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Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability

Erik M. Smith[1] *and Yves T. Prairie*
Département des Sciences Biologiques, Université du Quebec à Montréal, Case Postale 8888, succursale Centre-Ville, Montreal, Quebec H3C 3P8, Canada

Abstract

We investigated bacterial responses to variations in dissolved organic carbon (DOC) and nutrient availability by a comparative analysis of bacterial metabolism in lakes ranging from oligotrophic to eutrophic. Bacterial growth, respiration, and growth efficiency were quantified in lake water dilution cultures performed in 20 lakes located in eastern Quebec, Canada, which varied with respect to both DOC and nutrient concentrations. Intrinsic growth rates of the bacteria ranged from 0.1 to 1.4 d^{-1}, bacterial cell-specific respiration rates ranged from 0.4 to 7.2 fg C $cell^{-1}$ h^{-1}, and growth efficiencies ranged from 6.7% to 51.6%. These variations were unrelated to bulk DOC concentrations. Instead, growth rate and efficiency were positively related to total phosphorus concentrations. Specific respiration rate, on the other hand, decreased with increasing phosphorus concentrations, and the magnitude of respiration, on a per-cell basis, strongly influenced observed growth efficiencies. In a series of substrate enrichment experiments, additions of glucose alone failed to stimulate a response in growth rate, mean cell biovolume, or the potential biomass yield in dilution cultures, but all responded positively to phosphorus additions. Our results show that bacterial metabolism and the fate of DOC input to lake microbial communities are strongly dependent on phosphorus availability, rather than total carbon availability. Extreme oligotrophy appears to place high respiratory demands on the bacterioplankton, resulting in very low bacterial growth efficiencies and consequently greater DOC flow to CO_2 than to biomass available for transfer to higher trophic levels.

The organic carbon fueling bacterial metabolism in lakes originates from either in situ primary production (autochthonous carbon) or from terrestrial production that is carried into the lake from its watershed (allochthonous carbon). The relative importance of these two sources of carbon is highly variable among lakes. While autochthonous carbon tends to predominate in very eutrophic lakes, allochthonous carbon loading often greatly exceeds in situ primary production in oligotrophic lakes (Wetzel 1992). Input of allochthonous carbon thus represents a potentially large subsidy to the metabolism of lake communities.

Several lines of evidence argue that the total respiratory breakdown of organic matter exceeds that produced by in situ primary production in most lakes (e.g., del Giorgio and Peters 1994; Cole et al. 2000). This metabolic imbalance (net heterotrophy; total respiration > gross primary production) implies that allochthonous carbon must support a portion of the lake's total respiration. Indeed, it has recently been shown that, based on epilimnetic dissolved oxygen measurements, the net metabolic balance in lakes varies continuously along a gradient in dissolved organic carbon (DOC) concentration (Prairie et al. 2002). As DOC increases, lake metabolism becomes progressively more net heterotrophic and hence undersaturated with oxygen. The fact that bacteria are responsible for the bulk of the respiratory breakdown of organic matter suggests that DOC input plays an important regulatory role in lake metabolism through its influence on bacterial growth and respiration (Jansson et al. 2000).

Bacterial metabolism can be regulated by a number of different biotic and abiotic factors. The specific nature of this regulation is central to an ecological and biogeochemical understanding of the role that heterotrophic bacteria play in the transfer of energy and materials in aquatic ecosystems. Historically, research has focused on bacterial acquisition of organic carbon, which has traditionally been considered the primary limiting factor for bacterial growth in these ecosystems. Bacteria require mineral nutrients as well, however, and have high cellular requirements for both nitrogen and phosphorus, relative to carbon, as compared to phytoplankton (Fagerbakke et al. 1996). Bacteria can readily take up inorganic nitrogen and phosphorus and are effective competitors with primary producers for such nutrients (e.g., Currie and Kalff 1984). An increasing number of studies have documented the importance of nutrients, and particularly phosphorus, in controlling bacterial growth across a variety of aquatic ecosystems (e.g., Coveney and Wetzel 1992; Elser et al. 1995). Nutrient availability may thus place very substantial constraints on the consumption of allochthonous carbon by bacteria in oligotrophic lakes.

The objective of the present study was to test the hypothesis, suggested by the relationship between net metabolism and DOC concentration found by Prairie et al. (2002), that bacterial metabolism in lakes is regulated by DOC avail-

[1] To whom correspondence should be addressed. Present address: Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208 (smithe@biol.sc.edu).

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ability. To test this, we conducted a comparative analysis of bacterial metabolism in 20 lakes that varied in their DOC and nutrient concentrations. By intentionally selecting a set of lakes for which variations in DOC and nutrients were independent, we also tested the alternative hypothesis: bacterial metabolism and allochthonous DOC use are functions of nutrient availability. In a subset of these lakes, we also conducted a set of carbon and phosphorus enrichment experiments to test predictions based on the comparative analysis.

For this study we were particularly interested in potential variations in bacterial growth efficiencies along the changing resource base in these lakes. The link between allochthonous DOC and productivity at higher trophic levels within lakes depends, in part, on the relative balance between biomass production and respiratory loss at the first trophic step, that of the bacteria. Bacterial growth efficiency, a key variable describing the coupling (or lack thereof) between production and respiration, varies considerably in natural bacterial communities (del Giorgio and Cole 1998). These variations are likely not random, yet the extent to which these variations reflect the influence of changing resource availability, due to variations in allochthonous loading to lakes, remains unclear. While the regulation of bacterial growth and production rates has received a great deal of attention in the past, the factors that control bacterial respiration, and its relation to bacterial production (i.e., growth efficiency), remain poorly understood (Jahnke and Craven 1995; Williams 2000). This uncertainty has greatly hampered our understanding of the role of the bacterioplankton in aquatic carbon cycles.

Material and methods

Study sites and sampling scheme—We sampled the surface waters of 20 lakes located in the Eastern Townships region of Quebec, Canada, ~100 km due east of Montreal (45.50°N, 73.58°W). The topography of this area is characterized by the rolling hills of the Appalachian region. The watersheds of each lake are dominated by deciduous woodland (mean percentage forest cover = 74, range = 48–100), with the remainder being primarily small farm lots. The specific lakes were chosen to span as wide and near orthogonal a range of DOC and phosphorus concentrations as possible. Sampling was conducted from June to August of 2001. Each lake was sampled once during this period, with the exception of one (Lake Magog, which was sampled twice). The order in which the lakes were sampled was randomized.

Dilution culture experimental setup—Lake water dilution cultures (sensu Ammerman et al. 1984) consisted of adding a natural inoculum back to a volume of particle-free (0.2-μm filtered) lake water and then following the growth and respiration time course of the reintroduced bacterial assemblage. Surface water was collected from approximately 1–2 m depth with a Van Dorn sampler from the central, deepest portion of each lake. Lake water media were prepared by low-pressure (<7 psi, achieved by pushing water with N_2 gas) in-line filtration, first through a precombusted 142-mm diameter GF/F glass-fiber filter (Whatman) and then through a 0.2-μm pore-size filter capsule (Gelman Maxi Culture Capsule), which had been prerinsed with 10 liters of deionized water. Filters (GF/F) were replaced periodically to minimize disruption of cells retained on the filter. An inoculum from each lake was prepared by filtering lake water through a 0.8-μm pore-size filter (Gelman pleated capsule filter). All filtrations were done in the field, immediately after water collection. Filtered samples were kept cold and dark while transported to the laboratory, where the 0.2-μm media water was allowed to equilibrate to 20°C in a temperature-controlled environmental chamber. During this time, any media water that was undersaturated with O_2 by >15% was gently bubbled with air to achieve an initial condition of ~100% O_2 saturation in all cultures.

To begin the culture incubation, an inoculum of 0.8-μm filtered water was added (5% final volume) to the 0.2-μm filtered lake water and the combined sample was introduced into replicate incubation vessels. Each incubation vessel consisted of a silicon-stoppered 4-liter glass Erlenmeyer flask. The stopper was fitted with two 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-liter polyethylene collapsible cubitainer (VWR), which served as a reservoir to replace sampled water. The reservoir contained the same culture water as the Erlenmeyer flask. This setup allowed for sample volume removed from the flask to be 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. While the cubitainers are not completely gas impermeable, at O_2 concentrations near saturation (±15%) O_2 diffusion is undetectable and the total sample volume removed from the incubation vessel was kept below ~20%. All carboys, filtering devices, glassware, and tubing were acid-washed (24 h in 10% HCl) and rinsed with deionized water prior to each use. Cubitainers were initially subjected to repeated bathing in acid (10% HCl), then filtered lake water, then deionized water to minimize any plastic leachate from the polyethylene material from being introduced to the sample water. Dilution cultures from all lakes were performed in duplicate and incubated at 20°C in darkness. The rationale to incubate at a uniform temperature was to minimize any confounding effects of small but potentially significant temperature variations among lakes. Cultures were subsampled immediately upon setup ($t = 0$) for initial conditions and then subsampled at intervals of 4–8 h for ~36 h.

For 4 of the 20 lakes, dilution cultures included experimental enrichment treatments consisting of additions of carbon (as glucose) and phosphorus (as KH_2PO_4), both individually and in combination, as well as an unamended (control) treatment. Glucose and phosphate were added to achieve a threefold enrichment in anticipated ambient DOC and total dissolved phosphorous (TDP) concentrations, which were estimated a priori from long-term mean summer concentrations within the epilimnion of these four lakes (Prairie unpubl. data). Specific concentrations added were 6.3 μg P L^{-1}, 6.3 mg C L^{-1} for Bowker; 10.5 μg P L^{-1}, 19.1 mg C L^{-1} for Truite; 18.0 μg P L^{-1}, 7.8 mg C L^{-1} for Magog; and 25.1 μg P L^{-1}, 17.1 mg C L^{-1} for Aylmer. As a result of differences in long-term mean concentrations and actual in

situ concentrations at the time of sampling, these additions represented actual increases in P concentrations ranging from 1.9 fold (Bowker) to 5.3 fold (Aylmer) and actual increases in C concentration ranging from 2.9 fold (Magog) to 3.5 fold (Bowker).

Bacterial abundance and biovolume measurements—At each time point, a 10-ml subsample was taken for bacterial cell density and biovolume determinations. This was preserved with cold, particle-free 25% glutaraldehyde (1% final concentration) and subsequently frozen in liquid N_2 until all subsamples from an individual dilution culture could be processed together. Bacterial abundance was determined using a Becton Dickinson FACScan flow cytometer. Aliquots (2 ml) were stained with 2.5 μmol L^{-1} (final concentration) SYTO 13 (Molecular Probes). Green fluorescent 0.93-μm beads (Fluoresbrite Microspheres, Polysciences) served as the internal standard. Final bead concentration in the samples was $\sim 10^5$ ml^{-1}, with the concentration determined by epifluorescence microscopy. Subsamples from all time points within a given dilution culture were run together in a single series. For each measurement of bacterial abundance 10,000 events were quantified.

Mean cell biovolume was determined for a subset of bacterial samples ($n = 20$) representing a range of different lake water cultures and corresponding to those time points when cell growth started to enter stationary phase. A 2-ml aliquot was stained with 25 μg L^{-1} (final concentration) of 4,6-diamidino-2-phenylindole (DAPI) (Sigma Chemical) for 10 min and then filtered onto black 0.2-μm pore-size polycarbonate membrane filters (Poretics) under low vacuum. Bacterial images were visualized with an Olympus BX51 epifluorescence microscope equipped with a 100×/1.30 oil immersion objective and a Photometrics Cool Snap FX digital video camera system (Roper Scientific). Digital images were acquired with RSImage acquisition software (Roper Scientific). Resulting images had a resolution of 12.4 pixels μm^{-1}. Cell dimensions were recorded from these images with Scion image analysis software (Massana et al. 1997). A minimum of 200 individual cells from each sample was quantified, and overlapping cells were not included in the analysis. Cell biovolumes (V, μm^3) were then calculated according to the formula $V = 4\pi r^3/3 + (l - 2r)\pi r^2$, where l is cell length and r is cell radius. This formula approximates the form of a bacterial cell by assuming its shape to be a cylinder with two hemispherical caps. Carbon content per cell (CC, fg $cell^{-1}$) was then estimated from biovolume using the equation $CC = 218V^{0.86}$ (Posch et al. 2001).

Cell biovolumes were then estimated for the remaining majority of culture time points by deriving an empirical relationship between measured cell biovolumes and the mean green fluorescence of cells ($FL1_{cells}$), relative to that of the bead standard ($FL1_{beads}$), from the flow cytometric data (Gasol and del Giorgio 2000). For our data set these two measures were highly related by the regression equation, $V = 0.176 \times (FL1_{cells}/FL1_{beads}) + 0.009$; $r^2 = 0.88$, $n = 20$, $p < 0.001$.

O_2 measurements—At each sampling time point, subsamples for dissolved O_2 were collected in acid-washed (10% HCl for 24 h) 60-ml biological oxygen demand (BOD) bottles (Wheaton). Concentrations of dissolved O_2 were determined by automated Winkler titration of whole bottle samples using a Metrohm 716 DMS titrator equipped with a Metrohm Pt titrode and following the recommendations of Carignan et al. (1998). With this procedure, we were routinely able to achieve a precision of ~0.03% (measured as the coefficient of variation [CV] of replicate O_2 determinations).

Chemical analyses—Nutrient and DOC concentrations were measured in both in situ lake water samples taken at the time of water collection for dilution cultures and at the beginning ($t = 0$) of the dilution cultures. Concentrations of total phosphorus (TP) and total dissolved phosphorus (TDP, 0.2-μm filtered water) were measured by the molybdenum-blue method after persulfate digestion. Concentrations of total nitrogen (TN) and total dissolved nitrogen (TDN, 0.2-μm filtered water) were measured as nitrates after alkaline persulfate digestion. All colorimetric analyses were carried out on an Alpkem RFA300 or a Flow Solution IV autoanalyzer, and detailed methodologies are given in Cattaneo and Prairie (1995). Concentrations of DOC in 0.2-μm filtered sample water were measured by high temperature oxidation on a Shimadzu TOC analyzer after acidification.

Data calculations and statistical analyses—From the cell abundance, biovolume, and O_2 data of the dilution cultures, estimates of bacterial specific growth rate (μ, d^{-1}), cell-specific respiration rate (BR $cell^{-1}$, fg C $cell^{-1}$ h^{-1}), and bacterial growth efficiency (BGE, %) were derived. The value of 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. BR $cell^{-1}$ was estimated as the slope of the change in O_2 versus the change in cell density over the period of exponential growth divided by time. This rate was converted to C units assuming a respiratory quotient (RQ) of 1 (del Giorgio and Cole 1998). BGE was derived as the slope of bacterial production versus the sum of bacterial production plus respiration and expressed as a percentage. Bacterial production (BP) was taken as the change in cell abundance and cell carbon content over time. Bacterial respiration (BR) was taken as the change in O_2 over time converted to C units with an RQ of 1. Both measures were thus converted to a common unit, and linear regression allowed for the use of all data points during the dilution culture in the calculation of BGE, which was highly linear over the period of exponential growth. Total cell yield and total carbon yield during the dilution cultures were also calculated as the maximum cell density achieved at stationary phase and the maximum C biomass achieved (based on cell density and mean carbon content derived from biovolume estimates) at stationary phase, respectively. Slopes of the natural log of bacterial abundance versus time were estimated from ordinary least-squares regression, and all other slope estimates were derived from reduced major axis regressions, since the relative magnitudes of natural error in both axes were generally comparable. For comparisons among lakes, all data were log transformed to meet normality assumptions and to equalize variances. Relationships

Table 1. Characteristics of Eastern Township lakes sampled in the present study. TP is the total phosphorus, TDP is total dissolved phosphorus, TN is total nitrogen, TDN is total dissolved nitrogen, DOC is dissolved organic carbon, BA is total bacterial cell abundance. Dissolved pools for P, N, and C are defined as <0.2 μm. Concentration data represent samples collected from the epilimnion. ''nd'' = no data available.

Lake	Area (km^2)	Mean depth (m)	TP ($\mu g\ L^{-1}$)	TDP ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	TDN ($\mu m\ L^{-1}$)	DOC ($mg\ L^{-1}$)	BA ($\times 10^9\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)
Aylmer	32.4	7.0	16.5	5.9	613.5	526.8	9.1	1.7	nd
Bowker	2.5	23.9	9.1	6.9	346.8	207.1	2.5	0.7	0.7
Brome	14.7	4.0	13.7	7.0	473.0	357.7	3.3	2.3	nd
Brompton	11.7	11.0	4.9	4.9	541.2	123.9	4.8	1.0	nd
Denison	0.3	1.6	47.1	16.9	690.7	399.0	5.8	3.6	7.4
Libby	0.4	1.0	20.8	5.3	396.6	274.8	3.6	1.9	8.2
Lovering	4.9	9.8	16.5	9.8	436.9	337.7	6.2	1.5	1.8
Magog 1	11.5	7.5	24.4	15.1	470.0	235.0	3.7	2.4	3.6
Magog 2	11.5	7.5	25.8	19.5	483.4	323.9	4.1	2.7	nd
Massawippi	18.7	39.0	11.5	5.1	735.3	550.7	4.1	2.4	4.5
Memphremagog	90.0	18.2	23.5	16.7	410.0	297.4	3.4	2.0	4.3
Montjoie	3.3	8.0	13.1	7.5	377.5	260.0	5.3	1.9	1.6
Parker	0.2	3.6	18.5	9.6	623.8	385.9	7.8	1.3	nd
Simoneau	0.5	9.3	12.9	8.7	388.9	192.6	3.5	2.5	1.5
St. Georges	0.5	1.8	45.1	11.8	957.1	388.2	6.6	0.9	33.1
Stukely	4.0	13.1	6.1	4.4	302.3	267.6	3.7	2.5	1.8
Tomod	0.8	0.9	58.5	37.7	834.4	435.5	4.9	0.9	nd
Trois Lacs	2.4	1.3	36.9	13.7	681.0	438.2	8.1	5.0	6.6
Truite	2.4	3.3	31.7	5.8	448.9	477.4	8.5	2.2	2.9
Waterloo	1.5	3.0	45.5	5.5	698.5	321.1	4.6	1.4	20.3
Webster	0.1	1.3	26.1	5.9	525.1	302.5	3.9	2.7	7.8

among variables were investigated with simple correlation analysis and linear regression techniques. In some cases, relationships between a compound variable and one of its components were examined (e.g., BGE vs. BP), the statistical validity of which may be questioned. Given that BGE, BP, and BR correspond to clear and distinct conceptual entities, legitimate concerns arise only if measurement errors in both BP and BR are sufficiently large to artificially inflate the correlation between BGE and any of its components. We examined this possibility through a series of numerical simulations in which we added random noise to BP and BR of the magnitude observed in our samples (i.e., the variation seen among replicate samples), recalculated BGE, and examined its relationship with BP and BR. In all cases, the noise actually reduced the strength of the correlation between BGE and its component's variables. This implies that, for our data set, the strength of the reported correlations between BGE and BP or BR is probably underestimated.

The significance of treatment effects in the enrichment experiments was assessed using analysis of variance followed by means comparison using the Tukey–Kramer honestly significant difference (HSD) test at the $p < 0.05$ level of significance. All statistical analyses were performed using JMP 4.0 statistical software (SAS Institute).

Results

The lakes sampled were diverse with respect to both DOC and nutrient status (Table 1). Trends in nutrient status were apparent with respect to both total and dissolved fractions. Since it is only the dissolved fractions that are available as substrates for the bacteria in the dilution cultures, we focus on these fractions only. For both DOC and TDP concentrations, the slopes of the relationship between concentrations measured in situ in each lake and at $t = 0$ in each culture were not significantly different (at $\alpha = 0.05$) from 1 ($DOC_{t=0} = 0.99 \times DOC_{lake} + 0.09$, $r^2 = 0.99$, $p < 0.001$; and $TDP_{t=0} = 0.92 \times TDP_{lake} - 0.11$, $r^2 = 0.89$, $p < 0.001$), indicating that there were no systematic gains or losses in either DOC or TDP due to filtration and experimental setup. All subsequent analyses use DOC and TDP concentrations measured at $t = 0$ in the cultures. Concentrations of DOC varied from 2.5 to 9.1 mg C L^{-1}. Concentrations of TDP were more variable, ranging from 4.9 to 37.7 μg P L^{-1}. As concentrations of inorganic P in these lakes are consistently below the limits of detection during summer (Prairie unpubl. data), we consider TDP to represent primarily organically bound P. Importantly, variations in concentrations of DOC and TDP among lakes were independent ($r^2 = 0.007$, $p = 0.73$). There was a weak, but significant, relationship between DOC and TDN concentrations ($\log TDN = 0.5 \times \log DOC + 4.9$; $r^2 = 0.31$; $p < 0.01$).

In all cultures, the bacterial inoculum underwent an exponential increase in cell abundance and then exhibited signs of entering a stationary phase (an example of cell growth, O_2 consumption, and estimated mean cell biovolume during regrowth in a dilution culture is presented in Fig. 1). There was a large range in the magnitude of regrowth among dilution cultures from the 20 different lakes (Table 2). Intrinsic growth rates, μ, varied by an order of magnitude, from 0.14 to 1.41 d^{-1}. There was a general correspondence between μ and potential cell yield ($\log \text{cell yield} = 1.4 \times \log \mu + 14.3$; $r^2 = 0.50$; $p < 0.01$). This relationship, however, explained only 50% of the variability in potential cell yield,

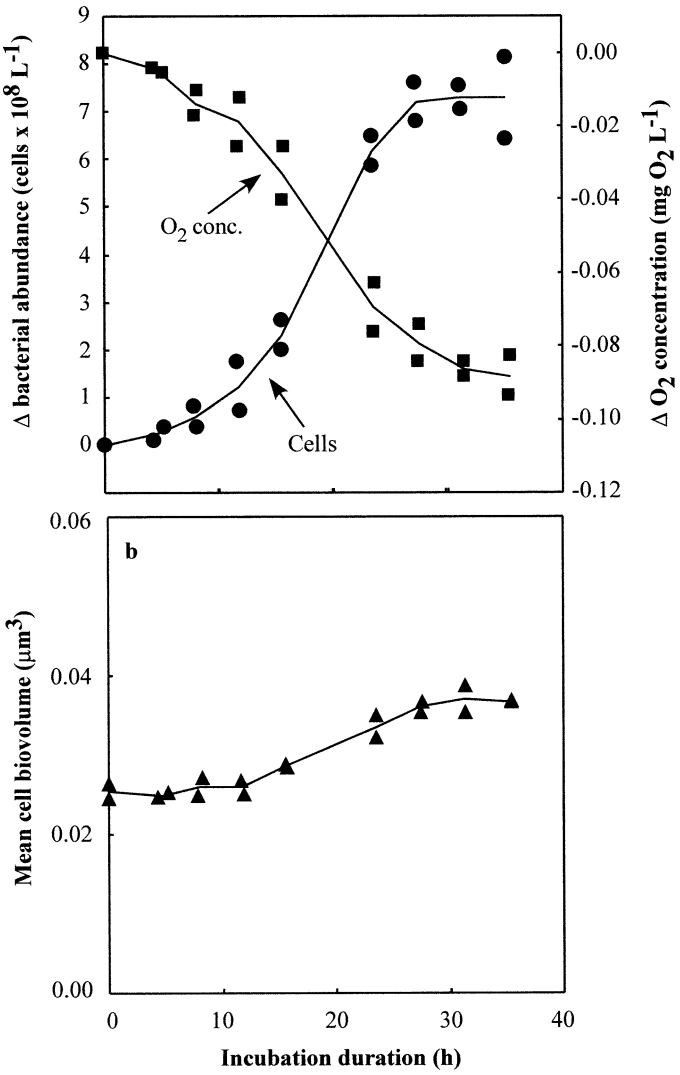

Fig. 1. Example time course of (a) increase in cell number, decrease in O_2 concentration, and (b) cell biovolume during regrowth in lake water dilution culture. The symbols represent the values for each individual culture, and the lines connect the means of the two replicate cultures.

which was substantially more variable than μ. Variability in mean cell biovolume was significantly correlated with both μ and cell yield ($r^2 = 0.44$ and 0.46, respectively), but was the least variable (CV = 20%) of all quantified parameters. When cell yield was converted to biomass units based on variations in mean cell biovolume, the resulting potential carbon yield showed the greatest variability (CV = 110%) of all variables quantified, ranging from 1.6 to 30.8 μg C L^{-1}.

Despite a significant relationship between increases in cell number and decreases in O_2 concentration in each culture ($p < 0.01$ in all cases), the slope of this relationship varied considerably among lakes. Variations in the slope of O_2 versus cell abundance (CV = 98%) were, in fact, greater than the variation in the slope of cell abundance versus time (CV = 68%). As a result, there was a high degree of variability in BR cell^{-1}, which ranged from 0.41 to 7.16 fg C cell^{-1} h^{-1}, assuming an RQ of 1. While variations in BP and BR among cultures were significantly related ($r^2 = 0.55$, $p < 0.01$), the variability in this relationship resulted in a large range in the resulting estimates of BGE, which varied from 6.7% to 51.6%.

Figure 2 presents the relationships between estimates of BGE and its two component terms, expressed as either integrated community rates (BP and BR) or cell-specific rates (BP cell^{-1} and BR cell^{-1}). BP showed a strongly significant, nonlinear, relationship to BGE, while there was no significant relationship between BGE and BR. When expressed on a per-cell basis, however, the pattern changed. BP cell^{-1} was related to BGE, but the strength of this relationship decreased substantially relative to that for BP. In contrast, the relationship between BR cell^{-1} was highly significant and explained the greatest amount of the variation in BGE.

Differences among the dilution cultures from the 20 lakes could best be explained by variations in TDP availability (Table 3). All measured parameters of the dilution cultures were significantly correlated to TDP concentrations at the beginning of the incubation. The correlation was weakest for mean cell biovolume, since it was the least variable of all parameters, and strongest for both BR cell^{-1} and BGE. The two latter variables displayed distinctly nonlinear relationships with TDP, however (Fig. 3). The negative relationship between TDP and BR cell^{-1} was best described by the power function, $y = 15.9x^{-1.09}$. The positive relationship between TDP and BGE was best described by the semilogarithmic function, BGE = 19.4 × log TDP − 17.5. In contrast, only the potential yield, expressed as either cell number or carbon biomass, was significantly related to DOC concentrations (Table 3). Variations in potential yields were also related to differences in TDN concentrations, since TDN and DOC are themselves significantly related to one another. Expressing TDP availability relative to DOC availability, as the atomic C : P ratio, did not improve our ability to predict variations in bacterial metabolism among lakes. Only BR cell^{-1} and BGE were significantly related to the C : P ratio, but in neither case was the degree of correlation greater than that for TDP alone.

Of the four lakes for which dilution cultures included substrate additions, three showed responses to enrichment. For these three lakes, response to phosphorus and glucose additions was variable. In general, additions of phosphorus alone produced significant increases in μ (Fig. 4a), mean cell biovolume (Fig. 4b), and potential biomass yield (Fig. 4c), whereas additions of glucose alone produced no significant increases in any of these parameters. There was, however, a significant interaction between phosphorus and glucose, with the combination producing increases in mean cell biovolume and biomass yield that were significantly greater than that of phosphorus alone. With respect to BR cell^{-1} and BGE, additions of phosphorus and glucose produced contrasting effects. BR cell^{-1} (Fig. 5a) showed significant increases with glucose addition but nonsignificant decreases with phosphorus addition. BGE (Fig. 5b) showed significant increases with phosphorus addition and significant decreases with glucose addition. For both BR cell^{-1} and BGE, treatments of phosphorus and glucose together produced responses that were intermediate to that of the individual additions. The

Table 2. Parameters derived from bacterial regrowth cultures for each lake. Growth rate is the slope of the natural log of cell abundance versus time, for the period of exponential growth. Cell biovolume is the mean biovolume of cells at the end of exponential growth. Cell yield is the maximum bacterial abundance achieved at the end of exponential growth. Carbon yield is the biomass achieved at the end of exponential growth, based on cell abundance and mean biovolume. BR cell^{-1} is the cell-specific respiration rate, calculated as the slope of Δ cells ver ΔO_2 (converted to C units) per time, for the period of exponential growth. BGE is the growth efficiency, calculated as the slope of bacterial production versus bacterial production plus respiration. *See Material and Methods* for details of all calculations. All lakes were sampled in 2001.

Lake	Date sampled	Growth rate (d^{-1})	Cell biovolume (μm^3)	Cell yield (cells $\times$ 10^9 L^{-1})	Carbon yield (μg C L^{-1})	BR cell^{-1} (fg C cell$^{-1}h^{-1}$)	BGE (%)
Aylmer	27 Aug	0.35	0.027	0.45	4.8	2.28	19.5
Bowker	8 Aug	0.54	0.027	0.16	1.7	2.83	9.3
Brome	27 Jun	0.37	0.034	0.42	5.1	1.19	28.0
Brompton	12 Jun	0.35	0.037	0.16	2.0	3.80	9.9
Denison	25 Jul	0.64	0.035	0.87	10.8	0.87	34.3
Libby	3 Jul	0.34	0.030	0.50	5.6	2.07	20.3
Lovering	27 Jul	0.34	0.026	0.25	2.6	0.83	28.4
Magog 1	30 Jul	0.23	0.031	0.53	6.1	0.57	35.2
Magog 2	22 Aug	0.69	0.035	0.77	10.1	1.04	32.4
Massawippi	30 Jul	0.23	0.029	0.16	1.8	2.81	11.0
Memphremagog	1 Aug	0.35	0.027	0.33	3.6	0.51	45.4
Montjoie	27 Jul	0.21	0.024	0.17	1.7	3.19	21.9
Parker	27 Jul	0.48	0.030	1.24	14.0	0.67	36.1
Simoneau	10 Jul	0.27	0.026	0.20	2.1	0.98	26.0
St. Georges	12 Jul	0.36	0.030	0.64	7.5	1.15	28.7
Stukely	28 Jun	0.15	0.029	0.15	1.6	7.16	6.7
Tomcod	12 Jun	0.82	0.043	2.21	30.8	0.41	51.6
Trois Lacs	25 Jul	1.41	0.049	1.67	25.3	1.28	32.1
Truite	14 Aug	0.91	0.027	0.42	4.4	1.63	17.9
Waterloo	3 Jul	0.22	0.030	0.34	3.9	2.02	12.1
Webster	1 Aug	0.14	0.023	0.22	2.1	2.69	13.6

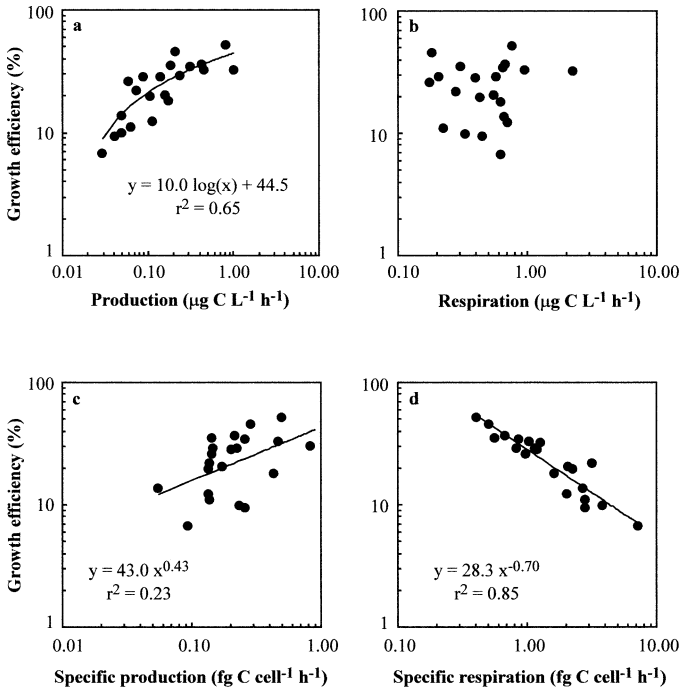

Fig. 2. Relationships between (a) integrated bacterial production rate and bacterial growth efficiency, (b) integrated bacterial respiration rate and bacterial growth efficiency, (c) cell-specific rate of bacterial production and growth efficiency, and (d) cell-specific rate of bacterial respiration and growth efficiency.

lake for which cultures showed no response to enrichment had the highest initial concentration of phosphorus (19.5 μg P L^{-1}) but a relatively low concentration of DOC (4.1 mg C L^{-1}).

Discussion

Heterotrophic bacteria, being chemoorganotrophs, must ultimately be dependent on a supply of organic carbon. Total DOC concentrations, however, failed to explain a significant fraction of the variance in bacterial growth rate, specific respiration, or growth efficiency among lakes. Instead, results of our comparative study demonstrate that pelagic bacterial metabolism and BGE varied as a function of phosphorus concentration (Table 3 and Fig. 3). Thus, despite the obvious role of allochthonous DOC in maintaining net heterotrophy in many lakes (Cole 1999), our results indicate that bacterial growth and attainable biomass in these lakes are primarily dependent on the absolute availability of phosphorus. It further appears, based on observed growth efficiencies, that energy flow and the fate of DOC use by bacteria (i.e., CO_2 versus biomass) are also tightly regulated by phosphorus supply.

Substantial variability in BGE is a common feature of natural bacterial assemblages (e.g., Biddanda et al. 1994; Jahnke and Craven 1995; Carlson and Ducklow 1996; Roland and Cole 1999). The range (6.7–51.6%) and mean (24.8%) for observed BGE values in the present study agree well with values reported previously for natural limnetic

Table 3. Pearson's correlation coefficients (r) for parameters of bacterial growth cultures versus initial concentrations of total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), total dissolved organic carbon (DOC), and the atomic C:P ratio of dissolved organic matter. Cell yield and biomass yield are maximum cell abundance and the maximum bacterial carbon achieved in each growth culture. All correlations were performed on log-transformed data ($n = 21$). Significant correlations are indicated with asterisks: *$p < 0.05$, **$p < 0.001$; ***$p < 0.001$.

	TDP	TDN	DOC	C:P ratio
Growth rate (d^{-1})	0.53*	0.34	0.43	−0.23
Mean cell biovolume (μm^3)	0.50*	0.13	0.18	−0.33
Cell yield (cells $\times 10^9$ L^{-1})	0.73***	0.50*	0.45*	−0.39
Biomass yield (μg C L^{-1})	0.73***	0.47*	0.44*	−0.40
BR $cell^{-1}$ (fg O_2 $cell^{-1}$ h^{-1})	−0.83***	−0.25	−0.13	0.66**
Growth efficiency (%)	0.83***	0.20	0.08	−0.56*

communities (del Giorgio and Cole 1998). Although the utility of BGE measurements has recently been questioned on the basis of potentially large uncertainties resulting from methodological concerns (Goldman and Dennet 2000), our results show that the observed variability in BGE across lakes was not random, but instead represents an ecologically meaningful pattern that is linked to the gradient in the trophic status of these lakes.

It has been suggested that a pattern of increasing BGE from oligotrophic to eutrophic environments may be linked to system productivity through systematic changes in the quantity and quality of organic carbon substrates available to the bacterial community (Biddanda et al. 1994; del Giorgio et al. 1997). Definitions of organic matter quality used in the literature tend to focus on the carbon to nutrient ratio of the organic resources (e.g., Goldman et al. 1987; Cimbleris and Kalff 1998). In this study, however, variations in BGE were unrelated to the C:N ratio of the initial organic matter pool (data not shown), and, while the relationship between BGE and bulk C:P ratio was moderately significant, this was less effective a predictor of BGE than was P alone (Table 3). The total pool of organic matter and the pool that is readily available for bacterial use are not, however, the same thing. It may be that the relationships observed across our study lakes reflected an underlying dependence of bacterial metabolism on an increasing fraction of labile, low C:P dissolved organic matter contained within the total organic pool. Responses of the bacterial assemblages to experimental enrichment (Figs. 4 and 5) generally corroborated the patterns seen among lakes and support the importance of phosphorus, per se, in driving variations in bacterial metabolism and BGE. Phosphorus limitation was apparent in three of the four lakes as significant increases in bacterial growth, potential biomass yield, and growth efficiency with the addition of phosphorus alone, but not with glucose alone. These results are consistent with previous studies demonstrating phosphorus limitation of bacterial production in individual lakes (Toolan et al. 1991; Coveney and Wetzel 1992; Gurung and Urabe 1999) and support the conclusion that the severity of phosphorus limitation has a profound effect on the magnitude of BGE in oligotrophic lakes.

Although our results do not corroborate directly the relationship between DOC concentration and lake metabolism (as oxygen undersaturation) that was previously observed for lakes of the same region (Prairie et al. 2002), the influence of organic carbon supply cannot be completely discounted in these lakes. Potential yield of bacteria in the dilution cultures, expressed as either cell number or carbon biomass, was significantly related to DOC concentrations (Table 3). In addition, for two of the four lakes subjected to experimental enrichment, it seems likely that carbon was either secondarily limiting or close to colimiting to the bacterial assemblage, since the combined glucose plus phosphorus treatment resulted in significantly higher potential biomass yields relative to that of phosphorus alone (Fig. 4). This is similar to the finding of colimitation of carbon and phosphorus that has been reported in a small dimictic lake (Carlsson and Caron 2001). An interesting exception to this, however, is Lake Magog, which had high TDP and low DOC but showed no significant response to either phosphorus or glucose enrichment. If bacterial metabolism in these lakes is primarily limited by phosphorus and secondarily limited by carbon, one would expect that glucose would have elicited a response in the cultures from this lake.

It is intriguing that, while no relationship between BR $cell^{-1}$ and DOC concentration was observed among lakes, experimental enrichment of glucose significantly increased BR $cell^{-1}$ in three of the four lakes (Fig. 5a). Since increases in BR $cell^{-1}$ were not accompanied by any positive increase in μ or biomass yield, the reason for this response is unclear. The use of energy in reactions not associated with growth has been shown to be highly variable in bacteria, especially when bacteria are growing under limiting conditions (Russel and Cook 1995). Perhaps the enhancement of respiration resulting from glucose enrichment reflects a strategy on the part of the bacteria to maintain the highest possible energy flow under conditions of constrained growth when presented with an easily usable source of energy (i.e., glucose). Since nutrients are available almost exclusively in organic-bound form in oligotrophic environments, their acquisition depends on enzyme-intensive processes that are energetically costly. Maintaining cellular energy flow would thus be advantageous for increasing growth should environmental conditions become more favorable (Morita 1997). In the lake comparison, BR $cell^{-1}$ was significantly greater in the most oligotrophic lakes and decreased with increasing TDP. This is consistent with previous reports of specific respiration in lakes (Cimbleris and Kalff 1998; Biddanda et al. 2001) and supports the idea that extreme oligotrophy entails a high energetic cost (likely due to the metabolic expenditures asso-

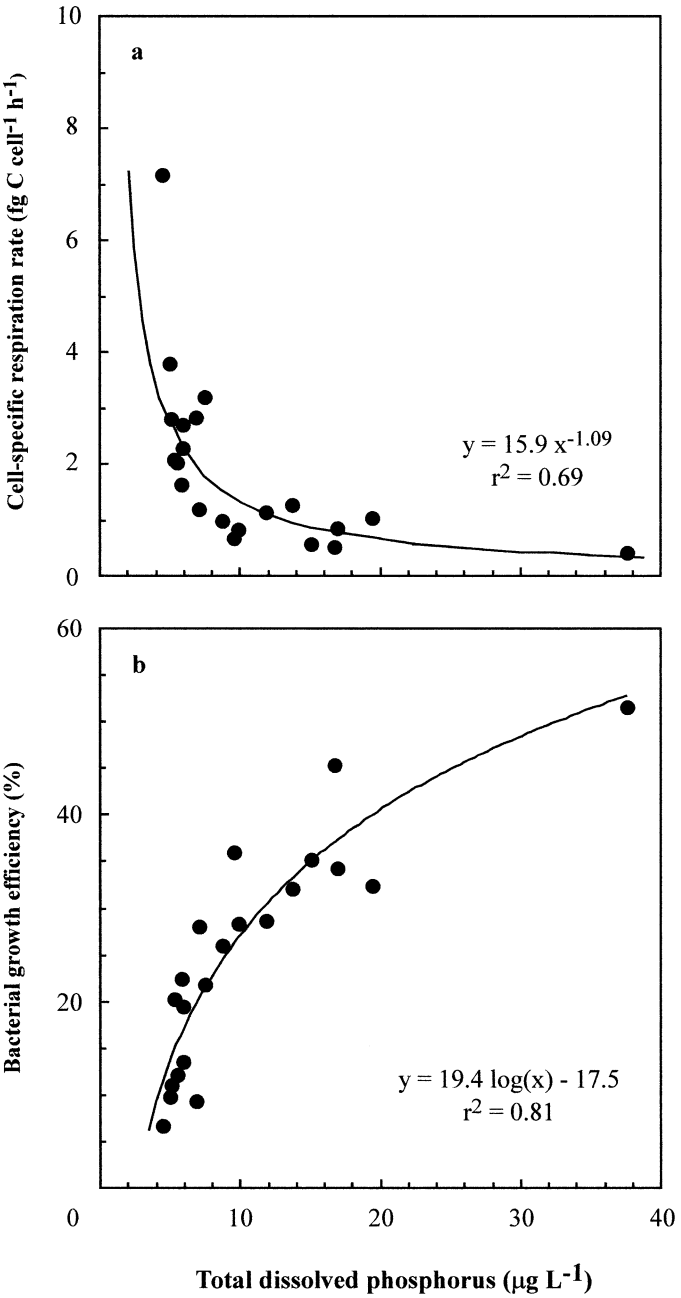

Fig. 3. Relationships between (a) cell-specific bacterial respiration and total dissolved phosphorus among lakes and (b) bacterial growth efficiency and total dissolved phosphorus among lakes.

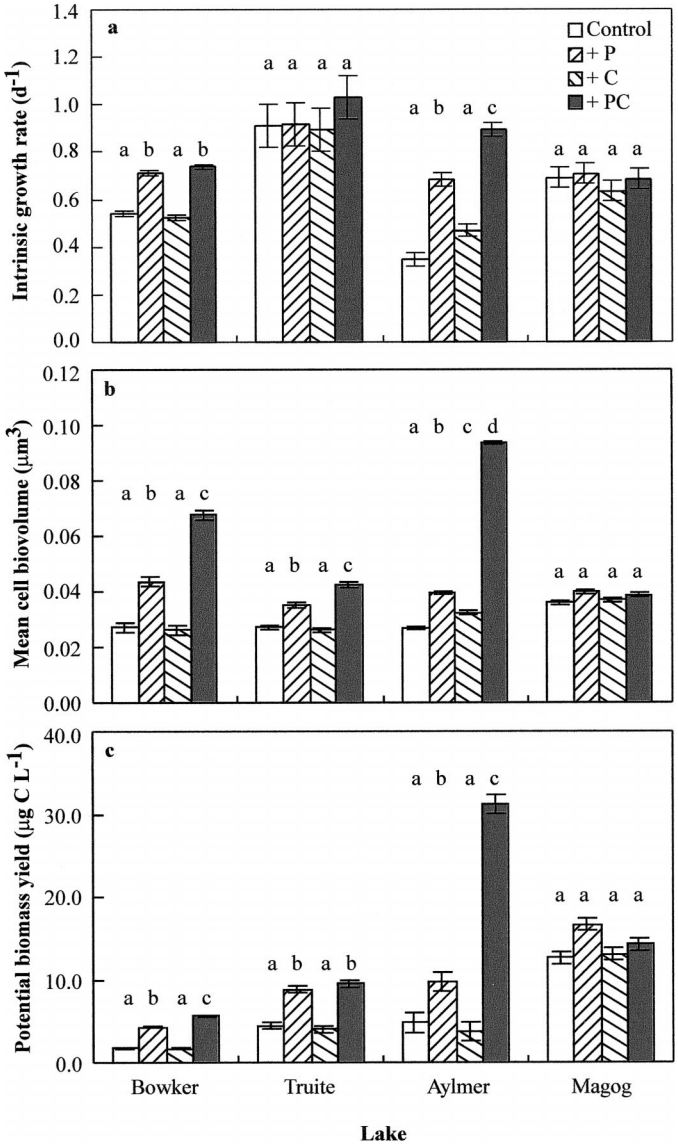

Fig. 4. Enrichment experiment treatment comparisons of (a) intrinsic growth rate, (b) mean bacterial cell biovolume, and (c) potential biomass yield during dilution cultures from each lake. Error bars represent ±1 standard error (SE) calculated using the pooled error estimate of error variance ($n = 2$). Treatment means with the same letter are not significantly different at the $p < 0.05$ level of significance, for within-lake comparisons only.

ciated with intensive enzyme synthesis) to the bacteria living in such dilute environments.

Variability in BGE results from the loose coupling between its two component terms, BP and BR. A significant relationship between BGE and BP, but not BR, as was seen in the present study (Fig. 2), has been reported previously (e.g., Roland and Cole 1999), although the evidence for a general relationship between BGE and BP is inconsistent (e.g., Søndergaard and Theil-Nielsen 1997). Across a range of aquatic ecosystems, BP has consistently been shown to be more variable than BR (e.g., del Giorgio et al. 1997). This has often led to the conclusion that variations in BGE are driven by those factors that regulate variations in BP. What has not been considered previously, however, is how these component terms relate to BGE when expressed on a per-cell basis, rather than as integrated terms. Results here suggest this can lead to very different interpretations (Fig. 2). BGE is significantly related to BP cell^{-1}, but the relationship is not as strong as that for BP. In contrast, BGE was not significantly related to BR but showed the strongest relationship of all with BR cell^{-1}. If bacteria tend to maximize growth at the expense of efficiency, however (Russell and Cook 1995; Vallino et al. 1996), one might then expect a

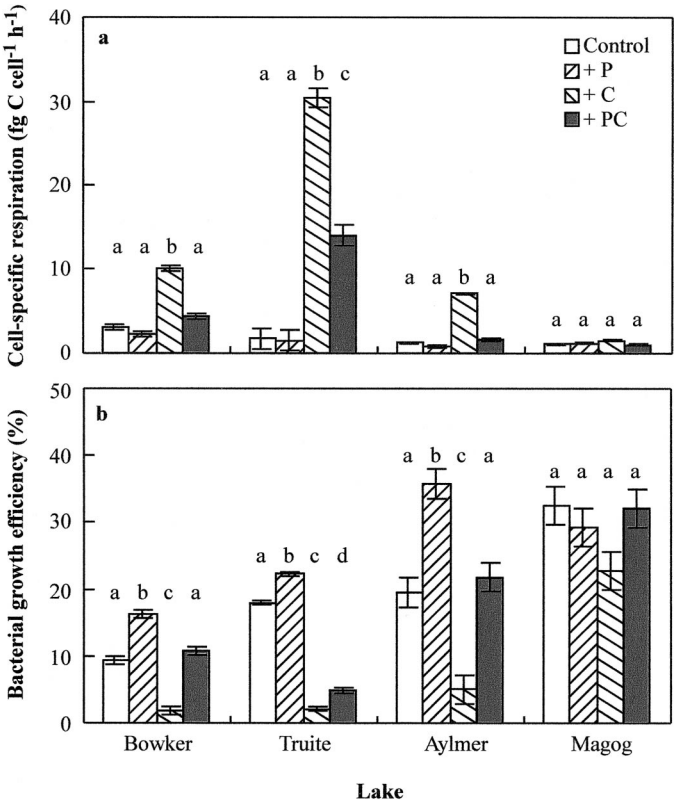

Fig. 5. Enrichment experiment treatment comparisons of (a) cell-specific respiration rate and (b) bacterial growth efficiency during dilution cultures from each lake. Error bars represent ±1 SE calculated using the pooled error estimate of error variance (n = 2). Treatment means with the same letter are not significantly different at the $p < 0.05$ level of significance, for within-lake comparisons only.

loose coupling between BGE and BP but a stronger coupling between BGE and BR at the level of the individual cell. There is presently very little research on variations in BR cell^{-1} in either freshwater (Cimbleris and Kalff 1998) or marine waters (Jorgensen et al. 1999). However, our results would argue that, while BR may often be rather invariant in aquatic ecosystems, BR cell^{-1} is highly variable and perhaps the more appropriate scale when considering how variations in respiration relate to BGE. Indeed, it may well be that the commonly observed lack of strong gradients in BR with increasing system productivity simply reflects the contrasting patterns of decreasing BR cell^{-1} (Biddanda et al. 2001; fig. 2a) and increasing bacterial abundance (e.g., Cole et al. 1993) as one moves from oligotrophic to eutrophic systems.

In this study we used lake water dilution cultures to explore variability in bacterial metabolism and BGE along gradients of DOC and TDP in lakes. This technique is not without its share of potential problems associated with enclosure effects, however. The isolation of the bacterial assemblage in dilution cultures uncouples the measured bacterial activity from potential in situ sources of the most labile substrates with the highest turnover rates, as well as from potential sources of regenerated nutrients. In addition, the phylogenetic composition may shift significantly over the course of long incubations (e.g., Massana et al. 2001). These methodological artifacts may make extrapolations of metabolic rates measured in such incubations to those occurring in situ potentially problematic. In a review of BGE measurements across a variety of aquatic ecosystems (del Giorgio and Cole 1998), however, no systematic differences were found between estimates of BGE obtained from either long-term or short-term incubations, and the range in BGE reported here agrees well with previous estimates for lake communities. The interpretation of metabolic responses by the bacteria in dilution cultures must nonetheless be limited to just those pools of resources present at the time of sampling, and we cannot say anything about the flux of potentially limiting resources supplied by an intact food web. If we consider the initial conditions of the cultures as a snapshot in time of available resources, however, results from these experiments provide fundamental information on how bacterial activity and growth efficiency vary across a changing resource base.

Estimates of BGE measured in these dilution cultures are also subject to some potential uncertainty associated with both an assumed respiratory quotient of 1, used to convert O_2 consumption to CO_2 production, and in the derivation of cellular carbon content. The choice of a respiratory quotient of 1 is likely of minor influence (del Giorgio and Cole 1998). Our estimates of cell carbon content are dependent on our estimates of cell biovolumes, which were derived from an empirical relationship between measured cell biovolumes and the mean green fluorescence of cells, relative to that of the bead standard, from the flow cytometric data. There is no theoretical reason to assume that nucleic acid content (DNA + RNA), as measured by the green fluorescence of SYTO-13, should necessarily exhibit a constant relationship with cell size, as opposed to, e.g., growth rate, or some other factor that affects staining efficiency. Nonetheless, the relationship we found was quite strong, as has been found by others (Gasol and del Giorgio 2000). More importantly, cell biovolumes were rather invariant among lake cultures (Table 2), and thus, in the end, a minor source of variation in the resulting estimates of BGE. If, for example, we double the value of cell biovolume at each time point in each culture, the resulting estimates of BGE increase by only 20–46%. In fact, employing a constant value for biovolume, as is frequently done in studies of BGE, changes the resulting relationship between BGE and TDP across lakes very little, with the correlation coefficient (Table 3) decreasing by only 6%. Finally, estimates of BP from our dilution cultures, which are derived as a product of cell abundance and mean cell biovolume, compare well with estimates of BP from short-term radiotracer techniques in these same lakes (P. A. del Giorgio pers. comm.).

While future investigations on the environmental control of bacterial metabolism and BGE would greatly benefit from techniques that avoid the separation of the bacterioplankton and subsequent lengthy incubations, our results demonstrate ecologically meaningful patterns in bacterial activity across gradients of TDP in these lakes and provide further insight to the regulation of BR cell^{-1} and its relationship to BGE. In this regard, it is important to note that dilution cultures produce the least ambiguous estimate of BR cell^{-1} of the currently available methodologies (Smith and Kemp 2003).

Results from the comparative study presented here lead us to conclude that the fate of DOC use by lake bacterial communities is strongly dependent on phosphorus availability. Extreme oligotrophy places high respiratory demands on the bacterioplankton at the level of the individual cell, resulting in very low bacterial growth efficiencies and consequently greater DOC flow to CO_2 than to biomass available for transfer to higher trophic levels. There is thus a need for increased research efforts that focus on the role of nutrients in organic matter use and bacterial metabolism, which should include rates of bacterial respiration as well as bacterial production.

References

AMMERMAN, J. W., J. A. FUHRMAN, A. HAGSTROM, AND F. AZAM. 1984. Bacterioplankton growth in seawater: I. Growth kinetics and cellular characteristics in seawater cultures. Mar. Ecol. Prog. Ser. **18:** 31–39.

BIDDANDA, B., M. OGDAHL, AND J. COTNER. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnol. Oceanogr. **46:** 730–739.

———, S. OPSAHL, AND R. BENNER. 1994. Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf. Limnol. Oceanogr. **39:** 1259–1275.

CARIGNAN, R., A. M. BLAIS, AND C. VIS. 1998. Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method. Can. J. Fish. Aquat. Sci. **55:** 1078–1084.

CARLSON, C. A., AND H. W. DUCKLOW. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquat. Microb. Ecol. **10:** 69–85.

CARLSSON, P., AND D. A. CARON. 2001. Seasonal variation of phosphorus limitation of bacterial growth in a small lake. Limnol. Oceanogr. **46:** 108–120.

CATTANEO, A., AND Y. T. PRAIRIE. 1995. Temporal variability in the chemical characteristics along the Riviere de l'Achigan: How many samples are necessary to describe stream chemistry? Can. J. Fish. Aquat. Sci. **52:** 828–835.

CIMBLERIS, A. C. P., AND J. KALFF. 1998. Planktonic bacterial respiration as a function of C : N : P ratios across temperate lakes. Hydrobiologia **384:** 89–100.

COLE, J. J. 1999. Aquatic microbiology for ecosystem scientists: New and recycled paradigms in ecological microbiology. Ecosystems **2:** 215–225.

———, M. L. PACE, N. F. CARACO, AND G. S. STEINHART. 1993. Bacterial biomass and cell-size distributions in lakes: More and larger cells in anoxic waters. Limnol. Oceanogr. **38:** 1627–1632.

———, ———, S. R. CARPENTER, AND J. F. KITCHELL. 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. Limnol. Oceanogr. **45:** 1718–1730.

COVENEY, M. F., AND R. G. WETZEL. 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. Appl. Environ. Microbiol. **58:** 150–156.

CURRIE, D. J., AND J. KALFF. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. **41:** 493–497.

DEL GIORGIO, P. A., AND J. J. COLE. 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. **29:** 503–541.

———, ———, AND A. CIMBLERIS. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature **385:** 148–151.

———, AND R. H. PETERS. 1994. Patterns in planktonic P-R ratios in lakes: Influence of lake trophy and dissolved organic carbon. Limnol. Oceanogr. **39:** 772–787.

ELSER, J., L. STABLER, AND R. HASSET. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: A comparative study. Aquat. Microb. Ecol. **9:** 105–110.

FAGERBAKKE, K. M., M. HELDAL, AND S. NORLAND. 1996. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. Aquat. Microb. Ecol. **10:** 15–27.

GASOL, J. M., AND P. A. DEL GIORGIO. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci. Mar. **64:** 197–224.

GOLDMAN, J. C., D. A. CARON, AND M. R. DENNETT. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C : N ratio. Limnol. Oceanogr. **32:** 1239–1252.

———, AND M. R. DENNETT. 2000. Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation. Limnol. Oceanogr. **45:** 789–800.

GURUNG, T. B., AND J. URABE. 1999. Temporal and vertical difference in factors limiting growth rate of heterotrophic bacteria in Lake Biwa. Microb. Ecol. **38:** 136–145.

JAHNKE, R. A., AND D. B. CRAVEN. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: A need for respiration rate measurements. Limnol. Oceanogr. **40:** 436–441.

JANSSON, M., A. K. BERGSTROM, P. BLOMQVIST, AND S. DRAKARE. 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. Ecology **81:** 3250–3255.

JORGENSEN, N. O. G., N. KROER, R. B. COFFIN, AND M. P. HOCH. 1999. Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. Aquat. Microb. Ecol. **18:** 247–261.

MASSANA, R., C. PEDROS-ALIO, E. O. CASAMAYOR, AND J. M. GASOL. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnol. Oceanogr. **46:** 1181–1188.

———, AND OTHERS. 1997. Measurement of bacterial size via image analysis of epifluorescence preparations: Description of an inexpensive system and solutions to some of the most common problems. Sci. Mar. **61:** 397–407.

MORITA, R. 1997. Bacteria in oligotrophic environments. Chapman and Hall.

POSCH, T., M. LOFERER-KROSSBACHER, G. GAO, A. ALFREIDER, J. PERNTHALER, AND R. PSENNER. 2001. Precision of bacterioplankton biomass determination: A comparison of two fluorescent dyes, and of allometric and linear volume-to-carbon conversion factors. Aquat. Microb. Ecol. **25:** 55–63.

PRAIRIE, Y. T., D. F. BIRD, AND J. J. COLE. 2002. The summer metabolic balance in the epilimnion of southeastern Quebec lakes. Limnol. Oceanogr. **47:** 316–321.

ROLAND, F., AND J. J. COLE. 1999. Regulation of bacterial growth efficiency in a large turbid estuary. Aquat. Microb. Ecol. **20:** 31–38.

RUSSELL, J., AND G. COOK. 1995. Energetics of bacterial growth: Balance of anabolic and catabolic reactions. Microb. Rev. **59:** 48–62.

SMITH, E. M., AND M. W. KEMP. 2003. Planktonic and bacterial respiration along an estuarine gradient: Responses to carbon and nutrient enrichment. Aquat. Microb. Ecol. **30:** 251–261.

SØNDERGAARD, M., AND J. THEIL-NIELSEN. 1997. Bacterial growth efficiency in lakewater cultures. Aquat. Microb. Ecol. **12:** 115–122.

TOOLAN, T., J. D. WEHR, AND S. FINDLAY. 1991. Inorganic phosphorus stimulation of bacterioplankton production in a mesoeutrophic lake. Appl. Environ. Microbiol. **57:** 2074–2078.

VALLINO, J., C. HOPKINSON, AND J. HOBBIE. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. Limnol. Oceanogr. **41:** 1591–1609.

WETZEL, R. G. 1992. Gradient-dominated ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia **229:** 181–198.

WILLIAMS, P.J.L.B. 2000. Heterotrophic bacteria and the dynamics of dissolved organic matter, p. 153–200. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley.

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