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**PROTOCOLS FOR MEASURING
BIODIVERSITY:**

Phytoplankton in Freshwater

by:

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Introduction

Phytoplankton are unicellular, microscopic, unattached plants found in freshwater and marine ecosystems. In comparison to many other biological organisms, phytoplankton are relatively homogeneously mixed throughout the water column. Because these microscopic organisms depend on light and nutrients, they populate the euphotic zone or the upper strata of freshwater lakes, reservoirs, and ponds. They optimize their residence in the photic zone by a number of mechanisms: controlling buoyancy using gas vacuoles, migration using flagella, increasing surface area/volume ratio to form resistance, and metabolic processes. Phytoplankton can be solitary or colonial and can range in size from $<1 \mu\text{m}$ to colonies that are $>500 \mu\text{m}$.

Like most plants, most phytoplankton are autotrophic; they contain chlorophyll pigment that enables them to fix solar energy by photosynthesis, converting carbon into an energy form transferable to other parts of the aquatic food web. However, some phytoplankton can be heterotrophic for short periods (e.g. dinoflagellates, cyanobacteria), by using dissolved organic substances, or they can be phagotrophic and use particulate organic matter.

There are thousands of species of phytoplankton, many of which remain undescribed. The cell coverings of phytoplankton can vary between and within taxonomic groupings. These coverings consist of simple plasma membranes; protective, ornamented thecae frustules; loricated structures; siliceous frustules; or cellulose. Species identifications are based on morphological features, cellular structure, color, size, and cell division, which are all visible under the light microscope. Preserved or living specimens can be identified; taxonomy can be enhanced with electron microscopy, especially for taxa that have external, recognizable, cell characteristics such as diatoms, chrysophytes, and desmids.

Phytoplankton have rapid turn-over times (in the order of days), and are sensitive indicators of environmental stresses. They are affected by physical, chemical, and biological factors, making them valuable in monitoring programs.

Abiotic Factors

Freshwater phytoplankton populations are seasonally variable (Hutchinson 1967) and are regulated by both chemical and physical factors. Nutrient concentrations, nutrient ratios (stoichiometry), and light are essential growth indicators. For example, nitrogen-fixing cyanobacteria tend to dominate in systems with N:P ratios $<5:1$ (Findlay et al. 1994), whereas chlorophytes tend to dominate in systems with higher N:P ratios. Dinoflagellates proliferate in freshwater lakes with low pH and low C:P ratios. Pearsall (1932) postulated that phytoplankton abundance in lakes of the English Lake District was related to nutrient concentrations. The most obvious demonstration of this hypothesis was the experimental eutrophication of Experimental Lakes Area (ELA) Lake 226 (Schindler 1974). Redfield (1934) noted the constant ratio of C:N:P in marine plankton (106:16:1), a ratio that remains widely accepted for marine systems. However, Redfield ratios are the exception rather than the rule for freshwater phytoplankton (Healey and Hendzel 1979; Hecky et al. 1993). In many stratified freshwater lakes and reservoirs, blooms of chrysophytes, chlorophytes, or dinoflagellates form at varying

depths throughout the euphotic zone where nutrient concentrations are high and these organisms can select optimum light levels (Fee 1976).

Physical characteristics of a lake, reservoir, or pond such as depth, volume, ratio of drainage area:lake surface area, and fetch distance can influence phytoplankton assemblages. In small shallow water bodies, the euphotic zone has a higher ratio of epilimnetic sediments:lake volume, allowing for more recycling of nutrients from the sediments than in large, deep lakes. (The epilimnion is the upper portion of a lake that is well mixed and has a uniform temperature.) In contrast, nutrient recycling in a large lake or reservoir may be influenced by internal mixing caused by wind action. As previously mentioned, phytoplankton rely on different mechanisms to remain in the water column. Flagellated organisms may occur in higher proportions in a small lake than a large one because less physical motion is available in a small lake to enable suspension.

Sampling Procedures

Sampling sites and frequency

Sampling sites for phytoplankton should be located a reasonable distance away from shore to eliminate contamination by periphytic (attached) species of algae. The depth of the euphotic zone can also be used in defining sampling location. The euphotic zone is defined by the maximum depth at which surface light is attenuated to 0.5%. It has been demonstrated that 0.5% is the minimum light level required for photosynthesis (Fee 1978).

Generally, twice the Secchi disc reading will be a reasonable estimate of the maximum depth of the euphotic zone. If the lake is thermally stratified, it is best to sample over the deepest location of the euphotic zone, which will reduce the probability of contacting the lake sediments and will also allow sampling of the different thermal strata (epilimnion, metalimnion, and hypolimnion). Bi-weekly to monthly sampling is necessary to capture the seasonal dynamics of phytoplankton, and to quantify their abundance and biomass. It may be necessary to increase sampling frequency during periods of blooms. Lakes should be sampled during mid-day to optimize light transparency. Although phytoplankton are relatively homogeneously mixed, uneven horizontal distribution (patchiness) can be a source of sampling error. Taking multiple samples from different stations and combining them to produce a composite sample can reduce this error.

Many types of field gear can be used to collect phytoplankton from freshwater lakes, reservoirs, and ponds. The type of gear selected depends on the information required to address the question being asked.

Qualitative sampling

For qualitative sampling of phytoplankton, a 10- μm Nitex® mesh phytoplankton net is recommended (Fig. 1). The net is 20 cm in diameter, 35 cm in length, and is fitted with a stopcock at the lower end to allow opening and closing. The mouth of the net has a

5-cm canvas collar fitted with a metal bridle that attaches to the sampling line. This type of net is commercially available from Geneq Inc. (Appendix 1). The net is lowered to a given depth, allowed to settle there for 15-30 sec, and then is slowly pulled to the surface. Pulling the net too fast will cause a bow wave and the net will be less efficient. The mouth of the stopcock is positioned into a sample-collecting bottle and the sample is then drained. This procedure may be repeated 3-4 times.

Qualitative net sampling will yield presence/absence information and can aid in the identification of rare species, but is not appropriate for accurate counting or biomass estimates. Numerous species or individuals can pass through even small mesh sizes, colonies can be disrupted by the net, and some fragile species may burst from excessive pressure. However, qualitative samples are excellent for taxonomic surveys because of the large number of specimens collected.

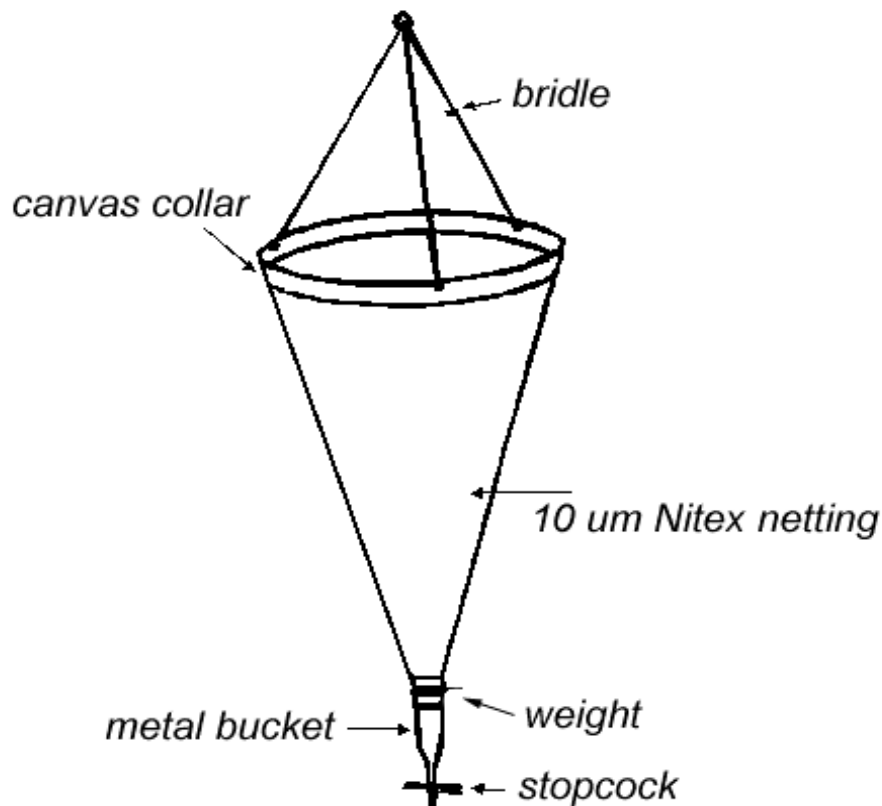


Figure 1. Phytoplankton net

Quantitative sampling

There are several reliable methods for obtaining quantitative phytoplankton samples,

which are unconcentrated samples taken from a known volume of water. The most common method is the use of a discrete-depth water-bottle sampler such as a Van Dorn, which samples a constant volume of water (1-6 L). The opened sampling apparatus is lowered to the desired depth, the trigger is released, and the sample is entrapped. The sampler is then brought to the surface, shaken, and a subsample is removed.

The use of an integrating sampler (Shearer et al. 1985) allows a sample to be obtained from a predetermined depth range (Fig. 2). This apparatus consists of a 1-L amber bottle attached to a weighted harness. A rubber stopper fitted with intake and outlet hoses is positioned in the mouth of the bottle. The intake hose is attached to a closing mechanism and opens when triggered. Technical information on the construction of this sampler can be found on the ELA web site (<http://www.umanitoba.ca/institutes/fisheries>).

The closed, integrating sampler is lowered to the bottom of the desired depth range (i.e. sampling 4-7 m, the sampler would be lowered to 7 m), the intake line is triggered open and the sampler is raised over the given depth range at a constant rate (1 m·10 sec⁻¹). The bottle is quickly repositioned at the bottom of the desired depth range and again raised at a constant rate.

This process is repeated until there are no air bubbles at the water surface, which indicates the bottle is full. The sampler is then retrieved into the boat, shaken, and a subsample is removed.

An integrated sample can also be collected from shallow water bodies by using a 3-6-cm diameter ridged, plastic pipe, 1-2 m in length. The pipe is gently inserted vertically into the water column and the top is capped off by covering it tightly with a hand. The pipe is lifted out of the water and is drained into a bucket. The water sample in the bucket is mixed well and a subsample is removed.

The above-mentioned methods all provide samples appropriate for accurate counts (i.e. number of cells·L⁻¹) and species identification. However, an integrated sampler allows an entire water column to be examined in fewer samples than for a discrete sampler, thereby reducing time-consuming microscopic analyses.

Preservation

Phytoplankton samples should be preserved by both acid iodine solution (Lugol's) and an acidified formalin solution (FAA; see recipes below) and stored in glass vials (opaque glass would increase shelf life) fitted with a polyethylene screw-cap lid. Both of

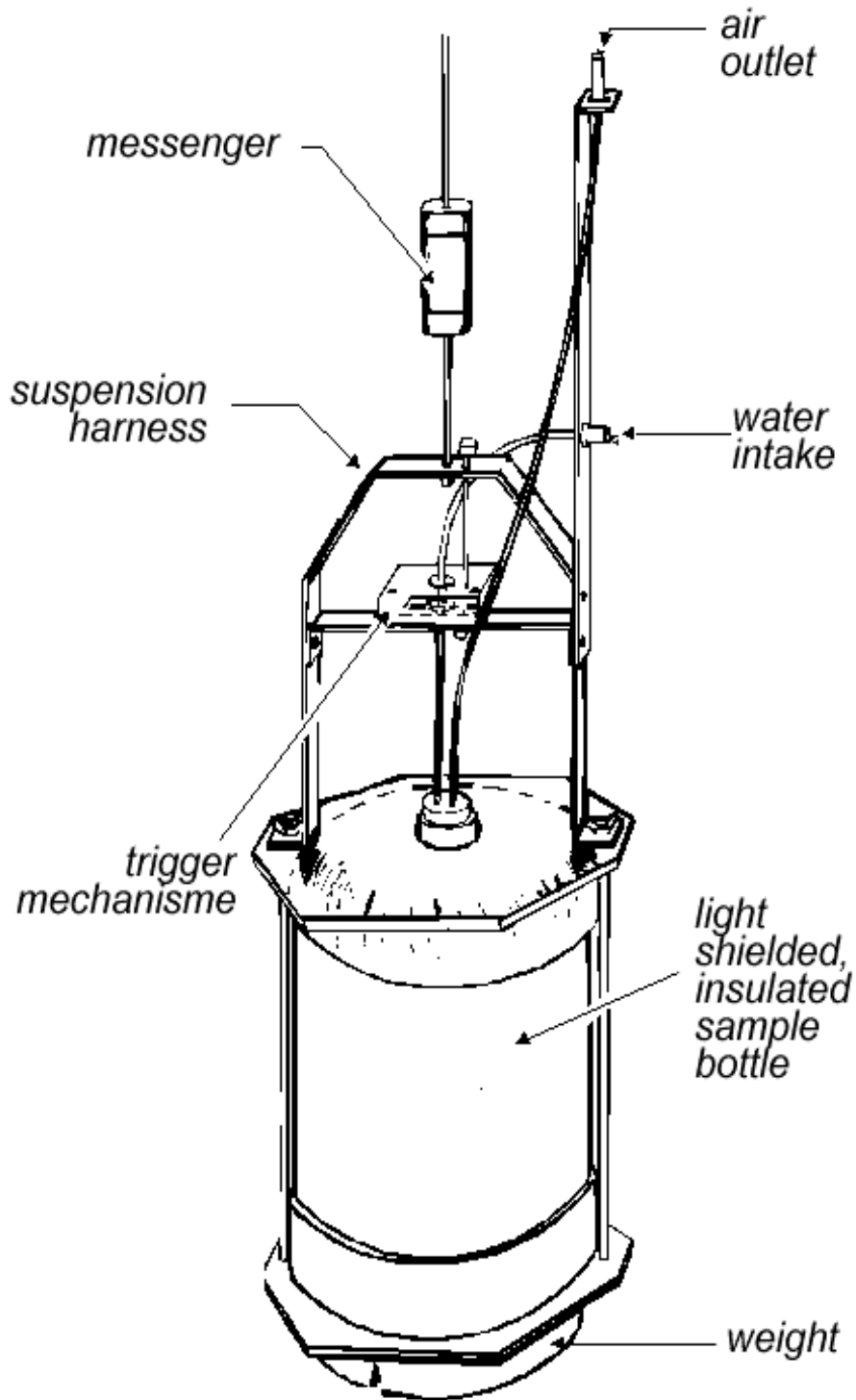


Figure 2. Integrating sampler (Reprinted from Shearer *et al.* 1985 by permission of J.A. Shearer, Freshwater Institute, Winnipeg, Manitoba)

the recommended preservatives have limitations. Lugol's solution has a shelf life that is affected by light. It is excellent for preserving chrysophytes but it makes the

identification of dinoflagellates difficult, if not impossible. FAA may cause thin-walled cells to burst. For best results, it is recommended that algal samples be preserved first with Lugol's (0.05-1% by volume) followed immediately by FAA (2% by volume). Color is an important taxonomic characteristic, especially for bluegreen algae. Formalin is a good preservative for green and bluegreen algae and dinoflagellates because cell color remains intact if samples are stored in the dark.

Lugol's solution can be prepared using 100 g I, 200 g KI, 200 mL glacial acetic acid, and 2000 mL of distilled water. Because it is light sensitive, this solution must be stored in a dark bottle. FAA is a solution of equal volumes of formaldehyde (37%) and glacial acetic acid.

Sample labelling and field notes

All samples and subsamples require accurate labeling. The label should contain information such as location, time, date, depth, type of sampling gear used, and the name of the collector. Complimentary field notes should be kept containing a description of the sampling site, type of land vegetation surrounding the sampling site, general geology of the area (e.g. granite vs. limestone), and pertinent information on other variables that were also sampled (e.g. air and water temperatures, wind speed and direction, and unusual odors).

Laboratory Procedures

An inverted microscope equipped with phase/contrast illumination; 10X, 40X, and 100X objectives; 12.5X eyepieces (10X or 16X are also available); and micrometer eyepieces (graduated in μm) is required to identify and count the specimens collected.

Photographic capability, although not necessary, can aid in confirming taxonomic decisions at a later date.

Identifications and counts for quantitative estimates are performed on preserved subsamples. Qualitative samples obtained using a phytoplankton net can be analyzed either live or preserved but they yield only presence/absence data.

Sample counting

The most common counting method used to quantify preserved phytoplankton samples is the Utermöhl (1958) technique as modified by Nauwerck (1963). (Staining is not required because samples have been preserved in Lugol's solution.) This method involves settling a known volume of sample into a counting chamber (Fig. 3). The density of phytoplankton will be dictated by the volume of subsample settled. If after settling a sample, the density of plankton is too great, a smaller volume of sample should be resettled. A simple counting chamber consists of three parts: (1) a bottom part, which is a piece of Plexiglas (40 mm² and 6 mm thick) with a 20-mm diameter hole drilled through it. A glass coverslip is glued (Pliobond glue) over the bottom of the hole; this part of the chamber holds 2 mL; (2) the top of the chamber, which is a column that can vary in length and volume (8 and 48 mL are shown). The top chamber is secured to the bottom with a thin film of stopcock grease. Samples require 4 h of settling time for every 6 mm of counting-chamber height (e.g. samples in a chamber 36

mm high would need to settle for at least 24 h); and (3) a cover glass, which is used to cap the top of the counting chamber after it has been filled with a sample. The cover glass is 3 mm thick and 35 mm in diameter. It is clear, ground glass that allows light to pass through. After settling is complete, the top portion of the chamber is slid off the bottom and a second cover glass is slid into place over the bottom chamber, which now contains all the phytoplankton that have settled. Chambers should be cleaned with alcohol before reuse to remove residue from previous samples.

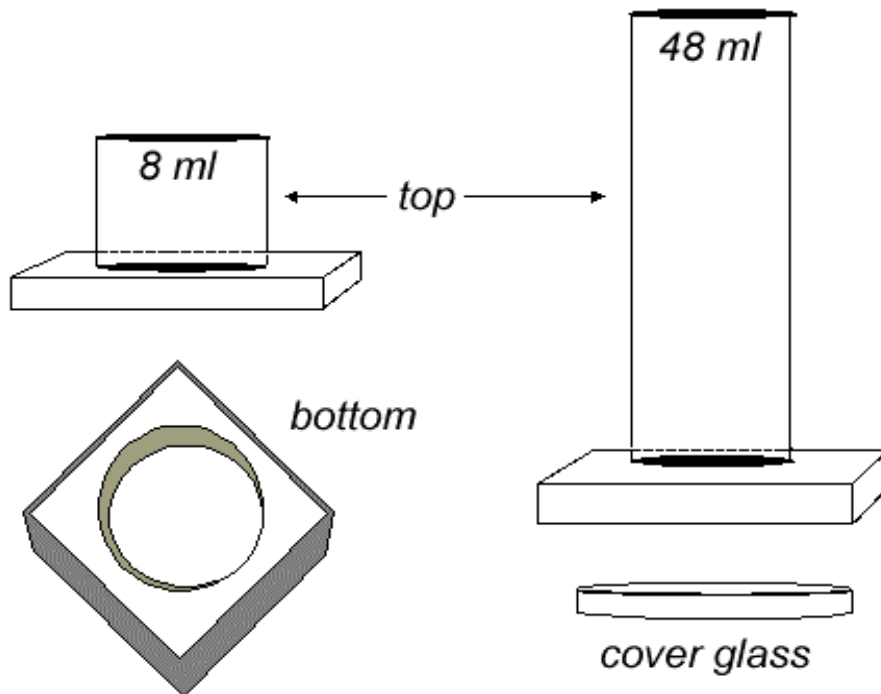
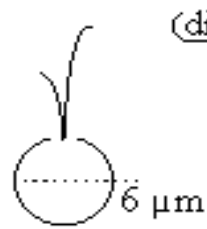


Figure 3.
Counting
chamber

The bottom chamber is then placed on the inverted microscope. Cells $>15\ \mu\text{m}$ are identified and counted using the 10X objective on transects that cover 50% of the chamber surface. Cells $<15\ \mu\text{m}$ are counted on a single transect, 200 μm wide, at the center of the counting chamber using the 40X objective. Cells must appear to be viable (i.e. chloroplasts intact). Cell fragments are not counted. Viable cells that are partially in a counting field on the right hand side are counted, but those on the left are omitted. For colonies, a small portion of the colony is counted, and the number of cells is then estimated. Filaments are counted individually. A minimum of 400-600 cells should be enumerated to assure that the count is representative of the sample. Cell counts are converted to wet-weight biomass by approximating cell volume. Estimates of cell volume for each species are obtained by routine measurements of 30-50 cells of an individual species and application of the geometric formula best fitted to the shape of the cell (Vollenweider 1968; Rott 1981). A specific gravity of 1 is assumed for cellular biomass. For accepted shapes and assignment of species see Rott (1981).

For example:



The diagram shows a spherical cell with a diameter of 6 μm. A horizontal dashed line across the sphere is labeled "6 μm". A vertical line from the top of the sphere to the center is also labeled "6 μm". A flagellum is shown extending from the top of the sphere. To the right of the diagram, the following calculations are shown:

$$\frac{(\text{diameter}) 6 \mu\text{m}}{2} = 3 \mu\text{m}$$
$$\text{cell volume} = \frac{4}{3} (\pi r^3)$$
$$= \frac{4}{3} (3.14 \times 27)$$

There are numerous types of devices used to tally counts. They range from a simple tab counter to voice recognition computer systems.

Identification

The premise of a biodiversity monitoring program is to observe changes at the lowest identifiable taxonomic level over time, so accurate species identifications are important. Identifications can be enhanced by using both light and electron microscopy, by examination of live and preserved specimens, and, in the case of diatoms, by examination of acid-cleaned specimens. However, identification of algae is extremely difficult and it is recommended that samples be processed by an experienced taxonomist.

Phytoplankton species are continually being described and classified. Therefore, it is necessary for those identifying phytoplankton to be abreast of current identification keys and those that are relevant to the geographic area in which sampling was done. Geitler (1932), Hubber-Pestalozzi (1941), Bourrelly (1966, 1968), Patrick and Reimer (1966, 1975), Komárek and Anagnostidis (1986), and Krammer and Lange-Bertalot (1986) are recommended keys for the identification of phytoplankton. The identifier should also have some knowledge of the life cycles of the different algae encountered and must be aware that species of phytoplankton can change size quickly as part of their reproductive phase.

Identifications can be checked/changed at a later time if subsamples are taken and archived. As a rough guide, 50-75 mL of each sample should be archived, and all unusual samples should be saved. Preservation methods described above should be followed.

Data Analysis

All analyses should be routinely recorded on a count sheet, which should allow for the following entries: sample identification, species names, numbers of cells counted, cell length and width, and drawings of unusual species (Table 1). Data should then be

stored in an electronic database; it is imperative that data entries be checked to eliminate unexpected errors. All variables required to transform the data into either biomass or number of cells·L⁻¹ should be saved with the count and identification data.

Phytoplankton data need to be summarized and plotted to enable seasonal, annual, and long-term analysis. This involves calculating the number of cells·L⁻¹ and the biomass of each species, summing species in the same taxonomic group to obtain group totals, and summing the totals of the different taxonomic groups to obtain a total biomass for each sampling date (Table 2).

Table 1: A phytoplankton count sheet containing all pertinent information

PHYTOPLANKTON COUNT SHEET								
# Species	Correction Factor ¹	Group	Species Code ²	Count	Length um	Width um	Cell Vol um ³	Species Name
1	200	1	1022	7	0	0	2600	Gomphosphaeria sp.
2	200	1	1078	9	251	6	7096.9	Planktothrix agardhil (Gom.) Anagnostidis
3	200	2	2113	1	0	0	3200	Pediastrum duplex Meyen
4	200	5	5540	12	30	6	848.2	Aulacoseira italica v subarctica (O.Mull)
5	200	5	5794	1	140	20	14660.8	Pinnularia flexuosa Cleve
6	200	6	6558	22	21	10	733	Cryptomonas erosa Ehrenberg
7	200	7	7632	4	34	34	20579.5	Gymnodinium sp.
8	200	7	7638	32	20	20	4188.8	Peridinium inconspicuum Lemmermann
9	7184	1	1065	16	6	4	50.3	Anabaena cylindrica Lemmermann
10	7184	1	1072	1	6	6	113.1	Heterocysts
12	7184	2	2105	21	10	6	188.5	Chlamydomonas spp.
13	7184	2	2112	4	4	4	33.5	Sphaerocystis schroeteri Chodat
14	7184	2	2115	8	4	4	11.2	Pediastrum tetras (Ehrenberg) Ralfs
15	7184	2	2121	14	8	4	67	Oocystis lacustris Chodat
16	7184	2	2127	3	10	10	174.5	Tetraedron minimum (Brunow) Hansgrig
17	7184	2	2131	4	6	4	33.5	Scenedesmus quadric auda v
18	7184	2	2132	12	8	4	44.7	Scenedesmus denticulatus Lagerhiem
19	7184	2	2136	12	5	5	65.4	Dictyosphaerium pulchellum Wood
20	7184	2	2138	68	49	1.8	83.1	Monoraphidium komarkovae (Nvg.) Komarkova
21	7184	2	2143	3	10	4	62.8	Monoraphidium minutum (Nag.) Komarkova-Le
23	7184	2	2178	1	12	12	301.6	Cosmarium sp.
24	7184	2	2201	21	3	3	14.1	Small greens
25	7184	4	4351	191	3	3	14.1	Small chrysophyceae
26	7184	4	4352	34	6	6	113.1	Large chrysophyceae
27	7184	4	4362	1	4	4	33.5	Kephyrion sp.
28	7184	4	4383	4	12	6	226.2	Dinobryon bavaricum Imhof
29	7184	4	4420	24	6	4	50.3	Gloeobotrys limneticus (G.M.Smith) Pasch
30	7184	5	5702	4	22	4	92.2	Achnanthes minutissima Kutzing
31	7184	6	6554	21	12	6	150.8	Rhodomonas minuta Skuja
32	7184	6	6568	6	8	4	44.7	Katablepharis ovalis Skuja

Lake: 223 Stratum: Epilimnion Date: 23 Jul 93 Start Depth: 0 End Depth: 3.75

¹ The correction factor is used to convert the number of cells in the settled subsample to the number of cells L⁻¹ (i.e., 10 cells in a 5 mL subsample = 10 cells * 200 (correction factor) = 2000 cells L⁻¹).

² Species are entered as numeric codes that are electronically linked to the species names.

149	5 Oct 93	15.6	33.5	0	14303	30.1	23.4	142.7	1675.5
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Table 2. Daily phytoplankton biomass (mg·m⁻³) for Experimental Lakes Area Lake 149, 1990-1993.

Figure 4 depicts how the summarized phytoplankton data can be graphically presented. Composition (lower panel) is presented as the percentage contributed by each of the seven taxonomic groups to the community on a daily basis. Total daily biomass (upper panel) is presented as g·m⁻³.

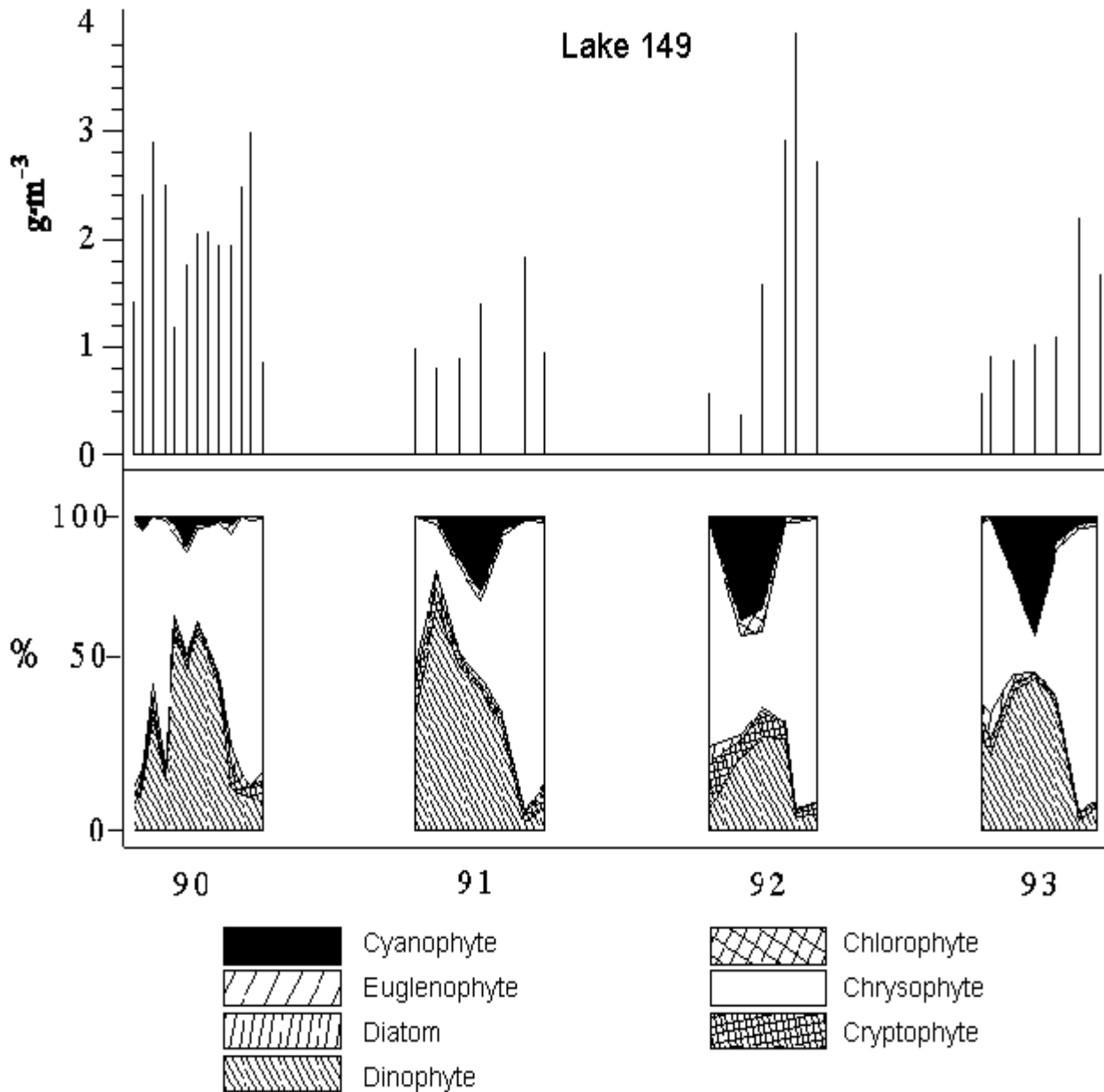
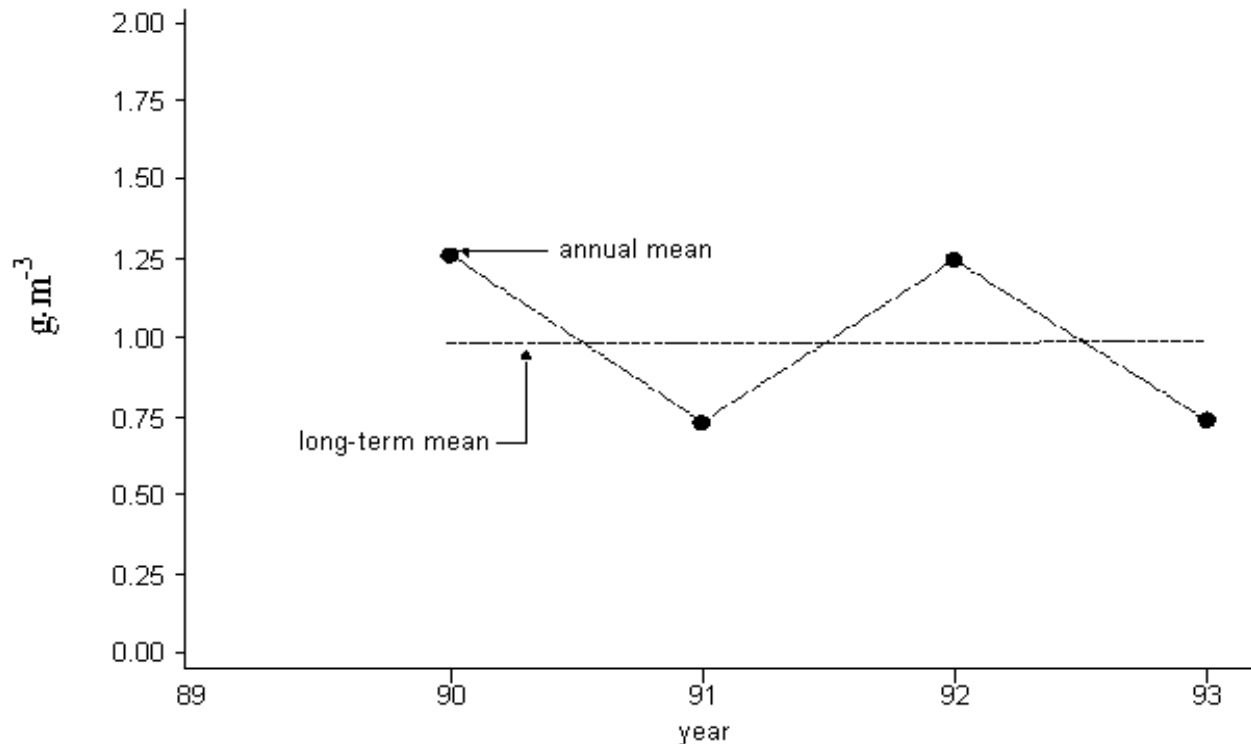


Figure 4. Phytoplankton biomass and composition for Experimental Lakes Area Lake 149 from 1990-1993.

Long-term changes in the biomass of a phytoplankton community can be assessed by comparing annual means to the long-term mean for each of the taxonomic groups (Fig. 5). The long-term mean is calculated by summing all annual means and dividing by the number of years involved.

Figure 5. Annual mean vs. long-term mean biomass for Experimental Lakes Area Lake 149.



Compositional and numerical species changes can be assessed by the use of a measure of species richness, a species diversity index, or a similarity index. However, the accuracy of the taxonomy will affect the final results. Species richness is simply the number of species encountered, and is based on presence or absence. It can be applied to both qualitative and quantitative samples. Washington (1984) recommends Simpson's Diversity Index for phytoplankton:

where s = the number of species in a sample, n = the number of individuals in a sample, and n_i = the number of individuals of species i in a sample. This index is biased by the dominant taxa.

Percent similarity analysis (Washington 1984) uses biomass and can also be applied to quantitative samples:

where s = total number of species in both samples, P_{1i} = proportion of the total biomass of species i in sample 1, and P_{2i} = proportion of the total biomass of species i in sample 2. This index allows direct comparison between two sites or stations, and also tends to favour the dominant species.

Quality Assurance/Quality Control (QA/QC)

A QA/QC program is necessary to instill credibility in a monitoring program. A QA/QC program is difficult to operate when dealing with algal communities because of the limited number of qualified taxonomists. However, the following checks can be put into place to increase the integrity of a monitoring program:

1. Constancy of identifications can be achieved if the same person analyzes all samples. This approach will facilitate subsequent changes to taxonomic identifications.
2. 10-15 % of the samples should be analyzed by other persons to ensure the accuracy of identifications and counts.
3. Replicate counts should be performed on selected samples at different times. The replicate count should be within $\pm 20\%$ of the first count.
4. Periodic analysis of check samples will also help to assure the quality of identifications. Check samples contain known species sent out to test the accuracy of identifications by a laboratory.
5. A good pictorial reference key and a reference collection of permanent slide material will ensure standard taxonomic identifications. Photographs or voucher specimens for every taxon identified should be archived in a nationally recognized herbarium.

Volunteer Involvement

Volunteers using the protocols described above will be able to sample phytoplankton both qualitatively and quantitatively. However, the identification of phytoplankton to species level is extremely difficult and should be only done by an experienced taxonomist.

Persons to contact for more information

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Ottawa, Ontario
K1P 6P4
E-mail: phamilton@mus-nature.ca

APPENDIX 1:

Sources of equipment and supplies for studies of phytoplankton biodiversity

Fisher Scientific
112 Colonnade Rd
Nepean, Ontario
K2E 7L6
1-613-226-8874

Geneq Inc.
7978 Jarry E.
Montreal, Quebec
H1J 1H5
1-514-354-2511

VWR Scientific
175 Hanson St.
Toronto, Ontario
M4C 1A7
1-800-932-5000

Marivac Ltd.
5821 Russell St.
Halifax, Nova Scotia
B3M 4C8
1-902-454-5544

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