Minimum Requirements for Sampling Benthic Algal Biomass in Streams[1]

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Abstract: Coefficients of variation for replicate samples of benthic chlorophyll from five sites on Ozark streams ranged from 40 to 80% for each site on a given day. To determine the attached algal biomass with a standard error of 20% of the true population mean requires a minimum of 4 to 17 samples depending on the site. To compare benthic algal data among sampling sites requires a quantification of the variability associated with sampling at each site and a calculation of appropriate sample size required for a specific degree of accuracy.

Key Words: Benthic algae, chlorophyll, variation, sample size, Missouri, streams, lotic.

Introduction

Algae attached to the substrate of streams account for much of the primary production within these aquatic systems and the planktonic algae in most flowing waters are derived from these attached algae (Swanson and Bachmann 1976, Welch 1980). Accurate measurements of benthic algae in streams are important for quantifying primary production and in assessing changes in the benthic community resulting from water pollution (Welch 1980). However, exact determination of the benthic algal biomass in streams is difficult because of patchy distribution. Patchy algal distribution results, in part, from spatial differences in current velocity, nutrient supply rate (velocity x nutrient concentration), temperature, light intensity, substrate type, and grazing by invertebrates and fishes (McIntire and Phinney 1965, Phinney and McIntire 1965, Prescott 1968, Welch 1980). The purpose of this study was to measure the variability associated with sampling the biomass of benthic algae within and among sampling sites in Missouri Ozark streams.

Study Sites

Five sites on four streams were chosen to represent the range of water quality found in Ozark streams (Table 1). Riffles were sampled for benthic algal concentrations on 26 July and 12 August 1980. Brushy Creek at Houston (Texas County) is a heavily shaded stream with high nutrient concentrations from municipal sewage input. The Big Piney River (location 1) near Cabool (Texas County) receives effluent from a lagoon system, but nutrient concentrations are moderate at Big Piney River (location 2) near Houston (Texas County). Both Spring Creek and the North Fork of the White River, which were sampled at Twin Bridges (Douglas County), have low nutrient concentrations.

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Table 1. Total phosphorus and nitrate nitrogen concentrations for the five Ozark stream sites on 27 July 1979.

Stream	Total P (mg/m^3)	NO_3-N (mg/m^3)
Brushy Creek	156	645
Big Piney (location 1)	275	535
Big Piney (location 2)	39	125
Spring Creek	8	112
North Fork of White River	17	86

Methods

Twenty rocks were selected sequentially from the stream bed at each site. Algae were scraped from a 4.5 cm^2 area on each rock and preserved in 90% acetone (Smart 1980). Samples were kept in the dark at 4°C until analysis. The samples were ground with a tissue homogenizer and chlorophyll *a* was measured by the method of Parsons and Strickland (1963). Chlorophyll *a* extractions are considered a valid indicator of benthic algal biomass (McConnell and Sigler 1959).

Results and Discussion

Benthic chlorophyll *a* measurements are extremely variable at each site (Table 2). One or two orders of magnitude separate the low and high chlorophyll concentrations of the 20 samples collected from each stream on a given day. Coefficients of variation range from 40 to 60% for the Big Piney and North Fork rivers and to 80% for Spring Creek. Replicates from Brushy Creek have a coefficient of variation from 50 to 80% (Table 2). The difference in Brushy Creek is likely because rocks large enough to sample were sparsely distributed, so different sections of the riffle are represented on the two sampling dates.

Using similar methodology, Perkins and Kaplan (1978) report replicate benthic samples of ATP from a mountain stream with coefficients of variation approaching 100%. The coefficient of variation for 55 replicated ($n=3$) benthic chlorophyll samples (area sampled $=0.33$ to 9.62 cm^2) from Iowa streams was usually between 40 and 80% (Swanson 1973). Tilley and Haushild (1975) reported coefficient of variations of about 20% for chlorophyll concentrations on glass substrates ($n=21$) in replicated Plexiglas racks. Other studies of benthic algae use multiple sampling, but do not report the variance of the replicates (Kobayasi 1961, Liaw and MacCrimmon 1978, Marker 1976). The average coefficient of variation for the density of benthic invertebrates in streams is comparable to values for our samples of benthic chlorophyll (Hornig and Pollard 1978, Egglishaw 1964).

The low sampling precision encountered in this study suggests that replicate samples are necessary to estimate the benthic algal biomass at a site. The number of samples required for a specific degree of accuracy at a given probability level can be made using the sample variability (Elliott 1977). Elliott (1977) considers a standard error of 20% of the true population mean as reasonable for samples of benthic invertebrates. To estimate benthic

Table 2. Variability in 4.5 cm² benthic chlorophyll *a* samples (n = 20) from five sites on Ozark streams.

Stream	Mean mg/m²	Range mg/m²	Standard Deviation	Coefficient[a] of Variation	Minimum sample size for the standard error to be within 20% of population mean assuming a random distribution.[a]
Brushy Creek					
7-29-80	50	4-170	41	82	17
8-12-80	84	22-181	42	50	6
Big Piney location 1					
7-29-80	370	86-595	143	39	4
8-12-80	308	123-669	153	50	6
Big Piney location 2					
7-29-80	154	33-312	86	56	8
8-12-80	111	29-226	53	48	6
Spring Creek					
7-29-80	49	7-140	39	80	15
8-12-80	56	5-154	41	73	14
North Fork of White River					
7-29-80	108	18-206	48	44	5
8-12-80	102	22-201	48	47	6

[a]Values similar assuming a negative binomial distribution.

algal biomass within this error, at a probability level of 0.05, the minimum sample size is the same for our data regardless whether a random or a negative binomial (clumped) distribution is used as the model (Table 2). Sample sizes range from 4 to 8 for the Big Piney and North Fork rivers to 13 to 15 for Spring Creek. The minimum sample size required for Brushy Creek depended on the sampling date (Table 2). Sample sizes would have to be quadrupled at each site to have the standard error be within 10% of the true population mean.

Data from this investigation show that the range of values for replicate samples from a site overlap among sites with low to high mean benthic chlorophyll concentrations. Because of this variation, estimates of algal biomass based on only a few samples would reduce the chance of detecting true differences among these sites. This suggests that one would first have to quantify the variability at each site and calculate (Elliott 1977) the appropriate sample size required to provide uniform accuracy to estimating the mean algal biomass at each site before values can be compared among sites.

Comparison of benthic algal data from the literature is difficult not only because of insufficient sample sizes, but also because methods are not standardized. Some investigators scrape algae from a known surface area on natural or artificial substrates and report the data as ATP, cell counts, biomass (weight), or chlorophyll *a* (Douglass 1958, Kobayasi 1961, Perkins and Kaplan 1978, Smart 1980). Others sample rocks from quadrats without measuring the actual surface on which the algae are growing (McConnell and Sigler 1959, Marker 1976).

Aquatic ecologists should consider these problems when estimating benthic algal biomass in streams and comparing values among sites.

Literature Cited

Douglas, B. 1958. The ecology of attached diatoms and other algae in a small stoney stream. J. Ecol. 46:295-322.

Egglishaw, H. J. 1964. The distributional relationship between bottom fauna and plant detritus in streams. J. Anim. Ecol. 33:463-476.

Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association. Scientific Publication 25. 160pp.

Hornig, C. E., and J. E. Pollard. 1978. Macroinvertebrate sampling techniques for streams in semi-arid regions. EPA-6001 4-78-040, USEPA, Las Vegas, Nevada.

Kobayasi, H. 1961. Chlorophyll content in sessile algal community of Japanese mountain river. Bot. Mag. Tokyo. 74:228-235.

Liaw, W. K, and H. R. MacCrimmon. 1978. Assessing changes in biomass of riverbed periphyton. Int. Rev. Ges. Hydrobiol. 63:155-172.

Marker, A. F. H. 1976. The benthic algae of some streams in Southern England I. Biomass of the epilithon in some small streams. J. Ecol. 64:343-358.

McConnell, J. W., and W. F. Sigler. 1959. Chlorophyll and productivity in a mountain river. Limnol. and Oceanog. 4:335-351.

McIntire, C. D., and H. K. Phinney. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35:237-258.

Parson, T. R., and J. D. Strickland. 1963. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Mar. Res. 21:155-163.

Perkins, M. A., and L. A. Kaplan. 1978. Epilithic periphyton and detritus studies in a subalpine stream. Hydrobiol. 57:103-109.

Phinney, H. K., and C. D. McIntire. 1965. Effect of temperature on metabolism of periphyton communities developed in laboratory streams. Limnol. Oceanog. 10:341-344.

Prescott, G. W. 1968. The algae: a review. Houghton Mifflin Company, Boston. 436pp.

Smart, M. M. 1980. Stream-watershed relationships in the Missouri Ozark Plateau Province. Ph.D. dissertation. University of Missouri, Columbia. 170pp.

Swanson, C. D. 1973. Algae and oxygen of some central Iowa streams. M.S. thesis, Iowa State University. Ames, Iowa. 94pp.

Swanson, C. D., and R. W. Bachmann. 1976. A model of algal exports in some Iowa streams. Ecology 57:1076-1080.

Tilley, L. J., and W. L. Haushild. 1975. Use of productivity of periphyton to estimate water quality. Jour. Water Poll. Control Fed. 47:2157-2171.

Welch, E. B. 1980. Ecological effects of waste water. Cambridge University Press. 337pp.