1989

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Recommended Citation

Shambaugh, Angela de, "Architecture and population dynamics of a subalpine benthic algal community" (1989). *Graduate Student Theses, Dissertations, & Professional Papers*. 7389.
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Architecture and Population Dynamics of a Subalpine Benthic Algal Community

By

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B.A., University of Montana, 1982

Presented in partial fulfillment of the requirements
for the degree of Masters of the Arts
University of Montana
1989

Approved by

Chairman, Board of Examiners

Dean, Graduate School

March 17, 1989
Date

4-13

Shambaugh, Angela de Ruiter, M.A. March 1989 Botany

Architecture and Population Dynamics of a Subalpine Benthic Algal Community (115 pp.)

Director: Richard P. Fritz-Sheridan RPFS

Benthic algal community structure was studied during one growing season (summer 1984) in a small subalpine lake in the Mission Mountain Wilderness, Montana. Preliminary investigations indicated that nitrogen fixation was occurring in the lake and that Cyanobacteria were present. Heterocystous Cyanobacteria are capable of nitrogen fixation, and thus can outcompete other algae in nitrogen-limited environments. Scanning electron microscopy, light microscopy, artificial substrates and acetylene reduction were used to determine whether benthic algal community structure, the spatial arrangement of individual algae to one another, was influenced by nitrogen limitation and the presence of Cyanobacteria.

The benthic community was dominated by non-heterocystous Cyanobacteria, but diatoms were also abundant. No three-dimensional architecture was identified by light or scanning electron microscopy although heterocystous Cyanobacteria were observed in the mat. Development of the benthic community upon the artificial substrates was initiated by individual diatoms and small pieces of the community carried to the surfaces. A secondary layer of filamentous algae and some stalked diatoms developed. Rudimentary tertiary layering was also observed. The mature community itself was an apparently random association of algae, detritus, and sand joined by overgrowth of algal and fungal filaments.

There appeared to be little succession occurring among algae in the mat. The same organisms were present throughout the observational period, although their densities were variable. Dominant genera were identical among the four sites and remained dominant throughout the study. The less abundant genera varied among sites. In general, the mat community was similar at the four sites.

Environmental conditions were similar among sites, nor did nutrient concentrations vary. Acetylene reduction was low, but highly variable. Because heterocystous Cyanobacteria were infrequent, low levels of reduction did not appear unusual.

The absence of heterocystous Cyanobacteria indicated that nitrogen limitation may not have been as severe as hypothesized. The low levels and large variability in acetylene reduction indicated that nitrogen fixation may not have been essential for survival. Nutrients available from decomposing detrital material may adequately supply the benthic algal community's growth requirements.

Acknowledgements

Many people were involved in the long process culminating in this manuscript. I can't thank them all, but a few deserve special thanks.

- Nat, thanks for the help with field and lab work, and putting up with me during this project.
- Dick Fritz-Sheridan, for providing the guidance I needed to finish this project while giving me "free rein."
- The University of Montana Biological Station for use of its facilities and equipment.
- George Starbuck and fellow employees of Aquatec, Inc. (South Burlington, Vermont) for the use of the computer facilities, their technical and moral support.

This research was supported in part by grants from the Sigma Xi Scientific Society and the Five Valley Audubon Society of Missoula, Montana.

Table of Contents

List of Tables

List of Figures

Architecture and Population Dynamics
of a Subalpine
Benthic Algal Community

1.0 Introduction

Algal communities have been investigated in great detail in the past, especially those of phytoplankton. Nutrition, composition, seasonal or temporal variation, and many other characteristics are described for phytoplankton in both marine and freshwater environments (see Wetzel 1983c for a review). This abundance of information regarding phytoplankton communities often cannot be applied to the evaluation of non-planktonic algal communities, e.g. periphyton, because of major differences in habitat. Lack of appropriate methodology and equipment to describe the physically more complex periphytic communities has hindered investigations into the relationship of these algae to one another and to their environment (Robinson 1983, Wetzel 1983a). In many studies, the periphytic material was scraped from the substrate for analysis (Gumtow 1955, Castenholz 1960, Jenkerson and Hickman 1983, Millie and Lowe 1983). This method allowed analysis of composition and seasonality of the periphytic communities, but could provide little information concerning community architecture, defined as the spatial arrangement of the elements within the periphytic community (Wetzel 1983a).

Understanding of periphytic communities began to expand beyond this rather superficial level of knowledge with the use of the scanning electron microscope (SEM), and sophisticated probes for measurement of physical conditions within the intact community. The SEM allowed investigators a view of the complexity of community architecture and, in conjunction with the use of artificial substrates, provided the means to elucidate its development (Hoagland et al. 1982, Hudon and Bourget 1981). Using microelectrodes, scientists were able to measure biological activity such as photosynthesis (Jorgenson et al. 1983) within the intact community. Significant progress has been made in recent years, but much more research is required before we can understand periphytic communities. The research described here provides further understanding of a benthic algal community, specifically describing seasonal composition, architecture, and the role nitrogen-fixing Cyanobacteria may have in the development of that architecture.

1.1 Importance of Nitrogen Fixation

Nitrogen fixation, the reduction of dinitrogen to biologically available ammonia, has been recognized in the last several decades as an important and beneficial biological phenomenon. The capability for nitrogen fixation is present in a wide variety of organisms, both terrestrial and aquatic. It has been demonstrated, for example, in the free-living photosynthetic bacterium <u>Rhodospirillum</u> (Stewart 1973), the nonphotosynthetic bacterium <u>Clostridium</u> (Skinner 1976), and the Cyanobacterium <u>Calothrix</u> (Jones and Stewart 1969).

Free-living nitrogen fixers are often said to contribute little to the global nitrogen cycle,but the Cyanobacteria are an exception to this rule (Bothe 1982). In an environment of limited nitrogen availability, nitrogen-fixing Cyanobacteria may represent a significant source of additional nitrogen, especially in systems of high nitrogen demand and low input (Stewart et al. 1977). Cyanobacteria provide significant nitrogen input in the Arctic tundra (Alexander 1974, Granhall and Lid-Torsvik 1975), temperate and tropical soils (Stewart et al. 1977), and aquatic systems (Flett et al. 1980).

Uptake of nitrogenous products released from Cyanobacteria has been documented for many organisms. Jones and Stewart (1969) demonstrated that labeled extracellular products of the marine Cyanobacterium _Calothrix_ _scopulorum_ were incorporated into the tissues of the two non-fixing algal genera _Ulva_ and _Porphyra_. Numerous lichen species are known to contain a symbiotic Cyanobacterium which supplies combined nitrogen to its host (Millbank and Kershaw 1970, Jordan 1972). _Azolla_ spp. also contain a symbiotic Cyanobacterium (Peters et al. 1981). The nitrogen-fixing capabilities of Cyanobacteria have been identified as important to many ecosystems as a whole, and further, may provide needed nitrogen for non-fixing organisms on an individual basis.

Within the confines of an algal community existing in an oligotrophic lake, the ability to fix atmospheric nitrogen into a usable form should provide a competitive advantage. In addition, competition for available nitrogen would exist among algae not capable of nitrogen fixation. There would be a distinct advantage for such non-fixing individuals to live in close proximity to one capable of fixation.

Paerl (1976) demonstrated that such relationships occur between phytoplankton and bacteria. Specifically, bacteria were found to prefer nitrogen-fixing heterocysts over non-fixing ones in blooms of Aphanizomenon flos-aquae and Anabaena circinalis. Heterocysts are deemed the major site of secretion by the Cyanobacteria. Paerl (1976) suggested that the uptake of amino acids released by the heterocysts occurred solely through the bacteria, and this uptake may actually stimulate nitrogen fixation. A similar physical relationship may be expected to occur between nitrogen-fixing Cyanobacteria and nitrogen-seeking algae or bacteria within a periphytic community.

1.2 Hypothesis and Objectives

The study lake, located in the Jim Lakes Basin of the Mission Mountain Wilderness, Montana, is oligotrophic with a benthic algal community consisting of a loose flocculent layer. Cyanobacteria formed a large portion of both the terrestrial algal community and the benthic mat (Dr. R. Fritz-Sheridan, personal communication; and personal observation). It is hypothesized that the heterocystous Cyanobacteria have a competitive advantage over, and also serve as a source of available nitrogen for, non-fixing algae under nitrogen-limited conditions. Therefore, the nitrogen-fixing Cyanobacteria might form the nucleus of an identifiable three-dimensional community in this habitat.

Several experiments were developed to test this hypothesis.

1. Confirmation of nitrogen fixation within the lake: Field experiments were designed to provide information about possible seasonal or locational trends of nitrogen fixation within the lake and to esti-

mate the importance of this process within the community.

2. Influence of four environmental factors upon the community: Light intensity, temperature, and the concentrations of nitrogen and phosphorus in the lake were monitored throughout the field season.

3. Identification of community architecture: This included observations of physical community development upon artificial substrates and the comparison of these to the natural community. Seasonal and location differences were investigated.

4. Identification of community structure: This provided data about the community and its dynamics as well as the importance of heterocystous Cyanobacteria. Homogenized communities from diverse locations were evaluated periodically throughout the season using similarity indices and visual evaluation of the light microscope data.

The data gathered through the above experimental objectives will provide insight into the influence of nutrient limitations on spatial architecture within algal communities. Cyanobacteria may play a large part in determination of community architecture, and the experiments will help identify the role of nitrogen-fixing Cyanobacteria.

2.0 Materials and Methods

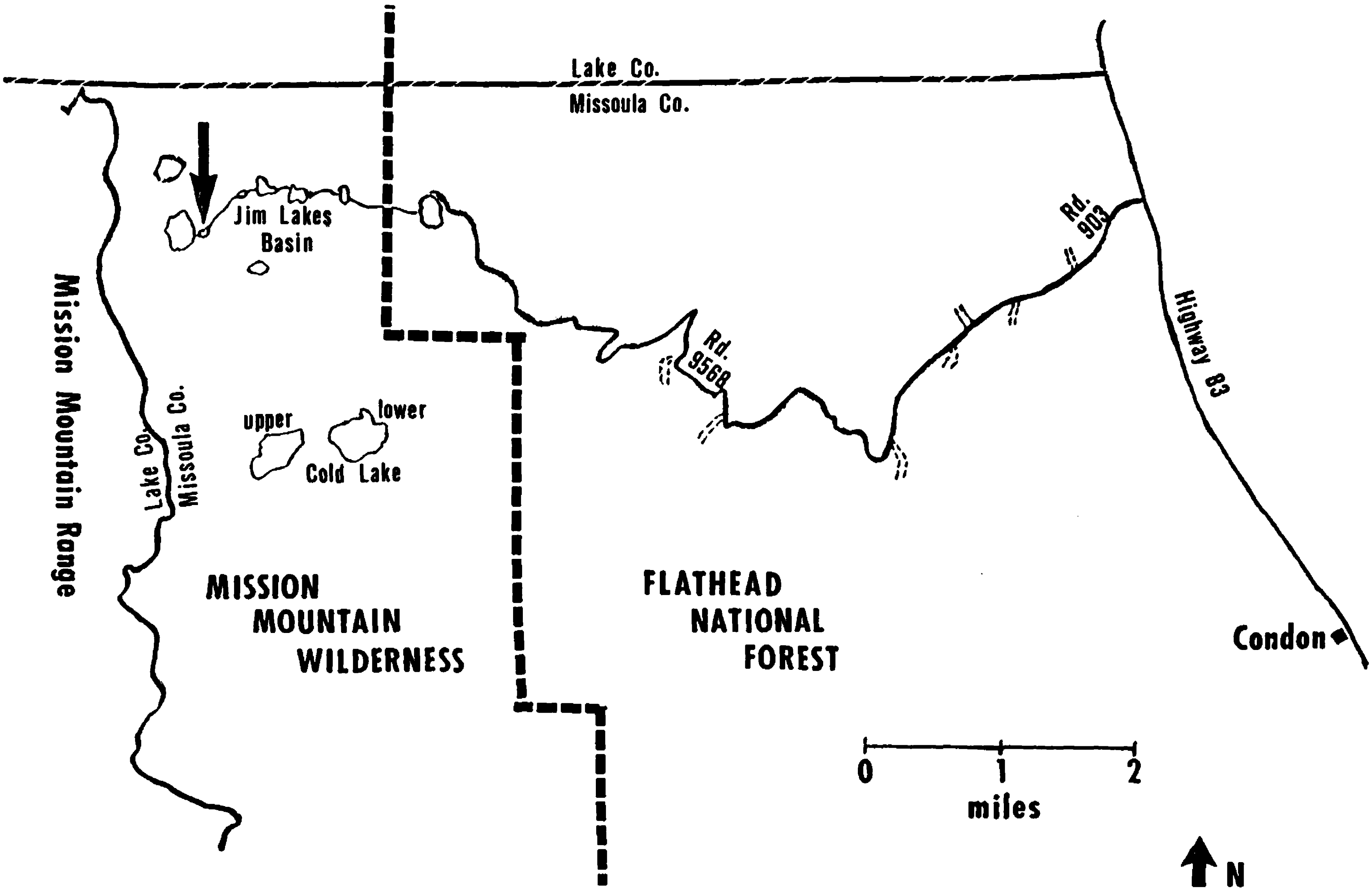
2.1 Study Area

The small, shallow lake (elev. 5560 ft.) chosen for this investigation of algal community structure is in the Jim Lakes Basin of the Mission Mountain Wilderness in northwestern Montana, near the town of Condon (Figure 1). Water flows into the lake from a larger deeper lake located to the west, and exits from the northern end. Water continued to enter the study lake from this stream throughout the experimentation period, but flow decreased considerably by mid-August. Water also entered the lake as rainfall from the frequent thunderstorms occurring throughout the period.

This study area was chosen for several reasons. First, it supported a luxuriant layer of benthic algae over a major portion of the lake bottom. The shallowness of the water allowed good algal growth and also access for sampling. Second, there was little growth of rooted aquatics in the lake. Patches of Isoetes spp. and also individuals of Ranunuculus spp. were noted scattered around the lake, but no emergent communities were observed. Except in these areas, the lake bottom consisted almost entirely of undisturbed benthic algal mat. Third, the remote location of this lake reduced the amount of interference and disturbance by humans which might have occurred at another more accessible lake.

The benthic community consisted of a loose flocculent material, easily disturbed by water movement. It remained greyish-green in color for the duration of the experimental period, except for small patches

Figure 1. Location of the study lake in the Mission Mountain Wilderness, Montana. The study lake is indicated by a large arrow at the upper left.


Lake Co.
Missoula Co.
Jim Lakes Basin
Mission Mountain Range
Lake Co.
Missoula Co.
upper
lower
Cold Lake
MISSION MOUNTAIN WILDERNESS
FLATHEAD NATIONAL FOREST
Rd. 9568
Rd. 903
Highway 83
Condon
0
1
2
miles
N

which were green or blue-green in color.

2.2 Field Sampling and Site Selection

Field sampling was conducted from early July to late September, 1984. Four sampling sites were established in the lake; each was thought to represent a different microhabitat (Table 1).

Because one aspect of this study was to observe formation and development of the benthic algal community in the lake, the first sampling period occurred as soon as possible after the spring thaw, July 4 to 6. The lake was no longer ice-covered, but the ground was still snow-covered in most areas. Subsequent trips were planned at one month intervals until snow prevented access to the lake. The first major snowstorm occurred on September 22, and the final samples were taken from the lake on September 30. At the end of the season, a total of four monthly sampling trips had occurred: July 4-6, July 25-27, August 24-26, and September 30. Another collection trip was made August 4-5 to replace data lost during the July 25-27 trip. In order to reduce disturbance to the especially fragile mat near site 2, this site was not measured during the August 4-5 trip. It was thought that sampling the site and reaching the island two weeks in a row would cause excessive damage to the benthic community. Each trip was planned to be two to three days in length, allowing time to gather sufficient data to characterize each sampling period. The early winter conditions prevented an extensive stay on September 30.

Table 1. Description of the four study sites chosen for investigation of benthic algal communities.

Site	Location	Depth	Special Features
1	northwest shore	1.0 m	-no aquatic plants -large baylike area -open shoreline -sedimentary bottom -algae are flocculent
2	small island in northwest portion of lake	> 2 m	-*Ranunculus* present -located near main channel -open shoreline -rocky bottom -algae are flocculent
3	south shore	< 1 m	-*Isoetes* present -directly east of inlet -trees near the shore -sediment bottom -algae firmly organized by August
4	east shore	1.0 m	-no aquatic plants -large shallow area -trees to water's edge -sediment bottom -algae loosely organized

2.3 Collection and Analysis Methodology

Four topics were evaluated in this investigation: nitrogen fixation, physical parameters, community composition and community architecture. The methodology for each is described separately below.

2.3.1 Nitrogen Fixation

Nitrogen fixation was measured daily during the monthly sampling trips. Individual sites were sampled in the morning and afternoon hours to obtain a good estimate of fixation occurring during the daylight hours. A modification of the acetylene reduction method (Fritz-Sheridan 1987) developed by Stewart et al. (1967) was used.

At each site, six to eight glass 8 ml blood serum vials were filled to two-thirds with pieces of the flocculent algal mat and water. Vials were sealed with rubber serum stoppers and 1 ml of acetylene gas injected into each. Vials were shaken gently to distribute the acetylene through the water and mat. Incubation was for a minimum of three hours *in situ*. Vials were placed horizontally in approximately 0.5 m of water. After the incubation period, the vials were shaken gently to distribute the ethylene evenly, and 1 ml of the gas removed from each vial with syringe. The syringes were sealed and transported back to the lab in the dark. Ethylene concentrations were analyzed within three days of collection using a Varian Aerograph gas chromatograph equipped with a Poropak R packed stainless steel column at 40°C. Chlorophyll *a* concentrations for all algae in each vial were determined spectrophotometrically (Shimadzu MPS-50L) by extracting in methanol for two days and applying the formula of Meeks and Castenholz (1971).

2.3.2 Physical Parameters

The physical factors monitored in this study were chosen because they influence levels of nitrogen fixation as well as algal growth. Four parameters were monitored: photon flux density, water temperature, and nitrogen and phosphorus concentrations in the water column. Photon flux density and water temperature were monitored at the four sites during acetylene incubation each day during the sampling trip. Photon flux densities ($umol \cdot m^2 \cdot s^{-1}$, PAR 400-700 nm) were measured at the water surface with a hand-held meter. Temperature was determined with a mercury thermometer, except for July 6. On this date, the thermometer was broken in transit, and temperatures had to be estimated. Water samples for nitrogen and phosphorus analysis were collected in acid-washed plastic bottles at the end of each monthly sampling trip, one bottle for each site. Bottles were transported in the dark back to the laboratory for analysis. Total phosphorus analyses were conducted within two days by Dr. R. Juday, (University of Montana) Department of Chemistry, utilizing methodology outlined by APHA (1985). Five ml subsamples for total nitrogen were frozen until analysis by nitrometer in the laboratory of Dr. N. Stark, (University of Montana) School of Forestry.

2.3.3 Community Composition and Architecture

Intact algal mat samples were collected from each site at the conclusion of the monthly sampling trips. These samples were used to determine species composition and also community architecture using SEM. Samples were transported back to the laboratory and fixed with 2 percent glutaraldehyde. Glutaraldehyde was chosen because it is an excellent fixative for specimens to be examined by SEM (Hoagland et al. 1982). For the analysis of composition, a composite sample for each site was made from several small pieces of mat weighing 3 to 4 grams (wet weight). This was homogenized in a minimum of 50 ml of water using a Branson Ultrasonic "Sonifier" Model L575 or a Whirltube test tube mixer. The amount of water used depended upon the concentration of material in the mat. From this homogenized sample, a minimum of three aliquots (0.5-1.5 ml) were taken using an automatic micropipette, placed in a Leitz plankton settling chamber, a small drop of Lugol's was added to enhance visibility, and stirred. After one hour, a coverglass was placed on the chamber and viable algae (those with intact internal structure) were counted using a Wild inverted microscope. A minimum of 15 fields, the minimum number required to achieve the equal variance assumption for statistical analysis, were counted at 200x in each chamber. To simplify the comparisons between samples, the total number of viable individuals observed was converted to numbe of algae/gram of intact mat (wet weight) using a modification of the procedure to obtain number/ml of phytoplankton (APHA 1985):

$$\text{number per gram} = \frac{C_x \times A_c}{F \times A_f \times (G/M \times V)}$$

where,

C_x = number of organisms counted (genus or species)

A_c = area of the settling chamber (530.93 mm^2)

F = number of fields counted

A_f = area of the field (mm^2)

G = grams of algal mat used, wet weight

M = water used for the dilution of algae (ml)

V = volume used in the settling chamber (ml)

For SEM examination of community architecture, small pieces of intact mat, preserved in 2 percent glutaraldehyde, were rinsed in Millonig's buffer (Millonig 1961) three times for 15 minutes each, stained for one hour in osmium tetroxide, and rinsed again in Millonig's buffer three times. The pieces were then run through a dehydration series of alcohol: 30, 50, 70, and 95 percent ethyl alcohol for ten minutes each. Finally, the samples were placed in 100 percent ethyl alcohol for three 10 minute rinses. A Samdri critical point dryer was used to dry the pieces, which were then attached to aluminum SEM stubs using colloidal silver paint. After drying one hour, the samples were coated with approximately 300 angstroms of gold-palladium using an SPI sputter apparatus. The sputter coating could be reapplied as necessary to achieve good resolution on the SEM. Coated samples were observed for community architecture using a Zeiss Novascan 30 scanning electron microscope. Micrographs were taken on

Polaroid Type 55 positive/negative Landfilm.

2.3.4 Artificial Substrate Colonization

Artificial substrates were glass and plastic microscope coverslips, 18 mm in diameter. The surface of the coverslips were roughened with fine grain Carborundum powder to facilitate the attachment of algae. Three coverslips were attached to a standard glass microscope slide using non-toxic aquarium sealer. Ten slides holding glass coverslips and ten holding plastic were placed on a platform at each of the four sites. Coverslips were wiped with alcohol to remove any prior inoculum and rinsed with lake water before being placed on site. Platforms were located in approximately 1 to 2 meters of water at each site. This allowed lake levels to drop without exposure of the slides and coverslips, and minimized the disturbance from people or animals. In addition, the floats were located 3 to 4 meters from the shore, reducing the chance of inoculation by particles disturbed from extremely shallow water areas by wave action. Floats were in place on July 6, 1984.

Two microscope slides of each type of coverslip, 12 total, were removed from each site at the end of every sampling period. Slides were placed in a slide box for transportation back to the laboratory. High humidity was maintained in the boxes by placing water-saturated pieces of foam rubber in each. At the lab, coverslips were immediately removed from the microscope slides, fixed in 2 percent glutaraldehyde, and stored in a refrigerator until prepared for SEM or light microscope analysis.

Coverslips were prepared for light microscopy by scraping the entire surface with a sharp razor blade to remove all attached cells. The scraped wet material was weighed, placed in a small amount of water (8-25 ml), and homogenized as described above for the intact mat. The procedure for analysis of the coverslip material follows that of the intact mat from this point. Organism numbers were determined per gram wet weight rather than number per mm surface area to allow better comparison of the natural and artificial communities.

Preparation of the coverslips for SEM observations followed the procedures outlined for the benthic samples except for the following. Because the layer of material on the coverslip was thin, the treatment time allotted for each step was reduced by two-thirds, i.e. five minutes in Millonig's buffer. After critical point drying, the coverslips were attached to the aluminum stubs with double-sided tape (3M Corporation). Silver colloidal paint was placed on all four sides, and used in addition to ground the coverslip to the stub. Sputter coating was achieved in the manner outlined above, as were the observations of the material.

2.3.5 Community Similarity Indices

Of particular interest was the comparison of the communities found at the four sites with one another and also over time. Two similarity indices were chosen for this purpose. Both are modifications of the index developed by Jaccard (1912) to determine the similarity of alpine vegetation based on presence or absence of individual species or genera:

$$\text{percent similarity (Jaccard)} = \frac{C}{A + B + C} \times 100$$

where,

C = number of genera common to the two communities being compared,

A = number of genera restricted to the first community,

B = number of genera restricted to the second community.

The modified indices chosen use quantitative data, e.g. biomass or cell number rather than the number of genera observed, to determine the percentage of similarity. These should be more sensitive to differences in communities because of their sensitivity to algal abundance. Gleason (1920) substituted data directly into Jaccard's index. Using the data available from the present study, the number of individuals observed in each genus, Gleason's index is

$$\text{percent similarity (Gleason)} = \frac{N_c}{N_a + N_b + N_c} \times 100$$

where,

N_c = number of individuals in the genera common to both communities,

N_a = number of individuals in the genera restricted to the first community,

N_b = number of individuals in the genera restricted to the second community.

Ellenberg (1956, as cited in Mueller-Dombris 1974) modified Jaccard's index to remove the double-weighting accorded to genera occurring in both communities. The index used in the present study is

$$\text{percent similarity (Ellenberg)} = \frac{N_c/2}{N_a + N_b + N_c/2} \times 100$$

where,

N_c = number of individuals in the genera common to both communities,

N_a = number of individuals in the genera restricted to the first community,

N_b = number of individuals in the genera restricted to the second community.

The two indices differ only in the elimination of double-weighting by Ellenberg. Rather than choose one or the other for this study, both were used for data analysis. It was of interest to note whether the double-weighting of common individuals would greatly affect the similarity of the communities when compared to an analysis where this did not occur.

Computer programs were written by Dick Lane(University of Montana Computer Center) to run the two indices using the numerical data gathered by light microscopy. An updated version of SPSSx, available to Mr. Lane, was used to perform average linkage cluster analyses of the percent similarity values.

3.0 Description of the Study Lake

Four physical parameters were measured at each site during the field season: photon flux density, water temperature, total nitrogen and total phosphorus. During the August 4-5 sampling trip, site 2 was not sampled. This was done to avoid excessive disturbance of the benthic community between the island and the shore. No information concerning light, temperature or nitrogen fixation was collected on this date at this site. On September 30, ice initially prevented access to sites 2 and 3. Because of this, nitrogen fixation, light and temperature were not measured. Algae and water samples were collected, however.

3.1 Photon Flux Density

Table 2 presents the average photon flux densities ($umol \cdot m^{-2} \cdot s^{-1}$, PAR) for each site during each sampling period. The highest PAR (1800 $umol \cdot m^{-2} \cdot s^{-1}$) were received by the lake early in the summer, generally July 4 to 27. After this period, average PAR dropped during August and September (to approx. 350 - 800 $umol \cdot m^{-2} \cdot s^{-1}$). The exceptionally low averages (approx. 460-590 $umol \cdot m^{-2} \cdot s^{-1}$) for all sites during the August 4 to 5 sampling period were due to a storm system that remained over the lake during the entire period. The skies were heavily overcast, and frequent rains occurred. The low values observed for August 24 to 26 were due to overcast skies. September 30 was cloud-free and photon flux densities were high at the two sites measured.

Table 2. Average light intensities (umol·m^{-2}·s^{-1}, PAR) for each collecting period by site. Maximum and minimum intensities observed at each site are indicated.

Date	Site	Maximum	Minimum	Average
7/6	1	1800*		
	2	1800*		
	3	1800*		
	4	1800*		
7/25	1	>1800	180	1058
	2	>1800	108	1083
	3	>1800	32	658
	4	>1800	108	774
8/4	1	666	414	540
	2			
	3	1080	108	594
	4	864	57	461
8/24	1	1296	396	720
	2	1080	522	738
	3	900	63	351
	4	1062	36	300
9/30	1	900	720	810
	2			
	3			
	4	828	65	446

* only one measurement was made

3.2 Water Temperature

Mean water temperatures (°C) for each site and sampling period are presented in Figure 2. Mean water temperatures at the various sites were within one or two degrees of each other during any single sampling trip. The range of temperatures observed over the day at any particular site during a single trip, however, varied by as much as seven degrees (i.e. site 3, August 24 to 27).

The overall seasonal variation in temperature also was predictable. Lowest temperatures occurred at the beginning and the end of the growing season, early July and late September respectively. Peak temperatures occurred in late July (20 to 22°C), slightly higher than those observed in early August (16 to 18°C). The daily range of observed temperatures was much larger in late August than in late July.

3.3 Total Nitrogen

Total nitrogen levels (ppm) in the water at each site over the growing season are presented in Figure 3. Nitrogen levels also were measured in the outlet stream, serving as an indication of overall nitrogen in water having passed through the lake. Nitrogen remained low during the entire observation period, never rising above 0.5 ppm (site 1, July 27). The lake outlet on July 27 was considerably higher than any other observations (1 ppm). Phosphorus, sampled from the same bottle, was also exceptionally high (0.188 ppm). Such anomalous values were considered due to sampling error and not included in the evaluations.

Figure 2. Mean temperature by site with observed range. Range is indicated by brackets.

Site 1 ▲

Site 2 ◇

Site 3 ▽

Site 4 □

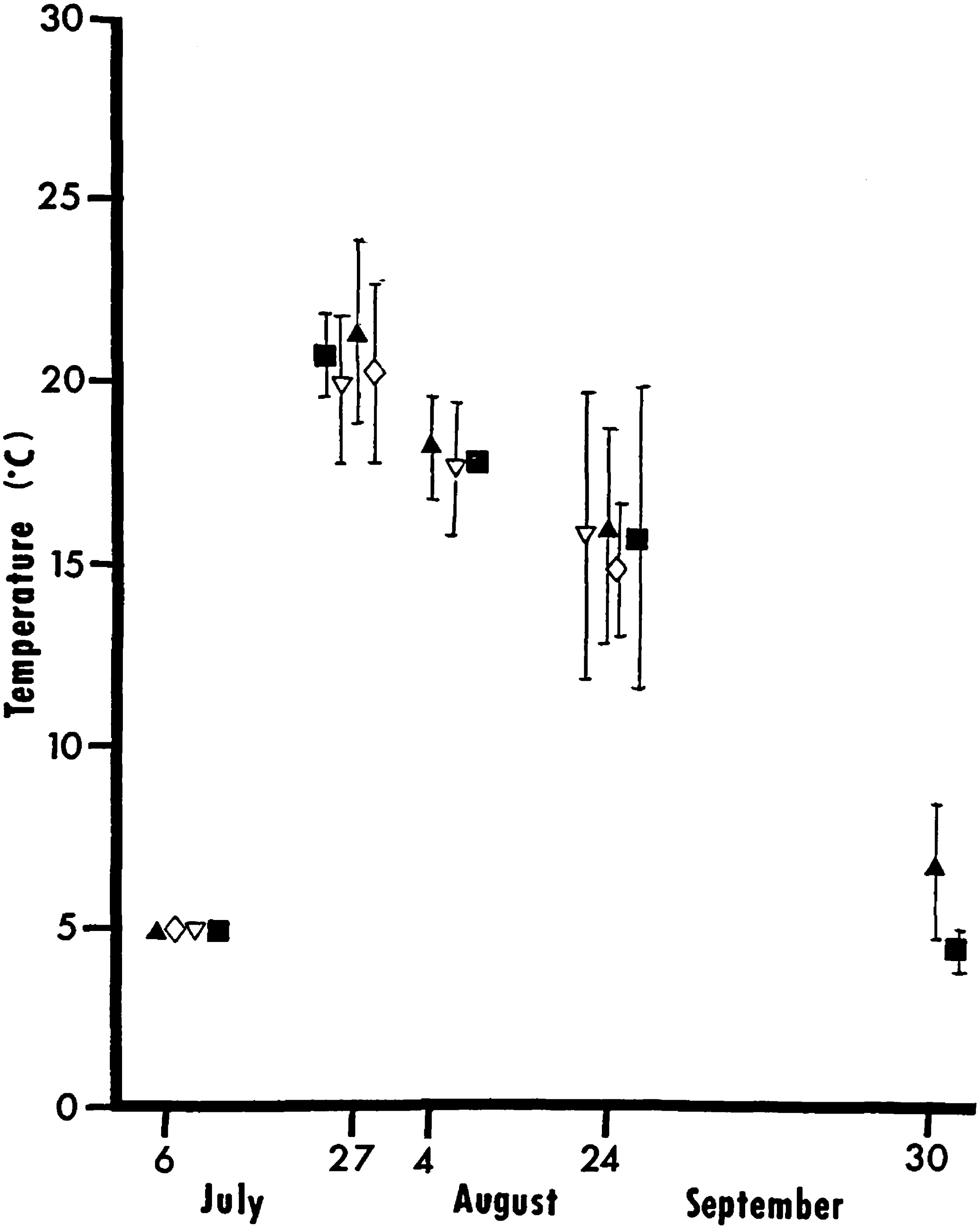

30
25
20
15
10
5
0
Temperature (°C)
6
27
4
24
30
July
August
September

Figure 3. Total nitrogen concentration of water (ppm) by site including outlet. A single sample was collected at each site per trip.

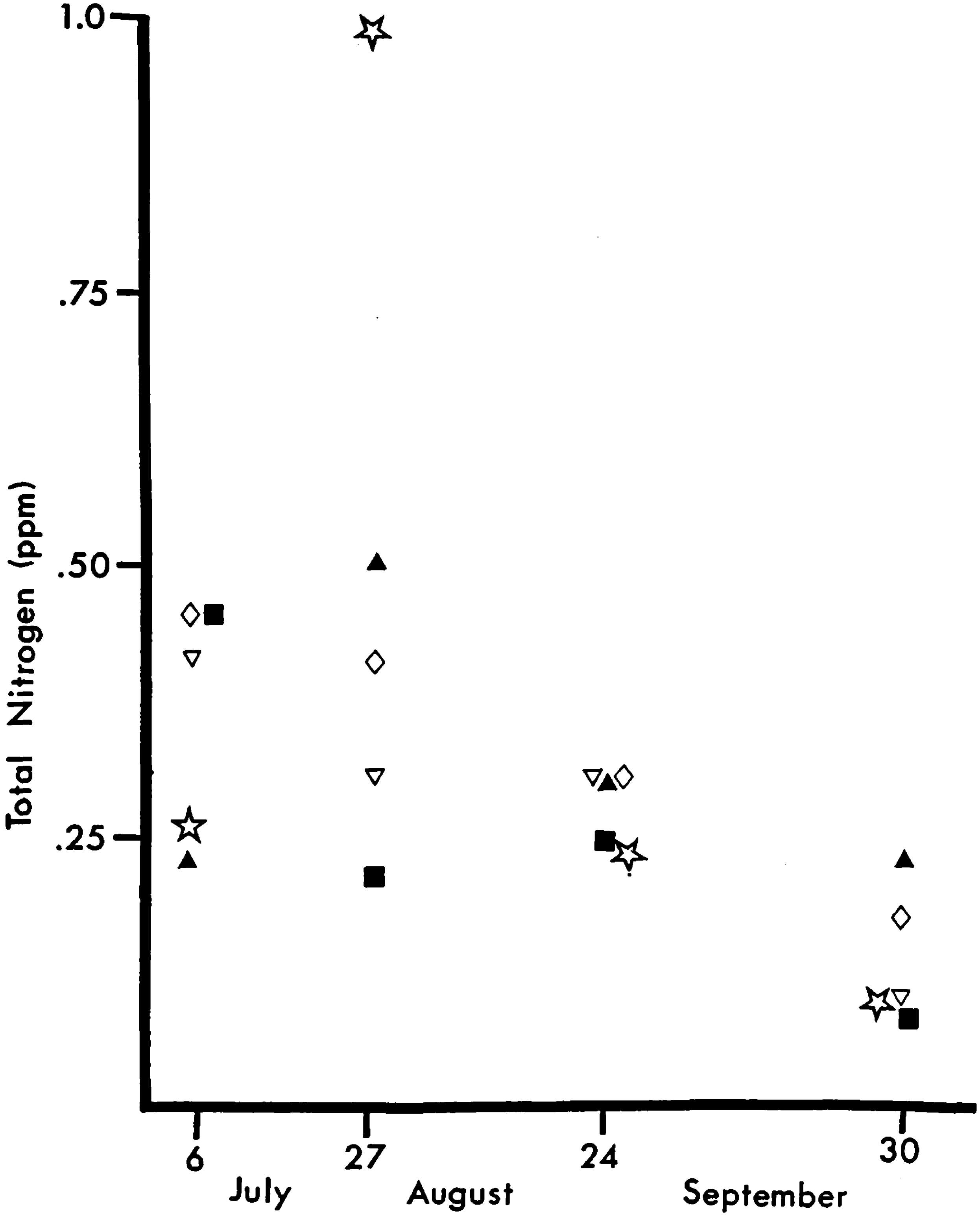
Site 1 ▲

Site 2 ◇

Site 3 ▽

Site 4 ■

Outlet ☆


Total Nitrogen (ppm)
1.0
.75
.50
.25
6
27
24
30
July
August
September

The lowest levels of nitrogen were generally found in the outlet sample, but all samples were characteristic of nitrogen levels in oligotrophic lakes, 0.307 to 1.63 ppm (Wetzel 1983c). Generally, the highest levels of nitrogen observed were at sites 1 and 2, the single exception being a low value at site 1 on July 6. Because of its location in the main channel of water flow through the lake, site 2 was expected to have slightly higher levels of nitrogen and this appeared to be true all through the period. The lower levels of nitrogen in the outlet waters were expected after passage through a shallow lake with high numbers of non-fixing algae.

The largest differences in nitrogen levels between sites occurred in the early part of the growing season. During this time, the levels varied by as much as 0.3 ppm (i.e. between site 1 and site 4 on July 27). At the end of the season, nitrogen levels were much more similar, varying at most by 0.1 ppm between sites.

For all sites, with the exception of site 1 and the outlet, highest levels of nitrogen were observed in early July, shortly after the spring thaw. Algal growth should be just beginning at this time, and nutrients added to the water column from material decaying during the winter would be at highest levels. As growth progressed during the summer, the nitrogen levels in the water column would be expected to drop. Site 1 is an exception to this, having exhibited rather large fluctuations in the early summer months. No explanation for this was found, perhaps only some type of sampling-induced error on July 6.

3.4 Total Phosphorus

Figure 4 presents total phosphorus levels in ppm for each sample site and the outlet during the experimentation period. A questionable level of 0.188 ppm was observed in the effluent on July 28 and for reasons outlined above, was not included in the evaluations. The remaining phosphorus values observed in the lake were very low, near the point of detection for the method used. Total phosphorus levels from 0.003 to 0.0177 ppm are considered to be characteristic of oligotrophic lakes (Wetzel 1983c). Although levels observed for site 1 on July 24 exceeded this range slightly, phosphorus levels were well within the range associated with oligotrophic conditions during the remainder of the field season.

Differences in observed phosphorus levels at sample sites during a single trip were very small (>0.003 ppm). Collections on July 6 and 27 were essentially identical (approx. 0.004 and 0.006 ppm, respectively). Differences in phosphorus levels between sites on August 24 were more distinct, i.e. 0.014 ppm between site 1 and the outlet. Levels of total phosphorus in the water were consistently low (oligotrophic levels) for each site throughout the period. This suggests that the lake can be considered phosphorus-limited during the entire summer season.

3.5 Nitrogen Fixation

Mean rates of acetylene reduction (nM $C_2H_4 \cdot$mg Chl$\underline{a}^{-1} \cdot hr^{-1}$) for the 1984 season are presented in Figures 5a and 5b. Temporal and loca-

Figure 4. Total phosphorus concentration of water (ppm) by site including outlet. A single sample was collected at each site per trip.

Site 1 ▲

Site 2 ◇

Site 3 ▽

Site 4 ■

Outlet ☆

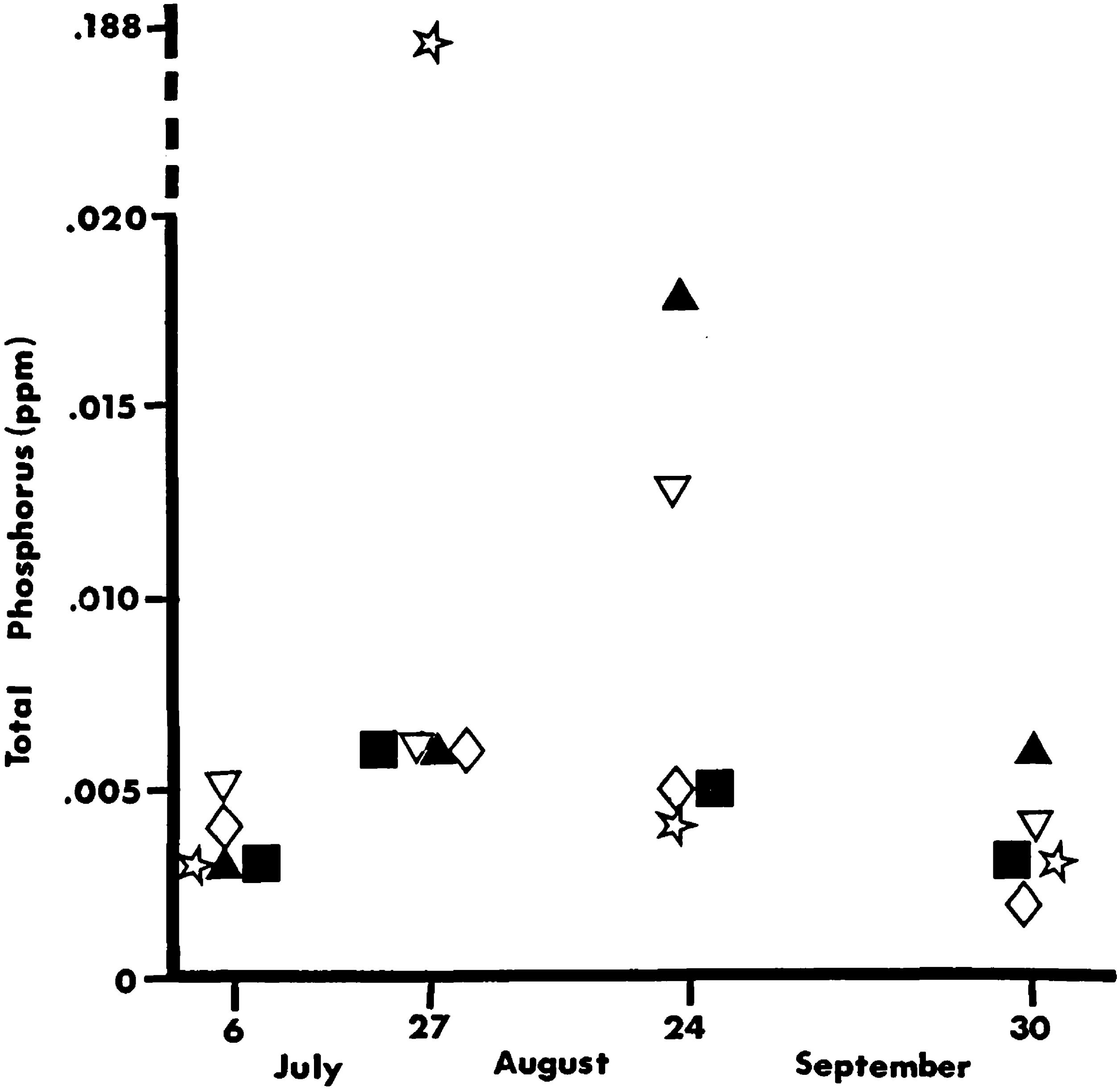

.188
.020
.015
.010
.005
0
Total Phosphorus (ppm)
6
July
27
August
24
September
30

Figure 5a. Acetylene reduction rates by benthic algal communities July to September 1984, at sites 1 and 2. Rates are presented as average values for the weekend indicated. Brackets indicate 1 standard deviation where calculated.

Morning ○

Afternoon △

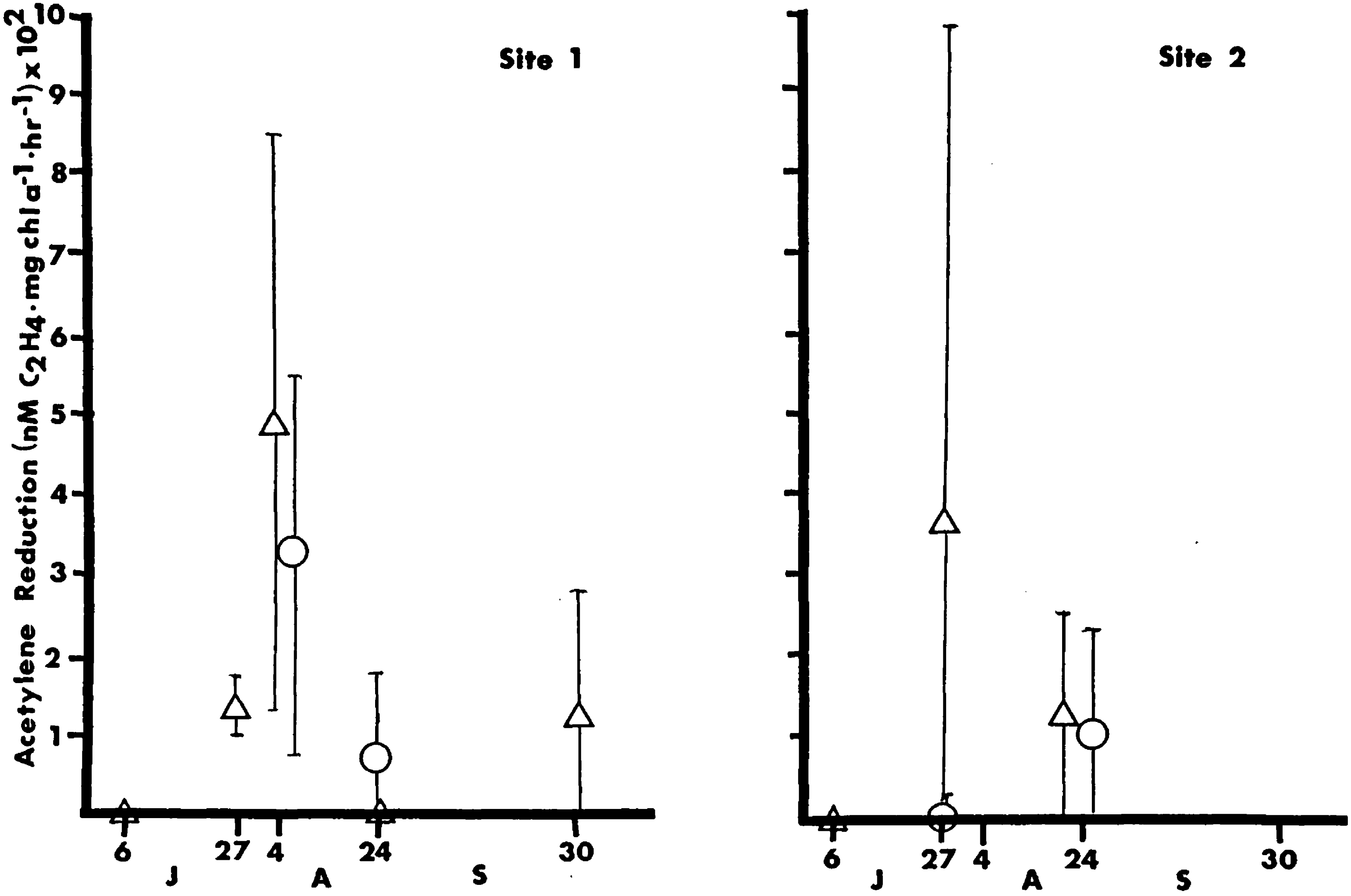
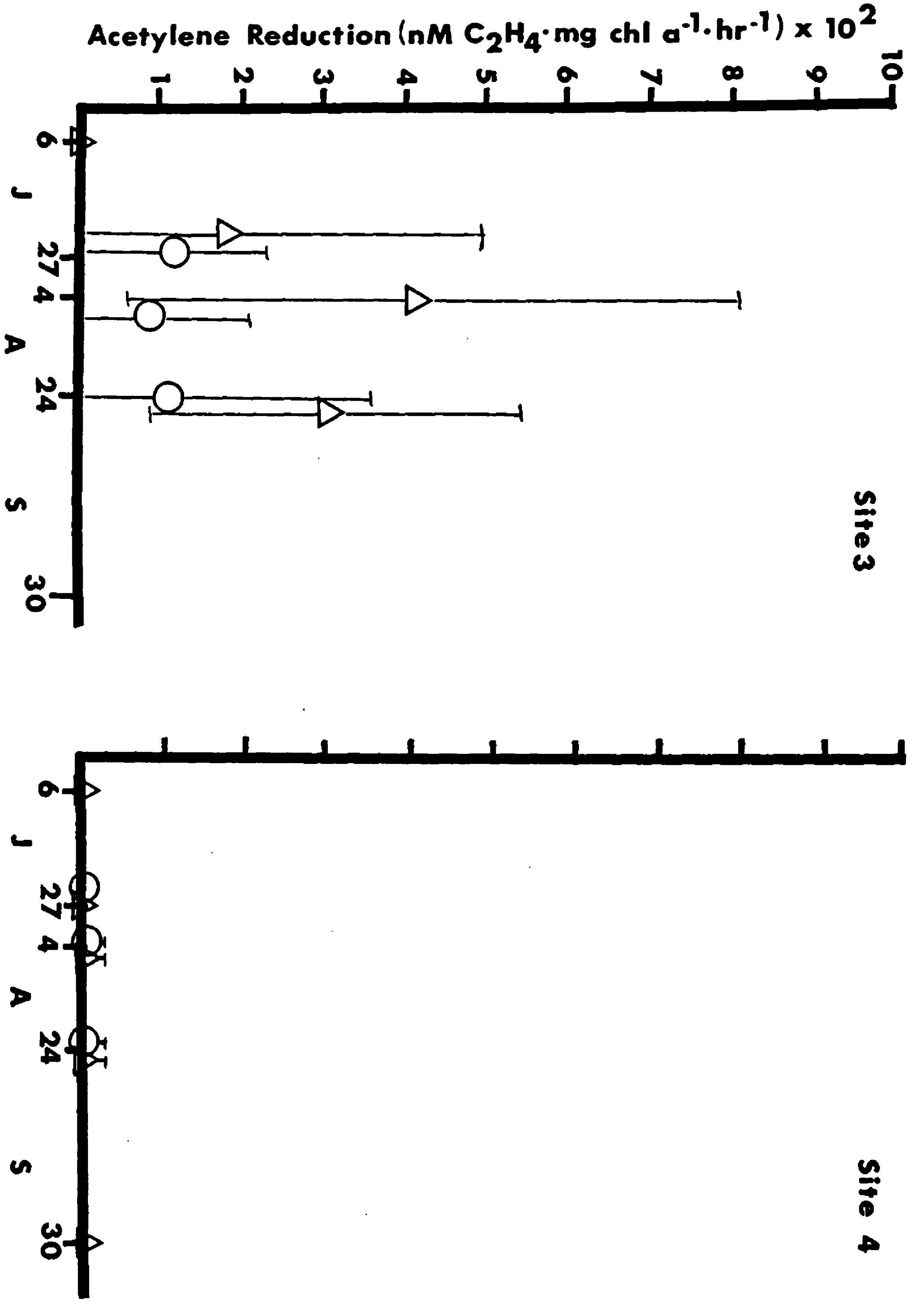

Acetylene Reduction (nM $C_2H_4 \cdot mg\ chl\ a^{-1} \cdot hr^{-1}) \times 10^2$
10
9
8
7
6
5
4
3
2
1
Site 1
6
27
4
24
30
J
A
S
Site 2
6
27
4
24
30
J
A
S

Figure 5b. Acetylene reduction rates by benthic algal communities July to September, 1984, at sites 3 and 4. Rates are presented as average values for the weekend indicated. Brackets indicate 1 standard deviation where calculated.

Morning ○

Afternoon △


Acetylene Reduction (nM C_2H_4·mg chl a^{-1}·hr^{-1}) x 10^2
1
2
3
4
5
6
7
8
9
10
Site 3
Site 4
6
J
27
4
A
24
S
30

tional differences remain identical whether viewed as nitrogen fixation or acetylene reduction.

Observed rates of reduction were highly variable both between and within sites (note the large standard error, particularly at site 2 on July 27). Later analyses showed that the occurrence of heterocystous Cyanobacteria was variable in the samples, and this would contribute to the variance observed in acetylene reduction (see Chapter 4). For this reason, average reduction rates were used to characterize the sites.

All sites had negligible acetylene reduction in early July. In late July, highest afternoon rates occurred at site 2 while those at site 1 and 3 did not appear to differ. Morning rates at site 2, however, were less than those at site 3. In early August, afternoon rates were similar at sites 1 and 3, but morning rates were lower at site 3. During late August, rates were similar at sites 1 through 3 with one exception. Afternoon reduction was much higher at site 3. Site 4, in contrast to the other sites, exhibited little to no acetylene reduction during the entire summer.

While large amounts of variability were identified, it was difficult to evaluate the significance of the observed differences. In spite of this, some trends in acetylene reduction, and therefore nitrogen fixation, could be identified. Except for site 4, where little to no fixation occurred, rates followed similar patterns between sites. In general, morning rates were lower than afternoon rates, but occasionally these were similar. At site 3 morning rates were similar all through the summer. Peak nitrogen fixation occurred during late July and early August.

3.6 Physical Factors and Nitrogen Fixation

Correlation coefficients for the regression analyses of acetylene reduction versus photon flux densities, temperature, and nutrient levels are presented in Table 3. When stations 1 to 4 were considered together, there was essentially no linear relationship between acetylene reduction and the four variables examined.

No linear relationships were evident when the sites were considered individually. Of the four environmental parameters evaluated, temperature had the most influence upon acetylene reduction, correlation coefficients up to 0.48. The other parameters had correlation coefficients generally lower than those for temperature.

3.7 Discussion

Acetylene reduction occurred at measurable levels in the study lake from early summer to late fall. No nitrogenase activity was observed during the spring sampling period (July 6). Rates varied with date and location in the lake. Heterocystous Cyanobacteria were observed at all locations, including those sites with low rates of nitrogenase activity. Apparently, the presence of nitrogen-fixing genera does not dictate continuous ethylene production.

Total phosphorus concentrations in the water column generally did not vary among sites or between sampling periods. Nitrogen concen-

Table 3. Correlation coefficients of acetylene reduction with light, temperature, nitrogen concentration and phosphorus concentrations.

All Sites (n=17), using average values for each sampling trip.

Variable	Coefficient
Light	-0.36
Temperature	0.51
Nitrogen	0.27
Phosphorus	0.21

Individual Sites

	Sites			
Variable	1	2	3	4
Light	-0.27	-0.36	0.21	-0.32
Temperature	0.31	0.44	0.48	0.03
Nitrogen	0.31	-0.07	-0.28	0.08
Phosphorus	-0.29	0.35	0.14	0.05
n =	8,10	6	9-11	9,10

tration was more variable. These elements are considered essential for algal growth and reproduction. Phosphorus deficiency has been documented to affect Cyanobacterial morphology (Livingston and Whitton 1983, Livingston et al. 1983) and physiology (Healey 1973, Healey and Hendzel 1975). Low levels of nitrogen are known in some species of Cyanobacteria to stimulate the formation of heterocysts and production of heterocysts has been shown to be dependent upon the absence of combined nitrogen and the presence of phosphate (Ogawa and Carr 1969).

The role of phosphorus in the lake was not clearly identifiable when the entire set of sampling data was considered. Phosphorus concentration did not vary significantly throughout the season, and thus no significant correlation with the highly variable acetylene reduction rate could be established. Nor was any relationship between nitrogen fixation and nitrogen concentration detected for the lake or the individual sites.

It is also possible that, while the water column appeared to be nitrogen- and phosphorus-limited based upon values provided by Wetzel (1983c), the benthic community under observation was not. Its location on the lake bottom, in close proximity to decaying materials, may have provided nutrients from a source unavailable to organisms inhabiting the water column (Wetzel 1983b). The flocculent material contained a great deal of detritus (personal observation) and may have provided enough nitrogen and phosphorus to support any algae growing in or on them. Mud from the Loosdrecht lakes, the Netherlands, was able to support growth of <u>Scenedesmus</u> <u>obliquus</u> without additional phosphate (Golterman et al. 1969) and benthic Cyanobacteria observed to incorporate labeled $^{15}NH_4$ absorbed from the sediments of an oligotrophic lake (Jansson 1980). The actual process of recyling nutrients such as phosphorus or nitrogen by detrital decomposers has not yet been sufficiently described, thus it is unclear how these nutrients become available for uptake by organisms such as algae (Pomeroy 1980).

The possibility exists that the benthic algal community was not experiencing nutrient limitations to the extent that water column values might suggest. Some degree of nitrogen deficiency seems likely because heterocyst formation and nitrogenase activity were observed. Increasing rates of acetylene reduction, which occurred later in the field season, may have been stimulated by decreases in nutrient availability from the sediment as it was depleted by algal growth. Because nitrogen and phosphorus concentrations of the sediment waters were not determined, it was impossible to confirm this theory for the benthic community. The variation in levels of acetylene reduction might possibly be the result of varying levels of nutrient sufficiency within the confines of the benthic material.

The other two environmental factors monitored in this study, light intensity and temperature, are known to have regulatory effects upon nitrogen fixation. Temperature was not anticipated to have inhibitory effects upon acetylene reduction during the investigation, and this seemed to be the case. Water temperature did not exceed 24°C, and the period of highest water temperature corresponded to the highest overall levels of fixation. Acetylene reduction also occurred in the lower range of observed water temperatures (see site 1, September 30).

Light was expected have a regulatory effect upon fixation rates, but this was not shown here. Nitrogen fixation was light-stimulated in all Cyanobacteria investigated and the duration of fixation depended on the size of the fixed carbon pool available from photosynthesis (Bothe 1982). Nitrogenase is extremely oxygen-labile (Bothe 1982) and high rates of oxygen production through photosynthesis might inhibit nitrogen fixation. High light intensities inhibited nitrogen fixation by Cyanobacteria of thermal springs (Wickstrom 1984). Heterocystous Cyanobacteria are morphologically and physiologically adapted to the presence of oxygen (Wolk 1981 and 1982, Bothe 1982), and the heterocystous forms are capable of nitrogen fixation under aerobic conditions while the non-heterocystous forms generally cannot.

While the occurrence of nitrogen fixation by unicellular Cyanobacteria is unusual, it has been documented for species of Gloeothece and Aphanothece under aerobic conditions (Bothe 1982). Analysis of the community composition in the lake showed that these two genera were abundant, comprising 8 to 17 percent of the total number of organisms observed during the season at site 4 alone (Chapter 4). If the unicellular genera found within the lake were capable of nitrogen fixation under aerobic conditions, their contribution might reach measurable levels. They would, however, be incapable of fixation at high light intensities because the vegetative unicells lack mechanisms for protecting nitrogenase from photosynthetic oxygen.

Light conditions in the lake were often low, especially in morning hours and at site 4. This might be conducive to nitrogen fixation by the unicellular Cyanobacteria. In addition, the flocculent nature of the mat itself might also aid nitrogen fixation by these algae. The colonies may be sheltered from direct light by the other algae in the community, but movement of the flocs due to wave action would provide them with enough light to survive. Heterocystous forms would not be able to fix nitrogen under low light conditions. They may, however, fix during the later morning and afternoon hours when the light intensity would be higher. This might explain the low acetylene reduction levels observed at site 4 throughout the summer. Heterocystous forms represented less than 1 percent of this community and low light due to shading may have prevented them from fixing appreciable amounts of nitrogen. The unicellular forms, however, were abundant and the light may have reduced the photosynthetic oxygen release enough to allow the them to produce the low levels of ethylene observed.

4.0 Quantitative Analysis of the Community

As anticipated, the mature benthic community was a very diverse and complex organization of cells, colonies, filaments, detritus and sand. SEM could provide only a superficial view of the mature mat and therefore quantitative analyses were conducted to supplement information gained by that method. Material for light microscope examination was prepared to provide an estimate of the number of organisms present in each gram of undisturbed benthic material. Comparisons of locational and seasonal variation were conducted using a standard analysis of variance (AOV) and Fischer's Least Significant Differences estimation as outlined by Ott (1984). A 0.05 level of significance was used in all analyses. In the following sections, the use of the word "significant" represents a level of $p<0.05$ associated with analysis of variance. Insignificant changes had probabilities greater than 0.05. Linear regression was also used in some analyses.

All numerical references in the following pages refer to the estimated average number of cells per gram of benthic material or to the percentage of the estimated average number of cells per gram of benthic material. The use of "estimated number of cells per gram of benthic material" is awkward and will not be employed.

4.1 Total Cell Densities

Cell densities fluctuated seasonally at all four sites (Figure 6). Site 1 exhibited a period of significantly higher standing crop late in July. Site 2 did not vary significantly at all during the investi-

Figure 6. Algal density as cell number per gram of benthic material during 1984. Symbols indicate mean values. Brackets represent 1 standard deviation.

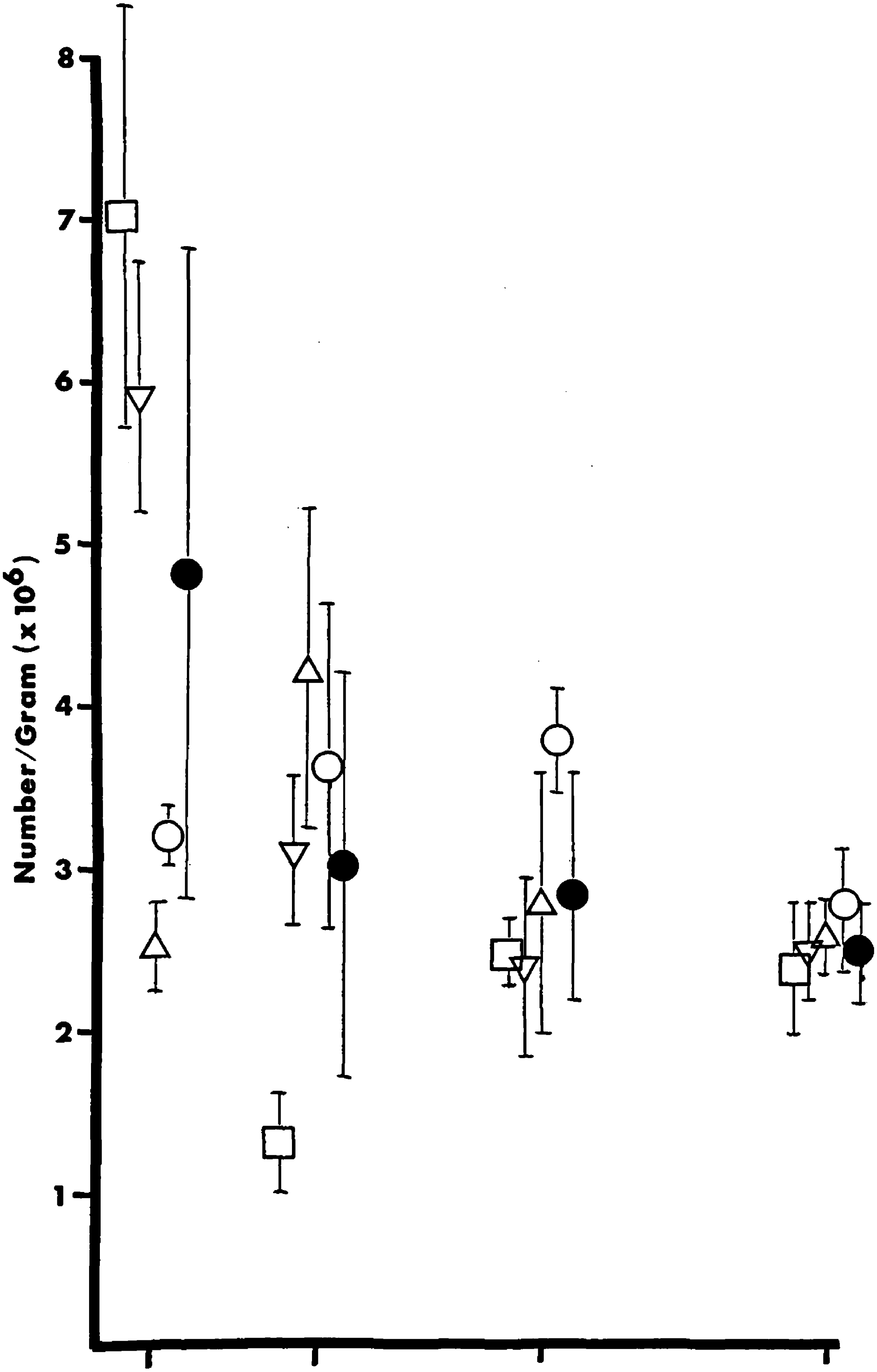
Site 1 △

Site 2 ○

Site 3 ▽

Site 4 □

Sampling trip ●


Number/Gram (x 10^6)
8
7
6
5
4
3
2
1
6
July
27
August
24
September
30

gation. Densities at sites 3 and 4 were more variable. Both sites had significant peak growth on July 6. Organism densities at site 3 decreased after this time and then remained stable. Algae at site 4 decreased to approximately 1 x 10^6 organisms per gram, the lowest population observed in the lake during this period. The population then increased significantly by August 24 and remained at this level for the remainder of the investigation.

There was a visible trend among the four sites during the summer of 1984. The highest densities and the largest differences in populations between sites occurred in early July. On July 6, sites 1 and 2 were not significantly different from one another, but had much lower densities than did sites 3 and 4. Differences between the sites became less pronounced as the summer progressed. Although densities at site 4 on July 27 were significantly less than all other sites and those of site 2 on August 24 were significantly higher, all sites had similar population densities by September 30.

It was hypothesized that cell densities might reflect seasonal changes in physical parameters within the lake. Correlation coefficients and scatterplots for the four parameters measured in 1984 versus density at each site did not show any relationship. Values were highest for light intensity and nitrogen concentration ($r=0.62$ and 0.69, respectively). Phosphorus and temperature correlation coefficients were low and indicated negative relationships ($r=-0.25$ and -0.30, respectively).

A major aspect of this investigation was the influence of nitrogen fixation by heterocystous Cyanobacteria upon algal community composition and structure. No similarity was seen between plots of density (Figure 6) and acetylene reduction rates (Figures 5a,5b). Site 4, for example, exhibited little to no acetylene reduction yet densities peaked at the same time and level as site 3 where acetylene reduction was measured. Periods of high acetylene reduction rates had been expected to correlate to algal densities. This did not appear to be the case, but cannot be confirmed by this study. If such an interaction exists, it may be too subtle to detect with the methodology employed in this study.

4.2 Composition

Organisms from three divisions were observed in large numbers: Chlorophyta, Chrysophyta and Cyanobacteria. Occasional representatives of the division Euglenophyta (Trachelomonas spp.) and Cryptophyta (Cryptomonas spp.) were observed in the material, but these were not encountered during analysis. Individual organisms were not considered from the functional standpoint of benthic or planktonic lifestyles. If a healthy phytoplanktonic organism was observed in the sample, it was considered a viable component of the community.

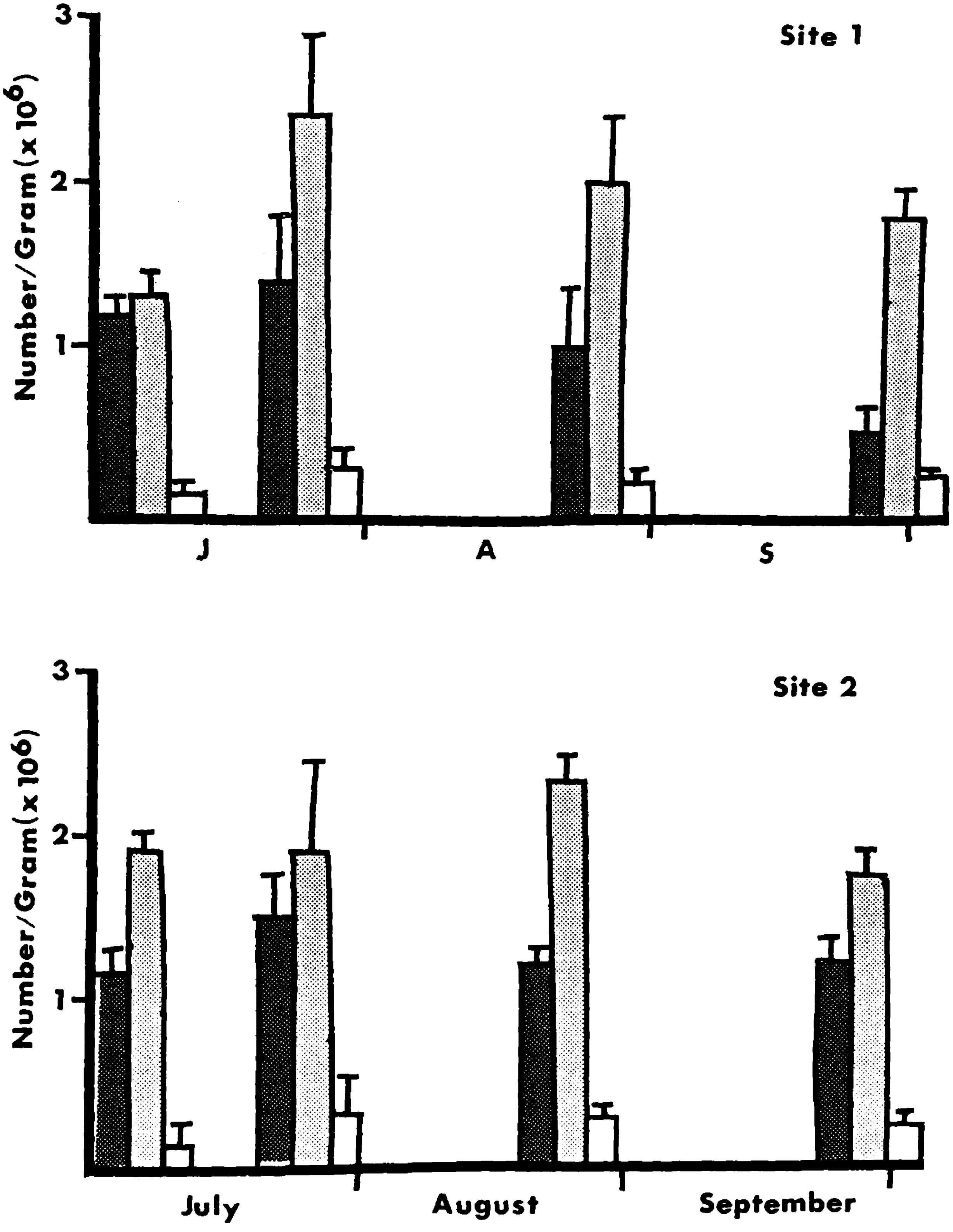
At all times and sites, Cyanobacteria dominated the community, followed by Chrysophytes and finally Chlorophytes (Figures 7 and 8). Site 2 was an exceptionally stable site when population numbers are considered. Based on AOV calculations, no significant differences in total density were detected during the summer. This uniformity was

Figure 7. Seasonal variation in algal number per gram benthic material and composition at sites 1 and 2. Brackets indicate 1 standard deviation.

Chlorophyta □

Chrysophyta ■

Cyanobacteria ░



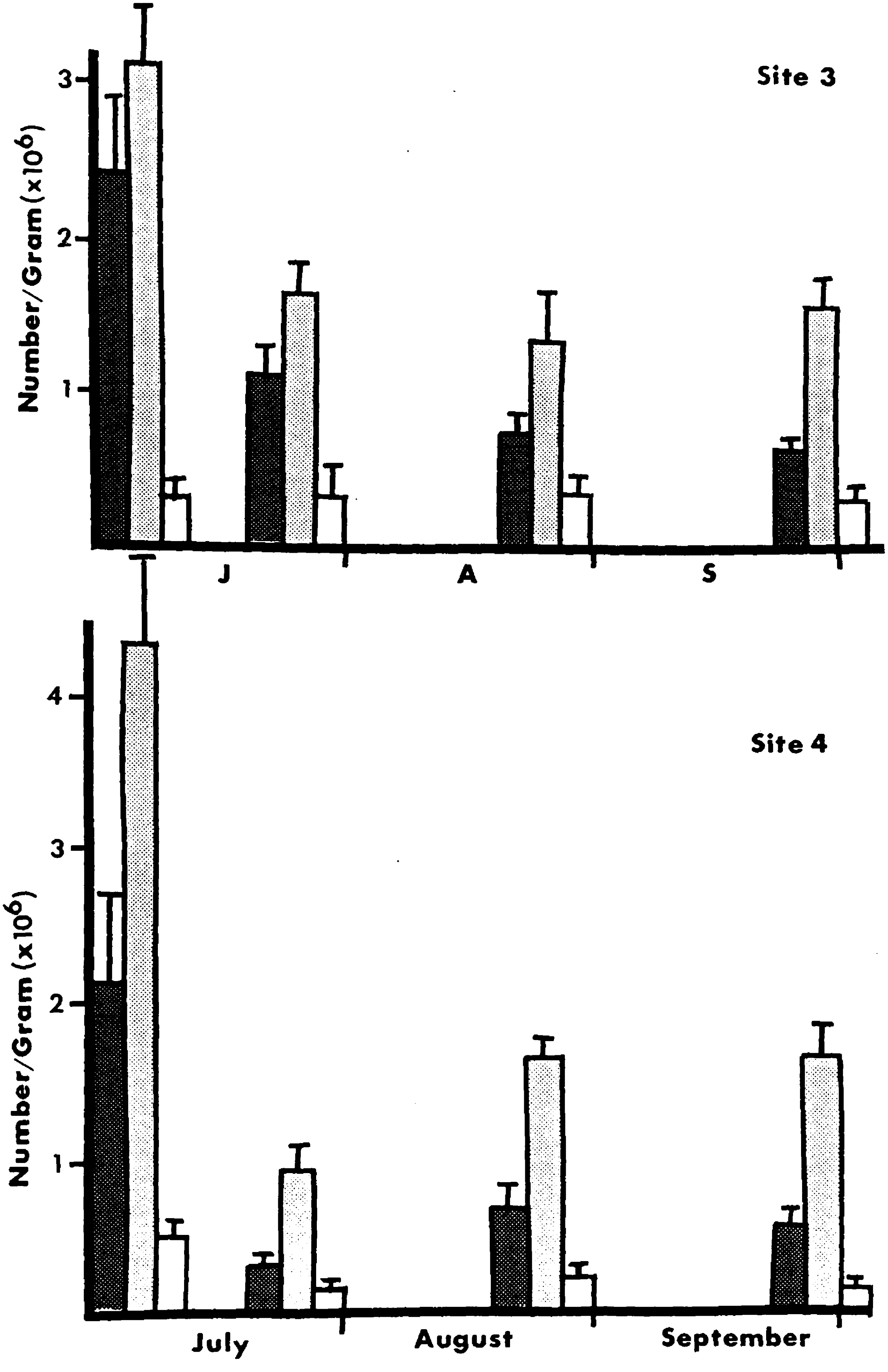
3
2
1
Number/Gram(x 10^6)
Site 1
J
A
S
3
2
1
Number/Gram(x 10^6)
Site 2
July
August
September

Figure 8. Seasonal variation in algal number per gram benthic material and composition at sites 3 and 4. Brackets indicate 1 standard deviation.

Chlorophyta □

Chrysophyta ■

Cyanobacteria ░


Site 3
Number/Gram (x10^6)
3
2
1
J
A
S
Site 4
Number/Gram (x10^6)
4
3
2
1
July
August
September

reflected in both the Chrysophytes and the Chlorophytes. In contrast, the Cyanobacteria exhibited slight but significant differences. September 30 samples at site 2 were lower in Cyanobacteria than August 24, while July 6 and 28 samplings did not differ significantly from either of these.

Several trends could be noted in the lake. Cyanobacteria reached the highest percentage of total density in late September at sites 1, 3 and 4. At site 2 this occurred in August. Cyanobacterial densities were different, peaking in July at sites 1, 3 and 4. Only at site 2 did the highest percentage of total density and the estimated peak populations of Cyanobacteria coincide in time of occurrence.

Chrysophyte populations peaked in July at sites 1, 3 and 4. At site 2 no significant differences in populations were detected between sampling dates. The percentage of total density represented by the division Chrysophyta at all sites was highest in July, in general the highest populations observed.

Members of the division Chlorophyta appeared to have no seasonal preferences. Benthic populations did not vary significantly at sites 1, 2 or 3 during the investigation. At site 4, the highest numbers of green algae were observed on July 6. The percentage of total density represented by this division generally did not change throughout the season.

Correlation coefficients were determined for percentage of total density and the population of each division versus the four measured physical parameters (Table 4). The Cyanobacteria showed little cor-

Table 4. Correlation coefficients of phylum composition with light, temperature, nitrogen concentration and phosphorus concentration. Values were combined from all sites for the analysis.

Variable	Phylum					
	Chlorophyta		Chrysophyta		Cyanobacteria	
	%	no.	%	no.	%	no.
Light (n=14)	0.73	0.29	0.03	-0.27	-0.40	-0.4
Temperature (n=14)	-0.47	0.11	0.55	-0.61	-0.26	0.4
Nitrogen (n=16)	-0.16	0.58	0.60	0.73	-0.50	0.5
Phosphorus (n=16)	0.41	0.12	0.12	-0.11	-0.32	-0.2

relation with any of the four parameters. The density of Chrysophytes correlated primarily with temperature and nitrogen concentration. Highest nitrogen concentrations occurred in September but highest temperatures occurred July 27 to August 4, approximately the period of largest standing crop for members of the division Chrysophyta. Correlations between the green algae and the four physical factors measured was highly variable. The low number of organisms observed from this division did not provide a sound base for the comparisons. The highest positive correlations, however, occurred with light and the percent of community composition.

4.3 Composition at the Generic Level

Each division is considered individually in this section. Taxonomic information for each site is presented by division (Figures 9-12). "Cells per gram of benthic material" has been shortened in the following sections to "cells per gram".

4.3.1 Chrysophyta

Similar Chrysophyte genera occurred at sites 1 - 4 (Table 5). With only one exception, _Chrysocapsa_, all Chrysophyte genera observed were diatoms. _Navicula_ was the dominant diatom although actual numbers varied between sites (Figures 9a, 10a, 11a, 12a). Maximum densities of this genus were observed on July 6 at sites 3 and 4, but on July 27 at sites 1 and 2. _Cymbella_ and _Fragilaria_ were the most abundant diatoms after _Navicula_, having 1 x 10^5 or more individuals per gram at

Table 5. Genera observed during the growing season in the benthic community.

Phylum	Site Where Present			
Chrysophyta				
Achnanthes	1	2	3	4
Centric diatom spp.	1	2	3	4
Cocconeis			3	
Cymbella	1	2	3	4
Diatoma	1	2	3	4
Epithemia	1	2	3	4
Fragilaria	1	2	3	4
Navicula	1	2	3	4
Ophephora	1	2	3	4
Pennate diatom spp.	1	2	3	
Pinnularia	1	2	3	4
Synedra	1		3	
Chrysocapsa	1	2		
Chlorophyta				
Ankistrodesmus	1			
Coelastrum	1			
Cosmarium	1	2	3	4
Crucigenia		2		
Gloeocystis	1	2	3	4
Nephrocytium			3	
Oocystis	1	2	3	4
Pediastrum	1	2	3	4
Scenedesmus	1	2	3	4
Staurastrum			3	
Stigeoclonium	1	2	3	4
Tetraedron	1	2	3	4
Tetraspora	1			4
Zygnema			3	
unknown colonies	1	2	3	4

Table 5. Continued

Phylum Cyanobacteria	Site Where Present			
Anabaena	1	2	3	4
Aphanocapsa	1	2	3	4
Aphanothece	1	2	3	4
Calothrix	1	2	3	4
Chroococcus	1	2	3	4
Coelosphaerium	1	2	3	4
Cylindrospermum	1	2		
Gloeothece	1	2	3	4
Gomphosphaeria	1	2	3	4
Lyngbya	1	2	3	4
Microcystis	1	2	3	4
Nostoc	1	2	3	4
Oscillatoria	1	2	3	4
Phormidium	1	2	3	4
Schizothrix	1			
Scytonema	1	2	3	4
Stigonema				4
Tolypothrix	1	2		
unknown genera	1	2	3	4

Figure 9a. Seasonal variation in generic composition of the Chrysophyta at Site 1.

Upper diagram:
- *Achnanthes* ●
- Centric spp. ▲
- *Cymbella* ■
- *Fragilaria* ○
- *Navicula* △
- *Ophephora* □

Lower diagram:
- *Diatoma* ◇
- *Epithemia* △
- Pennate spp. □
- *Pinnularia* ●

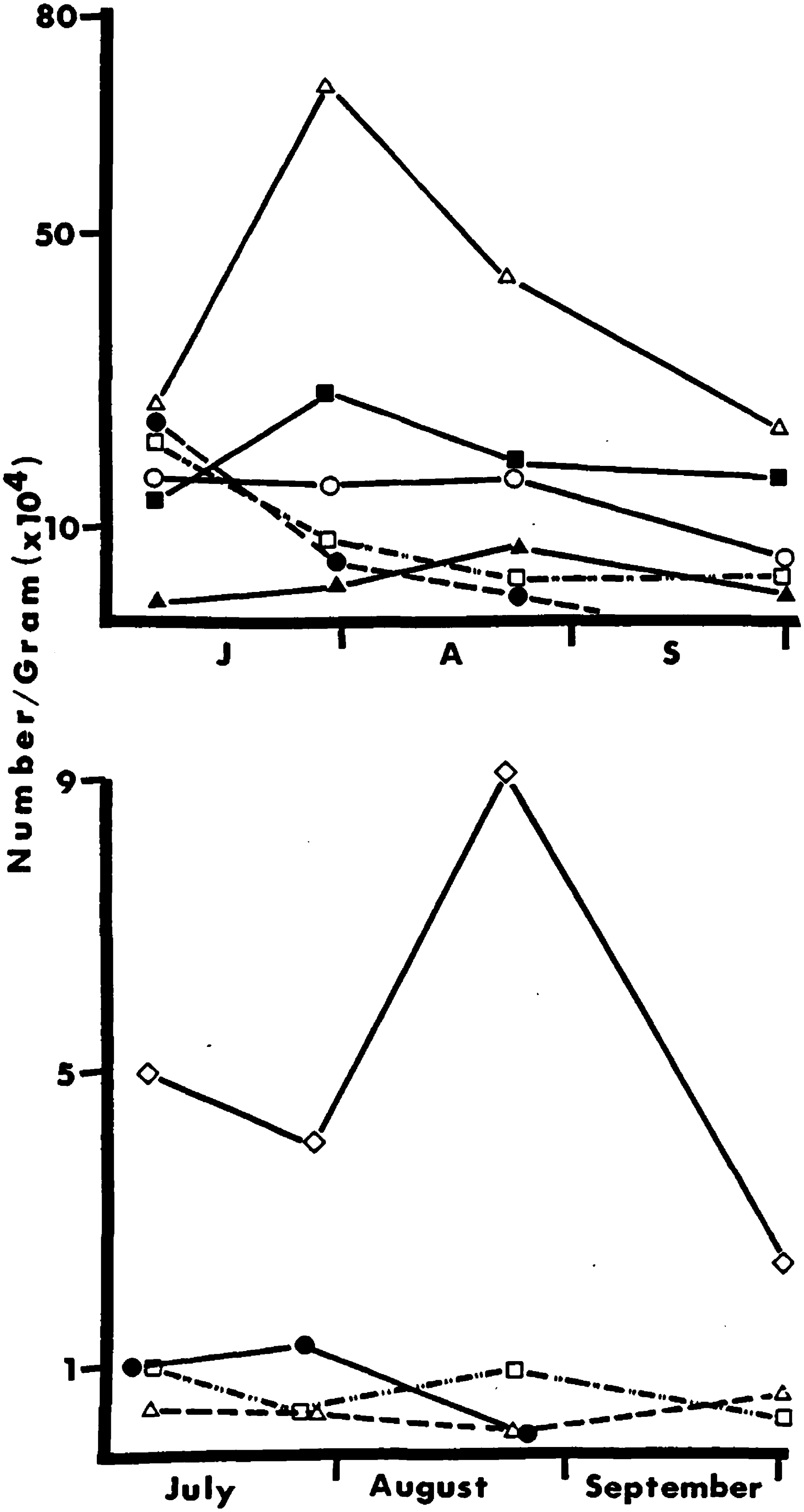

80
50
10
9
5
1
Number/Gram (x10^4)
J
A
S
July
August
September

Figure 9b. Seasonal variation in generic composition of the Chlorophyta at Site 1.

Upper diagram:
Gloeocystis ●
Oocystis ▲
Scenedesmus ■
Tetraedron ○
unknown colony △

Lower diagram:
Ankistrodesmus ●
Cosmarium ◇
Pediastrum ▼
Tetraspora △

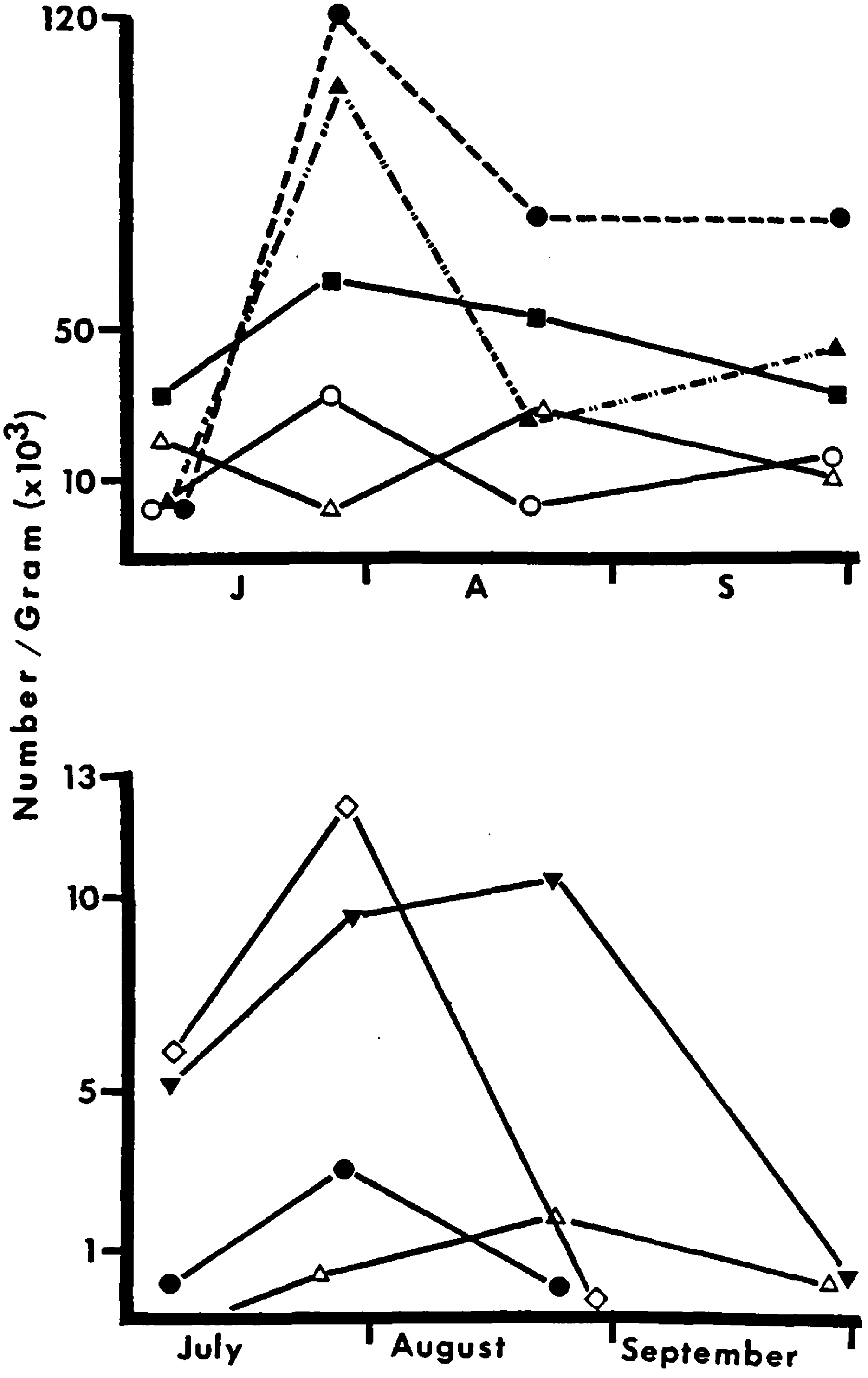

120
50
10
J
A
S
Number / Gram (x10^3)
13
10
5
1
July
August
September

Figure 9c. Seasonal variation in generic composition of the Cyanobacteria at Site 1.

Upper diagram:
- <u>Aphanothece</u> ●
- <u>Chroococcus</u> ▽
- <u>Gloeothece</u> ▲
- <u>Lyngbya</u> ○
- <u>Phormidium</u> ■

Lower diagram:
- <u>Anabaena</u> □
- <u>Aphanocapsa</u> ●
- <u>Calothrix</u> <
- <u>Coelosphaerium</u> ▲
- <u>Gomphosphaeria</u> △
- <u>Microcystis</u> ■
- <u>Nostoc</u> ◆
- <u>Oscillatoria</u> ▼
- <u>Scytonema</u> ◇

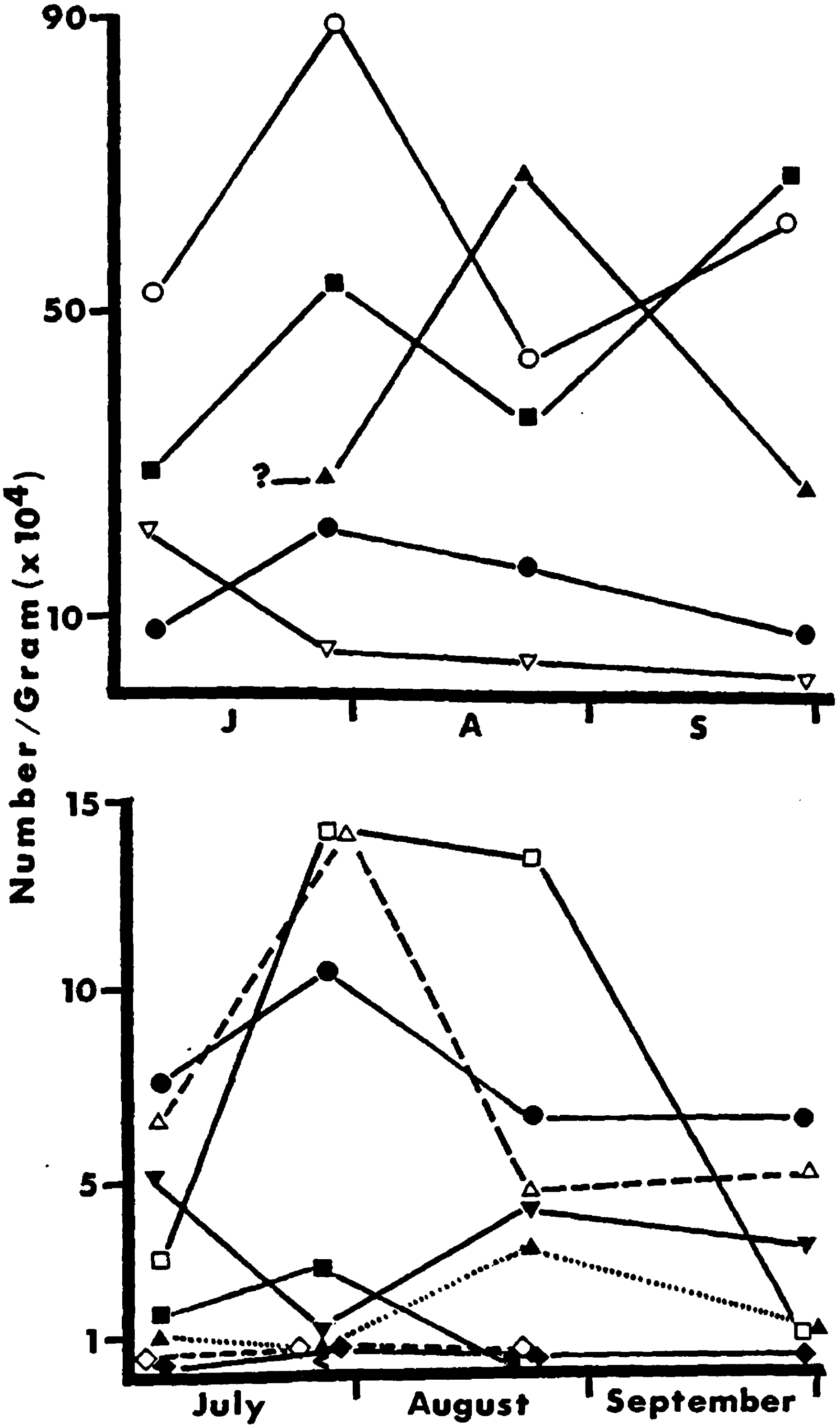
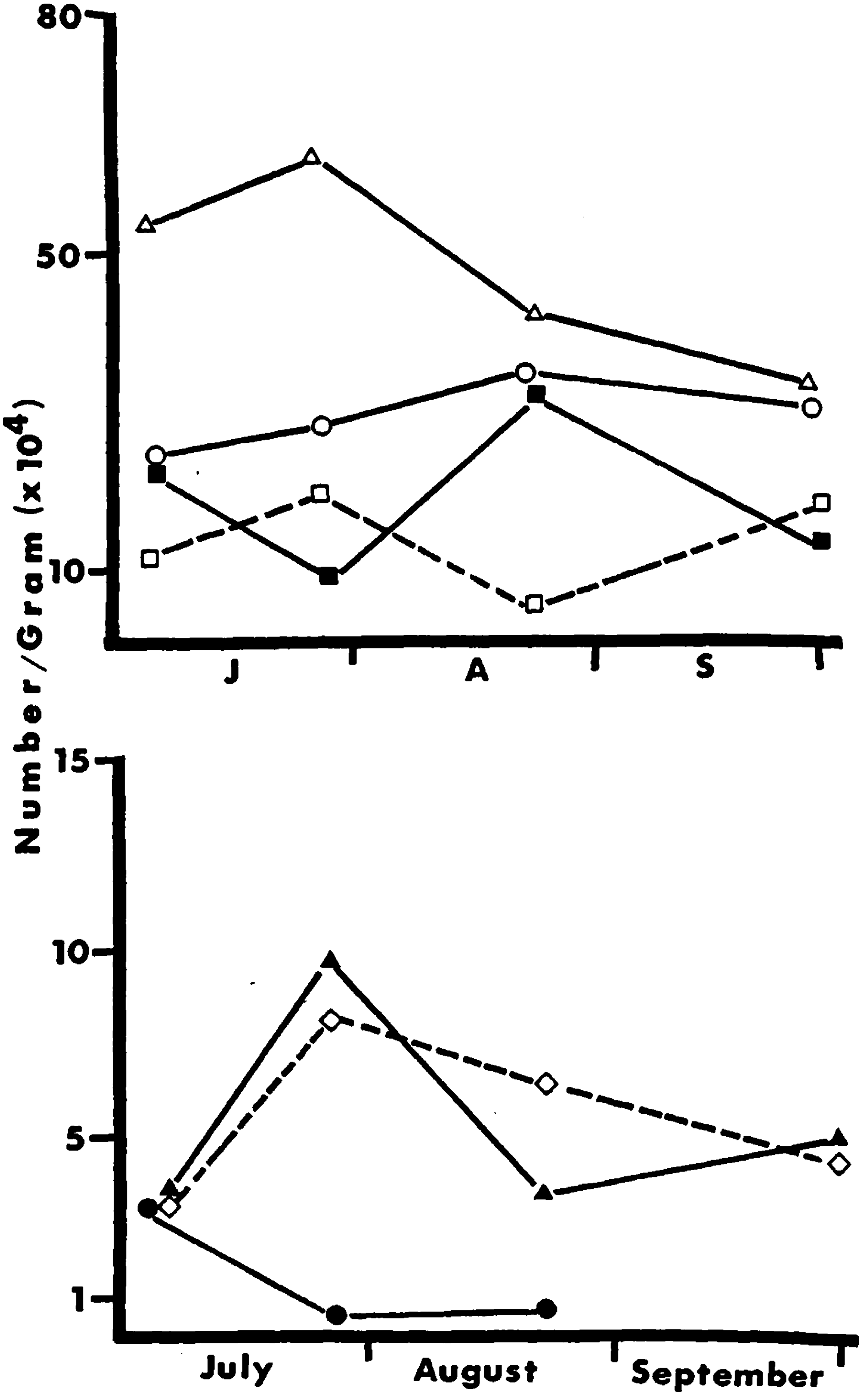

Number/Gram (x 10^4)
90
50
10
?
J
A
S
15
10
5
1
July
August
September

Figure 10a. Seasonal variation in generic composition of the Chrysophyta at Site 2.

Upper diagram:
Cymbella ■
Fragilaria ○
Navicula △
Ophephora □

Lower diagram:
Achnanthes ●
Centric spp. ▲
Diatoma ◇



80
50
10
J
A
S
Number/Gram (x 10^4)
15
10
5
1
July
August
September

Figure 10b. Seasonal variation in generic composition of the Chlorophyta at Site 2.

Upper diagram:
Cosmarium ◇
Gloeocystis ●
Oocystis ▲
Scenedesmus ■
unknown colony △

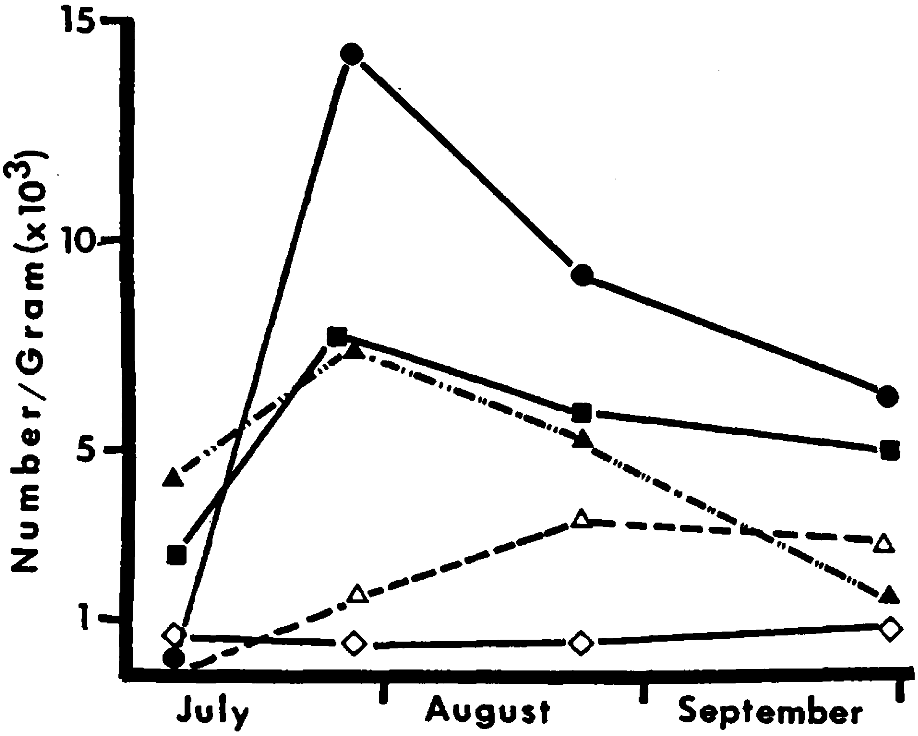

15
10
5
1
Number/Gram (x10³)
July
August
September

Figure 10c. Seasonal variation in generic composition of the Cyanobacteria at Site 2.

Upper diagram:
Anabaena □
Aphanothece ●
Gloeothece ▲
Lyngbya ○
Oscillatoria ▼
Phormidium ■

Lower diagram:
Aphanocapsa ●
Calothrix <
Chroococcus ▽
Coelosphaerium ▲
Gomphosphaeria △
Nostoc □
Scytonema ◇

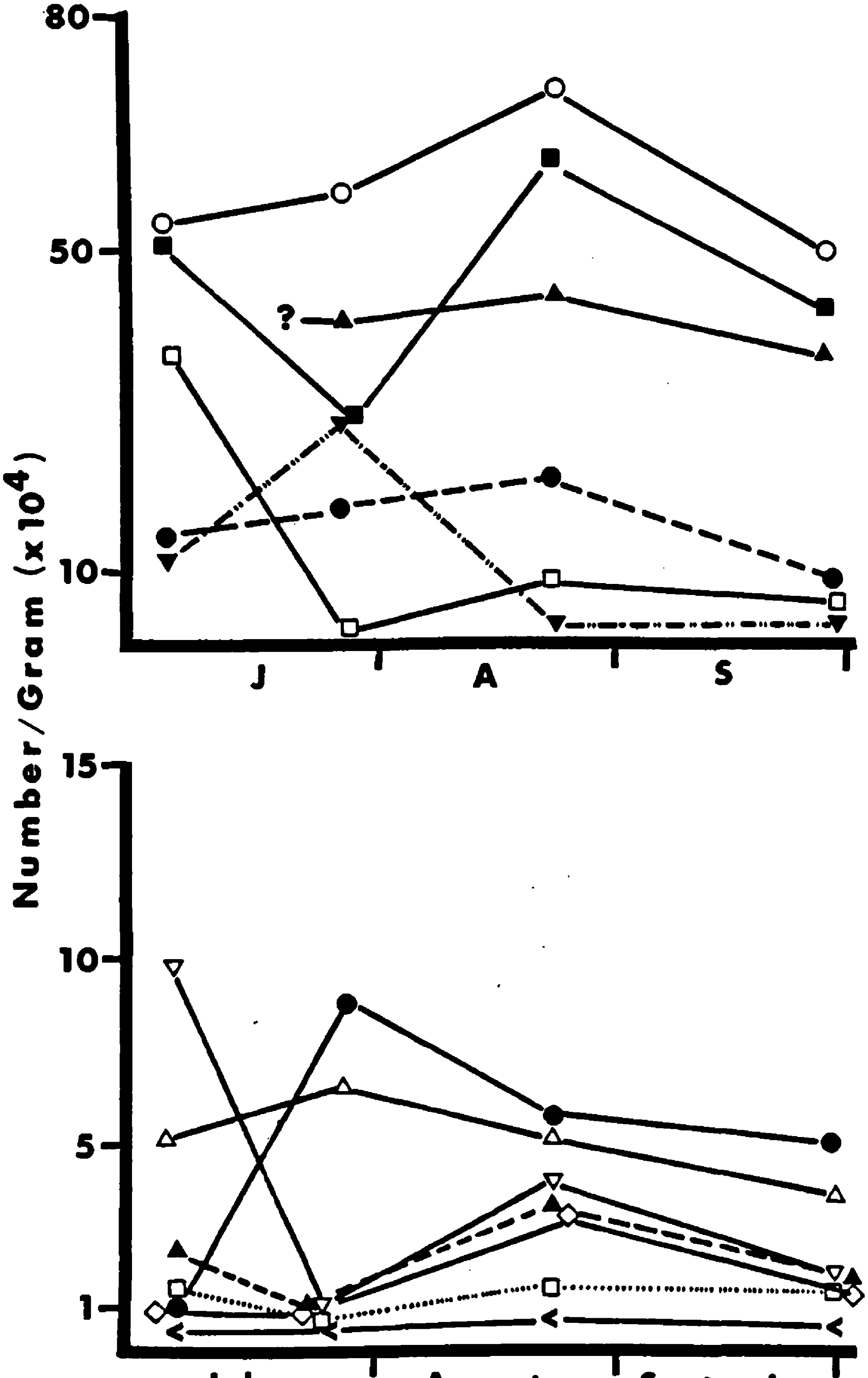

80
50
10
J
A
S
?
Number/Gram (x10^4)
15
10
5
1
July
August
September

Figure 11a. Seasonal variation in generic composition of the Chrysophyta at Site 3.

Upper diagram:
<u>Cymbella</u> ■
<u>Diatoma</u> ◇
<u>Fragilaria</u> ○
<u>Navicula</u> △
<u>Ophephora</u> □

Lower diagram:
<u>Achnanthes</u> ●
Centric spp. ▲
<u>Epithemia</u> △

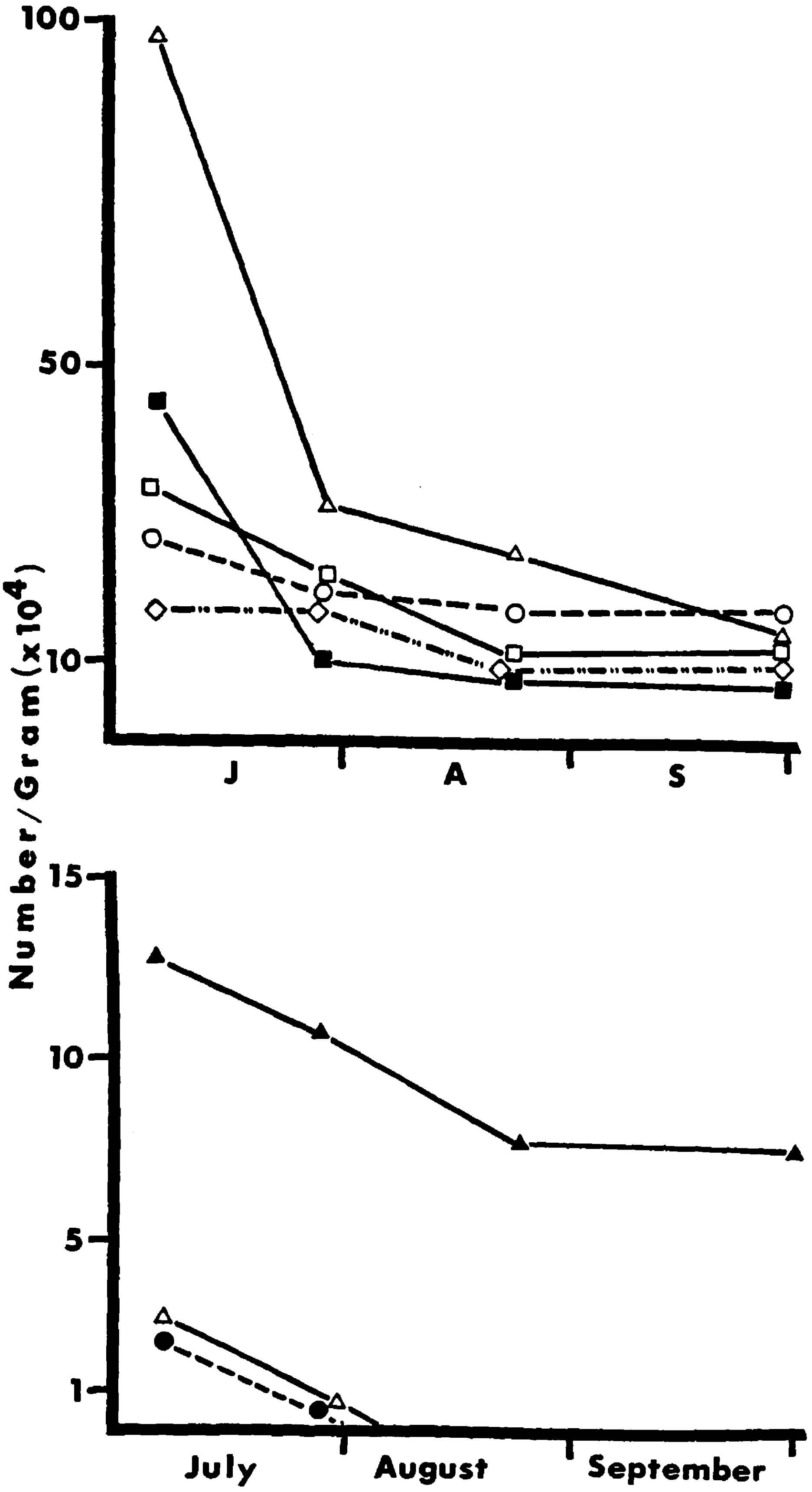

Number/Gram (x10⁴)
100
50
10
J
A
S
15
10
5
1
July
August
September

Figure 11b. Seasonal variation in generic composition of the Chlorophyta at Site 3.

Cosmarium ◇
Gloeocystis ●
Oocystis ▲
Pediastrum ▼
Scenedesmus ■
unknown colony △

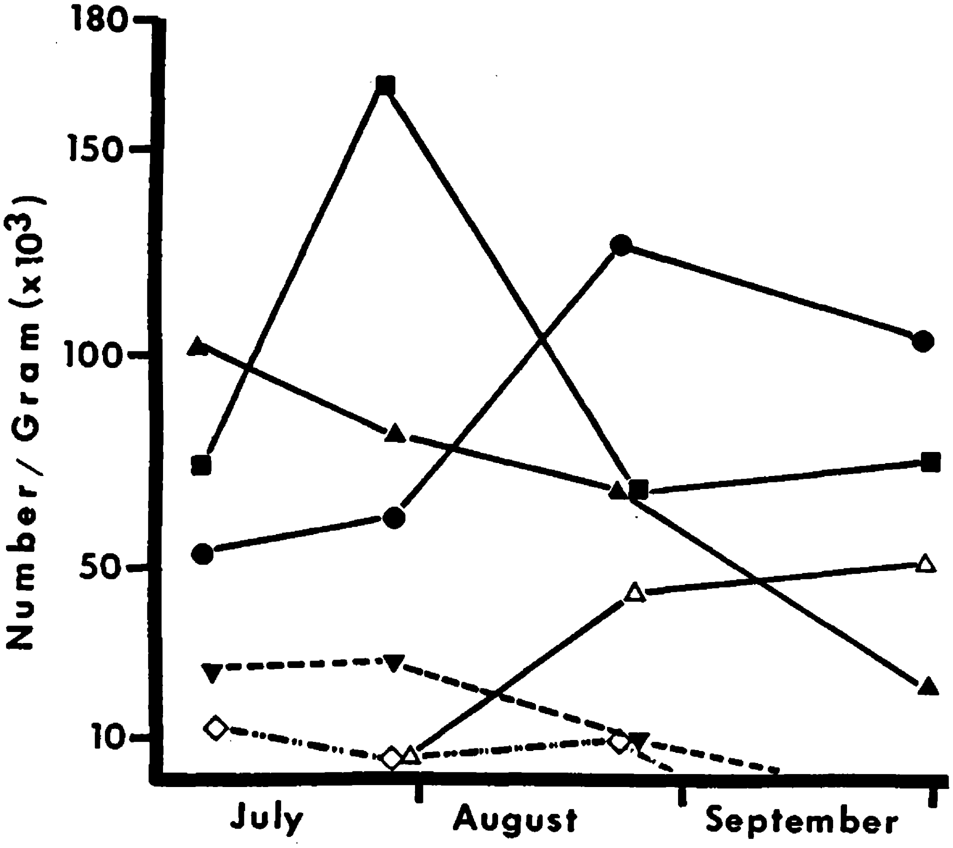

180
150
100
50
10
Number / Gram (x10^3)
July
August
September

Figure 11c. Seasonal variation in generic composition of the Cyanobacteria at Site 3.

Upper diagram:
Aphanothece ●
Gloeothece ▲
Lyngbya ○
Phormidium ■

Lower diagram:
Anabaena □
Aphanocapsa ●
Chroococcus ▽
Coelosphaerium ▲
Gomphosphaeria △
Microcystis ■

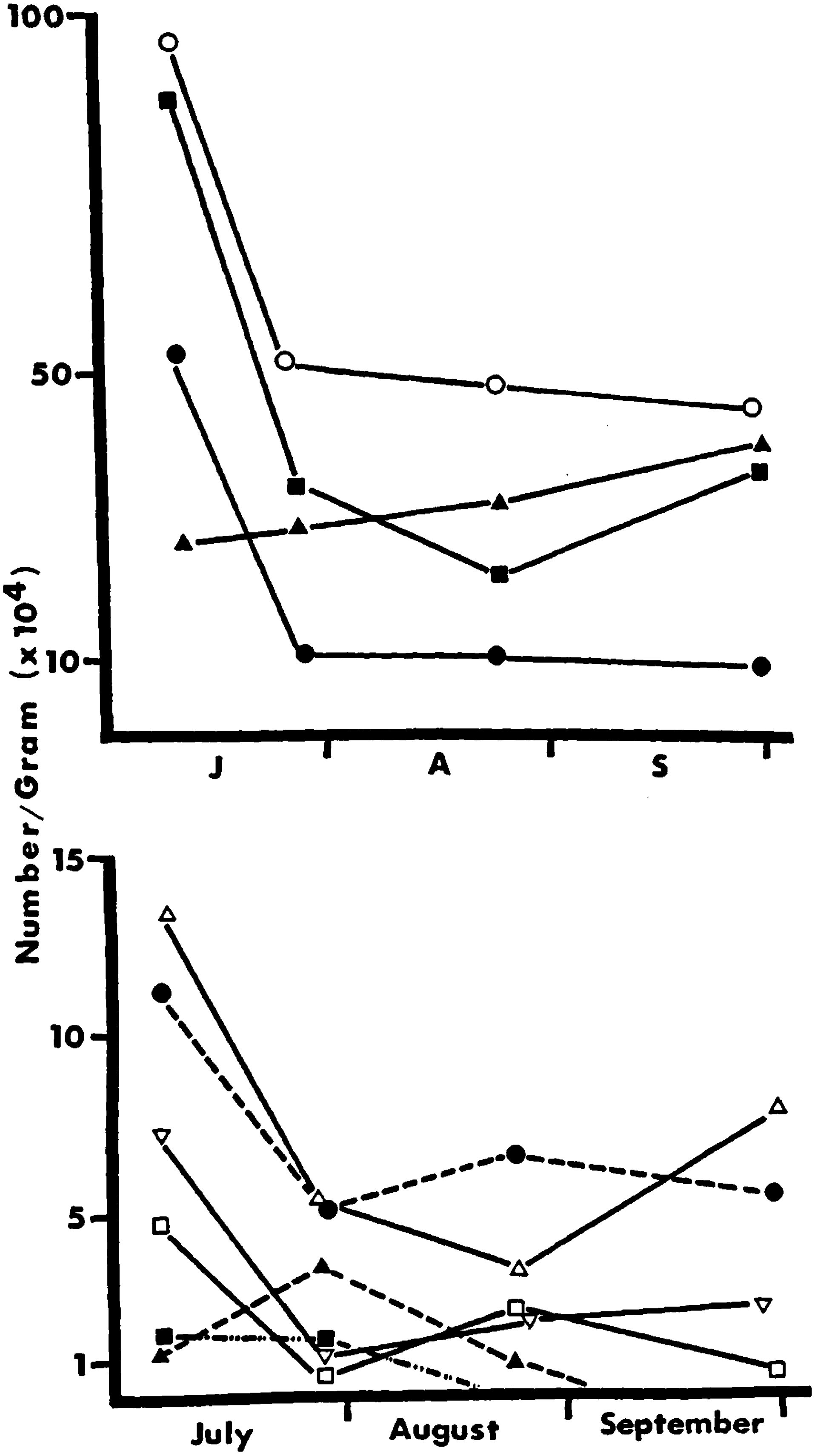
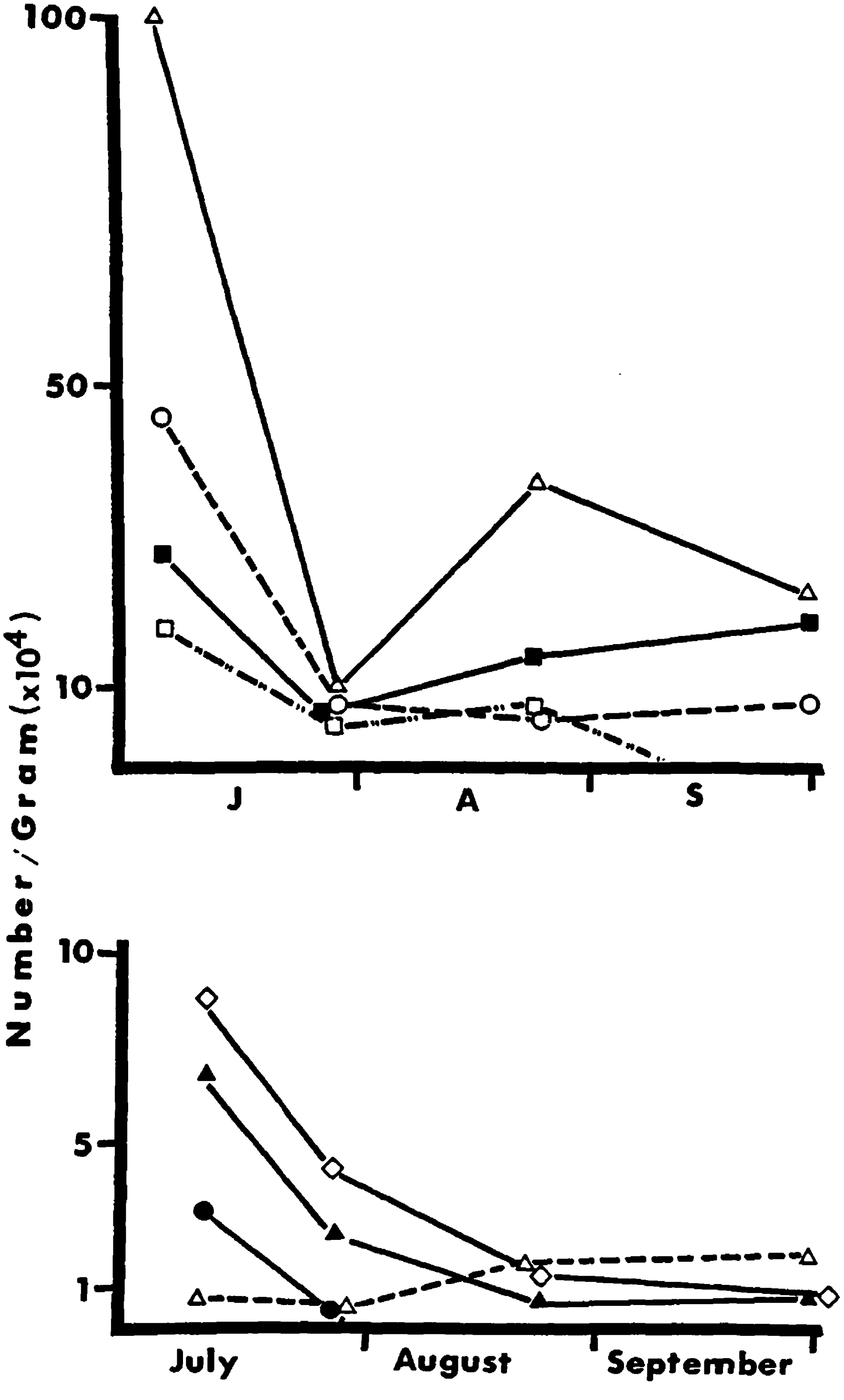

100
50
10
J
A
S
Number/Gram (x 10⁴)
15
10
5
1
July
August
September

Figure 12a. Seasonal variation in generic composition of the Chrysophyta at Site 4.

Upper diagram:
Cymbella ■
Fragilaria ○
Navicula △
Ophephora □

Lower diagram:
Achnanthes ●
Centric spp. ▲
Diatoma ◇
Epithemia △



Number / Gram (x10^4)
100
50
10
J
A
S
10
5
1
July
August
September

Figure 12b. Seasonal variation in generic composition of the Chlorophyta at Site 4.

Cosmarium ◇
Gloeocystis ●
Oocystis ▲
Pediastrum ▼
Scenedesmus ■
Tetraedron ○
unknown colony △

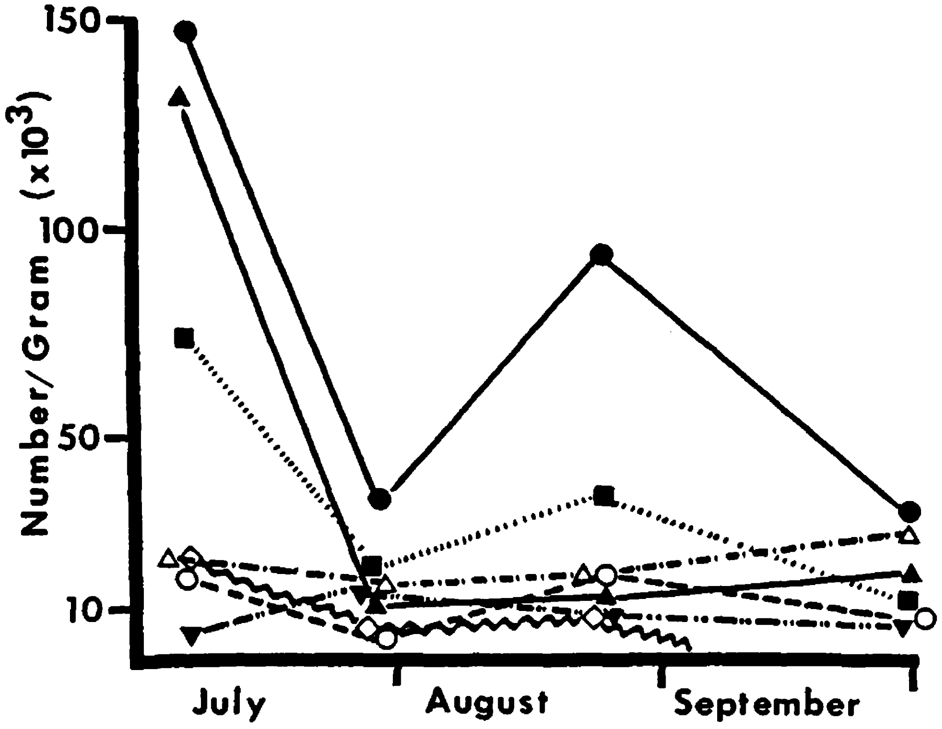
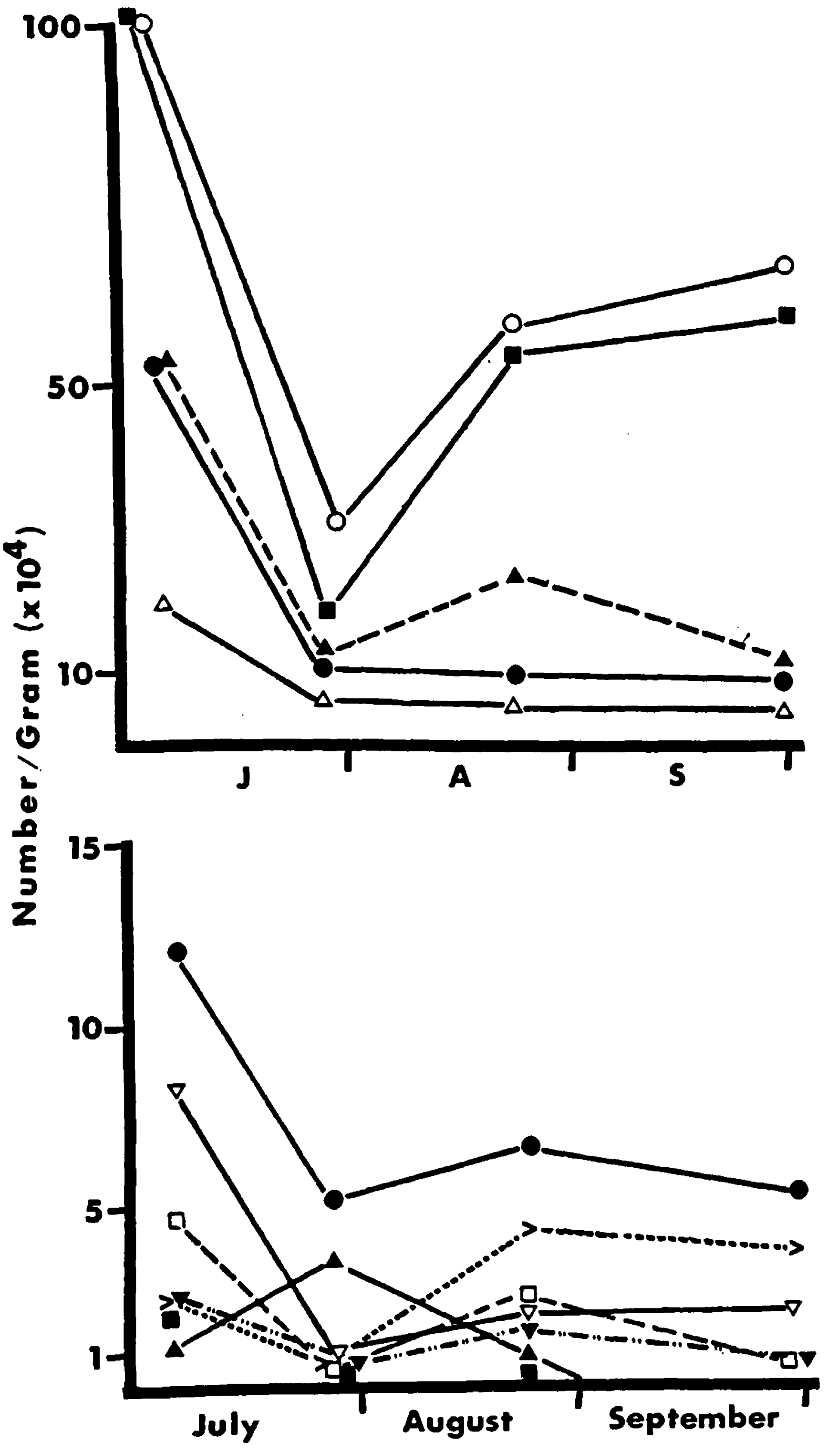

150
100
50
10
Number/Gram (x10³)
July
August
September

Figure 12c. Seasonal variation in generic composition of the Cyanobacteria at Site 4.

Upper diagram:
Aphanothece ●
Gloeothece ▲
Gomphosphaeria △
Lyngbya ○
Phormidium ■

Lower diagram:
Anabaena □
Aphanocapsa ●
Chroococcus ▽
Coelosphaerium ▲
Microcystis ■
Oscillatoria ▽
unknown colony >


Number/Gram (x 10^4)
100
50
10
J
A
S
15
10
5
1
July
August
September

each site during the season. *Ophephora* populations were generally slightly smaller than those of *Cymbella* and *Fragilaria*. *Achnanthes*, *Diatoma* and centric diatoms were usually less than 50,000 individuals per gram. These genera were present at all sites, but were highly variable in number. *Pinnularia*, *Epithemia*, *Synedra* and pennate diatoms each represented less than 10,000 organisms per gram. This was not surprising because these genera are often considered to be planktonic rather than benthic in habit (Prescott 1962). Population abundance observed during the investigation was highly variable between genera and also between the four sites, but common patterns could be identified.

4.3.2 Chlorophyta

High variability was noted among the Chlorophytes and therefore few clearly dominant genera were evident (Figures 9b, 10b, 11b, 12b). Densities were often very similar and hence less distinction between abundant genera was evident. *Gloeocystis*, for example, dominated at site 4 throughout the season, but was only seasonally dominant at the other three sites. *Scenedesmus* and *Oocystis* represented the next most abundant genera, but often their numbers did not differ from those of other green algae. Minor fluctuations in the consistently low numbers of genera such as *Tetraedron* occurred. Planktonic genera such as *Ankistrodesmus*, *Coelastrum* and small colonies of *Tetraspora* were noted periodically throughout the season. This perhaps represented seasonal succession in the phytoplankton community. Because the division

Chlorophyta represented less than 10 percent of the total algal density throughout the season, differences between sites may be due to chance rather than to actual variation in composition.

4.3.3 Cyanobacteria

The majority of the blue-green algae genera observed throughout the study period were present at all sites (Figures 9c, 10c, 11c, 12c). The exceptions were: Cylindrospermum and Tolypothrix, which occurred only at sites 1 and 2; Schizothrix only at site 1; and Stigonema only at site 4. Several unidentified Cyanobacteria also occurred at only one or two sites. These genera were present infrequently.

The dominant genera, tentatively identified as Phormidium and Lyngbya, as well as Gloeothece were common to all sites, though their relationship to one another varied between sites. Large differences in the importance of certain genera were noted between sites, i.e. Chroococcus was intermediately abundant at site 1 while consistently present in low numbers at all other sites. On the whole, Gloeothece, Phormidium and Lyngbya were very abundant; Aphanothece, Chroococcus, Anabaena, Gomphosphaeria and Aphanocapsa were moderately abundant; Oscillatoria, Coelosphaerium, Nostoc, Cylindrospermum, Scytonema and Calothrix were all low in number. All other genera were very low in number and infrequent. Again, differences in standing crop was highly variable between sites and genera, but several patterns could be identified.

Seven genera of heterocystous Cyanobacteria were observed within the lake: Anabaena, Calothrix, Cylindrospermum, Nostoc, Schizothrix, Stigonema, and Tolypothrix. From Figure 13a it can be seen that heterocystous Cyanobacteria formed less than 5 percent of the total density with only one exception. On July 6 at site 2, high numbers of Anabaena were observed, raising the presence of heterocystous Cyanobacteria to approximately 12 percent of the total benthic population. It was not apparent why this might have occurred. The population then crashed to lower levels by August.

Each site was unique when considered in terms of heterocystous Cyanobacteria. Percentage of total density (Figure 13a) and the number (Figure 13b) were similar and will not be considered separately. The communities were essentially identical in the late spring and early fall, with the exception of site 2. The communities were clearly different from one another only during periods of peak standing crop. When the anomalous value for site 2 on July 6 was excluded, a definite ranking with respect to number and percentage of total density represented by heterocystous Cyanobacteria could be distinguished. For the major portion of the year, site 1 appeared to have had highest number of heterocystous individuals, followed in decreasing order by sites 2, 3 and 4. There was no significant correlation between the abundance of heterocystous Cyanobacteria (Figures 13a,b) and rates of acetylene reduction (Figures 5a,b). The only similarity detected between the two sets of data was that site 4 had the lowest numbers of heterocystous individuals observed as well as the lowest reduction values during the entire study period.

Figure 13a. Percent of total number of algae per gram of benthic material represented by heterocystous Cyanobacteria.

Site 1 ▲

Site 2 ○

Site 3 ■

Site 4 △

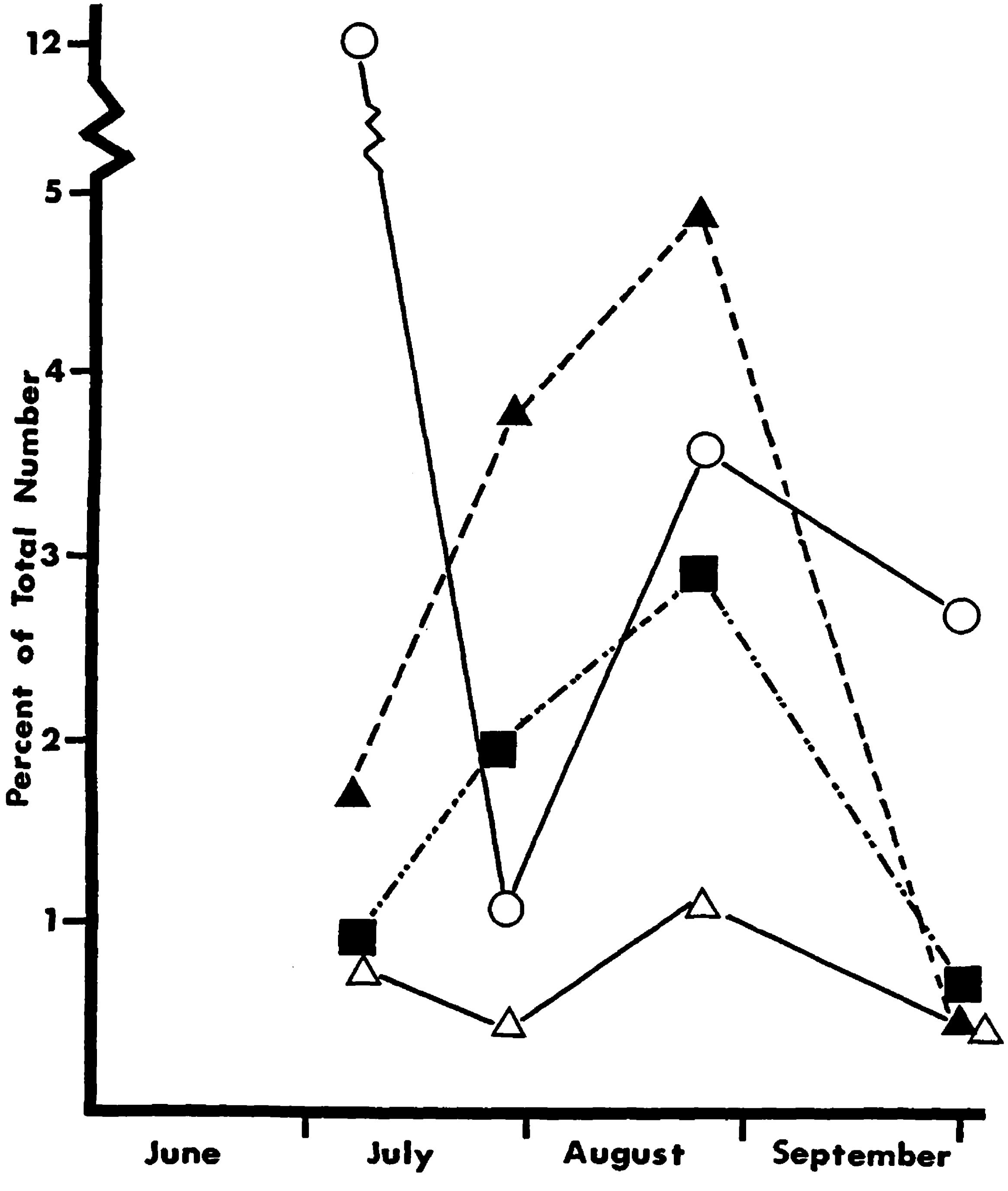

12
5
4
3
2
1
Percent of Total Number
June
July
August
September

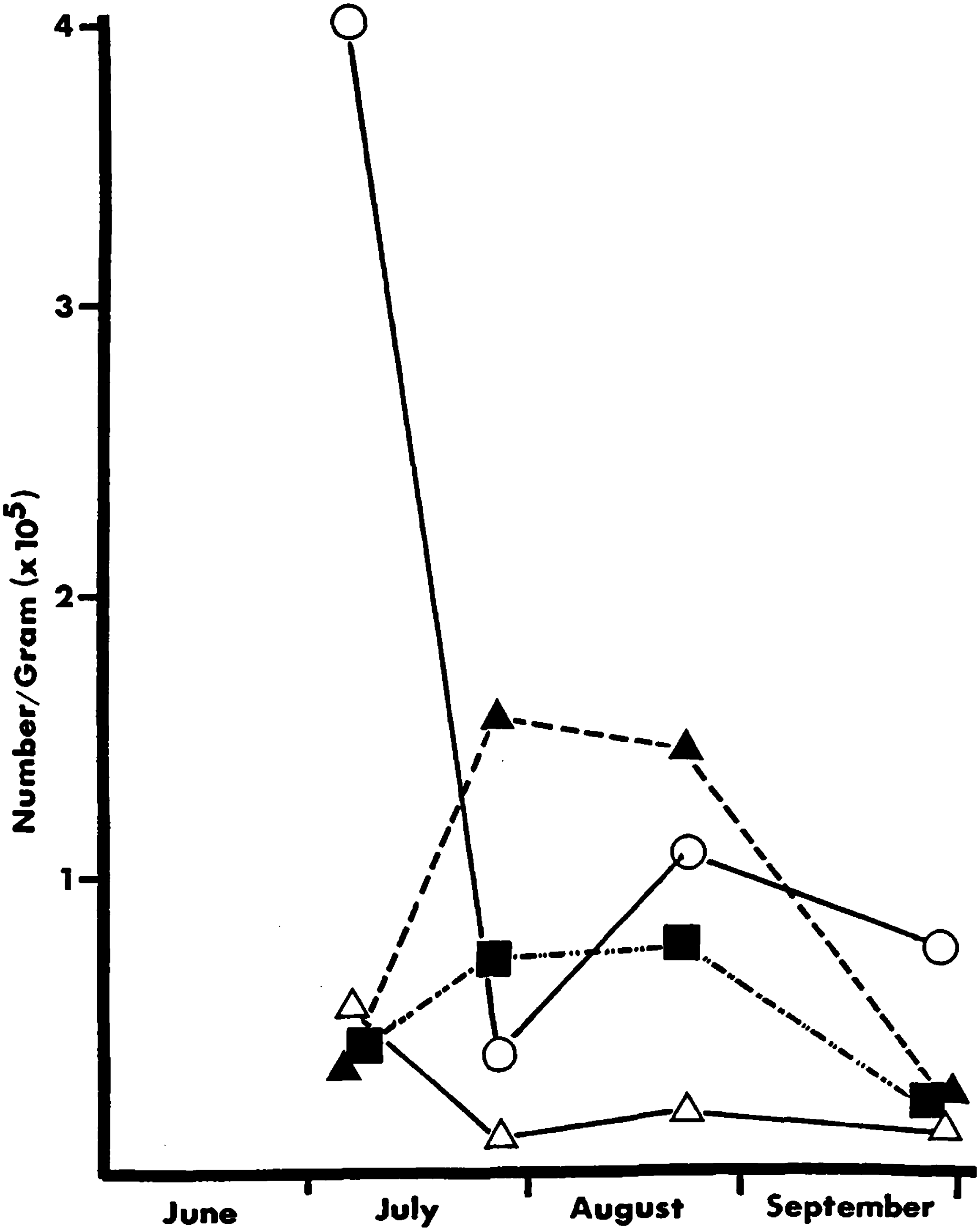
Figure 13b. Average number of filaments or colonies per gram benthic material of heterocystous Cyanobacteria.

Site 1 ▲

Site 2 ○

Site 3 ■

Site 4 △


4
3
2
1
Number/Gram (x 10⁵)
June
July
August
September

The distribution of heterocystous Cyanobacteria could be divided into two groups by site: sites 1 and 2 had Anabaena, Calothrix, Cylindrospermum, Nostoc and Tolypothrix; and sites 3 and 4 had Anabaena, Calothrix, and Nostoc. Anabaena was the only genus present at all sites during all observational periods. The other genera occurred periodically in low numbers.

4.4 Discussion

The benthic community in the study lake was dominated by members of the division Cyanobacteria. This division represented 50 percent or more of the total organism density in the community throughout the entire investigation. Genera of the division Chrysophyta were second in abundance to the division Cyanobacteria (composing 20 to 40 percent of the total density). The division Chlorophyta was the least abundant, less than 15 percent of the total number. An occasional Euglenophyte or Cryptophyte was also observed in the mat. These organisms are motile and most likely represent transient visitors. They did not appear to have a significant role within the benthic community.

The paucity of Chlorophytes within the benthic mat does not appear to be unusual. Stevenson et al. (1985) noted that green algae were not common in the sediments of 20 New Hampshire lakes. The Chlorophytes present in those lakes occurred as "filamentous clouds" attached at points above the sediments. In the present study large colonies of filamentous Chlorophytes were not observed. There were few attachment sites for such communities in the lake, and this might explain the overall lack of green algae. Stigeoclonium and Ulothrix were observed periodically in the material examined by light microscopy, indicating that Chlorophyte communities may occur somewhere in the lake. It appeared, however, that the division Chlorophyta did not have a large role in the benthic community. The majority of the genera observed are characterized as planktonic, at least in the mature state (Prescott 1962), i.e. Ankistrodesmus, Elakatothrix, Quadrigula, Scenedesmus, and Tetraedron. It is possible that these organisms settled out of the water column and were senescing in the mat. Perhaps they represent a passive component, occurring only randomly within the mat. Their presence might be of more importance from a nutritional standpoint, as a future source of nutrients, than as an integral structural component.

Stevenson et al. (1985) also noted that although the Cyanobacteria were dominant from a numerical standpoint, the Chrysophytes were dominant when biomass was considered. This might also be the case in the present study. The observed Cyanobacteria were often colonial forms composed of many small cells, i.e. Aphanocapsa or Gomphosphaeria, while the Chrysophytes were often large diatoms such as Navicula and Synedra. Because this study was concerned primarily with community architecture, cell biomass was not determined. If this had been considered, it is probable that the Chrysophyta would have had a greater biomass than the blue-green algae.

Numerical dominance of benthic algal assemblages by the Cyanobacteria is not unusual in subalpine oligotrophic lakes. Reuter (1983) observed that cyanobacterial species, especially several heterocystous ones, dominated the sublittoral zone of Lake Tahoe throughout the year. Loeb and Reuter (1981) surveyed five oligotrophic subalpine lakes in California and found all epilithic periphyton communities to be dominated by Cyanobacteria. Blue-green algae also dominated the epipelic algal assemblages of 20 New Hampshire lakes (Stevenson et al. 1985). Stevenson et al. (1985) hypothesized that the Cyanobacteria were better adapted for sequestering nutrients in low light environments. Low light conditions do not apply to all sites within the lake observed during the present study. It appears that factors other than light may also be important in determining algal dominance in this community.

When considered from an overall compositional viewpoint, very little difference existed between the observed genera at the four study sites over the summer. Exceptions to this occurred predominantly in the Cyanobacteria, where several genera were observed to occur in low numbers at sites 1 and 2, but were not present at sites 3 and 4. Instead, the primary differences between sites occurred at a numerical level, in the number of organisms represented by each division or species.

The greatest differences between sites when considering total densities occurred early in the growing season. Sites 3 and 4 had high numbers when compared to sites 1 and 2. As the season progressed, all sites became similar. At the generic level, differences are more pronounced. In general, dominant genera were dominant at all sites, although numbers might vary considerably. Large differences in organism numbers were observed among the rare algae. The same genera occurred at all sites for the most part, but the numbers were highly variable, and some genera were present only at one period during the entire experimentation period.

Seasonal succession of genera or species among the phytoplankton has been well documented (see Wetzel 1983c for a general review), but less so among the periphyton. Reuter (1983) indicated that Cyanobacteria were dominant in Lake Tahoe throughout the year while Chrysophytes and Chlorophytes became abundant on a more seasonal basis, achieving high numbers in the summer. Round (1960, 1961) found that the common pattern of Cyanobacteria and Chrysophyte abundance in several English lakes was a pronounced spring or early summer pulse with perhaps a period of autumn increase. He concluded that there was little evidence indicating that such changes at different times were due to different species.

Observations of individual genera identified in the present study indicated that recognizable succession of individuals on a numerical basis did not occur among non-planktonic individuals within the benthic mat. Patterns of abundance for all genera within a division were very similar. Planktonic and rare genera were the exception. They often occurred only once at each site in the season.

In general, heterocystous Cyanobacteria comprised less than 5 percent of the the Cyanobacteria observed in the benthic material. This is perhaps unusual. Loeb and Reuter (1981) examined epilithic periphyton of five oligotrophic lakes. In all five, the sublittoral biomass was dominated by Cyanobacteria with heterocystous forms most abundant. In contrast, the eulittoral zone was dominated by diatoms and filamentous green algae. Reuter (1983) found that the sublittoral epilithic periphyton community of Lake Tahoe was also dominated in biomass by Cyanobacteria, the majority of which were heterocystous forms. The eulittoral zone was dominated by non-nitrogen fixing stalked diatoms (see also Reuter et al. 1983).

Loeb and Reuter (1981) defined the eulittoral as the 'splash' zone and the sublittoral as from 'approximately one meter depth to the bottom of the photic zone'. They suggested that the epilithic eulittoral zone is dominated by species able to colonize rapidly, an important asset because of the instability of this zone. Colonization was not as important in the epilithic sublittoral, and hence they hypothesized slower growing blue-green algae would have an advantage in the sublittoral of oligotrophic lakes because of their ability to fix nitrogen.

The lake investigated in the present study is also oligotrophic, but very small and shallow. No splash or wave zone was identified beyond 1 meter from the shore. The sublittoral comprised nearly 90 percent of the lake and all samples were collected from there. The epilithic layer in the Loeb and Reuter study (1981) was dominated by heterocystous Cyanobacteria in all five lakes. In contrast, the flocculent material in the present study, an epipelic community, was dominated by non-heterocystous blue-greens. Apparently nitrogen fixation did not provide a competitive advantage in this sublittoral environment.

Epilithic periphyton are primarily dependent upon nutrient availability in the water column. Sloughing of material from the rock surfaces would eliminate most nutrient stockpiling near the periphyton. In a benthic mat however, detrital material, and hence nutrients, would collect and be retained within the community network. Water movement carried the flocs and their nutrient supply from place to place. The close proximity of nutrients, especially nitrogen, would eliminate the competitive advantage of nitrogen fixation. In this case, it would not be surprising if the benthic material had very few heterocystous Cyanobacteria.

The lack of heterocystous Cyanobacteria could be responsible for the low levels of nitrogen fixation observed in the lake. Those few organisms present would not need high levels of fixation to support growth. Site 4 had numbers of heterocystous forms similar to other sites, but samples exhibited little to no acetylene reduction. The observed acetylene reduction rates at the other sites may have been influenced by other sources such as bacteria. More experiments would be required to establish the rate of reduction pertaining to individual heterocystous Cyanobacteria.

The influence of specific environmental factors upon the composition and abundance of algae in the benthic communities was varied. Photon flux density and nitrogen concentration in the water appeared to be the two most important parameters affecting the community. Temperature had little correlation to composition or cell density. Phosphorus also had negligible influence on composition and population size. As stated previously, this nutrient was very limited within the lake system and therefore would be expected to impact the algal community to a great degree. The extent of this influence cannot be determined from the data available in this study, but it may be stated that the minimal spatial and temporal variation in phosphorus concentration documented within the lake could have little effect on variations in the benthic community's composition or density.

The influence of environmental parameters upon periphyton community structure in general is highly variable. It was therefore difficult to compare results from one study to another. The effect of environmental or physical parameters not measured can be of considerable importance. In general, increases in nitrogen and phosphorus in oligotrophic lakes would be expected to increase numbers of periphyton and phytoplankton (Wetzel 1983c and others). A related change in composition would also be expected as conditions regulating competition changed. Numbers of algae in 20 New Hampshire lakes of varying trophic states were either negatively or insignificantly correlated to total phosphorus concentrations (Stevenson et al. 1985), and it appeared that phytoplankton increased with increased phosphorus levels while periphyton decreased. In Elk Lake, British Columbia, a positive correlation was found between periphyton populations and temperature, nitrate and nitrite concentrations while orthophosphate concentrations were negatively correlated (Brown 1973a). In Brown's study, 80 percent of the variation in total periphyton cell number was explained by three of 15 variables (duration of substrate exposure to colonization, phosphate concentrations and hardness index). Because of the limited data base in the present study, specific relationships of the benthic community to light, temperature, nitrogen and phosphorus could not be identified to the degree found in other studies.

5.0 Community Architecture

The concept of community organization in benthic algal assemblages has been studied for many years (Fritsch 1929, Gumtow 1955, Patrick 1967). The communities developing on both natural and artificial substrates were analyzed using quantitative methods such as those of Sladeckova (1962). These involved the scraping of the algae from their substrate, resuspension and/or dilution of the scraped material, and finally enumeration with the light microscope. The information provided about the community in this manner was numerical and quantitative. The actual three-dimensional relationship of individual algal cells to one another, the architecture, could not be evaluated because the sampling technique destroyed the spatial aspects of the community. In addition, low magnification and limited depth of field allowed only a superficial view of the community by light microscopy. The scanning electron microscope (SEM) provided a new method for the investigation of algal community architecture by allowing the retention of actual physical structure during specimen preparation, and also the high magnification necessary to investigate relationships within the microcommunity. Even so, the complexity of the mature algal community often proved too difficult to understand or identify. The use of artificial substrates such as Plexiglas (Hoagland et al. 1982), aluminum SEM stubs (Korte and Blinn 1983), polyurethane (Cairns et al. 1983), and granite (Hamilton and Duthie 1984) overcame this problem somewhat by allowing the investigator to follow the development of an algal community from bare uncolonized surface to a fully mature community.

Observations of this "microsuccession" (Hoagland et al. 1982) greatly enhanced our understanding of the mature community in terms of the physical relationships between individuals over time.

The goal of the research presented in this section is to elucidate physical relationships occurring among individuals of a benthic algal mat, especially nitrogen-fixing genera of Cyanobacteria and non-fixing genera. To achieve this, natural mat communities and those grown on artificial substrates were observed and compared. SEM observation of sequential colonization on artificial substrates provided insight into the development of community architecture. Observation of the mature natural mat provided an opportunity for comparisons and testing the developmental theory deduced from the artificial substrates.

5.1 Artificial Substrate Colonization

After extensive observation of the coverslips representing all developmental stages at each site and also of the natural mat community, it became apparent that individual heterocystous Cyanobacteria did not have a significant role in development of community architecture in the lake. Only one colony of heterocystous Cyanobacteria could be identified as such, although occasional fragments were observed. Spherical structures (Figure 14a), possibly mucilaginous colonies of Nostoc, were observed infrequently and had several diatoms attached each time. Since the object cannot be conclusively identified as a cyanobacterial colony, it is impossible to relate the diatom attachment to nitrogen fixation. It is more likely the result

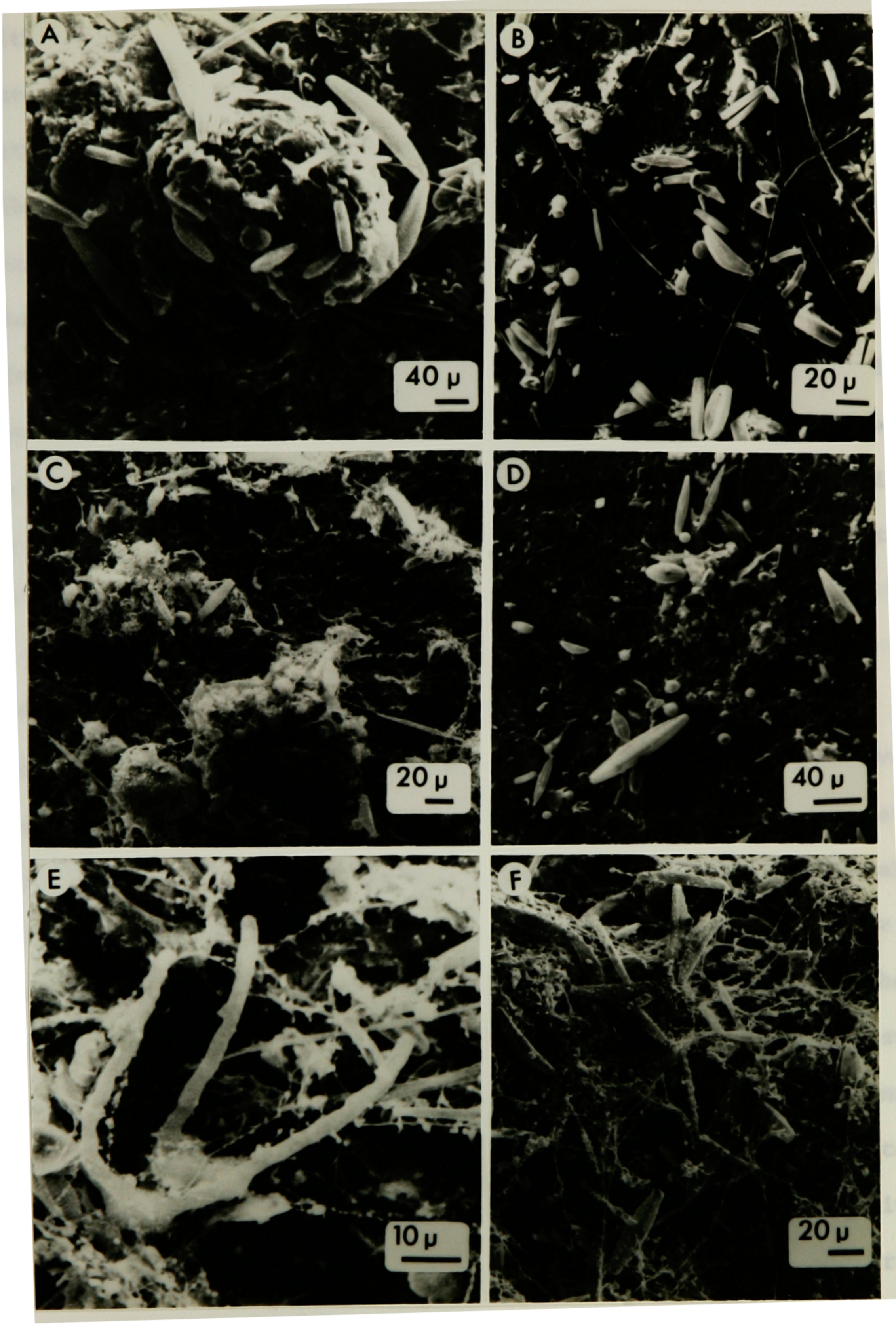
Figure 14. Scanning electron micrographs of community architecture and development. Bars indicate approximate size.

a) unidentified spherical colony

b) to d) initial stages of substrate colonization

e) heterocystous Cyanobacteria

f) diatoms obscured by expanded growth in the clumps


A
40 μ
B
20 μ
C
20 μ
D
40 μ
E
10 μ
F
20 μ

of competition for growing space.

Although the working hypothesis of this investigation seemed to be negated by these observations, there was identifiable community architecture in the initial stages of colonization. The following describes this architecture and its development. These observations are later used to interpret conditions within the natural mat. Visually, there were no apparent differences in colonization processes at the different sites. As a result, the observations were combined to form an overall scheme of community architecture development. The float at site 3 was vandalized and could not be used in analysis. The similarity of colonization at sites 1, 2, and 4 allowed the assumption that site 3 would also follow the same general pattern.

5.1.1 Month I (July 28)

This collection was made approximately three weeks after the coverslips were placed in the lake at the four sites. Initial colonization of the coverslips appeared to occur in two ways. First, individual algal cells, filaments, and also bacteria settled out of the water column and onto the surface of the coverslips. The majority of these were pennate diatoms such as Cymbella (Figure 14b), and several naviculoid types (Figure 14d). Also present were desmids, an occasional centric diatom, and unidentified filaments suspected to be non-heterocystous Cyanobacteria such as Lyngbya or Phormidium. The gelatinous material encompassing Cyanobacterial filaments and colonies hinders identification because SEM is capable only of examining surface structure. No identifying characteristics are visible through the gelatinous layers.

There were no mucilaginous pads or stalks on the diatoms observed. All were seen lying on the coverslips in a prone position without any visible means of attachment. The scalloped background in some photographs is the result of roughening of the glass surface. The plastic appears smoother.

The second method of colonization noted was the sedimentation of small clumps of intact algal mat out of the water column (Figure 14c, e). Extreme care had been taken to avoid disturbance of the natural mat around the platforms during the sampling periods. As indicated earlier, the mat was very flocculent and loosely associated in some portions of the lake. Because of its shallowness, the lake was easily disturbed by wind and pieces of the mat were often removed by the resulting water movement. Periods of high photosynthetic activity resulting in the formation of oxygen bubbles within the mat increased their buoyancy and also affected the rate of dispersal through the lake. Comparisons of these small clumps with portions of the intact mat removed from the same areas support the assumption that these clumps were from the mature mat. Clumps contained pennate diatoms such as <u>Cymbella</u>, desmids, filamentous algae, bacteria and detritus. They may be of varying size or complexity, but were easily distinguished from the small groups of individual cells forming after sedimentation from the water column. Growth and reproduction on the surface was evidenced by the formation of small monospecific colonies having from two to eight members. Growth also occurred within the clumps, apparent as the extension of fungal hyphae and/or algal filaments onto the coverslip surface from the center of the clump.

5.1.2 Month II (August 24)

Individual cells and clumps continued to settle out of the water column and onto the surface of the coverslips. In some cases, the clumps were sand grains or detrital material that had been colonized by algae.

Growth of clonal colonies had resulted in enlargement and overlapping of groups. In most cases, small portions of the coverslips remained uncolonized by single cells or by clumps. Previously settled clumps had continued to expand and many were now interconnected by the extended filaments or hyphae. The continued growth of the filaments in such a manner would eventually pull the individual clumps into a single network community.

Other evidence indicated that the clumps of mat were growing. Diatom frustules could be seen at the surface of many clumps during month I. Frustules were still visible in observations made on materials from month II, but they were obscured by growth within the clumps (Figure 14f). Evidently, the nonmotile diatoms adhere to the surface of the clumps and remain there as growth continues, becoming covered by expansion of the algae in the clump. New diatoms which became attached to the surface of the clump may also eventually be covered.

Cyanobacteria such as _Scytonema_ and _Schizothrix_, or possibly a

Chlorophyte such as Zygnema, had increased in abundance by month II (Figure 15a). All of these were observed in microscope counts made of coverslip materials. Also of interest was the occurrence of what appeared to be a mucilaginous layer on some portions of several coverslips. The layer appears to have developed after initial colonization of the substrate surface because individual diatoms could be seen protruding out from under it. Ferriera and Seeliger (1985) presented SEM micrographs of a similar layer which they described as "a mixed mucus-detritus crust" formed in part by mucilage production by Synedra. The layers seen in the present study may be the result of mucilage formation by diatoms, present in high numbers, but might also be an artifact of the preparation process. This layer did not appear on all coverslips collected at this time.

5.1.3 Month III (September 30)

Although colonization had been expected to occur throughout the field season, after 12.5 weeks of incubation, colonization was patchy (Figure 15b). It is possible that these sparser areas were sites of localized disturbances such as the sloughing of surface material noted by Roemer et al. (1984).

Growth of individuals had all but obscured the boundaries of monospecific groups on many portions of the coverslips. Filaments spread across the surface and through these groups. The resulting community looked more like an intact clump of mature mat than a monospecific group. Although not as physically complex as the mature mat, new

Figure 15. Scanning electron micrographs of community architecture and development. Bars indicate approximate size.

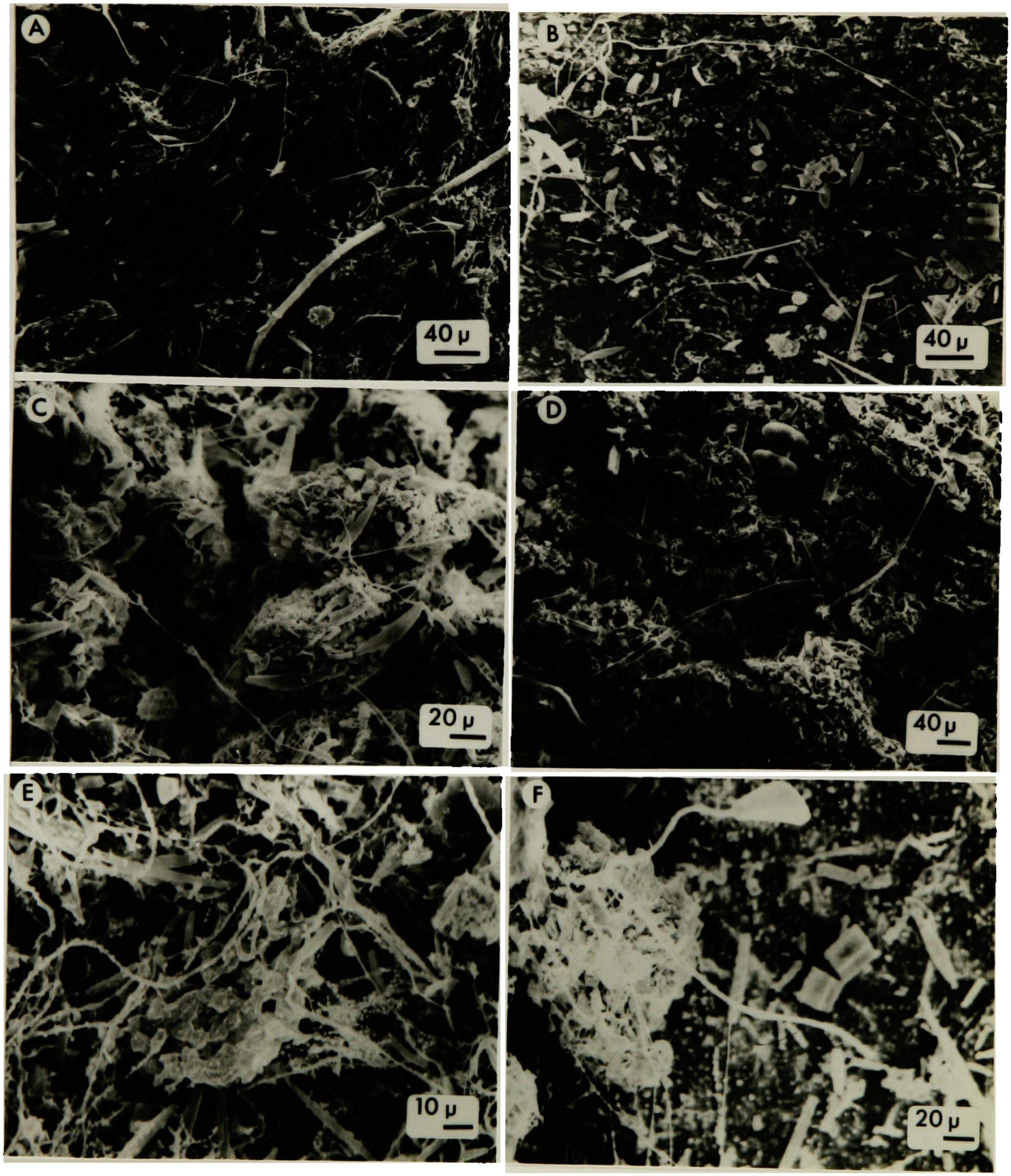
a) abundance of filament growth during month II

b) patchy colonization occuring during month III

c) and d) complex algal communities present on the substrates

e) occasional heterocystous Cyanobacteria were seen

f) stalked *Cymbella*


A
40 μ
B
40 μ
C
20 μ
D
40 μ
E
10 μ
F
20 μ

assemblages were recognizable as the foundations of a new community. The binding element appeared to be the large variety of filaments observed within the clumps. Their growth through clumps and small groups of cells served to bind the groups into a network which would eventually coalesce and develop into a mature mat community. In some areas, complex communities were visible. It is not likely these developed on bare substrate, but rather were flocs of material carried to the substrate surface by water movement. The complexities of physical relationships between individual algae were apparent in these communities (Figure 15c,d).

During the final period of growth on the coverslips heterocystous Cyanobacteria were not present in abundance, but an occasional trichome was seen (Figure 15e). Epiphytism was more pronounced, especially upon those filaments suspended above the clumps of mat, but it did not appear to be related to nitrogen fixation as many of the filaments were Oedogonium (Chlorophyta).

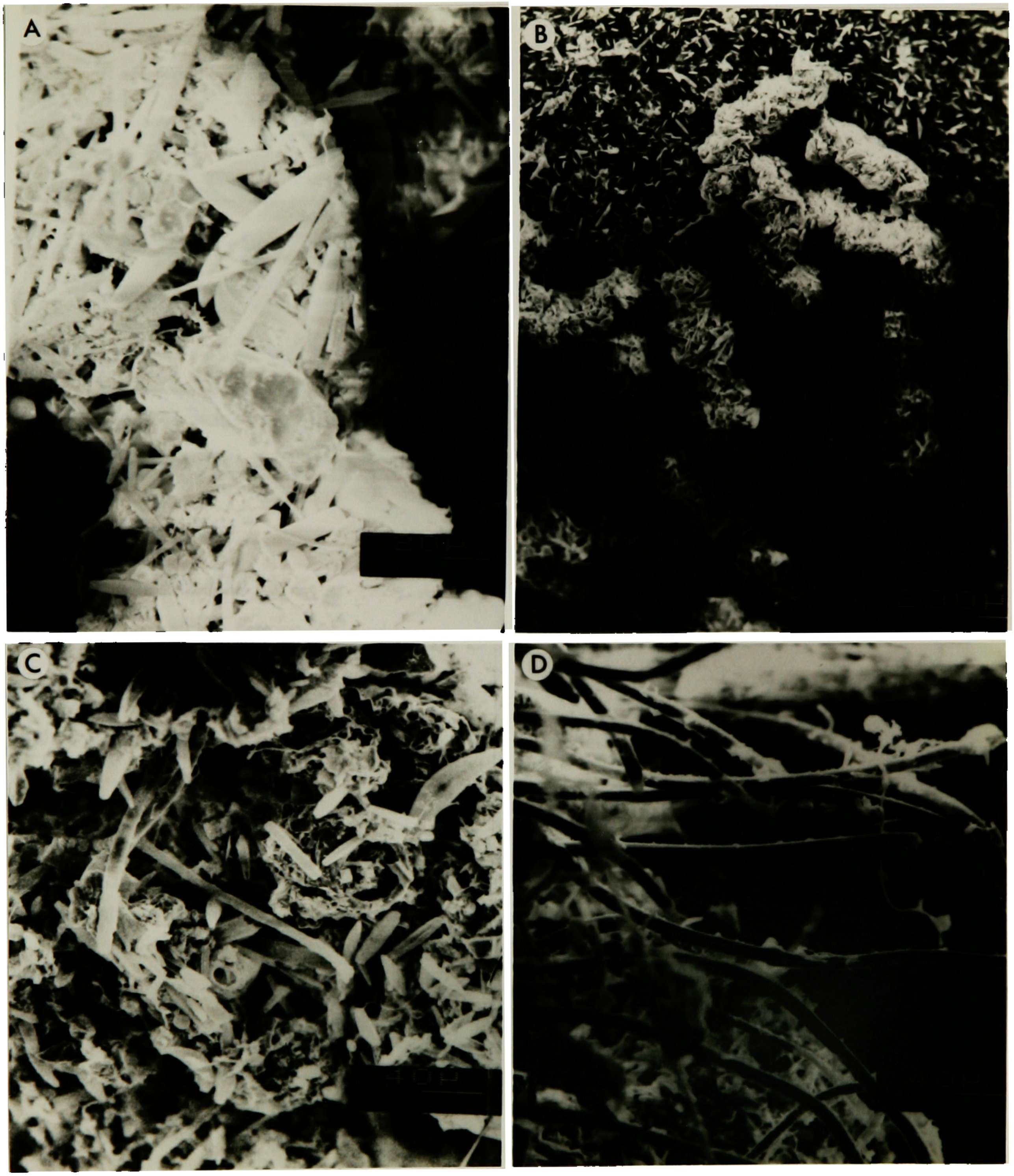
Although the majority of the pennate diatoms were still observed in a prone position, stalked Cymbella did appear on several coverslips (Figure 15f). Unfortunately, the stalks were often broken and the cells were lying on the coverslip, probably due to disturbance during transportation or preparation. The occurrence of stalked forms does indicate that the conditions on the surface were becoming more crowded, and it was advantageous for individual cells to rise above the surface of the coverslip (Hoagland et al. 1982). Finally, definite non-random algal associations were noted (Figure 16a,b). It is pos-

Figure 16. Scanning electron micrographs of community architecture and development. Bars indicate approximate size.

a) and b) non-random algal associations, possibly insect-related

c) internal view of the complex mat structure

d) external view of the complex mat structure


A
B
C
D

sible that these are fecal remains left by a grazing organism. Species composition of the diatoms appeared similar to that of undisturbed coverslips, but filamentous algae were not abundant and larger filaments were absent. Individual cells were more tightly clustered than those found elsewhere on the substrate, and large sand grains were visible. It is possible that the sand grains and diatoms would pass through the digestive tract and form these assemblages while the softer filaments would be digested. The observed compaction of the cells could be due to passage through the gut. Although not extremely abundant, these assemblages were observed on coverslips at each site.

5.2 Discussion

The spatial architecture of lake algae has been extensively described for communities on artificial substrates suspended in the water column (Hoagland et al. 1982), and epiphytic upon plants (Jenkerson and Hickman 1983, Meulemans and Roos 1985). Development of three-dimensional community architecture has been outlined (Hoagland et al. 1982): 1) development of an organic biofilm layer (detritus and bacterial mucilage) on the substrate surface, 2) initial colonization by unstalked or short-stalked diatoms forming an understory layer, 3) subsequent colonization by long-stalked diatoms or rosette-forming diatoms in an upper story and 4) colonization of the upper story by filamentous Chlorophyta and chain-forming diatoms from the water column. The complexity of the community increases over time. Meulemans and Roos (1985) found that the basal and intermediate layers formed the foundations of an epiphytic community, then the understory deteriorates as the upper story grows, leading to increased sloughing of the entire community.

The presence of a biofilm layer consisting of debris and bacterial mucilages was thought to be a prerequisite for algal colonization. Stevenson (1983) demonstrated that an agar layer, used to simulate a biofilm on artificial substrates made from ceramic floor tiles, enhanced attachment of stream algae by a factor of two. An organic film was noted by Hoagland et al. (1982) one week after substrate immersion in a Nebraskan reservoir and one week prior to observed diatom colonization. Steinman and McIntire (1986) observed no organic or bacterial film development on ceramic tiles in artificial laboratory streams, regardless of current speed, but they were able to document preferences for specific current regimes (high or low) by several diatom genera. Perhaps those genera preferring slower currents did so because of limited attachment capabilities without the presence of a biofilm layer.

The colonization rate in this study was very slow when compared with rates observed in other studies, and might be related to the lack of a biofilm. Biofilms are not necessary for algal attachment, but their presence may stimulate the colonization rate. Diatom attachment to the artificial substrates here occurred without the presence of a biofilm, but only slowly. Horizontal orientation of the coverslips simulated the natural conditions in the community and may also have helped the diatoms maintain their position. The extremely oligotrophic nature of the study lake may have also influenced the slow growth rate.

Colonization of bare substrates in many of the previously cited studies was achieved by individual bacteria, fungi and diatoms. Algae other than diatoms often did not appear until after the basal and sometimes the intermediate stories of the community had developed. In contrast, a large portion of substrate colonization in this study was accomplished by clumps of algal mat which settled out of the water column and onto the coverslip. This type of colonization is not reported in previous studies of lake communities probably because substrates are often positioned vertically, which would not allow retention of this material. Korte and Blinn (1983) noted that such "detrital microcosms" were important as early colonizers in a stream environment. Expansion could occur more rapidly from several hundred cells than from a few. Observations in the present study indicated that the primary mode of substrate colonization in this subalpine lake was the particulate material freed from the benthic mat by disturbance and easily dispersed. Rapid growth of filaments out of the flocs and onto the substrate surface served to anchor the material in place.

Diatom colonization also occurred in the manner described by Hoagland et al. (1982), but appeared to be rapidly overcome in some areas by growth expansion from the flocs. Development of the intermediate layer of stalked and rosette diatoms also occurred, but seemed restricted to those few areas not heavily covered by particulate material. The appearance of stalked Cymbella, for example, seemed less frequent in the present study than in those of Hoagland et al. (1982) or Roemer et al. (1984). The third story of filamentous algae or filamentous diatoms observed in earlier studies did not appear. Luttenton and Rada (1986) suggested that continued water disturbance by boat traffic on the Mississippi River hindered development of a third story in periphyton assemblages. This may be of importance in the present study as well. The lake was shallow and several of the summer storms were severe, possibly resulting in strong currents which could disrupt development of the secondary and tertiary levels of the community. Disturbance of the secondary layer and its development would effectively eliminate attachment possibilities for larger algae. In this community, the final stage of colonization was characterized by the occurrence of epiphytic diatoms which, due to their small size, may be the only ones capable of remaining attached during storms.

Observations of the intact mat showed an intricately interwoven and exceedingly complex community of filaments, diatoms and occasional desmids (Figure 16c). Rapid growth by the filaments appeared responsible for the cohesiveness of the community but it was apparent that the community was highly variable from clump to clump. Some exhibited heavy colonization by filaments such as Oscillatoria (Figure 16d), others lacked these entirely. Presumably those clumps having a high occurrence of filaments were those exposed to optimum growth conditions. Steinman and McIntire (1986) observed that the light intensity regime influenced the composition of laboratory stream periphyton assemblages. Filamentous Chlorophyta preferred the high light intensities, as did several genera of diatoms. Perhaps light intensity also influences growth of Oscillatoria and other filamentous Cyanobacteria in a similar manner.

Stalked diatoms were rare in the present study, but epiphytic ones occurred frequently on filaments extending above the clumps. A large portion of any individual clump was composed of detritus. Presumably the algae expanded outward beyond this material towards light and less crowded conditions. Nonmotile algae and bacteria would remain under the new layers, eventually decomposing and providing nutrients for their successors.

In conclusion, the colonization sequence in this community can be best described as: 1) initial colonization by individual diatoms and clumps of mature mat community deposited on the substrate surface; 2) formation of a secondary layer of filamentous algae extending outward from the clumps, along with some growth of stalked diatoms but mainly by those prostrate individuals already present; 3) formation of a rudimentary tertiary layer represented by small diatoms epiphytic upon the growing filaments and clump material; and 4) cohesion of separate mat clumps and diatom groups by overgrowth of the algal filaments. Organisms and detritus settling out of the water column would be caught within the developing network to form the complex community observed in the natural mat collections. Light intensity regime and chance disturbance by water movement are expected to have a significant effect upon the development of the final community architecture.

An organized structure did not appear to be maintained beyond the initial stages of colonization and succession, except perhaps on the outer portions of the mat clumps. Internal composition of the mat did not reveal any organization of individual cells. Compaction, continued disturbance by water movement, decaying of detritus and cells requiring more light for survival might obliterate any identifiable structure. Steinman and McIntire (1986) also noted the lack of organized structure in mature periphyton assemblages grown in laboratory streams.

The initial hypothesis of this investigation was that nitrogen fixation by heterocystous Cyanobacteria influenced the development of community architecture. A physical relationship was anticipated in which the nitrogen-fixing Cyanobacteria would serve as the nucleus for the developing community. Such a precise physical relationship could not be documented in the mat. Indeed, visible individual heterocystous Cyanobacteria were absent on most substrates. They did occur within the settling clumps of material. It appeared that while heterocystous Cyanobacteria do not have a significant role in the pioneering stages of community development, this did not eliminate the possible importance of nitrogen fixation in the mature community.

In some portions of the lake, late in the season, a more cohesive mat was observed. It was not investigated thoroughly, but in a cohesive community of this nature a type of stratified architecture might develop. This type of architecture, exhibiting a seasonal rotation of layering in Cyanobacterial mats, was described in great detail by Jorgensen et al. (1983), and also by Fagerburg and Arnott (1979). It was apparent from these studies that architecture was most readily identifiable in the upper zones of the mat, those containing living algae. Below this, the structure became obscured. An identifiable photosynthesizing layer of Cyanobacteria was recognized in some gelatinous benthic mats of Solar Lake, Sinai at a depth of 3 to 5 cm (Jorgensen et al. 1983). The ability of Cyanobacteria to survive within the benthic mats of the Sinai lake indicates that living Cyanobacteria could perhaps survive within the flocculent mat of the subalpine lake under observation in this study. The flocculent material does contain a large number of living cells, documented during the light microscopic examination of the mat. A subtle spatial relationship influenced by nitrogen fixation might exist among these algae, but it would require further investigation before this could be determined.

6.0 The Natural Community and Artificial Substrates

Identifying the development of the mature algal community and its architecture were of primary interest in this study. This was a difficult task because it required frequent and intensive sampling. Artificial substrates have proven to be an acceptable method of collecting information to describe the development of periphyton communities (Castenholz 1960, Brown 1976, Blinn et al. 1980). However, care must be taken when interpreting the results (Robinson 1983, Wetzel 1983).

The community under observation in the current study might be described as an epipelic community (growing in or on sediments). While some of the algae are capable of attachment to the sediments, others became trapped within the detrital network and were retained in the community although it may not be their preferred habitat. Some artificial substrates might induce a bias towards those algae capable of attachment. In this study, the coverslips were placed horizontally rather than vertically, and also placed close to the water/benthos interface to provide a benthic habitat similar to the natural one. Samples from September 30 represented the most mature artificial substrate communities and were used for comparisons to the natural community. The majority of the coverslips for September 30 had been prepared for SEM. For light microscopy, only two glass and one plastic coverslip were available for sites 1 and 2; and two plastic and one glass coverslip for site 4. Site 3 was vandalized and the data were not analyzed here.

6.1 Plastic versus Glass Substrates

No significant differences in total densities or phyletic composition were found between the two types of artificial substrates used at sites 1 and 2 (Figure 17). Site 4 was more variable. Significant differences, identified between replicate counts from the individual coverslips, were observed at this site in total density as well as the populations of Chrysophytes and Cyanobacteria. For example, plastic coverslip B had lower total density than did plastic A, while the glass coverslip did not differ from either plastic one.

6.2 Natural versus Artificial Communities

There were no significant differences between the total density for artificial and natural communities at sites 1 and 2. Density at these sites was between 1.9 x 10^6 and 2.5 x 10^6 organisms per gram of benthic material. Site 4 was exceptional because one plastic coverslip had significantly higher densities when compared with the other artificial and natural communities (3.68 x 10^6 and 2.1-2.9 x 10^6 organisms, respectively).

The Chrysophyte densities did not vary significantly between communities at site 1 (5.8 - 8.7 x 10^5 organisms) or site 2 (8.3 x 10^5 - 1.13 x 10^6 organisms). At site 4 significantly lower densities of Chrysophyta occurred in the mat samples (4.7 x 10^5 versus 1.13 - 1.84 x 10^6 organisms).

At site 1 Chlorophyte densities were significantly higher on the plastic coverslip (4.7 x 10^5 organisms). The natural community con-

Figure 17. Composition of natural and artificial substrates by number of cells per gram benthic material. Cryptophyta and Euglenophyta populations were observed to be less than 25,000 organisms per gram of material when present (symbols indicate presence only).

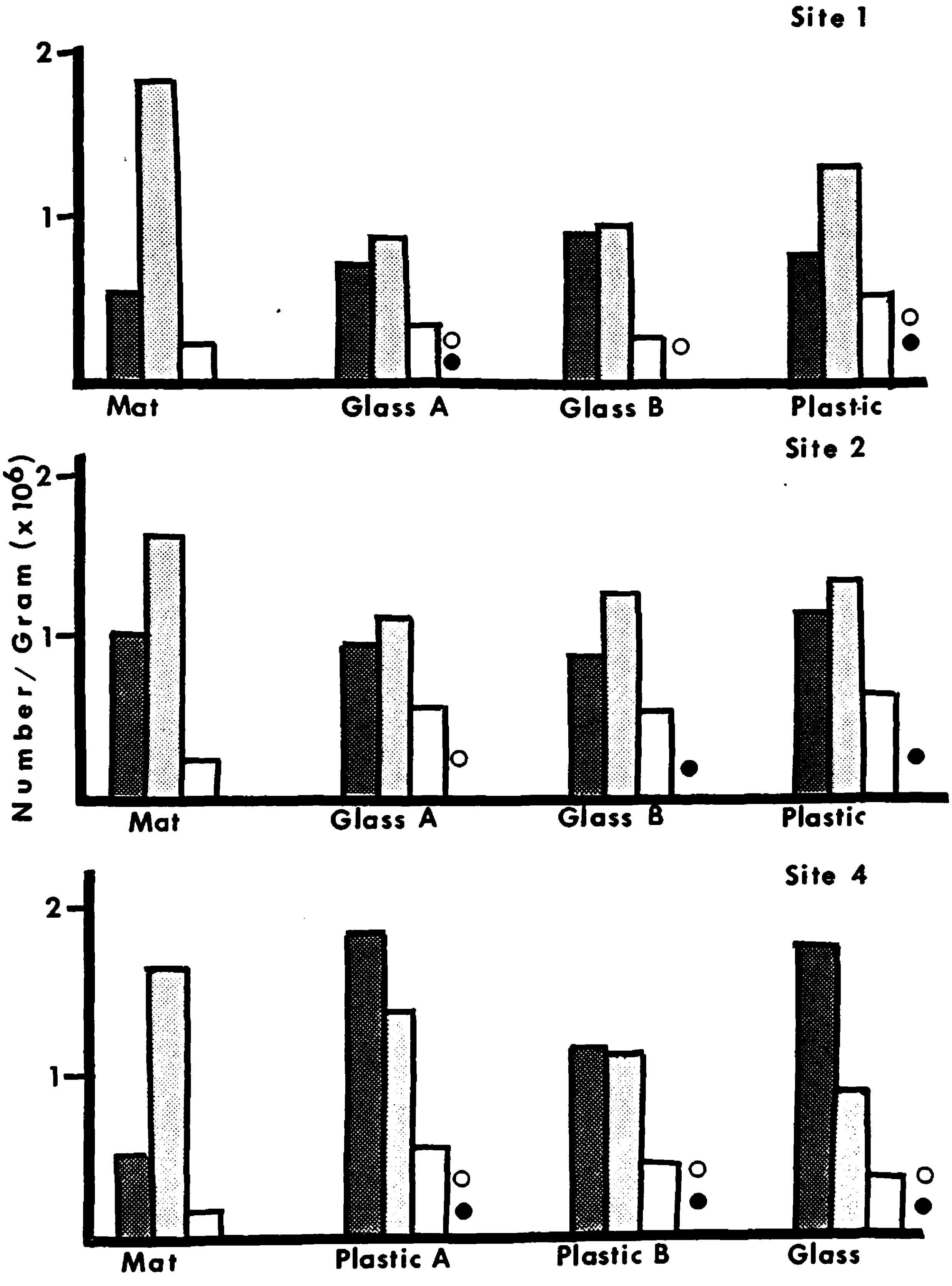
Chlorophyta ☐

Chrysophyta ▓

Cryptophyta ○

Cyanobacteria ░

Euglenophyta ●



Site 1
2
1
Mat
Glass A
Glass B
Plastic
Site 2
Number/ Gram (x 10^6)
2
1
Mat
Glass A
Glass B
Plastic
Site 4
2
1
Mat
Plastic A
Plastic B
Glass

tained the lowest densities of Chlorophytes (1.9 x 10^5 organisms) but this was not different from plastic coverslip B (2.4 x 10^5 organisms). At site 2, the artificial substrates did not differ significantly in the density of Chlorophytes (4.6 - 5.7 x 10^5 organisms). Lowest densities occurred in the mat (1.9 x 10^5 organisms), but were not different from the lowest artificial substrate values. At site 4, the mat had significantly lower green algae densities than the artificial substrates (9 x 10^4 and 3.5 - 5.3 x 10^5 organisms).

Cyanobacterial populations were variable between communities. At site 1, densities of blue-green algae did not differ from one another (8.3 x 10^5 to 1.2 x 10^6 organisms), but were significantly lower than the natural community (1.89 x 10^6 organisms). At site 2, Cyanobacterial densities on the artificial substrates were not different (1.03 - 1.27 x 10^6 organisms). The natural population was significantly denser than the smallest artificial substrate community (1.6 x 10^6 and 1.03 x 10^6 organisms, respectively). At site 4, the natural Cyanobacteria were significantly higher in density than the artificial substrate populations (1.7 x 10^6 vs. 8.3 x 10^5 to 1.3 x 10^6 organisms).

Finally, all coverslips were noted to have populations of two other divisions, Euglenophyta and Cryptophyta. These were represented by _Trachelomonas_ and _Cryptomonas_ spp., respectively. In general, these organisms formed less than 1 percent of the total density on the coverslips. In contrast, although occasionally seen in the mat, these organisms were not observed during any counts made of that material.

Community composition of the algae is important when considering similarities between natural communities and artificial substrates. As an example, Table 6 lists the genera in order of abundance present on both glass and plastic coverslips and in the natural mat community at site 1.

There were differences in community composition between the mat and the artificial substrates. Navicula and Cymbella were the most abundant Chrysophytes in the mat and on the artificial substrates. Epithemia and Centrales spp. were infrequent in both communities. Fragilaria was abundant in the mat, but among the least numerous genera in the substrate community. Ophephora and Diatoma were present in low numbers within the mat but they were not observed on the artificial substrates, whereas Synedra and Achnanthes were observed on the substrates but not in the mat. Infrequently occurring genera were observed in both communities.

The green algae comprised less than 10 percent of both the natural and artificial substrate-supported communities. This may be important when evaluating the similarity of the communities from a compositional viewpoint. Gloeocystis and Oocystis dominated in both natural and artificial communities. Intermediately abundant genera were also similar: Scenedesmus, Tetraedron, and an unknown colony. Infrequent genera were more variable. Several occurred on the substrates but not in the mat: Mougeotia, Cosmarium, Elakatothrix, Quadrigula, Lagerheimia, Ophiocytium, and Ankistrodesmus. Lyngbya and Phormidium dominated the Cyanobacteria populations in all communities. Gloeothece, Aphanothece and Aphanocapsa were common in all communities.

Table 6. Generic composition of natural and artificial substrates. Genera are listed in decreasing order by number of cells per gram of mat. Site 1 is presented as an example of the differences between substrate types.

Mat	Glass	Plastic
	CHRYSOPHYTA	
Navicula	*Cymbella*	*Cymbella*
Cymbella	*Navicula*	*Navicula*
Fragilaria	*Synedra*	*Achnanthes*
Ophephora	*Fragilaria*	Pennate spp.
Diatoma	*Epithemia*	*Synedra*
Centric spp.	Centric spp.	*Fragilaria*
Epithemia		
	CHLOROPHYTA	
Gloeocystis	*Oocystis*	*Oocystis*
Oocystis	*Gloeocystis*	*Mougeotia*
Scenedesmus	*Mougeotia*	*Cosmarium*
Tetraedron	*Cosmarium*	*Gloeocystis*
unknown colony	*Scenedesmus*	*Elakatothrix*
Coelastrum	unknown colony	*Scenedesmus*
Stigeoclonium	*Elakatothrix*	*Tetraedron*
	Tetraedron	unknown colony
	Quadrigula	*Ankistrodesmus*
	Lagerheimia	*Coelastrum*
	Ophiocytium	*Lagerheimia*
	Stigeoclonium	
	CYANOBACTERIA	
Phormidium	*Lyngbya*	*Phormidium*
Lyngbya	*Phormidium*	*Lyngbya*
Gloeothece	*Gloeothece*	*Anabaena*
Aphanothece	*Aphanothece*	*Aphanocapsa*
Aphanocapsa	*Aphanocapsa*	*Gloeothece*
Gomphosphaeria	*Anabaena*	*Aphanothece*
Oscillatoria	*Chroococcus*	*Chroococcus*
Chroococcus	*Coelosphaerium*	*Nostoc*
unknown	*Gomphosphaeria*	*Gomphosphaeria*
Anabaena		
Coelosphaerium		
Nostoc		

Gomphosphaeria was numerous in the mat, but low in number on the artificial substrates while Anabaena followed the opposite pattern. Oscillatoria, present in the natural communities, was not observed on the artificial substrates. Chroococcus and Coelosphaerium were minor components of both natural and artificial substrate communities. Nostoc was rare in the mat and not observed at all in the artificial substrate populations.

6.3 Community Similarity Indices

A description of the indices, developed by Gleason (1920) and Ellenberg (1956) is presented in section 2.3.5. The use of similarity indices was intended to supplement and/or confirm observations based on correlation coefficients, analysis of variance, Fischer's Least Significant Differences, and organism counts by light microscopic examination. In the following section, results from the application of the two community similarity indices are presented and evaluated with respect to one another and the information gathered using the other techniques. Artificial substrates from site 3 were not used in the previous statistical and descriptive evaluation because of vandalism to the float. In this section, the site 3 data was included in the analysis to determine if the vandalism had significantly impacted the artificial substrate community.

6.3.1 Percentage Similarity

Both indices generated a set of comparative similarity values for each pair of communities evaluated. The similarity values generated by both were generally higher than 75 percent (Table 7). Mat samples were more similar to one another, generally having 90 percent or higher similarity, than to artificial substrates, and vice versa. Lowest values were observed when mat samples were compared with the artificial substrates. This indicated that, although the mat and artificial substrates were similar, the natural communities resemble one another more than they resemble their artificial substrate partner.

The results generated by the two indices were very similar. Values generated by Gleason's index were on the average 4.3 percentage points higher than those generated by the application of Ellenberg's index to the same data set. Differences as high as 10 percentage points were observed. Ellenberg's index was designed to eliminate double-weighting of those taxa held in common by the two samples being compared. It was not surprising therefore that Ellenberg's index should have lower overall similarity values than Gleason's.

6.3.2 Dendrogram Evaluation

Average distance linkage was used to evaluate the similarity values calculated from each of the indices, and the generated dendrograms are presented in Figures 18 and 19. The two dendrograms were very similar despite the overall lower values of Ellenberg, but there were some noticeable differences. The sequence of samples varied slightly between the two dendrograms. Site 4-8/24 and site 3-7/28 were reversed and the data concerning the artificial substrates at site 4 were var-

Table 7. Similarity values generated by Gleason and Ellenberg indices. The following compares site 1 on July 6 with all other samples including artificial substrates. Values represent percent similarity between the two sites being compared.

Substrate	Date	Site	Gleason	Ellenberg
mat	7/6	2	99.0	98.1
"	"	3	99.3	98.5
"	"	4	99.2	98.4
"	7/28	1	99.5	99.0
"	"	2	98.7	97.5
"	"	3	98.6	97.2
"	"	4	99.4	98.8
"	8/24	1	99.2	98.3
"	"	2	98.9	98.0
"	"	3	94.9	90.3
"	"	4	94.9	90.4
"	9/30	1	94.6	89.7
"	"	2	94.4	89.4
"	"	3	94.4	89.4
"	"	4	90.4	82.5
glass	"	1	85.6	74.9
"	"	1	91.6	84.5
plastic	"	1	90.5	82.7
glass	"	2	93.1	87.1
"	"	2	93.2	87.2
plastic	"	2	93.7	88.2
"	"	3	89.6	81.2
"	"	4	94.2	89.1
"	"	4	89.4	80.8
"	"	4	85.9	75.3

Figure 18. Average linkage cluster dendrogram generated by the Ellenberg similarity index.

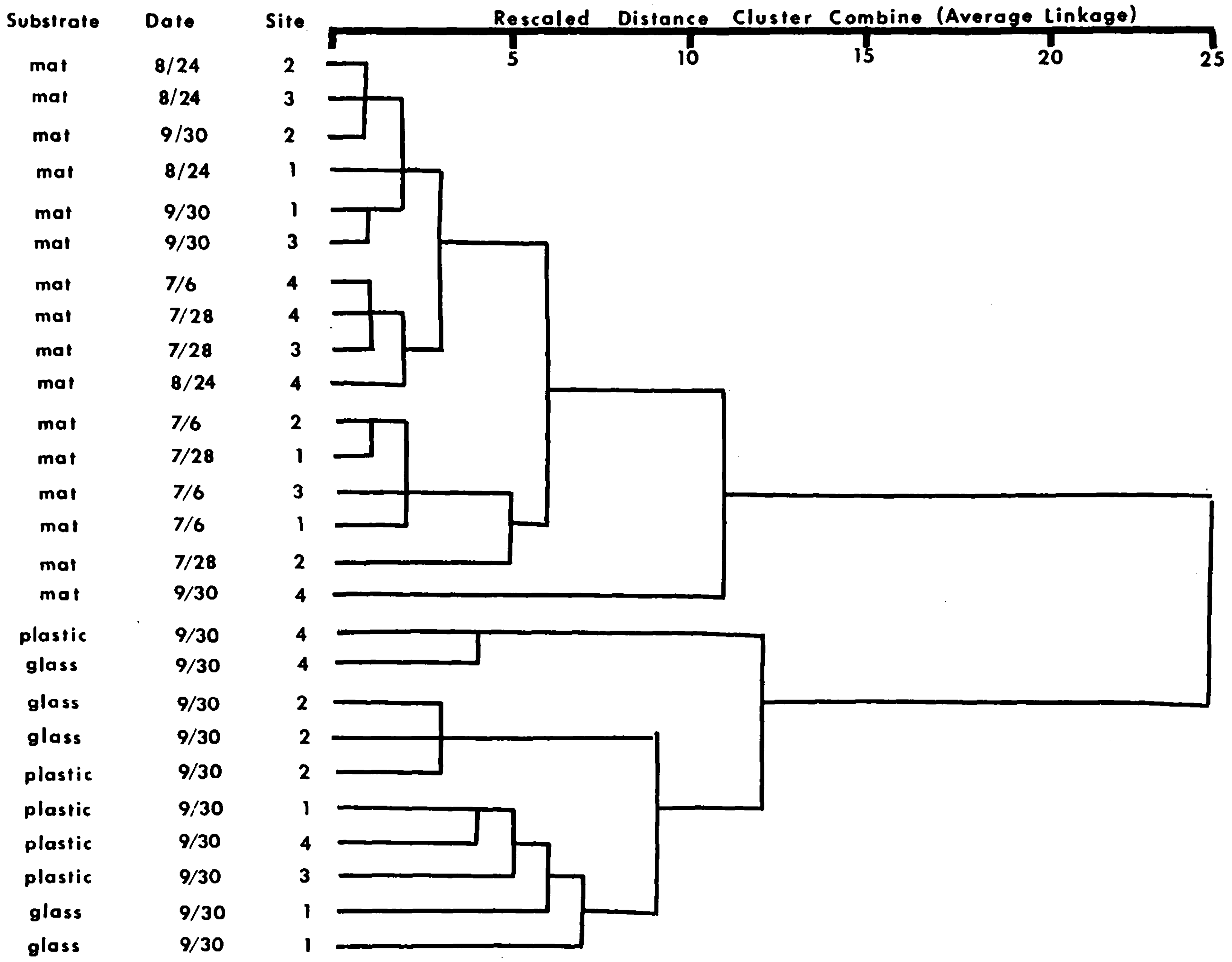
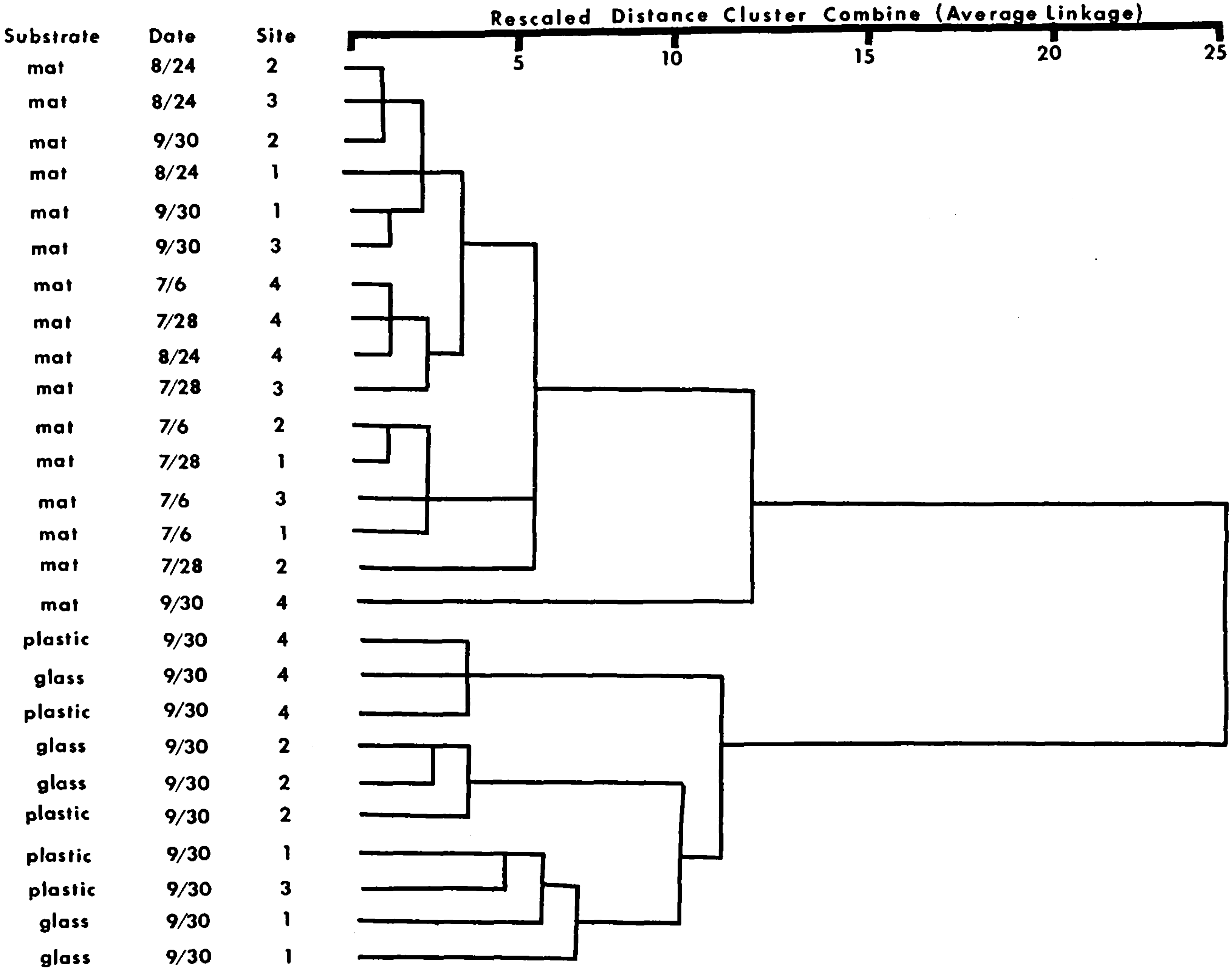

Substrate
Date
Site
Rescaled Distance Cluster Combine (Average Linkage)
5
10
15
20
25
mat 8/24 2
mat 8/24 3
mat 9/30 2
mat 8/24 1
mat 9/30 1
mat 9/30 3
mat 7/6 4
mat 7/28 4
mat 7/28 3
mat 8/24 4
mat 7/6 2
mat 7/28 1
mat 7/6 3
mat 7/6 1
mat 7/28 2
mat 9/30 4
plastic 9/30 4
glass 9/30 4
glass 9/30 2
glass 9/30 2
plastic 9/30 2
plastic 9/30 1
plastic 9/30 4
plastic 9/30 3
glass 9/30 1
glass 9/30 1

Figure 19. Average linkage cluster dendrogram generated by the Gleason similarity index.


Rescaled Distance Cluster Combine (Average Linkage)
5
10
15
20
25
Substrate Date Site
mat 8/24 2
mat 8/24 3
mat 9/30 2
mat 8/24 1
mat 9/30 1
mat 9/30 3
mat 7/6 4
mat 7/28 4
mat 8/24 4
mat 7/28 3
mat 7/6 2
mat 7/28 1
mat 7/6 3
mat 7/6 1
mat 7/28 2
mat 9/30 4
plastic 9/30 4
glass 9/30 4
plastic 9/30 4
glass 9/30 2
glass 9/30 2
plastic 9/30 2
plastic 9/30 1
plastic 9/30 3
glass 9/30 1
glass 9/30 1

iable. The re-scaled distance at which clusters formed was variable between the two. These do not appear to be major differences. The clusters that form and their relationship to one another were very similar in both cases. Evaluation of community similarity therefore will be presented using combined results from both dendrograms. Differences will be noted where they occur.

Clusters for the artificial substrate and natural communities were distinctly separated from each other. The two clusters joined at the re-scaled distance of 25. Within the artificial substrate cluster, there was a tendency for the substrates from a single site to cluster together. This was especially true for data from Gleason's index, where site 4 formed a distinct cluster; and site 2 clustered with itself before joining sites 1 and 3. Although substrates from a single site were similar to one another, they were not identical as can be seen from the distance required before they do cluster.

Among the natural communities this tendency for sites to cluster together was disturbed somewhat by the influence of "seasonality". With the exception of site 4-8/24 and site 4-9/30, early summer samples (from July 6 and July 28) tended to cluster together, with samples from the same site being more closely associated. The same trend generally occurred among the late summer samples (August 24 and September 30). Again, this was somewhat more apparent in the dendrogram drawn from the Gleason index. Sample site 4-9/30 was unique in both dendrograms. It remained separate until all the other mat samples had clustered.

6.4 Discussion

No significant differences in number or in composition could be detected arithmetically between glass and plastic coverslips at sites 1 and 2. Site 4 exhibited considerable variation, but this did not reflect preferential attachment of algae to glass or plastic. Cyanobacterial numbers at this site were significantly lower on the plastic coverslip, but this appeared to be a chance occurrence. No indication of this same preference could be found at the other sites. Differences in community composition and total organism number per gram due to the nature of the substrate could be ruled out in this investigation.

When the natural and artificial communities were compared, definite differences in the phyletic associations but not densities were observed. The Chrysophytes had similar densities on natural and artificial substrates. The green algae were most abundant on the artificial substrates while the Cyanobacteria were most abundant on the natural mat. Differences were detectable at the genus level. Although the most abundant organisms in each division were identical at the four sites, the less abundant species were very different. For example, filamentous green algae were numerous on the coverslips but were seen only rarely in the natural community. The fact that the dominant genera did not vary between artificial and natural communities appears related to the flocculent nature of the benthic community. As described in an earlier section, most of the substrate surfaces were colonized by flocs of benthic material rather than individual cells. Because the flocculent material represents the largest source of cells on the substrates, it was not surprising that dominant genera were identical in both the natural and artificial communities. If this were not the case, the proposed sequence of colonization would be questionable.

Selectivity of artificial substrates for specific divisions or only those algae capable of attachment had been suggested by many authors (i.e. Castenholz 1960 and Sladeckova 1962). Many Chrysophytes are capable of attachment to a variety of substrates, and generally are the dominant organisms observed in artificial substrate studies (Castenholz 1960, Brown 1973a and b, Hoagland et al. 1982; Roemer et al. 1984). Differences in generic composition of the Chrysophyte communities between the artificial substrates and natural communities can be explained partially by attachment ability. Fragilaria, Ophephora, and Diatoma, diatoms incapable of attachment, were abundant in the mat, but not on the artificial substrates. Their inability to attach themselves to the substrate could have prevented these diatoms from remaining on the coverslips. They might, however, become entangled among the filaments and detritus present in the benthic community and be retained. Hoagland et al. (1982) noted increasing numbers of such cells on artificial substrates only after the stalked or attached cells had developed enough suitable three-dimensional architecture to retain them. This type of three-dimensional architecture was noted only rarely on the coverslip surfaces employed in the present study. It was possibly inhibited by the water movement. This same water movement would remove any non-attached cells from the substrate surface in the same manner that dead cells do not remain on the surface

(Brown 1973a). Diatoms capable of attachment such as Synedra and Achnanthes were numerous on the substrates but not in the mat, thus the ability for attachment seems to be very important in determining habitat occurrence of the diatoms in the lake.

The higher numbers of Chlorophytes on the substrates when compared to the mat may also be related to survival strategies. A large number of the genera observed on the substrate but not in the mat are described as planktonic Chlorophyta (Prescott 1962), i.e. Quadrigula, Ankistrodesmus, Lagerheimia. After settling from the water column, these cells might be expected to survive longer on the more open surface of the coverslips where they would be less likely to be buried by continuing growth than in the mat community. Their method of retention on the substrate is unknown.

Sladeckova (1962) suggested that Cyanobacteria might not attach well to artificial substrates. This appeared to be true for the less abundant Cyanobacteria observed in this study. Many of those genera observed in the mat but not on the substrates were colonies of small cells. Although they may be planktonic, they prefer benthic or attached locations (Prescott 1962). This would reduce the probability of these genera being carried to the substrate independent of flocculent material. Oscillatoria generally is a benthic organism, and it was observed mainly in the mat community. However, it was rarely observed on the substrates in spite of the fact that colonization of substrates occurred mainly by such mat material. Natural communities containing numerous Oscillatoria would be more cohesive than communities lacking this genus because of the binding ability of this large trichome. Hence, these communities may be less prone to disturbance by water movement and would not be carried to the substrates.

On the basis of the dendrograms generated from the similarity values of Gleason's and Ellenberg's indices, it can be concluded that 1) the artificial substrate communities were distinctly different from the natural mat communities; 2) "seasonality" exerted considerable influence upon the similarity of natural communities; 3) samples from the same site were more similar to one another during a particular 'season' than they were to other sites; and 4) differences between glass and plastic substrates at the same site were generally insignificant, and 5) vandalism at site 3 did not greatly bias evaluation of the artificial community development. It is important to note that these are conclusions drawn from visual differences in the two dendrograms. Clustering of data may cause relationships to appear which may, in reality, be insignificant. Sample site 4-9/30 was clearly a unique sample when evaluated from the dendrograms, being the last of the natural mat samples to join the cluster. Yet when the similarity values from the indices were compared, this sample has 90.39 to 99.14 percent similarity with other mat samples when calculated by Gleason's index, and 83.3 to 98.3 percent similarity using Ellenberg's index. The designation of this sample as unique may be an artifact of the cluster method. It appears to be very similar to the other samples. When similarity values of 90 percent and above are generated by such indices it may be best to identify and evaluate all apparent trends with caution.

Kaesler and Cairns (1972) made similar observations in a limnological study of the upper Potomac River. They concluded the use of similarity indices could aid in reducing the amount of redundancy typically found in such studies by identifying important relationships, but did note that the clustering of results had several important disadvantages; i.e. distortion was introduced by averaging the similarity values and resulted in the production of artificial hierarchical structures. In a review of species diversity and similarity indices, Sheehan et al. (1984) pointed out that similarity values appeared to be more sensitive to differences in communities at low levels of stress than are diversity indices, and therefore "more indicative of structural differences."

Conclusions about community similarities made from the other mathematical and descriptive methods employed in this investigation can be briefly summarized: 1) no differences between artificial substrates and natural mat samples were noted in the total density per gram data. Significant differences occurred at the generic level. Genera were the same in both communities, but the numbers of each were variable. Less abundant genera were very different; 2) in early summer, differences between sites were more pronounced. Sites became increasingly similar in total density (i.e. not significantly different) as the summer progressed; 3) in general, sites 1 and 2 seemed more similar to one another than to sites 3 and 4, and vice versa; 4) no differences were detected between the glass and plastic substrates at sites 1 and 2, but differences were noted at site 4.

For the most part, conclusions reached using the similarity indicies were similar to those reached using the traditional statistical methods, but each provided a slightly different view of the same material. That of the similarity indices is perhaps the more superficial, because it is the result of one analysis rather than several. However, similarity indices identified a seasonal influence which was suspected but could not be confirmed by the other methods. Differences between artificial and natural communities illustrated by the dendrograms appeared to be of minor importance based on the other methods. This may be related to the high overall level of similarity between all sites. Employing the similarity indices identified small differences between the samples. They may have been too small to identify using the other methods or perhaps they were designated as unimportant. The presence of infrequent genera at one site or another might be expected to have such a result. The indices would identify these genera as important differences while the investigator may designate them a random chance occurrence.

7.0 Summary of Conclusions

The primary goal of this investigation was to characterize the benthic algal community of an oligotrophic subalpine lake located in the Jim Lakes Basin in northwestern Montana. Preliminary investigations by Fritz-Sheridan (personal communication) and myself had indicated measurable nitrogen fixation was occurring within the benthic community. Using this information, a hypothesis relating the nitrogen-fixing capabilities of Cyanobacteria to three-dimensional community architecture within the mat was developed. Specifically, it was hypothesized that in a nitrogen-limited environment, a three-dimensional architecture of non-fixing algal genera might develop around nitrogen-fixing Cyanobacteria. This investigation did not support the hypothesis. Nitrogen limitation within the mat could not be shown with certainty and no three-dimensional architecture centering around nitrogen-fixing Cyanobacteria could be detected. Nitrogen-fixing Cyanobacteria were observed in the mat, and it is possible that an architecture based upon their presence does exist at a level more subtle than that examined by the present investigation.

Despite the inability to confirm the proposed hypothesis, significant insight was gained in the areas of community architecture, composition and dynamics of a benthic algal mat in a subalpine oligotrophic lake. The following characterizes this benthic algal community.

The benthic algal community was dominated by non-heterocystous Cyanobacteria at all times during the investigation. Chrysophytes, especially the class Bacillariophyceae, were the second most abundant group found in the mat. The Chlorophytes formed less than 15 percent of the total number of organisms observed. An occasional Trachelomonas sp. or Cryptomonas sp. was also noted. In general, these uncommon genera and the majority of the Chlorophytes were transients or planktonic in nature. The bulk of the benthic community was comprised of Cyanobacteria and diatoms.

There appeared to be little succession over the summer in genera occurring in the mat. The same organisms were present throughout the summer though their standing crop were somewhat variable. Dominant genera were identical at all sites, and remained dominant throughout the season. The occurrence of less abundant genera varied between sample sites.

A definable community architecture was not observed in the mature benthic material collected during the season. There appeared to be no layering or organization within the mats at any of the sites. An apparently random association of algal cells, detritus and sand formed the benthic material observed. The non-cohesive nature of the mat was most likely responsible for this apparent lack of architecture. It was easily disturbed and thus could not provide the stability required for development of distinct architecture. The flocculent nature of the material also provided a rapid means of colonizing new substrates. Small pieces of benthic material were observed to be the primary means of colonization throughout the lake. The occurrence of distinct three-dimensional community architecture on the substrates employed in this study was greatly reduced because of this. A simple two-dimensional architecture and rudiments of tertiary structure were observed at all sites, but would be expected to be rapidly overcome by algal development from the flocs of benthic material transported to the substrates.

Environmental conditions were generally similar between sites. Generally, nutrient concentrations did not vary significantly between them. Phosphorus concentrations were uniformly very low and changed little during the field season. No correlations were found between phosphorus and algal community dynamics because of this. Nitrogen concentrations did vary during the investigation and were weakly correlated with variations in community structure. Nitrogen concentrations were low in the water column and might be expected to affect the algal community. The relative paucity of heterocystous blue-green algae indicated that nitrogen was not as scarce within the benthic community as had been hypothesized. It is likely that the benthic material served as a nutrient source for the algae within it. Detrital decay could provide a significant source of nutrients on a continual basis, and algae transported by wave action to new substrates would be equipped with all the nutrients necessary for growth.

Temperature was similar between sites throughout the investigation. Seasonal variation in temperature was observed, but did not correlate well to algal dynamics. Light appeared to be the single physical parameter measured during this investigation which varied between sites. Photon flux density may have been partially responsible for the small differences noted in composition between the four sites.

Sites 1 and 2, which had very open exposure to sunlight, were similar in composition and structure. Sites 3 and 4 were shaded by trees which grew almost to the water line and these were also very similar.

Based on the conclusions made from the present investigations, similarity indices are an excellent tool for characterization of several communities when used in conjunction with other methods. The index could be employed to identify similarities between sites, and therefore point out important relationships for further investigation. This would reduce unnecessary calculations and focus effort upon significant differences. Similarity index results should be interpreted cautiously when the similarity between sites is greater than 90 percent, as in the present study. Although subtle differences may become very distinct using this method, differences identified may also appear significant when they are not. In such cases similarity indices should be used only in conjunction with other methods, allowing a more balanced characterization of the communities under investigation.

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