Marine Biology (2004) 145: 1213–1225
DOI 10.1007/s00227-004-1406-7

RESEARCH ARTICLE

F. J. Jochem · P. J. Lavrentyev · M. R. First

Growth and grazing rates of bacteria groups with different apparent DNA content in the Gulf of Mexico

Received: 25 November 2003 / Accepted: 25 April 2004 / Published online: 30 June 2004

Abstract Growth rates and grazing losses of bacterioplankton were assessed by serial dilution experiments in surface waters in the Mississippi River plume, the northern Gulf of Mexico, a Texas coastal lagoon (Laguna Madre), southeast Gulf of Mexico surface water, and the chlorophyll subsurface maximum layer in the southeast Gulf of Mexico. Bacteria were quantified by flow cytometry after DNA staining with SYBR Green, which allowed for discrimination of growth and grazing rates of four bacteria subpopulations distinguished by their apparent DNA content and cell size (light scatter signal). Total bacteria growth rates (0.2–0.9 day^{-1}) were mostly balanced by grazing losses, resulting in net growth rates of −0.18 to 0.45 day^{-1}. Growth rates of DNA subpopulations varied within experiments, sometimes substantially. In most, but not all, experiments, the largest bacteria with highest DNA content exhibited the highest growth rates, but a relationship between DNA content and growth rates or grazing losses was absent. Small bacteria with the lowest DNA content showed positive growth rates in most experiments, sometimes higher than growth rates of bacteria containing more DNA, and were grazed upon actively. Low-DNA bacteria were not inactive and were an integral part of the microbial food web.

Communicated by O. Kinne, Oldendorf/Luhe

F. J. Jochem (✉)
Marine Biology Program, Florida International University,
3000 NE 151 Street, North Miami, FL 33181, USA
E-mail: frank@jochemnet.de
Fax: +1-305-919-5896

P. J. Lavrentyev
Department of Biology, University of Akron,
Akron, OH 44325, USA

M. R. First
Department of Marine Sciences, University of Georgia,
Athens, GA 30606, USA

Introduction

Bacteria play a key role in planktonic marine microbial food webs. They constitute an important share of plankton biomass, and their activity impacts ecosystem metabolism and function. Bacteria are a major component in aquatic carbon cycling and other biogeochemical processes (Ducklow 2000). A contemporary challenge in microbial ecology is to understand the functional role of phylogenetically or otherwise defined bacterial populations in natural ecosystems.

Marine bacterioplankton studies have improved with the development of new techniques. Culture-independent molecular tools now allow assessment of bacterial diversity of heterogeneous, natural populations, a task previously hindered by the minor number of cultivable marine bacteria (Giovannoni and Rappé 2000). The introduction of flow cytometry into the analysis of planktonic bacteria (Li et al. 1995; Marie et al. 1997) made bacteria counts faster, easier, and more precise (by higher number of counted cells; Venrick 1978) and provided feasibility for experiments involving a high number of counting samples to be processed. Good correlation between cytometric and microscopic bacteria counts has been shown (e.g. Marie et al. 1997; Yanada et al. 2000; Jochem 2001; Vaqué et al. 2001).

With the introduction of cytometric bacteria counts, which rely on DNA staining, it became obvious that bacterioplankton communities are composed of groups with different apparent DNA content (Li et al. 1995; Marie et al. 1997). Depending on the system under study, two to four bacteria groups distinguished by their combination of DNA content and cell size (light scatter signal) could be differentiated (Gasol and del Giorgio 2000 and references therein). Whereas coastal and eutrophic samples tend to have two groups, up to nine DNA clusters have been distinguished in coastal Mediterranean waters (Troussellier et al. 1999).

The ecological significance of bacteria groups with different DNA content has been set into the context of

bacterial cellular activity in the oceans. Although the actual value may depend on the technique applied, only a variable but small fraction of planktonic bacteria (from as low as 2% to >90%, but usually <50%) appears to be metabolically active (Smith and del Giorgio 2003). Based on micro- and mesocosm studies that exhibited high growth rates of bacteria with high DNA content, particularly under grazer removal and substrate addition (Gasol et al. 1995; Yanada et al. 2000; Vaqué et al. 2001; Jacquet et al. 2002), it was concluded that high-DNA bacteria represent the metabolically active fraction of bacterioplankton, whereas low-DNA bacteria were considered inactive or dead (Gasol and del Giorgio 2000). This interpretation is supported by lower cell-specific substrate uptake rates in low-DNA bacteria (Jellett et al. 1996; Lebaron et al. 2002) and a closer correlation of total bacterial production to high-DNA bacteria abundance than to total bacteria (Vaqué et al. 2001). The ratio of high- to low-DNA bacteria was suggested as an "active cell index" of bacterioplankton (Jellett et al. 1996). However, recent studies have revealed that low-DNA bacteria are not inactive (Grégori et al. 2001; Zubkov et al. 2001) and may exhibit metabolic activities and growth rates similar to high-DNA bacteria (Zubkov et al. 2001).

In the northern Gulf of Mexico and the Mississippi River plume (Louisiana shelf), four bacteria DNA groups corresponding to previously described groups were found (Jochem 2001). The aim of this study was to assess bacterial growth rates and grazing losses in the Mississippi River plume, the northern Gulf of Mexico, a Texas coastal lagoon, and the southeast Gulf of Mexico. Growth rates and grazing losses of the four DNA groups were established to reveal the heterogeneity of bacterial growth rates within bacterioplankton communities, assess if low-DNA bacteria can be considered inactive cells, and evaluate the role of DNA groups in the microbial food web.

Materials and methods

Growth rates and grazing losses of bacteria were estimated from serial dilution experiments (Landry and Hassett 1982) performed with surface samples in the Mississippi River plume (stations MRP; 20–29 May 2000), northern Gulf of Mexico (GoM; 18 June 2001), lower Laguna Madre (LLM; 28 June 2001), southeast Gulf of Mexico (stations 1–5; 2–7 May 2002), and chlorophyll subsurface maximum layer in the southeast Gulf of Mexico (stations A–D; 2–5 August 2002; Fig. 1).

Water samples were taken by CTD-mounted, metal-free Niskin water bottles and diluted (1.0, 0.8, 0.6, 0.3, 0.15 fractions) with sample water previously filtered through 0.2-μm 47-mm polycarbonate filters (<250 mbar). In the Mississippi River plume, dilutions were incubated in 100-ml polystyrene culture flasks (Corning Costar Corp., Cambridge, Mass., USA) submerged in a seawater-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, N.H., USA). For GoM and LLM experiments, 500-ml polycarbonate bottles were used. Experiments in the southeast Gulf of Mexico were incubated in 100-ml polycarbonate bottles placed in a seawater-cooled deck incubator with ambient light intensity simulated by black mosquito net. Experiments in the southeast Gulf were amended with f/20 nutrients, those in the northeast Gulf and Laguna Madre with 4 μmol l^{-1} NH_4Cl. All dilutions were performed in triplicate. Ten-milliliter subsamples were ta-

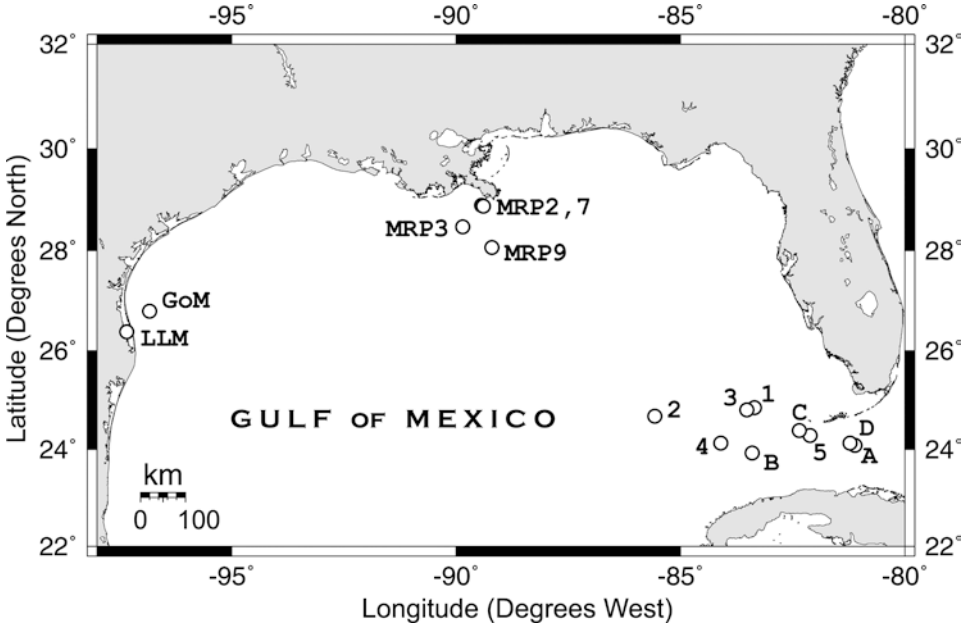

Fig. 1 Sampling stations in the Gulf of Mexico. *GoM* northern Gulf of Mexico, *LLM* lower Laguna Madre, *MRP* Mississippi River plume, *1–5* southeast Gulf of Mexico stations 1–5, *A–D* southeast Gulf of Mexico stations A–D

ken prior to incubation and after 24 h, fixed with 1% (final concentration) formaldehyde, and either stored at 4°C in the dark for up to 2 weeks (MRP, GoM, LLM) or frozen in liquid nitrogen and stored at –20°C (southeast Gulf of Mexico) until analysis.

Bacteria were quantified by flow cytometry after DNA staining (Marie et al. 1997; Jochem 2001). One milliliter of sample was incubated for 30 min at 37°C with 0.1 g l^{-1} RNAse (1:1 mix of RNAse A and B) before staining with SYBR Green I (10^{-5} dilution of commercial stock; Molecular Probes, Eugene, Ore., USA) in the presence of 30 m *M* potassium citrate. After 30 min of staining, samples were analyzed on a Becton Dickinson (San José, Calif., USA) FACSort flow cytometer at a sample flow rate of 0.2 µl s^{-1}. Measured sample volume was estimated from measurement time (50–300 s) based on repeated weight calibration of flow rates.

SYBR DNA fluorescence was detected in both the green FL1 (535 ± 15 nm) and the red FL3 (> 650 nm) fluorescence channels. Autofluorescence from cyanobacteria never inferred with SYBR Green fluorescence signals in the FL3 channel because chlorophyll fluorescence signals were always substantially higher than SYBR Green signals in this channel, and cyanobacteria were clearly separated from bacterial clusters. Side-angle light scatter (SSC) served as a proxy for bacterial cell size (Servais et al. 1999; Troussellier et al. 1999). All signals were recorded on a 4-decade log scale. Data were analyzed by WinMDI version 2.7 (Joseph Trotter, Scripps Research Institute, La Jolla, Calif.; freeware). DNA clusters of heterotrophic bacteria were counted by logical gating from both FL1 versus FL3 and SSC versus FL1 histograms, with gate borders set at the minima in frequency distributions of DNA fluorescence and light scatter and kept constant throughout experiment analysis (Fig. 2). Growth and grazing rates of bacteria were estimated from linear regressions of apparent growth rates (ln $[N_t/N_0]\times 1/t$ with N_0 and N_t as the initial and final abundance, respectively, and $t = 1$ day) versus sample dilution.

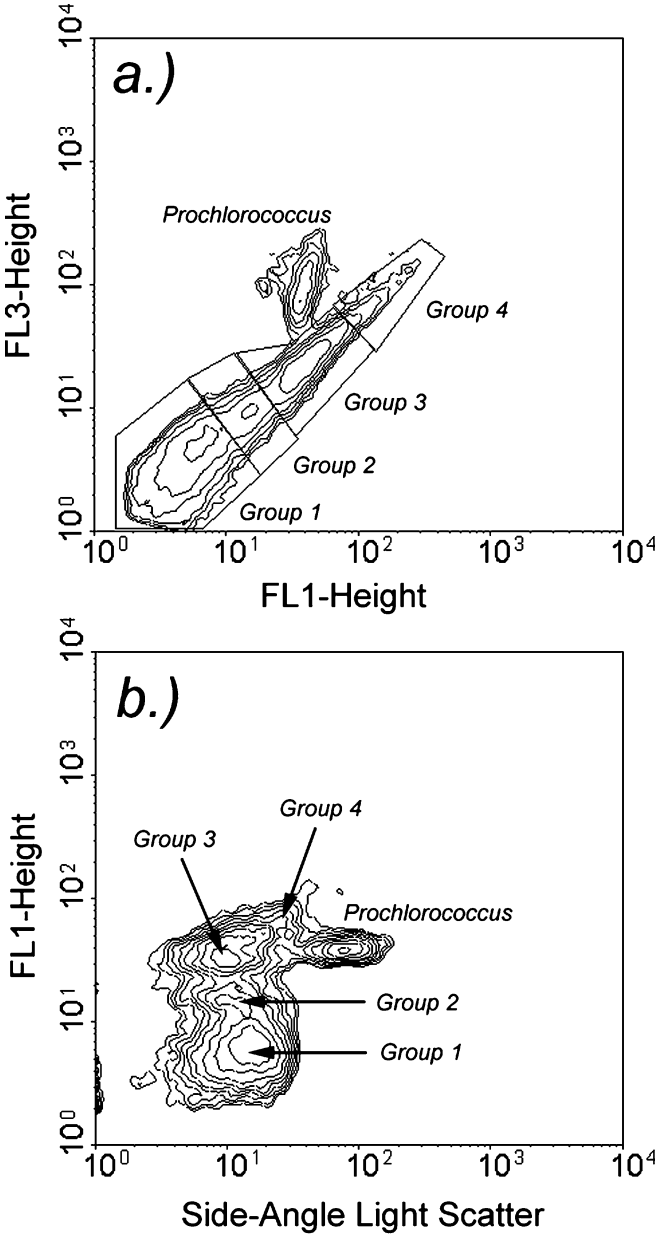

Fig. 2 Flow-cytometric signature of bacterioplankton in the northeast Gulf of Mexico. FL1 and FL3 (green and red fluorescence) both represent SYBR Green DNA fluorescence; side-angle light scatter is used as a proxy of cell size. *Prochlorococcus* are clearly separated from bacteria

Results

Table 1 summarizes locations, hydrographic conditions, phytoplankton chlorophyll concentrations, and bacteria abundances at the sampled stations. Both bacteria and phytoplankton abundance were highest ($1.2–1.5\times 10^6$ bacteria ml^{-1}; ca. 30 µg Chl. *a* l^{-1}) at low and mid-salinity stations in the Mississippi River plume. Dissolved inorganic nitrogen concentrations ($NH_4^+ + NO_3^- + NO_2^-$) decreased from 53 µ*M* at station MRP2 (8.5‰) to 18 µ*M* at station MRP7 (23.5‰) and were 0.27 µ*M* at the two full-salinity stations (Jochem 2001). High bacteria biomass and production in the Mississippi River plume has been related to riverine import of labile dissolved organic matter (Chin-Leo and Benner 1992). High bacterial abundance also occurred in the lower Laguna Madre (LLM), a shallow, coastal lagoon where NH_4^+ dominates inorganic nitrogen and phytoplankton often is restricted to the Texas Brown Tide pelagophyte *Aureoumbra lagunensis* and the coccoid cyanobacterium *Synechococcus* spp. All other stations exhibited low bacteria abundance ($2–4\times 10^5$ ml^{-1}) and phytoplankton biomass (< 0.5 µg Chl. *a* l^{-1}) typical for oligotrophic Gulf of Mexico waters. Except for LLM, bacteria abundance was correlated with phytoplankton abundance ($r^2 = 0.95$, $P < 0.0001$) as described in other marine systems (Gasol and Duarte 2000).

Table 1 Station locations and key environmental parameters

Station	Date	Latitude	Longitude	Sample depth (m)	Water depth (m)	Temp. °C	Salinity ‰	Chlorophyll a $\mu g\ l^{-1}$	Total bacteria $10^3\ ml^{-1}$
Mississippi River plume, May 2000									
MRP2	20 May	28°53.89′N	89°26.04′W	2	13	24.1	8.5	31.00	1561
MRP3	22 May	28°28.50′N	89°51.20′W	0	168	24.1	33.6	3.65	608
MRP7	27 May	28°52.79′N	89°23.62′W	2	8	26.5	23.5	29.20	1261
MRP9	29 May	28°04.04′N	89°11.80′W	0	1140	27.7	33.4	1.84	339
Coastal Texas samples, June 2001									
LLM	28 June	26°23.20′N	97°20.20′W	2		33.0	28.2	0.40	1202
GoM	18 June	26°48.00′N	96°50.00′W	8		29.0	33.0	0.20	299
SE Gulf of Mexico, Florida Strait, May 2002									
1	2 May	24°50.92′N	83°21.14′W	0	65	26.8	36.2	0.07	419
2	3 May	24°40.90′N	85°34.06′W	0	3780	27.6	36.3	0.10	415
3	5 May	24°48.39′N	83°31.42′W	0	65	27.4	36.2	0.08	383
4	6 May	24°07.81′N	84°05.87′W	0	2200	27.8	36.0	0.05	232
5	7 May	24°16.85′N	82°06.89′W	0	244	27.2	36.4	0.11	415
SE Gulf of Mexico, Florida Strait, August 2002[a]									
A	2 August	24°05.15′N	81°06.64′W	90	1025	26.5	36.5	0.22	199
B	3 August	23°55.76′N	83°23.96′W	66	1043	22.7	36.5	0.24	385
C	4 August	24°23.29′N	82°20.76′W	26	65	22.1	36.5	0.52	298
D	5 August	24°07.67′N	81°13.01′W	59	980	26.5	36.4	0.23	312

[a] All samples taken from depth of chlorophyll maximum

In all samples, four clusters of bacteria were distinguished by their apparent DNA content and cell size (light scatter; Fig. 2) as previously described for the Mississippi River plume and the northern Gulf of Mexico (Jochem 2001). Group 1 is assigned to bacteria with low light scatter (cell size) and lowest DNA content. Group 2 bacteria exhibited similar light scatter to group 1 but a higher DNA content. While group 3 bacteria, clearly distinguished from group 2 by their DNA fluorescence, exhibited similar light scatter signals to groups 1 and 2 bacteria in the Mississippi River plume, northwest Gulf of Mexico, and Laguna Madre, light scatter of group 3 was higher than for groups 1 and 2 in southeast Gulf samples. Group 4 bacteria represent cells with the highest light scatter and DNA signals. In the southeast Gulf samples, light scatter of group 4 cells was even higher, reaching light scatter signals similar to (the clearly distinguished) *Prochlorococcus* spp.

Small, low-DNA bacteria (group 1) were the most abundant bacteria in all Gulf of Mexico samples ($39.8 \pm 11.5\%$; mean ± SD). Their share was higher in the northern Gulf (GoM and MRP stations; $51.4 \pm 8.2\%$; Table 2, Fig. 3) than in the southeast Gulf ($33.4 \pm 7.8\%$). In the southeast Gulf, no significant difference in group 1 shares was evident between surface (stations 1–5) and subsurface (stations A–D; Table 2, Fig. 3) samples. In all Gulf samples, groups 2 and 3 exhibited similar shares to each other and among most stations ($25.4 \pm 3.0\%$ and $27.2 \pm 7.0\%$, respectively). Group 4 bacteria shares also were similar among all Gulf stations but only half to two-thirds of group 2 and 3 shares ($14.0 \pm 2.3\%$; Table 2). Lower Laguna Madre (LLM) exhibited a different composition of bacterioplankton, which may reflect different environmental conditions. Group 3 bacteria dominated the community (67.7%), and groups 1, 2, and 4 contributed 15.8, 11.8, and 4.6%, respectively (Table 2, Fig. 3).

Total bacterioplankton exhibited highest growth rates in the chlorophyll subsurface maximum in the southeast Gulf of Mexico ($0.75 \pm 0.11\ day^{-1}$). Growth rates in surface waters of the southeast Gulf (stations 1–5) were $0.51 \pm 0.18\ day^{-1}$, $0.42\ day^{-1}$ in the northern Gulf (GoM), $0.55\ day^{-1}$ in Laguna Madre, and 0.2–0.7 day^{-1} in the Mississippi River plume (Fig. 4). Growth rates of individual DNA groups fell both above and below the growth rate for total bacterioplankton in each experiment. Although all four DNA groups were present in all samples, not all groups computed significant serial dilution regressions in some experiments.

Table 2 Bacterioplankton composition by DNA subpopulations in different regions of the Gulf of Mexico (percent contribution to total bacteria abundance ± SD)

Region	Group 1	Group 2	Group 3	Group 4	Number
Mississippi River plume	51.4 ± 8.2	15.2 ± 1.5	25.0 ± 4.2	8.5 ± 3.3	4
Northern Gulf (GoM)	51.0	20.8	16.7	11.5	1
Laguna Madre (LLM)	15.8	11.8	67.7	4.6	1
SE Gulf of Mexico, surface	34.9 ± 9.7	25.5 ± 2.1	25.3 ± 9.2	14.3 ± 3.0	5
SE Gulf of Mexico, subsurface	31.5 ± 4.9	25.3 ± 4.3	29.6 ± 1.9	13.6 ± 1.4	4

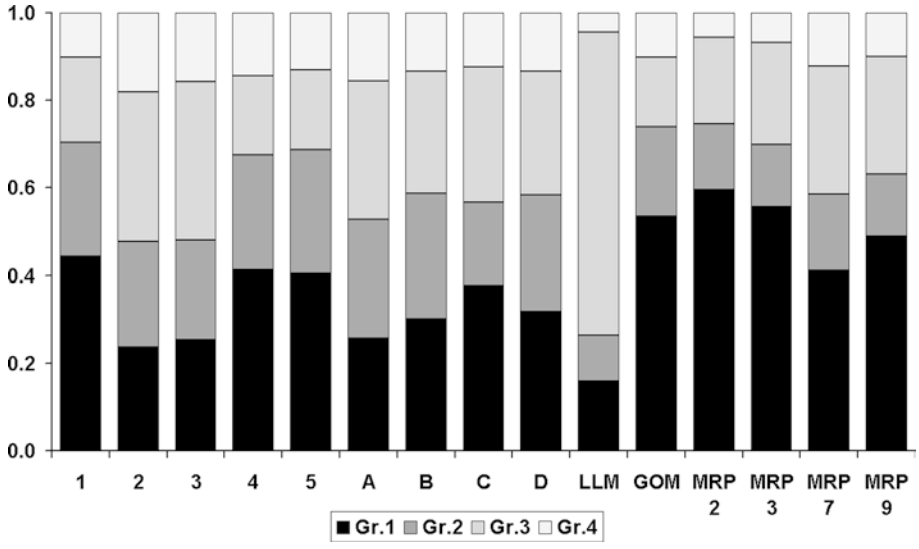

Fig. 3 Composition of bacterioplankton by DNA groups

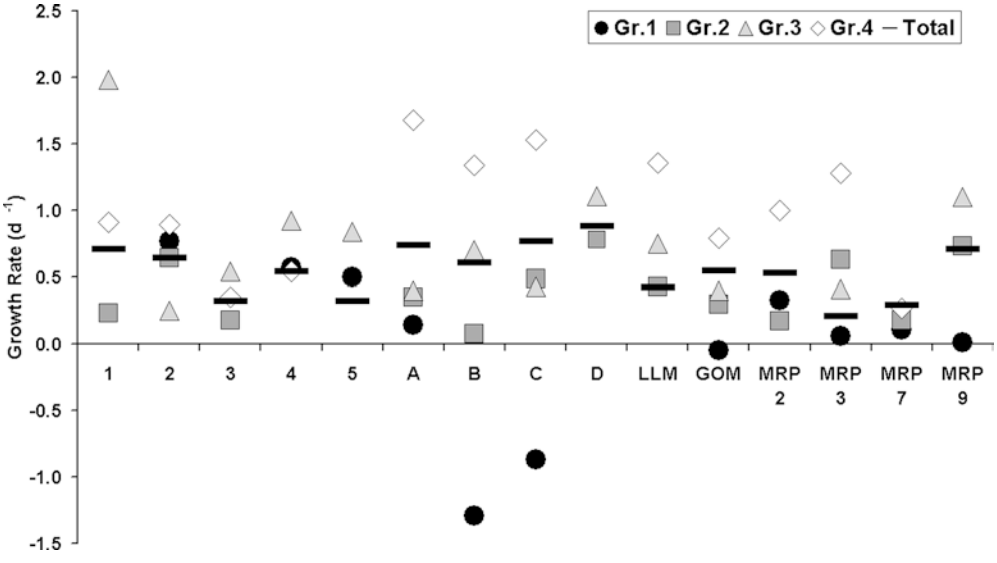

Fig. 4 Gross growth rates (per day) of total bacterioplankton (*bars*) and DNA groups (*symbols*). Some groups did not compute valid serial dilution results in some experiments

In most experiments in the Mississippi River plume, GoM, Laguna Madre and the subsurface chlorophyll maximum in the southeast Gulf of Mexico, group 4 bacteria with the highest DNA content exhibited the highest growth rates, mostly but not always followed by group 3 bacteria (Fig. 4) In surface waters of the southeast Gulf of Mexico, this trend was less obvious, and sometimes group 3 bacteria had higher growth rates than group 4 bacteria. Some experiments revealed group 2 bacteria having a higher growth rate than the more DNA containing group 3 bacteria (stations 2, C, MRP3, MRP9) or even group 4 bacteria (stations 3, 4). Group 1 bacteria exhibited the lowest growth rates among all subpopulations in 7 of 11 experiments where this subpopulation computed valid serial dilution results. In 3 experiments, group 1 bacteria had higher growth rates than group 2 or 3 bacteria. In the fourth experiment (station 5), only two of the four DNA groups computed significant serial dilution regressions; however, since the growth rates of both populations, including group 1 bacteria, were higher than the total population growth rate, the missing (not-computed) groups must have had a growth rate lower than the total and, thus, a lower than group 1 growth rate. In 7 of the 11 experiments, group 1 bacteria exhibited a positive growth rate.

Grazing losses of the total bacterioplankton amounted to 0.36 ± 0.06 day^{-1} in the Mississippi River plume, 0.37 day^{-1} in Laguna Madre, 0.45 day^{-1} at GoM, and 0.47 ± 0.06 day^{-1} in the subsurface layer of the southeast Gulf of Mexico. Total bacteria grazing losses in the surface layer of the southeast Gulf of Mexico varied, ranging from 0.16 to 0.69 day^{-1} (Fig. 5). Concomitant with highest growth rates, grazing rates were highest in the subsurface chlorophyll maximum in the southeast Gulf of Mexico. Total bacterioplankton grazing losses

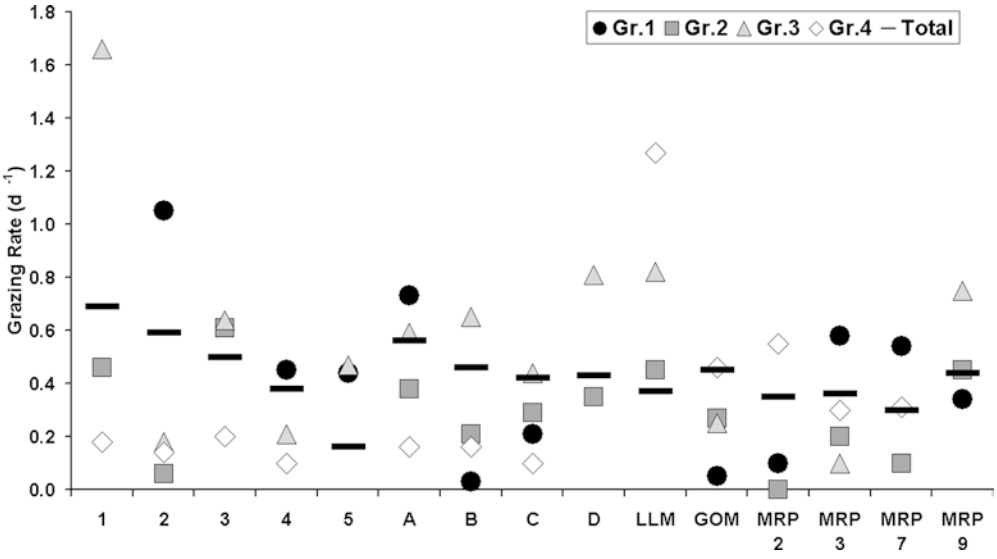

Fig. 5 Grazing rates (per day) of total bacterioplankton (*bars*) and DNA groups (*symbols*). Some groups did not compute valid serial dilution results in some experiments

were related to total population growth rates ($r^2 = 0.30$, $P = 0.03$, $n = 15$).

Grazing losses of DNA groups varied from undetectable to 1.66 day^{-1}. In most experiments in the southeast Gulf of Mexico (surface and chlorophyll subsurface maximum waters), group 4 bacteria experienced the lowest grazing loss (< 0.2 day^{-1}) among DNA groups (Fig. 5). In contrast, group 4 bacteria experienced high grazing losses of 0.3–1.27 day^{-1} in the northern Gulf of Mexico (GoM, MRP) and Laguna Madre. In half of the experiments in which group 1 bacteria computed valid serial dilution results, this group experienced the highest loss rates among DNA groups, and in all but one experiment, group 1 bacteria experienced a significant grazing loss. Loss rates of DNA groups were not related to either growth rates or initial abundance of DNA groups (regression analysis, $P > 0.05$; data not shown).

For most experiments, total bacterioplankton growth was balanced by loss rates, resulting in net growth rates of –0.18 to 0.45 day^{-1} (Fig. 6). Net growth rates of DNA groups differed substantially and tended to be highest for group 4 bacteria in most experiments. The chlorophyll subsurface maximum layer experiments in the southeast Gulf of Mexico exhibited the clearest trend: group 1 bacteria showed negative net growth rates (–0.6 to –1.3 day^{-1}), which were related to low growth rates rather than high grazing losses. In contrast, highest net growth rates of group 4 bacteria (1.2–1.5 day^{-1}) were related to low mortality rates. Groups 2 and 3 had net growth rates similar to each other that reflected close balance between growth and mortality rates. Negative net growth rates for group 1 bacteria also were found at three of four Mississippi River plume stations, GoM, and one of the southeast Gulf surface stations. These low net growth rates also were related to

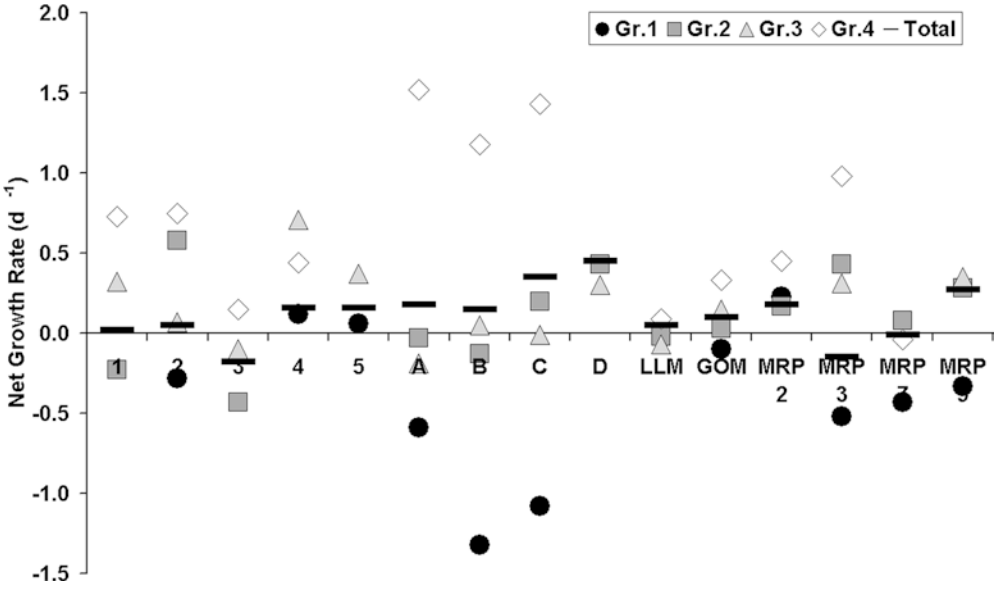

Fig. 6 Net growth rates (growth rate – grazing rate; per day) of total bacterioplankton (*bars*) and DNA groups (*symbols*). Some groups did not compute valid serial dilution results in some experiments

low growth and significant mortality rates, particularly at MRP3 and MRP7 where group 1 bacteria experienced the highest grazing pressure among all DNA groups. Groups 2 and 3 bacteria exhibited net growth rates between groups 1 and 4, but net growth rates of group 3 bacteria were not generally higher than those of group 2 bacteria.

Discussion

Cytometric analysis of bacterioplankton revealed the occurrence of bacteria groups distinguished by apparent DNA content and cell size. Although the number of clearly separated clusters seems to depend on the system under study, three to four subpopulations were usually recorded in offshore waters (Li et al. 1995; Marie et al. 1997; Jochem 2001; Zubkov et al. 2001; this study). It might be disputed whether observed differences in DNA fluorescence reflect different groups of bacteria or different stain penetration due to dye characteristics and physiological state of the populations (Li et al. 1995). Similar bacteria DNA types could, however, be distinguished by a variety of DNA dyes, including a suite of new cyanine dyes as well as DAPI, and from a variety of marine and freshwater systems ranging from oligotrophic high-mountain lakes to eutrophic reservoirs and from estuaries to open oceans (Gasol and del Giorgio 2000). The presence of varying shares of bacteria characterized by their different DNA content seems to be a ubiquitous, natural phenomenon with ecological meaning rather than a preparation artifact (Marie et al. 1997).

Not all clusters may be present at all times or stations. For example, Marie et al. (1997) found two of the three clusters described by Li et al. (1995), and a third cluster not described by the latter authors. Jochem (2001) did not find a particular cluster described by Li et al. (1995) and Marie et al. (1997) but did find an additional cluster not described by either. One cluster described by Marie et al. (1997), but not Li et al. (1995), was separated into two distinct clusters by Jochem (2001). Based on the occurrence of at least two clusters in all cytometric analyses of bacterioplankton, these clusters were termed "low-DNA" and "high-DNA" bacteria (Gasol and del Giorgio 2000). Among the four clusters presented in this study, groups 1 and 2 would fall into the low-DNA and groups 3 and 4 into the high-DNA categories.

The recurrent presence of four DNA groups at all study sites might present a surprising peculiarity of the Gulf of Mexico, and the number of discernible DNA groups may vary with environment. Samples from the Gulf Stream region off the Florida coast prepared by the same protocol exhibited only three DNA groups but samples from central Florida Bay exhibited seven groups (F.J. Jochem, unpublished data). More importantly, comparisons with other studies must be performed with caution since protocols with and without RNAse treatment prior to DNA staining were applied. DNA dyes used to assess the role of low- and high-DNA bacteria (SYBR Green I and II, PicoGreen, SYTO-13, To-Pro; Molecular Probes, Eugene, Ore.) stain both DNA and RNA (Haugland 2001). Studies reporting only two distinct bacterial cluster did not report use of RNAse (e.g. Gasol et al. 1999; Servais et al. 1999; Yanada et al. 2000; Vaqué et al. 2001; Lebaron et al. 2002). Studies reporting more than two DNA groups, however, applied RNAse prior to staining (Li et al. 1995; Marie et al. 1997; Troussellier et al. 1999; Jochem 2001; Zubkov et al. 2001; this study). The –RNAse/+RNAse fluorescence ratio in cultured bacteria of 1.89 to 5.48, with the highest ratios during exponential growth and the lowest ratio during stationary phase (calculated from Table 1 of Troussellier et al. 1999) corresponds to higher cellular RNA concentrations and RNA/DNA ratios in faster-growing bacteria (Kemp et al. 1993; Kerkhof and Ward 1993) and the RNA/DNA ratio of 1.8 reported in slow-growing bacteria (Kato 1994). In studies without RNAse treatment, fluorescence from RNA-dye complexes might obstruct differences in the true cellular DNA content. Therefore, these studies should refer to low- and high-nucleic-acid cells rather than low- and high-DNA cells (Lebaron et al. 2002), and the physiological and ecological significance of low-/high-nucleic-acid cells might differ from low-/high-DNA cells.

In all but the Laguna Madre experiments, low-DNA group 1 bacteria were the most abundant cluster, followed by group 2 and 3 bacteria with similar shares (25–30%) and the highest DNA group 4 bacteria with $<15\%$ of total bacteria abundance. Laguna Madre differed by group 3 bacteria contributing 68% of total bacteria. Group 1 bacteria are better adapted to oligotrophic conditions and exhibit decreasing shares toward the coast and riverine nutrient input (Li et al. 1995; Casotti et al. 2000). Although their dominance in the oligotrophic Gulf of Mexico waters complies with these reports, decreasing trends toward the river mouth in the Mississippi River plume were absent (Jochem 2001). Group 3 bacteria, in contrast, become dominant in coastal waters with riverine input (Casotti et al. 2000) and were the dominant fraction in the English Channel (Marie et al. 1997). Laguna Madre experiences low water exchange with the Gulf of Mexico (residence time >1 year; Shormann 1992). While high evaporation and low precipitation often cause hypersaline conditions (Armstrong 1987), strong rain events can lower salinity. Salinity at LLM during this study was 28.2‰, a relatively low value for the lagoon pointing toward recent freshwater runoffs, confirmed by cumulative rainfall data for adjacent Baffin Bay (Conrad Blucher Institute, Texas A&M University, Corpus Christi, Texas; http://dco.cbi.tamucc.edu).

Although serial dilution experiments have inherent problems related to whether the underlying assumptions (Landry and Hassett 1982) are met, a question not always straightforward to assess (Vaqué et al. 1994), they represent the most feasible technique to address microbial in situ growth and grazing rates. One primary

concern applying the serial dilution approach to bacterioplankton is whether bacterial growth in the dilutions is, in fact, grazer controlled, as demanded by the technique's assumptions, or substrate controlled, since natural substrate concentrations usually remain unknown in these experiments. Dilutions might alter the natural supply of organic substrates from phytoplankton exudation and grazer activity in the high dilutions, yielding underestimates of bacterial growth rates (Landry 1993). On the other hand, carbon enrichment from lytic release during the preparation of filtered seawater for the dilutions was suggested to cause overestimation of bacterial growth in the higher dilutions (Ferguson et al. 1984).

While bottom-up control is prevented in phytoplankton experiments by nutrient additions (Landry 1993), bottom-up control of bacterioplankton cannot be remedied easily by addition of organic substrates. Excess substrates might alter bacterial size and cell-specific activity (Ammerman et al. 1984) and through known size- and activity-selective protistan grazing preferences (Jürgens and Güde 1994; Gasol et al. 1995) might bias measured rates. Addition of organic substrates can also change the bacterioplankton community composition (Cottrell and Kirchman 2000). Whereas substrate limitation appears unlikely in the Laguna Madre and Mississippi River plume experiments due to high dissolved organic carbon (DOC) concentrations and DOC supply by seagrass beds (Ziegler and Benner 1999) and riverine inflow (Hernes and Benner 2003), respectively, its effect in the oligotrophic southeast Gulf of Mexico remains unknown. However, previous studies have shown a negligible effect of carbon enrichment on bacterial growth rates in serial dilution experiments (Tremaine and Mills 1987). Several studies have also confirmed that serial dilution results for bacterioplankton, including from oligotrophic oceanic waters, correlated well with widely accepted direct estimates of bacterial production (^{3}H-leucine, ^{3}H-thymidine uptake; Christian et al. 1982; Kirchman et al. 1982; Ducklow and Hill 1985; Brown et al. 2002). More important than the actual accuracy of the serial dilution experiments is, in the context of the present study, the direct comparison of bacterial growth and grazing loss rates of the distinguished DNA groups under identical experimental conditions.

Total bacteria growth rates of 0.2–0.9 day^{-1} are not unusual for marine systems, and, in most experiments, bacterial growth was balanced by microbial grazing, as reported commonly from other oceanic systems (Ducklow 2000). The experiments revealed, however, that growth rates of different DNA groups within any experiment vary, sometimes substantially. Although most techniques (e.g. thymidine, leucine, glucose incorporation) treat bacterioplankton as a homogeneous population, it is known that not all bacteria are equally active. The share of active bacteria may range from 2 to $>90\%$ but is usually $<50\%$. Although the actual fraction of "active bacteria" may depend on the technique used, bacterioplankton communities represent a continuum of cellular activity, from dead/inactive to highly active and proliferating cells (Smith and del Giorgio 2003).

Soon after their discovery, the contribution of low- and high-DNA bacteria was put into the context of active/inactive cells. Numerous incubation experiments, with and without substrate amendments, showed that abundance and contribution of high-DNA bacteria increased whereas low-DNA bacteria remained at low abundance or decreased (Li et al. 1995; Jellett et al. 1996; Gasol et al. 1999; Yanada et al. 2000; Vaqué et al. 2001). Using fluorescent dyes that differentiate between live and dead cells (based on membrane permeability differences), the number of high-DNA bacteria correlated with the number of cells labeled as "live" cells (Gasol et al. 1999). The share of high-DNA bacteria decreased in the presence of predators, which seems consistent with the known size- and activity-related grazing behavior of protists (Jürgens and Güde 1994; Gasol et al. 1995). Based on these results, it was hypothesized that high-DNA bacteria represent the active fraction of bacterioplankton, while low-DNA bacteria are dead and/or inactive cells (Gasol and del Giorgio 2000).

The interpretation of low- and high-DNA bacteria as inactive/active is, however, not that straightforward. Although the abundance of high-DNA bacteria correlated with the abundance of cells labeled as "live" cells (Gasol et al. 1999; see above), the abundance of low-DNA bacteria did not correlate with the abundance of cells labeled as "dead" in the same study. A combination of DNA and membrane potential staining of freshwater and Mediterranean bacterioplankton confirmed that high-DNA bacteria were active but rejected that low-DNA bacteria were inactive or dead (Grégori et al. 2001). Low-DNA bacteria also were active in the Celtic Sea, where they exhibited 4 times higher growth rates (0.4 day^{-1}) than high-DNA bacteria in surface waters, while growth rates of low- and high-DNA bacteria were similar (0.12 day^{-1}) in subsurface layers (Zubkov et al. 2001). In the northern Gulf of Mexico, low-DNA bacteria dominated bacterioplankton (average 46%, range 26–60% of total bacteria) in the euphotic zone; their abundance and contribution decreased below the subsurface chlorophyll maximum, where larger, high-DNA bacteria (average. 32%, range 22–55%) dominated the community (Jochem 2001). It appears surprising that presumably inactive low-DNA bacteria dominated the euphotic layer where substrate concentrations from phytoplankton production and riverine import of organic matter is highest (Lohrenz et al. 1999), while presumably highly active high-DNA bacteria dominated in deeper, substrate-poor layers.

The present study reveals that low-DNA bacteria were not inactive in various regions of the Gulf of Mexico. Group 2 bacteria exhibited positive growth rates in all experiments, and group 1 showed positive growth rates in most experiments, sometimes higher than growth rates of bacteria with higher DNA content. Low-DNA bacteria also were integral components of the microbial food web, experiencing loss rates balanc-

ing population growth. Loss rates of group 1 were sometimes higher than those of high-DNA bacteria, indicating that protistan grazers did not discriminate against low-DNA bacteria.

Protistan grazing is, however, not the only microbial process affecting bacterial populations. Viral attack has been discussed recently as another major impact on bacterioplankton communities. While some studies revealed that viral attack had a minor effect compared to protistan grazing activity (e.g. Bettarel et al. 2003; Choi et al. 2003), other studies revealed a major population control by viral lysis (e.g. Furhman and Noble 1995; Steward et al. 1996; Fisher and Velimirov 2002). Effects of viral attack appear to be more significant in eutrophic waters (Weinbauer and Peduzzi 1995). Originally designed to assess prey (bacterial, algal) loss rates related to protistan grazing activity, serial dilution experiments cannot distinguish prey population losses from viral infection and protistan grazing activity. Thus, the ultimate role of virus attack must remain unknown for the present study, as for most similar studies. Some caution must, therefore, be applied in interpreting presented loss rates as pure protistan grazing, but in any case, protistan grazing or viral lysis, results demonstrate that low-DNA bacteria are subjected to biological loss processes as much as bacteria with higher DNA content. Taking viral attack into account would further sustain the notion that low-DNA bacteria are not inactive or dead cells since viruses preferably attack metabolically active bacteria, as reflected by the correlation of viral to bacterial abundances and production (e.g. Weinbauer and Peduzzi 1995; Steward et al. 1996).

Whereas total bacterioplankton loss rates were related to total bacterioplankton growth rates, those of DNA groups were not. Grazing impact on DNA groups might be affected by grazer population composition. In the northern Gulf (MRP, GoM, LLM), group 4 bacteria were grazed more strongly (0.58 ± 0.40 day^{-1}) relative to growth rates and abundance than in the southeast Gulf (0.15 ± 0.04 day^{-1}), although they did not represent a large proportion of total bacteria abundance (they may have with respect to total biomass, though). Both GoM and LLM exhibited low concentrations of heterotrophic nanoflagellates (HNF), and micro-grazers were dominated by oligotrich ciliates (P.J. Lavrentyev, unpublished data). These ciliates may be better equipped to graze on large cells than HNF, or they may graze less on small cells because of physiological constraints.

The reasons for the negative growth rates of group 1 bacteria in the chlorophyll subsurface layer of the southeast Gulf of Mexico remain unknown. Negative growth rates may indicate that these small, low-DNA bacteria were indeed inactive, senescent, or subject to viral attack. They also may have been outcompeted by other bacteria groups in the subsurface layers. The predominance of group 1 bacteria in the surface layer corresponds to their higher surface area to volume ratio, which makes small bacteria better competitors for limited resources in oligotrophic surface layers (Button 1998). This competitive advantage may be lost in the subsurface chlorophyll maximum layer, which exhibits higher resource concentrations, both nutrients and perhaps DOC from phytoplankton production. Since group 1 bacteria contributed about a third of total bacteria abundance and were exposed to active grazing (loss rates of 0.03–0.73 day^{-1}), it is likely that they had positive growth rates prior to the study period to have sustained their share of the bacterioplankton community.

The interpretation of DNA clusters as active and inactive cells has been supported by substrate uptake experiments. Bacterial production, measured by thymidine or leucine uptake, correlated closer to high-DNA bacteria abundance than to total bacteria, and high-DNA bacteria accounted for the majority of substrate uptake (Jellett et al. 1996; Servais et al. 1999; Vaqué et al. 2001). Incubation with ^{3}H-thymidine or leucine and subsequent cell sorting by flow cytometry revealed 5–10 times higher cell-specific substrate uptake rates in high-DNA bacteria compared to low-DNA bacteria (Jellett et al. 1996; Servais et al. 1999; Lebaron et al. 2001). It was concluded that high-DNA bacteria represent the metabolically and biogeochemically active fraction of bacterioplankton, and the percent of high-DNA bacteria was suggested as an "active cell index" to which, rather than total bacteria abundance, biogeochemical process measurements should be related (Jellett et al. 1996; Gasol et al. 1999; Lebaron et al. 2001; Vaqué et al. 2001).

Since larger, high-DNA bacteria contain about 5 times more DNA (Jellett et al. 1996) and protein (Zubkov et al. 2001) compared to smaller, low-DNA bacteria, high-DNA bacteria also need to double 5 times more DNA and protein for a single cell division. Thus, to attain similar growth rates (doubling rates), cell-specific activity of high-DNA bacteria must be substantially higher than that of low-DNA bacteria. Higher cell-specific activity does not, therefore, imply that low-DNA bacteria are less active or inactive, and in this context activities should be related to DNA groups' biomass rather than abundance. In fact, high-DNA bacteria in subsurface layers of the Celtic Sea exhibited higher cell-specific methionine uptake rates, but biomass-specific uptake rates were not higher in high-DNA cells. In surface layers, both cell- and biomass-specific uptake rates of high-DNA bacteria were lower than those of low-DNA bacteria, disproving low-DNA bacteria to be inactive (Zubkov et al. 2001).

Relating biogeochemical process measurements to high-DNA bacteria rather than to total bacteria may be a misleading conclusion. While high-DNA bacteria may contribute more to total bacterial production/activity, smaller and/or slower-growing bacteria still can mediate significant biogeochemical processes. Certain bacteria groups, such as *Planctomycetales* (DeLong et al. 1993) or nitrifying bacteria (Ward 1986; Fenchel et al. 1998), are known for slow growth rates. Nevertheless, nitrifying bacteria are biogeochemically important in the

Mississippi River plume and can contribute substantially to total bacterial production (Pakulski et al. 2000). The better correlation of high-DNA bacteria to total bacterial production is not surprising and is an effect of cell size, not growth rate or activity. Since larger bacteria need to exhibit higher cell-specific activities to attain similar growth rates to smaller bacteria, larger cells must have a greater proportional contribution to total community activity.

This concept is demonstrated by an example using data from station 4. Group 1 bacteria were about 3 times as abundant as group 4 bacteria (95.8 vs 33.8×10^3 cells ml^{-1}) and exhibited a similar growth rate (0.57 day^{-1}) compared to group 4 (0.54 day^{-1}) and total bacteria (0.54 day^{-1}). Bacteria cells were not sized in the southeast Gulf of Mexico, but assuming bacteria sizes comparable to the northern Gulf of Mexico, cell volumes of group 1, group 4, and average volume of bacteria would amount to 0.014, 0.26, and 0.04 μm^3, respectively (Jochem 2001). The average bacterial cell volume of 0.04 μm^3 corresponds to average bacteria cell volumes reported from various oceanic systems (Ducklow 2000 and references therein). Converting bacteria cell volume and abundance into bacterial carbon biomass [picograms carbon per cell $=0.12\times$ volume$^{0.7}$; derived by Norland (1993) from data of Simon and Azam (1989)] results in 0.57, 1.58, and 2.93 $\mu g\ C\ l^{-1}$ for group 1, group 4, and total bacteria, respectively. The resulting ratio of cellular carbon biomass of group 1 and 4 bacteria (6.0 and 46.7 fg C $cell^{-1}$) of 7.8 corresponds to the protein and biomass ratios between low- and high-DNA bacteria reported previously (Zubkov et al. 2001; Lebaron et al. 2002). Average cellular biomass of 12.6 fg C $cell^{-1}$ lies within the published range (Ducklow 2000).

Based on the model of exponential growth, gross production can be derived from initial biomass and growth rate by $P=B_0\times e^{\mu}-B_0$ and amounts to 0.44, 1.13, and 2.10 $\mu g\ C\ l^{-1}\ day^{-1}$ for group 1, group 4, and total bacteria, respectively. While these values are not intended to represent in situ bacterial production, they allow comparison of the relative contribution of DNA groups to total production. Although group 4 bacteria accounted for only 15% of total bacteria in this experiment, they contributed 54% to both total bacterial carbon biomass and production. In turn, group 1 bacteria, representing 41% of total cells, contributed only 21% of total bacterial production. The difference in cell-specific production (33.4 fg C $cell^{-1}\ day^{-1}$ for high-DNA bacteria and 4.6 fg C $cell^{-1}\ day^{-1}$ for low-DNA bacteria; ratio 7.3) corresponds to a difference of 7.6 in cell-specific leucine uptake between small, low-DNA bacteria and high-DNA bacteria as reported by Lebaron et al. (2002). However, the biomass-specific production ($P/B=0.7$) did not differ among low-DNA, high-DNA (groups 1, 4), and total bacteria.

The interpretation of the presented results assumes that DNA groups are more or less discrete, and individual bacterial cells do not switch from one group to another by DNA replication or DNA reduction upon cell division (e.g. from one chromosomal set, 1N, to two chromosomal sets, 2N, or vice versa). Distances in DNA fluorescence peaks between DNA groups of about threefold appear too far to move individual cells from one DNA group to the next by DNA replication (1N to 2N). While some bacteria seem to remain in either 1N or 2N states independent of growth conditions, other species may produce multiple sets of chromosomes (up to 6N; Lebaron and Joux 1994; Åkerlund et al. 1995) that might move them among distinguished DNA groups. However, polyploid status in bacteria seems to depend on high-growth, high-substrate conditions (Åkerlund et al. 1995). These conditions are unlikely to be encountered in an oligotrophic natural environment such as the Gulf of Mexico, as supported by moderate overall and group-specific gross growth rates in this study. Furthermore, under conditions of polyploidy, 1N and 2N cells remain the majority of the populations (Lebaron and Joux 1994; Åkerlund et al. 1995), and a high degree of polyploidy among a natural, mixed population of bacteria with inherently different species-specific DNA content (e.g. Schut et al. 1993) would produce a continuous spectrum of cellular DNA fluorescence, which would prevent clear distinction of DNA groups as documented for all experiments in the Gulf of Mexico.

On the other hand, starvation has been suggested to readjust bacterial DNA content to 1N, or lower than 1N by DNA loss by leakage and/or degradation (Hood et al. 1986; Hoff 1989), which might move bacteria from a higher- to a lower-DNA group and, thereby, produce enhanced apparent growth rates in low-DNA groups. More recent studies revealed, however, that polyploid cells produced under high substrate conditions remained in polyploid status under prolonged starvation (Thorsen et al. 1992; Lebaron and Joux 1994), and starvation-induced reduction in DNA fluorescence occurred only after much longer incubation periods (several weeks) than experienced in the here-presented growth experiments (Lebaron and Joux 1994). Since DNA degradation or leakage would present a continuous process, it would, again, produce a continuous DNA fluorescence spectrum rather than distinct DNA group peaks. It appears unlikely, therefore, that positive growth rates of group 1 bacteria resulted from starvation of group 2 bacteria. Isolation of oligotrophic bacteria by low substrate dilution techniques also revealed that these cells possess a much lower (0.3–1 fg DNA $cell^{-1}$) DNA content than the average bacteria population (>4 fg DNA $cell^{-1}$; Schut et al. 1993). Low-DNA bacteria are more likely to present a natural, living component of marine bacterioplankton that may escape traditional culture attempts and are, therefore, little characterized physiologically and phylogenetically.

While cell cycles of individual species cannot be followed in natural, mixed populations due to lack of appropriate methodology, and changes of individual bacterial cells from one DNA group to another by

changes in ploidy cannot be excluded definitely, the little phylogenetic information available on bacteria DNA clusters (Eilers et al. 2000; Fuchs et al. 2000; Zubkov et al. 2001, 2002) is surprisingly consistent: large, high-protein and high-DNA bacteria were dominated by *Roseobacter* and γ-proteobacteria, whereas low-protein, high-DNA bacteria were largely composed of members of the *Cytophaga/Flavobacter* clade, and small, low-DNA bacteria by SAR86. Another group of small bacteria is the uncultivable SAR11 cluster of α-proteobacteria (Rappé et al. 2002), and members of the genus *Pseudoalteromonas* of the γ-proteobacteria are known for their large genome size (Lanoil et al. 1996). Flow sorting showed that high-DNA bacteria comprised a variety of large, curved, and S-shaped cells typical for γ-proteobacteria such as *Vibrio* spp., whereas low-DNA bacteria were small cocci (Jochem 2001; Jacquet et al. 2002). Although bacteria size and shape can change with growth phase and nutritional conditions, the flow-sorting results combined with phylogenetic studies suggest that DNA groups might comprise different consortia of bacteria. The phylogenetic composition of DNA groups might, however, change with time and location.

Experiments that lend the foundation for the active/inactive interpretation of bacterial DNA clusters involved long-term incubations (micro-, mesocosm) running for several days (Gasol et al. 1999; Lebaron et al. 1999; Troussellier et al. 1999; Vaqué et al. 2001). Substantial changes in bacteria community composition occur in incubations after as little as 20 h, favoring the growth of *Roseobacter* relatives of the α-proteobacteria, and γ-proteobacteria, while other α-proteobacteria, members of the *Cytophaga/Flavobacter* clade, and uncultured SAR86 bacteria decrease in abundance (Eilers et al. 2000; Fuchs et al. 2000; Massana et al. 2001). Long incubation experiments may be biased by selectively favoring certain groups of bacteria such as *Roseobacter* and γ-proteobacteria, which are similar to the few cultivable species of marine bacteria and have a high DNA content.

In contrast to long-term micro- and mesocosm experiments, this study addressed DNA-population-specific growth rates in relatively short incubations. Low-DNA bacteria in various regions of the Gulf of Mexico were growing actively, sometimes at rates similar to or higher than those of high-DNA bacteria. Low-DNA bacteria were an integral part of the microbial food web, showing loss rates similar to high-DNA bacteria. Conclusive clarification of whether cytometric DNA clusters of bacterioplankton host different bacterial consortia, if single species can shift between high- and low-DNA status, and which environmental and population dynamics regulate such switches remains in need of studies with high specificity and sensitivity. Combining cytometric flow sorting with molecular tools to assess bacterial diversity and identity may be a promising venue (e.g. Carlson et al. 2002). Consideration also has to be given to the protocol assessing DNA groups, that is, with or without RNAse treatment. Protocols without RNAse treatment might reflect the total nucleic acid content rather than the DNA content of cells. With RNA being about 5 times more abundant than DNA in fast-growing cells, a closer correspondence between high-nucleic-acid cells and bacterial production might be expected. As to the role of low-DNA bacteria (protocols with RNAse), however, this and other studies suggest that the interpretation of low-DNA versus high-DNA bacteria clusters as inactive and active cells might be oversimplified.

Acknowledgements We thank Ron Benner and John Lamkin for inviting FJJ to participate in the Mississippi River plume and southeast Gulf of Mexico cruises, respectively, and the captains and crews of R.V. "Longhorn" and "Gordon Gunter" for their hospitality and assistance at sea. Nutrient data from the Mississippi River plume by Mark McCarthy, and chlorophyll data by Kim Williams and CTD data by Elisabeth Johns and Ryan Smith from the southeast Gulf of Mexico are greatly appreciated. We are grateful to Mark McCarthy for editorial help. Work in the Mississippi River plume was supported by the Nancy and Perry Bass Endowment to the University of Texas Marine Science Institute. A Dean of the College of Arts and Sciences at FIU summer research grant supported work in the southeast Gulf of Mexico.

References

Åkerlund T, Nordström K, Bernander R (1995) Analysis of cell size and DNA content in exponentially growing and stationary phase batch cultures of *Escherichia coli*. J Bacteriol 177:6791–6797

Ammerman JW, Fuhrman JA, Hagström Å, Azam F (1984) Bacterioplankton growth in seawater I. Growth kinetics and cellular characteristics in seawater cultures. Mar Ecol Prog Ser 18:31–39

Armstrong NE (1987) The ecology of open-bay bottoms of Texas: a community profile. US Fish Wildl Serv Biol Rep 85(7.12)

Bettarel Y, Amblard C, Sime-Ngando T, Carrias JF, Sargos D, Garabetian F, Lavandier P (2003) Viral lysis, flagellate grazing potential, and bacterial production in Lake Pavin. Microb Ecol 45:119–127

Brown SL, Landry MR, Christensen S, Garrison D, Gowing MM, Bidigare RR, Campbell L (2002) Microbial community dynamics and taxon-specific phytoplankton production in the Arabian Sea during the 1995 monsoon seasons. Deep Sea Res II 49:2345–2376

Button DK (1998) Nutrient uptake by microorganisms according to kinetic parameters from theory as related to cytoarchitecture. Microbiol Mol Biol Rev 62:636–645

Carlson GA, Giovannoni SJ, Hansell DA, Goldberg SJ, Parsons R, Otero MP, Vergin K, Wheeler BR (2002) Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea. Aquat Microb Ecol 30:19–36

Casotti R, Brunet C, Aronne B, D'Alcala MR (2000) Mesoscale features of phytoplankton and planktonic bacteria in a coastal area as induced by external water masses. Mar Ecol Prog Ser 195:15–27

Chin-Leo G, Benner R (1992) Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. Mar Ecol Prog Ser 87:87–103

Choi DH, Hwang CY, Cho BC (2003) Comparison of virus- and bacterivory-induced bacterial mortality in the eutrophic Masan Bay, Korea. Aquat Microb Ecol 30:117–125

Christian RR, Hanson RB, Newell SY (1982) Comparison of methods for measurement of bacterial growth rates in mixed batch cultures. Appl Environ Microbiol 43:1160–1165

Cottrell MT, Kirchman DL (2000) Natural assemblage of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66:1692–1697

DeLong EF, Franks DG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Ducklow H (2000) Bacterial production and biomass in the oceans. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, pp 85–120

Ducklow HW, Hill SM (1985) The growth of heterotrophic bacteria in the surface waters of warm core rings. Limnol Oceanogr 30:239–259

Eilers H, Pernthaler J, Amann R (2000) Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Appl Environ Microbiol 66:4634–4640

Fenchel T, King GM, Blackburn TH (1998) Bacterial biogeochemistry. Academic Press, San Diego, Calif.

Ferguson RL, Buckley EN, Palumbo AV (1984) Response of marine bacterioplankton to differential filtration and confinement. Appl Environ Microbiol 47:49–55

Fisher UR, Velimirov B (2002) High control of bacterial production by viruses in a eutrophic oxbow lake. Aquat Microb Ecol 27:1–12

Fuchs BM, Zubkov MV, Sahm K, Burkill PH, Amann R (2000) Changes of community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. Environ Microbiol 2:191–201

Furhman JA, Noble RT (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. Limnol Oceanogr 40:1236–1242

Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar 64:197–224

Gasol JM, Duarte CM (2000) Comparative analyses in aquatic microbial ecology: how far do they go? FEMS Microbiol Ecol 31:99–106

Gasol JM, del Giorgio PA, Massana R, Duarte CM (1995) Active versus inactive bacteria: size-dependence in a coastal marine plankton community. Mar Ecol Prog Ser 128:91–97

Gasol JM, Zweifel U, Peters F, Fuhrman JA, Hagström Å (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65:4475–4483

Giovannoni S, Rappé M (2000) Evolution, diversity, and molecular ecology of marine prokaryotes. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, pp 47–84

Grégori G, Citterio S, Ghiani A, Labra M, Sgorbati S, Brown S, Denis M (2001) Resolution of viable and membrane-compromised bacteria in freshwater and marine waters based on analytical flow cytometry and nucleic acid double staining. Appl Environ Microbiol 67:4662–4670

Haugland RP (2001) Handbook of fluorescent probes and research products, 8th edn. (CD-ROM) Molecular Probes, Eugene, Ore.

Hernes PJ, Benner R (2003) Photochemical and microbial degradation of dissolved lignin phenols: implications for the fate of terrigenous DOM in marine environments. J Geophys Res Oceans 108(C9) 3291. DOI 10.1029/2002JC001421

Hoff KA (1989) Survival of *Vibrio anguillarum* and *Vibrio salmonicida* at different salinities. Appl Environ Microbiol 55:1775–1786

Hood MA, Guckert JB, White DC, Deck F (1986) Effect of nutrient depravation on lipid, carbohydrate, DNA, RNA, and protein levels in *Vibrio cholerae*. Appl Environ Microbiol 52:788–793

Jacquet S, Havskum H, Thingstad TF, Vaulot D (2002) Effect of inorganic and organic nutrient addition on a coastal microbial community (Isefjord, Denmark). Mar Ecol Prog Ser 228:3–14

Jellett JF, Li WKW, Dickie PM, Boraie A, Kepkay PE (1996) Metabolic activity of bacterioplankton communities assessed by flow cytometry and single carbon substrate utilization. Mar Ecol Prog Ser 136:213–225

Jochem FJ (2001) Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Aquat Microb Ecol 25:179–194

Jürgens K, Güde H (1994) The potential importance of grazing-resistant bacteria in planktonic systems. Mar Ecol Prog Ser 112:169–188

Kato K (1994) Planktonic bacterial DNA and RNA synthesis from algal extracellular products in a eutrophic lake. FEMS Microbiol Ecol 15:291–298

Kemp PF, Lee S, LaRoche J (1993) Estimating the growth rate of slowly growing marine bacteria from RNA content. Appl Environ Microbiol 59:2594–2601

Kerkhof L, Ward BB (1993) Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. Appl Environ Microbiol 59:1303–1309

Kirchman DL, Ducklow H, Mitchell R (1982) Estimates of bacterial growth from changes in uptake rates and biomass. Appl Environ Microbiol 44:1296–1307

Landry MR (1993) Estimating rates of growth and grazing mortality of phytoplankton by the dilution method. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis, Boca Raton, Fla., pp 715–722

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine microzooplankton. Mar Biol 67:283–288

Lanoil BD, Ciufettii LM, Giovannoni SJ (1996) *Pseudoalteromonas haloplanktis* has a complex genome structure composed of two separate genetic units. Genome Res 6:1160–1169

Lebaron P, Joux F (1994) Flow cytometric analysis of the cellular DNA content of *Salmonella typhimurium* and *Alteromonas haloplanktis* during starvation and recovery in seawater. Appl Environ Microbiol 60:4345–4350

Lebaron P, Servais P, Troussellier M, Courties C, Vives-Rego J, Muyzer G, Bernard L, Guindulain T, Schäfer H, Stackebrandt E (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat Microb Ecol 19:255–267

Lebaron P, Servais P, Agogue H, Courties C, Joux F (2001) Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? Appl Environ Microbiol 67:1775–1782

Lebaron P, Servais P, Baudoux AC, Bourrain M, Courties C, Parthuisot N (2002) Variations of bacterial-specific activity with cell size and nucleic acid content assessed by flow cytometry. Aquat Microb Ecol 28:131–140

Li WKW, Jellett JF, Dickie PM (1995) DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnol Oceanogr 40:1485–1495

Lohrenz SE, Fahnenstiel GL, Redalje DG, Lang GA, Dagg MJ, Whitledge TE, Dortch Q (1999) Nutrients, irradiance and mixing as factors regulating primary production in coastal waters impacted by the Mississippi River plume. Cont Shelf Res 19:1113–1141

Marie D, Partensky F, Jacquet S, Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl Environ Microbiol 63:186–193

Massana R, Pédros-Alió C, Casamayor EO, Gasol JM (2001) Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant processes. Limnol Oceanogr 46:1181–1188

Norland S (1993) The relationship between biomass and volume of bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis, Boca Raton, Fla., pp 303–307

Pakulski JD, Benner R, Whitledge T, Amon R, Eadie B, Cifuentes L, Ammerman J, Stockwell D (2000) Microbial metabolism and nutrient cycling in the Mississippi and Atchafalaya River plumes. Estuar Coast Shelf Sci 50:173–184

Rappé MS, Connon SA, Vergin KL, Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418:630–633

Schut F, de Vries EJ, Gottschal JC, Robertson BR, Harder W, Prins RA, Button DK (1993) Isolation of typical marine bacteria by dilution culture: growth, maintenance, and characteristics of isolates under laboratory conditions. Appl Environ Microbiol 59:2150–2160

Servais P, Courties C, Lebaron P, Trousselier M (1999) Coupling bacterial activity measurements with cell sorting by flow cytometry. Microb Ecol 38:180–189

Shormann DE (1992) The effects of freshwater inflow and hydrography on the distribution of Brown Tide in South Texas. Master's thesis, Department of Marine Sciences, University of Texas at Austin

Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213

Smith EM, del Giorgio PA (2003) Low fractions of active bacteria in natural aquatic communities? Aquat Microb Ecol 31:203–208

Steward GF, Smith DC, Azam F (1996) Abundance and production of bacteria and viruses in the Bering and Chukchi Seas. Mar Ecol Prog Ser 131:287–300

Thorsen BK, Enger Å, Norland S, Hoff KA (1992) Long-term starvation survival of *Yersinia ruckeri* at different salinities studied by microscopical and flow cytometric methods. Appl Environ Microbiol 58:1624–1628

Tremaine SC, Mills AL (1987) Tests of critical assumptions of the dilution method for estimating bacterivory by microeucaryotes. Appl Environ Microbiol 53:2914–2921

Troussellier M, Courties C, Lebaron P, Servais P (1999) Flow cytometric discrimination of bacterial populations in seawater based on SYTO 13 staining of nucleic acids. FEMS Microbiol Ecol 29:319–330

Vaqué D, Gasol JM, Marrasé C (1994) Grazing rates on bacteria: the significance of methodology and ecological factors. Mar Ecol Prog Ser 109:263–274

Vaqué D, Casamayor EO, Gasol JM (2001) Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bacteria with different DNA content. Aquat Microb Ecol 25:163–177

Venrick EL (1978) How many cells to count? In: Sournia A (ed) Phytoplankton manual. Monographs on oceanographic methodology 6. UNESCO, Paris, pp 167–180

Ward BB (1986) Nitrification in marine environments. In: Proser JI (ed) Nitrification. IRL Press, Oxford, pp 157–184

Weinbauer MG, Peduzzi P (1995) Significance of viruses versus heterotrophic nanoflagellates for controlling bacterial abundance in the northern Adriatic Sea. J Plankton Res 17:1851–1856

Yanada M, Yokokawa T, Lee CW, Tanaka H, Kudo I, Maita Y (2000) Seasonal variation of two different heterotrophic bacterial assemblages in subarctic coastal seawater. Mar Ecol Prog Ser 204:289–292

Ziegler S, Benner R (1999) Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. Mar Ecol Prog Ser 180:149–160

Zubkov MV, Fuchs BM, Burkill PH, Amann R (2001) Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. Appl Environ Microbiol 67:5210–5218

Zubkov MV, Fuchs BM, Taran GA, Burkill PH, Amann R (2002) Mesoscale distribution of dominant bacterioplankton groups in the northern North Sea in early summer. Aquat Microb Ecol 29:135–144