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Production and Turnover of Planktonic Bacteria in Two Southeastern Blackwater Rivers

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Production by attached and free-living planktonic bacteria in two blackwater rivers in the Southeastern United States was measured over a period of 14 months by using the rate of incorporation of [*methyl*-^{3}H]thymidine into DNA. Production rates and biomass dynamics were compared to determine the potential for in situ production to supply planktonic biomass. Bacterial production in these rivers was moderate and varied seasonally. Rates varied from 0.058 to 2.120 mg of C m^{-3} h^{-1} in the Ogeechee River and from 0.002 to 2.418 mg of C m^{-3} h^{-1} in Black Creek. Regressions of growth rate on various environmental variables showed that temperature and total dissolved organic carbon concentration were the best predictors of growth. Although attached bacteria were <21% of the total biomass, they accounted for up to 53% of the total production. Turnover times for attached bacteria ranged from <1 day to >3 years depending on season. Turnover times of free-living bacteria varied from 4.4 days to 11.8 years. Comparisons of biomass with production indicated that during most seasons, the majority of bacterial biomass in these rivers was of allochthonous origin. During summer, when water temperatures were high, bacterial growth in the river may have supplied a greater percentage of the standing stock of bacteria than allochthonous inputs.

The Satilla River, a large blackwater river in southeastern Georgia, exhibits high rates of secondary production of invertebrates (4). Much of this production is by filter feeders that have the capacity to capture particles as small as 0.0004 μm^3 (40). Sestonic bacteria are potentially an important trophic component of the observed secondary production in this type of river. Bacterial cell counts are unusually high, and bacterial biomass forms a large percentage of the seston in the Ogeechee River and Black Creek, two blackwater rivers in the coastal plain of Georgia (R. T. Edwards, Limnol. Oceanogr., in press). Factors affecting bacterial abundance in these rivers could exert control over a portion of invertebrate production by affecting seston food quality. In the present study, we estimated rates of bacterial production in two blackwater rivers and related bacterial growth to physical and chemical characteristics of the rivers. We calculated biomass turnover times to determine the relative importance of autochthonous and allochthonous bacterial sources.

The source of bacteria within these rivers is presently unknown. Cells could be growing at the expense of dissolved organic matter, which is present in high concentrations (J. L. Meyer, Arch. Hydrobiol., in press), or particulate organic matter, or they could be washed into the rivers from upstream or from the adjacent floodplains. Bacterial growth or activity has been related to primary production by planktonic algae (3, 6, 7, 26, 35) or submerged macrophytes (31) and concentrations of allochthonous organic matter exported to sediments (S. G. Findlay, J. L. Meyer, and R. Risley, Can. J. Fish. Aquat. Sci., in press). In the Ogeechee River and Black Creek, planktonic bacterial biomass dynamics were related to river stage and discharge (Edwards, Limnol. Oceanogr., in press), implying that allochthonous sources of bacteria were important.

Bacterial production was estimated by measuring incorporation of [*methyl*-^{3}H]thymidine ([^{3}H]TdR) into bacterial DNA. Growth rates and turnover times were calculated to examine the relative importance of allochthonous and autochthonous bacterial production. We attempted to separate attached and free-living bacteria with a sieve to examine relative production and turnover rates for each group. The susceptibility of bacteria to predation depends upon their size and attachment to particles (8, 11). Differences in activity between attached and free-living bacteria have been reported from many systems (2, 17, 21, 24). These phenomena could lead to differences in control and supply rates of bacterial biomass, depending upon the fraction eaten by consumers. The study was designed to find (i) the rates of bacterial production and the ways in which they vary between rivers and over time, (ii) whether bacterial production within the rivers is a significant source of bacterial carbon, and (iii) whether production rates and turnover times differ between attached and free-living cells.

MATERIALS AND METHODS

Study site. Samples for biomass and production measurements were taken in the Ogeechee River and Black Creek at sites in the Coastal Plain 18 km from Savannah, Ga. (32°08′ N, 81°21′ W). The Ogeechee River is a sixth-order river with headwaters in the piedmont of Georgia. Fourth-order Black Creek is entirely within the Coastal Plain. Physical and chemical characteristics are summarized in Table 1. More complete discriptions are given elsewhere (39; T. F. Cuffney, Ph.D. thesis, University of Georgia, Athens, 1984; Edwards, Limnol. Oceanogr., in press). Production rates and cell counts were determined on 11 dates from July 1982 to August 1983.

Biomass. Bacterial biomass was measured by acridine orange epifluorescent direct counting (19), combined with measurements of cell volume derived from digitizing microphotographs of the slide preparations (Edwards, Limnol. Oceanogr., in press). Cell volume was converted to carbon content by using a conversion factor of 0.22 g of C cm^{-3} (5). Cell count and biomass methods are described more completely elsewhere (Edwards, Limnol. Oceanogr., in press).

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TABLE 1. Physical and chemical characteristics of the Ogeechee River and Black Creek

River	Discharge ($m^3 s^{-1}$)			pH	Temp. (°C)	DOC (mg of $liter^{-1}$)		
	Mean	Max	Min			Mean	Max	Min
Ogeechee River	66.8	153.5	5.2	6.1–7.5	3–32	14	34	4
Black Creek	0.4	8.0	0.02	4.2–6.1	3–32	30	74	11

Production. Bacterial production was estimated by measuring rates of incorporation of [^{3}H]TdR into DNA (13, 30). Water samples were taken, in acid-washed polyethylene bottles, 0.5 m below the surface in the thalweg. The water sample was mixed gently, and then triplicate 20-ml subsamples were poured into sterile Whirlpak bags (NASCO) for [^{3}H]TdR uptake incubations. One set of replicates was gently poured through a stainless-steel sieve (mesh size, 4 μm) to remove attached cells prior to the isotope addition. Control samples were killed with buffered formaldehyde (4% final concentration). Formaldehyde-killed controls were used instead of zero-time controls because the time required to filter the sample frequently resulted in actual incubation times much greater than zero and because experiments comparing true zero-time controls with formaldehyde-killed controls showed no significant differences in counts recovered.

Unlabeled TdR was added at increasing concentrations to four sets of replicates to dilute the activity of the [^{3}H]TdR. A range of dilutions from 1:1 to 1:6 was used, depending upon the season and the expected uptake rate. [^{3}H]TdR (ca. 78 Ci $mmol^{-1}$; New England Nuclear Corp.) was then added to a final concentration of 50 to 63 nM, and the bags were closed and gently shaken. This was the minimal concentration at which curves of incorporation of counts versus added TdR concentration reached an asymptote.

The samples were returned to the river in a floating bag and incubated for 1 to 1.5 h depending on water temperature. The bag was gently agitated periodically to keep the contents stirred. Uptake of [^{3}H]TdR was linear for 1 h in the summer and up to 4 h in the winter.

Incubations were terminated by addition of formaldehyde, and the samples were filtered through 0.2-μm-pore-size polycarbonate filters, rolled, placed in test tubes, and frozen on dry ice for transport to our laboratory in Athens for further processing. Comparisons of frozen and unfrozen filters showed no difference in measured uptake rate with this technique.

In the laboratory, DNA was extracted from the filters with 8 ml of alkaline extractant (0.3 N NaOH, 25 nM EDTA, 0.1% sodium dodecyl sulfate) at 25°C for 15 min. The samples were then acidified with 0.8 ml of 3 N HCl and 1.6 ml of 50% trichloroacetic acid in an ice bath, to precipitate DNA. Carrier DNA was added and the samples centrifuged at 25,000 × *g* for 15 min at 4°C. The supernatant was discarded, and the pellet was washed with cold 5% trichloroacetic acid and centrifuged again. The pellet was then hydrolyzed in 3 ml of 5% trichloroacetic acid at 100°C for 1 h. After hydrolysis, the samples were centrifuged at 10,000 × *g* for 5 min, and 1 ml of the supernatant was removed for counting. Samples were counted in Scintiverse cocktail on a Beckman LS 1800 liquid scintillation counter. Counting efficiencies were determined by using an external standard and H-number calculations (22).

The total effective TdR pool size was estimated from dilution plots (13). Production calculations were made by using the incorporation into the undiluted samples after subtracting counts from the killed controls. TdR incorporation per unit volume of water was calculated in the following manner: nanomoles of TdR incorporated per hour = disintegrations per minute recovered per hour × [nanomoles of Tdr (total effective pool)/disintegrations per minute added as [^{3}H]TdR)]. TdR uptake was converted to cell production by using a factor of 2×10^{18} cells mol of TdR^{-1} (29). Cell production was converted to carbon production by using mean cell mass for the sampling date calculated as described previously (Edwards, Limnol. Oceanogr., in press).

DOC measurements. Concentrations of total dissolved organic carbon (DOC) and three molecular weight (MW) fractions were measured on a Dohrman DC-54 carbon analyzer (Meyer, in press). Total DOC was measured as all DOC passing an ashed Gelman AE glass fiber filter (nominal pore size, 0.3 μm). After filtration through a Gelman AE glass fiber filter, the fractions were separated by ultrafiltration through Amicon membranes. An Amicon PM 10 membrane was used to separate DOC with nominal MW of <10,000, and an Amicon YM2 membrane was used to separate DOC with MW of <1,000.

RESULTS

Production. Bacterial production rates varied from 0.028 to 2.120 mg of C $m^{-3}h^{-1}$ in the Ogeechee River and 0.002 to 2.418 mg of C $m^{-3}h^{-1}$ in Black Creek (Table 2). The highest rates in both rivers were found during the summer, and the lowest were measured during cold-water, winter conditions (Fig. 1). To determine which environmental variables were significantly related to bacterial growth during the study period, we used correlation, partial correlation, and multiple regression analysis. We used specific bacterial growth rates as the dependent variable in the analysis. Environmental variables used were temperature, total DOC concentration, hydrogen concentration ([H^+]), depth, discharge, and the concentrations of three MW fractions of DOC. Temperature and total DOC concentration were most consistently correlated with growth rate when each river was examined separately or when data from the two rivers were pooled (Table 3). Temperature and total DOC concentration were inversely correlated. When partial correlation was used to eliminate the effect of each variable on the correlation between growth rate and the other variable, the coefficients were significant (Table 3). The correlations between growth rate and [H^+], depth, or discharge had no significance that could not be explained by covariance with the other variables.

We found no consistent trends in the correlations between the individual DOC fractions and growth rates when the effects of temperature were partialled out by using data from the two rivers individually. When the data were pooled, the 1,000 to 10,000 MW and the >10,000 MW fractions were significantly correlated with growth ($P < 0.01$ and $P < 0.0001$, respectively), while the <1,000 MW fraction was not.

We used multiple regression on data from each river separately and both rivers combined to determine which

TABLE 2. Production, biomass and turnover times for the Ogeechee River and Black Creek

Date	River[a]	Mean cell production (cells liter^{-1} h^{-1}) (SE)[b]	C production (mg m^{-3} h^{-1})	Temp. (°C)	Biomass (mg of C m^{-3})	% Attached biomass	Total turnover time (days)
29 Jul. 1982	O	9.36×10^7 (4.51×10^6)	2.120	27	104.1	3.2	2.0
	B	2.40×10^8 (3.10×10^7)	2.418		30.6	20.7	0.5
23 Sept. 1982	O	8.50×10^6 (1.24×10^6)	0.158	22	169.1	4.0	44.5
	B	1.51×10^7 (2.45×10^6)	0.104		798.0	2.0	320.1
28 Oct. 1982	O	6.87×10^6 (2.91×10^5)	0.110	15.5	273.1	3.3	103.3
	B	2.44×10^7 (5.33×10^6)	0.172		98.2	8.5	23.7
3 Dec. 1982	O	5.12×10^6 (1.20×10^6)	0.092	10	ND[c]	ND	ND
	B	5.98×10^6 (9.53×10^5)	0.050		ND	ND	ND
21 Dec. 1982	O	1.77×10^6 (2.30×10^5)	0.028	8	1,142.3	1.1	1,669.0
	B	2.82×10^5 (2.20×10^4)	0.002		189.1	3.7	4,425.2
5 Feb. 1983	O	7.78×10^5 (2.30×10^5)	0.128	10	418.7	0.9	136.4
	B	3.04×10^6 (2.05×10^5)	0.021		117.5	3.5	230.3
17 Mar. 1983	O	2.83×10^7 (9.03×10^5)	0.521	12	186.0	2.4	14.9
	B	2.46×10^7 (9.20×10^5)	0.187		72.2	5.2	16.0
13 May 1983	O	9.02×10^6 (1.02×10^6)	0.162	21	297.8	4.1	76.2
	B	2.16×10^6 (1.41×10^6)	0.180		115.7	8.9	26.8
28 Jun. 1983	O	1.29×10^7 (2.50×10^6)	0.261	26	72.0	2.8	11.5
	B	3.30×10^7 (2.62×10^6)	0.318		62.8	14.5	8.2
28 Jul. 1983	O	9.79×10^7 (1.11×10^7)	1.755	27	216.1	2.4	5.1
	B	9.60×10^7 (2.21×10^7)	0.839		62.6	17.0	3.1
29 Aug. 1983	O	2.25×10^7 (1.08×10^6)	0.412	29	112.0	4.1	11.3
	B	3.65×10^7 (2.43×10^6)	0.338		64.4	12.9	7.9

[a] Abbreviations: O, Ogeechee River; B, Black Creek.
[b] Standard errors reported for cell production are for the three replicate incubations for each river sample.
[c] ND, Not determined.

combinations of variables best predicted growth rates (Table 4). To avoid inflated r^2 values, we eliminated combinations of independent variables that were correlated. Together, temperature and total DOC were good predictors of growth rate in each river separately and when data from both rivers were combined. Adding other variables to the regressions increased r^2 values very little.

We also examined the relationship between bacterial growth rates and primary-production rates in the Ogeechee River. Gross system primary production, measured by the diel oxygen change technique (34), was measured for the Ogeechee River concurrently with bacterial production (R. T. Edwards, Freshwater Biol., in press). The Pearson correlation coefficient between bacterial growth rates and primary production rates was not significant ($r = 0.20$; $P > 0.05$). Partialling out the effects of temperature or the three DOC fractions did not increase the correlation. Multiple regressions of bacterial growth rate on primary production and the variables used above were run for the Ogeechee River with data from all seasons as well as a subset including data from only warm months, when primary and bacterial production rates were highest. Regression coefficients for the primary production rates were not significant when combinations of primary production, DOC fractions, and temperature were used as independent variables to predict bacterial growth rates.

TABLE 3. Correlations between bacterial growth rate and temperature or total DOC in the Ogeechee River and Black Creek[a]

Area	Correlation between:			
	Temp	Temp (TDOC)	TDOC	TDOC (Temp)
Ogeechee River	0.55	0.89[b]	0.54	0.89[b]
Black Creek	0.42	0.82[b]	0.57	0.85[b]
Combined	0.37	0.65[c]	0.55	0.73[d]

[a] The variable partialled out is in parentheses.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P < 0.0001$.

Bacterial turnover. Turnover times for the total bacterial pool were calculated by dividing biomass by production rate. This is an indicator of the rate of supply of bacteria by growth relative to standing stocks or the time required for the population to replace itself. Turnover times for total bacterial biomass were generally long (Table 2), except during June through August. Biomass fluctuated widely, whereas production rates were more constant.

Attached versus free-living bacteria. Water was passed through a 4-μm sieve to separate free-living from attached cells for separate production measurements. Because of the small size of seston in these rivers, this treatment was only partially successful in separating attached bacteria from free-living cells. Epifluorescence direct counts showed that approximately 40% of attached bacteria passed the 4-μm

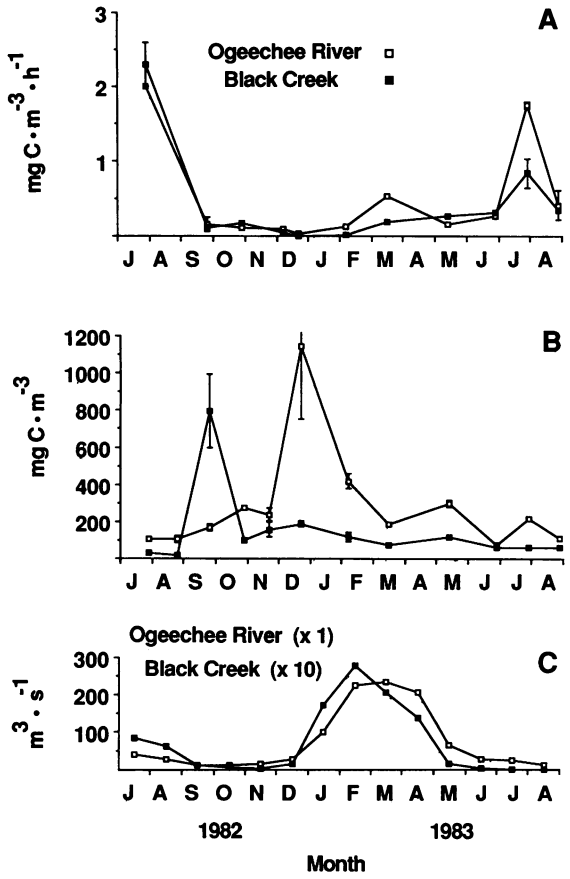

FIG. 1. (A) Bacterial production rates over the sampling period (± 1 standard error). Standard errors are from triplicate measurements on each sample. (B) Total bacterial biomass in the Ogeechee River and Black Creek (± 1 standard error) (Edwards, Ph.D. thesis). Standard errors are for triplicate samples. All error bars are symmetrical; the upper bar was truncated to maintain scale. (C) Mean monthly discharge in each river.

sieve. Total attached bacterial biomass values for the turnover calculations that follow were calculated from seston separations (Edwards, Limnol. Oceanogr., in press). Approximately half of this attached biomass was actually present in the sieved fraction during the TdR incubations; therefore, the biomass of cells actually responsible for the production in the >4-μm fraction was less than that measured, while that of the <4-μm category was slightly greater. Since turnover was calculated as biomass divided by production, turnover times in the >4-μm group were overestimated, while those in the <4-μm fraction were underestimated.

The differences between the two fractions were dramatic. Bacteria retained on a 4-μm sieve accounted for 13 to 53% of the production and only 1 to 21% of biomass. On several occasions, if the attached bacteria passing the sieve were growing at the same specific rate as the ones retained, most of the production in the <4-μm category would actually have been by the small percentage (<5%) of attached cells in that fraction. Turnover times for the bacterial carbon pool in the two fractions showed the same seasonal trends as those for the total bacterial pool (Table 5). Turnover times for bacteria in the >4-μm fraction were always shorter than those of the <4-μm group and varied from 0.3 days to >3 years. For the cells <4 μm, turnover times ranged from 4.4 days to >11 years.

TABLE 4. Regressions of growth rate on temperature and total DOC concentration in each river for total growth and growth within the fractions separated by a 4-μm sieve

Dependent variable	Regression[a] for:		
	Ogeechee River	Black Creek	Combined
Total growth	0.86[b]	0.78[c]	0.60[b]
>4-μm growth	0.40	0.55	0.29
<4-μm growth	0.66	0.57	0.48

[a] Values shown are r^2 for that model.
[b] $P < 0.001$.
[c] $P < 0.01$.

DISCUSSION

Production. Carbon production rates in the Ogeechee River and Black Creek were similar to those reported from a variety of other aquatic systems. Bell et al. (3) summarized bacterial production estimates made by a variety of methods for marine and lake environments. Marine production values ranged from 0.01 to 17.6 mg of C m^{-3} h^{-1}, with the majority ranging from <1 to 3 mg of C m^{-3} h^{-1}. Rates reported for oligotrophic lakes were 0.02 to 1.1 mg of C m^{-3} h^{-1}, and those for eutrophic lakes varied from 0.2 to 10 mg of C m^{-3} h^{-1}. Other studies reported values of 0.071 and 0.217 mg of C m^{-3} h^{-1} for a Danish Lake (38), 0.46 to 1.77 mg of C m^{-3} h^{-1} for a lake in Indiana (27), 0.6 to 17.6 mg of C m^{-3} h^{-1} for Georgia salt marshes (32), and 0.292 to 3.125 mg of C m^{-3} h^{-1} for the York River, Va. (9). These studies involved a variety of methods and assumptions and few measured production rates during seasonal or yearly periods, making comparisons tenuous. The summer production rates in the Ogeechee River and Black Creek (Table 2) were comparable to rates measured in marine environments and oligotrophic lakes and lower than those in eutrophic lakes. The winter

TABLE 5. Turnover times of cells in the sieved fractions

Date	River[a]	<4-μm turnover time (days)	>4-μm turnover time (days)
28 Oct. 1982	O	185	7.3
	B	30	7.1
21 Dec. 1982	O	ND[b]	ND
	B	4,328	1,126
5 Feb. 1983	O	180	5.0
	B	474	15.0
17 Mar. 1983	O	19	1.5
	B	26.7	1.9
28 Jun. 1983	O	16.4	1.0
	B	14.3	2.3
28 Jul. 1983	O	8.3	0.3
	B	4.4	1.3
29 Aug. 1983	O	17.5	1.2
	B	10.2	3.2

[a] Abbreviations: O, Ogeechee River; B, Black Creek.
[b] ND, Not determined.

rates were below those reported for other systems. It is surprising that production rates were not higher, since biomass values, which were 1 to 2 orders of magnitude higher than those for other freshwater systems, place these rivers in a category that Hobbie and Wright (20) considered eutrophic, highly productive systems.

The results of the statistical analysis of growth rates suggest that substrate availability, as indicated by total DOC concentration, and temperature are the primary determinants of bacterial growth in these systems. Those two variables explained 86 and 78% of the variation in growth rates in the Ogeechee River and Black Creek, respectively (Table 4). We have examined the role of native DOC in supporting bacterial growth in these rivers, and the results suggest that the lack of correlation between the <1,000 MW DOC fraction and growth observed here results from the relatively low proportion of that fraction in the total DOC (Meyer, in press) combined with the relatively rapid uptake of that fraction by native bacteria (J. L. Meyer, R. T. Edwards, and R. L. Risley, Microb. Ecol., in press).

Although the use of [^{3}H]TdR uptake to measure bacterial production has gained increased acceptance in recent years, it remains controversial. Recent studies suggest that in some systems, TdR uptake is not as directly coupled to production as was previously thought (10) or that the proportion of growing cells that are capable of taking up TdR is smaller than had been assumed (33). We extracted DNA to eliminate overestimation of [^{3}H]TdR incorporation by counting radioactivity in non-DNA cell constituents or incorporation by nonbacterial organisms. The inconsistent ratios of TdR incorporation to cell proliferation that have been reported (10) could be a consequence of failure to isolate the radioactivity in DNA. The percentage of cold-trichloroacetic-acid-insoluble radioactivity in DNA has not been measured sufficiently often to reliably assume that it is constant in all systems. Other organisms besides bacteria can incorporate exogenous TdR into non-DNA fractions. In addition, failure to extract the counts in DNA would explain why some investigators have observed TdR incorporation in the absence of increases in cell numbers. If significant proportions of the growing bacteria in these rivers are unable to incorporate TdR, we have underestimated production in these rivers. However, the main conclusions reported would remain valid even if production were underestimated considerably.

Source of bacteria. The data presented in this study support the hypothesis that bacterial biomass in these rivers is primarily of allochthonous origin (R. T. Edwards, Ph.D. thesis, University of Georgia, Athens, 1985). Although rates of carbon production during summer were comparable to those reported for other aquatic systems, in situ production was not great enough to produce the unusually high biomass during most of the year. Since residence times of the water in these rivers are measured in days, turnover times of weeks or years indicate that measured bacterial standing stocks were supported by inputs from outside the area studied.

The Ogeechee River and Black Creek appeared to be operating in two modes. During periods of low water and high temperatures, bacterial biomass was lowest and turnover times were several days, indicating that much of the biomass originated in the channel. Bacterial abundance was controlled by factors within the river such as substrate availability, temperature, and predation. Experiments in our laboratory showed that a portion of the DOC in these rivers will support bacterial growth (Meyer et al., in press). The correlations between total DOC and production further support the hypothesis that bacteria were growing at the expense of DOC.

During flood periods, when water levels and standing stocks were maximal, growth rates were lowest (Fig. 1) and turnover times were very long. Bacterial abundance at these times was presumably controlled by the hydrodynamics of river-floodplain interactions controlling material exchange. There are several possible sources of bacteria during flood periods: suspension of main-channel sediment bacteria, inputs from tributaries and their floodplains, and suspension of bacteria from the adjacent mainstem floodplain. Growth rates within the main channel sediments were of the same magnitude as water column rates (Findlay et al., in press) and were not adequate to supply the planktonic standing stocks, even assuming that all of the sediment growth was suspended. Black Creek is a tributary of the Ogeechee River, yet bacterial growth rates in Black Creek were not significantly higher than those in the Ogeechee River. For growth upstream to supply the large numbers of cells observed, tributary growth rates would have to be orders of magnitude higher than those measured in either river. For these reasons, we propose that the majority of bacterial biomass in these rivers is imported from the floodplains adjacent to the channels and from the headwater swamps from which many of the smaller tributaries originate.

Attached versus free-living bacteria. Because the free-living and attached cells are dissimilar in size and abundance, these groups may be grazed at different rates (11, 16, 23). Therefore, factors controlling the availability of bacterial biomass to consumers may vary with the fraction eaten. Few investigators have reported relative growth rates of attached and free bacteria, but many have examined uptake of various organic compounds by these groups. Although heterotrophically active cells, as measured by uptake of radiolabeled substrates or reduction of dyes by electron transport activity, are not necessarily producing biomass, these studies provide interesting insights. Frequently, despite their relatively small proportion of total biomass, attached bacteria account for most of the heterotrophic activity measured (18, 25). In some systems, there are more attached than free-living cells, and they account for most of the uptake (15), whereas in others, the free-living cells dominate the biomass and activity measured (3, 36).

In studies which involved the examination of heterotrophic activity by size fractionation alone, some investigators found the majority of activity in the smallest fraction, assumed to be free living (1, 23), whereas others found the highest activity in the larger fractions (35). Freshwater systems seem to have higher proportions of activity attributable to attached bacteria (2, 12, 25, 36) and a higher proportion of total biomass attached to particles than do marine systems. In systems for which actual growth rates have been measured for attached and free cells, a much smaller percentage of total production has been found in the attached cells than that found during the present study (3, 14, 28).

In the Ogeechee River and Black Creek, in situ production supplied a greater proportion of attached than free-living bacterial biomass, especially during warm conditions. To test whether temperature and DOC affected the attached and free bacteria differently, we regressed the growth rate of cells in the sieved fractions against temperature and the total DOC concentration (Table 4). The amount of variation in growth rate explained by the two variables was greater for bacteria of <4 μm in the Ogeechee River and when the two

rivers were combined. For Black Creek, there was no difference between the amount of variation explained for either fraction. The results suggest that there may be different factors regulating growth rates of free-living and attached bacteria, although the effects of such regulation are not consistent between rivers.

There appear to be two distinct populations of bacteria within these rivers; a small group of actively growing cells attached to particles and a much larger group of slowly growing or inactive free-living cells. Because we could not completely separate the free-living and attached cells, we cannot rule out the possibility that the free-living bacteria were virtually dormant at times. The greater production we found in the attached cells of >4 μm is consistent with the model proposed by Pedrós-Alió and Brock (37) to explain the relative numbers and activity of attached and free-living bacteria in nature. However, the consistently low percentages of attached cells observed in the Ogeechee River and Black Creek (Edwards, Limnol. Oceanogr., in press) are not consistent with their prediction of greater proportions of attached bacterial biomass in systems with high flushing rates.

Conclusion. Although in situ production of bacterial biomass may be significant during summer, allochthonous inputs of bacterial biomass are clearly the most important source of this potentially important food in these rivers during most of the year. This finding is unique to this study, and it remains to be seen whether this is a characteristic of lotic systems in general. Riparian zone-river interactions may be more important in secondary production dynamics of fine-particle filter feeders than the autochthonous production of bacteria within the channel.

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