Limnol. Oceanogr., 56(1), 2011, 1–16

doi:10.4319/lo.2011.56.1.0001

Coherent patterns in bacterial growth, growth efficiency, and leucine metabolism along a northeastern Pacific inshore–offshore transect

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Abstract

We investigated the patterns in bacterial growth, production, respiration, growth efficiency (BGE), and bacterial leucine respiration and C-to-leucine yield (i.e., conversion factor [CF]) along a transect off the coast of Oregon. Plankton respiration along the transect averaged 1.15 ± 0.16 μg C L^{-1} h^{-1}, peaking in the coastal upwelling region. The respiration in the filtered fraction, which was dominated by bacterial biomass, accounted for 79% of the total respiration. The different approaches that we used converged to an average BGE of 13% ± 1%, with peaks of over 20% in the more productive coastal areas and values declining to below 5% toward the oligotrophic gyre waters. There was overall coherence between the various aspects of bacterial C metabolism: communities with low BGE also tended to have low growth rates and high leucine-to-thymidine incorporation ratios. The patterns in BGE were mirrored at the single compound level, and in the most oligotrophic sites, bacteria tended to quickly respire a large fraction (20–75%) of the leucine that was taken up and had the lowest C-to-leucine yield, suggesting that the patterns in bulk BGE and growth also apply to individual substrates. Bacterial growth was a function of both C consumption and BGE; these two aspects of bacterial C metabolism do not necessarily covary, and they are regulated differently. The patterns in C consumption, growth, BGE, and leucine metabolism all reflect the basic physiological response of bacteria to energy limitation due to high maintenance costs associated with life in oligotrophy.

Our current understanding of oceanic bacterioplankton carbon metabolism and of the role of bacteria in global marine C cycles is based on a relatively small set of parameters that include the uptake and processing of selected substrates and the associated rates of synthesis of protein and nucleic acids, cell growth and replication, and the overall bacterial carbon consumption, biosynthesis, and respiration (Ducklow 2000; Gasol et al. 2008). Although some of these components (e.g., bacterial growth and bacterial biomass production and respiration, summarized in Table 1) have been extensively measured in marine systems; they have typically been analyzed separately in part because the methodological approaches and temporal measurement scales are very different between them. The result is that whereas the different aspects of bacterial carbon metabolism are no doubt functionally interconnected, the nature and strength of these connections are still not well understood (Gasol et al. 2008).

There are several reasons why it is important to assess how these components of metabolism are linked to each other. From a conceptual viewpoint, the nature and strength of the links that exist between these aspects of C metabolism may inform us about how bacterial communities deal with fluctuating and often challenging marine environments. Marine bacteria collectively maximize their survival and growth not by adjusting any single aspect of C metabolism but most likely by simultaneously modulating different aspects of their overall metabolism (Carlson et al. 2007; del Giorgio and Gasol 2008). For example, bioenergetic considerations would suggest that growth rate and bacterial growth efficiency (BGE) should be related to each other (del Giorgio and Cole 2000) and that both should in turn be related to the ratio of leucine to thymidine incorporation (Leu : TdR ratio), which provides an index of the relative importance of protein vs. nucleic acid synthesis and thus of the degree to which growth is balanced or unbalanced (Chin-Leo and Kirchman 1990; Kirchman 1992; Gasol et al. 1998). In turn, C processing and the subsequent growth should be linked to the underlying C availability and consumption (Church 2008). While some of these links have been explored theoretically (Vallino et al. 1996; Cajal-Medrano and Maske 2005) or experimentally (Linton and Stephenson 1978; Søndergaard and Theil-Nielse 1997), there are very few empirical studies that have addressed the ensemble of connections that exist within bacterial metabolic variables in marine environments.

There are practical implications as well concerning these potential links. These components of metabolism have been generally measured individually but have then often been used to infer other aspects of bacterial C metabolism and overall performance within the system, yet these extrapolations are problematic. For example, estimates of bacterial production (BP) have been extensively used to infer total bacterial C consumption in the oceans (Carlson and Ducklow 1996; Ducklow 2000; Gasol et al. 2008), but this

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Table 1. Variables related to C consumption by bacteria that are analyzed in this study as well as the time frame in which they are measured and the assumptions that they carry with them.

Variable	Method	Time frame	Assumptions
Total and bacterial respiration	Changes in O_2; converted to CO_2 production	24 h	Assume RQ of 1
Bacterial production (in situ)	Incorporation of ^{3}H-leucine	1–2 h	Standard CF (1.55 kg mol^{-1}) Empirical CF
Bacterial production (incubation)	(1) Incorporation of ^{3}H-leucine; converted to C production (different times or averages: t_0, t_{12}, t_{24})	1–2 h	Standard CF (1.55 kg mol^{-1})
	(2) Incorporation of ^{3}H-leucine as in (1)	1–2 h	Empirical CF
	(3) Biomass change in filtered samples	24 h	Cell : C conversion factor (20 fg cell^{-1})
Bacterial carbon consumption	BP + BR	24 h	BR calculated only in filtered samples BP chosen among different estimates
BGE	BP/(BP + BR)	24 h	BR calculated only in filtered samples BP chosen among different estimates
Protein vs. nucleic acid synthesis	Leucine : thymidine incorporation ratios	1–2 h	Only one tracer concentration used
Leucine metabolism (short term)	^{14}C-leucine incubations	3 h	
Uptake	= Leu assimilated + Leu respired		
assimilation		3 h	Leu in TCA precipitate after acidification
Respiration of ^{14}C-leucine		3 h	$^{14}CO_2$ in air after acidification
C-to-leucine yield (long term)	Total C produced per unit of leucine incorporated	3–5 d	Bottle growth comparable to in situ community
Bacterial growth rates	(1) In situ: leucine incorporation in unfiltered water divided by abundance (cell-specific leucine incorporation)	1–2 h	Depends on choice of CF
	(2) Changes in leucine incorporation in filtered water (i.e., growth in the absence of grazers) = "potential growth rates"	24 h	Insensitive to choice of CF

link is mediated by BGE, which is now recognized as being much more variable and less predictable than was originally assumed (Jahnke and Craven 1995; del Giorgio and Cole 2000). Studies that have attempted to measure BGE directly in ocean samples have reported low BGE values, usually in the range of 1% to 20% (Biddanda et al. 1994; Sherry et al. 2002; Alonso-Sáez et al. 2007). There also appears to be large spatial as well as temporal variability in BGE (Lemée et al. 2002; Reinthaler and Herndl 2005; Alonso-Sáez et al. 2007). The low values of oceanic BGE present the problem that, when combined to in situ measurements of BP, they result in estimates of total bacterial carbon consumption that often exceed local and regional estimates of primary production and organic carbon flow (del Giorgio and Cole 2000; Hoppe et al. 2006; Carlson et al. 2007), thus questioning the robustness of these estimates (Goldman and Dennett 2000). It is then of fundamental importance to better constrain BGE, growth, and C processing by bacteria and assess how the links between them vary across marine environmental gradients.

The approaches underlying some of the key variables discussed previously, such as BP, BGE, and growth rate, involve in one way or another measurements of substrate uptake, and leucine has been particularly widely used as a tracer in marine studies (Kirchman et al. 1985). A large volume of older literature converges to suggest that there is a large variability in the bacterial handling of individual substrates (including leucine and other amino acids), in terms of the proportion of the compound taken up that is respired vs. assimilated into biomass on both short and long terms (Crawford et al. 1974; Brittain and Karl 1990; Hollibaugh 1994), and that these variations in individual substrate handling are also a reflection of the overall physiologic state of bacteria (Hollibaugh 1994). In this regard, some recent evidence suggests that the proportion of leucine respired may covary with bulk BGE in the northern Atlantic (Alonso-Saéz et al. 2007), but the connections that exist between bulk metabolic processes and single-substrate processing, particularly of leucine, remain to be explored in a systematic manner.

A fundamental question, then, is the extent to which these key metabolic processes, both at the bulk and the single-substrate level, are connected and coherent with each other and, in turn, to what extent they reflect the collective response of the ambient bacterial communities to environmental forcing. In this article, we explore the connections that exist between four major categories of C metabolism: (1) bacterial C consumption, respiration, and production; (2) BGE; (3) bacterial growth; and (4) protein vs. nucleic acid synthesis. We further explore how short-term leucine metabolism (uptake, assimilation, and respiration) and long-term leucine metabolism (i.e., the relationship between leucine incorporation and biomass production in long-term incubations) relate to bulk metabolism. The study was carried out along an inshore–offshore transect in the Oregon coastal upwelling region that covers very distinct water masses and that has strong physical, chemical, and

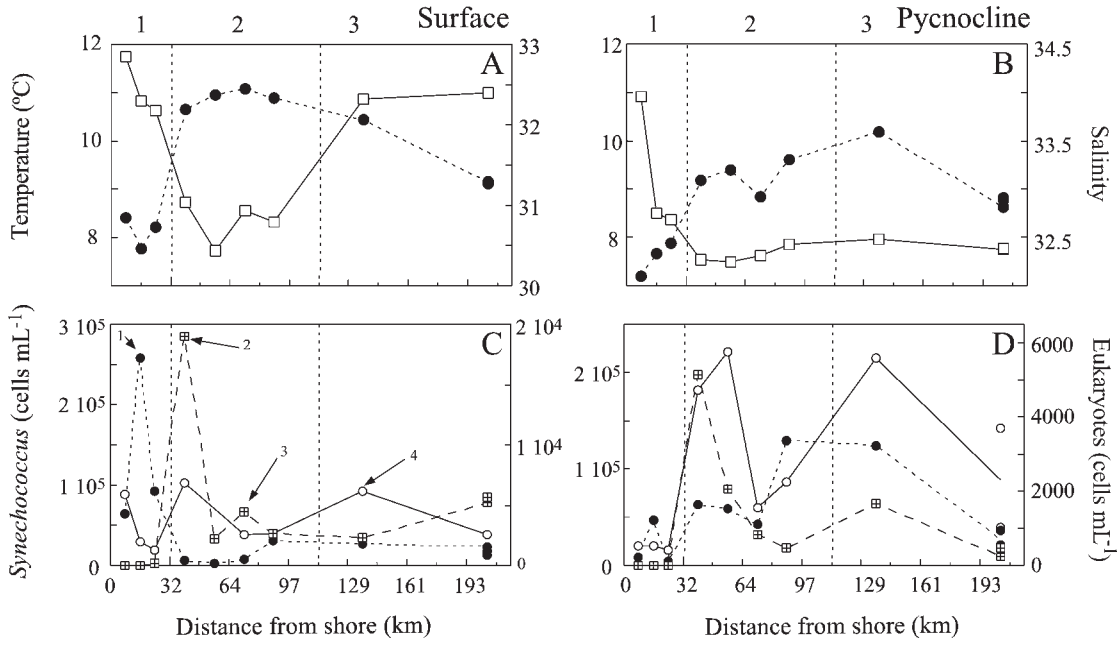

Fig. 1. Values of temperature (closed circle) and salinity (open square) in the samples collected (A) at the surface and (B) at the depth of the pycnocline. Abundances of *Synechococcus* (crossed square), picoeukaryotes (open circle), and small diatoms (closed circle) at (C) the surface and (D) the depth of the pycnocline. The arrows in the lower panels indicate dominance by one of these groups. The numbers at the top and the vertical lines identify the three main types of oceanographic regions as classified by nutrient and physical data.

biological gradients that, in turn, generate a large range in bacterioplankton activity (Sherr et al. 2001) and community composition (Longnecker et al. 2005; Bouvier and del Giorgio 2007). We followed the various aspects of bacterial C metabolism in both surface and pycnocline samples along this transect.

Methods

General experimental approach—The components of marine bacterial C metabolism targeted in this study are summarized in Table 1, together with the method used, the temporal scale of the measurement, and the underlying assumptions involved in each case. This comparison requires estimates that are as robust as possible and that the different components of metabolism are as independent as possible in terms of common underlying measurements so as to minimize methodological biases. This is particularly true for estimates of BP, which are at the base of the calculations of BGE and other parameters. We thus made a particular effort to better constrain BP by comparing several alternative approaches (*see* Table 1 and specific sections later in this article). We then used various combinations of the BP and bacterial respiration (BR) data to more effectively constrain both the magnitude and the spatial variability of bacterial carbon consumption and BGE along this transect. We further used two independent estimates of bacterial growth rate, one based on cell-specific leucine uptake in unfiltered samples and the other based on changes in leucine incorporation rates in incubations of filtered water. Finally, we compared the patterns in bulk metabolism with the patterns in leucine metabolism (short-term uptake, assimilation, and respiration of labeled leucine and the long-term C-to-leucine yield). Some of these metabolic variables were thus determined for the bulk, unfiltered water (i.e., Leu : TdR ratio or leucine metabolism), whereas others were measured from incubations of filtered samples (i.e., BR and BGE), and the time scale of these measurements varies significantly, from less than 1 h to more than 4 d. The experimental approach thus involved comparisons of metabolic processes in unfiltered and filtered samples as well as processes at contrasting temporal scales.

Sampling and sample preparation—Water samples were collected along the Newport sampling line off the Oregon coast in June 2002, spanning inshore shelf waters (44°39.1 N, 124°10.5 W) to an offshore station (44°38.9 N, 127°05.0 W), 240 km from the coast. The transect extended to the edge of the northern Pacific gyre and traversed distinct oceanic water masses, including cold, nutrient-rich coastal upwelling waters (region 1 in Fig. 1); low-salinity slope waters influenced by the plume of the Columbia River (region 2); and nutrient-depleted, offshore waters (region 3). Water samples for microbiological and plankton analyses were taken from the surface (10–20 m) and the pycnocline (35–65 m) at 11 stations along the transect using a conventional conductivity, temperature, and depth (CTD) rosette equipped with 5-liter Niskin bottles. Sensors on the CTD provided profiles of salinity, temperature, and in situ fluorescence at each station.

A portion of the water from each depth was set aside for chlorophyll *a* (Chl *a*), nutrient, and bulk metabolism analysis. Another portion was filtered to separate bacterioplankton from other planktonic components to measure BR and production. In preliminary experiments, we tested

a wide variety of filters commonly used to separate bacteria, including cellulose and polycarbonate membranes, glass fiber filters, and prefilters with pore sizes ranging from 0.6 to over 5 μm. We have concluded that the glass fiber 150-mm Millipore AP 15 filters (with a nominal pore size of approximately 1.2 μm) are the most effective in reducing the number of picoplankton organisms while maintaining most of the original free-living bacterial community structure: The filtration procedure allowed on average the passage of 84% of heterotrophic bacterial cells but retained 74% of Synecococcus and 87% of picoeukaryotes. In terms of metabolism, the filtered samples had on average 70% of BP rates measured in the bulk samples.

Incubation setup for respiration and BGE measurements—Respiration rates in unfiltered (total) and filtered (bacterial) samples were determined from declines in oxygen concentration in incubations using flow-through systems that were repeatedly sampled. In brief, either unfiltered or filtered water from each site was used to fill a 4-liter acid-washed Erlenmeyer flask and a 4-liter polycarbonate Cubitainer that had been acid washed and conditioned using aged seawater. The flask was connected to the bag by acid-washed silicone tubing so that a siphon was established. The flask was sealed with a Teflon stopper that had two ports: one used to connect the flask with the bag and another used as a sampling port. Once filled with sample water, the flask + bag system was immersed in water that was kept at in situ temperature using a recirculating water bath. Up to four such incubations could be set up and processed simultaneously. This system allows intensive sampling from the same water mass to establish detailed time courses for oxygen consumption and BP with minimal handling of the sample, thus reducing bottle effects. Samples were retrieved from the flask by the outlet port and allowing 5 mL of water to overflow through a sampling port before collecting the samples. Each flow-through system was sampled at times 0, 12, and 24 h. At each sampling time, a maximum of 30 mL of water were retrieved and used to determine O_2 concentration, bacterial abundance, and leucine uptake at each time point, as described later. The volume replaced at each sampling thus represents $< 1\%$ of the total volume of the incubation flask, and there is no detectable effect on gas concentrations in the incubation flask.

Total plankton and BR—Samples for oxygen concentration were taken directly from the flasks by inserting the outflow plastic tube into the bottom of a 5-mL glass tube and allowing the water to slightly overflow. Triplicate tubes were filled this way for every time point taken during the incubation. Each tube was poisoned with 8 μL saturated HgCl solution and then capped with a ground-glass stopper. The tubes were kept immersed in water at 10°C for later gas analysis in the lab. Previous work has shown that gas concentrations in the tubes remain stable for weeks (Sampou and Kemp 1994). Oxygen concentration in the samples was measured using membrane-inlet mass spectrometry within 2 weeks of collection. Briefly, the method is based on the spectrometric determination of the ratio of argon to oxygen in the sample after the gases in the sample have been allowed to diffuse through a permeable membrane and collected in a stream of helium (Kana et al. 1994). The oxygen concentration is then derived from this ratio by determining the solubility of argon corrected for salinity and temperature. The rates of oxygen consumption were calculated from the slope of the O_2-vs.-time relationship fitted to an ordinary least squares regression. Rates of oxygen consumption were converted to CO_2 production assuming a respiratory quotient (RQ) of 1 (del Giorgio et al. 2006).

BP and growth efficiency—Rates of BP were determined in the bulk water prior to filtration (in situ BP; Table 1) and in each of the time points used to determine respiration during the incubation of both the unfiltered and the filtered water (incubation BP). Rates of bacterial production were estimated from the uptake of ^{3}H-leucine (Perkin-Elmer Life Science Products; 170 mCi mmol^{-1}) following the centrifugation method of Smith and Azam (1992). The final leucine concentration in all experiments was 20 nmol L^{-1}, based on preliminary experiments. Three replicate tubes plus a killed control were incubated for 1 h for each measurement, and there were three measurements of leucine incorporation during the course of the respiration incubations. Rates of leucine incorporation were converted to rates of C production by applying the standard CF of 1.55 kg C mol leu^{-1} (Simon and Azam 1989; but *see* the following discussion for further details of conversions). We used these BP estimates based on the standard CF for all subsequent calculations of BGE and C demand. Bacterial growth efficiencies (BGE) were calculated as BP/BP + BR. As we describe in the "Results" section, we compared several different combinations of the previously mentioned production measurements to derive the most appropriate BP estimate to use for the calculation of BGE.

For comparative purposes, in the filtered water incubations we additionally calculated BP from the changes in bacterial abundance, determined using flow cytometry. We did not observe major differences in cell size (from the cytometric parameters) between samples or within samples during the short-term incubations based on our cytometric determinations, and we assumed a constant cell-to-carbon CF of 20 fg C cell^{-1}, which is intermediate between values that have been previously determined for shelf and slope stations in this area (Sherr et al. 1999).

We also measured ^{3}H-thymidine incorporation to determine the leucine to thymidine incorporation (Leu : TdR) ratio. These measurements were carried out only in the bulk, unfiltered water samples. Triplicate tubes plus a killed control were incubated for 1 h with 20 nmol L^{-1} ^{3}H-thymidine (Sherr et al. 1999), precipitated with cold trichloroacetic acid (TCA; Kirchman 1992), and stored frozen for later processing in the lab.

Growth rate estimates—We derived two separate estimates of bacterial growth. First, we calculated bacterial specific production (in pmol cell^{-1} h^{-1}) in bulk, unfiltered water samples by dividing the rates of leucine incorporation by cell abundance; we refer to this as "cell-specific leucine

incorporation'' rates. Second, an alternative index of growth that minimizes the dependency on BP is to treat the successive leucine incorporation measurements done during the incubations as a variant of a ''pulse-labeling'' experiment. In brief, rates of leucine incorporation typically increase during incubations of filtered water, and the slope of the relationship between the natural log-transformed rates of leucine incorporation vs. incubation time (in hours) provides an index of the growth rate of the assemblage in the absence of grazing (LaRock et al. 1988). Growth rates derived in this way integrate the changes that occur along an incubation within the same time span used to estimate BGE but do not share any common term with BGE or use any CF from leucine incorporation to biomass production, and thus allow a direct comparison between the two. We refer to these slopes as ''potential growth rates'' (expressed in h^{-1}).

Leucine respiration experiments—Uptake, assimilation, and respiration of uniformly labeled ^{14}C-leucine were measured following the protocol of Hobbie and Crawford (1969) with some modifications. Measurements were carried out in quadruplicate samples (4 mL) placed in 25-mL incubation Erlenmeyer flasks and one control, which was fixed with TCA (5% final concentration). L-[U-^{14}C-leucine] (ICN; 300 mCi $mmol^{-1}$; Amersham, CF3183) was added (60 nmol L^{-1}) to the samples, and the flasks were immediately closed with a rubber stopper fitted with a metallic cup holding a piece of filter paper (Whatman No. 1 chromatographic paper). After $\sim$ 3 h of incubation at the in situ temperature, 200 μL of H_2SO_4 (2N) were injected with a syringe through the stopper to stop the incubation. The Erlenmeyer flasks were then smoothly shaken for an hour to liberate the carbon dioxide, after which 1 mL of 2-phenylethylamine (Sigma) was injected through the stopper directly on to the filter paper. We waited 30 min to allow fixation of the carbon dioxide onto the filter before opening the Erlenmeyer flask. The filters were placed in a vial with Optisafe HiSafe-2 cocktail to estimate the respired fraction of ^{14}C-leucine. Triplicate samples of 1.2 mL were taken from the remaining liquid in each Erlenmeyer flask to determine the fraction of leucine assimilated into biomass after TCA fixation as described previously for BP. All samples were stored frozen and counted in the lab on return to port. For this component, we refer to leucine taken up as the sum of the leucine respired and that assimilated into biomass, and percent respired leucine is calculated relative to the total leucine taken up.

Note that we restrict the use of the term ''assimilation'' to the samples treated with ^{14}C-labeled leucine where we could distinguish the proportion of leucine taken up that was either respired or assimilated into biomass. For all the samples incubated with ^{3}H-labeled leucine or thymidine, for which we did not distinguish between the respired and assimilated fractions, we use the generic term ''incorporated'' to denote the tracer recovered in the TCA-precipitated fraction.

Long-term relationship between leucine incorporation and biomass production—We performed 16 duplicate experiments in order to determine the total yield of biomass per unit leucine incorporated. We gently filtered sample water through 0.6-μm polycarbonate filters (Millipore; DTTP) in order to remove predators. We then diluted the water (1 : 9) with 0.2-μm filtered (Millipore; GTTP) seawater and incubated the mixture in 2-liter acid-clean Nalgene polycarbonate bottles in the dark in water baths adjusted to the in situ temperatures (the same baths used for the respiration determinations; *see* the previous discussion). Subsamples were taken for leucine incorporation and bacterial abundance measurements at every 12–24 h until bacteria reached the stationary phase. The yield of biomass produced per unit leucine incorporated was computed with the cumulative method (Bjørnsen and Kuparinen 1991), which maximizes the use of the available data. The standard error (SE) for the duplicate bottles was low (average of 16%). We refer to these measurements as ''carbon-to-leucine yield,'' but these estimates also served to calculate a leucine-to-carbon empirical conversion factor (eCF). We used the carbon-to-leucine yield to explore patterns in leucine processing over long-term incubations, and we did not apply the eCF in the calculations of BP used for BGE and growth rates (where we used the standard CF), except for comparative purposes (*see* Table 1).

Cytometric enumeration of picoplankton—For flow cytometric analysis of cell abundance, 1-mL aliquots of all samples were fixed with 0.5% paraformaldehyde (final concentration), and then quick-frozen and stored at -80°C until run on a Becton Dickinson FACSCalibur flow cytometer back in the laboratory. Bacterial cells were enumerated after SYTO 13 staining (del Giorgio et al. 1996). For enumeration of small-sized phytoplankton, 500-μL subsamples were processed as described in Sherr et al. (2005). Populations of cyanobacteria (*Synechococcus*) and of photosynthetic eukaryotes were distinguished in unstained samples on the basis of differences in fluorescence in orange (cyanobacteria) and in red (eukaryotic phytoplankton) wavelengths.

Nutrients and chlorophyll—Inorganic nutrients were analyzed with a hybrid Technicon AutoAnalyzerII™ and Alpkem RFA300™ system (Gordon et al. 1994). Samples for chlorophyll were filtered onto Whatman GF/F filters, and the filters were stored frozen until processing on shore and then extracted for $>$ 12 h in the dark at -20°C using 90% acetone as solvent. Fluorescence was measured with a Turner Designs™ 10-AU fluorometer calibrated with purified Chl *a* (Sigma).

Statistical analyses—We used JMP software (SAS Institute). Model I regressions were used to compare either arithmetic or log-transformed data and Spearman rho nonparametric correlation coefficients to assess the relationships between variables.

Results

In situ conditions—We sampled the surface and the pycnocline of several stations along an inshore–offshore

Table 2. Position and characterization of the samples used in this study. Station name, maximum depth at the station, longitudinal position along latitude 44°39.09 (Newport GLOBEC line), depth of the sample, temperature (Temp), salinity (Sal), and nutrient concentrations (P for inorganic dissolved phosphorus, N + N for nitrate + nitrite, and Sil for dissolved silicate), Chl *a*, bacterial abundance, and whether we obtained measurements of the ratio leucine-to-thymidine incorporation rates (L : T), leucine respiration rates (LR) or whether we measured the C-to-leucine yield (CLY). BGE was measured in all the samples.

Station	Z max (m)	Longitude W	Depth (m)	Temp (°C)	Sal	P (μM)	N + N (μM)	Sil (μM)	Chl *a* (mg m^{-3})	L : T	LR	CLY	Bacterial abundance (cells mL^{-1})
Surface samples													
NH127	2884	127°5.992	10	9.1	32.4	0.84	5.53	8.7	0.52		*	*	6.37×10^5
NH127	2886	127°5.992	9	9.1	32.4	0.84	5.69	9.5	0.53	*	*	*	1.21×10^6
NH127	2885	127°5.991	10	9.2	32.4	0.85	5.52	9.2	0.45	*	*	*	8.36×10^5
NH85	2883	126°3	12	10.5	32.3	0.40	0.13	1.2	1.00	*	*	*	1.28×10^6
NH55	2865	125°22.005	10	10.8	30.8	0.29	0.00	5.0	0.29	*	*	*	6.55×10^5
NH45	710	125°6.997	10	11.1	30.9	0.29	0.01	5.3	0.21	*	*	*	1.23×10^6
NH35	442	124°53.004	13	10.9	30.5	0.22	0.07	6.0	0.21	*	*	*	1.19×10^6
NH25	296	124°38.997	14	10.7	30.9	0.30	0.36	3.8	0.49	*	*		1.13×10^6
NH15	91	124°24.706	14	8.7	32.1	1.32	10.89	17.6	2.10	*	*	*	6.99×10^5
NH10	82	124°17.712	13	8.2	32.2	1.44	13.48	18.5	4.90	*	*	*	6.91×10^5
NH5	60	124°10.605	9	8.4	32.9	1.21	11.64	16.3	17.86	*	*		1.86×10^6
Deep samples													
NH127	2885	127°6.003	60	8.8	32.4	0.94	6.68	9.8	1.27		*	*	1.73×10^6
NH127	2886	127°5.991	50	8.8	32.4	0.91	6.07	9.4	0.49				1.77×10^6
NH127	2885	127°5.979	60	8.8	32.4	0.93	6.41	9.6	0.35				1.40×10^6
NH85	2881	126°2.993	42	10.2	32.5	0.63	2.78	4.7	1.40	*	*		1.29×10^6
NH55	2863	125°22.002	40	9.7	32.4	0.79	4.40	7.9	0.23	*	*	*	5.99×10^5
NH45	715	125°7.04	40	8.8	32.3	0.89	6.93	8.9	0.30	*	*	*	8.54×10^5
NH35	442	124°52.992	35	9.3	32.2	0.63	2.46	7.3	0.67	*	*	*	1.37×10^6
NH25	296	124°38.975	36	9.2	32.3	0.69	3.94	7.4	0.74	*	*	*	1.89×10^6
NH15	91	124°24.711	40	7.9	32.7	1.50	17.36	21.4	0.43	*	*	*	1.70×10^6
NH10	82	124°17.725	36	7.6	32.7	1.48	17.35	20.6	0.70	*	*	*	1.51×10^6
NH5	60	124°10.605	35	7.2	33.9	0.38	0.81	2.7	0.80	*	*		1.25×10^6

gradient (Table 2). The offshore station (NH127) was sampled three times over 4 d, and since there was considerable variability between these samples, these data were treated as independent samples and included separately in all analyses. The transect covered nearshore upwelling surface waters (region 1, Sta. NH5, NH10, and NH15) with low temperature and high nutrients (Fig. 1A), characterized by high Chl *a* concentrations (Table 2); shelf waters influenced by the Columbia River plume, with lower salinities and higher temperatures and generally low N and P concentrations in the slope sites (region 2, Sta. NH25, NH35, NH45, and NH55); and the outer stations (region 3, Sta. NH85 and NH127), which had characteristically higher nutrient concentrations but low Chl *a* levels (Table 2). The pycnocline samples from these stations followed roughly these same trends (Fig. 1B), although nutrient and Chl *a* concentrations were more uniform within the pycnocline (Table 2). These regions differed in average Chl *a* concentration (Table 2) and also in the composition of the phytoplankton assemblage. The surface shelf waters were dominated by diatoms, and the slope waters had peaks of picocyanobacteria, whereas the outer stations were dominated by a mixed nanoeukaryote assemblage (Fig. 1C). The pycnocline samples also had marked shifts in autotrophic dominance, but picoeukaryotes were an important component at most stations (Fig. 1D).

Metabolic measurements—In order to estimate BGE, we had to measure BR and production and therefore had to physically separate bacteria from other planktonic components. As mentioned previously, filtration removed on average between 70% and 80% of autotrophic and heterotrophic pico- and nanoplankton, whereas over 80% of total bacteria passed through the filter. The filtered fraction is thus not composed of 100% bacteria since there is a small contribution from picophytoplankton in this fraction and there were bacteria retained in the filter as well, as indicated by losses of BP. Total bacterial metabolism is thus somewhat underestimated in the filtered fraction.

Total and BR and BP—Total respiration ranged from 0.5 to 3.5 μg C L^{-1} h^{-1} and 0.5 to 2 μg C L^{-1} h^{-1} in the surface and pycnocline samples, respectively, and was highest in shelf waters, coinciding with peaks of Chl *a* (Fig. 2A,B). No single autotrophic group explained the spatial variation in total respiration along the transect. Rather, the various peaks in total respiration were linked to peaks in different autotrophic groups. BR represented on average 79% of total respiration and was less variable than the latter along the transect in both the surface and the pycnocline samples (Fig. 2A,B).

We derived alternative estimates of BP for the incubations of the filtered fraction (used to estimate BGE together

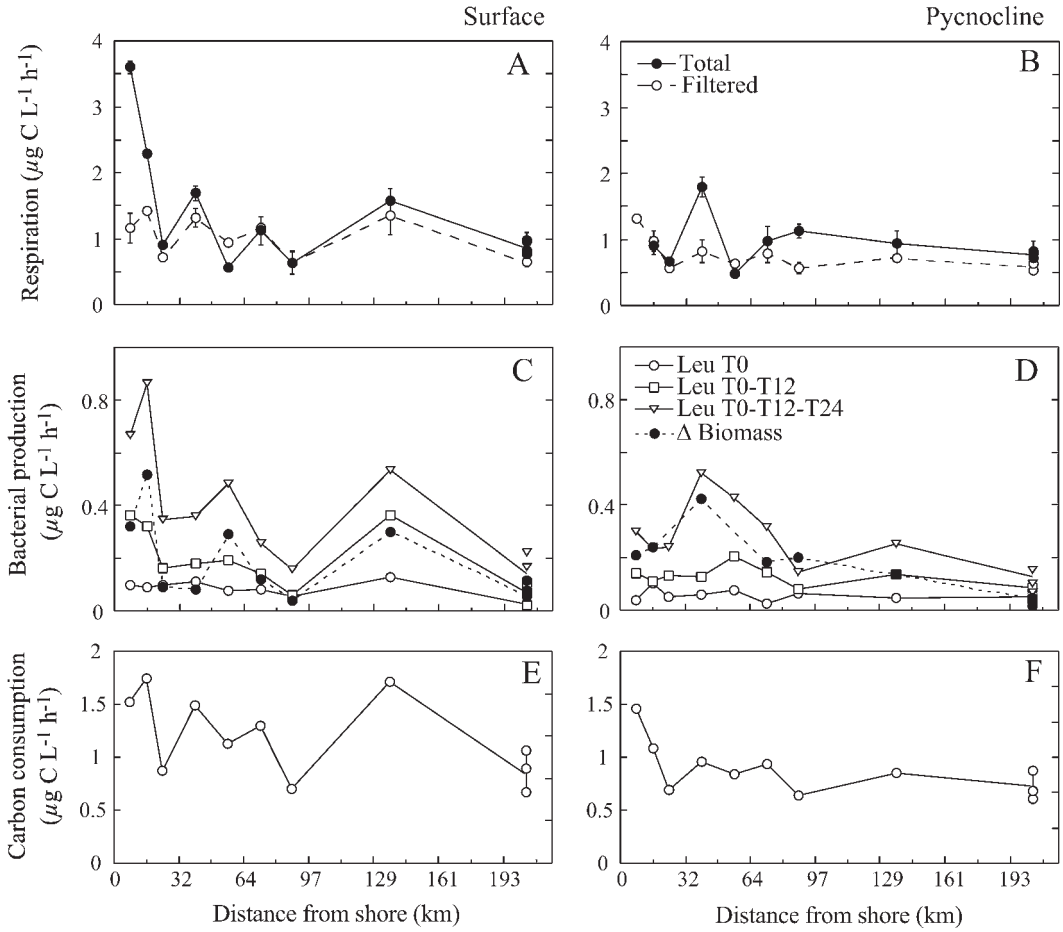

Fig. 2. Values of whole water (closed circles) and BR (open circles) in the samples collected at (A) the surface and (B) at the depth of the pycnocline. Different estimates of BP, based on initial leucine incorporation (open circles; Leu T_0), the integration of leucine incorporation from 0 to 12 h (open square; Leu T_0–T_{12}), the integration of leucine incorporation from 0 to 24 h (open triangles; Leu T_0–T_{12}–T_{24}), and the total biomass accumulated during the incubation (closed circles; Δ Biomass), (C) for the surface samples and (D) for the pycnocline samples. Bacterial carbon consumption calculated with the BR and the BP (using Leu T_0–T_{12}) data for (E) surface samples and (F) for pycnocline samples.

with BR) based on combinations of the measurements taken at different time points (all using the same standard CF); these alternative estimates of BP differed by up to fivefold, although the general spatial pattern was maintained, with higher BP rates associated with coastal waters with high Chl *a* levels (Fig. 2C,D). In general, the rates of leucine incorporation measured at the beginning of the incubations yielded uniformly low rates of BP, whereas by the end of the incubation at time 24 h, the rates had increased greatly, and thus the average BP calculated including these rates was very high. Rates calculated as a mean for the BP measurements at times 0 and 12 h were intermediate and agreed well with those derived from changes in biomass (Fig. 2C,D). Bacterial C consumption (computed as BR + BP) was narrowly constrained throughout the transect (Fig. 2E,F), ranging from 0.6 to 1.6 μg C L^{-1} h^{-1} in spite of Chl *a* values that ranged from 0.2 to 17.9 mg m^{-3}.

BGE—Figure 3 shows the calculated BGE based on BR and the different estimates of BP in the filtered fraction. Not surprisingly, there were large differences between the different estimates of BGE, although most showed a similar spatial pattern along the transect and between surface and pycnocline samples. The values of BGE derived using BP at T_0 were extremely low, never exceeding 10%, and showed very little spatial pattern. In contrast, BGE calculated by integrating the three BP measurements (T_0, T_{12}, and T_{24}) always exceeded 20% both in the surface (Fig. 3A) and in the pycnocline (Fig. 3B) and had greater spatial variability. BGE calculated integrating BP at T_0-T_{12} was intermediate and agreed to within 15% with BGE based on changes in biomass. These intermediate values of BGE peaked at around 25% in the upwelling areas and then declined to $< 10\%$ in the outer stations. Overall, BGE based on $BP_{T0–T12}$ (using the standard CF) and BGE based on BP calculated from changes in biomass were not significantly different from each other, and both were significantly different from BGE based on BP_{T0} and BGE based on $BP_{T0\text{-}T12\text{-}T24}$ (in all cases using the standard CF; Fig. 3C). For the purpose of subsequent comparisons, we will use the values of BGE derived using $BP_{T0\text{-}T12}$, and we will refer to this simply as

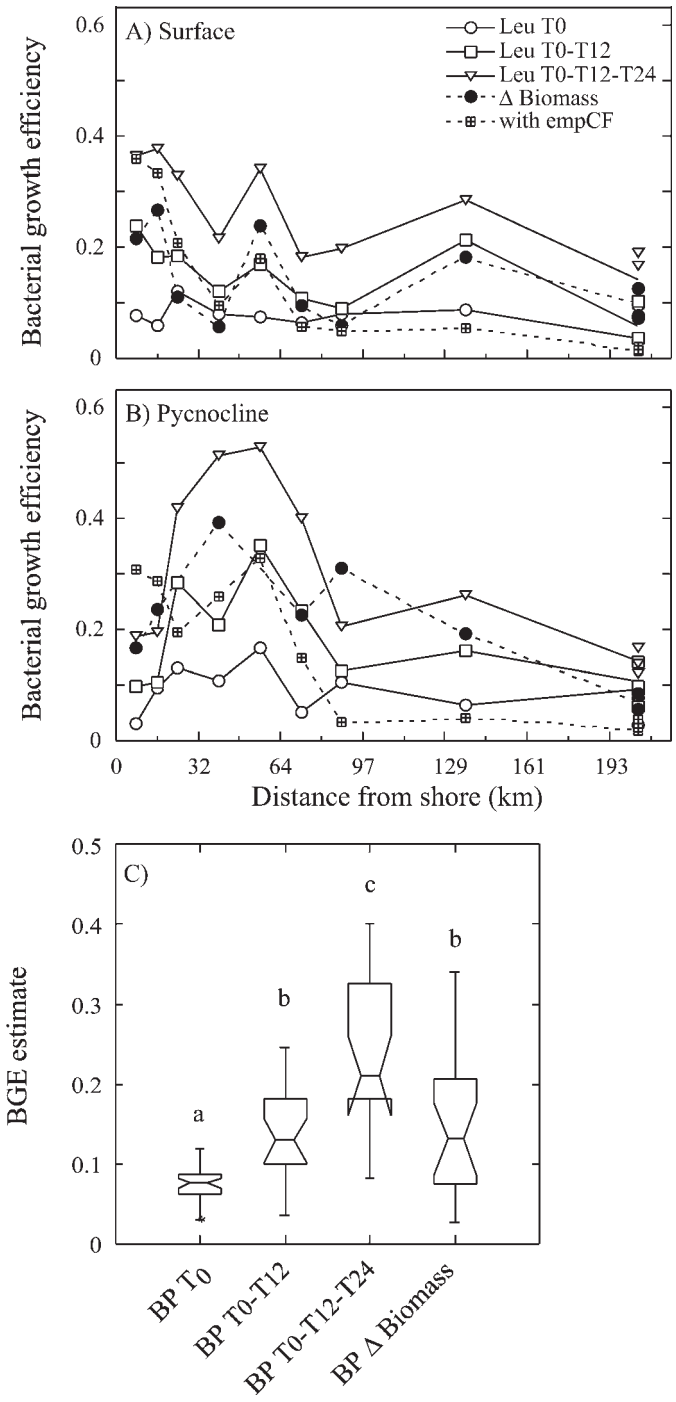

Fig. 3. Estimates of BGE, using different estimates of BP: based on initial leucine incorporation (open circles; Leu T_0), the integration of leucine incorporation from 0 to 12 h (open squares; Leu T_0-T_{12}), the integration of leucine incorporation from 0 to 24 h (open triangles; Leu T_0-T_{12}-T_{24}), the total biomass accumulated during the incubation (closed circles; Δ Biomass), and the integration of leucine incorporation from 0 to 12 h based on the empirical leucine-to-carbon CFs (crossed squares; eCF) for (A) surface and (B) pycnocline samples. Notch box-and-whisker plots of the distribution of BGE estimations based on the different combinations shown. The central horizontal line in each box represents the median of the distribution, whereas the other two horizontal lines contain 50% of the values between them. The end points of the inclined straight lines indicate the 95% confidence intervals for the median, whereas the whiskers indicate the total range of values without outliers (asterisks). The medians of two boxes with notches that do not overlap are significantly different at the 95% confidence level. Different letters on top indicate averages that are significantly different from each other according to a post hoc Tukey–Kramer test after a significant ANOVA ($p < 0.001$).

BGE. These estimates of BGE averaged 0.12 ± 0.02 (SE) in the surface samples, 0.14 ± 0.014 in the pycnocline, and 0.13 ± 0.01 for the entire data set and showed a distinct declining trend toward the outer stations, with some of the variation coinciding with local peaks of autotrophs (Fig. 3A,B).

Bacterial growth rates, leucine-to-thymidine incorporation ratio, leucine respiration, and C-to-leucine yield—Figure 4A,B shows the spatial pattern in the two estimates of growth rate: the cell-specific leucine incorporation and the potential growth rates. The average cell-specific leucine incorporation rates (an estimate of instantaneous growth rate in the unfiltered sample) in surface waters was 1.92×10^{-7} pmol Leu cell^{-1} h^{-1} (in surface, range 0.36–4.7×10^{-7}) and 0.78×10^{-7} pmol Leu cell^{-1} h^{-1} in the pycnocline (range 0.2–2.1×10^{-7}). The average potential growth rates (in filtered water incubations) were 0.094 h^{-1} (range 0.07–0.14) and 0.084 h^{-1} (range 0.04–0.14) in the surface and pycnocline, respectively, and were slightly less variable than the cell-specific leucine incorporation rates. These two indices of bacterial growth rate followed similar patterns along the transect, with peaks coinciding roughly with major variations in autotrophic biomass (*see* Fig. 1C,D) and a large decline toward open-ocean sites. In both cases, the average growth rates were lower for pycnocline samples, and the spatial pattern along the transect was at times very different between these two layers as we observed for the other processes we discuss later. For comparison, we have superimposed the BGE values discussed in Fig. 3 to show that there was strong covariation of growth and growth efficiency along the transect, particularly at the surface. We discuss this relationship in more detail later.

The Leu : TdR ratio averaged 9.8 (range 4–18) in surface waters and 10.5 (range 7–17) in the pycnocline (Fig. 4C,D), with no consistent trends along the transect. In contrast to the Leu : TdR ratio, the long-term carbon-to-leucine yield declined uniformly along the transect in both surface and pycnocline samples, from values exceeding 2.5 kg C mol^{-1} at the shelf stations to values below 0.5 kg C mol^{-1} at the outer stations.

Based on the ^{14}C-labeled leucine experiments, there was a general decline in the total leucine taken up (assimilated + respired) from the shelf to the outer sites as well as in the leucine assimilated into biomass in both surface and pycnocline samples (Fig. 4E,F). In surface samples, there was a general increase in the proportion of the leucine that was respired, from < 10% at the shelf to over 50% at the outer stations (Fig. 4E). The spatial pattern of the percent

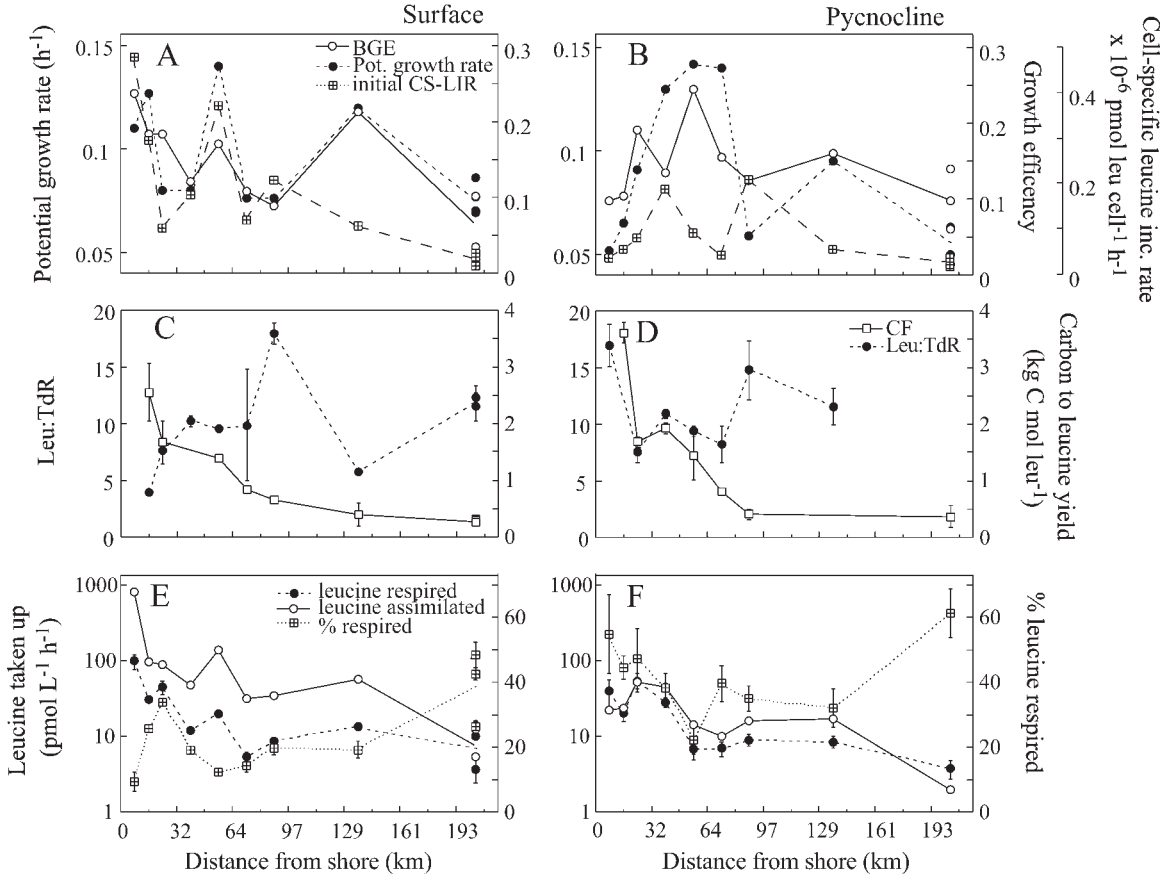

Fig. 4. Final bacterial growth efficiencies (open circles), potential growth rates measured in the filtered samples (closed circles), and cell-specific leucine incorporation rates (or instantaneous bulk growth rates; crossed squares) in (A) the surface and (B) pycnocline samples. Long-term carbon-to-leucine yields (open squares) and Leu : TdR incorporation ratios (closed circles) in (C) surface and (D) pycnocline samples. Leucine assimilated into biomass (open circles) or respired (closed circles) and percent leucine respired (crossed squares) in (E) surface and (F) pycnocline samples.

leucine respired was more erratic in the pycnocline, with high values exceeding 50% at both the shelf and the outer stations. Although average potential growth rates, BGE, and carbon-to-leucine yield were roughly similar between surface and pycnocline, as we have shown previously, the percent leucine respired was on average significantly greater in the pycnocline (42% ± 4 SE) than in the surface (25% ± 3 SE).

Overall, the rates of ^{14}C-leucine taken up (assimilation + respiration) were strongly correlated ($r = 0.90$, $p < 0.001$) with the rates of ^{3}H-leucine incorporation determined in parallel incubations in the ambient, unfiltered water (described in the preceding sections), suggesting a good agreement between these two alternative approaches. The median ^{14}C-leucine assimilation rate was 80% of the median ^{3}H-leucine incorporation, suggesting that the tritiated leucine may overestimate the actual carbon assimilated into protein.

Relationships between the metabolic variables—The target metabolic variables were related to each other in the direction that was initially hypothesized. The two indices of bacterial growth rate (cell-specific leucine incorporation in bulk samples and potential growth rates in filtered samples) were significantly positively correlated to each other (Table 3). In turn, both were positively correlated to BGE (Fig. 5A), and the potential growth rates were negatively correlated to the Leu : TdR incorporation ratio (Table 3; Fig. 5D). This indicates that bacterial assemblages capable of faster growth either in situ or during the incubations were those with higher BGE and with lower initial (ambient) Leu : TdR ratios (Fig. 5C). The percentage of leucine respired was negatively correlated to growth rates and to BGE (Fig. 5B), which suggests that slow-growing communities tended to have lower BGE and tended to respire a higher portion of the leucine that was taken up. The C-to-leucine yield was negatively related to BGE and the Leu : TdR ratio and positively related to both indices of growth, suggesting that the patterns in leucine processing in long-term incubations, such as the ones used to determine the leucine CF, are related to both ambient characteristics and short-term metabolic patterns.

Discussion

In this project we have attempted to constrain key aspects of marine bacterial C metabolism and better understand the connections that exist between them by

Table 3. Spearman's rho correlation coefficients between metabolic variables. *N* ranges from 16 to 22. Only correlations with $p < 0.05$ are shown. One asterisk indicates probabilities that are also below the Bonferroni-corrected probability equivalent to $p = 0.05$ (which for this set of correlations is $p = 0.002$). BP in the ambient water using the standard conversion factor.

	BP	BR	BGE	Leu : TdR	C-to-leucine yield	% leucine respired	Potential μ
BR	0.47						
Growth efficiency	0.52	—					
Leu : TdR	—	—	−0.83*				
C-to-leucine yield	0.56	—	−0.64*	−0.62*			
Percent Leu respired	−0.76*	—	−0.49	—	—		
Potential growth rate	0.49	—	0.70*	−0.60	0.60	−0.52	
Cell-specific BP	0.91*	—	0.51	—	0.58	−0.73*	0.49

analyzing their magnitude and patterns of variation along major marine environment gradients. At a conceptual level, we were further interested in exploring how different aspects of leucine metabolism, including both short term (i.e., leucine taken up and respired) and long term (fate of the leucine taken up and incorporated over longer time scales), relate to bulk aspects of bacterial C metabolism. Establishing these connections will improve our understanding of the regulation of bacterial carbon metabolism and, consequently, of the total carbon flux through oceanic bacterioplankton. From a practical standpoint, establishing the coherence (or lack thereof) in the patterns between these different components of bacterial C metabolism will reveal the robustness and ecological relevance of these measurements and help us further constrain these processes.

Methodological aspects of BR, BP, and BGE—The determination of BGE involves the measurement of two components, BP and BR (or, alternatively, changes in dissolved and particulate organic C and dissolved inorganic C), each of which requires assumptions and has characteristic weaknesses (del Giorgio and Cole 2000; Briand et al. 2004; Alonso-Saéz et al. 2007). These problems are particularly acute in open-ocean studies, where rates are

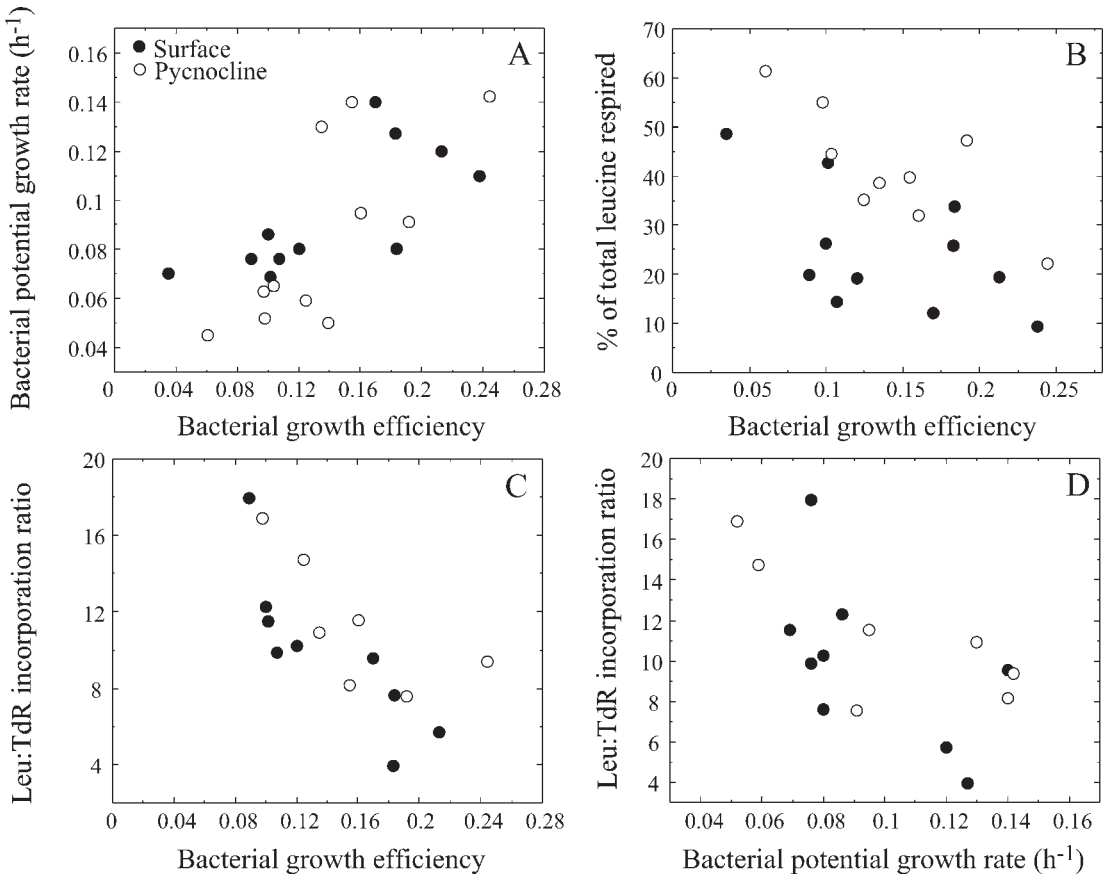

Fig. 5. Relationship between potential bacterial growth rates and growth efficiency (A), Leu : TdR incorporation ratios and potential bacterial growth rates (B), Leu : TdR incorporation ratios and BGE (C), and Leu : TdR incorporation ratios and potential growth rates (D). Surface and pycnocline samples are in different symbols.

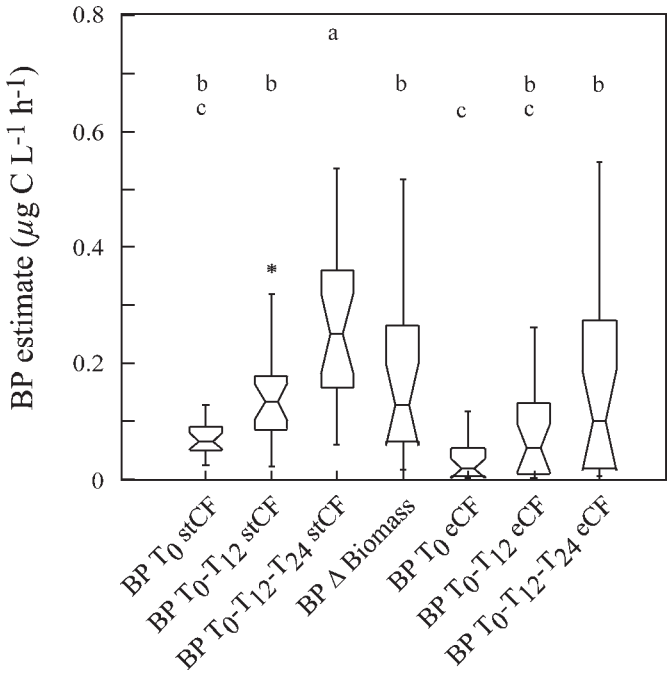

Fig. 6. Estimates of BP based on the use of the initial leucine incorporation measurements (Leu T_0), the integration of leucine incorporation from 0 to 12 h (Leu T_0-T_{12}), and the integration of leucine incorporation from 0 to 24 h (Leu T_0-T_{12}-T_{24}) using a standard CF of 1.55 kg C mol leucine^{-1} (stCF) or using the empirically determined CF (eCF). Also shown is the estimate of BP using the changes in bacterial biomass throughout the incubations (BP Δ Biomass).

difficult to obtain and often require long incubations that increasingly move samples away from in situ conditions (Pomeroy et al. 1994; Massana et al. 2001). BR measurements involve filtration, which disrupts the structure of the community, and also relatively long incubations (up to 24 h in our study). There is additional uncertainty associated with the conversion of O_2 to C rates, but most reported RQ values are in the range of 0.8 to 1.1 (P. del Giorgio and J. Aristegui unpubl.), and it is unlikely that this conversion will have a major effect on the patterns shown here.

BP measurements also have shortcomings, and in spite of the relative ease of the leucine incorporation measurements, the interpretation of these data, especially in the context of in vitro incubations, is far from simple. In order to better constrain these rates, we performed simultaneous measurements of leucine incorporation and of bacterial abundance at three points during all respiration incubations. Our results showed good agreement between the changes in biomass and leucine incorporation rates when only the first two time points (T_0 and T_{12}) were combined with the standard leucine CF (1.55 kg C mol^{-1} leucine; Fig. 6). Including the 24-h time point in the mean yielded a systematic overestimation of BP relative to the observed changes in biomass. This is also in agreement with previous results obtained in river, estuarine, and salt marsh samples (Apple et al. 2006; del Giorgio et al. 2006). As we discuss later, for a subset of the samples we determined the C-to-leucine yield, which can also be used as empirical leucine CFs, but use of these resulted in a systematic underestimation of BP for in vitro incubations, except when applied to the leucine incorporation rates measured at the final time point of the incubations.

Patterns in bulk bacterial C metabolism—With more or less intensity, the different aspects of bacterial C metabolism have been individually assessed in marine systems before, and the patterns that we found both corroborate and extend previous reports. The rates of plankton community respiration that we measured in this North Pacific transect agree well with those reported for other oceanic regions of similar primary productivity. For example, Robinson et al. (2002) reported that the regions with the lowest chlorophyll concentrations had an average respiration rate of 1.5 mmol O_2 m^{-3} d^{-1}, which compares well with the average of 15 μg C L^{-1} d^{-1} that we found at the edge of the North Pacific Gyre. Likewise, our BR measurements agree well with those reported by Sherry et al. (2002) for the subarctic northeastern Pacific and are well within the range of values reported in a recent comprehensive review by Robinson (2008). Bacterial (filtered) respiration represented on average 79% of the total respiration and was less variable than the latter; bacteria contributed over 90% in the least productive sites (exceptionally $>$ 100%, obviously a measuring problem) but less than 50% in areas with autotrophic peaks, also in agreement with previous reports (Biddanda et al. 1994; Robinson 2008). Overall, our results suggest that total planktonic respiration has a large and relatively constant bacterial component and a more variable algal or zooplanktonic component that may be locally important.

BGE was generally low ($<$ 15%), and there was substantial horizontal and vertical spatial variability. The magnitude and range of BGE agrees well with previous reports for coastal and open-ocean sites (del Giorgio and Cole 2000; Sherry et al. 2002; *see* Robinson 2008 for a recent synthesis of published data). Lemée et al. (2002) reported a wide range (1–44%) in BGE in one oligotrophic Mediterranean station during the year, with an overall average of 11%, similar to our overall average of 13%, and a similar range was reported by Reinthaler and Herndl (2005) for the North Sea. The molar ratio of leucine to thymidine incorporation has been shown to vary widely between systems (Chin-Leo and Kirchman 1990; Gasol et al. 1998; Hoppe et al. 2006) and also within a sample with time (Sherr et al. 1999), and our results are within the range reported. This variability has been interpreted as reflecting various degrees of uncoupling between protein and nucleic acid synthesis (Chin-Leo and Kirchman 1990; Torreton and Dufour 1996; Sherr et al. 2001), although Hoppe et al. (2006) have argued that the absolute values of the ratio that are associated with balanced growth probably differ between communities and regions. The range of values observed for the various metabolic variables was comparable between the surface and pycnocline layers, although their spatial patterns were often quite different, probably reflecting the characteristics of the water masses that coexist vertically along the transect. More important from the perspective of this study, surface and pycnocline data

all fit the same overall patterns between variables (e.g., Fig. 5), suggesting common underlying mechanistic links between them.

Links between components of bulk C metabolism—The previously discussed variables represent connected stages in the bacterial C metabolism and should be coupled through the overall cellular regulation pathways. In this regard, it has been postulated that growth efficiency and growth rate should be coupled (Vallino et al. 1996; del Giorgio and Cole 2000) such that high growth rates should generally coincide with higher growth efficiencies. On the other hand, it has generally been assumed that substrate consumption and growth should be coupled (Church 2008). In addition, cells with high Leu : TdR incorporation ratios are presumably processing carbon without this resulting in cell division and should thus have lower BGE than cells that are able to divert more of the carbon into cell growth and division (Gasol et al. 1998). Conversely, higher growth rates should be accompanied by higher rates of nucleic acid synthesis and thus lower leucine-to-thymidine incorporation ratios (Chin-Leo and Kirchman 1990).

Previous work has already shown connections between some of these components of bacterial metabolism. For example, Shiah and Ducklow (1997) also reported a negative relationship between the Leu : TdR ratio and specific growth rate, and a positive relationship between BGE and growth rate was reported for lake water cultures (Middelboe and Søndergaard 1993) and the Hudson River (del Giorgio et al. 2006). However, the collective links that exist between these components of bacterial metabolism have rarely, if ever, been explored.

We have shown here that there was overall coherence between the patterns in growth, growth efficiency and short- and long-term leucine metabolism. We found that the two estimates of growth rate, the Leu : TdR ratio and BGE, covaried significantly such that slow growth was associated with low BGE and high Leu : TdR, suggesting a direct connection between shifts in the ratio of protein to deoxyribonucleic acid synthesis and declines in the efficiency of biomass synthesis. The patterns of metabolism, both in situ and in incubations, might be influenced not just by resources and environmental conditions but also by intrinsic responses related to the community structure of bacterioplankton. Previous studies have shown major shifts in bacterial and microplankton community composition along this inshore–offshore transect (Longnecker et al. 2005; Sherr et al. 2005). Protistan bacterivory (Suzuki 1999) and viral infection (Bouvier and del Giorgio 2007) have also been shown to strongly influence community composition in this area; the latter may influence BGE at the community level as well (Motegi et al. 2009). The manipulation of samples (i.e., filtration) for metabolic measurements disrupts some of these biological interactions and may result in further shifts in community composition during in vitro incubations (Suzuki 1999; Gattuso et al. 2002).

Patterns in leucine metabolism and links to bulk metabolism—We did not seek here to explore in detail the practical and conceptual implications of the respiration of labeled substrates, which have been previously discussed (Brittain and Karl 1990; *see* the excellent discussion by Hollibaugh 1994), or the problems associated with the determination and use of leucine empirical CFs (Pulido-Villena and Reche 2003; Buessing and Marxen 2005); rather, we were interested in aspects of leucine metabolism as both indices of substrate processing and energy limitation, and our objective was to establish whether patterns in the uptake and processing of leucine had any counterpart in other aspects of bacterial C metabolism. Hollibaugh (1994) had already suggested over a decade ago that the patterns of metabolism of thymidine had not only practical implications (in terms of calculations of BP) but also fundamental ecophysiological interest because the metabolic fate of the incorporated thymidine probably reflects the overall nature and availability of organic substrates and nutrients as well as the physiological state and composition of the community.

Our results show that a significant fraction of the total leucine taken up by bacteria (30–70%) is respired, in agreement with earlier studies (Hobbie and Crawford 1969; Crawford et al. 1974; but *see* Suttle et al. 1991). The proportion of leucine respired declined with increasing total ^{14}C-leucine taken up. This pattern was not only present for ^{14}C-leucine but also relative to the ^{3}H-leucine incorporation rates measured in parallel but independent samples (details not shown), further confirming that this pattern is not an artifact of the ^{14}C-based technique. There have been previous reports of a similar pattern of increasing proportion of total leucine respired toward oligotrophy. For example, Jørgensen (1992) reported 20–50% of gross uptake respired based on $^{3}H_2O$ production, which agreed well with parallel measurements made of $^{14}CO_2$ production.

Our results also showed a clear declining trend in the long-term C-to-leucine yield toward the oligotrophic sites, similar to what has been reported for other marine samples (Bjørnsen and Kuparinen 1991; Pedrós-Alió et al. 1999; Alonso-Saéz et al. 2007). A recurrent observation in the type of long-term incubation used to determine eCF is that rates of leucine incorporation often tend to increase greatly as incubations progress. Yet these rates of leucine incorporation often do not have a counterpart in terms of biomass accumulation for marine (Ducklow et al. 1992; Pomeroy et al. 1994; Van Wambeke et al. 2008) or freshwater samples (Pulido-Villena and Reche 2003). These upshifts in apparent leucine incorporation have been linked to increases in the number and size of active cells and changes in bacterial cell-specific activity during incubations (Sherr et al. 1999; Briand et al. 2004) and to changes in bacterial community composition (Massana et al. 2001).

In our study, low eCF were generally associated with large upshifts in leucine incorporation toward the end of incubations, and the magnitude of this upshift relative to the initial rates was not random but was higher in samples from oligotrophic areas and deeper in the water column. This in fact explains much of the spatial pattern found in eCF. It is interesting to note that, in turn, these are the same samples that initially (i.e., in the short term) had a high percent of leucine respired. That the upshift itself is

may be linked to an increase in the proportion of leucine that is respired in the short term is a definite possibility that needs to be further investigated. Regardless of the underlying cause, it would appear that the magnitude of the long-term upshift might be linked to the degree of C or energy limitation that was initially present in the ambient waters. This conclusion is further supported by the observation that samples that had low long-term C-to-leucine yield tended to have low bulk BGE and growth rate (Table 3). These three levels of C metabolism, percent Leu respired, BGE (and growth rate), and the C-to-leucine yield are methodologically independent in that they share no common measurements and span the whole spectrum of time scales covered in this study: percent Leu respired corresponds to a scale of minutes to hours and BGE and growth to a scale of hours to a day, whereas the C-to-leucine yield corresponds to a scale of several days. This suggests that the factors that lead to low short-term growth efficiency may also lead to low long-term conversion efficiency of leucine and that these variables may indeed represent different expressions of the same basic type of resource limitation. In particular, our results would suggest that the long-term incubations tend to amplify features already present in the ambient samples such that the responses obtained are ecologically meaningful but the magnitude may not be applicable to ambient samples, and this appears to be particularly true for patterns in leucine metabolism.

These observations result in the somewhat paradoxical situation wherein the patterns in eCF appear to have an ecophysiological basis and reflect key aspects of bacterial C metabolism and resource limitation, yet the actual magnitude of these factors cannot be simply extrapolated to ambient processes and used to calculate BP. Our own results suggest that application of empirical eCF (= CLY)-to-leucine incorporation rates in samples of ambient water or from short-term incubations may result in large underestimations of BP. In this regard, not all studies have concluded that oceanic eCF must be low and variable. For example, Ducklow et al. (2000) concluded, on the basis of a C mass balance, that the CF should be relatively constant around 1.5 in the Ross Sea in order to close the C budget. Although the need to determine empirical CFs to derive robust estimates of BP from leucine incorporation has been repeatedly pointed out (Bell 1990; Buessing and Marxen 2005; Pulido-Villena and Reche 2003), our results suggest that CFs derived from long-term incubations may in fact yield biased BP results.

Ecological implications—From an ecological point of view, the covariation in these various aspects of bacterioplankton metabolism yields insight into the regulation of marine bacterial C metabolism. Along the transect and with depth, there was a much larger range in growth rate, both in the ambient samples and in incubations (Fig. 3), than in total C consumption (Fig. 2). This would imply that changes in growth must be associated with shifts in both BGE and in C consumption and not just to the latter, as has often been assumed. This is further supported by the positive relationship between BGE and the two indices of growth (Table 3) and the overall weak correlation between them and carbon consumption. This is similar to what was found in the Hudson River, where spatial variation in BP and growth could be explained only with a combination of carbon consumption and BGE (del Giorgio et al. 2006).

The question remains as to what determines the variation in BGE and C consumption in this marine area. Bacterial C consumption was remarkably constant along the transect, both in the surface and pycnocline samples (total range 0.5–1.7 μg C L^{-1} h^{-1}; Fig. 3), despite a variation in Chl *a* concentrations of two orders of magnitude (Table 1), and there was no clear trend toward open-ocean sites. In contrast, BGE varied almost 10-fold (0.03–0.3; Fig. 4), and there was a clear trend in surface waters of declining BGE toward open-ocean sites, punctuated by peaks that often corresponded to local changes in the abundance of autotrophs. In some of these local peaks of autotrophs, both C consumption and BGE increased in synchrony, whereas in other such features they showed contrasting responses, suggesting that local hot spots of primary production do not necessarily result in both higher BGE and higher C consumption. Interestingly, there was no trend in the pycnocline samples, and both BGE and C consumption were less variable along the transect, suggesting that the factors that influence the surface signal are rapidly modulated at depth.

Previous studies have concluded that marine bacterial growth may be limited by the amount and the nature of the C supply (Carlson and Ducklow 1996; Kahler et al. 1997; Cherrier and Bauer 2004) or by energy (Carlson et al. 2007; Van Wembeke et al. 2007), but the distinction between these two forms of limitation is not obvious. Most of the evidence points to the fact that bacterial C consumption tends to track C supply, at least over relatively large spatial and temporal scales (Ducklow 2000; Church 2008). Exceptions to this may occur, for example, when consumption of C may be transiently impeded by nutrient limitation or even grazing (Thingstad et al. 1997; Obernosterer et al. 2003; Gasol et al. 2009). But because the chemical nature of the available C varies spatially and temporally in marine systems, a given rate of bulk C consumption may result in very different energy yields, nutrients, and C precursors for biosynthesis (Linton and Stephenson 1978; del Giorgio and Cole 2000). In addition, the energetic demands of bacterial cells themselves are not constant such that for any given rate of C consumption, bacteria may experience different degrees of energy limitation (del Giorgio and Gasol 2008). These processes that uncouple C consumption from bacterial growth are expressed as variations in BGE, and low BGE may then result from any combination thereof: low energetic yield of the C consumed, nutrient limitation of the incorporation of this C into biomass, or high energetic demands of bacterial cells due to the prevailing environmental conditions.

In our study, bacterial C consumption had a weak but significant positive relationship with chlorophyll concentration, whereas neither BP, BGE, nor growth was related to chlorophyll, suggesting on the one hand a link between C consumption and C supply and on the other an uncoupling between C supply and growth. The spatial trends and the correlations found between the different

aspects of bacterial metabolism in our study all converge to suggest a pattern of increasing energy limitation from inshore to offshore samples and from surface to deep samples. Under conditions of energy limitation, a large fraction of the available carbon is channeled to adenosine-5′-triphosphate generation for maintenance and turnover functions rather than cell division, and this in turn is expressed as the high Leu : TdR ratios and low growth rates that we typically find associated with low BGE as well as with a higher percent of the Leu being respired.

The fact that growth efficiency based on the use of bulk organic matter mirrors the incorporation efficiency of an individual substrate (such as leucine, which is both highly reduced and a source of nitrogen) would suggest that the chemical or nutritional characteristics of the substrates consumed may be secondary to the overall physiological state of the community in determining the balance between catabolic and anabolic pathways. Furthermore, we observed no significant relationships between BGE and ambient nutrients, which was also true for the percent of leucine respired. Rather, we suggest that the particular energetic needs of bacterial communities in the different oceanic areas interact with the C availability (and thus the rates of C consumption) to determine the extent of energy limitation in these communities. In this regard, Carlson et al. (2007) have argued that BGE should decline along an axis of what they termed increasing environmental hostility, the latter potentially combining energy, C and nutrient limitation, ultraviolet (UV) penetration, and other forms of environmental stress that all result in increases in maintenance and repair costs. We suggest that environmental hostility increases toward the open-ocean sites, perhaps driven by a combination of overall dilution of resources (C and nutrients) and other factors, including increased UV penetration, such that the maintenance costs in oligotrophic areas may be on average higher relative to the available C supply (del Giorgio and Gasol 2008).

We postulate that in this northeastern Pacific transect, bacterial C consumption is directly limited by C supply, whereas BGE is linked to energy limitation driven mostly by the varying costs of survival and maintenance in the different oceanic regions; BGE (and other aspects of metabolism) may be additionally influenced by intrinsic responses related to community composition and structure. Growth clearly results from the interplay between these two factors. The notion of a single regulating factor of marine bacterioplankton growth is clearly an oversimplification (Saito et al. 2008) that does not capture the complexity of the regulation of bacterial activity in the ocean. For example, various types of nutrient limitation have been shown to influence BGE (Tortell et al. 1996; del Giorgio and Cole 2000). However, nutrients may profoundly influence bacterial growth even if they are not limiting from a stoichiometric point of view. For example, nutrient uptake could increase the overall energetic requirements of the cells and thus decouple biomass production from C consumption. Other environmental conditions and stressors may influence bacterial growth indirectly in the same manner, not by limiting any individual cellular process per se, but rather by influencing the overall energetic budget of the cells. It may be time to move beyond the paradigm of limitation of marine bacterial activity that considers only single, proximal factors in a purely stoichiometric manner (e.g., supply of organic matter, N, and P) toward an approach that incorporates the ensemble of energetic constraints imposed by the environment.

Acknowledgments

We thank the captain and crew of the R/V *Wecoma* and all other cruise participants for the great atmosphere on board, and C. Côté and B. Sherr for help with logistics and cruise preparation. Four anonymous reviewers provided thoughtful and constructive input and are acknowledged. This work was supported by the National Science Foundation grant OCE-0002236 to E.S. and P.d.G. and grant REN2001-5097-E/MAR of the Spanish Government to J.M.G. Writing of this manuscript was supported by grant CTM2008-03309/MAR to J.M.G. and a Visitor Fellowship of the Catalan Government to P.d.G.

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Associate Editor: Mikhail V. Zubkov

Received: 02 February 2010
Accepted: 13 July 2010
Amended: 07 September 2010