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# MEASUREMENT OF RECOVERY IN LAKES FOLLOWING PHOSPHATE MINING

GREAT SEAL OF THE STATE OF FLORIDA

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FLORIDA INSTITUTE OF PHOSPHATE RESEARCH

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# MEASUREMENT OF RECOVERY IN LAKES FOLLOWING PHOSPHATE MINING

## FINAL REPORT

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# PERSPECTIVE

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Phosphate mining in central Florida began about 1880 and has had a history of almost continuous growth since then. Mining began with dredging operations in the bed of the Peace River, then moved on-shore to exploit rich deposits in the river's watershed. Early land-based mining operations used high-pressure water to remove the overburden and to slurry the phosphate ore, which was washed into a sump and removed by centrifugal pumps to a washer plant.

The hydraulic pits were deep and steep-sided, but when they were abandoned and filled with water they created relatively circular lakes that harmonized well with central Florida's topography. Despite the fact that the basin morphology differed significantly from the typical bowl-shaped central Florida lake and that the pit configuration allowed for minimal littoral development, the lakes were productive and earned a reputation for supporting large game fish populations. The sites of these early hydraulic mining operations are now eagerly sought as lakefront homesites. The lakes are surrounded by rolling hills clothed by canopies of mature live oaks, making them practically indistinguishable from natural lakes. Hydraulically mined areas have been extensively developed for exclusive residential areas south of the city of Lakeland.

With the advent of electric draglines during the 1920's phosphate began to be mined in parallel excavations 200-300 feet wide and 2000 feet long. Overburden was cast into an adjacent cut in the most convenient manner, usually in high windrows. The pits were up to 60 feet deep and spoil windrows were narrow, steep, and unstable. The arrangement of water-filled pits separated by intervening spoil windrows is often referred to as a "finger lake." Where the mining cuts were sufficiently wide, many of the spoil piles were flattened and the areas reclaimed as "land and lakes." These areas have also become popular homesites, although the narrow, linear nature of the windrowed spoil severely restricts the size of the lots. Numerous subdivision south and east of the cities of Lakeland and Bartow have been developed along the edges of the finger lakes.

The current rules of the Department of Natural Resources dealing with mine reclamation contain precise guidelines for the reclamation of water bodies created by mining after 1975. Unlike the hydraulically mined pits, slopes at the bottom of lakes within 25 feet of the shoreline must not be steeper than 4:1 horizontal to vertical as measured from the lowest anticipated water line. In order to encourage the development of littoral vegetation, at least 25% of the lake surface must be within the zone of water fluctuation, or alternately, wetlands must be created adjoining the lake. In addition, at least 20% of the surface must fall within a zone between the annual low water line and the -6 feet annual low water to provide for fish bedding areas and submerged vegetation zones.

The rules for lake reclamation were promulgated to encourage the development of lakes more like natural Florida lakes with shallow, dish-shaped basins, a well-developed littoral area, and a large portion of the water column in the euphotic zone. Despite the fact that deep, steep-sided lakes have considerable merit for some forms of recreation and water quality improvement, little research has been carried out to weigh the costs and benefits of reclaiming pit lakes to a condition matching typical natural basin lakes.

Virtually no research had been conducted on reclaimed phosphate pit lakes prior to the establishment of the Florida Institute of Phosphate Research in 1978. Reid and Blake (1969) had studied a lake they named Phosphate Pit Lake, an old hydraulic excavation south of the community of Bradley in Polk County. They found that the lake was interesting biologically principally because the depauperate zooplankton community was limited to populations of calanoid copepods and cladocera. Following up on this initial survey, Reid and Squibb (1971) found that high concentrations of ions prevailed in deeper parts of the lake during most of the year.

Among the first projects supported by the Institute was a comparative study of reclaimed and natural lakes in central Florida. This project, "Ecological Considerations of Reclaimed Lakes in Central Florida's Phosphate Region" (Project #82-03-018), was performed by Environmental Science and Engineering, Inc. (ESE) of Tampa under the direction of Oliver Boody. The study was an in-depth evaluation of 12 phosphate pit lakes (some naturally reclaimed and some reclaimed with human subsidy) and 4 natural lakes in the mineralized region of central Florida. The study included data on basin morphology and hydrology, water and substrate quality, phytoplankton, zooplankton, fish, macroinvertebrates, and aquatic macrophytes for all lakes.

The water and substrate quality portion of the ESE study was supplemented by an investigation conducted by Post, Buckley, Schuh and Jernigan of Orlando ("Water Quality in Lakes in Central Florida's Phosphate Mineralized Region," Project 84-03-046). PBS&J's sampling provided additional data on the concentrations of 20 metals in the water and substrate for which the state has set water quality standards.

Despite the significant amount of information that was developed by these programs, however, the authors of ESE's final report noted that the investigation did not answer all scientific questions pertaining to reclaimed phosphate pit lakes. The results provided evidence that reclaimed lakes are dynamic systems and that in many respects they resemble newly-formed reservoirs on rivers. The similarity of pit lakes to reservoirs formed the philosophical foundation for the research that was carried out under the current project.

Because phosphate lakes are new additions to the landscape, they represent a natural laboratory of primary succession. In the majority of cases, the lakes occupy land that was previously upland and contained no lentic habitat. Therefore, all physical, chemical and biological processes that occur in the lakes must start from "scratch." The lakes tend to be eutrophic from their genesis as a result of elevated concentrations of phosphate from the mined ore, nitrogen compounds from organic material incorporated into the overburden during land clearing, and trace minerals returned to the biosphere from the subsoil. Biological introductions into these systems tend to be haphazard. Consequently, few phosphate pit lakes contain food webs as complex as those normally encountered in natural central Florida lakes. In addition, organisms finding their way to the lakes may be challenged by a system in constant flux as biogeochemical cycling stabilizes over a period of several years.

With considerable background in charting the successional processes in aquatic ecosystems, Dr. John Cairns, Jr. and his staff at Virginia Polytechnic Institute and State University proposed to investigate the rate of succession in phosphate pit lakes in Florida. They chose to use protozoans as indicator organisms because protists are sensitive to environmental conditions and VPI&SU researchers have amassed considerable data on changes that occur in protozoan communities as lakes age. They hoped to develop a relatively fast and inexpensive technique to quantify the successional process to give reclamation personnel and state regulators a tool to measure reclamation success.

The three lake reclamation projects that the Institute has supported to date have provided a foundation for understanding the limnological behavior and characteristics of small mine pit lakes in Florida. Important information is still lacking on some critical parameters exerting control on these ecosystems such as the hydrology of reclaimed watersheds. It is the Institute's intention to develop a comprehensive understanding of reclaimed drainage basins in order to allow regulators to write informed legislation for improving reclamation.

# ACKNOWLEDGEMENTS

The special efforts of a number of persons ensured the success of this project. Drs. David J. Robertson and David P. Borris of the Florida Institute of Phosphate Research, Mr. C. Milton Brown of W.R. Grace Agricultural Chemicals, and Mr. Robert Goodrich of IMC made major contributions to the selection of study sites. Dr. William H. Yongue, Jr. and Mr. Paul V. McCormick identified many of the biological samples. Dr. Eric P. Smith of the Statistics Department, VPI&SU, provided a great deal of counsel on statistical analyses. Robert B. Genter and Douglas H. Deutschman were responsible for most of the field water chemistry. Analyses at the VPI lab were assisted by Judy Alls and Janet Webster. The majority of data transcription and compilation were done by Kirk Treakle, Carol Guarino, Vicki Higginbotham, Macie Walker, and Barbara Wolter. Mr. Kenneth Prevatt of W.R. Grace greatly aided the collection of samples at the Hooker's Prairie site. Access to natural lakes was provided by Mr. Frank Wilson, Mr. Mike Gurr, and Mr. and Mrs. Boyce Polk. Mr. Al Pisaonechi of W.R. Grace aided in obtaining access to several lakes. Mr. Skip Hoffman of Regal Real Estate gave permission for sampling at Lake Christina. Judy Vest, Becky Allen and Angie Miller assisted in preparing this report.

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# EXECUTIVE SUMMARY

Surface mining of phosphate-bearing rock in central Florida annually affects approximately 6000 acres. Reclamation of these mined lands is directed by the Florida Department of Natural Resources under Chapter 16C-16 Mine Reclamation Rules of the Florida Administrative Code. Rules direct the reclamation of lakes, wetlands, and uplands. The reclamation of lakes is intended to provide ecosystems that will support fish and wildlife and that function in the same manner as natural or unmined lakes.

The research summarized in this report, "Measurement of Recovery in Lakes Following Phosphate Mining," was sponsored by the Florida Institute of Phosphate Research Applied Research Program. The purpose of the project was to apply a simple, direct method to the assessment of recovery of lakes reclaimed after mining. The specific objectives of the project were to (1) determine colonization rates and equilibrium species numbers for microbial communities developing on artificial substrates in natural, reclaimed, and unreclaimed lakes; (2) determine the effect of physical and chemical factors on microbial colonization rates and equilibrium species numbers in lakes; and (3) estimate the rate of recovery of lakes after mining.

A broad range of lake ages was selected for study. Several physical and chemical parameters were measured in water samples taken concurrently with biological collections in each lake studied. These analyses determined levels of nutrients and trace metals as well as commonly measured parameters. Polyurethane foam artificial substrates were placed in each lake and collected on an expanding time scale over a two-week period. The number and kind of protozoan species colonizing these substrates were determined. Relative abundances of species were estimated.

During the first sampling effort in March 1984, 10 lakes were studied. Reclaimed lakes ranged in age from less than 1 year to 7 years while unreclaimed lakes studied were over 60 years old. Two natural lakes were also studied. Chemical differences among lakes were very apparent. Potassium, hardness, alkalinity, conductivity, and total organic carbon separated lake types. However, chemical differences among lakes did not correlate with distributions of protozoan species in lakes. Species distributions appeared related to phosphate and aluminum concentrations. No clear pattern distinguishing reclaimed lakes from natural or unreclaimed lakes was apparent. Examination of the colonization process, however, showed that protozoan colonization was markedly reduced in very young lakes (<1 year old).

During the second sampling effort, 21 lakes were studied. These lakes included all the lakes studied in the first phase of the project and 11 additional lakes generally less than 4 years old. This added group of lakes included sampling of water in two active mine pits and two newly reclaimed (ca. 6 month old) lakes. Similar to results of the first sampling effort, lakes could be distinguished by composite factors based on conductivity, calcium, alkalinity, pH, nutrients, and aluminum. Selenium and aluminum levels were high, and pH's were low in the newly reclaimed lakes.

Natural and unreclaimed lakes and active mine sites could be distinguished by their protozoan communities. Examination of the photosynthetic portion of these communities further strengthened the differentiation. Reclaimed lakes spanned the range of variability for other lakes. Examination of protozoan species by functional group revealed differences between newly reclaimed lakes and other lakes. Protozoan colonization of artificial substrates was severely depressed in newly reclaimed lakes as compared with other systems studied, including active mine sites.

A variety of analyses, both chemical and biological, showed that newly reclaimed lakes (<6 months old) were very different from other lakes studied. Aluminum and selenium levels were generally high in these lakes, and pH's were generally low. Waters were typically turbid. Microbial communities were different in terms of colonization dynamics and functional group composition. Species numbers were generally low. Comparison of these lakes to older lakes showed that lakes greater than one year of age were very similar to all ages of reclaimed and unreclaimed lakes and that older reclaimed lakes spanned the variability present in the natural and unreclaimed lakes studied.

These studies suggest that recovery of the microbial component of lakes after reclamation is rapid and effective. Protozoan species represent intermediate steps between bacterial degraders and higher trophic levels. They have a variety of functions and, to some extent, represent the many functional groups found in the larger ecosystem. These species are generally sensitive to perturbations including toxic and nutrient inputs.

This investigation demonstrates that recovery can be documented by examining a portion of the microbial biota of reclaimed systems. It has not examined the relationship between community structure and system function. Further studies examining the processing of carbon and inorganic nutrients will be needed to determine if reclaimed ecosystems have functional attributes comparable to natural or unmined lake ecosystems in the same geographic area. Examination of the structure and dynamics of a complex subset of reclaimed ecosystems demonstrates the ability of the biota to integrate a number of important factors in community patterns. These factors have been identified to some extent by statistical analysis of physico-chemical factors but are limited by the complexity and adequacy of water sampling.

# 1. INTRODUCTION

## EFFECTS OF PHOSPHATE MINING

The Florida phosphate industry is a multimillion dollar industry which supplies a valuable product to American and international agricultual markets. While the addition of phosphate fertilizers to agricultural soils is a vital augmentation, the industry has often been criticized for its high demand for energy and water and for landscape degradation in strip mined areas (Blakely, 1973). Nevertheless, the industry made early, positive moves to reclaim mined areas. For example, American Cyanimid began reclamation of the Saddle Creek mines in 1949. Many of these early reclamation efforts were conducted on uncharted theoretical grounds but were nonetheless successful. A functioning system developed, although it was undoubtedly different from the original ecosystem.

There is a long history of public interest and debate concerning the protection and recovery of wetlands and mined areas in Florida. This interest is the culmination of a variety of concerns about protection of natural areas from development, maintenance of water tables, and use of wetlands as environmental filters. Environmental groups have applied considerable pressure to protection of natural areas (e.g., Alderson, 1983). There has been strong support for reestablishing waterways and comprehensive management of drainage basins even in areas where many wetlands are privately owned (e.g. Hartman, 1983). Concern about the development of artificial wetlands and the use of natural wetlands for treating municipal wastes has been a subject of study for many years.

These concerns are of special importance to the phosphate industry which mines over 6000 acres per year and uses over 100 billion gallons of water. While phosphate mining contributes to the development of native lands and consumes much water, mined lands are also potential reservoirs and artificial wetlands.

The purpose of the investigation described below was to develop a simple, direct, scientifically justifiable measure of biological development in reclaimed lakes. There is no generally recognized measure of reclamation, and, although there are many potential definitions, a simple, direct, predictive measure is needed to assess community development in reclaimed systems. We have not attempted to offer an index which will be all-encompassing, but have concentrated on integrating measures that assess lentic water quality. Assessment of reclamation of surrounding terrestrial communities will necessitate longer-term studies and will also be subject to less objective aesthetic factors. However, the relationship between water quality and the condition of the adjacent terrestrial system has been well established.

## RECOVERY OF ECOSYSTEMS

Defining recovery of damaged systems or verifying the reclamation of a once disturbed area is in an early developmental stage. The eastern deciduous forest is, for the most part, a second growth forest inexorably transformed by human settlement. While most observers consider the eastern forests to be "recovered", they are not identical to the ancestral woods. Human imports such as Dutch elm disease and chestnut blight have eliminated certain species. Yet a forest remains. Recovery is, then, not easily defined.

When one speaks of recovery in less familiar ecosystems, grasslands for example, agreement becomes more difficult. In aquatic systems, where most basic knowledge concerning management has yet to be uncovered, there is little agreement. Even very disturbed aquatic ecosystems will support certain tolerant species.

To verify recovery or reclamation, natural systems must be studied and acceptable similarities to natural systems must be attained. These similarities may be considered to be sufficient if measures of certain important variables fall within the measured variability of comparable natural systems.

Recovery of damaged systems or reclamation in newly created systems is essentially a colonization-succession phenomenon. As such, there will be considerable variation in colonization and succession within different groups of organisms. Estimates of colonization equilibrium run to extremely long times (e.g. hundreds of years) in some species (e.g. birds, MacArthur and Wilson, 1967; Haila, et al., 1982). Conversely, this may take only a few days or weeks in other groups (e.g., Maguire, 1977).

## PROJECT GOALS

The goal of this project was to apply a simple, direct method to the assessment of recovery of lakes reclaimed after mining. The project directed attention at a variety of physical-chemical factors interrelated with microbial community development in attempting to identify factors which contribute to accelerated recovery of reclaimed lakes.

## OBJECTIVES

The following were specific objectives relating to the assessment of recovery using microbial community development on artificial substrates. (1) To determine colonization rates and equilibrium species numbers of microbial communities developing on artificial substrates in natural, unreclaimed, and reclaimed lakes in central Florida's phosphate mining area. (2) To determine the effect of physical and chemical factors on microbial colonization rates and equilibrium species numbers in lake waters. (3) To estimate the rate of recovery of lake waters abandoned or reclaimed after mining.

## IMPACT OF STUDY

More accurate predictions of recovery costs and time will allow more precise predictions of mining costs and benefits relative to total site expenditures. At present there is no routine monitoring or measurement that can quickly compare ecosystems using complex biological communities. Management of systems to perpetuate target species is often productive for the target species but overly simplistic in approach to the total biological community. Managed habitats are typically expensive to maintain and lack many of the complex feedback controls that allow natural communities to fluctuate in response to environmental changes without collapsing. The methods used were designed to be inexpensive and rapid in obtaining results, standardized, and potentially applicable by relatively untrained personnel as routine measures.

## ADVANTAGES OF UTILIZING MICROBIAL COMMUNITIES

Microbial communities have a number of distinct advantages in the assessment of water quality.

1. Protozoans and other microbes are relatively easy to collect in the field without costly equipment. The organisms are small,, easily handled, and require only small containers for transport. In contrast, fish and macroinvertebrates are comparatively difficult to sample and handle.

2. Large numbers of samples can be collected without affecting the integrity of the indigenous biota. This can be a serious problem when collecting fish, large numbers of insect larvae, or other macroinvertebrates.

3. Protozoans have been shown to form complex species assemblages that have many of the characteristics of structured communities (Cairns, 1971). They are a diverse group with representatives of several trophic levels and are thus potentially capable of mimicking the responses of the entire natural community more accurately than any other group. Furthermore, because of their high reproductive rates and intimate contact with the environment (Cairns, 1974), protozoa are capable of manifesting a community-level response to environmental stress (such as a shift in dominance pattern of species occurrence) more rapidly than assemblages of higher organisms.

4. Although protozoans are affected by changes in the chemical, physical, and biological environment in the same general way as other organisms, their probably cosmopolitan distribution is shared with only a few other biological groups. Species with similar environmental requirements can occur together anywhere in the world; characteristic assemblages are likely to be found wherever ecological conditions are appropriate. In contrast, geographical isolation and endemism are necessarily important considerations when comparing assemblages of higher organisms.

Examination of microbial communities is often not done on artificial substrates during the early stages of the colonization process and often not until they are presumed to have reached a MacArthur-Wilson (1967) equilibrium condition. However, the colonization process may be as informative as the equilibrium condition (Cairns, et al., 1979). Similar results were obtained by Henebry and Cairns (1979) in surveying the South River upstream and downstream from a major source of organic input. This study also demonstrated the effectiveness of polyurethane artificial substrates as collecting devices. Approximately fifty percent more species were collected on artificial substrates than on natural substrates.

Intensive analysis of colonization dynamics in wetlands have revealed similar patterns in all systems studied. Plafkin et al. (1980) found direct relationships between trophic status of wetlands and colonization rate. Henebry et al. (1981) showed similar results for productive wetland habitats such as bogs and fens. which have rapid colonization while a complex swamp site demonstrated very great species diversity but slower colonization.

# 2. METHODS AND PROCEDURES

Confirmation of recovery or association of environmental measures with comparable values in undisturbed systems requires careful selection of the reclaimed areas studied and comparison of standard parameters with those of undisturbed, "control" systems. The methods described below were directed at achieving the following goals:

1. early selection of potential study areas
2. grouping of possible study sites based on known history, physical, and water chemistry similarities, and
3. concentration of research effort in potentially rewarding study sites with some background study.

## STUDY SITES

### Selection of Study Sites

Lakes chosen for study were selected after consultation with FIPR staff and industry representatives. For the initial (March 1984) phase of work, ten sites were selected (Table 1). An additional 11 sites were added to the initial group of lakes for the second (August-September) phase of the project. Sites were selected to give a broad range of ages for the first phase. The second group of lakes added to the study was selected to provide information concerning what appeared to be the critical recovery period based on preliminary results from the first sampling effort. Natural lakes selected as reference sites were estimated to be typical of lakes in the area. That is, the "cleanest" or least eutrophic lakes and the obviously stressed lakes in the region were excluded. Natural lakes selected were moderately to heavily settled along the lake shores. At least two lakes were selected for each age category with most age categories having three "replicate" lakes for the second phase of the project. The youngest mined lakes (newly reclaimed and active mine pits) and the oldest mined lakes (unreclaimed) were represented by only two lakes.

Lake ages were estimated initially based on reports by reclamation directors consulted. More accurate ages were obtained for lakes reclaimed under present reclamation laws. Ages were determined as the difference between the date of initiation of sampling and the earthmoving begun during reclamation activities. Dates for the completion of earthmoving and erosion repair were also obtained but were not used in age determinations. A complete list of sampled lakes and their ages is given in Table 2.

## Study Site Descriptions

Study sites selected for this project were all located in Polk County (Figure 1). Previously mined sites ranged in age from essentially zero to over 60 years. Additionally, water in two active mine cuts was examined.

Active Mine Sites. Water standing in two active mine cuts at the W. R. Grace Hooker's Prairie Mine was sampled. These sites, arbitrarily designated HPA and HPB, contained essentially surface water impounded and pumped as needed. Both sites were very steepsided. Water was routinely pumped into HPB, and the water at this site was very green and productive. Water at HPA was much more turbid and the sediment here was a highly leached, light gray sand-clay mixture. These-sites were sampled during the second phase (August-September) of the project.

Newly Reclaimed Lakes. Two sites at the W. R. Grace Bonny Lake site were reclaimed shortly before the second phase of the project. These sites, WRG-BL-SP(5) (GOA) and WRG-BL-SP(4) (GOB), were reclaimed less than six months prior to the second sampling effort. Some erosion repair and revegetation was still in progress at these sites. Lake GOA was still in the process of filling while GOB was still being regraded and repaired. Grass planted to stabilize the banks around GOA was luxuriant. The water at GOB was extremely turbid.

One-Year Old Lakes. Three lakes at the Grace Bonny Lake site were chosen for a group of lakes approximately one year old. Two lakes, GlB and GlC, were in the WRG-BL-SP(6) site. The third lake, GlA, was in the WRG-BL-SP(2) site. These lakes were 0.6-0.8 years old during the first phase and 1.1-1.3 years old during the second phase of the project. Only lakes GlA and GlB were sampled during the first phase.

Lakes GlA and GlB were very similar to lakes GOA and GOB during the initial sampling. Both lakes had a relatively lush growth of grass stabilizing the banks, but GlB was extremely turbid. Suspended matter was so dense that certain routine water chemistry determinations based on color changes were difficult to interpret. Water levels in both lakes increased dramatically between the first and second samplings, and turbidity decreased markedly in GlB prior to the second sampling effort.

Two-Year Old Lakes. Three lakes ranging in age from 2.0 to 3.4 years were grouped as "two-year old" lakes. Two of these lakes, G2A and G2B, were at the Grace Bonny Lake site in reclamation sites WRG-BL-12 and WRG-BL-SP(l) respectively. The third lake, denoted STB, was at IMC's South Tiger Bay reclamation site, IMC-NP-SP-1. Lakes G2A and STB were approximately the same ages (2.5 and 2.4 year during phase two sampling). G2A was 3.4 years during the same sampling period. All three lakes had extensive stands of emergent vegetation (chiefly Typha) along shorelines. These lakes were only sampled during the second phase of the project.

Four-Year Old Lakes. Three lakes estimated to be four years old were grouped in this category. These lakes included two lakes at the Grace Bonny Lake site, G4 (WRG-BL-10) and Lake 1215 (WRG-BL-9), which were 4.9 and 4.8 years old during the second project phase. The third lake in this group was North Triangle (NT, IMC-P-4) which was actually 6.25 years during the second phase. Only NT and 1215 were sampled during the first phase of the project.

Seven-Year Old Lakes. Lakes Law (LL) and Brown (LB) were designated as seven-year old lakes. Both were on W.R. Grace Property and were reclaimed in 1977 and estimated to be 7 to 7.5 years old. These lakes were sampled during the first phase of the project. Lakes Law and Brown were a nearly identical pair of small lakes. Both were steeper sided than younger lakes and lacked extensive Typha stands. A third lake was added to this group for the second sampling effort. This lake, G7 (WRG-BL-4), was 7.25 years old during the second phase of the project.

Unreclaimed Lakes. Two old, unreclaimed lakes were examined during both phases of the project. These lakes were estimated to have been mined at least 60 years prior to sampling. Both lakes had steep sides and were said to have been mined using older techniques (M. Lloyd, FIPR, personal communication). The first of these sites was a long (ca. 1.5 km) narrow lake that parallels state highway 37 just north of the town of Mulberry and was on W. R. Grace property. We designated this site "Long Lake" (LG). During the second project phase this lake was affected by highway construction along state route 37. A silt curtain was installed paralleling the highway. Sampling was restricted to the southeastern part of the lake which was apparently not affected by construction activities. Heavy backfilling and sediment intrusion was restricted to the northern half of the lake. The second lake in this group, Lake Christina (LK), is located near the intersection of state route 37 and county road 540A. Sampling was restricted to the southern portion of this lake to avoid disturbance of sampling devices.

Natural Lakes. Two natural lakes were selected for study during the first project phase. Lake Arietta is a large, shallow, circular lake. Previous studies had shown it to have low productivity (Boody et al., 1984). Its water was generally clear, and there were patches of emergent vegetation (chiefly rushes) along the shore. Eagle Lake was somewhat more productive and had several extensive Typha stands. A third lake was added to this group during the second phase of the project. Lake Shipp, located in the Winter Haven chain of lakes, was heavily stained and was nearly surrounded by homes with the exception of a small park along the southwestern shore. This lake had a few isolated stands of Typha where bulkheads and retaining walls had not been built. There has been some indication that this lake is a candidate for rehabilitation (Fernald and Patton, 1984).

## Physical-Chemical Measurements

Water samples were collected in conjunction with artificial substrate collections (described below). Water was collected in cubitainers and transported back to the field lab in ice chests. Maximum holding time was kept to 1 - 2 hours. Back in the field lab, a 1 L aliquot from each collection was immediately frozen for nutrient analysis. A 50 ml aliquot was placed in glass screw-cap test tubes and fixed with trace pure nitric acid to pH <2 for metal analysis. A 100 ml aliquot was placed in glass bottles, fixed with phosphoric acid to pH <2, covered with aluminum foil and capped for total organic carbon analysis. Nutrient, trace metal and total organic carbon analyses were performed at the VPI lab. The remaining water was used for field lab measurements to be carried out the same day as sample collection. Analytical methods outlined by USEPA (1983) are indicated by STORET number. Citations are given for methods used from other sources. An outline of the analyses carried out are shown in Table 3.

## Field Measurements

The following parameters were measured in the field each time water was collected: air and water temperature, dissolved oxygen, and conductivity. Four separate readings were taken for each parameter and an average reported. Both air and water temperature were measured using the thermistor of a conductivity meter and reported in°C (STORET NO. 00010). Dissolved oxygen was measured using the YSI Model 54A oxygen meter and reported in ppm (STORET NO. 00299). Conductivity was measured with a YSI Model 33 conductivity meter with a S-C-T probe and results were reported in umhos/cm (STORET NO. 00095). Meters were calibrated according to manufacturer's operator's manual each time the above measurements were made.

## Field Lab Measurements

Alkalinity, total hardness, and pH were determined on water samples brought back to the lab, each parameter being measured four times separately on each sample in order to obtain an average value (except with pH in which a median was reported). A Fisher-Accumet pH Controller Model 650 pH meter with a polymer body liquid-filled combination electrode was used and calibrated daily using commercially available pH buffers (STORET NO. 00403). Alkalinity was measured using unfiltered sample which was titrated with 0.01 M $H_2SO_4$ to a pH 4.5 end point and reported as mg $CaCO_3$/L (STORET NO. 00410). Total hardness was measured using unfiltered sample buffered with commercial $NH_4$-EDTA hardness buffer and titrated with 0.01 M EDTA using Eriochrome Black T as an indicator; results were given as mg $CaCO_3$/L (STORET NO. 00900).

## Measurements Made at VPI Lab

Nutrient, total organic carbon, and trace metal analyses were carried out at the VPI lab.

Nutrients. Sulfate (STORET NO. 00945): Sulfate concentrations on unfiltered samples were measured on a Dionex System 10 Ion Chromatograph using a Dionex Ion Pac guard column, an HPIC AS3 separator column, and an ASC-2 suppressor column. The eluent was 0.0024 M $Na_2CO_3$/0.0003 $NaHCO_3$ and separation was carried out using a flow rate of 3 ml/min. A 300 ul sample loop was used. Standards were run every time analysis was carried out.

Total Phosphate (STORET No. 00665): Total phosphase was determined by the ascorbic acid method on unfiltered samples after digestion with potassium persulfate.

Dissolved Ortho-Phosphate (STORET NO. 00671): Samples were filtered through a glass fiber filter and dissolved orthophosphate was measured as described for total phosphate without the digestion step.

Ammonia (APHA, 1981, pp. 360-361): Ammonia was measured on filtered samples using the phenate method.

Nitrite (STORET NO. 00615): Nitrite was measured on filtered samples following the diazotation of sulfanilamide coupling with N-(l-naphthyl)-ethylenediamine to form a colored azodye.

Nitrate + Nitrite (STORET NO. 00630): Nitrate was reduced to nitrite by passing the sample through a copper-cadmium reduction column. The resulting nitrite was measured as previously described.

Total Suspended Solids (APHA, 1981, pp. 92-92): A known volume of sample was evaporated in a tared porcelin casserole and dried to a constant weight. Casseroles were reweighed and the increase in weight was used to calculate mg suspended solids/L.

Total Organic Carbon (STORET NO. 00680): Total organic carbon was measured in samples fixed with phosphoric acid to pH <2 on a DC-54 Ultra Low-Level Total Organic Carbon Analyzer (Dohrman/Environtech. Inc.).

Trace Metal Analysis. Acidified water samples were analyzed by either direct aspiration into the flame of a Perkin-Elmer 603 Atomic Absorption Spectrophotometer or by injection into an HGA 2100 Graphite Furnace. Sample pretreatment was done according to methods in USEPA (1983). Table 4 lists STORET numbers, pretreatment required, method used and detection limits for all of the metals analyzed.

In addition to parameters determined from analysis, certain additional variables that have been shown to have biological meaning were created from the data set. Total nitrogen (designated TOTN in data reports) was taken as the sum of $NH_3$-N and $NO_3$-$NO_2$-N. This value is not strictly equivalent to other reports of total nitrogen since the fraction of nitrogen in living tissues was not determined for each sample. However, this value can serve as an additional indication of the relative availability of dissolved nitrogen compounds in lakes.

The relative amounts of dissolved nitrogen and phosphorus compounds were estimated from the ratio of TOTN and $OPO_4$. The ratio of dissolved nitrogen and phosphorus can reflect which nutrient is typically more limiting to algal growth. The ratio of dissolved nitrogen (as TOTN) to total organic carbon was also examined. This ratio can be used to compare the relative availability of nitrogen for heterotrophic assimilation in the water column.

## Colonization Processes

The invasion of artificial, introduced islands into aquatic ecosystems by microbrial species such as protozoans follows the theoretical prediction of the MacArthur-Wilson (1967) equilibrium theory of island colonization. Exposure of substrates and collection times were designed to produce data which will allow fitting of the model equation:

$$S_t = \hat{S}eq(1-e^{-Gt}) \quad (1)$$

where $S_t$ = species at time t,

$\hat{S}eq$ - equilibrium species numbers

G = rate fitted constant,

t = time.

For most systems, equilibrium is attained in 7-21 days although this may be considerably shorter if there is considerable flow in the case of streams, or if the water is rich in organics (Henebry et al., 1981). Sampling at intervals of 6, 12, and 18 hours may be indicated in highly productive or enriched systems (e.g., bogs, fens, marshes).

In general, substrates are suspended in the water column (e.g., Fig. 2) in sufficient numbers to allow 3-4 replicate islands to be collected at each sampling. Islands are removed from the water and harvested by squeezing the contents of the substrates into a sterile collecting jar or bags. The sample is allowed to settle and the species are then exhaustively identified by repeated subsampling. Experience has shown that 3 to 4 subsamples are sufficient to attain an asymptotic species number. At equilibrium this represents 85-95% of the available protozoan species which would be collectible by laborious means from natural substrates (e.g., Patrick et al., 1967). Natural substrate collections allow comparison of species numbers, functional groups (e.g., phytoflagellate algae, ciliates, amoebae) but yield little information about community function.

Colonization, on the other hand, is a vital functional characteristic. Microbial communities typically have a high rate of species turnover. Since the cells are naked and in intimate contact with the environment, the species present continually respond to changing environmental conditions. Exact environmental requirements of protozoans are difficult to determine and are generally quite broad (Cairns, 1965; Noland, 1925; Noland & Godjics, 1967). The group of species present may represent a vast array of possible permutations of available tolerant species (Cairns and Henebry, 1982). The coloniation process, specifically the equilibrium are reflective of local conditions. There is now an increasing body of evidence showing the utility of protozoan colonization as an environmental monitor (Henebry and Cairns, 1979; Cairns, et al., 1980; Buikema, et al., 1983; Cairns, et al., 1985).

Field Experiments. Polyurethane foam artificial sbustrates (5 x 6.5 x 7.5 cm) were placed in each lake studied. Substrates were anchored to weights on nylon lines and immersed at depths of approximately 30-50 cm in each lake. Sufficient substrates were placed to allow collection of 4 replicate substrates after 0.5 (12 h), 1, 3, 8, and 10 days of exposure. During Phase 1 of the project exposure time of 0.5, 1, 3, 10, and 14 days were used. Substrates collected after 0.5 d (12 hr ) exposure were placed in lakes in the evening and collected the following morning. Previous work has indicated that there is no difference in short-term night or day exposures (J. R. Pratt, N. B. Pratt, unpublished data).

Collection of substrates involved removing the substrate from the water and placing it immediately in a labeled, sterile whirlpak collecting bag. All collections were made in the forenoon and collected substrates were placed in insulated containers for transport to the field laboratory. Collection of water samples and other field collected data was made concurrent with substrate collections.

At the field laboratory, each collected substrate was harvested. Harvesting involved squeezing the contents of the substrate into the collecting bag. The substrate was then discarded. The collected contents were placed near a source of light and allowed to settle and respond to available light conditions. Subsamples were then removed for identification of protozoan species. All samples were examined live and species. identifications were complete within 24 hr, and generally within 12 hr, of collection for each sample.

During the first phase of the project, only presence of species in samples was recorded. During the second phase of the project, a relative abundance scale was used to estimate relative numbers of protozoan species in samples. This scale, based on that of Sramek-Husek (1956) used a five point scale to rate abundance as follows:

| Rank | Individuals/slide |
|---|---|
| 1 | 1 - 2 |
| 2 | 3 - 10 |
| 3 | 11 - 25 |
| 4 | 26 - 100 |
| 5 | >100 |

The results of the colonization experiments were fitted to the MacArthur-Wilson equilibrium model equation, $S_t=\hat{S}eq(1-e^{-Gt})$, by non-linear least squares regression analysis using Marquardt methods of estimation (Helwig and Council, 1979). Estimates of equilibrium species number (Seq) and colonization rate (G) were obtained. The regression was tested for significance according to Draper and Smith (1981).

## INTERACTION OF COMMUNITIES AND ABIOTIC FACTORS

### Factor Analysis

Factor analysis (principal components) was performed on the physicochemical data from the lakes. The most distinctive characteristic of this analysis is its ability to reduce a complex data set to a fewer number of new variables termed factors. Factor analysis techniques enable one to determine if some underlying pattern of relationships exists such that that data may be reduced to a smaller set of components that may be taken as source variables accounting for the observed interrelations in the data (Kim, 1975).

PCA uses transformations of a set of variables to form a new set of composite variables that are orthogonal (uncorrelated) to each other. No assumptions about the underlying structure of the variables is required (e.g., normality). The method iterates to what would be the best linear combination of variables. The first principal component may be viewed as the best summary of linear relationships exhibited in the data set. For normal purposes, the investigator retains only the first few components for futher rotation which simplifies the factor structure by moving the factor axes to a position for maximum clarity. The factors and environmental parameters are then correlated with the X and Y coordinates from an ordination to determine which parameters and factors are related to the distribution of samples (i.e., communities).

## Canonical Variate Analysis

Canonical variate analysis is a data reduction technique related to PCA and canonical correlation. This technique was used in this study to examine all physico-chemical parameters to determine those that contributed to distinguishing lakes. For example, many of the parameters from the March 1984 sampling period were similar for all lakes measured. Being similar, these add no new information to the understanding of differences in lake water chemistry or communities. This technique allows an examination of all parameters simultaneously and determination and elimination of those that do not appear important. The procedure is then repeated, but this time the parameters that do not appear important are omitted. If little separation occurs when the technique is performed using the omitted parameters, resulting in a stochastic distribution of samples, then one can with some confidence omit from further analysis the physico-chemical parameters deemed unimportant.

## Cluster Analysis

The purpose of cluster analysis is to place samples (and species) into groups or clusters suggested by the data, not defined *a priori*, such that objects in a given cluster tend to be similar to each other in some sense, and objectives in different clusters tend to be dissimilar. Cluster analysis is the first and simplest classification technique employed on the species by sample data collected in this study. With this technique, one is able to examine all samples at the same time and determine their relationship to other samples by examining their species composition. One is also able to do the converse, except that this is much less information for the scope of this study.

Cluster analysis is used to first examine the relationship among samples from a lake to determine if they form a cluster. For example, it would be informative if each lake formed a cluster, or natural lakes formed one cluster, reclaimed lakes in another, and unreclaimed lakes formed a third.

There are many algorithms available. One method that has thus far proved satisfactory has been the method of average linkage (SAS, 1984). This method basically examines the average distance between samples based on their species composition.

## Ordination

Ordination is a technique used to examine the overall similarities of the communities in the lakes under study. We used two ordination programs, Ordiflex and Decorana (Gauch, 1977; Hill, 1979). Ordiflex is a flexible computerprogram used to relate samples by their species composition. The placement of samples along two axes was examined for relationships to known environmental gradients to parameters (Gauch, 1977).

Ordination techniques separated lakes according to their species composition. Both relative abundance and presence/absence data were used. The ordination technique yielded a matrix of percentage similarities for relative abundance or for presence/absence data using the coefficient of distance algorithm.

Ordinations were performed to separate lakes according to their species composition and to reduce the complexity of the species data set. The results were plotted as scatter figures to show relative similarities by the distance between points. The coordinates of the samples on the scatter diagram were used to relate samples and species to underlying environmental gradients and to study patterns of communities as related to patterns of environmental factors. This last type of ordination allowed inference about environmental relationships of samples and species.

Reciprocal averaging ordination (RAO) was considered best to use with the type of data obtained in this study. It is often the first ordination technique used when the important environmental gradients are not known. RAO obtained sample scores from species scores and the converse by first assigning arbitrary species scores. The first iteration used weighted averages to obtain sample scores. The second iteration used the same procedure as the first, but began with the sample scores produced in the first iteration to obtain new species scores. This process continued until the scores converged to a unique solution (Gauch, 1977).

Ordinations were performed to search for patterns in the data. The X and Y coordinates for the samples (lakes) were then correlated with environmental parameters and factors (composite parameters) in an attempt to discern relationships between environmental parameters and the distribution of samples and species.

Detrended correspondence analysis (DCA) was also employed with the data gathered in the second phase of the study. It is considered a substantial improvement over reciprocal averaging ordination by avoiding the two main problems of RAO, that of the frequent arch distortion due to the dependency of the second axis on the first , and compression of axis ends (Hill, 1979).

PROTOZOAN FUNCTIONAL GROUPS

Lifestyles of common protozoan species have been described in the literature. On the basis of these reports, species were assigned to one of six functional groups: bacterivores, producers, non-specific feeders, algivores, saprovores, and raptors (Pratt and Cairns, 1985). A mean count of species in each functional group was calculated from four collections at each site on each sampling day. Then data for the last 3 sampling days were averaged yielding a single indicator of the distribution of protozoan species into functional groups for each lake. It was assumed that the later sampling days represented equilibrium numbers. This assumption was supported by ANOVAs comparing the square root transformed counts for each functional group over the last three sampling days. there were no significant differences between days (all $p>0.05$).

Contingency table analysis (e.g., Sokal and Rohlf, 1981) was used to compare functional group distributions between ages of lakes. In addition, Kaesler et al. (1978) suggested that similarity indices commonly used with taxonomic data may be equally useful for comparing community structure based on functional distinctions. Pinkham and Pearson's Coefficient of Similarity (B) was computed for each pair of lakes (Pinkham and Pearson, 1976). This index was calculated as:

$$B = \frac{1}{K} \sum \frac{\mathrm{Min}\,(X_{ia}, X_{ib})}{\mathrm{Max}\,(X_{ia}, X_{ib})} \qquad (2)$$

where K is the number of different functional groups in the two lakes, Min indicates the smaller of Xia or Xib, i.e. the numbers of species in the ith functional group for lakes a and b respectively, and Max indicates the larger of Xia and Xib. The Coefficient of Similarity should vary from 0 for very dissimilar distributions in functional groups to a value of 1 for perfect correspondence between samples. The resulting matrix of coefficients for all paired comparisons was then clustered using a nearest neighbor technique and a dendrogram was produced. The dendogram provides a visual indication of the degree of similarity between all samples. Finally, the hypothesis that lakes of the same age are more similar in functional group structure than are lakes of different ages was tested by sorting coefficients for paired comparisons into two groups. One group consisted of all coefficients for pairs of the same age. The other group included all coefficients for pairs of mixed age. A Wilcoxon Rank Sum test was used to compare the median similarities of these two groups. The null hypothesis was that same age lakes were not more similar than were lakes of different ages.

## DATA ANALYSIS

Results of the first sampling effort (March 1984) were used to evaluate the effectiveness of sampling and collecting methods and to identify variables which showed little difference among lakes or lake types. Since a broad range of lake ages and types were initially selected, it was generally accepted that parameters showing little variation would provide minimal information. Parameters which were difficult to evaluate (based on analytic methods) and which appeared to provide little information were eliminated from analysis during the second sampling effort. These parameters were only eliminated after factor analysis had shown that they added virtually no information even to analysis of composite variables generated in multivariate analyses. The analysis of mercury and chemical oxygen demand were eliminated on this basis.

The initial sampling of ten lakes was used to identify critical periods for recovery following lake reclamation. More intensive sampling of additional lakes within the supposed critical range was undertaken in the second sampling effort (August-September 1984). During this sampling, twenty-one lakes were studied. Seasonal differences were not considered to be of major interest for two reasons: only two seasonal periods were examined (spring, fall, neither an annual maximum or minimum) and young lakes were continuing to age between samplings confounding seasonal and aging comparisons. We have, therefore, restricted detailed analyses to within-season differences and have made only occasional comparisons between seasons.

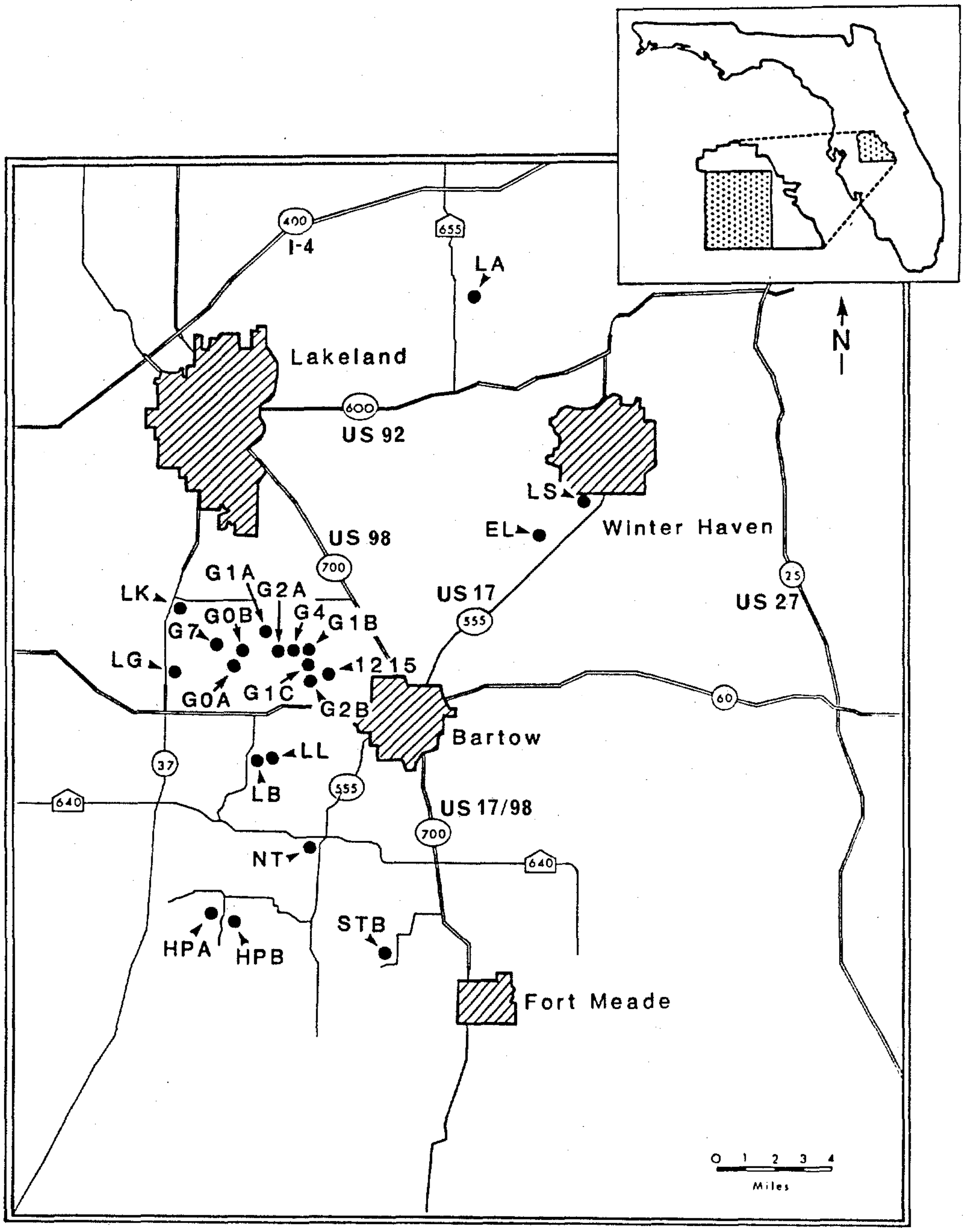


Fig. 1. Partial map of Polk County, Fla. (see insert) showing location of lakes sampled in fall 1984.

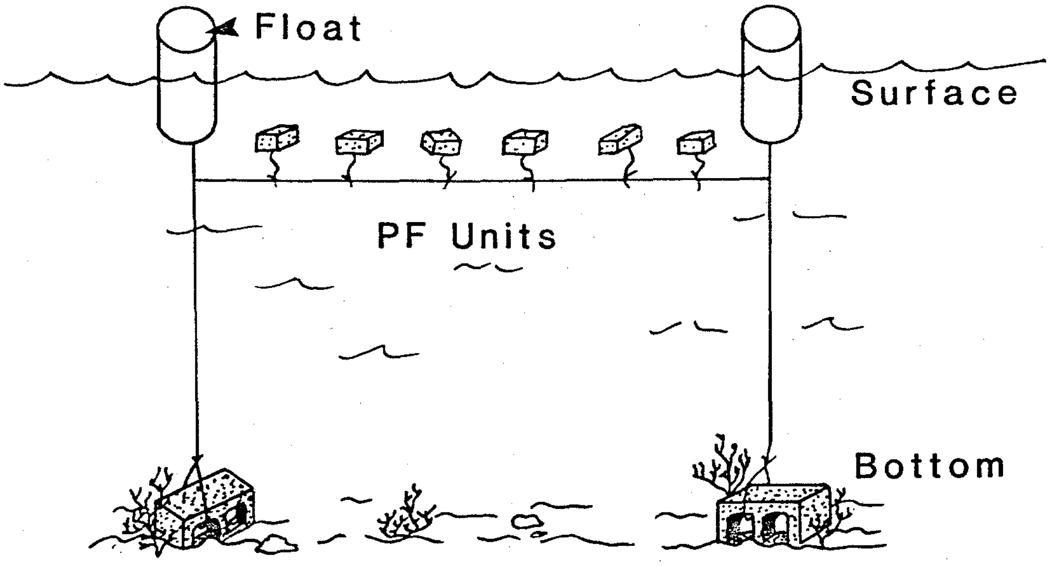


Fig. 2. Typical artificial substrate placement for surface water sampling in a lake or pond (from Cairns et al., 1979).

Table 1

Study sites for protozoan samples and lakes from Spring 1984 sampling. (Status: R = reclaimed, U = unreclaimed, N = natural).

| Site | Code | Number | Age | Status |
|---|---|---|---|---|
| WRGBLSP2 | G1A | 0 | < 1 yr | R |
| WRGBLSP6 | G1B | 9 | < 1 | R |
| Lake 1215 | L12 | 1 | 4 | R |
| North Triangle | NT | 2 | 4 | R |
| Lake Brown | LB | 4 | 3 | R |
| Lake Law | LL | 3 | 3 | R |
| Long Lake | LG | 8 | < 60 | U |
| Lake Christina | LK | 7 | < 60 | U |
| Lake Arietta | LA | 5 | --- | N |
| Eagle Lake | EL | 6 | --- | N |

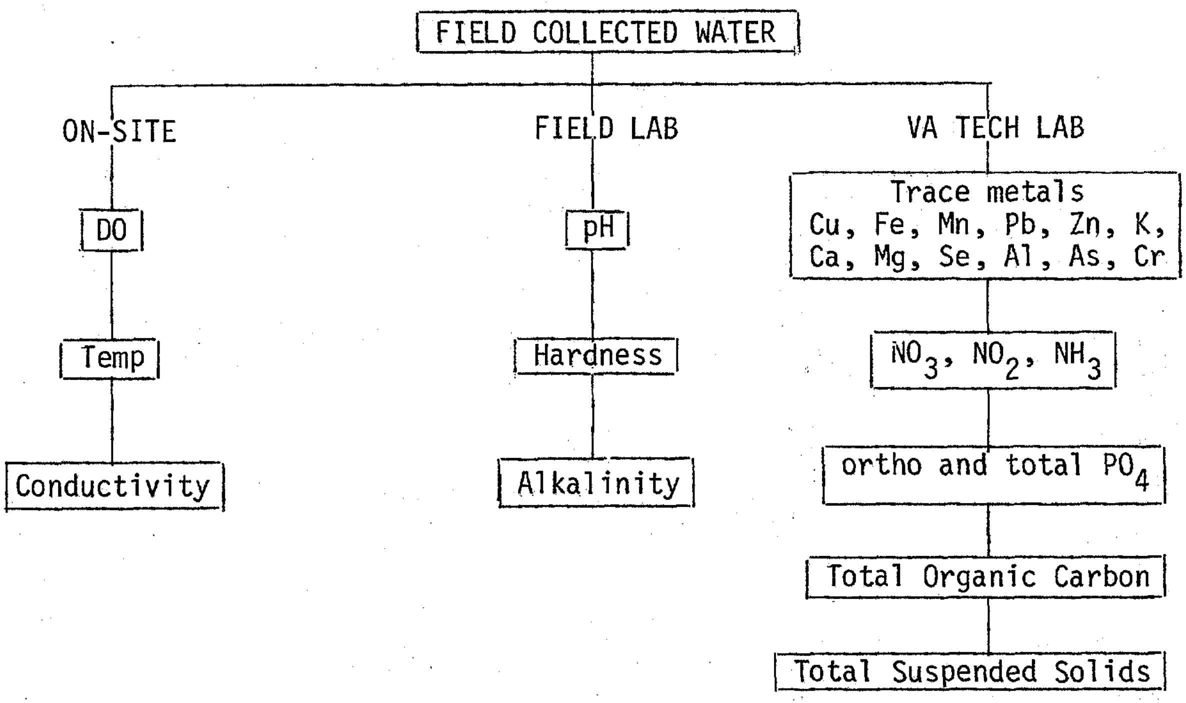
## Table 2

Study sites, lake codes, lake age, lake status, and authority for lakes sampled in Fall 1984. STATUS: A = active, R = reclaimed, U = unreclaimed, N = natural.

| Site | Code | Age March/September 1984 | Status | Reclamation designation/ Authority |
|---|---|---|---|---|
| Hooker's Prairie A | HPA | -/- | A | W. R. Grace |
| Hooker's Prairie B | HPB | -/- | A | W. R. Grace |
| GOA | GOA | -/0.5 | R | WRG-BL-SP(5) |
| GOB | GOB | -/0.5 | R | WRG-BL-SP(4) |
| G1A | G1A | 1.2/1.7 | R | WRG-BL-SP(2) |
| G1B | G1B | 0.8/1.3 | R | WRG-BL-SP(6) |
| G1C | G1C |  | R |  |
| G2A | G2A | 2.9/3.4 | R | WRG-BL-12 |
| G2B | G2B | 1.9/2.4 | R | WRG-BL-SP(1) |
| South Tiger Bay | STB | 2.0/2.5 | R | IMC-NP-SP-1 |
| G4 | G4 | 4.4/4.9 | R | WRG-BL-10 |
| Lake 1215 | 12 | 4.3/4.8 | R | WRG-BL-9 |
| North Triangle | NT | 5.7/6.2 | R | IMC-P-4 |
| Lake Brown | LB | est.7.0/7.5 | R | W. R. Grace |
| Lake Law | LL | est.7.0/7.5 | R | W. R. Grace |
| G7 | G7 | 6.7/7.2 | R | WRG-BL-4 |
| Long Lake | LG | >60 | U | W. R. Grace |
| Lake Christina | LK | >60 | U | Regal Real Estate |
| Lake Arietta | LA | - | N | Polk Co. |
| Eagle Lake | EL | - | N | Polk Co. |
| Lake Shipp | LS | - | N | City of Winter Haven |

Table 3

Outline of water collection and analysis.



## Table 4

A list of metals analyzed by Atomic Absorption Spectrophotometry, STORET numbers, pretreatment, method of analysis and detection limits.

| Metal | STORET NO. | Pretreatment | Method | Detection (mg/L) Limit |
|---|---|---|---|---|
| Aluminum | 01105 | None | Furnace | 0.003 |
| Arsenic | 01002 | $NiNO_3$ | Furnace | 0.001 |
| Calcium | 00916 | $LaCL_2$ | Flame | 0.010 |
| Chromium | 01034 | None | Flame | 0.050 |
| Copper | 01042 | None | Flame | 0.020 |
| Iron | 01045 | None | Flame | 0.030 |
| Lead | 01051 | None | Flame | 0.020 |
| Magnesium | 00927 | $LaCl_2$ | Flame | 0.007 |
| Manganese | 01055 | None | Flame | 0.050 |
| Potassium | 00937 | None | Flame | 0.040 |
| Selenium | 01147 | $NiNO_3$ | Furnace | 0.002 |
| Zinc | 01092 | None | Flame | 0.025 |

# 3. CHARACTERISTICS OF LAKES IN THE PHOSPHATE MINING REGION

## PHYSICAL-CHEMICAL CHARACTERISTICS OF LAKES

### Spring Sampling

Table 5 reports mean, standard deviation, maximum and minimum values for nitrogen, phosphorus and carbon from all lakes combined and reclaimed lakes. Table 5 also presents the same for unreclaimed lakes and natural lakes. Parameters differed among lake types: $NH_3$, $TPO_4$, and $OPO_4$ were somewhat less in the natural lakes than in the unreclaimed and reclaimed lakes. TOC did not appear to differ markedly among the lake types but was somewhat higher in reclaimed lakes.

Table 6 summarizes environmental parameters measured in the Spring 1984 sampling. Alkalinity, sulfate, calcium, potassium, and aluminum showed more obvious differences among lakes.

A stepwise selection procedure (Table 7) identified potassium, hardness, alkalinity, and total organic carbon as important variables in explaining lake variability with arsenic and conductivity contributing to a lesser degree.

Canonical variate analysis allowed elimination of several environmental parameters that appeared to be of little importance in distinguishing lakes. Table 8 summarizes the 18 environmental parameters remaining. A canonical variate analysis performed on the 18 remaining parameters (Table 8) separated primarily according to potassium concentrations with contributions from total organic carbon, hardness, alkalinity, and conductivity. Table 9 presents values for canonical variate axes 1 to 4. Axis 2 separated lakes along a strong alkalinity and a weaker hardness gradient. Lakes were separated along axis 3 by hardness, conductivity, and arsenic. Plots of axis 1 versus 2 (Figure 3) and axis 1 versus axis 3 (Figure 4) (see Table 10 for number interpretation) show the distributions of water samples along these composite axes.

Several observations can be made based on this analysis. First, natural lakes (numbers 5 and 6 on Figures 3 and 4) had similar characteristics and were separated from the other lakes along axis 1 (composed of primarily a potassium component). The remaining lakes (reclaimed, and unreclaimed) were not separated along axis 1, but were discriminated along axis 2 and 3 which have loadings composed of an alkalinity and hardness gradient. Another interesting observation was that lake GlB(9), a young reclaimed lake, appeared somewhat by itself along canonical axis 2, thus appearing to separate along an alklinity gradient (this separation shows up later in the protozoan data). This lake had been recently reclaimed and was very turbid during the spring sampling.

The factor analysis performed on the 18 important environmental parameters showed three important factors when a screen plot was examined. These three factors explain 64.5% of the 18 environmental parameter data set variability. Factor 1 (Table 11) was composed of loadings from hardness and conductivity with weaker contributions from sulfate, total suspended solids, and arsenic and accounted for 30.4% of the data set variability.

Factor 2 was primarily composed of total phosphate and ortho-phosphate with weaker loadings from aluminum and selenium. Factor 3 was derived from pH and alkalinity loadings with additional loadings from manganese, asrenic, and iron.

## Fall Sampling

Tables 12-16 summarize nitrogen, phosphorus, and carbon for all lakes during the Fall 1984 sampling. Natural lakes had the lowest phosphate values and active lakes the highest. Reclaimed lakes had high nitrogen values but also had high variability.

Tables 17-21 summarize the other environmental parameters measured. The newly reclaimed lakes were highest in mean values for hardness, conductivity, magnesium, aluminum, and selenium. The newly reclaimed lakes had the lowest protozoan species numbers. Natural lakes were lowest in calcium, highest in potassium, and had the highest equilibrium species number.

A stepwise selection procedure (Table 22) identified magnesium as the most important variable differentiating lakes with contributions from calcium, selenium, and sulfate. Ortho-phosphate and potassium could also be included in this analysis but several tolerance values fall below 0.10 when they were added. This pattern is also evident when only the most important 15 environmental parameters were examined in the stepwise procedure (Table 23).

Canonical variate analysis was utilized in an attempt to separate lakes using environmental parameters. Twenty parameters were used initially (Table 24). Thus several parameters were eliminated by using the criteria of lowest within canonical structure. Water and air temperature, ammonia, total organic carbon, and iron were removed from further analysis.

Table 25 presents canonical variate (within canonical structure) results for the 15 important environmental parameters used in separating lakes. Figure 5 shows the lake samples in environmental variable space. See Table 26 for letter identity as to lake and status. No pattern of lake separation was evident. However, there was good separation of lakes along axes 1 and 2 (Figure 5) and lesser separation along axis 1 and 3 and axis 2 and 3. Canonical axis 1 was primarily composed of a magnesium loading with contributions from calcium, conductivity, sulfate, selenium and alkalinity. Canonical axis 2 is formed by loadings of sulfate, calcium, alkalinity, and ortho-phosphate with weak loadings from magnesium, conductivity, and potassium. Canonical axis 3 is clearly composed of loadings of selenium, alkalinity, potassium and calcium.

Table 27 shows factor analysis results for the 15 most important environmental parameters. Three factors explain 68.71% of the data set variability. Factor 1 (37.35%) is a linear combination of conductivity and calcium with additional loadings from magnesium, total suspended solids, sulfate and hardness. Factor 2 (16.00%) is composed primarily of loadings from pH with contributions from alkalinity and nitrate-nitrite. Factor 3 is derived from a strong total phosphate component with contributions from aluminum and ortho-phosphate.

## MICROBIAL COMMUNITY CHARACTERISTICS

### Spring Survey

In the spring survey 537 protozoan taxa were identified (Table 28). Many (128, 23.8%) were found in only one sample. Fewer than 10% of the species were found in over 10% of the samples, and only 3 species were found in over 20% of the samples. The most common species included Monas spp., appearing in 45 of 49 pooled triplicate samples; Cryptomonas erosa (44) and Cyathamonas truncata were also common.

Results of a cluster analysis of the most commonly occurring 96 species (Figure 6), and the 100 next most common species (Figure 7), showed little pattern in these dendrograms with natural, reclaimed, and unreclaimed lakes intermixed. There did appear to be some grouping of protozoan samples from natural and unreclaimed lakes in the right half of each figure, with mostly reclaimed lakes to the left.

Figure 8 presents the results of reciprocal averaging ordination (RAO) of protozoan samples using presence/absence data of combined triplicate samples for each lake/date. The 96 most common species are also used in the ordination depicted. Other ordinations were performed, but were no more informative graphically and are not shown. As mentioned before, lake GlB(9) appears to be somewhat separated, appeared at the top of the diagram. All other samples appear distributed in a random fashion suggesting very little grouping of the protozoan samples in species space between natural, reclaimed and unreclaimed lakes.

Table 29 presents correlation values between ordination axes and physico-chemical parameters and factors. Environmental parameters instrumental in separating lakes, pH and potassium, were not correlated with any of the ordination axes, and thus, did not appear to be related to the distribution of communities. Several environmental parameters were related to the distribution of communities. These include nitrate for XORDl; AL and Factor 2 for YORDl; hardness, conductivity, nitrite, nitrate-nitrite, total-phosphate, alkalinity, and factor 2 for YORD3. Only correlations greater than $\pm 0.464$ ($p < 0.001$) are considered due to the large sample sizes and inflated p-values.

## Fall Survey

In the fall survey 892 protozoan taxa were identified (Table 30). Of these, 216 (24%) were found in only one sample. The frequency of common species is further documented in Table 30, although this summary is not strictly identical to that shown in Table 20 for the spring sampling. Quadruplicate samples were pooled and averaged during data compilation. Thus, if a species occurred in one or more of the quadruplicate samples taken on a given day in a given lake, it would be counted as occurring once for purposes of this data summary. The frequency of occurrence (and relative abundance) of species in samples was accounted for in data analysis but these differences are not reflected in Table 30.

Several species were common and these have generally been reported as common pioneer invaders in other studies (e.g., Henebry and Cairns, 1981). Cryptomonas erosa was found in all 105 pooled quadruplicate samples. This phytoflagellate is very common in freshwaters. Several bodonid flagellates (Boda spp. and Rhynchomonas nasuta) were also common. The most common ciliate was Ctedoctema acanthocrypta, and the most common amoeba was Naegleria gruberi which has been similarly reported all over the world (e.g., Page,1976).

Several cluster analysis procedures were used on the protozoan data using both the most common 495 species and the 106 species of phytoflagellate (photosynthetic protozoans). These analyses were uninformative reresulting in severe chaining of the samples with no apparent pattern.

Detrended correspondence analysis (DCA) was performed on the most common 495 species (those that occurred in 5 or more of the pooled (quadruplicate) samples). Two samples were identified as outliers and eliminated. These two outliers were LB day 1 and LK day 0.5. All other samples were again examined by DCA (Figure 9). There was broad dispersion of reclaimed lake protozoan samples and overlap of reclaimed lake samples with active, unreclaimed and natural lake samples. There was little overlap between protozoan samples from natural lakes and protozoan samples from unreclaimed lakes along axis 2 of the DCA.

The DCA axes were examined for validity. Axis 1 had an eigenvalue of 0.184; axis 2, 0.116; axis 3, 0.099; and axis 4, 0.087. Plotting eigenvalues versus axis number (a scree plot) showed that axis 1 is statistically sound. A sharp drop in eigenvalue occurred for axis 2 which was similar to axis 3 and 4. However, the residual value was larger than the tolerance value of the iteration between axis 2 and axis 3. Therefore, it was necessary to interpret the results of axis 3 and 4 with caution.

A plot of axis 4 against axis 1 revealed that newly reclaimed lakes (GOA and GOB) and the active mine sites (HPA and HPB) tended to separate from the other lakes.

Figure 10 is a plot of DCA scores for axis 1 versus axis 3 based only on phytoflaggelate species (106 species in 105 samples). Little overlap of active, unreclaimed, and natural lakes was found, but samples from reclaimed lakes were scattered throughout the distribution.

Reclaimed lake samples were removed from the analysis with active, unreclaimed, and natural lake DCA scores plotted (Figure 11). This supported the previous differences noted between active, unreclaimed, and natural lake protozoan community samples.

Table 31 shows correlation values for environmental parameters and factors for ordination axes (based on 103 samples and 495 species). Axis 1 correlated significantly with water temperatire, air temperature, nitrate-nitrite, and ortho-phosphate. Plots were examined for axis 1 and nitrate-nitrite which showed this correlation to be spurious largely due to lake GOA (newly reclaimed) being an outlier. Removal of this lake would no doubt remove any significant correlation. Axis 1 correlates weakly with ortho-phosphate (Figure 12) and does not correlate significantly with total phosphate (Figure 13). The correlation of ortho-phosphate to Axis 1 appears largely due to lake NT(M) being an outlier in both cases.

DCA axis 2 was correlated with water temperature, pH, alkalinity, hardness, dissolved oxygen, total organic carbon, calcium, magnesium, aluminum, iron, total nitrogen, and N:P ratio. From this point on, only strong correlations were examined due to possibility of spurious relationships. Sample pH had the highest correlation with axis 2. Lakes GOA and GOB (newly reclaimed) had the lowest pH (Figure 14). These lakes were quite different from the other lakes in this study. A plot of DCA axis 2 values against alkalinity showed that this significant correlation is largely due to extreme values in Lake NT(M) and an active lake HPB(B). Removal of these lakes would likely alter the nature of the correlation. Lake HPA, an active mine pit, had the highest values for iron. A scatter plot of DCA axis 2 and iron (Figure 15) shows a weak, yet real relationship between these variables. Additionally, a plot of axis 2 versus ortho-phosphate shows the correlation between axis 2 and ortho-phosphate is also due to high values of ortho-phosphate for lake NT(M) (Figure 16). Similarly DCA axis 3 versus total phosphate shows the distortion of the lake distribution due to lake NT.

Axis 4 was significantly correlated with many of the environmental parameters, Seq, G, factor 2 and factor 3. Axis 4 and pH were negatively correlated (Figure 17). This is apparently due to lakes GOA and GOB. If these lakes were removed, there would be a weak positive relationship. Similar distortions are indicated in correlation of axis 4 versus ammonia and nitrate-nitrite (Figures 18, 19). The relationship of aluminum to axis shows that the active mine sites (HPA and HPB) are outliers and that if removed the significant correlations would disappear. Selenium levels plotted against axis 4 shows that lakes HPB, GOA, and GOB drive the significant correlation values.

Significant correlations for nutrients versus axis 4 are primarily due to outlier values for lakes GOA and GOB Axis 4 versus factor 2 (Figure 20, pH and alkalinity, showing that lakes GOA and GOB are outliers). Other lakes show a strong positive relationship between axis 4 and factor 2. The weak relationship between axis 4 to factor 3 (total phosphorus, alumnum, and ortho-phosphorus) is strongly influenced by lakes GOB, GOA, HPA, and HPB.

There was a strong correlation of axis 4 with Seq. This demonstrates some relationship between sample location in species space and these two variables (Figure 21).

For relationships of phytoflagellates, correlations are shown between DCA axes and environmental parameters in Table 32.

Axis 1 correlated with pH, alkalinity, hardness, dissolved oxygen, G, Seq, total phosphorus, aluminum, iron, and selenium. Strongest correlations were between axis 1 and pH, G, and Seq, although G and Seq are highly related.

Axis 2 correlated with water and air temperature and lake age. Axis 3 correlates with pH, ammonia, nitrate-nitrite, total and ortho-phosphate, aluminum, iron, selenium, age, and Seq. Axis 4 correlates with orthophosphate only.

## Colonization of Artificial Substrates

Protozoan colonization of artificial substrates was rapid, and the numbers of species found at equilibrium were generally high for most lake types examined. The MacArthur-Wilson equilibrium model adequately described the colonization process in most instances (Table 33). Where the model failed to account for a significant or large portion of the measured variability in species numbers over time, colonization was typically extremely rapid. The rapidity of colonization is reflected in high estimates for the colonization rate parameter G. Time to 90% of equilibrium species number can be estimated by t 90% = 2.3/G. In most cases, G values ranged between 1 and 2 indicating that colonization equilibrium was approached at from 1 to 2 days. This pattern was maintained over both sampling periods. Species numbers were generally higher for samples from the Fall project period. The colonization model accounted for approximately 30 - 90% of the species-time variability for most sampled lakes.

The failure of the colonization model to account for a significant portion of the species-time data variability was especially apparent in lake GlB during the spring sampling and in lake GOB during the fall sampling. Both of these lakes were very turbid. Species numbers actually decreased after the first sampling in both lakes. the only other case of failure of the colonization model to adequately describe the colonization process was for lake LK during the spring sampling. Colonization in this system was extremely rapid, and species numbers were near their highest level after only 12 hr of colonization.

Species numbers in lakes increased rapidly with lake age (Figure 22) although this relationship is somewhat obscured by differences in species numbers between collecting periods. Differences between very young lakes (e.g., GOA, GOB) were consistent both in terms of estimates of equilibrium species number (Seq) and in terms of total species collected (Table 34). The exact timing of the critical attainment of microbial species equilibrium in lakes of a given "age" is complicated by the precision of age determination. Initiation of earthmoving was taken as the beginning of the aging process for reclaimed lakes. Earthmoving activities vary somewhat in duration and potential impact on reclaimed lakes but are generally limited to approximately 2 months of effort.

An estimate of the critical recovery period can be inferred from lakes studied during both collecting periods. For older lakes (i.e., those greater than 4 years) sampled during the first phase of research, equilibrium species number estimates increased by from approximately 10 - 100%. For example, $\hat{S}$eq estimates for Lake 1215 increased 58.5 to 96 between spring and fall 1984. $\hat{S}$eq's estimated for lakes Law and Brown increased from about 40 to over 80 in this period. Equilibrium species number increases for unreclaimed lakes and natural lakes were within the range for older reclaimed lakes. During the same period equilibrium species numbers increased from 38 to 70 for G1A and from 21 to 78 for G1B.

Equilibrium species numbers were consistently higher in the fall (end of wet-warm season) as compared to the spring (end of cool-dry season) for systems studied during both seasons. Estimates of colonization rate increased less consistently, but were also generally higher during the second sampling period. Increases in colonization rate reflect the increased productivity of the second sampling period, high water levels, and the higher temperatures in the systems studied.

Colonization parameters were correlated with several environmental parameters (Table 35). Those factors correlated with species numbers on artificial substrates were among factors distinguishing communities in the studied lakes. There was a strong relationship between equilibrium species number ($\hat{S}$eq) and two nutrient parameters ($NO_3NO_2$, $TPO_4$). In addition, there were strong negative correlations between $\hat{S}$eq and levels of aluminum and selenium and a strong positive correlation between $\hat{S}$eq and CTON (total organic carbon to total inorganic nitrogen) ratio. Several additional parameters were significantly correlated with $\hat{S}$eq, however these correlations were generally weak ($r<\sim0.5$). There were no strong correlations with colonization rate (G).

## Functional Group Analysis

A summary of the distribution of protozoan species in functional groups is shown in Table 36. Contingency table analysis of these data indicated no significant differences among lakes in functional group structure ($p > 0.995$). All protozoan communities were composed primarily of bacterivores, ranging from 56 to 78% of the total number of species. The next most common functional group was the producers that made up 7 - 34% of the total number of species. In all cases, these two functional groups accounted for the vast majority of species.

Results of analysis of Pinkham and Pearson's (1976) coefficient of similarity are presented in Figure 23. The first lakes to be distinguished as differing in functional group structure were G0B, 1215, and G0A. Of this group, G0A and G0B are newly reclaimed lakes. Lake 1215 had extremely low calcium levels compared to all other lakes studied. A more detailed comparison of the functional group structure of these lakes in comparison with typical lakes in other categories is shown in Figure 24. Lakes of the same age were more similar to each other than lakes of different ages

(Wilcoxon rank sum, z=2.034, p=0.0419). However, the differences were not large. The median coefficient of similarity for same-age pairs was 0.62 and for differing age pairs 0.54. Both GOA and GOB had extremely low species numbers. In contrast, Lake 1215 had the highest species numbers of fakes in the study. This difference in total species number may be the greatest factor separating these lakes from others studied. Distinctions based on total species number are discussed above.

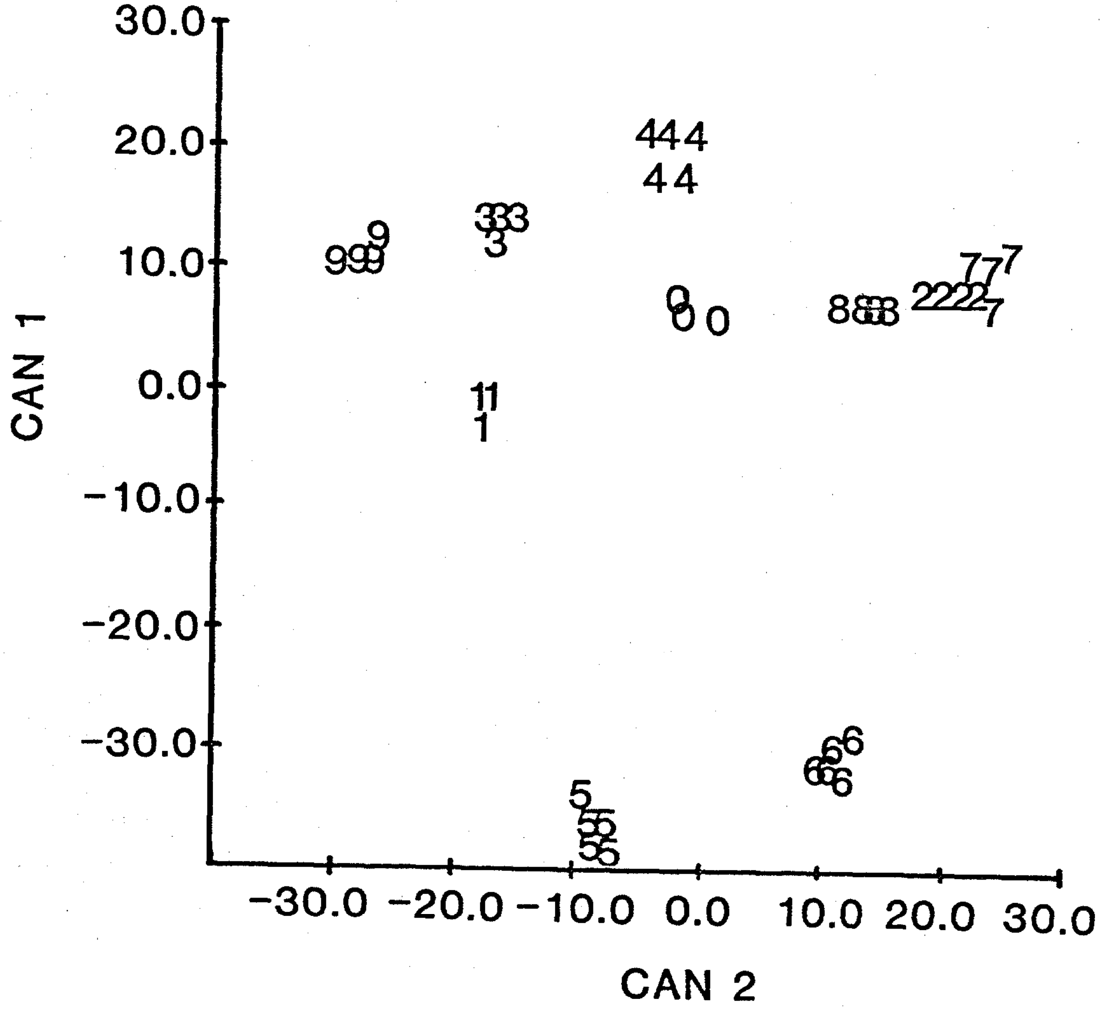


Fig. 3. Discriminant analysis of Florida lakes using 18 important environmental parameters from the March 1984 study period. See Table 1 for number interpretation. This figure is a plot of canonical axis 1 and 2.

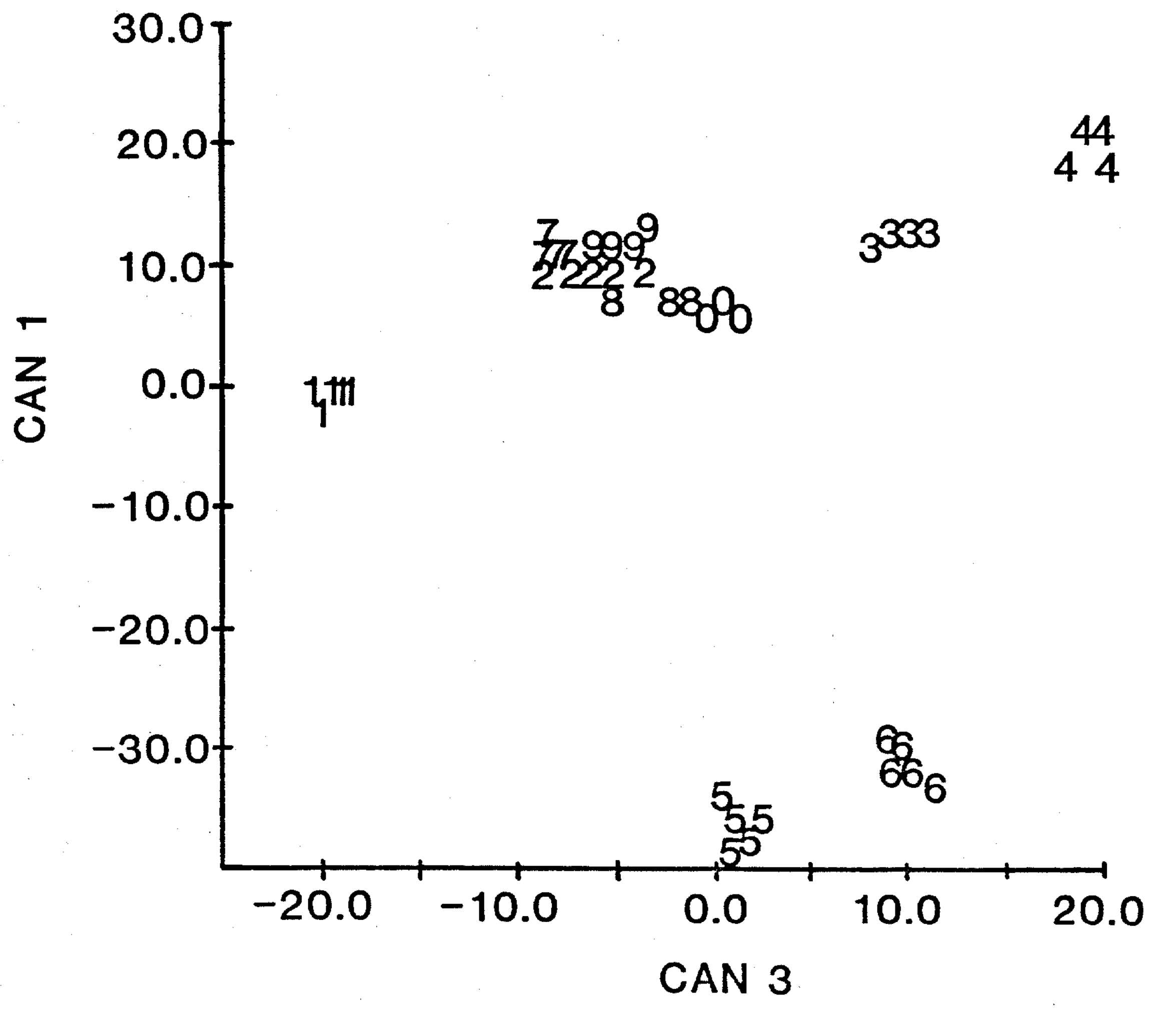


Fig. 4. Discriminant analysis of Florida lakes using 18 important environmental parameters from the March 1984 study period. See Table 1 for number interpretation. This figure is a plot of canonical axis 1 and 3.

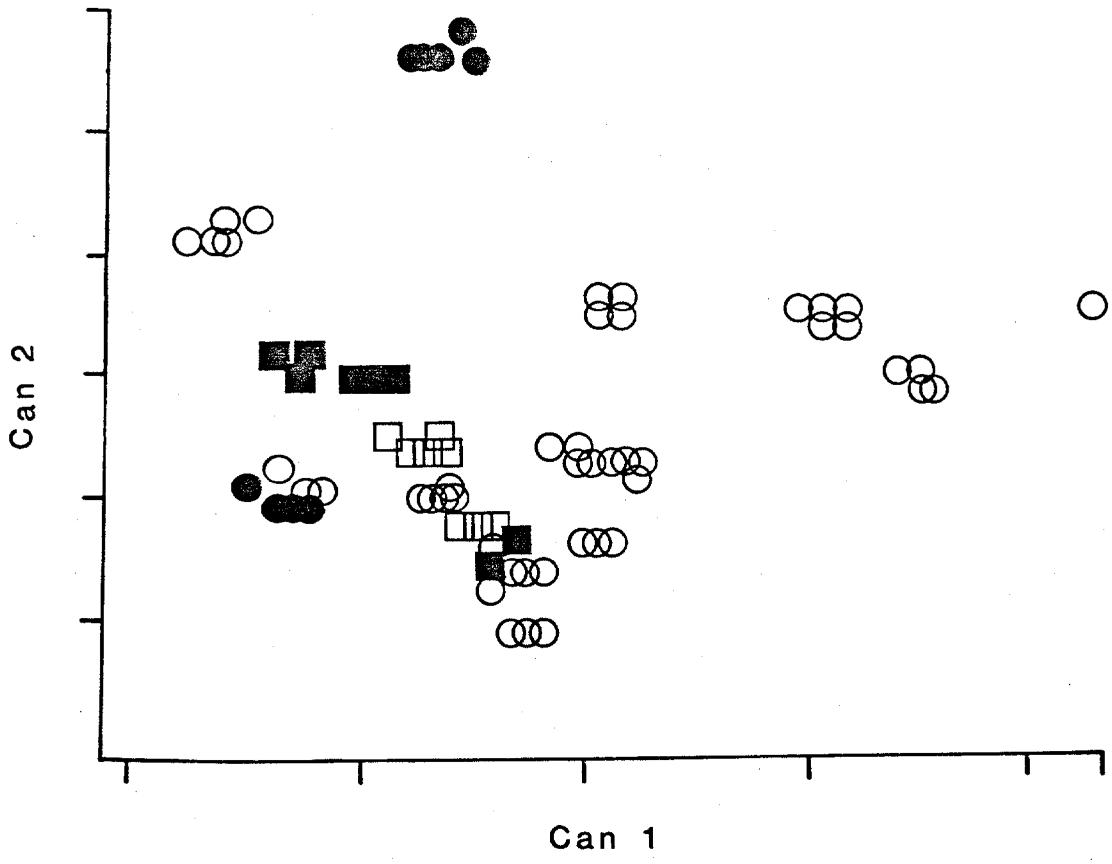


Fig. 5. Canonical variate analysis for all lakes using 15 important environmental parameters from Fall 1984 sampling. Dark circles = active mined pits, open circles = reclaimed lakes, dark squares = natural lakes, open squares = unreclaimed lakes (28 observations hidden).

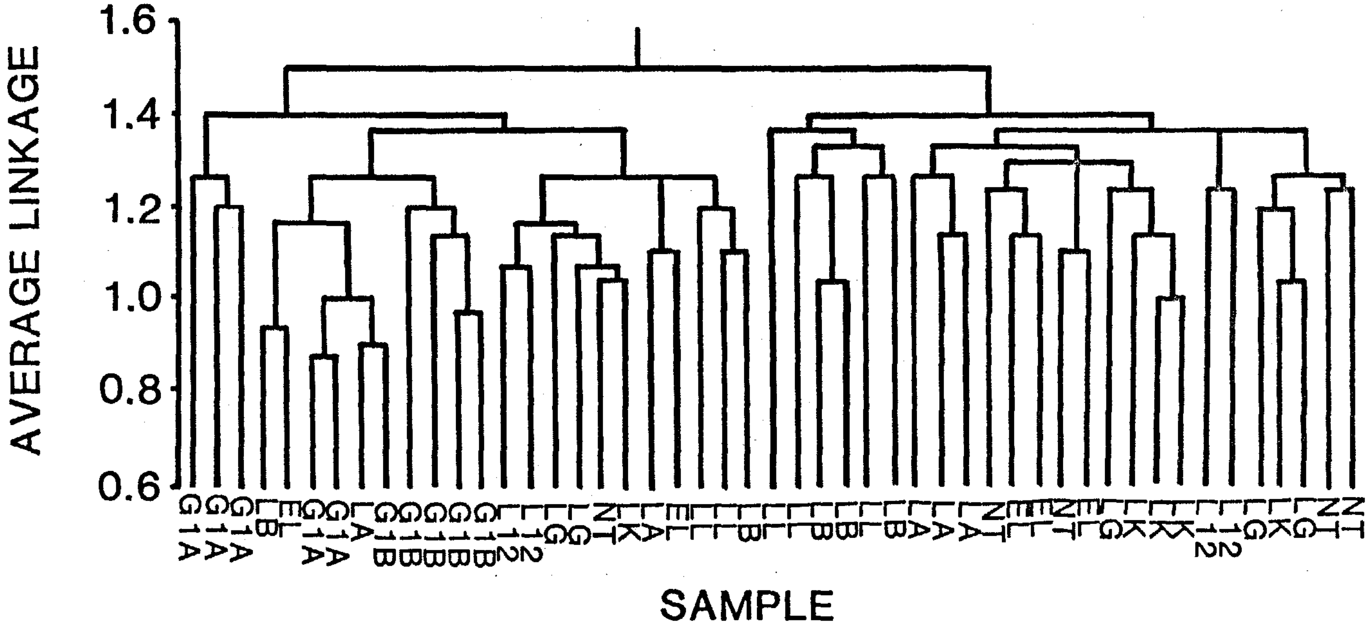


Fig. 6. Dendrogram of cluster analysis of pooled samples using the 96 most occurring species.

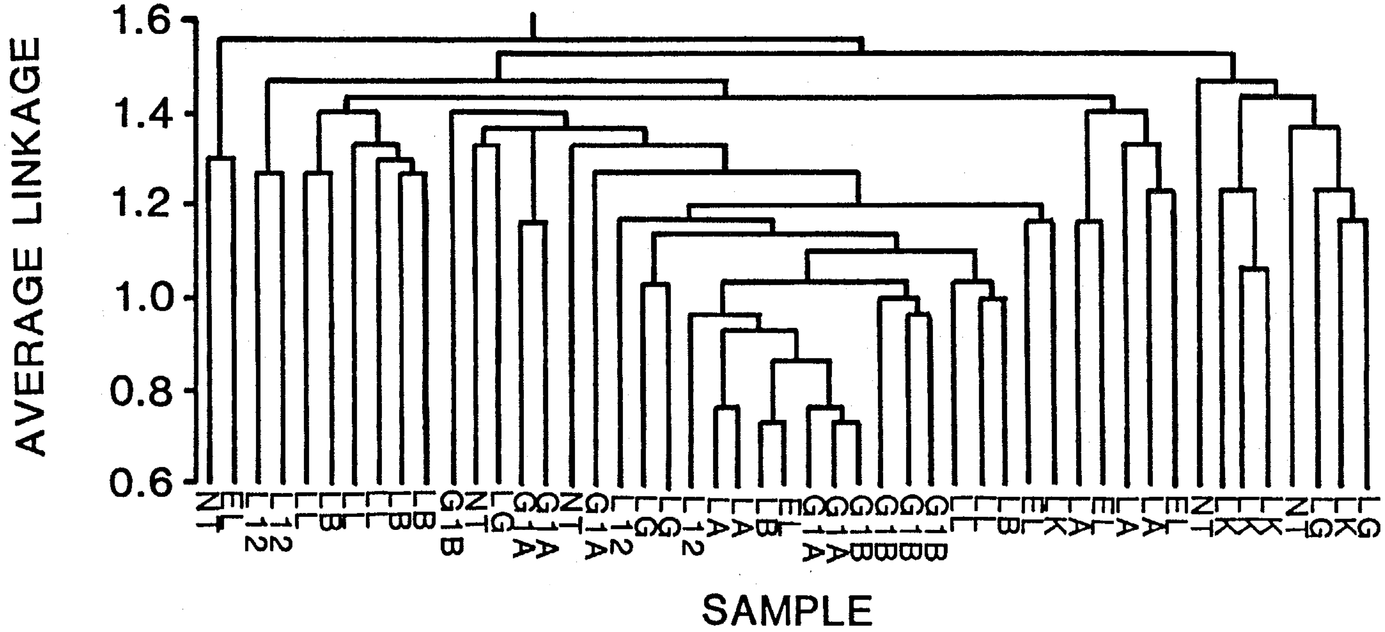


Fig. 7. Dendrogram of cluster analysis of pooled samples using the 100 species that occur in 11 to 24 of the samples.

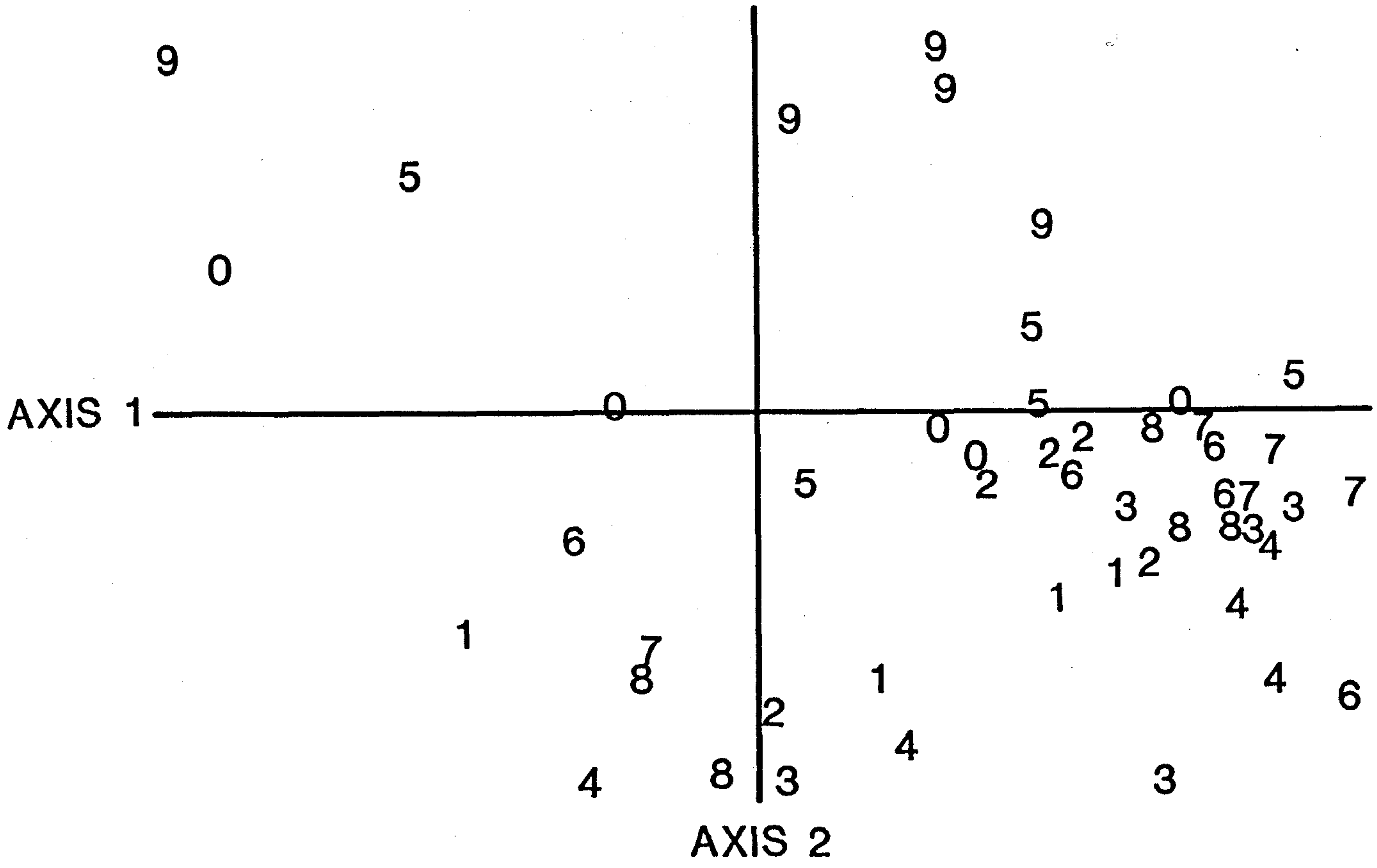


Fig. 8. Reciprocal averaging ordination of protozoan samples (in species space) using presence/absence data. The 96 most occurring species were used in this ordination in 49 pooled samples from the March 1984 study period.

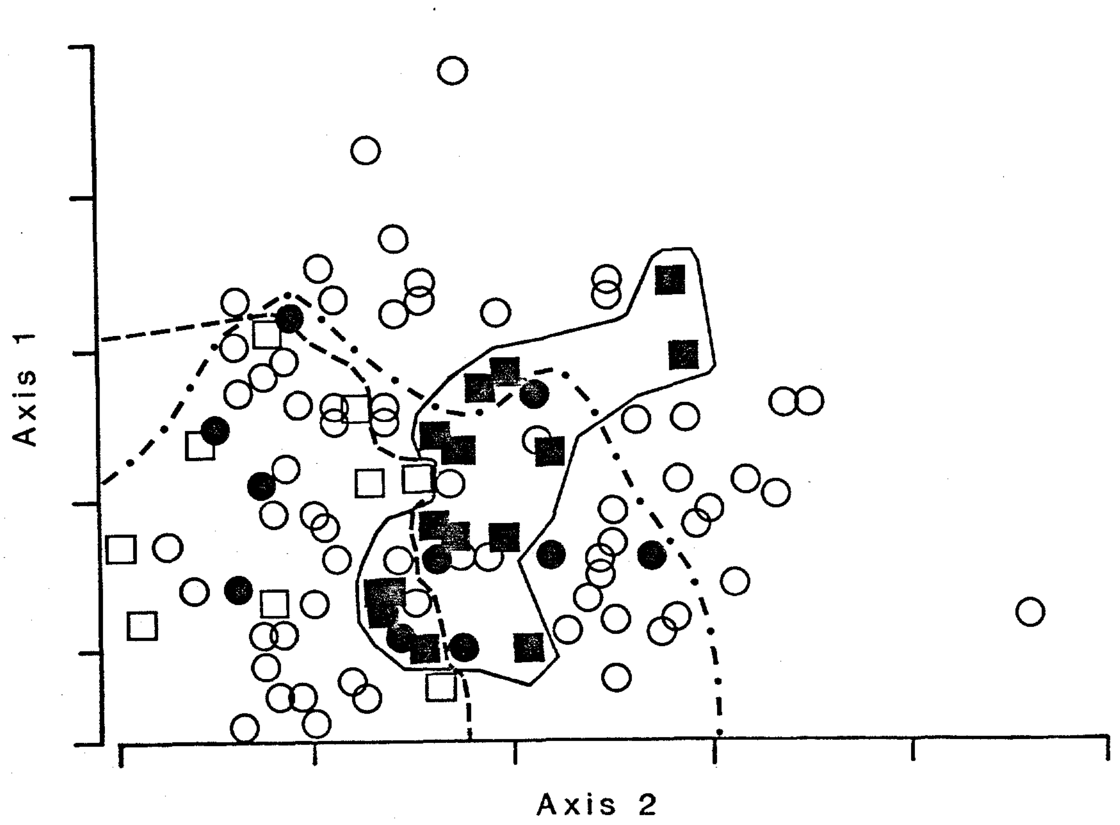


Fig. 9. Detrended correspondence analysis (DCA) for 103 protozoan samples (2 outliers discarded) and 495 species from Fall 1984 sampling. Dark circles = active mined pits, open circles = reclaimed lakes, dark squares = natural lakes, open circles = unreclaimed lakes (4 observation hidden).

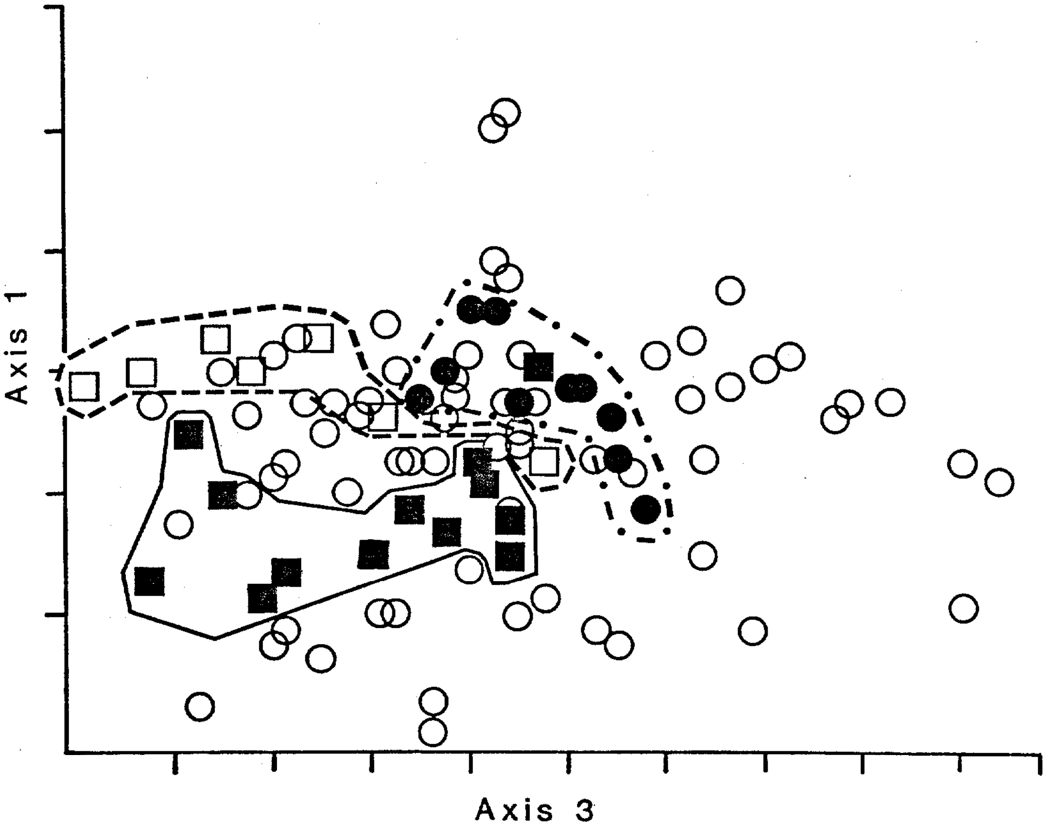


Fig. 10. DCA plots of axis 1 versus axis 3 for all samples using only phytoflagellate species from Fall 1984 sampling period. Dark circles = active mined pits, open circles = reclaimed lakes, dark squares = natural lakes, open squares = unreclaimed lakes (7 observations hidden).

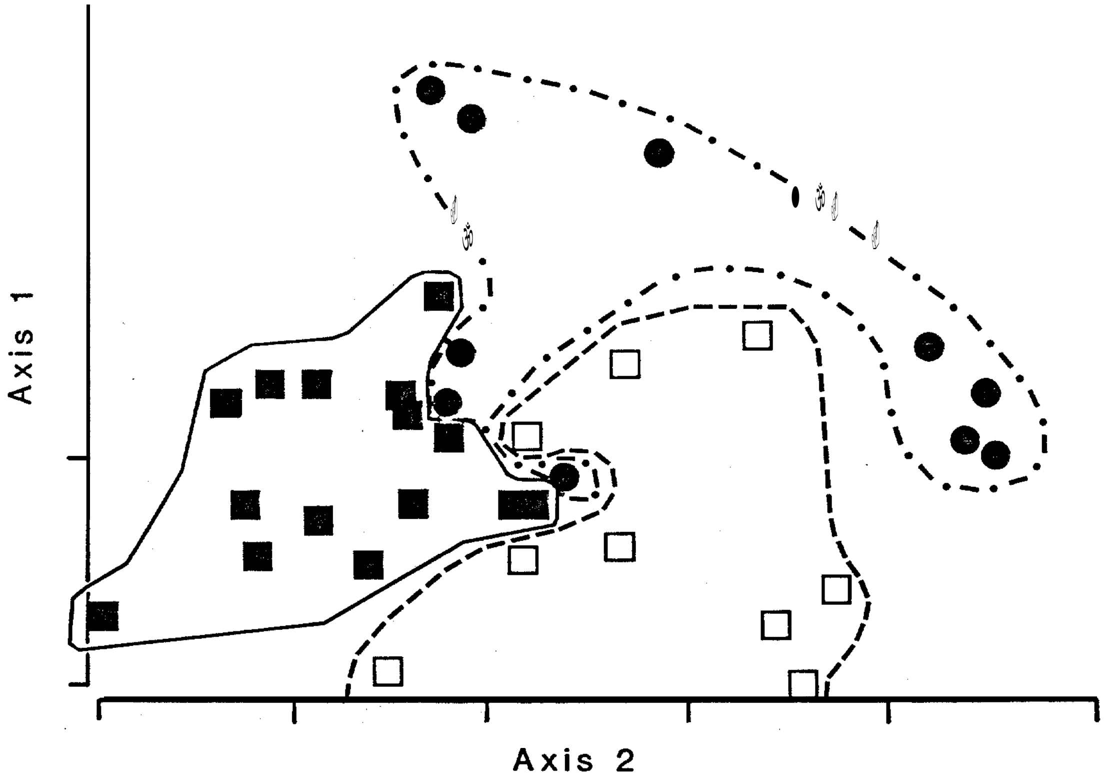


Fig. 11. DCA plots of axis 1 versus axis 2 for Fall survey without reclaimed lakes. Only phytoflagellates were used in this display. Dark circles = active mined pits, dark squares = natural pits, open squares = unreclaimed lakes (1 observation hidden).

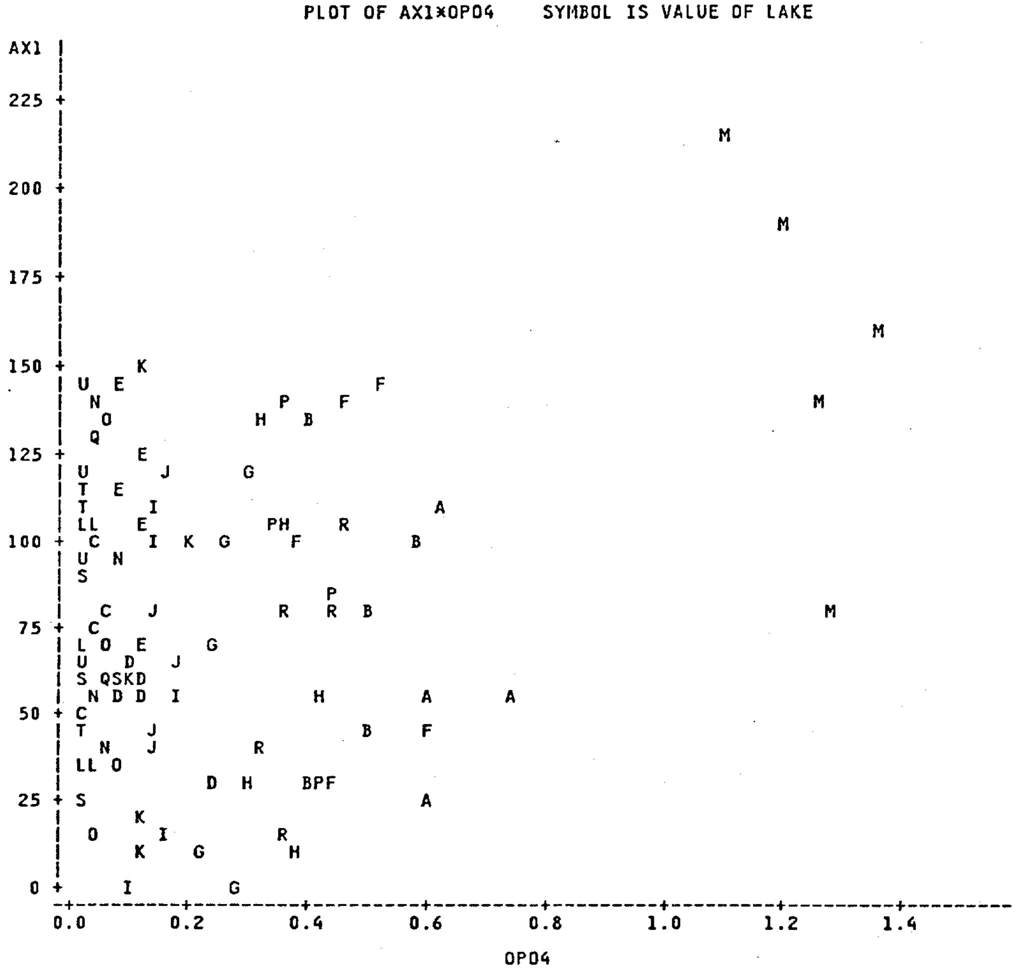


Fig. 12. The relationship of DCA axis 1 and ortho-phosphate in lakes from the Fall 1984 sampling. Letters identify lakes (10 observations hidden).

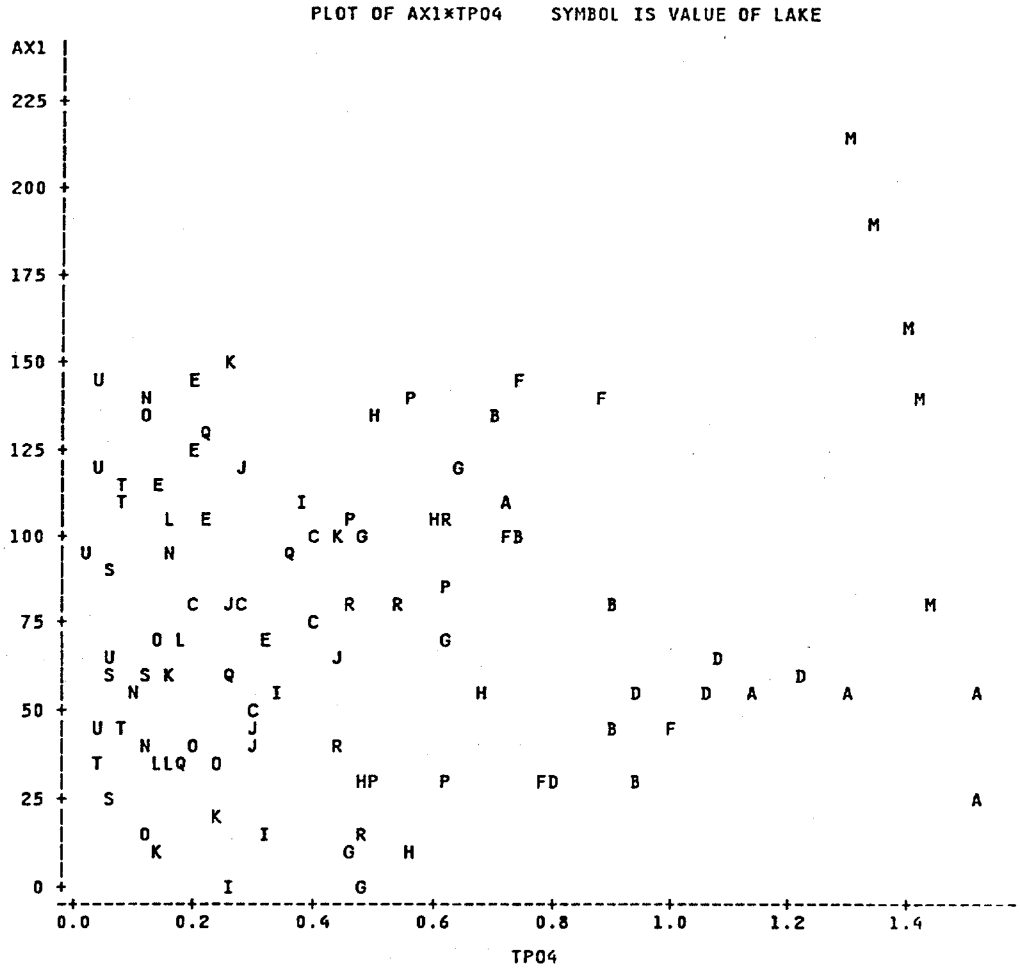


Fig. 13. The relationship of DCA axis 1 and total phosphate in lakes from the Fall 1984 sampling. Letters identify lakes (4 observations hidden).

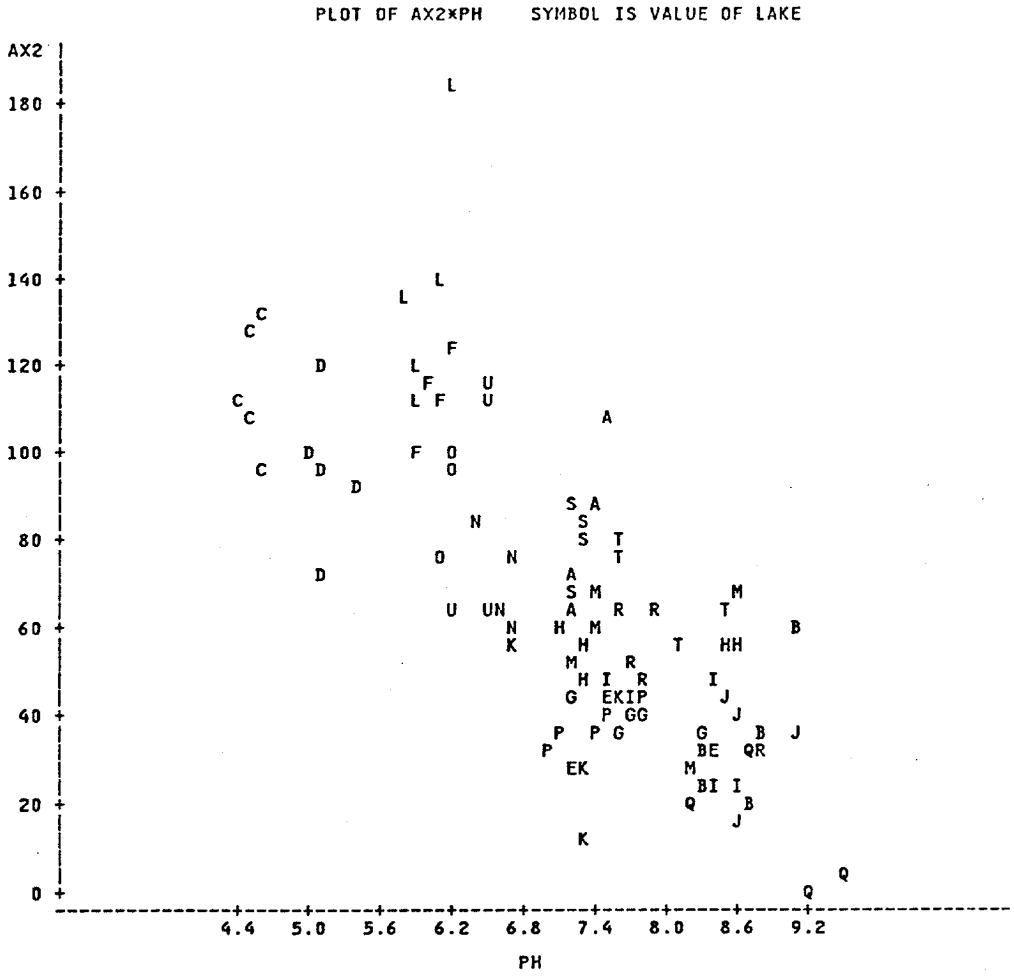


Fig. 14. The relationship of DCA axis 2 and pH in lakes from the Fall 1984 sampling. Letters identify lakes (11 observations hidden).

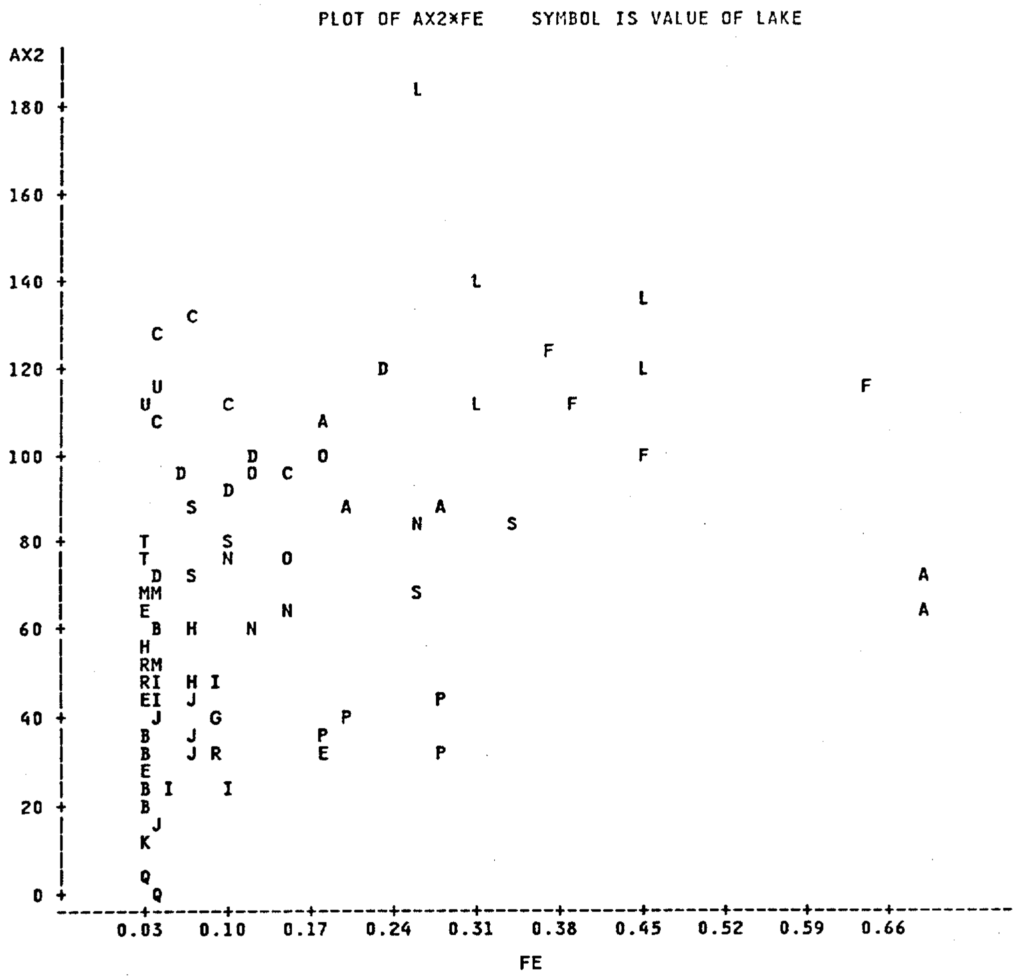


Fig. 15. The relationship of DCA axis 2 and iron in lakes from the Fall 1984 sampling. Letters identify lakes (27 hidden observations).

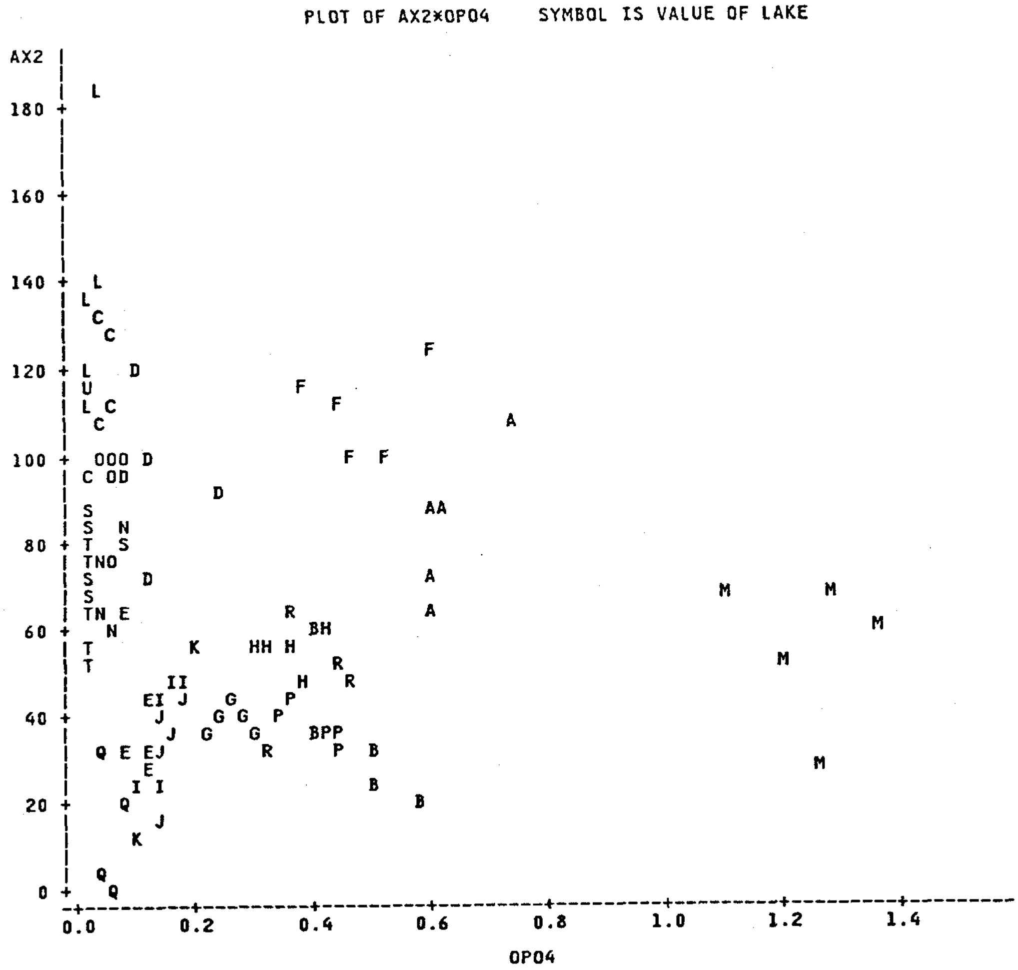


Fig. 16. The relationship of DCA axis 2 and ortho-phosphate in lakes from the Fall 1984 sampling. Letters identify lakes (8 observations hidden).

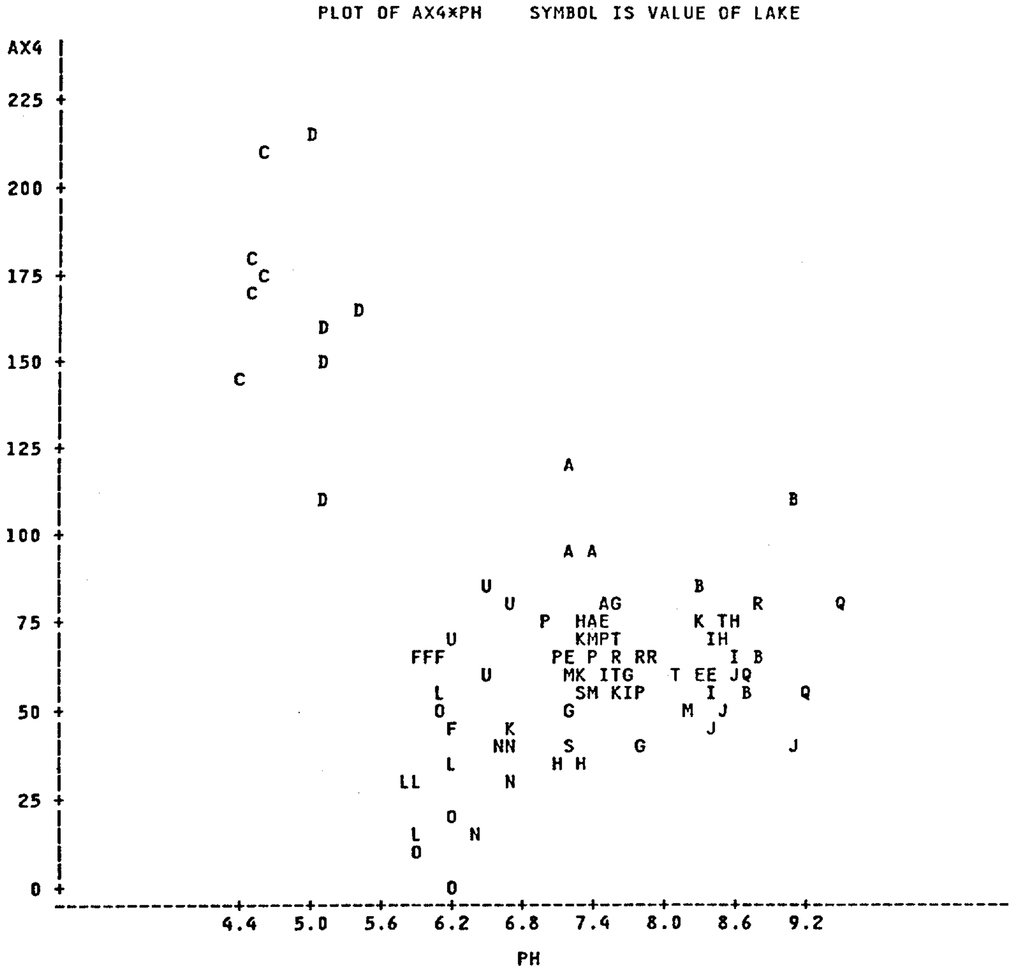


Fig. 17. The relationship of DCA axis 4 and pH in lakes from the Fall 1984 sampling. Letters identify lakes (14 observations hidden).

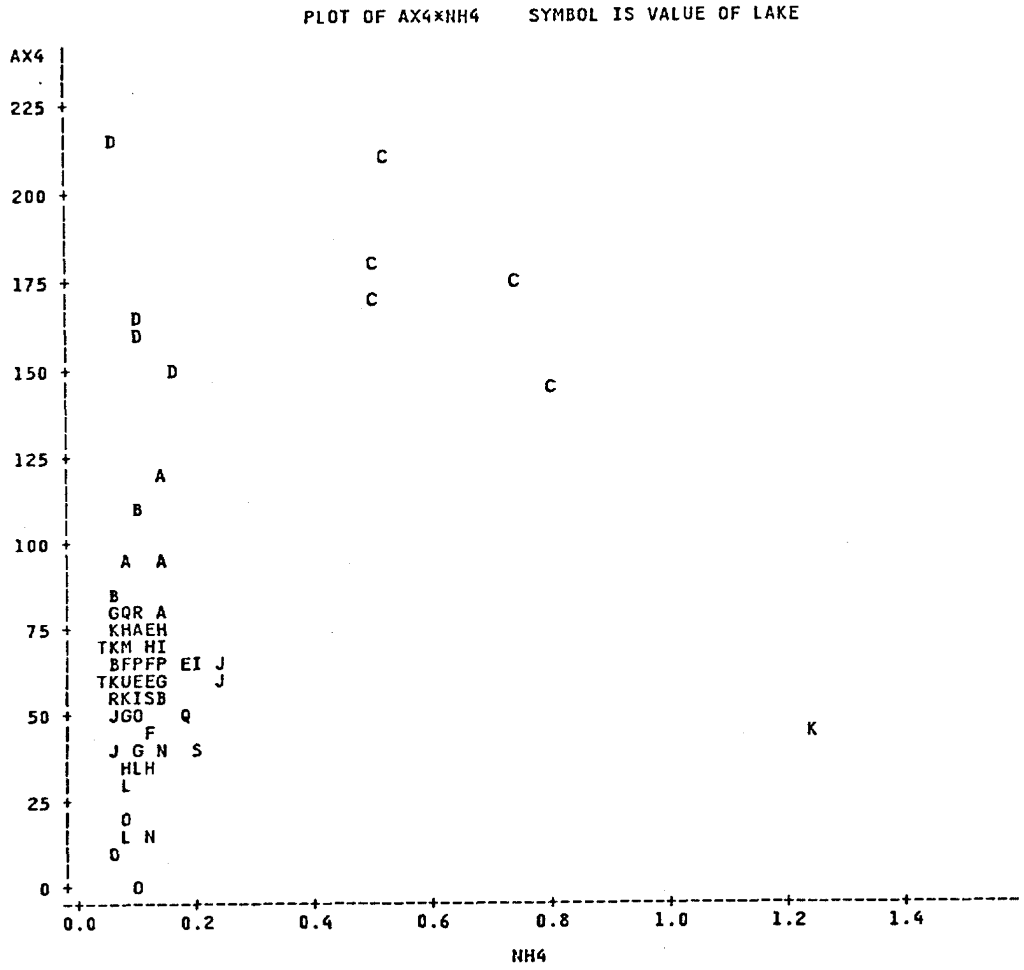


Fig. 18. The relationship of DCA axis 4 and ammonia in lakes from the Fall 1984 sampling. Letters identify lakes (41 observations hidden).

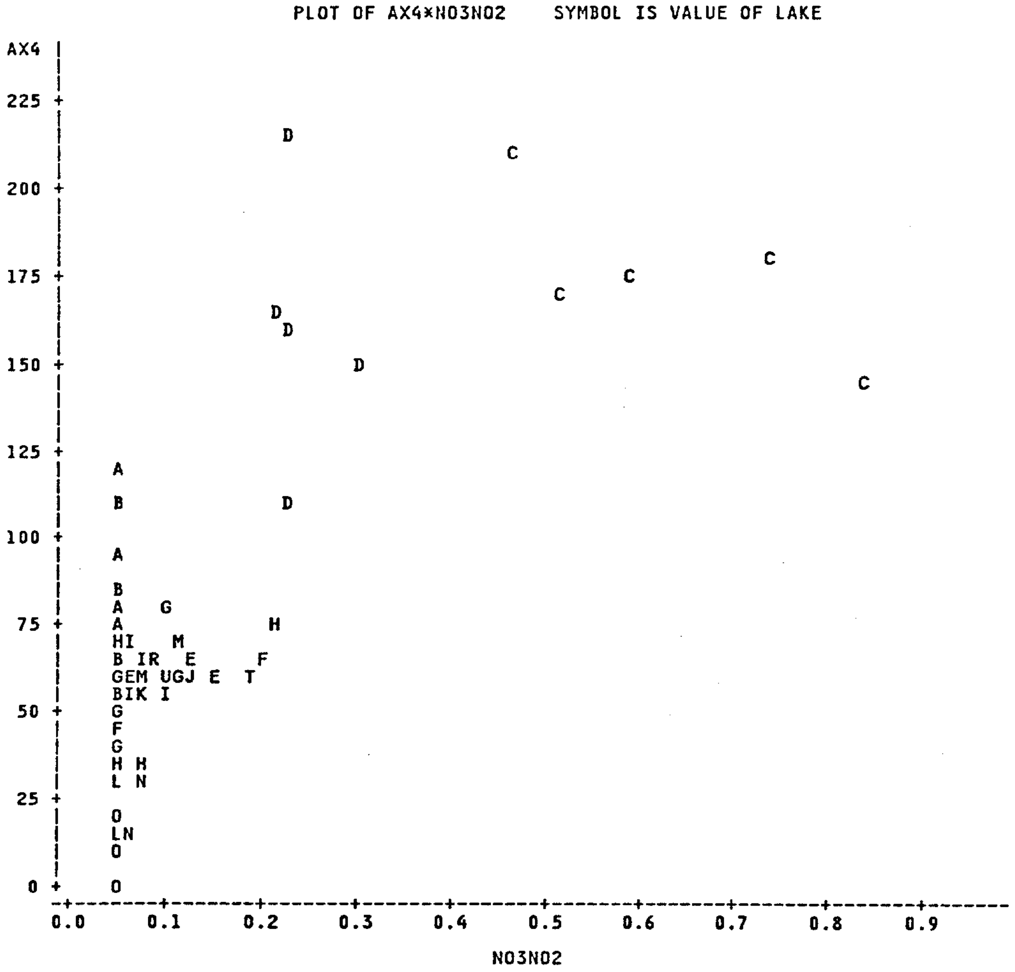


Fig. 19. The relationship of DCA axis 4 and nitrate-nitrite in lakes from the Fall 1984 sampling. Letters identify lakes (53 observations hidden).

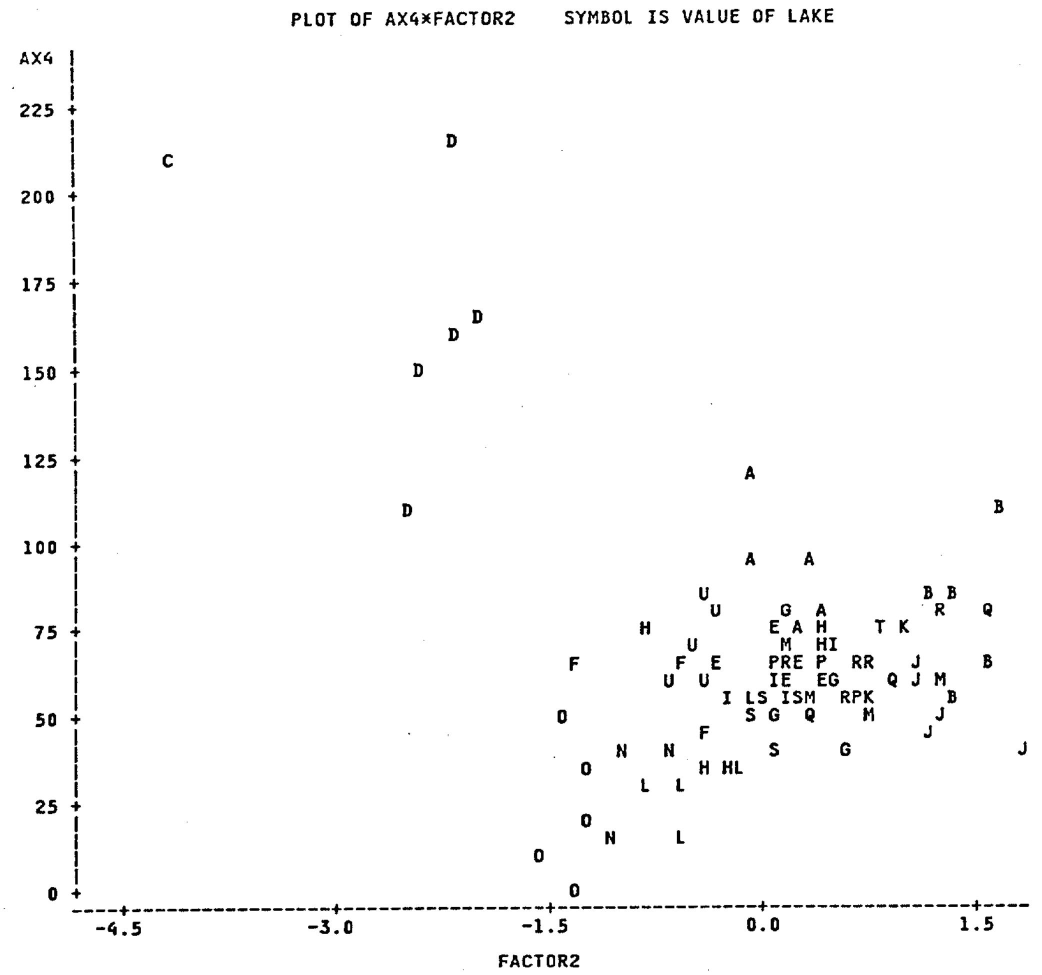


Fig. 20. Relationship between DCA axis 4 and factor 2 from Fall 1984 sampling. Letters identify lakes (see Table 26) (17 observations hidden).

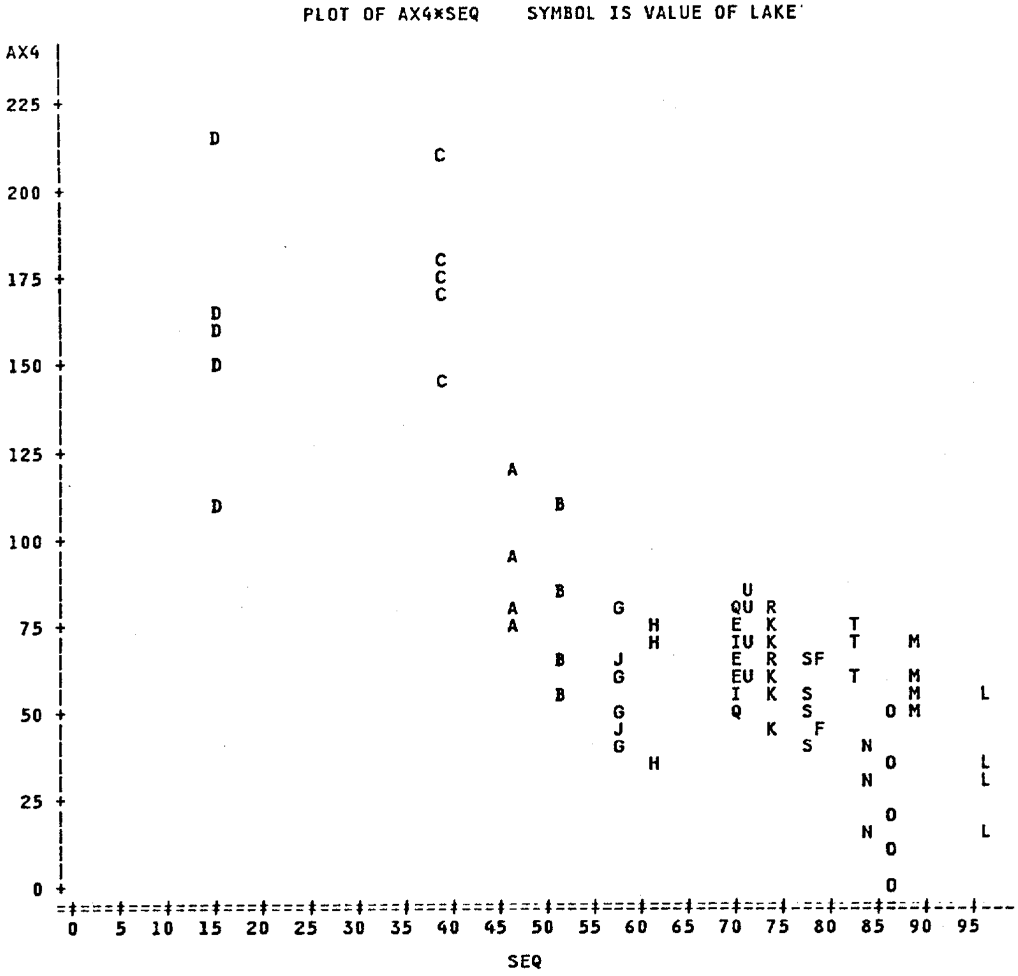


Fig. 21. The relationship of DCA axis 4 and the estimated species equilibrium number for lakes from the Fall 1984 sampling. Letters identify lakes (33 observations hidden).

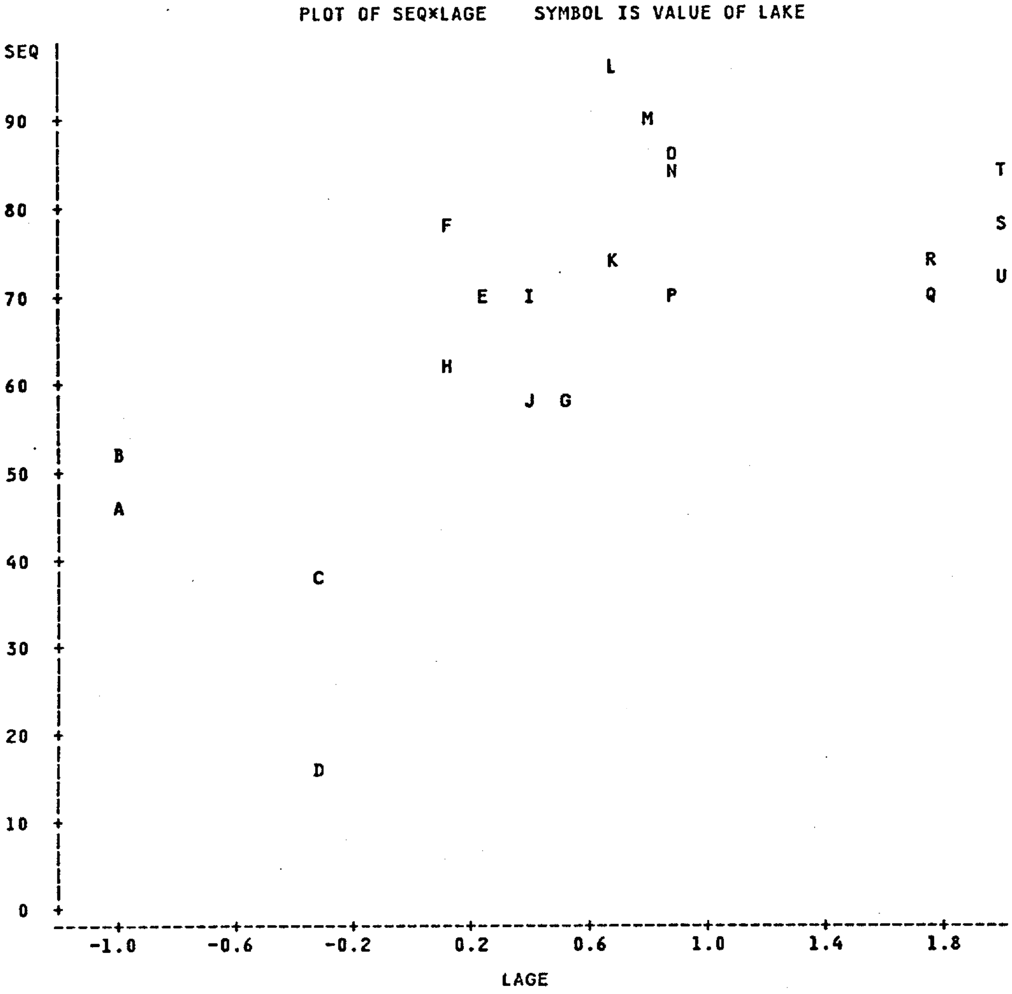


Fig. 22. The relationship of the estimated species equilibrium number to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (0 observations hidden).

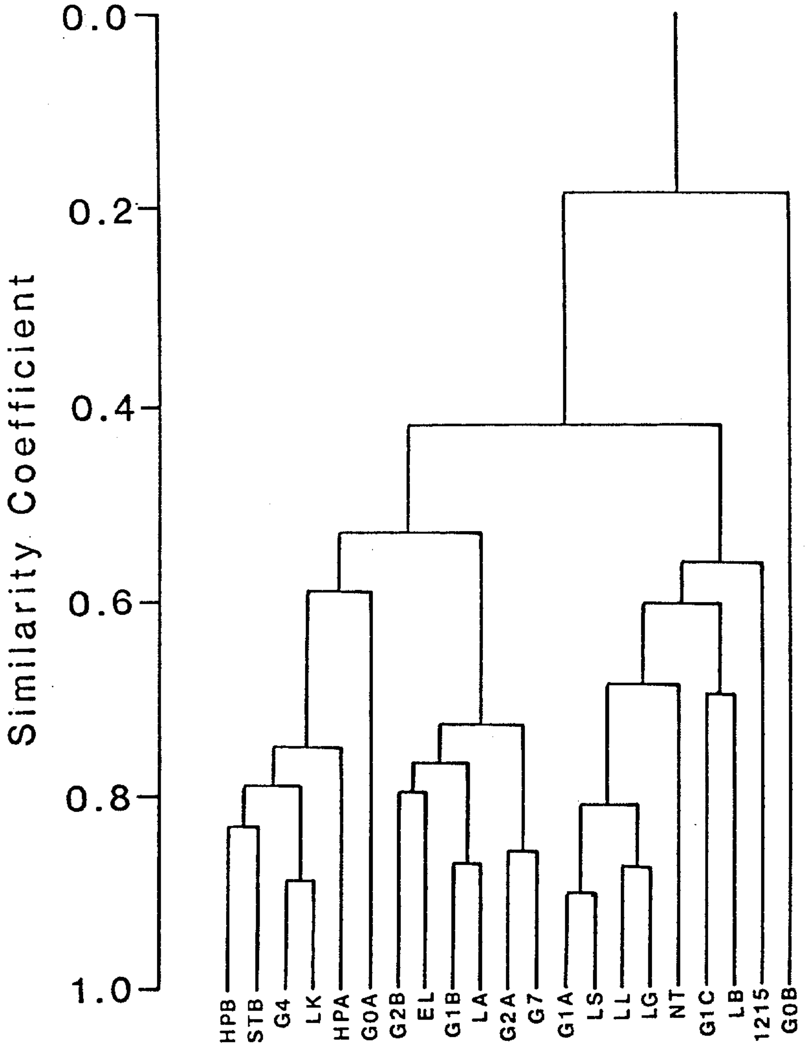


Fig. 23. Dendrogram based on matrix of Pinkham and Pearson similarity coefficients comparing distribution of protozoan species in functional groups among study lakes, Fall 1984.

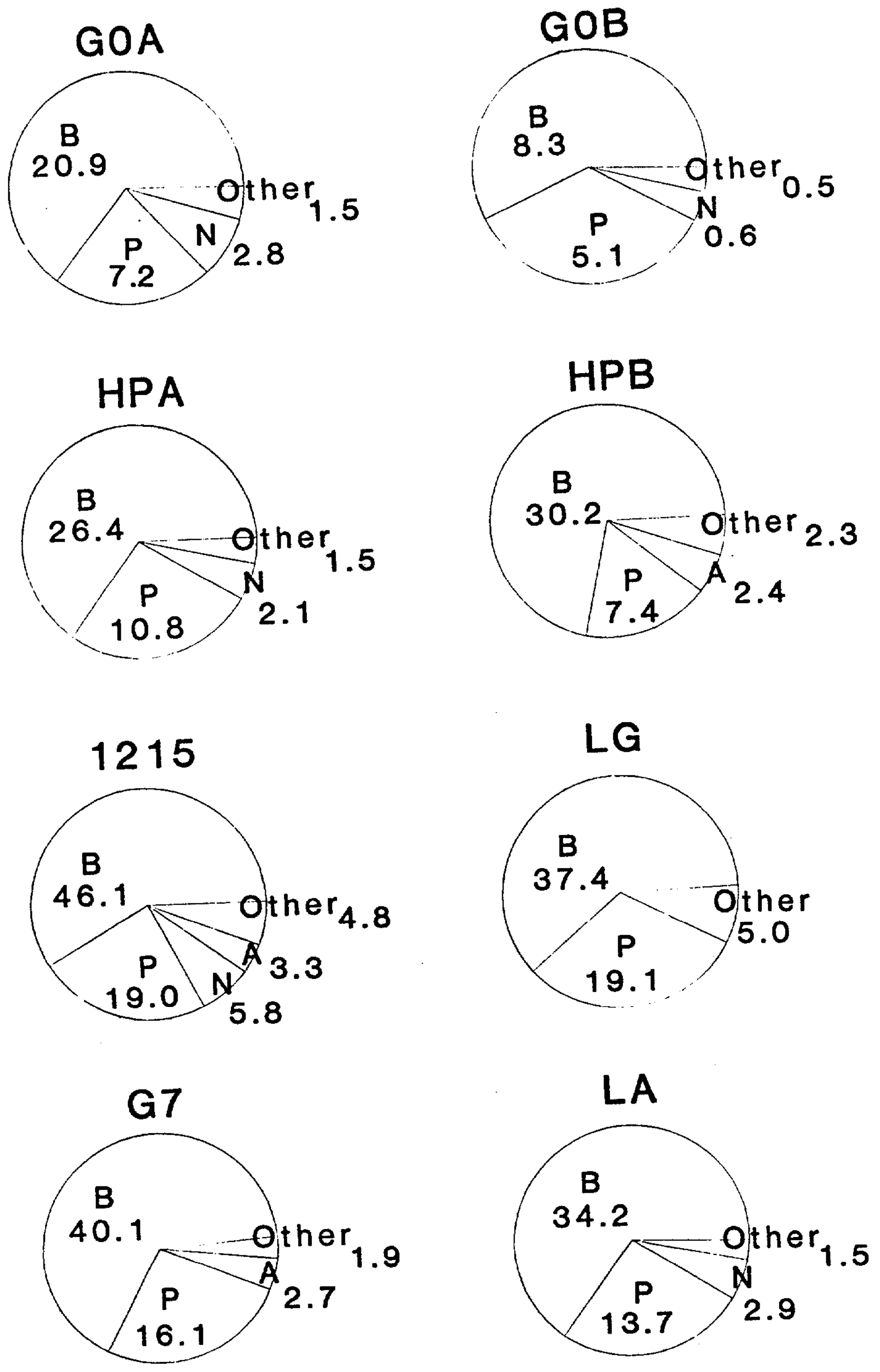


Fig. 24. Distribution of protozoan species into functional groups for selected lakes from Fall 1984 sampling. Numbers represent the average number of species within a functional group for equilibrium samples.

## Table 5

Mean, standard deviation, minimum and maximum values for nitrogen, phosphorus and carbon from Spring 1984 sampling. (Dash means values were below detection limits).

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| All | | | | | |
| $NH_3$(mg/l) | 47 | 0.110 | 0.191 | - | 1.240 |
| $NO_2$(mg/l) | 49 | 0.042 | 0.109 | - | 0.707 |
| $NO_3NO_2$(mg/l) | 49 | 0.145 | 0.316 | - | 2.160 |
| $TPO_4$(mg/l) | 48 | 0.449 | 0.952 | - | 6.130 |
| $OPO_4$(mg/l) | 49 | 0.302 | 0.556 | - | 2.020 |
| TOC(mg/l) | 50 | 8.908 | 3.154 | 3.496 | 14.471 |
| Reclaimed | | | | | |
| $NH_3$ | 28 | 0.120 | 0.236 | - | 1.240 |
| $NO_2$ | 29 | 0.061 | 0.137 | - | 0.707 |
| $NO_3NO_2$ | 29 | 0.174 | 0.404 | - | 2.160 |
| $TPO_4$ | 28 | 0.613 | 1.218 | 0.102 | 6.130 |
| $OPO_4$ | 29 | 0.430 | 0.689 | 0.025 | 2.020 |
| TOC | 30 | 10.292 | 3.019 | 4.617 | 14.471 |
| Unreclaimed | | | | | |
| $NH_3$ | 10 | 0.122 | 0.096 | - | 0.263 |
| $NO_2$ | 10 | 0.004 | 0.006 | - | 0.018 |
| $NO_3NO_2$ | 10 | 0.057 | 0.082 | - | 0.245 |
| $TPO_4$ | 10 | 0.356 | 0.186 | 0.163 | 0.620 |
| $OPO_4$ | 10 | 0.207 | 0.163 | 0.061 | 0.460 |
| TOC | 10 | 7.643 | 0.917 | 6.436 | 8.730 |
| Natural | | | | | |
| $NH_3$ | 9 | 0.066 | 0.091 | - | 0.279 |
| $NO_2$ | 10 | 0.026 | 0.036 | - | 0.105 |
| $NO_3NO_2$ | 10 | 0.149 | 0.817 | 0.008 | 0.221 |
| $TPO_4$ | 10 | 0.081 | 0.064 | - | 0.163 |
| $OPO_4$ | 10 | 0.028 | 0.026 | - | 0.064 |
| TOC | 10 | 6.020 | 2.558 | 3.496 | 9.144 |

# Table 6

Mean, standard deviation, minimum and maximum values for parameters measured during Spring 1984 sampling. (Dash means values were below detection limits).

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| ALL | | | | | |
| Age(yrs) | 40 | 18.075 | 24.602 | 1.000 | 60.000 |
| Air temp($^oC$) | 49 | 21.014 | 3.711 | 13.500 | 29.000 |
| Wa temp($^oC$) | 50 | 21.140 | 1.114 | 19.100 | 24.800 |
| pH | 50 | 7.484 | 0.953 | 6.200 | 9.600 |
| Alk(mg $CaCO_3$/l) | 50 | 33.674 | 25.478 | 3.500 | 85.800 |
| Hard(mg $CaCO_3$/l) | 50 | 87.660 | 39.461 | 12.000 | 165.000 |
| $DO_2$(mg/l) | 50 | 8.530 | 1.639 | 4.600 | 13.100 |
| Con(umhos CM) | 50 | 245.800 | 121.640 | 48.000 | 482.000 |
| $SO_4$(mg/l) | 49 | 76.666 | 61.433 | 6.190 | 208.000 |
| COD (mg/l) | 48 | 5.198 | 2.190 | 1.660 | 11.100 |
| Ca(mg/l) | 50 | 21.747 | 13.194 | 2.900 | 67.100 |
| Mg(mg/l) | 50 | 7.412 | 3.793 | 0.415 | 13.800 |
| K(mg/l) | 50 | 2.271 | 2.568 | 0.127 | 8.100 |
| TSS(ug/l) | 47 | 0.144 | 0.094 | 0.029 | 0.328 |
| Al(ug/l) | 50 | 1115.425 | 3517.058 | 0.173 | 20575.000 |
| As(ug/l) | 58 | 4.417 | 3.247 | - | 10.200 |
| Fe(mg/l) | 50 | 0.163 | 0.187 | - | 0.666 |
| Mn(mg/l) | 50 | 0.029 | 0.021 | - | 0.077 |
| Hg(ug/l) | 47 | 1.077 | 1.804 | - | 10.000 |
| Zn(mg/l) | 50 | 0.171 | 0.350 | - | 1.840 |
| Se(ug/l) | 50 | 32.740 | 12.805 | 13.200 | 39.800 |
| RECLAIMED | | | | | |
| Age | 30 | 4.100 | 2.426 | 1.000 | 7.000 |
| Air temp | 29 | 20.834 | 4.081 | 13.500 | 29.000 |
| Wat temp | 30 | 21.046 | 1.254 | 19.100 | 24.800 |
| pH | 30 | 7.420 | 1.031 | 6.200 | 9.800 |
| Alk | 30 | 28.556 | 24.463 | 6.200 | 85.800 |
| Hard | 30 | 91.450 | 49.605 | 12.000 | 165.000 |
| $DO_2$ | 30 | 8.410 | 1.899 | 4.600 | 13.100 |
| Con | 30 | 265.100 | 154.232 | 48.000 | 482.000 |
| $SO_4$ | 30 | 98.572 | 66.428 | 6.980 | 208.000 |
| COD | 28 | 5.08 | 2.281 | 1.660 | 11.100 |
| Ca | 30 | 23.428 | 13.974 | 2.900 | 67.100 |
| Mg | 30 | 6.995 | 4.446 | 0.415 | 13.800 |
| K | 30 | 1.029 | 0.521 | 0.127 | 1.990 |
| TSS | 27 | 0.159 | 0.110 | 0.029 | 0.328 |
| Al | 30 | 1756.574 | 4453.322 | 0.173 | 20575.000 |
| As | 28 | 4.207 | 3.795 | - | 10.200 |
| Fe | 30 | 0.233 | 0.197 | - | 0.866 |
| Mn | 30 | 0.029 | 0.217 | 0.002 | 0.077 |

Table 6. (con't)

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| Hg | 27 | 0.839 | 1.3371 | 0.0 | 5.950 |
| Zn | 30 | 0.106 | 0.228 | - | 1.810 |
| Se | 30 | 38.74 | 12.354 | 16.400 | 59.800 |
| UNRECLAIMED | | | | | |
| Age | 10 | 60.000 | 0.00 | 60.000 | 60.000 |
| Air temp | 10 | 21.350 | 3.916 | 15.000 | 25.500 |
| Wa temp | 10 | 21.380 | 0.862 | 20.000 | 23.000 |
| pH | 10 | 8.180 | 0.863 | 7.000 | 9.200 |
| Alk | 10 | 62.760 | 9.499 | 51.200 | 75.000 |
| Hard | 10 | 91.900 | 2.078 | 90.000 | 95.000 |
| $DO_2$ | 10 | 8.980 | 1.393 | 7.400 | 11.100 |
| Con | 10 | 207.500 | 5.911 | 199.000 | 219.000 |
| $SO_4$ | 9 | 27.262 | 21.597 | 6.190 | 66.900 |
| COD | 10 | 5.079 | 1.914 | 1.840 | 7.880 |
| Ca | 10 | 28.260 | 8.247 | 17.800 | 48.200 |
| Mg | 10 | 7.430 | 3.064 | 4.100 | 13.500 |
| K | 10 | 1.019 | 0.375 | 0.650 | 1.460 |
| TSS | 10 | 0.125 | 0.065 | 0.034 | 0.242 |
| Al | 10 | 193.110 | 75.414 | 47.00 | 313.000 |
| As | 10 | 4.895 | 1.612 | 3.280 | 7.370 |
| Fe | 10 | 0.082 | 0.146 | - | 0.487 |
| Mn | 10 | 0.018 | 0.011 | 0.002 | 0.032 |
| Hg | 10 | 1.188 | 1.397 | - | 4.390 |
| Zn | 10 | 0.231 | 0.572 | - | 1.840 |
| Se | 10 | 22.670 | 8.304 | 13.200 | 40.800 |
| NATURAL | | | | | |
| Air temp | 10 | 21.200 | 2.455 | 16.500 | 25.000 |
| Wa temp | 10 | 21.180 | 0.924 | 20.000 | 22.200 |
| pH | 10 | 6.980 | 0.608 | 6.300 | 7.800 |
| Alk | 10 | 19.940 | 16.694 | 3.500 | 36.500 |
| Hard | 10 | 72.050 | 14.365 | 57.500 | 89.000 |
| $DO_2$ | 10 | 8.440 | 0.885 | 7.000 | 10.000 |
| Con | 10 | 226.200 | 22.034 | 199.000 | 249.000 |
| $SO_4$ | 10 | 55.411 | 30.856 | 9.510 | 102.300 |
| COD | 10 | 4.451 | 2.201 | 1.720 | 7.140 |
| Ca | 10 | 10.191 | 6.760 | 3.560 | 19.000 |
| Mg | 10 | 8.638 | 1.735 | 5.860 | 10.300 |
| K | 10 | 7.251 | 0.689 | 6.180 | 8.100 |
| TSS | 10 | 0.124 | 0.065 | 0.0345 | 0.221 |
| Al | 10 | 114.294 | 85.877 | 7.340 | 249.300 |
| As | 10 | 4.515 | 2.973 | 0.0640 | 7.870 |
| Fe | 10 | 0.033 | 0.027 | - | 0.089 |
| Mn | 10 | 0.039 | 0.024 | - | 0.077 |
| Hg | 10 | 1.609 | 3.003 | - | 10.000 |
| Zn | 10 | 0.306 | 0.366 | 0.009 | 1.190 |
| Se | 10 | 24.790 | 5.366 | 17.400 | 35.000 |

## Table 7

Stepwise analysis results of physico-chemical variables and nutrient values for Spring 1984 sampling using 18 environmental parameters.

| Step | Variable Entered | Number In | Partial $r^2$ | F Statistic | Prob > F |
|---|---|---|---|---|---|
| 1 | K | 1 | 0.99 | 788.364 | 0.0001 |
| 2 | HARD | 2 | 0.99 | 426.713 | 0.0001 |
| 3 | ALK* | 3 | 0.98 | 320.786 | 0.0001 |
| 4 | TOC | 4 | 0.97 | 134.908 | 0.0001 |
| 5 | As | 5 | 0.94 | 57.479 | 0.0001 |
| 6 | COND | 6 | 0.92 | 41.644 | 0.0001 |
| 7 | Se | 7 | 0.50 | 3.164 | 0.0092 |
| 8 | Fe | 8 | 0.49 | 2.893 | 0.0157 |
| 9 | $DO_2$ | 9 | 0.50 | 2.913 | 0.0159 |
| 10 | $SO_4$ | 10 | 0.40 | 1.913 | 0.0968 |
| 11 | Mg | 11 | 0.40 | 1.913 | 0.0968 |
| 12 | $OPO_4$ | 12 | 0.42 | 1.864 | 0.1100 |
| 13 | $DO_2$ | 11 | 0.39 | 1.664 | 0.1556 |
| 14 | COD | 12 | 0.40 | 1.732 | 0.1385 |

*Falls below 0.10 tolerance after this point.

Table 8

Eighteen parameters that are important for separating lakes in the Spring 1984 sampling.

| Parameters of Major Interest | |
|---|---|
| Potassium | Total Organic Carbon |
| Alkalinity | Calcium |
| Conductivity | Magnesium |
| Hardness | Total Suspended Solids |
| pH | Aluminum |
| Dissolved Oxygen | Arsenic |
| Total Phosphate | Iron |
| Ortho-Phosphate | Manganese |
| Sulfate | Selenium |

## Table 9

Canonical variate analysis for all lakes using 18 important environmental parameters from the Spring 1984 sampling.

| | CAN1 | CAN2 | CAN3 | CAN4 |
|---|---|---|---|---|
| pH | 0.0188 | 0.0676 | -0.0394 | -0.0142 |
| ALK | 0.1666 | 0.5051 | -0.609 | 0.0513 |
| HARD | 0.2023 | 0.2370 | 0.5969 | -0.2305 |
| $DO_2$ | -0.0073 | 0.0057 | -0.0575 | -0.0056 |
| CON | 0.1177 | 0.0895 | 0.4984 | -0.2895 |
| $TPO_4$ | 0.0125 | -0.0100 | -0.0153 | 0.0511 |
| $OPO_4$ | 0.0183 | 0.0070 | -0.0248 | 0.0354 |
| $SO_4$ | 0.0386 | -0.0214 | 0.1345 | -0.0620 |
| TOC | 0.2026 | -0.0410 | 0.2291 | 0.3246 |
| Ca | 0.0429 | 0.0300 | 0.0434 | -0.0132 |
| Mg | 0.0013 | 0.0716 | 0.1475 | -0.0786 |
| K | -0.6110 | 0.0974 | 0.4423 | 0.1623 |
| TSS | 0.0152 | 0.0144 | 0.0484 | -0.0075 |
| Al | 0.0136 | -0.0397 | -0.0155 | 0.1146 |
| As | 0.0608 | -0.0525 | 0.3546 | 0.3484 |
| Fe | 0.0318 | -0.0597 | -0.0067 | 0.0233 |
| Mn | -0.0048 | -0.0183 | 0.1021 | 0.0335 |
| Se | 0.0188 | -0.0358 | -0.0981 | -0.0388 |

## Table 10

Identificiation of lake site, code, and sumbols for Spring 1984 sampling.

| Site | Code | Symbol |
|---|---|---|
| WRGBLSP2 | G1A | 0 |
| Lake 1215 | L12 | 1 |
| North Triangle | NT | 2 |
| Lake Law | LL | 3 |
| Lake Brown | LB | 4 |
| Lake Arietta | LA | 5 |
| Eagle Lake | EL | 6 |
| Lake Christina | LK | 7 |
| Long Lake | LG | 8 |
| WRGBLSP6 | G1B | 9 |

## Table 11

Factor analysis with varimax rotation of 18 important environmental parameters for all lakes from Spring 1984 sampling.

| | ROTATED FACTOR PATTERN | | |
|---|---|---|---|
| | FACTOR1 | FACTOR2 | FACTOR3 |
| pH | -0.00065 | -0.18858 | 0.83975 |
| ALK | 0.37607 | 0.07799 | 0.80835 |
| HARD | 0.93906 | -0.12030 | 0.21497 |
| $DO_2$ | -0.54679 | -0.16279 | 0.37241 |
| COND | 0.91616 | -0.11212 | =0.00907 |
| $TPO_4$ | 0.02590 | 0.87823 | -0.09149 |
| $OPO_4$ | 0.15570 | 0.90373 | 0.20312 |
| $SO_4$ | 0.80483 | 0.00380 | -0.23079 |
| TOC | 0.67578 | 0.46797 | -0.16813 |
| Ca | 0.67519 | -0.20304 | 0.29222 |
| Mg | 0.70649 | -0.09549 | 0.21785 |
| K | -0.06169 | -0.21840 | -0.27477 |
| TSS | 0.67621 | 0.09564 | 0.10750 |
| Al | -0.09056 | 0.74905 | -0.38637 |
| As | 0.62568 | -0.11269 | -0.56896 |
| Fe | -0.01296 | 0.20875 | -0.53130 |
| Mn | 0.50256 | -0.30701 | -0.57988 |
| Se | -0.32977 | 0.62167 | 0.10742 |
| | VARIANCE EXPLAINED BY EACH FACTOR | | |
| | 5.468140 | 3.15026 | 2.99774 |
| Variability explained(%) | 30.3 | 17.5 | 16.6 |
| Cumulative explained(%) | 30.3 | 47.8 | 64.5 |

## Table 12

Nutrient summary statistics for all lakes sampled in Fall 1984.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| $NH_3$ (mg/l) | 105 | 0.136 | 0.161 | 0.049 | 1.238 |
| $NO_3$-$NO_2$ (mg/l) | 105 | 0.099 | 0.132 | 0.050 | 0.841 |
| $TPO_4$ (mg/l) | 105 | 0.466 | 0.394 | 0.025 | 1.527 |
| $OPO_4$ (mg/l) | 105 | 0.248 | 0.290 | 0.025 | 1.368 |
| TOC (mg/l) | 105 | 8.666 | 2.575 | 3.040 | 16.010 |
| TOTN | 105 | 0.234 | 0.265 | 0.099 | 1.634 |
| NTOP | 105 | 3.665 | 6.786 | 0.097 | 39.680 |
| CTON | 105 | 54.150 | 26.526 | 3.733 | 121.386 |

Table 13

Nutrient summary statistics for natural lakes sampled in Fall 1984.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| $NH_3$ | 15 | 0.096 | 0.045 | 0.049 | 0.192 |
| $NO_3$-$NO_2$ | 15 | 0.063 | 0.037 | 0.050 | 0.188 |
| $TPO_4$ | 15 | 0.060 | 0.022 | 0.025 | 0.116 |
| $OPO_4$ | 15 | 0.029 | 0.016 | 0.025 | 0.089 |
| TOC | 15 | 7.547 | 2.783 | 3.220 | 10.410 |
| TOTN | 15 | 0.160 | 0.063 | 0.099 | 0.319 |
| NTOP | 15 | 6.169 | 2.826 | 1.382 | 12.760 |
| CTON | 15 | 52.702 | 27.933 | 17.219 | 105.151 |

## Table 14

Nutrient summary statistics for active mine pits sampled in Fall 1984.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| $NH_3$ | 10 | 0.098 | 0.033 | 0.050 | 0.139 |
| $NO_3$-$NO_2$ | 10 | 0.050 | 0.000 | 0.050 | 0.050 |
| $TPO_4$ | 10 | 1.038 | 0.320 | 0.693 | 1.527 |
| $OPO_4$ | 10 | 0.550 | 0.105 | 0.392 | 0.732 |
| TOC | 10 | 5.156 | 2.136 | 3.040 | 7.300 |
| TOTN | 10 | 0.148 | 0.033 | 0.100 | 0.189 |
| NTOP | 10 | 0.272 | 0.051 | 0.199 | 0.373 |
| CTON | 10 | 38.064 | 21.237 | 16.796 | 70.700 |

## Table 15

Nutrient summary statistics for unreclaimed mine pit lakes sampled in Fall 1984.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| $NH_3$ | 10 | 0.094 | 0.041 | 0.052 | 0.185 |
| $NO_3$-$NO_2$ | 10 | 0.055 | 0.013 | 0.050 | 0.091 |
| $TPO_4$ | 10 | 0.369 | 0.161 | 0.142 | 0.611 |
| $OPO_4$ | 10 | 0.218 | 0.185 | 0.030 | 0.459 |
| TOC | 10 | 8.642 | 0.943 | 7.190 | 9.940 |
| TOTN | 10 | 0.149 | 0.039 | 0.102 | 0.235 |
| NTOP | 10 | 2.019 | 1.917 | 0.230 | 5.105 |
| CTON | 10 | 61.629 | 16.098 | 30.596 | 86.373 |

Table 16

Nutrient summary statistics for reclaimed mine pit lakes sampled in Fall 1984 sampling.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| $NH_3$ | 70 | 0.155 | 0.193 | 0.050 | 1.238 |
| $NO_3$-$NO_2$ | 70 | 0.119 | 0.157 | 0.050 | 0.841 |
| $TPO_4$ | 70 | 0.485 | 0.363 | 0.108 | 1.440 |
| $OPO_4$ | 70 | 0.256 | 0.311 | 0.025 | 1.368 |
| TOC | 70 | 9.410 | 2.256 | 3.940 | 16.010 |
| TOTN | 70 | 0.274 | 0.316 | 0.100 | 1.634 |
| NTOP | 70 | 3.849 | 7.990 | 0.097 | 39.680 |
| CTON | 70 | 55.689 | 27.588 | 3.733 | 121.386 |

## Table 17

Physico-chemical and colonization summary statistics for all lakes for Fall 1984 sampling.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| pH | 105 | 7.212 | 1.185 | 4.400 | 9.500 |
| Air temp. | 105 | 24.868 | 2.199 | 21.000 | 31.500 |
| Wa temp. | 105 | 27.696 | 1.114 | 24.000 | 30.800 |
| Alk | 101 | 35.107 | 26.822 | 0.500 | 122.500 |
| Hard | 105 | 97.033 | 69.942 | 12.500 | 607.500 |
| $DO_2$ | 105 | 7.392 | 1.871 | 0.820 | 12.170 |
| Cond | 105 | 283.935 | 145.762 | 50.000 | 607.500 |
| $SO_4$ | 105 | 62.164 | 50.843 | 0.200 | 186.520 |
| Ca | 105 | 26.071 | 14.655 | 4.000 | 60.000 |
| Mg | 105 | 8.975 | 5.018 | 0.870 | 23.740 |
| K | 105 | 2.136 | 2.261 | 0.200 | 9.190 |
| TSS | 105 | 0.188 | 0.084 | 0.042 | 0.457 |
| Al | 105 | 608.370 | 894.336 | 95.200 | 4871.800 |
| Fe | 105 | 0.124 | 0.146 | 0.030 | 0.690 |
| Se | 105 | 7.562 | 8.864 | 2.000 | 38.770 |
| Age | 105 | 22.481 | 35.942 | 0.100 | 100.000 |
| Seq | 105 | 67.661 | 18.312 | 15.300 | 96.000 |
| G | 105 | 1.789 | 0.791 | 0.450 | 4.250 |

# Table 18

Physico-chemical and colonization summary statistics for natural lakes for Fall 1984 sampling.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| pH | 15 | 7.206 | 0.644 | 6.200 | 8.500 |
| Air temp. | 15 | 25.500 | 2.267 | 22.500 | 30.000 |
| Wa temp. | 15 | 27.653 | 1.435 | 26.000 | 30.000 |
| Alk | 15 | 27.353 | 17.111 | 4.000 | 44.600 |
| Hard | 15 | 69.306 | 14.088 | 55.000 | 91.200 |
| $DO_2$ | 15 | 7.354 | 0.650 | 6.550 | 8.800 |
| Cond | 15 | 259.800 | 37.750 | 211.000 | 317.000 |
| $SO_4$ | 15 | 37.972 | 15.808 | 10.800 | 54.700 |
| Ca | 15 | 14.216 | 6.902 | 4.750 | 20.500 |
| Mg | 15 | 9.026 | 3.225 | 4.320 | 11.660 |
| K | 15 | 7.108 | 1.703 | 4.330 | 9.190 |
| TSS | 15 | 0.159 | 0.035 | 0.110 | 0.240 |
| Al | 15 | 126.446 | 21.377 | 97.800 | 162.500 |
| Fe | 15 | 0.0076 | 0.094 | 0.030 | 0.340 |
| Se | 15 | 5.009 | 0.417 | 4.300 | 5.810 |
| Age | 15 | 100.000 | 0.000 | 100.000 | 100.000 |
| Seq | 15 | 77.060 | 5.114 | 71.000 | 83.100 |
| G | 15 | 1.300 | 0.635 | 0.450 | 1.880 |

## Table 19

Physico-chemical and colonization summary statistics for active mine pits for Fall 1984 sampling.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| pH | 10 | 7.990 | 0.728 | 7.200 | 9.100 |
| Air temp. | 10 | 23.800 | 1.932 | 21.000 | 26.500 |
| Wa temp. | 10 | 27.210 | 1.430 | 24.000 | 29.000 |
| Alk | 10 | 75.610 | 34.778 | 40.000 | 122.500 |
| Hard | 10 | 195.480 | 167.914 | 70.000 | 607.500 |
| $DO_2$ | 10 | 8.506 | 1.859 | 6.400 | 12.170 |
| Cond | 10 | 393.330 | 215.021 | 180.000 | 607.500 |
| $SO_4$ | 10 | 69.214 | 51.744 | 18.480 | 131.610 |
| Ca | 10 | 39.144 | 21.835 | 18.000 | 60.000 |
| Mg | 10 | 15.203 | 8.228 | 7.080 | 23.740 |
| K | 10 | 14.412 | 0.211 | 1.110 | 1.670 |
| TSS | 10 | 0.246 | 0.090 | 0.138 | 0.375 |
| Al | 10 | 1609.860 | 1456.115 | 176.500 | 3585.900 |
| Fe | 10 | 0.220 | 0.263 | 0.030 | 0.690 |
| Se | 10 | 21.874 | 16.144 | 5.310 | 38.770 |
| Age | 10 | 0.100 | 0.000 | 0.100 | 0.100 |
| Seq | 10 | 48.500 | 2.951 | 45.700 | 51.300 |
| G | 10 | 1.485 | 0.462 | 1.146 | 2.023 |

## Table 20

Physico-chemical and colonization summary statistics for unreclaimed mine pit lakes for Fall 1984 sampling.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| pH | 10 | 8.460 | 0.705 | 7.600 | 9.500 |
| Air temp. | 10 | 24.700 | 1.702 | 22.500 | 28.000 |
| Wa temp. | 10 | 28.170 | 0.727 | 27.100 | 29.500 |
| Alk | 10 | 52.770 | 2.53 | 50.000 | 56.200 |
| Hard | 10 | 88.970 | 12.870 | 72.500 | 103.700 |
| $DO_2$ | 10 | 7.185 | 2.420 | 2.840 | 10.820 |
| Cond | 10 | 253.460 | 21.949 | 229.700 | 279.00 |
| $SO_4$ | 10 | 21.538 | 18.232 | 3.450 | 42.020 |
| Ca | 10 | 25.775 | 2.209 | 22.000 | 29.000 |
| Mg | 10 | 7.623 | 1.745 | 5.730 | 9.480 |
| K | 10 | 1.083 | 0.217 | 0.850 | 1.400 |
| TSS | 10 | 0.170 | 0.053 | 0.118 | 0.312 |
| Al | 10 | 308.080 | 20.752 | 276.900 | 339.100 |
| Fe | 10 | 0.038 | 0.018 | 0.030 | 0.090 |
| Se | 10 | 4.261 | 0.742 | 3.290 | 5.430 |
| Age | 10 | 60.000 | 0.000 | 60.000 | 60.000 |
| Seq | 10 | 71.950 | 2.055 | 70.000 | 73.900 |
| G | 10 | 1.735 | 0.142 | 1.600 | 1.870 |

## Table 21

Physico-chemical and colonization summary statistics for reclaimed mine pit lakes for Fall 1984 sampling.

| Variable | N | mean | std. dev. | minimum | maximum |
|---|---|---|---|---|---|
| pH | 70 | 6.924 | 1.224 | 4.400 | 9.100 |
| Air temp. | 70 | 24.635 | 2.263 | 21.000 | 31.500 |
| Wa temp. | 70 | 27.707 | 1.024 | 25.700 | 30.800 |
| Alk | 66 | 28.059 | 22.602 | 0.500 | 80.000 |
| Hard | 70 | 90.062 | 44.757 | 12.500 | 190.000 |
| $DO_2$ | 70 | 7.271 | 1.942 | 0.820 | 11.620 |
| Cond | 70 | 277.832 | 153.649 | 50.000 | 590.000 |
| $SO_4$ | 70 | 72.145 | 54.573 | 0.200 | 186.520 |
| Ca | 70 | 26.785 | 13.977 | 4.000 | 57.750 |
| Mg | 70 | 8.267 | 4.497 | 0.870 | 18.850 |
| K | 70 | 1.324 | 0.908 | 0.200 | 3.580 |
| TSS | 70 | 0.188 | 0.090 | 0.042 | 0.457 |
| Al | 70 | 611.467 | 848.479 | 95.200 | 4871.800 |
| Fe | 70 | 0.133 | 0.134 | 0.939 | 0.640 |
| Se | 70 | 6.537 | 7.111 | 2.000 | 32.520 |
| Age | 70 | 3.707 | 2.560 | 0.500 | 7.500 |
| Seq | 70 | 67.771 | 20.575 | 15.300 | 96.000 |
| G | 70 | 1.930 | 0.866 | 0.680 | 4.250 |

Table 22

Stepwise analysis results of physico-chemical variables and nutrient values for Fall 1984 sampling using 20 environmental parameters (105 samples each).

| Variable entered | Number In | Partial $r^2$ | F Statistic | Prob> F |
|---|---|---|---|---|
| Mg | 1 | 0.99 | 1355.118 | 0.0001 |
| Ca | 2 | 0.98 | 297.412 | 0.0001 |
| Se | 3 | 0.98 | 234.011 | 0.0001 |
| $SO_4$ | 4 | 0.98 | 189.767 | 0.0001 |
| $OPO_4$ | 5 | 0.97 | 162.190 | 0.0001 |
| K | 6 | 0.96 | 102.315 | 0.0001 |
| Alk | 7 | 0.88 | 27.501 | 0.0001 |
| Al | 8 | 0.87 | 25.677 | 0.0001 |
| TOC | 9 | 0.83 | 18.532 | 0.0001 |
| COND | 10 | 0.78 | 13.310 | 0.0001 |

## Table 23

Stepwise analysis results of physico-chemical variables and nutrient values for Fall 1984 sampling using only 15 environmental parameters (105 samples each).

| Variable entered | Number In | Partial $r^2$ | F Statistic | Prob > F |
|---|---|---|---|---|
| Mg | 1 | 0.99 | 1355.118 | 0.0001 |
| Ca | 2 | 0.98 | 297.412 | 0.0001 |
| Se | 3 | 0.98 | 234.011 | 0.0001 |
| $SO_4$ | 4 | 0.98 | 189.767 | 0.0001 |
| $OPO_4$ | 5 | 0.97 | 162.195 | 0.0001 |
| K | 6 | 0.96 | 102.315 | 0.0001 |
| Alk | 7 | 0.88 | 27.501 | 0.0001 |
| Al | 8 | 0.87 | 25.677 | 0.0001 |
| Cond | 9 | 0.79 | 13.686 | 0.0001 |
| $NO_3NO_2$ | 10 | 0.65 | 6.606 | 0.0001 |
| pH | 11 | 0.63 | 6.087 | 0.0001 |
| $TPO^4$ | 12 | 0.49 | 3.435 | 0.0001 |
| TSS | 13 | 0.42 | 2.520 | 0.0025 |
| $DO_2$ | 14 | 0.39 | 2.171 | 0.0097 |

## Table 24

Canonical variate analysis for all lakes (21) using 20 environmental parameters from Fall 1984 sampling.

| | WITHIN CANONICAL STRUCTURE | | |
|---|---|---|---|
| VARIABLE | CAN1 | CAN2 | CAN3 |
| Water Temp. | 0.0007 | 0.0044 | 0.0075 |
| pH | 0.0324 | 0.1113 | 0.0579 |
| Alk | 0.2145 | 0.3497 | 0.2442 |
| Hard | 0.0662 | 0.0038 | 0.0523 |
| $DO_2$ | 0.0045 | 0.0157 | 0.0200 |
| Cond | 0.3731 | -0.178 | 0.030 |
| $SO_4$ | 0.3050 | -0.4869 | -0.0096 |
| Air Temp | -0.0068 | 0.0088 | -0.0021 |
| $NH_4$ | -0.0005 | -0.049 | 0.0072 |
| $NO_3$-$NO_2$ | -0.0087 | -0.0370 | 0.0310 |
| $TPO_4$ | 0.0270 | 0.1179 | 0.0518 |
| $OPO_4$ | 0.0877 | 0.2179 | -0.0271 |
| TOC | 0.0177 | -0.0208 | -0.0012 |
| Ca | 0.3851 | -0.1522 | 0.387 |
| Mg | 0.6998 | 0.2004 | -0.0306 |
| K | 0.0587 | 0.0664 | -0.3640 |
| TSS | 0.0728 | -0.0275 | 0.0139 |
| Al | -0.0195 | 0.0317 | 0.0506 |
| Fe | -0.0297 | -0.0078 | -0.0001 |
| Se | 0.2256 | 0.0768 | 0.3795 |

## Table 25

Canonical variate analysis for all lakes (21) using 15 important environmental parameters from Fall 1984 sampling.

| | WITHIN CANONICAL STRUCTURE | | |
|---|---|---|---|
| VARIABLE | CAN1 | CAN2 | CAN3 |
| pH | 0.0341 | -0.0916 | 0.0939 |
| Alk | 0.2249 | -0.2676 | 0.3587 |
| Hard | 0.0699 | 0.0127 | 0.0529 |
| $DO_2$ | 0.0051 | -010094 | 0.0248 |
| Cond | 0.3906 | 0.1922 | -0.0294 |
| $SO_4$ | 0.3198 | 0.4819 | -0.1685 |
| $NO_3$-$NO_2$ | -0.0086 | 0.0461 | 0.0186 |
| $TPO_4$ | 0.272 | -0.1027 | 0.0967 |
| $OPO_4$ | 0.0891 | -0.2270 | 0.0580 |
| Ca | 0.4042 | 0.2721 | 0.3328 |
| Mg | 0.7435 | -0.1981 | 0.0505 |
| K | 0.0628 | -0.1721 | -0.3439 |
| TSS | 0.0760 | 0.0327 | 0.0055 |
| Al | -0.0201 | -0.0167 | 0.0608 |
| Se | 0.2410 | 0.0405 | 0.3946 |

Table 26

Lake name, symbol, and status for all lakes sampled in Fall 1984. A = active, R = reclaimed, U = unreclaimed, and N = natural lakes.

| LAKE | SYMBOL | STATUS |
|---|---|---|
| HPA | A | A |
| HPB | B | A |
| GOA | C | R |
| GOB | D | R |
| G1A | E | R |
| G1B | F | R |
| G1C | G | R |
| G2A | H | R |
| G2B | I | R |
| STB | J | R |
| G4A | K | R |
| 1215 | L | R |
| NT | M | R |
| LB | N | R |
| LL | O | R |
| G7 | P | R |
| LK | Q | U |
| LG | R | U |
| EL | S | N |
| LS | T | N |
| LA | U | N |

## Table 27

Factor analysis with varimax rotation of 15 important environmental parameters for all lakes from Fall 1984 sampling.

| | ROTATED FACTOR PATTERN<br>FACTOR1 | FACTOR2 | FACTOR3 |
|---|---|---|---|
| pH | 0.4550 | 0.91116 | -0.01334 |
| Alk | 0.54882 | 0.71829 | 0.34683 |
| Hard | 0.82531 | 0.24645 | 0.06578 |
| $DO_2$ | 0.01910 | 0.43315 | 0.02150 |
| Cond | 0.96647 | 0.04468 | -0.09630 |
| $SO_4$ | 0.85160 | -0.34015 | -0.14470 |
| $NO_3$-$NO_2$ | 0.5900 | -0.62369 | 0.26501 |
| $TPO_4$ | 0.20379 | -0.02546 | 0.93099 |
| $OPO_4$ | 0.31681 | 0.21303 | 0.69538 |
| Ca | 0.92267 | 0.16189 | 0.07354 |
| Mg | 0.88854 | 0.31321 | 0.01359 |
| K | 0.07159 | 0.00002 | -0.42186 |
| TSS | 0.87527 | -0.02164 | 0.12157 |
| Al | -0.09392 | -0.35335 | 0.73711 |
| Se | 0.61177 | 0.06079 | 0.21393 |
| | VARIANCE EXPLAINED BY EACH FACTOR | | |
| EIGENVALUE | 5.602982 | 2.400547 | 2.304059 |
| Variability explained (%) | 37.3 | 16.0 | 15.36 |
| Cumulative variability explained (%) | 37.3 | 53.3 | 68.71 |

## Table 28

Species distribution and the 10 most common species found in the Spring 1984 sampling.

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SPECIES DISTRIBUTION

---

| 1. CATEGORY | Number of Species |
|---|---|
| Total study | 537 |
| Only one sample | 128 |
| Over 10% of samples | 53 |
| Over 20% of samples | 3 |
| **2. MOST COMMON SPECIES** | **Number of Samples** |
| *Monas* sp. | 45 |
| *Cryptomonas erosa* | 44 |
| *Cyathomonas truncata* | 40 |
| *Cyclidium glaucoma* | 39 |
| *Chlamydomonas globosa* | 37 |
| *Cinetochilium margaritaceum* | 37 |
| *Cryptomonas ovata* | 37 |
| *Acanthocystis aculeata* | 36 |
| *Trachelomonas volvocina* | 36 |
| *Cyclidium brandoni* | 34 |

## Table 29

Correlations of ordination coordinates with physico-chemical parameters and factors for Spring 1984 sampling.

| | WATER TEMP* | ALK | HARD | $DO_2$ | CON | $NH_3$ | $NO_2$ | $NO_3$-$NO_2$ | $TPO_4$ |
|---|---|---|---|---|---|---|---|---|---|
| X ORD1 | | | | $-0.398^{2}$ | $.287^{1}$ | $-.371^{1}$ | $-.485^{3}$ | | $-.379^{2}$ |
| Y ORD1 | | $-.317^{1}$ | $-.427^{2}$ | | $-.482^{2}$ | $.305^{1}$ | $.335^{1}$ | $.427^{2}$ | |
| X ORD2 | | | | $.292^{1}$ | | | | | |
| Y ORD2 | | $-.423^{2}$ | $-.401^{2}$ | | $-.394^{2}$ | | $.413^{2}$ | | $.332^{1}$ |
| X ORD3 | $.331^{1}$ | | | | | $-.306^{1}$ | $-.383^{2}$ | | |
| Y ORD3 | | | $.464^{3}$ | | $.509^{3}$ | $-.445^{2}$ | $-.479^{3}$ | $-.479^{3}$ | $-.527^{4}$ |

| | $OPO_4$ | $SO_4$ | TOC | Ca | Mg | TSS | Al | As | Fe |
|---|---|---|---|---|---|---|---|---|---|
| X ORD1 | | | | | | | | | $-.300^{1}$ |
| Y ORD1 | $.346^{1}$ | $-.321^{1}$ | | $-.392^{2}$ | $-.314^{1}$ | $-.324^{1}$ | $.566^{4}$ | | |
| X ORD2 | | | | | | $.301^{1}$ | | | |
| Y ORD2 | | | | $-.351^{1}$ | | $-.301^{1}$ | $.441^{2}$ | $-.291^{1}$ | |
| X ORD3 | | | | | | | $-.681^{4}$ | | |
| Y ORD3 | $-.450^{2}$ | $.335^{1}$ | | $.338^{1}$ | $.335^{1}$ | | | | |

| | Se | AGE | F1 | F2 | F3 | F4 |
|---|---|---|---|---|---|---|
| X ORD1 | | | | | | |
| Y ORD1 | $.305^{1}$ | | $-.323^{1}$ | $.478^{3}$ | | |
| X ORD2 | | | | | | $-.299^{1}$ |
| Y ORD2 | $.454^{2}$ | $-.524^{3}$ | $-.378^{1}$ | $.377^{1}$ | | |
| X ORD3 | | | | | | |
| Y ORD3 | $-.342^{1}$ | | $.329^{1}$ | $-.584^{4}$ | | |

Table 29. (con't)

pH and K - no sign correlations at all.

* .011 < 1 < .05; .0011 < 2 < .01; .0001 < 3 < .001; .0001 = 4

**Ord1 is a reciprocal averaging ordination using pooled presence/absence data for the 96 most occurring species (See Figure 8). Ord2 is the same using 100 species that occur in from 11 to 24 samples, excluding the most common species. Ord3 is a reciprocal averaging ordination using the number of times a protozoan occurs at a site on a day (from 0 to 4), using the 96 most common species.

## Table 30

Species distribution and 10 most common species found in Fall 1984 survey. The numbers of samples refer to the number of pooled quadruplicate samples (105 total).

| Species Distribution | |
|---|---|
| 1. CATEGORY | Numer of Species |
| Total study | 892 |
| Only one sample | 216 |
| More than 10% | 345 |
| More than 33% | 136 |
| More than 50% | 65 |
| 2. MOST COMMON SPECIES | Number of Samples |
| *Cryptomonas erosa*[1] | 105 |
| *Monas* sp.[1] | 101 |
| *Bodo rostratus* | 100 |
| *Ctedoctema acanthocrypta* | 97 |
| *Chlamydomonas subasymmetrica* | 95 |
| *Cyathomonas truncata*[1] | 93 |
| *Rhynchomonas nasuta* | 93 |
| *Naegleria gruberi* | 91 |
| *Aspidisca costata* | 91 |
| *Bodo variabilis* | 88 |

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[1] Very common in Spring survey.

## Table 31

Correlations of ordination coordinates for physico-chemical parameters and factors for Fall 1984 sampling for all types of protozoans (495 species).

|  | WA TEMP | pH | ALK | HARD | $DO_2$ | AIR TEMP | $NH_3$ | $NO_3$-$NO_2$ |
|---|---|---|---|---|---|---|---|---|
| AX1 | $-.269^{2}$ |  |  |  |  | $-.266^{2}$ |  | $.346^{3}$ |
| AX2 | $-.263^{2}$ | $-.767^{4}$ | $-.542^{4}$ | $-.216^{4}$ | $-.231^{4}$ |  |  |  |
| AX3 | $4.09^{4}$ |  |  |  |  |  |  |  |
| AX4 |  | $-.418^{4}$ |  |  | $.230^{1}$ |  | $.371^{4}$ | $.711^{4}$ |

|  | $TPO_4$ | $OPO_4$ | TOC | Ca | Mg | Al | Fe | Se |
|---|---|---|---|---|---|---|---|---|
| AX1 |  | $.297^{2}$ |  |  |  |  |  |  |
| AX2 |  |  | $0.307^{2}$ | $-.206^{1}$ | $-.322^{3}$ | $.305^{2}$ | $.455^{4}$ |  |
| AX3 |  |  |  |  |  |  |  |  |
| AX4 | $-.307^{2}$ |  | $-.269^{2}$ | $-.213^{1}$ |  | $.623^{4}$ |  | $.558^{4}$ |

|  | $S_{eq}$ | G | TOTN | NTOP | F1 | F2 | F3 |
|---|---|---|---|---|---|---|---|
| AX1 |  |  |  |  |  |  |  |
| AX2 |  |  | $.275^{2}$ | $.368^{4}$ |  |  |  |
| AX3 |  |  |  |  |  |  |  |
| AX4 | $-.780^{4}$ | $.494^{4}$ | $.580^{4}$ | $.569^{4}$ |  | $-.342^{3}$ | $.476^{4}$ |

1 = $.001 < p < .05$

2 = $.0011 < p < .01$

3 = $.00011 < p < .001$

4 = $p < 0.0001$

## Table 32

Correlations of ordination coordinates with physico-chemical parameters and factors for Fall 1984 sampling (phytoflagellates).

|  | WA TEMP | pH | ALK | HARD | $DO_2$ | AIR TEMP | $NH_3$ | G |
|---|---|---|---|---|---|---|---|---|
| AX1 |  | $.371^4$ | $.317^2$ | $2.16^1$ | $.309^2$ |  |  | $.391^4$ |
| AX2 | $.385^4$ |  |  |  |  | $.352^3$ |  |  |
| AX3 |  | $0.560^4$ |  |  |  |  | .230 | $.194^1$ |
| AX4 |  |  |  |  |  |  |  |  |

|  | TOTN | F1 | F2 | F3 | $NO_3$-$NO_2$ | $TPO_4$ | $OPO_4$ | Al |
|---|---|---|---|---|---|---|---|---|
| AX1 |  |  |  |  |  | $.247^2$ |  | $.193^1$ |
| AX2 |  |  |  |  |  |  |  |  |
| AX3 | $.367^4$ |  | $-.437^4$ | .528 | $.457^4$ | $.463^4$ | $.288^2$ | $.439^4$ |
| AX4 |  |  |  |  |  |  | $-.232^1$ |  |

|  | Fe | Se | AGE | Seq |
|---|---|---|---|---|
| AX1 | $-.360^3$ | $.242^1$ |  | $-.469^4$ |
| AX2 |  |  | $.291^2$ |  |
| AX3 | $.264^2$ | $.328^3$ | $-.324^3$ | $-.380^4$ |
| AX4 |  |  |  |  |

$1 = .011 << p < .05$

$2 = .001 < p < .01$

$3 = .00011 << p < .001$

$4 = p < 0.0001$

## Table 33

Protozoan colonization parameters for studied lakes. Estimates of equilibrium species number ($\hat{S}eq$) and colonization rate (G) are shown. Ages of lakes are for Spring 1984 (lesser value) and Fall 1984 sampling. Note: $r^2$=coefficient of determination for regression, p=significance, (-) indicates lake not sampled, NS=not significant.

| Site | Age(yr) | $\hat{S}eq$ | G | $r^2$ | p |
|---|---|---|---|---|---|
| HPA | Active | 45.7 | 2.02 | 0.28 | <0.06 |
| HPB | Active | 51.3 | 1.15 | 0.63 | <0.001 |
| GOA | - | | | | |
| | 0.5 | 38.5 | 2.06 | 0.43 | <0.001 |
| GOB | - | | | | |
| | 0.5 | 15.3 | 4.25 | 0.04 | >0.5 (NS) |
| G1A | 1.2 | 38.1 | 0.42 | 0.93 | <0.001 |
| | 1.7 | 70.0 | 1.72 | 0.75 | <0.0001 |
| G1B | 0.8 | 21.3 | 1.70 | 0.27 | >0.05 (NS) |
| | 1.3 | 78.3 | 0.68 | 0.73 | <0.0001 |
| G1C | 0.8 | - | - | | |
| | 1.3 | 61.7 | 2.35 | 0.14 | >0.05 (NS) |
| G2A | 2.9 | - | - | | |
| | 3.5 | 57.7 | 2.77 | 0.40 | <0.01 |
| G2B | 1.9 | - | - | - | - |
| | 2.4 | 70.5 | 1.99 | 0.71 | <0.0001 |
| STB | 2.0 | 0 | 0 | - | - |
| | 2.5 | 58.1 | 2.39 | 0.33 | <0.05 |
| G4 | 4.4 | - | - | - | - |
| | 4.9 | 74.0 | 1.24 | 0.84 | <0.0001 |
| 1215 | 4.3 | 58.5 | 0.96 | 0.95 | <0.001 |
| | 4.8 | 96.0 | 1.16 | 0.78 | <0.0001 |
| NT | 5.7 | 60.0 | 1.54 | 0.53 | <0.01 |
| | 6.2 | 89.1 | 2.20 | 0.49 | <0.001 |
| G7 | 6.7 | - | - | - | - |
| | 7.2 | 70.0 | 1.59 | 0.55 | <0.0001 |

Table 33 (con't)

| Site | Age(yr) | $\hat{S}eq$ | G | $r^2$ | p |
|---|---|---|---|---|---|
| LB | 7 | 40.2 | 0.77 | 0.82 | $< 0.001$ |
| | 7.5 | 83.9 | 1.65 | 0.55 | $< 0.0001$ |
| LL | 7 | 42.0 | 1.49 | 0.62 | $< 0.005$ |
| | 7.5 | 85.7 | 0.97 | 0.78 | $< 0.0001$ |
| LG | 60 | 66.3 | 0.70 | 0.74 | $< 0.005$ |
| | | 23.95 | 1.60 | 0.45 | $< 0.0001$ |
| LK | 60 | 51.5 | 5.14 | 0.02 | $> 0.75$ |
| | | 70.0 | 1.87 | 0.62 | $< 0.0001$ |
| LA | - | 47.4 | 0.70 | 0.91 | $< 0.001$ |
| | - | 71.0 | 1.88 | 0.29 | $< 0.05$ |
| EL | - | 63.3 | 0.91 | 0.90 | $< 0.001$ |
| | - | 77.4 | 1.57 | 0.80 | $< 0.0001$ |
| LS | - | - | - | - | - |
| | - | 83.1 | 0.45 | 0.75 | $< 0.0001$ |

Table 34

Total species number found in each of the twenty-one lakes from the Fall 1984 sampling.

| Lake Code | Total species |
|---|---|
| HPA | 249 |
| HPB | 246 |
| G0A | 205 |
| G0B | 112 |
| G1A | 322 |
| G1B | 344 |
| G1C | 312 |
| G2A | 306 |
| G2B | 351 |
| STB | 287 |
| G4A | 357 |
| 1215 | 410 |
| NT | 401 |
| LB | 361 |
| LL | 361 |
| G7 | 310 |
| LK | 315 |
| LG | 338 |
| EL | 344 |
| LS | 314 |
| LA | 338 |

## Table 35

Chemical parameters correlated with equilibrium species numbers in study lakes, Fall 1984.

| Parameter | r | significance |
|---|---|---|
| Aluminum | -0.68 | $p<0.0001$ |
| Carbon to nitrogen (CTON) | 0.52 | $<0.0001$ |
| Nitrate-Nitrite ($NO_3$-$NO_2$) | -0.51 | $<0.0001$ |
| Selenium | -0.51 | $<0.0001$ |

## Table 36

Distribution of protozoan species into functional groups from Fall 1984 sampling. Number of species in each functional group and percent of total are recorded for each lake.

| AGE: LAKE | BACTERI-VORES | PRODUCERS | NON-SPECIFIC | ALGI-VORES | SAPRO-TROPHS | RAPTORS | TOTAL |
|---|---|---|---|---|---|---|---|
| <0: | | | | | | | |
| HPA | 30 | 11 | 3 | 1 | 1 | 0 | 46 |
| | 65.16 | 24.14 | 6.61 | 1.94 | 2.16 | 0.00 | |
| HPB | 39 | 6 | 3 | 2 | 1 | 0 | 51 |
| | 75.80 | 11.74 | 6.20 | 3.85 | 2.41 | 0.00 | |
| 0: | | | | | | | |
| GOA | 23 | 7 | 3 | 1 | 0 | 0 | 35 |
| | 67.22 | 19.62 | 8.61 | 3.83 | 0.72 | 0.00 | |
| GOA | 8 | 5 | 1 | 0 | 0 | 0 | 14 |
| | 56.02 | 34.34 | 6.02 | 2.41 | 1.20 | 0.00 | |
| 1: | | | | | | | |
| G1A | 53 | 7 | 4 | 2 | 1 | 1 | 68 |
| | 78.16 | 10.92 | 5.89 | 2.45 | 1.84 | 0.74 | |
| G1B | 51 | 13 | 6 | 2 | 0 | 0 | 73 |
| | 70.29 | 17.44 | 0.49 | 3.10 | 0.23 | 0.46 | |
| G1C | 42 | 12 | 4 | 2 | 0 | 1 | 60 |
| | 69.47 | 19.99 | 6.17 | 3.35 | 0.18 | 0.64 | |
| 2: | | | | | | | |
| G2A | 42 | 12 | 2 | 3 | 0 | 0 | 60 |
| | 71.08 | 19.84 | 3.49 | 5.03 | 0.56 | 0.00 | |
| G2B | 48 | 16 | 3 | 2 | 0 | 0 | 69 |
| | 69.17 | 23.70 | 3.63 | 2.78 | 0.80 | 0.12 | |
| STB | 44 | 10 | 2 | 2 | 1 | 0 | 58 |
| | 75.68 | 16.83 | 2.73 | 5.02 | 1.15 | 0.58 | |
| 4: | | | | | | | |
| G4A | 51 | 15 | 3 | 4 | 1 | 0 | 74 |
| | 69.12 | 20.59 | 3.73 | 4.86 | 1.47 | 0.22 | |
| NT | 67 | 6 | 8 | 8 | 1 | 1 | 88 |
| | 75.85 | 6.98 | 8.77 | 6.32 | 0.75 | 1.25 | |
| 1215 | 59 | 20 | 8 | 4 | 3 | 2 | 97 |
| | 60.57 | 21.14 | 8.11 | 4.14 | 2.93 | 3.11 | |
| 7: | | | | | | | |
| LB | 54 | 22 | 6 | 4 | 0 | 1 | 85 |
| | 62.77 | 25.34 | 6.63 | 4.29 | 0.29 | 0.68 | |

Table 36 (con't)

| AGE: LAKE | BACTERIVORES | PRODUCERS | NON-SPECIFIC | ALGIVORES | SAPROTROPHS | RAPTORS | TOTAL |
|---|---|---|---|---|---|---|---|
| LL | 55 | 19 | 5 | 3 | 1 | 1 | 83 |
| | 66.43 | 23.15 | 6.01 | 3.01 | 0.70 | 0.70 | |
| G7 | 51 | 14 | 2 | 4 | 0 | 0 | 71 |
| | 71.68 | 19.74 | 2.70 | 5.05 | 0.59 | 0.24 | |
| >60: | | | | | | | |
| LK | 51 | 14 | 3 | 2 | 1 | 0 | 70 |
| | 71.95 | 20.00 | 3.91 | 2.60 | 1.30 | 0.24 | |
| LG | 49 | 20 | 2 | 3 | 1 | 1 | 75 |
| | 65.05 | 26.61 | 3.01 | 3.90 | 0.78 | 0.67 | |
| NATURAL: | | | | | | | |
| LA | 49 | 11 | 6 | 3 | 0 | 0 | 70 |
| | 70.20 | 16.33 | 8.10 | 4.65 | 0.24 | 0.48 | |
| LS | 51 | 16 | 4 | 2 | 1 | 1 | 74 |
| | 68.88 | 21.74 | 5.21 | 2.72 | 0.79 | 0.65 | |
| EL | 52 | 19 | 4 | 3 | 0 | 0 | 77 |
| | 67.06 | 24.05 | 4.98 | 3.36 | 0.11 | 0.45 | |
| TOTALS: | 967 | 275 | 79 | 53 | 14 | 9 | 1397 |

CHI-SQUARE:

$x^2 = 62.0$ d.f. = 100 Probability $\geq 0.995$

# 4. DISCUSSION

Recovery of lake ecosystems following mining and reclamation is a complex process. The habitat created must have appropriate characteristics for organism survival, and there must be an adequate supply of propagules from nearby species sources to effect the recovery of the biological community (Cairns and Dickson, 1977). For certain types of species, plantings or planned introductions (such as fish stocking) may be in order. Interestingly, although the microbial community (including bacteria, algae, and protozoa) are key mediators of the nutrient and carbon cycling processes, they are usually not considered for introduction.

A number of questions might be asked about the recovery process in newly created ecosystems (e.g., Bloom, 1980). For purposes of this investigation, we considered the following:

1. How do microbial communities differ in aging lakes?
2. What physical-chemical factors change as lakes age?
3. What effect do changes in physical-chemical factors have on lake microbial communities?

Additionally, a number of other important questions - not in the scope of this investigation - might be asked about recovery lakes. For example, do reclaimed lakes have functional attributes similar to natural lakes in the same area? Where do the colonizing species come from? Does wildlife play a role in lake recovery such as transporting species propagules or affecting nutrient balances? Can the recovery process be manipulated to produce more "natural" ecosystems?

We examined the initial questions by detailed analysis of the environmental and biological data collected during the fall of 1984 when a broad range of lakes were studied. To examine the relationship between age and biotic and abiotic factors, we assigned arbitrary ages to those systems which we could not accurately date. Unreclaimed lakes were taken to be 60 years old, although it is probable that they are actually older. Natural lakes were designated as 100 years old for convenience of examining the data. Placing a greater or more realistic geologic age on these lakes would have resulted in extended graphic displays which would have masked certain patterns that were apparent in the data. Actively mined sites were designated as 0.1 years such that the range of ages of lakes spanned only three orders of magnitude. In general, these arbitrary ages did not result in unusual skewing of the data. Age values were converted to the logarithm of the estimated age for purposes of displaying patterns in the data.

## CHANGES IN LAKE CHEMISTRY WITH AGE

Examination of water chemistry parameters revealed several changes during aging. It should be noted here that the number of replicate systems studied was not great and that a broader range of variability is likely. Many of the reclaimed lakes studied were in close proximity and probably shared many characteristics due to similar interactions between water and basin material. Alternatively, similarity of basin parent material for lakes reclaimed in the phosphate mineralized area of central Florida is probable. We examined several parameters in relation to values reported by Boody et al. (1984) in a previous extensive study of reclaimed and natural lakes in the mining region. In general, there was good correspondence between the environmental data of both studies. Since several of the same lakes were examined in both projects, comparisons of certain stable parameters further increased confidence in the measurements made. Some differences were noted and are detailed below. Many of the parameters which differed in lakes of differing ages were not significantly correlated with age. Often, only a few lakes showed aberrant values for chemical parameters measured. However, a preponderance of the evidence, based on repeated sampling of the same lakes, suggested several important differences in young reclaimed lakes.

The pH of newly reclaimed lakes (GOA and GOB) was lower than that for the other lakes studied (Figure 25). This pH difference may be related to chemical weathering processes of the basin parent material and is explained further below. Alkalinity was correspondingly low in newly reclaimed lakes (Figure 26) and was also generally high in the active mine sites (HPA, HPB). Alkalinities of several lakes were below Florida class III water quality standards of 20 mg/L. Alkalinity and pH were strongly correlated ($r=0.73$, $p<0.0001$). This relationship was not found by Boody et al. (1984) who sampled over all seasons.

There were strong, expected relationships among calcium, magnesium, hardness, conductivity, and sulfate (all $r>0.6$, $p<0.001$). Only sulfate was significantly related to lake age, but the factors tended to separate lakes in multivariate analyses. The relationship of these parameters to age is shown in Figure 27-30. Deviation of values for newly reclaimed lakes, active mine sites, and certain other lakes are obvious. Similar to previous findings, calcium and sulfate were strongly related ($r=0.78$, $p<0.001$). We postulated that in newly reclaimed lakes this relationship might also be involved in the production of low pH owing to the oxidation of pyrite, the concomitant production of sulfuric acid, the solubilizing of fluorapatite, and the precipitation of calcium sulfate (H. Barwood, FIPR, cited in Boody et al., 1984). This process forces phosphoric acid into solution. Phosphate ($OPO_4$) was only weakly correlated with pH ($r=0.20$, $p<0.0001$), and there was no correlation of pH and $TPO_4$.

Potassium levels were high (c.f. Wetzel, 1983) in natural lakes (Figure 31) but showed no relationship to lake age. While potassium levels are sometimes altered due to the incorporation of potassium in aquatic plant biomass, no such assumptions can be made for newly reclaimed lakes where the biota was very sparse.

The most surprising relationships between lake age and chemical factors were the high levels of aluminum and selenium in newly reclaimed lakes and active mine sites (Figures 32, 33). Aluminum solubilization is pH dependent (Cronan and Schofield, 1979). Selenium levels exceeded Florida class I and class III water quality standards (10 and 25 ug/L, respectively). There were strong correlations, as previously noted, between aluminum and selenium levels and protozoan species numbers in lakes. Selenium is known to be toxic in higher doses, although EPA criteria for chronic selenium toxicity (35 ug/L, USEPA, 1983) were only approached in some lakes. Our determination of selenium levels differs markedly from those of Boody et al. (1984). These differences may be due to differences in analytical methodologies.

Nitrogen nutrient values were generally high in newly reclaimed lakes (Figures 34-36), although these values may have been driven more by fertilizer runoff than by natural phenomena.

Phosphate levels showed some relationship to lake age (Figures 37, 38) with high levels of total phosphate recorded in newly reclaimed and active mine sites. Lake NT also had high phosphate levels, although this lake is still part of IMC's water recirculation system. Both this study and that by Boody et al. (1984) noted large fluctuations in water level in this lake. High nitrogen levels and low ortho-phosphate levels in newly reclaimed lakes also are documented in large NTOP values (Figure 39).

Factors separating newly reclaimed lakes return rapidly to levels comparable to older lakes. For example, elevated aluminum and selenium levels and lower pH's were also found in young lakes during the spring sampling period in lakes GlA and GlB. These values rapidly returned to levels within the variability of other sampled lakes in the six month period between samplings. Reclamation studies for lakes reclaimed after coal mining activities show much longer chemical instabilities. For example, Friedrich (1975) estimated that low pH in lakes reclaimed after surface mining of coal required ten years to return to normal values. This suggests that chemical differences in reclaimed phosphate pit lakes are transitory, although their effect on system function has not been evaluated. Previous studies in reclaimed lakes have not examined lakes as young as the lakes studied in the present research program.

## MICROBIAL COMMUNITY - ESTIMATION OF LAKE RECOVERY RATE

Lakes in the phosphate mining region of central Florida were very productive in terms of protozoan species richness. Species numbers exceeded those previously determined for temperate lakes and wetlands (e.g., Pratt and Cairns, 1985; Pratt et al., in press; Henebry and Cairns, 1984; Henebry et al., 1981) using comparable collecting techniques. Total species numbers approached numbers collected from many more samples in more heterogenous ecosystems (e.g., Pratt, 1984). The lowest values on total species collections were found at active mine sites and in newly reclaimed laeks. It is not possible to determine if comparable species pools are available in each ecosystem. The structuring of the local community may be due to lack of diversity in the local species pool due to the failure of certain species to colonize the system or may be due to the complex suite of environmental parameters affecting species abundance patterns.

Protozoan colonization in the studied lakes was typically rapid and resulted in large numbers of species at equilibrium. Colonization patterns were very similar for all lakes studied, except artificial substrate colonization was reduced and sometimes unpredictable in newly reclaimed lakes. Colonization was very rapid (c.f., Henebry and Cairns, 1984; Henebry et al., 1981), and generally reflected the advanced trophic state (Plafkin et al., 1980) of the area's lakes. In general, colonization was faster and resulted in greater equilibrium species numbers for fall collections as compared with spring.

Colonization in newly created lakes may have been affected by low levels of metal toxicants. Selenium and aluminum levels were consistently high in these lakes and pH's were consistently low. Protozoan colonization has been shown to be sensitive to comparative low levels of toxic materials (e.g., Cairns and Pratt, 1985; Niederlehner et al., 1985; Cairns et al., 1980) and the overall interaction of these factors may have resulted in depression of the colonization response. It is apparent from the chemical sampling data that potentially toxic concentrations of material may occur in newly created lakes. However, this study and previous examination of reclaimed lakes (e.g., Boody et al., 1984) in the region have shown excursions beyond state of Florida water quality standards for several potentially toxic materials. Despite this, apparently healthy communities may be found in older reclaimed lakes. In this study, levels of selenium and aluminum decreased with lake age. There were concomitant increases in pH suggesting that reclamation activities may produce a temporary disequilibrium in the aquatic community which is rapidly restored during the aging process. Documentation of lake recovery from low pH has recently been inferred from paleoecological studies using diatoms (Fritz and Carlson, 1982).

The composition of protozoan communities based on functional group structure was similar to that of other ecosystems examined (Pratt and Cairns, 1985) and showed that newly reclaimed lakes and active mine sites were significantly different from the other lakes studied. The functional group structure of most protozoan communities is remarkably similar, and differences found in the functional group structure of communities in this investigation were only obvious for the most and least diverse communities examined. However, examination of effects of the complex suite of interacting chemical factors showed good separation of non-reclaimed lakes studied. Functional group analysis added to the preponderance of evidence indicating consistent differences between young reclaimed lakes and other lake types. However, other parameters (such as colonization parameters) may be better indicators of community differences.

Community structure and colonization dynamics for protozoan communities have been previously related to system primary productivity (Henebry et al., 1981) and have shown exceptionally good response to environmental perturbations such as nutrient loading (Cairns et al., 1979; ANSP, 1984). It should not be inferred that all communities in older reclaimed and unreclaimed lakes are similar to natural communities. The broad range of tolerance (Cairns, 1982; Noland and Godjics, 1967) and the rapid turnover rate of protozoans (Schoener, 1983) make such determinations almost impossible to make. However, colonization dynamics and community structure are very similar in more general terms for all lakes two years old or older.

It seems clear that reclaimed lakes bracket the variability found in natural lakes and older, unreclaimed lakes in the mining region (Figure 40). The condition of the microbial community as determined by colonization analysis and examination of the inter-relation of community structure and water chemistry varies broadly among the lakes studied. However, newly reclaimed lakes and, to a lesser extent the disturbed surface water in active mine sites, are quite different chemically and biologically from other lakes in the region. Recovery of a microbial community comparable in terms of number of species, colonization rate, and functional group structure to those of older reclaimed, unreclaimed, and natural lakes in the region occurs within two years of the beginning of reclamation activity.

## WATER QUALITY CONSIDERATIONS

As noted previously by Boody et al. (1984) and Fernald and Patton (1984), several lakes in the mining region exceed Florida water quality standards for various factor. Notably, we found several lakes with pH's below standards although this appears to be a short-lived artifact of the reclamation process. Selenium levels were also higher than standards in newly reclaimed lakes. Several lakes had alkalinities below the 20 mg/L standard. We found isolated instances of oxygen depletion in certain lakes (for example, G4 had dissolved oxygen levels below 0.2 mg/L on one occasion). Careful water quality evaluation was not undertaken for several trace metals, although aluminum levels were generally high.

Despite certain water quality excursions, older reclaimed lakes, unreclaimed lakes, and natural lakes had very productive protozoan communities. This suggests that water quality variations are too small and too infrequent to produce significant impacts in the protozoan biota. It should be noted that microbial species such as protozoa respond rapidly to adverse water quality and also recover rapidly when water quality returns to within tolerance ranges. Whether less plastic populations are affected by apparently small water quality variations in reclaimed lakes is not known.

## SUMMARY AND CONCLUSIONS

Rules directing the reclamation of lakes on surface mined lands propose to create, by legislation and regulation, alternative ecosystems (Magnuson et al., 1980). These alternative ecosystems are quite different from the original terrestrial ecosystem which has been displaced by mining activities. It should be noted that creating alternative ecosystems differs substantially from the restoration of damaged systems (Bjork, 1982).

In the former case, the components of the new ecosystem must be assembled (or allowed to assemble) such that a "normal" functioning system develops. In the latter case, many of the components of the ecosystem are already present but are in disharmony. In restoration, effort must be expended to restore system function by changing abiotic conditions and/or altering the resident biota (e.g., Fox et al., 1977).

The purpose of this study was to apply a simple, direct method to the assessment of recovery in reclaimed alternative ecosystems. Specifically, the project was designed to determine microbial colonization rates and equilibrium species numbers on artificial substrates in reclaimed, unreclaimed, and natural lakes; to determine the effect of physical and chemical factors on microbial colonization; and to estimate the rate of recovery of lakes after mining and reclamation.

## Microbial Community

Protozoan colonization of artificial substrates was rapid and resulted in relatively high equilibrium species numbers. Reclaimed, unreclaimed, and natural lakes studied had comparable ranges of colonization rates and equilibrium species numbers. Newly reclaimed lakes (ca. 6 months old) had much lower species numbers than other lakes studied. Samples taken in late summer-early fall (August-September) had much higher species numbers than comparable samples taken in early spring (March). Microbial community colonization was strongly related to trace elements, especially aluminum and selenium levels in newly reclaimed lakes.

## Effects of Physical-Chemical Factors

Initial sampling of ten lakes in March revealed that natural lakes could be distinguished chemically from other lake types. Factor analysis indicated lakes varied primarily according to major cations, and to a lesser degree, according to phosphate levels. No meaningful differences in protozoan faunal composition could be inferred from the environmental data.

More extensive sampling of 21 lakes in August and September showed similar differentiation of lake types based on major ions, although differences were less clear. Additional differences in factors were noted for pH, alkalinity, and nitrate-nitrite. Additional differences were found in a factor based on phosphate and aluminum concentrations. Separation of protozoan community structure according to detrended correspondence analysis (DCA) showed that complex variables based on community composition overlapped broadly among lake types. Specific examination of the phytoflagellate group showed good separation of unreclaimed and natural lakes and active mine sites. Reclaimed lake samples spanned the range of variability for other lake types. Correlation of environmental parameters to DCA axes showed that pH, aluminum, and selenium values were related to community variables. Nutrient values were apparently of little importance in affecting community structure, Those samples with low pH and high aluminum and selenium values were from the two most recently reclaimed lakes.

## Recovery Rate of Mined Lakes

Examination of changes in important chemical factors (pH, aluminum, selenium) showed that reclaimed lakes older than approximately one year were generally similar. This rapid recovery was also reflected in microbial colonization patterns and in composition of protozoan functional groups. Microbial communities in reclaimed lakes greater than one year old were similar in colonization dynamics and functional group composition. Recovery as measured by microbial community characteristics was rapid and effective.

This investigation was not intended to make detailed examination of functional attributes of reclaimed ecosystems. The evidence presented here indicates that microbial communities are sensitive to unusual levels of chemical factors and recover quickly in aquatic ecosystems with high nutrient levels. This study was not designed to answer questions concerning the continued health and functioning of reclaimed ecosystems. On-going investigations will be needed to examine attributes of system function and to verify the adequacy of changing reclamation rules in directing the development of healthy ecosystems with qualities capable of sustaining wildlife and higher organisms of public interest and concern.

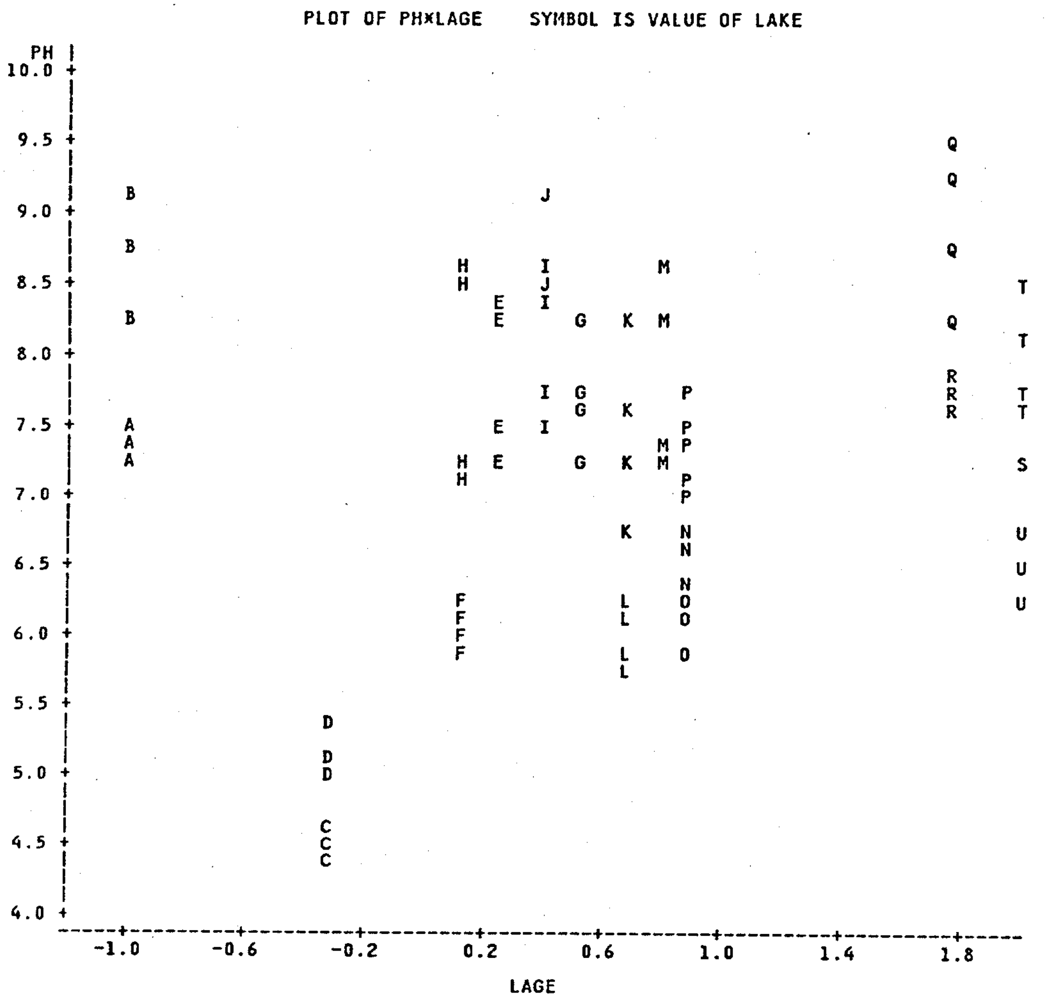

Fig. 25. The relationship of pH to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (33 observations hidden).

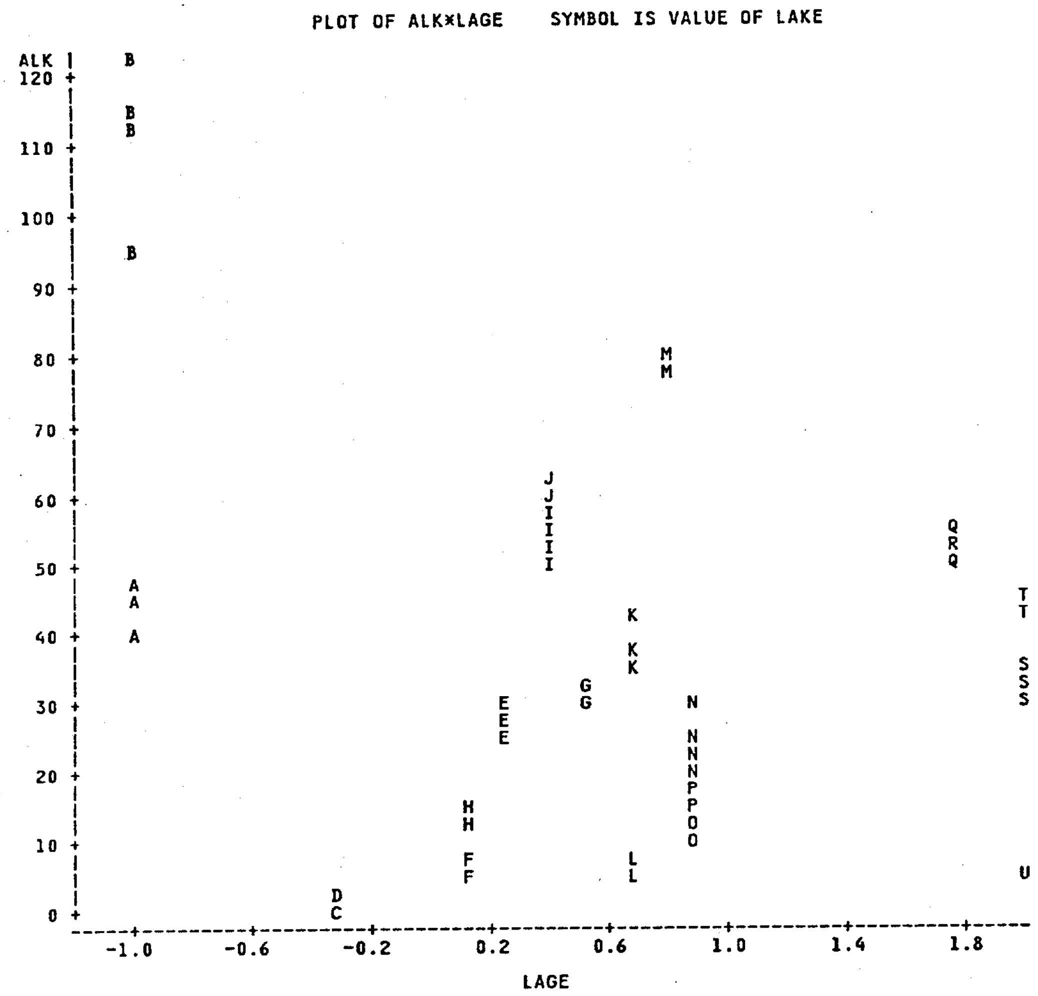

Fig. 26. The relationship of water alkalinity and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (60 hidden observations).

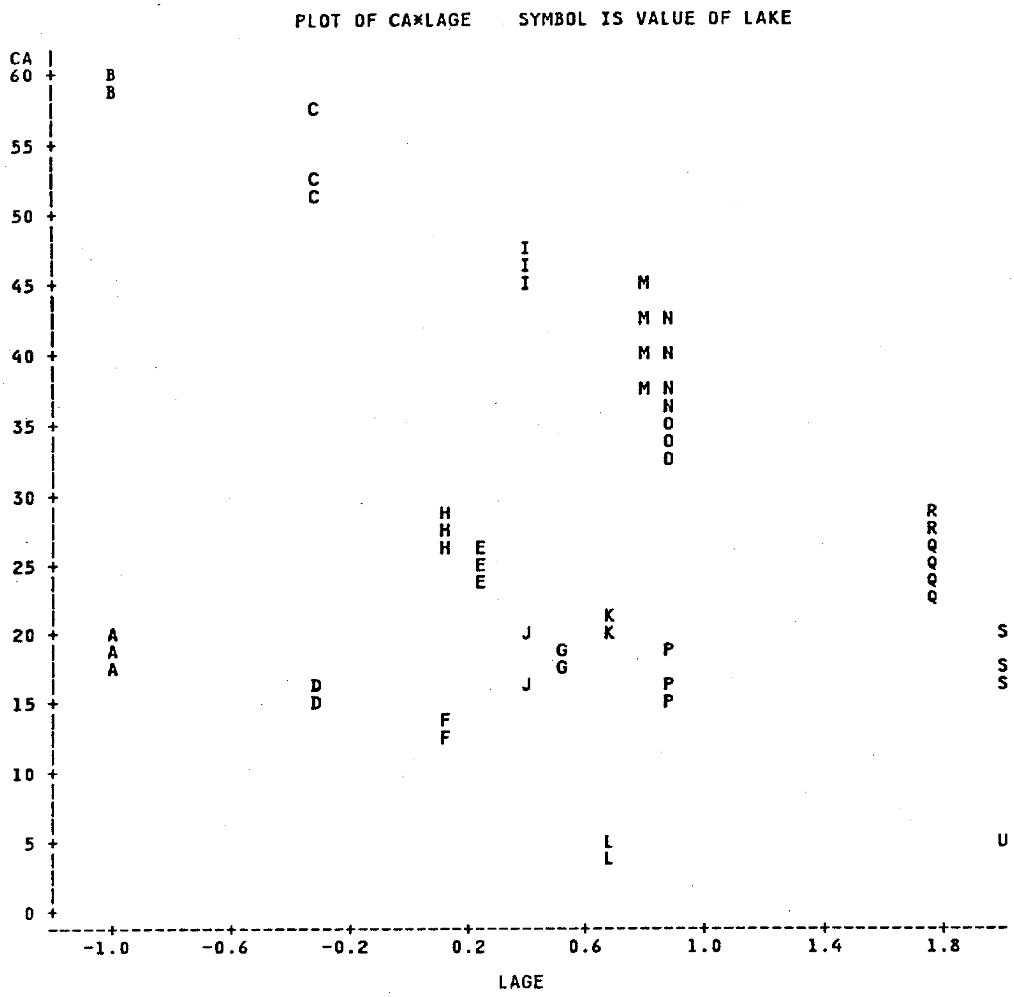

Fig. 27. The relationship of calcium and the logarithm of lake age for the Fall 1984 sampling. Letters identifying lakes (52 observations hidden).

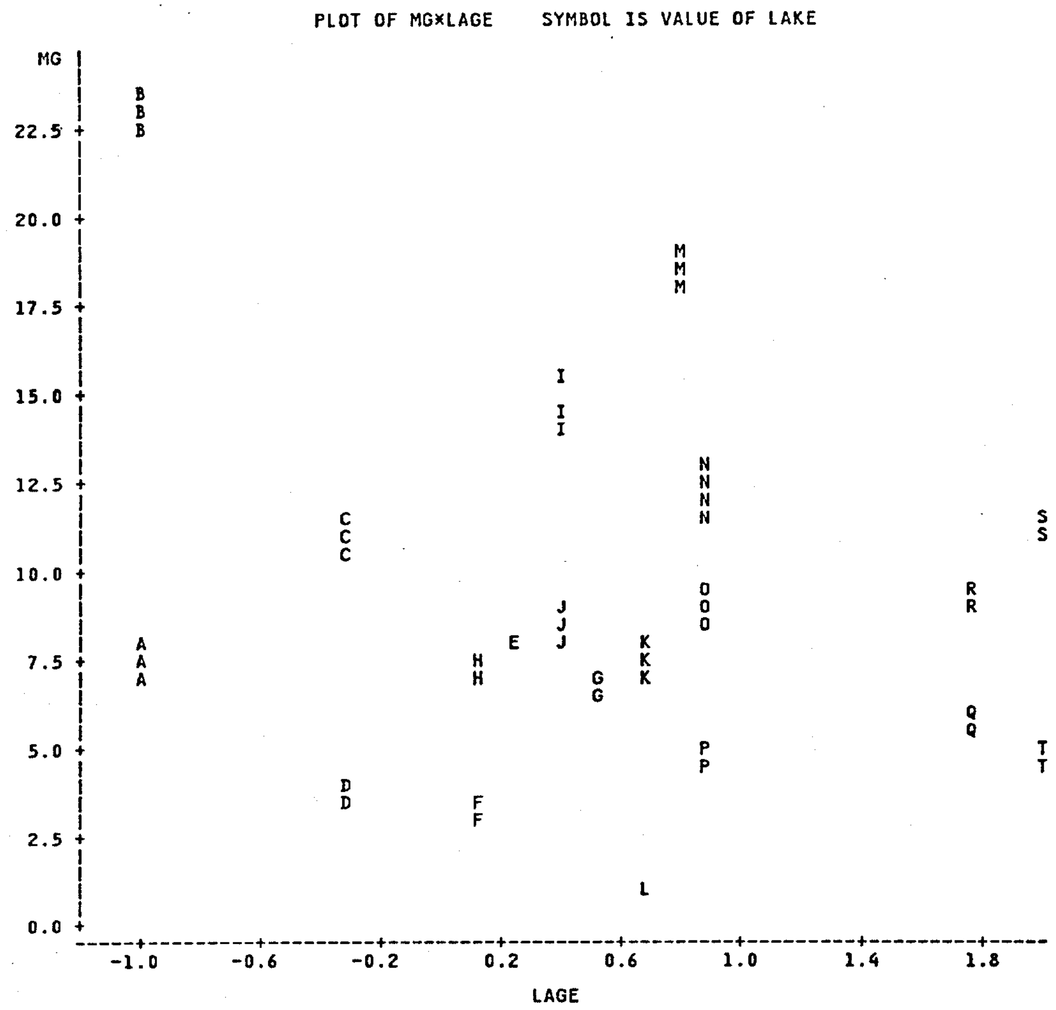

Fig. 28. The relationship of magnesium and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (57 observations hidden).

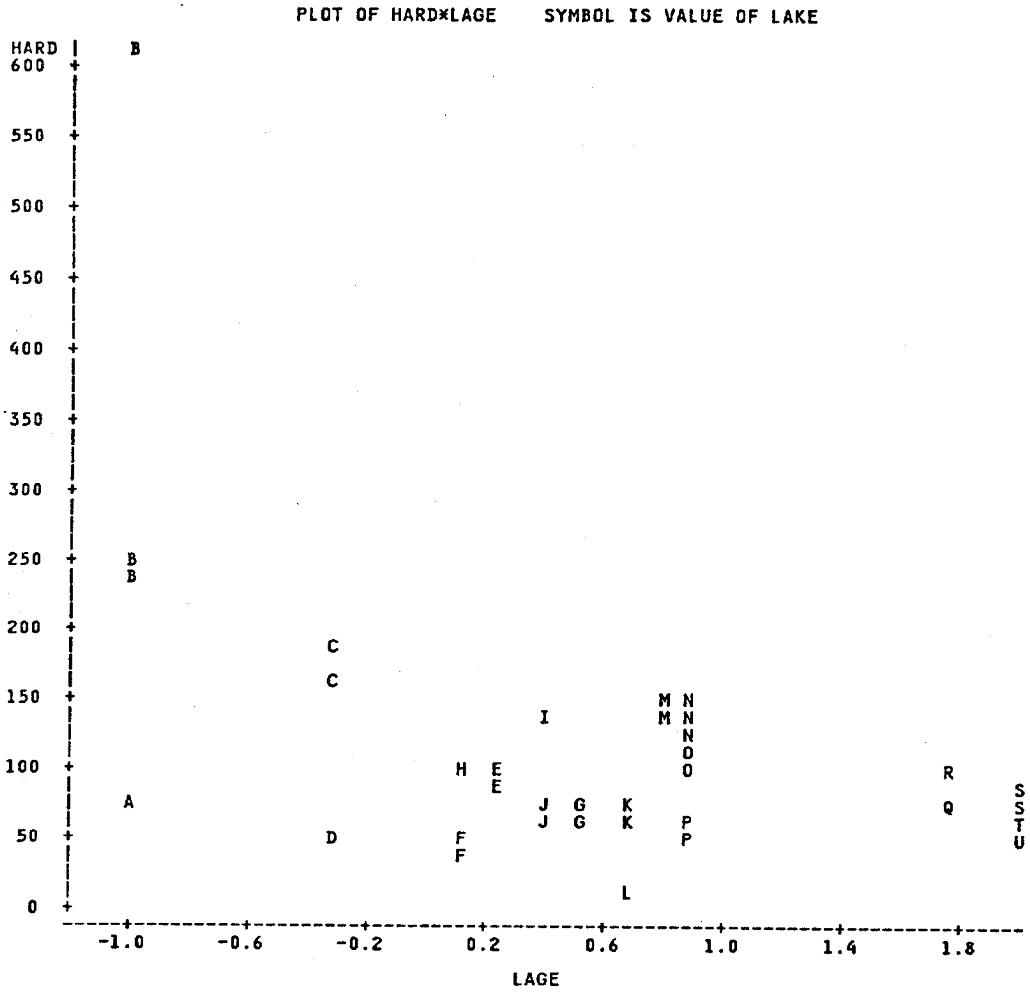

Fig. 29. The relationship of water hardness and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (70 observations hidden).

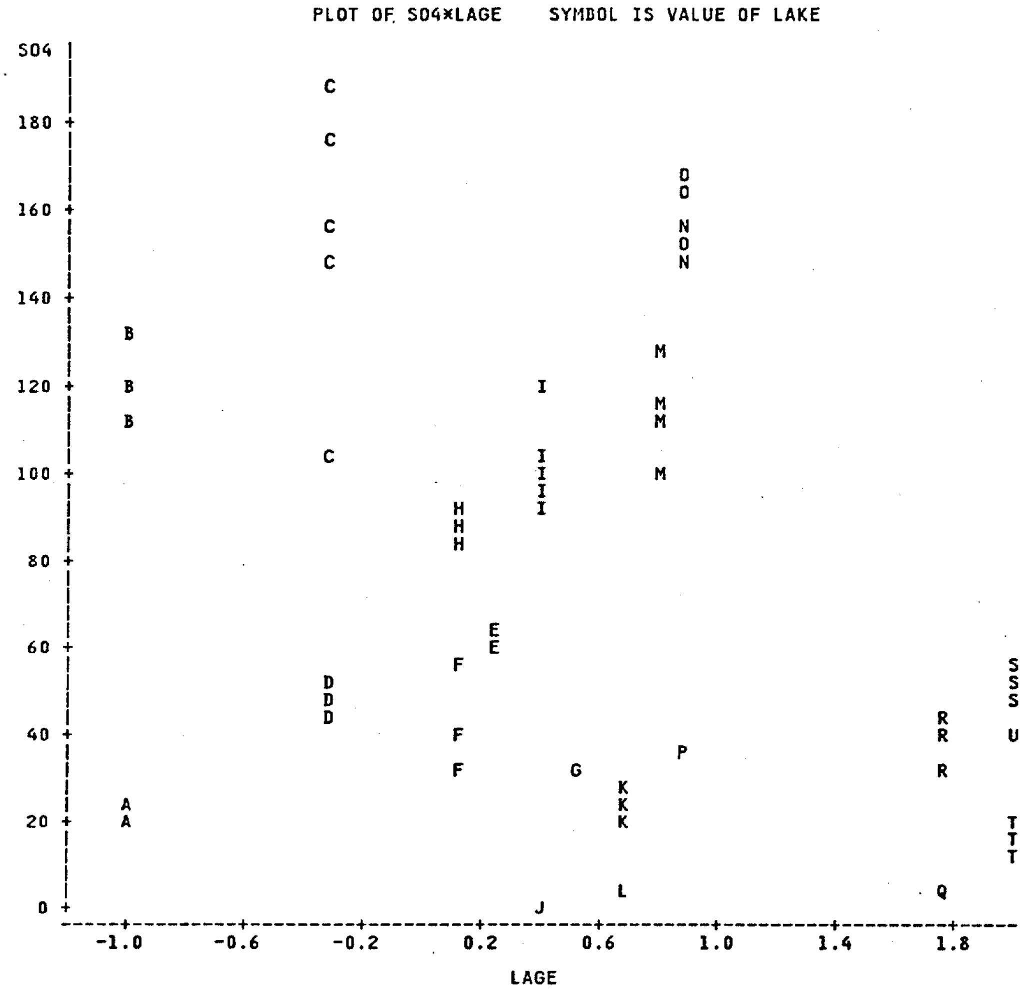

Fig. 30. The relationship of sulfate and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (53 observations hidden).

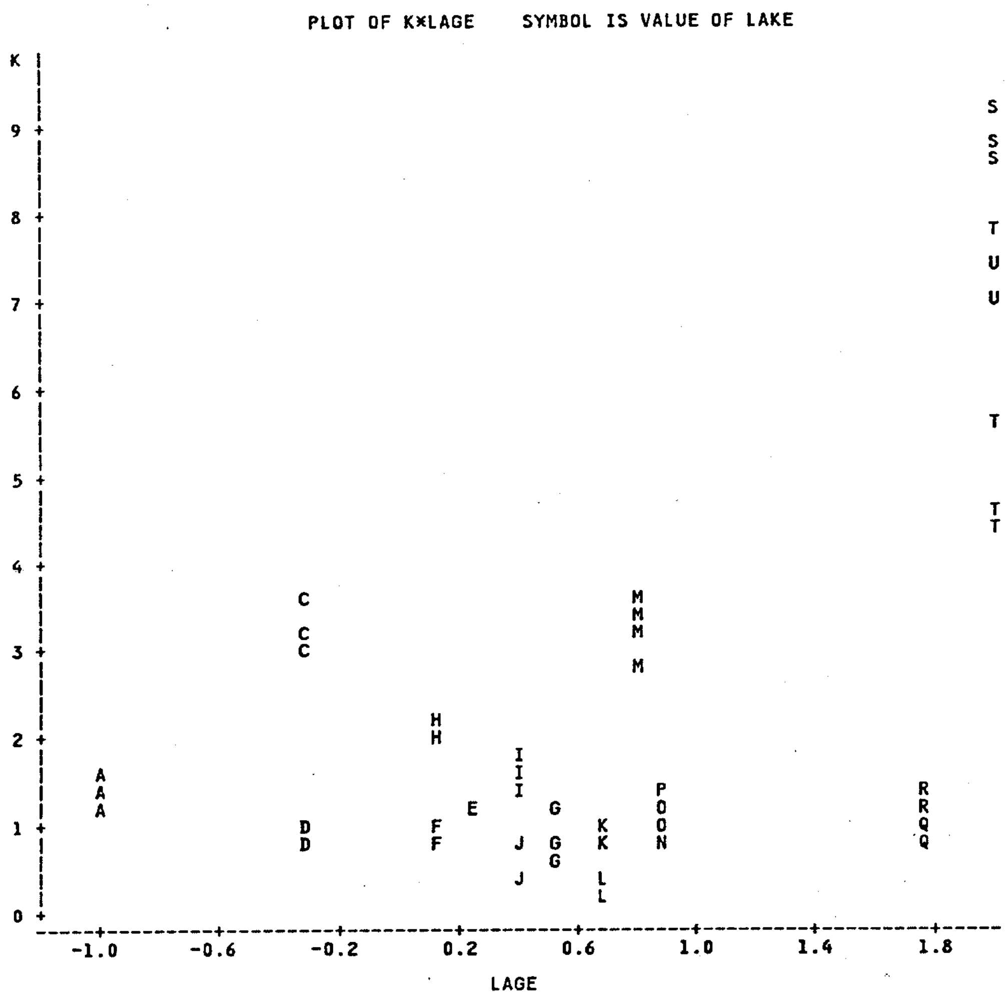

Fig. 31. The relationship of potassium to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (59 observations hidden).

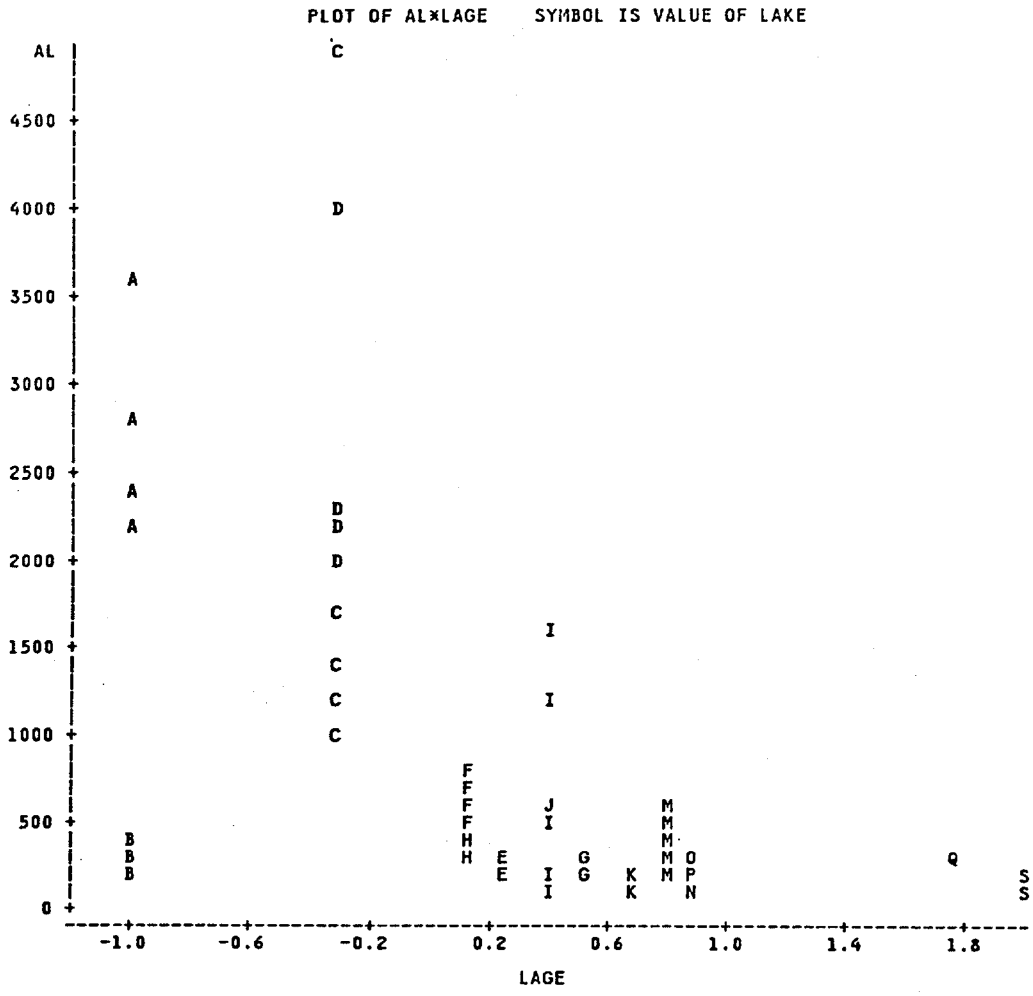

Fig. 32. The relationship of aluminum to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (64 observations hidden).

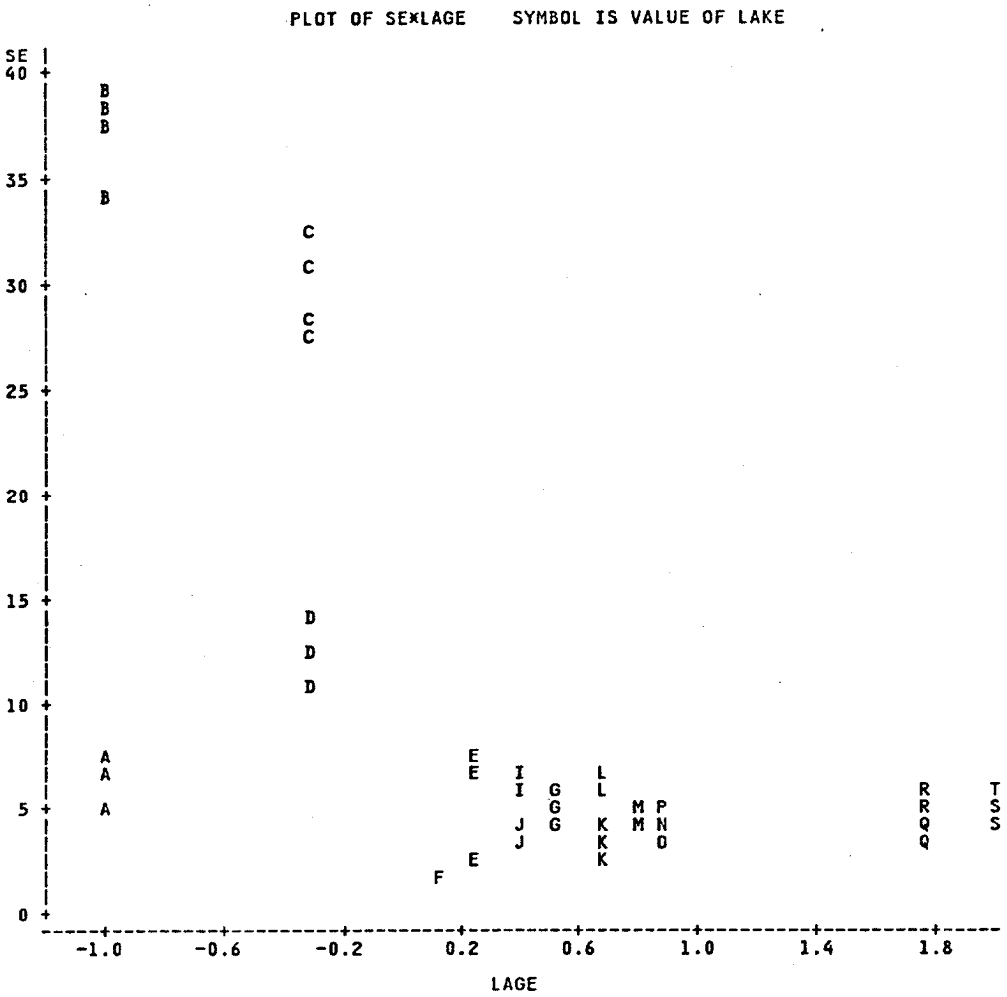

Fig. 33. The relationship of selenium to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (63 observations hidden).

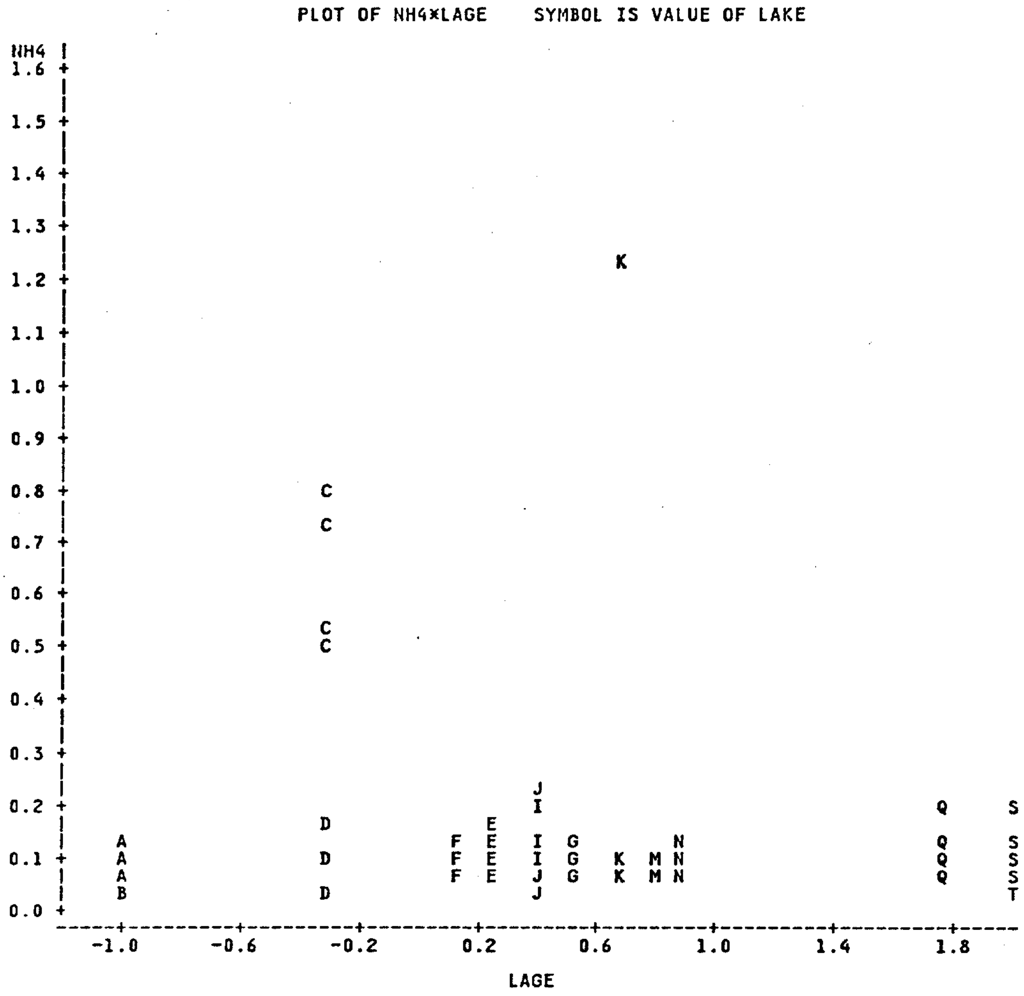

Fig. 34. The relationship of ammonia and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (61 observations hidden).

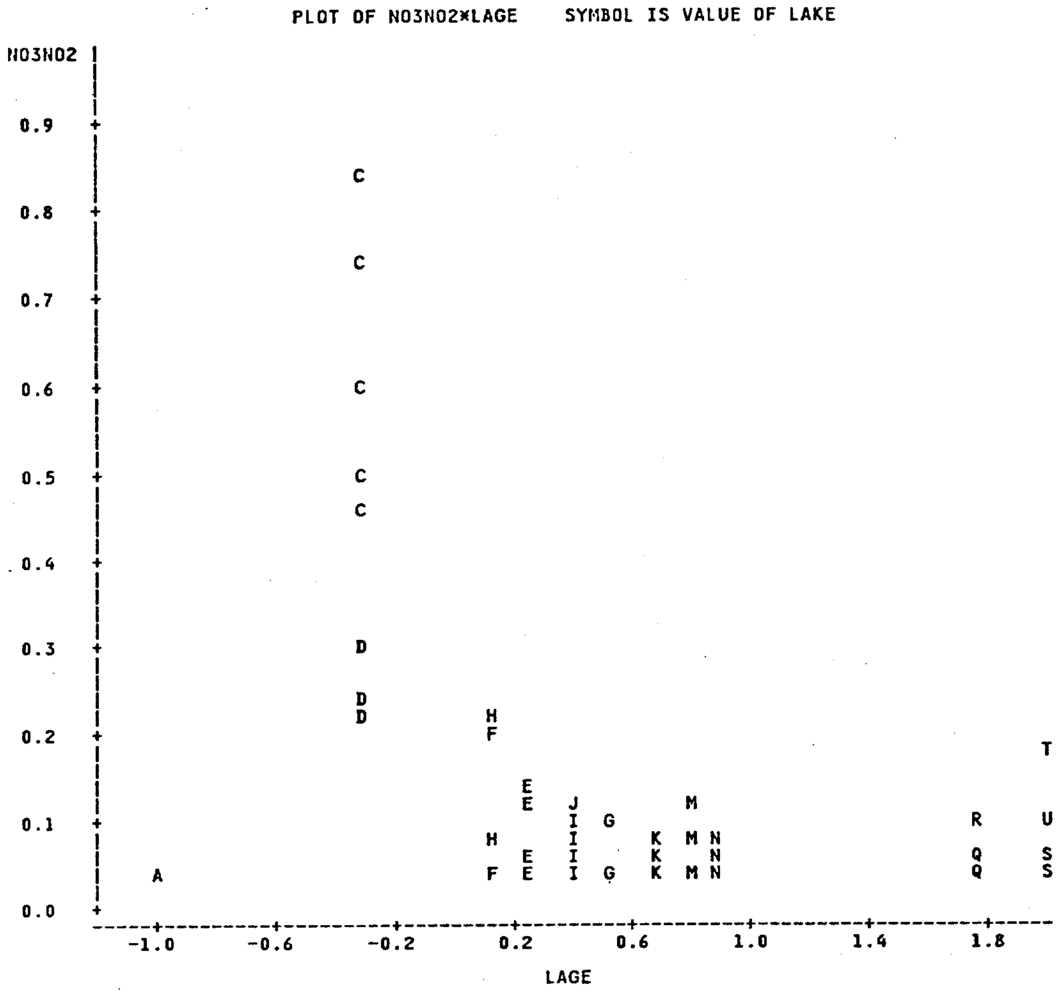

Fig. 35. The relationship of nitrate-nitrite and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (71 observations hidden).

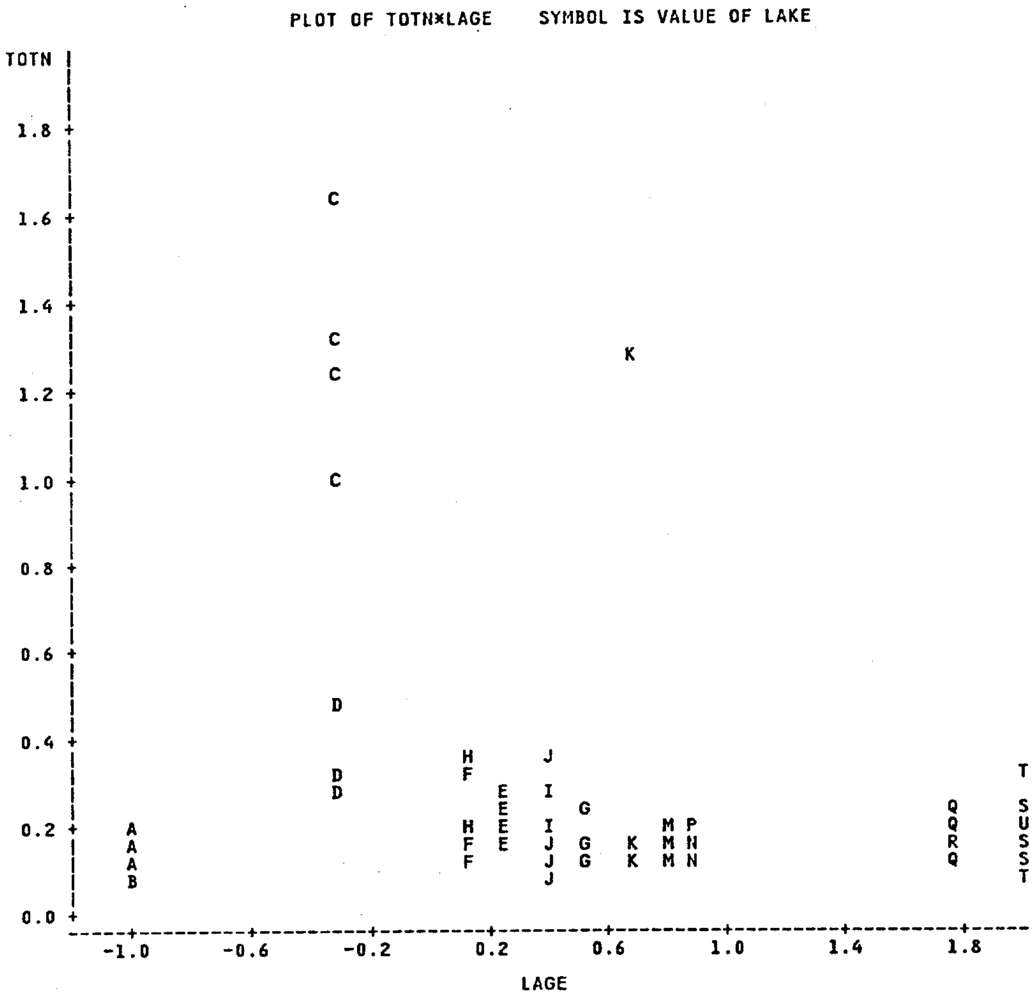

Fig. 36. The relationship of total nitrogen to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (57 observations hidden).

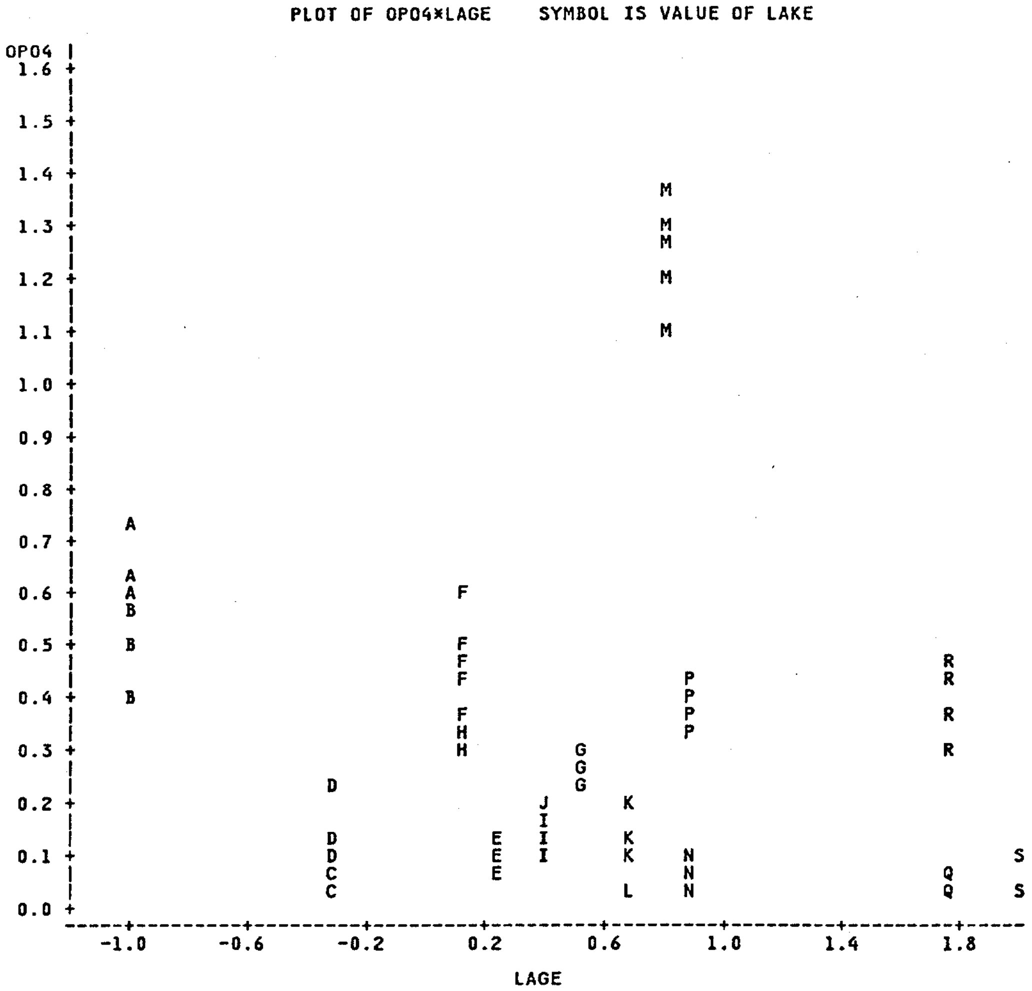

Fig. 37. The relationship of ortho-phosphate and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (53 observations hidden).

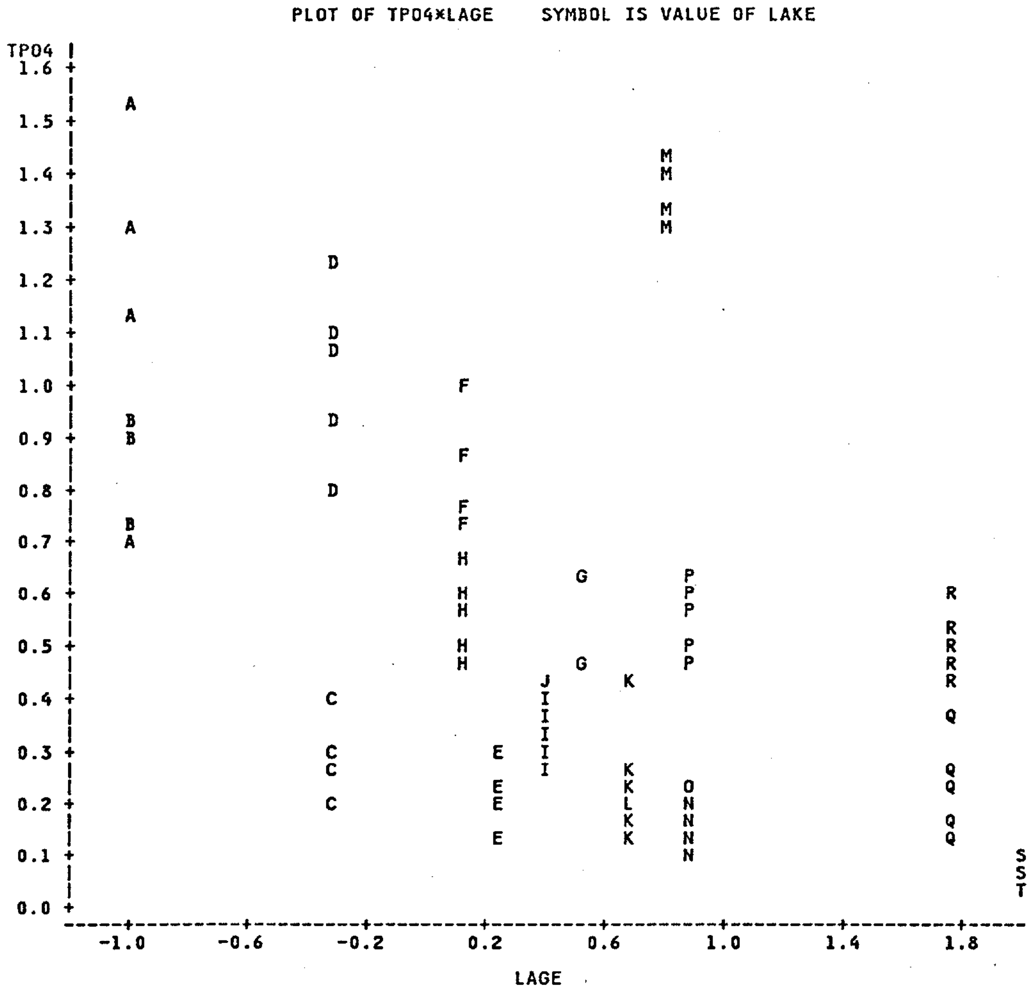

Fig. 38. The relationship of total phosphate and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (35 observations hidden).

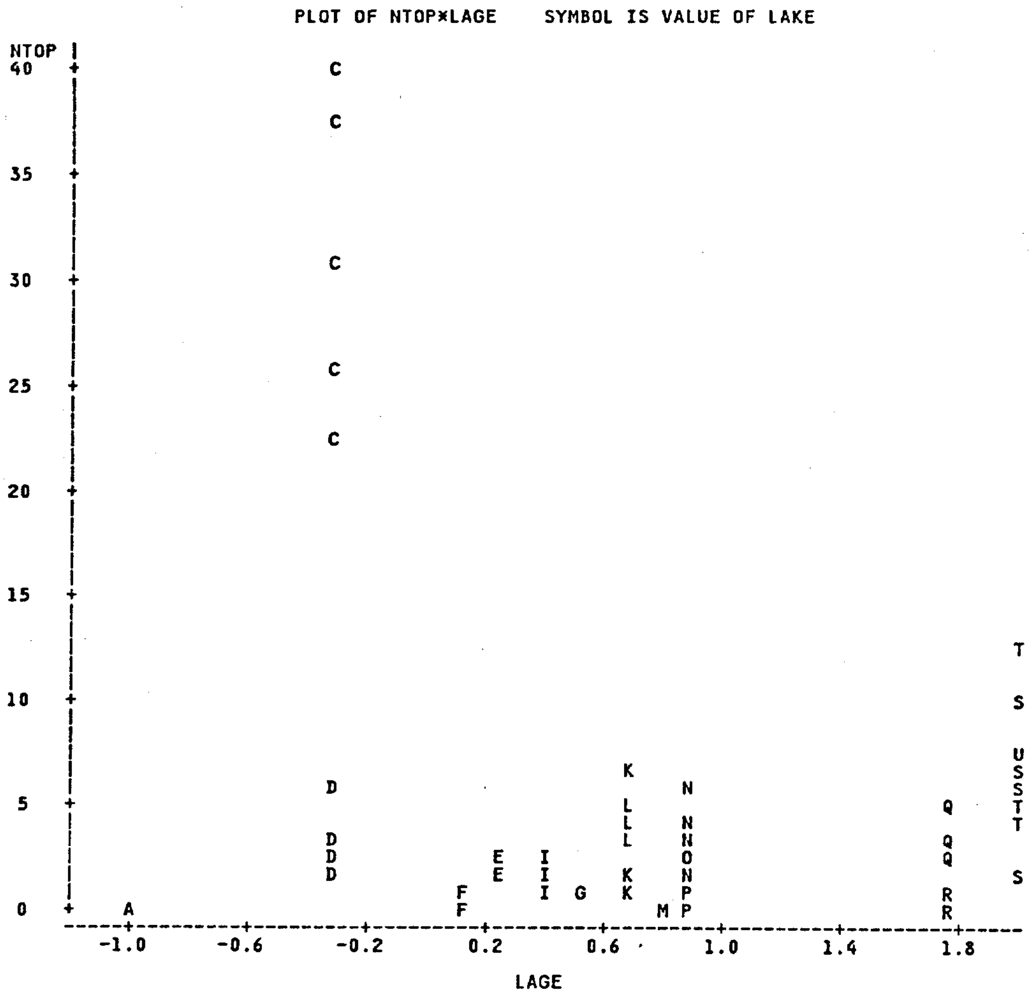

Fig. 39. The relationship of nitrogen to phosphorus ratio to the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (60 observations hidden).

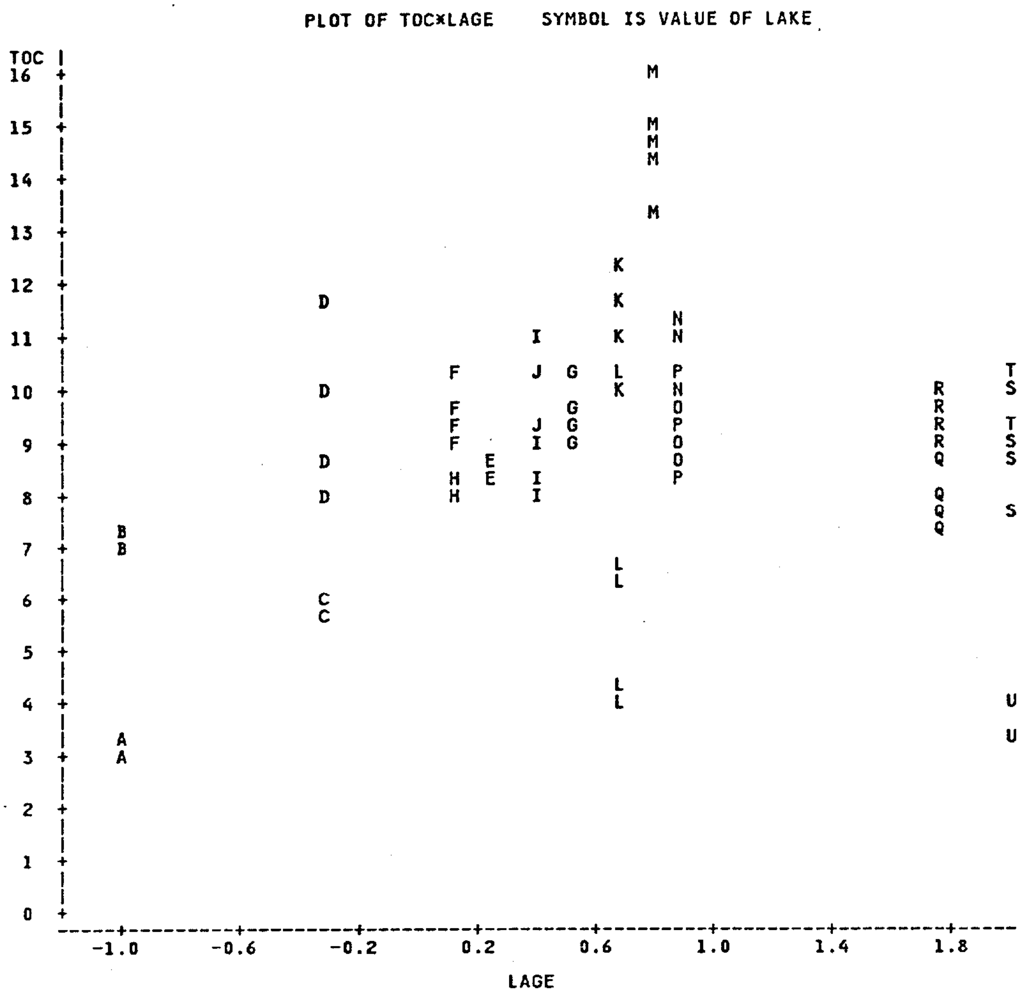

Fig. 40. The relationship of total organic carbon and the logarithm of lake age for the Fall 1984 sampling. Letters identify lakes (38 observations hidden).

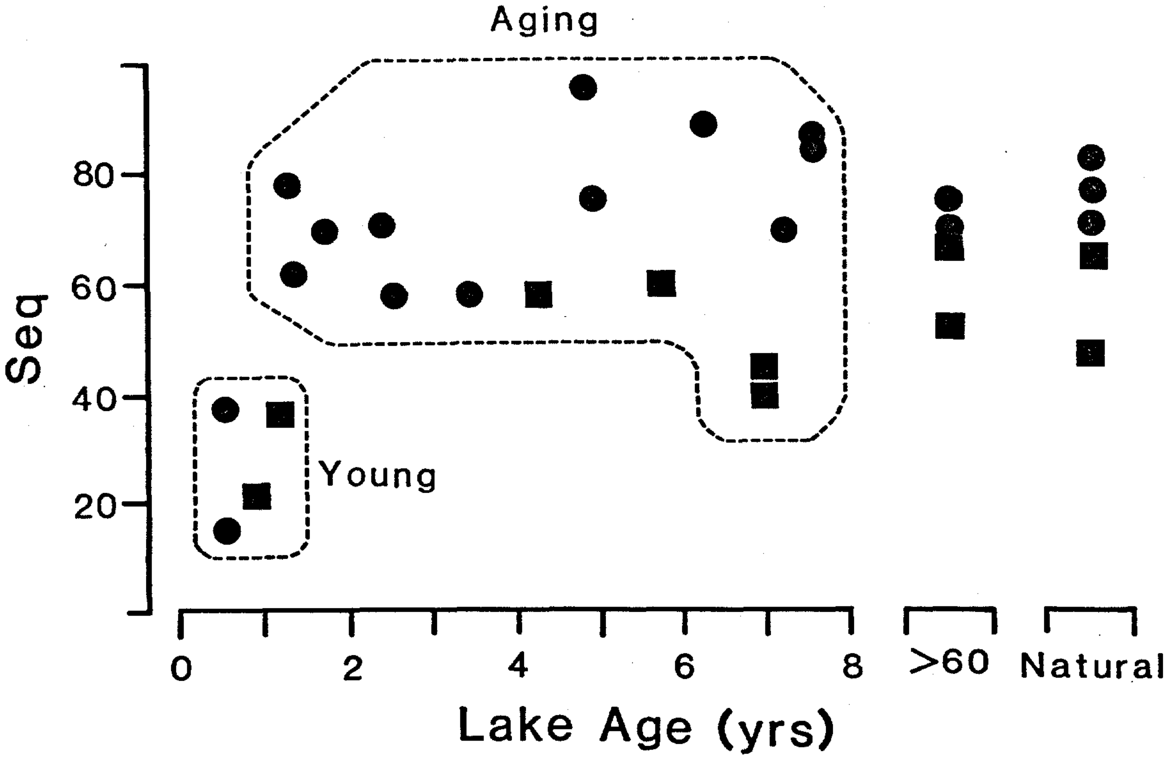

Fig. 41. Relationship of equilibrium species number for protozoans on artificial substrates and lake age. Squares are for Spring 1984 sampling, circles are for Fall 1984 sampling.

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