A QUANTITATIVE METHOD FOR THE COLLECTION AND MEASUREMENT OF STREAM PERIPHYTON[1]

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ABSTRACT

Two methods for the collection and estimation of stream periphyton production are presented.

The periphyton was collected on 1.4 dm^2 plexiglass plates attached to a horizontal crossbar. The crossbar was supported by a vertical post driven into the stream bottom or supported by a concrete block. The plates were removed from the stream and the established population estimated by determining the absorbency of the ethanol-soluble phytopigments and by gravimetric procedures.

A reliable estimate of the population can be made from the phytopigment absorbency values if the latter are corrected for deviations from the absorbency:concentration relationship and if the substrata are removed from the stream before the growth is too profuse. Confidence limits are given for the phytopigment density:weight of organic material relationship.

INTRODUCTION

Periphyton plays an important role in flowing waters because it is virtually the only primary producer in the ecosystem. Although this community is frequently subjected to adverse physical conditions such as high stream velocities or high turbidity levels, it is characterized by a very rapid recovery. Because of its ubiquitousness and rapid turn over it provides both food and shelter for the benthic fauna of a stream. Since the organisms involved are not equipped with a means of procuring the essential elements from the stream bed, the production of this community is also closely related to the characteristics of the water mass flowing by. Consequently, an evaluation of the periphyton community has long been recognized as a means of evaluating stream biodynamics.

When the periphyton of a stream is collected from the natural submerged objects, large variability results from difference in the texture and nature of the substratum. Because of this many workers have turned to the use of artificial substrata. Cooke (1956) gives a comprehensive review of the literature on the subject. The collected organisms are usually identified *in situ* and the characteristics of the community evaluated. Hooper, Ball and Hayne (ms) were the first to estimate periphyton production by using artificial substrata and the phytopigment extract method (Kreps and Verjbinskaya 1930, Harvey 1934, Manning and Juday 1941).

The methods described in this paper were used in an ecological study of the Red Cedar River, a productive warm-water stream which drains the south-central portion of the Lower Peninsula of Michigan. The stream is characterized by long pools or runs rarely exceeding four feet in depth which are separated by short riffle areas. The study was carried out by the authors over a 13-month period which included drought conditions, floods, and winter conditions when the stream was covered with as much as 12 inches of ice and 6 inches of snow. Qualitatively, the periphyton of the Red Cedar River consisted primarily of members of the Bacillariaceae. The dominant genera in the community varied with the season.

METHODS

The substrata used for the collection of periphyton consisted of plexiglass plates, 7

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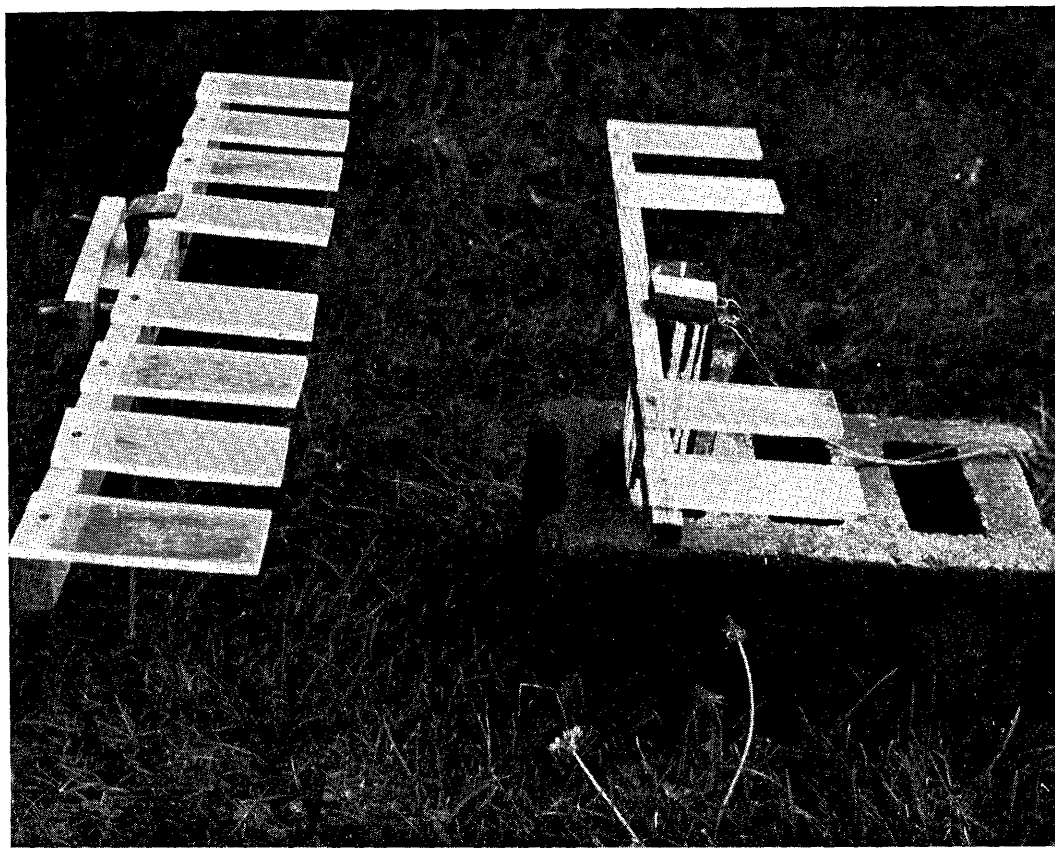

FIG. 1. Plexiglass substrata and supporting devices used for the collection of stream periphyton.

mm in thickness, having an exposed area of 1.4 dm^2 when attached to a horizontal crossbar. The crossbar was either bolted to a steel post which was driven into the stream bottom or supported from a vertical upright which was wedged into a concrete building block (Fig. 1). The exposure depth was maintained with relation to the water surface in order to compare the production potential of several areas of the stream.

The plexiglass substrata were recovered after the periphyton mat was plainly visible, but before the growth was dense enough to slough off the surface of the plate. The exposure periods ranged from one to three weeks depending upon the accrual rate of the periphyton.

The substrata were removed from the stream, placed in individual plastic bags, and then frozen to aid in the release of the growth from the plastic. This procedure also facilitated the extraction of phytopigments by rupturing the plant cells.

Two methods of laboratory treatment were used: the first, a method designed to use phytopigment density *per se* as an index of periphyton production; the second, designed to investigate the relationship between phytopigment absorbency and organic weight.

In the first method the periphyton growth was scraped from the substrata and allowed to stand in 95% ethanol for a minimum of 48 hours in total darkness. It was found that samples could be stored in this manner for as long as 30 days without a loss of phytopigments due to decomposition. The samples were filtered through glass wool and the volume of the filtrate adjusted to 50 ml by either dilution or evaporation. The density of the phytopigment solution was read on a Klett-Summerson colorimeter (4

cm solution depth) using the red filter (640–700 mμ).

In the second method, all of the macrofaunal components were first taken from the substrate. The periphyton was then removed by scraping and washing with 95% ethanol. The particulate matter was then separated from the solvent by filtration through a Gooch crucible. The crucible was dried to constant weight and the organic components determined by loss on ignition. The absorbency of the ethanol-soluble pigments was determined after the solution was diluted to a volume of 50 ml. The solvent was then evaporated from the sample and the weight of the organic residue determined by loss on ignition. The weight of the residue was then added to the weight of the particulate fraction of the sample to give an estimate of total organic weight.

When two consecutive readings of ± 0.5 mg were obtained after an interval of 12 hours, the sample was said to be at constant weight.

RESULTS

Experiments showed that the absorption of broad spectrum light (640–700 mμ) is not linearly related to the concentration of 95% ethanol extracts of phytopigment except at very low concentrations. The deviation from the Lambert-Beer Law becomes apparent at an absorbency of 0.20 when read on a Klett-Summerson colorimeter and increases proportionately with higher concentrations. Nonlinearity was also noted for measurements made with monochromatic light at the peak absorption wavelength. Inorganic solutions (Harvey Standards) having comparable absorbencies did not exhibit nonlinearity with either monochromatic or polychromatic light. Therefore, it is believed this effect may be due to interaction between the solvent and solute or to changes among the molecules.

The measured pigment absorbency may be corrected to correspond with the theoretical absorbency as related to concentration by constructing a correction graph (Fig. 2). This graph is made by plotting absorbency against concentration as determined by dilution. This portion of the curve corresponds to the line labeled experimental in Figure 2. The line labeled Lambert-Beer corresponds to the theoretical absorbency of the solution.

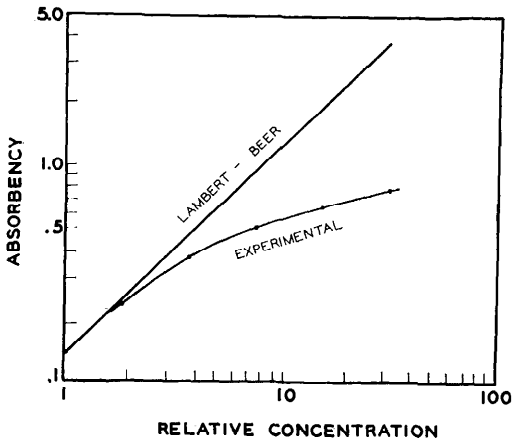

FIG. 2. A correction graph for adjusting measured phytopigment absorbency values to units related to concentration.

The correction graph is used in the following manner. The measured absorbency is found on the ordinate and is followed horizontally to intercept with the experimentally determined line; this intercept is then read vertically to intercept the extrapolated Lambert-Beer line; the absorbency unit on the ordinate opposite this intercept represents the corrected absorbency reading.

In order to avoid confusion between measured absorbency and corrected absorbency, the corrected absorbency will henceforth be designated as phytopigment units.

Experiments concerning the relationship between phytopigment units and organic weight showed that phytopigment units could be used to make quantitative estimates of organic weight when the value was less than 1.3 (Fig. 3). For samples having larger values the variations were too large to be useful as a quantitative technique. However, such samples are still valuable for comparative purposes.

It is believed that the increasing variation was due to the physical state of the algal

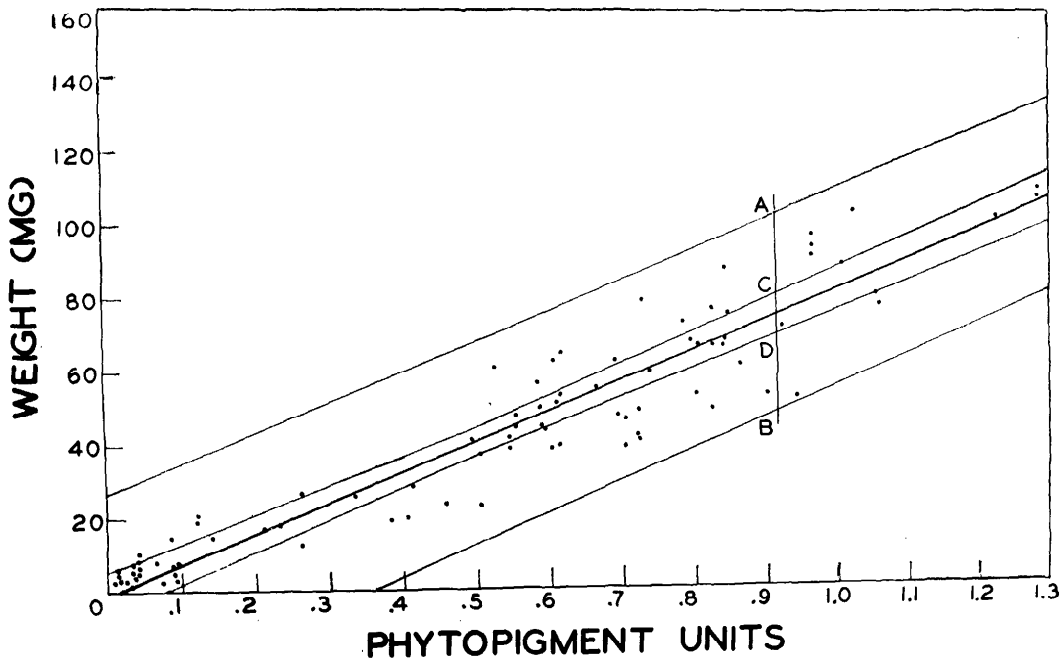

FIG. 3. The relationship between phytopigment units and periphyton weight. The 95% confidence regions are shown for estimates of $\hat{Y}$ (CD) and Y (AB).

colony. More specifically, the samples having the greatest variations were those which supported the most luxuriant growth of periphyton. Therefore, the increased variation is probably a combined effect of the death of some of the members of the colony and the accumulation of organic detritus on the surface of the substrate. For quantitative studies it is therefore important that the exposure period be short enough to avoid this condition.

The data presented in Figure 3 were collected from exposure periods ranging from one to three weeks depending upon the accrual rate of periphyton.

A linear regression was fitted to the phytopigment unit–weight relationship for values less than 1.3 (Fig. 3). The model used was Model 1 as given by Snedecor (1956). The sample regression equation for this model is

$$\hat{Y} = a + b\,X$$

where in this case

$\hat{Y}$ is the mean weight estimate
a is the intercept on the Y axis
b is the point estimator of the population slope
X is the observed phytopigment reading.

Using the computed constants the equation for predicting $\hat{Y}$ from X becomes

$$\hat{Y} = -1.72 + 82.37\ X.$$

Equations for computing a confidence region on the regression are given by Snedecor (op. cit.). The confidence region appears as curved borders on both sides of the regression. The width of the confidence region for any prediction made by X is dependent upon how far removed X is from $\bar{X}$ (the mean of the X's). Since all of the values of X in Figure 3 are near to $\bar{X}$ (0.518), the confidence region appears as two lines that are nearly parallel.

In Figure 3 a 95% confidence region is shown for estimates of Y (individual weights) and $\hat{Y}$ (mean weights). The maximum 95% confidence limit for any estimate Y made from phytopigment values ranging between 0 – 1.3 is ± 29.03 mg.

The minimum limit for any estimate of Y is found at $\bar{X}$, and in this particular case it is ± 28.43 mg. Similarly, the minimum limit on any estimate of $\hat{Y}$ is ± 3.05 mg and the maximum limit, ± 6.82 mg.

DISCUSSION

The two methods described for the collection and measurement of stream periphyton represent variations or adaptations of procedures utilized by other workers along with a statistical treatment of experimental data to relate the association between phytopigment density and the weight of organic material present on the collecting devices. The phytopigment density method represents a rapid technique utilizing relatively inexpensive laboratory equipment for the evaluation of periphyton production. This procedure may be used to determine the effects of organic enrichment on the periphyton community of a stream (Brehmer 1958) or to compare the production of areas within a stream system or to compare streams.

The relationship between the phytopigment density and the weight of organic material can be established to enable the investigator to place confidence limits on the data or to express the stream periphyton production in terms of grams/day/unit of surface area. In a stream where there are striking seasonal differences in the taxa of the periphyton community it might be desirable to compute a separate regression for each season. However, in this study one regression was found to be sufficient to make year-round predictions within the cited limits.

The method of collection can be used to study nutrient uptake by stream periphyton and has the advantage of providing the investigator with a sample of known state which is easily processed in the laboratory (Grzenda, Unpublished Report)[3].

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