# PHYTOPLANKTON SAMPLING WITH THE SEDGWICK-RAFTER CELL[1]

*Bernard J. McAlice*
Ira C. Darling Center for Research, Teaching and Service, and Departments of Oceanography and Zoology, University of Maine, Walpole 04573

ABSTRACT

Quantitative processing of large numbers of phytoplankton collections requires a sampling method that will yield precise and reproducible estimates of abundance within an acceptably short counting time. A two-stage sampling plan was developed, using the Sedgwick-Rafter cell, which satisfies these criteria. The sampling design is appropriate for larger phytoplankton species ($>$10–15 $\mu$) having relatively high population densities ($\geqslant 10^5$ cells/liter).

## INTRODUCTION

A major problem of the phytoplankton worker is the need for making estimates of standing crop that are precise, reproducible, amenable to statistical comparison, and not prohibitively time-consuming. Estimation of phytoplankton standing crop has two distinct phases which present dissimilar problems and which may be widely separated in time. The chief difficulty in the first phase is that of taking field collections that are sufficiently representative of the natural population to be considered as samples leading to standing crop estimates meeting the above criteria. The second phase is concerned with satisfying the same criteria for the populations in the collection bottles. The difficulties inherent in this phase must be resolved before any assessment of the natural population distribution can be made or the representativeness of the collections determined. Therefore, in the following discussion, it is the population in a collection bottle that is the one to be sampled.

Although other methods have been widely used, visual counting is still the most generally useful and informative means of estimating phytoplankton standing crops. Other techniques are subject to technical errors and other difficulties that impair their reliability. Information is also lost because the contributions of separate species are not revealed. This information is essential to inquiries about community structure and succession. The utility of species counts is often impaired, however, by inability to evaluate their precision. Counts differing by an order of magnitude may represent different population densities or may simply reflect counting imprecision. Statistical treatment of such data is often further encumbered by ignorance of the sampling distribution from which they were derived.

The work described here was undertaken to develop a sampling scheme that would furnish data of known precision within an acceptable counting time, using the Sedgwick-Rafter counting chamber.

This paper is based on portions of a Ph.D. thesis submitted to the Graduate School of Oceanography, University of Rhode Island. I am indebted to Drs. S. B. Saila, W. J. Hemmerle, D. M. Pratt, and T. J. Smayda for their criticism and advice.

## SAMPLING CHARACTERISTICS OF THE SEDGWICK-RAFTER CELL

### *Testing for Poisson distribution*

The collection population of any species is estimated by counting the number of occurrences of that species in all or part of one or more aliquots from the collection. It is usually assumed that the counting units of the species (individual cells of

[1] This research was supported in part by a National Science Foundation Graduate Fellowship. Contribution No. 15, Ira C. Darling Center for Research, Teaching and Service, University of Maine, Walpole 04573.

solitary forms, chains or colonies of colonial types) are Poisson distributed in 1) the collection container at the time a subsample is withdrawn and 2) in the counting chamber. The most common test for the Poisson distribution is a statistic due to R. A. Fisher (Fisher et al. 1922):

$$D = \frac{\sum_{i=1}^{k} (x_i - \bar{x})^2}{\bar{x}} = \frac{(n-1)s^2}{\bar{x}}. \qquad (1)$$

The statistic $D$, usually called the variance test, has the chi-square distribution with $k-1$ df. In comparison with the chi-square goodness-of-fit test, the variance test will more often correctly result in rejection of the null hypothesis of randomness (Cochran 1954). Potthoff and Whittinghill (1966) also pointed out the value of $D$ in testing the null hypothesis that the variance is equal to the mean.

Departure from randomness can be in the direction of either overdispersion ($\sigma^2 > \mu$) or underdispersion ($\sigma^2 < \mu$). In nature the latter is to be expected only in the case of organisms exhibiting some kind of mutual avoidance, such as territoriality; it is most unlikely to occur in natural phytoplankton populations. In the laboratory, however, there is no basis for predicting either the counting chamber or aliquot-to-aliquot distributions. Therefore, when sampling from collections both alternatives to randomness must be considered. The appropriate test is $H_0$: $\sigma^2 = \mu$ against $H_1$: $\sigma^2 \neq \mu$, and rejection criteria are $P(\chi^2) > 0.975$ and $P(\chi^2) < 0.025$.

*Previous work*

In making Sedgwick-Rafter cell counts it is common to assume a Poisson distribution of counting units in the cell, to count a few microscope fields in one or two aliquots, and to extrapolate the results to such units as "organisms per liter." Most workers make no attempt to assess the precision of their estimates or to set confidence limits on them. Where these things are done, they are usually based on an assumed distribution, the Poisson, which is in fact likely not to be present. This attitude persists despite several relatively critical evaluations of Sedgwick-Rafter cell sample statistics, which although not consistent in their conclusions have at least advertised the problem.

Gilbert (1942) and Uehlinger (1964) decided that the units were indeed randomly distributed in the counting chamber. Both used $D$ as the test statistic. Gilbert was dealing with densities of about 1 unit per field; many of Uehlinger's counts seem to have been on the order of 50/ml or $\leqslant$ 1/field. When the mean is this small the frequency functions for the Poisson and the common contagious series generate very similar distributions, and it is difficult to detect overdispersion unless the sample size is large (Cassie 1962). Serfling (1949) used a factorial design to evaluate the effects of species morphology and population density on the counting cell distributions and concluded from analyses of variance and chi-square goodness-of-fit tests that the units were not randomly distributed in the cell. Kutkuhn (1958) found that while some zooplankton species counts from Sedgwick-Rafter cell aliquots followed the Poisson distribution, others were much better approximated by the negative binomial.

*Experimental evaluation*

The workers cited above all used a technique for filling the Sedgwick-Rafter cell (basically that described in *Standard methods*), in which the 1-ml aliquot is pipetted into the chamber at one corner, under a cover glass. This is referred to here as the APHA (Amer. Public Health Ass. 1965) method. Ricker (1937) introduced the aliquot into an uncovered chamber and randomized it with several strokes of a teasing needle. These filling techniques might be expected to produce different distributions of organisms in the counting cell.

A 2 × 3 × 4 factorial experiment was designed and replicated 5 times. The three factors and the levels of each were: 1) two cell-filling methods, the APHA and that of Ricker (1937); 2) three dilutions, to relative densities of 1.0, 0.5, and 0.05, of a

TABLE 1. *Data summary and analyses of variance of the factorial experiment. Each datum is the mean of 75 Whipple field counts*

| | Filling method | | | | | |
|---|---|---|---|---|---|---|
| | APHA | | | Ricker | | |
| | Relative density* | | | Relative density* | | |
| Quadrat | 1.0 | 0.5 | 0.05 | 1.0 | 0.5 | 0.05 |
| 1 | 27.28 | 11.88 | 1.65 | 28.67 | 12.91 | 1.85 |
| 2 | 32.28 | 13.93 | 1.51 | 27.67 | 12.11 | 1.76 |
| 3 | 27.69 | 13.40 | 2.09 | 27.52 | 13.04 | 1.71 |
| 4 | 30.56 | 12.20 | 2.08 | 25.39 | 12.83 | 1.61 |

| | | Relative density | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1.0 | | 0.5 | | 0.05 | |
| Source of variation | df | MS | $F$ | MS | $F$ | MS | $F$ |
| Replicates | 4 | 4.967 | 4.431† | 1.829 | 2.801‡ | 0.069 | |
| Quadrats | 3 | 1.698 | | 0.355 | | 0.192 | 1.010 |
| Methods | 1 | 6.100 | 1.806§ | 0.087 | | 0.112 | |
| Quadrats × methods | 3 | 3.377 | 3.012‡ | 1.008 | 1.544 | 0.476 | 2.505 |
| Error | 588 | 1.121 | | 0.653 | | 0.190 | |

* Actual relative densities, calculated from the counts, were 1.0, 0.45, and 0.06.
† $P(F) < 0.01$.
‡ $P(F) < 0.05$.
§ Since significant interaction is present, the quadrats × methods mean square is used to test the significance of quadrats and methods mean squares.

preserved pure culture of *Skeletonema costatum*; 3) four equal quadrats in each Sedgwick-Rafter cell; these were numbered 1–4 clockwise from the corner at which the chamber was filled. Fifteen microscope fields were chosen randomly from each quadrat. Each field was defined by the boundaries of a Whipple eyepiece reticule having an area of 0.9612 mm$^2$ at 100× magnification. Diatom chains lying only partly within the field were included in the count if they crossed the distal or right-hand edges of the field; those crossing the proximal or left-hand edges were excluded. Each aliquot was withdrawn in a calibrated 1-ml pipet immediately after the collection had been mixed by inverting the bottle 15 times. The 30-aliquot counts (2 filling methods × 3 densities × 5 replicates) were made in random order.

A transformation could not be found that would stabilize the experimental variances over the full range of quadrat means obtained. The experiment was therefore

TABLE 2. *Examination of the quadrats × filling methods interaction. Each mean square has 1 df. The divisors are the error mean squares from Table 1, each with 588 df*

| | Relative density | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | | 0.5 | | 0.05 | |
| Treatment difference | MS | $F$ | MS | $F$ | MS | $F$ |
| Methods within quadrat 1 | 0.644 | | 0.538 | | 0.136 | |
| Methods within quadrat 2 | 7.499 | 6.693* | 2.165 | 3.315 | 0.223 | 1.174 |
| Methods within quadrat 3 | 0.027 | | 0.139 | | 0.383 | 2.016 |
| Methods within quadrat 4 | 8.060 | 7.193* | 0.271 | | 0.796 | 4.189† |
| Error | 1.121 | | 0.653 | | 0.190 | |

* $P(F) < 0.01$.
† $P(F) < 0.05$.

TABLE 3. *Overall means and variances based on aliquot means, factorial experiment*

| | Relative density | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | | 0.5 | | 0.05 | |
| | $\bar{x}$ | $s^2$ | $\bar{x}$ | $s^2$ | $\bar{x}$ | $s^2$ |
| APHA | 29.45 | 26.70 | 12.85 | 5.45 | 1.83 | 0.14 |
| Ricker | 27.31 | 13.42 | 12.72 | 5.14 | 1.73 | 0.07 |

treated as three separate $2 \times 4$ factorial designs for the analysis of variance using untransformed data. The data summary and results of the analyses of variance are shown in Table 1. The significant $F$-values for replicates at the two higher densities are of some interest as an illustration that real differences may occur between replicate aliquots from the same collection; they do not affect the interpretation of the other results. The only significant differences between treatments were found at relative density 1.0. Further examination of the quadrat–filling method interaction (Table 2) shows that the two filling methods yielded significantly different counts in quadrats 2 and 4. The quadrat means (Table 1) indicate that the APHA method tended to deliver more chains to quadrats 2 and 4 at the highest relative density and that this method may produce higher variability among the quadrats at all densities. This suggestion is supported by the data in Table 3, where the overall quadrat mean square is seen to be higher for the APHA method at each density although the difference is substantial only at the highest density.

The APHA method was tested further. From a 1-liter preserved collection of a natural population, four diatom species having comparable counting unit densities but different morphology were selected: *Skeletonema costatum*, a small chain-forming species; *Thalassiosira gravida* and *Chaetoceros lorenzianus*, considerably larger chain-formers, the latter bearing long setae; and *Rhizosolenia setigera*, a long, slender, solitary species. Aliquots were withdrawn as before, and the total number of units of each species in each quadrat was counted for four aliquots. The experiment was treated as a randomized block design. The results, analyzed separately for each species, are summarized in Table 4. The analysis of variance revealed no significant differences between quadrat totals, but *Rhizosolenia* showed significant differences between aliquot totals.

These results furnish a tenuous basis for preferring the open cell method. The method has certain inherent disadvantages, however. When the APHA technique is used with preserved material, virtually all the units settle to the bottom of the chamber within a few minutes, and enumeration is simplified since only a two-dimensional search is required. The aliquot is also protected from evaporation and from distur-

TABLE 4. *Data summary and analyses of variance of the 4-species experiment. Each datum is the sum of 4 aliquot counts*

| Species | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| *Chaetoceros lorenzianus* | 40 | 31 | 45 | 29 |
| *Rhizosolenia setigera* | 33 | 22 | 23 | 40 |
| *Skeletonema costatum* | 41 | 33 | 25 | 23 |
| *Thalassiosira gravida* | 40 | 32 | 44 | 40 |

| Source of variation | df | *Chaetoceros* MS | *Chaetoceros* F | *Rhizosolenia* MS | *Rhizosolenia* F | *Skeletonema* MS | *Skeletonema* F | *Thalassiosira* MS | *Thalassiosira* F |
|---|---|---|---|---|---|---|---|---|---|
| Aliquots | 3 | 1.06 | | 21.58 | 3.90* | 12.42 | 2.44 | 1.33 | |
| Quadrats | 3 | 14.23 | 1.13 | 18.42 | 3.33 | 16.92 | 3.33 | 6.33 | |
| Error | 9 | 12.56 | | 5.53 | | 5.08 | | 14.89 | |

* $P(F) > 0.05$.

bance by air currents. In the open cell method many smaller units are trapped in the surface film for a short or long time; the chamber must be searched in three dimensions. When the population density is high, the counting time for 30 or 40 Whipple fields can easily exceed 2 hr, during which there is often sufficient evaporation to dry the center of the chamber. The aliquot is also susceptible to disturbances caused by transient air currents, including those generated by the observer's breathing.

The APHA method is therefore recommended for use with preserved material, to decrease the counting time, and should be used for living material as well where population densities approach $10^6$/liter. For reliable counts of motile forms, the organisms must be narcotized or killed before counting, and the APHA method is appropriate.

Two assumptions made by most workers in making Sedgwick-Rafter cell estimates of collection populations were tested. These are that both 1) the total counts of several aliquots from a single collection and 2) the individual microscope field counts from a single aliquot conform to the Poisson distribution. Formally, in each case $H_0$: $\sigma^2 = \mu$ was tested against $H_1$: $\sigma^2 \neq \mu$. $D$ was the test statistic.

The aliquot-to-aliquot distribution was tested in six natural population collections. The number of occurrences of several species was counted in 4, 5, or 6 1-ml aliquots from each collection; the entire aliquot was counted in every case. The variance test was applied to the several series of individual species totals thus obtained. Figure 1 is a graphic presentation of the results. In sampling a Poisson distribution, when the sample mean square is plotted against the mean, all points should fall on or close to the 45° line $s^2 = \bar{x}$. Departure from this theoretical relationship is not significant unless a point falls on or outside of the upper $[P(\chi^2) < 0.025]$ or lower $[P(\chi^2) > 0.975]$ limit for the sample size used. Of the 25 sets of coordinates $(\bar{x}, s^2)$ obtained, 2 required rejection of the null hypothesis because of overdispersion; significant underdispersion was not detected. The Poisson distribution furnishes a generally adequate description of the aliquot-to-aliquot distribution of the counting units of individual species.

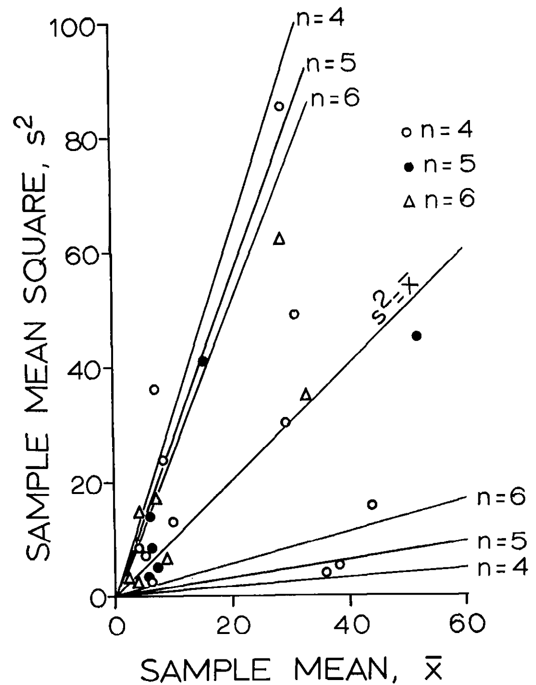


FIG. 1. Graphic variance tests of aliquot-to-aliquot distributions.

The 60 individual field counts obtained from each of the 30 aliquots in the factorial experiment, together with several sets of 20 and 30 Whipple field counts from Sedgwick-Rafter cell aliquots of natural populations, were used to test the distributions of counting units in the Sedgwick-Rafter cell. The results are shown in Fig. 2 for means less than 5 and in Fig. 3 for means greater than 5. Overdispersion is general for means greater than 10, and the null hypothesis must be rejected for counts in this range. The null hypothesis is also rejected for about half the counts having means between 5 and 10 but is accepted for most means less than 5. There are two possible interpretations of these results. If

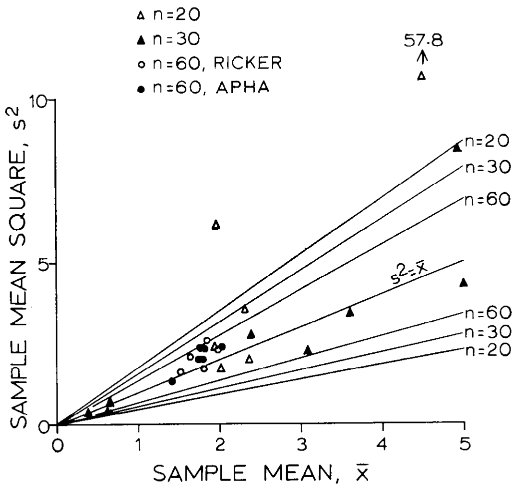


FIG. 2. Graphic variance tests of Whipple field distributions, means < 5.

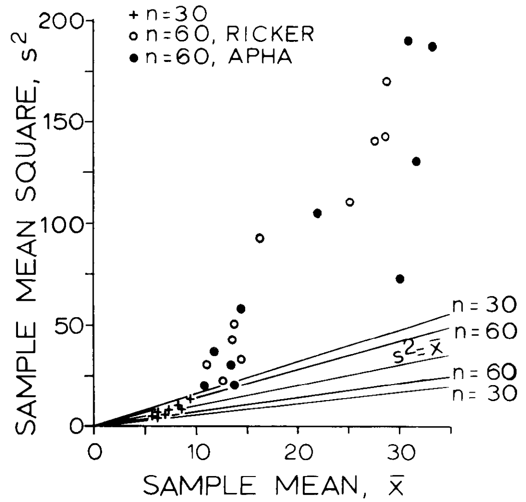


FIG. 3. Graphic variance tests of Whipple field distributions, means > 5.

the population density is so high that physical interference exists among the counting units, clumping can be expected to lead to overdispersion under these conditions, whereas it would not be present in sparser populations. Such interference was not noticeable at the very high population densities used in the factorial experiment. On the other hand, the difficulty of distinguishing between the Poisson and the common contagious distributions when the mean is small has been noted. Only 4 of the 25 points in Fig. 2 require rejection of the null hypothesis, but 17 of them have $s^2 > \bar{x}$. Significant underdispersion was not present in any of these counts.

The conclusion reached is that the Poisson distribution cannot usually be assumed for microscope field counts from Sedgwick-Rafter cell aliquots. It is also apparent that underdispersion is unlikely to occur and that the one-tailed test $H_0 : \sigma^2 = \mu$ against $H_1 : \sigma^2 > \mu$, with $P(\chi^2) < 0.05$, is the better choice. Attempts to generate a Poisson distribution of counts artificially entail either dilution of the collection so that the actual distribution is masked, or division of the chamber into a few large areas, each considered as a single field (Serfling 1949). In the latter case the probability that any given counting unit will be in a given area is no longer small and the Poisson is not appropriate.

## SAMPLING DESIGN

### *Transformations*

The counts obtained in phytoplankton sampling are usually nonnormally distributed and may exhibit marked instability of variance as well. This is true especially for microscope field counts of colonial forms. Even when field counts from a Sedgwick-Rafter cell or other aliquot are Poisson distributed, the sample means encountered are generally not high enough to justify the use of the normal approximation to the Poisson. In almost all such counts the sample mean square is proportional to, and usually larger than, the mean. Some transformation of the raw data, of the form

$$y_i = f(x_i) \tag{2}$$

is then needed to normalize the data or stabilize the variances, or both, before many of the parametric statistical tests based on the normal distribution can be used. The uses of such transformations, and which are best for various types of common experimental distributions, have

been extensively discussed in the literature (e.g., Anscombe 1948; Barnes 1952; Taylor 1953). For most purposes the single necessary and sufficient condition for the propriety of a transformation is that the transformed data meet the requirements of the statistical tests used. In analysis of variance the main requirement is stability of variances. Simple empirical transformations are as good as more complex ones having some theoretical nicety.

The negative binomial is a versatile distribution that can be fitted to a wide range of the unimodal, positively skewed frequency distributions so often encountered in phytoplankton work, since it approaches the Poisson at one extreme and the log series distribution at the other. However, to make comparisons between several data sets when the degree of contagion itself is not of primary interest, a common contagion parameter must be found for all the sets. This is a time-consuming iterative procedure whether the maximum likelihood method of Bliss and Fisher (1953) or the simpler technique of Anscombe (1948) is used. Furthermore, the common methods of estimating this parameter can yield unreliable results unless the sample size is very large (Shenton and Myers 1963).

Microscope field counts of total cells of chain-forming species can be more easily compared using the simple and effective empirical transformation

$$y_i = \log (x_i + 1). \quad (3)$$

For the more moderately skewed distributions of solitary cell or unit counts the change of variate

$$y_i = \sqrt{x_i + 1} \quad (4)$$

is effective.

Statistical tests and comparisons are performed on the transformed variates, but the conclusions are applicable to the raw data. Means established for the transformed distributions can be changed back to the raw scale, and rough comparisons of abundance estimates from diverse data sets can be made. Transformed means must

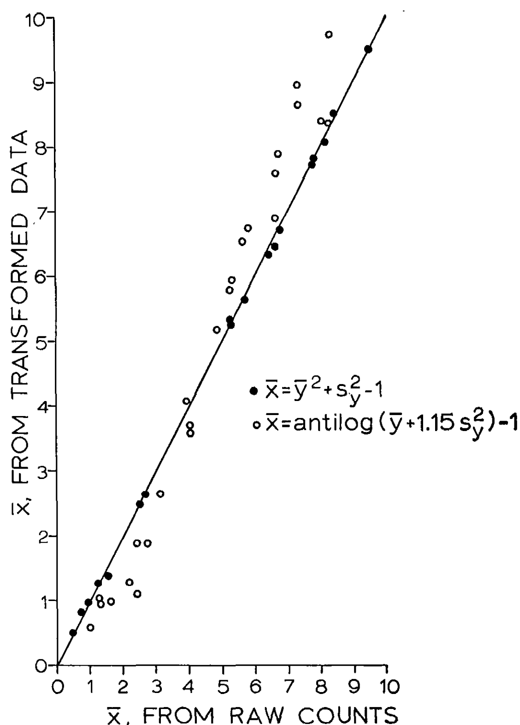


FIG. 4. Comparison of raw means with those derived from transformed data.

be adjusted in going back to the original scale so that they will be comparable to those obtained from the raw data (Barnes 1952). For the square root transformation, equation (4),

$$\bar{x} = \bar{y}^2 + s_y{}^2 - 1, \quad (5)$$

where $s_y{}^2$ is the sample mean square on the transformed scale. For the log transformation, equation (3), Barnes (1952) recommends

$$\bar{x} = \text{antilog}\,(\bar{y} + 1.15 s_y{}^2) - 1. \quad (6)$$

Equation (5) gives excellent estimates of the raw means, but equation (6) is not as good, since it tends to overestimate $\bar{x}$ when the mean is high and to underestimate it when the mean is low. This is illustrated by Fig. 4, where the means per field obtained from raw counts of 30 fields each from Sedgwick-Rafter cell aliquots of natural populations are plotted against

the means estimated from the transformed data. Each of the 43 points represents the mean calculated for a single species; the square root transformation was used for 18 sets of counts and the logarithmic transformation for the other 25.

### *Two-stage sampling*

Estimation of a phytoplankton population by viewing portions of one or more counting chamber aliquots from a collection clearly constitutes a two-stage sampling process. The samples, or primary units, are the counting chamber aliquots; these are subsampled by the elementary units, which may be microscope fields or other subdivisions of the counting cell. Two components contribute to the variance of the estimated population mean: the variation between elementary unit counts in each of the several aliquots and the variation between the estimated means derived from the series of aliquots. Knowledge of the relative importance of these two sources of variation will permit an allocation of sampling effort between primary and elementary units that will yield the greatest precision for any given counting time, and, conversely, can indicate the shortest counting time needed to attain any desired level of precision. (A third source of variability in plankton counts is the observer's counting error. This is difficult to assess without knowledge of the true population density, but it should be consistent for the same observer and thus affect all his counts equally.)

The notation used here follows Cochran (1963):

$n$ = the number of primary units in the sample;
$m$ = the number of elements in the subsample from each primary unit;
$\bar{y}'$ = the overall sample mean per element;
$s_1^2$ = the estimated variance among primary units;
$s_2^2$ = the estimated variance among elements within primary units.

The overall sample mean per element is an unbiased estimate of the true population mean per element; its variance is estimated by

$$s_{\bar{y}'}^2 = \frac{s_1^2}{n} + \frac{s_2^2}{nm}. \qquad (7)$$

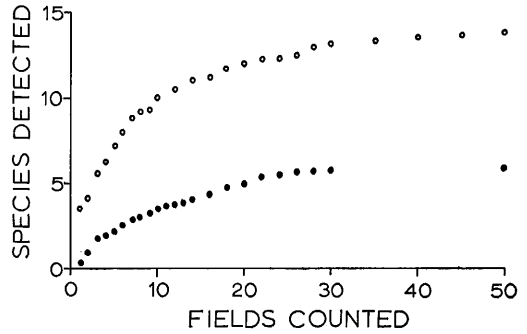


FIG. 5. Representative species-area curves for Whipple field counts.

The precision of the estimated mean is given by equation (7).

In theory, any desired level of precision can be attained by counting a sufficiently large number of aliquots. In practice there is usually a time constraint limiting the obtainable precision. After a preserved aliquot has been transferred to the Sedgwick-Rafter cell it is allowed to settle for about 15 min before enumeration. Actual counting time per element varies considerably, depending on the type of element (strip, microscope field, or arbitrary area), the population densities, and the ease with which the organisms present can be identified, but 1 min is a good average value for microscope field counts. The cost in minutes of sampling a collection can be expressed as

$$C = n(15 + tm), \qquad (8)$$

where $t$ is the time in minutes needed to count each element, including moving the microscope stage to each new position.

The optimum number of elements per primary unit for minimizing both $s_{\bar{y}'}^2$ and $C$ can be estimated following Cochran (1963) and Brooks (1955). The optimum value of $m$ was 25, using Brooks' Table 3.

One additional factor that must be con-

sidered in choosing a subsample size, $m$, is whether all or most of the species present are likely to be detected. Figure 5 shows species-area curves obtained from two preserved collections of natural populations. The points are the means of 20 replicate Sedgwick-Rafter cell counts from each collection. A count of 25 fields can be expected to reveal 80–90% of the species present and a count of 30 fields, 90–95%. The more conservative subsample size of 30 was therefore selected.

With $m$ established, the choice of $n$ was based on the decision that, if many collections are to be enumerated, the counting time for each collection should not greatly exceed 2 hr. Substitution in equation (8) gives $n = 2.7$. Taking $n = 3$, the average counting time for each collection is 135 min, an acceptable increase.

## DISCUSSION

The two-stage sampling scheme presented here used Sedgwick-Rafter cell subsamples to furnish precise and reproducible abundance estimates, within an acceptable counting time, for phytoplankton species having a population density of about $10^5$ or more per liter. Precise estimates of the abundances of sparser species require concentration of the collections. The configuration of the Sedgwick-Rafter cell does not permit the use of high-power microscope objective lenses, and identification of organisms smaller than 10–15 $\mu$ is difficult or impossible. The sampling design is thus in no sense universally applicable to problems of phytoplankton density estimation, and probably no design that must satisfy a reasonable counting time criterion can be. Acceptable sampling methods will vary, and the precision of the method used must be stated if meaningful comparisons with the results of others are to be made.

Both Kutkuhn (1958) and Uehlinger (1964) recommended the use of two-stage sampling to increase the precision of abundance estimates, but they did not attempt to optimize the distribution of effort between aliquots and microscope fields in the presence of an arbitrary time constraint. In this work such a constraint was imposed, and a two-stage sampling scheme was devised that gave maximum precision within the time limitation. Counts of either counting units or total cells are not normally distributed, so the data must be transformed to permit the application of normal statistical procedures.

Present knowledge of the dynamics of plankton populations furnishes no reasonable criteria for determining how much precision is enough, or too much. Statistical significance and ecological significance are not obviously related to one another. Two-stage sampling permits the attainment of any necessary level of precision in counting field collections, but the taking of truly representative field collections remains a problem.

## REFERENCES


AMERICAN PUBLIC HEALTH ASSOCIATION. 1965. Standard methods for the examination of water and wastewater, 12th ed. APHA. 759 p.

ANSCOMBE, F. J. 1948. The transformation of Poisson, binomial and negative-binomial data. Biometrika **35**: 246–254.

BARNES, H. 1952. The use of transformations in marine biological statistics. J. Cons., Cons. Perm. Int. Explor. Mer **18**: 61–71.

BLISS, C. I., AND R. A. FISHER. 1953. Fitting the negative binomial distribution to biological data, and note on the efficient fitting of the negative binomial. Biometrics **9**: 176–200.

BROOKS, S. H. 1955. The estimation of an optimum subsampling number. J. Amer. Statist. Ass. **50**: 398–415.

CASSIE, R. M. 1962. Frequency distribution models in the ecology of plankton and other organisms. J. Anim. Ecol. **31**: 65–92.

COCHRAN, W. G. 1954. Some methods for strengthening the common $\chi^2$ tests. Biometrics **10**: 417–451.

———. 1963. Sampling techniques, 2nd. ed. Wiley. 413 p.

FISHER, R. A., H. G. THORNTON, AND W. A. MACKENZIE. 1922. The accuracy of the plating method of estimating the density of bacterial populations. Ann. Appl. Biol. **9**: 325–359.

GILBERT, J. Y. 1942. The errors of the Sedgwick-Rafter counting chamber in the enumeration of phytoplankton. Trans. Amer. Micros. Soc. **61**: 217–226.

KUTKUHN, J. H. 1958. Notes on the precision of numerical and volumetric plankton estimates from small-sample concentrates. Limnol. Oceanogr. **3**: 69–83.

POTTHOFF, R. F., AND M. WHITTINGHILL. 1966. Testing for homogeneity. II. Biometrika **53**: 183–190.

RICKER, W. E. 1937. Statistical treatment of sampling processes useful in the enumeration of plankton organisms. Arch. Hydrobiol. **31**: 68–84.

SERFLING, R. E. 1949. Quantitative estimates of plankton from small samples of Sedgwick-Rafter-Cell mounts of concentrate samples. Trans. Amer. Microsc. Soc. **68**: 185–199.

SHENTON, L. R., AND R. MYERS. 1963. Comments on estimation for the negative binomial distribution, p. 241–255. *In* G. P. Patil [ed.], Proc. Int. Symp. Classical Contagious Discrete Distrib. Statist. Publ. Soc., Calcutta.

TAYLOR, C. C. 1953. Nature of variability in trawl catches. U.S. Fish Wildl. Serv., Fish. Bull. 83, p. 145–166.

UEHLINGER, V. 1964. Étude statistique des méthodes de dénombrement planctonique. Arch. Sci. **17**(2): 121–223.