

## Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean

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### Abstract

We present surface estimates of bacterial respiration, bacterial heterotrophic production (BHP), and bacterial growth efficiency (BGE), and their relationship with nutrient availability, along a trophic gradient from coastal upwelling waters to the open-ocean waters of the eastern North Atlantic. Bacterial respiration generally ranged between 10 and 30  $\mu\text{g C L}^{-1} \text{d}^{-1}$  and was relatively unaffected by nutrient enrichment. In contrast, BHP showed higher variability (more than one order-of-magnitude range) and was affected by carbon and/or phosphorus additions in different regions. Empirical bacterial carbon-to-leucine (Leu) conversion factors (CFs) (range, 0.02–1.29  $\text{kg C mol Leu}^{-1}$ ) decreased from the coast to the open ocean, largely influencing the BHP estimates in oligotrophic waters. We found high percentages of Leu respiration in oceanic waters (average 68% of Leu taken up by bacteria), possibly related to the low CFs found offshore. Empirical CFs were highly correlated to BGE (Pearson correlation coefficient  $r = 0.86$ ,  $n = 12$ ,  $p < 0.0004$ , log-log transformed), which varied between 1% in offshore waters and 56% in the upwelling waters. Empirical CFs could be critical not only for accurately constraining BHP, but probably also for predicting BGE in oceanic waters.

Planktonic heterotrophic bacteria are key players in the oceanic carbon cycle, recycling the large dissolved organic carbon reservoir (Hansell and Carlson 1998). However, methodological difficulties have limited a comprehensive understanding of bacterial carbon flux in the oligotrophic ocean. Even if bacterial respiration (BR) is the key parameter required to constrain both carbon remineralization and biogenic carbon export in the ocean (Rivkin and Legendre 2001), the available estimates of BR and bacterial growth efficiency (BGE) for oceanic waters are still very scarce. The precise measurement of these parameters is

particularly important in the open ocean, where most of the carbon that bacteria process is respired, and BGE can be as low as 1% (del Giorgio and Cole 1998).

The accurate measurement of bacterial heterotrophic production (BHP) is also of great importance for the determination of bacterial growth rates and energetics in marine waters. BHP has been extensively measured over most oceanic regions (e.g., Ducklow and Carlson 1992), but the conversion factors (CFs) required to convert the incorporation of leucine (Leu) or thymidine (TdR) to biomass accumulation are poorly constrained (Ducklow et al. 2002). The existing empirical CFs are often very different from the widely used theoretical estimates, especially in oligotrophic waters. However, few studies have tried to assess what factors control their variability in natural systems and to what extent the use of standard theoretical factors can seriously affect BHP estimates.

In this study, we examined the spatial variability in bacterial carbon flux in the northeast subtropical Atlantic across the transition zone between coastal waters, affected by the northwest Africa upwelling system and the central open-ocean waters of the North Atlantic subtropical gyre. BGE estimates had not yet been reported for this area. We assessed bacterial carbon cycling with two main objectives: (1) to constrain the range of variability of key parameters

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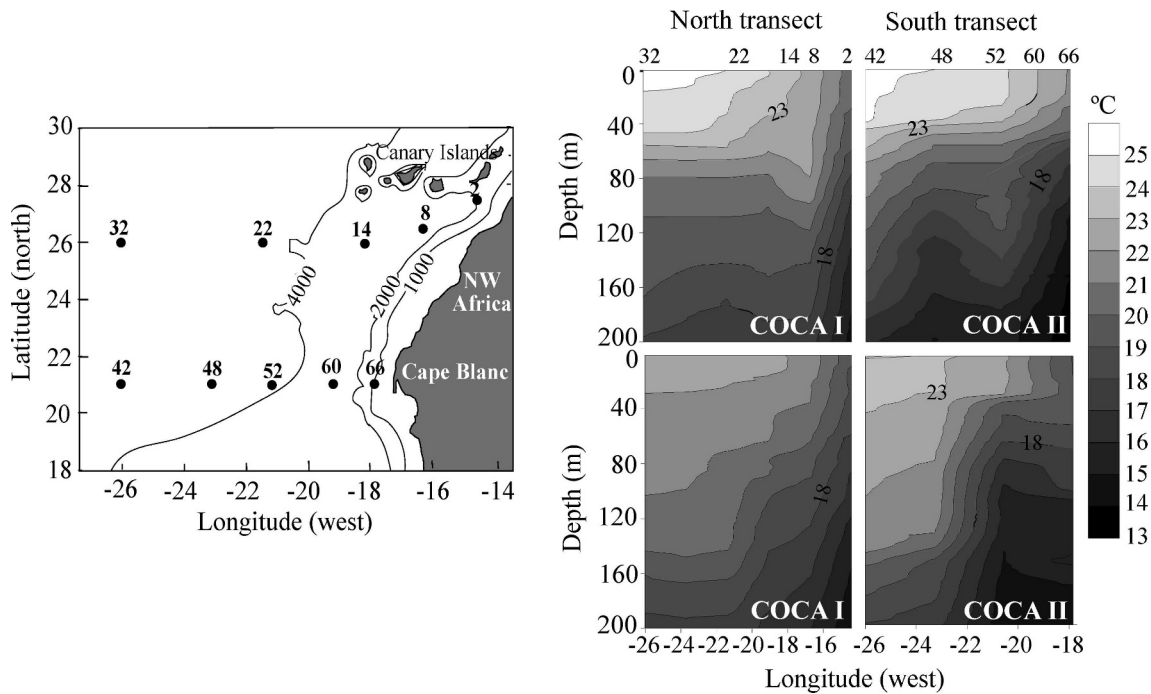


Fig. 1. (a) Map of the sampling area showing the station numbers and locations. (b) Depth profiles of water temperature ( $^{\circ}\text{C}$ ) along the north and southern transects during the COCA I and COCA II cruises. Station numbers indicate their position in the transects.

such as BR, BHP, BGE, and empirical carbon-to-Leu CFs, and (2) to study the relationship between these metabolic processes and their in situ potential controlling factors.

## Materials and methods

**Location and sampling**—The study was conducted along two transects extending from the northwest African coastal waters towards the open-ocean waters of the North Atlantic subtropical gyre (Fig. 1) during two cruises on board the *BIO Hespérides* (COCA I, 10 September 2003 and October 2002; COCA II, 20 May to 10 June 2003). The main study was performed with samples collected from surface water (5 m) in 10 open-water stations (depth  $>1,000$  m) during the COCA II cruise. Four or five additional depths were sampled throughout the euphotic zone (considered down to the depth reached by 1% of incident photosynthetically active radiation [PAR] light) at all stations for measurements of chlorophyll, bacterial abundance (BA) and BHP. During the COCA I cruise, the stations were sampled at a variable depth within the euphotic zone (25–85 m) for BGE and resource limitation experiments. At each station, temperature, salinity, and fluorescence were recorded with a CTD system (Idronaut MK-317 and Mark III-IOC) mounted on a General Oceanic rosette sampler equipped with 24 twelve-liter Niskin bottles.

**Chlorophyll, particulate organic carbon (POC), and particulate organic nitrogen (PON)**—Samples (250 mL) were filtered through Whatman GF/F filters to determine the chlorophyll *a* (Chl *a*) concentration. The filters were homogenized and kept refrigerated in the dark while

pigments were extracted in 90% acetone for  $\sim 1$  h. Fluorescence of the extracts was measured in a Turner Designs fluorometer.

Samples (2 liters) for POC and PON were filtered through combusted ( $450^{\circ}\text{C}$ , 12 h) 25-mm Whatman GF/F filters. The filters were wrapped in combusted aluminum foil and frozen at  $-20^{\circ}\text{C}$  until processed a few weeks later. In the laboratory, the filters were thawed and dried overnight at  $65^{\circ}\text{C}$  in a desiccator with HCl fumes to remove carbonates, and finally dried overnight in a desiccator with silica gel. Before analysis, samples were packed into ultraclean tin disks. The carbon analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer, according to the JGOFS protocol (UNESCO, 1994).

**BA and biomass**—BA was determined by flow cytometry. Samples (1.6 mL) were preserved with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration), left 10 min in the dark to fix, deep-frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ . The samples were later thawed, stained with Syto13 (Molecular Probes) at  $2.5 \mu\text{mol L}^{-1}$  (diluted in dimethyl sulfoxide) in the dark for a few minutes, and run through a Becton Dickinson FACScalibur cytometer with a laser emitting at 488 nm. Data were obtained in log mode until  $\sim 100,000$  events were acquired. Bacteria were detected by their signature in a plot of side scatter versus FL1 (green fluorescence) as explained in Gasol and del Giorgio (2000). Picocyanobacteria were discriminated in a plot of FL1 versus FL3 (red fluorescence). When prochlorophyte fluorescence was very low, they were first enumerated in an unstained sample, and their abundance was subtracted from the total bacteria

determined with Syto13 staining. Bacterial biomass was estimated from BA, assuming a conservative CF of  $12 \text{ fg C cell}^{-1}$  (Fukuda et al. 1998).

**Bacterial heterotrophic production**—BHP was estimated from the rates of protein and DNA synthesis determined by the incorporation of tritiated Leu or TdR, respectively, into cold trichloroacetic acid (TCA). Leu and TdR were each added at saturating concentration ( $40 \text{ nmol L}^{-1}$ ) to four experimental replicates of 1.2 mL. Duplicate controls were established with the addition of  $120 \mu\text{L}$  50% TCA before the isotope addition. The Eppendorf vials were incubated 2–3 h at temperatures as close as possible to the in situ in either water baths or temperature-controlled chambers. The incorporation was stopped with the addition of  $120 \mu\text{L}$  of cold 50% TCA to the Eppendorf tubes, and samples were kept frozen at  $-20^\circ\text{C}$  until processing. This was done following the centrifugation method of Smith and Azam (1992) with two runs ( $\sim 12,000 \text{ rpm}$ ) and aspiration of the water. Finally, 1 mL of scintillation cocktail was added to the Eppendorf tubes, and they were counted after 24–48 h on a Beckman scintillation counter; quenching correction used an external standard. The average coefficient of variation for the quadruplicate determinations was 15%.

We performed 13 experiments in order to determine the in situ carbon-to-Leu CFs for surface water communities. The water sample was gently filtered through  $0.6 \mu\text{m}$  polycarbonate filters (Millipore, GTTP), then diluted (1:9) with  $0.2 \mu\text{m}$  filtered (Acropack 1000, Pall) seawater, and incubated in 2-liter acid-clean polycarbonate bottles in the dark. Size fractionation was needed in order to effectively remove all predators. Dilution was used to further promote bacterial growth in the timescale of the experiments. Subsamples were taken for Leu incorporation and BA measurements every 12–24 h until bacteria reached the stationary growth phase. Factors were computed with the cumulative method (Bjørnsen and Kuparinen 1991), which maximizes the use of the available data.

**Bacterial respiration**—BR was measured by following changes in dissolved oxygen during dark incubations of filtered water. The water was gently filtered with a peristaltic pump through glass fiber filters with approximate pore sizes of  $0.6\text{--}0.8 \mu\text{m}$  and a diameter of 14 cm (AP1514250 Millipore) to increase the filtering surface, reduce pressure, and avoid cell breakage. The efficiency of filtration was analyzed during the COCA I cruise in five stations. We found that after filtration, we recovered on average ( $\pm\text{SE}$ )  $78\% \pm 5\%$  of total bacteria. Eight biological oxygen demand (BOD) bottles were carefully filled, and three replicate bottles were immediately fixed with Winkler reagents to determine the initial oxygen concentration. Four replicate bottles were incubated in the darkness at in situ temperature and fixed with Winkler reagents after  $\sim 24 \text{ h}$ . At each time step, one additional replicate bottle was used to determine BA and production as described above. The optimal incubation time (24 h) was set from previous measurements taken during the COCA I cruise, when samples were taken at 0, 12, 24, and 36 h from filtered and unfiltered water of five stations: Sta. 2 and Sta.

8 (80 m), Sta. 32 (30 m), Sta. 48 (50 m), and Sta. 60 (40 m). Dissolved oxygen measurements were made by automated Winkler titrations on the basis of colorimetric end-point detection (as described in Aristegui et al. 2005). The rate of respiration was determined by regressing  $\text{O}_2$  against time for the 0–24-hour interval. We assumed a respiratory quotient of 0.88 (Williams and del Giorgio 2005).

**Bacterial growth efficiency**—Bacterial growth efficiency (BGE) was estimated as the ratio of bacterial production to production plus respiration in the filtered seawater (bacterial fraction). For that calculation, BR was estimated as described in the previous section in 24-h incubations, and bacterial net production was estimated in five different ways: (1) in situ BHP based in Leu uptake using a theoretical CF ( $1.5 \text{ kg C mol Leu}^{-1}$ , which assumes no isotope dilution), (2) in situ BHP using the experimentally determined CF for each station, (3) BHP at time 0 of the incubation experiment (with the theoretical CF,  $1.5 \text{ kg C mol Leu}^{-1}$ ), (4) the integrated BHP in the 24-h incubation interval using the empirical CFs, and (5) the change in bacterial biomass, estimated from the increase in cell numbers along the incubation and using a conservative factor of  $12 \text{ fg C cell}^{-1}$  (Fukuda et al. 1998).

**Nutrient limitation assays**—During the first cruise (COCA I), we sampled five stations at a variable depth within the euphotic layer; the assays included experimental assessment of nutrient limitation of BHP and BR. For this purpose, four gas-tight bilaminated plastic bags were completely filled with unfiltered seawater ( $\sim 15 \text{ liters}$ ). Bubbles were carefully removed before starting the experiments. We established four treatments: inorganic additions (nitrate, ammonium and phosphate,  $0.5 \mu\text{mol L}^{-1}$  each), organic additions (glucose and acetate,  $1 \mu\text{mol L}^{-1}$  each compound,  $8 \mu\text{mol C L}^{-1}$  in total), inorganic plus organic additions, and an unamended control. The bags were incubated inside water baths adjusted to the in situ temperature. All treatments were sampled every day for BHP, BA, and oxygen concentration, and the data were integrated over the 96 h of incubation.

During the second cruise (COCA II), a simpler assay was designed where only the effect on BHP was measured. Samples were taken from surface waters at all stations. Well cell plates (Iwaki) were filled with subsamples (10 mL) of unfiltered seawater. Nitrate plus ammonium ( $0.5 \mu\text{mol L}^{-1}$  final concentration each), phosphate ( $0.5 \mu\text{mol L}^{-1}$  final concentration), glucose plus acetate ( $1 \mu\text{mol L}^{-1}$  final concentration each compound), and a mixture of all of them (at the same concentrations) were added as enrichment treatments. Two subsamples were left unamended as replicated controls. The plates were incubated at in situ temperature inside dark temperature-regulated chambers, and we measured BHP after 24–48 h.

**Leu respiration**—Respiration and incorporation into biomass of uniformly labeled  $^{14}\text{C}$ -Leu was measured in four replicated samples (4 mL) placed in 25-mL incubation Erlenmeyer flasks (Kimble/Kontes) and one control, which was initially fixed with TCA (5% final concentration). We

basically followed the protocol of Hobbie and Crawford (1969) with some modifications. L-[U- $^{14}\text{C}$ -Leu] (Amersham) was added ( $60 \text{ nmol L}^{-1}$ ) to the samples, and the Erlenmeyer flasks were immediately closed with a rubber stopper fitted to a plastic well containing a piece of filter paper (Whatman no. 1 chromatographic paper). After 2–4 h incubation at the in situ temperature,  $200 \mu\text{L}$  of  $\text{H}_2\text{SO}_4$  ( $2 \text{ mol L}^{-1}$ ) were injected with a syringe into the lateral arm of the Erlenmeyer flasks to stop the incubation. After 1 h, during which the Erlenmeyer flasks were smoothly shaken several times to liberate the carbon dioxide,  $200 \mu\text{L}$  of 2-phenylethylamine (Sigma) was injected into the plastic well inside the Erlenmeyer flasks until the filter paper was completely wet. We waited 30 min for fixation of the carbon dioxide onto the filter paper and opened the Erlenmeyer flasks. The filter papers were placed in a vial with Optisafe HiSafe-2 cocktail to estimate the respired fraction. Triplicate samples of 1.2 mL were taken from each Erlenmeyer flask to measure the incorporated fraction. We added  $120 \mu\text{L}$  of 50% TCA to the subsamples and processed them with the centrifugation method described above for BHP.

**Protein turnover**—We followed the Kirchman et al. (1986) pulse-chase approach to determine whether there was turnover of intracellular proteins in the bacterial assemblages. Surface samples were treated with  $100 \text{ mg L}^{-1}$  cycloheximide to inhibit eukaryotes and to ensure that the decrease in radioactivity was due to protein turnover and not due to grazer activity on labeled ingested bacteria. We also ran some parallel experiments in which water was filtered through  $0.8 \mu\text{m}$  with a hand-held syringe and a 25-mm Nuclepore filter, but we saw no differences between both ways of removing the eukaryote activity. After preincubation with cycloheximide for  $\sim 20$  min,  $^3\text{H}$ -Leu was added at  $40 \text{ nmol L}^{-1}$  and samples taken every 15 min for  $\sim 120$  min. Then a cold chase of  $100 \mu\text{mol L}^{-1}$  unlabeled Leu was added to the samples, and radioactivity in the bacterial protein was measured with the protocol explained above every 30–60 min for the next 5–7 h. Protein turnover is detected when there is a significant decrease in radioactivity after the addition of the unlabeled Leu. Results are expressed as percentage of the label recycled per hour.

## Results

**Regional oceanographic settings**—The sampling area comprised 10 stations distributed along two zonal sections (five stations at each section; Fig. 1). The northern section ( $26^\circ\text{N}$ ) extended offshore from the boundary of the coastal upwelling jet, near Cape Bojador, where an upwelling filament affected the station closest to the coast (Sta. 2) in both cruises. Sta. 8 and 14 were placed in the coastal transition zone between the coastal upwelling waters and the oceanic area. The southern section ( $21^\circ\text{N}$ ) extended from the Cape Blanc coastal upwelling waters to the open ocean, crossing the Cape Vert Frontal Zone. During the COCA II cruise, the upwelling core was located at  $21^\circ\text{N}$ , and its influence reached as far away as Sta. 52 (Fig. 1). The upwelling stations and the upwelling filament were

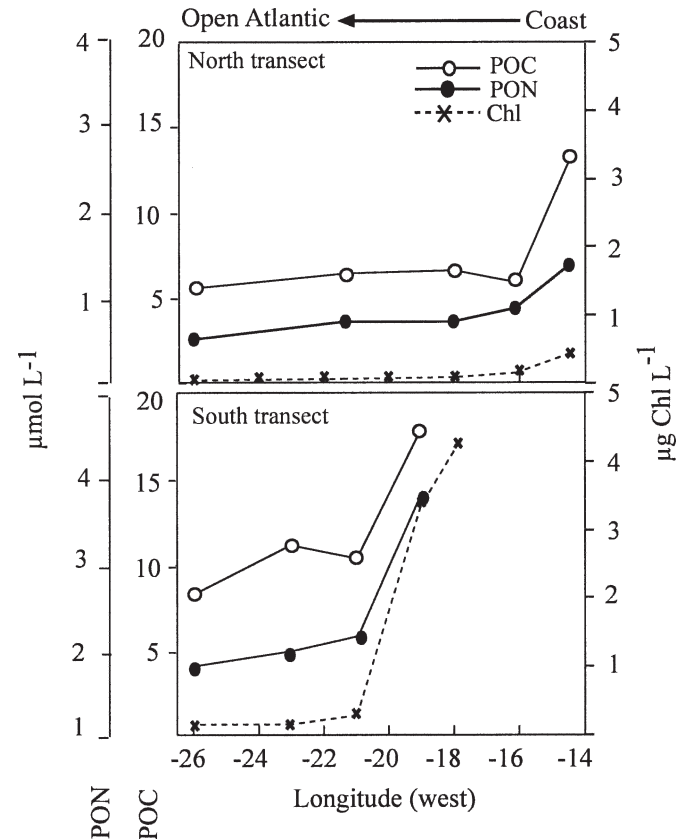


Fig. 2. Measurements of POC, PON, and Chl *a* at surface waters along both transects of COCA II cruise.

generally characterized by higher concentrations of surface Chl *a*, POC, and PON compared with the offshore stations (Fig. 2). During the COCA I cruise, the Cape Blanc upwelling center was placed at a more northern position, and its influence was thus restricted to the most coastal station (Sta. 66, Fig. 1).

**BA and heterotrophic production**—During the COCA II cruise, surface BA ranged  $0.41\text{--}5.65 \times 10^6$  bacteria  $\text{mL}^{-1}$ , being maximal at the stations closer to the Cape Blanc upwelling and lowest at the offshore stations (Fig. 3). Both estimates of BHP ( $^3\text{H}$ -Leu and TdR uptake) were highly correlated through the euphotic zone (one to six discrete depths per profile; Pearson correlation coefficient  $r = 0.89$ ,  $n = 25$ ,  $p < 0.00001$ , log-log transformed) and ranged  $15\text{--}331 \text{ pmol Leu L}^{-1} \text{ h}^{-1}$  and  $1\text{--}40 \text{ pmol TdR L}^{-1} \text{ h}^{-1}$ . The highest surface uptake rates were found at the offshore stations ( $159$  and  $139 \text{ pmol Leu L}^{-1} \text{ h}^{-1}$  in Sta. 32 and 42, respectively, and  $14 \text{ pmol TdR L}^{-1} \text{ h}^{-1}$  in both stations). On the other hand, the highest values of the empirical carbon-to-Leu CFs ( $0.7$  and  $1.29 \text{ kg C mol Leu}^{-1}$ ) were found at the stations closest to the upwelling, with values closer to the theoretical CF of  $1.55 \text{ kg C mol Leu}^{-1}$  (Table 1). The CFs calculated in the offshore stations were lower, averaging ( $\pm\text{SD}$ )  $0.14 \pm 0.07 \text{ kg C mol Leu}^{-1}$  (Table 1).

Applying the empirical CFs, the BHP estimates were generally higher in the upwelling stations (with the

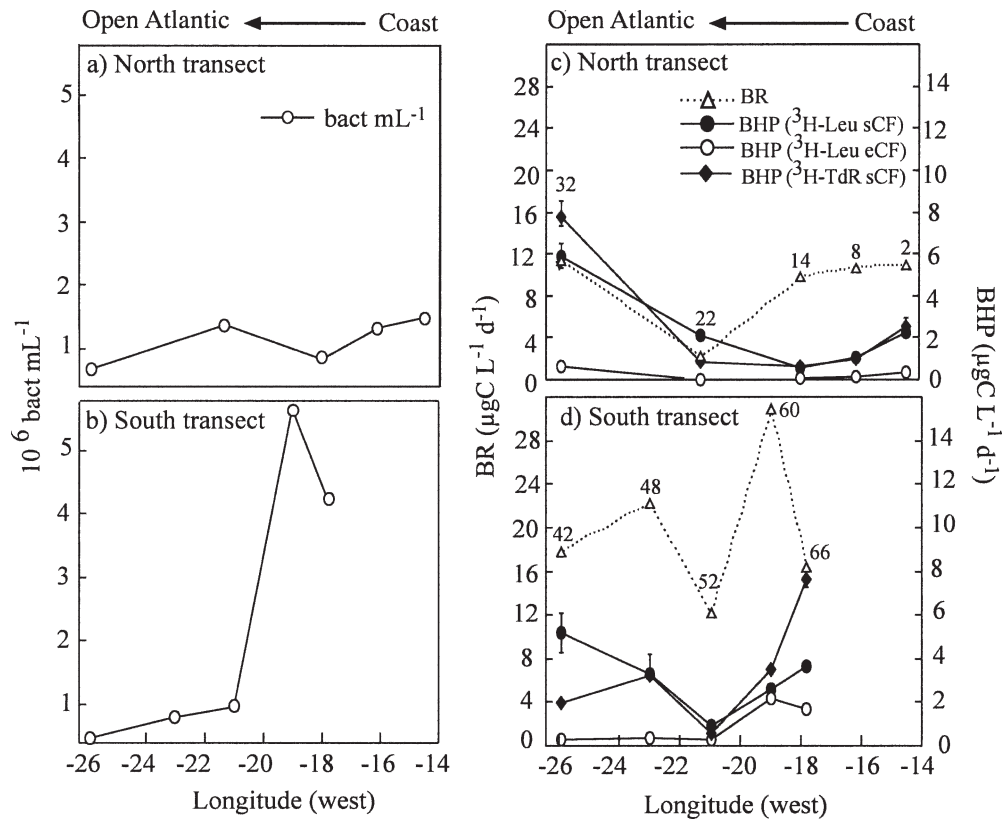


Fig. 3. (a, b) Estimates of bacterial abundance and (c, d) measurements of bacterial respiration (BR) and bacterial heterotrophic production (BHP) estimated from leucine (Leu) uptake (with standard,  $1.55 \text{ kg C mol Leu}^{-1}$ , and empirical conversion factors) and thymidine uptake (with standard conversion factor  $23 \text{ kg C mol TdR}^{-1}$ ) along the two transects (COCA II cruise). Station numbers are included above each corresponding data point in (c) and (d). eCF, empirical conversion factor; sCF, standard conversion factor.

exception of offshore Sta. 32, in the northern transect) and ranged between  $0.03$  and  $2.15 \mu\text{g C L}^{-1} \text{d}^{-1}$  (Fig. 3). In contrast, by using a theoretical factor of  $1.55 \text{ kg C mol Leu}^{-1}$ , the offshore stations at both transects yielded maximum BHP values (Fig. 3c,d), with estimates  $>5 \mu\text{g C L}^{-1} \text{d}^{-1}$ . This difference also affected estimates of integrated BHP in the euphotic zone, which were derived with a theoretical CF and that ranged  $24$ – $581 \text{ mg C m}^{-2} \text{d}^{-1}$ , whereas the range was much lower when the empirical CFs determined in the surface waters were used ( $1$ – $127 \text{ mg C m}^{-2} \text{d}^{-1}$ , Table 2). When we compared all the individual estimates in the euphotic zone, the values of BHP were only significantly higher in the upwelling area if empirical CFs were used (ANOVA  $p < 0.05$ , Tukey–Kramer post hoc test).

*BR, growth efficiency, and carbon demand*—During the COCA I cruise, we analyzed respiration rates of whole unfiltered seawater and the filtered bacteria-size fraction in five stations. The respiration rate in filtered samples averaged ( $\pm$ SE)  $78\% \pm 9\%$  of the total microplankton respiration. The optimal incubation time for respiration measurements was also determined in these experiments. In general, BHP increased during the incubation, whereas the

oxygen consumption rate was not significantly different from a constant decrease during the first 24 h.

During the COCA II cruise, BR was quite constant in the northern transect (around  $10 \mu\text{g C L}^{-1} \text{d}^{-1}$ , Fig. 3c) except for Sta. 22, which showed a lower respiration rate (Table 1). In the southern transect, BR was more variable and significantly higher, with an average value of  $20 \mu\text{g C L}^{-1} \text{d}^{-1}$  (Fig. 3d). The surface BR estimates did not differ significantly between the upwelling and the offshore stations (ANOVA test,  $p > 0.05$ ), although the limited data set (only a single measurement for each station) could probably affect the sensitivity of this analysis. BR was generally higher during the COCA I cruise than during the COCA II cruise, particularly in the northern transect stations (Table 1).

Surface and integrated estimates of bacterial carbon demand (BCD) followed the same pattern of BR and were, in general, rather constant (threefold range excluding Sta. 22; Tables 1 and 2). Similarly, the surface BCD values were higher during the COCA I cruise (range,  $18$ – $64 \mu\text{g C L}^{-1} \text{d}^{-1}$ ) compared with COCA II (range,  $2$ – $33 \mu\text{g C L}^{-1} \text{d}^{-1}$ , Table 1).

We calculated a range of BGE values for each station by using the BR and five different estimates of BHP (see

Table 1. In situ measurements of bacterial respiration (BR), leucine-based bacterial heterotrophic production (BHP), with a theoretical conversion factor (CF) of 1.55 kg C mol Leu<sup>-1</sup> and the empirical CFs determined for each station, and three BHP estimates obtained from the incubations and used to determine BGE: (1) initial BHP (CF 1.55 kg C mol Leu<sup>-1</sup>); (2) integrated BHP in 24 h of incubation calculated with empirical CFs, and change in bacterial biomass (BBM), estimated from cell abundance and a conservative factor of 12 fg C cell<sup>-1</sup> (Fukuda et al. 1998). BGE range was calculated using all BHP estimates and the BGE average was calculated with two BHP estimates (initial time and change in biomass; see text). Bacterial carbon demand (BCD) was estimated as BR + BHP in situ (with empirical CF). Zones are UPW, upwelling; NT, northern transect; ST, southern transect.

| Cruise  | Zone   | Stn. | Depth (m) | BR<br>( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) | Emp. CF<br>(kg C mol Leu <sup>-1</sup> ) | BHP in situ   |   | Integ. BHP<br>Emp. CF<br>( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) | $\Delta\text{BBM}$<br>12 fg C cell <sup>-1</sup><br>( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) | BGE range (%) | BGE avg. (%) | BCD<br>( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) |       |
|---------|--------|------|-----------|--|--|---|---|---|--|---------------|--------------|---|-------|
|         |        |      |           |  |  | Theor. CF<br>( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) | Emp. CF<br>( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) |   |  |               |              |   |       |
| COCA II | UPW    | 2    | 5         | 10.9   | 0.25                                     | 2.2   | 0.36  | 1.9   | —  | 3–17          | —            | 11.2  |       |
|         | NT     | 8    | 5         | 10.6   | 0.21                                     | 1.1   | 0.14  | 0.8   | 4.6  | 1–30          | 16           | 10.7  |       |
|         | NT     | 14   | 5         | 9.7  | 0.21                                     | 0.6   | 0.08  | 1.9   | 2.7  | 1–22          | 11           | 9.8   |       |
|         | NT     | 22   | 5         | 2.2  | 0.02                                     | 2.1   | 0.03  | 0.2   | 0.02   | 1–49          | 1            | 2.2   |       |
|         | NT     | 32   | 5         | 11.3   | 0.16                                     | 5.9   | 0.63  | 1.6   | 1.4  | 2–34          | 7            | 11.9  |       |
|         | ST     | 42   | 5         | 17.7   | 0.10                                     | 5.1   | 0.32  | 0.9   | 4.0  | 2–23          | 11           | 18.0  |       |
|         | ST     | 48   | 5         | 22.2   | 0.17                                     | 3.3   | 0.37  | 2.5   | 4.5  | 2–17          | 11           | 22.6  |       |
|         | UPW    | 52   | 5         | 12.1   | 0.42                                     | 0.9   | 0.26  | 4.0   | 4.4  | 2–27          | 17           | 12.4  |       |
|         | UPW    | 60   | 5         | 30.8   | 1.29                                     | 2.6   | 2.15  | 42.3  | 34.3   | 7–58          | 56           | 32.9  |       |
|         | UPW    | 66   | 5         | 16.4   | 0.70                                     | 3.6   | 1.65  | 6.7   | 2.2  | 9–29          | 17           | 18.0  |       |
|         | COCA I | UPW  | 2         | 80   | 63.9                                     | —   | 0.04  | —   | —  | 3.6           | <1–5         | 3   | 63.9* |
|         |        | NT   | 8         | 80   | 52.5                                     | —   | 0.48  | —   | —  | 2.8           | <1–5         | 3   | 53.0* |
| NT      |        | 32   | 30        | 45.4   | 0.14                                     | 0.51  | 0.05  | 7.3   | 2.4  | <–14          | 5            | 45.4  |       |
| ST      |        | 48   | 50        | 17.4   | 0.36                                     | 2.29  | 0.53  | 13.0  | 2.3  | 3–43          | 11           | 17.9  |       |
| ST      |        | 60   | 40        | 32.8   | 0.08                                     | 0.46  | 0.02  | 2.0   | 6.2  | <1–16         | 14           | 32.8  |       |
|         |        |      |           |  |  |   |   |   |  |               |              |   |       |

\* BHP with theoretical CF was used for calculation because empirical CFs were not estimated.

Table 2. Integrated values through the euphotic layer (1% PAR) of Chl *a*, bacterial biomass (BBM), bacterial heterotrophic production using the standard conversion factor (CF) 1.55 kg C mol Leu<sup>-1</sup> (BHP<sup>1</sup>) or the empirical CFs determined in each station at surface depth (BHP<sup>2</sup>), and bacterial carbon demand during the COCA II cruise. For the BCD calculation, we used the BR estimated for surface waters kept constant, and the BHP measurements with empirical CFs through the euphotic zone (range 3–6 discrete depths, average of 5). The depth corresponds to the limit of the euphotic layer.

| Sta. | Zone | Depth (m) | Integ. Chl <i>a</i> (mg Chl <i>a</i> m <sup>-2</sup> ) | Integ. BBM (g C m <sup>-2</sup> ) | Integ. BHP <sup>1</sup> (mg C m <sup>-2</sup> d <sup>-1</sup> ) | Integ. BHP <sup>2</sup> (mg C m <sup>-2</sup> d <sup>-1</sup> ) | Integ. BCD (mg C m <sup>-2</sup> d <sup>-1</sup> ) |
|------|------|-----------|--|-----------------------------------|---|---|--|
| 2    | UPW  | 73        | 32   | 1.31                              | 24  | 4   | 0.74   |
| 8    | NT   | 96        | 28   | 1.08                              | 325   | 44  | 1.00   |
| 14   | NT   | 97        | 33   | 1.13                              | 94  | 13  | 0.92   |
| 22   | NT   | 116       | 27   | 1.60                              | 103   | 1   | 0.25   |
| 32   | NT   | 114       | 9  | 0.76                              | 581   | 60  | 1.30   |
| 42   | ST   | 100       | 30   | 0.59                              | 379   | 24  | 1.70   |
| 48   | ST   | 85        | 27   | 0.81                              | 225   | 25  | 1.80   |
| 52   | UPW  | 50        | 20   | 0.77                              | 44  | 12  | 0.55   |
| 60   | UPW  | 57        | 54   | 1.03                              | 153   | 127   | 1.48   |
| 66   | UPW  | 60        | 91   | 1.25                              | 137   | 62  | 0.96   |

*Methods*), which yielded quite different results (Table 1). The calculation of BGE can use the in situ values of BHP, the initial BHP of the BR experiment, or the integrated BHP throughout the 24-h incubation. Furthermore, empirical or theoretical CFs can be used to convert Leu uptake into biomass production. Finally, net bacterial production can also be calculated from the increase in bacterial biomass during the BR determinations.

BHP determined at the initial time of the incubation (using the theoretical CF 1.55 kg C mol Leu<sup>-1</sup>) was in the same range than the in situ measurements (i.e., with water directly obtained from the oceanographic bottle) only when the in situ measurements were calculated with the empirical CFs. A remarkable exception was Sta. 60, which experienced a significant increase in both Leu and TdR-based BHP estimates after filtration of the seawater. Similar to in situ samples, Leu- and TdR-based BHP estimates were highly correlated in these samples at time 0 (Pearson  $r = 0.90$ ,  $n = 10$ ,  $p < 0.0004$  log-log transformed, TdR results not shown). BHP integrated during the 24-h incubations, or estimated from the change in BA assuming a conservative CF for marine bacteria of 12 fg C cell<sup>-1</sup> (Fukuda et al. 1998), yielded significantly higher values of biomass production than the initial Leu-based BHP.

The resulting ranges of BGE are quite wide because of the order-of-magnitude differences in the various approaches to calculate BHP. The median lower boundary of the range was 2%, whereas the median upper boundary of the range was 28% across stations during the COCA II cruise (Table 1). We chose to average the BGEs obtained by using the BHP at the initial time of the incubation and the change in biomass during the incubation as the most reliable estimation of BGE (see *Discussion*). Calculated in this way, BGEs were lower for the oceanic stations during the COCA II cruise (range, 1–11%) and higher toward the coast (range, 11–17% with a higher value of 56% at the upwelling Sta. 60). The values of BGEs in the five stations during the COCA I cruise were in the same range (3–14%).

*Protein turnover and Leu respiration*—The Leu-to-carbon CFs obtained (range, 0.02–1.29 kg C mol<sup>-1</sup>, Ta-

ble 1) were low compared with the standard theoretical factor. Intracellular protein turnover and respiration of the added Leu (instead of incorporation) by bacteria were studied as possible explanations for these low empirical factors found offshore.

Protein turnover was measured following the exponential decrease in radioactivity after addition of a Leu cold chase. We only found significant protein turnover, detected when the exponential decrease in radioactivity is statistically significant, at four stations (Table 3). Even at those stations, the rate of protein turnover was low (3–7% of Leu incorporated per hour), indicating that bacteria in our sampling area were not recycling their proteins at a significant rate. Thus, protein turnover was not accounting for the low CFs found in offshore waters.

On the contrary, the proportion of the Leu taken up by the cells that was respired (in a time period of 3 h) was high, especially in offshore stations (Fig. 4). We found a gradient of Leu respiration of <20% to 40% in the upwelling area (of the total Leu taken up by bacteria), compared with 60–80% in offshore waters. We observed an inverse relationship between the percentage of Leu respired and the Leu CF along the gradient, although it was not statistically significant at the  $p = 0.05$  level (Pearson  $r = -0.57$ ,  $n = 10$ ,  $p = 0.08$ ).

*Nutrient limitation bioassays*—Bacterial nutrient limitation was analyzed in both cruises, and the responses are reported as BHP and BR ratios relative to the unamended controls (Tables 4 and 5). During the COCA I cruise, we found BHP limitation (with always a more marked effect on TdR measurements) by inorganic nutrients at all sampling stations except Sta. 14, which showed a higher carbon limitation of BHP. BR was also stimulated by carbon at this station (Table 4). In the rest of the stations, BR was unaffected by the addition of organic nor inorganic nutrients, although at Sta. 42, BR increased by a factor of 2.4 with the addition of both resources simultaneously.

During the COCA II cruise, BHP was primarily stimulated by organic carbon additions in the stations

Table 3. Protein turnover rates calculated for the surface stations during the COCA II cruise with pulse-chase experiments. N, number of time points used to compute the rate of turnover; NS, not significant,  $p > 0.05$ . Zones are UPW, upwelling; NT, northern transect; ST, southern transect.

| Sta. | Zone | N  | Turnover rate ( $\text{h}^{-1}$ ) | SE    | $p$  | Turnover<br>(% of incorporation $\text{h}^{-1}$ ) |
|------|------|----|-----------------------------------|-------|------|---|
| 2    | UPW  | 12 | -0.078                            | 0.028 | 0.03 | 6.51  |
| 8    | NT   | 6  | 0.105*                            | 0.024 | 0.01 | No turnover                                       |
| 14   | NT   | 6  | 0.041                             | 0.017 | NS   | No turnover                                       |
| 22   | NT   | 6  | -0.053                            | 0.011 | 0.01 | 3.56  |
| 32   | NT   | 6  | 0.006                             | 0.021 | NS   | No turnover                                       |
| 42   | ST   | 6  | -0.003                            | 0.016 | NS   | No turnover                                       |
| 48   | ST   | 12 | -0.041                            | 0.019 | 0.02 | 4.78  |
| 52   | UPW  | 6  | -0.002                            | 0.009 | NS   | No turnover                                       |
| 60   | UPW  | 5  | -0.074                            | 0.011 | 0.02 | 7.19  |
| 66   | UPW  | 5  | 0.004                             | 0.044 | NS   | No turnover                                       |

\* Significant increase in label incorporated after addition of cold chase.

closer to the coast (upwelling area and the coastal Sta. 8 and 14). BHP was unaffected by N additions, but P enrichment resulted in significantly higher BHP at the offshore Sta. 22, in the northern transect (Table 4). The oceanic stations in the southern transect (Sta. 42 and 48) did not appear to be severely limited by either organic or inorganic nutrients. However, a strong response of BHP was found at the most oceanic station in the northern

transect (Sta. 32) when both organic and inorganic nutrients were added.

## Discussion

The results presented here identified BHP as a very poorly constrained component of bacterial carbon budgets, largely as a result of discrepancies between empirical and theoretical CFs. The region studied included the North Atlantic subtropical gyre and the northwest African coast upwelling, which differed greatly in dynamics as reflected in a decline in Chl *a*, inorganic nutrients, and POC concentration offshore from the upwelling zone (Barton et al. 1998; Fig. 2). Other parameters not specifically assessed in this study, such as bacterial biomass, also show large uncertainties because of the variability of CFs, as discussed below.

BR estimates (range, 2–64  $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) did not decline offshore and were comparable to values previously reported for oceanic waters such as the eastern Atlantic Ocean (average 26  $\mu\text{g C L}^{-1} \text{d}^{-1}$  taking 0.88 as respiratory quotient, Robinson et al. 2002a,b), the equatorial Atlantic (Morán et al. 2004), and the North Pacific subtropical gyre (average 8  $\mu\text{g C L}^{-1} \text{d}^{-1}$  taking 0.88 as respiratory quotient; Williams et al. 2004). Higher BR estimates and larger variability were found in the southern transect compared with the northern transect, matching a higher POC content in the surface waters at this latitude. This increase in POC is probably due to the influence of the carbon-rich Cape Blanc upwelling waters, which can be advected hundreds of kilometers offshore, as a result of the combined effect of the upwelling filament and the Cape Vert Frontal Zone (see review in Aristegui et al. 2006). The higher BR during the COCA I cruise than the COCA II cruise was consistent with warmer surface temperatures and higher dissolved organic carbon concentrations observed during the COCA I cruise (M. Calleja unpubl. data).

The BHP determinations showed the highest variability throughout the inshore-offshore gradient within a factor of 10 using the theoretical CF, or close to 30 using the empirical CF and excluding Sta. 22, which had an unusually low empirical CF. Leu and TdR incorporation

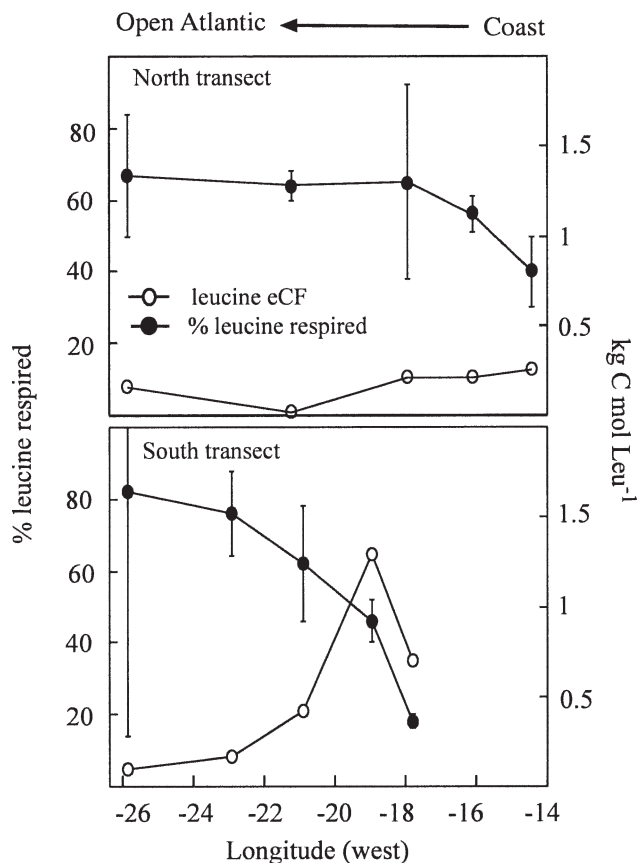


Fig. 4. Percentage of leucine (Leu) respiration (of the total Leu uptake: incorporation into biomass + respiration) and empirically derived carbon-to-Leu conversion factors (Leu eCF) in the COCA II cruise.



Table 4. Ratio of integrated bacterial heterotrophic production ( $^3\text{H}$ -Leu and  $^3\text{H}$ -TdR incorporation) and respiration (change in oxygen concentration,  $\mu\text{mol L}^{-1} \text{O}_2$ ) after different enrichments scaled to unamended control measurements, over 96 hour-long bioassays in the cruise COCA I. The stimulation diagnostic columns are qualitative interpretation of the results, taking a minimum ratio of 2 for a positive response to the addition. C, carbon enrichment (glucose and acetate  $1 \mu\text{mol L}^{-1}$  final concentration each); NP, nitrogen and phosphorus enrichment (ammonium, nitrate, and phosphate  $0.5 \mu\text{mol L}^{-1}$  final concentration each).

| Sta. | Enrich. | Leu-BP | TdR-BP | BR ( $\Delta\text{O}_2$ ) | BHP stimulation  | BR stimulation |
|------|---------|--------|--------|---------------------------|------------------|----------------|
| 8    | C       | 0.88   | 1.06   | 1.44                      | Inorganic        | No effect      |
|      | NP      | 1.44   | 2.23   | 1.57                      |                  |                |
|      | CNP     | 1.42   | 2.21   | 1.57                      |                  |                |
| 14   | C       | 1.80   | 5.31   | 4.59                      | C<br>(Inorganic) | C              |
|      | NP      | 1.44   | 2.32   | 1.20                      |                  |                |
|      | CNP     | 1.17   | 1.64   | 1.48                      |                  |                |
| 32   | C       | 1.12   | 2.13   | 1.00                      | Inorganic<br>(C) | No effect      |
|      | NP      | 1.64   | 9.95   | 1.11                      |                  |                |
|      | CNP     | 1.92   | 12.34  | 1.10                      |                  |                |
| 42   | C       | 1.12   | 1.00   | 1.23                      | Inorganic        | Co-stimulation |
|      | NP      | 1.46   | 2.78   | 1.07                      |                  |                |
|      | CNP     | 1.11   | 2.93   | 2.42                      |                  |                |
| 52   | C       | 1.09   | 1.04   | 1.10                      | Inorganic        | No effect      |
|      | NP      | 1.16   | 2.63   | 0.89                      |                  |                |
|      | CNP     | 1.31   | 2.30   | 1.00                      |                  |                |

rates were remarkably higher in offshore stations compared with the upwelling, a pattern that has been reported in other studies (Barquero et al. 1998). However, the empirical CFs showed an opposite trend; thus, the use of an standard CF for all stations yielded significantly higher BHP estimates in offshore waters compared with the use of empirical ones.

Surface BHP estimates in the oligotrophic, offshore stations derived by using empirical CFs were similar to other estimates reported for the equatorial Atlantic, where they used an empirical CF of  $0.73 \text{ kg C mol Leu}^{-1}$  (Morán et al. 2004). Our average ( $\pm\text{SE}$ ) integrated BHP in the euphotic zone with the empirical CFs was  $37 \pm 13 \text{ mg C m}^{-2} \text{ d}^{-1}$ , comparable to the values reported by Agustí et al. (2001) for the same area in the eastern North Atlantic by using an empirical CF of  $0.58 \text{ kg C mol Leu}^{-1}$ , and the values reported by Barbosa et al. (2001) off the northwest Iberian margin by using an empirical CF of  $0.48 \text{ kg C mol Leu}^{-1}$ . In contrast, the use of the theoretical CF would yield extremely high integrated BHP estimates,  $>300 \text{ mg C m}^{-2} \text{ d}^{-1}$  in our oceanic stations.

*Meaning of the empirical CFs and implications of Leu processing*—Simon and Azam (1989) calculated a theoretical carbon-to-Leu CF on the basis of constant cellular ratios of protein/dry weight and carbon/dry weight, and the measurement of an isotope dilution factor of 2 in coastal waters. Even if we assume no isotope dilution, the resulting CF ( $1.55 \text{ kg C mol Leu}^{-1}$ ) is higher than most of the CF calculated for open waters in our study (range,  $0.02$ – $1.29 \text{ kg C mol Leu}^{-1}$ ) as well as in others (e.g., Carlson and Ducklow 1996; Ducklow et al. 1999; Zubkov et al. 2000). We found a clear tendency for CFs to decrease from inshore to offshore stations, similar to those reported by Pedrós-Alió et al. (1999) in the Mediterranean Sea, and Sherr et al. (2001) in the northeast Pacific. These patterns along trophic gradients suggest that CF values are ecologically constrained and are not an artifact caused by the enclosure effect of regrowth incubations (Massana et al. 2001).

Despite the evidence that empirical CFs show a broad variability (e.g., 21-fold in Leu CF in the subarctic Pacific; Kirchman 1992), we are still far from understanding what

Table 5. Ratios of leucine-based bacterial production measurements after 24-h incubation with additions of phosphate alone (P,  $0.5 \mu\text{mol L}^{-1}$  final concentration), ammonium and nitrate alone (N,  $0.5 \mu\text{mol L}^{-1}$  final concentration each), carbon alone (C, glucose and acetate  $1 \mu\text{mol L}^{-1}$  final concentration each) and combined phosphate, nitrogen, and carbon enrichments (CNP) during the COCA II cruise. Values are relative to the unenriched controls. The diagnostic column is a qualitative interpretation of the results taking a minimum ratio of 2 for a positive response to the nutrient addition.

| Sta. | N               | P               | C                | NPC              | Diagnostic    |
|------|-----------------|-----------------|------------------|------------------|---------------|
| 8    | $0.87 \pm 0.14$ | $2.26 \pm 0.18$ | $32.59 \pm 1.74$ | $11.29 \pm 0.62$ | C (P)         |
| 14   | $1.05 \pm 0.14$ | $1.25 \pm 0.17$ | $3.27 \pm 0.42$  | $7.47 \pm 0.96$  | C             |
| 22   | $0.90 \pm 0.36$ | $4.46 \pm 0.36$ | $1.69 \pm 0.36$  | $5.48 \pm 0.36$  | P             |
| 32   | $1.03 \pm 0.13$ | $1.33 \pm 0.16$ | $0.91 \pm 0.12$  | $52.87 \pm 5.2$  | Colimitation  |
| 42   | $0.70 \pm 0.10$ | $1.03 \pm 0.10$ | $1.09 \pm 0.10$  | $1.76 \pm 0.10$  | No limitation |
| 48   | $0.81 \pm 0.01$ | $1.11 \pm 0.01$ | $0.93 \pm 0.01$  | $1.04 \pm 0.01$  | No limitation |
| 52   | $1.03 \pm 0.04$ | $0.92 \pm 0.04$ | $2.37 \pm 0.04$  | $2.86 \pm 0.04$  | C             |
| 60   | $1.19 \pm 0.06$ | $1.03 \pm 0.06$ | $3.88 \pm 0.07$  | $3.42 \pm 0.07$  | C             |
| 66   | $0.95 \pm 0.04$ | $1.04 \pm 0.04$ | $2.39 \pm 0.04$  | $1.90 \pm 0.04$  | C             |

controls these changes. For instance, Leu CF was unaffected by additions of organic compounds or ammonium in the subarctic Pacific (Kirchman 1992). Our results and previously reported CFs for oligotrophic open-ocean waters indicate that the theoretical CF can grossly overestimate BHP in oligotrophic waters. Isotope dilution has been repeatedly measured in different systems to constrain the upper limit of the empirical CF (Simon and Azam 1989; van Looij and Riemann 1993), but almost no studies have tried to assess the reasons for the low CF found in offshore waters. We examined two possible explanations for the low CFs from Leu incorporated into biomass production in oligotrophic waters:

1. Leu is incorporated into the cellular protein, but bacteria exhibit a significant protein turnover, leading to an overestimation of net incorporation. Proteins can be synthesized and continuously degraded (turnover) at high rates in selected growth conditions. If protein turnover occurs at a high rate, radioactivity can be incorporated into protein even if the net rate of protein synthesis is zero. Kirchman et al. (1986) estimated the protein turnover by marine bacteria in a salt marsh estuary and found rates between 0.012 and 0.311 h<sup>-1</sup>. We performed the same type of experiments in our sampling region and found rates in their lower range—between 0.04 and 0.08 h<sup>-1</sup> (Table 2). Hence, bacterial protein turnover is slow in the oligotrophic oceanic waters studied, so it is thereby unlikely to affect CFs.

2. An alternative explanation for the low CFs could be nonspecific incorporation of Leu into proteins and/or Leu catabolism. Although L-Leu has been usually shown to be incorporated only into the protein fraction (Kirchman et al. 1985), some discrepancies among macromolecular fractionation of labeled Leu or TdR in TCA precipitates have also been described (Torréton and Bouvy 1991). <sup>3</sup>H-Leu has been seen to precipitate in cold TCA even if it is not part of the protein fraction (Wicks and Robarts 1988; Hollibaugh and Wong, 1992). These authors showed that an ethanol rinse could remove a high proportion of <sup>3</sup>H-Leu (and also TdR) probably associated to cell membrane lipids as a storage mechanism. If Leu enters the cell but it is not used for protein biosynthesis, the radioactivity measured inside the cells can overestimate the biomass production calculated with the assumptions of theoretical CFs.

Kirchman et al. (1985) found that, on average, 90% of the Leu taken up by bacteria was incorporated into protein, and <20% was degraded to other amino acids in several aquatic systems. However, they also found that Leu degradation was much greater in less productive waters, reaching values of 49% of Leu degradation in two Gulf Stream samples. In studies measuring the uptake and respiration of specific <sup>14</sup>C- or <sup>3</sup>H-labeled amino acids, Leu is usually respired very slowly (Williams et al. 1976; Suttle et al. 1991), but higher percentages of Leu respiration (>20%) have also been described in freshwater environments (Hobbie and Crawford 1969; Sepers 1981). Jørgensen (1992) found some of the highest reported values of Leu respiration (78–91%) in two eutrophic lakes.

We suggest that a high percentage of the <sup>3</sup>H-Leu that enters the cell is not used for protein synthesis in energy-

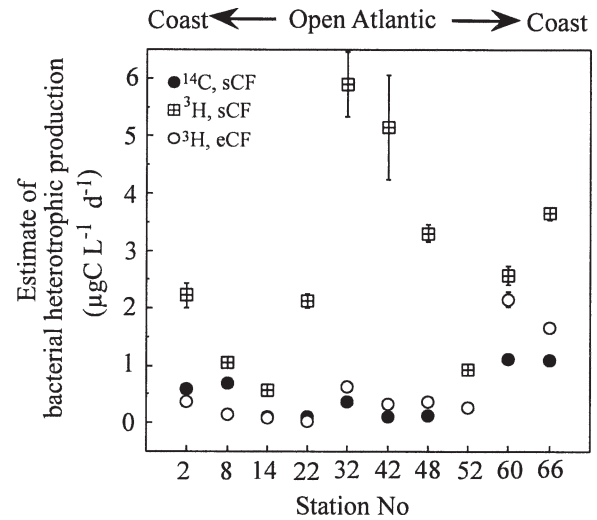


Fig. 5. BHP estimates based on carbon (<sup>14</sup>C)- or tritium (<sup>3</sup>H)-labeled Leu, and empirical or standard conversion factors. eCF, empirical conversion factor; sCF, standard conversion factor.

limited systems, even if it can be detected as biomass production in the timescale of the Leu incorporation radio assay (hours). After incorporation into the cell, Leu could be respired. This process would lead to a lower carbon-to-Leu CF than the theoretical one, which assumes that Leu is integrally incorporated into protein and used for biomass production.

In order to test this hypothesis, we measured the respiration and assimilation of <sup>14</sup>C-labeled Leu along the productivity gradient. We found an increasing gradient of respiration of Leu offshore, opposite to the pattern of decreasing CFs offshore, which is consistent with the idea that Leu catabolism could be related to the low CFs. Interestingly, BHPs estimated from the incorporation of <sup>3</sup>H-[4,5]-Leu were significantly higher than those measured with uniformly labeled <sup>14</sup>C-Leu by using the standard CF (1.55 kg C mol Leu<sup>-1</sup>) for both estimates (Fig. 5). This suggests that <sup>3</sup>H-Leu incorporation rate could overestimate biomass production even in instantaneous measurements (timescale of hours).

Because the concentration used for both substrates was saturating (40 nmol L<sup>-1</sup> for <sup>3</sup>H- and 60 nmol L<sup>-1</sup> for <sup>14</sup>C-Leu) and the incubation time (2–4 h) was similar, the differential molecular labeling is the most probable reason for the differences found. Jørgensen (1992) and Kuparinen (1984) found similar rates of incorporation of <sup>3</sup>H- and <sup>14</sup>C-labeled Leu and glucose, respectively. However, <sup>3</sup>H from different carbon position in substrates such as TdR (Hollibaugh 1994) or glutamic amino acid (Carlucci et al. 1986) have been shown to follow different metabolic pathways (incorporation to macromolecules or catabolism). If [4,5-<sup>3</sup>H] Leu (as it is usually used for routine BHP measurements) is degraded in the cell following the general catabolic pathway, the amino acid could initially be oxidized to CO<sub>2</sub> and acetyl-CoA (which can be further oxidized in the Krebs cycle) without losing most of the tritium signal. This could explain the differences between

$^3\text{H}$ - and  $^{14}\text{C}$  Leu-based BHP estimates because the use of uniformly labeled  $^{14}\text{C}$ -Leu could correct for Leu catabolism as the label would disappear as  $^{14}\text{CO}_2$ . In agreement with this hypothesis, BHP estimates with  $^3\text{H}$ -Leu were significantly higher than the  $^{14}\text{C}$ -Leu estimates by using the theoretical CF for both compounds (Fig. 5). On the contrary, BHPs calculated with  $^3\text{H}$ -Leu by using empirical CFs (that would correct for Leu catabolism) were in the range and positively correlated to the  $^{14}\text{C}$ -Leu incorporation-based BHP estimates by using the theoretical CF (Pearson  $r = 0.84$ ,  $n = 10$ ,  $p = 0.002$ ).

Clearly, more research should be done focusing on the metabolic pathway followed by [4,5]- $^3\text{H}$ -Leu into the cell in order to constrain present uncertainties on this measurement as an accurate estimator of BHP in oligotrophic systems. Further comparisons between the respiration of  $^{14}\text{C}$ -Leu and [4,5]- $^3\text{H}$ -Leu in oligotrophic systems, or the empirical CFs found for both compounds would help resolve this question. However, our results make clear that incorporation of [4,5]- $^3\text{H}$ -Leu can overestimate carbon production unless corrected with empirical CFs and emphasize the importance of estimating CFs in oceanic waters.

*BGE estimation along the productivity gradient in north-west subtropical Atlantic waters*—Because most of the carbon that bacteria consume in oceanic waters is generally respired, the understanding of BGE variability would be crucial to predict bacterial carbon flux. However, BGE calculation is not trivial, given the different timescales required for the BR and BHP measurements. BHP can be measured almost instantaneously (minutes to hours), but direct estimates of BR require a minimal incubation time of 24 h in oceanic waters (cf. Aristegui et al. 2005).

There is a high diversity of approaches for BGE calculation in open ocean and coastal marine waters in the literature (see *Web Appendix 1*: [http://www.aslo.org/lo/toc/vol\\_52/issue\\_2/0533.pdf](http://www.aslo.org/lo/toc/vol_52/issue_2/0533.pdf)), including different experimental settings (regrowth experiments or incubation of undiluted water), different methods for BHP and BR estimation, and CFs. All of these differences can greatly affect the BGE estimates. As an example, Briand et al. (2004) found higher estimates of BGE by using the increment of POC as BHP measurements compared with the use of TdR-based BHP in the same experiment, similar to our results.

Besides empirical CFs for BHP measurements, CFs for bacterial biomass determination (i.e., carbon content per cell or volume) show a rather wide range, which can also affect the uncertainty in BGE estimates. In this work, the average biomass CF calculated by Fukuda et al. (1988) for oceanic bacteria was used (i.e., 12 fg C cell $^{-1}$ ). This factor is lower than widely used factors in this type of studies (15–20 fg C cell $^{-1}$ ; see *Web Appendix 1*), but still higher than other values derived in different studies based on low carbon-to-volume factors (Carlson et al. 1999), including samples from our oceanic region (7 fg C cell $^{-1}$ , Zubkov et al. 2000). The use of this lower CF (7 fg C cell $^{-1}$ ) for our bacterial biomass measurements would have produced, on average, 25% lower BGE estimates.

Different choices of BHP estimations produced an order-of-magnitude range of possible BGE values for any one sample (Table 1), and it thus constitutes the factor of highest uncertainty for BGE calculation. Filtration had a moderate effect on BA and respiration. On the contrary, Leu uptake rates were highly affected at the initial time of the incubation, showing significantly lower estimates compared with those of in situ. This effect has been related to the uncoupling between the members of the microbial food web (Obernosterer et al. 2003). The application of the low empirical CFs found for these initial measurements is probably not correct because of the significant changes in bacterial activity after filtration and would yield unrealistically low values of BHP. Hence, we decided to use the theoretical CF (1.55 kg C mol Leu $^{-1}$ ) for the BHP estimate at time 0, in an attempt of applying the most reliable estimate of BHP for the BGE determination.

Because we had filtered our samples for the determination of BR, we considered that the in situ BHP (from unfiltered seawater) was not appropriate for the BGE estimate. We chose to use the average of the two most common ways of calculating BGE: the initial BHP, and the change in bacterial biomass throughout the incubation. The result allowed us to conclude that BGE was higher in the upwelling area (average 30%) compared with offshore waters (average 9%).

Higher BGEs in upwelling waters are in agreement with the large-scale correlations between Chl *a* and BGE (del Giorgio and Cole 1998) because higher growth efficiencies can be expected when bacteria are growing on phytoplankton exudates. However, BGE was independent of dissolved organic carbon or dissolved organic nitrogen concentrations, or C:N ratio of the dissolved organic matter pool in our samples (M. Calleja unpubl. data).

Our BGE values in the offshore stations (average 9%) are comparable to the average of published BGE estimates for open waters (12%; see *Web Appendix 1*). A similar tendency for lower growth efficiencies in oligotrophic relative to eutrophic waters has been reported in freshwater and marine systems (del Giorgio and Cole 1998) and could be associated to the increased maintenance costs relative to total carbon uptake in low-nutrient waters (Biddanda et al. 2001).

*Effect of nutrient availability on BGE*—It has been reported that BGE may be affected by substrate concentration and composition (Pomeroy and Wiebe 2001) and by inorganic nutrient availability (Kroer 1993). Del Giorgio and Cole (1998) pointed that phosphorus and iron, but not nitrogen, could control BGE in marine systems.

BHP can be limited by organic carbon (Kirchman 1990), nitrogen, or phosphorus (Pomeroy et al. 1995) in different oceanic regions. However, the factors controlling BHP could be very different from those that regulate BR (Smith and Kemp 2003) and very few studies have assessed the effect of nutrients on BR. We found that, independently of the factor limiting BHP, BR was generally unaffected by inorganic or organic additions, in contrast to the results of Pomeroy et al. (1995) and Obernosterer et al. (2003), who found BR to be primarily P-limited.

We found spatial and temporal variation in the type of BHP limitation. During the COCA I cruise, when the Cape Blanc upwelling waters affected a very reduced zone, most of the stations showed inorganic nutrient limitation of BHP (presumably phosphorus limitation because nitrogen alone never had any effect on BHP during the COCA II cruise). This agrees with other studies performed in the Atlantic Ocean, where phosphorus limitation of bacteria has been encountered (Cotner et al. 1997; Rivkin and Anderson 1997).

During the COCA II cruise, when the upwelling affected a much larger area, the coastal (Sta. 8 and 14) and upwelling-affected stations (Sta. 2, 52, 60, and 66) showed carbon limitation. The high inorganic nutrient concentrations supplied by the upwelled waters could have switched bacterial inorganic nutrient limitation to carbon limitation. In other nutrient-rich areas, such as Georges Bank (Caron et al. 2000), the California upwelling (Kirchman et al. 2000), or the Southern Ocean (Church et al. 2000), BHP has also been reported to be carbon limited.

In the oceanic stations, we found co-limitation or no significant limitation by inorganic or organic treatments. The lack of evidence for resource limitation is consistent with the idea of a tightly coupled microbial food web, and the suggestion of grazer control of bacterioplankton (Ducklow and Carlson 1992; Gasol et al. 2002), which would provide the organic and inorganic nutrients needed for growth. In agreement with this hypothesis, the protozoan grazing effect was generally higher at the offshore stations (average 123% of BHP in Sta. 32, 42, 48), whereas it was lower in the upwelling area (average 70%, Vaqué et al. unpubl. data).

BGE was highest at the coastal stations where carbon limitation (and no inorganic nutrient limitation) was found. This suggests that when supplied with inorganic nutrients, the organic carbon would be more efficiently converted into biomass. As an example, Sta. 22, the only station where clear phosphorus limitation of BHP was found in the COCA II cruise, bacteria showed the lowest growth efficiency and empirical CF of all stations sampled. Similarly (although in an estuarine system, Chesapeake Bay), Smith and Kemp (2003) found higher BGE in a carbon-limited zone (Upper Bay, 39%), lower BGE in a phosphorus limited zone (Lower Bay, 24%), and intermediate BGE in a resource sufficient zone (Mid Bay, 28%).

*Relationship between empirical CFs and Leu respiration with BGE*—The lower BGE and higher percentages of Leu respiration in the more oligotrophic areas are consistent with the concept of energy-limited and growth-constrained bacteria. Bacteria in oligotrophic waters may need a high energy flux in the cell in order to maintain the energization of cell membranes and their uptake systems active to react to the low nutrient inflow (Ishida et al. 1986) or whenever environmental conditions change (del Giorgio and Cole 1998). Indeed, cell-specific maintenance requirements appear to be higher in oligotrophic areas with very low concentration of organic substrates and nutrients (del Giorgio and Cole 1998).

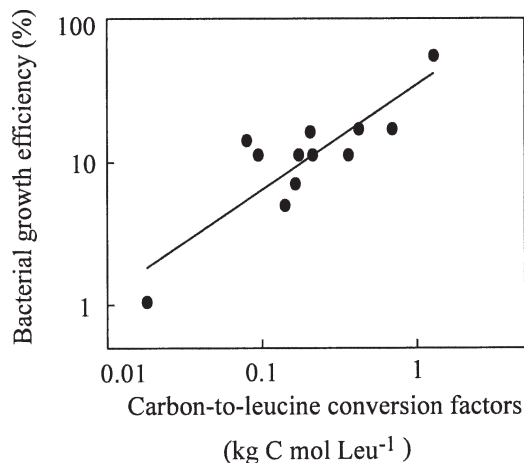


Fig. 6. Relationship between empirically derived leucine-to-carbon conversion factors and bacterial growth efficiency for data of cruises COCA I and II.

Leu respiration was very weakly correlated with total BR, and Leu incorporation efficiency was not significantly correlated to in situ BGE. Although the efficiency in the incorporation of single substrates has been assumed to be representative of in situ BGE in early studies, extrapolation from single compounds may lead to significant overestimation of natural BGE (del Giorgio and Cole 1998).

On the contrary, BGEs were highly correlated with empirical CFs (Pearson  $r = 0.86$ ,  $n = 12$ ,  $p < 0.0004$ , log-log transformed, Fig. 6) supporting the idea that both parameters reflect basically the same physiological processes. Because a theoretical CF ( $1.55 \text{ Kg C mol Leu}^{-1}$ ) was used for the BHP estimate in the calculation of the BGE, this relationship is not based on an autocorrelation. If low CFs were explained by Leu catabolism, as we suggest, then CFs would be measuring the amount of substrate incorporated that is assimilated into biomass, similarly to BGE. If this relationship is confirmed in subsequent studies, BGEs could be predicted from empirical CFs for oceanic bacterial communities.

In conclusion, bacterial communities in the upwelling-influenced stations were characterized by higher bacterial biomass, BGE, and significant carbon limitation. However, the patterns of BR and BHP were not as clear along the inshore-offshore productivity gradient. BR values showed low variability compared with BHP and were generally independent of inorganic or organic nutrient additions, suggesting that this is a rather resilient ecosystem function. The BCD estimates followed the pattern of BR, generally showing a narrow range of  $10\text{--}33 \mu\text{g C L}^{-1} \text{ d}^{-1}$ . The BHP estimates showed higher variability (up to 70-fold) and were highly sensitive to the use of empirical or theoretical CFs. This sensitivity affects the calculations of the growth rates and BGE estimates in open waters. Protein turnover did not significantly affect the low CFs found offshore. Leu respiration, on the contrary, was related to the CFs variability, pointing at Leu catabolism as the process responsible for the low CFs found in open waters. BGE ranged between 1% and 17%, although we found a very high value (56%) in an upwelling station. BGE estimates

were highly correlated with empirical CFs, suggesting that both parameters reflect similar metabolic processes and could be depressed under inorganic nutrient limitation. Our results show that the Leu-to-carbon CFs are ecologically relevant and necessary to derive accurate estimates of BHP in oligotrophic waters. Further demonstration of the consistency and mechanistic link between CFs and BGE could be instrumental in predicting bacterial carbon use in the ocean.

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## Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean

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Web Appendix 1. Review of BGE determinations in marine waters, with special interest in the methods used for determinations.

| Reference                     | Location                        | Type of experiment | BP method                                  | BR/BCD method                        | Biomass factors<br>(pg C $\mu\text{m}^{-3}$ fg C cell $^{-1}$ ) |      | Leu factor<br>(kg C mol Leu $^{-1}$ ) | TdR factor<br>(cells mol TdR $^{-1}$ ) | BGE range<br>(%) | BGE avg.<br>(%) |
|-------------------------------|---------------------------------|--------------------|--|--------------------------------------|---|------|---------------------------------------|--|------------------|-----------------|
| Griffith et al. (1990)        | Georgia Bight nearshore         | Nondiluted         | $^3\text{H}$ -TdR*                         | $\Delta\text{O}_2$ †                 | —   | 20   | —                                     | $1.18 \times 10^{18}$                  | —                | 6               |
|                               | Georgia Bight shelf             |                    |  |                                      |   |      |                                       |  |                  | 2               |
| Coffin et al. (1993)          | Santa Rosa Sound                | Nondiluted         | $\Delta\text{POC}$ ‡                       | $\Delta\text{O}_2$                   | —   | —    | —                                     | —                                      | 0.4–35           | 16              |
| Kroer (1993)                  | Santa Rosa Sound                | Regrowth           | $\Delta\text{POC}$                         | $\Delta\text{DOC}$                   | —   | —    | —                                     | —                                      | 26–33            | 30              |
|                               | Gulf of Mexico                  |                    |  |                                      |   |      |                                       |  |                  | 61              |
| Biddanda et al. (1994)        | Louisiana shelf Louisiana slope | Nondiluted         | $^3\text{H}$ -Leu§                         | $\Delta\text{O}_2$                   | —   | 20   | 2.3                                   | —                                      | 38–55            | 45              |
| Toolan (2001)                 | Massachusetts Bay               | Nondiluted         | $\Delta\text{POC}$ ‡                       | $\Delta\text{CO}_2$                  | —   | 15   | —                                     | —                                      | 0–69             | 24              |
|                               |                                 |                    |  |                                      |   |      |                                       |  |                  |                 |
| Gonzalez et al. (2003)        | Bay of Biscay                   | Nondiluted         | $^3\text{H}$ -Leu                          | $\Delta\text{O}_2$                   | —   | 20   | 0.77                                  | —                                      | 1–8              | 4               |
|                               |                                 |                    | $^3\text{H}$ -Leu¶                         |                                      |   |      |                                       |  |                  | 0.7–2           |
| Robinson et al. (2002)        | North Sea                       | Nondiluted         | $\Delta\text{POC}$ ‡                       | $\Delta\text{CO}_2$                  | —   | —    | —                                     | —                                      | 18               | 18              |
| Briand et al. (2004)          | New Caledonia                   | Nondiluted         | $\Delta\text{POC}$ ‡                       | $\Delta\text{O}_2$                   | —   | 20   | —                                     | —                                      | 1–42             | 21              |
|                               |                                 |                    | $^3\text{H}$ -TdR                          | $\Delta\text{O}_2$                   | —   | —    | —                                     | $2.9 \times 10^{18}$                   | 1–20             | 10>             |
| Reinthalder and Herndl (2005) | Southern North Sea              | Nondiluted         | $^{14}\text{C}$ -Leu¶                      | $\Delta\text{O}_2$                   | —   | 20   | 1.4                                   | —                                      | 5–28             | 19              |
| Shelf waters                  | Total                           |                    |  |                                      |   |      |                                       |  | 0.4–69           | 20              |
| Hansell et al. (1995)         | Sargasso Sea                    | Nondiluted         | $^3\text{H}$ -TdR#                         | $\Delta\text{CO}_2$                  | —   | 9    | —                                     | $1.7 \times 10^{18}$                   | 4–9              | 6               |
| Cherrier et al. (1996)        | Eastern North Pacific           | Nondiluted         | $\Delta\text{POC}$ ‡                       | $\Delta\text{DOC}$                   | —   | 19.6 | —                                     | —                                      | 1                | 1               |
| Kahler et al. (1997)          | Southern Ocean                  | Nondiluted         | $\Delta\text{POC}$                         | $\Delta\text{DOC}$                   | 0.28  | —    | 1.23                                  | $1.2 \times 10^{18}$                   | 26–30            | 28              |
| Sherry et al. (1999)          | Subarctic NE Pacific            | Nondiluted         | $^3\text{H}$ -TdR and $^{14}\text{C}$ -Leu | $\Delta\text{O}_2$                   | —   | 20   | 2.2                                   | $2 \times 10^{18}$                     | 4–31             | 12              |
|                               |                                 |                    |  |                                      |   |      |                                       |  |                  |                 |
| Lemée et al. (2002)           | NW Mediterranean                | Nondiluted         | $^3\text{H}$ -LIR                          | $\Delta\text{O}_2$                   | —   | 15   | 1.5                                   | —                                      | 1–43             | 11              |
| Kirchman et al. (1991)        | North Atlantic                  | Regrowth           | $\Delta\text{POC}$ ‡                       | $\Delta\text{DOC}$                   | 0.14  | 0.5  | —                                     | —                                      | 1.6–9            | 5††             |
| Carlson and Ducklow (1996)    | Sargasso Sea                    | Regrowth           | $\Delta\text{POC}$ ‡**                     | $\Delta\text{DOC}$                   | 0.12  | —    | —                                     | —                                      | 7–19             | 14              |
| Carlson et al. (1999)         | Southern Ocean                  | Nondiluted         | $\Delta\text{POC}$ ‡‡                      | $\Delta\text{CO}_2/\Delta\text{DOC}$ | 0.10  | —    | —                                     | —                                      | 9–38             | 20              |
| Open ocean                    | Total                           |                    |  |                                      |   |      |                                       |  | 1–43             | 12              |

\* In situ measurement.

† A total of 90% of oxygen consumption in the filtered treatment.

‡ Change of bacterial biomass.

§ Time (averaged).

|| Initial measurement (time 0) from the unfiltered fraction.

¶ Initial measurement (time 0) from the filtered fraction.

# Unspecified time.

\*\* BGE calculated taking into account log phase and total incubation.

†† Average BGE between two estimates with different carbon-to-volume conversion factors.

‡‡ Estimated either from change of bacterial biomass or derived from the mass balance between POC, DOC, and BR.

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