J. N. Am. Benthol. Soc., 1989, 8(1):74–84

Chemical composition and microbial activity of seston in a southern Appalachian headwater stream

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Abstract. Chemical composition and microbial activity of seston (i.e., fine particulate organic matter and associated inorganic material in transport) in a southern Appalachian headwater stream were evaluated to determine whether changes in microbial activity associated with decreasing particle size were related to changes in seston surface area and/or chemical composition. As seston particle size decreased from 500 to 10 μm, organic content measured as ash free dry mass decreased from 72.5% to 36.7%. Simultaneously, the nutritional quality of the organic fraction declined, as evidenced by increases in lignin and cellulose content and corresponding reductions in acid-detergent-soluble materials (simple carbohydrates, proteins, and lipids). Microbial activity, measured as mass-specific ^{14}C-glucose mineralization and ^{3}H-thymidine incorporation rates, increased significantly as particle size decreased, despite the reduced nutritional quality of smaller particles. Microbial biomass (ATP) and activity (glucose mineralization) per unit seston surface area were proportional to particle size over the 10–250-μm particle size range. Smaller particles of lower nutritional quality supported lower area-specific microbial biomass and activity. Production : biomass ratios of microorganisms associated with seston were low (1.21 to 6.08 × 10^{-9}/hr), suggesting that these microorganisms may have become inactive in response to the gradual decline in quality of detritus as it decomposed.

Key words: seston, lignin, cellulose, microbial biomass, microbial activity.

Organic matter budgets constructed for heavily shaded headwater streams show that 85 to >99% of the annual energy input to these systems comes from allochthonous organic matter (Fisher and Likens 1973, Hornick et al. 1981, Triska et al. 1982, Conners and Naiman 1984). Leaf litter constitutes a considerable portion of the organic matter inputs (e.g., 71%; Iversen et al. 1982), but outputs are generally dominated by fine particulate organic matter (FPOM; 0.45–1000 μm) and dissolved organic matter (DOM) (Iversen et al. 1982, Fisher and Likens 1973), which suggests that much of the particulate input to streams is processed very near the point of entry by a combination of physical and biological factors.

Upon entering streams, soluble components of leaves are quickly leached, resulting in a loss of up to 25% of leaf mass (Nykvist 1963, Kaushik and Hynes 1971, Petersen and Cummins 1974). Selective utilization by microbial and invertebrate consumers subsequently removes the polysaccharides, hemicellulose and cellulose, and the remaining particulate matter is composed chiefly of refractory plant structural compounds such as lignin (Triska et al. 1975, Suberkropp et al. 1976, Rosset et al. 1982, Paul et al. 1983). Although substantial evidence suggests that this pattern holds for the breakdown of coarse particulate organic matter (CPOM), little is known of the chemical composition and microconsumer activity associated with FPOM generated through the breakdown of CPOM. If a similar pattern occurs as FPOM is decomposed and reduced in size, one would predict small particles to be more refractory, i.e., contain more lignin per unit ash free dry mass (AFDM), contain more inorganic material per unit dry mass, and support lower levels of microbial activity. However, recent evidence suggests that this may not be the case. For example, Kondratieff and Simmons (1984, 1985) found that small (<25 μm) seston particles from the Dan River, Virginia, contained more protein and supported higher bacterial cell densities per unit surface area than larger particles. Also, Ward (1986) found that small particles of benthic FPOM contained less lignin than large particles and suggested that processing patterns for benthic FPOM may be obscured by the diverse origin

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of FPOM and the coexistence of particles in a variety of decompositional states. For example, small particles can be formed through the precipitation of DOM (e.g., Bowen 1984), and the chemical composition of these particles may differ from that of particles formed by comminution of dead leaves. Further evidence that CPOM processing patterns may not hold for FPOM comes from studies of microbial activity (respiration) associated with fine particles of detritus. Many investigators have shown that as particle size decreases, mass-specific respiratory rates for associated microorganisms actually increase (e.g., Odum and de la Cruz 1967, Fenchel 1970, Hargrave 1972, Petersen et al. 1989). Increased surface area : volume ratios of smaller particles have traditionally been used to explain increased microbial activity associated with smaller particles.

The primary objective of our study was to determine whether significant changes in chemical composition occur as seston is decomposed and reduced in size. Microbial biomass and associated activity (i.e., ^{14}C-glucose mineralization and ^{3}H-thymidine incorporation) were measured to illuminate relationships between seston chemical composition, seston surface area, and microbial activity.

Study Site

Seston samples were collected from a second-order tributary of Big Stony Creek (elevation = 850 m) in Jefferson National Forest, Giles County, Virginia, USA (latitude 37°25′N; longitude 80°31′W). This soft-water stream (hardness <30 mg/L) flows through a mature oak-hickory forest with a well-developed rhododendron (*Rhododendron*) understory. The average seston concentration at base-flow was 5.3 mg dry mass/L, and mean dissolved NO_3-N and PO_4-P concentrations during the study were 0.031 ± 0.006 (SD) mg/L and <0.010 mg/L, respectively (n = 11).

Methods

Sample collection

Seston was collected at base-flow and separated into four size classes (10–53, 53–106, 106–250, and 250–500 μm) for glucose mineralization experiments and analyses of chemical composition. Evaluations of seston chemical composition, glucose mineralization, and ATP concentration were restricted to FPOM >10 μm because of the difficulty of collecting sufficient quantity of very small particles. The three largest size classes were collected by passing stream water through a series of standard brass sieves (Fisher Scientific), and filtrate from the sieve series was passed through 10-μm plankton netting to obtain 10–53-μm particles. Four size classes were used in ^{3}H-thymidine incorporation experiments: 0.45–53, 53–106, 106–250, and 250–500 μm. Filtrate from the 53-μm sieve was passed through a 0.45-μm glass fiber filter (Gelman A/E) using low-vacuum filtration to obtain 0.45–53-μm particles. Particles were rinsed from the filter using filter-sterilized stream water.

Samples were collected for analysis of glucose mineralization rates, adenosine triphosphate (ATP) content, and chemical composition on three dates in January 1986 and approximately bimonthly thereafter through October 1986. ^{3}H-thymidine incorporation rates were measured in March, May, and June 1986.

Analysis of seston surface area

Surface areas of particles in each size fraction were measured for use in converting microbial biomass and activity estimates (expressed on a mass basis) to biomass and activity per unit seston surface area. At least 500 particles per size class were measured using compound and dissecting microscopes equipped with ocular micrometers. For each size fraction, a series of concentrated seston samples (1 ml) was placed on Metricel membrane filters to count the total number of particles per ml and to measure particle surface area. Replicate 1-ml samples were placed on tared, glass-fiber filters to determine dry mass (24 hr at 50°C) and AFDM (0.5 hr at 550°C) per ml. Mean dry mass and AFDM per particle were calculated as dry mass or AFDM per ml divided by the number of particles per ml. Mean surface area per unit seston mass was calculated as mean surface area per particle divided by mean dry mass and AFDM per particle. Visual inspection indicated that >95% of seston particles 10–250 μm were approximately spherical. Consequently, surface area calculations for these particles were based on measurements of particle diameter and an assumption of spherical geometry. Particles 250–500 μm were placed

in one of three categories based on overall geometry: (1) flat sheets (e.g., thin sheets of plant mesophyll cells); (2) cylinders; and (3) spheres. Appropriate dimensions of these particles were measured for surface area calculations. Particle sizes were analyzed on several dates, and means of these data were used in subsequent calculations.

Analysis of seston chemical composition

Lignin, cellulose, and acid-detergent-soluble material (ADSM) were analyzed using the permanganate lignin, cellulose, and ash procedure of Goering and Van Soest (1970). Samples (200–500 mg) were dried at 50°C, ground by mortar and pestle, placed in 500-ml round bottom flasks with 100 ml of acid detergent solution and 2 ml decahydronapthalene, and refluxed for 60 min to remove ADSM such as simple carbohydrates, lipids, and proteins. Lignin was oxidized in a saturated solution of potassium permanganate for 90 min and removed by rinsing with demineralizing solution, ethanol, and acetone. Cellulose was subsequently determined as loss on ignition at 550°C.

Analysis of microbial activity and biomass

Microbial activity was measured by quantifying the mineralization of ^{14}C-glucose (Williams and Askew 1968). Seston (5-ml subsamples) was pipetted into 25-ml Erlenmeyer incubation flasks, and trace quantities (0.050 ml; 0.1 μCi/ml) of uniformly labeled ^{14}C-glucose (345 mCi/mmol) were added to achieve final concentrations of 0.5 μg glucose/L. Flasks were sealed with rubber septa and incubated for 3 hr in a water bath at one of three test temperatures selected to approximate ambient stream temperature (Jan = 5°C; Mar–May = 10°C; Jun–Sep = 15°C; Oct = 10°C). Less than 10% of the added ^{14}C was respired during the experiment. Incubations were terminated by acidifying with 0.2 ml 6N H_2SO_4. Strips of phenethylamine-saturated chromatography paper (2 × 4 cm) suspended above the samples in filter cups (Kontes, Inc.) were used to trap $^{14}CO_2$ evolved during the experiments (Hobbie and Crawford 1969). Paper strips were transferred to 20-ml borosilicate glass scintillation vials containing 10 ml Scintiverse E (Fisher Scientific) and radioassayed using a Beckman Model LS-3105T Liquid Scintillation Counter. Counting efficiency and quench were determined using ^{14}C-$NaHCO_3$ and internal standards. Dry mass and AFDM of incubated samples were determined as described above. Filtered stream water (0.45-μm glass-fiber filter) incubated with ^{14}C-glucose was used to account for microbial activity associated with free (i.e., unattached) bacteria, and 5-ml distilled water samples were incubated to check for activity associated with airborne microbial contaminants and microorganisms attached to glassware. Controls killed with H_2SO_4 were used to check for abiotic release of $^{14}CO_2$. Oxygen and pH were monitored during incubations to ensure that conditions in the flasks remained constant. All incubations were initiated within 4 hr of sample collection.

Microbial ATP associated with seston was assayed using a modification of the technique employed by Holm-Hansen and Booth (1966). ATP was extracted from 2–5-ml concentrated seston subsamples in 15–18 ml boiling Tris buffer (92–95°C) for 5 min. Extracts were frozen at −4°C and later assayed using a Lab-Line Model 9140 ATP Photometer. ATP extracts (0.1 ml) were injected into 0.4-ml luciferin-luciferase enzyme solutions (Analytical Luminescence Laboratories, Inc.), and emitted light was measured over 6-s intervals. Enzyme solutions and ATP standards used to generate standard curves were prepared in Tris buffer. All ATP concentrations were corrected for recovery (86–91%), which was quantified by adding known quantities of ATP to replicate seston samples before extraction.

Analysis of microbial production

Bacterial production was estimated by quantifying ^{3}H-thymidine incorporation rates (Fuhrman and Azam 1980, Riemann et al. 1982). Samples were prepared as in the glucose mineralization experiments and incubated at the same temperatures for 1 hr with methyl-^{3}H-thymidine (0.5 ml; 13.75 μCi/ml) added to achieve final concentrations of 10 nM thymidine/L. Although ^{3}H-thymidine concentrations of 5 to 10 nM have generally proven adequate for estimating microbial production in aquatic systems (e.g., Riemann et al. 1982, Murray and Hodson 1985), incorporation rates were initially measured at thymidine concentrations of 5, 10, and 20 nM to insure maximal participation of ^{3}H-

TABLE 1. Mean surface area estimates for size-fractionated seston samples.

Size Fraction (μm)	n	Surface Area (μm²/particle)	Dry Mass (μg DM/ particle)	Surface Area (cm²/g DM)	Surface Area (cm²/g AFDM)	AFDM/ Surface Area (μg AFDM/ cm²)
10–53	507	1510	1	10,500	28,600	35
53–106	514	12,600	59	2100	4200	238
106–250	536	46,200	330	1400	2300	435
250–500	508	5,910,000	3700	16,000	22,100	45

thymidine in DNA synthesis. Because no significant differences were observed between 10- and 20-nM concentrations, 10-nM levels were used in subsequent experiments. Incubations were terminated by injecting 0.15 ml formaldehyde (final concentration = 1%) and formaldehyde-killed samples were used as controls. Flasks were immediately placed on ice, and cold 10% trichloroacetic acid (TCA) was added to remove unincorporated thymidine from seston particles and microbial cells. Samples were subsequently filtered onto Metricel membrane filters (0.45 μm nominal pore size), rinsed three times with cold 10% TCA, transferred to scintillation vials containing 10 ml Scintiverse E, and radioassayed.

Statistical analyses

Regression analyses were used to evaluate trends in microbial activity, microbial biomass, and chemical composition according to particle size. In these analyses, geometric means were used to estimate mean particle diameter for each size fraction.

Results

Seston surface area and particle size distribution

For the four particle size classes evaluated, seston surface area ranged from 5,910,000 to 1,510 μm²/particle (Table 1). Mean dry mass per particle ranged from 1 to 3,700 μg dry mass/particle. Although surface area per particle and dry mass per particle decreased as particle size decreased, surface area:dry mass and surface area:AFDM ratios were inversely related to particle size only for particles <250 μm. Seston particles in the 250–500-μm size class had much higher surface area:mass ratios than particles <250 μm. These large particles were distributed among three categories: 40.6% spheres, 20.3% cylinders, and 39.1% flat sheets. Flat particles accounted for 97.8% of the total surface area within this size class. Consequently, surface area:mass ratios for 250–500-μm particles were much higher than those for particles <250 μm, which were almost exclusively spherical.

Because the frequency distributions of particles in the 10–53, 53–106, and 106–250-μm size fractions approximated negative exponential curves (Fig. 1), geometric mean particle sizes were used in regression analyses. Although mean particle diameters and particle size distributions were not computed for the largest size fraction because of variations in particle morphology, geometric mean particle sizes were used in the 250–500-μm size fraction for consistency.

Seston chemical composition

As seston particle size decreased, the quantity and quality of organic material decreased significantly. Ash content was inversely related to particle size ($p = 0.0001$) and increased from 27.5% in 250–500-μm seston to 63.3% in 10–53-μm particles (Table 2). Correspondingly, the organic portion of seston (i.e., AFDM) decreased as particle size decreased, from 72.5% in the 250–500-μm fraction to 36.7% in the smallest seston size class.

The reduction in organic content associated with decreasing particle size was coupled with a significant decrease in nutritional quality. As particle size decreased from 500 to 10 μm, refractory plant structural compounds, such as lignin and cellulose, became more prevalent in the AFDM portion of seston, while the acid detergent soluble fraction, typically composed

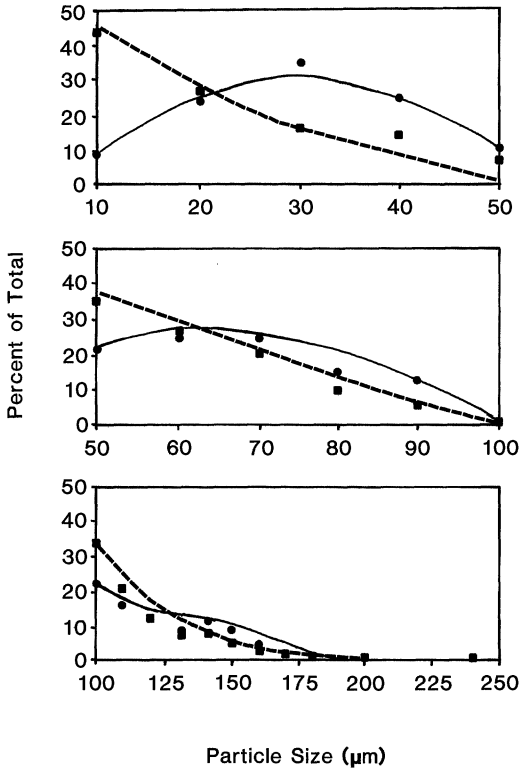

FIG. 1. Frequency (■) and surface area (●) distributions of 10–53, 53–106, and 106–250-μm seston particles expressed as a percent of total frequency and surface area for each size class.

of simple carbohydrates, lipids and proteins, declined (Table 2). Lignin content expressed as a percentage of AFDM was inversely related to particle size ($p = 0.0001$), ranging from 25.3% in the 250–500-μm size class to 54.3% in the 10–53-μm fraction. Cellulose content was also inversely related to particle size ($p = 0.008$) and ranged from 5.3 to 14.1% (% of AFDM). The acid detergent soluble fraction of seston decreased from 69.2 to 31.6% (% of AFDM) as particle size decreased from the 250–500 to the 10–53-μm size fraction.

Microbial activity

Glucose mineralization rates ranged from 235 to 810 ng glucose per g seston AFDM per hr (Fig. 2) and increased significantly ($r^2 = 0.21$, $p = 0.0001$) as seston particle size decreased from the 250–500 to the 10–53-μm size class on every sample date. However, glucose mineralization rates expressed on a surface area basis ranged from 11 to 156 pg glucose per cm^2 per hr and decreased significantly ($r^2 = 0.33$, $p = 0.0001$) as particle size decreased from the 106–250 to the 10–53-μm size fractions. In the 250–500-μm size fraction, glucose mineralization per unit surface area was much lower (11 pg glucose per cm^2 per hr) than that associated with 10–250-μm particles.

Microbial biomass

Estimates of viable microbial biomass (ATP) per unit seston surface area were consistent with the results of the glucose mineralization experiments. Although there was no significant correlation ($r^2 = 0.01$, $p = 0.67$) between ATP per unit AFDM and particle size, ATP per unit seston surface area decreased as particle size decreased ($r^2 = 0.31$, $p = 0.039$) over the particle size range from 250 to 10 μm (Fig. 2), just as glucose mineralization decreased over this size range. ATP levels per unit surface area were much lower in the 250–500-μm fraction, as were glucose mineralization rates.

Microbial production

^{3}H-Thymidine incorporation rates for 53–500-μm particles ranged from 3.15 to 11.9 × 10^{-14} g thymidine per g AFDM per hr and were inversely related to particle size ($r^2 = 0.83$, $p = 0.0001$; Fig. 2) within the 53–500-μm range. However, mean thymidine incorporation rates for 0.45–53-μm particles were much lower than rates associated with 53–106-μm particles.

Discussion

Seston surface area

Observed estimates of surface area : mass (Table 1) were similar to reported estimates of the surface area of detritus (Odum and de la Cruz 1967, Fenchel 1970, Hargrave 1972). One exception was the discrepancy between estimates of surface area for 250–500-μm seston and for 400-μm *Thalassia* detritus (Fenchel 1970). Apparently, this difference resulted from Fenchel's assumption that *Thalassia* particles were spherical, whereas most 250–500-μm seston particles from the Big Stony Creek tributary were flat.

TABLE 2. Ash, lignin, cellulose, and acid detergent soluble material (ADSM) in size-fractionated seston samples (mean ± 1 SE) and coefficients of determination (r^2) for regression against geometric mean particle size.

Size Fraction (μm)	n	% Ash (% of DM)	% Lignin (% of AFDM)	% Cellulose (% of AFDM)	% ADSM (% of AFDM)
10–53	24	63.3 ± 2.27	54.3 ± 4.6	14.1 ± 2.3	31.6
53–106	24	49.0 ± 1.73	50.4 ± 4.5	5.8 ± 0.5	43.8
106–250	24	40.4 ± 2.59	37.9 ± 3.5	5.3 ± 0.3	56.8
250–500	24	27.5 ± 3.07	25.3 ± 5.5	5.5 ± 1.1	69.2
		$r^2 = 0.72$ ($p = 0.0001$)	$r^2 = 0.35$ ($p = 0.0001$)	$r^2 = 0.16$ ($p = 0.008$)	

Microbial biomass and activity per unit seston surface area were extremely low in the 250–500-μm size fraction and did not fit the trends observed in the three smaller seston size classes. If one assumes that all 250–500-μm particles are spherical, surface area : mass ratios in this size fraction would be <2300 cm²/g AFDM and would fall in line with the surface area per unit mass trends observed over the 10–53, 53–106, and 106–250-μm particle size ranges (Table 1). Microbial biomass and activity estimates for the 250–500-μm particles would then fit the trends observed in the smallest three size fractions (i.e., microbial biomass and activity per unit surface area would then decrease as particle size decreases from 500 to 10 μm). Thus, the fact that 39.1% of the 250–500-μm particles were flat, with much higher surface area : mass ratios than smaller particles, was of critical importance in evaluating trends in microbial biomass and activity per unit seston surface area. Our findings suggest that generalizations regarding microbial respiration and seston decomposition rates based on particle diameter and the assumption of spherical morphology should be interpreted with caution.

Seston chemical composition

Assuming small seston particles are produced through the breakdown of larger particles and CPOM, one would predict smaller particles to be more refractory than the large particles from which they originated. In this study, changes in chemical composition associated with decreasing seston particle size supported this prediction. As particle size decreased, the quantity of organic matter per unit seston dry mass decreased significantly. Coupled with the decrease in organic content was a significant decrease in the quality of the AFDM fraction. Lignin and cellulose constituted 30.8% of the AFDM of 250–500-μm particles, while smaller and presumably more processed 10–53-μm particles were 68.3% lignin and cellulose. Correspondingly, the concentration of acid-detergent-soluble materials decreased as particle size decreased.

Lignin content, expressed as a percentage of AFDM, increased significantly as particle size decreased from 500 to 10 μm. Ward (1986) found similar lignin concentrations in benthic FPOM in three streams of the Oregon Cascades (mean lignin content = 45%). However, in contrast to the predicted trend of increasing lignin content for smaller size fractions, he found that lignin content generally decreased as particle size decreased. Our ability to distinguish relative changes in lignin content per unit AFDM among the various size fractions may be related to sampling a relatively homogeneous portion of the detritus pool in streams by limiting our samples to FPOM in transport (i.e., seston). Ward (1986) noted that the heterogeneous mixture of benthic FPOM, created by the input of organic matter from a variety of sources with differing processing regimes, might have obscured the predicted trend.

Cellulose content increased significantly as particle size decreased, but this trend was not as clearly defined as that observed for lignin. Percent cellulose did not change significantly ($p = 0.81$) as particle size decreased from 500 to 53 μm (mean cellulose content 5.27–5.80%), although cellulose content in the 10–53-μm size class was much greater (mean = 14.1%).

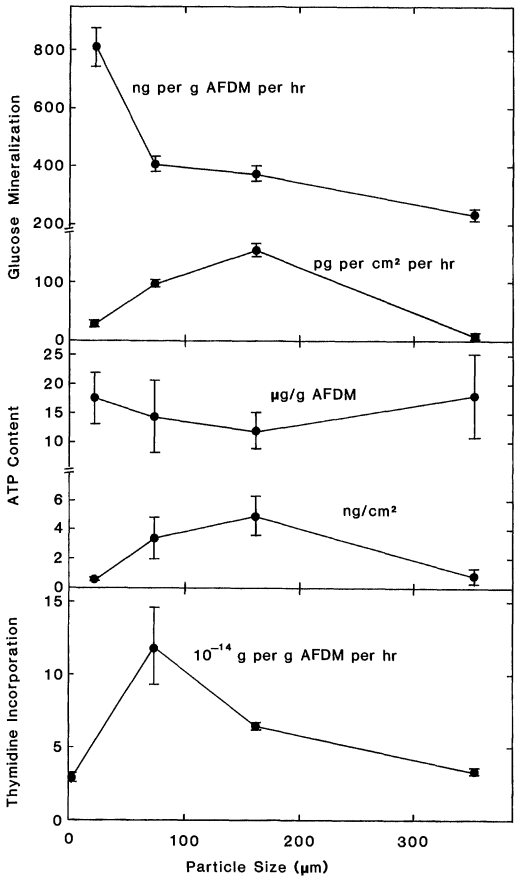

FIG. 2. Glucose mineralization rates, ATP, and thymidine incorporation rates on seston of various particle sizes. Error bars are ±1 SE.

Microbial activity

On every sample date, glucose mineralization rates expressed on an AFDM basis were inversely related to particle size. These results are similar to trends observed by other investigators of microbial activity associated with particulate matter in aquatic environments. For example, Odum and de la Cruz (1967), Fenchel (1970), Naiman and Sedell (1979a), and Petersen et al. (1989) observed inverse relationships between oxygen consumption and detritus particle size, and Hargrave (1972) found an inverse correlation between oxygen uptake rates and particle diameter for a variety of particles, including pebbles, sand, lake mud, and detritus. This trend has been attributed to the fact that smaller particles have greater surface area for microbial colonization per unit mass than larger particles of similar morphology (e.g., Hargrave 1972). In other studies, no correlation was found between microbial activity and detritus particle size. For example, Ward (1986) found no statistically significant differences in oxygen consumption in benthic organic matter ranging from 10 µm to 1 mm. Naiman and Sedell (1979b) found either positive correlations between oxygen consumption rates and particle size or no correlation for POM in transport in Oregon Cascade streams.

Since the quality of FPOM indicated by chemical composition appears to decrease as particles are processed, low levels of mass-specific microbial activity associated with small particles (e.g., Naiman and Sedell 1979b) might be attributed to these changes. In other cases where increased microbial activity per unit mass has been associated with smaller particles, the increased surface area : AFDM ratios of smaller particles apparently compensates for altered quality by providing greater surface area for microbial colonization. It appears that for seston, small particles supported greater mass-specific activity primarily as a result of increased surface area : mass ratios that provided greater surface area for microbial colonization. This idea is supported by our findings that small particles were significantly more refractory and supported lower area-specific microbial activity. Similar findings were recently reported by Petersen et al. (1989), who noted that microbial respiration associated with benthic detritus appeared to be controlled by nutritional quality, despite the overwhelming influence of surface area on mass-specific respiration rates.

Microbial biomass

Analyses of ATP per unit AFDM confirmed the suggestion that microbial activity was strongly influenced by the effects of surface area : mass ratios on microbial colonization. As particle size decreased from 250 to 10 µm, ATP per unit AFDM increased, even though organic content decreased. Regression analysis indicated that the relationship between ATP per unit AFDM and seston particle size was not statistically significant. However, Wallace et al. (1982) similarly reported that ATP levels associated with suspended FPOM 0.45–864 µm collected from Dryman Fork, North Carolina, increased as particle size decreased, ranging from 11.6 to 57.6 nM ATP/g AFDM (or 2.81–14.0 µg ATP/g

AFDM; our calculation). Thus, there is some evidence that smaller size fractions, with lower organic content, have greater microbial biomass and activity per unit AFDM, apparently because of increased surface area : mass ratios.

As particle size decreased in the 250–10-μm size range, microbial biomass per unit surface area decreased by 88% (Fig. 2). Concurrently, ash content increased 64% (Table 2) and the quantity of AFDM per unit surface area decreased from 435 to 35 $\mu g/cm^2$ (Table 1). Thus, because smaller particles contain less organic material per unit surface area and more refractory chemical compounds such as lignin (Table 2), they apparently support lower levels of area-specific microbial biomass. These data suggest that the chemical composition of seston exerts a significant degree of control over microbial colonization and activity. However, microorganisms have been shown to attach to particulate material to more efficiently use localized increases in DOM on particle surfaces (e.g., ZoBell 1943), and DOM may provide a substantial portion of microbial nutrition.

Patterns of microbial biomass and activity associated with the largest seston size fraction (250–500 μm) deviated slightly from those on 10–250-μm particles. Glucose mineralization rates and ATP concentrations per unit seston surface area for 250–500-μm seston were much lower than those associated with the 106–250-μm fraction even though the 250–500-μm particles were of higher quality. Low levels of microbial biomass and activity on the 250–500-μm particles may have resulted from macroinvertebrate grazing, though the fact that filter-feeding macroinvertebrates process a small fraction of the total seston load in rivers (McCullough et al. 1979a, 1979b, Benke and Wallace 1980, Parker and Voshell 1983) makes this unlikely. Another possibility is that these particles were recently generated from CPOM and had not had sufficient time for complete microbial colonization. Although it is possible that 250–500-μm seston is colonized slowly, there is no readily apparent reason why these particles should be colonized less rapidly than smaller particles. A third possibility is that significant quantities of microbial biomass were removed by grazing protozoans. Fenchel and Blackburn (1979) found that bacterial density increased by a factor of 2 to 10 when protozoa were experimentally excluded from microbial communities and suggested that bacterial density on detrital particles is controlled mainly by grazing of protozoa and other microfauna. Other studies (e.g., Caron 1987) have also shown that microflagellates can significantly affect bacterial density on planktonic particles. In the Big Stony Creek tributary, smaller particles may not support levels of bacterial production high enough to attract or support substantial grazing by protozoa, while larger, more labile particles may support extensive microbial production and greater protozoan grazing. Although microbial production on 250–500-μm particles was low, this estimate may reflect only instantaneous production by a microbial community that is periodically grazed by protozoans. We occasionally observed protozoans on 250–500-μm particles during the analyses of seston surface area but rarely, if ever, on smaller seston particles.

To roughly estimate the number of microbial cells on seston, we first converted ATP to microbial biomass using an ATP : carbon ratio of 1:250 (Hamilton and Holm-Hansen 1967). This conversion resulted in biomass estimates ranging from 2.95 to 4.48 mg carbon/g AFDM for the different particle size fractions. We then estimated cell densities using a conversion factor of 1.43×10^{-14} g carbon/cell (Bott et al. 1984, Lovell and Konopka 1985, Murray and Hodson 1985). Estimates of cell numbers ranged from 1.1 to 8.6 $\times 10^7$ cells/cm^2. These estimates are greater than other estimates of microbial densities on similar forms of detritus. Fenchel (1970) found approximately 3×10^6 cells/cm^2 on *Thalassia* detritus, and Tsernoglou and Anthony (1971) observed 0.3–1.4 $\times 10^6$ cells/cm^2 on freshwater sediments, and sand, pebble, and soil surfaces. Also, Kondratieff and Simmons (1985) found mean bacterial cell densities ranging from 3.0 to 8.3 $\times 10^6$ cells/cm^2 on seston particles from the Dan River, Virginia. Although our data suggest that seston particles are well colonized, such results should be interpreted with caution. Measurement of seston surface area is difficult, and the assumption of spherical geometry results in an underestimate of surface area for seston particles having irregular surfaces.

Microbial production

Thymidine incorporation rates were converted to production rates using conversion factors of 2.1×10^{18} cells produced per mole of thymidine incorporated, corrected for 20% non-DNA activity in the TCA insoluble mixture

(Fuhrman and Azam 1980, 1982, Riemann et al. 1982, Lovell and Konopka 1985, Murray and Hodson 1985), and 1.43 × 10^{-14} g carbon/cell (Bott et al. 1984, Lovell and Konopka 1985, Murray and Hodson 1985). Production rates ranged from 5.3 to 21.6 × 10^{-12} g carbon per g AFDM per hr. Dividing these production rates by microbial biomass estimated from ATP measurements gave P:B ratios of 1.21 to 6.08 × 10^{-9}/hr. There are several possible explanations for these extremely low production rates and P:B ratios. First, the various conversion factors may not be appropriate to our situation. However, even if each conversion factor is off by an order of magnitude, estimates of production and P:B ratios are still very small. Second, the thymidine technique we employed probably measures primarily bacterial production (J. L. Meyer, University of Georgia, personal communication). Much of the biomass on our seston particles may have been fungal and thus measured as biomass but not included in the production measurements. Third, most of the organisms associated with seston may have been inactive. Roszak and Colwell (1987) recently discussed the growing awareness that bacteria in oligotrophic environments frequently enter a state of inactivity in which they fail to divide (and synthesize DNA) while continuing to respire and synthesize RNA and protein. Apparently, reduced respiration and metabolism facilitate survival under low-nutrient conditions (Boylen and Ensign 1970, Meyer-Reil 1978, Nelson and Parkinson 1978, Novitsky and Morita 1978, Kurath and Morita 1983). In the present case, it appears that microbes associated with seston were not undergoing cell division. Instead, they appeared to be maintaining metabolic rates at levels indicative of low-nutrient conditions. Low microbial activity is probably a reflection of the refractory nature of seston and the low concentration of labile DOM and mineral nutrients in the water column. Particles may simply be colonized by microorganisms that attack CPOM and remain attached in relatively inactive forms when the nutritional value of the particle is exhausted. Also, seston particles are not perfect conglomerates of lignin, cellulose, and inorganic material. Many particles are almost exclusively inorganic, while a small portion of the seston pool is composed predominantly of more labile, organic material. For this reason, it seems logical that most microbial activity may be associated with a few labile particles that decompose much more rapidly than the remaining refractory particles.

Seston processing in streams

Several studies have shown that the breakdown of CPOM produces substantial quantities of FPOM. For example, Ward (1984) found that 33% of the dry mass of decaying pignut hickory leaves held in artificial streams was released as FPOM and that 71% of these fine particles were <53 μm. Despite the apparent input of large quantities of FPOM <53 μm, particles in this size range do not accumulate in streams to the extent one might expect based on decomposition estimates. For example, FPOM <53 μm represents approximately 7.5 to 15.6% of the benthic organic matter pool in streams (Naiman and Sedell 1979a) but amounts to 70–82.5% of all drifting organic matter (Sedell et al. 1978, Naiman and Sedell 1979b).

If small, labile particles of organic matter generated through the breakdown of CPOM are quickly entrained in the water column, substantial quantities of carbon may be transported downstream. However, from the limited data concerning the chemical composition of seston and benthic FPOM in streams, it appears that CPOM processing may be an efficient process that removes much of the labile portion of detritus. Fine particles of detritus contain relatively high concentrations of ash and refractory plant structural material (i.e., lignin) and may be of much less nutritional value to microbial and invertebrate consumers than CPOM.

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Received: 8 June 1988
Accepted: 9 December 1988