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# Microbial ammonium cycling in the Mississippi River plume during the drought spring of 2000

**FRANK J. JOCHEM*,†, MARK J. MCCARTHY AND WAYNE S. GARDNER**

THE UNIVERSITY OF TEXAS AT AUSTIN, MARINE SCIENCE INSTITUTE, 750 CHANNEL VIEW DRIVE, PORT ARANSAS, TX 78373, USA

†PRESENT ADDRESS: FLORIDA INTERNATIONAL UNIVERSITY, MARINE BIOLOGY PROGRAM, 3000 NE 151 STREET, NORTH MIAMI, FL 33181, USA

*CORRESPONDING AUTHOR: frank@jochem.net

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*Microbial potential uptake and regeneration rates of ammonium ($NH_4^+$) were studied along a salinity gradient (salinities 0.2–34.4) in the Mississippi River plume during an extreme drought in spring 2000. Chlorophyll concentrations up to 30 $\mu g\ L^{-1}$ were highest in the low- and mid-salinity regions (salinities 8.5–28.2) and comparable to records of other years but extended over smaller areas than during periods of normal river flow. Bacterial biomass (5.1–28.3 $\mu g\ C\ L^{-1}$) was at the low end of the range observed in normal flow years, decreased with distance from the river mouth and did not peak with chlorophyll. Heterotrophic nanoflagellate abundance (1.4–4.0 $\mu g\ C\ L^{-1}$) did not reflect phytoplankton and bacterial spatial distribution but peaked at 9.2 $\mu g\ C\ L^{-1}$ at salinity 8.5. Microbial $NH_4^+$ regeneration rates were estimated by $^{15}NH_4^+$ isotope dilution experiments for the whole microbial community, under light and dark conditions, and for the <2 $\mu m$ bacterium-dominated size fraction. Microbial $NH_4^+$ regeneration rates (0.018–0.124 $\mu mol\ N\ L^{-1}\ h^{-1}$) were low relative to previous reports and peaked at salinity 28. Total $NH_4^+$ regeneration rates were higher than those in the <2 $\mu m$ size fraction at only four stations, suggesting that bacterial mineralization was a significant component of $NH_4^+$ recycling in some parts of the river plume. Higher $NH_4^+$ regeneration in whole-water samples versus <2 $\mu m$ fractions provided evidence for microbial grazing in regions where chlorophyll and regeneration rates peaked and at two full-salinity stations.*

Biogeochemical cycles on the Louisiana shelf in the northern Gulf of Mexico are influenced by riverine freshwater inputs from the Mississippi River, which introduces dissolved organic carbon (DOC) and inorganic nutrients as substrates for bacteria and phytoplankton (Sklar and Turner, 1981; Chin-Leo and Benner, 1992; Lohrenz *et al.*, 1999). Nitrate loads have increased over the last 30 years (Turner and Rabalais, 1991; Justić *et al.*, 1993, 1995) and support dense phytoplankton blooms at intermediate salinities in the Mississippi River plume (Lohrenz *et al.*, 1990, 1999; Chin-Leo and Benner, 1992; Gardner *et al.*, 1997). Nitrogen can control primary production in shelf waters near the Mississippi River delta, although phosphorus and silicate limitation also has been observed at times (Sklar and Turner, 1981; Dortch and Whitledge, 1992). Long-term trends in the relative nutrient composition of Mississippi River water (Justić *et al.*, 1993, 1995) suggest that nutrient input ratios have approached Redfield values, leading to some ambiguity and temporal variability in nutrient-limitation status (Lohrenz *et al.*, 1999).

Hypoxic conditions over the Louisiana and Texas shelf have been linked to the nitrate load of the river plume (Scavia *et al.*, 2003). This relationship is complex mechanistically, however, because it involves nitrogen transformations and microbial food web interactions throughout the plume. Nitrate becomes depleted along the river-to-ocean mixing gradient through dilution and biological uptake (Bode and Dortch, 1996; Lohrenz *et al.*, 1999), whereas ammonium ($NH_4^+$) uptake and recycling rates increase at intermediate salinities (Gardner *et al.*, 1994, 1997; Bode and Dortch, 1996). Water-column nutrient recycling sustains phytoplankton production

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(Fahnenstiel *et al.*, 1995; Gardner *et al.*, 1997), and nitrification can affect oxygen consumption and nitrogen dynamics in the Mississippi River plume (Pakulski *et al.*, 2000).

Several factors suggest the role of an active microbial food web in the Mississippi River plume in shaping nitrogen regeneration rates and pathways, including the direct coupling of community $NH_4^+$ regeneration rates to phytoplankton distribution (Gardner *et al.*, 1994), active bacterioplankton communities coupled to phytoplankton distribution (Amon and Benner, 1998), high protozoan grazing pressure (Jochem, 2003) and nitrification (Pakulski *et al.*, 2000). Phytoplankton nitrogen can be transformed into bacterium-available dissolved organic nitrogen (DON) by direct algal exudation (Keil and Kirchman, 1991; Bronk and Glibert, 1993), viral cell lysis (Proctor and Fuhrman, 1990) or zooplankton grazing (Nagata and Kirchman, 1991; Bronk and Glibert, 1993). Bacteria using DON can act as net $NH_4^+$ producers (Cotner and Gardner, 1993; Haga *et al.*, 1995) or consume and produce $NH_4^+$ simultaneously (Tupas and Koike, 1991). Bacterial production in the Mississippi River plume appears to be linked to the production of low-molecular-weight DON by phytoplankton, which supplements riverine DOC as a carbon and nitrogen source (Cotner and Gardner, 1993; Gardner *et al.*, 1997; Amon and Benner, 1998). The role of heterotrophic bacteria as net $NH_4^+$ producers or consumers depends on the C:N ratio of the DOC and the production of low-molecular-weight DON (Goldman *et al.*, 1987; Gardner *et al.*, 1996). For example, while bacterial $NH_4^+$ regeneration amounted generally to ~10% of community $NH_4^+$ regeneration rates in the Chesapeake Bay, bacterial contribution increased to 40% of community regeneration rates during the decline of a diatom bloom (Glibert, 1982), which probably provided high amounts of low C:N DOC.

Bacteria can account for a substantial fraction of community $NH_4^+$ regeneration in some regions of the plume (Cotner and Gardner, 1993; Gardner *et al.*, 1994). Protozoa, which are major phytoplankton consumers in the plume region (Dagg and Ortner, 1992; Dagg, 1995; Fahnenstiel *et al.*, 1995; Strom and Strom, 1996), also may be important $NH_4^+$ producers (Keil and Kirchman, 1991; Dolan, 1997). The role of bacterial versus microzooplankton contribution to whole-community $NH_4^+$ regeneration has not been defined adequately in the Mississippi River plume. This study addresses (i) the effect of a drought spring on potential uptake and regeneration rates of $NH_4^+$ in the Mississippi River plume and (ii) the relative role of bacteria versus micro- and nanozooplankton grazers in facilitating pelagic nitrogen remineralization in the plume.

## METHODS

Ammonium recycling rates were assessed in surface waters (sampling depth of 1 m) at nine stations (Fig. 1) along a salinity gradient (0.2–34.4; Fig. 2) in the Mississippi River plume, sampled between 19 and 29 May 2000 aboard the R/V *Longhorn*. Stations with salinities ≤28 were located in water depths <6 m, and

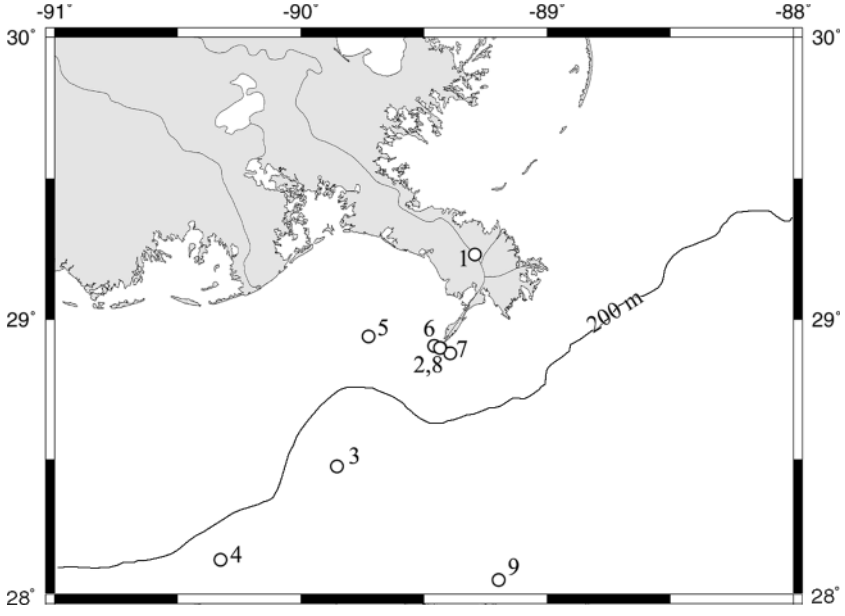


**Fig. 1.** Location of sampling stations in the Mississippi River plume, Louisiana shelf and Gulf of Mexico.

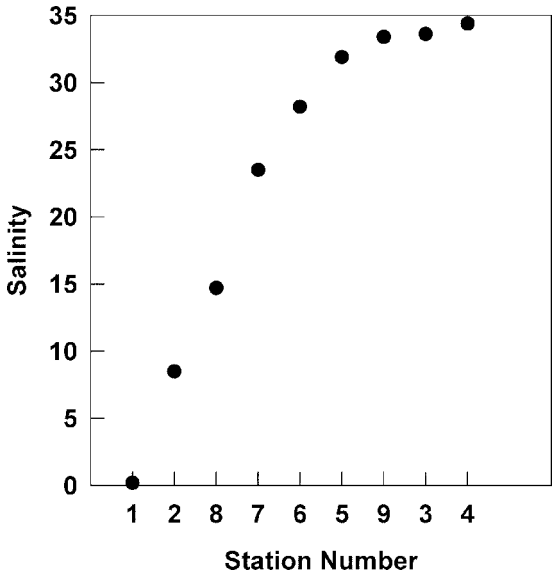


**Fig. 2.** Salinity gradient represented by the nine field stations in the Mississippi River plume.

station 5 (salinity 31.9) was located at 22 m water depth. Surface-layer sampling should represent shallow rather than deep-water column communities and processes. Since stations 3, 4 and 9 (with salinities >33) were located in deep water (>200 m), their subsurface populations and biogeochemical processes were probably different from those observed in surface water. Results represent surface communities that are affected directly by the river outflow.

Temperature and salinity were recorded by a Sea-Bird 911-Plus CTD equipped with a Sea Tec FL0500 fluorometer for *in situ* chlorophyll fluorescence. Since direct measurements of chlorophyll are not available, CTD chlorophyll fluorescence was converted to chlorophyll concentration by linear regression of fluorescence versus measured chlorophyll from a previous cruise using the same instrument ($r^2 = 0.78$; $P < 0.0001$; $n = 49$). Nutrient analyses (nitrate, nitrite and ammonium) were performed in the laboratory on thawed filtrates (0.2 μm pore size) of deep-frozen water samples using a Lachat QuikChem 8000 FIA autoanalyzer (triplicate analysis for each sample).

Samples for $NH_4^+$ regeneration experiments were taken by CTD-mounted, metal-free Niskin-type water bottles. Ammonium regeneration rates were quantified by $^{15}NH_4^+$ isotope dilution experiments. Water samples (70 mL) were transferred into Corning 25 $cm^2$ polystyrene culture flasks and amended with 99.8% $^{15}NH_4Cl$ (final concentration 4 μmol $L^{-1}$). Samples were submerged in a blue-shaded Plexiglas deck incubator to simulate the sunlight spectrum and reduce ambient light to about 50% incident irradiation (Lohrenz *et al.*, 1990). Water temperature in the incubator was stabilized by a microprocessor-controlled Neslab RTE bath/circulator. Parallel samples were pre-filtered through 47 mm 2.0 μm Nuclepore filters (less than −150 mbar), spiked with $^{15}NH_4^+$ and incubated as described above to assess the potential contribution of heterotrophic bacteria to total $NH_4^+$ regeneration. A third set of unfractionated samples was incubated in the dark (aluminum foil wrap). All incubations were performed in triplicate bottles.

Subsamples (10 mL) were collected immediately after $^{15}NH_4^+$ spiking and after 6 h of incubation and passed through a 0.2 μm nylon syringe filter. The first 3 mL of sample rinsed the filter and were discarded. The next 7 mL of filtrate were collected in clean 8 mL glass vials and frozen. Ammonium concentrations and atom% $^{15}NH_4^+$ were measured in the filtrates by direct-injection high-performance liquid chromatography using a cation-exchange column that fractionates $^{15}NH_4^+$ and $^{14}NH_4^+$ (Gardner *et al.*, 1993, 1995). Community $NH_4^+$ regeneration rates were calculated from changes in $NH_4^+$ concentrations and isotope ratios over time (Blackburn, 1979; Caperon *et al.*, 1979). Differences in $NH_4^+$ uptake and regeneration rates between total versus <2 μm and light versus dark incubations were deemed significant when the zero hypothesis of no differences was rejected by Student's *t*-test with $P > 0.05$.

Assessing biogeochemical processes using isotope addition is a compromise between tracer additions sufficient to resolve concentration changes and the biases of measured rates by the tracer additions themselves. Ammonium uptake rates are considered to be potential, but $NH_4^+$ regeneration rates should not be affected unless isotope tracers are exhausted within the incubation period. Total ammonium uptake within the incubation periods amounted to 0.2–0.48 μmol $L^{-1}$ and was only a small fraction of added $^{15}NH_4^+$ in our experiments, indicating that isotope dilution continued throughout the incubation period.

Bacterial $NH_4^+$ regeneration rates in pre-filtration experiments can be overestimated because of dissolved free amino acid (DFAA) release during size fractionation (Fuhrman and Bell, 1985) and subsequent mineralization of organic nitrogen by bacteria (Kirchman *et al.*, 1991; Haga *et al.*, 1995). Microzooplankton grazing also may be a substantial source of organic substrates, such as DFAA (Andersson *et al.*, 1985; Nagata and Kirchman, 1991; Ferrier-Pages *et al.*, 1998), which can increase bacterial production and $NH_4^+$ regeneration rates (Strom *et al.*, 1997). However, grazing pressure on bacteria was modest (<0.51 $day^{-1}$) during the present study (Jochem, 2003).

Heterotrophic bacteria were counted by flow cytometry. Samples were incubated for 30 min at 37°C with

0.1 mg $mL^{-1}$ of RNAse (1:1 mix of RNAse A and B) prior to staining with SYBR Green I ($10^{-5}$ dilution of commercial stock; Molecular Probes) for 20 min in the presence of 30 mM potassium citrate (Marie *et al.*, 1997; Jochem, 2001). Samples were analyzed on a Becton-Dickinson FACSort flow cytometer with a flow rate of 0.2 μL $s^{-1}$. The counting rate was <500 cells $s^{-1}$; more dense bacterial samples were diluted 1:10 with 0.2 μm filtered seawater. Measured sample volume for estimates of cells $mL^{-1}$ was calculated from measurement times (60–300 s) based on weight calibration of flow rates. All signals were recorded on a four-decade log scale, and data were analyzed using PC Lysys software (Becton-Dickinson, San José, CA, USA). Bacterial measurements were corrected for *Prochlorococcus* counts from unstained fixed samples measured on the same day, where *Prochlorococcus* spp. and bacteria were not separated in the cytometric analyses of SYBR Green-stained preparations due to the low chlorophyll autofluorescence of *Prochlorococcus* (Jochem, 2001). Bacterial cell counts were converted to carbon biomass by the conversion factor $1.042 \times V^{0.59}$ (Simon and Azam, 1989), assuming an average cell volume of 0.04 $\mu m^3$ (Jochem, 2001).

Heterotrophic nanoflagellates (HNF) were counted on black 0.8 μm pore-size Nuclepore filters after 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining. Sample volume for filter preparations was 20 mL, and one full diameter of the filter was counted using a ×100 objective. HNF were sized by a New-Porton G12 eyepiece graticule (Graticules Ltd, Tonbridge, UK), and HNF cellular biomass was computed as log (volume) × 0.939 – 0.665 (Menden-Deuer and Lessard, 2000).

## RESULTS

The Mississippi River plume extended into the Gulf of Mexico less during the extreme drought conditions of spring 2000 than during normal river-flow periods, and the entire salinity gradient occurred within short distances. Nevertheless, the sampled stations comprised a full gradient from salinity 0.2 within the river mouth to >33 at the offshore stations (Fig. 1 and 2). The river introduced high nitrate concentrations (77 μmol $L^{-1}$), which decreased along the salinity gradient to below detection at a salinity of >28 (Fig. 3). The $NH_4^+$ concentration in the river was 0.34 μmol $L^{-1}$, and maximum concentrations (1.5 μmol $L^{-1}$) occurred at a salinity of 8.5. Ammonium was below the detection limit (0.1 μmol $L^{-1}$) at salinities ≥28. Nitrite concentrations (0.6–0.8 μmol $L^{-1}$) were highest at mid salinities (15–24) and ~0.25 μmol $L^{-1}$ at offshore stations (Fig. 3).

Phytoplankton standing stocks (chlorophyll *a*) were high in low- and mid-salinity areas, exhibiting maximum concentrations (~30 μg Chl *a* $L^{-1}$) at salinities of 8 and 24 and decreasing to <2 μg $L^{-1}$ at salinities above 30 (Fig. 4). Bacterial biomass decreased along the salinity gradient from 28.3 μg C $L^{-1}$ at the river mouth to ~20 μg C $L^{-1}$ at salinity 28 and then to 5.1 μg C $L^{-1}$ at salinity 34.4. HNF showed a consistent biomass of 2.5–4 μg C $L^{-1}$ in the low- and mid-salinity range, except for a pronounced maximum (9.2 μg C $L^{-1}$) at salinity 8.5. HNF biomass was 1.4–2.3 μg C $L^{-1}$ at full-salinity (>30) stations (Fig. 4).

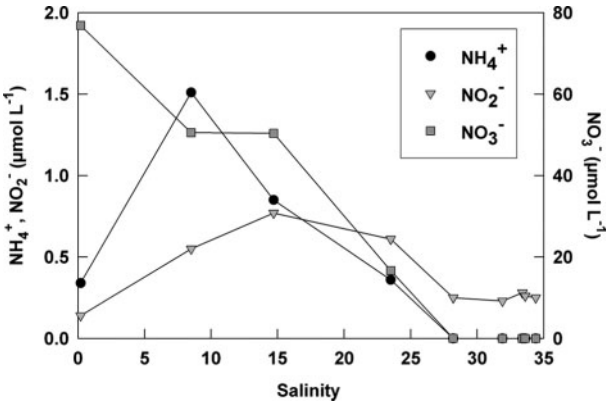


**Fig. 3.** Inorganic nitrogen (nitrate, nitrite and ammonium) concentrations (μmol $L^{-1}$) along the salinity gradient.

Total potential $NH_4^+$ uptake rates peaked in the chlorophyll-maximum area (0.17–0.36 μmol N $L^{-1}$ $h^{-1}$ at salinities of 8–28) and ranged from 0.03 to 0.08 μmol N $L^{-1}$ $h^{-1}$ at the river mouth and full-salinity stations (Fig. 5). Total potential $NH_4^+$ uptake rates correlated moderately ($r^2 = 0.54$; $P < 0.05$) with chlorophyll concentrations throughout the river plume but more strongly ($r^2 = 0.82$; $P < 0.05$) for stations with salinities ≥28 where nitrate was depleted. Potential $NH_4^+$ uptake rates in the <2 μm size fraction were significantly lower

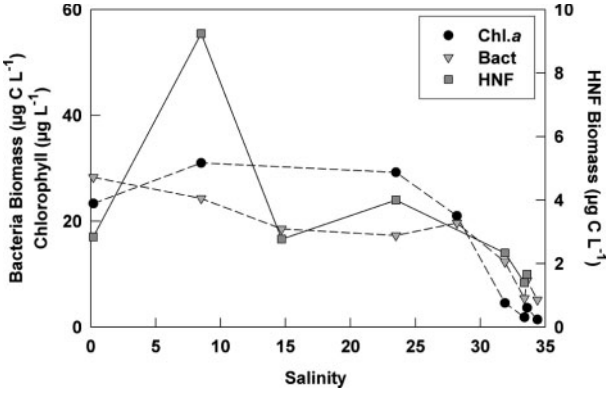


**Fig. 4.** Biomass distribution of phytoplankton [chlorophyll (μg $L^{-1}$)], heterotrophic nanoflagellates [HNF (μg C $L^{-1}$)] and bacteria (μg C $L^{-1}$) along the salinity gradient.

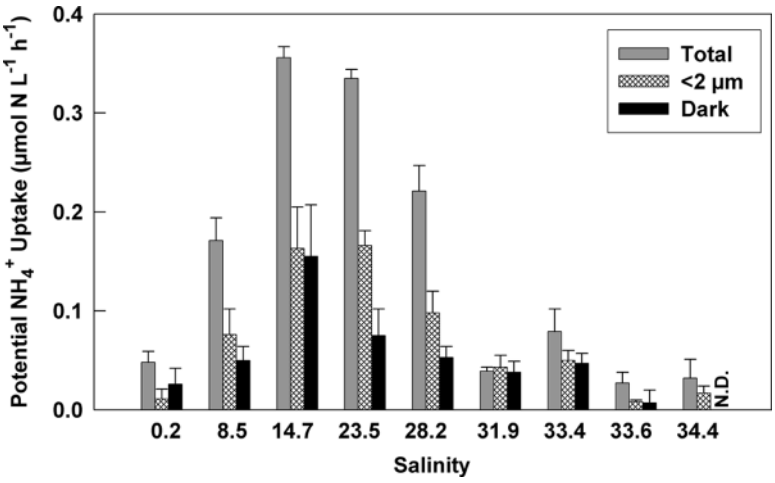


**Fig. 5.** Potential ammonium uptake rates (µmol N $L^{-1}$ $h^{-1}$) in whole-water light, <2 µm size-fractionated light and whole-water dark incubations along the salinity gradient. Error bars indicate standard errors of triplicate incubations. ND, not detectable.

than total $NH_4^+$ uptake rates in low- and mid-salinity regions that had the highest chlorophyll concentrations and uptake rates (Fig. 5). Organisms in this fraction accounted for 46 ± 2% of total $NH_4^+$ uptake for sites with salinities between 8 and 28. At full-salinity (i.e. >30) stations, picoplankton accounted for 64 ± 34% of total potential $NH_4^+$ uptake, but the difference between size fractions was not significant.

Potential $NH_4^+$ uptake rates were higher in light versus dark bottles, except at salinities 31.9 and 33.4 (Fig. 5); differences were statistically significant at salinities 8–28, 33.6 and 34.4. If dark $NH_4^+$ uptake rates are assumed arbitrarily to reflect bacterial uptake [based on light-dependent $NH_4^+$ uptake in phytoplankton; (MacIsaac and Dugdale, 1972; Kedong *et al.*, 1998)] comparison of dark and light incubations can reveal the bacterial contribution to total $NH_4^+$ uptake. Under N-replete conditions, as encountered at least in the low- and mid-salinity region of the study area, uptake of dissolved inorganic nitrogen (nitrate and ammonium) is coupled to the photoperiod; nitrogen assimilation becomes less dependent on concurrent photosynthesis under increasing N limitation (Raven, 1980; Clark and Flynn, 2002), and dark N assimilation has been shown to be most significant in N-starved cells (Amory *et al.*, 1991; Smith *et al.*, 1992; Clark *et al.*, 2002). Furthermore, N assimilation in darkness depends strongly on excess storage of carbon by previous photosynthesis (Clark *et al.*, 2002; Flynn *et al.*, 2002), which may be limited in the abundant pico- and nanophytoplankton (Jochem, 2003) by cell-size constraints. Under N-replete conditions as prevalent in the low- and mid-salinity regions of the river plume, excess carbon storage for later dark N assimilation also might be prevented, since photosynthetic products are utilized concomitantly to maintain maximum N assimilation (Clark and Flynn, 2002; Clark *et al.*, 2002). Thus, assuming dark uptake rates to represent bacterial uptake, bacteria accounted for 44 ± 26% of the total potential $NH_4^+$ uptake (dark/total uptake rates), a value that agrees with the relative rates of 46 ± 2% observed in the <2 µm size fraction versus the unfractionated samples. Within the picoplankton fraction, bacteria accounted for 76 ± 20% of $NH_4^+$ uptake (dark/<2 µm uptake rates) with a generally higher (88–94%) contribution at full versus mid salinities (45–95%). The river mouth station stands out by its dark/<2 µm $NH_4^+$ uptake ratio of 2.4, which may indicate the significance of particle-attached bacteria (excluded in the <2 µm fractionation).

Total $NH_4^+$ regeneration (0.018–0.124 µmol N $L^{-1}$ $h^{-1}$) peaked at salinity 28 and was lower in high versus low and mid salinities (Fig. 6). Comparison with standing stocks reveals that $NH_4^+$ regeneration rates did not follow regional distributions of HNF, bacteria or phytoplankton, except at the 8.5 salinity station with the highest nanophytoplankton and HNF biomass and the highest C consumption by microzooplankton (Jochem, 2003). Ammonium regeneration was significantly higher in the unfractionated samples than in the <2 µm size fractions at four stations (with salinities of 0.2, 28.2, 33.4 and 33.6; Fig. 6). Ammonium regeneration in the <2 µm size fraction followed (gross) bacterial production, as estimated from bacteria biomass and growth rates presented by Jochem (Jochem, 2003) (production calculated as $B_0 \times e^{\mu} - B_0$), in a logarithmic manner except at the river mouth station (Fig. 7). Regeneration rates in the microzooplankton dominated >2 µm size class (whole water – <2 µm), and whole-community results did not relate to pico- and nanophytoplankton and bacteria carbon biomass consumed by microzooplankton (Jochem, 2003) or to HNF biomass

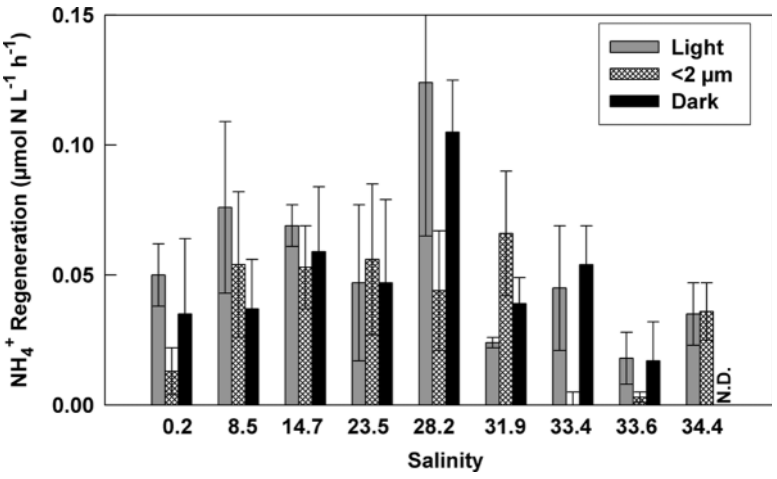


**Fig. 6.** Ammonium regeneration rates (µmol N $L^{-1}$ $h^{-1}$) in whole-water light, <2 µm size-fractionated light and whole-water dark incubations along the salinity gradient. Error bars indicate standard errors of triplicate incubations. ND, not detectable.

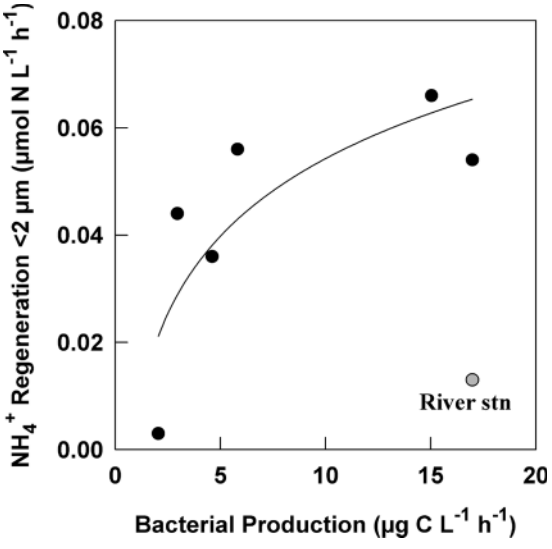


**Fig. 7.** Relation of ammonium regeneration rates (µmol N $L^{-1}$ $h^{-1}$) in the <2 µm size fraction to bacterial gross production (µg C $L^{-1}$ $d^{-1}$), as derived from bacteria biomass and growth rates (logarithmic regression, $r^2 = 0.66$, $P < 0.05$).

(data not shown). Significant differences in total $NH_4^+$ regeneration between light and dark bottles were lacking.

## DISCUSSION

### The drought spring river plume

Mississippi River discharge in May 2000 was 9000 $m^3$ $s^{-1}$ versus the normal river flow of 19,000 $m^3$ $s^{-1}$ (Amon and Benner, 1998; Lohrenz *et al.*, 1999; Hernes and Benner, 2003). The areal river plume was smaller than usual. Riverine nitrate loads were comparable to the long-term average (Bratkovich and Dinnel, 1992; Goolsby *et al.*, 2000) but lower than those from high river-flow periods (Turner and Rabalais, 1991; Pakulski *et al.*, 2000). DOC concentrations at the river mouth (268 µmol C $L^{-1}$; Hernes and Benner, 2003) were about 20% lower than those between July 1990 and 1993 (Benner and Opsahl, 2001).

High turbidity inhibits photosynthesis near the river mouth, despite high nutrient concentrations, and phytoplankton biomass and productivity usually peak at mid salinities (Lohrenz *et al.*, 1990, 1999; Gardner *et al.*, 1997). Diatoms dominate in the high phytoplankton biomass region at mid salinities (Fahnenstiel *et al.*, 1995; Lohrenz *et al.*, 1999). Peak chlorophyll concentrations were within previously reported ranges (Gardner *et al.*, 1997; Lohrenz *et al.*, 1999), but the position of the peak shifted toward lower salinities. This shift was surprising since water turbidity in the low- and mid-salinity range was high. Photosynthetically active radiation at 1 m water depth (around local noon) was <200 µE $m^{-2}$ $s^{-1}$ at salinities ≤28 (Hernes and Benner, 2003). The chlorophyll maximum in the May 2000 river plume may not have resulted from *in situ* phytoplankton growth but may be explained by hydrographic pumping along density fronts (Jochem, 2003). However, high eukaryotic nanophytoplankton abundance within the chlorophyll-maximum region was related to active population growth (Jochem, 2003). In contrast, chlorophyll concentrations at full-salinity stations (1.4–3.7 µg $L^{-1}$) were higher than those during normal river-flow years (0.02–0.8 µg $L^{-1}$; Strom and Strom, 1996; Pakulski *et al.*, 2000) despite lower dissolved inorganic nitrogen concentrations (Pakulski *et al.*, 2000).

Concurrent with lower-than-average DOC concentrations, bacterial abundance was low relative to previous

reports (Chin-Leo and Benner, 1992; Gardner *et al.*, 1994; Amon and Benner, 1998). Bacterial abundance and production are coupled in time and space to phytoplankton biomass and productivity (Amon and Benner, 1998). However, bacterial abundance decreased from the river mouth and did not peak with the mid-salinity chlorophyll concentration as expected. Protozoan grazers may have exerted top-down control on bacteria in the region of the chlorophyll peak and, thereby, limited the role of bacteria in nitrogen cycling in this region (Cotner and Gardner, 1993). In fact, bacterial net growth rates (growth rate – grazing loss) were lowest at salinities of 23.5 and 28.2 (Jochem, 2003). At salinities >30, bacterial standing stocks dropped along with phytoplankton biomass, suggesting that the general relationship of bacteria to phytoplankton abundance held for offshore regions, but other factors controlled bacterial distribution at low and mid salinities. In turbid estuaries, riverine DOC can enhance bacterial production in the absence of phytoplankton production and cause a decoupling of bacterial and phytoplankton production in various regions (Albright, 1983; Ducklow and Kirchman, 1983; Kirchman *et al.*, 1989), including the Mississippi River plume (Chin-Leo and Benner, 1992).

HNF abundance [1.4–9.2 μg C $L^{-1}$ corresponding to 1.4–2.7 × $10^3$ cells $mL^{-1}$; (Jochem, 2003)] was within the range reported for other marine systems (Fenchel, 1986; Sorokin, 1999) and the only previous report from the study area (Bode and Dortch, 1996). The spatial distribution did not reflect chlorophyll variation, but the HNF abundance peak at salinity 8.5 was related to the biomass maximum and high growth rates of predominantly eukaryotic nanophytoplankton (Jochem, 2003). HNF also shifted toward larger cells at this station, concomitant with a shift toward larger eukaryotes in the nanophytoplankton size fraction (Jochem, 2003).

The pattern of total potential $NH_4^+$ uptake rates (0.17–0.36 μmol N $L^{-1}$ $h^{-1}$) during this drought-affected period was comparable to that reported in previous reports from the Mississippi River plume (Gardner *et al.*, 1997) and generally followed chlorophyll concentrations. Potential $NH_4^+$ uptake rates, however, were lower than those observed within the chlorophyll maximum in summer 1991 (1.5–1.9 μmol N $L^{-1}$ $h^{-1}$; Bode and Dortch, 1996). The strong correlation of uptake rates with chlorophyll concentrations at ≥28 psu, where nitrate was depleted, suggests that ammonium was important for sustaining phytoplankton at offshore stations, while nitrate uptake may have been important for sustaining the chlorophyll peak, as has been previously reported (Bode and Dortch, 1996). Significant differences between whole-community and <2 μm $NH_4^+$ uptake rates also indicate a substantial fraction of nano- and microphytoplankton in the chlorophyll-maximum region of the plume.

Comparison of <2 μm light and whole-community dark uptake rates suggests that bacteria competed successfully for $NH_4^+$ in the picoplankton size fraction. These results agree with previous reports of a linear increase in $NH_4^+$ uptake rates with bacterial addition to plume samples under non-drought conditions (Cotner and Gardner, 1993). High abundance of nitrifying bacteria, suggested by the observed nitrite concentrations (0.6–0.8 μmol $L^{-1}$), may have contributed to bacterial $NH_4^+$ uptake rates. Active nitrification in conjunction with high water-column nitrite concentrations was reported for the mid-salinity region of the Mississippi River plume (Pakulski *et al.*, 2000).

Total $NH_4^+$ regeneration rates (0.018–0.124 μmol N $L^{-1}$ $h^{-1}$) were lower than those reported during non-drought summer conditions in the Mississippi River plume (Gardner *et al.*, 1993, 1997; Bode and Dortch, 1996) but comparable to rates from winter and spring (Cotner and Gardner, 1993; Gardner *et al.*, 1997), in Mobile Bay (Lehrter *et al.*, 1999) and other marine coastal systems (Lipschultz *et al.*, 1986; Hanson *et al.*, 1990; Gardner *et al.*, 2000). High $NH_4^+$ regeneration rates in summer were related to active primary production (Cotner and Gardner, 1993).

## Significance of grazers versus bacteria in $NH_4^+$ regeneration

Peak $NH_4^+$ regeneration rates and high chlorophyll concentrations at mid salinities are consistent with previous reports (Cotner and Gardner, 1993; Gardner *et al.*, 1997). Since pico- and nanophytoplankton abundance and grazed biomass were highest in the same region (Jochem, 2003), the spatial distribution of $NH_4^+$ regeneration rates agrees with the idea that microzooplankton grazing could have accounted for enhanced regeneration rates in the region of highest phytoplankton biomass. Planktonic protists are important $NH_4^+$ regenerators (Dolan, 1997), and microzooplankton can consume much of the primary production in the river plume (Dagg and Ortner, 1992; Fahnenstiel *et al.*, 1992). Large microzooplankton accounted for ~80% of annual chlorophyll loss in Mobile and Apalachicola Bays (Lehrter *et al.*, 1999; Mortazavi *et al.*, 2000). Meso- or microzooplankton grazing also may contribute to increased $NH_4^+$ regeneration rates within the region of the chlorophyll peak.

Pelagic nitrogen regeneration in the Mississippi River plume may relate to labile DON production by phytoplankton (Gardner *et al.*, 1997). Phytoplankton can exude DFAA during photosynthesis (Collos *et al.*, 1992; Obernosterer and Herndl, 1995; Rosenstock and Simon, 2001). Under light-limiting conditions such as those at the base of the euphotic zone, >90% of nitrate uptake was reported to be released as DON (Bronk and Ward,

1999). High turbidity in the low- and mid-salinity region of the river plume, coupled with low irradiance (<200 μE $m^{-2}$ $s^{-1}$), under high nitrogen concentrations might have stimulated increased DON release by phytoplankton. Increased release of DON by phytoplankton communities has been shown to increase $NH_4^+$ regeneration rates concomitantly (Varela *et al.*, 2003). Negative net growth rates of pico- and nanophytoplankton (Jochem, 2003) suggest that the phytoplankton community may have been in a poor physiological state. In addition, strong microbial grazing pressure on this community (Jochem, 2003) may have provided labile, low-molecular-weight and low C:N DON which could fuel bacterial $NH_4^+$ regeneration. Bacterial nitrogen remineralization rates increase if DOC concentrations are low, and bacteria are forced to metabolize low C:N DON for energy and release $NH_4^+$ (Goldman *et al.*, 1987). With background DOC concentrations lower than those in normal river-flow years, bacteria may have fulfilled their metabolic needs by using labile, low-molecular-weight DON, associated with primary production and grazing activity, which could have increased the relative contribution of bacterioplankton to total $NH_4^+$ regeneration (Gardner *et al.*, 1996).

Microzooplankton $NH_4^+$ regeneration rates did not correlate with HNF biomass or grazer-consumed pico- and nanophytoplankton biomass but may have been related to turnover rates of N-rich bacteria and other organisms. Grazing activity of large microzooplankton (ciliates and dinoflagellates) and copepods on microphytoplankton (diatoms) also may have contributed to the high total and microzooplankton $NH_4^+$ regeneration rates in the chlorophyll-maximum zone. Significant differences between potential $NH_4^+$ uptake rates in the total and <2 μm size fraction confirm the importance of microphytoplankton in the chlorophyll-maximum region.

Total community $NH_4^+$ regeneration was higher than bacterial $NH_4^+$ regeneration at the site with the highest total regeneration rates (salinity 28), the river mouth and two offshore stations. The highest-salinity (34.4) station was located closer to the Mississippi River plume than two stations with low bacterial $NH_4^+$ regeneration (salinities 33.4 and 33.6). These results agree with previous studies showing high bacterial contribution to $NH_4^+$ regeneration in the Mississippi River plume (Cotner and Gardner, 1993; Gardner *et al.*, 1994) and the English Channel during summer (Maguer *et al.*, 1999). In the present study, bacterial $NH_4^+$ regeneration rates correlated logarithmically with bacterial production rates (estimated from biomass and growth rates; Jochem, 2003). The lack of such correlation and low $NH_4^+$ regeneration rates in the <2 μm size fraction (26% of total $NH_4^+$ regeneration rates) in the river mouth may be explained by particle-attached bacteria, as also evidenced by $NH_4^+$ uptake rates, or by a high C:N ratio of riverine DOC and the lack of phytoplankton production in the river mouth. Low bacterial regeneration at offshore stations (salinities of 33.4 and 33.6) suggests increased protistan significance.

Light/dark experiments also serve as a useful tool for unraveling the importance of bacteria versus grazers in nitrogen regeneration. Light effects are less apparent for grazing and nutrient regeneration processes than for phytoplankton uptake, because heterotrophic organisms do not depend directly on light for energy. However, higher day than night protozoan grazing rates were reported from various aquatic systems (Weisse, 1989; Wikner *et al.*, 1990; Christoffersen, 1994; Dolan and Šimek, 1999), and recent evidence suggests that natural light stimulates phagotrophic nutrition in heterotrophic flagellates (Strom, 2001). A light-induced, simultaneous increase in $NH_4^+$ regeneration and grazing on *Synechococcus elongatus* was observed in Texas coastal waters (P. J. Lavrentyev and W. S. Gardner, unpublished results). Positive effects of light on $NH_4^+$ regeneration rates were documented in the Mississippi River plume, Lake Michigan and Lake Maracaibo (Gardner *et al.*, 1997, 1998, 2000), and these studies revealed a linear correlation between $NH_4^+$ regeneration rates and HNF:bacteria biomass ratios in the light but not in the dark (Gardner *et al.*, 2000). Thus, protistan grazing activity may produce differences in light/dark $NH_4^+$ regeneration rates in situations where $NH_4^+$ regeneration is related to grazing activity.

In the present study, $NH_4^+$ regeneration rates were higher in the light than dark only at salinity 8.5 (though not statistically significant), where HNF and grazed phytoplankton biomass (Jochem, 2003) were highest. Light $NH_4^+$ regeneration rates did not correlate with HNF:bacteria biomass ratios (data not shown), supporting the notion that light/dark differences in $NH_4^+$ regeneration rates may be more pronounced in systems where microzooplankton grazing dominates $NH_4^+$ regeneration. High bacterial rates relative to total $NH_4^+$ regeneration rates at most sites may explain the lack of light/dark differences in the present study. Microzooplankton $NH_4^+$ regeneration may have prevailed in July 1993 when light/dark differences were reported (Gardner *et al.*, 1997).

## CONCLUSION

Drought conditions and a condensed river plume affected biological and biogeochemical processes on the Louisiana shelf. Although maximum chlorophyll concentrations were comparable to previous years, the smaller plume and chlorophyll-maximum area decreased the

total productivity of phytoplankton and other organisms on the Louisiana shelf. Low community $NH_4^+$ regeneration rates, combined with a small plume surface area, decreased the extent and importance of areal nitrogen recycling, which may have decreased the water column and benthic productivity and alleviated bottom hypoxia during the summer of 2000 (Justić *et al.*, 2003; Scavia *et al.*, 2003).

Maximum $NH_4^+$ regeneration rates occurred in the chlorophyll-maximum area. High $NH_4^+$ regeneration and phytoplankton grazing loss rates in the chlorophyll-maximum region (Jochem, 2003) suggest the importance of protistan $NH_4^+$ regeneration in this part of the plume, which was supported by size-fractionation results. Size-fractionation experiments and the lack of correlation between $NH_4^+$ regeneration rates and HNF:bacteria biomass ratios agree with the idea that bacteria significantly contributed to $NH_4^+$ regeneration in the drought river plume outside the chlorophyll-maximum area.

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