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Bacterial Growth on Dissolved Organic Carbon from a Blackwater River

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Abstract. Different nominal molecular weight (nMW) fractions of DOC from a southeastern blackwater river were concentrated by ultrafiltration and added to sieved river water to assess each fraction's ability to stimulate bacterial growth. Bacterial growth was measured using change in bacterial biomass from direct counts and using ^3H -thymidine incorporated into DNA. Bacterial growth and amount of DOC used was greatest in the low MW enrichment (<1,000 nMW) and least in the intermediate MW enrichment (1,000–10,000 nMW). The high MW fraction (>10,000 nMW) supported more growth than did the intermediate MW fraction, apparently because of lower MW compounds complexed with a high MW refractory core. The low MW fraction of DOC from a clearwater mountain stream, a boreal blackwater river, and leachate from water oak and willow leaves also stimulated more bacterial growth than did other fractions. However, the high MW DOC from these other sources was not as biologically available as high MW DOC from a blackwater river. Bacteria converted blackwater river DOC to bacterial biomass with an efficiency of 31%. Bacteria produced at the expense of abundant riverine DOC provide a trophic resource for protozoa and higher levels of the microbial food web of a blackwater river.

Introduction

Dissolved organic carbon (DOC) is an abundant organic carbon source in natural waters (e.g., Ref. 47), yet it is incompletely characterized either with respect to its chemical composition (e.g., Ref. 44) or its availability to bacteria. Although there are numerous measures of bacterial growth on specific compounds (e.g., Ref. 3), these measures are difficult to relate to bacterial growth in nature because single compounds are such a small fraction of total DOC present (e.g., Ref. 24). There have been few attempts to quantify bacterial growth on the heterogeneous mixture of organics that comprise DOC in natural waters [9, 25].

The question of which fractions of the naturally occurring DOC are available to bacteria is of critical importance in evaluating the potential role of microbial food webs in natural waters. In marine planktonic systems, the link between

algal-produced DOC and bacterial growth is clear (e.g., Ref. 4). In rivers such as the blackwater river we are studying, where primary productivity is low [R. T. Edwards (1985) The role of seston bacteria in the metabolism and secondary production dynamics of southeastern blackwater rivers. PhD Thesis, University of Georgia] compared with allochthonous inputs [T. F. Cuffney (1984) Characteristics of riparian flooding and its impact upon the processing and exchange of organic matter in coastal plain streams of Georgia. PhD Thesis, University of Georgia], algal exudates probably support only a small fraction of observed bacterial production [18]. In these systems bacterial growth would more likely be at the expense of DOC leached from the surrounding watershed.

DOC leached from freshly fallen leaves is removed from the water by biotic processes in streams [11], and the lower molecular weight fractions (<10,000 nominal molecular weight) appear the most available to bacteria [23]. However, it is inappropriate to base predictions about bacterial uptake of riverine DOC from studies of fresh leaf leachate. Much of the labile DOC in sources such as leachate appears rapidly removed, leaving the refractory compounds to be transported downstream [48]. Hence, in the sixth-order blackwater river we are studying, one might expect that much of the labile DOC had already been removed.

Blackwater rivers characteristically have tea-colored waters due to the relatively high concentrations of humic substances leached from the sandy soils of their watershed [39]. In our studies of the Ogeechee River, a sixth-order blackwater river system in the southeastern United States, we have found high DOC concentrations (mean = 12.7 mg C/liter in the mainstream and 30.8 mg C/liter in a fourth-order tributary [28]). Although DOC concentration is commonly high in blackwater rivers, much of the DOC is humic substances that have been considered refractory to bacterial attack [6]. However, there is evidence that structurally complex aquatic humic substances of high molecular weight may stimulate microbial growth by increasing trace metal availability (e.g., Ref. 34) or by cometabolism [12, 13]. Humic substances in the soil, from which the aquatic material originates, have been demonstrated to stimulate bacterial growth (e.g., Ref. 45).

Bacteria are abundant in the water column of the Ogeechee River and Black Creek, with an annual average density of 1.5×10^{10} cells/liter [R. T. Edwards (1985) PhD Thesis, University of Georgia]. We are currently investigating possible sources of bacteria including suspension from the shifting sandy substrate [18], input from the extensive floodplain swamps, and water column production [R. T. Edwards (1985) PhD Thesis, University of Georgia]. Bacterial production in the Ogeechee River is high when water temperature is high. [R. T. Edwards (1985) PhD Thesis, University of Georgia]. Studies of this and other southeastern blackwater rivers have noted high secondary production rates for filter-feeding invertebrates [8, 38]. Since bacterial biomass produced at the expense of the abundant DOC is one possible energy source for the invertebrate filter-feeders in this river, we considered it important to examine the availability to bacteria of different apparent molecular weight fractions of DOC from the Ogeechee River and Black Creek, one of its tributaries. The results of those experiments are presented here.

Methods

We analyzed the ability of DOC from two southeastern blackwater rivers to stimulate bacterial growth in ten enrichment experiments. In these experiments, the DOC from 1 to 1.5 liter of Ogeechee River or Black Creek water was separated into three different nominal molecular weight (nMW) fractions and concentrated to 50 ml by ultrafiltration. We collected water from two similar fourth-order sites on Black Creek: just above its confluence with the Ogeechee River and several km upstream where Georgia Route 280 crosses it. Water samples were collected in March, May, July, and August 1983; February, April, May, June and July 1984; and January 1985. In May 1983 water from the sixth-order Ogeechee River was collected upstream of the point where Interstate 16 crosses the river, which is about 63 km from its mouth. These sites are more fully described elsewhere [28, 46].

To concentrate DOC, river water was first sterile-filtered twice through 0.22- μm Millipore filters (prerinsed with sterile water) to remove bacteria. It was then placed in a 200-ml stirred cell and filtered through an Amicon PM10 membrane (62-mm diameter at <10 psi of nitrogen) until 50 ml remained (>10,000 nMW fraction). The filtrate was then filtered through an Amicon YM2 membrane (<30 psi) until 50 ml remained (1,000–10,000 nMW) fraction. The <1,000 nMW fraction was obtained by freeze drying the YM2 filtrate and reconstituting it to 50 ml with sterile deionized water. The Amicon filters were sterilized by soaking in EtOH overnight and rinsed extensively with sterile deionized water before use. In addition, all ultrafiltration was done at 4°C to retard bacterial growth.

The three concentrates of different DOC fractions were added to three flasks of 300–350 ml of river water that had been passed through a 4- μm mesh sieve to remove large particles and large bacteriovores. A fourth flask (designated as “unenriched”) contained only sieved river water. The pH of all flasks was adjusted with NaOH or HCl to be the same as the pH of the unenriched flask, which ranged from 4.5 to 6.7 in different experiments. All flasks were incubated at 25°C for 72 hours. The river is at or above this temperature throughout the summer.

Samples for measurements of bacterial biomass and growth rate were removed from the flasks at 6 times during the incubation: 0, 6, 24, 30, 48, and 72 hours. Bacterial cells were counted after staining with acridine orange [22]. To determine the amount of bacterial carbon present, we photographed cells at different times during the incubation and used a digitizer to measure the length and width or diameter of at least 500 cells on the photographs to determine volume per cell. The accuracy of this digitizing technique has been verified with fluorescent latex spheres of known sizes [15]. Biovolume was then multiplied by $0.22 \times 10^{-12} \text{ g C}/\mu\text{m}^3$ [10] to calculate amount of bacterial carbon present.

Bacterial growth was also measured by determining the rate of incorporation of ^3H -TdR into bacterial DNA at each of the six sampling times. Triplicate ten ml aliquots were removed from the incubation flasks and 40 μCi tritiated thymidine (^3H -TdR; [Me- ^3H]TdR, 80 Ci/mmol, New England Nuclear) added to each aliquot prior to a one hour incubation at 25°C. The incubation was terminated by adding 0.25 ml buffered formalin and filtering through a 0.2- μm Nuclepore filter. DNA was extracted (0.3 N NaOH + 25 mM EDTA + 0.1% sodium lauryl sulfate, 25°C, 15 min), precipitated (0.8 ml 3 N HCl and 1 ml 50% TCA in an ice bath followed by centrifugation), hydrolyzed (3 ml 5% TCA at 100°C for 1 hour), centrifuged, and the supernatant counted in Scintiverse cocktail on a Beckman LS 1800 liquid scintillation counter. This is the optimal extraction procedure for bacteria from these blackwater rivers [16]. Measures of disintegrations per minute (DPM) incorporated were converted to nM thymidine incorporated using the specific activity of the isotope. No correction was made for isotope dilution [30]. To estimate the amount of thymidine incorporated into bacterial DNA over the 72-hour incubation, we plotted the amount of thymidine incorporated at each sampling time vs time and integrated under the curve.

In all experiments, the initial and final DOC concentration in each flask was determined using a Dohrman DC54 carbon analyzer. This instrument uses UV-catalyzed digestion in the presence of persulfate to measure DOC concentration with a precision of $\pm 4\%$ (standard deviation of 3 replicate standards). In several experiments, the ion content of water was determined on a Perkin-

Elmer plasma emission spectrograph. Total nitrogen concentrations [14] were measured in all treatments in six experiments.

Isotope dilution experiments [12, 30] were not routinely done in conjunction with the thymidine additions. In one experiment with Black Creek water, an isotope dilution series was done on each flask after 29- hours incubation to determine if dilution was different in each flask, since this could significantly affect our ability to compare results of thymidine uptake between treatments. From 1 to 10 nM unlabeled thymidine was added to each aliquot in a dilution series and the pool of unlabeled thymidine calculated from the dilution plots [16].

Although the river water used in the flasks was routinely sieved through a 4- μm sieve, bacteriivores (most conspicuously, flagellated protozoa) were not completely removed by this procedure. The growth of flagellated protozoa was documented by sampling at the beginning and end of one experiment, preserving the samples in Lugol's solution, settling a small subsample (10 ml) on a counting grid, and counting the settled material with an inverted microscope (400 \times). In another experiment we removed flagellated protozoa from river water by filtering it through a Whatman GF/C glass fiber filter before adding the concentrates to it. This procedure also removed some bacteria, particularly the more metabolically active attached forms [R. T. Edwards (1985) PhD Thesis, University of Georgia]. The concentrates used contained not only DOC but also dissolved ions. To determine if the observed treatment effects were a consequence of the DOC or the other ions added, in one experiment we passed riverwater through an ion-exchange resin (Fisher Rexyn 101H) prior to sterile filtering and concentrating it as described above.

To compare the results obtained with DOC from southeastern blackwater rivers with DOC from other sources, we followed the experimental procedure described above with DOC from four other sources: leachate from leaves of water oak (*Quercus nigra*) or willow (*Salix* spp.), and water from Hugh White Creek or Matamek River. Water oak and willow are common species in the Black Creek and Ogeechee River riparian zone. Hugh White Creek is a second-order clearwater stream in the Coweeta Hydrologic Laboratory in the southern Appalachians. Its DOC concentration ranges from <1 to 5 mg C/liter [27]. The Matamek River is a sixth-order boreal, blackwater river in Quebec with 11.3 mg C/liter average DOC concentration [32]. For the leaf leachate experiments, DOC concentrates were added to sieved Black Creek water as described above. For the experiments with Matamek River DOC, concentrates were added to sieved water from the Matamek River. Because bacterial densities are so low in water from Hugh White Creek ($\approx 10^4$ /ml, cf. 10^7 /ml in the Ogeechee and Black Creek), we added sediment bacteria from this stream to water before adding the DOC concentrate. We obtained the sediment bacteria by putting organically rich sediments in stream water, shaking vigorously, and then removing the sediment by centrifugation. Sediment bacteria were added to stream water to achieve bacterial densities of the same order of magnitude as observed in southeastern blackwater river water. Because DOC concentrations are so low in Hugh White Creek, the DOC concentrates in this experiment were made from a much larger volume of water (10 liters).

One interesting experimental result was the ability of the highest molecular weight fraction to stimulate bacterial growth (see below). To determine if this could be a consequence of lower molecular weight compounds complexed with high molecular weight material, we tried hydrolyzing the high molecular weight concentrate before it was added to river water in one experiment. Two 1-liter samples were concentrated to 50 ml with an Amicon PM10 filter (> 10,000 nMW), and the concentrates freeze-dried. One concentrate was hydrolyzed for 22 hours in 6 N HCl at 100°C, then diluted and neutralized to pH7 with NaOH. Both samples were diluted to 150 ml with sterile deionized water and concentrated to 50 ml using a PM10 filter. This dilution and filtering procedure was repeated three times to ensure that all material <10,000 nMW passed through the filter. The filtrate from the hydrolyzed material was then concentrated to 50 ml using a YM2 filter (1,000–10,000 nMW). The three concentrates (> 10,000 nMW, > 10,000 nMW hydrolyzed, hydrolysis products 1,000–10,000 nMW) were then used in the experimental procedure described above.

Efficiency of conversion of DOC to bacterial biomass was calculated using the data from cell counts and from thymidine uptake studies. Efficiency was calculated from cell counts as (maximum bacterial biovolume – initial bacterial biovolume) $\times 0.22 \times 10^{-12}$ g C/ μm^3 (from 10) \div change in DOC. We also calculated efficiency from the thymidine data by calculating carbon production rate at each sample time, plotting that vs time, integrating under the curve, and dividing that by

change in DOC. Carbon production at each time was calculated as [DPM incorporated] \times [specific activity of $^3\text{H-TdR}$ (Ci/mole)] \times [2×10^{18} cells/mole thymidine (from Ref. 31)] \times [average cell size at that time ($\mu\text{m}^3/\text{cell}$)] \times [0.22×10^{-12} gC/ μm^3 (from Ref. 10)].

Results

Blackwater River

Biomass Changes. Bacterial growth was observed in all flasks. In fact, we had to be extremely careful to maintain sterile conditions during the concentration phase of the experiments. If this was not done, large populations of bacteria would build up before the concentrates could be added to river water. Clearly there was biologically available DOC present.

In all flasks, both rods and cocci were present, and both increased in number over the course of the experiment, although the increase in rods was greater than the increase in cocci. Averaging over all experiments, cocci initially accounted for $19 \pm 2\%$ (mean \pm standard error used throughout the paper, $n = 59$) of bacterial volume, but by the end of the incubation period, they accounted for only $5 \pm 0.6\%$. Since the rods were much larger cells, the average biovolume of cells increased over the course of the experiment. However, no consistent change in average size of rods or cocci was observed over the course of an experiment. Averaging over all experiments, rods were $0.217 \pm 0.028 \mu\text{m}^3$ ($n = 14$), whereas cocci were $0.030 \pm 0.005 \mu\text{m}^3$ ($n = 14$). These sizes are within the range observed for bacteria taken directly from the river [R. T. Edwards (1985) PhD Thesis, University of Georgia].

The amount of bacterial growth observed was not equal in all flasks. In the data reported here we have used paired t tests and $\alpha = 0.05$ for all statistical comparisons of bacterial growth and DOC use in these different flasks. This was necessary because we combined experimental results from water collected on different dates. Hence, seasonal differences in DOC concentration and initial bacterial abundance increased the observed variability within treatments from experiments done on different dates. The only feasible way to make comparisons between treatments was therefore with paired t tests in which differences in growth between treatments were calculated for each date and combined over all dates in the t test. Although combining information from all seasons obscures potentially interesting and important seasonal differences in DOC availability, our replication was not adequate to assess these seasonal patterns.

Because initial bacterial densities were not the same in the different experiments, we have expressed growth as maximum bacterial biomass observed/initial bacterial biomass in Table 1. There was no significant difference between bacterial growth observed in unenriched flasks and growth observed in flasks enriched with intermediate MW (1,000–10,000 nMW) DOC (Table 1). Bacterial growth in flasks enriched with high ($>10,000$ nMW) or low ($<1,000$ nMW) MW DOC was significantly greater than in unenriched flasks. Growth in the low MW enrichment was significantly greater than that in the high MW enrichment. On the basis of this measure of bacterial growth, it appears that low MW DOC from these blackwater rivers supports the greatest amount of bac-

Table 1. The influence of different DOC enrichments on bacterial growth measured as maximum bacterial biovolume (μm^3)/initial bacterial biovolume (μm^3)^a

DOC source	Unenriched	Enriched		
		>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Ogeechee River or Black Creek	7.0 ± 2.1 (9)	14.8 ± 4.5 (10)	9.7 ± 3.4 (9)	24.4 ± 7.2 (8)
Matamek River	4.9 (1)	3.0 (1)	15.4 (1)	29.3 (1)
Hugh White Creek	5.4 (1)	4.3 (1)	12.0 (1)	16.4 (1)
Water oak leachate	9.2 (1)	12.4 (1)	10.0 (1)	50 (1)
Willow leachate	12.5 (1)	26.0 (1)	24.4 (1)	22.5 (1)

^a Values are mean ± standard error (number of experiments)

Table 2. The influence of different DOC enrichments on bacterial growth measured as change in bacterial C (ng) per μg DOC present^a

DOC source	Unenriched	Enriched		
		>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Ogeechee River or Black Creek	5.5 ± 1.0 (9)	7.8 ± 1.4 (10)	3.3 ± 0.9 (9)	21.4 ± 5.0 (8)
Matamek River	3.0 (1)	1.8 (1)	3.6 (1)	17.5 (1)
Hugh White Creek	14.5 (1)	9.0 (1)	12.0 (1)	28.0 (1)
Water oak leachate	3.0 (1)	2.1 (1)	3.8 (1)	18.0 (1)
Willow leachate	7.1 (1)	10.1 (1)	10.1 (1)	10.1 (1)

^a Values are mean ± standard error (number of experiments)

terial growth, high MW DOC supports somewhat less growth, and intermediate MW DOC does not support bacterial growth.

We reach the same conclusions when we express bacterial growth as change in bacterial carbon normalized for amount of DOC present in the different flasks (Table 2). This is a measure of bacterial growth per unit DOC present. Growth is greatest in the low MW enrichment, followed by the high MW enrichment. Growth in the intermediate MW enrichment was significantly less than growth in the unenriched flasks. In other words, each gram of intermediate MW DOC supported less bacterial growth than did a gram of DOC with all size fractions present.

Thymidine Incorporation. We can also compare bacterial growth in the different treatments by calculating the amount of thymidine incorporated into bacterial DNA over the course of an experiment (Table 3). Incorporation was significantly greater in all enriched flasks than it was in the unenriched flasks. Greatest incorporation was observed in the low MW enrichment, while intermediate rates were observed in the high MW enrichment, and incorporation was least in the intermediate MW enrichment. Because the amount of DOC added varied between enrichments, this comparison should be interpreted cautiously. To understand the differences in biological availability of the three molecular

Table 3. The influence of different DOC enrichments on bacterial growth measured as incorporation of thymidine into DNA over a 72-hour incubation period^a

DOC source	Unenriched	Enriched		
		>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Ogeechee River or				
Black Creek	1.38 ± 0.59 (9)	5.85 ± 2.12 (9)	2.81 ± 0.65 (8)	14.54 ± 4.69 (7)
Matamek River	0.19 (1)	0.19 (1)	0.58 (1)	2.22 (1)
Hugh White Creek	8.03 (1)	13.6 (1)	23.3 (1)	35.3 (1)
Water oak leachate	3.58 (1)	2.47 (1)	3.69 (1)	16.4 (1)
Willow leachate	0.78 (1)	1.07 (1)	1.28 (1)	2.43 (1)

^a Values are mean ± standard error (number of experiments) in units of nM thymidine/liter. Thymidine uptake was measured in water collected on all dates except April 1984

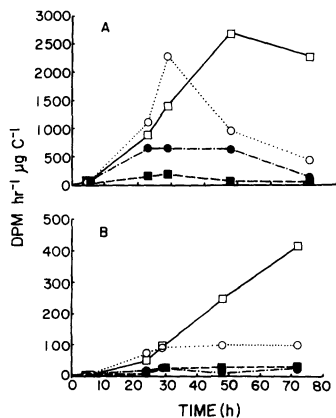


Fig. 1. Time course of thymidine uptake of bacteria in an experiment in which 4 μm -sieved (A) or GFC glass fiber-filtered (B) blackwater river water was enriched with high MW DOC ($\cdots\cdots\circ\cdots\cdots$), intermediate MW DOC ($-\cdots-\blacksquare-\cdots-$), low MW DOC ($-\square-$), or unenriched ($-\cdots-\bullet-\cdots-$). Large populations of protozoa were present in A but not B. Values are the means of three determinations of DPM of ^3H -TdR into DNA per hour per μg DOC initially present.

weight fractions, it is more appropriate to compare bacterial growth (in this case thymidine incorporation) per unit DOC present.

The time course of thymidine incorporation per unit DOC present over the period of two experiments is plotted in Fig. 1. The plot for most experiments looked like that in Fig. 1A, with peak incorporation occurring prior to the end of the incubation period. When protozoan predators were excluded (Fig. 1B), incorporation rates peaked at the end of the incubation period. Despite differences in pattern and absolute amount of thymidine incorporated in these two experiments, both showed greatest incorporation in the low MW enrichment, intermediate incorporation in the high MW enrichment, and least in the intermediate MW enrichment. In one experiment (Fig. 1B) there was little difference between control and intermediate MW enrichments, whereas in the other experiment (Fig. 1A) there was less incorporation in the intermediate MW enrichment.

Data on thymidine incorporation per unit DOC present is summarized for all experiments in Table 4. This measure of growth was significantly greater in the low and high MW enrichments than in the unenriched flasks. There was no significant difference between unenriched flasks and those enriched with

Table 4. The influence of different DOC enrichments on bacterial growth measured as thymidine incorporated into DNA per g DOC present^a

DOC source	Enriched			
	Unenriched	>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Ogeechee River or Black Creek	82 ± 29 (9)	210 ± 65 (9)	68 ± 24 (8)	316 ± 98 (6)
Matamek River	26 (1)	24 (1)	30 (1)	235 (1)
Hugh White Creek	1,830 (1)	2,280 (1)	2,020 (1)	5,090 (1)
Water oak leachate	112 (1)	42 (1)	76 (1)	447 (1)
Willow leachate	27 (1)	32 (1)	34 (1)	79 (1)

^a Values are mean ± standard error (number of experiments) in units of nM TdR/g C. Thymidine uptake was measured in water collected on all dates except April 1984

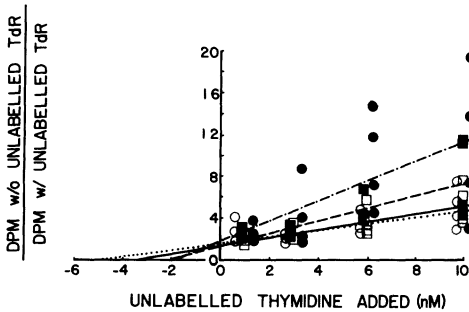


Fig. 2. Isotope dilution plot for bacterial incorporation of ³H-TdR into DNA in flasks enriched for 29 hours with high MW DOC (· · · · · ○ · · · · ·) ($r^2 = 0.467$), intermediate MW DOC (---■---) ($r^2 = 0.573$), low MW DOC (—□—) ($r^2 = 0.585$), or unenriched (—●—) ($r^2 = 0.401$). Each point represents one measurement, and the lines are least-squares regression fit to the 16 points. The intersection of the regression line with the horizontal axis gives the effective pool size.

intermediate MW DOC. This is the same result obtained with the other measures of bacterial growth described above.

These kinds of comparisons of thymidine incorporation between flasks are appropriate only if isotope dilution is the same in different flasks. If the specific activity of the thymidine differed greatly between flasks because of differences in amounts of unlabeled thymidine present either internally or in the medium, then one could not accurately compare rates of labeled thymidine incorporation. We checked this assumption of equal dilution in all flasks in one experiment (Fig. 2). Although there was some variation between flasks in the dilution plots, the differences in estimates of the size of the unlabeled pools were within the error limits of the measurements. Hence we feel confident in comparing thymidine incorporation between flasks.

Changes in DOC. Not only did the amount of bacterial growth observed differ between enrichments, the amount of DOC used also differed (Table 5). To make data comparable between experiments with different amounts of DOC added, we have expressed DOC use as the percent of added DOC that was removed during the course of the experiment, corrected for DOC use in the unenriched flasks. This was calculated as (change in DOC in enriched flask –

Table 5. Percentage of added DOC used during the course of a 72-hour enrichment experiment^a

DOC source	Type of DOC used (%)		
	>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Ogeechee River or			
Black Creek	21 ± 9 (6)	2 ± 2 (6)	54 ± 16 (4)
Water oak leachate	26 (1)	30 (1)	168 ^b (1)
Willow leachate	43 (1)	31 (1)	100 (1)
Matamek River	9 (1)	1 (1)	37 (1)
Hugh White Creek	0 (1)	31 (1)	86 (1)

^a Values are mean ± standard error (number of experiments) and were calculated as (Δ DOC enriched – Δ DOC unenriched)/(initial DOC enriched – initial DOC unenriched). Water for these experiments came from the Ogeechee in May 1983 and from Black Creek in March, July, and August 1983, February 1984 and January 1985

^b This is not an artifact. It merely means that some of the DOC present prior to enrichment was utilized when the low MW fraction was added but not used in the unenriched flask. Cometabolism [13] would be one possible explanation for this observation

Table 6 Nitrogen content of flasks enriched with different types of DOC Data are presented as μ M total N present and as molar N/C ratios^a

DOC source	Unenriched	Enriched		
		>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Black Creek				
Total N (μ M)	79 ± 8 (6)	109 ± 15 (4)	131 ± 12 (4)	196 ± 27 (4)
N/C (%)	0.24 ± 0.03 (6)	0.24 ± 0.02 (4)	0.20 ± 0.02 (4)	0.40 ± 0.06 (4)
Water oak leachate				
Total N (μ M)	—	103 (1)	122 (1)	152 (1)
N/C (%)	—	0.18 (1)	0.25 (1)	0.41 (1)
Willow leachate				
Total N (μ M)	—	95 (1)	78 (1)	146 (1)
N/C (%)	—	0.28 (1)	0.21 (1)	0.47 (1)

^a Values are mean ± standard error (number of experiments). Water for these experiments was collected in February, May, and June 1984, and January 1985

change in DOC in unenriched flask)/(initial DOC in enriched flask – initial DOC in unenriched flask). The greatest DOC removal was observed in flasks enriched with low MW DOC. DOC removal was intermediate in flasks enriched with high MW DOC, and not significantly different from zero in flasks enriched with intermediate MW DOC (Table 5).

Effects of Protozoan Grazers. Although river water was passed through a 4- μ m sieve before being used in these experiments, considerable growth of small flagellated protozoa (\approx 3- μ m diameter) was observed. In the one experiment in which we monitored this growth, there were initially 3,000 flagellates/ml

Table 7. Several measures of bacterial growth rate in a DOC enrichment experiment in which river water was passed through a deionizing column prior to concentrating it

Growth rate measure	Unenriched	Enriched		
		>10,000 nMW	1,000–10,000 nMW	<1,000 nMW
Final/initial biovolume	1.9	3.8	1.8	17.6
ng bacterial C/ μ g DOC	1.3	2.4	0.6	17.9
nM TdR/liter	0.4	7.0	2.2	11.0
nM TdR/g C	9	116	26	203

and 10 ciliates/ml. After 72 hours, flagellate density in the three enrichment flasks was 23,000, 25,000, and 32,000/ml, while ciliate densities had risen to 160, 105, and 310/ml. These protozoa appeared to have an impact on the bacterial community in the flasks. In 80% of the flasks bacterial biomass peaked prior to the end of the incubation period. When protozoan predators were excluded, bacterial biomass was maximum at the end of the incubation period in all flasks. Similarly, thymidine incorporation generally peaked before 72 hours (e.g., Fig. 1A), but was maximum at the end of the experiment when protozoan predators were excluded (Fig. 1B). Although protozoa clearly affected bacterial biomass present, their presence did not compromise the most important part of these experiments, namely the comparison of growth in the different enrichments. Regardless of whether protozoa were present or absent, bacterial growth was greatest in the low MW enrichment and least in the intermediate MW enrichment.

Differences in DOC and Dissolved Ions Between Treatments. Although DOC concentration differed between treatments, there was no relationship between DOC concentration and growth rate. No pattern was evident in plots of maximum biovolume/initial biovolume vs DOC concentration when all blackwater river experiments and all MW fractions were combined, or when each MW fraction was plotted separately.

Cation analyses were done for 5 of the 10 experiments with southeastern blackwater river DOC. The data from each experiment were analysed by ANOVA for 7 ions (Al, Fe, Ca, Mg, Na, K, and P) to determine if there were significant concentration differences between treatments. In general, concentration of the metals Fe and Al were greatest in the intermediate MW enrichment, and lowest in the low MW enrichment and unenriched flasks. The divalent cations Ca and Mg generally had highest concentrations in the low molecular weight enrichment with no consistent pattern among the other treatments. There was no consistent difference between treatments in Na, K, or P concentrations.

The results of total nitrogen analyses are summarized in Table 6. Total nitrogen concentration decreased in the order low MW enrichment > intermediate MW enrichment > high MW enrichment > unenriched. The N/C ra-

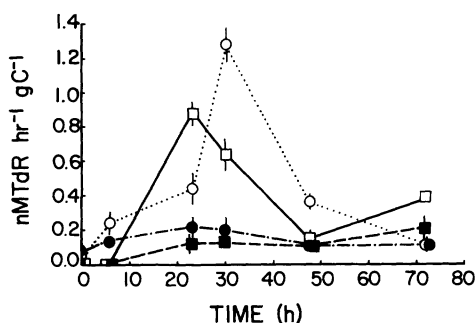


Fig. 3. Time course of thymidine incorporation into bacterial DNA in an experiment where blackwater river water was enriched with high MW DOC (· · · · · ○ · · · · ·), high MW DOC that had been hydrolyzed (--- ■ ---), DOC (1,000–10,000 nMW) released by hydrolysis of high MW DOC (— □ —), or unenriched (· · · · · ● · · · · ·). Values are means of ^3H -thymidine uptake per g DOC present, and vertical lines indicate \pm one standard error.

tio was considerably higher in the low MW enrichment than in any other flask and somewhat lower in the intermediate MW enrichment.

In one experiment, we removed dissolved ions prior to concentrating DOC to reduce differences in ion concentration between treatments. Concentration differences as described above for nitrogen, and for the metal and divalent cations were also observed in this experiment, presumably because these substances were complexed with dissolved organics and hence not removable by ion exchange. In addition, the pattern of differences in growth rate (Table 7) was the same as had been observed in the other experiments (Tables 1–4): the low MW DOC supported the greatest growth and the intermediate MW DOC the least growth.

Effect of Hydrolysis on High MW Compounds. One of the surprising results of these experiments was the ability of high MW DOC to stimulate bacterial growth. To determine if this could be due to low MW compounds that formed complexes with the high MW DOC, we hydrolyzed some high MW DOC concentrate and then added this refractory high MW core (with hydrolysis products $<10,000$ nMW removed) to sieved river water. Hydrolysis products 1,000–10,000 nMW were added to another flask, and nonhydrolyzed DOC was added to a third flask. Bacterial growth in these flasks was compared to that in an unenriched flask. The time course of thymidine incorporation rate per g DOC present is plotted in Fig. 3. A two-way ANOVA (time \times type of enrichment) of these data indicated that there was a significant interaction, i.e., that there was a difference in the pattern of thymidine incorporation over time in the different flasks. By calculating the area under the curves in Fig. 3, we can estimate thymidine incorporation per unit DOC in 72 hours. Incorporation was 10 nM TdR/g C in the unenriched flask and 9 nM TdR/g C in the flask enriched with hydrolyzed high MW DOC. In the flask with nonhydrolyzed high MW DOC, incorporation was 33 nM TdR/g C; in the flask enriched with hydrolysis products, incorporation was 26 nM TdR/g C. We conclude that, after hydrolysis, high molecular weight DOC lost its ability to stimulate bacterial growth, and that growth in the flask enriched with hydrolysis products was essentially the same as growth in the flask enriched with nonhydrolyzed high MW DOC. Hence, it appears that the biologically available portion of the high MW DOC is in fact low MW compounds complexed with it.

Table 8. Growth efficiency of bacteria growing on different natural DOC sources^a

DOC source	Growth efficiencies	
	Change in cell number (%)	³ H-TdR incorporation (%)
Ogeechee River or Black Creek	1.4 ± 0.4 (15) ^b	31 ± 10 (16) ^c
Hugh White Creek	1.7 (2) ^d	—
Oak leachate	1.0 ± 0.4 (3) ^e	11 ± 5 (3) ^e
Willow leachate	3.1 ± 0.1 (3) ^e	8.6 ± 2.3 (3) ^e

^a Efficiencies were calculated from either change in cell number or production (determined from measures of rate of incorporation of ³H-TdR into DNA) divided by change in DOC. Values are mean ± standard error (number of measurements). Data were combined from all experimental flasks where change in DOC was ≥ 1 mg C/liter

^b Data from 4 unenriched flasks, and 4, 2, and 5 flasks enriched with high, intermediate, and low MW DOC, respectively

^c Data from 5 unenriched flasks, and 5, 2, and 4 flasks enriched with high, intermediate, and low MW DOC, respectively

^d Data from one intermediate and one low MW enrichment

^e Data from one high, intermediate, and low MW enrichment

DOC from Other Sources

The experiments using DOC fractions isolated from sources other than southeastern blackwater rivers were not replicated. Hence a detailed comparison of bacterial growth in each size fraction is not appropriate since a statistical probability cannot be assigned to differences between treatments. However, two general trends are apparent in these data. The first is that DOC uptake (Table 5) and bacterial growth (Tables 1–4) were greatest in the low MW enrichment. This is the same pattern observed with Black Creek and Ogeechee River water. The second trend contrasts with the results obtained with southeastern blackwater river DOC: high MW DOC from other sources did not stimulate bacterial growth (Table 1–4), and in some cases (e.g., Matamek River, Tables 1–2; water oak leachate, Tables 2–4) appeared to retard it.

Bacterial Conversion Efficiency

These experiments were not expressly designed to measure the efficiency with which bacteria converted naturally occurring DOC to bacterial biomass; however, we have measured both bacterial growth and DOC use so we can calculate conversion efficiency. The one difficulty in this is that our measure of DOC use was not particularly sensitive. Because we were using water from blackwater rivers, initial DOC content was quite high (mean = 26.5 mg C/liter in unenriched flasks). Hence, we were trying to detect small changes in DOC against a relatively high background concentration. To try to minimize errors due to inaccuracies in DOC measurement, we have only used data from flasks where the change in DOC was at least 1 mg C/liter for calculating conversion effi-

ciencies. We calculated efficiency based on both biomass changes and on thymidine incorporation (Table 8). Both measures of efficiency are subject to error and are sensitive to the conversion factors used. They give us rather different measures of conversion efficiency. The efficiencies calculated from measured thymidine incorporation are probably most accurate since efficiencies calculated from changes in cell number are low because of extensive protozoan grazing.

Discussion

These experiments had one consistent result regardless of the bacterial growth measure used: low MW DOC from southeastern blackwater rivers was most available to bacteria, intermediate MW DOC was least available, and high MW DOC was intermediate in availability. When DOC was concentrated from other rivers or leaf leachate, the low MW fraction was also most available; however, the high MW fraction was generally less available than the intermediate MW fraction. Therefore, we conclude that high MW DOC from these blackwater rivers was more available to bacteria than was high MW DOC from a clearwater mountain stream, a boreal blackwater river, or fresh leachate of oak or willow leaves. High MW DOC leached from leaves of riparian bushes was also found to be unavailable to bacteria in a piedmont stream [23].

The molecular weight separations that form the basis of these studies of differential availability were obtained by ultrafiltration. Although this is a commonly used procedure (e.g., Ref. 43), the molecular weights obtained are relative and are influenced by the nature of the substances being filtered, particularly the charges they bear [1]. Although the PM10 membrane we used to concentrate high MW DOC performed appropriately when used with an aquatic fulvic acid from a southeastern blackwater river [1], it is important to recognize that these membranes are not absolute filters. Complex molecules of the same molecular weight may be either retained or may pass through the membrane, depending on a variety of factors such as their charge distribution [1]. Hence, the low MW concentrates used in these experiments may have been contaminated by higher MW compounds that passed through the membranes, and the intermediate MW concentrates may have had some contamination from lower MW compounds that were retained. However, this does not compromise our major conclusions: if high MW contaminants had not been present, bacterial growth on low MW compounds would have been even higher; if low MW contaminants had not been present, bacterial growth on intermediate MW DOC would have been even less.

The results of the hydrolysis experiments suggest that bacterial growth on the high MW DOC was at the expense of lower MW compounds complexed with a more refractory high MW core. In humic-stained waters of an Oregon river, 60% of sugars [42] and 96% of amino acids [26] were associated with the high MW humic acid fraction. Some of the high MW DOC in our experiments may also have been low MW DOC that had formed complexes with metals; the metal complexes would then be separated as high MW DOC by our ultrafiltration procedure [29]. Another possibility is that during the con-

centration procedure aggregates were formed that were too large to pass through the ultrafiltration membrane [1]. Regardless of the mechanism by which complexes were formed in DOC from southeastern blackwater rivers, bacterial exoenzymes were able to cleave them and to use the low MW compounds released. The same phenomenon was not observed in DOC from a boreal blackwater river, a clearwater mountain stream, or fresh leaf leachate. Either the complexes were not formed or they were more resistant to bacterial attack.

Others have observed evidence of bacterial growth at the expense of DOC from humic-stained waters [13, 36, 37, 40, 41], and in some cases bacterial biomass was correlated with humic or fulvic acid content of the water [13, 21]. In most environments low MW DOC appears the most susceptible to bacterial attack (e.g., Refs. 23 and 33; but see Ref. 20), as we observed in these blackwater rivers. Intermediate MW DOC from a softwater lake was considered to be more refractory than high or low MW DOC [2], although the criterion used was degree of photolysis when exposed to UV light rather than a direct measure of its availability to bacteria as we have used here.

We are interpreting the observed pattern of growth stimulation to be a consequence of differential availability of the organic matter as a growth substrate, since clear differences in DOC removal were observed between flasks. However, we cannot rule out the possibility that increased growth was a consequence of trace metal chelation by the added organics, although this has been observed previously with algae rather than bacteria [34]. The work with algae also showed that the lowest molecular weight fraction was most stimulatory [34]. It is less likely that the pattern was a consequence of differential binding of toxic metals by the added organic matter because DOC concentrations prior to enrichment were already high (mean \pm standard deviation = 26.9 ± 12.7 mg C/liter, $n = 12$). At these DOC concentrations there would be a few free ions of potentially toxic metals such as copper or aluminum, and adding more organic matter would have a minor effect on their toxicity.

Although the MW fractions had different concentrations of cations associated with them, the observed differences in bacterial activity could not be explained by variation in cation content. On the other hand, differences in nitrogen and especially nitrogen/carbon ratios between treatments were related to observed differences in bacterial growth (Table 6). The low MW fraction clearly had the most nitrogen-rich carbon and supported the greatest bacterial growth. The nitrogen content of the high MW DOC was somewhat greater than that of the intermediate MW fraction, and the high MW DOC supported more bacterial growth.

DOC from a sixth-order sampling site on the Ogeechee River is primarily of intermediate MW: 25% is high MW, 61% is intermediate MW, and 17% is low MW [28]. Differential bacterial uptake is one of several possible mechanisms causing this distribution. Data presented here have shown greatest uptake of low MW DOC and least uptake of the intermediate MW fraction, and hence that intermediate MW material could accumulate in the water. Bacterial activity has been implicated as the cause of observed molecular weight distributions in other aquatic ecosystems [13, 19].

Over a 72-hour period about half the low MW DOC was utilized by bacteria (Table 5). Had bacterial populations not been so heavily grazed by flagellates,

DOC use might have been greater. Although low MW DOC is a small fraction of total DOC in this river, its average concentration is 2 mg C/liter in the Ogeechee River and 4 mg C/liter in Black Creek; those concentrations are greater than total DOC in many clearwater streams (e.g., Ref. 27). There is, then, a sizeable stock of DOC available for conversion to bacterial biomass. This conversion is not particularly efficient. The 30% efficiency reported here for riverine DOC is considerably less than the 50–80% efficiencies generally recorded for bacteria growing on nonrefractory dissolved substrates [49], although 20–30% efficiency for marine bacteria growing on phytoplankton detritus has also been observed [5]. It is also less than the 53% efficiency noted for bacterial growth on leachate from Ogeechee River macrophytes [17], but far greater than the 0.02% estimated for benthic bacteria metabolizing DOC from a clearwater piedmont stream [9]. Further measures of conversion efficiencies for bacteria growing on complex natural substrates are needed before we will be able to reliably model the conversion from DOC to bacteria in riverine ecosystems.

The bacterial biomass produced from DOC in our experiments was consumed by flagellated protozoa, the next step in the “microbial loop” (sensu 4). Significant protozoan growth has been observed in other experiments where bacteria were grown on natural DOC [17, 35]. Similar grazing probably happens in the Ogeechee River where protozoan densities are high: annual average abundance is 1.6×10^3 flagellates/ml and 21 ciliates/ml (Leslie Carlough, University of Georgia, personal communication). In the river, bacteria may also be consumed by abundant filter-feeding invertebrates such as the net-spinning caddisfly *Macronema* and black flies (*Simulium* spp.), which are capable of capturing bacteria-sized particles [50] as well as protozoa. The links between these benthic invertebrates and higher trophic levels such as fish are well documented (e.g., Ref. 7). Whether bacterial biomass is produced at the expense of riverine DOC as we observed in these experiments or is washed in from the floodplain [R. T. Edwards (1985) PhD Thesis, University of Georgia], the microbial loop appears an important component of the trophic dynamics of the water column in this blackwater river.

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