated; or (4) some lakes are capable of receiving relatively large nutrient loadings without exhibiting common symptoms of eutrophication. (It is unlikely that hydraulic flushing rates could account for the discrepancies in the lakes studied.) Based on the information available, it appears that items 3 and/or 4 provide the most likely explanation for the anomalous results. Such lakes should be studied to determine why they do not appear to follow the expected pattern of loading versus condition. Such findings would have definite management implications.

Perhaps the best data available for evaluating the applicability of the nutrient loading-lake condition approach for Wisconsin lakes are shown in Figures 7 and 8 which were prepared by the Wisconsin Department of Natural Resources. Sufficient information was compiled to permit the preparation of a plot (Figure 7) of the type suggested by Vollenweider (1975). Based on this plot, a "relative P-loading" for each lake was

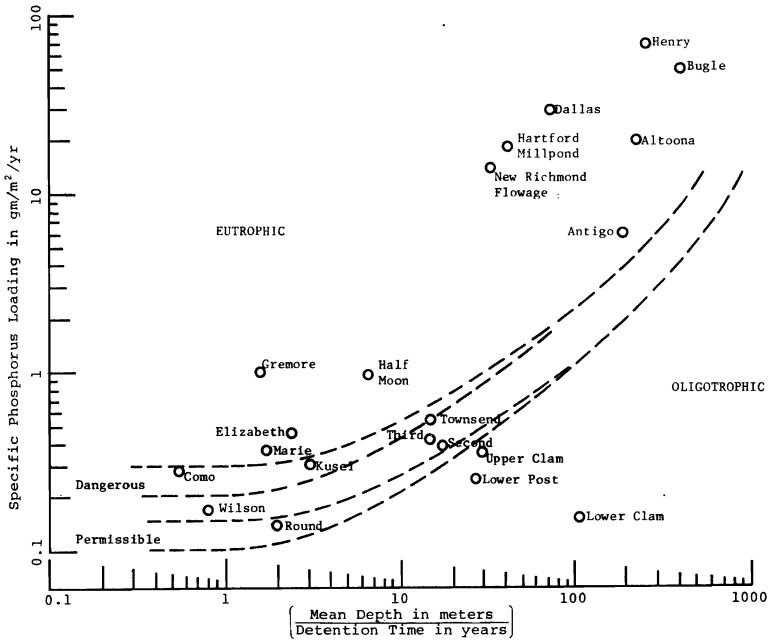

Figure 7. Phosphorus loadings for selected lakes in Wisconsin prepared by Office of Inland Lake Renewal of Wisconsin, Department of Natural Resources.

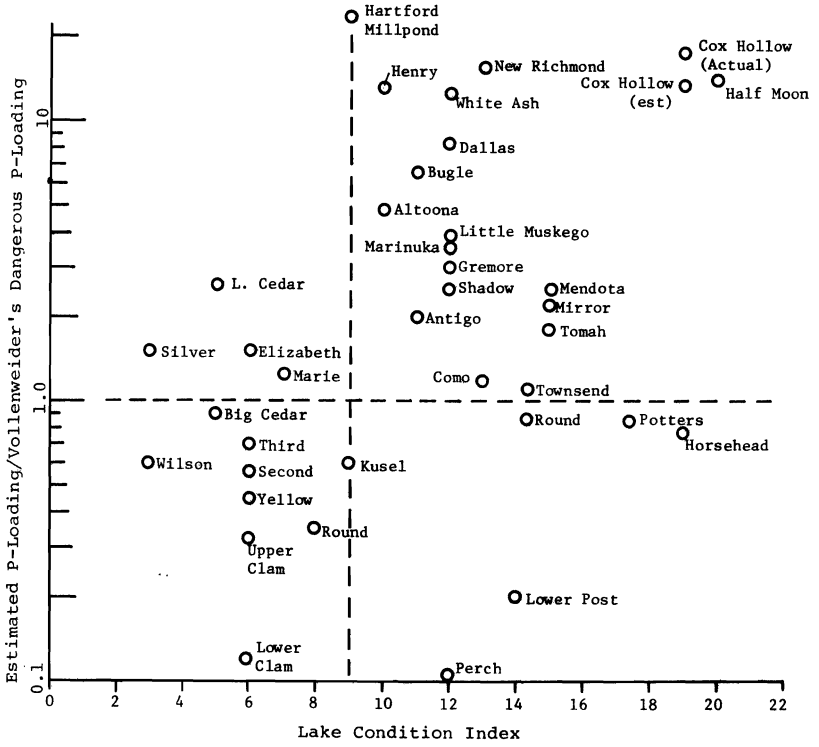

Figure 8. Relative phosphorus loading versus lake condition for selected lakes in Wisconsin prepared by: Office of Inland Lake Renewal of Wisconsin, Department of Natural Resources.

calculated by dividing the estimated P-loading by Vollenweider's "dangerous" level which corresponds to the mean depth and hydraulic detention time for each lake.

A plot of relative P-loading versus LCI is shown in Figure 8. The graph is divided into four quadrants by the lines, Relative Loadings = 1.0 and LCI = 9. If there exists a well-defined relationship between P-loadings and lake condition as defined by Vollenweider's input-output approach and the LCI methodology (as hypothesized in Figure 1), then it would be expected that in Figure 8 most points would fall within a band extending from the lower left to upper right. As shown in Figure 8, the plotted points follow the expected trend from lower left to upper right, with only 9 of 37 points falling in quadrants II and IV. However, data scatter ($r = 0.34$) prevented determination of a meaningful expression for relating P-loading to lake condition.

In summary, an approach was developed which provides an improved perspective of alternatives and priorities for water quality management in lakes. At this point, the objectives have been met only in part. Although the approach is felt to be conceptually sound, questions remain as to whether it provides a realistic perspective of actual field conditions, or whether the parameters used to depict lake loading-condition relationship will require refinement before the results can be used with confidence in a management context.

ACKNOWLEDGMENT

This work was supported in part by the Wisconsin Department of Natural Resources (133:9726) and the U.S. Environmental Protection Agency (R-801363).

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Comparison of Semi-continuous and Continuous Flow Bioassays

J. H. Reynolds, E. J. Middlebrooks, D. B. Porcella, and W. J. Grenney*

INTRODUCTION

Background

The use of bioassays for the assessment of biostimulation and toxicity has long been recognized. However, the challenge to develop a standard bioassay technique was not met until the Joint Industry-Government Task Force on Eutrophication initiated a coordinated effort on development of a standard algal bioassay procedure (Toerien et al., 1971). This effort resulted in the adoption of the "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971) and the development of a continuous flow (chemostat) bioassay procedure (Porcella et al., 1970; Toerien et al., 1971). Both methods have been used extensively in recent years with varying degrees of success.

However, very little effort has been devoted to development of a standard semi-continuous flow algal bioassay procedure. Because the semi-continuous flow culture is an approximation of a continuous flow system (Aach, 1952; Fencl, 1966), it seems appropriate to compare these two systems and to determine their compatibility.

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Objectives

The general objective of this paper is to compare semi-continuous and continuous flow algal bioassay methods. In addition, the paper will present the basic theory relating semi-continuous flow cultures to continuous flow cultures and compare the results obtained from a toxicity bioassay study which employed both techniques.

THEORY

Semi-continuous Flow Cultures

A semi-continuous flow culture is defined as being a culture with a fixed volume from which a withdrawal of cell mass, substrate, and metabolites occurs at regular time intervals. Fresh substrate is added to the culture to replace that which has been removed. The semi-continuous process is characterized by a variation of the substrate concentration and consequently of the specific growth rate of the organism as shown in Figure 1. The lower curve (solid line) in Figure 1 indicates the variation of

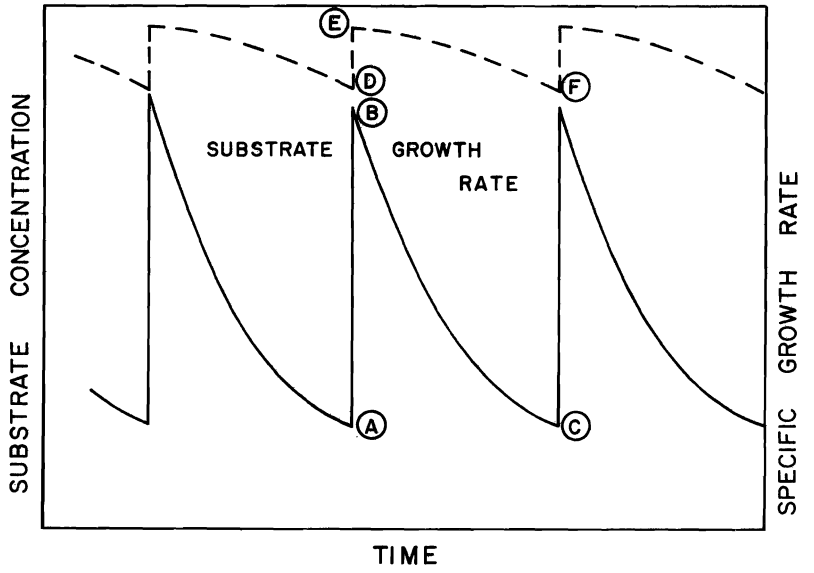

Figure 1. Schematic representation of substrate concentration and specific growth rate in semi-continuous cultures (Fencl, 1962).

substrate within the semi-continuous culture. At point A on the curve, the culture is at the point in time when a withdrawal is made from the culture. At this time, the substrate concentration is at a minimum. This corresponds to point D on the growth rate curve. The growth rate of the organism is also at a minimum due to the relatively low substrate concentration. Point B on the curve represents the concentration of substrate immediately after addition of fresh substrate. This is the maximum substrate concentration in the reactor and, thus, corresponds to point E on the growth rate curve. Point E represents the point at which maximum growth rate occurs. Between points B and C, substrate is gradually removed and the growth rate of the organism gradually declines (point E to F). Thus, in semi-continuous cultures, the specific growth rate of the organism and the substrate concentration is never constant or at "steady state." Rather, they fluctuate between two extremes. The magnitude of this fluctuation is dependent on the time interval between withdrawal and addition of substrate.

The effect of this fluctuation on cell mass is shown in Figure 2. If nutrients do not limit growth, the increase in cell mass with addition of substrate is similar to that for a batch culture in the exponential growth

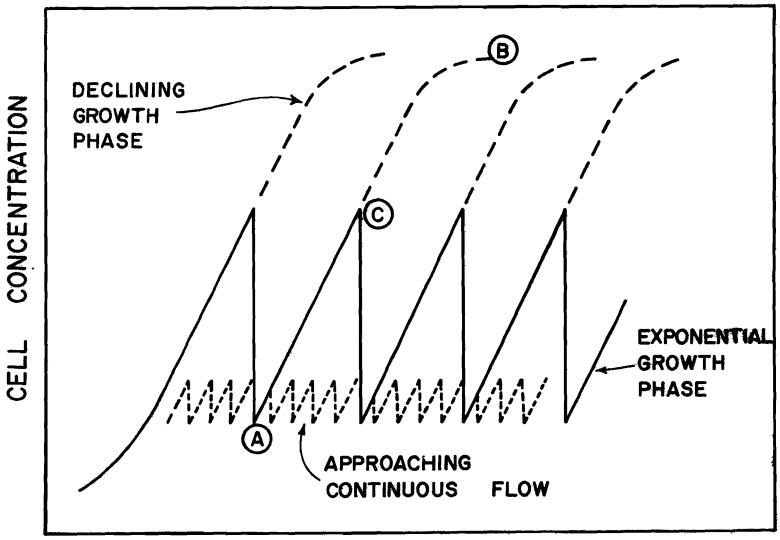

Figure 2. Schematic representation of cell mass variation in semi-continuous cultures (Fencl, 1966).

phase. This is represented between points A and C in Figure 2. However, if the interval between withdrawals is large compared to the maximum growth rate of the organism, or if substrate becomes limiting, cell production may decline as illustrated by point B.

For semi-continuous cultures to simulate continuous culture results, the withdrawal period must be small in comparison to the maximum specific growth rate of the organism and substrate concentrations must not be near limiting conditions. If this situation is achieved, the variation in cell mass will approach steady state as illustrated by the lower dotted curve in Figure 2, and the semi-continuous flow organism specific growth rate will approach the continuous flow organism specific growth rate (i.e., steady state value) for the same dilution rate.

Equation 1 (Fencl, 1966) describes the relationship between dilution rate, specific growth rate, and the number of withdrawals per time period.

$$D = n\left(1 - \frac{1}{e^{\frac{\mu}{n}}}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where

D = dilution rate, time^{-1}
n = number of withdrawals per unit time, time^{-1}
μ = organism specific growth rate, time^{-1}

Equation 1 is the fundamental formula, given the correlation between the dilution rate, D, and the specific growth rate, μ. This equation is plotted in Figure 3. The growth rate, μ, is plotted as an independent variable and the values of the dilution rate, D, and the number of withdrawals, n, are plotted as dependent variables. For different n, a nomogram of parabolic curves passing through the origin is obtained (Fencl, 1966).

As illustrated in Figure 3, the greater the number of withdrawals, the closer the dilution rate approaches the organism specific growth rate (assuming the organism specific decay rate, k_d, is very small and can be neglected). When the value of n reaches infinity, the dilution rate equals the organism specific growth rate. This is also the point at which a semi-continuous flow culture becomes a continuous flow culture.

Continuous Flow Cultures

A 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

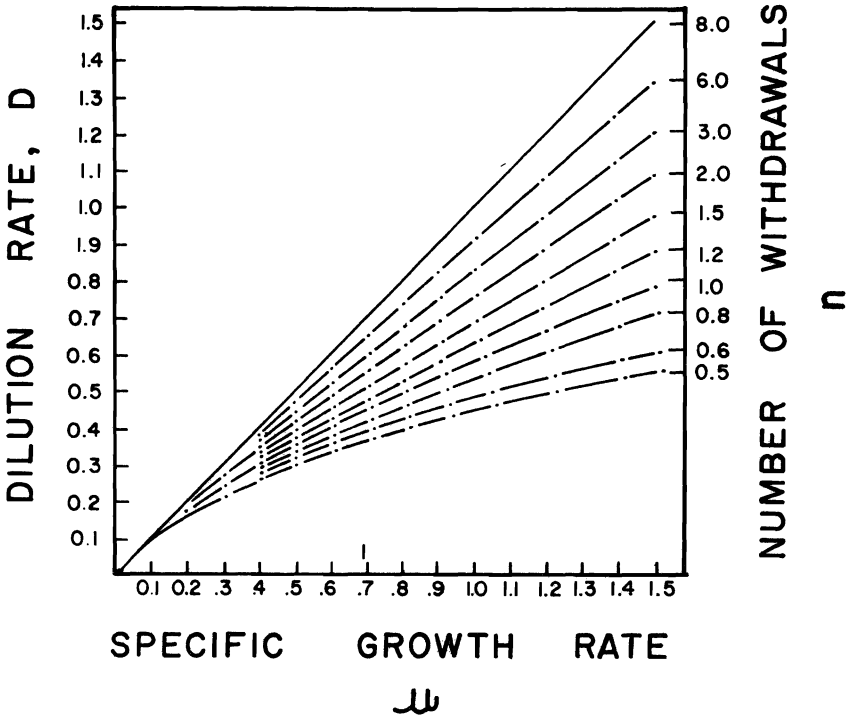

Figure 3. Nomogram expressing the dependence of the dilution rate, D, on the specific growth rate, μ, and the number of withdrawals (period) per unit time (after Fencl, 1966).

culture at a rate identical to the inflow rate of fresh medium, insuring 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 exponentially 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 exponentially 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.

The variation with time of the substrate concentration, S, cell mass, X, and organism specific growth rate, μ, in a chemostat at steady state for a constant dilution rate, D, is shown graphically in Figure 4.

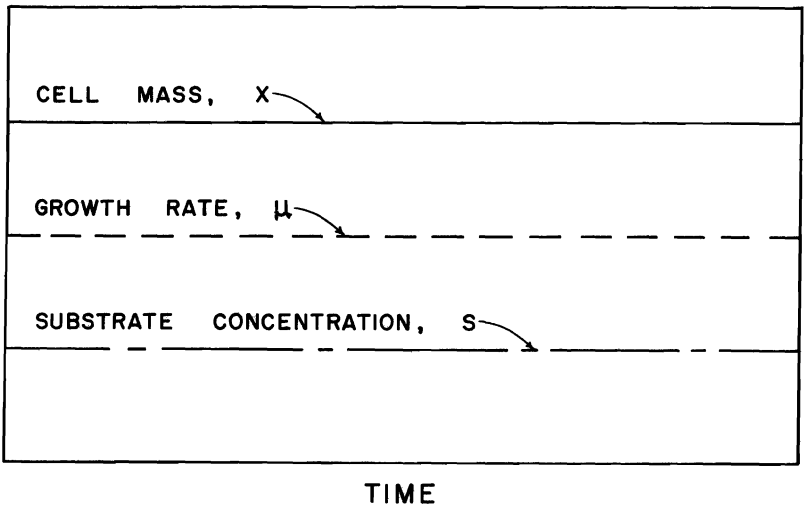

Figure 4. Theoretical variation in cell mass, X, organism specific growth rate, μ, and substrate concentration, S, in a continuous flow culture (chemostat) at steady state.

Theoretically, all of these parameters are constant at steady state. In practice, these parameters are allowed to vary within ± 10 percent of the steady state value (Porcella et al., 1970). This variation is due primarily to an inability to maintain exactly constant environmental conditions within the culture and also due to analytical techniques available for measurement of these parameters.

In general, the variation of these parameters in continuous flow cultures is significantly less that that observed in semi-continuous flow cultures.

The functional relationships which define a chemostat have been presented in detail by previous authors (Porcella et al., 1970; Toerien et al., 1971; Goldman, 1972) and will not be emphasized here. The following basic equations describing chemostat performance will be modified to include the effects of toxicants. The nomenclature of the equations is based on the "Unified Fundamental Symbols for Continuous Cultivation of Microorganisms" developed at the Second Symposium on Continuous Cultivation of Microorganisms held in Prague in 1962 (Malek et al., 1962). The expressions presented below for the cell concentrations in the chemostat (X_1), the limiting nutrient or substrate concentration (S_1) in the effluent, and the specific growth rate (μ) were developed from material balances for the chemostat.

$$X_1 = \frac{Y}{\mu\theta}(S_o - S_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$S_1 = \frac{K_s \left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\mu = \frac{1}{\theta} + k_d \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

in which

Y = net cell yield coefficient, or mass of organisms formed per mass of substrate used
μ = specific growth rate, time^{-1}
θ = mean residence time = V/F
V = volume of chemostat
F = flow rate, volume/time
S_0 = initial substrate concentration, mass/volume
S_1 = steady state substrate concentration, mass/volume
K_S = half saturation constant, concentration of substrate at which the growth rate is ½ of the maximum growth rate, μ, mass/volume
μ = maximum specific growth rate, time^{-1}
k_d = specific cellular decay rate, time^{-1}
X_1 = steady state cell concentration, mass/volume

Summary

Based on the foregoing discussion, it appears that semi-continuous flow cultures may nearly approximate conditions of a continuous flow (chemostat) system. In fact, it may be said that the continuous flow system is a special case of the semi-continuous flow system. However, the critical factor is the period of time between withdrawal of the culture content and addition of the fresh substrate.

TOXICITY EXPERIMENT

General

The following experiment will be used to illustrate the use of semi-continuous cultures in conjunction with continuous flow cultures. Although the experiment was not specifically designed to compare the use of semi-continuous and continuous flow techniques, it does provide data from which insights into the use of these two methods may be obtained.

The objective of the experiment was to develop a mathematical model to predict the effects of temperature on the toxicity of phenol to the green alga, *Selenastrum capricornutum* . Semi-continuous flow cultures were employed as an initial screening study to determine a workable range of phenol concentrations to be used in the later continuous flow experiments.

The semi-continuous flow experiments required limited space and capital investment. In addition, it was felt that by utilizing a relatively short time between withdrawals, that the results would approximate those from the continuous flow cultures.

Experimental Procedures

General. The original culture of *Selenastrum capricornutum* was obtained from the Pacific Northwest Environmental Research Laboratory of the Environmental Protection Agency, located at Corvallis, Oregon. Inoculum for both the semi-continuous and continuous flow cultures consisted of 10 ml of a 7 day old batch culture. The exact number of cells introduced into individual cultures was not rigidly controlled since these cultures would be measured at steady state conditions and not at a specified time period after inoculation. The cultures were considered to be at steady state when the range in variation in mean algal biomass (measured by cell count) was no more than ± 10 percent during a complete residence time.[1]

Semi-continuous flow cultures. The semi-continuous culture experiments were conducted in duplicate at 20°C, 24°C, and 28°C, and with phenol concentrations of 0, 20, 40, 60, 80, 100, and 120 mg/l. Temperature variation during the experiment was limited to ± 1°C.

The semi-continuous cultures were grown in a constant temperature environmental chamber,[2] in 500 ml Erlenmeyer flasks covered with steril tissue and 150 ml inverted Griffin beakers. Algal assay procedures (U.S. EPA, 1971) were employed with the following modifications. Each culture flask contained 250 ml of culture. One-third (83 ml) of that culture was withdrawn and replaced with fresh, sterile nutrient media every 24 hours (± 2 hours). Thus, the cultures remained at steady state with a mean residence time of 3 days.

The nutrient medium employed for the semi-continuous cultures was a modified PAAP solution (Porcella et al., 1970). The medium composition is shown in Tables 1 and 2. The original PAAP medium was modified by replacing $NaNO_3$ with NH_4Cl, to provide a final nitrogen concentration of 2.1 mg/l. Ammonium chloride was used to facilitate analysis of the growth limiting nutrient, nitrogen. Sodium bicarbonate was substituted for sodium carbonate to provide buffer capacity and also to provide essential carbon to sustain the algal growth. The final

[1]During the toxicant experiments this variation was slightly exceeded for a few cultures (±15 percent maximum).

[2]Sherer Controlled Environmental Lab, model CEL 37-14, Sherer-Gillet Co., Marshall, Michigan.

concentration of carbon in the medium was 80 mg/l. The medium was also buffered at pH 7.20 with a 0.03 phosphate buffer solution by varying the ratio of monobasic to dibasic sodium phosphate. This buffer was very satisfactory and pH variation was less than ± 0.10 pH units at steady state.

Cell counts, using a hemacytometer; pH measurements, using a Corning pH meter;[3] and optical density, read at 750 μm[4] with a 1 in. cell,

Table 1. Macronutrient composition of modified PAAP medium.

Components	Concentration mg/l	Essential Nutrient	Concentration mg/l
NH_4Cl	8.03	N	2.1
K_2HPO_4[a]	3.48	P	0.62
$MgCl_2$	19.00	Mg	9.68
$MgSO_4 \cdot 7H_2O$	49.00	S	6.37
$CaCl_2 \cdot 2H_2O$	14.70	Ca	4.01
$NaHCO_3$	571.43	K	1.56
$FeCl_3$	0.32	Fe	0.11
$Na_2EDTA \cdot 2H_2O$[b]	1.00	C	80.00

[a] 0.03 molar phosphate buffer was also added to control pH.

[b] Na_2EDTA = Disodium ethylene diamine tetra acetic acid.

Table 2. Micronutrient composition of modified PAAP medium.

Component	Concentration μg/l	Essential Nutrient	Concentration μg/l
H_3BO_3	618.40	B	110.00
$MnCl_2$	880.88	Mn	380.00
$ZnCl_2$	109.03	Zn	50.00
$CoCl_2$	2.60	Co	1.18
$CuCl_2$	0.03	Cu	0.01
$Na_2MoO_4 \cdot 2H_2O$	24.20	Mo	9.60
$Na_2EDTA \cdot 2H_2O$	7440.		

Note: The trace metals and EDTA were combined in a single stock mix at a level of 1000 times the final concentration.

[3] Corning Scientific Instruments pH meter, model 7.

[4] Bausch and Lomb, Spectronic 20, Rochester, New York.

were conducted daily on the 83 ml sample withdrawn from each culture. Upon reaching steady state the cultures were also analyzed for ammonia using the indophenol technique (Solorzano, 1969). Phenol determinations were made with a gas chromatograph[5] equipped with a flame detector and using isothermal operation procedures (Fisher Scientific Division, 1972).

During the experiment, attempts were made to limit bacteria; however, bacteria were present in the cultures. No attempt was made to determine the number of bacteria present. Because bacteria were present, the cell mass values for the semi-continuous flow cultures were calculated from the linear regression equation:

$$SS = 8.25 + 15.8\,(CC) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

in which

SS = cell mass in mg/l
CC = cell counts x 10^6 per ml

developed by Porcella et al. (1970).

Continuous flow culture experiments. The continuous flow experiments were conducted at 20°C, 24°C, and 28°C with pure phenol as the toxicant. The experiments involving pure phenol as the toxicant were conducted at hydraulic residence times approximately equal to 1 day, 1.5 days, and 2.0 days. Temperature variations in the chemostats during the experiments were less than ± 1.5°C.

The procedures employed during the continuous flow experiments are outlined by Porcella et al. (1970) except that air was not bubbled through the chemostat for mixing and to control pH; rather, pH was controlled by the buffer system used in the semi-continuous cultures. Complete mixing was provided by magnetic mixers (Goldman, 1972). The medium employed for the continuous experiments was the same as that used in the semi-continuous experiments, except that the ammonium-nitrogen concentration was increased to 4.2 mg/l to increase cell biomass.

Cell counts and pH measurements were conducted daily on a 30 milliliter sample withdrawn from each chemostate.

In addition, at steady state ammonium-nitrogen concentrations were determined using the indophenol technique (Solorzano, 1969) on a 50 ml sample withdrawn from each chemostat. Bacteria concentrations were determined by a spread plate technique (APHA, 1971; Post, 1973). Phenol measurements on the effluent from the 24°C chemostats were

[5]Hewlett-Packard Research Chromatograph, model 5750.

determined with the gas chromatograph (Fisher Scientific Division, 1972); however, the phenol concentrations in the effluent from the 20°C and 28°C chemostats were determined by the Direct Photometric Method (APHA, 1971). Suspended solids determinations were conducted by the technique described by Strickland and Parsons (1968).

Cell mass values (i.e., mg/l) used in the analyses of continuous flow data obtained from cultures with inhibitors at 20°C, 24°C, and 28°C were calculated from a linear regression equation of cell number versus cell mass which is shown in the results section of this paper. Semi-continuous cell mass values (i.e., mg/l) were calculated from an equation developed by Porcella et al. (1970).

Inhibition Model

The development of the kinetic inhibition model has been presented elsewhere (Reynolds et al., 1974a, Reynolds, 1974) and will not be presented in detail here. The model is based on Michaelis-Menten-Monod enzyme inhibition kinetics and describes the competitive inhibition of the organism by phenol. The linear form of the model is shown below.

COMPETITIVE INHIBITION

$$X_1 = \frac{Y S_o}{1 + \theta k_d} - \frac{\left(\frac{1}{\theta}\right) Y S_s}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} - \frac{\left(\frac{K_s Y}{K_I \theta}\right) I}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} \quad \ldots\ldots (6)$$

in which

X_1 = steady state cell concentration, mass/volume
Y = cell yield, mass organism formed per mass of substrate removed
θ = mean residence time, V/F, days
S_o = initial substrate or influent substrate concentration, mass/volume
k_d = specific cellular decay rate, time^{-1}
$\hat{\mu}$ = maximum specific growth rate, time^{-1}
K_s = half saturation constant, mass/volume
K_I = inhibitor constant, mass/volume
I = inhibitor concentration, mass/volume

The competitive inhibitor constant, K_I, in Equation 6 is a measure of the affinity of the inhibitor for the enzyme or organism. In practice, it is a measure of the toxicity of the particular toxicant to a specific organism.

The smaller the competitive inhibitor constant, K_I, the greater the toxicity of the toxicant to the organism. The value of the competitive inhibitor constant, K_I, may be obtained from the slope of a linear plot of steady state cell concentration, X_1, and inhibitor concentration, I, as shown in Figure 5.

Experiments conducted with a specific organism cell concentration and a series of inhibitor concentrations should reveal the type of inhibition exerted by the toxicant and the values for K_I, which will measure the strength of the toxicant. Experiments conducted at various temperatures should indicate if K_I is a function of temperature and should reveal the effect of temperature on the toxicity of a particular waste to a specific organism.

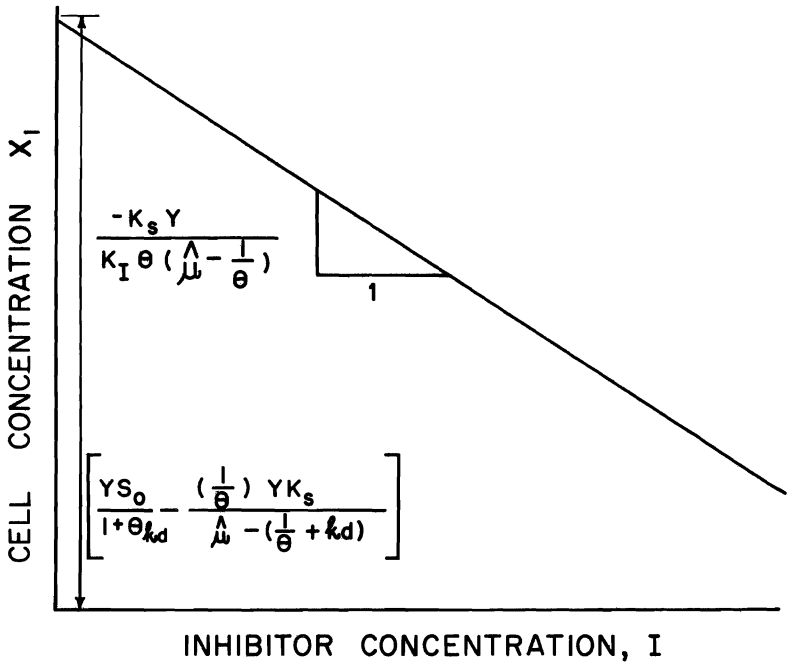

Figure 5. Linear plot of cell concentration, X_1, vs. inhibitor, I, for competitive inhibition.

Results

Semi-continuous flow culture experiments. Semi-continuous flow cultures of *Selenastrum capricornutum* (3 day residence time) were grown in phenol concentrations ranging from 0 to 120 mg/l at temperatures of 20°C, 24°C, and 28°C. The results of these experiments are summarized in Table 3. The individual data points presented in Table 3 represent an average of three samples measured during one complete residence time.

The data were fitted to Equation 6. A statistically significant correlation was obtained with the linear competitive inhibition equation, Equation 6. Results from the linear regression analyses for the competitive inhibition equation at 20°C, 24°C, and 28°C are shown in Figures 6, 7, and 8. The data points appear to be randomly distributed about the regression line and do indicate a significant amount of linearity.

The results of the linear regression analysis are summarized in Table 4. The maximum specific growth rate, μ, and the half saturation constant, K_S, employed for this particular analysis were obtained from the continuous flow experiments (Reynolds, 1974; Reynolds et al., 1974b).

The correlation coefficients for the linear regression analyses were all greater than 0.970. These values are substantially greater than the 1 percent significance level correlation coefficient of 0.798 and indicate an extremely high correlation of the data with the competitive inhibition equation (Equation 6).

Table 3. Cell concentration for semi-continuous experiments.[a]

Phenol Concentration (mg/l)	Cell count x 10^{-6}/ml[a]			Cell mass mg/l[b]		
	20°C	24°C	28°C	20°C	24°C	28°C
0	2.924	4.110	3.334	54.73	73.19	60.92
20	2.566	3.000	2.276	48.79	55.65	44.21
40	2.139	2.658	2.130	42.05	50.25	41.90
60	1.370	2.048	1.913	29.90	40.48	38.48
80	0.798	1.560	1.255	20.85	32.90	28.08
100	0.439	1.113	1.045	15.18	25.84	24.76
120	0.271	0.561	0.236	12.53	17.11	11.98

[a]Mean of the steady state measurements.

[b]Calculated from Equation 5.

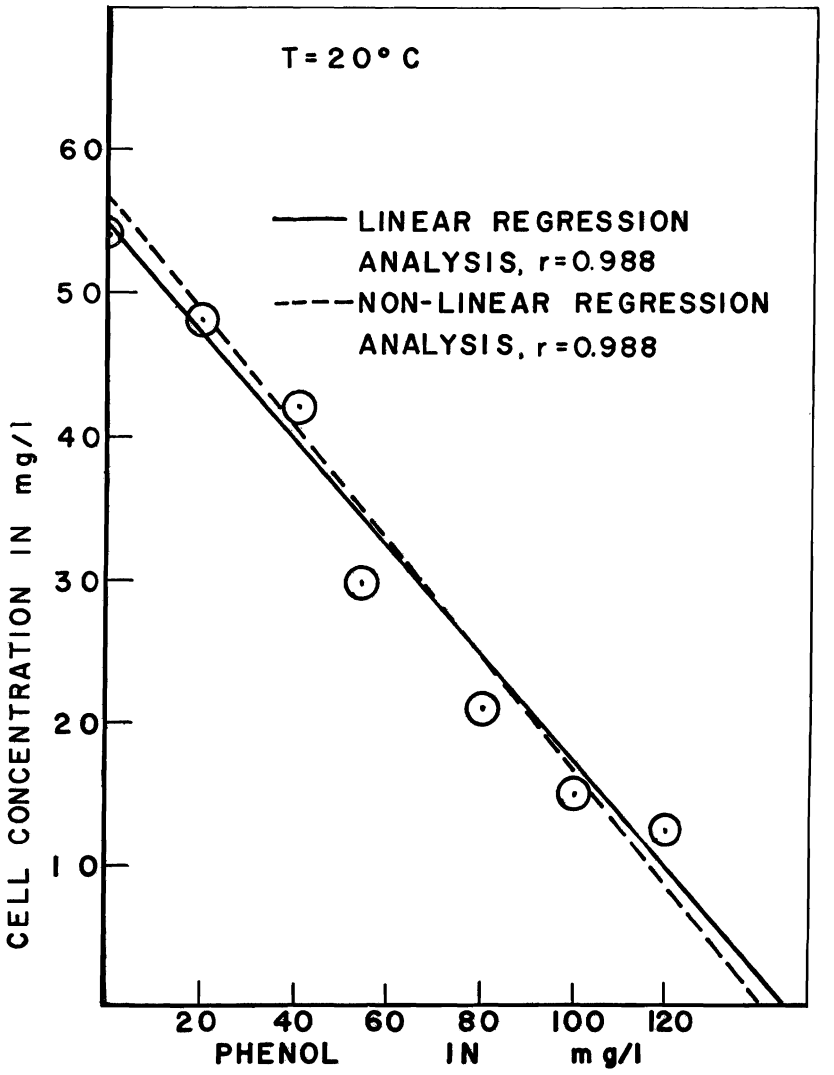

Figure 6. Cell concentration vs. phenol concentration for semi-continuous cultures at 20°C for the competitive inhibition model. Using linear regression and nonlinear regression analysis.

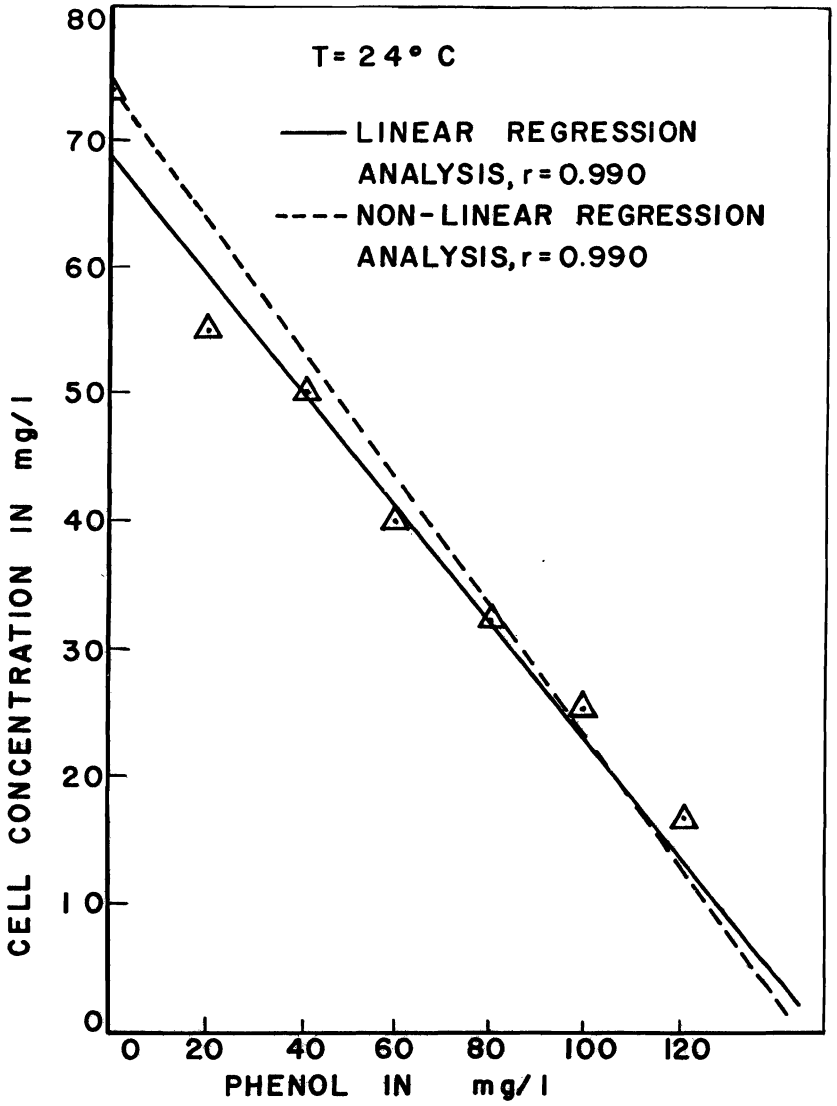

Figure 7. Cell concentration vs. phenol concentration for semi-continuous cultures at 24°C for the competitive inhibition model. Using linear regression and nonlinear regression analysis.

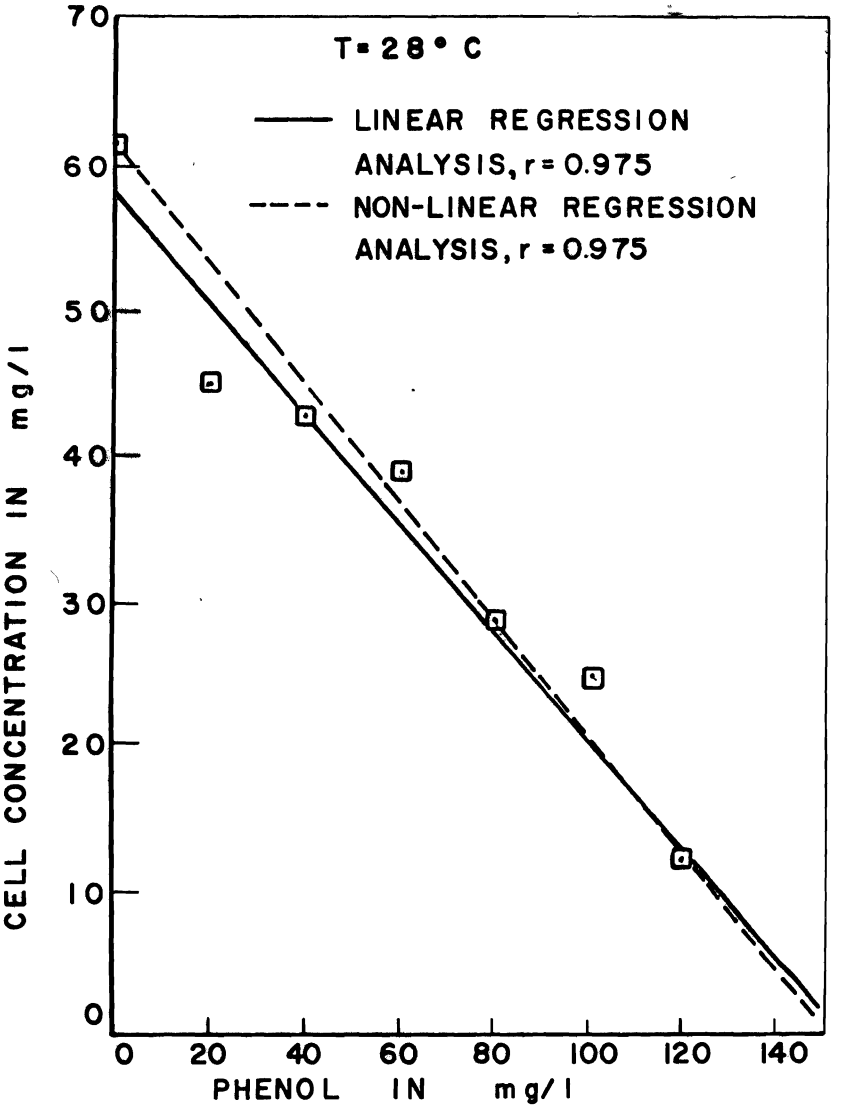

Figure 8. Cell concentration vs. phenol concentration for semi-continuous cultures at 28°C for the competitive inhibition model. Using linear regression and nonlinear regression analysis.

The competitive inhibition constants were calculated with two different assumptions. The first assumption was that the dilution rate in the semi-continuous flow culture was equal to the organism specific growth rate (assuming $k_d = 0$). Therefore, the organism specific growth rate, μ, equaled 0.33 day^{-1}. The other assumption was the Equation 1 was valid and that the organism specific growth rate, μ, was a function of the dilution rate (i.e. Equation 1). In the second case the organism specific growth rate, μ, equaled 0.40 day^{-1}.

A comparison of the competitive inhibition constants obtained from the two methods is shown in Table 5. In general, there was little significant difference in the values obtained. The percentage difference ranged from 1.6 percent at 20°C to 7.6 percent at 28°C. The values obtained from the assumption that the semi-continuous was indeed a continuous flow system produced smaller competitive inhibitor constant values and thus indicated the phenol to be more toxic than the results using the actual organism specific growth rate obtained from Equation 1. Both sets of inhibitor constant values indicate the same trend in toxicity; that is, phenol is more toxic at 24°C than at 20°C or 28°C.

Table 4. Semi-continuous culture linear regression analysis for the competitive (X_1 vs I) model using K_s and $\hat{\mu}$ determined from continuous culture experiments.

Temp. °C	Slope x 10^2	95% Confidence Interval for Slope x 10^2		Intercept	95% Confidence Interval for Intercept		Correlation[a] Coefficient
		Max.	Min.		Max.	Min.	
20	-0.384	-0.452	-0.315	55.042	59.117	50.967	-0.988
24	-0.438	-0.511	-0.365	68.475	72.861	64.089	-0.990
28	-0.356	-0.449	-0.264	57.141	62.710	51.572	-0.975

[a] 1 percent significance level correlation coefficient = -0.798.

Table 5. Comparison of semi-continuous flow, competitive inhibition constants, K_I, using specific growth rates of 0.33 day^{-1} and 0.40 day^{-1}.

Temp. °C	Inhibition Constants, K_I, in mg/l		Percent Difference (%)
	μ = 0.33 day^{-1}	μ = 0.40 day^{-1}	
20	0.121	0.123	1.6
24	0.083	0.087	4.6
28	0.145	0.157	7.6

In summary, assuming that the semi-continuous flow culture was exactly like the continuous flow system resulted in a conservative conclusion. However, the difference in the competitive inhibition constants obtained was less than 7.6 percent.

Continuous Flow Cultures. Three different concentrations of phenol were added to the continuous flow cultures at 20°C, 24°C, and 28°C. The correlation coefficients (Table 6) for the linear regression of Equation 6 are all significant above the 10 percent level except for the one day residence time at 24°C, and that particular correlation coefficient is significant at the 20 percent level. Figures 9 to 11 indicate the relationship between the linear regression curves and the measured data. The values for the competitive inhibition constants, K_I, are reported in Table 6. These values indicate the same relationship as those determined from the semi-continuous flow cultures. That is, the phenol is more toxic at 24°C than at 20°C or 28°C. However, the level of toxicity indicated by the continuous flow competitive inhibition constants is significantly greater than that indicated by the semi-continuous flow competitive inhibition constants (see Table 7).

Table 6. Linear regression analysis of continuous flow data using the linear competitive inhibition equation (Equation 6).

Temp. °C	Residence Time (Days)	Slope with 95% Confidence Limits	Intercept with 95% Confidence Limits	Correlation[a] Coefficient
20	1.07	-0.3683 ± 0.3969	46.058 ± 8.9296	0.943
	1.40	-0.9296 ± 0.5734	65.113 ± 12.9185	0.980
	1.92	-1.7778 ± 1.0874	95.372 ± 24.4699	0.980
24	1.01	-0.3257 ± 0.6097	63.292 ± 18.2903	0.852
	1.68	-1.0587 ± 1.2025	99.112 ± 36.0742	0.937
	2.00	-1.2531 ± 1.6851	103.270 ± 50.5516	0.915
28	1.12	-0.6142 ± 0.4096	72.189 ± 9.2157	0.977
	1.53	-0.7635 ± 0.8733	74.820 ± 19.6496	0.936
	2.26	-1.2608 ± 0.8618	94.785 ± 19.3897	0.976

[a] 10 percent significant value of the correlation coefficient is 0.910.

Table 7. Comparison of competitive inhibition constants, K_I, obtained from continuous and semi-continuous flow cultures.

Temp. (°C)	Residence Time, θ (Days)	Continuous Culture, K_I in mg/l Phenol	Semi-Continuous Culture, K_I in mg/l Phenol (μ = 0.40 day^{-1})	Semi-Continuous Culture, K_I in mg/l Phenol (μ = 0.33 day^{-1})
20	1.0	0.207		
	1.5	0.091		
	2.0	0.049		
	3.0		0.123	0.113
24	1.0	0.182		
	1.5	0.047		
	2.0	0.031		
	3.0		0.087	0.075
28	1.0	0.279		
	1.5	0.094		
	2.0	0.043		
	3.0		0.157	0.129

COMPARISON OF CONTINUOUS FLOW AND SEMI-CONTINUOUS FLOW INHIBITION CONSTANTS

It is difficult to compare the continuous flow and semi-continuous flow competitive inhibition constants directly, because of the limited data available from the semi-continuous cultures and the fact that the two systems were not run with equivalent residence times. However, sufficient data are available to make some general observations.

The semi-continuous flow cultures indicate that the phenol is less toxic than do the continuous flow cultures (i.e. semi-continuous flow K_I values are greater than continuous flow K_I values). This suggests that the semi-continuous flow cultures developed a tolerance to the phenol or that the uptake mechanism is not clearly revealed by the semi-continuous flow technique.

The continuous flow data indicates that the phenol is more toxic as the hydraulic residence time, Θ increases. Thus, the longer the organisms are in contact with the phenol the more toxic it becomes to them. Therefore, the notion that the organisms in the semi-continuous flow cultures are becoming more resistant is probably not valid.

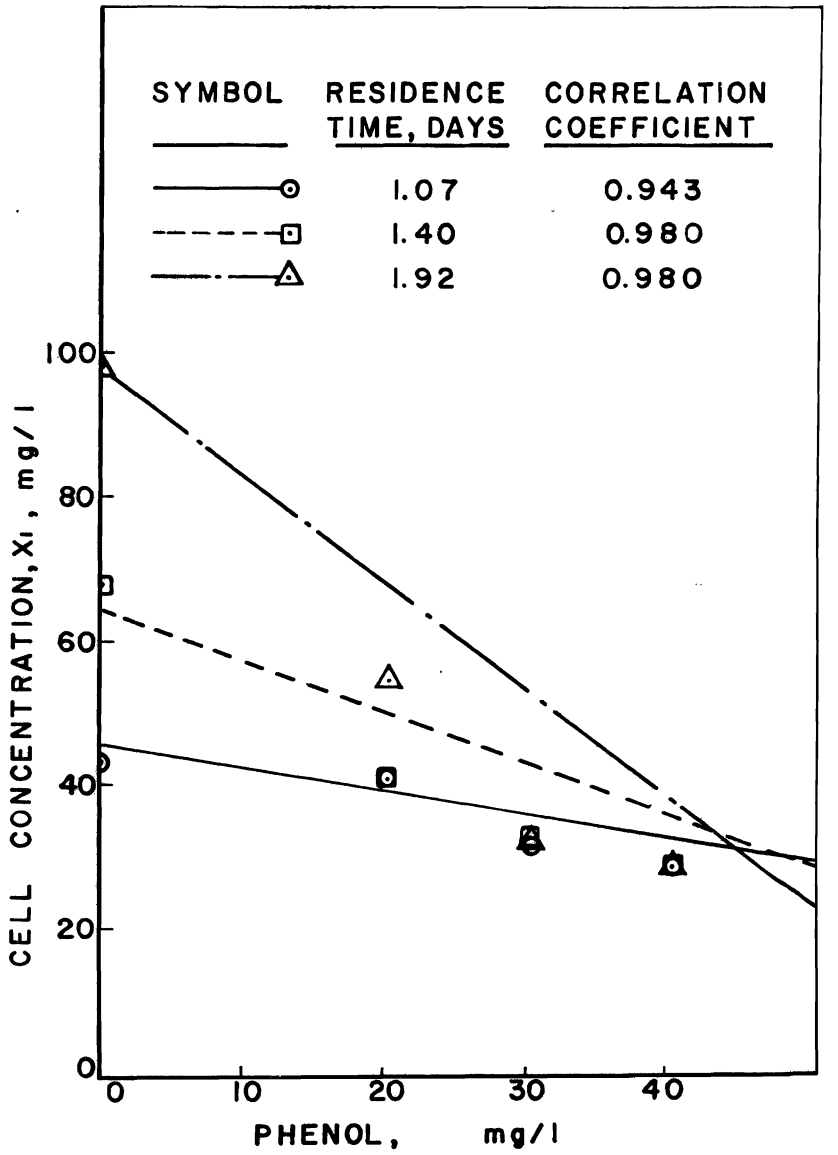

Figure 9. Linear form of competitive inhibition equation (X_1 vs. I) for continuous flow data at 20°C (Equation 17).

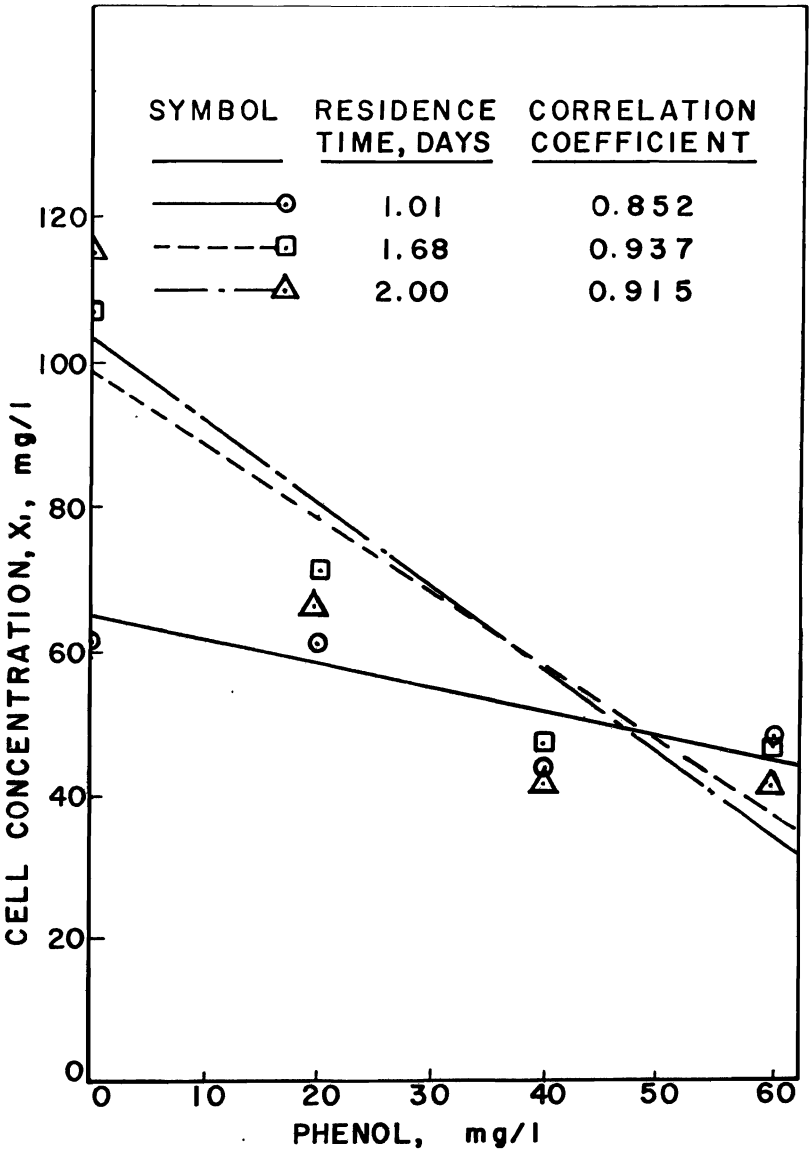

Figure 10. Linear form of competitive inhibition equation (X_1 vs. I) for continuous flow data at 24°C (Equation 17).

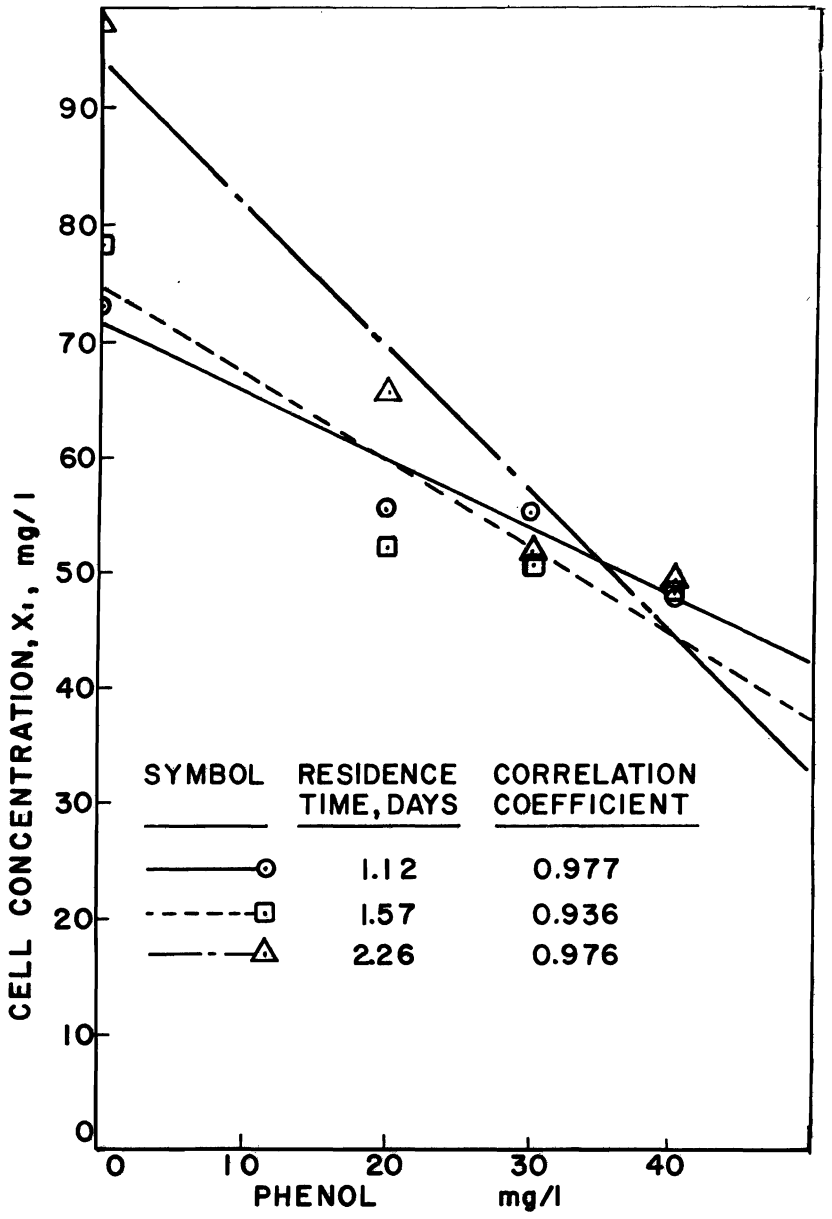

Figure 11. Linear form of competitive inhibition equation (X_1 vs. I) of continuous flow data at 28°C (Equation 17).

The larger K_I values obtained in the semi-continuous flow cultures may be explained by an artifact of the semi-continuous flow system when operated near nutrient limiting conditions. If the phenol reduces the ability of the organism to reproduce (which is supported by the continuous flow data), then the specific growth rate of the organism is reduced. However, if that growth rate is still large enough to permit the establishment of cell mass at the same level in the semi-continuous flow culture as an uninhibited growth rate, the semi-continuous flow data would not detect the effect. This concept is illustrated in Figure 12.

From point A to point B on the curve in Figure 12, growth rate is uninhibited by any toxicant. However, cell concentration reaches a maximum value which is determined by the concentration of the limiting nutrient. That is, the cultures are operating between points C and B in Figure 2. Thus, the cultures reach the steady state cell concentration in a fraction of the withdrawal period. If the growth rate is inhibited by toxicant as illustrated between points A and C in Figure 12, it takes the culture nearly the full withdrawal period to reach the maximum cell concentration. However, it does reach maximum cell concentration and the data would therefore indicate no toxicity when in fact the culture is severely inhibited.

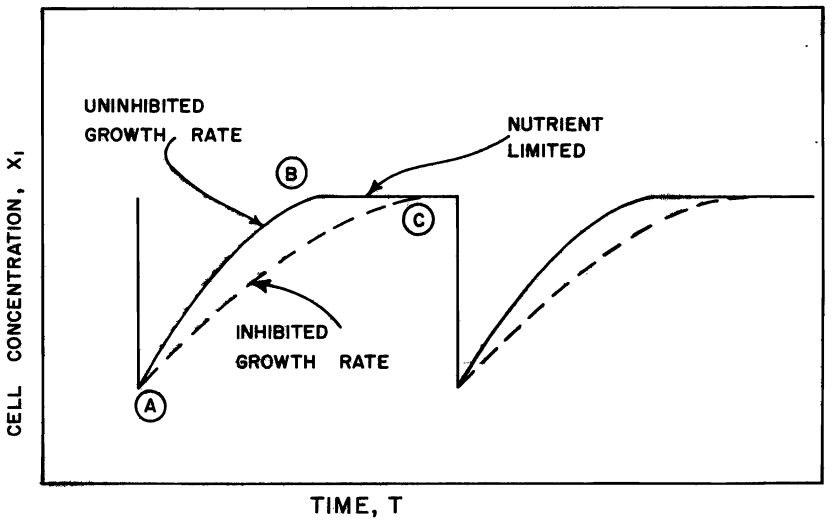

Figure 12. Effect of toxicant on growth rate in semi-continuous flow cultures.

This mechanism could explain the high competitive inhibition constants obtained from the semi-continuous flow cultures as compared to the continuous flow cultures. Malone et al. (1975) have additional data which support this argument. However, additional research is needed before a definite conclusion can be reached.

SUMMARY

Semi-continuous flow cultures are characterized by varying organism specific growth rate, substrate concentration, and cell concentration. Continuous flow cultures establish a steady state at which organism specific growth rate, substrate concentration, and cell concentration are theoretically constant. If the withdrawal period in a semi-continuous flow culture is sufficiently small, it will approximate a continuous flow culture. In fact, continuous flow cultures may be classified as a special case of semi-continuous culture where the number of withdrawals per unit time is infinity.

A comparison of data obtained from a specific phenol toxicity experiment conducted with both semi-continuous and continuous flow cultures do not appear to be in agreement. Competitive inhibition constants obtained from semi-continuous flow cultures, indicate less phenol toxicity to the alga *Selenastrum capricornutum* than inhibitor constants obtained from continuous flow cultures. This difference may be due to the variation in organism specific growth rate typically observed in semi-continuous cultures which may mask certain toxic effects.

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The Effects of Media Modifications Upon *Selenastrum Capricornutum* in Batch Cultures

R. F. Malone, K. A. Voos, W. J. Grenney, and J. H. Reynolds*

INTRODUCTION

The objective of this project was to develop a mathematical model which accurately predicted the growth of *Selenastrum capricornutum* under nitrogen limited conditions. An algal growth simulation program was developed to permit simulation of algal growth in batch, semi-continuous, and continuous cultures.

Continuous cultures and batch cultures maintained for purposes of model calibration did not exhibit typical algal growth patterns. Figure 1 illustrates a growth curve obtained from a batch culture of these initial experiments (double strength NAAM, carbon supplementation, and a 0.03 M sodium phosphate buffer in chemostat with zero flow). This growth curve more closely approximates a linear growth than the logarithmic pattern that would be expected (Bowman, 1974; U.S. Environmental Protection Agency, 1971). As illustrated in Figure 2, the specific growth rates exhibited by these cultures were low and erratic (attributed in part to temperature variations) and did not exhibit a typical pattern (U.S. EPA, 1971).

These initial observations prompted an investigation to determine a suitable medium for our calibration needs and to determine the factors

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leading to the atypical growth observed in earlier experiments. Areas of specific investigation are listed below:

1. Suitability of sodium bicarbonate as a carbon supplement.
2. Suitability of the phosphate buffer systems for pH control.
3. The degree of nitrogen limitation for the selected medium and nitrogen concentration.

EXPERIMENTAL METHODS

Cultures were maintained under batch conditions, at 24 ± 1°C, and under fluorescent lights providing a constant light intensity of 400 ± 40 foot-candles. Erlenmeyer flasks (500 ml) were filled with 325 ml of the appropriate media and covered with an inverted 150 ml Griffin beaker. Each flask was inoculated with 5 ml of a pure *Selenastrum capricornutum* culture which had been maintained under refrigeration.

Cultures were completely mixed by magnetic stirrers once a day before the removal of a 20 ml sample to be used for temperature, pH, and optical density measurements. These 20 ml samples were not returned to the algal

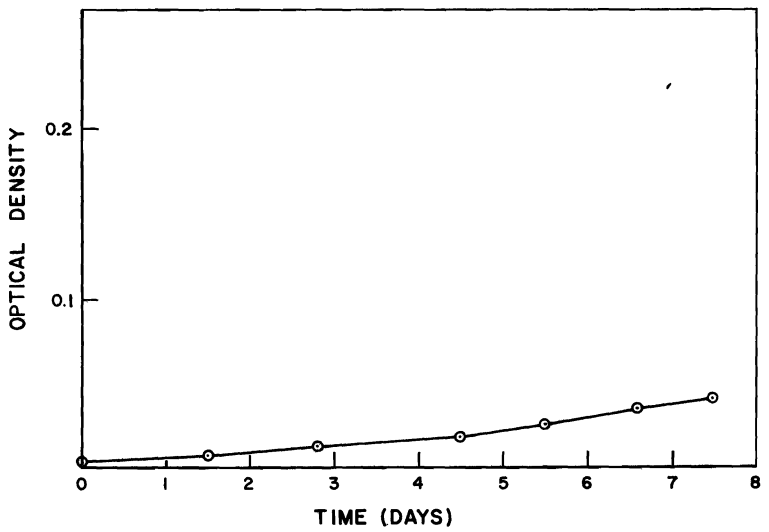


Figure 1. Growth curve batch no. 7, experiment no. 2.

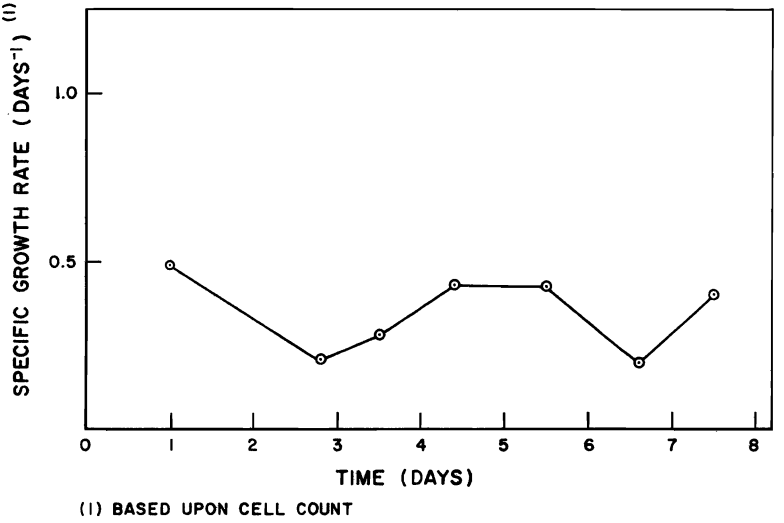


Figure 2. Specific growth rates batch no. 7, experiment no. 2

cultures minimizing contamination. pH measurements were made with a Corning Scientific Instruments, Model 7, pH meter. Optical densities were read at 750 nm on a Bausch and Lomb Spectronic 20. Optical density readings were taken on a 1/2 in. cell.

All glassware was washed and acid rinsed according to algal assay procedure bottle test (U.S. EPA, 1971).

The media used in all batch cultures were either a modified NAAM (U.S. EPA, 1971) of a modified PAAP media. The constituents contained in the media, as used here, are listed in Table 1 and Table 2. The sodium nitrate and the sodium bicarbonate levels were adjusted as required by individual experiments. In some cultures phosphate buffers were added in addition to the basic medium. pH control was accomplished by adjusting the ratio of monobasic to dibasic sodium or potassium phosphate.

The results presented here represent data collected from a series of experiments conducted over a 3 month period. All cultures were maintained in triplicate. The results presented here reflect an average of three cultures.

Table 1. Constituents of modified NAAM.

Compound	Concentration (mg/l)	Concentration (μg/l)
$NaNO_3$	25.50	
K_2HPO_4	1.04	
$MgCl_2 \cdot 6H_2O$	12.17	
$MgSo \cdot 7H_2O$	14.70	
$CaCl_2 \cdot 2H_2O$	4.41	
$NaHCO_3$	[a]	
H_3BO_3		185.5
$MnCl_2 \cdot 4H_2O$		415.6
$ZnCl_2$		3.3
$CoCl_2 \cdot 6H_2O$		1.4
$CuCl_2 \cdot 2H_2O$		1.1
$FeCl_3 \cdot 6H_2O$		160.0
$Na_2EDTA \cdot 2H_2O$		300.0
$Na_2MoO_4 \cdot 2H_2O$		7.3

[a]Adjusted to obtain desired C/N ratio.

Table 2. Constituents of modified PAAP.

Compound	Concentration (mg/l)	Concentration (μg/l)
$NaNO_3$	85.00	
K_2HPO_4	3.47	
$MgCl_2 \cdot 6H_2O$	40.57	
$MgSO_4 \cdot 7H_2O$	45.00	
$CaCl_2 \cdot 2H_2O$	14.70	
$NaHCO_3$	[a]	
H_3BO_3		618.3
$MnCl_2 \cdot 4H_2O$	1.39	
$ZnCl_2$		11.0
$CoCl_2 \cdot 6H_2O$		4.7
$CuCl_2 \cdot 2H_2O$		3.7
$FeCl_3 \cdot 6H_2O$		533.3
$Na_2EDTA \cdot 2H_2O$		1000.0
$Na_2MoO_4 \cdot 2H_2O$		24.33

[a]Adjusted to obtain desired C/N ratio.

EXPERIMENTAL RESULTS

Carbon Supplementation

Both NAAM and PAAP solutions are carbon limited and require an outside source of CO_2 to assure significant algal growth. In the algal assay procedure bottle test (U.S. EPA, 1971), it is recommended that CO_2 exchange be assured by adequate surface area to volume ratios. The chemostats to be used for the calibration experiments here do not conform to the surface area to volume requirements and, therefore, would be carbon limiting without some method of carbon supplementation.

The most common method of supplementing carbon and also regulating pH is aeration (Fuhs, 1969; Rhee, 1973; Droop, 1966; Eppley and Renger, 1974; Thomas and Dodson, 1972). Another method previously used (Reynolds et al., 1974) is the addition of sodium bicarbonate. Although this latter method does not provide for pH control, it was preferred for our modeling efforts since it eliminates the variabilities in air flow, carbon dioxide levels (Bowman, 1974) and evaporation with aeration.

The degree of carbon supplementation used is referred to by the elemental carbon to nitrogen mole ratio (C/N) that results from the addition of sodium bicarbonate to the modified PAAP medium (Table 1) or modified NAAM medium (Table 2). All C/N's presented here are based upon an unmodified level of nitrogen, not upon the actual concentration which may have been reduced. A C/N = 1 in our modified PAAP medium is equivalent to 84 mg/l of sodium bicarbonate.

Neglecting possible breakdown of Na_2EDTA (Provasoli and Pinter, 1959), the NAAM medium (U.S. EPA, 1971) and the PAAP medium (Toerien et al., 1971) have C/N = .5. It has been demonstrated (Figure 3) that a C/N = 3.4 is carbon limited as compared to a C/N = 7.1. In fact the possibility that the C/N = 7.1 might also be limiting has led to an experiment to test a series of carbon to nitrogen ratios.

The results of the addition of sodium bicarbonate to achieve C/N's of up to 45 show an inhibition of growth at higher sodium bicarbonate levels (Figure 4). The mechanism causing this inhibition is not known, but is believed to be a problem related to high total dissolved solids or precipitation phenomenon (Provasoli and Pinter, 1959). It appears that a C/N of 18 is a suitable level for carbon supplementation (Figure 4). Figure 5 compares the growth curves of cultures with aeration to those with a sodium bicarbonate level equivalent to a C/N = 18 (1.512 g/l of sodium bicarbonate). This figure illustrates that there is virtually no difference between carbon supplementation with sodium bicarbonate and aeration

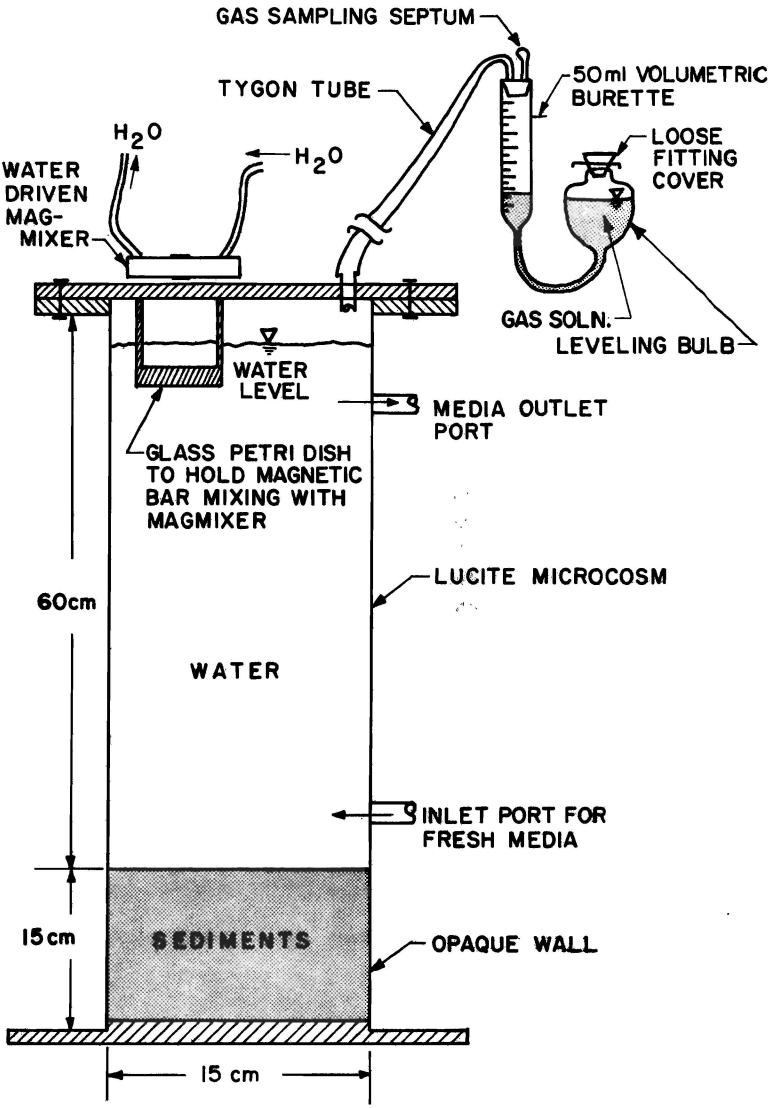


Figure 1. Schematic of a microcosm.

on the ports was tygon while tygon and glass were used for the gas phase tubing. The tygon tubing length was minimized where possible.

MATERIALS AND METHODS

Sediments

The microcosms were filled to a depth of 15 cm with lake sediment (2.25 liters). The cylinder walls around the sediments were painted black on the inside and the outside except for a strip which was taped. These opaque walls prevented growth of photosynthetic microorganisms in the sediments but the strip allowed observation of changes in the sediments as appropriate.

Sediments were collected at Hyrum Reservoir, Utah, at approximately the same sampling point as utilized by Drury et al. (1975) in a study of the eutrophication of Hyrum Reservoir. Enough sediments were collected for all the microcosms and to provide sufficient samples for further analysis. Sediments were collected in the afternoon using an Ekman dredge and placed in a polyethylene tank (see Keeney, 1974). Approximately 50 liters were collected. The morning after collection the collected sediments were vigorously stirred for 30-40 minutes in a polyethylene container to insure homogeneity of the samples. Then about 0.6 l of the mixed sediment was added randomly to each microcosm until the 2.25 l mark was reached. Each subsample was first weighted and then added to a microcosm. The final depth of sediment was 15 cm. Samples were also drawn at random for the analysis of the initial conditions.

At the end of the experiment run the microcosms were opened and overlying water removed. Nitrogen fixation was measured using acetylene reduction techniques. Then core samples were taken from the sediments. A 16 cm glass tube (2.5 cm inside diameter) was inserted into the sediments and capped using a rubber stopper. A small piece of glass tubing (5 mm) was inserted adjacent to the glass coring device. As a small positive pressure was being applied to the small glass tube, the coring tube was removed containing the core sample; this process relieved the negative pressure allowing the coring tube and sample to be easily removed. The coring tube and sample were then stoppered on the bottom and placed upright in a freezer and frozen. Cores were later sectioned for analysis into lengths of 0-1 cm, 1-3 cm, 3-5 cm, 5-10 cm, and greater than 10 cm. The remaining sediments in the microcosms were thoroughly mixed and replicate samples collected. These samples were then dried and analyzed to compare with the initial sediment analysis and to estimate any nutrient or metal loss during the experimental run. Sediments before and after the experiments were analyzed for Hg, P, avail P, N, soluble inorganic N, inorganic C, organic C, iron, and water content.

Water

Medium. Each day, 10 percent of the volume of water of each microcosm was removed and replaced with fresh media. The appropriate concentrated stock solutions were mixed carefully and completely in cooled aerated deionized water. By aerating the deionized water to assumed equilibrium, and recording the temperature and pressure, Henry's Law could be used to calculate the gas solubilities in the deionized water media for mass balance calculations.

The stock solutions were based on those described in the Algal Assay Procedures Bioassay (U.S. EPA, 1971), i.e. NAAM (Table 1). The addition of fresh media was made through the lower input port (Figure 1), using media which had been cooled approximately 5°C below the ambient temperature within the microcosm. At the same time, removal of effluent media from the upper port took place. The cooling was done to prevent mixing of fresh media with the media to be removed. It had been determined previously by dye studies that cooling the input media approximately 5°C below the microcosm temperature introduced a thermal gradient into the microcosm. This gradient was sufficient near the bottom to allow removal of the effluent media at the top port without including fresh media. However, within 20-30 minutes the microcosm was completely mixed with no apparent thermal gradient remaining and without a significant net change in temperature. Overall, it was assumed that the small temperature perturbation (initially $\leq$ 0.5°C) would have little effect on results.

Media exchange protocol. The media containers were color coded to minimize the possibility of a mixup in media additions. The exchange media were made up as follows:

a. 16 l (minus 416 ml to be added with the stock solution) of aerated, cooled deionized water were prepared and the non-variable nutrient solutions added.
b. The 16 l were divided into four 4 l portions (to a calibrated mark on the color coded container) and the variable solutions added. Each of the four 4 l portions were made up only as fast as it could be distributed to the appropriate microcosms. The variable solutions were color coded and added as follows: Yellow-blue was no nitrate and no mercury; yellow-red was no nitrate and plus mercury; green-blue was plus nitrate and no mercury; green-red was plus nitrate and plus mercury.

The date and time, barometric pressure, room temperature, effluent medium (exchange water) temperature, and any visual observations were recorded in a permanent notebook (Table 2).

Table 1. Medium constituents and concentrations.

Stock Sol'n		Compound	Conc. in Stock mg/l	Dil. in Feed D.W.	Element	Final Conc. in Microcosm µg/l			
						Hg		No Hg	
						I	II	III	IV
						Yellow Blue	Green Blue	Yellow Red	Green Red
A.	A_1	$NaNO_3$	1,821	1→1000	N	ϕ	300	ϕ	300
	A_2	$MgSO_4 \cdot 7H_2O$	12,167	10→1000	Mg	12,000	12,000	12,000	12,000
	A_3	$CaCl_2 \cdot 2H_2O$	8,070	10→1000	Ca	22,000	22,000	22,000	22,000
	A_4	KCl	7,181	1→1000	K	4,000	4,000	4,000	4,000
B.		K_2HPO_4	522	1→1000	P	93	93	93	93
C.	C_1	H_3BO_3	186	1→1000	B	33	33	33	33
		$MnCl_2$; $(MnO_2 \cdot 4H_2O)$	264(415)	1→1000	Mn	115	115	115	115
		$ZnCl_2$	33	1→1000	Zn	16	16	16	16
		$Na_2MoO_4 \cdot 2H_2O$	7.3	1→1000	Mo	2.9	2.9	2.9	2.9
	C_2	$CoCl_2$ $(CoCl_2 \cdot 6H_2O)$	0.8 (1.5)	1→1000	Co	0.37	0.37	0.37	0.37
		$CuCl_2$ $(CuCl_2 \cdot 2H_2O)$	0.01 (0.013)	1→1000	Cu	0.005	0.005	0.005	0.005
D.		$FeCl_3$ $(FeCl_3 \cdot 6H_2O)$	96 (160)	1→1000	Fe	33	33	33	33
		Na_2 EDTA$\cdot 2H_2O$	300	1→1000	Na_2 EDTA$\cdot 2H_2O$	300	300	300	300
E.		$NaHCO_3$	15,000	1→1000	C	2,145	2,145	2,145	2,145
F.		$HgCl_2$	67.7	1→1000	Hg	ϕ	ϕ	50	50

ϕ none added

Table 2. Data sheet for sampling protocal.

Date
Year _____
Month _____
Day _____
Time _____

Barometric Pressure _____
Room Temperature _____
Input Media Temperature _____

I _____
II _____
III _____
IV _____

Microcosm	Media Vol. Added	Gas Data	Effluent Media Temperature
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

Additional Comments:

Connecting the Media Lines

After the made-up nutrient medium had been thoroughly mixed, a syphon was established in the influent tube (tygon tubing 1/4″ I.D.) of the microcosm and secured with a pinch clamp. Prior to this tube being connected to the bottom orifice of the appropriate microcosm, the orifice was filled with medium to prevent gas bubbles entering the microcosm. effluent hose was connected to the top orifice and drained into a 1 liter graduated cylinder to measure the volume exchanged.

Gas Leveling

At this time while the lines (influent and effluent) were still clamped, the liquid levels in the gas-trap (buret and leveling bulb) were leveled and the water level (bottom of meniscus) of the buret recorded. Adjustments (gas production removal or helium addition) were sometimes necessary to properly level the two water levels and these were recorded (see Table 2).

Media Exchange

Then the gas trap was clamped off (this was necessary to prevent suction of the leveling fluid into the microcosm) and the syphon clamp released (influent media). Then the pinch clamps on the upper and lower orifices were released simultaneously. Approximately 890 ml of effluent was collected in the 1 liter graduated cylinder. Ideally, 900 ml were exchanged daily; however, a small leeway to balance the gas levels was needed due to a small pressure difference experienced in the microcosm during the medium exchange. Generally the medium exchanged was 890 and 910 ml.

Readjusting Gas Pressures

The pinch clamps on the upper and lower orifices were released simultaneously to avoid any undue pressure difference within the microcosm. The pinch clamp on the gas-trap was carefully removed. If there was an excessive pressure difference, the solution in the gas-trap could be drawn back into the microcosm. Once the pinch clamp had been removed from the gas trap, the water level in the buret was adusted to the original level (within < 0.1 ml) before media exchange. The water level in the buret was adjusted by opening the upper orifice pinch clamp to raise the water level in the buret. Opening the pinch clamp on the lower orifice resulted in lowering the water level in the buret.

Disconnection

A pinch clamp was secured to the influent syphon hose and both the inlet and outlet hoses removed. The volume of the effluent collected in the graduated cylinder was recorded. If it was an analysis day (every two weeks), the samples were placed in appropriate bottles for later analysis that day. The next microcosm with the same color code as the exchange solution (nutrient media) was then exchanged using the same procedure as described above.

For the Next Day

After the media had been properly exchanged, a polypropylene bottle was filled with deionized water to the 16 l mark (calibrated for 16 l minus 0.416 l for nutrient addition) and put in the refrigerator to cool. Aeration was carried out using a diaphragm pump. Any observations; new algal growth, unusual occurrences, etc., were recorded on the data sheets. It usually took 45-60 minutes to completely service the 16 microcosms and prepare for the following day.

Gases

Gas samples were collected bi-weekly through a septum located at the top of the buret in the gas trap system (Figure 1). It was assumed that the sample collected from the gas trap was a completely mixed sample; molecular diffusion alone was judged adequate to cause complete mixing in the system.

Gas samples were taken in a 2.5 ml disposable syringe and then the syringe needle was inserted into a labeled rubber stopper. The gas samples were analyzed immediately; time interval experiments were conducted to determine any possible leak or reaction parameters of the samples with the syringe or rubber stopper. No significant deviations or changes in the gas contents were indicated over a period as long as 24 hours.

The gas samples were analyzed on a Hewlett Packard 5750 research gas chromatograph equipped with a gas sampling valve delivering 0.5 cc of gas to the column for separation and detection. Instrument operating conditions were as follows:

Instrument-H-P 5750 Thermal Conductivity Detector

Columns-6 ft x $^1/_8$" o.d. stainless-steel containing 60-80 molecular sieve 5A (O_2, N_2, CH_4)
-6 ft x $^1/_8$" o.d. stainless-steel containing 100-120 Porapak S (CO_2, $CH_2 = CH_2$)

Carrier Gas-Helium

Flow Rates

Carrier Gas-35 ml/min.
Tank Pressure 55 psig

Temperature

Column-100 - 110°C
Detector-265°C
Injector Port-110°C

Known standards of all the gases detected and air samples were run on a routine basis in conjunction with the samples analyzed from the microcosms. The hydrocarbon gases were also verified using a flame detector on the H-P gas chromatograph. The area under each peak was calculated by the triangulation method and the mole fraction of each gas obtained. H_2S was detected by its odor but was not detected on the gas chromatograph; only great concentrations could have been detected with the analytical train used. Similarly, NH_3 could not be detected except at high concentrations; however the observed low water concentrations precluded significant gas phase concentrations.

Analytical Procedures

As indicated in the flow chart in Figure 2, there were a large number of analyses performed at the end of each interval ($\sim$ 14 days) on the effluent sample taken that day. A daily composite sample of the input media and deionized water was also run with the effluent samples of each interval to check on input concentrations of specific compounds.

Dissolved oxygen (Winkler), pH, temperature, and relative fluorescence of chlorophyll (Turner Fluorometer Manual, Model 110), were measured routinely. Methods utilized for nutrients and other materials were based on accepted procedures (APHA, 1971; EPA, 1971).

Data Analysis

All measurements were recorded in a permanent log book. Concentrations of specific chemical species were calculated, transferred to IBM cards according to a specified format, and utilized in calculating an elemental balance about the microcosm by computer program (see Porcella et al., 1975). Output from the program was plotted as a function of time and/or analyzed by ANOVA methods (Hurst, 1972) arising from the 2 x 2 x 4 design replicated with time.

The mass balance program computed input and output mass flows of materials or compounds in gas, liquid, or solid phase. The computed difference (net flux) between input and output was plotted on a daily basis or accumulated over time for the entire experimental period. For example, nitrogen input occurred as atmospheric gas (measured) and dissolved gas (N_2, computed using Henry's Law), organic nitrogen (soluble and particulate measured), ammonium (measured), nitrate (measured) and nitrite (measured); outputs were similarly measured. Mercury, phosphorus, and iron did not participate in the gas phase; CO_2 and O_2 were measured both in the atmospheric and dissolved gas phases. These net flux results were then analyzed statistically and plotted.

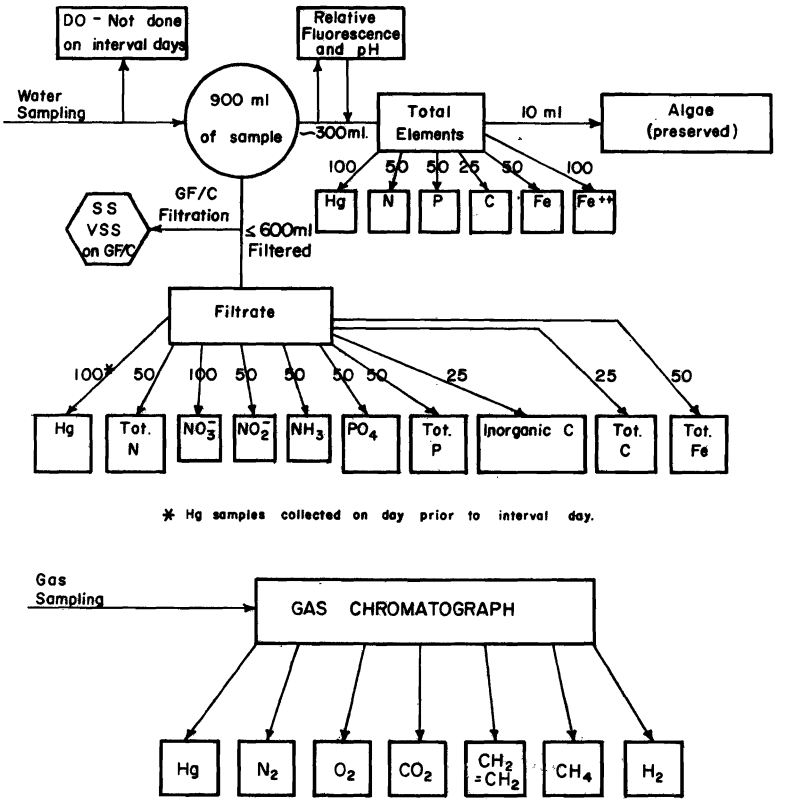


Figure 2. Analysis flow sheet (numbers represent ml of sample).

RESULTS

Statistical Analysis of the Net Flux of Specific Parameters

Although replicate experiments of the treatment in the experimental design were not made because of the great demand on analytical facilities and space, the experimental design allowed the analysis of variance and some judgments about the interactions between treatments. The analysis of variance for the 16 microcosms was based on the 2(NO_3-N) x 4 (light conditions) x 2 (Hg^{++}) experimental variations (treatments) and the 13 time intervals where the 22 parameters of nutrients and other chemicals were measured. In addition the calculated nutrient and gas balances for the same time intervals (10 parameters) were utilized in the analysis of variance to provide a grand total of 32 parameters. These 32 parameters were utilized to estimate effects and interactions between NO_3-N (A), light (B), Hg^{++} (C), and time (D) conditions of the experiment (A = 2, B = 4, C = 2, D = 13). The error mean square was estimated using the total combined conditions (ABCD, 36 degrees of freedom).

All statistical analyses were performed using a factorial design analysis of variance on a Burroughs 6700 computer with a STATPAC (Hurst, 1972) program (STATPAC/FCTCVR). The program calculates the mean square values of the data for the different combinations of variables and then the various F values for the combinations are estimated using the mean square of any combination as the numerator and the mean square of the overall combination (ABCD, equals the estimate of the error mean square) as the denominator (Hurst, 1974; Ostle, 1963). The F values were compared for the probability of erroneously rejecting the null hypothesis at the 1 and 5 percent levels for the different degrees of freedom for the different combinations (Hodgman, 1954).

The F values significant at either the 1 or 5 percent level for the 32 different parameters are listed in Table 3 for all the different combinations of the experimental variations. Neither NO_3-N nor Hg^{++} variations affected as many variables as light or time. This would be expected for statistical reasons (insufficient degrees of freedom) as well as experimental reasons. Experimentally, light would have a great effect because of the complete darkness variation (no photosynthesis) as contrasted with the lighted conditions (considerable photosynthesis). Also, changes would be expected for the time intervals as populations increased and decreased and as the microcosms matured and approached steady state.

The most sensitive parameters of response were primarily found among the nutrient and gas balance parameters. The total gas volume

Table 3. Significant effects and interactions on response parameters as affected by the experimental treatments.

Response Parameters	Number of Significant Occurrences	Levels of Significance for Different Treatments (Degrees of Freedom)[a]														
		All Experimental Combinations (207)	NO_3-N only (1)	Light only (3)	Hg^{++} only (1)	NO_3-N & Light (3)	NO_3-N & Hg^{++} (3)	Light & Hg^{++} (3)	NO_3-N, Light & Hg^{++} (3)	Time (12)	NO_3-N & Time (12)	Light & Time (36)	Hg^{++} & Time (12)	NO_3-N, Light & Time (36)	NO_3-N, Hg^{++} & Time (12)	Light, Hg^{++} & Time (36)
Unfiltered Total Phosphorus	7	1		1		1	1	1	1	1						
Unfiltered Total Nitrogen	7	1		1		1		1	1	1		1				
Unfiltered Total Carbon	8	1		1	5	1		1	1	1		5				
Unfiltered Total Iron	5			1		1	5	1		1						
Unfiltered Total Mercury	5	1	5	1	1			1								
Suspended Solids	9	1		1	1	1	1	1	1	1				5		
Volatile Suspended Solids	9	1		1	1	1	1	1	1	5				5		
Unfiltered Ferrous Iron	4			1		5		5		1						
Unfiltered Inorganic Carbon	8	1		1		1	5	5	1	1		1				
Unfiltered Total Organic Carbon	11	1		1	5	1		1	1	1		1		5	5	5
Filtered Orthophosphate-P	4	1		1						1		1				
Filtered Total Phosphorus	5	1		1		5				1		1				
Filtered Nitrite-N	0															
Filtered Nitrate-N	0															
Filtered Ammonium-N	5	1	5	1		5				1						
Filtered Total Nitrogen	6	1		1		5			1	1		1				
Filtered Total Carbon	7	1		1		1	5		1	1		5				
Filtered Inorganic Carbon	7	1		1		1	5		1	1		1				
Filtered Total Organic Carbon	4			1		1			5	1						
Filtered Total Iron	2					1				5						
Filtered Total Mercury	7	1	1	1	1		1	1	1							
Phosphorus Balance	10	1		1		1	1	1	1	1		1		1		5
Iron Balance	9	1		1	1	1	1	1	1	1				1		
Mercury Balance	4	1			1					1			1			
Nitrogen Balance	12	1	1	1	1	1	1	1	1	1		1			1	1
Carbon Balance	8	1		1	5	1		1	1	1		1				
Total Gas Volume Balance	14	1	1	1	1	1	1	1	1	1		1	1	5	1	1
Nitrogen Gas Weight Balance	12	1	1	1	1	1	1	1	1	1		1			1	1
Oxygen Gas Weight Balance	11	1		1	1	1	1	1	1	1		1			1	1
Carbon Dioxide Gas Weight Balance	11	1	1	1		1	1		1	1	1	1		1		5
Methane Gas Weight Balance	11	1	1	1	1	1	1		1	1	1	1		1		

[a] 1, 5 percent levels of significance. Greater than 5 percent are left blank.

balance provided the greatest numbers of significant responses to the different combinations, appearing at 14 of the 15 total combinations. The concentration of unfiltered total organic carbon (particulate carbon) was the only highly responsive parameter outside of the group of nutrient and gas balances. In general gases were the most sensitive parameters. Total gas volume, nitrogen gas, oxygen gas, carbon dioxide gas, and methane gas were all important indicators of interactions.

The gas phase responses indicate involvement of specific microbial functions which are greatly affected by the experimental variations. For example, oxygen and CO_2 would be expected to be greatly affected by NO_3-N (stimulation) and Hg^{++} (toxication) concentrations as well as by light. Nitrogen gas is affected by input processes such as denitrification and output processes (nitrogen fixation). Methane production is due to anaerobic breakdown of organic carbon compounds; but rapid utilization of methane gas in overlying, oxygenated waters must also be considered (Rudd et al., 1974). Similarly ethylene production in the sediments can be utilized in the water column by bacteria but at a greater rate than is methane (Flett et al., 1975).

Several parameters showed no response (nitrate and nitrite) while dissolved organic carbon, dissolved total iron, the mercury balance, filtered orthophosphate P, and unfiltered ferrous iron were relatively insensitive parameters of the effects of the different experimental variations. Some of this insensitivity was caused by analytical imprecision at the concentrations found in the microcosms; this was true for the dissolved organic carbon, total iron, and the unfiltered ferrous iron.

Specific Microcosm Response

Because of space and time limitations only the results of four microcosms will be discussed in this paper: Microcosms 1, 2 and 13, 14. Microcosms 1 and 2 were maintained in the dark continuously while 13 and 14 received constant light intensity on a 16 hr light, 8 hr dark cycle from the top. Microcosms 1 and 13 received no nitrate and 2 and 14 received 300 μg NO_3-N/l; mercury was not added to any of these four microcosms. Thus, Microcosms 1 and 2 were operated as dark anaerobic systems which typify the deep hypolimnetic anaerobic zones of eutrophic lakes, and 13 and 14 were operated as shallow, lighted littoral zones of eutrophic lakes. Temperature (23-25°C) and light conditions (200 ft candles) were typical of summer conditions; mixing and residence time (10 days) were intended to be representative of natural conditions. As occurs in natural lakes (Brock, 1966) and reported in earlier microcosm studies (Porcella et al., 1970), the sediments remained anaerobic irrespective of whether the overlying water was aerobic or anaerobic.

Gases

As was shown in Table 3, all experimental variables (light, nitrate-nitrogen, and mercury) produced statistically significant responses at that 99 percent level for total gas production. Thus, gas production was the most sensitive parameter of microcosm response.

Microcosms 1 and 2 were kept in the dark throughout the entire experiment except for a few minutes daily necessary for exchange of fresh nutrient media. As can be seen from Figures 3 and 4, the patterns of gas flux were essentially the same; the total gas volume decreased initially (8 - 40 days). After sediments and organisms had adjusted to the new conditons (muds were initially at $\approx 6°C$ when collected and then mixed thoroughly before being dispensed into the microcosms), net gas flux (production) began.

After this initial start-up-time Microcosm 1 had a rate of approximately 2.5 cc gas produced/day and Microcosm 2 had a rate of approximately 3.7 cc gas produced/day.

By 120 days, all dark microcosms had reached a more-or-less steady state condition. Microcosm 2 (nitrate added) produced the most gas (318.8 ml @ STP Table 4). Microcosm 1 (no nitrate) produced 87.9 ml @ STP. The data show definite stimulation effects of the nitrate. Microcosm 2 also produce the most nitrogen, methane, and carbon dioxide.

As these dark microcosms were anaerobic, there was no net oxygen (O_2) production but rather uptake of O_2 by the system. This occurred because reducing conditions existed. The continued loss of oxygen was possible because of utilization of the small quantities of oxygen entering the microcosms with the air saturated input nutrient media. Respiration or the ratio of CO_2 production to O_2 utilization was 0.77 for Microcosm 1 (mg CO_2/mg O_2 = 1047/1308) and was 1.2 for Microcosm 2 (1498/1241). Glucose respiration has a ration of 1.37 (44/32) indicating carbohydrate metabolism. If all the oxygen in nitrate were stoichiometrically available for glucose oxidation from denitrifying processes, the added O_2 in the nitrate over the period of study would add about 173 mg O_2 and change the respiration ratio to 1.06. CO_2 is more soluble in water than O_2 and kinetics of release are slower, so the CO_2 production is underestimated. The source of carbonaceous material was the sediments and a marked effect on the organic carbon concentration in the sediments was observed. All dark microcosms produced some ethylene (C_2H_4) although it was not observed until 70 or 80 days into the experiment. Fungi, etc., in soil systems have produced ethylene. The reason ethylene gas was not observed at an earlier date may have been due to utilization, high solubility in

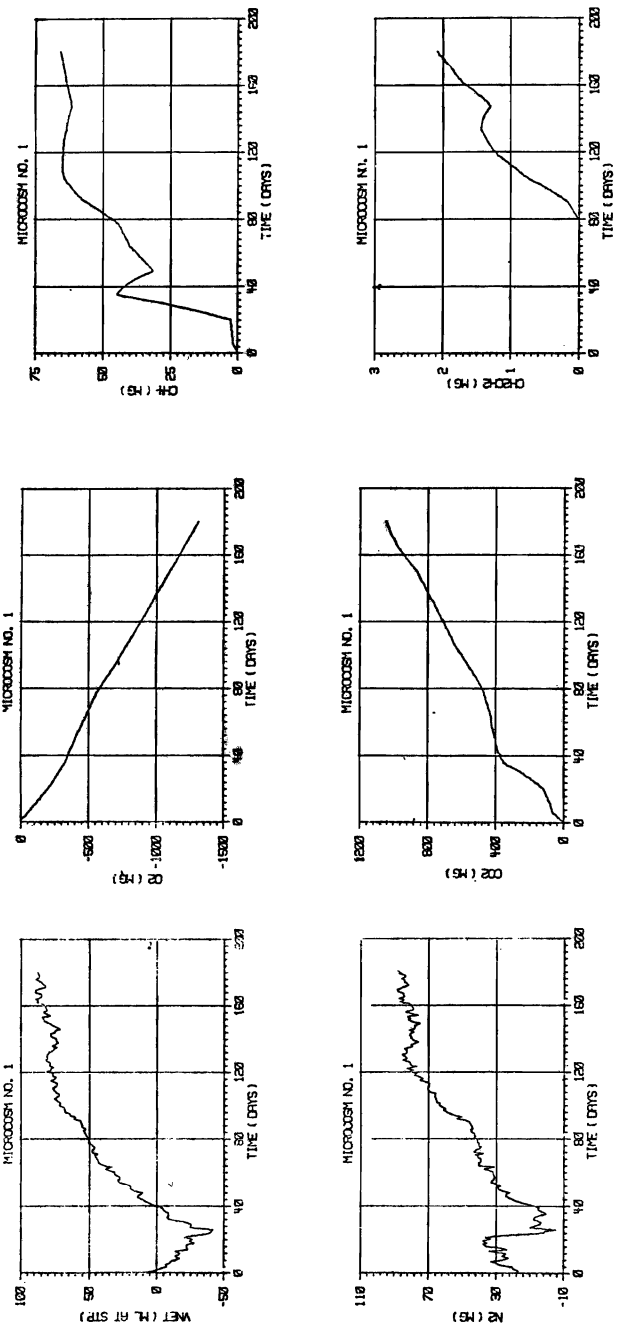


Figure 3. Mass balance of gases detected in Microcosm 1 accumulated over period of study. VNET is total gas production (ml at STP); other gases in mg.

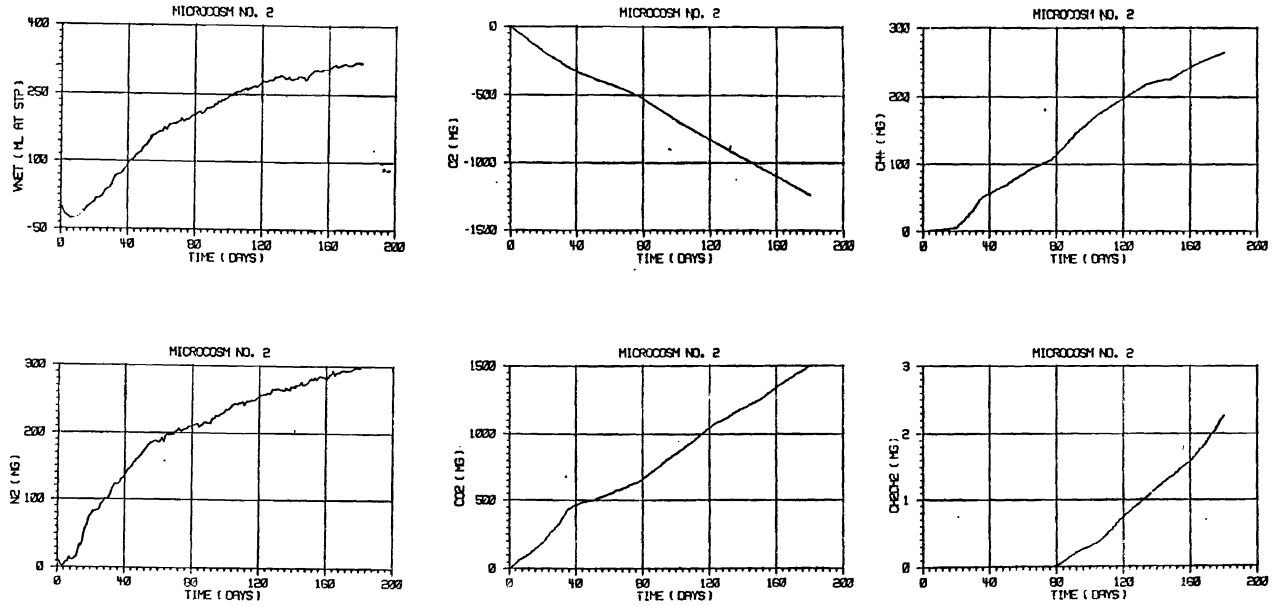


Figure 4. Mass balances of gases detected in Microcosm 2 accumulated over period of study. VNET is total gas production (ml at STP); other gases in mg.

Table 4. Cumulative net flux of gases in microcosm (187 days of study).

Micro-cosm	Gases, mg					
	Total Gas ml, STP	N_2	CO_2	O_2	CH_4	CH_2CH_2
1	88	88	1047	-1308	66	2.1
2	319	298	1498	-1241	265	2.3
13	1197	84	7.2	1382	22	0.24
14	1071	74	12.4	1128	25	0

water, or an equilibration stage. Apparently ethylene is a better substrate for aquatic bacteria than methane (Flett et al., 1975).

Microcosms 13 and 14 both produced more gas than the dark microcosms (Figures 5 and 6). This was expected because oxygen was the major gas produced and CO_2 would be derived from the bicarbonate system; increased light intensity would increase photosynthesis thus showing greater total gas production. There was considerably less methane production in these microcosms, as compared to the dark ones (anaerobic). This was due to (1) methanogenis perhaps occurring more favorably in the anaerobic microcosms, and/or (2) bacterial utilization of methane in aerobic overlying waters in the lighted microcosms (Rudd et al., (1974). The effects of nitrate did not appear to have the same types of effects in the horizontal light microcosms as the dark microcosms. After 14 days, the horizontal light microcosms had a high nitrogen gas production rate (i.e. 19 mg N_2/day over a 14 day period) and then leveled off or declined (Microcosms 5, 6, 14).

Carbon dioxide for the first 50 days was being used up (photosynthesis) or disspelled from the system. As the experiment proceeded, the pH increased in the light microcosms. This was the result of CO_2 from the alkalinity system being used by growing algae (Goldman et al., 1972). As the algae reached a maximum growth in proceeding to steady state, some began to die and decay. Thus CO_2 came back into the system by microbial degradation of the organic matter. Also during the first 50-day period, methane was actively produced. This process could result from anaerobic bacterial reduction of CO_2 or more likely from methanogenic fermentation of partially reduced forms of organic carbon compounds in the sediments.

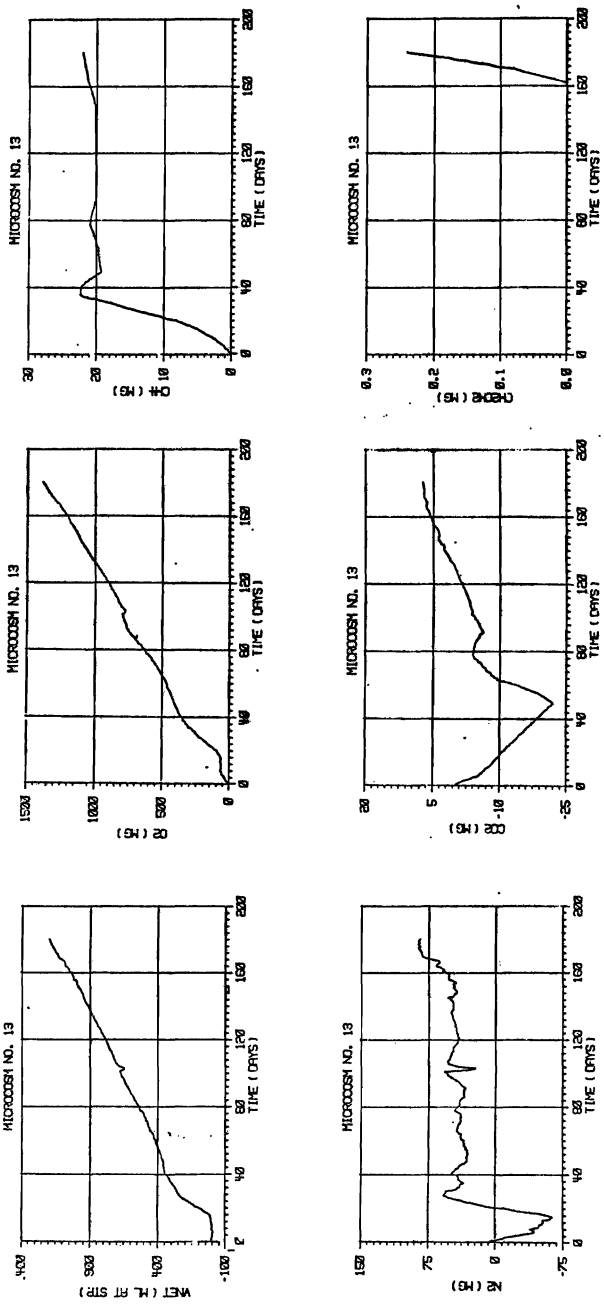


Figure 5. Mass balances of gas detected in Microcosm 13 accumulated over period of study. VNET is total gas production (ml at STP); other gases in mg.

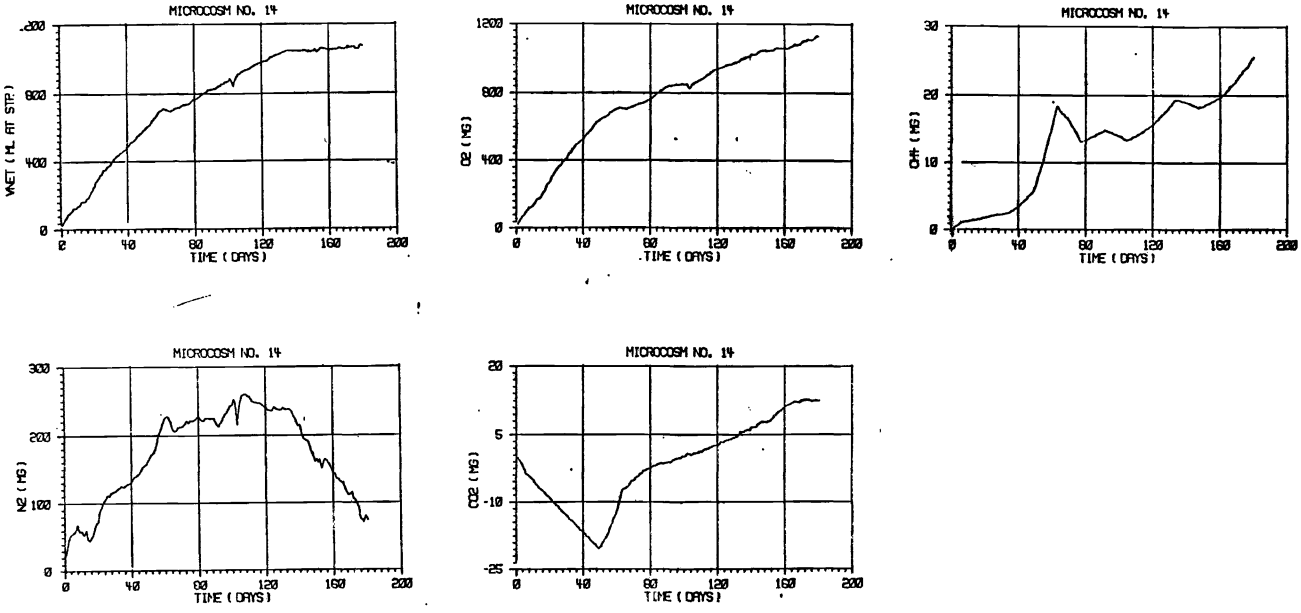


Figure 6. Mass balances of gas detection in Microcosm 14 accumulated over a period of study. VNET is total gas production (ml at STP); other gases in mg.

Oxygen Dynamics

Weekly measurements of dissolved oxygen (DO) in the effluents showed very low values for the dark microcosms (0.5-1.0 mg/l) and quite high values for the lighted microcosms (10-20 mg/l); saturation DO conditions in the temperature range of the microcosms (20-25°C) should have been between 7.8 to 7.1 mg/l DO. The DO concentrations in Microcosms 13 and 14 (Figure 7) essentially confirmed the patterns of responses noted for net flux shown in Figures 5 and 6. Mole fractions of oxygen in the atmospheres over the microcosms attained levels of 3 percent in Microcosms 1 and 2, 39 percent in 13, and 34 percent in 14. The atmospheric O_2 in Microcosms 13 and 14 indicated approximate equilibrium with the aqueous phase.

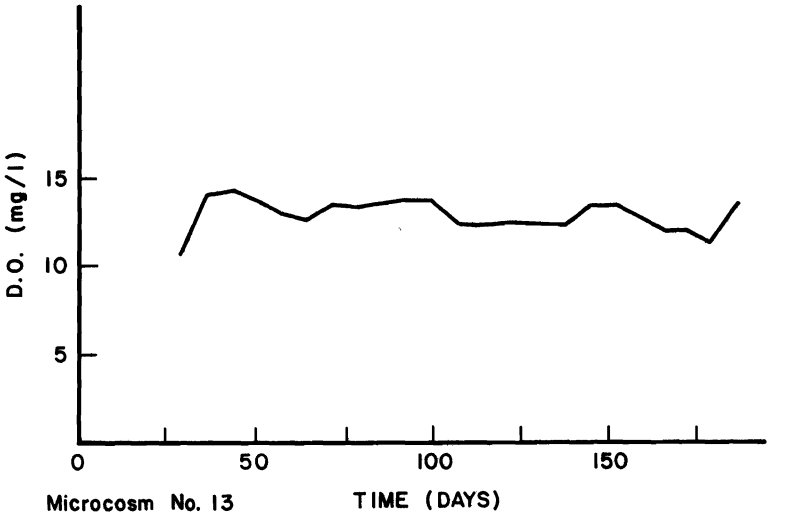
At the end of the experimental run for the microcosms, DO, inorganic carbon, and pH were observed at 2 hour intervals in the microcosms (Table 5). These data indicated that the weekly, once-daily measurements of DO were relatively good indicators of DO dynamics. DO, pH, and inorganic carbon in the dark microcosms did not vary over the 24 hour period because light input was essentially zero all the time (pH 6.6-7.0; inorganic carbon 8-12 mg/l). Minimum inorganic carbon was < 1 mg/l in Microcosms 13 and 14. The high pH's in this pair (9.6-10.0) further indicate possible carbon limitation.

Mass Balances of Elements

Elements were either accumulated in the microcosm system (input > output) or released from the microcosm (output > input) resulting in a negative or positive balance (Table 6), respectively. This was calculated using the mass balance computer program and determining the difference between the mass of nutrients in the microcosm liquid and gas phases before servicing on a given day and the mass of nutrients in the microcosm

Table 5. Diurnal productivity effects in lighted microcosm. Results of readings taken every two hours for 24-hour period beginning June 6, 1973.

			mg/l		
			ΔDO	Minimum Inorganic C	Maximum pH
13	Horizontal,	no N	1.0	< 1	9.9
14	16 hr light, 8 hr dark	N	1.7	< 1	9.6



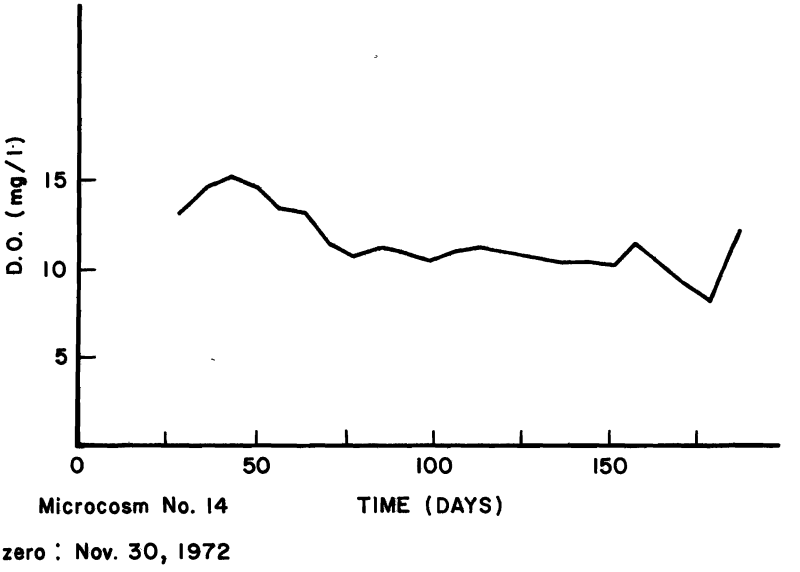


Figure 7. Effluent dissolved oxygen concentrations are above saturation in the lighted microcosms.

Table 6. Cumulative net flux of elements in microcosms (187 days of study). Negative values indicate an accumulation of element in the microcosm and positive values indicate a net loss from the system.

Microcosm	mg			
	C	N	P	Fe
1	1609	136	20	13.3
2	2119	294	22	16
13	394	84	-7.8	4.8
14	352	19	-7.2	6.9
Total input in liquid media	HCO_3^--C, 808	NO_3-N, 0, 50.5	P 16	Fe 5.6

after servicing on the pervious day. These data were then accumulated over the period of study (187 days).

Considerable output of all elements (C, N, P, Fe) occurred in the anaerobic microcosms. In the lighted microcosms less output of C, N, and Fe occurred and phosphorus was accumulated in the microcosm. The source of the increased output had to be the sediments. Thus, the major source of C was the sediments and this was reflected in the losses of organic carbon from initial sediment conditions (Table 7). Nitrogen output was greater than calculated even though no apparent loss from the sediments occurred.

Nitrogen input as nitrate was zero or was defined at a level equivalent to Sawyer's (1947) threshold concentration of 300 μg N/l and a loading rate of 4.38 g/m^2 yr, a value at the threshold of the eutrophic level defined by Vollenweider (1968). In dark Microcosm 2, nitrate quickly disappeared by denitrification; in the light microcosm it was quickly assimilated. Thus, nitrate was undetectable in all four microcosms.

Nitrogen fixation was measured at the end of the experiment using the entire microcosm as an incubation flask by acetylene reduction (corrected for *in situ* ethylene production). The results indicated that no measurable nitrogen fixation occurred in the dark microcosms but significant nitrogen fixation occurred in the lighted microcosms. Blue-green algal nitrogen fixation was implicated by the apparent photosynthetic relationship and the observation of heterocystous *Anabaena sp.* Nitrogen fixation was estimated to be 0.17 mg N_2/microcosm day in Microcosm 13 and 0.077 mg N_2/microcosm day in Microcosm 14. In the latter case the total nitrogen input was composed of

Table 7. Sediment characteristics of microcosms.

	Initial Conditions (Initial Mass of Element, g)	Final Condition in Microcosms			
		1	2	13	14
Sediment Wet Weight added, g.	-	2785	2779	2800	2816
Sediment Dry Weight added, g. (% Moisture = 68.8)	-	886	884	890	895
Total P, mg/g	1.16 (1030)	1.18	1.08	1.09	1.03
Avail. P, mg/g	0.057	0.058	0.049	0.054	0.053
Total N, mg/g	2.20 (1960)	2.13	2.17	2.02	2.13
Organic C, mg/g	22.7 (20200)	16	14	12	11
Inorganic C as $CaCO_3$, mg/g	208.0	218	228	214	218
Total Fe, mg/g	22.4 (21000)	20	19.8	19.4	19.2

about 20 percent nitrogen fixation and 80 percent nitrate in the input liquid. Compensation for low nitrogen input to Microcosm 13 resulted in an increased nitrogen fixation. In nitrogen budgets of eutrophic lakes fixation has been shown to be a significant fraction of the budget e.g. 40 percent in Clear Lake, California (Horne and Goldman, 1972). Nitrogen fixation could not be measured in the aqueous phase indicating that the heterocystous algae were scarce as plankton and were primarily present as benthic or wall growth. This was confirmed by the analysis performed at the end of experiment on the opened microcosms with water removed.

Although nitrogen in the sediments was apparently unchanged, core samples indicated that there was a redistribution from what had been originally present in the microcosms; greater N concentrations were observed in upper (1-5 cm) layers (3.2 to 2.6 mg/g) and lesser concentrations in lower (5-15 cm) strata (2.0 to 2.7 mg/g). The observation of significant sediment mixing as a result of gas bubble release from anaerobic fermentation processes in the sediments accounts for the redistribution, i.e. lighter materials (organic) rising to the surface of the sediment column.

Phosphorus output was evident, especially from the dark microcosms. Phosphorus was likely tied up with iron losses as these elements form precipitates and then dissolve under anaerobic and acid conditions. No change in sediment total phosphorus was observed but a measurable decrease in iron occurred. The reason for no measurable change in sediment phosphorus is still being investigated. The mass balance (Table 6) shows an obvious loss of phosphorus from the dark microcosms but as would be expected from the high productivity in the lighted microcosms, an accumulation in biomass occurred. Previous results have shown that all the phosphorus in the sediments is available for algal growth in microcosms (Porcella et al., 1970) and in algal assay studies (Wildung and Schmidt, 1973).

DISCUSSION

Aquatic microecosystems (microcosms) have been used to make measurements, perform bioassays, or develop information about aquatic processes. Because these systems are under controlled conditions, they allow characterization of specific processes and mineral or organic cyclings which could not be easily discerned in the field. Microcosms come in every shape and form and uses of resultant information usually define how complete and complex specific microcosms are. Systems analysis is another means of analyzing such complexities on a larger scale (Gillette et al., 1974). The microcosms described in this report were designed to allow complete chemical balances of important elements in microbially dominated sediment water systems.

Gas production in terms of quantity and composition showed the greatest responses to the major variation of dark and lighted microcosms. Also gas production seemed to be an excellent indicator of respiration and productivity as a function of organic matter in sediments and nutrient supply. Thus, the quantity and composition of gases produced in natural lake sediments under field conditions could be used as an indicator of trophic status as well as a level of organic degradation. For example, specific gases such as methane, ethylene, and other hydrocarbons might indicate the presence of specific microorganisms and processes (Dowdell et al., 1972; Gibson, 1964; Smith and Restall, 1971). In other cases sediment laden with heavy metals and resultant toxicity might prevent gas metabolism which would normally be expected given a high nutrient and organic content.

Another important aspect of the microcosm study was the significance of nitrogen fixation. In a strict sense in these systems, nitrogen apparently could not be made limiting because algal communities would develop which were dominated by blue-green nitrogen fixers (*Anabaena*) and which would fix sufficient nitrogen to ensure that some other factor became limiting. Thus, as has occurred in lakes (e.g., Clear Lake, Horne and Goldman, 1972) and would be expected to occur in the microcosms, the system will satisfy a nitrogen requirement so long as other factors are in relative abundance. The nitrogen fixation in the microcosms was associated with attached algae. It is possible that in lakes, significant nitrogen fixation could occur in attached algal communities as well as in the phytoplankton.

Iron and phosphorus interactions confirmed literature results, that iron and phosphorus dynamics are initimately linked (Wildung and Schmidt, 1973; Fitzgerald and Uttormark, 1974; Fillos and Swanson, 1975; Mortimer, 1941, 1942; Hwang et al., 1975; Syers et al., 1973). In anaerobic sediments the availability of sediment iron may be sufficient to always meet the needs of productivity even though many factors serve to limit its availability; this is because extremely low concentrations are required for algal growth.

The need to understand carbon (Goldman et al., 1972; Kerr et al., 1973), nitrogen (Keeney, 1973; Patrick, 1973) and phosphorus cycles (Syers et al., 1973) in natural aquatic systems arises from the ubiquity of nutrient sources in natural ecosystems, their possible role as limiting factors, and the need for adequate control mechanisms. That sediments act as a source of phosphorus (e.g., Porcella et al., 1970), the only element that so far has an indication of being controlled in lakes and reservoirs (e.g., see Edmondson, 1972; Porcella et al., 1972, for phosphorus; this paper for nitrogen; Schindler and Fee, 1974, for phosphorus and carbon) indicates the importance of evaluating sediments in terms of time and

quantity in acting as a source for eutrophication problems. Thus far, the microcosm experiments indicate the infeasibility of controlling eutrophication by limiting nitrogen inputs to eutrophic lakes. Field work has shown that controlling phosphorus can improve the quality of wastewaters (Porcella et al., 1972).

The microcosm approach is an excellent means for identifying parameters and interactions in sediment-water systems. Gas analysis, nitrogen fixation, dissolved O_2/CO_2 interactions, and nutrient cycling (carbon, nitrogen, phosphorus, and iron) were all major factors involved in respiration and photosynthetic activity in dark and light microcosms.

CONCLUSIONS

1. The following gases were detected in the microcosms: N_2, CO_2, O_2, CH_4, CH_2CH_2, H_2S. H_2S was too low in concentration to be detected except by its odor.

2. In the dark microcosms O_2 was rapidly utilized; thus, extremely low concentrations of O_2 occurred in the overlying water and atmosphere of the microcosms and anaerobic conditions occurred in the sediments.

3. Mass balances of gases around the sediment-water system of the microcosms indicated that: (a) Atmospheric nitrogen gas dynamics were not affected appreciably by nitrogen fixation. (b) Oxygen gas dynamics were especially responsive to benthic oxygen demand in the dark microcosms (> 300 mg O_2/m^2 day). And photosynthesis resulted in considerable oxygen input to the atmosphere in the lighted microcosms; partial pressures of up to 40 percent oxygen were observed. (c) CO_2 dynamics resulted in a pH range of about 6.5 to 7.0 in the dark microcosms and generally 9.5 to 10.0 in the lighted microcosms. (d) Methane production occurred in all microcosms after 40 days and apparently resulted from methanogenesis in the sediments. Thus sediments remained anaerobic even in the highly productive light microcosms. Methane utilization by water column bacteria may have been responsible for the observation that the lighted microcosms (aerobic) produced less methane than the dark microcosms (anaerobic). (e) Ethylene ($H_2C = CH_2$) production was not observed until late in the study (≥ 120 days of operation) and was detected in Microcosms 1, 2, and 13, but not 14. Highest concentrations were observed in the dark microcosms. Water column utilization of the ethylene in the aerobic microcosms by bacteria may have resulted in its disappearance.

4. Dissolved oxygen dynamics in lighted microcosms indicated that almost all the CO_2 was utilized driving the pH to 10 and lowering total dissolved inorganic carbon to values of 1 mg/l and less.

5. Nitrogen output from sediments was apparently limited and had an insignificant effect on sediment nitrogen even though it may have had a significant impact on the algal community. Nitrogen input to the sediments was appreciable and occurred in the upper layers primarily. Highest concentrations were observed in 0-1 cm layer but sediment mixing (from gas bubble release) must have occurred because typically the 1-5 cm layer had higher concentrations than the 5-15 cm layer or the initial sediment nitrogen concentration.

6. Nitrogen fixation (acetylene-ethylene) was observed to occur only in the lighted microcosms indicating involvement of blue-green nitrogen fixers. Significant populations of heterocystous *Anabaena*. in the wall growth indicated the probable source of the fixation. Nitrogen fixation by periphyton in natural systems is a likely significant source of nitrogen.

7. Lighted microcosms receiving no influent nitrate nitrogen produced essentially as much oxygen as those which did receive an input. Thus fixation was adequate to make up the nitrogen requirement so that some other factor was limiting algal growth.

8. Denitrification occurred in the plus nitrogen dark microcosms.

9. Nitrification could not be substantiated in any microcosm.

10. Organic iron and iron-phosphorus interactions controlled iron and phosphorus availability. These elements were in considerable excess relative to photosynthetic needs; sediments acted as a significant source of these elements.

ACKNOWLEDGMENTS

Many persons at the Utah Water Research Laboratory worked on providing the data for this paper and we gratefully acknowledge their help. The study was supported by Office of Water Research and Technology (Project No. B-081-Utah) and the Utah Water Research Laboratory.

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Effects of Water Hardness, Phosphorus Concentration and Sample Pretreatment on the Algal Assay Procedure— Bottle Test

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INTRODUCTION

Recent literature (Filip and Middlebrooks, 1975; Miller et al., 1974) and several discussions during the course of the Biostimulation and Nutrient Assessment Symposium suggest that the water sample pretreatment used affects the results of the algal assay procedure-bottle test (AAP).

In a recent survey of the waters of lakes in two states, New York and Indiana, we have also found significant effects of different sample pretreatments, apparently related to differences in water hardness.

METHODS

Surface samples were collected from 15 lakes in New York and 16 lakes in Indiana, beginning in early April 1975, at the time of ice-out and spring turnover. Routine biweekly sampling of surface and hypolimnion waters was initiated in a selected group of lakes in both states during the third week of June. Chemical analyses and algal assays were performed on samples kept on ice during the 2-3 days required for shipment to the laboratory.

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Algal assays on all samples were conducted using *Selenastrum capricornutum* (Printz) according to the published AAP (U.S. EPA, 1971). Both autoclaving followed by filtration through a 0.45 μm membrane filter and filtration alone were used as pretreatments. After autoclaving, prior to filtration, the samples were shaken (100 opm), for approximately 12 hours to restore equilibrium carbon dioxide levels and pH.

Since the status of phosphorus as an algal nutrient was of prime consideration in these studies, phosphorus (50 μg P/1 as K_2HPO_4), plus micronutrient salts (concentration as specified in AAP for the synthetic algal nutrient medium) were added to separate samples of the waters. Inoculated flasks containing the various lake waters with and without micronutrient salts additions served as fertility controls. Cell counts were made with an electronic particle counter on triplicate flasks and averaged.

In vivo measurements of chlorophyll *a* were made using an Aminco Fluoro-Colorimeter®. As used here this instrument measured the fluorescent response (663 nm, fluorescence units) of whole cells to ultraviolet light (430 nm). This was a simple and rapid method for monitoring gross changes in algal chlorophyll concentrations in the surface waters surveyed.

Water samples were analyzed chemically (U.S. EPA, 1974) prior to any pretreatment (untreated, U) and after autoclaving followed by filtration (A&F). The term, ortho-P, used below, refers to phosphorus passing through a 0.45 μm filter and analyzed by the ascorbic acid-phosphomolybdate blue method without persulfate treatment. Total dissolved phosphorus, TDP, is that which passed through a 0.45 μm filter and was analyzed similarly with persulfate treatment. Spot check analysis of water following filtration indicated no effect on soluble phosphorus forms, but a consistent reduction in the particulate P fractions, as would be expected.

RESULTS AND DISCUSSION

Results of analyses of water samples, including phosphorus concentration before and after autoclaving followed by filtration, are shown in Tables 1 through 4.

The data on these four tables are listed in order from highest to lowest fluorescence value of the untreated lake waters. The columns headed A&F:U refer to the ratio of the soluble phosphorus concentration (ortho-P or ortho-P and TDP) measured after autoclaving and filtration versus that measured in the untreated sample of water. A number less than unity in this column indicates loss of soluble phosphorus during the autoclaving-

Table 1. New York Lake waters, April 1975.

Lake	Date	Sample Type[a] (S or B)	Temp. (°C)	Fluor-escence	Ca + Mg Hardness as mg/l $CaCO_3$	Phosphorus				A&F:U Ratio
						Untreated		A & F		
						Ortho-P (μg/l)	TP (μg/l)	Ortho-P (μg/l)	TP (μg/l)	Ortho-P
Oneida	4/9	S	3	62	115	4	39	7	37	1.75
Onondaga	4/9	S	4	49	1024	212	396	22	91	0.10
Silver	4/3	S	3	45	103	4	22	6	32	1.50
Cayuga	4/8	S	4	30	171	7	52	7	53	1.00
Seneca	4/4	S	4	26	166	10	33	5	23	0.50
Otisco	4/9	S	3	25	152	2	18	3	33	1.50
Owasco	4/8	S	4	23	163	3	22	3	28	1.00
Conesus	4/3	S	3	21	153	4	17	4	30	1.00
Skaneateles	4/8	S	4	19	130	3	31	6	29	2.00

[a] S: Sample collected from surface.
B: Sample collected with VanDorn Bottle 5 feet from lake bottom.

Table 2. Indiana Lake waters, April 1975.

Lake	Date	Sample Type[a] (S or B)	Temp. (°C)	Fluor-escence	Ca + Mg Hardness as mg/l $CaCO_3$	Phosphorus				A&F:U Ratio
						Untreated		A & F		
						Ortho-P (μg/l)	TP (μg/l)	Ortho-P (μg/l)	TP (μg/l)	Ortho-P
Hogback	4/15	S	7	240	371	7	51	10	41	1.43
Cedar	4/16	S	12	230	209	6	109	11	83	1.83
Freeman	4/16	S	9	155	323	5	44	3	46	0.60
Morse	4/14	S	8	140	294	23	126	4	51	0.17
Geist	4/14	S	7	130	339	3	76	3	30	1.00
Salamonie	4/14	S	8	115	281	36	109	7	47	0.19
Shafer	4/16	S	9	115	331	12	54	2	29	0.17
Whitewater	4/17	S	10	110	305	28	76	4	41	0.14
Versailles	4/17	S	11	100	194	16	104	5	44	0.31
Silver	4/15	S	7	94	269	2	23	5	40	2.50
Tippecanoe	4/15	S	8	91	273	5	28	6	32	1.20
Mississinewa	4/14	S	9	65	400	64	106	5	33	0.08
Maxinkuckee	4/16	S	8	49	234	4	21	3	23	0.75
Wawasee	4/15	S	8	41	232	4	18	3	23	0.75
Gage	4/15	S	6	25	251	3	15	7	67	2.33

[a] S: Sample collected from surface.
B: Sample collected with VanDorn Bottle 5 feet from lake bottom.

Table 3. New York Lake waters, Summer 1975.

Lake	Date	Sample Type[a] (S or B)	Temp. (°C)	Fluor-escence	Ca + Mg Hardness as mg/l $CaCO_3$	Phosphorus						A&F:U Ratio	
						Untreated			A&F				
						Ortho-P (μg/l)	TDP (μg/l)	TP (μg/l)	Ortho-P (μg/l)	TDP (μg/l)	TP (μg/l)	Ortho-P	TDP
Onondaga	6/25	S	27	1000	850	5	25	271	17	138	150	3.40	5.52
Onondaga	9/17	S	19	590	1243	13	40	184	15	84	130	1.15	2.10
Onondaga	7/29	S	27	260	1107	3	21	82	6	46	513	2.00	2.19
Onondaga	8/27	S	22	250	1218	3	16	128	5	53	59	1.67	3.31
Oneida	8/27	S	22	51	145	72	78	100	78	91	94	1.08	1.17
Oneida	9/17	S	17	49	122	59	64	91	66	87	135	1.12	1.36
Cayuga	9/16	S	16	29	142	3	9	21	9	24	46	3.00	2.67
Cayuga	8/26	S	23	12	168	1	8	15	3	10	11	3.00	1.25

[a]S: Sample collected from surface.
B: Sample collected with VanDorn Bottle 5 feet from lake bottom.

Table 4. Indiana Lake waters, Summer 1975.

Lake	Date	Sample Type[a] (S or B)	Temp. (°C)	Fluor-escence	Ca + Mg Hardness as mg/l $CaCO_3$	Phosphorus						A&F:U Ratio	
						Untreated			A&F				
						Ortho-P (μg/l)	TDP (μg/l)	TP (μg/l)	Ortho-P (μg/l)	TDP (μg/l)	TP (μg/l)	Ortho-P	TDP
Big Center	8/4	S	27	550	211	3	34	201	37	201	283	12.33	5.91
Mississinewa	6/18	S	25	350	186	64	69	282	76	113	123	1.19	1.64
Big Center	6/19	S	21	310	277	47	69	254	103	250	280	2.19	3.62
Morse	8/5	B	27	250	216	9	21	375	47	116	511	5.22	5.52
Mississinewa	8/21	S	28	205	264	5	17	92	8	28	41	1.60	1.65
Mississinewa	8/2	B	29	185	290	39	45	208	29	73	79	0.74	1.62
Hogback	7/22	S	29	170	382	6	18	40	6	54	64	1.00	3.00
Long	7/22	S	28	165	422	6	17	42	3	36	49	0.50	2.12
Long	8/20	S	-	165	-	6	16	47	2	11	18	0.33	0.69
Morse	8/21	S	29	115	198	5	13	40	7	19	19	1.40	1.46
Morse	6/18	S+B	-	89	225	8	17	59	4	22	28	0.50	1.29
Gage	7/22	S	28	22	242	2	7	13	1	23	25	0.50	3.29

[a]S: Sample collected from surface.
B: Sample collected with VanDorn Bottle 5 feet from lake bottom.

filtration pretreatment process and conversely a number greater than unity indicates a net gain in soluble phosphorus as the results of sample pretreatment.

These A&F:U ratios were averaged for groups of both the Indiana and New York lakes selected according to high or low total hardness concentration (for Indiana, 194 ppm as $CaCO_3$ was the point of division; for New York, 171 ppm as $CaCO_3$ was the point of division). The mean A&F:U ratios for the spring and summer samplings are listed on Tables 5 and 6. A further subdivision of the lakes based on untreated lake water ortho-P concentration (above or below 11μg/l) was also made. The mean fluorescence values for the untreated lake waters of each group are shown in Tables 5 and 6.

Results of the April Sampling

The mean A&F:U ratios calculated for the New York and Indiana lake waters (Table 5) which had low ortho-P (<11μg/l) before treatment are not significantly different from unity, showing that there was not a significant decrease or increase in ortho-P. The A&F:U ratio calculated for the Indiana (hard) water which had a high ortho-P (>11μg/l) concentration before treatment was substanitally lower, indicating a loss of ortho-P during the autoclaving-filtration pretreatment.

The A&F:U ratio of 0.10 listed for the single New York lake water, which had a high ortho-P concentration before treatment, reflects the dramatic difference between Lake Onondaga and the other New York lakes. The total hardness of Lake Onondaga at the time of ice-out was >1000 ppm as $CaCO_3$ as shown in Table 1. The loss of ortho-P in the Onondaga water due to autoclaving followed by filtration was 90 percent.

This loss of total phosphorus due to autoclaving is mentioned in the AAP manual (U.S. EPA 1971). Filip and Middlebrooks (1975) point out that the pH rise accompanying autoclaving can cause precipitation. Futher, if the precipitate is filtered off, the phosphorus is permanently lost to the assay system.

Table 7 shows AAP results which demonstrate that the ortho-P removal due to autoclaving as shown by chemical assay is reflected in reduced growth of *Selenastrum* in 14 days. Columns headed by A/F/A in Table 7 give the algal growth response following a second autoclaving performed to insure sterility of the sample just prior to inoculation. The algal growth in the control samples of the hard water lakes was greatly reduced by the A/F/A pretreatment, compared with 0.45 μm filtration without any autoclaving. The P-availability for these samples may sometimes be further affected by the second autoclaving. The lack of a

Table 5. Ratios of ortho-P concentrations (A&F:U) for New York and Indiana Waters, Spring 1975.

	Untreated Water Ortho-P	Number of Lakes	Ratio (Mean ± 1 Std. Dev.) Ortho-P	Ca + Mg Hardness as mg/l (Mean ± 1 Std. Dev.)	Fluorescence (Units) (Mean ± 1 Std. Dev.)
New York	Low ($\leqslant 10$ µg/l)	8	1.28 ± 0.49	144 ± 25	32 ± 15
	High (> 10 µg/l)	1	0.10	1024	49
Indiana	Low ($\leqslant 10$ µg/l)	9	1.38 ± 0.70	278 ± 55	117 ± 79
	High (> 10 µg/l)	6	0.18 ± 0.08	300 ± 67	107 ± 25

Table 6. Ratios of ortho-P and total dissolved-P concentrations (A&F:U) for New York and Indiana waters, Summer 1975.

	Untreated Water Ortho-P	Number of Lakes	Ratio (Mean ± Std. Dev.)		Ca + Mg Hardness as mg/l $CaCO_3$ (Mean ± 1 Std. Dev.)	Fluorescence (Units) Mean ± (1 Std. Dev.)
			Ortho-P	Total Dissolved P		
New York	Low ($\leqslant 10$ µg/l)	2	3.00 ± 0.00	1.96 ± 1.00	155 ± 18	21 ± 12
	High (> 10 µg/l)	2	1.10 ± 0.03	1.27 ± 0.13	134 ± 16	50 ± 1
Indiana	Low ($\leqslant 10$ µg/l)	9	2.60 ± 3.95	2.77 ± 1.86	270 ± 85	192 ± 150
	High (> 10 µg/l)	3	1.37 ± 0.74	2.29 ± 1.15	251 ± 57	281 ± 86

Table 7. Growth response of *Selenastrum capricornutum* (cells/ml. x 10^6) in lake samples collected in April 1975.

Lake	Date Mo./Day	Chemical Assay					Algal Assay Bottle Best (14-Day Incubation)					
		Untreated			A&F		Filtered			A/F/A[a]		
		T.H.[b]	O-P µg/l	T-P µg/l	O-P µg/l	T-P µg/l	Control	Control + Minor (M.E.) Elements	Spike (50 µg P) + M.E.	Control	Control + Minor (M.E.) Elements	Spike (50 µg P) + M.E.
Cayuga, N.Y.	4/8	171	7	52	7	53	0.015	0.055	1.8	0.050	0.057	1.7
Onondaga, N.Y.	4/9	1024	212	396	22	91	2.5	6.5	7.8	0.036	0.20	0.28
Mississinewa Res., Ind.	4/14	400	64	106	5	33	1.7	1.7	4.4	0.064	0.092	0.35
Morse Res., Ind.	4/14	294	23	136	4	51	1.1	1.1	3.8	0.065	0.17	0.14

[a]Samples autoclaved followed by filtration and reautoclaved prior to inoculation.

[b]Total Ca and Mg hardness expressed as mg/l $CaCO_3$.

proportional growth response to the 50μg/l of ortho-P added to the hard water samples from both Indiana lakes and Lake Onondaga reflects this effect, i.e. non-availability of the added phosphorus due to precipitation.

The algal counts for both the filtered but not autoclaved and the A/F/A Cayuga Lake samples are equivalent and reflect the unchanged ortho-P in these samples.

The significant loss of ortho-P due to autoclaving pretreatment, reflected in the results of the AAP, could lead to an erroneous assessment of the algal growth potential and/or nutrient limitation status of such lakes, if taken at face value.

The soluble ortho-P precipitated during autoclaving of natural hard waters was usually not redissolved by re-equilibration at 24°C with the CO_2 in air, or by saturation for one hour with CO_2 at one atmosphere pressure.

Results of the Summer Samplings

During the summer sampling program, randomly selected samples were assayed using both filtration and autoclaving-filtration.

The mean A&F:U ratios computed for the summer lake waters (Table 6) clearly indicate that the April trend of ortho-P loss was no longer observed in the waters which had high ortho-P concentrations before treatment. None of the A&F:U ratios in Tables 3 and 4 match the low values of 0.10 and 0.18 ± 0.08 found for the April samples (Table 5). Generally speaking there was a net gain in ortho-P due to autoclaving. This increase seems to be associated with the higher fluorescence values obtained on these untreated waters. The mean fluorescence values in Table 6 reflect the higher algal biomass present in these summer samples compared with the April samples shown in Table 5. However, other AAP experiments, not reported here, suggest that much of the increased analyzable ortho-P was not available for *Selenastrum* growth during the 14-day incubation time, since the expected growth corresponding to the increased ortho-P analyses was not achieved. The TDP concentration of the summer samples also increased greatly when pretreatment included autoclaving. High biomass samples, when autoclaved, release soluble P which analyzes as ortho-P and TDP.

The linear correlation coefficient (+0.84) between raw water fluorescence and the A&F:U ratio for concentrations of ortho-P of summer Indiana samples (Table 4) was significant at the 95 percent level. The correlation coefficient for the New York summer group (Table 3) was +0.28, which is disappointingly low for reasons not understood.

The data from the two Lake Oneida summer samples in Table 3 indicate that relatively soft water lakes with high ($\geq 10\mu g/l$) untreated lake water ortho-P and relatively low (<150) untreated water fluorescence do not experience a loss of ortho-P during A&F pretreatment. In contrast, the April samples from Indiana lakes (Table 2), which also had high ortho-P and low fluorescence but higher hardness, showed significant loss of ortho-P due to A&F pretreatment.

Although the above comments pertain to problems accompanying autoclave pretreatment of water samples, 0.45 μm filtration pretreatment, also suggested in the AAP, is not without similar drawbacks. Appendix 5 of the AAP notes that "close to 67 percent of the total phosphorus of eutrophic water samples" may be removed by such filtration.

SUMMARY AND CONCLUSIONS

Water sample pretreatment by autoclaving and filtration may lead to erroneous results in the algal assay procedure-bottle test. Controlling factors include water hardness, phosphorus concentration and standing algal crop. Waters of high hardness (>200 ppm as $CaCO_3$) and high ortho-P (>10μg/l) may form a precipitate during autoclaving which effectively depletes available phosphorus, especially if the precipitate is filtered off. A substantial algal crop (>200 fluorescence units from *in vivo* chlorophyll *a*) in such waters, on the other hand, releases sufficient dissolved phosphorus during autoclaving to effect a net gain in soluble phosphorus concentration.

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A Bioassay Dilution Technique to Assess the Significance of Dredged Material Disposal

R. H. Plumb, Jr.*

INTRODUCTION

One of the potential problems at an open-water dredged material disposal site is that the nutrients associated with dredged sediment and its interstitial water may stimulate the growth of algae at the site. The basis for this concern is the relatively high levels of nitrogen and phosphorus that have been reported in the sediment. Unfortunately, the reported concentrations are generally bulk sediment analysis results that fail to consider the chemical form or availability of the chemical in question. However, there is a growing awareness of this problem, and investigations are in progress to evaluate an Elutriate Test (Lee and Plumb, 1974), a short-term leaching test, as a measure of sediment potential to release chemicals to surrounding water during disposal operations. Lee and Plumb (1974) suggested that bioassays are necessary in the Elutriate Test evaluation since the potential problems of algal stimulation are associated with nutrient availability and nutritional status of the algae in much the same way that chemical release is affected by chemical form and availability rather than total sediment concentration.

An appreciation of conditions at an open-water site makes it evident that the conventional bioassay methodology is not applicable to a discrete discharge operation. Prior to the discharge of dredged material, the site can be considered to have a uniform distribution of algae. Immediately after the disposal of dredged material, a portion of the population will be exposed to some high initial concentration. With the passage of time, the

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high initial concentration will be diluted by mixing with surrounding waters. Thus, the initially exposed algae will have been in contact with a high average concentration C_0 for a time interval t_1-t_0 and a lower average concentration C_1 for a time interval t_2-t_1. This step is not simply dilution, however, since the dilution water also contains algae that will be exposed to an average concentration C_1 for the time interval t_2-t_1. As the process continues, increasing numbers of algae will be exposed to the discharge but at decreasing concentrations.

It becomes apparent that the constant concentration and the exposure times of 96 hours to 2 weeks used in a standard bioassay are unrealistic and that results from such a test will only provide information on the availability of nutrients but not on the significance of the nutrients because the test conditions do not approximate those at a disposal site. Since open-water disposal is an intermittent event that produces some high initial concentration that is rapdily diluted with the passage of time, algal assays to assess the significance of open-water disposal must incorporate a dilution technique as a reasonable approximation to actual conditions.

APPROACH

The Elutriate Test (Lee and Plumb, 1974) is one of several procedures to be used to satisfy Section 404(b) of the 1972 Federal Water Pollution Control Act to evaluate the ecological impact of proposed dredged material disposal operations. The test specifies a ratio of one volume sediment to four volumes dredging site water as an approximation to the slurry discharged during hydraulic dredging, then a conventional bioassay of the filtrate.

As indicated in Figure 1, the concentration of a bioavailable constitutent in the standard elutriate would represent a maximum instantaneous concentration resulting from the discharge of dredged material that would be expected to decrease with time due to the combined effects of mixing, advection, and settling. Since the time required for dilution is short compared to the exposure time used in the conventional bioassay evaluation, the impact of the discharge would be less than the bioassay results would indicate. However, the use of a calculated average concentration would not necessarily provide a better estimate of the significance of a dredged material discharge since possible shock effects of the high initial concentration would not be considered (Figure 1).

The bioassay procedure discussed in this paper incorporates a serial dilution technique as a reasonable approximation of the changing concentration-exposure time relationship that would be expected

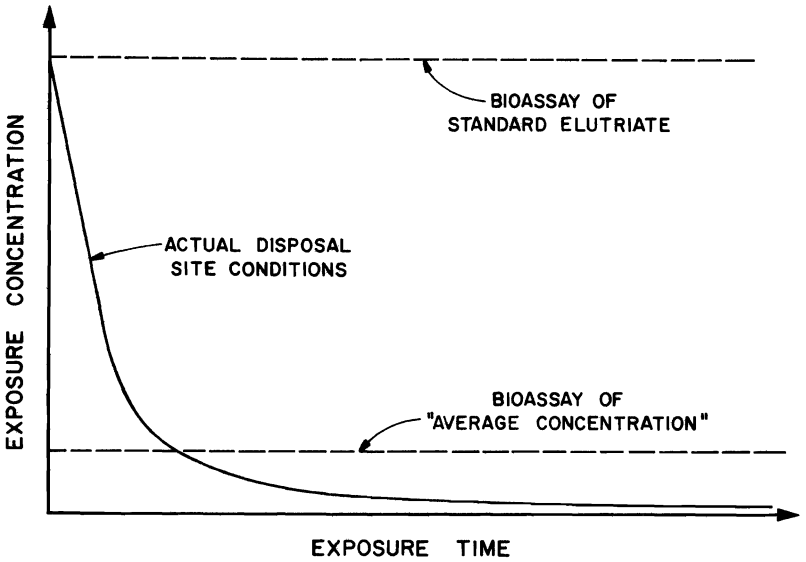

Figure 1. Schematic comparison of actual disposal site conditions with bioassay results of the standard elutriate and an average elutriate concentration.

following the discharge of dredged material at an open-water site. The implementation of the approach required the development of an expression for the expected rate of dilution. An empirical relationship between apparent dilution and time was obtained based on dye diffusion data collected by Carter and Okubo (1965). These studies were conducted over a three-year period with different types of dyes at different initial concentrations, in different water areas along the Florida coast, and at different times of the year. The Carter and Okubo (1965) data were transformed on the assumptions that the concentration at any time t was proportioned to the initial mass of dye released and the the concentration would decrease with time according to the equation:

$$C = M e^{-kt} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where:

$C = M e^{-kt}$
C = concentration at time, ug/l
M = mass of dye relased, ug
t = time since release, hours
k = diffusion coefficient -lm(1)/hr

The calculated diffusion coefficient k varies with time, but the transformed diffusion data could be described by the following equation:

$$\log k = -0.94 (\log t) + 1.26 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

with a correlation coefficient of 0.99 between log k and log t for 78 observations.

Equations 1 and 2 were then used to prepare a dilution curve from which the time intervals necessary for successive tenfold dilutions to occur were obtained (Figure 2). Equation 1 was used to calculate the expected concentration at 15 min. This value was selected as the "initial concentration" at an open-water disposal site because a discrete discharge would be expected to last approximately 30 min (Keeley, 1975). The necessary time intervals for successive tenfold dilutions to occur were then calculated to be 2, 15, 72, and 336 hr. That is, the concentration at 2-hr would be expected to be 0.1 of the 15-min concentration; the concentration at 15 hr would be expected to be 0.01 of the 15-min concentration, etc.

As a further approximation to open-water disposal, the water used for sequential dilution also contained algae. This follows from the fact that water at the disposal site not immediately affected by a discharge contains algae that will be influenced as a consequence of mixing and diffusion. However, these algae will be exposed to a lower concentration of the discharge for a different period of time.

PROCEDURES

Sediment samples and site water were collected from several locations in the Mobile, Alabama, Ship Channel in March 1975. This material was used to prepare standard elutriates as described by Keeley and Engler (1974). One volume of sediment was added to four volumes of site water and was shaken for 30 minutes. After a 1-hour settling period, the standard elutriate (filtrate) was obtained by centrifugation and filtration through a 0.45μ membrane filter. Filtered Mobile Harbor water was used as dilution water.

The experimental design consisted of duplicate flasks containing 100 ml of 100-percent standard elutriate (flasks A-1 and A-2) and eight flasks containing 90 ml of 0.45μ filtered site water (flasks B-1, B-2, C-1, C-2, D-1, D-2, E-1, and E-2). In addition, controls consisting of 100 ml site water and 100 ml of PAAP medium (U.S. EPA, 1969) in site water were prepared in duplicate. At the beginning of the experiment, each flask was inoculated with 200,000 cells per 100 ml solution.

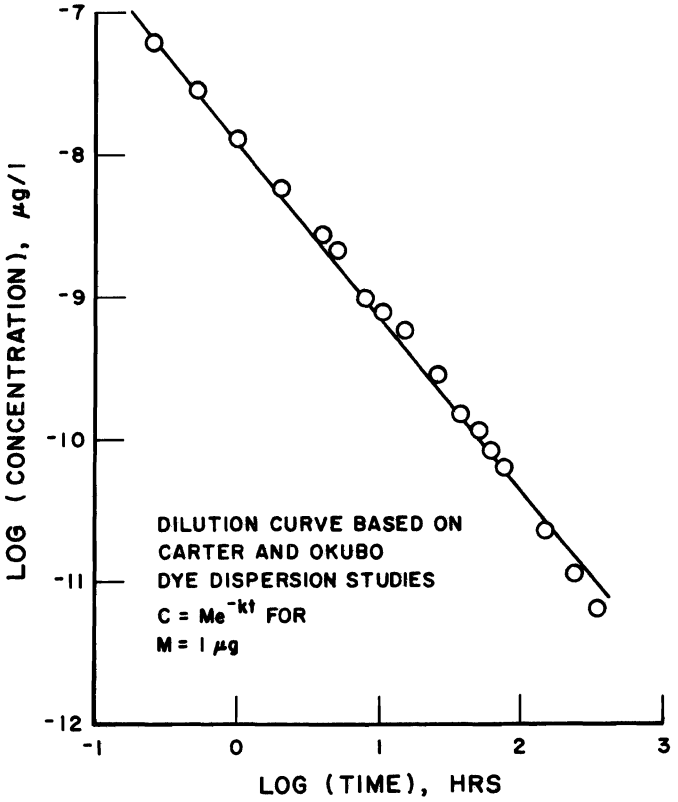

Figure 2. Concentration-time curve based on Carter and Okubo (1965) dye dispersion studies and a unit mass of 1 μg.

One hour after the experiment began, 10 ml of culture A (100-percent standard elutriate) was added to flask B to simulate a tenfold dilution. Three hours later (4 hours after the experiment began), 10 ml of culture B was added to culture C. Sequential tenfold dilutions from culture C to culture D and culture D to culture E were performed at 22 and 96 hours, respectively. The dilution times were selected based on an anlysis of dye diffusion data discussed earlier and were used as the best available estimate of what was likely to happen at an open-water disposal site.

Two types of algal cultures were used in the initial experiments described in this paper. One was *Dunaliella tertiolecta* grown in PAAP media and 8°/oo artificial sea water and the second was *Selenastrum capricornutum* grown in PAAP medium. The PAAP medium was prepared as needed by dilution of stock nutrient solutions (U.S. EPA, 1969).

RESULTS

Results of the *D. tertiolecta* bioassay are presented in Table 1. For the PAAP, site water, and 100-percent elutriate cultures, the reported cell counts were observed at the specified time after the start of the experiment. The results for culture labeled "Dilutant" were taken from different cultures depending on the elapsed time after the start of the experiment. The 24- and the 48-hour observations were taken from culture D in the sequential dilution series and the 96-, 192-, and 432-hour observations were taken from culture E in the same series. The reason for choosing these cultures is that, based on the dye diffusion studies of Carter and Okubo (1965), these cultures represent the extent of dilution to be expected at an open-water disposal site at the time the cultures were sampled.

PAAP medium cultures peaked at 1.5 million cells/ml between four and eight days and decreased to 800,000 cells/ml at day 18. The site water population reached approximately 250,000 cells/ml on day 4 and decreased slightly to 200,000 cells/ml at day 18. The algal population in the 100-percent elutriate cultures were similar to the site water cultures for the first eight days. However, by the eighteenth day, a significant reduction from 200,000 cells/ml to 10,000 cells/ml had occurred.

Another qualitative difference between the 100-percent elutriate cultures and the control cultures was apparent during the counting due to

Table 1. Dilution bioassay results of Mobil Harbor sediment elutriates.

Culture	Sample No.	Test Duration—Cell Counts				
		24 hr	48 hr	96 hr	192 hr	432 hr
PAAP	1	50	225	1,526	1,475	748
	2	60	195	1,428	1,308	848
Site Water	1	90	108	218	---	205
	2	50	120	278	220	198
100-Percent elutriate	1	10	115	248	240	7.5
	2	30	95	228	172	12.5
"Dilutant"	1	60	172	---	190	240
	2	50	110	245	272	198

Notes: All cell counts are the average of duplicate cell counts of duplicate subsamples from each culture.

The initial inoculum was 2×10^6 cells/100 ml.

Cell counts are expressed as 10^3 cells/ml.

the fact that *D. tertiolecta* is a motile organism. Samples were not fixed and the test organisms were seen to be motile in the PAAP and site water cultures but not in the elutriate cultures. This would suggest that the population in the elutriate cultures were under stress even though the population increased during the first eight days and that the stress resulted in a lower population on day 18. By comparison, the *D. tertiolecta* population in the dilution sequence maintained a motility similar to the site water cultures. Thus, even though the population was exposed to a toxic or inhibitory condition, there was no qualitative indication of stress and the final cell count on day 18 would suggest that no significant effect on the algal population would result from the open-water disposal of Mobile Harbor sediments.

Observed cell counts in all cultures after 432 hours are presented in Table 2. Maximum cell counts of 800,000 cells/ml were present in the PAAP growth medium cultures. The lowest cell counts, 10,000 cells/ml

Table 2. Dilution bioassay results of Mobil Harbor sediment elutriates after 432 hours.

Experimental Conditions	Culture	Cell Counts
100-percent elutriate from t = 0 to t = 432	A-1	7
	A-2	12
Site water from t = 0 to t = 1 and 10-percent elutriate from t = 1 to t = 432	B-1	22
	B-2	60
Site water from t = 0 to t = 4 and 1-percent elutriate from t = 4 to t = 432	C-1	215
	C-2	205
Site water from t = 0 to t = 22 and 0.1-percent elutriate from t = 22 to t = 432	D-1	202
	D-2	232
Site water from t = 0 to t = 96 and 0.01-percent elutriate from t = 96 to t = 432	E-1	240
	E-2	192
Site water from t = 0 to t = 432	SW-1	205
	SW-2	198
Site water plus PAAP from t = 0 to t = 432	P-1	748
	P-2	848

Notes: Cell counts are expressed as 10^3 organisms/ml. All counts are the average of duplicate counts of duplicate subsamples from each culture.

The initial inoculum in each culture was 2×10^5 cells.

All times (t) are given in hours.

Elutriate percentages are based on percentages to be expected following successive tenfold dilutions.

were observed in the 100-percent elutriate cultures (A), and the next lowest algal population occurred in the flasks containing 10-percent elutriate culture (B) for all but one hour of the experiment. These results are in agreement with unpublished results of Shuba (1975), who observed that 12-, 50-, 75-, and 100-percent solutions of Mobil Harbor elutriates were toxic to *D. tertiolecta*. Results from the 1-percent elutriate cultures (C), 0.1-percent elutriate cultures (D), and 0.01-percent elutriate cultures (E) were essentially the same as the site water cultures (SW).

The importance of considering the concentration-exposure time regime at an open-water disposal site in order to assess the significance of discrete discharges was further demonstrated by running a dilution bioassay of PAAP medium. The experimental setup was essentially the same as described above, except that complete PAAP medium was used in place of the standard elutriate; PAAP medium without phosphate addition (PAAP-P) was used as dilution water; and *Selenastrum capricornutum* was used as the test organism. The experimental design would be similar to discharging phosphorus-rich waste into a phosphorus-limited environment.

Results of the dilution bioassay, presented in Table 3, demonstrated that the highest cell counts were observed in complete PAAP medium (A and P) as would be expected. These values were approximately 2,000,000 cells/ml after 123 hours and 4,000,000 cells/ml after 291 hours. The 10-percent PAAP medium cultures (B) had cell counts approximately one order of magnitude lower than the complete PAAP medium cultures. Cell counts in the remaining cultures of the PAAP dilution sequence (C,D,E) were not different from the PAAP-P medium (H) used as a control.

DISCUSSION

All chemical constituents present in natural water systems are not equally available to aquatic organisms and one purpose of conducting bioassays is to determine what fraction of the total chemical concentration is available. However, as pointed out by Brown (1973), the concentration capable of producing some selected response is a function of the duration and nature of the exposure. This fact becomes apparent after examining any published list of toxicity data, such as that reported by McKee and Wolf (1963). Therefore, time of exposure is an important factor that must be considered in assessing the significance of a discrete discharge such as dredged material or an industrial spill. This is particularly true for those situations where the resultant concentrations persist for time periods that are short compared to those specified in bioassay procedures used to develop water-quality criteria.

Table 3. Results of a dilution bioassay of PAAP medium using *Selenastrum capricornutum*.

Experimental Conditions	Culture	Test Duration Cell Counts	
		123 hr	291 hr
PAAP media from t = 0 to t = 291	A	210	400
PAAP-P media from t = 0 to t = 1 and 10-percent PAAP media from t = 1 to t = 291	B	30	41
PAAP-P media from t = 0 to t = 4 and 1-percent PAAP media from t = 4 to t = 291	C	6.0	3.5
PAAP-P media from t = 0 to t = 22 and 0.1-percent PAAP media from t = 22 to t = 291	D	2.0	---
PAAP-P media from t = 0 to t = 100 and 0.01-percent PAAP media from t = 100 to t = 291	E	3.0	3.5
PAAP media from t = 0 to t = 291	P	240	400
PAAP-P media from t = 0 to t = 291	H	2.0	3.5

Notes: Cell counts are expressed as 10^4 organisms/ml.

All times (t) are given in hours.

PAAP media percentages are based on percentages to be expected following successive tenfold dilutions.

Brown (1973) has stated that the duration of a toxicity test should not be selected on convenience but should have some rational basis. Results presented in this paper demonstrate the importance of considering exposure time in order to assess the significance of a discrete discharge such as dredged material disposal in open water. Substances shown by conventional bioassay procedures to be inhibitory (Mobil Harbor elutriate) or stimulatory (PAAP medium) did not have a significant effect on the test population when the rate of dilution was simulated. Although the difference in results between conventional bioassay procedures and the dilution bioassay procedure can only be resolved with the collection of sufficient field data, the fact that disturbances due to dredging activities have become undetectable within 2 hours of disposal termination (May, 1973) is sufficient justification to consider the rate of dilution at a disposal site in order to assess the potential effects of open-water disposal.

The dilution equations developed in this work may not be strictly applicable to dredging activities because they are based on dye diffusion studies rather than suspended solids dispersion. However, the rate of dilution used in the experiments can probably be considered conservative because suspended solids would be removed from the water column faster than a soluble dye. Also, Carter and Okubo (1965) reported the maximum concentration remaining in the dye cloud at the time of sampling and not the change in concentration at a single point in space, which would further tend to make the rate of dilution that was used conservative.

The development of a single dilution equation in this study does not imply that the equation is universally applicable. The Carter and Okubo (1965) data set was utilized because they had sufficient data to define a dilution curve and because the data covered at time interval similar to that used in algal bioassays. As more data becomes available on open-water disposal and better equations are developed to describe the rate of dilution, the bioassay procedure can be modified but this will only require changing the time interval between successive dilution steps. In addition, if it is desirable to study the possible effects of a discharge in more detail and a dilution curve is available, dilution factors of two or five can easily be substituted for the factor of ten used in this study. It is also apparent that the conventional bioassay is only a special case in a dilution bioassay (initial concentration is specified with a dilution rate of zero).

One of the main objectives of bioassay procedures is the proper assessment of the hazard associated with any given pollutant (Brown, 1973). Because the hazard associated with a pollutant will be a function of the exposure concentration and the exposure time, it is suggested that the described dilution procedure would provide more representative estimates of the possible significance of open-water disposal of dredged material since the method provides the flexibility to consider site specific hydrodynamic factors that will affect exposure time and concentration. An estimated 290 million cubic meters of sediment are annually dredged from the nation's waterways (Boyd et al., 1972), and some type of regulatory decision must be made to place this material on land or at some other location in the waterway. It is felt that the dilution bioassay procedure will provide a more rational basis for deciding between the potential effects of open-water disposal and the higher costs of on-land disposal.

SUMMARY

A bioassay method has been proposed to assess the practice of open-water disposal of dredged material. The proposed method is based on nonlinear diffusion data observed in the field and approximates the changing concentration-time relationships that will exist at an open-water

disposal site. A dilution bioassay is necessary because a conventional bioassay will only indicate the bioavailability of constituents associated with dredged material and not the significance of the discharge because conventional bioassays are run for long periods of time compared to the duration of dredged material perturbations. Results demonstrated that inhibitory and stimulatory additions would not have a significant effect on algae when the rate of dilution at an open-water site is considered. It is anticipated that the proposed procedure could provide necessary information in determining the appropriate method of dredged material disposal.

ACKNOWLEDGMENT

The tests described and the resulting data presented herein, unless otherwise noted, were obtained from research conducted under the Dredged Material Research Program of the United States Army Corps of Engineers. Permission was granted by the Chief of Engineers to publish this information. Support during part of this investigation was obtained from the Institute for Environmental Sciences, University of Texas at Dallas, Richardson, Texas.

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Questions and Answers
Comments and Rebuttals

ANSWER TO QUESTION ON SINGULAR AND INTERACTIONS OF Ca, Mg, K, and S

T. Shiroyama

In the process of establishing the minimum requirements of Ca, Mg, K, and S, for *S. capricornutum* and *A. flos-aquae*, I did add beyond their minimum requirement of each nutrient in AAM in the following concentrations:

	Ca	S	K	Mg
S. capricornutum	2.00 mg/l	2.50 mg/l	0.50 mg/l	3.90 mg/l
A. flos-aquae	5.00 mg/l	10.0 mg/l	2.00 mg/l	6.00 mg/l

Each of the above concentrations for each nutrients did yield maximum algal growth, which indicate that at these higher concentrations there were no adverse effects of the response of the algae.

Af far as the interaction of these nutrients, it appears that at the higher concentratons the nutrients are independent of each other. We do use an enriched media (Gorham's Media) for our AAP algae quite routinely in which this media contains the following concentrations of the nutrients in question: Ca = 10 mg/l; S = 10 mg/l; K = 17 mg/l; and Mg = 7 mg/l. Even at these concentrations, we are able to obtain the maximum growth from all of our algae. I would assume that in natural waters, the algal response would be similar to that obtained with the artificial medium.

QUESTION ON THE EFFECTS OF MEDIA MODIFICATIONS UPON *SELENASTRUM CAPRICORNUTUM* IN BATCH CULTURES

Arthur R. Batchelder

When you tested the effects of various carbon to nitrogen ratios, you reported severe algal growth reduction at high ratios, especially when the C/N ratio was 45. However, because you achieved those ratios by adding $NaHCO_3$, have you considered the possibility that the reductions were caused by the exceptionally high Na concentrations? If it takes 1.512 g/l of $NaHCO_3$ for a C/N ratio of 18, then for a C/N ratio of 45, the Na concentration would be 3.78 g/l. That, plus the Na in the medium as $NaNO_3$, brings the Na concentration to 46 meq/l, which is about one-tenth the concentration of sea water.

The same reasoning might apply to growth reduction that occurred with the phosphate buffers, depending upon how much buffer was added. For example, the sodium phosphate buffer has a Na concentration of 1.7 g/l or about 74 meq/l.

You stated that "high total dissolved solids level can inhibit growth of some freshwater algal species." Considering the high Na and K concentrations, do you consider the solutions as "freshwater?"

It might be interesting to repeat the experiments using other freshwater algal species, such as *Anabaena*, or even some marine species.

ANSWER

R. F. Malone

It was not our intention to suggest that the high C/N ratios were responsible for the growth rate reductions (Figure 4) that we observed with high sodium bicarbonate levels. We suspect that either the high sodium levels and/or the high total dissolved solids levels resulted in the growth rate reductions, but we have no data at this time to support those suspicions. So we agree with you that sodium may be responsible for the slower growth observed at high sodium bicarbonate levels.

However, the same reasoning does not necessarily apply to the growth reductions that occurred with the phosphate buffers. In the case of the phosphate buffers, we have observed a decrease in both the maximum standing crop and growth rates with increasing buffer strength (Figures 7, 8, and 9). The former indicates a reduction in the available level of an essential nutrient. This is not the same pattern that we observe with high concentrations of sodium bicarbonate. Here a reduction in growth rate has been observed, but not in conjunction with a reduction of an essential

nutrient. This is best illustrated by a comparison of C/N = 18 and C/N = 27 on Figure 4.

So although the phospate buffer system and the method of carbon supplementation both have high levels of sodium it is doubtful that this element is responsible for the growth reductions we observed with the phosphate buffers. We believe it is much more likely that the phosphates are acting as chelation agents effectively removing essential metals. If the sodium or high total dissolved solids level have an effect, they are overshadowed by another mechanism.

There are a few numerical discrepancies due to lack of available information in your letter which should be noted. First, you stated that the level of Na for a C/N of 45 was 3.78 g/l; that is the level of $NaHCO_3$. The concentration of Na is 1.03 g/l; 46 meq/l of Na is correct. Secondly, the level of Na in a medium with carbon supplementation (C/N = 18) and a .03 M sodium phosphate buffer is about 91 meq/l. Forty-five meq/l of Na not 74 meq/l result from the phosphate buffer. The draft copy of our paper upon which you based your calculations listed the composition of the sodium phosphate buffer incorrectly as "(2.07 g/l NaH_2PO_4 and 4.02 g/l of $NaHPO_4$)." That has been corrected to read "(2.07 g/l $NaH_2PO_4 \cdot H_2O$ and 4.02 g/l of $NAHPO_4 \cdot 7\ H_2O$)" which reflects the crystaline forms actually utilized.

COMMENT ON DIFFERENT BIOASSAY RESULTS OBTAINED IN SAMPLING THE SAME LAKE SYSTEM, LAKE WYLIE, NORTH CAROLINA

D. B. Porcella

The basis for algal bioassaying techniques is that the population of an algal species will change in response to substances contained in a water sample. If several water samples are collected at the same site, at the same time and are prepared in the same way, the water samples should contain the same quantities and composition of substances. Then if the bioassay is performed under constant environmental conditions and standard analytical methods are utilized, similar results would be expected. It was the intent of the EPA publication "Algal Assay Procedures: Bottle Test" (EPA, 1971) to provide the first step in the standardization of appropriate procedures from the time of sampling to the completion of bioassaying. That publication culminated approximately three years of research and development on algal bioassay procedures. However, it was well understood that improvements in technique and further standardization was need to insure acceptable reproducibility and accuracy in the

bioassaying of substances in water. Two papers presented at this workshop gave contrasting results for the same water system (Gerhold, 1975; Weiss, 1975) and illustrated the need for further research and further standardization of bioassay procedures as well as a need for understanding how changes in procedure result in changes in bioassay results. This latter need results from procedure modifications caused by unavoidable constraints of cost, time, distance, and available laboratory facilities that all investigators experience at one time or another. The following discussion by Gerhold and Weiss relates to needed research and needed standardization.

COMMENT ON ALGAL NUTRITIONAL BIOASSAYS OF LAKE WYLIE, NORTH CAROLINA

R. M. Gerhold

The work presented in these two papers on Lake Wylie represents bioassays that were conducted on the same lake system at roughly the same sampling depth, with some common sampling locations by two separate laboratory teams working unbeknownst to the other, using and interpreting the Algal Assay Procedure-Bottle Test (AAP) in their own way and with some apparent time overlap (October, 1973-January, 1974) in sampling dates. The statements to follow are attempts to ferret out the reasons why we came to different conclusions of overall nutrient limitation (Weiss, nitrogen; Gerhold, phosphorus).

All of us who helped write and evaluate the AAP and who have had experience in its application understand the flexibility that is possible with its use. It is natural to expect that experimental designs and differences in data interpretation will occur in the hands of different workers, each with their own points-of-view, each with their own ideas of what information use of the AAP can or should provide. The varied papers from this meeting attest to this thesis. This is expected. But we were both unprepared for the opposite conclusions each of us came to on Lake Wylie! Hopefully, this exercise will help focus attention on published oversights and on the uses and interpretations of AAP that will enhance its usefulness in the future.

I have prepared a non-exhaustive comparison of the methodology and selected findings of both Dr. Weiss and myself in an attempt to spotlight major differences (Table 1). In my view, the major difference in methodology that might have led to different interpretations lies in the use of different wavelengths with a colorimeter in estimating biomass. An objective in the AAP testing should be to measure algal cells as suspended particles and to totally avoid the interference of the colors and absorbances that occur with algae in various stages of growth and that

Table 1. A comparison of Weiss and Gerhold AAP methodology.

CONDITION	WEISS	GERHOLD
Sample Pretreatment	Autoclaved, sometimes filtered[a] vs 0.45 μ MF filtration	Autoclaved, then CO_2-sparged to resolubilize precipitates and clarify the sample
Biomass Monitoring	Estimated by absorbance at 640 nm	Estimated by absorbance at 750 nm
Conversion From Absorbance To Biomass Estimates	Biomass of AAP synthetic nutrient medium used with its absorbance to calculate a factor to convert all bioassays of that incubation set to biomass	Biomass of unsupplemented lake water flasks employed with its absorbance value to obtain a factor to convert absorbances to biomass. Performed for each sample regardless of incubation-set. Biomass of AAP (and reference medium N- and P-response curves) estimated by application of an average of all factors for the incubation-set
Depth at Which Samples Collected	Secchi Depth (Varied from 0.6 to 1.5 m)	1 meter subsurface for all of 11 locations, 1 meter off bottom at one station
Sampling Frequency and Number of Samples Tested	Total, 32 samples: 19 in a "growing" season, April-November and 13 in a "winter" period, December-March. Work done July 1973 to January 1974	Total, 42 samples: 10 samples Oct. 8, 9, 1973 10 samples Jan. 7, 8, 1974 11 samples Apr. 8, 9, 1974 11 samples Jul. 8, 9, 1974 (32 in Weiss' "growing season," 10 in his "winter season")
Total Number of Sampling Stations	8	11

[a]Dr. Weiss, in his oral presentation, indicated that some samples were autoclaved, then bioassayed, whereas other samples, if "highly turbid" after autoclaving were then also filtered prior to bioassay to clarify them. There was no mention of a filtration step following autoclaving in the original manuscript.

Table 1. Continued.

CONDITION	WEISS			GERHOLD	
Number of Sampling Stations Located Near Same Spot	2 (It is not determinable from the Weiss report if these two samples were taken during the same period of time)			2	
Soluble N/P Ratios of L. Wylie Waters	Refer to Weiss Table No. 2:	Weiss's N/P Categories (p. 59) Lim. By–		Refer to Gerhold Tables 10-13:	Weiss's N/P Categories Limited By–
	"Growing Season" mean 8.8 (Apr-Nov)	P&N		Oct. '73 Mean 10.8 (6.6-18.1)	P&N
				Jan. '74 Mean 10.9 (8.8-13.9)	P&N
	"Winter Season" mean 11.2 Dec.-Mar.)	P&N		Apr. '74 Mean 21.0 (15.6-43.3)	P
				Jul. '74 Mean <24.5 (< 12.5 - <44.4)	P
Biomass in AAP Reference Medium	Grand Mean (p. 29) 114.8 (67.8-148.8)			Grant Mean (pp. 18-21) 161.2 (104.6-284.9) (Median 128.7)	
Mean Biomass in Upsupplemented Control Blanks (mg/l)	Weiss Tables 15, 16, 23:	Auto.	Filt.	Gerhold Tables 3-6:	Auto
	Jul. '73	5.9	1.0	Oct. 1973	0.6
	to	5.8	2.3	Jan. 1974	3.0
	Jan. '74	10.2	8.9	Apr. 1974	3.1
				Jul. 1974	16.2
Length of Incubation	14 Days			14 Days	

[a]Dr. Weiss, in his oral presentation, indicated that some samples were autoclaved, then bioassayed, whereas other samples, if "highly turbid" after autoclaving were then also filtered prior to bioassay to clarify them. There was no mention of a filtration step following autoclaving in the original manuscript.

result from the various nutritional states. It is unfortunate that we allowed AAP-1971 to be published with a range of acceptable absorbance wavelengths for estimating biomass (600-750 nm). Reference to published absorption spectra (Stewart, 1974; Lewin, 1962) for chlorophylls and other algal pigments reveals that major absorbance maxima can be largely avoided at either end of this range (almost totally avoided at the upper end) and that the range 640 to 690 nm includes most of the absorption maxima that occur in the 600 to 700 nm range. Dr. Weiss's use of the 640 nm wave length was correct insofar as the published method, but was an unfortunate choice inasmuch as it lies within the range of major chlorophyll absorption maxima. Thus, an algal culture supplemented with nitrogen could have exhibited enhanced greening that might not have reflected a true increase in turbidity (or estimated biomass) and might have led to a false conclusion that nitrogen was limiting.

It is known that the AAP test may show differences in nutrient limitation with varying depths within the water column of a stratified lake (unpublished data, Lake Mendota, Wisconsin, summer, 1970: P-limited in epilimnion; N and P limited in the thermocline; and N- limited in the hypolimnion). It is unlikely, however, that the sampling depths by the two of us could have led to such gross differences in bioassay results. Although Lake Wylie stratifies in the summer (epilimnion depth varies from 1-3 meters, roughly), Weiss's Secchi-Depth sampling (average 0.6 to 1.5 m), like Gerhold's (1 m), were taken from either the epilimnion or from reasonably well mixed waters. (I had one exception. At my only location where the sample was taken one meter off the bottom, one of the four quarterly samples was found to be wholly nitrogen-limited.)

Dr. Weiss's data, in one method of interpretation, suggests that, overall, phosphorus may, indeed have been limiting in his own samples: In eleven comparisons between the control growth and the nutrients found chemically, positive high correlations for the Lake Wylie samples were for phosphorus forms, whereas only two correlations were found for nitrate plus nitrite, and none for ammonia (Weiss, Table 23); In his filtered pretreatment samples, Dr. Weiss's data indicates four positive correlations with phosphorus, none for nitrogen in his Table 30; and two for phosphorus with none for nitrogen in his Table 32. Further, Dr. Weiss's chemical N/P ratios for Lake Wylie suggest that, according to his thesis, they may be in the range of P and N limitation when soluble nutrient forms are considered, or relatively more into the P-limited range if Total N/ Total P ratios are used. Since it is known that much insoluble P and N is available for the growth of algae, the ratios using total P and N forms may be more realistic in evaluating nutrient limitations. Indeed, most of Dr. Weiss's N/P ratios in the presented paper were shown as total nitrogen/total phosphorus (Weiss Tables 2,3,4,5,6,7,8,9,10) whereas

Tables 27-32 employed Inorganic-N/Sol-P ratios. His conclusions were based on the ratios of the soluble forms of both nutrients.

If, in fact the lake was marginally P-limited, with N running a close second, the wave-length problem could lead to conclusions of N-, rather than P-limitation. This casts some doubt on the advisability of widespread use of N/P ratios as the sole basis for decisions regarding nutrient limitations.

COMMENT ON FIELD EVALUATION OF THE ALGAL ASSAY PROCEDURE ON SURFACE WATERS OF NORTH CAROLINA

C. M. Weiss

Introduction

These two papers present data with estimates of nutrient limitation for Lake Wylie, North Carolina, based on the use of the algal assay. Their findings appear to be contradictory in that the primary limiting nutrient as reported by Gerhold was phosphorus and by Weiss nitrogen. These comments attempt to resolve this difference and to highlight problems associated with the use of the algal assay and its interpretation.

It should be noted that the statement made by Gerhold, in his introduction, that, "Lake Wylie, historically, has been characterized by excellent water quality and freedom from problems of eutrophication," is not entirely correct since substantial wastewater discharges from municipal and industrial sources have entered the upper waters of Lake Wylie from the urban areas of Belmont, and Mt. Holly, North Carolina. That problems of eutrophication have not occurred have in part been due to the apparent capacity of the lake for self purification as well as its relatively short retention time due to the operation of the hydroelectric turbines at Wylie Station. The pollutional loads in South Fork of the Catawba River have on occasion produced near bloom conditions with algal cell densities averaging 3200/ml as compared to an average of 1800 in the main channel (Weiss, Table 2).

Comparison of Methodologies

It has been demonstrated that laboratory variability may introduce into the assay procedure systematic effects which might obscure true growth response in either control or spiked samples. Gerhold reports that samples for assay were air freighted from North Carolina, to his laboratory near Chicago, in one gallon polyethylene containers and stored under refrigeration in the dark until processing was initiated. It should be noted

that no time of storage was reported which if prolonged could conceivably induce some change in nutrient quality. This would not be as critical with autoclaved samples as would be with filtered samples but even with the autoclaved pretreatment procedure undue length of storage could cause significant shifts in nutrient quantities that would be available for final assay. The assay samples described by Weiss were all autoclaved and/or filtered within 24 hours and then stored, refrigerated in the dark, until assayed. Another difference in procedure was that the reseeding protocol used by Gerhold was based on the innoculation of the entire unsupplemented reservoir of sample prior to placing in separate flasks for spiking whereas in investigations reported by Weiss the individual flasks were reseeded after spiking.

Perhaps a more important variable was the considerable variation in incubation temperature reported by Gerhold for his four sets of samples. During the incubation period of two weeks the range between maximum and minimum temperature varied from a low of 3.2°C to a high of 7.1°C, about the mean value for the period. The samples assayed by Weiss were all incubated at 24°C ±1°C and were routinely moved within the incubation shelves on a daily basis to ensure uniformity of temperature throughout the period of incubation. Variation in temperature of this magnitude has been shown to produce different growth rates for comparable samples.

Probably of most significance with respect to the estimates of nutrient limitation that are quite different and supposedly from the same lake is the fact that the sampling points are not directly comparable. As shown in Table 1 the stations sampled for assay in each of the two reports have been arranged according to their location and proximity to each other. Three sets of Gerhold stations Nos. 21, 22; 23, 24; and 25, 26; were located on side arms along the east shore of lower Lake Wylie. Weiss's stations were located along the center line of the main lake except for Station AC 2.2 in Allison Creek which was along the west shore of lower Lake Wylie. South Fork Station 3.0 of Weiss was close to South Fork Station 7 of Gerhold. Station 19 (Gerhold) was approximately coincident with Station 70.8 (Weiss) and Station 2 (Gerhold) was essentially coincident with the location of Station 83 or 83.1 of Weiss.

Comparison of Results

The results of the limiting assay for these stations as described by Weiss and Gerhold are shown in Table 2 with the quantity of control biomass grown in these samples without any nutrient addition. The baseline or original lake water quality for the stations in question is shown in Table 3. The results of the algal assays on Lake Wylie, by the two separate investigations, do not differ to the extent implied by the

Table 1. Station locations arranged in relative proximity and upstream order.

Weiss[a]	Gerhold
	25, 26 (Nivens Creek)
68.1	
AC 2.2 (Allison Creek)	23, 24 (Torrence Creek)
	21, 22 (Unnamed cove)
70.8	19
74	
78.9	
83,83.1[b]	2[b]
SF 3.0 (South Fork)	7 (South Fork)

[a]Station Nos. are river miles from Wateree Hydro or distance up a side arm from center channel.

[b]Opposite intake Allen Steam Station.

conclusions drawn from the two sets of data. In each instance differences in methodology and data interpretation as well as differences in location of samples generate valid answers which do not necessarily agree. As shown in Table 2 the cluster of stations used by Weiss to describe the quality of the lower Lake Wylie region and based on assays of 13 samples showed that the autoclaved samples were defined as nitrogen limited whereas the filtered samples were phosphorus limited with substantial difference in control biomass grown in each of the two pretreatment procedures of 5.8 and 1.0 mg/l respectively.

The importance of the pretreatment procedure is further highlighted when comparison is made to the Gerhold samples, taken from Stations 21-26. These were all in side arms of the lake and showed a consistent pattern of phosphorus limitation although in two instances individual samples were phosphorus and nitrogen limited. Generally the control biomass of these autoclaved samples were somewhat higher than was found by Weiss in his center channel series. However, as previously noted the considerable variability of temperature fluctuation in Gerhold's incubators could have substantially changed the quantity grown under the specific nutrient conditions. In the cluster of stations used by Weiss to describe the upper lake in the vicinity of the Allen Steam Station intake, nine assays showed in the autoclaved samples nitrogen limitation, whereas filtered samples were phosphorus limited. The biomass grown on the baseline nutrients compared almost exactly with that of Gerhold's sample from the similar location. Filtered samples showed a somewhat higher biomass than found downstream reflecting a higher baseline

Table 2. Comparison of limiting nutrient and control biomass Lake Wylie.

Weiss						Gerhold			
Station	Number of Samples	Limiting Nutrient		Mean Control Biomass mg/l		Station	Number of Samples	Autoclaved Only	
		A	F	A	F			Limiting Nutrient	Mean Control Biomass mg/l
						26	4	P	12
						25	3	P(3) P+N (1)	18
						24	4	P	19.2
						23	4	P	15
						22	4	P(3) P+N (1)	15.7
						21	3	P	20.6
68.1, 70.8 74, AC 2.2	13	N	P	5.8	1.0	19	4	P	15.7
78.9, 83, 83.1	9	N	P	5.9	2.3	2	4	P	5.7
SF 3.0	4	N	P	10.2	8.9	7	1	P+N	28

A – Autoclaved pretreatment.

F – Filtered pretreatment

nutrient level. These nutrient levels are compared in Table 3. The one sample taken in the South Fork by Gerhold (and the comparable station, SF 3.0 of Weiss) was phosphorus and nitrogen limited with a biomass of 28 mg/l grown on the baseline nutrients. Weiss found both autoclaved and filtered samples to be nitrogen limited and comparably little difference between autoclaved and filtered biomass, 10.2 and 8.9 mg/l respectively. This is generally the response of a nutrient rich system which is only marginally enhanced by the release of soluble nutrient materials on autoclaving.

The mean baseline nutrients (Gerhold) or original lake quality (Weiss) as arranged in Table 3 for the two locations lend themselves to direct comparison and confirm what the assays found. For instance at the Station 2 location of Gerhold and the station cluster of Weiss, 78.9, 83, and 83.1, comparable quantities of inorganic nitrogen were found. However, Weiss reported substantially more orthophosphate and soluble phosphorus with a ratio of inorganic nitrogen/soluble phosphorus of 8.2 This fits the nitrogen limited situation as described by the Weiss assays. Gerhold reported approximately the same inorganic nitrogen but less soluble phosphorus and consequently a higher inorganic nitrogen, soluble phosphorus ratio of 18.5 which confirms the phosphorus limited conclusion of his assays. Downstream in the vicinity of Gerhold Station 19 similar comparisons can be made. The ratio of inorganic nitrogen to soluble phosphorus as reported by Weiss was 12.1 and a P+N limitation by his algal assays. Gerhold again found a higher ratio of 16.6 which confirms his phosphorus limited situation. These ratios, as used above, were developed from the overall algal assays of North Carolina lakes that was reported by Weiss.

It is of interest to note that Miller, Maloney and Green (1974) reported for Lake Wylie (without reference to a specific sampling location) a phosphorus and nitrogen limitation for Wylie's waters, a control growth

Table 3. Mean values of baseline nutrients (Gerhold) original lake quality (Weiss).

Station	Number of Samples	μg/l				Ratio Inorg N/ Sol-P
		Inorg N	PO_4-P	Sol-P	T-P	
Weiss (78.9,83,83.1)	9	206	14	25	31	8.2
Gerhold 2	4	185	4	10	35	18.5
Weiss (68.1,70.8, 74, AC 2.2)	13	158	8	13	21	12.1
Gerhold 19	4	349	15	21	59	16.6

of 6.6 mg/l in their autoclaved sample and 1.9 mg/l in the filtered sample. If the assumption is made that this water sample had been taken somewhere in the downstream portion of the lake, it agrees very well with the biomass averages as reported by Weiss of 5.8 mg/l and 1.0 mg/l respectively for autoclaved and filtered samples from this same general location.

The conclusions to be drawn from this comparison is that the considerable inherent variability in the algal assay procedure requires particular care in maintaining a uniformity of methodology throughout the assay series. Temperature control in the incubator could very well be a major factor in improving reproducibility between runs. Also the inherent variability of a short retention time hydroelectric impoundment exaggerates the characteristics of a nonhomogeneous system. Therefore samples taken for algal assay can be used to define nutrient limitation only with marginal precision due to the variability in water quality.

GERHOLDS'S REBUTTAL

I agree that ideally, precise temperature control is a justifiable goal in algal assays where growth rate studies are performed, however, in both of our studies, growth rates in the logarithmic growth phase were not the study-parameter employed. Both of us employed the biomass of plateau growth achieved after a 14-day incubation as the end-point study-parameter, and both of us randomized our flasks' shelf positions to insure uniform lighting during the incubations. It is my contention that differences in incubation temperature within physiologically "healthy" ranges should not affect the conclusions from limiting-nutrient bioassays in batch culture. It has been demonstrated that *Selenastrum capricornutum*, the test organism employed in both of our studies, will grow over a range of more than twenty-two degrees centigrade (Reynolds, 1975). To my knowledge, it has never been demonstrated that the incubation temperature can govern the way a test organism perceives its environment as phosphorus or nitrogen limited. Reasonably reproducible plateau cell yields are achievable under nutrient-limited laboratory conditions so long as incubation temperatures and incident illumination are within physiological ranges for the test species and temperature or light stress is avoided. The measurement of growth rate-limiting nutrients in batch cultures, however, would be expected to require very precise temperature controls as well as precise daily estimations of biomass as compared to the single end-point estimation required for the bioassay as applied in these studies.

R. M. Gerhold

WEISS'S REBUTTAL
(Summary taken from Weiss, 1972)

Through the use of large replicate samples the growth of *Selenastrum capricornutum* in NAAM was examined to establish the degree of variability that might be expected from variation in growth conditions of light, temperature, position and other external variables that might be associated with an incubator room or chamber. Further growth comparison over a period of eight months was made using the standard NAAM control that was incubated with each lake water assay carried out between October 1971 and June of 1972. The assays were systematically grown in two incubator rooms and thus the inherent variability of each room was compared.

Large replicate series (@150 samples) showed considerable variability among different experiments even when performed under "identical" conditions. Analysis of the variation of these experiments indicates that the standard bottle test using NAAM as a control is too variable for the procedure to be considered as an absolute standard.

Within incubator rooms it was found that variations of temperature and illumination ranged between 22-27°C and 250 ft-c to 500 ft-c. By comparison of the growth response in the several light and temperature ranges within these limits it has been found that there was essentially no light effect but the temperature effect appears to be considerable in terms of ultimate biomass produced. Perhaps even more striking is the increase in nitrate utilization with increase in temperature within a narrow temperature range. In contrast unicellular weight decreased with increase in temperature.

The growth in NAAM controls, observed over a period of several months, showed that the degree of variability for one of the two incubator rooms was somewhat less than the other in each of the variables measured, differences were small but consistent. However, in the determinations of absorbance and dry weight the percent coefficient of variation was less than 10.

Conclusions

1. Large incubator rooms may have an unusually wide internal variation of temperature belying the name of "constant temperature." These temperature zones which may differ by as much as 5°C may materially affect the growth response of algal cultures under the standard conditions for assay. The recommended temperature limit of 24±2.0°C of the algal assay bottle test is probably too broad a range and should be limited to 24±0.5°C.

2. Any laboratory carrying out the algal assay should perform uniformity trials to calibrate their growth chambers and establish the form of the calibration curve for each chamber.

3. If physical limitations prohibit making a whole incubator room uniform, as established by uniformity trials, not just by light and temperature, the room should be divided into zones such that each zone is internally as uniform as possible. Assays should then be run in a "randomized complete blocks" design, with each zone containing one complete block (replication) of the assay.

R. M. Weiss

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