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Influence of Macrophyte Decomposition on Growth Rate and Community Structure of Okefenokee Swamp Bacterioplankton†

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Dissolved substances released during decomposition of the white water lily (*Nymphaea odorata*) can alter the growth rate of Okefenokee Swamp bacterioplankton. In microcosm experiments dissolved compounds released from senescent *Nymphaea* leaves caused a transient reduction in the abundance and activity of water column bacterioplankton, followed by a period of intense bacterial growth. Rates of [^{3}H]thymidine incorporation and turnover of dissolved D-glucose were depressed by over 85%, 3 h after the addition of *Nymphaea* leachates to microcosms containing Okefenokee Swamp water. Bacterial activity subsequently recovered; after 20 h [^{3}H]thymidine incorporation in leachate-treated microcosms was 10-fold greater than that in control microcosms. The recovery of activity was due to a shift in the composition of the bacterial population toward resistance to the inhibitory compounds present in *Nymphaea* leachates. Inhibitory compounds released during the decomposition of aquatic macrophytes thus act as selective agents which alter the community structure of the bacterial population with respect to leachate resistance. Soluble compounds derived from macrophyte decomposition influence the rate of bacterial secondary production and the availability of microbial biomass to microconsumers.

In the Okefenokee Swamp as well as other detritus-based wetland ecosystems, little of the vascular plant biomass is grazed while living; rather, most of it dies and is utilized in dissolved and particulate form by free-living and attached microorganisms. The substantial microbial biomass and high rates of production by free-living bacterioplankton in the Okefenokee Swamp (18, 19) suggest that the utilization of this dissolved organic carbon (DOC) by free-living bacteria is an important carbon flow, making this portion of the primary production available to the animal food web as bacterial biomass.

The rate of bacterial secondary production and thus the supply of bacterial biomass to bacteriovores is regulated at least in part by the quality and availability of DOC. DOC in freshwater wetlands is composed of a suite of compounds which range from labile substrates which are efficiently incorporated into bacterial biomass to refractory substances which are very poor substrates for bacterial growth. Moreover, the DOC may also contain bacteriostatic or bacteriocidal compounds which may inhibit the growth of natural bacterial populations. DOC released from algae and aquatic macrophytes, for instance, is known to inhibit the growth of laboratory cultures of certain species of bacteria (5, 13). Thus the net effect of DOC on the growth of bacterioplankton in a given aquatic system is dependent on the relative concentrations of labile, refractory, and inhibitory substances in the DOC, and the relative concentrations of these substances depends in part on the source(s) of the DOC.

The Okefenokee Swamp supports levels of microbial biomass in the water column and surface sediments similar to those in other organically rich aquatic ecosystems (18). Water column bacterial secondary production in the Okefenokee Swamp is substantial and highly seasonal and appears to be coupled to annual changes in temperature and the input of DOC from primary production (19). We initiated a series of microcosm experiments, using the white water lily (*Nymphaea odorata*), to investigate more directly the relationship between the quality of dissolved material derived from an aquatic macrophyte and water column bacterial secondary production. *Nymphaea* is a dominant plant in the aquatic macrophyte prairies of the Okefenokee Swamp and decomposes rapidly upon senescence (15; G. T. Auble, Ph.D. thesis, University of Georgia, Athens, 1982). Leaf biomass turns over several times during the growing season as old leaves senesce and new leaves grow from large underground rhizomes. Soluble compounds released during the decomposition of *Nymphaea* leaves, thus, represent a potentially important source of DOC to bacterioplankton throughout the growing season. Although essentially no information is available concerning the chemical composition of the DOC released in situ from decomposing *Nymphaea* leaves, we can assume that both growth-stimulatory and -inhibitory compounds are present. Previous studies have shown that *Nymphaea* leaves produce several types of bacteriostatic secondary metabolites including alkaloids, tannins, and saponins (25). Ethanol extracts of *N. tubersosa* have been shown, for instance, to inhibit the growth of pure cultures of the bacteria *Staphylococcus aureus* and *Mycobacterium smegmatis* (24).

Although these aquatic plants do produce compounds known to be inhibitory to pure cultures of nonaquatic bacteria, the net effect of DOC released from decomposing wetland plants on the growth of mixed aquatic bacterial populations will also depend on exposure time, the presence of resistant microorganisms, and the availability of nontoxic and, especially, growth-stimulating compounds in the DOC.

In this paper we present evidence that DOC released during the decomposition of *Nymphaea* leaves can affect the growth rate of the free-living bacterioplankton. In microcosm experiments dissolved compounds released from senescent *Nymphaea* leaves caused a transient reduction in the abundance and activity of water column bacterioplank-

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ton followed by a period of intense bacterial growth. These results underscore the importance of substrate quality as a mechanism which influences the rate of bacterial secondary production and thus the availability of microbial biomass to microconsumers in aquatic ecosystems.

MATERIALS AND METHODS

Sample sites. The Okefenokee Swamp, located in southeastern Georgia and northeastern Florida, is one of the largest freshwater wetlands in the United States. It is an acidic (pH 3.1 to 4.4), black-water, peat-accumulating environment consisting primarily of forested swamp and open marsh prairies. The major input of water to the swamp is precipitation, and flow through the watershed is slow. Evapotranspiration is the major mechanism of water loss from the system (4). Concentrations of inorganic micronutrients (e.g., nitrate, phosphate, ammonia) in the water column are very low; most of the nutrients in the ecosystem are sequestered in organic form as plant biomass or peat (22; Auble, Ph.D. thesis). Although the overall concentration of DOC in Okefenokee Swamp water is high (usually between 30 and 60 mg liter^{-1}), most of this material is probably refractory to microbial degradation consisting of tannins, fulvic acids, and humic acids.

All water and leaf samples were collected in the Chesser and Little Cooter aquatic macrophyte prairies. These prairies are open-water marsh areas dominated by aquatic macrophyte vegetation including white water lily (*N. odorata*), lady's hatpin (*Eriocaulon compressum*), golden club (*Orontium aquaticum*), and the sedge (*Rhynchospora inundata*). The mean monthly oxygen concentration at Little Cooter Prairie is 6.77 mg liter^{-1} and pH ranges from 3.81 to 4.07 (mean, 3.93; 3). Water levels at these sites can vary from 0 (during drought conditions) to over 60 cm.

Experimental design. Water samples were collected in 20-liter plastic carboys and stored at room temperature. All water samples were passed through a 110-μm mesh screen prior to use. Senescent *N. odorata* leaves were refrigerated after collection and forced-air dried at 50°C upon return to the laboratory. Macroscopic growths of epiphytes were not apparent on the surface of the leaves. Dried leaves of appropriate weight (stems removed) were added to microcosms containing 0.5 to 2 liters of swamp water and sampled at specified time intervals for thymidine incorporation, glucose uptake, and bacterial abundance. All microcosm experiments were conducted at ambient pH (approximately 3.9) and the addition of *Nymphaea* leaf fragments did not alter the pH of the microcosms. We did not routinely monitor oxygen concentrations during microcosm experiments, but bubbling microcosms with air for 24 h prior to and during experiments did not alter the inhibitory acitivity of *Nymphaea* leaf fragments. All microcosms were incubated in the dark at room temperature and stirred prior to sampling.

Thymidine incorporation. The incorporation of [^{3}H]thymidine into cold trichloroacetic acid (TCA)-insoluble material was used as an index of water column bacterial secondary production of particulate organic material (10, 11). Triplicate 10-ml water samples and Formalin-killed controls were incubated with 10 nM [*methyl*-^{3}H]thymidine (40 to 60 Ci mmol^{-1}; New England Nuclear Corp. or ICN Pharmaceuticals Inc.) at in situ temperatures for 0.5 to 2 h. Incubations were terminated by the addition of 10 ml of ice-cold 10% TCA and stored at 4°C until filtered. Samples were filtered on 25-mm-diameter (0.2-μm-pore size) filters (Millipore Corp. or Gelman Sciences, Inc.), washed twice with 5% ice-cold TCA, and then combusted in a biological oxidizer (OX-300; R. J. Harvey Co.) to oxidize the incorporated radiolabel to ^3H_2O. Tritiated water was collected in 14 ml of Scintiverse counting medium (Fisher Scientific Co.), and the samples were radioassayed in a Beckman LS 9000 liquid scintillation spectrometer. Quench corrections were made by using the sample channels ratio method.

Glucose turnover time. Glucose turnover times were determined by the incubation of triplicate 20-ml water samples for 30 min with 1.3 nM D-[6-^{3}H]glucose (New England Nuclear Corp.). Incubations were carried out in the dark at room temperature in sterile glass scintillation vials. All incubations were terminated by the addition of Formalin to a final concentration of 2%. Samples were then collected by filtration on 0.2-μm-pore size (25-mm-diameter) filters, rinsed twice with several milliliters of filtered swamp water, dried, combusted, and counted.

Bacterial abundance. Bacterial abundance was determined by epifluorescence microscopy of acridine orange-stained cells (14). Water samples were preserved with 2% formaldehyde and refrigerated until counted. Bacteria were filtered onto a 0.2-μm-pore size filter (Nuclepore Corp.) which had been previously stained with irgalan black (CIBA-GEIGY Corp.) and stained with 0.01% acridine orange. At least 10 randomly selected fields with >50 cells per field were counted on each filter, using an Olympus BHS microscope.

Leachate molecular weight fractionation. Two grams of dried leaves was placed in 500 ml of filter-sterilized swamp water and leached for 5 h at 4°C. The leachate was then filter sterilized, lyophilized, and suspended in distilled water. Low-molecular-weight-enriched (<500) and high-molecular-weight-enriched (>500) fractions of leachate were separated by using Diaflo ultrafiltration membranes (UM-05; Amicon Co.) fitted into a continuously stirred ultrafiltration cell. For microcosm experiments, leachate fractions were added to 500 ml of 110-μm screened swamp water at a concentration equivalent to the amount of soluble material which would have been released if 1 g of dried leaf material was placed in 1 liter of swamp water for 5 h. Incubations were placed in the dark and sampled every 6 h for a total of 48 h.

Nutrient agar petri plates. Okefenokee Swamp water (0.5 ml) was spread over the surface of nutrient agar (Difco Laboratories) petri plates (pH approximately 6.8), and the plates were incubated for 24 to 48 h at 20°C. After incubation the mixed bacterial flora was transferred to a culture tube containing several milliliters of sterile swamp water and the tube was vigorously mixed. The mixed bacterial suspension (0.1 ml) was then uniformly spread over another agar plate, sections of dried *Nymphaea* leaves were placed on the surface of the agar, and the plate was incubated at 20°C for 24 to 48 h to check for the release of inhibitory compounds.

RESULTS AND DISCUSSION

Inhibition of bacterial activity. In short-term experiments dissolved material released from *N. odorata* leaves had a net inhibitory effect on rates of bacterial activity. We noted in a series of preliminary experiments that glucose turnover time increased several hundredfold and that the rate of [^{3}H]thymidine incorporation into cold TCA-insoluble material (primarily DNA) decreased to approximately 15% of control rates 3 h after the addition of 1 g of dried *Nymphaea* leaves to 1 liter of Okefenokee Swamp water (Table 1).

These initial results suggested that substances released from *Nymphaea* leaves substantially altered rates of microbial activity. An alternate explanation was that unlabeled

thymidine and glucose were leached from the leaves, causing dilution of the specific activity of added radiolabel and the apparent decrease in microbial activity. However, other experiments suggest that this is not the case.

Previous characterization of the kinetics of glucose utilization by Okefenokee bacterioplankton has shown that even when the concentration of glucose in Okefenokee Swamp water is experimentally increased by nearly 10^6-fold (from a few nanomolar to approximately 1 mM) glucose turnover time typically increased by only 25- to 50-fold (18). Even if we were to assume that dried *Nymphaea* leaves were composed of 50% glucose and 1 g of dried leaf material was completely dissolved in 1 liter of swamp water, then the final concentration of glucose would be 2.5 mM; this would result in an increase in glucose turnover time on the order of 50-fold or less. It is thus highly unlikely that isotope dilution is significantly responsible for the much larger (several hundredfold) increase in glucose turnover time noted in Table 1.

Likewise, the decrease in the rate of thymidine incorporation which we observed in microcosms containing *Nymphaea* leaf fragments (Table 1) does not appear to be due to isotope dilution. We assayed the potential influence of isotope dilution on thymidine incorporation by determining the concentration of radiolabeled thymidine required to maximally label cold TCA-insoluble material and thus overcome isotope dilution. This approach has been widely used in studies of bacterial secondary production to estimate the concentration of radiolabeled thymidine required to saturate extracellular and intracellular thymidine pools (2, 10, 21). In Okefenokee waters, the addition of [^{3}H]thymidine at concentrations above 5 nM resulted in maximal labeling of cold TCA-insoluble material. Incubation of water samples with concentrations of [^{3}H]thymidine above 5 nM increased total incorporation minimally above the incorporation at 5 nM (Fig. 1). Thus concentrations of 5 nM [^{3}H]thymidine are sufficient to overcome any isotope dilution in unamended Okefenokee Swamp water. The rate of incorporation of [^{3}H]thymidine into cold TCA-insoluble material decreases substantially when *Nymphaea* leaf fragments are added to Okefenokee Swamp water (Fig. 1). This decrease in incorporation rate, however, is not due to isotope dilution of added [^{3}H]thymidine by substances released from the *Nymphaea* leaves; in the presence of 0.5 g (dry weight) of *Nymphaea* leaf fragments liter^{-1} concentrations of approximately 5 nM and above of [^{3}H]thymidine are still sufficient to produce maximal labeling of cold TCA-insoluble cellular material (Fig. 1). The decreases in rates of microbial activity we observed in the microcosm experiments are thus not caused by isotope dilution and appear, based on these kinetic studies, to result from the release of inhibitory compounds from *Nymphaea* leaf fragments.

TABLE 1. Short-term effect of *Nymphaea* leaf fragments on D-glucose turnover time and [^{3}H]thymidine incorporation by Okefenokee Swamp bacterioplankton[a]

Microcosm	Glucose turnover time (h)		Thymidine incorporation (pmol liter^{-1} h^{-1})	
	Before	After	Before	After
1	10.0	6,257	56.1	8.7
2	11.4	1,669	55.3	7.5
3	9.6	2,794	75.5	11.1

[a] Glucose turnover times and thymidine incorporation rates were measured before and 3 h after the addition of 1 g of dried *Nymphaea* leaf fragments to 1 liter of Okefenokee Swamp water. The reduced rates of glucose turnover and thymidine incorporation after the addition of *Nymphaea* leaves are significantly different (Student's t test, $P \leq 0.01$) from the rates before the addition of the leaf fragments.

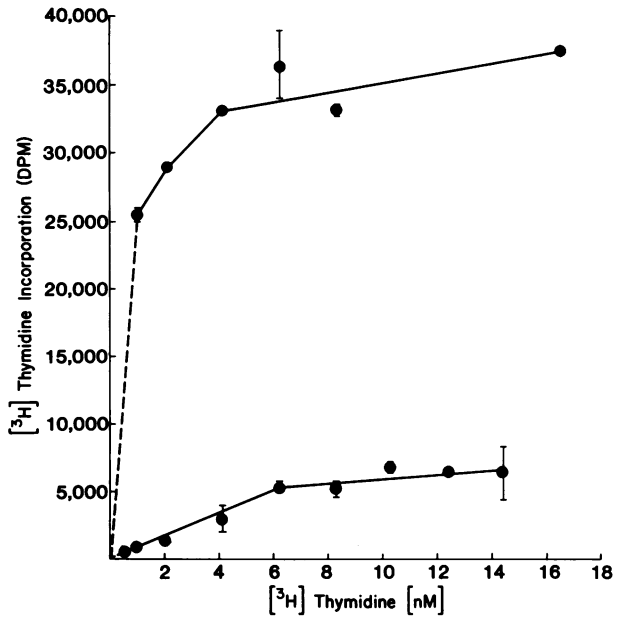

FIG. 1. Effect of increasing the concentration of [^{3}H]thymidine on rate of [^{3}H]thymidine incorporation into cold TCA-insoluble material in the presence and absence of *Nymphaea* leaf fragments. (Upper curve) No leachate added; (lower curve) 0.5 g [dry weight] of *Nymphaea* leaf fragments liter^{-1} added 3 h prior to assay for thymidine incorporation. Bars depict ± 1 standard deviation. Points without visible bars indicate that ± 1 standard deviation was less than the size of the point.

We have also corroborated the inhibitory activity of substances released from *Nymphaea* by assessing the impact of *Nymphaea* leaf fragments on the growth of mixed populations of Okefenokee Swamp bacteria on nutrient agar petri plates. *Nymphaea* leaf fragments consistently inhibited the growth of mixed populations of Okefenokee Swamp bacteria on nutrient agar petri plates (Fig. 2). The clear zones around the leaf fragments (Fig. 2) indicate areas in which dissolved substances diffusing from the leaf fragments have prevented growth of the mixed bacterial lawn. Nutrient agar is known to be a selective medium, and, thus, the agar plate experiments do not illustrate the response of the entire bacterial community. They do, however, illustrate the dramatic effect of leachable substances on that portion of the microbial community that can grow on nutrient agar. Moreover, several distinctive colony morphologies routinely occur on our experimental agar plates, indicating that the bacteriostatic effect is not restricted to a single group of microorganisms.

We investigated the relationship between bacterial secondary production by Okefenokee bacterioplankton and the concentration of *Nymphaea* leachate to assess the potential influence of inhibitory compounds released from decomposing *Nymphaea* leaves on water column bacterial production. Okefenokee bacterial populations are exposed to *Nymphaea* leachates in situ during the growing season. For example, in June of 1984 (the peak summer growing season) at the Little Cooter Macrophyte Prairie, live *Nymphaea* biomass reached 130.9 g (dry weight) m^{-2} and decomposing *Nymphaea* biomass reached 28.6 g (dry weight) m^{-2} (H. Greening, unpublished data). The mean water depth at Little Cooter

FIG. 2. Inhibition of the growth of mixed populations of Okefenokee Swamp bacteria on nutrient agar petri plates by *N. odorata* leaf fragments.

Prairie in June 1984 was 35 cm. The concentrations of decaying *Nymphaea* leaves was thus approximately 0.08 g (dry weight) liter^{-1}. The addition of 0.1 g (dry weight) of *Nymphaea* leaf fragments liter^{-1} to microcosms decreased thymidine incorporation by 64% (Fig. 3). Higher concentrations of leachate as might occur during periods of low water level and elevated *Nymphaea* mortality decreased thymidine incorporation by over 75% (Fig. 3). If, for example, during the onset of drought conditions water level decreased to 5 cm and *Nymphaea* leaf mortality doubled, then the concentration of *Nymphaea* leachate in the water column would reach 1 g (dry weight) liter^{-1}.

The net response of Okefenokee bacterial populations to *Nymphaea* leachates in situ would depend on several factors, including water level, the concentration of DOC released, the duration of the release event, the degradation or inactivation of inhibitory substances, and the rate of advective mixing. If rates of advective mixing are high (particularly when water levels are high), then it is likely that DOC released during *Nymphaea* decomposition is quickly diluted and leachate concentration in situ would be lower than in our microcosms. If rates of advective mixing are low (particularly during the peak growing season when water level is low), then leachate concentrations close to 0.1 g (dry weight) would occur in situ. Higher concentrations of *Nymphaea* leachates (1 g [dry weight] liter^{-1}) would occur during

drought periods associated with low water levels and mass mortality of *Nymphaea* leaves. A drought of this magnitude occurred in the summer of 1981, during which water levels over both macrophyte prairies slowly decreased to dryness.

Desiccation of *Nymphaea* leaves also occurs on a smaller scale in association with the formation of peat batteries. Peat batteries originate when large blocks of peat detach from the submerged sediments and float to the water surface. Battery formation occurs whenever the swamp is flooded and is a continuous source of desiccated vegetation. Rain events or the reflooding of these areas which contain dried aquatic macrophyte leaves would result in the release of substantial amounts of dissolved material from the rehydration of desiccated leaves.

Stimulation of bacterial activity. Inhibitory substances released from *Nymphaea* leaf fragments are able to reduce rates of microbial activity over short periods of time. Longer-term microcosm experiments with *Nymphaea* leachates were undertaken to determine the longevity of the antimicrobial effect. Leachates prepared from dried senescent *Nymphaea* leaves were separated into two size fractions enriched in <500- and >500-molecular-weight compounds,

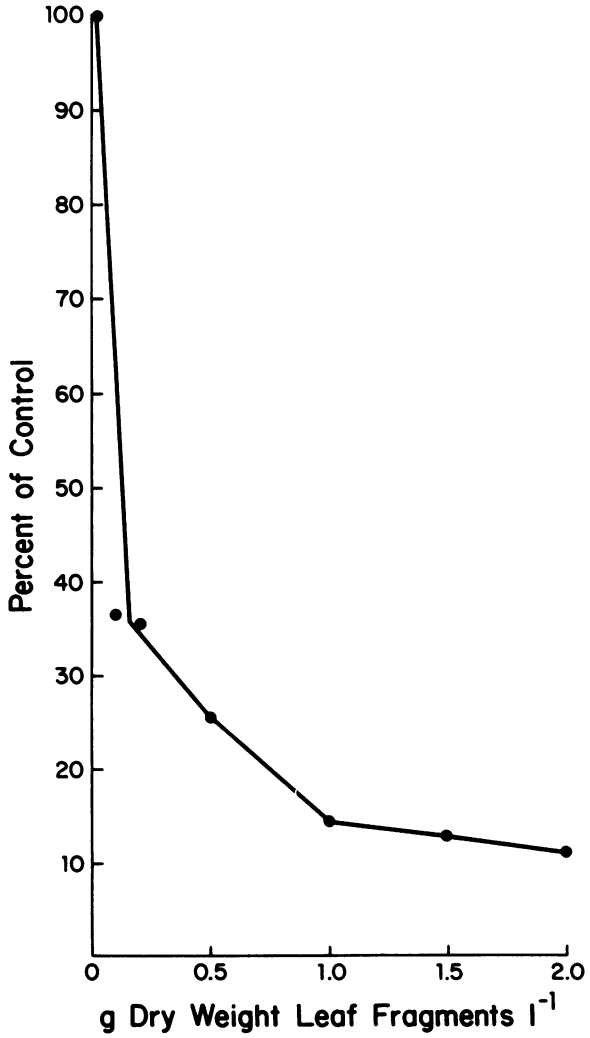

FIG. 3. Inhibition of [^{3}H]thymidine incorporation by increasing amounts of *Nymphaea* leaf fragments.

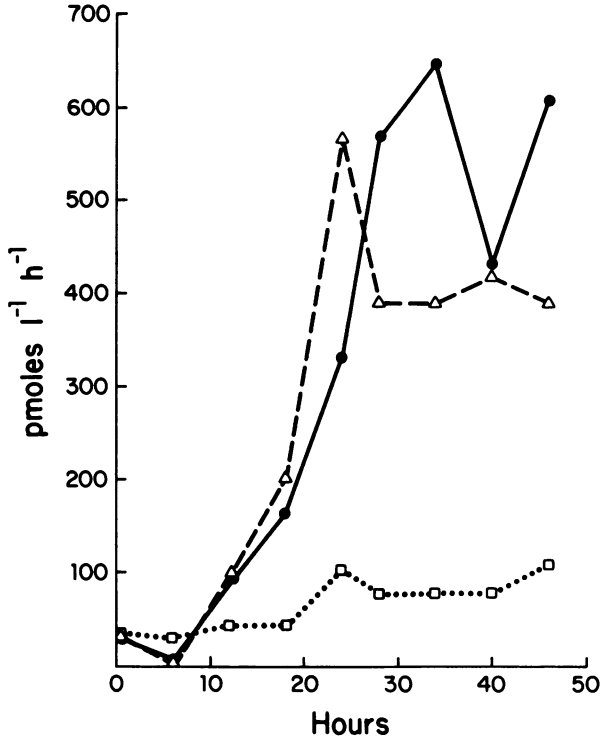

FIG. 4. Long-term effects of *Nymphaea* leachates on [^{3}H]thymidine incorporation by natural populations of Okefenokee bacterioplankton. Symbols: □, control; ●, <500-molecular-weight leachate fraction; △, >500-molecular-weight leachate fraction.

using Amicon ultrafiltration. The leachates were added to 500 ml of swamp water at a dose equivalent to the concentration of material which would have been released if 1 g of dried *Nymphaea* leaves were leached in 1 liter of swamp water for 5 h. We used high concentrations of leachate to mimic conditions of drought onset when the impact of dissolved material released from decomposing *Nymphaea* leaves would be the most apparent. Bacterial abundance and thymidine incorporation were monitored at 6-h intervals for 46 h in unamended control and leachate-treated microcosms.

Bacterial abundance and thymidine incorporation were significantly reduced (Student's t test, $P \leqq 0.05$) in both treated microcosms 6 h after leachate addition (Fig. 4 and 5). Abundance (acridine orange direct counts) decreased 38.3% in response to the <500-molecular-weight-enriched leachate fraction and 57.5% in response to the >500-molecular-weight-enriched leachate fraction. Rates of [^{3}H]thymidine incorporation decreased 91.2 and 85.6%, respectively. The reduction in the rate of thymidine incorporation is partially attributable to the lower bacterial abundance in leachate-treated microcosms but also represents a net change in activity on a per-cell basis. Thymidine incorporation per cell at the 6-h time point was 8.5×10^{-21} mol h^{-1} in the control microcosm and 1.2×10^{-21} and 2.8×10^{-21} mol h^{-1} in the <500-molecular-weight and >500-molecular-weight treatments, respectively. Peak bacterial abundance occurred between 24 and 28 h in both treatments (Fig. 5). Highest rates of thymidine incorporation were observed after 24 h in the >500-molecular-weight treatment (567×10^{-12} mol liter^{-1} h^{-1}) and 34 h in the <500-molecular-weight treatment (648×10^{-12} mol liter^{-1} h^{-1}).

The effective pore size of Amicon membranes may change

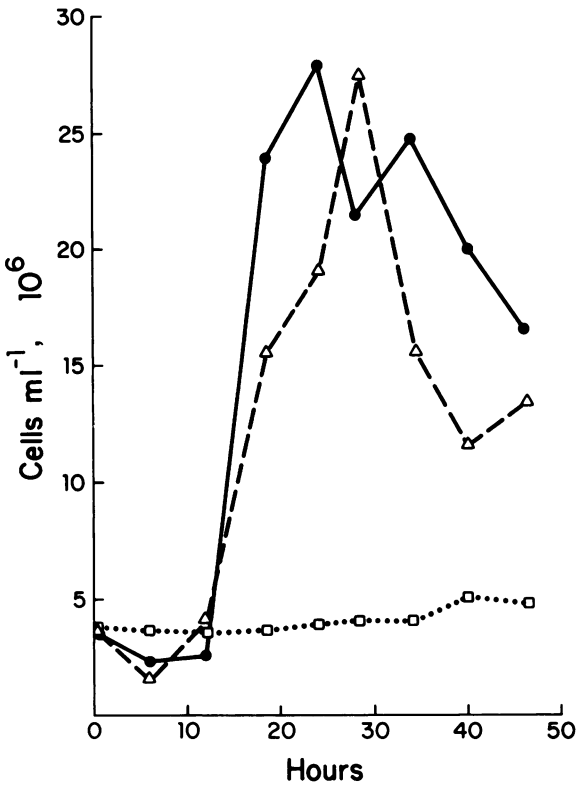

FIG. 5. Long-term effects of *Nymphaea* leachates on abundance of Okefenokee bacterioplankton. Symbols: □, control; ●, <500-molecular-weight leachate; △, >500-molecular-weight leachate fraction.

during filtration as a result of membrane-solute interactions (G. R. Aiken, Abstr. 48th Annu. Meet. Am. Soc. Limnol. Oceanogr. 1985, p. 1). We thus do not know if the inhibitory and growth-stimulating activity exhibited by both the high- and low-molecular-weight fractions is due to a real difference in molecular size or to inadequate separation of a single or several compounds. It is clear, however, that compounds which both inhibit and stimulate bacterial growth are present in *Nymphaea* leachates.

The decrease in bacterial abundance at the 6-h time point (Fig. 5) may be due to direct toxicity of leachate components and the subsequent autolysis of bacteria or a more general inhibition of bacterial growth which allows small bacterial predators (which would pass through the 110-μm prescreen) to deplete bacterial numbers. Several groups of high-molecular-weight compounds known to be present in *Nymphaea* could potentially exhibit inhibitory activity. These include alkaloids, flavanoids, tannins, saponins, or steroids (24, 25). Low-molecular-weight antimicrobial compounds, such a phenols, are also likely to be present in the *Nymphaea* leachates.

Resistance. The net effect of *Nymphaea* leachates on water column bacterial populations was to stimulate bacterial growth over several days, indicating that decaying *Nymphaea* leaves are a source of utilizable DOC, inorganic nutrients, or both to water column bacterioplankton. The recovery of microbial activity after the initial exposure to *Nymphaea* leachates suggests that the microbial population must acquire resistance to the inhibitory compound(s) or that the compound(s) must be inactivated by volatilization, microbial transformation, or binding to the water column DOC. If the observed recovery were the result of a shift in the bacterial population from nonresistant to resistant organisms, then one would expect that bacterial populations which were previously exposed to *Nymphaea* leachates would not exhibit reduced rates of thymidine incorporation when reexposed to leachate. Alternatively, if recovery were due to the detoxification of the inhibitory compound(s), then one would expect that, after several days of exposure to Okefenokee microflora, leachate removed from microcosms would not inhibit thymidine incorporation in microcosms containing previously unexposed bacterial populations.

Aged leachate preparations, however, were in fact as inhibitory as freshly prepared leachates. Aged leachates were prepared by leaching 3 g (dry weight) of *Nymphaea* leaves in 1 liter of Okefenokee Swamp water containing the natural microbial assemblage. After 24 and 72 h, 100 ml of leachate was removed from the microcosm, filter sterilized, and added to 100 ml of Okefenokee Swamp water, and the bacterial population was assayed for [^{3}H]thymidine incorporation. Both 24- and 72-h-aged leachates strongly inhibited bacterial production ([^{3}H]thymidine incorporation) by Okefenokee bacterioplankton which had not been previously exposed to *Nymphaea* leachate (Table 2). Thymidine incorporation by water column bacterioplankton was reduced by >88% between 1 and 6 h after the addition of the aged leachates and remained depressed by over 70% after 24 h (Table 2). The antimicrobial compound(s) present in *Nymphaea* leachates is thus not inactivated for at least 72 h, and the net stimulation of bacterial growth which we observed in the long-term experiments (Fig. 4 and 5) can be assumed to result from the growth of bacterial populations which are resistant to the inhibitory effect.

We corroborated this assumption by directly examining resistance acquisition in bacterial populations pre-exposed to leachate for 24 h (sufficient time for the recovery of activity, as demonstrated previously). Three grams (dry weight) of *Nymphaea* leaves was leached in 1 liter of Okefenokee Swamp water. After 24 h a 100-ml subsample of the leachate-treated microcosm was filtered on a 0.2-μm-pore size Nuclepore filter. Bacteria were gently washed off the filter and suspended in 400 ml of filter-sterilized unamended Okefenokee Swamp water. The suspension was divided into two 200-ml incubations, one of which served as a control and the other of which received *Nymphaea* leaf fragments (1 g [dry weight] liter^{-1}). Both microcosms were then sampled periodically for 24 h to determine effects on bacterial production. The transferred populations were completely resistant to inhibition. Moreover, after 6 h of reexposure to *Nymphaea* leaf fragments, thymidine incorporation in the experimental microcosm was elevated by 405% relative to the control value. Other [^{3}H]thymidine incorpo-

TABLE 2. Inhibition of [^{3}H]thymidine incorporation by Okefenokee Swamp bacterioplankton populations previously unexposed to *Nymphaea* leachates in response to treatment with aged *Nymphaea* leachate

Time (h)[a]	[^{3}H]thymidine incorporation (% of control)	
	24-h leachate	72-h leachate
1	12	2
3	6	8
6	5	8
24	30	19

[a] Time elapsed since the addition of aged leachates to experimental microcosms.

TABLE 3. Persistence of antimicrobial compounds in microcosms treated with *Nymphaea* leachates and maintenance of resistance to these compounds by the exposed bacterial population[a]

Day	Persistence of antimicrobial activity[b]			Maintenance of resistance[c]		
	[^{3}H]thymidine incorporation (pmol liter^{-1} h^{-1})		% change relative to control	[^{3}H]thymidine incorporation (pmol liter^{-1} h^{-1})		% change relative to control
	Control	Experimental		Control	Experimental	
3	14.7	2.1	−86	47.2	858.5	+1,819
9	14.3	2.9	−80	54.6	3,593.6	+6,582
27	18.8	0.3	−98	19.5	1,223.1	+6,272

[a] Two microcosms containing 2 liters of unamended Okefenokee Swamp water were incubated in the dark at 22°C. To one microcosm *Nymphaea* leaf fragments (3.0 g [dry weight] liter^{-1}) were added at time zero. These microcosms were subsequently used as sources of aged leachate and bacterioplankton populations which had or had not been previously exposed to *Nymphaea* leachate for 3, 9, or 27 days.

[b] A 100-ml portion of filter-sterilized contents of the leachate-treated microcosm (experimental) or untreated Okefenokee Swamp water (control) was added to 100 ml of unamended Okefenokee Swamp water, and the mixture was assayed after 6 h for [^{3}H]thymidine incorporation.

[c] After 3, 9, and 27 days of incubation, 100 ml of the contents of the leachate-treated microcosm was filtered on a 0.2-μm-pore size Nuclepore filter. The bacterial population was gently rinsed off the filter and suspended in 400 ml of unamended, filter-sterilized Okefenokee Swamp water. The suspension was partitioned into two 200-ml subsamples. *Nymphaea* leaf fragments were added to one of the subsamples (experimental) and the other subsample served as the control. Control and experimental treatments were assayed after 6 h for [^{3}H]thymidine incorporation rate.

ration values relative to the control were as follows, where time is time elapsed since addition of *Nymphaea* leaf fragments to experimental microcosms: zero time, 104%; 1 h, 105%; 3 h, 121%; and 24 h, 101%. The bacterial population which grew in response to the first exposure to *Nymphaea* leaf fragments was resistant to an inhibitory compound(s) released during the second exposure. *Nymphaea* leachates can thus act as selective agents which prevent the growth of nonresistant organisms and thereby modify the community structure of the bacterial population with respect to leachate resistance. The inhibitory compounds remained active in the microcosms for at least 27 days and bacterial populations maintained resistance to *Nymphaea* leachates throughout this period (Table 3).

Over the course of a year 20 experiments were conducted to investigate the susceptibility of natural Okefenokee bacterioplankton assemblages to *Nymphaea* leachates. Water samples were collected from randomly selected sites within the water lily habitat to get a rough approximation of the relative occurrence of bacterial populations susceptible or resistant to leachate. In each case [^{3}H]thymidine incorporation by Okefenokee bacterioplankton was initially inhibited by over 85% upon exposure to *Nymphaea* leaf fragments (1.5 g [dry weight] liter^{-1}) similar to the results presented in Table 1. Thus, using our sampling regime we have not found resistant populations in situ. This may be due in part to our inability to sample during periods of very low water level when resistant populations are likely to be most prominent. Alternately, if resistant organisms occur during periods of higher water level, they must lose their resistance over time, be diluted by advective mixing, or be removed from the water column.

Organisms might lose resistance in situ if *Nymphaea* leachates are detoxified (which is inconsistent with our results) or diluted to very low concentrations and thus no longer act as selective agents. Advective mixing could also disperse resistant organisms and thereby reduce the likelihood of sampling a parcel of water containing large numbers of these organisms. Resistant organisms could also be preferentially removed from the water column by microzooplankton grazers. We have noted that bacteria which grow on *Nymphaea* leachates exhibit mean cell volumes over twice those of Okefenokee bacterioplankton growing in situ. Ammerman et al. (1) suggested that large, fast-growing cells such as these may be preferentially eaten by microzooplankton predators and thus turn over faster than the bacterial population as a whole. Bacterial population turnover rates in Okefenokee macrophyte prairies average 4.9 day^{-1} during the warmer months of the year (19). If larger cells are preferentially taken by bacterial predators, then these cells would be removed from the water column at an even faster rate and probably would not reach large population densities in the field.

As discussed previously, *Nymphaea* leaves often undergo decomposition in the water column without prior dehydration. We initiated a series of microcosm experiments with freshly collected whole *Nymphaea* leaves to investigate leaching under these conditions. The addition of whole senescent *Nymphaea* leaves to 1-liter microcosms resulted in a reduction in the rate of [^{3}H]thymidine incorporation followed by a period of elevated activity. [^{3}H]thymidine incorporation as a percentage of the control was as follows at the times given, where time is time elapsed since the addition of *Nymphaea* leaf fragments to experimental microcosms: 1 h, 27%; 3 h, 21%; 6 h, 36%; 24 h, 39%; 48 h, 62%; and 52 h, 362%. These values are the means of three experiments. This is the same pattern we observed with dried leaf fragments and prepared leachates, although the duration of the inhibitory effect was longer in the presence of freshly collected whole senescent *Nymphaea* leaves, suggesting a slower but more sustained release under these conditions.

We have already presented evidence that during the onset of drought conditions inhibitory compounds could be released at concentrations sufficient to influence rates of water column bacterial production. Under these conditions, and during other periods of low water level, it is likely that bacteria resistant to the inhibitory compounds are the only organisms capable of utilizing the labile substrates also released during *Nymphaea* decomposition. It is much more difficult to assess the ecological impact of inhibitory compounds released from decomposing *Nymphaea* leaves during periods of higher water level and lower *Nymphaea* biomass. If rates of advective mixing are high under these conditions, then it is likely that the inhibitory compound(s) is diluted and that labile substrates are utilized at bulk water concentrations by nonresistant microorganisms. If rates of advective mixing are low, then the release of DOC may form a microzone in the immediate vicinity of the decomposing leaf where both inhibitory and labile compounds occur at elevated concentrations. In such a microzone, organisms which were resistant to the antimicrobial compounds would be able to utilize the labile substrates before they were diluted to

bulk water concentrations. Organisms with low-affinity (high K_t), high-capacity (high V_{max}) uptake systems for labile substrates such as glucose do occur in Okefenokee waters (18) and such organisms might be expected to efficiently use the elevated concentrations of labile substrates which would occur in microzones.

Ecological role of DOC release during aquatic macrophyte decomposition. Labile substances released during *Nymphaea* decomposition clearly represent a substantial input of DOC which can be utilized for growth by the free-living bacterioplankton. Dried *Nymphaea* leaves lose approximately 26% of their initial dry weight after only 24 h of leaching in Okefenokee water. This dissolved material stimulates bacterial growth over a period of several days and may be partially responsible for the high rates of bacterial secondary production which have been observed in aquatic-macrophyte-dominated areas of the Okefenokee Swamp (19). Consistent with our results, earlier reports indicate that natural bacterial populations incorporate radiolabeled leachates from other macrophytes (7, 9, 23) and exhibit growth when enriched with plant leachates (6, 26).

Several aquatic macrophytes are known to contain secondary compounds which exhibit antimicrobial properties (13, 16, 24). However, most of the previous work was carried out with pure cultures of target microorganisms, usually bacteria known to be pathogenic to higher organisms, and not necessarily important components of wetland bacterioplankton. Lalonde (16), for instance, found that several strains of phytopathic bacteria were susceptible to alkaloids isolated from the water lily *Nuphar*. He also noted that the bacteriostatic effect was species specific, as three bacterial strains isolated from the immediate vicinity of growing *Nuphar* plants were resistant.

McArthur et al. (17) noted differences in the ability of bacterial isolates from prairie streams to grow on plant leachates from various sources. Bacteria isolated from oak forest regions of the stream were able to grow on both oak and grass leachates, whereas bacteria isolated from grassland regions of the stream were able to grow only on grass leachates. These results suggest that natural bacterial populations can adapt to the plant leachates they most frequently encounter. Likewise, our results with *Nymphaea* leachates suggest that the composition of the water column DOC can alter the structure of the microbial community with respect to both acquisition of resistance to inhibitory compounds and the subsequent luxuriant growth of resistant organisms at the expense of labile leachate components.

The primary function of the inhibitory compounds produced by *N. odorata* in the Okefenokee ecosystem has not yet been determined. Aquatic macrophytes may produce antimicrobial compounds as antifouling agents which prevent the growth of periphyton (13), as bacteriostatic substances which prevent infection by phytopathic organisms, or as allelopathic agents directed against other organisms (12, 27). Harrison and Chan (13) demonstrated that extracts of eelgrass (*Zostera marina*) inhibited the growth of eight species of cultured microalgae including diatoms, dinoflagellates, and an autotrophic green flagellate. Secondary compounds released from aquatic macrophytes may also act as biochemical repellents to zooplankton (8, 20). Secondary compounds derived from aquatic macrophytes may thus influence the rates of activity and population dynamics of a wide variety of organisms at other trophic levels and with different ecological roles. We have presented evidence that DOC released during the decomposition of one aquatic macrophyte, *N. odorata*, contains inhibitory and stimulatory compounds which can substantially alter the rate of bacterial secondary production by free-living bacterioplankton.

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