

The fates and effects of riverine and shelf-derived DOM on Mississippi River plume/Gulf shelf processes

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Abstract

A variety of chemical and biological measurements provided complementary information on the dynamics of dissolved organic matter (DOM) during summer and winter cruises in the Mississippi River Plume/Gulf Shelf region. Non-conservative mixing of dissolved organic carbon (DOC) between the River and open Gulf was observed during the summer cruise, indicating a substantial input of DOC at intermediate salinities (15-30‰). Stable nitrogen isotope compositions of DOM isolated by ultrafiltration also indicated a source of freshly produced DOM at intermediate salinities during the summer, suggesting that phytoplankton were an important source of DOM in the plume. DOC mixing appeared to be fairly conservative during the winter cruise.

Calculation of areal bacterial demand for carbon indicated that bacteria consumed a substantial portion of the total carbon fixed by primary production in this region. The oxygen demand from growth of bacterioplankton in subsurface waters was sufficient to explain the occurrence of hypoxic conditions during the summer at stations where low oxygen levels were observed in bottom waters. Although more temporal data are needed to define seasonal trends accurately, bacterial activity, community respiration and nutrient regeneration rates were higher during the summer cruise than during the winter cruise. Rates of bacterial production, nitrogen regeneration and community respiration were highest at intermediate salinities in the plume, particularly during the summer. During both cruises, the proportion of total respiration and nutrient regeneration that were not accounted for by bacteria were consistently higher in the plume regions, where zooplankton grazing of particles may be relatively more important for nutrient regeneration than in regions where primary production was low.

Processes controlling the remineralization and fate of dissolved organic matter (DOM) in the Mississippi River Plume/Gulf Shelf (MRP/GS) region were studied to gain a fundamental understanding of the factors controlling productivity, hypoxia development and carbon transport and to fulfill the primary objectives of the Nutrient Enhanced Coastal Ocean Productivity (NECOP) program. The Mississippi River discharges $\sim 2 \times 10^{12}$ g of dissolved organic carbon (DOC) annually to the coastal waters of the Gulf of Mexico (Malcolm and Durum, 1976). In addition to this large load of dissolved organic materials the River also discharges dissolved inorganic nutrients that stimulate algal blooms in the plume region (Turner and Rabalais, 1991). Our studies in the MRP/GS region were focused on five primary objectives:

1. to determine the concentrations and origins of dissolved organic matter (DOM);
2. to determine the biological reactivity of DOM;
3. to determine the rates of ammonium regeneration and organic nitrogen utilization;

4. to determine the abundance and rates of production of heterotrophic bacteria; and
5. to estimate heterotrophic bacterial carbon, nitrogen and oxygen demand.

Materials and Methods

Study site and sampling procedures — An area of the northern Gulf of Mexico near the discharge of the Mississippi River was surveyed during two cruises of the NOAA vessel MALCOLM BALDRIDGE from July 18 to August 8, 1990, and from February 19-28, 1991. Nearly 70 percent of the river's discharge flows out of the Mississippi River delta. The remaining 30 percent enters the Gulf of Mexico through the Atchafalaya River, a large tributary of the Mississippi that flows into the Gulf west of the main delta. Samples were obtained near the outflows of the four major channels on the delta — the Southwest Pass, the South Pass, the Pass à l'Ouvre and the Main Pass, and at stations to the west and to the south of the delta on the Louisiana Shelf. Water samples were collected with a clean plastic bucket or with Niskin bottles mounted on a Neil Brown Mk III CTD rosette.

DOM isolation and characterization — Concentrations of dissolved organic carbon (DOC) were measured using a Shimadzu TOC 5000 analyzer with a Pt catalyst (0.5 percent Pt on alumina) at 680 °C (Sugimura and Suzuki, 1988). Samples were filtered through a Whatman GF/F filter, acidified and purged with ultra-high purity oxygen immediately prior to analysis.

Acknowledgements

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Standards were run several times each day, and the instrument and water blanks were evaluated during each cruise as described by Benner and Strom (1992).

Cross-flow ultrafiltration was used for the concentration and isolation of DOM from water samples (Benner, 1991). Water samples (100-200 l) were prefiltered through 0.2 μm pore-size filters and then through 1000 Dalton cutoff spiral wound polysulfone filters using an Amicon DC 10L ultrafiltration system. Concentrated DOM samples were diafiltered to remove sea salts and dried under vacuum in a Savant SpeedVac system. Stable carbon and nitrogen isotope compositions of the ultrafiltered DOM were measured using a sealed tube combustion method (Sofer, 1980). The isotope composition of a sample is defined as:

$$\delta^{\text{HI}} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3,$$

where $^{\text{HI}}$ is the heavy isotope of an element (^{13}C or ^{15}N) and R is the ratio of $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$ in the sample and the standard. The standard for $\delta^{13}\text{C}$ was PDB (0.0‰), and the standard for $\delta^{15}\text{N}$ was air (0.0‰).

Bacterial abundance and production — Bacterial abundance was measured using epifluorescence microscopy of DAPI stained samples (Porter and Feig, 1980). Samples were analyzed in duplicate, and bacteria in at least 10 fields were counted for each microscope slide. Bacterial production was estimated from rates of DNA and protein syntheses as measured by a dual-label method using [^3H]Thymidine (TdR) and [^{14}C]Leucine (Leu) (Chin-Leo and Kirchman, 1988). Triplicate water samples (10 ml) were incubated with 10 nM (final concentration) [^3H]TdR (specific activity of 84.1 Ci mmol $^{-1}$), and 20 nM (final concentration) [^{14}C]Leu (specific activity of 328.5 mCi mmol $^{-1}$) for 30 min. All radioactive substrates were from New England Nuclear (Boston, Mass.). The nucleic acid and protein fractions were separated in all samples as described by Chin-Leo and Benner (1991) to test for the possible non-specific incorporation of TdR into protein. Replicate measurements of bacterial abundance and bacterial production (TdR and Leu incorporation) differed by <10 and <5 percent, respectively. Abiotic absorption of the labeled substrates was estimated by measuring the incorporation of radiolabeled substrates in samples previously fixed with formaldehyde (10 percent final concentration). All samples were corrected for abiotic incorporation by subtracting the radioactivity in the formalin-killed controls.

Bacterial parameters were measured in surface waters and with depth. Depending on the depth of the water column, between three to eight samples were collected to sample surface waters, the pycnocline and bottom waters. In shelf waters (<100 m), rates of TdR and Leu incorporation were integrated over the depth of the water column to estimate bacterial production on an areal basis. A step-wise rectangular integration method that does not require interpolating between points was used to obtain conservative estimates. Rates of TdR incorporation were converted to rates of C production using factors determined empirically by comparing isotope incorporation with increases of

bacterial numbers and using a cell to C conversion value of 20 fg C cell $^{-1}$ (Lee and Furhman, 1988). Experiments to determine empirical conversion factors were performed in duplicate using Mississippi River plume water. Estimates of bacterial C production were also determined from rates of Leu incorporation using a conversion factor of 3.1 kg C mol $^{-1}$ of Leu (assuming an internal isotope dilution of 2) derived by Simon and Azam (1989), which is based on empirical confirmation of the assumptions of the Leu method and does not require a cell to C conversion factor.

Mesocosm experiments — Closed bottle incubations were conducted in the dark to differentiate heterotrophic processes from autotrophic ones and to compare the relative magnitude of heterotrophic activities at the respective sites. These experiments were conducted in 9-L glass bottles that were closed to the outside atmosphere and incubated for three to five days at *in situ* temperatures. For each experiment, duplicate bottles were set up for both filtered and unfiltered water samples. The filtered samples were prepared either by removing particles with a 1 μm pore-size filter (summer cruise) or by diluting (1:5) 3 μm pore-size filtered water with 0.2 μm pore-size filtered sea water from the same site (winter cruise). Both approaches decreased the quantitative importance of large organisms, i.e. zooplankton, large phytoplankton and detrital particles, relative to bacterial-sized particles. A second small reservoir, attached to each experimental bottle, provided makeup water for samples removed from the bottles without leaving an airspace. Each bottle was sampled at intervals of one or two times per day over the two- to five-day incubation interval. Concentrations of ammonium, dissolved oxygen, and carbon dioxide were monitored over time for each set of samples to provide an estimate of net mineralization rates for organic carbon and organic nitrogen in samples from the various sampling sites. Comparisons among sites, discussed here, are for accumulation or removal rates obtained over the whole incubation interval. In addition to measuring changes in dissolved organic components and mineralization products in the large dark bottles, additional indicators were used to assess organic matter turnover in the winter experiments, including: amino acid turnover, ammonium regeneration rates (as measured by isotope dilution), and process rate changes caused by bacterial manipulations (see below).

Ammonium and amino acid (and primary amine) concentrations were measured fluorometrically, after separation by cation exchange chromatography and reaction with o-phthalaldehyde/2-mercaptoethanol reagent (Gardner, 1978; Gardner and St. John, 1991). Amino acid turnover rates were determined by adding tracer amounts of a mixture of ^3H -labeled amino acids from algal protein hydrolysate (Amersham) to sea water and filtering 5 ml of sample through a 0.2 μm pore-size Millipore GS filter. After radioisotope addition, samples were filtered at various intervals from 0 to 30 minutes and dried. Biocount scintillation cocktail

(RPI) was added and samples were analyzed for radioisotope uptake on a LKB Model 1217 Rackbeta liquid scintillation counter. Counts were corrected to disintegrations per minute by calibration with quench curves and an external standard. Rates were calculated in the linear range of uptake as: $[DPM(t1) - DPM(t0)] / \text{Total DPM} * 60 / (t1-t0)$.

Ammonium regeneration rates were estimated from changes in ammonium concentration and in isotope ratios of added $^{15}\text{NH}_4$ using the Blackburn/Caperon Model (Blackburn, 1979; Caperon *et al.*, 1979). Isotope ratios for ammonium in N-15 isotope dilution experiments were measured with a new HPLC technique that was developed to determine isotope ratios directly on filtrates of experimental water (Gardner *et al.*, 1991).

For bacterial manipulations, microbes less than 1 μm in size were concentrated to examine the effect of these microbes on amino acid turnover rates and on ammonium regeneration rates (as measured by isotope dilution of added $^{15}\text{NH}_4$). Ten liters of seawater, from the sites where large bottle experiments were conducted, were pre-filtered through a Gelman Polypure TDC capsule filter (nominal pore-size 1 μm). The filtrate was placed in a Gelman 3.8 L stainless steel pressure vessel and pumped through Gelman Acroflux capsule (0.2 μm pore size) at 100 psi. The retentate was collected and passed through the filter 4-6 times to further concentrate microbes. This concentrate was added back to treatments, for amino acid turnover rate and $^{15}\text{N-NH}_4$ regeneration measurements, to increase heterotrophic bacterial concentrations from 7 to 29 percent above ambient concentrations.

Results and Discussion

Dissolved Organic Matter — During both the summer and winter cruises, concentrations of DOC were measured at the Head of Passes in the Mississippi River and across a salinity gradient extending in a westerly direction from Southwest Pass on the Mississippi Delta to shelf and slope waters of the Gulf of Mexico. During the summer, DOC concentrations ranged from 4 mg C l^{-1} in the River to 1 mg C l^{-1} in surface waters of open Gulf water (Fig. 1a). Mixing between these two end members was not conservative, as DOC concentrations were enhanced at intermediate salinities (15-30‰). Enhanced DOC concentrations at intermediate salinities coincided with high phytoplankton production in surface water (Lohrenz *et al.*, 1991 NECOP Workshop Abstract) indicating that primary production was a potentially significant source of DOC during the summer in this region of the plume. DOC concentrations were lower during the winter in both the river (3.25 mg C l^{-1}) and the open Gulf (0.75 mg C l^{-1}). Mixing between these two end members was linear during the winter (Fig. 1b), indicating a fairly conservative behavior of DOC.

Dissolved organic matter (DOM) was isolated from water samples during the summer and winter cruises by cross-flow ultrafiltration with 1000 Dalton cutoff

filters (Benner, 1991). The isolated material was subjected to a variety of isotopic and chemical analyses to study the fate of riverine DOM and to characterize the sources of DOM in shelf waters. The percentage of total DOC isolated by ultrafiltration decreased linearly from 45 percent in the river to ~27 percent in open Gulf waters. These spatial changes in the fraction of DOM recovered by ultrafiltration indicate that the average molecular size of riverine DOM is considerably greater than that of marine DOM. The percentage of DOC isolated at a given salinity was similar in summer and winter indicating that the average molecular size of the DOM did not vary temporally.

The completed analyses of stable carbon and nitrogen isotope compositions of the DOM samples collected during the summer cruise (Fig. 2) showed that stable carbon isotope ratios ($\delta^{13}\text{C}$) of DOM ranged from -25‰ in the river to -21.3‰ in the open Gulf. These ratios for ultrafiltered DOM are similar to ratios reported previously for bulk DOC in the river (-26 to -28‰) and the open Gulf (-22‰; Eadie *et al.*, 1978). Mixing between these two end members was approximately linear across the salinity gradient (Fig. 2a). The data for total DOC concentrations indicated a significant algal source of DOC at intermediate salinities, but the stable carbon isotope data do not indicate a significant input of algal-derived DOC above that expected for conservative mixing. However, we have preliminary data indicating that the inorganic carbon pool at intermediate salinities is depleted in ^{13}C by several parts per thousand relative to marine bicarbonate (~0‰), and the incorporation of this carbon into algal biomass would result in material that was isotopically "light" (-22 to -24‰) for algal-derived carbon. We therefore do not interpret the linear increase in stable carbon isotope ratios for DOC as completely conservative mixing between end members.

The stable nitrogen isotope ratios for the DOM isolated during the summer cruise demonstrate a strikingly different pattern across the salinity gradient (Fig. 2b). The $\delta^{15}\text{N}$ values for DOM from the River and the open Gulf were both near 3‰. No significant changes in isotopic composition across the salinity gradient would be expected if isotopic values resulted from conservative mixing between these two end members. Instead, $\delta^{15}\text{N}$ values increased significantly at intermediate salinities with maximal values near 9‰. It is evident that another source of organic nitrogen is contributing to the DOM pool at intermediate salinities. Cifuentes (1990 NECOP meeting, Miami, Fla.) reported a $\delta^{15}\text{N}$ value of ~10‰ for nitrate in the Mississippi River during the summer of 1990. It appears that riverine nitrate is incorporated into phytoplankton biomass at intermediate salinities and that DOM with a high $\delta^{15}\text{N}$ value (~10‰) is released from phytoplankton. We speculate that zooplankton grazing is the mechanism for the release of this DOM.

Heterotrophic bacterial abundance and production — Bacterial abundance and production were measured in water samples during the summer and winter

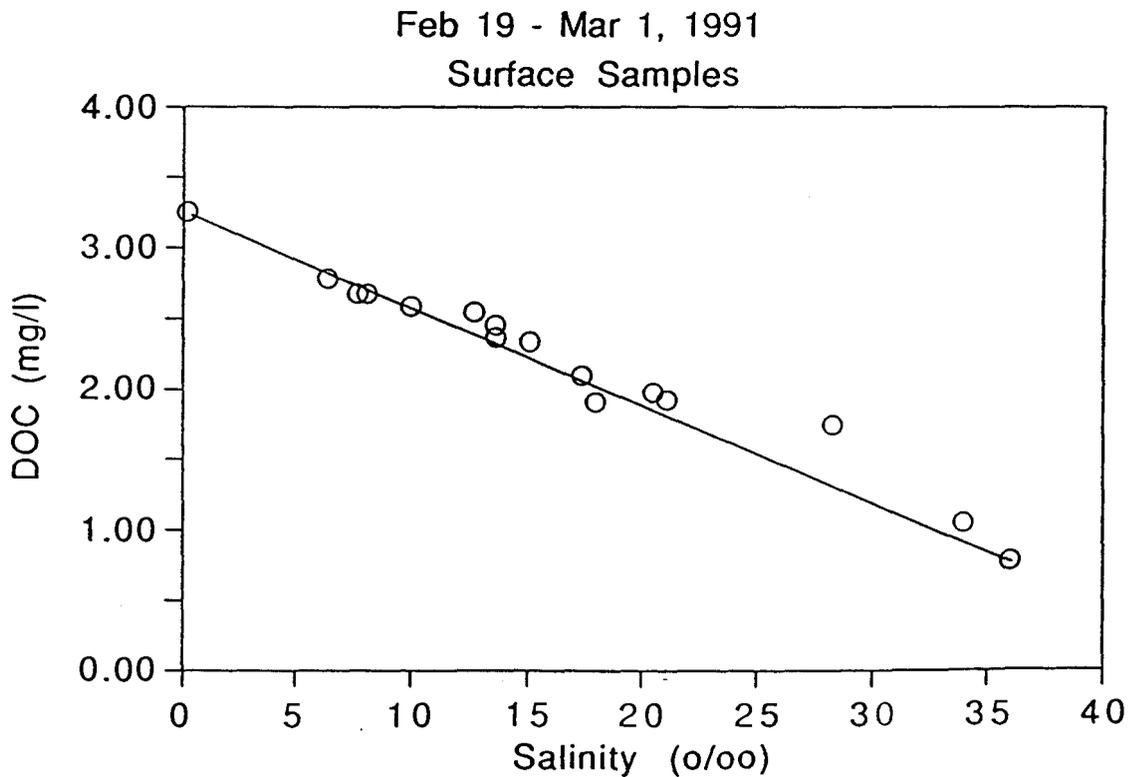
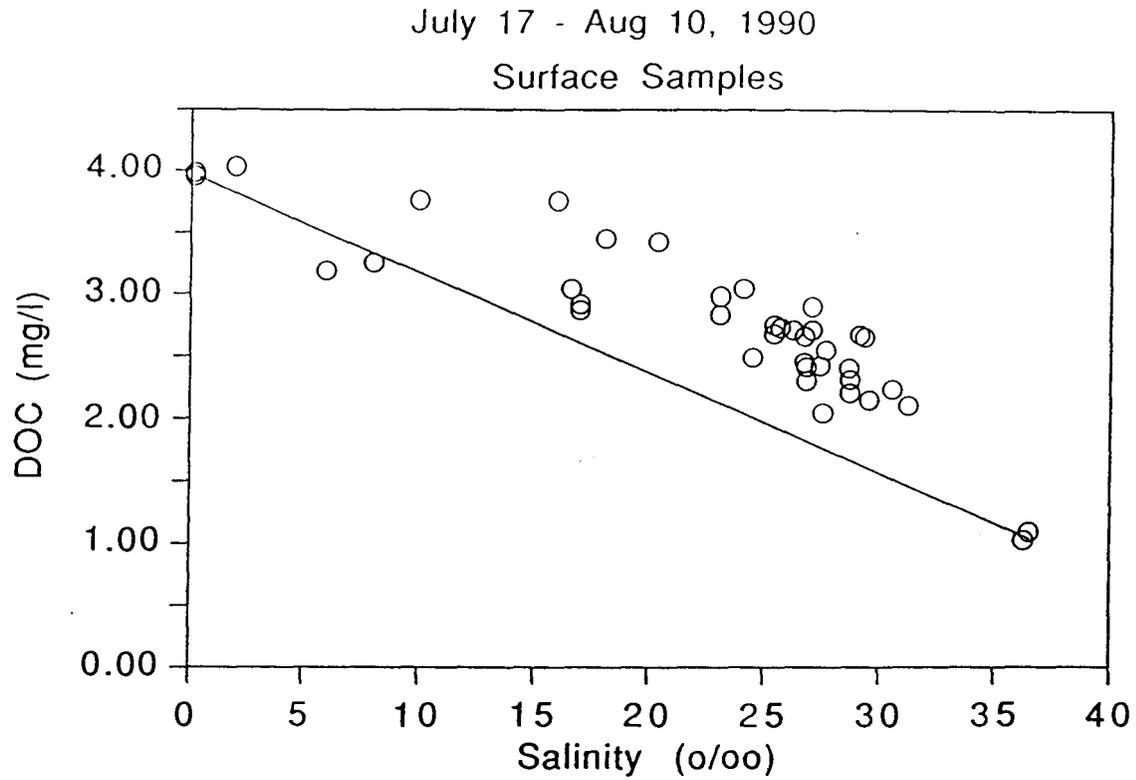


Figure 1. Concentrations of dissolved organic carbon (DOC) in surface water samples of varying salinity during the summer and winter NECOP cruises. The lines represent expected DOC values given conservative mixing between the freshwater and marine end members.

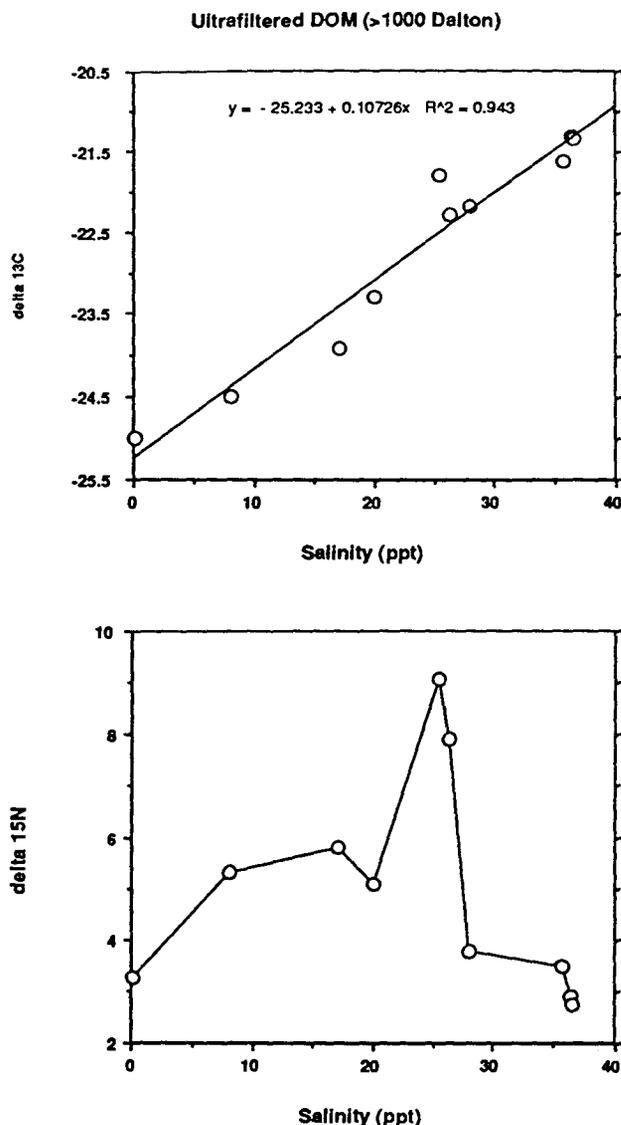


Figure 2. Stable carbon and nitrogen isotope compositions for ultrafiltered dissolved organic matter (DOM). A linear regression is shown for the stable carbon isotope data.

NECOP cruises. These data have been submitted for publication (Chin-Leo and Benner, 1991) and are summarized herein. Bacterial abundance ranged from 0.25 to 3.34×10^9 cells l^{-1} during summer and 0.36 to 1.09×10^9 cells l^{-1} in winter (Fig. 3a,d). During summer, maximal bacterial abundances occurred at intermediate salinities whereas during winter abundances were much less variable across the salinity gradient. Two independent indices of bacterial production, based on rates of DNA (thymidine incorporation) and protein synthesis (leucine incorporation), were measured. The magnitude of thymidine (TdR) and leucine (Leu) incorporation rates varied over the salinity gradient during summer and winter with maximal values occurring at intermediate salinities (Figs. 2b,c,e,f). Rates of TdR and Leu incorporation were significantly correlated during summer ($p = 0.0001$) and winter ($p = 0.0001$). The regions of maximal rates of TdR and Leu

incorporation in surface water coincided with regions of enhanced phytoplankton production (Lohrenz *et al.*, 1991 NECOP Workshop Abstract) and phytoplankton-derived DOM. Overall, rates of TdR and Leu incorporation were several-fold higher in summer than in winter, particularly at intermediate salinities.

Depth profiles of rates of TdR and Leu incorporation were measured at 12 locations on the Louisiana shelf during the summer and four locations during the winter. Depth-integrated estimates of bacterial production were derived from rates of TdR incorporation using an empirically-derived conversion factor (1.89×10^{18} cells mol^{-1}) and a cell to C conversion factor of 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1988). Depth-integrated rates of Leu incorporation were converted to estimates of bacterial production using the conversion factor (3.1 kg C mol^{-1}) determined by Simon and Azam (1989). During summer, integrated bacterial production on the shelf based on TdR incorporation varied from 238 to 740 mg C $m^{-2} d^{-1}$ with a mean value of 443 mg C $m^{-2} d^{-1}$ (Table 1). The corresponding estimates based on Leu incorporation ranged from 234 to 789 mg C $m^{-2} d^{-1}$ with a mean value of 462 mg C $m^{-2} d^{-1}$ (Table 1). Estimates of bacterial production during winter were about half of those during the summer with a mean value of 226 mg C $m^{-2} d^{-1}$ based on TdR incorporation and 277 mg C $m^{-2} d^{-1}$ based on Leu incorporation (Table 1).

Bacterial carbon metabolism and oxygen utilization — The potential role of bacteria in the cycling of C can be estimated from rates of bacterial production if the average bacterial growth efficiency is known. Mean integrated bacterial production in the Mississippi River plume based on TdR incorporation was 0.44 g C $m^{-2} d^{-1}$ in summer and 0.23 g C $m^{-2} d^{-1}$ in winter. Using the range of bacterial growth efficiencies (10 to 30 percent) determined during the NECOP cruises (see below), the amount of C necessary to sustain these rates of bacterial production was 1.48 to 4.43 g C $m^{-2} d^{-1}$ in summer and 0.75 to 2.26 g C $m^{-2} d^{-1}$ in winter. Estimates of phytoplankton production during July and August 1990 ranged from 2 to 10 g C $m^{-2} d^{-1}$ and during March 1991 ranged from 0.1 to 0.5 g C $m^{-2} d^{-1}$ (Lohrenz *et al.*, 1991 NECOP Workshop Abstract). It is obvious from a preliminary analysis of these data that bacterial production on the Louisiana shelf consumed a substantial fraction of the C fixed by phytoplankton during the summer of 1990. Using median values for algal and heterotrophic bacterial production we estimated that ~50 percent of the total phytoplankton production was consumed by bacterioplankton. During the winter of 1991 phytoplankton production alone could not support the estimated bacterial carbon demand, indicating that riverine-derived organic matter was an important source of substrate supporting bacterioplankton growth.

A recurrent feature of the Mississippi River plume ecosystem is the presence of low oxygen concentrations in bottom waters west of the main river delta during summer (Turner and Allen, 1982). These hypoxic conditions probably result from the heterotro-

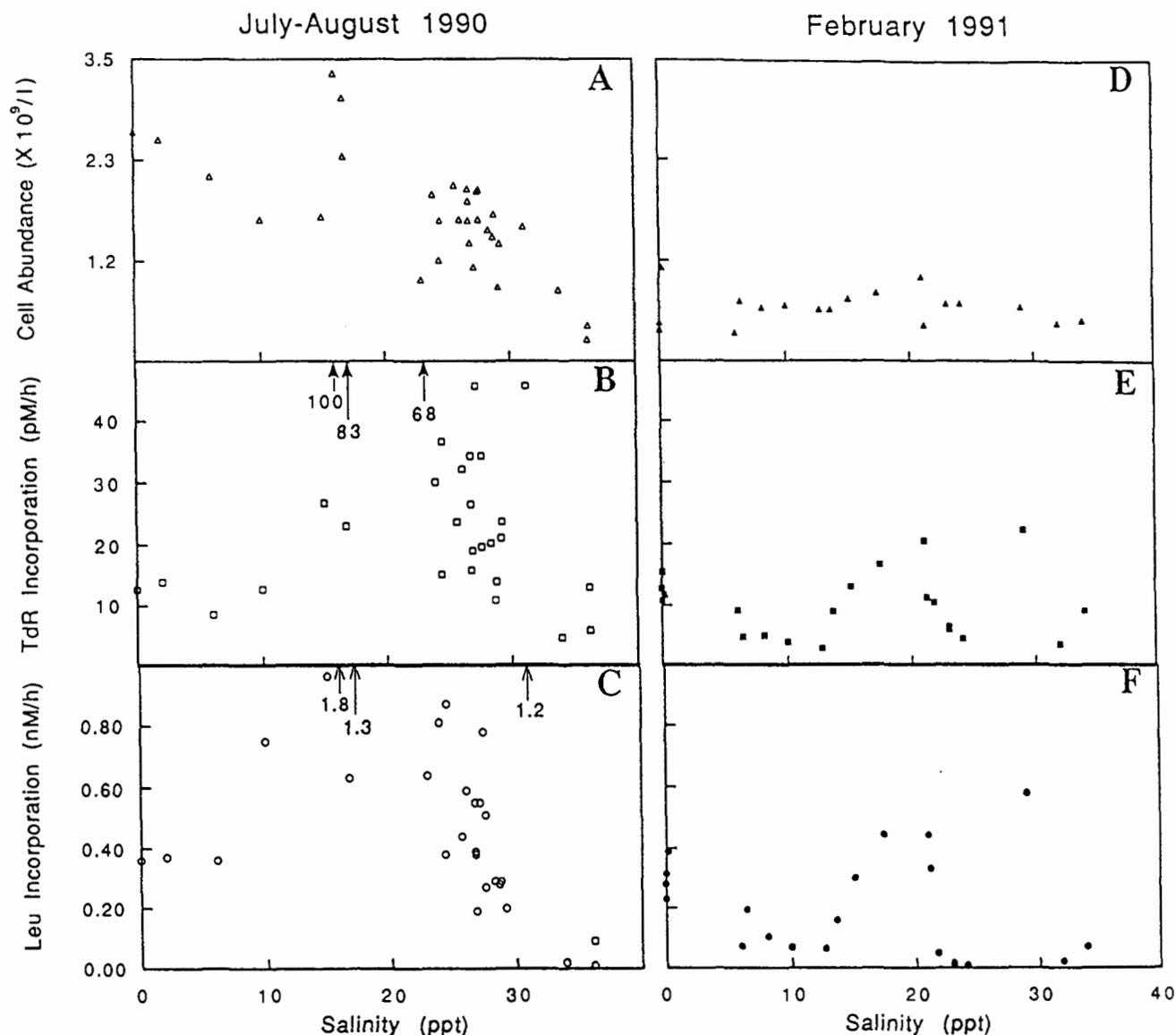


Figure 3. Scatter plots of surface water distributions during July and August 1990 of (A) cell abundance, (B) rates of TdR incorporation and (C) rates of Leu incorporation, and during February 1991 of (D) cell abundance, (E) rates of TdR incorporation and (F) rates of Leu incorporation.

phic utilization of organic matter in the water column and sediments and the lack of vertical mixing due to stratification. Rates of bacterioplankton C production can be used to estimate the O_2 demand by bacteria if bacterial growth efficiencies and respiratory quotients (RQ) are known. During the summer cruise four stations were surveyed where oxygen concentrations in bottom waters were $<1 \text{ ml l}^{-1}$. At these stations, we estimated the possible role of planktonic bacteria in the establishment of these hypoxic conditions by calculating the time that would be required for bacteria below the pycnocline to reduce the oxygen concentration from a saturated value ($4.51 \pm 0.13 \text{ ml l}^{-1}$) to the *in situ* levels. We assumed: (1) the pycnocline prevented any gas exchange with overlying waters and horizontal mixing was negligible; (2) a bacterial respiratory quotient of 1; (3) a range of bacterial growth efficiencies

from 10 to 30 percent. Using a 10 percent growth efficiency, bacterial oxygen demand would have reduced the concentration of oxygen from saturation to *in situ* levels in 13 to 28 d. Using a 30 percent growth efficiency, the range was from 40 to 84 d. If the bulk of the C supporting bacterial growth originates from the spring bloom, the oxygen demand from growth of planktonic heterotrophic bacteria in subsurface waters was sufficient to establish hypoxic conditions by summer.

Mesocosm experiments — Rates of dissolved oxygen consumption and carbon dioxide production (winter only) were measured during the mesocosm experiments to determine the total amount of organic matter that was oxidized and the relative lability of organic matter at various locations in the MRP/GS region. Rates of oxidation were usually higher in bottles with

Table 1 Depth integrated bacterioplankton C production on the Louisiana continental shelf. Estimates were calculated using rates of thymidine incorporation and an empirically determined conversion factor. Leucine incorporation rates were converted to rates of C production using the conversion factor determined by Simon and Azam (1988).

Date	Station	Latitude(W)	Longitude(N)	Surface Salinity (‰)	Depth (m)	Integrated Bacterial Production (mgC m ⁻² d ⁻¹)	
						TdR*	Leu#
27-Jul-90	HT-2	28° 50'	89° 49'	16	54	588	789
31-Jul	Anchor-2	28° 53'	89° 56'	18	36	399	591
29-Jul	HT-18	28° 48'	91° 31'	23	21	509	458
27-Jul	HT-8	28° 40'	90° 30'	24	18	362	441
21-Jul	J	28° 50'	89° 10'	24	99	740	719
29-Jul	HT-12	28° 36'	91° 00'	26	21	353	518
20-Jul	H	29° 17'	88° 52'	27	36	303	269
21-Jul	K	28° 50'	89° 22'	27	63	434	365
29-Jul	HT-20	28° 54'	91° 53'	27	20	238	328
21-Jul	I	28° 55'	89° 02'	28	75	614	374
29-Jul	HT-14	28° 27'	90° 59'	29	55	360	234
27-Jul	HT-10	28° 18'	90° 29'	29	56	411	457
Mean ± SD =						443 ± 144	462 ± 170
23-Feb-91	Anchor-1	28° 54'	89° 29'	6	29	102	148
26-Feb	HT-18	28° 40'	90° 29'	18	17	143	192
24-Feb	Miss. Can.-1	28° 41'	89° 40'	21	92	363	487
22-Feb	C	28° 46'	89° 30'	22	84	299	279
Mean ± SD =						226 ± 124	277 ± 151

*Estimated using a conversion factor of 1.89×10^{18} cells mole⁻¹ of thymidine and 20 fg C cell⁻¹ (Lee and Furhman 1987)
#Estimated using a conversion factor of 3.1 kg C mole⁻¹ of leucine (Simon & Azam 1988).

Table 2. Summary of rates of bacterial production, community respiration, and estimated bacterial growth efficiencies during mesocosm experiments conducted on the June/July 1990 NECOP cruise. Water samples were unfiltered (whole) or prefiltered through a 1 µm pore-size filter (filtered). Each number represents the mean of two replicate bottles.

Salinity (ppt)	Temperature (C)	Treatment	Bact. Production (µM C/h)	Respiration (µM O ₂ /h)	Bact. Growth Efficiency (%)
0	28	whole	0.038	NA	NA
		filtered	0.034	NA	NA
17	28	whole	0.112	1.30	8
		filtered	0.105	0.74	13
17	30	whole	0.062	0.35	15
		filtered	0.067	0.19	26
23	31	whole	0.102	0.74	12
		filtered	0.027	0.24	10
36	24	whole	0.033	0.06	35
		filtered	0.038	0.01	82

Table 3. Summary of rates of bacterial production, community respiration, carbon mineralization and estimated bacterial growth efficiencies during mesocosm experiments conducted on the February 1991 NECOP cruise. Water samples were unfiltered (whole) or diluted (1:5) with 0.2 μm pore-size filtered water (filtered). Each number represents the mean of two replicate bottles.

Salinity (ppt)	Temperature (C)	Treatment	Bact. Production ($\mu\text{M C/h}$)	Respiration ($\mu\text{M O}_2/\text{h}$)	Mineralization ($\mu\text{M C/h}$)	Bact. Growth Efficiency (%)
0	9	whole	0.058	0.19	0.11	24
		filtered	0.038	0.14	0.12	22
17	16	whole	0.065	0.56	0.61	10
		filtered	0.077	0.27	0.34	22
28	17	whole	0.059	0.33	0.43	15
		filtered	0.063	0.23	0.19	22
34	20	whole	0.047	0.10	0.23	32
		filtered	0.024	0.09	0.09	20

unfiltered water than in bottles containing filtered water (Tables 2, 3), indicating that detrital particles were important substrates for oxidation or that attached microflora and larger organisms were responsible for a significant fraction of the total respiration. Respiration rates ranged from 0.01 to 1.30 $\mu\text{M O}_2 \text{ h}^{-1}$ and were highest in plume waters of intermediate salinities (17 - 28‰) during both the summer (Table 2) and winter (Table 3). During the winter cruise both O_2 consumption and CO_2 production were measured as independent indicators of organic matter oxidation. Rates of O_2 consumption and CO_2 production were comparable and the overall mean respiratory quotient ($\Delta\text{CO}_2/\Delta\text{O}_2$) was 0.99 (excluding the data for the unfiltered bottle at 34‰ salinity).

Integrated estimates of bacterial carbon production did not show a consistent pattern of significantly higher rates of production in unfiltered water samples as was demonstrated with respiration rates (Tables 2, 3). These results suggest that most bacterial production was supported by dissolved organic matter rather than particulate materials. As with respiration rates, bacterial production was highest in plume waters of intermediate salinities. Estimates of bacterial growth or carbon conversion efficiencies were calculated as:

$$\text{Growth efficiency} = \text{bacterial production} / (\text{bacterial production} + \text{respiration})$$

assuming that the average respiratory quotient (RQ) was 1.0. Calculated growth efficiencies ranged from 8 to 35 percent (Tables 2, 3), excluding the high value of 82 percent determined for filtered Gulf water (36‰ salinity) during the summer. We did not observe any consistent spatial or temporal patterns in bacterial growth efficiencies. The average bacterial growth efficiency was 19 percent.

The patterns and rates of ammonium accumulation in the dark provided insights on the lability and chemical nature of dissolved organic nitrogen in the water samples from the respective sites and also provided information about whether bacteria and other hetero-

trophic organisms in the samples were net producers or users of dissolved ammonium. Results from the filtered and unfiltered samples were compared to provide insights on the relative importance of bacteria and larger heterotrophic organisms, respectively, in the mineralization process at the different sites and to provide comparisons between DOM and POM as substrate sources for the production of mineralized end products.

Three patterns of net ammonium accumulation or removal were observed — an increase in ammonium concentrations with time of incubation, no change in ammonium concentrations, or a decrease in ammonium concentrations. In experiments where accumulation of ammonium was observed, the rates of ammonium accumulation in the dark bottle experiments were generally linear with time of incubation. Samples showing no change in ammonium levels generally had no measurable ammonium either at the beginning or during the course of the experiments.

Duplicate-bottle ammonium regeneration rate results were generally in good agreement with each other (see SE bars on Figure 4) and results from duplicate treatments at each station were averaged to compare ammonium regeneration trends with salinity and to compare summer and winter trends (Fig. 4).

In July and August 1990, rates of ammonium accumulation for the river and offshore samples were generally $<10 \text{ nM h}^{-1}$, as compared to rates of about 30 to 150 nM h^{-1} at the plume stations (salinities of 17 to 23‰) (Fig. 4a). These results are consistent with the hypothesis that organic nitrogen recently fixed by phytoplankton is rapidly recycled in the plume. The relatively low rates at the other stations may reflect low concentrations and fluxes of organic nitrogen substrates because temperatures were similar at all the stations in the summer. Alternatively, low net rates at high salinity stations could reflect a greater microbial demand for inorganic nitrogen than was observed at the low salinity stations.

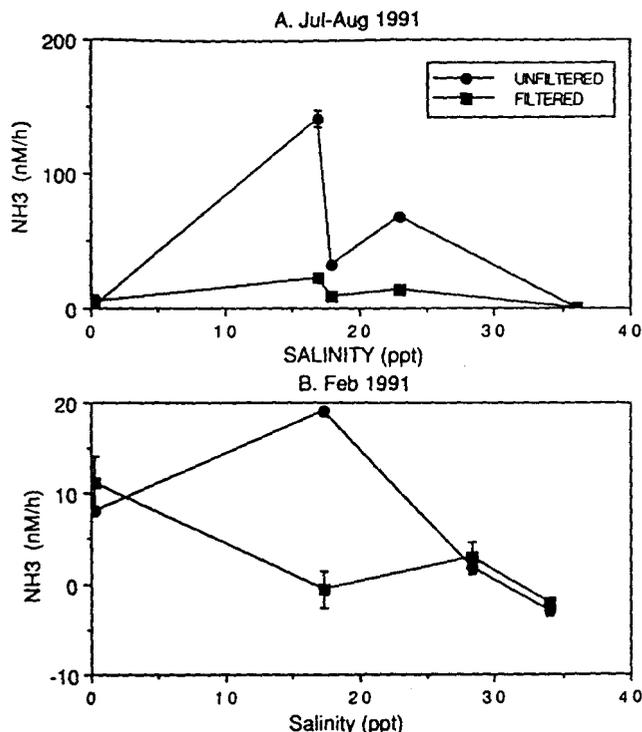


Figure 4. Net ammonium accumulation rates in the dark in water collected from different sites during the July and August 1990 and February 1991 cruises. For filtered treatments, water was either prefiltered through a 1 μm pore-size filter (July and August) or filtered (3 μm pore-size) water was diluted (1:5) with 0.2 μm pore-size filtered water (February). Error bars represent one standard error of the mean.

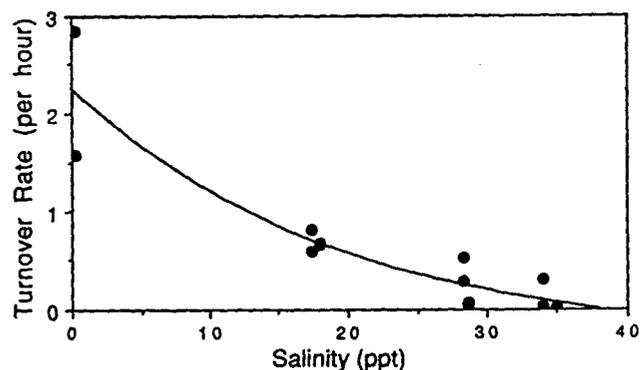


Figure 5. Amino acid turnover rates at different sites in the Mississippi River plume/Gulf shelf system during the February cruise.

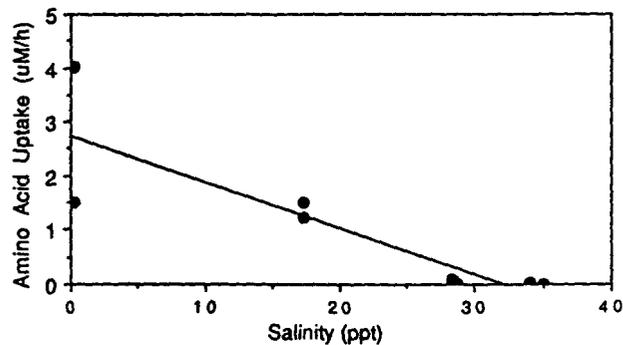


Figure 6. Amino acid uptake rates at different sites in the Mississippi River plume/Gulf shelf system during the February cruise. Uptake rates were determined by multiplying measured amino acid concentrations by turnover rates.

In contrast to results from the river and coastal plume samples, the samples from high-salinity stations (e.g. blue water station, and bottom water from hypoxic region) did not accumulate any ammonium during the course of our incubations. This result indicates either that ammonium was not produced in these waters or that any ammonium produced in the bottles was quantitatively removed by microbes. For example, if the bacteria use organic substrates with a high molar C:N ratio, i.e. greater than 7, they could be net consumers of ammonium. Alternatively, autotrophic nitrifiers may have used ammonium as an energy source and quantitatively removed it from solution.

The relative importance of the bacterial-sized fraction compared to larger particles in mineralizing organic nitrogen was dependent on the site of sampling. Ammonium accumulation rates in bottles containing filtered and unfiltered water samples were similar to each other in both the river and offshore stations. In contrast, the <1 μm size fraction accounted for only a relatively small fraction (20 to 25 percent) of total ammonium accumulation at the plume stations (Fig. 4). At stations where organic nitrogen mineralization was relatively high, only a small fraction of the total mineralization was attributed to microbes in the filtered bottles (Fig. 4). Thus, an important interaction was observed between sampling sites and the proportion of total ammonium regeneration accounted for by bacterial-sized particles. These results agree with those for respiration in the mesocosms and suggest that micro- or meso-zooplankton may account for a substantial portion of total nitrogen remineralization at the plume sites where primary production is relatively high.

Ammonium accumulation rates in the winter cruise (February 1991) ranged from negative values at the offshore station to near 20 nM h^{-1} for the unfiltered water at the near-river plume station (salinity = 17‰). Although rates at some stations were comparable to those sampled in the summer, those in the near-river plume were an order of magnitude lower (Fig. 4b). As was observed in the summer, differences between the unfiltered and filtered treatments were small in all samples except for the near-river plume station that had a relatively high ammonium accumulation rate in the unfiltered sample but a net uptake of ammonium (near zero) in the filtered sample.

In contrast to results from the summer cruise, water sampled from the river yielded ammonium accumulation rates (about 10 nM h^{-1}) that were higher than those from the high salinity (>20‰) stations. Except for the plume station (salinity = 17‰), which showed a large discrepancy in dark ammonium accumulation rates between the unfiltered and filtered samples, the rate of ammonium accumulation tended to decrease with increased salinity (Fig. 4b). Examination of amino acid turnover and uptake rates indicated patterns of bacterial activity consistent with the dark-ammonium accumulation rates in the diluted bottles. Amino acid turnover rates were highest in the river and decreased with

increasing salinity in the plume (Fig. 5). This result implies that the river had greater organic carbon demand than the plume if the assumption is made that DON composition and isotope dilution were comparable at all sites. The relationship of total amino acid uptake rate to station salinity (Fig. 6) generally resembled that for amino acid turnover (Fig. 5), indicating that the results from most of the sites were not influenced by isotope dilution during measurements. Amino acid turnover rates were also normalized for bacterial numbers, but again similar decreases were observed with increasing salinity (Fig. 7). These results imply that bacteria have a greater demand for amino acids in the river than in the plume and, if it is assumed that amino acids are representative of labile DON, they suggest that bacteria in the river and near-shore plume have the greatest DON demand of the sites examined.

These results imply that, at least in the winter, bacteria in the river and nearshore plume are more likely to remineralize available DON than are bacteria from offshore sites. However, DON cannot be remineralized to ammonium if it is not available for bacterial utilization. We examined the effect of increasing microbial concentration on amino acid turnover rates and on ammonium regeneration rates to get a relative idea of the size of the labile DON pool at three different sites. In these experiments, amino acid turnover and ammonium regeneration rates were likely indicative of the size of the labile organic nitrogen pool because changes in rates with changes in bacterial concentrations were consistent with results from the bottle experiments. In the river and at the second anchor station (salinity = 28‰), neither amino acid turnover rates (Fig. 8) nor total ammonium regeneration rates, measured in $^{15}\text{NH}_4$ isotope dilution experiments (Fig. 9), increased with increasing microbe concentrations. These results suggest that ambient DON concentrations were turning over at maximal rates and that ambient concentrations of labile DON were low. At the near-river plume station, however, the amino acid turnover rates (Fig. 8) and ammonium regeneration rates (Fig. 9) both increased hyperbolically with increased bacterial concentrations. These results indicate that ambient labile DON concentrations were sufficiently high to support greater microbial uptake rates than were observed at this site. The close agreement between the patterns of amino acid turnover (Fig. 8) and ammonium regeneration (Fig. 9) with increased bacterial abundances at the different sites is consistent with the idea that both of these measurements reflect the relative turnover of DON at the different sites.

In conclusion, the results suggest that heterotrophic bacterial production and nutrient regeneration processes in the study region both depend strongly on the recycling of fresh organic material produced by phytoplankton in the near-river plume. Organic matter delivered from the river may also support a significant fraction of bacterial activity, particularly in the winter when primary productivity in the plume is relatively low.

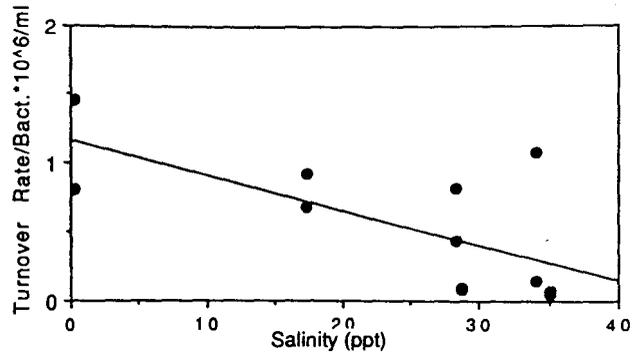


Figure 7. Amino acid turnover rates specific to bacterial numbers. Turnover rates were divided by ambient bacterial concentrations (cells per ml) to obtain y-values.

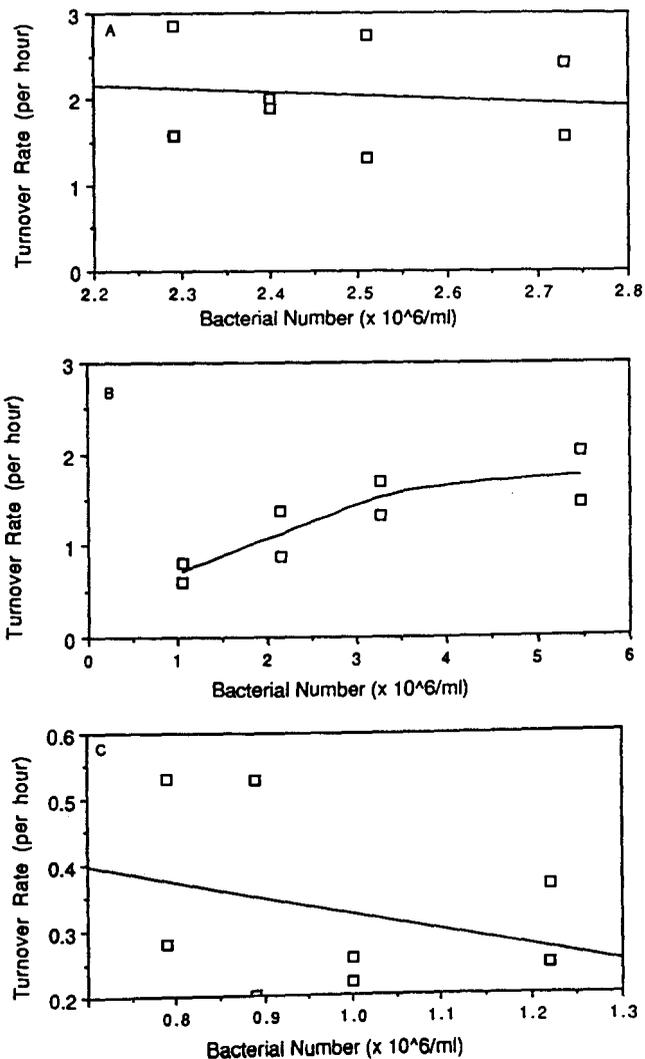


Figure 8. Amino acid turnover rates as a function of bacterial concentrations at the river site (A, salinity 0.3‰), first anchor station (B, salinity 17.3‰), and second anchor station (C, salinity 28.3‰) during the February 1991 cruise.

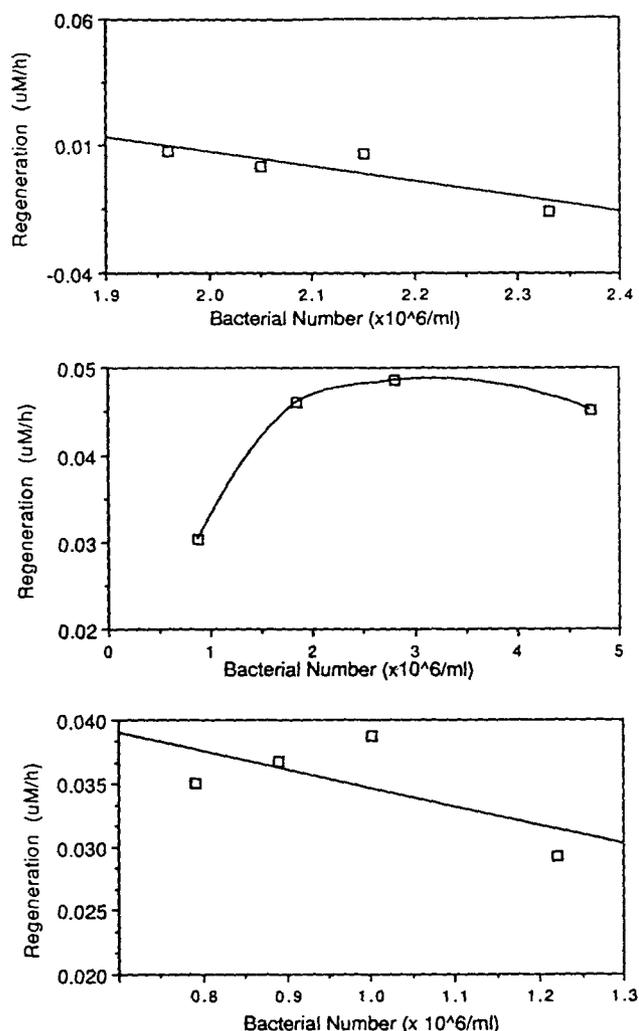


Figure 9. Ammonium regeneration rates as a function of bacterial concentrations at the river site (A, salinity 0.3‰), first anchor station (B, salinity 17.3‰), and second anchor station (C, salinity 28.3‰) during the February 1991 cruise.

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