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Harvey H. Harper III

University of Central Florida, [hharper@erd.org](mailto:hharper@erd.org)



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ECOLOGICAL RESPONSES OF LAKE  
EOLA TO URBAN RUNOFF

BY

HARVEY H. HARPER, III  
B.S., Florida Technological University, 1977

THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Sciences  
in the Graduate Studies Program of the College of Engineering  
at the University of Central Florida; Orlando, Florida

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## ABSTRACT

Lake Eola is a land-locked lake located in downtown Orlando, Florida. Its surface area is approximately 27.0 acres (11.0 Ha) and water depth is 2 to 3 feet (0.6 to 0.9 meters) near the shore and 22 feet (6.7 meters) toward the center.

Periodical water samples were collected from the lake and storm drains for various stormwater events and physicochemical parameters were analyzed to calculate loading rates from nutrients and heavy metals released to Lake Eola.

Algal bioassay studies were performed to investigate stormwater impacts on productivity. Periodical water samples were collected from the lake, mixed and filtered for limiting nutrient studies using various concentrations of N, P, and Fe. Unialgal species of Selenastrum, Chlorella and indigenous species were used and changes in chlorophyll "a" and biomass were measured. Results indicate that phosphorus or nitrogen can be limiting at some times of the year. However, the ratio of P:N can be more important than actual concentration of phosphorus and nitrogen separately. Similar algal bioassays were performed on a mixture of stormwater, coagulated stormwater and lake water at different ratios.

## ACKNOWLEDGEMENTS

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I cannot say enough about my special friend, Frank Marshall. His companionship and analytical outlook, both in the field and laboratory, were a constant source of rejuvenation during this research.

I would also like to thank Mrs. Sharon Darling for her invaluable aid in the final preparation of this manuscript.

To those who I hold close to me in life, I would like to express my sincere gratitude and love for the sacrifices they so unselfishly endured during the course of this research.



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## CHAPTER I

### INTRODUCTION

In recent years, the deterioration of the nation's lakes and streams has become increasingly obvious. Visual evidence of the destruction of our aquatic systems caused by rampant domestic and industrial growth is abundant. This destruction, once limited to small isolated localities, has spread throughout this nation until there are very few water bodies remaining which do not feel the pressure of man's existence.

Much research has been delegated to the quantity and quality aspects of pollution sources entering our lakes and streams. Virtually every type of pollution source known to man has been analyzed and its chemical constituents documented. However, relatively few studies have been conducted concerning the ecological impacts of these sources on receiving water bodies.

In order to gain a better insight into the impact of these sources, the National Eutrophication Research Program began investigations in 1968 to determine a method for assessing the effect of these compounds on aquatic ecology, and in particular, on algal production. The result was a publication, in 1969, entitled, Provisional Algal Assay Procedures in which a standard



procedure was described for conducting algal assays. Intensive research was conducted to improve and expand the understanding of results obtained in this procedure which culminated in 1971 with the publication of Algal Assay Procedure: Bottle Test. This document described processes which enabled investigators to define the stimulatory and/or inhibitory interactions of municipal, industrial and agricultural wastes upon algal productivity in natural waters. Further research and refinements led to the development in 1978 of the Selenastrum capricornutum Printz Algal Assay Bottle Test which extended these procedures into other applications in both eutrophication and toxicity problem areas. These procedures enabled researchers for the first time to predict natural algal responses to pollution inputs from carefully controlled and monitored laboratory experiments. The techniques described in these procedures in many instances are still being defined, and research is still badly needed in this area. It is hoped that techniques and procedures developed in the course of this research may answer some of the yet unresolved questions.

#### Scope and Objectives

This research deals with the ecological effects of urban runoff on Lake Eola. Lake Eola, which has been severely damaged by continuous stormwater inputs, is a focal point in the heart of downtown Orlando, Florida and is of interest mainly for the aesthetic value of the surrounding park land. A research project

was initiated during 1978 and funded jointly by the U.S. Environmental Protection Agency, Florida Department of Environmental Regulation, City of Orlando, and the Engineering and Industrial Experiment Station at the University of Central Florida to determine:

1. the nature and extent of pollutional loads from stormwater runoff to Lake Eola
2. the impact of these loadings on the lake's ecology
3. management techniques for reducing the effect of stormwater runoff, and
4. a proposed plan for the restoration of Lake Eola

As a portion of this project, a series of algal bioassays were begun to determine factors which limit algal productivity in Lake Eola and to determine the effect of stormwater runoff on the Lake. This investigation is designed, through the use of carefully controlled laboratory experiments, to provide an approximation of the in situ response which may be expected from a particular nutrient or stormwater addition. Since ordinary chemical analyses cannot distinguish between ions which are biologically available for growth and those which are not, the results of the research may be a fundamental tool in the selection and evaluation of optimum lake management techniques in Lake Eola.



## CHAPTER II

### LITERATURE REVIEW

#### Ecosystems

Life on earth has developed through time into a multitude of interconnected and interrelated functional units which are at the same time separate and unified. These units, called ecosystems, may be any area with a boundary through which the input and output of energy and materials can be measured and related to some environmental factor. One of the important features of an ecosystem is its flexibility. It is defined by function, not by some arbitrary criterion of scale, and therefore, may be studied at a variety of levels.

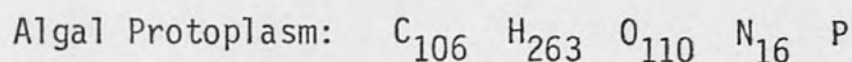
Energy is a common requirement of all organisms for maintaining themselves and for reproducing. The initial source of the energy used by an ecosystem is the sun. This energy is captured by green plants, combined with nutrients during photosynthesis, and stored in chemical form for use by the plant itself or by heterotrophs. Each time energy is stored in an organism, its movement is temporarily stopped until that organism serves as an energy source for another organism. This linear passage of energy through an ecosystem defines a food chain. A necessary part of every ecosystem are decomposers which derive energy

from waste products and in doing so, recycle nutrients through the system in a cyclic pattern.

Ecosystems have developed an inherent stability which enables them to withstand minor perturbations in their environment without permanently damaging the structure of the system. This stability is insured by: (1) controlling the rate of energy flow into the system, (2) controlling the rate of chemical cycling, and (3) by maintaining a diversity of species and food webs. As long as these natural systems are not overloaded, the stability of the ecosystem is maintained. However, man's alteration of his environment through mismanaged technological achievements as well as his sheer numbers is threatening many natural systems by affecting one or more of the above stabilizing factors. Of the three factors listed above, the most serious man-made threat to ecosystem stability, at least in terms of aquatic systems, is through alteration of nutrient influx and chemical cycling. Since nutrients are a common requirement of all organisms, the presence or absence of these compounds has been shown to regulate ultimate production in virtually every water system studied. However, nutrient inputs can often be accelerated greatly by man's activities, the result often being that the affected system can no longer self-regulate its internal processes, and stability is lost. This process of stability loss and the resulting rapid succession of communities which follows has been given the name of eutrophication.

### Algal Nutrient Requirements

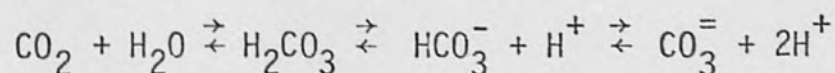
Stumm, et. al. (1972) proposed the following simplified empirical chemical composition of algal protoplasm:



In addition to the five macronutrients listed above, minute quantities of other elements such as sulfur, calcium, magnesium, sodium, potassium, as well as certain trace metals and vitamin complexes are essential for continued maintenance of a healthy organism. Each of these requirements is discussed in detail in the following sections.

#### Carbon

Carbon is derived from carbon dioxide, carbonates, bicarbonates or organic compounds. Since carbonates are generally present in relative excess in natural waters, carbon dioxide is usually available as the normal carbon source for photosynthesis. Algae in aquatic habitats live in a solution in which carbon is present in a variety of forms, the equilibrium depending on the hydrogen ion concentration, amount of excess base, the partial pressure of carbon dioxide in the atmosphere and the temperature. This relationship can be expressed by:



Below a pH of 5.0, only free  $CO_2$  will be present to any extent. Between pH 7-9, bicarbonate ions become dominant, and above a pH of 9.5, carbonate ions are in excess. Carbonate ions,

however, cannot be directly utilized by algae and may even have an inhibitory effect (Goldman, et. al., 1974).

It is generally considered that, when available, algae will use free  $\text{CO}_2$  in photosynthesis. However, at very high pH values, above 9.0, the absence of free  $\text{CO}_2$  may be an important ecological factor, causing, among other factors, a reduction in the number of species. Scenedesmus quadricauda has been shown to utilize both free  $\text{CO}_2$  and bicarbonate ions whereas Chlorella pyrenoidosa can only incorporate free  $\text{CO}_2$  (Round, 1973). Although free  $\text{CO}_2$  is necessary as a carbon source for virtually all algal species, an excess of carbon dioxide, above 10 percent, will actually inhibit growth. Algae grown under continuous illumination and in the presence of an adequate nutrient supply have a carbon content of 51-56 percent of the ash free dry weight, while 49.5-71.2 percent has been recorded for Chlorella grown under varying environmental conditions (Round, 1973).

When aquatic organisms exhibit a similar affinity for  $\text{CO}_2$  and  $\text{HCO}_3^-$  ions, utilization of bicarbonate generally occurs when the bicarbonate concentration exceeds that of  $\text{CO}_2$  by a factor of 10 (Wetzel, 1975). Because of the rapid diffusion of  $\text{CO}_2$  into natural waters, the concentration of this gas in most fresh waters is in approximate equilibrium with the atmosphere. Many alkaline hard water lakes with a pH greater than 8.5 may contain bicarbonate concentrations far in excess of 10 times the  $\text{CO}_2$



concentration, and bicarbonate utilization may predominate. However, an additional reaction is needed for  $\text{HCO}_3^-$  assimilation which is not required for  $\text{CO}_2$  assimilation. Active bicarbonate transport involves a dehydration process in the cytoplasm which is coupled with a similar stoichiometric excretion of a hydroxyl ion from the cell (Wetzel, 1975). Thus, incorporation of bicarbonate ions may occur, but only at the expense of metabolic efficiency. Because of the availability of various forms of carbon to photosynthetic organisms, Goldman et. al. (1974) suggested that it is unlikely that inorganic carbon is a growth-limiting nutrient in most natural waters.

### Nitrogen

Elemental nitrogen is readily available in dissolved form in most waters and can be utilized directly by some species of blue-green algae. However, members of other algal groups are forced to utilize inorganic nitrogen compounds, mainly in the form of nitrate, ammonium salts, and to a much lesser extent, nitrites. In addition to inorganic forms of nitrogen, certain organic nitrogen compounds can also be utilized in highly polluted waters. Round (1973) suggests that there may be a relationship between some of the products of animal excretions (ammonia, urea, uric and amino acids) and the growth of certain flagellates.

Although algae can utilize inorganic nitrogen in virtually any form, there is strong evidence to suggest that ammonia is the preferred source. However, this phenomenon may be pH related since preferential absorption of ammonia tends to decrease pH while assimilation of nitrate ions tends to raise the pH.

Normal growth requirements of nitrogen in cultures of Chloro-phyceae range between 6.5-8.3 percent of the ash-free dry weight, but under conditions of nitrogen starvation, this level can be greatly reduced (Round, 1973). Luxury uptake of nitrogen can be induced if algae are subjected to environments deficient in manganese, boron or zinc. Marine phytoplankton have been shown to contain nitrogen in proportion to carbon and phosphorus in a ratio of 7:42:1. Ryther and Dunstan (1971) came to the conclusion that due to the low nitrogen/phosphorus ratio in sewage and terrestrial runoff entering these waters and also because of a lack of nitrogen-reducing blue-green algae such as those found in inland lakes, nitrogen may be the critical limiting factor in coastal marine waters.

Although some members of the Cyanophyta can utilize atmospheric nitrogen, this process is not an absolute necessity for growth of most species, since their needs can be readily supplied by other sources. The chemical unreactivity of the covalent triple bonds of the  $N_2$  molecule insures that, although this chemical species may be used, its use is a very inefficient process. A small but adequate supply of molybdenum is required for the



process to occur.

### Phosphorus

Compounds containing phosphorus play major roles in nearly all phases of metabolism, particularly in energy transformation associated with phosphorylation reactions in photosynthesis. Phosphorus functions in the storage and transfer of a cell's energy by incorporation into ATP and is a main building block of nucleotides and nucleic acids. Phosphorus occurs in water as inorganic orthophosphate, which is the fraction immediately useful for autotrophic plants, as well as in organic combinations such as meta- or polyphosphates.

Soluble orthophosphorus is present in small quantities in natural waters. Low concentrations of this element may limit the growth of certain algal species and, in fact, Miller, et. al. (1974) using algal assays, conducted on water from 49 American lakes, found phosphorus limiting algal growth in 35 of the 49 lake assays. Phosphate concentrations as high as 100,000 and 850,000 times normal concentrations have been reported in species of Euglena and Spirogyra (Round, 1973). Certain algal species are known to absorb phosphorus in excess and can exist for some time in waters which have become phosphate deficient. A few specialized algal forms are able to utilize organic phosphate esters, such as glycerophosphates and pyrophosphates. Minimum cell nutrient quotas of nitrogen and phosphorus for selected

algal species are listed in Table II-1. The phosphorus requirement for most green-alga is 2-3 percent of dry cell weight.

Algae release phosphorus into the water during active growth mainly in the form of inorganic soluble phosphate, which in turn cycled rapidly (Wetzel, 1975). During algal decomposition, phosphorus is released in organic form and undergoes bacterial degradation. Bacteria then function as a nutrient pump, degrading the dissolved organic phosphorus to dissolved inorganic phosphorus which is then available for algae as well as bacterial assimilation. Studies by Rhee (1972) on the competition for phosphate between algae and bacteria have indicated that bacteria, because of a more favorable surface area to volume ratio, may serve to limit algal growth in certain systems.

### Sulfur

Sulfur is generally present in small quantities in all plant cells but is probably not a limiting factor for many algae under normal conditions. In most fresh waters, sulfur is present in the form of sulfate, but under the influence of strong reducing conditions, in the hypolimnion of certain lakes, for example, it may be converted into hydrogen sulfide. Sulfur is incorporated into cell mass during the synthesis of numerous organic compounds, and sulfur is known to exist in the vacuoles of certain cells. Strong evidence exists for a connection between divalent sulfur compounds and the assimilation of silica in diatoms (Round, 1973).

TABLE II-1

MINIMUM CELL NUTRIENT QUOTAS  
( $\mu\text{moles/cell}$ ) OF NITROGEN  
AND PHOSPHORUS FOR SELECTED ALGA

Organism	Nitrogen	Phosphorus	N:P Weight Ratio
<u>Asterionella</u> <u>Formosa</u>		$2.0 \times 10^{-9}$	-----
<u>Gymnodium</u>	$3.9 \times 10^{-7}$	$1.1 \times 10^{-8}$	15.8:1
<u>Dinobryon</u>	$1.8 \times 10^{-8}$	$0.5 \times 10^{-9}$	16.3:1
<u>Anabaena</u>	$1.0 \times 10^{-7}$	$2.5 \times 10^{-9}$	18.0:1
<u>Chlorella</u> <u>Pyrenoidosa</u>		$3.0 \times 10^{-9}$	-----
<u>Scenedesmus</u> sp.		$1.7 \times 10^{-9}$	-----

SOURCE: Lehman, J.J.; D.B. Botkin; and Gene E. Likens.  
"The Assumptions and Rationales of a Computer Model of Phyto-  
plankton Population Dynamics," Limnol. Oceanog. 20:3 (1975):  
343-64.

## Silica

Silica is an absolute requirement for diatoms and for certain species of flagellates. Although silica is often present in appreciable quantities in natural waters, it has been shown in certain instances to be growth limiting in those species which require this element. Low concentrations of silicate can be utilized by diatoms in natural habitats, but since assimilation of silicate is directly connected with formation of new cell walls, cell reproduction success may be affected. Some diatom species, such as Asterionella formosa, may exist at concentrations as low as 0.5 mg/l, while at least 25 mg/l are required for optimum growth of Fragilaria crotonensis (Round, 1973).

The cycle of silica is characterized, unlike other nutrient cycles, by an almost complete removal of assimilated silica from the system as diatom frustules accumulate within the sediments. The extent of this permanent loss depends on the morphometry of the lake basin and the percentage of the sediments which lie in the deep hypolimnion. Some of the more fragile forms may become dissolved and re-enter the water column, but for the most part, renewal of silica in an aquatic system must rely on inflow of water which has taken up silica during its transport to the lake.



### Calcium and Magnesium

Although calcium was at one time thought to be an absolute requirement for algae, researchers are now certain that, where it is required, the amount is small, with a minimal concentration around 5 mg/l. As a result, calcium is rarely limiting in aquatic systems. Calcium ions play an important role in the maintenance of cytoplasmic membranes and in wall structures, although strontium can substitute for calcium in certain species. Calcium is deposited as a calcite in the cell walls of many algae forming an integral part of the skeletal structure.

Magnesium, since it is a constituent of chlorophyll, is an absolute requirement for all pigmented algae. The minimum requirement for magnesium appears to be approximately 40 mg/l. Although Ca/Mg ratios are often important in regulating algal growth, many organisms can tolerate a wide range of ratios provided calcium and magnesium are present in sufficient amounts. There is also evidence to indicate that the ratio of monovalent to divalent ions may play an important part in determining the response of algae to these elements.

### Sodium and Potassium

Sodium appears to be a requirement for blue-green algae only. The increase of blue-green algae when waters become eutrophic, may, in part, be associated with an increase in sodium content. However, large amounts of sodium may be inhibitory, which may

account for the lack of blue-green algae in marine environments. A threshold level of 4 mg Na/l is required for near optimum growth of several green algal species (Kratz and Meyers, 1955).

Potassium is a requirement for all algae. Low potassium conditions can result in low rates of growth and photosynthesis with a high rate of respiration. Providing sufficient nutrients are available, the ratio of K to Na within the cell is independent of the ratio in the medium (Round, 1973).

#### Iron and Manganese

It has long been recognized that iron is essential to algal production. Iron is a key element in metabolism, being a constituent of the cytochrome molecule. A deficiency in iron will result in a decrease in photosynthetic rates. Iron exists in readily available form in all natural waters, although there is evidence to indicate that colloidal iron can also be utilized (Goldman, 1972). Iron deficiencies have been implicated in limiting productivity in certain northern oligotrophic lakes.

Manganese is present in all natural waters, and because of its role in nitrogen metabolism, it is probably a requirement of all algae. Photosynthesis and growth can be stimulated by an addition of manganese and decreased in a manganese deficient medium. Hutchinson (1957) suggests that seasonal variations of manganese in surface waters may play a part in regulating the composition of phytoplankton communities.



### Trace Elements

Minute concentrations of molybdenum, copper, vanadium and cobalt have all been shown to be essential for algae. Although trace amounts of each of these elements are essential, higher concentrations may act as poisons. Round (1973) suggests that concentration increases caused by autumnal circulations may be partly responsible for the composition of algal communities at this time. Molybdenum has been shown to be an essential element for the nitrogen fixing blue-green algae while cobalt or cobalt combined organically in vitamin B<sub>12</sub> has been shown to be essential for a large number of algae. Boron deficiency has been shown to produce a loss of pigment and reduction in growth in certain species, and iodine, as well as arsenic, have been shown to be essential in the growth of rhodophytes.

### Limiting Nutrients

Although originally developed by Liebig in the middle of the last century as the "Law of the Minimum", this fundamental environmental generalization of ecology was most consisely formulated by F.F. Blackman in 1905. He pointed out that an organism process that is dependent upon many distinct environmental factors for its operation will be limited by a single factor whose value is farthest from the process requirements. As developed originally by Liebig, this law only applied to a single nutrient limiting growth at any one time. Blackman, however,

was the first to realize that a succession of different limiting factors may affect a particular system. In his examinations of the effect of environmental variables upon assimilation of nutrients, he observed that when the rate of a function exhibits a transition from rapid increase to a stationary value, it becomes at once probable that another limiting factor has come into play.

This concept of limiting factors is central to ecology. The problem of defining limiting factors and predicting the effect that their alteration will have upon ecosystem organization is of key importance in formulating a predictive theory of ecology. This predictive ability can be important in predicting the impact of environmental alteration, such as the addition of urban stormwater to an aquatic system.

#### Limiting Nutrient Kinetics

Of the various kinetic models that are in current use for limiting nutrient identification, perhaps the most widely and successfully applied is the Monod relationship:

$$\mu = \hat{\mu} \left[ \frac{S}{K_S + S} \right]$$

where:  $\mu$  = specific growth rate,  $\text{day}^{-1}$

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

$S$  = limiting nutrient concentration,  $\text{mg/l}$ , and

$K_S$  = half-saturation coefficient (limiting nutrient concentration at  $\hat{\mu}/2$ ),  $\text{mg/l}$

At low values of  $S$ , which may often be experienced in aquatic systems, the Monod equation approximates a first-order equation in which the specific growth rate is linearly related to the limiting nutrient concentration. The equation then becomes:

$$\mu = \hat{\mu} \left[ \frac{S}{K_S} \right]$$

When  $K_S$  is very small in comparison with  $S$ , a zero-order relationship exists ( $\mu = \hat{\mu}$ ), in which the specific growth rate is at its maximum value and is no longer dependent on nutrient concentrations. External factors, such as light and temperature, then regulate growth. A typical Monod predicted relationship between limiting nutrient concentration and specific growth rate is shown in Figure II-1.

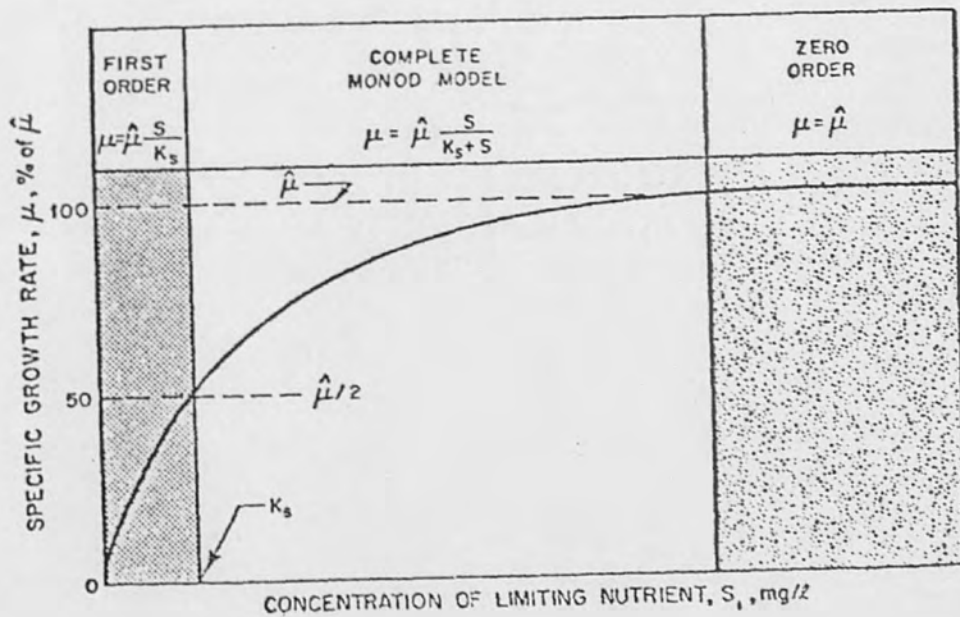


Fig. II-1. Relationship between limiting nutrient concentrations and growth rates as predicted by the Monod model.

If this model is to be useful in identifying limiting nutrients in natural waters, then the magnitude of  $K_S$  values must be determined. The  $K_S$  value indicates the approximate upper nutrient concentration at which the growth rate ceases to be proportional to that nutrient. Thus, for a nutrient to be limiting, its concentration must be approximately equal to or less than the  $K_S$  value. By comparing the  $K_S$  value for a particular nutrient with the actual concentration of that nutrient in a particular water system, it is possible to gain insight into the role of this nutrient in regulating algal growth. Half-saturation constants for selected nutrients and algal species are listed in Table II-2.

In addition to its use in identifying the nutrient which is limiting maximum yield or growth rate, the determination of  $K_S$  is potentially valuable in predicting relative successional patterns of different algal species in a nutrient-limited situation. The growth response of two different algal species to various concentrations of the limiting nutrient are presented in Figure II-2. As seen in this figure, alga A has both a lower  $\hat{\mu}$  and a lower  $K_S$  value than alga B, and as a result, at very low limiting nutrient concentrations the growth rate of alga A is greater than alga B. Alga A would have a competitive advantage over alga B in this case, and may actually eliminate alga B from the system. As the concentration of the limiting nutrient increases, the competitive advantage held by alga A is decreased until the growth



TABLE II-2

HALF-SATURATION CONSTANT ( $K_s$ ) FOR  
SELECTED NUTRIENTS  
AND ALGAL SPECIES

Phytoplankton Description	Half-Saturation Constant ( $K_s$ )		
	Nitrogen	Phosphorus	Carbon
Total Phytoplankton	0.025	0.005	----
Total Phytoplankton	0.025	0.010	----
Total Phytoplankton	0.025	0.002	----
Warm Water Species	0.07	0.015	0.03
Cold Water Species	0.01	0.02	0.04
Green Algae	0.015	0.0025	----
Small Cell Species	0.3	0.03	0.5
Large Cell Species	0.4	0.05	0.6

SOURCE: U.S. Environmental Protection Agency. Rates, Constants, and Kinetics Formulations in Surface Water Quality Modeling, Environmental Research Laboratory, Athens, GA (1978).

curves reach a common intersection (Point I) at which each species will be able to successfully compete for the supply of limiting nutrient. At higher limiting nutrient concentrations, alga B, because of its higher growth rate, should become the most successful competitor for the nutrient.

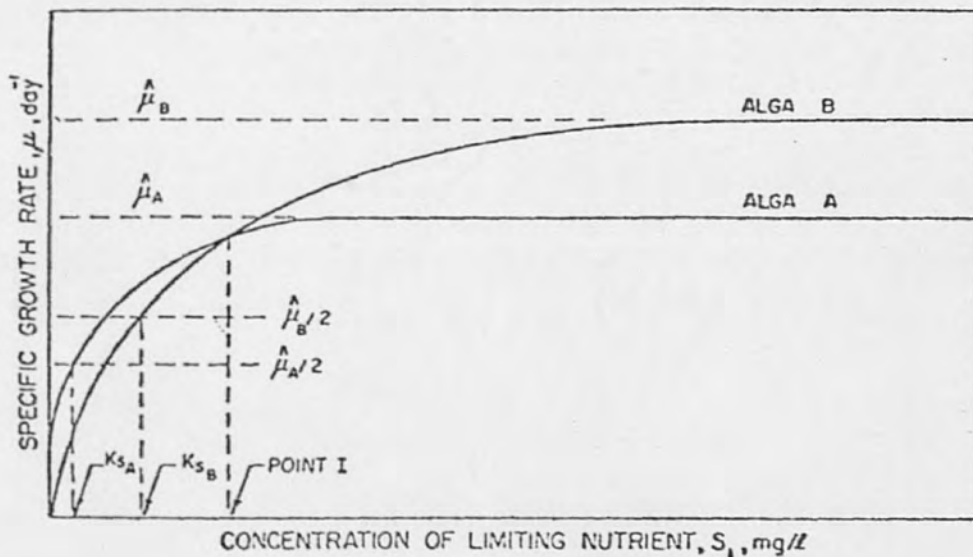


Fig. II-2. A comparison of Monod growth responses for two algae having different maximum growth rates and half-saturation coefficients.

### Influx of Nutrients into Aquatic Systems

Aquatic systems can receive nutrient inputs through a multitude of sources. Before man's technological era began, these sources were natural in origin and included such nonpoint source inputs as precipitation, runoff from forests and pasture lands, decaying vegetation and wastes from wild animals. However, technological advances and explosive population growth have resulted in a rapid increase in nonpoint sources such as drainage

from urban areas and return irrigation flows. On a mass loading basis, it was once thought that the effects of nonpoint sources were generally small compared with the magnitude of such point sources as municipal and industrial waste discharges. Additional information on the characteristics and magnitude of nonpoint sources, however, has led many researchers to question the validity of this assumption (Loehr, 1974).

To assess the relative importance and effect of nonpoint sources, information is needed on the magnitude and distribution of the inputs, the ultimate fate of the constituents desirable or undesirable, effects of the constituents, and any benefits or costs associated with control possibilities. Ecological impacts of nonpoint sources depend on the intensity as well as the seasonal distribution of the pollution source. Since overland flow and stream flow are the major transport agents for the nonpoint sources, information on time related changes in concentration and flow are essential for calculations of field rates. A comparison of nonpoint sources based solely on concentration units is often difficult because of the flow-dependent intermittent nature of the sources. Perhaps the best method of comparison is through the use of area yield rates, such as the quantity of nutrient per unit of drainage area per unit of rainfall or runoff. The constituents and relative magnitudes of a particular nonpoint input, urban drainage, are discussed in the next section.

### Characteristics of Urban Stormwater Drainage

During the past few years it has been recognized that urban stormwater runoff is not "rainwater" in terms of quality. Stormwater runoff typically contains substantial quantities of impurities, and in some locations, it has become a more serious source of pollutants than municipal wastes (Sartor, et. al., 1974). Street litter, gas combustion products, ice control chemicals, rubber and metals lost from vehicles, decaying vegetation, domestic pet wastes, fallout from industrial and residential combustion products, and chemicals applied to lawns and parks may be sources of contaminants in urban runoff. Lead, presumably from exhaust of internal combustion engines, may also be found in urban runoff. A comparison of area yields from urban and rural runoff sources is listed in Table II-3. As seen in this data, urban runoff produced higher concentrations of every parameter tested, and in the case of total  $PO_4$ , the urban runoff produced an area yield 19 times that found in rural runoff. Area loading rates for contributions of total nitrogen and phosphorus by various nonpoint sources are presented in Figure II-3.

Loehr (1974), in an investigation on the characteristics and constituents of urban runoff, found the major component of street surface contaminants to be inorganic mineral-like matter. The greatest portion of the pollutorial potential was associated with the fine solids fraction of street surface runoff. The quantity of contaminants per unit length of street was shown to



TABLE II-3

AREA YIELDS OF SELECTED CONSTITUENTS  
FROM URBAN AND RURAL RUNOFF SOURCES

Constituent	Area Yield (kg/yr/ha)	
	Urban Runoff	Rural Runoff
Total Organic Carbon	345	144
Total PO <sub>4</sub>	150	7.8
TKN	7.5	1.9
Nitrate N	13.3	5.0
Sodium	235	42.5
Potassium	133	21.7
Calcium	960	628
Magnesium	290	290

SOURCE: Loehr, Raymond C. "Characteristics and Comparative Magnitude of Non-point Sources." J. Water Pollution Control Federation 46:8 (1974): 1849-73.

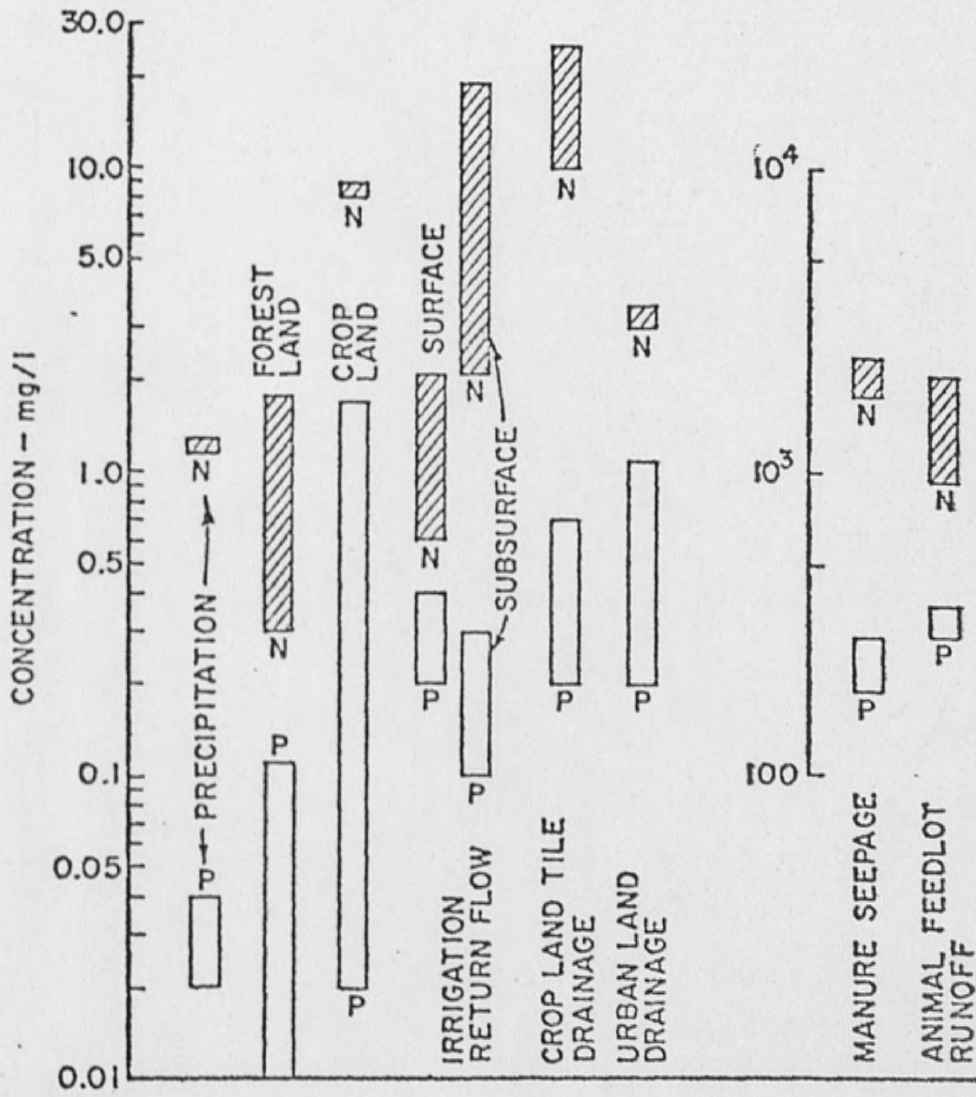


Fig. II-3. Comparison of nonpoint source effects

increase as the time interval since the last rain event or street sweeping increased, with the largest quantity of contaminants located within 6 inches of the curb. Runoff from residential streets was found to contain the highest concentrations of total phosphorus, runoff from arterial streets contained the highest concentrations of soluble phosphorus, with runoff from arterial highways containing the highest concentration of nitrogen. Mass loadings from urban stormwater runoff from commercial and residential areas surrounding Lake Eola are listed in Table II-4. In addition to the conventional water pollution parameters, constituents such as chlorinated hydrocarbons, organic phosphate compounds, heavy metals, and polychlorinated biphenyls were also found in urban runoff. Pathogenic organisms have also been isolated in urban runoff (U.S. EPA 1977) with the most concentrated pathogens being Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella and enteroviruses.

#### Phosphorus Inputs from Urban Runoff

Because of the importance of phosphorus in the nutrition of algae, the phosphorus contributions from point sources such as urban runoff has received much attention (Cowen and Lee, 1976). It has been estimated by Kluesener, et. al. (1974) that approximately 80 percent of the annual total P input to Lake Wingra in Madison, Wisconsin is from urban runoff.

TABLE II-4

MASS LOADINGS FROM URBAN STORMWATER RUNOFF  
FROM A 28 ACRE COMMERCIAL AREA AND A  
16.1 ACRE RESIDENTIAL AREA IN THE  
LAKE EOLA DRAINAGE BASIN

Constituent	Average Mass Loading (kg/ha-yr)	
	28 Acre Commercial	16.1 Acre Residential
SS	338	195
BOD	50	74.6
COD	296	442.3
TOC	123	138.5
TKN	4	1.8
NO <sub>3</sub> -N	6	2.2
OP-P	2	0.8
TP-P	3.5	2.2

SOURCE: Wanielista, Martin P.; Yousef A. Yousef; and Waldron M. McLellan. "Nonpoint Source Effects on Water Quality." J. Water Pollution Control Federation 49 (1977): 441-51.



Phosphorus transported by urban runoff occurs in both the soluble and insoluble forms. Kluesener, et. al. (1974) reported that approximately 58 percent of the total phosphorus contained in Cincinnati runoff samples was in the dissolved reactive state. The remaining 42 percent of total phosphorus occurred as particulate phosphorus, much of which was associated with the fine solids fraction of the particulate matter transported in the runoff. Sartor, et. al. (1974) reported a similar value of 56 percent of total phosphorus which was associated with particles less than 43 microns.

The availability of these phosphorus forms to photosynthetic organisms can be defined by the equation:

$$\begin{aligned} \text{available TP} &= \text{TSP} \times (\text{percentage of TSP available}) \\ &+ \text{PP} \times (\text{percentage of PP available}) \end{aligned}$$

where: TP = total phosphorus

TSP = total soluble phosphorus, and

PP = particulate phosphorus

However, since virtually all of the total soluble phosphorus will eventually be converted to orthophosphorus, the availability of all phosphorus forms can be calculated by the above equation provided the contribution by particulate phosphorus is known.

Cowen, et. al. (1976), using algal bioassays with Selenastrum as a test organism, reports an average estimated particulate phosphorus availability of 30 percent for Madison runoff. If this

figure is assumed reasonably accurate for other locations as well, the available total phosphorus from a stormwater source can be calculated by:

$$TP = TSP + PP (0.3)$$

#### Nitrogen Inputs from Urban Runoff

Because of the enriching effects of nitrogen to aquatic systems, concentrations of nitrogen in urban runoff are also of interest. Cowen, et. al. (1976) reported an inorganic concentration, including ammonia, nitrite and nitrate, of about 1.0 mg N/l in Cincinnati stormwater runoff with about 65 percent of the total N in the runoff present in the organic form. Kluesener and Lee (1976) found a similar organic nitrogen concentration of 77 percent in urban runoff entering Lake Wingra, Wisconsin.

Since inorganic nitrogen forms such as ammonia, nitrite and nitrate can be readily assimilated by algae in natural waters, the total amount of nitrogen in a stormwater runoff which may be available for aquatic growth will be equal to the amount of inorganic nitrogen present plus the percentage of organic nitrogen which may eventually become available. This relationship can be expressed mathematically as:

$$\begin{aligned} \text{Available Total Nitrogen} &= \text{Inorganic N} + (\text{Organic N}) \\ &\quad \times (\text{percentage of Organic N available}) \end{aligned}$$

The percentage of organic N available is a function of time and the rate at which microbial activities can mineralize organic N

into inorganic N. Cowen et. al. (1976) states that in tests of Madison urban runoff, an average of 70 percent total N, with a range of 57 to 82 percent, was present as algal-available nitrogen with bacterial mineralization of organic nitrogen comprising the major mechanism for increasing availability of these nitrogen sources. Since concentrations of organic nitrogen are often several times higher in urban runoff than inorganic sources, computation of the algal available N by addition of the inorganic sources may result in a gross under-estimation of available nutrient supplies.

#### Algal Bioassays

As seen in the preceding discussions on the availability of nitrogen and phosphorus in urban runoff, not all of the chemically measured nutrient forms are available for aquatic growth. To minimize problems which are often encountered in making predictions from chemical measurements alone, many researchers have resorted to the use of laboratory-conducted algal bioassay experiments to more accurately define possible growth responses to nutrient or waste additions.

Bioassays utilize the measurable response of living organisms to environmental variables. This response is an integration of the combined effects of ion solubility and ion availability to the test organism. Bioassays are more valuable than predictions based on chemical measurements alone because they

enable a distinction between biologically available ions and those not available. In many cases, chemical analyses would not make this distinction.

The response of a test alga to environmental conditions is directly affected by such physical parameters as light (Reynolds, et. al., 1975) and availability of CO<sub>2</sub> (Kuentzel, 1969), by chemical factors such as redox potential, biochemical transformations, complexation, sorption, total salts and ionic balance, hardness, acid-base equilibrium and solubility (Lee, 1973), as well as biological factors such as the presence of bacteria (Kuentzel, 1969). In fact, much of the early bioassay experimentation was conducted in an effort to define and minimize the effect of these variables. Bioassay methods have developed, however, over the past 10 years from a tentative procedure prepared by the National Eutrophication Research Program in March 1968 to the very sophisticated and well-designed present day assay methods as described in the Selenastrum capricornutum Algal Assay Bottle Test which was a major step in the clarification and standardization of assay methods. Many laboratory cultured algal species have been tested over this period including Selenastrum capricornutum, Microcystis aeruginosa and Anabaena flos aquae (Payne, 1974) as well as Stigeoclonium subsecundum (Trotter, et. al., 1976). Of all the algal species tested, Selenastrum capricornutum has been selected as a standard test organism because of its ease in culturing and enumeration (Payne, 1974 and U.S. EPA,



1978a) and because it produces a growth rate which is approximately twice that of the two blue-green species used. A more detailed discussion of bioassay techniques and procedures is given in later sections of this report.

### Practical Application of Bioassay Procedures

By far the most prolific application of bioassay procedures is the determination of the effects of a waste treatment discharge on the productivity of the receiving waters. Miller and Maloney (1971) considered the effects of secondary and tertiary wastewater effluents in algal growth in a lake-river system. Algal bioassays utilizing Selenastrum capricornutum as the test organism incubated in tertiary wastewater effluent would not support the growth of the test alga unless phosphorus was added despite the presence of all other nutrients. The results of this bioassay indicated that the installation of a full-scale advanced waste treatment plant capable of phosphorus removal would result in retardation of the eutrophication process in Shagawa Lake and would aid the eventual restoration of the lake. Miller, et. al. (1976), in an investigation of the effects of wastewater effluents on algal growth in a river system, found that algal production was regulated largely by the N:P ratio with maximum growth occurring at an N:P ratio of 11.3 to 1. It was further suggested that the N:P ratio may be useful in preliminary assessment of algal growth limitation in natural waters.

Waters containing N:P ratios less than 10 may be considered nitrogen limiting while those waters with N:P ratios greater than 10 may be phosphorus limited for algal growth. A similar study by Green (1975) upon the effects of municipal industrial and agricultural wastewater effluents upon phytoplankton production in the Snake River system found a high degree of correlation between the expected trophic state of a sampling site as predicted by its nutrient composition and the response obtained in algal bioassays. This indicates that the Algal Assay Bottle Test technique is sensitive to subtle changes of nutrient content in the various river waters assayed. Domel and Brooks (1974), using Chlorella pyrenoidosa and Chlamydomas reinhardtii as test organisms, tested the effects of detergent discharge of phosphorus on algal growth. In these experiments, no reduction of algal growth was observed even though a switch was made to non-phosphorus detergents in the study area. Only when effluents were tertiary treated so that reactive phosphorus levels were below 1.2 mg/l was algal growth significantly reduced.

#### Identification of Algal Growth-Limiting Nutrients

Another aspect of bioassay research is concerned with determining the trophic condition of various lakes throughout the country and predicting the effects of increasing or decreasing nutrient input into these lakes. Maloney, et. al. (1972), for example, conducted laboratory algal assays on waters from nine

Oregon lakes of varying water quality in which Selenastrum capricornutum was used as a test organism. Additions of nitrogen, phosphorus, and carbon, singly and in combination, were made to the waters and algal growth rates were determined. The addition of phosphorus alone greatly stimulated algal growth rates in four of the waters and the addition of nitrogen alone slightly stimulated algal growth in two of the waters. Three of the waters were capable of supporting relatively high algal growth rates without nutrient additions, and in one oligotrophic water, nutrient additions had no effect. In all cases, algal growth rates were directly proportional to the amounts of dissolved phosphorus in the waters, but there was no obvious correlation between algal growth rates and concentrations of nitrogen and carbon.

#### Bioassay Yields as a Predictor of Indigenous Populations

Because of the large number of factors known to affect laboratory bioassay results, few researchers have used laboratory bioassay yields as a predictive tool for estimating indigenous phytoplankton standing crops and chlorophyll "a" concentrations. Greene, et. al. (1978), however, suggests that under carefully controlled conditions, laboratory experiments can predict actual in situ concentrations with a high degree of correlation. Using data collected over a two year period with Selenastrum as a test organism in 18 separate bioassay experiments,

an equation predicting phytoplankton standing crop ( $r = 0.82$ ) was developed:

$$\text{indigenous population (mg/l)} = 1.07 \text{ (bioassay standing crop (mg/l))} - 0.04$$

A linear regression analysis of similar data of chlorophyll "a" concentrations and Selenastrum maximum yields resulted in the following equation ( $r = 0.91$ ):

$$\text{indigenous chlorophyll "a" (\mu g/l)} = 1.41 \text{ (bioassay standing crop (mg/l))} + 1.95$$

Although the data presented above is for a single lake system, it seems reasonable that similar relationships could be developed for other systems, provided a closely regulated bioassay procedure is followed.

In summary, it seems that, properly applied, algal bioassay experiments can be useful tools in predicting the response of aquatic systems to nutrient additions and pollutional inputs. Although bioassays have been conducted for many years, there are still many aspects of algal growth and behavior that are poorly understood. Research in these areas, especially in terms of predicting in situ responses from laboratory data, is badly needed.



## CHAPTER III

### FIELD AND LABORATORY EXPERIMENTATION

#### Lake Eola

Lake Eola is a small land-locked lake located in the heart of downtown Orlando. Physical characteristics of Lake Eola are listed in Table III-1 and water depth contours are shown in Figure III-1. Approximate contour areas and frustrum volumes in Lake Eola are listed in Table III-2. Contour areas drop off very little up to a depth of 5 feet, slightly more rapid to a depth of 10-15 feet, and then very rapidly below 15 feet. Approximately 73% of the total lake volume is located in the 0-10 foot frustrum layers. The lake receives stormwater runoff by way of storm sewers from a watershed of approximately 136 acres, composed of 78.2 acres of commercial and 57.8 acres of residential areas surrounding the lake (Figure III-2). A large fountain is located near the center of the lake. The natural shoreline of the lake has been replaced with a stone wall to prevent flooding of the adjacent parkland. Numerous small patches of rooted emergent macrophytes exist along this wall. No rooted submergent plants have been noted in the lake. The level of the lake is controlled by two drainage wells which drain into the underlying artesian aquifer. Since the level of the lake is

TABLE III-1

## PHYSICAL CHARACTERISTICS FOR LAKE EOLA, FLORIDA

Parameter	Quantity
Approximate Surface Area	109,270 m <sup>2</sup> 10.92 hectares (27.00 acres)
Approximate Volume	3.30 x 10 <sup>5</sup> m <sup>3</sup> 87.3 x 10 <sup>6</sup> gallons
Mean Depth	3.02 m (9.92 ft)
Maximum Depth	6.8 m (22.3 ft)
Length of Shoreline	1417 m (4650 ft)
Shoreline Development	1.21
Volume Development	1.72
Average Height Above Sea Level	26.8 m (88 ft)

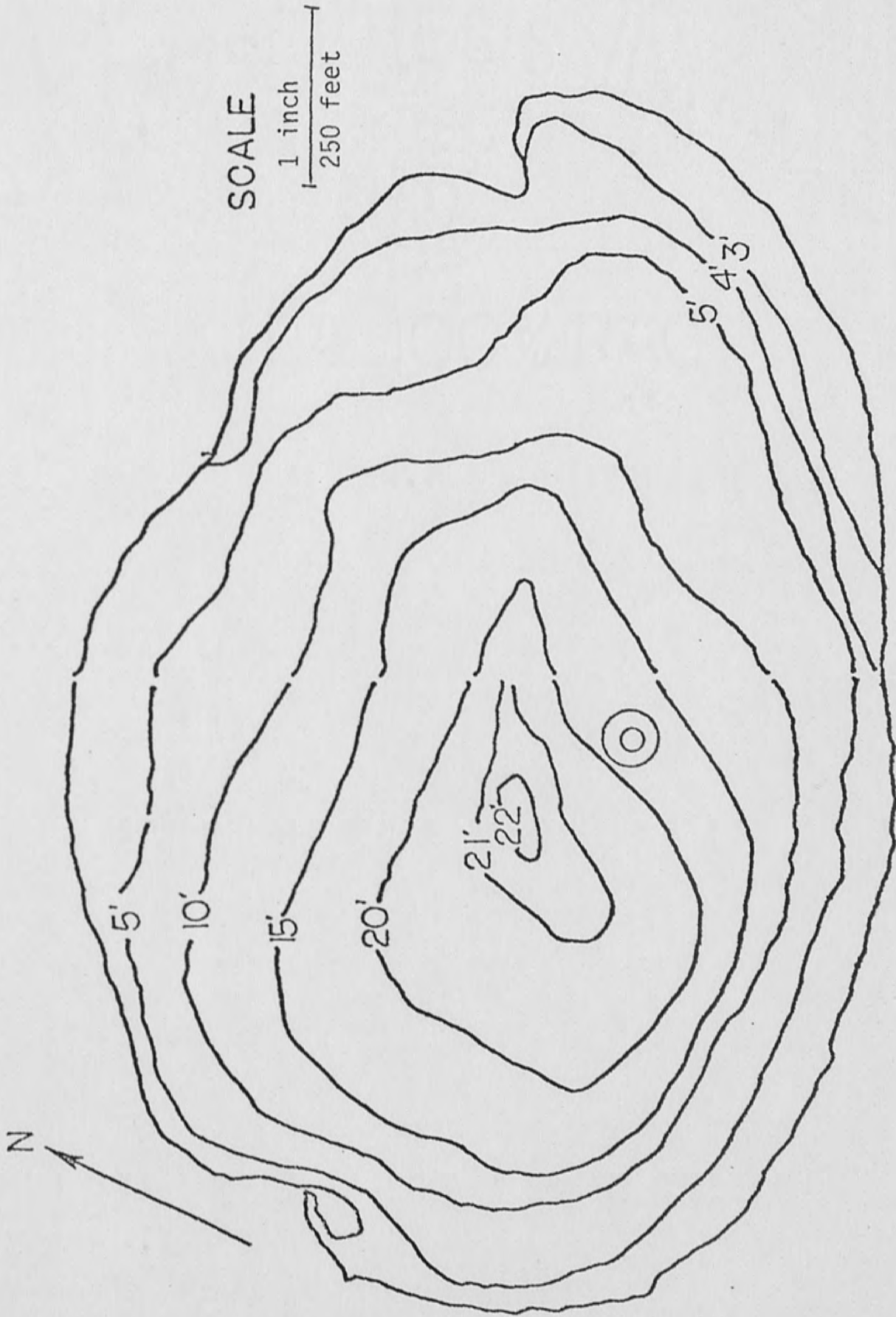


Fig. III-1. Water depth contours in Lake Eola. Contour data was collected while the lake was at a height of 88 feet above sea level.

higher than the piezometric surface, water flows readily down the wells. The piezometric surface is located approximately 57.0 feet above sea level (Wanielista, 1978). The level of the lake is maintained between 87.0 and 88.5 feet above sea level.

TABLE III-2

APPROXIMATE CONTOUR AREAS AND FRUSTRUM  
VOLUMES IN LAKE EOLA  
Contour Data was Collected While the Lake Was  
at a Height of 88.0 Feet Above Sea Level

Depth of Contour Below Lake Surface	Contour Area		Frustrum Volume		
	m <sup>2</sup>	ft <sup>2</sup>	m <sup>3</sup>	ft <sup>3</sup>	gal
0	109,224	1,175,712	--	---	---
3	103,391	1,112,932	97,222	3,432,966	25,678,586
4	95,717	1,030,325	30,348	1,071,629	8,015,781
5	72,607	781,566	25,656	905,946	6,776,472
10	45,135	485,847	89,733	3,168,533	23,700,623
15	27,922	300,559	55,678	1,966,016	14,705,792
20	11,158	120,110	29,783	1,051,673	7,866,510
21	1,679	18,074	1,957	69,092	516,808
22	106	1,138	272	9,606	71,853
TOTAL			330,649	11,675,458	87,332,425

Sources of pollution in Lake Eola include direct rainfall, stormwater runoff from the parkland surrounding the lake, and storm sewers which drain the urban watershed surrounding the lake. There are currently 11 active street drains which drain stormwater into the lake. A restoration of Lake Eola was undertaken in 1972 (Wanielista, 1973). At that time, the lake was partially drained and approximately 40% of the bottom was cleaned





of muck and silt and covered with relatively phosphorus free sand. Existing stormwater drains were also extended into the lake approximately at the 8-10 ft. water level. The lake was then refilled with water from the drainage wells. However, no efforts were undertaken to manage or treat the stormwater entering the lake, and now the water quality of Lake Eola is again questioned. Buildup of flocculant sediment matter is increasing rapidly. Large masses of algae can be seen floating along the shoreline, and fish and duck kills have been reported periodically during the summer months following heavy rain events. In addition, Salmonella, Shigella and Clostridium botulinum have been isolated from the water and shoreline sediments in the lake (Wanielista, 1979).

#### Site Selection

To establish a record of the water quality in Lake Eola, monthly water quality analyses were performed for a period of one year beginning July 1978. Initial samples were collected from 16 locations chosen randomly within the lake. After the initial sampling set, the number of locations was reduced to six fixed stations (Figure III-3) which were maintained throughout the sampling period. The six locations were divided into two groups and monitored alternately so that stations S1-S3 would be monitored one month and stations S4-S6 monitored the next. Station 1 was selected near a small island, which is used

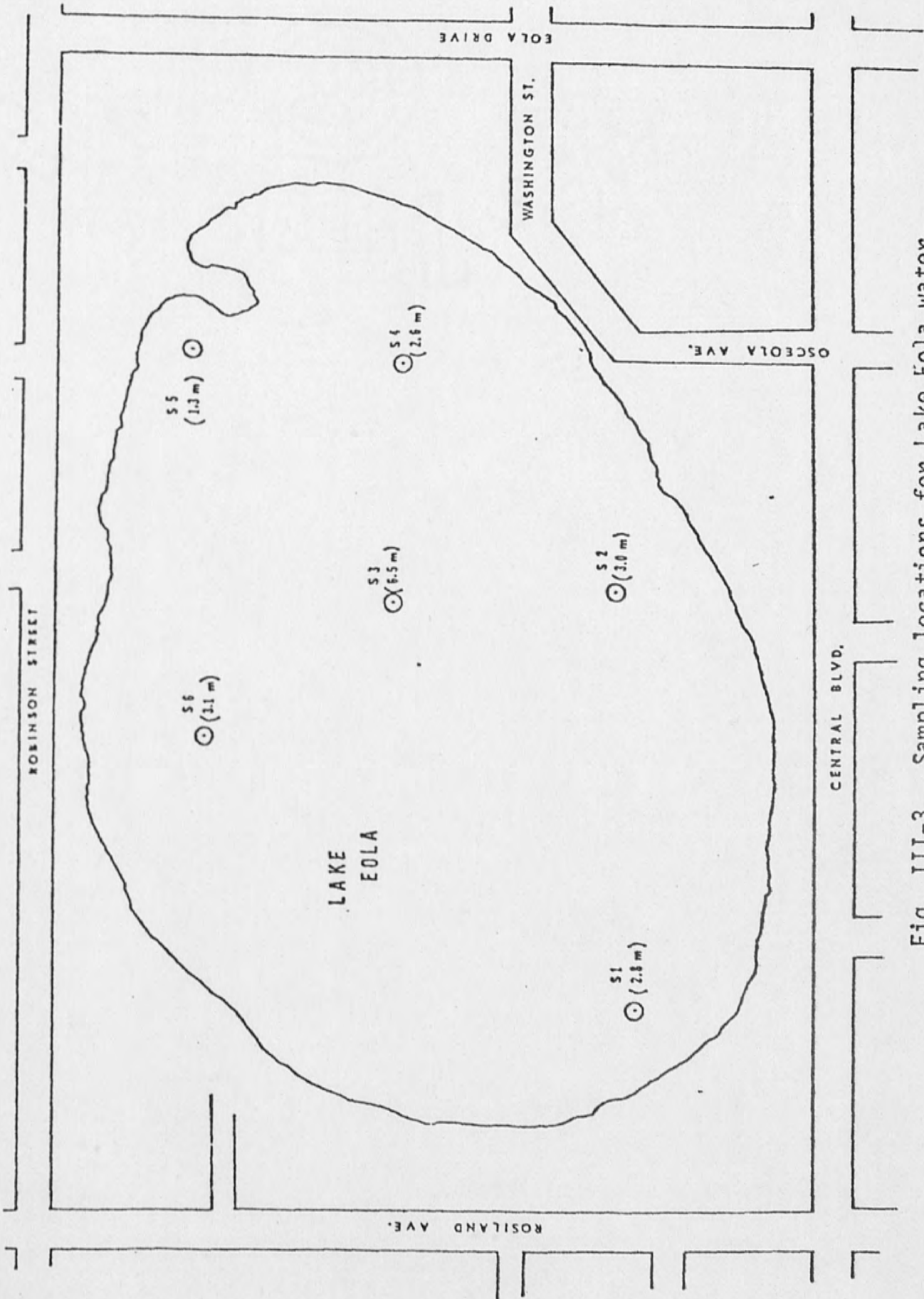


Fig. III-3. Sampling locations for Lake Eola water

primarily as a nesting area for birds and ducks, in order to determine if the waste products from these animals are sufficient to alter water quality in the vicinity of the island. Station 3 was selected near the large fountain to determine whether the fountain has an effect on water quality. Station 5 was chosen in a shallow silty area near shore. Stations 2, 4 and 6 were selected at random from the remainder of the lake.

#### Water Quality Analysis-Field Determinations

Dissolved oxygen and temperature profiles were recorded monthly at each of the three sampling locations which were monitored. Measurements were taken at 0.5 m intervals and at the bottom using a YSI Model 54A dissolved oxygen meter equipped with a remote sensing probe. Since station 3 was located in the deepest area of the lake, measurements of temperature and dissolved oxygen were recorded at this station each month. Secchi disk depth was also determined at each of the three stations. All Secchi disk determinations were conducted between 12 p.m. and 4 p.m. with the sun to the observer's back.

Water samples were collected at each of the three stations and returned to the Environmental Engineering Laboratory at the University of Central Florida for water quality analysis and for use in bioassay experiments. All samples were collected from the top 1 meter of the water column using a brass 2 liter Kemmerer water sampler and stored in 1 gallon polyethelene containers



which were completely filled to eliminate gas exchange. Samples were placed on ice in the dark for return to the laboratory.

#### Water Quality Analyses-Laboratory Determinations

The following determinations were performed on each sample collected: pH, turbidity, organic carbon, inorganic carbon, nitrate nitrogen, orthophosphorus and chlorophyll "a" along with an analysis of heavy metals which included: zinc, lead, chromium, nickel, copper, aluminum, iron, cadmium, arsenic and calcium. Total Kjeldahl nitrogen and BOD<sub>5</sub> were also determined on selected samples. Measurements of pH, turbidity, and chlorophyll "a" were performed within 4 hours of collection. Other analyses were conducted within the time specified by U.S. EPA in Methods for Chemical Analysis of Water and Wastes (1976).

Determinations of pH were performed with a Corning Model 12 Research pH meter equipped with a temperature compensation probe. Turbidity was measured with an H.F. Instruments Model DRT-150 Nephelometric Turbidimeter. Chlorophyll "a" concentrations were determined from a calibration curve using a Turner Model 111 Filter Fluorometer. The calibration curve was prepared by calculating chlorophyll "a" concentrations in water samples from Lake Eola using the trichromatic spectrometric acetone extraction method as described in Standard Methods for the Examination of Water and Wastewater (14th Edition) and comparing these values to relative fluorescence values. This calibration curve for

indigenous algal species in Lake Eola is shown in Figure III-4. All calibrations were conducted using the 1x and 3x fluorometer ranges since it was determined that switching between these two positions produced virtually identical results once the range multiplier factor had been applied. The 10x and 30x ranges, however, tended to underestimate chlorophyll "a" concentrations, compared to the 1x and 3x scales, and were not used in calibration or measurement procedures.

Determinations of orthophosphorus were performed using the ascorbic acid method as described in Standard Methods. The standard curve used in determination of orthophosphorus is shown in Figure III-5. Carbon analyses were performed using the combustion-infrared analysis technique with a Beckman Model 915 Total Organic Carbon Analyzer equipped with a Beckman Model 215A Infrared Analyzer. Determinations of total carbon and inorganic carbon were made with organic carbon determined by difference. Nitrate nitrogen was analyzed using an Orion Model 93-07 nitrate ion electrode with an Orion Model 801-A Digital Ionalyzer. Since nitrate nitrogen concentrations were generally less than 1 ppm, the low level technique as described by Orion in the ion electrode instruction manual was used. It was found, however, that the use of a low level ionic strength adjusting solution which is specified in this technique, would cause an overestimate of nitrate nitrogen concentrations in spiked lake water samples of approximately 0.40 mg/l per mg/l of nitrate nitrogen present at

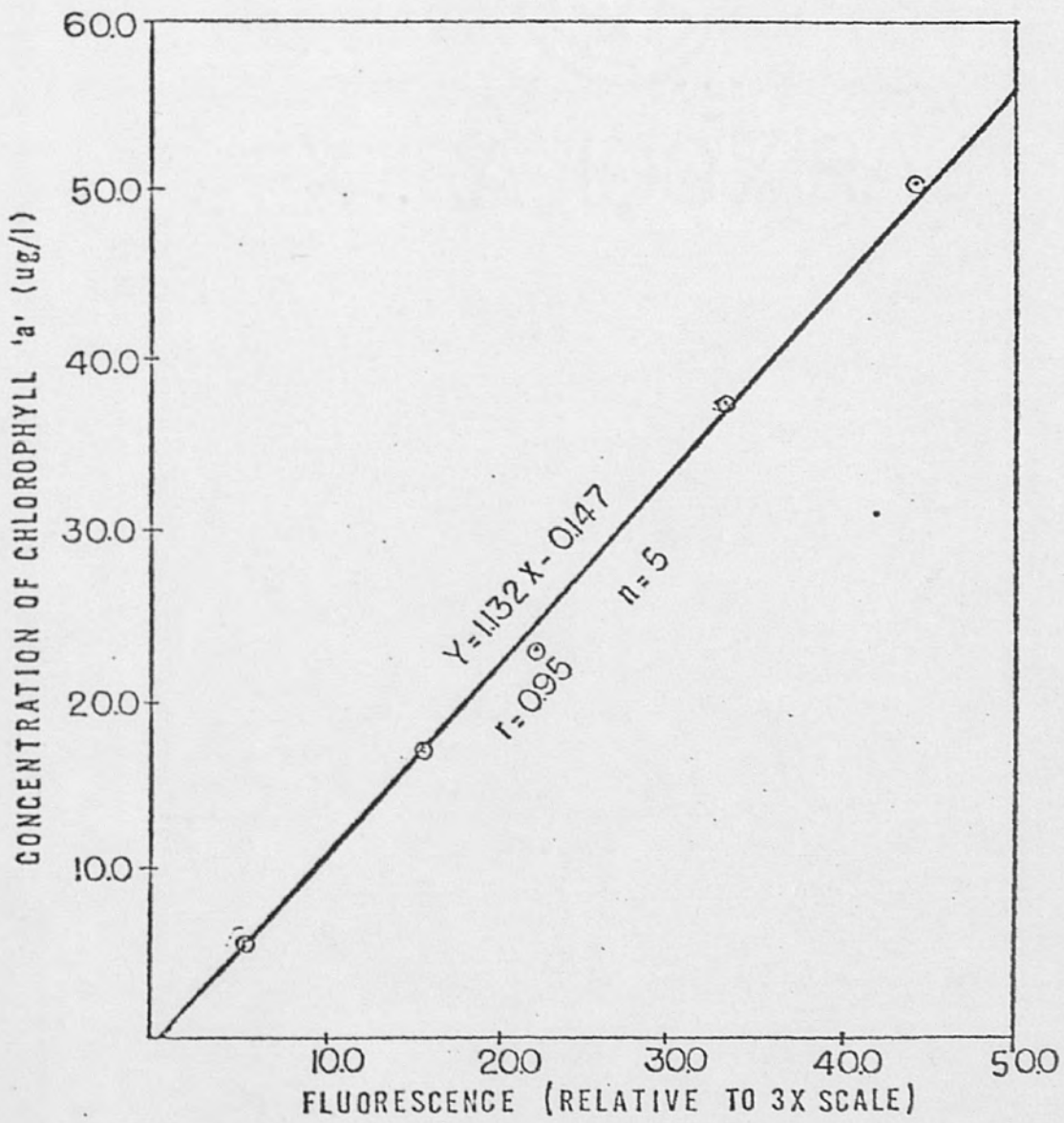


Fig. III-4. Relative fluorescence vs. chlorophyll "a" concentrations for indigenous algal species in Lake Eola.

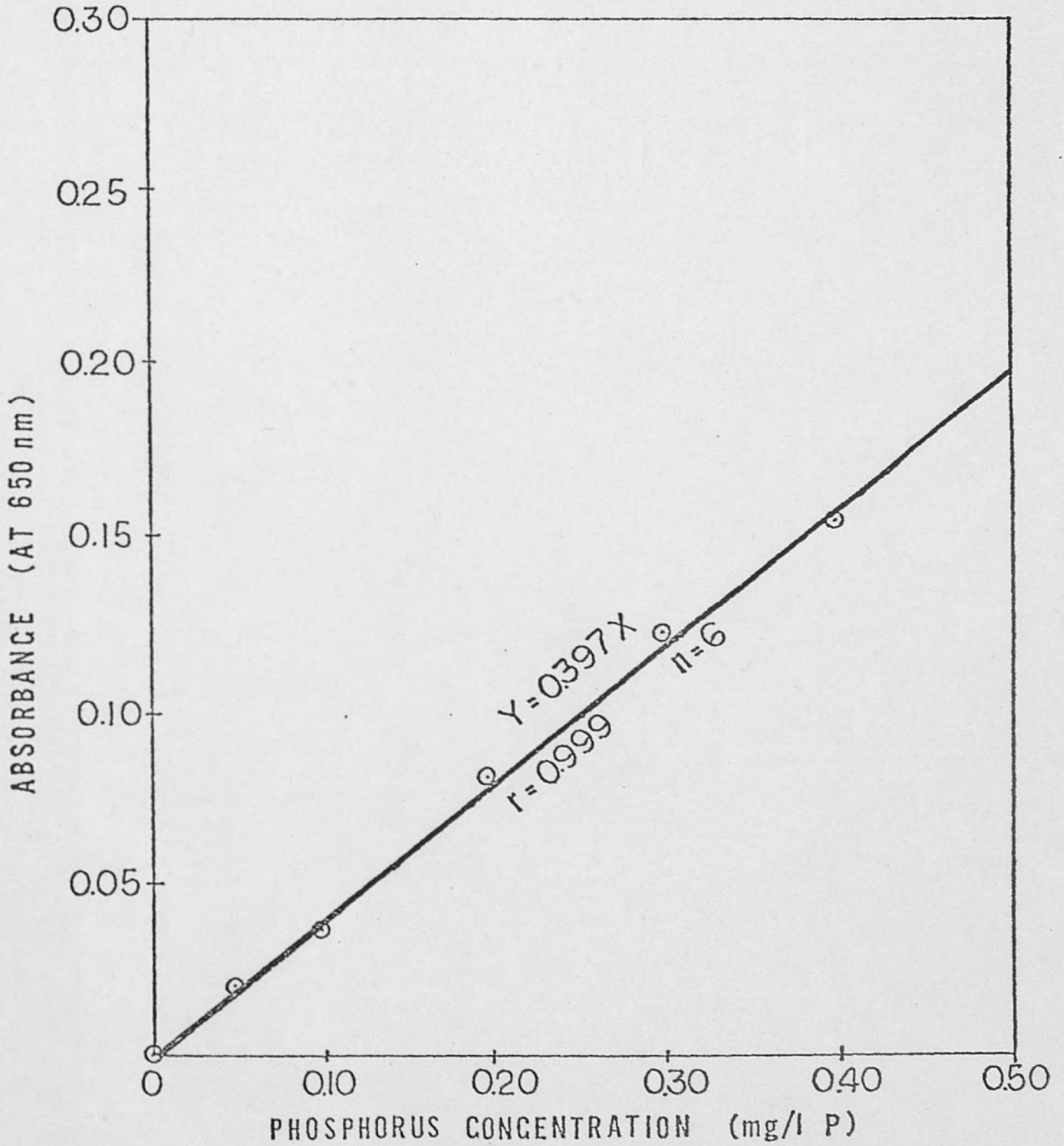


Fig. III-5. Standard curve for orthophosphorus using absorbic acid method on a Bauch and Lomb Spectronic 70 using a 1 cm cell.



concentrations of 0.5 mg/l nitrate nitrogen or greater. The substitution of the high level ionic strength adjustor for the low level ionic strength adjustor was shown to reduce this error to less than 0.05 mg/l per mg/l of nitrate nitrogen present in the range up to 2 mg/l. A typical nitrate nitrogen standard curve is shown in Figure III-6. Total Kjeldahl nitrogen and BOD<sub>5</sub> were determined as described in Standard Methods.

Heavy metal analyses were performed on concentrated samples using a Spectrometrics Incorporated Spectrospan III Plasma Emission Spectrophotometer. Samples were concentrated by adding 2 ml of concentrated HNO<sub>3</sub> to 100 ml of sample in a 250 ml Erlenmeyer flask and heating at 95° C until a volume of approximately 10 ml was achieved. The sample was then brought up to 20.0 ml with glass distilled water and stored in a covered polypropelene container for measurement. All glassware used in metal determinations was acid-washed before each use with a 1:1 solution of hot hydrochloric acid followed by 5 rinses in glass distilled water.

### Algal Bioassay Procedures

#### Sample Collection and Preparation

Lake water samples for use in algal bioassays were collected from Lake Eola using a brass 2-liter Kemmerer water sampler and stored in 1 gallon polyethelene containers. All containers were completely filled to eliminate gas exchange and were stored in the dark on ice to minimize biological and chemical changes in water

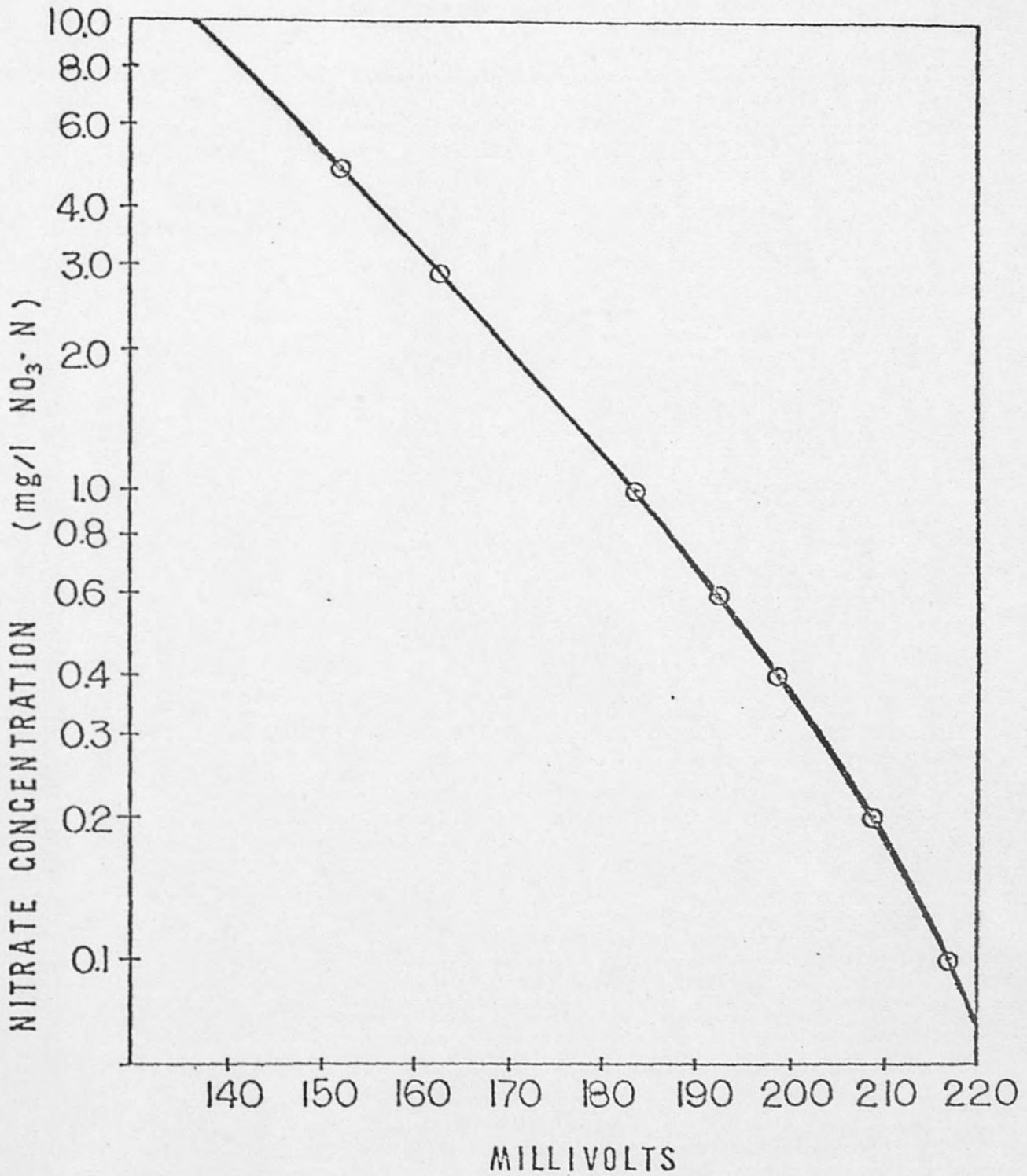


Fig. III-6. Typical nitrate nitrogen standard curve using an Orion Model 93-07 Nitrate Ion Electrode.

quality. Since the depth of the euphotic zone in Lake Eola was determined by Secchi disk measurements to be approximately 1 meter, all samples for use in bioassays were collected from the top 1 meter of the water column as suggested by U.S. EPA (1978). A composite water sample was prepared from lake water collected at 3 of the 6 fixed stations in the lake for use in bioassay experiments.

In order that a unialgal test species could be used in bioassay experiments, the indigenous algae in the sample must first be removed. This removal was achieved either by filtration followed by autoclaving or by autoclaving followed by filtration. Filtration involved passage of the sample through a 47 mm diameter Millipore acetate filter with a  $0.45 \mu$  pore size. Autoclaving was conducted at a pressure of  $1.1 \text{ kg/cm}^2$  (15 psi) and a temperature of  $121^\circ \text{C}$  for 10 minutes per liter of sample, provided the total sterilization period was not less than 30 minutes. Treated samples were stored in filled polyethelene containers at  $4^\circ \text{C}$  in the dark until needed. In no case was the storage period longer than 48 hours. In several instances, it was desirable to determine the effect of nutrient additions to indigenous algal species in Lake Eola. For these experiments, the lake composite sample was used as is without autoclaving or filtration of any kind. All samples for bioassay use were analyzed for the following constituents as described previously:

pH, organic carbon, inorganic carbon, nitrate nitrogen, total Kjeldahl nitrogen, and orthophosphorus, along with an analysis of heavy metals which included: zinc, cadmium, arsenic, nickel, copper, aluminum, iron and chromium.

#### Glassware Preparation

Glassware used as culture vessels or in the sample preparation was washed with a stiff bristle brush using Liqui-Nox non-phosphate detergent and rinsed thoroughly with tap water. All glassware was then rinsed in a 1:1 solution of hot hydrochloric acid followed by 5 rinses in glass distilled water, dried, and covered until used.

#### Test Alga and Inoculum Preparation

Two unicellular test alga were used as bioassay organisms: Selenastrum capricornutum Printz and Chlorella pyrenodosa. A concentrated Selenastrum culture was obtained from the Environmental Research Laboratory, Corvallis, Oregon. The Chlorella culture was obtained from Carolina Biological Supply Company, Burlington, North Carolina. A culture of Anabaena flos-aquae was also obtained from the Environmental Research Laboratory, but, because of the tendency of Anabaena to form into large flocculant clumps, it was not used as a bioassay organism. All algal cultures were stored in a dark refrigerator. Approximately 2 ml of a synthetic algal culture were aseptically transferred to 200



ml of a synthetic algal culture medium in a 1-liter Pyrex Erlenmeyer flask. The culture was incubated at  $24 \pm 2^{\circ}$  C under continuous "Cool-White" fluorescent lighting ( $400 \pm 10\%$  foot candles) and shaken continuously at 100 oscillations per minute. At approximately 2 week intervals, a routine stock transfer of 2 ml of algal culture was transferred to a fresh culture medium to maintain a continuous supply of cells for experimental work. The synthetic algal nutrient medium was a dilute medium prepared in the laboratory similar to that described by EPA (1978). Individual stock solutions of macronutrients were prepared at 1000 times the culture medium concentration while the micronutrients were combined into a single solution also at 1000 times recommended concentration. All chemicals used in preparation of the medium were reagent grade or better. One milliliter of each stock macronutrient solution and 1 ml of the stock micronutrient solution were combined with glass distilled water to form 1.0 liter of algal growth medium. The solution was filter sterilized by filtration through a 47 mm diameter Millipore acetate filter with a  $0.45 \mu$  pore size at a pressure of 1/2 atmosphere. Final pH of the prepared medium was found to be 7.50. Final concentrations of macroelements and microelements are listed in Table III-3.

TABLE III-3

CONCENTRATIONS OF MACROELEMENTS AND MICROELEMENTS IN  
THE PREPARED SYNTHETIC ALGAL MEDIUM

Element	Concentration of Element
Macroelements (mg/l):	
N (in the form of $\text{NaNO}_3$ )	4.200
Mg (in the form of $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ )	2.904
Ca (in the form of $\text{CaCl}_2$ )	1.202
S (in the form of $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ )	1.911
P (in the form of $\text{K}_2\text{HPO}_4$ )	0.186
K (in the form of $\text{NaHCO}_3$ )	0.469
C (in the form of $\text{NaHCO}_3$ )	2.143
Microelements ( $\mu\text{g/l}$ ):	
B (in the form of $\text{H}_3\text{BO}_3$ )	32.460
Mn (in the form of $\text{MnCl}_2$ )	115.374
Zn (in the form of $\text{ZnCl}_2$ )	1.570
Co (in the form of $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ )	0.354
Cu (in the form of $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ )	0.004
Mo (in the form of $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$ )	2.878
Fe (in the form of $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ )	33.051

## Algal Bioassay Methods

All bioassays were conducted in 1000 ml Erylenmeyer flasks containing 200 ml of sample. Flasks were incubated on an Eberbach shaker with a modified 3/4 inch plywood table top, 64 inches long and 32 inches wide, which was constructed to accomodate 66 Erylenmeyer 1 liter flasks. During the bioassay period, the shaker table was adjusted to provide 100 oscillations per minute. All flasks were fitted with foam plugs to allow gas exchange and prevent contamination. A constant temperature of  $24 \pm 2^{\circ}$  C was maintained in the incubation room. Constant illumination was provided by two "Cool-White" fluorescent lights which were adjusted to provide an illumination of  $400 \pm 10\%$  foot candles as measured adjacent to the flask at the liquid level. The incubation apparatus used in bioassay experiments is shown in Figure III-7.

Depending on the experimental design, it was often necessary to add an initial nutrient dosage to the flasks at the start of the experiment to determine the effect of these nutrients on algal productivity. Various concentrations of phosphorus, nitrogen, iron, and EDTA were added to selected flasks. Phosphorus was added in the form of a solution of  $K_2HPO_4$ , nitrogen as  $NaNO_3$ , iron as  $FeCl_3$  and EDTA as the sodium salt. Concentrated stock solutions of each nutrient were prepared so that, depending on the nutrient concentration desired in the flask, no nutrient

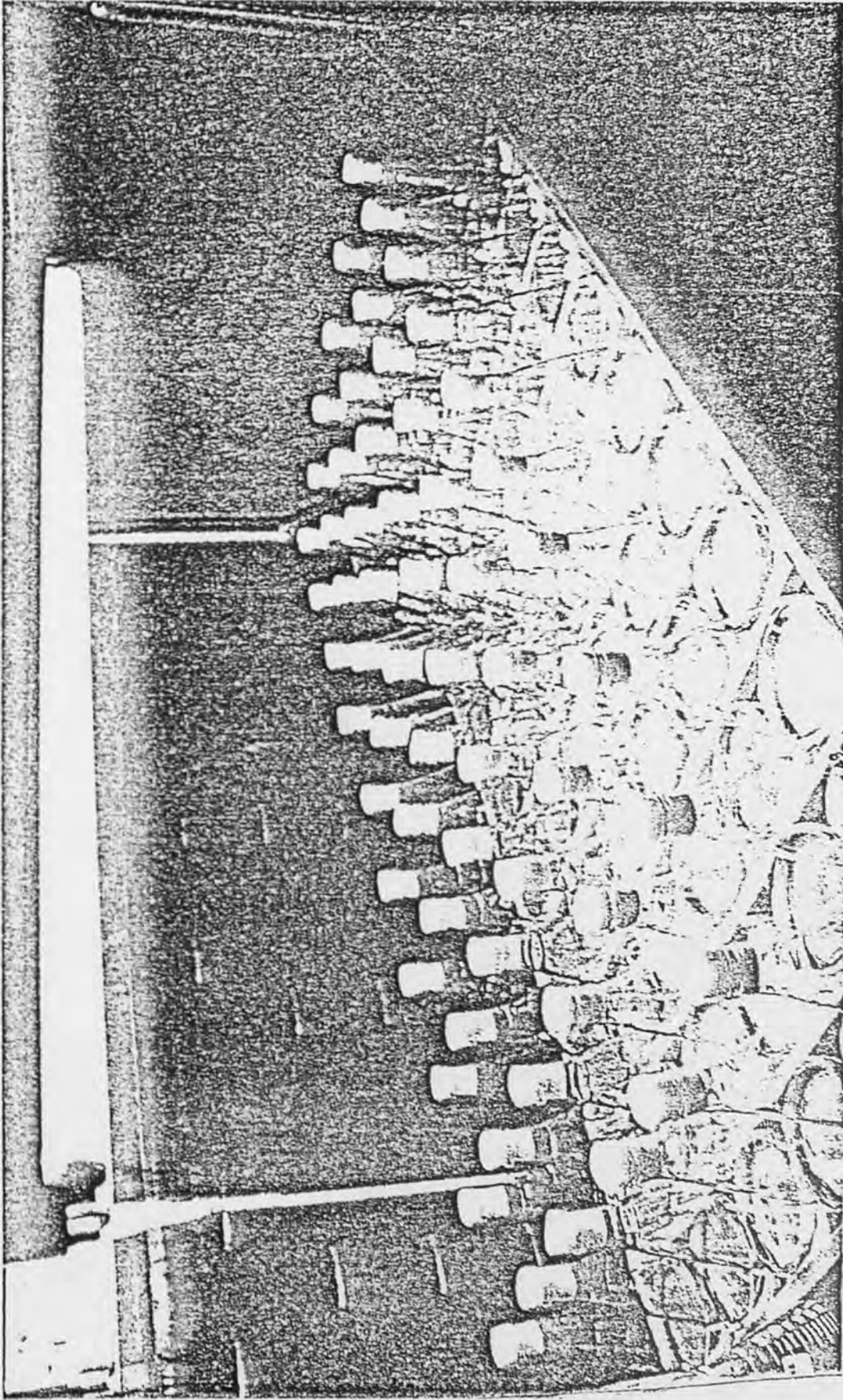


Fig. III-7. Bioassay incubation apparatus



addition would require a volume greater than 2 ml to be added to any flask. Disposable sterilized pipettes were used in all nutrient additions to avoid contamination. All nutrient spikes were added to flasks in triplicate, and the results of the three flasks were averaged. Glassware used as incubation vessels was permanently numbered so that any single flask which produced consistently higher or lower productivity responses could be detected and removed. All flasks were allowed to equilibrate under test conditions for 24 hours before inoculation with an algal species to allow time for the test media used to come to a constant temperature and also to allow for equilibrium in gas exchange. This procedure was shown to produce more consistent results among the flasks with a specified nutrient addition. All flasks were inoculated with 1 ml of a 14 day old algal culture to give an initial chlorophyll "a" concentration of approximately 10  $\mu\text{g/l}$ .

#### Standing Crop Determinations

Measurements of growth responses were performed by determination of in vivo fluorescence of chlorophyll "a" using a Turner Model 111 Filter Fluorometer equipped with a model 10-030 cuvette holder. The fluorometer was also equipped with a special photomultiplier tube with enhanced red sensitivity as recommended by Turner in Fluorometric Facts (1976) for in vivo chlorophyll determinations. The model 10-045 blue lamp was chosen as the light source

in combination with a model 5-60 primary excitation filter.

The emission filter used was a model 2-64 filter as recommended by Turner. Before measurement, each incubation flask was swirled to assure uniform mixing of the contents. A grab sample of approximately 4 ml from each flask was collected in a Pyrex 13 x 100 mm test tube for measurement in the fluorometer. All test tubes were inverted several times before measurement to assure uniform distribution of algae. Only the 1x and 3x fluorometer ranges were used, with all values recorded relative to the 3x range. All nutrient additions used in bioassay experiments were run in triplicate and results obtained in the three flasks were averaged for use in data analysis.

In order that the bioassay results could be expressed in terms of dry cell weight of cell mass per liter of solution, a calibration curve was prepared relating relative fluorescence to cell dry weight in mg/l. To prepare this curve, 14 Whatman 4.25 cm GF/A glass fiber filters were dried for 2 hours at 103<sup>0</sup> C. All filters were allowed to cool in a dessicator for at least one hour before weighing. Relative fluorescence of measured aliquots of an actively growing Selenastrum culture were measured with a fluorometer and then filtered at a pressure of 1/2 atmosphere. Filtration was followed with a 50 ml distilled water rinse of the filter funnel to transfer all algae to the filter and to wash nutrient salts through the filter. All filters were dried at 103<sup>0</sup> C for 24 hours, cooled in a dessicator for 24 hours, and

then weighed. A calibration curve was then prepared relating relative fluorescence measurements to algal cell dry weight. This calibration curve is shown in Figure III-8.

#### Luxury Uptake of Phosphorus

An experiment was conducted to determine if luxury uptake of phosphorus in an actively growing algal culture can occur in sufficient quantity to alter the results of short-term bioassays. Approximately 200 ml of synthetic algal medium (Table III-2) were placed into five 1-liter Erlenmeyer flasks with a 14 day old culture of Selenastrum and incubated on a shaker table at 100 oscillations per minute under a constant illumination of 400 ft-candles using "Cool-White" fluorescent lighting. The contents of each flask were combined daily into a single mixture in a 2-liter beaker. A portion of the combined mixture was filtered through a Whatman GF/A filter which had been washed with 100 ml of distilled water, dried, and weighed. The volume of culture filtered was dependent upon the concentration of algal cells present and was chosen in order to deposit the maximum amount of cell mass on the filter without clogging the filter. After filtration, the filters were dried at 103<sup>0</sup> C for 24 hours and reweighed. The filter paper was then mascerated with a tissue grinder and digested in an autoclave at 121<sup>0</sup> C at 15 psi using the persulfate digestion technique for phosphorus as described in Standard Methods. Concentrations of orthophosphorus were

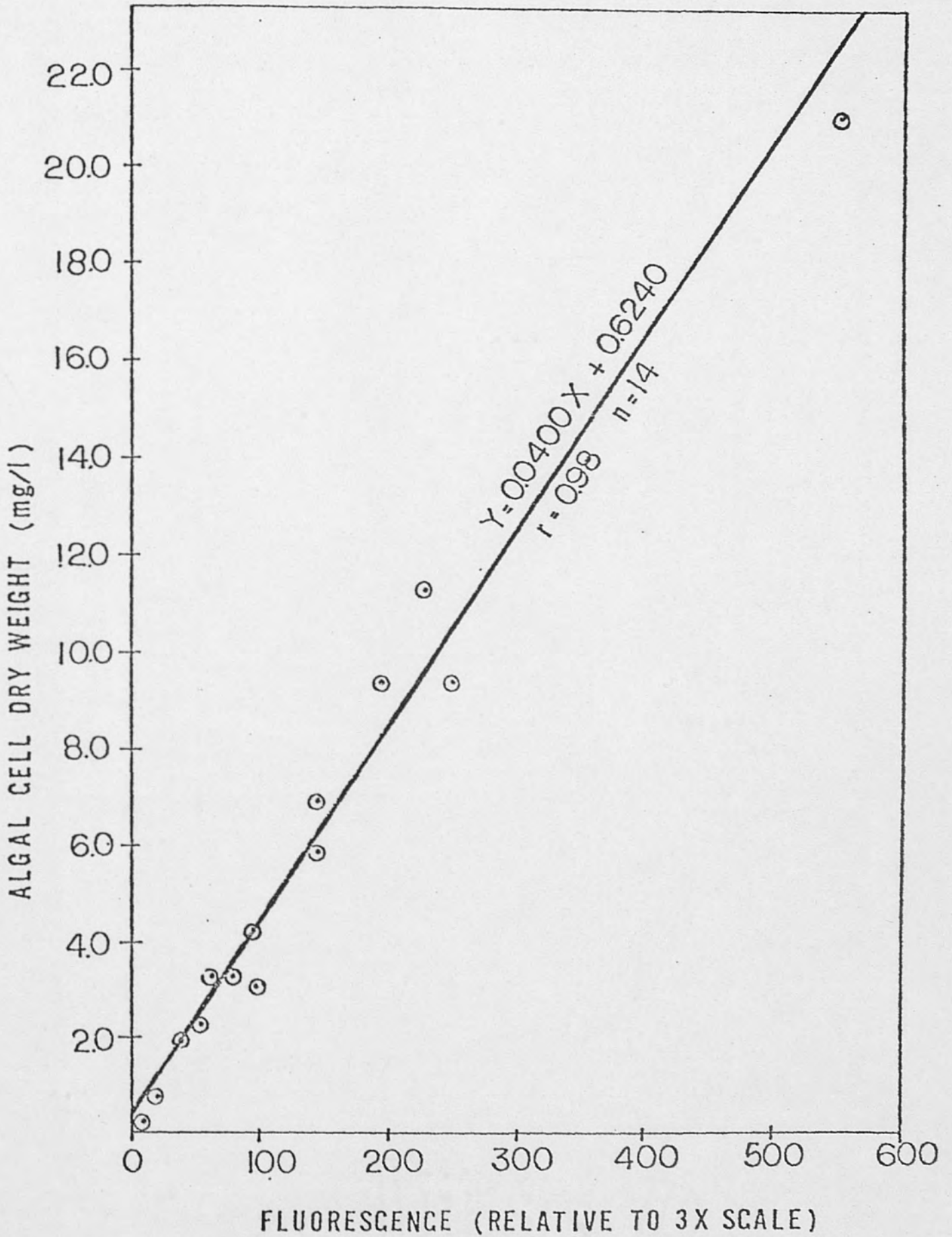


Fig. III-8. Correlation between relative fluorescence and algal cell dry weight for Selenastrum Capricornutum.



determined on neutralized samples using the ascorbic acid technique. The calibration curve used in these determinations is shown in Figure III-9. Concentration of phosphorus in mg per gram of dry cell mass was calculated and plotted as a function of incubation period. All glassware used in this experiment was acid-washed as described under "Glassware Preparation".

#### Relationship Between Organic Carbon and Cell Dry Weight

It has been reported in the literature that measurements of total organic carbon can be used to detect changes in algal production in biomass experiments. To determine the relationship between organic carbon in an actively growing algal culture and cell dry weight, a series of Selenastrum cultures grown in Lake Eola water were analyzed for organic carbon content on the 17th day of their incubation. All cultures were grown under the conditions described in "Algal Bioassay Methods". Organic carbon was measured on unfiltered samples using the combustion-infrared technique with a Beckman Model 915 Total Organic Carbon Analyzer. Fluorescence of each sample was measured and converted to cell dry weight using the standard curve shown in Figure III-7. Thirty-nine different samples were tested in this experiment, and the results are shown in Figure III-10. The relationship between cell dry weight and organic carbon appears to be a linear function in the range of values tested. This linearity is confirmed by the correlation coefficient of 0.947.

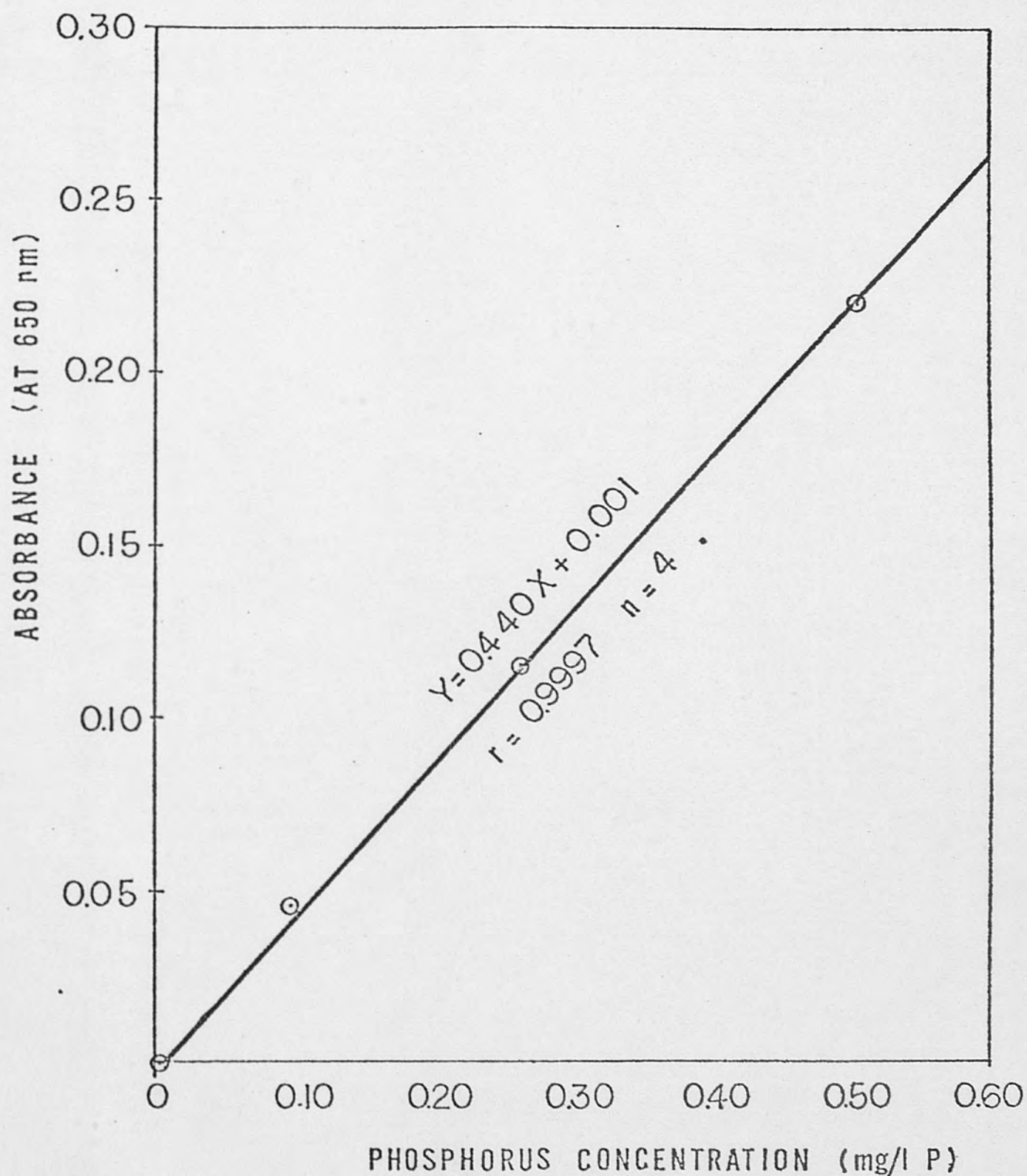


Fig. III-9. Standard curve for total phosphorus digested by persulfate digestion and measured using ascorbic acid method on a Bauch and Lomb Spectronic 70 with 1 cm cell.

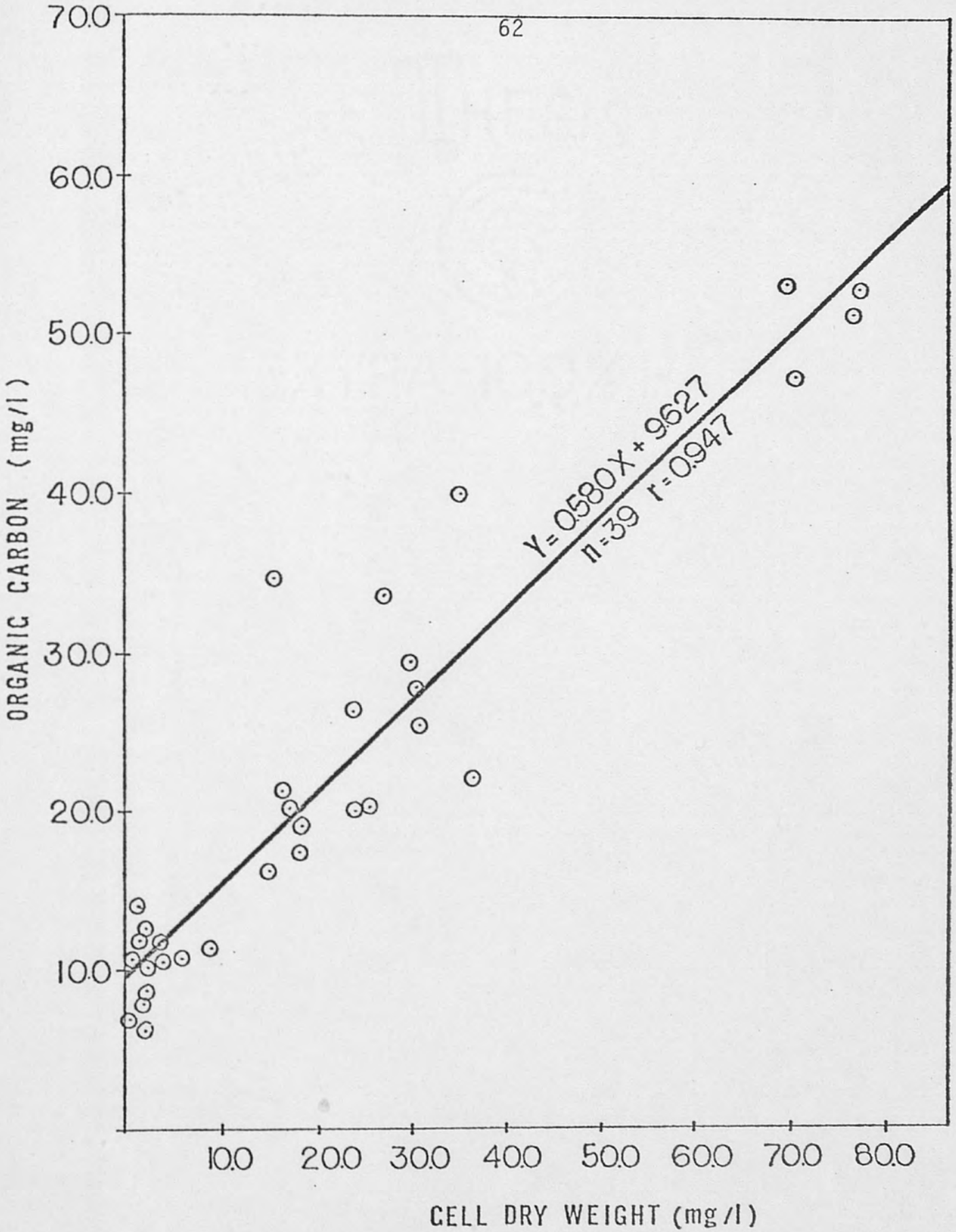


Fig. III-10. Relationship between cell dry weight and organic carbon in a Selenastrum Capricornutum culture incubated in Lake Eola water.

## CHAPTER IV

### LIMNOLOGICAL CHARACTERISTICS OF LAKE EOLA

To establish a record of the water quality in Lake Eola, monthly water quality analyses were performed at six fixed stations (Figure III-3) for a period of one year, beginning in July 1978. The six locations were divided into two groups and monitored alternately so that stations S1-S3 would be monitored one month and stations S4-S6 monitored the next. The results of the analyses are listed in Tables IV-1 to IV-3. Temperature measurements indicate little variation in lake water temperature between the three stations recorded on a specific date. Variations in temperature between stations were generally limited to  $1^{\circ}$  C or less. The highest temperature recorded during the test period was  $30.9^{\circ}$  C, occurring during August at stations S5 and S6. Lowest lake water temperature was measured at station S4 during February with a value of  $11.1^{\circ}$  C. Seechi disk measurements at stations S1, S2, S3 and S6 fluctuated little during the test period with recorded values between 1.0-1.3 meters. Stations S4 and S5 appeared to have consistently lower Seechi disk depths with values between 0.8 and 1.1 meters, the lowest values occurring during the period of September to December. Dissolved oxygen concentrations in the top 0.5 meters of the water column



TABLE IV-1  
 LIMNOLOGICAL PHYSICAL-CHEMICAL PARAMETERS FOR WATER SAMPLES  
 COLLECTED FROM TOP 0.5 METER IN LAKE EOLA

Date	Temp (°C)	Secchi Disk (m)	D.O. (mg/l)	pH	Turbidity (JTU)	Organic Carbon (ppm)	Inorganic Carbon (ppm)	NO <sub>3</sub> <sup>-</sup> -N (mg/l)	O.P. (mg/l)	BOD <sub>5</sub> (mg/l)	CHYL <sup>a</sup> (mg/m <sup>3</sup> )
Station #1											
7/10/78	---	---	---	---	3.5	7.2	13.7	0.10	0.005	---	31.4
7/25/78	29.6	---	7.5	8.89	6.0	8.7	15.9	0.10	0.040	3.9	19.4
8/28/78	---	1.3	---	8.86	5.5	10.8	17.6	---	---	2.1	9.0
11/08/78	---	1.1	---	8.84	5.0	---	---	---	0.024	---	22.8
1/05/79	14.9	1.2	9.4	8.55	4.5	---	---	---	0.011	---	31.3
3/13/79	18.2	1.0	10.3	8.61	5.1	4.5	21.8	0.36	0.021	---	30.2
4/30/79	23.7	1.3	11.6	9.15	3.8	14.5	38.6	0.24	0.013	---	19.0
7/10/79	28.8	1.1	8.8	8.93	4.7	5.1	16.7	0.33	0.021	---	16.2
Average	23.0		9.5	8.79	4.8	8.5	20.7	0.23	0.019	3.0	22.4
Station #2											
7/10/78	---	---	---	---	4.5	6.8	13.0	0.10	0.019	---	38.0
7/25/78	29.4	---	8.2	9.13	5.5	10.6	15.5	0.10	0.039	3.2	19.0
8/28/78	---	1.1	---	9.15	5.0	10.3	16.9	---	---	2.9	29.7
11/08/78	---	1.2	---	9.06	5.2	---	---	---	0.022	---	22.8
1/05/79	14.2	1.1	9.4	8.60	4.7	---	---	---	0.010	---	33.6
3/13/79	18.7	1.0	10.2	8.55	5.6	4.7	21.6	0.42	0.023	---	31.9
4/30/79	24.7	1.1	12.4	9.13	3.8	14.1	38.7	0.23	0.016	---	16.8
7/10/79	28.9	1.2	9.1	8.99	5.3	5.0	15.8	0.32	0.023	---	18.5
Average	23.2		9.9	8.87	5.0	8.6	20.3	0.23	0.022	3.1	26.3

TABLE IV-2  
 LIMNOLOGICAL PHYSICAL-CHEMICAL PARAMETERS FOR WATER SAMPLES  
 COLLECTED FROM TOP 0.5 METER IN LAKE EOLA

Date	Temp (°C)	Seechi Disk (m)	D.O. (mg/l)	pH	Turbidity (JTU)	Organic Carbon (ppm)	Inorganic Carbon (ppm)	NO <sub>3</sub> <sup>-</sup> -N (mg/l)	O.P. (mg/l)	BOD <sub>5</sub> (mg/l)	CHYL <sup>a</sup> (mg/m <sup>3</sup> )
Station #3											
7/10/78	---	---	---	---	4.1	8.4	13.7	0.10	0.015	---	41.4
7/25/78	29.0	---	8.7	9.04	5.8	7.7	15.5	0.10	0.039	4.0	24.6
8/28/78	---	1.0	---	9.09	5.6	10.3	16.9	---	---	4.6	20.1
11/08/78	---	1.1	---	9.11	5.8	---	---	---	0.025	---	24.3
1/05/79	13.9	1.2	9.3	8.72	4.7	---	---	---	0.012	---	35.8
3/13/79	18.0	1.0	10.7	8.66	5.9	4.7	21.6	0.51	0.023	---	35.2
4/30/79	23.9	1.2	10.6	9.12	3.8	13.1	40.6	0.22	0.028	---	16.8
7/10/79	29.0	1.1	9.0	9.03	4.7	6.1	15.8	0.30	0.023	---	19.0
Average	22.8		9.7	8.93	5.1	8.4	20.7	0.25	0.024	4.3	27.2
Station #4											
7/10/78	---	---	---	---	4.5	7.9	13.0	0.10	0.010	---	31.8
8/10/78	30.0	1.0	---	9.28	6.1	10.4	16.3	---	0.017	4.3	20.7
9/18/79	---	1.1	---	8.32	5.8	12.4	21.3	---	0.010	3.7	23.4
10/13/79	23.7	1.1	7.9	9.11	5.2	28.3	25.4	---	---	3.4	7.3
12/14/78	17.0	0.9	9.3	8.81	4.0	21.8	23.3	---	0.021	2.1	36.4
2/10/79	11.1	---	10.8	8.55	5.1	3.0	22.6	0.42	0.038	---	29.7
4/06/79	21.1	1.1	10.0	9.32	6.2	9.1	20.5	0.25	0.013	---	19.0
6/20/79	27.8	---	12.3	9.25	6.4	10.8	14.5	0.26	0.034	---	28.3
Average	21.8		10.1	8.78	5.4	13.0	19.6	0.26	0.020	3.4	24.8

fluctuated somewhat over the test period with values measured at individual stations between 7.5 and 12.6 mg/l. The averages of dissolved oxygen concentrations measured in Lake Eola on specific sampling dates are shown in Figure IV-1. A dissolved oxygen concentration of 8.2 mg/l was measured in July 1978 with a steady increase to 10.4 mg/l in March 1979. A decrease in dissolved oxygen concentration to 9.7 mg/l occurred during April 1978 but increased after that date, reaching a maximum value for the period tested of 12.2 mg/l in late June. Typical dissolved oxygen profiles for shallow, middle, and deep areas of Lake Eola are shown in Figure IV-2 and are listed in Tables IV-4 to IV-6. A slight decrease in dissolved oxygen concentration with increasing depth was noted in shallow regions of the lake during the summer months. This decrease was equivalent to approximately 1-2 mg/l over a depth of 1.5-2.0 meters. Temperature decreased 2-3<sup>0</sup> C over the same interval. In deeper portions of the lake (middle), oxygen concentrations decreased 2-3 mg/l over a 4 meter depth. Temperature decreased approximately 2-3<sup>0</sup> C over this depth with the surface temperature near 29.0<sup>0</sup> C. In the deepest regions of the lake, the oxygen concentration dropped rapidly, reaching 0.2 mg/l at a depth of 4.0 meters. In some instances, temperature decreased only about 3<sup>0</sup> C over the first 4.0 meters, but decreased another 4<sup>0</sup> C from 4.0 meters to the bottom at 6.5 meters. During the fall months, typical dissolved oxygen concentrations in shallow areas showed a decline with depth similar to

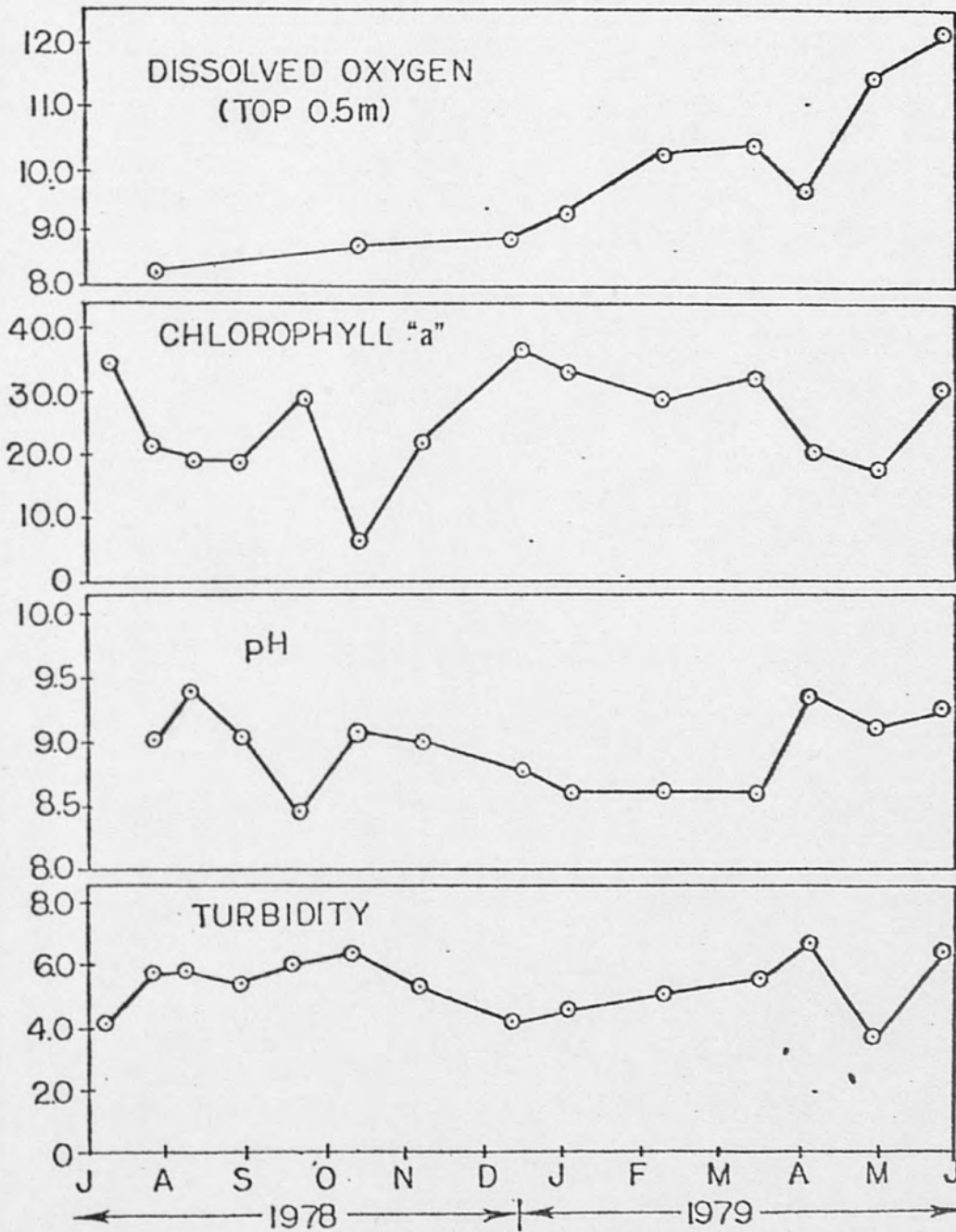


Fig. IV-1. Average physical chemical parameters of water samples collected from the top 0.5 m in Lake Eola.



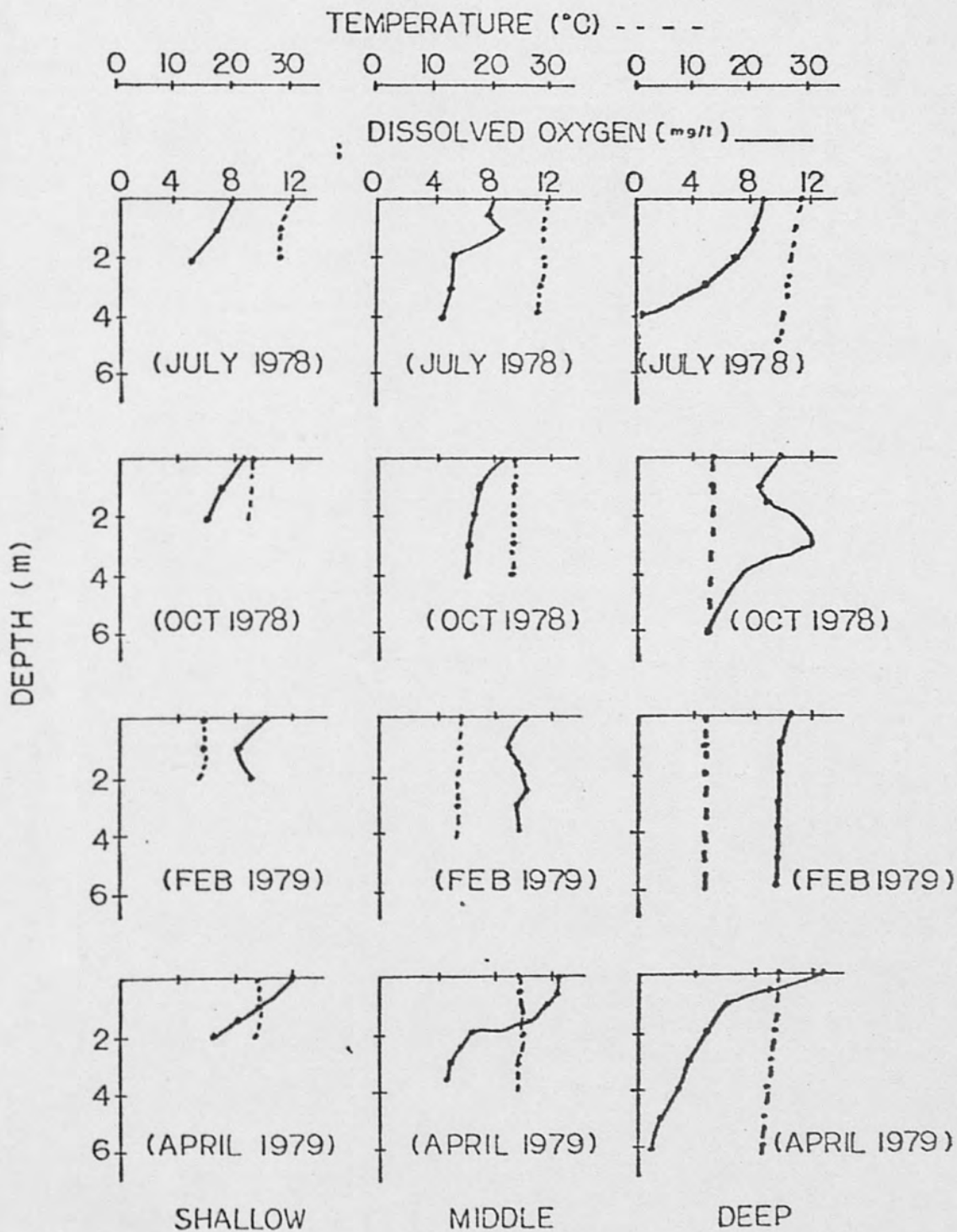


Fig. IV-2. Typical dissolved oxygen and temperature profiles by season and location in Lake Eola.

TABLE IV-4

TYPICAL DISSOLVED OXYGEN AND TEMPERATURE  
PROFILES IN LAKE EOLA FOR STATIONS S<sub>1</sub> AND S<sub>2</sub>

DEPTH (m)	DATE							
	7-25-78		1-5-79		4-30-79		7-10-79	
	Temp (°C)	D.O.	Temp (°C)	D.O.	Temp (°C)	D.O.	Temp (°C)	D.O.
Station 1:								
0.0	---	---	14.9	10.2	23.6	12.0	29.0	9.1
0.5	30.3	7.9	14.9	8.5	23.8	11.2	28.5	8.5
1.0	28.8	7.0	14.8	8.3	23.8	9.4	28.3	8.3
1.5	28.5	5.4	14.5	9.3	23.4	8.2	28.1	7.9
2.0	28.1	5.3	14.2	9.4	23.2	6.8	28.0	7.7
2.4 (B)	----	---	14.0	9.0	23.0	6.8	28.0	8.3
Station 2:								
0.0	----	---	14.2	10.2	24.6	12.2	28.9	9.1
0.5	29.8	7.9	14.2	8.6	24.8	12.5	28.9	9.0
1.0	29.0	8.5	14.2	8.9	24.7	11.5	28.9	8.9
1.5	----	---	14.0	9.6	24.7	10.6	28.3	8.5
2.0	28.1	5.3	13.8	9.9	24.3	6.3	28.2	8.4
2.5	----	---	13.7	10.2	23.8	5.2	28.0	8.1
3.0	28.0	5.3	13.5	9.5	23.7	4.8	27.9	6.9
3.5	----	---	13.5	9.3	23.2	4.2	27.9	4.9
4.0 (B)			13.5	8.6	----	---	----	----

TABLE IV-5

TYPICAL DISSOLVED OXYGEN AND TEMPERATURE  
PROFILES IN LAKE EOLA FOR STATION S<sub>3</sub>

DEPTH (m)	DATE							
	7-25-78		2-10-79		4-6-79		7-10-79	
	Temp (°C)	D.O.	Temp (°C)	D.O.	Temp (°C)	D.O.	Temp (°C)	D.O.
Station 3:								
0.0	---	---	12.0	10.4	21.0	10.8	29.0	9.1
0.5	29.3	8.8	12.0	10.1	21.1	9.6	29.0	8.9
1.0	28.7	8.6	12.0	9.9	21.1	8.0	28.8	8.2
1.5	---	---	12.0	10.0	21.0	7.6	28.5	7.8
2.0	28.2	7.2	12.0	9.6	20.9	6.8	28.2	7.5
2.5	---	---	11.9	9.6	20.8	6.5	28.0	7.4
3.0	28.0	4.9	11.8	9.3	20.8	6.1	27.9	6.4
3.5	---	---	11.8	9.1	20.8	6.0	27.8	4.5
4.0	27.0	0.2	11.8	9.1	20.0	5.3	27.6	1.2
4.5	---	---	11.8	9.1	19.3	2.9	27.2	0.3
5.0	24.9	0.1	11.8	9.1	19.0	2.2	27.0	0.4
5.5	---	---	11.8	9.0	18.8	1.3	26.8	0.3
6.0	22.8	0.1	11.8	8.7	18.5	0.8	26.5	0.2
6.5 (B)	---	---	11.7	---	18.0	0.6	26.2	0.2

TABLE IV-6

TYPICAL DISSOLVED OXYGEN AND TEMPERATURE  
PROFILES IN LAKE EOLA FOR STATIONS S<sub>4</sub>, S<sub>5</sub>, AND S<sub>6</sub>

DEPTH (m)	DATE							
	10-13-78		12-14-78		4-6-79		6-20-79	
	Temp (°C)	D.O.	Temp (°C)	D.O.	Temp (°C)	D.O.	Temp (°C)	D.O.
Station 4:								
0.0	23.6	8.7	17.0	9.3	21.0	10.2	27.8	12.0
0.5	----	---	17.0	9.2	21.1	9.7	27.8	12.5
1.0	23.8	7.1	17.0	9.2	21.1	8.1	27.3	10.0
1.5	----	---	----	---	21.0	7.2	27.0	8.6
2.0	23.7	6.9	17.0	9.3	20.8	6.7	26.8	5.9
2.5 (B)	23.6	6.4	17.0	9.3	20.6	6.3	26.7	5.1
Station 5:								
0.0	23.6	8.7	17.5	9.3	21.2	9.5	27.2	12.2
0.5	----	---	17.5	8.9	21.2	9.5	27.2	13.0
1.0	23.5	7.1	17.5	8.5	21.2	7.9	27.2	11.8
1.3 (B)	23.7	6.3	17.5	8.5	21.2	7.3	27.1	7.3
Station 6:								
0.0	23.8	8.7	17.0	8.4	21.1	10.4	27.4	12.2
0.5	----	---	17.0	8.4	21.2	8.0	27.5	12.9
1.0	23.9	7.4	17.0	8.4	21.2	6.7	27.6	11.6
1.5	----	---	----	---	21.2	5.9	27.6	11.2
2.0	23.8	7.7	17.0	8.1	21.2	5.6	27.3	10.8
2.5	----	---	----	---	21.2	5.4	27.0	4.4
3.0	23.7	8.0	17.0	8.0	21.2	5.2	26.7	3.3
3.5	23.7	7.9	----	---	20.5	4.7	26.3	2.7
4.0 (B)	----	---	----	---	20.3	4.4	25.4	2.1



that found during the summer. Average differences of 3-4 mg/l were found between the water surface and lake bottom. Temperature during this period was virtually isocline at all locations. Average lake surface temperature during this period was near 23<sup>0</sup> C. In the middle areas of the lake, a similar decline in oxygen concentration was noted with a 3-4 mg/l difference between the water surface and the sediments. The deep area of the lake experienced a slight decline in oxygen concentration from the surface to 1.0 m, after which dissolved oxygen increased to a depth of approximately 4.0 m and then decreased rapidly to the bottom. During the winter months, temperature was practically isocline with less than 1<sup>0</sup> C drop between the surface and the bottom. Surface temperature at this time was approximately 14<sup>0</sup> C. A similar isocline condition was found in dissolved oxygen in all areas of the lake with a decrease of only 1-2 mg/l between the top and bottom at all stations. In the spring both temperature and dissolved oxygen showed rapid increase in surface values. Average surface temperature was 23<sup>0</sup> C at this time with isocline conditions in the shallow and middle regions and a 2-3<sup>0</sup> C decrease between top and bottom temperatures in the deepest sections. Dissolved oxygen concentrations decreased rapidly with increasing depth in all sections of the lake, approaching 5 mg/l at a depth of 2.0 m and 0.5 mg/l at 4.5 m. The average percent saturations of dissolved oxygen in the top 0.5 m of Lake Eola are listed in Table IV-7. Average oxygen concentrations were

TABLE IV-7

AVERAGE TEMPERATURE, DISSOLVED OXYGEN AND  
PERCENT SATURATION OF DISSOLVED  
OXYGEN IN THE TOP 0.5m OF LAKE EOLA

Date	Temperature (°C)	Dissolved Oxygen (mg/l)	% Saturation
7/25/78	29.3	8.1	107.0
10/13/78	23.7	8.0	96.1
12/14/78	17.3	8.9	96.0
1/05/79	14.3	9.4	94.6
2/10/79	11.7	10.3	98.0
3/13/79	18.3	10.4	113.9
4/06/79	21.2	9.6	110.5
4/30/79	24.1	11.5	140.0
6/20/79	27.5	12.5	160.2
7/10/79	28.9	9.0	117.2

above saturation in the fall and winter. The highest saturation of oxygen occurred between March and June.

Average pH values for the six fixed stations (Tables IV-1 to IV-3) were very similar, ranging from 8.79 to 8.93. The highest pH recorded in the lake was 9.45 at station S5 in March 1979. The lowest value was 8.32, occurring at station S4 in September 1978. Average pH values in Lake Eola were typically above 9.0 during the spring and summer months and below this value during the fall and winter (Figure IV-1).

The turbidity at all stations in Lake Eola was relatively low with average values for the six stations between 4.8 and 5.9 and extreme values between 3.5 and 7.2. No significant differences were noted in turbidity measurements between the six stations. Average turbidity measurements appeared to be highest in the summer and spring and lowest in the fall and winter (Figure IV-1).

Concentrations of chlorophyll "a" were extremely varied over the test period. Extreme values measured at individual stations ranged between 6.2  $\mu\text{g/l}$  during October at station S6 to 41.4  $\mu\text{g/l}$  in July at S3. Average values for each location during the test period were very close, however, average values ranged from 22.4  $\mu\text{g/l}$  at S1 to 27.2  $\mu\text{g/l}$  at S3.

Organic carbon concentrations in Lake Eola also varied widely over the test period. Average lake concentrations were highest in the fall and late spring with a maximum value of 28.4 ppm reached in October (Figure IV-3). The lowest values were measured

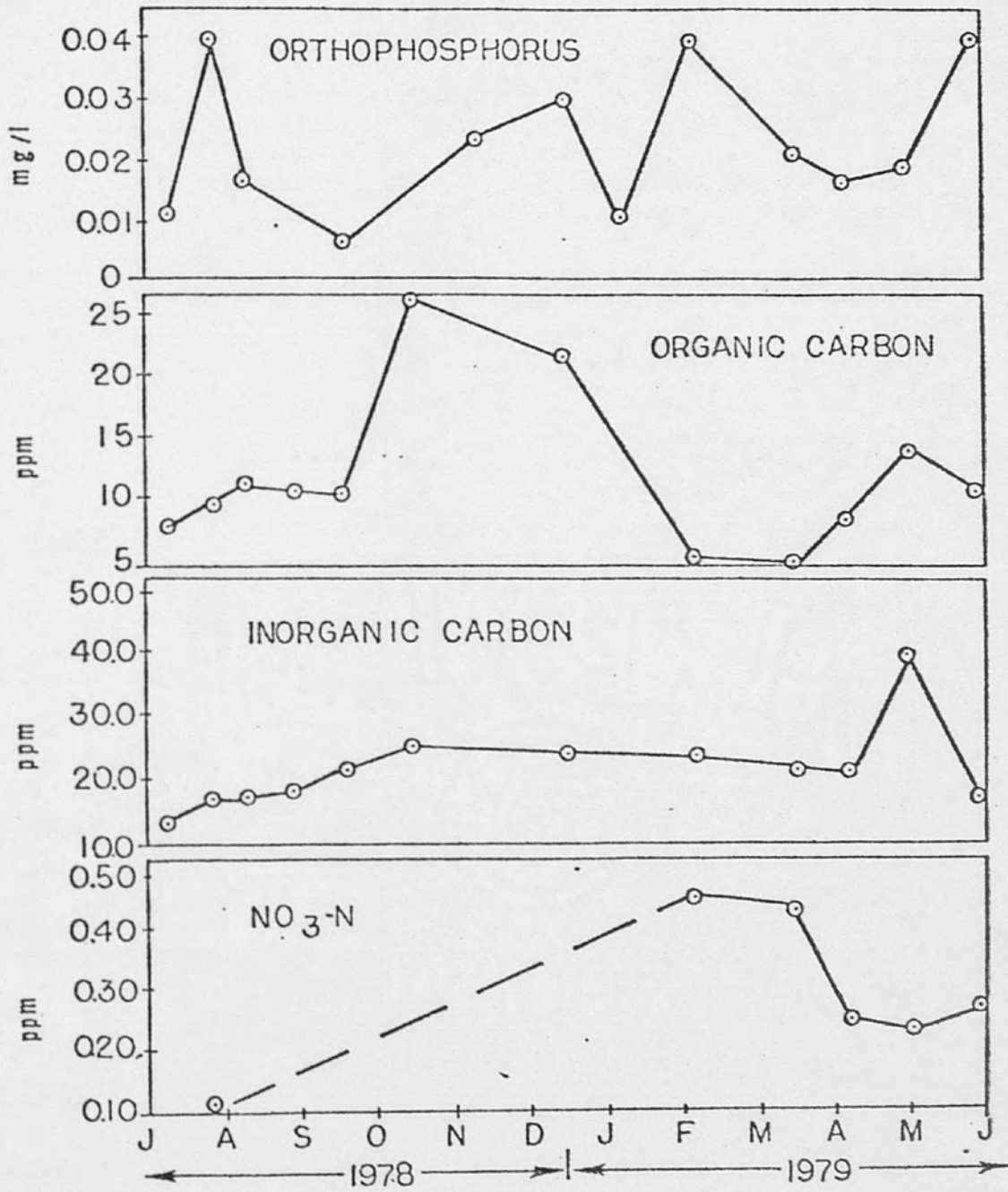


Fig. IV-3. Average Physical Chemical Parameters of water samples collected from the top 0.5 m in Lake Eola.



during winter and early spring with a minimum value of 4.6 ppm occurring in March 1979. Average values at individual sampling locations for the period studied seemed to indicate higher concentrations at locations S4, S5, and S6, than were found at S1, S2 and S3. The average concentration found at S4-S6, all located on the east end of the lake, was 12.8 ppm. Concentrations on the west end of the lake at stations S1-S3 were considerably lower with an average of 8.5 ppm.

Inorganic carbon concentrations in Lake Eola were also somewhat varied over the test period. Values ranged from a low of 12.3 ppm during July 1978 at station S5 to 40.6 ppm at station S3 in April. Concentrations of inorganic carbon appeared to be slightly lower at stations S4-S6 with a combined average of 19.4 ppm than at stations S1-S3 which had a combined average of 20.6 ppm.

Concentrations of nitrate nitrogen fluctuated mildly over the test period with the range of fluctuation between 0.10 and 0.52 mg/l. Average nitrate concentrations at individual stations were very close, however, ranging between 0.23 and 0.28 mg/l. Although data for this parameter is limited, it appears that average lake concentrations of nitrate were highest during the winter months with the lowest measured values occurring in July 1978 (Figure IV-3).

Orthophosphorus concentrations at individual stations fluctuated greatly over the test period. A maximum value of 0.041 mg/l was measured at stations S5 and S6 in February with a minimum of 0.010 mg/l occurring at various stations several times during the test period. Average orthophosphorus concentrations at each of the stations were very close. Values ranged from 0.019 mg/l at S1 to 0.024 mg/l at S3 and S6. Orthophosphorus concentrations appeared to be lowest during the late summer, early fall and early spring with maximum values occurring in early summer and winter (Figure IV-3).

Although the data is very limited, concentrations of  $BOD_5$  seemed to be very low. Average  $BOD_5$  at individual stations ranged from 3.0 to 4.3 mg/l.

Total metal concentrations measured in composite water samples collected in Lake Eola are listed in Table IV-8. No apparent trend is observed from the data presented, and metal concentrations seem to remain within a narrow range oscillating around an average value.

TABLE IV-8

TOTAL METAL CONCENTRATIONS IN COMPOSITE WATER SAMPLES  
COLLECTED FROM THE TOP 0.5 m IN LAKE EOLA

Date	Metal Concentration (mg/l)									
	Al	Fe	Pb	Cr	Zn	Cd	As	Ni	Cu	Ca
2/10/79	0.173	0.138	0.084	0.025	0.039	0.002	0.029	0.008	0.033	----
3/13/79	0.134	0.141	0.077	0.029	0.054	0.002	0.045	0.009	0.060	----
4/06/79	0.384	0.136	0.063	0.035	0.025	0.000	0.116	0.017	0.053	----
4/30/79	0.235	0.121	0.069	0.020	0.015	0.000	0.100	0.005	0.041	----
6/20/79	0.236	0.045	0.036	0.030	0.020	0.000	0.000	0.006	0.046	21.3
7/10/79	0.226	0.197	0.083	0.032	0.055	0.001	-----	0.005	0.057	25.1
Average	0.231	0.130	0.069	0.029	0.035	0.001	0.058	0.008	0.048	23.2

## CHAPTER V

### BIOASSAY EXPERIMENTATION

Numerous bioassay experiments were conducted to evaluate algal responses to nutrient changes in Lake Eola water and to study the impact of untreated and coagulated stormwater runoff on algal production. In this research, four different types of sample preparations were used: raw unfiltered lake water, water filtered through a 0.45 micron Millipore filter (F), water which was filtered then autoclaved (F/A), and water which was autoclaved and then filtered (A/F). In addition to indigenous algal species from Lake Eola, two different laboratory cultured test species were also utilized, Chlorella pyrenodosa and Selenastrum capricornutum Printz.

#### Uptake of Phosphorus by Actively Growing Algal Cells

The growth response of Selenastrum in a synthetic algal medium under constant illumination is shown in Figure V-1. Cell dry weight of Selenastrum in the culture increased exponentially with the maximum yield of approximately 120 mg/l of dry cell weight being achieved after 14 days. On the other hand, phosphorus concentrations in the algal cells, expressed in milligrams of phosphorus per gram of cell dry weight, decreased in an expo-



TABLE V-1

WATER QUALITY CHARACTERISTICS FOR LAKE WATER USED  
IN ALGAL BIOASSAY EXPERIMENTS FOR LAKE EOLA

Date and Treatment	pH	Turbidity (JTU)	Organic Carbon (ppm)	Inorganic Carbon (ppm)	NO <sub>3</sub> -N (ppm)	PO <sub>4</sub> -P (ppm)
Lake Water: 8/10/78 Composite F	9.34	5.9	11.8	13.8	----	0.017
10/16/78 Composite F/A	9.03	6.2	28.8	23.1	----	----
11/8/78 Composite F	8.94	5.3	----	----	----	0.022
12/14/78 Composite F	8.76	4.1	----	----	----	0.025
1/14/79 Composite F/A	8.37	0.1	----	22.0	----	0.011
2/21/79 Composite A/F	8.26	0.0	5.6	12.8	1.25	0.058
3/18/79 Composite A/F	8.86	0.0	1.7	15.3	0.54	0.021
Composite F/A	8.39	0.1	3.0	14.9	0.58	0.010
4/30/79 Composite	9.10	3.8	13.2	40.7	0.23	0.016
7/17/79 Composite	9.00	4.8	4.9	16.7	0.31	0.024

ponential fashion with increasing incubation period. Phosphorus concentration decreased from 18.1 mg of phosphorus per gram of cell mass on the second day to 6.8 mg of phosphorus per gram of cells on the fourth day. This concentration leveled off around 2.6 mg of phosphorus per gram of cell mass on approximately the 14th day, with only minor fluctuations noted around that value until the experiment was terminated on day 19.

#### Algal Responses to Nutrient Changes in Lake Eola Water

Several bioassay experiments were conducted to evaluate algal responses to nutrient changes in Lake Eola water. Water quality characteristics of the lake water used in these experiments are listed in Table V-1.

The results of a bioassay experiment conducted during August 1978 using Chlorella as the test species with varying concentrations of nitrogen and phosphorus are listed in Table V-2. The maximum yield obtained in this experiment (1.63 mg/l dry cell weight) was achieved after 11 days and occurred in the flasks to which a nitrogen spike of 1.0 mg/l nitrate nitrogen had been added. This yield represented an increase of 117% over the control flasks. The addition of nitrogen spikes in concentrations of 3.0 mg/l and 5.0 mg/l  $\text{NO}_3\text{-N}$  resulted in slightly lower maximum yields of 1.41 mg/l and 1.55 mg/l, respectively. The addition of phosphorus to lake water resulted in maximum yields which occurred on the 6th day of incubation for all concentrations of phosphorus spikes tested. These yields, however, were only slightly

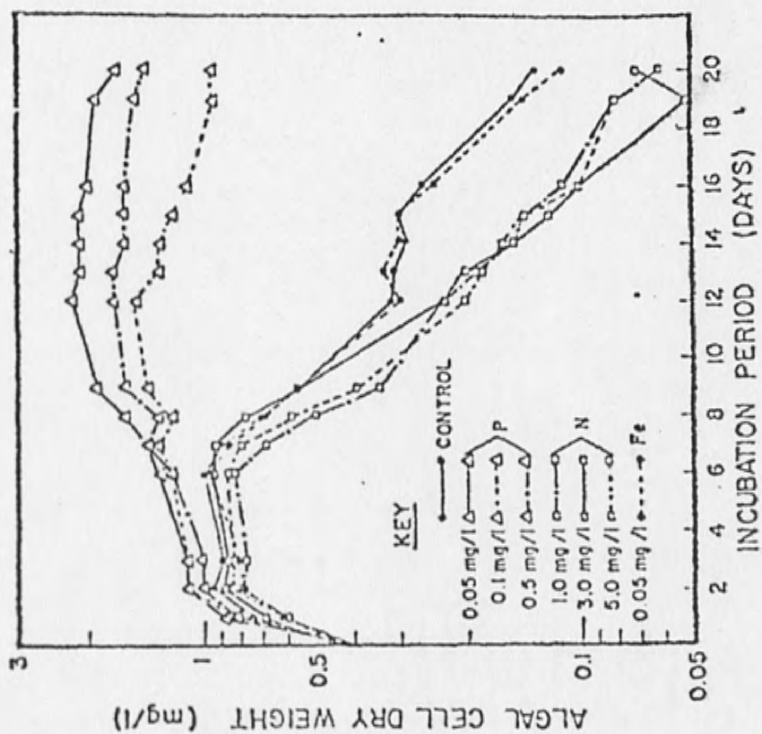


Fig. V-2. Responses of *Selenastrum* to various nutrients added to Lake Eola water samples collected 1/14/79. (Lake water filtered then autoclaved).

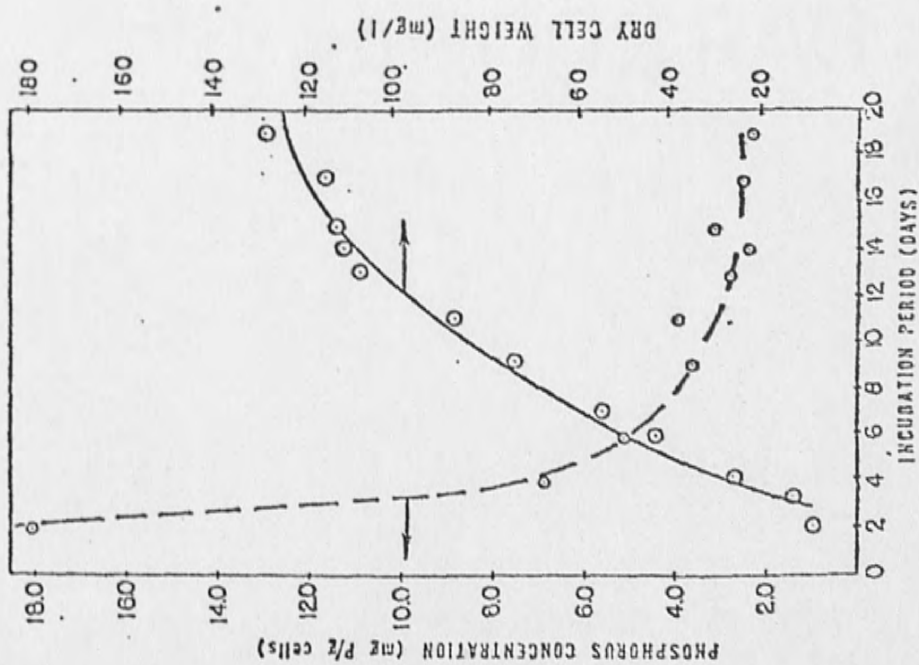


Fig. V-1. Phosphorus concentrations of *Selenastrum Capricornutum* as a function of incubation period.

TABLE V-2

GROWTH RESPONSES OF CHLORELLA TO VARIOUS  
CONCENTRATIONS OF NITROGEN AND PHOSPHORUS ADDED  
TO FILTERED LAKE EOLA WATER COLLECTED 8/10/78

Concentration of Nutrient Added to Lake Water	Average Cell Dry Weight (mg/l)						
	Initial	1 Day	3 Days	6 Days	8 Days	11 Days	12 Days
Lake Water Control	0.51	0.36	0.43	0.75	0.75	0.64	0.53
0.1 mg/l P	0.54	0.38	0.65	0.83	0.76	0.85	0.80
0.5 mg/l P	0.50	0.38	0.68	0.94	0.79	0.77	0.79
1.0 mg/l P	0.49	0.37	0.69	1.01	0.76	0.83	0.80
1.0 mg/l N	0.50	0.37	0.54	1.22	1.39	1.63	1.50
3.0 mg/l N	0.48	0.38	0.61	1.24	1.35	1.41	1.39
5.0 mg/l N	0.51	0.39	0.59	1.13	1.37	1.55	1.39



greater than the control with increases of 11%, 25%, and 35% for phosphorus spikes of 0.1 mg/l, 0.5 mg/l and 1.0 mg/l, respectively. The pH of the lake water used in this experiment was 9.34 which was the highest pH value found in a lake water sample used for bioassay experiments. Concentrations of orthophosphorus (0.017 mg/l) and inorganic carbon (13.8 ppm) were both lower in the water used in this bioassay than average values measured in Lake Eola.

The results of a bioassay performed with lake water samples collected during January 1979 are shown in Figure V-2. Selenastrum was used as the test organism. The greatest biomass yields in this experiment were obtained with a phosphorus spike of 0.05 mg/l P which was the smallest concentration of the phosphorus spikes tested. This nutrient addition resulted in a maximum yield of 2.38 mg/l of dry well weight after 12 days, representing an increase of 136% over the yield obtained in the control flasks. The addition of 0.5 mg/l P produced a yield of 1.80 mg/l dry cell weight after 12 days, an increase of 78% over the control. The addition of 0.1 mg/l P resulted in the lowest growth response of the phosphorus spikes tested. Maximum cell yield for this addition was 1.54 mg/l, occurring also on the 12th day, and representing an increase of 52% over the control. As seen in Figure V-2, the maximum yield in the control flasks occurred on day 6. However, after achieving this yield, the cell dry weight in the control flasks dropped off rapidly until on day 20, which was the

last day measurements were recorded, the concentration of cell dry weight had decreased by 88% to 0.12 mg/l. Flasks to which phosphorus had been added did not show this rapid decline, and instead, seemed to fluctuate within  $\pm 15\%$  of their maximum yields until the experiment was terminated. The addition of various concentrations of nitrogen to this lake water resulted in yields which were equal to or less than the control. A nutrient spike of 1.0 mg/l  $\text{NO}_3\text{-N}$  produced a maximum yield of 0.76 mg/l occurring on the 6th day, a decrease of 25% from the control value. The highest yield of the nitrogen spikes was obtained on the 6th day with an addition of 3.0 mg/l  $\text{NO}_3\text{-N}$ . Calculated cell dry weight for this addition was 0.90 mg/l which was a decrease of 11% from the control. The addition of a 5.0 mg/l nitrate nitrogen spike resulted in a somewhat lower maximum yield than did the addition of 3.0 mg/l  $\text{NO}_3\text{-N}$ . Maximum cell dry weight of 0.81 mg/l was achieved on day 6, representing a decrease of 20% from the control. It is interesting to note that, although the nitrogen spikes all produced a maximum yield on the 6th day which was near the control value, concentrations of cell dry weight in these flasks decreased after that time to lower values than did the control. After 20 days of incubation, the average cell mass concentrations in the nitrogen spiked flasks were only about 2% of the maximum yield they had obtained. The addition of an iron spike at a concentration of 0.05 mg/l produced a growth response which was virtually indistinguishable from the control. Maximum

yield was obtained on day 6 and was within 9% of the control. The pH of the filtered and autoclaved lake water used in this experiment was 8.37. The concentration of inorganic carbon was 22.0 ppm with an orthophosphorus concentration of 0.011 mg/l.

The results of an additional bioassay with Selenastrum as the test species using autoclaved then filtered lake water collected during February 1979 are shown in Figure V-3. The highest growth yields were obtained in this water using nitrogen spikes. Maximum yield was obtained after 18 days with a nitrate nitrogen spike of 3.0 mg/l which produced a cell dry weight of 21.0 mg/l, 108% higher than the control flasks. The addition of a 1.0 mg/l  $\text{NO}_3\text{-N}$  spike produced a yield in 15 days of 20.2 mg/l dry cell weight which was 100% greater than the control flasks and 4% less than the maximum yield for this experiment. A slightly lower yield was obtained after 18 days with a nitrogen spike of 5.0 mg/l (17.1 mg/l dry cell weight), but this yield was still 69% greater than the control and only 19% less than the maximum yield. The addition of phosphorus spikes to this lake water resulted in yields which were considerably less than the control. Maximum yield from a phosphorus spike was obtained after 10 days with a phosphorus addition of 0.05 mg/l. This corresponds to a total orthophosphorus concentration, including phosphorus background in the lake water, of 0.11 mg/l. The calculated cell yield obtained at this concentration was 6.9 mg/l which was 32% less than the control. The addition of phosphorus spikes in concentrations of

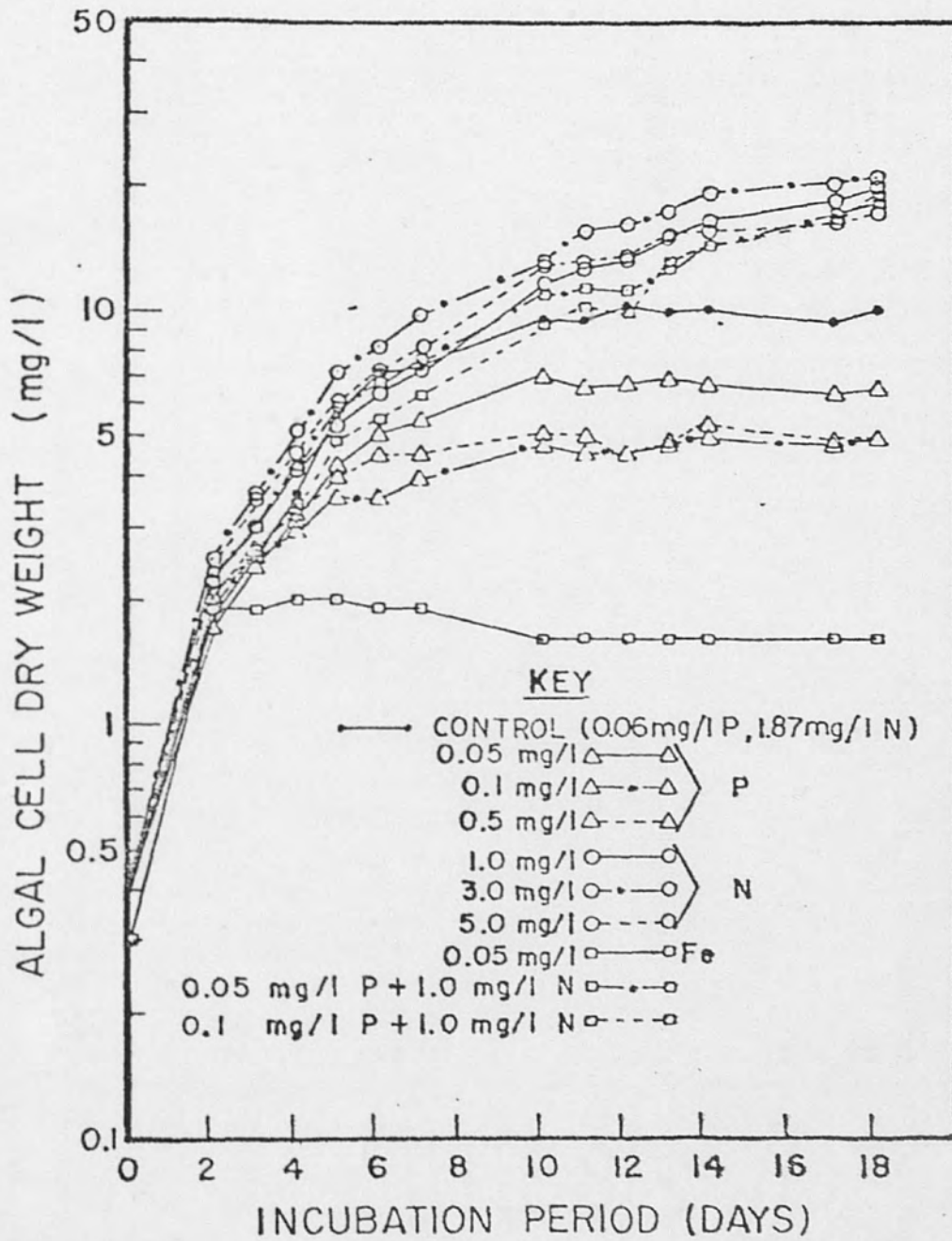


Fig. V-3. Responses of *Selenastrum* to various nutrients added to Lake Eola water samples collected 2/21/79. (Lake water autoclaved then filtered).



0.1 and 0.5 mg/l produced yields after 14 days of 4.9 and 5.3 mg/l dry cell weight. Total orthophosphorus concentrations in these flasks were 0.16 and 0.56 mg/l, respectively, including background concentration. These yields represented decreases of 51% and 48% of the control values for phosphorus spikes of 0.1 mg/l and 0.5 mg/l. The addition of a 0.05 mg/l iron spike produced the lowest yield of any nutrient tested in this assay. Maximum yield for this nutrient was obtained after 4 days at 2.0 mg/l dry cell weight which was only 20% of the control value. The addition of nitrogen and phosphorus spikes to this lake water in combination increased production greatly. When a spike of 0.05 mg/l P plus 1.0 mg/l N was added, maximum yield was obtained in 18 days with a final cell dry weight of 16.4 mg/l. This value represents an increase of 82% over the control and is only 9% less than the maximum yield obtained in this experiment, which occurred with a nutrient spike of 1.0 mg/l N. Similar results were obtained with a nutrient addition of 0.1 mg/l P and 1.0 mg/l N. Maximum yield obtained with this addition was 19.0 mg/l dry cell weight after 18 days, which is an increase of 89% from the control and only 6% less than maximum yield for this experiment. The pH of the autoclaved then filtered lake water used in this bioassay (Table V-1) was 8.26 which was somewhat lower than average pH values found in Lake Eola. Concentrations of organic carbon and inorganic carbon were also lower than average in situ lake values. The concentration of nitrate nitrogen in this water,

1.25 mg/l, was over twice as high as any other lake bioassay water used. Also, the orthophosphorus concentration of 0.058 mg/l was the highest background value measured in Lake Eola water used for bioassay experiments. Analysis of total metal concentrations in this water (Table V-3) indicate higher than average concentrations for water used in bioassay experiments of aluminum, lead, zinc, cadmium, nickel and copper.

The addition of EDTA to this same lake water collected in February 1979 was also tested, and the results are shown in Figure V-4. The addition of EDTA to the lake water control produced a maximum yield of 3.6 mg/l dry cell weight after 7 days. This represents a decrease of 64% from the lake water control without EDTA (Figure V-4). The largest maximum yield was obtained, as in the experiment without EDTA, with a nitrogen spike. The addition of EDTA, however, greatly increased the yield obtained in nitrogen spiked flasks. The largest maximum yield with EDTA occurred at a nitrogen spike of 5.0 mg/l compared to the maximum yield without EDTA, which was obtained with a 3.0 mg/l N addition. The yield obtained with the 5.0 mg/l spike + EDTA was 39.5 mg/l dry cell weight after 18 days. This corresponds to an increase of 88% over the largest maximum yield obtained in the same lake water (at a concentration of 3.0 mg/l) without EDTA. The addition of 1.0 mg/l and 3.0 mg/l N spikes resulted in very similar maximum yields of 33.8 mg/l and 33.4 mg/l after 14 and 18 days, respectively. These values were only 14% and 15% less than



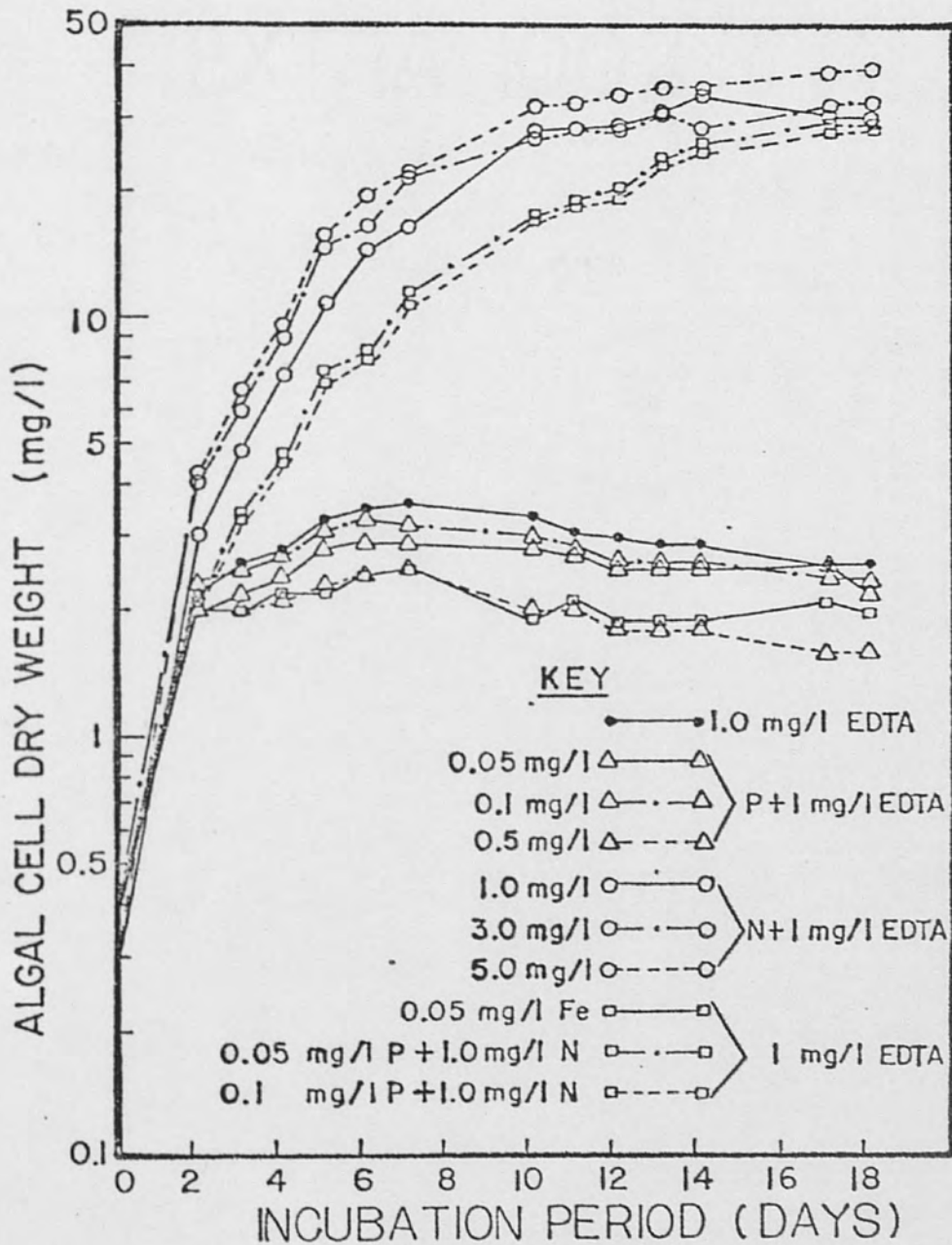


Fig. V-4. Responses of *Selenastrum* to various nutrients added to Lake Eola samples collected 2/21/79. (Lake water autoclaved then filtered).



the maximum yield obtained with a nitrogen spike. The addition of EDTA to flasks containing phosphorus spikes seemed to reduce maximum yields from the values obtained in flasks without EDTA. Maximum yield for a phosphorus spike was obtained at an orthophosphorus addition of 0.1 mg/l (0.16 mg/l total orthophosphorus, including background) after 6 days with a dry cell weight of 3.3 mg/l. This value is 8% less than the control + EDTA and 33% less than the same orthophosphorus concentration without EDTA. The addition of 0.05 mg/l and 0.5 mg/l phosphorus spikes produced similar yields of 2.9 mg/l and 2.4 mg/l of cell dry weight after 6 days. These values represent decreases of 58% and 55% of the yields obtained without EDTA. The addition of EDTA to an iron spike of 0.05 mg/l increased maximum yield from 2.0 mg/l dry cell weight in the flasks without EDTA to 2.5 mg/l dry cell weight with EDTA. The incubation period needed to reach this maximum yield was increased, however, from 4 days without EDTA to 7 days with EDTA. When EDTA was added to flasks containing combination spikes of nitrogen and phosphorus, increases in maximum yields were obtained. Maximum yield increased in flasks containing additions of 0.05 mg/l P + 1.0 mg/l N from 18.4 mg/l dry cell weight without EDTA to 29.3 mg/l with EDTA, an increase of 59%. Both of these yields occurred after 18 days. A nutrient spike of 0.1 mg/l P + 1.0 mg/l N produced a dry cell weight after 18 days of 28.3 mg/l which was an increase of 49% over the same flasks without EDTA. It is interesting to note that both additions of

nitrogen and phosphorus in combination resulted in yields which were slightly less than the yield obtained from the addition of the nitrogen spike alone.

A final limiting nutrient study was conducted with Selenas-trum as a test organism using filtered then autoclaved lake water collected in March 1979. Largest maximum yield was obtained with a phosphorus spike of 0.1 mg/l (Figure V-5). This produced a maximum yield of 4.7 mg/l dry cell weight after 17 days, an increase of 124% over the control. The addition of phosphorus at a concentration of 0.5 mg/l resulted in a somewhat lower yield of 3.2 mg/l dry cell weight after 8 days. This represented an increase of 52% over the control and a decrease of 32% from the highest maximum yield. The addition of nitrogen spikes of 3.0 mg/l and 5.0 mg/l resulted in yields of 2.3 mg/l and 2.2 mg/l dry cell weight. These values are very close to that observed in the control flasks. The pH of the composite lake water used in this experiment was 8.39 (Table V-1) which was somewhat lower than average lake water values found in Lake Eola at this time of the year. The concentration of inorganic carbon (16.7 ppm) was about average for water used in bioassay experiments. Background concentration of orthophosphorus in this water, 0.01 mg/l, was much lower than average in situ lake values, while the nitrate nitrogen concentration of 0.058 mg/l was slightly higher than average. Total metal concentrations in this filtered and autoclaved lake water were about average for water used in bioassays (Table V-3).

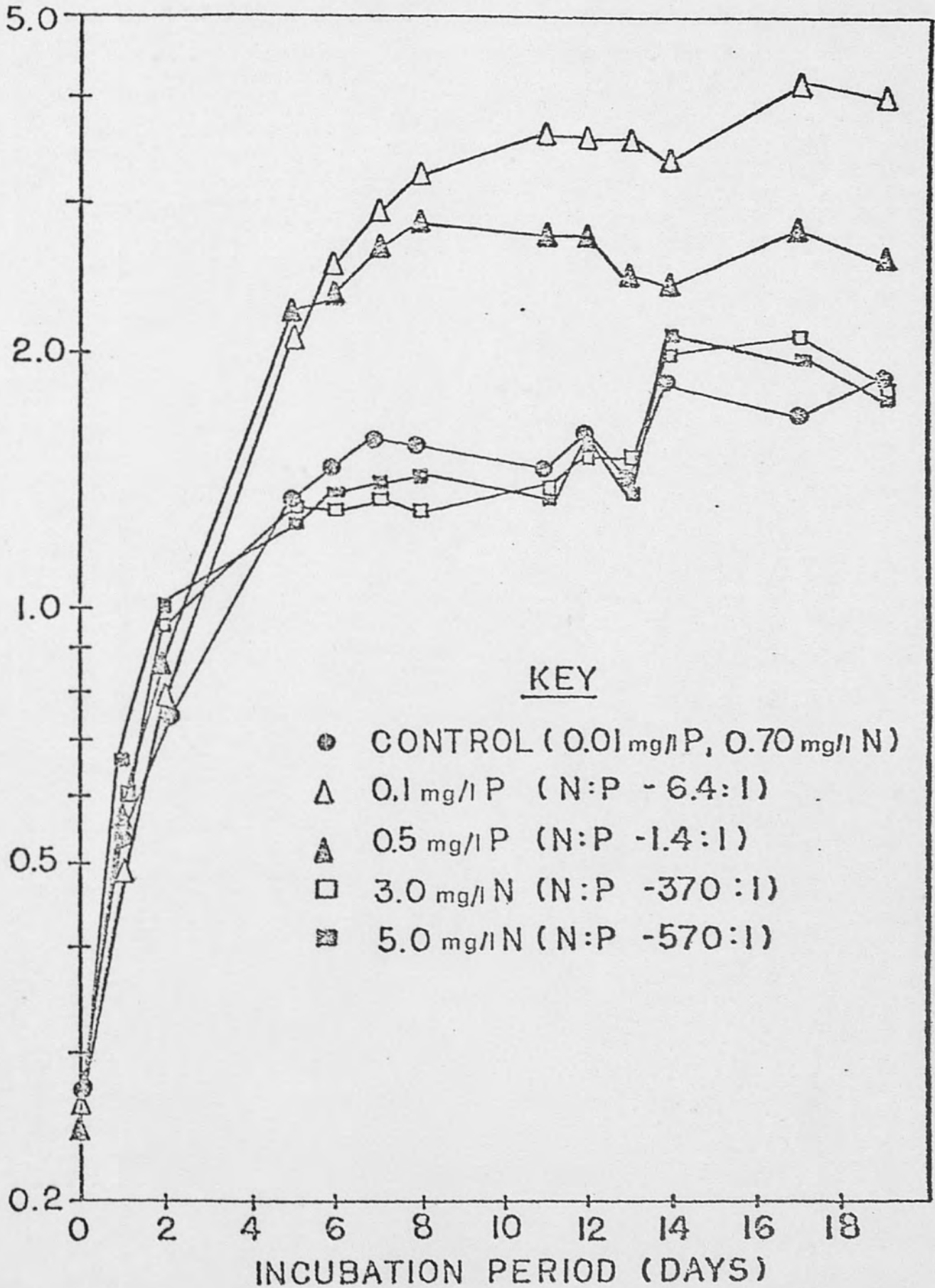


Fig. V-5. Responses of *Selenastrum* to various nutrients added to Lake Eola water samples collected 3/18/79. (Lake water filtered then autoclaved).

### Nitrogen to Phosphorus Ratio Experiments

A series of experiments were conducted with Selenastrum as the test organism using Lake Eola water collected March 18, 1979 to determine the effect of nitrogen to phosphorus ratios on algal productivity. The results of an experiment using autoclaved then filtered lake water are shown in Figure V-6. The highest maximum yield was obtained at an N:P ratio of 11.4:1. The yield obtained at this ratio was 86.0 mg/l dry cell weight after 19 days. An N:P ratio of 3.4:1 resulted in a maximum yield of 25.1 mg/l dry cell weight, 29% of the largest maximum yield. When the ratio was increased to 7.4:1, the yield increased to 76.7 mg/l which was 89% of the largest value. An N:P ratio of 15.3:1 decreased the yield somewhat to 78.5 mg/l after 19 days, 91% of the maximum value. When the N:P ratio was further increased to 21.3:1, the maximum yield was 84.9 mg/l representing 99% of the largest yield. The control flask, with an N:P ratio of 33.3:1, produced a maximum yield of 1.5 mg/l which was only 2% of the highest maximum yield.

When the same lake water was filtered then autoclaved and then tested under the same conditions and nutrient additions, the results were somewhat similar, although the maximum yields obtained were much lower than in the autoclaved then filtered flasks. Highest maximum yield in this experiment was obtained at an N:P ratio of 21.4:1 (Figure V-7). This yield was 40.6 mg/l dry cell weight, occurring after 19 days. An N:P ratio of 3.5:1



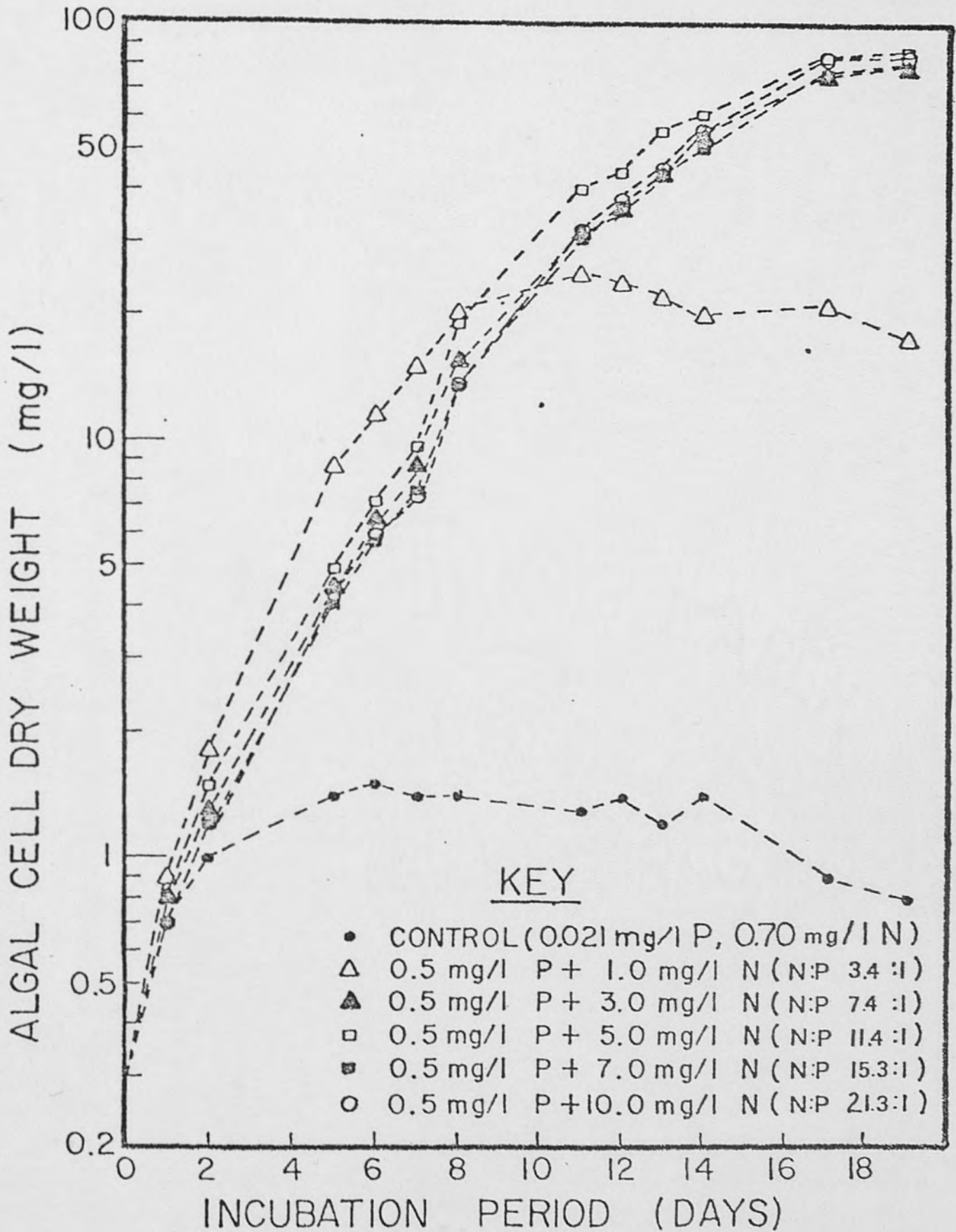


Fig. V-6. Responses of *Selenastrum* to various N:P ratios added to Lake Eola water collected 3/18/79. (Lake water autoclaved then filtered).

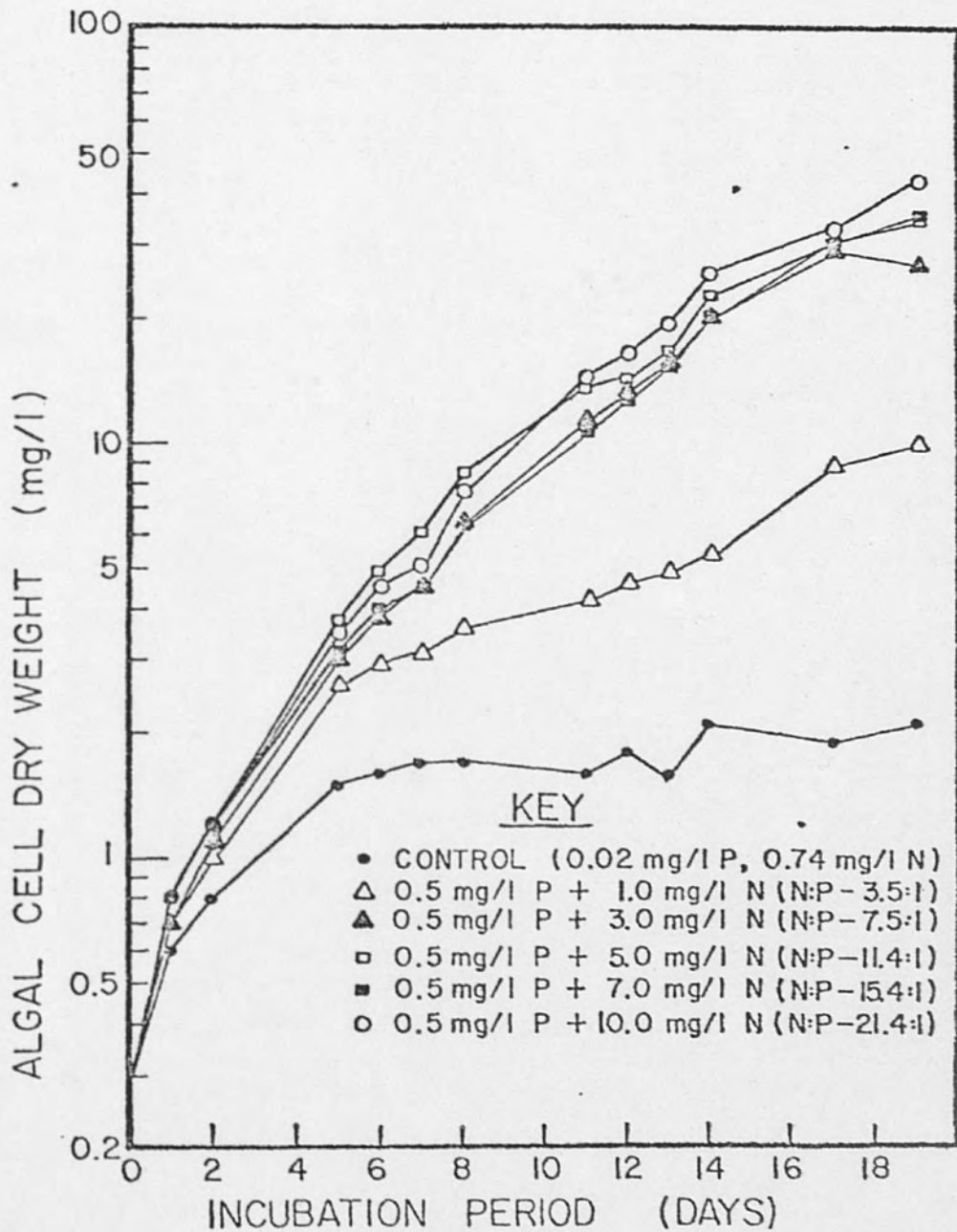


Fig. V-7. Responses of *Selenastrum* to various N:P ratios added to Lake Eola water collected 3/18/79. (Lake water filtered then autoclaved).

produced a maximum yield of 10.0 mg/l dry cell weight which was 25% of the maximum. An increase in the N:P ratio to 7.5:1 increased the yield to 29.2 mg/l after 17 days, 72% of the maximum.

A further increase in the N:P ratio to 11.4:1 resulted in an increase in observed yield to 33.3 mg/l, 83% of the maximum yield. The yield was increased to 84% of the maximum with an N:P ratio of 15.4:1 which produced a mean cell dry weight of 34.3 mg/l. Relative growth response of autoclaved then filtered and filtered then autoclaved lake water to changes in N:P ratios is shown in Figure V-8. Lake water which was filtered then autoclaved produced a yield approximately one-half that found with autoclaved then filtered samples. However, the range of N:P in which the greatest yields were obtained was virtually identical. The pH of the autoclaved then filtered lake water (Table V-1) used in these experiments (8.86) was slightly higher than the pH of the filtered then autoclaved water (8.39). Concentrations of inorganic carbon and nitrate nitrogen were similar between the two waters. However, the orthophosphorus concentrations in the autoclaved then filtered water was over twice as high as in the filtered then autoclaved water.

#### Effect of Stormwater Runoff on Algal Production in Lake Eola

To test the effect of stormwater runoff on algal productivity in Lake Eola, various concentrations of stormwater runoff collected on 10/16/78 were added to composite lake water samples. Chlorella

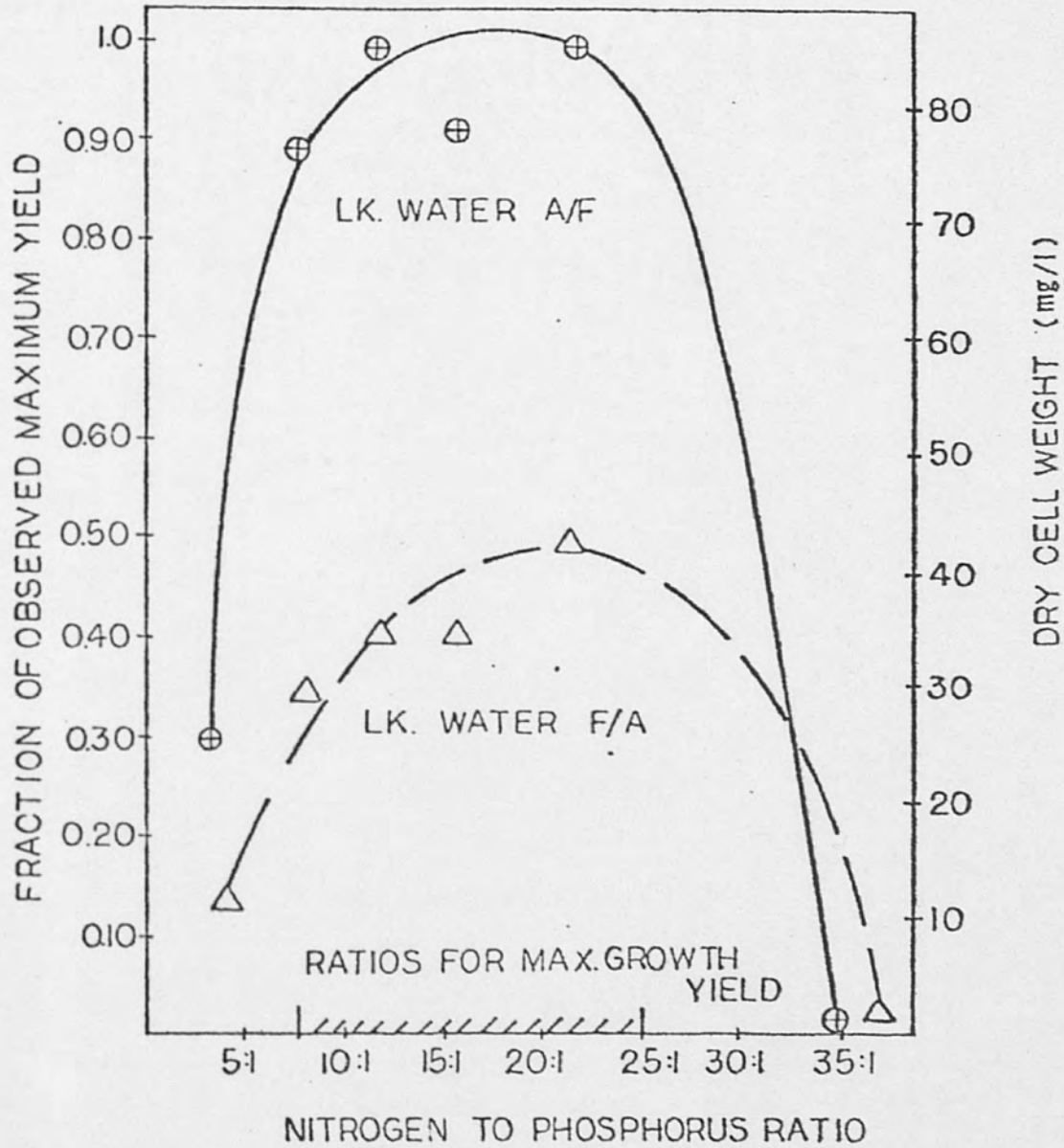


Fig. V-8. Changes in maximum growth yield in a *Selenastrum* culture incubated in Lake Eola water due to changes in nitrogen to phosphorus ratios. (Lake water collected 3/18/79).



pyrenoidosa was used as the test species, and both lake water and runoff were filtered then autoclaved. The lake water control for this experiment increased only slightly from an initial dry cell weight of 0.42 mg/l to 0.47 mg/l after 3 days (Figure V-9). After this time, the algal cell mass began to decline, reaching a minimum of 0.05 mg/l on the 14th day of incubation. When a 5% stormwater mixture was incubated, a maximum yield was obtained after 8 days of 1.09 mg/l dry cell weight. The algal population again began to decline slightly, although not as rapidly as experienced in the control flasks. Incubation of a 10% stormwater mixture produced a maximum yield, also on the 8th day, of 1.55 mg/l dry cell weight. The largest maximum yield was found with a stormwater concentration of 25%. The dry cell weight obtained at this concentration was 3.27 mg/l, also after 8 days. This yield was 111% greater than the 10% stormwater mixture, 200% greater than the 5% addition, and 596% higher than the control. After reaching this maximum yield, production dropped off somewhat but not as rapidly as was experienced in the control, 5%, and 10% mixtures. It should also be noted that a small initial decline in algal mass occurred at this concentration after 1 day. When a 50% mixture of stormwater and lake water was incubated, the maximum yield was obtained after 8 days and was calculated to be 1.58 mg/l dry cell weight. Cell mass dropped off after reaching this maximum to 0.57 mg/l on day 14. A large initial decline in cell mass was noted after 1 day with the initial value

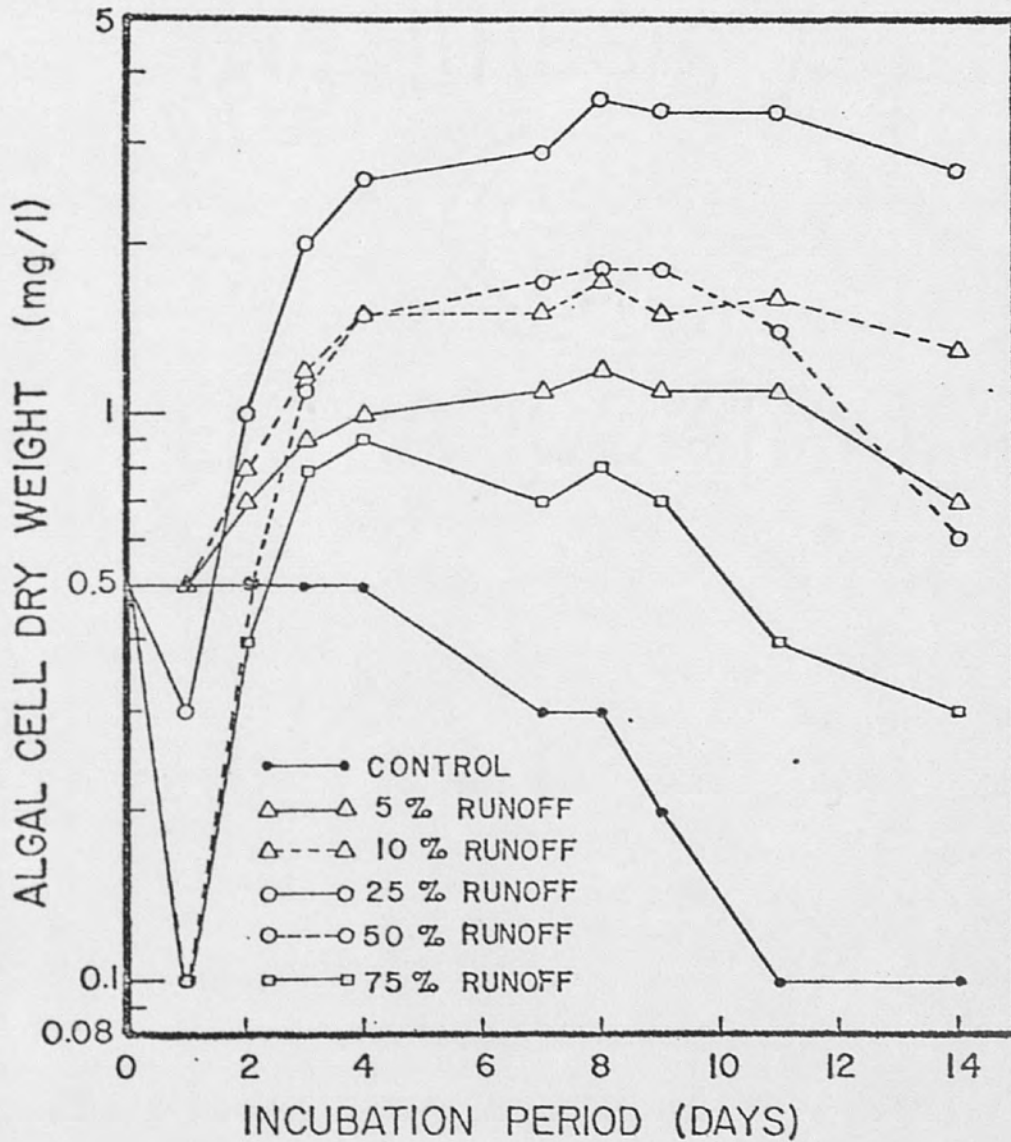


Fig. V-9. Responses of *Chlorella* to various concentrations of stormwater runoff and Lake Eola water collected 10/16/78. (Lake water and runoff filtered then autoclaved).

of 0.47 mg/l dropping to 0.09 mg/l . By the second day, the cell mass had recovered to slightly higher than the original value.

A 75% mixture of lake water and stormwater showed a rapid decline in cell mass to below detectable limits after only 1 day. The population recovered in 3 days, reaching a maximum yield of 0.80 mg/l in 4 days. Another decline in cell mass occurred after reaching this maximum yield to 0.25 mg/l on the 14th day. This value was only 31% of the maximum yield for this mixture. When a solution of pure stormwater runoff was tested, the same initial die-off to below detectable limits was experienced. Recovery to initial values was achieved in 2-3 days, and a maximum yield of 0.83 mg/l was obtained in 4 days. Die-off after achieving this maximum was rapid with a reduction to only 16% of the maximum value in 14 days. Chemical and physical characteristics for this stormwater runoff sample are listed in Table V-4. The pH of this sample was 7.49, much lower than average lake pH values. Turbidity of the sample was also high (65.0 JTU). Concentration of organic carbon in this runoff was 284.0 mg/l, which was about average for the stormwater used in bioassay experiments. The orthophosphorus concentration of 0.48 mg/l was much higher than concentrations normally found in Lake Eola water. Metal analysis of this stormwater (Table V-3) indicated very high concentrations of iron, lead, chromium, zinc, cadmium, copper, and calcium, as compared to average Lake Eola values presented in Tables IV-8 and

TABLE V-4  
 WATER QUALITY CHARACTERISTICS FOR STORMWATER USED IN  
 ALGAL BIOASSAY EXPERIMENTS FOR LAKE EOLA

Date and Treatment	pH	Turbidity (JTU)	Organic Carbon (ppm)	Inorganic Carbon (ppm)	NO <sub>3</sub> -N (ppm)	PO <sub>4</sub> -P (mg/l)
10/16/78 Composite Runoff F/A*	7.49	65.0	284.0	22.6	-----	0.48
11/8/78 Composite Runoff F**	8.30	28.0	218.0	9.4	8.9	0.65
12/14/78 Dry Weather Flow F	8.63	1.2	19.0	23.3	-----	0.041
4/28/79 Raw Runoff	6.97	112.0	308.2	59.0	6.9	0.291
Alum Coagulated (240 mg/l, pH = 5.5)	5.87	3.4	34.3	16.8	0.90	0.013
7/17/79 Raw Runoff	7.47	11.0	-----	-----	1.90	0.024
FeCl <sub>3</sub> Coagulated (58.5 mg/l, pH = 5.3)	6.25	-----	-----	-----	1.32	0.000

\* F/A Denotes the sample was filtered through a 0.45 μ Millipore filter then autoclaved.

\*\* F Denotes the sample was filtered through a 0.45 μ Millipore filter only.



V-3. Results of a chemical analysis of the lake water used in this experiment are listed in Table V-1. The pH of this lake water was 9.04 which is very near average lake values. The organic carbon content was 28.8 mg/l, which was the highest value found in any lake water used in bioassay experiments.

Another experiment testing the effect of various concentrations of filtered stormwater runoff on algal production was conducted in November 1978 using Chlorella as the test species. Incubation of a Chlorella culture in filtered lake water as a control produced a maximum yield of 3.39 mg/l dry cell weight after 5 days (Table V-5). This represented an increase of 688% over the initial value for that concentration. After reaching this maximum yield, however, cell mass dropped off rapidly, reaching a minimum of 0.34 mg/l on the 10th day. A mixture of 5% stormwater produced a cell yield of 5.34 mg/l after 5 days, an increase of 57% over the control. Cell mass dropped off rapidly after reaching this maximum, although not as low as the control. A 10% mixture reached a maximum yield after 5 days of 3.59 mg/l, an increase of only 6% over the control. Cell die-off in this mixture was less rapid than the previous concentrations, with a value of 1.48 mg/l dry cell weight after 11 days. The largest maximum yield of any stormwater concentration tested was obtained with a 25% mixture of runoff and lake water. This yield of 6.33 mg/l was obtained after 10 days, an increase of 87% over the control yield. A slight decline in cell mass to 5.94 mg/l occurred

TABLE V-5

GROWTH RESPONSES OF CHLORELLA TO VARIOUS  
CONCENTRATIONS OF FILTERED LAKE EOLA WATER AND  
FILTERED STORMWATER RUNOFF COLLECTED 11/8/78

Runoff Concentration	Average Dry Cell Weight (mg/l)						
	Initial	1 Day	5 Days	6 Days	7 Days	10 Days	11 Days
Lake Water Control	0.43	0.81	3.39	2.49	1.57	0.34	0.39
5% Runoff	0.40	0.55	5.34	2.77	1.16	1.31	1.15
10% Runoff	0.38	0.62	3.59	3.42	2.17	1.71	1.48
25% Runoff	0.35	0.61	2.05	2.03	2.17	6.33	5.94
40% Runoff	0.37	0.61	1.96	1.47	1.51	5.21	4.52
50% Runoff	0.34	0.61	1.91	2.46	2.31	3.51	3.92
75% Runoff	0.32	0.56	2.43	2.51	2.01	2.51	3.24
100% Runoff	0.37	0.53	3.02	3.78	4.36	4.39	5.44

on the 11th day. A 40% mixture of stormwater produced a maximum yield also on the 10th day of 5.21 mg/l. This represented an increase of 54% over the control. A mixture of 50% runoff produced a gradual increase in cell mass reaching a maximum value of 3.92 mg/l on the 11th day, an increase of 16% over the control. A similar increase in cell mass was noted at a stormwater concentration of 75%, reaching a maximum of 3.24 mg/l on day 11. A more rapid increase in cell mass was observed in the 100% runoff flasks, obtaining a maximum yield of 5.44 mg/l after 11 days, representing an increase of 60% over the control. Analysis of the stormwater used in this bioassay revealed large concentrations of organic carbon, nitrate nitrogen, and orthophosphorus (Table V-4). Relatively high concentrations of iron and lead were also found (Table V-3).

To test the effectiveness of chemical coagulants in reducing the productivity responses of Lake Eola water to stormwater runoff, an experiment was conducted using various concentrations of untreated runoff and coagulated runoff in combination with unfiltered lake water. The results of this experiment are shown in Figure V-10. The untreated lake water control showed only a slight increase in cell mass during the incubation period, increasing from 0.59 mg/l dry cell weight initially to 0.83 mg/l dry cell weight on day 5. When a 5% mixture of lake water and untreated runoff were incubated, algal cell mass increased steadily from an initial value of 0.60 mg/l to 1.59 mg/l after 11

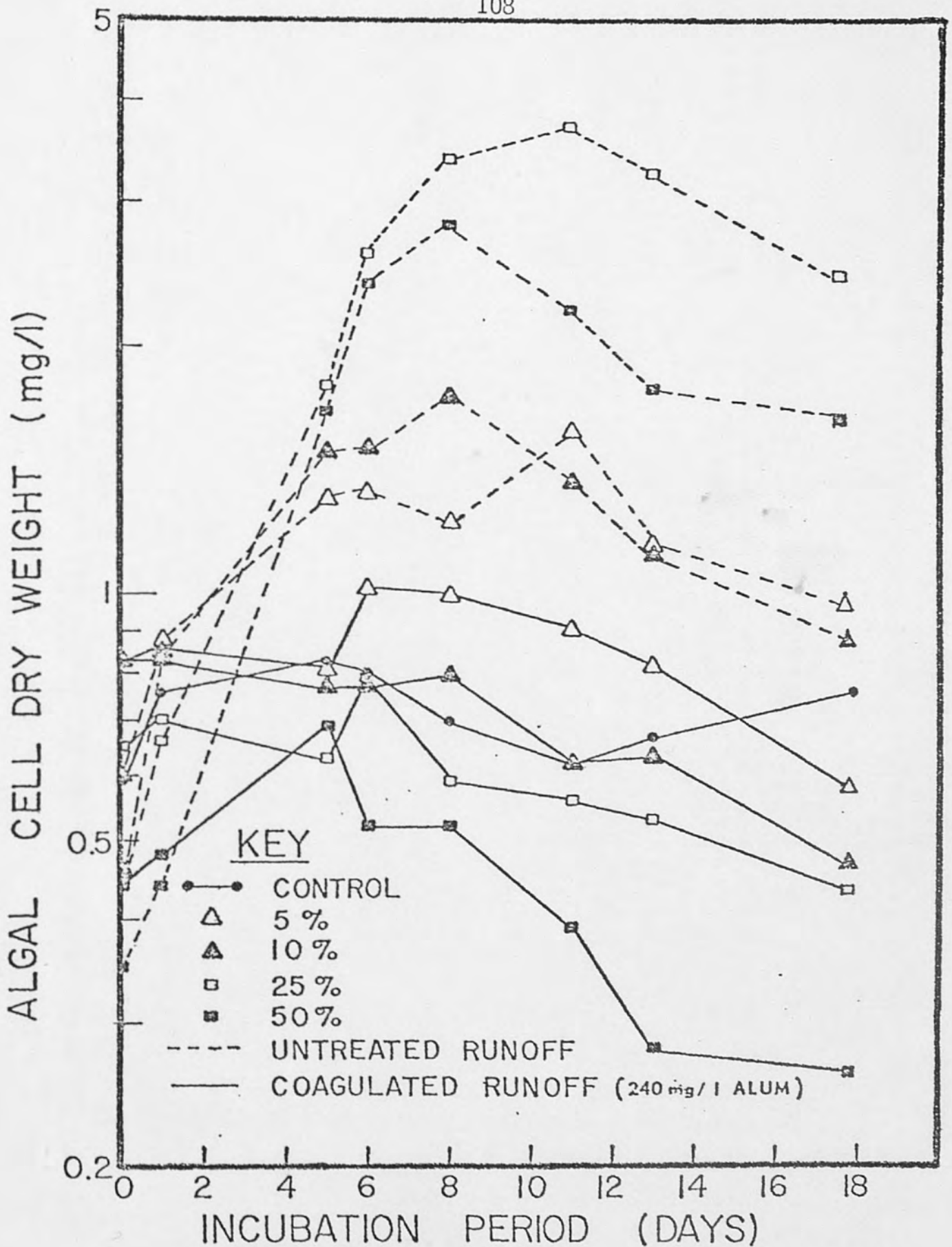


Fig. V-10. Responses of indigenous algal species in Lake Eola to various concentrations of stormwater runoff & coagulated runoff. (Water samples collected 4/30/79, stormwater collected 4/28/79).



days. After reaching this maximum value, the cell mass decreased to 0.94 mg/l after 18 days, corresponding to a reduction of 40% from the maximum value. A mixture of 10% runoff produced a maximum yield of 1.80 mg/l after 8 days and then fell to 0.85 mg/l dry cell weight by the end of the incubation period. This corresponded to a decrease of 53% from the maximum value. The largest cell yield in this experiment was obtained with a 25% mixture of stormwater runoff. This yield occurred after 11 days and reached 3.72 mg/l dry cell weight. This value was 348% higher than the control, 134% higher than the 5% mixture, and 107% higher than the 10% mixture. When the stormwater runoff concentration was increased to 50%, the maximum cell yield decreased to 2.83 mg/l. The value was 24% less than the maximum value. After reaching this value, the cell mass died off slightly, and after 18 days, was only 58% of its maximum value for this concentration. Chemical analysis of this stormwater revealed high concentrations of organic carbon and inorganic carbon (Table V-4). The pH of this runoff was relatively low considering average in situ lake pH values. A nitrate nitrogen concentration of 6.9 mg/l was found. Orthophosphorus concentration was relatively high with a value of 0.291 mg/l.

To test the effectiveness of a coagulant in reducing the potential algal productivity of stormwater, a sample of stormwater was coagulated with 240 mg/l alum at a final pH of 5.5. Various concentrations of this coagulated stormwater were incubated with

lake water, and the results are shown in Figure V-10. The incubation of a 5% coagulated stormwater mixture produced the largest maximum yield obtained using coagulated stormwater additions. This yield was obtained after 6 days and reached a cell mass of 1.04 mg/l. This value was only 25% greater than the control compared to the maximum yield using untreated runoff (at a concentration of 25%) which increased growth 348% over the control. The addition of coagulated runoff in concentrations of 10% and 25% resulted in identical cell yields of 0.79 mg/l, although the 10% addition reached its maximum on day 8 while the 25% addition reached its maximum on day 6. This value was 20% less than the control. The incubation of a mixture of 50% runoff reduced the yield to 0.69 mg/l, occurring after only 5 days. After this value was reached, algal cell mass dropped off rapidly until, on day 22, the cell mass was 0.26 mg/l, only 38% of the maximum value. Chemical analysis of this coagulated runoff revealed relatively low concentrations for both organic and inorganic carbon, as well as orthophosphorus (Table V-4).

The pH of this coagulated runoff was 5.87, which was the lowest pH of any water used for bioassay experiments. Metal analysis of the coagulated runoff revealed that coagulation had been effective in reducing the concentration of every metal tested with the exceptions of aluminum, arsenic, and calcium (Table V-3).

An additional coagulation experiment was conducted to test the effectiveness of  $\text{FeCl}_3$  as a chemical coagulant in reducing

algal productivity. The results of this experiment are listed in Table V-6. The highest maximum yield in this experiment occurred in the untreated lake water control flasks. A maximum cell dry weight of 0.91 mg/l was obtained after 7 days, representing an increase of 102% over the initial value of 0.45 mg/l. After reaching this mass, cell dry weight dropped off rapidly to 0.28 mg/l after 17 days. A 5% mixture of stormwater runoff and lake water produced a maximum yield of 0.88 mg/l after 7 days, a value which was only 3% less than the highest maximum yield for the control. Cell mass for this concentration, however, dropped off less rapidly after reaching the maximum value than did the control flasks. A mixture of 10% stormwater resulted in a maximum yield of 0.75 mg/l after 3 days, declining to 0.35 mg/l in 17 days. Although the maximum yield for this concentration was less than the control, the final cell mass of 0.35 mg/l after 17 days was higher. A 25% mixture of stormwater runoff produced a cell yield between that observed with the 5% and 10% mixtures. Maximum yield of 0.81 mg/l was obtained after 7 days, declining to 0.27 mg/l on the 17th day. A mixture of 50% runoff reached a maximum yield of 0.75 mg/l after 7 days, declining to 0.44 mg/l on day 17. This decline was the least rapid of any of the stormwater mixtures tested.

To test the effect of coagulation of this stormwater on reducing algal production in Lake Eola water, a stormwater sample

TABLE V-6

GROWTH RESPONSE OF INDIGENOUS ALGAL SPECIES  
IN LAKE EOLA TO VARIOUS CONCENTRATIONS OF  
COMPOSITE STORMWATER RUNOFF AND COAGULATED  
STORMWATER RUNOFF COLLECTED 7/17/79

Type	Runoff Concentration	Average Cell Dry Weight (mg/l)					
		Initial	3 days	7 days	10 days	14 days	17 days
Composite Runoff	Lake Water Control	0.45	0.79	0.91	0.71	0.46	0.28
	Lake Water & 5% Runoff	0.48	0.74	0.88	0.61	0.52	0.47
	Lake Water & 10% Runoff	0.41	0.74	0.71	0.48	0.28	0.35
	Lake Water & 25% Runoff	0.40	0.76	0.81	0.67	0.51	0.27
	Lake Water & 50% Runoff	0.33	0.71	0.75	0.71	0.39	0.44
Coagulated Runoff	Lake Water Control	0.45	0.79	0.91	0.71	0.46	0.28
	Lake Water & 5% Runoff	0.35	0.51	0.39	0.25	0.15	0.05
	Lake Water & 10% Runoff	0.34	0.41	0.45	0.29	0.15	0.11
	Lake Water & 25% Runoff	0.27	0.23	0.27	0.34	0.89	0.65
	Lake Water & 50% Runoff	0.18	0.12	0.09	0.05	0.35	0.31

NOTE: Runoff Water was coagulated using 58.5 mg/l  $\text{FeCl}_3$  and final pH was 5.3.



was treated with a dosage of 58.5 mg/l  $\text{FeCl}_3$  at a final pH of 5.3. The results of a bioassay performed with various concentrations of this coagulated runoff are listed in Table V-6. All mixtures of coagulated stormwater runoff and lake water resulted in lower cell dry weights than the control flasks, and with the exception of the 25% mixture, produced a lower cell dry weight than the corresponding value obtained with untreated runoff. A 5% mixture of coagulated runoff and lake water produced a maximum yield of 0.51 mg/l after 3 days which was 42% less than the untreated yield at this concentration although the untreated yield required 4 more days to obtain. After reaching this maximum, cell mass dropped off rapidly, reaching a value of 0.05 mg/l after 17 days, only 10% of the maximum yield for this concentration. Incubation of a 10% mixture resulted in a maximum yield of 0.45 mg/l after 7 days, 40% less than the untreated sample for this mixture. Cell yield at this mixture also dropped off after reaching the maximum, with a value of only 0.11 mg/l on day 17. A 25% mixture of this coagulated stormwater and lake water produced the highest maximum yield of the untreated mixtures, reaching 0.89 mg/l after 14 days. This value was 10% higher and was reached 7 days later than the corresponding untreated sample. Die-off in this mixture was not as rapid, decreasing only 27% to 0.65 mg/l on the 17th day. A 50% mixture of coagulated runoff resulted in an initial decline in cell mass from 0.18 mg/l to 0.05 mg/l after 10 days. After

that time, however, cell yield increased to 0.35 mg/l on the 14th day. Chemical analysis of the lake water used in this bioassay revealed concentrations of inorganic carbon, orthophosphorus, and nitrate nitrogen very near average in situ lake values for year (Table V-1). The pH of this lake water sample was 9. Total metal concentrations in the lake water used in this experiment were higher than average for aluminum, iron, lead, chromium, zinc, and copper (Table V-3). Water quality characteristics of the stormwater and coagulated stormwater used in this experiment are listed in Table V-4. Coagulation of the stormwater reduced the pH from 7.47 in the untreated sample to 6.25 after treatment. Coagulation was also successful in reducing orthophosphorus concentrations from 0.024 mg/l to below detectable limits. A reduction in nitrate nitrogen was also noted. Coagulation with  $\text{FeCl}_3$  reduced concentrations of aluminum, lead, zinc, and arsenic (Table V-3), while concentrations of iron, chromium, nickel, copper and calcium were increased.

To determine if dry weather storm sewer flow has any effect on algal productivity in Lake Eola, a composite sample of lake water and a sample of dry weather storm sewer flow were both filtered through a 0.45  $\mu$  millipore filter and then inoculated with a Chlorella culture. The results of the incubation of various concentrations of this base flow are listed in Table V-7. The lake water control flasks produced very little growth with a

TABLE V-7

GROWTH RESPONSES OF CHLORELLA TO VARIOUS CONCENTRATIONS  
OF DRY WEATHER STORM SEWER FLOW AND  
LAKE EOLA WATER COLLECTED 12/15/78

Concentration of Dry Weather Flow Used	Average Cell Dry Weight (mg/l)					
	Initial	3 days	6 days	10 days	12 days	16 days
Lake Water Control	0.65	0.67	0.68	0.45	0.41	0.31
Lake Water + 5% Dry Flow	0.63	0.52	0.52	0.53	0.43	0.21
Lake Water + 10% Dry Flow	0.64	0.53	0.50	0.29	0.25	0.15
Lake Water + 25% Dry Flow	0.63	0.44	0.36	0.30	0.22	0.17
Lake Water + 50% Dry Flow	0.66	0.54	0.62	0.40	0.37	0.15
Lake Water + 75% Dry Flow	0.65	0.20	0.18	0.08	0.07	0.06
100% Dry Flow	0.72	0.44	0.22	0.10	0.07	0.03

cell mass increase of only 0.03 mg/l after 6 days. After this time, the cell mass began to decline, reaching a minimum of 0.31 mg/l after 16 days. A mixture of 5% dry weather flow did not produce an increase in algal cell mass over the initial value of 0.63 mg/l and finally declined to 0.21 mg/l after 16 days. A 10% mixture of base flow and lake water resulted in a growth response similar to the 5% mixture with the exception of a slightly smaller terminal value. The growth response of a 25% mixture showed a more rapid decline in cell mass than did the 5% and 10% mixtures. Growth response in the 50% mixture did not decline as fast as the previous mixtures, and in fact, seemed to be increasing after 6 days. After that time, however, cell mass dropped off rapidly. A very rapid initial decline was observed in the 75% mixture with a final cell mass after 16 days of only 0.06 mg/l, 9% of the initial value. A similar decline was observed in pure dry weather flow with a terminal value of 0.03 mg/l. Chemical analysis of the dry weather flow used in this experiment (Table V-4) indicated a very low phosphorus concentration of 0.041 mg/l. The pH of this water was 8.63 which is only slightly less than average lake values. Concentrations of organic and inorganic carbon were slightly higher than average in situ lake values.



## CHAPTER VI

### DISCUSSION

From all indications, both visually and analytically, Lake Eola appears to be a lake in severe ecological distress. Persistent algal blooms exist virtually year round. Populations of the macroscopic algae Chara and the filamentous green algae, Spirogyra, became so dense during the summer months along the shoreline that in many instances it became very difficult to launch the small Jon Boat used in this research. Floating masses of dead algae and fish and their accompanying odor are a common occurrence in Lake Eola. With the exception of areas near the shoreline, the bottom of the lake is covered with an accumulation of loose flocculant partially decomposed organic matter which is easily disturbed. The loose nature of this material makes it difficult for rooted submergent plants to exist and with the exception of a very small area near the shoreline, no rooted plants of any kind were seen in Lake Eola. In areas near the center of the lake, this organic matter, subjected to long periods of anoxic and reducing conditions, has formed into sapropel complete with the characteristic hydrogen sulfide smell.

Limnological Characteristics of Lake Eola

Concentrations of dissolved oxygen in Lake Eola, although usually at or above saturation near the surface (Table IV-7), drop periodically during the spring and summer months to 1 mg/l or less at depths of 4 meters or greater (Figure IV-2 and Table IV-5). Determinations of Secchi disk depth in Lake Eola averaged approximately 1 meter. If the ratio between euphotic zone depth and Secchi disk depth is assumed to be 3.0 (Cole, 1975), then the euphotic zone depth in Lake Eola would be approximately 3 meters. This depth corresponds with data shown in Figure IV-2, where concentrations of dissolved oxygen are seen to drop rapidly below 3-4 meters during spring and summer months. Below this depth, oxygen is consumed rapidly by decomposition processes. Although measurements of dissolved oxygen have indicated that the deeper areas can become oxygenated somewhat by wave action during windy periods, it seems reasonable to assume that the areas in Lake Eola below 4-5 meters deep remain anoxic during much of the spring and summer. Besides releasing large quantities of  $H_2S$  and  $CO_2$ , these conditions can also cause release of considerable quantities of phosphorus which may then become available for further algal production. This release has been demonstrated in Lake Eola to be as high as 250 mg of orthophosphorus/ $m^2$  (Marshall, 1930). If this release is conservatively considered to be limited to areas of 4.5 meter depth or more, the area under this depth would be approximately 30,000  $m^2$  (Table III-2) and the

expected release under anoxic conditions may be as high as 7500 g of phosphorus. A release of this magnitude, if mixed into the water column by wave action, could increase average in situ orthophosphorus concentrations by as much as 0.023 mg/l P.

Measurements of pH in Lake Eola are indicative of the tremendous rate of algal production. The average hydrogen ion concentration in Lake Eola during the research corresponded to a pH value of 8.86. At this pH, approximately 96% of the inorganic carbon present would exist in the bicarbonate state. The remaining 3% would be in carbonate form with only a minute percentage of free  $\text{CO}_2$  within the first few meters in Lake Eola. Thus, it appears that algal production is depleting this source almost as fast as it enters the water. After the free  $\text{CO}_2$  is utilized, those photosynthetic organisms which are capable of utilizing bicarbonate ions also begin to do so. It is not unreasonable to assume that the composition of algal communities in Lake Eola is determined and regulated not only by seasonal variations but also by the type of inorganic carbon compounds present. As seen in Figure IV-1, pH was typically lower in fall and winter months when algal production would be expected to decrease and higher in the spring and summer when production is at a maximum. Turbidity increases during spring and summer are also indicative of this increased production.

Nutrient data for Lake Eola, in many cases, does not follow typical predicted cyclic patterns (Figure IV-3), which is to be

expected considering the irregularity in both quantity and quality of the pollution source. Concentrations of orthophosphorus fluctuated between 0.01 and 0.04 mg/l during the test period. Although concentrations would normally be expected to reach minimum values during the highly productive spring and summer months, peaks in phosphorus were found in August and May, presumably due to stormwater additions and/or phosphorus release from bottom sediments. Organic carbon was lowest in the winter months as expected due to decreases in algal production and stormwater inputs with a corresponding increase during the spring and summer. However, a very large increase in organic carbon was measured during October and November and was presumed to be due to mixing of sediment material caused by circulation increases typical of the fall season. Inorganic carbon appeared to be lower during the spring and summer as algae utilized this substrate as a food source with slight increases during the nonproductive winter months. A two-fold increase in concentration was recorded in April 1979 presumably due to stormwater inputs. Although the data on nitrate nitrogen is limited, it appears that nitrate concentrations experienced decreases during spring and summer as algae utilization increased.

#### Sample Preparation for Bioassay Use

Two of the most discussed and variable aspects of bioassay research involve sample preparation and the selection of a test



organism. In this research, four different types of sample preparations were used: raw unfiltered lake water, water filtered through a 0.45 micron Millipore filter (F), water which was filtered then autoclaved (F/A), and water which was autoclaved and then filtered (A/F). In addition to indigenous algal species from Lake Eola, two different laboratory cultured unialgal test species were also utilized, Chlorella pyrenodosa and Selenastrum capricornutum Printz. Both of these species are solitary, non-motile green algae which possess a wide tolerance towards environmental conditions and occur in waters of diversified composition (EPA, 1978). A summary of experimental conditions for bioassay studies conducted during this research is listed in Table VI-1.

The validity of results obtained from a bioassay experiment hinge upon the ability of the experiment to predict or mimic in situ algal responses to nutrient additions. Since these responses are an integration of the combined effects of ion solubility and ion availability to the test organism, any factor which alters these conditions, such as sample treatment, may bias bioassay results. Chemical analyses conducted during this research indicate that autoclaving, filtration followed by autoclaving, and autoclaving followed by filtration all result in significant decreases in concentrations of organic carbon, inorganic carbon, and orthophosphorus. However, nitrate nitrogen concentrations show substantial increases due to these treatments as presented in Table VI-2. Similar results have been reported by Filip and Middlebrooks

TABLE VI-1

## SUMMARY OF EXPERIMENTAL CONDITIONS FOR BIOASSAY STUDIES

Date	Lake Water Treatment	Test Organism	Runoff %	Nutrients Added mg/l			EDTA Added mg/l
				N	P	Fe	
8/10/78	Composite F	<u>Chlorella</u>	0	1- 5	0.1-1.0	0	0
10/16/78	Composite F/A	<u>Chlorella</u>	0- 75	0	0	0	0
11/08/78	Composite F	<u>Chlorella</u>	0-100	0	0	0	0
12/14/78	Composite F	<u>Chlorella</u>	0-100 (dry weather)	0	0	0	0
1/14/79	Composite F/A	<u>Selenastrum</u>	0	1- 5	0.05-0.5	0.05	0
2/21/79	Composite A/F Composite A/F	<u>Selenastrum</u>	0 0	1- 5 1- 5	0.05-0.5 0.05-0.5	0.05 0.05	0 1
3/18/79	Composite A/F Composite F/A	<u>Selenastrum</u>	0 0	1-10 1-10	0.5 0.5	0 0	0 0
4/30/79	Composite Composite	Indigenous Indigenous	0- 50 (untreated) 0- 50 (alum coagulated) (untreated)	0	0	0	0
7/17/79	Composite Composite	Indigenous Indigenous	0- 50 0- 50 (Fe Coagulated)	0 0	0 0	0 0	0 0

TABLE VI-2

PERCENT CHANGE IN WATER QUALITY PARAMETERS  
DUE TO SAMPLE TREATMENT PRIOR TO BIOASSAY USE

Sample Treatment	# of Samples	Percent Change Due to Treatment					O.P.
		pH	Organic Carbon	Inorganic Carbon	NO <sub>3</sub> -N		
Filtered	2	-1.5	-12.5	- 1.5	+ 7.5	-58.0	
Autoclaved	2	+1.0	-53.0	-16.0	+17.0	-21.5	
F/A	3	+3.3	-40.0	-38.0	+48.0	-39.0	123
A/F	4	+1.0	-36.5	-35.3	+25.0	-18.0	

(1975) in tests on mesotrophic pond water. They suggested that oxidation attributable to the physical disturbance of the sample as it passes through the filter probably accounts for higher nitrate levels in treated samples. It seems reasonable that increases in temperature and pressure during autoclaving may also be responsible for chemical changes of various parameters in water samples. Oxidation of organic carbon, nitrogen and phosphorus may occur altering the composition and quantity of these nutrients while at the same time, slightly increasing the pH. Autoclaving of the sample either before or after filtration, besides substantially reducing concentrations of organic carbon, inorganic carbon, and orthophosphorus can also cause reductions in concentrations of toxic heavy metals by precipitation during autoclaving (Filip and Middlebrooks, 1975). These precipitates may be extremely resistant to resolubilization under bioassay conditions (Environmental Protection Agency, 1978). Oxidation of organic wastes may also occur rendering these compounds less toxic. It appears that autoclaving of a sample tends to produce conditions which may enhance algal production by increasing the availability of some nutrients and at the same time decreasing toxicity of organic compounds and heavy metals. As seen in Table VI-3, the maximum yields obtained in test waters which were autoclaved then filtered are much larger than any other type of treatment. This difference may be a result of a combination of several factors. First, a micronutrient necessary for growth may have been present



TABLE VI-3

## SUMMARY OF SAMPLE CHARACTERISTICS AND EXPERIMENTAL RESULTS FOR NUTRIENT LIMITATION BIOASSAYS USING LAKE EOLA WATER

Test Organism	Sample Characteristics				Maximum Cell Yield (mg/l)	Limiting Nutrient	Concentration of Limiting Nutrient Producing Max. Yield Added
	Date Sample Collected	Sample Treatment	Background PO <sub>4</sub> -P (mg/l)	Background NO <sub>3</sub> -N (mg/l)			
<u>Chlorella</u>	8/10/78	Filtered	0.017	--	1.63	N	1.0 mg/l N
<u>Selenastrum</u>	1/14/79	F/A	0.011	--	2.38	P	0.05 mg/l P <sup>125</sup>
<u>Selenastrum</u>	2/21/79	A/F	0.058	1.25	21.0	N	3.0 mg/l N
<u>Selenastrum</u>	2/21/79	A/F (EDTA Added)	0.058	1.25	39.5	N	5.0 mg/l N
<u>Selenastrum</u>	3/18/79	F/A	0.010	0.58	4.70	P	0.1 mg/l P

in minimal quantity in the water column in relation to the needs of the organism. As a result, most of this element which is in a form useable by algae may already be incorporated in biomass. The lake water samples which were prepared by autoclaving followed by filtration were collected during February when the highest levels of chlorophyll "a" were measured in Lake Eola (Figure IV-1). Autoclaving of the sample may have released this nutrient making it available for growth. Second, heavy metals which are precipitated during autoclaving are removed when filtration follows autoclaving. However, when autoclaving follows filtration, these precipitates are not removed. The resolubilization of some of these compounds may produce an inhibitory effect in samples which are filtered then autoclaved.

While autoclaving may result in substantial changes in the chemical composition of water containing large quantities of organic particulate matter, filtration alone produces, with the exception of orthophosphorus, very little change in sample composition. As seen in Table VI-2, pH, organic carbon and inorganic carbon decreased slightly during filtration with nitrate concentrations experiencing a slight increase. Concentrations of orthophosphorus, however, were reduced by 58% compared to the raw lake water sample. Similar results were found by Filip and Middlebrooks (1975). Raw lake water samples probably contain some particulate phosphorus which is converted to orthophosphorus under the acidic conditions of the ascorbic acid technique and

serves to increase the measured orthophosphorus concentration in the raw water. Measurement of orthophosphorus in the filtered sample resulted in a substantially lower value, indicating, perhaps erroneously, that filtration removes orthophosphorus. It seems clear from these results that filtration alone produces the least alteration in chemical composition especially when dealing with a very eutrophic water containing large quantities of suspended organic particles.

From the results obtained during this research, it appears that autoclaving of a sample is not a suitable treatment technique for use in studies where the effects of heavy metals or complex organic compounds are to be determined. In these cases, filtration only should be used. If, on the other hand, a relatively clean oligotrophic water is to be assayed and it is desirable to determine the amount of algal biomass that can be grown from all nutrients in the water, including those contained in filterable organisms and other particulate matter, then autoclaving of the sample seems to be the best treatment technique. It also appears that autoclaving of a lake water sample, either before or after filtration, and the subsequent use of a unicellular algal species under laboratory conditions in a bioassay experiment will not produce results which are indicative of the amount of biomass that will be produced, under the same nutrient conditions, in the lake itself. Although autoclaving the sample will probably not change

the nutrient which is indicated to be limiting growth, as a general rule, samples which are autoclaved will produce a much larger standing crop than filtered samples.

### Selection of a Test Organism

From the standpoint of modeling in situ algal responses in bioassay experiments, the sample preparation technique which alters water quality the least will be the preferred method. The use of raw lake water containing indigenous algal species then appears to be the optimum method for predicting algal responses in a particular system. The major advantage of using raw water is that the chemical composition of the lake water sample used in the bioassay remains essentially unchanged. Indigenous algal populations have developed, over time, a tolerance to environmental conditions in their particular habitat. Organisms which are continually subjected to large loadings of organic wastes or heavy metals may evolve, through natural selection, an immunity to concentration levels of these compounds which would be toxic to laboratory cultured species. A bioassay using this cultured species may predict algal die-off at certain concentrations of pollutant while actual in situ conditions would be much different.

### Indigenous Algal Species

The use of raw lake water, however, possesses several distinct disadvantages. First, the composition of indigenous algal species in any given aquatic environment is varied and complex.



Representatives of Chlorophyta, Cyanophyta, Chrysophyta, Euglenophyta, Rhodophyta and Phaeophyta may and probably will be present, representing a wide spectrum of sizes and habitats. Members of these phyla may be unicellular, colonial, filamentous, motile, non-motile, encased in a sheath, or encased in a gelatinous coating. Because of this variation in composition, measurement of production in indigenous populations is often difficult. Indirect determinations by electronic particle counting using a Coulter Counter is the preferred method specified by the Environmental Protection Agency for all bioassay work (Greene, personal communication). However, measurement of indigenous populations by this method would first require large clumps and filaments within the sample to be broken up either by mechanical or sonic means. Certain algal forms are more resistant to these procedures than others resulting in a suspension of uneven composition which could cause clogging of the aperture tube or selective passage of particles. Gravimetric techniques, although not affected by the composition of the culture, are prohibitively time consuming as well as being subject to inherent large experimental error. Measurement of in vivo fluorescence of algal chlorophyll "a" is perhaps the best suited detection method for indigenous species. While a fluorometer will not accurately measure large filaments or colonial forms, somewhat reproducible results may be obtained if the sample is first broken up slightly by a mechanical mixer or by vigorous shaking. Fluorometric measurement of chlorophyll

"a" is a very sensitive method which can be quickly performed. However, the chlorophyll "a" to cell mass ratio may vary considerably with growth in natural waters having different chemical composition (Round, 1973) and may cause a bias in bioassay results. These problems can be minimized by preparing separate cell mass vs. chlorophyll "a" fluorometric calibration curves for each algal species and natural water used. Although electronic particle counting is the preferred method for measurement of algal production, fluorometric determinations, because of their sensitivity and rapidity, can be very useful in bioassay work and may be the only valid method for use with indigenous populations.

The second disadvantage of using indigenous species involves a basic assumption implied in their use. A succession of algal populations normally occurs in a lake over time until the species which are best suited for growth in that particular environment have become dominant. When these species are subjected to the conditions of a bioassay experiment which invariably involve different temperatures, light intensities, and nutrient availabilities, the natural result is either for the community to be unable or slow in responding to nutrient additions or for a different succession to occur in species composition to a community more suited to the test environment. When this occurs, the main advantage of using indigenous species, that of closely mimicking natural responses, is lost and compounded by increased difficulty in production measurement. It was noted during this research that the

results obtained within the triplicate flasks of a single nutrient addition were extremely variable when indigenous species were used. This variation was sometimes as large as 100-200% and reflects the complex nature of natural systems. These variations often make interpretation of bioassay results from indigenous populations difficult at best.

#### Unicellular Algal Species

To avoid the problems of enumeration and slow growth response associated with the use of raw lake water containing indigenous algal species, most algal bioassay researchers have resorted to the use of an easily measured and cultured unialgal test species. Of the many species tested in bioassay work, Selenastrum has surfaced as the most common assay organism and is now the only species recommended for use by the Environmental Protection Agency (Greene, personal communication). The distinctive crescent moon shaped cell configuration makes microscopic examination of a sample for contamination by other algal species a very simple process. No problems of any kind were experienced during this research in culturing or maintaining a Selenastrum culture. Chlorella, which was also used in these experiments, is easily cultured but lacks the characteristic cell shape which makes enumeration of Selenastrum a simple task. No differences in growth response were detected during this research between Chlorella and Selenastrum. Unialgal cultures of the blue-green

alga, Anabaena and Microcystis as recommended in PAAP, were also obtained for use in preliminary bioassay experiments, however, neither one of these species were judged suitable for bioassay work. Microcystis simply refused to grow in the synthetic algal medium and a satisfactory culture of the species was never obtained. Anabaena could be easily cultured but tended to clump together in tight masses which were resistant to resuspension, making fluorometric measurement of this species virtually impossible. Anabaena also required reculturing in fresh algal medium on a weekly basis to prevent population die-off. Selenastrum, on the other hand, did not require this frequent reculturing.

It appears, then, that if a unialgal species is to be used in bioassay research, that Selenastrum is the preferred organism. Enumeration of this species is a simple task either by electronic particle counting or in vivo fluorescence. The selection of a single test species, such as Selenastrum, for use in most bioassay experiments would make interpretation and comparison of results obtained in different laboratories a much easier task.

#### In Situ Predictions from Bioassay Experiments

An important and often puzzling aspect of bioassay research involves the prediction of possible in situ responses from the results obtained in a laboratory experiment. The most useful predictive model is one in which the expected chlorophyll "a" concentration in a lake, as a result of nutrient or stormwater



additions, could be calculated from the cell yield obtained in a bioassay experiment. A summary of bioassay results obtained in control flasks with no nutrient additions is listed in Table VI-4. This data represents changes in cell mass other than those actually measured in the lake due to the type of test organism used and the conditions of the bioassay. Since bioassay experiments are subjected to a constant illumination as well as optimum nutrient availability due to oscillation of the flasks, the ultimate yield obtained in these control flasks was somewhat larger than the actual standing crop of algae in Lake Eola itself, as measured by the concentration of chlorophyll "a". If it is assumed that the concentration of chlorophyll "a" actually measured in a lake is the maximum concentration that can occur under the given environmental conditions, then a ratio can be developed between the actual in situ chlorophyll concentration and the increased yield obtained in control flasks due to the conditions of the bioassay. However, the response obtained in a bioassay experiment is a direct result of the type of sample treatment and test organism used and only those experiments utilizing the same treatment type and test organism should be used for predictive purposes.

As seen in Table VI-3, the only type of sample preparation and test organism combination which produced similar ratios is the use of raw water containing indigenous species. The average chlorophyll "a" to maximum yield ratio for this combination was

TABLE VI-4

SUMMARY OF BIOASSAY RESULTS OBTAINED IN CONTROL FLASKS CONTAINING LAKE EOLA WATER WITH NO NUTRIENT ADDITIONS

Date of Sample Collection	Sample Treatment	Maximum Cell Yield Obtained in Control Flasks (mg/l)	Test Organism	In Situ Conc.		Ratio of CHYL "a"/Max Yield	Ratio of Cell Mass Experimental / In Situ
				CHYL "a" ( $\mu\text{g/l}$ )	Cell Mass mg/l		
8/10/78	F	0.75	<u>Chlorella</u>	19.4	0.66	25.9	1.1
11/08/78	F	3.39	<u>Chlorella</u>	23.3	0.79	6.9	4.3
10/16/78	F/A	0.47	<u>Chlorella</u>	6.7	0.23	14.3	2.0
1/14/79	F/A	0.90	<u>Selenastrum</u>	33.6	1.14	37.7	0.8
3/18/79	F/A	2.10	<u>Selenastrum</u>	32.4	1.09	15.4	1.9
2/21/79	A/F	10.10	<u>Selenastrum</u>	28.2	0.96	2.8	10.5
4/30/79	none	0.83	Indigenous	17.5	0.59	21.1	1.4
7/17/79	none	0.91	Indigenous	17.9	0.61	19.7	1.5

20.4. Therefore, the chlorophyll "a" concentration expected in Lake Eola from a particular nutrient or stormwater addition in a bioassay experiment using untreated lake water containing indigenous species could be calculated by multiplying the maximum cell yield in mg/l by a factor of 20.4. Stated in other terms, laboratory bioassay experiments conducted during this research using indigenous species resulted in increases in the standing crop of between 1.4 and 1.5 times those levels actually measured in Lake Eola. However, the lack of the other sample treatment and test organism combinations to produce consistent ratios does not indicate that these are not suitable predictive tools. Since sample treatment, other than filtration only, was shown to significantly affect water quality, it is expected that these results will be considerably different from actual in situ responses in eutrophic waters such as Lake Eola. Filtered samples, however, when used with the same test organism, should produce results consistent with results obtained using indigenous species. Since only two tests were conducted using water which was filtered only, the failure of this treatment to produce consistent results deserves further research.

#### Effect of Nutrient Additions to Lake Eola Water

A summary of nutrient addition bioassay experiments using Lake Eola water is listed in Table VI-5. As seen in this data, both nitrogen and phosphorus were able to stimulate algal production

TABLE VI-5

SUMMARY OF NUTRIENT ADDITION BIOASSAY  
EXPERIMENTS USING LAKE EOLA WATER

Date Collected	Sample Characteristics			Maximum Experimental Yield (mg/l)	Predicted Maximum Yield (mg/l)	Nutrient Addition Producing Maximum (Yield)	N:P Ratio at Maximum (Yield)	Test Organism
	Treatment	PO <sub>4</sub> -P (mg/l)	NO <sub>3</sub> -N (mg/l)					
8/10/78	Filtered	0.017	----	1.63	10.97	1.0 mg/l N	-----	<u>Chlorella</u>
1/14/79	F/A	0.011	----	2.38	39.35	0.05 mg/l P	-----	<u>Selenastrum</u>
2/21/79	A/F	0.060	1.25	21.00	38.70	3.0 mg/l N	71:1	<u>Selenastrum</u>
2/21/79	A/F (EDTA Added)	0.060	1.25	39.50	38.70	5.0 mg/l N	104:1	<u>Selenastrum</u>
3/18/79	F/A	0.010	0.58	4.70	65.15	0.1 mg/l P	6.4:1	<u>Selenastrum</u>



in Lake Eola on certain dates. Although the data is limited, it appears that stimulation of a particular water by nitrogen or phosphorus may be related to the background phosphorus concentrations in the sample at the beginning of the bioassay. Whenever the phosphorus concentration before nutrient additions was approximately 0.02 mg/l or less, algal production was stimulated by a phosphorus addition. Above this value, nitrogen was shown to stimulate growth.

While nitrogen was able to produce a short-term stimulation on algal production in certain bioassay experiments using a uni-algal species in Lake Eola water, it will probably not be a limiting resource when considered over a period of years. In Lake Eola, as in most lakes, the major new source of phosphorus is the watershed. Nitrogen and carbon, although also obtained from the watershed, both have considerable inputs from the atmosphere. If an excess of phosphorus, relative to carbon, is added to a water, algal growth will deplete the available carbon before phosphorus becomes limiting. This will lower the partial pressure of carbon dioxide in the water, and additional carbon dioxide will diffuse into the water from the atmosphere allowing complete utilization of phosphorus until it becomes a limiting resource (Schindler, et. al., 1972; Schindler and Fee, 1974). Molecular nitrogen will follow a similar diffusion pattern into waters when nitrogen fixers reduce its concentrations. Since blue-green algae are a major component of most eutrophic systems, it is reasonable

to assume that nitrogen will rarely limit long-term algal production in these waters.

A close examination of the data indicates that the N:P ratio may, under certain conditions, be more influential in regulating algal production in Lake Eola than the actual concentration of nitrogen or phosphorus. The fact that N:P ratios can play an important part in determining the potential biomass in a system is clearly demonstrated in Figures V-6 through V-8. In these experiments, an optimum weight ratio of total soluble inorganic nitrogen (TSIN) to orthophosphorus was found to be between 3.4 and 21.4. Chiaudani and Vighi (1974) report optimum weight ratios between 4.5 and 9 in studies on Italian lakes. However, the data presented in Table VI-5 indicates that maximum yields were obtained in nutrient addition bioassays with N:P ratios as high as 104. As stated previously, growth response of an organism will be affected largely by the availability of ions in its environment. It seems reasonable to assume that when a nutrient is present in a minimal quantity that a point will be reached in terms of the concentration of a particular nutrient at which the availability of the nutrient will become so low that further growth will be limited by ion availability rather than by its relationship to other ions. It is this reduction in ion availability that may explain the seemingly erratic results for N:P ratios reported above. In the bioassay experiment conducted using lake water collected on 2/21/79, maximum yields were obtained with N:P ratios

between 71 and 104. Evidently, the concentration of phosphorus was sufficient to stimulate growth but was not present in sufficient quantity to produce an excess of ion availability to the test organism. It appears then that a threshold level of phosphorus exists below which the growth of an organism is regulated solely by the concentration of phosphorus present, assuming all other essential nutrients are also present. Above this threshold value, phosphorus is in relative excess and the organism is no longer limited by ion availability alone. As concentrations of ions increase, organisms are virtually surrounded by an abundance of nutrients. Since some molecules have greater affinities for binding sites on the cell membrane than others, these binding sites may become blocked by certain compounds, limiting further nutrient uptake. In an environment with an excess of nutrients, cell growth will be optimum at a nutrient ratio where nutrients needed for growth can be readily taken up by the cell in approximate quantities necessary for growth. As seen in Table VI-5, an orthophosphorus concentration of 0.060 mg/l was not sufficient to provide excess ion availability. However, when the concentration is increased to 0.110 mg/l, as was the case in the experiment using lake water collected on 3/18/79, optimum growth occurred at a weight ratio of 6.4:1 which is within the range reported previously. It appears then that the threshold concentration within Lake Eola above which sufficient phosphorus ions are available so that growth may be limited by the relative abundance of these ions

rather than their actual concentrations is between 0.060 and 0.110 mg/l  $\text{PO}_4\text{-P}$ . In other words, when the concentration of orthophosphorus in Lake Eola is approximately 0.10 mg/l or less, algal production is regulated by the concentration of orthophosphorus alone. Above this concentration, it is assumed that an excess of phosphorus is available, and algal growth is regulated by the N:P ratio. However, since concentrations of orthophosphorus recorded in Lake Eola during this research were 0.04 mg/l or less, it can be concluded that, except during periods of heavy phosphorus loadings by stormwater inputs or by long periods of anoxia, algal production in Lake Eola is limited by the concentration of phosphorus alone.

#### Effect of Stormwater Runoff on Algal Populations

A summary of stormwater addition bioassay experiments using Lake Eola water is listed in Table VI-6. As seen in this data, maximum yields were produced in virtually every case with a mixture of 25% stormwater runoff, resulting in increases of 87-731% over control flasks. If the ratio of in situ chlorophyll "a" to maximum cell yield using indigenous algal species is assumed to be 20.4 as previously developed, the maximum cell yield of 3.72 mg/l obtained using lake water collected on 4/28/79 would correspond to a chlorophyll "a" concentration in Lake Eola of 76  $\mu\text{g/l}$  as a result of this storm event. An algal cell concentration of this magnitude would quickly utilize available nutrient supplies and



TABLE VI-6

SUMMARY OF STORMWATER ADDITION EXPERIMENTS  
USING LAKE EOLA WATER

Sample Characteristics			Stormwater Conc. Producing Maximum Yield	Test Organism	# of Days to Reach Maximum Yield	Percent Increase Over Control		
Date Collected	Treatment	PO <sub>4</sub> -P					NO <sub>3</sub> -N	Maximum Experimental Cell Yield (mg/l)
10/16/78	F/A	0.480	--	3.27	25%	Chlorella	8	596%
11/08/78	F	0.650	--	6.33	25%	Chlorella	10	87%
4/28/79	None	0.291	6.90	3.72	25%	Indigenous Species	11	731% 14
4/28/79	Alum Coagulated	0.013	0.90	1.04	5%	Indigenous Species	6	8%
7/17/79	None	0.024	1.90	0.88	5%	Indigenous Species	7	-3%
7/17/79	FeCl <sub>3</sub> Coagulated	0.000	1.32	0.89	25%	Indigenous Species	14	-2%

be reduced to a much lower value in a short time. However, this reduction would occur largely at the expense of increased sediment buildup.

If Figure V-9 is considered a typical algal response to stormwater additions, it can be seen that the addition of stormwater in any concentration, even 100% stormwater runoff, resulted in an increase in algal production over the control. However, when stormwater was added in concentrations of 25% or greater, the growth curve was typified by an initial die off of algal populations, the magnitude of which being a function of runoff concentration. This die-off is due largely to the presence of toxic elements in the stormwater which is discussed, along with its implications, in a later section. If a typical 1/2 inch storm fell on the Lake Eola watershed, the corresponding runoff, if completely mixed throughout the water column, would represent an addition of only 2% to the total lake volume. At this concentration of stormwater runoff, the effect on algal production would be greatly minimized. However, the time necessary for the stormwater to mix throughout the entire lake, depending on weather conditions, is probably on the order of several days. It seems reasonable to assume then that stormwater concentrations of 25% or greater may exist near stormwater outfalls for at least 1 day and that the die-off predicted at this concentration would occur in these areas.

Effect of Coagulated Runoff on Algal Growth

One of the techniques under consideration for the restoration of Lake Eola includes chemical coagulation of a portion of the runoff to remove phosphorus and heavy metals. Alum,  $\text{Al}_2(\text{SO}_4) \cdot 18 \text{H}_2\text{O}$ , was selected as a coagulant for test purposes. An alum dosage of 240 mg/l at a final pH of 5.5 was determined through jar tests to provide optimum phosphorus removal. Coagulation under these conditions resulted in a reduction of 96% of orthophosphorus and 87% of nitrate nitrogen (Table VI-6). With the exception of aluminum and arsenic, coagulation also reduced concentrations of every heavy metal tested.

As seen in Table VI-6 and Figure V-10, coagulation of the stormwater resulted in substantially lower growth responses, as compared with uncoagulated test flasks at every concentration of stormwater tested. The maximum percent increase in algal production over the control flasks was reduced from 731% using untreated stormwater to 8% in a coagulated sample. Using the in situ chlorophyll "a" to maximum experimental yield ratio for indigenous species of 20.4, the maximum cell mass obtained in the treated flasks correspond to a chlorophyll "a" concentration in Lake Eola of only 21.4  $\mu\text{g/l}$ . It should be noted that, even though an input of stormwater has occurred to the lake, the maximum production predicted by this addition is less than the average in situ chlorophyll "a" concentrations in Lake Eola measured during this research (Tables IV-1 to IV-3).

An interesting result of these coagulation experiments is that the untreated sample required 11 days to reach its maximum yield while the coagulated sample obtained a maximum in only 6 days. Greene, et. al. (1976) suggests that a growth lag in algal response is often experienced in waters containing toxic compounds. Certain algal forms are able to produce extracellular substances which can form chemical complexes with the growth inhibiting substance. Anabaena, for example, over a period of days, may produce a polypeptide which forms a non-toxic complex with copper, iron, and phosphorus. The absence of this characteristic growth lag in the coagulated samples suggests that sufficient toxic elements had been removed by this process so that algal forms were no longer inhibited. Thus, it seems that coagulation of storm-water not only removes nutrients and limits algal production but it also produces a product which is less toxic to aquatic organisms.

#### Significance of Dry Weather Flow

To determine if dry weather storm sewer flow has any effect on algal production in Lake Eola, various concentrations of storm-water base flow and lake water were incubated with Chlorella as the test organism. The results of this experiment are listed in Table VI-7. As seen in this data, dry storm sewer flow was not able to stimulate algal growth at any concentration tested and, in fact, seemed to inhibit production. It seems reasonable, then,



to assume that the effect of this input on the algal production in Lake Eola is negligible.

#### Physical-Chemical Effects of Stormwater Additions

Average water quality characteristics of urban stormwater collected from the Lake Eola drainage basin are listed in Table VI-7. As seen in this data, considerable variation exists in water quality characteristics between individual storm events with most parameters listed experiencing a 10-fold range of concentrations over the eight storm events tested.

Also, more than half of the nutrients released to the lake in stormwater runoff appear to be in dissolved form. Calculations of the mass loadings from these storm events are presented in Table VI-8. Mass loading averaged 8.3, 1.1, 8.5 and 0.03 kg/ha/cm of rainfall for suspended solids, BOD<sub>5</sub> and orthophosphorus, respectively. Assuming a drainage basin area of 55 hectares and an average rainfall of 128 cm, the total soluble orthophosphorus released to the lake would equal 211 kilograms/yr. Similarly, loadings from suspended solids, BOD<sub>5</sub> and TOC will equal to 48,432, 7744 and 59,480 kilograms per year, respectively. If all the suspended solids are transported to areas of the lake deeper than 6.5 meters, a buildup of solids approximately 0.15 meter deep will accumulate per year in these areas of the lake. This material, in addition to rapidly decreasing available water depth in the lake, also creates a significant oxygen demand during its

TABLE VI-7

AVERAGE WATER QUALITY CHARACTERISTICS OF URBAN STORMWATER  
COLLECTED FROM LAKE EOLA DRAINAGE BASIN

Date	Rainfall (inch)	pH	Spec. Cond. $\mu\text{mho/cm}$	Alk.	Turb JTU	SS mg/l	BOD <sub>5</sub> mg/l	TOC mg/l		Nitrogen mg/l-N		Ortho- Phosphorus mg/l-P
								Total	Diss.	TKN	$\text{NO}_3^-$	
7/11/78	0.18	6.9	---	34.8	26.9	142	10.0	33.4	26.6	4.35	1.33	0.44
7/14/78	0.11	7.8	209	59.6	18.9	77	22.0	38.2	30.1	2.14	0.55	0.25
7/25/78	3.20	7.6	66	134	31	129	5.7	21.8	10.2	0.65	0.42	0.10
8/03/78	0.04	7.6	71	25	24	88	9.0	30.6	13.1	--	0.62	0.15
8/10/78	0.43	7.3	38	15	14	74	4.6	18.9	10.1	0.65	0.29	0.13
8/15/78	0.18	7.5	193	69	44	134	16.3	178	137	1.04	3.20	0.67
10/12/78	0.10	7.4	256	65	65	275	16.4	284	248	3.30	0.39	0.28
11/07/78	0.04	8.3	337	95	28	27	18.3	218	206	3.29	8.90	0.53

TABLE VI-8

## MASS LOADINGS FROM LAKE EOLA BASIN

Date	Mass Loading, kg/ha-cm			
	SS	BOD <sub>5</sub>	TOC	Soluble Ortho-Phosphorus
7/11/78	18.5	2.6	4.1	0.06
7/14/78	4.4	0.6	6.0	0.02
7/25/78	8.9	0.3	1.0	0.02
8/03/78	9.3	0.9	3.6	0.02
8/10/78	4.5	0.3	1.6	0.03
9/15/78	5.9	2.0	20.0	0.02
10/12/78	13.0	1.0	18.0	0.02
11/07/78	1.8	1.1	13.4	0.03
Average	8.3	1.1	8.5	0.03
Calculated Loadings* (kg/yr)	58,432	7,744	59,840	211

\*Assumes a drainage basin area of 55 ha and average rainfall of 128 cm/yr.

decomposition and it may be responsible for anoxic conditions experienced during spring and summer months.

Of the dates on which stormwater was collected, the lowest concentrations of virtually every stormwater parameter seemed to occur during the months of July and August. These months are typically characterized by frequent and intense storm events. Since the dry period between storms is very short, the accumulation of transportable material in contributing areas of the watershed is minimized, and nutrient input into the lake per storm is reduced. However, since storm events occur almost daily during this period, the total input of nutrients over this rainy season will be very large although it will be stretched out over a period of several months. The frequent and intense nature of these storms serves to reduce concentrations of toxic heavy metals and organics while at the same time supplying the lake with a constant supply of phosphorus, nitrogen and carbon. This continuous supply of nutrients with reduced concentrations of toxic elements combined with warmer water temperatures during these summer months provides enriched conditions for algal growth, and one would expect the most rapid rate of algal growth to occur during this time. However, because dilution of algal populations by stormwater and the constant removal of the upper layers on the lake via the drainage well are constantly lowering these populations, the highly enriched growth which is occurring during this period is not reflected by in situ chlorophyll "a" concentrations.



In contrast to the enhanced algal growth conditions experienced during the summer rainy months due to stormwater events, runoff entering the lake after prolonged periods of drought may produce severe toxic effects on aquatic life in Lake Eola. Contaminants have been allowed to accumulate within the watershed over this period, and when a storm event occurs, the mass loading to the lake is many times larger than that experienced during a rainy period. This large influx of toxic and oxygen demanding wastes can be lethal to many forms of aquatic life. Evidence of such a phenomenon was recorded in March, 1979, when a rain event occurred after a 6 weeks dry spell. Concentrations of organic carbon as high as 400 mg/l were measured in stormwater runoff entering the lake during this event. Two days after this event, dissolved oxygen concentrations had been reduced from saturation near the surface to 4 mg/l at a depth of 1 meter and to virtually zero below 2 meters. Numerous large-mouth bass averaging 2-3 pounds were also found floating in the water, and large masses of dead filamentous algae had accumulated in thick mats over much of the lake's surface. The color of the water itself was changed from its characteristic blue-green tint to a gray-green appearance. Secchi disk depth was reduced to less than 0.5 meters. After approximately 5 days, conditions began to improve, and after 10 days physical conditions in the lake, as determined by dissolved oxygen profiles and Secchi disk measurements, had returned to near normal values for this time of year. However, it should not be

inferred that the lake had returned to the same ecological condition as before the storm event. The damage caused to the lake system by increases in sediment buildup and loss of animal species are difficult to document and can never be regained.

### Heavy Metal Toxicity

Although the algal production measured in these bioassays would certainly correspond to eutrophic conditions, it appears the production in Lake Eola may actually be partially inhibited by toxic elements in the stormwater runoff. The growth response of Selenastrum in a synthetic algal medium is shown in Figure V-1. Since this medium is by design a phosphorus limited medium (EPA, 1978), then algal growth should continue until all available phosphorus has been utilized by organisms. A total of 120 mg of dry cell weight per liter of Selenastrum were produced from the 0.186 mg/l of phosphorus contained in the synthetic algal medium. From this relationship, it can be calculated that 0.01 mg/l of phosphorus will produce, assuming all other essential nutrients are present in sufficient quantities needed for growth, 6.45 mg/l of Selenastrum cell mass. Accordingly, the 0.017 mg/l of phosphorus present in the lake water sample collected on 8/10/78 should have produced 10.97 mg/l of dry cell mass assuming no toxic effects existed and all other nutrients necessary for growth were present. As seen in Table VI-3, none of the bioassay experiments with the exception of the one in which EDTA was added, were able to produce the maximum predicted yield.

The fact that the addition of EDTA was responsible for increasing the experimental maximum yield strongly suggests the presence of heavy metal stress in Lake Eola. The additions of EDTA to a lake water sample collected on 2/21/79 resulted in a two-fold increase in cell yield (Figure V-4 and Table VI-5). An inhibition of 47% was calculated for this experiment between control flasks incubated with and without EDTA. EDTA may act to stabilize ferrous iron, increasing the availability of this ion for aquatic growth while at the same time suppressing heavy metal toxicity by chelation (Miller, et. al., 1976). However, since the addition of iron to this experiment in control flasks without EDTA did not stimulate algal production, it was concluded that the increase in production caused by EDTA was due largely to suppression of heavy metal toxicity. Toxic levels of selected heavy metals to Selenastrum along with average Lake Eola water and stormwater levels are listed in Table VI-9. It can be seen that normal background concentrations of copper and zinc in Lake Eola itself are sufficient to cause continuous incipient inhibition of algal production in certain species. Average heavy metal concentrations in stormwater collected near Lake Eola indicate an algicidal level of copper with a zinc concentration sufficient to cause complete inhibition. Although no information is listed for toxic levels of lead, the average concentration of 1500  $\mu\text{g/l}$  of lead found in stormwater runoff at Lake Eola can be assumed to exhibit almost certain algicidal effects. Experiments reported by Shiroyama (1976) and Greene



TABLE VI-9

TOXIC LEVELS ( $\mu\text{g}/\text{l}$ ) OF HEAVY METALS TO SELENASTRUM ALONG WITH AVERAGE LAKE EOLA WATER AND STORMWATER CONCENTRATIONS

Element	Incipient Inhibition	Complete Inhibition	Algicidal	Lake Eola	Stormwater
Cu	50	90	300	48	513
Zn	30	120	700	35	423
Cd	50	80	650	1	25
Pb	--	---	---	69	1,580
Cr	--	---	---	29	46
As	--	---	---	58	94
Ni	--	---	---	8	30
					152

SOURCE: L. Bartlett and F. Rabe, "Effects of Copper, Zinc and Cadmium on Selenastrum Capricornutum," Water Research, Volume 8, March 1974, pp. 179-185.



(1975) on the toxicity of zinc to Anabaena and Selenastrum indicate that a zinc concentration corresponding to the average Lake Eola concentration of 0.048 mg/l was sufficient to reduce the yield of Anabaena and Selenastrum to only 20-40% of the maximum yield. Similar concentrations of lead and arsenic are also present in Lake Eola water and may exhibit similar exhibitory effects. Synergistic effects due to the simultaneous presence of various heavy metals may increase this toxic effect and further limit algal growth. Greene further suggests that ionic strength may be a prime factor in regulating zinc toxicity upon the growth of Selenastrum. Sensitivity of the test alga to zinc appears to be inversely proportional to the ionic strength of the test substrate. Ion pair formation with some of the more common cations present in a receiving water such as calcium, magnesium or sodium may be a significant process by which the availability of zinc in waters of high ionic strength is altered. In the absence of synergistic constituents, the factor of  $2.72 \pm 20$  percent when multiplied by the ionic strength ( $\mu\text{mhos/cm}$ ) of a test substrate should indicate the level of zinc in  $\mu\text{g/l}$  that would inhibit 95% of growth in the test organism. If the average specific conductivity of Lake Eola water is assumed to be 300  $\mu\text{mhos/cm}$  (Wanielista, 1976), then the zinc concentration which would inhibit 95% of growth in this system would be approximately twice the average stormwater concentration of 423  $\mu\text{g/l}$ . It seems reasonable that, although the concentrations of zinc in Lake Eola may not produce this 95%

inhibition in algal production, the concentrations of zinc found in stormwater, even disregarding any synergistic effects, are sufficient to cause significant inhibition in Lake Eola.

Since limiting algal production is the essence of a lake restoration project, it would seem that the heavy metal inhibition found in Lake Eola is not totally undesirable. However, the algicidal and inhibitory effects of these toxic compounds do contribute to other serious problems in Lake Eola. As seen in Figure V-9, the response of a test organism to concentrations of stormwater runoff larger than 25% is often an initial die-off of a portion of the cells with the magnitude of this die-off being a function of the composition of the stormwater entering the lake. Although a storm event of sufficient magnitude to produce a concentration of stormwater in the lake of 25 percent is unlikely, these concentrations do occur near stormwater outfalls. Density gradients due to the large amount of dissolved and suspended solids in stormwater runoff may make water surrounding these areas somewhat resistant to mixing and allow sufficient time for algicidal effects to occur. Perhaps the major problem associated with this die-off is an increase in sediment buildup. With the exception of areas near the shore, the bottom of Lake Eola is covered with a thick layer of silt and flocculant material, increasing in depth as one nears the center of the lake. Besides contributing large quantities of phosphorus and other metals (Marshall, 1980) during periods of anoxia (Marshall, 1980) and creating a continuous

oxygen demand, the consistency of this material makes it impossible for rooted submergent plants to develop. The absence of these plants severely limits the fauna that can develop within the lake creating a system characterized by low diversity and a corresponding lack of stability. Benthic organisms, which also play an important role in regulating decomposition and release of sediment material, are limited to only a few specialized forms. The increasing buildup of heavy metals within the lake also makes the problem of biological magnification very real. Lake Eola is commonly used as a winter home for many species of water fowl. If these animals feed on organisms within the lake heavy metal concentrations may build up within their bodies posing serious health problems to hunters who may shoot these birds for food on their return north.

## CHAPTER VII

### SUMMARY AND CONCLUSIONS

During the course of this research, monthly water quality analyses were performed in Lake Eola and bioassay experiments were conducted to determine the effect of nutrients and stormwater additions on algal productivity in this lake system. Coagulation of stormwater to remove nutrients and limit algal production was also studied. From the results obtained in this research, the following conclusions were reached:

1. The input of stormwater into Lake Eola has severely damaged this aquatic system. Persistent algal blooms exist virtually year round. Bottom sediments have become covered with a layer of loose flocculant material and anoxic conditions exist in deep areas of more than 4 meters deep during the spring and summer.
2. Bioassay research can be a useful tool in predicting algal responses to pollution loads as long as the proper combination of test organism and sample treatment which best represent the aquatic system are used.
3. Bioassay preparation techniques other than filtration only were found to decrease concentrations of organic carbon, inorganic carbon, orthophosphorus, and heavy metals, while at the same time increasing nitrate nitrogen concentrations.
4. Autoclaving of a water sample is not a suitable treatment technique for use in bioassay studies where the effects of heavy metals or complex organic compounds are to be determined. In these cases, filtration only should be used.
5. The use of indigenous algal species in a bioassay experiment may encounter problems in enumeration and can be characterized by a slow growth response.



However, the use of these species can be a useful tool especially in systems where continued exposure to pollutants has produced a certain amount of biological resistance.

6. When the concentration of orthophosphorus in Lake Eola is less than 0.10 mg/l, algal production is regulated by the addition of orthophosphorus alone. Above this concentration, it appears that an excess of phosphorus is available, and algal growth is regulated by the N:P ratio. However, in most cases the concentration of orthophosphorus in Lake Eola water is below 0.04 mg/l, and algal production is limited by the concentrations of added phosphorus alone.
7. Although nitrogen was able to stimulate algal production in limited bioassay experiments, it will probably not be a limiting resource in Lake Eola when considered over a period of years due to the large numbers of nitrogen-fixing blue-green algae which are characteristic of eutrophic systems.
8. Additions of stormwater runoff to Lake Eola water in any concentration will result in an increase in algal production. A mixture of 25% stormwater runoff will produce the largest standing crop.
9. Coagulation of stormwater runoff with alum will reduce concentrations of both orthophosphorus and nitrate nitrogen by 80-95%. Certain heavy metals will also be removed.
10. Coagulation of stormwater runoff will significantly reduce the growth potential of this pollution source while at the same time producing a produce which is less toxic to aquatic organisms.
11. Dry weather storm sewer flow has a negligible effect on algal production in Lake Eola.
12. Maximum algal growth in bioassay experiments generally occurred after 6-11 days of incubation. However, when stormwater runoff is added to Lake Eola water, a growth lag is often experienced which may extend the time required to reach maximum yield to as many as 18 days.
13. Continuous stormwater inputs into Lake Eola during the rainy season will produce greatly enhanced algal

growth due to the constant input of nutrients and dilution of toxic components. Inputs of stormwater after a long dry spell may inflict serious toxic effects on aquatic life.

14. Concentrations of copper and zinc in Lake Eola itself are sufficient to cause incipient inhibition of algal production. Average stormwater runoff concentrations of copper, zinc and lead are sufficient to produce complete inhibition or algicidal effects.

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