APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1988, p. 718–727
0099-2240/88/030718-10$02.00/0

Vol. 54, No. 3

Seasonal and Diel Variability in Dissolved DNA and in Microbial Biomass and Activity in a Subtropical Estuary

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Received 31 August 1987/Accepted 21 December 1987

Dissolved DNA and microbial biomass and activity parameters were measured over a 15-month period at three stations along a salinity gradient in Tampa Bay, Fla. Dissolved DNA showed seasonal variation, with minimal values in December and January and maximal values in summer months (July and August). This pattern of seasonal variation followed that of particulate DNA and water temperature and did not correlate with bacterioplankton (direct counts and [^{3}H]thymidine incorporation) or phytoplankton (chlorophyll *a* and $^{14}CO_2$ fixation) biomass and activity. Microautotrophic populations showed maxima in the spring and fall, whereas microheterotrophic activity was greatest in late summer (September). Both autotrophic and heterotrophic microbial activity was greatest at the high estuarine (low salinity) station and lowest at the mouth of the bay (high salinity station), irrespective of season. Dissolved DNA carbon and phosphorus constituted 0.11 ± 0.05% of the dissolved organic carbon and 6.6 ± 6.5% of the dissolved organic phosphorus, respectively. Strong diel periodicity was noted in dissolved DNA and in microbial activity in Bayboro Harbor during the dry season. A noon maximum in primary productivity was followed by an 8 p.m. maximum in heterotrophic activity and a midnight maximum in dissolved DNA. This diel periodicity was less pronounced in the wet season, when microbial parameters were strongly influenced by episodic inputs of freshwater. These results suggest that seasonal and diel production of dissolved DNA is driven by primary production, either through direct DNA release by phytoplankton, or more likely, through growth of bacterioplankton on phytoplankton exudates, followed by excretion and lysis.

The presence of extracellular DNA in freshwater and marine environments has been known for some time (22, 26, 30, 31). Early studies were devoted to the detection of dissolved DNA (2, 30) rather than the spatial and temporal distribution of dissolved DNA in aquatic environments. Recently, a simplified and sensitive method of testing for dissolved DNA on the basis of the fluorescence of Hoechst 33258-DNA complexes has been developed (5) and used to determine the distribution of dissolved DNA in the eastern Gulf of Mexico (6). Offshore and subsurface waters possessed the lowest dissolved DNA values, with concentrations increasing shoreward and in estuarine plumes. Dissolved DNA from most marine environments possessed a wide range of molecular sizes (0.12 to 35.2 kilobases [4, 6]) and was turned over rapidly (turnover times of ≤1 day [28]).

Little information exists on the temporal and spatial distribution of dissolved DNA in estuarine environments. The goal of the present study was to investigate seasonal, diel, and spatial variability in dissolved DNA concentrations in a subtropical estuary and to relate its abundance to the activity and biomass of autotrophic and heterotrophic microbial populations.

MATERIALS AND METHODS

Sampling sites and description of the Tampa Bay estuary. Tampa Bay is a subtropical estuary with a watershed of 2,235 mi^2 (5,789 km^2), a mean discharge rate of 43.54 m^3/s, and a diurnal tide range of 0.6 to 0.85 m (36). One of three stations which was sampled monthly for 15 months was located in the mouth of the Alafia River (labeled AR in Fig. 1), a river with an average discharge velocity of 10.75 m^3/s (36) and phosphate concentrations which were often in excess of 100 μM (23). A second station was located near the center of the bay (Fig. 1, CB) and a third was located at the mouth of the bay (Fig. 1, MB). Surface waters were collected between 0800 and 1100 h, and all incubations were initiated within 2 h of collection.

Diel studies were performed on samples collected off the dock of the University of South Florida Marine Science Laboratory in Bayboro Harbor, St. Petersburg. Samplings were taken every 4 h for 52 h.

Determination of dissolved DNA and microbial parameters. The amount of dissolved DNA was determined in 0.2-μm filtrates which were collected under mild vacuum (≤150 mm Hg [1 mm Hg = 133.3 Pa]) by the method of DeFlaun et al. (5). Dissolved DNA values were corrected for non-DNA-fluorescing material by DNase I treatment. Correction for losses in handling were accounted for in DNA-spiked replicate samples. Filtration at ≤150 mm Hg has been shown to result in minimal cell lysis, as determined by dissolved chlorophyll *a* measurements (5). In the seasonal study, microbial parameters were determined in unfiltered water and in the <1-μm fraction which was generated by vacuum prefiltration through a 1-μm Nuclepore filter (Nuclepore Corp., Pleasanton, Calif.) at ≤150 mm Hg. The amount of particulate DNA was determined by the Hoechst 33258 method of Paul and Myers (29). Chlorophyll *a* was determined fluorometrically in methanol extracts of Whatman GF/F-filtered samples, as described by Holm-Hansen and Riemann (12) and by us (27). Bacterial abundance was determined by epifluorescence direct counts after staining with 10^{-5} M Hoechst 33342 (Calbiochem-Behring, La Jolla, Calif.) or diamidinophenyl indole (DAPI; Aldrich Chemical Co., Inc., Milwaukee, Wis. [24]). Primary production was

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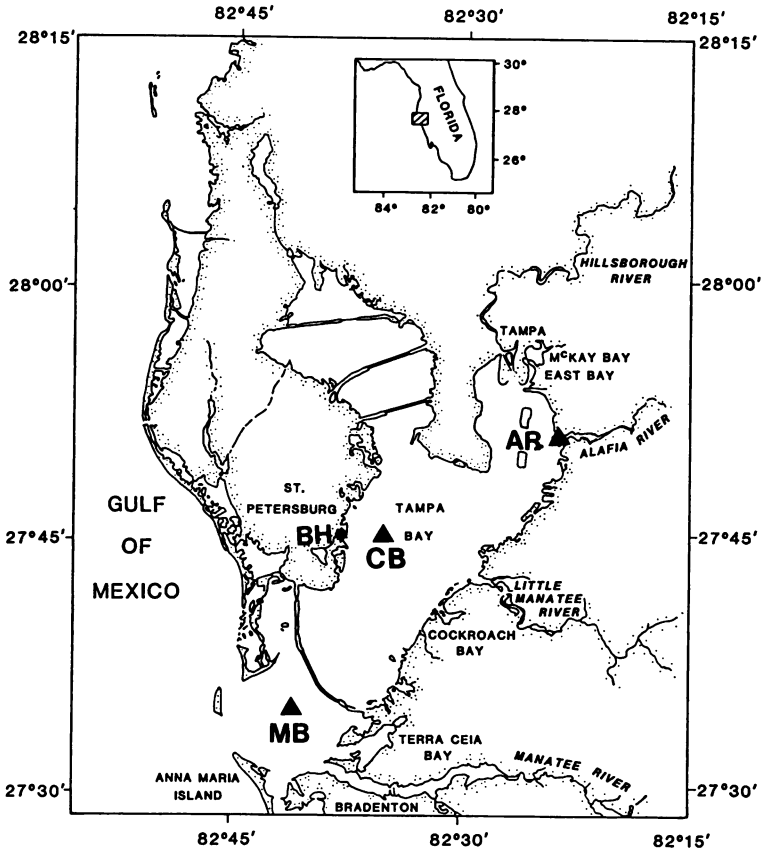

FIG. 1. Location of stations sampled for the seasonal and diel studies. Abbreviations: AR, Alafia River Station; CB, center-of-the-bay station; MB, mouth-of-the-bay station; BH, Bayboro Harbor.

determined by the ^{14}C method, with modifications suggested by Carpenter and Lively (3). These modifications include incubation in polycarbonate flasks, the use of high purity $NaH^{14}CO_3$ (Amersham Corp., Arlington Heights, Ill.), and sampling at several time points (total incubation time, <2 h) to assure linearity of uptake. Samples were incubated in ambient daylight under two layers of neutral density screening (≅33% incident irradiation) during the seasonal study or under a fixed illumination (71 microeinsteins/m^2 per s) in the laboratory for diel studies. Total CO_2 was determined by the titration method, as described by Strickland and Parsons (38). [^{3}H]thymidine incorporation into trichloroacetic acid-insoluble material was used as a general indicator of bacterial activity (9). [*methyl*-^{3}H]thymidine (50 to 70 Ci/mmol; ICN Radiochemicals, Irvine, Calif.) was added to seawater for a total radioactivity of 0.4 μCi/ml and a concentration of ~5 nM. Samples (usually 2.0 ml) were filtered with time onto 0.2-μm Nuclepore filters, and the filters were washed with 5.0 ml of 5% trichloroacetic acid. Since no molecular fractionation or isotope dilution analysis was performed, no calculation of DNA synthesis or bacterial production can be made, and results are expressed in moles of thymidine incorporated per liter per hour or per cell per hour.

For both primary productivity and bacterial heterotrophic activity, the <1-μm fraction was generated by filtration after incubation to prevent artifacts in activity caused by filtration.

Dissolved organic carbon was determined by the persulfate oxidation method of Fredericks and Sackett (8). Salinity was determined by refractometry. Dissolved organic phosphorus, total soluble phosphorus, and soluble reactive phosphorus determinations were kindly performed by Gabriel A. Vargo, University of South Florida, St. Petersburg, by the method of Solorzano and Sharp (37).

RESULTS

Seasonal study of Tampa Bay. Figure 2 displays the salinity of the three stations as a function of monthly sampling over the project period. The Alafia River station possessed the lowest and most variable salinities (7 to 28‰), being strongly influenced by runoff from storm events. The center-of-the-bay station possessed an intermediate salinity (25 to 31‰), while the mouth of the bay possessed the least variable salinity (29.5 to 34.7‰). In general, there is less rainfall from mid-February to the end of June, and the rainier months are July through February in the Tampa Bay area.

The terrestrial input of dissolved organic carbon (DOC) is clearly evident in the Alafia River water (Fig. 3). DOC values ranged from 3.41 mg of C per liter to over 15 mg of C per liter, with maximal values in spring (April 1985 and March 1986) and minimal values in winter months. Center-of-the-bay DOC values (Fig. 3) ranged from 2.46 to 4.26 mg of C per liter, and mouth-of-the-bay stations ranged from 1.2 to 2.83 mg of C per liter, with the lowest values in December. The distribution of DOC values at these three stations (i.e., lowest at the mouth of the bay and highest in the Alafia River) was also reflected in nearly every other parameter measured over the study period.

Figures 4, 5, and 6 show the distribution of dissolved

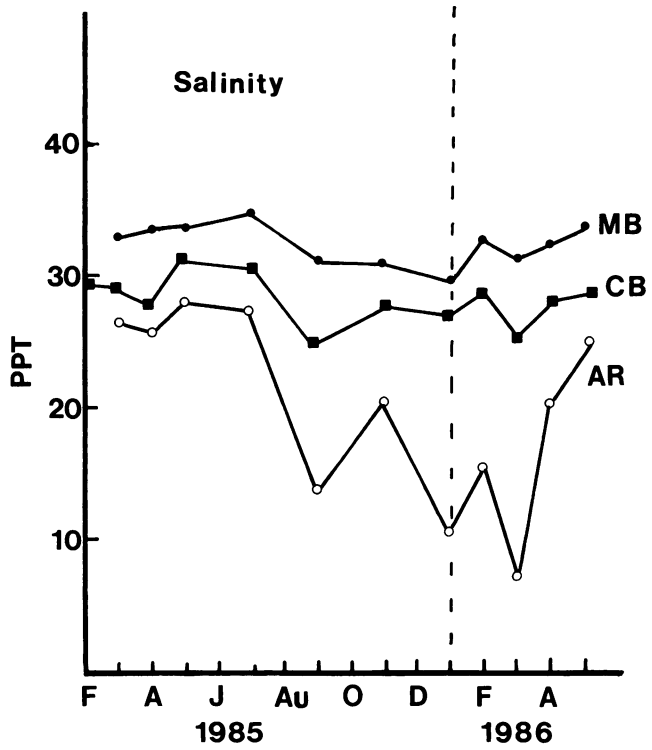

FIG. 2. Salinity at the three stations as a function of time of year. Abbreviations: F, February; A, April; J, June; Au, August; O, October; D, December. Other abbreviations are the same as those in the legend to Fig. 1. The dashed line represents the end of 1985 and the beginning of 1986.

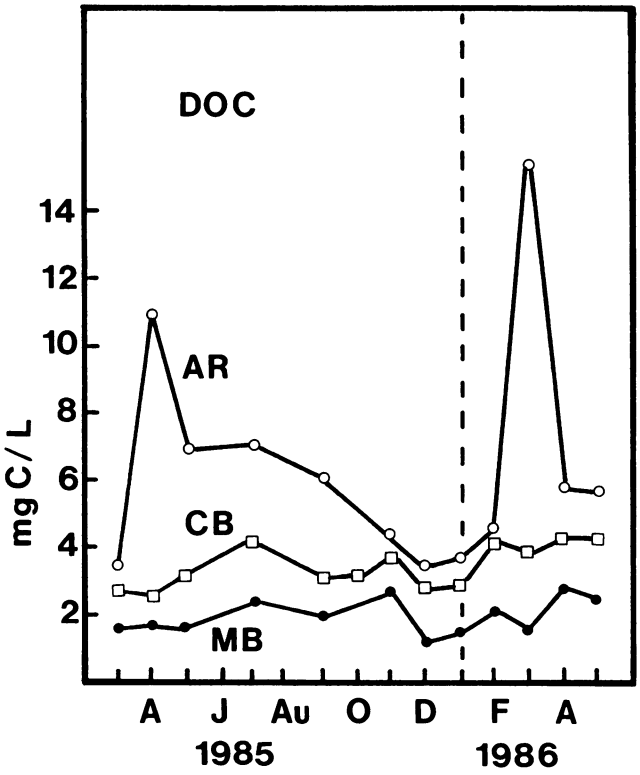

FIG. 3. DOC content at the three stations as a function of time of year. Abbreviations are the same as described in the legend to Fig. 1.

DNA, temperature, and all other microbial parameters which were measured at the three stations. Seasonal changes in water temperature in Tampa Bay (14.5 to 32.5°C) were less extreme than those found in many temperate estuaries. However, strong seasonal effects were apparent in most parameters which were measured. Dissolved DNA values in the Alafia River ranged from 1.81 to 19 μg/liter, with minimal values in December and maximal values in July 1985 and April 1986. Approximately 2 miles up the river from the Alafia River Station, we measured the highest dissolved DNA value thus far measured, 43.8 μg/liter on 22 May 1985.

Similar seasonal trends were observed for dissolved DNA at the other two stations, where maxima occurred in summer months and minima occurred in December or January. Dissolved DNA values ranged from 4.1 to 15 μg/liter and 2.2 to 10.4 μg/liter for the mid- and mouth-of-the-bay stations, respectively.

Of all parameters measured, the distribution of dissolved

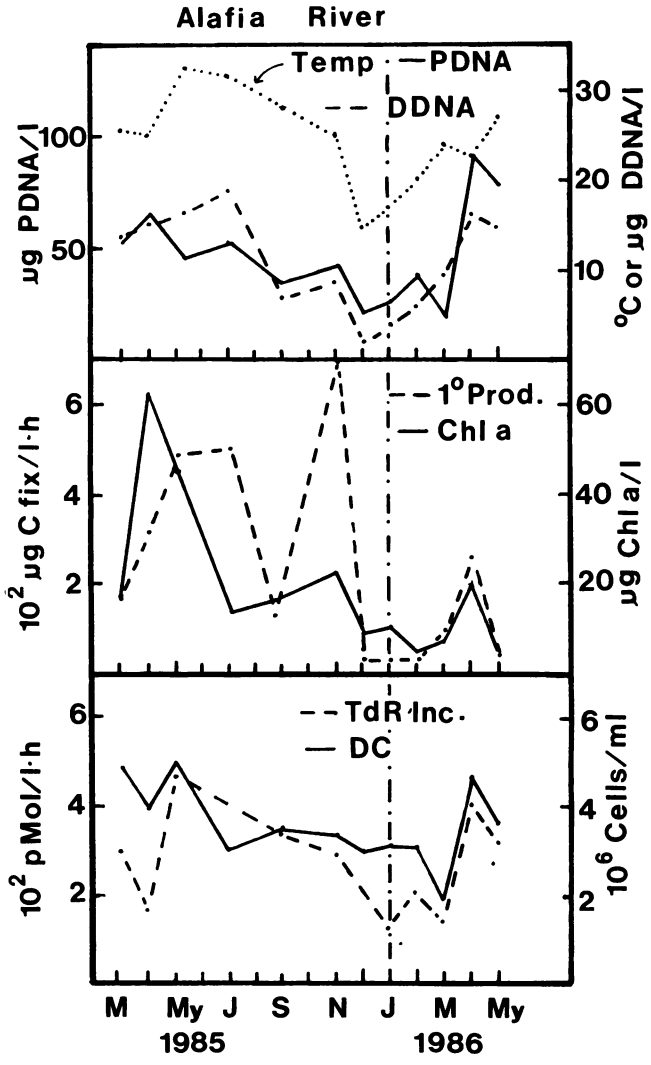

FIG. 4. Water temperature (Temp), dissolved (DDNA) and particulate DNA (PDNA), and microbial activity and biomass as a function of monthly sampling at the Alafia River station in Tampa Bay. Abbreviations: 1° Prod., primary productivity; Chl a, chlorophyll *a*; TdR Inc., thymidine incorporation; DC, bacterial direct counts; M, March; My, May; J, July; S, September; N, November; J, January. Vertical dashed line divides 1985 from 1986.

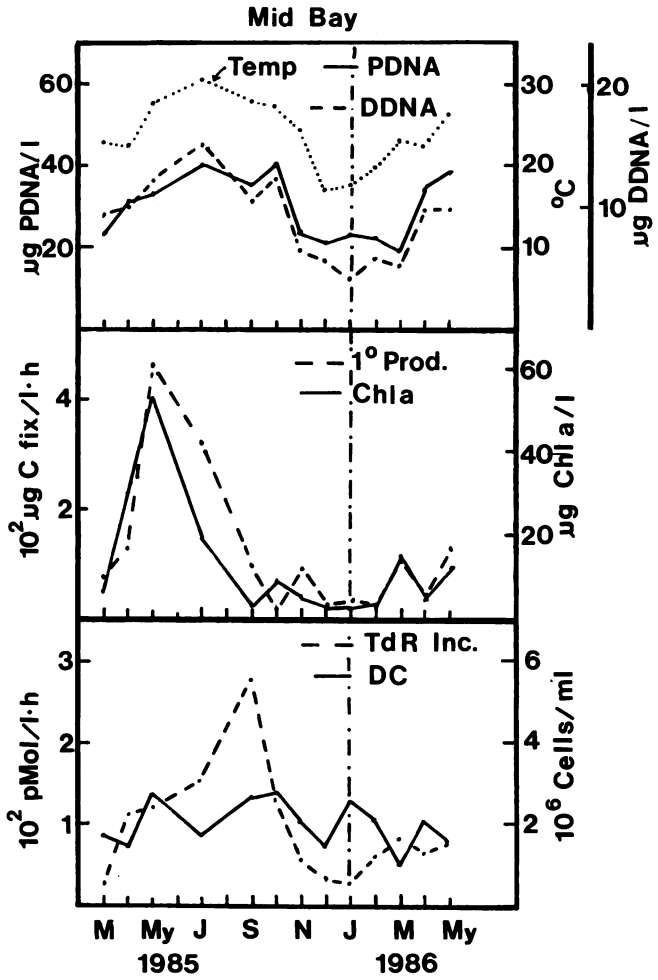

FIG. 5. Water temperature, particulate and dissolved DNA, and microbial activity and biomass at the center-of-the-bay station. Abbreviations are the same as described in the legend to Fig. 4.

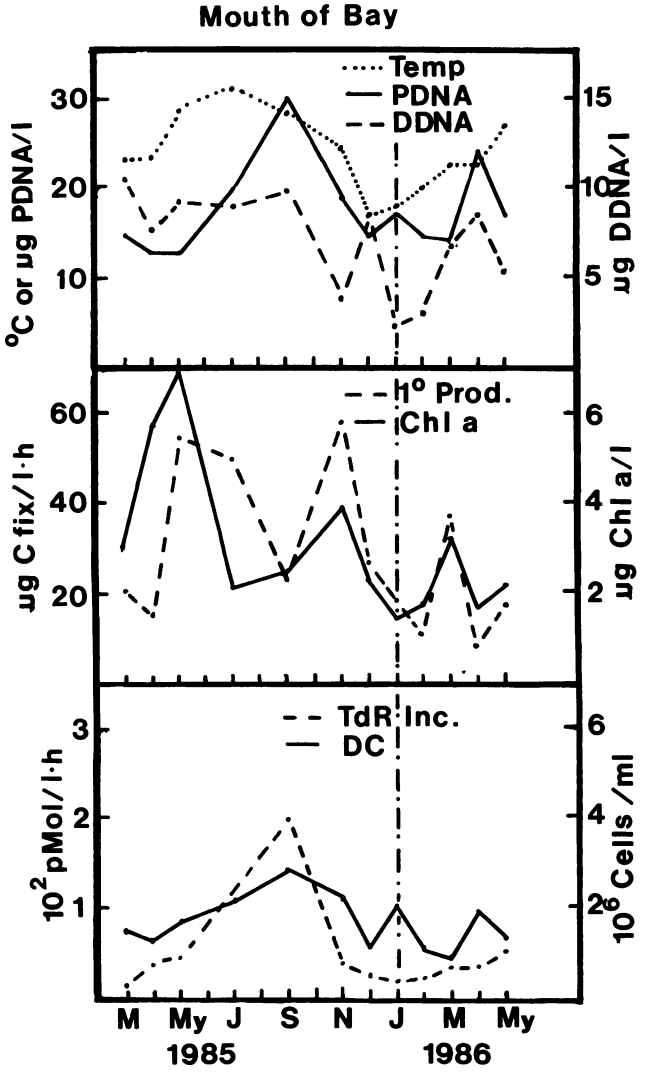

FIG. 6. Water temperature, dissolved and particulate DNA, and microbial biomass and activity at the mouth-of-the-bay station as a function of monthly samplings. Abbreviations are the same as described in the legend to Fig. 4.

DNA most closely followed that of particulate DNA and temperature (Fig. 4, 5, and 6). Particulate DNA values in the Alafia river and mid-bay stations ranged from 20 to 91 µg/liter and 18 to 41 µg/liter, respectively. Particulate DNA at the mouth-of-the-bay station ranged from 12.5 to 31 µg/liter, with a maximum in September that correlated with the peak in thymidine incorporation.

Autotrophic microbial biomass measured as chlorophyll *a* in the Alafia River ranged from 4.2 to 62.3 µg/liter, which was indicative of a hypertrophic environment. Chlorophyll *a* at the mid- and mouth-of-the-bay stations ranged from 2.6 to 54 and 1.4 to 6.9 µg/liter, respectively. Spring and fall maxima (usually April to May and November and March of the second year) were evident at all the stations, even though

TABLE 1. Percentage of microbial biomass and activity occurring in the 0.2-to-1-µm size fraction

Location	Mean % ± SD (range) of:				
	Particulate DNA	Chlorophyll *a*	Primary production	Direct counts	Thymidine incorporated
Alafia River	37.02 ± 8.9 (26.9–54.3)	2.64 ± 1.48 (0.6–5.3)	5.29 ± 6.82 (0.81–21.6)	71.5 ± 9.36 (50.9–84.2)	70.9 ± 13.1 (55.1–100)
Center of bay	45.03 ± 16 (26.2–68.8)	4.91 ± 5.5 (0.7–9)	6.78 ± 6.29 (0–11.45)	86 ± 8.78 (67.5–100)	78.3 ± 11.86 (62–100)
Mouth of bay	59.03 ± 10.84 (48.5–71.4)	5.95 ± 3.2 (2.75–12.1)	12.36 ± 7.65 (3.69–28.7)	86.1 ± 10.99 (61.6–98.4)	85.6 ± 12.6 (61.5–100)

TABLE 2. Correlation between parameters measured during seasonal studies at three stations in Tampa Bay (n = 35)[a]

Parameter	Correlation between parameters											
	DDNA	PDNA	AODC	PP	LOGTHY	DOC	CHLA	TEMP	LOG	PP	THY	SAL
DDNA	1.0000											
PDNA	0.7185	1.000										
AODC	0.4905	0.7818	1.0000									
PP	0.4721	0.4348	0.5034	1.0000								
LOGTHY	0.4524	0.7020	0.7963	0.4996	1.0000							
DOC	0.3643	0.3977	0.3941	0.3156	0.4650	1.0000						
CHLA	0.5470	0.4558	0.4891	0.7469	0.4310	0.4201	1.0000					
TEMP	0.6638	0.3226	0.2605	0.4287	0.3676	0.2214	0.3766	1.0000				
LOG PP	0.5055	0.4788	0.5168	0.8630	0.5813	0.4360	0.7509	0.4396	1.0000			
THY	0.5441	0.7652	0.8589	0.5506	0.8405	0.2757	0.4257	0.4315	0.5586	1.0000		
SAL	0.0812	−0.2627	−0.4201	−0.1422	−0.5487	−0.6226	−0.0685	0.2104	−0.3105	−0.3662	1.000	

[a] Abbreviations: DDNA, dissolved DNA; PDNA, particulate DNA; AODC, direct counts; PP, primary productivity; LOGTHY, log rate of thymidine incorporation; CHLA, chlorophyll a; LOG PP, log rate of primary productivity; THY, rate of thymidine incorporation; SAL, salinity.

the relative seasonal magnitude of these peaks varied with the stations (Fig. 4, 5, and 6). A November and April peak in primary production was observed at all three stations, with the Alafia River possessing the greatest production (30 to 725 μg of C per liter per h).

Bacterial direct counts varied at all three stations, with little evidence of a seasonal pattern. The greatest bacterial density was found in the Alafia River (1.86×10^9 to 4.93×10^9 cells per liter), followed by the mid-bay (0.94×10^9 to 2.7×10^9 cells per liter) and the mouth-of-the-bay (0.85×10^9 to 2.2×10^9 cells per liter) stations.

Thymidine incorporation values were usually greatest at all three stations in the late summer months (in September for the mid-bay and mouth-of-the-bay stations), with minima in December and January. Values ranged from 125 to 412 pmol/liter per h for the Alafia River, from 24 to 275 pmol/liter per h for the mid-bay station, and from 20 to 199 pmol/liter per h for the mouth of the bay.

A summary of the size distribution of microbial biomass and activity measurements appears in Table 1. For every parameter, as one proceeds down the estuary, a numerically greater proportion of the activity occurs in the <1-μm fraction, although not all differences are statistically significant. For bacterial activity, the Alafia River and mouth-of-the-bay stations were significantly different by analysis of variance and multiple range testing ($0.02 < P < 0.05$), whereas the mid-bay station was not significantly different from either of the other stations. The bacteria in the Alafia River were larger than those at either the mouth-of-the-bay or the center-of-the-bay station ($0.0005 < P < 0.001$). Nearly three-fourths of the bacterial activity and direct counts were <1 μm.

A small proportion of the microautotrophic population was contained in the <1-μm fraction at all three stations. Of the three stations, the mouth-of-the-bay station contained a significantly larger proportion of the primary production ($0.005 < P < 0.01$) and chlorophyll a ($0.025 < P < 0.05$) in the <1-μm fraction. Over 50% of the particulate DNA was in the <1-μm fraction at the mouth of the bay; it was a significantly greater proportion than that at the Alafia River or the center of the bay ($P \leq 0.005$).

Table 2 displays the results of multiple correlation analyses of the parameters measured during the monthly samplings. Dissolved DNA correlated best with particulate DNA (r = 0.72), followed by temperature (r = 0.66). The highest correlation coefficient obtained for all parameters was between bacterial direct counts and thymidine incorporation (r = 0.86). Other highly significant correlations occurred between primary production and chlorophyll a and particulate DNA and bacterial direct counts.

Specific rates of microbial activity for autotrophic and heterotrophic populations are displayed in Table 3. There was a significant decrease in specific heterotrophic activity as one proceeded down the estuary ($0.001 < P < 0.0025$). Thus, bacteria had greater cellular activity rates in the mouth of the Alafia River than at either of the other stations.

Dissolved DNA was a larger percentage of the particulate DNA at the mouth of the bay than at the Alafia River station ($0.005 < P < 0.01$) and averaged 32% of the particulate DNA for all stations. The average percent dissolved DNA with respect to DOC was 0.29 ± 0.16%, and the percent DNA carbon with respect to DOC was 0.11%. Thus, dissolved DNA constituted a relatively small proportion of the DOC in this estuary.

TABLE 3. Specific activities of microheterotrophic and microautotrophic populations and percent dissolved DNA of DOC and particulate DNA

Location	Mean ± SD (n):			
	Dissolved DNA/particulate DNA (%)[a]	Dissolved DNA/DOC (% [wt/wt])[b]	Primary product/chlorophyll a (μg of C/μg of chlorophyll a per h)[c]	Thymidine incorporated/cell (10^{-20} mol/cell per h)[d]
Alafia River	23.5 ± 11.0 (12)	0.22 ± 0.13 (12)	12.86 ± 10.5(12)	6.64 ± 1.99 (11)
Center of bay	31.6 ± 6 (14)	0.278 ± 0.104 (14)	10.81 ± 7.71 (14)	4.89 ± 3.21 (12)
Mouth of bay	42 ± 19.5 (12)	0.375 ± 0.2 (12)	9.70 ± 5.49 (13)	2.70 ± 1.79 (11)

[a] The mouth-of-the-bay sample was significantly different from that from the Alafia River ($0.005 < P < 0.01$).
[b] Not significantly different ($P > 0.025$).
[c] No significant difference among all three environments ($P > 0.25$).
[d] All of the environments were significantly different ($0.001 < P < 0.0025$).

TABLE 4. Dissolved inorganic and organic phosphorus at selected samplings

Sampling location and date	DIP (μg of P/liter)[a]	DOP (μg of P/liter)[b]	P in dissolved DNA[c]	
			μg of P/liter	% DOP
Alafia River				
1/23/86	891 ± 0	79.4	0.37	0.47
2/28/86	864.1 ± 2	8.05	0.60	7.5
3/19/86	1048 ± 15	ND[d]	0.21	ND
4/24/86	881 ± 28	ND	1.6	ND
5/22/86	829 ± 0.4	19.2	1.48	7.7
Mid bay				
1/23/86	274 ± 1.8	17.36	0.41	2.4
2/28/86	268 ± 0	4.65	0.58	12.4
3/19/86	255 ± 0.4	25.4	0.53	2.1
4/24/86	283 ± 0.4	ND	0.98	ND
5/22/86	291 ± 0.4	ND	0.95	ND
Mouth of bay				
1/23/86	155 ± 3.1	15.91	0.22	1.4
2/28/86	95 ± 0	5.27	0.30	5.7
3/19/86	93.9 ± 2.6	8.68	0.67	7.7
4/24/86	103.4 ± 0.7	3.72	0.85	22.8
5/22/86	81.7 ± 0.7	26.0	0.52	2.0

[a] DIP or soluble reactive phosphorus.
[b] DOP, Difference between total soluble phosphorus and soluble reactive phosphorus.
[c] Calculated by assuming a P:DNA ratio of 0.1 (wt/wt).
[d] ND, The difference between total soluble phosphorus and soluble reactive phosphorus was ≤0.

During samplings in 1986, we were fortunate to have dissolved phosphorus determinations performed on water samples from the three stations (Table 4). The highest dissolved inorganic phosphorus (DIP) values were found at the Alafia River station (mean, 902 ± 85 μg of P per liter or 29 ± 2.7 μM P). The mid-bay station possessed approximately one-third this concentration (274 ± 14 μg of P per liter or 8.8 μM P), whereas the mouth of the bay contained less than half the center-of-the-bay concentration (106 ± 29 μg of P per liter or 3.4 ± 0.94 μM P). These values indicate that the Alafia River was a source of DIP for the bay. DOP concentrations were one to several orders of magnitude below this level (Table 4) and did not show the spatial differences found for the DIP values. The percentage of DOP that was DNA phosphorus (assuming a DNA phosphorus content of 10%) ranged from 0.47 to 22.8% (mean, 6.56 ± 6.5%). If the highest and lowest values are discarded, the mean DNA phosphorus is 5.43 ± 3.73% of the DOP, indicating that this fraction is usually <10% of DOP.

The results of the diel studies performed in Bayboro

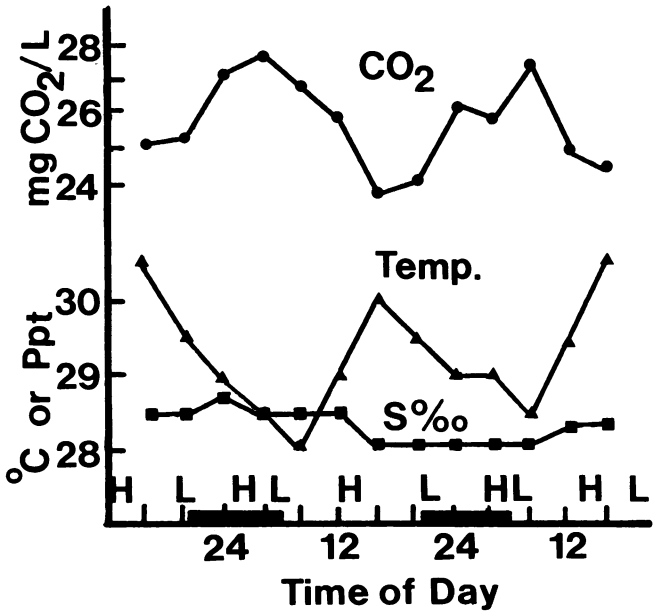

FIG. 7. CO_2, water temperature (Temp.), salinity (S‰), and occurrence of high and low tides (H and L, respectively) for the diel study of June 1986 (dry season).

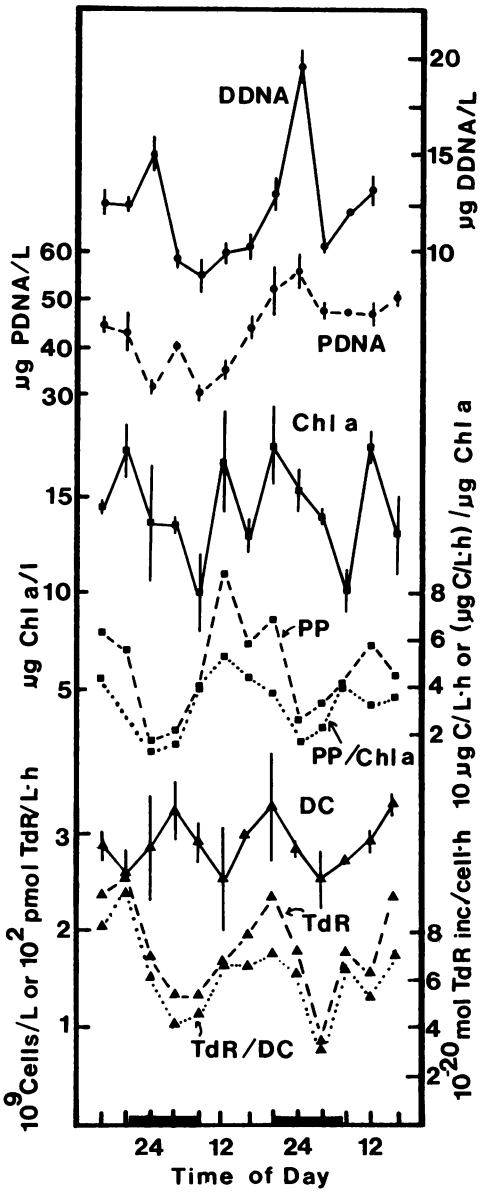

FIG. 8. Diel study performed in Bayboro Harbor during the dry season (June 1986). Black bars indicate nighttime samplings. Abbreviations: PP, primary productivity; PP/Chl a, specific rate of primary production; TdR, thymidine; TdR/DC, cellular rate of thymidine incorporation. All other abbreviations are the same as in the legend to Fig. 4.

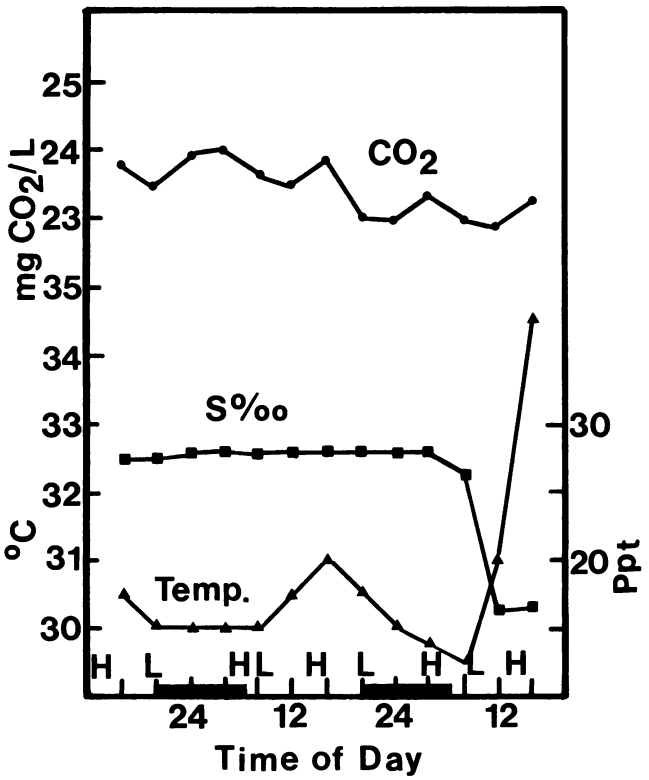

FIG. 9. Carbon dioxide, water temperature, salinity, and tidal occurrence in Bayboro Harbor during the wet season (August 1986). Abbreviations are the same as in the legend to Fig. 7. Note the sharp decrease in salinity during the last 8 h.

Harbor (Fig. 1) appear in Fig. 7 through 10. The diel study conducted in the end of the dry season was characterized by stable salinity and is depicted in Fig. 7 and 8. Surface water temperature showed diel variability with a maximum at 1600 h. Carbon dioxide showed the inverse of this pattern, due in part to temperature effects on solubility and consumption by phytoplankton populations (Fig. 7). The occurrence of the high and low tides appears on this figure, but data on the magnitude at each sampling were not available.

Dissolved DNA showed clear maxima at midnight, with minimal values at 0400 or 0800 h (Fig. 8). Particulate DNA values reached a maximum at the second midnight sampling but showed no diel periodicity. Chlorophyll *a* values decreased during the dark periods and increased during the light periods. Primary productivity also showed maxima during daylight hours (at noon), with minima at midnight. This finding was not due to ambient illumination, since all primary production incubations were performed at a fixed illumination. The specific rate of primary production (μg of C/μg of chlorophyll *a* per h) also followed this pattern, indicating a diel periodicity in the photosynthetic efficiency of the microautotrophic population.

Bacterial direct counts showed some indication of cyclic variability, but the magnitude of the change was seldom greater than the variability in the measurement.

Bacterial activity showed strong diel periodicity, with maxima at 2000 h and minima at 0400 to 0800 h. This variability was due to changes in activity of the bacterial population as indicated by specific rates of thymidine incorporation (moles of thymidine incorporated per cell per hour; Fig. 8).

The results of the diel study which was done in the wet season (August 1986) appear in Fig. 9 and 10. This study was disturbed by a large freshwater input during the last 8 h (Fig. 9), when salinity decreased from 28 to 16‰ in less than 4 h, and temperature increased by 4.5°C. The source of fresh water was not determined, but could have been from a creek or storm sewers which empty into Bayboro Harbor. This fresh water contained its own microbial flora, since bacterial direct counts increased over fivefold (to over 7×10^9 cells per liter) and chlorophyll *a* increased over 10-fold (Fig. 10). This influx of fresh water was low in dissolved DNA (a threefold decrease) but rich in particulate DNA (a sevenfold increase). Before this event, two midnight peaks in dissolved

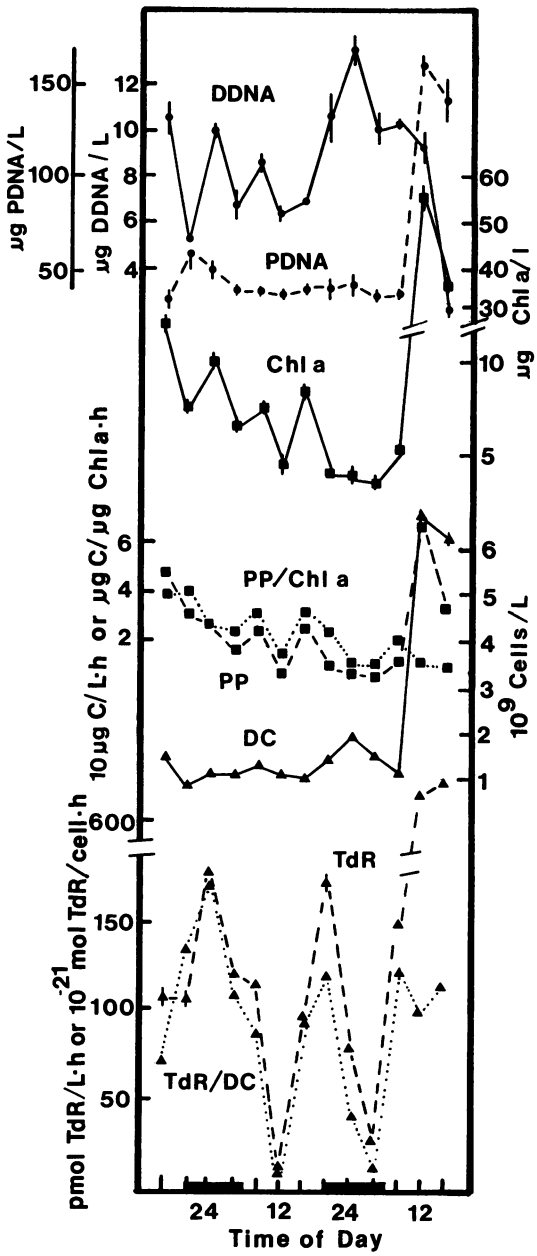

FIG. 10. Diel study performed in Bayboro Harbor during the wet season (August 1986). Abbreviations are the same as in the legend to Fig. 8. Note the dramatic increase in most parameters during the last 8 h, which coincides with the salinity decrease shown in Fig. 9.

DNA occurred, although the magnitude of the peaks was less than that observed during the dry season. Primary production increased greatly during the freshwater event, mainly because of the increased algal population and not because of changes in activity of existing phytoplankton populations.

Thymidine incorporation showed the greatest diel periodicity (Fig. 10), with maxima at midnight on the first night and at 2000 h on the second night. Cellular rates of thymidine incorporation followed this pattern except during the low-salinity event, when increases in bacterial numbers would explain the increases in bacterial activity.

DISCUSSION

Tampa Bay is a eutrophic subtropical estuary that receives phosphate stress through rivers like the Alafia (36). Although DIP values were high in the mouth of the Alafia during our study period (up to 1 mg of P per liter or 32 μM), U.S. Geological Survey records indicate that there were DIP levels as high as 88 mg of P per liter in 1967, with DIP values which have only been below 10 mg of P per liter since 1970 (36). Thus, comparable subtropical estuaries receiving this magnitude of nutrient loading are not commonly found.

The Alafia River station, with its high phosphate input, also had the highest values for most microbial parameters which were measured in this study. Maximal values for primary production and chlorophyll *a* were found at this station in 9 of 12 samplings. This finding is in contrast to those at other estuaries (i.e., the Chesapeake Bay, the Bristol Channel), where high estuarine sites had lower chlorophyll *a* and primary production than midestuarine stations, even though nutrient values were higher, owing to limited light penetration from turbidity (11, 14). The turbidity in these temperate estuaries was caused by silt and clay particles, which are less common in Florida rivers, owing to the Florida carbonate platform (36).

The spring and fall peaks in primary production and chlorophyll *a* and corresponding decreases in summer and winter are features which are common to most temperate estuaries (19, 33, 39, 41) and Tampa Bay has been described as a possible geographic boundary between tropical and temperate coastal environments (20).

Dissolved DNA values were found to correlate most closely with particulate DNA values and temperatures. Our observation of a summer (July) peak in dissolved DNA may be due in part to senescence of spring phytoplankton blooms. Senescence of diatom populations resulted in production of high-molecular-mass dissolved organic matter (>300,000 daltons [32]). Natural phytoplankton populations have been shown to rapidly release small (<500 daltons) material, followed by delayed release of higher-molecular-mass (>500 daltons) material (16), with ≥50% of the material released being >500 daltons. Minear (22) reported release of DNA by cultures of *Chlamydomonas* spp. We have demonstrated release of ^{14}C-labeled RNA and proteins, but not DNA, from incubations of natural populations of phytoplankton with $^{14}CO_2$ (28).

Bacterioplankton may also be a source of dissolved DNA. A *Pseudomonas aeruginosa* strain was shown to produce ~8 mg of extracellular DNA per ml, a concentration much greater than that contained (per milliliter of culture fluid) in the bacterial cells (10). This DNA was shown to be active in transformation. Production of extracellular DNA in estuarine and offshore environments by natural populations of bacterioplankton has been reported previously (28). The rate of production of the dissolved macromolecular fraction was less than 10% of the total thymidine incorporation. In offshore coastal and oceanic environments, dissolved DNA correlated with bacterial activity and abundance (6). In the present study, dissolved DNA correlated poorly with either phytoplankton or bacterioplankton biomass or activity measurements; but it did correlate with particulate DNA and temperature. Multiple linear regression analyses (stepwise solution; Human Systems Dynamics software, Northridge, Calif.) indicated that only temperature and particulate DNA were required as independent variables to predict dissolved DNA values ($r = 0.87$; $n = 35$). Unlike offshore environments, where phytoplankton constitutes <10% of the particulate DNA pool and bacterioplankton composes 70 to 90% of the particulate DNA (25), both phytoplankton and bacterioplankton make significant contributions to the particulate DNA pool in estuaries. Thus, the spatial and temporal distribution of dissolved DNA most likely reflects a synergistic input from both microautotrophic and microheterotrophic genetic pools.

An increase in cellular activity of the microheterotrophic population was found from the mouth of the bay up into the estuary. The augmented input of DOC and the greater phytoplankton production in the upper estuary might account for this increase in bacterial activity. This increase in cellular activity also was reflected in an increase in average cell size (or a decrease in the percentage of the <1-μm fraction) of bacterial direct counts and in the size distribution of thymidine incorporation in the Alafia River. Similar observations have been made on the size distribution of bacteria in the Fraser River estuary (British Columbia, Canada), where attached bacteria represent a larger proportion of the microbial population at 0‰ salinity (60%) than at 26‰ (15 to 39%; [1]). The smaller size of the microautotrophs in the mouth-of-the-bay sample was probably caused by a larger proportion of autotrophic picoplankton at this station (17), which dominates tropical oceanic environments.

Dissolved DNA constituted a relatively small proportion of the DOC in Tampa Bay (0.29 ± 0.16% [wt/wt] or 0.11% [wt/wt] C) but a relatively larger proportion of the DOP (6.56 ± 6.51%). This finding may be due to the more refractory nature of the DOC pool compared with the DOP pool. Owing to the high levels of phosphate in Tampa Bay, the DOP pool is a relatively small proportion of the total soluble phosphorus (mean, 7.3 ± 8.9%). Minear (22) found that DOP constituted 35 to 94% of the soluble phosphorus in lake water. Of the high-molecular-mass DOP compounds (≤30,000 daltons), dissolved DNA composed 38 to 49% (22). Minear (22) found 4 to 30 μg of dissolved DNA per liter in these lakes, which represented 7.8 ± 4% (range, 3 to 12.5%, $n = 4$ [our calculations]) of the total DOP compounds, values which were in close agreement with our estimates for Tampa Bay.

Diel periodicity in dissolved DNA, primary production, and bacterial activity were clearly evident in the dry season study, but less evident in the wet season, when pulses of fresh water dominated the microbial ecology. Diel periodicity in estuarine microautotrophic populations is a common occurrence (7). Malone (18) found that larger phytoplankton (net plankton, mostly diatoms) showed greater diel variability in photosynthetic rate than did smaller phytoplankton (nannoplankton, mostly chlorophytes).

Reports of diel periodicity in microheterotrophic estuarine populations are less common. Riemann et al. (34) found slight diel periodicity in bacterial activity and biomass but a

larger effect on cellular rates of thymidine incorporation. Riemann and Søndergaard (35) found only minor periodicity in heterotrophic activity after 13 diel studies in coastal oceanic stations and lakes. Winn and Karl (40) found greater adenine incorporation from 0800 to 1600 h than from 2000 to 0400 h. Diel periodicity in phosphatase activity (13) and exoenzyme activity in sediments (21) have been reported. Diel periodicity in the production of dissolved free amino acids in lakes has been noted, with minimum production times which corresponded to maximal heterotrophic bacterial utilization (15).

Diel periodicity observed in dissolved DNA production lagged approximately 4 h behind the maximum in bacterial activity and 12 h behind the maximum in primary production. One explanation of these data is that bacterial activity responds to diel periodicity in phytoplankton activity but lags behind the latter by 4 to 8 h, being dependent upon release of DOM by phytoplankton. Bacterial growth is then kept in check, perhaps by grazers, which cause the release of dissolved DNA that lagged 4 h behind the bacterial production peak. An alternate explanation is that the pulse in dissolved DNA is of phytoplankton origin but represents the delayed release of macromolecules observed by Lancelot (10).

ACKNOWLEDGMENTS

This work was supported by National Science Foundation awards OCE-8415605 and BSR-8507343 and Gulf Coast Charitable Trust Fund scholarships to M.F.D. and W.H.J.

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