NITROGEN FIXATION AND MULTIVARIATE ANALYSIS OF PHYTOPLANKTON AND ENVIRONMENTAL DATA FROM LAKE HEFNER, OKLAHOMA

By

MEL ALLEN MCFARLAND

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Thesis Approved:

Dale Tolz
Thesis Adviser

Jerry Wilhm

P. L. Claypool

Norman N. Durham
Dean of the Graduate College

PREFACE

The objectives of this study were to (1) estimate the contribution of nitrogen fixation to the nutrient budget (2) identify environmental variables influencing nitrogen fixation and (3) identify summer phytoplankton assemblages of Lake Hefner.

The author would like to express appreciation to Dr. Dale Toetz for direction, advice, and analysis of this study. I also recognize with gratitude his help in editing, teaching and research.

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CHAPTER I

INTRODUCTION

Water from water supply reservoirs must meet health standards and and customer preferences. Problems of taste and odor also require attention. A lake providing recreation has additional aesthetic and functional requirements. Lake Hefner, which supplies water for Oklahoma City, has a history of taste and odor problems (Toetz, 1982; Silvey et al., 1959) and also provides sailing, boating, and fishing (Summers, 1978). The control of blooms of blue-green algae often associated with taste and odor in Lake Hefner could alleviate taste and odors in finished water, reduce water quality problems, and improve the appearance of the lake.

Control of algal growth through manipulation of nutrient supply requires knowledge of both nitrogen and phosphorus budgets. Estimation of nitrogen fixation becomes essential to both the nutrient budget and to control measures in the special case of a large phosphorus supply which can stimulate nitrogen fixation (Horne and Goldmann, 1972). In such a case, removal of phosphorus, but not nitrogen, would be required if nitrogen were being fixed.

The importance of an accurate estimate of nitrogen

fixation cannot be overstated. The contribution of nitrogen fixation to the nutrient budget of a lake is potentially significant. Nitrogen fixation accounted for 43% of the nitrogen budget of Clear Lake, California (Horne and Goldman, 1972). Blooms of blue-green algae, including potential nitrogen fixers, are almost annual occurrences in eutrophic lakes (Wetzel, 1975).

Nitrogen (N) and phosphorus (P) are the two elements in shortest supply relative to demand in lakes (Wetzel, 1975). The supply of new nitrogen via nitrogen fixation can be crucial to the nutrient budget of a lake (Horne and Fogg, 1970; Torrey and Lee, 1976). The calculated annual budget of a lake yields gross amounts of nitrogen and phosphorus entering and leaving a lake. Undersupply of nitrogen relative to phosphorus may result in higher rates of nitrogen fixation. Ratios between N:P can be used as an indication of nitrogen of phosphorus limitation.

Nitrogen fixation can be compared to the nutrient budget to quantify the percentage of nitrogen contribution in this manner. This contribution can be considered on the basis of all nitrogen supplied during the entire year, or strictly for nitrogen supplied from all sources during the summer.

Calculated areal loading rates for N and P are useful also in lake modeling. Loading rates can be used to estimate lake trophic status or to predict summer chlorophyll _a_ and total P concentrations (Jones and Bachmann, 1976;

Mikalski et al., 1975).

In nitrogen-limited systems, nitrogen fixation may account for the dominance of blue-green algae (Fogg, 1975). The seasonal pattern of phytoplankton succession, however, also involves other changes in the environment. Light, temperature, pH, and silica also help determine successional patterns (Fogg, 1975; Shapiro, 1973). Diatom population crashes in late spring may correlate closely with silica depletion (Fogg, 1975). There is not, however, any one hypothesis that explains differences in successional patterns between lakes. As Schindler (1975) noted, even nearly identical lakes do not exhibit the same patterns in the same year.

Nitrogen fixation also varies in response to environmental variables. Nitrogen fixation is suppressed by high light intensities, high dissolved oxygen concentration and ammonia (Ward and Wetzel, 1980; Bone, 1972; Carr and Whitton, 1973). Nitrogen fixation may also be influenced by nutrient concentrations and pH (Shapiro, 1973; Horne and Goldman, 1972; Vanderhoef et al., 1974).

Factor analysis can be used to examine the relationships among variables and patterns of variation in multivariate data sets (Gnanadesikan and Wilk, 1969; Seal, 1974). Principal axis analysis, a type of factor analysis, yields results similar in interpretation to principal components analysis (Helwig and Council, 1979). Each principal axis is defined by an eigenvalue and an eigenvector. The greatest

amount of variation in the data is found along the first axis. Each succeding axis accounts for less and less variation (Gnanadesikan and Wilk, 1979). Factors resulting from principal axis analysis can be used to investigate the relationships (patterns) among the variables. From these patterns, one can describe more general lake phenomena, and perhaps infer causal relationships.

Nutrient budget information, results of factor analysis, and limnological measurements can assist in understanding the taste, odor, and aesthetic problems in Lake Hefner. The amounts and ratios of nutrients supplied to the lake provide one possible control of taste and odor-causing algae. Other possible control methods may be evident from the factor analysis results. Desirable assemblages of algae could be selected for in lake management if casual relationships could be inferred. Likewise, environmental data correlated with a desirable factor could lead to a lake management plan.

The objectives of this study were (1) to estimate the contribution of biological nitrogen fixation to the nutrient budget of Lake Hefner, Oklahoma, (2) to clarify the relationships between nitrogen fixation and nutrient loading rates, and between nitrogen fixation and other environmental variables and (3) to describe summer phytoplankton assemblages.

CHAPTER II

A REVIEW OF SELECTED LITERATURE

In considering the relationships between productivity and environmental parameters, one can focus on nutrient limitation. Ahlgren (1980) compared single and multiple nutrient limitation theories with results from chemostat culture. Other investigations have centered on phosphorus and to a lesser extent nitrogen, since these elements are those most likely to limit productivity in lakes (Wetzel, 1975). Specific instances of limitation by other elements exist where nitrogen and phosphorus are present in sufficient amounts (Fogg, 1975). The actual limits to productivity may be the result of a single element being in limited, with modifications due to other scarce nutrients (Wetzel, 1975; Ahlgren, 1980).

Literature on nitrogen fixation in lakes and the background biochemistry of nitrogen fixation will be reviewed here. Some aspects of multivariate analysis applicable to the research will also be discussed.

Nitrogen fixation is carried out by a non-specific, oxygen sensitive enzyme that can be separated into two fractions (Bone, 1971; Burris, 1969). In its active form, nitrogenase reduces N_2, acetylene, cyanide, and other

substrates chemically similar to N_2 (Hardy and Jackson, 1967; Hardy and Burns, 1968; Schöllhorn and Burris, 1966). Reaction pathways, rate constants, inhibitors and possible binding sites have also been identified (Burris, 1969; Hwang and Burris, 1968; Hardy et al., 1968; Koch and Evans, 1966; Stacy et al., 1977). The reduction of acetylene to ethylene, intensively studied by Dilworth (1966), Schöllhorn and Burris (1966), and Schöllhorn and Burris (1967), is now used as an indirect measure of nitrogen fixation.

Cellular studies have identified several important factors in nitrogen fixation. Nitrogen fixation has requirements for ATP and carbon skeletons (Stewart, 1973; Fay, 1976; Winkenbach and Wolk, 1973); hence, it is closely tied to photosynthesis. Photorespiration may inhibit fixation by competition for ATP (Lex et al., 1972; Ganf and Horne, 1975). Heterocyst development is suppressed by sufficient levels of inorganic N, primarily ammonia (NH_4^+) in most aquatic systems and requires light for initiation (Bradley and Carr, 1977; Ogawa and Carr, 1969). Nitrogen fixation usually occurs in the heterocyst in blue-green algae, where the enzyme is protected from inactivation by oxygen (Haury and Wolk, 1978; Bone, 1971). Nitrogen fixation also has a direct relationship to heterocyst frequency (Jewell and Kulasooriya, 1970; Horne and Goldman, 1972).

Nitrogen fixation occurs in many types of aquatic ecoysystems (Mague et al., 1974; Alexander and Schell,

1973; Torrey, 1978; Brezonik and Harper, 1969; Brooks et al., 1971) including eutrophic lakes such as Lake Erie (Howard et al., 1970) and Lake Erken (Lannergren et al., 1974). Nitrogen fixation has also been documented for Clear Lake, California (Horne et al., 1972) and Green Bay, Wisconsin (Vanderhoef et al., 1974).

Nitrogen fixation in lakes may vary in several ways. Nitrogen fixing blue-green algae contain gas vacuoles that provide buoyancy within the water column (Walsby, 1972). Changes in algal distribution with depth through vertical movement can alter rates of nitrogen fixation. Water circulation patterns due to wind may lead to patchy distribution of algae across a lake surface (Wetzel, 1975). Nitrogen fixation also varies with light intensity, productivity, and time of day (Ward and Wetzel, 1980; Duong, 1972; Lannergren et al., 1974; Peterson et al., 1977; Vanderhoef et al., 1975).

Multivariate analysis is highly useful in investigating data sets with large numbers of observations, n, on many variables, p. Reduction in dimensionality of n points in p space can retain sufficient detail while simplifying interpretation (Gnanadesikan and Wilk, 1969). In general, a linear reduction in dimensionality in multivariate analysis is achieved by describing the dispersion of the points. This is done through orthogonal linear coordinates that have progressively smaller sample variances associated with each succeding coordinate (Gnanadesikan and Wilk, 1969). Often,

the analysis is carried out on the correlation matrix instead of the raw data. The correlation matrix is constructed of the correlations of each variable with every other variable.

The analyses that can be carried out, dependent in part on the computer program, include principle components, factor analysis, cannonical correlation, and minimal residual analysis (Seal, 1964). Orthogonal or oblique rotations of the cordinate axes may be used in factor analysis to arrive at more interpretable solutions. Orthogonal rotation leaves factors independent from each other, allowing easier interpretion of results (Helwig and Council, 1979).

Recently, factor analysis has been applied to biological situations (Sokal et al., 1961; Allen, 1971; Allen and Koonce, 1973; Levandowsky, 1972). Sokal et al. (1961) were able to obtain meaningful factors from measurements of both physical and biological variables. Phytoplankton periodicity and ecological strategies of various algal groups have also been explained by use of this technique (Allen and Koonce, 1973; Levandowsky, 1972). Bartell et al. (1978) also described phytoplankton periodicity using factor analysis. Data transformations are sometimes used when working with biological data because of initially low correlations or abnormal distributions (Allen and Koonce, 1973; McIntire, 1973; Heywood et al., 1980). Cluster analysis and principle components analysis have also been used to describe plankton assemblages and their variation (Brown, 1969; Bruno and Lowe, 1980; McIntire, 1973).

CHAPTER III

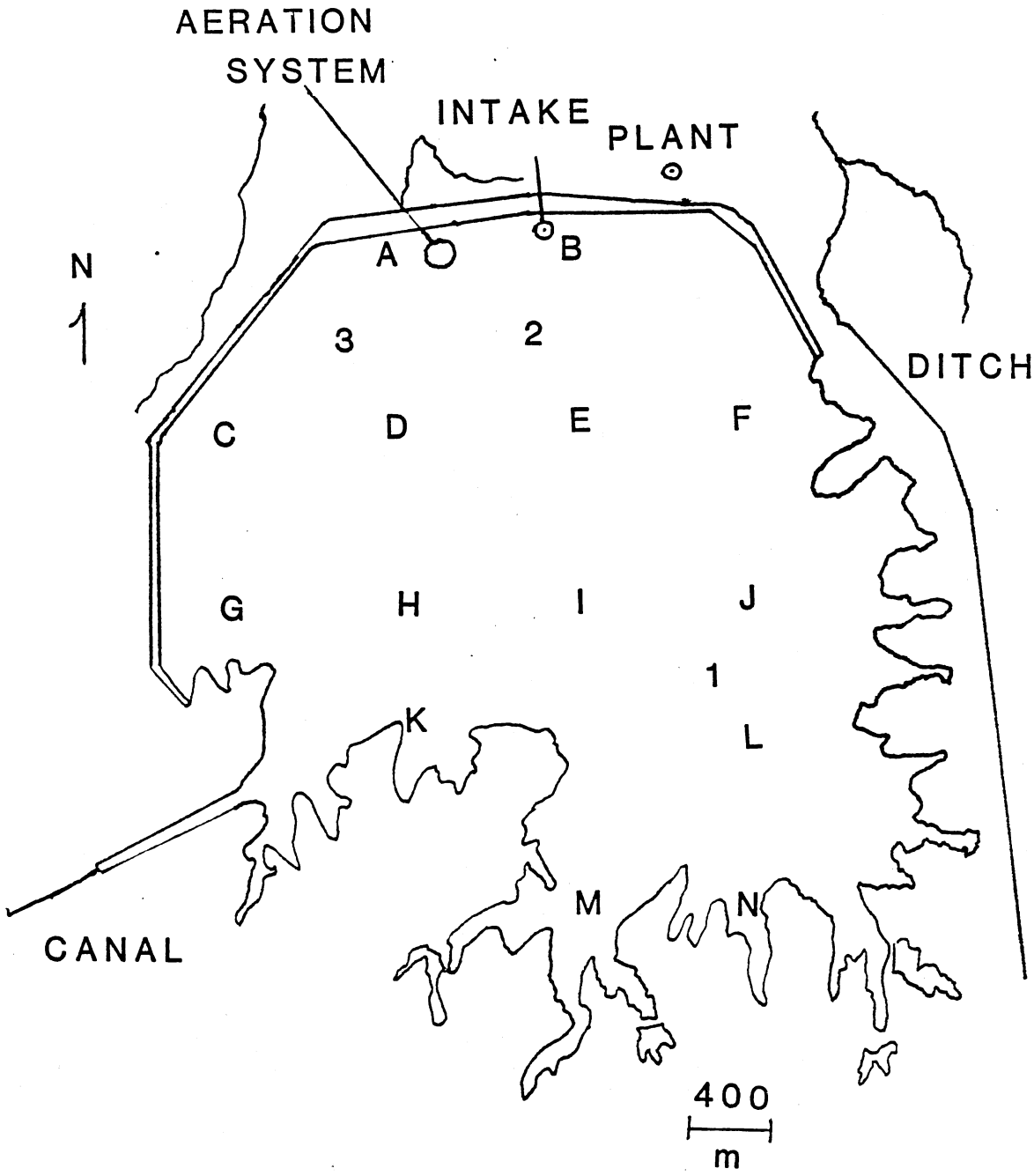
DESCRIPTION OF STUDY SITE

Lake Hefner is a terminal offset water supply reservoir located in Oklahoma City (OKC), Oklahoma County (Figure 1). Most of the water entering the lake is taken from the North Canadian River (NCR), by a 11.6 km gravity feed canal. Water from the NCR is the result of watershed runoff and upstream releases from Canton Reservoir (Toetz, 1982). Maximum possible flow in the canal is 1500 cfs (Anon, 1952). Low summer flows are due to alluvial seepage into the canal (Anon, 1952) and intentional intake to prevent summer fish kills (Toetz, 1982).

Lake Hefner was formed by impounding of Bluff Creek in 1947 (Gomez and Grindstead, 1973). The dam is a 5.63 km horseshoe-shaped earth-and-fill construction with a clay core and rises 32 m above the thalweg at its highest point (Anon, 1952).

The major geologic formation in the Bluff Creek area is the Hennessey Shale, a Permian redbed. It consists of interbedded siltstones and sandstones with a total thickness of approximately 76 m and a regional dip of 7.6 m/km to the west (Anon, 1952). Soil thickness in the Bluff Creek area is only 30-90 cm (Toetz, 1982); thus, Lake Hefner is

Figure 1. Location Map of Lake Hefner, Oklahoma and Sampling Stations


AERATION
SYSTEM
INTAKE
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A
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CANAL
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m

situated primarily on bedrock.

Lake Hefner has a surface area of 1044 ha at normal pool elevation, a mean depth (z) of 8.84 m, and a maximum depth (z_{max}) of 28.6 m (Gomez and Grindstead, 1973). The watershed is only 1317 ha (Toetz, 1982). Relative depth, is 0.79 (Hakanson, 1981). Most larger lakes have a relative depth of approximately 0.12; thus, Lake Hefner is a relatively deep lake in relation to its surface area (Hakanson, 1981). The ratio $z:z_{max}$, an indication of basin shape (Hutchinson, 1957), is 0.308. A value of 0.33 to 0.50 is typical for bowl-shaped lakes with easily eroded bedrock. Ratios below 0.33 may occur in shallow lakes with deep holes (Hutchinson 1957). Lake Hefner contains extensive shallow areas in its western and southern ends (See Appendix A), but it is quite deep along the creek bed. The shoreline development ratio is 3.2 and the pool length is 5.63 km (Gomez and Grindstead, 1973). Water residence time is 1.96 years (Toetz, 1982), which is high in comparison to most reservoirs.

A rather unique feature of Lake Hefner is the aeration system. The system uses two positive displacement compressors, each with a maximum capacity of 5.66 $m^3 \cdot minute^{-1}$. These compressors are housed on the dam, and air is introduced at 26 m through thirteen 2.54 cm I.D. plastic pipes (Hearn, personal communication). The immediate area affected by this system is shown in Figure 1.

Two types of stations were established on Lake Hefner.

Stations for measuring horizontal and diel nitrogen fixation were located at the intersections of 0.80 km (0.50 mile) lines. These lines corresponded to the bisected grid lines on the Britton (SE) Quadrangle, U.S. Geological Survey, 1951 (1975 revision). All intersections which were mapped on the lake were used, except for those stations in shallow portions of the lake (depth <3 m). These stations correspond to stations A-N (Figure 1). Stations were located on the lake by visual sighting of two or more landmarks on shore. Where landmarks were not easily visible, 55 gallon drums were placed at the proper location to insure repeated sampling from the same location on the lake.

Three additional stations were established for measuring vertical and aphotic variation in nitrogen fixation, and for measurement of physical, chemical, and biological parameters. Stations 2 and 3 were in water at least 18 m, but as far away from the lake aeration system as possible. These stations were located visually and by depth readings and marked with buoys (Figure 1). Station 3 was subsequently relocated visually after the loss of its buoy on July 1. Station 1 was located in shallow water of approximately 7 m at the south end of the lake (Figure 1), where a metal platform was set into the lake bottom.

CHAPTER IV

MATERIALS AND METHODS

Nitrogen Fixation (Fixation)

Estimates of Variability

Variability in the horizontal distribution of nitrogen fixation was measured on July 4, 17, and 31, August 7 and 22, and September 5, 1981. Fourteen stations, A through N (Figure 1), were sampled on each date using a 1.27 cm x 3.05 m PVC pipe lowered vertically until the top of the pipe was flush with the surface of the water. The tube was then stoppered and brought to the surface. Two of these samples were thoroughly mixed to form a composite sample of approximately 6 liters. One to 2 liters of the composite was filtered through a 10 μm plankton net (Carolina Biological Supply Co.) resulting in a volume of about 20 ml. These concentrated samples were placed in serum bottles, and the final volume was adjusted to 50 ml with filtrate. Two concentrated samples from each station, together with six concentrated samples poisoned by the addition of 2 ml of 50% trichloroacetic acid (TCA) prior to exposure to acetylene (controls), were prepared for acetylene reduction analysis. All samples were incubated at Station 1 in a large flat wire

basket at 0.5 m for 2 h.

The vertical distribution of nitrogen fixation was measured on 17 dates at Station 1 and on 18 dates at Station 2 (Appendix B). Samples were taken with a non-toxic Kemmerer bottle at each station from the surface, 0.5, 1, and 3 m. Two 50 ml concentrated samples were prepared per depth as above. Three 50 ml unconcentrated samples of lake water were also prepared per depth. Concentrated and unconcentrated control samples per depth were poisoned by addition of TCA prior to acetylene addition. Final volume was 50 ml. All samples were incubated at the same depth as sampled in flat wire baskets at each station. Incubation was for 2 $\pm$ 0.50 h, between 1000 and 1400 h CDT at Station 2, and between 1100 and 1500 h at Station 1.

Diel nitrogen fixation experiments were conducted on June 17, July 1, 14 and 28, and August 12 and 29 at stations B, M, E, L, N, and A, respectively (Figure 1). Sampling stations were selected using a random digits table (Snedecor and Cochran, 1980). At each sampling station, two tube samples were taken and mixed to yield a composite sample. Two concentrated samples were prepared by filtration of 2 liters of composite sample through the plankton net. Concentrated sample volume was adjusted to 50 ml with filtrate before preparation for acetylene reduction. Three unconcentrated subsamples were also taken from the composite sample. Two unconcentrated composite subsamples poisoned with TCA were included as controls. All samples were incubated as

near as possible to the station in a wire basket at 0.5 m for 2 h. The first sampling began at approximately 0800 h. Four or five sampling periods followed at 2-2.5 h intervals, with the last sampling period after sundown.

Vertical distribution of nitrogen fixation in the aphotic zone was measured on the same six dates in which diel variation in nitrogen fixation was measured. Water at stations 2 and 3 was sampled using a non-toxic Kemmerer to obtain discrete samples from 6, 12, and 18 m. On July 14 and 28, two concentrated subsamples and two controls were prepared from each sample. Two liters of water were filtered for each concentrated subsample. Final volume in the serum bottles was 60 ml. On June 17, July 1, and August 12 and 28, two unconcentrated and two control subsamples were prepared per depth for acetylene reduction measurements. Final volume was 50 ml on June 17, and 60 ml on all other dates. Sample volume was increased to reduce gas exchange and the risk of implosion of the septa due to pressure differences. Unconcentrated samples were poured directly from the Kemmerer to avoid turbulence and gas exchange. The possibility of large changes in dissolved oxygen content due to sample handling was low except for 18 m samples taken on July 1. All samples were shielded from light and reincubated at the depth sampled at station 2. The duration of incubation was 21.5-27.2 h.

Acetylene Reduction Preparation

The acetylene reduction technique used was modified from Stewart et al. (1967). Serum bottles with a volume of 72 ml when stoppered were used for all experiments. Samples for acetylene reduction were placed in the bottles, stoppered with rubber septa, and 5 ml of air was withdrawn by syringe. Purified acetylene (Matheson Gas Co.) purged of acetone by passage through water was carried into the field in a septum stoppered glass reservoir. This gas had 70 ppm ethylene as a contaminant. Five ml of acetylene (C_2H_2) was injected by syringe into all sample bottles. The resultant partial pressure in the bottles made flushing with N_2-free gas unnecessary (Granhall and Lundgren, 1971; Stewart et al., 1971). The bottles were then shaken thoroughly for 30 sec to insure solution of the acetylene in the water. Timing of incubation began immediately after shaking. Acetylene reduction in all experimental samples was ended by addition of 2 ml TCA. Experimental and control samples were then sealed with Silicone Sealant (Maclanburg-Duncan Co.) and stored at room temperature until analysis.

Quantification of ethylene (C_2H_4) in control and experimental bottles was accomplished using a Hewlett-Packard 5750 gas chromatograph fitted with a 2 m x 0.32 cm 80/100 mesh Porapak R column, a flame ionization detector, and a Hewlett-Packard 7128A strip chart recorder. Oven and detector temperatures were 50-70°C and 115°C, respectively. From 2-6 ml boiling water was injected into all

sample bottles to insure positive pressure, and a 1 ml gas sample withdrawn for injection. The carrier gas was high purity nitrogen (Zero Gas, Matheson Gas Co.) to reduce background noise. Calibration curves were prepared on each day that ethylene was analyzed. Additional curves were also prepared whenever machine settings were changed. Ethylene standard gas for calibration was either 423 or 2099 ppm ethylene in nitrogen (Matheson Gas Co.). The ethylene concentration calculated from peak heights (n=1 except n=2 when peak height >12 mm) was converted to nanomoles C_2H_4 present in the entire air space within the bottle. This amount was corrected for incomplete transfer of ethylene to the vapor phase (Flett et al., 1976) using the equation:

$$\% \text{ transfer of ethylene} = 100/[1+(\alpha A/B)] \qquad (1)$$

where A = volume of the aqueous phase (ml), and B = volume of the vapor phase (ml). Temperature was assumed to be 25°C, thus alpha was 0.108 (Landolt-Bornstein, 1923).

Bottle Leakage

The loss of C_2H_4 from sealed serum bottles over time was determined using an axenic culture of Anabaena flos-aquae, University of Texas, (UTEX 1444), grown in nitrogen-free media (Miller et al., 1978). The culture contained approximately 1.8×10^6 cells$\cdot$ml^{-1}. Forty, 72 ml serum bottles were prepared with 50 ml culture solution. Twenty control bottles were poisoned with 2 ml TCA prior to acetylene exposure. All bottles were stoppered, prepared

for acetylene reduction, and incubated in a E-30B Percival growth chamber at 19.23×10^4 watts$\cdot$cm$^{-2}\cdot$sec^{-1} (400 foot candles) of continuous light (Miller et al., 1978) at 25°C for 2 h. Five each of pre-selected experimental and control bottles were analyzed immediately (t_o). Five more bottles were analyzed each month for 3 months.

Summer Nitrogen Loading

Nitrogen contributed by biological nitrogen fixation was calculated using the average rates of C_2H_2 reduced$\cdot$ liter$^{-1}\cdot$ h^{-1} (R) for stations 1 and 2 at each depth in the euphotic zone (surface, 0.5, 1, and 3 m). All calculations were carried out separately for concentrated and unconcentrated samples.

Rates calculated for all samples held for more than 5 days before analysis by gas chromotography were corrected for leakage of C_2H_4 from the serum bottles. The average (n=5) percent of C_2H_4 remaining at time t in serum bottles incubated for the leakage experiment was first plotted against time. The data points suggested a curvilinear relationship, thus the $\log_{10}$ (average percent C_2H_4 remaining) was plotted against time. The equation that describes the best fit least-squares line is:

$$Y_t = (-\ 0.008509) - 0.004628t, \qquad (2)$$

where r = (-0.9942). The percentage of C_2H_4 remaining

at time t was then antilog(Y_t). The reciprocal of antilog(Y_t) yielded a correction factor (CF) for adjustment of C_2H_4 remaining to that amount originally present:

$$CF = 1/\text{antilog}(Y_t) \quad (3)$$

For any experimental sample analyzed t days after incubation, the amount of C_2H_2 originally reduced was:

$$R' = CF \times R; \quad (4)$$

where R' = rate of reduction of C_2H_2 $\cdot liter^{-1}$ $\cdot hour^{-1}$ corrected for ethylene leakage.

The acetylene reduction rate, R', was converted to a rate of nitrogen fixation, mg $N_2 \cdot m^{-3} \cdot hour,^{-1}$ (M). A ratio of 3 moles C_2H_2 reduced for each 1 mole of N_2 fixed was assumed. The equation relating nanomoles C_2H_2 reduced to mg N_2 fixed, (M) is:

$$\text{mg } N_2 \text{ fixed} \cdot m^{-3} \cdot h^{-1} = R' \times 0.00933 \quad (5)$$

$$M = R' \times 0.00933 \quad (6)$$

The depths of incubation (surface, 0.5, 1, and 3 m) were assumed to be representative of the lake strata 0.00-0.25 m, 0.25-0.75 m, 0.75-2.00 m, and 2.00-5.00 m, respectively. The average lake elevation for each month and the lake volumes in m^3 at the elevation of each stratum were used to obtain the volume in m^3 of each stratum (L) for each month. The mg N_2 fixed $\cdot h^{-1}$ in a particular stratum

(G) equals the volume of that stratum multipled by mg N_2 fixed$\cdot m^{-3} \cdot h^{-1}$:

$$G = M \quad L \tag{7}$$

Expansion of G to an amount of nitrogen fixed per day was accomplished using the average variation in nitrogen fixation during the day represented by a smooth curve fitted to the diel data. The assumptions implicit in the drawing of this curve are that nitrogen fixation was negligible during the night, and that the rate of fixation rapidly increased after sunrise. The entire area under the curve was intergrated, as was the area under each hourly section of the curve. Division of the areas represented by each hour into the entire area under the curve gave percentages (P) of daily fixation that each hour represented. Division of P into unity gave an expansion factor (E) to calculate mg N_2 fixed$\cdot day^{-1}$ in a stratum from mg N_2 fixed$\cdot h^{-1}$ per stratum (G):

$$E = 1/P \tag{8}$$

$$\text{mg } N_2 \text{ fixed} \cdot day^{-1} = E\ G \tag{9}$$

This rate of daily nitrogen fixation, mg $N_2 \cdot day^{-1}$ was assumed to be the same for 2-3 days both before and after the actual measurement of fixation in the euphotic zone. The amount (S) of N_2 fixed per stratum in the five or seven day period represented by an experiment equals mg

$N_2 \cdot day^{-1}$ in equation (9) multiplied by the days represented by the experiment (D):

$$S = mg\ N_2 \cdot day^{-1} \cdot D \qquad (10)$$

The amounts of N_2 fixed per stratum per time period from (10) were summed for all strata in the euphotic zone. This yielded the total amount of N_2 fixed in the lake per experimental period. The N_2 fixed were summarized for all experimental periods at each station. This grand total represents the amount of nitrogen fixed in the lake during the summer.

Physical Measurements

Light penetration was measured in two ways. Secchi disk depth was measured on July 15, 17, and 20, and August 5, 6, 13, and 20 at stations 1-3, and on July 25 at station 3. Light penetration was measured on eleven dates (Appendix B) at stations 1-3, on August 30 at Station 2, and on June 20 and August 30 at Station 3 using a model 268 WA 310 submarine photometer with deck cell (G. M. Mfg. and Instrument Corp.). Measurements were made at the surface, 0.5, and 1 m, and at 1 m intervals until no light was detected. Temperature was measured on 23 dates (Appendix B) at stations 1-3, and on June 17, July 1, 14, and 28, and August 12 and 29 at Station 2 using a model 4041 Hydrolab or a model 57 YSI thermistor. Measurements were made at 0.5 m and at 1 m intervals from surface to bottom. Data on wind speed and

duration were obtained from NOAA, Climatological Data for Oklahoma (1981) taken at the Chickasa Experiment Station.

Chemical Analysis

Samples for nutrient analysis, ammonia (NH_4^+), nitrate (NO_3^-), soluble reactive phosphorus (SRP), and total phosphorus (TP), were taken with a brass Kemmerer bottle at stations 1-3 on 23 dates (Appendix B). Nitrite (NO_2^-) was measured on June 25 and 30, July 4, 5, 15, 17 and 20 at stations 1-3. All samples were taken with a brass Kemmerer at the surface, 0.5, 1, and 3 m at stations 1 and 2, and at 1 m at Station 3. Samples were stored in acid-washed polypropylene bottles, and were preserved by adjustment to pH 2 with 2 ml concentrated H_2SO_4. All samples were then placed on ice inside coolers and held at 4°C until analysis.

Analysis for nitrate was accomplished by the cadmium-column reduction method (Strickland and Parsons, 1968). Nitrite was analyzed by the sulphanilamide method (Strickland and Parsons, 1968). Nitrite values were sufficiently low to justify reporting nitrate without correction for nitrite. Total phosphorus, SRP, and Kjeldahl nitrogen analysis followed EPA (1974). Ammonia was determined by the phenylhypochlorite method (Solórzano, 1968). EPA quality control samples were included in all nutrient determinations except for Kjeldahl nitrogen.

A model 4041 Hydrolab was used to obtain dissolved

oxygen (DO), pH, and conductivity profiles with readings at 0.5 m and at 1 m intervals from surface to bottom. Dissolved oxygen and pH were measured on 23 dates (Appendix B) at stations 1-3. Dissolved oxygen was also measured on June 17, July 1, 14, and 28, and August 12 and 29 at Station 2. Conductivity was measured on June 20, 25, and 30, and July 4 and 5 at stations 2 and 3, and on June 30 and July 4 and 5 at Station 1. Hydrolab pH readings were calibrated with pH 7 and 11 buffers. DO was calibrated using Winkler oxygen readings (Lind, 1979). Conductivity was calibrated using potassium chloride standard solutions. When a Hydrolab was unavailable, DO profiles were obtained using a Winkler-calibrated model 57 YSI oxygen probe. Duplicate surface samples were taken at stations 1-3 for measurement of pH. The pH of each sample was read at the Lake Hefner Water Treatment Plant (WTP) using a Corning combination electrode and an Orion pH meter. Calibration was with pH 7 and 11 buffers. Conductivity was not measured when the Hydrolab was unavailable.

Two surface samples for chlorophyll _a_ were taken from stations 1-3 on 24 dates (Appendix B). Samples were taken in polypropylene bottles and placed on ice in the dark. Five hundred ml of each sample was filtered onto a 4.25 cm diameter 934-AH Whatman glass filter. The filters were placed in centrifuge tubes, and 10 ml of 2:1 chloroform-methanol solution (v/v) was placed in each tube. Chlorophyll _a_ was extracted in the dark for 4 h. Fluoresence

was measured after extraction on a model 111 Turner fluorometer fitted with Wratten 47B and 26 excitation filters, and a Wratten 2A emmission filter. Calibration curves were prepared for the 30X and 10X settings using purified chlorophyll *a* (Sigma Chemical Co.)

Phytoplankton

Phytoplankton samples were taken on 23 dates at stations 1-3 (Appendix B). Two vertical tows were made at each station from 5 m to the surface using a 10 μm plankton net. Samples were placed in small plastic or glass bottles and preserved with 1 ml Lugol's solution per 100 ml of sample. In the laboratory, one well-mixed 2 ml aliquot from each plankton tow was filtered onto a 0.22 μm pore glass Millipore filter, cleared with immersion oil, and mounted on glass slides with Permount (Greeson *et al.*, 1977). Algae were identified using Prescott (1978), Whitford and Schumacher (1973) and Patrick and Reimer (1966).

Anabaena, *Aphanizomenon flos-aquae*, *Microcystis aeruginosa*, *Merismopedia glauca*, and three other algae taxa were enumerated using random field counts (Greeson et al., 1977). These counts were converted to density estimates assuming random distribution on the filter. A correction factor, density in a filtered sample of known volume/density of plankton tow samples was used to calculate the actual density of organisms$\cdot ml^{-1}$ lake water. All other algae were identified to species and their

presence or absence recorded.

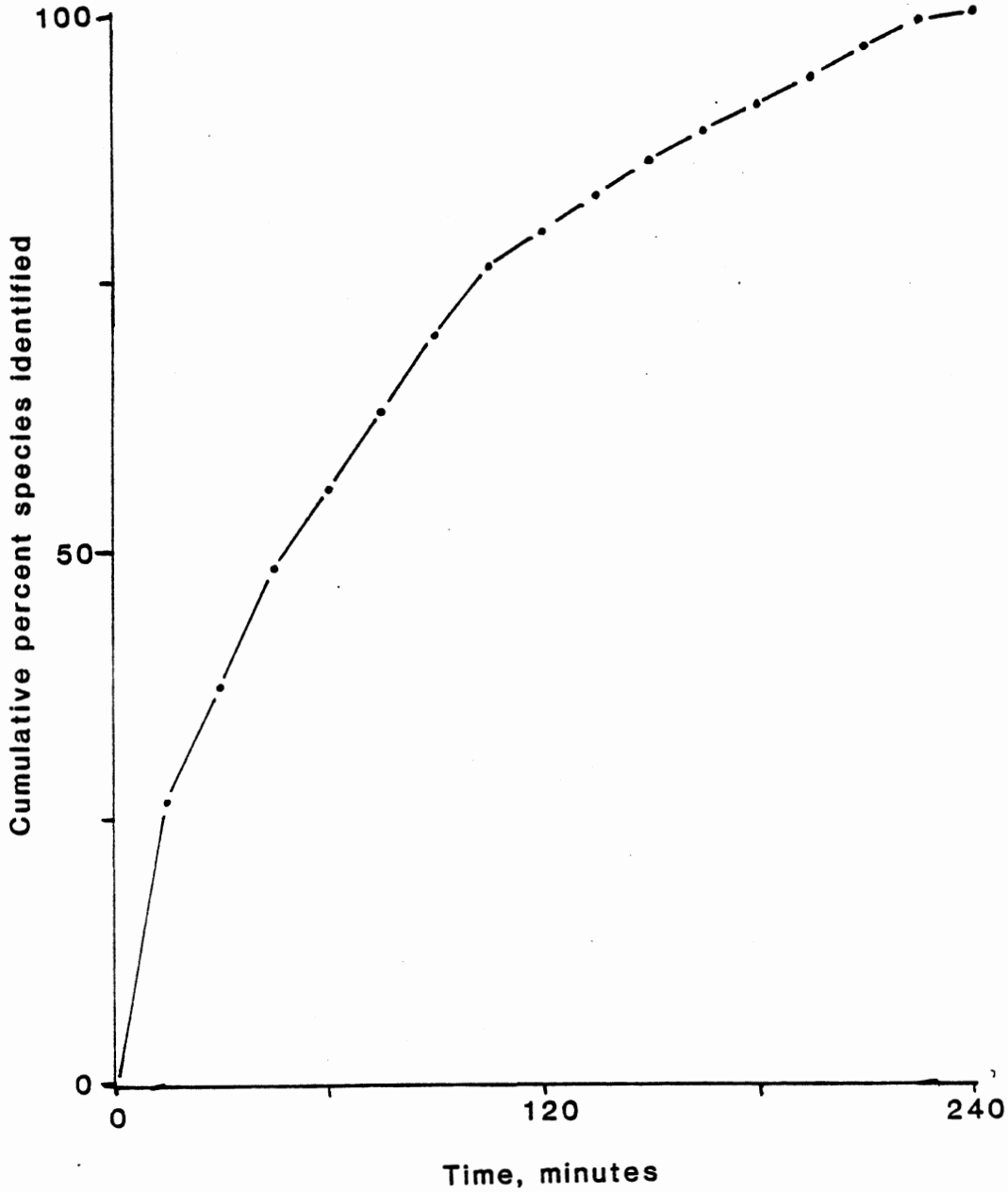
Presence-absence information was used in multivariate analysis of the phytoplankton community (Allen and Koonce, 1973). Presence of a species was recorded if a species was encountered during a 2.5 h examination of each slide. A 2.5 h examinantion period was chosen to minimize time spent on an individual slide, while identifying most (86.3 %, 66 species, n=2) of the algae present (Figure 2).

This examination procedure yielded a presence-absence data set containing observations on 143 species. Extremely rare species may actually weaken an analysis; thus, all species with two or less occurences were dropped (Allen and Koonce, 1973; Austin and Greig-Smith, 1968). The resultant data set contained 107 species, and was used in the phytoplankton factor analysis.

Statistical Analysis

Analysis of variance of the horizontal nitrogen fixation results from all six dates was accomplished using dates as blocks. The station and bottle values used in the calculations were means (n=12 and 2, respectively). One value for station A on July 4 and 17, and one value for station B on July 17 were estimated using $X = (aT + bB - S)/[(a - 1)(b - 1)]$, where a = number of treatments, b = number of blocks, T = the sum of items with the same treatment as the missing item, B = the sum of items with the same block as the missing item, S = the sum of all observed items, and X =

Figure 2. Cumulative Percent Species Identification as a Function of Time


100
50
0
Cumulative percent species identified
0
120
240
Time, minutes

estimate (Snedecor and Cochran, 1980). Dummy values were used for missing values not being calculated, and the entire process was iterated until the estimates did not change. The degrees of freedom of the residual term was reduced by three.

Regression analysis of acetylene reduction rates in concentrated versus unconcentrated samples was performed by SAS 1979.5 on an IBM 370 computer. The GLM procedure (General Linear Model) was used. The rate of reduction in concentrated samples was the independent variable. Procedure PLOT was used to graph the data points for each station. Factor analysis was also performed on the IBM computer using SAS 1979.5. The procedure FACTOR analyzed Spearman correlation coefficients generated by procedure CORR for each variable in the environmental and phytoplankton data sets. Initial estimates of communality were all 1.000. The PRIN (principal axis) and VARIMAX (variamax rotation) options were specified for both data sets. The factor retention criterion used was an eigenvalue of 1.000 for the environmental data set, and 70 percent of the total variation for the phytoplankton data.

No iteration was used. A factor loading was considered significant at a value of 0.50 for the environmental data and at 0.30 for the phytoplankton data (Kerlinger, 1979).

The Score option was specified and together with procedure SCORE produced factor scores for each combination of retained factor and observation. The Plot option was

specified and this generated plots of each possible two factor combination for each data set.

CHAPTER V

RESULTS

Variability in Nitrogen Fixation

Horizontal

Since the horizontal distribution of nitrogen fixation was assumed to vary widely, horizontal variation in nitrogen fixation was measured. The mean acetylene reduction rates varied from 0-4.90 nanomoles·liter^{-1}·h^{-1} (Table I). The summer mean rates for each station varied from 0.07-0.91 nanomoles·liter^{-1}·h^{-1} (Table I). An analysis of variance (ANOVA) showed a variation among stations and dates (Table II).

When the stations were ranked by their mean rate for the summer, stations A, G, H, and K are grouped as stations with low rates of fixation. All other stations occurred in a group with higher fixation rates as revealed by the ANOVA ($P<0.05$) and by tests of pairs of stations using the Least Significant Difference (LSD) method (Snedecor and Cochran, 1980). Tests between pairs of stations using LSD indicated significant differences ($P<0.05$) only when a station from the low group and a station from the high group comprised the test pair. Test pairs from within the high or low

TABLE I

HORIZONTAL DISTRIBUTION OF ACETYLENE REDUCTION IN COMPOSITE SAMPLES, IN NANOMOLES C_2H_2 REDUCED·LITER^{-1}·h^{-1}, AS MEANS FOR EACH DATE* AND FOR THE SUMMER**

Station	7/14	7/17	7/31	8/7	8/22	9/1	Summer Mean
A	0	0	0.02	0.05	0.27	0.10	0.07
B	0.04	0	0.02	0.09	0	2.04	0.36
C	0.03	0.45	0.02	0.14	4.14	0.66	0.91
D	0.42	1.74	0.01	0.05	0.18	0.16	0.43
E	1.56	1.21	0.24	0.05	0	0.48	0.59
F	0.03	0.22	0.08	0.01	4.90	0.16	0.90
G	0	0	0.02	0.12	0.38	0.20	0.12
H	0.09	0	0.08	0.02	0.20	0.04	0.07
I	1.39	0.67	0.12	0.07	0.13	0.82	0.53
J	0.03	1.36	0.04	0.01	0.87	0	0.38
K	0.02	0.02	0.02	0.01	0.27	0.30	0.11
L	0	1.34	0.08	0.05	2.57	0.18	0.70
M	0.09	0.96	0.08	0.19	2.35	0	0.61
N	0.03	0.67	0.02	0.09	2.46	0.12	0.56

*n=2

**n=12

TABLE II

ANALYSIS OF VARIANCE OF ACETYLENE REDUCTION RATES AT STATIONS A - N

Source	df.	Sum of Squares	Mean Square	F
Dates	5	5.980	1.196	14.419*
Stations	13	2.762	0.212	2.562**
Residual	63	5.226	0.0829	
Bottles (subsamples)	83	22.272	0.268	

* $P<0.005$

** $P<0.05$

groups showed no significant differences. Of the group with low rates, stations G, H, and K were in shallow water of 3-5 m near the canal outfall, and Station A was the nearest station to the lake aeration system (Figure 1).

Significant differences among dates ($P<0.005$) also existed as indicated by the F-ratio (Table I). Significant temporal variation is expected when measuring cyclic phenomena. A large variation among bottles may be the result of technique in handling samples despite the fact that these samples were concentrates of composite samples.

Aphotic

Rates of nitrogen fixation in the aphotic zone of Lake Hefner were low. Many experimental samples contained no more ethylene than controls. No difference existed on a volume basis between concentrated and unconcentrated samples from different dates (Table III). Septa on some samples, despite reduction in the air space within the serum bottles and application of sealant before incubation, were pushed inside the serum bottles by water pressure. Also, some bottles broke during incubation, probably due to movement of the buoy (Table III). Because of the low rates of fixation and missing samples, rates of fixation in the aphotic zone were not included in the calculation of nitrogen loading due to biological nitrogen fixation.

TABLE III

AVERAGE NITROGEN FIXATION IN THE APHOTIC ZONE OF LAKE HEFNER, SUMMER 1981, IN NANOMOLES C_2H_2 RECUCED·LITER^{-1}·h^{-1} (n=2)

Depth (m)	6/17	7/1	7/14	7/28	8/12	8/9
			Station 2			
6	0	0.020	0.015	0.005	0	0
12	0.090	0.020	0.006	0	0.080	0
18	0.050	0.015	0*	0.002	0.290*	0
			Station 3			
6	0	0.120	0.003	0.030	0	0
12	0.050	0.100	B	0	0.025	0.020
18	0	B	B	0	B	B

* One Sample Only
B - Broken or Imploded Bottles

Sample Type

The possibility existed that there were differences between rates of nitrogen fixation calculated from data for concentrated and unconcentrated samples, regardless of depth of incubation. Linear regressions using the average concentrated rates as the independent variable and the average unconcentrated rate from each depth for each date showed a definite relationships between the two types of samples. For stations 1 and 2:

$$Y_1 = 1.097 + 1.168\ X_1, \text{ and} \quad (11)$$

$$Y_2 = 1.574 + 1.561\ X_2, \quad (12)$$

where Y_i = unconcentrated rates X_j = concentrated rates. The coefficients were $r_1 = 0.6533$, and $r_2 = 0.8116$. The relationship was stronger for station 2 samples than for station 1 samples.

The rate of nitrogen fixation in unconcentrated samples was significantly higher (slope greater than 1.000) than the rate in concentrated samples at Station 2. This may be the result of either real differences between types of samples, or it may reflect the difficulty in measuring differences between unconcentrated samples and controls. Use of the rates of nitrogen fixation by concentrated samples would represent a conservative estimate of nitrogen fixation.

Vertical

Variation of nitrogen fixation with depth was erratic.

Fixation was depressed at the surface in concentrated and unconcentrated samples on less than half of the dates on which it was measured (Figure 3).

The maximum rate of fixation was usually between 0.5 and 1 m. A representative curve is shown in Figure 4. A minimum occurred at 0.5 m on July 20, however (Figure 4).

Maxima in the rates of acetylene reduction not shown in Figure 4 occurred at 3 m on July 15 and September 20. There were also occasional minima at 0.5-1 m. Thus, although the nitrogen fixation rate occasionally exhibited a typical bust-shaped distribution of rate with depth, only certain components of this relationship were common throughout the summer.

Light attenuation was rapid under light intensities that varied from 900-7600 microamps at Station 1. Extinction coefficients ranged from 1.06-2.10. A typical light attenuation curve is shown in Figure 4. Varying light regimes would directly influence vertical variation in nitrogen fixation.

Changes in the overall rate of nitrogen fixation in the euphotic zone are best seen in the rate of daily fixation of N_2 (Figure 5). This daily rate is equal to the summation of equation 9, (page 20) for all four depths in the euphotic zone. The daily rate of nitrogen fixation varied from 0.6 on August 30 to 32.6 nanomoles$\cdot$liter$^{-1}\cdot$h^{-1} on June 25th. These peaks were correlated with peaks in the density of Anabaena. Concentrated samples showed that daily fixation

Figure 3. Comparison of Nitrogen Fixation at Two Depths (0 and 0.5 m) for Concentrated and Unconcentrated Samples During 1981. A. Averages of Concentrated Samples (n=2). B. Averages of Unconcentrated Samples (n=3)

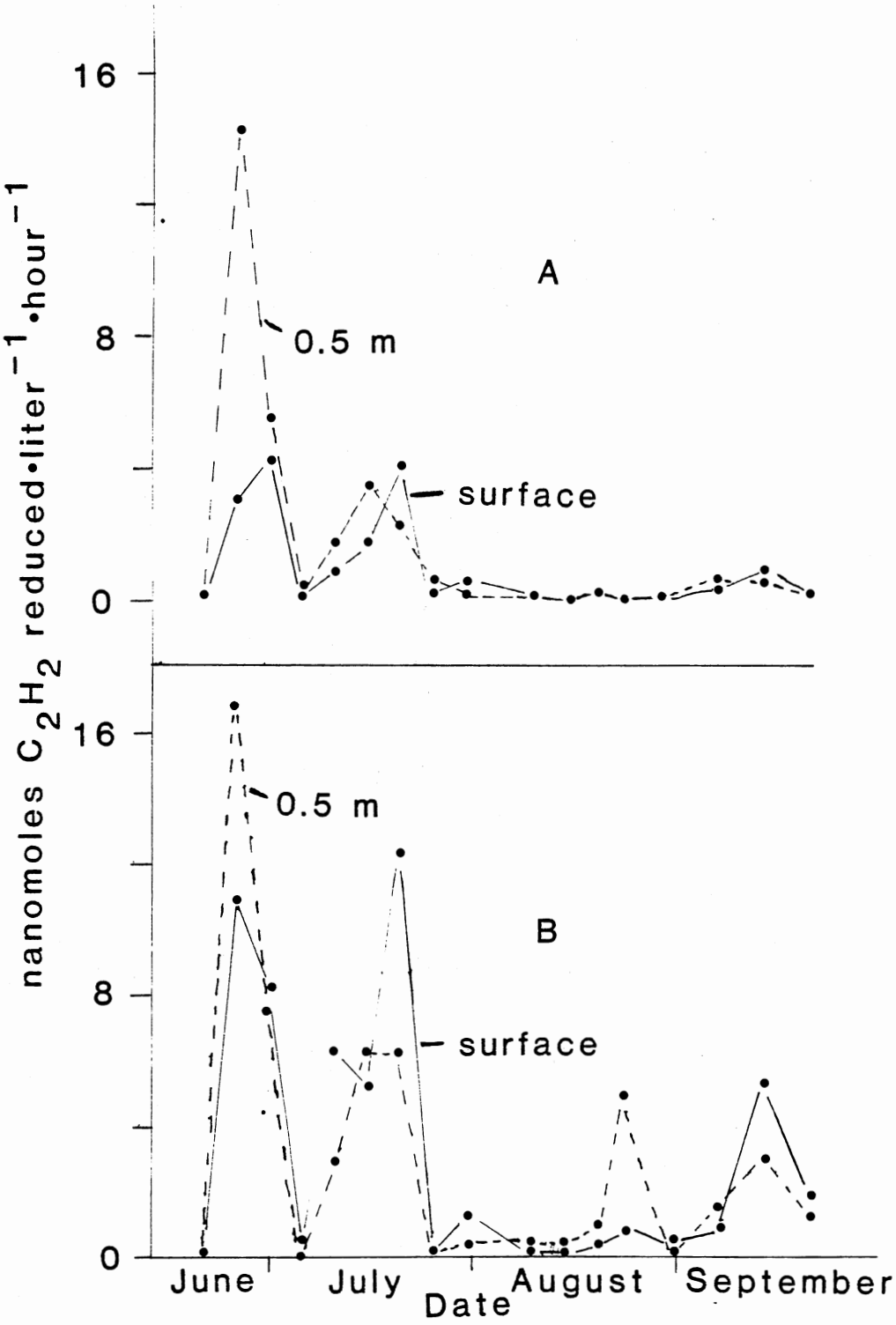

16
8
0
A
0.5 m
surface
16
8
0
B
0.5 m
surface
nanomoles C_2H_2 reduced•liter^{-1}•hour^{-1}
June
July
August
September
Date

Figure 4. The Relationship of Acetylene Reduction in Concentrated Samples to Depth and Light Attenuation

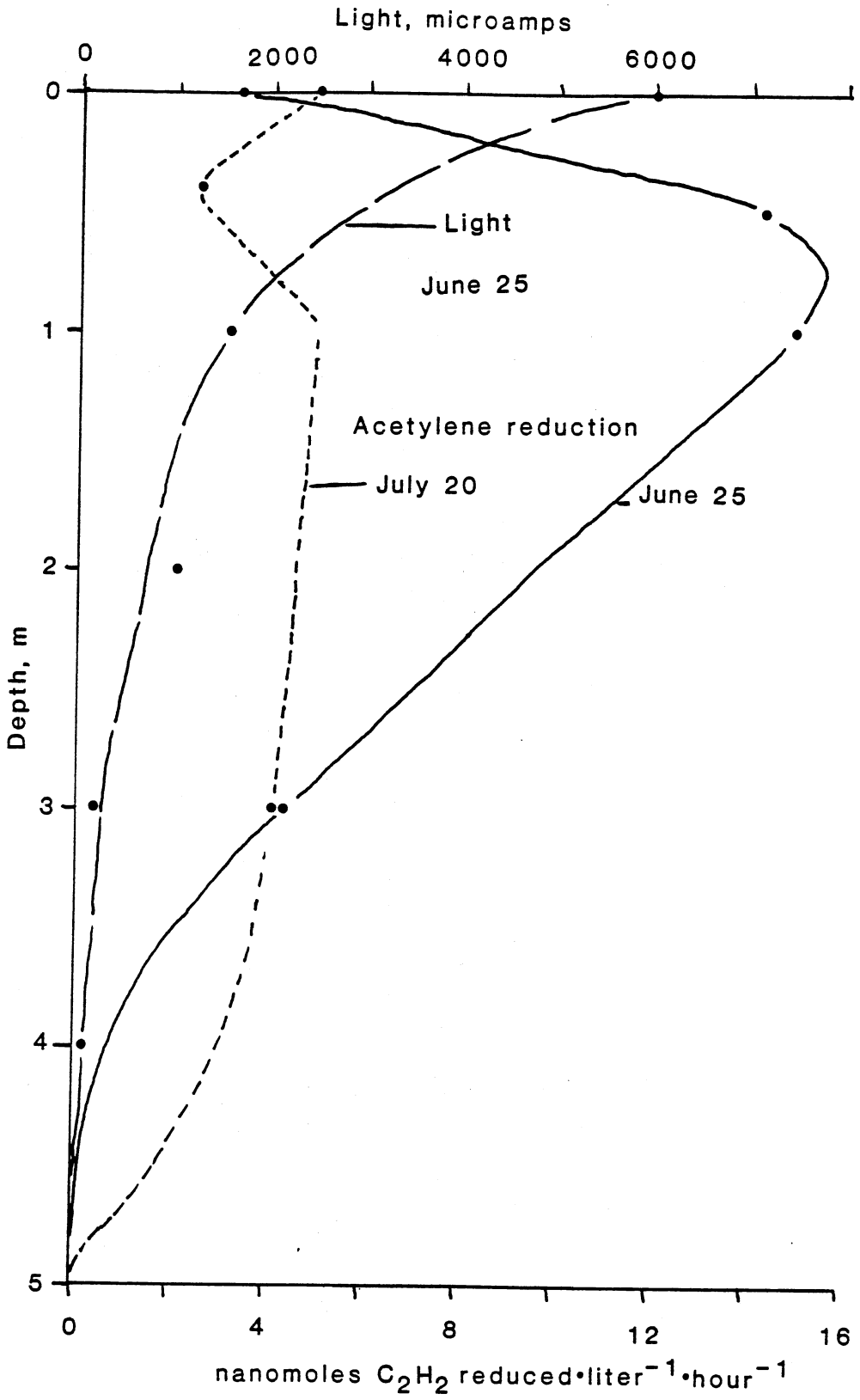
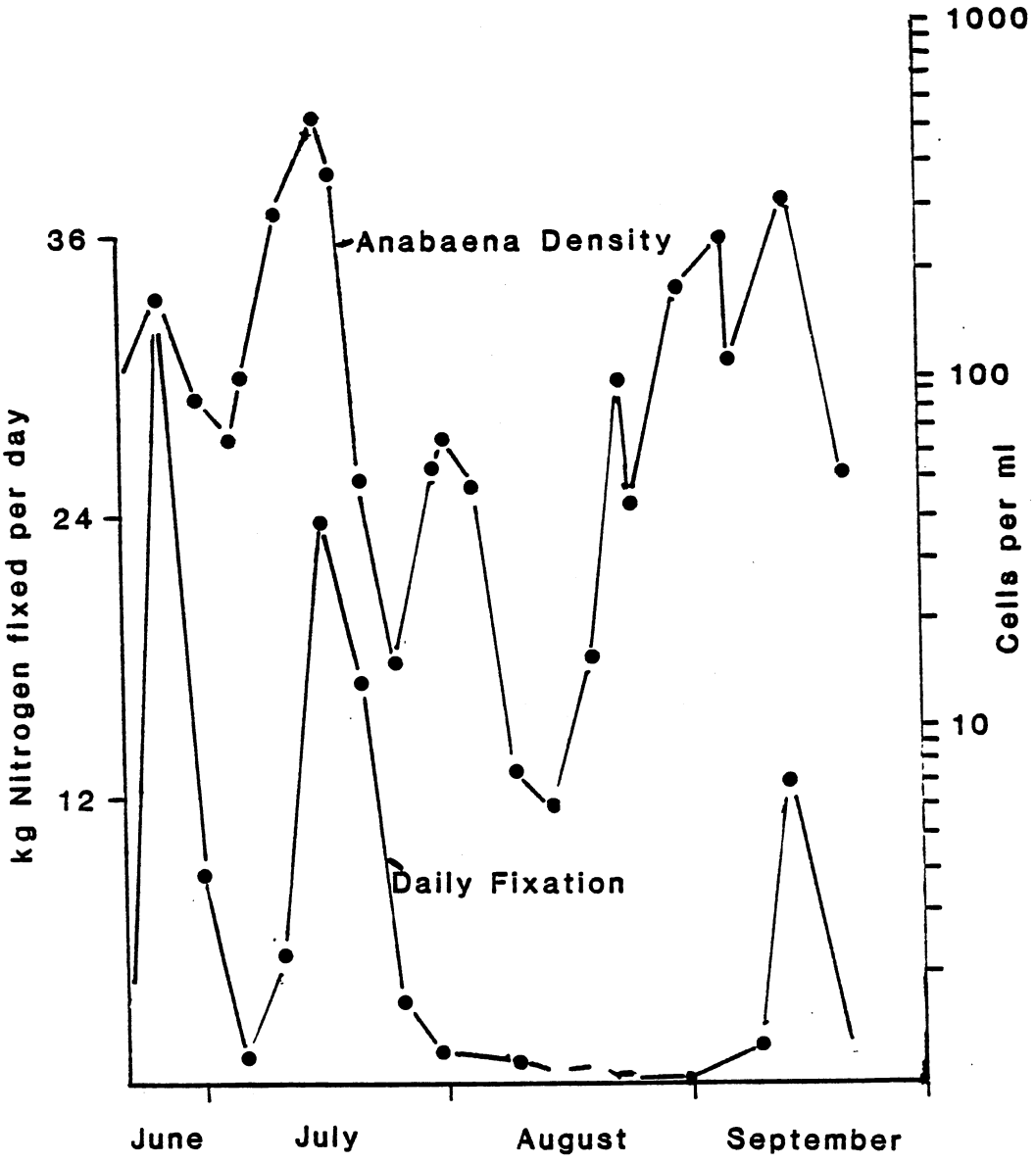

Light, microamps
0
2000
4000
6000
Light
June 25
Acetylene reduction
July 20
June 25
Depth, m
0
1
2
3
4
5
0
4
8
12
16
nanomoles C_2H_2 reduced·liter^{-1}·hour^{-1}

Figure 5. Plot of Anabaena Density and Daily Fixation Versus Date, Summer, 1981


1000
100
10
Cells per ml
36
24
12
kg Nitrogen fixed per day
Anabaena Density
Daily Fixation
June
July
August
September

rates were high on June 25, July 15 and 20, and on September 13. The unconcentrated samples showed the same pattern of nitrogen fixation activity at a higher level of activity (Figure 6).

Diel

Diel variation in nitrogen fixation was erratic from date to date. Fixation in concentrated samples, as a percent of the total for that date (July, 1), ranged from 46.3% in the morning, to 2.5-11.7% in the early afternoon and late evening (Figure 7B). This suggests midday supression of nitrogen fixation. Other dates, however, do not show this pattern (Figure 7A and B). The only commonality is the low percentage of fixation found in the night samples, as would be expected of an energy-dependent process. Fixation in unconcentrated samples as a percent of the total showed even wider variation, again with no common pattern to the variation during the day, and will not be considered further.

The aim of measuring diel variation was to derive one, or perhaps two, sets of data with which to expand fixation in the euphotic zone during a particular time of day to an amount fixed during the entire day. Given the high variability present in the data, points representing averages of the percent fixation in concentrated samples at each time period were plotted and line of best fit was drawn through the data. The line of best fit from diel nitrogen fixation

Figure 6. Daily Fixation in Unconcentrated Samples, Summer 1981, in kg N_2 Fixed·Day^{-1}

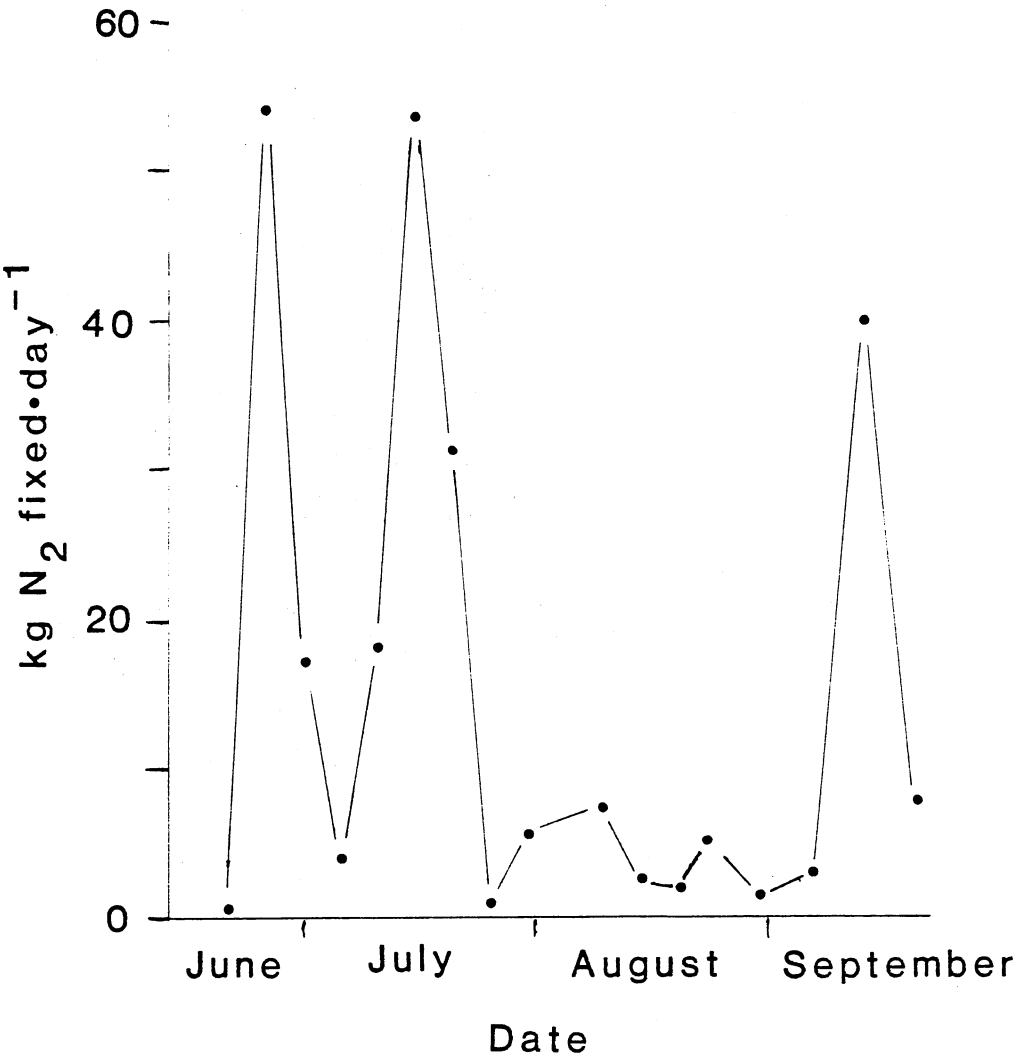
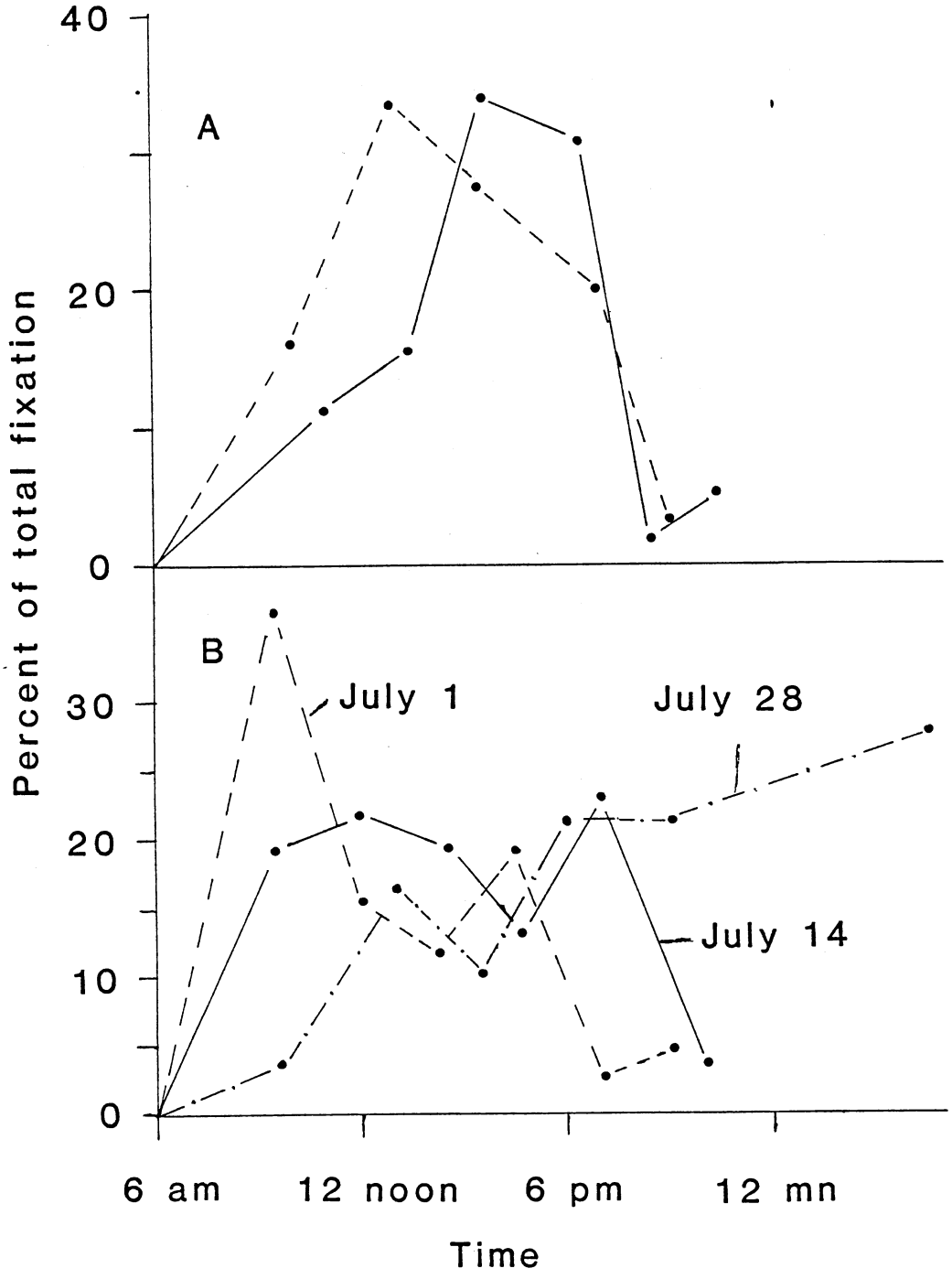

60
40
20
0
kg N_2 fixed•day^{-1}
June
July
August
September
Date

Figure 7. Percent of Total Nitrogen Fixation as a Function of Time of Day. A. August 12 (———) and 29 (---). B. July 1 July 14 and July 28


40
20
0
A
B
30
20
10
0
July 1
July 28
July 14
Percent of total fixation
6 am
12 noon
6 pm
12 mn
Time

rates was used to expand hourly rates to daily rates.

Nutrient Budget

The nitrogen and phosphorus budgets for Lake Hefner, Oklahoma, for 1980 have been previously calculated (Toetz, 1982). The nitrogen budget for 1981 is similar (Table IV). Nitrogen inputs are dominated by the canal flow, and to a lesser extent by precipitation. Nitrogen losses are dominated by water plant withdrawls. Relative phosphorus inputs and withdrawls were similar to nitrogen, but input of phosphorus by precipitation was much lower. Losses of nitrogen due to denitrification and voltilization of ammonia were assumed to be negligible.

The input of nitrogen via biological nitrogen fixation as estimated using concentrated samples (Table IV) differs from that reported by Toetz (1982) for 1981 due to correction for loss of C_2H_4 over time. The corrected estimate, 588 kg N_2, is 28% larger and it is equivalent to the summation of equation (10) for all strata. The percentage contribution to the nitrogen budget is small (0.69%). The contribution of nitrogen fixation to the nitrogen budget calculated from unconcentrated samples is 1122 kg N_2. The percentage contribution, however, is still relatively small (1.3%). An intermediate estimate of the contribution of fixation to the nitrogen budget is 1%.

During 1981 the ratio of nitrogen to phosphorus (N:P) in the incoming water was 5.23:1 and the N:P ratio in

TABLE IV

PRELIMINARY TOTAL NITROGEN AND PHOSPHORUS BUDGETS FOR LAKE HEFNER, JANUARY 1, 1981 TO DECEMBER 31, 1981*

Influx	Phosphorus	Nitrogen
Canal (Spring, 1981)	11,579	51,489
Canal (Summer/Autumn, 1981)	1,388	4,159
Precipitation (bulk)	611	14,550
Groundwater	8	74
Watershed	?	?
Biological nitrogen fixation**	NA	1,222
Total	13,586	71,079
Outflux		
Water treatment plant	3,542	34,690
Groundwater	16	147
Volatilization and denitrification	NA	0
Fish harvest	42	530

Influx:outflux

*Toetz (1982)(pers. comm.). Biological nitrogen fixation. **Estimate for summer of 1981 using unconcentrated samples All values are kg. NA=does not apply to this element

outgoing water was 9.82:1.

Twice as much nitrogen and 3.8 times as much phosphorus entered the lake than left via the water treatment plant. The loading rates for nitrogen and phosphorus, using a lake surface area of 1044 ha, were 6.81 and 1.30 $gm \cdot m^{-2} \cdot year^{-1}$, respectively.

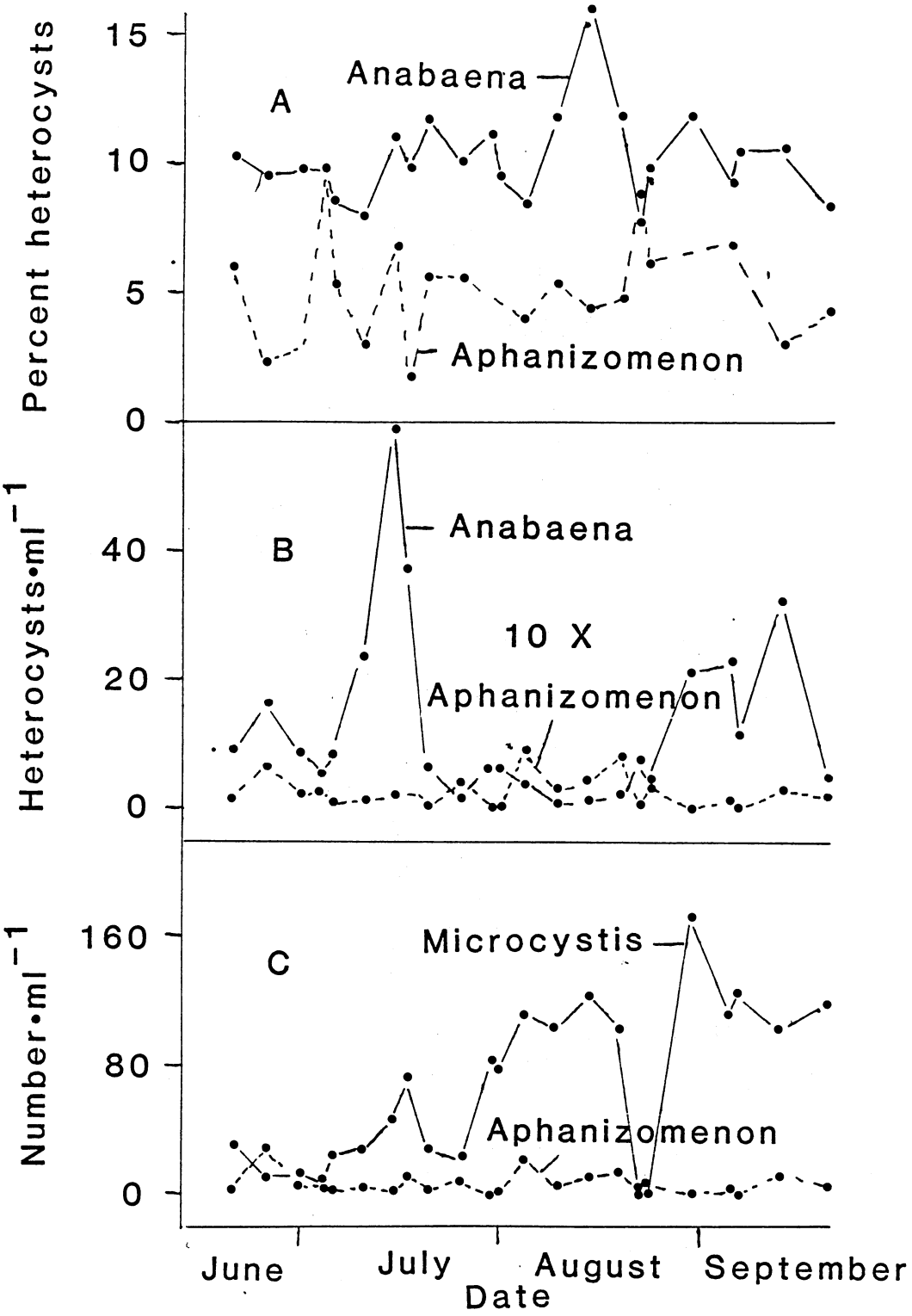
Phytoplankton Densities

Densities calculated from plankton hauls were corrected for sampling efficiency. Slides were made from concentrated samples from the nitrogen fixation experiments (July 17, Station B; July 25, August 30 and September 13, Station 2). These samples were known volumes of water poured directly through the plankton net. The density in the concentrated samples was 3.74 times as great as in the plankton hauls (n=10), indicating an efficiency for the plankton net of only 26.7%. Algal densities estimated from random field counts for the plankton hauls were then corrected for lack of efficiency in sampling with the plankton net.

Density of Anabaena during the summer ranged from 6.0 $cells \cdot ml^{-1}$ on August 14 to 538 $cells \cdot ml^{-1}$ on July 15 (Figure 5). These densities are much lower than reported for 1980 (Toetz, 1982). Anabaena heterocyst density varied closely with cell density (compare Figures 5 and 8). Heterocysts as a percent of all Anabaena cells ranged from 7.5 to 16.1% (Figure 8). Changes in Anabaena densities and daily nitrogen fixation were similar (Figure 5).

Figure 8. Blue-green Algal Densities and Percent Heterocysts, Summer 1981, Station 2

(A) Percent Heterocysts of *Aphanizomenon* and *Anabaena* (B) Heterocysts ml^{-1} of *Anabaena* and 10 x Heterocysts ml^{-1} of *Aphanizomenon* (C) Cells ml^{-1} *Aphanizomenon* and Colonies ml^{-1} *Microcystis*


Percent heterocysts
15
10
5
0
A
Anabaena
Aphanizomenon
Heterocysts•ml^{-1}
40
20
0
B
Anabaena
10 X
Aphanizomenon
Number•ml^{-1}
160
80
0
C
Microcystis
Aphanizomenon
June
July
August
September
Date

The changes in density of the other dominant blue-green alga, Microcystis aeruginosa, differed considerably from Anabaena (Figure 8). The greatest densities of Microcystis occurred in August and in September when the numbers of Anabaena were low. The complete disappearance of M. aeruginosa on August 22 and 23 may be an artifact of preservation, as many small, single cells were visible on these slides, suggesting that the colonial mucilage had been destroyed. Densities of Microcystis were also low in the early summer when Anabaena densities were high.

Aphanizomenon flos-aquae was observed rarely during random field counts. Its density ranged from 0 - 27 cells·ml^{-1} during the summer (Figure 8). The percent heterocysts for A. flos-aquae in 1981 (Figure 8) was higher than reported by Toetz (1982) for 1980, but it is still low in comparison to Anabaena. The heterocyst density of Aphanizomenon was also low (Figure 8).

Dynamics of Environmental Parameters

In contrast to the wide variation in phytoplankton densities, the concentrations of nutrients (NO_3^-, SRP, TP) remained fairly stable during the summer (Figure 9). Mean nitrate concentrations were generally low and stable (Table V). Mean total P and SRP concentrations were somewhat more variable (Table V). Nitrate, NH_4^+, TP, and SRP were rarely absent from the epilimnion. Nitrite was low in the surface waters (Table V), and was not measured past

Figure 9. Nutrient Concentrations at 1 m, Station 2, Summer 1981 as N or P

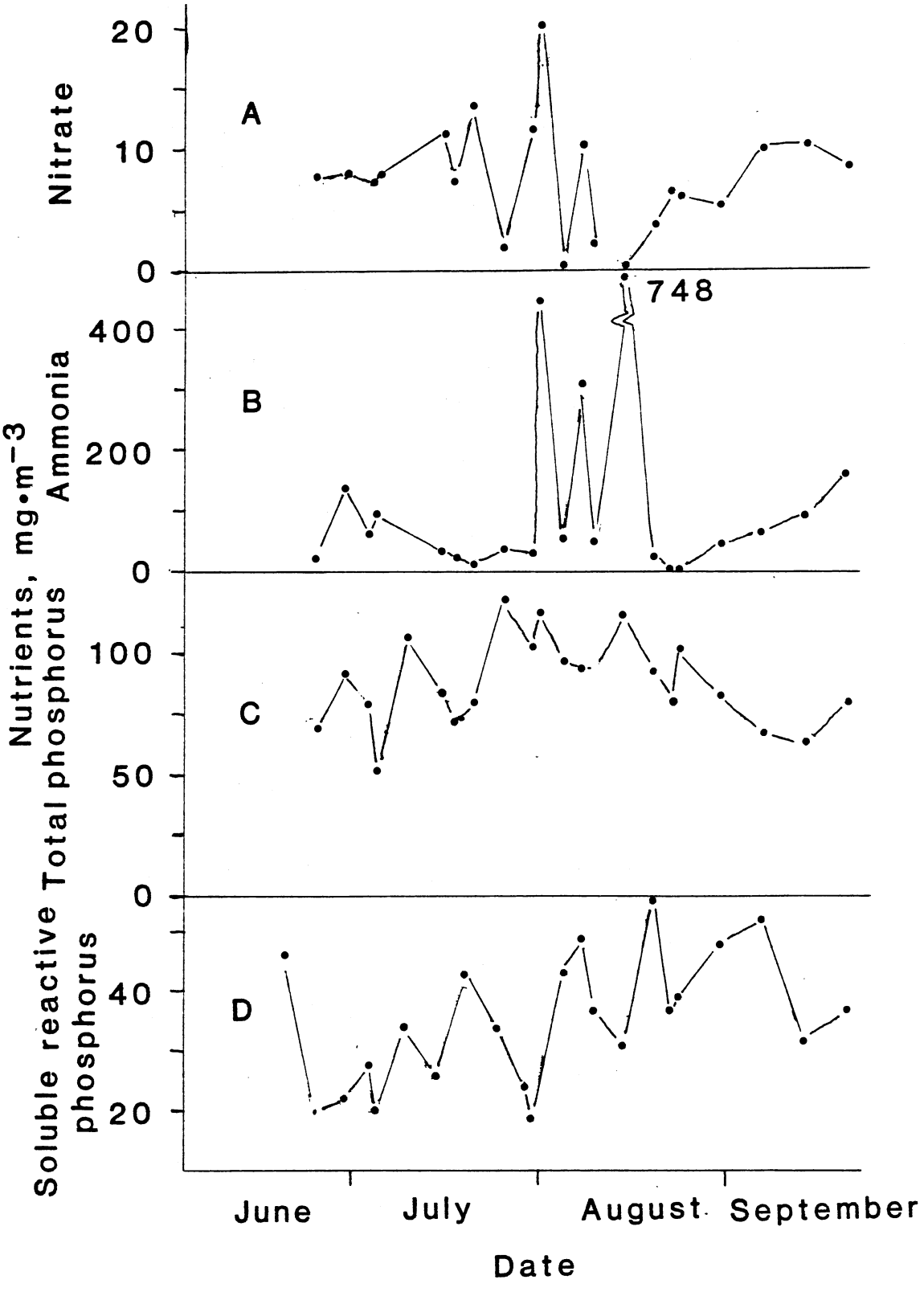

Nutrients, mg•m⁻³
Nitrate
Ammonia
Total phosphorus
Soluble reactive phosphorus
A
B
C
D
20
10
0
400
200
0
748
100
50
0
40
20
June
July
August
September
Date

TABLE V

MEANS AND STANDARD DEVIATIONS FOR SELECTED PARAMETERS, LAKE HEFNER, SUMMER 1981, μg LITER^{-1}

Measurement	Station		
	1	2	3
Nitrate-N(NO_3^-)	11.7+11.0	8.3+5.1	9.1+6.0
Nitrite-N(NO_2^-)	3.2+1.8	3.4+2.1	3.4+2.1
Ammonia-N(NH_4^+)	114+128	115+177	86+102
Kjeldahl-N	821+354	777+238	628+197
Total Phosphorus (TP)	92+13.5	88+18.5	93+15.0
Soluble Reactive Phosphorus (SRP)	37+16	36+11	38+11
Chlorophyll *a*	28.2+16.4	30.6+15.0*	28.0+13.6
Extinction coefficient	1.48±0.28	1.43±0.36	1.46±0.31
Secchi Disk (m)	0.82+0.09	0.87+0.10	0.86+0.11

*Does not include value of 295 μg·liter^{-1} for September 13, 1981

July 5. Total Kjeldahl nitrogen varied by roughly a factor of two during late summer (Table V). Only NH_4^+ exhibited wide variations (Figure 9). Up to 1 month storage before analysis and difficulity in exact pH adjustment may account for some of the extremely high NH_4^+ values.

The pH at Station 2 ranged from 8.4 -9.4. The pH at the surface and temperature at 1 m, respectively, were also quite stable during the summer (Figure 10). The majority of pH measurements were made in the morning and early afternoon. On June 25, pH was measured later in the afternoon and pH had increased from 8.6 to 9.0. The measurement of later pH samples at the water treatment plant may have obscured productivity-related pH changes. Temperature at 1 m ranged from 22.0°C on September 20 to 29.0°C on July 14 and 15 (Figure 10). At 18 m, temperature ranged from 21.8°C on September 20 to 26.8°C on July 28.

A marked variation existed in chlorophyll *a* during the summer. Chlorophyll *a* was low during late June (Figure 11). The value of 295 mg $liter^{-1}$ on September 13 may represent algae that surfaced during a brief calm period. The mean concentration of chlorophyll *a* present at station 2, excluding September 13, was 30.6 mg $liter^{-1}$ (Table V).

Lake Hefner showed a more variable stratification pattern than expected for a normal warm monomictic lake. Temperature stratification (at high hypolimnetic temperatures) was most evident on July (Figure 11A). Although the abso-

Figure 10. Season Variation of Selected Parameters at Station 2, Summer 1981

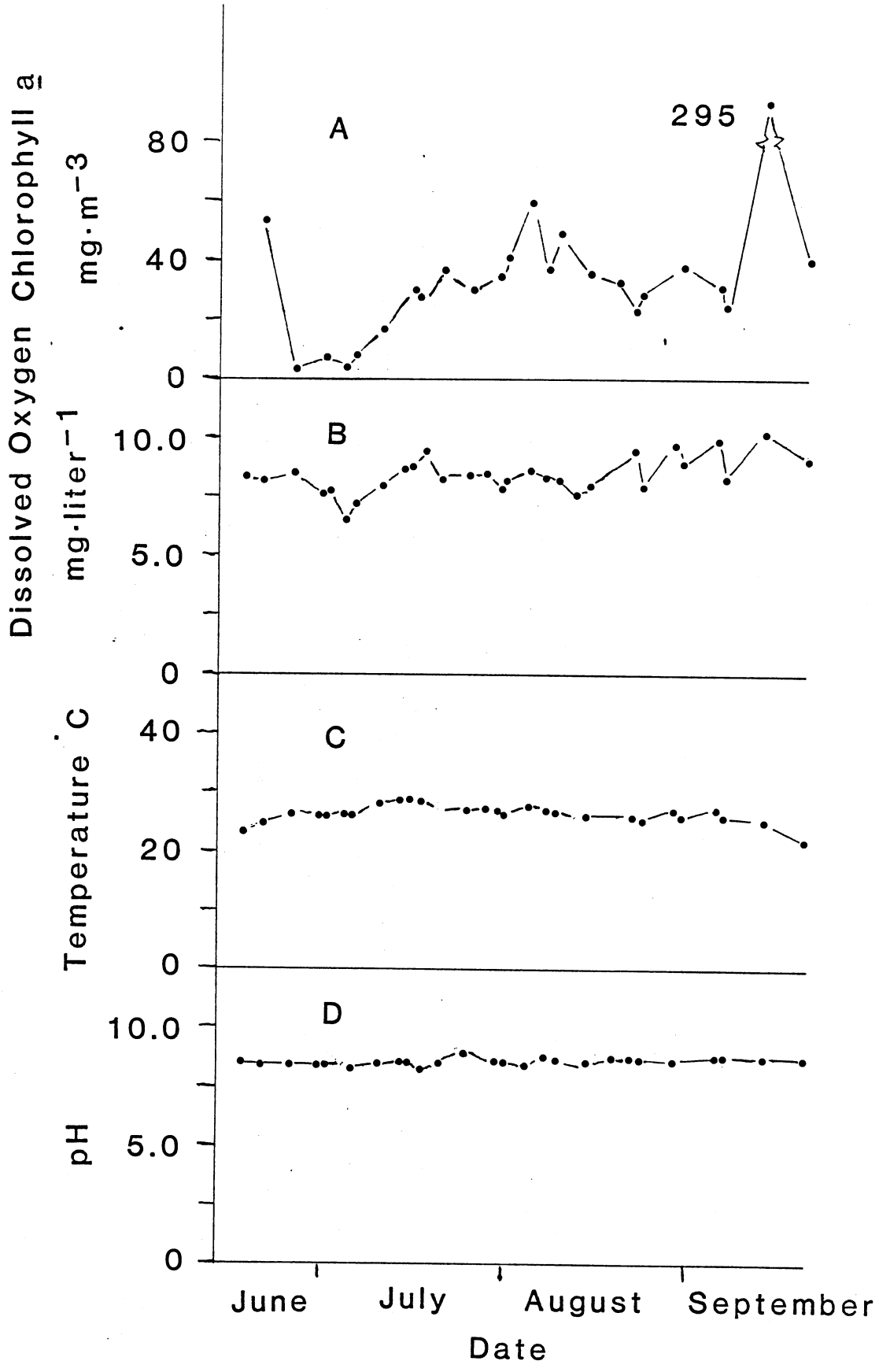
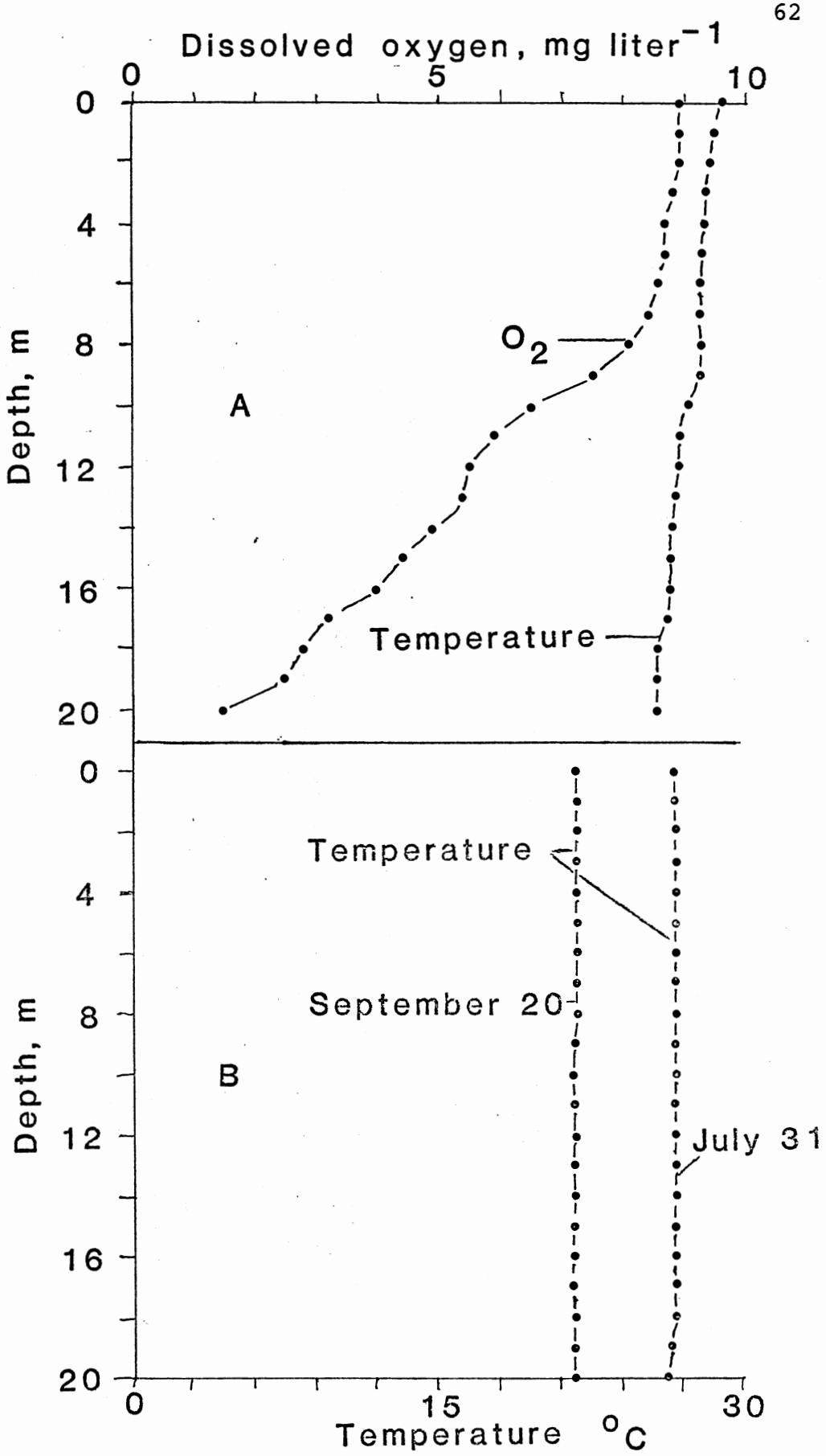

Dissolved Oxygen Chlorophyll a
mg·m⁻³
80
40
0
A
295
mg·liter⁻¹
10.0
5.0
0
B
Temperature °C
40
20
0
C
pH
10.0
5.0
0
D
June
July
August
September
Date

Figure 11. Dissolved Oxygen and Temperaturae Profiles for Station 2. A - Dissovled Oxygen and Temperatures, July 15. B - Temperature Profiles, July 31 and September 20


Dissolved oxygen, mg liter⁻¹
0
5
10
0
4
8
12
16
20
Depth, m
O2
A
Temperature
0
4
8
12
16
20
Depth, m
Temperature
September 20
B
July 31
0
15
30
Temperature °C

lute temperature difference was small, density differences were sufficient to produce a clinograde DO curve (Figure 11A). Periods of mixing occurred in late July and late September (Figure 11B), when Lake Hefner became isothermal. Summer stratification was thus weak during 1981.

Light was rapidly attenuated in Lake Hefner. A typical plot of light intensity versus depth (Figure 4) showed that only 1% of the surface light intensity (I_o) was available below 2 m. That light was rapidly extinguished is also born out by the mean Secchi disk depth which ranged from 0.82 to 0.87 m and the mean extinction coefficients (n" in $\log_{10}$), which ranged from 1.48 to 1.43 (Table V). The maximum depth of euphotic zone was 4.25 m on June 25 (n"=1.058).

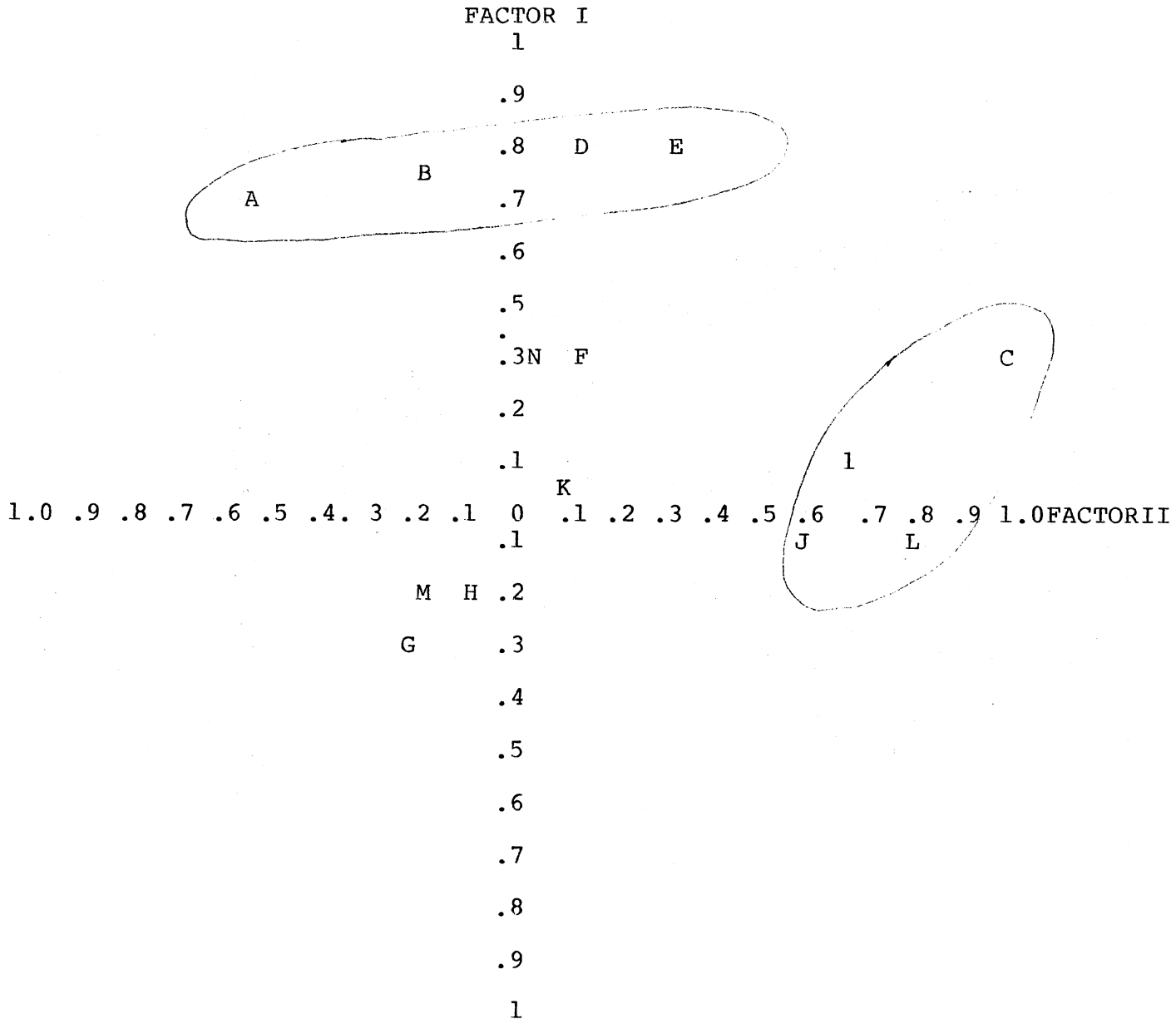
Principal Axis Factors

Environmental Data

The factor analysis of the environmental data yielded five factors which accounted for 81.6% of the variations present in the data set before rotation. Rotation adjusted the factor axes so that graphical identification of the factors and their biological interpretation were simplified (Appendix D and Figure 12). The first three factors after rotation accounted for 55.7% of the total variation and were the most easily interpretable. The factor loadings that produced Figures 12 and 13 are presented in Appendix C.

Figure 12. Environmental Factors I and II After Rotation, Station 2

Time =A Temp =B DO =C Delt =D Delo =E Nitrate =F
Ammonia =G Tphos =H Ophos =I Chla =J Light =K
pH =L Wind =M Nfix =N


FACTOR I
1
.9
.8
D
E
B
A
.7
.6
.5
.3N
F
C
.2
.1
1
K
1.0 .9 .8 .7 .6 .5 .4. 3 .2 .1 0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0FACTORII
.1
J
L
M
H
.2
G
.3
.4
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.6
.7
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.9
1

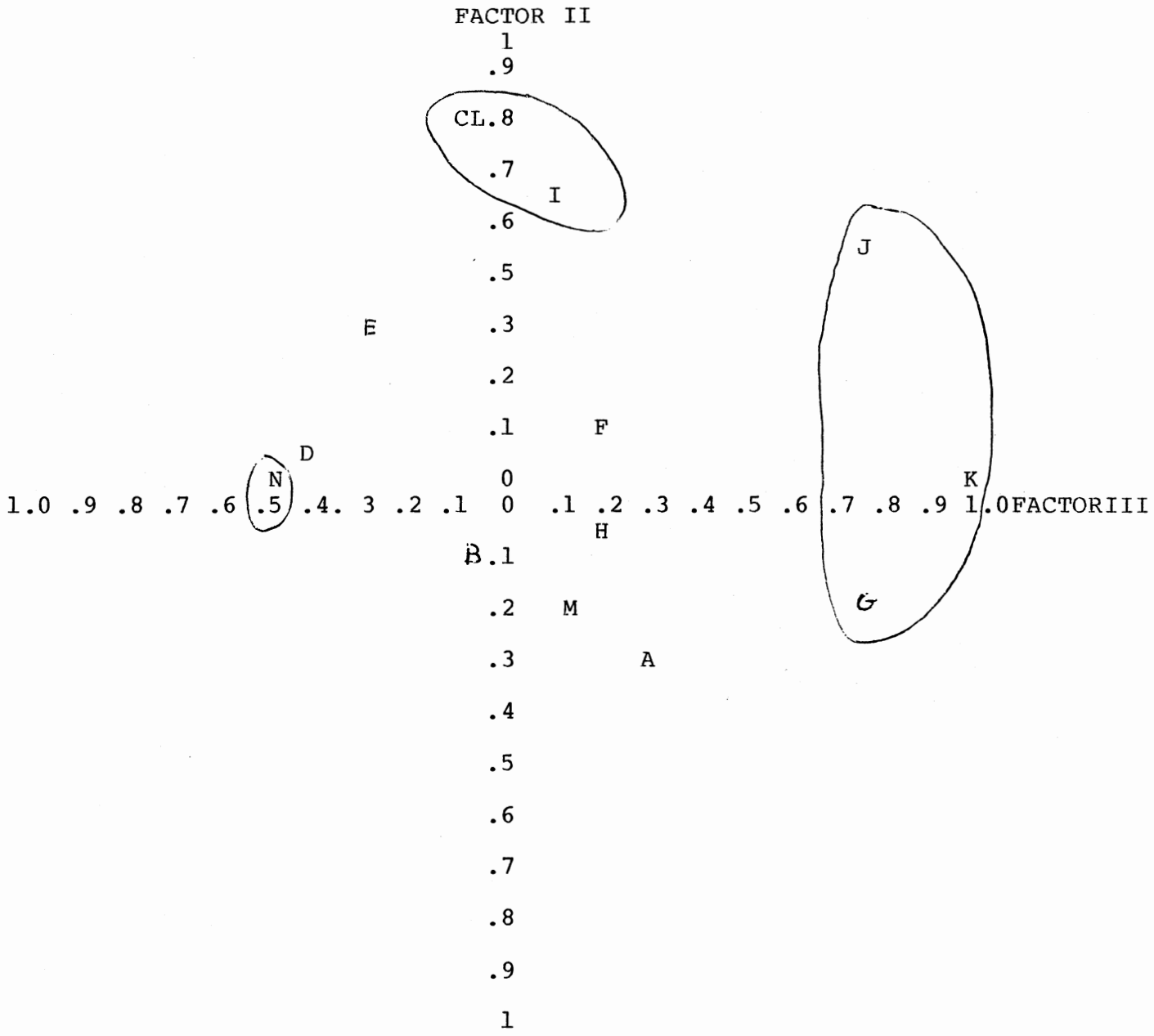
Factor I consisted of time, temperature,and the differences between 1 and 15 m for dissolved oxygen and temperature, respectively Δ DO, and Δ T (Figure 12). No significant negative loadings existed for Factor I after rotation. Factor I accounted for 20.7% of the variation. These measurements describe an independent stratification variable. Stratification would then be associated with a large Δ T and Δ DO, high surface temperature, and late sampling (time of day). The thermal structure of Lake Hefner did change from day to day. No nutrients are associated with Factor I. Factor I and Factor II are related as shown in Figure 12.

Factor II consisted of dissolved oxygen, chlorophyll a, SRP and pH (Figure 15). Again, no significant negative loadings existed after rotation, and the rotated Factor II accounted for 16.6% of the variation. Factor II could best be described as a measure of productivity in a classical limnological sense. Dense algal populations (high chlorophyll a values), when photosynthesizing rapidly, would take up the majority of free CO_2 and increase pH. Dissolved oxygen would also increase. The presence of SRP as a part of Factor II might be influenced by a relationship between high levels of SRP and high rates of productivity.

Variables that loaded positively on Factor III included ammonia, chlorophyll a, and light (extinction coefficient). Factor III also included a negative component, daily nitrogen fixation (Figure 13). Factor III accounted

Figure 13. Environmental Factors II and III After Rotation, Station 2

Time =A Temp =B DO =C Delt =D Delo =E Nitrate =F
Ammonia =G Tphos =H Ophos =I Chla =J Light =K
pH =L Wind =M Nfix =N

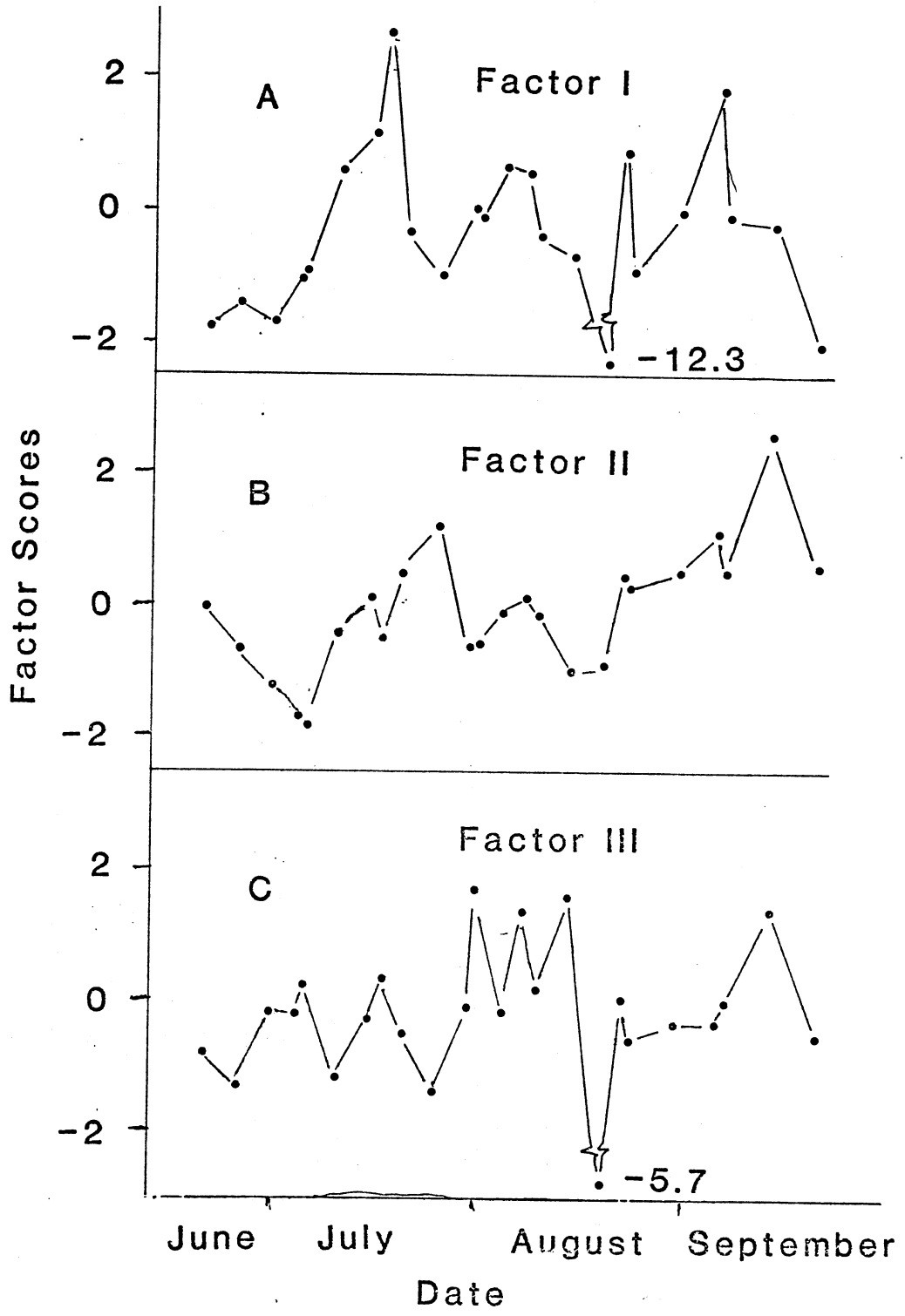

FACTOR II
1
.9
CL.8
.7
I
.6
.5
E
.3
.2
.1
F
D
N
0
K
1.0 .9 .8 .7 .6 .5 .4. 3 .2 .1 0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0FACTORIII
H
B.1
.2
M
.3
A
.4
.5
.6
.7
.8
.9
1
J
G

for 18.5% of the variation after rotation. Factor III describes variables involved in nutrient cycling. Rapid light extinction was associated with high ammonia values. If a high extinction coefficient is due to other than actively nitrogen fixing algae, this would further strengthen the association between light and the reductant and carbon skeletons needed for nitrogen fixation (Ward and Wetzel, 1980). Although the ammonia levels were insufficient to supress heterocyst development (See Figures 9A and 9B), high levels of ammonia would be expected only between periods of bloom development (Horne et al., 1972).

Unstandardized factor scores by date may be readily used to identify dates with particularly high or low values of significant variables. Factors scores for Factor I were lowest in late June and on September 20, indicating the least stratification on these dates (Figure 14A). The values of -12.3 and -5.7 on August 19 were the result of missing data (Figure 14A and C). Stratification was most pronounced on July 17 (Figure 14A).

Factor II scores exhibited a steady decline from late June to July 5, marked by decreased cholorphyll _a_ concentrations (Figure 14B). Chlorphyll _a_ also influenced high Factor II scores on July 25 and September 13 (Figure 14B). High scores on July 31, August 7 and 14, and September 13 for Factor III are primarily the result of high ammonia values (Figure 14C). Comparsion of the scores for Factors I, II, and III also points out the independence of

Figure 14. Comparison of Standardized Factor Scores by Date for Factors I, II, and III


A
Factor I
2
0
-2
-12.3
B
Factor II
2
0
-2
C
Factor III
2
0
-2
-5.7
Factor Scores
June
July
August
September
Date

the factors as there are no common patterns.

Phytoplankton Assemblages

Factor analysis of presence-absence data may result in delineation of some aspects of community structure and algal strategies, but a large percentage of variation often remains unexplained. In the present study many species are accounted for by the first three factors (Figures 15 and 16), but these same factors account for just 29.0% of the total variation. The first five factors accounted for 43.0% of the variation before rotation. At the retention criterion of 70.0% of the variation, 11 factors were retained. The rotation of these eleven factors, as with the earlier rotation of the environmental factor axes, more clearly defined those plankton assemblages in an interpretable fashion. The first three factors, however, now accounted for only 24.1% of the variation.

Pediastrum duplex, Ceratium hirudinella, Coscinodiscus sp., Stephanodiscus hantzschia, and Anabaena sp. were present in every sample and thus had zero correlation coefficients. These species graphed at the origin in every plot of one factor with another. Although these species contributed significantly to the phytoplankton, they had no role in this particular analysis.

All factors, unlike Factors I and II from the environmental data, had both significant positive and negative loadings. Fewer species were negatively loaded on each

Figure 15. Ordination of Phytoplankton Factor I with Factor II

Green1 =A Green2 =R Green3 =C Green4 =D Green5 =Q
Green6 =F Green7 =G Green8 =O Graeen9 =N Green10 =J
Green11 =K Green12 =L Green13 =M Green14 =N Green15 =O
Green16 =O Green17 =Q Green18 =R Green19 =N Green20 =T
Green21 =U Green22 =V Green23 =W Green24 =R Green25 =Y
Green26 =Z Green27 =A Green28 =R Green29 =O Green30 =O
Green31 =Q Green32 =F Green33 =G Green34 =O Green35 =N
Green36 =J Green37 =K Green38 =L Eug1 =M Cera =N
Diat1 =O Diat2 =O Diat3 =Q Diat4 =R Diat5 =N Diat6 =T
Diat8 =V Diat9 =W Diat10 =R Diat11 =Y Diat12 =Z
Diat13 =A Diat14 =R Diat15 =C Diat16 =D Diat17 =Q
Diat18 =F Diat19 =G Diat20 =O Diat21 =N Diat22 =J
Diat23 =K Diat24 =L Diat25 =M Diat26 =O Diat27 =O
Diat28 =A Diat29 =Q Diat30 =R Diat31 =O Diat32 =T
Diat33 =M Diat34 =M Diat35 =W Diat36 =X Diat37 =Y
Diat38 =Z Diat39 =A Diat40 =M Diat41 =L Diat42 =O
Diat43 =M Diat44 =F Blg1 =O Blg2 =H Blg3 =O
Blg3 =O Blg4 =L Blg5 =K Blg6 =L Blg7 =M
Blg8 =O Blg9 =O Blg10 =A Blg11 =Q Blg12 =R
Blg13 =O Blg14 =T Blg15 =M Blg16 =M Blg17 =W
Blg18 =X Blg19 =Y Blg20 =Z UK9 =A UK10 =M
UK11 =L. See Appendix E for specific taxanomic identification.

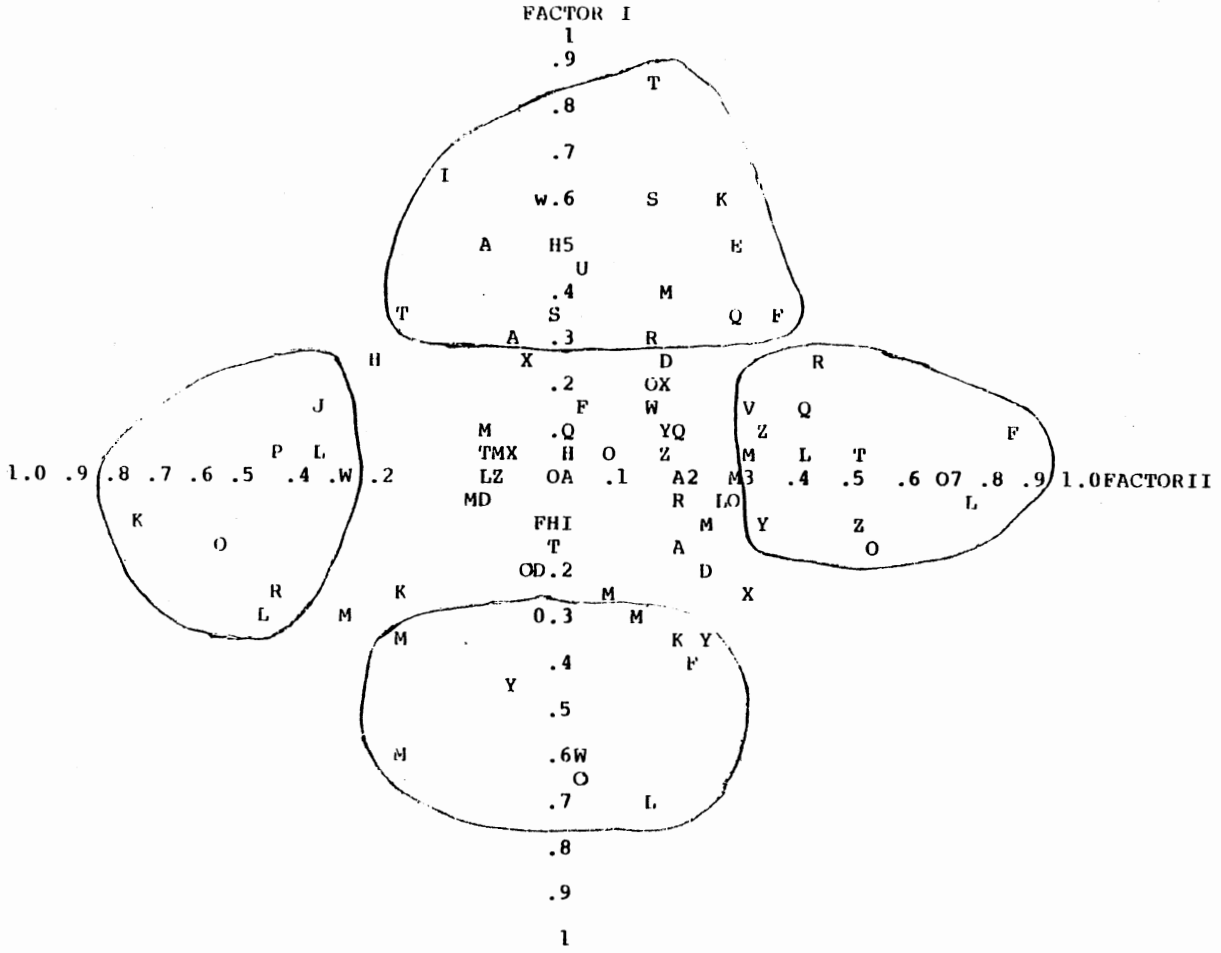
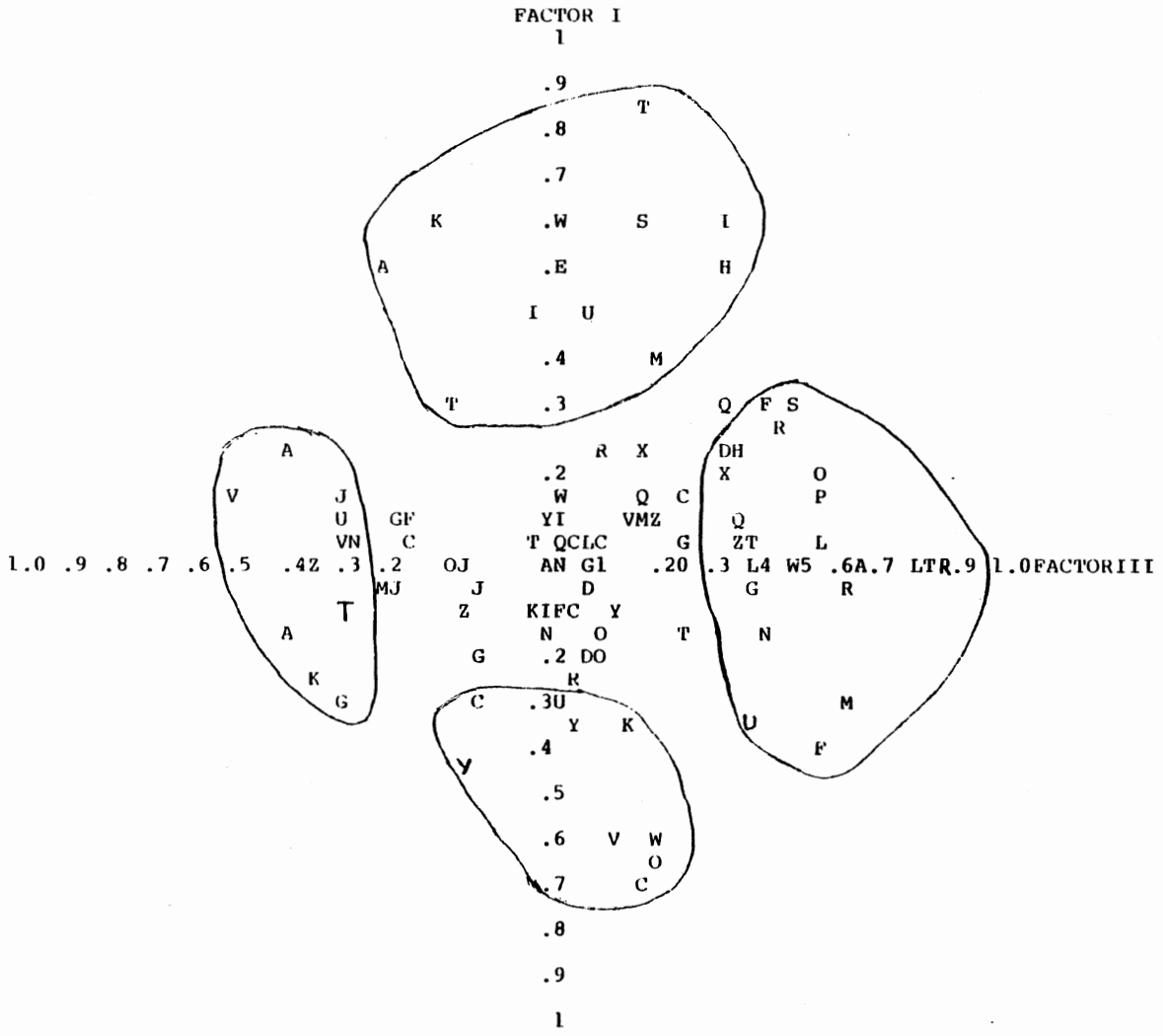

FACTOR I
1
.9
.8
.7
.6
.5
.4
.3
.2
1.0 .9 .8 .7 .6 .5 .4 .3 .2
1.0 .9 .8 .7 .6 .5 .4 .3 .2 1.0FACTORII
.8
.9
1

Figure 16. Ordination of Phytoplankton Factor I with Factor III.

Green1 =A Green2 =R Green3 =C Green4 =D Green5 =Q
Green6 =F Green7 =G Green8 =O Graeen9 =N Green10 =J
Green11 =K Green12 =L Green13 =M Green14 =N Green15 =O
Green16 =O Green17 =Q Green18 =R Green19 =N Green20 =T
Green21 =U Green22 =V Green23 =W Green24 =R Green25 =Y
Green26 =Z Green27 =A Green28 =R Green29 =O Green30 =O
Green31 =Q Green32 =F Green33 =G Green34 =O Green35 =N
Green36 =J Green37 =K Green38 =L Eug1 =M Cera =N
Diat1 =O Diat2 =O Diat3 =Q Diat4 =R Diat5 =N Diat6 =T
Diat8 =V Diat9 =W Diat10 =R Diat11 =Y Diat12 =Z
Diat13 =A Diat14 =R Diat15 =C Diat16 =D Diat17 =Q
Diat18 =F Diat19 =G Diat20 =O Diat21 =N Diat22 =J
Diat23 =K Diat24 =L Diat25 =M Diat26 =O Diat27 =O
Diat28 =A Diat29 =Q Diat30 =R Diat31 =O Diat32 =T
Diat33 =M Diat34 =M Diat35 =W Diat36 =X Diat37 =Y
Diat38 =Z Diat39 =A Diat40 =M Diat41 =L Diat42 =O
Diat43 =M Diat44 =F Blg1 =O Blg2 =H Blg3 =O
Blg3 =O Blg4 =L Blg5 =K Blg6 =L Blg7 =M
Blg8 =O Blg9 =O Blg10 =A Blg11 =Q Blg12 =R
Blg13 =O Blg14 =T Blg15 =M Blg16 =M Blg17 =W
Blg18 =X Blg19 =Y Blg20 =Z UK9 =A UK10 =M
UK11 =L. See Appendix E for specific taxanomic identification.


FACTOR I
1
.9
.8
.7
.4
.3
.2
1.0 .9 .8 .7 .6 .5 .4Z .3 .2 1.0FACTORIII
.3 L4 W5 .6A.7 LTR.9
.20
1
.9
.8
.7
.6
.5
.4
.3
.2
.1
1

factor than were loaded, however, because orthogonal rotation minimizes negative loadings. After rotation, Factor I accounted for 8.4% of the variation. Factor I consisted of summer species that were found in the majority of the samples. These species had significant positive loadings (Figure 15). A smaller number of negatively loaded species, present primarily in the first few plankton hauls, represented a late spring community (Figure 15). The summer species composition was mixed, being dominated by green algae, but also including a number of blue-green algae, diatoms, and a species of flagellate. The late spring group was almost equally divided between green algae and diatoms. A species list corresponding to the abbreviated notation of numbered algae given in the legends is presented in full in Appendix E.

Factor II can best be described as a mid-summer diatom assemblage. After rotation, Factor II accounted for 7.5% of the variation. The positively loaded species included in Factor II also include some diatoms with loadings slightly below the criterion of 0.30 for significance (see Figure 15). An exact break point was difficult to identify for this factor. The positive component of Factor II is overwhelmingly diatoms, whereas the negative portion is predominantly green algae. These green algae had a very scattered occurence in the presence-absence data.

Factor III, accounting for 8.2% of the variation after rotation, identified a second diatom assemblage (Figure 16).

The positively loaded species in Factor III were again predominantly diatoms, but five green and three blue-green species including Microcystis aeruginosa were included. The small number of negatively loaded species for Factor III were three green algae, two blue-green algae, and one unidentified species.

Interpretation of factor scores was complicated by the large number of species contributing to each score. Also, in the computation of the factor scores matrix a factor scoring is multiplied with the original observations. Many factor scoring coefficients in the matrix were zero. Only the green algae had consistently non-zero scoring coefficients. In light of these facts, factor scores will not be discussed.

CHAPTER VI

DISCUSSION

Limnological Characteristics

The values measured in the lake during 1981 were typical of an eutrophic lake. Although dissolved oxygen was often depleted in the hypolimnion, it was rarely competely absent probably due to lake aeration. In lakes similar to Hefner, stratification can break down at elevated temperatures (Cole, 1979). Microstratification also occurred at these elevated temperatures (Pastorok et al., 1981). The aeration system was apparently more successful in destratifying the lake thermally than it was in supplying oxygen to the water. Localized destratification with respect to DO together with slow mixing throughout the summer is typical of lakes with underpowered aeration systems (Pastorok et al., 1981; Lorenzen and Fast, 1977).

Also typical of an eutrophic lake were the epilimnetic nutrient levels. Ammonia levels were high at times during the summer. High concentrations of Kjeldahl nitrogen, total phosphorus and chlorophyll *a* indicated considerable seston in the water column. Nitrite, NO_3^-, and NH_4^+ were undetectable in some samples in late July and early August,

however. The high ammonia values should be viewed with some scepticism as they may be the result of analytical error or they may be measurements of other nitrogen species in addition to NH_4^+.

Trophic Indices and Models

The trophic indices that have been developed by other workers can be used to further classify Lake Hefner. In Carlson's (1977) trophic system based on chlorophyll a, total phosphorus and Secchi disk, Lake Hefner consistently ranks between 60 and 70. This is eutrophic by his definitions. The use of nutrient loading rates in relationship to mean depth and retention time (Mikalski et al., 1975) classifies Lake Hefner in the eutrophic region despite a high residence time. Removal of 77 and 90% of the phosphorus load would place Lake Hefner in mesotrophic and oligotrophic loading conditions, respectively (Mikalski et al., 1975). The Secchi disk-chlorophyll a relationship given by Mikalski et al. (1975) does not include any lakes with Secchi disk and chlorophyll values similar to Lake Hefner, but it would appear to be highly eutrophic in their model.

Three trophic models relating phosphorus, nitrogen, and chlorophyll a have been developed. The Jones and Bachmann (1976) summer total phosphorus predictive equation overestimates summer chlorophyll a values by 80% for Lake Hefner. Their model for predicting total phosphorus, from phosphorus loading rates, sedimentation rates, mean depth and

retention time underestimates total phosphorus in Lake Hefner (Jones and Bachmann, 1976). Another equation using phosphorus and nitrogen loading rates to predict chlorophyll a (Ahl, 1975), greatly underestimates the summer chlorophyll a values observed for Lake Hefner. The assumptions underlying the model of Jones and Bachmann (1976) may not exactly apply to Lake Hefner. The sedimentation rate may be slower than their model implies. Also, errors in mean depth and retention time may have lowered the total phosphorus estimate.

Compensation Hypothesis

The hypothesis that blue-green algae will compensate for undersupply of nitrogen relative to phosphorus might be interpreted as predicting that significant nitrogen fixation will occur in Lake Hefner (i.e. 20 - 40% of total N input). This was not the case in Lake Hefner. Exactly what nutrient ratios yield the highest rates of nitrogen fixation are still not clear. The results of Vanderhoef et al. (1974) suggest that, at in-lake N:P ratios below 3:1, high rates of nitrogen fixation will occur.

Significant nitrogen fixation did not occur in Lake Hefner. The low percentage contribution by nitrogen fixation is similar to the results of Horne and Fogg (1970) for Lake Windermere and Torrey and Lee (1976) for Lake Mendota. Other workers have found much higher rates of nitrogen fixation and percentage contribution to the

nutrient budget (Horne and Viner, 1971; Horne and Goldman, 1972; Granhall and Lundgren, 1971). With most of the nutrient loading occurring in the spring and fall, however, even this low percentage contribution may be significant in the summer (Horne and Fogg, 1970).

The smaller amounts of nitrogen and phosphorus exported via the water treatment plant reflect the interaction of the long residence time and lower nutrient concentrations in the outflow. A higher ratio of N:P in the outflow could have been the result of differences in sedimentation rates for nitrogen and phosphorus, or the result of movement of nitrogen from the sediments.

Nitrogen fixation itself was probably not suppressed by the levels of nitrate and ammonia found during the summer, i. e., heterocysts were never absent. Suppression of heterocyst development requires approximately 5 mg $\cdot liter^{-1}$ of ammonia which is higher than measured in Lake Hefner (Carr and Whitton, 1973). Rather, the low rates of nitrogen fixation were probably the result of low absolute numbers of nitrogen fixing algae, Anabaena ssp. and Aphanizomenon flos-aquae. Other algae known to fix nitrogen under controlled conditions were not found in Lake Hefner (Stewart et at., 1978; Peterson et al., 1977). Also, rapid internal rates of supply of nitrogen (Toetz and Cole, 1980) may have been sufficient to allow successful competition by algae other than blue-green algae. Many of the blue-green algae species identified were not capable of

fixing nitrogen, suggesting that nitrogen fixation was not a decisive competitive advantage in Lake Hefner. Blue-green algae, as obligate phototrophs, have a much less flexible cellular metabolism that may place them at a disadvantage when nutrients are not limiting, but they have a superior inorganic metabolism (Carr and Whitton, 1973).

The observation that Lake Hefner is an eutrophic lake with a fairly low, variable rate of nitrogen fixation agrees with the results of Rusness and Burris (1970), Tison et al. (1977), and Horne and Goldman (1972). Tison et al. (1977), after investigating Washington lakes of varying degrees of eutrophication, suggested that rates of nitrogen fixation would be highest in mesotrophic lakes. Horne and Goldman (1972) demonstrated that large blooms of nitrogen fixing algae in Clear Lake, California, were associated with large releases of phosphorus from anoxic lake sediments. Rusness and Burris (1970), also working with lakes with varying degrees of eutrophication, found a low, variable rate of fixation present from July to September in an eutrophic lake. The vertical and seasonal distribution of nitrogen fixation in Lake Hefner is most similar to the results of Peterson et al. (1977) and Rusness and Burris (1970). Thus, even with a low N:P loading ratio, Lake Hefner should not be expected to exhibit high, sustained rates of nitrogen fixation.

Variability in Nitrogen Fixation

Variation in nitrogen fixation with respect to station, time, and depth paralleled some previous research. Two groups of stations differed from each other when the horizontal distribution of nitrogen fixation was measured. These stations were associated with nearness to the canal outfall and the aeration system. Although Granhall and Lundgren (1971) demonstrated one case where one station was sufficient, sampling for variation in nitrogen fixation in lakes similar to Lake Hefner would be more accurate if two stations were used. Gradients in turbidity could also affect nitrogen fixation (Kennedy et al., 1980).

The findings of Vanderhoef et al. (1974), who observed that nutrient gradients in a lake were associated with gradients in nitrogen fixation are not applicable. The lack of differences among stations with respect to the other parameters (Table V) may be the result of thorough mixing of the epilimnion by high wind (Anon, 1952). One station should be sufficient for measurements nutrients, dissolved oxygen, and chlorophyll _a_ in Lake Hefner under most conditions.

Variation in nitrogen fixation in time and space was expected on the basis of previous research. High rates of fixation at depths near the compensation point have been reported (Lannergren et al., 1974; Peterson et al., 1977), and have been attributed to algal relocation at lower depths

and the use of carbon skeletons and reductant left from previous light exposure (Ward and Wetzel, 1980). Variation of nitrogen fixation with depth is a complex issue involving light intensity, algal density, pO_2, and photosynthesis (Peterson et al., 1977). The surface depression of nitrogen fixation may have been due to either vertical migration, inhibition of photosynthesis at high light intensities, actual cell death, or high oxygen levels. Although a DO was rarely extreme, oxygen levels within the bottles were not measured and there may have been a bottle effect due to entrapment of DO.

Diel variation in nitrogen fixation was also quite erratic. This variation was expected to show either a smooth curve response or a general midday depression. The latter case was observed on July 1, when the highest absolute rate of nitrogen fixation occurred. The lack of agreement with the midday depression hypothesis (Peterson et al. 1977 and Vanderhoef et al. 1975) or with the smooth response (Rusness and Burris, 1970) may be the result of changes in insolation which were not measured. Low rates of nitrogen fixation throughout August 12 may have been the result of a thundershower and extensive cloudiness on that day. Torrey and Lee (1976) found that rainfall could reduce nitrogen fixation considerably. Also, six different stations were sampled for diel variation in nitrogen fixation. The combined effects of different stations, changes in insolation, and low absolute rates of nitrogen fixation yielded a data

set that was difficult to interpret. Averaging the results by hourly intervals and using the curve plotted through those averages yielded better data for the expansion of hourly nitrogen fixation rates to daily rates of nitrogen fixation (Horne and Viner, 1971).

Principal Axis Factor Analysis

Principal axis analysis was more successful in terms of the percentage of the variation explained by the first three factors in providing a clear picture of the environmental data. This is not suprising in view of the large number of species included in the phytoplankton analysis. The factor analysis of the environmental data gives a larger overall picture of the dynamics of Lake Hefner than is possible by considering the individual variables. Factor I identified a stratification variable composed of weighted values for four variables time, temperature, ΔDO, and ΔT. That these variables co-varied is somewhat evident even in the raw data. The factor scores for Factor I then pointed out dates on which Lake Hefner was either highly stratified or well mixed. On September 20, the lake was isothermal, cooler than in summer, DO was high throughout the water column, and the measurements were made earlier in the day. The reality of variables constructed through a technique that forces orthogonal, linear relationships upon any data might be questioned. The use of Spearman correlation coefficients to construct the correlation matrix was one method of dealing

with variables that were potentially non-linear. This also dealt with the wide range of values found in some of the variables. A test of the reality of the factors derived from the environmental data would consist of remeasurement of the variables in a similar system. The existence of similar factors would constitute substantial proof. Such measurements are lacking at the present time, however.

The results of principal axis factor analysis of the phytoplankton data were similar to past research. The data format and method of analysis were patterned closely after the work of Allen and Koonce (1973). The main differences were in the number of samples, the exact type of factor analysis performed, and inclusion of other variables in their analysis. The interpretations of the results are similar to Allen and Koonce (1973) in substance. The phytoplankton Factor I resembles their first factor in that these algae are the persistent members of the assemblages. Factors II and III may represent fast-growing, highly grazed species of diatoms which provide a better food source than blue-green algae. However, the diatoms may also have been present due to physical events in the lake related to the destratification that occurred during late July. Changes in nutrient availability or turbulence may have allowed the diatoms to survive.

The failure of blue-green alage to load highly on any one factor may be partially due to the sampling method. Also, the five species that did not appear in any of the

factors due to their presence in all samples included one of the most predominant blue-green algae, Anabaena sp. Logarithmic transformations of algal densities would have allowed inclusion of these species in the analysis. Also, the predominance in numbers of species of green algae and diatoms may have entered into the failure to extract a "blue-green" factor. A design problem affecting the whole phytoplankton analysis was the small number of samples relative to the number of species identified. This could only have been overcome by more intensive sampling, a longer sampling period, or inclusion of only the most common algae. Some combination of these approaches might have also been useful. The larger number of species with few observations may have also entered into the lack on non-zero scoring coefficients.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The results of this investigation warrant some conclusions with respect to the original purposes. 1) it is not likely that control strictly of the blue-green algae will result in the elimination of taste and odor problems in the finished water since many other nuisance algae are present. 2) given the possibly high rates of nitrogen recycling and the in-lake changes in the nutrient ratios, a nutrient loading ratio of 5.2 is insufficient to cause high rates of nitrogen fixation in Lake Hefner except during brief periods. 3) factor analysis is useful in examining relationships among variables and in identifying phytoplankton assemblages. 4) one or two stations are sufficient to characterize events in Lake Hefner. 5) Lake Hefner nutrient dynamics are dominated by canal flow and water plant withdrawals. 6) lake aeration and destratification were partially successful and led to slow circulation of the lake. 7) the most meaningful information on diel variation was gathered by sampling during periods when nitrogen fixation rates were substantial and measurements at these times will provide a more accurate picture of the daily pelagic cycle of nitrogen fixation than sampling on arbitrarily chosen

sampling dates.

Factor analysis showed that the close association of light, pH, chlorophyll a and DO could form the basis of other questions. Is carbon fixation actually being measured by Factor II? What would be the effects of changes in light, pH, or dissolved oxygen on chlorophyll a or productivity? Factors I and III also pose questions. Is the negative correlation of nitrogen fixation to NH_4^+ in Factor III real? Are epilimnetic nutrient concentrations independent of stratification for Factor I? A more general question concerns the effectiveness of the aeration system in maintaining DO in the hypolimnion, and how this might be related to distance from the system. Is wind actually unrelated to the stratification factor?

Having described the phytoplankton, the effects of zooplankton composition, grazing rates, and assimilation efficiency on the phytoplankton could be considered. Recent evidence suggests a significant influence by the zooplankton on phytoplankton community structure (Lynch and Shapiro, 1981). Also, a systems dynamics approach might be possible by including the trophic structure of the fish populations, much as Lane and Levin (1977) have suggested, in order to fully understand the effects of nutrient enrichment. Last, given the difficulity of applying existing trophic models to Lake Hefner, one could attempt to model the trophic status of this lake, or southwestern lakes in general, with respect to nutrient loading rates, phosphorus and chlorophyll a concentrations.

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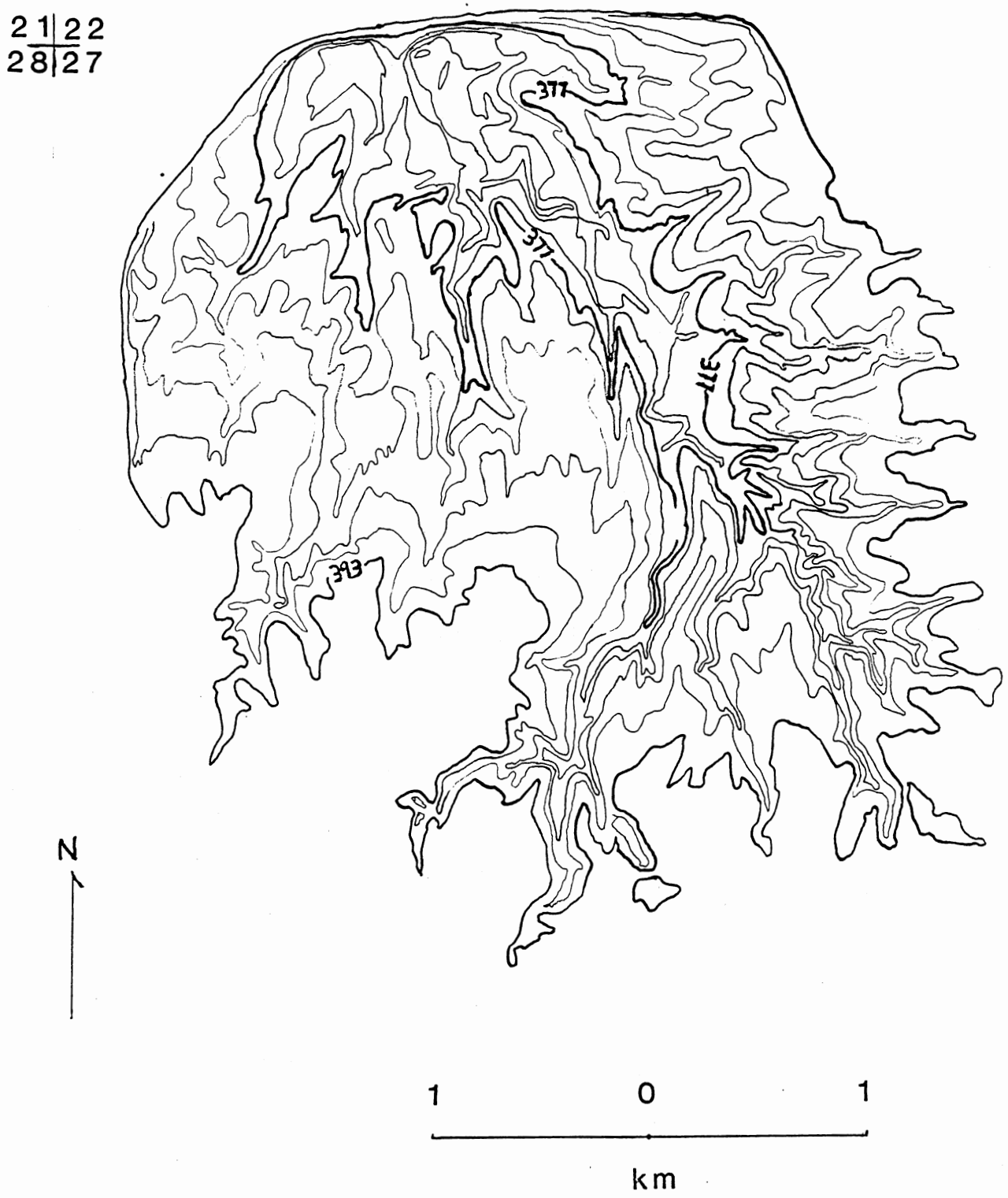
APPENDIX A

MORPHOMETRIC MAP OF LAKE HEFNER, OKLAHOMA, WITH CONTOUR INTERVALS OF 3.05 METERS

Figure 17. Morphometric Map of Lake Hefner, Oklahoma, With Contour Intervals of 3.05 Meters


21 22
28 27
377
371
311
393
N
1 0 1
km

APPENDIX B

SAMPLING EVENTS BY DATE AND TYPE

FOR LAKE HEFNER

TABLE VI

SAMPLING EVENTS BY DATE AND TYPE
FOR LAKE HEFNER

Summer, 1981
Day of Week

	S	M	T	W	T	F	S
June	14	15	16	17	18	19	20 * A
	21	22	23	24	25 A,C	26	27
	28	29	30	1	2	3	4 B
July	5 A	6	7	8	9	10 A	11
	12	13	14	15 A	16	17 B	18
	19	20 A	21	22	23	24	25 z A,C
	26	27	28	29	30 A,C	31 B,C	1
August	2	3	4 A,C	5	6	7 B**,C	8
	9 A,C	10	11	12	13	14 A,C	15
	16	17	18	19 A***	20	21	22 B,C
	23 A,C	24	25	26	27	28	29
	30 A	31	1	2	3	4	5 B
September	6 A	7	8	9	10	11	12
	13 A	14	15	16	17	18	19
	20 A						

A Vertical Variation plus Factor analysis measurements
B Factor analysis only
Note: Factor analysis measurements consisted of chlorophyll a, nutrients, dissolved oxygen, temperature, pH, and phytoplankton samples
C Light penetration
* Station 1 was not sampled, and stations 2 and 3 were not sampled for NO_3, NH_4, or total phosphorous
** Dissovled oxygen and temperatures were not measured
*** Phytoplankton samples were not taken

APPENDIX C

FACTOR LOADINGS FOR FACTORS I THROUGH V FROM ENVIRONMENTAL DATA

ROTATED FACTOR PATTERN

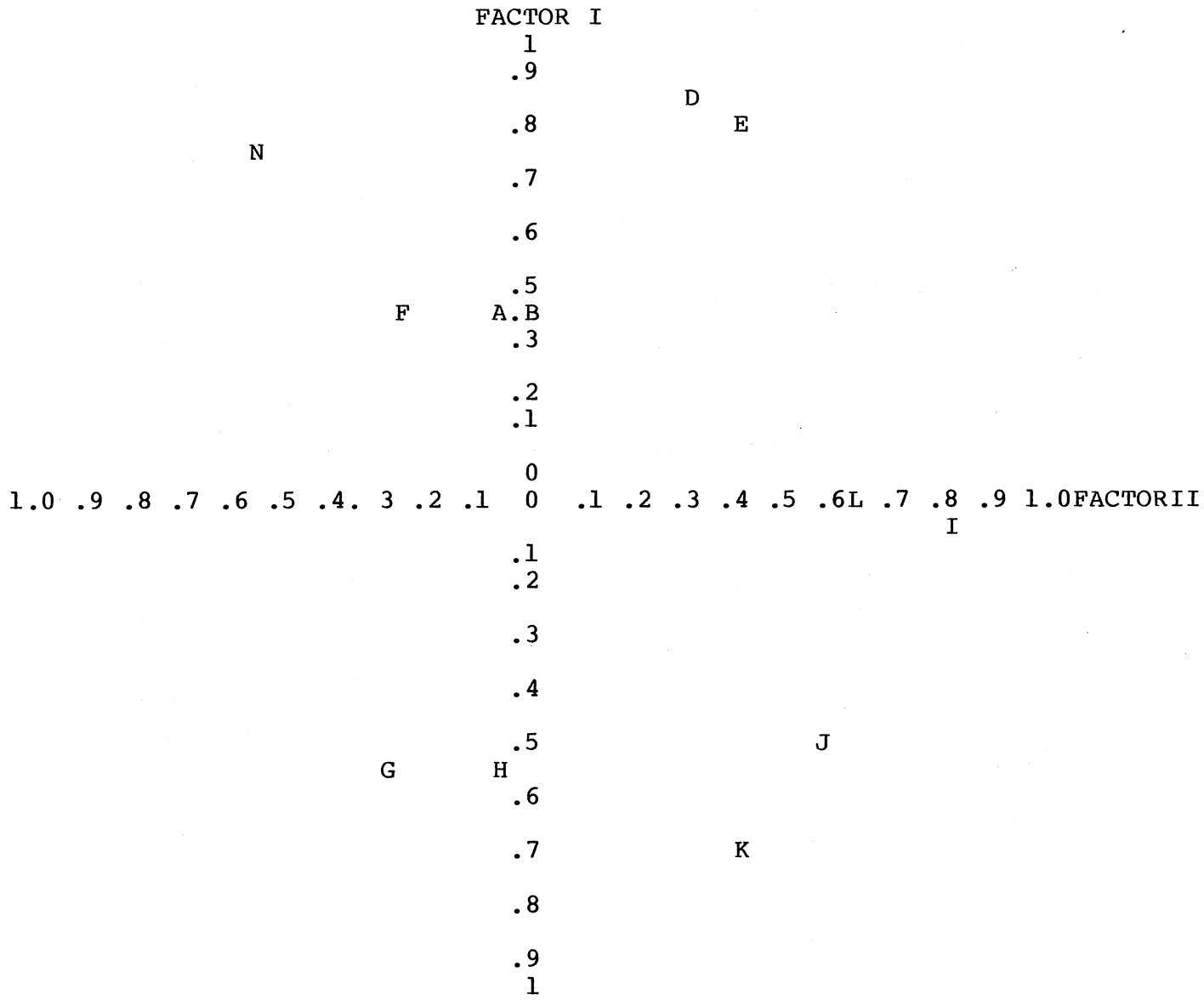
	FACTORI	FACTORII	FACTORIII	FACTORIV	FACTORV	
Time	0.71144	-0.27158	0.24726	0.00815	-0.22981	Military Time
Temp	0.76114	-0.10122	-0.01379	0.16045	0.55582	Temperature
DO	0.66040	0.79445	-0.09280	0.16324	-0.15740	Dissolved Oxygen
Delt	0.81809	0.12529	-0.40384	-0.02855	-0.19103	Delta Temperature
Delo	0.79628	0.28480	-0.27626	0.06927	0.35602	Delta Oxygen
Nitrate	0.27724	0.10002	0.16996	0.16997	0.35602	Nitrate At One Meter
Ammonia	-0.26391	-0.22411	0.77793	0.22370	-0.17251	Ammonia At One Meter
Tphos	-0.21029	-0.07561	0.19124	-0.07254	0.85919	Total Phosphorus One Meter
Ophos	0.07849	0.65618	0.09400	-0.50659	-0.02467	Ortho Phosphorus One Meter
Chla	-0.15290	0.58701	0.64870	0.055389	0.25160	Chlorophyll A At Surface
Light	0.06420	0.07200	0.96866	-0.28248	0.31323	Extinction Coefficient
pH	-0.15892	0.78931	-0.04944	-0.10051	-0.04425	pH At Surface
Wind	-0.18957	-0.19810	0.08661	0.69106	0.20880	Wind Miles
Nfix	0.30028	0.04984	-0.48198	0.81731	-0.14684	Integrated Nitrogen Fixation

APPENDIX D

ORDINATION OF FACTORS I AND II FROM ENVIRONMENTAL DATA FOR STATION 2 BEFORE ROTATION

Figure 18. Ordination of Factors I and II From Environmental Data for Station 2 Before Rotation

Time =A Temp =B DO =O Delt =D Delo =E Nitrate =F
Ammonia =G Tphos =H Ophos =I Chla =J Light =K
pH =L Wind =M Nfix =N


FACTOR I
1
.9
D
.8
E
N
.7
.6
.5
F
A.B
.3
.2
.1
0
1.0 .9 .8 .7 .6 .5 .4. 3 .2 .1 0 .1 .2 .3 .4 .5 .6L .7 .8 .9 1.0FACTORII
I
.1
.2
.3
.4
.5
J
G
H
.6
.7
K
.8
.9
1

APPENDIX E

SPECIES LIST FOR PHYTOPLANKTON

SPECIES LABELS

Label	Species
Green 1	Ankistrodesmus falcatus
Green 2	Botrycoccus braunii
Green 3	Closteriopsis longissima
Green 4	Coelastrum monus
Green 5	Coelastrum cambricum
Green 6	Cosmarium sp. 1
Green 7	Cosmarium sp. 2
Green 8	Cosmarium sp. 3
Green 9	Cosmarium sp. 4
Green 10	Crucigenia sp.
Green 11	Kircheriella lacustris
Green 12	Pandorina morum
Green 13	Pediastrum simplex
Green 14	Pediastrum duplex var.gracillimum
Green 15	Pediastrum duplex var.gracillimum
Green 16	Pediastrum duplex
Green 17	Quadrigulla closteriodes
Green 18	Quadrigulla lacustris
Green 19	Scendesmus quadricauda
Green 20	Sphaerocystis
Green 21	Staurastrum leptoclodum
Green 22	Staurastrum manfeldtii
Green 23	Staurastrum tetracerum
Green 24	Staurastrum sp. 1
Green 25	Staurastrum paradoxum
Green 26	Tetraedon constrictum
Green 27	Closterium sp. 1
Green 28	Closterium sp. 2
Green 29	Closterium sp. 3
Green 30	Closterium sp. 4
Green 31	Closterium sp. 5
Green 32	Closterium sp. 6
Green 33	Closteridium sp.
Green 34	Unknown Green sp. 1
Green 35	Unknown Green sp. 2
Green 36	Unknown Green sp. 3
Green 37	Unknown Green sp. 4
Green 38	Unknown Green sp. 5
Eugl	Euglena sp.
Cera	Ceratium hinudinella
Diat 1	Amphora ovalis
Diat 2	Amphora pediculus
Diat 3	Bacillaria paradoxa
Diat 4	Caloneis amphora
Diat 5	Coscinodiscus sp.
Diat 6	Cyclotella meneghiana
Diat 7	Cyclotella stelligera
Diat 8	Cyclotella sp. 1
Diat 9	Cyclotella comata
Diat 10	Cymbella sp.1

Diat	11	Cymbella naviculiformis
Diat	12	Diatoma vulgare
Diat	13	Diploneis oblongella
Diat	14	Diploneis puella
Diat	15	Fragillaria sp. 1
Diat	16	Fragillaria crotonensis
Diat	17	Rhoicosphenia curvata
Diat	18	Gyrosigmia obtusatum
Diat	19	Hantzschia virgata
Diat	20	Melosira granulata
Diat	21	Melosira granulata var. angustissima
Diat	22	Navicula elginensis
Diat	23	Navicula placentula
Diat	24	Navicula cryptocephala
Diat	25	Navicula sp. 1
Diat	26	Navicula sp. 2
Diat	27	Navicula pupila
Diat	28	Navicula sp. 3
Diat	29	Navicula viridula
Diat	30	Navicula sp. 4
Diat	31	Nitzschia acicularis
Diat	32	Nitzschia ganderheimensis
Diat	33	Nitzschia obtusa
Diat	34	Nitzschia apiculta
Diat	35	Nitzschia palea
Diat	36	Nitzschia sp. 1
Diat	37	Opephora martii
Diat	38	Nitzsonia frustrula
Diat	39	Stephanodiscus hantzschii
Diat	40	Surirella ovata
Diat	41	Synedra acus
Diat	42	Synedra sp. 1
Diat	43	Synedra sp. 2
Diat	44	Unknown diatom
BLG	1	Chroococcus sp. 1
BLG	2	Coelosphaerium naegelianum
BLG	3	Anabaena flos-aquae
BLG	4	Anabaena spiroides
BLG	5	Anabaena sp. 1
BLG	6	Anabaena sp. 2
BLG	7	Aphanizomenon flos-aquae
BLG	8	Aphanocapsa sp. 1
BLG	9	Arthrospira jenneri
BLG	10	Merismopedia glauca
BLG	11	Merismopedia major
BLG	12	Oscillatoria sp. 1
BLG	13	Oscillatoria sp. 2
BLG	14	Oscillatoria sp. 3
BLG	15	Oscillatoria sp. 4
BLG	16	Gloecapsa sp. 1
BLG	17	Microcystis aeruginosa
BLG	18	Microcystis flos-aquae
BLG	19	Microcystis firma
BLG	20	Unknown blue-green

UK	9	Unknown flagellate 1
UK	10	Unknown flagellate 2
UK	11	Unknown species

VITA 2

Mel Allen McFarland

Candidate for the Degree of

Master of Science

Thesis: NITROGEN FIXATION AND MULTIVARAIATE ANALYSIS OF PHYTOPLANKTON AND ENVIRONMENTAL DATA FROM LAKE HEFNER, OKLAHOMA

Major Field: Zoology

Biographical:

Personal Data: Born in Houston, Texas, 1958, the son of Mr. and Mrs M. G. McFarland.

Educational: Graduated from Crockett High School, Auston, Texas, in 1976; received the Bachelor of Science degree in Biology from Baylor University in June 1980; completed requirements for Master of Science degree at Oklahoma State University in May, 1983.

Professional Experience: Warehouse Supervisor, Reynolds Distributing Co., 1978-79; Salesman Payless Cashways, 1980; Research Assistant, Oklahoma State University, 1980-1981; Teaching Assistant, Oklahoma State University, 1981-1982.