An Evaluation of Methods of Concentrating and Counting the Phytoplankton of Bear Lake, Utah-Idaho

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AN EVALUATION OF METHODS OF CONCENTRATING AND COUNTING THE
PHYTOPLANKTON OF BEAR LAKE, UTAH-IDAHO
by
William J. Clark

A thesis submitted in partial fulfillment
of the requirements for the degree
of
MASTER OF SCIENCE
in
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Logan, Utah
1956
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I wish to acknowledge the counsel and guidance of Dr. William F. Sigler in all phases of the study. Thanks are due to Dr. Rex Hurst for statistical guidance, and to Earl Smart for help in collecting many of the samples.

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William J. Clark
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INTRODUCTION

The phytoplankton, or plant plankton, live in the open water throughout their life cycle and obtain the necessary nutrients from the water. They are important contributors to the total plant production of lakes and ponds.

Phytoplankton populations are rarely stable. There are usually large fluctuations in abundance from season to season throughout the year. Considerable work has been done on the relationship of these population fluctuations to the concurrent changes in environmental conditions and in amounts of nutrients present. This work has added much to our knowledge of the life processes of the algae. It has also advanced our as yet only general understanding of the relationship of plant production in aquatic environments to the animal production based on it; and of the plant production to the nutrients and environmental conditions present.

Measurements of the phytoplankton population level are an integral part of these studies. Reliable estimates of the total phytoplankton production are not attained since it is not yet possible to determine the number of generations produced during the year in natural environments. The population studies consist of a series of estimates of the standing crop taken at intervals throughout the year. A number of water samples sufficient to represent the body of water under study are collected, which on large or irregular lakes may involve an extensive sampling program. The plant content of the water samples has been determined
by extraction of the chlorophyll or other pigments; by determining the
difference between dry and ash weights of the total suspended material;
and by enumeration, microscopically because of their small size, of the
individual phytoplankton cells. The pigments vary in composition and
amount among the various taxonomic groups of the algae, making correla-
tion of pigment content and plant production difficult. Dry and ash
weight determinations include the debris with the living cells, since
separation is not possible. The enumeration method, though more com-
plicated, deals with the living cells only and is the method most used.
The cells vary considerably in size from species to species and the
numbers are converted to units of cell volume for a final population
index, using appropriate conversion factors for each species.

The phytoplankton cells are rarely so dense that microscopic exam-
ination of a water sample as collected could give accurate informa-
on either the types of phytoplankton present or their abundance. Some
method of concentrating the water sample must be used to increase the
number of cells per unit volume of water examined.

Before a phytoplankton study can be initiated concentration methods
and methods of determining the number of cells per unit volume of the
concentrate must be chosen which will function adequately with the
phytoplankton forms present. No data were available prior to this
study from which such a choice of methods for use on Bear Lake could be
made.

It has been the purpose of this study to evaluate concentration
methods and counting procedures, and to designate those thought to be
most applicable for a quantitative study of the Bear Lake phytoplankton.
Since population estimates were not an objective, the plankton counts
have not been converted to volumetric units.
BEAR LAKE

General description

Bear Lake is a large oligotrophic lake lying half in south-east Idaho and half in north-east Utah, at an elevation of 5923 feet. It is approximately rectangular in shape, 19 miles long and 8 miles wide at the widest point. Soundings made concurrent with this study showed a maximum depth of 210 feet, with 15 percent of the lake less than 25 feet deep and 52 percent deeper than 100 feet.

During 1954 and 1955 surface water temperatures reached 70 degrees Fahrenheit for only a week or so in August. Definite temperature stratification occurred in late April or early May of both years, with the epilimnion deepening during the year, reaching a depth of 60 to 70 feet before fall overturn began in late October or early November.

The lake is used in part as a reservoir. Excess flow of the Bear River is diverted into Bear Lake during winter and spring through a canal at the north end of the lake. Water is pumped out of Bear Lake and returned to Bear River during the summer. It is possible to lower the lake 21 feet below the maximum elevation of 5923 feet by pumping. The maximum change in water level during any one year rarely exceeds 4 feet (from records of Utah Power and Light Co. pumping plant).

The phytoplankton population

Two important characteristics of the phytoplankton population are the general small size of the cells (table 1), and the small number of diatoms, usually less than 5 percent. The small size of the cells made use of the normal high dry microscope objective (400 X) necessary
for differentiation. Recognition of classification characteristics was difficult even under oil immersion (1000 x) for several forms. No attempt was made to compile a check list of phytoplankton species present in the lake.

In a specific study of the phytoplankton population definite knowledge of the species concerned would be important. In this study of techniques it was considered that the primary objective was recognition of the forms present as separate entities, so that they could be followed through the separation and enumeration processes. Tentative identifications were made of the more abundant forms (Smith 1933, Prescott 1951). Over the period of the study these forms comprised better than 90 percent of the phytoplankton present. None of the rare or occasional forms gave evidence of concentration or enumeration problems.
Table 1. Tentative classification, general description and maximum abundance of the more abundant phytoplankton forms in Bear Lake from samples collected January 1954 through August 1955

Division Chlorophyta

Family Oocystaceae

Ankistrodesmus falcatus (Corda) Ralfs
Cells single, needle shaped, 1.5 to 2u wide and 30-40u long. Maximum abundance 2,100 cells per milliliter (ml.) of lake water.

Ankistrodesmus spiralis (Turner) Lemmermann
Cells as above but loosely spiraled. Maximum abundance 460 cells per ml.

Lagerheimia sp.
Cells single, oval, 6-10u long, 4-5u wide, setae at ends. Maximum abundance 640 cells per ml.

Oocystis parva West and West
Cell oval to spindle shaped, single, or two cells enclosed by old mother cell wall. Single cells 10-12u long and 5-6u wide. Maximum abundance 420 cells per ml.

Oocystis pusilla Hansgirg
Cell oval to rectangular, single, or two cells in old mother cell wall. Cells 9-11u long, 4-5u wide. Maximum abundance 450 cells per ml.

Selenastrum sp.
Single cell, lunate with rounded ends 10-15u between the points, 5-6u wide. Maximum abundance 80 cells per ml.

Dictyosphaerium sp.
Cells in pairs in enlarged ends of old mother cell walls, pairs may be joined to form groups of 4, 6, or rarely 8. Individual cells oval, 5u long, 2u wide. Maximum abundance 170 cells per ml.

Division Chrysophyta

Order Pennales
All diatoms were from this order and were treated as a single group. The largest found was 72u long, most were under 50u long. Maximum abundance 180 cells per ml.

Order Chrysomonadales

Family Ochromonadaeae

Dinobryon sp.
Single or in short chains, lyre shaped lorica 25-30u long, 15-20u wide. Maximum abundance 160 cells per ml.
Division Cyanophyta

Family Oscillatoriaceae

*Lyngbya contorta* Lemmermann
A spiral filament 2μ in diameter up to 40μ long. Maximum abundance 1400 filaments per ml.

Family Chroococcaceae

*Dactylococcopsis* sp.
Cells elongate spindles, 20-25μ long, 6-8μ wide, single or paired, in large gelatinous envelope. Maximum abundance 180 cells per ml.

*Chroococcus* sp.
Cells small, single, 2-3μ in diameter. Maximum abundance 900 cells per ml.
CHOICE OF COUNTING CHAMBER

The determination of plankton numbers involves collection and concentration of a water sample, the estimation of the number of plankton per unit volume of concentrate, and calculation from this of the number per unit volume of the original sample. In some extreme cases the cells may be abundant enough to permit counting without concentration, though this was never the case in Bear Lake. In any case the volume counted is very small compared to the sample volume, and must be accurately determined.

The samples used for evaluation of the concentrating and counting methods were collected with a 3 liter Kemmerer water sampler at many depths and locations, from January 1954 through August 1955. The samples used for evaluation of the counting chambers were collected January through August 1954 and were concentrated with a Foerst plankton centrifuge. The concentration methods will be discussed in detail later.

Sedgewick-Rafter cell

The Sedgewick-Rafter cell is probably the most common plankton counting chamber in general use today. It consists of a rectangular rim of brass or glass one millimeter (mm.) thick, with inside dimensions 50 x 20 mm., which is cemented to a regular microscope slide. When capped with a coverslip, a volume of one cubic centimeter is enclosed (Welch 1948, p. 281). It was not possible to focus the standard high dry objectives over the entire depth of the Sedgewick-Rafter cell.
Since the high magnifications were required for the Bear Lake phyto-
plankton the standard Sedgewick-Rafter cell was not useable. Shallower
cells could be constructed in the same manner but it would be difficult
to maintain an accurate and even cell depth. It has also been shown by
Serfling (1949) that random distribution of cells is not achieved in
the Sedgewick-Rafter cell, a condition which should be avoided if
possible because of the sampling problems it generates. For these
reasons it was decided to investigate other possible counting methods
before considering the shallow Sedgewick-Rafter cell.

Drop and coverslip

Lackey (1938) published a procedure which has been used extensively,
utilizing a standard microscope slide and coverslip. In this method a
dropper or pipette is calibrated to determine the number of drops per
milliliter (ml.). One drop of the concentrate is placed on a slide and
covered with a coverslip of known area. By determining the size of the
area counted, the fraction of the volume counted can be calculated.
The area counted may be the area of the field of view, or it may be a
fraction of that area with divisions of the field provided by an ocular
micrometer.

The Whipple ocular micrometer, which provides a large square sub-
divided into many smaller squares, is most often used. The size of the
area chosen is determined with a stage micrometer. A given number of
fields, or paths of definite length, are then counted. The method has
the advantage of use of readily available standard equipment, and per-
mits the use of either low or high power objectives.

There are disadvantages, however. Some of the drop squeezes out
from under the coverslip, which gives some error in calculating the
actual volume counted. Evaporation effects soon cause currents which move the cells and tend to concentrate them at the edge of the coverslip. Lackey states that the first error could be minimized by use of a small drop and a light #1 coverslip. For the second error, caused by evaporation effects, he recommends quick completion of the count. He counted two complete paths across the coverslip at right angles to each other and passing through the center of the coverslip. If it were necessary for quick completion not all species were counted on each slide, since he repeated the count on 10 slides from each sample. One of the problems planned for investigation here was the effect of various numbers of fields or strips counted per slide. It was not possible to increase the number of strips counted without serious interference from evaporation effects. Single fields could not be used because repeated trials showed that there were gross variations in cell density from one area of the coverslip to another, despite care in placing the coverslip. This would be partially compensated for when entire strips were counted as Lackey did. If single fields were used, however, the unequal distribution would radically increase the variation of the counts and require many more fields to be counted for a given degree of precision than with an even distribution. For these reasons the method was rejected.

**Inverted microscope**

One method commonly used in Europe but not in this country requires that an inverted microscope be used to examine the bottom of a cell into which the plankton have been precipitated by treatment with a saturated solution of iodine in potassium iodide (Huttner 1953, p. 110). The special microscope necessary was not available and the method could not be tested. However, as will be discussed, it was later determined
that the precipitation treatment was not effective with the Bear Lake phytoplankton.

**Haemacytometer**

The small size of the phytoplankton cells involved suggested that a haemacytometer might be used as the counting chamber. It has been used by others for phytoplankton work (Lund 1950, Ryther 1954, and Brook 1954). A standard Spencer Bright Line haemacytometer was obtained. This cell is constructed from a single piece of glass. The counting areas and coverslip supports are formed by grinding. The coverslip is supported 0.1 mm. or 100 microns (u) above two 6 mm. x 12 mm. counting plateaus. The counting plateaus are separated from each other and from the coverslip supports by an H-shaped trough. A drop of the concentrate is introduced at the edge of the coverslip with a dropper or pipette. Capillary action draws the sample under and fills the counting chamber. A nine square mm. area, 3 mm. x 3 mm. is scribed on each plateau with Spencer's improved Neubauer ruling. In this ruling the four corner mm. are each divided into 16 squares. Each of the 16 squares covers a volume 0.625 x 10^-5 ml. (from booklet of the American Optical Co. accompanying the haemacytometer). The high dry magnification of the microscopes used just covers one of these 1/16 square mm. areas.

It appeared from initial trials of the haemacytometer that the cells were evenly distributed over the counting plateau. Evaporation effects were much slower to appear than with the slide and coverslip, though evaporation still limited the number of fields that could be counted in a differential count of many species. If only one or two species were being counted all of the 64 available fields could be
counted on one slide without difficulty. In most cases the evaporation did not cause serious cell movement until the water front was almost to the area counted.

Since the scribed area was some distance from the edge of the coverslip it was decided to test whether or not there might be a distribution gradient from the edge inward. A Whipple ocular micrometer was inserted and the field size adjusted to equal one of the 1/16 mm. areas of the regular scribed area. The number of cells of the 13 commonly occurring species were counted in 10 fields, with each species recorded separately. Five of the fields were the regular 1/16 sq. mm. squares in the scribed area, and 5 were Whipple fields at the edge of the coverslip. This was repeated for 5 slides, and a paired comparison made (Snedecor 1946, p. 44) utilizing the two sets of totals for each organism, one from the edge of the slide and one from the regular counting area (Table 2). It was concluded that there was no distribution gradient and that the regular counting area was representative of the sample introduced.

The haemacytometer seemed to best fit the requirements for a counting chamber and was used throughout the rest of the study. Standard procedure consisted of thoroughly mixing the concentrate by alternate filling and expelling with a dropper and then introduction of a drop at the edge of the coverslip. A count was then made of the number of cells in each of a designated number of the 1/16 sq. mm. squares. The squares counted were chosen at random on each slide from the 64 available. In some cases all of the commonly occurring species were counted; in others only one or two species were used, depending on the purpose of the count. In every case the species were recorded separately.
Table 2. Paired comparison to test for differences in distribution of phytoplankton cells on the counting plateau of a haemacytometer. The test was between the scribed counting area and an area near the edge of the counting plateau at the point of introduction of the sample.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total cells in 25 fields</th>
<th>Difference ((X_1 - X_2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scribed area (X_1)</td>
<td>Edge of plateau (X_2)</td>
</tr>
<tr>
<td>Dinobryon</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Ancistrodesmus falcatus</td>
<td>151</td>
<td>143</td>
</tr>
<tr>
<td>A. spiralis</td>
<td>34</td>
<td>46</td>
</tr>
<tr>
<td>Dictyosphaerium</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Chroococcus</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>Lagerheimia</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>Daicylococonsis</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Lyneta</td>
<td>61</td>
<td>47</td>
</tr>
<tr>
<td>Selenastrum</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Cocystis musilla</td>
<td>51</td>
<td>46</td>
</tr>
<tr>
<td>O. narva</td>
<td>88</td>
<td>83</td>
</tr>
<tr>
<td>Chlorella</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Diatoms</td>
<td>32</td>
<td>28</td>
</tr>
</tbody>
</table>

\[ t = \frac{(X_1 - c)}{\sqrt{\frac{S^2}{n}}} \]

\[ t^* = 0.106 \text{ with 12 d.f. giving } P \text{ greater than } .5 \]

* See p. 14 for statistical notation
If more than one slide were used the required number of fields was distributed evenly over the slides. For example, when 30 fields were required they were distributed in one case as 6 fields on each of 5 slides.
COUNTING PROCEDURES

Collections used for evaluation of the counting procedures and concentration methods were made August 1954 through August 1955. The study involved the simultaneous evaluation of concentration and enumeration methods. The enumeration methods are presented first, but counts made throughout the study on concentrates from several methods were utilized.

Statistics were calculated for 225 counts from 70 concentrates prepared during the period given above.

The term "count" as used here refers to the data derived from enumerating one species over a given number of fields. For example: if 20 fields were examined and 5 species were considered, the numbers of each species were tabulated and treated separately, giving 5 counts.

For each count the total number of organisms and the mean number per field (\( \bar{x} \)), were determined, and the following statistics calculated:

\[
\text{Variance } S^2 = \frac{\sum x^2 - (\sum x)^2}{n - 1}
\]

(1)

signifies summation

\( x \) stands for the number of cells in each individual field

\( n \) is the number of fields counted

\[
\text{Standard deviation } S = \sqrt{S^2}
\]

(2)

\[
\text{Standard error } S_{\bar{x}} = \frac{S}{\sqrt{n}}
\]

(3)

\[
\text{Coefficient of variation } C_v = \frac{S}{\bar{x}}
\]

(4)
95 percent confidence limits to \( \bar{x} \)

\[
m = \bar{x} \pm t_{0.05} \frac{s}{\sqrt{n}} \tag{5}
\]

\( m \) is the true mean, of which \( \bar{x} \) is an estimate

The statistic \( t \) is taken at the chosen confidence level from prepared tables such as Table 3, page 65, of Snedecor (1946).

It was expected that there would be some error in calculating the confidence limits using the statistics given. They are based on a normal distribution. The distribution from counts of cells in the counting chamber was expected to approximate the Poisson. What was desired, however, was an indication of the general level of precision. Snedecor (1946) indicates that the requirement of normality could be considerably relaxed, and Cochran (1947) in reviewing the problem of the effect of non-normality states:

"The consensus from these investigators is that no serious error is introduced by non-normality in the significance levels of the F and two tailed t tests."

The nature and magnitude of the actual error involved in this case will be discussed later.

The following statement is appropriate concerning the confidence limits obtained by formula (5): Unless a 1 in 20 chance has occurred, the true mean lies within the calculated limits.

It must also be pointed out that under these conditions the true mean will lie outside the given limits in approximately 5 percent of the cases, strictly due to chance.

**Distribution of cells in the haemacytometer**

According to Lancaster (1950), Poisson first used this distribution, which carries his name, in 1837; and Abbe' derived the same laws for the distribution of cells over the haemacytometer in 1878. Student
(1907) whose paper is often quoted as a basis for this distribution does not mention the term "Poisson distribution" in discussing his analysis of the distribution of yeast cells in the haemacytometer, and Lancaster states that he was apparently unaware of the previous work.

If the phytoplankton cells in this study were randomly distributed over the counting area of the haemacytometer the counts would be from a Poisson series. Bliss (1953) gives the following formula for testing for agreement with the Poisson:

\[ \text{chi square} = \frac{(n - 1) \sum s^2}{x} \]  

with \( n - 1 \) degrees of freedom

This formula was applied to 66 counts in which all fields were counted on the same slide. Eight of these 66 counts, or 11.8 percent, exceeded the 5 percent level of chi square, as read from a table such as Table 9.2, p. 190, in Snedecor (1946).

Seven of the 66 counts were of an organism that often appeared in pairs, though each cell was counted separately. This would be expected to increase the variance associated with these counts, and these 7 counts did contribute 3 of the 8 significant results.

When 155 counts in which the fields counted were distributed over several slides were tested in the same manner, 38, or 24.5 percent, departed significantly from the Poisson. Again those cells which appeared in twos, threes or fours contributed significantly more of the departures. Twenty of the 56 counts of multiple cell forms, or 35.7 percent, departed significantly from the Poisson as against 18 of the 99 counts of single cell forms, or 18.2 percent.

Five percent of the counts can be expected to be significant entirely due to chance, because of the level of chi square chosen. The
5 percent level of chi square is that value of chi square which would be exceeded 1 time out of 20 strictly due to chance, even if no real difference existed. There remains in both cases a percentage which must be attributed to actual deviation from a Poisson distribution. Many of those counts which do deviate from the Poisson might be best fitted by the negative binomial discussed by Bliss (1953). This was not tested. It is apparent however that the Poisson best approximates the general distribution, and judging from the extent of agreement to the theoretical distribution, the cells are quite randomly distributed over the counting area.

**Error involved in applying statistics of the normal curve**

It was expected that there would be some error from applying the statistics of the normal curve to counts from a presumably Poisson distribution. To investigate the nature and magnitude of this error the raw data from 35 counts were transformed by the following formula (Snedecor 1946, p. 446):

\[ X_t = \sqrt{X + 0.5} \]  

(7)

\( X = \) the observed number of cells per field  
\( X_t = \) the transformed value

In the Poisson, the mean and variance are equal. This transformation should give a new set in which the two are independant, and to which the normal statistics can be legitimately applied.

The calculations involved in formulas (1) through (5) were done for the transformed data. The 95 percent confidence limits calculated from this transformed data were then re-transformed by reversal of the process of formula (7), and the limits compared with those calculated from the original data (Table 3).
Table 3. Comparison of 95 percent confidence limits as percent of the mean calculated from non-transformed data with those calculated from the same data after transformation to make the variance and mean independent.

<table>
<thead>
<tr>
<th>Number of fields counted</th>
<th>Total organisms counted</th>
<th>Mean number of organisms per field</th>
<th>95 percent confidence limits as percent of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transformed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>0.55</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.70</td>
<td>55</td>
</tr>
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<td></td>
<td>31</td>
<td>1.55</td>
<td>43</td>
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<td></td>
<td>42</td>
<td>2.10</td>
<td>48</td>
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<td>0.73</td>
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<td>219</td>
<td>7.30</td>
<td>16</td>
</tr>
<tr>
<td>64</td>
<td>445</td>
<td>6.95</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>476</td>
<td>7.43</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>613</td>
<td>9.57</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>696</td>
<td>10.87</td>
<td>8.8</td>
</tr>
<tr>
<td>72</td>
<td>21</td>
<td>0.29</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0.36</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>0.71</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>1.18</td>
<td>23</td>
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<tr>
<td></td>
<td>174</td>
<td>2.41</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>193</td>
<td>2.68</td>
<td>10.8</td>
</tr>
</tbody>
</table>
The general trend was toward underestimation of the true limits when non-transformed data were used. This was particularly true when the total number of organisms counted was low, though they still gave a useful approximation of the true limits. When more than 100 organisms were counted there was in general very little error. There appears to be no serious objection to the use of limits calculated from the non-transformed data in establishing the general level of precision. When fine differences are important more refined methods must be employed.

Sub-sampling of concentrate

Since the entire concentrate was not counted, the question of accurate sub-sampling was examined. Simple analysis of variance determinations were made on counts in which several slides were used, each slide being a separate sub-sample of the concentrate (Table 4). The data were transformed by formula (7) to remove any effect of non-normality. Two organisms showed significant difference between slides in the first series of tests, *A. falcatus* and *Q. musilla*. Other tests were then made, which gave replicates of all the more abundant organisms and additional replicates of the two species which originally showed significance. Additional significant results were obtained with these two species. In 3 of 6 tests of *A. falcatus* and 2 of 4 tests of *Q. musilla* the sub-sampling was apparently not equal. The remainder of the organisms were apparently being adequately sub-sampled.

As an additional check the analyses for *A. falcatus* were pooled, as were the analyses for *A. spiralis* and *Q. musilla*. The pooled analyses again showed significant difference between slides for *A. falcatus* and *Q. musilla*. The difference between slides was not significant in the pooled analyses for *A. spiralis* where none of the individual analyses were significant.
Table 4. Analyses of variance to test for significant difference between sub-samples (slides) taken from the same concentrate. Mean squares significant at the 5 percent level of F are marked with one asterisk, those significant at the 1 percent level with two asterisks.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Category</th>
<th>Degrees of freedom</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ankistrodesmus falcatus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slides</td>
<td>5</td>
<td>0.356*</td>
<td>0.104</td>
</tr>
<tr>
<td>Fields</td>
<td>30</td>
<td>0.100</td>
<td>0.245</td>
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<tr>
<td>Total</td>
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<td></td>
</tr>
<tr>
<td>Slides</td>
<td>5</td>
<td>0.836*</td>
<td></td>
</tr>
<tr>
<td>Fields</td>
<td>24</td>
<td>0.264</td>
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</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slides</td>
<td>5</td>
<td>0.108</td>
<td>0.034</td>
</tr>
<tr>
<td>Fields</td>
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<td>0.064</td>
<td>0.124</td>
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<tr>
<td>Total</td>
<td>23</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>0.291**</td>
<td></td>
</tr>
<tr>
<td>analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slides</td>
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<td></td>
</tr>
<tr>
<td>Fields</td>
<td>138</td>
<td>0.155</td>
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</tr>
<tr>
<td>Total</td>
<td>168</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankistrodesmus spiralis</td>
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<td></td>
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<tr>
<td>Slides</td>
<td>5</td>
<td>0.070</td>
<td>0.168</td>
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<tr>
<td>Fields</td>
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<td>0.136</td>
<td>0.143</td>
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<tr>
<td>Total</td>
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</tr>
<tr>
<td>Slides</td>
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<td>0.270</td>
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<tr>
<td>Fields</td>
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<td>0.193</td>
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<tr>
<td>Total</td>
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</tr>
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<td>Pooled</td>
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<tr>
<td>Slides</td>
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<td>0.161</td>
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<tr>
<td>Fields</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lagerheimia</td>
<td>Slides</td>
<td>5</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>Fields</td>
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<td>0.230</td>
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<td>Total</td>
<td>35</td>
<td>0.175</td>
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<td>Cocystis parva</td>
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<td>0.150</td>
</tr>
<tr>
<td></td>
<td>Fields</td>
<td>30</td>
<td>0.265</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Cocystis musilla</td>
<td>Slides</td>
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<td>0.150</td>
</tr>
<tr>
<td></td>
<td>Fields</td>
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<td>0.278</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>0.143</td>
</tr>
<tr>
<td></td>
<td>Slides</td>
<td>5</td>
<td>0.679*</td>
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<td>Fields</td>
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<td>0.199</td>
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<tr>
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<td>Total</td>
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Table 4. (Cont.)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Category</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
</tr>
</thead>
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<td>Oocystis musilla</td>
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<td>20</td>
<td>0.402*</td>
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<tr>
<td>Pooled analysis</td>
<td>Fields</td>
<td>78</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td><em>Selenastrum</em></td>
<td>Slides</td>
<td>5</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>Fields</td>
<td>24</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td></td>
</tr>
<tr>
<td><em>Dictyosphaerium</em></td>
<td>Slides</td>
<td>5</td>
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</tr>
<tr>
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<td>Fields</td>
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<td>Total</td>
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<td>0.279</td>
</tr>
<tr>
<td><em>Dinobryon</em></td>
<td>Slides</td>
<td>5</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>Fields</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
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<tr>
<td><em>Lyngbya</em></td>
<td>Slides</td>
<td>5</td>
<td>0.170</td>
</tr>
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<td>Fields</td>
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<td>0.225</td>
</tr>
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<td></td>
<td>Total</td>
<td>35</td>
<td>0.117</td>
</tr>
<tr>
<td><em>Dactylococcus</em></td>
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<td>Fields</td>
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<td>Total</td>
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<td>0.101</td>
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<td><em>Chroococcus</em></td>
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<td></td>
<td>Fields</td>
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<td>0.247</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>
The reasons for this difficulty in sub-sampling were not clear. *A. falcatus* appeared as a single cell, lighter than many of the other forms, so that it should have remained in suspension in the mixed concentrate. *Q. musilla* appeared sometimes as a single cell and sometimes as a pair of cells together. The paired condition might have been expected to increase the variance of the counts but should not have effected the sub-sampling. *Dictyosphaerium*, which appeared in units of 2 and 4 and consequently would be even more variable, showed no similar significance.

A comparison was made to determine if the counts which showed sub-sampling significance were those which showed significant difference from the Poisson distribution. No such correlation existed.

Cells of the genus *Ankistrodesmus* are reported as solitary or clustered, often twined about one another (Prescott 1951 and others). Neither *Ankistrodesmus falcatus* nor *A. spiralis* were observed clustered or twined together in this study. This arrangement might have been destroyed during separation, or when mixing the concentrate for counting. If the clustering had persisted to the counting chamber the distribution would have been significantly different from the Poisson. As stated above, this relationship does not exist.

In making population estimates of these two organisms counts should be spread over several slides to obtain the most accurate estimate of the true abundance in the concentrate. This should probably be a general practice with all organisms to minimize the effect of possible unequal sub-sampling.

**Precision of counts**

Student (1907) determined that if a certain degree of accuracy was found by counting $M$ squares, and the liquid counted was diluted to
q times its bulk, then qM squares must be counted to be as accurate as before. He states as follows:

"So that the same accuracy is obtained by counting the same number of particles, whatever the dilution, or, to look at it from a slightly different point of view, whatever be the size of the unit of area adopted."

By accuracy he was referring to what is termed here precision, or the reproducibility of the counts. Accuracy, as used here, refers to the relationship of the population estimates to the true population level. These estimates are subject to many errors in addition to counting errors.

There are few attempts in the plankton literature to equate precision to the total number of organisms counted. In most cases the number of fields counted is used. There is of course, for any one concentrate, a direct relationship between the number of fields covered and the total number of organisms counted. However, no comparison is possible between concentrates of different densities.

The general references give little information. No recommendation to count either a specific number of cells or fields is given in "Fresh-water Biology" (Ward and Whipple 1918). In "The Microscopy of Drinking Water", 4th Edition (Whipple et al. 1927, pp. 97 and 101) a count of 10 or 20 squares is given as usually sufficient. It is stated that examination of many samples had shown that the Sedgewick-Rafter counting method was usually precise within 10 percent. No correlation with total organisms counted was made. In "Limnological Methods" (Welch 1948, p. 287) 10 fields selected at random are recommended, with 10 fields each in two different counting cells giving greater accuracy. No recommendation on specific counting procedures is given in "Fundamentals of Limnology" (Ruttner 1953). "Standard
Methods for the Examination of Water Sewage and Industrial Wastes*, 10th Edition (Anon. 1955, p. 449) recommends not less than 5 and preferably 10 fields, with 10 as the recommended number of cells per field. This in effect gives a recommendation of 50 organisms as a minimum and 100 as a preferred number counted. They further state that for specific studies it may be necessary to increase the number of fields tenfold, which if taken literally would give total counts of 500 to 1,000 organisms.

The plankton literature, with a few exceptions, reflects this same lack of statistical substantiation. Allen (1921) concludes that with care the extreme deviation could probably be kept within ± 25 percent and the mean deviation within ± 10 percent. His data for these estimates show total organisms enumerated per count from 215 to 2500. He states that his general intention however is to carry all enumerations to 50 individuals or colonies, but to stop at one-eighth of the slide in any event. He reasons that because of the time necessary to complete the longer counts they are not practical as standard procedure; that it is more important to do many counts with less precision.

Ricker (1937) uses Poisson statistics in his counts of zooplankton. He counts total numbers of organisms per cell, and establishes confidence limits for these totals.

His table is not applicable when a number of fields are counted, since it makes no provision for the precision with which the mean number of organisms per field is calculated.

The probable error, which corresponds to the 50 percent confidence limits; and the standard error, which corresponds to approximately the 68 percent confidence limits, are sometimes used. The probable error
equals 0.675 of the standard error, and twice the standard error closely approximates the 95 percent confidence limits used in this study. These relationships are used to convert these statistics for comparative purposes. Littleford, et al. (1940) give the equivalent of 95 percent confidence limits, as percent of the mean, of 3.5 percent for 30 fields, 2.8 percent for 40 fields and 5.9 percent for 50 fields. These are calculated for densities of 1800, 550, and 140 organisms per ml. It is not clear from their data however whether this refers to concentrate density or lake density. If it refers to concentrate density the total organisms counted were 26, 10 and 3 respectively. If the figures refer to lake density there is a choice of concentration methods, the plankton trap and the centrifuge. Based on their descriptions of procedure, the trap net would have resulted in total counts of 22,400, 9000 and 2900 organisms; and the centrifuge of 6100, 2500 and 800 organisms. The latter figures correspond fairly well with data from other studies and the first set appears to be too low. However in the absence of definite information their results must remain in question as regards the comparison of precision and total cells counted. Gilbert (1942) refers to the relationship of total numbers and precision but gives no specific examples.

Chu (1942) gives counts of 3, 5, 8, and 10 fields, with calculated limits equivalent to 95 percent confidence limits of 7.6, 16, 2.3 and 1.9 percent. The corresponding total organisms counted are approximately 600, 1000, 1600, and 2000.

A comparison of confidence limits and total organisms counted was made for 225 counts from this study (Figures 1, 2, and 3). The very
Figure 1. The relationship of total organisms counted and 95 percent confidence limits as percent of the mean, for 49 counts of 20 fields per count
Figure 2. The relationship of total organisms counted and 95 percent confidence limits as percent of the mean, for 108 counts of 24 fields per count.
Figure 3. The relationship of total organisms counted and 95 percent confidence limits as percent of the mean for 68 counts; of which 9 were of 18 fields, 21 of 25 fields, 12 of 30 fields, 6 of 36 fields, 4 of 64 fields, and 12 of 72 fields.
close correlation of precision and total number of organisms counted was obvious. The number of fields seemed to have little effect. In the literature cited good precision was associated with large total counts where data were available, while Chu (1942) in particular gains his high level of precision with very few fields per count.

It seems clear that total organisms counted and not total fields counted must be the criterion used for establishing general levels of precision, where these levels are expressed as percent of the mean.

The one sobering aspect for researchers is the large number of cells which must be counted for high levels of precision.

Moore (1952) attacks the problem of measurement of precision by considering the relationship of the coefficient of variation (or variability), \( C_v \), to the confidence limits. He presents a graph giving the relationship of number of fields counted and \( C_v \) at several levels of precision, from ±5 percent to ±30 percent, and gives a formula for direct calculation of \( C_v \) from the plankton counts.

\[
C_v = \sqrt{\frac{\sum x^2}{\sum x^2/n} - n} \quad (8)
\]

His levels of precision are calculated for the 68 percent confidence limits. In other words the true mean would be within the limits given, unless an approximately 1 in 3 chance has occurred. Doubling the limits of Moore's graph gives those approximately corresponding to the 95 percent confidence level used in the present study.

The confidence limits and coefficient of variation have a linear mathematical relationship expressed by the following formula, not presented by Moore:
\[ \text{CL} = \frac{t}{n} \text{C}_V \]  

(9)

where:

- \( \text{CL} \) = confidence limits as percent of the mean
- \( \text{C}_V \) = coefficient of variation in percent
- \( n \) = number of fields counted
- \( t \) = tabular value at \( n - 1 \) degrees of freedom and the confidence level desired. For the 95 percent confidence limits as used in this study \( t_{0.05} \) at \( n - 1 \) degrees of freedom would be used.

A line representing the relationship of confidence limits and \( \text{C}_V \) can be quickly calculated for any desired number of fields counted by substituting the correct values for \( n \) and \( t \) in formula (9), calculating confidence limits for two or three values of \( \text{C}_V \), then connecting the points with a straight line.

The confidence limits associated with a given \( \text{C}_V \) can be read within one or two percent from such a graph, more accurately than is possible from the graph presented by Moore.

Where the statistics necessary for calculation of the \( \text{C}_V \) using formula (4) are not available the short cut formula (formula 8) given by Moore would permit a considerable saving of time and calculation.
According to Ruttner (1953, p. 93), Johannes Muller, who is better known as a physiologist, first discovered the community we now call plankton, probably prior to 1850. He sampled the surface waters of the North Sea with nets of silk bolting cloth such as was used for flour milling. Nets of this silk are still being used for plankton sampling. The mesh openings of this silk are of fairly uniform size and retain their shape well under pressure. Welch (1948, p. 355) gives the aperture size of #25 silk, the finest made, as 0.064 mm. or 64 microns (μ), and that of #20 silk as 0.076 mm. or 76 μ. These sizes are most commonly used for plankton work. Actual working apertures will be somewhat smaller than those given, since the silk shrinks when first wet. A comparison of these aperture sizes with the dimensions of the Bear Lake phytoplankton (Table 2) made it plain that the silk nets could not be expected to retain a significant number of the cells. Kemmerer, et al. (1924), in the only published reference to the Bear Lake phytoplankton, give data from plankton net hauls made during a two or three day survey of the lake. They did not sample the water passing through the net. Ruttner states that the inefficiency of the nets for sampling phytoplankton was recognized by Kofoid in 1897 and Lohmann in 1908. Ricker (1933) points out the many problems in obtaining quantitative data from tows of the plankton net, even for larger organisms. Raymond (1937) centrifuged water which had passed through a #25 silk net and states that only 8 percent of the cells were retained by the net.
Despite this evidence that the plankton net is not a quantitative phytoplankton sampling method it is still much used in phytoplankton work. The prospects of simultaneous collection and concentration of the sample are hard to resist.

Concentration by plankton net was not attempted on the Bear Lake phytoplankton.

**Centrifuge**

According to Ruttner (1953), Lohman in 1908 used a centrifuge to study the material passed by the plankton nets. Juday (1916) describes the use of a clinical centrifuge. His specifications were 3500 R.P.M. and a tube volume of 15 ml. Lackey (1938) used a clinical centrifuge at 2500 R.P.M. He reports that examination of the decanted water showed almost all organisms retained, with the exception of a few minute green algae. He specifically lists an *Ankistrodesmus* species as one lost. He made no quantitative study of the extent of the loss. Littleford, *et al.* (1940) compare results obtained by clinical centrifuge, and find the Foerst centrifuge samples to be 30 percent higher. Birge and Juday (1922) pioneered the use of the continuous flow centrifuge for plankton work by using a large machine designed for purification of paints and varnishes. This machine left a residue of over 5 liters in its bowl. It was considered adequate for their purpose, which was to obtain sufficient sample for chemical analysis, and for which they centrifuged up to 1500 liters. Juday (1926) describes a Foerst electric plankton centrifuge, which was an outgrowth of the use of the larger machine. Essentially the same instrument is described by Welch (1948, pp. 256-257). It consists of a vertically mounted motor with the shaft extending above the motor. A bowl is mounted on the end
of the shaft and covered by a housing. The water sample is fed through the top of the housing to the center of the bowl at a rate of 7 to 10 minutes per liter. The plankton and debris are deposited in the bowl and the water spins out into the housing from which it is drained off through a tube. The motor speed is varied from 3600 to 20,000 R.P.M. by a rheostat. At 20,000 R.P.M. the centrifuge is rated as removing 98 percent of the algae on the first run, and most of the remaining 2 percent on a second run. Welch states that some algae are resistant to centrifuging, but lists only *Aphanizomenon*, of which about 50 percent would be removed during the first run.

A Foerst centrifuge was obtained for use during this study. It was found however that the centrifuge being sold was of the fixed speed type, rated by the manufacturer at 15,000 R.P.M., considerably below the speed of the machine described by Welch. It appears from a reference found later that this change in R.P.M. has been in effect for some time. Kraatz (1940) reports on a machine similar to the one obtained by us. He gives the R.P.M. at just under 15,000 with the cup empty. At a flow rate of 7 minutes per liter he lists *Coelosphaerium* and *Anabaena* lost to a large degree, *Microcystis* almost as much, *Aphanocapsa* somewhat less and *Aphanizomenon* usually not at all. In one test he centrifuged one liter 5 times. *Coelosphaerium* and *Anabaena* are listed present in the overflow after the fifth run.

During this project water samples were run into the centrifuge from a three liter wash bottle suspended above. The rate of flow slowed somewhat as the water level lowered, but flow rates were always adjusted when the wash bottle was full.

As an initial test, a water collection of 12 liters was divided into two 6 liter aliquots. The first aliquot was run at the flow rate
of 5 minutes per liter and then re-run at 10 minutes per liter. The second aliquot was run first at 7 minutes per liter and then re-run at 10 minutes per liter.

Cell density was very low, 16 fields were counted in each case and the total number of organisms counted per species varied from 1 to 40. The diatoms were apparently completely removed, even at 5 minutes per liter. None were seen in either re-centrifuged sample. Dictyosphaerium appeared in the overflow at 5 minutes per liter, but not at 7 minutes per liter. All the other species counted, A. falcatus, A. spiralis, Lagerheimia, Dactylococcus, Lyngeya, Selenastrum, Q1 musilla and Q. parva were carried over. For 5 minutes per liter, the lowest percent carry over was for Q. musilla with 11 percent, the highest Lagerheimia with 56 percent. For 7 minutes per liter, other than Dictyosphaerium which was previously mentioned, the lowest percentage of carry over was again Q. musilla with 4 percent, the highest Q. parva with 50 percent, and Q. musilla was the only species below 20 percent. The average carry over for all species was 34 percent at 5 minutes per liter and 28 percent at 7 minutes per liter.

As a further check a sample was run at 10 minutes per liter and then re-run at 15 minutes per liter. Three species were tested; A. falcatus went over 21 percent, A. spiralis 20 percent and Lagerheimia 51 percent. Precision was only moderate, with 20 to 90 organisms counted. From this data and the report of Kraatz (1940) it was concluded that the Foerst plankton centrifuge at 15,000 R.P.M. was not adequate for the Bear Lake phytoplankton. The clinical centrifuge also was eliminated on the basis of the lack of efficiency reported by Lackey (1938) and Littleford, et al. (1940).
**Filtration**

Ordinary laboratory filter papers are composed of a mat or network of fibers, and retention is accomplished throughout the depth of the paper. Removal of the plankton would be complete, but recovery of any large percent of the cells from the filter would be impossible.

In the Sedgewick-Rafter sand filter method the water sample is strained through a layer of fine sand held in a small funnel by a circle of silk bolting cloth. The plankton are retained in the sand. The sand is then mixed with a known amount of water in a small beaker, allowed to settle for a few seconds, and then the wash water containing the plankton cells is poured off (Anon. 1955). Juday, writing in Ward and Whipple (1918, p. 83), reports that there is considerable loss of organisms in this method, with many of the smaller forms passing between the sand grains. He also reports that the filter soon becomes clogged and the rate of flow decreases rapidly. Since the sand is supported in the funnel by a silk bolting cloth disc, the grains must be very large compared to the phytoplankton cells from Bear Lake, and the problem reported by Juday would be at a maximum. Separation of the Bear Lake phytoplankton by sand filter was not attempted.

Molecular or membrane filters, which are composed of cellulose compounds, have several properties which differentiate them from the regular laboratory filter papers. The openings in the membrane filter are formed during the chemical reaction of formation of the membrane. The size of the openings can be controlled during manufacture from 0.005 to 3.0 microns. Objects larger than the pore size are held on the glazed surface of the membrane instead of penetrating into the filter as with regular filter papers, and can be washed off if desired. Vacuum filtration is necessary with membrane filters (Anon. 1952).
According to Goetz and Tsuneishi (1951) membranes of this general type have been used for at least 60 years. The technique of controlling pore size was well developed in the 1930's. The early membranes were delicate and difficult to use, and required extensive boiling to prepare them for use. During World War II German bacteriologists had many of their laboratories destroyed by bombing. They developed methods of bacterial analysis utilizing the membrane filters which were at least as efficient as standard methods, much faster, and did not require extensive laboratory facilities. Because of the potentialities in bacteriological warfare detection this new method was investigated after the war by Dr. Goetz, Associate Professor of Physics at the California Institute of Technology, under the auspices of the Joint Intelligence Objective Agency of the Armed Forces. Research contracts were given to the Institute and the manufacture and use of the membrane filter for bacteriological work was investigated.

The publication of the results of this work (Goetz and Tsuneishi 1951) and subsequent general availability of the membranes, generated renewed interest in the membrane filter. Membrane filters had been used for phytoplankton work in a few cases prior to the war. Riley (1939, 1940) mention use of such filters for concentrating plankton samples, but describe neither the filters nor the methods. Cole and Knight-Jones (1949) state that collodion membranes have been successfully employed for 15 years to concentrate sea water for estimation of nannoplankton flagellates and algae at Conway, England. Their method involves filtering the water sample, addition of 1 ml. of filtered water to the surface of the membrane, mixing the organisms into the 1 ml. of water by brushing with a fine sable brush, and removal of the 1 ml. of water, plus the organisms, with a pipette.
Membrane filters are presently being manufactured in this country by two companies. Carl Schleicher and Schuell Co., Keene, New Hampshire, market them under the trade name Ultra Filters. The Millipore Filter Corp., Watertown, Mass., market them under the trade name MF Millipore Filters.

Goldberg, et al. (1952) report the use of Millipore Filters 50 mm. in diameter with an average pore size of 0.45μ in studying marine microplankton and suspended organic and inorganic matter. Their method involves straining a water sample of one or two liters through the filter, fixing and staining the organisms while on the filter, clearing the filter and mounting half of it on a microscope slide. They report the filter as practically transparent when cleared with cedar oil. This method was considered to be too complicated and time consuming for routine plankton work, and would present the additional problem of identification of preserved material.

It was decided to try a method involving removal of the cells from the filter for counting. At this point in the study the Millipore Filter Co. offered filters of 0.45 or 0.8μ effective pore size. Schleicher and Schuell Co. offered a series of porosities, with their coarse grade membrane filter rated at a pore size range of 0.75 to 3.0μ in their bulletin (Anon. 1952) and at 0.5μ average pore size and 1.2μ maximum size in a separate price list. In reply to a letter the company stated that the figures in the price list were a change in specification resulting from electron microscope studies. It was thought that filter clogging would be a primary problem. Clogging effects could be reduced by using the largest pore size and filtering area possible. Filters 150 mm. in diameter were decided on as the largest that could
be handled easily. The coarse grade filters from Schleicher and Schuell Co. had the largest available pore size (1.2μ). A supply of coarse grade membrane filters 150 mm. in diameter was obtained from Schleicher and Schuell Co.

Membrane filters require a holder in which they can be securely clamped to prevent vacuum leakage. Commercial filter holders were available, but seemed needlessly expensive. A holder of plastic and copper was constructed for under $15 which performed very satisfactorily throughout the study (Figure 4). A hole 15 mm. in diameter was cut in the center of an 8-inch square of one-half inch plastic. A shoulder was cut around the hole so that a disc of fritted glass 125 mm. in diameter could be mounted flush with the surface, as support for the filter. A funnel of copper was constructed and bolted to the one-half inch plastic. The flat head bolts were countersunk and the bolts and surfaces coated with gasket seal before assembling. A hole 125 mm. in diameter was cut in the center of an 8-inch square of one-fourth inch plastic. A section of plastic tubing with an inside diameter of 125 mm. was cemented over the hole in the one-fourth inch plastic.

In operation, a sheet of filter paper was placed over the fritted glass. The membrane filter was placed on the filter paper and the top section clamped on. The paper clamps illustrated were first used as a temporary arrangement, but functioned well enough that more complicated clamps were not needed. Vacuum was supplied by a Cenco pressure vacuum centrifugal pump. A trap bottle was placed in the line to protect the pump.

The flow rate of the coarse filter is given at from 1 to 10 seconds for 100 ml. to pass through 100 sq. cm. of filter surface, at a differential pressure of one atmosphere (about 15 lbs/sq. inch) (Anon. 1955).
Figure 4. Membrane filter holder
The effective area of the filter in the holder was about 120 square cm. Using the Genco pump, a liter of filtered water would pass through a new filter in 15 to 25 seconds. Filter time for actual samples varied according to plankton density and turbidity, with turbidity apparently the more important factor.

After storms the water near shore contained considerable suspended matter, and during spring and fall overturn this was present throughout the lake. Under these conditions the third liter of a three liter sample would often take 15 to 20 minutes to pass through the filter. The periods of high turbidity were infrequent. For the great majority of the samples 3 liters would pass through the filter in less than 2 minutes, and a 6 liters in less than 5 minutes. Filtering was always stopped by breaking the vacuum at the instant the last water left the surface of the filter.

The brush and pipette method given by Cole, et al. (1949) for removal of cells from the filter did not function well. There were always many cells in a second wash. Removal was next attempted by swirling the filter face down in a petri dish containing 10 ml. of distilled water. This was not effective. A second, third, and even fourth swirling always gave additional cells. Brushing the filter down with a small artist's brush using 10 ml. of distilled water was attempted but did not remove all of the cells. A hand atomizer was obtained, and the filter was washed down with it after brushing. This gave better results, but up to 25 percent of the cells were still recovered in a second wash. It was thought that a more powerful atomizer might solve the problem. A fortuitous combination of available materials provided one which was very satisfactory (Figure 5). A Perkin-Elmer
Figure 5. Pressure atomizer
Flame Photometer sample atomizer with a slightly damaged tip was obtained. The rubber bulb from a battery syringe was placed over the funnel of the atomizer. The lip of the funnel engaged a groove inside the neck of the bulb. The atomizer was used with the bulb down, with air pressure provided from the pressure line of the Genco pump. The bulb was filled with plankton free water and a fine hard spray could be turned on and off by squeezing and releasing the bulb. This control of the amount of water used was important in keeping concentrate volumes to a minimum (7 to 11 ml.), and thus providing the maximum counting density.

As finally perfected, the process of removal of the cells from the filter was as follows:

1. Removal of the filter from the holder and cupping in the hand over a petri dish
2. Wash down with the pressure atomizer
3. Brush down with a small, fairly stiff, good quality artist's brush, utilizing the water from step 2
4. A final wash down with the atomizer

The first wash apparently removed the diatoms practically 100 percent. Only an occasional cell would be seen in a second wash.

Better than 95 percent of the cells of the more abundant forms were consistently removed on the first wash. Very often 98 to 99 percent would be removed. In one test, 10 percent of *A. falcatus*, 20 percent of *Lagerheimia*, and 5 percent of *Q. musilla* remained in the second wash. This was the least efficient removal tested, and it considerably exceeded the general level. There seemed to be a tendency for an absolute as well as a relative number of cells to remain on the filter. For the least abundant species, where less than 10 cells would be seen during the count of the first wash, there would very often be 1 to 3 cells
counted in the second wash. This gave some rather high percentages (up to 30 percent) in the second wash for the least abundant forms but a very low absolute number. Greater numbers of the cells of abundant species would appear in the second wash, but these constituted a very small percentage of the total. A third wash invariably contained only an occasional cell. If proper technique was used, better than 95 percent of all cells could be removed with one wash of the filter.

It was possible to use the same filter for several samples, the exact number depending primarily upon the turbidity of the water. There was no deterioration of the filter. It gradually became clogged by fine suspended material which reduced the flow rate below the practical level. The number of samples per filter varied from 3 to 12.

The filters were dry when received. Once wet however they were kept wet as per the manufacturers directions. Shrinking and distortion appeared when the filters were allowed to dry out.

One comparison was made between the filter and the centrifuge. A sample was mixed and divided into two aliquots. One was concentrated by centrifuging at 10 minutes per liter, and the other was filtered. The estimate from the centrifuged sample for *A. falcatus* was 50 percent of the estimate from the filtered sample. Precision was on the order of 10 to 15 percent.

When field work was almost completed, Schleicher and Schuell Co. supplied some samples of coated, or backed, filters. These coated filters were stiffer and much easier to hold while removing the cells. A separate filter paper support was not necessary with the coated filters.

At about this same time, Millipore Filter Corp. announced availability of filters with larger pore size than the previous 0.8u maximum.
They supplied a trial shipment of their R. A. grade with pore size of 1.2μ. Their filters were more expensive ($1.85 each) than those from Schleicher and Schuell Co. ($0.60 each). They were considerable more brittle. Several were torn during handling and use, something that did not occur with the S. and S. filters. This would add further to the cost because of less re-use. The M. F. filters had a much faster filtration rate. A liter of filtered water would pass in less than 5 seconds, as compared to 15 to 25 seconds for the S. and S. filters. A trial run was made with a moderately turbid sample, and there seemed to be less effect of the turbidity than with the S. and S. filters. With either make of filter, the actual filtering time was a small fraction of the total time involved in setting up apparatus, handling and labeling samples, etc.

The fragility and cost of the M. F. filters might be balanced in their greater efficiency for turbid samples; however, the regular coarse grade S. and S. membrane filter appeared to be the best choice for general use on Bear Lake.

Sedimentation with iodine in potassium iodide

A 500 ml. water sample was treated in a 500 ml. graduated cylinder with a saturated solution of iodine in potassium iodide until a wine-yellow color was obtained, as recommended by Ruttner (1953, p. 110).

The sample was allowed to stand for 48 hours, and then the top 400 ml. carefully siphoned off. The two parts of the sample were then put through the membrane filter and counts made on each. With the small water samples used, cell densities would be expected to be low. More than 10 total organisms per count were found only for A. falcatus, O. pusilla and O. parva. The counts from the two parts were added for
each of these organisms and that percent of the total number which was found in the decanted 400 ml. calculated. These percents were; for *A. falcatus*, 58 percent, for *Q. musilla*, 31 percent, and for *Q. narva*, 63 percent. On the basis of these results, the sedimentation technique was not considered further.
SUMMARY AND CONCLUSIONS

It was the purpose of this study to evaluate methods of concentrating and enumerating the phytoplankton of Bear Lake. The methods were judged for suitability of use in a sampling program aimed at quantitative estimates of the standing crop.

The plankton population was found to be composed of very small cells, with diatoms comprising less than 5 percent of the population, by number.

Choice of counting chamber

The Sedgewick-Rafter cell was rejected as a counting chamber. The high dry microscope objectives (100 X) necessary for differentiation of the phytoplankton forms could not be focused over the entire depth of the cell.

Evaporation effects and uneven distribution of cells under the coverslip caused rejection of the slide and coverslip method of enumeration.

The inverted microscope method was not applicable. The required precipitation of the cells with iodine-potassium iodide was not effective.

A haemacytometer was found to be an adequate counting chamber. Evaporation effects were slow in appearing, and caused no serious limitation of the counts. It was determined that there was no gradient of cell density from the point of filling of the haemacytometer to the regular counting area.
Counting procedures

Counts from the haemacytometer were found to vary little from the Poisson distribution. It was determined that if at least 100 organisms were counted, there was little error in applying statistics of the normal curve to the counts, for determination of confidence limits as a measure of precision. For counts with lower numbers of organisms, the error was greater but the limits were still useful.

It was found that sub-samples of the concentrate varied significantly for 2 of the 11 organisms tested.

It was concluded that several sub-samples should be counted from each concentrate to minimize sub-sampling errors.

It was shown that there was a very direct relationship between the total number of organisms per count and the level of precision as measured by the 95 percent confidence limits. The number of fields over which the organisms were counted had little or no effect from 18 fields to 70 fields per count. The relationship below 18 fields per count was not tested.

It was concluded that the general level of precision of the counts could best be determined by choice of the associated total number of organisms counted. From the graphs in the present study, it was concluded that for 95 percent confidence limits of \( \pm 50 \) percent or better, 50 organisms must be counted; for limits of \( \pm 25 \) percent or better, at least 100 organisms; for \( \pm 10 \) percent or better, at least 400 organisms.

A linear mathematical relationship exists between the coefficient of variation and the confidence limits. It was concluded that the coefficient of variation could be calculated directly and the confidence limits read within one or two percent from a graph of the 2 factors.
Concentration methods

Cells of the Bear Lake phytoplankton were found to be too small for use of the plankton net.

There was found to be significant loss of cells with centrifuge methods.

Precipitation with iodine-potassium iodide solution was not effective.

Filtration through a membrane filter with pores of .5 to 1.2u was found to be an effective method of concentration. Ninety-five percent or more of the cells were removed from the filter by washing with a pressure atomizer and brushing with an artist's brush. For samples of 3 to 6 liters, filtration time was 2 to 5 minutes, with a maximum of 30 minutes for turbid samples. The combination of speed and efficiency made the membrane filter concentration method by far the most desirable.
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