SEDGWICK-RAFTER CELL COUNTS: A PROCEDURAL ANALYSIS

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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
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 (H₂O:1000 ml.: I₂-10 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)