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# Growth rate of the major phylogenetic bacterial groups in the Delaware estuary

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*Abstract*

The phylogenetic composition of bacterial communities varies along the salinity gradient of estuaries, one notable pattern being the prevalence of alpha- and beta-proteobacteria in salt- and freshwater regions, respectively. We tested the hypothesis that bottom-up forces (substrate supply) control these and other changes in bacterial community composition in the Delaware estuary. We measured the biomass and growth rate of four major phylogenetic groups of bacterioplankton (alpha-, beta-, and gamma-proteobacteria and the *Cytophaga-Flavobacter* cluster) in low- and high-salinity regions of the estuary by the dilution culture approach combined with fluorescent in situ hybridization with rRNA-targeted probes. Group-specific growth rates were highly variable depending on location and season and could far exceed (up to nearly fourfold) the growth rates of total bacteria. The phylogenetic groups that exhibited the highest growth rates included alpha- and gamma-proteobacteria at both low- and high-salinity stations and beta-proteobacteria at a low-salinity station. These data help to explain the high abundance of beta-proteobacteria in the freshwater region, but bottom up controls appear to account only partly for the variability in bacterial communities. The relationship between production and biomass suggested that bacterial communities at the low-salinity site were controlled by substrate supply, whereas bacterial mortality appeared to be more important at the high-salinity site. Our data demonstrate that group-specific growth rates are useful parameters for examining the competitive advantages of individual groups and the mode of regulation of bacterial communities in estuarine environments.

Recent studies that used molecular approaches have begun to reveal variations in time and space of the composition of bacterial communities that could not be adequately distinguished by conventional techniques (Amann et al. 1995; Giovannoni and Rappé 2000). Generally, marine systems are dominated by alpha-proteobacteria and *Cytophaga*-like bacteria, whereas beta-proteobacteria are often the dominant group in freshwater systems (Glöckner et al. 1999; Kirchman 2002). In estuarine systems, drastic compositional shifts in the major phylogenetic groups along the salinity gradient have been documented. One notable pattern is the prevalence of alpha- and beta-proteobacteria in salt- and freshwater regions, respectively (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2003).

Community structure in aquatic systems is likely dependent on the availability of organic and inorganic resources (bottom-up control) and the mortality caused by grazers and viruses (top-down control). Bottom-up control could affect community structure because even broad phylogenetic groups of bacteria appear to differ in uptake of specific organic components (Cottrell and Kirchman 2000; Kirchman et al. 2004; Malmstrom et al. 2004). Other lines of evidence indicate that different phylogenetic groups might respond differently to pulsed inputs of nutrients. In coastal marine environments, studies have suggested that gamma-proteobacteria are an ''opportunistic'' group that responds quickly to the abrupt increase in the concentration of labile dissolved organic material (DOM) (Eilers et al. 2000; Beardsley et al. 2003), whereas bacteria in the alpha-proteobacterial SAR11 cluster appear to favor low-nutrient regimes (Morris et al. 2002). In contrast, Fuchs et al. (2000) found that both gamma- and alpha-proteobacteria grew rapidly in dilution cultures with minimized resource competition and grazing pressure. These differences in the uptake of DOM and in growth characteristics of different phylogenetic groups provide a basis for explaining the abundance of individual groups (community composition) in different environments. Differences in mortality rates because of protozoan (Pernthaler et al. 1997) and metazoan (Jürgens et al. 1999) grazers and viral infection (Øvreås et al. 2003) could also influence the relative abundance of different phylogenetic groups. Little is known as yet about the relative importance of these forces in determining the makeup of bacterial communities in aquatic systems.

One of the key variables for clarifying the mechanisms underlying the variations in community composition is the growth rate of each subgroup because this rate variable is a sensitive indicator of bottom-up controls such as substrate stress and limitations. At equilibrium, a bacterial subgroup that grows fastest is expected to dominate the community if mortality (top-down control) does not differ among subgroups. Alternatively, if a subgroup that grows fastest represents a minor component of the community, one could infer that the fast-growing group is selectively eliminated, the community is in transition (not at equilibrium), or both. Previous studies have examined the growth of some major

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Table 1. Physical and chemical parameters at the sampling stations located in the Delaware Bay estuary. —, not determined.

| Station (location) | Date | Salinity | Water temperature (°C) | Chl *a* ($\mu$g L$^{-1}$) | $NO_3$ ($\mu$mol L$^{-1}$) | $PO_4$ ($\mu$mol L$^{-1}$) |
|---|---|---|---|---|---|---|
| River | 10 Jun 01 | 0.1 | 21.5 | 15.60 | 57.3 | 1.33 |
| (40°7.67′N, | 15 Aug 01 | 0.1 | 27.6 | 6.05 | 77.4 | 3.50 |
| 74°49.28′E) | 4 Dec 01 | 0.1 | 11.9 | 2.26 | — | — |
| Bay | 10 Jun 01 | 26.5 | 20.9 | 5.34 | 1.3 | 0.50 |
| (38°54.95′N, | 15 Aug 01 | 28.5 | 23.1 | 6.60 | 1.4 | 0.82 |
| 75°6.00′E) | 4 Dec 01 | 29.8 | 12.8 | 7.46 | — | — |

phylogenetic groups of bacteria in freshwater (Jürgens et al. 1999) and coastal seawater environments (Eilers et al. 2000), but the relationship between relative abundance and growth of specific bacterial groups remains unclear. Recently, Cottrell and Kirchman (2004) examined the relationship between community composition and the fraction of bacteria assimilating [$^3$H]-thymidine (an index of bacterial growth) along the salinity gradient of the Delaware estuary. Their data revealed that the dominance of beta-proteobacteria declined with the decrease in the fraction of beta-proteobacteria assimilating [$^3$H]-thymidine, but no such a trend was observed for other major phylogenetic groups. Thus, both bottom-up and top-down forces appear to affect bacterial community composition in the estuarine environment, although factors that affect variations in the mode of community regulation have yet to be elucidated.

Our objective was to use a dilution culture technique in combination with fluorescent in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes (Amann et al. 1995) to determine the specific growth rate of major phylogenetic groups of bacteria at the fresh- and saltwater endpoints of the Delaware estuary. We tested the hypothesis that some bacterial groups exhibit higher growth rates than other groups depending on environmental conditions and, more specifically, that the variation in the relative abundance of alpha- and beta-proteobacteria could be explained by their growth rates (i.e., by bottom-up forces). Our data demonstrate that the group-specific growth rate is a dynamic parameter that helps in evaluating ecological traits of individual groups and mechanisms by which bacterial communities are regulated.

## Materials and methods

Sampling was carried out aboard the R/V *Cape Henlopen* during the three transect cruises in the Delaware Bay estuary (9–13 June 2001, 14–18 August 2001, and 3–6 December 2001). For incubation experiments, surface-water samples were collected with a 10-liter Niskin CTD rosette sampler at two stations: one at the site of river inflow (River Station) and the other at the mouth of the Bay (Bay Station), representing fresh- and saltwater regions of the estuary. Table 1 summarizes the physical and chemical parameters at the time of water sampling at each station. Sample water was filtered through either 0.2- or 0.8-$\mu$m pore-size polycarbonate filters (Whatman) by gentle filtration (<80 mm Hg). One hundred milliliters of the 0.8-$\mu$m filtrate and 900 ml of the 0.2-$\mu$m filtrate were mixed and contained in an acid-washed 1-liter polycarbonate bottle. Gloves were worn and care was taken to minimize contamination. Incubations were carried out in the dark at the in situ temperature (12–28°C).

For the microscopic analysis of bacteria, subsamples were fixed with 2% (final conc.) paraformaldehyde solution. After fixation at 4°C for 24 h, bacteria were filtered onto a 0.2-$\mu$m pore-size polycarbonate filter (Whatman) and stored at −20°C until analysis. Bacterial community composition was determined by the FISH method with rRNA-targeted oligonucleotide probes (Cottrell and Kirchman 2000). The hybridization solution contained 2.5 $\mu$g ml$^{-1}$ probe, 0.9 mol L$^{-1}$ NaCl, 20 mmol L$^{-1}$ Tris-HCl (pH 7.2), 5 mmol L$^{-1}$ ethylenediaminetetraacetic acid (EDTA), 0.01% sodium dodecyl sulfate, and the concentration of formamide determined to achieve specificity for the bacterial groups targeted by the different probes (Zarda et al. 1997; Eilers et al. 2000): Eub338 (5′-GCTGCCTCCCGTAGGAGT-3′, formamide = 30%; Amann et al. 1990) for eubacteria, Alf968 (5′-GGTAAGGTTCTGCGCGTT-3′, formamide = 30%; Glöckner et al. 1999) for alpha- proteobacteria, Bet42a (5′-GCCTTCCCACTTCGTTT-3′, formamide = 30%; Manz et al. 1992) for beta-proteobacteria, Gam42a (5′-GCCTTCCCACATCGTTT-3′, formamide = 30%; Manz et al. 1996) for gamma- proteobacteria, CF319a (5′-TGGTCCGTGTCTCAGTAC-3′, formamide = 35%; Manz et al. 1996) for the *Cytophaga-Flavobacter* cluster, and a negative control probe (5′-TAGTGACGCCGTCGA-3′, formamide = 30%; Karner and Fuhrman 1997) for nonspecific probe bindings. All probes were commercially synthesized and labeled with Cy3 (Operon). Although the probe sequences were designed on the basis of the best coverage of the database at the time of the studies, some of the sequences do not perfectly match the sequences of aquatic clones in the growing database. The estimates of cell counts of individual groups could be biased if a probe recognizes cells outside of the targeted phylogenetic group. However, the probes of this study have been used by many previous studies to demonstrate patterns in the distribution and ecological traits of different phylogenetic groups in estuaries and other aquatic habitats. It is necessary to use the same probes to compare results and to explain these patterns. After hybridization, the samples were transferred to a wash solution containing 20 mmol L$^{-1}$ Tris-HCl (pH 7.2), 5 mmol L$^{-1}$ EDTA, 0.01% sodium dodecyl sulfate, and the concentration of NaCl appropriate for the probe (Zarda et al. 1997; Eilers et al. 2000).

Bacterial cells were detected by semi-automated microscopy and image analysis according to Cottrell and Kirchman (2003), with modifications in the optical setup. A CCD cam-

era (Hamamatsu Orca: C-4742-95-12NR) was mounted on a fluorescence microscope (Olympus BX50) equipped with a ×100 UPlanApo oil immersion objective and a 100-W mercury lamp. Cy3 and 4′,6-diamidino-2-phenylindole (DAPI) images were acquired with the use of excitation filters (OMEGA optical: XF1074 for Cy3, XF1005 for DAPI) and a band-pass emission filter/dichroic mirror cartridge that was designed to capture the fluorescence of Cy3 and DAPI (OMEGA optical: XF59). Excitation filters were mounted on a wheel (Prior Scientific) that was controlled by Scope Pro (Media Cybernetics). Exposure time was optimized manually to restrict background Cy3 counts obtained by the negative control probe to be less than 1% of DAPI counts. The image analysis was conducted with Image Pro (Media Cybernetics) to determine cell counts and volume as described (Cottrell and Kirchman 2003). For each sub-group, 10 microscopic fields (1,164.9 $\mu m^2$ per field) were examined. The number of DAPI-positive cells detected in each field was 219 ± 63 (mean ± SD). The counts per field of Cy3-positive cells varied depending on the relative contribution of each subgroup; for the dominant group, the average counts were 50 ± 22 cells per field. The cell abundance of each subgroup was estimated by multiplying the ratio of hybridized cell counts to DAPI-positive counts with the estimate of the abundance of DAPI-positive cells. Cell volume was determined as a geometric mean for each subgroup by using DAPI-stained images that corresponded to hybridized cells. The biomass for each sub-group was estimated from an allometric equation that relates cell volume to carbon (Simon and Azam 1989). Bacterial growth rate was calculated assuming exponential growth. Production rate was estimated as a product of the growth rate and the biomass at the beginning of the incubation period (corrected for a 10-fold dilution). The error of calculated parameters (e.g., production in the dilution experiments) was estimated by standard propagated error equations (Bevington 2003). For each experiment, we collected samples at 12-h intervals for 72 h. Cell abundance generally increased exponentially during the initial 24–36 h, followed by a decrease in growth rate (presumably because of substrate depletion) and, on occasion, in cell abundance (presumably because of grazing by protists that passed through 0.8-$\mu$m pore-size filters; data not shown). In order to minimize the potential effects of prolonged incubation, we used the data collected after 12 h of incubation for the calculation of growth rate and production rate, except for an experiment conducted at Bay Station on 4 December 2001 when the data collected after 36 h of incubation were used because no change in total cell abundance was detected with a shorter incubation. Note that growth and production rates estimated by this method are conservative because small flagellates and viruses that pass through the 0.8-$\mu$m pore-size filter would cause bacterial mortality during the incubations.

In addition to the dilution culture approach, we measured the total bacterial production rate from the rate of thymidine (TdR) incorporation by the centrifugation method (Kirchman 2001). We used a $2.2 \times 10^{18}$ cells $mol^{-1}$ TdR conversion factor that was previously determined in the Delaware Bay (Kirchman and Hoch 1988). Cell-to-carbon conversion factors were obtained for each sample as described above.

Table 2. Comparison of bacterial production rates estimated by the dilution culture experiments and those from TdR incorporation. ND, not detected. Errors of production rate are standard errors for triplicate measurements (TdR method) or estimated by propagation error equations (SE, $n = 10$) for the dilution culture method.

| | | Production rate ($\mu g$ C $L^{-1}$ $d^{-1}$) | |
|---|---|---|---|
| Station | Month | TdR | Dilution culture |
| River | June | 50.2±1.6 | 49±31 |
| | August | 70.9±0.9 | 55±24 |
| | December | 105.1±6.5 | 47±24 |
| Bay | June | 6.9±0.1 | 70±46 |
| | August | 63.8±0.9 | 99±56 |
| | December | 5.1±0.2 | ND |

## Results

*Environmental characteristics of the sampling sites*—Experiments were carried out at two stations representing low-salinity (0.1, River Station) and high-salinity (26.5–29.8, Bay Station) regions of the Delaware estuary. At both the River and Bay Stations, concentration of chlorophyll *a* (Chl *a*; 2.3–15.6 $\mu g$ $L^{-1}$) and inorganic nutrients (nitrate, 1.3–77.4 $\mu mol$ $L^{-1}$; phosphate, 0.5–3.5 $\mu mol$ $L^{-1}$) were generally high (Table 1), indicating the productive nature of the estuary. The concentrations of inorganic nutrients in June and August were higher at River Station than at Bay Station. Water temperature varied in the range of 12–28°C (Table 1). Total bacterial abundances (<0.8-$\mu$m fraction) in the sample waters used for the dilution culture experiments varied in the range of $1.9 \times 10^6$ and $3.6 \times 10^6$ cells $ml^{-1}$. The abundances tended to be lower at River Station (mean ± SE, $2.1 \pm 0.2 \times 10^6$ cells $ml^{-1}$ for June, August, and December) than at Bay Station ($3.1 \pm 0.3 \times 10^6$ cells $ml^{-1}$). Bacterial production rate with the TdR method ranged from 5.1 to 105.1 $\mu g$ C $L^{-1}$ $d^{-1}$ (Table 2). Bacterial production rates tended to be higher at River Station (75.4 ± 16.0 $\mu g$ C $L^{-1}$ $d^{-1}$) than at Bay Station (25.3 ± 19.3 $\mu g$ C $L^{-1}$ $d^{-1}$) for June, August, and December.

*Growth of total bacteria and individual bacterial groups*—In the dilution cultures, total bacterial abundance usually increased substantially (2–6-fold) over 12 h, although bacterial abundance changed little, even with prolonged incubation (36 h) in the December experiment with water collected at Bay Station. Percentages of cells detected by the Eub338 probe at the beginning of the incubation were 66 ± 2% and 45 ± 3% for River and Bay Stations, respectively (Table 3), whereas at the end of the incubation period, they were 72 ± 3% at River Station and 57 ± 11% at Bay Station. At River Station, each phylogenetic group increased as a percentage of total cell counts during the incubation, except for the *Cytophaga-Flavobacter* cluster in August and for gamma-proteobacteria in December (Table 3). The percentage of beta-proteobacteria decreased in all incubations of water from Bay Station, whereas percentages of the other phylogenetic groups increased during the incubation. The total fraction of bacteria accounted for by the four groups

Table 3. Percentage of bacterial groups detected by each probe at the beginning (Initial) and at the end of the incubation (End). Bacteria were classified by rRNA-binding oligonucleotide probes specific for all bacteria (Eub), alpha-proteobacteria (Alpha), beta-proteobacteria (Beta), gamma-proteobacteria (Gamma), and the *Cytophaga-Flavobacter* cluster (CF).

| Station | Month | Sampling[a] | Total abundance ($\times 10^6$ cells $ml^{-1}$) | % total bacterial abundance | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Eub | Alpha | Beta | Gamma | CF | Total of four phylogenetic groups |
| River | June | Initial | 0.24 | 68 | 1 | 22 | 1 | 2 | 26 |
| | | End | 0.55 | 67 | 9 | 40 | 2 | 11 | 62 |
| | August | Initial | 0.12 | 62 | 10 | 24 | 13 | 10 | 57 |
| | | End | 0.81 | 74 | 12 | 39 | 21 | 4 | 76 |
| | December | Initial | 0.19 | 69 | 6 | 25 | 3 | 17 | 51 |
| | | End | 0.85 | 76 | 20 | 27 | <1 | 19 | 66 |
| Bay | June | Initial | 0.36 | 40 | 9 | 9 | 11 | 9 | 38 |
| | | End | 0.86 | 70 | 12 | 1 | 40 | 13 | 66 |
| | August | Initial | 0.30 | 50 | 9 | 6 | 6 | 9 | 30 |
| | | End | 1.73 | 67 | 24 | 3 | 6 | 5 | 38 |
| | December | Initial | 0.26 | 46 | 47 | 11 | 3 | 10 | 71 |
| | | End | 0.46 | 33 | 23 | 1 | 11 | 29 | 64 |

[a] The incubation period was 12 h, except for December experiments, in which samples were incubated for 36 h.

(summed percentage of the four group probes) was generally lower than the percentage of Eub338-positive bacteria with only two exceptions (Table 3).

We calculated growth rates of individual bacterial groups and total bacterial communities from the increase in cell abundance during the incubation. Growth rates of the individual phylogenetic groups could far exceed (up to fourfold for alpha-proteobacteria at River Station in June) growth rates of bulk communities (Fig. 1A). In one case (River Station in June), growth rates of all groups were substantially higher than total community growth rates, suggesting that unidentified bacteria grew much more slowly than the bacterial phylogenetic groups that we examined (Fig. 1A). At River Station (Fig. 1A,C,E), growth rates of alpha- and beta-proteobacteria were high in all experiments; the average growth rates ($\pm$SE, $n = 3$) were $4.9 \pm 0.9\ d^{-1}$ and $3.3 \pm 0.3\ d^{-1}$ for alpha- and beta-proteobacteria, respectively. Growth rates of the *Cytophaga-Flavobacter* cluster were high in June ($5.1 \pm 0.2\ d^{-1}$) and December ($3.2 \pm 0.2\ d^{-1}$) but low in August ($1.0 \pm 0.4\ d^{-1}$). Gamma-proteobacteria exhibited high growth rates in June ($3.1 \pm 0.5\ d^{-1}$) and August ($3.8 \pm 0.3\ d^{-1}$), but the increase in cell abundance was negligible in December. Generally, the composition of bacterial communities at the end of the incubation of River Station water differed little from that at the beginning of the incubation period; both communities were dominated by beta-proteobacteria (Table 3).

At Bay Station (Fig. 1B,D,F), the seasonal variability of growth rates of individual bacterial groups was generally greater than that at River Station; the range of growth rates for each group was 0–5.5, 0–2.1, 1.3–4.3, and 1.1–2.5 $d^{-1}$ for alpha-, beta-, and gamma-proteobacteria and the *Cytophaga-Flavobacter* cluster, respectively. The bacterial group with the highest growth rate in June was gamma-proteobacteria ($4.3 \pm 0.2\ d^{-1}$), whereas in August, it was alpha-proteobacteria ($5.5 \pm 0.3\ d^{-1}$). All groups grew slowly (or negligibly) in December. In contrast with River Station, growth rates of beta-proteobacteria were relatively low at Bay Station, resulting in low contributions (1–3%) of beta-proteobacteria to bacterial communities at the end of the incubation (Table 3). Generally, the bacterial community changed more during the incubation in Bay Station experiments than in River Station experiments.

*Changes in the average cell volume of individual bacterial groups*—In order to examine the variability of the average cell volumes of individual bacterial groups, three-way analysis of variance was performed, with sites (River and Bay Stations), seasons (June, August, December) and incubation time (before and after incubation) as factors. The average cell volume of the phylogenetic bacterial groups varied between sites and among seasons ($p < 0.05$), except that beta-proteobacteria did not differ significantly ($p > 0.05$) between the sites. In contrast, for all phylogenetic groups, cell volumes did not change significantly ($p > 0.05$) during the incubation. The phylogenetic groups did not differ significantly in average cell volume ($p > 0.05$).

*Biomass and production rates of bacteria belonging to different phylogenetic groups*—We measured the biomass and the increase in biomass of individual phylogenetic groups in dilution cultures in order to estimate the carbon production rate of each group. In June, bacterial biomass at River Station was dominated by beta-proteobacteria (Fig. 2); this group alone accounted for 29% of total bacterial biomass (Table 4). The dominance of beta-proteobacteria was also observed in August and December, although the contributions of other phylogenetic groups, such as gamma-proteobacteria (August) and the *Cytophaga-Flavobacter* cluster (December), were also high (>15%) in these months. Total biomass of the four phylogenetic bacterial groups accounted for 35–65% of total bacterial biomass (Table 4). The phylogenetic groups with the highest biomass also had the highest production rate (Fig. 2); the largest fraction (32–44%) of total bacterial production was accounted for by beta-proteobacteria (Table 5). The second largest fraction of total pro-

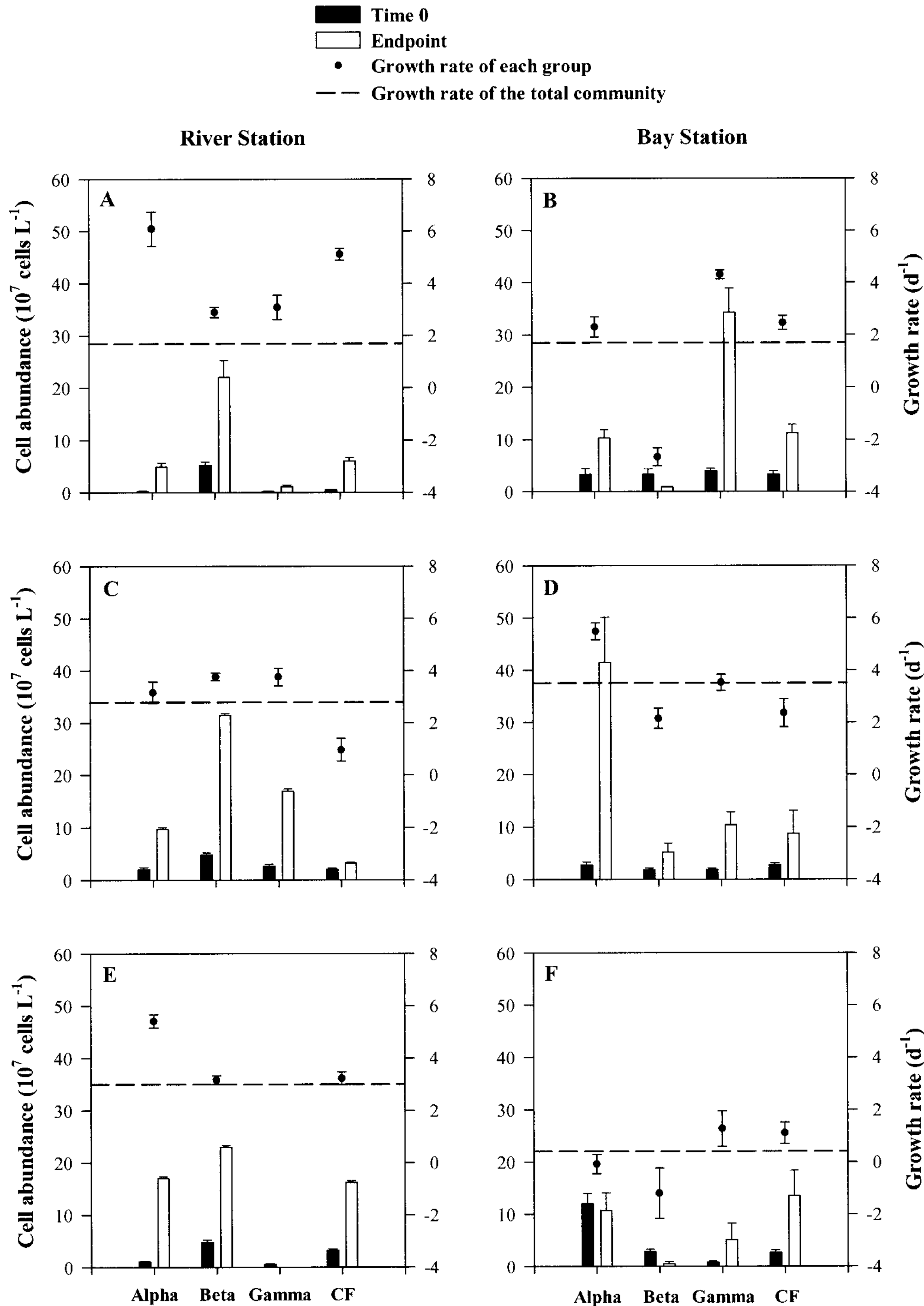


Fig. 1. Changes in the abundance and growth rate (mean ± SE, $n$ = 10) of alpha-, beta-, and gamma-proteobacteria and the *Cytophaga-Flavobacter* cluster (CF) in dilution cultures prepared from sample waters collected at River Station in (A) June, (C) August, and (E) December and at Bay Station in (B) June, (D) August, and (F) December. Closed and open bars represent abundance at the beginning (Time 0) and the end of the incubation (Endpoint), respectively. The growth rate for gamma-proteobacteria in the December experiment at River Station is not presented because this phylogenetic group was not detected at the end of the incubation.

duction was due to either gamma-proteobacteria (20% in August) or the *Cytophaga-Flavobacter* cluster (29% in December). The contribution of alpha-proteobacteria to total bacterial production was relatively low (<15%) throughout this study. Total production of the four phylogenetic bacterial groups accounted for 59–76% of total bacterial production.

At Bay Station in June and August, the four phylogenetic groups examined here accounted for nearly equal percent-

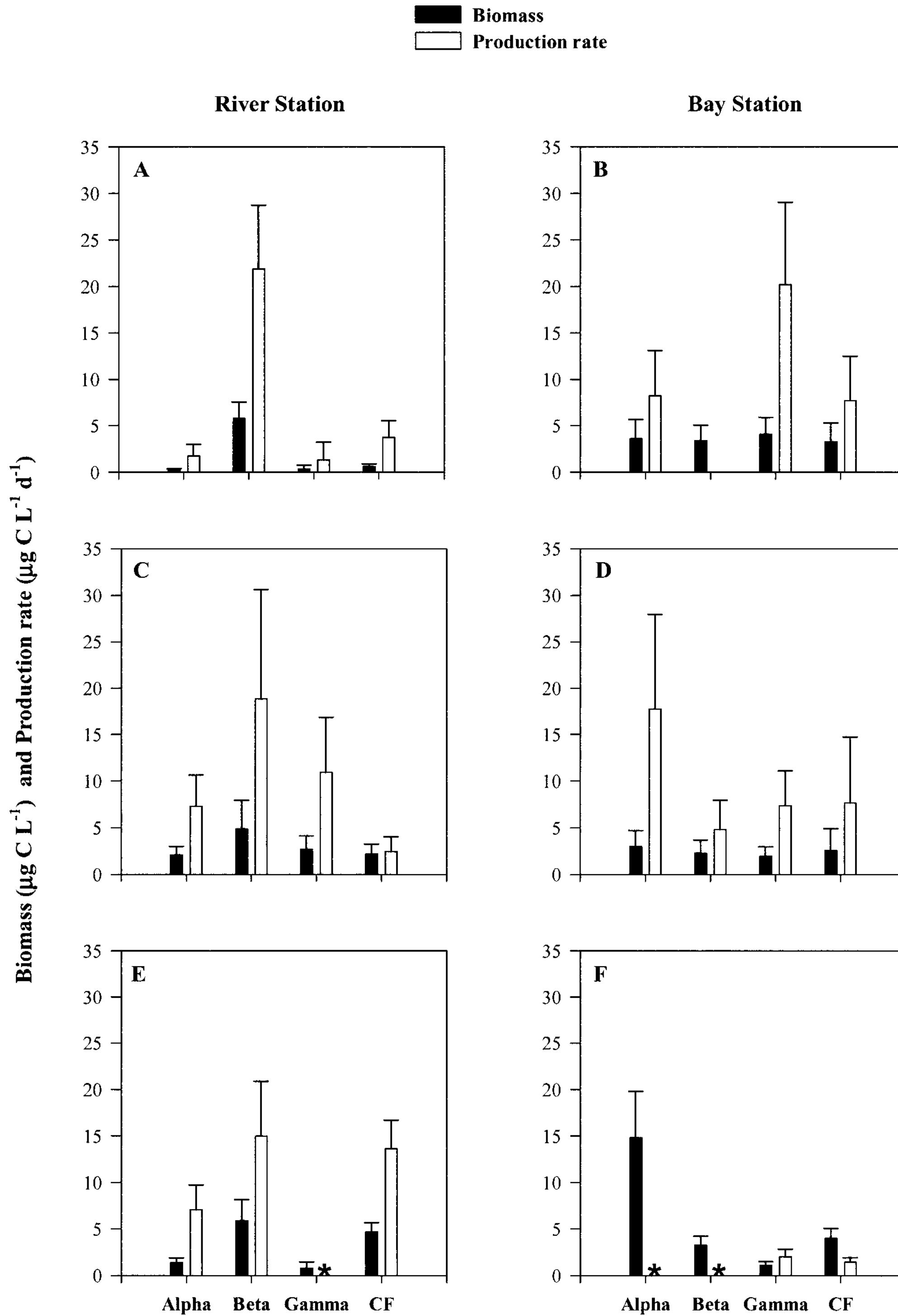


Fig. 2. Biomass and production rate (±SE, $n$ = 10) of alpha-, beta-, and gamma-proteobacteria and the *Cytophaga-Flavobacter* cluster (CF) at River Station in (A) June, (C) August, and (E) December, and at Bay Station in (B) June, (D) August and (F) December. Biomass at the beginning of the incubation is presented. An asterisk (*) indicates that the production rate could not be calculated because an increase in biomass was not detected during the incubation.

ages (11–13% in June and 8–12% in August) of total bacterial biomass, and the total of the four phylogenetic bacterial groups accounted for 40–47% of total bacterial biomass (Fig. 2; Table 4). In contrast, the relative contribution of the phylogenetic groups to total production varied substantially, and the total production of the four groups accounted for 52% and 39% of total bacterial production in June and August, respectively (Fig. 2; Table 5). Gamma-proteobacteria contributed the most (29%) in June, whereas alpha-proteobacteria accounted for the largest fraction (18%) of total pro-

Table 4. Contribution of each phylogenetic bacterial group to total bacterial biomass at the beginning of the incubation. The abbreviations are explained in Table 3.

| Station | Month | Total biomass ($\mu$g C L$^{-1}$)* | % total bacterial biomass | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Eub | Alpha | Beta | Gamma | CF | Total of four phylogenetic groups |
| River | June | 2.01 | 76 | 1 | 29 | 2 | 3 | 35 |
| | August | 1.83 | 65 | 12 | 26 | 15 | 12 | 65 |
| | December | 1.99 | 72 | 7 | 30 | 0 | 24 | 61 |
| Bay | June | 3.12 | 48 | 12 | 11 | 13 | 11 | 47 |
| | August | 2.46 | 55 | 12 | 9 | 8 | 11 | 40 |
| | December | 2.64 | 55 | 56 | 13 | 4 | 15 | 88 |

* The total biomass calculated from the counts and volume of total DAPI-positive cells.

duction in August. The contribution of the *Cytophaga-Flavobacter* cluster was moderately high in both June and August (11% and 8%, respectively). Beta-proteobacteria were a negligible (June) or a minor (5% in August) contributor to total bacterial production. Production of bacterial biomass in the dilution cultures was unmeasurable in December.

*Comparisons of thymidine and dilution methods*—Production rates from the thymidine method and the dilution method agreed within <25% in three out of six cases (Table 2). Two of the three cases of disagreement were in December when rates were low. In December at Bay Station, low production estimated by the TdR method (5.1 $\mu$g C L$^{-1}$ d$^{-1}$) was not detectable by the dilution approach. Large differences were observed in June at Bay Station (6.9 $\mu$g C L$^{-1}$ d$^{-1}$ by TdR vs. 70 $\mu$g C L$^{-1}$ d$^{-1}$ by dilution) and in December at River Station (105.1 $\mu$g C L$^{-1}$ d$^{-1}$ by TdR vs. 47 $\mu$g C L$^{-1}$ d$^{-1}$ by dilution).

## Discussion

By dividing the bacterioplankton ''black box'' into a few major groups on the basis of broad phylogenetic affiliation (i.e., at a level of division or class), recent studies have begun to show that each group often exhibits distinctive ecological traits in terms of biogeographical distribution (Glöckner et al. 1999), substrate uptake (Cottrell and Kirchman 2000; Kirchman et al. 2004; Malmstrom et al. 2004), and predation avoidance (Pernthaler et al. 1997; Jürgens et al. 1999). Although these broad phylogenetic groups consist of several species and strains (the true units of natural selection), they are still potentially useful in ecological studies, particularly in ecosystem and biogeochemical modeling. In addition, results from the major group-level analysis could provide a guide for identifying the key subgroups at finer phylogenetic levels because we still do not know which bacterial species in the major groups are most important.

We found that some phylogenetic groups of bacteria have growth rates much higher than others. This result supports the hypothesis that some groups have competitive advantages over co-existing groups depending on environmental condition. The phylogenetic groups with the highest growth rates include alpha- and gamma-proteobacteria at both low- and high-salinity stations and beta-proteobacteria at the low-salinity station. Although the reason why growth rates vary remains unclear, one possibility is the quality and quantity of DOM; both vary greatly in estuarine environments such as the Delaware Bay (Mannino and Harvey 2000). Even broad bacterial groups, such as those examined by this study, appear to differ in the uptake of specific DOM compounds (Cottrell and Kirchman 2000; Kirchman et al. 2004).

A few previous studies have reported growth rates of bacterial groups in marine and freshwater environments (Table 6). Consistent with our data, these studies have generally shown that group-specific growth rates can be much greater than those of total communities. Notably, gamma-proteobacteria are often reported to grow most rapidly in dilution cultures (Eilers et al. 2000; Fuchs et al. 2000; Beardsley et al.

Table 5. Contribution of each phylogenetic bacterial group to total bacterial production. The abbreviations are explained in Table 3. ND, not detected.

| Station | Month | % total bacterial production | | | | | |
|---|---|---|---|---|---|---|---|
| | | Eub | Alpha | Beta | Gamma | CF | Total of four phylogenetic groups |
| River | June | 60 | 4 | 44 | 3 | 8 | 59 |
| | August | 71 | 13 | 34 | 20 | 4 | 71 |
| | December | 70 | 15 | 32 | 0 | 29 | 76 |
| Bay | June | 66 | 12 | 0 | 29 | 11 | 52 |
| | August | 63 | 18 | 5 | 8 | 8 | 39 |
| | December | ND | ND | ND | ND | ND | ND |

Table 6. Specific growth rate of bacterial groups in aquatic systems. The groups include eubacteria (Eub), alpha- (Alpha), beta- (Beta), and gamma- (Gamma) proteobacteria; and the *Cytophaga-Flavobacter* cluster (CF). Total specific growth rate is the growth rate of DAPI-positive cells. —, no data reported.

| System | Treatment | Specific growth rate of bacterial group ($d^{-1}$) | | | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | Total | Eub | Alpha | Beta | Gamma | CF | |
| Lake | Filtration (5- or 8-$\mu$m filter) | 1.1 | 0.9 | 1.4 | 1.7 | 1.3 | 2.7 | Jürgens et al. 1999 |
| Estuary | Dilution (salinity, 0.1) | 1.7–3.0 | 1.7–3.2 | 3.2–6.1 | 2.9–3.8 | 0.0–3.8 | 1.0–5.1 | This study |
| | Dilution (salinity, >26.5) | 0.4–3.5 | 0.2–4.1 | 0.0–5.5 | 0.0–2.1 | 1.3–4.3 | 1.1–2.5 | This study |
| | Dilution (14°C) | 2.3 | 3.2 | 2.6 | 0.0 | 4.0 | 1.4 | Fuchs et al. 2000* |
| | Dilution (24°C) | 2.3 | 3.8 | 3.9 | 0.0 | 4.6 | 2.3 | Fuchs et al. 2000* |
| Ocean | Filtration (1.2-$\mu$m filter) | 0.5 | 1.0 | 1.0 | — | 1.1 | 0.5 | Eilers et al. 2000† |
| | Filtration (1.2-$\mu$m filter) + substrate addition | 0.6 | 1.1 | 0.5 | — | 1.8 | 0.9 | Eilers et al. 2000† |
| | Dilution | 1.9 | — | — | — | 2.5–2.9‡ | — | Beardsley et al. 2003§ |

* Growth rates were calculated from the data presented in Fuchs et al. (2000, fig. 2).
† Growth rates were calculated from the data presented in Eilers et al. (2000, fig. 1).
‡ Probes specific at the genus level of *Alteromonas, Pseudoalteromonas,* and *Vibrio* were used.
§ Growth rates were calculated from the data presented in Beardsley et al. (2003, figs. 1, 3).

2003), a pattern that is partly consistent with our data; gamma-proteobacteria exhibited the highest (June and December at Bay Station) or nearly the highest (August at River Station) growth rates. However, depending on the site and season, our data also show that the growth rates of other groups, including alpha-proteobacteria (August at Bay Station and June and December at River Station) and the *Cytophaga-Flavobacter* cluster (June at River Station), are higher by >1.5-fold than those of gamma-proteobacteria. These data suggest that the faster growth rate of gamma-proteobacteria in dilution cultures is not a general rule in aquatic systems.

Potential problems of the dilution culture and other bottle confinement experiments include the selective enhancement of specific bacterial groups, particularly when the incubation period is long (>24 h; Ferguson et al. 1984; Eilers et al. 2000; Fuchs et al. 2000). Bottle confinement effects could be a consequence of multiple processes, including artificial enrichment of substrates during filtration and handling, effects of interfaces (walls) on bacterial activity, and changes in initial microbial compositions (e.g., elimination of particle-attached bacteria) because of selective filtration (Ferguson et al. 1984). To what extent these factors affect bacterial production might vary depending on the environment and incubation period. In our experiments, however, we suggest that perturbation of the organic substrate regime was minimal because care was taken to minimize the filtration pressure and contamination, and the incubation period was kept relatively short (12 h, except for December). In support of this hypothesis, we detected no significant changes in average cell volume of bacterial cells during the incubation, suggesting that physiological changes were minimal. Furthermore, production rates estimated by the dilution method generally agreed well with those derived by the TdR method. Thus, enhancement of bacterial production because of artificial changes in substrate conditions appears to have been minimal in our experiments with the use of productive estuarine waters.

The inability to detect dormant bacteria could impose another limitation on our experimental approach. The lower growth rate of bulk (DAPI-positive) communities might be due to the presence of dormant or inactive cells that are difficult to detect with the oligonucleotide probes (Karner and Fuhrman 1997). Consequently, some of the apparent increase in relative abundance of specific groups might be ascribed to increased ribosome content and thus enhanced detection by FISH (Klappenbach et al. 2000). However, changes in the percentage of Eub338-positive bacteria during the incubation were usually much smaller (range 1.0–1.3-fold) than changes in the percentages detected by the group-specific probes (about 4-fold, range 1.6–9.0-fold) for the phylogenetic groups with the highest growth rate (Table 3). Thus, it is unlikely that increases in detectability explain the rapid increase in cell abundances of individual bacterial groups during incubation.

Our data indicate that growth rate, and thus bottom-up factors, in part explain the relative abundance of beta-proteobacteria in the estuary. The growth rates of this group always exceeded the growth rate of the total community at the River Station site, where beta-proteobacteria accounted for a large fraction (24 ± 2%) of total cell abundance, whereas corresponding rates were either low (60% of the total community growth rate) or negative at the Bay Station site, where beta-proteobacterial abundance was low (11 ± 6%). In contrast, no such relationship between growth rate and numerical dominance was observed for alpha-proteobacteria and other groups. Consistent with our data, Cottrell and Kirchman (2004) found a significant positive correlation between the fraction of cells assimilating [$^3$H]-thymidine and the numerical dominance for beta-proteobacteria, but not for other groups. These results suggest that bottom-up controls

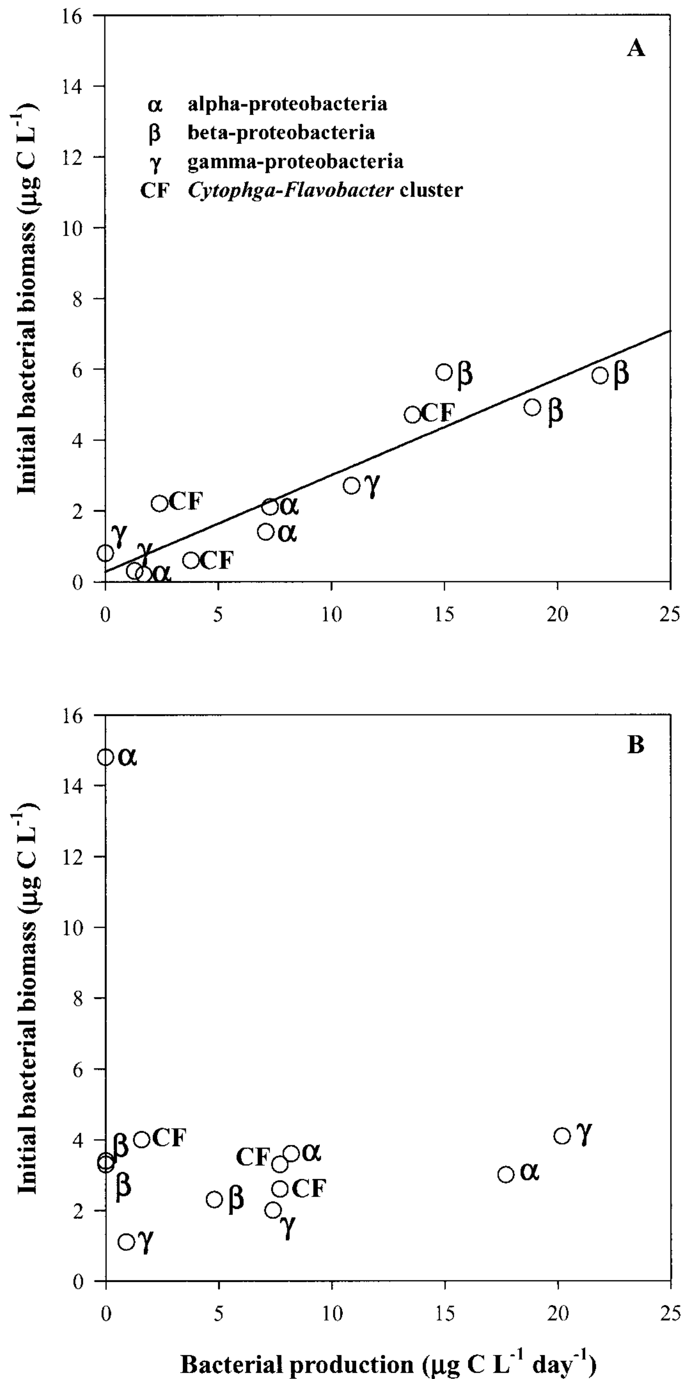


Fig. 3. The relationship between biomass at the beginning of the incubation and production at (A) River Station and (B) Bay Station. Individual plots represent data for individual phylogenetic groups. All the data collected in different months are pooled. The linear regression equation for data collected at River Station is Biomass = 0.27 (±0.03 SE) × Production + 0.28 (±0.39) ($n$ = 12, $r^2$ = 0.86, $p < 0.001$). The Pearson correlation between biomass and production was not significant ($p$ = 0.49) for the data collected at Bay Station.

partially explain the distribution of beta-proteobacteria in the estuary, but our data also indicate that other controls, such as top-down factors, are probably important.

To gain more insight into the relative importance of bottom-up and top-down forces in determining community composition, the relation between biomass and production for each phylogenetic group was examined for each region (Fig. 3). This analysis is based on the concept originally developed by Billen et al. (1990), who postulated that bacterial production can be taken as an index of the flux of the limiting resource (generally, organic carbon) available to bacteria and that the regulation of bacteria by resource and mortality can be assessed by examining how biomass changes along a resource gradient. As suggested by Pace and Cole (1994), the greater the resource (bottom-up) control, the steeper the slope in graphs of biomass versus the resource gradient (biomass production). In contrast, high mortality (top-down) control leads to a less steep slope.

Our results indicate that bacterial biomass ($B$) increases with increasing bacterial production ($P$) at River Station ($B = 0.27P + 0.28$, $r^2 = 0.86$, $n = 12$, $p < 0.001$), whereas there was no significant correlation ($p = 0.49$) between $B$ and $P$ at Bay Station. On the basis of the theoretical considerations discussed above, these results suggest that bacterial communities in the freshwater region were controlled by substrate supply, whereas top-down factors (grazing and viral lysis) were more important in regulating bacterial communities in the seawater region. This analysis leads us to hypothesize that not only bacterial community composition but also the regulation of community structure in fresh- and seawater regions of the estuary differ. This regulation could shift because of ecological and biogeochemical gradients, including the abundance and grazing activity of heterotrophic protozoa and concentrations and compositions of DOM (Mannino and Harvey 2000). Clearly, we need more data to clarify the link between environmental variables and controls of bacterial community structure in estuarine environments.

Bacterial growth rate is the fundamental parameter that links biomass and production and is a sensitive indicator of the substrate (DOM) regime for bacterial communities. We demonstrated that dilution culture–based measurements of the growth rate of different groups of bacteria, detected by FISH at a broad phylogenetic level, provide valuable information regarding group-specific characteristics of bacterial growth and the possible mode of the regulation of bacterial communities in estuarine environments. The data presented in this manuscript are among the first to demonstrate the spatiotemporal variability in growth rates of phylogenetically distinct subgroups of bacterioplankton. Results demonstrated also a clear difference in the mode of regulation of bacterial communities between freshwater and seawater sites of the estuary. Even though the geographic and seasonal coverage is not high enough to resolve smaller scale (e.g., weekly) variability, we believe that the data set presented in this manuscript has its own value. These results have important implications for understanding microbial dynamics and biogeochemical cycling in aquatic systems such as the Delaware estuary.

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