FRESHWATER ALGAL POPULATIONS AND WATER QUALITY

by

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ABSTRACT

Periphyton communities on vertically oriented artificial substrata at four sampling stations in the shallow (1.83 to 2.75 m) littoral region of Elk Lake (Vancouver Island, B.C.) were quantitatively sampled over a six month period from August 1967 to January 1968. Statistical methods were employed to determine the degree of variability in community structure between station locations (and exposure periods) and the significance of this variation in relation to fluctuations in environmental data (15 variables) concurrently monitored.

An exposure-frame supporting 50 x 75 mm glass slides was devised to replicate sampling at monthly and overlapping time intervals (TS) of 27 to 135 days at each station. Periphyton species numbers, numbers of individuals, relative abundances and species diversity (Shannon-Weaver index H") were computed from count data obtained using permanent membrane filter preparations. While count estimates (cells or colonies/mm^2) were within recommended error limits and most organisms were randomly distributed on the filter surfaces, analysis of the experimental design indicated that the counting stage contributed the largest variance component with percent error of 17 and 30% for total and individual species counts. By contrast, variance due to sampling (5-15%) and filtering (0-5%) was usually lower for both.

Of the 29 periphyton taxa counted, 28 were diatoms and 20 of these had constancy values of 100%. Four species, *Achnanthes minutissima*, *Cocconeis placentula*, *Fragilaria crotonensis*, and *F. virescens*, composed >50% of total cell populations in all periphyton samples and the latter two species also dominated the net planktonic diatom assemblage.

Elk Lake, a second order warm monomictic lake of enriched eutrophic condition, is typical of the Insular Lowland Limnological Region. In general, normal seasonal fluctuations in physico-chemical variables were found, their variation being effected to some extent by warm dry summer weather followed by heavy rainfall, high flush rates and flood conditions.

Whereas the four stations were similar in measured physico-chemical variates, periphyton species numbers and species composition, as well as plankton species composition and relative abundances, statistically significant station variation occurred in total cell numbers, individual species populations and species diversity of periphyton communities in each of the 11 different TS's. While the temporal variation in cell numbers and species diversity was statistically related to fluctuations in measured physico-chemical variates (total and orthophosphate, calcium and total hardness) and length of slide exposure, the station variation within the lake for any one TS was not.

Since species diversity of the periphyton decreased with increasing exposure duration and increasing total cell populations, station variation in periphyton communities over the same exposure periods and under the same measured physico-chemical conditions may be due in part to species interaction as hypothesized for two species, *Achnanthes minutissima* and *Cocconeis placentula*, which appeared to compete for substrate area. Another factor inducing station variability was the occurrence of species more typically found in the plankton. Species such as *F. virescens* and *A. formosa* appeared to settle out into the Elk Lake periphyton following the November breakdown of thermal stratification.

While periphyton reflect the sum total of environmental conditions within a lake, these communities are so sensitive to change in ecological

variables that more refined experimental and statistical designs, including more frequent and accurate measurements of physico-chemical variates are necessary to elucidate causal relationships. Similarly, the close relationship between net plankton and periphyton requires that both habitats be studied simultaneously in periphyton studies. Analysis of H" indicates that it is less appropriate and useful than commonly believed.

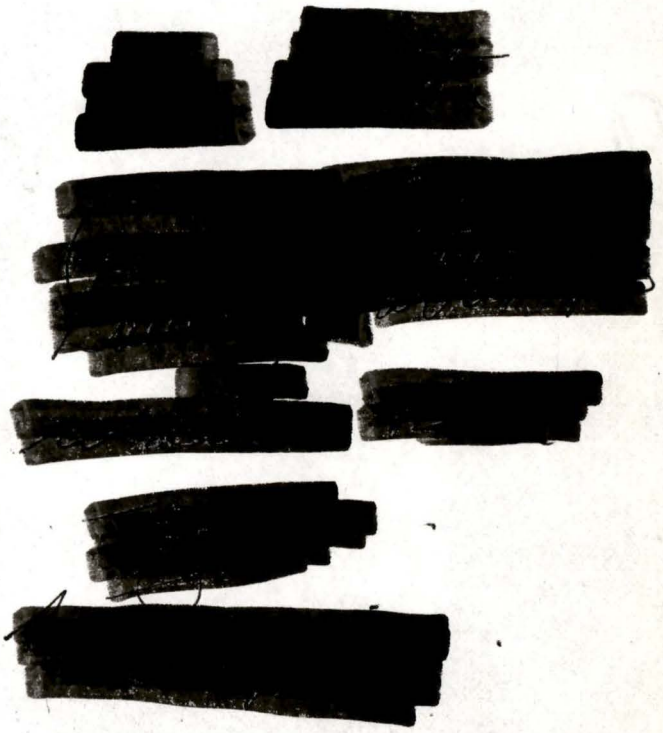

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Until recently the study of freshwater periphyton communities in North America lagged behind that of the plankton and even the benthos. The variability in structure of these attached populations and the wide variety of natural substrata hindered the development of sampling methods so that early investigations were often restricted to qualitative descriptions of species compositions. Consequently, these studies did not fully exploit the potential value of periphyton communities in the evaluation of water quality or the characterization of freshwater environments. However, difficulties incurred in the examination of periphyton microbiota on natural substrata were subsequently mitigated to some extent by the immersion of artificial substrata, a quantitative periphyton sampling method with wide application (*e.g.*, Cooke 1956; Sladeckova 1962; Kevern *et al.* 1966; Patrick 1968; Ball *et al.* 1969; Nelson *et al.* 1969).

The contribution of periphyton communities to total energy production in shallow lakes and ponds has since been established (Castenholz 1961; Maciolek & Kennedy 1964; Wetzel 1963, 1964, 1965; Dickman 1968b; Moss 1969a) and the use of these communities has also contributed to the development of methods for the biological assessment of water quality or pollution levels in both lotic (Patrick & Strawbridge 1963; Williams & Mount 1965; Weber & Raschke 1966; Arthur & Horning 1969) and lentic habitats (Sladeckova 1966; Neal *et al.* 1967; Dickman 1969b). Nevertheless, studies employing a rigorous quantitative approach to the biotic composition of the periphyton and its diversity, within a natural water body, correlated with environmental variables are lacking or

confined to lotic periphyton communities (Patrick *et al.* 1954; Hohn & Hellerman 1963; Cushing 1967; Flemer 1970). Sophisticated quantitative and statistical methods have been generally restricted in application to laboratory periphyton populations maintained under controlled or simulated natural conditions (McIntire 1966, 1968a, 1968b 1969; McIntire *et al.* 1969).

Since it was my belief that such a study of lentic periphyton within a natural water body might aid in assessing slight changes in water quality (as a result for instance of low levels of pollutants or early stages of eutrophication), a program of investigation was undertaken to examine quantitatively and statistically, algal periphyton on artificial substrata in a number of lower Vancouver Island lakes (British Columbia, Canada). The purpose of this program was to establish the degree of variability in structure of the periphyton communities by species numbers, species composition, and numbers of individuals, and the significance of this variability (if present) in relation to physico-chemical conditions concurrently monitored.

Because the present work constitutes the basis of a larger program of investigation on Vancouver Island, the emphasis has been placed here on the discussion and interpretation of preliminary results from Elk Lake, only one of the study lakes. The methods employed in the study, including periphyton sampling methods and possible objective methods for analyzing and processing the large quantities of data obtained, have been published (Brown 1969; Brown & Austin 1971) and are included here in slightly modified form as Appendices I and II.

In this study, the term periphyton or "*Aufwuchs*" refers primarily to the microflora found on glass slides, but is used in the general sense to include all organisms (except the rooted macrophytes) which occur attached to (but not penetrating into), or associated with a submerged surface or object (Young 1945; Wetzel 1964). No attempt has been made to separate the associated true benthic or planktonic organisms from the more typically attached or "true periphyton" (Sladeckova 1962; Round 1964) species, except where specifically indicated.

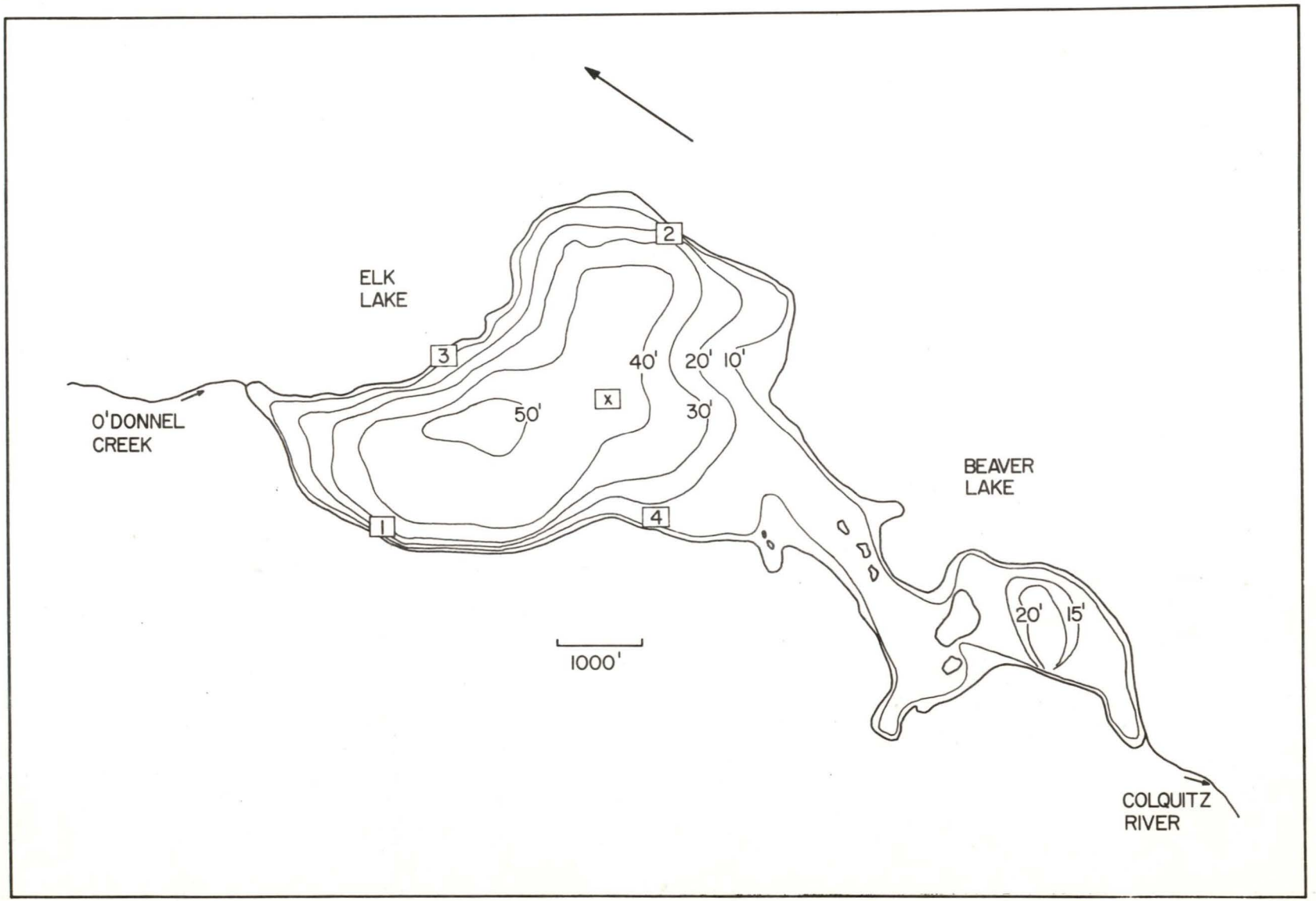
STUDY AREA AND METHODS

DESCRIPTION OF THE STUDY SITE: ELK LAKE

Elk Lake, situated in the Insular Lowland limnological region of British Columbia (Northcote & Larkin 1956, 1963; Larkin & Northcote 1958), is an open drainage lake formed after the most recent period of glaciation in an eroded hollow of drift mantle (Clapp 1912, 1913; Armstrong *et al.* 1965). The largest lake on the Saanich Peninsula (southern Vancouver Island), it forms part of the main drainage system, Colquitz River, and drains 1,146.56 ha of residential, light agricultural, and recreational park lands. Elk Lake is largely spring-fed with one inflow, O'Donnel Creek (Fig. 1), and drains southerly, being connected by a natural channel to the south basin. This smaller basin, known as Beaver Lake, is significantly different from the north basin, or Elk Lake, and is treated here as a separate, distinct water body. The entire Elk-Beaver Lake system is drained by Colquitz River, emptying via an estuarine inlet (see Waldichuk 1968, 1969) into Juan De Fuca Strait and the Pacific Ocean. A flow rate of 0.8 cu m/min is typical for the Colquitz River outlet in May, however this flow is considerably reduced in summer months and may dry up completely.

Elk Lake, described in some detail elsewhere (Brown 1969; Appendix I, Table 20), is a relatively small, eutrophic lake with a surface area of 207.8 ha. Morphometric features, including a low mean depth of 7.63 m, a shoreline development ratio of 1.9, and a percent littoral development of 58%, indicate the importance of the littoral region to the overall productivity of this lake. The eutrophic status of Elk Lake is further enhanced by edaphic factors. These are characterized

Figure 1. A contour map of Elk Lake, British Columbia showing the location of the four periphyton sampling stations, and the midlake sampling station [X] (Scale: 1000 ft = 305 m; contour intervals of 10 ft or 3.05 m).


ELK
LAKE
O'DONNEL
CREEK
BEAVER
LAKE
COLQUITZ
RIVER
1
2
3
4
X
50'
40'
30'
20'
10'
20'
15'
1000'

geologically by sedimentary deposits, eroded from insular mountains and overlain by glacial till or interglacial sediments, and expressed in T.D.S. readings of 105-120 p.p.m. and relatively high measured nutrient concentrations. Furthermore, the climate acts as a modifying influence, causing summer thermal stratification and hypolimnion oxygen deficits, fall mixing, and a typical absence of winter ice formation. Increased concentrations of dissolved substances may be expected during warm, dry summer months when evaporation may exceed inflow, while increasing input in winter months during periods of moderate precipitation (mean annual precipitation, 76 cm) may be expected from drainage run-off and inflows.

The interaction of these morphometric, edaphic and climatic factors is reflected in the eutrophy of Elk Lake and a predominantly cyanophyte-diatom plankton population indicative of nutrient enriched waters.

LOCATION OF SAMPLING STATIONS

Four periphyton sampling stations (Fig. 1), located between 3.05 and 6.10 m of the shoreline at a depth of 1.83-2.75 m, were established in Elk Lake on level, sandy to sandy-mud bottoms. The aquatic macrophytes, *Elodea canadensis* Michx., *Potamogeton amplifolius* Tukerm., *P. foliosus* Raf., *Ceratophyllum demersum* L. (syn. *S. validus* Vahl.) and *Lemna minor* L. are found in varying densities in the littoral zone around the lake perimeter. These plants support dense epiphytic diatom populations and thick entangled growths of filamentous green algae such as *Spirogyra crassa* Kütz.

An additional mid-lake or open-water sampling station was established at a depth of 14.64 m for the purpose of recording limnological data such as temperature profiles and water transparencies.

PHYSICO-CHEMICAL METHODS

The following collections and physico-chemical determinations were made at each periphyton sampling station and, where applicable, these were made both at the water surface and at the depth of the sampled periphyton communities. Air and water temperatures were recorded in °C using a calibrated thermistor unit (Simpson Thermometer, Model 389-3L, Simpson Electric Co., Chicago, Illinois) and pH was measured with a Rigosha® pH comparator (Rigosha & Co., Ltd., Tokyo, Japan). Water for dissolved oxygen determinations was collected in a Kitahara water bottle and allowed to flow into 300 ml glass stoppered bottles. The azide modification of the iodometric method for dissolved oxygen (American Public Health Association 1965) was used, titrations were completed in the laboratory, and results expressed as ml D.O./l and percent saturation (after Mortimer 1956). The water bottle was also used to collect one liter water samples for chemical analyses. These samples, taken in polyethylene containers, were kept under refrigeration until analyzed within a maximum 24-hr period.

In the laboratory the following chemical analyses of the water samples were performed according to the methods described for use with a Hach laboratory kit (Hach Chemical Co., Ames, Iowa, U.S.A.): copper, total hardness, calcium hardness, magnesium hardness, nitrate-nitrogen, nitrite-nitrogen, orthophosphate, total phosphate, and sulfate. Concentrations were recorded in mg/l. Only single determinations were made for each water sample, but regular duplication of tests were run as a procedure and reagent check.

Comprehensive field observations were recorded on each sampling date. Temperature profiles and transparency readings, using a standard 20 cm

diameter Secchi disk, were made at the mid-lake station. Meteorological data were supplied by the Canadian Department of Transport, Meteorological Branch weather station at Victoria, B.C.

PERIPHYTON SAMPLING METHODS

The experimental periphyton collection method, sample preparation and counting procedures employed in this study are fully described and statistically assessed in Appendix II. Only essential outline details of the sampling program are given here.

A special periphyton collection device was constructed to support 23 vertical and 36 horizontally exposed 50 x 75 mm glass slides. These frames were designed to sit completely submerged on the lake bottom in the littoral zone with the slides raised 30.5 cm from the lake bottom. A single exposure-frame was placed at each of the four Elk Lake sampling stations and, at regular intervals, samples of 4 replicate vertical and 4 replicate horizontal slides were collected from all stations. Each slide was preserved in 230 ml of a 4% formalin solution and permanent Millipore® filter preparations (0.45±0.02μ pore size; Millipore Filter Corp., Bedford, Mass., U.S.A.) were made of subsample aliquots.

Counts, at a magnification of x313 in 30 random Whipple micrometer fields for each filter, were made of the number of periphyton taxa and the numbers of individuals per species. For colonial or filamentous diatoms such as *Asterionella* sp., counts were made of the number of distinct cell aggregates, colonies, or structural units as well as the number of individual cells. However, cell counts for some larger multicellular green and filamentous blue-green algae were not recorded and these species were tabulated as present or absent. Results

for the vertical slides only are presented here and given as the numbers of species or individuals per sq. mm of original slide surface area.

After preliminary field experimentation, periphyton samples were collected in Elk Lake at approximately one month intervals as well as overlapping time intervals. The field collection dates and eleven coded time series (TS) of slide samples for the four periphyton sampling stations are given in Table 1.

As shown in Appendix II, analysis of the count data indicated that (1) most organisms were randomly distributed on the filters, (2) count estimates of total cells were more reliable than those of individual taxa, although all count estimates were generally within recommended error limits, and (3) differences in counts between slide sample replicates were not significant. Further analysis illustrated that of the three stages of the whole quantitative sampling method, counting, filtering, and sampling, the greatest variance component was introduced into the experimental design during counting. The magnitude of the variance however, was comparable to that reported for other aquatic studies and related experimental designs.

Additional biological samples, including plankton (Brown 1969) and natural substrata samples of *Aufwuchs* communities, were taken at each station and sampling date but will not be discussed here unless in support or clarification of the vertical slide periphyton data.

ANALYSIS OF THE DATA

Two blocks of data were simultaneously collected from the field and laboratory work. One block of data consisted of physico-chemical variables measured at two depths at four different periphyton sampling

Table 1. Eleven coded time series of slide exposure for Elk Lake.

Time Series* (TS)	Starting Date	Collection Date	Days Exposed
1	3 Aug 1967	5 Sept 1967	33
2	3 Aug 1967	9 Oct 1967	67
3	3 Aug 1967	19 Nov 1967	108
4	3 Aug 1967	16 Dec 1967	135
5	5 Sept 1967	9 Oct 1967	34
6	5 Sept 1967	19 Nov 1967	75
7	9 Oct 1967	19 Nov 1967	41
8	9 Oct 1967	16 Dec 1967	68
9	19 Nov 1967	16 Dec 1967	27
10	19 Nov 1967	19 Jan 1968	61
11	16 Dec 1967	19 Jan 1968	34

* A time series is defined as a specific group of slide samples from all four sampling stations, immersed for a distinct period of time and are subsequently referred to in the text as TS01, TS02, ... , TS11.

stations on six different sampling dates at approximately one month intervals. The second block of data represented the vertical slide periphyton count data from the same stations and sampling dates (11 TS x 4 stations x 4 vertical slide replicates x 1-6 filters x 30 Whipple fields; for all periphyton taxa). Initial qualitative assessment of the complete data set illustrated the magnitude and complexity of the data and suggested that standard statistical methods might be useful as a means for consolidating the information and detecting general relationships or underlying trends obscured by the data in their original form.

Subsequently both the physico-chemical and the periphyton data were treated statistically. While the environmental variables were expressed in raw data form or simple averages, the periphyton count data were expressed in essentially three different forms: (1) individual species counts, (2) total cell counts, and (3) species diversity indices. Since graphical analysis illustrated mean count values were generally proportional to their respective standard deviations, and counts of less than one or zero also occurred, a logarithmic transformation, $\log_e (x+1.0)$, was performed on all count data (after Barnes 1952; Pearce 1965; Angel 1969). Apart from the calculation of species diversity, transformed count data were used for all simple statistical computations.

Using both the environmental and the periphyton count data, appropriate *t*-tests and analyses of variance (anova) were performed in order to test the significance of differences in measured variates between depths, stations, and sampling dates. Lastly, multiple regression analysis was used to interrelate the two data sets. While the

sequence of procedures used was essentially straight forward and is explained in the text, it is necessary to enlarge here on procedures used in computation of (1) species diversity indices and (2) multiple regression analysis.

(A) Species diversity

Structure may be defined as the partitioning of individuals amongst the various species components of a collection, a community, or any multispecific assemblage of organisms. The variation in community structure (or organization), as a function of time or space, may be quantitatively assessed by diversity indices, mathematical expressions which assume a maximum when all individuals belong to different species and a minimum value (no diversity) when all individuals belong to a single species.

Hence, in order to describe periphyton community structure and summarize the vertical slide data, species diversity was calculated after the following indices, H (Brillouin 1962), H' (Shannon-Weaver 1964), and H" (Pielou 1966a, 1966b, 1966c) derived from information theory and defined by

$$H = (1/N) \left(\log_2 N! - \sum_{i=1}^{s} \log_2 N_i! \right) \qquad (1)$$

$$H' = - \sum_{i=1}^{s} P_i \log_2 P_i \qquad (2)$$

$$H'' = - \sum_{i=1}^{s} (N_i/N) \log_2 (N_i/N) \qquad (3)$$

The notation used is that of Pielou (1966a, 1966b, 1966c 1967) where N is the total number of individuals in s species, N_i is the number

of individuals in the ith species with $\sum_{i=1}^{s} N_i = N$; P_i or $P_i \tilde{\sim} N_i/N$ is the proportion (probability of occurrence) of the total numbers of individuals that belong to the ith species with $\sum_{i=1}^{s} P_i = 1$, and the information content or species diversity is expressed in units of bits/individual cell/mm^2. While logarithms to any base may be used, species diversity in the present study was computed using logarithms to the base 2 to conform to the practice in information theory where information is expressed in binary digits or bits.

The greatest possible value of H", H''_{MAX}, which would occur if the individuals were evenly distributed among the species, was calculated as follows (after Pielou 1966a, 1969)

$$H''_{MAX} = -\sum_{i=1}^{s} \frac{1}{s} \log_2 \frac{1}{s} = \log_2 s \qquad (4)$$

where s species are present in the same proportion, 1/s. The ratio,

$$J'' = H''/H''_{MAX} \qquad (5)$$

where H" is the observed diversity and H''_{MAX} the maximum attainable, was calculated as a measure of evenness, or the degree to which the proportions of the species approach equality. Similarly, an alternative measure of diversity (MacArthur 1965)

$$E = 2^{H''} \qquad (6)$$

was calculated where $2^{H''}$ may be viewed as the minimum number of E, equally abundant species which would yield the observed diversity calculated, H".

Despite the wide application of diversity indices, some confusion exists in the literature regarding the choice of the most appropriate

formulae and their limiting assumptions. Few persons, other than Pielou (1966a, 1966b, 1966c, 1967, 1969) have considered the distinctions between the indices, their proper use and implications (Hairston *et al.* 1969; Wilhm 1968, 1970; Dickman 1968a, 1968c) and it remains to be proven whether these differences are important in the context of most study applications.

In addition to those indices of equations (1) to (6), other indices of species diversity were also computed and compared for the entire periphyton data set. However, the interpretation of these data and the full discussion of the relative merits of different indices constitutes a study in itself; for the purposes of this report, results using H" only were chosen for presentation here because the use of Shannon-Weaver's formula is more widespread in the literature and the count data were in a form amenable to direct computation. Hence, substituting sample ratios N_i/N for P_i of Shannon-Weaver's formula, H", $H"_{MAX}$, J", and E were calculated [1,2] for all slide data where sample values of s were used. These formulae were computed in the same manner and applied in apparently the same context as in other related studies (Dickman 1968a, 1968c, 1969b; Goldman *et al.* 1968; McIntire & Wulff 1969; Platt & Subba Rao 1970).

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[1] Diversity was calculated using cell counts only, and not numbers of unit structures such as filaments or colonies (after Margalef 1967).

[2] All diversity indices were calculated using computer programs written in FORTRAN by E.M. Hagmeier and D.J. Thomson.

(B) Multiple regression analysis

In order to investigate the relative importance of measured physico-chemical variables to the variation in structure of periphyton communities over the entire sampling program, multiple regression analyses were run using both total cell counts and species diversity, H", as the dependent variables. The following independent environmental variables were used: copper (mg/l), pH, water temperature ($^{\circ}$C), dissolved oxygen (ml/l), sulfate (mg/l), total hardness (mg/l $CaCO_3$), magnesium hardness (mg/l $CaCO_3$), calcium hardness (mg/l $CaCO_3$), total phosphate (mg/l), orthophosphate (mg/l), nitrate-nitrogen (mg/l), nitrite-nitrogen (mg/l), percent oxygen saturation (%), air temperature recorded on station ($^{\circ}$C), and slide exposure duration in number of days.

In all cases, the dependent variable, total cells/mm^2, was logarithmically transformed, while environmental data and species diversity values were untransformed (after Barnes 1952; Cassie 1963). A square root transformation of total cell counts was discarded after analysis showed the logarithmic transformation to be more consistent with the assumptions of regression that variates are normally distributed and linearly related to each other.

Based on preliminary statistical analysis of the data (to be discussed), separate multiple regression analyses were run for each station and averages were chosen as the most appropriate data points to represent all independent variables except length of slide immersion. These mean values were computed as follows: (1) for all one-month series the average was of four values, surface and frame depth determinations on the collection dates representing day "one" of the immersion duration and the day on which the slides were removed from the frames;

(2) for two-month series, the means are of six comparable values, representing the first and last days and the intermediate collection date on which monthly slides were sampled; and (3) lastly, for the four-month increasing interval immersions, mean values were of 4, 6, 8 and 10 values, respectively.

Similarly, the dependent variable in these analyses, represents in all cases, the mean value of four slides. These eleven means (of TS01, 02, 03, ... , 11; see Table 1) were used instead of the four slides per sample or 44 data points per station because the four slide replicates do not represent true repeated runs in the context of multiple regression but rather repetition of the same reading (P. Konkin, personal communication; see Draper & Smith 1966, p. 28). A true replication in the sense of multiple regression and the predictive nature of the regression equation would entail a complete duplication of the entire experiment at some subsequent point in time, such as a day, week, month, or year later. However, use of the eleven mean values per station was not inconsistent with the aims of this study since the variation between the replicates in any slide sample was found to be negligible (Appendix II). In a complete replication of the experiment, the means might be expected to be different but variation within any sample could be expected to be comparably small, *i.e.*, the slide values will change from year to year (or any other realistic unit change in time), but variation about the mean within each sample of four slides would be consistently small. Hence mean values are truly representative of the four slide values within any one sample.

The regression analysis was performed in a stepwise fashion, in that independent variables (X_k, k = 1,2, ... 15) were added to the

regression equation in decreasing order of their importance, or in decreasing order of the percentage change in the dependent variable (Y_i, i = 1,2,...n; where n = 11) accounted for by the inclusion of each successive predictor variable entered in the equation. The computer program used was an update version (June 26, 1969) of the BMD02R (Dixon 1967) stepwise multiple linear regression program of the Health Sciences Computing Facility, U.C.L.A. Full descriptions of the stepwise multiple regression procedures are given in Efroymson (1960), Draper and Smith (1966), and Spurr and Bonini (1967).

The criteria used for acceptance or rejection of those variables incorporated into the final predictive equation, as calculated by the stepwise procedure, is largely dependent on the study aims. Statistical tests may be used, or interpreted, so as to retain as many or as few variables as possible after the final predictive equation has been estimated by the stepwise regression program. In the present study, it was felt best that only a minimum number of independent variables be retained in the final equations. Hence, five main criteria, based on the following statistics were applied at each step in all regressions and used as a basis for selecting the "best" regression equations:

(1) to determine whether the amount of variation in Y accounted for by linear regression was significantly greater than that not accounted for, the F-test for significance of regression ($P \leq 0.05$) was used where the mean square (MS) due to linear regression is tested over the MS due to residual variation, used as an error MS (Draper & Smith 1966, p. 24; Sokal & Rohlf 1969, p. 421);

(2) the significance of regression coefficients, or the null hypothesis that sample regression coefficients, b, come from populations with

parametric values $\beta = 0$, was tested using a *t*-test with n-k degrees of freedom (df) ($P \leq 0.05$) where each regression coefficient is tested over its respective standard error and k is the number of independent variables included in the regression at that particular step (Spurr & Bonini 1967, p. 608);

(3) to test the significance ($P \leq 0.05$) of the difference in multiple correlation coefficients (R's) between successive steps in the regression program, an F-test was used as described by Guilford (1965, p. 403);

(4) residuals, the difference between what is actually observed, Y_i, and what is predicted, $\hat{Y}$, by the regression equation, or the amount of variation in Y_i not ascribed to linear regression (the observed errors if the linear model is correct), were tested graphically (see Draper & Smith 1966, Chapt. 3, p. 86-103) to determine whether any discernible pattern in their distributions was apparent in violation of the error assumptions (*i.e.*, errors are independent, with a mean of zero and a constant variance σ^2, and are normally distributed); and

(5) from step to step in the regression program, before and after each new independent variable was added to the model, the increase in the square of the multiple correlation coefficient [R^2 = (Regression sum of squares (SS))/ (total SS corrected for mean)] which measures the proportion of total variation about Y explained by regression, was simultaneously compared with the standard error of the estimate, s ($s = \sqrt{\text{residual MS}}$), which decreases as the predictive equation becomes more precise (Draper & Smith 1966, p. 117).

As will be shown, the above statistical criteria represent ideal conditions, modifications of which were considered where there was some doubt as to the ecological significance or usefulness of a

predictive equation. A realistic approach to the solution and understanding of problems of applied multiple regression analysis is presented by Draper and Smith (1966), who point out that many comparable decisions and assumptions regarding regression must, in the final analysis, be arbitrarily made on the basis of prior knowledge, study aims, and/or economic considerations.

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL AND METEOROLOGICAL CONDITIONS

(A) Mid-lake station

Environmental variables, measured during the study at the open-water station, are given in Figure 2 along with selected meteorological data.

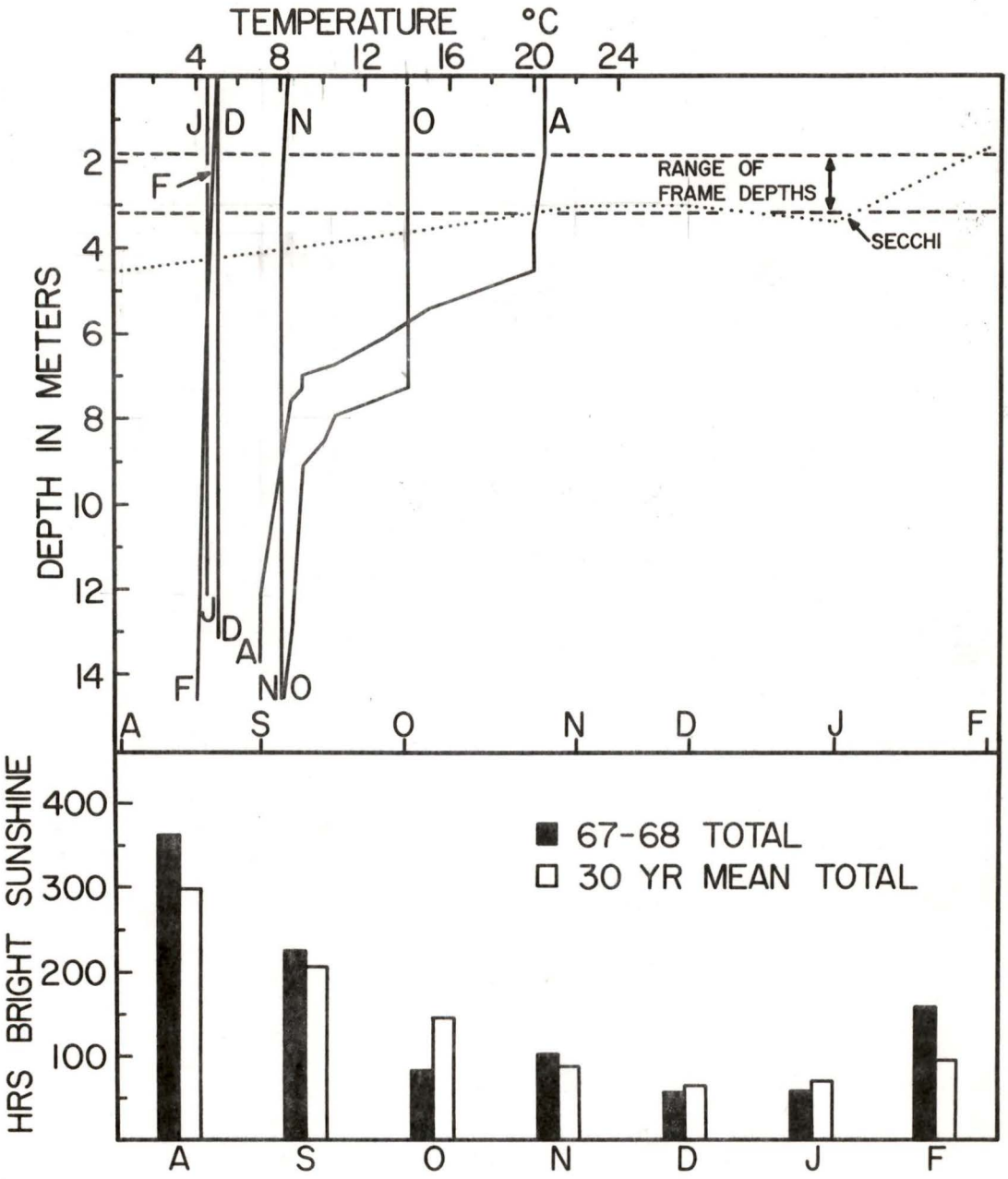
Temperature profiles illustrate the presence of a thermocline and a steady increase in thickness of the epilimnion from 4.6 m on August 3, to 7.4 m on October 9.

Summer stratification terminated between the October 9 and November sampling dates, with full autumnal circulation complete by November 19.

Elk Lake does not become thermally stratified under normal winter conditions. December and January are typically the windiest months of the year. From October to February, prevailing winds are from the North, with frequent gale force winds from the southeast or southwest, preceding and following Pacific storms. Little protection of the main water body is afforded by the watershed topography and surrounding mixed conifer and deciduous forests. Any temporary stratification is broken down by wind driven circulation in all but the deepest basin (see Fig. 1). Although winter air temperatures are mild, the season is typified by fog and cloud conditions, and little heating of the lake occurs by direct solar radiation throughout the winter. In particularly wet, rainy years, such as during this study, the heavy run-off also cools the surface waters of the lake.

The temperature of the surface water at the mid-lake station followed closely the seasonal trend in recorded hours of bright

Figure 2. Secchi disk transparency readings and temperature profiles recorded at the Elk Lake mid-lake sampling station from August, 1967 to February, 1968. The maximum range of frame depths at the four periphyton sampling stations over the entire sampling period is given by the two solid horizontal lines. Monthly total numbers of hours bright sunshine recorded at the main Victoria weather office over the same time period, as compared with the 30 year mean total values, are represented in the histogram.

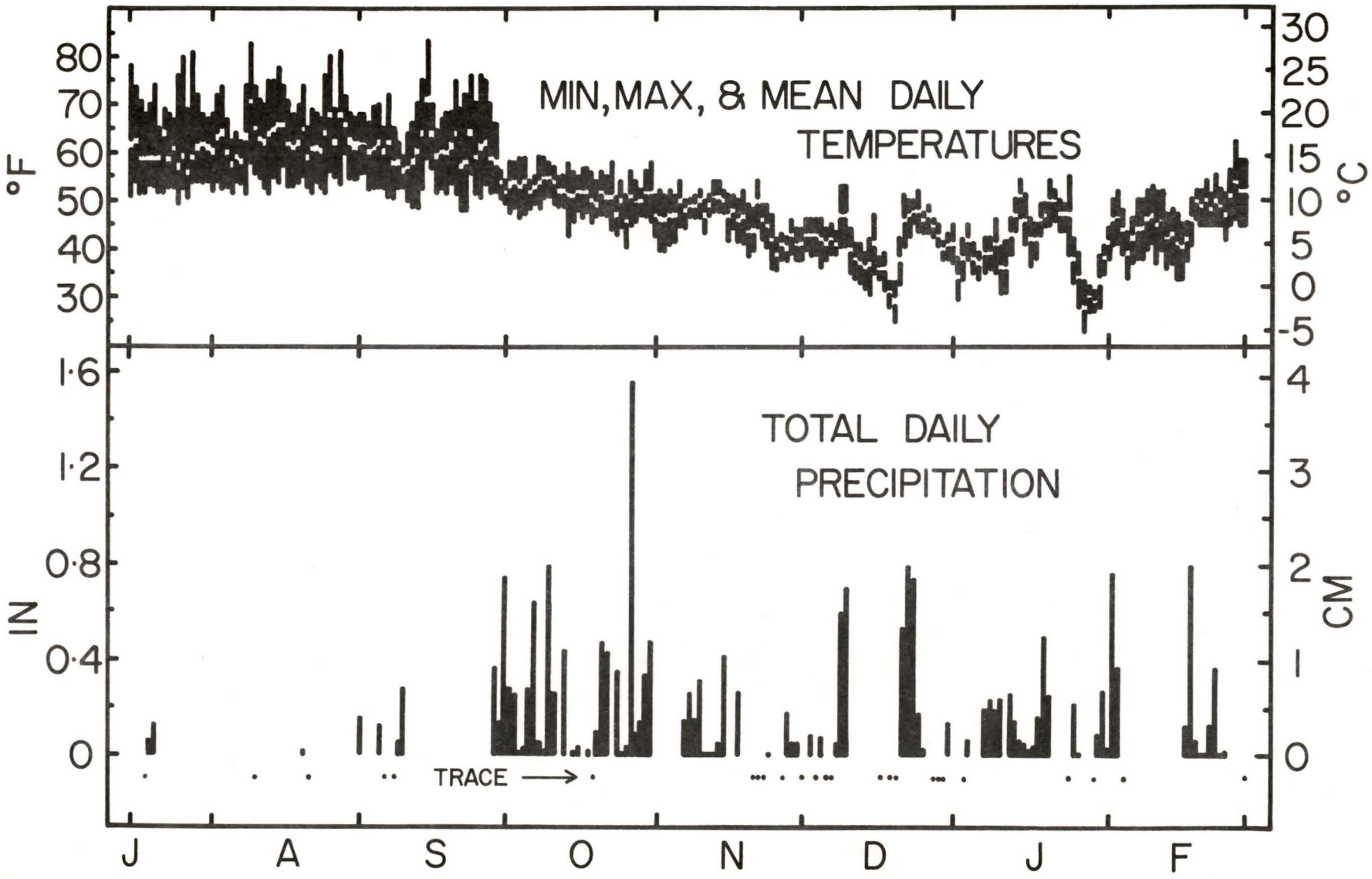

TEMPERATURE °C
4
8
12
16
20
24
J
D
N
O
A
F
J
D
A
N
O
F
RANGE OF
FRAME DEPTHS
SECCHI
DEPTH IN METERS
2
4
6
8
10
12
14
A
S
O
N
D
J
F
HRS BRIGHT SUNSHINE
400
300
200
100
67-68 TOTAL
30 YR MEAN TOTAL
A
S
O
N
D
J
F

sunshine; maximum and minimum temperatures of 21.5°C and 4.5°C were recorded on the September 5 and January 19 sampling dates, respectively. As of February 24 (1968) no ice had formed on the lake and it remained free until the following winter when atypical weather conditions resulted in an ice and snow cover of up to 20 cm from mid December, 1968 until late January of 1969. The effect of these unusual conditions was still evident in early May of the following spring (1969) when Elk Lake would normally be thermally stratified. At this time the temperature profile at the mid-lake station was quite erratic with signs of a weak stratification commencing and a thermocline forming at a depth of about 3 m.

Meteorological data revealed a number of unusual features in that August was abnormally dry and warm with a near record number of 364.7 hours bright sunshine, a higher value than that of July which typically has the maximum total per month in any 12-month period. October, on the other hand, was the wettest on record with 24.79 cm total precipitation (Fig. 3) and a record low number of 82.9 hours sunshine (Fig. 2). As a result, the lake level increased and remained in flood from late November until mid March when it began to return to normal level.

Secchi disk readings gradually decreased from a maximum depth of 4.6 m on August 3, to 3.1 m on November 19, coincident with full circulation and rainfall (Fig. 2 and 3). Increased turbidity due to the flood condition of the lake, the sediment load of the heavy run-off and a plankton bloom decreased transparency to a minimum depth of 1.7 m on February 24. Such an inverse relationship between Secchi disk transparency and rainfall is not uncommon (Wetzel 1964) and has been found, along with decreased temperature and light, to correlate

Figure 3. Histograms representing the minimum, maximum and mean daily air temperatures and the total daily precipitation recorded at the Victoria weather office from July 15, 1967 to February 29, 1968. A total of 63.3 cm total precipitation was recorded at Victoria from August 1, 1967 to February 29, 1968, compared with a total of 85.7 cm recorded at the Elk Lake sub-station, over the same period.


MIN, MAX, & MEAN DAILY TEMPERATURES
°F
80
70
60
50
40
30
30
25
20
15
10
5
0
-5
°C
TOTAL DAILY PRECIPITATION
IN
1·6
1·2
0·8
0·4
0
4
3
2
1
0
CM
TRACE
J
A
S
O
N
D
J
F

well with a rapid winter decline in primary productivity (Clear Lake, California; Goldman & Wetzel 1963). Elk Lake transparency readings also reflected, in general, the trend in recorded hours of bright sunshine (Fig. 2) except for the low Secchi reading in February, a month which conversely had a record high total of 161.3 hours sunshine. In contrast, most European workers have reported higher transparencies during winter months than in summer months (Hutchinson 1957) when plankton populations pulse and the resultant accumulation of organisms in the epilimnion tends to decrease transparency (Willen 1966). Benson (1967) found similar seasonal fluctuations in transparency for Lake Washington, with the decrease due to seston commencing earlier in the spring months. However, throughout the current study, some degree of plankton "bloom" was maintained in Elk Lake (Brown 1969) and it is thus likely that the suspended material in run-off was more important in causing the reported differences in Secchi readings. On other occasions, the limit of Secchi disk transparency in early May, under comparable plankton conditions, has been recorded at a depth of 4.9 m which corresponds with the August reading in Figure 2.

The importance of run-off in decreasing Secchi readings was supported by a transparency reading of 5.2 m on October 23, 1969. This value, 1.5 m greater than the October, 1967 reading, was indicative of the effect of rainfall and run-off on the latter, as a "bloom" condition was also in effect on the 1969 sampling date but the run-off was unusually light and the lake was not in flood.

(B) Periphyton sampling stations

To test the null hypothesis that there were no statistically significant differences between those physico-chemical measurements made at the surface and those made at the station depths between stations or within the lake on any one particular periphyton field collection date, a paired-observation *t*-test (Li 1964, p. 108) was used. This was also used to test the difference at each station between the surface and bottom measurements determined over all field collection dates or throughout the sampling period. Results of these *t*-tests indicated that the differences between those physico-chemical measurements made at the surface and those, determined from water samples, taken at the frame depths were not statistically significant ($n = 4$ or $n = 6$ at $P \leq 0.01$) for all variables except one. The one significant difference ($P \leq 0.05$) occurred between surface and frame depth determinations of total phosphate at the four periphyton sampling stations in October and will be discussed later.

Eventhough the measured differences between surface and frame depth environmental variables were not statistically significant, it does not necessarily follow that these differences were not biologically significant. Heterogeneity in the vertical distribution of physico-chemical conditions in the water columns of lakes and ponds of various sizes and depths is a well documented occurrence (*e.g.*, Ahl 1966; Moss 1969b, 1969c; Happey 1970a, 1970b), although the biological significance is often not adequately understood. Few studies have included statistical analyses of such differences which are particularly important when examining periphyton microenvironments and the unresolved relationships between plankton and periphyton communities. However,

until Elk Lake plankton collections, sampled at surface and frame depths, are quantitatively analyzed, and/or evidence to the contrary is found, the statistical results of the *t*-tests will be accepted and only the frame depth physico-chemical measurements are presented here unless otherwise stated.

A three-way classification anova (without replication, Hartley 1962; Sokal & Rohlf 1969) was used as the basis of a computer program to determine whether there were any significant differences in the Elk Lake physico-chemical variables between the six sampling dates or the four station locations. Results of this analysis (Table 2) indicate that throughout the study period from August 3, 1967 to January 19, 1968 there were no significant differences in physico-chemical characteristics (12 factors combined, *i.e.*, copper, pH, water temperature, dissolved oxygen, sulfate, total hardness, calcium hardness, magnesium hardness, total phosphate, orthophosphate, nitrate-nitrogen, and nitrite-nitrogen) between the four Elk Lake periphyton sampling stations as pooled over the six sampling dates. That is, for the 12 measured variables in combination, all four stations were similar in their environmental characteristics. There was no significant interaction between stations and dates, or between stations and physico-chemical features which was interpreted to mean that measured environmental features were not dependent upon station location. To confirm these results a one-way classification analysis of variance, run on each separate variable, was used to test the null hypothesis that there was no significant difference between the four periphyton sampling stations for any one measured environmental variable over the entire sampling period. None of the individual

Table 2. Results of a three-way classification analysis of variance run on the Elk Lake physico-chemical data (see text).

SOURCE OF VARIATION	df	SS	MS	F
A stations	3	11.5385	3.8462	1.1054
B dates	5	91.2905	18.2581	5.2474**
C physico-chemical measurements[1]	11	79089.0625	7189.9140	2066.4116**
FIRST ORDER INTERACTION				
A x B	15	52.5683	3.5045	1.0072
A x C	33	75.0426	2.2740	0.6535
B x C	55	2404.1225	43.7113	12.5628**
SECOND ORDER INTERACTION				
A x B x C (error)	165	574.1037	3.4794	
TOTAL	287	82297.5625		

[1] Twelve measured environmental variables (combined) as given in Table 3.

** Significant at $P < 0.01$.

anova were statistically significant ($P \leq 0.05$ or $P \leq 0.01$), suggesting that statistically the data for the four stations could be pooled or averaged without loss of information.

The significant difference between physico-chemical factors as shown in Table 2, was largely inherent in the different units of measurement (*i.e.*, pH units, mg/l, $^{\circ}$C) and different numerical levels of concentration for the 12 individual variables.

Table 2 also shows that there was a significant difference in physico-chemical features of the lake between sampling dates and significant interaction between sampling dates and environmental characteristics. Results for individual variables in Table 3 show that there was a significant temporal difference for all variables measured except calcium hardness and orthophosphate; monthly concentrations of total inorganic phosphate were found significantly different only at the 5% level of probability.

The statistical analysis and interpretation of the physico-chemical data provide, for each individual environmental factor, information indicating the time of significant fluctuations when discontinuities may be reflected in the biota. As in all applications of statistics to living systems, it must be emphasized that statistical significance does not necessitate biological significance or a direct causal relationship and, in this study, does not preclude at least two basic reservations. Firstly, the periphyton may react gradually to gradual variations in any one, or any combination of environmental variables whether measured or not (*e.g.*, time-lag, accumulation, or synergistic effects). Secondly, the measurement of those physico-chemical

Table 3. Results of simple one-way classification analyses of variance used to test the null hypothesis that there was no significant difference between sampling dates for any one measured environmental variable over the four Elk Lake periphyton sampling stations. Differences between monthly means (A, S, O, N, D, J) were ranked and tested by Duncan's New Multiple Range Test (D.N.M.R.T.) (Li 1964; Edwards 1967). Letters underscored by the same unbroken solid line are not significantly different at $P \leq 0.01$.

SOURCE OF VARIATION	df	SS	MS	F	D. N. M. R.[1]
Copper					
Among Samples	5	.238020	.047604	27.0520**	N D J S O A
Within Samples	18	.031675	.001759		
Total	23	.269695			
pH					
Among Samples	5	3.583750	.716750	70.6931**	N J D O S A
Within Samples	18	.182500	.010139		
Total	23	3.766250			
Water Temperature					
Among Samples	5	1201.755	240.3500	1371.2519**	J D N O S A
Within Samples	18	3.155	.1728		
Total	23	1204.905			
Dissolved Oxygen					
Among Samples	5	51.041983	10.208397	16.9421**	S O D A N J
Within Samples	18	10.845800	.602544		
Total	23	61.887783			
Sulfate					
Among Samples	5	195.875	39.1750	11.4194**	O J N S D A
Within Samples	18	61.750	3.4306		
Total	23	257.625			
Total Hardness					
Among Samples	5	306.30208	61.260416	7.4036**	A S N O J D
Within Samples	18	148.93750	8.274306		
Total	23	455.23958			
Calcium Hardness					
Among Samples	5	42.70833	8.541666	.8482	J S O D A N
Within Samples	18	181.25000	10.069444		
Total	23	223.95833			

Table 3. Continued.

SOURCE OF VARIATION	df	SS	MS	F	D. N. M. R.[1]
Magnesium Hardness					
Among Samples	5	436.718750	87.34375	5.2592**	A S N O D J
Within Samples	18	298.937500	16.60763		
Total	23	735.656250			
Total Phosphate					
Among Samples	5	.474750	.094950	3.3518*	O A D J S N
Within Samples	18	.509900	.028328		
Total	23	.984650			
Orthophosphate					
Among Samples	5	.115633	.023127	2.5175	O S N J A D
Within Samples	18	.165350	.009186		
Total	23	.280983			
Nitrate-nitrogen					
Among Samples	5	245.169766	49.033953	61.0321**	N D J O A S
Within Samples	18	14.461403	.803411		
Total	23	259.631169			
Nitrite-nitrogen					
Among Samples	5	.000416	.000083	4.9168**	N J D S O A
Within Samples	18	.000304	.000017		
Total	23	.000720			

[1] Monthly mean values are arranged, from left to right, in order of increasing magnitude.

* Significant at $P \leq 0.05$ but not at $P \leq 0.01$.

** Significant at $P \leq 0.01$.

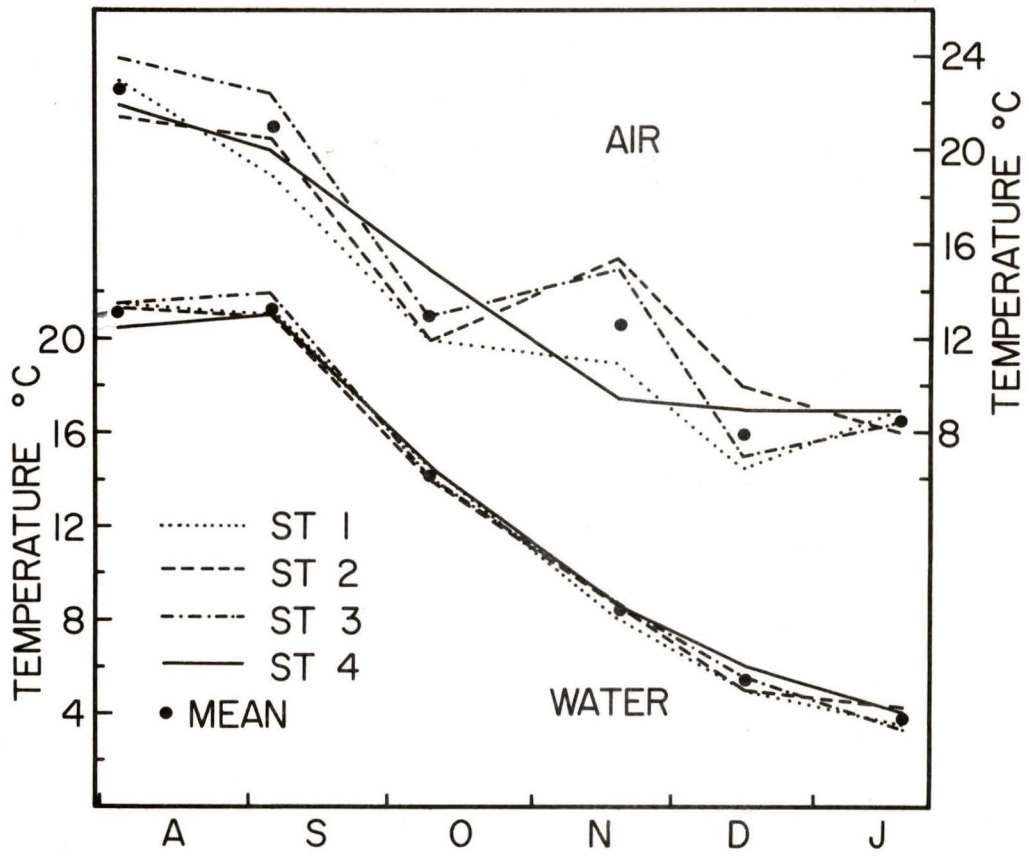
characteristics chosen for study may not have been as refined, or as frequent, as is necessary to elucidate a biologically relevant change. The physico-chemical measurements provide information on the nature of the environment at a particular point in time, while the periphyton populations---species composition and changes in numbers of individuals---represent an integrated, historical response to, or summation of, past and present environmental conditions and change (see Cairns *et al.* 1968). Nevertheless, the preliminary statistical analyses identify defined discontinuities at which a biological response may be anticipated, and are a first step in data interpretation and consolidation.

(a) Temperature and Secchi disk transparency

Both air and water temperatures (Fig. 4) decreased with the seasonal decrease in light intensity. In shallow lakes or ponds, epilimnion temperatures closely reflect ambient air temperatures (Efford 1967; Moss 1969b) and in some cases, water temperatures to a depth of 2 m may remain higher than mean monthly air temperatures except during icing conditions (Ahl 1966). This was found to be true for Elk Lake, where, throughout the study, frame depth water temperatures were higher than the mean monthly air temperatures recorded at both the Victoria (Fig. 3) and Elk Lake meteorological stations. However, from November 19 onward, the mean water temperatures were considerably less (2.6-5.4 ^{o}C) than the mean air temperatures recorded concurrently at the sampling stations (Fig. 4).

On any one of the six sampling dates the differences in depths of the four periphyton exposure-frames were never greater than 0.41 m, and throughout the study period all frames were situated within a

Figure 4. Air and frame depth water temperatures measured at the four periphyton sampling stations in Elk Lake from August 3, 1967 to January 19, 1968.


AIR
WATER
ST 1
ST 2
ST 3
ST 4
• MEAN
TEMPERATURE °C
TEMPERATURE °C
4
8
12
16
20
8
12
16
20
24
A
S
O
N
D
J

maximum depth range of 1.8-2.7 m and were therefore above the thermocline (Fig. 2). Hence, periphyton organisms were in competition with plankton and littoral benthos for nutrients and oxygen available in the epilimnion during stratification. With thermocline breakdown, the periphyton was still in competition with, but possibly at an advantage over, the plankton. The periphyton, fixed in position, was not subject to movement by wind or mixing currents which might carry the plankton out of the euphotic zone. In addition, the flushing action of run-off (Oglesby 1969; Dickman 1969a) would tend to remove certain components of the plankton population from the lake into the drainage outlet (Colquitz River) and, unlike catastrophic storm conditions (Hargraves & Wood 1967), would not be severe enough, in Elk Lake, to seriously abraid periphyton communities.

From August to January, the frames also remained at depths within the limit of Secchi disk visibility (Fig. 2). Secchi disk readings at the periphyton stations, where run-off, turbulence, and wind-driven plankton accumulation might decrease transparency, were never less than the depth of the arificial substrata. Without vertical profile photometric readings it was not possible to determine the actual amount of light reaching the frame depth periphyton communities in Elk Lake. However, it is possible, on the basis of Secchi readings (and other information about the lake) and the extrapolation of data from the literature, to make some statement concerning the relative amount of light reaching the frame depths and the probability of its becoming a limiting factor during this study.

Pyrroheliometer readings in the general northwest region (Benson 1967; Efford 1967; Dickman 1968a) compare well with calculated values

given by Hutchinson (1957) for sea-level stations at latitudes of 45-50° and show an annual pattern of light intensity with maximum values in June and minimum values in December. It is assumed that Elk Lake measurements of solar radiation would not be grossly different in magnitude, and that the annual pattern of intensity is identical.

The Elk Lake data implied that sufficient surface light was available throughout the sampling duration to sustain large standing crops of plankton (although photosynthetic rates may have fluctuated) as evidenced by the "bloom" conditions which persisted, even during decreased hours of sunlight in December and January, and low Secchi readings in February. It is probable that some phytoplankton species were shade tolerant and that the seasonal succession in species composition was partially due to natural succession of species adapted to different low light intensities and durations. Nevertheless, there was sufficient surface light to maintain the standing crop, and the seasonal decrease in light intensity and duration did not appear to be limiting to the Elk Lake plankton population as a whole.

Secchi disk transparency has been found to represent the depth to which 15% (Lake Huron; Beeton 1957), 12.5% (Lake Washington; Benson 1967), or more conservatively, 5% (Hutchinson 1957) of the surface light penetrates (see also Edmondson 1956; Verduin 1956, 1962; Anthony & Hayes 1964), while the compensation depth is generally given as the depth to which approximately 1% of the surface light penetrates (Talling 1962). Thus the compensation depth is usually greater than the limit of Secchi disk transparency. Furthermore, Ruttner (1963) states that the natural *Aufwuchs* community is apt to become more heterotrophic in function where light and temperature are limiting

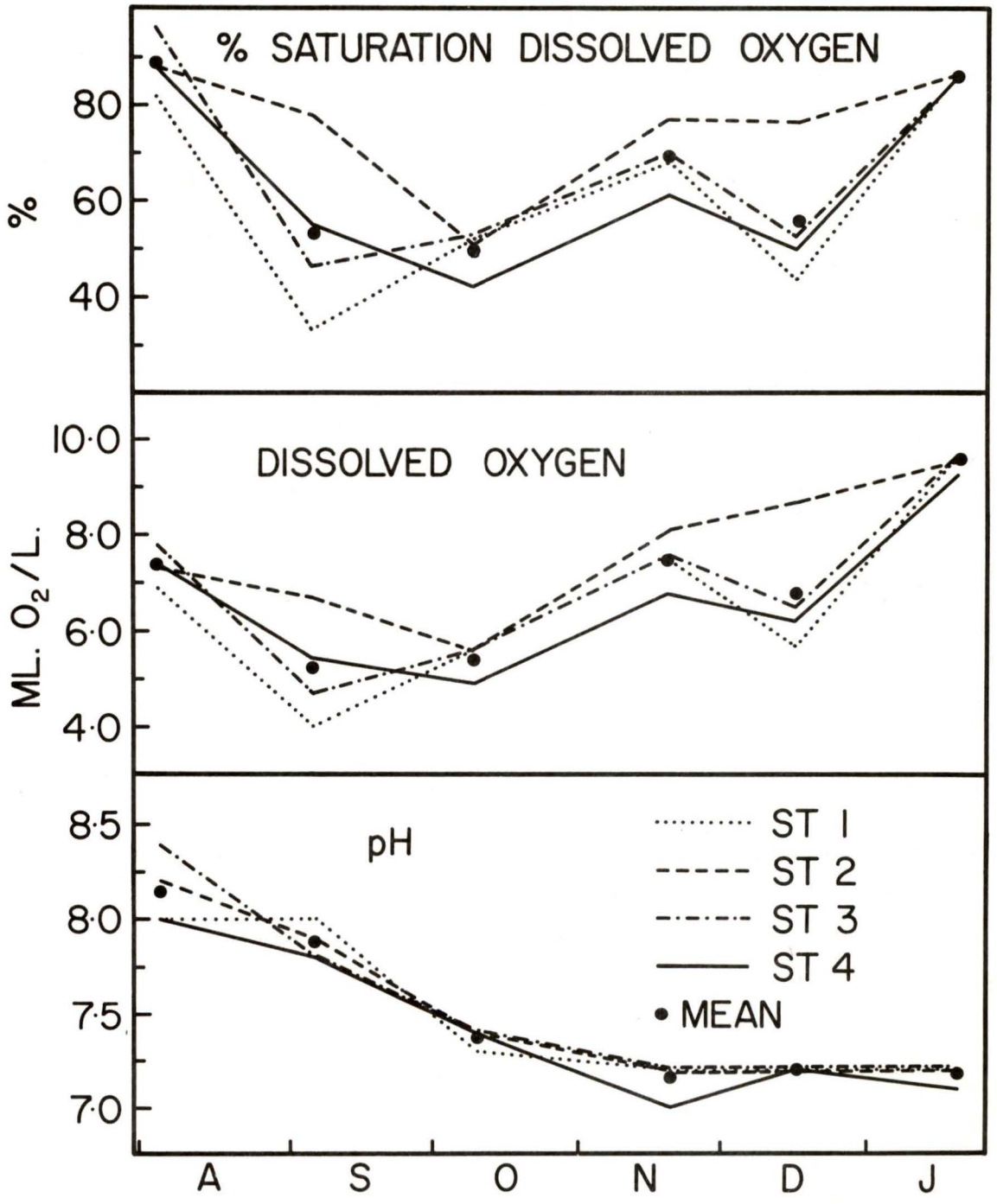
(see also Neal *et al.* 1967) especially at and below the thermocline depth. As will be shown, the majority of periphyton organisms found at the frame depths (above the thermocline) in this study, are autotrophic. Therefore, in view of the foregoing discussion, and assuming surface light was adequate, sufficient light must have penetrated to the frame depths to enable autotrophic maintenance of the periphyton standing crop communities. There is no reason to suspect that the compensation depth for these organisms was less than the Secchi transparency depths, and finally, no reason to suspect any gross differences in light penetration between stations on any one date.

(b) Dissolved oxygen and pH

Concentrations and percentage saturations of dissolved oxygen are given in Figure 5. Although the epilimnion was supersaturated to a depth of 6 m in May 1967, supersaturation never occurred at frame depths (1.8-2.7 m) during the study period. Similarly, there was also no evidence of serious oxygen depletion or anaerobic conditions at these depths.

While the concentration of oxygen was highest in January, the percent saturation was greatest in August when photosynthetic activity of the phytoplankton and macrophytic vegetation was probably maximal for the period of study. There was a slight decrease in recorded dissolved oxygen in September during stratification, due possibly to the oxidizable organic matter of plankton die-off of preceeding plankton population pulses. Dissolved oxygen then increased with overturn which was coincident with the first evidence of flooding. The greatest

Figure 5. Graphs showing the frame depth dissolved oxygen and pH fluctuations at the four periphyton sampling stations in Elk Lake from August 3, 1967 to January 19, 1968. Percent saturation values for dissolved oxygen were calculated (after Wetzel 1966) from an enlarged nomogram given in Mortimer (1956).


% SATURATION DISSOLVED OXYGEN
%
80
60
40
DISSOLVED OXYGEN
ML. O_2 / L.
10·0
8·0
6·0
4·0
pH
8·5
8·0
7·5
7·0
ST 1
ST 2
ST 3
ST 4
MEAN
A
S
O
N
D
J

variation in dissolved oxygen between stations occurred in December when concentrations reached 6.65 ml/l at station 2. Continued photosynthetic activity, cooler water temperatures, and wind-driven circulation and turbulence of waters effected an increase in oxygen concentration and percentage saturation at all four stations in January.

The annual fluctuation in dissolved oxygen concentrations is largely a physical phenomenon due to the temperature dependent solubility of the gas; any deviations in this pattern of inverse correlation with temperature are commonly attributable to biological activity. For example, in productive lakes such as Elk Lake, photosynthetic rates of large standing crops of phytoplankton and macrophytic vegetation during thermal stratification and the oxidation of decomposing organic matter, prior to and immediately after, fall circulation are important factors producing temporary variations in dissolved oxygen concentrations.

There was no recorded evidence of a hypolimnion oxygen deficit in Elk Lake during summer stratification in 1967. However, it is likely that this was an atypical condition, as other lakes in the same limnological region develop clinograde oxygen curves to the extent that summer mortalities of fish populations, associated with oxygen depletion in deeper hypolimnetic waters, commonly occur (Northcote & Larkin 1956, 1963). Although summer fish kills have not been reported for Elk Lake, it may be assumed that oxygen depletions do occur, as found on October 23 in 1969 when a clinograde oxygen curve occurred in the region of the deepest basin ($\sim$ 17 m, Fig. 1). At this time the epilimnion extended to a depth of 10.5 m, oxygen concentrations decreased sharply in the thermocline region with 0% oxygen saturation first evident at 13.7 m, and anaerobic conditions continued to the bottom. pH also

decreased from a maximum of 7.6 in the epilimnion to a minimum of 7.1 in the hypolimnion.

Recorded values of pH (Fig. 5) were not positively correlated with dissolved oxygen concentrations to the same extent as observed in other lakes. Values ranged from a pH of 8.4 to 7.0, decreasing with the seasonal decrease in temperature (Figs. 3 and 4), sunlight and transparency (Fig. 2), and inversely, to some extent with rainfall (Fig. 3). Similar correlations were reported for Clear Lake (California), where the decrease in pH also corresponded with a decrease in primary production rates of phytoplankton (Goldman & Wetzel 1963). High pH values frequently reflect increased photosynthetic activity (Goldman & Carter 1965; Duthie 1968). The highest pH values and greatest variation between Elk Lake stations occurred on the August sampling date. Consistent differences in pH between sampling stations were also reported by Duthie (1968) only during summer stratification. In addition to vertical differences, horizontal variation in pH in the epilimnion, particularly at the surface in summer months, is not uncommon; patchiness in the distribution and local accumulation of plankton and occurrence of littoral macrophytic vegetation can cause daily spatial fluctuations in pH due to different photosynthetic rates (Bartsch 1960; Verduin 1962; Wetzel 1966).

After fall overturn, from November to January, all Elk Lake stations were nearly identical in pH values. This absence of variation between sampling stations may be attributed to complete mixing throughout the water column. Furthermore, the general seasonal decrease in pH was also likely a result of photosynthetic activity slowed by decreases in light and temperature. Some of this seasonal drop in pH may also be attributed

to the mixing of breakdown products, mainly carbon dioxide, hitherto accumulating and trapped in the deeper hypolimnetic region by the thermocline barrier.

Recorded pH values for Elk Lake were neutral to slightly alkaline and the small fluctuation with season was indicative of the good buffering capacity characteristic of medium-hard water drainage lakes.

(c) Total hardness, calcium hardness, and magnesium hardness

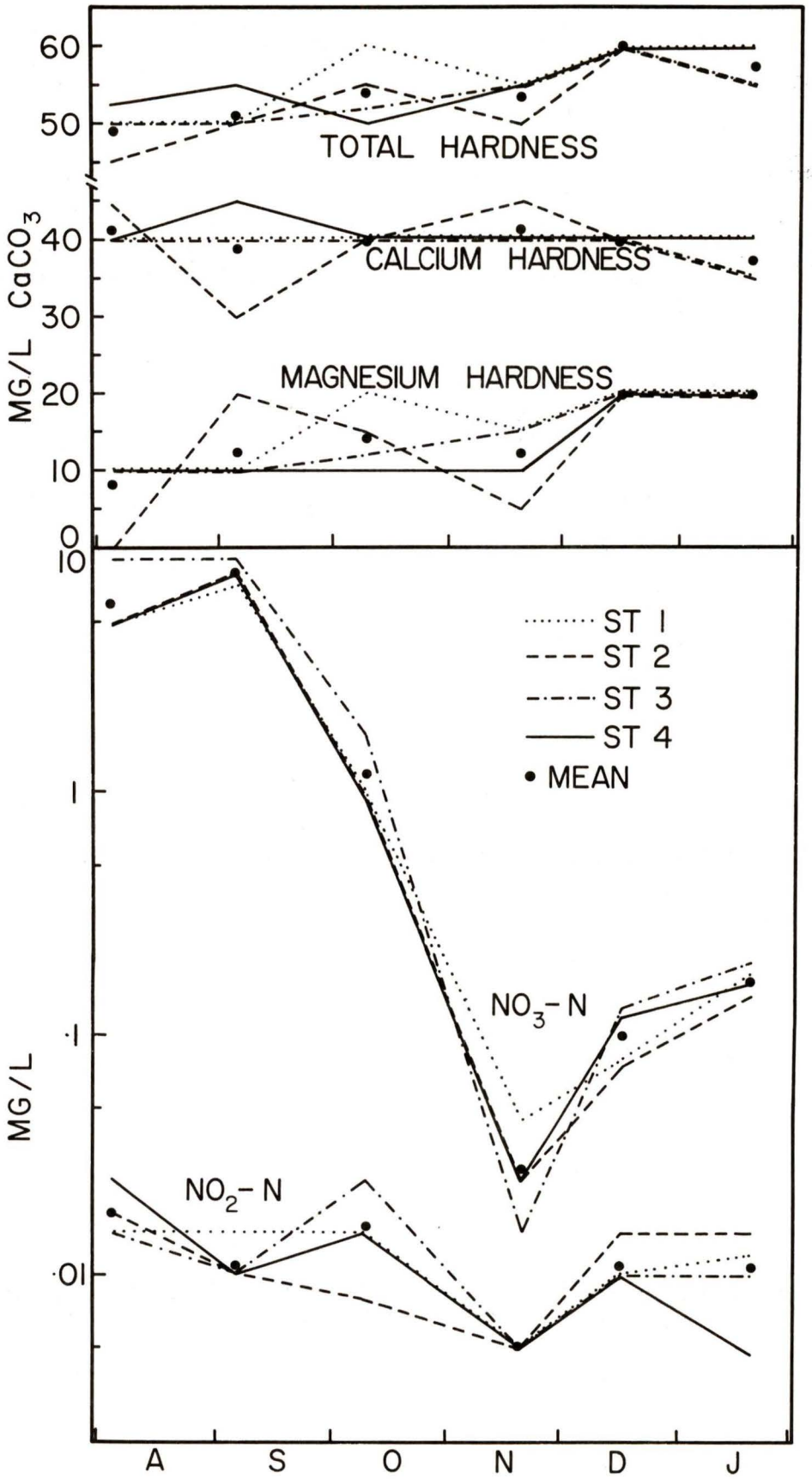
Values for Elk Lake total, calcium and magnesium hardness, expressed in mg/l calcium carbonate, are given in Figure 6.

Throughout the sampling duration total hardness ranged between 60.0 and 45.0 mg/l $CaCO_3$. Mean values for the four stations increased gradually from a low of 49.4 mg/l $CaCO_3$ on August 3, to a maximum concentration of 60.0 mg/l $CaCO_3$ on December 16, falling off slightly in January to 57.5 mg/l $CaCO_3$. Between August and November, there was a considerable amount of variation between stations with a maximum difference of 10.0 mg/l $CaCO_3$ on October 9. This variation between stations was largely a reflection of the concurrent fluctuations in magnesium hardness.

Mean concentrations of calcium hardness ranged from 37.5 to 41.3 mg/l $CaCO_3$ with little variation between sampling dates, whereas there was considerable (but not statistically significant) variation between stations primarily attributable to station 2 measurements.

Magnesium hardness ranged between 0 and 20.0 mg/l $CaCO_3$, fluctuating erratically between stations until December and January when concentrations became stabilized at all stations at 20.0 mg/l $CaCO_3$. Mean magnesium hardness values increased gradually from August to October,

Figure 6. Elk Lake frame depth fluctuations in total hardness, calcium hardness, magnesium hardness, nitrite-nitrogen and nitrate-nitrogen concentrations at the four periphyton sampling stations from August 3, 1967 to January 19, 1968. Hardness is expressed in mg/l calcium carbonate and nitrogen concentrations are plotted on a logarithmic scale to more clearly illustrate the small differences between the four stations from November to January. Exceptionally high nitrate-nitrogen concentrations are shown during warm, dry weather in August and September.


60
50
40
30
20
10
0
MG/L CaCO3
TOTAL HARDNESS
CALCIUM HARDNESS
MAGNESIUM HARDNESS
10
1
·1
·01
MG/L
ST 1
ST 2
ST 3
ST 4
MEAN
NO3-N
NO2-N
A
S
O
N
D
J

dropped slightly on November 19 and increased to a maximum on December 16, remaining constant at that value in January.

Elk Lake fluctuations in total, calcium, and magnesium hardness were most similar to those documented by Tucker (1958) who found that total hardness and calcium concentrations reached maxima during winter months, with minimum values in summer and early autumn; no definite seasonal pattern being evident in magnesium concentrations, which fluctuated erratically throughout the year. Conversely, he found that bicarbonate (methyl-orange-alkalinity) and pH reached high values in summer and early autumn, and minimum values in winter months (see also Mann 1958; Goldman & Wetzel 1963) with only slight pH fluctuations in hard waters. This reverse effect was particularly evident in ponds with high concentrations of dissolved organic matter. His data suggested the seemingly contradictory fluctuations in bicarbonate were inversely related to, and somewhat dependent on variations in sulfate concentrations.

The overall, gradual increase in total hardness in Elk Lake was concurrent with a decrease in pH (Fig. 5) as well as temperature (Fig. 4). The precipitation of calcium carbonate was likely a modifying influence on the Elk Lake fluctuations as suggested in other studies (Tucker 1958; Wetzel 1960). In addition, it is also possible that there was some dilution, causing a decrease in hardness concentrations due to the heavy rainfall, as this was found to be true in ponds (Tucker 1958) where pH, bicarbonate alkalinity, and calcium decreased after heavy rainfall (see also Goldman & Wetzel 1963; Prophet 1966). A similar dilution effect was also documented for Green Lake (Washington), where flushing decreased concentrations of total alkalinity and total hardness (Oglesby 1968, 1969).

Elk Lake hardness concentrations were commensurate with the T.D.S. readings, slightly alkaline pH, and plankton flora (primarily cyanophyte-diatom), all characteristic of medium-hard water drainage lakes. The range of Elk Lake pH values suggests the presence of bicarbonate (Moore 1939; Golterman 1969) and it is likely that there was a continuous supply of the anion through the reported spring inflow near station 1.

No sampling was undertaken to determine marl deposition, but macrophytic vegetation such as *Ceratophyllum demersum*, *Potamogeton amplifolius*, and *Elodea canadensis* had visible, thin surface-crust carbonate deposits. Other resident genera of the Elk Lake flora, including phytoplankton and periphyton organisms, and *Chara*, are regarded as potential lime formers (Young 1945; Wetzel 1960). However, precipitation of carbonate compounds in Elk Lake was not extensive, and the lake cannot be classified as a "marl" lake.

Calcium and magnesium are essential element requirements of most algae and a positive relationship between calcium hardness and productivity has been documented (Rawson 1960; Williams 1964). Bioassays have been conducted with lake inflows by adding Millipore-filtered stream water (to remove phytoplankton) to cultures of a lake's natural phytoplankton population (Goldman & Carter 1965). An induced significant or non significant stimulation in primary productivity can be assayed by relative photosynthetic uptake of ^{14}C. On March 9, 1968, J.S. Griffiths (unpublished data) performed a similar bioassay (after Goldman & Carter 1965) to assess the effects of the O'Donnel Creek inflow waters on the primary productivity of natural Elk Lake phytoplankton populations. It was found that the nutrient enriched inflow waters induced an increase in ^{14}C assimilation. These results suggested that the increased uptake by

the phytoplankton may have been due to, among other things, the higher carbonate alkalinity (phenothalein-alkalinity) reading of 54.0 mg/l $CaCO_3$ in the inflow waters as compared with a reading of 35.0 mg/l $CaCO_3$ in the lake water.

(d) Nitrite-nitrogen and nitrate-nitrogen

Nitrite-nitrogen was measured in Elk Lake because of suspected mild organic pollution from agricultural run-off, inflows and seepage from faulty septic tanks. However, concentrations were expected to be low because epilimnion waters were well-oxygenated (Fig. 5) and potable drinking waters rarely have concentrations in excess of 0.10 mg/l NO_2-N (A.P.H.A. 1965).

Irregularly distributed trace concentrations of nitrite-nitrogen (Fig. 6) were found in the lake, ranging between 0.005 and 0.025 mg/l, with the lowest mean value of 0.005 recorded on November 19 when variation between stations was nil. These concentrations were not sufficiently high to indicate organic pollution and were comparable to those of other productive lakes where nitrite is produced through nitrate reduction by phytoplankton. In Elk Lake, nitrite fluctuations were erratic and did not appear closely related to the nitrate-nitrogen values, however minima for both were reached in November. Nitrite-nitrogen also showed a slight increase during early winter under conditions of complete circulation.

Peak mean nitrate-nitrogen values for Elk Lake (Fig. 6) were reached during the present study in September at 7.99 mg/l. Concentrations fell sharply to 1.16 mg/l on October 9, and then decreased to a mean value of less than 0.50 mg/l in November, remaining at low levels through January, 1968. Relative to the magnitude of the extremely high nitrate-

nitrogen values recorded in August and September, the variation between mean values from November to January was negligible (see Table 3), although mean concentrations in December and January were twice that in November (0.005 mg/l NO_3-N). Greatest variation between stations occurred on the August sampling date during stable thermal stratification. Graphed nitrate-nitrogen concentrations do no indicate a clear relationship with other environmental data, except that the values follow the same general trend as temperature (Fig. 4) and pH (Fig. 5), decreasing with the advent of winter, heavy rainfall, and fall overturn.

The greater part of nitrogen compounds present in any lake are derived from the soil, entering by influents, whereas the major loss of these ions from open lakes is through natural drainage outflows (Hutchinson 1957; Mackenthun 1962; Frink 1969; McCarty 1970). Maximum amounts of inorganic nitrate tend to be present at the end of winter or at vernal circulation and this has been documented for many lakes where high winter nitrate concentrations are usually followed by low summer values concurrent with maximal photosynthetic activity (*e.g.* Ahl 1966; Benson 1967; Duthie 1968). In some lakes, extremely rich in nutrients, no seasonal trend is evident and considerable amounts of ammonia and nitrate may always be present in solution (Lund 1965).

Precipitation and run-off leach nitrogen from the soil, particularly agricultural lands rich in humus and other organic matter (Owens & Wood 1968). Hence, lakes receiving effluents from such typically poorly drained soils as those which occur in the northwest corner of the Elk Lake watershed, are often enriched in nitrate (Fogg & Horne 1966). Commonly, nutrient retention in lakes is lowest in winter during high

flow, whereas the greatest retention of inorganic nitrogen compounds occurs in summer during low flow rates and high biological activity (Mackenthun 1962; Goering & Neess 1964. However, this enrichment may not always occur. Inflow nitrate concentrations may remain higher than those of the receiving water body throughout the year (Moss 1969c) and in the same water body, nitrate-nitrogen may increase during the growing season (Happey & Moss 1967), the pattern in fluctuations varying from year to year. Increases in nitrate from enriched inflows may not always be reflected in higher measured nitrate values found in the receiving water body due to immediate assimilation by algal populations and macrophytic vegetation. For example, *Elodea canadensis*, a hydrophyte well established in Elk Lake, has been shown capable of removing nitrate as fast as it was formed in a eutrophic pond (Hutchinson 1957). Similarly, nitrate may remain in the water column for a longer period of time when attached algal development and bottom sediments are limited and the nitrate demand thus reduced (Dickman 1968a).

In contrast to the usual seasonal pattern in nitrate fluctuations, Douglas (1958) found nitrate concentrations of a small stream tended to be higher during dry weather and lower during wet. Similarly, the Elk Lake data were unusual in that during the period of investigation extremely high nitrate-nitrogen values were recorded in the dry, warm months of August and September. Minimum concentrations occurred concurrent with overturn, heavy run-off and winter months, while the standing crop of phytoplankton did not vary appreciably in quantity. Prior to artificial flushing and dilution, nitrate-nitrogen fluctuations in in another small, eutrophic lake also showed summer peaks (Oglesby 1968, 1969) similar to that found in Elk Lake.

The low nitrate, as measured in Elk Lake from November to January, inferred that there was no appreciable increase in the lake water concentrations due to potentially higher nitrate values in the O'Donnel Creek inflow or other drainage inlets swollen by heavy precipitation. However, a subsequent comparison of both creek inflow and lake waters in March, 1968 indicated that the inflow waters had a nitrate-nitrogen concentration four times greater than that of the lake waters which had then reached a level of 0.880 mg/l NO_3-N. In addition, it was found that the creek waters (also high in carbonate alkalinity as previously discussed) stimulated carbon assimilation of the Elk Lake phytoplankton which was then dominated by *Anabaena flos-aquae* (Lyngb.) Bréb. Thus, although the Elk Lake standing crop of phytoplankton was high, photosynthetic rates were not optimal for the predominantly cyanophyte-diatom assemblage present at that time. The difference between the high nitrate-nitrogen in O'Donnel Creek and the comparatively low lake concentration may be accounted for by a combination of factors, such as, biogenic uptake, reduced retention due to high flushing rates, and also, loss to the lake sediments as refractory material (see Frink 1969). It would appear that in addition to the large resident waterfowl population and leaves from deciduous trees such as *Alnus rubra* Bong., *Salix* spp., and *Pyrus fusca* Raf. that border the lake perimeter, the O'Donnel Creek inflow is an important source of nitrogenous compounds resulting in high nitrate-nitrogen values in Elk Lake.

It is difficult to interpret the seasonal pattern in Elk Lake nitrate-nitrogen concentrations except in terms of weather conditions, *i.e.*, an uncommonly heavy fall precipitation, preceded by an unusually warm and dry summer. The fluctuations in nitrate-nitrogen were probably a result

of firstly, a concentrating of nitrate in summer months due to evaporation and reduced flushing rates or high retention, coupled with a releasing and recycling of nutrients from decomposing organic matter (from large standing crops of algae and macrophytic vegetation) in marginal sediments. In August and September, this lead to very high nitrate-nitrogen values, probably in excess of that immediately required to meet biological demands so that nitrate remained in the water column. However, this in turn was followed by dilution or high flushing rates and low retention in late fall and early winter, simultaneously modified by biogenic utilization, recycling, overturn, and a net loss of nitrate to both the sediments and outflow, all of which lead to comparatively low nitrate-nitrogen concentrations in November, December and January despite the possible increase that could have occurred by leaching and run-off.

Concentrations of nitrate-nitrogen and nitrite in another study lake, Sooke Lake, (Appendix I) followed similar trends. The Sooke lake pattern in nitrate-nitrogen fluctuations occurred under comparable weather conditions, the only conspicuous, major environmental factor common to both lakes which are so vastly different in all other respects such as morphometry, trophic condition, standing crop biomass size, nutrient levels, *etc.* It remains to be determined through further investigation whether annual fluctuations in nitrate values in both lakes may be more typical under normal weather conditions.

(e) Total phosphate and orthophosphate[1]

Measured phosphate was present in Elk Lake in concentrations less than 1.0 mg/l (Fig. 7) throughout the study period. These concentrations were within the range of values considered normal for most fresh waters (Hutchinson 1957), and would not be considered limiting by limnological standards (Mackenthun 1962; Verduin 1966). Mean total phosphate values fluctuated with no definite seasonal pattern, increasing to 0.500 mg/l in September, dropping to 0.110 mg/l in October, and increasing again in November to 0.500 mg/l. December and January mean values were stable at 0.300 mg/l. Considerable variation in frame depth total phosphate concentrations occurred between stations, these differences being minimal only on the August and October sampling dates.

In October the surface total phosphate determinations at the four periphyton sampling stations were statistically greater than those measured concentrations at the frame depths. This may have been due to the death of cyanophytes in surface waters, coincident with decreasing fall temperatures and light, which frequently results in a release of measureable quantities of nutrients (Hammer 1964; Benson 1967; Fitzgerald 1970), as well as a reduced uptake of phosphate. It is also possible that the difference was due to a reduction of phosphate in solution at the frames because of increased uptake and demand by natural periphyton

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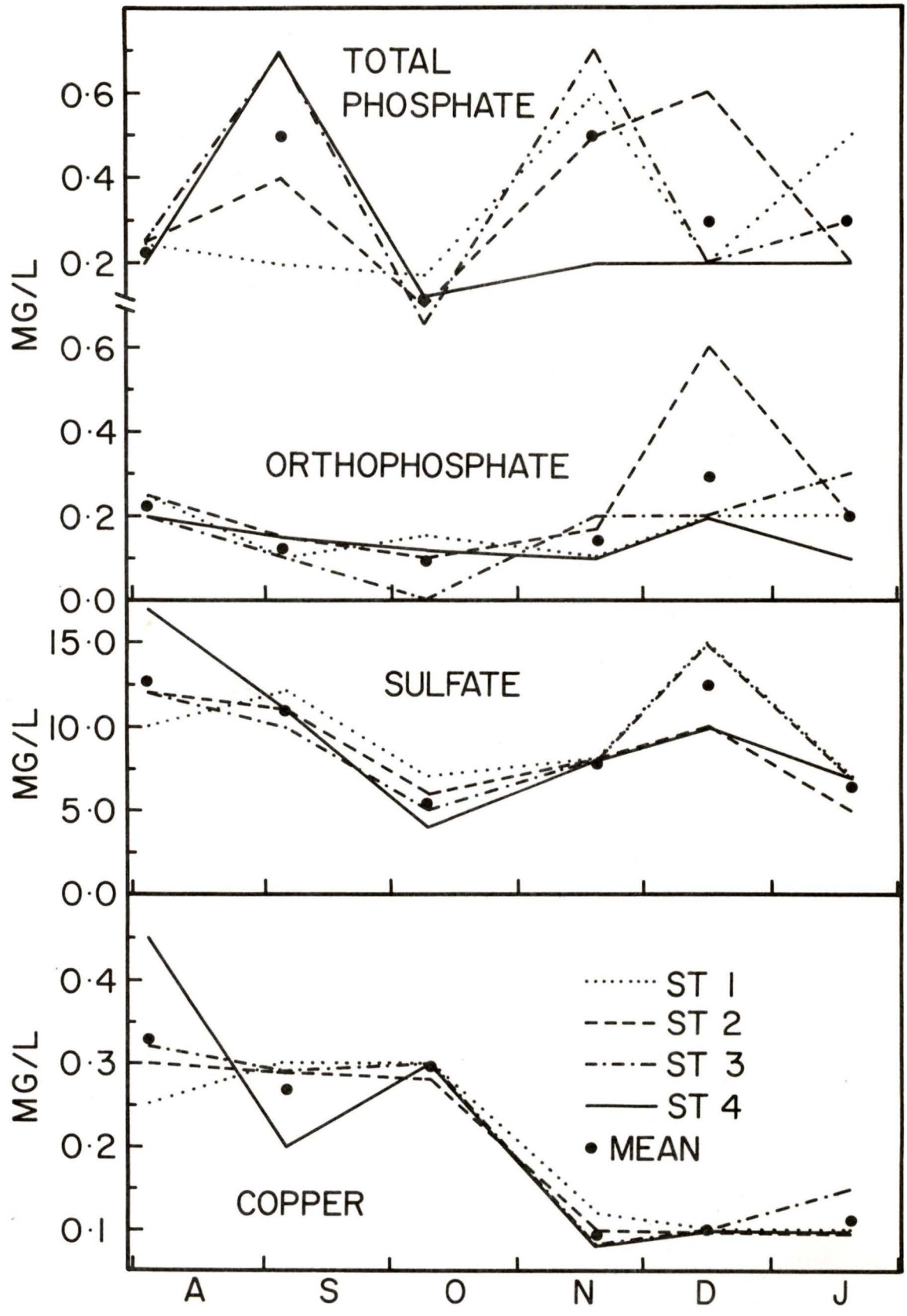
[1] The two inorganic phosphate fractions reported here, dissolved orthophosphate and total dissolved phosphate (condensed or polyphosphates plus orthophosphate), may include unknown amounts of sestonic (or particulate) and colloidal phosphates hydrolyzed by the chemical reagents used in the method of phosphate analysis (S. Olsen 1967 and personal communication).

Figure 7. Graphs illustrating fluctuations in Elk Lake frame depth concentrations of total inorganic phosphate, orthophosphate, sulfate, and copper at the four periphyton sampling stations from August 3, 1967 to January 19, 1968.


TOTAL
PHOSPHATE
ORTHOPHOSPHATE
SULFATE
COPPER
MG/L
0·6
0·4
0·2
0·0
15·0
10·0
5·0
0·3
0·1
ST 1
ST 2
ST 3
ST 4
MEAN
A
S
O
N
D
J

and macrophytes in the littoral region, an effect modified by thermal stratification (see Dickman 1968a). Alternatively, the difference may also have been caused by rainfall and immediate entry of enriched surface run-off. However, in view of the fact that the significant vertical profile difference in October was an isolated incident, and that erratic fluctuations were shown in both surface and frame depth concentrations, no further significance is attributed to these particular differences in recorded total phosphate.

Station differences in orthophosphate concentrations are illustrated in Figure 7. After a high of 0.178 mg/l in August, mean values decreased to a minimum of 0.083 mg/l in October. A peak mean orthophosphate concentration of 0.325 mg/l was reached in December, while values dropped again in January to a mean of 0.138 mg/l. Orthophosphate did not significantly vary in concentration with season (Table 3) and did not appear closely related to the total phosphate values, although a zero measurement of the former was recorded at station 3 on October 9, coincident with the lowest mean recorded concentrations of both phosphate fractions.

Similar to fluctuations in nitrate, inorganic phosphate commonly decreases in concentration concurrent with increased photosynthetic activity and large algal standing crops during spring and summer growing seasons (Lund 1965; Cushing 1967; Shapiro 1970). A typical seasonal pattern was recorded for Lake Washington (Benson 1967), where inorganic phosphate was high in concentration in surface waters throughout the winter months and exhibited a rapid decline in spring, reaching a minimum value in June. Summer concentrations were low but erratically fluctuating until November, when high phosphate values recurred with fall overturn. A somewhat similar trend can be seen in the Elk Lake

orthophosphate values where concentrations gradually decreased until October when the excessive rainfall commenced. Coincident with overturn in November, orthophosphate increased, reaching a maximum for the study in December. January values were low but higher concentrations were recorded in March, 1968.

The rapid loss of both dissolved inorganic phosphate and nitrate from the water column, documented in many studies (Hutchinson 1957, 1967; Dickman 1968a; Keup 1968; Smith 1969), necessitates frequent sampling over long study periods to establish, and accurately evaluate, seasonal cycles. Recorded fluctuations in the Elk Lake phosphate levels were in all probability influenced by the rapid assimilation and turnover rates of inorganic phosphate (Rigler 1964, 1966, 1968), as well as adsorption and release of phosphorous from enriched bottom sediments (see Hutchinson 1957; Harter 1968; Frink 1969; Wentz & Lee 1969; Hynes & Greib 1970). Since algae tend to take up and store inorganic phosphate in amounts greater than that required for immediate physiological needs (Maloney 1966; Goldman 1968), it has been suggested that total phosphate concentrations may be a more indicative measurement of the true fertility of a given water body (Lund 1965).

In early March (1968) the orthophosphate concentration of the inflow was 0.350 mg/l compared with a very high concentration of 1.700 mg/l in the lake, whilst the converse was true for nitrate-nitrogen which was also higher in magnitude than the phosphate in both waters. Since phosphate, unlike nitrogen, is tightly bound to inorganic soil particles and is not leached by rainfall to the same extent, surface run-off and drainage inflows are typically more rich in nitrates than phosphates (Oglesby & Edmondson 1966; Owens & Wood 1968) as was evident in the Elk Lake inflow, O'Donnel Creek.

In previous years phosphate fluctuations in the Colquitz River outflow and downstream drainage system of Elk Lake were unusual in that there was a marked increase in concentrations during spring and summer (Waldichuk 1968, 1969). It was suggested that this was due to an unknown waste effluent input of phosphorus or a release of nutrients from decaying vegetation in the sediments. Although Waldichuk (1969) discounted the concentrating effects of evaporation in this instance, it is still possible that evaporation was a modifying influence as was found with salinity and other nutrient measurements in the same system. In summer months, precipitation is low and despite sewage treatment plant inflows, Colquitz River flow is visibly reduced in volume and velocity. The effects of dilution on phosphate concentrations have been documented in other drainage systems (Duthie 1968; Keup 1968; Oglesby 1968, 1969).

It is probable that the phosphate levels in Elk Lake were also subjected to some modification due to evaporation and dilution as previously discussed with regard to nitrate-nitrogen, although not to the same extent as the latter. Rapid assimilation and luxury consumption of phosphate would tend to keep a large part of the available nutrient organically bound in the metabolic pool or possibly bound in the sediments, and not present, to the same extent as nitrate, in the lake water itself where concentrations of dissolved inorganic phosphate might be influenced by dilution and evaporation factors. The high recorded nutrient levels in Elk Lake suggest the lake will continue to support large standing crops of macrophytes, phytoplankton, and natural periphyton, particularly in the shallow littoral region. The effects

of expanded urbanization and agricultural development in the watershed, expressed in these high nutrient values within the lake, can be expected to grow increasingly more acute as conveyed directly through the enriched O'Donnel Creek inflow and indirectly in surface run-off.

(f) Sulfate

Sulfate concentrations recorded for Elk Lake (Fig. 7) were within the reported range of 3 to 30 mg/l ordinarily found in open lakes (Hutchinson 1957). Mean sulfate values in Elk Lake gradually decreased from a high of 12.8 mg/l on August 3, to a low value of 5.5 mg/l on October 9. A concentration of 8.0 mg/l was recorded at all four periphyton sampling stations in November, concurrent with thermal mixing. Mean values of sulfate then increased to 12.5 mg/l in December, dropping off again in January to a concentration of 6.5 mg/l. The greatest amount of variation in sulfate concentrations between the stations occurred in the August and December water samples when similar high values were recorded.

In ponds where sulfate concentrations were high in spring and low in autumn, an inverse correlation between annual fluctuations of sulfate and bicarbonate was found closely related to the winter oxidation of sulfide to sulfuric acid (Mann 1958; Tucker 1958). The development of sulfuric acid was also thought to occur to a limited extent in Clear Lake, where sulfate concentrations were inversely related to primary productivity values of phytoplankton, eventhough bicarbonate simultaneously increased (Goldman & Wetzel 1963). Without further study it is difficult to propose a detailed explanation of the sulfate pattern found in Elk Lake, although variations in concentration must have been largely

dependent upon photosynthetic activity and bacterial metabolism, as well as flushing rates. It is improbable that the sulfuric acid phenomenon was operative to any appreciable extent in Elk Lake because of the slight inverse relationship sulfate displayed with dissolved oxygen concentrations and percent oxygen saturations (Fig. 7; see Mann 1958).

In Elk Lake, during this study, there was no indication of the presence of hydrogen sulfide gas. This was to be expected since water samples were taken at exposure-frame depths where the water was shown to be well-aerated, even during stratification. No bottom samples were made in the deeper hypolimnetic regions at the mid-lake station where bacterial, anaerobic reduction of sulfate to hydrogen sulfide would be most likely to occur. However, *Beggiatoa* was present in fresh plankton tow net samples taken at stations 3 and 4 on September 5, and at station 4 on October 9. Such bacterial organisms are important in the oxidation of hydrogen sulfide and the metabolism and recycling of sulfur compounds, of particular importance in highly productive lakes with oxygen deficits. Although Elk Lake has not yet reached a stage of eutrophy where fish kills or the generation of hydrogen sulfide and methane gases are of critical significance, sludge deposition due to the build up of hydrogen sulfide and consequent sulfur accumulation, can soon be expected to reach serious levels now expressed in other small lakes in the lower Vancouver Island region (*i.e.*, Saanich Peninsula). Such conditions are commonly aggravated by urbanization and agricultural activities within the watershed.

(g) Copper

In Elk Lake, copper concentrations varied from 0.450 to 0.080 mg/l (Fig. 7). Mean copper concentrations decreased slightly from a high value of 0.330 mg/l in August to 0.270 mg/l in September, increasing slightly again in October to 0.295 mg/l. Concurrent with overturn in November, mean copper values decreased sharply to 0.095 mg/l, showing a gradual increase to 0.113 mg/l in January. The greatest variation in concentrations between stations occurred on the August and September sampling dates, and most of this variation can be attributed to station 4 measurements.

The decrease in copper content which occurred with fall overturn in Elk Lake was also evident in Sooke Lake. This pattern in copper fluctuation is in conflict with other studies where copper concentrations were found to increase throughout summer stratification and fall circulation (Hutchinson 1957). These studies showed that in addition to high concentrations of the organic or colloidal fraction, large amounts of ionic and sestonic copper were also present at the autumnal peak. Total copper concentrations were found to be directly related to bicarbonate and the rate of decrease in epilimnion temperature, and inversely correlated with rainfall. The relationship with alkalinity was thought to indicate that some of the copper was derived from basin sediments; while the temperature effect was held to be a result of increased copper uptake by littoral vegetation in spring (rising temperatures), followed by a release again during decomposition of decaying plants with autumn decreases in temperature. Hutchinson (1957) further suggested that the temperature effect may also have been a result of the oxidation of the mud surface, liberating copper at overturn which had previously

been held as copper sulfide under conditions of reduction at the mud surface. However, the graphed mean copper concentrations for Elk Lake (Fig. 7) varied directly with decreasing pH (Fig. 5) and temperature (Fig. 4), and showed only a slight negative relationship with rainfall (Fig. 3) and total hardness (Fig. 6). Thus the Elk Lake data did not suggest a releasing of copper from the sediments with overturn, or from decaying littoral macrophytes, although it is possible any release may have been masked by the unusually heavy precipitation. Inflows did not contribute any demonstrable quantities of copper resulting in increased copper concentrations within the lake. In fact, the rainfall and resultant run-off, common to both Elk and Sooke lakes, may have had a diluting effect due to abnormally high flushing rates. Therefore the fluctuations in copper found in Elk Lake (and Sooke Lake) were unusual in that concentrations were inversely related to rainfall, positively related to temperature and pH, and decreased during late thermal stratification, reaching a minimum at autumnal overturn.

Although Hutchinson (1957) found no evidence to indicate that the seasonal pattern of ionic copper in hard waters was regulated by the precipitation of copper carbonate, he did cite examples of copper sulfate being combined as an insoluble precipitate, causing a decrease in pH. Oxidized compounds of copper are highly insoluble and tend to accumulate in bottom muds (Oglesby & Edmondson 1966). It is therefore possible that the decrease in copper with overturn in Elk and Sooke lakes may be explained by the precipitation of an oxidized form of copper concurrent with thermal mixing and reoxygenation of the hypolimnetic waters, although a simultaneous fall in calcium carbonate (Fig. 6) as

well as pH (Fig. 5) might have been expected. On the other hand, copper, released from bottom sediments by oxidation of the mud surface following thermal stratification, could have been taken up immediately by the algae and macrophytic flora at overturn. It is unlikely, however, that this alone could account for decreases in copper as large as that found in both Sooke and Elk lakes. Other factors such as chelation and adsorption on allochthonous matter from run-off may also have accounted, in part, for the decrease in copper with fall overturn reported here.

To the author's knowledge, there have been no published reports indicating that naturally occurring copper concentrations have been found limiting to resident lake biota. Hutchinson (1957) states that 0.050 mg/l is a higher amount of ionic copper than would be expected to occur in the trophogenic zones of uncontaminated, untreated lakes. Copper concentrations found for Elk Lake, a treated lake, were also higher than those values reported elsewhere (Wetzel 1966; Oglesby 1968, 1969). Differences between the Elk Lake values and those of other studies may be due in part to the use of different methods of chemical analysis, as supposedly susceptible plankton genera occurring in Elk Lake did not appear limited in growth, and phytoplankton populations flourished throughout the study period. It is also possible, however, that chelation of the heavy metal ions may have reduced the toxicity of the high recorded copper concentrations (see Kraus 1962; Wetzel 1966).

Copper sulfate has been applied, with varying degrees of success, to many water bodies as a panacea for eradicating nuisance blooms of phytoplankton (*e.g.*, Hutchinson 1957; Mackenthun 1962; Oglesby & Edmondson 1966; Wetzel 1966). The effectiveness of its application is

largely dependent on the alkalinity of the water; in hard or medium-hard waters such as Elk Lake, the ionic copper combines with carbonate to form an insoluble precipitate which accumulates in lake bottom sediments. Copper sulfate is generally applied at a rate to ensure an end concentration of at least 1.0 mg/l, however, the actual concentrations used in Elk Lake are uncertain (W.H. Warren, R.P. Finegan, and H.P. Carsner, personal communications). No records are available to indicate the immediate, short-term effectiveness in Elk Lake of the applications of copper sulfate and other herbicides (2,4-D, sodium arsenite, aquathol; see Brown 1969, Appendix I), but it would appear that there has been little or no long-term benefit in abatement of the cyanophyte blooms, as these still persist in Elk Lake despite high measured copper values. Nevertheless, it is possible that the build up of copper compounds has reduced the benthic fauna (see Hutchinson 1957) either in quantity or in diversity, and that the species composition of the resident algal flora has been modified since the time of initial algicide application. None of the macrophytic vegetation present during the current study appeared to have been adversely affected. It is reasonable to suppose that this additional source of copper compounds was the prime reason for the relatively high copper concentrations found in Elk Lake. Treatment of a lake with copper sulfate will result in a striking build up of copper in the sediment, either as a precipitate of basic carbonate or in organic combination (Hutchinson 1957); similar sediment accumulations occur with sodium arsenite applications (Mackenthun 1965; Chamberlain & Shapiro 1969).

PERIPHYTON POPULATIONS

(A) Qualitative features

(a) Species composition

Four taxonomic groups were represented on the filter preparations of the Elk Lake vertical slide periphyton: Cyanophyta, Chlorophyta, Bacillariophyta and Protozoa. Elk Lake periphyton, or *Aufwuchs* communities, consisted almost entirely of algae with the occasional associated heterotrophic microorganism. A total of 41 different periphyton species were found (Table 4), and of these, 29 taxa were counted whilst the remaining 12 species (3 blue-green and 9 green algae) were tabulated as present or absent. The enumerated organisms included 28 diatoms (3 centric and 25 pennate forms) and one amoeboid protozoan (Class Sarcodina, *Difflugia* sp.).

It is probable that the total number of 41 different taxa found was an underestimate of the actual number of species originally present on the artificial substrata prior to formalin fixation. Minute, fragile organisms such as microflagellates, chrysophytes, and protozoans are not well preserved in formalin and would likely be destroyed in the subsequent filter preparation (see Appendix II). For example, *Vorticella, Phacus, Micrasterias, Euglena,* and *Mallomonas caudata* were present on the unfixed, fresh horizontal slide samples. However, other workers, using comparable preparation methods, have reported good preservation of most organisms (Ehrlich 1955; McNabb 1960; deNoyelles, Jr. 1968), and in the current investigation, delicate taxa such as *Microcystis aeruginosa* and *Selenastrum westii* appeared relatively unchanged from the natural condition and were readily recognized on the cleared filters.

Table 4. Alphabetical list of Elk Lake periphyton taxa found on Millipore filters as prepared from preserved vertical glass slides throughout the sampling period (August, 1967 to January, 1968). Twelve of the total of 41 different species were tabulated as present or absent (*) while the remaining 29 were enumerated on the cleared filters. (The species were coded by number to facilitate analysis.)

Code No.		Taxon
19		*Achnanthes minutissima* Kütz.
22		*Amphora ovalis* Kütz.
28	*	*Ankistrodesmus* sp.
33		*Asterionella formosa* Hassall
38	*	*Cladophora glomerata* (L.) Kütz.
01		*Cocconeis placentula* Ehr.
02		*Cocconeis placentula* var. *lineata* (Ehr.) V.H.
24	*	*Coleochaete orbicularis* Pringsheim
39		*Cymatopleura solea* (Bréb.) W. Sm.
05		*Cymbella* sp.[1]
21		*Difflugia* sp.
13		*Epithemia sorex* Kütz.
03		*Fragilaria crotonensis* Kitton
04		*Fragilaria virescens* Ralfs
34	*	*Gloeotrichia echinulata* (J.E. Sm.) P. Richt.
15		*Gomphonema acuminatum* var. *coronatum* (Ehr.) Baben.
16		*Gomphonema olivaceum* (Lyngb.) Kütz.
40		*Gyrosigma* sp.
17		*Melosira italica* (Ehr.) Kütz.
18		*Melosira varians* Ag.
32	*	*Microcystis aeruginosa* Kütz.
09		*Navicula* sp.
10		*Navicula cryptocephala* Kütz.
31		*Navicula pupula* Kütz.
36		*Nitzschia tryblionella* Hantzsch.
14		*Nitzschia vermicularis* (Kütz.) Grun.
30	*	*Oscillatoria* sp.
11		*Pinnularia* sp.
07		*Pinnularia gibba* Ehr.
08		*Pinnularia viridis* (Nitz.) Ehr.
29	*	*Quadrigula* sp.
31	*	*Selenastrum Westii* G.M. Sm.
23	*	*Spirogyra* sp.
26	*	*Spirogyra crassa* Kütz.
20		*Stephanodiscus niagarae* Ehr.
25	*	*Stigeoclonium tenue* (Ag.) Kütz.
12		*Surirella* sp.
27		*Synedra radians* Kütz.
06		*Synedra ulna* (Nitz.) Ehr.
41		*Tabellaria fenestrata* (Lyngb.) Kütz.
35	*	*Zygnema* sp.

[1] Likely includes two of the following species: *C. turgida*, *C. ventricosa*, and/or *C. affinis*.

The method was not deleterious to diatom frustules which were excellently preserved.

The paucity of enumerated taxa representative of the Protozoa and Rotifera in the Elk Lake periphyton, as noted by Sladeckova (personal communication), can also be partially attributed to filter preparation and slide fixation. The presence of invertebrate fauna in periphyton communities is however, heavily dependent upon environmental and experimental conditions such as, trophic status, substratum, exposure position and depth of substrata, *etc.* The faunal component of the total biomass found on glass slides from other, more oligotrophic lakes studied (Sooke and Buttle lakes; see Appendices I and II) was greater and more diverse than that in Elk Lake, eventhough the experimental design and methods of sample preparation used in all study lakes were essentially identical.

Microscopic examination of living material on horizontally exposed slides from Elk Lake revealed, in addition to the occasional gastropod snail, a few nematodes, ciliates, rotifers, *Vorticella* sp., *Phacus* sp., and some copepods. However, the distribution and occurrence of these organisms was irregular, numbers were sparse, and were not related to month of exposure, length of submergence, or the taxonomic composition and abundance of the floral component of the periphyton. Prepared filters of the fixed vertical slides did not indicate the presence of any of these organisms, although there is no other evidence suggesting they are exclusive to horizontal surfaces.

Benson (1967), using glass slides suspended in Lake Washington, found a larger representation of invertebrates (protozoans, hydras, chironomids) than found in Elk Lake periphyton. However, again, aside from the environmental characteristics of the lake itself, which may be

conducive to invertebrate development, his substrata were suspended in a pelagic situation, in vertical profile series to a maximum depth of 3 m where consumer organisms would likely be more common. Thus the lack of periphyton fauna found on vertically exposed glass slides in Elk Lake may have been a consequence of at least two factors, the method of filter preparation, and/or environmental conditions characteristic of the Elk Lake littoral zone. Since only a few consumer organisms from the lower invertebrate phyla were present on the glass substrata or prepared filters, the periphyton of the littoral region of Elk Lake can be considered as essentially a population of primary producers, similar to that documented for other lakes (Castenholz 1960; Maciolek & Kennedy 1964; Wetzel 1964; Dickman 1968b; Szczepanski 1968).

Fungal components of the periphyton assemblage were sparse with some water moulds, *Saprolegnia* sp. and *Achlya* sp., occurring particularly in those samples immersed for longer time periods with consequently greater detritus deposits and higher biomass accumulation. It is probable that there were other genera (*e.g.*, chytrids), distorted and lost during filter preparation and/or undetected due to the study emphasis on the algal components. However, fungi, like the bacteria, never became major constituents of stabilized periphyton communities on the glass slides.

Regardless of experimental conditions, diatoms were present in significant numbers in all samples. Approximately 24 different species occurred with some regularity, and together dominated the standing crop expressed in cell numbers. Many other investigators, using various sample preparation and counting methods, have documented an abundance of diatom taxa in periphyton communities found on different substrate

types (Butcher 1932; Brook 1955a; Gumtow 1955; Castenholz 1960; Maciolek & Kennedy 1964; Eaton & Moss 1966; McIntire 1968a, 1968b; compare with Neal *et al.* 1967). Similarly, the dominant proportion of pennate forms to centric diatom taxa, as found in the Elk Lake periphyton, is not uncommon (Weber & Raschke 1966), and even the generic composition bears striking resemblance to those diatom assemblages found elsewhere in many different water bodies.

Twenty of the Elk Lake periphyton taxa (all diatoms) had a constancy[1] value of 100%, occurring in all samples (4 stations x 11 TS = 44 samples of glass slides in total). The remaining 21 taxa had constancy values less than 100%, and of these organisms, 2 taxa, *Zygnema* sp. and *Gyrosigma* sp., each occurred only once (station 1, TS02 and station 2, TS03, respectively).

Some of the species listed in Table 4 can be classified as planktonic as well as periphytonic. Twelve periphyton taxa were definitely common to the net plankton samples (Brown 1969; Appendix I, Table 2), and an additional 4 were dubious plankton components because of incomplete identification to species and rare occurrences in the net plankton (*e.g.*, *Stigeoclonium* sp., *Coleochaete* sp., and *Spirogyra* sp.).

In summary, the method of filter preparation adopted in this study was designed to evaluate the abundance of those taxa that provided a significant contribution by cell number to the community biomass. That is, the biomass contribution of any taxon too rare to appear in Table 4 was negligible in that it was not observed on the filters.

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[1] Constancy is defined here (after Cooke & Hirsch 1958) as the number of occurrences of a taxon in samples, expressed as a percentage of the total possible number of samples (*i.e.*, 44 samples). A sample typically consists of four glass slides immersed for a given length of time during the sampling program.

(b) General short-term successional stages

Within the first week of immersion all slides became slippery to the touch with an initial formation of bacterial slime (see Henrici 1936, 1939; Cooke 1956). Whereas bacteria were present on all slide samples throughout the study, they were never dominant in any time series and were always quickly succeeded in abundance by algae after one week's immersion. Principal colonizing algae were the diatoms, *Cocconeis placentula, C. placentula* var. *lineata, Navicula* sp., *N. cryptocephala, Achnanthes minutissima, Gomphonema olivaceum, G. acuminata* var. *coronatum, Fragilaria crotonensis, F. virescens,* and *Melosira italica,* which occurred on all slides sampled.

The first five of these pioneering or opportunistic species were commonly attached to the glass slides by one entire side of the frustule, laying firmly appressed to the slide surface. *Gomphonema* spp., *Cymbella* sp., and sometimes, *Achnanthes* sp., were attached by elongate gelatinous or mucilaginous stalks, while occasionally *Navicula* and *Fragilaria* species were found to be attached by means of short gelatinous pads. Often species of *Fragilaria, Melosira,* as well as, *Asterionella formosa* and *Stephanodiscus niagarae* had no visible means of attachment and were presumably entangled and trapped in the samples. Some of the taxa enumerated, *e.g., Navicula cryptocephala, Nitzschia* spp., and *Amphora ovalis,* possess the capacity for movement (Harper & Harper 1967; Harper 1969) although this was not observed.

Most of the above diatom species were well established in all one-month immersion samples. However, in TS01, *Pinnularia* sp. and *Synedra ulna*

were only present at station 1, and the former species did not occur at the other three stations until some time in November, being absent in samples of TS05. These slides, immersed for 34 days between September and October, also contained specimens of the cyanophyte, *Gloeotrichia echinulata,* a more typical component of the plankton (Young 1945; Brown 1969; see Appendix I, Table 21). In samples of TS09, immersed for 27 days from November 19 to December 16, *Leptothrix ochraceae* Kütz. was found on fresh slides (all of which were a yellow-brown color), suggesting the presence of iron compounds as similarly reported by Sladeckova (1966) for glass slides immersed in a Czechoslovakian reservoir.

Slides exposed for approximately two month intervals or longer were coated with a slippery, felt-like covering characteristic of diatom dominated periphyton communities. This coating tended to grow darker in color with increasing exposure durations when occasional tufts of green algae were also evident. For example, *Spirogyra crassa* occurred on all slides of TS02 and increased slightly in numbers on those of TS03; *Stigeoclonium tenue* was present in TS06 and increased in TS10 samples at all but station 3. Additional greens (*Cladophora glomerata, Coleochaete orbicularis*) and blue-greens (*Oscillatoria* sp., *Microcystis aeruginosa*) were also observed but were never abundant enough to develop a continuous growth over more than a few isolated mm of slide surface. Mucilaginous tracks of gastropod snails were more frequent on long-term exposure slides, particularly on those horizontally oriented.

Thus, aside from the initial colonization by bacteria, the only other "community type" clearly distinguishable was that of the diatoms. With increasingly longer exposure durations (*e.g.,* TS03, TS06, TS10), scattered settlement of certain species of green and blue-green algae

occurred, although these organisms never became abundant even after 135 days of slide immersion. On the basis of cursory observations only, these periphyton communities, on vertically oriented glass slides, were found comparable in species composition, successional pattern, and physiogamy to those on horizontal slides and the natural substrata sampled from the Elk Lake littoral region during this study.

(B) Quantitative features

As a preliminary analysis of the Elk Lake periphyton data, attached populations from vertically exposed glass slides were compared in a number of ways. Results presented here illustrate the variation in size of the total periphyton populations (cells or organisms/mm^2) at each of the four stations in relation to exposure period. The diversity of these populations is discussed and the contribution of certain individual species to the total populations in each situation is shown compared with fluctuations in comparable plankton species populations. Finally, the variation in population size of selected individual species, at each of the four sampling stations, is analyzed, and general relationships with environmental data are discussed.

(a) Total cell populations

Although it is possible that the occurrence of very rare species in samples may have been a random phenomenon with respect to station location, it was assumed that the species pool and colonization rates were essentially identical for all four sampling stations and that periphyton communities which developed at each station were primarily a manifestation of the physico-chemical properties of the lake water and ambient meteorological conditions prior to, and during any particular slide exposure period. Therefore in view of the similarities expressed in measured physico-chemical characteristics of the four sampling stations, concomitant similarities in periphyton communities were expected.

(i) Variation by site and exposure period: statistical analysis

As anticipated, Elk Lake periphyton communities were alike in some respects, but dissimilarities did occur. For example, the results of simple anovas, used to test the null hypothesis that there were no significant differences in total cell numbers/mm^2 (logarithmically transformed) between the four stations in any one time series, were all significant ($F_{\alpha = 0.01}(3, 12) > 5.9526$). Similar results were obtained using comparable data for total numbers of structural units or filaments/mm^2 (and for the 29 individual taxa counts). Thus, for any period of exposure, total counts at the four stations were statistically different. Again, when total counts at each station were tested for similarity over the 11 time series, significant differences were found ($P < 0.01$) between counts of different slide exposure periods. Mean values were then ranked and their differences tested for each station as shown in Table 5.

Hence, while all stations were statistically similar in measured physicochemical properties over the six sampling dates, and species composition was essentially the same, total numbers of cells or organisms sampled from these stations (over the 11 time series) were not. The variation in total counts between stations was greater than that within station slide replicates. These results suggest, among other things, that; (1) a greater number of sampling stations or a fixed number taken at random locations on successive occasions may be necessary to define spatial variation in the littoral region in biological terms, *i.e.*, the spatial variation in periphyton cannot be assumed to be as abrupt as found by point sampling by station in location (unless a specific inflow or source of pollutants or such is known) and may be a continuum necessitating many stations to delimit; and (2) conclusions and generalizations

Table 5. Summary of statistical tests illustrating the differences in total cell counts between the 11 different exposure periods for each of the four periphyton sampling stations. Values given here are average total cell counts/mm^2; those means scored by the same solid line are not significantly different at $P \leq 0.01$ as tested by D.N.M.R.T.

TS	STATION 1	TS	STATION 2	TS	STATION 3	TS	STATION 4
11	98.45	11	109.58	11	85.78	11	99.97
09	196.90	09	179.89	09	140.72	09	120.82
01	446.11	10	229.86	10	186.01	10	197.18
05	502.02	05	319.68	01	255.75	05	257.48
07	531.19	08	336.85	08	367.46	08	465.11
10	599.98	01	442.34	05	402.44	07	525.19
02	943.81	07	935.43	02	779.47	01	544.07
08	1135.24	02	1085.72	03	1006.31	06	865.67
06	1283.15	06	1406.97	07	1058.76	03	917.65
03	1362.04	04	1496.88	06	1287.96	02	1015.37
04	1662.42	03	1546.03	04	1390.75	04	1231.76

concerning the whole lake, based on findings from single sampling stations, particularly those located in the littoral region, may not be entirely justified in many instances and care must be taken in their interpretation.

In view of the sampling methods used, and within the statistical framework applied, it may be concluded that differences in total counts between the stations in Elk Lake were not due to chance alone and within any one time series or over the 11 different periods of slide exposure, these differences cannot be attributed to recorded differences in measured physico-chemical variables between the stations. Previously, some evidence based on periphyton count data suggested that station 4 was not as similar as the others in environmental features (Brown & Austin 1971; Appendix II) although this was not confirmed by the statistical analysis of measured physico-chemical variables. It is of interest to note that Castenholz (1961) reported large variations in production of periphyton on glass plates located at widely spaced, but evidently similar sampling stations within individual study lakes in Washington (see also Maciolek & Kennedy 1964; Ewing & Dorris 1970).

In view of the statistical results using total count values, periphyton data were examined in greater detail employing cell counts for each of the 29 different species. Further analysis (Table 6) indicated that over the study period there were statistically significant ($P < 0.01$) main effects on the counts due to station location, slide exposure time period, and periphyton species. Significant first order interactions of stations x time series, stations x periphyton species, and time series x periphyton species also occurred, indicating

Table 6. Results of a three-way classification analysis of variance (with replication, i.e., four slides) run on the Elk Lake count data for 29 periphyton taxa. Count data (cells/mm^2) were logarithmically transformed.

SOURCE OF VARIATION	df	SS	MS	F
A stations	3	21.0937	7.0312	398.2659**
B time series	10	794.2734	79.4273	4498.9414**
C periphyton taxa[1]	28	9039.4726	322.8381	18286.2852**
FIRST ORDER INTERACTION				
A x B	30	57.0703	1.9023	107.7531**
A x C	84	144.6835	1.7224	97.5620**
B x C	280	1444.5859	5.1592	292.2307**
SECOND ORDER INTERACTION				
A x B x C	840	233.4609	0.2660	15.0683**
Within (Error)	3828	67.5820	0.0176	
TOTAL	5103	11792.2226		

[1]The 29 periphyton taxa are listed in Table 4.

**Significant at $P < 0.01$.

that none of the three factors were independent in their effects on the counts. That is, the magnitude of cell counts (for 29 taxa combined) was dependent on station location and period of slide immersion (*i.e.*, season and/or duration), as well as on periphyton species. Each of these three factors had differential effects on the count values; the stations over time series did not react the same, nor did individual species over time series, or species over stations.

An example of the latter differential effect is shown by mean count values (n = 11 means) for two of the species, *A. minutissima* and *C. placentula*. Over the time series, mean values (in cells/mm^2) for both species show that *Achnanthes* was at least three times greater in abundance than *Cocconeis* (102.33 cells/mm^2) at station 1. At station 2 both species were almost equal in mean abundance (126.14 and 179.60 cells/mm^2, respectively), but at the other stations means were inversely related so that at station 4, *Achnanthes* had a mean count value of 62.55 compared with a mean of 184.14 cells/mm^2 for *Cocconeis;* conversely, at station 3 the latter had a mean of 36.21, while the mean value of *Achnanthes* was 258.85 cells/mm^2. A simple plot of this interaction clearly illustrated that the effects of station location on the abundance of these two diatoms was not the same for each station. Interpretation of this interaction relates to earlier findings where, on a few occasions, significant differences in counts for these two species were found between the four replicate slides of a sample (Brown & Austin 1971; Appendix II). That is, not only are counts of *A. minutissima* significantly different between stations, but occasionally, by χ^2 analysis, they have been found different between the slides of a given station sample. It was suggested that variation in these counts was the result

of species interaction based on competition for available substrate area (Brown & Austin 1971; Appendix II).

There is no reason to suspect that this explanation should not be applied to the station differences and the significant interaction of the two species. The complexity of differentiating effects of biotic interaction from those conditioned by abiotic features is again illustrated, for it is possible that the variation in numbers of these two diatoms may be dependent only on substrate area available and direct competition, while, on the other hand, minute differences in the physico-chemical microenvironment, not detected by methods of analysis used here, may predispose one species to increase in numbers at the apparent expense of the other in a fashion which appears random with respect to station location.

Because first order interactions were significant, plots were made of all interactions, and simple main effects were calculated (after Winer 1962; Kirk 1968). All results of these analyses were significant ($P < 0.01$) except for those of the four species, *Nitzschia tryblionella, Cymatopleura solea, Gyrosigma* sp., and *Tabellaria fenestrata,* evaluated over both stations and slide exposure periods. These species were the least abundant occurring taxa throughout the study, and these statistical results suggest their numerical abundance on the vertical slides was not affected by station location or period of slide immersion. Unlike the remaining 25 enumerated taxa, the occurrence of individuals of these four rare species was in all probability, random with respect to station location and time series.

Finally, the significant second order interaction (Table 6), *i.e.,* stations x time series x periphyton species, suggests the

interaction of stations x periphyton species differs depending on the particular period of slide immersion, or the interaction of time series x periphyton species differs dependent on station location, *etc.;* each first order interaction may be dependent on the level of the third factor. Tests for simple simple effects (Winer 1962; Kirk 1968) were not made for this aspect of the study, although their evaluation may provide for further grouping of species in future analyses when the second order interaction may be examined in more detail.

These results are in contrast with those of the similar analysis of physico-chemical data (Table 2) where no significant main effect due to station location was found, and where the effects of sampling dates and physico-chemical variable measurements were shown to be independent of station location.

Thus, on the basis of statistical analysis to this point, the most important finding is that significant differences between stations for total cells or for 25 individual species counts, within any one slide immersion period or over the 11 time series, cannot be attributed to variation in the physico-chemical properties between these stations as measured with the given methods. This variation between stations in periphyton counts may be a consequence of biotic factors such as species interaction and competition, or it may be an artifact of point sampling in time and space (*i.e.*, an artifact of the experimental design and methods used). However, with regard to these periphyton taxa, station values cannot be pooled and must be expressed individually. It is possible, although not highly probable, that the same non significant station differences in occurrence of the four rare species may have been related to measured physico-chemical characteristics, although it is

more likely that these species maintained such small populations in the lake that individuals occurred in slide samples by chance alone.

It must not be forgotten that statistics are applied here chiefly as a means for data grouping and interpretation, and that the important relationship discussed above may be an example of biological significance. That is, the variation in physico-chemical properties between stations was statistically non significant, but may have been biologically relevant in terms of the response elicited from the periphyton.

Although it was not of prime concern at this stage of the study program, some attempt was made to differentiate the effects of length of slide immersion from seasonal effects (*i.e.*, responses to seasonal variation in physico-chemical properties), as confounded by the fact that unequal periods of immersion occur at different and overlapping seasons. Further complications arise because significant count differences occur between stations within each time series, and because sloughing and colonization rates may not be constant as assumed. Preliminary results of simple analyses indicate that in some cases seasonal effects are more important, while on other occasions, length of slide immersion appears to be of more significance in relation to fluctuations in cell counts.

If number of days of immersion were the most important factor determining the magnitude of cell counts, we would expect TS04, the period of longest immersion or 135 days, to have the highest mean total count value, while TS09 the shortest exposure duration of 27 days would be expected to have the least number of cells/mm^2. Such is not the case, as illustrated in Table 5. The complexity of the problem can be appreciated by examining the mean station values for total cell counts in each

time series (Table 5). For example, comparison of mean values for TS01, TS05, and TS11, all periods of equal exposure duration (*i.e.*, 33 or 34 days, see Table 1), illustrates station differences. At station 1, mean total count values for TS01 and TS05 are not significantly different, whereas at stations 2 and 4, mean values for TS01 are significantly greater than those of TS05. Conversely, TS01 has a significantly smaller total cell count than TS05 slide samples at station 3. By contrast, TS11 mean count values were significantly lower than those of all other time series, at all four stations. Thus, differences in these particular mean count values between time series (and/or stations) cannot be attributed to the duration of slide immersion.

Slide samples of TS02, immersed for 67 days over the combined durations of TS01 and TS05, had significantly higher mean count values than either of the three equal exposure periods at all stations. The increase in cell numbers during this exposure period must be attributed, in part, to the longer period of slide immersion. At station 1 the sum of mean total counts for TS01 and TS05 is almost equal to the mean value of TS02. However, at stations 2,3 and 4, the comparable sums are less (by 121.28 to 323.70 cells/mm^2) than TS02 mean values. The differences may be due in part to different colonization rates and the time necessary to "acclimatize" pristine slide surfaces of TS01 and TS05 samples. In addition, the increase in cells on TS02 slides may represent a greater addition of cells due to reproduction, as opposed to settling and accumulation of cells.

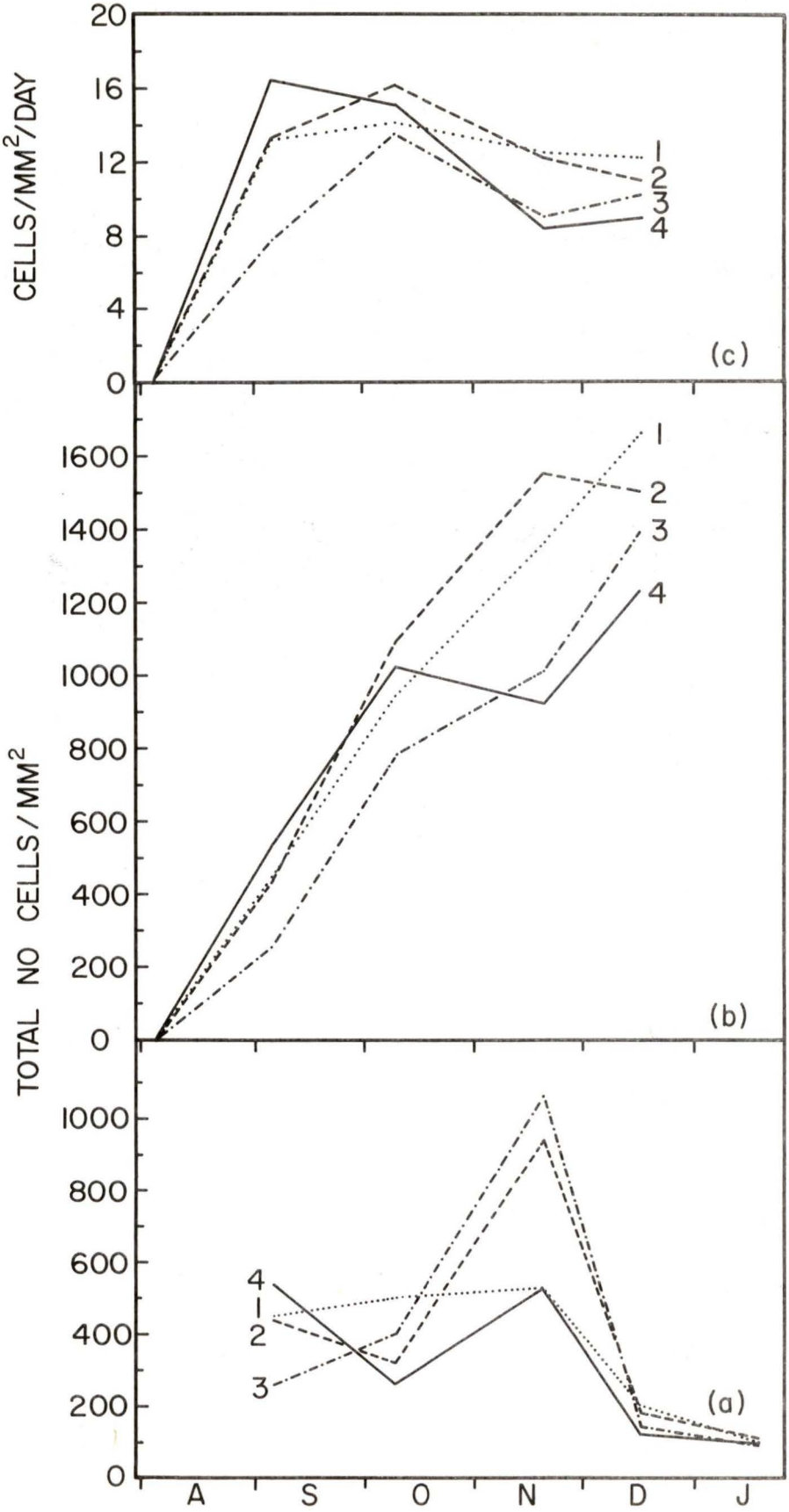
Other similar examples of conflicting evidence are apparent and it therefore appears that attempts to isolate seasonal and time effects require further study. Brief reconsideration of this problem is made in the final section of this presentation.

(ii) Variation by site over similar exposure periods

Whereas total cell counts were significantly different between stations and between certain exposure periods (Tables 5 and 6), in samples of approximately one month's duration (*i.e.*, TS01, 05, 07, 09, 11) these counts illustrated similar trends at all four stations (Figure 8) reaching maximum mean values in TS07 samples, immersed for 41 days, between October 9 and November 19. While this exposure duration was the longest of the five comparable intervals, the increase in cell numbers was also coincident with overturn, flooding, increasing concentrations of dissolved oxygen and total phosphate, and decreasing concentrations of nitrate-nitrogen, pH, and temperature. In this group of samples stations 1 and 4 appear more similar to each other in overall trend than stations 2 and 3, which were in turn, more similar to one another. At all stations, minimum values occurred in TS11 samples. This exposure period, the shortest duration in the group (27 days), occurred from December to January when the above physico-chemical factors were essentially similar in trend as during the TS07 immersion period, with the exception that light was decreasing and thermal mixing was in effect throughout the entire TS11 exposure duration.

In Figure 8 total cell counts/mm^2 are also plotted against increasing exposure intervals of 33, 67, 108 and 135 days duration (*i.e.*, TS01, 02, 03, 04) from August to December. Again a similar trend occurred at all four stations; an increase in cell density occurred with increasing length of substrate exposure. However, in this group of samples, stations 1 and 2 were more similar to each other than to stations 3 and 4. In addition, the range between mean station count values increased as exposure increased in duration, particularly after 67 days. As there were no

Figure 8. Changes in mean density or total cells/mm^2 of periphyton communities at each of the four sampling stations (a) over one month exposure periods where slide immersion durations ranged from 27 to 41 days (TS01, 05, 07, 09, 11) and (b) over increasing exposure durations of 33 to 135 days (TS01, 02, 03, 04). Mean "average growth rates" (c) are also given for each station over the increasing exposure durations.


CELLS/MM²/DAY
TOTAL NO CELLS/MM²
(c)
(b)
(a)
1
2
3
4
A
S
O
N
D
J

significant differences in measured physico-chemical variates between stations at this time, it may be concluded that species interaction and export factors such as grazing and sloughing (Castenholz 1960, 1961) were becoming of greater significance with increasing age of the developing periphyton communities, thus precipitating the recorded station differences in total count values. Kevern *et al.* (1966) also reported greater variation in periphyton standing crop biomass (g/m^2) determinations between slide replicates with increasing exposure periods up to 35 days in length (see also Patrick *et al.* 1954).

(iii) Estimates of periphyton growth or production

The rate of colonization (Weber & Raschke 1966), growth rate (Benson 1967), or accrual rates for attachment materials (Newcombe 1949, 1950) have generally been examined in relation to production estimates or productivity studies (Castenholz 1960; Sladeckova 1962, 1966; Sladecek & Sladeckova 1964; Grzenda & Ball 1968; Dor 1970). The wide variability in reported results from different water bodies is evidence of both the variety of sampling methods and the many different environmental factors which may affect recorded estimates, *e. g.*, trophic status of the water body, season, substrate type, duration of exposure or age of the periphyton community, *etc.* In this study, as in others employing the unmodified slide sampling method (Sladecek & Sladeckova 1964; Dickman 1969b), there were no reliable determinations of all export or reduction factors, hence increments in standing crop expressed in total cells/mm^2 serve as estimates of periphyton community growth or net *in situ* production. These values are equivalent to the average growth rate calculated by Kevern *et al.* (1966) and defined as the

standing crop at time t divided by the time elapsed between placement of the substrata and time t.

In Elk Lake, cell density appeared occasionally to be increasing linearly with corresponding increases in length of slide exposure, particularly at station 1 (Figure 8). Similarly, logarithmic plots of the entire data set for the study suggested neither consistent exponential or sigmoidal increases in total cell counts from different time series and stations. It was felt that the sampling durations were of too great a time interval to obtain representative and reliable estimates of true production rates. However, comparison of average growth rates (calculated after Kevern *et al.* 1966), as depicted in Figure 8, illustrate general trends. For example, the maximum average growth rates do not always correspond to the time series of maximum total count values and this again illustrates, that with increasing age of the communities, losses due to reduction factors tend to be greater than increments in attachment or community growth. In those samples illustrated in Figure 8, maximum average growth rates occurred at stations 1, 2, and 3 (*i.e.*, 14.1, 16.2, and 11.6 cells/mm^2/day, respectively) after 67 days exposure between August 3 and October 9; at station 4, a maximum average growth rate of 16.5 cells/mm^2/day occurred after 33 days exposure between August 3 and September 5. At stations 2 and 3, the maximum average growth rate for all exposure periods occurred on slides of TS07 immersed 41 days between October 9 and November 19, while at station 1, the maximum occurred in TS06 after 75 days immersion between September and November, and at station 4 in TS01 as discussed. Sharp declines in average growth rates occurred in all those samples exposed from November 19 on.

Whereas some workers employing artificial substrata to estimate production have assumed the accumulation or growth rate of *Aufwuchs* communities to be linear (Newcombe 1949, 1950; Castenholz 1960; Sladeckova 1962), it appears reasonable to expect linear growth only under certain periods of initial development (King & Ball 1966; Kevern *et al.* 1966). Weber and Raschke (1966), investigating periphyton slide communities in the Ohio River in the fall interval between October and November, sampled vertical slides at increasing intervals of 1, 4, 7, 15 and 32 days exposure. They found that following a brief lag during the first few days of exposure, the number of diatom cells on vertical glass slides increased exponentially, reaching a maximum of 15000/mm^2 in 32 days. Since plankton diatom counts near the sampler remained constant over the same time interval, they concluded that the rapid increase on slides indicated that colonization resulted primarily from the division of cells which had become attached during the lag phase. They suggested that a linear increase in attached diatom cells would have resulted if the colonization of slides resulted principally from the gradual deposition of drifting cells. Because there was a decline in the growth rate at 32 days, they assumed the diatom populations were established within 15 days exposure. Similarly, King and Ball (1966) found river *Aufwuchs* production stabilized, after a near constant arithmetic rate of increase, at about 15 days when the new growth equaled the organic matter sloughed off.

Intuitively, considering the complex effects of environmental conditions and the factor of a finite substrate area, it seems more reasonable to assume that growth is sigmoid, or at least not entirely linear, and that it reaches an asymptotic stabilization value in time

(Benson 1967; McIntire & Phinney 1965) where growth renewal or increments in standing crop are equal to export losses due to reduction factors (Neal *et al.* 1967; McIntire 1968b), especially since available substrate area and species interaction become of increasing importance with age of the developing communities. Increments in periphyton/unit area/unit time represent the net sum of both increments in settled material and increments in growth of the communities, as well as increment losses in organic matter due to reduction factors. Although some variability occurred between stations, Elk Lake periphyton communities tended to reach maximum average growth rates or "equilibria" between production and reduction factors after exposure durations of the following lengths; (1) after 67 days, between August 3 and October 9; (2) after 75 days, between September 5 and November 19; (3) after 41 days, between October 9 and December 16; and (4) after 27 days, between November 19 and January 19. In general, periphyton production factors appeared predominant prior to the November sampling date; subsequently, lower maximum growth rates were attained indicating the possible effects of overturn, decreasing light and temperature, and seasonally poor growing conditions (*i.e.*, reduction factors were predominant).

(b) Species diversity

Since the structure or organization of any multispecific assemblage of organisms is dependent on total numbers of species and individuals, as well as on the relative abundance of the various component species, the variation in community structure as a function of environmental factors represents a more complete and meaningful index of response of the entire system than do estimates of standing crop or total cell numbers alone. Mathematical expressions of structure, such as diversity

indices, facilitate objective comparisons of variations in structure and permit quantitative summarization of data. Their use thereby obviates the alternative prospect of examining each individual constituent species population in relation to environmental change, a formidable operation, the results of which are extremely complex and difficult to interpret meaningfully.

Because the structure of lotic *Aufwuchs* communities has been found sensitive to environmental conditions (*e.g.*, Butcher 1932; Patrick *et al.* 1954; Hohn & Hellerman 1963), and diversity indices have been reported useful in describing these biotic responses (Patrick 1967, 1968; McIntire *et al.* 1969), it was felt species diversity might similarly reflect periphyton responses to small fluctuations in measured physico-chemical variables between sampling stations and exposure periods in Elk Lake.

(i) Variation by site and exposure period: statistical analysis

Species diversity[1] values (H") for the periphyton communities were compared between stations and time series in the same manner as were the physico-chemical variates and total numbers of individuals. As shown in Table 7, there were significant differences in species diversity between stations and between exposure periods. The significant interaction,

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[1] Although diversity is dimensionless, in that units of measurement cancel, it is not dimensionless in the sense that while H" (or H') will be the same whether counts are per mm^2 or per cm^2, H will differ, since H is dependent on N. I have therefore expressed diversity as diversity (in bits)/individual cell/mm^2.

Table 7. Results of a two-way classification analysis of variance (with four slide replicates) used to test the differences between stations and between slide exposure periods in Elk Lake periphyton species diversity, H".

SOURCE OF VARIATION	df	SS	MS	F
A stations	3	0.8654	0.2884	41.6947**
B time series	10	5.4553	0.5455	78.8437**
FIRST ORDER INTERACTION				
A x B	30	8.4587	0.2819	40.7503**
Within (Error)	132	0.9133	0.0069	
TOTAL	175	15.6928		

** Significant at $P < 0.01$.

stations x time series, indicates that fluctuations in species diversity over the 11 exposure periods varied with station location (Fig. 9). Since species diversity was dependent on station location and period of exposure, and the pattern in species diversity over time series was not consistent between stations, data could not be pooled. Significant differences between the mean species diversity values for each time series are illustrated in Table 8 for the four different stations.

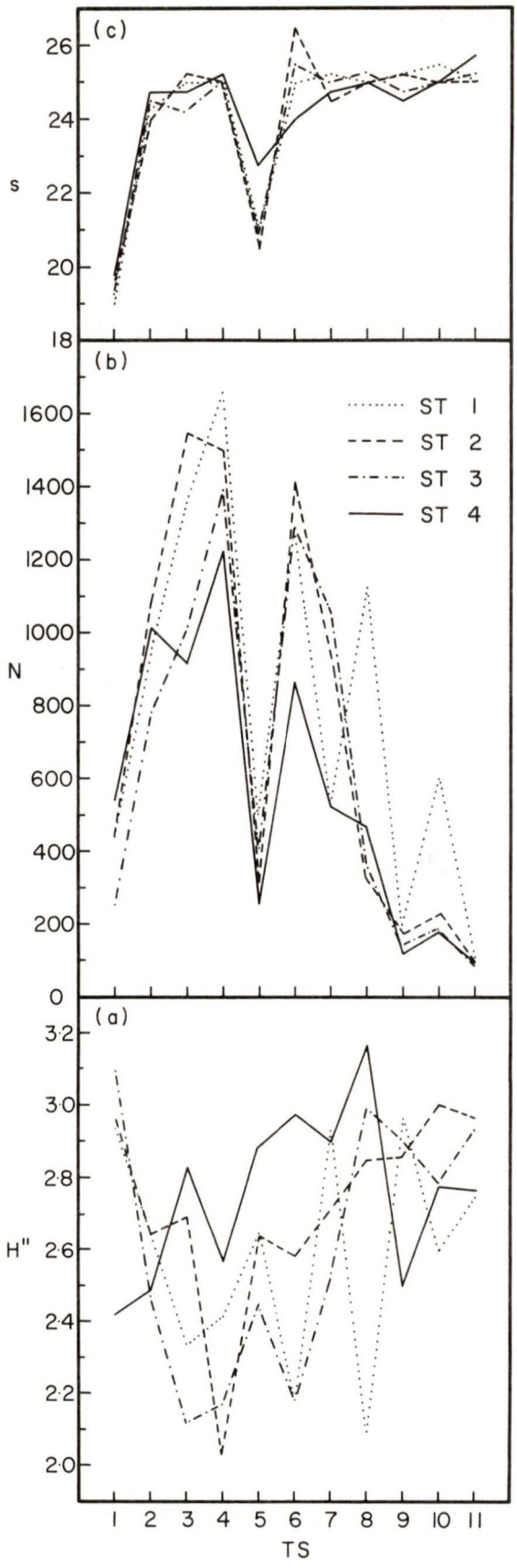
These results may be compared with those comparable analyses of the physico-chemical data (Tables 2 and 3) and the count data for 29 periphyton taxa (Tables 5 and 6). It is shown that while stations were similar in measured physico-chemical features and species composition, they were significantly different in cell numbers (and structural units or organisms) and species diversity. Hence station differences in biological variates cannot be statistically attributed to concurrent variation in measured environmental variables between the four stations. However, within any one station, significant fluctuations occurred in periphyton counts, diversity values, and physico-chemical variables over the 11 time series or six sampling dates (Tables 3, 5, and 8), so that there remains the possibility that the variation in numbers of cells/mm^2 and H" over the study period may be related to measured environmental change. The relationship however, is not readily interpretable at this stage of the analysis. No obvious, consistent pattern occurs in either the ranked order of the various mean values, or in the grouping of these means by significance of their differences.

A plot of the interaction, station x time series, for total cells, number of species, and species diversity H" (Fig. 9) illustrates the variation between stations at each TS, irrespective of its duration in

Table 8. Summary of statistical tests illustrating the differences in periphyton species diversity between the 11 different slide exposure periods for each of the four periphyton sampling stations. Data given here are mean values of H" in bits/individual/mm^2; those means scored by the same solid line are not significantly different at $P < 0.01$ as tested by D.N.M.R.T.

TS	STATION 1	TS	STATION 2	TS	STATION 3	TS	STATION 4
08	2.0957	04	2.0254	03	2.1225	01	2.4222
06	2.1781	06	2.5805	04	2.1698	02	2.4955
03	2.3352	02	2.6389	06	2.1911	09	2.5130
04	2.4240	05	2.6423	05	2.4472	04	2.5694
10	2.6036	03	2.6935	02	2.4699	11	2.7664
05	2.6520	07	2.7120	07	2.5338	10	2.7799
02	2.6526	08	2.8470	10	2.7882	03	2.8301
11	2.7479	09	2.8555	09	2.9042	05	2.8925
07	2.9423	01	2.9563	11	2.9350	07	2.9008
01	2.9444	11	2.9692	08	2.9940	06	2.9752
09	2.9680	10	2.9950	01	3.0982	08	3.1669

Figure 9. Graphs illustrating the interaction, stations x time series (TS), for (a) species diversity, (b) total cells, and (c) species numbers.


(c)
S
26
24
22
20
18
(b)
ST 1
ST 2
ST 3
ST 4
N
1600
1400
1200
1000
800
600
400
200
0
(a)
H''
3·2
3·0
2·8
2·6
2·4
2·2
2·0
1 2 3 4 5 6 7 8 9 10 11
TS

number of days. Whereas there appears to be a general trend of decreasing cell numbers at all stations from August to January, the relationship with species diversity is less clear; it is not always consistent between stations, but in general species diversity appears to be increasing over the comparable time series from August to January. When species numbers are plotted in the same manner, there is very little variability between all four stations, and the pattern of their distributions is closely related to that of the cell counts, except that after TS07 the number of species, s, does not decrease as does N but remains relatively stable at approximately 25 species (the maximum number of s counted from any one individual slide was 27). Similar plots of E, equally abundant species and J" show the same pattern in fluctuation as H" at each station. Further examination of Figure 9 suggests that species diversity decreases as cell numbers increase and this is best demonstrated by the samples of TS01 to TS04 at stations 1, 3, and 2. Although the relationship is not always consistent, subsequent analyses (Table 15) showed that there was a significant decrease in H" with an increase in N or total cells for stations 1 ($R = -0.784$, $P < 0.01$), 2 ($R = -0.731$, $P < 0.05$) and 3 ($R = -0.865$, $P < 0.01$); while at station 4 there was a very weak but also negative correlation ($R = -0.152$, not significant at $P < 0.05$).

While correlation is discussed in greater detail in subsequent sections, it is necessary to point out here that the negative relationship expressed between decreasing species diversity and increasing total cells or individuals[1] may be due, in part, to the concurrent positive

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[1] Unlike H (equation (1)), the value of the index H" is not dependent on the size of N, but rather on the ratios N_i/N and s.

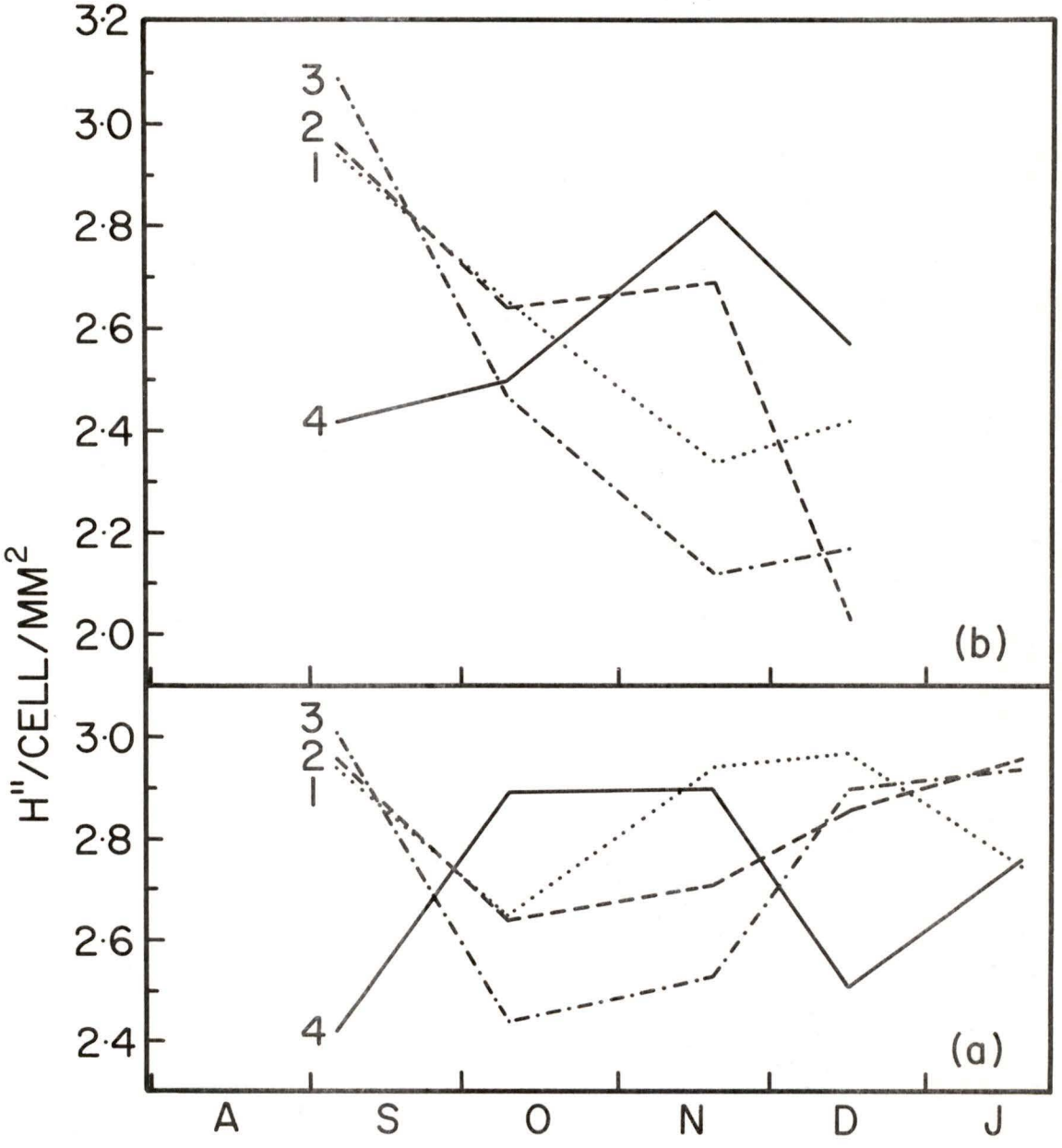
correlation between increasing N and increasing age of the developing communities (Fig. 8), since H" is also negatively related to length of slide exposure (Fig. 10). However, in these communities, decreasing species diversity with increasing N is commensurate with the concept of limiting factors. As numbers of individuals increase, species interaction intensifies (Margalef 1958) so that competition for a finite area of substrate surface results in an increase in individuals of some species and a consequent decrease or elimination of individuals of those species less able to compete.

Over the entire sampling program, H" values ranged between 2 and 3 bits/cell/mm^2 at all four stations. This range in variation was small but not unusual (Dickman 1969b; Ewing & Dorris 1970). Margalef (1967) reported an upper bound of about 4.5 bits/individual for most aquatic populations. Earlier he (Margalef 1964) reported ranges of 1-2 bits/individual cell for eutrophic lakes and 3-4 bits/individual cell for dystrophic and oligotrophic lakes based on published phytoplankton count data. While the range in magnitude of diversity values may be of interest to ecologists in general, it is not the actual values of H" or any other diversity index which are of prime importance here, but rather the variation and change in diversity with respect to other variables.

(ii) Variation by site over similar exposure periods

Compared with total cell fluctuations (Fig. 8), there was considerable variability in mean species diversity between the four stations over the one-month exposure periods (Fig. 10). Only stations 2 and 3 had similar patterns of change over the August to December period; while

Figure 10. Changes in mean diversity/individual cell/mm^2 for periphyton communities at each of the four sampling stations (a) over one month exposure periods where slide immersion durations ranged from 27 to 41 days (TS01, 05, 07, 09, 11) and (b) over increasing exposure durations of 33 to 135 days (TS01, 02, 03, 04).


3·2
3·0
2·8
2·6
2·4
2·2
2·0
3
2
1
4
(b)
3·0
2·8
2·6
2·4
3
2
1
4
(a)
H''/CELL/MM²
A
S
O
N
D
J

station 4 variation in H" was exactly opposite. In these samples of approximately one month's duration, at all stations H" increased over the TS07, 41 day, interval between October 9 and November 19, during which time overturn occurred and total cells reached maxima, suggesting that diversity does not always decrease as standing crop increases. Despite differences in length of exposure and cell density, the mean H" values for station 4 remained fairly constant over the TS05 and TS07 time series, indicating that in this instance community structure remianed stable while both age and total number of individuals underwent change. Whereas an overall increase in species diversity with the advent of winter was suggested by Figure 9, it is not clearly shown in Figure 10. However, for three of the stations, the slope of change in diversity between December and January is positive. An increase in diversity of periphyton communities from TS09 and TS11, exposed after overturn, was expected since complete thermal mixing provides opportunity for colonization by "new" species introduced from the entire water column rather than from the epilimnion only. Similarly, increased run-off, inflow and flood conditions may also introduce "new" colonizing species from O'Donnel Creek and swollen drainage ditches.

While no comparable data are available for periphyton, Margalef (1967, 1968) reported that maximum diversity of the entire plankton population generally occurs in summer when that of component populations or taxonomic groups such as diatoms or net phytoplankton is generally at a seasonal minimum. Since the count data in this study represents essentially attached diatoms only, then a summer low in species diversity might be quite reasonable (see Figures 9 and 10). Ewing and Dorris (1970) found summer maxima in pond phytoplankton and Goldman *et al.* (1968)

found phytoplankton diversity/individual decreased between August 18 and October 6 in Lake Maggiore (Italy). In the same lake at more than three depths (to a maximum depth of 30 m), species diversity values showed a significant positive correlation with silicate concentrations over the entire study period from May 25 to December 7, and significant negative correlations with blue-green biomass and light during the spring and summer.

In the samples of increasing exposure durations of 33, 67, 108 and 135 days, mean diversity/individual cell decreased between August and December at stations 1, 2 and 3, while station 4 values were again generally opposite in trend. In these samples, stations 1 and 3 appeared more similar in their changes; however, visual comparison of Figures 8 and 10 shows no consistent pattern of station grouping in total cells and diversity/individual over exposure periods. A decrease in diversity over increasing time intervals of this length is expected in view of the concurrent increase in total cells, since competition between species and limiting substrate area reduce diversity (Margalef 1958, 1967; MacArthur 1965). Substituting volume ratios, V_i/V, for N_i/N in Shannon-Weaver's formula (3), Dickman (1969b) found mean diversity of periphyton communities increased on vertical slides exposed for increasing intervals, reaching a maximum after 21 days exposure in Lago Banolas (Spain). Diversity dropped sharply after the fourth week, an exposure duration comparable to the period of shortest duration or 27 days, used in Elk Lake.

(iii) Components of diversity: evenness and species numbers

Diversity embodies two components (Pielou 1969; Sager & Hasler 1969) the number of species and evenness. A collection is said to have high diversity if it has many species and their abundances are fairly even (absolute evenness is seldom attained in biological samples, MacArthur 1965), or conversely, low diversity when the species are few and their abundances are uneven. Hence values of H", calculated for the periphyton, were dependent on s and evenness; this relationship is discussed here in light of the study aims and computed H" values.

While the number of periphyton taxa, s, per slide ranged from 18 in 453.54 individuals or cells/mm^2 (station 1) to 27 in 1440.94 individuals/mm^2 (station 2), the number of equally common species for communities from all stations ranged from 3.86 to 9.04. This wide disparity between values of s and E is illustrated by a TS01 slide sample of station 1 (Table 9) where 7.61 E equally abundant species would have given the same diversity, H" = 2.9284, as the s or 18 unequally common periphyton species observed in the counting procedure. This slide is illustrative of all slide samples from Elk Lake. The large differences between the observed number of species (s) in the slide samples and the calculated numbers of equally common (E) species indicate that N_i/N ratios for Elk Lake periphyton species were far from being even. Since, as will be discussed, four periphyton taxa accounted for 66.00% to 91.60% of the total cell counts in all vertical slide samples, a large number of the observed species of Elk Lake were represented by few individuals, *i.e.*, were rare. In the example cited (Table 9), slide 1 of station 1 TS01, nine species had a relative abundance or $N_i/N < 1\%$ of the total cell count for the 18 observed s and

Table 9. Example data from station 1 periphyton slide samples illustrating the differences between observed s and computed E values, and the simultaneous change in H''_{MAX} and J" values. (Each row represents one slide only.)

TS	S	E	N	H"	H''_{MAX}	J"
01	18	7.61	453.54	2.9284	4.1699	0.7022
02	24	6.10	950.42	2.6098	4.5849	0.5692
03	25	5.01	1381.32	2.3269	4.6438	0.5010
04	25	5.38	1692.86	2.4295	4.6438	0.5231
05	21	6.09	530.54	2.6072	4.3923	0.5935
06	25	4.51	1302.73	2.1757	4.6438	0.4685
07	25	7.40	556.61	2.8893	4.6438	0.6221
08	25	4.21	1136.61	2.0758	4.6438	0.4470
09	25	7.02	233.69	2.8125	4.6438	0.6056
10	26	5.64	490.15	2.4974	4.7004	0.5313
11	25	6.96	85.08	2.8001	4.6438	0.6029

together these nine species represented only 5.37% of the total numbers of cells, N. In this same slide, three species, *Cocconeis placentula*, *Fragilaria crotonensis*, and *F. virescens* each had N_i/N values of 20-24% of the total and together their individual cells represented 67.48% of the total N.

McIntire and Wulff (1969) also found observed s values much greater than computed E values in periphyton communities of marine diatoms. They attributed this difference to the presence of many rare species represented in the cell counts by only one specimen, and cited an example where 26 of 65 counted species (40%) were represented by only one cell each. Their examination showed that many of these rare individual diatom frustules were dead, washed in from a freshwater river inflow. Hence, they concluded that Shannon-Weaver's formula for species diversity was appropriate for their data, since it deemphasizes the importance of rare species, while those species with relative abundances of about 37% contribute the most to the magnitude of computed values of H" (see Shannon-Weaver 1964; Wilhm 1968).

However, I cannot agree that these conclusions apply to the Elk Lake data. Firstly, the distribution of relative abundances found in these periphyton communities is representative of conditions found in eutrophic lakes, *i.e.*, few species with many individuals vs. many species having few individuals per species in dystrophic or oligotrophic lakes as shown by Margalef (1964) using Brillouin's equation (1). The dominance in cell number of one or a few species in the periphyton of an enriched lake may be equated with similar conditions in the plankton, or in phytoplankton blooms (Margalef 1967) and is indicative of a successional stage in community development or species interaction and internal

correlations in response to environmental stimuli (Margalef 1958). Secondly, it has been argued in terms of general ecosystem theory that non-living biomass may be as important as living material in organization of ecosystems (Margalef 1968; Patt & Subba Rao 1970), and therefore perhaps the significance of dead frustules in the marine periphyton communities of McIntire and Wulff's (1969) study should not have been deemphasized. For example, aside from any contribution to the recycling of nutrients, the occurrence of a dead diatom frustule on a periphytic substrate[1] must influence colonization and induce competition for available substrate area thereby effecting community structure which should, in turn, be reflected in indices of diversity if they are to be representative of real ecological phenomena.

As mentioned previously, evenness or J" follows the same pattern as H" (*i.e.*, J" $\propto$ H") but not that of s shown in the same graph (Figure 9). The change in s appears to have little affect on species diversity values computed as H" and this may be further illustrated by the station 4 slides samples for the entire study, where s varied from 19 to 26, while $\log_2$ s varied from 4.2479 to 4.7004; a range of 7 species but a very small change in $H"_{MAX}$. Similarly, these 7 species were "rare" species so that their N_i/N ratios were also small. As depicted in Figure 11, H" is positively correlated with both evenness and the number of species. However, the change in evenness has a greater effect on H", since H" is significantly correlated with J" ($R = 0.9671$ at $P < 0.01$) but not with s ($R = 0.3415$ at $P > 0.05$), and J" is not

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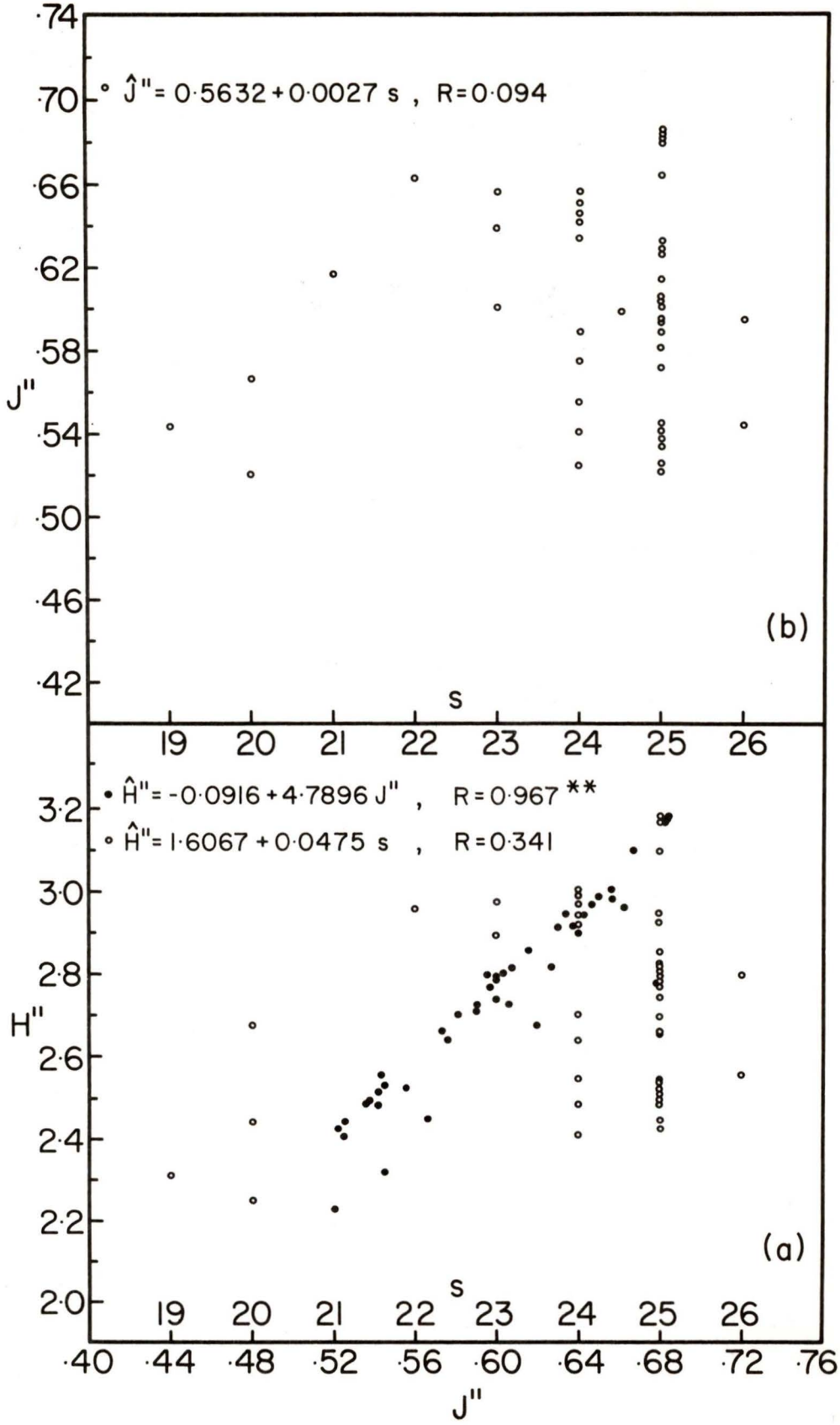
[1] Patrick *et al.* (1954) reported that dead diatoms are rarely found on glass slides since dead frustules do not remain attached; similar observations were made in the present study.

Figure 11. Example data for station 4 periphyton slide communities illustrating the relationship between species diversity (H") and the two components of diversity, species richness (s) and evenness or equitability (J") (**, significant at $P < 0.01$).


° Ĵ" = 0·5632 + 0·0027 s , R = 0·094
J"
·74
·70
·66
·62
·58
·54
·50
·46
·42
(b)
s
19
20
21
22
23
24
25
26
• Ĥ" = -0·0916 + 4·7896 J" , R = 0·967 **
° Ĥ" = 1·6067 + 0·0475 s , R = 0·341
H"
3·2
3·0
2·8
2·6
2·4
2·2
2·0
(a)
s
19
20
21
22
23
24
25
26
·40
·44
·48
·52
·56
·60
·64
·68
·72
·76
J"

significantly correlated with s (R = 0.0941 at P > 0.05). Station 4 samples are typical for all slide data, where similar relationships were also demonstrated. The insignificant correlation between J" and s may be attributed to the minor effect of the rare species on evenness and also to the limited range in values of s between the slide samples of different time series. As also found by Sager and Hasler (1969), samples with a large or even moderate range in numbers of species have a relatively narrow range in values of J"

Others have discovered similar problems with the interpretation and application of H" diversity values (Dickman 1968a, 1968c; Wilhm 1968; Sager & Hasler 1969) and although modifications to the index have been devised, none have solved the problem of rare species (or those species with N_i/N ratios in excess of 37%) which make little contribution to the H" index values calculated. Recently, Sager and Hasler (1969) computed H" values (using both numerical and volumetric estimates of P_i) for phytoplankton communities of three extremely different Wisconsin lakes and found that the wide variation in values of Shannon-Weaver's index between the lakes was largely attributable to evenness or equitability as expressed in the 10-15 most abundant species and not due to the variability in s, *i.e.*, phytoplankton species in excess of the 10-15 most abundant ones (maximum s = 40) had little effect on the value of H".

Hence, as demonstrated here and by Sager and Hasler (1969), as well as others (Dickman 1968a; Margalef 1967), the index H" is insensitive to the contribution to diversity made by rare species. Sager and Hasler (1969) suggest that because of this characteristic, the incomplete censuses provided by most plankton sampling methods (as well as periphyton

sampling methods) incurs small error (see Pielou 1966c 1967, 1969) provided some critical number of species is exceeded in the samples. In addition, since the counting error incurred by rare species is generally larger than that for more abundant taxa (Brown & Austin 1971; Appendix II) it may also be argued that the lack of sensitivity to rare species is adequate reason alone for using H".

However, in spite of these considerations, the relevance of the contribution of rare species to the Elk Lake periphyton communities cannot be assessed on the basis of Shannon-Weaver's diversity index H" (or H'). In this context the index H" has not adequately summarized the count data and can be said to have arbitrarily weighted those species of greater relative abundance so that in many slide samples less than one half the species have been taken into account and truly represented. Since these "rare" species constitute a very real part of each Elk Lake periphyton sample (in addition to phytoplankton samples; Sager & Hasler 1969) they likewise must make a significant contribution to the organization of these communities which are subject to and reflect modification by environmental factors within the lake. Hence, species diversity, as measured here by H", has indicated that in terms of evenness, H" is negatively correlated with N and exposure duration. This supports the contention that with increasing exposure duration and simultaneous increases in periphyton cells, competition and species interaction become increasingly important, so that diversity decreases. In addition, the H" values computed for the Elk Lake periphyton compare well with other species diversity values computed from phytoplankton samples of eutrophic lakes (Margalef 1964; Sager & Hasler 1969). However, the relationship between diversity and measured physico-chemical variables

is not clear; the change in periphyton communities with measured environmental factors within the lake is also not clear and, if anything, is more complex than indicated by standing crop or total cells. Indeed, it is only the lack of evenness demonstrated quantitatively by the diversity indices, which is a clearly representative characteristic of the Elk Lake periphyton, a feature typical of nutrient enriched lakes where few species are generally dominant.

The Shannon-Weaver index and its relation to physico-chemical variates is discussed in more detail in a subsequent section on correlation.

(c) Individual species populations

To effect brevity and simplicity, only data for the most abundant individual periphyton taxa are presented here. The count data of other, less abundant species, such as the diatom *Asterionella formosa*, will also be included in discussions illustrating the possible relationships between plankton and periphyton communities.

Throughout the Elk Lake study, the most abundant periphyton taxa by cell number, in decreasing order of abundance, were the following diatom species: *Achnanthes minutissima* (having the greatest number of cells per sq mm of slide surface area in 17 of 44 possible samples), *Fragilaria crotonensis* (11/44), *Fragilaria virescens* (9/44), and *Cocconeis placentula* (7/44). Each of these taxa had a constancy value of 100% and together the four species had a total percentage abundance by cell number of 66.0 to 91.6%.

Patrick (1968) has found that under identical slide exposure periods and controlled, similar ecological conditions, very similar diatom communities develop with little variation in structure and near

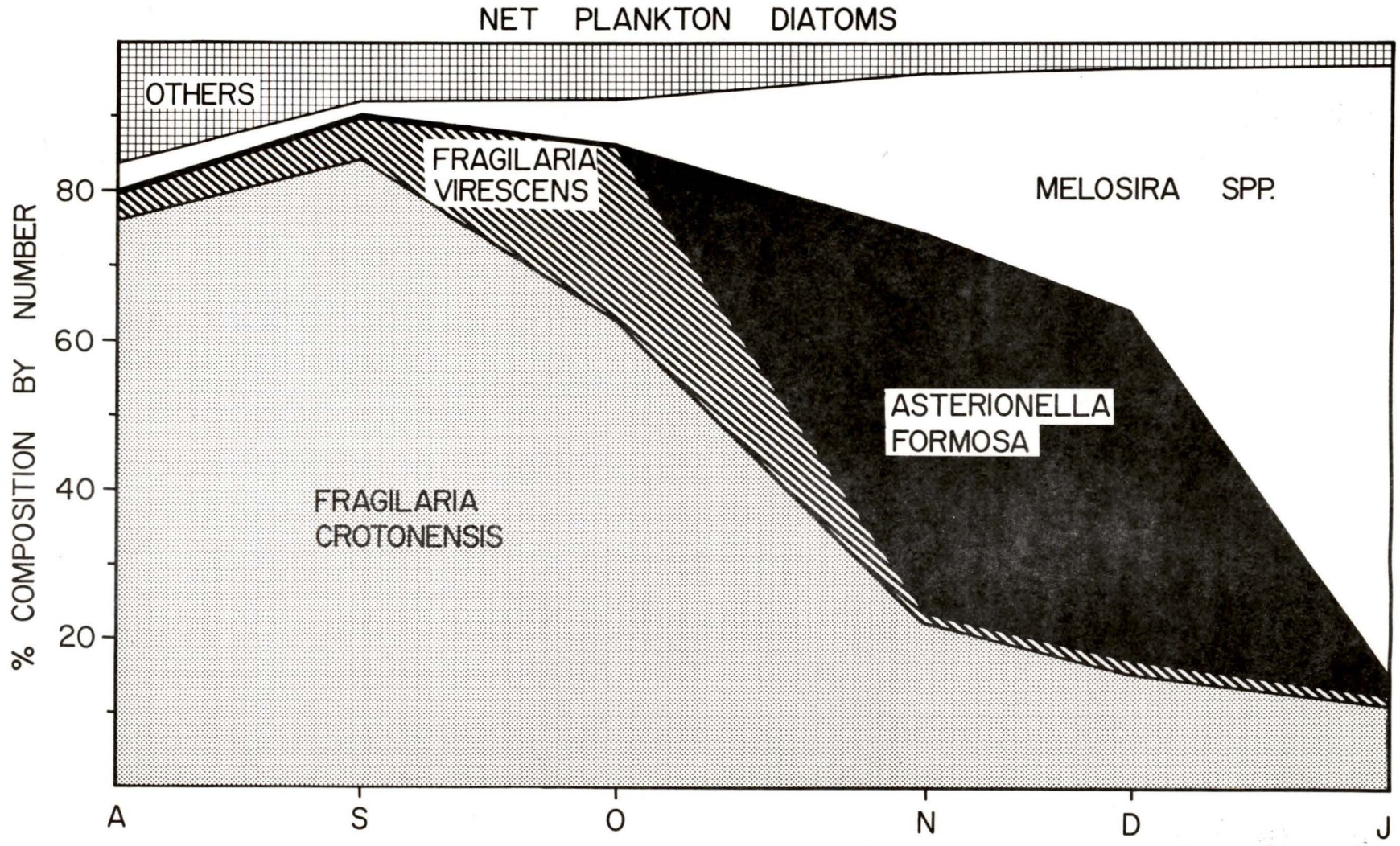
steady-state features. The lack of variation in structure was largely attributed to the occurrence of species having high percentage abundance values which are common to different communities. Thus the Elk Lake data are not unusual with respect to the large number of constants, and the four species whose individuals represent greater than 50% of total cell numbers in all samples, particularly in view of the similarities shown in physico-chemical features at the four sampling stations.

The one faunal component of the enumerated Elk Lake periphyton, *Difflugia* sp., had a constancy value of 100%, occurring in all samples, but always represented less than 1% of the total standing crop (in cell numbers) of any slide sample. This species did not appear to be an important grazer and numbers were not obviously related to any measured environmental variables.

(i) Succession and interaction between net plankton and periphyton

The interaction between phytoplankton and benthic algal associations has been the subject of a number of investigations (*e.g.*, Lund 1949, 1954, 1955; Round 1964; Moss 1969a, 1969c). However, little direct examination of comparable interaction with periphyton communities has ensued (Sladeckova 1966; Weber & Raschke 1966), eventhough Castenholz suggested as early as 1961 that concurrent study of plankton and attached algal production might be of some benefit in characterizing the primary production of a lake. In Elk Lake, a number of diatom species were found common to both communities, hence fluctuations in planktonic species were examined (see Figure 12) to determine whether any relationship with the corresponding periphyton populations could be shown. These data, from Hyrax mounts of incinerated net plankton samples previously described

Figure 12. The seasonal succession of planktonic diatoms, prepared from Hyrax mounts of surface tow net samples, and expressed as percentage abundance of total diatom cells or specimens present. Percent values represent the mean of samples from the four sampling stations and letters indicate sampling dates.


NET PLANKTON DIATOMS
% COMPOSITION BY NUMBER
80
60
40
20
OTHERS
FRAGILARIA VIRESCENS
MELOSIRA SPP.
ASTERIONELLA FORMOSA
FRAGILARIA CROTONENSIS
A
S
O
N
D
J

(Brown 1969; Appendix I), illustrate the seasonal pattern of succession of the most abundant species populations constituting the diatom component of the plankton. Throughout this study, four species dominated the planktonic diatom assemblage and were also present in the periphyton: *Fragilaria crotonensis, F. virescens, Asterionella formosa,* and *Melosira* spp. (*M. italica* and *M. varians*).

Similarity between the sampling stations was strikingly illustrated by the fact that the seasonal pattern of succession of planktonic diatoms which occurred at each of the four stations was identical (<±5% difference). *F. crotonensis* was most abundant from August to October. The population reached a maximum percentage abundance in September, comprising 76% of the diatom specimens, and thereafter gradually decreased in numbers, representing a low of 11% of the total diatom population in January. *F. virescens* developed a small peak in percentage abundance in October, but never became a dominant population in the plankton. Cells of *A. formosa* accounted for less than 1% of the total number of diatom individuals from August to October, but increased sharply in importance in November (52% abundance). Comparably high numbers were maintained in December, but this species decreased in importance again in the January net tows. After steadily increasing from August onward, *Melosira* spp. became dominant in January with a maximum percentage abundance value of 82%.

This overall seasonal pattern in succession of planktonic diatoms is comparable to that documented for other lakes. *F. crotonensis,* apparently correlated with temperature (see Hutchinson 1967), generally reaches its peak development during high temperatures in summer months; populations decrease in the autumn, settling out after fall overturn. Similarly, when nitrate is depleted, *Fragilaria* may be out-competed

by *Anabaena circinalis* Raben. (Hutchinson 1944), or other blue-greens able to fix nitrogen. In the Elk Lake plankton, *F. crotonensis* reached its maximum percent abundance when high nitrate-nitrogen values were recorded, and its decline in importance coincided with sharp decreases in measured nitrate-nitrogen concentrations and the onset of isothermal conditions.[1] Following overturn, corresponding increases in the periphyton population of *F. crotonensis* occurred[2] (Figs. 13 and 14).

Seasonal fluctuations in *Melosira italica* (subsp. *subarctica* O. Müll.) have been found largely dependent on an inverse correlation with high light intensities, a high sinking rate, and an ability to live on bottom sediments in the dark or under anaerobic conditions (Lund 1954). Loss through the outflow during flood conditions may also be a modifying factor, while fungal parasitism and grazing are not considered of major importance (Lund 1955). Elk Lake data for *Melosira* spp. (Fig. 12) are not in disagreement with Lund's (1954, 1955, 1965) findings in English lakes where the loss of thermal stratification at fall overturn generally results in a sharp increase in plankton populations of *M. italica* subsp. *subarctica*. Lund attributed this increase

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[1] Similarly, Benson (1967) found *Fragilaria bidens* Heib. was the only periphyton species in Lake Washington whose decline in numbers correlated with nitrate depletion.

[2] Unlike Benson's (1967) findings, that cells of *A. formosa* and *F. crotonensis* present in the periphyton of Lake Washington were empty shells or had reduced and faded chromatophores, cells of these species occurring in the Elk Lake periphyton did not appear damaged and chromatophores retained their normal coloration.

to the resuspension of filaments by turbulent mixing currents from bottom sediments where they remained alive in a resting stage condition during stratification. Concomitant with this increase and reappearance of *Melosira* in the plankton at overturn, a sharp decrease occurs in the numbers of *Melosira* cells present on the bottom.

A comparable decrease in Elk Lake periphyton populations of *Melosira* spp. was not so clearly defined. Table 10 shows that *M. varians* reached its average maximum development in numbers of cells/mm^2 in TS01 and TS02 samples of the one, two, and four month sample series, decreasing gradually thereafter so that minima occurred after the November turnover. Numbers of *M. italica,* however, appeared to be more closely related to the length of slide exposure, did not decrease with overturn, and percent abundance was maximal after November in all but the four month series. It is reasonable to assume that not all those cells present in the periphyton during thermal stratification were released to the plankton at overturn because all exposure-frames were located within the epilimnion and were subjected to epilimnion mixing currents prior to the breakdown of thermal stratification. There is some question as to whether *Melosira* cells present on slides at the frame depths were actually in a resting stage condition as waters were well oxygenated at these depths, and frames were at depths well above the compensation point. Further, some *M. italica* filaments present in samples of TS06 at stations 2 and 3 were observed in a state of vegetative reproduction, and chromatophore conditions characteristic of the resting stage (Lund 1954, 1955) were not observed. It seems more probable that a major part of the planktonic increase in *Melosira* spp., especially *M. italica,* was due to an inoculuum of cells resuspended from bottom sediments of deeper hypolimnetic regions of the

Table 10. Change in mean cell numbers/mm^2 (4 slides/station) and mean percentage abundances (16 slides, all stations) of two diatom species, *Melosira italica* and *Melosira varians*, over the 11 different exposure periods at the four Elk Lake periphyton sampling stations.

	Melosira italica						*Melosira varians*						
	$\bar{X}$ cells/mm^2 (n=4)				$\bar{X}$ (n=16)		$\bar{X}$ cells/mm^2 (n=4)				$\bar{X}$ (n=16)		
TSST	1	2	3	4	cells/mm^2	%abundance	1	2	3	4	cells/mm^2	%abundance	INTERVAL DAYS
	A. "ONE MONTH" EXPOSURE PERIODS												
01	10.6	20.4	13.9	16.5	15.3	3.62	11.1	9.4	7.4	8.2	9.0	2.14	$A_3 - S_5$ 33
05	8.8	4.5	5.2	5.0	5.9	1.52	8.9	5.0	5.4	3.1	5.6	1.45	$S_5 - O_9$ 34
07	17.9	31.4	23.7	12.2	21.3	2.80	3.2	5.9	10.7	5.1	3.8	0.50	$O_9 - N_{19}$ 41
09	16.2	12.4	12.3	9.4	12.6	7.88	2.2	1.3	0.7	0.6	1.2	0.76	$N_{19} - D_{16}$ 27
11	5.6	7.7	7.6	10.6	7.9	7.99	0.4	0.5	0.5	0.4	0.5	0.48	$D_{16} - J_{19}$ 34
	B. "TWO MONTH" EXPOSURE PERIODS												
02	13.5	13.0	8.4	8.1	10.7	1.13	6.3	6.2	4.1	5.1	5.4	0.57	$A_3 - O_9$ 67
06	13.8	21.4	20.3	16.9	18.1	1.52	6.0	7.4	2.2	6.3	5.5	0.46	$S_5 - N_{19}$ 75
08	28.6	32.1	27.3	46.0	33.5	5.82	6.2	3.2	1.4	2.5	3.3	0.58	$O_9 - D_{16}$ 68
10	18.3	12.9	12.6	17.3	15.3	5.04	2.5	1.4	0.9	1.0	1.5	0.48	$N_{19} - J_{19}$ 61
	C. "FOUR MONTH" INCREASING EXPOSURE PERIODS												
01	10.6	20.4	13.9	16.5	15.3	3.62	11.1	9.4	7.4	8.2	9.0	2.14	$A_3 - S_5$ 33
02	13.5	13.0	8.4	8.1	10.7	1.13	6.3	6.2	4.1	5.1	5.4	0.57	$A_3 - O_9$ 67
03	21.6	26.4	12.3	17.6	19.5	1.61	5.5	7.2	2.3	4.5	4.9	0.40	$A_3 - N_{19}$ 108
04	59.5	35.1	37.9	32.0	41.1	3.17	4.4	4.1	3.8	4.0	4.1	0.31	$A_3 - D_{16}$ 135

lake, rather than from the frame depth periphyton communties.

The development of a fall pulse in planktonic populations of *Asterionella formosa*, a species which sinks at a slower rate than *M. italica* (Lund 1954), is not uncommon and has been well documented in English lakes (Lund 1949, 1950; Lund *et al.* 1963). Unlike the meroplanktonic species *M. italica*, *A. formosa* is holoplanktonic (Fogg 1965). Cells of the latter species are derived from sheltered bays or inflows; hence live cells are always present in the open water and able to increase in numbers if sufficient nutrients and light are available. Littoral and profundal deposits receive cells from the plankton but no resting stages have been observed and these deposits are not returned to the plankton. Hence periphyton communities in Elk Lake reflect the plankton fluctuations of *Asterionella*, and show an increase with overturn (Figs. 13 and 14). The decrease in the autumnal maximum of *A. formosa* in the plankton may be due to a number of factors, *e.g.*, decrease in light and temperature, loss to bottom sediments, loss through the outflow during flood conditions and chytrid parasitism (Canter & Lund 1949, 1951). All of these conditions were evident in Elk Lake at the time of the decline in the fall peak of planktonic *Asterionella*.

It is of interest to compare the pattern of species succession in Elk Lake planktonic diatom populations with comparable successional patterns of species illustrated in the periphyton communities. Although other species were also common, two diatom species, *F. crotonensis* and *F. virescens*, were important dominant populations in both communities, each of which was largely represented and dominated by only four species. Station differences in the successional pattern of the net plankton were essentially non-existent; in sharp contrast with station patterns in

Table 11. Successional patterns of dominant species in all slide samples expressed in mean numbers of cells/mm^2 and mean percentage abundance of the total cell populations where n=4 slides and figures are rounded correct to one decimal place. Four periphyton taxa are represented by abbreviations: *Cocconeis placentula*, *Achnanthes minutissima*, *Fragilaria crotonensis*, and *F. virescens*.

	STATION 1			STATION 2			STATION 3			STATION 4		
ST. TS	taxon	$\bar{X}$ cells/ mm^2±S.D.	$\bar{X}$ % abund.	taxon	$\bar{X}$ cells/ mm^2±S.D.	$\bar{X}$ % abund.	taxon	$\bar{X}$ cells/ mm^2±S.D.	$\bar{X}$ % abund.	taxon	$\bar{X}$ cells/ mm^2±S.D.	$\bar{X}$ % abund.
01	*C.p.*	107.9±5.5	24.2	*C.p.*	119.9±22.6	27.1	*F.v.*	73.3±3.5	28.7	*C.p.*	261.2±43.9	48.0
02	*A.m.*	327.0±9.6	34.7	*A.m.*	316.8±8.8	29.2	*A.m.*	329.4±18.4	42.3	*C.p.*	434.9±74.9	42.8
03	*A.m.*	690.1±14.9	50.7	*F.v.*	477.6±45.1	30.9	*A.m.*	570.9±25.9	56.7	*C.p.*	363.0±121.7	39.6
04	*A.m.*	749.5±11.6	45.1	*C.p.*	951.6±48.5	63.6	*A.m.*	817.0±27.9	58.7	*C.p.*	640.8±63.8	52.1
05	*A.m.*	194.2±22.8	38.7	*A.m.*	96.6±68.7	30.2	*A.m.*	178.8±56.6	44.2	*F.v.*	84.0±8.9	32.6
06	*A.m.*	712.6±12.9	55.5	*F.v.*	458.8±13.5	32.6	*A.m.*	621.0±11.1	48.2	*F.v.*	221.2±10.4	25.6
07	*A.m.*	159.1±19.9	30.0	*F.v.*	292.7±0.7	31.3	*F.v.*	448.8±23.8	42.4	*A.m.*	140.3±2.1	26.7
08	*A.m.*	719.8±17.4	63.4	*F.c.*	123.3±8.5	36.6	*A.m.*	90.3±9.2	24.6	*F.c.*	113.6±6.1	24.4
09	*F.c.*	68.0±20.4	34.6	*F.v.*	65.0±17.6	36.2	*F.v.*	45.8±9.7	32.6	*F.c.*	57.8±6.3	47.8
10	*A.m.*	288.4±10.7	48.1	*F.c.*	76.1±6.1	33.1	*F.c.*	83.6±19.1	45.0	*F.c.*	88.4±5.2	44.8
11	*F.c.*	42.7±6.16	43.4	*F.c.*	40.6±4.3	37.0	*F.c.*	34.3±2.7	40.0	*F.c.*	43.6±2.3	43.6

periphyton species succession over the 11 sampling intervals where considerable variation in dominant species populations occurred between the four stations (Table 11).

(ii) Succession and interaction within the periphyton

When the one-month periphyton samples (*i.e.*, TS01, 05, 07, 09, 11) where examined, the seasonal succession in percent abundance of the five taxa, *A. minutissima, F. crotonensis, F. virescens, C. placentula* and *Asterionella formosa,* was found to be reasonably similar at the four sampling stations, with some modification in pattern of the seasonal dominants (Figs. 13 and 14). *Cocconeis* decreased from a maximum percent abundance peak in the August-September samples at all stations, and remained steady at less than 10% abundance from November to January. *Fragilaria virescens* displayed a slightly different successional pattern between stations, but decreased in importance on all slide samples immersed for 34 days between December 16 and January 19. This species constituted a larger proportion of the total population in these periphyton samples than it did in the plankton over the same period. Periphyton populations of *F. crotonensis* also displayed an opposite pulse to counterpart plankton populations, as shown in Figures 13 and 14 where it steadily increased in importance, reaching a peak at all stations in January. These data reflect the fluctuations in planktonic populations (Fig. 12) and illustrate a settling of *F. crotonensis* cells from the plankton after autumnal overturn. The periphyton species population of *Achnanthes* was dominant at stations 1, 2, and 3 in the September-October samples, while it peaked later in November at station 4. The contribution of this population decreased in importance at all

Figure 13. Succession in dominant periphyton species on those slides of TS01, 05, 07, 09, and TS11, samples immersed for successive intervals of approximately one month's duration from August 3 to January 19 at stations 1 and 2 in Elk Lake. Plotted values are expressed as mean percentage abundances of total cell counts from four slides and letters indicate sampling dates.

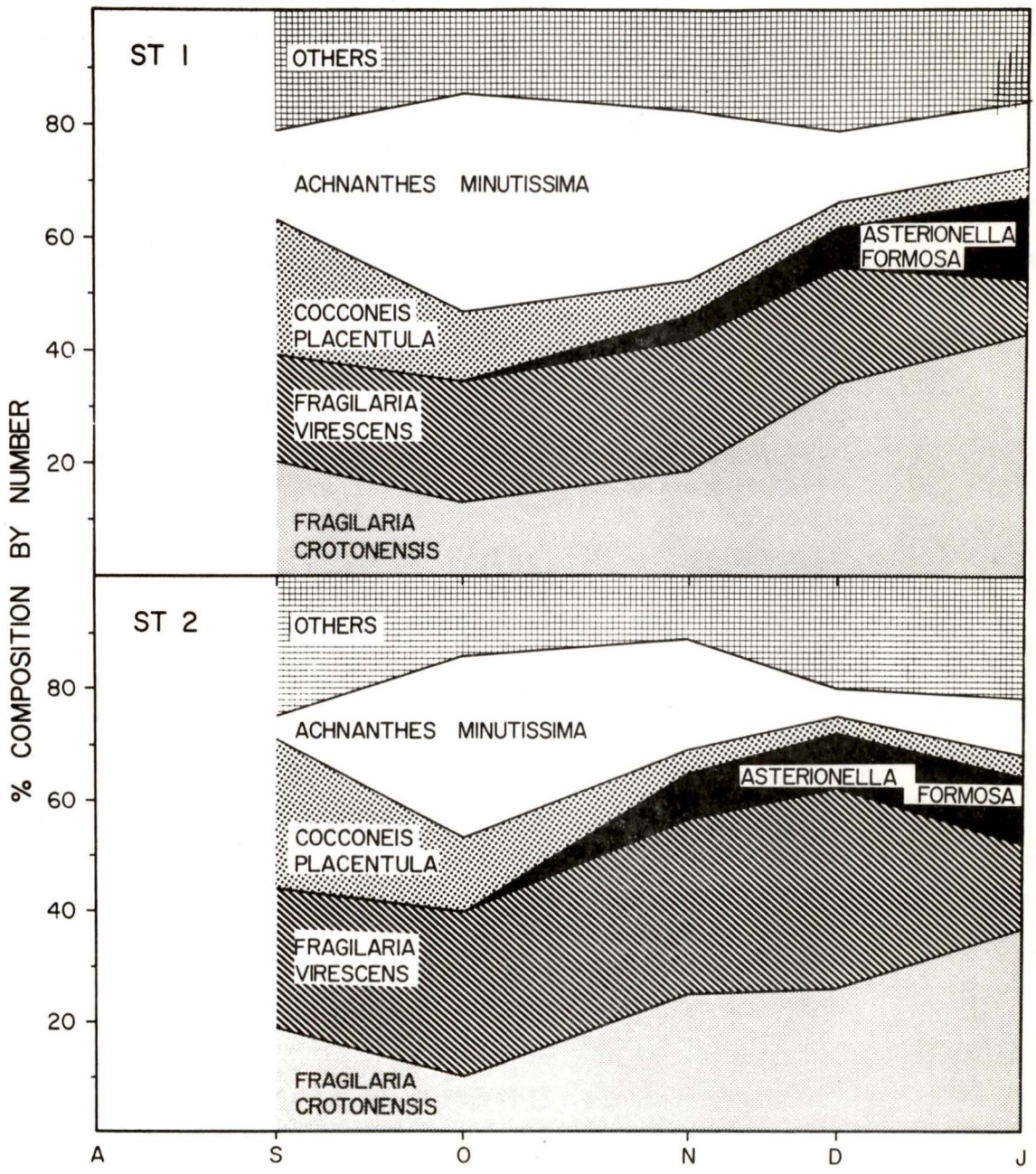
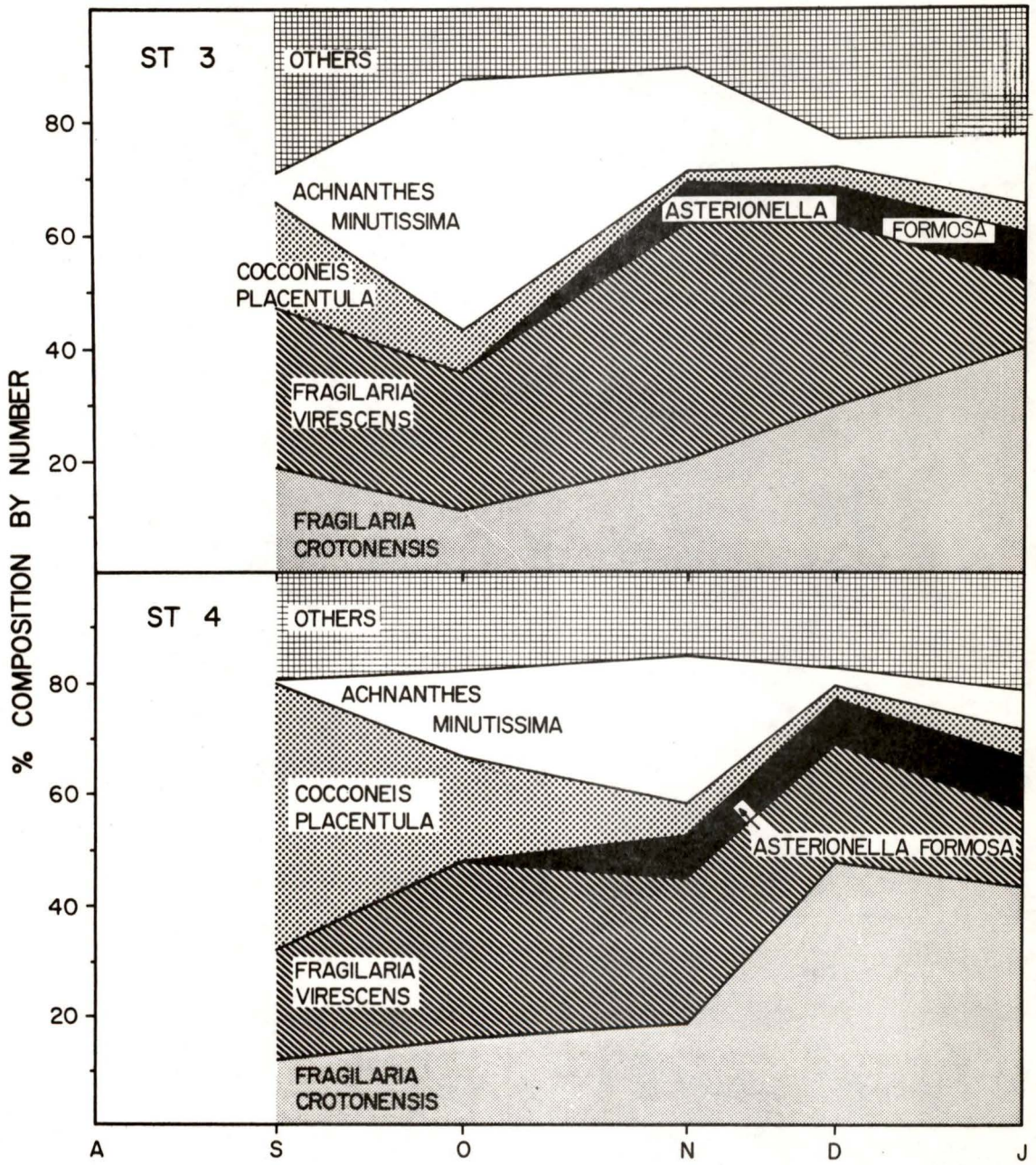

ST 1
OTHERS
ACHNANTHES MINUTISSIMA
ASTERIONELLA FORMOSA
COCCONEIS PLACENTULA
FRAGILARIA VIRESCENS
FRAGILARIA CROTONENSIS
ST 2
OTHERS
ACHNANTHES MINUTISSIMA
ASTERIONELLA FORMOSA
COCCONEIS PLACENTULA
FRAGILARIA VIRESCENS
FRAGILARIA CROTONENSIS
% COMPOSITION BY NUMBER
80
60
40
20
A
S
O
N
D
J

Figure 14. Succession in dominant periphyton species on those slides of TS01, 05, 07, 09, and TS11, samples immersed for successive intervals of approximately one month's duration from August 3 to January 19 at stations 3 and 4 in Elk Lake. Plotted values are expressed as mean percentage abundances of total cell counts from four slides and letters indicate sampling dates.


ST 3
OTHERS
ACHNANTHES
MINUTISSIMA
ASTERIONELLA
FORMOSA
COCCONEIS
PLACENTULA
FRAGILARIA
VIRESCENS
FRAGILARIA
CROTONENSIS
ST 4
OTHERS
ACHNANTHES
MINUTISSIMA
COCCONEIS
PLACENTULA
ASTERIONELLA FORMOSA
FRAGILARIA
VIRESCENS
FRAGILARIA
CROTONENSIS
% COMPOSITION BY NUMBER
80
60
40
20
A
S
O
N
D
J

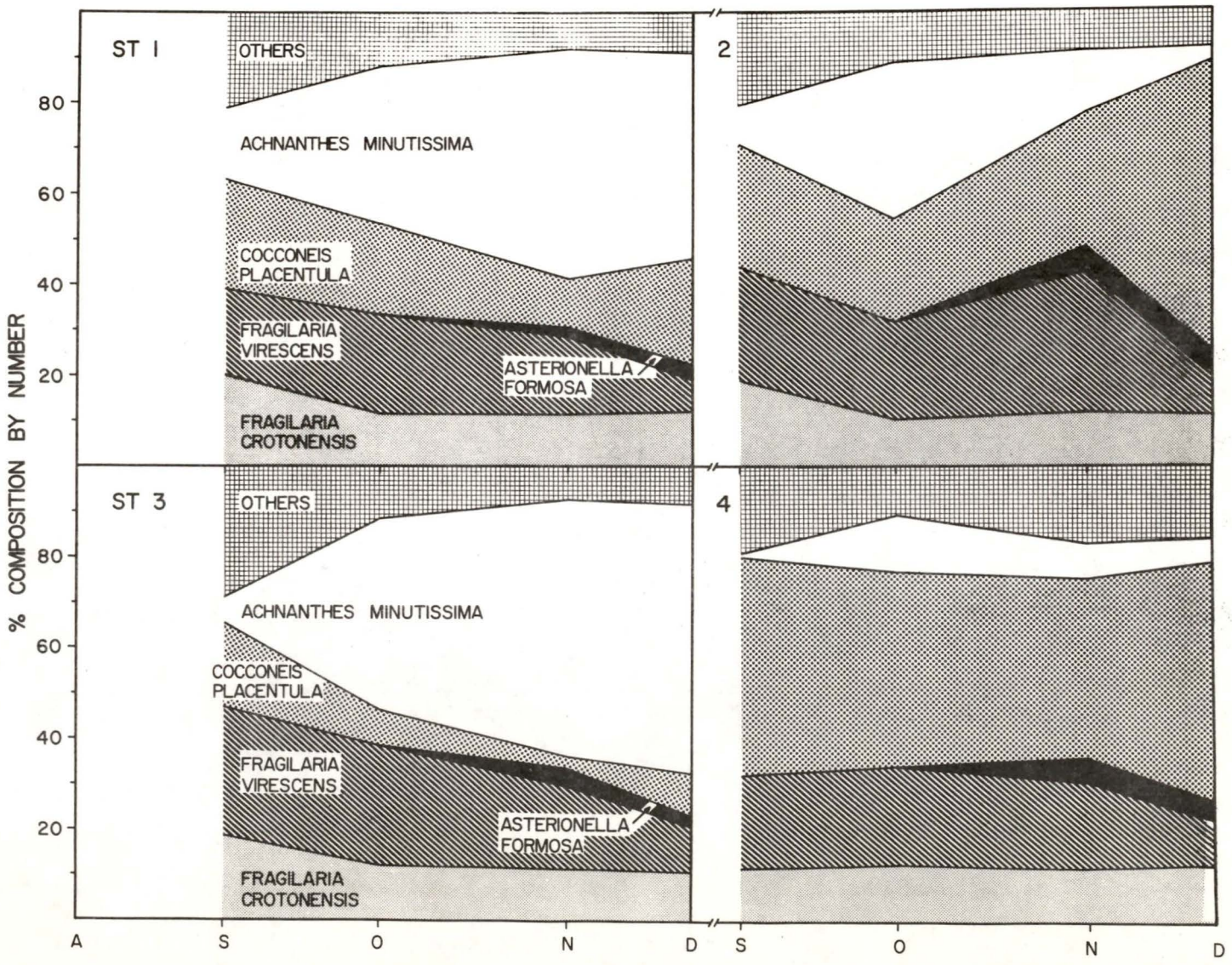
stations in the December and January (TS09 and TS11, respectively) samples. *Asterionella formosa* did not appear in periphyton samples until the November collection date, although it was present as early as August in the net plankton. The proportion of *Asterionella* cells on these slides increased slightly, representing a maximum amount of ~10% of the total periphyton population in January. These data suggest that not all cells of the planktonic *Asterionella* population settled out on the slides following its pulse in the plankton or the advent of complete thermal mixing. Possibly, some cells were lost to the outflow, Colquitz River, due to heavy precipitation and flood conditions.

In summary, the one-month immersion slide samples illustrate that with regard to the three species common to both net plankton and periphyton (as shown in Figs. 12, 13 and 14, and Table 10), a decrease in cell numbers and percent abundance in planktonic populations is partly reflected by an increase in importance in the periphyton populations. Settlement from the plankton, commencing with the breakdown of thermal stratification largely accounts for this inverse relationship, although in the case of *Melosira italica*, it is suggested that resuspension of cells from bottom sediments more likely leads to the increase in numbers and percentage importance in the plankton population. *Asterionella*, however, does not appear in the periphyton to the extent one might expect judging from its relative abundance in the net plankton. This may be due in part to high flush rates under flood conditions, and the slow sinking rate of *Asterionella* colonies. The more typical periphyton species, on the other hand, appear to be inversely related to one another and this might be a possible competition or "initial-colonization" effect, but ultimately in December and January, *Achnanthes* and *Cocconeis*

are represented by relatively equal, residual populations of small importance.

In light of the above findings, the successional pattern of species populations in other periphyton samples were also examined to enable comparisons and provide a wider basis for interpretation. The seasonal succession of the same five periphyton taxa in samples of TS01, 02, 03, and 04, immersed from 33 to 135 days between August and December (Fig. 15), differed somewhat from that expressed in the monthly samples. *F. crotonensis* did not increase in importance with the advent of winter as it did in the monthly samples; the population remained relatively stable, representing 10-20% of the total number of cells throughout the four month period. The successional pattern of *F. virescens* was, however, quite similar to that in the monthly samples, although here at station 3, it did not peak in November, and at all stations showed a decrease in percent abundance in December. *A. formosa* again appeared for the first time in the November samples and expressed an identical build up at all stations in both sample series, except comparatively, the populations were more reduced in importance in the four-month samples. In contrast, *A. minutissima* and *C. placentula*, displayed a much different successional pattern, as opposed to the corresponding monthly patterns. At stations 1 and 3 fluctuations in *Achnanthes* were quite similar. The latter species increased in importance as the period of slide exposure increased in length, reaching a November maximum percentage abundance at station 1 and a December maximum at station 3. Concurrently, at station 4, *Achnanthes* never became dominant, and dropped off as at station 2 where it reached a peak in October and then steadily declined in importance. Populations of *C. placentula* increased, as shown in Figure 15,

Figure 15. Succession of dominant Elk Lake periphyton species on those slides of TS01, 02, 03, and 04, samples immersed for increasing exposure durations of 33, 67, 108, and 135 days between August 3 and December 16 at the four sampling stations. Plotted values are expressed as mean percentage abundances of total cell counts from four slides and letters indicate sampling dates.


ST 1
OTHERS
ACHNANTHES MINUTISSIMA
COCCONEIS PLACENTULA
FRAGILARIA VIRESCENS
ASTERIONELLA FORMOSA
FRAGILARIA CROTONENSIS
2
ST 3
4
% COMPOSITION BY NUMBER
80
60
40
20
A
S
O
N
D

between November and December at all stations, differing sharply from the monthly samples where it decreased between November and December. In the four-month immersion series, stations 1 and 3 were more similar to each other with respect to the relative importance of *Cocconeis* where the percent contribution of the populations decreased between September and November, increasing again slightly in December. At stations 2 and 4, cells of *Cocconeis* represented a relatively large proportion of the total over the entire sampling period, becoming dominant in December. Interaction or competition for colonization space on the slides appears to be operative again between *Cocconeis* and *Achnanthes* populations, for when *Achnanthes* reached maximum percentage importance, *Cocconeis* was depressed as at stations 1 and 3, while the reverse appears true at station 4, and to a lesser extent at station 2.

Percentage composition by cell number of the four-month slide samples illustrates further differences between the dominant periphyton biota at the four stations. Species of *Asterionella* and *Fragilaria,* planktonic constituents of developing slide communities, appear to maintain only low, relatively constant levels of importance over these series of slide samples. Competition with true periphytonic species for substrate area, as well as a possibly constant settlement/sloughing-off balance (Knudson 1957) may account for these values being low and constant, relative to those of monthly exposure periods. Concomitantly, the two more typical periphyton species, *Cocconeis* and *Achnanthes,* again illustrate a possible competition and/or replacement relationship, *i.e.,* whichever species is able to colonize and establish a growing population first, becomes dominant. Differences in successional

patterns occurring at the sampling stations between the sets of monthly and cummulative four-month exposure periods may be due in part to differences in colonization rates dependent on season and physiological conditions of the colonizing populations, in rates of sloughing-off, in time periods necessary for acclimation of substrate surfaces, in the availability of various species populations for "seeding on", *etc.* However, it would appear that with increasing length of slide immersion, substrate surface area available for attachment becomes an increasingly important limiting factor and probably reaches a critical level under some of the experimental conditions imposed in this study. In these situations of limiting substrate area it is suggested by the data, that species interaction becomes of greater significance in determining station differences, than measured physico-chemical conditions. Thus in the samples depicted in Figure 15, competition for available substrate between *C. placentula* and *A. minutissima* accentuated station differences at the expense of planktonic species which were subjected in monthly samples, to essentially identical environmental conditions. Intuitively, one would expect these "differences" (station) and the effects of species interaction to reach a maximum and eventually stabilize or come to equilibrium much like a climax state (Blum 1956b) so that long-standing communities, such as those on natural substrata in Elk Lake, should be similar in structure and if not would reflect a limiting abiotic environmental condition. This would particularly apply to epiphytic communities where different macrophytic species have been found to support characteristic periphyton communities (Miller 1936; Young 1945; Knudson 1957; Douglas 1958; Tippett 1970). For although the epilithic and epiphytic "subcommunities" share many common species (Round 1964), natural vegetation

cannot be considered inert surfaces, having been in association with aquatic organisms for eons during which time, opportunity has existed for the development of adaptations to inhibit or promote growth of periphyton and associated microbiota (Edmondson 1944; Jorgensen 1957; Benson 1967; Quade 1969). A slide, immersed in the water for 135 days or less, would support a community of much less maturity than that which has evolved over eons (*i.e.*, epiphytic periphyton on macrophytes), hence irregular differences between stations, within and across seasons, which are not clearly related to water chemistry or other environmental variables, may be an expression of species interaction such as competition (see Margalef 1958).

Species of *Cocconeis* and/or *Achnanthes* are common constituents of periphyton communities (*e.g.*, Douglas 1958; Castenholz 1960; Weber & Raschke 1966; McIntire 1968a; Tippett 1970). However, to my knowledge, there has been no evidence citing a possible competitive interaction occurring between the two species populations. More often, these taxa are reported to form separate, distinct communities; the amount of substrate area available is frequently discussed but only as a limiting factor related to the general processes of colonization and succession. In English rivers, *Cocconeis placentula*, described by Butcher (1931, 1932) as a "summer encrusting community", was found to form distinct glass slide communities from mid-June to the end of September, or later. These communities were indicative of oligosaprobic lotic zones or eutrophic conditions (Butcher 1947, 1949; Weber & Raschke 1966). Douglas (1958) found *C. placentula* to be largely epiphytic in nature and "patchy" in distribution in a small English stream. Small growths occurred on stones in late summer and autumn, irrespective of weather conditions

and a limited number of observations suggested higher populations of *Cocconeis* were supported on glass slides than were apparent on stones[1]. Furthermore, numbers of an *Achnanthes* species group, which included *A. minutissima*, increased from April to June, but these increases were not correlated with temperature or light. Irregular fluctuations in numbers of *Achnanthes* spp. occurred on moss and permanent rock indicating "patchy" distributions; while some station differences on stones were attributed to grazing by herbivorous insect (caddis) larvae. Douglas (1958) concluded that fluctuations in attached diatom populations occurring at different stations or seasons could not be correlated with changes in water chemistry.

Similar erratic fluctuations in numbers of diatoms on different substrate types have been reported for Abbot's Pool (Tippett 1970). Much of this irregularity or variation can be attributed to the invalid comparison of macrophytic surfaces with inert surfaces (Knudson 1957; Douglas 1958; Benson 1967), substrate orientation, history of exposure, and location of sampling stations, *etc.* However, it remains that populations of *Achnanthes affinis* Grun. and *Cocconeis placentula* were generally irregularly fluctuating in numbers from sample to sample, regardless of season. A peak on four week glass slides of *A. affinis* between March and June, followed by a summer peak of *C. placentula* between July and September may have been strictly a seasonally induced succession in dominant species as suggested by Tippett (1970) or, the pattern may have been influenced by competition for substrate area by the two species.

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[1] In contrast, Tippett (1970) found *C. placentula* to be an important winter component of epiphytic communities on *Fontinalis antipyretica* Hedw. in Langford Spring, "although hardly present on the slides".

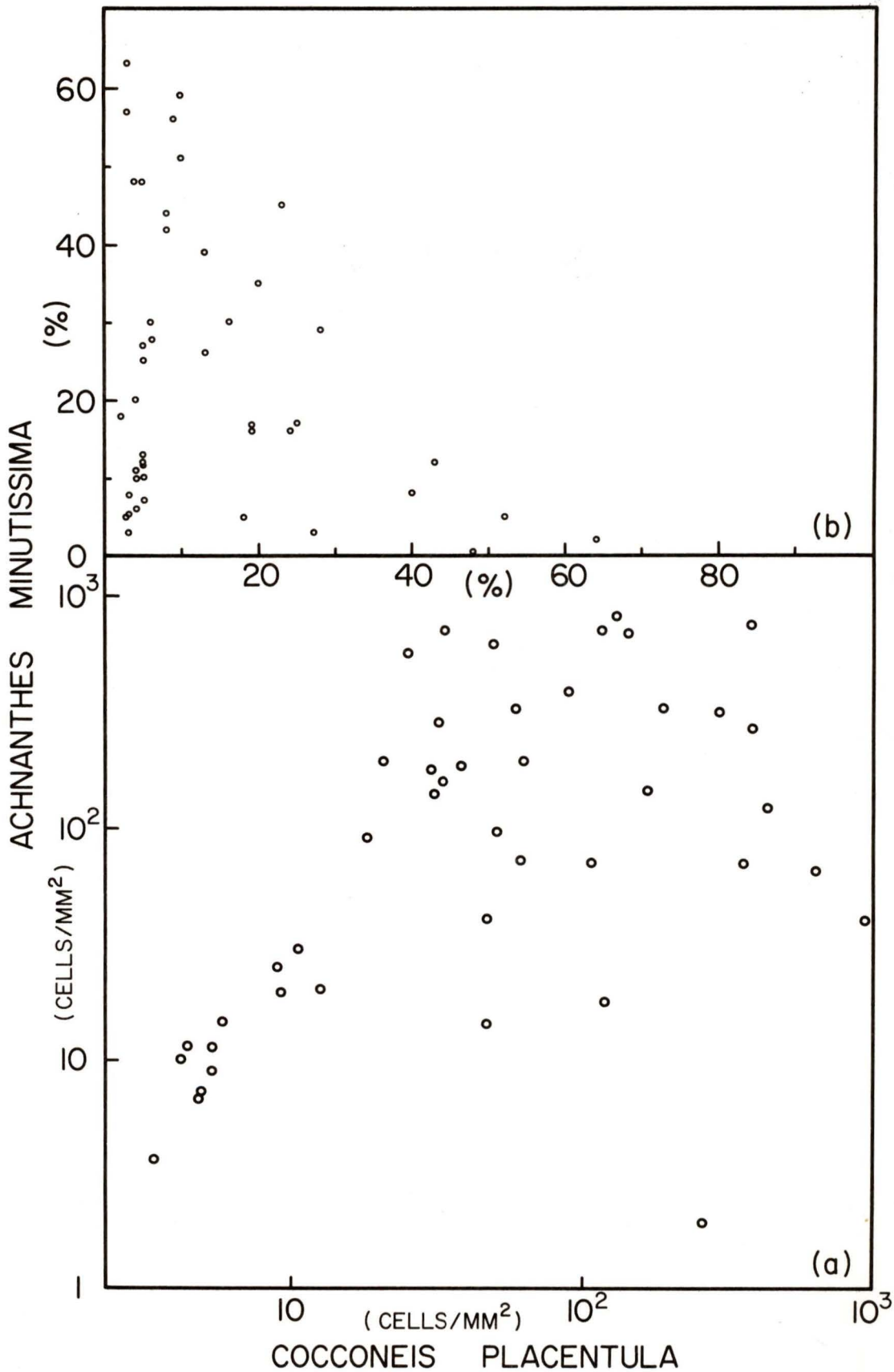
On the basis of the data presented the possibility of species interaction, such as competition cannot be entirely dismissed.

For example, Tippett (1970) found *Achnanthes affinis* in Abbot's Pool, increased from 47% abundance on the two week slides to 63% on the four week slides, whereas in Langford Spring, *A. affinis* decreased from 55% on two week slides to 10% on the four week slides. At the same station and over the same slide immersion periods, *A. lanceolata* (Breb.) Grun. increased from 29% to 81% illustrating the possibility of a competition induced succession in dominants dependent on available substrate area.

It is thus evident that erratic variations in species populations of *Achnanthes* and *Cocconeis*, between sampling stations or water bodies, or different substrata, regardless of season, and on occasion, with no apparent relation to environmental factors such as water chemistry, are not uncommon. The only example of species interaction which might account for this irregular fluctuation in numbers was reported by Douglas (1958) who attributed some station variation in populations of *Achnanthes* spp., epilithic on stones, to grazing by insect larvae. However, although grazing is an important factor in reduction of biomass often characterized by sharp, irregular fluctuations in periphyton populations (see Dickman 1968b), no evidence was found in the present study to indicate that fluctuations in any species populations of vertical slide communities were related to grazing. Similarly, Castenholz (1961) reported that grazing, common on natural substrata, was rare on lake and marine glass plates where 2% or less of the total biomass constituted animal material.

Figure 16 shows the correlation between numbers of *C. placentula* and *A. minutissima* throughout the study period in Elk Lake. *Cocconeis* is

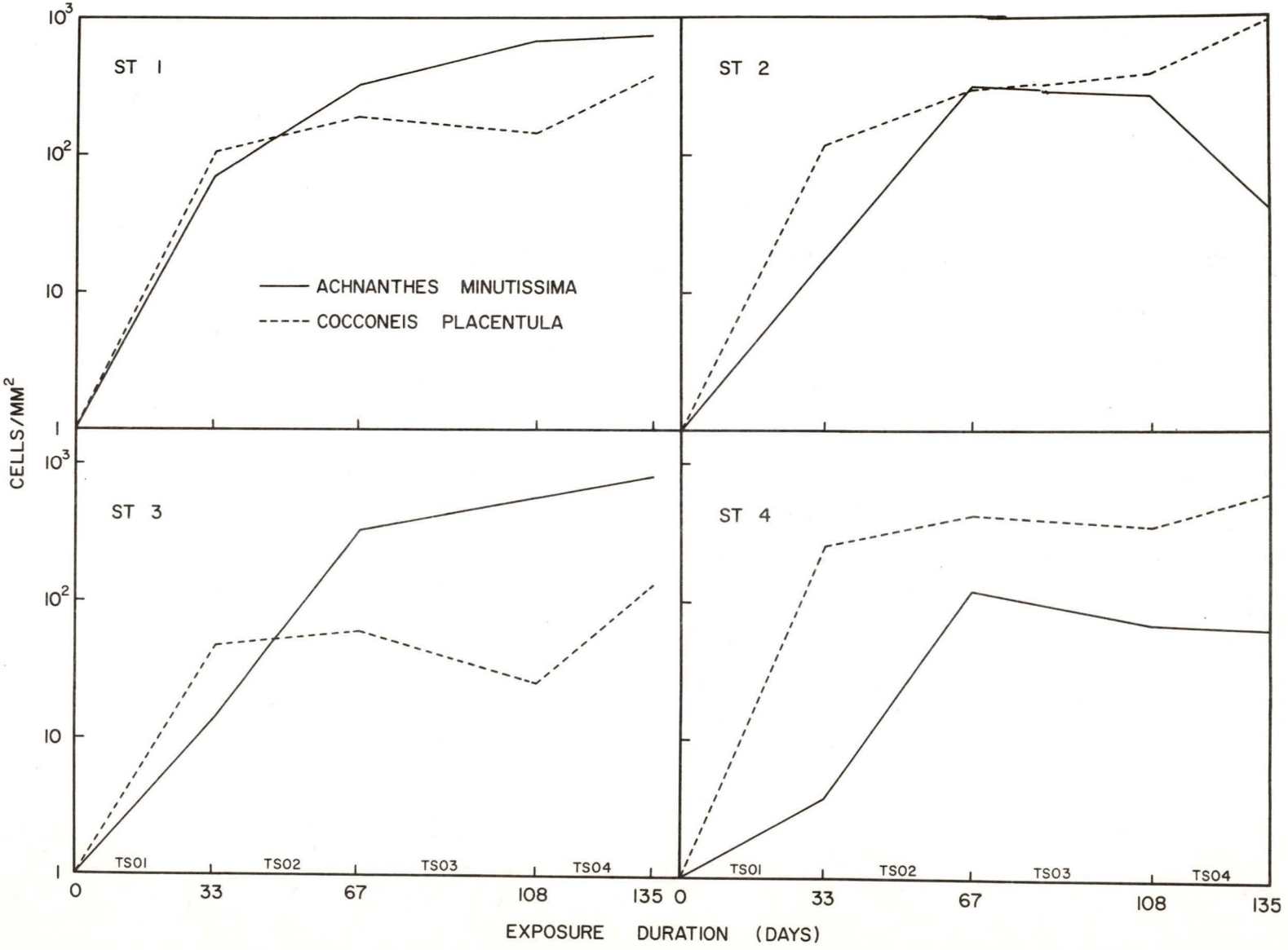
Figure 16. Graphs illustrating the correlation between *Achnanthes minutissima* and *Cocconeis placentula* for the 11 different time series at the four different stations. Plotted values are expressed as (a) mean numbers of cells/mm^2 and (b) mean percentage abundances of total cell populations (all data points represent the mean of four slide values).


ACHNANTHES MINUTISSIMA
(%)
60
40
20
0
(b)
20
40
(%)
60
80
10^3
10^2
10
1
(CELLS/MM^2)
(a)
10
(CELLS/MM^2)
10^2
10^3
COCCONEIS PLACENTULA

seen to increase in direct relation to numbers of *Achnanthes*. However when the same data is expressed as percentage abundance, a negative correlation is apparent, illustrating that when *Achnanthes* is dominant, *Cocconeis* generally represents a lesser proportion of the total slide population, although considerable variation occurs between stations as shown earlier. It is possible that the apparent competition for surface area between these two species may be an artifact of the artificial substrate, however there is no reason to suggest that a similar interaction should not occur on other inert natural surfaces. It may be that in the epilithic habitat, the nature of the substrate surface is such that it affords a competitive advantage to one or the other species so that the end result of succession on these surfaces is the occurrence of a community dominated by only one species and leads to the general finding of irregular fluctuations in these species between sampling stations irrespective of season. Some unknown physico-chemical factor may also afford similar advantage to one species population. The relationship between these two species in Elk Lake with increasing slide exposure durations is further illustrated in Figure 17. The evidence does not indicate a direct competition for space and complete replacement of one species by the other, but with increasing immersion time, space becomes more limiting, species interaction is intensified, and station differences are accentuated.

Despite the reporting of planktonic forms in periphyton communities (Round 1964; Sladeckova 1962), I did not expect to find (on the basis of the literature) the very close relationship in Elk Lake between species of the littoral plankton and periphyton, where at least 12 taxa were common to both communities; 5 diatom species of which dominated the

Figure 17. Plots illustrating the relationship, in cells/mm^2, between *Achnanthes minutissima* and *Cocconeis placentula* at the four stations over increasing exposure periods of 33, 67, 108, and 135 days between August 3 and December 16. All data points, plotted on a logarithmic scale, represent mean values of four slides.


ST 1
ST 2
ST 3
ST 4
ACHNANTHES MINUTISSIMA
COCCONEIS PLACENTULA
CELLS/MM²
10³
10²
10
1
TS01
TS02
TS03
TS04
0
33
67
108
135
EXPOSURE DURATION (DAYS)

planktonic diatom populations while simultaneously representing populations of considerable importance in the vertical slide communities. Even in lotic habitats, where the interdependence of the two communities may be expected (Butcher 1932; Douglas 1958), Weber and Raschke (1966) found glass slides, colonized by "drifting" organisms, supported periphyton communities the composition of which was distinctly different from the plankton. The plankton consisted primarily of centric diatoms while periphyton communities were dominated by pennate forms. Patrick *et al.* (1954) noted the occurrence of typical planktonic diatoms such as *Asterionella formosa* on diatometer slides immersed in rivers. In a study of Lake Erie plankton (see Hohn 1966), periphyton communities on glass slides and styrofoam contained three characteristic planktonic species, *Melosira ambigua, M. granulata,* and *Fragilaria capucina,* which together constituted only 5% of the total attached diatom population. Concurrent sampling revealed the same three species accounted for 99% of the plankton population present at the same time. However, in Lake Washington, Benson (1967) found species of genera such as *Melosira, Synedra, Fragilaria, Anabaena, Oscillatoria,* and *Phormidium,* were common to both the periphyton and plankton communities and appeared to be adapted for life in both habitats.

It has been suggested by various authors (Sladeckova 1962; Round 1964) that attached microbiota or "true periphyton"---epiphytic, epipelic, or epilithic periphyton (Wetzel 1964, 1965)---should be differentiated and analyzed separately from associated organisms of the "pseudo-periphyton" or metaphyton which occur together in sample data collections. However as found in Elk Lake, vertical slide periphyton communities of littoral regions contain species common to at least the net plankton of

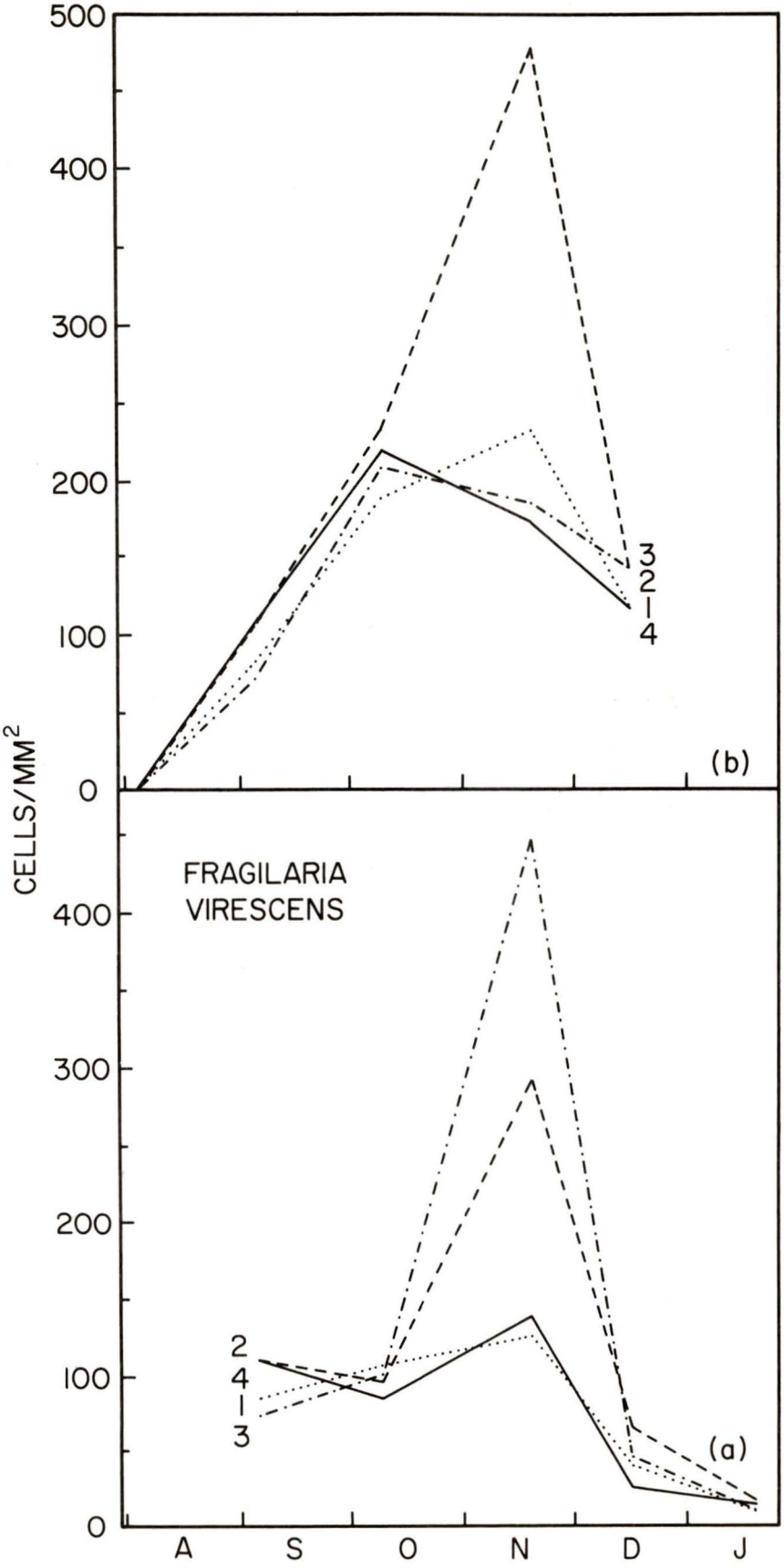
the same sampling locations. These species constitute a considerably large proportion of the total cells present in both communities and their occurrence on glass slides cannot be denoted accidental. Furthermore, in view of comparable findings in the pelagic region of Lake Washington (Benson 1967), this relationship cannot be attributed to a condition of sampling peculiar to the littoral region where wave action may cause mixing of species populations from the planktonic and benthic habitats.

Aside from the discovery of the close interrelation between the diatoms of the plankton and the periphyton in Elk Lake, there are at least two other reasons why one might be advised to sample both communities in other situations: (1) those species common to planktonic and periphytonic habitats, and apparently identical, may in fact represent different ecotypes (Knudson 1953, 1957; Fogg 1965; Hutchinson 1967) or different physiological strains adapted for life in specific habitats; and (2) fluctuations in numbers of individuals in the periphyton may be heavily dependent on similar fluctuations in the plankton with regard to plankton utilization and depletion of nutrients, shading of periphytonic substrates, production of growth inhibitors, *etc.* (Jorgensen 1957; Knudson 1957; Wetzel 1964; Benson 1967; Moss 1969a).

(iii) Variation in cells, filaments and filament lengths: *Fragilaria virescens*

Fluctuations in numbers of the colonial or filamentous diatom, *Fragilaria virescens*, are illustrated in Figure 18, where station differences are shown. In the one-month immersion slide samples, numbers of cells/mm^2 reached maxima at all stations on the November collection date in samples of TS07 immersed for the longest period of 41 days, while

Figure 18. Graphs illustrating the standing crop in mean numbers of cells/mm^2 of *Fragilaria virescens* in (a) one month exposure periods of TS01, 05, 07, 09 and 11 between August 3 and January 19, and in (b) slide samples of successively increasing exposure durations of 33, 67, 108, and 135 days between August 3 and December 16. Differences between the four periphyton sampling stations are shown.


500
400
300
200
100
0
CELLS/MM²
3
2
1
4
(b)
FRAGILARIA
VIRESCENS
400
300
200
100
0
2
4
1
3
(a)
A
S
O
N
D
J

F. virescens composed less than 5% of the plankton population at this time (Figure 12). Similar peaks in the periphyton are shown with total cells/mm^2 in Figure 8, however *F. virescens* peaked in percentage importance in TS07 samples only at station 2 (Fig. 13). The height of the maxima attained was dependent on station location, for stations 1 and 4 reached similar mean peaks of 125.02 (S.D. = ±10.36) and 137.99 (±8.62) cells/mm^2, while stations 2 and 3 reached higher peaks of 292.72 (±0.72) and 448.77 (±23.89) mean cells/mm^2, respectively. The dependence on station location reflectes corresponding similarity in fluctuations of total cell populations also dependent on station location (Fig. 8). Maxima in numbers of *F. virescens*, occurring as temperature and light decreased, were coincident with loss of thermal stratification and a sharp decrease in recorded nitrate-nitrogen concentrations occurring at all stations. In the four-month increasing interval samples, *F. virescens* also peaked in numbers/mm^2 on the November collection date after 108 days immersion at stations 2 and 1, but peaked earlier in the 67 day immersion slide samples, collected in October at stations 3 and 4. Greatest variation between stations occurred in samples of TS03, immersed for 108 days between August 3 and November 19.

Little is known of the relationship between the numbers of cells per colony of colonial or filamentous diatoms such as *F. virescens*, and environmental parameters. Knudson (1957) found the number of cells per colony of the epiphytic diatom, *Tabellaria flocculosa* var. *flocculosa* to be "very variable" in three English lakes. She presumed the length of colonies to be dependent on factors such as growth rate, consistency of the mucilage, and amount of water disturbance; colonies of 100 or more cells in length occurred under "favourable conditions". Earlier,

Canter and Lund (1948, 1951, 1953) found a decrease in the number of cells per colony of the planktonic diatom, *Asterionella formosa*, indicative of chytrid epidemics in English lakes. They observed that while number of cells per colony may not always be indicative of diatom vigour, high numbers typically indicated a capacity for rapid increase in population numbers provided light or other physical conditions were not limiting. Flood conditions or changes in lake level did not affect the average number of cells per colony of *A. formosa*.

In Elk Lake periphyton communities, the average number of cells per colony of *F. virescens* were quite variable (Table 12), and not clearly related to any measured environmental factors or chytrid parasitism. In one-month samples, maximum colony or unit[1] length occurred at stations 3 and 4 in TS07 samples immersed 41 days between October and November, while at stations 1 and 2 average lengths were greater in TS09 samples exposed later in the season from November 19 to December 16 or 27 days. As light intensity and duration were less under exposure conditions of TS09 than TS07, it appears that decreasing light had little effect on colony length; similarly, length of exposure was not positively correlated with colony length in these samples. In the two-month exposure series, the maximum number of cells per colony of *F. virescens* occurred at all four stations in TS06. In these samples, immersed for 75 days from September 5 to November 19, colonies consisted of almost twice the number of cells as observed in other two-month samples. Unlike peaks in cell numbers (Fig. 18) and the number of organisms or colonies

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[1] It is probable that colonies were of greater length prior to filter preparation, thus "colony" as used here actually refers to the unit or organism counted on the filter as previously described.

Table 12. Data illustrating station and time series differences in mean numbers of filaments, colonies, or structural units/mm^2 and cell length of filaments (cells/filament/mm^2) of *Fragilaria virescens* in Elk Lake periphyton samples (where n = 4 slides and figures are rounded correct to one decimal place).

ST	1		2		3		4	
TS	units /mm^2	unit length	units /mm^2	unit length	units /mm^2	unit length	units /mm^2	unit length
A. "ONE MONTH" EXPOSURE PERIODS								
01	7.9	(10.5)	13.4	(8.2)	10.6	(6.9)	10.2	(10.8)
05	8.9	(12.0)	7.1	(13.4)	9.1	(11.1)	7.6	(11.0)
07	8.2	(15.2)	16.7	(17.5)	21.5	(20.9)	7.4	(18.6)
09	2.5	(16.5)	3.4	(19.0)	2.7	(16.9)	1.6	(16.6)
11	1.0	(8.9)	1.5	(10.9)	1.3	(7.8)	1.3	(10.2)
B. "TWO MONTH" EXPOSURE PERIODS								
02	15.6	(12.1)	18.8	(12.4)	14.7	(14.9)	14.5	(11.1)
06	11.8	(18.6)	18.7	(24.6)	13.3	(26.1)	10.9	(20.3)
08	4.9	(13.5)	5.6	(12.0)	5.4	(15.5)	5.2	(11.9)
10	2.9	(14.5)	3.5	(12.5)	2.4	(10.6)	2.1	(8.4)
C. "FOUR MONTH" INCREASING EXPOSURE PERIODS								
01	7.9	(10.5)	13.4	(8.2)	10.6	(6.9)	10.2	(10.8)
02	15.6	(12.1)	18.8	(12.4)	14.7	(14.9)	14.5	(11.1)
03	10.9	(21.4)	19.5	(24.7)	8.2	(22.7)	8.6	(20.0)
04	10.1	(11.6)	11.4	(12.4)	10.8	(13.2)	8.1	(13.1)

(Table 12) which were dependent on station location, with increasing exposure duration (*i.e.*, TS01, 02, 03, 04), maximum colony length of *F. virescens* occurred at all stations in those samples immersed for 108 days between August 3 and November 19 (TS03). The sharp decrease shown after 135 days exposure reflects similar trends in numbers of cells/mm^2 of *Fragilaria virescens* and suggests a possible limiting nutrient factor such as nitrate-nitrogen. A seasonal response to a decrease in light intensity and duration appears unlikely, however peak colony length did occur on all occasions after thermal mixing and whilst the planktonic population of *F. virescens* remained negligible in percentage importance.

INTERCORRELATIONS

In order to relate biological and environmental data, correlation coefficients (R) were calculated between the independent environmental variables and between these variables and dependent biological variables. On this basis an effort was made to describe station differences and/or similarities in ecological relationships by multiple regression analysis which was used to investigate the relative importance of several measured ecosystem components to standing crop or total cells and structural characteristics of periphyton communities. Because of the great variability in methodology employed and the scope of the various investigations, it is difficult to compare species diversity, standing crop, or estimates of production for Elk Lake littoral periphyton with data from other lakes of the world. Comparisons from the literature are included here with caution due to differences in substrate type and degree of community stabilization.

(A) Correlation analysis

(a) Correlation of environmental factors

Correlation coefficients, each calculated from 11 pairs of data points per station, for the 15 different environmental or X-variables used in the multiple regression analyses are given in Tables 13 and 14.

Whereas previous analysis has shown the four stations to be statistically similar, in that no significant differences occur between stations in recorded values of physico-chemical variables on any one sampling date or over the entire study period, comparison of the correlation coefficients illustrates certain differences in environmental features

Table 13. Correlation coefficients (R), calculated from 11 different pairs of data points per station, among the 15 environmental variables measured in the study and used in multiple regression analysis. The upper half of the matrix contains correlation coefficients for station 1, while the bottom half represents those computed for station 2. Coefficients, calculated correct to three figures, are truncated here to two figures to conserve space. Abbreviated variables are: copper, pH, water temperature, dissolved oxygen, sulfate, total hardness, calcium hardness, magnesium hardness, total phosphate, orthophosphate, nitrate-nitrogen, nitrite-nitrogen, percent oxygen saturation, air temperature, and length of exposure duration in days.

St.1 / St.2	Cu	pH	WT	DO	SO_4	T-H	Ca-H	Mg-H	T-PO_4	o-PO_4	NO_3-N	NO_2-N	% O_2	AT	Days
Cu		86**	96**	-90**	-07	-92**	44	-89**	-65*	-81**	88**	92**	-20	90**	17
pH	94**		95**	-71*	35	-94**	15	-82**	-53	-46	99**	86**	16	98**	15
WT	99**	96**		-86**	12	-98**	40	-93**	-58	-65*	96**	88**	-04	98**	20
DO	-91**	-74**	-89**		04	-81**	-55	84**	64*	85**	-73**	-71*	53	-76**	-17
SO_4	61*	80**	65*	-29		-12	-63*	09	-19	40	32	05	30	23	-07
T-H	-96**	-95**	-97**	82**	-70*		45	96**	43	58	-95**	-81**	-02	-98**	-21
Ca-H	-41	-35	-41	50	13	28		-68*	07	-50	17	11	-35	31	26
Mg-H	-72*	-75**	-72*	52	-77**	83**	-29		33	63*	-83**	-70*	08	-90**	-25
T-PO_4	-83**	-84**	-82**	70*	-47	77**	67*	38		55	-55	-70*	33	-50	-08
o-PO_4	-86**	-68*	-83**	92**	-17	82**	49	53	70*		-50	-70*	65*	-50	-05
NO_3-N	93**	99**	95**	-72*	81**	-94**	-37	-72*	-82**	-65*		88**	13	-98**	14
NO_2-N	64*	84**	67*	-29	93**	-70*	-10	-64*	-68*	-22	84**		-01	85**	07
% O_2	-58	-31	-55	86**	21	43	52	12	41	80**	-28	20		13	03
AT	96**	94**	97**	-82**	74**	-98**	-20	-86**	-70*	-76**	93**	69*	-44		19
Days	21	17	19	-20	12	-22	09	-27	-15	-22	14	07	-13	21	

* Significant at $P \leq 0.05$ but not at $P \leq 0.01$
** Significant at $P \leq 0.01$

Table 14. Correlation coefficients, calculated as in Table 13, for 15 environmental variables measured at station 3 (upper half of matrix) and at station 4 (lower half of matrix). Abbreviations are given in Table 13.

St.3 / St.4	Cu	pH	WT	DO	SO_4	T-H	Ca-H	Mg-H	$T\text{-}PO_4$	$o\text{-}PO_4$	$NO_3\text{-}N$	$NO_2\text{-}N$	% O_2	AT	Days
Cu		97**	96**	-68*	08	-93**	50	-89**	-69*	-61*	97**	73*	01	95**	19
pH	98**		94**	-60*	25	-87**	49	-84**	-53	-48	97**	64*	13	96**	19
WT	96**	95**		-80**	-01	-97**	66*	-96**	-60*	-71*	97**	56	-12	98**	19
DO	-44	-39	-63*		34	80**	-77**	86**	57	80**	-71*	-30	67*	-73**	-10
SO_4	62*	71*	51	18		23	-24	24	39	70*	14	07	54	04	-05
T-H	-87**	-82**	-95**	77**	-25		-62*	98**	70*	83**	-92**	-59	18	-94**	-20
Ca-H	85**	92**	91**	-45	56	-79**		-76**	-23	-69*	49	-13	-34	66	24
Mg-H	-89**	-86**	-97**	75**	-30	99**	-84**		63*	86**	-88**	-45	23	-94**	-22
$T\text{-}PO_4$	73**	85**	78**	-29	73**	-60	93**	-66*		66*	-56	-82**	27	-51	-15
$o\text{-}PO_4$	-31	-23	-46	65	46	66*	-37	64*	-07		-56	-28	44	-67*	-19
$NO_3\text{-}N$	95**	98**	96**	-44	66*	-84**	96**	-88**	89**	-30		66*	-05	96**	13
$NO_2\text{-}N$	93**	90**	80**	-15	70*	-66*	70*	-68*	62*	-07	84**		04	50	06
% O_2	20	26	-01	77**	69*	23	12	19	26	52	20	45		-01	11
AT	98**	98**	98**	-51	61*	-90**	91**	-93**	81**	-36	98**	86**	13		20
Days	21	15	19	-19	04	-24	04	-22	-01	-10	12	18	-07	18	

* Significant at $P \leq 0.05$ but not at $P \leq 0.01$
** Significant at $P \leq 0.01$

between the sampling stations. For example, at all stations the length of slide exposure, or age of the sampled periphyton communities, was not significantly correlated with any other measured physico-chemical variables. Similarly, at the four sampling stations, slide exposure duration was negatively correlated with total phosphate and orthophosphate, but positively related to both nitrite- and nitrate-nitrogen concentrations. Another relationship common to all stations occurred between dissolved oxygen concentrations and nutrients, where dissolved oxygen was negatively related to nitrate-nitrogen (all significant, except at station 4) and significantly positively correlated with orthophosphate concentrations. In contrast, the age of sampled periphyton communities was negatively correlated with sulfate fluctuations at stations 1 and 3, and positively correlated at stations 2 and 4. In addition, calcium hardness, a variable found to have no significant temporal variation in concentrations between the six sampling dates, was significantly correlated with different variables at each of the four separate stations.

At least three points of significance arise from examination of the correlations. Firstly, the relationships expressed between any two environmental variables by these correlation coefficients may not always be exactly identical to those suggested earlier by graphical display of the variation in values expressed over the six sampling dates. This is due in part to the use and/or method of calculation employed in computing the 11 mean values for physico-chemical variables used in the regression analysis, but more largely attributable to the consideration of all samples together, including those exposure periods of different length, overlapping in exposure duration as well as season. Environmental variates, averaged over time and/or depth, are however, commonly used

in multiple regression and correlation analysis (Cushing 1967; Goldman *et al.* 1968) and are used here as well.

A second point is one of a statistical nature with particular bearing on the interpretation and application of multiple regression analysis. As the matrices of correlation coefficients indicate (Tables 13 and 14), many of the physico-chemical or X-variables are clearly not independent of one another. As a consequence, the reliability of individual regression coefficients may be affected. For example, if two variables, air and water temperature, vary directly together, their separate effects on the independent variable will be difficult to distinguish. However, as a check, the reliability of each regression coefficient may be assessed by examination of its standard error, a statistic which is sensitive to co-linearity of the X-variables and increases in value as the correlation between X_1 and X_2 approaches unity. Alternatively, the X-variable considered of less importance may be eliminated from the analysis. Intercorrelations may also cause reduction in the value of the multiple correlation coefficient which attains a maximum value when correlations of Y with X_1 are large (Table 15) and intercorrelations among X_1 and the other X_k variables are small. However, while co-linearity of X-variables may affect the reliability of individual regression coefficients, the predictive precision of the overall regression coefficient may not be altered in that the standard error of the estimate $\hat{Y}$ may not increase (Spurr & Bonini 1967).

The effects of high correlation between independent variables and the ramifications in interpretation of affected multiple regression results, although generally referred to by statisticians (*e.g.*, Guilford, 1965; Draper & Smith 1966; Spurr & Bonini 1967), are seldom considered in

ecological studies since X-variables of ecological data (particularly environmental data; see Buzas 1969), are seldom independent and it would be unrealistic to expect otherwise. While Cassie (1969) offers no direct solutions to ecologists, he suggests graphical and time series analysis might be applicable to problems of this nature (see also Margalef 1965). More often, biologists using multiple regression methods (Margalef 1965, 1968; Goldman *et al.* 1968; Haertel, *et al.* 1969; Platt & Subba Rao 1970) have seemingly found it unnecessary to dwell on this apparent conflict, employing environmental variables for which high intercorrelations exist. While the occurrence of high intercorrelations between independent variables does not invalidate the use of multiple regression or correlation analyses, results should be interpreted with care (Cassie 1959a; 1960, 1963, 1969; Buzas 1969) as attempted here.

A third point of interest, the significance of which will become clearer when regressions are discussed, may be illustrated by consideration of the fluctuations in orthophosphate concentrations throughout the study. Previous statistical analysis has shown that there were no significant differences in orthophosphate concentrations between the four stations over the six sampling dates. Similarly, unlike all but one other variable, there were also no significant differences in recorded values between the six sampling dates (Table 3). Comparable statistical analysis, using the 11 mean values for orthophosphate as calculated for the different periphyton samples considered here, also indicates no significant difference between station concentrations; however there is a statistically significant difference between concentrations calculated for the 11 different TS's ($F_{(10, 33)} = 13.447$, significant at $P \leq 0.01$) which may be interpretable as a type of temporal difference.

Again, this is due in part to the computational method used to establish the 11 mean values, to the consideration of unequal exposure periods overlapped in time, and to the increase in sample size.

(b) Correlation of environmental factors, total cells, and species diversity

Correlation coefficients between the dependent and independent variables used in multiple regression analysis are given in Table 15 where stations may be compared. A significant overall increase in total cells/mm^2 with increasing length of slide exposure occurred at all stations, although the correlations were slightly stronger at stations 1 and 4. Similarly, total cells were positively related to copper concentrations, pH, water and air temperatures, and concentrations of nitrite- and nitrate-nitrogen; X-variables which exhibit significant positive intercorrelations at all stations (Tables 13 and 14). These variables all tended to decrease throughout the study from seasonally high values in August, September, and early October (Figures 4, 5, 6 and 7) in much the same manner as total cells/mm^2 which generally reached minimum values on those slides immersed from December 16 to January 19. (Figure 8). The positive relationship expressed between numbers of periphyton cells and copper concentrations is surprising in view of its known toxicity to algae (Hutchinson 1957). However, this apparent correlation is an example of the effects of positive intercorrelation with the other variables which fluctuate seasonally in the same direction over the 11 different samples. Similarly, the positive correlation between total cells and nitrate concentrations at all stations is probably also due to the extremely high and unusual concentrations recorded in August and September (Figure 6). One would normally expect nitrate to

Table 15. Correlation coefficients among the dependent and independent variables used in the stepwise multiple regression analysis and calculated from 11 different pairs of data points per station. Abbreviations as in Table 13.

Dependent Variables (Y)	St.	Independent Variables (X) Cu	pH	WT	DO	SO_4	T-H	Ca-H	Mg-H
Total cells[1]	1	.522	.345	.501	-.556	-.289	-.517	.637*	-.618*
	2	.658*	.501	.622*	-.692*	.189	-.657*	-.082	-.608*
	3	.594	.544	.681*	-.594	-.429	-.745**	.710*	-.791**
	4	.533	.429	.565	-.609*	-.032	-.686*	.318	-.652*
H"	1	-.113	.051	-.035	.174	.212	.002	-.156	.050
	2	-.360	-.247	-.344	.450	-.068	.278	.139	.197
	3	-.285	-.207	-.364	.430	.501	.461	-.368	.472
	4	-.366	-.434	-.275	-.180	-.757**	.098	-.276	.125

Dependent Variables (Y)	St.	$T\text{-}PO_4$	$o\text{-}PO_4$	$NO_3\text{-}N$	$NO_2\text{-}N$	$\%\ O_2$	AT	Days	Total Cells
Total cells	1	-.197	-.454	.363	.337	-.226	.446	.786**	
	2	-.439	-.770**	.459	.160	-.570	.630*	.712*	
	3	-.462	-.813**	.534	.172	-.090	.689*	.623*	
	4	.116	-.534	.411	.415	-.322	.516	.785**	
H"	1	.139	.255	.036	-.100	.296	.029	-.659*	-.697*
	2	.238	.362	-.228	-.009	.451	-.314	-.748**	-.691*
	3	.330	.608*	-.271	-.118	.265	-.337	-.704*	-.744**
	4	-.447	-.470	-.381	-.461	-.512	-.351	.061	.114

1. Total cells /mm^2 were logarithmically transformed.
* Significant at $P \leq 0.05$ but not $P \leq 0.01$
** Significant at $P \leq 0.01$

decrease in the trophogenic zone during summer months coincident with high levels of primary productivity and assimilation, particularly if nitrate is the limiting nutrient---a condition apparently not present in Elk Lake, at least not during the early part of the study. In Lake Maggiore (Italy), Goldman, *et al.* (1968) found nitrate concentrations were inversely correlated with phytoplankton productivity and standing crop biomass by volume of diatoms. In periphyton communities of Lake Washington, Benson (1967) found the decline of *Fragilaria bidens* (an important genera of the Elk Lake periphyton) correlated with nitrate depletion in late May.

Table 15 also shows that total cell counts were negatively correlated with dissolved oxygen concentrations which, in turn, were significantly negatively correlated with water temperature at all stations (Tables 13 and 14), indicating the temperature dependency of oxygen solubility. While all stations showed negative correlations between total cells and levels of magnesium and total hardness, station differences were illustrated by correlations with calcium hardness, where a significant positive relationship occurred only at stations 1 and 3. Eventhough the actual "cause" of these station differences is unknown, the differences illustrated in magnitude between the coefficients of the same sign were probably due more to variations in cell numbers per station, than to differences in calcium hardness fluctuations which were not statistically different between stations or between sampling dates. The positive relationship between estimates of primary production, including standing crops of freshwater periphyton communities, and water hardness is well documented (Rawson 1960; Hohn & Hellerman 1963; Williams 1964; Goldman *et al.* 1968). The lack of a similar relationship

at station 2 is puzzling and probably related to the small, but not statistically significant, differences in station 2 fluctuations of calcium hardness compared to those at the other stations as depicted in Figure 6. Finally, at all stations, total cells were negatively correlated with orthophosphate concentrations, a relationship not uncommon for both phytoplankton and periphyton communities (Cushing 1967; Ball *et al.* 1969; Platt & Subba Rao 1970). At stations 2 and 3, the significant negative correlation coefficients between orthophosphate and total cells represented the strongest correlations between total cells and X-variables at these two stations, the importance of which will be seen on examination of multiple regression results (Tables 16 and 17).

It is apparent upon examination of Table 15 that correlations between total periphyton cells and environmental variables are much more consistent, in magnitude and sign, between stations than comparable correlations with diversity indices. This may be expected since diversity indices embody more information and are structural characteristics expressing community organization as opposed to the non structural variable, total cells which is an estimate of standing stock. For example, two stations may be very similar in total cell counts of periphyton, the structural characteristics of which may be quite distinct. Although there are both clear similarities and dissimilarities between the stations, station 4 stands out as being very different from the other three stations in its structural relationship with measured environmental variables.

Species diversity was negatively related, at all stations, to copper concentrations, pH (with the exception of a very weak positive correlation

at station 1), and temperature; a reflection of the intercorrelations between X-variables (Tables 13 and 14), and also the negative relationship with total cell counts which, in turn, were positively correlated with copper, pH, and temperature.

There was a similar non significant negative correlation between periphyton species diversity and concentrations of nitrate- or nitrite-nitrogen, while at stations 1, 2, and 3, the relationship with inorganic phosphate concentrations was positive. In contrast to this, Platt and Subba Rao (1970) found species diversity during a marine diatom bloom of approximately one month's duration, was negatively correlated (but not statistically significant) with concentrations of both nitrate and phosphate. In Marion Lake, plankton species diversity decreased concomitant with decreased nutrient levels during early stages of bloom succession, while after about two weeks, diversity values increased and phosphate remained constant at low concentrations (Dickman 1968a). Ewing and Dorris (1970) found the size of pond phytoplankton populations was not strongly correlated with nutrient concentrations and the species diversity of these communities did not parallel nutrient levels. Correlations between nutrients and species diversity were both negative and positive, with strong correlations observed in only a few ponds, all of which were not significantly different in mean concentrations of nitrate, nitrite, ammonia or phosphate.

Relationships expressed in Elk Lake between periphyton species diversity and nutrient concentrations are confounded by other X-variable intercorrelations, by unusual seasonal fluctuations, and by the fact that species diversity is more clearly related to length of slide exposure. The importance of time and of different time scales in interpretation

of diversity becomes increasingly apparent, considering that under conditions of high nutrient levels in eutrophic lakes such as Elk Lake, overall biotic diversity is low compared to that of nutrient poor oligotrphic lakes (Margalef 1964), while seasonally the species diversity in both lakes will fluctuate.

There was a significant trend of decreasing species diversity with increasing slide exposure duration or age of the Elk Lake periphyton communities at all stations, except station 4 where there was a very weak but positive correlation. Platt and Subba Rao (1970) also found a decrease in species diversity with age of a marine diatom bloom, and this is in agreement with Margalef's (1958, 1967, 1968) ecosystem theories of succession where a differential multiplication of the species components may eventually result in a lowering of the index of diversity as succession proceeds. On the other hand, Pielou (1966a) considers that during succession of terrestrial forest communities, species diversity decreases, while pattern diversity increases, *i.e.*, spatial patterning becomes more complex, the tendency for pure single species clumps or segregation decreases and individual species become more randomly mingled in their spatial distributions. Succession supposedly has a direction progressing from a state of low complexity to one of increased complexity and stability, or climax condition, if applicable. Margalef (1968) also states that in some instances, diversity may initially increase and then decrease toward the final stage of succession, or as Dickman (1968a) found with induced nutrient enrichment, freshwater plankton succession appeared to consist of two stages. For the first two weeks after nutrient enrichment, diversity decreased as standing crop increased; while in the second stage, diversity increased during

the two to seven week interval following initial fertilization coincident with decreased standing crop or total cell numbers. The relationship of species diversity with age of a community is thus very dependent on the time scale and the type of community investigated and is not amenable to general interpretation. The Elk Lake data is most comparable with that of Dickman (1969b) who found that freshwater periphyton communities reached a maximum species diversity after three weeks of immersion and dropped sharply in the fourth week.

In general, Margalef (1958, 1968) suggests, in his hypothesis of ecosystem dynamics, that biomass and primary productivity both increase during succession along with species diversity; simultaneously, the ratio of primary productivity to total biomass drops and diversity is negatively correlated with turnover. Estimates of standing crop of freshwater phytoplankton, such as chlorophyll a, are positively related to species diversity, carbon uptake (mg $C/m^3/hr$) and production per unit biomass (mg C/g C/hr), while diversity is negatively related to carbon uptake and production (Margalef 1964, 1965). However, in the present study, the association between species diversity and standing crop was closely related and interdependent with that between species diversity and age of the periphyton communities. In Elk Lake, a decrease in species diversity occurred with increasing size of the total cell populations of periphyton communities due to: (1) the increase in total cell counts with increasing exposure duration (estimates of standing crop which, in turn, were negatively correlated with species diversity); and (2) the limiting effect of available slide surface area coincident with increasing length of slide exposure, so that certain species (*e.g., C. placentula, A. minutissima*) were able to out-compete others and thereby

increase at the expense of those species unable to find suitable substrate area for colonization. The end result is a community of low species diversity, consisting of a small number of species, dominated by one or two species with many individuals (see Margalef 1958).

These results appear to be similar to those of Yount (1956) who found that under conditions of high productivity in Silver Springs (Florida), diatoms and chlorophyll rapidly accumulated on glass slides which were characterized by a rapid decrease in species variety as the density of the populations increased (see also Dickman 1969b). Platt and Subba Rao (1970) also found species diversity negatively correlated with all measures of phytoplankton standing stock, including particulate carbon, cell density, and chlorophyll a. They attributed this apparent contradiction with Margalef's (1968) general theory, that increases in biomass are associated with increases in diversity, to be due to the consideration of different time scales (see also Dickman 1968a).

It follows from these observations and previous discussion, that there are problems associated with general application of Margalef's (1958, 1965, 1968) ecological theories and the interpretation of diversity indices as applied to different studies and community types. Firstly, the concept of time and the use of different time scales has been discussed by Platt and Subba Rao (1970) and Dickman (1968a). An example of time differences on a seasonal scale is illustrated by the data of Goldman *et al.* (1968) for Lake Maggiore where from May 25 to August 18, low plankton species diversity was positively correlated with low primary productivity; however, in contrast, during the fall period from August 18 to October 6, primary productivity per unit biomass

increased as biotic diversity decreased. A second consideration of importance are the anomalies associated with comparisons of diversity indices based on partial ecosystem components with those indices of diversity calculated from data of larger components of the entire system, (see Dickman 1968a, 1968c; Wilhm 1968; Platt & Subba Rao 1970). As Margalef (1968) points out, those species groups belonging to partial niches, such as the diatoms or dinoflagellates, are poor indicators of the overall organization of the total ecosystem; for example, diversity of the diatoms may drop in the summer when diversity of the whole phytoplankton community is at a maximum. While indices based on different species groups or trophic levels may give insight into dynamic properties of the total populations, they may also lead to erroneous conclusions if consideration is not given to the possibility of niche differentiation. A third point of consideration is inherent in the nature of the diversity indices themselves and is due to the fact that H", the maximum likelihood estimator of Shannon's H', depends only on the number of species as well as their relative proportions, while Brillouin's H also depends on the size, or N, of the collection (see also Pielou 1966a, 1966b, 1966c; Goldman *et al.* 1968; Wilhm 1968, 1970). Hence, comparison of different indices between samples of different size from different studies may be misleading. Also comparisons of samples of different sizes with the same index may be equally misleading.

Further examination of the correlation coefficients between diversity indices and measured environmental variables (Table 15) suggests that station 4 was ecologically different from the other three stations in periphyton community structure. Unlike the other stations, species diversity at station 4 showed a weak positive correlation with

total cell standing crop, while a non significant weak correlation of positive sign occurred with increasing exposure duration. Similarly, diversity per individual was negatively correlated with all nutrient determinations recorded at station 4. The only significant correlation with species diversity at station 4, suggests that species diversity showed a significant increase as sulfate concentrations decreased, or vice versa, and the reasons for this are unknown (see Figure 7). It may only be concluded that statistically non significant differences in environmental variables occurring at station 4 were ecologically significant in inducing differences in the structural organization of the periphyton communities not apparent at the other three stations.

Eventhough statistically significant station differences do occur in standing crop, the relationships expressed with environmental variables are quite similar between stations. The differences which do occur between stations are clearly manifested in the diversity indices which express the sum total of species interaction under statistically similar abiotic features. Even under these conditions, it is only station 4 which appears radically different in its association with measured environmental features.

(B) Multiple regression analysis

On the basis of the preceeding correlation analyses, multiple regression was run in an effort to select those variables of significance accounting for variation in standing crop estimates or differences in the structural organization of periphyton communities at the four sampling stations. Emphasis was placed on the description of variation and the ordering of environmental variables as will be discussed.

(a) Regression of total cell populations against environmental factors

As a first step in data interpretation, relationships between measured environmental variables and periphyton total cell count/mm^2 were examined using stepwise multiple regression analysis. Regressions, performed with the linear model, considered the data for each individual station for the entire study program. The X-variable, slide exposure duration, was omitted from the analyses for the initial run.

For all stations these regressions were terminated after five steps or less, when the final predictive equations accounted for a total of 56-83% of the variation in Y_i, and the F levels for sequential and partial F-tests (see Draper & Smith 1966) were insufficient for further computation. However, the regression MS's were not significantly greater than the residual MS's ($P \leq 0.05$) after the second step and the standard error of the estimate increased with each succeeding step from this point on.

The significant X-variables of greatest importance in terms of total cell fluctuations were as follows: at station 1, calcium hardness accounted for 40.60% of the variation in total cell counts, while comparably, at stations 2 and 3, orthophosphate accounted for 59.30 and 66.13% of the variation, and finally, at station 4, total hardness was associated with 47.11% of the changes in total cells throughout the study. Nevertheless, even with the inclusion of only those variables with significant regression coefficients, the $\hat{Y}$'s were never closer than ±10% of the $\overline{Y}$'s at each station; a level of precision considered inadequate.

Examination of the residuals also revealed discrepancies with the error assumptions. For each station, a plot of the residuals against the observed values for total cells/mm^2 illustrated a definite pattern.

High values of Y_i were systematically underestimated while low Y_i's were always overestimated, a condition indicative of the absence of a variable representative of a time effect; a necessity if the data is to satisfactorily fit the assumed linear model. Hence these analyses were discarded.

Subsequent re-examination of the same data, using the stepwise multiple regression procedures and including the X-variable, exposure duration, is illustrated in Table 16 where the results are summarized. In this manner, an attempt was made to describe the environmental relationships and, where possible, to establish a satisfactory prediction equation for variations in total cells/mm^2 which would be valid for all vertical slide periphyton samples at each separate station, irrespective of the differences in length of slide exposure (or age) and/or season of immersion of these sample communities.

Regressions, performed with the linear model, considered the data for each individual station, through time, for the entire study program (Table 16). Multiple correlation coefficients (R) may be interpreted as the square root of the portion of the variance in the dependent variable which has been accounted for by the independent variables included in the analysis to that particular step. That is, at station 2 after three steps in the regression, $(0.9710)^2$ X 100 = 94.45% of the variance in total cell counts has been accounted for and this variance is interpretable in terms of orthophosphate, length of slide exposure duration, and calcium hardness. At all four stations, 7 independent ecological variables accounted for at least 95% of the variation in total cells/mm^2 throughout the study, although in each case not all 7 were significant in their contribution to the predictive

Table 16. Summary of the stepwise multiple regression of total cells/mm^2 against 15 independent variables at each station (n=11) according to the linear model:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_{15} X_{15} + \epsilon$$

where Y, the dependent variable is logarithmically transformed. R, the multiple correlation coefficient is given along with the standard error of the estimate which measures the closeness with which the predicted values agree with the observed values, and R^2, the coefficient of multiple determination which indicates the proportion of variance in the dependent variable mathematically accounted for by the inclusion of independent variables to a particular step in the regression. Abbreviations as in Table 13.

STATION 1	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
	Days**	Days**	Days**	Days**	Days**	Days**	Days**
		Ca-H*	Ca-H*	Ca-H*	Ca-H	Ca-H**	Ca-H*
			NO_2-N	NO_2-N	NO_2-N	NO_2-N*	NO_2-N*
Variable				% O_2	% O_2	% O_2	% O_2
					Cu	Cu*	Cu*
						DO*	DO*
							T-PO_4
R	0.7859	0.9015*	0.9330	0.9377	0.9452	0.9863	0.9891
s.e. of est.	0.5658	0.4199	0.3733	0.3894	0.4006	0.2265	0.2330
R^2	0.6176	0.8128	0.8705	0.8793	0.8935	0.9728	0.9784
ANOVA	F=14.537** (1,9)	F=17.367** (2,8)	F=15.683** (3,7)	F=10.923** (4,6)	F=8.388* (5,5)	F=23.808** (6,4)	F=19.393* (7,3)

Table 16. Continued.

STATION 2	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Variable	$o\text{-}PO_4$**	$o\text{-}PO_4$** Days**	$o\text{-}PO_4$** Days** Ca-H*	$o\text{-}PO_4$** Days** Ca-H* Cu	$o\text{-}PO_4$ Days** Ca-H Cu $NO_2\text{-}N$	$o\text{-}PO_4$ Days** Ca-H Cu $NO_2\text{-}N$ D.O.	$o\text{-}PO_4$ Days* Ca-H Cu $NO_2\text{-}N$ D.O. $T\text{-}PO_4$
R	0.7700	0.9486**	0.9719*	0.9728	0.9729	0.9763	0.9818
s.e. of est.	0.6328	0.3329	0.2649	0.2816	0.3076	0.3220	0.3264
R^2	0.5930	0.8999	0.9445	0.9463	0.9466	0.9532	0.9639
ANOVA	F=13.111** (1,9)	F=35.947** (2,8)	F=39.705** (3,7)	F=26.412** (4,6)	F=17.717** (5,5)	F=13.567** (6,4)	F=11.441* (7,3)

Table 16. Continued

Station 3	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Variable	$o\text{-}PO_4$**	$o\text{-}PO_4$** Days**	$o\text{-}PO_4$** Days** % O_2	$o\text{-}PO_4$** Days** % O_2 Ca-H	$o\text{-}PO_4$** Days** % O_2 Ca-H $T\text{-}PO_4$	$o\text{-}PO_4$** Days** % O_2 Ca-H $T\text{-}PO_4$ $NO_2\text{-}N$	$o\text{-}PO_4$ Days % O_2 Ca-H $T\text{-}PO_4$ $NO_2\text{-}N$ Mg-H
R	0.8132	0.9394**	0.9592	0.9714	0.9734	0.9739	0.9753
s.e. of est.	0.5809	0.3630	0.3198	0.2901	0.3065	0.3397	0.3818
R^2	0.6613	0.8824	0.9201	0.9437	0.9476	0.9485	0.9512
ANOVA	F=17.570** (1,9)	F=30.025** (2,8)	F=26.881** (3,7)	F=25.134** (4,6)	F=18.082 (5,5)	F=12.284* (6,4)	F=8.357 (7,3)

Table 16. Continued.

STATION 4	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Variable	Days**	Days**	Days**	Days**	Days**	Days**	Days**
		T-H**	T-H**	T-H**	T-H*	T-H	T-H
			$T\text{-}PO_4$*	$T\text{-}PO_4$	$T\text{-}PO_4$	$T\text{-}PO_4$	$T\text{-}PO_4$
				SO_4	SO_4	SO_4	SO_4
					$NO_2\text{-}N$	$NO_2\text{-}N$	$NO_2\text{-}N$
						AT	AT
							Ca-H
R	0.7849	0.9376**	0.9700*	0.9704	0.9725	0.9791	0.9797
s.e. of est.	0.5731	0.3411	0.2548	0.2737	0.2892	0.2824	0.3213
R^2	0.6161	0.8791	0.9410	0.9416	0.9457	0.9586	0.9598
ANOVA	F=14.444** (1,9)	F=29.093** (2,8)	F=37.201** (3,7)	F=24.195** (4,6)	F=17.418** (5,5)	F=15.427** (6,4)	F=10.231* (7,3)

* Significant at $P \leq 0.05$ but not at $P \leq 0.01$
** Significant at $P \leq 0.01$

value of the regression equations. Three variables, pH, water temperature, and nitrate-nitrogen, did not appear to account for any of the variation here although they were significantly intercorrelated with other X-variables as previously discussed (Tables 13 and 14). Of the remaining 12 variables accountable for some variation, four---sulfate, total hardness, magnesium hardness, and air temperature---were included in these regressions only once (three of these occurrences were at station 4). These four variables, excepting total hardness, accounted for less than 2% of the variation in total cell counts at the sampling stations. Similarly, four included variables, calcium hardness, total phosphate, nitrite-nitrogen, and number of days were common to the four stations.

Differences between estimated and empirical values, the residuals, express the variation in total cell counts not accounted for by linear regression. Plots of residuals for the 15 independent variables, the dependent variable and the computed $\hat{Y}$ values, showed that the residuals were apparently independent of each other and uniform in their scatter. That is, graphical evaluation suggested that the unexplained variation in total cells was not related to any systematic variation in the independent variables as there was no apparent continuity or discernible pattern in distribution of the residuals.

While the variable, age or exposure duration, was significant in its contribution under all significant F-tests for regression (Table 16), many other variables were not. Hence, in order to establish which environmental variables might possibly be excluded from further analysis without seriously jeopordizing the predictive value of the regression equations, and secondly, in the interests of simplicity, facilitating

data interpretation, I arbitrarily selected for closer examination, those equations complying with as many of the statistical criteria as possible (as previously discussed, p. 17-20). By complying with as many of the statistical criteria as possible, the regression was terminated at all stations after the inclusion of two or three variables at steps 2 and 3 (Table 16). This is not to suggest or infer that other environmental variables are ecologically unimportant in predicting Y, or that the relationships under investigation are uncomplicated. However, the complexity already encountered to this point in the study necessitates that the data be simplified if conclusions of any relevance are to be reached without further unwarranted expenditure of time in computation, analysis, and interpretation.

Hence, on the basis of the above criteria, the stepwise regression program was re-run for each station using information derived from Table 16. The resultant significant environmental variables associated with at least 80% of the variation in total cell counts are given in Table 17 with the appropriate regression equation data.

At stations 1 and 3, two independent variables were associated with a total of 81.28% and 88.24%, respectively, of the variation in Y_i, whereas 94.45% and 94.10% of this variation was ascribed to fluctuations in three environmental variables at stations 2 and 4. The standard error of estimate was within 8.82% and 5.89% of the mean (n = 11) of total cells at stations 1 and 3, while the inclusion of a third significant variable at stations 2 and 4, increased the level of precision of the sample estimates ($\hat{Y}$'s) to within 4.23% and 4.25%, of the respective mean station responses or $\bar{Y}$'s. The X-variable, slide exposure duration, was of significant importance at all four stations. It can

Table 17. Regression equation constants, extracted from Table 16 data, showing the environmental variables which significantly account for a total of at least 80% of the variation in total cells/mm^2 of vertical slide periphyton at the four stations in Elk Lake.

Station	Variable	Regression Coefficient	S.E. of Regression Coefficient	R	ΔR^2
1	Exposure Duration	0.01677**	0.00402	0.7859*	0.6176
	Ca Hardness	0.32193*	0.11147	0.9015**	0.1952
	(Constant term	- 8.00097)		Total:	0.8128
2	Orthophosphate	-11.84008**	1.63603	0.7700	0.5930
	Exposure Duration	0.01412**	0.00258	0.9486**	0.3069
	Ca Hardness	0.15156*	0.06389	0.9719**	0.0446
	(Constant term	1.60261)		Total:	0.9445
3	Orthophosphate	-10.30532**	1.77682	0.8132*	0.6613
	Exposure Duration	0.01326**	0.00342	0.9394**	0.2212
	(Constant term	7.01956)		Total:	0.8824
4	Exposure Duration	0.01551**	0.00247	0.7849	0.6161
	Total Hardness	- 0.19661**	0.03245	0.9376**	0.2630
	Total Phosphate	- 5.21414*	1.92513	0.9700**	0.0619
	(Constant term	17.37839)		Total:	0.9410

*Significant at $P \leq 0.05$ but not at $P \leq 0.01$

**Significant at $P \leq 0.01$

be seen that stations 2 and 3 were similar (5 common independent variables as entered in Table 16) in terms of the relative importance of various environmental effects on total cell populations of periphyton communities, whereas in these respects, stations 1 and 4 were more similar to each other (4 common variables) than to the other stations. At stations 2 and 3, orthophosphate accounted for 59.30% and 66.13% of the variation in total cells/mm^2, but was not related to any of the variation at stations 1 and 4 (see also Table 15). Slide exposure duration, the second most important predictor variable at stations 2 and 3 was associated with, respectively, 30.69% and 22.12% of the variation in total cell counts. By contrast, at stations 1 and 4, the duration of slide exposure accounted for 61.76% and 61.61% of the change in total cells throughout the study.

Plots of the residuals for the new regressions of the four stations also revealed no discernible pattern in their distributions and the variation in Y_i not accounted for by linear regression was assumed to be random. At station 4, seven of the 11 Y_i's were over-predicted but the negative residuals were well scattered in their distribution and the deviation did not appear systematic.

Therefore, in addition to duration of slide immersion, the fluctuations in concentrations of four chemical variates of water quality, *i.e.*, orthophosphate, total phosphate, calcium hardness, and total hardness, accounted for greater than 80% of the observed variation in total cells of periphyton/mm^2 of vertical glass slides at the four stations. It follows, under the given experimental conditions of this study, and assuming the linear model to be correct, that considerable time and effort might have been conserved by eliminating from the program measurements of the other 10 physico-chemical variables without consequent

loss of any apparent significant information necessary to statistically predict or define the variations in total periphyton populations in terms of fluctuations in measured environmental variables.

The similarity expressed between regressions of total cell counts of stations 2 and 3, and that between stations 1 and 4 (Tables 16 and 17) is reflective of that already domonstrated in total cell fluctuations (Fig. 8), in fluctuations of *F. virescens* (Fig. 18) and other individual species populations, and in indices of diversity (Fig. 10). In view of the fact that comparable data of the physico-chemical measurements were not statistically different between stations, the importance of subtle, but non statistically significant, variation in ecologically relevant variables is indicated by the regression analyses.

(b) Regression of species diversity against environmental factors

For each station, diversity indices were regressed against a variety of environmental variables using the linear model and stepwise multiple regression. In general, the interrelationships between indices of species diversity and physico-chemical variates were not as well defined or as consistent between stations as those expressed by regression analyses between total cell populations and the same environmental variables.

In the first analysis, regressions were run using Shannon-Weaver's H" (as in equation (3)) against 15 independent physico-chemical variables, including exposure duration. These analyses (Table 18) terminated at each station after 6 or 7 variables had been entered in the regression, accounting for approximately 69% to 88% of the variation in H"; however, only three of the variables entered (exposure duration, orthophosphate, and sulfate) at any of the stations had significant regression coefficients.

Table 18. Summary of the stepwise multiple regression of Shannon-Weaver's diversity index, H", against 15 independent environmental variables for periphyton communities at each sampling station (n=11) in Elk Lake (as in Table 16).

STATION 1	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Variable	Days*	Days* % O_2	Days* % O_2 Mg-H	Days* % O_2 Mg-H NO_2-N	Days* % O_2 Mg-H NO_2-N Cu	Days* % O_2 Mg-H NO_2-N Cu Ca-H	Days* % O_2 Mg-H NO_2-N Cu Ca-H o-PO_4
R	0.6588	0.7310	0.7472	0.7797	0.8582	0.8945	0.9404
s.e. of est.	0.2421	0.2329	0.2424	0.2468	0.2216	0.2158	0.1896
R^2	0.4340	0.5344	0.5584	0.6079	0.7365	0.8000	0.8843
ANOVA	F=6.900* (1,9)	F=4.590* (2,8)	F=2.950 (3,7)	F=2.325 (4,6)	F=2.795 (5,5)	F=2.667 (6,4)	F=3.276 (7,3)

Table 18. Continued.

STATION 2	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
	Days**	Days**	Days**	Days*	Days*	Days
		% O_2	% O_2	% O_2	% O_2	% O_2
			o-PO_4	o-PO_4	o-PO_4	o-PO_4
Variable						
				NO_3-N	NO_3-N	NO_3-N
					NO_2-N	NO_2-N
						pH
R	0.7477	0.8276	0.8388	0.8578	0.8586	0.8594
s.e. of est.	0.1908	0.1711	0.1774	0.1809	0.1977	0.2204
R^2	0.5591	0.6848	0.7035	0.7358	0.7372	0.7386
ANOVA	F=11.413**	F=8.692**	F=5.537*	F=4.177	F=2.805	F=1.884
	(1,9)	(2,8)	(3,7)	(4,6)	(5,5)	(6,4)

Table 18. Continued

STATION 3	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Variable	Days*	Days*	Days*	Days*	Days*	Days*
		$o\text{-}PO_4$*	$o\text{-}PO_4$	$o\text{-}PO_4$	$o\text{-}PO_4$	$o\text{-}PO_4$
			SO_4	SO_4	SO_4	SO_4
				$NO_3\text{-}N$	$NO_3\text{-}N$	$NO_3\text{-}N$
					$T\text{-}PO_4$	$T\text{-}PO_4$
						AT
R	0.7042	0.8505*	0.8694	0.8846	0.9093	0.9110
s.e. of est.	0.2665	0.2094	0.2103	0.2144	0.2096	0.2322
R^2	0.4959	0.7233	0.7558	0.7825	0.8268	0.8299
ANOVA	F=8.852* (1,9)	F=10.458** (2,8)	F=7.222* (3,7)	F=5.398* (4,6)	F=4.775 (5,5)	F=3.252 (6,4)

Table 18. Continued

STATION 4	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
	SO_4**	SO_4**	SO_4*	SO_4*	SO_4	SO_4	SO_4
		Ca-H	Ca-H	Ca-H	Ca-H	Ca-H	Ca-H
			DO	DO	DO	DO	DO
Variable							
				Days	Days	Days	Days
					$o\text{-}PO_4$	$o\text{-}PO_4$	$o\text{-}PO_4$
						T-H	T-H
							NO_2-N
R	0.7568	0.7797	0.7883	0.7984	0.8020	0.8050	0.8335
s.e. of est.	0.1595	0.1621	0.1702	0.1799	0.1956	0.2171	0.2335
R^2	0.5728	0.6079	0.6214	0.6374	0.6432	0.6480	0.6946
ANOVA	F=12.065** (1,9)	F=6.201* (2,8)	F=3.830 (3,7)	F=2.637 (4,6)	F=1.802 (5,5)	F=1.227 (6,4)	F=0.975 (7,3)

*Significant at $P \leq 0.05$ but not at $P \leq 0.01$
**Significant at $P \leq 0.01$

Increasing the number of variables included (from step to step) in the regression by one resulted in a significant increase in the multiple R only at step 2 of the analysis of station 3 data. At stations 1 and 4 a significant percentage of the variation in species diversity could only be accounted for by linear regression on the physico-chemical variables entered up to step 2. At station 2, the F-test for regression was significant ($P \leq 0.05$) up to step 3, while the same test was significant up to step 4 at station 3. Under these circumstances, it was felt that the analysis could not be used to obtain a reliable predictive equation for each station which would satisfy the previously stated statistical criteria. Hence the data is presented in summary form (Table 18) for comparative purposes only and to illustrate the ordering of the entered variables. It may be concluded that exposure duration was the most important of the measured variables in accounting for the variation in H" at stations 1, 2, and 3; while sulfate concentrations were of greater importance at station 4 in accounting for changes in species diversity of periphyton communities developed in this particular location of the lake (see also Table 15).

Surprisingly, graphical analysis of the residuals did not suggest the occurrence of any non random variation in $\hat{Y}$ not accounted for by the regression at any of the four stations.

Comparison of the ordering of environmental variables in Table 18 does not lead to any obvious grouping of the stations on the basis of similarity in variables entered into the regression analyses. The lack of similarity between stations 2 and 3 or 1 and 3, in light of their similar patterns in distribution shown in Figure 10, suggests, among other things, that (1) many factors effect diversity and the combination of

environmental factors need not be consistent, (2) the statistically non significant variation in measured physico-chemical variates was biologically significant, (3) ignoring station 4, only exposure duration is important in determining species diversity in periphyton samples of this study, or (4) the variables measured in this study have little effect on the change in H". As previously noted (Table 15), station 4 appears distinctly different from the others in the regression analysis; the significance of sulfate concentrations to periphyton species diversity is not clear. Throughout the entire sampling period there was little variation in actual values of H" at all stations. Mean values ($\bar{Y}$) for stations 1 and 3 were similar at 2.5949 (±0.3052 S.D.) and 2.6049 (±0.3561) with less variation occurring at station 1; while mean species diversity values of 2.7196 (±0.2726) and 2.7557 (±0.2315) occurring at stations 2 and 4 were slightly higher, indicating that these two station populations were more even in their species distributions throughout the study.

The simple linear relationship between H" and exposure duration may be expressed for three of the stations by the following equations: (1) station 1, $\hat{Y}=2.9591-0.0059X_1$; (2) station 2, $\hat{Y}=3.0889-0.0060X_1$; and (3) $\hat{Y}=3.0592-0.0073X_1$, where X_1= exposure duration in number of days. The comparable relationship expressed between diversity and sulfate concentrations (X_2) at station 4 is described by the equation $Y=3.6105-0.1021X_2$.

Since correlation analysis (Table 15) showed H" to be significantly correlated with N or total cell populations at stations 1, 2, and 3, the regression analyses were re-run for all stations where total cells (log transformed) was the sixteenth independent X-variable. The results

of the regression analysis for station 4 were exactly the same as that given in Table 18, indicating that at this station the change in H" could be more readily associated with variation in the physico-chemical variates, sulfate, calcium hardness, dissolved oxygen, exposure duration, orthophosphate, total hardness and nitrite-nitrogen, than with change in total cells. On this basis it may be concluded that substrate area was not limiting at station 4 to the same extent as at other stations; it appears to have had little or no bearing on inducing species interaction and reducing diversity. Examination of Figure 9 illustrates that total cell populations at station 4 were generally lower than those occurring at other stations. If the size of these populations did not reach a critical level where space became limiting, diversity would not have been effected; this may account for some of the unexplained variation at station 4 previously shown in Figure 10.

The results were not similarly affected at the other three stations. For example, at stations 1 and 3, total cells accounted for 48.58% and 55.40% of the variation in H" (Table 19) compared with 43.40% and 49.59% accounted for by exposure duration in Table 18. At station 2, exposure duration, the first variable entered accounted for 55.91% of the variation (as also in Table 18) in H", whereas total cells, the fifth variable entered, accounted for 4.04% of the variation. At all stations, the only significant increase in the multiple R occurred at step 2 in the station 1 analysis when magnesium hardness was entered. Similarly, aside from sulfate at station 4, only three variables, total cells, magnesium hardness, and exposure duration, had significant regression coefficients whereas the analysis included 7 or more variables at each station. The first three steps of the multiple regression analyses

Table 19. Summary of first three steps of stepwise multiple regression of periphyton species diversity, H", against 16 X-variables, including total cell populations (logarithmically transformed), at stations 1, 2 and 3 in Elk Lake (symbols and statistical tests as in Table 16).

STATION	1			2			3		
STEP	1	2	3	1	2	3	1	2	3
VARIABLE	Total Cells**	Total Cells** Mg-H*	Total Cells** Mg-H* NO_2N	Days**	Days** % O_2	Days** % O_2 o-PO_4	Total Cells**	Total Cells Days	Total Cells Days SO_4
R	0.6967	0.8484*	0.8819	0.7477	0.8276	0.8388	0.7443	0.8142	0.8654
s.e. of est.	0.2308	0.1807	0.1720	0.1908	0.1711	0.1774	0.2507	0.2312	0.2133
% of Y	8.89	6.96	6.62	7.02	6.29	6.52	9.62	8.87	8.19
R^2	0.4858	0.7198	0.7778	0.5591	0.6848	0.7035	0.5540	0.6629	0.7490
ANOVA	F=8.488* (1,9)	F=10.274** (2,8)	F=8.169 (3,7)	F=11.413** (1,9)	F=8.692** (2,8)	F=5.537* (3,7)	F=11.178** (1,9)	F=7.865* (2,8)	F=6.962* (3,7)

* Significant at $P \leq 0.05$ but not at $P \leq 0.01$
**Significant at $P \leq 0.01$

for stations 1, 2 and 3 are summarized in Table 19. The interchanging of the position of total cells with exposure duration in these analyses in largely due to the fact that exposure duration is also significantly positively correlated with total cell populations at all stations (Table 15). While the addition of total cells to the analysis may change the position of exposure duration and other variables in the analysis, the particular variables entered in the regression equations at each station remained remarkably constant, and no significant increase occurred in the percentage variation in H" accounted for.

Regression analyses for species diversity were run a third time, where the sixteenth X-variable included was raw total cell counts. However, since the results of these analyses lent little information to further facilitate interpretation of the relationships between species diversity and environmental data, they are not included here.

(c) Interpretation of multiple regression results

I believe that multiple regression has been applied in similar studies of aquatic communities in basically two different contexts: the predictive and the descriptive. The predictive application of multiple regression analysis is best illustrated by Table 17, and by the work of McIntire (1968a). In this context, the results of the stepwise procedure are subject to further statistical analysis in an effort to make the final equations statistically reliable, *i.e.*, to ascertain a good predictor equation the use of which will give reproducible results in subsequent experimentation. In this approach the linear model may be tested for lack of fit and further modification may be undertaken to develop new, more realistic models. The descriptive

context, is best illustrated by the work of Goldman *et al.* (1968), and Platt and Subba Rao (1970) where a minimum number of statistical tests are applied to the computer output of stepwise multiple regression analyses. The linear model provides guidelines for further experimentation, acts as a variable screening device, and serves to summarize (Draper & Smith 1966) and objectively order the experimental variables in terms of their apparent importance in relation to the dependent variables. This approach is illustrated here by Tables 16 and 18 (modified after Goldman *et al.* 1968) where a summary of all the data is presented with a minimum amount of subsequent analysis. In the present study, those variables of Table 17 represent statistically reliable results on the basis of the data collected. Re-running of the experiment can be expected to yield comparable results in that changes in exposure duration, phosphate concentrations, and hardness values should account for a large amount of the variation in total cells, and these variables should be of equal importance if the same variables measured here, are chosen for measurement in subsequent experiments. Conversely, the entire data set of Tables 16 and 18 provide screening, enable comparison of station differences, *etc.*, but do not provide statistically reliable regression equations which can be numerically applied in subsequent analyses to predict periphyton standing crop or species diversity. In view of the problems of replicating an experiment such as this, or justifiably comparing results with other published accounts, multiple regression is best applied in studies of this nature in a descriptive context until such time as the ecological meaning of correlation and other interrelationships is clearly defined; in ecological studies, multiple regression analysis generally has a wider application

in a descriptive context.

It should be noted that regression analysis does not provide cause and effect information. In the present study, when an X-variables is said to account for a certain percentage of the variation in Y, it means that given the linear model, and the measured experimental data, that a percentage of the change in Y can be predicted on the basis of, is associated with, or can be described in terms of the change in an environmental variable. However, other variables added to the analyses might better describe the variation in Y. Similarly, increasing the number of X-variables in the analysis will increase the multiple R or R^2, *i.e.*, if adding other variables such as Secchi disk and compensation point causes an increase in R^2, it does not necessarily mean that light causes a decrease in diversity. However, if other evidence has been recorded substantiating this relationship, the results of regression analysis may be used to corroborate the findings.

In the present study, multiple regression analysis has shown that at least 80% of the variation in standing crop, expressed as total cells of periphyton/mm^2, can be statistically described and reliably predicted in terms of two or three measured environmental factors at all four stations. In addition, the ordering of X-variables suggests that the relationships between total cells and measured physico-chemical variates are more similar between stations 2 and 3, than with the other stations, although common variables are included in the regression analyses for all four stations. Of the independent variables measured in the study and used in regression, exposure duration is of greatest importance in predicting total cell populations at all stations. The remaining variables entered into the regressions are approximately in the following decreasing order of importance: orthophosphate, calcium hardness, total

phosphate, percent oxygen saturations, nitrite-nitrogen, magnesium hardness, dissolved oxygen, sulfate, copper, and air temperature. Some of these are significantly intercorrelated (Table 15).

Conversely, the relationship between structure of the same periphyton communities, as expressed by species diversity or H", and the measured environmental data is not so readily summarized. Considerable station differences occur in entered variables of statistical significance and station 4 appears to be distinctly different from the other three. Reliable predictive equations are not feasible. Similarly, variables entered in the regression analysis are seldom statistically significant and are unable to statistically describe more than 72% of the variation in species diversity at any station. The variables of greatest importance, associated with the change in H" are exposure duration and total cell populations at stations 1, 2, and 3; and sulfate at station 4. Other variables of some importance in the regression analyses are, in approximate decreasing order as follows: orthophosphate, magnesium hardness, percentage oxygen saturation, calcium hardness, dissolved oxygen, nitrate-nitrogen, total phosphate, total hardness, nitrite-nitrogen and pH.

SUMMARY

1. Elk Lake (the lake studied) is, on the basis of general characteristics (morphometry, water chemistry and biota), a small, relatively eutrophic, warm monomictic second order lake, similar to other lakes in the Insular Lowland Limnological Region.

2. Three sets of data were collected: one of physico-chemical variates, one of periphyton counts, and one of net plankton which consisted of presense and absence data as well as estimates of relative abundance for individual diatom taxa. Field collections were made at approximately one month intervals from August 1967 to January 1968 at four stations in the littoral region. Physico-chemical variates were measured at two depths.

3. With the exception of a higher surface total phosphate concentration at all stations in October, physico-chemical factors showed no variation between depths, or between stations, at any one time. Seasonal changes were marked, however, in all variables except calcium hardness and orthophosphate. Turnover was completed by the middle of November, and during the study period transparency, temperature, oxygen, pH, nitrite and nitrate decreased, and hardness increased. Phosphate and copper fluctuated irregularly, and with no clear pattern. The changes observed were normal for a lake of this type, except for those of nitrate, due possibly to a concentration effect related to high summer temperatures and evaporation, followed by dilution due to fall flooding. Clear signs of organic pollution were absent.

4. Cluster analysis of net plankton indicated that differences between lakes were greater than those found within lakes, and that of the latter, seasonal differences were distinctive, while station differences were not.

Net plankton thus closely reflect differences in environmental factors. A close relationship was found to exist between net plankton and periphyton. One quarter of the species of the periphyton are found in the net plankton, and is supplied from it. It follows that both need to be studied simultaneously in periphyton studies.

5. Periphyton is best sampled by the submersion of glass slides in suitably designed exposure-frames. Of the three stages of the experimental design (sampling, filtering and counting), greatest variance is introduced by counting; thus the counting of a minimum of 30 Whipple fields is likely not excessive. Differences in depth of the exposure-frames (1.83 to 2.75 m), within the euphotic zone of the littoral are not significant. Colonization of slides is such that near stabilization of the resultant communities is reached in about four weeks, and that a submersion time of about a month is quite satisfactory. Cell counts are best made normal in distribution by means of a $\log_e (x + 1)$ transformation. When this is done, variance is least in total cell counts, greater in species counts, and greatest in conversion to diversity indices.

6. The Elk Lake periphyton biota is composed of 41 species of diatoms, chlorophytes, cyanophytes, and protozoans. Diatoms dominated both in number of species (28), and in standing crop. Twelve species of the periphyton also occurred in the net plankton. After one week's submersion in Elk Lake, slides were covered with a thin bacterial slime, but by the second week this was replaced by a diatom community made up of species of *Cocconeis, Navicula, Achnanthes, Gomphonema, Fragilaria,* and *Melosira,* that thereafter grew thicker, but changed little in species composition. Growth of the periphyton community was logistic,

reaching equilibrium after about 75 days, between September 5 and November 19, but after only about 27 days, between November 19 and January 19. Equilibrium is attained through sloughing off of older parts of the community, followed by recolonization. *Achnanthes* and *Cocconeis* appear to undergo a form of competetive exclusion, in that either species may dominate at equilibrium, a consequence of factors that are not clearly understood.

7. Periphyton communities of one month's duration show highest standing crops in November, a consequence it appears of turnover in the weeks preceding. Prior to turnover, the diatom component of the net plankton is dominated by *Fragilaria crotonensis*, while the periphyton is dominated by *Achnanthes minutissima* and *Cocconeis placentula*. Following turnover, *Asterionella formosa* and *Melosira* spp. replace *Fragilaria* in the net plankton, and *Fragilaria* and *Asterionella* increase in percentage abundance in the periphyton. It appears that with turnover, *Melosira* is resuspended from the lake bottom, and that *Asterionella* and *Fragilaria* settle out of the plankton and enter the periphyton. These relationships do not appear however in periphyton communities exposed for durations of up to 135 days, which are dominated throughout by *Cocconeis* and *Achnanthes*.

8. The above patterns are common to all stations. The use of a multifactorial analysis of variance indicated however that significant differences existed between stations in total cells, individual species counts, and diversity indices; this in spite of the fact that no such differences were found in the physico-chemical variables, or the net plankton. It follows that the detailed responses of the periphyton to subliminal environmental changes are both more accur-

ately measured or more variable, and much more local in effect. In almost all cases, stations 2 and 3 were most similar, while stations 1 and 4 show lower degrees of similarity to each other. These differences may be related to differences in solar exposure or to positioning in the lake, or possibly to local water currents.

9. Multiple regression has been used in aquatic studies in both a predictive and a descriptive manner. Both applications have been made here, but the predictive is dangerous in view of the present lack of understanding of the true dynamics of aquatic ecosystems. Co-linearity (interdependence) of the independent variables, and the tendency to assume cause and effect relationships from correlations are common in the literature, and doubtless have led to error. In the present study, positive seasonal correlations were found between total cells and copper, pH, temperature, nitrate and nitrite, and negative ones with oxygen, hardness, and orthophosphate.

10. Stepwise multiple regression demonstrated however that of these, more than 80 percent of total variation in total cells at all four stations could be accounted for by length of exposure duration of the periphyton communities, phosphate (ortho and total), and hardness (calcium and total). It is reasonable to assume that these are at least among the primary factors limiting periphyton growth.

11. Diversity indices are much used in current limnological studies. Of the indices, diversity in the form of Shannon-Weaver's H" has had widest application, and is emphasized here. In general, H" in Elk Lake periphyton tends to decrease as total cells increase, thus decreasing with succession and the season, and most likely indicates reduction in species due to increased competition. Its range is from

2 to 3 bits/cell/mm^2, indicating a condition intermediate between Margalef's oligotrophy and eutrophy. Two derivatives of H", J" and E follow the same general pattern. Analyses of diversity indices suggest they are in reality far less useful than commonly believed. H" fails to measure either the effect of change in the total numbers of cells (N), or of the two thirds of those species rarest in the sample. Periphyton samples (at least those from Elk Lake) are characterized by many rare species, and thus lose much information in their transformation to values of H". Further, the values obtained vary with the time scale employed, and the extent to which the biota treated exploit the total niche hyperspace. In all analyses, the use of diversity indices gave more variable and less consistent results than did either total cells or individual species counts.

12. Both physico-chemical data and net plankton counts showed significant changes with season, but not by station, whereas periphyton counts (as total cells, individual species counts, and diversity) showed real differences between stations as well. The increased variability found in periphyton samples could be due either to the greater amount of information they carry, or to greater amounts of error inherent in their collection. The relatively crude methods employed, however in collecting and testing for physico-chemical factors, and of sampling for net plankton, together with the relatively low variance found within periphyton samples taken at a single time and station suggest that they are in fact far more sensitive estimators of subliminal environmental factors than the others. Since further, the periphyton include the sum of effects operative over the whole of the period of substrate colonization, they appear

to constitute a refined, sensitive indicator of long term variation in environmental parameters and of the general ecologic condition of the system.

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APPENDIX I

Grouping Plankton Samples By Numerical Analysis[1]

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[1] The following account represents the main text of a paper by the author published under the same title in the journal *Hydrobiologia* (see p. 176).

CONTENTS

INTRODUCTION

Any detailed investigation of the periphyton necessitating the enumeration of taxa, tends to yield large quantities of data which are relatively complex and not readily interpreted by traditionally subjective methods of data analysis alone. Such methods of sample data comparison and manipulation typically entail lengthy descriptive interpretation which, although quite essential, does not lend itself to unbiased comprehensible communication of biological data or concepts (to non biologists) and hence such important information is apt to be omitted from consideration when water quality criteria or standards are established.

To date little statistical treatment and almost no numerical analysis of periphyton data has been reported in the literature. As a result, an attempt was made to investigate an objective method of sample data comparison and to evaluate this method using qualitative plankton data from two Vancouver Island lakes with a view to assessing both its application to periphyton results, and its wider implications in problems of lake classification. The plankton data, unlike the periphyton, were easier to obtain (already in a usable table form) and provided an ideal test case for improving objective methods of data manipulation.

SAMPLE DATA COMPARISON

Increased computer accessibility has led to the advancement of numerical methods of sample data analysis and comparison. While traditional plankton studies (well reviewed by Marsh 1903; Birge & Juday 1922; Lund 1965; Hutchinson 1967; and others) have become more quantitative, little change has occurred in the actual data manipulation or presentation. A literature search revealed few plankton investigations which employed computer techniques and numerical methods of sample comparison. Whittaker and Fairbanks (1958) used quantitative data and an index of percentage similarity (Whittaker 1967) to identify copepod community types in the Columbia Basin region. Fager and McGowan (1963) also used an index of affinity between taxa (Fager 1957) to distinguish zooplankton species groups in the North Pacific. Other workers have employed alternative objective methods of data analysis such as diversity indices (Margalef 1961; Patten 1962; Hulburt 1963), correlation coefficients (Williamson 1961), and multivariate analysis (Cassie 1963) to plankton data but as yet these methods are relatively new in their application and their use is not widespread.

In view of recent advances in the fields of numerical ecology (Koch 1957; Kontkanen 1957; Dahl 1960; Greig-Smith 1964; McIntosh 1967) and numerical taxonomy (Sneath & Sokal 1962; Sokal & Sneath 1963; Sokal 1965), it was decided to apply a similar analysis to plankton data and assess its application in grouping samples on the basis of biotic similarity.

To measure the degree of floristic similarity or relationship between two different stands of vegetation, plant ecologists have developed

coefficients of association[1]. The original coefficient, Jaccard's Coefficient of Community (Williams 1949; Greig-Smith 1964), is defined by the equation

$$CC = \frac{C}{n_1 + n_2 - C} \quad x \quad 100 \qquad (7)$$

where n_1 is the number of species in the first sample, n_2 is the number of species in the second sample, and C is the number of species common to both. The higher the percent value of the Coefficient of Community (hereafter CC), the closer the similarity between the two samples.

The purpose of this aspect of the present study is to give the results of a numerical analysis of qualitative data from plankton tows taken in two lakes, and to discuss these results in terms of the utility this approach may have in subsequent limnological studies. Jaccard's CC was used because of its simplicity and the availability of a suitable computer program. It was felt that this coefficient would be adequate for initial investigation.

It should be emphasized that this study employed presence and absence data alone. The use of qualitative or quantitative data in objective methods of classification remains to be clarified (Sorensen 1948; Kershaw 1957, 1964, 1967; Fager 1963; Greig-Smith 1964; Lambert & Dale 1964). In view of this controversy, the choice of coefficients for anlysis and the type of data to be used should be dictated by the aims of each separate ecological investigation.

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[1] Coefficients of association are also known as coefficients of similarity or relationship, or matching coefficients and are distinct from coefficients of correlation and distance (Sokal & Sneath 1963). The terms association, similarity and relationship are used interchangeably here.

LOCATION AND DESCRIPTION OF STUDY AREAS

The location and parameters of the two lakes are listed in Table 20. Both lakes are used as public water supplies and are subject to some drawdown. Water level fluctuations in Sooke Lake are quite severe, causing some shoreline erosion.

Elk Lake, situated in a well established residential area with some agricultural development, is used heavily for recreation purposes. A program of aquatic "weed" control using chemical herbicides (*e.g.* sodium arsenite, Aquathol, copper sulfate, and 2,4-D) was in effect for at least 4 years between 1962 and 1966. In contrast, Sooke Lake occurs in a relatively undisturbed watershed where some logging is permitted, but the general public is denied access. Copper sulfate has been applied.

Elk Lake is very eutrophic, being in a state of more or less perpetual phytoplankton bloom throughout the year, while Sooke Lake may be considered much more oligotrophic, having only 1 or 2 slight and short term blooms per year.

Table 20. Location and parameters of Sooke and Elk lakes.

	Sooke Lake	Elk Lake
Location	48° 30' - 48° 35' N lat. 123° 45' - 123°40' W long. 32.18 km N.W. of Victoria, British Columbia, Canada	48° 30' - 48° 35' N lat. 123°25' - 123°20' W long. 12.07 km N.W. of Victoria, British Columbia, Canada
Limnological Region[1]	Coast and Insular Mountain	Insular Lowland
Elevation (above sea level)	173.24 m	59.78 m
Watershed Area	7,249.50 ha	1,146.56 ha
Surface Area	428.09 ha	207.77 ha
Maximum Depth	61.00 m	18.30 m
Mean Depth	19.95 m	7.63 m
Volume	86,565,094.71 cu m	15,819,509.25 cu m
Shoreline Perimeter	20,923.00 m	9,662.40 m
T.D.S.[2]	53 ppm	105 ppm

[1] Based on Northcote and Larkin's (1956) classification system for lakes of British Columbia.

[2] Recordings of total dissolved solids were made on May 14, 1955 in Elk Lake and on May 17, 1958 in Sooke Lake by the Provincial Department of Recreation and Conservation, Fish and Wildlife Branch. More recent TDS recordings of 120 ppm for Elk Lake (December 14, 1969) and 60 ppm for Sooke Lake (February 4, 1970) were obtained using a Beckman® RA-2A condictivity cell.

SAMPLING PROCEDURE

Five stations were established in the littoral region of Sooke Lake within 9 m of the shoreline. These were sampled over a 4-month period at 36-day intervals. Similarly, in Elk Lake four littoral stations within 7 m of the shoreline were sampled at approximately 34-day intervals over a 6-month period.

The samples were taken using a standard 10-inch (25.4 cm) diameter plankton net of No. 25 bolting silk. The net was towed from a boat at approximately 5-8 km p h for about 5 minutes. These surface tows were to serve as species checklists for a quantitative plankton investigation to follow. Each sample was split immediately upon collection, one half was preserved in 4% formalin and the other kept fresh to facilitate laboratory identification.

This method of collection, although it proved satisfactory, was not initially designed for numerical analysis. Quantitative samples of large volumes of lake water would give more reliable results.

In the laboratory, the fresh samples were scanned first and then five 0.05 ml. aliquots of each well-shaken preserved sample were examined under the microscope. An attempt was made to take all the net organisms into account. Algae were identified to species where possible and zooplankters were identified at least to Class. When the organisms defied accurate identification, they were photographed, described in detailed note form and given appropriate "names" (Dickman 1968).

Lists of the net organisms, indicating presence (x) and absence were prepared for each station and collection date (Tables 21 and 22).

Table 21.

ELK LAKE

Plankton net organisms	Date / Station	A 1234	S 1234	O 1234	N 1234	D 1234	J 1234
Anabaena circinalis		xxxx	xxxx	xxxx	xxxx		
Anabaena flos-aquae		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Anabaena limnetica		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Aphanizomenon flos-aquae		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Aphanocapsa sp.		x x	xxx	xxxx	xxxx	xxx	xx
Asplanchna sp.		xxxx	x			x	xx
Asterionella formosa				xxxx	xxxx	xxxx	xxxx
Botryococcus braunii		xxxx	xxxx	x xx	x xx	xx	xx x
Brachionus sp.		x		x		x	xxx
Ceratium hirundinella		xxxx	xxxx	xxxx	xx		
Chytrids		x	xx	x x	xxxx	xxxx	xxxx
Closterium sp.					x	x	x
Coelosphaerium naegelianum		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Coleochaete sp.			x				
Copepoda		xxxx	xxxx	xxxx	xxxx	xxxx	xxx
Cosmarium obtusatum		x	x				
Daphnia sp #1		xx x	xxx	xx x	xxxx	xx	x x
Dictyosphaerium pulchellum		x	x x	x x	xxxx	x xx	xx
Difflugia sp.		xx x	x	x	xx		x
Dinobryon divergens						xx	xxxx
Epistylis sp.		xxxx	xxxx	x	x	x	
Eudorina elegans		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Fragilaria crotonensis		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Fragilaria virescens		xxx	xxxx	xxxx	xxxx	xxx	xx
Gloeotrichia echinulata		xxxx	xxxx				
Gonium sp.			x	x		x	
Keratella cochlearis		xxxx	xxxx	xxxx	xxxx	x xx	xxxx
Keratella cochlearis var. *tecta*						x	
Mallomonas caudata			x	xxxx	xxxx	x	x xx
Melosira sp.		xxx	xxxx	xxxx	xxxx	xxxx	xxxx
Merismopedia sp.		x					
Microcystis aeruginosa		xxxx	xxxx	xxxx	xxxx	x xx	xxxx
Nauplius (Copepoda)		x					
Notholca sp.		x				x	x xx
Oocystis Borgei					x		xx
Oscillatoria sp.		xx	x x	xx x			
Pandorina morum		xxxx	xxxx	xxxx	xxx	x xx	xxx
Pediastrum Boryanum		x x		x	xx	x x	x
Phacus sp.		x			x x		x

Table 21. Continued.

Plankton net organisms	Date Station	A 1234	S 1234	O 1234	N 1234	D 1234	J 1234
Pinnularia sp.		x					x
Pleodorina californica						x	
Polyarthra trigla		xxxx	xxx	xxxx	xxxx	xxxx	xxxx
Quadrigula sp.			xxxx	xxxx			
Scenedesmus sp.		x					
Selenastrum bribraianum			x		x		
Spirogyra sp.		xxx	x x	x	x		
Staurastrum sp. #1		xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
Stephanodiscus niagarae		xxx	xxxx	xxx	xxxx	xxxx	xxxx
Stigeoclonium sp.		x		x			
Surirella sp.		x		xx x	x x	x	
Synura uvella							xxxx
Trichocera sp.		xxx	xxxx	xxxx	xx		
Ulothrix sp.		x					
Volvox aureus		xxxx	xxxx	xxx	xxxx	x x	xxxx
Vorticella sp.		xxxx	xxxx	xxxx	x	xx	x x

Table 22.

SOOKE LAKE

Plankton net organisms	Date / Station	A 12345	O 12345	N 12345	D 12345
Actinosphaerium-like sp.		x	xxxxx	xxxxx	xxxxx
Amphora sp.		x			
Anabaena flos-aquae		xxxx	xxxxx	xxxxx	xx xx
Anabaena limnetica		x			
Aphanocapsa sp.		xxxxx	xxxxx	xxxxx	xxxx
Aphanothece sp.		xxxxx	xxxxx	xxxxx	xx xx
Asplanchna sp.		x		x	
Asterionella formosa		xxxxx	xxxxx	xxxxx	xxxxx
Asterococcus sp.		xxxxx			
Botryococcus braunii		xxxxx	xxxxx	xxxxx	xxxx
Brachionus sp.				x	x
Bulbochaete sp.		x			x
Ceratium hirundinella		xxxxx	xxxxx	xxxx	x
Chaetosphaeridium globosum			x		
Characiopsis sp.		xx x	xxxx	xxxxx	x
Chroococcus limneticus		xxxxx	xxxxx	xxxxx	x xx
Chrysocapsa planctonica		xxxxx	xxxxx	xxxxx	x xx
Closterium sp.		x x	x	x	x
Cocconeis placentula				x	
Coelastrum cambricum				x	
Coelosphaerium-like sp.		xxxxx	xxxxx	xxxxx	xx
Copepoda		xxxxx	xxxxx	xxxxx	xxxxx
Cosmarium obtusatum		x xxx	x xx		x
Cyclotella sp.					x
Cymbella sp.			x x		
Dactylococcopsis sp.		x			
Daphnia sp. #2		xxx x	x x x	x xx	xxxxx
Dictyosphaerium pulchellum		xxxxx	xxxxx	xxxxx	x
Difflugia sp.		x xx	x	x	
Dinobryon divergens		xx	xx	xxxxx	xxxx
Epithemia sp.		x	x x	x x	x x
Eudorina elegans			x x		
Eunotia pectinalis			x x		
Fragilaria virescens		x		x	x x
Gloeocapsa punctata			xx		
Gloeocystis sp.		xxxxx	xxxxx	xxxxx	xx xx
Gloeotrichia echinulata		xxxxx	x	x	
Gomphonema acuminatum var. *coronatum*		x			x
Gonatozygon sp.			x	xx	
Gonium sp.			x x		
Hydrachna halacarus			x		
Kellicottia longispina		xxx	xxxxx	xx	x x
Keratella cochlearis			x	x	

Table 22. Continued.

Plankton net organisms	Date / Station	A 12345	O 12345	N 12345	D 12345
Mallomonas caudata		x	xxxxx	xxxxx	xxxxx
Melosira sp.		xxx	xxxxx	xxxxx	xxxx
Merismopedia sp.		x		x xx	
Micrasterias sp.		xxx		x	
Microcystis aeruginosa		x	x		
Monostyla sp.			x		
Mougeotia sp.		x	xxx	x	xxxx
Navicula sp.			x x x	x	
Netrium sp.				x	
Nostoc sp.		x			
Oocystis Borgei		x xx	x	x	
Oscillatoria sp.		xxxxx	xxxxx	xxxxx	
Pediastrum Boryanum		xx		x	x
Pedinella sp.		xx xx	xxxxx	xxxxx	xxxx
Peridinium sp.		xx	xxxxx	xxxxx	xxxx
Phormidium sp.		x	x		
Pinnularia sp.		xx xx	x xxx	x xx	x xx
Polyarthra trigla		xxxxx	xxxxx	xx	
Quadrigula sp.			xx	xx xx	x
Scenedesmus sp.				x	
Spirogyra sp.		x		x	x x
Spondylosium lutkemulleri		x	x	xxxxx	x xx
Staurastrum sp. #1		xxxxx	xxx x	xxxxx	xx x
Staurastrum sp. #2		xxxxx	xxxx	xxxxx	x xx
Stephanodiscus niagarae			x	x x	xx
Surirella sp.		xxx	xxxxx	xxxxx	xxxx
Synedra radians		xxxxx	xxxxx	xxxxx	xxxxx
Synedra ulna			xx	xx x	xx xx
Synura uvella			xxxxx	xxxxx	x xx
Tabellaria fenestrata		xxxxx	xxxxx	xxxxx	xxxxx
Tabellaria flocculosa		x	x	xxxx	xxxx
Tolypothrix sp.		x	xxx		xxx
Tribonema sp.		xx	x		
Trichocera sp.					x
Ulothrix sp.			xx	xx	x
Vorticella sp.		xxxxx	xxxxx	xxxxx	x xx
Xanthidium sp. #1		xxxxx	xxxxx	xxxxx	
Xanthidium sp. #2		xxxxx	xxxxx	x xx	x
Xanthidium sp. #3		xxxxx	x x	x	x x
Zygnema sp.		x	x		xxx

NUMERICAL ANALYSIS

Plankton taxa, listed alphabetically and given code numbers (Table 23) to facilitate analysis, represented a possible total of 100 different characters to be compared per sample. Samples were numbered as in Table 24.

The percent similarity for all possible combinations of pairs of samples was computed using a program designed by H. Jacobsen, formerly of the University of Victoria[1]. This program uses the weighted pair-group method of cluster analysis (Sokal & Sneath 1963) and Jaccard's CC.

In view of the obvious data variation, it was decided to forego the usual final shaded matrix[2] and construct a dendrogram directly from the clustering results. A matrix of the raw, unclustered CC's occurs in Table 25. The data suggested that the samples would show more variation in biotic composition (or less similarity and therefore lower CC's) over the sampling period than from station to station in any one lake. Therefore the samples were arranged as closely as possible into sequential site-date order in the dendrogram (see Table 24 and Figure 19).

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[1] Current address: 16 St. Francis Drive, Chico, California, U.S.A.

[2] Normally the CC's calculated by the computer program are arranged in a matrix or trellis diagram. The similarity values are then divided into class intervals, each class being represented by a different degree of shading with the highest similarity class being the darkest. The order of the samples is manipulated until the highest similarity values are located along the diagonal. Triangular area of dark shading occur, indicating groups of samples which have the highest percent similarity. The order of the samples on the diagonal of this final shaded matrix determines the sample order in the dendrogram which is prepared from the clustering results (Kontkanen 1957; Sokal & Sneath 1963; Sokal 1966; Colless 1967; Moss 1967).

Table 23. Numbered alphabetical list of the possible total number of plankton net organisms or sample characters and their constancy values (*i.e.*, number of times a taxon occurred in a set of samples expressed as a percentage of the total number of samples in the set).

Taxa Code No.	Constancy (%) E+S 44	E 24	S 20	Lake Sample Total	Plankton Net Organisms
1	36.4		80		*Actinosphaerium*-like sp.
2	2.3		5		*Amphora* sp.
3	36.4	67			*Anabaena circinalis* Raben.
4	95.5	100	90		*Anabaena flos-aquae* (Lyngb.) Bréb.
5	56.8	100	5		*Anabaena limnetica* G.M. Smith
6	54.5	100			*Aphanizomenon flos-aquae* (Lyngb.) Ralfs
7	84.1	75	95		*Aphanocapsa* sp.
8	43.2		95		*Aphanotheca* sp.
9	22.7	33	10		*Asplanchna* sp.
10	81.8	67	100		*Asterionella formosa* Hassall
11	11.4		20		*Asterococcus* sp.
12	86.4	79	95		*Botryococcus braunii* Kütz.
13	18.2	25	10		*Brachionus* sp.
14	4.5		10		*Bulbochaete* sp.
15	65.9	58	75		*Ceratium hirundinella* (O. Müll.) Duj.
16	2.3		5		*Chaetosphaeridium globosum* (Nordst.) Kleb.
17	29.5		65		*Characiopsis* sp.
18	40.9		90		*Chroococcus limneticus* Lemmer.
19	40.9		90		*Chrysocapsa planctonica* (W. & W.) Pasch.
20	38.6	71			Chytrids
21	18.2	13	25		*Closterium* sp.
22	2.3		5		*Cocconeis placentula* Ehr.
23	2.3		5		*Coelastrum cambricum* Arch.
24	38.6		85		*Coelosphaerium*-like sp.
25	54.5	100			*Coelosphaerium naegelianum* Unger
26	2.3	4			*Coleochaete* sp.
27	97.7	96	100		Copepoda
28	22.7	8	40		*Cosmarium obtusatum* Schmidle
29	2.3		5		*Cyclotella* sp.
30	4.5		10		*Cymbella* sp.
31	2.3		5		*Dactylococcopsis* sp.
32	38.6	71			*Daphnia* sp. #1 (small)
33	34.1		75		*Daphnia* sp. #2 (large)
34	68.2	58	80		*Dictyosphaerium pulchellum* Wood
35	31.8	38	25		*Difflugia* sp.
36	43.2	25	65		*Dinobryon divergens* Imhof
37	25.0	46			*Epistylis* sp.
38	15.9		35		*Epithemia* sp.
39	59.1	100	10		*Eudorina elegans* Ehr.
40	4.5		10		*Eunotia pectinalis* (Kütz.) Raben.
41	54.5	100			*Fragilaria crotonensis* Kitton

Table 23. Continued.

Taxa Code No.	Constancy (%) E+S 44	E 24	S 20	Lake Sample Total	Plankton Net Organisms
42	54.5	83	20		*Fragilaria virescens* Ralfs
43	4.5		10		*Gloeocapsa punctata* Naegeli
44	43.2		95		*Gloeocystis* sp.
45	34.1	33	35		*Gloeotrichia echinulata* (J.E.Sm.) P. Richt.
46	4.5		10		*Gomphonema acuminatum* var. *coronatum* (Ehr.) Raben.
47	6.8		15		*Gonatozygon* sp.
48	11.4	13	10		*Gonium* sp.
49	2.3		5		*Hydrachna halacarus* O. Müll.
50	27.3		60		*Kellicottia longispina* Kellicott
51	56.8	96	10		*Keratella cochlearis* Gosse
52	2.3	4			*Keratella cochlearis* var. *tecta* Gosse
53	65.9	54	80		*Mallomonas caudata* Iwanoff
54	90.9	96	85		*Melosira* sp.
55	11.4	4	20		*Merismopedia* sp.
56	9.1		20		*Micrasterias* sp.
57	56.8	96	10		*Microcystis aeruginosa* Kütz.
58	2.3		5		*Monostyla* sp.
59	20.5		45		*Mougeotia* sp.
60	2.3	4			Nauplius (Copepoda)
61	9.1		20		*Navicula* sp.
62	2.3		5		*Netrium* sp.
63	2.3		5		*Nostoc* sp.
64	11.4	21			*Notholca* sp.
65	18.2	13	25		*Oocystis Borgei* Snow
66	52.3	29	75		*Oscillatoria* sp.
67	47.7	88			*Pandorina morum* (O. Müll.) Bory
68	27.3	33	20		*Pediastrum Boryanum* (Turp.) Menegh.
69	40.9		90		*Pedinella* sp.
70	36.4		80		*Peridinium* sp.
71	9.1	17			*Phacus* sp.
72	4.5		10		*Phormidium* sp.
73	36.4	8	70		*Pinnularia* sp.
74	2.3	4			*Pleodorina californica* Shaw
75	79.5	96	60		*Polyarthra trigla* Ehr.
76	34.1	33	35		*Quadrigula* sp.
77	4.5	4	5		*Scenedesmus* sp.
78	4.5	8			*Selenastrum bribraianum* Reinsch
79	25.0	29	20		*Spirogyra* sp.
80	22.7		50		*Spondylosium lutkemulleri* Gron.
81	93.2	100	85		*Staurastrum* sp. #1 (*gracile*-like)
82	38.6		85		*Staurastrum* sp. #2 (*megacanthum*-like)
83	61.4	92	25		*Stephanodiscus niagarae* Ehr.

Table 23. Continued.

Taxa Code No.	Constancy (%) E+S 44	E 24	S 20	Lake Sample Total	Plankton Net Organisms
84	4.5	8			*Stigeoclonium* sp.
85	54.5	29	85		*Surirella* sp.
86	45.5		100		*Synedra radians* Kütz.
87	20.5		45		*Synedra ulna* (Nitz.) Ehr.
88	38.6	17	65		*Synura uvella* Ehr.
89	45.5		100		*Tabellaria fenestrata* (Lyngb.) Kütz.
90	22.7		50		*Tabellaria flocculosa* (Roth) Kütz.
91	15.9		35		*Tolypothrix* sp.
92	6.8		15		*Tribonema* sp.
93	31.8	54	5		*Trichocera* sp.
94	13.6	4	25		*Ulothrix* sp. (*aequalis*-like)
95	47.7	88			*Volvox aureus* Ehr.
96	79.5	71	90		*Vorticella* sp.
97	34.1		75		*Xanthidium* sp.#1
98	31.8		70		*Xanthidium* sp.#2 (*Arthrodesmus*-like)
99	22.7		50		*Xanthidium* sp. #3 (*antilopaeum*-like)
100	11.4		25		*Zygnema* sp.

Table 24. Numbered order of samples as used in the computer program.

Sooke Lake Station	1	2	3	4	5	Elk Lake Station	1	2	3	4
Sampling date						Sampling date				
Aug. 28, 1967	1	2	3	4	5	Aug. 3, 1967	21	22	23	24
Oct. 2, 1967	6	7	8	9	10	Sept. 5, 1967	25	26	27	28
Nov. 6, 1967	11	12	13	14	15	Oct. 9, 1967	29	30	31	32
Dec. 11, 1967	16	17	18	19	20	Nov. 19, 1967	33	34	35	36
						Dec. 16, 1967	37	38	39	40
						Jan. 19, 1968	41	42	43	44

Table 25. Upper half of matrix showing percentage similarity (Jaccard's CC) between all possible pairs of samples prior to cluster analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
1	1.00	.743	.625	.519	.609	.542	.522	.581	.545	.491	.542	.435	.440	.510	.467	.235	.468	.275	.360	.458	.191	.185	.180	.192	.189	.160	.184	.180	.196	.173	.163	.196	.160	.132	.176	.160	.122	.130	.170	.125	.100	.167	.115	.154
2		1.000	.684	.529	.659	.521	.568	.634	.558	.500	.553	.477	.511	.553	.512	.242	.388	.250	.367	.500	.196	.212	.184	.220	.192	.188	.239	.184	.224	.176	.191	.250	.163	.180	.204	.188	.149	.159	.200	.152	.125	.196	.140	.157
3			1.000	.577	.711	.510	.587	.614	.651	.577	.571	.643	.563	.540	.500	.216	.469	.286	.392	.521	.180	.196	.192	.226	.222	.196	.220	.192	.255	.208	.200	.255	.173	.212	.189	.220	.208	.170	.208	.163	.137	.180	.130	.189
4				1.000	.655	.468	.500	.466	.545	.524	.468	.456	.483	.492	.431	.135	.458	.236	.371	.450	.217	.286	.226	.274	.231	.210	.250	.226	.258	.219	.233	.279	.230	.242	.222	.230	.220	.190	.241	.203	.180	.237	.210	.242
5					1.000	.509	.547	.569	.600	.625	.593	.592	.527	.654	.560	.200	.473	.260	.455	.519	.153	.246	.164	.233	.210	.167	.207	.183	.237	.197	.211	.259	.207	.241	.200	.207	.196	.143	.196	.200	.155	.193	.186	.241
6						1.000	.673	.739	.633	.569	.564	.529	.585	.593	.529	.256	.528	.313	.509	.577	.115	.169	.127	.138	.172	.129	.148	.127	.217	.197	.169	.217	.167	.161	.161	.186	.136	.123	.155	.158	.136	.172	.148	.180
7							1.000	.727	.652	.554	.547	.644	.667	.673	.574	.250	.540	.372	.520	.560	.143	.180	.155	.207	.203	.158	.200	.175	.255	.211	.204	.278	.179	.214	.214	.222	.167	.154	.189	.170	.167	.185	.158	.193
8								1.000	.682	.667	.600	.636	.660	.633	.674	.263	.531	.390	.510	.617	.170	.186	.182	.193	.232	.185	.208	.161	.288	.241	.235	.288	.208	.222	.222	.255	.196	.184	.220	.200	.196	.240	.164	.222
9									1.000	.635	.702	.714	.660	.667	.674	.231	.563	.326	.480	.652	.148	.186	.161	.193	.232	.185	.208	.204	.264	.241	.212	.264	.164	.179	.179	.208	.151	.137	.196	.200	.173	.216	.164	.200
10										1.000	.625	.627	.589	.596	.660	.204	.564	.308	.441	.582	.177	.209	.206	.234	.270	.230	.250	.226	.322	.279	.276	.300	.230	.262	.242	.271	.241	.169	.263	.246	.241	.281	.210	.283
11											1.000	.696	.647	.720	.660	.256	.620	.340	.538	.745	.193	.206	.183	.194	.250	.186	.207	.203	.281	.237	.211	.259	.167	.180	.200	.207	.155	.143	.196	.179	.196	.236	.167	.200
12												1.000	.689	.696	.707	.278	.587	.375	.565	.682	.132	.133	.145	.158	.218	.170	.192	.167	.275	.226	.220	.275	.170	.208	.185	.216	.180	.143	.204	.184	.180	.200	.148	.231
13													1.000	.680	.617	.238	.612	.419	.560	.667	.158	.194	.190	.180	.217	.172	.193	.169	.268	.224	.218	.246	.193	.207	.207	.236	.182	.192	.226	.185	.204	.222	.172	.228
14														1.000	.696	.256	.528	.340	.569	.640	.133	.226	.145	.194	.210	.167	.207	.164	.259	.217	.232	.281	.207	.220	.200	.207	.175	.143	.196	.200	.175	.214	.186	.241
15															1.000	.242	.521	.375	.500	.609	.132	.193	.167	.179	.264	.192	.216	.167	.300	.250	.245	.275	.216	.208	.208	.265	.204	.191	.229	.234	.229	.277	.192	.255
16																1.000	.289	.292	.297	.282	.059	.048	.054	.050	.075	.056	.056	.054	.108	.108	.121	.108	.116	.111	.111	.118	.094	.103	.094	.133	.094	.091	.118	.111
17																	1.000	.487	.630	.674	.167	.183	.179	.169	.207	.182	.161	.200	.236	.236	.231	.214	.182	.196	.218	.226	.170	.180	.216	.173	.216	.260	.140	.196
18																		1.000	.462	.405	.098	.104	.116	.133	.182	.146	.146	.116	.220	.190	.211	.190	.205	.195	.195	.237	.189	.171	.222	.229	.222	.216	.146	.195
19																			1.000	.583	.127	.186	.121	.133	.169	.143	.123	.121	.218	.175	.212	.196	.164	.179	.158	.208	.173	.160	.173	.154	.196	.240	.143	.200
20																				1.000	.123	.125	.117	.129	.203	.138	.138	.155	.211	.190	.182	.211	.158	.153	.172	.200	.145	.132	.167	.170	.167	.208	.138	.193
21																					1.000	.611	.710	.647	.629	.733	.677	.606	.618	.571	.594	.571	.529	.500	.636	.529	.485	.484	.531	.412	.485	.563	.368	.459
22																						1.000	.694	.641	.625	.622	.667	.605	.575	.575	.553	.575	.463	.512	.590	.500	.462	.350	.462	.400	.357	.450	.395	.442
23																							1.00	.639	.714	.774	.719	.697	.611	.657	.636	.611	.528	.500	.676	.571	.486	.485	.625	.500	.486	.559	.447	.541
24																								1.000	.615	.706	.758	.686	.605	.525	.629	.605	.568	.579	.667	.568	.528	.405	.571	.500	.410	.474	.415	.538
25																									1.000	.788	.686	.667	.771	.771	.657	.771	.686	.605	.743	.735	.600	.432	.600	.528	.514	.541	.405	.525
26																										1.000	.800	.719	.727	.727	.767	.727	.636	.600	.750	.687	.594	.500	.700	.515	.545	.625	.385	.514
27																											1.000	.667	.676	.629	.656	.676	.500	.514	.647	.543	.457	.412	.545	.429	.417	.486	.385	.474
28																												1.000	.611	.611	.636	.611	.486	.500	.676	.528	.444	.324	.576	.500	.368	.472	.375	.425
29																													1.000	.765	.750	.818	.629	.639	.735	.727	.588	.500	.687	.514	.543	.571	.425	.513
30																														1.000	.750	.818	.629	.686	.735	.727	.636	.500	.588	.559	.543	.571	.425	.513
31																															1.000	.750	.710	.667	.719	.710	.613	.469	.667	.633	.563	.645	.432	.571
32																																1.000	.676	.735	.788	.781	.636	.457	.636	.559	.543	.571	.462	.553
33																																	1.000	.750	.750	.742	.759	.500	.645	.724	.645	.625	.543	.697
34																																		1.000	.758	.750	.828	.471	.606	.625	.559	.543	.556	.611
35																																			1.000	.750	.656	.471	.767	.625	.606	.588	.514	.568
36																																				1.000	.700	.548	.645	.613	.645	.625	.500	.600
37																																					1.000	.552	.655	.679	.600	.633	.500	.656
38																																						1.000	.500	.419	.607	.586	.455	.515
39																																							1.000	.679	.600	.690	.500	.559
40																																								1.000	.621	.655	.613	.733
41																																									1.000	.750	.594	.656
42																																										1.000	.576	.636
43																																											1.000	.697
44																																												1.000

The next step was to determine the level of similarity in the dendrogram at which samples could finally be grouped objectively. Preston (1962a, 1962b) concluded from his analysis of the Resemblance Equation[1] that a similarity value of 73% would give good species groups, distinguishing between biotic homogeneity and heterogeneity. Hagmeier and Stults (1964), investigating the geographical distribution of North American mammals, found that Preston's similarity value of 73% corresponded to a CC value of 62.5%[2]. On this basis they distinguished provinces as "areas of relatively homogeneous fauna, separated from other similar areas by a coefficient of community of at most 62.5%". In a later paper, Hagmeier (1966), correcting for statistical error, proposed the use of a CC value of 65% and considered all values lying between 60 and 65% as suspect. Popham (1968) in an investigation of sub-littoral pelecypod associations found a CC value of 62.5±2.5% to overestimate the number of associations suggested by traditional studies. From analysis of the shaded matrix he reasoned that on the basis of current sampling techniques, a similarity value of 35% might be more useful for benthic ecologists.

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1 $RE = (n_1/N)^{1/z} + (n_2/N)^{1/z} = 1$

where N is the total number of species in two samples or areas, or $N=n_1 + n_2 - C$, as in equation (7). The exponent, 1/z, is the reciprocal of the "degree of similarity" between the biotas compared (Hagmeier & Stults 1964; Peters 1968).

2 Preston's (1962) value of faunal dissimilarity, z = 0.27, may also be expressed as degree of similarity, 1 - z=0.73, and was expressed in percentage form by Hagmeier and Stults (1964), as, S = 100(1-z)= 73%, equivalent to a CC value of 62.5% (62.5 ± 2.5%, Hagmeier 1966).

It remains to be determined whether or not there is one similarity value which may be applied objectively to all situations in both terrestrial and aquatic ecology. Current work in numerical analysis suggests that ecologists, like numerical taxonomists (Rogers & Tanimoto 1960), can rely on intuition and background knowledge to choose the "cutting off" levels according to the aims of each separate investigation. At present the final groupings are best tested by comparison with those obtained in other fields of ecological research and with those arrived at by traditional means of analysis. Numerical analysis is used mainly in support of existing assumptions and is not without inherent errors itself (Sokal & Sneath 1963; Sokal 1965; Popham 1968). The limitations of this technique must be clearly understood. In many cases the prime importance of its application, aside from enabling the manipulation of large quantities of data, lies in the new and alternate hypotheses that the method of data arrangement suggests. It is likely that in some ecological studies there may be no need to determine a critical similarity value. In this event the application of yet another method of analysis might elucidate an otherwise obscure relationship.

Although it was not essential to the study aims, a critical level of 60-65% similarity was used here to compare the results with those obtained in terrestrial and benthic studies.

RESULTS AND DISCUSSION

The results of the numerical analysis are summarized in Figures 19 and 20. In view of the preliminary nature of this study and the collection technique used, the interpretation of these results is not detailed.

SPATIAL AND TEMPORAL VARIATIONS IN BIOTIC SIMILARITY

The sample order in the dendrogram was supported by the graphs in Figure 20. These graphs illustrate that there was a greater change in species composition (lower CC's) per sample with time than with station location. In Elk Lake the mean biotic similarity between stations at any one time ranged between 77% and 58%, whereas with season (or time) the mean CC values were lower and ranged between 16% and 56%. Similarly in Sooke Lake the spatial variation in mean similarity at each collection date ranged between 68% and 56%, while the mean CC values ranged between 59% and 39% similarity with time. In lakes of this size and character the horizontal surface distribution of plankton is, on the whole, relatively homogeneous when compared with seasonal variation in species composition. This was found to be the case particularly in Elk Lake. A quantitative analysis of the net plankton diatoms indicated that the four stations were almost identical in species composition and relative abundance on any one field collection date although there was a definite seasonal pattern of succession both in composition and abundance common to all four stations.

Elk Lake stations (Fig. 20,C) were more constant (higher CC's) in biotic composition over time than those established in Sooke Lake

Figure 19. Dendrogram showing relationships between samples. The mean CC's or percent similarities are the result of cluster analysis. The solid horizontal lines show the critical region between 60 and 65%.

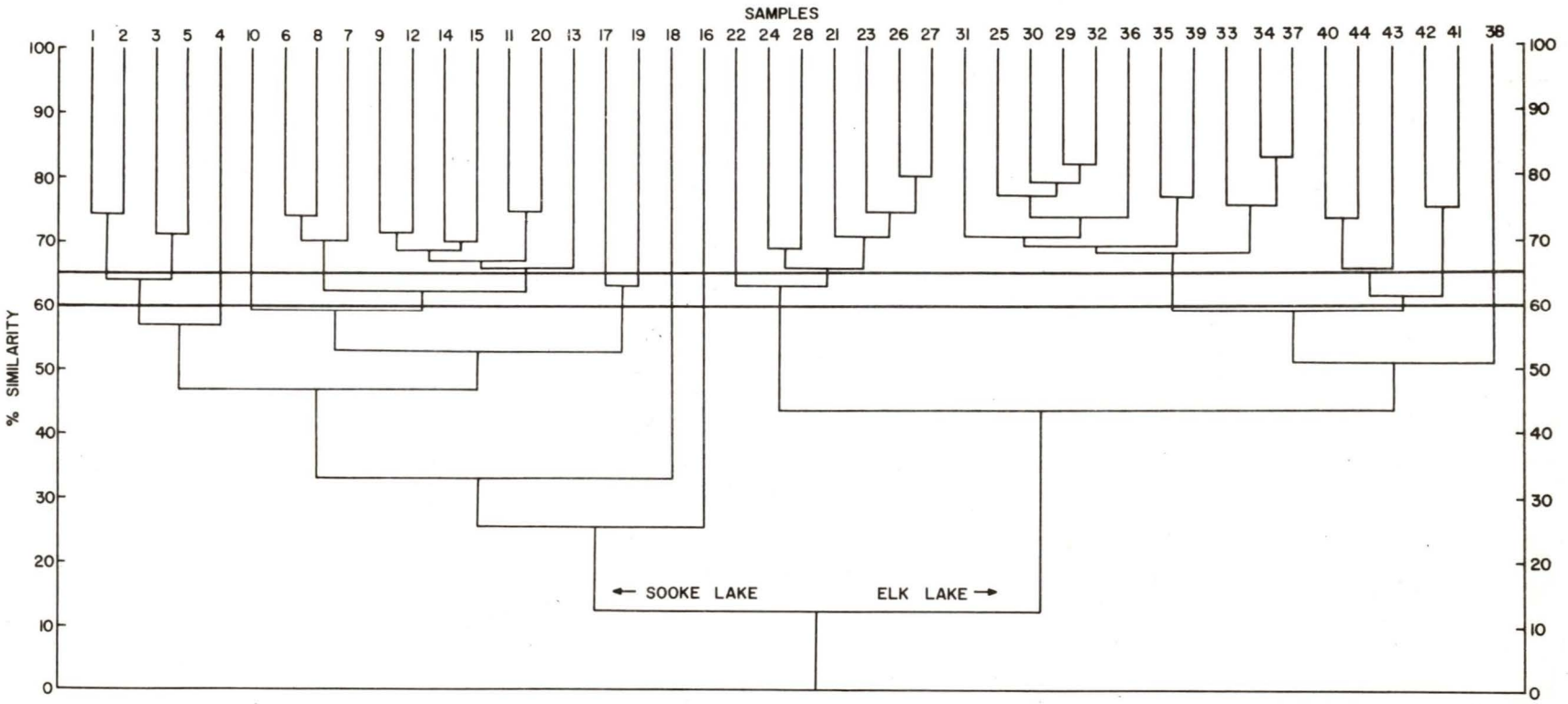
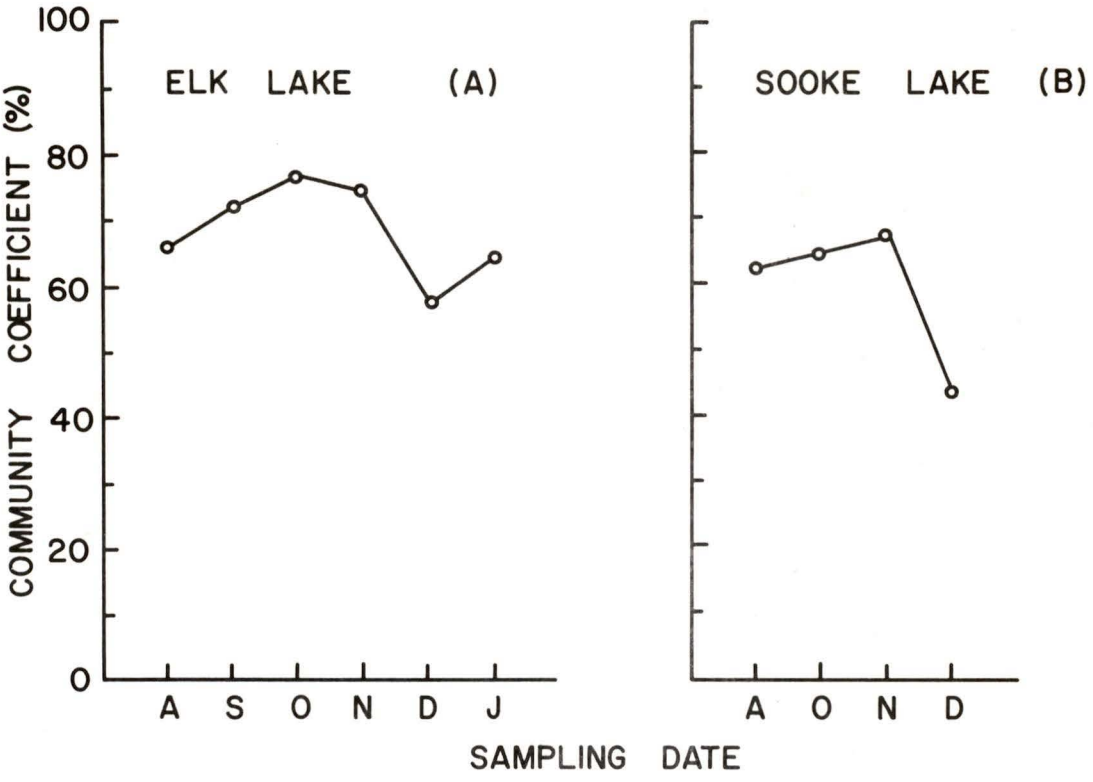
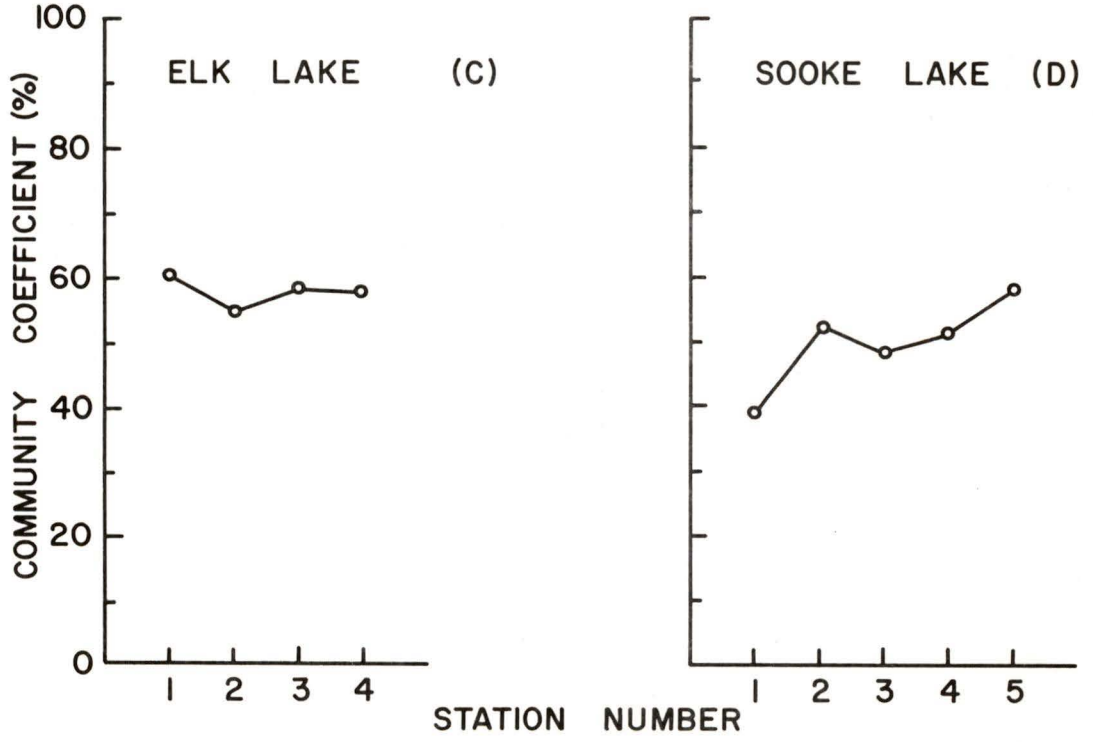

SAMPLES
1 2 3 5 4 10 6 8 7 9 12 14 15 11 20 13 17 19 18 16 22 24 28 21 23 26 27 31 25 30 29 32 36 35 39 33 34 37 40 44 43 42 41 38
% SIMILARITY
100 90 80 70 60 50 40 30 20 10 0
← SOOKE LAKE
ELK LAKE →

Figure 20. Graphs showing the percent similarities between samples as they vary with time and station location. Graph (A) was prepared by averaging all possible combinations (six) of the CC values for the four Elk Lake stations at each separate date and plotting these values against the six sampling dates. Similarly in Graph (B) of Sooke Lake the averages are of ten possible combinations of CC values between five stations for the four separate sampling dates. Graphs (C) and (D) show the average CC's for each separate station over the entire sampling period.


ELK LAKE (A)
SOOKE LAKE (B)
COMMUNITY COEFFICIENT (%)
100
80
60
40
20
0
A S O N D J
A O N D
SAMPLING DATE


ELK LAKE (C)
SOOKE LAKE (D)
COMMUNITY COEFFICIENT (%)
100
80
60
40
20
0
1 2 3 4
1 2 3 4 5
STATION NUMBER

(Fig. 20,D). This may be due to the smaller size of Elk Lake and a greater number of inflows in Sooke. Water level fluctuations in Sooke Lake were more severe and seasonal environmental changes were also more extreme because of its higher elevation. In Elk Lake, station 2 had the largest variation in plankton composition over the 6-month sampling period. This suggests that this particular station was under the influence of different modifying environmental factors such as colonization from epiphytes on aquatic angiosperms, drainage outflows, *etc.*, and was therefore not as comparatively similar as the other three stations. In Sooke Lake only station 5 appeared relatively constant in composition in comparison with the higher mean CC's of all four stations in Elk Lake. These results lend support to the practice of establishing permanent sampling locations (Spodniewska 1967) and suggest that Elk Lake requires fewer stations than Sooke Lake. Elk Lake had consistently higher similarity values in species composition although an analysis of variance (Table 26) indicated that the differences between station mean CC's within each lake were not statistically significant.

Graphs (A) and (B) of Figure 20 show that the stations in both lakes had the least similarity in species composition in December. Here the CC's had the greatest change over any sampling interval. In Sooke Lake the mean CC value for December was statistically different (Table 26) from those of the other sampling dates, while the December values for Elk Lake differed significantly from those of November but not those CC values of January. The biological significance of this decrease in similarity is difficult to interpret and may have been a natural differential species response of the plankton populations to a

Table 26. Analyses of variance illustrating the significant differences in CC's between sampling dates and the non significant differences between sampling stations (as plotted in Figure 20). Mean values were ranked, from left to right, in order of increasing magnitude and their differences tested by Duncan's New Multiple Range Test (Li 1964).

SOURCE OF VARIANCE	df	SS	MS	F	D.N.M.R.[1]
(a) ELK LAKE					
Among Sample	5	.1581	.0316	8.6226**	D J A S N O
Within Sample	30	.1100	.0036		
Total	35	.2682			
(b) SOOKE LAKE					
Among Sample	3	.3587	.1195	14.5763**	D A O N
Within Sample	36	.2953	.0082		
Total	39	.6541			
(c) ELK LAKE					
Among Sample	3	.0256	.0085	0.8422	
Within Sample	56	.5679	.0101		
Total	59	.5935			
(d) SOOKE LAKE					
Among Sample	4	.1211	.0302	2.1977	
Within Sample	25	.3445	.0137		
Total	29	.4656			

1 Mean values underscored by the same solid line are not significantly different at $P < 0.01$.

** Significant at $P < 0.01$.

seasonal decrease in temperature and light. It may also have been a result of autumnal circulation concordant with heavy rainfall which left both lakes in flood condition at this time (see Dickman 1969). Other factors which would influence the surface distribution of plankton such as exposure to wind (Langford & Jermolajev 1966) and number of inflows must also be considered.

The low mean CC value in Sooke Lake on December 11 was largely influenced by the low number of species (11) present in the station 1 sample (Table 22). It is interesting to note that if the stations are plotted in a north-south direction (*i.e.*, station 1, 3, 2, 4, 5) in the direction of the flow and prevailing winter winds, and the number of taxa per station plotted similarly (*i.e.*, 11, 20, 38, 37, 39), there is an increase in the number of different species at the south end of the lake so that in December, station 5 samples contained more than three times as many plankton taxa as did those of station 1. A sharp increase in plankton taxa occurred in samples of station 2 which is situated near the mouth of the major inflow, Rithet Creek. These data suggest there was an increase in allochthonous matter being washed into Sooke Lake, possibly due to the scouring action of high flush rates in Rithet Creek. This relationship, while not as clearly defined in the other monthly samples from Sooke Lake, was not apparent in any of those from Elk Lake. The effect of wind and flow on the surface distribution of plankton populations would be more pronounced in Sooke Lake than in Elk Lake due to differences between the two lakes in such parameters as shape of the lake basin, direction and degree of flushing, time and elevation of freezing temperatures, number of inflows, direction of prevailing winds, *etc.*

DENDROGRAM SAMPLE GROUPINGS

The dendrogram (Fig. 19) shows that Elk Lake samples did not cluster with any of those from Sooke Lake (at least not above the lowest cluster value of 12.5% similarity) despite the presence of 38 common plankton net organisms at one time or another during the sampling. On this basis the two lakes were distinctly different over the entire sampling period, as their morphometric characteristics and histories would suggest. Hagmeier's (1966) critical level of 62.5 ± 2.5% similarity gave 16 sample groupings; 10 in Sooke Lake and 6 in Elk Lake. This appears to be an overestimation but longer sampling and more detailed analysis is necessary to determine the homogeneity of station locations and the validity of these groupings.

Since little is known regarding the environmental requirements of individual plankton species in general, it is still very difficult to distinguish plankton communities on the basis of differential species (Table 23) or species groups. However, it is possible to characterize broad community-types on the basis of species composition, constants, and dominant species.

A total of 83 different taxa were found in the 20 net plankton samples collected from Sooke Lake, while in the 24 samples from Elk Lake, 55 different taxa were identified (Tables 21 and 22). Hence Sooke Lake was shown to have a more diverse plankton population over a 4-month period than Elk Lake over a 6-month period, *i.e.*, Elk Lake was more constant seasonally in species composition. This partially accounts for the order in the dendrogram which shows that Sooke Lake samples were better fitted to the site-date order (eventhough they tended to

cluster at lower values of percent similarity) than samples from Elk Lake which tended to be integrated and overlapped in species composition with season, while samples generally had higher CC's. Of the 38 taxa common to the two lakes, none had 100% occurrence in all samples, but four of these taxa or sample characteristics, *Anabaena flos-aquae*, *Staurastrum* sp. #1, *Melosira* sp., and Copepoda, had constancy values greater than 90% (Table 23).

Within Elk Lake, 7 taxa occurred in all net plankton samples from August to January: *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, *Coelosphaerium Naegelianum*, *Fragilaria crotonensis*, *Staurastrum* sp. #1, and *Eudorina elegans*. These species, commonly found together, are generally considered characteristic of medium-hard or hard-water drainage lakes, and indicative of nutrient enriched or eutrophic conditions (Hutchinson 1967). Conversely, in Sooke Lake, only four taxa had 100% constancy values, occurring in all samples from August to December: *Asterionella formosa*, *Synedra ulna*, *Tabellaria fenestrata*, and Copepoda. These species, together with the large number of desmids (Table 22) and more diverse species composition, indicate the oligotrophic nature of Sooke Lake.

CONCLUSIONS

Numerical analysis has given a preliminary grouping of samples based on qualitative plankton data and thereby has laid the groundwork for subsequent more refined studies. Further relationships may be shown by measuring environmental variables and correlating these with the sample groupings as shown in the dendrogram. Jacobsen (personal communication) suggests that more information may be obtained by combining both quantitative and qualitative data. He recommends grouping the environmental data with the presence and absence information of the species lists for each sample and computing distance doefficients (Sokal & Sneath 1963). The addition of this quantitative data would enhance the analysis by increasing the number of characters to be compared per sample. If there is a sufficient number of measured environmental parameters, it is also possible to apply a coefficient, group the samples, and compare the resulting dendrogram (Sokal & Rohlf 1962) with that obtained by using simple species lists or quantitative plankton data.

These methods might be applicable to lake classification which in turn could be correlated with degree of eutrophication and used in programs of resource management and pollution control. The results could also be used to indicate whether the morphometry and morphology of a lake under intensive study necessitate sampling numerous stations and to evaluate the feasibility of using only one station per sampling date in extensive surveys. The dates and extent, or rate of seasonal biotic variation as determined by environmental factors might also be detected by a similar method of numerical analysis.

Despite the problems of population instability and irregularity encountered in plankton studies, it is possible to apply research methods developed in the field of terrestrial plant ecology. Numerical analysis provides a means of classification by which observations may be arranged or organized in such a way as to elucidate meaningful patterns of relationships. Some modification of the method used in this study will likely be employed in more detailed investigations of the periphyton and phytoplankton of these two lakes which will follow. These studies will include analysis of physical and chemical variables.

SUMMARY

Routine plankton tow collections were made at established sampling stations in two lakes of distinctly different morphometric parameters, Sooke and Elk lakes, situated on lower Vancouver Island (B.C., Canada). Species lists were prepared for all samples: 20 samples taken at 36-day intervals over a four month period from five stations in Sooke Lake and 24 samples taken at 34-day intervals over a six month period from four stations in Elk Lake. A method of numerical analysis was applied to these presence and absence data using Jaccard's Coefficient of Community (CC) to compare all samples on the basis of their biotic similarity.

Cluster analysis of the CC's for all possible combinations of pairs of samples (44 x 44 matrix) illustrated that samples from the two lakes were well segregated, *i.e.*, Sooke Lake samples clustered with those of Elk Lake only at the lowest cluster value of 12.5% similarity, despite the presence of 38 taxa common to the net plankton of both lakes during the study. Using a critical value of 62.5±2.5% similarity, 16 different sample groupings (10 in Sooke Lake, 6 in Elk Lake) were obtained. Further analysis of the data, showed that there was a greater change in species composition (lower CC's) per sample with time than with station location in both lakes, although Elk Lake stations were more constant in biotic composition over time than those of Sooke Lake, due possibly to the larger size of the latter, and effects of current, number of inflows, and allochthonous plankton taxa. Within each lake, no statistically significant differences occurred between station mean CC's, however there were significant temporal variations with lowest mean CC values being recorded in December in both lakes.

The method was considered useful, and possible alternative applications of similar methods in other limnological investigations were discussed.

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APPENDIX II

A Method of Collecting Periphyton in Lentic Habitats With Procedures for Subsequent Sample Preparation and Quantitative Assessment[1]

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[1] Appendix II represents the main text of a paper by the author and A.P. Austin, accepted for publication under the same title in the journal, *Int. Revue ges. Hydrobiol.* (see p. 176).

CONTENTS

INTRODUCTION

The recent literature illustrates a renewed interest in the role of periphyton microbiota as the principal primary producers in rivers, streams, ponds and the littoral zones of shallow lakes. Quantitative periphyton sampling methods have thus undergone some concurrent improvement, particularly with reference to productivity studies and the biological assessment of pollution.

As collection devices, a wide variety of artificial substrata have been found useful in systematic and life history investigations; in quantitative studies of productivity, standing crop, and colonization rates; in the assessment of periphyton population dynamics; and in the study of environmental limiting factors (*e.g.*, Castenholz 1960, 1961; Neal *et al.* 1967; Beers & Neuhold 1968; Grzenda & Ball 1968; Cairns, Jr. *et al.* 1969). The biotic selectivity of these substrata has afforded much discussion and studies comparing the alleged differences in selectivity between artificial and natural substrata have resulted (Hohn & Hellerman 1963; Albin 1965; Hohn 1966). However, the majority of published data do not present conclusive objections to the use of artificial substrata and none of the studies, to date, have reported the differences between periphyton settlement on substrata which have had exactly similar gross shapes and exposure histories (Benson 1967). It seems reasonable to accept the statement by Cooke (1956) that there is no one universal substratum capable of sampling all organisms of a given habitat. Usually, the substrata employed are chosen for case of handling, availability, and facility of subsequent observation, and glass has proved most popular. Thus standard glass microscope slides were used in this study to facilitate comparison

of data with published accounts of typical periphyton communities.

Numerous methods have been used to expose substrata upon which periphyton biota may develop (Cooke 1956; Sladeckova 1962; Wetzel 1964). The purpose of this aspect of the present study is to give details of design and construction of a new collection cage or frame to support glass (or other) substrata in lentic habitats, and to give details of sample collection, preparation and counting methods, illustrated by some preliminary experimental data. Existing exposure apparatus was reviewed but was generally found lacking when field tested for the following requirements: to remain stable and fixed on the lake bottom for relatively long periods of time; to include both horizontally and vertically exposed glass slides, plus some samples of natural substrata; and to remain completely submerged, undetected and hence undisturbed by vandals.

The English term, "periphyton", is most commonly equated with the German term *Aufwuchs*, although definitions vary considerably (Young 1945; Ruttner 1963). A broad definition, including dependent non-sessile forms, is adopted here and used interchangeably with *Aufwuchs* to refer to all organisms (except rooted macrophytes) which occur attached to (but not penetrating into), or associated with a submerged surface (Cooke 1956; Sladeckova 1962; Round 1964; Wetzel 1964).

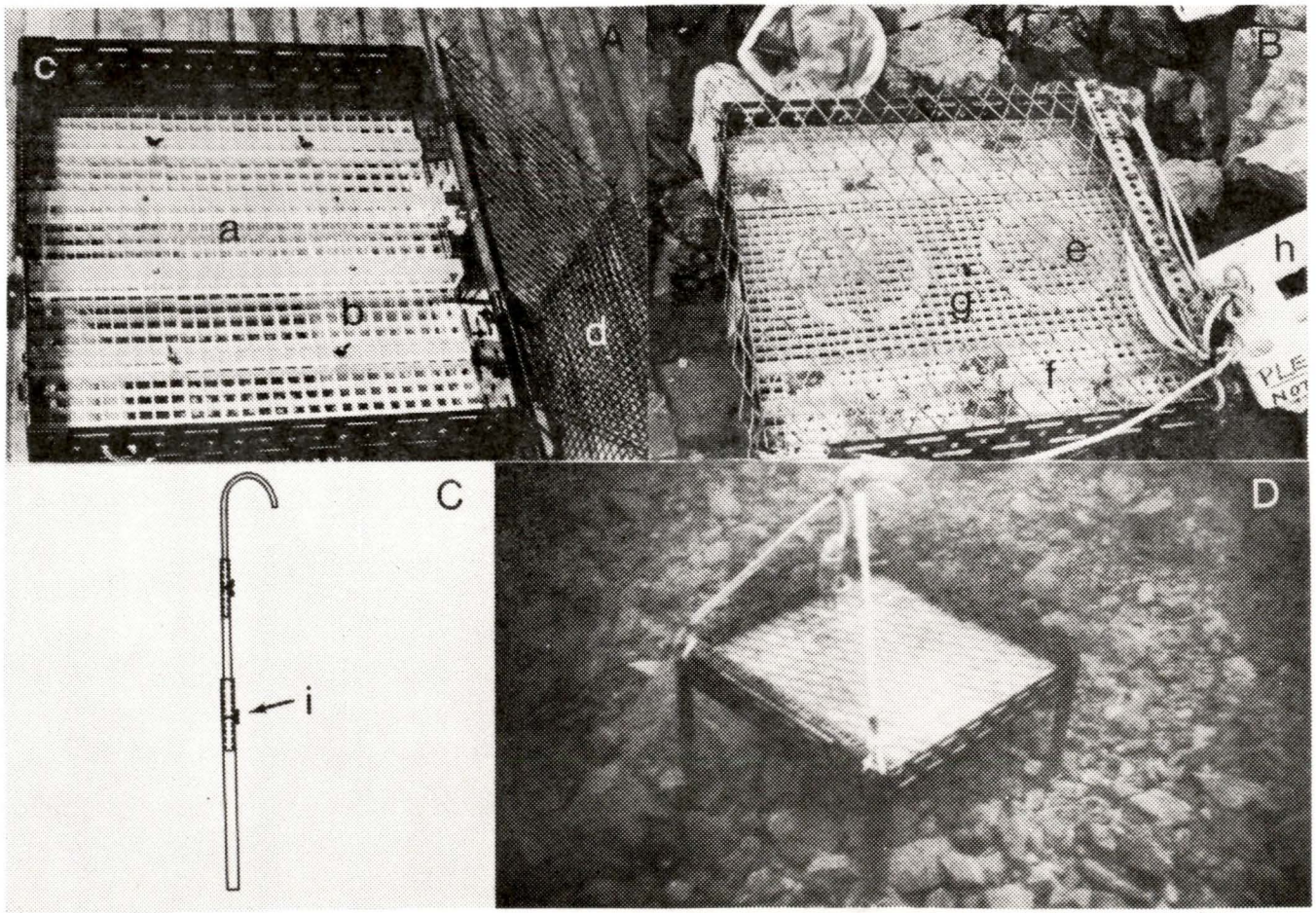
MATERIALS AND METHODS

PERIPHYTON EXPOSURE-FRAME: DESIGN AND CONSTRUCTION

To meet the sampling requirements of a program of investigation of littoral periphyton communities in lower Vancouver Island (B.C., Canada) lakes it was found necessary to design exposure-frames to support large, 50 x 75 mm, glass microscope slides (Fig. 21). The frame, made of slotted steel Dexion® (Dexion Ltd., Weston, Ont., Canada), was essentially an open, shallow (8.9 cm deep) box-like structure (46.0 cm square) which rested on four legs (30.5 cm high) bolted to the box corners. A plastic grid with 16 sq. mm openings formed the bottom of the box. The upper surface of this plastic base supported 36 horizontal and 23 vertically oriented glass slides. The box was covered with a hinged screen top, and to facilitate location in the field, a styrofoam float was attached. To minimize undesirable effects of wind, wave action, and siltation, the periphyton exposure-frame was designed to sit completely submerged, with the slides in position 30.5 cm from the lake bottom (Fig. 21,D). The frame legs were equipped with flat, triangular "feet", preventing the frame from sinking into soft sediments. The construction materials provided the weight and strength of support required and yet restricted the natural circulation of water and penetration of light as little as possible.

Within each frame the vertical slides were located in a single row, 16 mm apart, down the center of the plastic grid and supported on either side by two grooved plexiglass runners similar to the arrangement in a standard slide box (Fig. 21,A-a). Both surfaces of each vertical slide were used in the periphyton analysis giving a total

Figure 21. Exposure-frames designed to support artificial substrata for studies of littoral periphyton communities in lower Vancouver Island lakes. A. Exposure-frame used in Elk and Sooke lakes to support 23 vertical (a) and 36 horizontal (b) large glass slides (c, Dexion framework; d, hinged wire mesh lid). B. Larger (61 cm square) exposure-frame used in Buttle Lake to support 22 vertical (e) and 28 horizontal (f) glass slides (g, unoccupied grid space to support natural substrata; h, styrofoam float and nylon line). C. Telescoping tubular pole of aluminum used to raise frame to surface (i, wing-nut and bolt used to extend or retract pole length; not to scale). D. Underwater photograph of exposure-frame in field position at a periphyton sampling station.


A
B
C
a
b
d
e
f
g
h
C
i
D

exposure area of 6500 sq. mm per slide, somewhat less than the actual slide area due to a 5 mm strip at each end obscured by the support slot. On each side of this row of vertical slides were two rows of 9 horizontal slides each, occurring side by side with no spacing between (Fig. 21, A-b). Only the upper surfaces of the horizontal slides were used in the analysis, the bottom surfaces being wiped clean, and hence the total exposure area of each of these slides was 3250 sq. mm.

This particular frame design, used in Sooke and Elk lakes, was a modification of an earlier model used in Buttle Lake (Fig. 21, B) which differed in arrangement of slides and in its larger size (frame box: 61.0 cm square). Here, double rows of 7 horizontally exposed glass slides per row were situated on both sides of two slotted plexiglass cylinders, each of which held 11 vertically exposed slides in wheel or spoke-like formations. The unoccupied grid space between the vertical and horizontal slides enabled the exposure of various natural substrata such as small pebbles which rested in place by virtue of their weight. All other dimensions and construction materials of this frame were similar to that described above, the increased size and weight being necessary to ensure that the frames remained stable during any periods of wind generated water movement as well as wide fluctuations in drawdown (0-13 m) in the lake sampled. For use in other lakes, this frame was modified because of its cumbersome size. The resulting frames supported more horizontal slides (36 *v* 28) with greater surface exposure areas (3250 sq. mm *v* 3200 sq. mm per slide), but forfeited space for natural substrata which were then sampled at frame depths by a SCUBA diver or by Eckman dredge when the former was not available.

Typically, within each lake, one frame was placed at each selected periphyton sampling station and they were maintained as close as possible to a constant depth of about 2.1 m. These frames were serviced from a small boat and a telescoping pole of tubular aluminum with a hook on one end was used to grapple for the styrofoam float (Fig. 21, C). Even at this shallow depth there were periods of heavy run-off and/or high plankton bloom during which the floats were not visible even through a water telescope. At such times the frames were located by SCUBA divers. All frames were carefully handled in transit to the surface, care being taken to disturb the water as little as possible. At any one station, slides were exchanged and the frame returned to its original location, the whole operation being completed within a maximum of 10 minutes.

ADDITIONAL PERIPHYTON COLLECTION DEVICES

Other, inexpensive methods for exposing substrata were tested and evaluated under field conditions prior to exposure-frame construction. Cork and rubber stoppers with 25 x 75 mm glass slides and various sized coverslips (Newcombe 1950; Sladeckova 1962, 1966; Benson 1967) were immersed in the littoral zones of Elk Lake and a shallow marine inlet. These devices were suspended individually at different depths, or in a vertical series, attached to an anchored line. However, these devices, typically suspended in pelagic regions, were not suited to shallow littoral regions where considerable damage and loss of equipment was found to occur, Benson (1967), using similar arrangements, also reported breakage of line and loss of slides. Large numbers of such suspended devices at each station were required, in my studies, in

order to obtain a reasonable sample size (4 slides or coverslips), exposure area, and number of samples throughout the sampling program. In addition, a rather complicated arrangement of floats and anchor weights was necessary to accomodate for drawdown fluctuations which, despite precaution, fouled the lines during the immersion period. The equipment was subject to repeated vandalism and much breakage by passing pleasure craft. The amount of field apparatus and the time required for servicing was prohibitive and furthermore, periphyton samples overlapping in time were not feasible. Although this collection method was found useful for periphyton distribution studies, in vertical profile in certain pelagic situations, it was abandoned for my study purposes.

Some method of slide attachment, not requiring stoppers (Maciolek & Kennedy 1964), or better the immersion of plastic tapes (Neal *et al.* 1967), might be suited to vertical profile studies and perhaps amenable to modification for use in littoral zones (Austin, unpublished data).

Concurrent pilot studies were also undertaken using wooden slide boxes with the tops and bottoms removed and replaced with a fine mesh fiberglass cloth (similar to the Bissonette slide rack described by Miller 1936; Welch 1948; Sladeckova 1962). Although the mesh controlled grazing by larger herbivores, and the rack supported a greater number of slides (a maximum of 25, 25 x 75 mm), the disadvantages were found to be similar to those described above with the additional problems of clogged mesh. Slide staining carriers were also used under field conditions with comparable results.

During all pilot projects the styrofoam used for floats was sampled (after Hohn & Hellerman 1963) to assess its utility as a periphyton

sampling substrate. However, preliminary studies indicated that styrofoam was selective almost exclusively for diatoms: hence styrofoam samples were used only to supplement slide material for taxonomic identification of the diatoms.

SUBSTRATE IMMERSION AND SAMPLING PROCEDURES

The duration of substrate exposure favored by different workers is quite variable, ranging from 24 hours (Wilson 1925; Henrici 1936) to 335 days (Yount 1956), depending upon the particular environment, biota investigated, and study aims. My experience indicates that preliminary exploration of exposure time should be undertaken to determine the optimum duration necessary. After pilot trials, an exposure time of approximately one month was found most suitable and collections were subsequently made at monthly intervals.

In the present studies the number of coded slides in each frame was sufficient to enable the collection of slide samples exposed for monthly intervals as well as overlapping "time series" (TS); the latter being a specific group of slide samples from all stations within a lake, immersed for a distinct period of time. These samples which overlapped in time (see Miller 1936; Brook 1955; Cooke 1956) were collected in order so that standing crop, successional development, accrual rates, amounts sloughed off, and climax conditions (if they existed) could be determined. The immersion period of samples ranged from 27 to 135 days.

Four vertical and 6 horizontal slides were removed at each collection date from each frame, for each time series. However, only 8 replicate slides (4 vertical, 4 horizontal) per TS and station were used for the

laboratory data analysis and the two remaining slides stored for further exigency. All slides removed during the present studies were replaced with clean, new slides coded with a diamond marker.

QUANTITATIVE SAMPLING OF SLIDE PERIPHYTON: FILTER PREPARATION

In order to correlate small changes in physico-chemical variables with changes in periphyton community development and structure, within various water bodies, it was necessary to determine the total number of individual organisms as well as the taxonomic composition of *Aufwuchs* communities developing on slides. Therefore, a numerical counting method was employed instead of mass quantitative methods such as gravimetric determinations, productivity or respiratory measurements, and pigment or other chemical extractions (*e.g.*, Wetzel 1963, 1964; McIntire 1968a, 1968b; Grzenda & Ball 1968; Ball *et al.* 1969; Nelson *et al.* 1969; McIntire *et al.* 1969; Moss 1969).

An added advantage of the glass slide method is the feasibility of direct microscopic examination and counting of attached organisms *in situ* (Yount 1956). The use of fresh material also facilitates identification of species and the distinction between live and dead cells, but necessitates immediate counting, special care of specimens in transport to the laboratory (Nielson 1953; Cooke 1958), as well as critical immersion duration, and usually sacrifices permanent reference collections. In local studies one slide from each frame for each field sampling date was kept fresh for immediate laboratory identification. For the main analysis the extent of growth and structured layering of the communities prevented direct identification and counting on the slides *in situ*

(see Wilson 1925; Miller 1936; Brook 1955; Castenholz 1960). In many cases stalked and filamentous forms overlapped adpressed frustules when slides were mounted with coverslips. Therefore, in the field, each slide to be enumerated was removed from the frame and immediately placed in a separate labelled bottle containing 230 ml of a formalinized distilled water solution[1]. Following transportation to the laboratory, these slides were stored in the dark for later analysis.

In the laboratory a sharp metal spatula[2] was carefully used to thoroughly scrape the periphyton from both surfaces of each vertical slide, or the upper surface of each horizontal slide, into its container of 230 ml 4% formalin. Microscopic examination of these scraped slides showed that few organisms remained attached. There was no significant difference in the amount of material attached to opposite sides of the vertical slides, although Sladeckova (1962) noted that with long immersion, the side facing the sun supports consistently heavier periphyton growth.

Each bottle containing the periphyton scraped from one slide was well shaken and equal aliquots withdrawn by pipette. These subsamples were then vacuum filtered onto HA Millipore® filters (0.45 ± 0.02 μ pore size) of 25 mm diameter (Millipore Filter Corp., Bedford, Mass., U.S.A.)

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1 Other preservatives or fixatives such as merthiolate, thimerol (Williams 1964; Weber 1967), and Lugol's solution (Lund *et al.* 1958) may be used depending on the desirability of a staining procedure prior to counting (Goetz & Tsuneishi 1951; Goldberg *et al.* 1952; de Noyelles, Jr. 1968). The simple 4% formalin solution used in this study proved quite satisfactory.

2 Similar scraping procedure was documented by Cooke (1958), Williams and Mount (1965), King and Ball (1966), Weber and Raschke (1966), and Castenholz (1967).

until a total of 30 ml was filtered; the volume of individual aliquots being dependent upon the density of periphyton. If the concentration of organisms or suspended matter was high, 6 filters of 5 ml each were made; if concentration was moderate, 3 filters of 10 ml each or 2 filters of 15 ml each were made. With experience the optimum aliquot volume was readily determined.

Each filter with the periphyton organisms adpressed to its surface was rendered transparent and mounted with Permount and a large No. 1, 35 x 50 mm coverslip on a labelled 50 x 75 mm glass slide. The 1-propanol and xylol solutions used to dehydrate and clear the filters were also regularly filtered and microscopic examination of these residues revealed that few cells were lost during filter preparation.

TAXONOMIC IDENTIFICATION AND PERIPHYTON ENUMERATION

All organisms present on the filters were identified to species where possible, using fresh material. Organisms that defied accurate taxonomic identification were photographed, described, and given appropriate "names" or codes (after Hargraves & Wood 1967; Dickman 1968a). Hyrax mounts (Weber 1966; Patrick & Reimer 1966) were also prepared to aid in identification of diatoms.

For each filter a count was made of the number of individuals of each taxon present in 30 random Whipple micrometer fields at a magnification of x 313. Diatoms such as *Fragilaria* sp. and *Melosira* sp. were treated somewhat differently in that counts were made of the number of distinct ribbon-like aggregates as well as the number of individual cells (after Cooke 1958; Dickman 1968a). Cell counts of large multicellular green and filamentous blue-green algae were not made, and only their presence

or absence was recorded. The majority of the counting was done soon after filter preparation and most cells retained their contents and colour despite the relatively harsh chemical treatment during filter preparation. Live and dead cells were not enumerated separately (see Parr 1962; Weber 1966; Weber & Raschke 1966) but care was taken to count only those organisms with visible, intact cellular contents (Weber 1966; Londo 1967; Dickman 1968a).

Organisms enumerated in a total of 30 Whipple fields per filter for each original vertical slide were calculated as number per ml, number per sq. mm, and number per 230 ml or 6500 sq. mm. Comparable calculations were made for horizontal slides, but only vertical slides will be dealt with in detail. Final count estimates reported here are numbers of individual colonies and cells or organisms per sq. mm of original vertical slide surface area. These values were calculated for each individual taxon and also totalled across all taxa combined for each slide, by means of a computer.

The simple formula used in these calculations for each slide was as follows:

$$\bar{N} = \frac{C}{N} \sum_{i=1}^{N} \frac{n_i}{w_i \; v_i} \qquad (8)$$

where; "$\bar{N}$" is the calculated number of cells, individuals or colonies per ml; "C" is the conversion factor or the total actual filtering area of the filter divided by the area of one Whipple field; "N" is the total number of filters per slide; "n_i" is the number of individuals or colonies counted on the ith filter; "w_i" is the number of Whipple fields counted in, on the ith filter; and "v_i" is the volume of each aliquot filtered

through the ith filter. To convert this value ($\bar{N}$) to the number per sq. mm of original slide surface area, it was multiplied by the total volume of the bottle (230 ml) and divided by the total area of the slide (6500 sq. mm).

For each station and particular TS, the values for each taxon and total for all taxa combined were summed and averaged over the four replicate slides per sample and the means and standard deviations calculated.

STATISTICAL ANALYSIS OF THE COUNT DATA

In view of its considerable volume it was not practical to subject the sample count data to complete statistical treatment. However, to give an indication of the overall reliability of count estimates, representative periphyton samples from each lake were subjected to extensive statistical analyses and inferences about the complete data sets for separate lakes were based on these results. The vertical slide data given here were taken from exposure-frames at four periphyton sampling stations in Elk Lake. Essential details of lake morphometry and the field sampling program were given by Brown (1969).

The analysis of count data was essentially in four parts: (1) filter count distributions were tested for randomness in order to determine the necessity of counting entire filter surfaces; (2) statistical measures were applied to illustrate the variability or precision of count estimates; (3) the variation within samples, between replicate filter and slide counts, was tested for statistical significance; and (4) an analysis of the variance of the rountine experimental method was used to permit the determination of, among other things, the optimum

method design. These analyses were made for examples of both individual and total species count estimates.

A chi-square test for randomness (Ricker 1937; Lund *et al.* 1958; Greig-Smith 1964) was run on the total filter counts for one complete Elk Lake time series, TS01, the slides of which were immersed for 33 days from August 3 to September 5, 1967. The results for two stations only, are given in Table 27, and show that random distribution according to the Poisson law was not disproved for the majority of the data; the same hypothisis was not rejected for 99.2% of the chi-square analyses run on periphyton total species counts for 52 filters in this TS.

Many investigators (Littleford *et al.* 1940; Goldberg *et al.* 1952; Moore 1952; Ballantine 1953; Ehrlich 1955; Ecker & Lockhart 1959) assumed random distribution for all taxa on the filter surface, or within counting chambers and cells (unlike Holmes 1962). However, Kutkuhn (1958) found certain plankton genera failed to distribute themselves randomly in cell mounts. Those species departing significantly from Poisson and exhibiting contagion could be accounted for by a negative binomial distribution. Since both species composition and the total periphyton counts were important in this study, additional chi-square tests were run on individual taxa counts. An example illustrating that the hypothesis of random distribution was not entirely rejected for 18 periphyton species, is given in Table 28. Comparable results were found for the 29 taxa enumerated from periphyton communities in Elk Lake. Randomness was only disproved by this method when individual cells of colonial organisms such as *Asterionella formosa* Hass. or *Melosira italica* (Ehr.) Kütz. were tested instead of discrete units or numbers

Table 27. Tests of fit of Poisson distribution and related sample statistics for total periphyton species counts enumerated in 30 random Whipple micrometer fields on prepared filters from two sampling stations in Elk Lake (Code No. E-TS01-5/9/67-33-1 & 3). Counts are of individual colonies or discrete units.

Station	Slide	Filter	$\bar{x}$	s^2	s	$P=\frac{100C_v}{\sqrt{n}}$	$s^2/\bar{x}$	$X^2_{(df29)}$
1	V003	10A	23.43	20.4609	4.5233	2.8999	0.8731	25.3214
		10B	24.13	20.1195	4.4854	3.3933	0.8336	24.6740
		10C	23.93	21.0298	4.5858	3.4982	0.8787	20.8022
	V009	10A	23.03	16.9298	4.1145	3.2614	0.7350	21.3154
		10B	24.10	14.9896	3.8716	2.9330	0.6219	18.0373
		10C	23.63	18.9988	4.3587	3.3672	0.8039	23.3131
	V015	10A	27.53	14.2574	3.7759	2.5038	0.5178*	15.0169*
		10B	26.76	8.8667	2.9441	2.0081	0.3238**	9.3910**
		10C	27.56	10.8747	3.2976	2.1840	0.3944**	11.4401**
	V019	10A	25.56	26.8057	5.1774	3.6972	1.0484	30.4054
		10B	25.70	23.3896	4.8362	3.4357	0.9101	26.3929
		10C	25.63	27.0678	5.2026	3.7056	1.0559	30.6228
3	V001	10A	13.83	16.7643	4.0944	5.4038	1.2118	35.1445
		10B	13.93	16.0643	4.0080	5.2518	1.1529	33.4354
		10C	15.06	16.6850	4.0847	4.9497	1.1074	32.1150
	V005	10A	11.80	10.0965	3.1775	4.9163	0.8556	24.8135
		10B	12.10	8.3689	2.8929	4.3650	0.6916	20.0578
		10C	12.36	10.9298	3.3060	4.8808	0.8838	25.6307
	V010	10A	12.63	15.0678	3.3817	5.6097	1.1927	34.5883
		10B	13.53	15.9126	3.9890	5.3815	1.1758	34.0985
		10C	13.90	16.3689	4.0458	5.3141	1.1776	34.1510
	V019	10A	9.13	5.3609	2.3153	4.6283	0.5869	17.0218
		10B	10.76	6.8057	2.6087	4.4238	0.6321	18.3457
		10C	10.13	4.7402	2.1772	3.9227	0.4677*	17.0218

* significant at $P \leq 0.05$ but not at $P \leq 0.01$

** significant at $P \leq 0.01$

Table 28. Tests of fit of Poisson distribution and related sample statistics for counts of 18 periphyton taxa enumerated on one filter preparation (Code No. E-TSO1-5/9/67-33-1-V003-10A). Counts are of individual colonies or discrete units in 30 random Whipple micrometer fields.

Taxon	T	$\bar{x}$	$P=\frac{100C_V}{\sqrt{n}}$	$s^2/\bar{x}$	$X^2_{(df29)}$
Achnanthes minutissima Kütz.	144	4.80	10.7304	1.6580*	48.0833*
Amphora ovalis Kütz.	7	0.23	39.4365	1.0886	31.5714
Cocconeis placentula Ehr.	269	9.86	5.3395	0.8438	24.4729
Cocconeis placentula var. *lineata* (Ehr.) V.H.	12	0.40	22.7429	0.6206	18.0000
Cymbella sp.[1]	11	0.36	27.6875	0.8432	24.4545
Difflugia sp.	5	0.16	41.5227	0.8620	25.0000
Fragilaria crotonensis Kitton	39	1.30	14.8217	0.8567	24.8461
Fragilaria virescens Ralfs	27	0.90	17.9477	0.8697	25.2222
Gomphonema acuminatum var. *coronatum* (Ehr.) Baben.	11	0.36	27.6875	0.8432	24.4545
Gomphonema olivaceum (Lyngb.) Kütz.	25	0.83	18.2700	0.8344	24.2000
Melosira italica (Ehr.) Kütz.	7	0.23	39.4365	1.0886	31.5714
Melosira varians Ag.	6	0.20	37.1391	0.8275	24.0000
Navicula sp.	11	0.36	30.6201	1.0313	29.9090
Navicula cryptocephala Kütz.	10	2.73	11.3590	1.0580	30.6829
Nitzschia vermicularis (Kütz.) Grun.	5	0.16	50.5146	1.2758	37.0000
Pinnularia gibba Ehr.	4	0.13	47.3432	0.8965	26.0000
Stephanodiscus niagarae Ehr.	8	0.26	30.7941	0.7586	22.0000
Surirella sp.	3	0.10	55.7086	0.9310	27.0000
Total number of organisms	703	23.43	2.8999	0.8731	25.3214

[1]May include two of the following species: *C. turgida*, *C. ventricosa* and/or *C. affinis*.
*Significant at $P \leq 0.05$ but not at $P \leq 0.01$.

of colonies. Similar results have been reported for colonial forms by Gilbert (1942) and Lund *et al.* (1958).

Another test for randomness, Blackman's coefficient of dispersion or the variance: mean ratio, $s^2/\bar{x}$ (Greig-Smith 1964), approaches the value of one if the counts are distributed in a Poisson series with the mean equal to the variance (see also Moore 1952; Kutkuhn 1958; Cassie 1959a, 1959b). Any deviations from unity indicate clumping or underdispersion. With few exceptions (Tables 27 and 28), these tests showed no significant departure from randomness for the Millipore filter counts (unlike Sanford *et al.* 1969), giving essentially the same results as the chi-square analyses (see Cassie, 1959b).

For each count of 30 Whipple fields, the coefficient of variation ($C_v = s/\bar{x}$) and the percentage error of the mean (P) (after Moore 1952)[1] was calculated to provide a measure of the variability of count estimates obtained for both individual taxa and all taxa combined (Tables 27, 28, 29; Fig. 22). The distribution of C_v values obtained for each total species count taken from the 52 filters of TS01 is given in Figure 22 which shows that a C_v of less than 0.30 was found in 98% of these counts.

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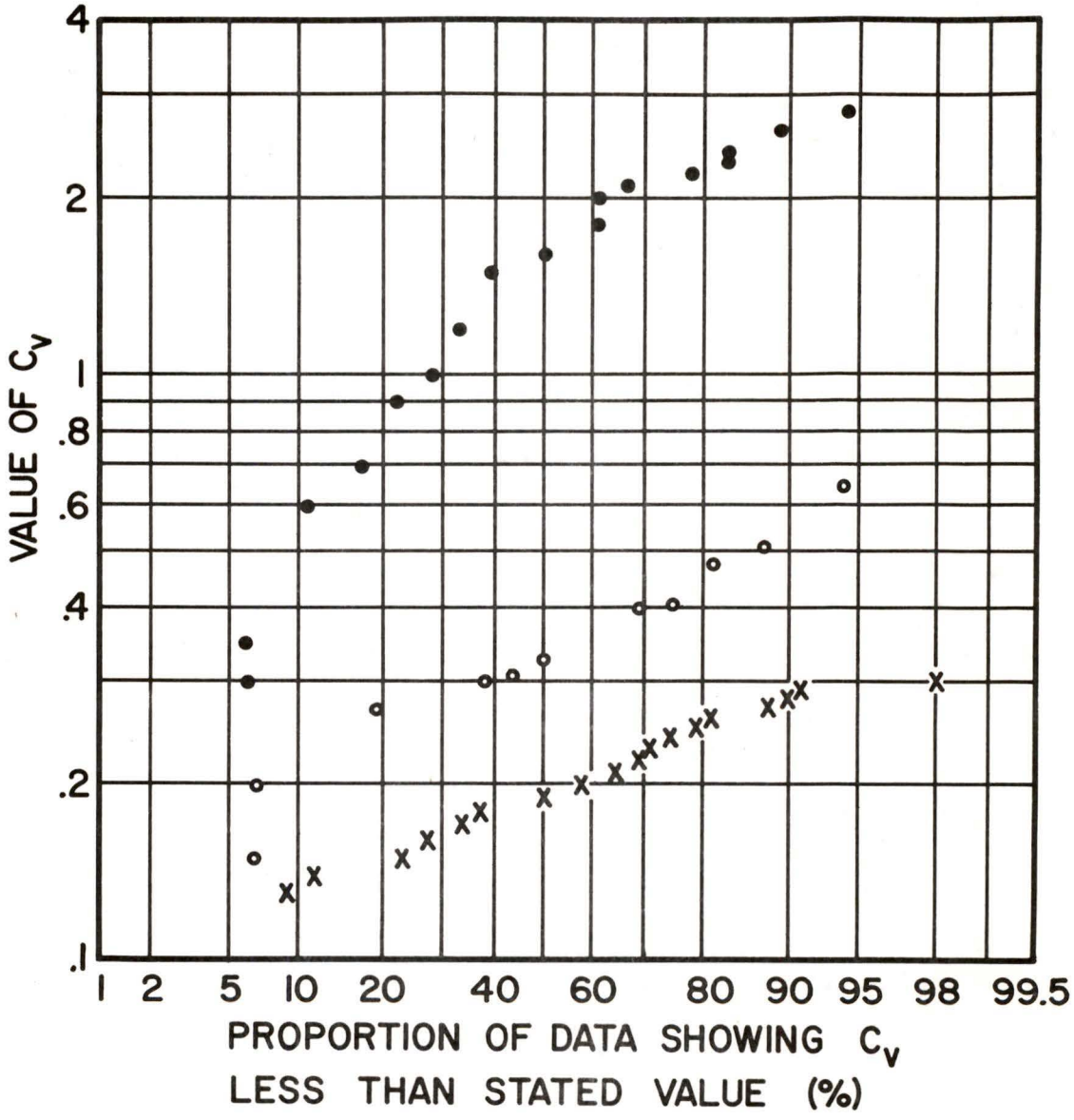
[1] The coefficient of variation, given as $s/\bar{x}$ by Moore (1952), is usually multiplied by 100 and given in percent in most standard statistical texts. C_v is expressed here in both decimal and percent form. Similarly, the percentage error, $P = \frac{100C_v}{\sqrt{n}}$, as given by Moore (1952) differs from that given by Sokal and Rohlf (1969) where the standard error of the C_v is represented as $S_{cv} \approx \frac{C_v}{\sqrt{2n}}$ (see also Simpson *et al.* 1960). However, Moore was followed in the present study to enable comparison of the results with comparable count data for freshwater plankton.

Table 29. Example filter count data for one Elk Lake periphyton species, *Cocconsis placentula* Ehr., showing percentage errors, extended count values and related sample statistics from two different time series at station 1.

TS	St.	Slide	Filter	Total	$\bar{x}$	s	$P = \frac{100C_v}{\sqrt{n}}$	Number per mm^2 vertical slide
01	1	V003	10A	296	9.86	2.8855	5.3394	108.7
			10B	297	9.90	2.8929	5.3350	109.1
			10C	296	9.86	2.9211	5.4054	108.7
		V009	10A	271	9.03	3.6149	7.3062	99.5
			10B	276	9.20	3.6426	7.2288	101.4
			10C	272	9.06	3.5421	7.1328	99.9
		V015	10A	299	9.96	2.9998	5.4951	109.8
			10B	297	9.90	3.1769	5.8589	109.1
			10C	297	9.83	2.5875	4.8042	109.1
		V019	10A	311	10.36	2.6971	4.7501	114.2
			10B	303	10.10	0.9948	1.9982	111.3
			10C	312	10.40	4.1447	7.2761	114.6
11	1	E360	60A	95	3.16	1.5104	8.7087	5.8
		E362	60A	82	2.73	2.1323	14.2434	5.0
		E363	60A	76	2.53	1.2793	9.2202	4.7
		E364	60A	93	3.10	1.9887	11.7127	5.7

The percentage error of the mean values (Table 27) did not exceed a maximum of ±5.4% of the true mean count values, or as Moore (1952) showed, there is approximately a 95% probability that the observed mean counts for 30 micrometer fields fall within ±2P of the true mean for each entire filter. These data for total species estimates were in sharp contrast with similar values calculated for individual taxa where a C_v less than 0.30 was found in only 5% of the counts from one filter, and 38% of 16 arbitrarily chosen filter counts for one species, *Cocconeis placentula* Ehr. (Fig. 22). Although results of further analyses were not always consistent, single species counts had generally higher percentage errors (Tables 27, 28, 29) than total species counts (Table 27), especially when the mean number of organisms per micrometer field was less than one. Larger filtered volumes or an increase in the number of field counts per filter would likely have decreased the variation of these count estimates, although it is possible that despite the non significant results of chi-square tests and Blackman's ratio, they may have been more readily fitted to negative binomial distributions which would account for a certain amount of the count variability detected here for individual species estimates. Kutkuhn (1958) and Moore (1952) provide formulae for estimating the optimum number of fields necessary to achieve a desired level of precision for count estimates. However, despite disappointingly large percentage error values in individual periphyton species estimates, the majority of the counts during this study compared well with the reported precision achieved in other counting schemes (Littleford *et al.* 1940; Moore 1952; Kutkuhn 1958; Lund *et al.* 1958; McNabb 1960). Further time spent to improve the reliability of individual species count estimates would not have been commensurate

Figure 22. Distribution of coefficient of variation (after Moore 1952) in form of a probability plot, illustrating the lower C_v values calculated for total species counts per Whipple field (x, 52 filters of TS01; Table 27), compared with those C_v values for 18 individual periphyton species field counts on one filter (o, Table 28) and those for 16 filter counts of one species, *Cocconeis placentula,* Ehr. (●, Table 29).


4
2
1
.8
.6
.4
.2
.1
VALUE OF C_v
1 2 5 10 20 40 60 80 90 95 98 99.5
PROPORTION OF DATA SHOWING C_v
LESS THAN STATED VALUE (%)

with the reduced variability attained. For example, a count of 50 Whipple fields for filter V003-10A (Table 28) would only reduce the percentage error from ±53% for 94% of the species counted in 30 fields, to ±30% error for a probable 75% of the 18 species counts (given a maximum C_v = 2.9). In view of the small sample: lake ratio (in terms of volume or substrate area suitable for periphyton colonization), the importance of species extremely low in count density to the total periphyton population structure within the lake remains in question.

In all filter counts of TS01, the last "new" periphyton species encountered was enumerated, on the average, in the 17th field (±6), indicating that a total of 30 fields included a sufficiently large area to ensure inclusion of representatives of all taxa present on each filter.

For the most part, total and individual periphyton species counts (means and totals) were consistently similar between filters and between the four replicate vertical slides of any one sample. Preliminary analyses of variance (after McNabb 1960) (*e.g.*, Table 30) run on filter counts showed that the variation between filters, within samples, was not statistically significant. However, because the number of filters per slide was not always constant (2-6), individual filter counts tended to be unequally weighted in some instances, and the unequal sample sizes were more difficult to handle statistically. Furthermore, after preliminary statistical treatment, in order that time might be conserved, only count totals for the entire 30 fields were tallied, on a counter, rather than the counts per individual field. It was thus decided to pool the filter data for each slide and run further statistical tests on the extended counts, *i.e.* numbers per sq. mm of original substrate. Therefore, to test the null hypothesis that there were no significant differences

Table 30. Analysis of variance between filter counts. Two examples of preliminary analyses illustrating that the variation between filter counts within samples of four replicate vertical slides was not statistically significant.

SOURCE OF VARIATION	df	SS	MS	F
a) TSO1-Station 1, *Cocconeis placentula* Ehr.				
Between filters	11	69.4082	6.3098	0.6949 ns
Within filters	348	3160.9666	9.0832	
Total	359	3230.3748		

$$C_v = \frac{\sqrt{9.0832} \times 100}{9.7916} = 30.78\%$$

b) TSO1-Station 2, total species counts				
Between filters	11	189.6880	17.2444	1.5591 ns
Within filters	348	3848.8667	11.0599	
Total	359	4038.5547		

$$C_v = \frac{\sqrt{11.0599} \times 100}{22.8899} = 14.53\%$$

in total species counts and all individual species counts, between the four slides of any one sample, a chi-square test was used where the mean of the four slides was used as the expected value (Drs. D.J. Ballantyne & R.E. Odeh, University of Victoria, personal communications). Of a total of 1,337 different chi-square analyses run on this data, only 33 tests gave significant (d.f. = 3) results, and of these, 15 were from tests run on the number of cells of the colonial diatoms, *Fragilaria crotonensis* Kitton, *F. virescens* Ralfs, and *Melosira italica* (Ehr.) Kütz. The significance of tests on cells of these species was to be expected in view of the deviation from randomness which their distributions showed, as previously discussed. Of the remaining 18 significant chi-squares, 9 occurred within samples taken from periphyton sampling station 4, with stations 1, 2, and 3 each having only three similar results.

These results suggest that station 4 in Elk Lake may not have been as similar in environmental factors influencing the colonization and growth of periphyton communities as the other three stations, although earlier work (Brown 1969) indicated that for the plankton at least, station 2 was not as similar as the other stations. In addition, significant results for all chi-square tests occurred for only five of a possible 29 different taxa enumerated: *Cocconeis placentula* Ehr. (9); *Achnanthes minutissima* Kütz. (6); *Fragilaria virescens* Ralfs (1); *Synedra radians* Kütz. (1); and *Navicula cryptocephala* Kutz. (1). The latter three were considered unimportant chance deviations due primarily to very low densities. However, it is of interest to note that the two most important species in this connection, as well as being the most

abundant taxa in periphyton communities throughout the study, *C. placentula* and *A. minutissima,* may be considered "true periphyton" (Sladeckova 1962) organisms in that they are firmly attached to the substrate surface along one entire side of the frustule and are thus highly dependent upon substrate area available for colonization. Chi-square results were not inconsistent with earlier interpretations of the data which suggested that a competition and replacement relationship between these two species was operative on the glass slides. The data indicated that, given the same environmental conditions as measured, and assuming the reproductive rates for *Achnanthes* and *Cocconeis* were approximately equal, as was found for planktonic populations in another British Columbian lake (Marion Lake; Dickman 1968a), then whichever species was able to colonize and establish a growing population first, became dominant on that slide and out-competed the other for space. A competition and replacement relationship between these two species and possibly other less abundant taxa would account, in part, for some of the variability in abundance of these two taxa in their distributions on four slides from the same TS and station. In view of the suggested importance of micro-habitat fluctuation to the distributions of these two species, the proximity of long term immersion slides with viable populations capable of colonizing adjacent slides would be an important factor contributing to the variability within samples. A greater number of replicate slides per sample would be necessary to establish more precise count estimates for these species.

Finally, an analysis of variance of the experimental design used to estimate the periphyton populations was undertaken as described by Davies (1963) (detailed examples of similar analyses were given by

Eaton and Moss 1966 and Happey 1970). The aim of this analysis was to establish the components of variance introduced at each stage of the sampling procedure and determine which stages might best be replicated to improve reliability of periphyton population estimates in future experiments, without unduly increasing the time required to process the data.

Table 31, illustrating the results of two such analyses, shows that the greatest source of variation was that of counting. As expected, the counting error for a single species (30%) was considerably greater than that for all taxa combined (17%) in the same sample. Nevertheless, these counting errors, in addition to the variances of the whole methods, were still within the ±50% standard error suggested by Lund *et al.* (1958) as adequate for most phytoplankton count estimates, where each new generation involves a population increase of 100%. However, *Cocconeis* may be expected to increase at a slower rate than most rapidly growing planktonic species, or more precisely, at a rate less than once every five days as estimated for a planktonic population of *Cocconeis* (75 reproductive cycles per year; Dickman 1968a). It is probable that most true periphytonic species, like epipelic populations (Eaton & Moss 1966), increase, on the whole, at a rate relatively slower than comparable planktonic populations and therefore periphyton count estimates should be more precise than those acceptable for planktonic populations. In contrast to the variance attributable to counting (Table 31), little variance was due to sampling and filtering. The mathematical artifact, or negative variance obtained at the filtering stage, is commonly interpreted as zero variance.

Table 31. Analyses of variance in estimation of littoral periphyton populations of Elk Lake.

Quantity estimated[1] by the mean square	Variance at each stage	Variance due to	Stage
$\sigma_c^2 + C\sigma_f^2 + CF\sigma_s^2$	$\frac{\sigma_c^2}{CF} + \frac{\sigma_f^2}{F} + \sigma_s^2$	σ_s^2	Sampling
$\sigma_c^2 + C\sigma_f^2$	$\frac{\sigma_c^2}{C} + \sigma_f^2$	σ_f^2	Filtering
σ_c^2	σ_c^2	σ_c^2	Counting

Stage	Variance σ^2	Standard Deviation σ	C_v
	TS01-St. 1-Total Species[2]		
(S) Sampling	2.8919	1.7005	6.78 %
(F) Filtering	-0.4772	0	0
(C) Counting	18.7686	4.3322	17.28 %
Whole Method	3.0996	1.7605	7.02 %
	TS01-St. 1-*Cocconeis placentula* Ehr.[3]		
(S) Sampling	0.2449	0.4949	5.05 %
(F) Filtering	0.2928	0.5411	5.53 %
(C) Counting	9.0832	3.0138	30.18 %
Whole Method	0.2482	0.4982	5.09 %

[1] S = number of slide replicates per sample; F = number of filters per slide; C = number of counts per filter.
[2] Mean of all counts = 25.06 individuals; S = 4; F = 3; C = 30 .
[3] Mean of all counts = 9.79 individuals; S = 4; F = 3; C = 30 .

It thus appears that the greatest variance was introduced into the experimental design during counting, while filtering and sampling each accounted for less than 10%. These results are in contrast with similar analyses of comparable designs (Eaton & Moss 1966; Happey 1970), where the largest variance component was due to sampling. However, the difference may be partially explained on the basis of data assimilation and interpretation, and the numerical value given to C. In this design, counts were recorded separately in each of 30 random Whipple fields (C^1 or w_i = 30), whereas Happey (1970), for example, recorded a count only after one complete transect of a cell mount (C = 1). Four complete transects would likely include a counting area much larger than that included by 30 fields, but yield a C value much lower than 30 (C = 4). The variance due to sampling would be much greater if C were not large.

In view of the relatively small substrate area sampled by each vertical glass slide and the heterogeneity in natural micro-habitats, it was expected that sampling variation would be quite large due to the inclusion at the sampling variance stage, of both variance due to sampling technique and natural variation in populations affected by habitat differences. The chi- square results confirmed this in 33 different slide samples for individual species, and the analysis of variance of TS01, station 3 total species counts (Table 32) shows the variance due to sampling was, in this case, a more realistic 15%, greater than that shown in Table 31.

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[1] C, after the notation used by Happey (1970), is from Tables 31 and 32 and is not from expression (8).

Table 32. Analysis of variance and method designs. 252

Stage	Variance σ^2	Standard Deviation σ	C_v
	TS01-St. 3-Total Species[1]		
(S) Sampling	3.2576	1.8048	14.52 %
(F) Filtering	0.1060	0.3256	2.62 %
(C) Counting	9.6426	3.1052	24.96 %
Whole Method	3.4001	1.8439	14.83 %

Design No.	S	F	C	Overall Variance		Standard Deviation	C_v
1.	1	1	1	$\sigma_s^2 + \sigma_f^2 + \sigma_c^2$	13.0062	3.6064	29.00 %
2.	2	1	1	$\frac{\sigma_s^2}{2} + \frac{\sigma_f^2}{2} + \frac{\sigma_c^2}{2}$	6.5031	2.5501	20.51 %
3.	1	2	1	$\sigma_s^2 + \frac{\sigma_f^2}{2} + \frac{\sigma_c^2}{2}$	8.1319	2.8516	22.93 %
4.	1	1	2	$\sigma_s^2 + \sigma_f^2 + \frac{\sigma_c^2}{2}$	8.1849	2.8609	23.01 %
5.	¼	1	1	$4\sigma_s^2 + 4\sigma_f^2 + 4\sigma_c^2$	52.0248	7.2128	58.01 %
6.	1	1	¼	$\sigma_s^2 + \sigma_f^2 + 4\sigma_c^2$	41.9340	6.4756	52.08 %
7.	1	1	½	$\sigma_s^2 + \sigma_f^2 + 2\sigma_c^2$	22.6488	4.7590	38.27 %
8.	3	2	1	$\frac{\sigma_s^2}{3} + \frac{\sigma_f^2 + \sigma_c^2}{6}$	2.7105	1.6463	13.24 %

[1] Mean of all counts = 12.43 individuals; S = 4; F = 3; C = 30 .

Note that Table 31 shows the individual components of variance; that is, for TS01, station 1, *Cocconeis placentula,* the variance due to sampling (σ_s^2) was 3.2576, variance due to filtering (σ_f^2) was 0.1060, and variance due to counting (σ_c^2) was 0.6426. This is not equivalent to the variance at each subsampling stage which is a cumulative phenomenon given by the estimated variance formulae as modified from Happey (1970). Similarly, the overall variance (sum), $\sigma_s^2 + \sigma_f^2 + \sigma_c^2$, is not the same as the variance of the whole method, given by the same formula used to calculate the variance at the sampling stage (see Eaton & Moss 1966; Happey 1970).

It is clear that increasing the number of replicates at any stage of subsampling will increase the precision of the method. Table 32 illustrates the components of variance in count estimates of total periphyton species for TS01, station 3, and the changes in the overall variance attributable to alterations in the number of slide, filter and Whipple field replicates (after Eaton & Moss 1966). Increasing or decreasing the number of replicate slides per sample has the greatest effect on the overall variance. Duplicating the slides (design 2), *i.e.,* increasing the number of slides per sample from 4 to 8, resulted in almost 50% reduction in the overall variance with a corresponding reduction in the standard error of about 30%. A sample size of one slide (design 5) resulted in a four-fold increase in the overall variance. Duplication of the number of replicates at the filtering and counting stages (designs 3 and 4) increased the reliability to about the same extent as increasing slide replicates per sample, while reducing the number of Whipple fields counted per filter to about 7-15 (designs 6

and 7) increased overall variance considerably. Clearly, increasing both the number of slides and filters (design 8) resulted in a very reliable method of estimating the periphyton populations. However, the practicalities involved in a long term survey requiring 12 slides per sample and 6 filters per slide would in most cases be prohibitive for routine analyses.

EVALUATION AND DISCUSSION

Experimental results to date indicate that the exposure-frame described is useful for quantitative sampling of periphyton communities in littoral regions. Despite drawdown fluctuations and wind-generated undertow currents in these shallow zones, the frames remained upright and stable over lengthy immersion periods, and none suffered vandalism. There was no corrosion evident after a two year immersion period in freshwater, while only minor corrosion of the Dexion framework was apparent after one year in an alternatingly hypo-/hyper-saline marine inlet, and the potential exists for future use of these frames in relatively shallow subtidal marine situations.

Each frame supported sufficient slides to permit long term investigation, although the lakes sampled required at least 30 days slide immersion for the establishment of relatively stable communities. In studies requiring sampling at more frequent intervals, reloading of frames at designated intervals would be necessary to ensure a number of slides adequate to effect overlapping sampling periods without reducing the number of slide replicates per sample. There was no clear evidence that settlement of colonizing organisms on clean new slides was dependent upon proximity of other long term immersion slides. However, this may warrant further study and statistical assessment in view of the competition-replacement relationship suggested between the opportunistic species *Achnanthes minutissima* Kützz., and *Cocconeis placentula* Ehr. So that recruitment on the glass slides is not influenced by periphyton film communities developed on the supporting frame itself, all frames should be either removed and completely

scrubbed down, or replaced with clean substitutes at regular predetermined intervals. Due to the economical cost of production (about $15 per frame), the holding of new or used frames in reserve is not prohibitive. In addition, the fact that only one exposure-frame per station is required allows an increase in the number of sampling stations per lake. Reported conclusions reached in periphyton studies have too often been based on results from single sampling stations only. Moreover, in contrast to suspended open-water substrata (*e.g.*, Maciolek & Kennedy 1964; Sladeckova 1966; Benson 1967), the exposure-frame places the substrata in a more realistic and ecologically relevant position in relation to the position of most natural substrata (see Wetzel 1965), which typically occur in the littoral zone.

In general, the present studies required both horizontal and vertical slides in relatively large numbers and other methods assessed could not be easily modified to accomodate these. Alternative methods were not suitable for long immersion periods and the exposure of natural substrata; stakes and visible stoppers or floats tended to entangle and foul suspension lines in addition to attracting repeated acts of vandalism.

During the experiments reported here, Elk Lake periphyton or *Aufwuchs* communities consisted almost entirely of algae with the occasional associated beterotrophic microorganism. A total of 41 different species were found, and of these, 29 taxa (28 diatoms; one amoeboid Protozoan) were counted whilst the remaining 12 organisms (3 blue-green and 9 green algae) were tabulated as present or absent. The species composition of the predominantly diatom flora which developed on the glass slides was very similar to that found on natural substrata of Elk Lake (macrophytes and small pebbles), particularly with respect to the more abundant

taxa such as *Cocconeis placentula* Ehr., *Achnanthes minutissima* Kütz., *Navicula cryptocephala* Kutz., *Fragilaria* spp., *Gomphonema* spp., and *Synedra* spp. Generally, the species composition of glass slide communities from Elk Lake compared well with that of similar periphyton communities reported from both natural and many artificial substrata (Butcher 1932; Brook 1955; Gumtow 1955; Whitford 1956; Castenholz 1960; Hohn & Hellerman 1963; Round 1964). However, a quantitative study of periphyton community structure on both glass and natural substrata would be necessary to determine the selectivity of substrata type, as well as the possible release of toxic metal ions from the frame construction materials. Furthermore, even though species composition was essentially identical on vertical and horizontally exposed slides, the possibility of predator preference for a specific substrate orientation, and the influence of increased deposition of detritus on horizontal slides warrant further investigation. Such environmental variables as grazing or physico-chemical limiting factors, could be profitably investigated under controlled laboratory conditions using *in situ* glass slide periphyton communities, freshly removed from exposure-frames.

Although the preparation of permanent Millipore filters sacrificed some small Protozoans and microflagellates, the majority of the periphyton community components were well preserved without undue distortion and two year old filter preparations remain in excellent condition. More tests for random distribution of taxa may lead to modification of counting procedures for different lakes, *i.e.*, increasing Whipple fields, filtered volumes, and filters per slide. Failure of tests of significance may indicate that the numerical abundance of individual taxa is insufficient, rather than random distribution, and conformity to a negative binomial

distribution should be investigated for those species consistently displaying departure from randomness and contagion on the filters. Actual counts of the most common periphyton taxa encountered revealed little statistically significant variation between individual counts on successive filter preparations, as illustrated by the preliminary analysis of variance examples in Table 30, and the chi-square tests between slide replicates. Few periphyton studies (if any) involving count estimates, have included investigation of individual species distributions and estimates of the precision of count data. However, as in the present case, it is not uncommon in plankton studies to find that the variability of individual species components is greater than that of the whole population (Kutkuhn 1958; Hopkins 1963; Angel 1969). Modification of experimental design, as shown in Table 32, is necessary to improve individual count precision, even though most species count estimates here were within the ±50% standard error limit for phytoplankton (Lund *et al.* 1958).

Recently developed methods for making permanent slide mounts (Dickman 1968b; de Noyelles, Jr. 1968; Sanford *et al.* 1969) may possibly be adapted for use in periphyton studies of this nature. It is conceivable that much of the time consuming, tedious work of sample preparation and periphyton enumeration may be alleviated in the future by means of a Coulter counter (Cushing *et al.* 1963; Mulligan & Kingsbury 1966) or scanning microscope photometer (Zimmer 1970). However, until such time as this equipment becomes economically feasible for routine analysis, methods similar to the one described here will continue to be used for estimating population sizes of aquatic microorganisms. The sampling design used in Elk Lake, gave reasonably reliable periphyton

count estimates, and the precision of the whole method did not exceed 15%. The counting error (17-30%) was comparable to that quoted by Eaton and Moss (1966) using similar subsampling procedures for estimation of epipelic algal populations (maximum 23%) and by Happey (1970) for estimation of benthic and planktonic populations of small cocoid and flagellate green algae (maximum 26%).

To the author's knowledge, no other periphyton investigations to date, have involved an analysis of the components of variance of the whole quantitative sampling method. Given the Elk Lake data and the interpretation shown, then contrary to comparable results of Eaton and Moss (1966) and Happey (1970), the greatest variance in the sampling design was attributable to the counting stage (see Moore 1952; Kutkuhn 1958), rather than the sampling stage. Hence studies using the exposure-frame should be first modified at the counting stage to improve the reliability of the overall sampling method, and as shown, the described experimental design may be variously modified to meet the needs of many different investigations. Similarly, periphyton collection methods developed for lotic habitats (Patrick *et al.* 1954; Hohn & Hellerman 1963; Weber 1966; Weber & Raschke 1966; Cushing 1967) might be amenable to modification for periphyton collection in studies of littoral regions in lakes, and work continues on the design and construction of more suitable exposure-frame methods, and substrate types, as well as counting procedures.

Before the part played by periphyton organisms in aquatic ecosystems can be accurately assessed, precise reproducible methods for quantitative sampling must be developed, and designed with particular attention to natural micro-habitat variability as considered with comparable plankton

sampling methods (Cassie 1959b; Hopkins 1963; Baker 1970). Until the structure of natural periphyton communities under different environmental conditions is known, conclusions about limiting factors and biological pollution indicators, drawn from laboratory experiments, are largely meaningless when used in interpreting field conditions. Aside from artificial substrata, few other quantitative sampling methods have been investigated for determining periphyton community structure (McIntire 1968a; McIntire *et al.* 1969; Moss 1969). Despite a recent increase in the number of periphyton investigations, basic quantitative information concerning efficacy of substrate type, orientation, area, length of immersion, number of sample replicates, counting procedures, *etc.*, remain largely unexplored or ignored.

The enumeration of individual taxa, in addition to total species counts, was necessary in the Elk Lake studies. Quantitative assessment of community structure or percentage species composition by cell number revealed differences between stations within Elk Lake not evidenced by total numbers. Just how meaningful these differences in community structure are in terms of physico-chemical variables and biological interaction, remains speculative and warrants further work.

SUMMARY

An exposure-frame was designed and constructed to quantitatively sample the littoral periphyton *Aufwuchs* communities in lentic habitats, using artificial substrata (namely large glass microscope slides), in both horizontal and vertical orientation. Within the study lakes, one frame was placed at each selected periphyton sampling station and immersed replicate slide samples were removed at monthly and overlapping time intervals. Collected slides were placed in individual bottles containing measured volmes of formalinized distilled water. In the laboratory, after removal of periphyton organisms from the slides, subsample aliquots were vacuum filtered onto Millipore filters and permanent slide mounts prepared using standard methods. Individual taxa and all species combined were enumerated on each filter and the numbers of organisms per sq. mm of original glass substrate area calculated, along with standard statistical measures.

Statistical analysis of count data was illustrated using vertical slide periphyton data from four sampling stations in Elk Lake (B.C., Canada). The distribution of count data was tested for randomness by chi-square analysis and the variance: mean ratio. Results indicated that the hypothesis of random distribution was not disproved according to the Poission law for the majority of the experimental data presented. To measure the precision of count estimates, the coefficient of variation and percentage errors were calculated, and results showed that total periphyton taxa counts were generally more reliable than individual species estimates. The differences in extended count estimates between the four slides of any one sample were assessed by means of a chi-square

test and found non significant for the majority of the data. An analysis of the experimental design used to estimate the periphyton populations indicated the largest variance component was introduced during counting whilst little variance or error was attributable to either the filtering or the sampling stages of the method. Experimental data were given to illustrate the changes in overall variance of the whole method which can be expected when different experimental designs involving modification of the numbers of slide, filter and count replicates are considered for possible use in future investigations.

Certain alternative quantitative periphyton sampling methods were assessed under field conditions prior to exposure-frame construction and disadvantages of these methods were noted. The exposure-frame and experimental design used in studies of Vancouver Island lakes were evaluated and generally found suitable for quantitative estimates of littoral periphyton populations. Possible modifications of frame design and sampling procedures were suggested and the results of some Elk Lake data analyses discussed.

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