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Photo- and heterotrophic pico- and nanoplankton in the Mississippi River plume: distribution and grazing activity

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The abundance of pico- and nanophytoplankton, bacteria and heterotrophic nanoflagellates, and grazing rates on phototrophic pico- and nanoplankton and bacterioplankton were assessed along a salinity gradient (0.2–34.4) in the Mississippi River plume in May 2000. Grazing rates were established by serial dilution experiments, and analysis by flow cytometry allowed differentiation of grazing rates for different phytoplankton subpopulations (eukaryotes, Synechococcus spp., Prochlorococcus spp.). Grazing rates on phytoplankton tended to increase along the salinity gradient and often approached or exceeded 1 day^{-1}. Phytoplankton net growth rates (growth – grazing) were mostly negative, except for positive values for eukaryotic nanoplankton in the low-salinity, high-chlorophyll region. Grazing pressure on bacteria was moderate (~0.5 day^{-1}) and bacteria gained positive net growth rates of ~0.3 day^{-1}. Eukaryotic nanophytoplankton were the major phototrophic biomass and protozoan food source, contributing 30–80% of the total consumed carbon. Bacteria were the second most important food source at 9–48% of the total consumed carbon. Synechococcus spp. and Prochlorococcus spp. remained an insignificant portion of protozoan carbon consumption, probably due to their low contribution to the total pico- and nanoplankton biomass. Group-specific grazing losses relative to standing stocks suggest protozoan prey preference for eukaryotes over bacteria. Protozoan grazers exerted a major grazing pressure on pico- and nanophytoplankton, but less so on bacteria.

INTRODUCTION

Dense phytoplankton accumulations at salinity fronts occur in estuarine areas (Pingree, 1978; Parsons *et al.*, 1981; Dagg *et al.*, 2003). High and variable input of fresh water in river-dominated estuaries creates surface salinity gradients associated with high spatial variability in inorganic and organic nutrient concentrations, water turbidity, and phytoplankton biomass and production (Cloern, 1996; Lohrenz *et al.*, 1999). Intense phytoplankton blooms at intermediate salinities occur in the Mississippi River plume (Lohrenz *et al.*, 1990, 1999; Chin-Leo and Benner, 1992; Gardner *et al.*, 1997). These blooms are supported by high riverine import of inorganic nutrients but are located downstream of the river mouth due to the high turbidity in the low-salinity region (Lohrenz *et al.*, 1990, 1999). Anthropogenic eutrophication of the Mississippi River, which drains ~41% of the continental USA, has increased the river nutrient load over the last 30 years (Turner and Rabalais, 1991; Justíc *et al.*, 1993, 1995) and promotes extensive bottom-water hypoxia during summer (Pakulski *et al.*, 1995).

Protozoan plankton, comprising heterotrophic nanoflagellates (HNF), ciliates and heterotrophic dinoflagellates, form an important component of secondary producers in marine ecosystems (Reckermann and Veldhuis, 1997; Brown *et al.*, 2002) and are responsible for the consumption of large amounts of primary production (Sherr and Sherr, 1994). Mesozooplankton can be a significant source of mortality for estuarine phytoplankton (Dam and Peterson, 1993), but most studies indicate protozoan microzooplankton as the major consumers of estuarine primary production (McManus and Ederington-Cantrell, 1992; Gallegos *et al.*, 1996).

Mississippi River water also imports high amounts of dissolved organic matter (DOM) into the coastal marine

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system, which in turn supports substantial bacterioplankton populations and production (Chin-Leo and Benner, 1992; Gardner *et al.*, 1994; Amon and Benner, 1998). Protozoan plankton, predominantly HNF, are the major consumers of bacteria (Fenchel, 1986; Kuipers and Witte, 2000; Sakka *et al.*, 2000) and form an important link in channeling bacterial production to higher trophic levels (Stoecker and Capuzzo, 1990; Sanders *et al.*, 1992).

Whereas the predominant role of protozoan grazing on phytoplankton has been well established (Dagg and Ortner, 1992; Fahnenstiel *et al.*, 1992, 1995; Dagg, 1995; Strom and Strom, 1996), grazing losses of specific pico- and nanoplankton groups (i.e. eukaryotes, *Synechococcus* spp., *Prochlorococcus* spp., heterotrophic bacteria) and their relative significance as protozoan food source have not been addressed in the Mississippi River plume. Analysis of serial dilution grazing experiments (Landry and Hassett, 1982) by flow cytometry allows differentiation of the role of these microbial populations (Reckermann and Veldhuis, 1997; Brown *et al.*, 1999, 2002; Kuipers and Witte, 1999).

METHOD

Grazing rates of heterotrophic bacteria and phototrophic picoplankton were assessed by serial dilution experiments at nine stations along a salinity gradient (0.2–34.4) in the Mississippi River plume and on the outer Louisiana shelf in May 2000 (Figure 1). Temperature and salinity were recorded by a Sea-Bird 911-Plus CTD equipped with a Sea Tec FL0500 fluorometer for *in situ* chlorophyll (Chl) fluorescence. Fluorescence values were calibrated against spectrophotometrically measured Chl *a* to convert *in situ* fluorescence to Chl *a* concentrations. Nutrient analyses (nitrate, nitrite, ammonium) were performed in the laboratory on deep-frozen water samples on a Lachat QuickChem autoanalyzer.

Surface samples for grazing experiments were taken by CTD-mounted Niskin-type water bottles. Five nominal dilutions (100, 80, 60, 40 and 20% of original sample) were prepared in triplicates with 0.2-µm-filtered sea water from the sampling site. Dilutions were incubated for 24 h in Corning 25 cm^2 polystyrene culture flasks (Corning Costar Corp., Cambridge, MA) submerged in a sea water-containing deck incubator with blue-shaded Plexiglas walls to simulate the sunlight spectrum and cooled to ambient water temperature by a microprocessor-controlled Neslab RTE bath/circulator cooler (Neslab Instruments, Newington, NH). Subsamples of 10 ml were taken immediately prior to and at the end of incubations, fixed by 1% (final concentration) formaldehyde and stored at 4°C in the dark until analyses. Growth and grazing rates were computed from linear regressions of apparent growth rates ($\ln[N_t/N_0]$) versus true dilutions as

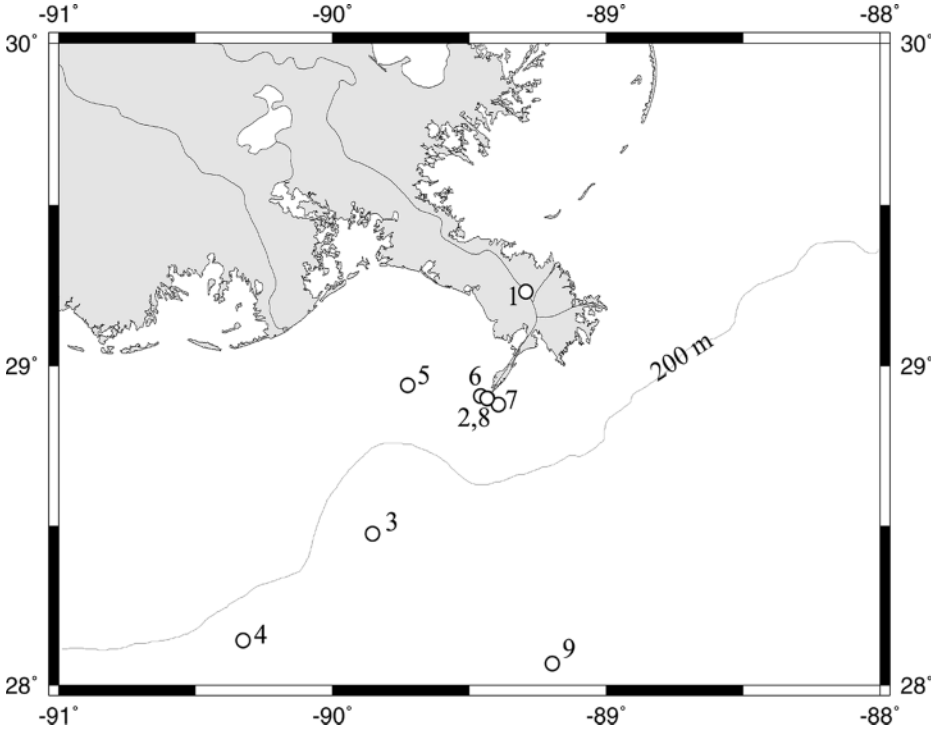

Fig. 1. Location of sampling stations in the Mississippi River plume and on the Louisiana shelf, Gulf of Mexico.

derived from cell abundance in dilutions compared with the original sample (Figure 2).

Both heterotrophic bacteria and phototrophic pico- and nanoplankton (*Prochlorococcus* spp., *Synechococcus* spp., small eukaryotic algae) were counted by flow cytometry. Phytoplankton were counted directly from fixed, unstained samples. For bacterial counts, samples were incubated for 30 min at 37°C with 0.1 g l^{-1} RNAse (1:1 mix of RNAse A and B) prior to staining with SYBR Green I (10^{-5} dilution of commercial stock; Molecular Probes, Eugene, OR) 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} for bacteria and 1 µl s^{-1} for phytoplankton. The counting rate was maintained at <500 cells s^{-1}; bacterial samples that were too dense were diluted 1:10 with 0.2-µm-filtered seawater. The measured sample volume for estimates of cells per milliliter was calculated from measurement times (60–300 s) based on weight calibration of flow rates.

The different subpopulations of phytoplankton were distinguished by their autofluorescence of Chl (FL3, >650 nm) and phycoerythrin (in the case of *Synechococcus* spp.; FL2, 585 ± 15 nm) and their side-angle light scatter (SSC) as a proxy of cell size (Troussellier *et al.*, 1999). Owing to its broad emission spectrum, SYBR DNA-fluorescence was detected both in the green FL1 (535 ± 15 nm) and the red FL3 (>650 nm) fluorescence channels. SSC served as a proxy for bacterial cell size. All signals were recorded on a four-decades log scale. Data were analyzed by PC Lysys software (Becton-Dickinson, San José, CA). In near-surface samples, where *Prochlorococcus* spp. and bacteria were not separated in the cytometric analyses, bacteria measurements were corrected for *Prochlorococcus* counts from unstained fixed samples measured on the same day.

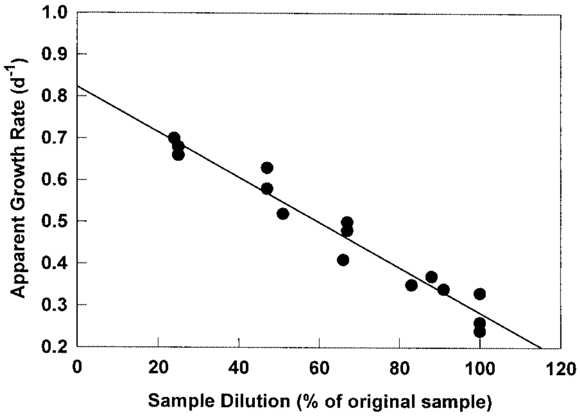

Fig. 2. Example result of serial dilution experiment (station 2, eukaryotes). Apparent growth rates ($\ln[N_t/N_0]$, where N_0 and N_t are abundance at time zero and after 24 h) are plotted versus true sample dilutions. Negative slope represents grazing rate (day^{-1}) and intercept growth rate (day^{-1}). All dilutions were incubated in triplicates.

HNF were counted on black 0.8-µm-pore-size Nuclepore filters after DAPI (4′,6-diamidino-2-phenylindole) 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 computed as log(volume) × 0.939 – 0.665 (Menden-Deuer and Lessard, 2000). Pico- and nanoplankton biomass was computed from cytometric cell counts and conversion factors of 60 fg C $cell^{-1}$ for *Prochlorococcus* (Charpy and Blanchot, 1998), 200 fg C $cell^{-1}$ for *Synechococcus* (Charpy and Blanchot, 1998; Liu *et al.*, 1999) and 4000 fg C $cell^{-1}$ for small eukaryotic algae (Montagnes and Franklin, 2001). Bacterial cell counts were converted to carbon biomass using the conversion factor $1.042 \times V^{0.59}$ (Simon and Azam, 1989), assuming an average cell volume of 0.04 μm^3 (Jochem, 2001).

RESULTS

Owing to extreme drought conditions in spring 2000, the Mississippi River plume in May 2000 extended substantially less than in years of normal river flow and the salinity gradient occurred within a relatively small distance (Figure 1). Nevertheless, the sampled stations comprised a full salinity gradient from 0.2 within the river mouth to >34 at the offshore stations. The river water introduced high nitrate loads (77 µmol l^{-1}) into the coastal waters, which decreased along the salinity gradient to below the detection limit at >28. The ammonium load of the Mississippi River water was moderate (0.34 µmol l^{-1}), and maximum concentrations of up to 1.5 µmol l^{-1} occurred at a salinity of 8–14. Ammonium was below the detection limit at salinities 28. Nitrite concentrations were highest (0.6–0.8 µmol l^{-1}) at mid-salinities (15–24) and still measurable (~0.25 µmol l^{-1}) at the full-salinity, offshore stations. Total dissolved inorganic nitrogen (DIN) decreased from 77.3 µmol l^{-1} at the river mouth to 0.3 µmol l^{-1} at salinities of >28 (Figure 3).

Phytoplankton standing stocks (Chl *a*) were high in the low- and mid-salinity range (20–30 µg l^{-1}), exhibiting maximum concentrations between 8 and 24, and rapidly decreasing to 1.4–4.5 µg l^{-1} at salinities >30 (Figure 3). Abundance of small eukaryotic phytoplankton was highest at low-salinity stations with a maximum of 22×10^3 cells ml^{-1} at a salinity of 8.5 and decreased to $0.2–1.0 \times 10^3$ cells ml^{-1} at full salinities (Figure 4a). *Synechococcus* spp. were also most abundant in the low-salinity area, reaching a maximum of 30.6×10^3 cells ml^{-1} at a salinity of 14.7; they occurred at ~10^3 cells ml^{-1} at the river mouth and in

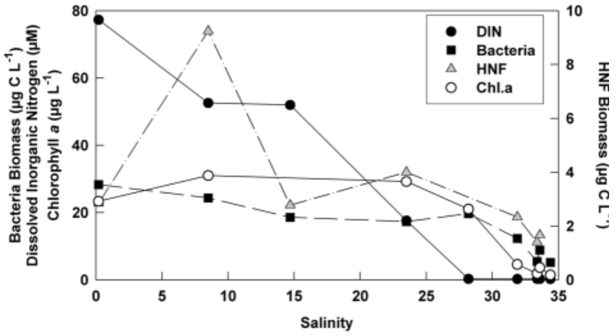

Fig. 3. Dissolved inorganic nitrogen (DIN; µmol l^{-1}), chlorophyll *a* (Chl *a*; µg l^{-1}) and bacterial and HNF biomass (µg C l^{-1}) along the salinity gradient.

the >28 region but at only 30 cells ml^{-1} at the 34.4 salinity station. *Prochlorococcus* spp. were absent at salinities <23. Their abundance decreased from 43.5×10^3 cells ml^{-1} at a salinity of 23.5 to $0.6–1.3 \times 10^3$ cells ml^{-1} at full salinities but 18.1×10^3 cells ml^{-1} at the 33.6 salinity station (Figure 4a).

Eukaryotes were the dominant pico- and nanophytoplankton biomass fraction at all stations, reaching up to 88 µg C l^{-1} at a salinity of 8.5 (Figure 4b). *Synechococcus* spp. reached up to 6.1 µg C l^{-1} at their highest abundance at a salinity of 14.5, but even here remained a small fraction compared with the eukaryotic biomass of 32.4 µg C l^{-1} and only 16% of the total pico- and nanophytoplankton biomass. At the river mouth and high-salinity stations, picocyanobacterial biomass amounted to 0.14–0.81 µg C l^{-1} and presented 0.5–14% of total pico- and nanophytoplankton biomass. Despite their high numerical abundance, *Prochlorococcus* spp. remained a small fraction of the pico- and nanophytoplankton biomass at all stations, providing up to 2.6 µg C l^{-1} and 3–24% of total biomass (Figure 4b).

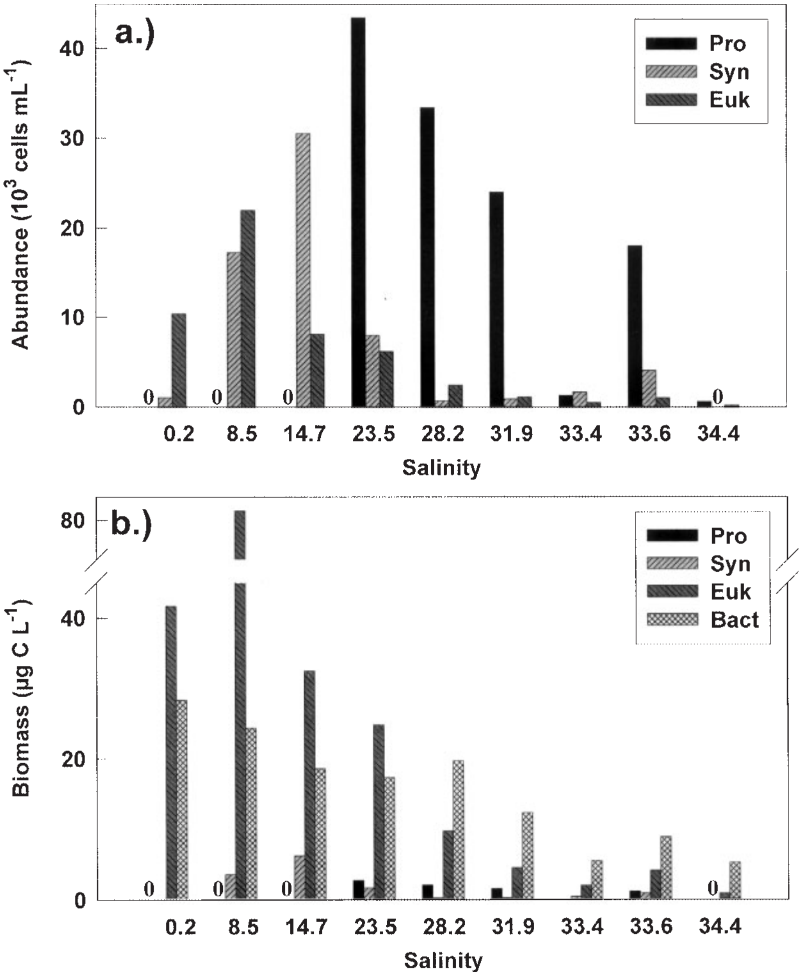

Fig. 4. Abundance (**a**; 10^3 cells ml^{-1}) and biomass (**b**; µg C l^{-1}) of pico- and nanoplankton along the salinity gradient. Zeros indicate absence of *Prochlorococcus* spp., or of *Synechococcus* spp. at a salinity of 34.4.

Bacterial biomass decreased along the salinity gradient from 28.3 μg C l^{-1} at the river mouth to 5.1 μg C l^{-1} at a salinity of 34.4 (Figure 3), corresponding to a decrease in cell numbers from 2.0×10^6 cells ml^{-1} at the river mouth to 0.4×10^6 cells ml^{-1} at offshore stations (Jochem, 2001). At salinities >28, bacterial biomass exceeded pico- and nanophytoplankton biomass (Figure 4b).

HNF occurred at 1.4–2.7×10^3 cells ml^{-1}. HNF biomass amounted to 2.8–4.0 μg C l^{-1} in the low- and mid-salinity range, except for a pronounced maximum of 9.2 μg C l^{-1} at the 8.5 salinity station and 1.4–2.3 μg C l^{-1} at full-salinity stations (Figure 3). HNF biomass increased with bacterial biomass (Figure 5a), but was correlated more strongly to pico- and nanophytoplankton biomass (Figure 5b). HNF in the 4.0–5.7 μm size class were most abundant at most stations, but the biomass of larger HNF was higher in the Chl maximum region and at the 31.9 salinity station (Figure 6). Most notable was the 'bloom' of >8 μm HNF of 5.1 μg C l^{-1} at the 8.5 salinity station, where they contributed 55% of the total HNF biomass. HNF in the 1.4–2.0 μm size class remained insignificant at all stations (0.6–5.7% of total HNF biomass), and no HNF <1.4 μm were detected.

Grazing rates on pico- and nanophytoplankton increased along the salinity gradient, often exceeding 1 day^{-1} at the high-salinity stations (Figure 7a). *Synechococcus* spp. was grazed more than other phytoplankton groups at low and high salinities (except at the 34.4 salinity station). Grazing of *Synechococcus* was highest at the lower end of the plume; low grazing rates at a salinity of 34.4 are probably related to low *Synechococcus* abundance, which stimulated grazing on larger food items. Grazing of phototrophic eukaryotes was highest in waters of a salinity of >23 with extreme grazing pressure at the highest salinities. Grazing pressure on eukaryotes in the region of their biomass maximum at low salinities was only moderate (0.5 day^{-1}). *Prochlorococcus* spp. grazing rates were near or above 1 day^{-1} and highest at the 34.4 salinity

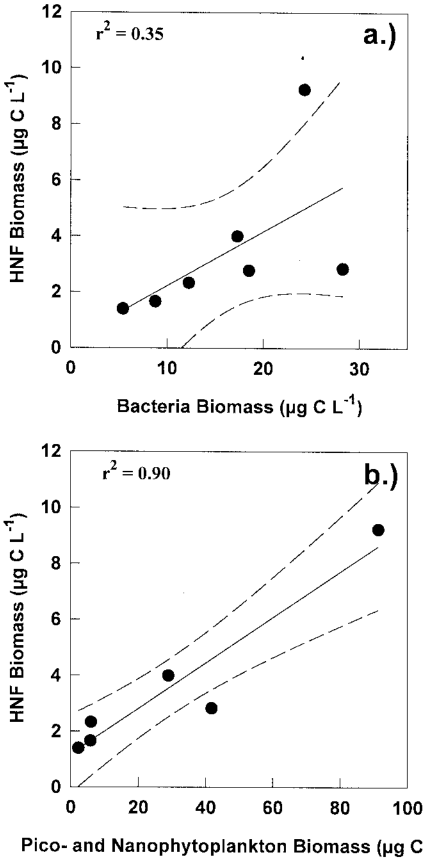

Fig. 5. Relationship between HNF biomass and (**a**) bacterial and (**b**) pico- and nanophytoplankton biomass (μg C l^{-1}).

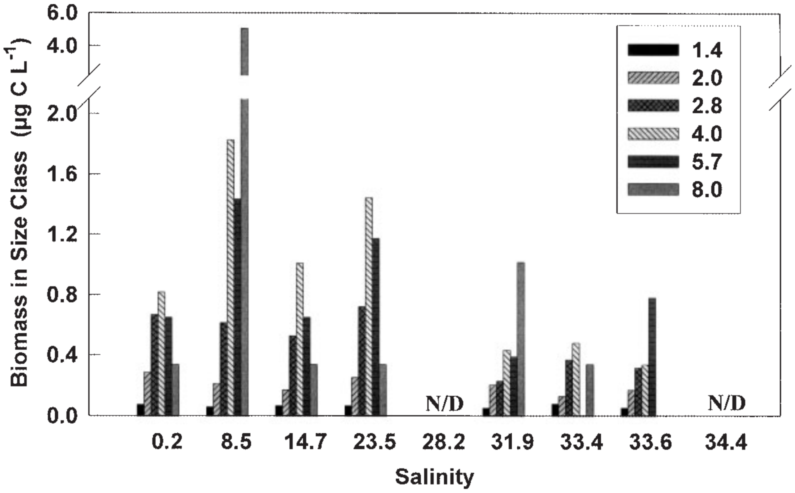

Fig. 6. Biomass of HNF (μg C l^{-1}) in size classes listed in micrometers. No data (N/D) are available for the 28.2 and 34.4 salinity stations.

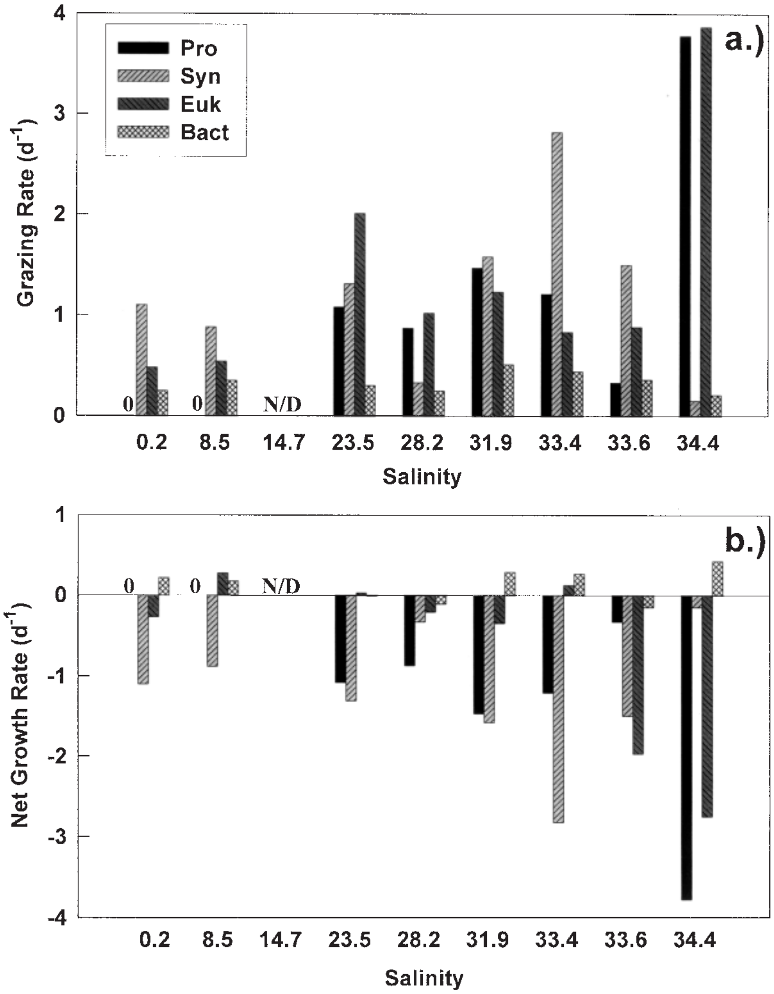

Fig. 7. (**a**) Grazing rates (day^{-1}) on and (**b**) net population growth rates (day^{-1}) of pico- and nanophytoplankton and bacterioplankton along the salinity gradient. N/D, no data. Zeros indicate the absence of *Prochlorococcus* spp.

station. Grazing rates on bacteria (0.21–0.51 day^{-1}) were less variable and lower than phytoplankton grazing rates (Figure 7a).

Computing population net growth rates from grazing and growth rates revealed a net loss of all phytoplankton populations at most stations (Figure 7b). The phototrophic prokaryotes had strongest net loss rates, which increased along the salinity gradient. *Synechococcus* spp. net growth rates amounted to –0.15 to –2.8 day^{-1}, and *Prochlorococcus* spp. net growth rates to –0.3 to –3.8 day^{-1}. Phototrophic eukaryotes gained a positive net growth of 0.3 day^{-1} in their biomass maximum at a salinity of 8.5, exhibited moderate loss rates of –0.1 to –0.3 day^{-1} at mid-salinities and high loss rates (–2.0 to –2.8 day^{-1}) at the highest salinities. Bacteria showed positive net growth rates of 0.2–0.4 day^{-1} at most stations (Figure 7b).

Phototrophic eukaryotes were the most important food source for microzooplankton in the low- and mid-salinity region (Figure 8). Grazed eukaryotic phytoplankton carbon in this region amounted to 20–50 μg C l^{-1} day^{-1}, presenting 48–200% of their standing stock and ~80% of the total consumed pico- and nanoplankton carbon. At high-salinity stations, grazed eukaryotic carbon amounted to 0.2–5.5 μg C l^{-1} day^{-1}, representing 83–390% of their standing stocks and ~30% of total consumed carbon. *Synechococcus* spp. grazed biomass constituted up to 3 μg C l^{-1} day^{-1} and contributed 0.3–5.2% to the total consumed pico- and nanoplankton carbon, but 26% at the 33.4 salinity station. Ingested *Prochlorococcus* spp. (0.1–2.8 μg C l^{-1} day^{-1}) accounted for 2.6–15% of total carbon ingestion. For both *Synechococcus* spp. and *Prochlorococcus* spp., the daily carbon consumption represented >100% of their standing stocks. Bacterial carbon grazed (1.1–8.5 μg C l^{-1} day^{-1}) represented 21–51% of their standing stocks and 9–48% of the total consumed pico- and nanoplankton carbon.

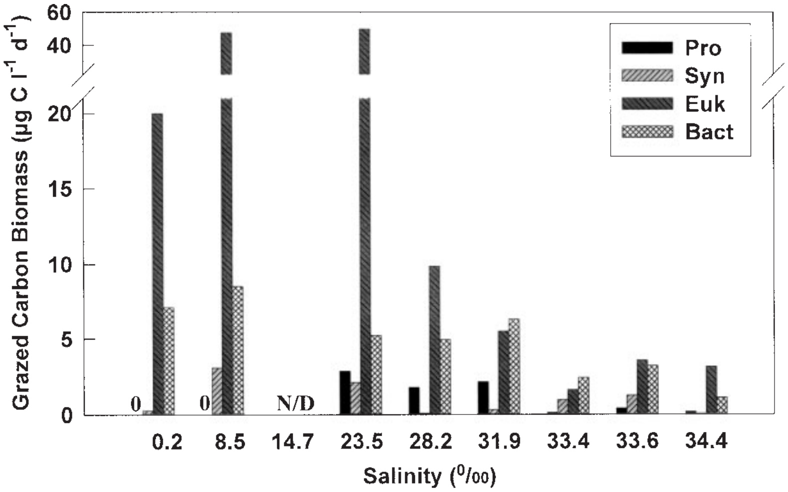

Fig. 8. Phytoplankton and bacterial carbon biomass consumed by microzooplankton (µg C l^{-1} day^{-1}) along the salinity gradient. Zeros indicate absence of *Prochlorococcus* spp. N/D, no data.

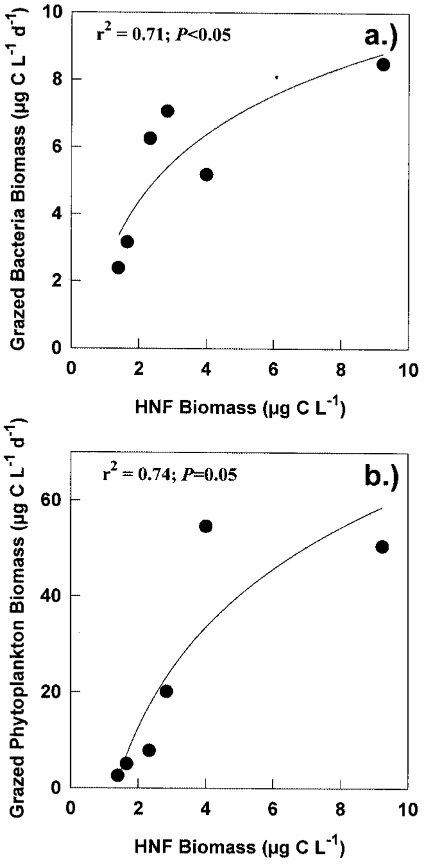

Fig. 9. Relationship of consumed (**a**) bacterial biomass and (**b**) phytoplankton biomass (µg C l^{-1} day^{-1}) with the abundance of heterotrophic nanoflagellates (HNF; µg C l^{-1}). Logarithmic model I regressions indicated by lines.

Both bacterial and phytoplankton carbon consumed by microzooplankton followed HNF abundance, but more in a logarithmic than linear fashion (Figure 9).

DISCUSSION

Since the aim of this study was directed towards impacts of the Mississippi River loading on microbial abundances and food web interactions, sampling was restricted to the surface layer (sample depth of 1 m). In offshore, deep waters of the northern Gulf of Mexico, subsurface Chl maxima occur (Jochem, 2001) and may host different assemblages of phytoplankton and bacteria exhibiting different food web interactions. Stations of a salinity of ˇ28.2 were located over water depths of <6 m, and station 5 (a salinity of 31.9) over 22 m water depth, so that surface layer sampling may be considered representative for the shallow-water communities and processes except those directly at the sediment–water interface. Stations 3, 4 and 9 (a salinity of >33) were located over deep water (>200 m), and their subsurface populations may have exhibited different process rates and abundances. Therefore, results presented in this study should be seen as only representing surface layer populations directly affected by river outflow.

Abundance and distribution of microorganisms

Estuarine areas accumulate phytoplankton at salinity fronts (Dagg *et al.*, 2003). The Chl maximum at intermediate salinities is consistent with previous reports from the Mississippi River plume (Lohrenz *et al.*, 1990, 1999; Pakulski *et al.*, 1995; Gardner *et al.*, 1997). Maximum phytoplankton biomass at mid-salinities in the presence of high nutrient concentrations in the river mouth has been explained by light limitation of primary production due to high water turbidity (Lohrenz *et al.*, 1990, 1999). The phytoplankton maximum at a salinity of 8–25 was located further upstream of the plume as compared with previous studies (Lohrenz *et al.*, 1999). With 9000 $m^3 s^{-1}$ compared with an average of 19 000 $m^3 s^{-1}$ (Hernes and Benner, 2003), the river outflow in May 2000 amounted to only 47% of the long-term average, and the region of the Chl maximum seems to shift upstream in years of low river flow (Gardner *et al.*, 1997). The low river flow did not affect maximum Chl concentrations (~30 $\mu g\ l^{-1}$), which were within the previously reported range of 20–40 $\mu g\ l^{-1}$ (Gardner *et al.*, 1997; Lohrenz *et al.*, 1999), although lower concentrations (<10 $\mu g\ l^{-1}$) were reported from certain years (Lohrenz *et al.*, 1999; Pakulski *et al.*, 2000). River flow may, however, have affected total phytoplankton biomass on the Louisiana shelf since maximum Chl concentrations were limited to a much smaller regional extent than in normal river flow years.

Large phytoplankton, namely diatoms, sometimes dominate in the high-phytoplankton-biomass region at mid-salinities (Fahnenstiel *et al.*, 1995; Lohrenz *et al.*, 1999), but studies on the distribution of phototrophic pico- and nanoplankton have been lacking for the Mississippi River plume. Pico- and nanophytoplankton also exhibited highest abundances in the high-phytoplankton-biomass region. Assuming a C:Chl ratio of 50, as has been applied to Mississippi River plume and northern Gulf of Mexico estuarine areas (Dagg, 1995; Lehrter *et al.*, 1999; Mortazavi *et al.*, 2000), the maximum abundance of pico- and nanophytoplankton of 91 $\mu g\ C\ l^{-1}$ converts to 1.82 μg Chl *a* l^{-1}, a contribution of only 6% to total Chl, which indicates a predominance of microphytoplankton in the high-Chl region during this study.

The cytometric analyses of the grazing experiments, which are inherently limited to small cells, may have missed the consumption of larger phytoplankton in the high-Chl region of the Mississippi River plume. However, they provide insight into the little studied dynamics of the plume microbial food web, and grazing of larger phytoplankton (diatoms) is largely restricted to the classical phytoplankton–mesozooplankton food chain. At high-salinity, offshore stations, pico- and nanoplankton are a major fraction of the phytoplankton. The <2 µm size class contributed 50–80% of total Chl *a* in the open northern Gulf of Mexico (F. J. Jochem, unpublished data), and phytoplankton <8 µm in size contributed 95–97% of total Chl at 'fully oligotrophic' stations in the lower end of the Mississippi River plume (Strom and Strom, 1996). However, the pico- and nanophytoplankton biomass of 0.8–6.1 $\mu g\ C\ l^{-1}$, equivalent to 0.02–0.12 μg Chl *a* l^{-1} assuming a C:Chl ratio of 50, would constitute a fraction of total Chl not higher than in the Chl maximum area of the plume. Chlorophyll concentrations at full-salinity stations in the lower plume in May 2000 (1.4–3.7 $\mu g\ l^{-1}$) were >10 times higher than reported from the lower plume in normal river flow years [0.015–0.2 $\mu g\ l^{-1}$; (Pakulski *et al.*, 1995; Strom and Strom, 1996)]. It seems that despite high salinities, environmental conditions in the highly condensed plume in the spring drought of 2000 at these stations did not reflect true oligotrophic, open-ocean characteristics and limited the role of small phytoplankton. These conditions may have caused the low or negative growth rates of pico- and nanophytoplankton (see below), which are best adapted to true oligotrophic conditions, and may have been more favorable for larger phytoplankton.

Nevertheless, the abundance of phototrophic eukaryotes and *Synechococcus* spp. at the lower end of the plume was comparable to open Gulf of Mexico waters (F. J. Jochem, unpublished data) and other oligotrophic, warm-water systems (Partensky *et al.*, 1999). The absence of *Prochlorococcus* spp. at salinities <23 is consistent with these organisms being restricted to full-salinity, oligotrophic waters, and cell concentrations were below those found in the open Gulf of Mexico and other warm-water oceans (Partensky *et al.*, 1999; F. J. Jochem, unpublished data). The abundance of HNF was within the range reported from other marine studies (Fenchel, 1986; Sorokin, 1999). Their spatial distribution did not reflect the variation in Chl, but the pronounced peak at the 8.5 salinity station can be related to the biomass maximum of eukaryotic nanophytoplankton.

Bacterial abundances were in the lower range of previous reports from the Mississippi River plume (Chen-Leo and Benner, 1992; Gardner *et al.*, 1994; Amon and Benner, 1998), which may be related to the low river flow and concomitant low import of dissolved organic carbon (DOC) substrates. DOC concentrations at the river mouth of 268 μmol^{-1} (Hernes and Benner, 2003) were ~20% below concentrations found during four studies between July 1990 and 1993 (Benner and Opsahl, 2001). Bacterial abundance and production are closely coupled in time and space to phytoplankton biomass and productivity in the Mississippi River plume (Amon and Benner, 1998) and other marine systems (Cole *et al.*, 1988; Gasol and Duarte, 2000). In May 2000, bacterial abundance at mid-salinities was not enhanced, but decreased from the river mouth towards offshore. At salinities above 30, bacterial standing stocks fell markedly along with lower phytoplankton biomass, suggesting that the general relationship of bacterial abundance to phytoplankton abundance held for the offshore regions, but that other factors controlled the distribution of bacteria at low and mid salinities. In estuaries, riverine input of DOM can enhance bacterial production in the absence of phytoplankton production and cause a decoupling of bacterial and phytoplankton production (Albright, 1983; Ducklow and Kirchman, 1983; Kirchman *et al.*, 1989). Decoupling of bacterial and primary production at low and mid salinities has also been documented for the Mississippi River plume (Chin-Leo and Benner, 1992).

Microbial growth and grazing rates

Phytoplankton >8 µm in size grew faster than cells <8 µm in size in the lower part of the Mississippi River plume (Strom and Strom, 1996). At the biomass maximum of eukaryotic nanoplankton (8.5 salinity station), 'small' and 'large' nanoplankton populations could be clearly distinguished in the cytometric analyses (data not shown). The growth rate for 'large' cells of 1.60 day^{-1} was higher than that for 'small' cells (0.45 day^{-1}). The grazing rates on both populations did not differ greatly (0.44 and 0.58 day^{-1}), and 'large' cells showed a net growth rate of 1.16 day^{-1} while 'small' cells exhibited a negative net growth rate of –0.13 day^{-1}. High net growth rates of the larger nanophytoplankton seem to have sustained the eukaryotes' biomass maximum at this 8.5 salinity station.

The high growth rate of 'large' cells but the decline in 'small' cells is also consistent with the increased abundance and contribution of larger-sized HNF in this region. Growth rates of protozoa are often closely linked to the growth rates and abundance of their prey (Rivier *et al.*, 1985; Montagnes *et al.*, 1996; Weisse, 1997). A shift in the pico- and nanophytoplankton community towards larger cells is, therefore, most likely to cause a shift towards larger protozoa that are better suited to prey on larger cells. Increased abundance of larger protozoa will also increase the grazing pressure on smaller HNF and further decrease their abundance (Reckermann and Veldhuis, 1997).

Protists are effective consumers of pico- and nanoplankton (Hansen *et al.*, 1994; Verity *et al.*, 1996), and microzooplankton have the greatest grazing impact on phytoplankton in the Mississippi River plume (Dagg and Ortner, 1992; Fahnenstiel *et al.*, 1992; Dagg, 1995). HNF are the primary consumers of bacterioplankton (Fenchel, 1986; Kuipers and Witte, 2000; Sakka *et al.*, 2000), but they also feed on *Synechococcus* spp. (Caron *et al.*, 1991; Reckermann and Veldhuis, 1997) and small eukaryotic algae (Parslow *et al.*, 1986). However, HNF are not the only predators on pico- and nanoplankton as ciliates and phagotrophic dinoflagellates can exert a high grazing pressure as well (Rassoulzadegan *et al.*, 1988; Šimek *et al.*, 1996, 2000). Ciliates and phagotrophic dinoflagellates removed 80–230% of primary production in the lower, high-salinity Mississippi River plume in May 1993 (Strom and Strom, 1996). Since no data for ciliate distribution are available, the picture of the microbial food web in the Mississippi River plume must remain incomplete with respect to the larger microzooplankton for this study.

Grazing experiments revealed that protozoa exerted a high grazing pressure on pico- and nanophytoplankton, resulting in negative phytoplankton net growth rates at most stations. Grazing rates in serial dilution experiments may be biased if basic assumptions of this technique are violated. Nutrient limitation of phytoplankton growth, particularly in the most diluted bottles due to lower grazing and nutrient recycling, can cause grazing rates to be overestimated and phytoplankton growth rates to be underestimated (Landry and Hassett, 1982), which may account for the observed negative phytoplankton net growth rates. Nutrient limitation is a likely factor at the high-salinity, oligotrophic stations. Nutrient limitation occurred in grazing experiments performed in the lower part of the Mississippi River plume at salinities >32 (Strom and Strom, 1996). High concentrations of DIN make nutrient limitation unlikely in the low- and mid-salinity region, however, unless phosphate limitation occurred. Although nitrogen limitation has been suggested as controlling primary production in shelf waters near the Mississippi River plume (Sklar and Turner, 1981), phosphate limitation has been evidenced for the upper part of the plume (Smith and Hitchcock, 1994; Lohrenz *et al.*, 1999), but inorganic phosphate is rapidly recycled in this region (Ammerman and Glover, 2000). Long-term trends in the relative nutrient composition of the Mississippi River water (Justíc *et al.*, 1993, 1995) suggest that nutrient input ratios have approached Redfield values, leading to ambiguity and temporal variability as to which nutrient potentially limits productivity (Lohrenz *et al.*, 1999).

Grazing rates of >1 day^{-1} are well within the capabilities of protozoan grazers (Murrell and Hollibaugh, 1998; Garcia Pamanes and Lara Lara, 2001). Although grazing and growth rates of phytoplankton are often closely coupled, especially when grazing rates approach or exceed 1 day^{-1} (McManus and Ederington-Cantrell, 1982; Wikner and Hagström, 1988; Reckerman and Veldhuis, 1997; Brown *et al.*, 2002), daily phytoplankton grazing losses of >100% of their standing stocks do occur occasionally (Fahnenstiel *et al.*, 1995; Gallegos *et al.*, 1996). Protozoan grazing rates increase with prey abundance (Brown *et al.*, 2002), and high grazing rates on high phytoplankton biomass can persist even after the phytoplankton specific production decreases due to physiological limitations of phytoplankton growth.

The serial dilution approach has not been used often to assess growth and grazing rates of heterotrophic bacterioplankton. The experiments in the Mississippi River plume indicated that bacteria had a positive net growth of ~0.3 day^{-1}. High ambient nutrient concentrations and import of DOC substrates by the river water may prevent substrate limitation of bacteria in nature and in the dilution experiments in this region. Bacteria may also depend less on protozoan nutrient recycling in the incubation bottles, and potential exudate production by phytoplankton was included in the incubations. Carbon enrichment effects in grazing experiments have been shown to be negligible (Tremaine and Mills, 1987), and bacterial production estimates from serial dilution experiments were consistent with those from [^{3}H]thymidine incubations (Brown *et al.*, 2002).

Bacterial production, based on their growth rate and abundance ($B_0 \times e^{\mu} - B_0$), of 2.1–17.0 μg C l^{-1} day^{-1} was at the lower range of previous reports (Chin-Leo and Benner, 1992), which can be related to the lower river flow and lower DOC input as compared with high-river-flow years. If HNF are assumed to be the primary consumers of bacteria (Sakka *et al.*, 2000), HNF ingestion rates, based on HNF abundance and estimated grazing rates, amounted to 5–13 bacteria HNF^{-1} h^{-1}, which is within the range of HNF ingestion rates reported from field studies (Bjørnsen, 1988; Šimek *et al.*, 1990; Weisse and Scheffel-Möser, 1991; Wieltschnig *et al.*, 1999; Cleven and Weisse, 2001). Because of their size being similar to bacteria, *Prochlorococcus* spp. probably present another food source for HNF (Kuipers and Witte, 2000). Including *Prochlorococcus* spp. as HNF prey does not change the estimated ingestion rates greatly (5–14 cells HNF^{-1} h^{-1}).

Low and negative net pico- and nanophytoplankton growth rates in the upper plume may also have been related to limitation of photosynthesis by extreme water turbidity (Lohrenz *et al.*, 1990, 1999). Photosynthetically active radiation at 1 m water depth was <200 μmol m^{-2} s^{-1} at salinities <28, but increased up to 1200 μmol m^{-2} s^{-1} at higher salinities (Hernes and Benner, 2003). The Chl maximum at mid-salinities may not have been the result of active *in situ* algal growth but due to advection leading to 'packing' of phytoplankton at density fronts (Franks, 1992). Periodic advance and relaxation of the plume front over time scales of 2–6 h have been reported (Govoni and Grimes, 1992). Such processes may gain higher importance in the spatial concentration or dispersal of plankton during times of small plume extent. Low or negative phytoplankton growth rates in this region may reflect poor physiological status of cells and severe light limitation of photosynthesis in organisms usually acclimated to high photon flux densities. Owing to hydrographic forces, peak biomass in estuaries may not be observed where local conditions are most favorable to phytoplankton production, and inherently unproductive areas may be regions of high biomass accumulation (Lucas *et al.*, 1999). The peak of eukaryotic phytoplankton in the low-salinity area was, however, supported by active algal growth, exhibiting positive growth rates and positive net growth rates, particularly in the larger nanophytoplankton (see above).

Protozoan food preference

Protozoa consumed more phytoplankton carbon than bacterial carbon. The predominance of phytoplankton carbon in the protozoan diet was not only related to the higher availability of phytoplankton carbon, but specific grazing rates on phytoplankton were also higher than on bacteria. Both the ingested phytoplankton and bacterial biomass increased with HNF abundance, but in a logarithmic rather than a linear way, suggesting that HNF grazing was prey limited at high HNF abundance (e.g. at the 8.5 salinity station).

Eukaryotic phytoplankton were by far the most important protozoan food source throughout the Mississippi River plume. At higher salinities, bacteria also became important as a food source (25–48% of the total ingested pico- and nanoplankton carbon). The higher importance of bacteria at the offshore stations was due to the decrease in eukaryotic phytoplankton biomass, and consumed bacterial carbon in absolute terms (μg C) was higher at low and mid salinities than offshore. Despite their high numerical abundance, *Prochlorococcus* spp. and *Synechococcus* spp. did not present a major food source for protozoa. Comparing the share of distinguished pico- and nanoplankton prey populations of total standing stocks to their share of the total ingested carbon (Figure 10) reveals that grazing relative to their abundance was not equal among groups. Eukaryotes were always ingested preferentially, and bacteria were consumed less than other prey groups. *Prochlorococcus* spp. and *Synechococcus* spp. seem to be favorable prey (steeper slope in Figure 10), but their significance in protozoan nutrition in the Mississippi River plume was restricted by their low available biomass.

Protozoa exhibit prey preferences when given the choice. Most ciliates prefer larger cells (algae) to bacteria (Sherr and Sherr, 1991; Kivi and Setälä, 1995), whereas nanoflagellates prefer smaller prey (Sherr and Sherr, 1991). Within restricted size ranges, large prey is mostly grazed more than small prey (Gonzalez *et al.*, 1990; Premke and Arndt, 2000). But size selection alone cannot fully explain the prey preference in protozoa (Monger and Landry, 1991; Hall *et al.*, 1993). Chemosensory abilities of protozoa are known, and heterotrophic flagellates can discriminate fluorescently labeled from unlabeled or live from dead prey (Nygaard *et al.*, 1988;

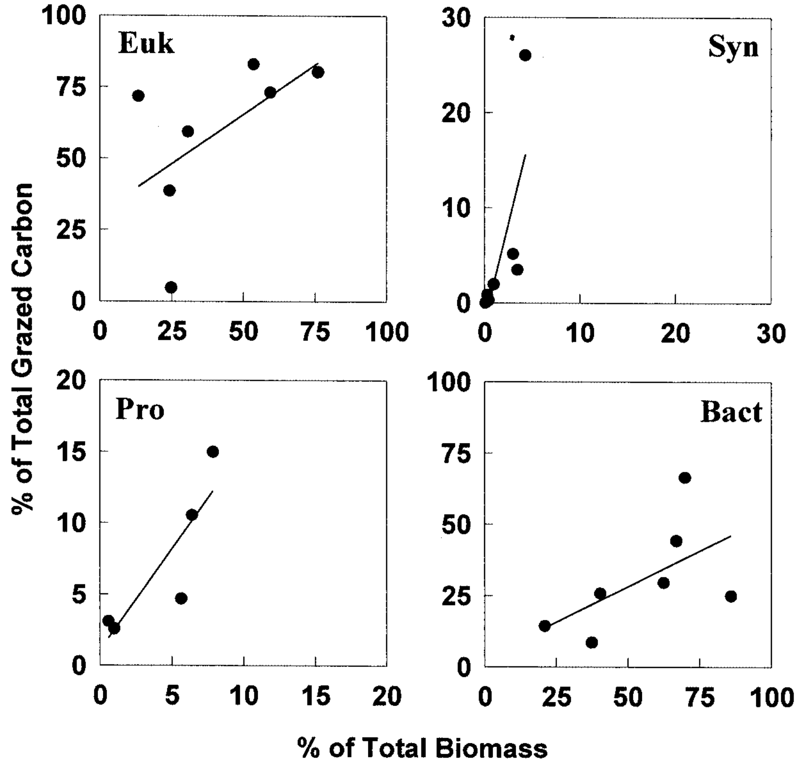

Fig. 10. Relationship of the percent of picoplankton groups of total consumed carbon biomass with their percent of the pico- and nanoplankton biomass.

Nygaard and Hessen, 1990; Landry *et al.*, 1991; Mischke, 1994). Laboratory experiments revealed that certain phagotrophic dinoflagellates showed a preference for diatoms over dinoflagellates, but other species preferred dinoflagellates to diatoms (Naustvoll, 2000). Nanoflagellates and oligotrich ciliates preferred *Synechococcus* spp. to *Prochlorococcus* spp. (Christaki *et al.*, 1999; Monger *et al.*, 1999). HNF prefer pico- and nano-sized algae to bacteria (Mischke, 1994; Safi and Hall, 1999), but bacteria to *Synechococcus* spp. (Caron *et al.*, 1991). Cyanobacteria seem to be of poor nutritional value (Caron *et al.*, 1991; Schmidt and Jónasdóttir, 1997) and can pass through the digestive tracts of zooplankton undigested and alive (Pfannkuche and Lochte, 1993).

The preferential consumption of eukaryotic pico- and nanophytoplankton and the disproportionally low grazing on bacterioplankton in the Mississippi River plume are consistent with current knowledge on prey preferences among planktonic protozoa. Preferential grazing on active over inactive bacteria has been documented (Gonzalez *et al.*, 1990; Del Giorgio *et al.*, 1996), but bacterial production computed from growth rates consistent with previous reports of bacterial production in the Mississippi River plume (see above) and active bacterial remineralization of ammonium (W. S. Gardner and F. J. Jochem, unpublished data) suggest that the bacterioplankton community was active. The lack of prey preferences among pico- and nanoplanktonic prey in oligotrophic oceans (Reckermann and Veldhuis, 1997) may suggest that prey selection is more pronounced where food is plentiful, but grazing becomes indiscriminate when food resources are low.

CONCLUSIONS

Whereas eukaryotic nanophytoplankton showed active growth in the low-salinity region, pico- and nanophytoplankton growth was low or negative at mid and high salinities, and strong protozoan grazing impact further decreased populations. The high turbidity in the plume may have light-limited phytoplankton growth, particularly in the more oceanic phototrophic prokaryotes. The high Chl biomass extending through the low- and mid-salinity region may have resulted more from hydrographic pumping at salinity fronts in this small river plume rather than from *in situ* growth. Growth and distribution of larger phytoplankton were not considered in this study, however, and higher Chl concentrations in the mid-salinity region could have partly resulted from larger phytoplankton (Fahnenstiel *et al.*, 1995). Bacteria had low grazing losses, positive net growth rates and played a dominant role in nitrogen recycling (F. J. Jochem and W. S. Gardner, unpublished data). However, protistan grazers exerted a major grazing pressure on pico- and nanophytoplankton. The unusual hydrographic conditions during the extreme spring drought of 2000 may have affected the distribution and activity of the microbial food web in the river plume. Comparisons with normal river flow years and flood years are needed to clarify the effect of river discharge on the quantity and quality of microbial food webs in the river plume.

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