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Techniques of Water-Resources Investigations
of the United States Geological Survey

Chapter A4

METHODS FOR COLLECTION AND ANALYSIS OF AQUATIC BIOLOGICAL AND MICROBIOLOGICAL SAMPLES

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Revised 1987
Book 5

LABORATORY ANALYSIS

PHYTOPLANKTON

Introduction

Phytoplankton are unicellular algae existing as single cells, colonies, chains, or filaments that generally are transported passively (some forms are active swimmers) by currents and turbulent mixing. Morris (1967) divides the planktonic algae into nine taxonomic divisions, including the blue greens (Cyanophyta), greens (Chlorophyta), diatoms (Bacillariophyta), dinoflagellates (Pyrrophyta), and five other divisions of flagellates. The range of sizes among phytoplankton cells or colonies is diverse (ranging from about 1 to about 1,000 m) and has been partitioned into four size classes by Wetzel (1975): macroplankton (more than 500 μm), netplankton (50–500 μm), nannoplankton (10–50 μm), and ultraplankton (less than 10 μm). Physiological processes of planktonic algae can profoundly affect (and indicate) the productivity and quality of natural water. Their photosynthetic assimilation of carbon dioxide and production of organic matter provide a (the) primary food source for other trophic levels, including harvestable species; they also affect the concentration of dissolved gases (carbon dioxide, oxygen), inorganic nutrients (nitrogen, phosphorus, silica, and trace elements), and dissolved organic substances. Phytoplankton blooms can severely affect water quality, either through the production of toxins that lead to fish kills or threats to human health or through the decomposition of organic matter that can deplete oxygen.

Integrated studies of aquatic ecosystems need to include measurements of phytoplankton biomass and composition. Measurement of bulk constituents [chlorophyll *a*, adenosine triphosphate (ATP), and particulate organic carbon or nitrogen] can be used as indices of biomass, while particle counters can provide information about size distribution. However, these methods have interferences from nonphytoplankton particulate matter (detritus, bacteria, microzooplankton, and sediment). The only method of determining the species composition of phytoplankton communities is by microscopic enumeration and identification. Although time consuming and laborious, this method can offer valuable information. Knowledge of species composition can indicate the causes of seasonal changes in biomass, can be useful as tracers for different water masses, and can indicate stresses imposed by pollutants that may not be evident from measurements of biomass alone. Estimates of cell size and measurements of cell-size distribution also can provide an accurate measurement of phytoplankton biomass [as biovolume, which can be converted to carbon (Strathman, 1967)].

Collection

There is no single best method for collecting and enumerating phytoplankton samples because phytoplankton types and abundance differ spatially and temporally. Therefore, it is necessary to choose a sampling strategy and method most consistent with the goals of a given water-quality study. For example, frequent collection of a depth-integrated sample at one representative site may be appropriate for a monitoring study; whereas, a detailed spatial grid may be more appropriate for assessing the effects of a point source of a pollutant. Sampling in those areas having the greatest environmental variability or having rapid temporal change needs to be intensified. Sournia (1978) has compiled a detailed manual of phytoplankton methods that includes a discussion of sampling strategy and statistical analyses.

A phytoplankton sample consists of a volume of water (usually 100 mL to 1 L) that is stored in a graduated polyethylene or glass bottle. Dissolution of weakly silicified diatoms is minimized in bottles made of soft glass (Banse, 1974). To ensure maximum correlation of results, the sample site and method used need to correspond as closely as possible to those selected for chemical and microbiological sampling. If a living sample is to be examined, it can be maintained at 3 to 4 °C for 24 hours or it can be kept cool and darkened for 3 to 4 hours. Extended storage requires use of a preservative. Two preservatives commonly are used:

1. To each 100 mL of sample, add 3 mL 34 to 70 percent aqueous formaldehyde solution (100 percent formalin), 0.5 mL 20 percent detergent solution, and 0.1 mL cupric sulfate solution. This preservative maintains cell coloration and is effective indefinitely but may distort the cell shape of species and cause loss of flagella.
2. Lugol's solution using acetic acid (Rodhe and others, 1958) will stain cells (and other organic particles) brownish yellow and will maintain cell morphology of flagellates. To each 100 mL of sample, add 1 mL Lugol's solution having 10 percent acetic acid.

Phytoplankton samples can be collected using a water-sampling bottle, depth-integrating sampler, net, or pump. Most water-sampling bottles consist of a cylindrical tube that has stoppers at each end and a closing device that is activated by a messenger. The bottle is lowered into the water in the open position to a desired depth, tripped, and retrieved. Most common examples of bottles are the Kemmerer (fig. 10), Van Dorn-type (fig. 11), the Nansen, the Fjarlie, and the Niskin. These bottles are available in a variety of sizes,

having capacities from 0.2 to more than 30 L, and are constructed of brass, clear acrylic, or polyvinyl chloride. Advantages of water-sampling bottles include these features: (1) Quantitative samples can be collected that include nannoplankton and ultraplankton; (2) samples of a known volume can be obtained from a precise depth; (3) bottles can be hung in arrays to collect simultaneous samples at a variety of depths; and (4) bottles are light and do not require auxiliary equipment. However, they are difficult to handle in strong currents.

Depth-integrating samplers are used to collect quantitative samples representative of a cross section of a stream or the water column of a lake, reservoir, stream, or estuary (Schröder, 1969; Lewis and Saunders, 1979; Wetzel and Likens, 1979). The simplest depth-integrating sampler is a length of garden hose or flexible tubing that is weighted on one end (Lund, 1949). The weighted end is lowered through the desired sampling depth of the water column, and the open end then is pinched off to secure the sample within the hose as it is raised to the surface.

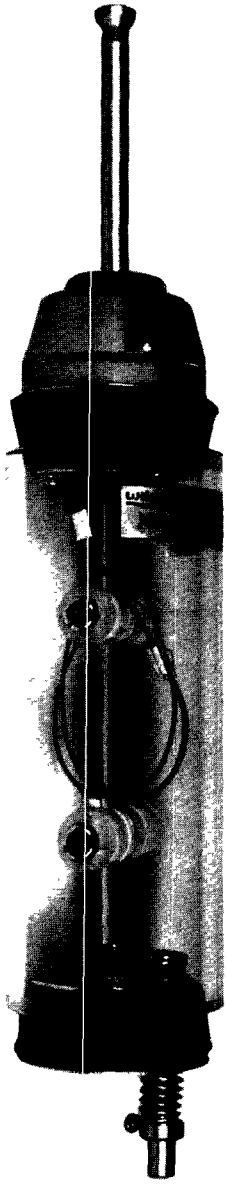


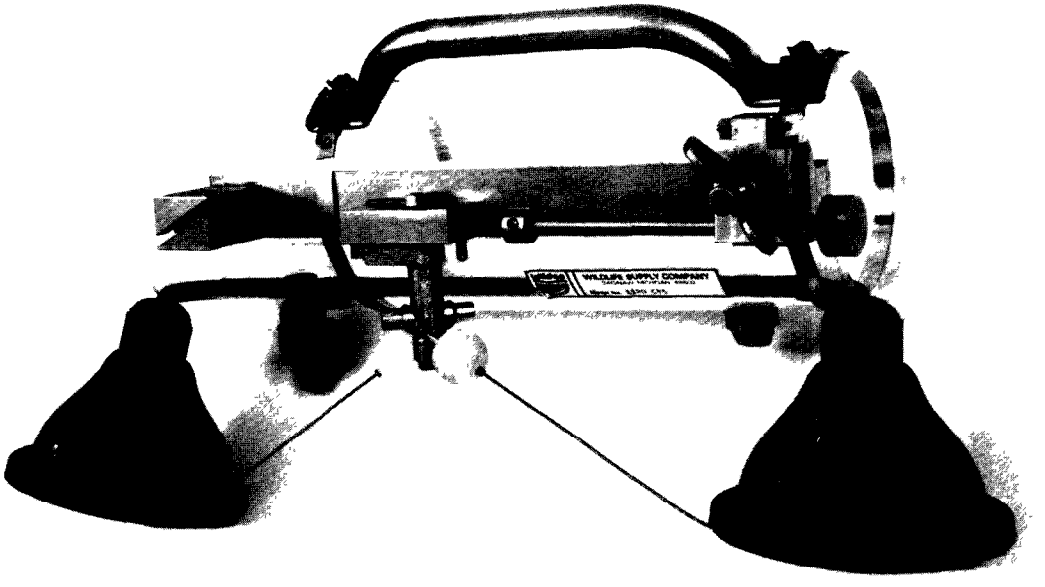
Figure 10.—Kemmerer water-sampling bottle. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

A sampler, such as the D-77 sampler (fig. 12), can be used for depth-integrating sample collection. This sampler is made of aluminum or bronze and has a built-in cap and nozzle that can be sterilized and will collect a 3-L sample. A depth-integrating sampler designed specifically for collecting phytoplankton is described in Fee (1976). This sampler is a modification of the Van Dorn-type water-sampling bottle and has release mechanisms to clamp the sample-inflow and air-escape hoses. The sample-inflow hose goes to the bottom of the sampler, and the air-escape hose to the bottom of the cap. The sampler is lowered to the desired depth, a messenger is released, and the release of the two hoses starts the sampler. For stream sampling, the equal-transit method developed by Guy and Norman (1970) is useful. In this method, the standard suspended-sediment sampler is used to collect samples at a number of equally spaced verticals in the cross section. Samples collected in each vertical are composited into a single sample that has been discharge-weighted and is representative of the entire cross section.

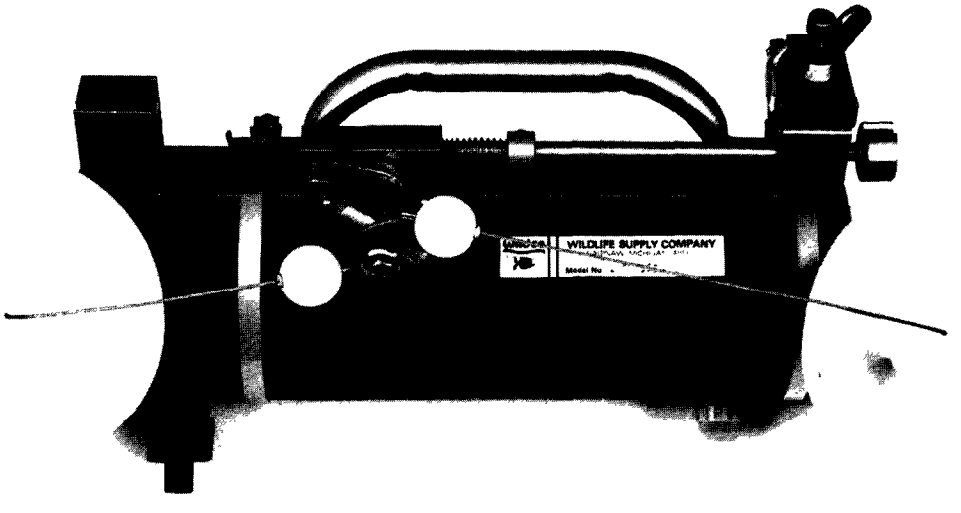
Advantages of depth-integrating samplers include these features: (1) Quantitative samples that include nannoplankton and ultraplankton can be collected; (2) samples of a known volume can be obtained; (3) these samplers provide the only means of collecting a truly representative sample of phytoplankton within a water column or in a stream cross section; and (4) many are light and can be used without auxiliary equipment. However, sample collection may be time consuming with the use of some of these samplers, and some are heavy and require auxiliary equipment. In addition, these samplers may not be adequate for use during high flow.

Plankton nets have been used widely as sampling devices in phytoplankton investigations because they enable filtration of a large volume of water; however, nets selectively retain only the largest phytoplankton cells. Margalef (1969) assumed that only 10 percent of all algal cells are retained by nets having a mesh size of 40 μm. However, phytoplankton collection using nets may be appropriate for qualitative studies of large planktonic algae. Most qualitative samplers are cone-shaped nets that are towed slowly from a bridle and that funnel trapped material into a bucket (fig. 13). Nets come in a variety of mesh size, have openings ranging from 0.5 μm to 5 mm, and usually are constructed of woven synthetic filaments (monofilament nylon or polyester) that resist chemicals and have stable mesh geometry. Nets can be towed vertically, horizontally, or obliquely to collect integrated samples. Closing nets, such as the Birge sampler (Welch, 1948), can be lowered to a selected position, activated, and then closed by messenger to sample only at a specific depth.

Advantages of nets include these features: (1) They provide a simple means of collecting qualitative samples of macroplankton, netplankton, and some nannoplankton; (2) they can be adapted with a flowmeter for collecting semi-quantitative samples; (3) the mesh size can be chosen, within limits, to collect planktonic algae of selected sizes; (4) large species are collected; and (5) nets are relatively inexpensive and easy to operate from a small boat. Disadvantages include these features: (1) They do not collect quantitative samples; (2) they exclude ultraplankton and some nannoplankton (these



A



B

Figure 11.—Van Dorn-type water-sampling bottle: (*A*) Alpha bottle; (*B*) Beta bottle. (Photograph courtesy of Wildlife Supply Co., Saginaw, Mich.)

forms often constitute a majority of phytoplankton biomass); (3) they are not suitable for collection in very shallow water or water having large algal populations; and (4) clogging by vascular plants, detritus, and dense populations of algae can be a problem, particularly with fine-mesh nets.

Pumps can be used to collect qualitative or quantitative samples of phytoplankton (Aron, 1958; Fee, 1976; Schemel and Dedini, 1979). The basic design consists of a centrifugal (impeller) or reciprocating (piston or diaphragm) pump connected to a hose that is lowered to the sampling depth, a base, and a collecting net and bucket. The centrifugal pumps probably are least damaging to algae. Quantitative samples can be collected by measuring the flow rate of the pumped stream using either a volume register or a calibrated container. Advantages of pumps include these features: (1) Quantitative samples of macroplankton, netplankton, and some nannoplankton can be collected quickly; (2) discrete samples from known depths can be collected; (3) the sampling hose can be moved during sampling to collect a depth-integrated sample; and (4) the pumps can be used in shallow water. In addition, pumps are good for point samples but may induce erroneous respiration and productivity values. Disadvantages include these features: (1) Pumps usually are bulky, expensive, and require an electrical source; and (2) they may break algal chains and colonies or physiologically stress planktonic algae.

Precision

The precision of estimated phytoplankton cell densities is essential for comparing estimated population densities in different samples; however, calculation of the exact precision of population estimates is difficult for two reasons. First, accurate statistical analysis requires knowledge of the frequency distribution of algal cells in nature, in aliquots of samples, and in counting chambers. Second, most sampling programs involve multiple stages of subsampling (for example, onsite population → sample → aliquot → microscopic field). Each stage of subsampling adds a new component of variability to the data (Venrick, 1978). If the distribution of phytoplankton cells is random (that is, conforms to a Poisson distribution), then the precision of cell counts can be estimated from the formulas in the following paragraphs. Departures from a random distribution are common, usually because of clumping or aggregation, and can be determined using the chi-squared test (Lund and others, 1958). Assuming that phytoplankton cells are not densely aggregated in counting chambers, the following procedures can provide reasonable estimates of counting precision (Venrick, 1978).

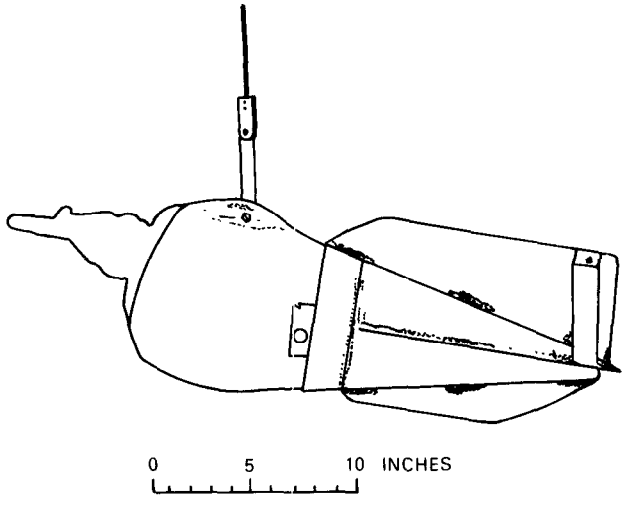


Figure 12.—D-77 depth-integrating sampler. (Sketch courtesy of St. Anthony Falls Hydraulic Laboratory, Minneapolis, Minn.)

If phytoplankton are counted in n random microscopic fields of only one aliquot from one sample, then the precision of only the mean number of cells in that one aliquot can be estimated. This may not represent the overall precision of a multilevel sampling program, and it certainly overestimates the precision of population estimates when phytoplankton are spatially heterogeneous. When the number of cells enumerated per chamber is small (less than 50), the confidence limits for a count can be estimated using figure 14. If more than 50 cells are enumerated per chamber, Venrick (1978) suggests using the normal approximation, where confidence limits around the total count (at the $1-\alpha$ level of significance) are indicated by

$$\Sigma x \pm z\alpha \sqrt{\Sigma x},$$

where

Σx is the total count of cells; and

$z\alpha$ is the normal variate (tabulated in most statistics texts).

Precision increases in proportion to the square root of the total number of cells counted, as listed in table 12. This table can be used to determine the number of cells that should be enumerated for a desired level of precision. For example, if 100 cells are enumerated, we can say with 95-percent certainty that the true count does not vary from the mean estimated count by more than 20 percent. Enumeration of 400 cells ensures a precision that is within 10 percent of the mean count.

In the instance where replicate chambers are enumerated from one or more aliquots from one or more samples, total variance of counts from all subsampling stages can be estimated. Venrick (1978) recommends use of the studentized normal variate (t) when the mean number of counts per chamber $\bar{x}$ ($\bar{x}=\Sigma x/N$) is greater than 50. Confidence limits around the mean thus are

$$\bar{x}=t\alpha,\ N-1\ \sqrt{\bar{x}/N},$$

where N is the number of chambers enumerated.

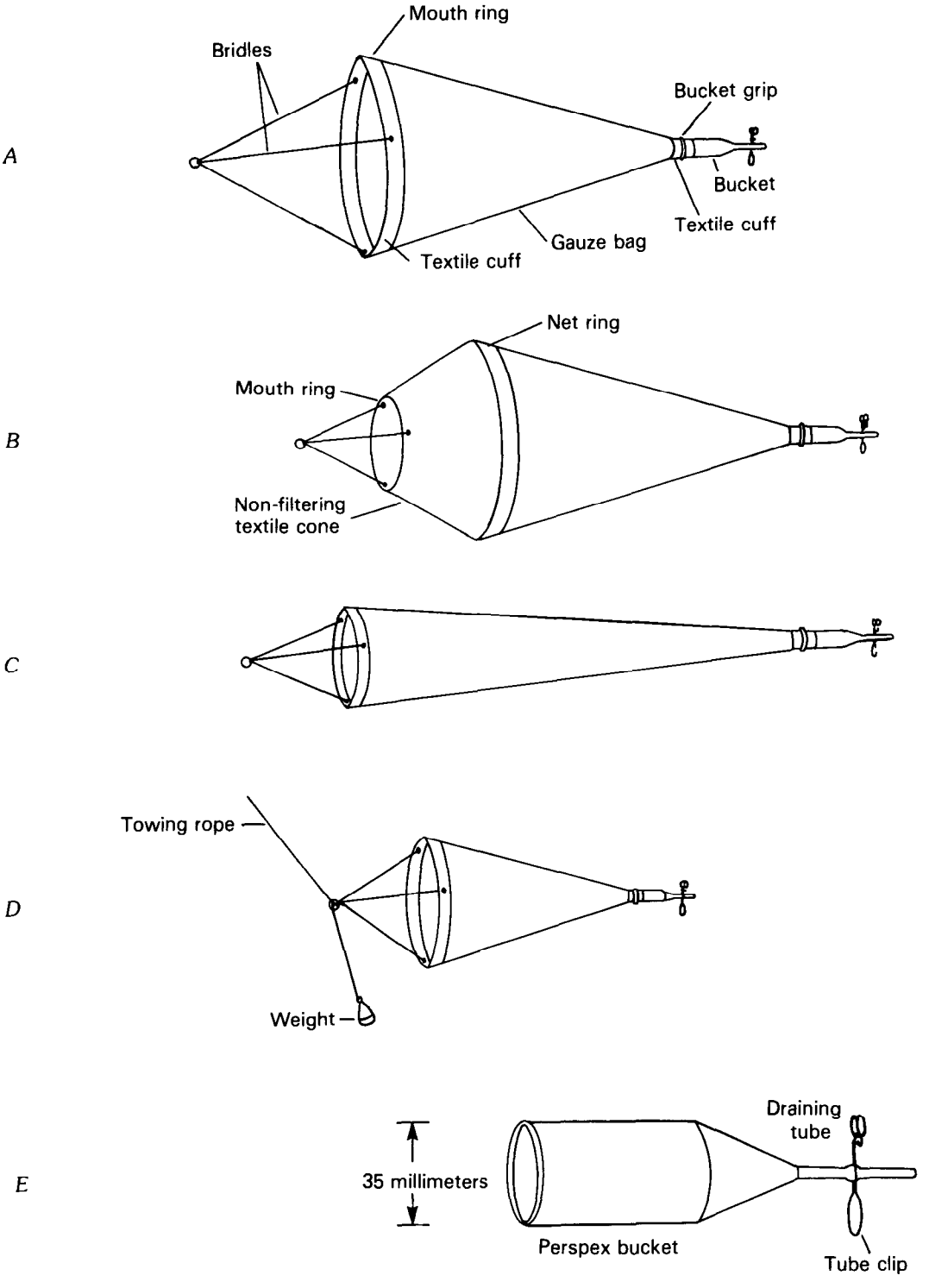


Figure 13.—Phytoplankton sampling nets and accessories: (*A*) Standard net. The length of standard nets normally is 2 to 3 times the mouth diameter. (*B*) Fine-mesh net that has decreased mouth diameter. A tapering non-filtering textile sleeve is inserted between the large net ring and the smaller mouth ring. (C) Extra long, fine-mesh standard net. (*D*) Standard net attached to the towing rope, and a weight in front of the mouth. (*E*) Plankton-collecting bucket made of clear perspex material. Diameter of the bucket is 30 to 100 millimeters (here 35 millimeters); length of the cylindrical part is 50 to 200 millimeters (here 65 millimeters). The bucket is attached to the net tail by textile tape or a specially made metal grip (from Sournia, 1978; reproduced by permission of UNESCO).

Table 12.—*Approximate 95-percent confidence limits for the number of cells counted, assuming a random distribution (from Lund and others, 1958)*

[Precision is the maximum expected departure from the count, expressed as a percentage of the count]

Number of cells counted	95-percent confidence limit[1]	Precision (percent of the count)
4	0-8	±100
16	8-24	±50
25	15-35	±40
100	80-120	±20
400	360-440	±10
1,600	1,520-1,680	±5

[1]For some colonies, the confidence limits in terms of number of cells can be calculated by finding the confidence limits for the complete count of phytoplankton, and then multiplying these by the mean number of cells per colony in these same phytoplankton (Lund and others, 1958).

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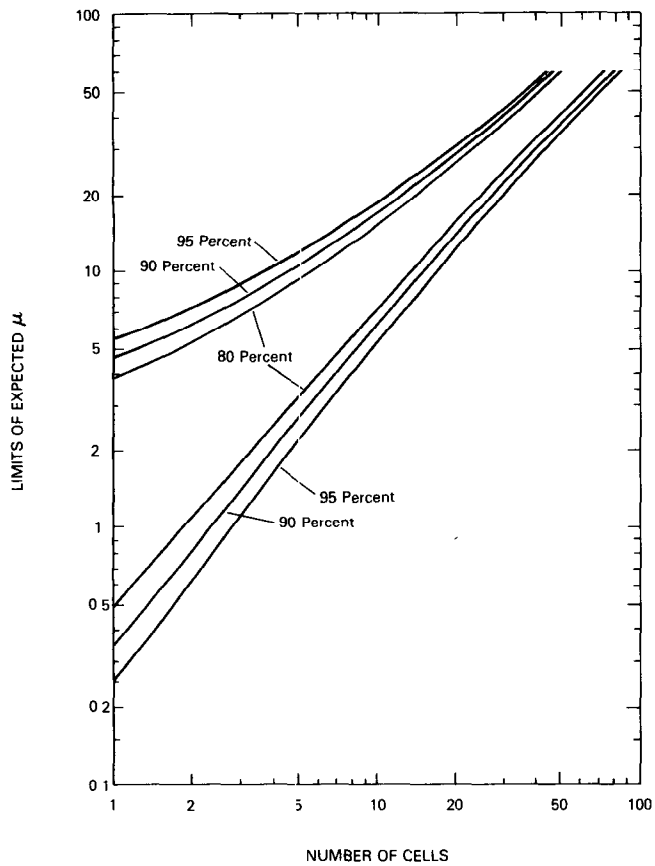

Figure 14.—Limits of expectation of phytoplankton population means, based on single estimates of abundance from a Poisson distribution, at three levels of significance: 95, 90, and 80 percent (from Sournia, 1978; reproduced by permission of UNESCO).

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Counting-cell method

(B-1505-85)

Parameter and Code:
Phytoplankton, total (cells/mL): 60050

Aliquots from phytoplankton samples that previously may have been concentrated or diluted are placed in one of four different counting cells and then examined under a conventional light microscope. Each counting cell is appropriate for a specific range of cell sizes. The Sedgwick-Rafter cell is most appropriate for enumerating macroplankton and netplankton; the Palmer-Maloney cell is appropriate for nannoplankton; and the Hemacytometer and Petroff-Hausser cells are most efficient for enumerating ultraplankton. Efficient counting schemes may require use of two different counting-cell types to ensure inclusion of both large and small phytoplankton.

The counting-cell method is one of several procedures for determining the concentration of phytoplankton. The method is performed easily and provides reasonably reproducible data when used with a calibrated microscope equipped with an eyepiece measuring device, such as the Whipple ocular micrometer (American Public Health Association and others, 1985).

The counting-cell method is much less time consuming than the membrane-filter method. The disadvantage of the method is that the Sedgwick-Rafter counting cell, for example, does not provide for use of a high-power microscope objective. However, the kinds of phytoplankton present in a sample may be determined by high-power magnification prior to using this counting cell.

The Sedgwick-Rafter cell is too thick to use with high-power microscope objectives. Observation of fine structure necessary for identification of some phytoplankton thus is not possible. Furthermore, counting of individual cells, especially filamentous species, is limited. Thinner walled counting cells, which can be used with high-power objectives, are available commercially. Most common is the biomedical hemacytometer, a single piece of thermal- and shock-resistant glass that has an H-shaped trough forming two counting areas. Raised supports hold a cover glass the proper distance above the counting areas. Most hemacytometers have a slight recession on the underside of the chamber to decrease the possibility of accidentally scratching the viewing area and have a thin, metallized deposit on the ruled area to enhance contrast. The primary disadvantage of the hemacytometer, in contrast to the Sedgwick-Rafter cell, for phytoplankton enumeration is that counts are more time consuming, and large cells are not distributed evenly.

1. Applications

The method is suitable for all water.

2. Summary of method

An aliquot of a thoroughly mixed phytoplankton sample is placed in a counting cell (chamber) and examined microscopically. The number of algal cells present in random fields is counted. The density of phytoplankton in the sample, as cells per milliliter, is calculated.

3. Interferences

The enumeration and identification of phytoplankton is impaired by large concentrations of suspended sediment or detritus that obscure micro-organisms. Previously used sample bottles and counting cells must be scrubbed thoroughly to remove adherent diatoms and other materials.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Balance*, that has an automatic tare.

4.2 *Centrifuge*, either swing-out or fixed-head cup-type, 3,000 to 4,000 r/min, 15- to 50-mL conical or 100-mL pear-shaped *centrifuge tubes* and simple *siphoning* or *suction device* to remove excess fluid after centrifugation.

4.3 *Counting cells for conventional microscope.*

4.3.1 Sedgwick-Rafter counting cell (fig. 15*A*) and cover glass, 50×20×1 mm.

4.3.2 Palmer-Maloney cell (fig. 15*B*), and 22-mm No. 1½ cover glass.

4.3.3 Hemacytometer (fig. 15*C*), 0.1 mm deep, having Improved Neubauer ruling, and cover glasses.

4.3.4 Petroff-Hausser cell (fig. 15*D*), 0.02 mm deep, having Improved Neubauer ruling.

4.4 *Microscope*, either conventional light microscope or equivalent. Bright field condensor and objectives are required, and phase contrast is desirable, particularly for taxonomic examination. A series of objectives needs to be available (10×, 20×, and 40×), and 100× phase-contrast oil-immersion objectives need to be available for examination of ultraplankton. The microscope needs to be equipped with a movable mechanical stage that has vernier scales.

4.5 *Pipet*, Pasteur, 1 mL, disposable.

4.6 *Sample containers*, glass or graduated polyethylene bottles and screwcaps, 100 mL to 1 L.

4.7 *Stage micrometer*, 2-mm scale divided into 200× 0.01-mm units mounted on 25×75-mm slide.

4.8 *Water-sampling bottle*, or *nets*. Depth-integrated samplers are discussed in Guy and Norman (1970) and in Wetzel and Likens, (1979).

4.9 *Whipple disc* placed in one ocular of the microscope.

5. Reagents

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Ethyl alcohol*, 90 percent, for cleaning counting slides.

5.5 *Formaldehyde cupric sulfate solution.* Mix 1 L 40 percent aqueous formaldehyde containing 10 to 15 percent methyl alcohol with 1 mL cupric sulfate solution.

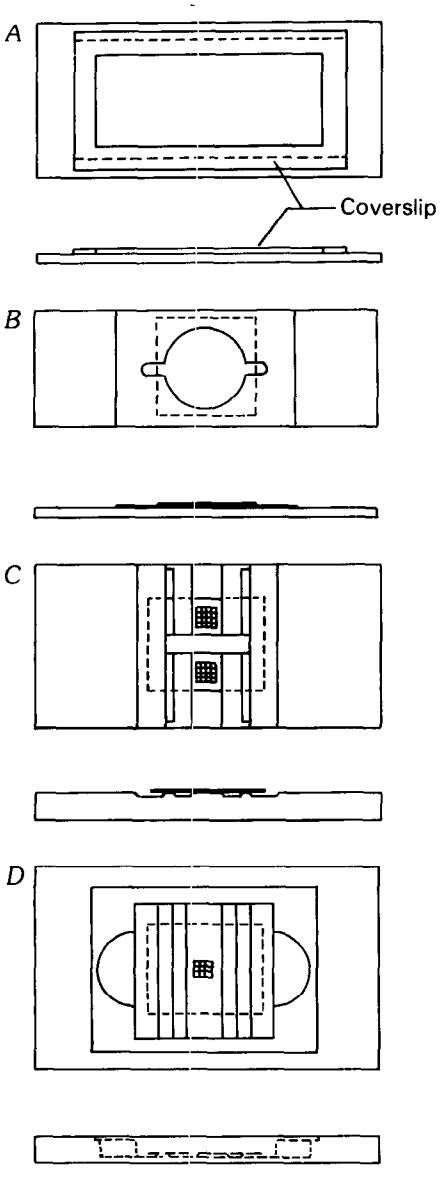

Figure 15.—Phytoplankton counting cells: (*A*) Sedgwick-Rafter; (*B*) Palmer-Maloney; (*C*) Hemacytometer; and (*D*) Petroff-Hausser (from *Sournia, 1978; reproduced by permission of* UNESCO).

5.6 *Lugol's solution plus acetic acid.* Dissolve 10 g iodine (I_2) crystals and 20 g potassium iodide (KI) in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior to use, and store in an amber glass bottle (Vollenweider, 1974).

6. Analysis

Phytoplankton samples need to be examined under two different magnifications: low power (80× to 200×) to ensure inclusion of large, usually rare, species; and high power (200× to 1,000×, using oil immersion, if possible) to facilitate identification and to ensure inclusion of ultraplankton. Phytoplankton in the entire slide mount often can be counted using low magnification, but random fields need to be selected at high magnification until a sufficient number of units (cells, filaments, chains, or colonies) have been enumerated for the desired level of precision. Use of a Whipple disc in one ocular will demarcate the microscopic field into a defined, easily viewed grid of 100 squares. When making the counts, enumerate only forms that lie completely inside the grid and those that intersect two of the outer grid borders. If a large number of colonies or filaments appear within the field, determine the average number of cells in an average-size colony or filament and multiply by the number of colonies or filaments present. Count only viable cells, those having protoplasm or pigments. Identify all forms to some predetermined taxonomic level (species level is preferred); count and describe unidentifiable cells.

The volume of original, unconcentrated sample to be examined will vary, depending on sediment content and density of phytoplankton; the volume commonly will range between 25 mL (for eutrophic water or water that has large suspended-sediment concentrations) and 100 mL (for oligotrophic water). Net samples may not require further concentration.

6.1 A variety of counting cells, as well as a conventional light microscope, have been used to enumerate phytoplankton samples. The four types described here (fig. 15) vary in the volume of sample they hold and in the depth of the sample chamber. Therefore, each is suited to a particular size and abundance of planktonic algae. The smaller cells are ruled to enable easy calculation of cell density from tallies within the chamber grid. The Sedgwick-Rafter cell (McAlice, 1971) has a rectangular chamber 1 mm deep that holds 1 mL. The Palmer-Maloney cell (Palmer and Maloney, 1954) has a circular chamber 0.4 mm deep that holds 0.1 mL. Hemacytometers, having Improved Neubauer ruling (Guillard, 1973), are 0.1 mm deep and have two counting grids composed of nine 1-mm squares (sample volume thus is 0.0018 mL). The Petroff-Hausser cell is 0.02 mm deep, has one chamber that has Improved Neubauer ruling, and holds 0.00018 mL; it is designed for cells of bacterial dimension.

6.2 If phytoplankton abundance is sufficiently great to impede enumeration, dilute samples (serially, if necessary)

using distilled water. More often, samples collected using a water-sampling bottle must be concentrated to ensure a sufficient density of phytoplankton on counting cells to enable statistically reliable estimation of population abundance. Concentrate samples by settling or centrifuging.

6.3 Allow the sample to settle in the sample container for 4 hours per centimeter of depth to be settled. After settling, weigh the sample container on an automatic tare balance.

6.4 Carefully siphon the supernatant to avoid disturbance of the settled material. Place sample container and remaining sample on balance and weigh. The decrease in weight (in grams) is equivalent to the number of milliliters of supernatant removed. Use the same method to obtain the volume of concentrate. Use centrifugation to concentrate either live or preserved samples. Using a swing-out or fixed-angle cup-type centrifuge, spin balance samples in 15- to 50-mL conical tubes at about 1,500 r/min (200 × gravity) for 20 to 30 minutes. Siphon a measured volume of supernatant and then disperse the phytoplankton concentrate in the remaining volume of water.

6.5 Use of the Sedgwick-Rafter and Palmer-Maloney cells is similar. With the counting cell on a flat surface, place a No. 1½ cover glass across the cell. Thoroughly mix the sample, remove a 1-mL (0.1 mL for Palmer-Maloney) aliquot using a large-bore Pasteur pipet and transfer the aliquot to the counting cell. Place the cover glass over the counting cell and allow the phytoplankton to settle. Carefully place the cell on the mechanical stage of a calibrated microscope, and enumerate phytoplankton as described in 6. Because neither of these counting cells is ruled, enumeration is facilitated by use of a Whipple disc.

6.6 To fill a hemacytometer, place a clean cover glass onto the counting-chamber supporting ribs. Using a smooth-tipped pipet, place a drop of homogenized sample in the V groove of the metal surface at the edge of the cover glass. The sample will be drawn rapidly into the space between the cover glass and the ruled area of the cell. Any overflow will draw phytoplankton into the moat, and the chamber will have to be cleaned and refilled. Allow phytoplankton to settle and examine the ruled counting area using low power (80× to 200×) to ensure an even distribution of phytoplankton over the grid. Count using high power (200× to 1,000×) and tally cells in a sufficient number of grid squares to ensure the desired level of precision.

6.7 Wash all counting cells using 90-percent ethyl alcohol or phosphate-free detergent and then distilled water.

7. Calculations

The following procedure will provide estimates of phytoplankton population density from tallied counts of algal cells from subsamples enumerated on microscopic slides or counting cells.

7.1 If the sample has been collected by net or if a bottle sample has been either diluted or concentrated by centrifugation-siphoning, calculate the concentration factor, c (volume of water represented by a volume of processed sample). The factor f corrects for the volume of preservative added:

$$f = \frac{\text{Volume of water collected} + \text{Volume of preservative added}}{\text{Volume of water collected}};$$

$$\text{Net sample } c = \frac{\text{Volume of water passed through the net}}{\text{Volume of preserved sample}} \times f; \text{ and}$$

$$\text{Bottle sample } c = \frac{\text{Volume of water collected}}{\text{Final volume of concentrated or diluted sample}} \times f.$$

7.2 For ruled counting cells, calculate the area, a (square millimeters), represented by one microscopic field (or Whipple disc grid) using a stage micrometer. This needs to be done for each magnification used for enumeration. For example, if enumeration is done using a Whipple disc at 125×, a=0.49 mm^2.

7.3 For unruled counting cells, calculate the area, A (square millimeters), that the sample covers on the counting cell or membrane filter. For the Sedgwick-Rafter cell, A=1,000 mm^2; for the Palmer-Maloney cell, A=250 mm^2.

7.4 Sum the total number of units, T (cells, colonies, or filaments × number of cells per colony or filament), tallied within n microscopic fields:

$$T = \sum_{i=1}^{n} x_i,$$

where x_i is total number of units counted in field i.

7.5 For unruled counting cells, calculate the total volume, v (milliliters), of the original sample represented by n microscopic fields:

$$v = c \times n \times a/A \times V,$$

where V is the volume (milliliters), of preserved sample that was settled, filtered, or placed directly into a counting cell.

7.6 For ruled counting cells (hemacytometer), calculate the total volume, v (milliliters), of original sample represented by n 1-mm squares of the hemacytometer:

$$v = c \times n \times 0.0001,$$

where the volume of sample represented by one square is 0.0001 mL.

7.7 Calculate the population density, D (cells per milliliter), of phytoplankton in the original sample:

$$D = T/v.$$

8. Reporting of results

Report phytoplankton density to two significant figures.

9. Precision

See "Precision" subsection in the "Phytoplankton" section.

10. Sources of information

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Inverted-microscope method

(B-1520-85)

Parameter and Code:
Phytoplankton, total (cells/mL): 60050

1. Applications

The method is suitable for all water.

2. Summary of method

The inverted-microscope method enables the observation of the phytoplankton in an aliquot of water at high-power magnification without disrupting or crushing delicate phytoplankton. Phytoplankton are concentrated by settling to the bottom of a vertical-tube sedimentation apparatus (Utermohl, 1958; Lovegrove, 1960; Hasle, 1978). Lund and others (1958) reported that all known phytoplankton can be settled. After settling, an aliquot of phytoplankton sample is poured into a phytoplankton counting cell or sedimentation apparatus (fig. 16). The phytoplankton settle onto a microscope cover glass that forms the bottom of the sedimentation apparatus, and the settled phytoplankton are observed from beneath, using an inverted microscope. Because this method enables use of the high-powered dry and oil-immersion objectives on the microscope, ultraplankton can be identified and enumerated.

3. Interferences

The enumeration and identification of phytoplankton is impaired by large concentrations of suspended sediment or detritus that obscure micro-organisms. Previously used sample bottles and counting cells must be scrubbed thoroughly to remove adherent diatoms and other material. Convection currents and air bubbles in the sedimentation tube can interfere with settling of phytoplankton.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Balance*, that has an automatic tare.

4.2 *Cover glass*, 22-mm diameter, No. 1 and No. 1½.

4.3 *Inverted microscope.*

4.4 *Pipet*, serological, 1 mL.

4.5 *Plankton counting cell*, 26×76-mm glass slide that has a 12-mm circular hole, covered by cementing No. 1½ cover glass to slide, and a No. 1½ cover glass for top of cell.

4.6 *Rubber cement*, for attaching cover glass to the counting cell.

4.7 *Sample containers*, glass or graduated polyethylene bottles and screwcaps, 100 mL to 1 L.

4.8 *Sedimentation apparatus*, of the type described by Lovegrove (1960) and Hasle (1978), consisting of a sedimentation tube that connects to a counting cell and a bottom cover glass (fig. 16).

4.9 *Stage micrometer*, 2-mm scale divided into 200× 0.01-mm units, mounted on 25×75-mm slide.

4.10 *Water-sampling bottle*, or *nets*. Depth-integrated samplers are discussed in Guy and Norman (1970) and in Wetzel and Likens (1979).

4.11 *Whipple disc*, placed in one ocular of the microscope.

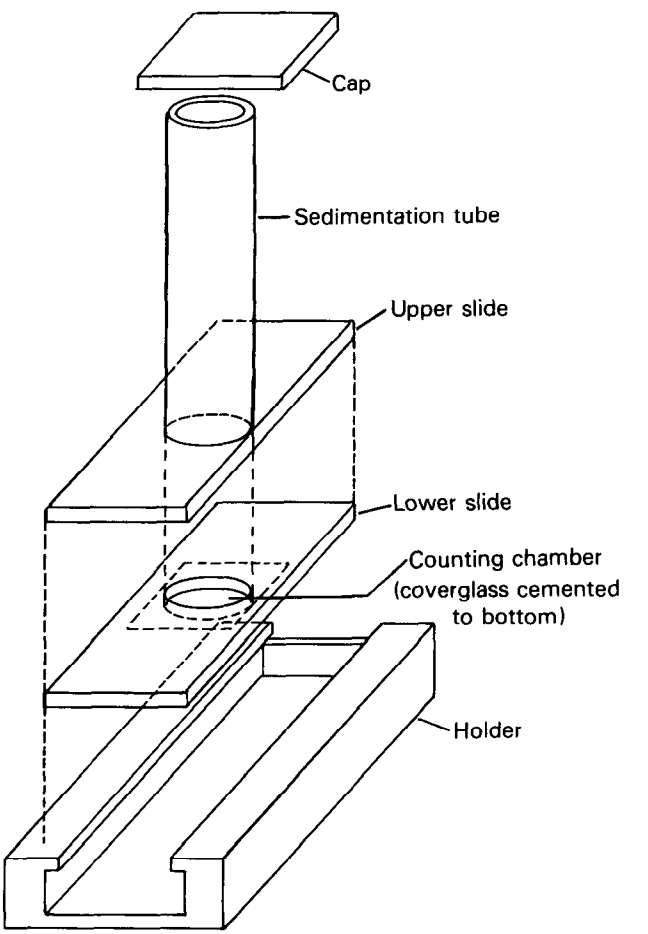

Figure 16.—Phytoplankton counting cell and sedimentation apparatus (modified from Lovegrove, 1960).

5. Reagents

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Formaldehyde cupric sulfate solution.* Mix 1 L 40 percent aqueous formaldehyde containing 10 to 15 percent methyl alcohol with 1 mL cupric sulfate solution.

5.5 *Lugol's solution plus acetic acid.* Dissolve 10 g iodine (I_2) crystals and 20 g potassium iodide (KI) in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior to use; store in an amber glass bottle (Vollenweider, 1974).

6. Analysis

Phytoplankton samples need to be examined using two different magnifications: low power (80× to 200×) to ensure inclusion of large, usually rare, species; and high power (200× to 1,000×, using oil immersion, if possible) to facilitate identification and to ensure inclusion of ultraplankton. Phytoplankton in the entire slide mount often can be counted using low magnification, but random fields need to be selected at high magnification until a sufficient number of units (cells, filaments, chains, or colonies) have been enumerated for the desired level of precision. Use of a Whipple disc in one ocular will demarcate the microscopic field into a defined, easily viewed grid of 100 squares. When making the counts, enumerate only forms that lie completely inside the grid and those that intersect two of the outer grid borders. If a large number of colonies or filaments appear within the field, determine the average number of cells in an average-size colony or filament and multiply by the number of colonies or filaments present. Count only viable cells, those having protoplasm or pigments. Identify all forms to some predetermined taxonomic level (species is preferable); count and describe unidentifiable cells.

The volume of original, unconcentrated sample to be examined will vary, depending on sediment content and density of phytoplankton; the volume commonly will range between 25 mL (for eutrophic water or water that has large suspended-sediment concentration) and 100 mL or more (for oligotrophic water). Net samples may not require further concentration.

6.1 If using the sedimentation apparatus (fig. 16), proceed to 6.5. If using the plankton counting cell, proceed as follows. If concentration is necessary, allow the sample to settle undisturbed in the sample container for 4 hours per centimeter of depth to be settled. After settling, weigh the sample container on an automatic tare balance.

6.2 Carefully siphon the supernatant to avoid disturbance of the settled material. Place sample container and remaining sample on the balance and weigh. The decrease in weight (in grams) is equivalent to the number of milliliters of supernatant removed. Use the same method to obtain the volume of concentrate.

6.3 Mix the concentrated sample well (but not vigorously) and pipet an appropriate volume into each of two plankton counting cells. Slide cover glass into place.

6.4 Place the plankton counting cell on the mechanical stage of a calibrated microscope. Proceed to 6.10.

6.5 To prepare the sedimentation apparatus, cement a No. 1 cover glass to the bottom of the lower slide to form the bottom of the counting cell (fig. 16). When dry, remove the excess rubber cement from the inside of the counting cell using a knife.

6.6 Test for leaks. Coat the underside of the upper slide (fig. 16) with vacuum grease and press onto the lower slide to form a watertight seal. Assemble the sedimentation apparatus and fill with distilled water so the meniscus bulges slightly above the top of the sedimentation tube. Slide the cap over the top to seal the tube. Let stand overnight and check for water loss in the morning.

6.7 If no leaks are detected, thoroughly mix a sample by inverting it at least 40 times, and then fill the sedimentation apparatus and apply the cap as described in 6.6 (Note 1). Allow 4 hours settling time per 1 cm of sedimentation-tube length. The volume of sample is dependent on the density of phytoplankton. In oligotrophic water, 100 mL or more of sample may be required; in eutrophic water, 25 mL or less of sample may be sufficient. The 25-mL volume is most commonly used. Dilute the samples if necessary.

Note 1: Air bubbles on the sides of the sedimentation tube can be prevented if the water sample and the sedimentation apparatus are at the same temperature when the sample is added. The apparatus needs to be maintained at a constant temperature to avoid convection currents, which can interfere with settling.

6.8 After settling, isolate the phytoplankton in the counting cell from the remainder of the sedimentation apparatus. To separate the sedimentation tube and upper slide from the lower slide and counting cell (fig. 16), move the sedimentation tube to one side, dividing the water column. Remove the tube cap and siphon or pipet off the supernatant. Remove the empty sedimentation tube.

6.9 Remove the lower slide that has the counting cell from the holder (fig. 16). Place the cap over the top of the counting cell to form a closed cell. If an air bubble remains under the cap, move it to one side of the cell and carefully add distilled water to fill the void. Replace the tube cap and put the slide on the inverted microscope.

6.10 Three basic procedures exist for microscopically enumerating and identifying concentrated phytoplankton samples. Although specific materials and methods vary between these procedures, the general counting procedure is identical.

7. Calculations

The following procedure will provide estimates of phytoplankton population density from tallied counts of phytoplankton cells from subsamples enumerated on microscopic slides or counting cells.

7.1 If the sample has been collected by net or if a bottle

sample has been either diluted or concentrated by centrifugation-siphoning, calculate the concentration factor, c (volume of water represented by a volume of processed sample). The factor f corrects for the volume of preservative added:

$$f = \frac{\text{Volume of water collected} + \text{Volume of preservative added}}{\text{Volume of water collected}};$$

$$\text{Net sample } c = \frac{\text{Volume of water passed through the net}}{\text{Volume of preserved sample}} \times f; \text{ and}$$

$$\text{Bottle sample } c = \frac{\text{Volume of water collected}}{\text{Final volume of concentrated or diluted sample}} \times f.$$

7.2 For ruled counting cells, calculate the area, a (square millimeters), represented by one microscopic field (or Whipple disc grid) using a stage micrometer. This needs to be done for each magnification used for enumeration. For example, if enumeration is done using a Whipple disc at 125×, $a=0.49$ mm^2.

7.3 For inverted-microscope counting cells that have a bottom plate that has a diameter of 25 mm, the area is $A=491$ mm^2.

7.4 Sum the total number of units, T (cells, colonies, or filaments × number of cells per colony or filament), tallied within n microscopic fields:

$$T=\sum_{i=1}^{n} x_i,$$

where x_i is total number of units counted in field i.

7.5 For unruled counting cells, calculate the total volume, v (milliliters), of the original sample represented by n microscopic fields:

$$v=c\times n\times a/A\times V,$$

where V is the volume (milliliters), of preserved sample that was settled, filtered, or placed directly into a counting cell.

7.6 Calculate the population density, D (cells per milliliter), of phytoplankton in the original sample:

$$D=T/v.$$

8. Reporting of results

Report phytoplankton density to two significant figures.

9. Precision

See "Precision" subsection in the "Phytoplankton" section.

10. Sources of information

Guy, H.P. , and Norman, V.W., 1970, Field methods for measurement of fluvial sediment: U.S. Geological Survey Techniques of Water-Resources Investigations, bk. 3, chap. C2, 59 p.

Hasle, G.R., 1978, The inverted-microscope method, *in* Sournia, Alain, ed., Phytoplankton manual: Paris, UNESCO, Monographs on Oceanographic Methodology 6, p. 88-96.

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Wetzel, R.G., and Likens, G.E., 1979, Limnological analyses: Philadelphia, W.B. Saunders, 357 p.

Permanent-slide method for planktonic diatoms

(B-1580-85)

Parameter and Code: Not applicable

This method enables preparation of permanent mounts using a minimum of time and equipment. Numerous alternative methods for clearing diatom frustules (cell walls) and mounting exist in the literature. Alternative methods for clearing include nitric acid digestion of tissue on the slide (Knudsen, 1966), sulfuric acid and potassium permanganate (Hasle and Fryxell, 1970), hydrochloric acid (HCl) (Cupp, 1943), and potassium permanganate and HCl (Hasle, 1978). Hydrogen peroxide and potassium permanganate (Von der Werff, 1953), hydrogen peroxide and ultraviolet light (Swift, 1967), and hydrogen peroxide after mild heating (Wong, 1975) also have been used for tissue digestion. The reader is referred to the original papers for the details of these procedures.

1. Applications

This qualitative method is suitable for all water. Advantages of the method are that a permanent mount is prepared, and clearing of the cells enhances observation of frustule detail. The method, therefore, is important in the taxonomic study of diatoms.

2. Summary of method

The diatoms in a sample are concentrated, the cells are cleared, and a permanent mount is prepared. The mount is examined microscopically, and the number of diatom taxa is calculated from strip counts.

3. Interferences

3.1 Inorganic particulate matter, including salt crystals, interferes with mount preparation but can be decreased by sample washing.

3.2 The method does not distinguish living from dead diatoms.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Balance*, that has an automatic tare.

4.2 *Centrifuge*, either swing-out or fixed-head cup-type, 3,000 to 4,000 r/min, 15- to 50-mL conical or 100-mL pear-shaped *centrifuge tubes*, and simple *siphoning* or *suction device* to remove excess fluid after centrifugation.

4.3 *Cover glasses*, 18×18 or 22×22 mm, No. 1½, and *microscope slides*, glass, 76×22 mm.

4.4 *Forceps*, curved tip.

4.5 *Graduated cylinders*, plastic, of sufficient capacity (100 and 500 mL, and 1 L are convenient sizes) for measuring known volumes of water samples.

4.6 *Hotplate*, thermostatically controlled to 538 °C. It is convenient to have a second hotplate for operation at about 93 to 121 °C as described in 6.8.

4.7 *Microscope*, conventional light microscope, or equivalent. Bright field condensor and objectives are required, and phase contrast is desirable, particularly for taxonomic examination. A series of objectives needs to be available (10×, 20×, and 40×), and 100× phase-contrast oil-immersion objectives need to be available for examination of smaller sized diatoms. The microscope needs to be equipped with a movable mechanical stage that has vernier scales.

4.8 *Pipets*, 1-mL or 10-mL capacity, sterile.

4.9 *Sample containers*, glass or graduated polyethylene bottles and screwcaps, 100 mL to 1 L.

4.10 *Water-sampling bottle*, or *nets*. Depth-integrated samplers are discussed in Guy and Norman (1970) and in Wetzel and Likens (1979).

4.11 *Whipple disc*, placed in one ocular of the microscope.

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Cupric sulfate solution*, saturated. Dissolve 21 g cupric sulfate ($CuSO_4$) in 100 mL distilled water.

5.2 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.3 *Distilled or deionized water.*

5.4 *Formaldehyde cupric sulfate solution*. Mix 1 L 40 percent aqueous formaldehyde containing 10 to 15 percent methyl alcohol with 1 mL cupric sulfate solution.

5.5 *Immersion oil*. Cargille's nondrying type A.

5.6 *Lugol's solution plus acetic acid*. Dissolve 10 g iodine (I_2) crystals and 20 g potassium iodide (KI) in 200 mL distilled water. Add 20 mL glacial acetic acid a few days prior to use; store in an amber glass bottle (Vollenweider, 1974).

5.7 *Mounting medium* (table 13). Generally, mounting media should have a refractive index different than that of diatom frustules. Diatom frustules have a refractive index of approximately 1.15 (Reid, 1978).

6. Analysis

6.1 If the sample contains great numbers of phytoplankton, as typically occurs in eutrophic water, dilute the sample. To dilute, thoroughly mix 50 mL sample with 50 mL distilled water (1:1 dilution) and proceed to 6.2. If microscopic examination reveals a concentration of phytoplankton still too numerous to count, thoroughly mix 50 mL 1:1 dilution with

Table 13.—*Synthetic mounting media in general use for permanent mount of planktonic diatoms*

[Adapted from Reid, 1978; reproduced by permission of UNESCO; --, not available]

Media	Refractive index, n	Solvent	Other information
Aroclor	1.63	Xylene.	Good for diatoms.
Clearax	1.67	Xylene, acetone.	Good for diatoms.
Clearmount	1.51	Xylene, benzene, toluene, alcohol, dioxan, and other solvents.	Conserves stains.
Euparal	1.48	Xylene, alcohol.	Mixture of natural and synthetic resins; can be used immediately after 95-percent alcohol application; intensifies hematoxylin stains.
Hyrax	1.63	Xylene, benzene, toluene.	Expensive; good for diatoms (Hanna, 1930).
Naphrax	1.72	Xylene, toluene, acetone.	Good for diatoms (Fleming, 1943, 1954).
Permount	--	Toluene.	Conserves stains: does not yellow.
Pleurax	1.75	Alcohol.	Good for delicate diatoms. Procedure for mixing in Hanna (1949).

50 mL distilled water (1:4 dilution). Make additional dilutions as appropriate.

6.2 If concentration is necessary, allow the sample to settle undisturbed in the sample container for 4 hours per centimeter of depth to be settled. After settling, weigh the sample container on an automatic tare balance.

6.3 Carefully siphon the supernatant to avoid disturbance of the settled material. Place sample container and remaining sample on balance and weigh. The decrease in weight (in grams) is equivalent to the number of milliliters of supernatant removed. Use the same method to obtain the volume of concentrate.

6.4 If the sample was collected from seawater or saline lakes, wash the sample, using distilled water, at least three times to ensure that the permanent mounts are not obscured by salt crystals. Add about 10 mL distilled water to the concentrate in the centrifuge tube, gently shake the tube to suspend the residue, fill the tube with distilled water, and centrifuge for 20 minutes. Decant the supernatant fluid and repeat the washing process two more times.

6.5 Place two or three drops of the concentrate on each of three or four cover glasses.

6.6 With the concentrate side up, place the cover glass on a hotplate and heat, slowly at first to prevent splattering, to about 538 °C (a higher temperature will melt diatom valves) for 30 minutes.

6.7 Remove cover glass from the hotplate and cool.

6.8 Place a drop of mounting medium (table 13) on a microscope slide and heat at about 93 to 121 °C for 3 to 4 minutes.

6.9 Invert the cover glass, concentrate side down, on the heated medium. Apply slight pressure to the cover glass (for example, with a pencil eraser) until visible air bubbles disappear. Remove slide from hotplate and allow to cool. If bubbles still are present under the cover glass, heat the slide and gently apply additional pressure to the cover glass. Label slide to identify sample.

6.10 Examine the slide using the 1,000× objective (oil immersion). Count and identify diatom taxa found in several lateral strips the width of the Whipple disc. *Identify and*

tabulate 200 to 300 diatom cells, if possible. Generally, at least 100 individuals of the most common species should be enumerated. Ignore frustule fragments. Thin-walled forms, such as *Rhizosolenia eriensis* and *Melosira crenulata*, may be difficult to observe when using this method (Weber, 1966, p. 3). If a microscope that has a mechanical stage is used, recording of the x and y coordinates of lateral strips or individual cells enables investigators to later recheck and verify identification (Wong, 1975).

7. Calculations

$$\text{Percent occurrence of each species} = \frac{\text{Number of diatoms of a given species}}{\text{Total number of diatoms tabulated}} \times 100.$$

8. Reporting of results

Report percentage composition of diatoms to the nearest whole number. Report taxa and number of diatoms per taxa.

9. Precision

No numerical precision data are available.

10. Sources of information

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Wetzel, R.G., and Likens, G.E., 1979, Limnological analyses: Philadelphia, W.B. Saunders, 357 p.

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ZOOPLANKTON

Introduction

The zooplankton are the animal part of the plankton. In general, they predominantly are composed of free-living, nonphotosynthetic protozoa, rotatoria, and crustacea. They are found in a variety of aquatic habitats, although usually they are absent or occur in small numbers in rapid streams. Zooplankton are important contributors to aquatic ecosystem metabolism because they are grazers of phytoplankton and bacteria and are important predators. Fish and certain invertebrate groups also use zooplankton as a food source. Zooplankton, therefore, can have a substantial effect on the structure and functioning of aquatic ecosystems.

Zooplankton characteristically have patchy distributions in aquatic ecosystems. They are rarely distributed randomly or uniformly. Additionally, vertical differences in zooplankton abundance on a daily and seasonal basis commonly are observed and are caused by the diurnal vertical migration of certain groups of zooplankton in response to changes in illumination. The fact that zooplankton are heterogeneous in their areal and vertical distribution must be considered in any investigation of the zooplankton. No single method of sampling can sample conclusively and accurately the entire zooplankton community.

Collection

There are several methods available for the collection of zooplankton. These methods are grouped into two categories based, in part, on the size of the zooplankton being collected. Zooplankton smaller than 200 μm are considered microzooplankton; this includes protozoa and small rotifers (Tranter and Fraser, 1968; Tonolli, 1971). They are readily collected by water-sampling bottles, water cores or tubes, and water pumps, followed by concentration of the sample onsite or in the laboratory. Collection also is facilitated by the use of plankton traps. Larger zooplankton, including the crustacea and larger rotifers, can be collected using various equipment that filter the zooplankton from the water through a net (Tonolli, 1971). These devices include unmetered tow nets (Wisconsin- or Birge-type), metered tow nets (Clarke-Bumpus sampler), and plankton traps (Schindler-Patalas trap).

There are several types of net mesh and sizes available for use in net sampling devices. The choice of mesh size and net design depends on the abundance of the zooplankton and the towing speed of the net. Nets of 202-μm mesh generally are used during U.S. Geological Survey studies. Smaller net sizes can be used for the purpose of collecting microzooplankton; however, clogging becomes an important factor using mesh sizes less than 65 μm (Steedman, 1976). Although the collector need not be restricted to the 202-μm mesh size, the mesh size used needs to be reported when presenting zooplankton results.

Detailed collection methods are discussed in Tranter and Fraser (1968), Schwoerbel (1970, p. 37-52), Edmondson and Winberg (1971, p. 1-20), Lind (1979, p. 100-115), and Wetzel and Likens (1979, p. 161-166). The study objectives need to be considered when selecting appropriate methods of collection. However, to ensure maximum correlation of results, the sample sites and methods used for zooplankton need to correspond as closely as possible to those selected for other biological, microbiological, and chemical sampling.

Water-sampling bottles can be used to collect a sample representative of the zooplankton density at a particular depth in ponds, lakes, reservoirs, estuaries, and deep rivers. This method is appropriate for collection when information on the vertical distribution of all zooplankton (including microzooplankton) is required. Water-sampling bottles, which enable collection, cause minimal disturbance of water passage into the bottle, and minimize avoidance reactions by the zooplankton, are desirable (Tonolli, 1971). Van Dorn-type water-sampling bottles, or equivalent, are an adequate collection device for zooplankton.

Depth-integrating samplers are used to collect a sample representative of the entire flow of a stream (Guy and Norman, 1970; Goerlitz and Brown, 1972). For small streams, a depth-integrated sample, or a point sample, at a single transverse position located at the centroid of flow may be adequate. Depth-integrating samplers are discussed in Guy and Norman (1970).

Following collection, the contents from the water-sampling bottle or depth-integrating samplers are poured through an appropriate monofilament screen cloth (202 μm could be used, but it will enable microzooplankton to pass through), which retains the zooplankton for identification and enumeration or for biomass determinations. The advantage of water-sampling-bottle collection is negated by filtering the zooplankton through an inappropriate screen cloth that damages them or through a mesh size that lets microzooplankton pass through (Tonolli, 1971).

A sampling tube or water core can be used when information about the vertical distribution of all zooplankton (including microzooplankton) is not required. One limitation of this method is that good swimmers can avoid capture. This

collection device consists of a weighted thin-walled rubber or plastic tube, having a closing device for collection of a relatively large vertical column of water and its associated zooplankton.

To collect a sample, the flexible tube is lowered to the desired depth. The sampling core is retrieved by pulling on a rope that is connected between two rings about 10 cm apart at the base of the tube. Pulling on the rope closes the tube. The advantage of this method is that the entire water column can be sampled using a relatively simple device (Tonolli, 1971, p. 4). Following collection, the contents are filtered through an appropriate mesh-size monofilament screen cloth (less than or equal to 202 μm), which retains the zooplankton for identification and enumeration or for biomass determination.

The advantage of the water-pump method is that it easily collects large volumes of water from various depths. However, the problem of avoidance by larger zooplankton may be encountered (Tonolli, 1971). A hand pump or electric pump is attached to a relatively large diameter tube, which in turn is weighted at the bottom. The tube is lowered to a preselected depth and flushed with a volume of water three times the tube's volume to eliminate water that entered the tube during lowering. A known quantity of water then is pumped and filtered through an appropriate mesh-size monofilament screen cloth (less than or equal to 202 μm), which retains the zooplankton for identification and enumeration or for biomass determination.

Unmetered plankton nets are useful in qualitative investigations of the zooplankton when complete quantitative data are not required. It is a fairly simple technique that permits relative comparisons of zooplankton communities (Tonolli, 1971). The entire water column is sampled easily by using plankton nets in vertical hauls. Wisconsin-type (open) (fig. 17*A*) and Birge-type (closed) (fig. 17*B*) plankton nets are examples of the nets suitable for this method. The zooplankton are collected by lowering the net to a known depth and raising it at a constant speed to the surface. Wisconsin-type plankton nets may become clogged and lose sampling efficiency during long retrieval. Birge-type plankton nets that can be closed at a preselected depth by dropping a messenger are advantageous for these conditions. In general, a large ratio of filtering surface to mouth-opening area decreases clogging. Therefore, long nets are more efficient than short nets. After retrieval, the filtering cone then is cleared of zooplankton by rapidly lowering and raising the net in the water, without submerging the net opening, and then bringing the net completely out of the water. Alternatively, the filtering cone of the plankton net can be cleared by repeated washing using water. These procedures concentrate the zooplankton in the removable plankton bucket, located at the bottom of the net. The zooplankton are washed from the plankton bucket into a sample container for identification and enumeration or filtered through an appropriate mesh-size monofilament screen cloth for biomass determination.

The volume of water (V) filtered through the Wisconsin- and Birge-type nets is calculated as $V=\pi r^2 d$, where r=radius of the mouth of the net and d=tow length through the water column (entire length of tow for the Wisconsin-type net and length of tow before closing for the Birge-type net). This assumes that the filtering efficiency of the net is 100 percent. The actual efficiency of the net generally will be less than 100 percent (Tonolli, 1971).

The Clarke-Bumpus plankton sampler is a metered tow net that enables quantitative sampling of the zooplankton in either horizontal or vertical tows (fig. 17*C*). This device consists of a net and flowmeter mounted on a horizontal frame. The net is opened and closed using a messenger. By knowing the initial and final reading on the counter of the flowmeter, the volume of water that has passed through the net can be determined (Schwoerbel, 1970, p. 45; Tonolli, 1971, p. 6-12). Thus, the Clarke-Bumpus plankton sampler has an advantage over the Wisconsin-type net or Birge-type net, because the exact volume of water passing through the net is known. However, clogging can become important when samples are collected from water that has dense zooplankton populations, because of the large volumes filtered by the Clarke-Bumpus plankton sampler (Tonolli, 1971; Wetzel and Likens, 1979).

When collecting a sample, the initial reading of the flowmeter is recorded. The sampler is lowered to the selected depth, and the net is opened by dropping a messenger. After towing the sampler for a known interval of time or distance, the net is closed using another messenger, and the net is retrieved. The final reading on the flowmeter then is recorded. The net is washed, and the zooplankton are concentrated into the removable bucket. The zooplankton then are washed from the plankton bucket into a sample container for identification and enumeration or filtered through an appropriate mesh-size monofilament screen cloth for biomass determination.

For horizontal hauls, a moving boat is required. Also, a clinometer and cable depressor are necessary to ensure that the haul is collected at a known depth. Further detailed discussion of the use of this device is presented by Tonolli (1971).

Plankton traps are used for point sample collection of the water column when information about the vertical distribution of the zooplankton is required. This method is suitable for capture of microzooplankton and larger zooplankton. There are two basic types of plankton traps, those requiring a messenger for closing [Juday trap, (fig. 17*D*)] (Juday, 1916) and one that does not [Schindler-Patalas trap (fig. 17*E*)] (Schindler, 1969). The Juday trap is lowered to a predetermined depth and closed by a messenger. The trap then is retrieved, and the water drains through an attached plankton bucket, concentrating the zooplankton. The Schindler-Patalas trap, constructed using transparent

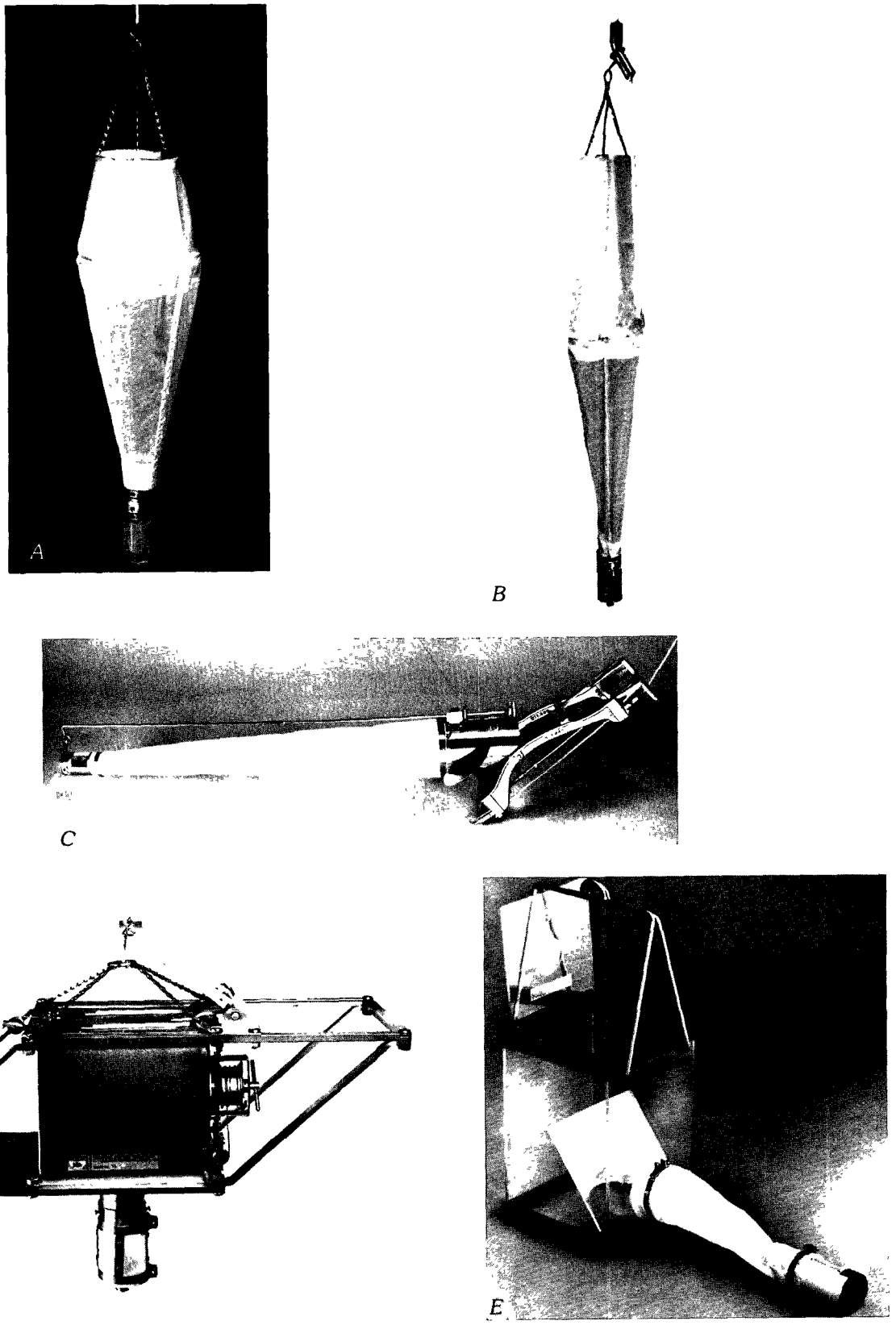

Figure 17.—Zooplankton collecting devices: (*A*) Wisconsin-type (open) plankton net; (*B*) Birge-type (closed) plankton net; (C) Clarke-Bumpus plankton sampler; (*D*) Juday plankton trap; (*E*) Schindler-Patalas plankton trap. (Photographs courtesy of Wildlife Supply Co., Saginaw, Mich.)

Plexiglas, has two swinging lids that facilitate collection by lowering to a predetermined depth and then raising the trap to the water surface. A mesh-covered hole in the top lid enables the contents of the trap to be filtered through the attached net. The contents of the net are washed readily into the detachable plankton bucket (Schindler, 1969). Once the zooplankton have been concentrated in the plankton bucket of either the Juday trap or the Schindler-Patalas trap, the zooplankton are washed into a sample container for identification and enumeration or filtered through a 202-μm (or less, to include the microzooplankton) mesh-size monofilament screen for biomass determination. The advantages of the Schindler-Patalas trap are that it does not have a messenger activated tripping system, filtering occurs during raising, and it is less subject to the avoidance reactions by zooplankton encountered using water-bottle samplers, tow nets, and metal traps because it is transparent.

Samples collected for biomass determination on mesh-size monofilament screen cloth are handled as follows. Wash the screen cloth containing the zooplankton by dipping in distilled water several times, place in a plastic bag or other suitable sample container, and preserve onsite by freezing using dry ice. Keep frozen until gravimetric determinations can be made (Committee on Oceanography, Biological Methods Panel, 1969, p. 57). Additional information about sample preparation onsite prior to biomass determination is presented in Beers (1976, p. 74-76).

Samples collected for identification and enumeration are narcotized using an appropriate agent. A simple method is the addition of a commercial soda water (10-15 percent of total sample volume) to the sample, resulting in carbon dioxide excess. Narcotization prevents contraction and distortion of the zooplankton when fixed by use of a preservative that enables ready identification in the preserved state (Steedman, 1976). Following narcotization, preserve the samples by using neutralized formaldehyde (approximately 2–4 percent of total sample volume) solution (5 percent formalin). Add several drops of glycerin (approximately 5 percent of total sample volume) to the sample to prevent drying during storage. If samples collected for biomass determination cannot be kept frozen, preserve using 2 percent neutralized formaldehyde solution, but use the selected sample-preservation method consistently throughout the study.

For identification and enumeration and for biomass determinations, label the sample to indicate the volume of water filtered or to indicate the information needed to determine the volume. For example, record the length of a vertical net haul and the diameter of the net opening. Also, the date and site location should be included, the order of collection when replicate sampling is used, and collection device and mesh size of any screen cloth used.

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Counting-cell method

(B-2501-85)

Parameter and Code:
Zooplankton, total (organisms/m^3): 70946

1. Applications

The method is suitable for all water.

2. Summary of method

Samples of the zooplankton community are collected, preserved, and examined microscopically for numbers and types of zooplankton per unit volume of water sampled.

3. Interferences

Suspended materials in the water and abundant algae may interfere with the collection and microscopic examination of zooplankton.

4. Apparatus

Methods and equipment for the collection of zooplankton and their examination for identification and enumeration are described briefly in this section and are described in more detail in Welch (1948), Tranter and Fraser (1968), Schwoerbel (1970), Edmondson and Winberg (1971), Steedman (1976), Lind (1979), Wetzel and Likens (1979), and American Public Health Association and others (1985). Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Beaker*, 250-mL capacity, for use as a mixing vessel for zooplankton samples.

4.2 *Clarke-Bumpus plankton sampler* that has 202-μm mesh netting. An impeller at the net opening registers the volume of water filtered through the net. The Clarke-Bumpus plankton sampler is used most often for horizontal tows, but it also may be used for vertical tows (fig. 17*B*).

4.3 *Counting cells*. A petri dish, half, that has etched grid on the bottom, is a convenient open counting cell. The construction of large-volume counting cells is discussed in Edmondson (1971, p. 131). *Open counting cells* are used for counting subsample aliquots larger than 1 mL. *Closed counting cells* are used for smaller subsamples. *Sedgwick-Rafter counting cells*, 50×20×1 mm and cover glass are used in counting small samples. Small organisms (less than 10 μm) are identified more easily and counted using thinner counting cells, such as the Palmer-Maloney cell or standard medical hemacytometer (Edmondson, 1971).

4.4 *Graduated cylinders*, plastic, of sufficient capacity (100 and 500 mL and 1 L are convenient sizes) for measuring known volumes of water samples.

4.5 *Microscope*, binocular, flat-field, zoom lens, and illuminator for the smaller zooplankton. For the larger zooplankton, a binocular wide-field dissecting microscope is adequate.

4.6 *Nylon monofilament screen cloth*, 202-μm mesh opening.

4.7 *Piston or Hensen-Stempel pipet*, 4-mm diameter or 5-mL capacity, for obtaining subsamples from zooplankton samples. A 1-mL Hensen-Stempel pipet is convenient for use with Sedgwick-Rafter counting cells.

4.8 *Plankton nets*, Wisconsin-type, open, or Birge-type, closing. The closing plankton nets have greater sampling flexibility in deep-water bodies because they can be closed at any selected depth (fig. 17*A*).

4.9 *Plankton trap* (Juday type), a 10-L closing box, attached plankton bucket that has 202-μm mesh openings and that has messenger closing (fig. 17*C*), or transparent Plexiglas type that does not require messenger closing [Schindler-Patalas type (fig. 17*D*)].

4.10 *Sample containers*, glass or plastic bottles, vials, or sealable plastic bags. However, bags are subject to leakage during prolonged storage.

4.11 *Sampling tube* or water core, a weighted thin-walled rubber or plastic tube that has a closing device for collecting a relatively large vertical column of water and its associated zooplankton (Edmondson and Winberg, 1971, p. 4).

4.12 *Spatula*, for stirring samples.

4.13 *Water pump*, and attached rubber or plastic hose. Water is pumped through a net having a mesh size of 202 μm to retain the zooplankton (Committee on Oceanography, Biological Methods Panel, 1969, p. 48).

4.14 *Water-sampling bottle*, Van-Dorn type. Depth-integrating samplers are described in Guy and Norman (1970).

4.15 *Whipple disc*, placed in one ocular of the microscope.

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Detergent solution*, 20 percent. Dilute 20 mL liquid detergent, phosphate free, to 100 mL using distilled water.

5.2 *Distilled or deionized water.*

5.3 *Formaldehyde solution*, 2 percent. Dilute 5 mL 37 to 40 percent aqueous formaldehyde solution (formalin) to 100 mL using distilled water (Note 1).

Note 1: Commercial formaldehyde solution is slightly acid and may be neutralized by maintaining a small deposit of sodium or calcium carbonate in the stock bottle.

5.4 *Glycerin*, used to prevent drying of stored zooplankton samples.

5.5 *Narcotizing agent* (soda water, Schweppes, Canada Dry, or equivalent).

6. Analysis

6.1 Empty the contents of the entire sample into a graduated cylinder and adjust the volume to some convenient value, such as 50, 100, or 200±5 mL, by adding preservative solution. Because of the difficulty in examining the zooplankton in formalin preservative, tap water also can be used.

6.2 Pour the suspension in the graduated cylinder into an appropriate size beaker. Stir the contents of the beaker irregularly, using a spatula to produce a random distribution of the zooplankton in the beaker. Take a subsample from the beaker for counting.

6.3 Count the zooplankton as in 6.4 or 6.5. Use the taxonomic keys in Edmondson (1959), Needham and Needham (1962), and Pennak (1978) to identify the different taxa of zooplankton for qualitative analysis and for the calculations of percent species composition.

6.4 *Closed counting-cell method—Sedgwick-Rafter method.*

6.4.1 With the counting cell on a flat surface, place the cover glass across the cell. Take a subsample as described in 6.2 by removing a 1-mL aliquot using a Hensen-Stempel pipet and transfer the aliquot to the cell. As the cell fills, the cover glass often will rotate slowly and cover the inner part of the cell, but the cover glass must not float above the rim of the cell. Allow the cell to stand for 15 to 20 minutes so the contents will settle.

6.4.2 Carefully place the counting cell on the mechanical stage of a microscope calibrated using a Whipple disc. Count the entire contents of the cell at 100 × magnification. Alternatively, count several horizontal transects where the percent of the total contents of the cell is determined by the use of the Whipple disc. Count at least two subsamples from the beaker using the cell. The Sedgwick-Rafter method is not suitable for some large zooplankton because they do not fit in the cell under a cover glass.

6.5 *Open counting-cell method.* In this method, the entire contents from the beaker are counted. Using the etched or painted guidelines on the bottom of the Sedgwick-Rafter counting cell, count the zooplankton in random sections to determine an average density. A binocular wide-field dissecting microscope is adequate to count the zooplankton. Take care not to disturb the placement of the zooplankton in the open cell when counting, or the counting process will have to be started again. Several drops of liquid detergent can be added to the open-cell subsample to decrease surface tension and prevent floating of the zooplankton on the surface. The open counting-cell method enables easy access to the subsample contents to enable manipulation of individual zooplankton for easier identification or removal for closer examination using a binocular flatfield microscope.

6.6 If the sample is to be retained, proceed as follows: After counting of the sample has been completed, return all of the sample to the beaker and allow to settle overnight. Remove enough of the supernatant liquid to enable the return of the sample contents to the original sample container. Add preservative to ensure the integrity of the sample.

7. Calculations

7.1 Sedgwick-Rafter method:

$$\text{Total zooplankton per cubic meter} = \frac{\text{Zooplankton per cell} \times \text{Volume of sample (milliliters)}}{\text{Volume of water sampled (liters)}} \times \frac{1{,}000\ \text{L}}{\text{Cubic meters}}.$$

7.2 Open counting-cell method, section counts:

$$\text{Total zooplankton per cubic meter} = \frac{\text{Average count per section} \times \text{Number of sections} \times \text{Total volume of concentrated sample (milliliters)}}{\text{Volume of counting cell (milliliters)} \times \text{Volume of water sampled (liters)}} \times \frac{1{,}000\ \text{L}}{\text{Cubic meters}}.$$

7.3 Percent taxon composition in sample

$$= \frac{\text{Number of zooplankton of a particular taxon}}{\text{Total number of zooplankton of all taxa}} \times 100.$$

8. Reporting of results

Report zooplankton densities as total number of organisms per cubic meter to two significant figures.

9. Precision

No numerical precision data are available.

10. Sources of information

American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1985, Standard methods for the examination of water and wastewater (16th ed.): Washington, D.C., American Public Health Association, 1,268 p.

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Gravimetric method for biomass

(B-2520-85)

Parameters and Codes:
Zooplankton, dry weight (g/m^3): 70947
Zooplankton, ash weight (g/m^3): 70948

1. Applications

The method is suitable for all water.

2. Summary of method

Samples of the zooplankton community are collected from known volumes of water. The dry weight and ash weight are determined, and the weight of ash-free matter, an estimate of organic weight per unit volume of the water sampled, is calculated.

3. Interferences

Suspended materials in the water may interfere with sample collection. Inorganic matter in the sample will cause erroneously large dry and ash weights. Nonliving organic matter, as well as living plant and bacteria material, in the sample will cause erroneously large dry and ash-free weights.

4. Apparatus

Methods and equipment for the collection of zooplankton for biomass determination have been described in the "Collection" subsection of the "Zooplankton" section and are presented in more detail in Tranter and Fraser (1968), Schwoerbel (1970), Steedman (1976), Wetzel and Likens (1979), and American Public Health Association and others (1985). Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Balance*, capable of weighing to at least 0.1 mg.

4.2 *Beaker*, 250-mL capacity, for use as a mixing vessel for zooplankton samples.

4.3 *Clarke-Bumpus plankton sampler* that has 202-μm mesh netting. An impeller at the net opening registers the volume of water filtered through the net. The Clarke-Bumpus plankton sampler is used most often for horizontal tows, but it also may be used for vertical tows (fig. 17*B*).

4.4 *Desiccator*, containing silica gel or anhydrous calcium sulfate.

4.5 *Drying oven*, thermostatically controlled for use at 105 °C.

4.6 *Forceps*, stainless steel, smooth tip, or tongs.

4.7 *Graduated cylinders*, plastic, of sufficient capacity (100 and 500 mL and 1 L are convenient sizes) for measuring known volumes of water samples.

4.8 *Muffle furnace*, for use at 500 °C.

4.9 *Nylon monofilament screen cloth*, 202-μm (or appropriate size for collecting microzooplankton) mesh opening.

4.10 *Piston or Hensen-Stempel pipet*, 4-mm diameter or 5-mL capacity, for obtaining subsamples from zooplankton samples.

4.11 *Plankton nets*, Wisconsin-type, open, or Birge-type, closing. The closing plankton nets have greater sampling flexibility in deep-water bodies because they can be closed at any selected depth (fig. 17*A*).

4.12 *Plankton trap* (Juday type), a 10-L closing box, attached plankton bucket (202-μm mesh openings or appropriate size for collecting microzooplankton), and messenger closing (fig. 17*C*), or transparent Plexiglas type that does not require messenger closing [Schindler-Patalas type (fig. 17*D*)].

4.13 *Porcelain crucibles.*

4.14 *Sample containers*, glass or plastic bottles, vials, or sealable plastic bags. However, bags are subject to leakage during prolonged storage.

4.15 *Sampling tube* or water core, a weighted thin-walled rubber or plastic tube that has a closing device for collecting a relatively large vertical column of water and its associated zooplankton (Edmondson and Winberg, 1971, p. 4).

4.16 *Spatula*, for stirring samples.

4.17 *Water pump*, and attached rubber or plastic hose. Water is pumped through a net that has a mesh size of 202 μm to retain the zooplankton (Committee on Oceanography, Biological Methods Panel, 1969, p. 48).

4.18 *Water-sampling bottle*, Van-Dorn type. Depth-integrating samplers are described in Guy and Norman (1970).

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Distilled or deionized water.*

5.2 *Dry ice*, for freezing zooplankton samples onsite for transport back to the laboratory.

6. Analysis

Detailed information about various biomass-determination methods are presented by Beers (1976) and Ruttner-Kolisko (1977). Biomass determination by gravimetric methods is

presented in the following paragraphs. Determinations need to be made on replicate samples when available or at least two subsamples if only one sample is available.

6.1 Place the zooplankton sample in a graduated cylinder, and if necessary, add distilled water to make up to a known volume. Pour the suspension into a beaker. Stir the contents using a spatula to ensure random distribution of the zooplankton.

6.2 Obtain the tare weight of a crucible that has been heated at 500 °C for 20 minutes and cooled to room temperature in a desiccator.

6.3 Place a known volume, using a large Hensen-Stempel pipet or equivalent, of the zooplankton suspension into the tared crucible and dry to a constant weight in an oven at a temperature no higher than 105 °C. Cool the crucibles containing dried zooplankton to room temperature in a desiccator before weighing. Weigh as rapidly as possible to decrease moisture uptake by the dry residue. Use these values to calculate dry weight.

6.4 Place the crucible containing the dried residue in a muffle furnace at 500 °C for 1 hour. Cool to room temperature.

6.5 Moisten the ash using distilled water and again oven-dry at 105 °C to a constant weight as in 6.3. Use these weight values to calculate ash weight.

7. Calculations

7.1 Entire sample used:

$$\text{Dry weight of zooplankton (grams per cubic meter)} = \frac{\text{Dry weight of residue and crucible (grams)} - \text{Tare weight of crucible (grams)}}{\text{Volume of water sampled (liters)}} \times \frac{1{,}000\ \text{L}}{\text{Cubic meters}}.$$

7.2 If subsample used:

$$\text{Dry weight of zooplankton (grams per cubic meter)} = \frac{\left(\text{Dry weight of residue and crucible and subsample residue (grams)} - \text{Tare weight of crucible (grams)}\right) \times \frac{\text{Volume of suspension (liters)}}{\text{Volume of subsample (liters)}}}{\text{Volume of water sample (liters)}} \times \frac{1{,}000\ \text{L}}{\text{Cubic meters}}.$$

7.3 Ash weight of zooplankton (grams per cubic meter)

$$= \frac{\text{Ash weight of residue and crucible (grams)} - \text{Tare weight of crucible (grams)}}{\text{Volume of water sample (liters)}} \times \frac{1{,}000\ \text{L}}{\text{Cubic meters}}.$$

7.4 Ash-free, or organic weight, of zooplankton (grams per cubic meter)

= dry weight of zooplankton (grams per cubic meter)

− ash weight of zooplankton (grams per cubic meter).

8. Reporting of results

Report biomass of zooplankton to two significant figures.

9. Precision

No numerical precision data are available.

10. Sources of information

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