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Regulation of bacterial growth efficiency in a large turbid estuary

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ABSTRACT: Bacterial secondary production transforms organic C from the environment into new bacterial biomass. Bacterial respiration generates energy and converts assimilated organic C into CO_2. Two decades of research have given us a good understanding of the magnitude and regulation of bacterial production in pelagic ecosystems, but much less is known about bacterial respiration. Bacterial growth efficiency [BGE = BP/(BP + BR)] relates measurements of bacterial production and respiration. Recent reviews demonstrate a large range in BGE among and within systems; the regulation of this variance is not well understood. We made direct measurements of both BP and BR over a full seasonal cycle in the Hudson River, New York, and in a series of manipulative experiments. BGE was well correlated with BP and ranged from 0.04 to 0.66, with a majority (69%) between 0.2 and 0.5. BR and BP were correlated ($r = 0.65$; $p < 0.0001$) but BR was less variable than BP. Thus, much of the variation in BGE could be explained by the variation in BP. The relationship (based on 24 h bioassays) between BP and BGE fit a rectilinear hyperbola [BGE = 0.10 + 0.68BP(5.21 + BP)] and explained 70% of the variation in BGE ($p < 0.001$). During the relatively long incubation (24 h) required to measure BR, conditions diverge from ambient. BP, BR and BGE all increase during this incubation period. We used the relationships between BGE and BP and BR (above) to calculate realistic ambient estimates of BGE from short-term measurements (<1 h) of BP. Based on this approach, modeled BGE for the Hudson averaged 0.16 ± 0.05 (range = 0.07 to 0.23), about 50% lower than the values based on 24 h bioassays. Using this relationship we estimate pelagic BR in the tidal, freshwater Hudson River to be between 176 and 229 g C m^{-2} yr^{-1}.

KEY WORDS: BGE · Bacterial metabolism · Production · Respiration · Estuary · River · Ecosystems

INTRODUCTION

In pelagic ecosystems, bacteria process a large amount of the organic C that enters from either photosynthesis or allochthonous inputs. Among systems, bacterial secondary production (BP) averages 20 to 30% of planktonic primary production and is as large as the secondary production of all zooplankton (Cole et al. 1988). Because BP can be an important link between dissolved organic C and higher consumers (Pomeroy 1974, Ducklow et al. 1986, Carlson & Ducklow 1996, Kato 1996), we have learned a great deal about both its magnitude and its regulation. The production of new bacterial biomass, however, is a transfer of organic C from one pool (dissolved organic carbon [DOC], usually) to another (bacterial biomass). While this internal, organic-to-organic pool transfer is of interest with respect to the food web, it is not particularly useful to biogeochemists interested in flows of C into and out of an ecosystem (Jahnke & Craven 1995). BP, in fact, is unconstrained by the inputs of organic C; given certain assumptions about growth efficiency, BP can actually exceed the sum of net primary production and allochthonous inputs (Scavia 1988, Strayer 1988, Cole & Caraco 1993).

Unlike BP, bacterial respiration (BR) is a conversion of organic to inorganic C and is absolutely constrained by the inputs of organic C. BR is often needed in carbon budgets of ecosystems but is rarely measured directly due to methodological difficulties. Where BR has been directly measured, it has been found to be a very large term in the C budget of the system

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(Schwaerter et al. 1988, Biddanda et al. 1994, Jahnke & Craven 1995, Pomeroy et al. 1995, del Giorgio et al. 1997). Since BR is difficult to measure, researchers usually measure BP (from the uptake of ^{3}H-thymidine or leucine) and estimate BR using assumed values of bacterial growth efficiency (BGE).

The problem with this approach is that reported values of BGE exhibit a large range, from <0.1 to >0.6 (Cole & Pace 1995, Jahnke & Craven 1995, Pomeroy et al. 1995, Carlson & Ducklow 1996, del Giorgio & Cole 1998). This range leads to a 13-fold uncertainty in estimating BR at the extremes from a given value of BP. Further, since we do not understand what regulates either BR or BGE, it is not often clear which value of BGE would be appropriate to a given environment.

In the present study, we measured BP and BR in the tidal, freshwater portion of the Hudson River to examine how BGE varied seasonally in this system. In addition, we performed a series of manipulative experiments with Hudson water to determine what factors could regulate BGE. Finally, using both the seasonal and experimental data, we developed a predictive model for BGE and compare this model to what is known from other systems.

STUDY SITE

The lower Hudson River is a tidal, fresh-to-oligohaline river in eastern New York State in the USA. The river has an average depth of about 9 m and the freshwater portion is vertically well mixed due to tidal and wind action (Caraco et al. 1997, Raymond et al. 1997). Since the freshwater input to this river is relatively low compared to its volume, the water residence time is long. Highest inputs of freshwater occur in March and April and lowest inputs are between August and September (Cooper et al. 1988). During the summer period the average water residence time is ~100 d. The water is nutrient-rich with NO_3 concentrations usually >50 µM and PO_4 concentrations varying seasonally between 0.1 and 1 µM (Cole et al. 1992). The system is moderately turbid (6 to 12 NTU), containing a large amount of suspended solids (averaging ~20 to 50 mg l^{-1}, Cole et al. 1991).

The tidal freshwater portion of the Hudson River Estuary has been particularly well studied regarding food-web dynamics. Phytoplankton primary production is low due to the deep mixing and high turbidity of the system (Cole et al. 1991, 1992). Prior to the invasion of the zebra mussel in 1992, algal biomass reached peak levels in summer of ~40 µg chl *a* l^{-1}. Following the zebra mussel invasion, however, peak chlorophyll levels have not exceeded 4 µg chl *a* l^{-1} (Strayer et al. 1996, Caraco et al. 1997). Metabolism in the Hudson is dominated by heterotrophic activity, and system respiration exceeds primary production (Howarth et al. 1996, Raymond et al. 1997). Similarly, bacterial secondary production exceeds primary production by a factor of about 5 (Findlay et al. 1991, 1992).

METHODS

Seasonal samples. Samples were taken at bi-weekly intervals from September 1996 to September 1997 at a single station near Kingston-Rhinecliff, New York. This station, located at river km 144, is the site of much of the intensive seasonal work on the Hudson River (see Caraco et al. 1997). Subsurface water samples were taken in 10 l Nalgene PVC containers and transported back to the laboratory (20 min) in a cooler at ambient temperature. Field measurements of temperature and dissolved oxygen were made with a YSI model 1000 meter; conductivity and turbidity were measured with a YSI TLC model 2000 meter and a turbidimeter HF Scientific model DRT 100B, respectively.

Upon arrival in the lab the water was passed through a GF/D glass-fiber filter. This coarse filtration removed essentially all of the chl *a* and heterotrophic flagellates and reduced bacterial numbers by only 16 ± 9%. Identical results were obtained in a test using a 0.8 µm Nucleopore filter but filtration times were much slower. The filtered samples were kept, in the dark at *in situ* temperature, in 2 l containers for measurements of bacterial parameters (BP, number and biomass) at time zero (instantaneous measurement) and after 24 h (bioassay approach). BR was measured in separate parallel incubations (below).

Manipulative experiments. In a series of simple experiments we varied conditions expected to alter BP, and examined the effects on BR and BGE. Experiments were conducted to test the effects of initial bacterial abundance, concentration of inorganic nitrogen, phosphorus and DOC, and temperature on the bacterial parameters. Water samples from the Hudson River were collected in triplicate, filtered (above) for each experiment and maintained at laboratory temperature for 48 h prior to any other manipulations. For the temperature experiments, samples were incubated at 5, 10, 15, 25 and 30°C. For the bacterial dilution experiments we generated a range of bacterial abundance (0.5×10^9 to 2.5×10^9 cells l^{-1}) by combining the GF/D-filtered water with Hudson water filtered through 0.45 µm membrane filters (Millipore HA). In both the inorganic nutrient and organic carbon experiments, experimental samples were prepared using 1 part of the GF/D-filtered water and 9 parts of the de-ionized water to which 900 µM of $NaHCO_3$ had been added to match the alkalinity and ionic strength of the Hudson.

For the carbon manipulations, glucose was added at 0.36 mg C l^{-1} and 3.6 mg C l^{-1}. For the nutrient manipulations we added NH_4Cl (0.5, 5.0 and 25 µM) and KNO_3 (4.5, 45 and 225 µM). A phosphorus gradient was obtained by adding KH_2PO_4 at 0.1, 1.0 and 5.0 µM. We varied these nutrients alone and in combination. All manipulated samples were kept in the dark at *in situ* temperature, in 2 l containers for measurements of bacterial abundance and BP at time zero (instantaneous measurement) and after 24 h. Samples for dissolved oxygen were fixed at time zero and after 24 h.

Bacterial parameters. ***Abundance:*** Samples for bacterial abundance were placed in scintillation vials and preserved with buffered formalin (2% final concentration). The preserved samples (1 ml) were stained with acridine orange and passed through 0.2 µm pore size Nucleopore filters according to Hobbie et al. (1977). Samples were counted at 1250× with an Olympus BHT microscope, and at least 10 fields of >30 bacteria $field^{-1}$ were counted.

Production: We used the Smith & Azam (1992) microcentrifuge modification of the [^{3}H]-leucine method (Kirchman et al. 1985) to estimate BP. In order to determine the concentration of labeled leucine required to saturate leucine uptake, 3 saturation experiments were run by measuring uptake over a leucine concentration series ranging from 12 to 88 nM (Fig. 1a). The expected Michaelis-Menten function defined saturation to occur at about 60 nM. Water samples (1.5 ml, 4 replicates and 1 blank) were incubated with 59 nM final concentration of [^{3}H]-leucine (47.5 Ci $mmol^{-1}$, New England Nuclear [NEN]) at *in situ* temperature for 60 min. Incubations were ended by adding 0.3 ml of 50% TCA. Zero-time controls (blank) were fixed with 50% TCA immediately after adding labeled leucine. Following incubation, the samples were centrifuged (14 000 rpm [17 000 × *g*]; 10 min) and the supernatant was discarded. Subsequently, 1.5 ml of 5% TCA were added and the samples were centrifuged for 10 min at 14 000 rpm; afterwards, the supernatant was sucked out. A scintillation cocktail (Scintiverse BD) was added, the centrifuge tubes were placed in scintillation glass vials, and the radioactivity was determined using a Beckman LS1801. Quench was determined from the relationship between H number and counting efficiency (Horrocks 1977). BP was calculated from the disintegration per minute (dpm) incorporated to protein according to Simon & Azam (1989).

On 3 dates the extent of isotope dilution (ID) was measured by adding a concentration series of unlabeled leucine along with our labeled addition (Moriarity 1986, Chrost 1990). Unlabeled leucine was added at 5 concentrations ranging from 0 to 221 nM. The ID experiments revealed a relatively low contribution of exogenous unlabeled leucine (Fig. 1b). The 3 experiments all gave ID values near 2 (July, 2.69 ± 0.34; September, 2.05 ± 0.24; November, 2.56 ± 0.39). Based on these results we used an ID of 2, in agreement with the value reported by Simon & Azam (1989).

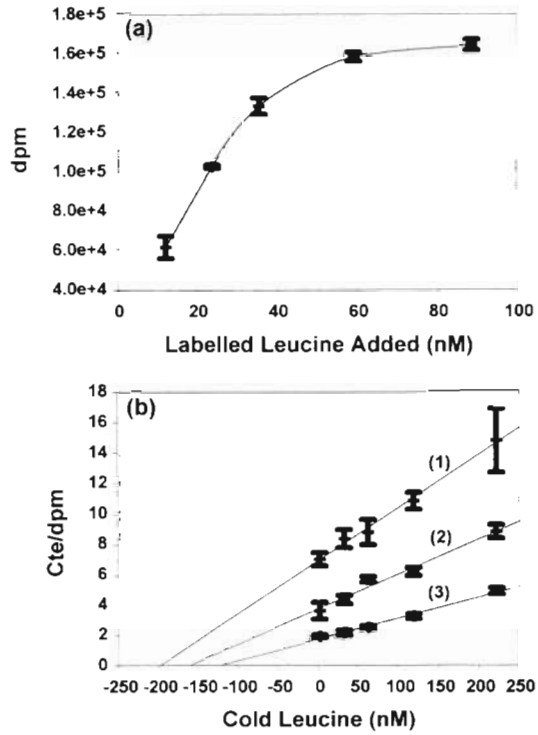

Fig. 1. (a) Kinetic saturation curve. Mean (with SD) of DPM in protein is shown as a function of added leucine concentration. (b) Three isotope dilution plots showing how the estimate of the *x*-intercept was obtained. Values plotted on the *y*-axis are a constant divided by the disintegrations per minute (dpm)

During 24 h incubations (bioassay approach) bacteria grow, generally, at an exponential rather than a linear rate. This means that to estimate cumulative BP during the incubation from instantaneous measurements of the rate leucine uptake, one has to integrate values of BP incubations at time zero and 24 h. Thus:

$$\mathrm{BPI}\ (\mu g\,C\,l^{-1}\,d^{-1}) = [(\mathrm{BPT1}/k)e^{kT1}] - [(\mathrm{BPT0}/k)e^{kT0}] \quad (1)$$

where BPI is the integrated bacterial production, BPT0 is the instantaneous bacterial production at time zero (T0), BPT1 is the instantaneous bacterial production at the end of the incubation (after 24 h, T1) and k is the slope of the increment in production [(lnBPT1 – lnBPT0)/T1]. The integrated BP was calculated for both seasonal and experimental set samples.

Respiration: BR was estimated as dissolved oxygen consumption in BOD bottles (5 replicates for each time). The time-zero control samples were fixed immediately with Winkler reagents. Another set of 5 bottles was incubated in the dark at *in situ* temperature for 24 h. Dissolved oxygen was determined by a spec-

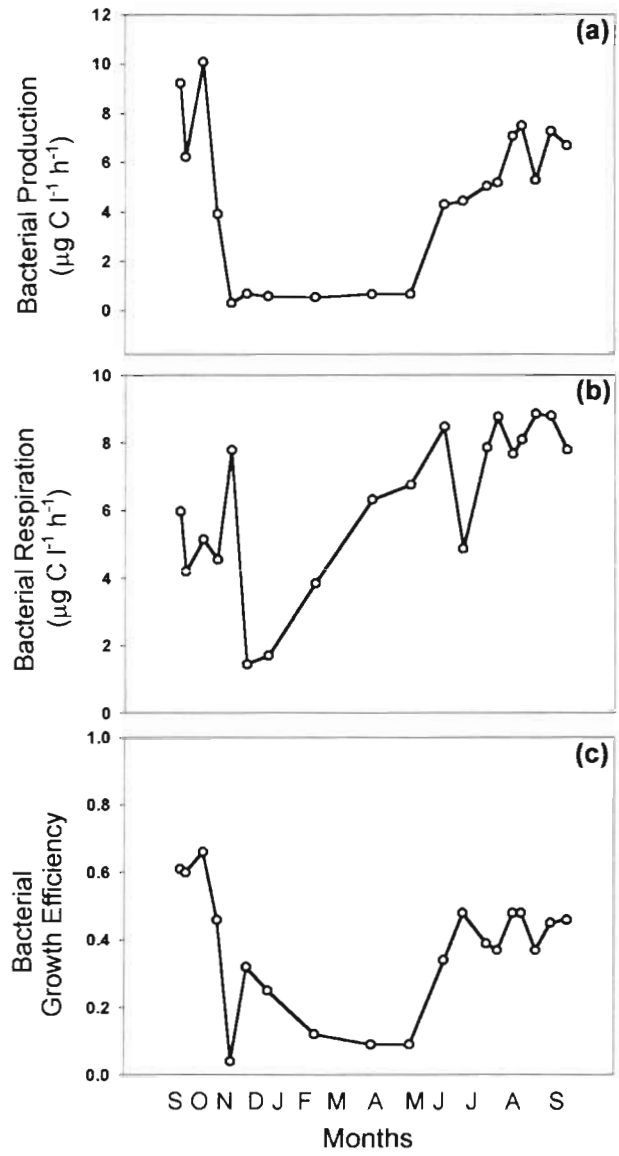

Fig. 2. Seasonal changes in (a) integrated bacterial production (integrated 24 h bioassay), (b) bacterial respiration and (c) bacterial growth efficiency

trophotometric modification of the Winkler technique (Reynolds 1972, Duval et al. 1974, Ashton & Twinch 1985, Roland et al. 1999). The dissolved oxygen consumption (mg DO l^{-1} h^{-1}) between the 2 measurements was converted to carbon respired (µg C l^{-1} h^{-1}) assuming a respiratory quotient (RQ) of 1.

RESULTS

Seasonal trends

Integrated (24 h bioassay) BP showed strong variation in the seasonal cycle (0.5 to 10 µg C l^{-1} h^{-1}; Fig. 2a). Highest values were found in late summer and early autumn and lowest values in the winter. The 24 h bioassay values of BR showed a similar trend in the seasonal cycle (Fig. 2b). Maximum values were obtained during the warmer months (~8 µg C l^{-1} h^{-1}) and minimum values in the winter (2 to 4 µg C l^{-1} h^{-1}). BR was less seasonally dynamic (coefficient of variation, CV = 37%) than BP (CV = 70%). In the seasonal cycle bacterial growth efficiency ranged from ~0.1 during the winter up to ~0.5 during the summer months (Fig. 2c).

Experimental results

The experimental manipulations were successful in inducing changes in both BP and BR and therefore in generating a range in BGE (Table 1). The initial density of bacteria had the least affect on all 3 parameters (Fig. 3a). The addition of a labile C substrate as glucose (Fig. 3b) and alteration of temperature (Fig. 3c) had the greatest effects. In the inorganic nutrient addition experiments (Fig. 3d), the ranges of both BP and BR spanned the ranges we observed in the seasonal cycle. As with the seasonal cycle, we observed a greater range in BP than in BR, with the exception of the DOC addition experiment.

Table 1. Analyses of variance within treatments for bacterial production (BP), bacterial respiration (BR) and bacterial growth efficiency (BGE). *t*-test (normal distribution) and Mann-Whitney test (non normal distribution) were used to test the occurrence of differences among treatments (significant [S] or not significant [NS]). The Student-Newman-Keuls test was used to verify the significance of the difference with p = 0.05. The coefficient of correlation (r) indicates the response of each bacterial parameter to the treatment

Treatment	BP	BR	BGE
Dilution	NS; $p = 0.964$; $r = 0.66$	NS; $p = 0.821$; $r = 0.97$	NS; $p = 0.901$; $r = 0.91$
DOC	S; $p < 0.001$; $r = 0.99$	S; $p < 0.001$; $r = 0.67$	S; $p < 0.001$; $r = -0.03$
Temperature	S; $p < 0.001$; $r = 0.92$	S; $p < 0.001$; $r = 0.99$	S; $p < 0.001$; $r = 0.69$
Nitrogen	S; $p < 0.001$; $r = 0.99$	NS; $p = 0.165$; $r = 0.64$	S; $p = 0.003$; $r = 0.92$
Phosphorus	S; $p < 0.001$; $r = 0.97$	NS; $p = 0.505$; $r = 0.79$	S; $p = 0.024$; $r = 0.94$
N+P	S; $p < 0.001$; $r = 0.97$	S; $p < 0.001$; $r = 0.66$	S; $p < 0.001$; $r = 0.91$

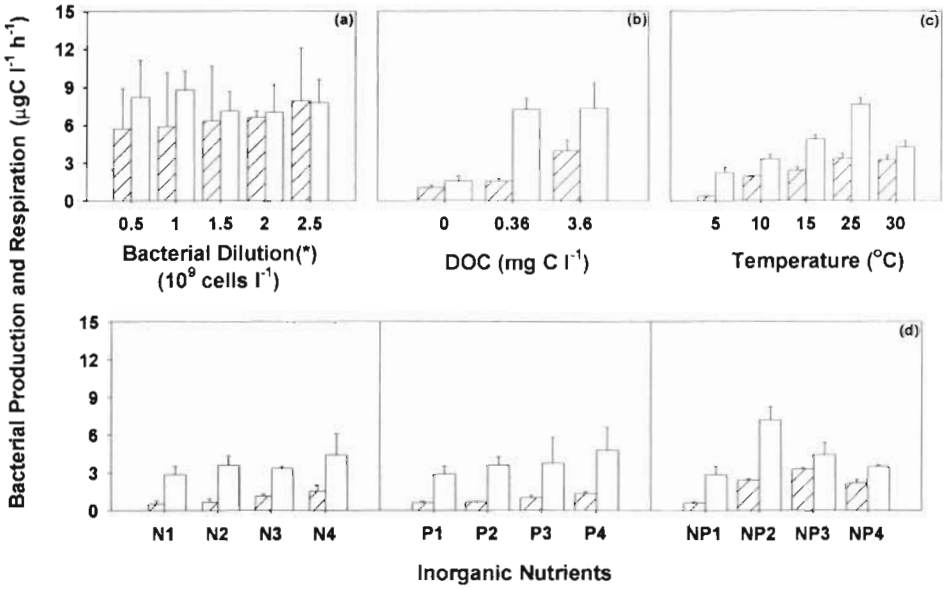

Fig. 3. Experimental change in integrated bacterial production (24 h bio-assay, cross-hatched bars) and bacterial respiration (open bars). Average values and standard deviation, according to the experimental gradients, are shown. (a) Bacterial dilution (initial bacterial cell numbers are given on the *x*-axis); (b) dissolved organic carbon; (c) temperature; and (d) inorganic nutrients

There were some intriguing non-linearities in the experimental results. For example, BR peaked at a temperature of 25°C rather than at the highest temperature (30°C). When we added both N and P, both BR and BP peaked at moderate rather than high additions. Clearly, BP and BR did not react in identical ways to the manipulations. This is illustrated in Fig. 4, which simply shows the experimental results arranged from lowest to highest BGE. Highest BGE occurs in the high levels of dilution, temperature and combined nutrient addition experiments (near 0.5) and lowest in low levels of nutrient addition experiments (<0.2).

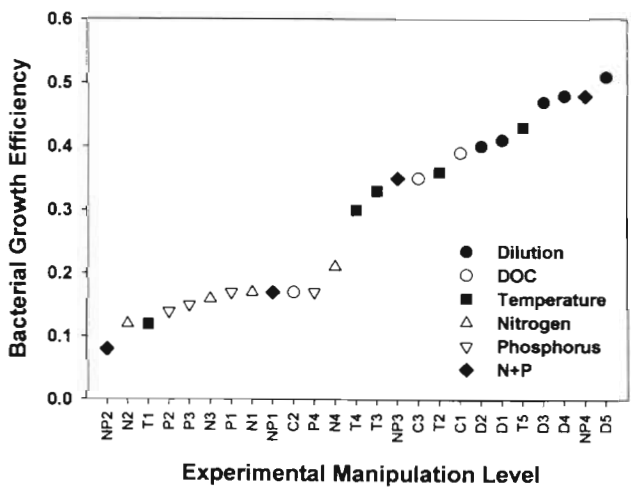

Fig. 4. Relationship between bacterial growth efficiency (BGE) and experimental treatment. The data were arranged in rank order from lowest to highest BGE. Experimental manipulation: D = bacterial dilution; C = DOC; T = temperature; N = nitrogen; P = phosphorus; NP = nitrogen and phosphorus. Numbers (1 to 5) represent the level of treatment (see Fig. 3)

Relationships

We combined the data from the seasonal cycle and the experiments to investigate the relationships between BP, BR and BGE. On a log-log scale BR was correlated to BP (Fig. 5a; $r^2 = 0.42$; $p < 0.001$). However, while BP varied by nearly 3 orders of magnitude, BR varied by only 1. Further, bacterial respiration was not correlated to BGE (Fig. 5b; $p > 0.15$). As a consequence of the lack of correlation between BR and BGE, BP and BGE were strongly correlated (Fig. 5c; $r^2 = 0.72$; $p < 0.001$). The CV of BGE, including both seasonal and experimental sets ($n = 88$), was 43%, and the range was between 0.04 and 0.66; 69% of the measurements had BGE between 0.2 and 0.5 (Fig. 5c). The relationship between BGE and BP fit a hyperbolic model which explained 72% of the variation in BGE data and the BGE maximum, according to the model, is 0.64 (Fig. 5c).

DISCUSSION

Both the seasonal cycle and the experimental manipulations provided some similar insights as to the possible regulation of BGE. In both the field and the experiments, BGE varied within the same range (<0.05 to near 0.6). Although BP and BR (the 2 components needed to calculate BGE) tended to covary in both the field and the experiments, they did exhibit some individualistic behavior, particularly in the experiments. For example, BR was more strongly stimulated than BP in the glucose addition experiment. In every experiment and every sample from the field, BR exceeded BP by a substantial amount. Clearly, in this system, the

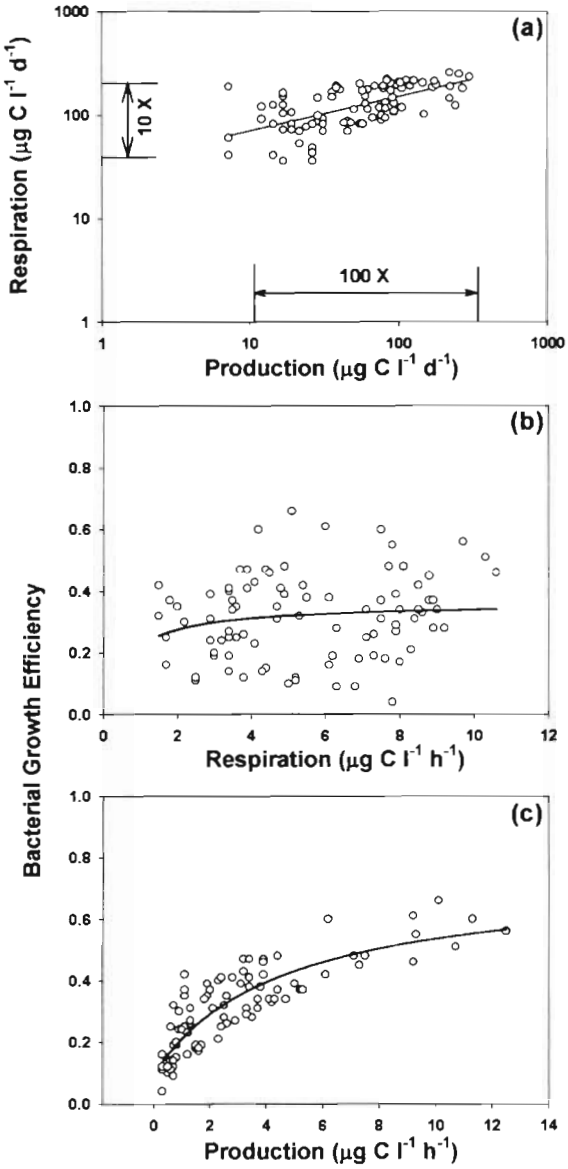

Fig. 5. Relationships between (a) integrated bacterial production (24 h bioassay) and respiration, (b) bacterial respiration and bacterial growth efficiency and (c) integrated bacterial production (24 h bioassay) and bacterial growth efficiency. Both seasonal and experiments data sets were used in these plots. The regression for (a) is BR = 2.66 + 8.7BP/(4.90 + BP) with N = 88, r = 0.65, $p < 0.0001$, and that for (c) is BGE = 0.09 + 0.640BP/(4.52 + BP) with N = 88, r = 0.85, $p < 0.0001$. Statistical facts for (b) are not included because they are not significant

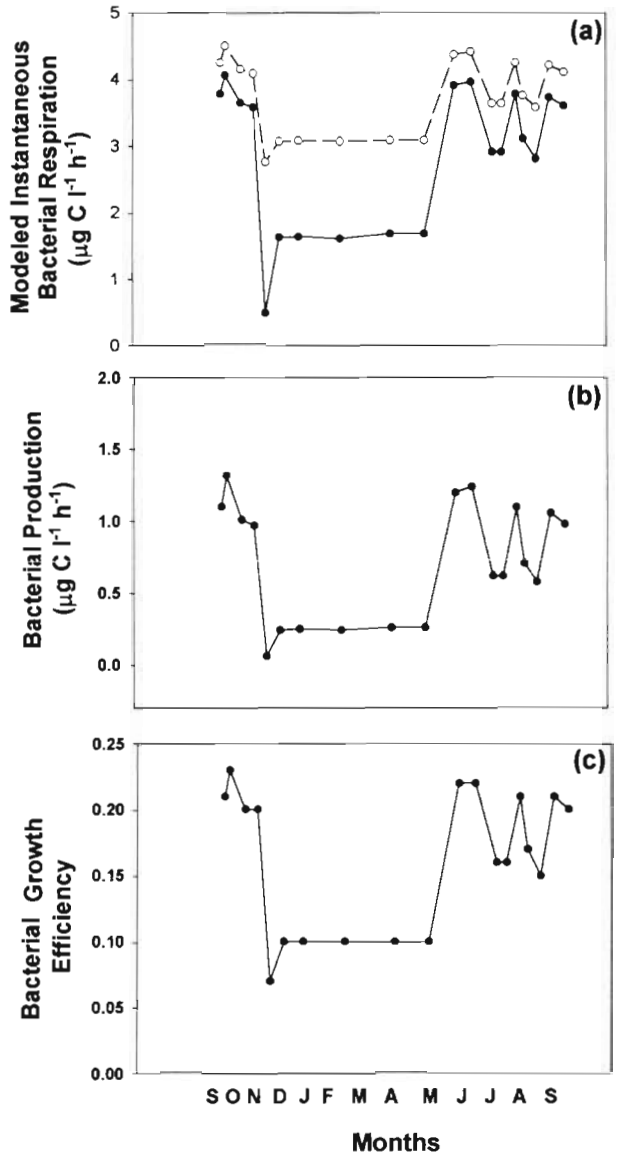

Fig. 6. Seasonal variation. (a) Bacterial respiration estimated from the model in Fig. 4a (○) and from the model in Fig. 4c (●). (b) Instantaneous bacterial production and (c) bacterial growth efficiency estimated from the model in Fig. 4c. For (a) and (c), instantaneous bacterial production data were used

respiratory loss of C is larger than the net assimilation of organic matter into bacterial biomass under almost any conditions.

Both parts of the study showed that BR was more conservative than BP. Over the entire range of observations, BP varied by a factor of more than 100, while BR varied by a factor of only 10. Further, while BP and BR were correlated, BR and BGE were not. The result of these relationships is that BGE is easily predictable from BP measurements alone, at least in the Hudson River. The hyperbolic shape of the relationship between BP and BGE (e.g. Fig. 5c) is a consequence of the way in which BP and BR are related and is not simply an autocorrelation. Because the BP term occurs in

both the numerator and denominator, the relationship is driven to an asymptote if BR is constant, has low variance compared to BP or is unrelated to BP. If BR were linearly related to BP, the result would be a constant BGE, not the hyperbolic relationship with BP that we observed. For example, if BR were always twice BP, BGE would always be 0.33 and would not vary systematically with BP. If BR were a positive exponential function of BP, BGE would be a negative exponential function of BP.

In our study the small dynamic range of BR in comparison to that of BP drives the hyperbolic relationship. The relationship is useful in that it suggests a way of estimating appropriate values for BGE in the absence of direct or extensive measurements of BR. BR is difficult to measure. A potential drawback of most measurements of BR, and therefore BGE, is the long time frame needed. While BP can be measured in a 0.5 h incubation, obtaining a reliable measurement of BR (or of DOC decline) usually takes many hours. Since bacterial cells typically grow during these experiments, the long time frame compromises the estimate of BGE. Our study reveals 2 relationships that could conceivably be used to estimate BR and BGE at a time scale comparable to the BP measurement.

Since BR and BP are correlated in the 24 h integrated measurements it is reasonable to expect this relationship (e.g. Fig. 5a) to be similar for an instantaneous measurement of BP. This relationship is not perfect; plotted on an arithmetic scale r^2 is only 0.42. Nevertheless, it provides a clear improvement over applying some arbitrary value of BGE to the measured BP value in the estimation of BR. Using this approach and the instantaneous leucine-based measurements of BP, we estimate annual BR in the Hudson to average 3.7 µg C l^{-1} h^{-1} or about 226 g C m^{-2} yr^{-1}.

Since BR is less dynamic than BP, BGE is basically driven by variation in BP and is well correlated to it (e.g. Fig. 5c). If we assume that the hyperbolic relationship in Fig. 5c is also true for instantaneous BP, we can compute an instantaneous value of BGE for each value of BP (Fig. 6c). Looked at this way, instantaneous BGE would vary from around 0.2 during the warmer months to values near 0.1 during the winter. The resulting respiration calculated from this cycle of BGE and BP is shown in Fig. 6a. It generally agrees with BR based on the BR-BP relationship when high, but diverges somewhat during the winter. Using this approach, BR in the Hudson would be 2.9 µg C l^{-1} h^{-1} or about 176 g C m^{-2} yr^{-1}.

Our study attempted to overcome a problem with some of the prior literature on BGE. In many studies, a 'mixed' time scale is used in which BP is measured instantaneously at the beginning of the experiment and BR is measured as briefly as possible, usually on the time scale of more than 6 h, often as long as 24 h (Coffin et al. 1993, Biddanda et al. 1994, Carlson & Ducklow 1996). Some researchers have avoided this problem by measuring the decline of DOC to get total assimilation and bacterial POC production; these can be used to obtain BP. In this case, the time scales are correct, but tend to be long (e.g. Sondergaard & Theil-Nielsen 1997).

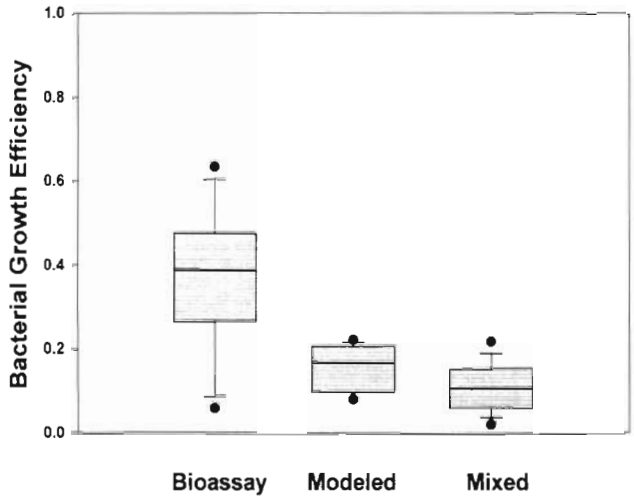

Fig. 7 Comparison of measured bacterial growth efficiency (BGE) estimated assuming 3 distinct combinations of BP and BR. Bioassay BGE: both integrated BP and BR (bioassay BGE); field BGE: instantaneous BP and model (Fig. 4c); Mixed BGE: instantaneous BP and integrated BR

For the 'mixed' time scale approach, if there is growth during the long incubation, this procedure overestimates BR and underestimates BGE. Using instantaneous BP and 24 h BR, the annual average BGE in the Hudson would be 0.11 (Fig. 7), which is lower than that obtained by using the above models. Calculating BR from the relationship between instantaneous BP and 'mixed' BGE gives an annual average value for BR which is considerably higher than the other approaches (5.29 µg C l^{-1} h^{-1} or 324 g C m^{-2} yr^{-1}). This is very close to the value (318 g C m^{-2} yr^{-1}) obtained by Findlay et al. (1991), who used thymidine-based estimates of BP and a 'mixed' approach to obtain BR.

As methods for the measurement of dissolved oxygen improve (Williams et al. 1982, Carignan et al. 1998, Roland et al. 1999), it may be possible to greatly shorten the time needed to estimate BR to the point where BP and BR can be measured on the same time scales, and perhaps BR could become a routine measurement.

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