Quantitative Determinations of Algal Density and Growth

There are four major techniques for counting algae. Two of these are used when counting algae in mixtures, as from field sampling or competition experiments, while the other two are used when counting unialgal samples, such as in growth or bioassay experiments. In each technique pair, one is "high-tech," requiring an expensive instrument, whereas the other is relatively "low-tech" and could be used in remote locations.

I. Counting algae in mixed assemblages.

A. "Low-tech" method--Sedgewick-Rafter chamber. This chamber is constructed from a thin brass plate from which a precise internal area has been cut; the plate is epoxied to a glass slide. Large, rectangular glass cover slips form the top of the chamber. The chamber's dimensions are 50X20X1 mm, with an area of 1000 mm^2, and a volume of 1.0 ml. We have found that the chambers may have a greater depth than 1 mm with the layer of epoxy and hold a greater volume than 1.0 ml; therefore, we will measure the sample added to the chamber.

To fill the Sedgewick-Rafter chamber, place the cover slip on top of the chamber, but at an oblique angle, so that the chamber is only partially covered. Watch the instructor's demonstration.

Use a pipette to measure 1.2 ml of well-mixed sample and fill the chamber, then gently nudge the cover slip so that it covers the chamber completely. The cover slip should not float, nor should there be any air bubbles; the former results from over-filling, the latter from under-filling the chamber. Now, let the sample settle for at least 15 minutes before beginning to count algae (the time can be used for calibrating the counting grid--see next paragraph).

Counts are done with the 4X or the 10X objectives of the compound microscope (depth of field and lens length preclude the use of higher magnification objectives). A Whipple disk is inserted into one of the ocular lenses in order to provide a sample grid. It is necessary to first determine the area (A) of the Whipple field for each set of ocular and objective lenses used. This is accomplished with a stage micrometer. At 4X the width of the grid field is _____mm; the length is _____mm. The area (A) is thus _____mm.

Now place the Sedgewick-Rafter chamber on the microscope stage. Observe 10 randomly-chosen fields, counting every alga within the boundaries of the Whipple grid field and every other alga lying on a boundary line. The average number of cells is N. The density of algae (D = cells/ml) in the original sample is calculated:
D = <u>N X A Sedgewick-Rafter/A Whipple</u>
             Volume
Find the average value of D for each alga in the sample provided to you.

For maximum statistical accuracy with the least time expenditure, it has been recommended to count 2 Whipple fields in each of 12 chambers (Woelkerling, et al., 1974. Hydrobiologia 48: 95-107). However, in our case we have streamlined the process due to time constraints.

Rinse out your chamber, then refill it with the mixed field sample provided. Do not attempt to count the algae, but note the difficulties involved in trying to accurately identify small cells (since you can't use the 40X objective lens). One strategy for doing this might be to do a preliminary survey of the community from a regular slide, identifying the major taxa (using the 40X or 100X lenses if necessary). Counts could then be done, since you now have a mental catalogue of the small forms.

I B. "High-tech"--Inverted microscope method. The expensive component here is the inverted microscope, whose great advantage is that settling chamber depth does not preclude use of high magnification objective lenses. Special "Utermöhl" chambers can be purchased, but they are expensive. Chambers can be constructed from large cover slips, plastic bottle caps, and plastic syringe barrels. If a long focal-length lens is available, chambers may be constructed by gluing syringe barrels to glass slides. A measured volume of sample is added to the chamber and allowed to settle for at least an hour. Time periods as long as 24 hours are preferred, especially if small algae are present in the sample (these will settle only very slowly). A total of 20 random Whipple field should be counted (follow procedures described above for determining the area (A) of the Whipple field for the magnification used, usually 400X). For counting procedures that can be used to achieve increased accuracy, see Sandgren and Robinson (1984), Br. J. Phycol. 19: 67-72.

II. Counting unialgal samples (unicells, small colonies, or relatively short filaments).

A. "Low-tech" method--hemacytometer. The hematocytometer was developed for counting cells in blood samples (now this is mostly done with electronic paticle counters). There are two delicate, mirrored surfaces that must not be scratched. There are special thick cover slips in the box with hemacytometer; do not throw these away; do not use a regular cover slip with the hemacytometer. Make sure that the mirrored surfaces and special cover slip are clean by wiping them gently with a lens tissue (NOT LAB TISSUES) moistened with water. Now, place the cover slip on top of the two mirrored surfaces, and use a pipette to introduce a drop of well-mixed algal suspension beneath the cover slip. The entire mirrored surface area should be wet, but liquid should not spill into the adjacent channels. Allow to settle at least 15 minutes.

Each mirrored surface has a grid etched upon the surface. Each grid is composed on 9 squares, each 1 mm along the sides. These 9 squares are further subdivided into small

areas. See the magnified, printed version posted on the bulletin board. The chamber is 0.1 mm deep. Each 9 mm^2 grid holds exactly 0.0009 ml of sample. You have the choice of counting the algae in the entire grid; counting algae in only one of the 9 squares, then multiplying by 9; or counting algae in an even smaller area, and multiplying accordingly. Counts of about 30 cells per unit area are desirable for accuracy. If there are more than 30 cells per unit, you will find it hard to keep track of whether you counted them or not. In this case, dilute the sample, or count algae in a smaller-sized unit and multiply. Repeat the process ten times and determine a mean. If the mean number of cells in the whole grid area were 3×10^3, then the density of cells in the original sample would be 3.3×10^5 cells/ml.

B. "High-tech" method--electronic particle counter (e.g. Coulter counter). In spite of relatively high cost, an electronic particle counter is highly recommended for doing growth or bioassay studies that require many counts and high accuracy. In addition, the instrument will provide particle size/biovolume distributions. The principle of operation is that particles, suspended in an electrolyte solution, are sized and counted by passing them though an aperture having a particular path of current flow for a given length of time. As the particles displace an equal volume of electrolyte in the aperture, they place resistance in the path of the current, resulting in current and voltage changes. The magnitude of the change is directly proportional to the volume of the particle; the number of changes per unit time is proportional to the number of particles in the sample. When opened, the stopcock introduces vacuum into the system, draws sample through the aperture, and unbalances the mercury in the manometer. The mercury flows past the "start" contact and resets the counter to zero. When the stopcock is closed, the mercury starts to return to its balanced position and draws sample through the aperture. As the column of mercury flows past the "stop" position, it initiates electronic counting and when it passes the "stop" contact, the count ceases. The distance traveled by the mercury column can be calibrated to provide a reproducible sample volume.

The electrolyte used is 1% NaCl which has been filtered to remove particulates. Samples are dispersed into electrolyte in special sample beakers. The dilution factor is determined experimentally, and is designed to yield the best distribution.

The control settings are to be done only by an expert. You should not change the threshold controls, amplification settings, aperture/current setting, bandwidth selector, separate/locked switch, gain control, vacuum control regulator, stirring motor rheostat, or manometer without seeking the assistance of an expert operator.

Place the sample to be analyzed on the platform of the sample stand. Open the control stopcock and watch the readout return to zero and the pattern appear on the oscilloscope screen. The unit will count all particles above the size determined with the threshold dial, then display the count on the numeric readout. Write the count down.

Variously-sized aperture tubes are available for use in counting variously-sized particles; the aperture size is chosen to match that of particles. Aperture tubes are quite fragile and expensive. They should be handled only by an expert operator. In using an aperture of 50 um of less to count very small particles, extreme care must be taken to reduce background counts. Electrolyte should be filtered at least 3 times. When large particles are to be counted, care must be taken to reduce error due to settling.

III. Growth rate and generation time determinations.

In comparing the results of bioassay experiments, or growth of various algae under the same or varying conditions, it is necessary to determine growth rates experimentally. Cell density data are usually obtained with a hemacytometer or electronic particle counter.

Growth curves are prepared from data obtained by sampling cultures at intervals, such as once per day, depending upon the growth rate of the alga. Plots of number of cells against time (in days) can be made, and from these curves can be calculated specific growth rate or growth constant (u) and division or generation time (tg).

A typical growth curve will show a lag phase, an exponential or log phase, and a stationary or plateau phase, where increase in density has leveled off. In the stationary phase, growth is likely limited by resources such as light or nutrients. Growth rate (u) is calculated with the following equation:

$$u = \frac{\ln X2 - \ln X1}{t2 - t1}$$

where X1 and X2 are densities at times t1 and t2.

Division time (tg) is calculated with the following equation (Guillard, 1973):

$$tg = 0.6931/u$$

Useful references:

Schoen, S. 1988. Cell counting. In, Lobban, et al. (eds), Experimental Phycology. A Laboratory Manual. Cambridge University Press, Cambridge, pp. 16-22.

Guillard, R.R.L. 1973. Division rates. In, Stein (ed), Handbook of Phycological Methods, V. 1, Cambridge University Press, Cambridge, pp. 289-312.