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AN ABSTRACT OF THE THESIS OF Michael Patrick Mulvey for the Master of Science in Biology presented September 17, 1986.

Title: Nitrogen Limitation of Phytoplankton Growth in an Oligotrophic Lake.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

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Blue Lake, Jefferson County, Oregon, has high summer surface phosphorus concentrations (ca. 30 ug/l) yet is oligotrophic (summer Secchi depth is 11 to 16 meters). Nutrient enrichment experiments done with 1000 l polyethylene enclosures indicate nitrate limitation of phytoplankton growth. Basin morphology may be an important

factor in nutrient cycling in this lake. The lake has a maximum depth of 95.7 meters with an average depth of 42.7 meters. The lake basin has steep sides with only 4% of the lake bottom less than 3.3 meters deep. The lake basin is of recent volcanic origin.

In contrast, Suttle Lake, which is immediately downstream from Blue Lake, is moderately eutrophic (Secchi depth 1.7 meters) and supports much larger populations of phytoplankton, including nitrogen fixing cyanophytes. Suttle Lake is shallower and more subject to wind mixing.

NITROGEN LIMITATION OF PHYTOPLANKTON GROWTH IN AN OLIGOTROPHIC LAKE

by

MICHAEL PATRICK MULVEY

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University

1986

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CHAPTER I

INTRODUCTION

Questions Examined in This Thesis

Blue Lake and Suttle Lake are neighboring bodies of water in Oregon's Cascade Range. The difference in the quality of these two lakes is striking. The water in Blue Lake is very clear with sparse phytoplankton populations (Secchi depth 16 meters) while Suttle Lake has much denser phytoplankton growth (Secchi depth 5.2 meters).

These lakes would be expected to have similar phytoplankton populations since they are in the same drainage basin, experience the same climate, and seem to have comparable levels of most major phytoplankton nutrients, with the exception of iron. Iron is a nutrient needed in trace amounts that is much lower in Blue Lake than in Suttle Lake. The levels of the major nutrients, particularly phosphorus, suggest that Blue Lake should be much more productive than it is and have phytoplankton populations characterized by nitrogen fixing species.

The question examined in this thesis is why Blue Lake is so clear. The hypothesis is that Blue Lake is a

nitrogen limited system due to the scarce supply of iron. This question was investigated using nutrient additions to lake water with natural phytoplankton populations enclosed in large plastic tubes to which nitrogen fixing cyanophytes had been added. The tubes were incubated *in situ* and phytoplankton growth response was observed. The stimulation of phytoplankton growth in a tube would be evidence that the nutrient added to that tube was limiting growth in the lake.

CHAPTER II

BACKGROUND INFORMATION

An Overview of Nutrient Limitation of Phytoplankton Growth

Lakes can be classified on a trophic scale that ranges from ultraoligotrophic to hypereutrophic. A major interest in limnology is to be able to understand what controls the trophic status of lakes. The type and abundance of organisms in a lake are a result of the interaction of the biochemistry and physiology of the organisms with the many environmental variables of the lake ecosystem. A key environmental variable is nutrient availability. Liebig's "Law of the Minimum" states that the growth of any particular species of organism will be determined by the abundance of the substance that is least abundant in relation to the needs of the organism (Odum,1971). Thus, total abundance of all species of phytoplankton will be determined by the availability of one or more limiting nutrients.

Even though lakes are very complex and varied systems, it now is widely accepted that phosphorus is the nutrient that is critical in controling the abundance and character

of most lake phytoplankton populations (Schindler, 1977). In comparison to other major nutrients, (i.e. carbon, hydrogen, oxygen and nitrogen) usually phosphorus is least available in proportion to the requirements of the phytoplankton and therefore commonly limits the biological activity and determines the trophic status of lakes. Unfortunately, human activity in a watershed tends to increase lake phosphorus through erosion, agricultural runoff and sewage pollution (Goldman and Horne, 1983). Phosphorus is an extensively studied element in limnology and it has attracted the wide attention of aquatic ecologists as well as others interested in water quality management. Lake phosphorus inputs are used in models to predict productivity (Vollenweider and Kerekes, 1980). Reducing phosphorus inputs is typically a key part of any lake restoration program (Wetzel,1978,p 643).

Behavior of Phytoplankton Nutrients

In order to grow and reproduce phytoplankton require a number of nutrients from their environment. Any of these nutrients could potentially limit growth. Carbon, hydrogen, oxygen, nitrogen and phosphorus are the major structural components that make up the cell and are referred to as the macronutrients since they are needed in relatively large quantities.

Phytoplankton need a source of inorganic carbon for photosynthesis. Inorganic carbon (CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}) is soluble in water so that diffusion from the atmosphere is usually more than adequate to meet the needs of growing phytoplankton populations. Carbon limitation in natural systems is unlikely even though it is the nutrient required in the greatest quantity. (Stagnant sewage treatment lagoons might be an exception.)

Nitrogen is needed by phytoplankton for amino acids, and is therefore required in relatively large quantities (about 5% total nitrogen by dry weight, Goldman and Horne, 1983). Inorganic nitrogen species (such as nitrate and ammonia) usable by eukaryotic phytoplankton are water soluble and are typically derived from sources outside the lake via surface runoff as well as internal recycling from the decomposition of nitrogen containing sediment and organic matter, and from zooplankton excrement. In very productive systems where the available nitrogen has been exhausted relative to other nutrients, particularly phosphorus, the abundant atmospheric nitrogen (N_2) can be fixed by some cyanophytes. The atmosphere is 78% molecular nitrogen but is in a form that is unavailable to eukaryotic phytoplankton. Nitrogen fixation will in time remove the nitrogen limitation, causing limitation by some other nutrient (Schindler, 1977).

Phosphorus is important in cells as essential components in membranes, nucleic acids and high energy intermediates. Unlike nitrogen, phosphorus has no gaseous phase so that there is no phosphorus equilivent to nitrogen fixation. Phosphorus available to phytoplankton is in the form of dissolved free phosphate and organic phosphate. Phosphate tends to be adsorbed to sediments and clay minerals, thus exporting this nutrient from the water column to the lake sediments where it is unavailable to the phytoplankton. Natural phosphorus sources are from the weathering of phosphorus containing rock in the drainage basin and from recycling of phosphorus from lake sediments. Phosphorus containing minerals are often geochemically scarce in rock. Anthropogenic sources of phosphorus in lakes are from runoff from agricultural lands, sediment from errosion, and sewage and other waste water. Detergents can be a rich source of phosphorus. Even though phytoplankton require smaller amounts of phosphorus compared to the other macronutrients already discussed, it is typically the growth limiting nutrient due to its slight availability and behavior in lake systems (Goldman and Horne, 1983, Chapter 9).

In addition to the major nutrients there are a large number of elements needed in smaller quantities that could also limit phytoplankton growth. These include iron,

silicon, calcium, magnesium, sodium, potassium, sulfur, chlorine, and trace metals such as manganese, copper, zinc, molybdenum, nickel and cobalt. Although natural concentrations are usually quite dilute, most of these biologically important elements are available in excess in proportion to the other nutrients, so that they are generally not growth limiting and will not be discussed further here. However, there can be exceptions, particularly if the watershed is unusually deficient in one or more of these elements, or if there is a high demand for a particular element.

One trace nutrient that is sometimes limiting is silicon. Biologically available silicon in aquatic systems is probably in the form of dissolved silicic acid, $Si(OH)_4$. Diatom frustules are hydrated amorphous silica, SiO_2, formed by the condensation and polymerization of silicic acid. Extensive diatom growth can sometimes deplete available silicon causing it to become growth limiting for diatoms. Silica is sparingly soluble under the conditions found in most natural lakes and its behavior in the aquatic environment is different than most other nutrients in that it moves in a one way flow from weathered rock in the watershed to the organisms to the lake sediments. Recycling accounts for very little of the silica budget of a lake. Diatoms are much more sensitive to silicon

limitation than other types of phytoplankton and silicon depletion can lead to a shift in the population composition toward species other than diatoms (Lewin, 1962, and, Werner, 1977).

Although iron is considered a micronutrient it is needed in fairly large quantities compared to other micronutrients. Iron is an essential part of many enzymatic pathways including photosynthesis, cellular respiration and nitrogen fixation. Iron in lakes can exist as a number of different chemical forms. Dissolved iron can be present as dissolved inorganic compounds, as a chelated organic complexes and as free ions. In well oxygenated water, the ferric (Fe+3) form will predominate rather than the ferrous (Fe+2) form. Ferric iron forms insoluble compounds that can rapidly precipitate iron from the water column to the sediments where it is unavailable to phytoplankton. In addition, phosphate ions tend to be adsorbed to these iron compounds and are also precipitated out of solution along with the iron. In lakes with anoxic hypolimnia or sediments the reducing environment will reduce the insoluble ferric iron to soluble ferrous iron, thus recycling the iron and phosphorus from an insoluble to soluble form. Iron limitation has been demonstrated in cyanophyte blooms of eutrophic lakes that have low N/P ratios (Elder and Horne, 1977; Wurtsbaugh and Horne, 1983;

Swain, 1984). In these lakes nitrogen fixation by cyanophytes accounted for a considerable part of the nitrogen budget. Nitrogen fixing cyanophytes have a higher nutritional requirement for iron than other phytoplankton due to the iron contained in the nitrogenase enzyme.

In summary, the chemical behavior of the elements involved in aquatic environments, drainage basin geochemistry, lack of a large atmospheric reservoir of the element, as well as the nutritional requirements of the phytoplankton all lead to phosphorus limitation in lakes, provided that nitrogen fixation can take place in conditions of low N:P ratio. Lakes with high phospohorus and low nitrogen that are not limited by some other nutrient favor the growth of nitrogen fixing cyanophytes that do not have to rely on combined forms of nitrogen for growth.

Phosphorus and the Primary Productivity of Oregon Lakes

The basic chemical, biological and geographic features of over 200 Oregon lakes were surveyed from 1981 to 1984 by the Clean Lakes Program in Oregon (Section 314 of Public Law 92-500). As expected, the primary productivity of Oregon lakes, as measured by chlorophyll *a* concentration, correlates well with phosphorus concentration (Figure 1). This is in general agreement with the widely held axiom of

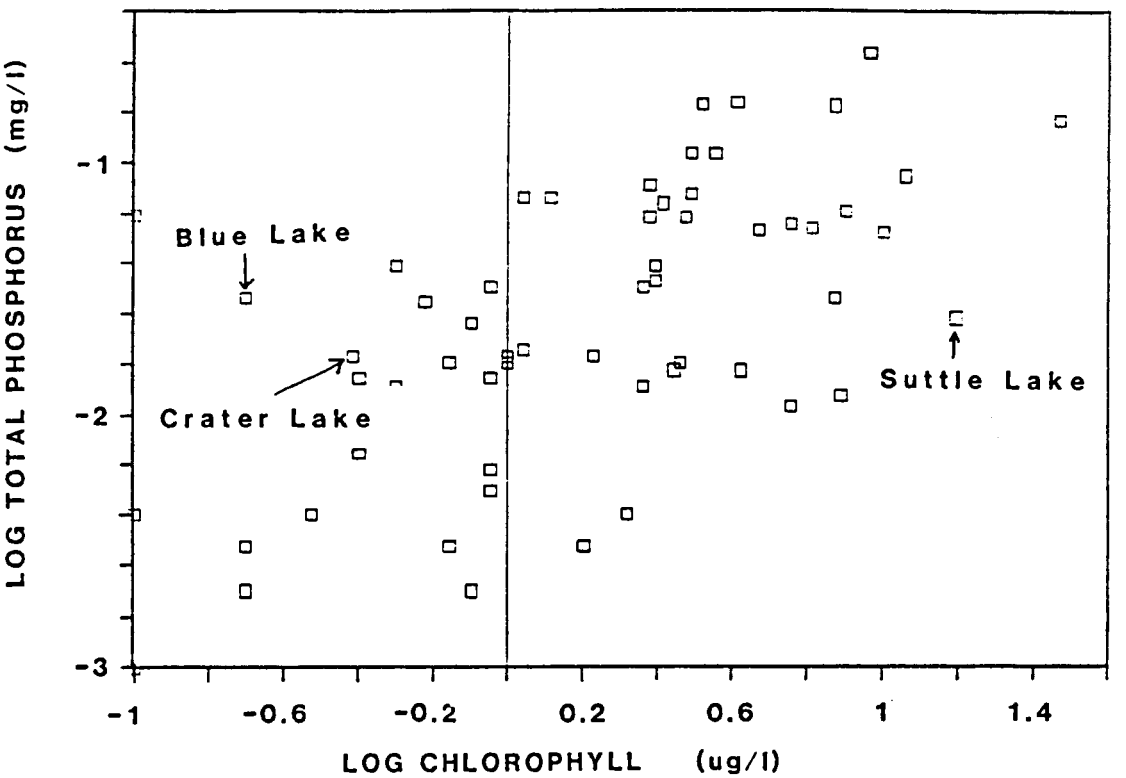


Figure 1. The correlation between primary productivity in selected Oregon Lakes (measured by chlorophyll*a* concentration) and total phosphorus concentration (data from Johnson, et.al., 1985).

phosphorus limitation of lake productivity already discussed. However, there are a few Oregon lakes that appear to be exceptions in that they have high phosphorus concentrations and low N/P ratios, yet have low chlorophyll a concentrations and lack the expected nitrogen fixing cyanophytes. These include Crater Lake and Blue Lake (Jefferson County). These lakes are located in the Cascade Range in areas of recent volcanic activity. These lakes are also characterized by low iron concentrations compared to more fertile lakes with similar phosphorus content, such as Suttle Lake. Filterable iron concentration in Suttle Lake was observed to be 27 nmole/l, compared to observed values of only 13.7 nmoles/l in Blue Lake and 6.5 nmole/l in Crater Lake (Collier, unpublished data). Suttle Lake is immediately downstream from Blue Lake. Seasonally dense blooms of nitrogen fixing Anabaena sp. have been observed in Suttle Lake.

A comparison of central Oregon Cascade volcanic rock indicates that the unusual geochemistry of this area could produce a scarcity of iron in the Blue Lake basin. The rocks of the major eruptive episodes in this area become progressively richer in sodium and progressively poorer in other elements, particularly iron (McBirney and White, 1982). The volcanic events that formed the Blue Lake basin are one of the more recent ones in the volcanic history of

the area (Scott, 1974).

The hypothesis of this research is that Blue Lake is a nitrogen limited lake, and that nitrogen fixing cyanophytes are missing from Blue Lake due to the short supply of available iron needed to synthesize the nitrogenase enzyme.

Description of Blue Lake

Blue Lake is a small, deep alpine lake located in the Cascade Mountains 23 miles (14.3 km) northwest of Sisters off US Highway 20, and 3.5 miles (2.2 km) east of the crest of the Cascade Range (Figures 2 and 3). The lake is a maar formed by an explosion of steam when ground water came in contact with hot subsurface volcanic rock. The basin is the result of at least three explosions that formed overlapping craters along an axis N 25 E. Radiocarbon dating of burried charred wood places these events that formed the basin at 3460 +/-250 years ago (Taylor, 1965). Prior to the events that formed Blue Lake, the entire watershed had been glaciated late in the Pleistocene Epoch. Suttle Lake was formed by damming with terminal and lateral moraines. The south shore of Blue Lake is a steep, narrow rock ridge that is about 300 feet (91.4 m) above the water level and about 150 feet (45.7 m) above the adjacent topography. (Baldwin, 1981; Scott 1974). There are no perennial surface stream

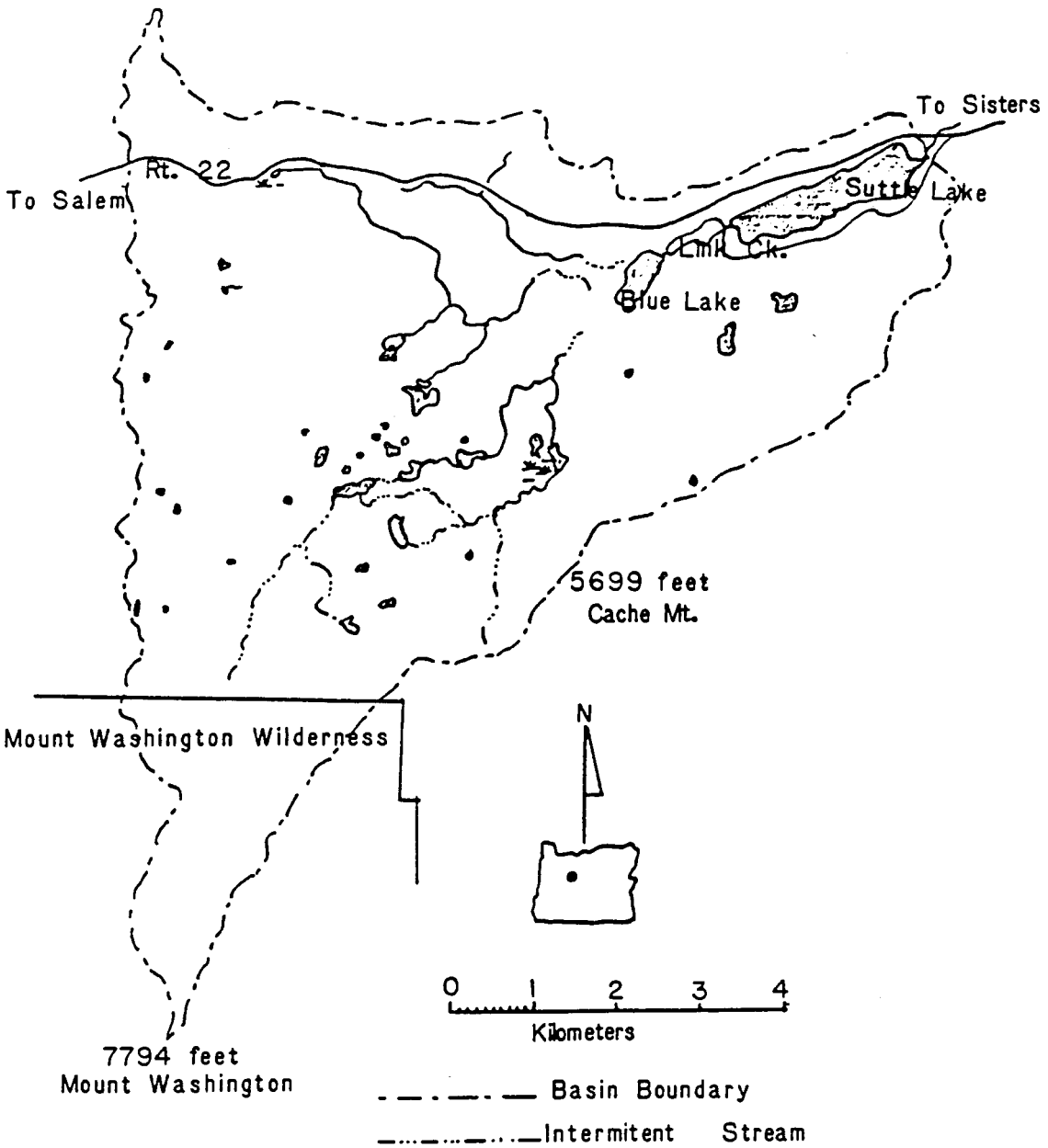


Figure 2. The Blue Lake-Suttle Lake drainage basin.

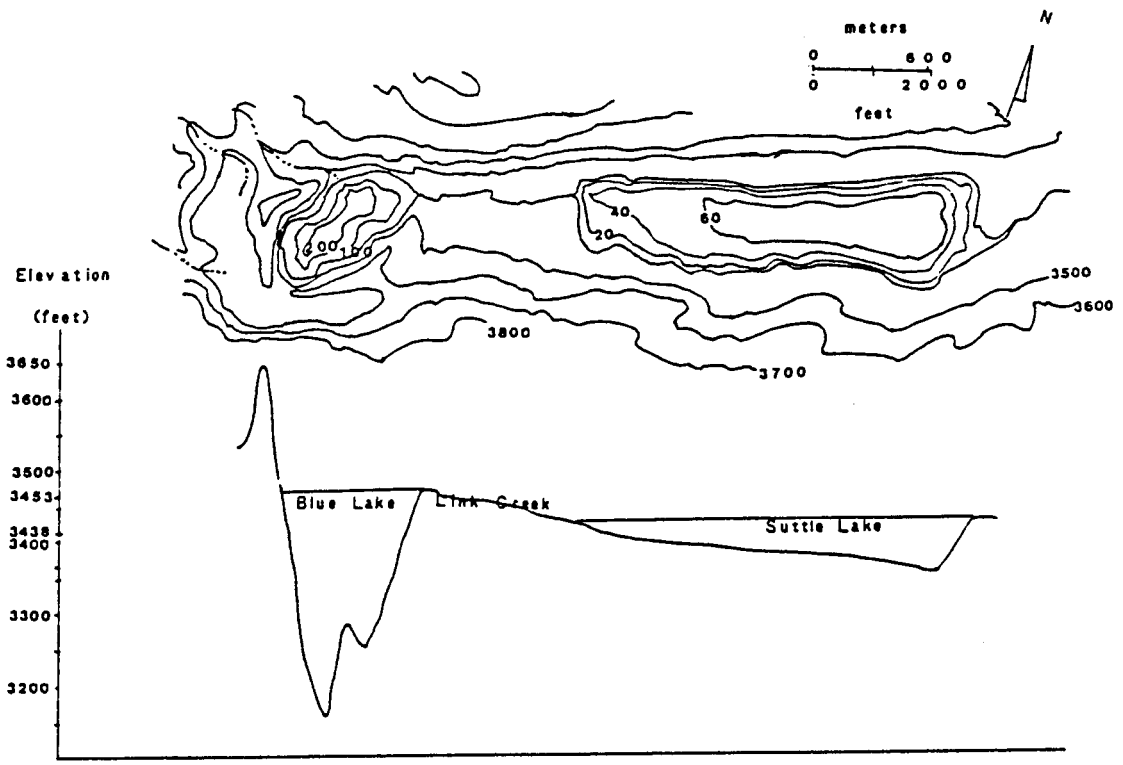


Figure 3. Bathymetry of Blue and Suttle Lakes. Elevation of data is given as feet above sea level. To convert to meters multiply by 0.3048 ft/m. The contour interval is 100 feet in Blue Lake and surrounding land, and 20 feet in Suttle Lake.

flows into Blue Lake. The source of the majority of the water in the lake is thought to be a large, subsurface spring about 240 feet (73 m) below the water surface near the southeastern shore of the lake (Johnson, et. al., 1985).

The Blue Lake-Suttle Lake drainage basin is within the Deschutes National Forest with the southwest portion lying in the Mount Washington Wilderness. In the Blue Lake drainage basin 95% of the land cover is forests, with several hiking trails and horse trails. Corbett Memorial State Park at the southern end of the lake has access by hiking trail only. At the northern end of Blue Lake there is a private home, Blue Lake Resort, with cabins, campgrounds, a boat dock, boat rental, and a small swimming pond. The lake is well stocked with fish and is popular with fishermen.

Blue Lake flows into Suttle Lake via 0.5 mile (0.3 km) Link Creek. Suttle Lake receives considerably more recreational use than Blue Lake. It is ringed with several National Forest campgrounds, hiking trails, a resort and marina, and church owned camps. The lake is popular with water skiers.

In contrasting the limnological features of these two neighboring lakes, Blue Lake is distinctly oligotrophic with an orthograde dissolved oxygen profile (Secchi depth =

16 meters) while Suttle Lake tends towards eutrophy (Secchi depth = 5.2 meters)(Table 1). Blue Lake is much deeper with little littoral area (maximum depth = 95.7 meters, shoal area = 4%) compared to shallower Suttle Lake (maximum depth = 22.9 meters, shoal area = 10%) (Johnson, et.al., 1985).

TABLE I

LIMNOLOGIC FEATURES OF BLUE AND SUTTLE LAKES

FEATURE	BLUE LAKE	SUTTLE LAKE
LAKE MORPHOMETERY		
LAKE AREA (acres/hectares)	54/22	253/102
LAKE DEPTH-MAXIMUM (meters)	95.7	22.9
LAKE DEPTH-MEAN (meters)	42.7	13.5
VOLUME (cubic hectometer)	9.31	13.79
SHOAL AREA (area < 3.3m deep)	4%	10%
RETENTION TIME (months)	2.4 (approx.)	3.5
DRAINAGE BASIN CHARACTERISTICS		
DRAINAGE BASIN AREA (sq. km.)	43	53
LAND USE		
FOREST	95.0%	93.0%
ROCK OUTCROP	3.0%	3.3%
RANGE	1.0%	1.0%
WATER	1.0%	2.7%
ELEVATION (feet/meters)	3453/1052.5	3438/1047.9
WATER QUALITY DATA		
SECCHI DEPTH (meters)	16.0	5.2
TEMPERATURE (C)	13.5	19.5
CHLOROPHYLL a (ug/l)	0.2	25.1
NO3 (micromolar)	0.26	0.56
PO4 (micromolar)	1.3	0.64
N/P ratio	0.20	0.88
pH	6.9	7.9
Mo (nanomolar filtered)	2.5	1.33
Mo (nanomolar unfiltered)	2.2	1.31
Mn (nanomolar filtered)	2.9	2.4
Mn (nanomolar unfiltered)	5.7	13.5
Fe (nanomolar filtered)	13.7	27

Temperature data 8/25/85. Nutrient data 8/29/85. All data except nutrient and temperature from Johnson, et.al.,1985. Blue Lake data 7/21/82. Suttle Lake data 11/7/82. Lake retention times D. Johnson, personal communication. Metals data from R. Collier, unpublished data.

CHAPTER III

EXPERIMENTAL DESIGN AND METHODS

Experimental Design

Field studies were conducted at Blue Lake from September 6, to September 28,1984, and from August 28 to September 12, 1985. The basic experimental design involved adding nutrients to natural populations of phytoplankton in *in situ* incubation inclosures innoculated with Suttle Lake water in which a nitrogen fixing *Anabaena* sp. was the most abundant species. The enclosures used were an adaptation of devices described by a number of investigators, including: Strickland and Terhune, 1961; Goldman, 1962; Gerhart and Likens, 1975; Lean, et.al.,1975; Liao and Lean, 1978; Landers, 1979; Wurtsbaugh and Horne, 1983; Schelske, 1984; and Swain, 1984.

The enclosures were transparent 0.003 gauge polyethylene tubes (from the Cellocraft Bag Co., Portland, OR) measuring 4 meters long and 0.6 meter in diameter after filling. They were installed in the northwest side of the lake (See Figures 4 and 5). This area was chosen because it appeared to be most wind protected and was furthest from

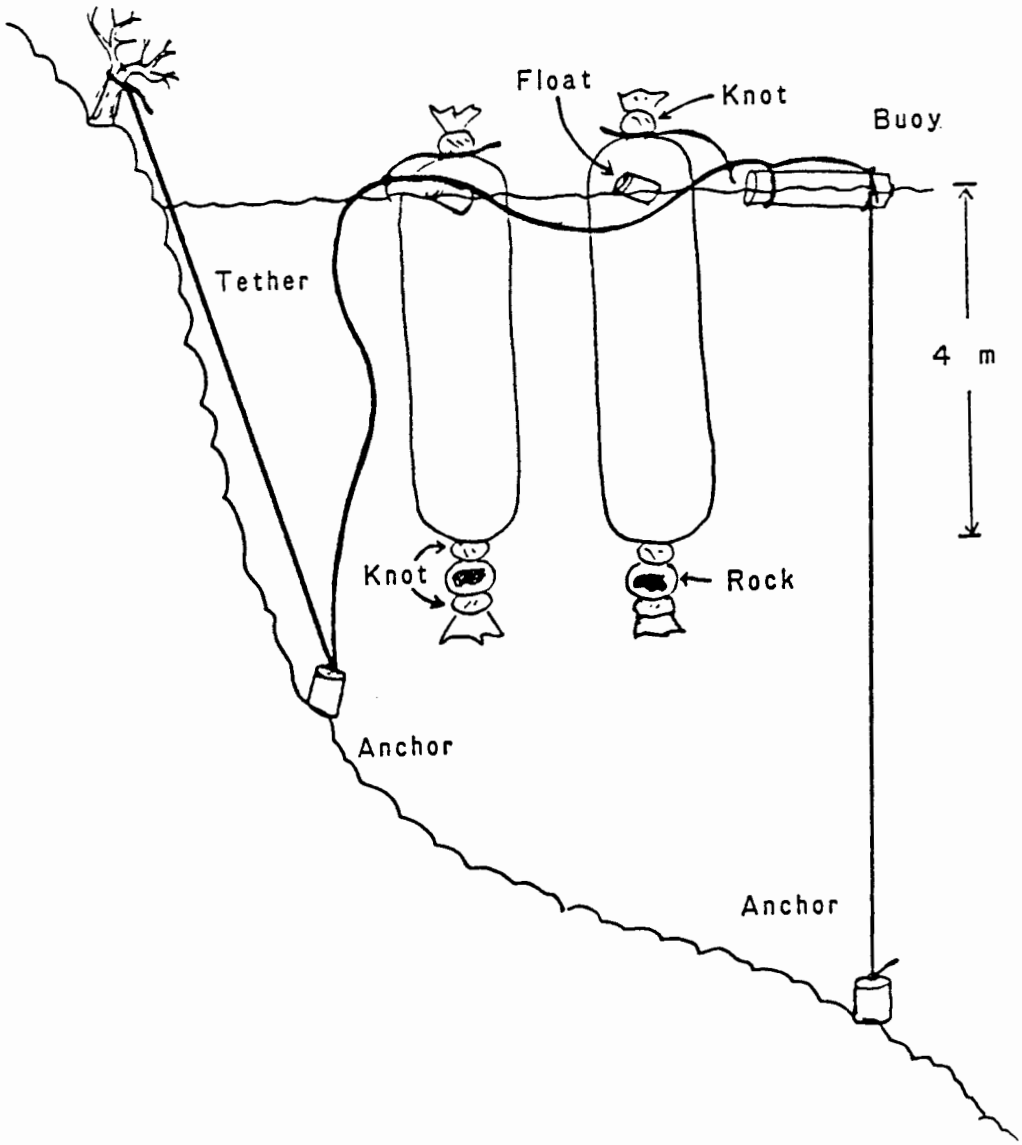


Figure 4. Cross section of the treatment enclosures used in Blue Lake.

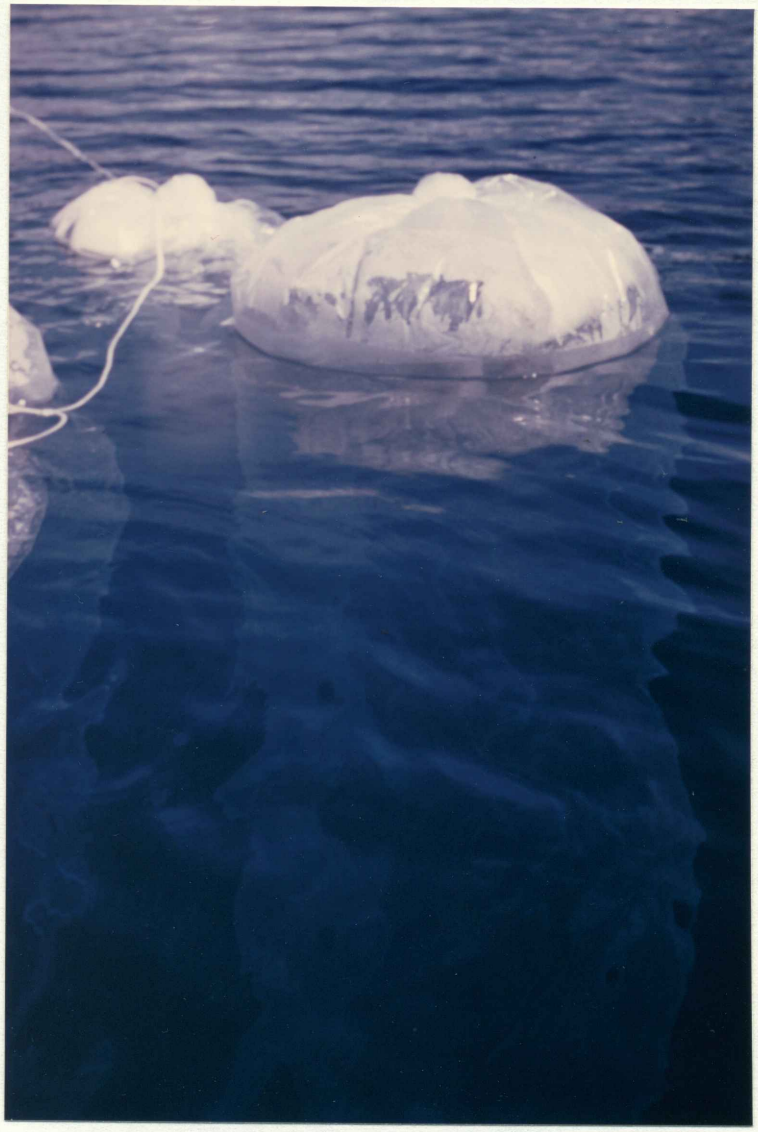


Figure 5. A treatment enclosure in Blue Lake. Note how clear the water is.

the boat dock. Each tube held approximately 1000 liters of water. Tubes were knotted at one end and weighted with a rock. The rock was between two knots in the plastic tubing so that it was not in contact with the enclosed water. The tubes were filled by towing them behind a row boat. The untied end of the bag was held open by folding it over a plastic hoop. Care was taken to keep the the hoop completely submerged to exclude possible floating particulate contamination. An empty 0.5 gallon (1.9 l) polyethylene bottle was placed in each tube as a float. The open top end of the tube was knotted closed and tethered to an anchored line. The tubes floated vertically and had some limited freedom of movement by the wind so that the relative position of any one tube in the group randomly changed.

Treatments: Nutrient Additions and Inoculations

Various nutrient solutions and Anabaena sp. from Suttle Lake were added to the filled enclosures. All treatments were duplicated. In 1984 the inoculum was collected at the Lake Creek outflow of Suttle Lake. Anabaena sp. was the most numerous species (294.5 cells/ml) in the inoculum. Thirteen less abundant species were also observed. These were Cyclotella sp. (93 cells/ml) and two species of Synedra (51 and 25 cells/ml). Fragilaria sp.,

Fragilaria crotonensis, Cocconeis sp., an unidentified small pennate diatom (25 cells/ml each), Navicula sp., Asterionella formosa, Ceratium sp., Scenedesmus sp., Epithemia sp., and Nitzschia sp. (12 cells/ml each) were also present but in low numbers. Each tube received approximately 1.9 liters of this inoculum.

In 1985 the innoculum was collected by pulling a plankton net through a dense surface bloom of Anabaena at the western end of Suttle Lake. This inoculum was far more concentrated than the previous inoculum. The Anabaena was too dense to determine cell concentration from sampling directly. However, the Anabaena contributed by the inoculum was sufficiently diluted to permit accurate counting of Anabaena in the experimental tubes. The initial Anabaena concentration in the tubes ranged from 264 to 1450 cells/ml. Other less abundant species also present in the inoculum were Cyclotella sp. (321 cells/ml), two species of unidentified pennate diatom (104 cells/ml each) and Staurastrum sp. (211 cells/ml). The cells in the 1985 inoculum were very dense and poorly preserved in the sample collected for counting, making enumeration difficult and less accurate. The concentrated algae were placed in a 20 liter plastic carboy. The carboy was shaken to distribute the cells evenly prior to taking aliquots. Each tube received 1 liter of this inoculum.

Duplicate tubes were treated with different nutrient additions. In the 1984 study the treatments were as follows: a high iron treatment, a low iron treatment, a combined nitrate and phosphate treatment, and a control treatment. In the 1985 study the treatments were as follows: an iron treatment, a nitrate only treatment, a phosphate only treatment, a combined nitrate and phosphate treatment, and a control treatment. For the control treatments a volume of distilled water equal to that of the nutrient addition solution was added. (See Table II for information on the concentrations of the nutrient additions.)

Iron added to the tubes in 1984 was FeSO4 dissolved in 2 liters of distilled water acidified with 10 drops of H2SO4. Dissolved oxygen was removed from the water by bubbling with nitrogen gas for one hour before and 5 minutes after adding the FeSO4 to prevent oxidation of the iron. The solution was stored in tightly stoppered brown glass jugs.

The iron treatment in 1985 was chosen to approximately double the total iron concentration and increase the filterable iron by approximately 15 times. Ambient Blue Lake iron concentrations are 100 nanomole/l total iron, 13.7 nanomoles/l filterable iron (Collier, unpublished data). Iron was added in the form of chelated Fe-EDTA.

TABLE II

NUTRIENT SPIKES ADDED TO THE TUBES AND AMBIENT LAKE NUTRIENT CONCENTRATIONS

1984: ONE LITER SOLUTIONS

TREATMENT	CHEMICAL	FINAL CONCENTRATION
High Iron	21g $FeSO_4-7H_2O$	49.5 ug Fe/l
Low Iron	4.3g $FeSO_4-7H_2O$	10.1 ug Fe/l
Nitrate plus Phosphate	100 mg $NaNO_3$ 15 mg KH_2PO_4	73 mg NO_3/l & 10 mg PO_4/l

1985: FOUR LITER SOLUTIONS

Iron	0.0296 g $FeSO_4-7H_2O$	100 nmoles Fe/l
Nitrate only	100mg $NaNO_3$	73 mg NO_3/l
Phosphate only	15 mg KH_2PO_4	10 mg PO_4/l
Nitrate and Phosphate	same as the separate spikes	

AMBIENT NUTRIENT CONCENTRATIONS IN BLUE LAKE

Nitrate	0.016 mg NO_3/l	(0.26 nmoles/l)
Phosphate	0.124 mg PO_4/l	(1.30 nmoles/l)
Iron-total	5.58 ug Fe/l	(100 nmoles/l)
Iron-filterable	0.76 ug Fe/l	(13.7 nmoles/l)

Nitrate and phosphate data: 8/29/85.
Iron data: R.Collier, unpublished data.

Sampling

The tubes and the lake were periodically sampled for nutrients and phytoplankton at 1 meter depth using a peristaltic pump (Masterflex, Cole-Plamer Instrument Co.) in 1984 and a 1 liter polyethylene bottle attached to an aluminum rod in 1985. Nutrient samples were placed in 125 ml linear polyethylene bottles (Nalgene) and algal samples were placed in 130 ml plastic bottles. Sampling was done between 10:00 AM and 2:00 PM. Phytoplankton samples were preserved at the time of collection with 1 ml of Lugol's solution (Standard Methods, 1979).

Depth profile sampling was done in the 1985 study using an acid washed van Dorn bottle (Scott Instruments, Seattle, Wa). Light transmission in the water column was measured using a light meter (spherical sensor, model LI-185B, Li-cor, Inc.).

Plasticware and Glassware Cleaning

All glassware and nutrient sample bottles were washed in phosphate-free detergent (Extran 1000, EM Science Inc.), rinsed six times in hot tap water, soaked in 4% HCl overnight (or longer), rinsed six times with deionized distilled water (Nanopure II, Barnstead), inverted and allowed to air dry. Clean bottles were stored under plastic to prevent dust contamination. Phytoplankton

sample bottles were detergent washed and hot tap water rinsed.

Phytoplankton Analysis Methods

Either 100 ml or 10 ml of the sample was filtered through a 0.45 micrometer membrane filter (Type HA, Millipore Corp) and allowed to air dry in a dust and draft free area. The actual filtering area was 1320.25 square millimeters. Approximately 1 square centimeter of the filter was permanently mounted on a microscope slide with medium viscosity immersion oil (which made the filter transparent) and covered with a glass coverslip. The edges of the coverslip were painted with clear nail polish ("Clear Ice", Covergirl Nail Slicks, Noxell Corp.). The cells per milliliter and the species composition were determined at 1000X with an oil immersion Neoflur objective lens (Zeiss) using the technique described in Standard Methods (1979, section 1002F.4). It was modified slightly in that fifty fields per slide were examined rather than thirty fields.

The technique used to determine the cell concentration and species abundance is a statistical estimation, not an actual count of the cells present. Random microscope fields were sampled and only the presence or absence of a particular species was noted. It is most accurate if the

organism occurs in 70 to 90% of the fields exaimed. The more rare species may be missed entirely while abundant species that exceed 100% saturation of the fields will be underestimated. The technique also becomes increasingly less accurate if an organism occurs in more than 90% of the fields sampled.

Phytoplankton productivity was also measured at the end of the 1985 study by the uptake of radioactive carbon using a Beckman LS 9000 Scintillation counter (Parsons, et.al., 1984). Bag and lake samples were incubated in polycarbonate flasks for three hours at the lake surface. The sky was cloudy and the water temperature was 10 C.

Nutrient Analysis Methods

All nutrient solution spikes and reagents were made with deionized distilled water (Nanopure II, Barnstead) and analytical reagent grade chemicals.

Chemical analysis for nitrate and phosphate were done in the field at the Suttle Lake campground on August 23 and 25, 1985, and in the lab for all other dates in 1985. Attempts to do nutrient analysis in the field were unsucessful in the 1984 study. Analysis for nitrate and phosphate was done within 12 hours of sampling.

Analysis for nitrate concentration was done using the method described by Jones, 1983. This method requires that

the sample be constantly agitated with spongy cadmium granules for 90 minutes. In the lab this mixing was done using an orbital shaker (No. 3590, Labline Instruments, Inc.) and in the field using a spring powered baby rocker (Swyngomatic, Grayco Children's Products, Inc.).

Analysis for soluble reactive phosphate was done using the method described by Parsons et.al., 1984.

Both nitrate and phosphate methods are colorimetric methods. A Spectronic 100 (Bausch and Lomb) was used for analysis done in the lab and a battery operated spectrophotometer (DR-EL/4, Hach Chemical Corp.) was used for field work. Comparable levels of accuracy and precision were obtained using both sets of equipment.

CHAPTER IV

RESULTS

Phytoplankton

The addition of nitrate, either alone or in combination with phosphate, resulted in rapid increases in the phytoplankton growth. All other treatments did not (Figures 6 and 7). The predominant species was the pennate diatom *Synedra mazaemensis*, which is a species naturally occuring in Blue Lake. *Anabaena* did not grow under any of the treatments.

Phytoplankton succession for a few representative treatment bags indicate that in all cases the dominant species was *Synedra mazaemensis* (Figures 8 and 9).

A number of other species were also present in low numbers in the phytoplankton samples. For clarity, only the predominant species have been presented in the phytoplankton succession examples (see Figures 8 and 9). Low frequency species have been included in the total cell concentrations. The non-dominant species present in the nitrate plus phosphate treatment enclosure on day 35, 1984, (Figure 8) were the following: an unidentified pennate diatom (65 cells/ml), *Synedra* sp. (51 cells/ml), *Achnanthes*

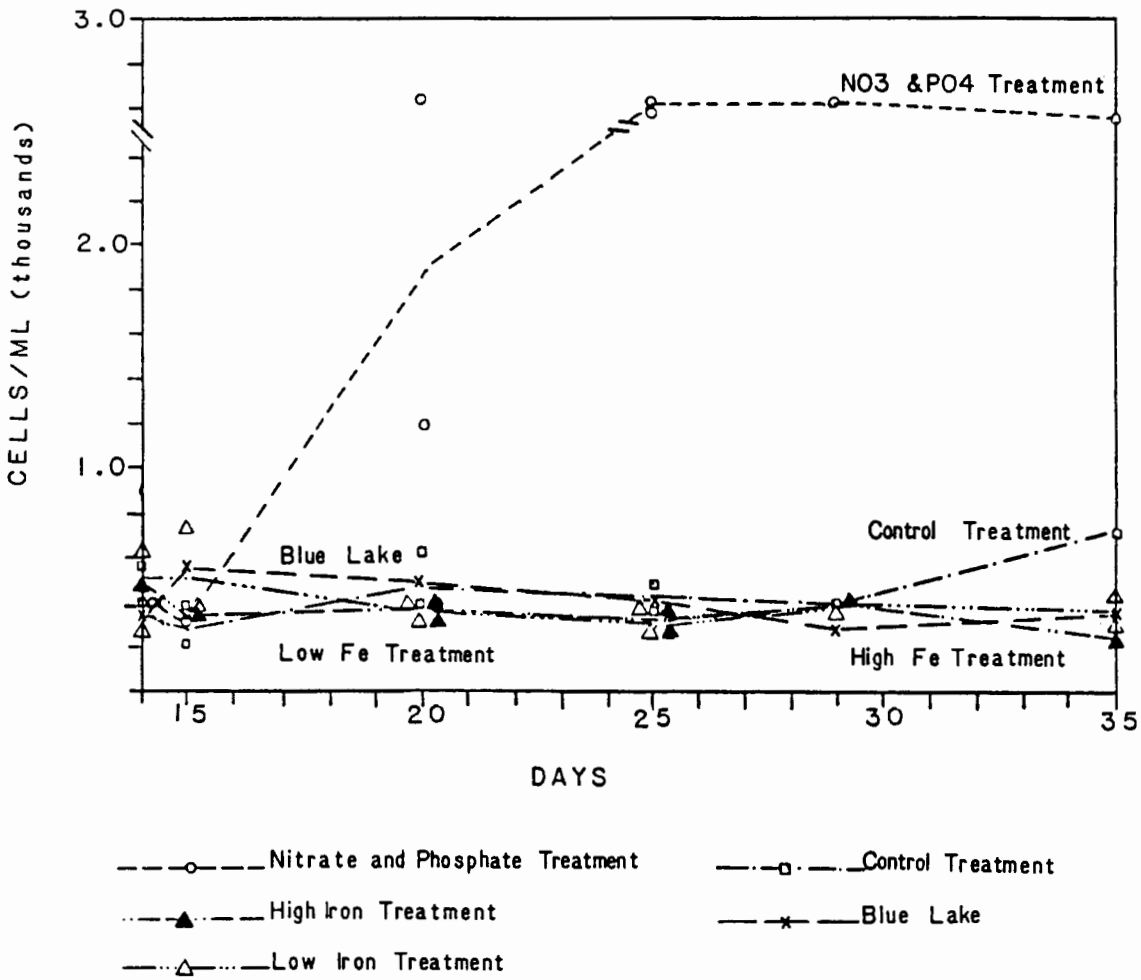


Figure 6. Total phytoplankton, all treatments: 1984. Cell concentrations greater than 2450 cells/ml are off scale and cannot accurately be estimated with the method used.

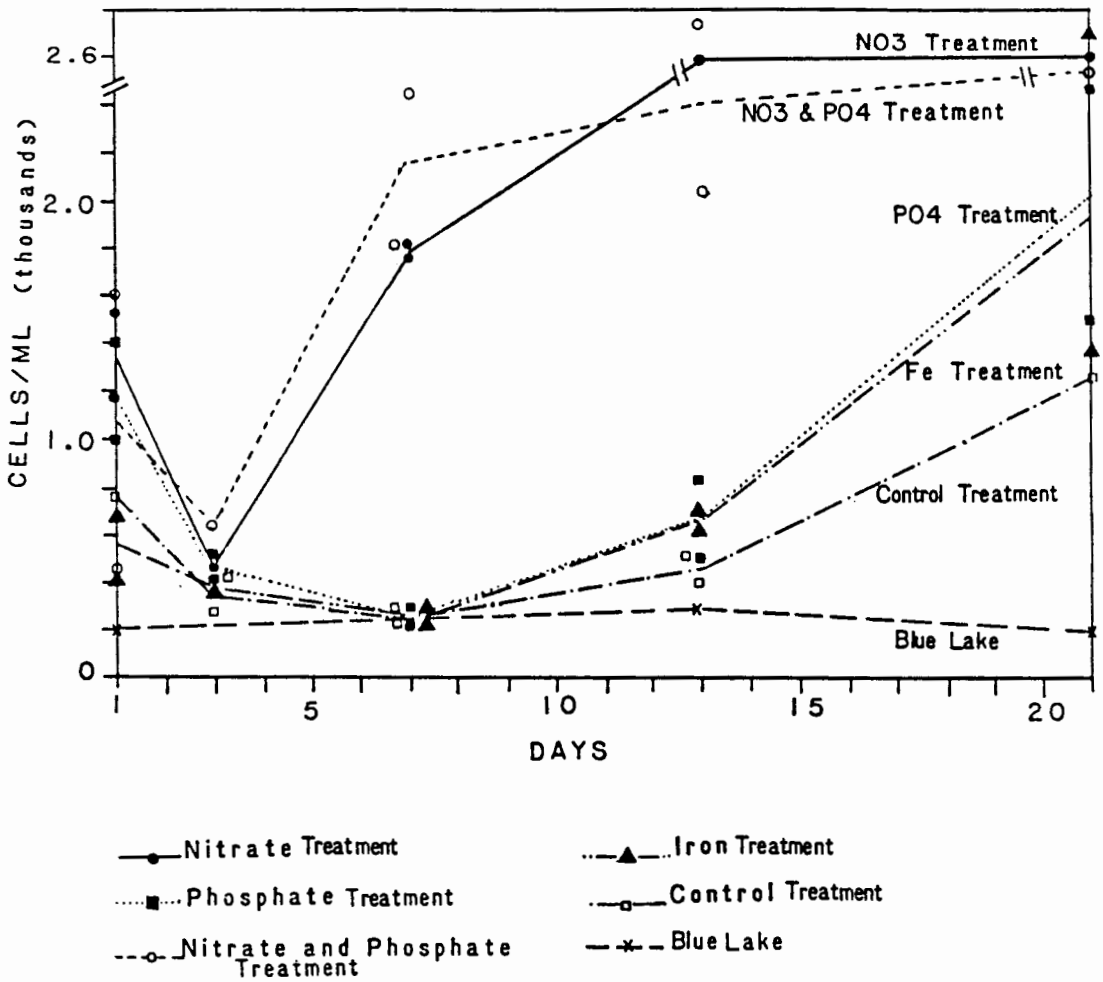


Figure 7. Total phytoplankton, all treatments: 1985. Cell concentrations greater than 2450 cells/ml are off scale and cannot accurately be estimated with the method used.

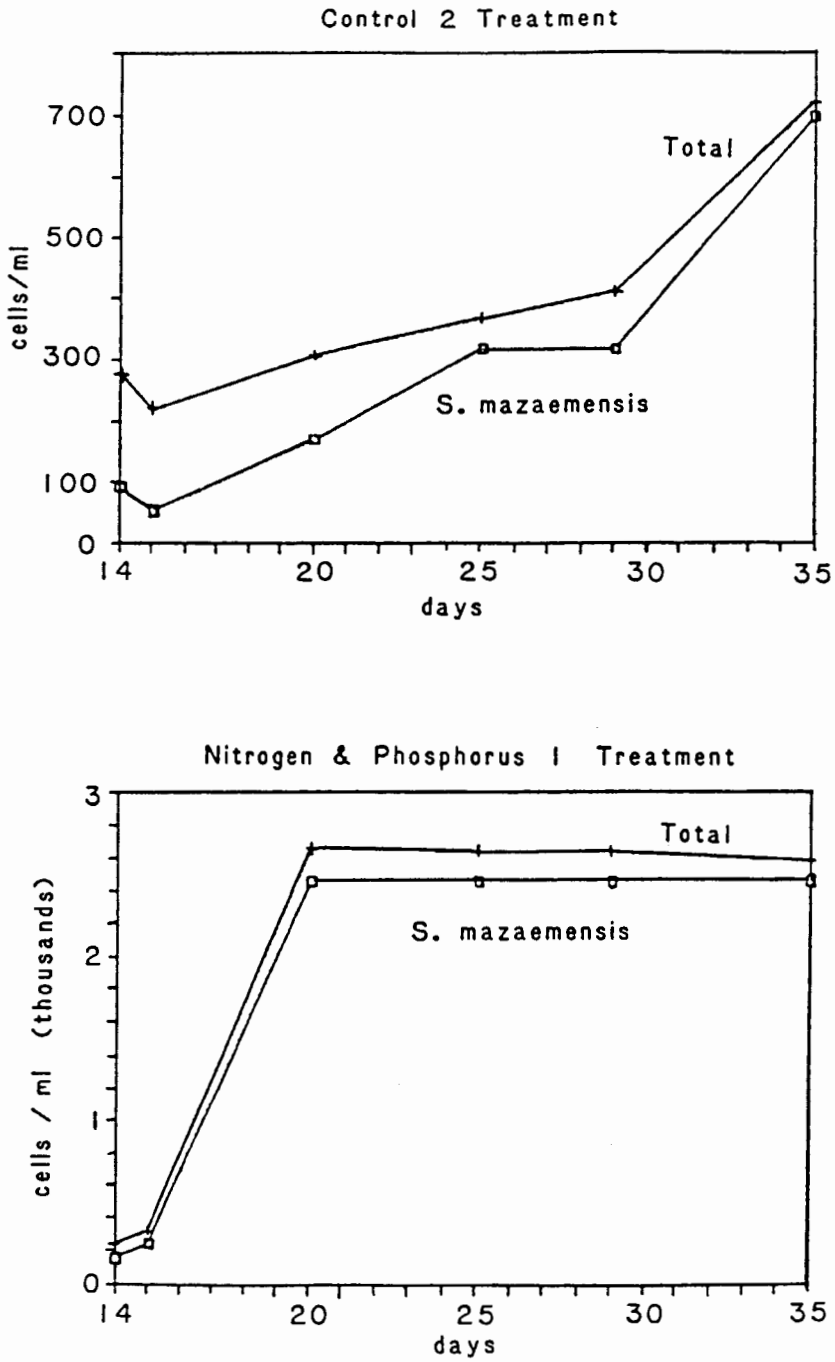


Figure 8. Algal succession in treatment enclosures: 1984. These two examples are representative of all the treatment enclosures. Anabaena sp. was below dectection in all enclosures. Note that the scale on the vertical axis is not the same in the two graphs.

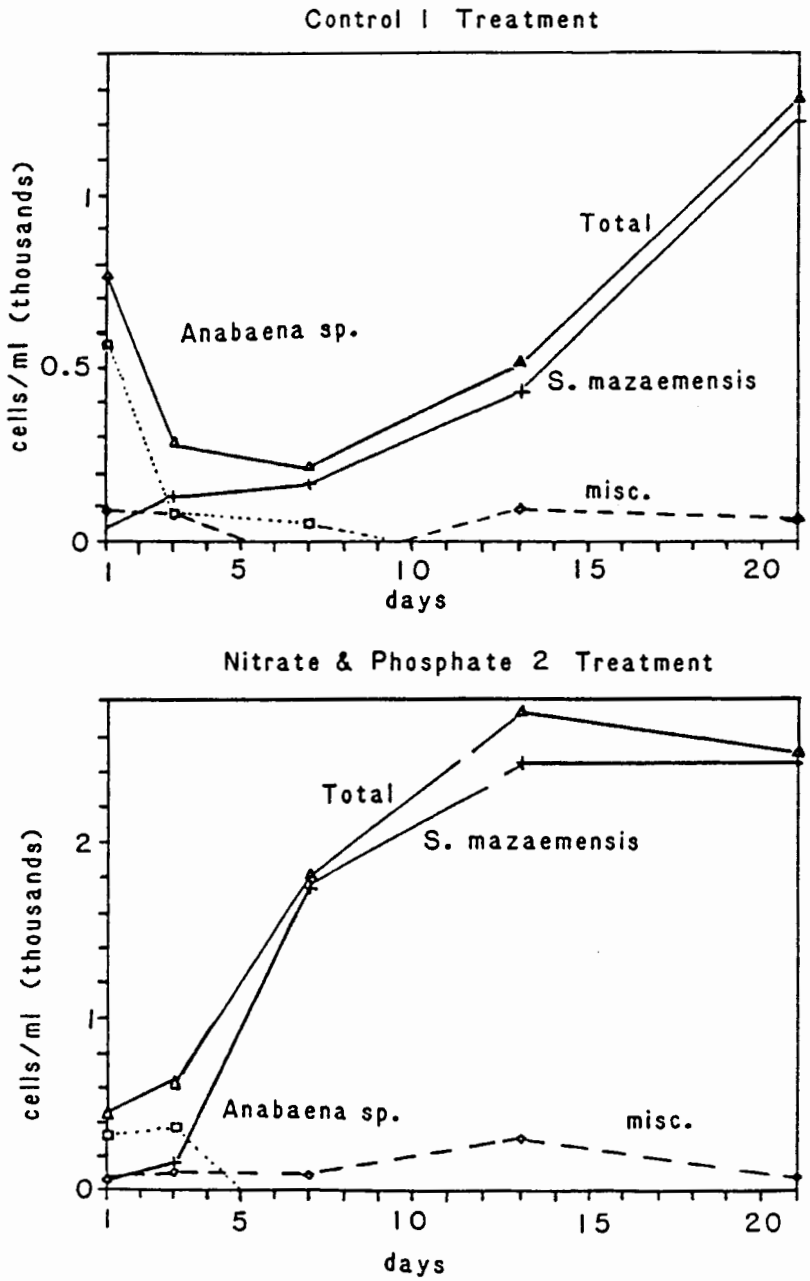


Figure 9. Algal succession in treatment enclosures: 1985. These two examples are representative of all the treatment enclosures. Anabaena sp. was detectable in all enclosures at first but then decreased rapidly. Note that the two graphs have different scales for the vertical axis. Cell/ml concentrations greater than 2450 are off scale and not accurate.

sp. (25 cells/ml), Asterionella formosa (12 cells/ml), and Nitzschia sp. (12 cells/ml). These five species constituted less than 6% of the sample. On day 21, 1985, (Figure 9) the non-dominant species in the nitrate plus phosphate treatment enclosure were the following: Asterionella formosa (12 cells/ml), and two species of unidentified pennate diatoms (51 cells/ml and 12 cells/ml). These three species constituted less than 3% of the sample. These two examples are representative of the less numerous species present in the other treatment enclosures in species of phytoplankton present and the approximate abundance.

No significant amount of Anabaena was detected in the treatment bags in the 1984 study. The concentration of the Anabaena in the inoculum was 295 cells/ml, making the final concentration of Anabaena in the treatment bags approximately 56 cells/ml. The theoretical limit of detection of the technique used is approximately 12 cells/ml for the volume of sample that was filtered. The inoculum Anabaena should have been detectable if the cells remained viable and in the water column.

The inoculum used in the 1985 study was much richer in Anabaena. Anabaena was detected in all treatment tubes on day one but rapidly decreased, and was not present in any of the samples by day 13 (Figure 9). The data

indicates that the treatments did not receive the same initial dose of Anabaena. Initial concentrations in the enclosures ranged from 265 cells/ml to 1450 cells/ml. Evidently, shaking the carboy was not adequate to evenly mix the inoculum.

Nutrients

As would be expected, the nitrate and phosphate levels rose sharply in the tubes that had received those nutrient additions (Figures 10 and 11). The initial nitrate levels in the nitrate treatment enclosures and in the nitrate plus phosphate treatment enclosures were approximately 3 times that of the control enclosure. The initial phosphate levels in the phosphate treatment enclosures and in the nitrate plus phosphate treatment enclosures were approximately 1.3 times that of the control treatment. The elevated levels of these nutrients in the enclosures decreased gradually with time. The nitrate treatment caused a gradual decrease in the phosphate concentration in a ratio of approximately 16 N : 1 P (Figure 11). Nitrate analysis was not done on all of the samples on day 20 due to an unexpected shortage of cadmium necessary for this analysis (Figure 10).

Nutrient values are reported as control normalized ratios (treatment bag concentration/control bag

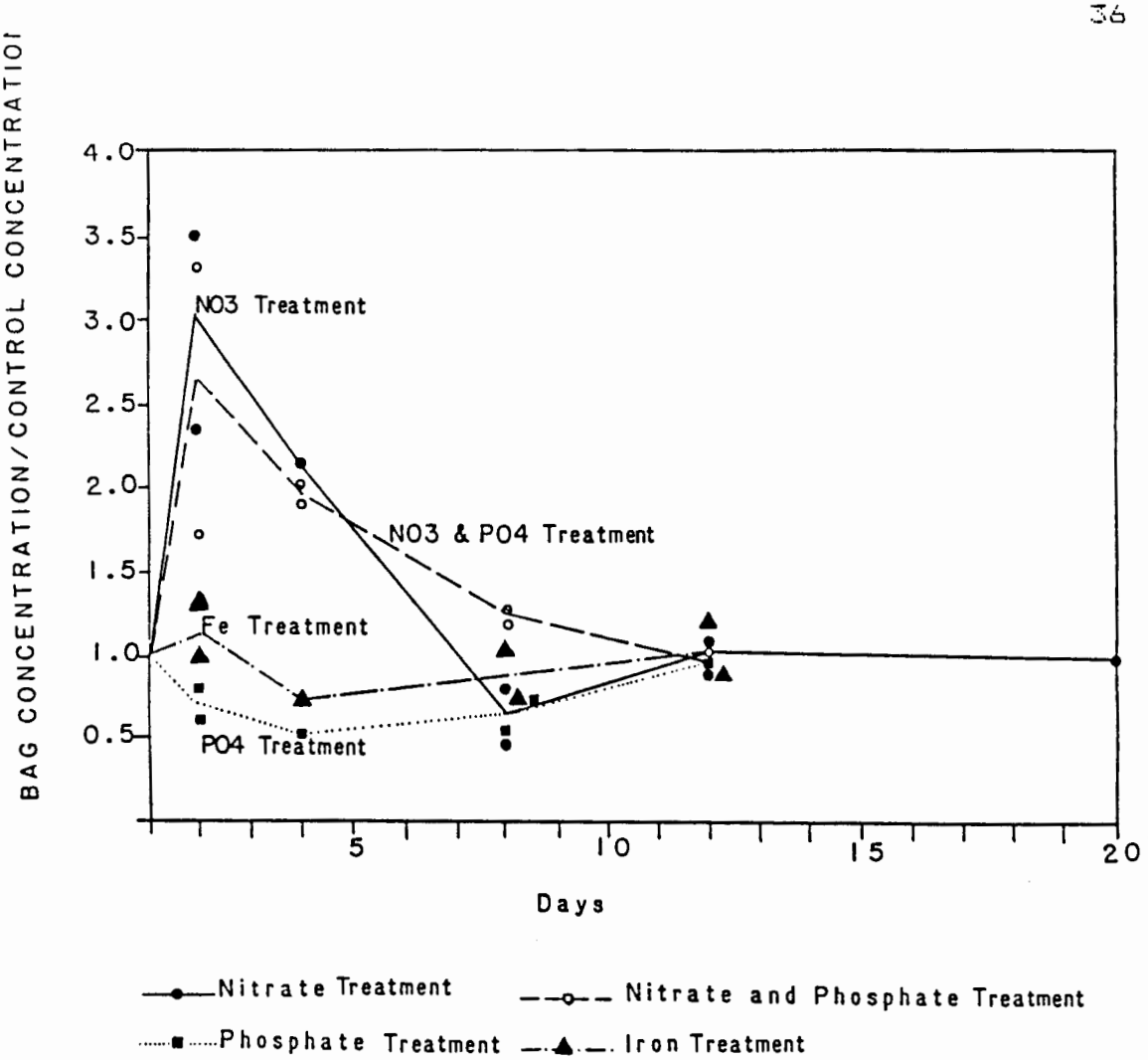


Figure 10. Nitrate concentrations in the treatment enclosures: 1985.

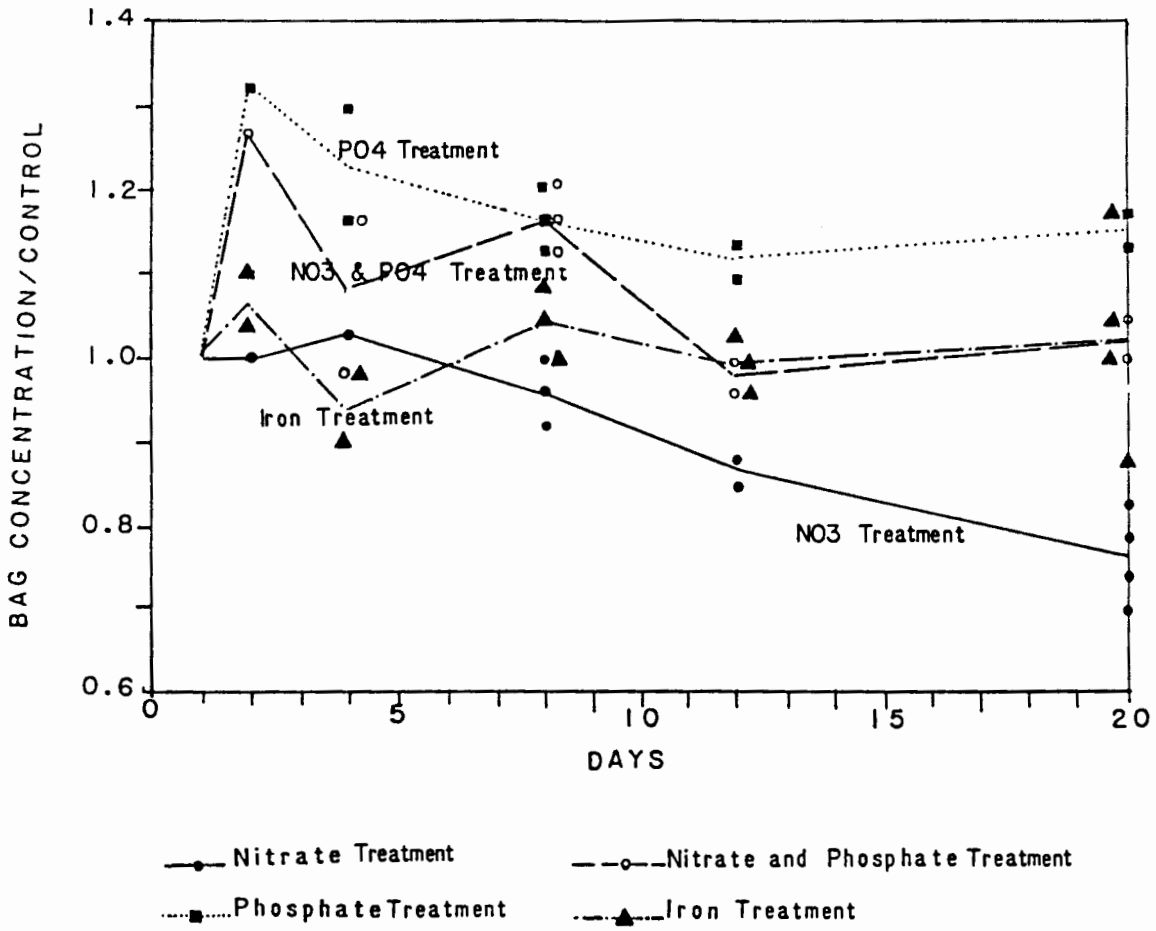


Figure 11. Phosphate concentrations in the treatment enclosures: 1985.

concentration) to reduce the effects of variations between sampling days that are most probably artifacts not related to the treatments themselves.

C-14 Uptake

Fixation of radioactive carbon also confirms that phytoplankton growth was stimulated by nitrogen addition and not by other nutrient spikes (Figure 12). C-14 fixation increased by an average of 75% in tubes treated with nitrate.

Depth Profile

The depth profile of the phytoplankton of Blue Lake indicates that there are three distinct phytoplankton communities in August and September (Figure 13). Synedra mazaemensis dominates only the surface waters to a depth of 10 meters. Between 12 and 45 meters there is an Asterionella formosa and Tribonema sp. community. Starting at 45 meters Melosira italica and Stephanodiscus astrea predominate. Total phytoplankton are maximum at 60 meters with most of these being M. italica. The environment at this depth is 5.1 C and only 0.02% of incident surface light (Figure 14). This would seem to be a rather dark and cold environment for photosynthetic organisms.

Apparently, it is not unusual for stratified, low

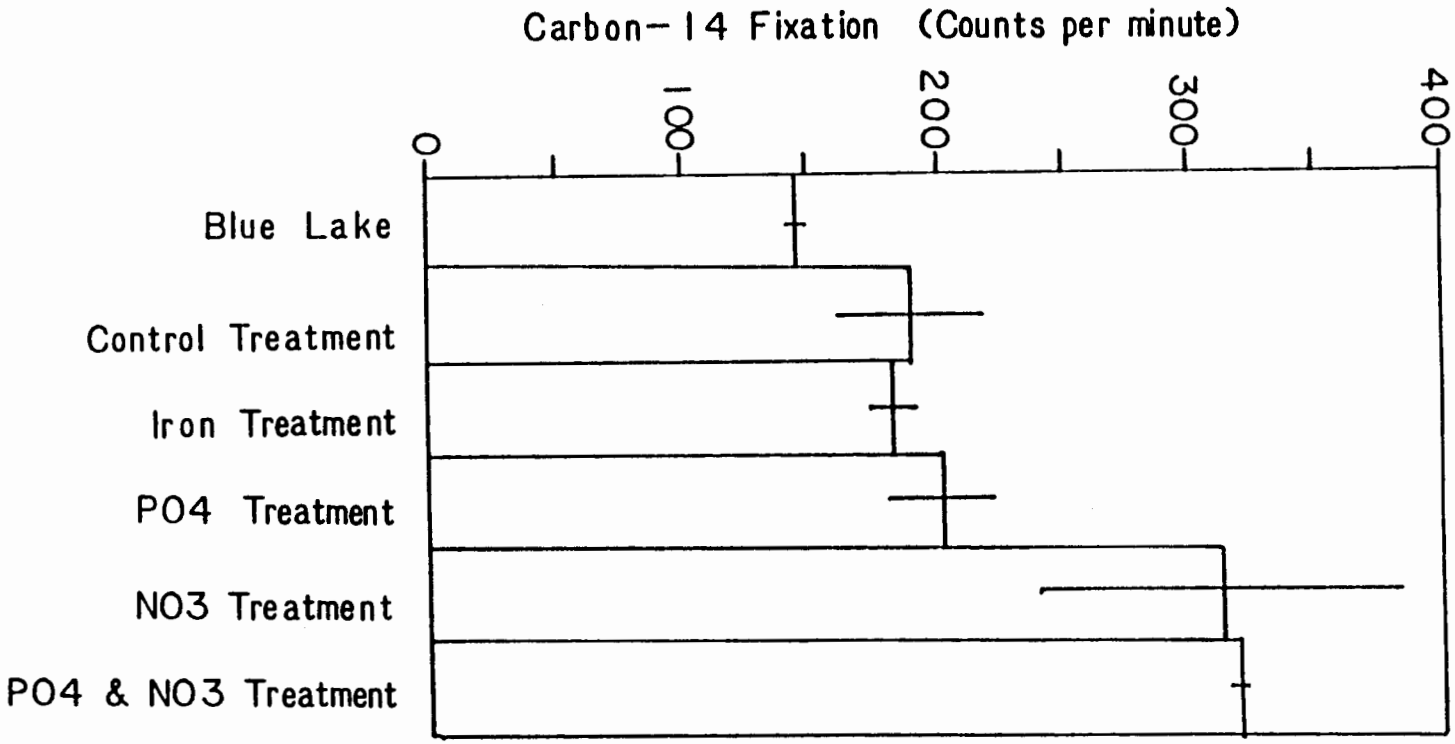


Figure 12. Radioactive carbon fixation in the treatment enclosures and Blue Lake. The vertical lines represent the range of variation of the replicates.

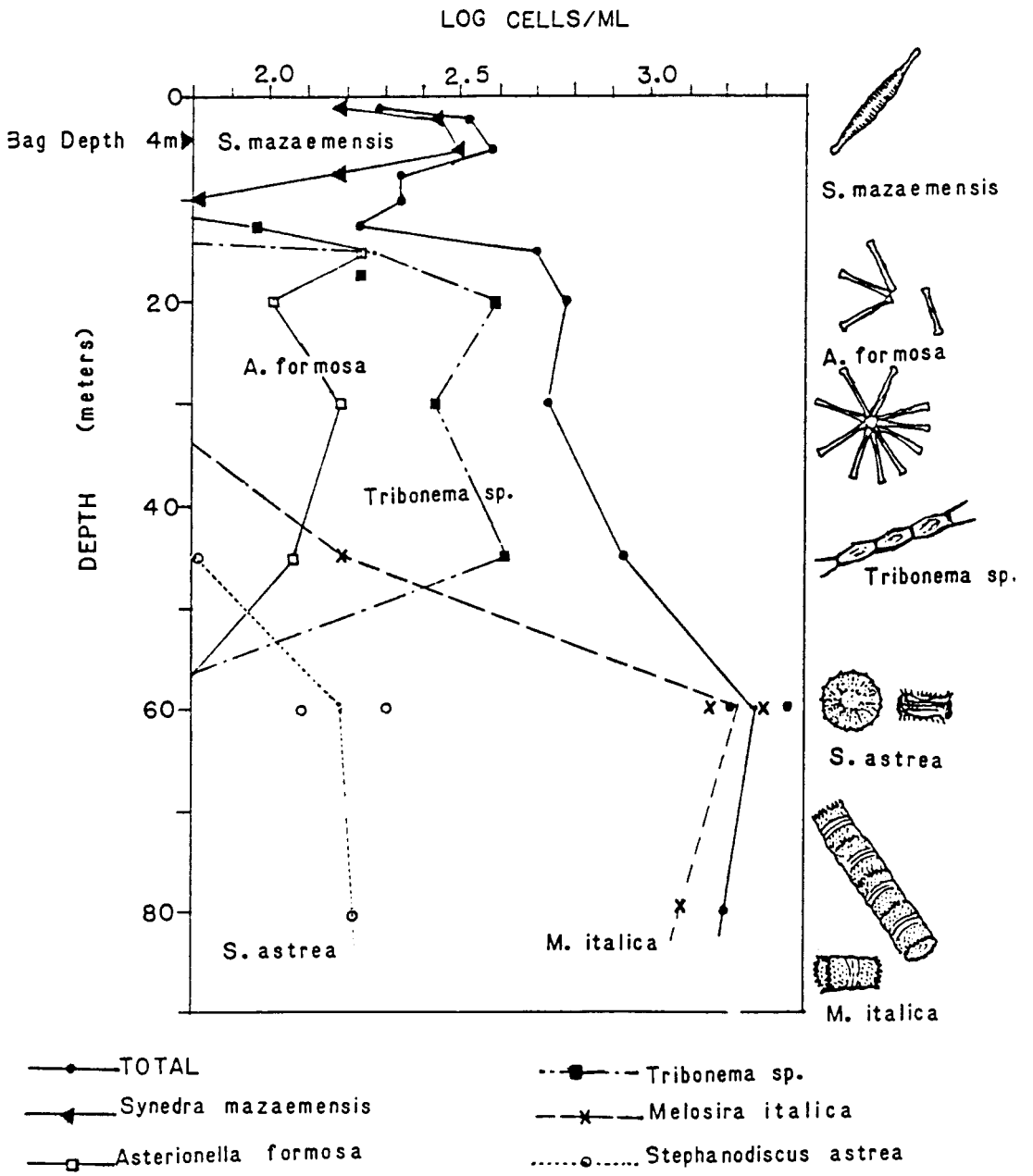


Figure 13. Phytoplankton populations vertical profile of Blue Lake.

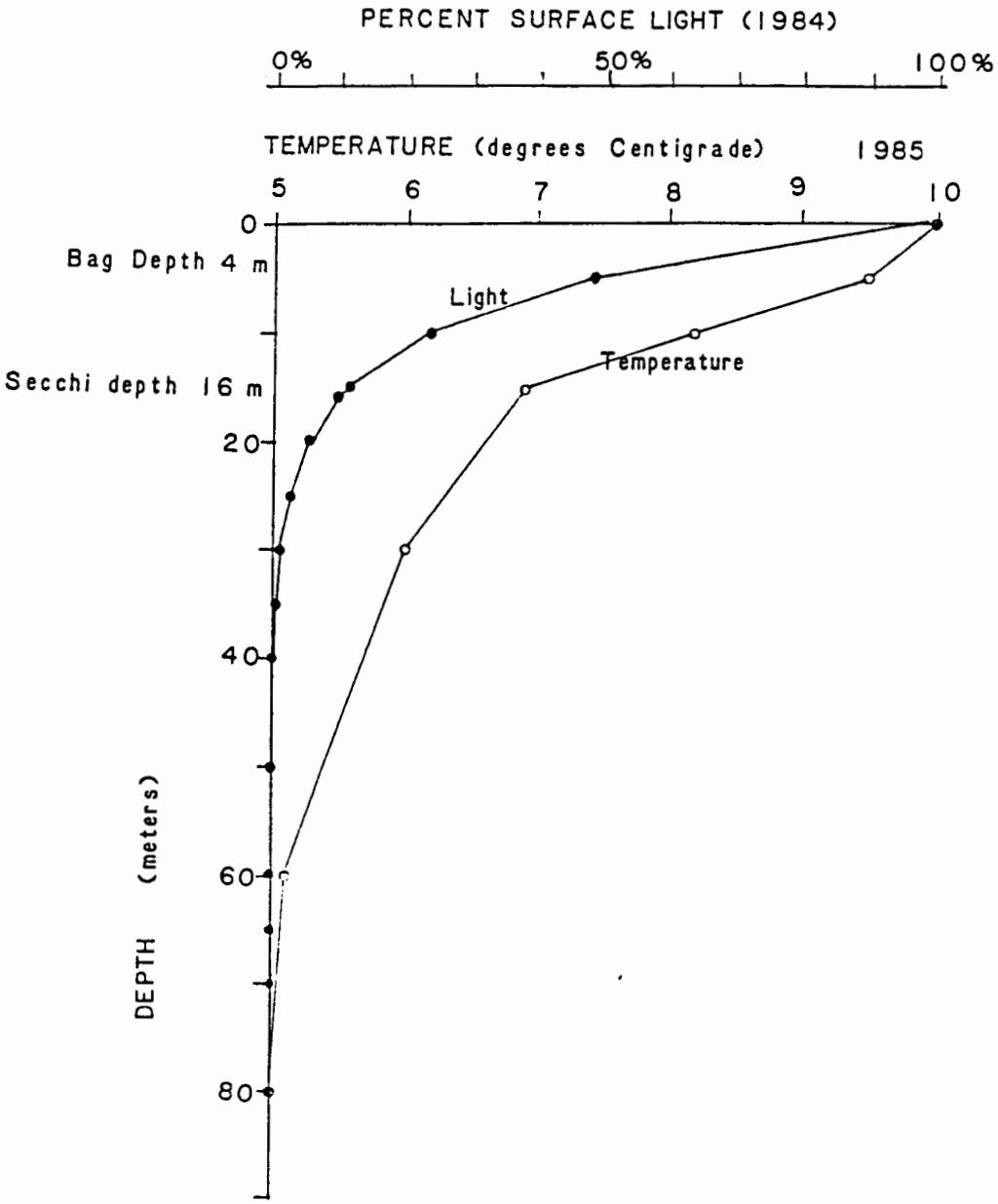


Figure 14. Light and temperature vertical profile of Blue lake. Light data is from 1984 and is estimated from light extinction in top 7 meters. Temperature data is from September, 1985.

productivity lakes to have deep phytoplankton maxima at depths well below the 1% of incident light level. Larson and Geiger (1980) have described two deep phytoplankton maxima in Crater Lake at 80 to 120 meters and at 180 to 200 meters, as well as a surface phytoplankton population. The surface population was predominantly Nitzschia gracilis, the mid-depth maximum was dominated by Tribonema sp.. This mid-depth population is at the bottom of the photic zone. The deepest Crater Lake populations were dominated by Stephanodiscus hantzschii. Priscu and Goldman (1983) describe a deep phytoplankton maximum at approximately 20 meters in oligomesotrophic Castle Lake, California. This maximum was predominantly diatoms in the spring and dinoflagellates in the summer. These authors list several mechanisms and advantages for maintaining deep populations. Deep water phytoplankton may be heterotrophic to to some extent, be shade adapted with increased chlorophyll per cell, and be less vulnerable to zooplankton grazing. Lower temperatures at depth would reduce respiration costs to balance reduced photosynthesis at low light levels.

The vertical profile of nitrate and phosphate show that phosphate increases slightly with increasing depth while nitrate is somewhat variable (Figure 15). Nitrate levels are especially variable above the thermocline (about 15 meters, see Figure 14).

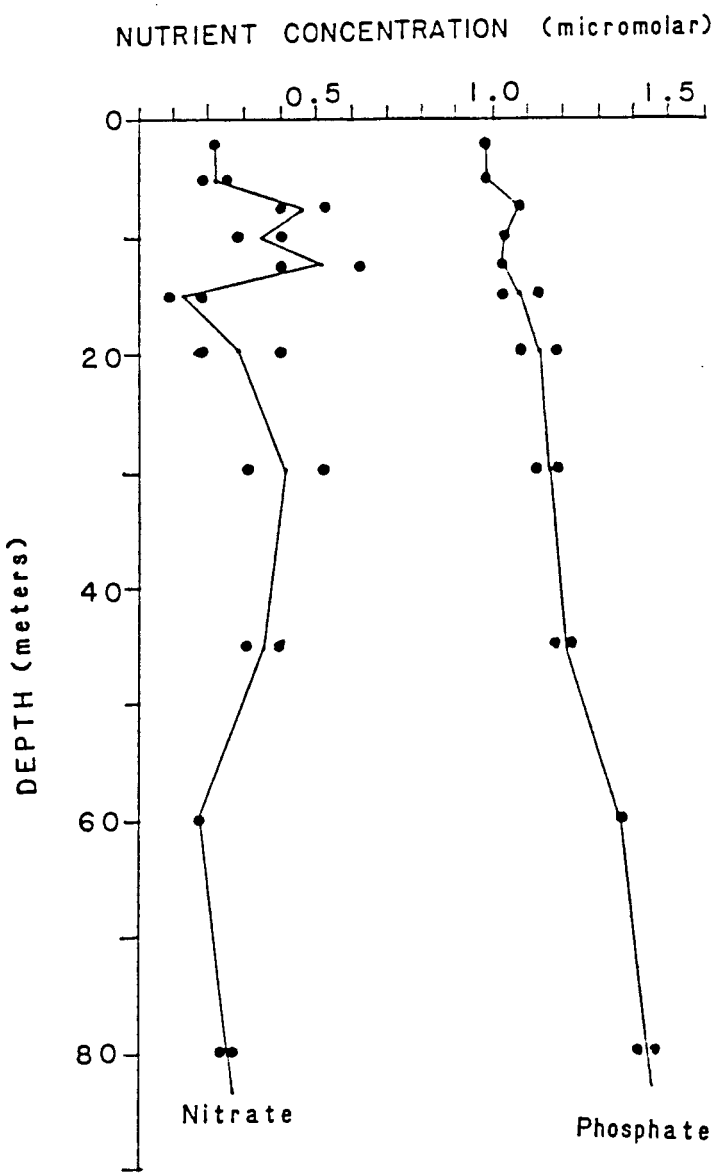


Figure 15. Nitrate and phosphate vertical profile of Blue Lake, September, 1985.

CHAPTER V

CONCLUSIONS AND DISCUSSION

Conclusions

Phytoplankton growth is clearly nitrogen limited in Blue Lake in the experiments reported here. This is definitely true for the surface populations in late summer and is probably true throughout the year and at all depths. It is unlikely that the seasonal change from phosphorus limitation to nitrogen limitation that is seen in many productive lakes due to nitrogen depletion by the phytoplankton would occur in an oligotrophic lake like Blue Lake. Since phytoplankton at all depths experience greater phosphate concentrations than nitrate concentrations, it is likely that the deeper phytoplankton populations are also nitrogen limited (provided that they are not light limited).

The absence of nitrogen fixing cyanophytes has not been explained. The addition of iron alone does not seem to be adequate to stimulate cyanophyte growth. Evidently some other physical or chemical factor (or both) is limiting cyanophyte growth.

Discussion: The Cyanophyte Limitation Question

A number of factors may be the cause of the lack of success in growing cyanophytes in these experiments. These include: temperature, limitation of nitrogen fixation by some nutrient other than just iron, and the vertical water flow patterns in Blue Lake.

Future studies at Blue Lake should examine the possibility of molybdenum limitation by adding molybdenum to enclosures alone and in combination with iron. The nitrogenase enzyme contains molybdenum as well as iron (Brock, et.al., 1984). A factor that has been shown to influence molybdenum nutrition of phytoplankton is the sulfate concentration of the water. High sulfate levels in coastal and estuary waters has been shown to inhibit molybdate uptake by phytoplankton causing decreased rates of nitrogen fixation by cyanophytes (Howarth and Cole, 1985). The sulfate concentration of Blue Lake is low compared to other Oregon lakes. The sulfate concentration is less than 0.1 mg/l (Johnson, et.al., 1985) and the molybdenum concentration is 2.335 nmoles/l (R. Collier, unpublished data). The S:Mo molar ratio is at the most 445. The average freshwater molar ratio is 60,000 (Howarth and Cole, 1985). This indicates that sulfate inhibition of molybdate metabolism is an unlikely reason for the absence of nitrogen fixing cyanophytes in Blue Lake.

Another valuable treatment would be to add all the known trace metals possible to enclosures. If such a treatment stimulated growth then limitation by some trace metal would be demonstrated and save time in testing many alternative nutrients individually.

Temperature may be a significant factor in determining the speciation of phytoplankton populations of Blue Lake. A summary of reported phytoplankton temperature optima by Hutchinson (1967, p 431), indicates that a species related to the Anabaena of Suttle Lake has the warmest temperature optimal range of the organisms surveyed. The reported optimal temperature ranges for Anabaena flos-aguae were from 11.6 to 21 C. Surface temperatures in Blue Lake were at the lower end of this range and below during the studies reported here. In 1985 surface temperatures were in a generally decreasing trend during the study. The surface temperature decreased from 13.0 C at the beginning on August 23 to 10.0 C at the end of the study on September 12. Suttle Lake had a surface temperature of 19.5 C on August 25, 1985 during the dense Anabaena bloom.

Continuous culture studies done by R.L. Kiesling (personal communication) using Anabaena flos-aguae in competition with non-nitrogen fixing cyanophytes found that both N:P ratio and temperature were critical in determining the dominant species. Cultures were grown at a range of

N:P ratios at 24, 17, and 10 C. This temperature range spans the Blue Lake - Suttle Lake temperature differences. Colder temperatures required lower N:P ratios for A. flos-aquae to dominate. At higher temperatures A. flos-aquae dominated all cultures except the one with the highest N:P ratio. Nitrogen fixation is a physiologically costly process. Perhaps Blue Lake is too cold for nitrogen fixers to be able to grow fast enough to compete with eukaryotic phytoplankton even with an apparently favorable N:P ratio. These data were presented at the June 1985 meeting of the American Society of Limnology and Oceanography in a paper titled "Lake Superior phytoplankton community responses to changes in resource supply: blue-green algae responses to a nitrogen:phosphorus supply gradient", by R.L. Keisling, Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan, 48109.

Another factor that may be adversely affecting the success of surface cyanophytes is the unusual water flow dynamics of Blue Lake. The major inflow of water into the lake is thought to be from a spring 240 feet below the surface of the water. The mean residence time of the water in the lake is approximately 2.4 months, which is a rather short period of time. The pattern of water movement in the lake is that the inputs are very deep and the out flows are at the surface via Link Creek at the opposite shore. The

rate of flushing is fairly fast. Perhaps surface populations of Anabaena are being skimmed away faster than they can grow. In the 1985 study (Figure 7) all enclosures showed elevated phytoplankton growth compared to Blue Lake towards the end of the study. Perhaps this is because the enclosures prevented the captured phytoplankton from being flushed from the surface waters.

Discussion: Experimental Design

It is possible that the enclosures themselves produced artifacts in the results. During the early part of both studies the control treatment phytoplankton were not considerably different in species composition and abundance from those in the unenclosed lake. This is at the same time that the phytoplankton in the nitrate added enclosures had shown markedly stimulated growth. This has been taken as evidence that the artifacts produced by the enclosures are most likely minimal compared to the magnititude of the effects induced by the experimental treatments.

The main advantage of the enclosures is that it easily and precisely duplicates the broad range of constantly changing environmental variables that the phytoplankton experience in the natural environment. This allows for the selective manipulation of only one variable at a time in a natural setting that is not feasibly duplicated in a

laboratory setting with algal culture experiments. However, there are a number of potential disadvantages that should be noted since they can produce artifacts. For example, the enclosed phytoplankton do not receive the same level of turbulent mixing as do those in the lake. Boyce (1974) cautions that the presence of a thermocline and temperature stratification inside the enclosure should not be used as evidence of a mixed epilimnion. Reduced mixing would mean that the enclosed phytoplankton do not experience the natural pattern of light and temperature. Reduced turbulence may influence species succession, selecting against denser species that rely on mixing to maintain their position in the water column and in favor of more buoyant types. Also, the enclosure may reduce or eliminate significant variables such as nutrient inputs from the environment and the effects of zooplankton grazing. It is important that these effects be kept in mind since they can produce changes in species composition and abundance independently of any experimental treatment.

Carbon limitation of the enclosed phytoplankton was not felt to be a problem in this study. A study of Carty Reservoir by Jim Sweet used the same design of enclosures made from the same material that was used in this study. Carty reservoir is a far more productive lake than Blue Lake. No increase in dissolved oxygen was found inside

the enclosure. This indicates that either the polyethylene is adequately gas permeable or that the net production and consumption of this gas was balanced in the short term. This was found to be true even if the bag material was doubled. (Jim Sweet, personal communication).

CHAPTER VI

SUMMARY

Nutrient enrichment experiments using 1000 liter polyethylene *in situ* enclosures found evidence of nitrogen limitation of phytoplankton growth in oligotrophic Blue Lake. Typically, the phytoplankton populations of nitrogen limited lakes are characterized by nitrogen fixing cyanophytes which are not found in Blue Lake. The absence of cyanophytes was thought to be due to iron limitation, although this was not demonstrated. Evidently some other nutritional and/or physical factor is limiting cyanophyte growth. Low water temperature, water flow dynamics, molybdenum nutrition, or other trace metals may be involved in determining the success of cyanophytes in Blue Lake.

The clear, pristine waters of oligotrophic lakes are rare recreational and esthetic resources. Learning more about what causes these lakes to be so low in productivity will help us to better manage and conserve them. The research presented in this thesis demonstrates that one oligotrophic lake, Blue Lake, is nitrogen limited rather than phosphorus limited, as is more typical.

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