Hydrobiologia vol. 57, 2, pag. 103-109, 1978

EPILITHIC PERIPHYTON AND DETRITUS STUDIES IN A SUBALPINE STREAM[1]

Michael A. PERKINS[2] & Louis A. KAPLAN[3]

Division of Environmental Studies University of California Davis, California 95616

[1] This work was supported by a grant from the National Science Foundation/RANN GI-22. C. R. Goldman, Principal Investigator.

[2] Present Address: Department of Civil Engineering, University of Washington, Seattle, Washington 98195

[3] Present Address: Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19143

Received June 28, 1977

Keywords: Periphyton, detritus, ATP, SEM

Abstract

The accumulation of epilithic periphyton in Ward Creek, a permanent stream within the Lake Tahoe basin, California, was measured weekly at three stations from July through September, 1972. Subsamples were analyzed for total carbon and adenosine triposphate content. The mean total carbon content at three stations over the period of investigation was 0.508 ± 0.263 mg carbon cm^{-2}. Live biomass, as estimated from ATP measurements, averaged 0.121 ± 0.115 mg carbon cm^{-2}. It was estimated that approximately 76% of the organic carbon accumulating on rock substrates was present as detritus. Scanning electron microscopy of rock substrates suggested that much of this detrital accumulation may consist of diatom stalk materials.

Introduction

The predominately heterotrophic nature of stream ecosystems has received considerable attention over the past few years. Conceptual models relating to the origin, accumulation, and utilization of organic matter in lotic systems have been developed for specific locals (Boling, *et al.*, 1975; Cummins, 1974; McIntire, 1973; Sedell *et al.*, 1973; Fisher & Likens, 1972). Since the investigations of Nelson & Scott (1962) it has become increasingly evident that the accumulation and utilization of detrital material in streams plays a major role in the functioning of these systems (Madsen, 1972; Calow, 1975).

In the investigation of the structure and function of heterogeneous epilithic accumulations, techniques which yield qualitative and quantitative information regarding the composition of these accumulations is useful. Traditional gravimetric techniques for the determination of dry weight and ash-free weight have been criticized because the information yielded does not reveal the relationship between the autotrophic and heterotrophic components of the community (Sladeckova, 1962). A further objection to gravimetric techniques is that they fail to distinguish between the accumulation of living and detrital materials.

Various improvements in the estimation of periphyton biomass have included chlorophyll determinations (McConnell & Sigler, 1959), incorporation of phosphorus-32 (Nelson *et al.*, 1969), determinations of elemental ratios (McMahon *et al.*, 1974), organic matter and coloric content (McIntire & Phinney, 1965; Madsen, 1972), and determinations of biochemical content (Calow, 1975). Weber (1973) has supported the use of the autotrophic index to describe the relationship between the autotrophic and heterotrophic components of the periphyton community. These refinements however do not fully satisfy the aforementioned criticisms of gravimetric procedures, particularly in relation to distinguishing between the living and detrital components of the community.

Holm-Hansen & Paerl (1972) have suggested several criteria which should be satisfied if a particular cellular constituent is to serve as an estimator of microbial biomass. They also indicated that adenosine triphosphate

Dr. W. Junk b.v. Publishers - The Hague, The Netherlands

(ATP) appeared to satisfy these requirements. The utility of the determination of ATP for the estimation of microbial biomass has been demonstrated for a variety of biological systems (Holm-Hansen & Paerl, 1972; Holm-Hansen, 1973; Ausmus, 1973; Karl & LaRock, 1975). Its application would appear to be particularly promising in studies where a distinction must be made between the accumulation of living and detrital materials.

Various forms of microscopy have been a valuable addition to gravimetric techniques in the study of epilithic accumulations. Patrick *et al.* (1954), studied the species composition of diatom communities but the techniques employed eliminated consideration of spatial associations and obscured the role of other algal components of the community. Munro & Brock (1968) used fluorescene microscopy with transmitted and incident light to observe bacteria and algae on sand grains as did Madsen (1972) with detritus. The scanning electron microscopy (SEM) of periphyton on deciduous leaves by Suberkropp (Cummins, 1974) and on macrophytes by Allanson (1973) also permit the qualitative assessment of representative samples of the intact periphyton community.

In this work presented here, the epilithic periphyton community of Ward Creek was studied to quantitatively distinguish between the living and detrital components of the community using ATP analysis and to qualitatively inspect the spatial relationships of autotrophs and heterotrophs using SEM.

We wish to acknowledge Dr. H. W. Paerl and J. Pangborn who assisted with various aspects of the scanning electron microscopy and Dr. O. Holm-Hansen for discussions relating to the determination and interpretation of adenosine triphosphate. We would also like to thank Drs. R. L. Leonard and E. B. Welch for critically reviewing the manuscript and Valerie Stout for technical assistance in its preparation.

Methods and materials

The epilithic accumulations on natural substrates in Ward Creek were sampled weekly at three stations from July through September, 1972. Riffle areas having comparable bottom characteristics were chosen as sampling sites in an effort to reduce variation related to substrate type.

Systematic sampling techniques were utilized to withdraw representative samples of the extant epilithic accumulations at each station. The width of the creek was measured at each station and a nylon line was strung across the creek to establish the sampling transect. The first sample was selected randomly nad, depending upon the width of the creek, 3 to 5 additional samples were selected at uniform distances from this initial point. Metal rods suspended from the line were used to designate the location of sampling points.

Samples were withdrawn from the rock substrates using a 50 cc syringe modified to hold a nylon bristle brush on the piston end (Stockner & Armstrong, 1971). The device cleaned an area of 5.3 cm^2. Samples scraped from the rock substrates were transferred to 250 ml widemouth jars. Materials adhering to the brush were washed into the jar using prefiltered stream water delivered from a wash bottle.

Within one hour of collection, the volume of each sample was measured, thoroughly mixed, and 10 to 20 ml subsamples were filtered for total carbon and ATP analysis. Subsamples for total carbon analysis were filtered through precombusted glass fiber filters (Whatman GF/C). The filters were analyzed for their carbon content using the combustion technique of Armstrong, Goldman & Fujita (1971). Subsamples for ATP analysis were filtered onto 0.45 μm membrane filters (HA Millipore), immediately transferred to boiling Tris buffer (0.02 M, PH 7.75) and extracted for five minutes. Subsequent analysis was accomplished using an ATP Photometer (JRB Incorporated, La Jolla, California) to monitor the luciferase mediated assay. The procedure employed was essentially that of Holm-Hansen & Booth (1966). ATP values were converted to cellular organic carbon using a factor of 250 (Holm-Hansen, 1970; Holm-Hansen & Paerl, 1972; Holm-Hansen, 1973).

Samples for study with scanning electron microscopy (SEM) were chipped from the substrates using a rock hammer, tied in cheese cloth, and prepared by the critical-point drying and mounting technique outlined by Paerl and Shimp (1973).

Results

The results of the analyses for total carbon and ATP-carbon along each transect are shown in Figure 1.

It was evident that considerable variation in the accumulation of epilithic carbon between sample dates existed along each transect. Given the heterogeneous nature of streambed substrates, a fairly high degree of variation between samples collected along any one transect might be expected. Indeed, such variation was observed

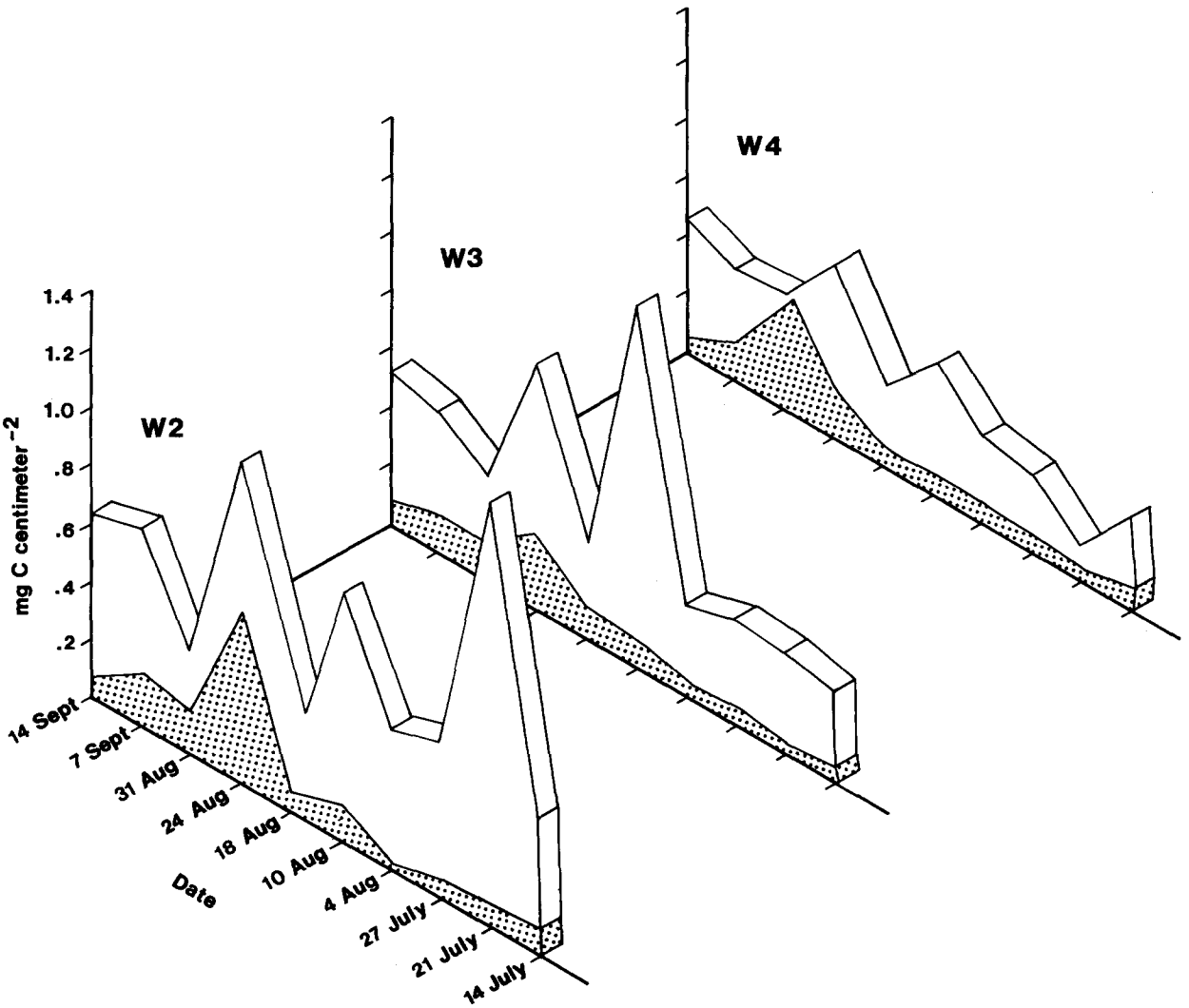

Fig. 1. Variation in the total and ATP carbon content of samples of the epilithic accumulations taken across three transects in Ward Creek. Shaded area represents ATP-Carbon.

in the samples collected, the coefficient of variance (100 x s/x) was often approaching 100%. An analysis of variance run on a logarithmic transformation of the data indicated that the variation occurring between sample dates was not significantly greater than that occuring between samples taken along any given transect. Hence, the temporal patterns shown in Figure 1 may be questionable. However, the results of these determination did indicate that substantial differences existed between the accumulation of live and detrital carbon. Further, it was evident that the variation occurring in the total carbon accumulation was more closely related to variation occurring within the detrital component. Analysis of the data indicated a highly significant, direct correlation between the accumulation of total carbon and detrital carbon ($r = 0.90$, 33 df).

The mean values (± 1 S.D.) for the total carbon content of the samples collected at the three stations were: W2 = 0.650 ± 0.328; W3 = 0.505 ± 0.234; W4 = 0.370 ± 0.126 mg carbon centimeter^{-2}. Combining the three stations, the overall mean total carbon content of the samples collected over the ten week period was 0.508 ± 0.263 mg carbon centimeter^{-2}. The mean ATP-carbon content (± 1 S.D.) at the three stations were W2 = 0.143 ± 0.157; W3 = 0.122 ± 0.086; W4 = 0.098 ± 0.096 mg carbon centimeter^{-2}. The overall mean ATP-carbon content was 0.121

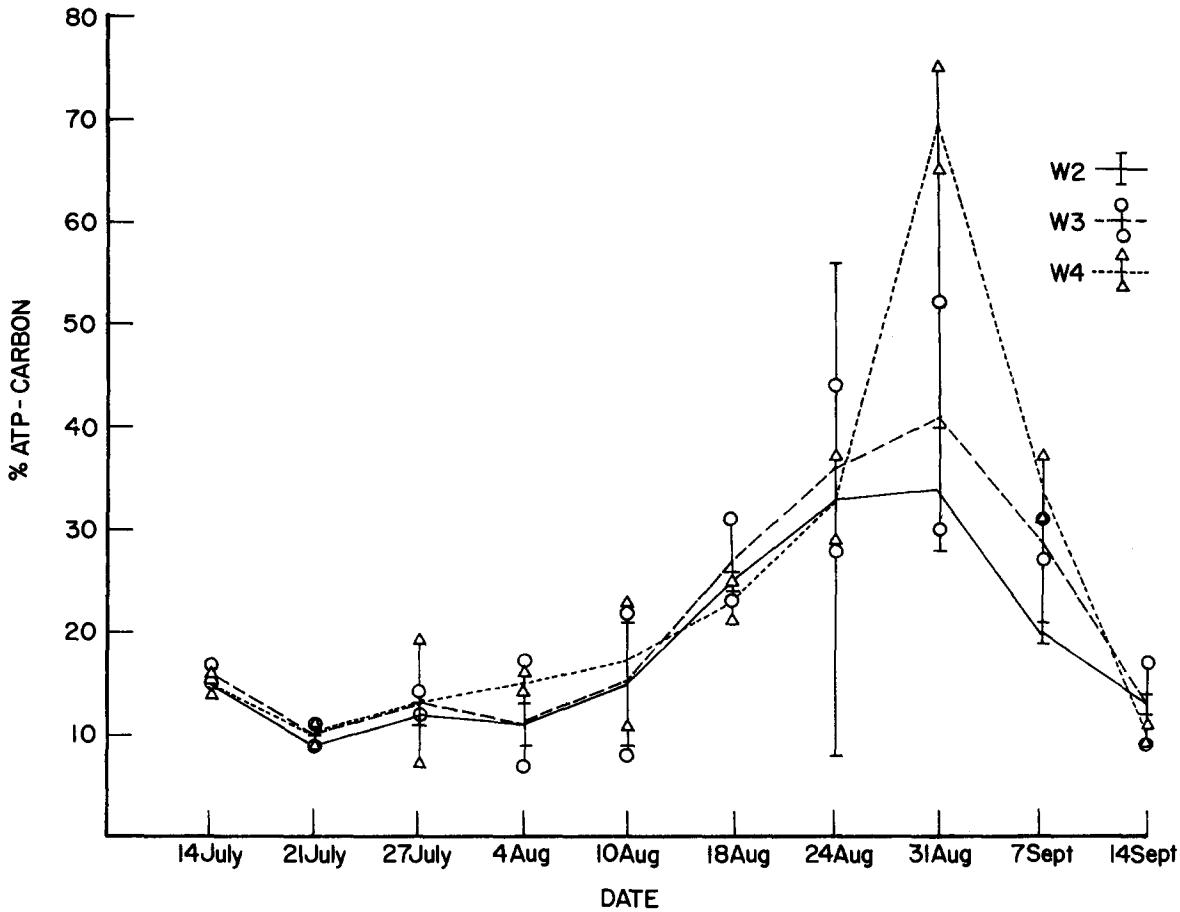

Fig. 2. ATP-carbon content of the periphyton at the three stations expresses as a percent of the total organic carbon accumulation. Confidence intervals represent two standard deviations from the mean.

± 0.115 mg carbon centimeter^{-2}. The accumulation of epilithic detritus, calculated as the difference between total and ATP-carbon, averaged 0.388 ± 0.236 mg carbon centimeter^{-2} for the samples collected over the ten week period. This represented approximately 76% of the carbon content of the samples collected from Ward Creek over the period July to September.

In contrast to total carbon and ATP-carbon values for the transects (Fig. 1), ATP-carbon expressed as a percentage of the total content showed consistency between the transects for any specific date (Fig. 2). The percent ATP-carbon at station W2 ranged from a low of 9.5% in the samples collected on 21 July to a high of 34% in those samples taken on 31 August. Minimum and maximum values at Stations W3 and W4 occurred on the same dates as those for W2 with values of 11% and 41%, and 10% and 70%, respectively. All three transects showed little change during July, a rapid rise toward a peak in late August followed by a decline in September.

Samples taken for SEM were clearly indicative of the complex nature of the epilithic accumulations in Ward Creek (Fig. 3). Of particular interest were the associations of diatoms embedded in a fibrillar matrix thought to be composed predominately of diatom stalk materials (Fig. 3a). This was particularly evident in samples collected from associations which were dominated by *Gomphonema* sp. (Fig. 3b).

Initially we had hoped that the use of SEM would allow for further elucidation of the heterotrophic component of these accumulations. However, rarely were we able to clearly discern what might be interpreted as heterotrophic biomass. In those samples where epilithic bacteria were

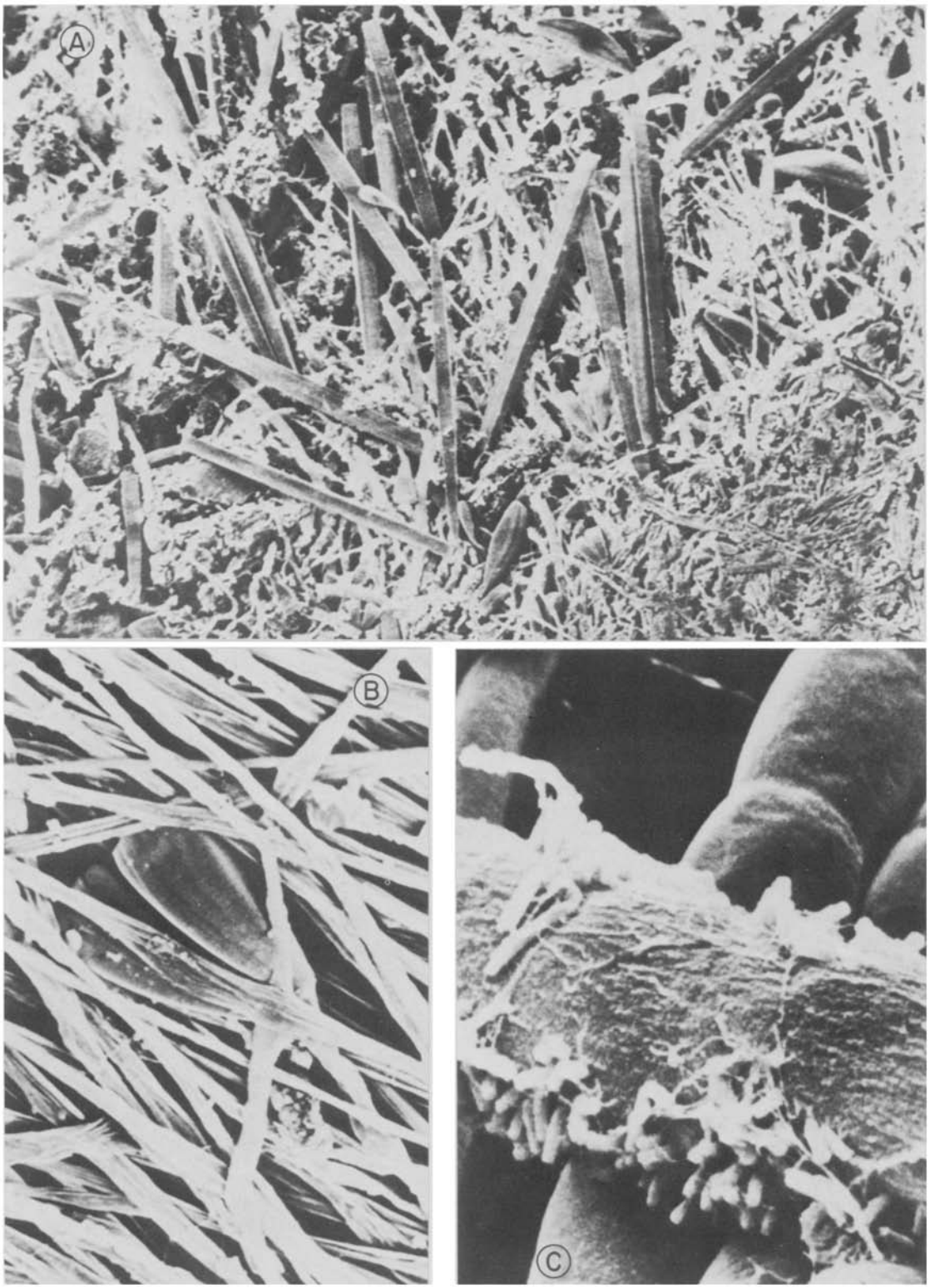

Fig. 3. Scanning electron micrographs of representative samples of the epilithic accumulations in Ward Creek.
a. low magnification micrograph of an association dominated by the diatoms *synedra* sp. and *Gomphonema* sp. (500 x).
b. *Gomphonema* sp. showing extensive production of stalk material (650 x).
c. filaments of Ulothrix sp. showing the occurrence of epiphytic bacteria (6500 x).

evident their populations appeared to be relatively small compared to the algal biomass (Fig. 3c).

Discussion

The importance of epilithic detrital accumulations as a food base for invertebrate grazers has been emphasized by Madsen (1972). Considerable significance was given to bacteria and fungi as substrate preconditioners, organic trapping agents, and as primary food sources. The results of our work on Ward Creek indicate that the ratio of living to detrital carbon within epilithic accumulations varies over time. Clearly, in studies addressing the trophic potential of periphytic accumulations it is necessary to have some quantitative assessment of their ratio. Further, in regard to the measurement of carbon flux and inorganic nutrient interactions involving periphytic accumulations, the biomass responsible for the observed activity must be known.

Estimates of periphyton biomass in streams have typically employed gravimetric techniques or chlorophyll determinations, both of which may be subject to some ambiguity in interpretation as 'live' accumulation.

Chlorophyll, while relatively easy to measure, does not remain constant relative to cellular carbon under varying light regimes or physiological states. Interpretational problems may also arise due to the presence of degradation products. However, perhaps the major objection of chlorophyll determinations are that they fail to account for the heterotrophic component of the developing community.

Dry weight and ash-free weight determinations would tend to overestimate actual biomass in the presence of detrital carbon accumulations. The error involved may be considerable in systems such as Ward Creek where detrital carbon was estimated to account for approximately 76% of the organic material accumulating on rock substrates. The utility of the combined ATP-SEM approach is suggested in regard to this detrital carbon accumulation.

Ward Creek is a relatively open stream flowing through a coniferous forest and as such, there was no input of leaf materials comparable to that found in streams of deciduous forest systems. In view of the apparent lack of substantial allochthonous carbon inputs, the accumulation of detrital carbon was somewhat surprising. The SEM observation of epilithic accumulations in Ward Creek suggested that they were composed largely of diatom stalk materials. This production of stalk materials, which are thought to be composed predominantly of polysaccarides (Huntsman & Sloneker, 1971), would represent an autochthonously derived input of detrital carbon and may account for the observed accumulations in Ward Creek.

While there are recognized deficiencies in the use of ATP as an estimator of microbial biomass, primarily in regard to the constancy of the ratio of ATP to cellular carbon, the inclusion of the heterotrophic component in the biomass estimate is clearly beneficial. The results of this study demonstrate the potential usefulness of ATP determinations for distinguishing between live and detrital carbon accumulation and for assessing the relative physiological state of the developing epilithic community. By comparing temporal patterns in the variation in ATP relative to total organic periods of high biological activity may be distinguished from periods of senescenes.

References

Allanson, B. R. 1973. The fine structure of the periphyton of Chara sp. and Potamogeton natens from Wytham Pond, Oxford and its significance to the macrophyte-periphyton metabolic model of R. G. Wetzel and H. L. Allen. Freshwat. Biol. 3: 535-542.

Armstrong, R., Goldman, C. R. & Fujita, D. K. 1971. A rapid method for use estimation of the carbon content of seston and periphyton. Limnol. and Oceanogr. 16: 137-139.

Ausmus, B. S. 1973. The use of the ATP assay in terrestrial decomposition studies. Bull. Ecol. Res. Comm. (Stockholm). 17: 223-234.

Boling, R. H., Goodman, E. D., Van Sickle, J. A., Zimmer, J. O., Cummins, K. W., Petersen, R. C. & Reice, S. R. 1975. Towards a model of detrital processing in a woodland stream. Ecology 56: 141-151.

Calow, P. 1975. On the nature and possible utility of epilithic detritus. Hydrobiologia 46: 181-189.

Cummins, K. W. 1974. Structure and function of stream ecosystems. Bioscience 24: 631-1641.

Fisher, S. G. & Likens, G. E. 1972. Stream ecosystems: Organic energy budget. Bioscience 22: 33-35.

Holm-Hansen, O. 1973. The use of ATP determinations in ecological studies. Bull. Ecol. Res. Comm. (Stockholm) 17: 215-222.

Holm-Hansen, O. 1970. ATP levels in algal cells as influenced by environmental conditions. Plant and Cell Phisiol. 11: 689-700.

Holm-Hansen, O. & Booth, C. R. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. and Oceanogr. 11: 510-519.

Holm-Hansen, O. & Paerl, H. W. 1972. The applicability of ATP determination for estimation of microbial biomass and metabolic activity. Mem. 1st. Idrobiol. 29 Suppl: 149-168.

Huntsman, S. A. & Sloneker, J. H. 1971. An exocellular polysaccaride from the diatom Gomphonema olivaceum. J. Phycol. 7: 261-264.
Karl, D. M. & LaRock, P. A. 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Board Can. 32: 599-607.
Madsen, B. L. 1972. Detritus on stones in small streams. Mem. 1st. Ital. Idrobiol. 29 Supl: 385-403.
McConnell, W. J. & Sigler, W. F. 1959. Chlorophyll and productivity in a mountain river. Limnol. and Oceanogr. 4: 335-351.
McIntire, C. D. 1973. Periphyton dynamics in laboratory streams: A simulation model and its implications. Ecol. Monogr. 43: 339-420.
McIntire, C. D. & Phinney, H. K. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35: 237-258.
McMahon, R. F., Hunter, R. O. & Russell-Hunter, W. D. 1974. Variation in aufwuchs at six freshwater habitats in terms of carbon biomass and carbon : nitrogen ratio. Hydrobiologia 45: 391-404.
Munro, A. L. S. & Brock, T. D. 1968. Distinction between bacterial and algal utilization of soluble substances in the sea. J. Gen. Microbiol. 51: 35-49.
Nelson, D. J. & Scott, D. C. 1962. Role of detritus in the productivity of a rock-outcrop community in a Piedmont Stream. Limnol. and Oceanogr. 7: 396-413.
Nelson, D. J., Kevern, N. R., Wilhm, J. L. & Griffith, N. A. 1969. Estimates of periphyton mass and stream bottom area using phosphorus-32. Water Res. 3: 367-373.
Paerl, H. W. & Shimp, S. L. 1973. Preparation of filtered plankton and detritus for study with scanning electron microscopy. Limnol. and Oceanogr. 18: 802-805.
Patrick, R., Hohn, M. H. & Wallace, J. H. 1954. A new method for determining the pattern of the diatom flora. Notulae Naturae. 259: 1-12.
Sedell, J. R., Triska, F. J., Hall, J. D., Anderson, N. H. & Lyford, J. H. 1973. Sources and fates of organic inputs in coniferous forest streams. Contributation 66, Coniferous Forest Biome, Int. Biol. Prog. Oregon State Univ. 23 pp.
Sladeckova, A. 1962. Limnological investigation methods for the periphyton ('Aufwuchs') community. Bot. Rev. 28: 286-350.
Stockner, J. G. & Armstrong, F. A. J. 1971. Periphyton of the experimental lakes areas, Northewestern Ontario. J. Fish. Res. Bd. Canada. 28: 215-229.
Weber, C. I. 1973. Recent developments in the measurement of the response of plankton and periphyton to changes in their environment. pp. 119-138. in Bioassay techniques and environmental chemistry. ed. G. E. Glass, Ann Arbor, Michigan.