Hydrobiologia vol. 48, 2, pag. 95-107, 1976

SEDGWICK-RAFTER CELL COUNTS: A PROCEDURAL ANALYSIS

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Received April 28, 1974

Keywords: Statistical analysis of algal counts. Sedgwick-Rafter cell.

Abstract

Information in the existing literature on some aspects of the collection and statistical analysis of Sedgwick-Rafter cell data appears contradictory, confusing, or absent. Using data from an experimental phytoplankton population as a basis, an investigation of S-R cell procedure has been undertaken with the following conclusions: 1) settling time depends upon the type of preservation and the composition of the sample; 2) the field counting technique gives more accurate data and is less time consuming than the strip counting technique; 3) making fewer counts on each of a greater number of S-R cells gives more accurate results than making a greater number of counts on one or several S-R cells; 4) nonparametric methods offer a more convenient and nearly as efficient a means of detecting statistically significant differences as compared with parametric methods. A method is presented for optimally allocating counts within and among S-R cells for getting an estimator with the greatest precision in the least time.

Introduction

The use of Sedgwick-Rafter (S-R) cells for estimating the standing crop of phytoplankton and in measuring algal growth rates by counting cell divisions appears to be widespread, and descriptions of the techniques involved have been included in a number of methodological handbooks and review papers (e.g., APHA 1971, Guillard 1973, Lund & Talling 1957, Welch 1948). According to the APHA (1971, page 734), the S-R cell offers the advantage of being '...easily manipulated and provides reasonably reproducible information...'; it suffers, however, from the limitation that the high magnifications needed for counting nannoplankton and ultraplankton are difficult to achieve with ordinary microscopes due to S-R cell design, and other procedures (see Guillard 1973, Schwoerbel 1970) have been proposed for these purposes.

During preliminary work on the role of desmids (Desmidiales, Chlorophyta) in Wisconsin lake communities, it quickly became apparent that the directions given in the methodological handbooks for S-R cell use are not explicit in some respects and that information on these points from other sources appears contradictory, or confusing, or altogether absent. Among questions that have arisen are the following:

1. Should at least 15 minutes settling time be allowed prior to counting (APHA 1971) or is 3-5 minutes sufficient (Guillard 1973)?
2. Does the field counting technique yield results that are better than, comparable to, or poorer than strip counting data in terms of accuracy and efficiency?
3. If the field counting technique is employed, how many S-R cells should be examined and how many fields per cell should be counted? (The APHA (1971) states that 10 or more random fields should be counted but makes no mention of the number of S-R cells to be examined; Welch (1948) recommends counting at least 10 fields in each of 2 cells; McAlice (1971) suggests examining 30 fields in each of 3 cells; Kutkuhn (1958) proposes enumerating 10 fields in each of 4 cells, and Guillard (1973, page 300) says: 'Count enough fields to get the precision desired.')
4. What is the most efficient way of obtaining S-R data

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given arbitrarily defined standards of accuracy or arbitrarily set time limits for the examination of individual samples? McAlice (1971) considers only time but not accuracy in his cost analysis, and to our knowledge, no one else has undertaken a complete cost analysis.

Another problem concerns methods used to detect statistically significant differences between two or more samples. Those apparently few investigators (e.g., Ballentine 1953, Gilbert 1942, Littleford *et al.* 1940) who have employed statistical evaluations have used parametric procedures on the assumption that the distribution of the data is approximately normal. Kutkuhn (1958), McAlice (1971), and Serfling (1949), however, have demonstrated that this assumption cannot always be made. Furthermore, transforming data to approximate a normal distribution apparently is not always possible (Kutkuhn 1958, page 73). In view of these facts, the question arises as to whether nonparametric procedures, which are less distribution-dependent, offer a satisfactory alternative to parametric procedures for detecting statistically significant differences.

The present study has been undertaken 1) to gather information which hopefully will help to answer the four questions raised above concerning S-R cell use and 2) to discuss the use of parametric and nonparametric procedures in testing S-R cell data for statistical significance.

II. Materials and Methods

The experimental population employed in this investigation has been prepared by mixing aliquots of three unicellular desmids *(Micrasterias laticeps* Nordstedt, *Netrium digitatus* (Ehrenberg) Itzigsohn and Rothe, *Staurastrum leptacanthum* Nordstedt), one filamentous desmid (*Sphaerozosma* sp.), and one colonial chlorococcalean alga (*Scenedesmus quadricauda* (Turpin) Breb.) and then preserving the mixture with Lugol's solution (H_2O-1000 ml.; I_2-10 gm.; KI-5 gm.) or with FAA (10:7:2:1::95% ethanol:distilled water:formalin:acetic acid). All taxa represent clonal isolates from Wisconsin lakes.

Using pipettes with a bore diameter of 1 mm., aliquots of the test population were extracted from the preserved sample (which was constantly being mixed with a magnetic stirrer) and were pipetted into S-R cells according to directions given in APHA (1971). Once the algae had settled, data were obtained at 100x total magnification by the field counting method using a Whipple micrometer (APHA op. cit.). Five randomly selected (see Guillard 1973, page 300) Whipple grid areas were tallied in each of 100 different S-R cells for a total of 500 counts. Each count included the total population and the numbers of individual plants (a filament or colony is one plant) of each of the five component taxa. Organisms touching or crossing the upper and the right hand boundaries of the Whipple grid were included in the tallies while those touching or crossing the lower or left hand boundaries were excluded from the tallies.

In this study, the time required to prepare a S-R cell for counting (excluding settling time; see discussion below)

Table 1. List of mathematical symbols.

Symbol	Definition
A	Estimated component of variance due to differences among S-R cells
CV	Coefficient of variation
k	Number of available S-R cells
M	Settling time for a S-R cell
m	Number of fields counted per S-R cell
m*	Integer just smaller than the calculated value of m
m* + 1	Integer just larger than the calculated value of m
n	Number of S-R cells studied
p	Half-width of confidence interval, expressed as fraction of mean
s	Time required for preparing, filling and cleaning a S-R cell
s_f	Time required for filling a S-R cell
T	Total time spent for a S-R cell analysis of a population
t	Time required for making a single Whipple grid tally
$t_{.05,n-1}$	Student's t at 5% significance level with n-1 degrees of freedom
V	Estimated variance of mean
W	Estimated component of variance due to differences among counts within S-R cells
$\bar{x}$	Mean of nm measurements
$\sigma^2_{\bar{x}}$	True variance of mean
σ^2_A	True component of variance due to differences among S-R cells
σ^2_W	True component of variance due to differences among counts within S-R cells

averaged 1 min; the time required to clean an S-R cell after use (see Guillard 1973, page 297 for cleaning instructions) averaged 2 min; and the time required to examine one Whipple grid area also averaged 2 min. Since similar times were required in counting raw plankton samples from Wisconsin lakes (unpublished data), the above times have been employed in making efficiency determinations.

The coefficient of variation (CV) of the mean has been employed as a statistical measure of the accuracy and reproducibility of a given counting regime. A low CV means greater accuracy and reproducibility than a high CV. A detailed summary of mathematical formulae and calculations appears in Appendix I at the end of the paper and a complete list of mathematical symbols appears in Table 1.

III. Procedural Results

A. Settling Time & Counting Technique

Settling time appears to depend upon the type of fixative used and upon the type of algae present. The iodine in Lugol's solution apparently facilitates settling (Guillard 1973), probably because of its high atomic weight and by speeding gas release from cells, whereas FAA does not appear to offer such advantages. In tests conducted on the experimental population and on some raw plankton samples from Wisconsin lakes, plants preserved with Lugol's solution required a maximum settling time of 7 min. while FAA preserved material required a maximum settling time of 10 min. Waiting for 15 min as recommended by APHA (1971) appears unnecessary in the samples tested. Observations made during these experiments suggest that taxa with large surface to volume ratios (e.g., certain species of *Staurastrum*) or taxa with gas vacuoles (e.g., certain Cyanophyta) settled more slowly than most other taxa.

Superficially, the strip counting technique would appear to offer more reliable results than the field counting technique since a much greater area of the S-R cell is counted. Closer investigation, however, reveals that the field counting technique is not only far less time consuming but is also more reliable (i.e., it results in a smaller coefficient of variation).

Consider the nature of a strip count involving the use of a Whipple grid whose width is 0.7 mm. at 100x magnification (this equals the grid width for the microscope employed in this study). Since the length of an S-R cell is 50 mm., each strip contains 71.4 Whipple grid areas, and if one follows APHA recommendations, 2-4 strips or 142.8-285.6 Whipple grid areas would be counted. Assuming 2 minutes are required to count each Whipple grid area, the counting of two strips would take 286 minutes (4 hours, 46 minutes) and the counting of four strips would take 571 minutes (9 hours, 31 minutes). In contrast, a field counting technique involving two Whipple grid tallies on each of 12 different S-R cells takes only 84 minutes (1 hour, 24 minutes), including the time necessary to prepare (1 minute) and clean (2 minutes) each slide. Moreover, the coefficient of variation (CV) for the above field counting regime on the experimental population examined during this study was 5.83%, while the comparable CV for the strip counting method was 13.33% for a 2 strip count and 13.27% for a 4 strip count (see Table 2).

Thus, the 2/12 field counting method requires less than a third (0.29) the time and gives a CV less than one half (0.43) as large as the two-strip counting technique. Therefore, the former appears to be by far the preferred method.

Table 2. Coefficients of variation (CV) of the mean of the experimental population for various combinations of n and m.

n	m	nm	CV	Comments
1	10	10	14.9	
1	24	24	13.9	
1	71.4	71.4	13.5	
1	142.8	142.8	13.3	
1	285.6	285.6	13.3	
2	10	20	10.5	Recommended by Welch (1948)
2	12	24	10.3	
3	30	90	8.0	Recommended by McAlice (1971)
4	6	24	7.9	
4	10	40	7.4	Recommended by Kutkuhn (1958)
5	5	25	7.3	
6	4	24	7.0	
8	3	24	6.4	
10	2	20	6.4	
10	3	30	5.8	
12	2	24	5.8	
15	1	15	6.5	
15	2	30	5.2	
15	3	45	4.7	
20	1	20	5.7	
24	1	24	5.2	

B. The Counting Regime

Since, as indicated above, recommendations for S-R cell counting regimes vary considerably in the existing literature, studies have been undertaken to develop a general procedure for deciding upon a particular counting regime and to determine which, if any, of the recommended schemes is to be preferred based on a comparison of coefficients of variation of the mean.

A S-R counting regime has two components: n, the number of slides studied, and m, the number fields per slide counted. To determine the coefficient of variation for a given combination of n and m, it is necessary to estimate the variance of the mean, V, which is given by the expression

(1) $V = A/n + W/nm$

where A is that component of the variance due solely to differences in algal densities among the various S-R cells and W is that component of the variance due solely to differences in the counts within a given S-R cell (Cochran 1953, chapter 10; see appendix I for further details on A and W). Once V has been determined, the coefficient of variation can be calculated from the formula

(2) $CV = 100\sqrt{V}/\bar{x}$

where $\bar{x}$ is the mean of the nm measurements.

Using A = 8.06, W = 21.6, and $\bar{x} = 21.5$ (calculated for the experimental population with n = 100 and m = 5 as described under 'Materials and Methods'), the CV values for different combinations of n and m have been calculated and are summarized in Table 2. The results strongly indicate that, in general, making fewer counts on each of a greater number of S-R cells gives more accurate results (based on CV values) than making a large number of counts on one or several S-R cells. Consider, for example, making a total of 24 counts. The CV value for 24 counts on one S-R cell is 13.9, for 6 counts on 4 cells, CV = 7.9; and for 2 counts on 12 cells, 5.8. The analysis also indicates that the regimes recommended by Kutkuhn (1958), McAlice (1971), and Welch (1948) provide less reliable data than does a regime involving 2 counts on each of 12 S-R cells. Moreover, the Kutkuhn and McAlice schemes involve total counts of 40 and 90 respectively, and this

Table 3. Cost analyses giving optimum n and m with time or precision limiting and with t = 2 minutes (A = 8.06, W = 21.6, $\bar{x} = 21.5$, s = 3, and M = 10).

	T	CV	p	m	n
$(k - 1)s \geq M$ or $k \geq 5$					
	63	6.7	.16	2	9
	126	4.8	.10	2	18
k = 2					
	59	7.6	.21	4	5
	158	4.6	.10	4	14
k = 1					
	63	9.9	.42	4	3
	294	4.6	.10	4	14

[a] The limiting value, time or precision, is underlined. If time, T, is limiting, it is made as close to 60 minutes as possible with balanced sampling. Precision is measured by the quantity p, where the 95% confidence limits are $\bar{x} \pm p\bar{x}$. If precision, p, is limiting, it is made as close to .10 as possible.

means that they require much more time to gather data than does a 2/12 regime.

McAlice (1971) got m = 25 from Table 3 of Brooks (1955) and increased this to m = 30 to insure detection of less frequently occurring species. The increasing m from 25 to 30 is of questionable value not only because of the difficulties involved in getting reliable data at the species level (see Kutkuhn 1958) but also because McAlice (1971) himself recommends the technique only for taxon populations $\geqslant 10^5$ cells/l. Moreover, the detection of taxa depends on the total number of fields examined (i.e. nm), not just on m, and increasing n will, therefore, have the same effect as increasing m with the added advantage of increasing precision. The use of Table 3 of Brooks (1955) is discussed below in connection with part *C*, efficiency.

A and W, and, hence, CV will vary for different samples and different techniques. However, greater variability in algal density among the S-R cells (or relatively larger A) will only increase the advantage of studying more slides. On the other hand, less variability in algal density among the S-R cells (or relatively smaller A) would favor using fewer slides. But the experimental population was well mixed, making unlikely, for all practical purposes, the drastic reduction in A that would be necessary to favor studying only 2 to 4 S-R cells, as recommended in the literature.

C. Efficiency

The cost of estimating algal density with S-R cells in terms of time and accuracy depends not only upon the values of A and W but also upon the following factors:

1. The desired precision of the mean (e.g., the value of CV);
2. The time required for preparing and cleaning of S-R cells (denoted by 's');
3. The time required for making an individual Whipple grid tally (denoted by 't');
4. The time available for making the total number of counts (denoted by 'T'; T = ns + nmt); and
5. The settling time (denoted by 'M').

Based on results of work on the experimental population

Table 4. Cost analyses giving optimum n and m with time or precision limiting and with t = 1 minute (A = 8.06, W = 21.6, $\bar{x}$ = 21.3, s = 3, and M = 10).

	T	CV	p	m	n
$(k-1)s \geq M$ or $k \geq 5$					
	60	5.7	.13	3	10
	90	4.7	.10	3	15
k = 2					
	64	6.3	.16	7	6
	124	4.5	.10	7	12
k = 1					
	57	9.2	.39	6	3
	228	4.6	.10	6	12

[a] The limiting value, time or precision, is underlined. If time, T, is limiting, it is made as close to 60 minutes as possible with balanced sampling. Precision is measured by the quantity p, where the 95% confidence limits are $\bar{x} \pm p\bar{x}$. If precision, p, is limiting, it is made as close to .10 as possible.

as well as on some raw plankton samples from Wisconsin lakes, values of $s = 3$ minutes (i.e., 1 minute for filling and 2 minutes for cleaning), $t = 2$ minutes, and $M = 10$ minutes appear to be reasonable estimates. Moreover, if $k \geq 1 + M/s_f$ where k is the number of S-R cells available and s_f is the filling time, the settling time can be ignored (see Appendix I for further details and for cases where M cannot be ignored.).

Knowing the values for s, t, A, and W, and assuming M can be ignored, the optimum value of m can be determined from the equation

(3) $m = \sqrt{Ws/At}$

Commonly the integer value of m will equal one. However, A and W, and hence m, will vary with different techniques of S-R cell preparation and with samples of differing composition as shown in Appendix I. It is important, therefore, to get data that allow estimating both A and W to see if the optimum m changes substantially. Consequently one should consider making $m = 2$ even when formal analysis gives $m = 1$ so as to allow some measure of variation within slides.

McAlice (1971) selected an initial m of 25 by consulting Table 3 of Brooks (1955). This implies that McAlice used only one S-R cell ($k = 1$) and thus could not use his 15 min settling time productively. Our results indicate that settling time can be ignored (and thus efficiency increased) if $k \geq 1 + M/s_f$ as shown above. McAlice implies he had a W/A ratio between 10.5 and 184.2. For our experimental population $W/A = 2.7$, and (based on our values for M, s, and t), Table 3 of Brooks (1955) gives a range for m of 1 to 4; and this is consistent with our results and recommendations. Thus, the difference between McAlice's recommendation of $m = 30$ and our recommendation of $m = 2$ or 3 stems from our assuming more than 1 S-R cell is available and from our having greater variability among S-R cells. Our Table 7 allows for choosing the optimum m for any number of S-R cells and for a range of W/A ratios.

The value of n, unlike m, is dependent upon both CV and T, and hence one cannot arbitrarily set limits on both precision (CV) and time (T). If time is the more important and, therefore, limiting factor, the optimum value of n for a specified T is given by the equation

(4) $n = T/(s + mt)$

whereas if precision is the more important and, therefore, limiting factor, the optimum n is given by the equation

(5) $n = (A + W/m)/V$

where $V = (\bar{x} \cdot CV/100)^2$ for a specified value of CV. An alternative way of determining n where precision is defined in terms of confidence intervals rather than CV is presented in Appendix I. Tables 3 and 4 summarize results of cost analyses for the experimental population given different restrictions of time and precision.

IV. Parametric vs. Nonparametric Tests

The choice of parametric or nonparametric procedures in testing for statistically significant differences depends both upon the nature of the data and upon the relative advantages and limitations of the two approaches. As a basis for discussion, consider the data in Table 5, which

Table 5. Summary of S-R cell data for five samples from the experimental population using a $m = 2$, $n = 12$ counting regime. Samples 1 to 3 were taken from the original population; samples 4 and 5 were taken from the original population after dilution and concentration, respectively.

Sample and organism	Sums of two counts on each Sedgwick-Rafter cell											
	1	2	3	4	5	6	7	8	9	10	11	12
Sample 1												
Micrasterias	3	3	3	3	0	4	0	2	1	3	5	0
Netrium	1	2	1	1	1	1	1	4	1	2	2	0
Scenedesmus	22	37	17	23	13	21	32	27	22	21	26	17
Sphaerozosma	2	4	5	5	2	2	0	2	3	4	4	2
Staurastrum	8	9	7	16	10	13	15	11	6	12	12	11
TOTAL	36	55	33	52	26	41	48	46	33	42	49	30

Table 5 continued

Sample 2												
Micrasterias	3	2	4	3	5	2	3	4	1	4	4	4
Netrium	0	0	0	0	1	0	2	2	2	1	0	1
Scenedesmus	16	29	26	22	24	28	26	21	29	42	32	18
Sphaerozosma	3	1	2	2	1	1	3	1	2	2	6	2
Staurastrum	19	9	6	17	12	16	15	8	8	19	16	13
TOTAL	41	41	38	44	43	47	49	36	42	68	58	38
Sample 3												
Micrasterias	1	1	5	2	1	4	12	1	2	2	2	2
Netrium	2	0	1	0	0	1	1	1	2	0	0	0
Scenedesmus	34	22	31	27	29	27	18	23	29	26	15	17
Sphaerozosma	5	2	2	3	7	5	1	5	7	1	4	3
Staurastrum	9	9	13	14	16	9	8	11	13	13	13	9
TOTAL	48	34	52	46	53	46	30	41	53	42	34	31
Sample 4												
Micrasterias	1	1	0	1	2	1	1	3	0	2	2	2
Netrium	0	0	0	0	0	0	1	0	2	1	0	0
Scenedesmus	3	5	10	9	10	7	9	13	10	12	11	11
Sphaerozosma	1	2	1	2	1	0	2	3	2	0	1	0
Staurastrum	2	11	7	2	3	5	4	5	4	2	3	5
TOTAL	7	19	18	14	16	13	17	24	18	17	17	18
Sample 5												
Micrasterias	5	2	8	6	1	5	2	2	1	1	5	3
Netrium	0	3	1	1	1	0	0	1	1	1	1	1
Scenedesmus	35	26	21	25	23	30	29	22	21	34	32	32
Sphaerozosma	7	7	6	7	6	5	4	3	2	3	3	3
Staurastrum	7	19	18	20	13	17	13	14	9	9	14	13
TOTAL	54	57	54	59	44	57	48	42	34	48	55	52

was generated from the experimental population by means of a $m = 2$, $n = 12$ counting regime, and assume one wishes to know whether significant differences occur among the population densities of the samples for the total population and for each organism.

Because parametric procedures are applicable only to

approximately normally distributed data, and because such data cannot always be assumed for S-R cell work (Kutkuhn 1958, McAlice 1971, Serfling 1949), tests such as the Lilliefors test (Conover 1971, p. 302) must first be conducted to determine if the data are normally distributed. For cases where the test indicates non-normal distribution, efforts can be made to transform the data to an approximately normal distribution (see Kutkuhn 1958, McAlice

Table 6. Results of parametric and nonparametric analyses of data in Table 5.

	Lilliefors test			Probabilities associated with test statistics	
				True status of null hypothesis that no differences exist	
Organism	Best transformation[a]	Probability	Statistical test[b]	True[c]	False[d]
Micrasterias	logarithm (x + 2)	>>.20	Parametric	.093	.011
			Nonparametric	.071	.036
Netrium	logarithm (x + .1)	>.20	Parametric	.052	.069
			Nonparametric	.11	.11
Scenedesmus	logarithm (x + .1)	>>.20	Parametric	.52	$5.6 \cdot 10^{-11}$
			Nonparametric	.48	$6.7 \cdot 10^{-6}$
Sphaerozosma	logarithm (x + 1)	>.20	Parametric	.14	.000016
			Nonparametric	.099	.00019
Staurastrum	logarithm (x + .1)	>>.20	Parametric	.41	$4.2 \cdot 10^{-9}$
			Nonparametric	.35	$1.7 \cdot 10^{-5}$
TOTAL	logarithm (x + .1)	>>.20	Parametric	.43	$9.5 \cdot 10^{-4}$
			Nonparametric	.67	$2.3 \cdot 10^{-6}$

[a] Lilliefors tests were made on 36 observations from a single population (samples 1, 2, and 3 of Table 5) using the original data and the following transformations: $\sqrt{x}$, $\sqrt{x + .5}$, $\sqrt{x + 1}$, $\ln(x + .1)$, $\ln(x + .5)$, $\ln(x + 1)$, $\ln(x + 2)$. Under "Best transformation" is the transformation giving the closest approximation to a normal distribution.

[b] The parametric test is the one-way analysis of variance with data transformed using the transformation listed under "Best transformation." The nonparametric test is the Kruskal-Wallis test, with the probability associated with the test statistic (corrected for ties) estimated using a chi-square approximation.

[c] Analysis based on samples 1-3 in Table 5, all of which represent aliquots of the same population.

[d] Analysis based on samples 3-5 in Table 5, each of which represents a different population.

1971), but such transformations are not always possible (e.g., Kutkuhn 1958, page 73), especially in cases where the means approach zero. For the samples in Table 5, transformations appear to be possible in all cases, once the proper transformation functions have been determined (see Table 6).

Nonparametric procedures, in contrast, are less distribution-dependent. As a result they offer two distinct advantages: 1) they can be used for a wider range of data, and 2) they can be applied directly without any preliminary testing or transforming. Furthermore, the nature of the data may be such that nonparametric tests are the only ones which can be applied.

Where the data are approximately normally distributed or can be transformed, the choice between nonparametric or parametric tests for S-R cell analysis also involves a consideration of the asymptotic relative efficiency (A.R.E.) of particular nonparametric tests with their parametric counterparts. If only two samples are involved, one can test for statistically significant differences by using either the parametric t-test or the nonparametric Mann-Whitney test (or the Wilcoxon rank sum test). If more than two samples are involved, one can test for statistically significant differences by using either the parametric F test or the nonparametric Kruskal-Wallis test. In both cases, the A.R.E. of the nonparametric test relative to the parametric test for normally distributed data is 0.955 (Conover 1971, pages 235 and 262). Roughly this means that if a parametric test of a given power and level of significance requires 96 observations on each population, the nonparametric test of the same power and level of significance would require 100 observations on each population. Thus, nonparametric tests are almost as powerful as their parametric counterparts.

Analyzing the same data (Table 5) parametrically and nonparametrically illustrates the above point (see Table 6). As shown there, results of nonparametric tests agree with those of parametric tests in all cases in showing or not showing significance at the 5% and 1% levels. When the null hypothesis that no differences exist among samples

Table 7. Summary of equations to be used in cost analyses for various conditions of k.

Equation for	k = 1	k > 1 but (k - 1)s < M	(k - 1)s $\geq$ M
T =	$n(s + M) + nmt$	$M - 2(k - 1)s + ns + nmt$	$ns + nmt$
V =	$A/n + W/nm$	$A/n + W/nm$	$A/n + W/nm$
m =	$\sqrt{W(s + M)/At}$	The larger of the quantities $\sqrt{Ws/At}$ or $[M/(k - 1) - s]/t$	$\sqrt{Ws/At}$
n (time limiting)	$T/(s + M + mt)$	$[T - M + 2(k - 1)s]/(s + mt)$	$T/(s + mt)$
n (precision, defined by CV, limiting)	$(A + W/m)/V$	$(A + W/m)/V$	$(A + W/m)/V$
n (precision, defined by confidence interval, limiting)	$t^2_{.05,n-1}\ (A + W/m)/(p\bar{x})^2$	$t^2_{.05,n-1}\ (A + W/m)/(p\bar{x})^2$	$t^2_{.05,n-1}\ (A + W/m)/(p\bar{x})^2$

is true (i.e., among samples 1-3 in Table 5), the probabilities from the nonparametric and the parametric tests are similar and indicate no significant differences. When the null hypothesis that no differences exist among samples is not true (i.e., among samples 3-5 in Table 5), the probabilities from the nonparametric tests are all larger than those from the parametric tests. Thus, the nonparametric test is more conservative. However, for probabilities in the range .05-.01 (e.g., for *Micrasterias* and *Netrium* in Table 6) the tests give equivalent results. The only dramatic differences occur for probabilities much less than .001 (e.g., for *Scenedesmus* and *Staurastrum* in Table 6), but since both tests give overwhelming evidence of real differences among the samples, the differences in probabilities are of no practical importance.

Assuming normally distributed data, two conclusions can be drawn from the above: 1) nonparametric tests are nearly as effective as parametric tests in detecting significant differences; and 2) nonparametric tests are slightly more conservative than parametric tests; i.e., any significant difference detected by a nonparametric test will also probably be detected by the parametric counterpart, whereas in a few borderline cases only the parametric test will show significant differences.

In view of the facts that nonparametric procedures can be applied in all situations without testing for the presence of approximately normal distributions and without attempting to make transformations, and because they are nearly as efficient as their parametric counterparts (indeed, they can be even more efficient where the data is not normally distributed; see Conover 1971), nonparametric tests generally appear to be more satisfactory than parametric tests for analyzing Sedgwick-Rafter cell counts.

Acknowledgments

Sincere thanks are due Mr. William Blobner for assistance in gathering data on the experimental population used in this study. This investigation was supported in part by funds awarded to the senior author by Grant No. 133-9129 from the Wisconsin Department of Natural Resources and Grant No. 140374 from the Wisconsin Alumni Research Foundation. Computing costs were supported by Wisconsin Alumni Research Foundation Grant No. 140265 to the second author.

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Appendix I

This appendix presents details for the derivation of the formulae used in the main body of the paper. In addition, it provides a method for determining which integer to use when the calculated m is not a whole number, and it indicates how a cost analysis can be performed when the number of Sedgwick-Rafter cells, k, is limited. The mathematical symbols used are summarized in Table 1. The material presented, though independently derived, is an extension of that in Cochran (1953, chapter 10).

A. Derivation of Formulae

Let n be the number of slides studied, m, the number of

counts made on each slide, and x_{ij}, count j on slide i, where $j = 1, ..., m$, and $i = 1, ..., n$. One can use the mean of all the counts,

$$\bar{x} = \sum_{i=1}^{n} \sum_{j=1}^{m} x_{ij}/nm,$$

to estimate the density of algae if the volume represented is known. The variance of the mean is a measure of the precision of the estimator. The smaller the variance, the better the estimator. The variance of the mean is

$$(1) \qquad \sigma^2_{\bar{x}} = \sigma^2_A/n + \sigma^2_W/nm$$

where σ^2_A is that component of the variance due solely to differences among densities on different slides and σ^2_W is that component of the variance due solely to differences among counts within a given slide (Cochran, 1953). Using a preliminary set of data (x_{ij}; $i = 1, ..., n$; $j = 1, ..., m$), one can estimate the components of variance with statistics from an analysis of variance testing for differences among the slides:

$$(2) \qquad W = \hat{\sigma}^2_W = MS_W$$

$$(3) \qquad A = \hat{\sigma}^2_A = (MS_A - MS_W)/m$$

where

$$(4) \qquad MS_W = \sum_{i=1}^{n} \sum_{j=1}^{m} (x_{ij} - \bar{x}_i)^2/(nm - n)$$

with

$$(5) \qquad \bar{x}_i = \sum_{j=1}^{n} x_{ij}/m$$

and

$$(6) \qquad MS_A = \sum_{i=1}^{n} (\bar{x}_i - \bar{x})^2/(n - 1)$$

are mean squares measuring variation within and among slides respectively (Sokal and Rohlf, 1969). Using the estimator of variance components one can estimate the variance of the mean by

$$(7) \qquad V = A/n + W/nm$$

The coefficient of variation can then be determined from

$$(8) \qquad CV = 100\sqrt{V}/\bar{x}$$

If the total number of counts to be made, nm, is fixed and the relative costs of making a slide and of making a count on a slide are ignored, the variance of $\bar{x}$ is minimized by making n as large as possible or, equivalently, making m as small as possible, i.e., $m = 1$. Setting $m = 1$, however, will not always give the most precise estimator of algal density if time is limited.

To determine the best choice of n and m the cost, in time, of collecting the data must be considered. Let T be the total time spent studying a sample; then

$$(9) \qquad T = ns + nmt$$

where s is the time needed to make a slide and t is the time required to make a count on a slide. In practice, slides need a minimum settling time, M, before counting can begin. Thus, after the first slide is made one must wait at least M units of time before starting to count. If this time can be used to make additional slides (i.e., if $(n-1)s \geqslant M$), M can be ignored in the cost equation, and this has been done in equation 9.

If one assumes $(n-1)s \geqslant M$, then the optimum choice of m and n is such as to minimize V (equation 7) and T (equation 9) simultaneously. To obtain the desired minimization, equation 7 indicates that m and n should be made larger and equation 9 indicates the reverse. Also, if the total number of counts, nm, is fixed, equation 7 indicates that n should be increased and equation 9 indicates the opposite. Clearly a compromise is necessary.

If the total time for analysing a sample is fixed, T is constant, and using equation 9, n can be expressed as a function of m, namely $n = T/(s + mt)$. Substituting this expression for n in equation 7 gives

$$(10) \qquad V = [A(s + mt) + W(s + mt)/m]T.$$

To get the value of m which minimizes the estimated variance of the mean, V, the derivative of V with respect to m is set equal to zero, and the equation is solved for m:

(11) $$dV/dm = (At - Wsm^{-2})/T = o$$

and

(12) $$m = \sqrt{Ws/At}$$

Substituting expression 12 in d^2V/dm^2 shows that the positive root of Ws/At minimizes V.

Thus, the best m is independent of both V and T and depends only upon two ratios, namely the within to among slide variation and the time needed for making a slide to the time needed for making a count. Equation 12 is intuitively reasonable, since the number of counts per slide, m, increases as either the variability within a slide, W, or the cost of making a new slide, s, increases.

Once m is known, the value of n can be determined by solving equation 9 for n if a given time, T, is specified, i.e.,

(13) $$n = T/(s + mt)$$

or by solving equation 7 for n if a given degree of precision, V, is specified, i.e.,

(14) $$n = (A + W/m)/V.$$

An alternative way of having precision determine n is to use confidence intervals rather than coefficients of variation. The number of slides, n, must be such as to give 95% confidence limits on the mean approximately equal to $\bar{x} \pm p\bar{x}$, where p is an arbitrary number, e.g., p = .1. If the data are approximately normally distributed, the 95% confidence limits are $\bar{x} \pm t_{.05,\, n-1}\ s_{\bar{x}}$, where $t_{.05,\, n-1}$ is Student's t at the 5% significance level with n - 1 degrees of freedom and $s_{\bar{x}}$ is the standard deviation of the mean (Sokal and Rohlf, 1969). Estimating $s_{\bar{x}}$ by $\sqrt{V}$ gives

(15) $$p\,\bar{x} = t_{.05,\, n-1}\ \sqrt{V}$$

or

(16) $$V = p^2\,\bar{x}^2/t^2_{.05,\, n-1}$$

Substituting expression 16 in equation 7 and solving for n gives

(17) $$n = t^2_{.05,\, n-1}\ (A + W/m)/(p^2\,\bar{x}^2).$$

Substituting values of Student's associated with different values of n until the right-hand expression of equation 17 approximately equals the n of Student's t gives the required n.

B. The Integer Value of 'm'

Since practical considerations dictate that the same number of counts, m, be made on each Sedgwick-Rafter cell, it will be necessary to convert the calculated value of m (equation 12) to either the integer just smaller, m*, than or just larger, m* + 1, than m itself. The choice of m* or m* + 1 depends on which minimizes the estimated variance of the mean, V. Let V_0 and V_1 be the variances obtained by substituting m* and m* + 1 respectively into equation 10. If $V_0 < V_1$ the integer m* is preferred, whereas if $V_0 > V_1$ the integer m* + 1 is preferred. These relations are equivalent to choosing m* if m* (m* + 1) is greater than Ws/At and m* + 1 otherwise.

C. Cost Analysis for a Limiting 'k'

In cases where the number of available Sedgwick-Rafter cells, k, is limited such that (k - 1)s < M, the settling time, M, cannot be used fully to make new slides. Therefore, M must be taken into consideration in computing the cost in time of analyzing a sample, and equation 9 must be modified accordingly. If only one S-R cell is available (k = 1), the settling time can be included in the cost of making a slide, and equation 9 becomes

(18) $$T = n(s + M) + nmt$$

and the cost analysis is as in part A of the appendix with s + M substituted for s.

If k > 1 but (k - 1)s < M, equation 9 becomes

(19) $$T = s + M - (k-1)s + k(mt + s) - ks + (n/k - 1)[\text{Positive}\,(M - (k-1)(mt + s)) + k(mt + s)]$$

where the function 'Positive' takes on the value zero or [M - (k - 1) (mt + s)] if the latter expression is negative or positive, respectively. The function Positive [(M - (k - 1) (mt + s)] represents time wasted to allow for settling in the interval between the end of counting and refilling one batch of k slides and the start of counting and refilling the next batch. Optimization requires that this time either be eliminated or put to use.

Since the time cannot be eliminated because the slides must settle, and since the time cannot be used to make additional slides because k is limited, the only alternative

is to spend the time making more counts on each of the available slides. Therefore, m must be made large enough to make the function Positive $[M-(k-1)(mt+s)]$ zero, that is

(20) $$M-(k-1)(mt+s)\leqslant 0$$

or

(21) $$m\geqslant [M/(k-1)-s]/t.$$

Hence, one need only consider the simpler version of the cost function represented by equation 19 where Positive $[M-(k-1)(mt+s)]$ is zero:

(22) $$T+M-2(k-1)s+ns+nmt$$

Equation 22, however, is equivalent to equation 9 with T replaced by $T-M+2(k-1)s$. Therefore, the optimum m is the same as before and can be computed from equation 12 with the restriction that m must also satisfy equation 21.

A summary of the sequence of equations to be used for various conditions of k appears in Table 7.