MICROBIAL ECOLOGY

Microb Ecol (2003) 46:33–42
DOI: 10.1007/s00248-002-0004-9

Exploring Bacterioplankton Growth and Protein Synthesis to Determine Conversion Factors Across a Gradient of Dissolved Organic Matter

E. Pulido-Villena[1], I. Reche[2]

[1] Departamento de Biología Animal y Ecología, Universidad de Granada, 18071 Granada, Spain
[2] Instituto del Agua, Universidad de Granada, 18071 Granada, Spain

Received: 5 October 2002; Accepted: 16 January 2003; Online publication: 21 May 2003

ABSTRACT

The effect of bacterial specific growth rates of abundance (μ) and protein synthesis (*b*) on conversion factor (CF) variability was explored in order to provide an alternative approach to the controversial application of just one universal CF to field data. Nine regrowth cultures (RCs) were set up from very diverse aquatic ecosystems, controlling temperature and adding N and P to avoid mineral limitation and force organic carbon limitation. The values of μ varied one order of magnitude from 0.26 to 3.34 d^{-1}, whereas *b* values varied two orders of magnitude from 0.28 to 34.87 d^{-1}. We found no relationships between μ or *b* values and the dissolved organic carbon (DOC) concentration or the dissolved organic matter (DOM) quality indexes assayed. Abundance and protein synthesis increased exponentially and synchronously in four RCs, leading to balanced growth ($\mu = b$). In contrast, abundance and protein synthesis increased logistically in the other five RCs and *b* values were significantly higher than μ values, leading to unbalanced growth ($\mu \neq b$). CFs ranged from 0.0062 to 0.0576×10^{18} cells mol $leucine^{-1}$ with an average of 0.0305×10^{18} cells mol $leucine^{-1}$. CFs obtained in RCs with balanced growth were generally higher than CFs obtained in RCs with unbalanced growth and were not alike, impeding the establishment of an upper limit for CFs. A positive and significant relationship ($n = 8$, $p < 0.01^{**}$, $r^2 = 0.71$) was found between CFs and DOC concentration (CF ($\times 10^{18}$ cells mol $leucine^{-1}$) = 0.0104 + 0.0094 DOC (mM)) when the value for the most productive system was excluded. This function permits the estimation of site-specific CFs based on DOC concentration instead of the controversial use of a single CF for different systems.

Introduction

Bacterioplankton constitute the trophic level with most organisms and control one of the largest C fluxes from dissolved organic matter (DOM) to microplankton in

Correspondence to: I. Reche; E-mail: ireche@ugr.es

many aquatic ecosystems, modeling their trophic structures [3, 8]. In addition, these organisms regenerate and/or consume nitrogen (N) and phosphorus (P) [18, 52], thereby influencing biogeochemical processes [10].

Scientific recognition of the prominent role played by bacterioplankton in aquatic ecosystems has followed methodological advances such as the introduction of epifluorescence microscopy to determine their abundance [19, 34] and the use of radioisotopes to measure their production [16, 43]. The extensive use of these techniques has required the translation of radioisotope data into abundance and carbon units, leading to the development of numerous conversion factors (CFs). Most of these have been obtained by relating increases in bacterial abundance to the incorporation rates of radioisotopes into macromolecules (^{3}H-thymidine in DNA or ^{3}H-leucine in proteins) in cultures [22]. However, the large variability of the CFs reported has made their general application to field data controversial.

Sources of CF variability include methodological differences in the measurement of radioisotope incorporation (filtration vs microcentrifugation) [26], in the setting up of bacterial cultures (dilution vs filtration, mineral and organic nutrient additions, etc.) [11, 22, 24, 46], and in the performance of calculations (integrative, derivative, etc.) [14]. CF variability can also derive from processes related to the synchronization between radioisotope incorporation and cell production [14].

Under optimal resource conditions, bacterioplankton grow exponentially and optimize DNA duplication with respect to protein metabolism, maximizing reproduction and showing balanced growth (synchronous increases of cells and radioisotope incorporation rates) [14, 41]. Because CFs are obtained by relating increases in abundance to increases in protein synthesis, these conditions are expected to yield the maximum (upper limit) of cell production per protein unit and, therefore, a CF potentially extrapolative among systems where resources are not limited. In contrast, under limited conditions bacterioplankton present logistic growth and protein synthesis may be favored in order to maximize survival rather than reproduction. This leads to unbalanced growth (cell division and radioisotope incorporation is uncoupled) [14, 41], and this situation is expected to yield variable, lower, and site-specific CFs.

Bacterioplankton resources are dissolved organic carbon (DOC), organic and inorganic forms of N and P, vitamins, and trace metals [9, 38, 47, 48]. A strong limitation (bottom-up control) of bacterioplankton by the organic substrate has been underlined in numerous across- systems studies [4, 17, 32]. Bacterial growth is constrained not only by the quantity of the organic substrate but also by its quality, which determines the efficiency of carbon incorporation into new biomass in relation to respiratory losses [2, 27, 45, 48]. Although an unequivocal and broadly used definition of DOM quality has not been established, several properties such as molecular size, humic content, aromaticity, and elemental composition have been considered indicators of DOM quality [1, 2, 31, 45, 49]. DOM chemical characterization requires the processing of large volumes of water, and the isolation procedures are laborious. For this reason, considerable scientific effort has focused on the development of accurate and simple indexes to characterize DOM. Some of these indexes have been derived from the optical properties of DOM, such as its absorbance or fluorescence [13, 28, 44].

In the present study, we tested hypotheses that optimal organic-substrate conditions (high DOC concentration of high quality) yield balanced (exponential) growth and consequently an upper limit of CFs and that more restrictive organic-substrate conditions (low DOC concentration of low quality) yield unbalanced (logistic) growth and lower CFs. We obtained a linear function between DOC concentration and CFs that allows estimations of site-specific CFs.

Materials and Methods

Study Sites and Sampling

Nine aquatic ecosystems were selected in an effort to include organic substrates with a wide range of DOC concentration and DOM quality. Eight systems are in the southeast of Spain and one (Old Man McMullen pond (OMM)) in Norfolk, CT, USA. The systems are located in very diverse settings: alpine (Caldera Lake, CA), agricultural (Bermejales Reservoir, BE), coastal (Honda Lagoon, HO), karstic (Grande, Dulce and Salada lakes, GR, DU, SA), alkaline-peaty (Padul P1 and Padul P0 ponds, PP1, PP0), and conifer forest (OMM). Each lake was sampled once at a central station during spring or summer of 1999 (except OMM, summer 1996). Underwater photosynthetically active radiation (PAR) was measured at 0.2-m depth intervals with a LiCor (LI 193SA) quantum sensor (except OMM). Water samples were collected at a depth of 50% PAR to avoid intense DOM photobleaching, which modifies DOM optical properties [37]. Samples were taken for analysis of DOC and optical properties of DOM, and for setting up regrowth cultures (RCs). Water for the RCs (2 L) was stored at approximately 4°C in the dark for 2 to 3 h until its use at the laboratory.

DOM Optical Characterization

Triplicate samples from all systems except the alpine lake were prepared for DOC analysis by filtering lake water through precombusted Whatman GF/F filters. The filtrates were collected in combusted (>2 h at 500°C) flasks, acidified with HCl (final pH < 2), stored at 4°C in the dark until analysis, and measured with a Shimadzu Total Carbon Analyzer TOC-5050. Duplicate samples from the alpine lake were similarly prepared and the filtrates were measured with a TOC-5000 equipped with a Shimadzu platinized-quartz catalyst for high sensitivity analysis.

Triplicate samples for absorbance and fluorescence were filtered through Whatman GF/F filters. Absorbance scans from 250 to 700 nm were measured in 10-cm quartz cuvettes using a Perkin Elmer Lambda 40 spectrophotometer connected to a computer equipped with UV-WINLAB software (except for water from OMM pond). Absorbances at the specific wavelengths of 250 nm (A_{250}) and 440 nm (A_{440}) were expressed as Napierian absorption coefficients (a_{250} and a_{440}) in m^{-1} [30]:

$$a_{250,440} = \frac{2.303 A_{250,440}}{l} \quad (1)$$

where l is the optical pathlength in metres. Molar absorption coefficients at 250 nm (ϵ_{250}) and 440 nm (ϵ_{440}) in $m^2 mol^{-1}$ were also calculated as:

$$\varepsilon_{250,440} = \frac{a_{250,440}}{C} \quad (2)$$

where C is the concentration of DOC in mM [5].

Fluorescence emission spectra from 370 to 650 nm (excitation at 370 nm, slit width of 0.5 nm) were measured in a Perkin Elmer LS50B spectrofluorometer using 1-cm quartz cuvette (rinsed twice with the sample) except for water from OMM pond. Fluorescence at 450 nm (F_{450}) was expressed as quinine sulfate units (QSU), where 1 QSU = 1 μg L^{-1} of quinine sulfate dissolved in 0.1 N H_2SO_4. Five standards (1, 2, 5, 10, and 20 μg L^{-1}) were used for the calibration curve. Milli-Q water was used as sample blank and 0.1 N H_2SO_4 as standards blank [42]. The ratios of the fluorescence at 450 nm with respect to 500 nm (F_{450}:F_{500}) were also determined.

Three indexes were used as surrogates of DOM quality: ϵ_{250}, ϵ_{440}, and F_{450}:F_{500}. Absorption at longer wavelengths is related to compounds of high molecular weight enriched in chromophores, whereas absorption at the shorter wavelength is related to molecules of lower weight [13, 44]. Therefore, molar absorption coefficients at 250 nm (ϵ_{250}) can be considered as an indicator of the relative contribution of small molecules (absorbing at short wavelengths) to the total DOC pool. Conversely, molar absorption coefficients at 440 (ϵ_{440}) can be considered as the relative contribution of humic acids (large molecules enriched in chromophores) to the total DOC pool [12, 36]. The F_{450}:F_{500} ratio can be considered an index of the origin of fulvic acids (terrestrial vs autochthonous) [28]. A ratio of ≈1.4 is typical for terrestrially derived fulvic acids, and a ratio of ≈1.9 is typical for microbially derived fulvic acids (from degradation of algae and bacteria). Microbially derived fulvic acids have fluorophores with a more sharply defined emission peak occurring at lower wavelengths compared with fluorophores of terrestrially derived fulvic acids.

Regrowth Cultures (RCs)

To determine the specific growth rates of abundance (μ) and (^{3}H-leucine)-protein synthesis (b), and the conversion factor (CF) of each system, regrowth cultures (RCs) were performed in triplicate (Grande, Dulce, Salada, Padul P1, Padul P0, OMM) or duplicate (Bermejales, Honda, Caldera). Each RC consisted of water filtered by Whatman GF/F and amended with mineral nutrients [50]. This filtration removed a variable fraction of *in situ* bacteria (22–97%, mean value 65%), and all bacterivores and phytoplankton (visual examination). To avoid potential limitation of mineral nutrients and to force organic carbon limitation, inorganic N (NH_4Cl) and P (KH_2PO_4) (final concentration of 10 μmol L^{-1} and 1 μmol L^{-1}, respectively) were added to all RCs. All RCs were incubated at 25°C in the dark for several days.

Aliquots from each triplicate (GR, DU, SA, PP1, PP0, OMM) or duplicate (BE, HO, CA) RC were sequentially taken (in most cases every 24 h) to measure (^{3}H-leucine)-protein synthesis rate and bacterial abundance. Bacterial abundance was determined by epifluorescence microscopy using DAPI fluorochrome stain [34]. At least 450 cells in 30 random fields were counted per filter. (^{3}H-Leucine)-protein synthesis was measured using, a microcentrifugation technique [43]; 5 μL of L-[4,5-^{3}H]leucine with a S.A. of 141 Ci $mmol^{-1}$ or 157 Ci $mmol^{-1}$ (Amersham) was added to 1.5-mL aliquots from each RC yielding a final concentration of 23.6 nM or 21.2 nM, respectively. Samples were incubated for 1h. Incubations were stopped by the addition of trichloroacetic acid (50%). Three replicates and two blanks were prepared for each sampling time.

Specific Growth Rates of Bacterial Abundance (μ)

Changes in bacterial abundance throughout incubation were fitted to exponential (Equation 3) and logistic (Equation 4) functions. The fits and parameters were obtained using the Nonlinear Estimations Module of the STATISTICA (V. 5.0) software package. This module uses a very efficient algorithm (quasi-Newton) that approximates the second-order derivatives of the loss function in order to guide the search for the minimum (i.e., for the best parameter estimates, given the respective loss function). The specific growth rates of abundance (μ) in d^{-1} were fitted to:

$$N_t = N_0 e^{\mu t} \quad (3)$$

and

$$N_t = \frac{k_N}{1 + e^{(a_N - \mu t)}} \quad (4)$$

where N_0 is initial abundance in cell ml^{-1}, N_t is abundance in cell ml^{-1} at time t in days, k_N is the carrying capacity of the RC, and a_N is a constant derived from the fits.

Table 1. Concentration of dissolved organic carbon (DOC), optical characterization (a_{250}, a_{440}, and F_{450}) and quality indexes (ε_{250}, ε_{440}, and F_{450}:F_{500}) of dissolved organic matter (DOM) at the initial conditions in each regrowth culture (RC)

RCs from	Landscape	Chl-a ($\mu g\ L^{-1}$)	DOC (mM)	a_{250} (m^{-1})	a_{440} (m^{-1})	ε_{250} ($m^2\ mol^{-1}$)	ε_{440} ($m^2\ mol^{-1}$)	F_{450} QSU	F_{450}:F_{500}	Peak
Caldera (CA)	Alpine	0.3	0.04 ± n.a.	2.7 ± 0.1	0.2 ± 0.0	68.0	4.04	1.4	1.82	431
Bermejales (BE)	Agricultural	1.6	0.36 ± 0.04	5.6 ± 0.1	0.3 ± 0.0	15.8	0.72	2.0	1.79	422
Grande (GR)	Karstic	0.4	0.68 ± 0.03	18.4 ± 0.2	0.3 ± 0.0	27.0	0.40	14.5	1.77	448
Honda (HO)	Coastal	5.8	1.23 ± 0.03	59.7 ± 0.3	1.8 ± 0.0	48.2	1.42	56.8	1.79	439
OMM (OMM)	Conifer forest	2.7	1.45 ± 0.02	57.8 ± n.a.	11.6 ± 0.0	39.8	7.03	n.d.	n.d.	n.d.
Dulce (DU)	Karstic	0.7	1.72 ± 0.05	68.6 ± 0.6	1.3 ± 0.0	40.0	0.75	55.3	1.81	443
Padul P1 (PP1)	Alkaline-peaty	3.2	2.42 ± 0.01	109.0 ± 3.2	4.5 ± 0.1	45.1	1.86	272.3	1.67	453
Salada (SA)	Karstic	0.9	3.79 ± 0.01	105.6 ± 2.6	1.8 ± 0.1	27.8	0.48	56.9	1.81	442
Padul P0 (PP0)	Alkaline-peaty	1.1	3.80 ± 0.02	126.2 ± 6.6	11.8 ± 0.8	33.3	3.11	454.4	1.59	449

Specific Growth Rates of (^{3}H-Leucine)-Protein Synthesis (b)

Changes in (^{3}H-leucine)-protein synthesis during incubation were also fitted to exponential (Equation 5) and logistic (Equation 6) functions, using the procedure described above. The specific growth rates of (^{3}H-leucine)-protein synthesis (b) in d^{-1} were calculated from the fits as:

$$L_t = L_0 e^{bt} \tag{5}$$

and

$$L_t = \frac{k_L}{1 + e^{(a_L - bt)}} \tag{6}$$

where L_0 and L_t are (^{3}H-leucine)-protein synthesis rate in pmol leucine $L^{-1}h^{-1}$ at initial and t times in days, respectively, K_L is the carrying capacity of the RC, and a_L is a constant derived from the fits.

Calculation of Conversion Factors (CFs)

The CFs in cells mol leucine^{-1} were obtained by the integrative method [24] (Equation 7):

$$CF = \frac{(N_f - N_0)}{\int_{t_0}^{t_f} Leu\ dt} \tag{7}$$

where N_0 and N_f are initial and final abundance in cell ml^{-1} of the RC, and $\int Leu\ dt$ is the definite integral of the (^{3}H-leucine)-protein synthesis function from initial (t_0) to final time (t_f). The solutions of the definite integrals for the exponential (Equation 8) or logistic (Equation 9) fits were calculated using the following equations:

$$\int_{t_0}^{t_f} Leu\ dt = \frac{L_0}{b}\left(e^{bt_f} - e^{bt_0}\right) \tag{8}$$

$$\int_{t_0}^{t_f} Leu\ dt = \frac{k_L}{b}\ln\left(\frac{e^{bt_f} - e^{a_L}}{e^{bt_0} - e^{a_L}}\right) \tag{9}$$

Results

Organic Substrate

In the different RCs, DOC concentration varied almost two orders of magnitude, from 0.04 mM in the alpine lake (CA) to 3.80 mM in one of the alkaline-peaty (PP0) systems (Table 1). DOM optical characteristics were also very variable; absorption coefficients ranged from 2.7 to 126.2 m^{-1} at 250 nm (a_{250}) and from 0.2 to 11.8 m^{-1} at 440 nm (a_{440}), and fluorescence at 450 nm (F_{450}) varied from 1.4 to 454.4 QSU (Table 1). The indexes selected to estimate DOM quality (ϵ_{250}, ϵ_{250}, and F_{450}:F_{500}) are also shown in Table 1. The highest value of ϵ_{250} was observed in the RC from the alpine lake (CA). DOM from this system likely includes a high proportion of small molecules. By contrast, the RC from the system located in a conifer forest (OMM) presented the highest value of ϵ_{440}, suggesting a high contribution of humic acids to the total DOC pool. According to the F_{450}:F_{500} index results, DOM in RC from the alpine lake (CA) appeared to contain the highest proportion of organic carbon of autochthonous (phytoplankton) origin, whereas DOM in RC from one of the alkaline-peaty systems (PP0) appeared to contain the highest proportion of organic carbon of terrestrial origin.

Specific Growth Rates of Abundance (μ) and (^{3}H-Leucine)-Protein Synthesis (b) and Conversion Factors (CFs)

In four of the nine RCs (BE, OMM, SA, and PP0) exponential fits explained higher percentages of the variance (r^2) of abundance and (^{3}H-leucine)-protein synthesis increases in comparison to logistic fits (Table 2). Figure 1A depicts an example of exponential fits of abundance (solid line) and (^{3}H-leucine)-protein synthesis (dotted line) in RCs from Salada lake. In the other five RCs (CA, GR, HO, DU, and PP1) logistic fits explained higher percentages of the variance (r^2) of the abundance and the (^{3}H-leucine)-protein synthesis increases in comparison to exponential fits (Table 2). Figure 1B shows an example of logistic fits of abundance (solid line) and (^{3}H-leucine)-protein synthesis (dotted line) in RCs from Padul P1 pond.

Table 2. Selection of exponential (E) or logistic (L) fit according to the highest value of the explained variance(r^2)[a]

RC	Abundance					Protein synthesis						CF ($\times 10^{18}$ cells mol leu^{-1})
	Fit	r^2	k_N (Cell ml^{-1})	a_N	$\mu \pm$ s.e. (d^{-1})	r^2	K_L (pmol leu lh^{-1})	a_L	$b \pm$ s.e. (d^{-1})	Growth type	
Caldera (CA)	L	0.999	4,311,010	1.2	2.13 ± 0.06	0.906	2174	5.76	20.30 ± n.c.	U	0.0185
Bermejales (BE)	E	0.933		1,117,058.8	0.33 ± 0.06	0.621		720.49	0.36 ± 0.19	B	0.0223
Grande (GR)	L	0.999	6,736,223	2.6	0.73 ± 0.04	0.982	4260	8.57	13.74 ± n.c.	U	0.0062
Honda (HO)	L	0.787	20,508,872	−0.8	1.17 ± 0.88	0.578	995	0.54	22.71 ± n.c.	U	0.0576
OMM (OMM)	E	0.999		437,734.7	0.26 ± 0.00	0.979		213.31	0.28 ± 0.06	B	0.0210
Dulce (DU)	L	0.654	4,953,390	0.6	0.66 ± 0.77	0.988	2455	2.62	1.40 ± 0.56	U	0.0136
Padul P1 (PP1)	L	0.999	3,808,646	1.5	3.34 ± 0.14	0.801	1056	1.97	34.87 ± n.c.	U	0.0394
Salada (SA)	E	0.695		2,272,645.0	0.31 ± 0.14	0.906		531.00	0.31 ± 0.07	B	0.0539
Padul P0 (PP0)	E	0.968		1,325,009.0	0.36 ± 0.05	0.954		523.23	0.35 ± 0.07	B	0.0419

s.e., standard error; n.c., not computed by the software

[a] Values of the specific growth rates, carrying capacities and constants of abundance (μ, K_N, and a_N) and protein synthesis (b, K_L, and a_L) derived from the fits. Growth type (balanced (B) or unbalanced (U)) determined by the concordance between μ and b, and the values of conversion factors (CFs)

Specific growth rates of abundance (μ) varied more than one order of magnitude, from 0.26 d^{-1} in RC from OMM pond to 3.34 d^{-1} in RC from PP1 (Table 2). The specific growth rates of (^{3}H-leucine)-protein synthesis (b) ranged more than two orders of magnitude from 0.28 d^{-1} in RC from OMM pond to 34.87 d^{-1} in RC from PP1 lake (Table 2). The carrying capacities of abundance (k_N) varied from 3.8×10^6 to 20.5×10^6 cell ml^{-1} and those of protein synthesis (k_L) from 996 to 4260 pmol leu L^{-1} h^{-1} (Table 2). Carrying capacities of abundance (k_N) and protein synthesis (k_L) were positively correlated ($n = 4$, r = 0.98, $p = 0.018$) when the values for Honda Lagoon were excluded. In this last system, bacteria reached the highest k_N and the lowest k_L value (Table 2).

In RCs where the increases of abundance and protein synthesis were best fitted by exponential functions (BE, OMM, SA, and PP0), the growth was balanced (μ was not significantly different from b) (Table 2 and Fig. 2). By contrast, in RCs where the increases of abundance and protein synthesis were best fitted by logistic functions (CA, GR, HO, DU, and PP1), the growth was unbalanced (μ was significantly different from b) (Table 2 and Fig. 2). In all RCs with unbalanced growth, the specific growth rates of protein synthesis (b) were considerably higher than the specific growth rates of abundance (μ) (Fig. 2).

CFs varied almost one order of magnitude from 0.0062 to 0.0576×10^{18} cells mol leu^{-1} incorporated into proteins, with an average of 0.0305×10^{18} cells mol leu^{-1} (Table 2). CFs obtained from RCs with balanced growth ($\mu \approx b$) were not alike and varied from 0.0210 to 0.0539×10^{18} cells mol leu^{-1} (Table 2 and Fig. 3). The CFs obtained from RCs with unbalanced growth ($\mu \neq b$) were more variable, ranging from 0.0062 to 0.057×10^{18} cells mol leu^{-1} (Table 2 and Fig. 3). The median of CFs in RCs with balanced growth was higher that in RCs with unbalanced growth (Fig. 3), although the overlap was significant. The segregation between CFs grouped by balanced and unbalanced growth was more evident when the CF from Honda Lagoon was not included in the unbalanced growth group (Fig. 3).

Relationships between μ, b, and CFs and the Organic Substrate

The setup of the RCs, with constant incubation time and temperature, addition of inorganic nutrients (N and P), and absence of predators guaranteed that μ and b were largely controlled by the DOC concentration and/or DOM quality.

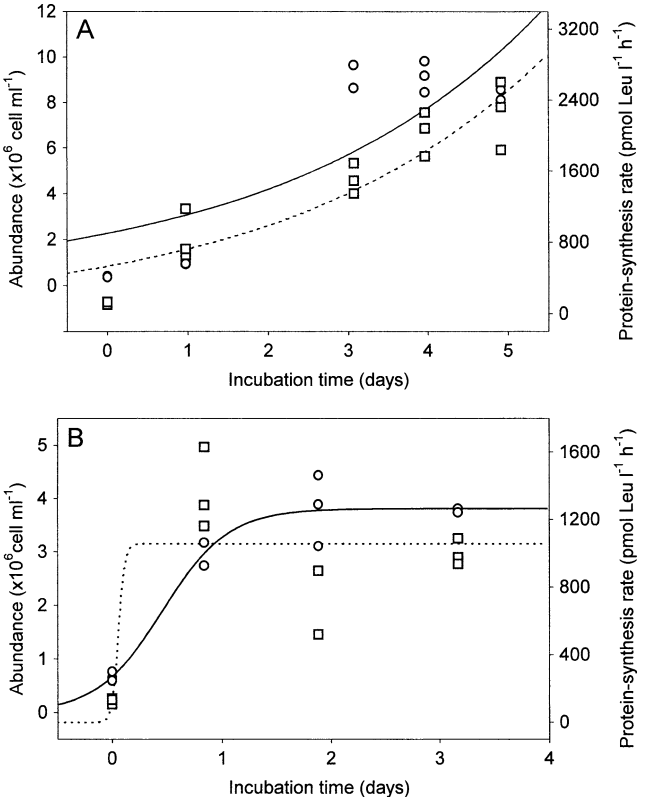

Fig. 1. Example of exponential (A) and logistic (B) fits of abundance (solid line) and (^{3}H-leucine)-protein synthesis (dotted line) in the regrowth cultures from Salada Lake and Padul P1pond, respectively. Solid and dotted lines represent the nonlinear functions estimated, and circles and squares are the replicates of abundance and protein synthesis, respectively.

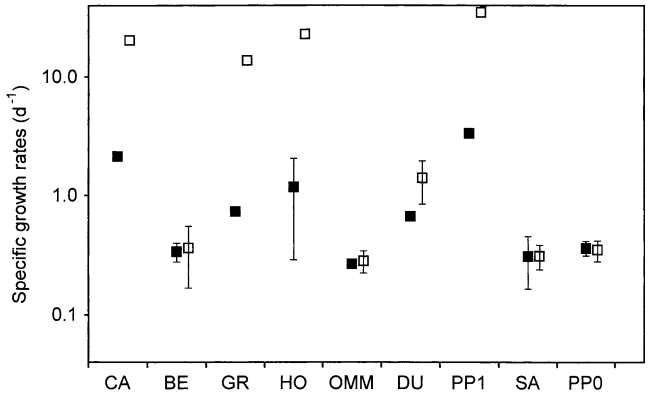

Fig. 2. Comparison of the specific growth rates of abundance (μ) (dark squares) and protein synthesis (*b*) (empty squares) in all regrowth cultures. Error bars represent standard errors of μ and *b* (also in Table 2). Note the logarithmic scale.

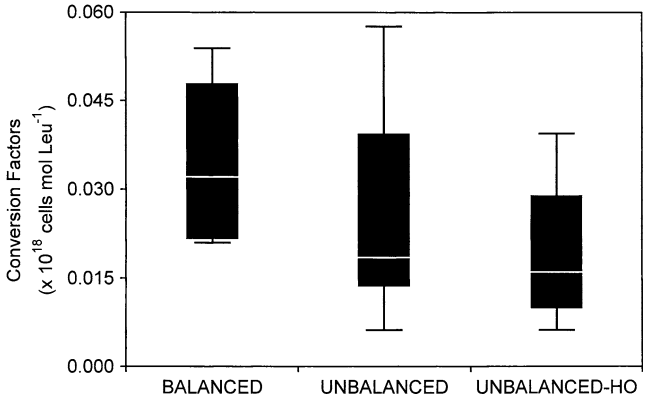

Fig. 3. The median (horizontal line), the 25–75% (black box), and the maximum and minimum values (whiskers) of the conversion factors grouped by balanced, unbalanced, and unbalanced-HO growth (except the value of the RC from Honda Lagoon).

A set of regression analyses was performed in order to assess whether specific growth rates of abundance (μ) and protein synthesis (*b*) variability were dependent on DOC quantity and/or DOM quality. We observed no significant relationships between DOC concentration or any of the DOM quality indexes assayed (ϵ_{250}, ϵ_{440}, and F_{450}:F_{500}) and μ or *b*.

A positive and significant ($n = 8$, $p < 0.01^{**}$, $r^2 = 0.71$) relationship was observed between the DOC concentration and the CFs when the value of the RC from Honda Lagoon was excluded (Fig. 4). The linear function obtained between the DOC concentration and the CFs was:

$$\text{CF } (\times 10^{18} \text{ cells mol leu}^{-1}) = 0.0104 + 0.0094 \text{ DOC(mM)}$$

We found no significant relationships between the CFs and the DOM quality indexes assayed ϵ_{250}, ϵ_{440}, and F_{450}:F_{500}.

Discussion

Bacterial Growth and Organic Substrate

Robust correlations between DOC concentration (from both autochthonous and allochthonous sources) and bacterial biomass or production have been reported [9, 48], suggesting a strong control of bacterial growth by organic substrate in natural systems. This field work was corroborated by experimental bioassays in which organic C was manipulated [11, 22, 23]. However, it has proven difficult to establish general rules regarding DOC control over bacteria growth, because of the variability (glucose, amino acids, proteins, etc.) of organic substrates added in

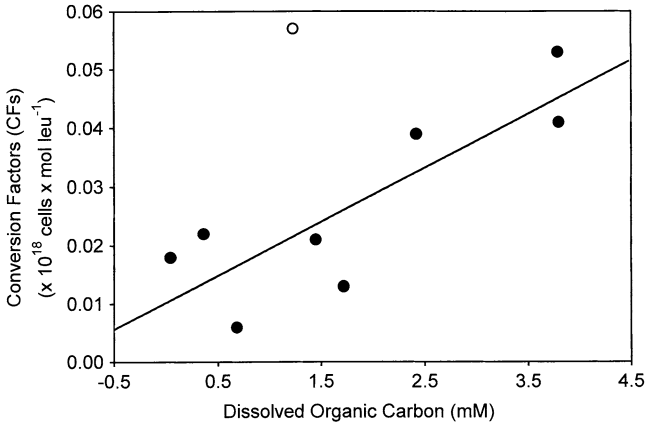

Fig. 4. Scatterplot of conversion factors (CFs) in relation to DOC concentration and the relationship between both parameters when CF from Honda Lagoon was excluded (empty circle).

the experiments, the heterogeneity of indigenous DOC, and potential multiple interactions with mineral resources (N, P, Fe) [7, 25, 35].

In the present study, neither the specific growth rates of abundance (μ) nor the specific growth rates of protein synthesis (*b*) were found to be related to DOC concentration. This absence of any relationship may be due to the considerable variability in DOM quality among the systems studied (Table 1). Thus, quantity could have been compensated by quality, masking a simple and positive relationship.

In general, an organic substrate of high quality has a low aromatic content [45], molecules of small mean size [29], or a low C:N ratio [18, 27, 20]. Nevertheless, some high-molecular-weight compounds (e.g., polysaccharides) can also be efficiently used by bacteria, whereas low-molecular-weight compounds (e.g., by-products of previous degradation) can be refractory [1, 2]. These properties are not necessarily independent. For instance, DOC with low molecular weight usually also has a low C:N ratio [29], and autochthonous DOM (derived from algae or macrophytes) contains few aromatic compounds [45], and maximum growth rates are supported in both cases.

The specific growth rates of protein synthesis (*b*) were more variable (two orders of magnitude) than the rates of abundance (μ) (one order of magnitude) (Table 2). In all RCs with unbalanced growth, the *b* values were markedly higher than μ values (Fig. 2). These results suggest that cell division was more constrained than protein synthesis across the RCs. Protein synthesis appears to increase more rapidly than cell duplication in order to maximize survival under unfavorable environmental conditions [14, 41]. Thus, cell division could be considered as a secondary process: bacteria first maximize survival via protein synthesis and then enter cell division only when environmental conditions are adequate. Therefore, specific growth rates of protein synthesis (first step) reflected more accurately the variability of the organic substrate (two orders of magnitude).

On the other hand, the concordance between the carrying capacities of protein synthesis (k_L) and abundance (k_N) in all RCs except HO (Table 2) suggests a dependence of cell abundance on protein synthesis at carrying capacity. In the RC from Honda Lagoon, the very low k_L relative to the k_N suggests that protein synthesis could have been underestimated.

Probably, in this productive system, the concentration of ^{3}H-leucine used was not enough to repress the isotope dilution. In eutrophic systems, a ^{3}H-leucine concentration greater than 40 nM is usually necessary to reach the saturation level [21].

Conversion Factors

In the present study, CFs varied almost one order of magnitude (Table 2) and were lower than values reported in published studies that used filtration [6, 14, 22, 23, 33, 46, 51] or microcentrifugation [15, 39] techniques. The discrepancy with the former studies may be because the filtration method recovers less ^{3}H-leucine-proteins compared with microcentrifugation [26, Reche unpublished data] yielding higher CFs. The lower CFs in our study than in the scant studies that also used microcentrifugation [15, 39] may be related to our use of integrative rather than cumulative or modified derivative calculation methods.

The RCs with balanced growth (Fig. 2) yielded CFs that ranged twofold (Table 2), impeding the establishment of an upper limit for CFs. This finding suggests that under optimal conditions with exponential growth, bacterial assemblages did not produce an identical number of cells per protein unit, underlining the possible impact of bacterial community composition.

Although CF variability was influenced by temperature and mineral nutrient availability in previous studies [22, 23, 40], these factors were controlled in the regrowth cultures. All cultures were standardized by the addition of N and P and by incubation at constant time and temperature. Therefore, the variability obtained in this study should be mostly related to the organic substrate. In fact,

we found a significant positive relationship between CFs and DOC concentration when the CF from HO was excluded (Fig. 4). This CF was excluded because it likely was an overestimation. At carrying capacity conditions the ratio of cell abundance to protein synthesis in the RC from HO was very high in relation to the other RCs with unbalanced growth (Table 2). This fact suggests an underestimation of protein synthesis that could be related to isotope dilution.

High DOC concentration appears to promote both protein-synthesis and cell division in balanced growth, leading to higher CFs (Figs. 3 and 4). In contrast, low DOC concentration appears to promote protein synthesis (high *b* values) but not cell division in unbalanced growth, which leads to lower CFs (Figs. 3 and 4). DOC concentration explained more than 70% of the CF variance. Unexplained variance could be related to iron availability, virus infections, and different bacterial community composition, among other uncontrolled parameters.

Using the function obtained in this study to estimate specific-site CFs instead of taking a standard value can have important consequences for the functional interpretation of natural systems. For instance, *in situ* bacterial production (BP) of Grande (GR) and Salada (SA) lakes are 147.25 and 1914.56 pmol $L^{-1}h^{-1}$, respectively (Reche et al., unpublished data). If a CF from the literature is applied, e.g., 0.087×10^{18} cells mol leu^{-1} [39], 12.81×10^6 and 166.57×10^6 cells $L^{-1}h^{-1}$ is obtained. This calculation procedure maintains the 13-fold difference obtained in the ^{3}H-leucine incorporation. However, if we apply CFs derived from the function obtained in the present study, the BP is 2.47×10^6 cells $L^{-1}h^{-1}$ for GR and 88.07×10^6 cells $L^{-1}h^{-1}$ for SA. This calculation procedure increases to 36-fold the difference obtained in ^{3}H-leucine incorporation.

Natural systems are substantially more complex than the RC conditions, including multiple interactions with mineral nutrients, viruses, predators, and environmental stresses, among others. Nevertheless, the linear function obtained between DOC concentration and the CFs offers an alternative approach to estimate site-specific CFs depending on their DOC concentrations. The degree to which this relationship can be extrapolated across systems remains to be established by further studies that consider more controlling factors. Factorial analysis of organic substrate, mineral nutrients, temperature, and other variables could provide more complex and accurate functions that describe the patterns of CF variability and replace the use of a single CF for all aquatic ecosystems.

Acknowledgments

We thank Dr. J.M. Conde-Porcuna, Dr. P. Carrillo, and J.A. Delgado for their assistance in the field. DOC samples were kindly analyzed in the labs of Dr. M.L. Pace (samples from all systems except the alpine lake) and Dr. R. Sommaruga (samples from the alpine lake). We are grateful to Dr. Michael L. Pace and two anonymous reviewers for insightful comments and to Richard Davies for assistance with the English version. This research was supported by Projects AMB 99-0541 and EVK-CT1999-00046.

References

1. Amon RMW, Benner R (1994) Rapid cycling of high-molecular-weight dissolved matter in the ocean. Nature 369:549–552
2. Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41:41–51
3. Azam F, Fenchel T, Field JG, Grey JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263
4. Billen G, Servais P, Becquevort S (1990) Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Arch Hydrobiol Beih 207:37–42
5. Braslavsky SE, Houk KN, Verhoeven JW (1996) Glossary of terms used in photochemistry. International Union of Pure and Applied Chemistry
6. Chin-Leo G, Kirchman DL (1988) Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. Appl Environ Microbiol 54:1934–1939
7. Church MJ, Hutchins DA, Ducklow HW (2000) Limitation of bacterial growth by dissolved organic matter and iron in the southern ocean. Appl Environ Microbiol 66:455–466
8. Cole JJ (1999) Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. Ecosystems 2:215–225
9. Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar Ecol Prog Ser 43:1–10
10. Cotner JB, Biddanda BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5:105–121
11. Coveney MF, Wetzel RG (1988) Experimental evaluation of conversion factors for the [^{3}H]thymidine incorporation assay of bacterial secondary productivity. Appl Environ Microbiol 54:2018–2026

12. Cuthbert ID, del Giorgio P (1992) Toward a standard method of measuring color in freshwater. Limnol Oceanogr 37:1319–1326
13. De Haan H (1972) Molecule-size distribution of soluble humic compounds from different natural waters. Freshwat Biol 2:235–241
14. Ducklow HW, Kirchman DL, Quinby HL (1992) Bacterioplankton cell growth and macromolecular synthesis in seawater cultures during the north Atlantic spring phytoplankton bloom, May, 1989. Microb Ecol 24:125–144
15. Ducklow HW, Carlson C, Smith W (1999) Bacterial growth in experimental plankton assemblages and seawater cultures from the *Phaeocystis antarctica* bloom in the Ross Sea, Antarctica. Aquat Microb Ecol 19:215–227
16. Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66:109–120
17. Gasol JM, Duarte CM (2000) Comparative analyses in aquatic microbial ecology: how far do they go? FEMS Microbiology Ecology 31:99–106
18. Goldman JC, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol Oceanogr 32:1239–1252
19. Hobbie JE, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228
20. Hunt AP, Parry JD, Hamilton-Taylor J (2000) Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. Limnol Oceanogr 45:237–241
21. Jørgensen NOG (1992) Incorporation of ^{3}H-leucine and ^{3}H-valine into protein of freshwater bacteria: uptake kinetics and intracellular isotope dilution. Appl Environ Microb 58:3638–3646
22. Kirchman DL (1992) Incorporation of thymidine and leucine in the subarctic Pacific: application to estimating bacterial production. Mar Ecol Prog Ser 82:301–309
23. Kirchman DL, Hoch MP (1988) Bacterial production in the Delaware Bay estuary estimated from thymidine and leucine incorporation rates. Mar Ecol Prog Ser 45:169–178
24. Kirchman DL, Ducklow HW (1993) Estimating conversion factors for the thymidine and leucine methods for measuring bacterial production. In: PF Kemp, BF Sherr, EB Sherr, JJ Cole (eds) Handbook of Methods in Aquatic Microbial Ecology CRC Press, Boca Raton, FL, pp 513–517
25. Kirchman DL, Meon B, Cottrell MT, Hutchins DA (2000) Carbon versus iron limitation of bacterial growth in the California upwelling regime. Limnol Oceanogr 45:1681–1688
26. Kirschner AKT, Velimirov B (1999) Modification of the ^{3}H-leucine centrifugation method for determining bacterial protein synthesis in freshwater samples. Aquat Microb Ecol 17:201–206
27. Kroer N (1993) Bacterial growth efficiency on natural dissolved organic matter. Limnol Oceanogr 38:1282–1290
28. McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe DT, Andersen DT (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46:38–48
29. Meyer JL, Edwards RT, Risley R (1987) Bacterial growth on dissolved organic carbon from a blackwater river. Microb Ecol 13:13–29
30. Miller WL (1998) Photochemical principles and experimental considerations. In: DO Hessen, LJ Tranvik (eds) Aquatic Humic Substances. Ecology and Biogeochemistry. Springer-Verlag, Berlin pp 125–143
31. Moran MA, Hodson RE (1990) Bacterial production on humic and nonhumic components of dissolved organic carbon. Limnol Oceanogr 35:1744–1756
32. Pace ML, Cole JJ (1994) Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microb Ecol 28:181–193
33. Pomeroy LR, Sheldon JE, Sheldon Jr WM (1994) Changes in bacterial numbers and leucine assimilation during estimations of microbial respiratory rates in seawater by precision Winkler method. Appl Environ Microbiol 60:328–332
34. Porter KG, Feig LS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
35. Reche I, Pace ML, Cole JJ (1998) Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. Microb Ecol 36:270–280
36. Reche I, Pace ML, Cole JJ (1999) Relationship of trophic and chemical conditions to photobleaching of dissolved organic matter in lake ecosystems. Biogeochemistry 44:259–280
37. Reche I, Pulido-Villena E, Conde-Porcuna JM, Carrillo P (2001) Photoreactivity of dissolved organic matter from high mountain lakes of Sierra Nevada, Spain. Arct Antarct Alp Res 33:426–434
38. Rivkin RB, Anderson MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. Limnol Oceanogr 42:730–740
39. Sherr EB, Sherr BF, Sigmon CT (1999) Activity of marine bacteria under incubated and in situ conditions. Aquat Microb Ecol 20:213–223
40. Shiah FK, Ducklow HW (1994) Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnol Oceanogr 39:1243–1258
41. Shiah FK, Ducklow HW (1997) Bacterioplankton growth responses to temperature and chlorophyll variations in estuaries measured by thymidine:leucine incorporation ratio. Aquat Microb Ecol 13:151–159
42. Skoog A, Wedborg M, Fogelqvist E (1996) Photobleaching of fluorescence and the organic carbon concentration in a coastal environment. Mar Chem 55:333–345

43. Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar Microb Food Webs 6:107–114
44. Strome DJ, Miller MC (1978) Photolytic changes in dissolved humic substances. Verhan Inter Verein Limnol 20:1248–1254
45. Sun L, Perdue EM, Meyer JL, Weis J (1997) Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnol Oceanogr 42:714–721
46. Torréton JP, Dufour P (1996) Bacterioplankton production determined by DNA synthesis, protein synthesis, and frequency of dividing cells in Tuamotu Atoll lagoons and surrounding ocean. Microb Ecol 32:185–202
47. Tortell PD, Maldonado MT, Price NM (1996) The role of heterotrophic bacteria in iron-limited ocean ecosystems. Nature 383:330–332
48. Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb Ecol 16:311–322
49. Tranvik LJ (1990) Bacterioplankton growth on different molecular weight fractions of dissolved organic carbon from humic and clear waters. Appl Environ Microbiol 56:1672–1677
50. Tranvik LJ (1998) Degradation of dissolved organic matter in humic waters by bacteria. In: DO Hessen, LJ Tranvik (eds) Aquatic Humic Substances. Ecology and Biogeochemistry. Springer-Verlag, Berlin, pp 125–143
51. Tulonen T (1993) Bacterial production in a mesohumic lake estimated from [^{14}C]Leucine incorporation rate. Microb Ecol 26:201–217
52. Vadstein O, Olsen Y, Reinersten H (1993) The role of planktonic bacteria in phosphorous cycling in lakes–sink and link. Limnol Oceanogr 38:1539–1544