

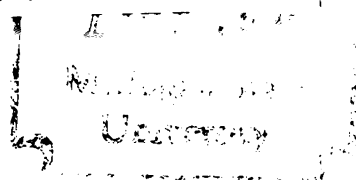


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of the requirements for

Ph.D. degree in Botany

Major professor

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DIFFERENTIAL EFFECTS OF GROWTH AND LOSS PROCESSES IN
CONTROLLING NATURAL PHYTOPLANKTON POPULATIONS

by

WILLIAM GRADY CRUMPTON

A DISSERTATION

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ABSTRACT

DIFFERENTIAL EFFECTS OF GROWTH AND LOSS PROCESSES IN CONTROLLING NATURAL PHYTOPLANKTON POPULATIONS

by

William Grady Crumpton

An investigation was made of factors controlling algal succession in a small, oligotrophic lake during summer stratification. Weekly measurements were made of growth rate, sedimentation rate and population density for each of the dominant phytoplankton species. Weekly diel measurements were made of zooplankton grazing rates using ^{14}C labeled algae. Cyclotella michiganiana was the dominant alga through the end of June at which time Cyclotella comensis began to increase, becoming the dominant by August. In August, high grazing pressure caused the rapid declines of both C. michiganiana and C. comensis which were followed by an increase of Sphaerocystis Schroeteri. The combined effect of greater growth rates and lower loss rates of C. comensis resulted in its dominance over of C. michiganiana. In contrast, the C. comensis - S. Schroeteri succession clearly resulted from differential mortality alone. It is likely that the importance of losses due to sedimentation and/or grazing is large in many lakes and that interspecific competition may be less important in actually controlling seasonal succession.

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INTRODUCTION

The dynamics of algal succession are of fundamental importance in the ecology of freshwater lakes and, accordingly, much effort has gone toward delineating factors which contribute to the rise and decline of phytoplankton populations. The results of such study now make it apparent that while losses due to grazing, sinking, and other processes may at times be quite substantial, their impact will vary for different populations and conditions. In addition, losses may have less apparent effects upon the size of a rapidly reproducing population than upon that of a stationary one. In order to assess the actual impact of various processes, it is necessary to conduct studies in which population increases due to growth and decreases due to grazing, sinking, and other processes are determined simultaneously.

Unfortunately, such comprehensive investigations are rare and most studies address community level processes, which reveal very little regarding the populations involved. If phytoplankton ecologists are to realistically address algal succession, it is imperative they initially investigate population level processes. Primary production is obviously dependent upon species characteristics as are sedimentation and other transport processes. Indeed, grazing, even by filter feeders, is a much more discriminatory process than is often realized (Porter 1977; Infante 1978; Starkweather and Gilbert 1978). Many other processes seem to display a high degree of specificity as to the phytoplankton affected (e.g. death due to parasitism or physiological extremes). If successional changes are to be understood, the differential effects of the above processes upon individual phytoplankton populations must be considered.



The need for such consideration has not escaped previous investigators, and attempts have been made to address the dynamics of individual phytoplankton populations. The work of Lund (1949, 1950, 1954) is a well-known example of excellent investigation along these lines. More recently, Knoechel and Kalff (1975, 1978) have utilized autoradiography in an effort to determine the relative importance of growth rates and loss rates in algal succession. However, neither these nor other investigations have combined actual measurements of growth rates and loss rates to delineate phytoplankton population dynamics.

Such basic information from field systems is essential to an understanding of the structure and function of natural communities. Recent laboratory and theoretical investigations greatly increased our understanding of certain major processes (Dugdale 1967; Greeney, et al. 1973; Droop 1974; O'Brien 1974; Tilman 1977). However, an understanding of these individual processes provides limited insight unless we know the frequency, intensity, and duration of their operation in nature.

DESIGN AND METHODOLOGY

The objective of this study was to construct a detailed population growth equation for each of several major species of phytoplankton in Lawrence Lake, a small (4.96 ha., $z_m = 12.6$ m, $\bar{z} = 5.9$ m), oligotrophic lake in southwestern Michigan (for a more detailed description, see Wetzel et al. 1972). The components of the equation may be conceptualized as:

$$\bar{r} = \mu - g - s - p - f \quad (1)$$

where $\bar{r}$ = predicted increase

μ = growth (by cell division)



g = mortality due to grazing

s = loss due to sedimentation

p = mortality due to parasitism or physiological
extremes

f = flushing losses

are expressed as instantaneous rates for each population. The logistical barriers to such an investigation are of course enormous and a primary reason for the lack of previous study. This investigation was limited to a consideration of epilimnetic populations during summer stratification in 1979 and specifically to the diatom-green algal succession which is a prevalent feature of this lake. Temperature profiles were obtained twice weekly in order to track the epilimnetic-metalimnetic boundary. Simultaneous estimates of growth rates and loss rates were obtained for each phytoplankton population studied. Actual rates of increase (r) were obtained independently based on population changes. With these data, it is possible to assess the relative importance of these processes in phytoplankton population dynamics.

Estimates of phytoplankton population size and r :

Replicate phytoplankton samples were collected weekly from a central station using an integrating tube sampler and were preserved with 0.4% Lugol's solution. The samples were processed and the phytoplankton counted using the technique described in Appendix B. Using a combination of field and strip counts, between 100 and 1000 cells of each species were counted for each sample and 95% confidence limits calculated as in Lund, Kipling, and Le Cren (1958). Instantaneous daily rates of increase (r) between successive sampling periods were calculated from

$$N_7 = N_0 e^{r_7} \quad (2)$$

where N_0 and N_7 represent population estimates for successive, weekly samples.

Estimates of μ and p :

In this study, I used dialysis chambers (Appendix A) suspended in situ to estimate growth rates of natural phytoplankton populations at weekly intervals. The use of these chambers was critically evaluated experimentally and in Lawrence Lake. Exchange rates of dissolved material was very rapid and estimates of growth rates were not statistically significantly different from those of natural populations under the conditions of this study (Appendix A).

Triplicate dialysis chambers were filled with lake water from which the dominant grazers had been removed by filtration through 153- μ m mesh Nitex® netting. An aliquot of this water was preserved with 0.4% Lugol's solution for estimates of initial cell numbers (C_0 below). The chambers were suspended at the depth of mean epilimnetic light intensity using a surface-tethered buoy to provide agitation of the chambers via wave action. After four days of incubation, the chambers were collected and the incubated samples were preserved with 0.4% Lugol's solution. Phytoplankton counts were made as described above on initial (C_0) and final (C_4) samples. Instantaneous growth rates (μ) were calculated from

$$C_4 = C_0 e^{\mu_4} \quad (3)$$

For each incubation, physiological death (p) was estimated based on direct microscopic evaluations of changes in numbers of dead cells. Cellular and subcellular integrity were the criteria used to distinguish

living from dead cells. Cells were considered viable unless there was visible damage to their cell walls or plastids.

Based on the work of Haney and Hall (1975), two sampling periods were chosen for measuring zooplankton grazing rates. Daytime (2-3 hours past midday) and nighttime (2-3 hours past sunset) grazing rates (5 replicates each) were measured at weekly intervals using ^{14}C -labeled, log-phase cultures of Chlorella pyrenoidosa of a mean cell diameter of 5 μm . Zooplankton were collected from the epilimnion using vertical tows with a calibrated net (153- μm mesh size) and immediately transferred to a feeding suspension of unfiltered lake water with ca. 10^3 cells ml^{-1} of labeled C. pyrenoidosa (1-2 dpm cell $^{-1}$). The effect of this collection and transfer was evaluated by comparison with a modification of Haney's (1973) technique. No significant difference was found between collection rates estimated by the two methods (Table 1). After a six-minute feeding interval, the zooplankton were collected on 153- μm mesh netting, narcotized with carbonated water, transferred to liquid scintillation vials and preserved with 95% ethanol. These entire samples were later processed using tissue solubilizers (Packard Soluene 350) and their radioactivities determined with liquid scintillation radioassay. Counting efficiencies were obtained from a quench curve constructed using internal standards. For controls, zooplankton were killed with ethanol prior to the feeding incubation and then processed as were the other samples. Based on the volume of water from which the zooplankton were collected and on their feeding activity, grazing rates were calculated as % volume cleared hr^{-1} . Daytime and nighttime hourly rates were assumed constant for daytime and nighttime periods, and were used to calculate instantaneous rates of mortality due to grazing (g).

Table 1. Comparison of the net concentration and transfer technique with Haney's (1973) in situ technique.

Species	Relative collection rate (+ S.D.; n = 3)	
	Net concentration	Haney chamber
<u>Daphnia pulex</u>	4.2 + 0.4	4.4 + 0.4
	2.3 + 0.2	2.0 + 0.2
<u>Daphnia galeata mendotae</u>	1.6 + 0.2	1.5 + 0.2
Calanoid copepods	0.34 + 0.04	0.37 + 0.05



For biomass estimates, triplicate zooplankton samples were collected as described above, narcotized with carbonated water and preserved with 95% ethanol. Total zooplankton volume (μl zooplankton l^{-1}) was measured for each sample using a graduated column. Zooplankton were not counted, but representative samples were examined and the major forms identified.

Estimates of s and f:

Current methodology does not allow an in situ measure of species-specific sinking rates. Therefore, in this study, I estimated sinking losses from the epilimnion during a specified interval using sedimentation traps placed just below the epilimnetic-metalimnetic boundary. The placement of the traps was adjusted twice weekly to the gradually changing epilimnetic-metalimnetic boundary.

The traps used in this study were cylinders with a height:width ratio of 23 (43 cm long x 1.9 cm dia.). The critical work of Hargrave and Burns (1979) indicates that traps of this design collect with 95-100% efficiency in water moving less than 5 cm sec^{-1} . Thus, when suspended in metalimnetic layers, as in this study, such traps should provide reasonable estimates of sedimentation.

Each week, triplicate sedimentation traps were suspended just below the epilimnetic-metalimnetic boundary and harvested the following week. The traps were initially filled with filtered ($0.5\text{-}\mu\text{m}$) lake water, and a layer of Lugol-NaCl solution was placed in the bottom of each trap to preserve sedimenting organisms. The material collected was assumed to represent cumulative loss from the epilimnion per surface area of trap opening. Instantaneous rates of loss due to sedimentation (s) were calculated from



$$S = \int_0^7 sN_t dt \quad (4)$$

where $N_t = N_0 e^{rt}$ = population size at each instant and S = total number of cells sedimented volume⁻¹ during the 7-day trapping period.

Integrating and solving,

$$s = (rS)/(N_7 - N_0) \quad (5)$$

Base flow of Lawrence Lake inlet streams is very low compared to epilimnion volume and flushing losses could only be significant during storm events. Flushing losses (f) were calculated assuming that all precipitation impinging on the drainage basin went through the epilimnion. At no time did flushing losses calculated in this manner reach more than a few per cent and they are thus assumed to be insignificant.

RESULTS AND DISCUSSION

From spring through midsummer, Cyclotella michiganiana and C. comensis strongly dominated the phytoplankton in terms of numbers, biomass and production. In August there was a precipitous decline of Cyclotella followed by an increase of Sphaerocystis Schroeteri (Figure 1A). These three species comprised >90% of the total phytoplankton from mid-June through September, and the following analyses are restricted to their dynamics.

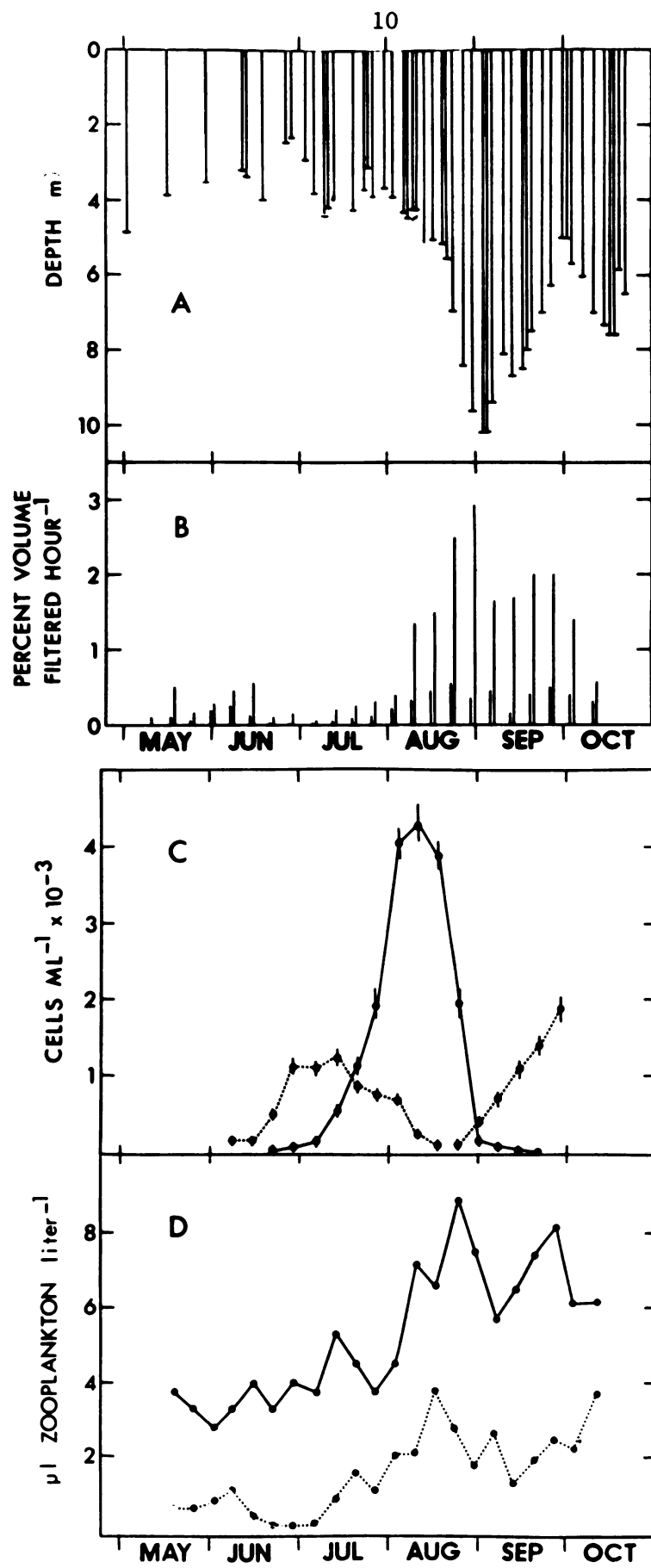
The decline of Cyclotella and subsequent increase of Sphaerocystis is strikingly mirrored by changes in Secchi depth water transparency (Figure 1B). Data shown in Figure 1C suggests that grazing pressure was a major factor in the Cyclotella-Sphaerocystis succession. Grazing rates were relatively low through June, reached a peak in August during

Figure 1A. Population curves for Cyclotella michiganiana (-----), Cyclotella comensis (————), and Sphaerocystis Schroeteri (- - - -). Error bars denote 95% confidence limits.

Figure 1B. Transparency (Secchi depth) in meters.

Figure 1C. Grazing rates as % of epilimnion filtered hr^{-1} . The first of each pair of bars represents daytime rates; the second represents nighttime rates. Each bar presents mean of five replicates (average C.V. = 22%).

Figure 1D. Daytime (-----) and nighttime (————) epilimnetic zooplankton biomass. Each point represents mean of three replicates (average C.V. = 10%). Dominant zooplankton were copepods (>90%) from May through July and Daphnia spp. (>90%) from August through October.





the decline of Cyclotella and remained high for the duration of the study. These seasonal patterns in grazing were related to quantitative changes in the zooplankton community (Figure 1D). In addition, the seasonal increase in grazing pressure in early August (Figure 1C) corresponded to a shift from a copepod community to one dominated by Daphnia pulex and D. galeata mendotae.

It is obvious that there was a striking diel periodicity in grazing pressure, with consistently higher rates at night (Figure 1C). It is also obvious that this diel pattern was related to increases in zooplankton biomass at night, presumably due to migration from deeper strata (Figure 1D). However, the well established nocturnal increase in feeding activity of individual zooplankton (Haney and Hall 1975) must also have contributed to the higher grazing pressure at night and it is not possible to separate the relative contribution of that effect.

Estimates of *b*, *s*, and *r* are available for each population. However, grazing rates are based on collection of Chlorella and are thus not independent estimates for each population. Cyclotella michiganiana and C. comensis are small centric diatoms whose diameters ranged from 8-16 μm and from 4-10 μm respectively in this study. Gut analyses showed that both Cyclotella species were readily ingested by Lawrence Lake zooplankton. While some selectivity is likely, the methods employed do not allow separate estimates of grazing mortality for these two populations. It is therefore necessary for the current study to assume both Cyclotella were grazed at the measured rates. In contrast, Sphaerocystis Schroeteri is a large colonial green alga, and colonies commonly exceeded 40 μm in diameter. Gut analyses showed no ingestion by Lawrence Lake zooplankton (including Daphnia pulex and D. galeatae

mendotae) during the period of this study. Sphaerocystis is certainly ingested by large Daphnia (Porter 1976, 1977) and gut analyses of Daphnia collected from Lawrence Lake in October, 1979 showed substantial ingestion at that time. However, since ingestion was not found during the period studied here, g is set equal to zero for Sphaerocystis.

In the successional pattern shown in Figure 1A, C. michiganiana was the dominant alga through the end of June at which time C. comensis began to increase. During July, C. michiganiana declined slightly while C. comensis continued to increase becoming the clear dominant by August. With the increased grazing pressure in August, there was a rapid decline of both C. michiganiana and C. comensis followed by an increase of S. Schroeteri.

Returning to the conceptual framework established earlier (Eq. 1), it is now possible to critically evaluate the roles of growth, grazing and sedimentation in the dynamics of these phytoplankton populations. Physiological death was never sufficiently significant to measure for any of these populations and, as stated above, flushing losses were negligible. The population growth equations can thus be simplified to

$$\bar{r} = \mu - g - s \quad (6)$$

for C. michiganiana and C. comensis, and to

$$\bar{r} = \mu - s \quad (7)$$

for S. Schroeteri.

These rate equations and corresponding population curves are plotted for each population in Figures 2, 3 and 4. Growth rates of C. michiganiana (Figure 2) declined initially, but showed no clear trend after late June. However, sedimentation losses were quite large and



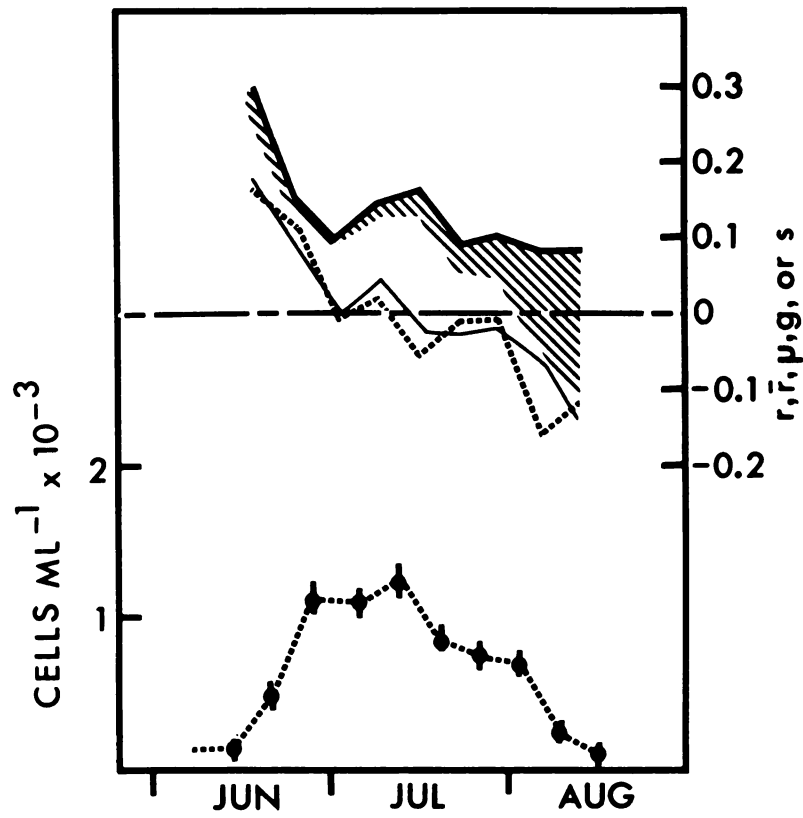


Figure 2. Population curve (lower figure, ----) and rate budget (upper figure) for Cyclotella michiganiana showing μ (—), g (hatched area), $\bar{r}$ (—), s (open area between hatching and $\bar{r}$), and r (----).



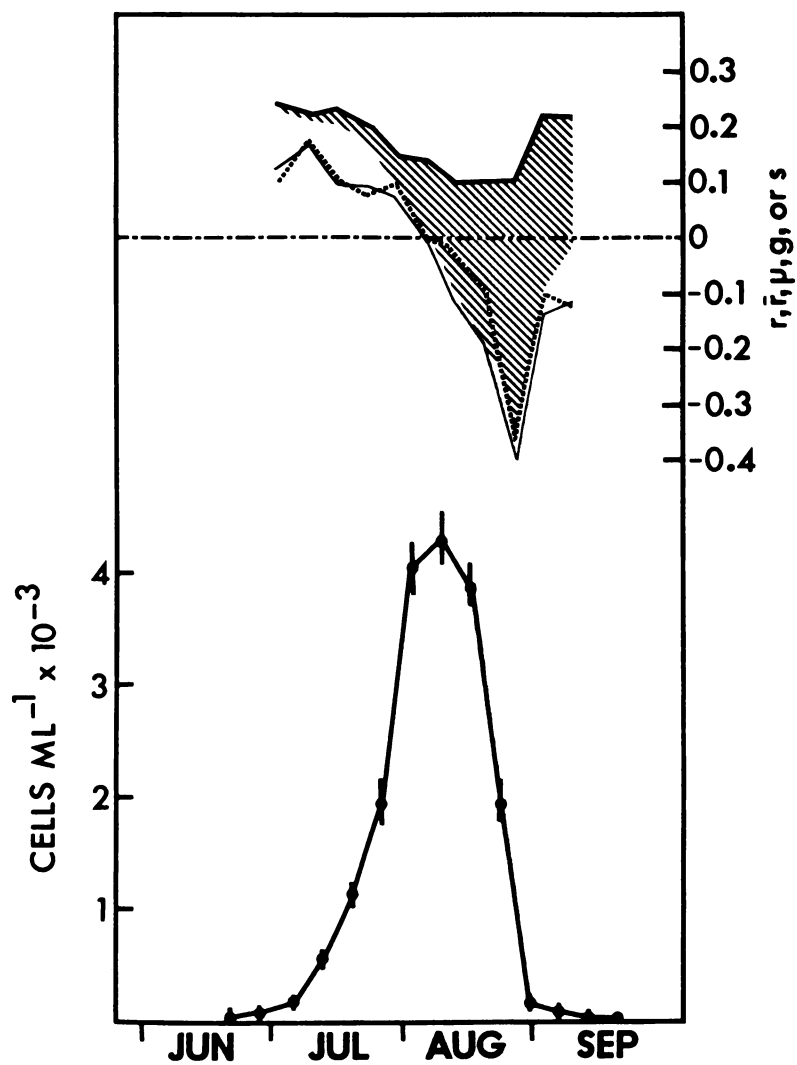


Figure 3. Population curve (lower figure, —) and rate budget (upper figure) for Cyclotella comensis showing μ (—•—), g (hatched area), $\bar{r}$ (—), s (open area between hatching and $\bar{r}$), and r (----).

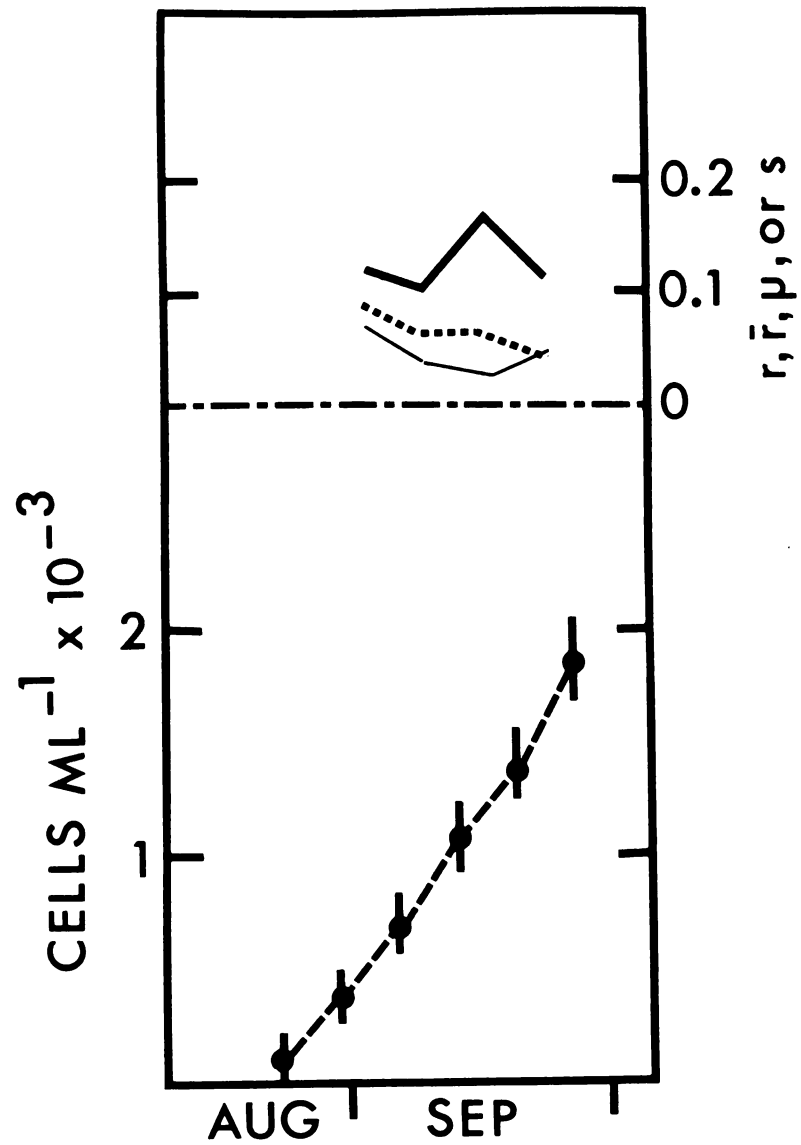


Figure 4. Population curve (lower figure, - - -) and rate budget (upper figure) for Sphaerocystis Schroeteri showing μ (—), $\bar{r}$ (---), s (open area between μ and $\bar{r}$), and r (----).

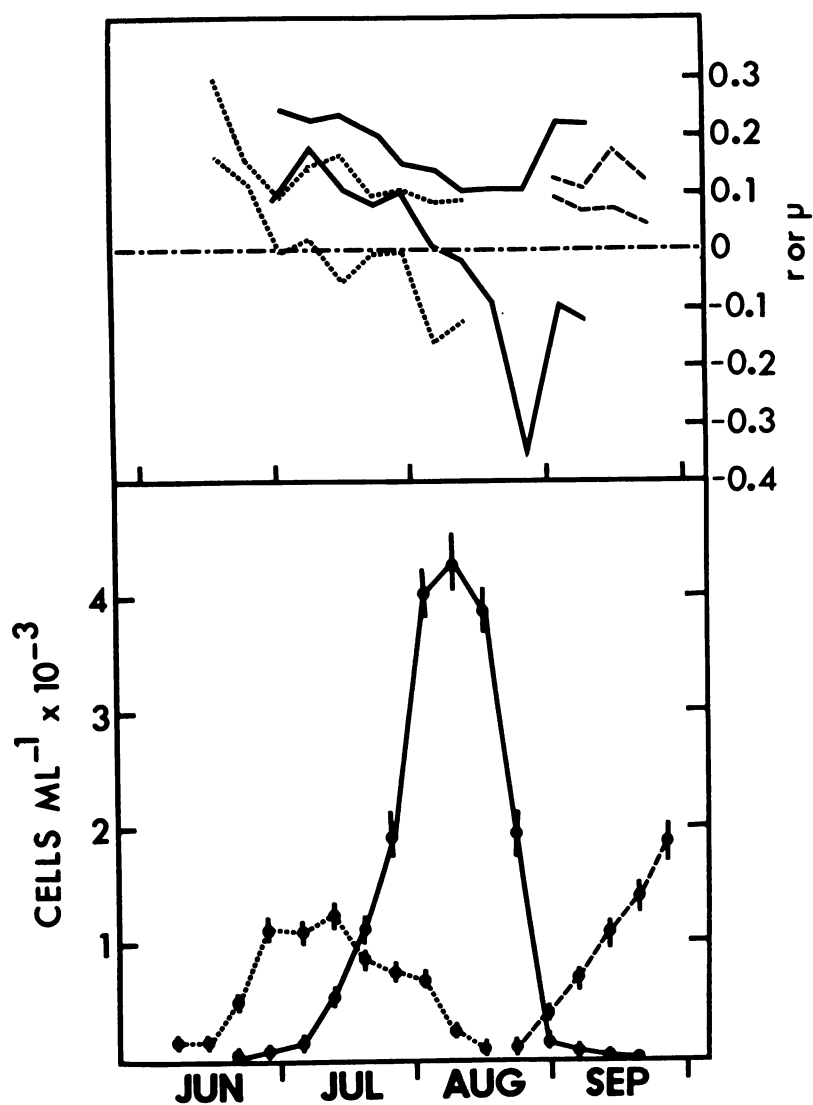


Figure 5. Population curves (lower figure) and simplified rate budgets (upper figure) for Cyclotella michiganiana (----), Cyclotella comensis (—) and Sphaerocystis Schroeteri (- - -). For each rate budget, the upper line represents μ and the lower line represents r .



effectively offset growth, so that $\bar{r}$ was near zero from late June through July. In August, additional losses due to grazing reduced $\bar{r}$ to less than zero. Growth rates of C. comensis (Figure 3) declined through most of its growth period but stabilized and then increased as grazing pressure (and presumably nutrient regeneration) increased in August. Sedimentation losses were occasionally large but generally insufficient to offset growth. Consequently, $\bar{r}$ remained positive until August when high grazing losses reduced $\bar{r}$ of C. comensis to well below zero. Growth rates of S. Schroeteri (Figure 4) showed no clear trends, but sedimentation losses (the only significant loss suffered) never offset growth so that $\bar{r}$ was always positive during the study period.

Given the close correspondence between $\bar{r}$ and r in Figures 2, 3, and 4, plots of μ and r may be used to examine the role of growth and loss processes in the seasonal succession of these phytoplankton. The difference between μ and r represents net loss (d), such that

$$r = \mu - d \quad (8)$$

These rates and corresponding population curves are plotted in Figure 5. Although C. comensis had consistently higher growth rates than C. michiganiana, it is obvious that their succession was not simply due to differential growth. C. comensis was smaller than C. michiganiana and, as might be expected, suffered lower sedimentation losses. It is the combined effect of higher growth rates and lower loss rates of C. comensis that led to the observed pattern. In contrast, the C. comensis-S. Schroeteri succession clearly resulted from differential mortality.

These results have interesting implications regarding the relative



importance of growth and loss processes in phytoplankton population dynamics and thus community structure. In particular, these results contradict the argument that mortality due to sedimentation and grazing is generally insignificant and that competition is the major force controlling phytoplankton populations. For example, the idea that the epilimnion is a continuously, vigorously mixed layer is widespread in aquatic ecology. As a result, losses due to sedimentation are generally thought to be insignificant for the smaller phytoplankton. However, in Lawrence Lake, temperature profiles indicated that periods of epilimnetic stagnation were quite common, particularly in midsummer. During these times, sedimentation losses were quite large and a major force affecting community structure. Most likely, this loss is true for a majority of lakes since, particularly in temperate, continental regions, midsummer includes a number of rather long periods of very low winds and very high heat inputs. As a result, periods of epilimnetic stagnation are probably accompanied by substantial losses of phytoplankton by sedimentation.

Another widespread idea is that mortality due to grazing is relatively unimportant in phytoplankton community structure. It is generally conceded that if grazing pressure were sufficiently high, it could alter community structure, but it is argued that grazing pressure is generally low. However, in Lawrence Lake, it is obvious that grazing mortality was a major factor affecting phytoplankton population dynamics and resultant community structure during the midsummer period investigated. While it is not reasonable to generalize on the importance of grazing from this single detailed study, similar results of grazing effectiveness exist in the literature at this time. Studies

which conclude that grazing is insignificant rarely involve actual measurements of grazing rates. In contrast, when grazing rates have been measured directly, the results have consistently indicated grazing to be of significance at certain times of the year.

Competition for nutrients or other resource limitations have long been suggested as important factors in the seasonal succession of phytoplankton, although the argument has recently been formalized (Kilham and Tilman 1979). Field demonstration of the importance of competition in phytoplankton population dynamics is sorely lacking. Based on growth rate data of this study, it is possible to speculate on the importance of competition in the Cyclotella michiganiana - C. comensis succession in Lawrence Lake.

In order for resource competition to affect succession, growth rates must be affected. Although the effects of competition might be delayed because of internal nutrient stores, unless these effects finally appear as reduced growth rates, no operative role can be argued for competition. An analysis of the growth rate curve of C. comensis suggests possible intraspecific competition. As the population increased, growth rates steadily declined. However, with the onset of high grazing pressures and presumably high rates of nutrient regeneration in August, growth rates first stabilized and then increased to near maximum. However, an analysis of growth rate curves (Figure 5) shows no depression of Cyclotella michiganiana growth rates by C. comensis. Although interspecific competition may have been involved, its effects did not appear as modifications in growth rate before loss processes eliminated C. michiganiana from the community. The implications of these results seem obvious; namely it can not be



assumed, and should not be expected, that such highly dynamic systems as lakes will remain stable for sufficiently long time periods for competitive displacement to occur. This lack of stability is especially true when the effects of competition are delayed due to internal nutrient stores which are commonplace in planktonic algae.

Clearly, despite much effort the field of phytoplankton population dynamics and succession is little understood. However, future developments will profit most from carefully executed investigations of natural populations in concert with detailed mechanistic studies in the laboratory and appropriate theoretical advances.



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APPENDIX A

DIALYSIS CHAMBERS FOR THE MEASUREMENT OF PHYTOPLANKTON GROWTH RATE IN SITU

ABSTRACT

A dialysis/cage culture technique is described and its use for estimating growth rates of natural phytoplankton populations in situ is critically evaluated. Samples from which grazers had been eliminated were incubated in situ in Plexiglas chambers with Nuclepore polycarbonate membrane side walls. Growth rates of phytoplankton in these chambers were corrected for measured grazing and sedimentation losses and compared with actual rates of increase of natural populations. No significant differences were found between the two estimates.



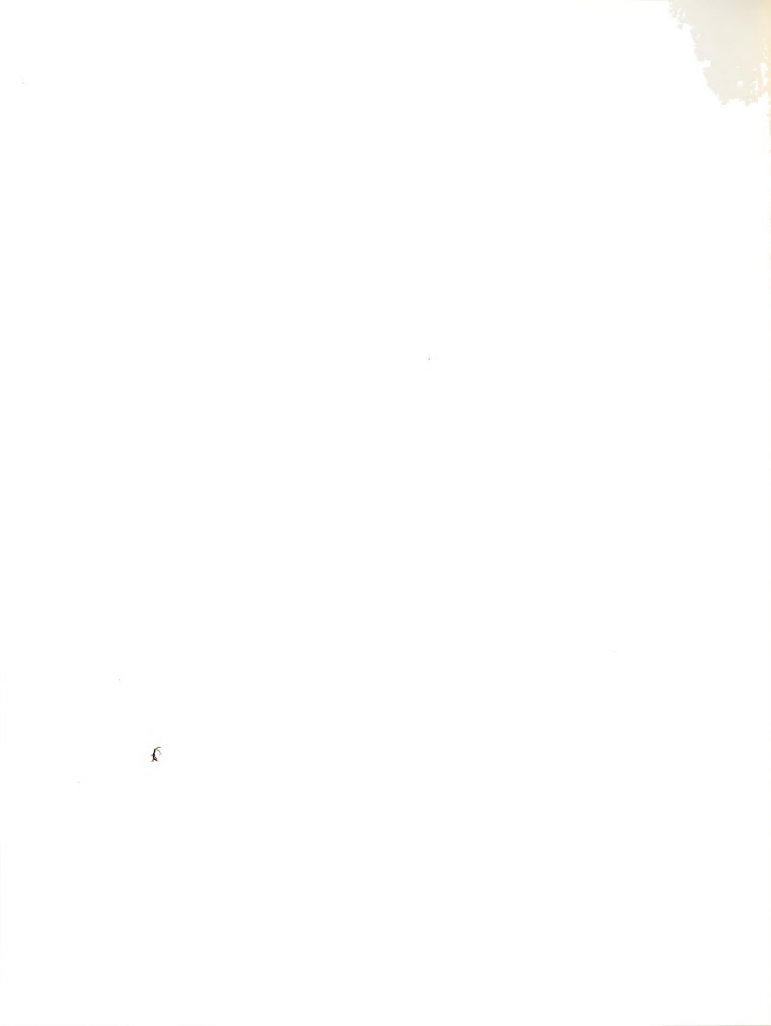
A major obstacle to the study of phytoplankton population dynamics has been the lack of suitable methodology. Species-level productivity measurements have been a particular problem, and as a result, phytoplankton have commonly been considered a single, homogeneous entity in terms of productivity. Recent advances using track autoradiography held promise of extending the ^{14}C -uptake technique to the consideration of species-level productivity (Knoechel and Kalff 1976). Unfortunately, the fixatives required for autoradiography cause large and variable losses of ^{14}C (Silver and Davoll 1978, Brock 1978, Crumpton and Wetzel unpublished data). In addition, the ^{14}C technique suffers from such inherent problems as bottle effects, the uncertain relationship between ^{14}C -uptake and particulate carbon fixation, and difficulties in estimating nighttime respiration and excretion losses.

Given this bleak outlook for meaningful estimates of ^{14}C -uptake, a more suitable and more direct measure of growth rate might be obtained from changes in population size. Estimates of growth rates based on changes in population size have been attempted only rarely for phytoplankton due to the uncertainties introduced by grazing, sinking, and other loss processes. In order to calculate growth rates from population changes, these losses must either be eliminated or measured.

One very promising approach which has been poorly exploited is the use of dialysis or cage cultures suspended in situ. With this approach, grazing and transport losses can be eliminated and mortality from other processes estimated from changes in numbers of dead cells. The growth rate of each population can thus be estimated based on changes in the number of living and dead cells over a specified time interval. Despite a long history in the study of micro-organisms (reviewed by Schultz and

Gerhardt 1969), dialysis/cage culture has seldom been applied to the study of aquatic algae and only recently extended to the study of mixed natural populations. This area has been recently reviewed by Jensen, Rystad and Skoglund (1972), Sakshaug and Jensen (1978) and Eide, Jensen and Melsom (1979), although the works of Braune (1966a & b, 1970a & b, 1972, and 1975), Pierre (1969), Owens et al. (1977) and Laake (1978) were apparently overlooked. While previous studies have provided considerable information on algal growth in dialysis/cage culture, none has critically evaluated the use of cage cultures for estimating growth rates of natural populations. In this paper, I describe an improved dialysis/cage culture chamber and critically evaluate its use for estimating growth rates of natural phytoplankton populations in situ.

Dialysis/cage culture chambers (Figure 1) were constructed from cast Plexiglas tubing (7.6cm OD, 0.64cm wall thickness). In case of slight irregularities in the Plexiglas stock, the components of each chamber were cut as adjoining sections. Holes were drilled through as shown and fitted with stainless steel or nylon machine screws. A combined sampling-port-suspension-point was made by tapping a 0.64cm hole through the central section of the chamber and fitting it with a nylon machine screw. These chambers are similar in design to those described by MacFeters and Stuart (1972). However the MacFeters and Stuart chambers were designed for use with axenic cultures of bacteria, and although they have been used in phytoplankton study (Owens et al. 1977; G. A. McFeters personal communication), they are not ideally suited for that purpose. For applications where sterile conditions are not required, the chambers described above are more suitable. These



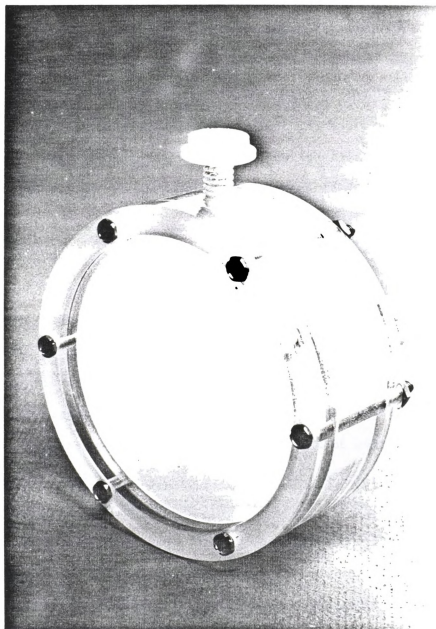


Figure 1. Dialysis Chamber

chambers are easier to fill and empty and their thinner walls cause less light attenuation and spectral modification.

For assembly (Figure 1), the inside face of each retainer ring is wetted and pressed to a 76mm dia., 1.0- μ m pore size Nuclepore polycarbonate membrane. The membrane will adhere to the ring and these units are then carefully positioned on each side of the central section (Figure 1). A dissecting needle or thin wire is then run through each screw hole, puncturing the membranes to allow the screws to be inserted smoothly. The screws are then inserted and tightened with moderate pressure.

Filling the chambers is a simple matter of adding sample through the port provided and sealing the chamber with the nylon screw. Flow through 1.0- μ m and smaller pore size membranes is relatively slow and handling time is sufficient for most purposes. Chambers may be transported or held for longer periods if immersed in water.

Diffusion rates into the chambers were measured using ^{14}C -glucose. Triplicate chambers were suspended in a 10-l tank of continuously mixed water containing 100- μCi ^{14}C -glucose. Duplicate 100- μl samples were taken from each chamber at appropriate intervals and their activities determined. Transport followed first order kinetics with a half time of ca. 1 hour.

The chambers were tested for leakage of algae as follows. Triplicate chambers were filled with dense cultures of Chlorella pyrenoidosa (mean cell diameter ca. 5- μm) and placed in separate, sealed flasks of media. These flasks were shaken vigorously for ca. 48 hours and the external media examined for algal cells using the method of Utermöhl (1958). No cells were found in any instance.

Field studies were conducted in Lawrence Lake, Michigan to evaluate the use of the dialysis/cage culture chambers for estimating growth rates of natural phytoplankton populations. Population growth in the chambers was corrected for losses due to grazing and sedimentation and compared with actual changes of epilimnetic populations. Growth rates, grazing losses (g), and sedimentation losses (s) were measured as part of a larger study extending from May through October 1979 (main body of this thesis). Grazing and sedimentation losses were lowest for Cyclotella michiganiana during June and for Cyclotella comensis during July. These periods were chosen to evaluate the use of dialysis/cage culture chambers for estimating growth rates for these populations since the effects of errors in grazing and sedimentation measurements would be minimal at these times. However, the test must still be considered conservative since some errors are derived from these other measurements.

Replicate phytoplankton samples were collected twice weekly from a central station using an integrating tube sampler and were preserved with 0.4% Lugol's solution. The samples were processed and the phytoplankton counted using the technique described in Appendix B. Using a combination of field and strip counts, between 100 and 1000 cells of each species were counted for each sample and 95% confidence limits calculated as in Lund, Kipling, and Le Cren (1958).

At weekly intervals, six replicate dialysis chambers were filled with lake water from which the dominant grazers had been removed by filtration through 153- μ m mesh Nitex® netting. An aliquot of this water was preserved with 0.4% Lugol's solution for estimates of initial cell numbers (C_0 below). The chambers were suspended at the depth of mean



epilimnetic light intensity using a surface-tethered buoy to provide agitation of the chambers via wave action. On day four and again on day seven, three chambers were collected and the incubated samples were preserved with 0.4% Lugol's solution. Phytoplankton counts were made as described above on initial (C_0) and incubated (C_t) samples. Instantaneous growth rates (μ) were calculated from

$$C_t = C_0 e^{\mu t}$$

A common problem with any incubation is failure to duplicate the vertical movements and thus light regime of actual phytoplankton populations. The importance of such failure is unsettled due to conflicting reports (cf. Jewson and Wood 1975 with Harris and Piccinin 1977 and Marra 1978). However, the photoinhibitory effect often observed in productivity studies is in many cases an artifact of subjecting phytoplankton to extended periods of high light intensity when they would otherwise have spent only a short time under such conditions. Samples which are moved through the light-depth gradient during incubation provide the most realistic estimate of in situ productivity for epilimnetic populations. However, a point exists on the light-depth profile where the productivity of a stationary sample approximates that of the mean for the epilimnion, and in Lawrence Lake, this point is near the depth of mean epilimnetic light intensity (Wetzel, unpublished data). Despite numerous implicit assumptions, incubating samples at this depth represents the most reasonable compromise.

Growth rates (μ) obtained using dialysis/cage culture chambers were combined with estimates of instantaneous grazing (g) and



sedimentation losses (s) and used to predict population density according to

$$\bar{N}_t = N_0 e^{(\mu - g - s)t}.$$

These predicted population densities ($\bar{N}_t$) and actual population densities (N_t) are compared for Cyclotella michiganiana in Figure 2 and Cyclotella comensis in Figure 3. The correspondence of the two curves is striking in each case. These results demonstrate that, under certain conditions, dialysis/cage culture can indeed be used to estimate growth rates of natural phytoplankton populations. The limitations to such use are at present unclear, but at least for epilimnetic phytoplankton populations the potential is great. The approach has been used in an investigation of the differential effects of growth and loss processes in a diatom-green algal succession in Lawrence Lake, Michigan (main body this thesis).

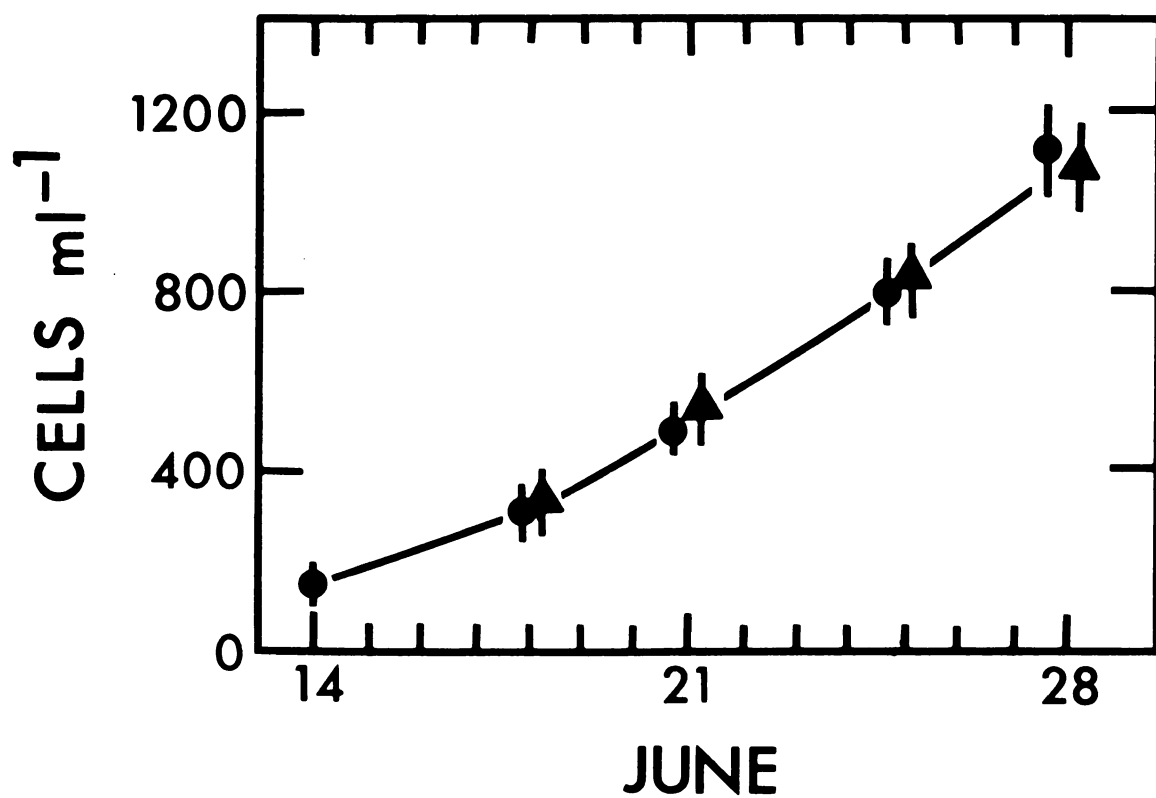


Figure 2. Predicted (o) and observed(●) densities for Cyclotella michiganiana. Error bars represent 95% confidence limits on phytoplankton counts. Predicted densities have additional variance due to errors in estimates of g (mean C.V. ca. 20%) and s (mean C.V. ca. 15%).



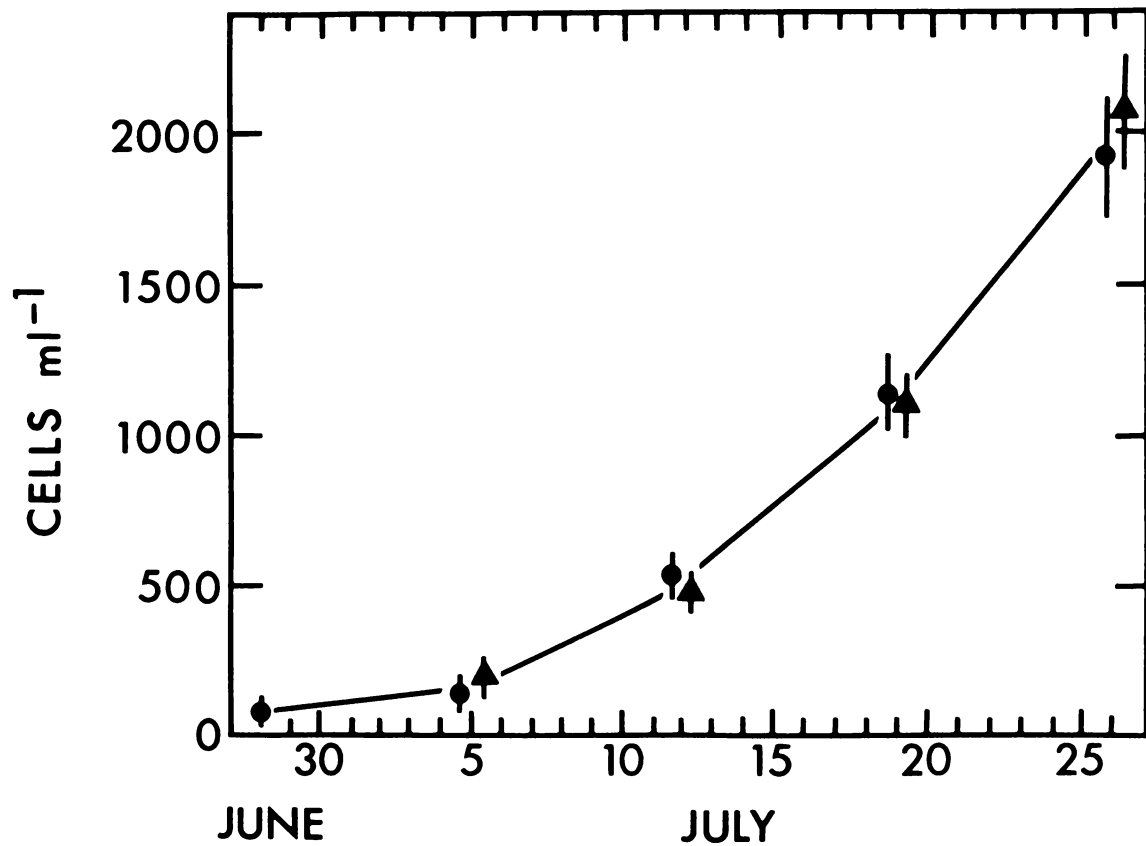


Figure 3. Predicted (o) and observed (●) population densities for Cyclotella comensis. Error bars denote 95% confidence limits on phytoplankton counts. Predicted densities have additional variance due to errors in estimates of g (mean C.V. ca. 20%) and s (mean C.V. ca. 15%).



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APPENDIX B

A METHOD FOR THE PREPARATION OF PERMANENT, QUANTITATIVE MOUNTS OF PHYTOPLANKTON FOR CRITICAL MICROSCOPY

ABSTRACT

A method is described for the preparation of permanent, quantitative mounts of phytoplankton for critical microscopy. Preserved phytoplankton are settled onto cover glasses (or slides), dehydrated using ethanol vapor substitution, and mounted in one of several ethanol-soluble resins. The resulting slides are permanent, quantitative and of unsurpassed optical quality. Resolution is excellent and, in most cases, contrast so high as to eliminate the need for phase shift or other contrast enhancement.

The inverted microscope technique of Utermöhl (1958) has allowed quantitative analyses of even the most delicate μ -plankton, and as a result, has become the standard by which other techniques are judged. Nonetheless, the Utermöhl technique does suffer a number of shortcomings. Among the most crucial are the need for a suitably equipped inverted microscope, the lack of a permanent record, and the poor optical quality provided by long working distance condensers.

The obvious need for a better technique has not escaped previous investigators, and several have attempted improvements. Sanford et al. (1969) developed a settle-freeze method which has received some application but probably lacks the accuracy necessary for general acceptance. Sanford et al. (1969) reported unexplained discrepancies between the settle-freeze method and that of Utermöhl (1958). Although they do not discuss the possibilities, spillage during slide preparation and morphological artifacts resulting from freezing are potential sources of error.

A more promising approach was introduced by Coulon and Alexander (1972) and later improved by Knoechel and Kalff (1976). This method is less manipulative than that of Sanford et al. (1969) and contains fewer potential sources of error. In addition to the well known advantages of gentle sedimentation, the approach provides permanent and quantitative mounts (Coulon and Alexander, 1972; Knoechel and Kalff, 1978; this study). However, the water-soluble mounting media used by previous investigators leave much to be desired. This problem was recognized by Coulon and Alexander (1972), who suggested the possibility of mounting in alcohol- or xylene-soluble media. In addition, previous investigators have settled phytoplankton directly onto slides despite

the considerable optical advantages of settling them onto cover glasses.

In this paper, I describe the preparation of quantitative, resinous mounts of phytoplankton for critical microscopy. Preserved phytoplankton are settled directly onto cover glasses (or slides) using the sliding-chambers developed by Knoechel and Kalff (1976). The supernatant is then removed and the algae are dehydrated using ethanol vapor substitution. Once in ethanol, the algae can be mounted in any of several ethanol-soluble resins. The resulting slides are permanent, quantitative, and of excellent optical quality. While the entire process takes several days, only 5-10 minutes actual work is required per slide.

I acknowledge the helpful discussions of E. F. Stoermer, R. Knoechel and A. J. Stewart, and the machining skills of W. Wetzell. J. Kalff kindly provided sample chambers from which our design was taken.

The settling chambers (Figure 1) are based on the design of Knoechel and Kalff (1976). With this design, it is possible to settle phytoplankton directly onto cover glasses rather than slides, a considerable advantage for critical microscopy. It is a simple matter of inverting the bottom plate to use cover glasses rather than slides. These chambers are easily constructed from Plexiglas® stock, or may be obtained from Total Plastics Inc., 5271 Wynn Road, Kalamazoo, Michigan 49001. This firm now has complete specifications and will manufacture chambers to order at a unit price of approximately \$35.

The vapor chambers are essentially thin layer chromatography desiccators manufactured from 1/4" (6.4mm) Plexiglas® (Figure 2). A final cut across the face of the chamber provides a squared surface for sealing the door. Studs are tapped into the face and the door secured

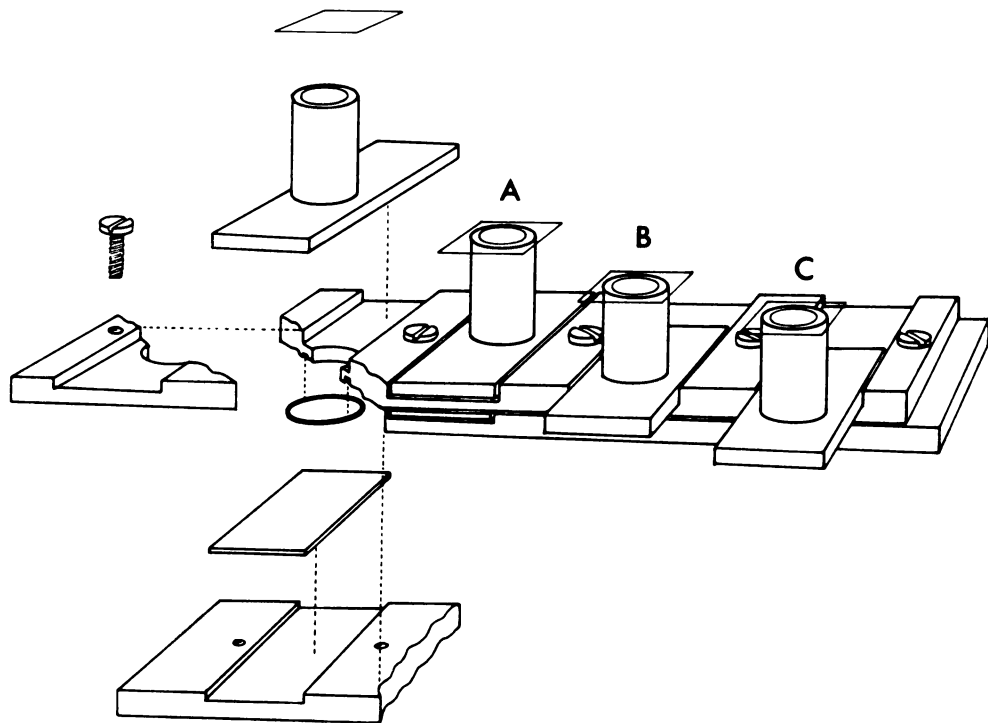


Figure 1. Settling chambers showing positions for (A) normal filling and settling, (B) filling for nonturbulent rinse and (C) dumping overlying water.

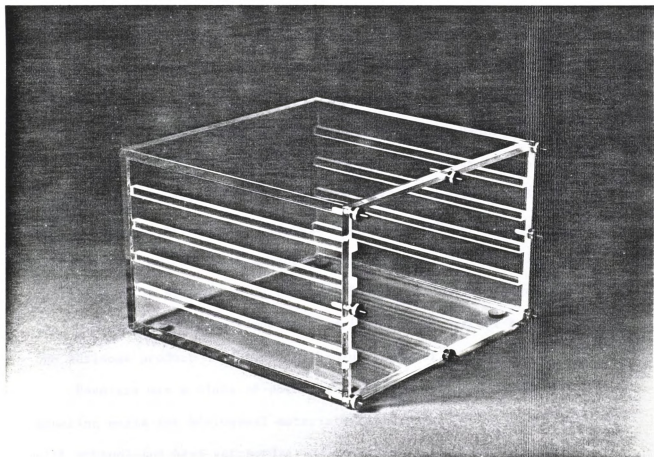


Figure 2. Empty vapor chamber.

with knurled nuts. A layer of silicone grease or a neoprene gasket between the door and the chamber face provides an inert and airtight seal. These chambers are available from Total Plastics Inc. at a unit price of approximately \$50.

Euparal, pleurax (Hanna 1949, von Stosch 1974), and novolacs (Appendix C) have all proven to be useful mounting media. Euparal is a proprietary mixture based on gum sandarac. It is miscible with 95% ethanol, has a refractive index of 1.48, and may be obtained commercially.

Pleurax is a synthetic resin first described by Hanna (1949). It is miscible with 95% ethanol and has a refractive index of 1.75. Pleurax is not available commercially but its synthesis (von Stosch 1974) is relatively simple. In this procedure, 110 parts phenol, 40 parts sulfur, and 0.1 part anhydrous sodium carbonate are refluxed at 180-190°C until all sulfur is reacted (5-9 hrs.). In my preparations (as in Hanna 1949), excess phenol is driven off by further heating and the resinous product dissolved in absolute ethanol.

Novolacs are a class of phenolic resins recently suggested as mounting media for biological materials (Appendix C). They are miscible with ethanol and have refractive indices greater than 1.6. I have successfully used Union Carbide® BRPB 5215, a commercially available novolac which is miscible with 60% ethanol and has a refractive index of 1.65. In my preparations, the stock resin is recrystallized from ethanol to remove excess phenol, and the dried product is dissolved in absolute ethanol.

Chambers are assembled as shown in Figure 1 using either slides or #1 1/2 cover glasses (the bottom plate is inverted when using cover

glasses). For extremely critical work, it is best to use only premeasured cover glasses of appropriate thickness (0.17mm for most objectives). Each chamber is filled with preserved sample, and capped with a cover glass (Figure 1A). Alternatively, for reasons discussed below, fresh samples may be used and preservative added directly to the chamber. If leakage is a problem, a layer of silicone grease may be placed between the towers and their guide grooves. As with any settling chamber it is imperative that a filling tube (Utermöhl 1958) or some means be used to ensure the random distribution of settled organisms. The filled chambers are placed in a moist atmosphere and the organisms allowed to settle for at least 4 hr per cm of chamber height. A moist atmosphere is necessary to prevent evaporative loss and possible leakage. After settling is complete, the overlying water is removed by sliding the chamber towers just past the edge of the chamber plates (Figure 1C). I routinely follow this with a non-turbulent rinse of deionized water for those samples where crystals might later form (high concentrations of Lugol's, CaCO_3 , or NaCl). For rinsing, each chamber is positioned as shown in Figure 1B, filled with deionized water and recapped. The towers are then very carefully slid back into position over the settled material. After allowing time for diffusive mixing and for settling of any resuspended organisms, the overlying water is again removed as described above.

The settling chambers are next placed into vapor chambers (Fig. 2) and the towers carefully removed from the chamber plates. An open dish of absolute ethanol (minimum of 15 ml per sample) is placed inside the vapor chamber and the door immediately sealed. At least 72 hrs are allowed for vapor substitution, after which the settling chambers are

transferred to shallow desiccators. In order to ensure that the distribution of settled organisms is not affected, cover glasses are positioned over each depression during the transfer. The cover glasses are then slid away and the excess ethanol evaporated (takes 2-3 hours), being careful not to dry out the organisms. Just as each sample approaches dryness, the cover glass is slid back over the depression to prevent further evaporation. In a dry atmosphere, the concentration of ethanol on the organisms will not fall below the azeotrope of 95.6%, assuming at least this concentration to start. Alternatively, settling chambers may be left in the vapor chamber and the dish of ethanol replaced with silica gel. The distribution of settled organisms is well protected, but subsequent steps become more difficult and time consuming. Once the excess ethanol has been removed, chambers are disassembled and the organisms mounted in a suitable resin. Curing is dictated by the nature of the sample and resin so that no set procedure can be recommended.

Euparal and the novolac BRPB 5215 both give excellent preservation of cellular and subcellular structures. In addition, contrast is sufficient in either that flagella, plastids, pyrenoids, pectic processes, and cell walls can be distinguished without resorting to phase-shift or other contrast enhancement techniques. In BRPB 5215, diatom frustules are also clearly visible, but in euparal, contrast is insufficient without enhancement. Euparal has the additional disadvantage that mounts remain fluid and thus relatively delicate while BRPB 5215 mounts soon harden sufficiently to endure considerable manipulation. Also, if for some reason mounts contain less than 95% ethanol, BRPB 5215 mounts are still usable while euparal mounts become

milky and near worthless. Euparal does have the advantage that it has been in use for many years and is known to be relatively permanent whereas novolacs have only recently been introduced as mounting media. However, novolacs are inert compounds and have proven extremely durable as lacquers and varnishes. If care is taken to remove excess phenol from the stock resin, BRPB 5215 mounts can be expected to last indefinitely.

Pleurax gives excellent results with diatoms but with very little else. In many cases, overall plastid structure is obscured, apparently by increased prominence of substructures. This may be the result of increased contrast of these substructures at higher refractive indices or it may be that pleurax acts as a clearing agent. In addition, many structures, including flagella and some cell walls and processes, lack sufficient contrast in pleurax. In either case, structures needed for identification of many algae are not readily visible in pleurax.

The accuracy of the sliding-chamber technique has been demonstrated by previous investigators (Coulon and Alexander 1972, Knoechel and Kalff 1978). In order to test the accuracy of the procedures described here and to further test the general approach, comparisons were made between this technique and that of Utermöhl (1958). Samples were taken from two small, hardwater lakes and preserved with 0.4% Lugol's solution. Four replicate Utermöhl chambers and four replicate slides were prepared for each sample. Using a combination of field and strip counts, at least 100 of each of the dominant species were counted for each replicate. The results (Table 1) demonstrate no significant difference in accuracy between the two techniques.

The most desirable technique is one which makes it possible to

Table 1. Mean number of organisms $\text{ml}^{-1} \pm \text{S.D.}$ of four replicates.

	Utermöhl	Slides
Little Mill Lake - August 1979		
<u>Cyclotella comta</u>	99 $\pm$ 19	93 $\pm$ 11
Lawrence Lake - October 1978		
<u>Dinobryon divergens</u>	747 $\pm$ 53	787 $\pm$ 58
- June 1979		
<u>Cyclotella michiganiana</u>	1100 $\pm$ 80	1160 $\pm$ 50
- September 1979		
<u>Rhodomonas minuta</u>	570 $\pm$ 60	600 $\pm$ 60

observe algae in near natural condition with not only intact cellular and subcellular structures but also intact colonies and chains. Problems often arise when samples have been preserved for any length of time. Among other things, gelatinous linkages are weakened and the vigorous agitation needed to homogenize stored samples can disrupt colonies and especially loosely bound chains of diatoms. As a result, valuable information is lost, leaving an incomplete and unrealistic picture. Solitary cells are certainly not ecologically equivalent to those in colonies or chains, differing with regard to nutrient dynamics, sedimentation, grazing, and parasitism. Fortunately, it is relatively simple to overcome this problem. Rather than preserving samples and later subsampling, fresh samples are added directly to settling chambers and then preserved or else freshly preserved samples are used. This may also be done with Utermöhl chambers but their expense and impermanency make such use impractical. In contrast, the technique described above is ideally suited for such application and is a considerably more practical approach to exacting phytoplankton analyses.



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APPENDIX C

NOVOLACS: SYNTHETIC RESINS SUITABLE FOR MOUNTING BIOLOGICAL MATERIALS¹

ABSTRACT

Novolacs are transparent, highly refractive ($n_D > 1.6$), alcohol-soluble resins. A commercially available novolac (Union Carbide® BRPB 5215, $n_D^{20} = 1.65$) was used successfully in preparing mounts of freshwater algae and microcrustacea. Additional applications for these resins are apparent.

¹ For further information including availability contact Union Carbide Corporation, 270 Park Avenue, New York, N.Y. 10017.

Novolacs are transparent, highly refractive ($n_D > 1.6$), phenolic resins soluble in alcohol, acetone, esters, ethers, and to some extent hydrocarbons (Roff & Scott, 1971). These polymers are permanently fusible with melting points above 80°C. Since commercial production began in 1910-1912 (as Bakelite), novolacs have been used as lacquers and varnishes, and as bonding agents in filler-reinforced plastics.

Beyond their current applications, the solubility and optical characteristics of novolacs suggested their potential utility in the preparation of biological materials for light microscopy. To explore this potential, I selected a commercially available resin (Union Carbide® BRPB 5215)¹ soluble in 60% to 100% ethanol. Its refractive index was determined by immersion in reference liquids ($n_D^{20} = 1.65$). The commercial product contains free phenol and other contaminants which I remove by recrystallizing the novolac from ethanol with excess water. The precipitate is collected by centrifugation, dried, and dissolved in absolute ethanol.

The solubility and optical characteristics noted are useful in preparing many kinds of biological materials for light microscopy. I have had considerable success mounting fresh-water algae and microcrustaceans in BRPB 5215. In my preparations, preserved organisms are dehydrated using ethanol vapor substitution (Sanford et al. 1969) and mounted directly in a drop of resin-ethanol solution with a cover glass. Curing is dictated by the nature of the sample, but if allowed to sit at room temperature, mounts harden at their edges within a few hours. Although the permanency of these mounts is unknown, novolacs have proven to be durable lacquers and varnishes. If one is careful to remove excess phenol from the stock resin, problems with deterioration

seem unlikely. All that can be said at present is that mounts of diatoms prepared one year ago and mounts of green algae and cryptomonads prepared six months ago have not deteriorated in any noticeable aspect. Preservation of cellular and subcellular structures is excellent and contrast is sufficient that flagella, plastids, pyrenoids, pectic processes, frustules, and cell walls can be distinguished without resorting to phase-shift or other contrast enhancement techniques. In addition to excellent contrast and resolution, these mounts possess useful fluorescent properties. Background in blue-light fluorescence is acceptable and autofluorescence of many algae is preserved (E. F. Stoermer, personal communications).

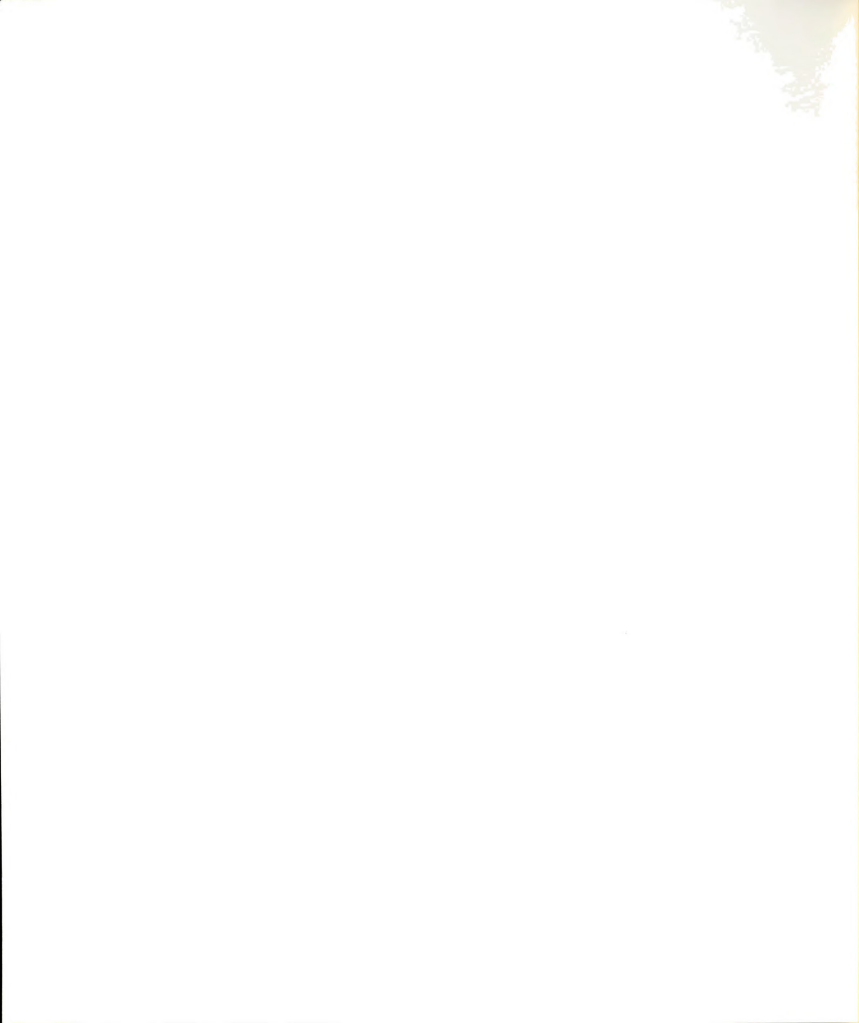
No commercial mounting media combines the solubility and optical characteristics of BRPB 5215. Of those available, only euparal is soluble in ethanol and unfortunately is both eutectic and has a much lower refractive index ($n_D = 1.48$) than BRPB 5215.



LITERATURE CITED

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