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# Planktonic and bacterial respiration along an estuarine gradient: responses to carbon and nutrient enrichment

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ABSTRACT: Previous observations have revealed that rates of plankton community respiration vary consistently along the main salinity gradient of Chesapeake Bay, USA, with maximum values occurring in the mesohaline region and significantly lower rates in both the oligohaline and polyhaline regions. This study tested the hypothesis that spatial variations in respiration are the result of differential resource limitation of the bacterial communities along the land-sea gradient. Effects of organic carbon and inorganic nutrients on microbial (<3 µm) respiration rate were assessed in short-term (6 h) enrichment bioassays. Responses of bacterioplankton to enrichment were also investigated by means of re-growth culture experiments. Both sets of experiments were conducted during summer in 3 regions along the main salinity gradient of the estuary. Within the re-growth cultures, there was little variation in, or effect of enrichment on, specific growth rates, which were roughly 2 $d^{-1}$ in all cultures. In contrast, estimates of cell-specific respiration varied 2-fold along the salinity gradient (from 3.3 to 6.9 fg $O_2$ $cell^{-1}$ $h^{-1}$ in the Upper and Mid Bay regions, respectively) and showed significant responses to substrate enrichment. Resulting estimates of bacterial growth efficiencies exhibited similar variability, ranging from 20.4 to 41.3%, with spatial variations in efficiencies apparently related to nutrient concentrations. Results of substrate enrichment in both short-term bioassays and bacterioplankton re-growth cultures demonstrated a pattern of stimulation by organic carbon in the Upper Bay and by inorganic nutrients in the Lower Bay, with no significant response to substrate enrichment in the Mid Bay. Patterns of bacterioplankton response to enrichment in the nutrient-poor polyhaline region suggested a propensity for the bacterial community to maximize growth, rather than efficiency, via changes in specific respiration rates.

KEY WORDS: Respiration · Bacterioplankton · Nutrient effects · Growth efficiency · Estuaries



## INTRODUCTION

Respiration is the essential counterpart to primary production and likely represents the largest sink for organic matter in aquatic ecosystems. Traditionally, ecological research has focused on primary production, with little emphasis on respiration. While there is now a growing number of studies describing variability in the balance between production and respiration within pelagic communities (e.g. Duarte & Agustí 1998), and a plethora of information on the factors affecting rates of primary production (e.g. Valiela 1995), very limited information exists as to what may influence variations in plankton community respiration (Sampou & Kemp 1994, Pomeroy et al. 1995). The controls on respiration may be quite different from those that regulate production and, thus, affect carbon flow within ecosystems in very different ways. Understanding what factors influence the magnitude and variabil-

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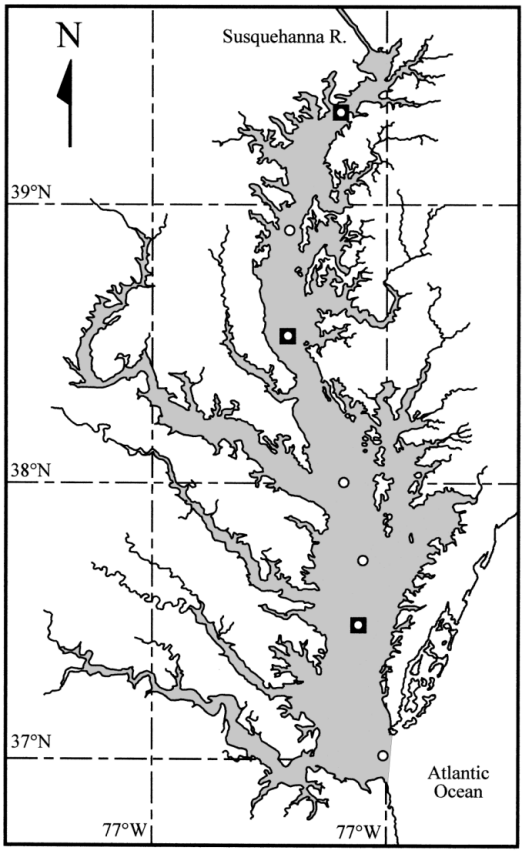


Fig. 1. Sampling locations of Chesapeake Bay (shaded area) for measurements of ambient respiration rate (○) and experimental enrichment studies (■)

ity of respiration is critical to fully understanding the role of pelagic biota in global carbon cycling.

Bacteria are responsible for much of the total respiration in planktonic communities (Williams 1984, Biddanda et al. 2001). The flow of organic carbon through the bacterial community can, however, vary from close to 0–>100% of *in situ* primary production (del Giorgio et al. 1997). This would suggest that factors that affect bacterial metabolism might greatly influence the balance between that fraction of organic matter that is respired within the plankton community and that which remains available for export or transfer to higher trophic levels. Until recently, most studies investigating the limitation of bacterial activity have been based on measures of bacterial production rates, without direct measurements of respiration (Jahnke & Craven 1995). Studies that have simultaneously quantified bacterial production and respiration indicate highly variable relationships between the two (Chin-Leo & Benner 1992, Biddanda et al. 1994). Information on the factors limiting bacterial production (e.g. Shiah & Ducklow 1994) may, therefore, be of little help in understanding what regulates bacterial respiration.

As a consequence of inputs and mixing of fresh and oceanic water, estuarine ecosystems are characterized by strong gradients in concentrations of organic carbon and inorganic nutrients (Day et al. 1989). Pronounced variability in respiration rates along a salinity gradient appears to be a common occurrence in estuarine ecosystems (Jensen et al. 1990, Chin-Leo & Benner 1992, Iriarte et al. 1997). These variations in respiration may strongly affect the fate of autochthonous and allochthonous organic matter transported between estuaries and the coastal ocean. Previous studies in the Chesapeake Bay revealed that plankton respiration rates were consistently highest in the mesohaline region, with significantly lower rates in adjacent oligohaline and polyhaline regions (Smith & Kemp 1995, Smith 1998). The objective of the present study was to test the hypothesis that these spatial variations in respiration result from bacterial resource limitation along the salinity gradient. The research approach involved experimental enrichments of organic carbon and inorganic nutrients on: (1) the short-term (6 h) respiration response of the microplankton (<3 µm) community; and (2) the specific physiological response of the bacterioplankton community as assessed by re-growth cultures (sensu Ammerman et al. 1984).

## MATERIALS AND METHODS

**Respiration rates.** A survey of total plankton community respiration rates was conducted at 7 stations along the main salinity gradient of Chesapeake Bay, USA, from August 4 to 12, 1998 (Fig. 1). Respiration rates were based on standard dark-bottle $O_2$ consumption rates. $O_2$ concentrations were determined by automated Winkler titration of whole bottle samples using a photometric end-point detection system (Smith & Kemp 1995). Incubations were performed in 300 ml borosilicate glass biological oxygen demand (BOD) bottles (Wheaton), which were acid washed and then rinsed with deionized water prior to use.

Water samples were obtained at each station from surface waters (1 to 2 m below air-sea interface) during morning hydrocasts (07:00 to 10:00 h) using an array of 10 l Niskin bottles mounted on a General Oceanics rosette. Immediately after completion of the sampling hydrocast, water was gently combined, via siphon, from several Niskin bottles into a low density polyethylene (Nalgene) carboy (50 l) to ensure homogeneity of the sample. Water was then siphoned from the carboy into the BOD incubation bottles, which were filled and allowed to overflow to twice the sample volume, and then capped with ground-glass stoppers. Four replicate bottles were fixed with Winkler reagents immediately after filling for initial $O_2$

concentrations, while 4 other bottles were incubated in removable opaque sleeves and incubated in a shipboard flowing-seawater incubator at *in situ* temperatures (±1°C) for 6 h. Within the deck incubator, BOD bottles were allowed to roll freely under the ship's motion, which helped to minimize the potential for settling of particulates during incubation. At the end of the incubation, bottles were fixed with Winkler reagents for final $O_2$ concentrations. Respiration rates were calculated as differences in $O_2$ concentration between initial and final replicate bottles, over the duration of the incubation.

**Enrichment bioassays.** Three stations where chosen to represent the oligo-, meso- and polyhaline regions of the main salinity gradient in Chesapeake Bay during summer. These stations are designated as Upper Bay (39° 22.1′ N, 76° 08.0′ W), Mid Bay (38° 30.0′ N, 76° 12.1′ W) and Lower Bay (37° 29.8′ N, 76° 02.8′ W) (Fig. 1).

Water samples from the surface (1 to 2 m depth) mixed layer were collected from morning hydrocasts, as described above. Immediately after completing the hydrocast, sample water (10 l) was gently subjected to gravity-fed, reverse filtration (Williams 1981, Sampou & Kemp 1994) through a 142 mm diameter, 3 µm pore-size, polycarbonate membrane filter (Poretics). Filtration was generally completed within 1.5 h after initial sampling. The 3 µm filtrate was used for subsequent bioassay measurements of respiration rates. The resulting concentrate of sample water was discarded.

Upon completion of filtration, the 3 µm filtrate was partitioned and 4 treatments were administered: +N treatment, 50 µM additions of $NH_4$ as $(NH_4)_2SO_4$; +P treatment, 5 µM additions of $PO_4$ as $KH_2PO_4$; +C treatment, 500 µM additions of C as glucose; and +NPC treatment, all 3 additions in combination. Incubations were conducted in 60 ml borosilicate glass BOD bottles, with 8 replicates for each treatment and for untreated 'control' water.

Respiration rates in enrichment bioassays were measured as changes in dissolved $O_2$ between initial (n = 4) and final (n = 4) bottles. Incubations were conducted in a flowing-seawater deck incubator at *in situ* temperatures (±1°C) for a period of 6 h. Dissolved $O_2$ concentrations were determined by Winkler titration, as described above.

**Bacterial re-growth experiments.** ***Sampling and culture preparation:*** Surface water samples were collected at the same locations with the same protocol as used for the enrichment bioassays. Bacterial re-growth cultures (sensu Ammerman et al. 1984), involve adding a natural inoculum back to a volume of particle-free (0.2 µm filtered) Bay water, and then following the growth and respiration time-course of the reintroduced bacterial assemblage. Seawater media were collected at each station and prepared for incubations by low-pressure (<7 psi) in-line filtration, first through a pre-combusted and pre-rinsed 142 mm diameter GF/F glass fiber filter (Whatman) and then through a 0.2 µm pore-size filter (Gelman Maxi Culture Capsule, which had been pre-rinsed with 10 l of ultrapure [18 Megohm] deionized water). Filters (GF/F) were replaced periodically to minimize disruption of cells retained on filter. Following 0.2 µm filtration, an inoculum (5% final volume) of 0.8 µm filtered (Gelman pleated capsule filter) sample water was added. C enrichment involved adding glucose to achieve an addition of 500 µM of C, while P enrichment was accomplished by adding $KH_2PO_4$ to achieve an addition of 5 µM of P.

At each station, duplicate treatments and controls were incubated in silicon-stoppered 4.5 l glass serum bottles (Kimax). The stoppers were fitted with 2 glass tubes: one tube was connected to a clamped silicon tube, which served as a sampling port, while the other tube was connected to a 4 l polyethylene collapsible cubitainer (VWR), which served as a reservoir to replace sampled water. The reservoir contained the same culture water as in the serum bottle. This set-up allowed for sample volume removed from the serum bottle to be simultaneously replaced from the reservoir, which would progressively collapse as the sample was drawn, thereby preventing the introduction of a gas headspace that would confound the dissolved $O_2$ measurements. All carboys, filtering devices, glassware and tubing were acid-washed (24 h in 10% $H_2SO_4$) and deionized-water rinsed prior to use. Cubitainers were initially soaked in acid (10% $H_2SO_4$) for 2 d and bathed for 2 d in ultrapure deionized water. This procedure was repeated 3 times to minimize any plastic leachate from the polyethylene material of the cubitainer from being introduced to the sample water. Re-growth experiments were incubated in the dark in a shipboard flowing-seawater incubator at ±1°C of *in situ* temperature. Both replicate re-growth cultures were sub-sampled immediately for initial conditions ($t$ = 0), and then sub-sampled at approximately 8 h intervals for 2 d. Total volume sampled was kept to <20% of the initial culture volume.

***$O_2$ and bacterial abundance measurements:*** Subsamples for dissolved $O_2$ were collected in 60 ml BOD bottles and concentrations of dissolved $O_2$ were determined by Winkler titration, as described above. Immediately after $O_2$ sample collection, a 10 ml sub-sample was taken for bacterial cell density. This was preserved with particle-free 25% glutaraldehyde (1% final concentration) and stored in liquid $N_2$ (e.g. Sherr et al. 1999). Upon completion of the cruise (within 6 d after collection), bacterial samples were thawed and an aliquot was stained with acridine orange (0.01% final concentration; Hobbie et al. 1977) and filtered through

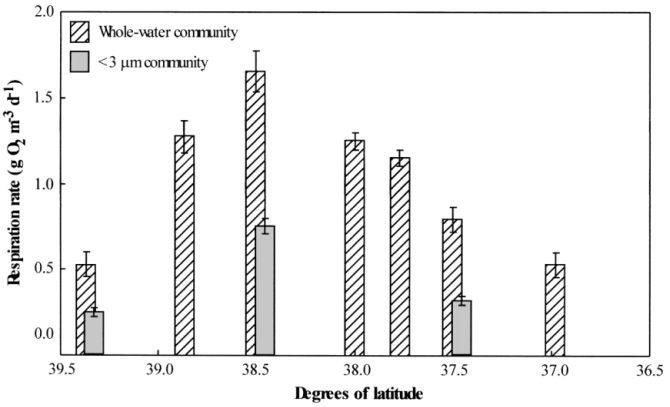


Fig. 2. Distribution of total whole-water plankton community and microplankton community (<3 µm size-fraction) respiration rates with latitude along the central axis of Chesapeake Bay. Error bars represent ±1 SE (n = 4)

25 mm diameter, 0.2 µm pore-size black polycarbonate filters (Poretics) under low vacuum. Filters were mounted in immersion oil (Resolve) on slides and enumerated at 1612× with a Zeiss Axiophot epifluorescence microscope equipped with a 100 W mercury lamp, blue 450 to 490 excitation filter and LP520 barrier filter. For each slide preparation, at least 10 random fields were counted, with a total of 300 to 500 cells counted per slide.

***Data calculations and analyses:*** From the raw $O_2$ and cell density data, estimates of bacterial specific growth rate (µ, $d^{-1}$), cell-specific respiration ($R$ $cell^{-1}$, fg $O_2$ $cell^{-1}$ $h^{-1}$) and growth efficiency (BGE, %) were derived. µ was estimated as the slope of the natural log of bacterial abundance versus time, starting at $t = 0$ and until growth showed evidence of entering stationary phase. $R$ $cell^{-1}$ was estimated as the slope of the change in $O_2$ per time versus the change in cell density per time over the duration of exponential growth. BGE was derived as the slope of the linear regression of bacterial production versus the sum of bacterial production plus respiration. Bacterial production was taken as the change in cell abundance, assuming a cellular carbon content of 20 fg C $cell^{-1}$ (Lee & Fuhrman 1987). Respiration was converted to C units assuming a respiratory quotient of 1 (del Giorgio & Cole 1998). Both measures were thus converted to a common unit and linear regression techniques allowed the use of all data points during re-growth in the calculation of BGE. Total $O_2$ consumption and bacterial yield (relative decrease in $O_2$ and increase in cell numbers, respectively) over the course of the re-growth experiment were also computed. These values were normalized to incubation duration ($t_{final}$), to account for minor differences (<5%) in incubation length among experimental units. The term 'bacterial yield' is used here to refer to the total production of cell over the course of the re-growth culture. Slopes of natural log of bacterial abundance versus time were estimated using ordinary (Model 1) least-squares linear regression, all other slope estimates were derived from geometric mean (Model 2) linear regressions. Analyses of all treatment effects, for both respiration bioassays and re-growth cultures, were determined by ANOVA followed by $t$-tests at a 0.01 probability level. All statistical analyses were performed using the JMP statistical software package (SAS Institute).

## RESULTS

### Spatial pattern of respiration rates

Whole water respiration exhibited a 3-fold range in rates, from 0.53 ± 0.07 to 1.66 ± 0.12 g $O_2$ $m^{-3}$ $d^{-1}$ and exhibited a pattern of highest rates at intermediate distances along the length of the estuary (Fig. 2). Respiration rates within the <3 µm size-fraction (microplankton respiration) at the 3 stations sampled were similarly variable, ranging from 0.23 ± 0.01 to 0.74 ± 0.02 g $O_2$ $m^{-3}$ $d^{-1}$, and were significantly different among stations (ANOVA, $p < 0.01$). These <3 µm respiration rates represented 43.9 to 46.0% of the corresponding whole water rates.

### Nutrient enrichment bioassays

The 3 stations chosen for enrichment experiments encompassed a range of environmental conditions along the salinity gradient (Table 1). Concentrations of dissolved organic carbon (DOC), phytoplankton biomass (as indexed by chlorophyll *a*, chl *a*) and bacterial abundance all followed a pattern among the 3 stations similar to that of respiration, with highest values at the Mid Bay station and lowest values generally at the Upper Bay station. Total dissolved inorganic nitrogen (DIN) and phosphorus (DIP), on the other hand, both showed consistently decreasing concentrations with increasing salinity.

The short-term (6 h) response of microplankton respiration to additions of inorganic nutrients and glucose differed substantially among the three stations. At the Upper Bay station, respiration rates were not significantly different from controls for either of the nutrient (+N or +P) treatments, but increased to roughly 150% of the control rate in the +C and +NPC treatments (Fig. 3). Although both +C and +NPC treatments were significantly different from the control treatment ($p < 0.01$), respiration rates for these 2 treatments were not significantly different from each other. At the Mid Bay

Table 1. *In situ* conditions in the upper mixed layer of each station at the time of sampling for enrichment experiments. See text for specific station locations and dates. DOC = dissolved organic carbon, DIN = $NH_4 + NO_2 + NO_3$, DIP = $PO_4$

| Station | Temp. (°C) | Salinity (psu) | Bacteria ($10^9$ cells $l^{-1}$) | Chl *a* (µg $l^{-1}$) | DOC (µM) | DIN (µM) | DIP (µM) |
|---|---|---|---|---|---|---|---|
| Upper Bay | 27.4 | 2.8 | 5.0 | 4.38 | 286.6 | 36.20 | 0.60 |
| Mid Bay | 25.9 | 13.1 | 8.4 | 9.10 | 313.1 | 0.39 | 0.17 |
| Lower Bay | 26.5 | 17.4 | 6.9 | 4.88 | 234.1 | <0.01[a] | 0.02 |

[a]Below limits of detection

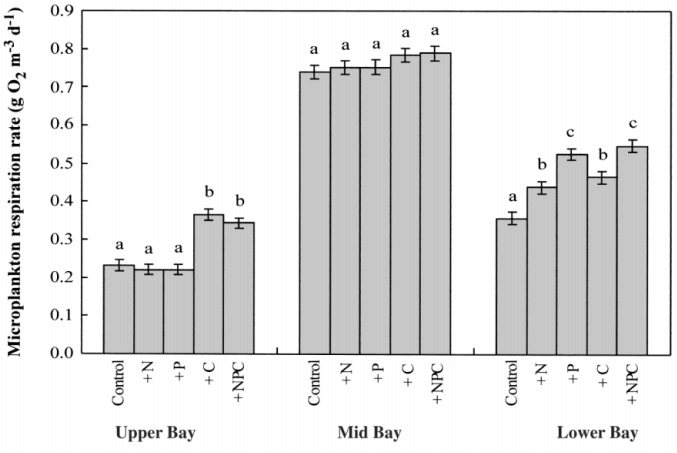


Fig. 3. Treatment comparisons of short-term enrichment effects on microplankton (<3 µm size-fraction) respiration rate at the Upper Bay, Mid Bay and Lower Bay stations. Error bars represent ±1 SD, calculated using a pooled estimate of error variance (n = 4). Treatment means with the same letter not significantly different at the $p < 0.01$ level of significance, for within station comparisons only

station, there were no significant short-term responses in respiration to any of the enrichment treatments (Fig. 3). Although there was a suggestion of a small response to C treatment at this station, it was not significant at the $p < 0.05$ level. In contrast, significant increases in respiration rate occurred in all the enrichment treatments at the Lower Bay station (Fig. 3). Microplankton respiration response ranged from 123 (+N) to 154% (+NPC) of the mean value observed in the control treatment. Among the individual amendments, the +P treatment exhibited the greatest response in respiration rate (148% of the control) and this response was not significantly different than that of the +NPC treatment, suggesting no additive effect of the treatments in combination.

## Bacterial re-growth cultures

In all cultures, the bacterial assemblage showed a significant increase in cell numbers before exhibiting signs of entering a stationary phase (data for the control treatments at each station are shown as examples in Fig. 4). Increases in bacterial density were always accompanied by commensurate decreases in $O_2$ concentration. Indeed, the 2 measures were highly correlated in all experiments, with correlation coefficients (r) of −0.97 or better. Initial bacterial densities in the 3 cultures were roughly 3 to 4% of measured *in situ* cell densities. Initial $O_2$ values were generally similar to *in situ* concentrations, and within ±15% of saturation values, although filtration had the effect of slightly increasing $O_2$ content of sample water.

Comparisons of unamended control treatments indicate the different behaviors of the bacterial assemblages at each station (Table 2). Estimates of µ ranged from 1.7 to 2.3 $d^{-1}$, but values were not significantly different among stations (at the $p < 0.01$ level). µ did not covary with the final yield of cell numbers, however. Cell yield was significantly higher at the Mid Bay

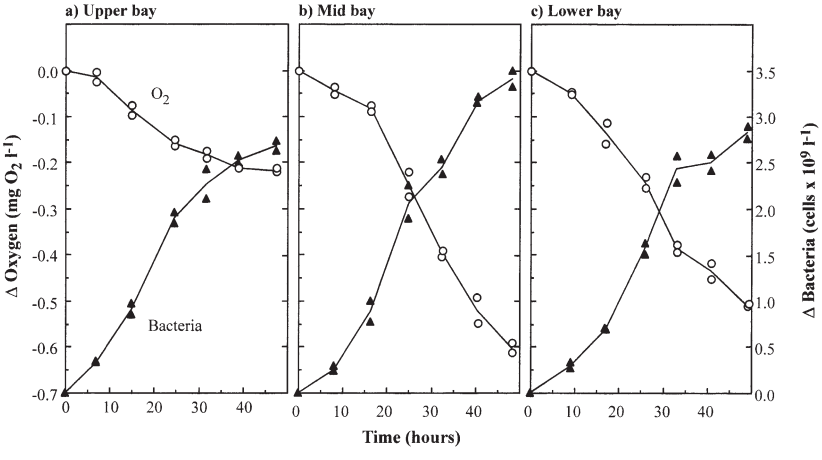


Fig. 4. Changes in bacterial abundance (▲) and $O_2$ concentration (○) versus incubation time in unamended control cultures at the (a) Upper Bay, (b) Mid Bay and (c) Lower Bay stations. Individual symbols represent data from each replicate culture, while solid lines connect mean values

Table 2. Characteristics of unamended control cultures in bacterial re-growth experiments. Growth rate, μ, calculated as the slope of natural log of bacteria versus time, for the period of exponential growth until stationary phase. Cell-specific respiration rate, $R$ cell$^{-1}$, calculated as the slope of $\Delta O_2$ versus Δbacteria per time, for the period of exponential growth. See 'Materials and methods' for details. Values in parentheses are standard deviations of the 2 replicate cultures

| Variable | Upper Bay | Mid Bay | Lower Bay |
|---|---|---|---|
| Incubation duration (d) | 1.98 | 2.02 | 2.05 |
| Initial cell concentration ($10^9$ cells l$^{-1}$) | 0.18 (0.01) | 0.30 (0.05) | 0.26 (0.01) |
| Change in cell concentration ($10^9$ cells l$^{-1}$) | 2.68 (0.01) | 3.41 (0.12) | 2.88 (0.01) |
| Growth rate (d$^{-1}$) | 2.34 (0.08) | 2.04 (0.26) | 1.72 (0.01) |
| Initial $O_2$ concentration (mg $O_2$ l$^{-1}$) | 5.88 (0.03) | 7.59 (0.01) | 6.19 (0.01) |
| Change in $O_2$ concentration (mg $O_2$ l$^{-1}$) | −0.22 (0.01) | −0.60 (0.02) | −0.51 (0.01) |
| Cell-specific respiration rate (fg $O_2$ cell$^{-1}$ h$^{-1}$) | 3.34 (0.07) | 6.87 (0.1) | 5.03 (0.11) |

station compared with either the Upper or Lower Bay stations. Total $O_2$ consumption also varied significantly among stations. This resulted from differences both in cell yield and respiration rate per bacterial cell among stations. Estimates of bacterial cell-specific respiration, $R$ cell$^{-1}$, ranged from 3.34 to 6.87 fg $O_2$ cell$^{-1}$ h$^{-1}$ and exhibited significant ($p < 0.01$) spatial variability among stations.

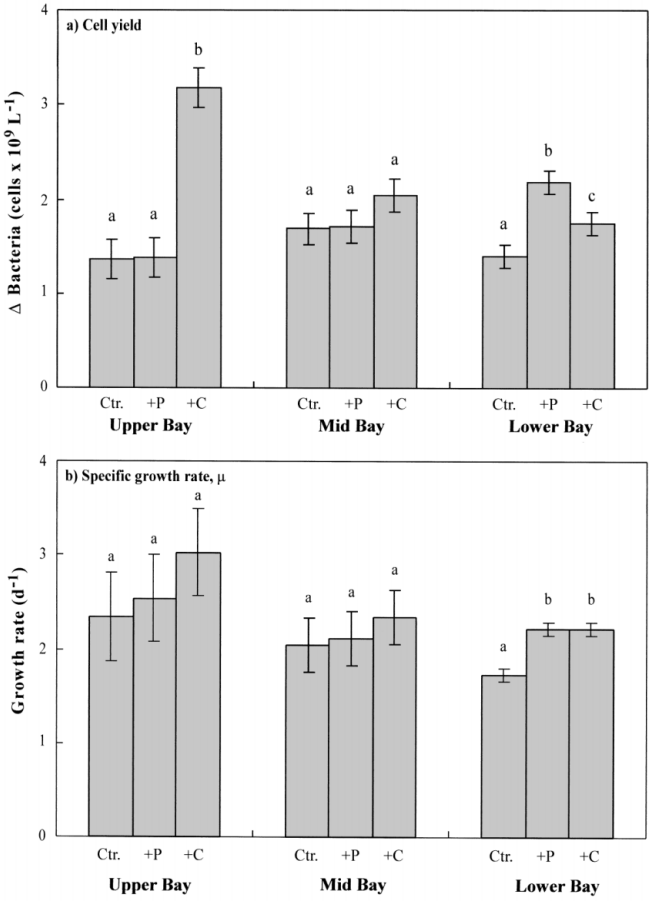


Fig. 5. Treatment comparisons of (a) cell yield and (b) specific growth rate, μ, during re-growth cultures at each station. Error bars represent ±1 SD, calculated using a pooled estimate of error variance (n = 2). Treatment means with the same letter not significantly different at the $p < 0.01$ level of significance, for within station comparisons only

Differences among stations in the effects of experimental enrichment on bacterial re-growth cultures were essentially the same as those observed in the respiration bioassays (Fig. 3), both with respect to patterns in growth (Fig. 5) and respiration (Fig. 6). For logistical reasons, the responses to the +N treatment were not included, however, in the re-growth study. The omission of N enrichment was based on results of a preliminary experiment the previous summer (data not shown), which were consistent with the observation that respiration responses were more pronounced for P than for N (Fig. 3). In general, the +C treatments produced a significant response for both growth and respiration at the Upper Bay station, while both +C and +P treatments produced responses at the Lower Bay station. In contrast, neither substrate addition elicited a significant response in either growth or respiration at the Mid Bay station.

Specifically, in the Upper Bay the +C treatment produced the largest effect on cell yield, which increased from 1.36 ± 0.04 to 3.18 ± 0.14 × $10^9$ cells l$^{-1}$ d$^{-1}$ with C addition (Fig. 5a). μ, while more variable, showed no significant increase with C addition (Fig. 5b). Similarly, the +C treatment showed a significant increase in total $O_2$ consumption (Fig. 6a), but estimated R cell$^{-1}$ rates did not differ between the control and +C treatments (Fig. 6b). The effects of the +P treatment were not significantly different (at the $p < 0.01$ level) from the control treatment for any of the measured response variables at this station.

For the Lower Bay station, +P and +C treatments resulted in contrasting effects. Both treatments significantly increased total cell yield (Fig. 5a), relative to that of the control, although this effect was significantly greater for the +P treatment (2.18 ± 0.08 × $10^9$ cells l$^{-1}$ d$^{-1}$) than for the +C treatment (1.73 ± 0.04 × $10^9$ cells l$^{-1}$ d$^{-1}$). These 2 treatments also produced an increase in growth rate (Fig. 5b), although in this case,

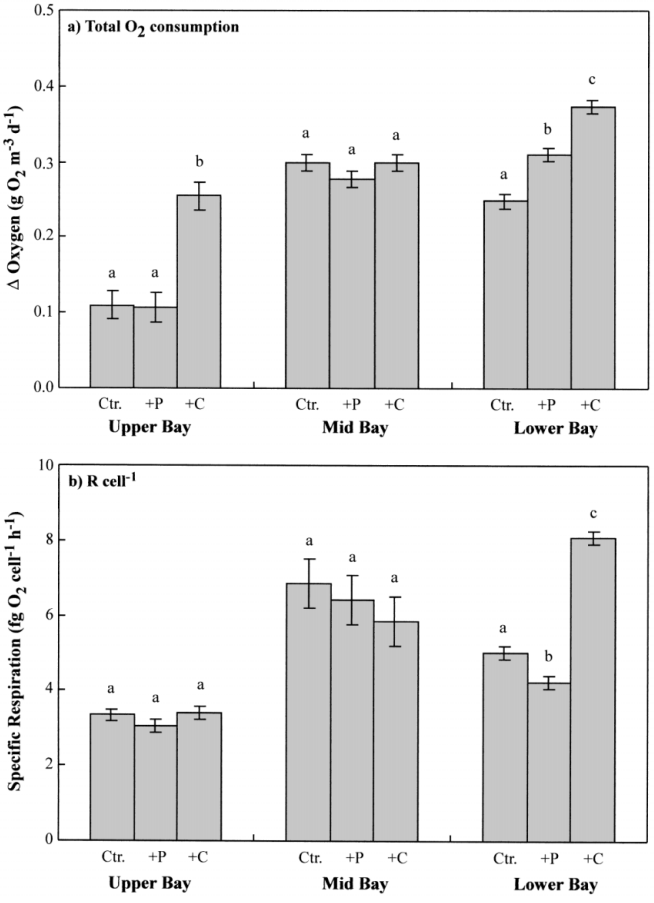


Fig. 6. Treatment comparisons of (a) total $O_2$ consumption and (b) cell-specific respiration rate, $R$ cell$^{-1}$, during re-growth cultures at each station. Error bars represent ±1 SD, calculated using a pooled estimate of error variance (n = 2). Treatment means with the same letter not significantly different at the $p < 0.01$ level of significance, for within station comparisons only

both treatments resulted in similar relative increases. Total $O_2$ consumption exhibited significant increases in response to both treatments (Fig. 6a), but with a pattern opposite that for total cell yield (compare with Fig. 5a). In this case, total $O_2$ consumption was significantly greater in the +C treatment (0.37 ± 0.01 mg $O_2$ $l^{-1}$ $d^{-1}$) than in the +P treatment (0.31 ± 0.01 mg $O_2$ $l^{-1}$ $d^{-1}$). Consequently, cell-specific respiration rates (Fig. 6b) increased dramatically relative to controls with C addition (8.07 ± 0.08 fg $O_2$ cell$^{-1}$ $h^{-1}$), but decreased significantly with P addition (4.23 ± 0.03 fg $O_2$ cell$^{-1}$ $h^{-1}$).

The tight relationship between changes in $O_2$ concentration and bacterial abundance (above) allowed us to estimate values of BGE, calculated as 100 times the slope of the regression (Model 2) of production versus production + respiration. Overall, estimates of BGE ranged from 20.4 to 41.3% (Table 3), with values in the unamended control treatments decreasing from the Upper (39.3%) to Lower (24.1%) Bay stations. Substrate enrichment resulted in no significant effects on BGE at either the Upper or Mid Bay stations. At the Lower Bay station, however, there was a significant increase in BGE with +P treatment (27.7 ± 0.6%) and a significant decrease in BGE with +C treatment (20.4 ± 0.5%), relative to controls (24.1 ± 0.8%).

Table 3. Bacterial growth efficiencies (BGE) calculated as 100 times the slope of the geometric mean regression of production versus (production + respiration). See 'Materials and methods' for details. Also given are the standard errors (SE) of the regression slopes, the number of data points in each regression (n) and the regression coefficient ($r^2$). All regressions are significant at the $p < 0.001$ significance level

| Stn/Treatment | BGE (%) | SE (%) | n | $r^2$ |
|---|---|---|---|---|
| **Upper Bay** | | | | |
| Control | 39.3 | 0.8 | 14 | 0.99 |
| +P | 41.3 | 1.8 | 14 | 0.97 |
| +C | 40.0 | 0.7 | 14 | 0.99 |
| **Mid Bay** | | | | |
| Control | 27.8 | 1.3 | 14 | 0.97 |
| +P | 26.9 | 1.1 | 14 | 0.98 |
| +C | 27.2 | 1.0 | 12 | 0.98 |
| **Lower Bay** | | | | |
| Control | 24.1 | 0.8 | 14 | 0.99 |
| +P | 27.7 | 0.6 | 14 | 0.99 |
| +C | 20.4 | 0.5 | 14 | 0.99 |

## DISCUSSION

Most of the allochthonous loading to the main stem of Chesapeake Bay occurs via the Susquehanna River at the head of Upper Bay region (see Fig. 1) and this results in decreasing nutrient concentrations with increasing salinity (Harding 1994; Table 1). Associated with this nutrient gradient is a strong trend of decreasing ratios of allochthonous to autochthonous sources of organic C input moving down the length of the estuary (Kemp et al. 1997). Along this physio-chemical gradient, there is also a distinct spatial trend of net heterotrophy (primary production < total community respiration) in the Upper Bay, relatively balanced metabolism in the Mid Bay and pronounced net autotrophy (primary production > total community respiration) in the Lower Bay (Smith & Kemp 1995, Kemp et al. 1997). The 3 stations chosen for experimental enrichment studies thus represent the range of trophic conditions experienced, not only in Chesapeake Bay, but also in estuarine systems in general (Smith & Hollibaugh 1993).

A pattern wherein maximum plankton community respiration rates occur at intermediate distances along the salinity gradient (Fig. 2) appears to be a consistent characteristic for Chesapeake Bay (Smith 1998), as well as other coastal systems (e.g. Chin-Leo & Benner 1992). In the Chesapeake, the distribution in respiration rates is similar to that observed for phytoplankton productivity (Kemp et al. 1997, Harding et al. 2002), such that spatial distributions of plankton community production and respiration rates tend to be well correlated to each other (unpubl. data). This would suggest that the low rates of respiration in both the Upper and Lower Bay are simply a response to decreased *in situ* primary productivity in these same regions. However, a temporal comparison of rates within the different regions (Smith & Kemp 1995) revealed that plankton community production ($P_p$) and respiration ($R_p$) in the Upper Bay were strongly correlated ($r^2 = 0.82$) even though both rates were minimal in this region, and often times $R_p > P_p$. In contrast, for the Lower Bay, $P_p > R_p$, but the 2 rates were poorly correlated ($r^2 = 0.21$) and the decrease in mean $R_p$ from the Mid to Lower Bay was much greater than the corresponding decrease in $P_p$. From this, one must conclude that variations in $R_p$ are not simply a function of spatial differences in $P_p$.

Combined results of experimental enrichment in both respiration bioassays (Fig. 3) and re-growth experiments (Figs. 5 & 6) offer a strong basis for explaining these contrasts in respiration rate and their coupling to primary production, in regions experiencing widely differing environmental conditions. On one hand, enrichment bioassays, which have long been used to investigate factors limiting phytoplankton growth (e.g. Hecky & Kilham 1988) and more recently, bacterial activity (e.g. Pomeroy et al. 1995), provide a short-term index of physiological state in relation to substrate availability. On the other hand, re-growth methods, which have been widely used for determining bacterial growth, respiration and substrate utilization (e.g. Carlson et al. 1999, Raymond & Bauer 2000), as well as for the determination of conversion factors used for leucine and thymidine incorporation to bacterial production (Kirchman & Ducklow 1993), offer an integrated longer-term measure of specific bacterial activity under different environmental conditions. Although the re-growth technique is not without potential problems associated with 'enclosure effects' (del Giorgio & Cole 1998), including possible changes in bacterial species composition (Suzuki 1999, Massana et al. 2001), it provides the most unambiguous estimates of cell-specific bacterial respiration rates. This is because, in contrast to ambient waters where many bacteria are virtually inactive (e.g. Sherr et al. 1999), re-growth cultures, by definition, contain only active cells. Here, we used the combination of short-term bioassays and longer-term re-growth cultures to provide a relatively robust approach to investigate resource limitation for heterotrophic microbial communities under contrasting environmental conditions.

Experimental bioassay enrichments in the Upper Bay strongly suggest that heterotrophic bacterioplankton activity during summer was significantly limited by availability of organic C, rather than inorganic nutrients. This is surprising because concentrations of river-borne allochthonous DOC tend to be relatively high in this region (e.g. Kemp et al. 1997) and several studies have shown estuarine bacteria are capable of utilizing riverine DOC inputs (e.g. Hopkinson et al. 1998). Previous observations of net heterotrophy for the Upper Bay plankton also suggest the importance of allochthonous C in support of $R_p$ (Smith & Kemp 1995). Results of the present study indicate that heterotrophic microplankton may, however, have a limited ability to consume and respire the allochthonous material available during the summer. When presented with a readily utilizable substrate, such as glucose, short-term respiration rates within the microplankton quickly increased (Fig. 3). This is consistent with the report that bacterial production in Chesapeake Bay shifts from temperature limitation to resource limited at temperatures above roughly 20°C (Shiah & Ducklow 1995). In a similar fashion, the strong correlation reported between $P_p$ and $R_p$ in Upper Bay plankton suggests heterotrophic metabolism can respond readily to even small increases in autochthonous production (Smith & Kemp 1995). C limitation of heterotrophic metabolism has also been reported for the oligohaline reaches of the Mississippi River estuary, where *in situ* respiration rates were also minimal (Chin-Leo & Benner 1992). In the present study, results of re-growth cultures corroborated those from the enrichment bioassays, providing further physiological insights on regulating mechanisms. Glucose addition in re-growth cultures resulted in equivalent >2-fold increases in both total cell yield (Fig. 5a) and total $O_2$ consumption (Fig. 6a), but had no effect on cell-specific rates (Figs. 5b & 6b). This suggests C limitation of the ecosystem's carrying capacity for bacteria. That is to say, the addition of C substrate did not increase the bacterial community's per capita rate of consumption, but rather its total attainable biomass.

Ambient rates of both total plankton and microplankton respiration rates were highest in the Mid Bay (Fig. 2). In this region, typical of highly productivity environments, substrate enrichment failed to simulate any significant responses in either the respiration bioassays (Fig. 3) or the re-growth cultures (Figs. 5 & 6), suggesting that the heterotrophic microplankton in this region were relatively replete with respect to these

resources. During summer, rates of plankton community production and respiration in the Mid Bay tend to be at their seasonal maximum, with rates that are both closely balanced and dominated by the smaller (<3 µm) size class of organisms (Smith & Kemp 2001). Thus, the lack of evidence for resource limitation, at least on the time scales of the present experiments, is consistent with the idea of a tightly coupled microbial food web, and the suggestion of grazer control on bacterioplankton in this portion of the Bay (Ducklow & Carlson 1992).

In the Lower Bay, bioassay enrichment with N, P and C all stimulated microplankton respiration (Fig. 3), with the greatest response to individual additions observed in the +P treatment. These results are not consistent with the idea of a single limiting nutrient (sensu Liebig 1840), but can readily be explained by recognizing that the microbial community sampled is diverse and heterogeneous (e.g. Bouvier & del Giorgio 2002). Different taxonomic and functional groups of the bacterioplankton within a given sample may respond differently to different substrate enrichments. Nonetheless, the results indicated that overall microplankton respiration was most limited by inorganic nutrients, and most affected by P addition. P limitation of heterotrophic bacterial activity has also been reported for other coastal environments (e.g. Zweifel et al. 1993, Pomeroy et al. 1995, Thingstad et al. 1998), for lakes (e.g. Gurung & Urabe 1999) and for open ocean waters (e.g. Cotner et al. 1997). Heterotrophic bacteria tend to have high P requirements relative to phytoplankton (e.g. Bratbak 1985), and the ability of bacterioplankton to utilize and respire *in situ* primary production might be limited by the availability of nutrients, and P in particular, in relatively dilute estuarine waters, such as the polyhaline region of Chesapeake Bay.

From the effects of enrichment in the re-growth cultures, it appears that there was a substantial interaction between organic C utilization and the ability of bacterioplankton cells to meet P demands in the Lower Bay region. Organic C use in bacteria goes not only to the anabolic reactions of biomass synthesis, but also to the catabolic reactions that generate energy (i.e. ATP). Bacterial utilization of energy in reactions not associated with growth has been shown to be highly variable and can be particularly large when growth itself is constrained (Russell &Cook 1995). Cell-specific respiration ($R$ cell$^{-1}$) is a measure of that energy utilization. The increase in R cell$^{-1}$ suggests that the added glucose was going toward energy production rather than used in 'luxury uptake' for the production of storage compounds (cf. Baxter & Sieburth 1984). It has been posited that maintaining the highest possible energy flow under conditions of constrained growth is advantageous for the resumption of growth should environmental conditions become more favorable (e.g. Morita 1997). It would seem likely that in a nutrient-poor environment, when given an easily utilizable source of energy (i.e. an addition of glucose), bacteria would increase their energy flux to maximize ability to scavenge nutrients from a dilute environment by, for example, increasing membrane-associated active transport systems. Increased energy availability would be particularly useful in facilitating organic P assimilation because hydrolytic breakdown of organic P is an enzyme-intensive process (Ammerman 1991).

Patterns of bacterial growth among stations and in response to enrichment differed from those seen for respiration. In fact, µ showed no significant differences among stations ($p > 0.01$), averaging approximately 2 d$^{-1}$. This value is similar to the mean growth rate (1.75 d$^{-1}$) from a survey of estuarine/coastal waters, which also revealed that growth rates in these environments tended to exceed those of either fresh or marine waters (White et al. 1991). Results of the present study suggest that the bacterioplankton were capable of growing at uniformly high rates throughout the Bay. Variations in measures of respiration, by comparison, were much larger and displayed a spatial pattern similar to that seen for ambient respiration of the total planktonic community (cf. Figs. 2 & 6). While it has been suggested that, in general, bacterial production is more variable than bacterial respiration (e.g. del Giorgio & Cole 1998, Roland & Cole 1999), just the opposite was seen for the spatial differences in this study, a result also observed for bacterioplankton in the Gulf of Mexico (Biddanda et al. 1994). Cell-specific rates reported here are similar to those estimated (1.3 to 6.3 fg $O_2$ cell$^{-1}$ h$^{-1}$) in re-growth cultures from Gulf of Mexico coastal waters (Jørgensen et al. 1999).

The relative variability between rates of bacterial production and respiration resulted in pronounced differences in BGE. Estimates of BGE varied from 24 to 40% (Table 3). These values are within the range reported for other estuarine systems (del Giorgio & Cole 1998) but slightly higher than those reported for the Choptank River (del Giorgio & Bouvier 2002), a less productive sub-estuary of the Chesapeake Bay. The values of BGE reported here are perhaps subject to some uncertainty, given the use of a fixed value for bacterial C content. While 20 fg C cell$^{-1}$ (Lee & Fuhrman 1987) is the value most commonly employed (Ducklow & Carlson 1992), some variation in C cell$^{-1}$ has been reported for natural bacterioplankton (e.g. Zweifel et al. 1993). For example, our estimate of 28% for BGE at the Mid Bay station (Table 3) would have been 15 or 43% if extreme values of 10 or 40 fg C cell$^{-1}$, respectively, were used instead of 20 fg C cell$^{-1}$.

Estimates of BGE nonetheless help summarize interpretations of the bacterial limitation discussed above. Variations in BGE also showed behavior different from either of the individual component terms, with a significant decreasing trend with increasing distance down Bay. Although rates of bacterial metabolism were lowest at the Upper Bay station and appeared to be limited by C availability, the efficiency with which the bacterial community grew was maximal. Organic substrate quality alone thus did not regulate BGE (del Giorgio & Cole 1998). The only variable measured in our study that exhibited a comparable trend to that seen for BGE was the decrease in nutrient concentrations with increasing salinity (Table 1). This supports the hypothesis that the availability of nutrients, more so than C, tends to control BGE in both freshwater (e.g. Benner et al. 1995) and coastal (e.g. Zweifel et al. 1993, Pomeroy et al. 1995) ecosystems. At the Lower Bay station, addition of P significantly increased BGE over that of the controls, while glucose addition significantly decreased BGE. These contrasting patterns derive largely from the substantially different responses in $R$ cell$^{-1}$ between the 2 treatments (Fig. 6b). These results thus support the prediction that bacteria will maximize growth at the expense of efficiency (Vallino et al. 1996) and suggest that plasticity in $R$ cell$^{-1}$ is a key factor in this regard.

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